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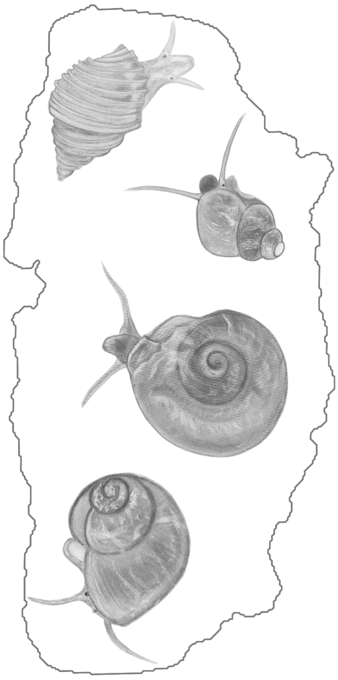

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Torsten Hauffe: Processes of eco-evolutionary metacommunity assembly in insular ecosystems with special emphasis on ancient Lake Ohrid

“Any sufficiently advanced technology is
indistinguishable from magic”
— Arthur C. Clarke, *Hazards of Prophecy*

Dedicated to my mother

Contents

Part I

Synthesis

1 | Rationale

In the fields of ecology and evolution, a widespread phenomenon is scale dependence where inferred patterns or processes change upon an increase in spatial, temporal, or taxonomic scale[1–4]. In fact, to understand how community level processes scale up and imprint evolutionary dynamics is considered to be one of the major challenges of eco-evolutionary research[5,6]. Insular ecosystems are suitable systems to study the eco-evolutionary intersection due to ecological, evolutionary, and geological processes occurring on similar timescales[7].

Island Biogeography Theory (IBT[8–10]) postulates that available niche space regulates insular biodiversity through a diversity-dependent feedback on immigration, speciation, and extinction. Available niche space is the difference between maximum species richness of an insular ecosystem (i.e., carrying capacity), mainly determined by area and topography, and its current richness. Area and topography change with the life cycle of an insular ecosystem, and therefore IBT suggests that available niche space peaks at a moment in time and induces a hump-shaped trajectory of species richness and endemism through time[10]. Moreover, IBT proposes the mechanism of how available niche space affects immigration, speciation, and extinction through, in a very broad sense, competitive interaction among species for ecological resources and/or geographical space[5]. This competitive interaction process occurs on short time scale and at the level of the metacommunity (i.e., local communities in different patches linked by the dispersal of interacting species[11]). It should therefore also shape metacommunity structure by affecting the assembly of local communities, which itself is pivotal for diversification[12].

Simulation studies confirmed the causal relationship between the life cycle of an insular ecosystem and biodiversity trajectories driven by ecological processes at the metacommunity level[13–15]. However, there is limited empirical evidence supporting the ‘competitive interaction shape evolutionary dynamics’ notation derived from the same taxon of a single insular ecosystem with a reconstructed environmental history and inferred long-term biodiversity patterns and short-term metacommunity processes[16]. This thesis seeks for evidence on whether trajectories of species richness and endemism through time are shaped by short-term processes determining the co-existence of species.

Firstly, I review the metacommunity processes suggested by evolutionary studies of insular ecosystems and whether those could be demonstrated for the taxa of interest. Secondly, I complement this by designing biodiversity scenarios conditioned on the limnological history of the Balkan Lake Ohrid, its gastropod richness and endemism, and different metacommunity assembly processes in order to compare the emerging biodiversity trajectories with empirical ones and known assembly processes of gastropod communities. This will help to understand whether competitive interaction among species for ecological resources and/or geographical space shape insular richness and endemism through time as IBT predicts. Lastly, I discuss challenges in causally linking metacommunity processes with long-term biodiversity patterns and propose methodological approaches to achieve this. This review and the suggested methodology contribute to the aim of ‘inferring the driving forces of biotic evolution in Lake Ohrid’[**17**][1].

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[1] Bold references indicate my participation

2 | Paper summary

Seven papers evaluate processes of mollusk metacommunity assembly and whether these influence evolutionary dynamics as proposed by theories of island biogeography. Two reviews provide the approach and background to link the eco-evolutionary dynamics with the limnological history of Lake Ohrid.

Hauffe *et al.* Spatially explicit analysis of gastropod biodiversity in ancient Lake Ohrid. *Biogeosciences* **8**, 175–188 (2011).

> Finding: The first detailed species richness map of an ancient lake showed evolutionary hotspots linked to hydrological features. Correlative analyses demonstrated how ecological processes shape gastropod communities through species sorting along environmental gradients but also indicated ecological neutral dispersal limitation.
>
> Contribution: Lead author; conceiving the study; material collection; data analyses.

Hauffe *et al.* Assembly processes of gastropod community change with horizontal and vertical zonation in ancient Lake Ohrid: a metacommunity speciation perspective. *Biogeosciences* **13**, 2901–2911 (2016).

> Finding: A novel process-based model of community assembly through dispersal limitation, environmental filtering, and competitive interaction revealed a vertical and horizontal structure in process importance.
>
> Contribution: Lead author; conceiving the study; material collection; developing and performing the novel statistical analysis.

Albrecht *et al.* Sediment core fossils in ancient Lake Ohrid: testing for faunal change since the Last Interglacial. *Biogeosciences* **7**, 3435–3446 (2010).

> Finding: Major drivers and processes of mollusk community assembly remained unchanged over periods of substantial environmental change, which may mitigate extinction risk.
>
> Contribution: Participating in drafting the research question; material collection and species identification; analyses of paleoenvironmental conditions; authoring sections on community composition and discussion.

Albrecht *et al.* Mollusc biodiversity in a European ancient lake system: lakes Prespa and Mikri Prespa in the Balkans. *Hydrobiologia* **682**, 47–59 (2011).

> Finding: Lake Ohrid shows biogeographic affinity with the Western Balkan, constituting the main source of species immigration.
>
> Contribution: Material collection and species identification; data analyses; participating in paper writing.

Albrecht *et al.* Invaders versus endemics: alien gastropod species in ancient Lake Ohrid. *Hydrobiologia* **739**, 163–174 (2014).

> Finding: Invasive species may serve as model organisms for colonization processes. Here, they demonstrated that immigration into Lake Ohrid is rare but ongoing. It occurs without strong competitive interactions with the incumbent fauna and is restricted to areas of environmental change.
>
> Contribution: Material collection, species identification, and indicating the invasion; participating in paper writing.

Schreiber *et al.* The role of barriers and gradients in differentiation processes of pyrgulinid microgastropods of Lake Ohrid. *Hydrobiologia* **682**, 61–73 (2011).

> Finding: Environmental gradients shape genetic and morphological divergence among populations and species, suggesting ongoing ecological speciation.
>
> Contribution: Material collection and identification; analyzing the relationship among lake depth, shell shape, and genetic structure; authoring sections on geometric morphometrics.

Föller *et al.* Constant diversification rates of endemic gastropods in ancient Lake Ohrid: ecosystem resilience likely buffers environmental fluctuations. *Biogeosciences* **12**, 7209–7222 (2015).

> Finding: The most species rich taxon of Lake Ohrid diversified with a constant rate during the entire limnological history. Although ecological non-neutral processes shape the metacommunity, we detected no imprint of them on evolutionary dynamics.
>
> Contribution: Material collection and identification; discussing diversification analyses and participating in paper writing.

Wilke *et al.* Scientific drilling projects in ancient lakes: integrating geological and biological histories. *Global and Planetary Change* **143**, 118–151 (2016).

> Finding: This methodological review summarizes the interdisciplinary progress in linking geological and biological approaches to understand how endemic biodiversity in ancient lakes unfolds over time.
>
> Contribution: Authoring section on biological diversification and contributing to chapters about paleolimnological reconstruction and character evolution over time.

Wagner *et al.* The environmental and evolutionary history of Lake Ohrid (FYROM/Albania): interim results from the SCOPSCO deep drilling project. *Biogeosciences* **14**, 2033—2054 (2017).

> Finding: The second review focuses on how the environmental history of Lake Ohrid over the last 637 ka might have affected the evolution of its biota. It builds upon the methods reviewed in paper 8 and integrates the conclusions of papers 1–7.
>
> Contribution: Co-authoring sections on evolutionary history of the lake's biota.

3 | Eco-evolutionary metacommunity assembly in insular ecosystems

3.1. Introduction

The study of island biota as nature's laboratory fueled theory building in the fields of ecology, evolution, and their intersection[18,19]. Eco-evolutionary dynamics are the reciprocal impact of ecological interactions, phenotypic evolution, and lineage diversification on another[20]. According to a bottom-up order (i.e., from individuals organized in local communities to species richness and endemism of a system), the most relevant theories for the eco-evolutionary understanding of insular biodiversity are: the Unified Neutral Theory of Biodiversity and Biogeography (UNTB[21]), the Metacommunity Speciation Model (MSM[12]), and Island Biogeography Theory (IBT[8–10]). Stacked together, these theories suggest that on a long timescale the life cycle of an insular ecosystem induces a hump-shaped trajectory of species richness and endemism through time by modulating on a short timescale competitive interactions among species for ecological resources and/or geographical space. However, all these theories involve necessary simplification and narratives of real-world processes.

I review whether in insular ecosystems the processes shaping the co-existence of species in local communities, including competitive interaction, imprint the species richness and endemism trajectories through time. For this, I (i) summarize how individual-level processes theoretically scale up to shape macroevolutionary dynamics, (ii) compile confirmatory and contradictory evidence for such a mechanistic upscaling derived from simulation experiments and compared this with empirical studies from several insular ecosystems, putting specific emphasis on gastropods of the model system Lake Ohrid (Box 1), and (iii) outline future avenues for enhancing the understanding of the consequences of metacommunity processes on short timescales on evolutionary dynamics over long timescales.

3.2. Dynamics of insular biodiversity

Unified Neutral Theory of Biodiversity and Biogeography

Through analytical solutions or simulations, the UNTB describes species co-existence by a simple, ecological neutral setting where (i) all individuals of all species in a metacommunity share the same probability of dying, reproducing, dispersing, or speciating (i.e., the same fitness), and (ii) consequently no species prefers certain environmental conditions. The random death of individuals and their replacement by an individual from the same, different, or new species ('ecological drift'[21]) generate patterns of biodiversity (e.g., species richness and species-abundance-distribution) and biogeography (e.g., β-diversity[22] and species area relationship) that are (i) astonishing similar to the ones observed in real-world data, and (ii) immutable with further birth-death events (i.e., equilibrium dynamic). Equilibrial species richness is higher and faster reached by high speciation rate[23].

Importantly, the good fit of UNTB models to biodiversity and biogeographical patterns does not mean species-specific characteristics do not exist. Instead, it highlights that existing ecological properties may not imprint summary statics of biodiversity[24]. In other words, we infer seemingly neutral processes behind biodiversity patterns but this might be due to not tracing all relevant and informative facets of biodiversity.

For instance, the picture of high fit changes when we look at diversification dynamics (i.e., speciation minus extinction). When species richness reaches equilibrium, corresponding to a net diversification rate of zero, we see at the same time an unrealistic high turnover rate (i.e., extinction·speciation^{-1})[23,25,26]. This is because less abundant species are more likely to go extinct[21] and every new species starts with one or few individuals, causing a high number of generated 'incipient' species of a short evolutionary lifespan. This contradicts Van Valen's[27] *Law of Constant Extinction* where the probability of extinction is constant throughout the age of a species. Relaxing the neutral assumption by including mild, evolvable selection results in realistic turnover[25] and demonstrates, as proposed by the *Red Queen* hypothesis[27], how microevolutionary biotic processes scale up to macroevolutionary patterns.

This mild selection scenario, as most variants of the UNTB, features a constant number of individuals over time (i.e., the metacommunity size). Population growth until saturation due to the depletion of renewable resources initially justified this zero-sum assumption[21]. Whereas many authors criticized this assumption[24,28], realistic patterns of biodiversity emerge even without this zero-sum constraint[29,30]. In contrast, only an increase in metacommunity size yields realistic turnover during species diversification[24,31]. However, ever-growing communities do not cumulate in equilibrial species diversity[21].

In summary, it is important for the bottom-up understanding of insular species richness and endemism that species equilibrium, as well as realistic spatial community differentiation and diversification dynamics, may emerge from neutral dynamics, altough not all of these properties together can.

Metacommunity Speciation Model

The MSM[12] provides the link between processes determining the co-existence of species on short timescales (i.e., local community assembly) and speciation processes on a longer, evolutionary timescale. In general, the UNTB features the same connection: geographical barriers or sheer spatial distance limiting the dispersal of individuals result in variation in community composition across the metacommunity and genetic divergence that may result in speciation when dispersal rates are low enough[12,21]. The MSM includes, in addition to the ecological neutral processes of no interspecific differences in dispersal ability, non-neutral processes of community assembly related to the ecological niche of species and/or a trade-off between life history traits (i.e., between competitive and dispersal ability).

Adopting Hutchinson's[32,33] concept of the fundamental ecological niche, the sorting of species along environmental gradients according to their ecological niche causes variation in community composition (the 'environmental filtering' metaphor[34,35]). If, as the UNTB also assumes, ecological resources are somewhat finite, species will compete for them[12], with species expanding in population sizes on expense of others. This differs from the UNTB perspective by not being stochastic where all species are ecologically equal. Instead, competition strength depends on the match between the species' ecological niche and the environmental conditions. With by definition species-specific niches[32,33], eventually a single species may take over the whole patch (i.e., the species sorting paradigm[11]). However, such a species loss might be compensated by immigration due to source-sink dynamics (i.e., the mass effect paradigm[36]). On evolutionary timescales, the sorting of species along environmental gradients may cause diversifying selection and adaptation of species' traits to environmental conditions[37,38]. The speciation defining reproductive isolation might be then (i) a by-product of the accumulation of genetic incompatibilities due to this diversifying selection and adaptation of species' traits[38,39], or (ii) a direct consequence of selection on 'magic traits' that are related to local adaptation and reproductive isolation[40,41].

However, in addition to this 'niche mediated' ecological speciation through adaptive divergence, ecological speciation might be also driven by community composition[12]. On a fitness landscape[40], different local optima in competitive and dispersal ability may drive ecological speciation through adaptive shifts among populations. In the 'community mediated' ecological speciation model, competition induces such shifts through adaptation towards (i) higher dispersal ability enabling movements to patches unoccupied by competing species, or (ii) higher competition strength leading to an advantage over local competitors[27]. A 'jack-of-all-trades' scenario with one global optima in competitive and dispersal ability is, however, unlikely. This trade-off between dispersal and competition results in interspecific differences and in the metacommunity paradigm of patch dynamics[11], where local species richness is limited by dispersal and the balance between colonization and extirpation dynamics drives spatial community structure.

Eventually, the processes of dispersal limitation, environmental filtering, and competitive interaction shape metacommunity structure with dispersal being the key component[12]. With high dispersal rates, species quickly sort along environmental gradients and avoid competition through emigration, which is a community adaptation through immigrantion and emphasizes the role of ecology in community assembly[42]. In contrast, with low dispersal rates, populations may adapt to local environmental conditions, resulting in communities shaped through evolutionary processes. These extremes translate into a hump-shaped relationship between dispersal and speciation rate. Intermediate dispersal rates do no exhibit extensive gene swamping and prevent genetic divergence. However, they enable some species sorting along environmental gradients and hence stimulating ecological speciation, which is supposedly faster than geographical speciation and located at the lower end of the dispersal rate gradient[37].

Because of limited geographical and/or niche space[21,43], on short timescales the species richness of a metacommunity is, in principle, bounded, regardless of the main assembly process, and therefore should also be bounded on longer timescales. However, the height of the bound (i.e., the carrying capacity of the ecosystem) and how fast it is reached depend on the metacommunity assembly process and the associated speciation process. Under strictly neutral dynamics of dispersal limitation, the height of the richness bound and the velocity of approaching it hinge on dispersal rate, with lower dispersal rates causing higher limits and a slower approaching. In case of environmental filtering or competitive interaction dynamics, dispersal rate is also crucial because, by its hump-shaped relationship with speciation rate (see above), it determines how fast a carrying capacity might be approached. However, the species richness limit of the metacommunity depends on the assembly process due to its role in ecological speciation. With communities assembled through the trade-off between dispersal ability and competitive strength, the upper limit of species richness does not differ from the one of strictly neutral dynamics when the dispersal rate equals the mean across all species in the competitive interaction scenario[11,12]. On macroevolutionary timescales, communities assembled through environmental filtering may have the highest limit of species richness of all assembly dynamics. This is because the corresponding processes of ecological speciation increase the utilized niche space of a community due to species' adaptation and niche packing[44–46]. No other speciation and assembly processes feature such an evolutionary characteristic. Theoretically, the limit of species richness may constantly increase over time and will never be reached. However, the contemporary take on adaptive speciation postulates two scenarios of how: (i) the access to new ecological opportunity stimulates divergence in species traits[**47**,48–51], or (ii) the evolution of a key innovation in species traits grants access to new ecological opportunity[52,53]. In both scenarios, species accumulation through time decreases.

In summary, processes of metacommunity assembly inform on the process of speciation and vice versa. Depending on the assembly processes, different trajectories of species richnesses through macroevolutionary timescales emerge.

Island Biogeography Theory

I refer to Island Biogeography Theory (IBT) in a wide sense, starting with MacArthur's and Wilson's seminal work ([8,9]; but see[54] for preceding work), integrating the extension of Whittaker *et al.*[10] into the *General Dynamic Model of island biogeography* (GDM), and I restrain from semantic differentiating between *Core Island Biogeography Theory*[18] or *Equilibrium Theory of Island Biogeography*[8]. The initial development of the IBT was largely sparked by increasing knowledge on population and metacommunity level processes, mainly isolation-by-distance and density-dependence[18,54,55], which both were latter formalized by the UNTB and the MSM. IBT now primarily represents a concept of species richness and endemism on longer evolutionary timescales[19]. Moreover, the principles of IBT do not only apply to oceanic islands alone but also to other insular ecosystems such as sky mountains, habitat islands, springs, or lakes[7,18,56,57].

The principal events determining species richness and endemism of an insular ecosystem (i.e., its regional species pool) are immigration, extinction, and speciation (see UNTB). Immigration probability decreases with increasing isolation of the insular ecosystem and lower dispersal ability of potential colonists. The area and topographic complexity of the insular ecosystem define its niche space and with this the upper limit of species richness the system may maintain. A greater unoccupied niche space (i.e., the difference between total species richness of an insular system and its carrying capacity) increases immigration and ecological speciation rates but decreases extinction rates[15]. With equal rates of species gain and loss, the insular ecosystem attains a state of a dynamic equilibrium, characterized by no net change in species richness[9]. However, area and topographic complexity change in a predictable manner over the life cycle of an insular ecosystem with three phases: a growing phase, a time of maturity featuring greatest extent and highest topographic complexity, and a ceasing phase with an eventual vanishing of the system due to erosion, subsidence, and downcutting processes. The change in carrying capacity over time, induced by the varying area and topographic complexity, feeds back on immigration, extinction, and ecological speciation rates, which consequently vary over time and cause the trajectory of species richness to peak shortly after the phase of maximum insular maturity[10]. According to this GDM paradigm, equilibrium diversity may never be reached on oceanic islands because (i) the geological evolution outpaces rates of species gain until reaching maximum insular maturity, and (ii) during the ceasing phase, extinction rate exceeds speciation and immigration[13,15,58].

Depending on the main process of metacommunity assembly, diversity-through-time trajectories differ (see MSM). With communities assembled through environmental filtering and the thereby resulting 'niche mediated' ecological speciation and niche packing through adaptive processes[12,39], species richness accumulates faster than in the ecological neutral scenario of dispersal limitation and geographic speciation. This is caused by the higher pace of ecological speciation and the weaker feedback on rates of species gain and loss due to the by niche packing elevated carrying capacity. Communities shaped by competitive interactions on short timescales and 'community mediated' ecological speciation on longer timescale also build up species richness quickly. However, the carrying capacity is approximately equal to the ecological neutral scenario because no niche packing occurs, but instead trait evolution along the dispersal/competition axis. As consequence, the richness trajectory should mirror the carrying capacity curve more closely because the fast ecological speciation is not falling behind the geological evolution of the insular ecosystem as much as the geographic speciation does in the ecological neutral scenario. Nevertheless, the insular ecosystem does not attain equilibrium diversity[15].

In addition, the mechanisms and processes inherent to IBT may also provide the theoretical background for adjusting our expectancies on the impact of changing environmental parameters through time on species richness and endemism. The key point is that not only area and topography determine the carrying capacity of an ecosystem but, to some extent, also additional environmental characteristics might be involved. For instance, according to the water-energy hypothesis[59], rising temperature and/or precipitation increases primary production and eventually the species richness that an ecosystem can support. The resulting larger empty niche space stimulates ecological speciation via cladogenesis and reduces extinction, eventually boosting richness and endemism. The expected strength of the effect depends on ecosystem state: if there is plenty of empty niche space, a further increase will not promote speciation much. However, change in environmental parameters through time could also affect the build-up of biodiversity in different ways than mediated through carrying capacity, for instance, by metabolic scaling[**47**,60,61] or by intensifying the effect of geographical barriers[62]. This *Court Jester* model of abiotic control over species diversification[1,63] is opposed to the microevolutionary *Red Queen* model of biotic interaction shaping biodiversity[27,64].

In summary, the ontogeny of insular ecosystems shapes the build-up of species richness and endemism through time with a differential importance of community assembly processes distinctively imprinting these trajectories via their pivotal role on speciation dynamics. Competition processes for geographical space and/or ecological resources differ in their outcome on short-term metacommunity structure and long-term biodiversity trajectories. Additionally, the strength of competition influences both, with low competition typically resulting in a strong community-environment relationship and high species richnesses.

3.3. Lack of knowledge

Do ecological processes shape evolutionary dynamics in insular ecosystems?

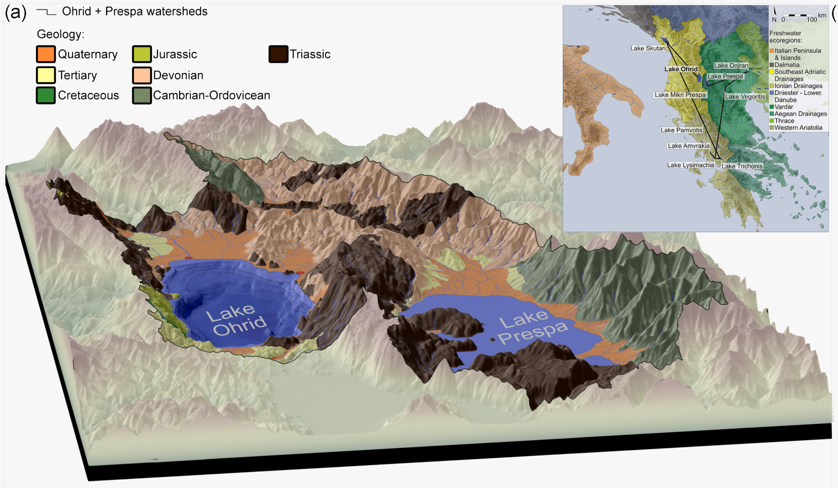
Although the causal link among ecosystem ontogeny, changing carrying capacity, metacommunity assembly processes, and the feedback on immigration and diversification is appealing, evidence for these theorized interrelationships is rare for several reasons. First, we know the detailed ontogeny only for a few insular ecosystems[65,66]. The second cause is the inherent complexity due to the involved interacting processes and mechanisms[15]. Third, temporal and spatial scales on which processes and mechanisms operate are vastly different from the scale of their resulting macroevolutionary patterns. Scale dependence, where patterns or processes seem to change upon an increase in spatial, temporal, or taxonomic scale[1–4], is a widespread phenomenon in ecology and evolution and one of the major challenges of eco-evolutionary research[6,67]. Fourth, processes, such as environmental filtering or competitive interaction, cannot be directly observed, except for, maybe, unicellular organisms monitored in cell cultures[68]. Inferring processes from observed patterns is conceptually and methodologically challenging[35,69,70]. Of particular concern is the case when several metacommunity processes generate the same biodiversity trajectory (many-to-one matching[71]). Fifth, there are few studies on the very same taxa that investigate metacommunity assembly processes on short timescales and evolutionary dynamics on longer timescales[7].

In summary, little is known about whether the postulated iconic trajectories of species richness and endemism over the life-cycle of an insular ecosystem[15] indeed emerge from population and metacommunity level processes. Recent reviews on IBT[7,72] highlighted the lack of knowledge on how these processes upscale over time.

Box 1 Lake Ohrid

The Balkan Lake Ohrid (Fig. 1) is a suitable study system potentially advancing our understanding of whether and how population and metacommunity processes imprint biodiversity patterns emerging through time. Whereas several publications provided a comprehensive site description of Lake Ohrid[73–80], here the focus lays on its characteristics mitigating some of the major obstacles in our process understanding, namely limited knowledge on ecosystem ontogeny, the scale dependence, and taxonomic scope.

Within the interdisciplinary Scopsco (Scientific Collaboration on Past Speciation Conditions in Lake Ohrid) research program, the age of Lake Ohrid was estimated through two independent approaches. On the one hand, dated molecular phylogenies of several endemic animal taxa[**17**,81–84] suggested maximum ages of 3–10 million years (Ma) and minimum ages of 1.2–2.7 Ma, which are within the range previously suggested by geologists[81,85]. On the other hand, using seismic surveys and bathymetric data, a reconstruction of the tectonic and sedimentary history of Lake Ohrid unraveled that the initial opening of the lake basin occurred during the Alpine orogeny in the Late Miocene and suggested no major paleolake deposits as potential precursor of Lake Ohrid[78]. Ongoing lithological analyses of sediment cores, covering the upper 560 m of the 700 m thick basin infill, revealed a succession of (i) fluvial deposits, (ii) peat layers produced by bogs and punctuated by fluvial deposits, followed by (iii) uninterrupted lacustrine deposits[76]. This suggests a single major infill of the lake basin, however, the exact age of this event is still unknown. Continuous lacustrine biogenic and terrigenous sedimentation started at ca. 1.3 Ma[77], which roughly corresponds to the first cladogenetic events in several endemic taxa of Lake Ohrid[84,86]. Seismic analyses, however, pointed to an age of 1.9 Ma for the oldest, mainly fluvial, sediments[78].



(b)

Surface area	358 km²
Elevation	693 m a.s.l.
Length x width (maximum)	30.4 x 14.7 km
Depth (mean maximum)	150 293 m
Volume	50.7 km³
Watershed (+ Lake Prespa)	2,600 km²
Water residence time	36 years
Water input	Total 44.8 m³/s
Precipitation	20%
Surface inflow	16%
Groundwater inflow	
- Prespa-fed	17%
- Surface springs	13%
- Sublacustrine springs	34%
Water output	Total 44.8 m³/s
Evaporation	31%
Surface outflow	33%
Groundwater outflow	36%

Fig. 1 Characteristics of Lake Ohrid. (a) Geographical position, biogeographic affinity, and geological features. (b) Limnological properties. Modified from[77,87,88,80].

This hemipelagic condition appears to be continuous without major alterations due to environmental change[76]. Although lake level drops with a magnitude of 150 m were demonstrated[77,89], Lake Ohrid remained deep enough to maintain a relatively constant volume of the ecosystem-vital photic zone and provided constant benthic area in the littoral and sublittoral zones. Both, the initial ontogeny without preceding paleolakes and the following constancy in volume and area over an evolutionary significant amount of time correspond to the first two stages of the GDM scenario of island evolution. However, there is no evidence for Lake Ohrid to be in the third, vanishing stage of declining area and complexity. This simplifies the explanation of community assembly and species diversification in Lake Ohrid through IBT mechanisms.

The continuous hemipelagic condition of Lake Ohrid does not signify a complete lack of environmental fluctuation. Sediment cores obtained through the Scopsco program testified environmental change mostly in synchrony with glacial/interglacial cycles[90] and a general trend towards drier and warmer conditions[77]. In addition to these periodic and gradual changes, Lake Ohrid also experienced several short-term (‘pulse’) disturbance events such as fallout of volcanic tephra[91] or landslides causing mass wasting deposits[92,93]. However, there is no evidence for a catastrophic impact of these events on the biota of Lake Ohrid, such as increased extinction or irreversible community turnover[17,94].

In comparison to other oceanic islands or ancient lakes, Lake Ohrid is comparable young and small. This strengthens the known effect of islands to homogenize the spatial and temporal scale of community assembly and evolutionary processes[7]. The small size enabled a comprehensive biodiversity inventory. With the history of biodiversity research at Lake Ohrid starting in 1894, the flourishing period 1930–1970 starring Hadžišće, Radoman, and Stanković, and the regained interest during the 21st century[85], its species richness and endemism got increasingly well known. Currently, more than 300 endemic eukaryotic species are formally described[17], among them 75 gastropod species[95,96,97], qualifying

Lake Ohrid as a freshwater richness hotspot[98,99]. However, the species inventory is still incomplete and species either new for science or previously unknown for the lake are discovered regularly[95,96,100–104]. While early community studies focused mainly on biomass and depth gradients[73], more recent investigations recorded species identities on horizontal and vertical scale[88,96,105,106,107–109].

Utilizing these records, we could infer metacommunity assembly processes and link those to evolutionary dynamics shaping biodiversity trajectories through time. Across most taxa, all evolutionary events inherent to IBT are documented. Extinction seems rare[17,94,110,111], although this might be simply due to the limited power to estimate extinction rates in relatively species-poor phylogenies[112,113]. In fact, preliminary analyses of the species-rich fossil record of diatoms indicated several extinction events[114]. Intralacustrine cladogenetic speciation is probably the most thoroughly documented type of event across taxa, as shown in several crustaceans[82,103,115–118], diatoms[119], gastropods[17,84,86,120], and leeches[81], resulting in endemic and monophyletic clades of high species richness (i.e., ancient lake species flocks).

In addition to cladogenetic speciation, budding and anagenetic speciation may occur but do not imprint phylogenetic signatures on global scale[121]. Because insular ecosystems are an immigration sink and the source populations do not go extinct upon colonization, several authors inferred budding or anagenetic speciation through the presence of single endemic species[58,122]. Recently, a debate revolved around whether anagenesis and cladogenesis are useful terms in general[123] and for insular biogeography in particular[124,125]. However, although extinction invalidates the operational criterion of a single endemic species by pruning a species flock to a single lineage, molecular phylogenies may suggest anagenetic or budding speciation for crustaceans[83], bivalves[126–128], gastropods[129,130], and fish[131] of Lake Ohrid.

Immigration is indicated by all colonizing lineages, either increasing non-endemic species richness or subsequently speciating into species flocks or single endemics. Relevant for insular biodiversity dynamics is whether colonization rate decreases over time through diversity-dependent feedback of the carrying capacity[8,10]. In fact, the ancestor of most species flocks colonized Lake Ohrid relatively early during its limnological history[17,82,84,119,120], supporting this mechanism. In contrast, single endemics, or their ancestors, may have immigrated just as recently as 0.5–0.9 Mya[103,119,128,129,131]. Tracing the biogeographic affinity of the Lake Ohrid gastropod fauna may inform about colonization sources. Comparing the dominance of incumbent families with ceased paleolakes[132] and extant genera with those of present-day lakes[87,133] suggested a Western Balkan origin. There is an ongoing debate on whether invasive species are informative about ongoing ecosystem colonization and the existence of diversity limits because typically little time has passed since their immigration and long-term consequences thereof are still unknown[43,134]. Lake Ohrid harbors less invasive species than other Balkan lakes[127,135] and the spatially restricted occurrence of two invasive gastropod species in areas under the highest anthropogenic pressure[135] did not seem to have affected local mollusk communities[95].

3.4. Evidence for ecological processes shaping evolutionary dynamics

Simulation experiments are one way to circumvent all aforementioned issues of inherent complexity, differences in scale, unobservability of processes, and divergence in taxonomic scope. Unlike correlative models without a necessary causal relationship (e.g., regression analysis utilizing empirical observations), mechanistic or process-based models generate biodiversity patterns through an explicit causal mechanism[136–138], such as species dispersal[**139**]. Hence, they do not fit a statistical model to empirical data but we can compare either (i) those empirical observations with the simulated biodiversity patterns, where a high similarity suggests an importance of the generating causal mechanisms, or (ii) the differential impact of multiple processes on biodiversity patterns. Process-based models of eco-evolutionary dynamics are either (i) analytical, where a set of equations formalize eco-evolutionary processes and a given input of parameters determines an immutable outcome, or (ii) stochastic, where a causal connection among a series of interlinked eco-evolutionary processes generates stochastically varying biodiversity patterns[137]. The latter allows for considerable more complex scenarios that cannot be formulated in an analytical framework and basically most studies in the field of the UNTB rely on this approach[24,137]. However, process-based models of eco-evolutionary dynamics of insular ecosystems used both methodologies but no study could include the full range of potentially interacting eco-evolutionary processes.

Analytical[15] and stochastic simulation studies[13,14,140] revealed an influence of island ontogeny through implicit diversity-dependent mechanisms on temporal trajectories of species richness, endemism, and rates of immigration, speciation, and extinction. Because fossil records are rare for geological active insular ecosystems[141], we cannot compare richness trajectories of extant taxa alone with these theoretical prediction (but see[142]), but may contrast the predicted rates of species in- and output resulting from these simulations with the ones inferred from phylogenies of extant taxa. However, these and other studies[10,13–15,140] displayed rate trajectories in units of immigration, extinction, or speciation events·time^{-1}. In contrast, MacArthur & Wilson[8,9] as well as paleo- and neontological diversification studies typically utilize the unit events·lineage^{-1}·time^{-1}[**17,47,143**,144–147]. The former formalization seems biased because, with a constant event probability an increasing number of species will always result in more events. Normalizing rates per lineage drastically reduces the iconic hump-shaped trajectory of immigration, speciation, and extinction rates (Fig. 2). However, since rate trajectories are an emerging pattern of the GDM simulations[13,15,140] extracted a posteriori, the principal mechanisms generating biodiversity and their relationship with island ontogeny are not invalidated. Instead, it demonstrates how challenging it might be to infer ontogeny-driven rate variation. Valente *et al.*[140] showed this by evaluating under which combinations of island ontogeny phases and immigration, speciation, and extinction rates we could correctly identify rate variation. In fact, individual simulated phylogenies could only testify variation in speciation rates during the ceasing phase of the insular ecosystems and by high rates of cladogenetic speciation. However, extinction and variation in its rate could not be demonstrated, which is a known limitation of phylogenies of exclusively extant species[112,113,**148**].

Whereas these studies[15,140] focused on macroevolutionary patterns and the difficulties in inferring those from phylogenies, some stochastic simulations[13,14,150,151] explicitly modeled

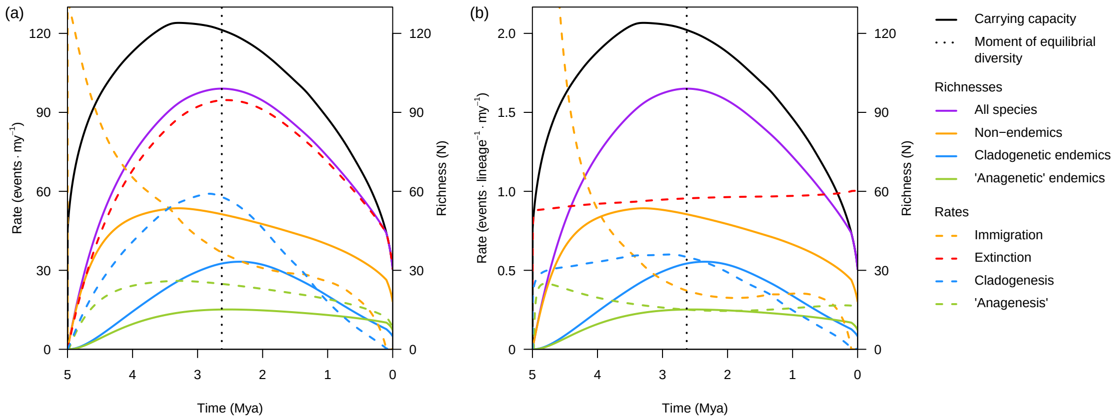


Fig. 2 Biodiversity and key rates through the life cycle of insular ecosystems. Rates of species in- and output (a) showing the characteristic hump-shaped trajectory of the General Dynamic Model of island biogeography[10,15], and (b) decreasing upon normalizing rates per lineage, as in the Equilibrium Theory of Island Biogeography[8,9] and several paleo- and neontological diversification studies (e.g.,[145–147,149]).

metacommunity assembly processes on short timescales and their consequences on niche evolution, patterns of macroevolutionary richness, and rate dynamics over broad timescales. This showed how diversification-driven local richness decreases through the sequential order of colonization events across an insular ecosystem, but also how differential competitive interactions within local communities may eventually overrule initial richness patterns on long timescales[151]. Empirical richness patterns of the Canaries[65] and of Lake Ohrid (Box 1), where the limnological oldest areas such as the feeder spring area[85] present the highest gastropod richness[**88**], may exemplify the importance of colonization order. Remarkably, realistic patterns of species richness through time, trait evolution, and species-abundance-distribution emerged only in simulation settings that feature competitive interaction, life history trade-offs, and niche-based environmental filtering[13,14]. This suggests that exclusively ecological neutral processes, such as dispersal limitation and geographic speciation, previously identified as the most important process for community assembly[16,**96**] and diversification[152,153] in insular ecosystems, are not sufficient for generating their biodiversity patterns.

All these simulations[13–15,140,151] parameterized carrying capacity and diversity-dependent dynamics. By contrast, niche evolution may mitigate diversity-dependent effects by effectively increasing the carrying capacity of local communities and the insular ecosystem[150]. However, this evolutionary strategy also seems to eventually end at the richness bound that is typical only for insular ecosystems characterized by extensive periods of no major geological change[10]. Aguilée *et al.*[150] could demonstrate how biotic (*Red Queen*) and abiotic (*Court Jester*) processes at the community level differentially imprinted species diversification during three successive phases. Adaptive radiation, limited in its rate by resource availability, and community assembly through the matching of diverging traits and local environmental conditions characterized the first phase of a rapid, but declining net diversification rate. By contrast, the second phase of slower and relatively constant positive diversification is mainly driven by geographic speciation through dispersal limitation at the community level and features a significantly decreasing net diversification upon strong competitive interactions structuring communities[150]. The third phase of constant species richness displayed the dynamics and mechanisms proposed by the UNTB with prevalent geographic speciation generating a high number of short-living incipient species resulting in a high turnover rate. Whereas no other factor influenced the turnover rate itself, the upper bound of species richness on long timescales increased by local community level parameters and processes such as high resource availability, decreasing competitive interaction due to evolutionary niche packing, and less catastrophic environmental events.

Summarizing, simulation studies of insular biodiversity dynamics[13,14,140,150,151] suggested that ecological neutral processes alone, such as dispersal limitation and competition for geographical space acting on short timescales and at the local level, cannot generate the observed hump-shaped trajectory of species richness and endemism through the life cycle of insular ecosystems[10,142,154–156]. Moreover, they showed the differential impact of community assembly processes on richness and diversification rates on the path to equilibrium dynamics, which may never be reached for insular ecosystems where the geological evolution outpaces biological dynamics (see above[10,15]). Environmental filtering is always of importance for community assembly and imprints diversification dynamics strongly during the early growing phase. By contrast, dispersal limitation and competitive interaction played a significant role during the following phases of diversity build-up and equilibrium diversity, each of which are characterized by a constant positive and zero net diversification rate, respectively[150]. Edward O. Wilson suggested this succession from environmental influenced community assembly through adaptive niche packing to competitive interaction already in 1969[18]. However, such a transition from the *Court Jester* to the *Red Queen* paradigm is often obscured in analyses of empirical data over longer time frames that lack a time-stratification in drivers of diversification[1,**157**,158,159] and exemplifies the scale-dependence of eco-evolutionary patterns and processes[2–4].

Although simulations of insular biodiversity dynamics demonstrated that diversification rates are often seemingly unaffected by island ontogeny and rate variation could mainly be estimated from dated molecular phylogenies during the ceasing phase of the insular life-cycle[140], several empirical studies found either increasing[160] or declining[51,58,144,161–166] speciation rates for insular species flocks or entire island assemblages[167,168]. Lim & Marshall[142] found rate variation for several Hawaiian taxa by conditioning on island ontogenies and extant richnesses. Most likely because of the adverse effect of active tectonics of true oceanic islands on fossil preservation (but see landsnails on the Canaries[141]), inference from fossil records is performed mainly on regional and continental scales[169]. However, fossil remains testified how the building of isolated mountains in combination with climatic change drove the colonization and diversification of mammals[149]. In contrast, the aforementioned cases of declining speciation dynamics were typically attributed to high ecological opportunity and/or vacant niche space upon island formation, followed by diversity pile-up that increases competitive interactions among species for declining niche space. This, together with the rapid initial eco-morphological divergence in species traits and their association with environmental gradients (reviewed in[**148**]), qualify several taxa like the Caribbean lizards[170–172], Madagascan vangas[162,163], Hawaiian spiders[173], or Tanganyikan cichlids[174,175] as text book examples of ecological speciation[39] and adaptive radiation[44,46,176].

In contrast to the abovementioned studies, constant rates of insular diversification are rarely reported (‘File drawer problem’, but see[**17**,177–179]) or served only as comparison to variable rate dynamics[161,180]. Such a pattern of seemingly constant rates may emerge also by expansion of the insular ecosystem (Fig. 2[13–15,140]) and/or during periods of community assembly through dispersal limitation causing geographic speciation[150]. Moreover, for a number of studies supporting the GDM paradigm of biodiversity being determined by ecosystem ontogeny, the rate dynamics are unknown. Those studies utilized the space-for-time substitution[14], which instead of tracing richness and endemism through time of an single evolving island, quantifies these biodiversity measures for a set of islands from an archipelago with different ages and area (i.e., a proxy for carrying capacity). They showed a hump-shaped relationship of species richness and endemism with age and an increase with area[10,154–156,181]. This pattern may emerge by constant or varying diversification rates (Fig. 2b), both implying different processes of diversification and metacommunity assembly[150].

The terminology for co-occurring species and the required scale of their spatiotemporal, phylogenetic, and functional interrelationship changed considerably over time[182]. In contrast to the recent consensual understanding of (meta)community[11,182], in IBT the term community typically denotes an island-specific set of species[7,10,18,181], which can only be assembled through evolutionary processes. However, in order to evaluate how, for instance, dispersal or environmental differences within the insular ecosystem affect evolutionary processes requires studying the structuring of species within and among local communities.

Studies of insular metacommunity structuring typically used correlative approaches where a statistically significant relationship between community composition and environmental parameters is interpreted as signature and strength of the environmental filtering process[107,109]. Alternative or additional explanations (e.g., competitive interactions or dispersal limitation) are often not evaluated because these processes cannot be directly observed (see lack of knowledge). Nevertheless, a hypothesis about which spatial structure of the metacommunity should emerge under dispersal limitation can be tested in correlative analysis through spatial variables together with environmental parameters[**183**,184]. Such an approach suggested that amphibian and reptile communities of Madagascar are assembled through environmental filtering and dispersal limitation[185,186], which is inconsistent with the revealed decline in diversification rate[165]. Moreover, these spatial analyses implied higher speciation in peripheral areas[185], as predicted by the MSM[12], effectively reversing the hypothesized mid-domain effect known from other areas[**139**,187,188]. With a similar correlative approach, Hauffe *et al.*[**88**] demonstrated that upon discarding the typical strong effect of water depth on aquatic organisms[16,**105**,107,189], dispersal limitation is of higher importance than environmental filtering for gastropod community assembly in Lake Ohrid. Fossilized mollusk communities from the last interglacial period (ca. 120 kya) were highly similar to present-day ones at similar location, suggesting the persistence of dispersal limitation through short–medium timescales[**105**]. This process seems to translate into the constant diversification rate of some of those gastropods over long timescales[**17**] and is equivalent to the eco-evolutionary dynamic suggested by mechanistic simulations during phases of little ecosystem expansion[13,150]. Results of these simulations imply a high importance of geographic speciation, which contrasts with the suggested importance of ecological speciation for at least some of the gastropod species[**106**]. However, empirical studies suggested an initial step of spatial isolation as crucial for ecological speciation[39]. In eco-evolutionary research, the omnipresent scaling issues[1–4] may conceal the signal of ecological speciation.

Community assembly studies of other Lake Ohrid taxa than gastropods[**88**] did not quantify the relative importance of environmental versus dispersal processes[107–109] and, in general, the simultaneous inference of all major processes of metacommunity assembly (i.e., dispersal limitation, environmental filtering, and competitive interactions) is in its infancy[136]. This severely impedes the pleas to test for metacommunity assembly processes[72,181] that are implicitly assumed to drive the macroevolutionary patterns of island biogeography[9,10]. Islands as model systems were long at the forefront of theoretical and methodological progress[18]. For instance, the seminal work of Diamond[69] on bird communities of the Bismarck archipelago stimulated the development of methods to test for competitive interactions ('Checkerboards'), whereas Webb *et al.*[190] proposed an approach to disentangle environmental filtering and competitive interactions through trait or phylogenetic clustering (but see[191,192] for issues and alternatives). Utilizing the latter method, corroborated filtering during the assembly of Hawaiian spider[173] and plant communities[193], which according to predictions derived from eco-evolutionary simulations for young islands[150], is in agreement with their increasing diversification rate due to ongoing island expansion[142].

The magnitude of decline in speciation rate of Caribbean anoles lizards decreases with island area and smaller islands featured equilibrial diversity[144]. Populations of these smaller islands diverged mainly via neutral isolation-by-distance effects, whereas environmental differences additionally drove divergence on larger islands[194]. Due to the low species richness, there is little evidence for competitive interactions structuring communities on smaller islands, but the evolution of distinct ecological roles upon island colonization suggested an effect of it[171,195]. By contrast, competitive interactions preventing the co-occurring of morphological similar species shaped lizard communities on Hispaniola, but to a lesser extent than environmental filtering and dispersal limitation[196]. These metacommunity assembly processes matched only partly the predictions derived from simulation studies[13,15,150]. Whereas we would expect competitive interaction and dispersal limitation as assembly processes in the case of diversity-dependent decline in speciation rate and some imprint of environmental filtering during non-equilibrial diversity, the environmental influence on metacommunity assembly and population divergence[194,196] was stronger than anticipated.

Van der Plas *et al.*[197] suggested a mechanistic trait-based method for identifying the relative importance of dispersal limitation, competitive interaction, and environmental filtering for metacommunity assembly. In the case of Tanganyikan cichlids, metacommunities were mainly assembled by the first two processes[16]. According to the MSM, these processes should correspond to geographic speciation with an induced decline in diversification rate via competition for remaining niche space[12,150]. In fact, the dated molecular phylogeny of cichlids evidenced such a diversity-dependent rate pattern[51]. Hauffe *et al.*[**96**] extended this method for inferring metacommunity assembly processes to work with phylogenetic distances instead of species traits. They revealed a high importance of dispersal limitation and environmental filtering, whereas competitive interaction played a minor role in structuring gastropod communities. This result was consistent with the previous correlative analysis where the latter process could not be evaluated[**88**] and with no obvious effect of invaders on the resident gastropod fauna[**95**]. As, over long timescales, weak competitive interaction may represent the situation of plenty available niche space and goes hand in hand with a higher species richness than under strong competitive interaction[15,150], the processes identified by studying metacommunity assembly may explain the evolution of the high endemic gastropod richness of Lake Ohrid (Box 2).

Box 2 Biodiversity scenarios for the Lake Ohrid gastropods

Using (i) deterministic equations representing the mechanisms and processes inherent to the GDM[15], (ii) a simplified version of the reconstructed limnological history of Lake Ohrid[77,78,A. Francke pers. comm], (iii) fundamental community properties shown by the gastropods of Lake Ohrid (e.g., species-frequency-distribution[88]), and (iv) as target the species richness and endemism of its extant gastropod diversity, I sketched distinct scenarios of how this diversity may have evolved over time (Fig. 3). The submodel approach[13] of selectively switching off key mechanisms and processes of the GDM resulted in distinguishable rate and diversity trajectories. This defines operational criteria to inform on the relevant drivers of diversification and contributes to one of the main goals of the Scopsco project[17].

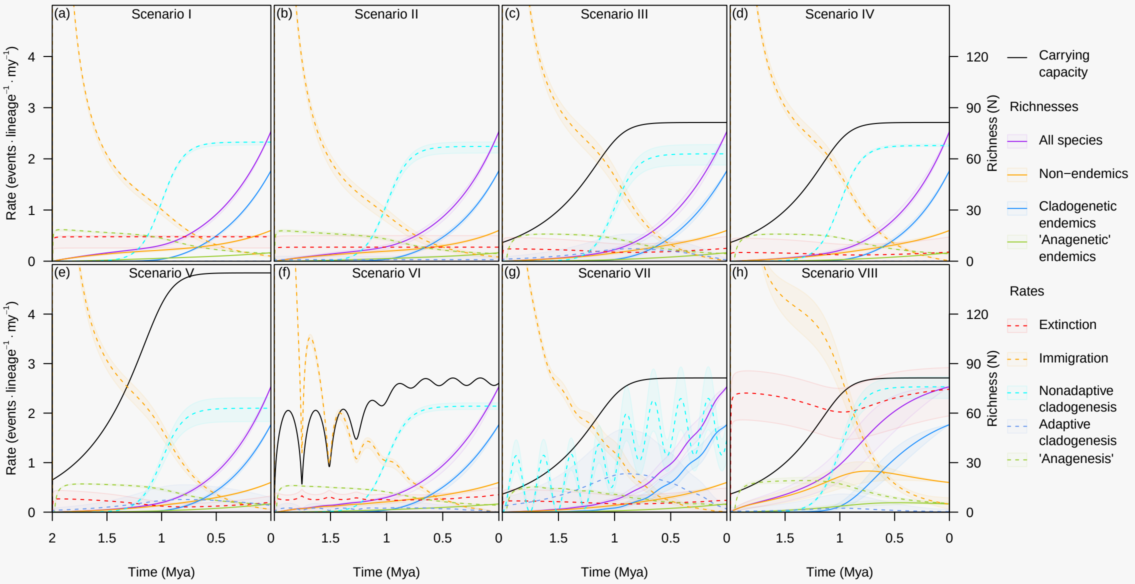


Fig. 3 Alternative scenarios of eco-evolutionary dynamics. They representing the central question of this synthesis on what drives gastropod diversification and metacommunity assembly in Lake Ohrid with Scenario III receiving most support through several studies.

Scenario I represents a Null model devoid of any eco-evolutionary dynamics, including only unbounded immigration, geographical speciation, and background extinction (Fig. 3a). Scenario II adds ecological speciation but without imposing a carrying capacity (Fig. 3b). Scenario III includes all processes and mechanisms of the GDM, whereas Scenario IV lacks ecological speciation (Fig. 3c,d). Importantly, scenarios III and IV feature extant species richnesses close to the carrying capacity and therefore high importance of competitive interactions. In contrast, Scenario V does not include competition by considering species richnesses far from the theoretical carrying capacity (Fig. 3e). The *Red Queen* Scenario VI (Fig. 3f) includes an additional dependency of the carrying capacity on environmental condition (e.g., glacial-interglacial cycles), while the *Court Jester* Scenario VII (Fig. 3g) features environmentally fostered geographic speciation[62]. Whereas all these scenarios are parameterized with the low extinction rate typical for Lake Ohrid species[17,94,105], Scenario VIII (Fig. 3h) differs by a higher extinction rate from Scenario III. These scenarios did not encompass all possible, partly unrealistic, combinations of processes and mechanisms and some are not compatible with the observed gastropod diversity.

3.5. Promising avenues

A robust evaluation of the reciprocal effect between metacommunity assembly processes and diversification dynamics, as predicted by the eco-evolutionary nexus of UNTB, MSM, and IBT, requires (i) a better sampling of local communities in order to infer assembly processes, as for most insular ecosystems species distributions are only known with relatively low spatial and temporal resolution (but see[65,**94,96**,185]), (ii) a higher taxonomic coverage of speciation and extinction times from the entire insular biota, (iii) the scoring of species traits to infer, for instance, niche packing or dispersal/competition trade-offs, which influences processes of community assembly, on short and long timescales and the upper limit of species richness an ecosystem can support, and (iv) an extension of our analytical toolbox. Eco-evolutionary dynamics could be readily inferred with a high-quality fossil record. However, fossil records are rare for geological active insular ecosystems but for extant taxa the first three points are mainly a matter of effort and funding. Therefore, I focus on the needed extension of the eco-evolutionary toolbox using information from extant taxa.

To understand the impact of the evolutionary history and niche evolution of insular species on community assembly, we need a unifying approach separating the effects of environment, species traits, phylogeny, and the spatial configuration of sampling sites on community composition. Hierarchical regression models[198,199] can quantify how (i) spatial distances among communities contribute to their β-diversity, (ii) the prevalence of all individual species in local communities depends on environmental factors, thereby approximating their ecological niche, and (iii) differences in these niches can be attributed to either species-specific traits (i.e., divergent adaptation) or phylogenetic relatedness (i.e., niche conservatism[200]). The explained variance in community composition can then be partitioned (e.g.,[**88,183**]) by spatial configuration, environmental factors, species traits, and phylogeny to inform on their individual importance in structuring communities. This offers the potential for moving beyond the current attempts of utilizing largely distinct methods for separately and consecutively estimating the effect of evolving traits on the ecological niche of species, biotic interaction, and the sorting of species along environmental gradients, which eventually results in the observable community composition. In this way, the effect of adaptation on endemism and community structure over time, as suggested by simulations[13,14,150], could be tested.

On long timescales, several studies utilizing dated molecular phylogenies could demonstrate the decrease in net diversification rate predicted by IBT[51,144,161–166]. However, diversification of endemics is only one part of the insular biodiversity dynamics and only few studies jointly estimated immigration and diversification rates for entire island biota, including non endemic species, instead of for a single monophyletic taxon alone[58,122,167,168,201]. Implementing the possibility to estimate shifts in immigration, extinction, anagenetic, and cladogenetic speciation rates in models for island community assembly and diversification[58], Hauffe *et al.*[**202**] demonstrated an increase in immigration rate for the fishes of the ancient Lake Biwa (Japan), potentially caused by a tectonically induced increase in lake depth and area. A diversity dependent decline in speciation rates could not be detected, because either (i) the lake is fundamentally non-equilibrial, (ii) species richness is far from carrying capacity (i.e., high available niche space), or (iii) the expansion of Lake Biwa compensates for the decline in niche space with increasing diversity. This contrasts with the diversity-dependent decline in immigration and speciation rate of the Caribbean anoles lizards[144].

Whether varying environmental parameters through time influenced insular diversification dynamics (*Court Jester* scenario; Box 2) is largely unknown. Utilizing dated molecular phylogenies requires ca. 100 species to identify such an environmental imprint[158]. However, with the exception of cichlids[153], most species flocks of insular ecosystems feature not enough species. Several authors[58,203,204] imposed one speciation and extinction rate on several phylogenies not connected in a single evolutionary history (Fig. 4). This would augment the number of species and increase the statistical power to detect variation in diversification rates. Adapting single-clade, environmental dependent diversification models[205,206] to several phylogenies is then straightforward by fitting a single common correlation coefficient between an environmental parameter and a collective diversification rate for all individual phylogenies (Fig. 4). However, preliminary analyses suggest no influence of changing environmental parameters through time on gastropod diversification in Lake Ohrid.

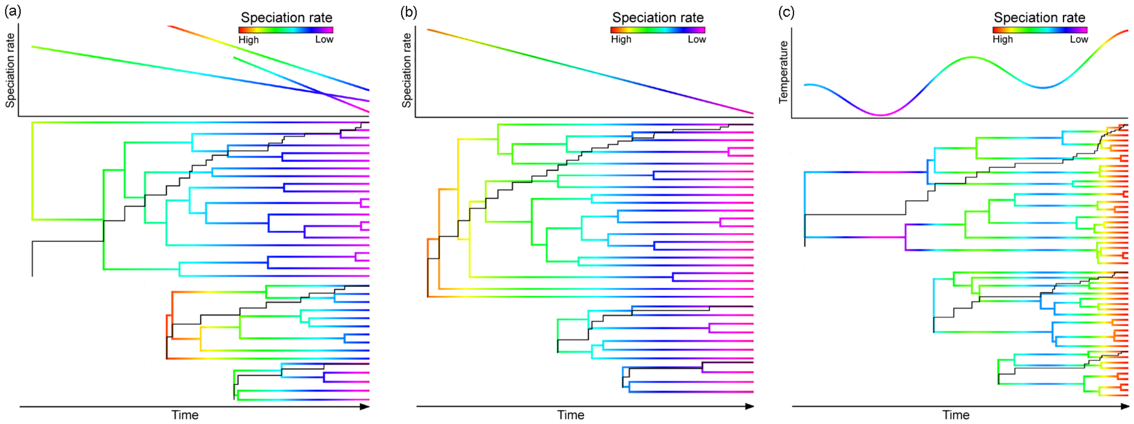


Fig. 4 Phylorate and lineage-through-time plots. (a) Changes in speciation rates over time for individual phylogenies. A novel, statistical more powerful approach of fitting (b) a single varying speciation rate for a set of dated phylogenies of island endemic taxa, and (c) a correlation of a single speciation rate with environmental change over time.

The aforementioned approaches are phenomenological ones where, for interpretation, parameters correlated with the pattern of interest are substituted by biological processes. For instance, a metacommunity structured by environmental gradients suggests environmental filtering[**88**,185,186] or a decline in speciation rate implies increasing competitive interaction[67,207]. By contrast, a truly integrative, process-based approach for evaluating how community assembly processes give rise to macroevolutionary diversity trajectories is considerable more challenging due to the inherent complexity of interacting processes, the different timescales on which these operate, and the general infancy of process-based biodiversity models[136,137,208,209]. A potential solution could be the combination of (i) already existing mechanistic simulations of biodiversity patterns through dispersal, interaction of individuals, and species' evolution (i.e., niche change or diversification by range fragmentation[150,210]) with (ii) Approximate Bayesian Computation (ABC[211,212]) for inferring process variables (e.g., dispersal distance, interacting strength, or demographic parameters). Out of a large set of simulations, ABC parameterizes process variables that generate biodiversity patterns most similar to the observed ones ('target'). Having its roots in population genetics, this versatile approach also served to detect how lake level changes influenced cichlid diversification[153] and depth-dependent gastropod community assembly[**96**]. However, the computational burden for such an integrative, process-based approach would be high.

3.6. Conclusions

This dissertation addressed whether and how temporal trajectories of species richness and endemism of insular ecosystems are shaped by short-term processes determining the distribution and co-existence of species and their subsequent influence on immigration, speciation, and extinction dynamics. Simulation experiments and empirical studies on several insular ecosystems suggest a differential upscaling of metacommunity assembly processes over time, resulting in distinct macroevolutionary diversification dynamics. This offers the possibility to infer mechanisms of species co-existence from evolutionary dynamics and vice versa. However, when metacommunity assembly processes strongly change through time, for instance from environmental filtering upon colonizing an emerging insular ecosystem to competitive interaction during filling of niche space, the signal of the early dynamics vanishes. The same way, extinction may conceal the traces of older evolutionary or colonization events from rate trajectories. Hence, for a causal understanding of how biodiversity unfolds over the ontogeny of an insular ecosystem, a combination of inferring the differential importance of metacommunity assembly processes and diversification rate is advisable.

For the gastropods of Lake Ohrid, such an approach suggested that mainly dispersal limitation and constant geographic speciation with an intermediate influence of environmental filtering and ecological speciation resulted in a gradual build-up of biodiversity through time. With limited importance of competitive interactions for metacommunity assembly and due to the low extinction rate, diversity increase seems not to be limited by a time-variable carrying capacity, even with a carrying capacity close to the extant gastropod richness (Fig. 3c,e,h). Importantly, only with the feedback of carrying capacity on species accumulation, a substantial, initially high rate of ecological speciation and a strongly declining immigration rate emerge (Fig. 3c). This scenario is consistent with the finding for Lake Ohrid gastropods and suggests that their eco-evolutionary dynamic adheres to the principles of island biogeography. The exact reason for the high dispersal limitation and the low extinction rate resulting in this dynamic is currently unknown, but conserved species traits and ecosystem properties may play a role.

In addition to the integrative discussion of eco-evolutionary metacommunity assembly, this synthesis outlined how methodological progress may (i) enable to estimate the temporal trajectories and drivers of these assembly dynamics, and (ii) improve our mechanistic understanding of how processes of metacommunity structuring on short timescales imprint these temporal trajectories on longer timescales. This contributes to the Scopsco aim of ‘inferring the driving forces of biotic evolution in Lake Ohrid’.

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Part II

Publications

4 Spatially explicit analysis of gastropod biodiversity in ancient Lake Ohrid

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Spatially explicit analysis of gastropod biodiversity in ancient Lake Ohrid

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Abstract. The quality of spatial analyses of biodiversity is improved by (i) utilizing study areas with well defined physiogeographical boundaries, (ii) limiting the impact of widespread species, and (iii) using taxa with heterogeneous distributions. These conditions are typically met by ecosystems such as oceanic islands or ancient lakes and their biota. While research on ancient lakes has contributed significantly to our understanding of evolutionary processes, statistically sound studies of spatial variation of extant biodiversity have been hampered by the frequently vast size of ancient lakes, their limited accessibility, and the lack of scientific infrastructure. The European ancient Lake Ohrid provides a rare opportunity for such a reliable spatial study. The comprehensive horizontal and vertical sampling of a species-rich taxon, the Gastropoda, presented here, revealed interesting patterns of biodiversity, which, in part, have not been shown before for other ancient lakes.

In a total of 284 samples from 224 different locations throughout the Ohrid Basin, 68 gastropod species, with 50 of them (= 73.5%) being endemic, could be reported. The spatial distribution of these species shows the following characteristics: (i) within Lake Ohrid, the most frequent species are endemic taxa with a wide depth range, (ii) widespread species (i.e. those occurring throughout the Balkans or beyond) are rare and mainly occur in the upper layer of the lake, (iii) while the total number of species decreases with water depth, the proportion of endemics increases, and (iv) the deeper layers of Lake Ohrid appear to have a higher spatial homogeneity of biodiversity. Moreover, gastropod communities of Lake Ohrid and its feeder springs are both distinct from each other and from the surrounding waters. The analysis also shows that community similarity of Lake Ohrid is mainly driven by niche processes (e.g. environmental factors), but also by neutral processes (e.g. dispersal limitation and evolutionary histories of species). For niche-based mechanisms it is shown that large scale effects such as type of water body or water depth are mainly responsible for the similarity of gastropod communities, whereas small scale effects like environmental gradients affect gastropod compositions only marginally. In fact, neutral processes appear to be more important than the small scale environmental factors, thus emphasizing the importance of dispersal capacities and evolutionary histories of species.

1 Introduction

Biodiversity, the variation among living organisms or ecosystems (UNEP, 1992), is a multi-factorial concept. Ecosystem diversity can be assessed by a combination of α-diversity (i.e. species richness at one location), β-diversity (i.e. variation in species assemblages among locations), and γ-diversity (i.e. the total number of species of the ecosystem; Whittaker, 1972). Biodiversity is known to be heterogeneous in time (e.g., Gaston, 2000; Rohde and Muller, 2005; Weir, 2006) and space (e.g., Diniz-Filho and Bini, 2005; Buckley and Jetz, 2008). In general, spatial distribution of biodiversity is explained by two partly overlapping concepts. On the one hand, it is thought to be shaped both by large scale factors such as latitudinal gradient, altitude, water depth, and peninsula or bay effects, and by smaller scale factors such as habitat heterogeneity, barriers to dispersal, predation, competition or mutual stimulation (Gaston and Spicer, 2005). The second concept assumes that differences in community composition are caused by: (i) purely biological interactions, such as competition and mutualism, (ii) neutral processes like speciation, extinction or dispersal limitation of species,

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or (iii) processes based on the species' niche, acting via environmental factors such as climate (Legendre et al., 2005).

Unfortunately, our knowledge of biodiversity patterns is strongly biased towards marine or terrestrial habitats (Gaston and Spicer, 2005), and to conspicuous taxa like vertebrates (e.g., Abell et al., 2008; Strayer, 2006). Thus, our level of understanding of patterns in freshwater invertebrate distribution remains limited, raising the need for comprehensive studies (Hof et al., 2008).

To enable a spatial analysis of freshwater biodiversity, some prerequisites should be fulfilled: (i) ideally the area studied should be well defined based on natural entities rather than geopolitical ones, (ii) the majority of the studied taxa should occur exclusively in the study area to reduce the impact of widespread species on biodiversity estimates and to avoid scaling problems (Albert et al., 2010), and (iii) the taxon of interest should be characterized by high biodiversity and in-homogenous distribution to diminish the correlation between local and regional diversity relationships (Gaston and Spicer, 2005).

The first prerequisite is typically met by ancient lakes, that is, water bodies that have continuously existed for at least hundred thousand years. They often show a high degree of biodiversity and have inspired numerous studies of both fossil (Williamson, 1981; Van Boxclaer et al., 2008; Harzhauser and Mandic, 2008) and extant lake biota (Wilson et al., 2004; Herder et al., 2006). Cohen (1994) emphasized that the species richness we see in ancient lakes today could be a temporary snapshot. Lake-level fluctuations and changes of basin morphology with considerable effects on lake biota, for example, are well documented for ancient lakes of the east African Rift Valley (e.g., Cohen et al., 2007; Scholz et al., 2007; Schultheiß et al., 2009, 2011), Lake Baikal (Kashiwaya et al., 2001), or the Caspian Sea (Reid and Orlova, 2002; Grigorovich et al., 2002). Comparable data about hydrological changes in the European ancient Lake Ohrid have been published only recently (Belmecheri et al., 2009; Lindhorst et al., 2010).

Lake Ohrid is a graben lake of bathtub shape with a maximum depth of 289 m, a surface area of 358 km^2 and a volume of 55 km^3 (Matzinger, 2006b). Hence, it is one of the smallest ancient lakes of the world. Nevertheless, the Ohrid Basin has a complex geological and limnological structure. Different bedrock types, active tectonics (for details see Hoffmann et al., 2010), steep-sided mountain ranges, and water-discharge by lake-side and sublacustrine spring fields (Matzinger et al., 2006a) cause the characteristics of different habitats (Fig. 1). Some workers have suggested that these complex features are partly responsible for a high number of ecological niches and the outstanding degree of biodiversity (Radoman, 1985; Albrecht and Wilke, 2008). In the latter regard, Lake Ohrid differs from many other old Balkan lake systems (Albrecht et al., 2009; Marková et al., 2010; Trajanovski et al., 2010).

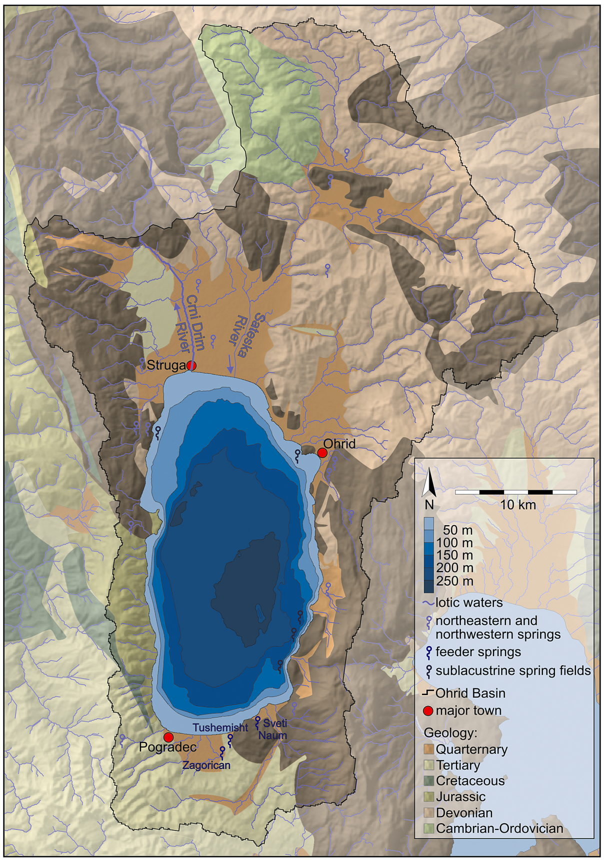

Fig. 1. Map of the Ohrid Basin showing major hydrological and geological features. Map adapted from Hoffman et al. (2010).

While Lake Ohrid, indeed, possesses a relatively isolated basin, thus fulfilling criterion (i) for spatial analysis of freshwater biodiversity, criteria (ii) and (iii), addressing taxonomic requirements, are met by representatives of its gastropod fauna. The vast majority of Lake Ohrid's gastropod species is morphologically relatively easy to distinguish and gastropods probably constitute the best studied invertebrate taxon in the lake (e.g., Poliński, 1929; Hubendick and Radoman, 1959; Radoman, 1985; Hauswald et al., 2008; Wilke et al., 2009). Recently, even the first fossil molluscs have been reported from the Last Interglacial period (Albrecht et al., 2010). Moreover, there is growing evidence for vertical and horizontal gradients and barriers within the Ohrid Basin (Albrecht et al., 2006, 2008; Wysocka et al., 2008; Trajanovski et al., 2010), possibly promoting parapatrical or even allopatrical speciation events (Albrecht and Wilke, 2008). Despite these interesting findings, a comprehensive picture of the spatial distribution of gastropod biodiversity within Lake Ohrid is still missing. However, this is not a problem typical for Lake Ohrid. In fact, we have no knowledge of any ancient lake in the world where a species-rich invertebrate taxon has been sampled in a manner allowing for statistically sound studies of the spatial distribution of biodiversity. Reasons include the often large size of ancient lakes, their, in part, limited accessibility (both physically and politically), and the lack of infrastructure for lake-wide biodiversity studies.

In this regard, Lake Ohrid constitutes a rare exception. Not only does the relatively small size of the lake allow for comprehensive sampling but the lake is also readily accessible, and infrastructure for lake-wide biodiversity studies can be regarded as excellent. Given this unique opportunity for a comprehensive horizontal and vertical sampling of a species-rich taxon over a whole ancient lake basin, we are using information from 224 collecting points for gastropods from Lake Ohrid to:

i. provide a comprehensive assessment of Lake Ohrid's gastropod diversity,

ii. identify potential faunal subdivisions of the Ohrid Basin utilizing a multivariate framework,

iii. provide a spatially-explicit description of biodiversity patterns within the lake proper, and

iv. assess the differential contribution of neutral versus niche based processes.

2 Material and methods

2.1 Zonation of the Ohrid Basin

The basin of Lake Ohrid (Fig. 1) has a size of 1605 km^2 (calculated based on SRTM-3 data from the year 2000) and consists of the lake itself with different depth layers as well as different zones in the surroundings, i.e. lotic waters, pools, and springs.

2.1.1 Lake Ohrid

The vertical subdivision of worldwide lakes is typically based on the degree of benthic photic production with the littoral being the zone of macrophytic vegetation and the profundal being the layer without photic production. In deep oligotrophic lakes such as Lake Ohrid, it is difficult to infer an exact zonation based on biotic factors. Thus, we here adopted a strictly hydrological approach based on lake-level fluctuations and the location of the summer thermocline.

Surface Layer

The Surface Layer is the upper vertical zone in Lake Ohrid down to approximately 5 m depth. It is affected by wave action (Stanković, 1960), intra-annual lake-level fluctuations (which today are regulated to only 1 m; Popovska and Bonacci, 2007), and high seasonal temperature variations (Stanković, 1960). The vegetation of the Surface Layer consists mostly of microalgae and extensive reed belts at the northern and southern shores (Watzin et al., 2002). Based on prevailing abiotic conditions, such as sublacustrine spring fields, substrate type and differences in geology (Hoffman et al., 2010), a horizontal zonation of the Surface Layer has been suggested (Albrecht and Wilke, 2008).

Intermediate Layer

The Intermediate Layer between 5–25 m water depth is summer-stratified by a relatively steep temperature decline and an increase of dissolved oxygen. It consists of a partly continuous belt of *Chara* algae (Lindhorst et al., 2010), which supposedly impedes the migration of benthic invertebrates (Albrecht et al., 2006; Sell et al., 2007; Wysocka et al., 2008; Trajanovski et al., 2010). The shell zone below the *Chara*-belt is characterized by wide *Dreissena* beds (e.g., Wilke et al., 2010) and sparse macrophytes (Stanković, 1960).

Deep Layer

The upper boundary of the Deep Layer is characterized by the end of the summer thermocline at ∼25 m (Watzin et al., 2002) and relatively high oxygen saturation (Stanković, 1960). As the first zone of definite fine-grained sediment deposition (Stanković, 1960), the substratum consists mainly of silt and mud (Vogel et al., 2010), and sporadic oxygen-depletion influences organic matter preservation (Holtvoeth et al., 2010). Below the 50 m isobath, there is no benthic photic production (Stanković, 1960).

2.2 Springs

Springs represent an important hydrological system in the Ohrid Basin as they are responsible for a considerable part of its water balance. They can be found within the lake (i.e. sublacustrine spring fields) or along the margins. Many of them are located in the northwest and northeast of the lake, but the most powerful springs are the feeder spring complexes of Sv. Naum (Macedonia) and Tushemisht/Zagorican (Albania) in the south. They form spring lakes of up to 5 m depth (Kunz, 2006). Mainly supplied by neighbouring Lake Prespa, the feeder-springs are characterized by different water chemistry than the mainly precipitation-fed springs in the northeast and northwest (Matzinger et al., 2006a). Thus, we here distinguish between the southern feeder-springs and the northeastern/northwestern springs.

2.2.1 Lotic waters and pools

Many of the northeastern and northwestern springs give rise to creeks and small, often artificial pools. Due to the steep-sided basin shape, the lotic waters are mainly short, shallow and often subject to seasonal desiccations. The two main lotic waters in the area are the effluent Crni Drim River and the affluent Sateska River (Fig. 1). The latter was artificially diverted into Lake Ohrid in 1962 (Matzinger et al., 2006b).

2.3 Gastropod sampling

Gastropods from the Ohrid Basin were collected during seven field trips carried out between May 2003 and September 2009. Individuals were obtained by hand collecting from hard substrata in shallow waters or from stones and rocks lifted during snorkelling. Soft substratum and plant material was sieved. Deeper parts of Lake Ohrid down to 60 m were sampled using a triangular dredge from small boats or from the Hydrobiological Institute Ohrid (HBI) research vessel. Data on sampling locations were recorded with a GPS device and stored in a database in UTM format. All materials are deposited at the University of Giessen Systematics and Biodiversity Collection (UGSB). Species determination and nomenclature for gastropod samples follows Poliński (1929), Hubendick and Radoman (1959), Radoman (1983), Bodon et al. (2001), and Albrecht et al. (2006, 2008). A total of 284 samples from 224 different collecting points were obtained during field work. Note that our non-quantitative sampling does not allow for a statistical sound comparison of abundances of species. Thus we had to rely on strict presence/absence information of gastropod occurrences at each collecting point.

2.4 Statistical analysis

2.4.1 Community composition and species richness

Number of species, proportion of endemics, and species frequencies are important characteristics of ecosystems and local subdivisions, and may give clues as to the underlying abiotic constraints and evolutionary histories (Cooper and Purvis, 2010).

First, species collecting frequencies were estimated and species were classified into the categories rare, common, and frequent (see Sect. 3.1 for details). We used the rarefaction method for each species at each depth layer because of unbalanced collecting frequencies in the three different depth zones. Species frequency and standard deviation was obtained for the lowest number of collecting points ($N = 34$, Deep Layer) by random site accumulation of one layer with 4999 permutations. To test for faunal subdivision of the different layers within the Ohrid Basin, we compared gastropod community compositions. Species compositions within a layer should be more similar than those of communities from different layers. Our hypothesis of distinct gastropod communities based on presence/absence data and Bray-Curtis dissimilarities was tested by a one-factorial, permutational analysis of variance (PERMANOVA; Anderson, 2001) with 4999 permutations. For explorative data analysis, we used non-metric multidimensional scaling (NMDS; Kruskal and Wish, 1978) to transform similarity of species composition into an illustratable low-dimensional figure. Water depth and species richness of the collecting points were fitted to the ordination of the lake proper and statistical testing was achieved by 4999 permutations.

Differences in mean species richness of distinct layers were tested by a one-factorial PERMANOVA with 4999 permutations.

2.4.2 Species co-occurrence

Distinct gastropod communities might be the result of species aggregation or segregation. We used the C-score, which measures the degree of species co-occurrence (Stone and Roberts, 1990), standardized it to values between 1 (maximum segregation) and 0 (maximum aggregation), and classified all 2278 species pairs into 22 bins according to their C-score. A null model distribution of species pairs was calculated by permuting the presence/absence matrix 1000 times with the constraint of preserving species richness per collecting point and species occurrence frequency. Within one bin, species pairs were ordered according to their specific Z-score, which is $(C_{\text{observed}} - C_{\text{null model}}) \cdot \text{StDev}^{-1}$. Subsequently, the number of species pairs $(N_{\text{species pairs observed}} - N_{\text{upper 95\% CL of species pairs null model}})$ with the highest Z-score within every bin was calculated, which are considered to be aggregated. Detailed information about this Bayes CL-criterion, the most conservative one to detect co-occurrence, can be found in Gotelli and Ulrich (2010). The analysis was conducted with Pairs 1.1 (Ulrich, 2008).

2.4.3 Analyses of spatial patterns

Horizontal and vertical variations of species richness in Lake Ohrid were compared utilizing a heat map generated by the R package spatstat 1.17-5 (Baddeley and Turner, 2005). Three ESRI shapefiles (ESRI, Redlands California) were imported corresponding to the three bathymetrically different layers (see above). All collecting points with their species richness were allocated to the appropriate bathymetric layer and species richnesses among collecting points were interpolated using a Gaussian kernel weighting of species richnesses within a radius of 2 km.

2.4.4 Explanatory variables of community composition

Variance partitioning (see Peres-Neto et al., 2006 for methological details) have been shown to be an adequate method to assess whether niche or neutral based processes drive biodiversity (Smith and Lundholm, 2010). In order to explain the similarity of gastropod communities in Lake Ohrid, we partitioned the variance into two fractions, one accounting for niche based and one for neutral processes. Niche processes summarize the influence of environmental factors and were classified into a fraction of three large scale predictors like collecting depth and into a fraction of eigth small

Table 1. Environmental and spatial predictors explaining the variance of 156 gastropod communities of Lake Ohrid. All environmental predictors were arcsin- or z-standardised and chosen by stepwise selection using a Bray-Curtis distance based redundancy analysis. Note that adding predictors to our model was always carried out with $p < 0.05$ and only the significance level for dropping the predictor is given.

Predictor	Type	Source	α	Sum R^2_{adj}
C/N	continuous	Vogel et al. (2010)	NS	
Total inorganic carbonate	continuous	Vogel et al. (2010)	NS	
Silt	continuous	Vogel et al. (2010)	NS	
Geological age	categorical ordered	Hoffmann et al. (2010)	NS	
Collecting depth	continuous	This study	∗∗∗	0.226
Substrate forming bedrock	categorical	European Soil Portal (2008)	∗	0.261
Collecting depth:Slope	continuous	This study	∗∗∗	0.284
PCNM2	continuous		∗∗∗	0.303
PCNM1	continuous		∗∗∗	0.320
PCNM3	continuous		∗∗∗	0.333
PCNM8	continuous		∗∗	0.345
Slope	continuous	Nearest neighbour interpolation of ArcMAP 9.3 (ESRI, Redlands California) using 10 000 equidistant points of isobaths map (Albrecht and Wilke, 2008)	∗∗∗	0.354
PCNM7	continuous		∗∗	0.362
Chlorophyll-*a*	continuous	Vogel et al. (2010)	∗∗	0.370
Total organic carbonate	continuous	Vogel et al. (2010)	∗∗∗	0.378
PCNM59	continuous		∗	0.385
PCNM6	continuous		∗	0.391

NS: not significant, ∗ $p < 0.1$, ∗∗ $p < 0.05$, ∗∗∗ $p < 0.01$, ∗∗∗∗ $p < 0.001$

scale variables such as chlorophyll-*a*. All tested environmental predictors are shown in Table 1. Until recently, an analysis of the contribution of the third fraction, neutral processes, was hampered by the limited quality of variables that describe the distribution of community similarity via spatial autocorrelation (Smith and Lundholm, 2010). These spatial descriptors are now obtained by principal coordinates of neighbouring matrices (PCNM; Borcard and Legendre, 2002). This approach computes a diagonalized matrix of Euclidean distances for the point coordinates by truncating all pairwise distances above a fourfold threshold value (Borcard and Legendre, 2002). All collecting points above this value are not considered as neighbours and may receive different community similarities in consecutive simulations. According to Blanchet et al. (2008), we first tested for a significant ($\alpha < 0.05$) prediction of the full model with all PCNMs with 4999 permutations. We used a constrained ordination (distance based redundancy analysis; db-RDA) because it allows the use of non-euclidean distances (Legendre and Anderson, 1999) like the here utilized Bray-Curtis dissimilarities of gastropod communities. Moreover, though this analysis is strictly linear, it allows for the utilization of interaction terms of explanatory variables. Then, significant environment and spatial variables were selected via stepwise model selection, which extended the forward selection suggested by Blanchet et al. (2008). This approach utilizes a double stop criterion: p-values of variables obtained by a maximum of 999 permutations and the adjusted coefficient of determination (R^2_{adj}) of the model, which penalizes additional variables and stops if additional variables do not increase the R^2_{adj} any further (Blanchet et al., 2008).

According to Peres-Neto et al. (2006), the partitioning of variance into fractions should be unbiased, that is, fractions with more degrees of freedom have to be penalized. The resulting R^2_{adj} allows for a direct comparison of predictor fractions. In order to obtain non-negative values for shared fractions and to perform the recommended redundancy analysis (Peres-Neto et al., 2006), we utilized a square root transformation of the semi-metric Bray-Curtis dissimilarities to meet the assumption of strict Euclidian distances (Legendre and Anderson, 1999).

All analyses were carried out using the R 2.12 statistical environment (R Development Core Team, 2009) and the vegan 1.18-20 package (Oksanen et al., 2011).

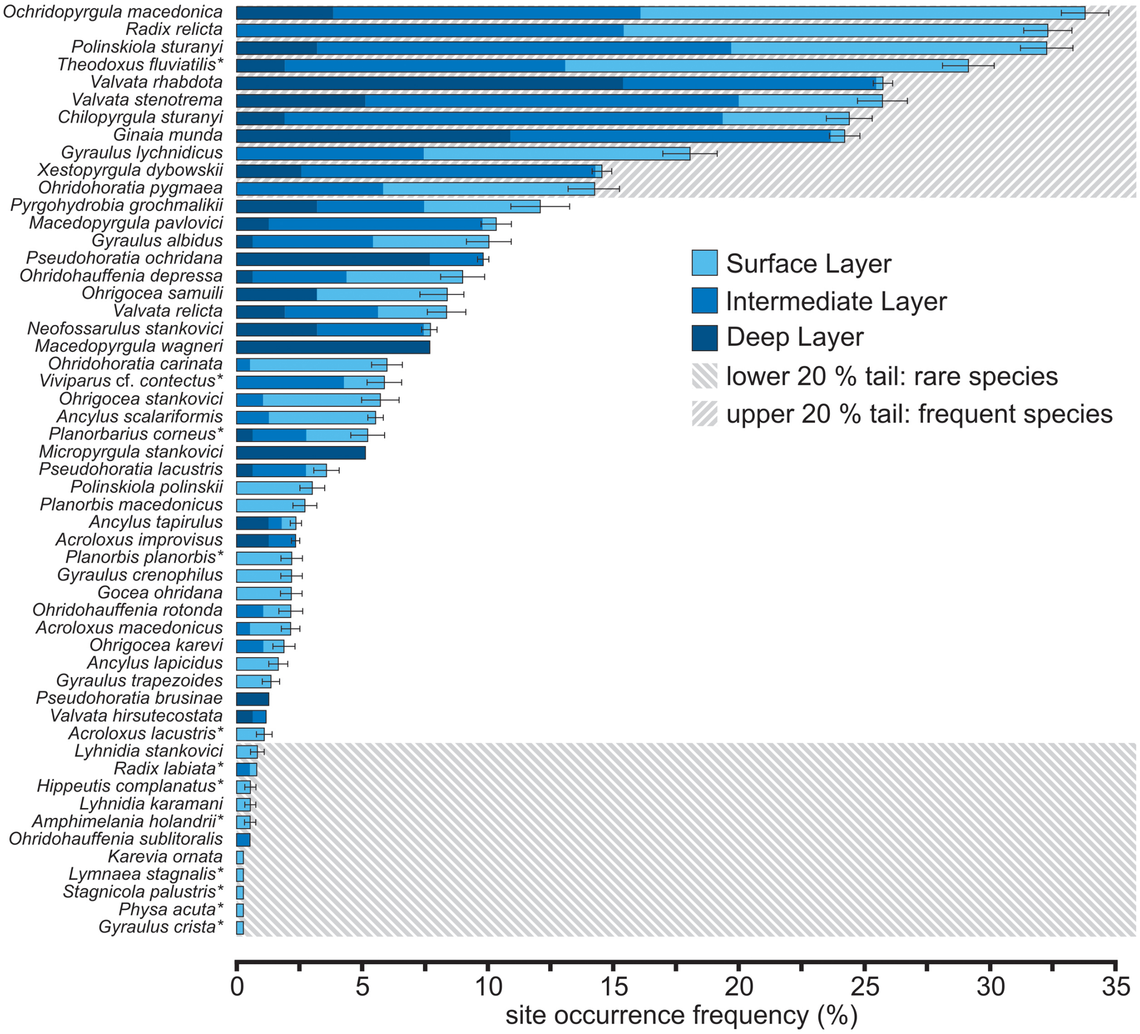

Fig. 2. Plot of frequency and standard deviation of species sites occurrence of gastropod species found in Lake Ohrid. Depth layers are colour-coded and widespread species are marked by asterisks.

3 Results

3.1 Species occurrence frequency and endemism

A total of 68 gastropod species was found in the Ohrid Basin with 50 of them (73.5%) being endemic. Following the species based rarefaction of the lake species (Fig. 2), we applied the criteria of Williams et al. (1996) to classify taxa according to frequency. Those species that account for the top 20% quantile were considered to be frequent species and the same proportion at the lower tail as rare. Frequent species were found at up to one third of all sampling points; common species at 1–12% and the 11 rare species at less than 1% of all sites. Moreover, most of the rare gastropod species were non-endemics. No gastropod specimens were found in Lake Ohrid below 60 m water depth or in the high mountain springs of its basin.

3.2 Community composition and species richness

Explorative analyses of gastropod community similarities showed differences both within the Ohrid Basin (Fig. 3a) and within Lake Ohrid itself (Fig. 3b). The two-dimensional ordination of all collecting points revealed a Kruskal stress value of 19.39, indicating marginal congruence with the original data structure (Kruskal and Wish, 1978). A partitioning into Lake Ohrid, feeder springs, lotic waters, and northeastern/northwestern springs was supported by a significant zonation effect on community composition ($F_{3,220} = 21.48$, $P < 0.001$). The three-dimensional NMDS of the Lake Ohrid communities had a stress value of 15.4. Vector fitting showed a highly significant correlation between ordination structure and both collecting depth ($R^2 = 0.79$, $P < 0.001$) and species richness ($R^2 = 0.48$, $P < 0.001$). The

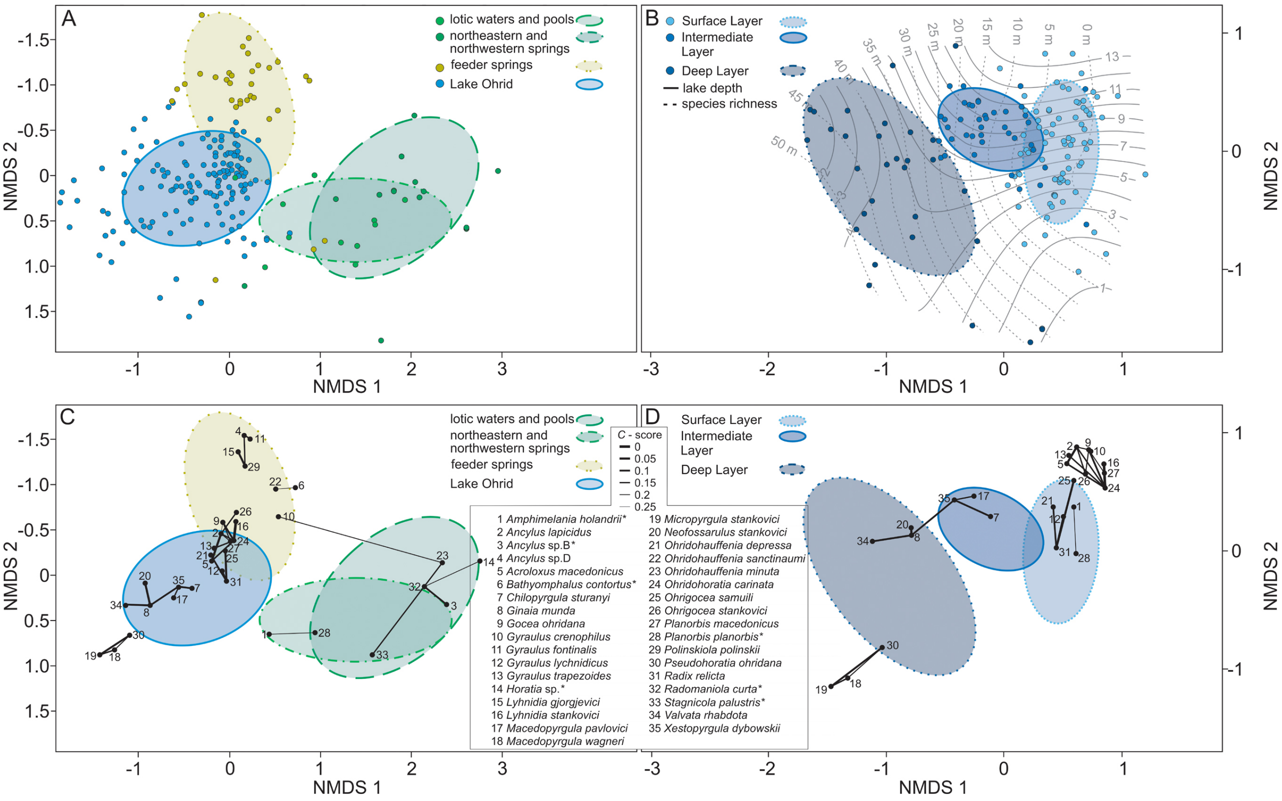

Fig. 3. NMDS plots of gastropod communities (Bray-Curtis dissimilarity). **(A)** Two-dimensional solution within the Ohrid Basin showing little similarity among horizontal zones. **(B)** Vector fitting of collecting depth and species richness for the three-dimensional solution within Lake Ohrid showing community turnover correlated with collecting depth. **(C)** Species joint plots of the ordination within the Ohrid Basin, and **(D)** within Lake Ohrid indicating non-random co-occurrence of species by line connection. Widespread species are marked by asterisks and line widths correspond to the degree of species co-occurrence.

differences between species assemblages in the three lake layers were also supported by a significant PERMANOVA result ($F_{2,153} = 29.18$, $P < 0.001$).

The different layers are characterized by different numbers of widespread, endemic, and layer endemic gastropod species (Fig. 4a). The three different layers of Lake Ohrid harbour more species and endemics than outside water bodies. The northeastern and northwestern springs as well as the feeder-springs revealed the highest proportion of point endemic species.

Species richness (Fig. 4b) differed among the zones of the Ohrid Basin ($F_{3,220} = 15.68$, $P < 0.001$) as well as among the depth layers of the lake ($F_{2,153} = 19.73$, $P < 0.001$). The Intermediate Layer, for example, showed the highest alpha diversity with a mean of 8.8 ± 1.4 gastropods per collecting point, whereas the lotic waters and northern springs harboured the lowest number of species (2.5 ± 0.6 and 3 ± 0.8, respectively).

3.3 Species co-occurrence

Out of 2278 species pairs analysed, 31 showed a higher co-occurrence frequency than expected by chance. All of these species pairs occurred in the same zone or layer. (Fig. 3c and d). Of these 31 pairs, 30 pairs involved endemic species and one pair Palaearctic widespread species. No pair of species recently invading Lake Ohrid Basin (i.e. not known from previous studies) and endemic gastropods could be detected.

3.4 Spatial analysis

Species richness maps (Fig. 4c and d) showed a relatively homogeneous distribution of gastropod species in the Deep and Intermediate Layer, whilst the highest variation occurred in the Surface Layer.

Acknowledging that we did not apply a statistical model for identifying places with elevated degrees of endemic biodiversity ("hotspots" sensu Prendergast et al., 1993), candidate places as shown in Fig. 4d are the area around Veli Dab (southeastern shore) of the Surface Layer as well as areas of

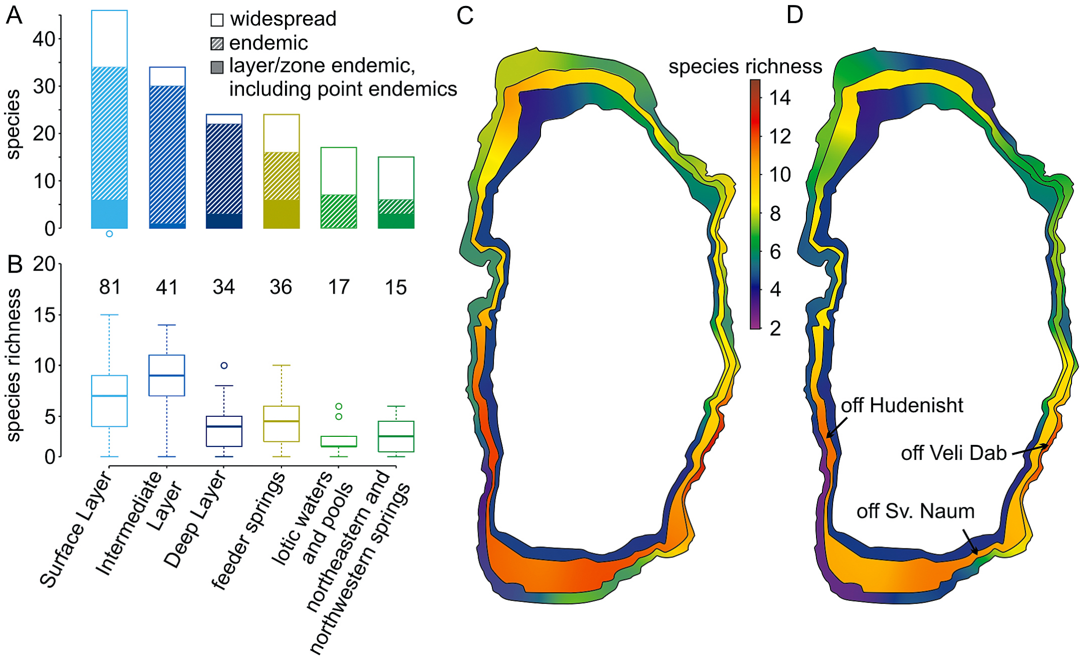

Fig. 4. Zonation and layer-specific analyses of gastropod species richnesses in the Ohrid Basin. **(A)** Bar plots of species compositions. **(B)** Quartile box plots of species richness including median, upper and lower quartiles (boxes) and 1.5 interquartile range whiskers (numbers above plots indicate the total number of species in the respective layer/zone). **(C)** Species richness map based on all species. **(D)** Species richness map based on endemics. Candidate hotspots of endemic species richness are indicated by arrows. Because of the strong slope at the eastern and western tectonic faults, the depth layers could have a width of less than 10 m, and were thus stretched for better visibility.

the Intermediate Layer off Sv. Naum (southern shore) and off Hudenisht (southwestern shore).

3.5 Explanatory variables of community composition

A total of 75 PCNMs were constructed and the significance test of the full spatial modal was passed ($P < 0.05$). Altogether, the significant spatial (Fig. 5) and environmental variables explained 39.1% of the variance of gastropod species composition of Lake Ohrid (Table 1). The variance inflating factors of all variables were lower than the critical heuristic value of 10, indicating that they are not correlated. The environmental variables explained more than double the variance than spatial predictors (Fig. 6). Moreover, large scale environmental variables had a higher explanatory power than small scale factors, which in turn contributed less to the similarity of gastropod communities than purely spatial predictors. In general, the shared fractions among our predictor classes explain only a small proportion of variance of the gastropod assemblages.

4 Discussion

Our results suggest the existence of distinct gastropod communities both within the different zones of the Ohrid Basin and within the different depth layers of the lake. These communities seem to be explained by environmental variables with a significant contribution of neutral processes. The rivers and northeastern and northwestern springs are species poor, the southern feeder-springs show moderate mean species richness, and all zones of the lake proper are species rich, except for the Deep Layer. However, all three depth layers are almost exclusively inhabited by endemics. While the Intermediate Layer has the highest mean alpha diversity, the highest variation of alpha diversity can be found in the Surface Layer.

4.1 Endemism and species frequency

The total number of 68 gastropod species and the total number of 50 endemic species found in our study are only slightly lower than those reported by Radoman (1985), that is, 72 and 56 species, respectively. It should be noted that the numbers published by Radoman are based on approximately 30 years of field work. The six endemic species not rediscovered in our recent sampling are known to have been described from empty shells, are only sporadically released

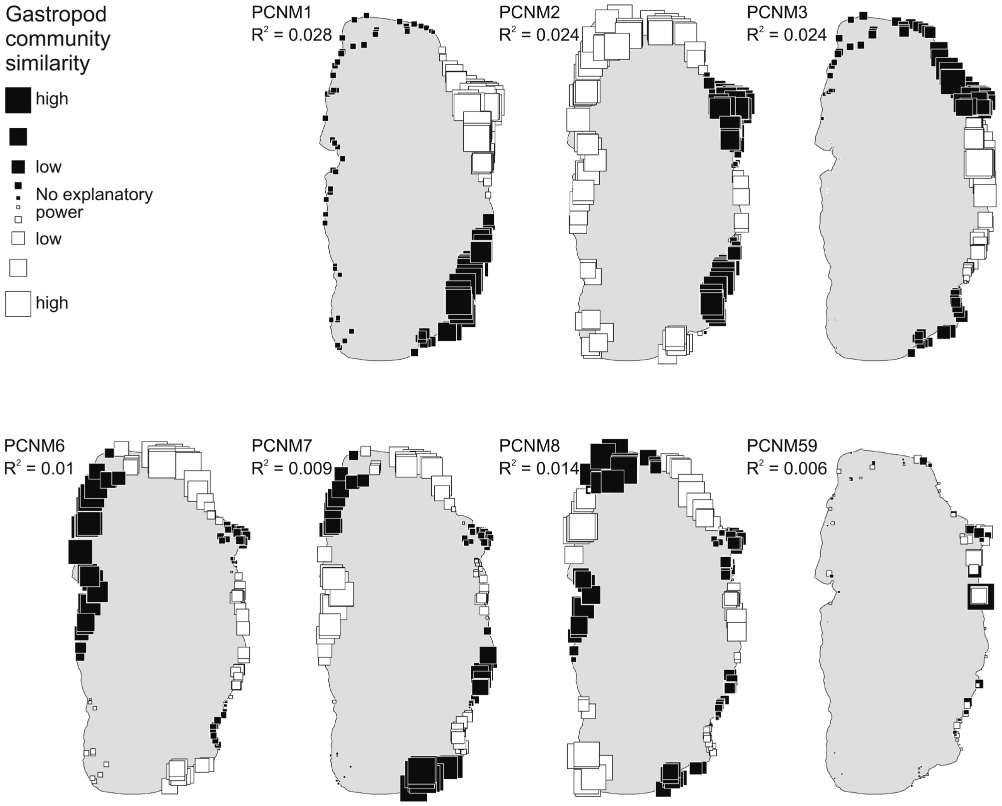

Fig. 5. Spatial prediction of community similarities of Lake Ohrid utilizing PCNM eigenfunctions. Six broad and one fine scale vectors explain 11.5% of the variation of gastropod communities. Within each eigenfunction, groups of equally-coloured squares indicate similar gastropod communities. Increasing square size shows higher similarity (note that small squares may not have sufficient explanatory power). Higher eigenfunctions represent increasingly smaller spatial scale predictions. Therefore, the frequency of alternating groups of black and white squares increases.

from subterranean springs, or are rare Deep Layer species. Accordingly, the rate of endemism revealed in our study (73.5%) is also lower than the 78% suggested by Radoman (1985). In addition, we collected three widespread species previously unknown to the Ohrid Basin, which further reduces the rate of endemicity. On the other hand, recent molecular studies (Albrecht et al., 2006, 2008) revealed the existence of two new cryptic feeder spring species. Albrecht and Wilke (2008) emphasized the lack of knowledge concerning the evolutionary history of many taxa and that future molecular studies will probably result in the discovery of more new endemic species in Lake Ohrid (also see Schultheiß et al., 2008 for a molecular study on pea-clams and Marková et al., 2010 for cryptic phylogenetic subdivision in a cyprinid species). In terms of the total number of endemic gastropod species, Lake Ohrid is only outnumbered by ancient lakes Baikal, Tanganyika and the Malili lakes. However, taking surface area into account by applying the normalized endemic species area index (Albrecht and Wilke, 2008), Lake Ohrid surpasses other ancient lakes in terms of endemic gastropod biodiversity (Albrecht et al., 2009).

In Lake Ohrid, a high proportion of gastropod species are endemics (Fig. 4a), many of them being common (Fig. 2) and relatively unconfined regarding their depth preferences. Interestingly, the non-endemics are almost exclusively rare species and occur mostly in the Surface Layer. In fact, there appears to be a positive correlation between species frequency and depth range.

In contrast to previous reports (e.g., Hadžišče, 1956; Stanković, 1960; Radoman, 1983), an obvious shift in the frequency of endemic species occurrences at sites could not be detected. However, a shift in species depth range appears to be possible. Whereas we were unable to find specimens below depths of 60 m, occurrences at up to 100 m depth were reported by Radoman (1985).

Moreover, an increasing number of widespread species are observed living in areas of the highest anthropogenic pressure in the western (Lin Peninsula) and northeastern (Ohrid Bay, Sateska Estuary) parts of the lake (Fig. 4d, also see Kostoski et al., 2010). However, no community disassembling due to either widespread-/widespread- or widespread-/endemic species pairs could yet be observed (Fig. 3c and d).

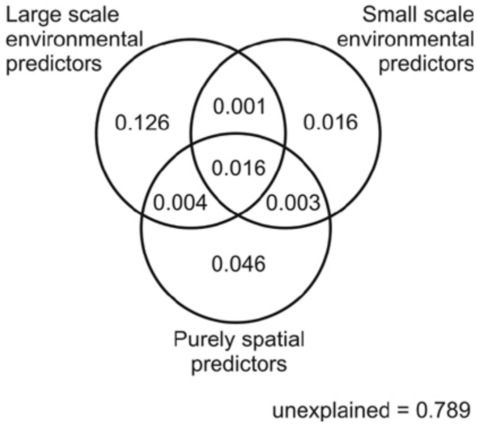

Fig. 6. Venn-diagram showing the partitioning of the explained variance of gastropod community compositions in Lake Ohrid into fractions of predictors and their combinations. The figure indicates that large scale environmental predictors (i.e. collecting depth, slope, and collecting depth:slope) alone can explain most of the similarity between gastropod communities. This is followed by purely spatial predictors (i.e. 7 PCNMs) and small scale environmental predictors (i.e. chlorophyll-*a*, total organic carbonate, and substrate forming bedrock). Note that the square root of the community dissimilarity measure was used. Therefore the adjusted explained variance is lower than given in Table 1. For detailed explanations see the Material and method section.

This is probably due to the only recent onset of eutrophication of Lake Ohrid (Matzinger et al., 2006a) and the still limited invasion of Lake Ohrid by additional widespread species (Fig. 2). In contrast to Lake Ohrid, an increase in abundances of widespread gastropod species at the expense of endemic species has been shown in ancient Lake Malawi (Genner et al., 2004), the Caspian Sea (Grigorovich et al., 2002), and potential ancient lakes in the Balkans (Albrecht et al., 2009).

4.2 Faunal subdivision of the Ohrid Basin

In general, little faunal overlaps were observed among different zones or layers (Fig. 3). Hence, our results suggest, on the one hand, a good separation of lake gastropod communities from the surrounding watershed, whilst, on the other hand, a fair degree of separation among communities of feeder springs versus northwestern/northeastern springs and lotic waters (Fig. 3a). As originally proposed by Boss (1978) and refined by Wilke et al. (2010), a common feature of ancient lakes is eco-insularity. Accordingly, well adapted ancient lake organisms may out-compete most invading species but are probably inferior outside the native lake. Hence, little faunal overlap is expected in the case of eco-insularity. The recent lake shore, however, may not necessarily constitute the “island” border and there are examples showing effects of eco-insularity not only at the lake but also at the watershed level (Glaubrecht and Rintelen, 2008; Schultheiß et al., 2009). In Lake Ohrid, the situation appears to be more complex. Whilst we do see eco-insularity on a basin scale (Albrecht et al., 2009), we also see evidence for patterns within the basin: eco-insularity of the lake proper and eco-insularity of the feeder-springs.

While these horizontal zones are well reflected in a distinct species distribution, the differentiation of gastropod communities relative to vertical depth layers is less pronounced. This may be due to less distinct transitions of environmental factors among the different layers of Lake Ohrid. However, spatial gradients of sediment features have been shown before (Vogel et al., 2010) and different substrata are likely to affect species richness and distribution (Kershner and Lodge, 1990; Michel, 1994). In fact, the present study strongly suggests that today’s gastropod composition of Lake Ohrid was mainly driven by environmental conditions with a partial but significant contribution of purely neutral mechanisms. The similarity of gastropod communities of Lake Ohrid is mostly explained by large scale factors like water depth, resulting in a high turnover of species assemblages (Table 1). Interestingly, the neutral drivers of biodiversity showed a marginal higher explanatory power of community composition than small scale environmental factors. According to Legendre et al. (2005) and Gotelli and McGill (2006), these neutral processes suggest a limited dispersal of species or the evolution of new species in spatially distinct areas, thus the evolutionary histories of species appears to matters and it is reflected in their distribution in Lake Ohrid.

4.3 Hotspots of species richness

The Intermediate Layer showed both the highest number of species per collecting point but also the lowest number of species exclusively restricted to a single depth layer. Whereas annual water level fluctuation and wave action restrict plant and bacteria growth in the Surface Layer (Stanković, 1960), the Intermediate Layer is relatively undisturbed, yet enough light reaches this part (Stanković, 1960). Therefore, this photic zone is known to be the most bioproductive layer in Lake Ohrid (Tocko and Sapkarev, 1978). Similar patterns of elevated species richness in Intermediate Layers were observed in ancient lakes Tanganyika (Michel, 1994), Baikal (Sitnikova, 2006) and Titicaca (Dejoux, 1992). In contrast, the Caspian Sea shows a rather continuous decline of species richness and density with increasing water depth (Parr et al., 2007). However, elevated species richness in shallow parts is suggested to be a general feature of ancient lakes (Martens, 1997). No comprehensive species richness study of other taxa has been conducted in Lake Ohrid so far. Analyses of phytoplankton and Chironomidae abundances suggest a similar peak value at the Intermediate Layer, whereas Oligochaeta, Amphipoda and especially Ostracoda show their highest densities in deeper waters (Stanković, 1960; Mikulič and Pljakic, 1970).

Interestingly, the species richness map (Fig. 4c) shows relatively uniform distributions of alpha diversity for the Deep and Intermediate Layers. The Surface Layer, however, is characterized by a rather heterogeneous species richness distribution. Of the three suggested biodiversity hotspots, the area around Veli Dab, appears to be outstanding in terms of point endemism. This hotspot, comprising an area of less than 1 km^2, exclusively harbours >10% of Lake Ohrids' endemic gastropod biodiversity (Fig. 4a; also see Wilke and Albrecht, 2007), including most point endemics of Lake Ohrid, which are highly aggregated (Fig. 3d). These taxa are known to require a specific environment, the so-called "littoral interlithon", that is, a patchy system of porous stones or rocks often associated with sublacustrine spring fields (e.g., Hadžišče, 1956; Albrecht and Wilke, 2008).

5 Conclusions

This spatially comprehensive study of a large taxon revealed interesting patterns of biodiversity in the Ohrid Basin. The total number of 68 species of gastropods here reported with 73.5% of them being endemic does not substantially differ from the numbers generated decades ago. We also did not find significant differences in the frequency of species occurrences at sites. We do, however, see a moderate trend indicating a decrease in endemic species and an increase of widespread species. Interestingly, many endemic species are very common (Fig. 2) and relatively unconfined regarding their depth preferences. In contrast, non-endemic species are mostly rare species, typically being restricted to limited areas of the surface layer. However, so far, community disintegration due to widespread species invading Lake Ohrid could not be shown.

Little faunal overlap was observed in the NMDS analysis, indicating strong effects of eco-insularity for the Ohrid Basin. Moreover eco-insularity appears to act on two spatial scales – on the level of the whole watershed and on the level of the lake proper/feeder springs.

Variation of community assemblages is likely to be explained by hierarchically structured effects acting on different scales. Large scale effects such as type of water body and lake depth do, indeed, cause broad differences, while small scale effects like environmental gradients typically cause minor differences in biodiversity distributions on a smaller spatial scale. However, a significant proportion of community variation seems to be driven by the dispersal capacity and evolutionary history of the species.

Alpha diversities of the Deep and Intermediate Layers show relatively uniform spatial distributions. In contrast, only the Surface Layer is characterized by heterogeneous species richnesses. Moreover, all point endemics of the lake proper reported in our study are to be found in a single biodiversity hotspot at the rocky southeastern shore. This site harbours >10% of Lake Ohrids' endemic gastropod species.

While our study shows a strong correlation between the hydrological features of the Ohrid Basin and the spatial distribution of gastropod diversity, future studies should focus on the underlying evolutionary processes generating the extraordinary high degree of biodiversity within Lake Ohrid.

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5 | Assembly processes of gastropod community change with horizontal and vertical zonation in ancient Lake Ohrid: a metacommunity speciation perspective

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Assembly processes of gastropod community change with horizontal and vertical zonation in ancient Lake Ohrid: a metacommunity speciation perspective

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Abstract. The Balkan Lake Ohrid is the oldest and most diverse freshwater lacustrine system in Europe. However, it remains unclear whether species community composition, as well as the diversification of its endemic taxa, is mainly driven by dispersal limitation, environmental filtering, or species interaction. This calls for a holistic perspective involving both evolutionary processes and ecological dynamics, as provided by the unifying framework of the "metacommunity speciation model".

The current study used the species-rich model taxon Gastropoda to assess how extant communities in Lake Ohrid are structured by performing process-based metacommunity analyses. Specifically, the study aimed (1) to identifying the relative importance of the three community assembly processes and (2) to test whether the importance of these individual processes changes gradually with lake depth or discontinuously with eco-zone shifts.

Based on automated eco-zone detection and process-specific simulation steps, we demonstrated that dispersal limitation had the strongest influence on gastropod community composition. However, it was not the exclusive assembly process, but acted together with the other two processes – environmental filtering and species interaction. The relative importance of the community assembly processes varied both with lake depth and eco-zones, though the processes were better predicted by the latter.

This suggests that environmental characteristics have a pronounced effect on shaping gastropod communities via assembly processes. Moreover, the study corroborated the high importance of dispersal limitation for both maintaining species richness in Lake Ohrid (through its impact on community composition) and generating endemic biodiversity (via its influence on diversification processes). However, according to the metacommunity speciation model, the inferred importance of environmental filtering and biotic interaction also suggests a small but significant influence of ecological speciation. These findings contribute to the main goal of the Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO) deep drilling initiative – inferring the drivers of biotic evolution – and might provide an integrative perspective on biological and limnological dynamics in ancient Lake Ohrid.

1 Introduction

Ancient Lake Ohrid on the Balkan Peninsula (Fig. 1) is the oldest and most speciose freshwater lacustrine system in Europe (Albrecht and Wilke, 2008; Neubauer et al., 2015). Recently, an International Continental Scientific Drilling Program (ICDP; Wagner et al., 2014) has been conducted in the lake within the research initiative Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO). One of the major goals of this collaborative project is to infer the drivers of speciation by linking the geological and biotic evolution of the lake over space and time. Geological, limnological, and paleontological data from sediment cores are being complemented with phylogenetic and molecular clock data derived from DNA information of extant endemic species.

Though the sediment and molecular data are still being analyzed, first results indicate that climatic, geological, and/or environmental changes over time may have had little direct effect on speciation and extinction processes in selected endemic biota of Lake Ohrid (Föller et al., 2015). In-

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stead, such changes potentially affected abundances of paleospecies and community compositions over time (Albrecht et al., 2010; Belmecheri et al., 2009; Jovanovska et al., 2016; Reed et al., 2010; Wagner et al., 2014). Horizontal and vertical (bathymetric) environmental gradients may have not only shaped the structure of extant Ohrid communities (Hauffe et al., 2011; Lorenschat and Schwalb, 2013), but also fostered species divergence (Schreiber et al., 2011; Wysocka et al., 2008). Speciation and extinction, in turn, may have also affected the spatial structure of extant communities (Hauffe et al., 2011).

For Lake Ohrid, these results question the direct effect of climatic, geological, and/or environmental factors on diversification and call for a holistic perspective involving evolutionary processes (e.g., speciation) and ecological processes affecting community composition (e.g., dispersal limitation, species interaction).

Such a mechanistic and unifying eco-evolutionary framework – the metacommunity speciation model – was recently proposed by Hubert et al. (2015). It considers how a metacommunity (i.e., a network of local communities linked by dispersal of multiple interacting species; Leibold et al., 2004) is affected by speciation and vice versa.

In principle, a local community is structured by three nonexclusive consecutive processes (e.g., Leibold et al., 2004; van der Plas et al., 2015): (1) dispersal of individuals to a patch previously unoccupied by the species, (2) environmental filtering that permits the establishment of species, whose ecological niche fits the local environmental conditions, and (3) species interaction including competitive exclusion among resident and colonizing species because of shared resources, predators, and/or pathogens.

From a theoretical point of view, all of these three processes might affect speciation (Hubert et al., 2015). However, their individual contribution and spatial dynamics in Lake Ohrid remain little understood. The latter is particularly true for the bathymetric structure of Lake Ohrid. The deep lake has a distinct horizontal eco-zonation (reviewed in Albrecht and Wilke, 2008). This leads to the question of whether the individual importance of the three processes mentioned above gradually changes with lake depth or whether there are abrupt changes related to transitions between eco-zones. Conceptually, a differentiation between gradual and abrupt changes in structuring processes among eco-zones may shed light on how limnological complexity and stability affect community assembly and diversification processes.

Gastropods are a suitable model taxon to study community structuring processes as they represent the most species-rich animal group in Lake Ohrid with 74 described species, 56 endemic to the lake and its catchment (Albrecht et al., 2014; Albrecht and Wilke, 2008; Hauffe et al., 2011; Radoman, 1985). In the current study we therefore focus on the question of how extant gastropod communities are assembled by performing process-based metacommunity analyses. Our working hypothesis is that, in general, dispersal limitation plays a crucial role in structuring communities.

Our specific aims are as follows:

1. To identify the relative importance of the three community assembly processes (dispersal limitation, environmental filtering, and species interaction) in shaping the lakes' gastropod community composition. We simulated community assembly and measured the respective simulation steps corresponding to these three processes.
2. To test whether the importance of these individual processes changes gradually with lake depth or whether they are distinctively related to eco-zones. We first performed an automatic detection of eco-zones based on community composition and then inferred whether the relative importance of the three community assembly processes was better predicted by either lake depth or delineated eco-zones.

This study will help to infer the drivers of community assembly and speciation in Lake Ohrid by providing an independent and unifying view of eco-evolutionary processes and their relationships to ecosystem characteristics. The novel process-based model presented here may also serve as a basis for studies of the community composition and their underlying assembly processes in (paleo)species communities of Lake Ohrid, other ancient lake systems, or elsewhere.

2 Material and methods

2.1 Gastropod sampling and taxonomic classification

The current study is based on gastropod community compositions taken from Hauffe et al. (2011), which were supplemented with additional field data obtained from 2010 to 2011. In total, we analyzed 264 localities of Lake Ohrid and its feeder springs. Gastropods were collected by hand picking, sieving, or dredging from small boats or the research vessel of the Hydrobiological Institute Ohrid. The latter enabled sampling depths of down to 70 m, thus covering the entire bathymetric range of gastropods in Lake Ohrid.

Though phylogenetic relationships among endemic gastropod taxa of Lake Ohrid are increasingly well characterized, a complete picture is still lacking. We therefore classified taxa in five hierarchical levels: species, genus, species flock, family, and superfamily. Note that we used the level "species flock" for endemic clades that comprise more than one genus (Föller et al., 2015; Wilke et al., 2007). Species flocks typically evolved within the lake and are common in Lake Ohrid. For critical taxa, here we used the taxonomic assignments of Bodon et al. (2001), Hubendick and Radoman (1959), and Radoman (1983). We calculated pairwise taxonomic distances between the 66 collected gastropod species using the taxonomic classification above. This method was described by Clarke and Warwick (1998) and is

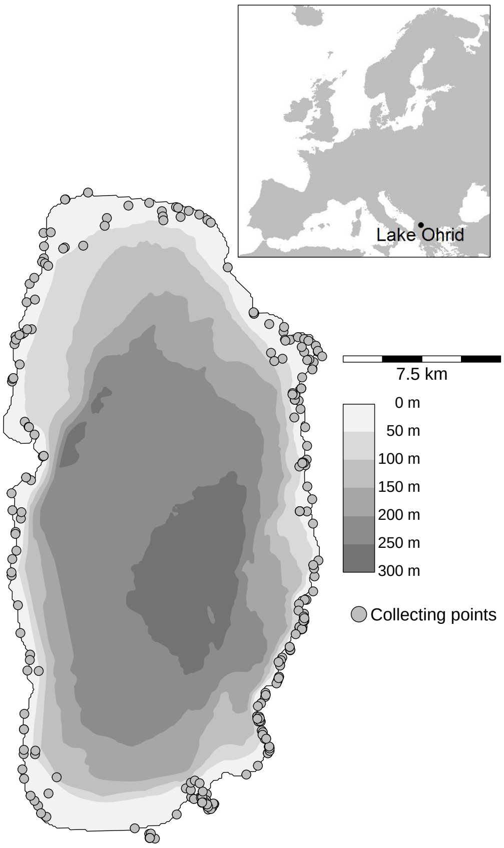

Figure 1. Bathymetric map of Lake Ohrid (Albania, Macedonia) with sampling localities of gastropod communities studied.

implemented in the package vegan v2.2-1 (Oksanen et al., 2015) for the R v3.1.2 statistical environment (R Core Team, 2015).

2.2 Simulation of individual community assembly processes

Processes of community assembly can be inferred by three distinct approaches. First, environmental parameters and measures of spatial autocorrelation are linked to community composition and may represent proxies for estimating indirectly the importance of processes such as environmental filtering (Sharma et al., 2011) and dispersal limitation (Legendre et al., 2005). Second, a comparison of the phylogenetic structure or trait distribution of a community with a null model of the expected structure or distribution informs one about the prevailing importance of environmental filtering or species interaction (Webb et al., 2002). Third, a simulation method of stepwise community assembly (STEPCAM) infers the relative importance of all three processes (van der Plas et al., 2015). It prunes the regional species pool to the size of an observed community by sequentially removing species according to specific simulation processes for dispersal limitation, environmental filtering, and biotic interaction.

The simulation requires a presence–absence matrix of community composition and species characteristics such as traits or their taxonomic/phylogenetic relationship (Fig. 2a). Here we extended the original STEPCAM approach, which only considers continuous species traits, and now allow a mixture of continuous and categorical species traits resulting in matrices of pairwise distances between species (Fig. 2a). This generalization also permits the use of phylogenetic or taxonomic distances. These distances are typically strongly correlated with trait divergence among species and used in analyses of community assembly (Emerson and Gillespie, 2008; Webb et al., 2002).

Trait or taxonomic characteristics of a community define the target of the STEPCAM model: if the values of trait/taxonomic indices of a simulated community approximate the observed ones, we assume that the performed species removal steps during the simulation reflect the relative importance of the three assembly processes for the observed community (see specific aim 1). The similarity between simulated and observed communities ($\mathrm{Fit}_{\mathrm{total}}$; van der Plas et al., 2015) is the sum of absolute differences between four indices (i.e., richness, evenness, divergence, and mean distance to the community centroid; Fig. 2b) based on traits (Villéger et al., 2008) or taxonomic/phylogenetic distances (Helmus et al., 2007). However, calculating $\mathrm{Fit}_{\mathrm{total}}$ is problematic because the scale of the utilized indices differs: "evenness", for example, is bounded between 0 and 1, whereas "richness" lacks an upper limit. Therefore, van der Plas et al. (2015) standardized the trait indices by dividing them by the standard deviation (SD) of the respective index, calculated for all observed communities. For large differences in species richness or few sampled communities, this approach can be biased because some indices are not independent of species richness (Villéger et al., 2008) and the SD is less precisely estimated in case of small sample sizes. We estimated all SDs using an initial simulation of 500 communities based on observed species richness.

STEPCAM simulates dispersal limitation by randomly removing species with a probability inversely related to their relative frequency in the regional species pool (Fig. 2c). Dispersal-limited species are therefore less likely present in the simulated community (van der Plas et al., 2015).

Environmental filtering and species interaction depend on the ecology of the species (i.e., its niche). These ecological requirements are assumed to be reflected by morphological features, which are typically under selection, or effected by the relatedness of species. Environmental filtering therefore predicts that species of a community show similar morphological traits or are closely related because environmental conditions only allow for the establishment of species that are adapted to the specific habitat (Webb et al., 2002). Pruning species most distant to the morphologically or taxonom-

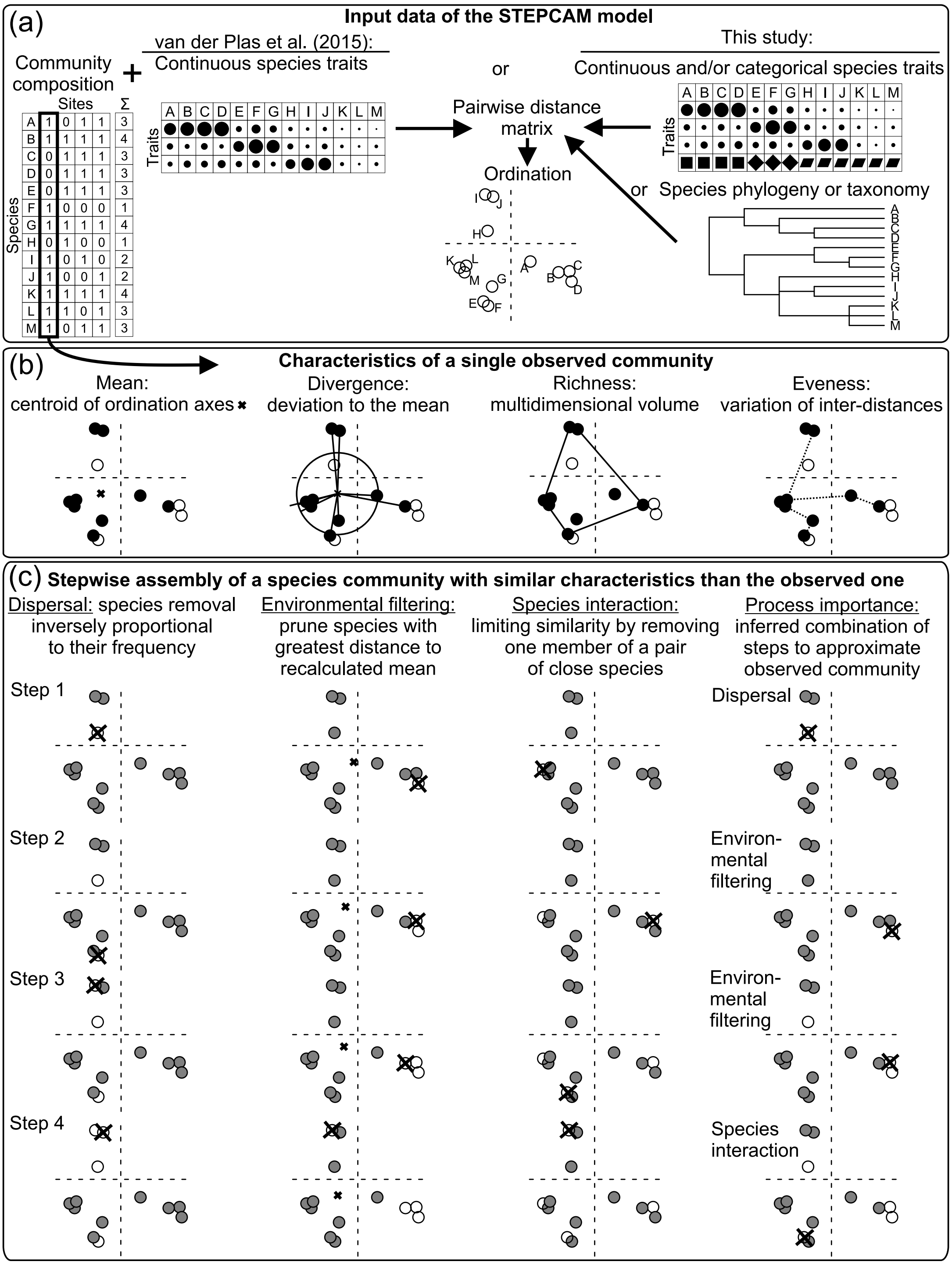

Figure 2. Illustration of the distance-based stepwise community assembly model (STEPCAM). **(a)** The model is parameterized by species' frequencies and either functional species traits, a species phylogeny, or a hierarchical taxonomic classification. **(b)** Functional or taxonomic/phylogenetic indices (for details see Villéger et al., 2008) characterize the focal species community. **(c)** These index values are the targets for the modeled community. A simulation starts with all species of the regional species pool, removes stepwise species according to the three processes dispersal limitation, environmental filtering, and species interaction, and stops when the same number of species as in the focal community is reached. Approximate Bayesian computation within a sequential Monte Carlo framework infers the combination of simulation-steps that generates a species community with minimum differences between observed and generated index values. The number of removal steps provides information on the importance of the respective assembly process.

ically mean of the generated community simulates environmental filtering (Fig. 2c).

Community composition driven by species interaction assumes that species with similar niches do not co-exist because they share the same resources, predators, or pathogens (Webb et al., 2002). Therefore, this process is simulated by removing one of two species from the regional species pool that show the highest similarity in their traits or taxonomy (Fig. 2c).

Following van der Plas et al. (2015), here we utilized approximate Bayesian computation within a sequential Monte Carlo (ABC-SMC) framework for inferring the number of species removals needed per simulation step, which results in a simulated community that approximates the observed community.

Our distance-based community simulation was built on the R package STEPCAM v1.0 (Janzen and van der Plas, 2014). We improved the algorithm for runtime and a commented, fully reproducible analysis including the taxonomic distance and presence–absence matrix of gastropod community composition is available in the Supplement. The STEPCAM target for the assembly simulation for each of our 264 sampled communities was a final acceptance rate of 1 in 10 000 simulated communities.

2.3 Testing for gradual vs. distinct changes among eco-zones

For testing whether the importance of individual structuring processes changes gradually with lake depth or discontinuously with eco-zone shifts (see specific aim 2), reliable information for the spatial distribution of these zones is necessary.

Previous assessments of the vertical distribution of eco-zones in Lake Ohrid were based on analyses of habitat types, limnological features, and/or typical species assemblages (Albrecht and Wilke, 2008; Hauffe et al., 2011). In contrast, only a rough approximation of the horizontal dimension exists so far (Stanković, 1960).

Therefore, we first performed an automatic detection of eco-zones using a spatially constrained clustering of ecological networks (Miele et al., 2014). The analysis, implemented in the R package geoclust v0.2.0 (Miele et al., 2014), is based on the similarity of gastropod community composition and their geographical connectivity in horizontal (x and y) and vertical (z) dimensions. Community composition similarity was calculated using Bray–Curtis distances and the vegan package. Similarities were then arcsin transformed to ensure normality, as required for ecological networks. The three-dimensional spatial network was based on vertical (bathymetric) connectivity and the cost distance between collecting points along isobaths. The latter approach had to be used because intralacustrine dispersal of benthic organisms is not well approximated by straight line distances (Heino et al., 2015a). We explored the robustness of our analysis by varying the number of directly connected neighbors from 15 to 264 with an increment of 10, ensuring a fully linked spatial network. For the vertical scale, a connectivity threshold of 8.2 m was determined by a multivariate community correlogram with 9999 permutations in vegan, showing that similarity between gastropod communities decreases significantly if their bathymetric distance exceeds this threshold.

In a final step, we used a Bayesian generalized linear model (BGLM; R package MCMCglmm v2.21; Hadfield, 2010) to test whether our multivariate response, i.e., the relative importance of the three community assembly processes, was better predicted by either lake depth or delineated eco-zones (see specific aim 2). As decision criterion between the two competing BGLMs, we utilized the deviance information criterion (DIC), a Bayesian measure of model fit (Spiegelhalter et al., 2002). In both BGLMs, we included species richness and its interaction with lake depth or eco-zones as additional predictor because process importance may co-vary with species richness. Prior to model fitting, normality of the $[0, 1]$ scaled multivariate response was improved by an additive planar transformation, implemented in the compositions v1.4-1 package (van den Boogaart et al., 2014) for R.

3 Results

3.1 Relative importance of the three community assembly processes

Using our ABC-SMC-based STEPCAM approach, we obtained a posterior distribution for the relative contribution of the three community assembly processes (dispersal limitation, environmental filtering, and biotic interaction) in shaping the lakes' gastropod communities. Averaging the posterior distributions over all 264 gastropod communities, dispersal limitation achieved the highest relative importance with 80.1 % (95 % bootstrapped confidence interval (CI): 77.0–83.0), followed by environmental filtering (mean 11.5 %; CI: 9.4–13.8), and species interaction (mean 8.4 %; CI: 7.2–9.7).

3.2 Testing for gradual vs. distinct changes among eco-zones

Prior to testing whether the importance of the three individual processes changes gradually with lake depth or discontinuously with eco-zones shifts, we performed an automatic detection of eco-zones using a spatially constrained clustering of ecological networks. Applying a range of 1 to 10 groups and different numbers of directly connected neighbors in the spatial network, our delineation of eco-zones consistently revealed that seven groups of gastropod communities showed the highest likelihood. These communities relate to seven eco-zones (southeastern (SE) upper littoral 1–3, non-SE upper littoral, lower littoral, upper sublittoral, lower sublittoral). The horizontal and vertical distribution of these eco-zones based on 65 neighbors is shown in Fig. 3 (zone terminology roughly follows Stanković, 1960).

Finally, we used two BGLMs to test whether the relative importance of the three community assembly processes was better predicted by either lake depth or delineated eco-zones. In general, assembly processes were explained both by depth and eco-zones. However, according to the rule of thumb of Bayesian model selection (Bolker, 2008), the difference in DIC greater than 10 decisively favored eco-zones over lake depth (DIC values of −861 vs. −753, respectively). For a visual presentation of the eco-zone-dependent relative importance of dispersal limitation, environmental filtering, and biotic interaction, see Fig. 4.

4 Discussion

In the current study, we addressed the question of how extant gastropod communities are assembled by performing a process-based metacommunity analysis. More specifically, we attempted to identify the relative importance of three previously suggested community assembly processes (dispersal limitation, environmental filtering, and species interaction) and tested whether their importance changes gradually with lake depth or discontinuously with eco-zone shifts. Given that geographical isolation has been stressed as an important driver for speciation in Lake Ohrid (reviewed in Albrecht and Wilke, 2008) and adhering to the metacommunity speciation model, our working hypothesis was that dispersal limitation also plays a crucial role in structuring communities. We, indeed, could show that dispersal limitation had the strongest influence on gastropod community composition in Lake Ohrid. However, it was not the exclusive assembly process but acted together with the other two processes – environmental filtering and species interaction (Fig. 4). In fact, the relative importance of the three community assembly processes varied both with lake depth and eco-zones (Fig. 4), though the processes were better predicted by delineated eco-zones.

In the following, these principal findings are interpreted in the context of how ecosystem features influence community assembly. We also discuss the implications of our three community assembly processes for patterns and processes of speciation in endemic gastropods of Lake Ohrid. As structuring processes appear to be strongly related to eco-zones, we first address the result of our automatic eco-zones identification.

4.1 The eco-zones of Lake Ohrid

Previously, six horizontal and four vertical eco-zones have been suggested for Lake Ohrid (Stanković, 1960; Albrecht and Wilke, 2008). However, as they were largely based on an a priori differentiation into horizontal and vertical features, here we automatically delineated eco-zones using a three-dimensional approach (i.e., involving the three spatial axes x, y, and z).

The zonation inferred (Fig. 3) resembles, in large parts, the one previously proposed. However, it appears to be slightly more complex in terms of horizontal and vertical structuring. Moreover, the zones show a considerable degree of overlapping, reflecting the patchy nature of some habitats in the lake (see Albrecht and Wilke, 2008). The strong vertical structure largely corresponds to vertically arranged biotopes in Lake Ohrid, such as the "*Chara* belt" in the lower littoral and the "shell zone" in the upper sublittoral (Albrecht and Wilke, 2008). Note that in this study we could not identify a potential fifth vertical zone, the profundal, as the only species of gastropod previously reported from there has not been found in years (Hauffe et al., 2011).

Interestingly, we do not see any significant horizontal structuring within deeper vertical zones. However, within the upper littoral (i.e., a zone typically extending from a water depth of 0 to 3 m), a strong horizontal structure is evident. One eco-zone ("non-SE upper littoral zone") comprises all shallow water gastropod communities from the northeastern, northern, western, and southwestern parts of the lake. Three additional eco-zones can be found in the southeastern upper littoral ("SE upper littoral zones 1–3"). This area, comprising major spring fields within the lake, has long been known for its high degree of point-endemism (Albrecht et al., 2006; Albrecht and Wilke, 2008; also see Hauffe et al., 2011 Fig. 3a for compositional similarities among these communities).

The emergent pattern of partly overlapping horizontal/vertical eco-zones, indeed, confirms that the gastropods of Lake Ohrid adhere to the metacommunity concept.

4.2 Relative importance of community assembly processes

Our results suggest that gastropod communities of Lake Ohrid are substantially structured by the dispersal limitation process (i.e., restricted specimen exchange; mean importance across all communities ∼ 80 %; see Fig. 4). Environmental filtering and biotic interaction were of lower importance (12 and 8 %, respectively). However, the individual importance of these three processes depends on lake depth and even more on individual eco-zones (Fig. 4). The importance of dispersal limitation is, for example, relatively low in the SE upper littoral 1 (i.e., the shallowest and most species-rich zone in Lake Ohrid) and in the lower sublittoral (i.e., a deep and relatively species-poor zone in Lake Ohrid). The opposite is true for environmental filtering and, to a lesser extent, for species interaction (see Fig. 4). This interesting relationship shows that the relative importance of the three community structuring processes does not depend on species richness or lake depth per se. The latter might also explain why eco-zones (i.e., three spatial dimensions) have a higher explanatory power than lake depth (only one spatial dimensions).

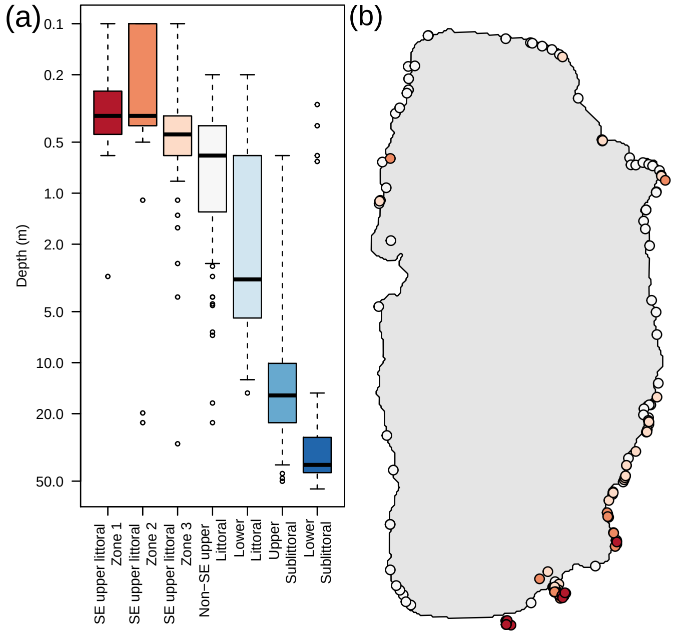

Figure 3. Sampling sites and eco-zonation of 264 gastropod communities of ancient Lake Ohrid and its feeder springs. Eco-zones were detected by network analysis weighting links among sampling sites by their geographically proximity and compositional similarity. **(a)** Vertical (bathymetrical) extension of the eco-zones. Due to the spatial density, plots of horizontal distribution of clusters were divided into **(b)** upper littoral, and **(c)** lower littoral and sublittoral.

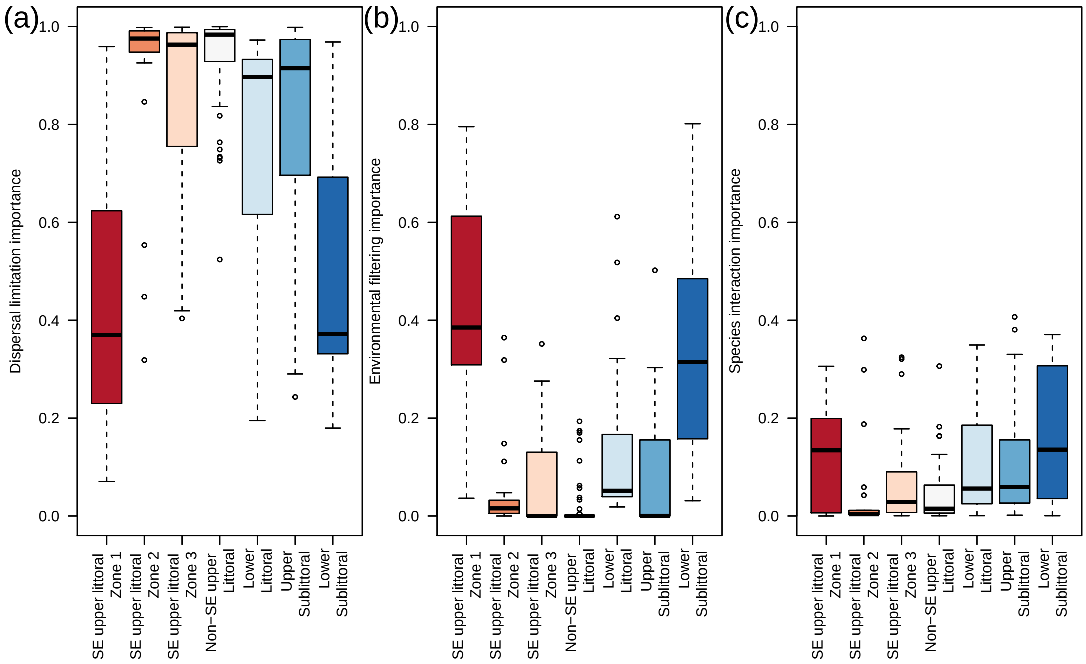

Figure 4. Box plots showing the eco-zone-dependent relative importance of **(a)** dispersal limitation, **(b)** environmental filtering, and **(c)** biotic interaction in structuring gastropod communities. Colors correspond to Fig. 1. These differences in process importance due to eco-zones are statistically supported by a multivariate Bayesian generalized linear model.

Interestingly, the clearly demonstrated high importance of dispersal limitation for maintaining species richness in Lake Ohrid through its impact on community structure stands in contrast to some other studies on Lake Ohrid biota. In fact, previous investigations on gastropod (Schneider et al., 2014), ostracod (Lorenschat et al., 2014), diatom (Reed et al., 2010), and fungal communities (Čomić et al., 2010) suggested a strong environmental control (i.e., environmental filtering) of community composition. However, this is not surprising as other processes were not considered in these studies.

In studies that did consider more than one process, the picture was more complex and also showed, in addition to environmental filtering, the influence of dispersal limitation and/or species interaction. This concerns not only gastropod communities in Lake Ohrid (Hauffe et al., 2011) and in other ancient lakes such as Lake Tanganyika (Meyer et al., 2011), but also communities in freshwater systems in general (Heino et al., 2015b).

In a comparable study, Janzen et al. (2016) investigated cichlid communities in Lake Tanganyika. Interestingly, the individual importance of the assembly processes inferred is very similar to ours, with dispersal limitation being the dominant process. In contrast to our study, they did not find a correlation of process importance with environmental characteristics. The reasons for this difference remain unclear, though differences in the life history of the study taxa and in the spatial extant of the study area (Janzen et al., 2016 performed a local study) may play a role.

As the importance of assembly processes often depends on spatial scale (Meyer et al., 2011) and resolution (Münkemüller et al., 2014), the sensitivity of the STEPCAM approach to spatial data aggregation should generally be considered. However, as the spatial resolution in our study is relatively high and as dispersal and movement rates of freshwater gastropods are generally low (Kappes and Haase, 2012; Michel et al., 2007), here we refrained from data aggregation. Note that the occurrence of invasive species, which often have high dispersal rates, could have affected the STEPCAM analysis. However, only two recent gastropod invasions of limited spatial extensions are known from Lake Ohrid (Albrecht et al., 2014) and should therefore not have biased our analysis.

4.3 Metacommunity implications for gastropod speciation

If we accept that species divergence and community assembly can be unified in an eco-evolutionary framework because both are based on similar processes (Hubert et al., 2015), then the inferred importance of processes here allows for a fresh look into the diversification mode of Lake Ohrid's endemic gastropods. Research on species divergence, in general, and in ancient lakes, in particular, has traditionally focused on geographic speciation (Cristescu et al., 2010; Schluter, 2001; also see Albrecht and Wilke, 2008 for a review on geographic speciation modes in Lake Ohrid). Adaptive diversification along environmental gradients, however, highlights the additional role of ecology in driving species divergence in ancient lakes (Schön and Martens, 2004).

Our results support this perspective of non-exclusive geographic and ecological speciation in Lake Ohrid. Though the inferred high importance of dispersal limitations in structuring gastropod communities implies a dominant role of geographic speciation in the lake (also see Albrecht et al., 2006; Kilikowska et al., 2013; Trajanovski et al., 2010; Wysocka et al., 2014), the demonstrated importance of environmental filtering and species interaction suggests a small but significant influence of ecological speciation.

The fact that ecological speciation appears to be more important both in the SE upper littoral 1 (i.e., a shallow and species-rich zone) and in the lower sublittoral (i.e., a deep and species-poor zone) potentially implies a bimodal contribution of ecological speciation in generating biodiversity over time.

4.4 Methodological implications and outlook

For many questions concerning processes in eco-evolutionary biology, such as dispersal- or selection-dependent species diversification (Pigot et al., 2010) and community assembly (Rosindell et al., 2015), no analytical solutions are (yet) available. However, the outcome of processes can be simulated by utilizing a wide range of parameter values representing them. The set of parameters resulting in a simulated pattern that best resembles the observed one is then assumed to approximate the processes in question. Our newly developed distance-based STEPCAM approach is a relatively simple model that could be easily extended by making, for instance, dispersal limitation spatially or temporally explicit, but at the risk of overparameterization.

Moreover, we suggest further exploration of the STEPCAM approach using different taxa in Lake Ohrid, for instance in another species-rich group, the diatoms. A combined study of extant and paleo-communities derived from multiple sediment cores would allow for inferring the relative importance of dispersal limitations, environmental filtering, and species interaction over space and time.

5 Conclusions

Our study builds on a prediction of the metacommunity speciation model: if geographic speciation is the prevailing mode of species divergence, as suggested by many evolutionary studies, then dispersal limitation should also structure communities. The results of our analysis corroborate this hypothesis for gastropods in Lake Ohrid. The inferred importance of environmental filtering and biotic interaction in community assembly also suggests a small but significant influence of ecological speciation on diversification dynamics; an aspect that previously could not be quantified. Moreover, based on a novel automated detection of eco-zones in Lake Ohrid, we show that these eco-zones do influence the relative importance of community assembly processes, whereas former studies could only show that these limnological features give rise to distinct community composition. These findings contribute to the main goal of the SCOPSCO initiative – inferring the drivers of biotic evolution – and provide an integrative perspective on biological and geological dynamics in ancient Lake Ohrid.

Moreover, our novel distance-based STEPCAM complements the growing eco-evolutionary toolbox that aims to disentangle patterns and processes.

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6 Sediment core fossils in ancient Lake Ohrid: testing for faunal change since the Last Interglacial

Christian Albrecht, Hendrik Vogel, **Torsten Hauffe** & Thomas Wilke

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Sediment core fossils in ancient Lake Ohrid: testing for faunal change since the Last Interglacial

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Abstract. Ancient Lake Ohrid is probably of early Pleistocene or Pliocene origin and amongst the few lakes in the world harbouring an outstanding degree of endemic biodiversity. Although there is a long history of evolutionary research in Lake Ohrid, particularly on molluscs, a mollusc fossil record has been missing up to date.

For the first time, gastropod and bivalve fossils are reported from the basal, calcareous part of a 2.6 m long sediment succession (core Co1200) from the north-eastern part of Lake Ohrid. Electron spin resonance (ESR) dating of mollusc shells from the same stratigraphic level yielded an age of 130 ± 28 ka. Lithofacies III sediments, i.e. a stratigraphic subdivision comprising the basal succession of core Co1200 between 181.5–263 cm, appeared solid, greyish-white, and consisted almost entirely of silt-sized endogenic calcite ($CaCO_3 > 70\%$) and intact and broken mollusc shells. Here we compare the faunal composition of the thanatocoenosis with recent mollusc associations in Lake Ohrid. A total of 13 mollusc species (9 gastropod and 4 bivalve species) could be identified within Lithofacies III sediments. The value of sediment core fossils for reconstructing palaeoenvironmental settings was evaluated and the agreement between sediment and palaeontological proxies was tested.

The study also aims at investigating major faunal changes since the Last Interglacial and searching for signs of extinction events.

The combined findings of the ecological study and the sediment characteristics suggest deposition in a shallow water environment during the Last Interglacial. The fossil fauna exclusively included species also found in the present fauna, i.e. no extinction events are evident for this site since the Last Interglacial. The thanatocoenosis showed the highest similarity with recent Intermediate Layer (5–25 m water depth) mollusc assemblages. The demonstrated existence of a mollusc fossil record in Lake Ohrid sediment cores also has great significance for future deep drilling projects. It can be hoped that a more far reaching mollusc fossil record will then be obtained, enabling insight into the early evolutionary history of Lake Ohrid.

1 Introduction

Ancient lakes, that is, extant lakes that have continuously existed for a hundred thousand or even for millions of years, are well recognized as hotspots of freshwater biodiversity (e.g. Martens, 1997). The few worldwide ancient lakes also provide invaluable archives of tectonic (Müller et al., 2001), climate (Scholz et al., 2007), environmental (Wagner et al., 2009), and evolutionary histories (Schwarzer et al., 2009), spanning time frames from the Miocene to the Holocene. Though most current research focuses on extant taxa, palaeontological research in ancient lakes has a long and rich history, and important evolutionary concepts such as the theory of the punctuated equilibrium (e.g. Williamson, 1981; but see van Boxclaer et al., 2008) are derived from ancient lake research. The question of long-term stability vs. rapid changes as ultimate cause for diversification events is among the most intensively discussed issues in ancient lake research (e.g. van Boxclaer et al., 2008). Among fossilized taxa, the molluscs often figure prominently in many lake sediments, thus reflecting a long and diverse evolutionary history because of their excellent preservation potential (e.g. Taylor, 1988; Tracey et al., 1993; Wesselingh, 2007). Fossilized molluscs have a number of advantages over other taxa: (i) determination is typically possible down to the genus or even species level, (ii) they frequently belong to the most conspicuous fossils, (iii) though lacustrine fossil assemblages may vary in quality mainly depending on abiotic factors such as water chemistry (van Damme and

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Pickford, 2003), they may occur as preserved shells, molds, or casts. Therefore, they are often used as valuable indicators of palaeoecological conditions such as water temperature (Goodwin et al., 2003), lake-level fluctuations (Filipov and Riedel, 2009), depth information and oxygen conditions (Magyar et al., 2006), salinity (e.g. Mischke et al., 2010), and primary productivity (Langlet et al., 2007). They also serve as important proxis for environmental changes (e.g. Harzhauser and Mandic, 2008).

For example, a number of Neogene palaeolakes exist on the Balkans that are characterized by a high degree of mollusc diversity and endemism (Harzhauser et al., 2008). These lakes are excellent archives of past climate changes with records mirroring even decadal to centennial shifts of the respective palaeolimnological system (Harzhauser et al., 2008; Lirer et al., 2009). The excellent fossil records of those lakes provided insights into, for example, the geological evolution of the eastern Mediterranean (e.g. Rust, 1997), climate and environmental change (Owen et al., 2010), patterns and processes of radiations, and biogeographical patterns (Harzhauser and Mandic, 2010). There is a well-known modern counterpart to those long-lived Neogene lakes, the ancient Lake Ohrid (Macedonia, Albania; Fig. 1). This lake has been in the focus of evolutionary biologists for more than 100 years because of its outstanding biodiversity and degree of endemism (reviewed in Albrecht and Wilke, 2008). Over the past years, our knowledge of evolutionary histories of important recent taxa has increased rapidly (Albrecht et al., 2006, 2008; Hauffe et al., 2010; Hauswald et al., 2008; Schultheiß et al., 2008; Wysocka et al., 2008; Trajanovski et al., 2010; Wilke et al., 2010). Although probably of early Pleistocene or Pliocene origin, the exact age and origin of Lake Ohrid remain unknown, though a number of hypotheses as to its geological and limnological history have been proposed (reviewed in Albrecht and Wilke, 2008). Major progress has recently been made with respect to modern limnological and hydrological studies (Matzinger et al., 2006a, 2007; Vogel et al., 2010a), as well as palaeolimnological studies in the lake (Wagner et al., 2009). New insights are now available into environmental dynamics and their impact on sedimentation in Lake Ohrid over the last glacial-interglacial cycle (Vogel et al., 2010b) and on changes of the hydrological budget of the lake (Leng et al., 2010; Lindhorst et al., 2010). Stratigraphic and chronological precision has been enhanced by radiocarbon, luminescence, and electron spin resonance dating, and in particular tephrostratigraphy (Vogel et al., 2010c; Lindhorst et al., 2010; Sulpizio et al., 2010).

Although fossil diatom and ostracod records have been studied using sediment successions from the pelagic parts of Lake Ohrid (Wagner et al., 2009; Belmecheri et al., 2009) a similar study for the littoral parts is missing to date. Littoral parts of the lake are particularly interesting for studies of their mollusc faunal assemblages, as they not only harbour a high degree of endemic representatives (e.g. Hauffe et al., 2010), but also because these shallow parts are particularly vulnerable to environmental changes (Vogel et al., 2010a; Kostoski et al., 2010). The lack of mollusc fossils, however, has been a very unfortunate situation given the rich fossil mollusc assemblages of Neogene (Harzhauser et al., 2008) and Pleistocene lakes (Frogley et al., 2007) on the Balkans and thus the potential for supraregional comparisons.

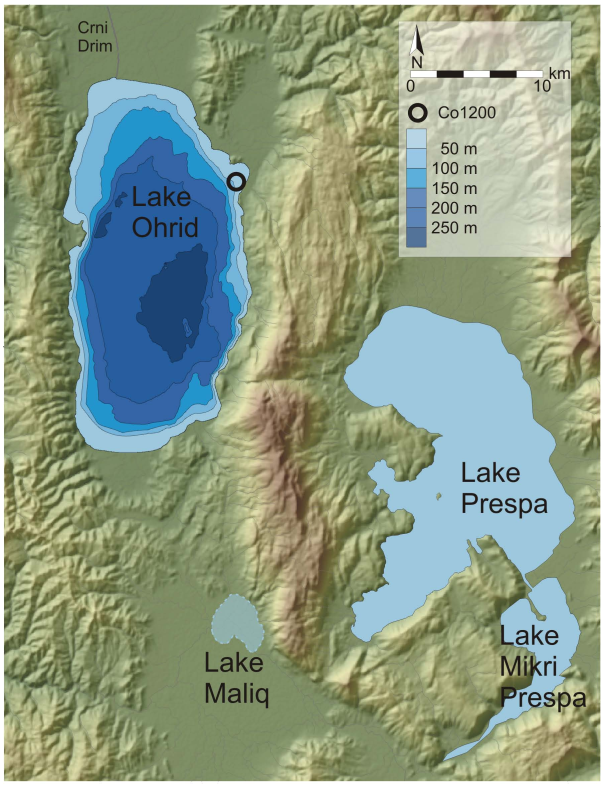

Fig. 1. Location of lakes Ohrid and Prespa and former Lake Maliq in the Macedonian-Albanian-Greek border zone. Shape and topographic setting of Lake Ohrid, bathymetry of Lake Ohrid in 50 m contour intervals with the location of coring site Co1200. Note that only the approximate location of former Lake Maliq is shown.

Numerous long sediment records of up to 15 m length have been recovered from hemi-pelagic sites in Lake Ohrid (Roelofs and Kilham, 1983; Wagner et al., 2008a, b; 2009; Belmecheri et al., 2009; Vogel et al., 2010b, c). However, sediment records from shallower littoral parts have only recently become available (Lindhorst et al., 2010). Combined hydroacoustic and lithological data from these shallow water sites revealed significant lake level fluctuations, which led to the formation of widespread terrace levels below the present water level during marine isotope stages (MIS) 6 and 5 (Lindhorst et al., 2010).

Here we report on the first record of gastropod and bivalve fossils discovered in the basal, calcareous part of a 2.6 m long sediment succession (core Co1200) from the north-eastern part of Lake Ohrid (Fig. 1). Electron spin resonance (ESR) dating of mollusc shells from the same stratigraphic level was used for chronological constraints. We here utilize statistical faunal comparisons of the thanatocoenosis with modern mollusc associations in Lake Ohrid in order to:

i. evaluate the value of sediment core fossils for reconstructing palaeoenvironmental settings,

ii. test for agreement between sediment and palaeontological proxies,

iii. test for major faunal changes since the Last Interglacial,

iv. search for signs of extinction events.

2 Materials and methods

2.1 Site description

Lake Ohrid (Ohridsko Ezero (Macedonian), Liqeni i Ohrit (Albanian)) is part of the European lake group called Dessaretes, located on the Balkan Peninsula (Fig. 1). The most outstanding of them is the oligotrophic and karstic Lake Ohrid, a steep-sided tectonically active graben situated in the South Adriatic-Ionian biogeographic region (Banarescu, 1991). The lake has a surface area of 358 km^2 and a maximum depth of 289 m (Matzinger et al., 2007).

Lake Ohrid is an oligomictic lake with complete mixing occurring roughly every seven to ten years (Hadzisce, 1966 in Matzinger et al., 2006a, b). Secchi disk transparency varied between 9 and 17 m in the year 2005 (Naumoski et al., 2007). The water balance of the lake shows average in- and output rates of approximately 37.9 $\mathrm{m}^3\,\mathrm{s}^{-1}$ (Matzinger et al., 2006b). Today's water input of Lake Ohrid is characterized by inflows from karstic aquifers (∼53%), direct precipitation on the lake surface (∼23%), and river inflow (∼23%). The river discharge was even lower before the River Sateska was artificially diverted into the lake in 1962 (Matzinger et al., 2006a). A specific characteristic of Lake Ohrid's water balance is the high inflow from karstic springs with sublacustrine (49%) and surface springs (51%) contributing almost equally to the inflow balance (Matzinger et al., 2006a). The output occurs via the River Crni Drim (2/3) and through evaporation (1/3). The catchment area of Lake Ohrid is relatively small with 2600 km^2 (including Lake Prespa; Matzinger et al., 2007). Though, Lake Ohrid can still be classified as oligotrophic, progressing eutrophication has recently been noted (Matzinger et al., 2007).

2.2 Core recovery

Sediment core Co1200 was recovered in autumn 2007 from the north-eastern part of Lake Ohrid (Fig. 1) where hydro-acoustic surveys indicated a sub-aquatic terrace at a water depth of 32 m (Lindhorst et al., 2010). The surface sediments and deeper sediments were collected using a 0.6 m long gravity corer and a 3 m long percussion piston corer, respectively (both UWITEC Co.). The 3 m long core segments were subdivided into 1 m long segments in the field.

2.3 Sediment analysis

After longitudinal splitting of the cores, one core half was used for high-resolution X-ray fluorescence (XRF) scanning using an ITRAX core scanner (COX Ltd.), equipped with a Mo-tube set to 30 kV and 30 mA and a Si-drift chamber detector. Scanning was performed at 2.5 mm resolution and an analysis time of 20 s per measurement. The obtained count rates for Ti, K, and Ca can be used as estimates of the relative concentrations for these elements (Croudace et al., 2006).

Sub-sampling was performed on the same core half used for XRF-scanning at 2 cm intervals. The water content (WC) for each sample was determined from the weight difference between wet and freeze-dried samples. Aliquots of the freeze-dried subsamples were ground to <63µm using a planetary mill for subsequent biogeochemical analyses, which was done at 6 cm resolution. Total carbon (TC) concentrations, were measured with a Vario Micro Cube combustion CNS elemental analyzer (VARIO Co). Samples for total organic carbon (TOC) analysis were pre-treated with HCl (10%) at a temperature of 80 °C to remove carbonates and then analyzed using a Leco CS-225 carbon-sulfur detector (LECO Co). The amount of total inorganic carbon (TIC) was determined from the difference between TC and TOC. The carbonate ($CaCO_3$) content was calculated from TIC under the assumption that TIC solely originates from $CaCO_3$.

2.4 Dating

In order to develop a chronological framework for core Co1200, radiocarbon, electron spin resonance (ESR) dating, and tephrostratigraphy were applied. Radiocarbon dating was performed on plant macrofossils from 6 and 13 cm depth in core Co1200 by accelerator mass spectrometry (AMS) at the Leibniz Laboratory for Radiometric Dating and Isotope Research in Kiel, Germany. The obtained ages were calibrated into calendar years before the present (cal yrs BP) using the CalPal-2007 online and the CalPal2007_HULU calibration curve (Danzeglocke et al., 2008).

ESR dating was performed on mollusc shells extracted from a horizon containing large quantities of well-preserved fossil mollusc specimens at a depth of 230–262 cm. ICP-mass spectrometry was applied in order to determine the radionuclide contents of the surrounding sediment and of the molluscs themselves. The ESR measurements were carried out using an additive dose protocol for multiple aliquots (Schellmann et al., 2008).

Core Co1200 contained two peculiar horizons at 40–38 cm and 85.5–120.5 cm, which consisted almost entirely of volcanic glass shards (tephra). From these horizons about

1 cm^3 was washed and sieved. The >40µm fraction was embedded in epoxy resin and screened for glass shards and micro-pumice fragments using scanning electron microscopy (SEM). Energy-dispersive-spectrometry (EDS) analyses of glass shards and micro-pumice fragments was performed using an EDAX-DX micro-analyser mounted on a Philips SEM 515 (operating conditions: 20 kV acceleration voltage, 100 s live time counting, 200–500 nm beam diameter, 2, 100–2, 400 shots per second, ZAF correction).

2.5 Sampling mollusc fauna

Materials on recent molluscs from the Ohrid Basin, including ponds, springs, artificial lakes, streams and rivers were collected during several field trips carried out between May 2003 and September 2009 (see Hauffe et al., 2010). Individuals were collected by hand from hard substrata in shallow waters or from stones and rocks lifted from depths down to 5 m by snorkeling. Deeper parts down to 60 m were sampled using a dredge from small boats or from the research vessel of the Hydrobiological Institute in Ohrid (HBI). Locality information (georeferenced with Garmin handheld GPS devices) and collection details were recorded for a total of 156 sampling points. All materials are deposited at the permanent DNA, shell and tissue collection of the University of Giessen, Systematics and Biodiversity Group (UGSB).

The abundance of the fossil species was estimated by counting recognizable complete or near complete shells. Species that were represented by less than 5 specimens were regarded as rare, 5 to 50 specimens as common, and more than 50 specimens as frequent. Bivalve valves were counted as halves. The classification scheme of the species is described in Hauffe et al. (2010).

2.6 Analyses of faunal similarity and ecology statistics

A comparative analysis of the Co1200 thanatocoenosis and recent gastropod assemblages was carried out to identify the recent depth range of the assemblage with the highest similarity to the thanatocoenosis. Bray-Curtis dissimilarities were calculated for pairwise comparisons of the fossil assemblages with the 156 recent gastropod assemblages (Hauffe et al., 2010), utilizing the *R* package vegan 1.18 (Oksanen et al., 2010). These distances were calculated for three different habitats (depth zones) according to Albrecht and Wilke (2008) and Hauffe et al. (2010). 81 points were included for the shallow sandy or rocky Surface Layer (0–5 m), 41 for the Intermediate Layer (5–25 m), characterized by the *Chara* belt and *Dreissena* beds, and 34 for the sandy and silty Deep Layer (25–50 m). Due to the unequal group size and non-normal distribution of the dissimilarities, a non-parametric analysis of variance (PERMANOVA, Anderson, 2001) was carried out with the function adonis of the vegan package. Given that the thanatocoenosis was used for multiple comparisons, which leads to an increase of the degrees of freedom, a Bonferroni adjustment for the Type I error inflation was carried out.

A non-metric multidimensional scaling (NMDS) was performed because of its robustness as compared to other ordination methods. In order to estimate a possibly depth range, we apply a posteriori vector fitting to the ordination result. The vector fitting algorithm of the *R* package vegan is able to handle the unknown depth information, thus, can provide an estimation of the depth range and depth layer of the fossil gastropod assemblage.

Quartile box plots of the recent depth range of the nine gastropod and three bivalve species present in Co1200 were calculated to compare the inferred habitat with recent bathymetric preferences of the individual species. This allows cross-checking for consistency of the results of the potential habitat analyses. *Pisidium subtruncatum* was excluded from this comparison due to its rare recent records. Unequal numbers of recent collecting points in the different depth layers were balanced by sampling without replacement. The lowest number of collecting points (Deep Layer, N = 34) was utilized to sample 1000 times a set of 34 random collecting points of each of the different depth layers. Our procedure tested whether the particular species was found at those random points. If one species was not found at one of those points, this record was excluded from the subsequent analysis. Otherwise, the depth information of the collecting point was used to calculate the box plots of the depth distributions of the species.

For horizontal and vertical comparison of the thanatocoenosis of core Co1200 and recent gastropod communities in ancient Lake Ohrid, a dissimilarity map was obtained by the *R* package spatstat 1.17–5 (Baddeley and Turner, 2005). Due to the clear identification of at least three bathymetrically differentiated habitats, three ESRI shapefiles (ESRI 2008) were imported for the different zones by using the *R* package maptools 0.7–29 (Lewin-Koh and Bivand, 2009) and the collecting points were allocated a priori to the appropriate bathymetric zone. To mitigate the effects of unequal point distribution, the function smooth.ppp was applied with a Gaussian kernel weighting of a radius of 2 km. Each of the 156 collecting points (see above) was attributed with the respective pairwise Bray-Curtis distances. Then, the dissimilarity at the spatial interval between two collecting points was estimated by a Gaussian smoothing factor.

3 Results

3.1 Lithology and chronology of core Co1200

Individual core segments of core Co1200 were correlated using XRF data, and lithological core descriptions, leading to a composite core of 2.63 m length. Based on sedimentological and geochemical data and lithological peculiarities three different lithofacies can be distinguished in the Co1200 sediment succession (Fig. 2).

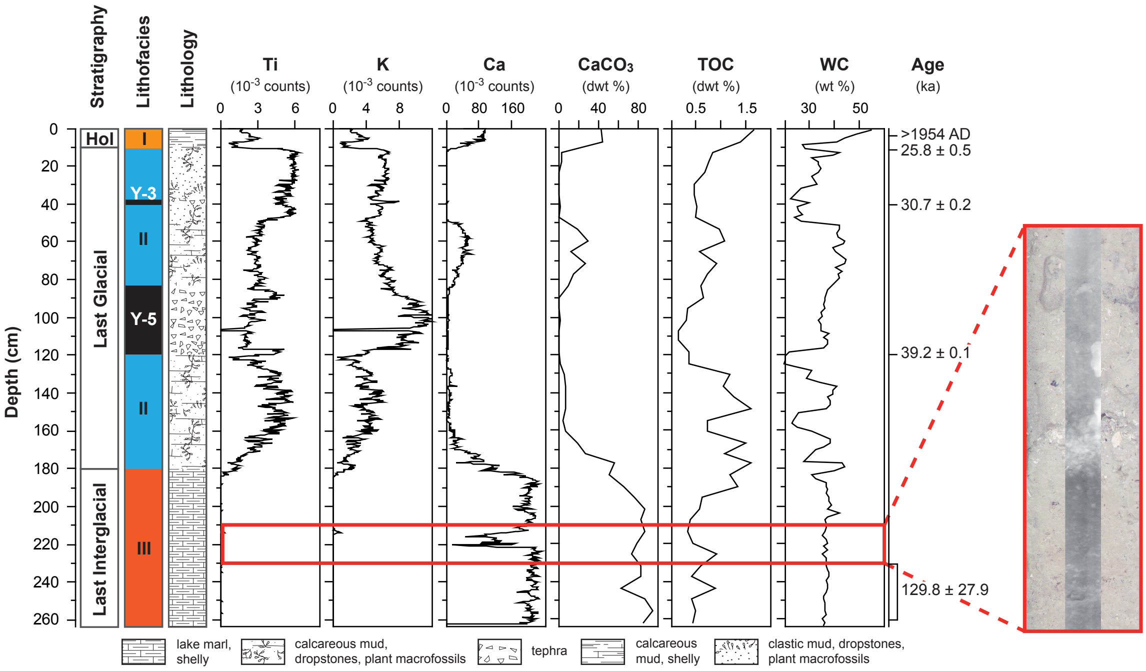

Fig. 2. Rough stratigraphic framework (Hol = Holocene), lithofacies, lithology, Ti-, K-, Ca-intensities, $CaCO_3$, total organic carbon (TOC) dry weight percentages, water content (WC), and age control points of core Co1200. The red framed optical and radiographic images to the right show a horizon at 220 cm which consisted almost entirely of intact and broken mollusc fossils. Fossil mollusc specimens were solely extracted from the Lithofacies III sediment succession.

Lithofacies I between 0–13 cm is light-brown in colour and composed of massy calcareous ($CaCO_3$ >40%) clayey silt and contains complete bivalve shells. TOC concentrations of up to 1.7% can be explained by finely dispersed organic matter (OM) as well as small leaf and shaft fragments from *Chara* algae. Radiocarbon dating of plant macrofossils from Lithofacies I at 6 cm depth yielded a modern age (>1954 AD) probably as a result of contamination with recent organic and plant material from bioturbation or from disturbance during coring of these soft and water saturated uppermost sediment section.

The transition from Lithofacies I to II is characterised by a 2 cm thick sand layer between 13 and 16 cm with an apparent erosive base. These findings in combination with hydroacoustic data from this site indicate a primarily erosive mass movement process (Lindhorst et al., 2010). Support for an erosional process and thus a hiatus in core Co1200 comes from radiocarbon dating just below the sand layer at 13 cm, which yielded an age of 25.82 ± 0.5 cal kyrs BP.

Lithofacies II between 16 and 181.5 cm appears dark-grey and consists of massy clastic clayey-sandy silts with frequent occurrences of larger granules. Small shell fragments and larger intact *Chara* fragments are abundant. Except for the succession between c. 50–90 cm $CaCO_3$ and TOC concentrations are lower, whilst Ti and K intensities are significantly higher compared to Lithofacies I sediments. This is well correlated to higher amounts of clastic detritus. Two tephra horizons occur in Lithofacies II sediments at 38–40 (OT0700-1) and 85.5–120.5 (OT0700-2) cm. Geochemical and morphological investigation of glass shards from both tephra deposits allowed a correlation of the upper OT0700-1 tephra to the Y-3 tephra layer (Sulpizio et al., 2010), dated at 30.7 ± 0.2 ka (Sulpizio et al., 2003), and of the lower OT0700-2 to the Campanian Ignimbrite (CI)/Y-5 eruption of the Campi Flegrei Caldera (Sulpizio et al., 2010), dated to 39.2 ± 0.1 ka (De Vivo et al., 2001).

The transition from Lithofacies II to Lithofacies III sediments is abrupt and occurs within a few centimetres. These transitional centimetres contain gravel and sand with no observable grading, and reworked mollusc shells. Therefore it seems likely that the deposition of this transitional part occurred in a high energy erosive environment, which probably caused a hiatus in core Co1200 at the transition of Lithofacies II and III.

Lithofacies III sediments comprising the basal succession of core Co1200 between 181.5–263 cm appear massy, greyish-white, and consist almost entirely of silt-sized endogenic calcite ($CaCO_3$ >70%) and intact and broken mollusc shells. Single horizons consisting almost entirely of mollusc shells and shell fragments are frequently interspersed in the fine grained calcite matrix. As indicated by extremely low Ti and K intensities, clastic matter is almost absent. Low TOC

Table 1. List of mollusc taxa found in core Co1200. Semi-quantitative abundances of identifiable shell remains in the core, recent distribution, endemicity and abundance in Lake Ohrid are given. Fossil abundances: Less than 5 – rare, 5 to 50 – common, above frequent. For more details on recent occurrences of Gastropoda see Hauffe et al. (2010). Frequency of recent Bivalvia is according to Albrecht et al. (unpublished data).

Taxon	Abundance in core	Recent distribution	Recent occurence in Lake Ohrid
Gastropoda			
Chilopyrgula sturanyi (Brusina, 1896)	frequent	Endemic to Ohrid Basin	frequent
Ginaia munda (Sturany, 1894)	rare	Endemic to Lake Ohrid	frequent
Ochridopyrgula macedonica (Brusina, 1896)	common	Endemic to Ohrid Basin	frequent
Xestopyrgula dybowskii (Polinski, 1929)	rare	Endemic to Lake Ohrid	frequent
Polinskiola sturanyi (Westerlund, 1902)	common	Endemic to Lake Ohrid	frequent
Gyraulus lychnidicus Hesse, 1928	common	Endemic to Lake Ohrid	frequent
Planorbarius corneus (Linnaeus, 1758)	rare	Widespread	common
Radix relicta Polinski, 1929	common	Endemic to Ohrid Basin	frequent
Valvata stenotrema Polinski, 1929	frequent	Endemic to Ohrid Basin	frequent
Bivalvia			
Dreissena presbensis Kobelt, 1915	frequent	North/Central Balkans	frequent
Pisidium edlaueri Kuiper, 1960	common	Endemic to Lake Ohrid	common
Pisidium subtruncatum Malm, 1855	rare	Widespread	rare
Pisidium s. recalvum Kuiper, 1960	rare	Endemic to Lake Ohrid	common

(<1.2%) originates from finely dispersed OM and few leaf and shaft fragments of *Chara* algae. ESR dating of mollusc shells collected between 230–262 cm yielded a modeled age of 130 ± 28 ka (Lindhorst et al., 2010).

3.2 Fossil mollusc composition

A total of 13 mollusc species (9 gastropod species and 4 bivalve species; Fig. 3, Table 1) could be identified within Lithofacies III sediments. Generally, the calcified parts of the shells were well preserved, allowing in most cases for identification (Fig. 3). However, no remnants of organic shell components were found. The fossil fauna exclusively included species also found in the present fauna, i.e. no extinction events are obvious at the core site since the Last Interglacial. No morphological disparities between the shells of the fossil and recent assemblages could be observed either. No ambiguities of the fossil species compositions itself were shown, i.e. very similar recent assemblages exist.

The most abundant species in the core assemblage were *Valvata stenotrema*, *Chilopyrgula sturanyi* and *Dreissena presbensis*. The endemic pea-clam *Pisidium edlaueri* was common whereas two pea-clams, *Pisidium subtruncatum* and *Pisidium subtruncatum recalvum*, were rare. Among the rare gastropods were *Planorbarius corneus*, *Xestopyrgula dybowskii*, and *Ginaia munda*. *Gyraulus lychnidicus*, *Radix relicta, Polinskiola sturanyi* and *Ochridopyrgula macedonica* completed the assemblage as common members (Table 1).

Fig. 3. Pairwise comparison of representative fossil mollusc specimens of core Co1200 (left specimens) and recent analoga from Lake Ohrid (right specimens). Scale bar is 2 mm.

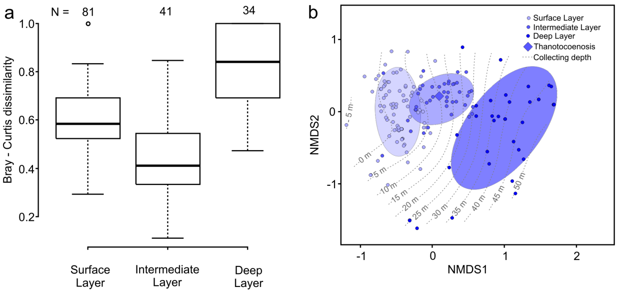

Fig. 4. **(a)** Quartile box plots of Bray–Curtis dissimilarities of the fossil and 156 recent gastropod assemblages. Assemblages from the Intermediate Layer habitats showed the significantly ($F_{2,153}$ = 52.44, $P < 0.05$) lowest pairwise Bray–Curtis dissimilarity to the fossils of core Co1200. **(b)** Three-dimensional NMDS plot of gastropod communities (Stress value 15.4). According to vector fitting, the thanatoecoenosis is well nested within the ellipse with one standard deviation of the Intermediate Layer and the depth estimation equals 10 m.

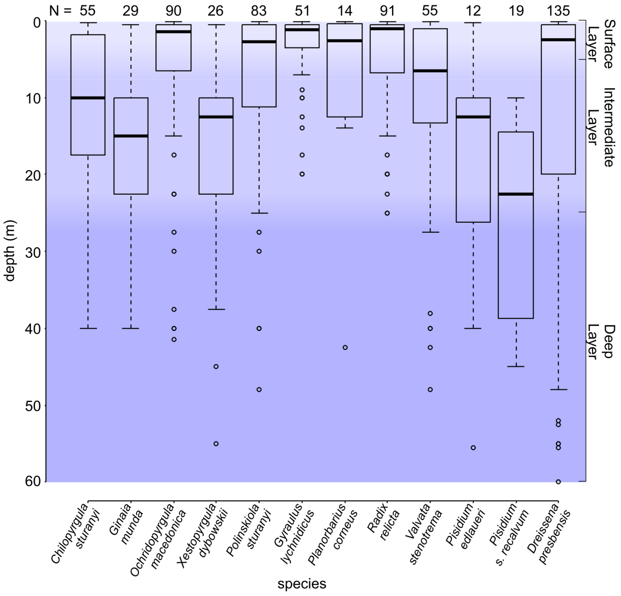

Fig. 5. Quartile box plots of observed depth ranges of recent members of the thanatocoenosis after balancing for unequal numbers of collection points. The 50% range of 11 of 12 species matches the depth range of the Intermediate Layer, where recent collecting points showed the highest similarity to the thanatocoenosis. The depth range of the Deep Layer was not covered by any of the species and the depth range of the Surface Layer by 6 of the 12 mollusc species.

3.3 Faunal similarity and depth range analysis

To distinguish between the three potential habitats, pairwise Bray-Curtis dissimilarities were calculated between the fossil and 156 recent gastropod assemblages and classified into three potential habitat zones (Fig. 4a). The non-parametric anova (PERMANOVA) with Bonferroni adjustment ($F_{2,153}$ = 52.44, $P < 0.05$) supported the significant differences between the three assignments. The Intermediate Layer showed the lowest Bray-Curtis distances to the fossil composition of core Co1200. The Surface Layer revealed a lower similarity and 50% of the Deep Layer dissimilarities did not overlap with 50% of the Intermediate Layer distances. The three-dimensional NMDS had a stress value of 15.4. According to vector fitting, the thanatoecoenosis is well nested within the ellipse with one standard deviation of the Intermediate Layer, and the depth estimation equals 10 m (Fig. 4b).

The 50% range of 11 of 12 species matches the depth range of the Intermediate Layer (Fig. 5), where recent collection points showed the highest similarity to the thanatocoenosis. The depth range of the Deep Layer was not covered by any of the occurring species, and the depth range of the Surface Layer by 6 of the 13 mollusc species.

The dissimilarity map (Fig. 6), which is based on pairwise Bray-Curtis distances between the fossil and the recent gastropod assemblages, had the lowest overall values for the Intermediate Layer (represented mainly by blue to green colours). The highest dissimilarity for this habitat zone was calculated for the sandy southern and north-eastern section. The Surface Layer is represented by green to yellow colours, with areas of higher dissimilarity at the south eastern shore. These dissimilarities are exceeded by the Deep Layer, predominantly appearing in orange to red colours. Drawing arbitrary transects perpendicular from the shore to the Deep Layer, the Intermediate Layer always reveals the lowest dissimilarity to the thanatocoenosis.

4 Discussion

4.1 Record characteristics and palaeoenvironment

Despite the large dating error of the ESR age of 130 ± 28 ka measured on bivalve and gastropod shells from Lithofacies III, we assume that deposition of calcareous sediments and mollusc fossils found therein took place under warm climate conditions during the Last Interglacial (probably MIS 5e). This assumption is supported by stratigraphic and paleoenvironmental constraints suggested by Lindhorst et al. (2010), and furthermore by investigations of sediment successions from pelagic sites at Lake Ohrid where carbonate sedimentation/preservation is restricted to interglacial periods (Vogel et al., 2010b). The peculiar sediment and geochemical characteristics in combination with the finding of intact mollusc shells in Lithofacies III and the fact that the sediments were recovered from a submerged terrace level at a water depth of 32 m point to deposition in a relatively low energy shallow

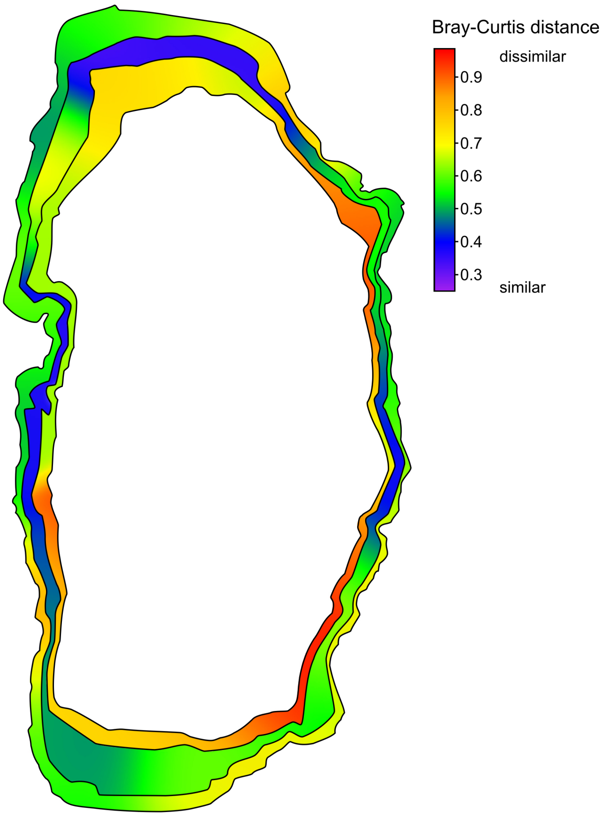

Fig. 6. Similarity map of the thanatocoenosis of core Co1200 and recent gastropod communities in ancient Lake Ohrid. Three habitat zones are shown: Surface Layer (0–5 m), Intermediate Layer (5–25 m), and Deep Layer (25–50 m). Note that the extents of the zones are not to scale with Albrecht and Wilke (2008).

water environment of the Intermediate Layer (Lindhorst et al., 2010).

All species identified are assumed to still occur in Lake Ohrid. Therefore, a direct comparison of recent and fossil faunas is also possible within the context of bio-indication. The basic assumption of bio-indication is that niches and ecological requirements remain unchanged over the time frame of interest. As no changes in shell morphology could be observed, it can be assumed that no major shifts associated with adaptation to, for example, a specific niche have occurred. Such shifts, however, can occur rather rapidly in ancient lakes (Wilke et al., 2007).

The core Co1200 fauna is well nested in many of the 156 recent assemblages studied. Redeposition of mollusc shells by wave action or wind-induced currents from shallower sites or different parts of the lake cannot be completely ruled out. However, the excellent preservation of all shells recovered and the characteristics of the surrounding sediment facies imply that redeposition did not occur or that relatively short transport and rapid deposition in a calm environment took place. Thus, the overall depositional environment and sedimentary facies imply that taphonomic processes seem to be negligible.

4.2 Comparison of recent and Last Interglacial fauna

As noted in the introduction, the mollusc fauna of Lake Ohrid is extraordinarily endemic. The Co1200 fauna is likewise characterized by the dominance of endemic species with the exception of *Planorbarius corneus* and the bivalve species *Dreissena presbensis* and *Pisidium subtruncatum* (Albrecht et al., 2007, but see Wilke et al., 2010 for a discussion on the genetic peculiarities of Lake Ohrid *D. presbensis*). In this respect, i.e. dominance of endemics, the Last Interglacial fauna of Lake Ohrid does not generally differ from Neogene faunas of the Balkans (Harzhauser and Mandic, 2008).

The highest similarity in thanatocoenosis was found with recent species compositions occurring in the Intermediate Layer in depths between 5–25 m. The Intermediate Layer is the habitat zone with the highest overall gastropod diversity in Lake Ohrid (Hauffe et al., 2010). Thus the general habitat (depth) range indicated by the faunal composition fits well with the sediment characteristics of Lithofacies III and the stratigraphic and paleoenvironmental constraints outlined by Lindhorst et al. (2010).

None of the species recovered are characterized by particular adaptations to substrate or food, i.e. all species are rather opportunistic within the Ohrid fauna (see Hauffe et al., 2010). Judging from current habitat conditions and observations on where the recent species occur most often, a habitat with sandy-gravel substrate is most likely for the Co1200 fauna. This correlates accurately to the sediment features of Lithofacies III. Additional support for the suggested depth range (5–25 m) comes from leaf and shaft fragments of *Chara* remains in Lithofacies III. The absence of intact *Chara* algae specimens implies that deposition of Lithofacies III at site Co1200 did not take place within the *Chara* belt and that the mollusc community lived on open substrate rather than in the *Chara* belt itself.

4.3 Faunal turnover and extinctions

Whilst acknowledging that only a subset of the recent fauna was found in the thanatocoenosis, it is important to note that no particular faunal turnover has occurred since the Last Interglacial. Moreover, none of the species occurring in Eemian times became extinct. No changes in relative frequency were recognizable. This is somewhat remarkable, since faunal shifts and extinctions have been recognized for this major climatic change on the Balkans (Frogley and Preece, 2004). A possible explanation for the lack of major fauna change in Lake Ohrid is that this deep oligotrophic lake can buffer major environmental changes better than other lakes in the area (Leng et. al., 2010; Wagner et al., 2010; Wilke et al., 2010).

Species extinctions are a major drawback in reconstructions of evolution and particular diversification rates. In the absence of a useful fossil record, it becomes extremely difficult and most often impossible to fully reconstruct evolutionary histories of recent taxa (Etienne and Apol, 2009). On the other hand, comparisons of fossil and recent faunas may lead to very interesting insights into factors generating biodiversity within a given hydrological setting. Such a study revealed the importance of major Pleistocene lake-level fluctuations on faunal evolution in Lake Malawi (Schultheiss et al., 2009). Lake Ohrid has the potential for similar studies now that fossil records are available. These records were not restricted to Lithofacies III of Co1200, but (less well preserved) shell material was present in Lithofacies II. Another core (Co1201) yielded mollusc material in Lithofacies II and IV, the latter dated to the penultimate glacial (Lindhorst et al., 2010). Recently, Holocene mollusc material has been recovered from cores drilled at the margins of Lake Ohrid (N. Hoffmann, personal communication, 2010).

4.4 Potential of Lake Ohrid mollusc fossil record

It has been shown recently that the sediments of Lake Ohrid are excellent archives for palaeoenvironmental reconstructions (Leng et al., 2010; Wagner et al., 2009, 2010; Vogel et al., 2010b; Reed et al., 2010). They cover the last glacial/interglacial cycle and yielded, for example, interesting insights into volcanic ash dispersal and climate change in the central northern Mediterranean region (Wagner et al., 2008a; Sulpizio et al., 2010; Vogel et al., 2010c). The new mollusc fossil record opens a valuable biological perspective for palaeolimnological and evolutionary reconstructions, particularly in respect to the role of environmental stability in generating biodiversity. In this context comparisons of fossil faunal assemblages and recent compositions can be enlightening. In Lake Pamvotis in Greece, for example, there are marked differences in the Plio-Pleistocene fossil compositions and the Holocene fauna. These differences were discussed in the context of lake-level and associated habitat changes (Frogley and Preece, 2004, 2007). Community changes did not only occur at the species level but major taxa shifts took place. This is obviously not the case for Lake Ohrid molluscs, at least not for the time frame since the Last Interglacial. The same holds true for the ostracode record (Belmecheri et al., 2009).

In the future, lake-level fluctuations and potential associated faunal changes in the Ohrid basin could be traced using additional sediment records from sites outside or inside the recent lake. The plains in the North and South of the present lake were flooded during phases of lake-level highstands (N. Hoffmann, personal communication, 2010) and may thus provide potential fossil mollusc records. The most promising information can be expected from foreset and terrace structures in the southern part of the basin dating back several 100 ka (Lindhorst et al., 2010). These structures may provide potential fossil mollusc records for investigations of community changes, which may date back to the earliest stages of lake formation. The methodology outlined in this paper would also be applicable to such records.

Though many Neogene palaeolakes exist on the Balkan Peninsula (Harzhauser and Mandic, 2008), extant Lake Ohrid is among the few systems where both fossil and recent mollusc assemblages can be directly compared. Other such lakes include Lake Pamvotis and potentially Lake Dojran. Such comparisons may also help in recognizing recent faunal changes caused by increasing human impact and are thus important for establishing conservation strategies (Kostoski et al., 2010).

5 Conclusions

The first recovery of mollusc fossils from sediment cores in Lake Ohrid reported here opens a whole new perspective on evolutionary research in this famous ancient lake in particular, and to interdisciplinary research resulting from lake drilling in general. Given the well studied recent mollusc fauna, the approach outlined in this study might be applicable to future extended analyses using sediment records from already identified and significantly older foreset structures or terrace levels of Lake Ohrid. These records may shed more light on the impact of significant climatic and environmental change, on radiations, and causes for endemic biodiversity in this unique lake system. This system covers several Pleistocene glacial-interglacial cycles and perhaps even dates back to the Pliocene. Adding compatible data from Lake Prespa and former Lake Maliq would help to unravel the complex faunal evolution of the Dessarete Lake System. It can be hoped that a more longterm mollusc fossil record will be obtained during future deep drilling campaigns, enabling insight into the early evolutionary history of Lake Ohrid.

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7 Mollusc biodiversity in a European ancient lake system: lakes Prespa and Mikri Prespa in the Balkans

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SPECIATION IN ANCIENT LAKES

Mollusc biodiversity in a European ancient lake system: lakes Prespa and Mikri Prespa in the Balkans

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Abstract The spatial distribution of (endemic) biodiversity in ancient and potentially ancient lakes in Europe is poorly understood. Examples include Lakes Prespa and Mikri Prespa in the Central Balkans. Utilizing information of the most species-rich taxon in these lakes, the Mollusca, we therefore attempt to statistically assess and visualize the spatial distribution of biodiversity, to analyse biogeographical patterns, and to carry out a conservation assessment. We estimate that at least 40 (sub)species (29 gastropod and 11 bivalve taxa) occur in the lakes. For both lakes combined, 37.5% of the mollusc taxa are endemic. In general, the mollusc richness in Lake Mikri Prespa is lower than in Lake Prespa and less heterogeneously distributed. The highest species richness can be seen on the western and south eastern shores of Lake Prespa. Based on the presence/absence of genera, a minimum spanning tree analysis supports the sister lake relationship of both lakes, which, in turn, are most closely related to lakes in the western Balkans and not to nearby Lake Ohrid. The IUCN red list assessment revealed (A) a tendency towards mollusc faunal change, (B) a contemporary decline and potential loss of mollusc diversity, and (C) that all endemic species are of conservation concern.

Keywords Lake Mikri Prespa · Lake Prespa · Biogeography · Endemism · Extinction · Species decline · Conservation

Introduction

Many ancient and potentially ancient lakes in Europe harbour a high number of endemic species but, at the same time, face anthropogenically induced ecosystem stress that ultimately impacts all taxa (e.g., Crivelli & Catsadorakis, 1997; Kostoski et al., 2010; Skarbøvik et al., 2010). While the most famous European ancient lake, Lake Ohrid, has received considerable attention, both in terms of biodiversity research and conservation needs (Albrecht & Wilke, 2008), other lakes have been less studied and largely neglected (Albrecht et al., 2009a). Examples include lakes Prespa and Mikri Prespa in the Prespa region. The region is located on the Balkan Peninsula, in the border area between the Republic of Albania (from hereon called Albania), the Former Yugoslav Republic of Macedonia (Macedonia), and the Hellenic Republic (Greece). The Prespa lakes are well

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recognized as wetlands of international importance (Crivelli & Catsadorakis, 1997) and are of particular relevance to waterbirds (Löffler et al., 1998).

Lake Prespa is a transboundary shallow lake with few deeper holes of up to 58 m water depth (Albrecht & Wilke, 2008). On a European scale, the lake is among the very few large lakes situated at a high altitude (849 m a.s.l.) (Noges et al., 2008), causing distinct climatic conditions. The main tributary is the River Golema in Macedonia (Fig. 1). No surface outlet exists but there are karstic underground connections to neighbouring Lake Ohrid (Matzinger et al., 2006a). The lake is subject to significant annual lake-level fluctuations (Popovska & Bonacci, 2007). The latter authors also reported a lake-level decrease of more than 7 m between 1965 and 1996. The physiography of the lake is characterized by shallow sandy beaches in the north, east, south, and southwest of Lake Prespa, whereas the western coast is comparably steep and rocky (Online Resource 2 Fig. 1).

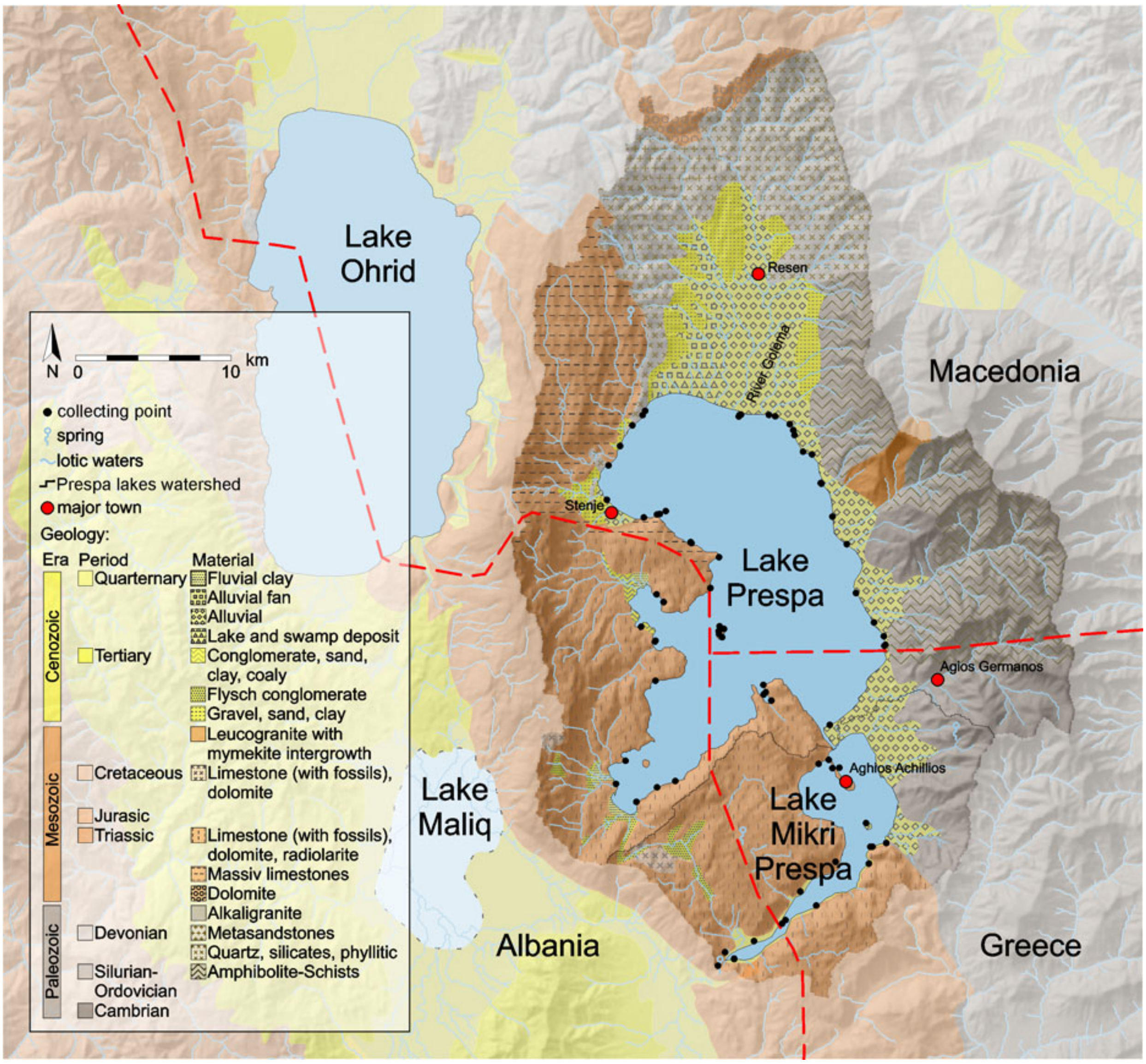

Fig. 1 Map of the Prespa region showing major hydrological and geological features. Collection sites are marked for field surveys conducted between 2003 and 2010. Geological data provided by N. Hoffmann

In addition, two rocky islands exist within the lake. Macrophytes (reed belts) are restricted to the northern edge of the lake.

Lake Mikri Prespa is today directly connected to Lake Prespa and drains into the latter via a canal (Koula canal, Online Resource 2 Fig. 1f). The canal is also used to keep the water level of Lake Mikri Prespa relatively constant throughout the year. Lake Mikri Prespa is relatively shallow and characterized by extensive wetlands on its margins, particularly in the north and east. The Albanian gulf is nowadays extensively covered with macrophytes. Characteristics of both lakes are summarized in Table 1.

The hydrological system of the Prespa lakes has been greatly impacted during the last decades by irrigation, water extraction, and regulation (Hollis & Stevenson, 1997; Matzinger et al., 2006a). Canal constructions in Albania, the diversion of the River Agios Germanos Torrent and general irrigation in the Prespa Basin have altered the hydrological setting (Parisopouios et al., 2009). They are also responsible for the loss of natural habitats along the shorelines such as wetlands or wet woodlands.

Several authors also report on increasing eutrophication in Lake Prespa (e.g., Matzinger et al., 2006a; Wagner et al., 2010) and Lake Mikri Prespa (Koussouris & Diapoulis, 1989; Stevenson & Flower, 1991; Petaloti et al., 2004), indirectly also affecting Lake Ohrid via underground discharge (Matzinger et al., 2006b).

Besides limnology and hydrology, the (endemic) biodiversity of the Prespa lakes has also been subject to intensive studies, particularly in the species-rich Mollusca. Malacological research started as early as 1894 (Sturany, 1894). Later, Urbanski (1939), Hadžišče (1955, 1958a, b), Radoman (1956), Schütt (1974), Meier-Brook (1979), Maassen (1980), Kuiper (1987), Reischütz & Stummer (1989), Korniushin et al. (2000), and Korniushin (2004), worked on mollusc materials from Lake Prespa.

Some endemic gastropod species in the Prespa lakes show shell characteristics typical for ancient lake environments, such as the possession of keels (Online Resource 2 Fig. 2) (Albrecht & Wilke, 2008). However, despite these original works, no comprehensive biodiversity treatment of molluscs exists to date, and both the distribution of biodiversity within the lakes and biogeographical relationships with other Balkan lakes are not fully understood. In fact, despite recent attempts to

Table 1 Summarized geographical, physiographical, and hydrological characteristics of lakes Prespa and Mikri Prespa compiled from LandSat 7 GIS data following conventions of Wetzel & Likens (1991)

	Lake Prespa	Lake Mikri Prespa
Location	40.763°N–41.007°N; 20.898°E–21.119°E	40.691°N–40.809°N; 21.029°E–21.125°E
Surface area	254 km^2	53 km$^{2\ c}$
Altitude	849 m asl^b	853 m asl^c
Length (maximum)	25.7 km	13.6 km
Width (maximum)	21.85 km	6.5 km
Depth (mean)	14 m^b	4.1 m^c
Depth (maximum)	58 m	8.4 m^c
Volume	3.6 km$^{3\ b}$	0.22 km$^{3\ c}$
Watershed	1130 km$^{2\ d}$	265 km$^{2\ d}$
Residence time	11 a^b	3.4 a^c
Surface water temperature (maximum)	22°C^c	28°C^c
Vollenweider (P/N)	Mesotrophic–eutrophic/mesotrophic^c	Mesotrophic–eutrophic/mesotrophic^c
Climate type	Csa (Koeppen)	Csa (Koeppen)
Annual precipitation	757 mm^a	738 mm^a
Mean January temperature	5.1°C^a	2.9°C^a
Mean July temperature	19.8°C^a	19.8°C^a

Other data are adopted from [a] Hijmans et al. (2005), [b] Matzinger et al. (2006b), [c] Zacharias et al. (2002), and [d] Vogt et al. (2007)

enhance our understanding of biogeographical processes on a regional scale using different taxa and proxies (Griffiths & Frogley, 2004; Korniushin, 2004; Lipej & Dulčić, 2004; Reed, 2004; Hauswald et al., 2008), our knowledge of patterns of freshwater biogeographical zones in the Balkans remains largely biased towards fish and drainage networks (e.g., Banarescu, 2004). Lakes Ohrid and Prespa are commonly placed in the southeastern Adriatic Drainage/south Adriatic-Ionian region (Banarescu, 2004; Abell et al., 2008), whereas other authors dispute such a linkage and place these lake in either biogeographical zone (e.g., Wilke et al., 2010).

In this paper we therefore perform state-of-the-art biodiversity and biogeographical analyses in order to:

(A) assess the mollusc biodiversity in both Prespa lakes and visualize its spatial distribution, and
(B) analyse biogeographical relationships to other lakes in the Balkans.

These data are then used to:

(C) carry out a conservation assessment and to suggest necessary conservation measures.

Materials and methods

Materials

Molluscan materials from the Prespa lakes were collected during several field trips carried out between May 2003 and September 2010. Hand collection was done from hard substrata in shallow waters or from stones and rocks lifted from depths down to 5 m by snorkeling. Deeper parts of the littoral and depths down to 50 m were sampled using a dredge from small boats. Locality information (georeferenced with Garmin handheld GPS devices) and collection details were recorded for all 70 sampling points (Fig. 1). Materials are deposited in the University of Giessen Systematics and Biodiversity collection (UGSB).

Our own collection data were supplemented with data from the literature (Online Resource 1 Tables 1 and 2).

Diversity measures and biogeographical analyses

Faunistic data were analyzed for a total of 70 collecting points. The spatial variation of gastropod diversity in the Prespa lakes was visualized using heat maps generated by the R package (R Development Core Team, 2009) spatstat 1.17-5 (Baddeley & Turner, 2005). Each collecting point was marked with its alpha diversity and degree of endemism, and respective values between collecting points were estimated using a Gaussian smoothing factor with a radius of 1.5 km. A possible correlation between gastropod richness and degree of endemism was tested by a Spearman rank correlation test (R Development Core Team, 2009).

Faunal relationships between the Prespa lakes and other lakes in the area were assessed using gastropod biodiversity information of 10 major Balkan lakes. A minimum spanning tree analysis on faunal similarities of a total of 61 gastropod genera was performed using the vegan package (Oksanen et al., 2010) for the R statistic environment. Bray–Curtis similarity was used as similarity measure. The minimum spanning tree was plotted on a topographic map to visualize the biogeographical relationships inferred.

Conservation assessment

A Red List Assessment of the endemic species of the Prespa lakes was carried out following the IUCN Red List Categories and Criteria version 3.1 (IUCN, 2001). This system consists of a total of nine categories with seven categories displaying an increased threat or risk of extinction: Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, Extinct in the Wild, and Extinct.

The remaining two categories include Data Deficient (meaning that there is not enough information about this taxon to make an assessment) and Not Evaluated (taxa that were not assessed).

Results

Mollusc diversity and endemism

A literature survey together with our own field sampling indicates that a total of 51 nominal mollusc taxa is mentioned for the Prespa lakes. Of these, we consider at least 40 species (29 gastropods and 11 bivalves) to, indeed, occur in the lakes (Online Resource 1 Tables 1 and 2; Online Resource 2 Fig. 2). Bank (2006) listed a total of 112 gastropod and 25 bivalve species (and subspecies) for the entire freshwater fauna of Greece. Thus, the Prespa lakes are home to 26% of the Greek freshwater gastropod diversity and 44% of the bivalve diversity (29% of total freshwater mollusc fauna). For the entire Albanian fauna, Dhora (2002) acknowledged 123 freshwater gastropod and 24 bivalve species. Thus, 24% of the Albanian gastropod and 46% of the bivalve fauna is represented in the lakes. According to Stankovic-Jovanovic & Stojkoska (2001), 102 species and subspecies of aquatic gastropods occur in Macedonia. This means that 28% of the total fauna can be found in the Prespa lakes.

Lake Mikri Prespa was believed to be less species-rich than Lake Prespa with a total number of 33 taxa mentioned (27 gastropod and 6 bivalve taxa). 17 taxa are now considered as present and another 3 as valid but with no recent records (Online Resource 1 Table 1). The cumulative list of Lake Prespa contains 47 taxa (30 gastropods and 17 bivalves) of which 36 are considered valid with recent records for 30 of them.

Our field work resulted in four new records for Lake Mikri Prespa and in eight new records for Lake Prespa. In addition, two invasive species are present: *Ferrissia wautieri* (Lake Prespa) and *Haitia acuta* (= *Physa acuta*) (Lake Mikri Prespa).

Of the 40 taxa considered valid in the lakes, 17 (42.5%) have restricted ranges, i.e., in either or both of the lakes or in parts of the Balkan Peninsula. The dreissenid species *Dreissena blanci* and *D. presbensis* have southern and south-central Balkan distributions, respectively. The pea-clam *Pisidium maasseni* is endemic to the lakes, but recently has only been found in Lake Prespa. The following four gastropod species occur endemically in both lakes Prespa and Mikri Prespa: *Bithynia prespensis*, *Marstoniopsis malaprespensis*, *Marstoniopsis macedonica*, and *Planorbis presbensis*.

For the individual lakes, the picture is as follows: a single species is exclusively distributed in Lake Mikri Prespa, *Prespolitorea malaprespensis*. Another taxon, *Malaprespia albanica* from Lake Mikri Prespa, proved to be monogenetic with *Albaniana albanica* (mitochondrial gene COI, Schreiber et al., unpublished data). Both taxa had been sampled from their respective type localities. Thus, a synonymization appears justified and the distribution of the species is Mikri Prespa and one adjacent spring. In contrast, Lake Prespa is inhabited by seven endemic taxa: *Prespolitorea valvataeformis*, *Vinodolia lacustris*, *Pyrgohydrobia prespaensis*, *Valvata* sp. 2, *Radix pinteri*, *Planorbarius corneus arabatzis*, and *Gyraulus stankovici*. Note that the status of *Planorbarius corneus arabatzis* is disputed (Fehér & Eröss, 2009).

With 10% (2 out of 20 species), the degree of mollusc endemism in Lake Mikri Prespa is much lower than in Lake Prespa (20% or 7 out of 36 species). For both lakes combined, 37.5% of the occurring mollusc taxa are endemic (15 out of 40).

By relating the number of endemic species to the surface area of each water body, an area-balanced endemic species/lake surface index can be calculated (Albrecht et al., 2009a). The resulting $\log N_{\mathrm{endemic\ species}}/\log A_{\mathrm{surface\ area}}$ index based on area data provided in this study (Table 1) is 0.175 for Lake Mikri Prespa and 0.349 for Lake Prespa.

In general, the gastropod richness in Lake Mikri Prespa is lower than in Lake Prespa and less heterogeneously distributed. The highest species richness can be seen on the western and south eastern shore of Lake Prespa (Fig. 2). The Spearman correlation test ($r_s = 0.52$, $P < 0.001$) showed that the degree of endemism is higher in areas with high species richness. A notable exception with a moderate degree of endemism and moderate gastropod richness is the rocky western shore of Lake Prespa.

Biogeographical patterns

The minimum spanning tree analysis (Fig. 3) supports the sister lake relationship of both lakes. Based on presence/absence of genera, the two lakes are most closely related to the western Balkan Lake Vegoritis and Lake Dojran. Interestingly, the Prespa lakes and the neighbouring Lake Ohrid are situated at the opposite ends of the minimum spanning tree, hence they possess the highest dissimilarity as far as their gastropod genera are concerned.

At the species level *Marstoniopsis macedonica*, *Dreissena prespensis*, and *Dreissena blanci* suggest an eastern Balkan (Aegean) affinity of the Prespa populations based on molecular studies (Table 2).

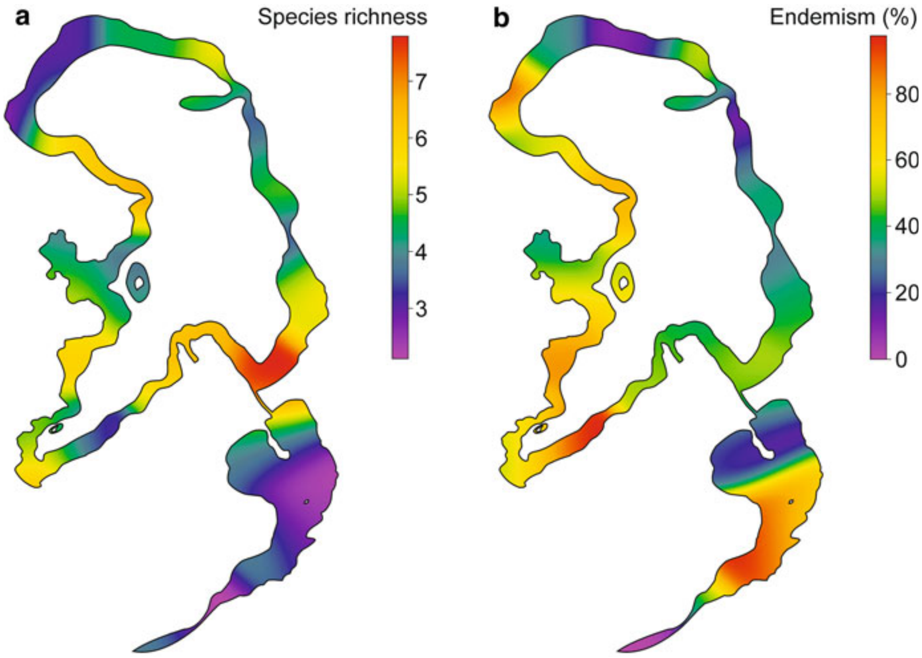

Fig. 2 Distribution of gastropod diversity within the Prespa lakes based on a total of 70 collecting points (**a** species richness, **b** endemism). **a** Lake Mikri Prespa shows less species richness than Lake Prespa. The highest alpha diversity was observed at the southeastern shore of Lake Prespa. **b** The highest percentage of endemic gastropod species was found in Lake Mikri Prespa and on the western shores of Lake Prespa

Only *Radix relicta* and *Pyrgohydrobia prespaensis* have been shown to be directly related to Lake Ohrid endemic congeners. The endemic *Pisidium maaseni* is closely related to Ohrid populations of *Pisidium edlaueri* whereas *Pisidium* cf. *casertanum* might be the only species endemic to both the Prespa lakes and Lake Ohrid (also see Schultheiß et al., 2008).

Conservation assessment

Of the endemic taxa of the Prespa lakes described, some have been found in very restricted areas, some in very low numbers. *Vinodolia lacustris* could not be relocated, though the type locality has been visited several times.

For some endemic species, historic records suggest occurrence in both lakes but they could only be found in one of the two lakes during our recent surveys. Examples include *Gyraulus stankovici* and *Pisidium maasseni*, which were not found in Lake Mikri Prespa.

Endemic species are among both the most frequent (e.g., *Prespolitorea malaprespensis*) and the rarest species (e.g., *Malaprespia albanica*) in Lake Mikri Prespa (Fig. 4b). In Lake Prespa, the situation is somewhat different (Fig. 4a). Here, five endemics are among the most frequent species (at least 25% site occurrence frequency).

The IUCN red list assessment revealed that all endemic taxa of the Prespa lakes are of conservation concern. Five species fulfil the criteria for being critically endangered. Another five species have been assessed as endangered. Further two are considered to be vulnerable (Table 3).

Among the most important threats facing Lake Prespa and its indigenous fauna is eutrophication related to intensive agriculture practice and settlements in the immediate and wider surroundings. An increased sediment load as the result of erosion processes in the watershed adversely impacts bivalves and their filtration ability. Other consequences from agricultural practices, e.g., apple cultivation, are pollution of the lakes with pesticides and herbicides, and particularly the excessive abstraction of water. The latter practice worsens the distressing loss of water caused by climatic extremes such as unusually dry summers and winters with less rainfall in the last decades. Observed drops in water level can be abrupt (Online Resource 2 Fig. 3).

Habitat changes, e.g., extensive macrophyte growth, being related to eutrophication, are obvious, particularly in shallow bays (Online Resource 2 Fig. 3f). Overgrown soft substrates become unsuitable for unionid mussels and also diminish the oxygen availability within the substrate. Changes can be observed in altered biofilm cover of rocks along the shorelines. Those changes can also result from herbicides that

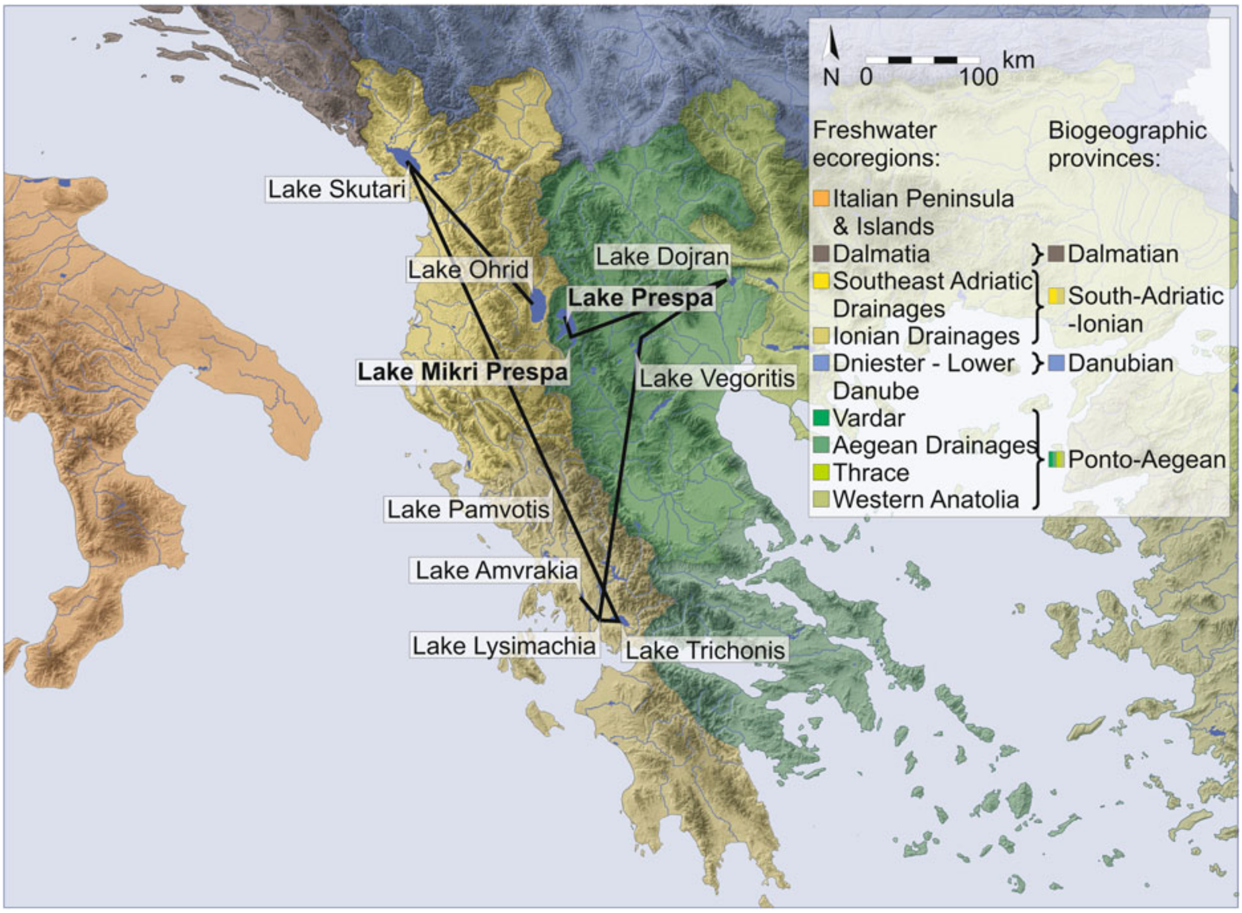

Fig. 3 Map of the Balkan Peninsula with the respective modified biogeographical provinces (Banarescu, 2004) and ecoregions (Abell et al., 2008), and a minimum spanning tree showing biogeographical relationships based on 61 gastropod genera

might impact the diatom communities, which serve as a food source for many gastropod species. Sometimes these rocky shorelines are already buried under organic sediments or mud (Online Resource 2 Fig. 3c). Limnological changes of the system result in lowered oxygen availability, particularly in deeper parts of both lakes. The intentional introduction of alien fish species, particularly in Lake Mikri Prespa, is also a matter of concern. In fact, the number of non-native fishes today approximately equals the native fish number in Lake Prespa (Talevski et al., 2010).

Discussion

Mollusc diversity and endemism

The Prespa lakes harbour a high number of mollusc species and genera compared to many other Balkan lakes. However, the lakes have slightly less overall taxonomic diversity than lakes Trichonis, Pamvotis, Skutari, and particularly Ohrid (Albrecht et al., 2009a). It has already been noted that the extent of faunistic studies varies greatly among those lakes. Thus, numbers presented for various other Balkan lakes should still be regarded as preliminary. In the Prespa lakes, a higher species richness was observed for Lake Prespa (Fig. 2a), with a slight tendency to more species-rich assemblages along the rocky shores. At Lake Prespa, the shallow eastern shore is habitat for mostly cosmopolitan gastropod species, whereas the western cliffy bays harbour rock dwelling microgastropods in low abundances.

Despite the generally low endemic species richness (Fig. 4), the degree of endemism (Fig. 2b) may be high in parts of Lake Mikri Prespa. Habitat heterogeneity is often seen as a proxy for species diversity in ancient lakes (Martens, 1997). As the

Table 2 Biogeographical relationships of the Prespa lakes based on molecular studies of phylogenetic relationship of mollusc species

Taxon	Prespa lakes species	Phylogenetic relationship	Biogeographical affinity	Reference
Gastropoda				
Marstoniopsis spp.	*Marstoniopsis macedonica*	Closely related to *M. graeca*	Aegean (Vardar ecoregion), but also closely related to European species	Szarowska (2006), Wilke, unpublished data
Valvata spp.	*Valvata piscinalis* (*Valvata* sp. 1)	Greek lakes and German populations	Unclear	Hauswald et al. (2008)
	Valvata sp. 2	Prespa endemic	Unresolved	Hauswald et al. (2008)
Gyraulus spp.	*Gyraulus* (*Carinogyraulus*) *stankovici*	Closely related to widespread *G. albus* and not to the endemic *Carinogyraulus* spp. of the Ohrid Basin	Widespread	Geertz (2009), Albrecht, unpublished data
Radix spp.	*R. pinteri*	Sister species to *R. relicta*	Lake Ohrid	Albrecht et al. (2008)
	R. auricularia	European and Balkan populations	Widespread	Albrecht et al. (2008)
Pyrgohydrobia spp.	*Pyrgohydrobia prespensis*	Closely related to *Pyrgohydrobia* spp. of the Ohrid Basin	Lake Ohrid	Wilke et al. unpublished data
Bivalvia				
Pisidium spp.	*P. maaseni*	Unresolved relations to *P. edlaueri*, *P. nitidum*	Potentially Lake Ohrid affinity	Schultheiß et al. (2008)
	P. subtruncatum		Widespread	Schultheiß et al. (2008)
	P. henslowanum		Widespread	Schultheiß et al. (2008)
	P. personatum		Widespread	Schultheiß et al. (2008)
	P. cf. *casertanum*	Sister species to *P. subtruncatum*; potentially distinct species of Prespa lakes and Lake Ohrid	Unclear	Schultheiß et al. (2008)
	P. casertanum Type II	Closely related to European populations	Widespread	Schultheiß et al. (2008)
Dreissena spp.	*D. presbensis*	Populations in northern and central Greek lakes	Aegean (Vardar, Thrace, Ionian drainages ecoregions)	Albrecht et al. (2007), Wilke et al. (2010)
	D. blanci	Southern and central Greek lakes	Aegean (Ionian drainages, Vardar ecoregions)	Albrecht et al. (2007), Wilke et al. (2010)

Prespa lakes have less habitat diversity than Lake Ohrid, since they are much shallower and more homogeneous, they offer fewer niches to be occupied.

Endemism in freshwater habitats is subject to intense studies (e.g., Dudgeon et al., 2006) and, as endemism can occur on different spatial scales, narrow-range taxa have been of particular interest to researchers (e.g., Ponder & Colgan, 2002). On a Balkan scale, Lake Prespa ranks among the lakes with a high degree of mollusc endemism, greatly outnumbered only by Lake Ohrid. Lake Mikri Prespa belongs to the lakes with a relatively low degree of mollusc endemism, resembling, e.g., lakes Skutari or Vegoritis. Considering this area-balanced endemicity

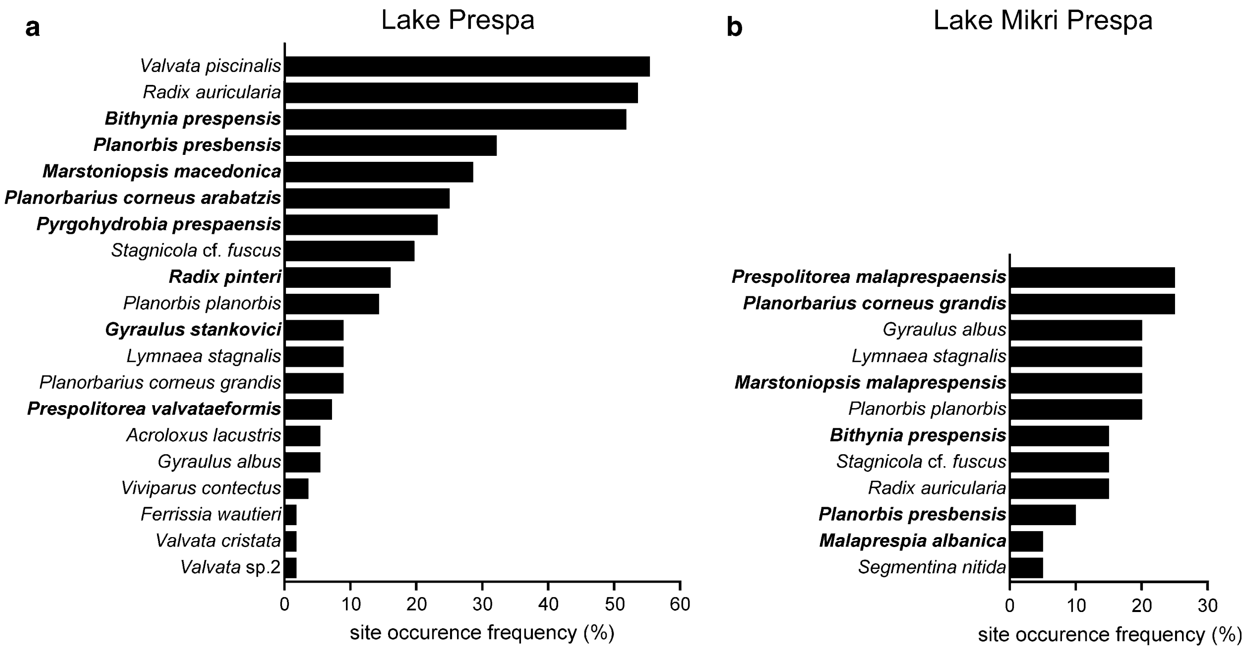

Fig. 4 Frequency distribution of gastropod taxa found during the field survey conducted between 2003 and 2010. **a** Lake Prespa ($N = 50$ localities). **b** Lake Mikri Prespa ($N = 20$ localities). Endemic species are shown in bold

Table 3 IUCN Red List assessment of endemic taxa of the Prespa lakes

Assessed species	Category	Criteria
Bithynia prespensis	VU	D2
Malaprespia albanica	CR	B1ab(ii,iii) + 2ab(ii,iii)
Prespolitorea malaprespensis	CR	B1ab(ii,iii)
Prespolitorea valvataeformis	CR	B1ab(ii,iii)
Marstoniopsis malaprespensis	CR	B1ab(ii,iii)
Marstoniopsis macedonica	EN	B1ab(iii)
Vinodolia lacustris	CR	B1ab(ii,iii)
Pyrgohydrobia prespaensis	EN	B1ab(iii)
Radix pinteri	EN	B1ab(iii)
Planorbis presbensis	VU	D2
Gyraulus stankovici	EN	B1ab(iii)
Pisidium maasseni	EN	B1ab(iii)

The assessment followed the IUCN Red List Categories and Criteria version 3.1 (IUCN, 2001). Categories: *CR* critically endangered, *EN* endangered, *VU* vulnerable. Note that subspecies and undescribed species were not assessed

index (Albrecht & Wilke, 2008; Albrecht et al., 2009a), Lake Prespa not only belongs to the lakes with high mollusc endemism on the Balkan scale, but even exceeds lakes like Malawi and Titicaca. In contrast to Lake Prespa, Lake Mikri Prespa, however, is not characterized by a high index value for endemic species/lake surface area.

Biogeography and faunal origins

The faunal relationships of the Prespa lakes based on mollusc data turned out to be complex and not conclusive in terms of a close Ohrid–Prespa relationship or western Balkan (Adriatic) vs. eastern Balkan (Aegean) biogeographical affinities. Recent studies (e.g., Albrecht & Wilke, 2008; Albrecht et al., 2009a) suggest a closer biogeographical connection between Lake Prespa and the lakes of the Vardar region (Fig. 3). It seems that Aegean relationships prevail among the phylogeographical studies with adequate resolution. Wilke et al. (2010), therefore, suggested modifying the borders of the Vardar ecoregion to include lakes Prespa and Mikri Prespa. Taxa with a Balkan-wide distribution such as the gastropod family Bithyniidae might provide more insight into biogeographical patterns and processes among Balkan lakes (Glöer et al., 2007). Such studies can reveal new insights as recently shown in the *Barbus* fishes, where the presumed endemic Lake Prespa *Barbus prespensis* turned out to be much more widespread in the Adriatic basin (Marková et al., 2010).

Although detailed palaeostratigraphic studies must still be conducted in the Prespa lakes, previous phylogeographical studies (e.g., Wilke et al., 2010) indicate a minimum age of mollusc populations in Lake Prespa of 110,000 years. Moreover, shell beds consisting of predominantly *Dreissena* sp. have been found in recent lake sediment drilling cores. They date back to approximately 70,000 years BP (H. Vogel, personal communication, 2010). Longer drilling cores would help unravel the history of the Prespa lakes as they did with the sister Lake Ohrid (Wagner et al., 2010). Only such data will eventually allow a discussion of stable or unstable lake conditions with regard to species diversity and endemism.

Conservation status of mollusc fauna

The endemic mollusc species of many of the Balkan lakes were only described a few decades ago. The decline and potential loss of endemic mollusc diversity in lakes in Montenegro, Albania, Macedonia, and Greece has been noted by Bouchet et al. (1999) and Regnier et al. (2009). The urgency of conservation measures was highlighted by Albrecht et al. (2006) as lakes in circum-Mediterranean countries are under ever increasing anthropogenic pressure (Álvarez-Cobelas et al., 2005). Major causes are dramatic changes in water levels due to massive extraction for agribusiness. At the same time, the eutrophication-level of most lakes has increased during the past decades due to a combination of water use, fishery, pollution, toxification, and climatic extremes, especially during summer (e.g., Kostoski et al., 2010). These factors lead to direct or indirect habitat destruction and loss. As a consequence, communities change and eventually complete food webs become disrupted. Highly adapted and specialized species cannot cope with these environmental changes that may happen very quickly in some cases, e.g., during a single season.

From a conservation point of view, it is necessary to assess the current status of the endemic species of the Prespa lakes based on recurrent survey data. The outcomes of the recent assessment (Table 3) are alarming and reiterate the call for urgent conservation activities repeatedly outlined more than a decade ago (Hydrobiologia Special Issue Prespa edited by Crivelli & Catsadorakis, 1997).

Changes are recognizable in the whole ecosystem, for example, by the presence of invasive fish species (e.g., Talevski et al., 2010). Invasive gastropod species also become more and more important in ancient lakes as outlined for *Haitia acuta* in Lake Titicaca (Albrecht et al., 2009b), a species that today is also present in Lake Mikri Prespa. The observation of more opportunistic species as compared to former listings for the Prespa fauna also fits in this picture. These circumstances and the reported decline in endemic gastropods should trigger efforts to safe this sensitive lake ecosystem.

A critical point in all conservation efforts related to Balkan lakes is public awareness of the uniqueness of these ecosystems, which may help to increase the acceptance of, e.g., management plans for controlled water extraction. Sewage treatment systems should be installed along the actual lakes and all tributary systems. Agricultural practices should become more sustainable with reduction of fertilizer and pesticide use as the ultimate goal (Catsadorakis & Malakou, 1997; see also Kostoski et al., 2010 for a discussion on the Lake Ohrid Basin). However, all these practices can only be effective if concerted action plans are implemented in the immediate future. A first step has been taken by the establishment of the trilateral Prespa Park. It is also clear that more intensified conservation activities leading to sustainable land use practices in the Prespa watersheds are necessary in order to avoid further biodiversity loss and further ecosystem degradation in the unique Prespa lakes.

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8 Invaders versus endemics: alien gastropod species in ancient Lake Ohrid

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SPECIATION IN ANCIENT LAKES 6

Invaders versus endemics: alien gastropod species in ancient Lake Ohrid

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Abstract Species invasions into ancient lakes are an important but little understood phenomenon. At ancient Lake Ohrid, a systematic assessment of invasive mollusc species using morphological and genetic data was conducted from 2003 to 2012. Two globally invasive gastropod species, *Physa acuta* and *Ferrissia fragilis*, have recently been discovered at 4 out of 386 sites. These sites are anthropogenically impacted. The invasive species co-occur with endemics. Phylogenetic analyses of populations from native and invaded ranges of both species confirmed their identities and provided insights into their invasion histories. Accordingly, *P. acuta* is genetically more diverse than *F. fragilis*. Both species are currently present in a considerable number of lakes on the Balkan Peninsula. Possible future trends in Lake Ohrid and the Balkans are discussed and further spread of both species is likely. Given the ongoing environmental change in Lake Ohrid, the number of observations of non-indigenous or other widespread species will probably rise in the coming years and such species and their impact on native species should be carefully monitored. Moreover, ancient lakes with recurrent invasions of alien species might serve as interesting model systems for the study of important topics of invasion biology.

Keywords *Physa acuta* · *Ferrissia fragilis* · *Ferrissia wautieri* · Alien species · Non-native species · Invasion

Introduction

Species invasions into ancient lakes are an important but little understood phenomenon. Some cases of invasive species in ancient lakes serve as textbook examples of ecological disasters following an introduction that happened decades ago (Goudswaard et al., 2008). The extent and possible impact of other introduction events became known only recently (e.g., Herder et al., 2012). This is particularly true for small benthic and inconspicuous invertebrate species. Molluscs, for example, are important components of the macrozoobenthos of all ancient lakes (e.g., Albrecht et al., 2012). However, very few recent gastropod invasions have been documented, among them *Melanoides tuberculata* O.F. MÜLLER, 1774 in Lake Malawi (Genner et al., 2004) and *Physa acuta*

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Draparnaud, 1805 in Lake Titicaca (Albrecht et al., 2009a). Note that, the latter species is often attributed to the genus *Haitia* or *Physella* (but see Wethington & Lydeard, 2007). Interestingly, at about the same time *P. acuta* was introduced in the South American Lake Titicaca, it was also reported as invasive species in the European ancient Lake Ohrid (Albrecht et al., 2009b). In fact, this was the first record of an invasive mollusc species in Lake Ohrid, although the lake is historically among the best studied ancient lakes in terms of molluscs (Albrecht & Wilke, 2008; Hauffe et al., 2011). In particular, gastropods represent a major component of the macrozoobenthos of Lake Ohrid (Albrecht & Wilke, 2008) and historically 72 species of which 56 were considered as endemic (78 %) had been described from the lake (Radoman, 1985).

However, the introduction of non-indigenous species into the Lake has recently become of concern (Kostoski et al., 2010). The increase in number of invasive species has been attributed, in part, to the increasing anthropogenic pressure on the lake resulting in habitat transformations.

Given the recent discovery of the first invasive mollusc species in Lake Ohrid (i.e., *P. acuta*), and the "creeping biodiversity crisis" of the lake (Kostoski et al., 2010), the present study aims at a systematic assessment of invasive mollusc species in Lake Ohrid based on morphological and genetic data. The specific goals are:

(1) A lake-wide assessment of invasive mollusc species in Lake Ohrid including habitat and mollusc community assessment; and
(2) the reconstruction of the respective invasion histories in a global and regional Balkan context.

This is to the best of our knowledge the first comprehensive assessment of invasive molluscs in any of the ancient lakes worldwide. It can thus serve as case study and an outline for comparative discussions, potentially triggering similar studies in other ancient lakes.

Materials and methods

Sampling design

Materials from Lake Ohrid and its watershed were collected and faunal assessments were carried out during several field trips conducted between 2003 and 2012. Individuals were obtained by hand collecting from hard substrata in shallow waters or from stones and rocks lifted from depths to 5 m by snorkelling. Deeper parts of the littoral and sublittoral to 60 m were sampled using a dredge from small boats or from the research vessel of the Hydrobiological Institute in Ohrid (HBI). At Lake Ohrid, a total of 386 localities (285 in the lake proper, 101 in the basin; Fig. 1) have been surveyed for the presence of invasive species. The presence of invasive species was checked by morphological/anatomical examination and genotyping. For invasive species identified, materials from the respective type localities were included in this study (Table S1). Both habitat state relative to overall habitat conditions in Lake Ohrid (see Kostoski et al., 2010) and mollusc communities (using the methodology described in Hauffe et al., 2011) have been assessed for the localities from which invasive species had been identified.

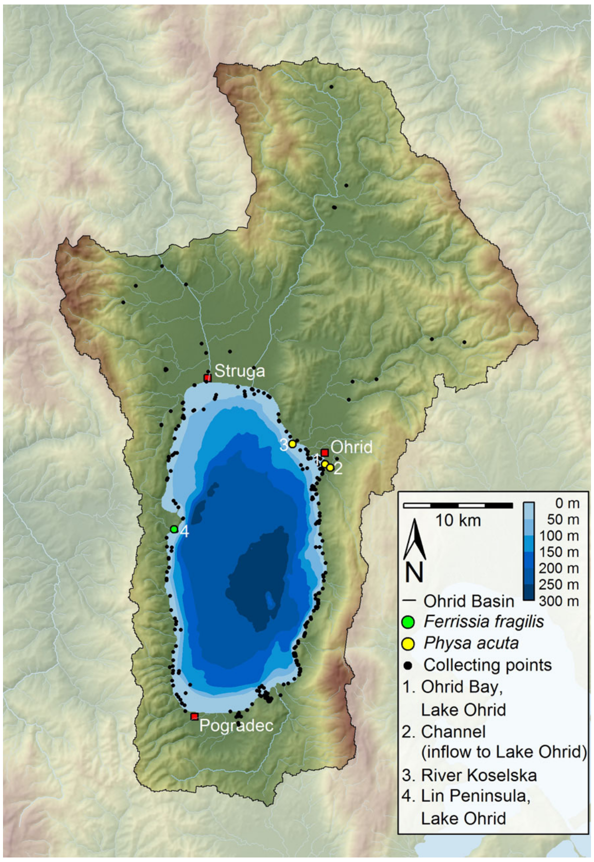

Fig. 1 Map of Lake Ohrid and its basin (Macedonia, Albania) with sampling localities (2003–2012) and the known occurrences of invasive mollusk species

Extensive field survey data from the Dessarete lakes (Albrecht & Wilke, 2008) and other lakes and occasionally other limnic systems of the Balkan Peninsula with repeated sampling in a total of 20 lakes, were analyzed and the respective faunistic literature was reviewed. We also conducted expert interviews in order to trace records of invasive species in Balkan lakes and thus to eventually assess the invasion histories of the invasive species reported from Lake Ohrid.

DNA sequencing

DNA was isolated from individual snails following the protocol described in Albrecht et al. (2004). Standard primers (Folmer et al., 1994) were used for amplifying a fragment of the cytochrome *c* oxidase subunit I (COI) gene with a target length of 655 bp (excluding 51 bp primer sequence). Sequences (forward and reverse) were generated either on a Long Read IR2 4200 sequencer (LI-COR) using the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing kit (Amersham Pharmacia Biotech) or on an ABI 3730 XL sequencer (Life Technologies) using a Big Dye Terminator Kit (Life Technologies). All sequences could be unambiguously aligned using ClustalW implemented in BioEdit 7.0.8.0 (Hall, 1999).

Phylogenetic analyses

For each invasive species identified we conducted individual tree building analyses. First the best-fit model of sequence evolution was inferred for each data set based on the Akaike Information Criterion by conducting dynamical likelihood ratio tests in jModelTest 0.1.1 (Posada, 2008). Then we tested the degree of saturation for each dataset using the entropy-based approach of Xia et al. (2003) as implemented in DAMBE 5.2.9 (Xia & Lemey, 2009) with the input parameter for invariable sites taken from jModelTest.

Phylogenetic reconstructions using Bayesian inference were done with the software package MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). During two independent runs, every 100th tree was sampled and individual analyses were terminated when final average standard deviations of split frequencies in MrBayes reached values of near or <0.01.

Table 1 List of non-indigenous freshwater mollusk species in Europe that could potentially invade Lake Ohrid

Species	Native range	Presence on Balkan Peninsula
Gastropoda		
Melanoides tuberculata	Southeast Asia	No data
Potamopyrgus antipodarum	New Zealand	Yes (Radea et al., 2008)
Ferrissia fragilis	North America	Yes
Physa acuta	North America	Yes
Pseudosuccinea columella	North America	Yes
Planorbella duryi	North America	Yes
Planorbella/*Helisoma* spp.	North America	No
Gyraulus chinensis	Southeast Asia	Yes
Gyraulus parvus	North America	Yes
Menetus dilatatus	North America	No data
Bivalvia		
Sinanodonta woodiana	East Asia	Yes
*Dreissena polymorpha**	Ponto-Caspian	Yes (Wilke et al., 2010)
Dreissena bugensis	Ponto-Caspian	No
Corbicula fluminalis[a]	Asia	No data
*Corbicula fluminea**	Asia	Yes

Species belonging to the 100 most invasive species in Europe are marked with an asterisk (DAISIE, 2009) Presence on the Balkan Peninsula refers to data provided by Fauna Europaea (Bank, 2012) if not otherwise stated. Species present in Lake Ohrid (this study) are bold

[a] Note that the taxonomic status and identity of *Corbicula fluminalis* is debated

Results

Lake Ohrid assessment of invasive species

Prior to our survey, we identified 10 gastropod and 5 bivalve candidate invasive species (Table 1). The majority of these species (10 of 16) are known to be already present on the Balkan Peninsula. From these potentially invasive species, two were found to be currently present in Lake Ohrid: *P. acuta* and *Ferrissia fragilis* (TRYON, 1863). The former species occurred in 3 out of 386 surveyed localities (0.8 %), the latter one in 1 locality (0.3 %; Table 2).

Table 2 Lake Ohrid mollusk communities with either *Ferrissia fragilis* or *Physa acuta* (bold) presence, based on field records from 2009 to 2011

Species	Locality 1	Locality 2	Locality 3	Locality 4
Physa acuta	**x**	**x**	**x**	
Ferrissia fragilis				**x**
*Radix relicta**	x	x		
Radix labiata	x			
Planorbis planorbis	x	x		x
Planorbarius corneus	x			
*Gyraulus albidus**	x			x
*Carinogyraulus lychnidicus**	x			
*Ochridopyrgula macedonica**	x			x
*Polinskiola sturanyi**				x
*Valvata stenotrema**	x			
*Ohridohoratia pygmaea**	x			
*Ohrigocea stankovici**	x			
Theodoxus fluviatilis	x	x		
*Pyrgohydrobia grochmalickii**			x	
*Ohridohauffenia rotonda**		x		
Dreissena prespensis	x	x		x
Total	13	6	2	6

Lake Ohrid basin endemics are marked with an asterisk

The first thriving population of *P. acuta* was discovered in Lake Ohrid in May 2009. The population was found in the Ohrid Bay in close proximity to the channel leading to the Hydrobiological Institute Ohrid (41.10558°N, 20.80746°E; locality 1 in Fig. 1; also see Table S1) as well as in the channel itself (41.10362°N, 20.80946°E; locality 2 in Fig. 1). In 2010, the species has been recorded alive from a further location in the greater Ohrid Bay area (River Koselska; 41.12209°N, 20.77105°E; locality 3 in Fig. 1). All three places are impacted by human activities such as boating or fishing and contamination. The water quality is also worse than in most less influenced regions of Lake Ohrid. This is mostly due to sewage inflow. *P. acuta* has been found again during repeated monitoring conducted in 2010 and 2011.

Ferrissia fragilis was, for the first time, discovered at a site on the Albanian shore of Lake Ohrid opposite to Ohrid town (Lin Peninsula; 41.05138°N, 20.64190°E; locality 4 in Fig. 1, also see Table S1). It has hitherto been found exclusively at this place. This site is characterized by eutrophication caused by inflow of sewage and nutrient input from adjacent agricultural land.

In all four sites in which these invasive species were recorded, they co-occurred with endemic species, though at different degrees (Table 2). The most diverse mollusc community was found at locality 1 where *P. acuta* was associated with 12 other native and endemic species. *P. acuta* co-occurred with 5 other species in locality 2. In locality 3, however, only one additional (endemic) species was found. An unspecific littoral community (5 native and endemic species) characterized locality 4, where the population of *Ferrissia* thrives.

Phylogenetic analyses

The phylogenetic analyses of the *Physa* dataset included 114 specimens of a total of ten species, including 32 newly generated sequences from Balkan localities (for details see Table S1; Fig. 2). The *Ferrissia* dataset comprised two species with a total of 58 specimens of which 50 were obtained from GenBank (Fig. 3). Topotypic material for *Physa acuta* (River Garonne, France, AY282589) and *Ferrissia wautieri* (Lago di Mergozzo, Italy)—a species likely synonymous with *F. fragilis*—as well as new material of *F. rivularis* from the USA was included in the analyses.

Both under the assumption of a symmetrical as well as an asymmetrical tree, the Xia test indicated only

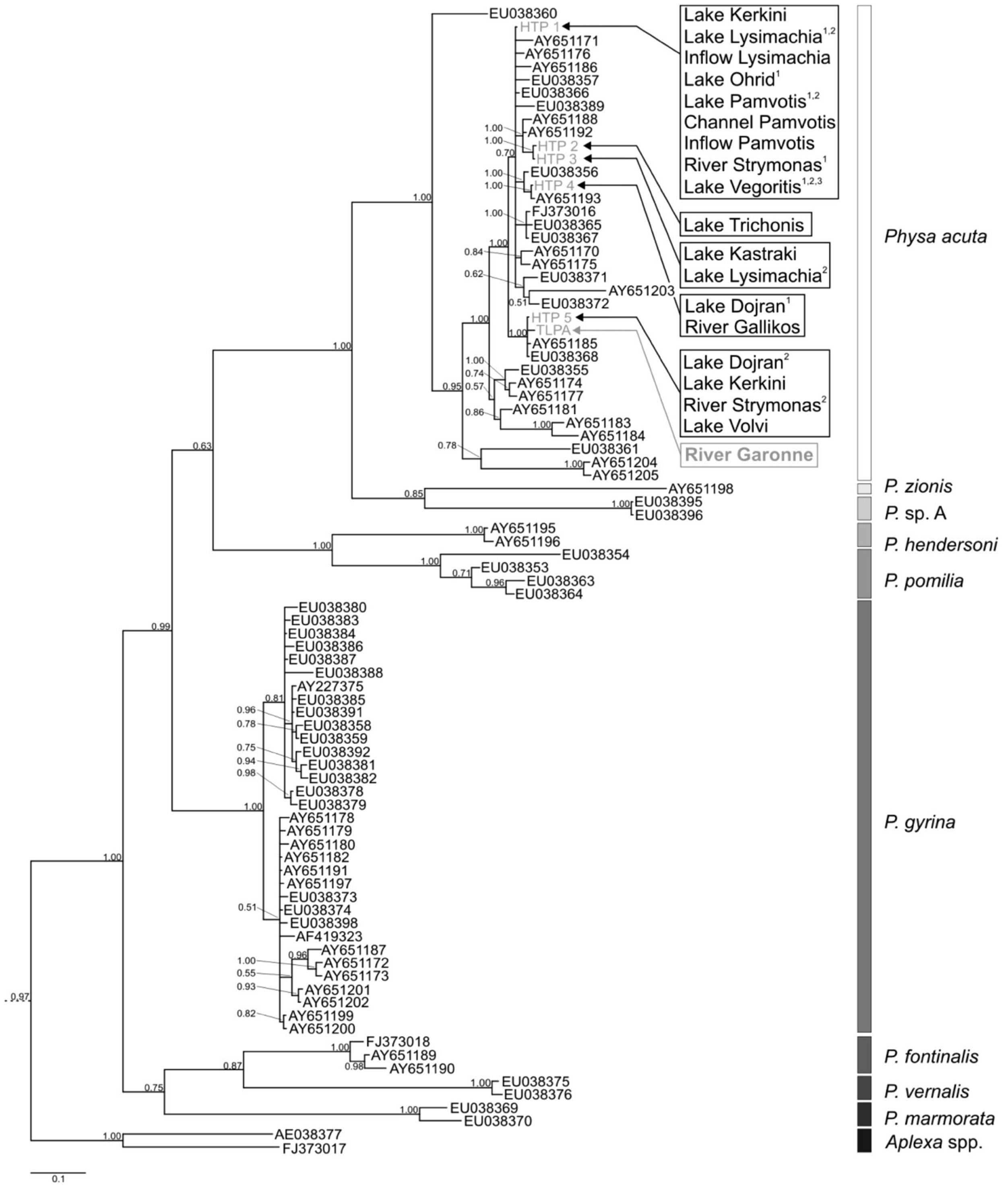

Fig. 2 Bayesian inference phylogram of Physidae based on 114 sequences of the COI gene under the GTR + I + G model. The outgroup taxa were a posteriori removed from the tree. They belong to the Hygrophila families Acroloxidae (*Acroloxus lacustris*), Planorbidae (*Ancylus fluviatilis*), and Lymnaeidae (*Lymnaea stagnalis*) used in a previous study (Albrecht et al., 2009a). Topotypes of *Physa acuta* are marked in bold. Clade assignment of Physidae follows Wethington and Lydeard (2007, Fig. 5a). Superscript numbers at particular localities refer to sampling sites listed in Table S1

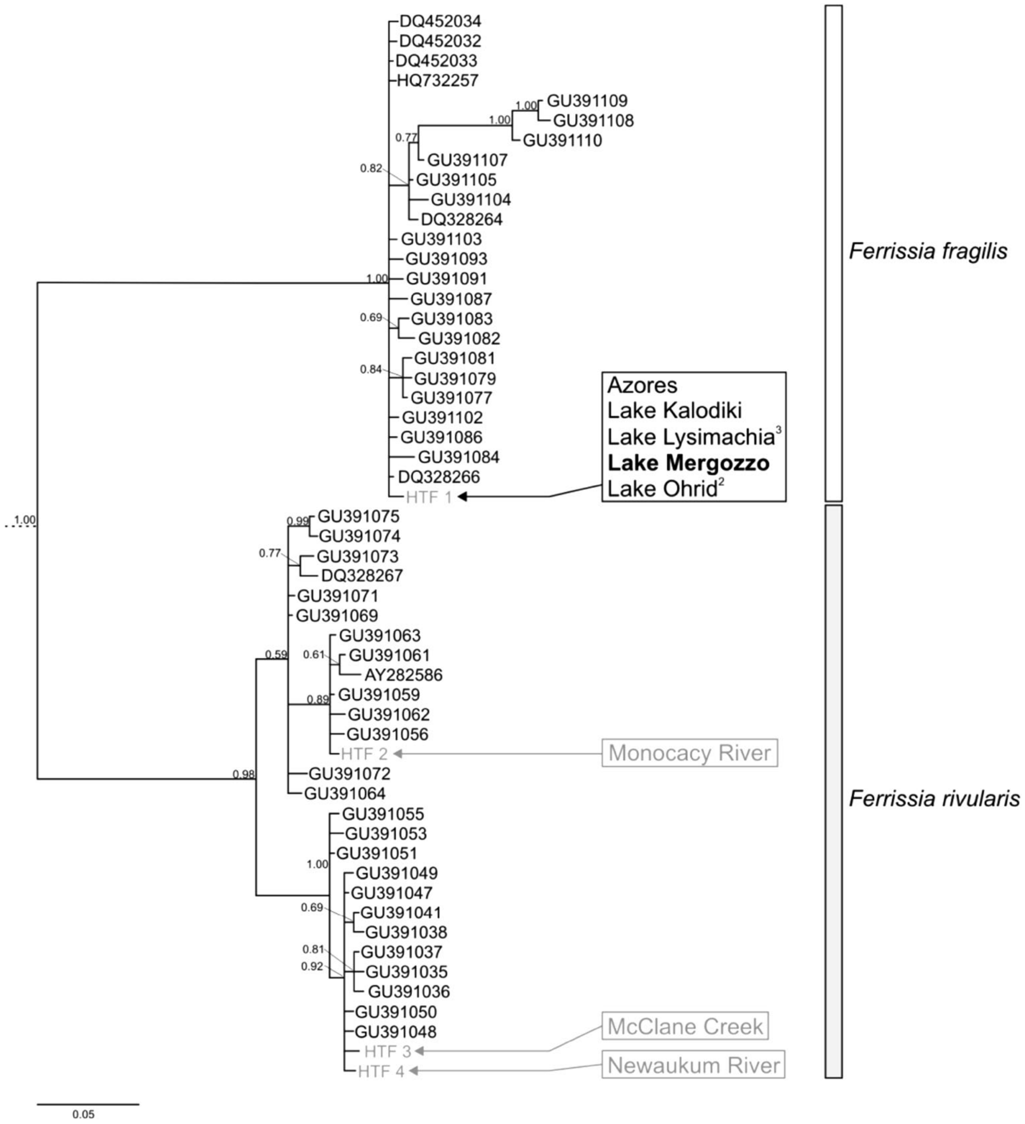

Fig. 3 Bayesian inference phylogram of *Ferrissia* spp. based on 58 sequences of the cytochrome *c* oxidase I gene under the TIM1 + G model. Topotypes of *Ferrissia wautieri* are marked in bold. The outgroup taxa were removed a posteriori. They belong to the Hygrophila families Acroloxidae, Planorbidae, and Lymnaeidae (from Albrecht et al., 2009a). Superscript numbers at particular localities refer to sampling sites listed in Table S1

little saturation in the *Physa* (GTR + I + G model) and *Ferrissia* (TIM1 + G model) data sets.

All morphologically identified *Physa acuta* specimens nested within the well-supported *P. acuta* clade (Bayesian posterior probability (BPP) 1.0, Fig. 2). A total of five different haplotypes were found in seven natural and two artificial lakes as well as four riverine systems on the Balkans. The Lake Ohrid haplotype (HTP1) is widespread on the Balkans and occurs also in specimens from Lakes Lysimachia, Pamvotis, Kerkini and Vegoritis, and the River Strymonas. *Physa acuta* populations from Lake Trichonis share a unique haplotype (HTP2), whereas there are two different haplotypes from the neighboring Lake Lysimachia (HTP1 and 3). HTP3 also occurs in Lake Kastraki. Lake Dojran specimens possessed haplotypes 4 (also River Gallikos) and 5. Haplotype 5 (Lakes Dojran, Kerkini, Volvi and River Strymonas) is closely related to haplotypes from the type locality of *Physa acuta* in France (Fig. 2; Table 1).

The *Ferrissia* specimens genotyped from lakes Ohrid, Lysimachia, Mergozzo, Kalodiki, and the

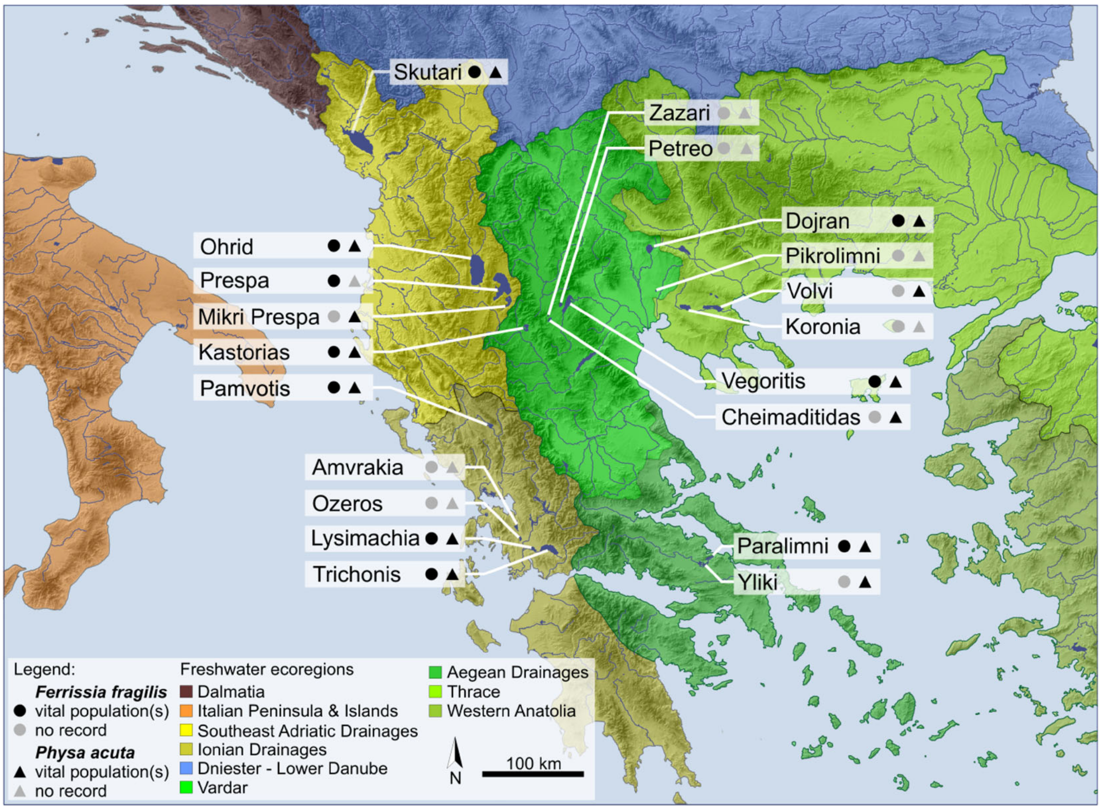

Fig. 4 Records of *Physa acuta* and *Ferrissia fragilis* in natural Balkan lakes based on field work carried out from 2003 to 2012, expert interviews and the literature (see Table S2 and S3 for details)

Azores (San Miguel Island; GU391101; Raposeiro et al., 2011) constitute a single haplotype (Fig. 3). They cluster within a well-supported *Ferrissia fragilis* clade (BPP 1.0) together with representatives from North America, Europe, and Asia. Thus nominal European *Ferrissia wautieri* (Lake Mergozzo) is genetically (COI) not different from *Ferrissia fragilis* and therefore all European populations studied so far belong to the invasive *F. fragilis*.

Invasion histories

Physa acuta and *Ferrissia fragilis* are currently present in a considerable number of lakes on the Balkan Peninsula, including Lake Ohrid. From the major natural lakes of the Balkans considered here, the first record of *P. acuta*—to our knowledge—dates back to 1985 when it was reported from Lake Dojran (Stanković, 1985). *Physa acuta* today occurs in at least 13 (65 %) of the 20 studied lakes (Fig. 4). No *Physa* populations have been found in lakes Amvrakia, Petreo, and Zazari. Lakes Koronia and Pikrolimni were dried out during the field campaign in 2007, but no empty shells of *Physa* were found.

Ferrissia fragilis is at least present in ten of the surveyed lakes (50 %). All records were made after the year 2000, with the notable exception of Lake Vegoritis from where it already had been reported in 1962 (as *F. wautieri*; Schütt, 1985). All literature records of *Ferrissia wautieri* are considered here as representative of *F. fragilis* following the results of the genetic characterization of topotypic material of *F. wautieri* (see above). Details on first records and current status of both species in Balkan lakes can be found in the supplement (Tables S1, S2).

Discussion

Invasive species of Lake Ohrid

Hitherto, Lake Ohrid appeared to harbour fewer invasive species than other Balkan Lakes (Albrecht et al., 2009c, 2012). Given the presence of two globally invasive gastropod species reported here, it becomes obvious that highly impacted littoral parts such as the Ohrid Bay or areas near the Lin Peninsula have lately become prone to molluscs invasions. The quality of the mollusc fauna present in Lake Ohrid has thus changed significantly. Whereas hitherto the fauna was made up of widespread European or Balkan species and endemics (at various levels of endemicity; Hauffe et al., 2011), we now also see globally invasive species in the lake. Lake Ohrid thus shares the fate of Lake Malawi (Genner et al., 2004), Lake Titicaca (Albrecht et al., 2012), Lake Tahoe (Wittmann et al., 2012) and the Caspian Sea (Heiler et al., 2010) from which recently globally invasive molluscs species have been recorded. Of the total number of currently known gastropod species in Lake Ohrid (76), 56 are endemics to either the lake or its basin (73.7 %), 18 are widespread (23.7 %) and two are globally invasive species (2.6 %). Given our intensive surveys conducted during the last decade on the malacofauna of Lake Ohrid, we do consider the invasion of both species as, indeed, very recent events, especially in the case of the distinct *P. acuta*. The situation of *F. fragilis* might be a bit different in as much as it is a tiny limpet-like species. It could potentially be mistaken for an *Acroloxus* species, a genus of freshwater limpets present in Lake Ohrid with three to four species. In contrast to the long known presence of *P. acuta* in Europe, *F. fragilis* represents a case of a cryptic invader. Being also of North American origin, it was only recently shown to be conspecific with some populations of a *Ferrissia wautieri* (Walther et al., 2006). The latter species had been considered to be a European native by some authors (Falkner & Von Proschwitz, 1998; Raposeiro et al., 2011; Kadolski, 2012), but was questioned by others (Son, 2007; Marrone et al., 2011). No consensus could be reached due to the lack of modern data of topotypic material prior to this study.

It can only be speculated why Lake Ohrid has received both invasive species in a somewhat delayed manner compared to the other Balkan lakes (see below). Though slightly polluted at few sites, Lake Ohrid is still a relatively isolated oligotrophic lake (Matzinger et al., 2006) and thus far less frequented by migrating waterbirds compared to most other Balkan lakes. This is important, since waterbirds, especially Anatidae have been repeatedly suggested to be vectors of both *P. acuta* (e.g., Van Leeuwen et al., 2013) and *F. fragilis* (e.g., Kappes & Haase, 2012). It is impossible to predict whether (i) any of the two species will become permanent members of the malacocoenoses of Lake Ohrid and (ii) whether they will remain restricted to few affected sites. However, based on knowledge of other invaded regions (Bousset et al., 2004), there is no reason to assume that the populations might be of temporary nature only. Further monitoring must clarify the persistence of those populations and their impact on native mollusc communities. Whereas the competitive power of *P. acuta* has been shown repeatedly (e.g., Zukowski & Walker, 2009), no such observations are published for *Ferrissia fragilis* so far.

In the case of *P. acuta*, a new family of pulmonates is now present in Lake Ohrid. This is interesting since there are other families of gastropods (Bithyniidae) which are enigmatically absent though abundant otherwise on the Balkans (Glöer et al., 2007). However, there is data accumulating supporting far more pronounced seasonal or maybe long-term fluctuations in the presence or absence of species or even higher taxa in the lake.

Invasion histories

Most major freshwater habitat types are prone to *P. acuta* and *F. fragilis* invasions (Kappes & Haase, 2012; Van Leeuwen et al., 2013). Though earlier faunistic reviews of the Balkans reported *P. acuta* only from Croatia, Bulgaria (Jaeckel et al., 1957), and Greece (Käufle, 1930), recent comprehensive lists have recognized it also for Romania (Glöer & Sîrbu, 2006), Albania (Fehér & Erőss, 2009), and Montenegro (Glöer & Pešić, 2008). Reports for Macedonia, Serbia, and Bosnia and Herzegovina are scarce and apparently restricted to dam lakes or polluted running waters. Natural Balkan lakes, which have been subject to considerable malacological research, may serve as models to demonstrate an invasion trend obvious. *P. acuta* has been reported from Lake Skutari in 2002 by Dhora (2002) whereas a survey 5 years earlier (Jovanović, 1997) did no yield records of *P. acuta*. In all Balkan countries concerned and particularly in Greece, *P. acuta* and *F. fragilis* are present in many rivers and dam lakes (Albrecht & Wilke, unpublished data). This was not the case 25–30 years ago (e.g. Frank, 1983) when only scattered records were known from continental Greece. In Lake Pamvotis, no *P. acuta* was found in 1990 (Reischütz & Sattmann, 1990). In 2002, the first record was reported and the species presence has been confirmed ever since (Frogley & Preece, 2004, 2007). Lake Vegoritis was apparently free of *P. acuta* in 1985 (Schütt, 1985), new field data at the very same localities yielded vital populations 20 years later (Table S1). The situation in lakes Trichonis and Lysimachia was lately reviewed (Albrecht et al., 2009c). The first records of *Physa acuta* from these lakes date back to August 2002 (Reischütz & Reischütz, 2003). It is clear that some recent records in the Balkan lakes (except for Lake Ohrid, see above) may reflect rather delayed recognition due to more intense research efforts and absence in some lakes might likely be due to lower sampling intensity or simply a matter of chance.

Physid gastropods are known to be phenotypically plastic and thus some species are cryptic with misidentifications being a common problem (e.g., Anderson, 2003). Though the presence of other physid species in the lakes studied cannot be excluded completely, the demonstrated conspecifity of morphologically similar *Physa* spp. with *P. acuta* (Dillon et al., 2002), and the long invasion history in the Mediterranean is a strong argument in favor of exclusive *P. acuta* invasions into the lakes of the Balkans. Earlier reports of *P. acuta* in the fossil record of the Balkans (e.g., Schütt, 1987) predate the recognition of the species as a global invader and have to be treated with great caution.

For *Ferrissia*, our data have important implications for an ongoing discussion on the origin of European populations of this genus. All recent populations genotyped turned out to represent *F. fragilis*, an invader from North America (Walther et al., 2006, 2010; Marrone et al., 2011; Raposeiro et al., 2011). The conspecifity of this species with *F. wautieri* could not be tested prior to this study. This strongly supports the hypothesis that most if not all recent European populations are deriving from rather young invasion events. However, further research is necessary, especially in circum-Mediterranean regions. As already pointed out by Marrone et al. (2011), topotypic material from another species *F. clessiniana* (Jickeli, 1882) should be genotyped to finally clarify the confusing systematic situation of European populations of *Ferrissia*. Given the fact that *F. fragilis* is a cryptic invader, it is likely that the presence of this species in some Balkan lakes remains unrecognized. The fact, however, that only a single haplotype was found so far hints to a relatively recent invasion from a single or only few sources. The situation concerning *Physa* is different since we do have a higher haplotype diversity on the Balkan scale, with more than a single haplotype in some of the lakes. Such a pattern rather indicates multiple introductions from different sources and/or older or more diverse source populations.

Conclusions

Invasive molluscs such as *P. acuta* and *F. fragilis* can thrive in disturbed habitats and under fluctuating ecological circumstances. Given the current environmental situation in almost all Balkan lakes, the invasion success of at least *P. acuta* might be directly linked to human activities. It is unpredictable whether the presence of such invasive species is of only temporary nature and what competitive impact the invaders will have on native and particularly on the endemic gastropod faunas of Balkan lakes. At least for the time being, invasive molluscs species coexist with natives and endemics, as outlined in detail for Lake Ohrid. In fact, in this lake, there is not yet evidence for a competitive displacement. The general question in the Balkan lakes, however, is whether the habitat alteration (eutrophication etc.) is rather of superior importance in impacting native species as compared to the direct influence the invaders might have on other species. It is likely that these species will further spread all over the Balkans via active and/or passive dispersal. For the time being, only concerted international action plans can eventually enhance the environmental situation in the lakes affected and thus potentially slow down the further spread of *P. acuta* and *F. fragilis*. This is of particular importance for ancient Lake Ohrid where largely intact ecosystem conditions together with a relative remoteness have delayed the arrival of invasive molluscs species. Given the ongoing environmental change in Lake Ohrid, the number of observations of non-indigenous or other widespread species will probably rise in the coming years and such species and their relationship to native species should be carefully monitored. In most other lakes, the already reported decline and loss of endemic molluscs diversity will most likely continue and coincide with increasing reports of invasive molluscs species. Ancient lakes with their outstanding biodiversity and the recurrent invasion of alien species might serve as interesting model systems for the study of important topics of invasion biology such as the diversity–invasibility hypothesis.

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9 The role of barriers and gradients in differentiation processes of pyrgulinid microgastropods of Lake Ohrid

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SPECIATION IN ANCIENT LAKES

The role of barriers and gradients in differentiation processes of pyrgulinid microgastropods of Lake Ohrid

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Abstract Ancient Lake Ohrid is characterized by vertical (bathymetrical) zones within the lake, presumably promoting allopatric speciation due to barriers or parapatric speciation along gradients. Examples within the lake include the belt of *Chara* algae as well as the shell zone, both presumably impeding migrations of benthic invertebrates. Three potential cases of vertical differentiation leading to distinct depth forms have been reported for the gastropod subfamily Pyrgulinae (Caenogastropoda: Hydrobiidae): *Ginaia munda* ssp., *Macedopyrgula* spp. and *Ochridopyrgula macedonica* ssp. Based on DNA data of the COI gene from a total of 145 specimens, this article aims at investigating the vertical differentiation within these depth forms and thus patterns of speciation in Lake Ohrid. An initial morphometric analysis showed a clear correlation of shell shape and collecting depth for *Ginaia munda* ssp. and *Macedopyrgula* spp. This morphological trend is largely reflected in the genetic structure of the respective taxa. The data presented here indicate the existence of strong gradients of abiotic and biotic factors in Lake Ohrid rather than distinct barriers. Therefore, parapatric speciation may be the predominant form of differentiation of benthic invertebrates in the lake. Incomplete lineage sorting, hybridization and phenotypic plasticity possibly caused by epigenetic mechanisms are discussed as possible reasons for the incongruence between geno- and phenotype observed in few specimens of *Ginaia munda* ssp. and *Macedopyrgula* spp. For the third taxon, *Ochridopyrgula macedonica* ssp., morphometric and genetic analyses revealed only weak support for the previously proposed depth forms. However, a horizontal differentiation of lake and spring populations was revealed instead, and parapatric and allopatric differentiations are discussed in this taxon.

Keywords Hydrobiidae · Pyrgulinae · Ancient lake · Phylogeography · Speciation

Introduction

Due to their relative isolation, ancient lakes have become prime systems for studying speciation processes (e.g. Brooks, 1950; Martens et al., 1994; Rossiter & Kawanabe, 2000). Examples include sympatric speciation within cichlids of Lake Victoria (Magalhaes & Seehausen, 2010), parapatric speciation

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of Baikalian amphipods (Takhteev, 2000), and allopatric speciation of gastropod species flocks in the central lake systems of Sulawesi (Glaubrecht & von Rintelen, 2008).

Whereas speciation in sympatry occurs in populations with overlapping geographical ranges and free migration (Coyne & Orr, 2004; Bolnick & Fitzpatrick, 2007), allopatric speciation requires spatial barriers to gene flow where populations are characterized by (i) no geographical overlap, (ii) no exchange of migrants and (iii) complete environmental barriers preventing dispersal (Bolnick & Fitzpatrick, 2007). Finally, populations subject to parapatric speciation (i) show adjoining ranges and (ii) exchange a moderate number of migrants (Bolnick & Fitzpatrick, 2007).

An ancient lake, which, at least in theory, provides opportunities for all three modes of speciation, is the European Lake Ohrid, situated on the Balkan Peninsula. This oligotrophic and karstic lake is a Graben lake of oblong shape at 693.7 m a.s.l. (Matzinger et al., 2006). Its depth averages 155 m with a maximum at 288.7 m (Matzinger et al., 2006). Lake Ohrid has a surface area of 358 km^2 and a volume of 54.9 km^3 (Matzinger et al., 2006). The lake is characterized by a high degree of endemic biodiversity, particularly in invertebrates (reviewed in Albrecht & Wilke, 2008). By applying the normalized endemic species area index (Albrecht & Wilke, 2008), which takes surface area into account, Lake Ohrid, for example, exceeds all other ancient lakes in this metric of endemic gastropod diversity (Albrecht et al., 2009).

This elevated gastropod diversity has been attributed primarily to intralacustrine speciation due to horizontal and vertical barriers or gradients in Lake Ohrid and its watershed, presumably promoting allopatric and/or parapatric differentiation (Albrecht & Wilke, 2008). Different types of horizontal structuring within Lake Ohrid and its watershed are reported (reviewed in Albrecht & Wilke, 2008), leading to faunal subdivisions, for example, of gastropod communities (Hauffe et al., 2011).

Within the lake, biotic and abiotic features may be responsible for a vertical zonation. Radoman (1985) and Albrecht & Wilke (2008) used the degree of benthic photic production, suggesting the following zones: an upper rocky or sandy part (Littoral I), the belt of Characea algae (‘*Chara* belt’; Littoral II), the shell zone (Sublittoral I), a sandy and silty part (Sublittoral II), and the Profundal (Fig. 1). They also suggested the existence of barriers for migration of invertebrates within the lake. The most often discussed barrier in Lake Ohrid is a lake-wide belt of macrophytic algae, the so-called *Chara* belt. The actual belt typically occurs between 3 and 20 m depth, but varies throughout the lake in its depth range and width (Radoman, 1985; Trajanovska, 2002). It may act as barrier for some species because it presumably impedes migration due to the dense net of these macrophytic algae (Albrecht et al., 2006; Albrecht & Wilke, 2008), because of anoxic or toxic conditions in the interstitial water at the base of the *Chara* algae (Berger & Schagerl, 2003), and because of a potentially high predation pressure (Trajanovski et al., 2010).

The lower end of the *Chara* belt merges into another potential barrier: the shell zone. It consists of deposited mollusc shells and dense colonies of live *Dreissena* individuals. These bivalve specimens are usually attached to empty shells or sparse stones (Radoman, 1985). Ranging typically from 20 to 35 m depth (Albrecht & Wilke, 2008), this zone forms a lake-wide belt running parallel to the shore line (Stanković, 1960). Its barrier effect might be caused by a potentially high predation pressure (Trajanovski et al., 2010) and a lack of suitable substrate for many invertebrates due to the high density of *Dreissena* specimens.

While both *Chara* belt and shell zone may constitute unsuitable habitats for some species, they are inhabited by other species, resulting in a system of closely related taxa living above, within and/or below these barriers (e.g. Hadžišče, 1956; Hubendick, 1960). Three potential cases for such depth forms in the microgastropod subfamily Pyrgulinae were suggested by Radoman (1983) (for the phylogenetic position of the Balkan pyrgulinids see Wilke et al., 2007).

The first example comprises the nominal gastropod subspecies *Ginaia m. munda* (Sturany, 1894) and *G. m. sublitoralis* Radoman, 1978. The former taxon is known to occur in the Littoral of Lake Ohrid and the latter in the Sublittoral, especially in the shell zone (Radoman, 1978). Morphologically, the two depth forms differ mainly in the number of keels. Whereas *G. m. munda* possesses an average of 13 keels, the keel number of *G. m. sublitoralis* averages around 20 (Radoman, 1978) (Fig. 2).

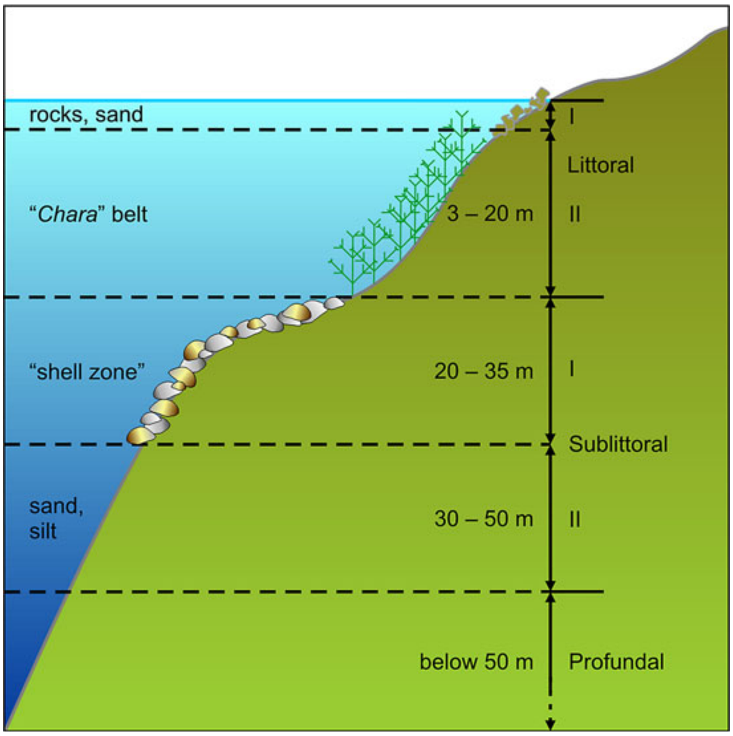


Fig. 1 Model of the vertical zonation of major habitat types in Lake Ohrid. Figure modified from Albrecht & Wilke (2008)

The second case involves the genus *Macedopyrgula* with the species *M. pavlovici* (Polinski, 1929) occurring in the Littoral down to 30 m depth, especially in sandy parts and in the *Chara* belt, and *M. wagneri* (Polinski, 1929) occurring in the Sublittoral and Profundal between 40 and 100 m depth (Polinski, 1932). The deep water form of *Macedopyrgula* possesses a more slender shell and the space between the two keels is concave in contrast to the shallow water form, which has a more plane interspace (Polinski, 1932) (Fig. 2).

The subspecies of *Ochridopyrgula macedonica* are the third example with *O. m. charensis* Radoman, 1978 mainly inhabiting the *Chara* belt in 5–20 m depth and *O. m. macedonica* (Brusina, 1896) occurring in the littoral shore zone of the lake above 5 m depth (Radoman, 1983). The latter subspecies also occurs in the feeder spring complexes of Sveti Naum and Zagorican (Radoman, 1983). Morphologically, the two forms differ in that *O. m. charensis* is more slender than *O. m. macedonica* (Radoman, 1978).

Despite the fact that all these pairs of depth forms were described based on morphological differentiations of populations inhabiting different vertical zones, the distinctness of these forms in terms of population structure remains unknown. Moreover, effects between adjacent zones, that is, distinct barriers versus gradients promoting allopatric speciation and parapatric speciation, respectively, are poorly understood.

Here, we used DNA sequence data of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) together with morphometric information of *Ginaia munda* ssp., *Macedopyrgula* spp. and *Ochridopyrgula macedonica* ssp. in order to:

1. test for an explanatory effect of depth on shell characters,
2. investigate the role of vertical barriers/gradients for genetic differentiation of populations and species, and
3. discuss potential driving forces of differentiation in these taxa.

Materials and methods

Sampling and preliminary work

We collected gastropods all around Lake Ohrid at 370 localities during eight fieldtrips between 2003

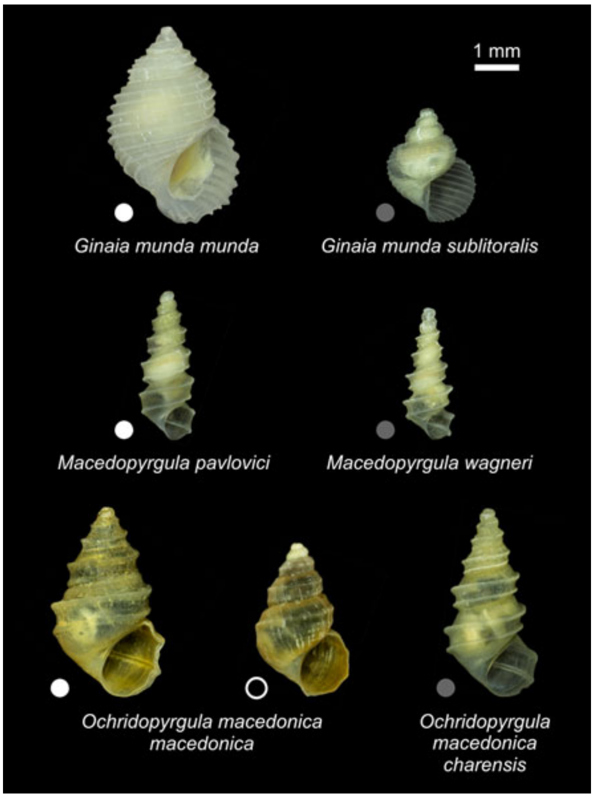


Fig. 2 Photographs of ethanol-preserved specimens of pyrgulinid taxa studied in Lake Ohrid. Note that the colour code shown applies to all figures in the present study: *white*—shallow water specimens; *dark grey*—deep water specimens; *black*—spring specimens (only *Ochridopyrgula macedonica* ssp.)

and 2010. Specimens of *Ginaia munda* ssp. ($N = 54$), *Macedopyrgula* spp. ($N = 46$), and *Ochridopyrgula macedonica* ssp. ($N = 45$) were used for further analyses from 65 localities at Lake Ohrid and its southern feeder springs of Sveti Naum (Fig. 3; for locality details, see Appendix—Supplementary material). Specimens were picked by hand from stones and hard substrates in shallow waters down to 1.5 m depth. Stones from the lake bed were lifted up during snorkeling down to a depth of 5 m and by scuba diving between 3 and 30 m depth. Greater depths of down to 65 m were sampled with a dredge from the research vessel of the Hydrobiological Institute Ohrid or from smaller boats.

Sampling depths were mainly determined either by using the echo sounder of the research vessel or a handheld echo sounder (Echotest II, Plastimo). The recorded depth ranges for transects were averaged for further analyses (for depth ranges, see Appendix—Supplementary material).

All specimens were transferred immediately into 80% ethanol and determined in the laboratory based on the morphological and ecological characters suggested by Radoman (1983). Shells were photographed for documentation and morphometrics in apertural view in a standardized way as described by Van Bocxlaer & Schultheiß (2010) prior to DNA isolation.

Morphometric pre-analysis

Though detailed morphological analyses are beyond the scope of this work, we conducted a pre-analysis in order to test a correlation of shell shape and collecting depth. As traditional morphometrics (e.g. the measurement of diameter or height) may only account for a part of the morphological variation of an individual, we here used geometric morphometrics, that is, the analysis of the position of specific landmarks or the shape of specimens.

We conducted Fourier analyses of shell outlines (see Van Bocxlaer & Schultheiß, 2010, for a justification of using this outline approach). First, digital colour photographs of adult individuals were transformed into black/white images. Utilizing the software packages tpsUtil 1.46 (Rohlf, 2010b) and tpsDig 2.16 (Rohlf, 2010a), the outline of each shell was converted into 300 equidistant points. The Cartesian coordinates of these points along the outline were standardized by using the PAST 2.04 software package (Hammer et al., 2001), resulting in equal area of the shape of specimens, then transformed into Elliptic Fourier coefficients and respective pairwise Euclidean distances were used in order to conduct an ordination. Note, that allometry, or the change of growth rates at different ontogenetic ages influences the shell shape of gastropods (Hollander et al., 2006) and life-history traits might be more important than previously expected (Urdy et al., 2010 and references therein). Since we cannot account for the ontogenetic age, we attempted to minimize the influence of shell size by showing and testing only the variance, which is not accounted for by the factors shell height and width. Therefore, we did not derive any factor of height and width, instead we performed a redundancy analysis ordination with these two factors as co-variables, constraining them on the first axes. Thus,

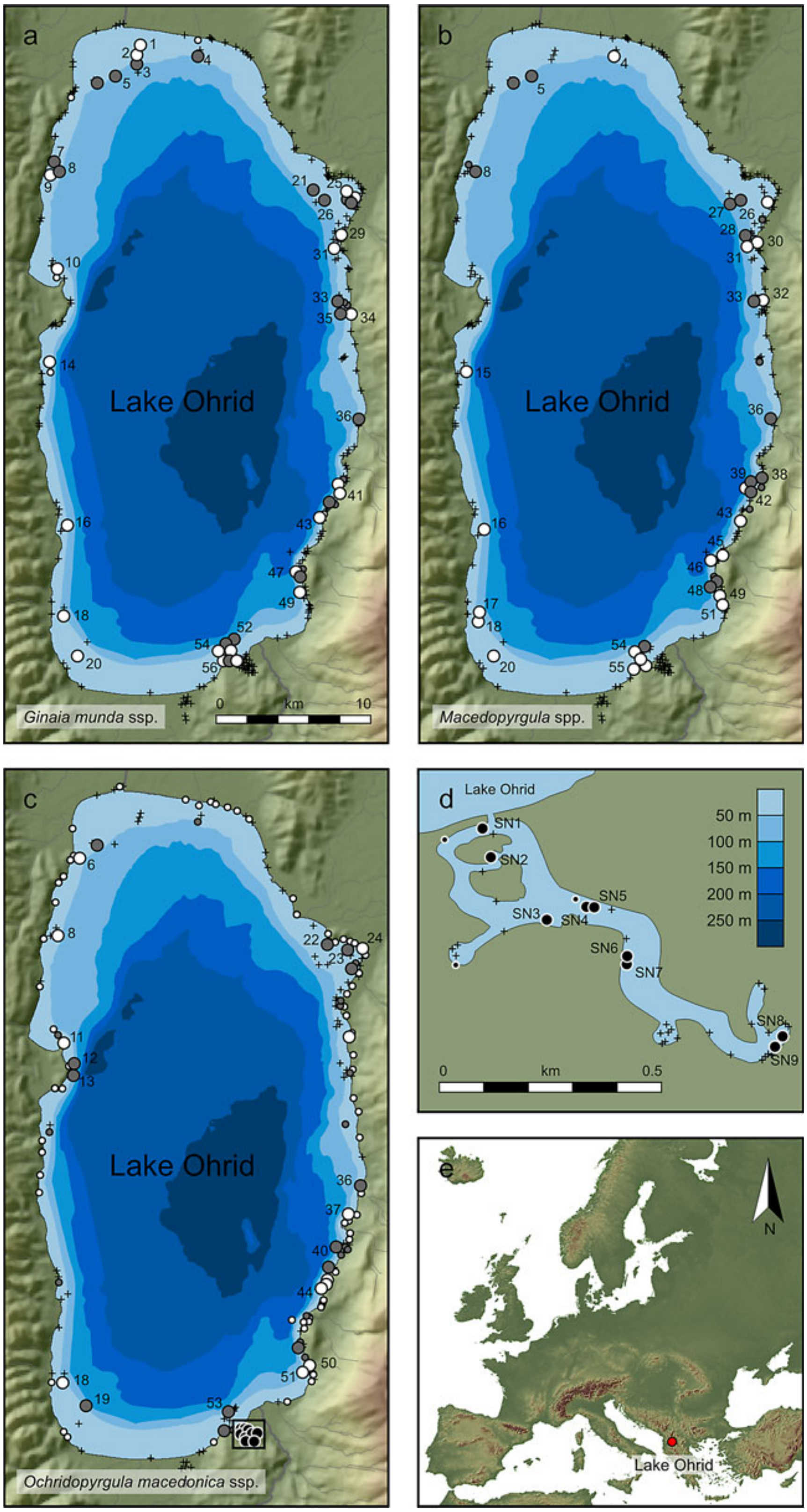


Fig. 3 Locality maps showing the sampling sites of *Ginaia munda* ssp. (**a**), of *Macedopyrgula* spp. (**b**) and of *Ochridopyrgula macedonica* ssp. around the lake (**c**) and at the feeder spring complex of Sveti Naum (**d**). Map (**e**) shows the location of Lake Ohrid on the Balkan Peninsula. *White symbols*—respective shallow water form; *dark grey symbols*—respective deep water form; *black symbols*—spring specimens (only *Ochridopyrgula macedonica* ssp.). *Big circles*—localities of morphologically analysed and/or sequenced specimens; *small circles*—localities with occurrence of the respective taxon, but unused in this study; *crosses*—localities without record of both respective depth forms. Localities of sequenced specimens are numbered following the locality table in the Supplementary material

the plotted unconstrained principal component axes show only the variance, which is not explained by shell height and width. Collecting depth was fitted to this remaining variance via thinplate spline regression and the significance of the depth effect was tested by 10,000 permutations. The latter analysis was carried out using the vegan 1.18-20 package (Oksanen et al., 2011) for the R 2.12 statistical environment (R Core Development Team, 2009).

DNA isolation, amplification and sequencing

The shells of individual specimens were cracked and the whole specimen was used for DNA isolation following the CTAB protocol of Wilke et al. (2006). A fragment of the protein-coding COI gene was amplified with the primers LCO1490 and HCO2198 as described by Folmer et al. (1994). PCR amplification was performed with an initial denaturation step at 95°C for 1 min, followed by 30 or 35 amplification cycles (denaturation at 95°C for 30 s, annealing at 52°C for 30 s and elongation at 72°C for 30 s), depending on the concentration of genomic DNA. The reaction ended with a final extension step at 72°C for 4 min. Sequences (forward and reverse) were determined using the LICOR DNA sequencer Long ReadIR 4200 (Lincoln, NE, USA) and the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

The protein-coding sequences, which are free of insertions and deletions, were aligned unambiguously using the default settings in ClustalW (version 1.4; Thompson et al., 1994) as implemented in Bioedit 7.0.9.0 (Hall, 1999). The first and last 10 base pairs (bp) were cut-off, because they were difficult to read, resulting in a 638 bp long overlapping fragment. All sequences are available from GenBank (Accession numbers JN398511–JN398653; EF379286; EF379287).

Network analyses

For inferring the genetic differentiation within respective pairs of depth forms, statistical parsimony haplotype networks were constructed using the program TCS 1.21 (Clement et al., 2000).

In order to test the relationship between the position of haplotypes in the statistical parsimony network and their collecting depth, first the position of each haplotype was assessed by Cartesian coordinates (details not shown here). Afterwards a regression analysis was carried out using the respective coordinates and associated depth information. The applied thinplate spline regression method utilizes a generalized additive model implemented in the R statistical environment, which penalizes for increasing number of knots of the thinplate splines, thus resulting in an adjusted regression coefficient. Note that due to the use of averaged sampling depths for transects (see above), the resulting isobaths should be treated with some caution.

A Mantel test (Mantel, 1967) was used as additional and independent test for the correlation between genetic distances and vertical distribution utilizing a genetic distance matrix calculated with the program MEGA ver. 4.1 (Tamura et al., 2007) under the K2P model. The test itself was carried out in the R statistical environment and the significance was tested based on 4,999 permutations.

Test for genetic structure

In order to assess the vertical genetic structure of all three taxa, we assigned the respective haplotypes to the morphologically defined shallow and deep water populations. We performed hierarchical Analyses of Molecular Variance (AMOVA) as implemented in Arlequin 3.5.1.2 (Excoffier & Lischer, 2010) on the basis of a distance matrix of pairwise differences and tested the significance based on 10,000 permutations of the original data set. Note that the same approach was applied to test the horizontal genetic structure of lake and spring haplotypes of *Ochridopyrgula macedonica* ssp., which became evident in our network analyses.

Results

Morphometric pre-analysis

As mentioned in the 'Materials and methods' section, we utilized shell height and shell width as co-variables for redundancy analyses (Fig. 4, top) to mitigate the problem of allometric growth. By fitting the collecting depth only to the variance not explained by shell height and width, respective effects on shell similarity could be reduced considerably ($r^2 < 0.001$). For *Ginaia munda* ssp. ($N = 67$) and *Macedopyrgula* spp. ($N = 65$), the remaining variance could be well explained by the collecting depth ($r^2_{\mathrm{adj}} = 0.385$, $r^2_{\mathrm{adj}} = 0.364$, respectively, each $P < 0.001$). This depth effect was lower in the case of *Ochridopyrgula macedonica* ssp. ($N = 49$; $r^2_{\mathrm{adj}} = 0.192$, $P < 0.001$).

Network analyses

The *Ginaia munda* ssp. network analysis resulted in 22 haplotypes ($N = 54$; Fig. 4a, bottom). The two depth forms of this species are well separated by at least eight mutational steps. Exceptions include one specimen of the morphological deep water form found in greater depths but possessing a 'shallow water' haplotype and three specimens of the morphological shallow water form found in shallow water but possessing 'deep water' haplotypes. Nonetheless, both the Mantel test ($r_M = 0.633$) and the thinplate spline regression ($r^2_{\mathrm{adj}} = 0.676$) showed a high correlation between genetic differences and collecting depth. Accordingly, *G. m. munda* occurs above and *G. m. sublitoralis* below the mean of the thermocline at around 25 m depth (Watzin et al., 2002).

The *Macedopyrgula* spp. network analysis yielded 26 haplotypes ($N = 46$; Fig. 4b, bottom). Specimens of the two subspecies are typically separated by at least five mutational steps. However, three specimens occurring in greater depths and possessing a deep water morphotype showed haplotypes typical of the shallow water form. The Mantel test as well as the regression analysis revealed a high correlation between genetic differences and collection depth, as suggested by values of $r_M = 0.528$ and $r^2_{\mathrm{adj}} = 0.743$. A depth differentiation is observable with specimens of *M. pavlovici* occurring above and specimens of *M. wagneri* occurring below the 25 m mean of the thermocline.

The *Ochridopyrgula macedonica* ssp. network analysis yielded 20 haplotypes ($N = 45$; Fig. 4c, bottom). Although the 45 specimens could be assigned morphologically to the two depth forms, a genetic separation between them is not apparent. Likewise, no significant correlation between genetic distances and depth distances was shown by the Mantel test ($P = 0.24$), which is also reflected by the low correlation of network position and collecting depth of 0.226. Remarkably, all 17 specimens of *O. m. macedonica* collected from the feeder spring complex of Sveti Naum share two closely related and distinct haplotypes, which were not found in lake specimens. Moreover, the genetic diversity within the lake is relatively high in contrast to the very low variation in spring specimens.

Test for genetic structure

As outlined in the 'Materials and methods' section, we performed two sets of AMOVA analyses to assess the vertical genetic structure of all three pairs of depth forms as well as the horizontal genetic structure in *Ochridopyrgula macedonica* ssp.

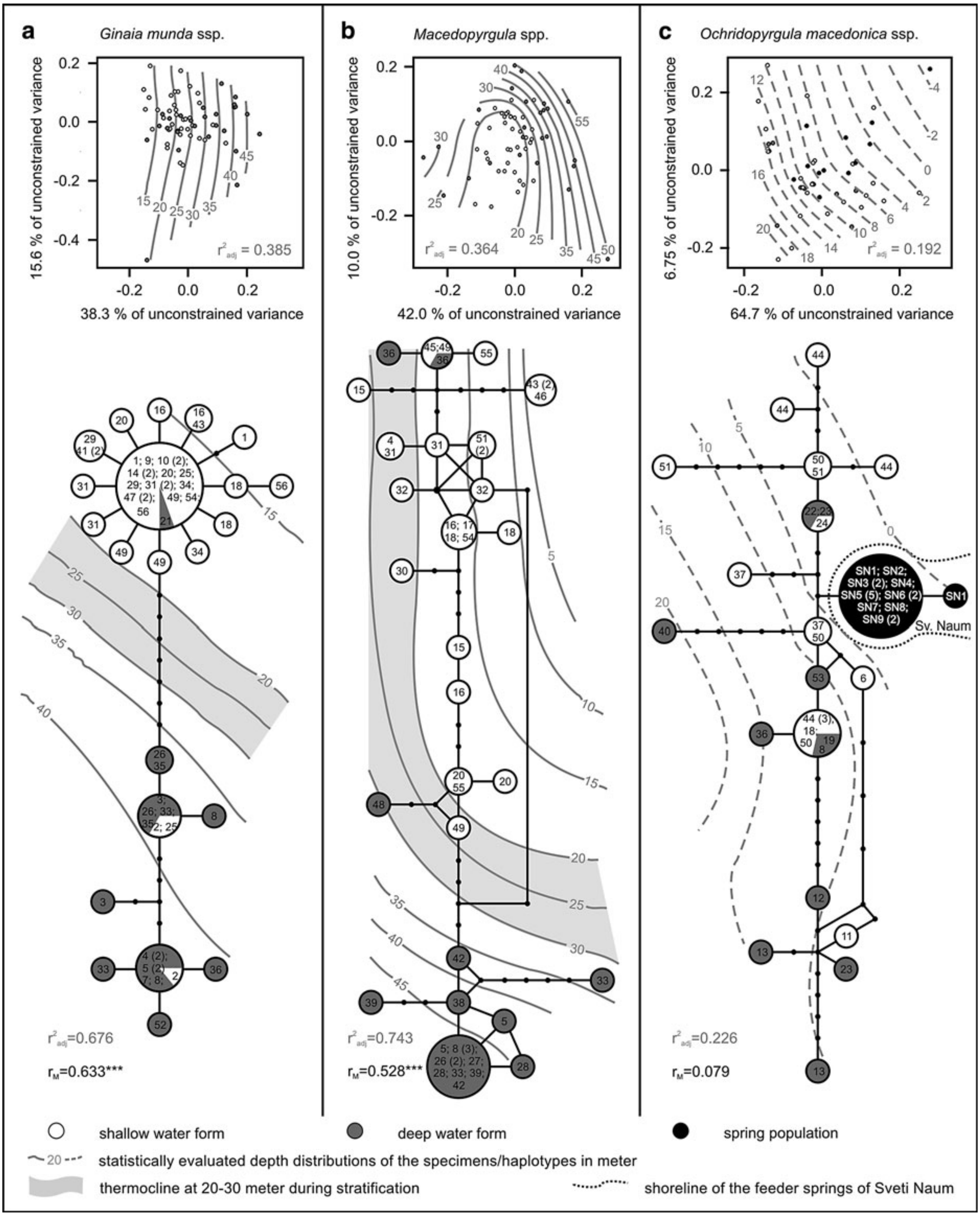
All AMOVAs revealed significant differences between the respective depth forms of the three taxa studied (Table 1). The highest degree of differentiation can be found in *Ginaia munda* ssp., followed by *Macedopyrgula* spp. and *Ochridopyrgula macedonica* ssp. However, the results of the AMOVA comparing lake and spring forms of *Ochridopyrgula macedonica* ssp. (Table 1) indicate that in this taxon, a horizontal differentiation may better explain the genetic structure observed than a vertical differentiation.

Discussion

Vertical differentiation of taxa

Vertical barriers in Lake Ohrid impeding the migration of benthic invertebrates and causing allopatric speciation have been discussed for more than 50 years (e.g. Hadžišče, 1956; Hubendick, 1960; Radoman, 1985; Albrecht & Wilke, 2008; Wysocka et al., 2008). Especially, the *Chara* belt (3–20 m) has commonly been suggested to constitute an environmental barrier against the expansion of invertebrate benthic taxa (Hubendick, 1960; Radoman, 1985).

Our analyses, both the pre-test of depth correlated morphological variation and the COI network suggest a rather minor role of the *Chara* belt for the differentiation of the pairs of species studied here. In fact, the presence of the shell zone (20–35 m) does considerably better explain the spatial separation of *Ginaia munda* ssp. and *Macedopyrgula* spp. (Fig. 5). The depth range of the shell zone, however, fits well


a
Ginaia munda ssp.
15.6 % of unconstrained variance
38.3 % of unconstrained variance
r²adj = 0.385
r²adj=0.676
rM=0.633***
b
Macedopyrgula spp.
10.0 % of unconstrained variance
42.0 % of unconstrained variance
r²adj = 0.364
r²adj=0.743
rM=0.528***
c
Ochridopyrgula macedonica ssp.
6.75 % of unconstrained variance
64.7 % of unconstrained variance
r²adj = 0.192
r²adj=0.226
rM=0.079
SN1; SN2; SN3 (2); SN4; SN5 (5); SN6 (2) SN7; SN8; SN9 (2)
SN1
Sv. Naum
shallow water form
deep water form
spring population
statistically evaluated depth distributions of the specimens/haplotypes in meter
thermocline at 20-30 meter during stratification
shoreline of the feeder springs of Sveti Naum

◀ **Fig. 4** Redundancy analysis biplots (*top*) and COI statistical parsimony haplotype networks (*bottom*) of specimens of *Ginaia munda* ssp. (**a**), *Macedopyrgula* spp. (**b**) and *Ochridopyrgula macedonica* ssp. (**c**). To show the explanatory power of depth on shell similarity, the collecting depth of the specimens was fitted to the dimension reduction of the unconstrained variance. Note that the Redundancy analysis biplots account for different shell size via the co-variables shell width and height. Specimens/haplotypes are colour-coded according to the respective taxa as in Fig. 2 and numbered according to sampling sites (see Fig. 3 or Appendix—Supplementary material for details). *Numbers in parentheses* represent the number of individuals from the same sampling locality sharing an identical haplotype. *Areas of circles* representing haplotypes found are proportional to the number of specimens sharing the respective haplotype. Missing haplotypes are indicated by *black dots*. The *grey solid and dashed lines* represent the statistically evaluated depth distributions of the haplotypes in meter based on the mean sampling depth with the respective value of r^2_{adj}. The *light grey area* represents the thermocline between 20 and 30 m during stratification of Lake Ohrid (Watzin et al., 2002). The results of the Mantel test (r_M values) showing the correlation between genetic distance and vertical distribution distance. The significance level is indicated by asterisks: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

the depth range of the thermocline at 20–30 m (Watzin et al., 2002). Therefore, it remains unclear whether the shell zone, the thermocline or a combination of both could be responsible for the morphological and genetic differentiation of *Ginaia munda* ssp. and *Macedopyrgula* spp. The influence of shell belts on benthic communities was also discussed in other ancient lakes, for example in Lake Tanganyika (McGlue et al., 2010). Moreover, differential effects of temperature on shell morphologies in shallow and deep water individuals were experimentally shown for freshwater molluscs like the quagga mussel (Peyer et al., 2010).

In addition, several other abiotic factors are known to promote vertical segregation in ancient lakes such as substrate, water movement, light penetration, food availability, hydrostatic pressure and oxygen (Martens et al., 1994; Michel, 2000; Park & Downing, 2000; for respective morphological consequences see Urdy et al., 2010). All these factors are likely intercorrelated with water depth and thus may affect the differentiation of the taxa studied as well. Note, however, that one pair of depth forms studied here, *Ochridopyrgula macedonica* ssp., appears to be unaffected by the shell zone/thermocline as almost all specimens studied occur above the respective depth ranges (Fig. 5).

An interesting finding in the cases of *Ginaia munda* ssp. and *Macedopyrgula* spp. is that shallower and deeper water populations are characterized by distinct shell morphotypes (Fig. 2). While the morphological differentiation is largely reflected by genetic structure (Fig. 4, Table 1), some haplotypes of the shallow water forms may be found in specimens occurring in deeper waters and possessing a deep water phenotype and vice versa (Fig. 4). In other words, shell morphology does reflect vertical distribution, whereas genetic structure of our single mitochondrial marker does not always.

Several processes might account for this pattern such as (a) incomplete lineage sorting, (b) hybridization and (c) epigenetic effects. Incomplete lineage sorting due to the persistence of ancestral genotypes in an extant population (Avise, 2000) could explain the occurrence of deep water haplotypes in shallow

Table 1 Results of the analyses of molecular variance (AMOVA) for the vertical structure of *Ginaia munda* ssp., *Macedopyrgula* spp., and *Ochridopyrgula macedonica* ssp. within Lake Ohrid and the horizontal structure of lake versus spring populations of *Ochridopyrgula macedonica* ssp.

Approach	Source of variation	*Ginaia munda* ssp.		*Macedopyrgula* spp.		*Ochridopyrgula macedonica* ssp.	
		Var. comp.	Variation (%)	Var. comp.	Variation (%)	Var. comp.	Variation (%)
Vertical structure (shallow vs. deep water populations)	Among groups	3.61	68.85**	1.82	46.30**	0.56	20.79**
	Within groups	1.63	31.15**	2.11	53.70**	2.14	79.21**
Horizontal structure (spring vs. lake populations)	Among groups					0.94	34.83**
	Within groups					1.76	65.17**

See text for details. *Abbreviation*: *Var. comp.* variance components

Statistically significant results are marked with asterisks: $* P < 0.05$; $** P < 0.001$

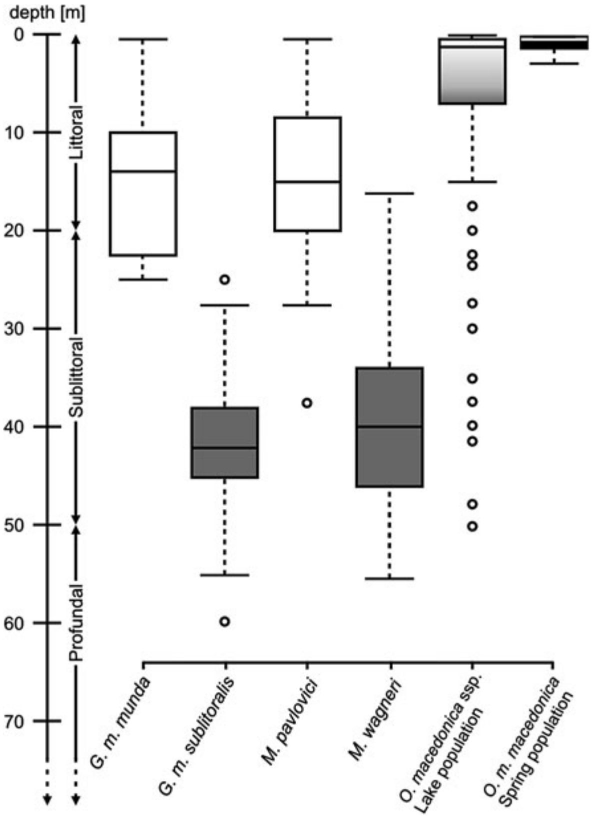


Fig. 5 Quartile Box plots with 1.5 interquartile ranges of the vertical distributions of the studied depth forms in Lake Ohrid. Depth ranges were calculated using the averaged depth information of all localities where the respective taxa were found (Fig. 3): 31 localities for *Ginaia munda munda*, 19 localities for *G. m. sublitoralis*, 22 localities for *Macedopyrgula pavlovici*, 22 localities for *M. wagneri*, 139 localities for the lake population of *Ochridopyrgula macedonica* ssp. and 13 localities for the spring population of *O. m. macedonica*

waters and vice versa. Though our sampling was not designed to estimate the exact amount of ancestral polymorphism, the studied taxa are generally well separated, especially *Ginaia munda* ssp. (Fig. 4). Therefore, incomplete lineage sorting may play only a minor role (also see Funk & Omland, 2003).

Hybridization, defined as occasional mating between otherwise distinct species and an associated transfer of parental alleles to hybrid offsprings (Funk & Omland, 2003), may also explain the patterns observed (Seehausen, 2004). Introgressive hybridization (backcrossing of hybrids into parental populations) has been discussed as a potential cause of incongruence between phenotype and genotype (e.g. Funk & Omland, 2003; also see Köhler & Deein, 2010; von Rintelen et al., 2010 for examples in gastropods). Acknowledging that only a limited number of specimens have been studied, they all could easily be assigned to the respective depth forms based on morphological characters, and intermediate phenotypes are completely missing. This lack of intermediate phenotypes indicates against major effects of hybridization. However, final evidence has to come from future studies utilizing nuclear markers (e.g. Cristescu et al., 2010; Glaubrecht, 2011).

Environmentally triggered epigenetic effects, that is, changes in gene expression without changes in the underlying DNA sequences (Van Speybroeck, 2002, also see West-Eberhard, 2005; Gilbert & Epel, 2009) could play a considerable role after the migration of deep water specimens into shallow waters and vice versa. Female specimens would maintain their mtDNA haplotypes and pass them on to their offspring. At the same time, epigenetic effects would change the phenotype of the migrants or of the subsequent generations based on a response to environmental factors (Bossdorf et al., 2008) in the respective depth zone such as water temperature, water pressure, substratum, predation or dietary modifications (e.g. Trussell & Smith, 2000; Feinberg, 2007; but also see Russo & Patti, 2005).

Overall, the processes that cause the incongruence between phenotype and COI genotype in some specimens here remain unknown and are presumably multi-factorial. However, epigenetic effects causing phenotypic plasticity are likely, though effects of hybridization and/or incomplete lineage sorting cannot be excluded.

Horizontal differentiation of *Ochridopyrgula macedonica* ssp.

Our data point towards a hitherto unknown horizontal structure of *Ochridopyrgula macedonica* ssp. separating the lake populations from the populations of the Sveti Naum spring complex (Fig. 4c). Given the low genetic distance between lake and spring haplotypes and the lack of variation within the spring population, a recent establishment of this population from lake populations seems to be likely. Horizontal differentiation between lake and spring taxa (particularly the southern feeder springs, Fig. 3d) in the Lake Ohrid region has been reported before for other gastropod taxa (e.g. Radoman, 1985; Albrecht et al., 2006, 2008) and other invertebrates (e.g. Sywula et al., 2003; Trajanovski et al., 2010). In fact, some of these taxa within the spring complex constitute distinct, endemic species. As the genetic differentiation within *Ochridopyrgula macedonica* ssp. is less distinct, the taxonomic status of the spring population remains unclear.

Conclusion

This phylogeographical study of pairs of closely related gastropod taxa revealed distinct patterns of differentiation in Lake Ohrid. Both, *Ginaia munda* ssp. and *Macedopyrgula* spp. show a vertical differentiation of shell morphotypes and COI haplotypes, probably caused by differentiation processes along gradients of abiotic factors (such as water temperature) or biotic factors (such as predation pressure in the shell zone). These suggested gradients together with the demonstrated permeability of previously proposed barriers within the lake make allopatric speciation less likely and parapatric speciation may be assumed (Gavrilets et al., 2000; Doebeli & Dieckmann, 2003).

Regarding the horizontal differentiation of lake and spring populations in *Ochridopyrgula macedonica* ssp., our data are not conclusive as to whether the observed structure reflects allopatrical or parapatrical processes. On the one hand, the feeder springs of Lake Ohrid today constitute an isolated environment, thus implying allopatric differentiation. On the other hand, the springs are close to the lake (Fig. 3d) and were repeatedly inundated in the past, as shown by fossil ostracods found outside the lake (N. Hoffmann, pers. comm., 2010), thus implying parapatric speciation.

Investigations of other taxa with potential horizontal and vertical differentiations (e.g. the gastropod genera *Carinogyraulus*, *Polinskiola*, *Ohrigocea*) are required to test the assertions made in this study.

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10 Constant diversification rates of endemic gastropods in ancient Lake Ohrid: ecosystem resilience likely buffers environmental fluctuations

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Constant diversification rates of endemic gastropods in ancient Lake Ohrid: ecosystem resilience likely buffers environmental fluctuations

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Abstract. Ancient lakes represent key ecosystems for endemic freshwater species. This high endemic biodiversity has been shown to be mainly the result of intra-lacustrine diversification. Whereas the principle role of this mode of diversification is generally acknowledged, actual diversification rates in ancient lakes remain little understood. At least four types are conceivable. Diversification rates may be constant over time, they may fluctuate, rates may be higher in the initial phase of diversification, or there may be a pronounced lag phase between colonization and subsequent diversification. As understanding the tempo of diversification in ancient lake environments may help reveal the underlying processes that drive speciation and extinction, we here use the Balkan Lake Ohrid as a model system and the largest species flock in the lake, the non-pyrgulinid Hydrobiidae, as a model taxon to study changes in diversification rates over time together with the respective drivers.

Based on phylogenetic, molecular-clock, lineage-through-time plot, and diversification-rate analyses we found that this potentially monophyletic group is comparatively old and that it most likely evolved with a constant diversification rate. Preliminary data of the SCOPSCO (Scientific Collaboration On Past Speciation Conditions in Lake Ohrid) deep-drilling program do indicate signatures of severe environmental/climatic perturbations in Lake Ohrid. However, so far there is no evidence for the occurrence of catastrophic environmental events. We therefore propose that the constant diversification rate observed in endemic gastropods has been caused by two factors: (i) a potential lack of catastrophic environmental events in Lake Ohrid and/or (ii) a probably high ecosystem resilience, buffering environmental changes. Parameters potentially contributing to the lake's high ecosystem resilience are its distinct bathymetry, ongoing tectonic activities, and karst hydrology.

The current study not only contributes to one of the overall goals of the SCOPSCO deep-drilling program – inferring the driving forces for biotic evolution in Lake Ohrid. It might also enhance our understanding of how ecosystem resilience, in general, may promote relatively constant diversification rates in isolated ecosystems. However, we encourage future studies testing hypotheses about the lack of catastrophic events in Lake Ohrid. These studies should be based on high-resolution data for the entire geological history of the lake, and they should potentially involve information from the sediment fossil record, not only for gastropods but also for other groups with a high share of endemic taxa.

1 Introduction

Ancient lakes represent key ecosystems for the world's endemic freshwater biodiversity (Brooks, 1950; Martens et al., 1994; Martens, 1997; Rossiter and Kawanabe, 2000). Two hypotheses have been suggested for the underlying processes generating their often high levels of species richness. Originally, ancient lakes were considered to be evolutionary refugia that accumulate immigrating elements from extralimital areas during periods of environmental changes ("reservoir function"). Accordingly, distantly related "relic" species may have colonized a lake at different times and possibly from different geographic areas (e.g., Hauswald et al., 2008; Wilson et al., 2004). However, with the advance of

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molecular techniques, several researchers noted that many endemic species are considerably younger than the lake they inhabit. Hence, they suggested that the high endemic biodiversity in ancient lakes is predominantly a result of intralacustrine diversification ("cradle function") (e.g., Martens, 1997; Salzburger et al., 2005; Sherbakov, 1999).

Though the principle role of the cradle function is hardly disputed today, rates of diversification in ancient lakes remain little understood (e.g., Cristescu et al., 2010; Martens et al., 1994). As ancient lakes are considered to be comparatively stable systems (Martens, 1997), originally diversification rates (i.e., speciation minus extinction rates) were assumed to be constant over time. However, in the past decades, several factors, typically related to environmental change, have been proposed to alter the tempo of diversification in species flocks. The most renowned theory, punctuated equilibrium, suggests little net evolutionary change during periods of environmental stability (Gould and Eldredge, 1977; but see, e.g., Pennell et al., 2014; Van Bocxlaer et al., 2008). This equilibrium might be "punctuated" during phases of rapid environmental change. Another theory suggests that diversification rates can be higher in the initial phase of diversification (particularly in groups that diversify through an adaptive radiation) and may decline once niche spaces becomes successively occupied (e.g., Purvis et al., 2009; Schluter, 2000). This may happen after a lake first came into existence or after the occurrence of major environmental events such as volcanic ash deposits, severe lake-level drops, and desiccation or salinization events (Cristescu et al., 2010; Kroll et al., 2012; Salzburger et al., 2014). A forth theory proposes the opposite, i.e., the existence of a pronounced lag phase between colonization and onset of diversification (e.g., Cristescu et al., 2010).

However, these scenarios have rarely been tested in ancient lake environments due to the lack of appropriate candidate lakes and suitable model taxa. Criteria for a candidate lake would be a long and continuous existence, providing sufficient time for repetitive cladogenesis, as well as a good knowledge of its paleo-limnological history, enabling a link between geological and biotic evolution. The model taxon, in turn, should be monophyletic, permitting unbiased calculations of diversification rates; species rich, thus providing sufficient power for evolutionary analyses; and reasonably old, allowing for studying the effect of environmental changes on speciation rates over an extended period of time.

Of the few ancient lakes in the world, even fewer fulfil the above criteria. Some lakes, despite being old, have gone through a series of major environmental events, and the respective endemic species are often comparable young, as observed in Lake Malawi (e.g., Schultheiß et al., 2009, 2011) and Lake Titicaca (Kroll et al., 2012). Other lakes such as Lake Baikal (e.g., Ivanov et al., 2013) and Lake Tanganyika (e.g., Salzburger et al., 2014; Scholz et al., 2007) might be sufficiently old but lack a continuous paleo-limnological record. In fact, one of the very few ancient lakes enabling a link between geological and biotic evolution throughout its existence is the Balkan Lake Ohrid (Wagner et al., 2014). It is the oldest freshwater lake in Europe and perhaps the most speciose in the world when considering lake size (Albrecht and Wilke, 2008). Though the exact age of the lake remains controversially discussed, biological data suggest an age of no older than 2–3 million years (My) (e.g., Albrecht et al., 2006; Trajanovski et al., 2010; Wysocka et al., 2013). Newer sedimentological and seismological data obtained during the recently conducted SCOPSCO (Scientific Collaboration On Past Speciation Conditions in Lake Ohrid) deep-drilling project in Lake Ohrid revealed a minimum lake age (deep-water conditions) of ca. 1.2 My (Wagner et al., 2014), and an age of its oldest sediments of approximately 2.0 My (Lindhorst et al., 2015). This time frame of 1.2–2.0 My for the origin of extant Lake Ohrid is also considered in the current study.

Besides its relatively well characterized limnological history, Lake Ohrid also harbors a high number of endemic animal species. So far, at least 185 taxa have been described (Albrecht and Wilke, 2008; Pešić, 2015; Stocchino et al., 2013; Wysocka et al., 2013). In addition, there is a rich protist flora. Diatoms alone account for 789 taxa, with 117 of them being endemic to the lake (Levkov and Williams, 2012). Accordingly, a number of more than 300 endemic eukaryotic species for ancient Lake Ohrid is conceivable. The majority of the animal groups form relatively old species flocks in several higher taxa, including crustaceans (Wysocka et al., 2008, 2013, 2014), leeches (Trajanovski et al., 2010), and gastropods (Albrecht et al., 2006; Wilke et al., 2007, 2009). In fact, gastropods represent the most speciose animal group in Lake Ohrid, with 74 species described, 56 of which are endemic to the lake and its catchment (Albrecht and Wilke, 2008; Albrecht et al., 2009, 2014; Hauffe et al., 2011; Radoman, 1985). The largest share of this diversity is held by snails of the family Hydrobiidae (Caenogastropoda: Truncatelloidea), including 13 pyrgulinid and 27 other endemic species (Radoman, 1983). The latter group comprises the nominal genera *Dolapia*, *Gocea*, *Lyhnidia*, *Ohrigocea*, *Ohridohauffenia*, *Ohridohoratia*, *Polinskiola*, *Pseudohoratia*, *Strugia*, and *Zaumia* (see Fig. 1). Pending a formal classification of this potentially monophyletic taxon, it is henceforth called the "non-pyrgulinid Hydrobiidae". Given that this group probably represents the largest species flock in Lake Ohrid (see also Radoman, 1983), it appears to be a suitable candidate taxon to study speciation processes in this ancient lake.

Therefore, the major goal of the present study is to test for changes in diversification rates over time and to assess the underlying drivers. In order to achieve this objective, three specific goals are addressed.

i. Based on molecular-clock analyses, we estimate the age of the most recent common ancestor (MRCA) of this group as a baseline for our temporal studies.

Figure 1. Selected representatives of genera belonging to the endemic non-pyrgulinid Hydrobiidae from Lake Ohrid. Scale bar: 1 mm.

ii. Utilizing lineage-through-time (LTT) plot and diversification-rate analyses, hereinafter we assess changes in diversification rates over time.

iii. If deviations from a constant diversification model are inferred, we finally attempt to link environmental and climatic fluctuations derived from the SCOPSCO program to these biotic changes.

Given that Lake Ohrid has long been considered to be a relatively stable system with considerable ecosystem resilience (sensu Stankovic, 1960), our working hypothesis is that there are no significant changes in diversification rates over time in the lake's non-pyrgulinid Hydrobiidae.

The current study will complement paleontological evidence of evolutionary processes obtained from the SCOPSCO high-resolution sediment record and thus contribute to one of the overall goals of the deep-drilling program – inferring the driving forces for biotic evolution in this fascinating ancient lake. It might also enhance our general understanding of how environmental change alters the tempo of diversification in isolated ecosystems and how ecosystem stability may buffer such changes.

2 Material and methods

2.1 Sampling

Hydrobiid gastropods were collected during field trips to Lake Ohrid and other waterbodies in the Balkan region between 2003 and 2011 (Fig. 2; see Table 1 for details). The collection methods followed those described in Schreiber et al. (2012) and included hand collecting, snorkeling, sieving, and dredging from small boats or the research vessel of the Hydrobiological Institute Ohrid. Samples were preserved in 80 % ethanol and determined in the laboratory to species level based on Radoman (1983).

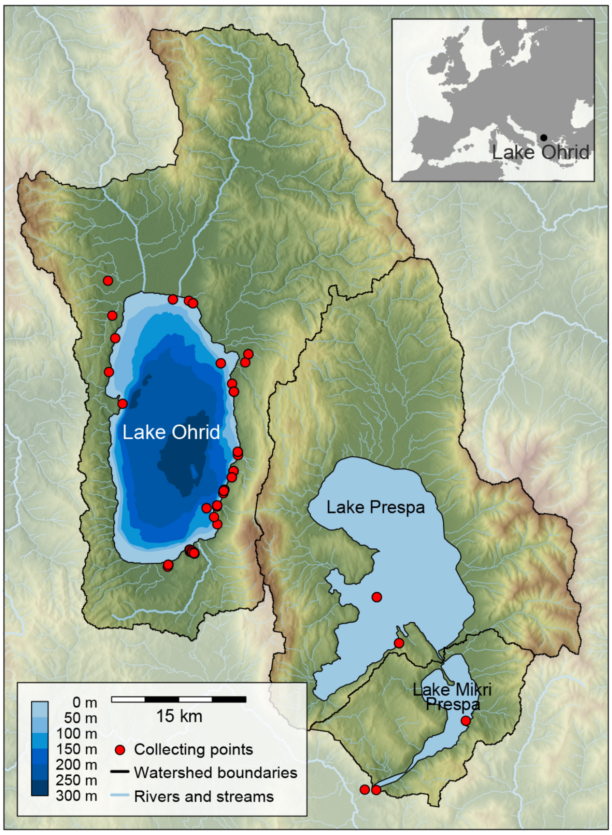

Figure 2. Sampling sites for non-pyrgulinid Hydrobiidae in lakes Ohrid, Prespa, and Mikri Prespa, and their watersheds.

2.2 DNA isolation, PCR amplification, and DNA sequencing

Genomic DNA was isolated from whole specimens using the CTAB protocol described in Wilke et al. (2006). Voucher specimens and digital images were deposited in the University of Giessen Systematics and Biodiversity collection (UGSB). Fragments of the mitochondrial genes for cytochrome oxidase c subunit I (COI) and large subunit rRNA (LSU rRNA or 16S rRNA) were amplified using the universal primers LCO1490 (Folmer et al., 1994) and COR722b (Wilke and Davis, 2000) as well as 16Sar-L and 16Sbr-H (Palumbi et al., 1991), respectively (for PCR conditions see Schreiber et al., 2012). Subsequent Sanger sequencing was conducted either on a Long Read IR2 4200 sequencer (LI-COR, Lincoln, NE, USA) using the Thermo Sequenase fluorescent labelled primer cycle sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) or on a ABI 3730 XL sequencer (Life Technologies, Carlsbad, CA, USA) using the Big Dye Terminator Kit (Life Technologies, Carls-

Table 1. Taxa studied including locality information, collection and DNA voucher details, and NCBI GenBank accession numbers.

Genus	Species	Locality	Latitude	Longitude	UGSB voucher no.	DNA voucher no.	GenBank accession no. COI	 16S rRNA	Reference
Albaniana	*albanica*	Albania, Ventroku, cave spring	40.67295	20.97403	UGSB 10730	12073	KU170805	KU170882	This study
Belgrandia	*mariatheresia*	Italy, Fonte di S. Cassiano	–	–	UGSB 17130	2332	KU170806	–	This study
Daphniola	*exigua*	Greece, large spring at Agia Paraskevi, Tembi Valley, N of Larisa	–	–	–	–	JF916470	–	Falniowski and Szarowska (2011)
	graeca	Greece, Dafne Spring	39.891083	22.607222	UGSB 17132	4238	KU170807	–	This study
	louisi	Greece, Athens, spring at Kessariani	37.960729	23.798555	UGSB 17133	4239	KU170808	–	This study
Gocea	*ohridana* 1	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10710	10493	KU170809	–	This study
	ohridana 2	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10709	10332	KU170810	KU170883	This study
	ohridana 3	Macedonia, Lake Ohrid, Veli Dab	40.97097	20.78604	UGSB 10681	4299	KU170811	–	This study
Grossuana	*codreanui*	Bulgaria, Jasenovo	–	–	–	–	EF061920	–	Szarowska et al. (2007)
	delphica	Greece, Delphi, Kastalia Spring	38.483056	22.505278	–	–	EF061922	–	Szarowska et al. (2007)
	serbica	Serbia, Raska River Spring at Sopocani Monastery	43.115833	20.370833	–	–	EF061921	–	Szarowska et al. (2007)
	sp.	Greece, E of Volos, Oros Pilion, spring E of Anilion	–	–	–	–	KC011768	–	Falniowski et al. (2012)
	vurliana	Greece, spring of Louros River	–	–	–	–	EF061923	–	Szarowska et al. (2007)
Islamia	*hadei*	Greece, Peloponnese, N Taigetos Mts., W of Sparta, spring at Dhiaselo	–	–	–	–	JF916473	–	Falniowski and Szarowska (2011)
Lyhnidia	*gjorgjevici* 1	Macedonia, feeder springs of Sveti Naum	40.91208	20.74213	UGSB 10746	12499	KU170812	–	This study
	gjorgjevici 2	Macedonia, feeder springs of Sveti Naum	40.91208	20.74213	UGSB 10747	12500	KU170813	–	This study
	gjorgjevici 3	Macedonia, Sveti Naum, small lake with springs	40.91029	20.74791	UGSB 10695	10286	KU170814	KU170884	This study
	gjorgjevici 4	Macedonia, Sveti Naum, small lake with springs	40.91029	20.74791	UGSB 10696	10287	KU170815	KU170885	This study
	stankovici	Macedonia, Lake Ohrid, Trpejca	40.95583	20.76396	UGSB 10697	10291	KU170816	KU170886	This study
Malaprespia	*albanica*	Albania, Lake Mikri Prespa, spring in the lake	40.67258	20.9892	UGSB 10731	12075	KU170817	KU170887	This study
Ohridohauffenia	*depressa* 1	Macedonia, Lake Ohrid, in front of Hotel Desaret, village Pestani	41.00927	20.80544	UGSB 10716	10507	KU170818	KU170888	This study
	depressa 2	Macedonia, Lake Ohrid, beach at Gorica Hill	41.08105	20.7974	UGSB 10711	10495	KU170819	KU170889	This study
	minuta 1	Macedonia, spring Studenicišta	41.10251	20.81491	UGSB 10712	10497	KU170820	KU170890	This study
	minuta 2	Macedonia, spring Bej Bunar	41.11085	20.81905	UGSB 10714	10501	KU170821	KU170891	This study
	minuta 3	Macedonia, spring Bej Bunar	41.11085	20.81905	UGSB 10715	10502	KU170822	KU170892	This study
	rotonda	Macedonia, Lake Ohrid, at camping site “Livadište”	41.12643	20.64184	UGSB 10713	10500	KU170823	KU170893	This study
	sanctinaumi 1	Macedonia, feeder springs of Sveti Naum	40.91414	20.74147	UGSB 10738	12358	KU170824	–	This study
	sanctinaumi 2	Macedonia, feeder springs of Sveti Naum	40.91061	20.74748	UGSB 10717	10510	KU170825	KU170894	This study
Ohridohoratia	*carinata* 1	Macedonia, Lake Ohrid, Veli Dab	40.97439	20.78746	UGSB 10698	10299	KU170826	KU170895	This study
	carinata 2	Macedonia, Lake Ohrid, Trpejca	40.95868	20.77802	UGSB 10699	10305	KU170827	KU170896	This study
	pygmaea 1	Albania, feeder springs of Tusemisht	40.89874	20.71325	UGSB 10749	12508	KU170828	–	This study
	pygmaea 2	Albania, feeder springs of Tusemisht	40.89686	20.7127	UGSB 10748	12507	KU170829	–	This study
	pygmaea 3	Macedonia, feeder springs of Sveti Naum	40.91019	20.74793	UGSB 10750	12510	KU170830	–	This study
	pygmaea 4	Macedonia, Sveti Naum, spring lake at island with restaurant	40.91393	20.74226	UGSB 10739	12359	KU170831	–	This study
	pygmaea 5	Macedonia, Sveti Naum, spring lake at island with restaurant	40.91393	20.74226	UGSB 10740	12360	KU170832	–	This study
	pygmaea 6	Macedonia, Sveti Naum, springs at pumping station, near chapel	40.9113	20.74744	UGSB 10741	12362	KU170833	–	This study
	pygmaea 7	Albania, feeder springs of Tushemisht	40.89885	20.7136	UGSB 10755	12863	KU170834	–	This study
	pygmaea 8	Albania, Lake Ohrid, south of Lin peninsula	41.06055	20.65186	UGSB 10756	12865	KU170835	–	This study
	pygmaea 9	Macedonia, Lake Ohrid, in bay south of Gorica Hill	41.07304	20.79991	UGSB 10758	12867	KU170836	–	This study
	pygmaea 10	Macedonia, Lake Ohrid, in bay south of Gorica Hill	41.07304	20.79991	UGSB 10757	12866	KU170837	–	This study
	pygmaea 11	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16183	20.7451	UGSB 10742	12439	KU170838	KU170897	This study
	pygmaea 12	Macedonia, Lake Ohrid, Veli Dab	40.97439	20.78747	UGSB 10759	12868	KU170839	–	This study
	pygmaea 13	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16235	20.74395	UGSB 10718	10689	KU170840	KU170898	This study
	pygmaea 14	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16183	20.7451	UGSB 10743	12440	KU170841	KU170899	This study
	pygmaea 15	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16183	20.7451	UGSB 10744	12442	KU170842	KU170900	This study
	pygmaea 16	Macedonia, Lake Ohrid, northwestern bay, near Podmolje	41.16429	20.7396	UGSB 10745	12444	KU170843	KU170901	This study
Ohrigocea	*samuili* 1	Macedonia, feeder springs of Sveti Naum	40.91287	20.74483	UGSB 10752	12514	KU170844	–	This study
	samuili 2	Macedonia, Lake Ohrid, south of Pestani	41.09239	20.63361	UGSB 10687	10153	KU170845	KU170902	This study
	samuili 3	Macedonia, Lake Ohrid, Veli Dab	40.97439	20.78747	UGSB 10688	10154	KU170846	KU170903	This study
	samuili 4	Macedonia, Lake Ohrid, bay south of Gradishte	40.99323	20.79958	UGSB 10689	10156	KU170847	KU170904	This study

Table 1. Continued.

Genus	Species	Locality	Latitude	Longitude	UGSB voucher no.	DNA voucher no.	GenBank accession no.		Reference
							COI	LSU rRNA	
	samuili 5	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10692	10170	KU170848	KU170905	This study
	stankovici 1	Macedonia, feeder springs of Sveti Naum	40.91029	20.74791	UGSB 10751	12512	KU170849	–	This study
	stankovici 2	Albania, feeder springs of Tushemisht	40.89874	20.71325	UGSB 10753	12517	KU170850	–	This study
	stankovici 3	Macedonia, feeder springs of Sveti Naum	40.91298	20.74455	UGSB 10754	12518	KU170851	–	This study
	stankovici 4	Macedonia, spring at Sveti Naum	40.91135	20.745	UGSB 10690	10164	KU170852	KU170906	This study
	stankovici 5	Macedonia, Lake Ohrid, Sveti Stefan	41.07306	20.79985	UGSB 10691	10166	KU170853	KU170907	This study
	stankovici 6	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10694	10173	KU170854	KU170908	This study
	stankovici 7	Macedonia, Lake Ohrid, lake bank at Veli Dab	40.97324	20.78668	UGSB 10693	10172	KU170855	KU170909	This study
Polinskiola	*polinskii* 1	Macedonia, feeder springs of Sveti Naum	40.91061	20.74748	UGSB 10725	10730	KU170856	KU170910	This study
	polinskii 2	Macedonia, feeder springs of Sveti Naum	40.91061	20.74748	UGSB 10724	10729	KU170857	KU170911	This study
	polinskii 3	Macedonia, Lake Ohrid, Trpejca	40.95868	20.77802	UGSB 10726	10734	KU170858	KU170912	This study
	polinskii 4	Macedonia, Lake Ohrid, southeastern part of the lake	40.9396	20.7783	UGSB 10729	10739	KU170859	KU170913	This study
	polinskii 5	Macedonia, Lake Ohrid, Trpejca	40.95752	20.77771	UGSB 10727	10735	KU170860	KU170914	This study
	sturanyi 1	Macedonia, Lake Ohrid, bay south of Gradishte	40.99323	20.79958	UGSB 10722	10727	KU170861	KU170915	This study
	sturanyi 2	Macedonia, Lake Ohrid, in bay south of Gorica Hill	41.07304	20.79991	UGSB 10728	10737	KU170862	KU170916	This study
	sturanyi 3	Macedonia, Lake Ohrid, bay south of Gradishte	40.99323	20.79958	UGSB 10723	10728	KU170863	KU170917	This study
Prespolitorea	*malaprespensis*	Greece, Lake Mikri Prespa, at the former Hydrobiological Institute Mikrolimni	40.74215	21.10763	UGSB 10737	12089	KU170864	KU170918	This study
	valvataeformis 1	Macedonia, Lake Prespa, southern shore of Golem Grad	40.86655	20.98989	UGSB 10736	12088	KU170865	KU170919	This study
	valvataeformis 2	Macedonia, Lake Prespa, southern shore of Golem Grad	40.86655	20.98989	UGSB 10735	12087	KU170866	KU170920	This study
	valvataeformis 3	Macedonia, Lake Prespa, southern shore of Golem Grad	40.86655	20.98989	UGSB 10734	12086	KU170867	KU170921	This study
	valvataeformis 4	Greece, Lake Prespa, bay on cliff-like coast	40.82032	21.01939	UGSB 10733	12085	KU170868	KU170922	This study
	valvataeformis 5	Greece, Lake Prespa, bay on cliff-like coast	40.82032	21.01939	UGSB 10732	12084	KU170869	KU170923	This study
	valvataeformis 6	Greece, Lake Prespa, bay on cliff-like coast	40.82032	21.01939	UGSB 10686	10118	KU170870	KU170924	This study
Pseudohoratia	*brusinae*	Macedonia, Lake Ohrid, in front of Ohrid Bay	41.10156	20.78236	UGSB 10700	10313	KU170871	KU170925	This study
	lacustris 1	Macedonia, Lake Ohrid, 2–5 m depth at northern part of the lake	41.16569	20.71836	UGSB 10702	10316	KU170872	KU170926	This study
	lacustris 2	Macedonia, Lake Ohrid, 2–5 m depth at northern part of the lake	41.16569	20.71836	UGSB 10703	10319	KU170873	KU170927	This study
	ohridana 1	Macedonia, Lake Ohrid, south of Gradishte	40.98687	20.79729	UGSB 10707	10330	KU170874	KU170928	This study
	ohridana 2	Macedonia, Lake Ohrid, in front of Ohrid Bay	41.10156	20.78236	UGSB 10701	10314	KU170875	KU170929	This study
	ohridana 3	Macedonia, Lake Ohrid, in front of Hotel Desaret, village Pestani	41.01262	20.80513	UGSB 10705	10327	KU170876	KU170930	This study
	ohridana 4	Macedonia, Lake Ohrid, southeastern part of the lake	40.94696	20.77385	UGSB 10706	10328	KU170877	KU170931	This study
	ohridana 5	Macedonia, Lake Ohrid, south of Gradishte	40.98687	20.79729	UGSB 10708	10331	KU170878	KU170932	This study
	ohridana 6	Macedonia, Lake Ohrid, in front of Hotel Desaret, village Pestani	41.01262	20.80513	UGSB 10704	10326	KU170879	KU170933	This study
Strugia	*ohridana* 1	Macedonia, small spring near Sum Spring	41.18417	20.63181	UGSB 10761	12923	KU170880	–	This study
	ohridana 2	Macedonia, spring near road to Kalishta	41.14912	20.63773	UGSB 10760	12922	KU170881	–	This study
Trichonia	*kephalovrissonia*	Greece, spring at Thermo	–	–	–	–	EF070619	–	Szarowska (2006)

bad, CA, USA). In total, we sequenced 65 specimens of 17 nominal species of the endemic non-pyrgulinid Hydrobiidae. For comparison, we also analyzed 20 specimens of 15 closely related species occurring in lakes Prespa and Mikri Prespa as well as in surrounding waterbodies (Fig. 2; see Table 1 for locality details, UGSB collection numbers and GenBank accession numbers).

2.3 Preliminary genetic analyses

The protein-coding COI sequences were unambiguously aligned in BioEdit 7.0.9.0 (Hall, 1999), resulting in a final alignment of 638 base pairs (bp). The 16S rRNA sequences were aligned using the secondary structure model for the Hydrobiidae suggested by Wilke et al. (2013), resulting in a final alignment of 462 bp including gaps (an internal fragment of 45 bp was removed since no reliable alignment could be achieved for this region).

Prior to the phylogenetic analyses, our data set of non-pyrgulinid Hydrobiidae was supplemented with sequences of closely related species. We first performed searches with BLASTN 2.2.32 (Zhang et al., 2000) against the National Center for Biotechnology Information (NCBI) nucleotide database as well as against the nucleotide database of the Wilke lab, which contains DNA information for more than 300 hydrobiid species. A preliminary phylogenetic analysis was conducted in order to identify the hydrobiid groups that are most closely related to our endemic non-pyrgulinid Hydrobiidae. These preliminary analyses (details not shown) indicated that endemic species from the sister lakes Prespa and Mikri Prespa (Fig. 2) as well as from other Balkan waterbodies are the closest relatives to the Ohrid taxa. These taxa were included in our final data set (see Table 1).

2.4 Phylogenetic inference and molecular-clock analyses

The main phylogenetic analyses using Bayesian inference were performed in BEAST v. 1.8.0 (Drummond and Rambaut, 2007). Best-fit substitution models were estimated using jModelTest 0.1.1 (Posada, 2008) based on the Akaike information criterion. The models suggested for the COI and 16S rRNA fragments were GTR+I+Γ and GTR+I, respectively. We tested the COI data set for substitutional saturation using the test by Xia and Xie (2001) as implemented in DAMBE 5.0.23 (Xia and Xie, 2001). The value for the proportion of invariant sites ($\text{Pinv} = 0.46$) was obtained from the jModelTest output. The observed saturation was significantly lower than the critical values ($p < 0.001$), suggesting that this partition can be used for further (molecular-clock) analyses. Note that we did not test for saturation in the 16S data set as, within the family Hydrobiidae, the 16S gene is more conservative than the COI gene (Wilke et al., 2001, 2013).

We first ran two unconstrained analyses in BEAST, one under the strict-clock and one under the relaxed-clock model, using relative rates for both partitions. These initial runs, however, revealed an extremely low effectivity sample size for the prior and posterior distributions, suggesting that the runs might have been over-parameterized due to the complex GTR+I+Γ model (see, e.g., Grummer et al., 2014; Slager et al., 2014). Hence, the less complex HKY+I+Γ and HKY+I models were used for the final analyses for the COI and 16S rRNA partitions, respectively.

For calibrating the molecular clock, an external trait-specific clock rate of $1.57 \pm 0.45\,\%\,\text{My}^{-1}$ for the HKY+I+Γ model was utilized for the COI portion of our data set (Wilke et al., 2009). This rate was established for small, dioecious, subtropical or tropical Protostomia with a generation time of approximately 1 year – all of these criteria apply to our non-pyrgulinid Hydrobiidae.

The final analyses (two strict-clock and two relaxed-clock runs) with a total of 85 sequences were run for 100 000 000 generations each, sampling every 5000 generations. The resulting log and tree files for each strict-clock and relaxed-clock run were combined using LogCombiner v. 1.8.0 (BEAST) with a 50 % burn-in. The maximum clade credibility (MCC) tree was identified based on the posterior distribution (20 000 trees). Information from the post-burn-in posterior distribution including mean node ages and 95 % highest posterior densities (HPDs) was summarized using TreeAnnotator v. 1.8.0 (BEAST; no additional burn-in). A Bayes factor (BF) analysis of the likelihoods of both runs (strict-clock vs. relaxed-clock model) was performed in Tracer 1.5 (Rambaut and Drummond, 2007; 1000 bootstrap replicates) in order to determine the best-fitting clock model (see Newton and Raftery, 1994; Suchard et al., 2001).

The BF analysis, which compares the likelihoods of both BEAST runs (strict- vs. relaxed-clock model), showed a decisive support for the relaxed-clock model with a BF of 45.61 ($\ln P_{\text{relaxed}} = -5213.40$ vs. $\ln P_{\text{strict}} = -5318.42$).

2.5 Lineage-through-time plot and diversification-rate analyses

In a first explorative analysis, LTT plot analyses were conducted using the packages ape v. 3.3 (Paradis et al., 2004) and phytools v. 0.4-56 (Revell, 2012) for the R statistical environment 3.2.1 (R Core Team, 2015) in order to examine whether major deviations from a constant diversification rate can be directly observed over time. In this way, all but the endemic non-pyrgulinid Hydrobiidae from Lake Ohrid were pruned. In a second step, a LTT plot was generated for the MCC tree plus a 95 % confidence interval based on the posterior distribution.

However, generating LTT plots and detecting changes in the slope is an explorative approach and might lead to misinterpretations (see Stadler, 2011). Therefore, potential shifts in diversification rates over time were analyzed using the R package TreePar v. 3.3 (Stadler, 2015) by testing a maximum of three shifts for 10 trees randomly sampled from the posterior distribution. This package implements a birth–death-shift model (Stadler, 2011), which allows changes in speciation and extinction rates along a phylogeny for a given time frame and for pre-defined time intervals. Shifts were analyzed along the pruned tree with default settings and a time interval of 0.1 My. The sampling fraction was set to $17/27 = 0.63$ according to the actual number of species included divided by the number of nominal species described. Results (log likelihoods of different runs, i.e., constant diversification rate vs. 1 shift, 1 shift vs. 2 shifts, and 2 shifts vs. 3 shifts) were compared by applying likelihood ratio tests in order to examine whether shifts in rates explain the tree significantly better than a constant diversification rate (indicated by p values > 0.95; see Stadler, 2011, 2015).

3 Results

3.1 Phylogenetic inference and molecular-clock analyses

The relaxed-clock MCC tree (Fig. 3) shows that the endemic non-pyrgulinid Hydrobiidae from Lake Ohrid likely form a monophyletic group (Bayesian posterior probability (BPP) = 0.87). The potential sister to the Lake Ohrid group is a monophyletic group containing endemic non-pyrgulinid Hydrobiidae species from its sister lakes, lakes Prespa and Mikri Prespa, and their catchments. The split from a MRCA for these two groups is supported by a BPP of 1.0. The closest relatives to the Ohrid/(Mikri) Prespa group are other Balkan hydrobiids (BPP = 1.0) previously classified into the nominal subfamilies Belgrandiellinae, Belgrandiinae, and Horatiinae (see Wilke et al., 2014).

The molecular-clock analyses indicates an age for the MRCA of the endemic non-pyrgulinid Hydrobiidae from Lake Ohrid (i.e., the onset of diversification within this group) under the favored relaxed-clock model of 1.75–3.76 My (95 % HPD; see Fig. 3). Under the inferior strict-clock model, the upper value is slightly lower at 1.75–2.68 My.

3.2 Diversification-rate analysis

The 95 % LTT plot does not suggest major deviations from a constant diversification rate (Fig. 4a). This finding is supported by the TreePar (birth–death-shift) diversification-rate analysis. Accordingly, the likelihood ratio tests suggest for 8 out of 10 random trees a constant rate over time. Only in two of the random trees is a single shift at an age of 0.1 My detected (Fig. 4b and Table 2).

4 Discussion

4.1 The Ohrid non-pyrgulinid Hydrobiidae: an old species flock

Our phylogenetic analyses indicate that the non-pyrgulinid Hydrobiidae form a potential monophyletic, speciose, and endemic clade, and thus, by definition, represent a species flock (Greenwood et al., 1984; Schön and Martens, 2004). Therefore, in situ diversification appears to be a main process in this group. The latter conclusion even holds under the assumption of a non-monophyly of the Ohrid taxa.

The age estimates obtained from the molecular-clock analysis revealed that the Lake Ohrid flock potentially started to diversify before extant Lake Ohrid came into existence (i.e., in a pre-lake/paleo-lake phase or in other waterbodies of the Ohrid Graben system such as rivers or springs; see Figs. 3 and 4a). In fact, karst springs have previously been proposed as potential ancestral habitats for other invertebrate flocks inhabiting Lake Ohrid such as the pulmonate snail genus *Ancylus* (Albrecht et al., 2006), leeches of the genus *Dina* (Trajanovski et al., 2010), and the isopod genus *Proasellus* (Kilikowska et al., 2013). This may also be the case for the species flock studied here, given that karst springs are the dominant habitat of its closest relatives outside lakes Ohrid, Prespa, and Mikri Prespa. However, testing this hypothesis is beyond the scope of the current study and probably would require a denser sampling, more precise limnological information about the early stage of Lake Ohrid, and more specific approaches such as the reconstruction of ancestral waterbodies as well as better calibration points for molecular-clock analyses.

4.2 The Ohrid non-pyrgulinid Hydrobiidae: constant rate of diversification over time

As mentioned in the Introduction, at least four modes of tempo of speciation in ancient lake species flocks are conceivable. Diversification rates may (1) be constant over time, (2) fluctuate (“punctuated equilibrium”), (3) be higher in the initial phase of diversification, or (4) show a pronounced lag phase between colonization and subsequent diversification.

As understanding the temporal frame of speciation in Lake Ohrid is of considerable importance for reaching one of the main goals of the Ohrid scientific deep-drilling program – inferring the driving forces for biotic evolution – the second specific goal of the current study is to estimate diversification rates over time. Given that Lake Ohrid has long been regarded as a relatively stable system (Stankovic, 1960), our working hypothesis assumes no significant changes in diversification rates over time in the lake’s non-pyrgulinid Hydrobiidae.

Interestingly, our LTT plot suggests that the species flock most likely evolved with a constant diversification rate. This finding is supported by our diversification-rate analysis, in-

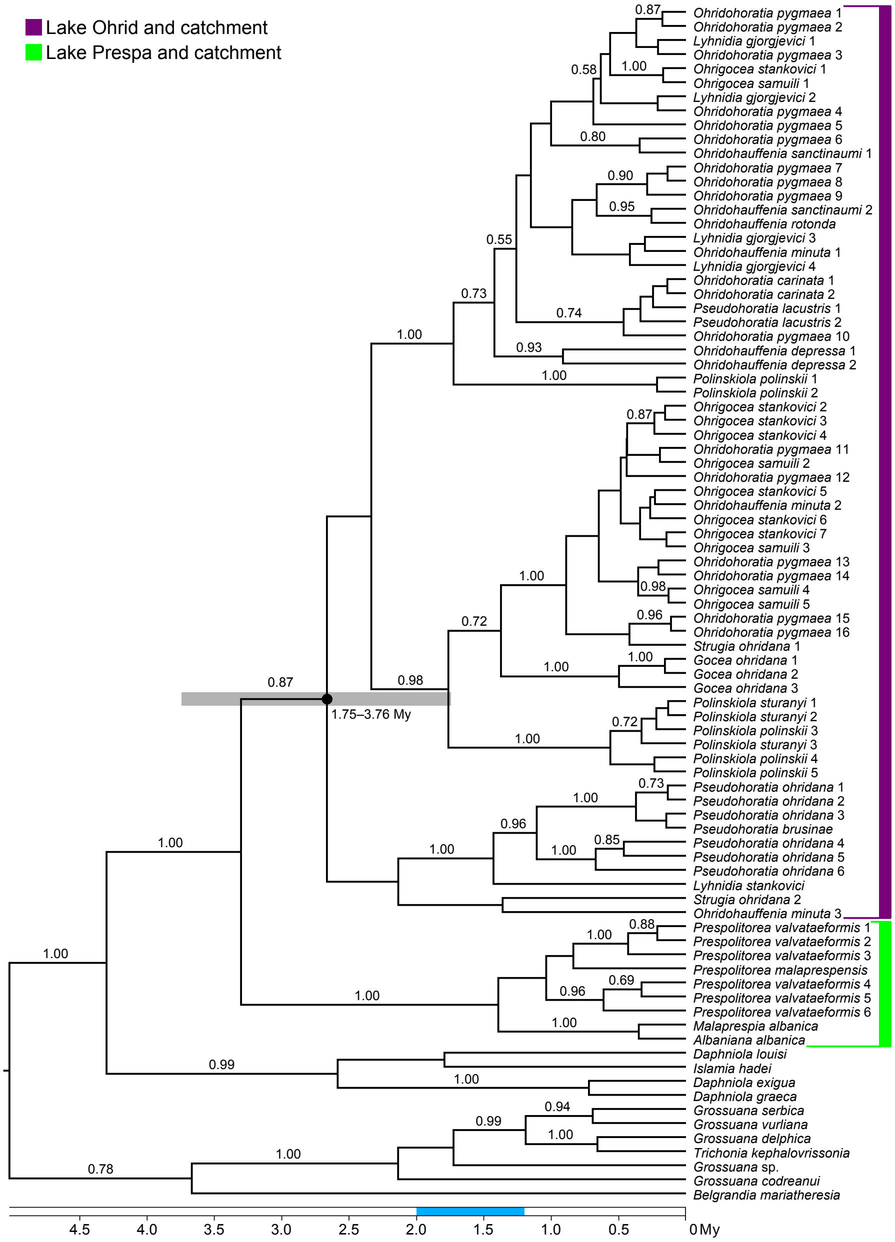

Figure 3. MCC tree based on a relaxed-clock BEAST analysis for non-pyrgulinid Hydrobiidae from the Balkans. Posterior probabilities > 0.5 are shown at the respective branches. The grey bar indicates the 95 % HPD for the age of the MRCA of the Ohrid endemics. The blue bar in the timescale ranging from 1.2 to 2.0 My shows the assumed age of Lake Ohrid.

dicating a single recent shift (drop of rates) at 0.1 Ma for 2 out of 10 random trees (i.e., a time frame in which incomplete lineage sorting may play a role; Fig. 4b and Table 2). Therefore, our initial working hypothesis – a constant diversification rate – cannot be rejected. However, we cannot rule out a type II error here due to poorly resolved phylogenetic trees or insufficient sampling size. As for the quality of the phylogenetic tree used for the LTT plot and the subsequent diversification-rate analyses, our analyses, indeed, revealed some poorly supported (especially recent) nodes (Fig. 3). However, timetree studies are relatively robust against phylogenetic uncertainties (see, e.g., Morvan et al., 2013) as a poorly supported topology does not necessarily affect the timing of rapid speciation events (e.g., Pagel, 1999). We are therefore confident that the low BPP support of some nodes

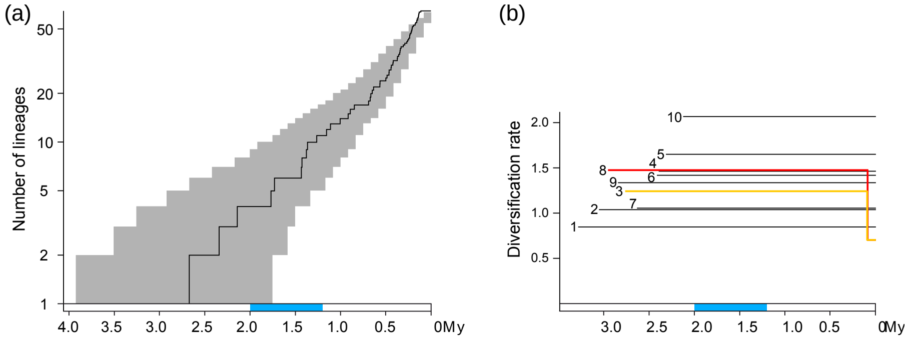

Figure 4. (a) LTT plot for the endemic non-pyrgulinid Hydrobiidae in Lake Ohrid. The plot is based on a relaxed-clock BEAST analysis, with the black line showing the BEAST MCC and the shaded area indicating the 95 % confidence interval based on 20 000 post-burn-in BEAST trees. The blue bar ranging from 1.2 to 2.0 My shows the assumed age of Lake Ohrid. **(b)** Diversification rates obtained from the diversification-rate analyses (TreePar); trees with a single shift in diversification rates at 0.1 My (trees nos. 3 and 8) are highlighted in red and yellow.

Table 2. Reduced output of the diversification-rate analyses (TreePar). P values > 0.95 indicate that a single shift in rates explains the tree significantly better than constant diversification rates, that two shifts explain the tree significantly better than a single shift, or that three shifts explain the tree significantly better than two shifts (see main text for details).

Random tree no.	Max. age (My)	Rate shifts	Log likelihood	P value	Shift (Ma)
1	3.29	0 vs. 1	45.882 vs. 42.043	0.947	
		1 vs. 2	42.043 vs. 38.749	0.914	
		2 vs. 3	38.749 vs. 37.060	0.663	
2	3.06	0 vs. 1	46.292 vs. 45.073	0.514	
		1 vs. 2	45.073 vs. 43.192	0.712	
		2 vs. 3	43.192 vs. 42.478	0.301	
3	2.77	0 vs. 1	36.879 vs. 32.362	0.971	1 shift: 0.1
		1 vs. 2	32.362 vs. 30.577	0.688	
		2 vs. 3	30.577 vs. 28.505	0.754	
4	2.40	0 vs. 1	28.083 vs. 26.171	0.712	
		1 vs. 2	26.171 vs. 25.507	0.278	
		2 vs. 3	25.507 vs. 23.337	0.773	
5	2.32	0 vs. 1	18.364 vs. 14.942	0.923	
		1 vs. 2	14.942 vs. 12.978	0.731	
		2 vs. 3	12.978 vs. 10.751	0.784	
6	2.42	0 vs. 1	31.850 vs. 29.342	0.829	
		1 vs. 2	29.342 vs. 27.932	0.580	
		2 vs. 3	27.932 vs. 26.219	0.670	
7	2.64	0 vs. 1	31.770 vs. 29.237	0.833	
		1 vs. 2	29.237 vs. 28.266	0.415	
		2 vs. 3	28.266 vs. 27.571	0.292	
8	2.96	0 vs. 1	34.733 vs. 30.793	0.951	1 shift: 0.1
		1 vs. 2	30.793 vs. 28.986	0.694	
		2 vs. 3	28.986 vs. 27.431	0.625	
9	2.85	0 vs. 1	34.152 vs. 31.644	0.829	
		1 vs. 2	31.644 vs. 29.433	0.781	
		2 vs. 3	29.433 vs. 27.109	0.801	
10	2.13	0 vs. 1	31.492 vs. 28.169	0.916	
		1 vs. 2	28.169 vs. 25.885	0.794	
		2 vs. 3	25.885 vs. 23.883	0.739	

in our tree had no significant influence on the outcome of our hypothesis testing.

Moreover, we also think that our conclusions are not affected by a non-monophyly of the Ohrid group (see random tree no. 2) as the split between Prespa and Ohrid taxa is, in any event, older than 2 My and thus does not affect intralacustrine diversification rates within the Lake Ohrid clade.

As for the sampling size (i.e., 17 out of 27 nominal species studied), the high diversity of evolutionary lineages found in our phylogenetic analyses indicates that our sampling design likely recovered most major evolutionary lineages within this group. Our sampling includes the majority of genera described except for *Dolapia* (which some authors included in the genus *Ohrigocea*) and *Zaumia*. We also included a variety of specimens collected at various types of habitats and type localities across the lake and its surroundings (see Fig. 2). Moreover, the diversification-rate analysis does account for incomplete sampling, and we did infer a single rate shift in two of the random trees tested in the present study. Thus, the method used seems to have enough power to detect deviations from a constant diversification rate in our data set.

4.3 Ecosystem resilience of Lake Ohrid

If we assume that the rate of diversification in the non-pyrgulinid Hydrobiidae from Lake Ohrid is constant, linking environmental/climatic fluctuations to changes in tempo of diversification becomes impossible (see specific goal iii).

However, an important question is whether a non-constant diversification rate could not be demonstrated because Lake Ohrid never experienced massive environmental and/or climatic changes or whether the lake has a high ecosystem resilience that might buffer such perturbations. Preliminary data of the SCOPSCO deep-drilling program based on core catcher data for the last 1.2 My and high-resolution data for the last 640 thousand years (ky) so far do not indicate the occurrence of catastrophic environmental events (Francke et al., 2015; Wagner et al., 2014), i.e., events that lead to sudden drastic regime shifts (sensu Scheffer and Carpenter, 2003; Scheffer et al., 2001) and thus potentially to mass extinction. By comparison, such events have been observed in other ancient lakes, including Lake Titicaca (Kroll et al., 2012; Lavenu, 1992) and Lake Malawi (Cohen et al., 2007; Scholz et al., 2007; Schultheiß et al., 2009, 2011). We do, however, see signatures of severe environmental/climatic perturbations in Lake Ohrid, including significant lake-level drops (Lindhorst et al., 2010), volcanic ash deposits (Sulpizio et al., 2010; Wagner et al., 2014), and glacial–interglacial cycles (Lézine et al., 2010; Reed et al., 2010; Wagner et al., 2014).

Therefore, we think that the lack of changes in tempo of diversification of Lake Ohrid's non-pyrgulinid Hydrobiidae might potentially be a result of two factors: (i) either Lake Ohrid never experienced catastrophic environmental events that resulted in the extinction of all or most of its endemic taxa and thus caused a "reset" of diversification processes or (ii) Lake Ohrid possibly has a high ecosystem resilience that buffers environmental changes and potentially mitigates extinction events. Note that the two factors might not be mutually exclusive as it has been shown that a loss of resilience may set the scene for a catastrophic-event-induced switch to an alternative state (reviewed in Scheffer et al., 2001).

The second scenario is supported by the mollusk (Albrecht et al., 2010) and diatom fossil records (Cvetkovska et al., 2015; Jovanovska et al., 2015) for the past ~ 100 ky. For instance, the Campanian Ignimbrite Y5 tephra influx 39.6 ky ago (see Leicher et al., 2015) altered the water chemistry of Lake Ohrid and increased the content of silica, which in turn amplified diatom growth rates. However, it did not cause severe changes in diatom community structures or even extinction events (Jovanovska et al., 2015).

The suggested high ecosystem resilience of Lake Ohrid might be sustained by several factors, including the lake's bathymetry (deep lake with steep flanks, allowing habitats to move vertically with lake-level changes; Lindhorst et al., 2010), ongoing moderate tectonic activities (compensating sedimentation; Hinderer and Einsele, 2001), and its peculiar limnology (the lake is fed by numerous karstic sublacustrine springs, locally buffering environmental changes; Matzinger et al., 2006).

Although we think that the patterns inferred from mtDNA sequencing data of extant taxa are highly informative, future analyses utilizing additional (nuclear) markers may help better resolve some of the basal relationships of Ohrid taxa. Moreover, the temporal resolution is still limited, the error rate for time estimates is relatively high, and some important processes such as extinction events are difficult to infer from extant organisms (see also Rabosky, 2010, for a discussion). Therefore, we encourage future paleontological studies on endemic species using the sediment cores gained during the SCOPSCO deep-drilling campaign. This concerns, for example, diatoms and ostracods. Moreover, besides the main core "DEEP", obtained from the deepest part of Lake Ohrid, several other cores were retrieved in shallower parts of Lake Ohrid. The latter appears to be relatively rich in mollusk fossils (see Wagner et al., 2014), which could potentially be used to directly study extinction and speciation events (for a proof of principle see Albrecht et al., 2010).

5 Conclusions

Our molecular-clock analyses indicate that the non-pyrgulinid Hydrobiidae of ancient Lake Ohrid represent an old endemic group, which is characterized by a constant rate of diversification. We propose that this constant rate has been caused by two factors: (i) a possible lack of catastrophic environmental events in Lake Ohrid and/or (ii) a high ecosystem resilience, buffering environmental changes. Parameters potentially contributing to Lake Ohrid's ecosystem resilience are its distinct bathymetry, ongoing tectonic activities, and karst hydrology. These findings are not only of interest for one of the overall goals of the SCOPSCO deep-drilling program – inferring the driving forces for biotic evolution in Lake Ohrid. They might also enhance our understanding of how ecosystem resilience, in general, may promote a relatively constant diversification in highly isolated ecosystems.

However, high-resolution sedimentological data are currently only available for the last 640 ky. Therefore, we encourage future studies specifically testing hypotheses about the lack of catastrophic events in Lake Ohrid based on high-resolution data for the entire geological history of the lake, and potentially involving information from the sediment fossil record not only for gastropods but also for other groups with a high share of endemic taxa.

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11 Scientific drilling projects in ancient lakes: integrating geological and biological histories

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Invited review article

Scientific drilling projects in ancient lakes: Integrating geological and biological histories

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ABSTRACT

Sedimentary sequences in ancient or long-lived lakes can reach several thousands of meters in thickness and often provide an unrivalled perspective of the lake's regional climatic, environmental, and biological history. Over the last few years, deep-drilling projects in ancient lakes became increasingly multi- and interdisciplinary, as, among others, seismological, sedimentological, biogeochemical, climatic, environmental, paleontological, and evolutionary information can be obtained from sediment cores. However, these multi- and interdisciplinary projects pose several challenges. The scientists involved typically approach problems from different scientific perspectives and backgrounds, and setting up the program requires clear communication and the alignment of interests. One of the most challenging tasks, besides the actual drilling operation, is to link diverse datasets with varying resolution, data quality, and age uncertainties to answer interdisciplinary questions synthetically and coherently. These problems are especially relevant when secondary data, i.e., datasets obtained independently of the drilling operation, are incorporated in analyses. Nonetheless, the inclusion of secondary information, such as isotopic data from fossils found in outcrops or genetic data from extant species, may help to achieve synthetic answers. Recent technological and methodological advances in paleolimnology are likely to increase the possibilities of integrating secondary information. Some of the new approaches have started to revolutionize scientific drilling in ancient lakes, but at the same time, they also add a new layer of complexity to the generation and analysis of sediment-core data. The enhanced opportunities presented by new scientific approaches to study the paleolimnological history of these lakes, therefore, come at the expense of higher logistic, communication, and analytical efforts. Here we review types of data that can be obtained in ancient lake drilling projects and the analytical approaches that can be applied to empirically and statistically link diverse datasets to create an integrative perspective on geological and biological data. In doing so, we highlight strengths and potential weaknesses of new methods and analyses, and provide recommendations for future interdisciplinary deep-drilling projects.

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Contents

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1. Introduction

The vast majority of the world's lakes has existed or will exist for up to a few ten thousand years (e.g., Brooks, 1950). Primarily due to sediment infill, they become progressively shallower and subsequently vanish. Ancient or long-lived lakes, on the contrary, exist for over 100,000 years (100 ky), sometimes millions of years (My) (Brooks, 1950; Gorthner, 1994; Martens, 1997). They typically occur in settings where sedimentation rates are low or balanced by subsidence (Cohen, 2012). Accordingly, most of today's ancient lakes are oligotrophic and situated in active tectonic graben settings, karst systems or impact craters with low sediment supply from the catchment.

Because of the long-term availability of accommodation space (Jervey, 1988), sediment sequences in ancient lakes can reach several hundreds to thousands of meters in thickness (e.g., Scholz et al., 1993, 2011; Lindhorst et al., 2015). Lake deposits contain material that mostly derives from the lake proper and the catchment area and, hence, provide an unparalleled perspective of the lake's history through time (O'Sullivan, 2004). Combining the paleolimnological records from different lakes permits the reconstruction of continental and global environmental, and climatological histories. It is this potential, captured in the often continuous lacustrine sedimentary archives, that has inspired several deep-drilling projects in ancient lakes (reviewed in Cohen, 2012; Fig. 1).

However, over the past decades, drilling operations became increasingly interdisciplinary, as data bearing on physical, chemical, biochemical, and biological research questions can also be obtained from sediment cores. Because of a wealth of new information, scientists from different fields, such as sedimentology, climatology, geochemistry, paleolimnology, paleontology, biochemistry, microbiology, evolutionary biology, physics, and modeling, currently aim to use ancient lakes as paradigms to interactively look into natural phenomena from various angles, emphasizing the need for truly interdisciplinary collaborations (sensu O'Sullivan, 2004; Birks and Birks, 2006).

Multidisciplinary and interdisciplinary studies enable a more holistic approach to scientific problems, provide excellent opportunities for hypothesis-driven research, and are likely to have greater success in generating a widespread interest in the broader scientific community. However, these projects pose several challenges for the diverse science teams. The interests of the various groups involved need to be aligned; participants may lack the required knowledge of other disciplines; traditions and common practices may differ widely between disciplines. Finally, larger teams increase the challenge to communicate and coordinate efforts effectively. The various goals of individual teams call for compromises on several levels, such as drill site selection, subsampling strategies, and choice of analyses (see Section 2.1.1). Life scientists are typically not familiar with drilling operations and often lack basic geological knowledge whereas earth scientists may not be acquainted with biochemical or biological procedures. More practically, the difficulty arises that life scientists do not know exactly how to retrieve the archives they hope to study, and that earth scientists cannot evaluate applicability and performance of biological methods. Similar problems persist on smaller scales, and given the rapid advancement of many of the individual fields, specialists may even struggle with methodological innovations in their field over the often year-long duration of deep-drilling projects, involving the planning, the actual drilling campaign, and the interpretation of the final datasets. These issues are also relevant for core storage, which may affect geological and biological properties differently. Sedimentologists are typically acquainted with long-term changes in sediments after core retrieval, but others may draw erroneous conclusions when linking biological and geological data without accounting for potential contamination, drilling artifacts, decay processes, and other complications (see Section 2.1.2). In general, greater logistic, communicative, and administrative efforts are required with increasing complexity of interdisciplinary projects, and drilling methods may have to be optimized to guarantee the required data quality.

Perhaps the most challenging task, however, is to integrate the diverse datasets various teams collect from drilling cores. These datasets typically have differences in resolution, data quality, and dating uncertainty, but combining them is required to answer interdisciplinary questions. While the physical linkage of information directly obtained from sediment cores is, in most cases, relatively straightforward due to the

Fig. 1. Map showing location of ancient lakes with a presumed age of >1 My at which deep drilling has been done (Cohen, 2012; Russell and Bijaksana, 2012).

stratigraphical constraints on the data, the challenge grows when primary data, i.e., data generated from sediment cores or in boreholes, are to be linked with external (secondary) data, i.e., data obtained independently of the drilling operation. Examples of secondary data sources include stable isotope information from fossils found in outcrops (see Section 2.1.6) or genetic information from extant species (see Section 3.3).

Here we review the types of geological and biological data that can be obtained from ancient lake drilling projects (Section 2) and the methods that can be used to analyze these data against the backdrop of the abovementioned practical and analytical challenges (Section 3). Acknowledging the increasing number of approaches and analyses that can be applied to drilling data, we narrow our focus on data and methods that have a high potential towards integrating geological and biological data and for hypothesis-testing related to interdisciplinary questions. We also provide a retrospect on how the actual drilling operation and conditions of sediment-core storage can affect data and subsequent multi- and interdisciplinary analyses. Although this review focuses on extant ancient lakes, some of the information given is also applicable to lakes from the past and even young lakes.

Our aim is to provide scientists from various disciplines with a background to strengthen interdisciplinary approaches to ancient lake drilling projects. We thus explain data acquisition and analyses in broad terms and provide information as to the underlying fundamental principles that may be equally useful for earth and life scientists. Given this scope, we refrain from detailed discussions that are constrained to a specific field, nor do we provide a historic overview of drilling operations for which other reviews exist (Cohen, 2012).

As such, this review intends to encourage scientists from diverse disciplines to join scientific deep-drilling projects, and to utilize these unique records of global change during the earth's history for understanding current and future changes on a planetary scale.

2. Data and methods

2.1. Geological data and methods

2.1.1. Site selection and drilling strategies

Careful consideration of the drill site(s) and the drilling strategy are a prerequisite to optimize the chances that the goals of a deep-drilling project can be reached. Scientific objectives are the foremost criteria for the selection of drill sites and strategies, but financial and time constraints also have an distinct impact. The extensive infrastructure needed and the shipping of highly specialized gear are important cost factors of deep drilling (Fig. 2).

Given a certain budget, the costs for logistics, including the transport of the drilling equipment to and from the lake and daily operational expenses (e.g., labor costs, fuel consumption, daily shuttle of drill team and cores), define the number of drilling days. Technical failures, weather delays, or lithologies that are challenging to drill are difficult to predict and may further reduce available drilling time. In contrast, unexpected drilling progress may provide the opportunity to drill deeper than anticipated or to add additional sites, addressing for example 2nd or 3rd order scientific objectives. For these reasons, a well-calculated budget, which includes sufficient contingency to cover unforeseen costs and/or delays, may significantly increase the chances for success. Finally, site selection may also be influenced by the time required for daily commutes of the drill teams to and from the drill site.

Fig. 2. Deep lake drilling system of DOSECC (USA) in operation at Lake Ohrid. In this case, the equipment had to be shipped from Salt Lake City (USA) to this inland lake on the Balkans.
Photo credits: T. Wilke.

Experience has shown that the one-way commute time should not exceed 1.5 h when working in 12 h shifts.

Most of the scientific criteria for site selection and drilling strategies are reviewed in Cohen (2012). Catchment characteristics, such as the location of inlets, providing terrigenous, clastic, and organic sediments to the basin, the bathymetry and surrounding topography, and the presence of undisturbed successions may affect site selection. However, bathymetry, catchment topography, and the location of inlets may have changed over time, especially in tectonic basins. Most commonly, the site selection is therefore based on data from reflection-seismic surveys, providing information about bathymetry and the three-dimensional sub-bottom sediment structure, as well as the stability of sediments in target areas. For most scientific questions, areas with undisturbed sedimentation are preferred, but for others, such as the reconstruction of earthquake history, a focus on disturbed successions may be beneficial (e.g., Takemura et al., 2013). Moreover, most drilling campaigns focus on the depocenter of a lake. If biological questions are of interest, such a drilling strategy will almost certainly omit valuable information of the nearshore benthic communities where most of the biodiversity is concentrated (see also Section 2.2.2). However, during initial lake phases or massive drops in lake level, shallow water species may have lived nearby the depocenter and thus be preserved in the old sediments just overlying the basement rock (e.g., Wagner et al., 2014a). Coring in shallower or littoral regions faces other problems, such as obtaining discontinuous or incomplete records of biotic evolution, hydrological characteristics, and catchment processes. In these environments, sediments from the initial lake phase or low-level periods will be missing.

The drilling strategy also includes criteria, such as the number of drill sites, their priorities, or the number of holes per site needed for a composite core. As a general rule, the site with the highest scientific priority should be drilled first, as unforeseeable technical or weather issues may delay the coring progress. However, it may be advisable to start with a site of secondary priority, for example, if the sedimentary characteristics are poorly known or if the main drill site requires high logistic efforts. In many campaigns, the initially retrieved sediment cores have a poorer quality compared to cores drilled at later stages of the operation, because drill and science teams need to develop communication and drilling strategies (e.g., the choice of drill tools) suitable for the encountered lithologies. As core recovery in a single borehole can be as low as 10% (see also Section 2.1.2), parallel coring is needed to fill the gaps. Sometimes 3–4 holes are required to obtain 100% or close to 100% recovery at one site (resulting in a so-called composite core).

In some cases, the drilling strategy may need to be adapted to effectively address specific scientific questions or deviating lithological intervals. For example, in studies focusing on subsurface microbial activity, contamination by drilling fluids needs to be minimized and samples may need to be taken directly upon recovery of the cores (see Section 2.2.3). If the exact depth of the recovered sediments is not of highest priority, the hole of the first core drilled for subsurface biosphere studies can be used for borehole logging (Section 2.1.3) and to obtain initial information about lithological characteristics at depth. This facilitates adaptation of drilling strategies for the subsequent holes and sites to be drilled.

In summary, several criteria affect site selection and drilling strategies. Because of the specifics of each lake, these criteria need to be balanced carefully based on the exact setting encountered. Meticulous preparation, including the collection of site-specific information, such as data on sediment structure from seismic surveys and lithological characteristics from pilot coring surveys may serve to construct a drilling strategy that can then be evaluated against scientific objectives, budget, logistic requirements, and the available time.

2.1.2. Coring techniques and data recording

The main lake coring tool is a piston-type corer, which allows penetration under hydraulic power into generally cohesive sediments of various aggregation up to a pre-determined length. This tool permits collection of the least 'contaminated' or 'disturbed' type of core as it is pushed into the formation ahead of any disturbance caused by the rotational drill bit and sediments are collected before they get into contact with drilling fluids. Before the actual drilling, the piston corer is sealed with a piston to avoid contamination of the drill string with surface lake water and cuttings or cavings, which may be in the borehole before and during the core collection, to enter the coring tube.

In soft to firm clays, sample recovery can be 100%. The slight underpressure caused by the piston avoids shearing of sediment packages in front of the corer during penetration, but it leads to expansion of the core, particularly at its top and may promote degassing. In more granular sediments, compression rather than expansion is common due to grain packing. Moreover, as the drill string is frequently advanced to the next firing point by the length of recovery rather than the length of the core barrel, there can be overlap of 'strata' between two core runs, with the top of a subsequent sample consisting of cavings from the previous sample. It is not easy to completely clean the borehole in this type of sediment and the coarser and less cohesive it is, the more likely it will be that full recovery of the section will not fill the core barrel due to packing and dewatering occurring. Again, recalibration of any subsample depth with regard to the composite borehole log and marker horizons will ensure that correct depths for correlation with other scientific data results are achieved. Although the core length may not reflect the full sampled length, the stratigraphic coverage typically will be complete and essentially free from other types of mechanical disturbance and fluid mixing. Therefore, piston cores allow for the best stratigraphic resolution of all drill-sampling method.

Other frequently used lake drilling tools are the extended nose corer and the alien corer. Both of those tools require a rotation while coring. The core is cut and collected into the core barrel in close proximity to the drill bit while fluid discharge is required to keep the drill bit cool and clear of cuttings. This makes it difficult to allow the core an undisturbed passage into the core barrel, and results in artifacts by the drilling and the use of drilling fluid. These artifacts depend also on the lithology of drilled sediments, but there may be mixing of drilling and formation fluids, or selected portions of the core may be washed away, and disturbance to the structure and stratigraphy of the core may also occur.

The extended nose corer is used when the material in the stratigraphic section is non-cohesive or friable but becomes too coarse or compacted to be able to operate a piston corer for any reasonable length into the subsurface. To use this corer, the sediment needs to be still soft or granular enough to allow extending a thin diamond bit nose ahead of the main bit and main fluid flush when progressing the hole. Ideally, the core will enter the extended bit section without too much interference or influence from the rotation and drilling fluid. If there is any movement of the drill string due to heave of the platform while drilling, then discs of core and cuttings may also occur and show up as bands in the core. Recovery is unlikely to reach 100% even under ideal conditions.

The alien or rotary core barrel is essentially a method of collecting cores from hard, cohesive sediments. It may selectively wash away material that is too soft or non-cohesive before it can be properly cut to enter the core barrel. It may also induce drilling fractures in otherwise good quality rock. There is always an interaction between the drilling and formation fluids as the core is being cut and collected. Recovery will range from poor (<10%) to excellent (100%), depending on lithology, weather conditions, and type of drill bit. However, good recovery does not equate to quality core for laboratory analyses given the earlier highlighted artifacts. The length of the recovered core may not be a good reflection of the length of the stratigraphic section cored. Harder material is most likely to be recovered while soft material can be crushed or washed away, thus reducing its occurrence in the recovered stratigraphy. In these cases, composite borehole log interpretation, utilizing borehole geophysical and multi-sensor core logger (MSCL) data, is important to indicate what sections are recovered and how they are to be placed in the actual stratigraphy. Stratigraphic resolution with this type of corer will be very good in hard materials but will quickly diminish where uncemented, friable, or non-cohesive sediments occur in the section. Subsamples of such cores, hence, need to be carefully extracted.

In general, core portions used for bulk analyses should be disassembled and cleaned of foreign materials before use. If, in later years, bulk samples are made available for analyses by scientists that were not part of the drilling team, it is important that the core's full history is known, so that anomalous or unexpected results can be interpreted in the light of possible artifacts of the drilling, storage or preparation of the material. Detailed drilling and core interval logs should be held with the composite geological core log and identify, as a minimum, the type of drilling and coring tools used, and details of any drilling mud/fluid utilized. The latter information is important to eliminate geochemical anomalies in scientific results. For example, contact of long-deposited sediments with current lake water may leave a signature in the stored core. Although the drilling mud will not directly affect fossil material (Sections 2.2.1, 2.2.2), unless there are secondary reactions during storage, it may affect microbial activity, metabolites, and decay products as some types of drilling mud contain guar, which is a food source. For microbiological studies (see Section 2.2.3), tracers used to detect 'contamination' need to be documented. Moreover, if microspheres were utilized, they may still be present in some samples.

Subsamples of sediment cores from scientific drilling projects are increasingly being used for multidisciplinary studies in a much wider scientific aspect than that of the original project and thus particular emphasis should be placed on understanding the circumstances in which the original data were collected and records were archived (for a review see Cohen, 2012). Any subsample from a core section needs to have the depth of the core section as an unmistakable criterion, as correlation depths derived from the correlation of cores from parallel holes (so-called core composites) may change with results from ongoing measurements (e.g., high resolution logging). Ideally, these core composites are recalibrated to borehole logging data prepared from a number of boreholes at the same site. However, significant marker horizons (e.g., tephra deposits), being used as the correlation basis for the composite borehole log are not always available.

Storage in controlled and cool temperatures will minimize moisture loss, bacterial activity (see Section 2.2.3) and ancient DNA decay (see Section 2.2.6), and allow physical property measurements to be extended by a few months. Cooler temperatures will also slow down any secondary chemical reactions or existing core alteration.

Drilling operation and coring methodology define initial core quality and determine the degree of physical or chemical 'contamination' that may be anticipated. Archiving and storage imprints, further characteristics on the core, and accurate subsampling interpretation require a full tracking record of the core from collection to interpretation with subsample positions clearly archived in relation to the final composite borehole log. These logs should also show where subsamples have been taken from and regularly updated as new information is generated.

2.1.3. Borehole logging

Borehole logging is the process of measuring physical, chemical, and structural properties of penetrated geological formations via tools that are lowered into a borehole on a wireline cable. It provides in situ information about the physical properties of the rock or sediment strata and groundwater. Borehole logs deliver a continuous record that provides information on the lithological changes with a precision of decimeters to a few centimeters. Since it allows depiction of actual depth and petrophysical characteristics, information from borehole logging is often used in combination with seismic reflection data to construct geological models. In addition, the combination of downhole logging data and petrophysical datasets from several drill holes and cores from the same site are essential to construct a composite lithological log. More recent applications include the derivation of paleoclimatic indicators and cyclostratigraphic analyses.

The main components of logging equipment are a surface unit, winch, cable, and logging tools equipped with variable detectors and/or sensors. The surface unit is used to control the measurements, including the movement of the tool in the borehole. It also provides the energy supply to the tool and records, displays, and stores the data generated in the borehole. The depth of the measurement in the borehole is independently recorded by a gauge on the winch. To allow depth correlation between all logging runs, each tool is equipped with a gamma ray sensor, which records the formation's natural gamma radioactivity caused by its occurring contents of uranium (U), thorium (Th), and potassium (K).

A number of tools have been developed over the recent decades that maximize the number of physical parameters that can be measured in slim boreholes. The equipment, field application, and analytical methods have been described by, e.g., Ellis and Singer (2007) and Rider and Kennedy (2011). The most important tools/physical aspects of borehole logging in a lake drilling project are spectral gamma ray (natural gamma ray plus contents of U, Th, and K), magnetic susceptibility, resistivity, acoustic velocity, vertical seismic profiling (VSP), dipmeter, and caliper (borehole diameter and orientation). Furthermore, tools that register the density, neutron porosity, and the content of a selection of geochemical elements of the drilled formations are available. These tools emit ionizing radiation and contain either radioactive sources or neutron accelerators. The regulations for the import and export of these tools are complex and differ from country to country, which regularly limits their use in ancient lake drilling projects. Tools based on optical methods like video cameras or optical televiewers exist as well, but they cannot usually be operated in lake drillings because the drilling fluids are not translucent.

The various downhole logging methods together with a sensor configuration adapted to the measurement conditions are used to obtain data from a limited, irregular rock volume. The vertical and radial extent of this volume is influenced by the borehole diameter, the physical properties of the content of the borehole, the ratio of the borehole diameter to the diameter of the tool, the position of the tool in the borehole, and the design of the tool (detector size, electrode spacing, transmitter-receiver spacing, radioactive source-detector spacing). Thus, each tool has a characteristic depth resolution and an average radial depth of investigation under the given conditions.

The logistical effort involved with transport and installing logging equipment at the drill site can be high, particularly when the gear has to be transported to a floating drilling barge. These barges are typically not equipped with heave compensation, so that logging operations need to be conducted during appropriate weather conditions. Final decisions about holes to be logged during or at the end of drilling operations can change quickly and are dependent on the overall progress of the drilling operation.

Downhole logging measurements in ancient lake drilling projects are typically made in unconsolidated sediments. Due to the specific sensor requirements, most physical parameters have to be measured in an open hole. To reduce the impact of potential borehole wall collapses, logging is performed in borehole sections. Their number and individual length (down to 30 m) need to be defined in close cooperation with the drilling supervisor and leads to a significant prolongation of the logging time. The logging speed depends on the tools/sensors used, and has a large influence on the quality of the data. Typical logging speeds are between 60 m h^{-1} and 600 m h^{-1}. The sampling interval also influences data quality. It is typically 5–10 cm, with a vertical resolution of about 20 cm.

The quality of the borehole logs are dependent on the measurement conditions and the depth resolution of the tool. The conditions of logging are determined primarily by the borehole geometry, the physical properties of the drilling fluid (density, electrical resistivity, pH, neutron braking, and absorption properties), and the properties and size of the flushed and invaded zone. Borehole logging is thus an important and versatile tool in ancient lake drilling projects and applications include:

i) Lithological classification of the strata penetrated by a borehole. Characteristic physical rock parameters, especially when combined, can be used to determine or confirm the lithology of the rocks or sediment successions. The most useful parameters for this are gamma radiation, magnetic susceptibility, resistivity, acoustic velocity, and caliper.

ii) Site-to-site/hole-to-hole correlation of lithological units. Correlation of lithological units are crucial for obtaining core-composites and for linking stratigraphic positions across boreholes (see Section 2.1.2). Most suitable are gamma-radiation and magnetic susceptibility. Furthermore, magnetic susceptibility data has the potential to identify tephra layers that are important chronostratigraphic marker horizons in sediment successions.

iii) Paleoenvironmental/paleoclimatic reconstructions. Paleoclimatic indicators were derived from physical properties of the sediments from lakes El'gygytgyn (Nowaczyk et al., 2013), Van (Baumgarten and Wonik, 2014), and Ohrid (Baumgarten et al., 2015). Sediment records from these lakes display strong shifts in physical and chemical properties with depth that are tied to different environmental/climatic states.

iv) Cyclostratigraphic analyses. Contrasting physical properties and therefore changes in the sediment characteristics can trigger cyclic changes in the logging data. Cyclostratigraphic analyses in lakes based on physical parameters from cores and/or boreholes were conducted by Nowaczyk et al. (2013), Baumgarten and Wonik (2014), and Baumgarten et al. (2015). Under favorable sedimentation conditions, results from cyclostratigraphic analyses based on downhole logging data even allow estimates of varying sedimentation rates and establishment of reliable age-depth relationships (see Section 2.1.5).

v) Time-depth conversions. Measurements of acoustic velocities in boreholes are useful for the interpretation of shipborne seismic reflection surveys. Therefore, for the geological modeling based on seismic data, downhole logs (acoustic velocity and VSP) can provide essential information necessary to develop time-depth conversions for seismic datasets.

vi) Inference of structural/tectonic features. Important information needed for tectonic and structural geology investigations include the dip angle, strike direction, and angle of formations. These parameters can be inferred in the borehole using a dipmeter tool. Layers with thicknesses as small as 1–2 cm can be detected.

vii) Hydrogeological and geotechnical parameters (e.g., clay content, porosity). Data registered from the acoustic velocity logging tool can be used to determine porosity. Assumptions about the lithology and fluid properties based on local knowledge or other measurements have to be made to estimate porosity. Spectral gamma ray logs may help to distinguish between different clayey sediments based on their U, Th, and K contents. Acoustic velocity logs via the compression modulus provide indirect information about the storage coefficient of the rock or sediment successions.

Due to technical difficulties, micro-resistivity imagers and nuclear magnetic resonance (NMR) equipment have not been applied in slim boreholes so far (for details of the methods see, e.g., Kenyon, 1997; Coates et al., 1999; Dunn et al., 2002). With the former method, detailed, high-resolution spatial data providing structural and textural information can be obtained from the measurement of resistivity with a large number of electrodes (Lovell and Parkinson, 2002). Using a NMR tool, a real time permeability log may be possible. Also, the application of interpretative methods for hydrocarbon exploration and basement rock data (e.g., multivariate statistics) to ancient lake drilling projects can provide further possibilities to combine geophysical data from boreholes with other geophysical and geochemical datasets towards more holistic analyses in the future.

2.1.4. Sediment-core data

Standard measurements on sediment cores can be separated into those conducted on whole core sections, on split core surfaces, and on discrete samples (Fig. 3).

Whole core measurements are typically performed just after drilling on site and/or shortly before core opening in the laboratory. For these purposes, multi-sensor core loggers (MSCL), which can be described as a logging bench unit, are commonly used. MSCL systems allow stepwise measurements of geophysical parameters at centimeter resolution and are commonly equipped with variable sensors/detection systems for magnetic susceptibility, gamma ray density (GRAPE), P-wave velocity, natural gamma spectroscopy, and resistivity (e.g., Weber et al., 1997). These datasets provide a first, rough overview about changes in sediment composition (density, detrital/magnetic mineral content, U-, Th-, K-concentrations) before the cores are opened and sediments are exposed to the atmosphere. They are useful to establish hole-to-hole correlations, to identify gaps in the core recovery, and to assist adjusting the drilling strategy. P-wave velocity data are needed to establish time/depth conversions for acoustic and seismic reflection datasets, thus allowing for more sophisticated interpretations of seismic units and their correspondence to changes in lithology. Furthermore, obtaining these whole core measurements is a prerequisite for geomicrobiological sampling (see also Section 2.2.4), which needs to be undertaken in the field shortly after core recovery and consumes entire core sections. Hence, if geomicrobiological datasets need to be tied to a stratigraphy/chronology or compared to other analyses obtained on overlapping intervals later on, this can only be achieved using whole core MSCL datasets. MSCL datasets can be biased by cracks in the sediments due to gas expansion or by incompletely filled liners, such as is common when using rotational drilling tools (see Section 2.1.2).

Macroscopic lithological core descriptions on split core surfaces, supported by microscopic smear slide analyses of major biogenic and minerogenic sediment components, are used to define major lithologic (i.e., pelagic sediments, mass wasting deposits, tephra layers) and sedimentary/stratigraphic units. This information is not only important to determine changes in the depositional environment but also allows more targeted lithotype-specific subsampling strategies to be developed. Detailed and spatially highly resolved (down to 0.2 mm) geochemical analyses on split core surfaces can be performed using X-ray fluorescence (XRF) core scanners. Newer systems allow the analysis of major and minor elements heavier than Al at high precision and permit, besides inference of relative changes in concentration, also quantification upon calibration (e.g., Russell et al., 2014). Relative changes in intensities as well as ratios of specific elements are useful indicators for changes in sediment composition (e.g., Melles et al., 2012), sediment provenance (e.g., Kylander et al., 2013), redox conditions (e.g., Naeher et al., 2013; Costa et al., 2015), diagenetic overprinting, grainsize/depositional processes (e.g., Bloemsma et al., 2012; Francke et al., 2016), and to identify the occurrence of tephra or cryptotephra layers (e.g., Vogel et al., 2010a; Leicher et al., 2016). The quality of the data depends on the analytical time and on several sedimentological characteristics, such as surface smoothness, water content, or grain size distribution. XRF scanning of marine and lacustrine core sequences has become a standard method to obtain a rapid, spatially highly resolved overview of changes in element composition, but the method is time consuming at high resolutions. Requiring an analysis time of up to several hours per meter

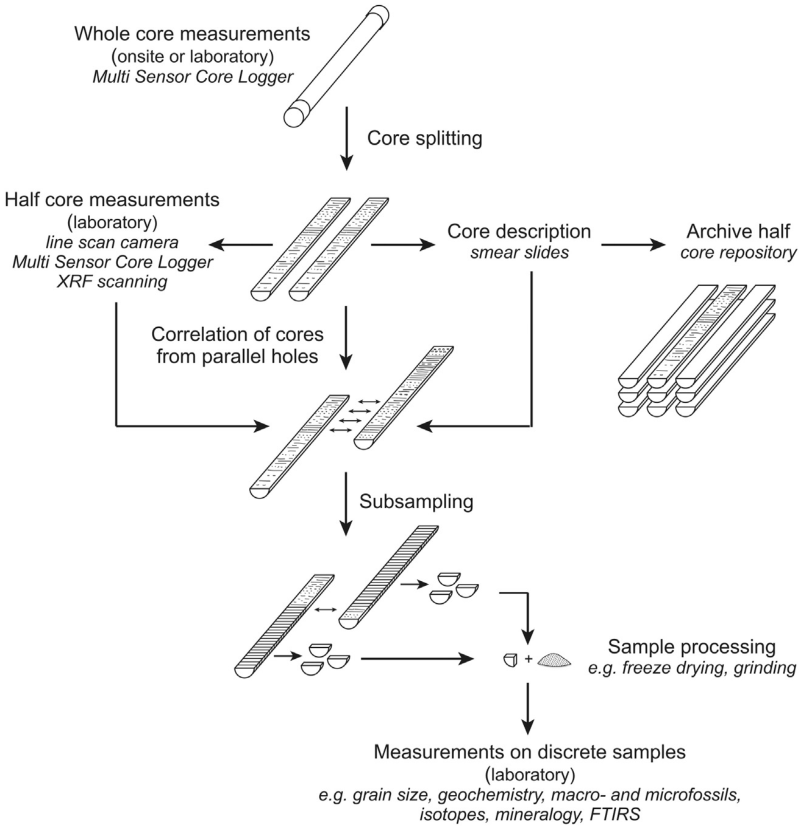

Fig. 3. Generalized scheme of core processing for basic sedimentological analyses. Individual steps and analytical methods may vary across projects.

sediment core, the scanning of multiple sediment sequences from one site with a total length of hundreds of meters may take several weeks. Moreover, due to the high scanning resolution, the amount of data produced is immense and often requires smoothing or filtering in order to facilitate the identification of major trends of element variation in sediment cores and to reduce background noise. Therefore, careful selection of the resolution based on other data, e.g., reduction of resolution if a section of sediments appears homogeneous, for example, due to bioturbation or mass movement processes, can significantly reduce measurement time and facilitate data handling.

Analytical methods on discrete samples comprise a broad range of granulometric, geochemical, and biological methods. Much variation exists in the amount of preparation and time that are required to apply individual methods, e.g., sample preparation or identification of micro- and macrofossils can be very time consuming. Significant differences also exist in the required amount of sediment needed to perform each analysis. Some geochemical methods require only a few milligrams of powdered material, but others, such as paleontological studies, may need several grams of sediment or more. Both labor-intensity and availability of material affect the sampling resolution, with the separation between subsequent samples usually varying from one centimeter to several decimeters.

Granulometric analyses are used to obtain information on sediment transport history and energy (fluvial, aeolian, pelagic, gravity driven). The measurement of grain-size distributions can be done relatively fast with a laser particle size analyzer for sand- to clay-sized fractions, as are common in lacustrine sediments. However, sample preparation is necessary to extract information on transport and depositional processes unbiased by autochthonously derived sediment components. Sample preparation can be labor-intensive if removal of organic matter, carbonates (authigenic, biogenic), biogenic silica from diatoms, phytoliths, or sponge spicules is required. Standard geochemical analyses often comprise the measurement of total organic carbon (TOC), total inorganic carbon (TIC), total nitrogen (TN), total sulphur (TS), and require only relatively low amounts of powdered material. TOC and TN concentrations are useful indicators that provide estimates on changes in intralacustrine productivity (e.g., Wagner et al., 2009; Vogel et al., 2010b) and/or changes in the supply of terrestrial organic matter (e.g., Meyers, 2003). However, TOC and TN concentrations in the sediment are not only influenced by their initial fluxes but also by the degree of post-burial remineralization, which in most settings is controlled by lake mixis and the availability of oxygen in the hypolimnion and top sediments (e.g., Melles et al., 2007). TIC concentrations are a measure of the amount of carbonate, which can be present in biogenic, endogenic, detrital, and/or authigenic form in lacustrine sediments. Calcite and aragonite (both $CaCO_3$) are the predominant carbonate phases in most freshwater settings and changes in their concentrations are usually driven by temperature, productivity, detritic influx, and hydrological variations (e.g., Kelts and Talbot, 1990; Wick et al., 2003; Wagner et al., 2009; Vogel et al., 2010b). However, other carbonate phases, such as siderite ($FeCO_3$), dolomite ($CaMg(CO_3)_2$), and/or ankerite ($Ca(MgFeMn)(CO_3)_2$), can also be present in certain settings (e.g., De Decker and Last, 1988; Stevens et al., 2012; Lacey et al., 2015). Moreover, the measurement of isotopes, such as carbon or oxygen isotopes (see Section 2.1.6), require relatively small quantities of sediment if the concentration of biomineralized carbon or oxygen is sufficient.

A new analytical method that has been applied in recent deep-drilling projects for the analysis of discrete samples is Fourier transformed infrared spectroscopy (FTIRS). FTIRS is a relatively fast and cost efficient method, which requires small sample amounts and can be used to infer absolute concentrations of biogenic silica (bSi), carbonate (TIC), and organic matter (TOC) with a single measurement (Vogel et al., 2008; Meyer-Jacob et al., 2014a). The low processing time might be important for the continuous analysis of long drilling sequences (e.g., Meyer-Jacob et al., 2014b) and for high-resolution studies of specific time slices in these sediment records (e.g., Cunningham et al., 2013). Further applications of FTIRS involve the determination of relative changes in the abundance of different carbonate phases (Lacey et al., 2016).

Overall, the combination of information from individual proxies and future progress in the development of analytical methods as well as scanning and logging techniques will significantly help to improve the study of environmental changes from sedimentary records in ancient lakes.

2.1.5. Age-depth models

Creating a reliable and robust chronological framework is fundamental for drilling studies across disciplines and, hence, also for the synthesized interpretation of paleoenvironmental, climatological, and biological data. Age-depth relationships in non-marine records are commonly established by combining absolute chronological information from radiometric and magnetic dating methods, and from varve counting with chronostratigraphic information derived from comparisons of a proxy response to a reference record (e.g., Nowaczyk et al., 2013; Stockhecke et al., 2014; Francke et al., 2016). Similar to dendrochronology (tree ring counting), varve counting provides robust age-depth control points as varves consist of thin (millimeter scale), characteristic seasonal summer and winter deposits (so-called laminea). However, the preservation of varves in lacustrine sediments may depend on several factors, such as the absence of sediment-dwelling organisms or the presence of anoxic bottom water conditions.

Obtaining absolute ages from radiometric dating techniques (Table 1) or techniques that utilize radiometrically induced lattice effects in certain mineral phases requires the presence of suitable/datable materials in the studied sediment sequence (reviewed in Bradley, 2014). In addition, the different dating methods cover different time ranges. Consequently, various radiometric dating techniques are typically used in combination and choices depend on the age range covered in a core, the anticipated/estimated age of a specific dating point, and the presence of suitable materials. Whereas the relative error of some of these techniques is small, absolute uncertainties increase in the deeper sediment record and obtained dates can be biased by a variety of physical and chemical effects. Nevertheless, dates from radiometric methods provide the most precise chronological tie points, and are therefore introduced as 1st order constraints in age-depth calculations.

Additional 1st order constraints can be obtained by correlating the chemical composition of tephra layers to the volcanic eruption of which the age is known or to tephra layers with identical composition found in other, well-dated sediment sequences (e.g., Wulf et al., 2008; Sulpizio et al., 2010; Vogel et al., 2010a; Leicher et al., 2016). However, an unequivocal correlation of tephra layers to known eruptions based on their chemical composition is, due to the often encountered similarity in glass shard chemistry of ejectas from different eruptions, not always straightforward. In recent studies, these limitations have been partly overcome by additional measurement of the trace element and isotope chemistry of glass shards (e.g., Sulpizio et al., 2013; Insinga et al., 2014; Albert et al., 2015; Tomlinson et al., 2015).

Further independent age control can be provided by paleomagnetic data. Polarity reversals and excursions of the earth's magnetic field are common in the geological record (Merrill et al., 2006). Moreover, the intensity and vector (secular variation) of the earth's magnetic field varies within magnetic chrons. As magnetic particles will be aligned during and after settling on the lake floor, their orientation in the sediment core depends on the direction and strength of the ambient magnetic field during or shortly after deposition (depositional remnant magnetization, DRM). Alignment of particles during deposition is, however, not always an instantaneous process and different factors, such as the lock-in depth of DRM (i.e., the sediment depth where magnetic particles are aligned to the ambient magnetic field, important for sedimentation rates of <10 cm ky^{-1}; Roberts and Winklhofer, 2004) and early diagenetic formation of ferrimagnetic minerals (important in low oxygen environments) should be considered (see Tauxe, 1993; Roberts et al., 2013 for more details). Age information from well-dated, paleomagnetic reference records can be transferred to the analyzed sediment sequence, if similar magnetic features were identified (Peck et al., 1996; Frank et al., 2002; Nowaczyk et al., 2013). A number of magnetic reversals and excursions have been dated using absolute radiometric dating methods (Laj and Channell, 2009 and references therein). This is, however, not yet the case for the entirety of these events in the geological history. In some cases, the chronology of the paleomagnetic reference records is based on synchronization of marine benthic oxygen isotope data of either individual or stacked records with orbital parameters, standard oxygen isotope stacks (SPECMAP and LR04), or ice-core records (GISP2). Where absolute age control of paleomagnetic events based on radiometric methods is absent, ages derived from magnetostratigraphy should not be regarded as absolute age control points.

Correlation of a proxy response measured in a sediment record to orbital parameters or to the global benthic foraminifera oxygen isotope stack LR04 (Lisiecki and Raymo, 2005), which primarily displays ice-volume/sea-level changes during the Quaternary, is a chronostratigraphic method that is often used to refine age-models when independent age control points are sparse. At Lake Ohrid, for example, variations in the winter season length and the intensity of local insolation during summer trigger changes in the primary productivity and mixing in the lake (Francke et al., 2016). These variations affect the TOC content of Lake Ohrid's sediments, which allow tuning the TOC concentrations with the Earth's orbital parameters (i.e., local summer insolation and winter season length). At Lake El'gygytgyn, various stratigraphic parameters, which are related to the redox conditions at the lake floor and to the climatic conditions in the surroundings of the lake, show variation synchronous with the northern hemisphere insolation and the global benthic isotope stack LR04 (Nowaczyk et al., 2013).

Tuning of a proxy response against reference records requires a thorough understanding of the processes that shape the proxy response to interpret possible leads and lags compared to the reference dataset (Prokopenko et al., 2006). This is particularly important in lake studies as proxy/forcing relationships are strongly dependent on site characteristics. Ideally, leads and lags between proxy responses and reference records should be tested on horizons for which absolute time markers are available in both the studied and reference records. Zanchetta et al. (2016) showed that tuning against an absolutely-dated reference record from the vicinity of the study site can improve the understanding of a proxy response compared to insolation and ice-volume reference records. Furthermore, this approach may enable the identification of the synchronicity or asynchronicity of climate events compared to the

Table 1
Selected absolute dating techniques for sediment cores. ESR = electron spin resonance; OSL = optically stimulated luminescence. ^{10}Be refers to burial dating.

	^{14}C	Ar/Ar	U/Th	U/Pb	OSL	ESR	^{10}Be
Material	Organic matter/carbonate fossils	Volcanic glass	Endogenic/authigenic carbonates	Endogenic/authigenic carbonates	Quartz/feldspar	Carbonates	Quartz
Age range (My)	0–0.05	~0.01–4600	0–0.5	1–4600	0.001–0.2	0.001–0.2	~0.01–4
Precision (%)	1–5	5	1	0.1–1	~10	~10	10–20

reference records and other, absolutely-dated regional records. The feasibility of such endeavors, however, depends on the availability of suitable reference records in the region.

Chronological constraints adopted from tuning against a reference record comprise two potential errors: uncertainties introduced by the tuning and the uncertainty of the age model of the reference record. Therefore, these tie points are not independent and introduced as 2nd order constraints (e.g., Nowaczyk et al., 2013; Francke et al., 2016). For example, the chronology of the global benthic isotope stack LR04, which is frequently used as reference record (see above), comprises uncertainties in a range of ±4–40 ky for a timeframe of 0–5.3 My, as the age model of LR04 is based on tuning the benthic isotope data to the 21 June insolation at 65°N (Lisiecki and Raymo, 2005). For records younger than 1 My (a relevant range for most lake sediment studies), the error of LR04 is estimated close to ±4 ky.

Once a sufficient number of data points have been obtained via the outlined methods, a quantitative age-depth model can be calculated by interpolation between the individual age control points (Fig. 4). For this purpose, various interpolation methods, such as linear interpolation, linear regression, polymodal interpolation, or smooth spline interpolation, can be used. Considering the sedimentological characteristics, expected sedimentation rates, and other stratigraphic information, including the position of event layers (mass wasting deposits, tephra layers) and hiatuses, forms the basis for deciding which interpolation method is most appropriate. Linear interpolation implies abrupt changes in the sedimentation rate at each age control point (sensu Blaauw, 2010; Blaauw and Christen, 2011), which, in most cases, is not supported by the lithological characteristics and stratigraphic information from the studied sediment succession. Polymodal or smooth spline interpolation methods calculate more gradual changes of sedimentation rates and are often more suitable for age-depth modeling. Non-linear changes in sedimentation rates between two age points are incorporated in new age-depth modeling software, e.g., Bacon v2.2 (Blaauw and Christen, 2011), which has been applied only recently for age-depth modeling on long lacustrine sediment sequences (e.g., Shanahan et al., 2013; Francke et al., 2016). It uses Bayesian statistics and Markov chain Monte Carlo iterations to infer the accumulation history based on a priori assumptions about the sedimentation rate and its variability over geological time (Fig. 5). Another advantage of this approach is that uncertainties of tuning and independent age points can directly be included into the age-depth modeling (Fig. 5).

In summary, age-depth models, which reveal the relationship between increasing sediment depth and age (Fig. 5), are a prerequisite to integrate and interpret biological and geological data. However, as each dating technique has its specific limitations, their respective uncertainties should be considered during age-depth calculations (Blaauw and Heegaard, 2012).

2.1.6. Stable isotopes

The analysis of stable isotope ratios in mineralized components from lake sediments (Leng and Marshall, 2004), are well established in paleoclimatology, paleolimnology, and limnogeology since the early work of McCrea (1950) and Urey et al. (1951). Stable isotopes (in particular $\delta^{18}O$ and $\delta^{13}C$; Leng and Marshall, 2004) are incorporated into a number of different components that precipitate or grow in lake waters and subsequently get deposited within the sediments, for example shelly materials, fine grained calcium carbonate crystals (a chemical precipitate called endogenic carbonate), and diatoms (siliceous algae, see also Section 2.2.2). Paleoclimate studies commonly use changes in the isotope compositions throughout the sediment succession to infer changes in either temperature ($\delta^{18}O$) or the isotopic composition of lake water ($\delta^{18}O/\delta^{13}C$). With $\delta^{18}O$, the latter could be a function of changes in/or at the source of water to the lake (changes in moisture source) or the precipitation/evaporation balance. By looking at changes in $\delta^{18}O$ through time, and depending on the characteristics of the lake in question, it is possible to reconstruct an aspect of paleoclimate for a particular location. With $\delta^{13}C$, changes are usually ascribed to the source of the dissolved carbon ion or changes in productivity of the aquatic plants and phytoplankton, which preferentially utilize ^{12}C (Leng and Marshall, 2004).

In ancient lakes, where the isotope composition of the lake water has been demonstrated to reflect mean annual precipitation, it has been shown that a signal of climate change can be determined from the analysis of biogenic calcite from the shells of benthic ostracods, which live below the thermocline and are thus not affected by seasonal shifts in temperature or evaporation. For endorheic lakes, the isotopic composition of the carbonate has been shown to reflect lake level, most significantly between glacial-interglacial cycles. In Lake Ohrid, for example, these cycles suggest that the lake has been subject to hydroclimate fluctuations on orbital and millennial timescales (Lacey et al., 2016).

Despite an increase in the application of stable isotope techniques in paleolimnology, interpreting stable isotope results can be challenging. These challenges may be general or specific to a core, lake, or basin. The vast majority of studies focus on oxygen isotopes because their interpretation can be linked directly or indirectly to climate change. Factors that have an influence on the oxygen isotope composition of the lake water ($\delta^{18}O_{lakewater}$) are given in Fig. 6. The oxygen isotope composition of water in hydrologically open lakes ($\delta^{18}O_{water}$) will dominantly reflect the isotopic composition of the precipitation received by the lake ($\delta^{18}O_{precipitation}$ or δp). Many studies have shown that the $\delta^{18}O$ signature of mean annual precipitation varies globally between regions and covariation in δp (and δD) defines a global meteoric water line (Craig, 1961). Outside the tropics, where 'amount' effects (relating to intense precipitation events) are common, and δp varies systematically with mean annual temperature (Clark and Fritz, 1997; http://isohis.iaea.org/GNIP), δp thus broadly correlates with latitude and altitude of a site (Bowen and Wilkinson, 2002 and references therein). The global relationship between changes in δp with temperature is referred to as the 'Dansgaard relationship' (after Dansgaard, 1964).

In many lacustrine environments it cannot be assumed that either the modern or past $\delta^{18}O_{lakewater}$ reflect that of mean annual precipitation. The residence time of water in the lake and modification of water compositions by catchment and lake processes are particularly important to consider as evaporation will affect the water composition. The size of a lake in comparison to its catchment is important because the isotope composition of rain and snowfall are very variable on short

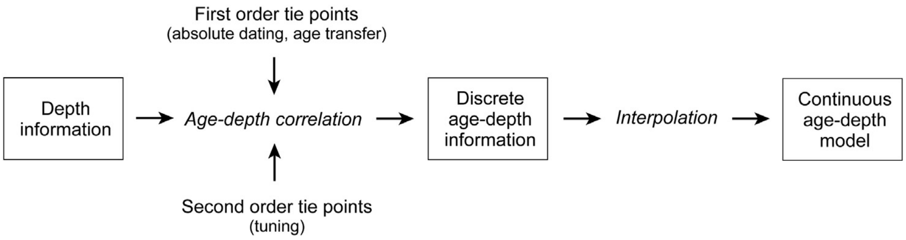

Fig. 4. Flow chart illustrating the required steps to establish an age-depth model.

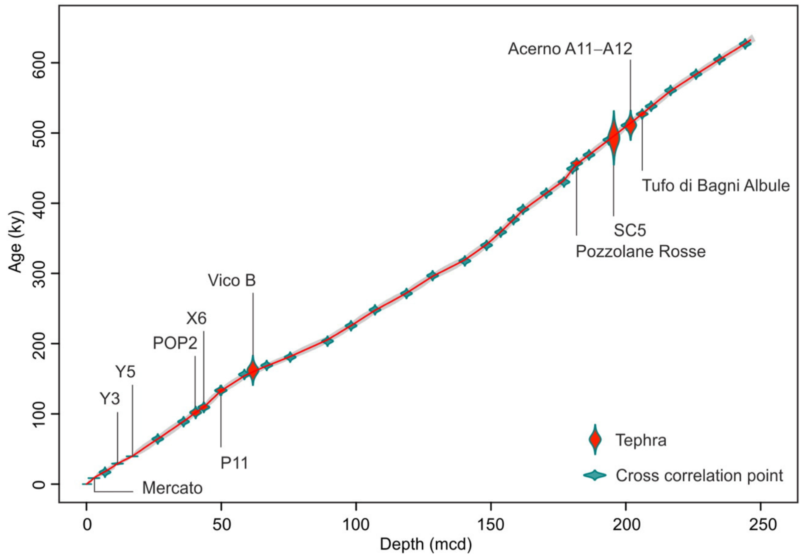

Fig. 5. Age model of the DEEP site sediment sequence from Lake Ohrid (Macedonia, Albania) down to 247.8 m composite depth (mcd) corresponding to an age of 637 ky. Modified from Francke et al. (2016).

time scales: a lake therefore needs to be big enough and well enough mixed for its isotope composition to 'average out' the short-term variation and reflect mean annual precipitation. The greatest degree of variation in the isotope composition of rainfall occurs on the time scale of hours to days. Seasonal variation in precipitation is likely to be much more significant in small, short residence time lakes as these tend to have $\delta^{18}O_{water}$ values that are regularly displaced by later precipitation. However, if the inference of intra-seasonal fluctuations is of interest, growth increments analyses of the isotope composition of molluscan shells will often provide intraseasonal information (Kaandorp et al., 2005; Taft et al., 2012; Leng and Lewis, 2014).

At temperate latitudes, monthly mean rainfall $\delta^{18}O$ typically have an overall range of 2–8‰. The range increases with continentality of the site. At such sites, winter rainfall has significantly more negative $\delta^{18}O$ than its summer equivalent. If a lake is very small in relation to its catchment (with residence times of < 1 year), winter rainfall will be physically displaced by summer rainfall and thus $\delta^{18}O_{water}$ will be influenced by seasonal variation. Therefore, deep ancient lakes with their long water residence time are particularly useful for isotope studies. The precise isotope composition of lake water at any time will depend on the amount of rain in the different seasons and the degree of mixing of winter and summer rainfall. Even in lakes with relatively long overall residence times, surface waters may have isotope compositions that reflect summer rainfall rather than mean annual precipitation if the waters become stratified.

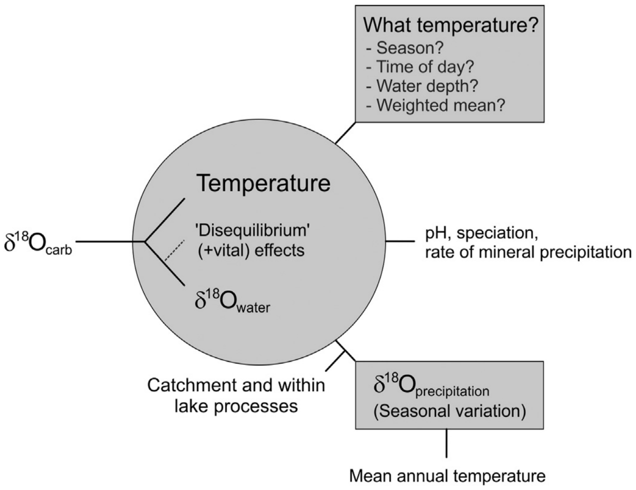

Fig. 6. Overview of factors that can influence the isotope composition of a lacustrine carbonate or biogenic silica ($\delta^{18}O$). For equilibrium mineral precipitation, the oxygen isotope composition of the mineral is controlled only by the temperature and by the isotope composition of the lake water from which the mineral precipitated. If a mineral is precipitated in isotope equilibrium, paleotemperature equations can be used to estimate past temperatures and their changes. Other factors, such as pH, mineral speciation, and rates of mineral precipitation, may affect the fractionation relationship. Modified from Leng and Marshall (2004).

The $\delta^{18}O$ of components within lake sediments contain information on temperature, and there are many empirically derived 'paleotemperature equations'. During equilibrium precipitation, carbonates for example, have $\delta^{18}O$ compositions that decrease by about 0.24‰ for each 1 °C increase in temperature (Craig, 1965). There is a number of paleotemperature equations for the equilibrium precipitation. For example, Kim and O'Neil (1997) proposed an equilibrium fractionation relationship, which was reordered by Leng and Marshall (2004). Assuming that equilibrium precipitation has occurred, the interpretation of oxygen isotope data in terms of paleotemperatures requires an understanding of two processes that have opposing effects on the composition of a carbonate or silicate precipitate. At intermediate and high latitudes, the oxygen isotope composition of mean annual precipitation correlates directly with change in temperature with a gradient of approximately $+0.6‰\,°C^{-1}$ (Dansgaard, 1964). Opposing this effect from being transferred directly into the sediment record, the equilibrium isotope fractionation between carbonate and water has a gradient of around $-0.24‰\,°C^{-1}$. For many lake records, the carbonate response to temperature will be dominated by the change in the isotope composition of precipitation and effectively 'damped' by the opposing effect of mineral-water fractionation. In this case, the measured carbonate values will covary with temperature – with an increase of $\sim 0.36‰°C^{-1}$ (Eicher and Siegenthaler, 1976). This is reasonable for the paleoclimatic interpretation of many lakes but it implicitly assumes that $\delta p/dT$ always changes according to the Dansgaard relationship.

Evaporation has a major influence on the oxygen isotope composition of any standing water body. For closed (terminal) lakes, where water loss is mainly through evaporation, lake waters tend to have high $\delta^{18}O$ (Fig. 6). Measured $\delta^{18}O$ (and δD) values are always higher than those of average precipitation as the lighter isotopes of ^{16}O and (^{1}H) are preferentially lost to evaporation. Isotope records from such lakes show large swings in composition as the ratio of the amount of precipitation to evaporation (P/E) changes with climate. Any interpretation of the isotope records from a lake must take into account the hydrology of the lake and likely changes in hydrology that may have occurred in the past.

Endogenic carbonates are still the most commonly utilized materials for stable isotope analysis. They are formed by photosynthetic utilization of CO_2 and resultant calcium carbonate supersaturation (often mistakenly referred to as authigenic carbonate). In most temperate and high-latitude regions, endogenic carbonates are precipitated mainly in the summer months during periods of maximum phytoplankton productivity (Leng et al., 1999b, Teranes and McKenzie, 2001). In mid-latitude and tropical lakes, phytoplankton growth may occur throughout the year although other mechanisms, such as supersaturation, may also cause continuous carbonate precipitation (e.g., the Dead Sea aragonite; Niemi et al., 1997). More commonly in the tropics, carbonate precipitation is related to phytoplankton blooms associated with annual lake-water mixing and nutrient availability (sensu Lamb et al., 2002). The advantage of using endogenic carbonate in stable isotope studies is that it provides an integrated climate signal for the whole sample, which may be time-averaged over several years, depending on sedimentation rate. However, there are some potential problems. It can be difficult to distinguish between authigenic (diagenetic carbonate formed within the sediment) and allogenic (detritus derived from the terrestrial environment) carbonates, especially in karstic regions, so there is always the possibility of contamination of the isotope signal from a washed-in component (sensu Hammarlund and Buchardt, 1996). Some carbonates do not precipitate in equilibrium with their environments. Disequilibrium effects (often called 'vital effects' in biogenic materials) have been attributed to rates of precipitation, pH effects, incorporation of metabolic fluids, and growth in microenvironments not typical of the water body as a whole (Leng and Marshall, 2004). Also, there are several endogenic carbonate minerals that could precipitate out in a lake and each mineral has its own mineral-water fractionation (see above). In freshwater systems, calcite ($CaCO_3$) usually forms. However, with increasing evaporation, other forms can occur, such as aragonite ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$) (e.g., Lake Bosumtwi; Talbot and Kelts, 1986). Physically separating different carbonate minerals is not easy, although respective protocols are available (e.g., Dean et al., 2015). Diatom silica $\delta^{18}O$ is being increasingly utilized in paleolimnology, and many of the issues are the same as with carbonates. However, the main consideration is that almost pure diatom samples are required as extraction techniques will liberate oxygen from all the components in the sediment (Leng and Barker, 2006).

Overall, isotope geochemistry is an essential part of paleoclimatological and (paleo-)limnological research. Over the last few decades, the analysis of isotopes in carbonate materials has contributed significantly to our understanding of a broad array of environmental change research. Nevertheless, advances are still to be made. These include better preparation, analysis, and interpretation of some of the less routine materials (other than carbonate), such as biogenic silica and chironomid chitin (Leng and Henderson, 2013). There is also the up and coming field of determining the excess of ^{13}C–^{18}O bonds in clumped isotopes (Leng and Henderson, 2013). Bulk carbon and nitrogen isotope ratios within organic materials are also frequently analyzed but do not provide specific information and, therefore, are not further discussed here. Moreover, paleoenvironmental studies targeting paleohydrology and biome dynamics increasingly use carbon and hydrogen isotope ratios of specific organic sources and compounds, such as leaf waxes and algal lipids (for details see Section 2.2.4).

2.2. Biological and paleontological data and methods

2.2.1. Macrofossils

Macrofossils include all remains of organisms from the remote past large enough to be visible without a microscope. Although fossils are widely reported from lake sediment cores, obtaining macrofossils is often a hope, though rarely an objective of drilling campaigns. The larger individual fossils are, the smaller the chance that such fossils will be picked up in a sediment core. The diameter of drill cores (typically 48–85 mm) sets a strong constraint on the possible recovery of macrofossils and their size. Even fossils with a maximum linear size of 2–4 cm unlikely fall within the categories of exquisite preservation and complete recovery. An additional difficulty for the recovery of macrofossils is that cores are typically cut and observed perpendicular to bedding planes, so that macrofossils may easily be overlooked. Recovery of macrofossils from a drilling core may also require disturbing the sediments more than is desirable at the early stages of core documentation (see Section 2.1.2). To overcome this issue, CT- or radiographic images can be obtained of core sections during the core documentation procedure just after opening a core.

Macrofossils may be obtained from sediment cores as complete body fossils, such as mollusks, plant leaves, or isolated biomineralized parts, such as bones, teeth, charophyte oogones, seeds, plant cuticles, and wood fragments (Taviani et al., 2000; Reinthal et al., 2011; Cohen, 2012; Pepe et al., 2013, Mazzini et al., 2015). Lake sediments regularly yield fossils with exceptional preservation (e.g., Richter and Wedmann, 2005; Smith, 2012), and sometimes the temporal resolution is extraordinary, i.e., centennial to even annual scales (Bell et al., 2006; Hunt et al., 2008; Van Bocxlaer and Hunt, 2013). The choice of drilling sites affects the chances to recover macrofossils as well as fossil preservation (reviewed in Cohen, 2012). For example, drilling in the deeper waters of a meromictic lake is unlikely to yield many macrofossils because the benthic habitats at these sites can usually be expected to have been inhabitable for benthic life for most of the lake's history (see Section 2.1.1), although remains of pelagic organisms, such as lake sardines (Cyprinidae) may end up in such cores (Reinthal et al., 2011). Hence, macrofossils from nearshore habitats generally can be found only in low abundances in offshore cores. Drill sites relatively close to the shores, in general, provide greater potential to recover macrofossils (Harzhauser et al., 2013). However, such sites usually contain large proportions of sand and gravel (indicative for greater water energy and poorer preservation potential), and are indicative of greater sediment redeposition, which makes them undesirable targets for paleoclimate studies and difficult to drill (see also Section 2.1.1). Mid-lake topographic highs are often selected as disturbance can be anticipated to have been limited, but such sites are unlikely the most suitable target of drilling from the perspective of macrofossil recovery. If recovered, macrofossils may serve several research goals, and because of the practical limitations of finding macrofossils, we discern here between goals that can be reached with a low number of specimens and those that require the sampling of larger numbers of macrofossils.

Single or scarce macrofossil finds may provide valuable information for the study of paleoenvironments or organismal evolution. They can also facilitate dating analyses, either directly if the fossil provides datable substances (e.g., charcoal, shells, and seeds of terrestrial plants), via the study of ancient amino acids (see Section 2.2.5), or if the macrofossil can be placed into a biostratigraphic framework for the studied basin. It can also be used indirectly if the fossil can be inserted with reasonable constraint into a time-calibrated phylogeny of extant taxa (see Section 3.3). Moreover, macrofossils may provide unique opportunities for paleolimnological reconstructions, either via isotope studies (see Section 2.1.6) or via reconstructions directly based on the fossils' properties or habitat characteristics (see Section 3.1). Beyond calibrating the molecular clock (see Section 3.3), macrofossils may give a good insight into organismal evolution including information on how long the taxon is present in the basin, on ancestral character states (see Section

3.4), and on morphological adaptation by comparing the morphology of the fossil to that of modern populations. In the most fortunate case, the fossil may be exceptionally preserved so that attempts to isolate ancient amino acids or DNA can be undertaken (see Sections 2.2.5, 2.2.6).

However, most biological goals relate to community changes over time or morphological evolution, and thus require sampling multiple taxa or individuals per interval (see also Section 3.1). As indicated above, chances to recover macrofossils in substantial numbers relate first to the size of such fossils, but furthermore also to preservation potential (e.g., $CaCO_3$-bearing fossils will corrode when deposited below the local carbonate-compensation depth), general abundance, and lifestyle (e.g., benthic vs. pelagic). Therefore, most of the macrofossils that can be used for goals requiring abundant finds are only marginally larger than microfossils (e.g., fish teeth, or small mollusks; see Section 2.2.2). Continuity of the recovery of such fossils throughout a core relates primarily to the preservation potential under changing chemical conditions (dissolution, corrosion, and abrasion diminish preservation potential) and the patchiness of a taxon's occurrence. Patchy taxa can be expected to occur with great numbers at few intervals, whereas more evenly distributed taxa would occur in smaller numbers throughout more intervals within a sediment core. The abundance of the target taxon per interval of sediment is the main determinant of the resolution at which the taxon's macrofossils can be studied. For example, if an interval of 2 cm of sediment yields on average 40 seeds, but only 2 fish teeth and 4 mollusks, then the core can be analyzed in 2 cm intervals for plant remains, but perhaps only in 10+ cm intervals for the study of fish teeth and mollusks, which affects the power to discover trends (see the simulation in Fig. 7).

Upon detecting macrofossil remains in lacustrine sediment cores, the specimens are usually picked out, identified and/or counted prior to further analyses, such as dating or the study of biomarkers, isotope and element geochemistry. The advantage of using macrofossils for the latter studies is that analyses may be performed on a single fossil and may yield data on, e.g., seasonal fluctuations. This procedure allows a more accurate signal to be obtained than when individuals are pooled or a sample of bulk organic remains is analyzed, because these latter samples provide averaged signals.

Identifications of macrofossils may be challenging because taphonomic processes, such as time averaging, sorting, and post-mortem pooling (e.g., deposition of two taxa with fine-scaled habitat differences in the same assemblage), may considerably alter the amount of variation observed in a fossil assemblage in comparison to that in a modern population (e.g., Bell et al., 1987; Bush et al., 2002). Furthermore, chronospecies or taxa with character states intermediate to those of two or more modern species remain a problem. They result in the poor applicability of identification keys and potentially in doubtful identifications—much of these aspects are inherent to fossils and hence, apply to microfossils as well. Initial exploratory analyses may be conducted to examine occurrence data (counts) belonging to different groups of taxa (e.g., endemics vs. non-endemics), or to calculate biodiversity or community estimators/indices to compare a number of target assemblages (e.g., faunal and floral compositions before, during, and after a climatic or geological event). If qualitative observations suggest potential morphological changes, measurements may be taken to accurately document these changes through time. Such measurements can range from traditional caliper measurements to studies of size and shape with fractal dimensions or geometric morphometrics. The choice for a particular method typically depends on the complexity of the signal, the time required to document/measure a single specimen, and the total number of specimens to be studied. ‘Targeted macrofossils’ may also be studied with more time-consuming 3D scanning methods and/or biogeochemical analyses (e.g., to document chemical composition, to study diagenetic processes, or to get information on an environmental proxy).

After exploratory analyses, more in-depth statistical and time-series studies can be undertaken. Fossils (mainly microfossils) encountered in sediment cores are regularly used for analyses of community composition, often in relation to environmental change (e.g., Cohen et al., 2007; Kröpelin et al., 2008; Harzhauser et al., 2013; see also Section 2.2.2) or to document morphological change and evolution in the fossil record (e.g., Pearson and Ezard, 2014 and references therein). For studying shape/community changes related to environmental change or organismal evolution, statistical and time-series analyses provide a useful framework to explore and test relationships between predictor variables and organismal change, or to fit models of morphological evolution. Major determinants of the power of such time-series approaches to discover ecological patterns will be the strength of the association between the predictor variables and organismal/community change, the range of values of the predictor variable observed throughout the core, and the variation in the dependent variable. To reliably document patterns of morphological evolution in a fossil lineage, a major determinant of analytical power will be the ratio of variation within individual samples to the changes between consecutive samples. For example, fewer specimens per interval will be required to document a strong morphological trend in a time series with limited within-sample variation than for a time series with the same trend and great within-sample variation. Additionally, as mentioned, the number of sampled intervals throughout the core for which all required data are available and, hence, the length of the time series is a determining factor of analytical power (Fig. 7). To assess the feasibility of time-series analyses with drill core data, detailed analyses of the anticipated patterns of change and the various components that contribute to the variation in fossil assemblages may be required. Explorations examining variation in homologous or analogous modern faunas and floras may be required to understand

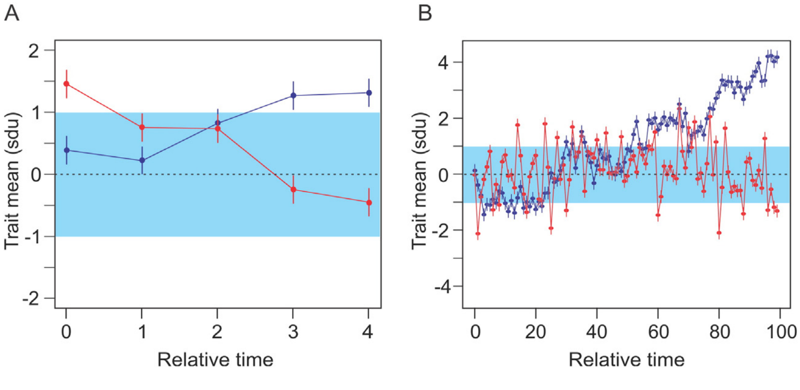

Fig. 7. The feasibility of detecting ecological or evolutionary signals from drilling data depends on the length of a time series (here expressed as relative time). Simulated time series of a morphological trait (scaled in within-sample standard deviation units; sdu) are represented in A) and B). The trait indicated in blue was simulated with a model of directional change, the red trait with a model of morphological stasis. Parameters were identical for each model in A and B. Only over an extended period of time (B), morphological stasis and directional change can be distinguished (blue area = 2 sdu). Modeling was performed in R3.3.1 (R Development Core Team, 2015) with the paleoTS package (Hunt, 2006, 2012).

how different sources of variation contribute to the total variation observed in fossil assemblages. Dieleman et al. (2015) presented such an exploration for the study of fossil cichlid teeth from the African crater Lake Challa.

Offering suggestions on how future lake drilling campaigns could be designed optimally for the study of macrofossils is not easy. First, strategies depend on the group of macrofossils that is specifically targeted. Second, each ancient lake has unique ecosystems that differ from those of other lakes and the design of the program needs to be adjusted to the specific target lake. Third, the multidisciplinary nature of many drilling operations may regularly weaken the feasibility of obtaining a continuous fossil record due to compromises in site selection (Cohen, 2012; see also Section 2.1).

Nevertheless, promising subprojects based on macrofossils can be constructed, and the following aspects can augment the potential of such programs. Drilling campaigns usually aim to retrieve multiple cores and one (or some) of the drilling locations may be selected where abundant macrofossils can be expected (e.g., Wagner et al., 2014a). Moreover, fossil-bearing outcrops may be present in the basin, and these too may contain a rich archive of faunal and environmental history. An outstanding example is the mollusk fauna from the Turkana Basin (Williamson, 1981, 1985; Van Bocxlaer et al., 2008). However, this example also highlights that interpretations of outcrop data are highly dependent on the available stratigraphic control. Integrating data from lake drilling and the study of fossiliferous outcrops would be a powerful approach to eliminate the weaknesses of individual strategies, and hence to solidify the documentation of the lake's natural history. Specific hypotheses that were constructed based on the study of the modern fauna and that are testable with macrofossils from sediment cores can provide good objectives for a drilling strategy that maximizes the potential to recover macrofossils.

Several future trends in data generation and analyses specifically geared to macrofossils are conceivable. An important starting point is the diameter of the core. Bigger diameters would increase the potential to obtain macrofossils, however, they would also increase the chance of technical difficulties. Core scanning (i.e., CT-scanning) in a way that allows the non-invasive detection of various macrofossils on bedding planes based on biomineral concentrations, density contrasts, or shape would be invaluable to select targeted parts of the cores for detailed examination, with minimal disturbance to other parts. Furthermore, more automated methods in fossil retrieval and visualization would help and speed up the development of goals based on large numbers of fossils. Automated identification, measurement, and digitization would greatly facilitate the study of macrofossils, but the desirability of such developments ranges beyond the drilling communities at large (e.g., Houle et al., 2003).

2.2.2. Microfossils

Lake sediments are also valuable archives of microfossils, i.e., remains or traces of organisms from the past that require a microscope for study. Typical microfossils in sediment cores include pollen from seed plants, single-celled protists with biomineralized shells (e.g., calcareous dinoflagellate cysts and siliceous diatoms), as well as small shelled invertebrates, such as ostracods and micromollusks. As the latter share many characteristics with macrofossils (see Section 2.2.1), this review focuses mainly on the discussion of diatom and pollen microfossils, which have great potential for environmental studies based on lake cores.

Diatoms (phylum Bacillariophyta) are photosynthetic eukaryotes. Their silica shell ('frustule') preserves exceptionally well, making diatoms one of the most abundant microfossil taxa in lacustrine systems (Gross, 2012). Moreover, the frustules often display species-specific ornamentation, providing important information for identification and classifications (Round et al., 1990).

Ancient lakes regularly display an exceptional diatom biodiversity (Cocquyt, 1998; Levkov et al., 2007; Mackay et al., 2010; Kulikovskiy et al., 2012; Wagner et al., 2014a) with the total number of morphotypes often exceeding 1000 (Levkov and Williams, 2012). Most diatom species are benthic; only few species are euplanktonic and spend their entire life cycle in the water column (Stoermer and Edlund, 1999; Khursevich and Prokopenko, 2009; Snyder et al., 2013; Recasens et al., 2015).

Current diatom research in ancient lakes strongly focuses on the reconstruction of paleoenvironments and past environmental fluctuations. One of the most frequently used approaches is the chronological assessment of changes in species composition and relative abundances of the dominant species at various temporal scales (orbital, millennial, centennial, or pluriannual). These community studies are typically undertaken at low taxonomic resolutions (i.e., species from one or several genera are combined) and with a relatively large number of valves to be counted per sampled sediment interval (e.g., 400–600). As habitat characteristics and ecological preferences of many diatom species are well characterized, the collective diatom community found in a sediment sample then becomes an important source for analysis, e.g., with paleoecological transfer functions (see Section 3.1).

However, given that diatom communities in ancient lakes are often dominated by endemic species, approaches based on transfer functions may be problematic due to the lack of analogues in the existing calibration sets. In fact, given the old age of many diatom fossils in ancient lakes, they may not even have extant analogues in the lake (= supralimital evolution; Wesselingh, 2007; Mackay et al., 2010). Nonetheless, some attempts have been made to establish modern diatom calibration sets for ancient lakes (e.g., Lake Baikal; Mackay et al., 2003). These attempts try to overcome the limitation of missing modern analogues by examining modern species compositions along environmental/spatial gradients within the lake. Moreover, some studies suggest that species with similar size and comparable ultrastructural features have similar ecological preferences (e.g., Winder et al., 2009), and this assumption may also be used in establishing calibration sets. In combination with other proxies (e.g., biogeochemical data derived from the same sediment record), these new approaches enable a more reliable paleoenvironmental interpretation of diatom communities (Bradbury, 1999).

Another focus of research on diatoms obtained from ancient lake sediment cores is the assessment of a lake's primary productivity (sensu Battarbee, 1986). As diatoms are important primary producers, their concentration is a good indicator for productivity and epilimnetic nutrient availability (Zhang et al., 2016). Productivity data have been used, for example, to identify successive glacial and interglacial stages (Rioual and Mackay, 2005; Melles et al., 2012; Meyer-Jacob et al., 2014b). This approach is especially promising if environmental fluctuations are not anticipated to have caused significant changes in diatom species composition (e.g., Cvetkoska et al., 2016).

However, diatom concentrations in drill cores may be affected by taphonomic processes, such as differential dissolution (Ryves et al., 2006). Parameters like low temperature, high pH, high detrital mineral content, and grazing, may (selectively) decrease valve preservation, and can lead to the loss of specific taxa, morphological details on individual frustules, or even the entire assemblage (Mackay, 2007; Reed et al., 2010). Moreover, biases in diatom concentrations may result from inwash of specimens from rivers, or from the loss of specimens via the lake's outflow (O'Sullivan, 2004). In addition, diatom concentrations do not account for size differences in taxa or changes in sediment accumulation rates. Therefore, the biovolume-accumulation rate, i.e., diatom concentration divided by sediment-accumulation rate and corrected for size differences between taxa (O'Sullivan, 2004), could be a more powerful proxy for a lake's productivity (Rioual and Mackay, 2005).

Several other aspects complicate interpretations of diatom records from ancient lakes (Bradbury, 1999). Besides the taxonomical and ecological uncertainties mentioned above, only a fraction of the actual (endemic) biodiversity is known. Moreover, recent changes in diatom species and genus concepts have led to an introduction of >5000 new names (Fourtanier and Kociolek, 2011). Even specialists with several years of experience have difficulties keeping up with these changes and, more importantly, to unify their taxonomic opinions with those of colleagues. Finally, diatom analyses are time consuming, thus limiting the number of samples that can be analyzed. Therefore, samples from sediment cores are often subdivided and analyzed in parallel by several researchers, creating a potential bias in observations. Moreover, species within common planktonic genera, such as *Cyclotella* and *Stephanodiscus*, differ in morphological features that are difficult to assess with a light microscope, potentially increasing determination errors. The problem of taxonomic accuracy is further increased by the cryptic nature of some closely related diatom species, on the one hand, and by a partially high phenotypic plasticity, potentially driven by environmental factors, on the other hand (e.g., Cvetkoska et al., 2012; García et al., 2012). These problems are progressively addressed via molecular analyses and by cultivation experiments, which have shown that diatoms can change morphological features during cultivation (e.g., Abarca et al., 2014; Rose and Cox, 2014). Nonetheless, diatom studies can provide very valuable insights into the primary productivity of ancient lakes, and into the interpretation of past environmental changes (see Section 3.1).

Pollen produced by terrestrial seed plants is frequently deposited in lacustrine systems by wind (‘pollen rain’), riverine inflow, and animals (e.g., O'Sullivan, 2004). Similar to diatoms, pollen is often well-preserved in long sediment cores. The high resistance to degradation under anoxic/hypoxic conditions is due to the polymer sporopollenin, which is a component of the outer wall (= exine) of pollen grains (Brooks and Shaw, 1978).

The pollen deposited in sediment cores from ancient lakes is an important archive of past environmental and climatic changes. Pollen is typically present throughout the sediment core, thus enabling continuous analyses over long time scales. Moreover, their deposition is usually only affected to limited extent by hydrological and chemical changes within the lake, and, hence, pollen records typically provide accurate reflections of terrestrial events near the studied lake (O'Sullivan, 2004). Finally, pollen deposition in sediment cores may permit the reconstruction of regional changes as pollen rain is often dispersed over long distances. Thus, the record may contain the signal of both local and regional drivers of environmental change (e.g., Wagner et al., 2014a).

As the composition and provenance of pollen in lake cores are often complex due to varying pollen productivity and dispersal rates (Faegri et al., 1989), significant efforts have been made to understand the extent to which these records represent past vegetation covers (e.g., Davis et al., 2013; Schüler et al., 2014; Trondman et al., 2015; see also Section 3.1) and, hence, how such records should be interpreted. Pollen is generally identified to the genus level, because the pollen of many closely related species cannot be distinguished using transmission light microscopy. Subsequent paleoreconstructions are generally done using pollen diagrams, which utilize information on pollen composition, concentration, and influx values (Berglund and Ralska-Jasiewiczowa, 1986). Past vegetation covers and floral compositions can then be related to the underlying climatic drivers. Tzedakis et al. (1997), for example, observed a close correlation between herbaceous vegetation and ice volume at a global scale during glacial intervals, whereas forest physiognomy and development appeared closely related to changes in temperature and humidity during interglacials. This study and following work (Tzedakis et al., 2001) reinforced evidence for a broad correspondence between climate signals provided by pollen data in long lacustrine records and oxygen isotopes from marine cores. Other pollen records have allowed short-term vegetation oscillations to be lined to centennial-scale climatic events on various smaller scales (e.g., Dansgaard et al., 1993; Broecker, 1994; Bond and Lotti, 1995). Pollen records from cores of ancient lakes that have a well-established chronology can thus provide a better understanding of climate forcing from local to global scales over several glacial/interglacial cycles (Litt et al., 2014; Sadori et al., 2016).

Other objectives of pollen analyses in long and continuous sediment records are the reconstruction of species dynamics, dating of extinction events (see also Section 3.2), and the inference of possible refuge areas. Bertini (2010), for example, could show that extinction events following climate changes did not occur synchronously across ecosystems. However, geographically-related records, in general, may show somewhat different vegetation dynamics. Part of the problem is that many previous sediment records have not been studied with a high temporal resolution and/or lack a precise chronological control.

Given the challenges and limitations outlined above for diatom and pollen microfossil analyses from sediment cores, the following recommendations for future deep-drilling projects are suggested:

i) Microfossil studies should be hierarchically structured. As analyses from long records are very laborious and time-consuming, the first target should be to produce low/medium resolution data (e.g., skeleton pollen diagrams with key pollen curves, Sadori et al., 2016 or stratigraphic diatom diagrams, Cvetkoska et al., 2016). This enables a preliminary chronological alignment with major environmental and climatic fluctuations. Only then, high-resolution diatom and pollen studies should be conducted.
ii) The temporal sampling design should adhere to the main question and time scale of the respective project. However, in case of uncertainties about the temporal resolution required, subsampling should be set up in a way that samples for higher resolution studies are available even if a first analysis is to be conducted in lower temporal resolution. This is because resampling of sediment cores that are already in long-term storage may be difficult and/or costly.
iii) Sediment subsampling should be coordinated (see Section 2.1.4). In order to be able to combine microfossil data sets and link them to other biotic and abiotic data generated in multidisciplinary deep-drilling projects, samples should, whenever possible, be taken from the same sample depth.
iv) Protocols for sample preparation and microfossil identification to be used by all scientists involved in the project have to be implemented. A ‘taxonomy working group’ should be established that elaborates and shares the taxon list with all investigators, defines diagnostic characters for problematic taxa, solves taxonomic disputes, and implements quality control procedures (e.g., Munro et al., 1990).
v) Microfossil studies should involve specialists in climate reconstruction and empirical modeling at an early stage to improve data quality and the power of subsequent analyses.

We expect to see considerable advances in microfossil biodiversity research in the future, partly driven by ongoing deep-drilling projects in ancient lakes. Comparative molecular investigations of recent taxa, particular for diatom analyses, may help clarify systematic problems, mainly in respect to cryptic species and species with high phenotypic plasticity (Kermarrec et al., 2013). Robust species-level phylogenies could then be used to statistically identify those morphological character states or combinations thereof that have a high diagnostic power. These characters could possibly also be applied to paleo-morphotypes.

As to future pollen research, a better link to studies of plant macrofossils could be established (Birks and Birks, 2000; see also Section 2.2.1). Macrofossils can often be identified with more taxonomic precision than pollen, and in the context of lake drilling, they may provide supplementary information, particularly on water plant communities (Birks and Birks, 2000; Sadori et al., 2010), and taxa that produce very little or no pollen. Integrated paleobotanical analyses may allow a more comprehensive picture of paleoenvironmental changes in ancient lakes and their watersheds to be created.

Another promising development is the 3D reconstruction of microfossils. Whereas scanning electron microscopic 3D approaches are still costly and time consuming (e.g., Mansilla et al., 2015), light microscopic 3D solutions are currently offered by several companies.

Bioinformatic advances in image analyses have also led to improved automatic identifications of diatoms (e.g., Jalba et al., 2005; Mosleh et al., 2012; Kloster et al., 2014) and pollen (Guru et al., 2013; Holt and Bennett, 2014; Marcos et al., 2015), potentially reducing processing time and identification biases. Future improvements may further increase the performance of these approaches beyond their current application as preliminary sorting tools.

2.2.3. Subsurface biosphere

Over the past decades, marine and continental drilling projects have shown a growing interest in understanding the role of microbes in the complex chemical reactions occurring in the sediments and or the sediment/water interface (Konhauser, 2007). Microbial activity in the water column of modern lakes and oceans is well known in comparison to effects of microbial life in sediments, but despite controversy on specifics, scientists generally agree that the impact of the deep marine biosphere on global biochemical cycles is massive (Whitman et al., 1998; Kallmeyer et al., 2012). As a result, there has been a substantial increase in investigations dealing with microbial activity in freshly retrieved sediments (D'Hondt et al., 2002; Inagaki, 2010). This hidden microbial mass, as well as the study of the interactions between microbes and sediments in the marine environment, is known as 'deep-biosphere research'. Analogously, the study of active microbes in lake sediments through scientific drilling have been labeled as 'subsurface biosphere research'. Many microbes in this subsurface biosphere grow extraordinarily slowly and under extreme conditions (Røy et al., 2012). Their study is critical to understand the physiological abilities and biogeochemical impact of subsurface life within the sedimentary column.

Subsurface biosphere studies have only recently been implemented in continental deep-drilling projects (Vuillemin et al., 2010, 2013a, 2013b, 2014a, 2014b; Glombitza et al., 2013; Thomas et al., 2014, 2015; see also Ariztegui et al., 2015 for a detailed description of the different sites).

The systematic study of the subsurface biosphere in lacustrine sediments will allow geologists and biologists to identify the magnitude and impact of microbes during early diagenesis on both sediments and biological remains. Geomicrobiological studies permit the identification of signatures of former microbial activity recorded in the sediments as well as investigating their impact in biogeochemical cycles. Some open questions are:

i) What are the source, composition, and global significance of subsurface communities in lacustrine basins?
ii) What is the impact of environmental change on subsurface biodiversity? Do changes in environmental conditions generate changes in microbiomes population diversity and density?
iii) How does the diversity and activity of microbial life vary with depth, geochemistry, sediment composition, and age?
iv) How does the diversity of active and non-active microbes relate spatially across the lacustrine basin at given time slices?
v) How do microbes resolve the paucity of nutrients and energy as well as the limits of life in the subsurface of lakes under contrasting physicochemical conditions?
vi) What is the influence of subsurface communities on paleoenvironmental and paleoclimatic proxies, minerals, and hydrocarbon reservoirs? How do they alter sediments and hence the data and interpretations of other workers on deep-drilling cores?

Cell counts in recent continental deep drilling in mesosaline Lake Potrok Aike (Patagonia, Argentina) and alkaline Lake Van (Turkey) indicate variable microbial activity at sediment depths reaching down to 100 mcd (Vuillemin et al., 2010; Kallmeyer et al., 2015). Additionally, investigations in hypersaline Dead Sea sediments (Levantine region) have shown a dominant Archaea population down to 200 mcd (Thomas et al., 2014, 2015). These results indicate that further research is critical to obtain more detailed information about the fine-scale mechanisms controlling microbial life in the deep subsurface of ancient lake sediments while answering several of the aforementioned questions.

Compared to their marine counterparts, the study of lacustrine sediments is often logistically easier. However, the implementation of subsurface biosphere studies in lake systems has required new sampling techniques that provide the needed aseptic conditions to avoid contamination. Whereas most ocean drilling vessels have a dedicated laboratory for geomicrobiological sampling on board, the size and configuration of lake drilling platforms prevent setting up a comparable sampling laboratory with conditions of asepsis. Therefore, special on-shore facilities are required to solve this problem. Core sections are transported to this laboratory as frequently as possible, depending on the logistics of the drilling (e.g., distance to the shore, frequency of drilling, and crew rotation; see also Section 2.1.1). Moreover, in situ sampling procedures allow recovery of aseptic samples as well as determining the presence of active microbes (Fig. 8; for details see Vuillemin et al., 2010).

Ideally, a geomicrobiological study should be planned well ahead of the start of a drilling operation. It is preferable to have a dedicated core for microbiological and geochemical studies. This will secure the best possible sampling conditions but can cause problems of hole-to-hole correlation because the microbiological results have to be tied with other sedimentological, biological, and geochemical parameters as well as core chronologies. To overcome this issue of missing stratigraphic information, petrophysical properties (whole core MSCL; see Section 2.1.4) of cores dedicated for geomicrobiological research can be measured in the field, or borehole logging can be used (see Section 2.1.3). These petrophysical or logging datasets can then be used to generate at least a rough hole/core-to-hole/core correlation and help to embed the geomicrobiological datasets into the common stratigraphic framework and other continuously generated datasets from other holes/cores of the same site.

In some cases it may, for logistical and/or budgetary reasons, not be possible to dedicate an entire drill core to microbiological investigations. If this is the case, there is the possibility to sample the core catchers for microbiological studies. When working with core catcher materials, special care should be taken to avoid contamination. A recent study that utilized core catcher samples for microbiological analyses from the Dead Sea ICDP sediment cores and followed appropriate sampling protocols, has proven to produce reliable results (Thomas et al., 2014, 2015).

The significance and validity of the results of subsurface biosphere studies is largely dependent on the quality, speed, and prevailing conditions of the initial sampling. Hence, it is critical to attain a methodological standardization for all lake and ocean deep-drilling sites in order to be able to compare results between different campaigns and environments. A generalized protocol would allow to reduce the impact of contamination issues, to determine the best method to accomplish on-site cell counting, to choose the appropriated sampling methods for further molecular characterization, and to design a proper strategy for sample archiving. Due to the different nature of each drilling project, a protocol for standardized biological sampling, processing, and analysis would be a significant accomplishment. Recently, the development of genomics and the emergence of high-throughput DNA sequencing technologies have been opening up new possibilities including the expansion of databases, which contain crucial information to define the metabolic pathways of different microbes (see also Section 2.2.3). The latter combined with laboratory culture experiments will be critical to constrain the impact of active microbes on, for example, the carbon cycle and diagenetic processes in the sediments.

As in ocean research, a main challenge for the development of subsurface biosphere studies in lacustrine settings is to communicate the

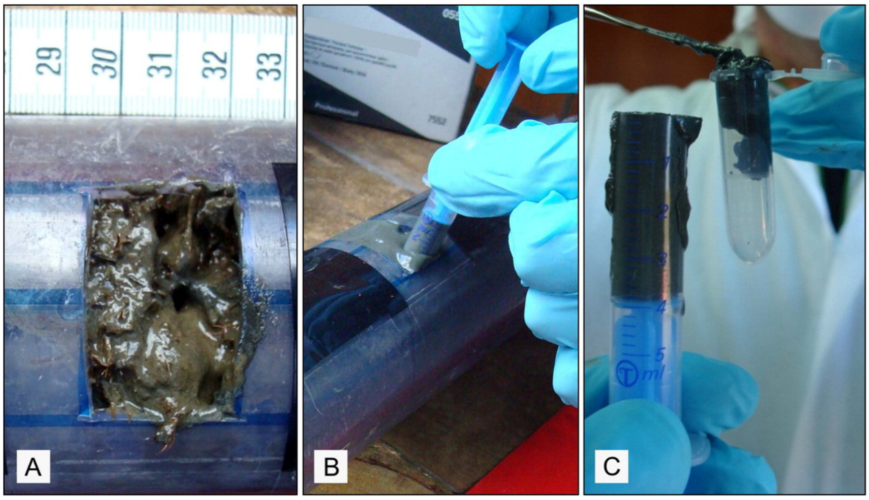

Fig. 8. Subsurface biosphere sampling from sediment cores obtained from Lake Potrok Aike (Patagonia, Argentina). A) Sampling window cut in the core under aseptic conditions. B) Methane sampling. C) DNA sampling.
Photo credits: A. Vuillemin and D. Ariztegui.

potential of these investigations to the broader scientific community participating in deep-drilling projects. It is also important to involve geomicrobiologists in discussions about the effects of an active biosphere on the subsurface environment and sediment composition. Paleoclimatological reconstructions largely depend on the use of a variety of petrophysical, geochemical, and biological proxies. Proxy responses are typically interpreted to reflect the environmental/climatic conditions during the time of deposition. However, organic compounds (ancient DNA, see Section 2.2.6; biomarkers, see Section 2.2.4) as well as element and isotope compositions (see Section 2.1.6) may be altered by microbially induced processes long after deposition, thus biasing and complicating the interpretation of proxies. A precise understanding of the influence of different microbial communities on compositional changes after burial is of vital importance for a better understanding of proxy responses and their interpretation.

2.2.4. Biomarkers

Biomarkers are source-specific organic molecules, i.e., they are synthesized by living organisms in aquatic or terrestrial settings for specific organic tissue types, e.g., cell membranes or protective and supporting tissue, or to fulfill specific functions, e.g., energy storage or pigmentation. The presence of such compounds in lacustrine sedimentary records is a testimony that, in the past, certain groups of organisms occurred in aquatic and terrestrial habitats of the catchment of the studied lake basin. In ancient lakes, occurrences of organisms may be affected by geological processes, such as tectonic subsidence or uplift, changing catchment topography, and material fluxes within the watershed. This, in turn, can be detected by biomarkers. Very few individual biomarkers are species-specific, and there is considerable overlap in biomarker profiles of large groups of organisms. Still, many biomarkers can be chemotaxonomically assigned to groups of organisms that characterize a specific habitat. Furthermore, organic matter pools of living and decaying biomass (aquatic/terrestrial vegetation, sinking particles/plant litter, soil organic matter) produce equally specific combinations of biomarkers, i.e., an organic geochemical fingerprint that can be interpreted to represent an ecosystem (e.g., Holtvoeth et al., 2016). Thus, ‘source-specific’ may refer to a specific organic matter pool, a group of organisms, or to individual species. The most fundamental distinction as to the sources of organic matter in sedimentary records that biomarkers can provide is between aquatic and terrestrial plant biomass, based on the fact that vascular plants (higher land plants) require structurally supportive and protective tissues that are not present in non-vascular, aquatic plants. Some other biomarkers indicate highly specific adaptions of their source organisms to environmental conditions. For example, pigments of anaerobic phototrophic bacteria indicate past anoxia in the photic zone of the water column when found in lacustrine sediments (Hanisch et al., 2003; Castañeda and Schouten, 2011).

The association of biomarkers to specific ecological functions or conditions highlights a fundamental principle behind many biomarker applications in paleo-environmental research. Variations in the biomarker inventories of sediments over time document changing fluxes of organic matter from various sources in response to largely climatically controlled environmental parameters, e.g., temperature and the supply of moisture. Factors such as catchment topography and lake bathymetry also determine organic matter pools through, for example, soil thickness and stability, weathering and erosion rates, runoff modes, the extent of the littoral zone, all of which also affect organic matter degradation during storage, transport, and deposition. Thus, the two main approaches in biomarker studies are to infer i) sources of organic matter and ii) environmental parameters (Table 2).

Both approaches are frequently applied in paleoenvironmental studies as ecosystem functions are adjusted to environmental parameters. In tropical and subtropical settings, for example, information on moisture supply and evapotranspiration, which can be detected by plant wax δ^2H, can be combined with carbon isotope data of the same plant wax δ^{13}C (e.g., Berke et al., 2012) to trace the abundance of C4 vegetation through time. Carbon and hydrogen isotope records indicating shifts in climatically controlled hydrology and types of organic matter can then be used for climate modeling (Aichner et al., 2015).

Lipid biomarkers provide highly specific proxy data that support and validate data from other organic sediment components, in particular, palynological and bulk organic matter data (see Section 2.2.2). Relatively fast and cost-efficient bulk geochemical proxies, such as carbon to nitrogen ratios (C_{org}/N_{tot}), bulk organic carbon isotopes ($\delta^{13}C_{org}$) or hydrogen and oxygen indices (HI, OI) from Rock-Eval pyrolysis, are useful tools to explore aspects of environmental variability. Although bulk proxies provide limited environmental information, they do have the potential to indicate major changes in sources and/or fluxes of organic matter (see Meyers, 2003 for a review). Furthermore, bulk analyses facilitate the formulation of detailed hypotheses and the design of targeted, high-resolution biomarker studies. A popular strategy for paleoenvironmental analyses therefore is to compare biomarkers with proxy data from palynology and bulk organic geochemistry. Examples include studies of East African lakes, combining pollen data with compound-specific carbon and hydrogen isotope data, lignin composition, and lake surface temperature data (Tierney et al., 2010; Berke et al., 2012).

Table 2
Biomarker-based approaches that determine sources of organic matter and environmental parameters, with examples of relevant literature.

Parameters	Molecular evidence	References
Organic matter source		
Aquatic biomass (phytoplankton/algae, zooplankton, macrophytes, bacteria, archaea)	- Chemotaxonomic compounds, incl. membrane lipids, storage lipids, pigments - Compound-specific stable isotopes ($\delta^{13}C$, $\delta^{2}H$, $\delta^{15}N$)	Volkman et al. (1998), Meyers (2003), Castañeda and Schouten (2011)
Terrestrial vegetation (woody/non-woody vegetation, angiosperms/gymnosperms, C3/C4 plants)	- Chemotaxonomic compounds and compound distributions, incl. membrane lipids, leaf waxes, lignin phenols - Compound-specific stable isotopes ($\delta^{13}C$, $\delta^{2}H$, $\delta^{15}N$)	Meyers (2003), Castañeda and Schouten (2011)
Soil organic matter (belowground biomass, incl. degraded plant debris, roots, bacteria, archaea, fungi)	- Biomarkers from soil microbial organisms (membrane lipids) - Biomarkers from root material (suberin monomers)	
Burned biomass	- Pyrogenic compounds	Denis et al., 2012
Environmental parameters		
Lake surface temperature (LST)	- Alkenone unsaturation index (Uk_{37}, from C_{37} alkenones of haptophyte algae) - Tetraether index (TEX_{86}, from glycerol dialkyl glycerol tetraethers/GDGTs of aquatic archaea)	Castañeda and Schouten (2011), Schouten et al. (2013)
Mean annual air temperature (MAT)	- Methylation and cyclisation of branched archaeal tetraethers (MBT, CBT)	
Moisture source (hydrology)	- Compound-specific hydrogen isotopes ($\delta^{2}H$)	Huang et al. (2002, 2004), Sachse et al. (2004)
Soil pH	- MBT, CBT	Weijers et al. (2007), De Jonge et al. (2014)

A basic problem is that biomarkers, as any organic substance left behind by a deceased organism, are affected by microbial and physicochemical degradation, which can occur before or after deposition in the sediments. Nitrogen- and oxygen-containing compounds, such as carbohydrates or amino acids, are prone to microbial degradation (see also Section 2.2.3), whereas molecules based on hydrocarbons, such as lipids, may preserve source-specific information over geological timescales, i.e., over hundreds of millions of years (e.g., Marynowski et al., 2011; Izart et al., 2012; Rohrssen et al., 2013). The oldest deep-time context from which biomarkers have been interpreted dates back ~1.6 billion years (Pawlowska et al., 2013). Microbial degradation may selectively alter the relative amounts of lipid biomarkers of different recalcitrance or that are bio-accessible, e.g., in a clay mineral matrix. In order to minimize diagenetic bias, biomarker proxies are generally based on ratios or distributions of compounds of the same compound class that also likely derive from similar source tissue types.

In the following, we discuss some practicalities of biomarker approaches, focusing on the recovery of paleoenvironmental records from ancient lakes, and including strategic considerations in order to gain maximum and reliable information from biomarker studies.

Lipid biomarkers are principally extracted from sediment samples by organic solvents through the application of a range of extraction techniques, in particular sonication, accelerated solvent extraction (ASE), and microwave-assisted solvent extraction (MAE), all of which are fast methods for high sample throughput (Camel, 2000; Kornilova and Rosell-Melé, 2003). Although time consuming and using greater quantities of solvent, Soxhlet extractions are a suitable option for the study of sediments with very low organic matter content. Once extracted, the lipids can be identified and quantified by gas chromatography–mass spectrometry (GC-MS). In cases where differences in ionization during GC-MS analyses may lead to quantitative bias, a standard gas chromatograph fitted with a flame ionization detector (GC-FID) is used for quantification instead. A typical application is the quantification of haptophyte-derived alkenones, which can provide information on paleo-surface water temperatures (Uk_{37} LST proxy, Table 2). Larger molecules, such as bacterial bacteriohopanepolyols (BHPs) or archaeal glycerol dialkyl glycerol tetraethers (GDGTs), which also provide temperature proxies for lake surface waters as well as for soils, are analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS). Lignin phenols used for vegetation reconstructions or terrestrial input can be analyzed efficiently by pyrolysis-gas chromatography–mass spectrometry (Py-GC-MS; e.g., Ishiwatari et al., 2006). For compound-specific stable isotope analyses ($\delta^{13}C$, $\delta^{2}H$, $\delta^{15}N$), compound classes or individual compounds can be isolated either through standard flash chromatography or automated preparative methods (prepGC, prepHPLC).

However, a single lab is rarely able to produce the complete suite of biomarker proxy data. One possibility to increase material- and cost-efficiency of multi-proxy biomarker studies is to design a coordinated approach and to distribute splits of the total lipid extracts (TLEs) for various applications, rather than bulk sediment samples.

The inter- and intra-habitat heterogeneity of biomarkers leads to the fundamental problem that biomarker-based proxies often are not interchangeable between investigated lake basins. Therefore, it is highly recommended to include a survey of the modern biomarker sources within the catchment of an environmental archive (biogeochemical fingerprinting) when planning biomarker-based paleoenvironmental research. In this context, it is crucial to correctly identify the major sources of sedimentary organic matter. For example, rather than the living vegetation itself, plant litter is a major source of terrigenous organic matter. This is important as the biomarker composition of plant litter is already altered compared to living biomass due to degradation processes that begin as soon as a plant dies. Soils are another major organic matter pool within many lacustrine basins. Furthermore, as they accumulate over time, soils integrate the biogeochemical signature of the changing vegetation cover and its degradation products and of belowground biomass (root material plus fungal and bacterial biomass). Thus, biogeochemical fingerprinting of the major organic matter pools facilitates the correct identification of the sources of the organic matter in lake sediments and helps assess potential input of pre-aged material, which can lead to considerable bias in high-resolution records (Douglas et al., 2014). A large uncertainty also affects the interpretation of stable isotope data, in particular, for hydrogen isotopes (Sachse et al., 2012). Determination of the carbon and hydrogen isotope compositions of biomarkers from the major organic matter pools in the modern environment of a lacustrine catchment is needed in order to improve the understanding of the impact of hydrological changes on compound-specific isotope compositions (see Wilkie et al., 2013 for the Lake El'gygytgyn drilling project). For paleotemperature reconstructions, a surface sediment-surface water calibration is highly recommended due to the many factors that can bias biomarker-based temperature proxies in lacustrine settings.

Unless sediment cores are stored in a freezer, microbial breakdown processes continue to alter organic matter in the sediments. Samples for biomarker studies should therefore be taken from the cores at the earliest possible occasion and then be frozen (ideally at −80 °C) or freeze-dried as soon as possible to prevent further microbial degradation. Even after freeze-drying, cold storage is advisable in order to preserve labile biomarkers, such as mono- and poly-unsaturated fatty acids.

During sampling and storage, it is crucial to avoid contamination with organic compounds derived from petroleum products such as lubricants and plastics (see also Section 2.1.2). Polyethylene (PE), which is commonly used for soft plastics, such as sampling bags or the lids of sample containers, releases a series of compounds, in particular branched alkenes with quaternary carbon atoms (BAQCs), which can contaminate even freeze-dried sediment during prolonged storage (Brocks et al., 2008; Holtvoeth, unpublished data). Samples should be transferred into furnaced glass jars or vials, or into containers lined with combusted foil (aluminum foil heated to 450 °C for at least 4 h).

The study of biomarkers preserved in lacustrine sediments has developed strongly ever since their potential for paleo-environmental reconstructions was recognized and developed from the late 1960s onwards (e.g., Cranwell, 1973; Brooks et al., 1976). This is largely due to the rapid advance of analytical technology and the increase in analytical capacity. Promising analytical methods are established using advanced detectors for mass spectrometry, such as a quadrupole time-of-flight mass detector (GC/Q-TOF) or orbitraps, increasing resolution and precision in the detection of molecular fragments. Moreover, high-temperature GC-MS applications extend the range of GC-amendable compounds towards high-boiling biomarkers (e.g., wax esters, triacylglycerides, GDGTs; Sutton and Rowland, 2012). A dynamic field with great potential for biogeochemical fingerprinting of archaeal and bacterial organic matter sources and reconstructions of microbial ecology is the analysis of compounds derived from bacteriohopanepolyols (BHPs; Talbot et al., 2003; Zarzycki and Portka, 2015) and of intact polar lipids (IPLs; Rethemeyer et al., 2010; Tierney et al., 2012; Buckles et al., 2014). A recent and entirely different approach to the use of biomarkers as indicators of ecosystem change is the application of advanced statistical programs integrated in the analytical software for identifying the key variables in biomarker screening data that may include hundreds of compounds in an environmental sample (lipidomics, environmental metabolomics; Bundy et al., 2009). Finally, genetic research targeting enzymatically controlled molecular adaptions of organisms to certain environmental conditions is fundamentally changing the conventional interpretation of biomarker data. Rather than linking a biomarker to the presence of a certain organism or groups of organisms, it can instead be assigned to a specific ecological niche (e.g., Welander et al., 2012). This change in view is important for the understanding of the geochemical fingerprint that past ecosystems left behind and opens up new possibilities to interpret assemblages of biomarkers that, individually, had been regarded as unspecific.

2.2.5. Ancient amino acids

Towards the end of the 19th century, the first identification of the likely remains of proteins in fossils paved the way for their use in the earth sciences. Amino acids, the building blocks of proteins, are found in all living tissues and can be preserved in subfossil biominerals, such as shells, as well as in sediment. Analysis of these ancient amino acids has proved important for three main avenues of research: dating (via amino acid geochronology), species identification (via paleoproteomics), and to assess the integrity of the organic matter for other biomolecular studies (e.g., aDNA).

Amino acids can be used for dating purposes because of amino acid racemization (AAR), i.e., the time-dependent breakdown of proteins (and their constituent amino acids) in fossils. Spanning an age range from 10 years ago up to as long ago as a few million years, the method is applicable to the whole of the Quaternary Period (see Lowe and Walker, 2015 for a recent review). Advances in chromatography, preparation methods, and the choice of material for dating have greatly improved the accuracy of the methods, and demonstrate the technique's potential for developing regional Quaternary chronologies around the world (e.g., Penkman et al., 2011; Wehmiller, 2012). Identification of endogenous amino acids in Cretaceous and Paleogene samples (Miller and Hare, 1980; Penkman et al., 2013) opens up the opportunity to use other protein degradation reactions to date material over much longer timescales.

The 20 naturally-occurring amino acids all have a central carbon atom (the α-C) with four attached groups: an amino group (NH_3), a carboxylic acid group (COOH), hydrogen (H), and a side chain (R) that defines the type of amino acid. In glycine, the side chain is H, but for all other amino acids, the α-C has four different groups (Fig. 9). The four distinct groups connected by single bonds make the α-C a chiral center, meaning that it can exist as two stereoisomers: the *laevo* (L-form) and *dextro* (D-form), named after the optical activity of glyceraldehyde. In living organisms, proteins are almost exclusively made from the L-form. However, this dominance of one form is thermodynamically unstable, so after death, a spontaneous racemization reaction occurs to balance the abundance of both forms. The extent of AAR is analyzed by gas or liquid chromatography and recorded as a D/L value. AAR continues until a dynamic equilibrium is reached (usually $D/L = 1$). First applied to fossil shells (Hare and Abelson, 1968), AAR geochronology measures the extent of this degradation in fossils as an index of relative age (an aminostratigraphy), which can provide calibrated ages in combination with known-age samples or detailed temperature records (Fig. 10). This then may allow correlation of deposits with the marine oxygen isotope stage (MIS) record (for lacustrine deposits see McCoy, 1987; Bowen et al., 1989; Magee et al., 1995; Oviatt et al., 1999; Kaufman, 2003a; Ortiz et al., 2004; Penkman et al., 2011), to a sub-MIS level for at least the Late Pleistocene.

Protein degradation consists of a series of chemical reactions that are dependent on time, but also on environmental factors (e.g., pH, availability of water, temperature), which can confound the time signal. These difficulties in AAR's early applications have led to a focus on analyzing 'closed-system' protein from fossil samples (Towe, 1980), where the fraction of protein analyzed is physically or chemically shielded from the environment. The chemically-isolated 'intra-crystalline' fraction found in mollusk and egg shells forms such a closed system, meaning that the AAR within this fraction is solely dependent on time and temperature, and therefore predictable (Brooks et al., 1990; Sykes et al., 1995; Penkman et al., 2008). AAR has been particularly successful in dating carbonate fossils (shells, eggshells, foraminifera, ostracods) and in long-lived biominerals (e.g., corals), providing age information *within* an individual sample (Hendy et al., 2012). In subfossil samples, the different proteins break down at different rates, so analyses are undertaken on monospecific samples (usually individual mollusk shells, a few mg in weight). Labs performing AAR have developed dating frameworks for a large number of commonly occurring species, but tests can be undertaken on additional species to examine whether they would be suitable for AAR dating. The crystal phase of calcite (e.g., opercula, eggshell, ostracods) are more stable over longer timescales and are

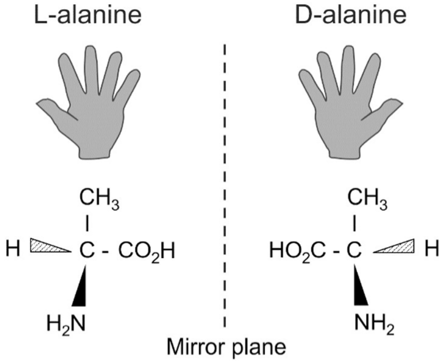

Fig. 9. Most amino acids have no plane of symmetry, just like hands, so their mirror images are non-superimposable and therefore distinct from each other. The breakdown of left-handed molecules to the right-handed form over time provides a mechanism for estimating age of fossil material.

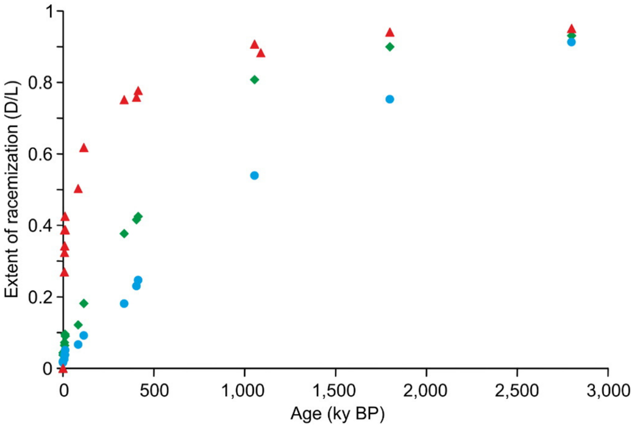

Fig. 10. The increase in racemization in the opercula of the snail species *Bithynia tentaculata* with age for the free amino acid (FAA) aspartic acid (Asx; red symbols) and the total hydrolysable amino acids (THAA) valine (Val; blue symbols) and alanine (Ala; green symbols) from British deposits with independent geochronology. Asx racemizes rapidly and is therefore most valuable for separating sites younger than MIS 7 in these temperate deposits. Val, in contrast, racemizes more slowly and is able to differentiate between sites back to the Pliocene, but provides poorer resolution for young sites. Utilizing multiple amino acids with different rates of degradation therefore enables greater time depth and age resolution.
Image modified from Penkman et al. (2011).

therefore preferred for material of Early and Middle Pleistocene age (Penkman et al., 2011).

The rate of breakdown towards D/L equilibrium in the intra-crystalline fraction is still affected by temperature, so comparative frameworks need to be applied from regions with a broadly similar temperature history. However where age control is available, the extent of racemization can then be used to estimate the effective diagenetic temperatures (Kaufman, 2003b). Published amino acid data are now being archived by NOAA and are freely available at http://www.ncdc.noaa.gov/paleo/aar.html.

The advent of soft-ionization mass spectrometers made protein sequence identification more routine, and this was soon applied to fossil material (Ostrom et al., 2000). The ordering of the amino acids in a peptide chain (its sequence) can be diagnostic of the species from which it came, and therefore identification of specific ancient proteins informs on the past biota (Buckley and Wadsworth, 2014). While the evolutionary picture from proteins is not as detailed as that from aDNA (as changes in the peptide sequences are significantly slower), proteins are significantly more stable than DNA (see Section 2.2.6), allowing identification of peptide sequences (and hence phylogenetic information) from material where aDNA is not recoverable (Cappellini et al., 2011). Focusing initially on Pleistocene bones and shell, this technique has primarily been applied to terrestrial deposits, but the excellent preservation of organic material in lake sediments (often due to anoxic conditions) lends itself to the expansion of paleoproteomics to lacustrine material.

For all biomolecular analyses, it is critical to identify and exclude contamination. One advantage of protein analysis is that (unlike aDNA) proteins do not have to be amplified for detection, while the predictable nature of protein degradation enables identification of modern contamination (e.g., Buckley et al., 2008). Amino acid analysis of sedimentary material provided a useful tool for identifying the presence of original biomolecules, and hence helped authenticate some of the oldest DNA sequences yet recovered (Willerslev et al., 2007).

A non-specialist can collect material and/or sediment samples in the field, and samples should be stored at or below room temperature. Biominerals for AAR dating are typically obtained from wet-sieved residues of sediment samples. Development of better preparative and analytical methodologies is allowing analysis of smaller and more degraded samples, while protein databases to match recovered sequences against are ever-growing, enabling more accurate species identifications. Amino acids and proteins are present in geological samples, and our abilities to use the geochronological and biological information they hold are advancing rapidly.

2.2.6. Ancient DNA

The analysis of ancient DNA (aDNA), i.e., DNA of long-dead specimens (Krause, 2010), has become an emerging field in evolutionary biology and paleoecology during the last decades (e.g., Hofreiter et al., 2001; Pääbo et al., 2004; Willerslev et al., 2014; Hagelberg et al., 2015; Birks and Birks, 2016). Ancient DNA provides a unique opportunity to assess paleo-biodiversity, and to unravel past evolutionary and environmental processes by comparing genetic information of fossil and extant organisms. Moreover, as the nucleotide sequence of DNA fragments ('DNA sequences') can be digitally encoded in a standardized way and stored in public databases, newly generated DNA sequences are directly comparable to previously generated data.

Ancient DNA may be isolated from (parts of) specimens preserved in sediment cores (incl. mummified tissues, bones, teeth, or other biomineralized material) or, more commonly, as 'sedimentary aDNA', i.e., organismal DNA that has been released into the water or directly into the sediment, often in the absence of visible fossils (Parducci et al., 2013, 2015; Pansu et al., 2015). However, aDNA is often of poor quality compared to DNA from samples of extant organisms as DNA molecules exponentially degrade in smaller fragments after the death of an organism (Hofreiter et al., 2001; Allentoft et al., 2012). In addition, microorganisms may digest aDNA and even introduce mutations (Hofreiter et al., 2001; Krause, 2010; Allentoft et al., 2012). These microbial effects are of particular concern for sedimentary aDNA analyses, as the respective DNA molecules are largely unprotected against the environment. Other factors determining degradation are, among others, time, environmental temperature, oxygen content, pH, sediment type and composition, and presence of free water (Allentoft et al., 2012; Hagelberg et al., 2015). Once a critical sequence length of approximately 15–25 nucleotides is reached, these DNA fragments lose their unique fingerprint and can no longer be used for comparative studies.

The two main methods to decode aDNA are PCR-directed sequencing and direct sequencing. For PCR-directed sequencing, short oligonucleotides ('primers') have to be designed that selectively bind to a complementary aDNA target region. The advantage of this method is that a specific region (e.g., a particular gene or part of a gene) can be targeted, that the number of read errors is relatively low, and that contamination (e.g., through microbial DNA) is limited by the use of taxon-specific primers. Disadvantages are that the aDNA fragments targeted have to be relatively long (typically several hundreds of nucleotides) and the need to design specific primers, which is often difficult in the absence of reference data. In contrast, direct sequencing does not require specific primers and thousands or millions of short aDNA fragments can be directly encoded using 'next generation sequencing' platforms (e.g., Metzker, 2010; Mardis, 2011). These high-throughput methods work well with highly degraded aDNA fragments (i.e., with fragments as short as 25 nucleotides; Storvall et al., 2013), but the numerous individual aDNA reads have to be assembled using reference databases, which so far cover mainly 'model' organisms. Moreover, as no taxon-specific primers are used, contamination with exogenous DNA may constitute a major problem even though parts of these contaminations can be recognized a posteriori using bioinformatic tools (e.g., Schmieder and Edwards, 2011).

Despite numerous methodological advances, aDNA analyses from lake-core materials remain problematic. Reasons are the small amount of available material, the highly degraded nature of aDNA from sediment cores, read errors, contamination, and incomplete reference databases (sensu Krause, 2010; Pedersen et al., 2013; Birks and Birks, 2016). Therefore, aDNA from lake sediments is currently mainly used for biodiversity assessments of Late Pleistocene and Holocene communities

(Bissett et al., 2005; Anderson-Carpenter et al., 2011; Boessenkool et al., 2014; Pansu et al., 2015), particularly as complementary information to fossil data (sensu Jørgensen et al., 2012; Parducci et al., 2013).

Though respective analyses from ancient lake cores are still lacking, we expect to see more paleo-biodiversity assessments based on aDNA analyses in future deep-drilling projects. Given that shallow areas in ancient lakes are typically more biodiverse than deep sites, we suggest to retrieve aDNA samples from near-shore sediment cores (although temperature and O_2 content in these areas might be higher, thus accelerating degradation; see also Sections 2.1.1 and 2.2.1. about the disadvantages of near-shore sites and Cohen, 2012 for issues related to geological conditions in general). A principle problem is contamination with exogenous DNA. Therefore, cores have to be sampled under aseptic conditions and sedimentary aDNA sampling is ideally done directly in the field. However, previous studies have shown that aDNA can also be isolated from lake sediments after long-term refrigeration at 4 °C (Bissett et al., 2005). Respective procedures of field sampling are similar to those used in deep-biosphere studies (Ariztegui et al., 2015; see also Section 2.2.3). For this reason, coupling aDNA and subsurface biosphere studies in ancient lake drilling projects is advisable.

Future studies will probably use direct high-throughput sequencing, perhaps extending the time frame of analyses to the Middle Pleistocene. Moreover, the usefulness of high-throughput approaches may also increase once more extensive reference DNA datasets are established that include genetic information on both fossil and extant species. We therefore recommend to couple future aDNA studies in ancient lake drilling projects with genetic and genomic studies on extant species (see also Section 3.3).

2.3. Data accessibility and storage

Long-term core and sample storage as well as data accessibility and curation are of fundamental importance in light of the immense financial, logistic, labor, and intellectual efforts associated with scientific drilling projects. Over the last decades, core repositories (e.g., Bremen Core Repository (BCR), Bremen, Germany; Kochi Core Center (KCC), Kochi, Japan; National Lacustrine Core Facility (LacCore), Minneapolis, USA), equipped for core processing and particularly designed for the long-term storage of cores, samples, and data were established in order to service the scientific drilling community and to guarantee the accessibility of samples, relevant drilling metadata, and initial core descriptions (ICD) for extended periods. Project parties of scientific drilling projects are conventionally obliged to generate ICD data, which typically encompasses whole core and split core MSCL data, split core surface imaging, visual core descriptions, smear slide analysis, and core correlation points (Fig. 3). ICD data is crucial for subsequent sample selection by those directly involved, or others that intend to study samples after the moratorium has ended (commonly two years after drilling).

All relevant metadata and initial datasets generated on-site during drilling and laboratory-based core processing should, by default, be uploaded into dedicated, specific databases such as the ICDP Drilling Information System (DIS) for archival in professionally managed server facilities. In order to assure globally unique identifiers (sample IDs) for long-term traceability between samples and data, International Geo Sample Numbers (IGSNs; http://www.geosamples.org/aboutigsn) for each type of sample generated should be registered and assigned appropriately through the System for Earth Sample Registration (SESAR, http://www.geosamples.org/) by each project. Furthermore, fundamental datasets along with datasets published in scientific publications by scientific drilling project parties have to be made available through online databases such as PANGAEA (http://www.pangaea.de/) and/or NOAA National Geophysical Data Center Index to Marine and Lacustrine Geological Samples (http://www.ngdc.noaa.gov/mgg/curator/curator.html) in accordance with ICDP and national funding agency rules.

However, most of the data storage and data sharing policies outlined above only apply to primary data generated from sediment cores. In contrast, there are still no generally accepted rules for storing and sharing secondary data and materials obtained during drilling campaigns. As a minimum, geological and biological voucher materials should be deposited in a freely accessible and internationally recognized scientific collection. Moreover, data should be made available through major public databases such as NCBI's GenBank (http://www.ncbi.nlm.nih.gov/genbank), the Paleobiology Database (https://www.paleobiodb.org), or the citable Dryad Digital Repository (https://datadryad.org).

3. Integrating geological and biological data

In Section 2, we have shown that many different types of data, both geological and biological, can be obtained from drilling campaigns. Despite this rich variety of data types, lake drilling long remained the domain of earth scientists. As a result, many geological and paleolimnological analyses are well established and have been reviewed abundantly before (e.g., Cohen, 2003; O'Sullivan, 2004). In comparison, the use of organismal approaches to sediment-core data for questions related to environmental and evolutionary biology are newer, and therefore we focus here on data analysis methods that fall within this scope. We start with paleolimnological reconstructions as a nexus of environmental inquiry between strictly geological and biological approaches, then discuss the consequences of environmental change for biological diversification processes, and finally deal with aspects of the natural history of biota, i.e., timing evolutionary events and tracing character evolution.

3.1. Paleolimnological reconstruction

A major goal of scientific drilling projects in ancient lakes is the reconstruction of their paleolimnology and paleoclimate (Cohen, 2012). Sediment records revealed, for example, regional climatic and environmental expressions of Milankovitch/glacial-interglacial cycles (e.g., Hooghiemstra, 1989; Kashiwaya et al., 2001; Wagner et al., 2014a) and their extremes in tropical and polar realms (e.g., Cohen et al., 2007; Melles et al., 2012).

Traditionally, paleolimnological reconstructions are primarily based on micro- and macrofossils, both from sediment cores (primary data) and outcrops (secondary data) (see Sections 2.2.1, 2.2.2). However, modern reconstructions also integrate sedimentological, geochemical, and isotope information, thus enabling a more comprehensive assessment of paleoconditions in a given lake and its watershed. These conditions include, for example, past lake-level fluctuations (Magyar et al., 2006; Filippov and Riedel, 2009; Lyons et al., 2015), oxygen conditions (Costa et al., 2015), salinity (Mischke et al., 2010), water temperature (Goodwin et al., 2003; Castañeda and Schouten, 2011; Recasens et al., 2015), or primary productivity (Langlet et al., 2007; Recasens et al., 2015). Paleolimnological reconstructions often provide a well-informed picture about past regional (e.g., Bergner and Trauth, 2004; Stager et al., 2009) or local environmental conditions (e.g., Mourguiart et al., 1998). Although the resolution and the indicative power of paleolimnological reconstructions largely depends on the availability and quality of the respective fossil record, preserved biological remains sometimes reflect past climate and environmental changes even on the scale of decades or years (e.g., Ghinassi et al., 2015).

Analytical approaches for paleolimnological reconstructions range from qualitative over semi-quantitative to quantitative analyses. Qualitative inference is typically based on linking ecological and environmental information using indicator species, whereas quantitatively inspired analyses utilize estimated changes in fossils proportions and involve a link between environmental and biotic information (Cohen, 2003; Birks et al., 2012; see also Section 2.2). These approaches usually employ models ('paleoecological transfer functions', Fig. 11) that correlate modern species assemblages (i.e., composition or abundances) and their environmental characteristics ('calibration'; Cohen, 2003; Juggins and Birks, 2012). Subsequently, fossils are used to infer paleoenvironmental conditions based on the revealed assemblage-environment relationship. Transfer functions require a reference data set (= 'training set'), i.e., a data matrix that contains information on the distribution of recent species, either found in the water column or in surface sediments, together with information on the environmental variables that drive these distributional patterns. Ideally, a training dataset should be produced under controlled laboratory conditions, subjecting an organism or community (e.g., phytoplankton) to a range of environmental conditions for establishing a causal link between the environmental (independent) variable and a set of response (dependent) variables (Saros et al., 2012). However, because of the often complex relationship between environmental and distributional data, these models are typically built with data from a range of modern lakes.

For ancient lakes, the training of transfer functions might be restricted to the lake itself due to the lack of analogues for endemic taxa (see also Section 2.2.2; Mackay et al., 2003).

Whereas it is typically straightforward to produce environmental and biological datasets from extant lakes, it is more difficult to obtain the necessary fossil record from sediment cores. Of concern are site selection (i.e., drilling shallow or deep sites; see also Section 2.1.1), continuity of records, as well as data quality and resolution. The latter aspects are particularly important because they strongly affect the applicability of transfer functions. It is also important to account for diagenetic and taphonomic processes that might mask relationships and/or mislead interpretations (see Sections 2.2.1, 2.2.2). Environmental reconstruction based on transfer functions and sediment proxies can give conflicting information for the interpretation of the same paleoenvironment (Cohen, 2003). Therefore, it is necessary to assure that there is, indeed, a causal link between the physico-chemical variable and the bioindicators' response (Juggins, 2013). Strategies have to be developed to implement uncertainty in paleolimnological reconstructions. Another challenge is the need to disentangle individual and joined effects of multiple causal factors in the species-environment relationship (Juggins, 2013). Moreover, the species-environment relationship should be almost constant over time (i.e., no niche shift; for a critical assessment of transfer functions see also Juggins, 2013).

Limited systematic knowledge and taxonomic confusion, in general, are potential sources of misinterpretation of communities, meta-communities, and related ecological measures. For example, the biological relevance of morphological characters for species delimitation in the fossil record (e.g., diatoms, ostracods, mollusks) should be studied in extant analogues ideally using an integrative taxonomic approach based on phylogenetic relationships and character evolution analyses (see Section 3.4). New methods to be developed should also identify and estimate the range of effects of diagenetic and taphonomic processes on the records used for paleolimnological reconstructions (Birks et al., 2012).

Undoubtedly, we will see several technical and methodological advances in the future, including improved dating of sediment records (e.g., Shanahan et al., 2013; Zanchetta et al., 2016; see also Section 2.1.5), high-resolution datasets (Lacey et al., 2015), and high-throughput technologies (Tolu et al., 2015), allowing a more efficient production of long-term datasets that can be used for paleolimnological reconstructions (see also Seddon et al., 2014).

The application of new proxies for paleolimnological reconstructions, such as biomarkers, is rapidly increasing as the costs for analytical instrumentation are diminishing (Section 2.2.4). By now, a broad range of proxies has been established for lacustrine sediment records. These markers allow tracing of surface water temperature, mean annual air temperature, and moisture supply as key climatic factors in habitat dynamics (reviewed in Castañeda and Schouten, 2011). Paleothermometry based on estimations of racemization rates of different amino acids is another direction of analytical developments (Collins and Demarchi, 2014; see also Section 2.2.5). Further aspects of ecosystem change that can be targeted through biomarker proxy applications are photic zone anoxia or the dynamics of the terrestrial surroundings,

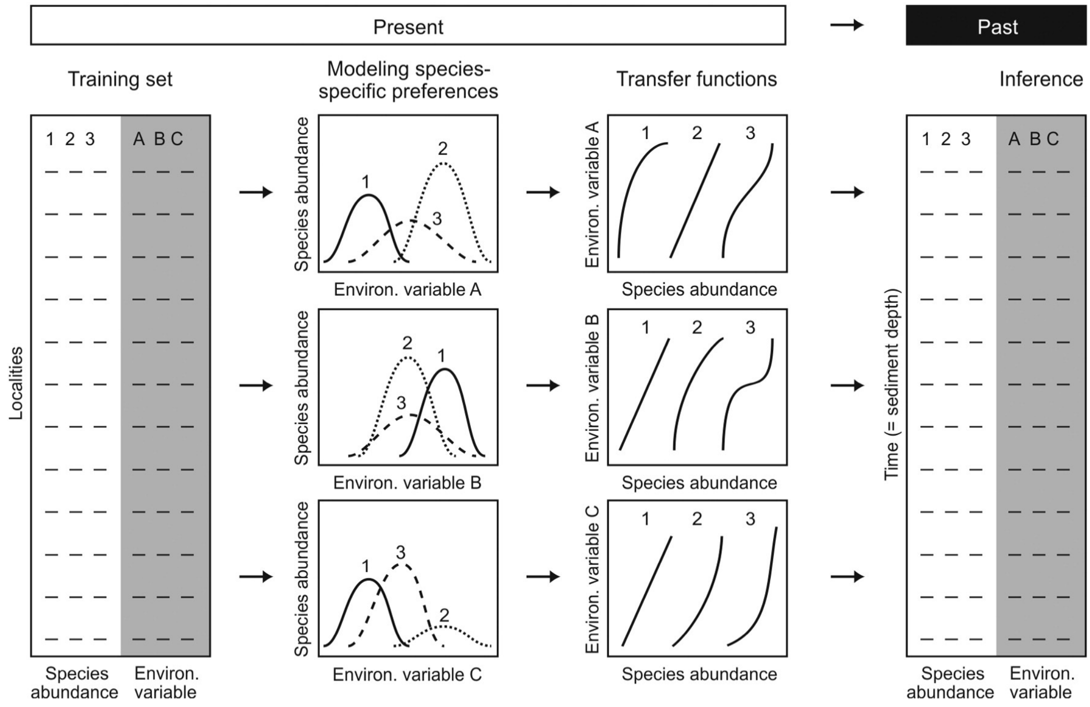

Fig. 11. Flow of transfer function development for paleolimnological reconstructions. Species abundances (Arab numerals) and environmental variables (letters) are measured in extant assemblages. These data are used to model species-specific curves of environmental preferences, which then can be utilized to develop and refine predictive functions of environment-abundance relationships using fossil assemblages from time-resolved sediment-core samples in the lake of interest.
Modified from Fritz et al. (1999).

including vegetation change, biomass burning, and soil erosion (see Section 2.2.4), all of which affect the trophic state of a lacustrine ecosystem.

Despite exiting results derived from integrating geological and biological data for paleolimnological reconstructions, there are many questions remaining how past ecosystem dynamics have shaped extant ancient lakes. Future drilling projects might also aim at identifying early ecosystem warning signals for catastrophic regime shifts, and they may attempt to identify common environmental signals in multiple records spanning different spatial and temporal scales. Methods should be implemented that address problems of time lead and lag, conflicts between datasets derived from the various proxies used, data uncertainties, and spatial autocorrelation.

3.2. Consequences of environmental change: a biological diversification perspective

The often extraordinary endemic species richness and high morphological disparity of ancient lake taxa have inspired evolutionary biologists to unravel the underlying mode, tempo, and drivers of biological diversification. Increasingly, non-biologists, such as geologists and climatologists, are also interested in the balance between speciation and extinction events – the two sides of biological diversification. Their reasoning is straightforward: linking environmental and evolutionary data in space and time may help to infer and possibly even quantify the consequences of past geological and/or climatic change. Moreover, information on past speciation and extinction events can be used to assess if and how an ecosystem can buffer such perturbations, i.e., its ecosystem resilience. Finally, an understanding of how abiotic factors have driven diversification processes in the past might help to predict the impact of future environmental changes on the biotic world under various global change scenarios (Condamine et al., 2013; Lawing and Matzke, 2014).

Indeed, integrating chronologically constrained geological and biological datasets from sediment cores has become a standard in interdisciplinary drilling projects. Increasingly, these data are supplemented with secondary data such as fossil or stable isotope information from outcrops (see Sections 2.1.6, 2.2.1, 2.2.2) or with genetic information from extant species (Section 3.3). Three main, non-exclusive objectives are of potential interest:

i) Inferring the drivers of diversification; of concern are, for example, potential effects of changes in temperature, lake-level fluctuations, and tephra depositions on speciation and/or extinction events (e.g., Schultheiß et al., 2009; Brown et al., 2010; Wagner et al., 2014c; Jovanovska et al., 2016).
ii) Inferring the tempo of speciation; of relevance are whether, for example, environmental perturbations affected changes in diversification rates over time and/or triggered radiations (e.g., Day et al., 2008; Sherbakov, 1999; Schön and Martens, 2011; Wysocka et al., 2014).
iii) Inferring the mode of speciation; of interest are, for example, whether intralacustrine barriers or lake-level associated vicariance events have triggered allopatric speciation (Trajanovski et al., 2010; Koblmüller et al., 2011; Schreiber et al., 2012) or whether environmental changes opened new ecological niches, thus promoting ecological speciation (e.g., Schön and Martens, 2004; Herder et al., 2008; Young et al., 2009; Muschick et al., 2012, 2014).

Previous studies, for example, have shown that lake-level changes in ancient lakes had very different effects on the lake's endemic taxa. Depending on the magnitude of lake-level changes, the ecosystem resilience of the respective lake, and the resistance of its biota, these changes may have caused extinction events across taxa (e.g., Schultheiß et al., 2009, 2011), selective extinction in some but speciation in other taxa (e.g., Kroll et al., 2012; Takahashi and Moreno, 2015; Weiss et al., 2015), or no visible effect on patterns of diversification (e.g., Föller et al., 2015).

However, linking geological and biological data from drilling projects, particularly in the context of hypothesis testing, is not always straightforward (see also Section 3.4). This issue is particularly true when secondary data are used, which are not per se chronologically constrained with the primary sediment core data. For example, linking a sequence of diversification events inferred from DNA phylogenies to a series of lake phases (e.g., Kroll et al., 2012) is challenging because of uncertainties in the timing of both datasets. Furthermore, even when a correlation can be demonstrated, it does not imply causality. In addition, drilling projects largely focus on the generation of abiotic data, which may not affect tempo and mode of diversification directly. Instead, abiotic changes possibly act indirectly through biotic drivers such as changes in character states (Hansen, 2014; Cantalapiedra et al., 2014; Salzburger et al., 2014; see also Section 3.4) or community structures (Hauffe et al., 2016). Moreover, to unravel the consequences of environmental change (sensu Condamine et al., 2013; Lawing and Matzke, 2014), it is not only important to understand if abiotic drivers are involved, but also to what extent. These issues, however, are not unique to scientific deep-drilling projects (Rabosky and McCune, 2010).

In the following, we therefore exemplify recent developments of statistical approaches that allow testing of specific hypotheses about drivers of diversification. Depending on the type of dataset to be linked in drilling projects, three categories of analyses can be distinguished.

The first set of methods (secondary + secondary datasets) is based on dated molecular phylogenies and tests for deviation from a constant diversification rate (see also Section 3.3 and Morlon, 2014). In particular, the methods of Stadler (2011a, 2011b), Morlon et al. (2011), and Rabosky (2014) allow detecting distinct shifts and a non-linear time-dependence of diversification rates. These molecular data can be complemented by another set of secondary data, i.e., biotic factors hypothesized to drive diversification such as habitat use or changes in morphological character states (see also Section 3.4 and the review of Ng and Smith, 2014).

The second set of methods (secondary + primary datasets) uses dated molecular phylogenies in combination with abiotic factors that can be directly obtained from the sediment core and/or the borehole. Though the idea that abiotic parameters may drive diversification ('Court Jester hypothesis'; Barnosky, 2001) is conclusive, to the best of our knowledge, only two approaches exist for testing the influence of abiotic factors on speciation and extinction events (Condamine et al., 2013; Cantalapiedra et al., 2014). The method of Cantalapiedra et al. (2014), for example, enables the identification of the relative importance of abiotic versus biotic drivers, their consequences on diversification rates, and the change of rates over time. Previous studies have indicated an interplay of present-day abiotic factors, such as area and depth of the African Great Lakes, and biotic characteristics, such as mating behavior, in determining the probability of cichlid lineages to form intralacustrine species flocks (Wagner et al., 2012).

The third set of methods (primary + primary datasets) for inferring drivers of biological diversification uses dated origination and extinction events obtained from fossils, their biotic features, and abiotic conditions. The estimation of diversification rates based on fossil occurrence including taphonomic uncertainties is constantly improving (Foote, 2000), and the influence of abiotic drivers on these rates can be assessed using standard regression techniques (e.g., Neubauer et al., 2015). However, an alternative framework suggested by Silvestro et al. (2014) enables a simultaneous inference of diversification rates and identifies how these rates are shaped over time by biotic or abiotic factors. This allows including various factors such as morphological changes, standing species richness for diversity-dependent diversification, and environmental changes.

Whereas these three sets of methods have largely improved our ability to test evolutionary hypotheses based on data derived from interdisciplinary deep-drilling projects, they all share the same major limitations and pitfalls:

i) Typically only the influence of a single abiotic or biotic driver of diversification can be estimated due to the current lack of tests for multiple drivers (Rabosky and McCune, 2010; Morlon, 2014).
ii) Most analyses require a large set of species to correctly identify the effect of a potential driver (Davis et al., 2013). Though many ancient lake species flocks are relatively species rich, the statistical power for smaller flocks might not be sufficient. For such cases, simulations have been suggested (Rabosky and Goldberg, 2015). Another option to increase the power is to test for a common signal across species groups by combining the phylogenetic information from several species flocks (i.e., phylogenetic meta-analyses; Adams, 2008).
iii) Constraining primary and secondary datasets from drilling projects is often challenging because they are independently dated. Moreover, the dating uncertainties of primary datasets from sediment cores (see Section 2.1.5) may be lower by orders of magnitudes than those of some secondary datasets (Wilke et al., 2009; see also Section 3.3).
iv) Environmentally-triggered diversification events frequently occur with a time lag (Stadler, 2011b; Dynesius and Jansson, 2014), making a direct link of geological and biological datasets difficult. A solution for this problem could potentially come from the field of epidemiology where the spatial extent and duration of disease outbreaks and their causes are analyzed (e.g., Liang et al., 2010). In interdisciplinary drilling projects, similar approaches (e.g., marked point pattern analyses; Ripley, 1976) could be used to identify abiotic drivers and the temporal extent of their influences on species diversification.
v) The ability to estimate extinction rates based on phylogenies remains difficult (Rabosky, 2010) and the incorporation of the fossil record has been advocated (Quental and Marshall, 2010). However, as macrofossils are rare in sediment cores (Section 2.2.1), statistical approaches would need to be applied that enable inferring extinction rates based on phylogenies alone (e.g., Morlon et al., 2011).
vi) Another problem involves the suitability of the model taxa studied. Many ancient lakes harbor a high number of endemic species, often with diverse niches and different life styles (e.g., benthic vs. planktonic and generalist vs. specialist). However, baseline studies are necessary to verify that the candidate taxa, indeed, represent suitable model systems for the questions of interest in terms of, for example, monophyly and species richness (for details of sampling requirements and potential sampling biases see Section 3.3).

In general, the candidate abiotic and biotic drivers to be studied in interdisciplinary deep-drilling projects have to be selected based on the specific scientific objectives. Apart from ‘standard’ abiotic factors such as lake-level changes, desiccation, and salinization events, parameters such as temperature and productivity (Condamine et al., 2013) have been proposed to influence diversification events and rates. Biotic drivers might be even more diverse. The underlying mechanisms and causality of some biotic drivers are well understood (e.g., the pharyngeal jaw of cichlids, Salzburger et al., 2014; depth preferences, Stelbrink et al., 2015), whereas the influence of species richness and composition on diversification is controversially discussed (e.g., Day et al., 2008; Harmon and Harrison, 2015; Hubert et al., 2015; Rabosky and Hurlbert, 2015). Also, the timeframe of evolution has to be considered when linking geological and biological datasets. Some ancient lakes such as lakes Baikal (e.g., Müller et al., 2001) and Ohrid (e.g., Albrecht and Wilke, 2008) are considered to harbor many old species or groups of species. Other lakes, though being old, experienced a series of major environmental perturbations, and the respective endemic species are often comparably young. Examples are Lake Malawi (e.g., Genner et al., 2007), the Caspian Sea (e.g., Dumont, 1998), and Lake Titicaca (e.g., Benavides, 2005; Kroll et al., 2012). Due to the young age of many taxa and problems of incomplete lineage sorting (for details see Section 3.3), phylogenetic relationships may be difficult to ascertain. In such cases, the use of primary data, such as fossils from the sediment core, is recommended for the study of old and/or long-term diversification processes, though sufficient data are rarely available.

3.3. Molecular dating

The ability to reconstruct older evolutionary events based on aDNA from sediment cores (see Section 2.2.6) remains limited. However, the lack of aDNA may be compensated for using DNA information from extant specimens. During organismal evolution, mutations become fixed over time (= substitutions) in the genome. Comparing these substitutions using DNA sequences of individuals that share a common ancestor may allow to reconstruct past evolutionary events, potentially driven by ecological, environmental, and/or geological processes (sensu Avise, 2000). A large ancestral population of lacustrine animals may, for example, become separated into two geographically separated subpopulations due to a severe lake-level lowstand. These resulting subpopulations might then evolve independently. After time, the two subpopulations thus become genetically distinct and potentially even new species. By comparing the substitutional patterns of the extant subpopulations/species using statistical approaches, the genetic structure of the common ancestor of these populations/species at the time of population subdivision (= ‘most recent common ancestor’) can be reconstructed. Under certain conditions, these divergence events might be time-calibrated using a methodology called molecular dating. This approach is based on the molecular clock hypothesis (Zuckerkandl and Pauling, 1965), which relates nucleotide or amino acid substitutions to time. In other words, knowing the number of substitutional differences between extant populations/species may allow for calculating divergence time and thus the age of the underlying event that potentially drove the separation of the ancestral population.

Today, molecular dating is widely used in evolutionary biology to infer such past evolutionary events. However, the accuracy and even the applicability of molecular dating have long been subject to controversy (e.g., Takahata, 2007; Wilke et al., 2009; Ho, 2014; Ho and Duchêne, 2014). Substitution rates may, for example, vary among taxa, with life history traits, and/or over time, making a precise dating of evolutionary events challenging. These rates are gene-specific and their correct inference depends on a number of additional issues such as incomplete lineage sorting and substitutional saturation. Moreover, results from molecular dating analyses can easily be misinterpreted if, for example, the sampling design of extant species is insufficient (e.g., Wilke, 2004; Wilke et al., 2009; Friedman et al., 2013). For most of these problems, test statistics and mathematical solutions are available, which have made molecular dating statistically sound. However, a problem that continues to exist is that the molecular clock has to be calibrated in order to be able to calculate absolute times. This can be done using calibration points or bounds from externally derived dates such as ages of fossil occurrences and biogeographical events (e.g., vicariance events resulting from the closure of the Isthmus of Panama or the Mediterranean Salinity Crisis; Ho et al., 2015). Alternatively, calibration can be achieved via external clock rates that are gene- and often also taxon- or trait-specific (reviewed in Wilke et al., 2009). All of these different calibration methods have in common that uncertainties introduced by the calibration process are often an order of magnitude larger than mathematical issues.

The two main approaches that are currently used for molecular dating are molecular clock and coalescence analyses. The former typically calculates divergence times between species or groups of species by estimating the number of substitutions that occurred along the respective branches of a phylogenetic tree (Fig. 12). The latter models the timing of demographic and spatial expansion events of populations (e.g., mismatch analyses; Rogers and Harpending, 1992), or past changes in population sizes (e.g., Bayesian Skyline Plots; Ho and Shapiro, 2011).

Both molecular clock and coalescence analyses have been used extensively in ancient lake studies including lakes Baikal (e.g., Sherbakov, 1999; Koskinen et al., 2002; Fazalova et al., 2010), Tanganyika (e.g., Nevado et al., 2013; Koblmüller et al., 2015), Malawi (e.g., Genner et al., 2010; Schultheiß et al., 2011), and Titicaca (e.g., Kroll et al., 2012). However, despite the high potential of molecular clock approaches in providing temporal information, their application in ancient lake drilling projects remains limited. Wagner et al. (2014b), for example, found a temporal correspondence between a lake-level low stand in Lake Prespa on the Balkan as inferred from sediment-core data, the Late Pleistocene Toba eruption, and a spatial expansion in the lake's most abundant mussel species. Moreover, Föller et al. (2015) used lineages-through-time plots (i.e., a visualization of the number of accumulated evolutionary lineages over time inferred from molecular clock analyses; Harvey et al., 1994) and diversification-rate analyses in an attempt to link major environmental events inferred from the deep-drilling campaign in Lake Ohrid to changes in diversification rates of endemic species.

Certainly, molecular clock analyses will gain more importance in future deep-drilling projects. However, given the 'vagaries' (Ayala, 1997) of the molecular clock, we recommend a careful planning of molecular dating approaches. This concerns the choice of methods, molecular markers, calibration means, and taxa. The latter requires especially thorough planning because sampling in ancient lakes can be expensive and time consuming, and incomplete sampling may bias molecular dating results. As a rule of thumb, molecular clock analyses in ancient lakes work best with large, monophyletic groups of endemic species – so-called species flocks (e.g., Schön and Martens, 2004). They typically evolved within the lake and these taxa are thus likely to reflect its environmental, ecological, and geological history.

Research on the behavior of the clock, calibration means, and data basis is continuing at high pace. Of interest for drilling projects in ancient lakes are, for example, newly developed models that enable improved fossil calibrations (e.g., Heath et al., 2014; Gavryushkina et al., 2015). However, notwithstanding the progress that will be made in the future, confidence intervals of molecular dating results will likely remain wide. It is therefore important to consider these uncertainties in all conclusions made based on molecular clock or coalescence approaches in order to avoid misinterpretations (reviewed in Hipsley and Müller, 2014; Warnock et al., 2015).

3.4. Tracing character evolution

Understanding character (= 'trait') evolution of a species' individual characters over time, such as changes in morphological/anatomical traits, ecological niches, functional roles in ecosystems, reproductive modes, or changes in geographic distributions, is of great relevance for linking geological and biological histories in ancient lakes. Tracing character evolution ideally involves groups of species that originated within the lake (species flocks) and which are comparatively old, thus allowing for inferring character evolution along the lake's entire geological history.

Studies of character evolution can be done utilizing two main approaches. The first involves the direct observation of character-state change using chronological fossil information, often supplemented with other primary information obtained from the sediment cores. Examples include stratigraphic series of gastropods (Williamson, 1981; Van Bocxlaer and Hunt, 2013) and diatoms (e.g., Khursevisch, 2006). However, continuous, high quality fossil information is typically not available (see Section 2.2.1) or only for selected groups of microfossils (Section 2.2.2). In the latter case, secondary data have to be used, i.e., information on characters states of extant species together with a hypothesis about their evolutionary relationships (typically a phylogenetic tree).

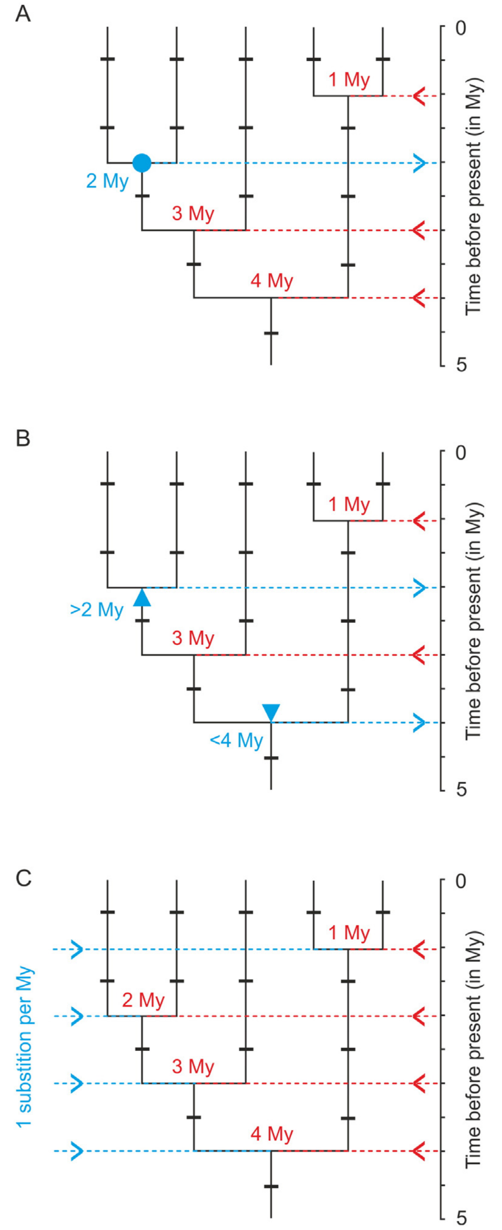

Fig. 12. Methods for calibrating molecular clock trees. Substitutions along the branches of a phylogenetic tree are shown as black rectangles. A) Calibration with point(s) from externally derived dates. In this example, the known age of a divergence event (blue circle and blue dashes line) is used to estimate the timing of three other divergence events in the tree (red dashed lines). B) Calibration with bounds from externally derived dates. In this example, the known minimum and maximum ages of two divergence events (upward and downward pointing blue arrows, respectively, and blue dashes lines) are used to estimate the timing of two other divergence events in the tree (red dashed lines). C) Calibration with a known external molecular clock rate. In this example, a fixed external clock rate of 1 substitution per My and derived depth of nodes in the tree (blue dashed lines) are used to calculate absolute divergence ages (red dashed lines). For reasons of clarity, error bars of calibration uncertainties are not shown.

There are two particular interests in tracing character evolution along a phylogenetic tree. The first involves 'ancestral-state reconstruction', i.e., the reconstruction of either categorical or continuous character states back in time (sometimes also referred to as 'character mapping' or 'character optimization'; see Fig. 13).

The second interest concerns modeling tempo (i.e., trait divergence per time) and mode (e.g., adaptive, non-adaptive, divergence, convergence) of mainly continuous character evolution. Tempo and mode may vary considerably over time and are potentially driven by extrinsic factors (sensu Schluter, 2000; Coyne and Orr, 2004). This offers the possibility to test the effects of geological, climatic, and/or environmental changes in ancient lakes inferred from sediment cores on species traits over time. Thereby, a major goal of interdisciplinary deep-drilling projects can be persued – understanding the consequences of environmental change for the biotic evolution of ancient lake taxa as well as the capacity of an ancient lake to buffer such perturbations.

Three testable models are particularly useful for ancient lake studies (Fig. 14). In the simplest model ('Brownian motion'; Edwards and Cavalli-Sforza, 1964), trait evolution proceeds as a random walk through trait space, implying that extrinsic factors have no effect on character evolution (Fig. 14A). Because of its neutral characteristic, it is often used as null model to compare to empirical data. The 'early burst model' describes a rapid trait evolution early on, after which the increase in disparity slows down (Blomberg et al., 2003; Harmon et al., 2010; Fig. 14B). It can, for example, be used to test whether the formation of an ancient lake triggered the onset of adaptive radiations (sensu Schluter, 2000). The third, the 'Hansen model', allows species to be influenced by past environmental events and the trait mean converges to an optimum over time (Fig. 14C; Hansen, 1997). This model focuses on the relationship between environment and the pull towards an optimal character state ('selection'; see Hansen, 2014).

Potential changes over time in tempo and mode of character evolution can be visualized by disparity-through-time plots (Harmon et al., 2003; see Muschick et al., 2012 for an example from Lake Tanganyika), i.e., a calculation of the mean relative disparity at each node (Fig. 14).

However, tracing character evolution is subject to several pitfalls and challenges. Phylogeny-based analyses of character evolution through time can be biased as morphological exuberant species may be more prone to extinction (Huang et al., 2015). The effects of missing extinct species on phylogeny-based character analysis has not been studied comprehensively yet (but see Albert et al., 2009; Slater et al., 2012). However, fossil species may provide complementary insights into character evolution (e.g., Van Bocxlaer and Hunt, 2013; Benson et al., 2014) and could be used to fine-tune or even verify models of character evolution (Betancur-R et al., 2015). Another challenge is that information on species traits and detailed information on morphology, ecological attributes, and distribution of the taxa of concern is often sparse. Quality issues include sampling completeness, taxonomic coverage, the presence of cryptic species, and DNA marker choice, all affecting the quality of phylogenetic trees (node support, resolution) and subsequent state reconstructions (ambiguous states).

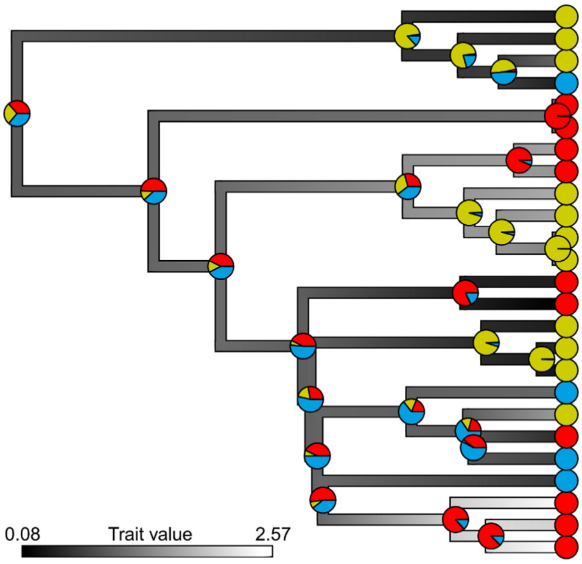

Fig. 13. Tracing character ('trait') evolution along a phylogeny. Either categorical (pie charts) or continuous (grey-step gradient) character states can be estimated along the branches of a phylogeny solely based on trait information of extant species. Uncertainties of character tracing are here exemplified for the categorical states by the proportions of the pie charts, but uncertainty can also be estimated for continuous traits.

Examples of tracing character evolution in ancient lakes include studies on the effect of lake origin on changes in morphological traits. Gonzalez-Voyer and Kolm (2011), for example, showed two periods of increased morphological disparity in Lake Tanganyika, the older was related to the initial colonization after lake origination but the younger one could not be explained by limnological history. Tracing character evolution was also used to infer trophic specialization and coloration patterns of endemic invertebrates of the ancient Malili Lakes of Sulawesi, Indonesia (von Rintelen et al., 2004, 2010) and to uncover convergent evolution in ancient lakes (e.g., Meixner et al., 2007; Young et al., 2009). The latter phenomenon has been puzzling evolutionary biologists for years (e.g., Mahler et al., 2013) and might be of eminent importance for the interpretation of fossil records from sediment cores. Other applications include reconstructing the colonization history of ancient lakes over time (e.g., Van Bocxlaer et al., 2015; Daniels et al., 2015), habitat or niche occupation of ancient lake species such as the colonization of rocky habitats in Lake Tanganyika by cichlids (Koblmüller et al., 2004), or the bathymetric range evolution of limpet gastropods in lakes Baikal (Stelbrink et al., 2015) and Ohrid (Albrecht et al., 2006).

From a methodological point of view, there are three trends in tracing character evolution that might be of importance for future deep-drilling projects. First, phylogenetic uncertainties (Sorenson et al., 2014; Shi and Rabosky, 2015) and character variance caused by measurement errors or intraspecific variation (Revell, 2012; Clavel et al., 2015) need to be considered. Second, shifts in the tempo (i.e., different rates of trait divergence; Eastman et al., 2011; Thomas and Freckleton, 2012) or mode (e.g., from neutral divergence to adaptation; Clavel et al., 2015) of character evolution over time are to be identified, which may help mitigating erroneous ancestral-state reconstruction (King and Lee, 2015). Third, characters may not evolve independently of each other but co-vary or even constrain one another. Therefore, multivariate evolutionary models that simultaneously use a set of characters with several states each are being designed (Freckleton, 2012; Mahler et al., 2013; Adams and Collyer, 2015; Clavel et al., 2015).

Whereas most earlier attempts did not integrate geological and evolutionary data from ancient lakes at once, such integration will become increasingly important in analyses of character changes. Integrated approaches would allow to test whether geological or environmental changes previously inferred from deep-drilling campaigns had an effect on patterns of character change in species (e.g., Danley et al., 2012; Lyons et al., 2015; Stelbrink et al., 2015). Potential drivers of previously inferred changes in species traits can also be studied with data that are subsequently generated from deep-drilling campaigns (e.g., von Rintelen et al., 2010 for Lake Towuti and Trajanovski et al., 2010 for Lake Ohrid).

For future deep-drilling projects, we expect to see more explicit attempts to link geological and biological histories. As more phylogenetic data become available, future comparative analyses across taxa will help shed light on general evolutionary processes in ancient lakes that affect entire communities (e.g., Salzburger et al., 2014). If common temporal signals in phylogenies can be detected (e.g., simultaneous character or rate changes across taxa; O'Meara et al., 2006), a more straightforward link between environmental changes and evolutionary patterns may be established. These signals could also help to understand the biological consequences of environmental change in ancient lakes, even when fossil information is absent.

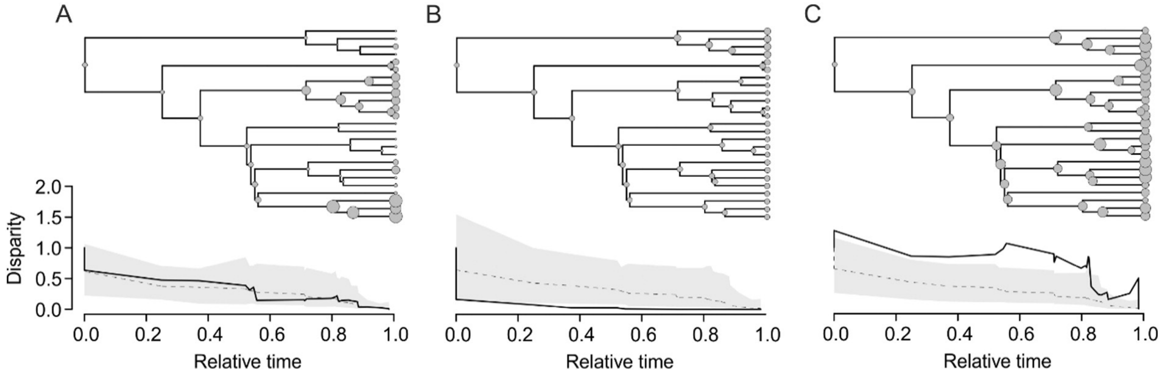

Fig. 14. Simulation of three modes (A–C) of trait evolution (grey circles) showing their signatures in the respective disparity-through-time plots (trait divergence $\sigma^2 = 0.3$). Observed traits at the tips of the phylogeny are used to estimate the 95% confidence interval over time (grey area). Deviation of the observed disparity (solid black line) from this expectancy may indicate periods of environmental influence on evolution or changes in selection strength. A) Brownian motion, which is a random walk model and the null expectancy in evolutionary studies. B) Early burst model of trait evolution in which the accumulation of disparity decreases over time. C) Hansen model of adaptive trait evolution with trait mean approaching an optimum.

4. Conclusions

1) Over the past years, scientific drilling projects in ancient lakes became increasingly interdisciplinary and have intensified the use of secondary data, i.e., data obtained independently of the drilling operation. Comprehensive interdisciplinary projects enable a more holistic view on scientific problems and provide excellent opportunities for hypothesis-driven research.
2) One of the most challenging tasks for answering novel research questions in deep-drilling projects is to link diverse datasets with different resolutions, different data qualities, and potentially different age uncertainties to solve complex problems.
3) Careful consideration of drill sites and drilling strategies are a prerequisite to optimize the chances that the goals of a deep-drilling project can be reached. Meticulous preparation, including the collection of site-specific information from pilot studies may serve to construct a strategy for collecting primary and secondary data that can then be evaluated against scientific objectives, budget, logistic requirements, and the available time.
4) Accurate subsampling and data interpretation requires a full tracking record of the core. Moreover, sediment subsampling should be coordinated and samples should, whenever possible, be taken from the same sample depth to aid data integration.
5) Data analyses should be hierarchically structured. As studies from long records are very laborious and time-consuming, the first target should be to produce low/medium resolution data. Only then, high-resolution analyses are to be conducted.
6) Though many new methods and analyses (e.g., analysis of isotopes in organic materials, high throughput image analyses and high throughput DNA sequencing, low-concentration biomarker analyses, studies of ancient amino acids and ancient DNA, molecular dating) are of high potential for integrating diverse datasets, their weaknesses in terms of resolution, quality of data, and practicability have to be considered.
7) Whereas the physical linkage of primary information obtained from sediment cores is, in most cases, straightforward due to the chronological constraints on the data, integrating secondary data and/or interpretations into a consistent representation of the natural history of ancient lakes remains challenging.
8) Moreover, in most previous deep-drilling projects, geological and biological data were linked empirically. However, recent statistical developments enable a better integration of diverse types of datasets and the testing of hypotheses based on specific null models.
9) For future deep-drilling campaigns we expect to see more explicit attempts to statistically link geological and biological histories aided by methodological advances in data generation (e.g., automated methods of fossil retrieval, visualization and identification) and data analyses (e.g., a better integration of uncertainties in age-depth calculations).
10) Interdisciplinary projects should include earth and life scientists, statisticians, and modelers in the planning phase, to establish clear communication strategies, to align interests, and to discuss data requirements.
11) Finally, the interdisciplinary character of modern deep-drilling projects not only requires a constant adaptation to methodological innovations, but also targeted scientific training components. Therefore, thematic workshops, network training events and/or field schools, particularly for early stage researchers, should be an integrative part of interdisciplinary deep-drilling campaigns.

Glossary

Accommodation space Available space for accumulation of sediments.

Adaptive radiation Rapid diversification of species accompanied by adaptation into various niches. The term is used both to describe an evolutionary process as well as the result of this process.

Age-depth model Synthetic model that explains the relationship between sediment depth and sediment age in depositional environments.

Allopatric speciation (= *geographical speciation*) Speciation due to the evolution of (geographical) reproductive barriers in populations that prevent or interfere with gene flow.

Amino acid racemization Spontaneous reaction describing the interconversion between the chiral forms of an amino acid.

Aminostratigraphy Relative dating framework based on the extent of amino acid racemization in subfossil biominerals.

Anagenesis Directional evolutionary change from an ancestor species to a descendant species without lineage splitting (see also chronospecies).

Ancestral-state reconstruction Reconstruction of ancestral phenotypic or genetic states of species along a phylogenetic tree based on information of extant taxa.

Ancient lake A lake that has continuously existed for >100 ky or even >1 My. The meaning of the term is not universally accepted. Some authors use this term synonymously with 'long-lived lake'. Others use the term ancient lake only for extant long-lived lakes.

Ancient lake species flock Species rich, monophyletic group of endemic taxa that typically evolved within the lake (i.e., intralacustrine).

Bioindicator Extant species that are used to infer the present ecological conditions of an ecosystem.

Biomarker, *sedimentary* Source-specific organic molecules ('molecular fossils').

Biovolume-accumulation rate Diatom concentration divided by sediment-accumulation rate and corrected for size differences between taxa.

Borehole logging Process of measuring physical, chemical, and structural properties of penetrated geological formations using logging tools that are lowered into a borehole on a wireline cable.

Bottleneck, *genetic* Sudden decrease in population size, which potentially reduces the genetic variation within a population.

Coalescence analysis Population genetic analysis that relates patterns of genetic diversity in an extant population to its demographic history.

Chronospecies Arbitrary divisions of a single evolutionary lineage, defined on the basis of morphological change within the lineage (see also anagenesis).

Cladogenesis Evolutionary branching of an ancestor species into two or more descendant species.

Composite core Layer-to-layer correlation of core segments from multiple boreholes drilled at the same drill site, i.e., best-case scenario of a continuous, undisturbed sediment profile.

Convergence Similarities that have arisen independently in two or more organisms that do not share a common ancestry.

Cyclostratigraphy Study of stratigraphic records of astronomically forced climate cycles.

Depocenter Location of the thickest deposit in a sedimentary basin.

Disparity Ratio of morphological variation among subclades and the entire phylogeny. High disparity is a result of high within and low among subclade variation, suggesting clade-independent filling of morphospace.

Divergence time Time since separation of descendent taxa from a most recent common ancestor.

Endemism Characteristic of a taxon that is restricted to a geographic location (such as an ancient lake).

Evolution, *biological* Change in heritable traits of populations from generation to generations.

Evolution, *biotic* Gradual change in the structure, composition, or dynamics of biological objects or systems.

Evolution, *geological* Gradual change in the structure, composition, or dynamics of geological objects or systems.

Global benthic isotope stack Stack of 57 benthic marine $\delta^{18}O$ records reflecting global ice volume and deep ocean temperature for the past 5.3 My, often used as stratigraphic reference record.

Hiatus Discontinuity ('break') in the stratigraphic succession.

Incomplete lineage sorting Phenomenon that not all genetic lineages are segregated at the time of species splitting.

Interdisciplinarity Integration of two or more scientific disciplines.

Intralacustrine Within a lake.

Lineages-through-time plot Plot showing the accumulation of lineages through time in a time-calibrated phylogeny.

Lake proper Lake body excluding peripheral water bodies or effluents/affluents.

Long-lived lake A lake that has continuously existed for >100 ky or even >1 My. Some authors use this term synonymously with the term 'ancient lake'.

Macrofossils Remains of organisms from the remote past large enough to be visible without a microscope.

Magnetic susceptibility A measure of the degree of magnetization of a material after the application of a magnetic field.

Microbiome The entire microbial population within a specific environmental niche.

Microfossils Microscopically small remains of organisms from the remote past.

Molecular clock A concept that correlates number of nucleotide or amino acid substitutions (i.e., mutations fixed in the genome) to time.

Multidisciplinarity Concurrent combination of two or more scientific disciplines.

Phylogenetic tree Graphical representation of evolutionary (genealogical) relationships of several species or other units, which are assumed to have a common ancestor.

Pollen rain The cloud of airborne pollen produced by plants.

Proxy Measured variable used to model or generate the value of a variable that is typically more difficult to obtain.

Radiation, *evolutionary* Event of rapid cladogeneses.

Relaxed clock A dating approach that relaxes the assumption of a single substitution rate within a phylogeny and allows rates to vary across the branches.

Resilience, *ecosystem* Ability of an ecosystem to resist disturbances.

Speciation Evolutionary process leading to new species.

Species flock In ancient lakes, monophyletic group of endemic species that evolved intralacustrine.

Stable isotope An isotope of an element that does not tend to decay over time.

Substitution rate Here used in terms of number of fixed mutations per site and time unit.

Subsurface biosphere Term used to designate the active microbial life in lacustrine sediments in analogy to deep biosphere that refers to the marine environment.

Tephra Pyroclastic material ejected from a volcano including fragmented rocks and smaller particles.

Trait An inherited morphological, molecular, or ecological characteristic of a species.

Trait-specific clock A single molecular clock rate of a specific gene that can be assigned to a range of taxa that share similar biological and life history characteristics that are supposedly affecting rate heterogeneity.

Transfer function, *paleoecological* Model that correlates modern species assemblages and their environmental characteristics to fossil assemblages for reconstructing past environmental conditions.

Watershed Catchment area of a drainage basin.

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The environmental and evolutionary history of Lake Ohrid (FYROM/Albania): interim results from the SCOPSCO deep drilling project

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Abstract. This study reviews and synthesises existing information generated within the SCOPSCO (Scientific Collaboration on Past Speciation Conditions in Lake Ohrid) deep drilling project. The four main aims of the project are to infer (i) the age and origin of Lake Ohrid (Former Yugoslav Republic of Macedonia/Republic of Albania), (ii) its regional seismotectonic history, (iii) volcanic activity and climate change in the central northern Mediterranean region, and (iv) the influence of major geological events on the evolution of its endemic species. The Ohrid basin formed by transtension during the Miocene, opened during the Pliocene and Pleistocene, and the lake established de novo in the still relatively narrow valley between 1.9 and 1.3 Ma. The lake history is recorded in a 584 m long sediment sequence, which was recovered within the framework of the International Continental Scientific Drilling Program (ICDP) from the central part (DEEP site) of the lake in spring 2013. To date, 54 tephra and cryptotephra horizons have been found in the upper 460 m of this sequence. Tephrochronology and tuning biogeochemical proxy data to orbital parameters revealed that the upper 247.8 m represent the last 637 kyr. The multi-proxy data set covering these 637 kyr indicates long-term variability. Some proxies show a change from generally cooler and wetter to drier and warmer glacial and interglacial periods around 300 ka. Short-term environmental change caused, for example, by tephra deposition or the climatic impact of millennial-scale Dansgaard–Oeschger and Heinrich events are superimposed on the long-term trends. Evolutionary studies on the extant fauna indicate that Lake Ohrid was not a refugial area for regional freshwater animals. This differs from the surrounding catchment, where the mountainous setting with relatively high water availability provided a refuge for temperate and montane trees during the relatively cold and dry glacial periods. Although Lake Ohrid experienced significant environmental change over the last 637 kyr, preliminary molecular data from extant microgastropod species do not indicate significant changes in diversification rate during this period. The reasons for this constant rate remain largely unknown, but a possible lack of environmentally induced extinction events in Lake Ohrid and/or the high resilience of the ecosystems may have played a role.

1 Introduction

Systematic limnological studies started in the early 20th century and were first carried out in Europe, for example at Lake Geneva (e.g. Forel, 1901), a number of lakes in Germany (e.g. Thienemann, 1918), and at Lake Ohrid on the Balkan Peninsula (reviewed in Stanković, 1960). These initial studies focused on hydrological data, such as temperature, dissolved oxygen, and bottom morphology, and on biological data, such as the distribution and ecology of lake biota. Analytical and technological advances in the following decades facilitated a more comprehensive understanding of the interactions between catchment dynamics, hydrology, and the living world of lakes. This led to the establishment of new institutions, such as the Hydrobiological Institute at Lake Ohrid in 1935 (Stanković, 1960).

Besides analyses in extant lakes, early scientists were also interested in studying past changes in lake systems, and palaeolimnology, a sub-discipline of limnology, was established in the 1920s. This field started with the collection of sediment cores from lakes to interpret stratigraphic data on plant and animal fossils as a record of the lake's history (National Research Council, 1996). Particularly with the establishment of radiometric dating methods in the 1950s and 1960s, palaeolimnological studies developed into a powerful tool for long- and short-term reconstructions of the climatic and environmental history of lakes and their catchments.

One of the most important developments in palaeolimnological work has been the formation of a multi-national continental drilling program – the International Continental Scientific Drilling Program (ICDP). The Potsdam Conference, conducted in 1993, defined the scientific and management needs for the ICDP and declared Lake Ohrid, Europe's oldest freshwater lake, as an ICDP target site.

One of the most outstanding characteristics of Lake Ohrid, besides its presumed old age, is its high degree of endemic biodiversity. With more than 300 described eukaryotic endemic taxa (Föller et al., 2015), Lake Ohrid belongs to the most biodiverse ancient lakes, i.e. lakes that have continuously existed for >100 kyr (Albrecht and Wilke, 2008). If its surface area is taken into account, it may have the highest endemic biodiversity amongst all lakes worldwide. Though Lake Ohrid has long been considered to be of Tertiary age, estimates vary considerably, between ca. 2 and 10 Ma (reviewed in Albrecht and Wilke, 2008). Likewise, its limnological origin remains poorly understood, and hypotheses include palaeogeographical connections to former marine or brackish water systems and a de novo formation from springs and/or rivers (see also Albrecht and Wilke, 2008, for further information and references therein).

The unique characteristics of Lake Ohrid, together with the lack of knowledge regarding its origin, precise age, and limnological/biological evolution, provided the main motivation to establish an international scientific deep drilling project. Its continuous existence over a long timescale together with an extraordinary degree of endemic biodiversity made Lake Ohrid an ideal "natural laboratory" to study the links between geological and biological evolution and to unravel the driving forces of speciation, leading to the interdisciplinary project Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO). The four major aims of the SCOPSCO project are to (i) obtain more information on the age and origin of Lake Ohrid, (ii) unravel the regional seismotectonic history including effects of major earthquakes and associated mass-wasting events, (iii) obtain a continuous record containing information on Quaternary volcanic activity and climate change in the central northern Mediterranean region, and (iv) evaluate the influence of major geological events on biotic evolution and the generation of the observed extraordinary degree of endemic biodiversity (Wagner et al., 2014). Based on several site surveys and studies conducted between 2004 and 2011, an ICDP drilling campaign at Lake Ohrid was carried out in spring 2013 using the Deep Lake Drilling System (DLDS) from the Drilling, Observation and Sampling of the Earth's Continental Crust (DOSECC) consortium. In total, more than 2100 m of sediments were recovered from four drill sites, with a maximum penetration of 569 m below lake floor (b.l.f.) at the main drill site (DEEP) in the central part of Lake Ohrid (Fig. 1).

Subsampling and analyses are ongoing, but initial, detailed results of geological and biological investigations of the upper 247.8 m (637 ka) of the DEEP sediment sequence and newer results from biological studies on the extant fauna of Lake Ohrid were recently published in a special issue in the journal *Biogeosciences* (Wagner et al., 2015). The aim of this paper is to review and synthesise the results of the 14 individual papers of this special issue and to complement them with information from former and new studies in order to provide a comprehensive overview on progress towards achieving the four main aims defined for SCOPSCO.

2 Site information

Lake Ohrid is a transboundary lake shared between the Former Yugoslav Republic of Macedonia (FYROM) and the Republic of Albania (Fig. 1). The lake is located at 693.5 m above sea level (a.s.l.) and has a maximum length of 30.4 km (N–S), a maximum width of 14.7 km (W–E), a surface area of 358 km^2, and a tub-shaped bathymetry with a maximum water depth of 293 m, a mean water depth of ~ 151 m, and a total volume of 50.7 km^3 (Fig. 1; Popovska and Bonacci, 2007; Lindhorst et al., 2012). Water loss occurs by evaporation (13.0 $\mathrm{m}^3\,\mathrm{s}^{-1}$) and by the artificially controlled surface outflow in the northern part of the lake, river Crni Drim, which flows into the Adriatic Sea. Outflow rates vary between 22.0 $\mathrm{m}^3\,\mathrm{s}^{-1}$ (Popovska and Bonacci, 2007) and 24.9 $\mathrm{m}^3\,\mathrm{s}^{-1}$ (Matzinger et al., 2006, and references therein), depending on seasonal and long-term variations in water level of up to ~ 1.5 m between 1950 and 2000 (Popovska and Bonacci, 2007). The total water loss can be averaged to $\sim 36.5\,\mathrm{m}^3\,\mathrm{s}^{-1}$ and is balanced by water input from surface and sublacustrine springs, direct precipitation, and rivers. Published data of the annual precipitation in the watershed of Lake Ohrid vary between 698.3 and 1194.0 $\mathrm{mm\,yr}^{-1}$, with higher precipitation at higher altitudes and an average of 907 $\mathrm{mm\,yr}^{-1}$ (Popovska and Bonacci, 2007). The average monthly rainfall is highest in winter, with a maximum in November and December, and lowest between June and September. The lake level, however, is highest in June due to snowmelt input and lowest in October and November, before the start of autumn rainfall (Popovska and Bonacci, 2007). The seasonal and long-term variations in water budget allow only an approximation of the water input from the various sources. Direct precipitation and river inflows (45 %) as well as surface and sublacustrine karst springs (55 %) contribute to the overall water input (Matzinger et al., 2006). The river Sateska, which was previously a direct tributary of the Crni Drim, was artificially diverted into Lake Ohrid in 1962 and is today the largest surface river inflow with a contribution of ~ 15 % of the total inflow of Lake Ohrid (Matzinger et al., 2006; Popovska and Bonnacci, 2007). The karst springs are located primarily along the eastern shoreline of the lake (Fig. 1) and karst waters originate in almost equal proportions from mountain range precipitation and via outflow from Lake Prespa, located ~ 10 km to the east and ~ 155 m higher in altitude (Matzinger et al., 2006). Calculating the ratio between the volume of Lake Ohrid (50.7 km^3) and its outflow ($\sim 23.5\,\mathrm{m}^3\,\mathrm{s}^{-1}$) results in a theoretical water residence time of ~ 70 years (Matzinger et al., 2006; Popovska and Bonacci, 2007). This theoretical residence time is reduced to ~ 45 years, when evaporation is taken into account and calculated with the total water output or input ($\sim 36.5\,\mathrm{m}^3\,\mathrm{s}^{-1}$). However, the real water residence time is probably much higher, as sporadic mixing intervals or incomplete mixing, variations in wind stress, or kinetic effects of inflow water entering may affect the lake's hydrology (Ambrosetti et al., 2003). For example, Lago Maggiore in Italy was classified as a holo-oligomictic lake prior to 1970, when the upper 150–200 m of the water column mixed every winter and complete mixing occurred irregularly every few years (Ambrosetti et al., 2003). This is similar to Lake Ohrid today (Matzinger et al., 2006) and the real residence time at Lago Maggiore is higher by a factor of 3 to 4 than the theoretical residence time (Ambrosetti et al., 2003).

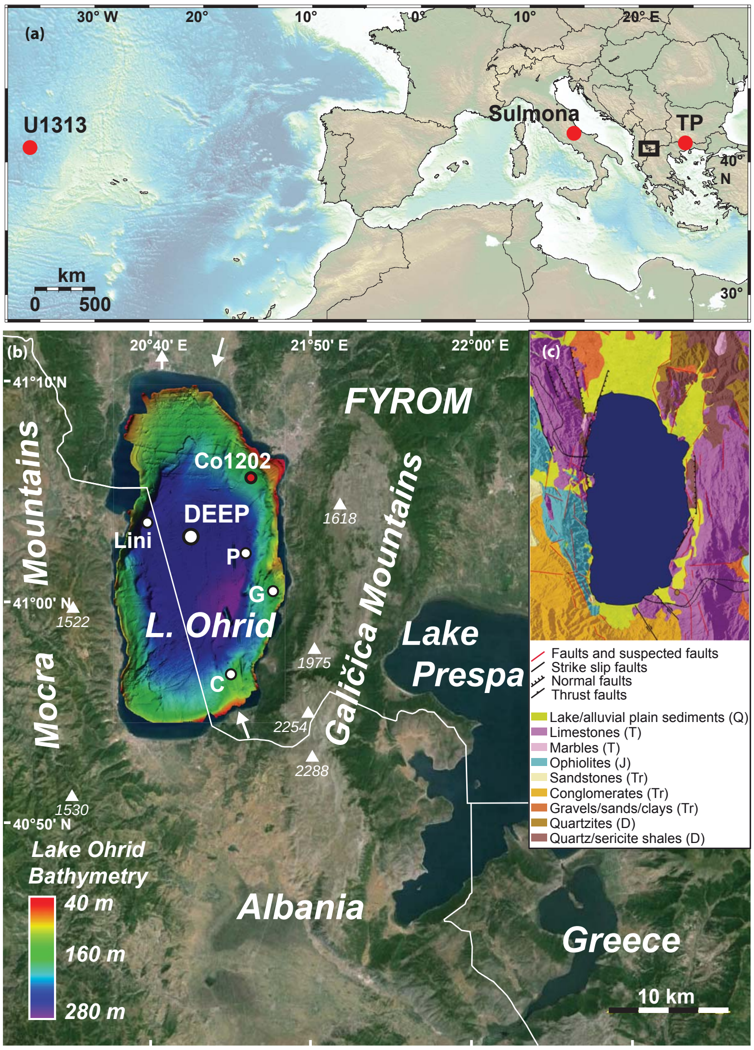

Figure 1. (a) Location of Lake Ohrid (black rectangle) on the Balkan Peninsula at the border of the Former Yugoslav Republic of Macedonia (FYROM) and the Republic of Albania. Other records mentioned in the text are indicated by red dots (core U1313 in the North Atlantic, Sulmona basin in Italy, Tenaghi Philippon (TP) in Greece). **(b)** Map of the area of lakes Ohrid and Prespa and bathymetric map of Lake Ohrid (from Lindhorst et al., 2015). Coring locations of piston core Co1202 (red; Vogel et al., 2010a) and ICDP sites (white) are shown, with DEEP and Lini sites mentioned in the text. Secondary ICDP sites P (Pestani), G (Gradiste), and C (Cerava) are not mentioned in the text. **(c)** Geological map of the Lake Ohrid catchment (modified from Lindhorst et al., 2015).

Physical and chemical characteristics of Lake Ohrid have been provided in several publications and annual reports (e.g. Watzin et al., 2002; Matzinger et al., 2006; Jordanoski et al., 2004, 2005; Naumoski et al., 2007; Schneider et al., 2014). Average total phosphorous (TP) concentrations of $< 10\,\mathrm{mg\,m^{-3}}$ and Secchi depths ranging between 7 and 16 m characterise the pelagic zone of Lake Ohrid as oligotrophic. These oligotrophic conditions explain why bottom water oxygen concentrations of above $4\,\mathrm{mg\,L^{-1}}$ are recorded even during years without complete overturn (Matzinger et al., 2006). The surface water temperature varies between ∼ 25 °C in summer and ∼ 7 °C in winter, while bottom water temperatures are ∼ 6 °C throughout the year. The boundary between epilimnion and hypolimnion is between 30 and 50 m, depending on the season. The pH decreases from 8.6–8.9 in surface waters to 7.9–8.4 in bottom waters. The specific conductivity is around $200\,\mathrm{\mu S\,cm^{-1}}$ in surface waters and around $150\,\mathrm{\mu S\,cm^{-1}}$ at 50–200 m water depth and increases again in deeper waters. The concentration of Si is lowest in the trophogenic surface waters, where it is taken up by diatoms, and increases gradually to $< 2\,\mathrm{mg\,L^{-1}}$ in bottom waters (Stanković, 1960). The littoral part of the lake exhibits a slightly higher trophic state (Schneider et al., 2014). These meso- to slightly eutrophic conditions in relatively shallow waters might be due to a direct input of nutrients from the catchment, higher temperatures, and increasing anthropogenic pollution over the last several decades (Kostoski et al., 2010; Schneider et al., 2014). The macrophytic flora in the littoral part of Lake Ohrid can be subdivided into different belts, with *Chara* species in water depths between 3 and 30 m, *Potamogeton* species in shallow waters, and a discontinuous belt of *Phragmites australis* along the shore (Albrecht and Wilke, 2008; Imeri et al., 2010).

The vegetation in the catchment of Lake Ohrid can be categorised along altitudinal belts (see Filipovski et al., 1996; Matevski et al., 2011). Grasslands and agricultural land are encountered in the littoral zone and the lowlands surrounding the lake, followed by forests dominated by different species of both deciduous and semi-deciduous oaks (*Quercus cerris*, *Q. frainetto*, *Q. petraea*, *Q. pubescens*, and *Q. trojana*) and hornbeams (*Carpinus orientalis*, *Ostrya carpinifolia*) up to 1600 m a.s.l. Mesophilous/montane species such as *Fagus sylvatica*, *Carpinus betulus*, *Corylus colurna*, *Acer obtusatum*, and *Abies borisii-regis* dominate at higher altitudes up to 1800 m a.s.l. Due to intense grazing, the timberline is between 1600 and 1900 m a.s.l. Reforestation is now slowly replacing the existing alpine pasture lands and grasslands at and above this altitude (Matevski et al., 2011). Sparse populations of several *Pinus* species, considered to be Tertiary relics, are located in the wider region of Lake Ohrid (Sadori et al., 2016). Em et al. (1985) considered the Ohrid-Prespa region to be a refugial area with remains of vegetation of other species (e.g. *Pinus heldreichii*, *Quercus trojana*, *Juniperus excelsa*, *Aesculus hippocastanum*, *Genista radiata*).

The highest mountains in the Lake Ohrid watershed, which encompasses 1002 km^2 sensu stricto and 2393 km^2 including the Lake Prespa catchment, reach 1532 m a.s.l. in the Mocra Mountains to the west, and 2288 m a.s.l. in the Galičica Mountains to the east of the lake. The average altitude of the Lake Ohrid watershed is 1109 m a.s.l. About 12 % of its watershed is located at an altitude above 1500 m a.s.l. (Popovska and Bonacci, 2007). Intensely karstified Triassic limestones and Devonian siliciclastic bedrock dominate in the south-eastern, eastern, and north-western catchment (e.g. Wagner et al., 2009; Lindhorst et al., 2015). Ultramafic metamorphic and magmatic rocks including ophiolites of Jurassic and Cretaceous age crop out in the west. The plains at the northern, north-eastern, and southern lake shore are covered by Quaternary sediments.

3 Material and methods

3.1 Field work

3.1.1 Seismic and hydro-acoustic surveys

Seismic and hydro-acoustic surveys were carried out on Lake Ohrid between 2004 and 2009. Parametric sediment echosounder profiles span > 900 km in length and were collected at operating frequencies between 6 and 12 kHz (SES-96 light in 2004 and SES 2000 compact in 2007 and 2008, Innomar Co.). These frequencies allowed up to 60 m of penetration into the sediments at a vertical resolution of ~ 20 cm. Over 500 km of profiles were collected by multichannel seismic surveys using a Mini GI Air Gun (0.2 L in 2007 and 0.1 L in 2008) and a 16-channel 100 m long streamer. The Mini GI Air Gun operated at frequencies between 150 and 500 Hz and allowed a maximum penetration of several hundred metres at a vertical resolution of ~ 2 m. A multibeam survey in 2009, using an ELAC Seabeam 1180 sonar system, was used to acquire detailed bathymetric information of the lake floor below ~ 20 m water depth. More detailed information on the technical specifications of the seismic and hydro-acoustic systems, their settings, the location of the individual profiles, and the operational logistics can be found in Wagner et al. (2014) and Lindhorst et al. (2015).

3.1.2 Coring and on-site analyses

Several gravity and piston coring campaigns were carried out from local research vessels or small floating platforms (UWITEC Co.) on Lake Ohrid between 2004 and 2011. Whereas surface sediments collected by gravity corer throughout the basin were used to reconstruct the recent settings and the most recent history of Lake Ohrid (e.g. Matzinger et al., 2007; Wagner et al., 2008a; Vogel et al., 2010c), piston cores with a maximum penetration of ~ 15 m b.l.f. were collected from the lateral parts of the lake, where the water depth did not exceed 150 m (e.g. Wagner et al., 2008b, 2009; Belmecheri et al., 2009; Vogel et al., 2010a, b). These piston cores enabled a reconstruction of the environmental, climatic, and tephrostratigraphic history of the lake back to ~ 140 ka and provided fossil records of pollen (Wagner et al., 2009), molluscan faunas (Albrecht et al., 2010), and diatom floras (Reed et al., 2010).

Based on the site surveys, five primary target sites in Lake Ohrid were proposed for the SCOPSCO ICDP project. One of these sites, Lini (Co1262; Fig. 1), was cored in 2011 using a UWITEC platform and piston corer at 260 m water depth. Although the Co1262 sediment sequence reached only 10.08 m b.l.f., this is the most complete Holocene sequence retrieved to date. Studies on the core material contributed to a better understanding of the tectonic activity (Wagner et al., 2012) and the Late Glacial to Holocene environmental history of the region (Lacey et al., 2015; Zhang et al., 2016).

The remaining four sites were cored in spring 2013 using the DLDS (Wagner et al., 2014; Francke et al., 2016). At the main site, the DEEP site in the central part of the Lake Ohrid basin, six holes (5045-1A to 5045-1F) were drilled with a maximum depth of ~ 569 m b.l.f. (Fig. 1) and an average distance of ~ 40 m between the individual holes (for details see Francke et al., 2016). In total, ~ 1500 m of sediment cores were recovered, cut into up to 1 m long segments, and stored in a reefer at 4 °C before being shipped to the University of Cologne, Germany, for further processing.

On-site analyses during the 2013 deep drilling campaign included borehole logging, core scanning for magnetic susceptibility (MS), and sedimentological and palaeobiological core catcher analyses. Borehole logging was carried out with various probes at all four drill sites. The logging tools comprised MS, dipmeter, resistivity, borehole televiewer, spectral gamma ray (SGR), and sonic. While SGR was run through the drill pipe in order to prevent caving of sediments into the drill hole, all other tools were run in 40–50 m long open-hole sections except for the uppermost 30 m b.l.f., which was kept open with drill pipes to allow re-entry of other probes. Details of the borehole logging tools, logging speed, and vertical resolution are given in Baumgarten et al. (2015). Check shots were recorded for hole 5045-1C, allowing a very good seismic-to-core correlation for the DEEP site.

In order to determine volume-specific MS on the sediment cores and to carry out preliminary core correlation, all cores were scanned on site at a resolution of 2 cm with a Bartington MS2C loop sensor (10 cm internal diameter) mounted on a multi-sensor core logger (MSCL, Geotek, UK). Smear slide analyses of core catcher material (~ 3 m resolution) from holes 5045-1B and 5045-1C were used for on-site diatom analyses (Wagner et al., 2014).

3.1.3 Laboratory work

The geological work carried out on the gravity and piston cores from the site surveys and on the cores obtained during the ICDP drilling campaign comprises a broad suite of analytical methods. It includes lithological description after core opening, measurement of the geophysical properties, and granulometric, geochemical, mineralogical, and rock-magnetic analyses. These analyses are carried out on whole core sections, on split core surfaces, and on discrete samples (see Wilke et al., 2016) and are described in detail in several individual publications (Matzinger et al., 2007; Wagner et al., 2008a, b, 2009, 2012; Belmecheri et al., 2009, 2010; Holtvoeth et al., 2010, 2016; Leng et al., 2010; Lindhorst et al., 2010; Matter et al., 2010; Vogel et al., 2010a, b; Lacey et al., 2015, 2016; Francke et al., 2016; Just et al., 2016; Leicher et al., 2016). Dating of the sediment successions was mainly based on radiocarbon dating as well as tephrostratigraphic and tephrochronological work. Tuning of sediment proxies to orbital parameters, such as summer insolation and winter season length, or to other records has only been carried out on the sediment sequence from the DEEP site (Baumgarten et al., 2015; Francke et al., 2016; Zanchetta et al., 2016). Optical and geochemical information was used for a correlation of the DEEP core sequences and led to a composite profile of 584 m composite depth (mcd) (Francke et al., 2016, and unpublished data). Some of the sediment sequences were also studied for their fossil diatom, pollen, ostracod, or mollusc compositions. The sample preparation for the micro- and macrofossil analyses and the determination of the taxa are described in detail in the individual publications (Belmecheri et al., 2009, 2010; Wagner et al., 2009, 2014; Albrecht et al., 2010; Reed et al., 2010; Cvetkoska et al., 2016; Sadori et al., 2016; Zhang et al., 2016).

Information on interspecific relationships between Ohrid endemics and Balkan species, and on the drivers of speciation processes and community changes was derived from extant taxa by conducting molecular phylogenetic, lineage-through-time plot, and diversification-rate analyses (for details see Föller et al., 2015, and references therein), as well as modelling of community assembly processes (see Hauffe et al., 2016).

4 Results and discussion

4.1 Age and origin

4.1.1 Age

At the start of the SCOPSCO project, the age and origin of Lake Ohrid were poorly constrained. Previous geological and biological age estimates varied from 2 to 10 Ma (summarised in Albrecht and Wilke, 2008). Our new results allow for more precise age estimation. Based on SGR from borehole logging, MS from core logging, and total inorganic carbon (TIC) analyses on core catcher samples from the DEEP site, and by comparing these data with global climate records such as the benthic isotope stack LR04 (Lisiecki and Raymo, 2005), a minimum age of 1.2 Ma has been proposed for the permanent lake phase of Lake Ohrid (Wagner et al., 2014). This minimum age is supported by the results from more detailed studies of the uppermost 247.8 mcd of the DEEP site sequence, which covers the last 637 kyr, according to an age model derived from tephrochronology and tuning of biogeochemical proxy data to orbital parameters (Francke et al., 2016). The high-resolution data allow a better understanding of proxy variation over time and show that high TIC characterises interglacial periods and very low TIC represents glacial periods, as previously inferred from studies on core catcher material (Wagner et al., 2014). Indeed, a prominent TIC maximum at ~ 368 m b.l.f. in the core catcher samples from the DEEP site was presumed to represent the Marine Isotope Stage (MIS) 31 at 1.081–1.062 Ma (Wagner et al., 2014), which is regarded as one of the warmest interglacials during the Middle Pleistocene Transition (MPT; e.g. Melles et al., 2012) between 1250 and 700 ka (Clark et al., 2006). The lithology of the DEEP site sediment sequence indicates that lacustrine, hemipelagic sediments comprise the upper ~ 430 m b.l.f., whereas littoral and fluvial sediments dominate below (Wagner et al., 2014). The transition from fluvial or littoral facies to hemipelagic sediments most likely indicates the onset of full lacustrine conditions in Lake Ohrid. Five TIC maxima below the presumed MIS 31 maximum and above the fluvial or littoral facies (see Wagner et al., 2014) could represent five additional interglacials, which would place the onset of hemipelagic sedimentation within MIS 41 and refines the minimum age of Lake Ohrid to ca. 1.3 Ma.

An age estimation for the onset of lacustrine sedimentation in the Lake Ohrid basin has been derived from comparing seismic and chronological information from core Co1202 recovered in the north-eastern part of the lake (Fig. 1). Tracking seismic reflectors from this coring location (~ 2 km from the DEEP site) to the central part of the lake allowed for the transfer of chronological information of the core into the basin centre (Lindhorst et al., 2015). In addition, the strength of the reflectors was correlated with chronological information and glacial–interglacial cycles derived from pollen analyses at Lake Ioannina (Tzedakis, 1994), 200 km to the south of Lake Ohrid. Based on this information, an average sedimentation rate of 0.43 mm yr^{-1} was calculated for the last 450 kyr in the basin centre (Lindhorst et al., 2015). Using this sedimentation rate for the maximum sediment fill of ~ 800 m b.l.f. observed in the basin centre resulted in an age of 1.9 Ma for the onset of sedimentation (Lindhorst et al., 2015). At the DEEP site a somewhat lower average sedimentation rate of 0.39 mm yr^{-1} can be calculated for the upper 247.8 mcd or for the last 637 kyr (Francke et al., 2016). Sediment compaction with increasing sediment depth (see Baumgarten et al., 2015) may have caused further lowering of the calculated sediment accumulation rate downward and also would lead to older ages compared to those based on a constant sedimentation rate of 0.43 mm yr^{-1}. However, lacustrine, hemipelagic sediments only form the upper ~ 430 m b.l.f. of sediments at the DEEP site, which represents only half of the maximum sediment fill equivalent to ~ 800 m b.l.f. As the underlying littoral and fluvial sediments most likely have significantly higher sedimentation rates, the extrapolated age of 1.9 Ma for the onset of hemipelagic sedimentation can be regarded as a tentative maximum age, assuming there were no major phases of erosion and/or non-deposition.

Overall, based on this new geological information, the minimum and maximum age of Lake Ohrid can be restricted to ca. 1.3 and 1.9 Ma, respectively. More precise age estimation will be obtained by ongoing tephrostratigraphic work and palaeomagnetic analyses, which may reveal the existence of major reversals in the Earth's magnetic field, such as the Jaramillo (1.075–0.991 Ma), Cobb Mountain (1.1938–1.1858 Ma), or Olduvai (1.968–1.781 Ma) subchrons (Nowaczyk et al., 2013, and references therein).

These estimates of 1.3–1.9 Ma correspond well to evolutionary data for endemic Lake Ohrid species obtained prior to the drilling campaign. Based on genetic information from extant endemic species and molecular-clock analyses, the onset of intralacustrine speciation in various groups of Lake Ohrid endemics ("ancient lake species flocks") started between 1.4 Ma for the limpet genus *Acroloxus* (Albrecht et al., 2006) and 2.0 Ma for the endemic *Salmo trutta* trout complex (Sušnik et al., 2006) and the *Dina* leech flock (Trajanovski et al., 2010). Assuming that the origin of Lake Ohrid predates the onset of intralacustrine speciation events, the latter authors suggested that the minimum age of Lake Ohrid is approximately 2.0 Ma. However, they were not able to explain why the species flocks investigated differed in their time of origination and why some of the flocks were as young as 1.3 Ma. A potential explanation is now provided by the initial results of the SCOPSCO deep drilling campaign, which indicate that persisting lacustrine conditions with pelagic or hemipelagic sedimentation established between 1.9 and 1.3 Ma ago. The period of lake establishment and persisting lacustrine conditions may have comprised up to several hundred thousand years, which in turn might have given rise to most species flocks in Lake Ohrid.

4.1.2 Origin

There is a broad consensus that the 40 km long and N-S-trending Ohrid graben basin developed as part of the Alpine orogeny during a transtensional phase in the Late Miocene, followed by an extensional phase since the Pliocene (e.g. Cvijić, 1911; Aliaj et al., 2001; Dumurdzanov et al., 2004; Reicherter et al., 2011; Lindhorst et al., 2015). There is little consensus on the limnological origin of the lake itself, however. Albrecht and Wilke (2008) summarised four related hypotheses. Three of these hypotheses favour an origin as part of a marine ingression or a brackish-water lake system during the Miocene: the Mesohellenic Trough hypothesis, the Tethys hypothesis, and the Lake Pannon hypothesis. A fourth hypothesis postulates a de novo origin, i.e. that Lake Ohrid formed in a dry polje fed by springs during the Pliocene or Pleistocene. The latter is supported, in part, by the known existence of substantial active karst aquifers (Matzinger et al., 2006) and the seismic data, which indicate that Lake Ohrid formed in a relatively narrow and elongated valley (Lindhorst et al., 2015). Moreover, sediments at the base of the DEEP site sequence are formed by gravel, which is overlain by alternating peat layers, sand horizons, and fine-grained sediments, and contain a relatively shallow, obligate freshwater diatom flora (Wagner et al., 2014). These sediments indicate very dynamic environments, ranging from fluvial to slack water conditions, with varying shallow water conditions, and support, in combination with the presumed Pleistocene age of Lake Ohrid, the de novo hypothesis of lake formation.

4.2 Sediment architecture and basin development

In addition to information on the formation of the Ohrid basin, the hydro-acoustic data sets from Lake Ohrid can also provide knowledge on mass transport deposits (MTDs) and on long-term lake-level change.

The evaluation of the seismic and hydro-acoustic data sets indicated that MTDs are only observed during the last ca. 340 ka in Lake Ohrid (Lindhorst et al., 2016). Older MTDs are not covered by the seismic profiles or may be masked by multiple reflections below 250–300 m sediment depth in the central part of the basin. Five major MTDs are detected during MIS 9, 7, and 6. Since ca. 80 ka, the number of MTDs increased, but this is accompanied by a trend of decreasing MTD volume. Due to the restricted vertical resolution of the seismic data sets, the age control of the MTDs is relatively imprecise. Nevertheless, it seems that the occurrence of MTDs is not driven by or a response to glacial–interglacial cyclicity, as they occur during glacials, interglacials, and their respective transitions. Although MTDs are detected throughout the entire basin (Lindhorst et al., 2016), they cluster along the major faults in the south-eastern and north-western part of the basin and are probably the result of fault activity and major earthquakes (Lindhorst et al., 2012; Wagner et al., 2012). Hence, MTDs in the Ohrid basin apparently have a rather limited spatial extent and are not accompanied by basin-wide suspension clouds or turbidites. MTDs with a maximum thickness of < 3 cm are observed in the DEEP site record, with clusters in MIS 8, late MIS 6, and MIS 2 (Francke et al., 2016). The thickness of these MTDs is significantly below the vertical resolution of the seismic data.

The hydro-acoustic data can also provide information about the tectonic history of the basin with respect to lake-level fluctuations. The minimum water depth can be estimated from measuring the depth difference of individual reflectors between their largest depth in the basins and the minimal depth of occurrence at the lake margins. The minimal depth of occurrence for individual reflectors may be a real reflection termination, but in most cases individual reflectors cannot be traced further up because the shallowest areas of the lake basin are not covered by the seismic and hydro-acoustic survey or reflectors could not be traced to the shallower parts due to faults (Fig. 2). In a second step, linking these reflectors to the chronological information from the DEEP site provides chronological information for the minimum water depth. Tracing a reflector from ~ 275 m b.l.f. at the DEEP site, i.e. a reflector located below the existing age model, supposes a minimum water depth of 300 m (Fig. 2). Reflectors at the MIS 16/15 (~ 240 m b.l.f.) and the MIS13/12 boundaries (~ 190 m b.l.f.) suggest minimum water depths of 300 m as well, thus exceeding the present-day water depth of 293 m (Fig. 2). The minimum water depth was reduced to 225 m during MIS 9 (~ 140 m b.l.f.), to 200 m during MIS 7 (~ 100 m b.l.f.), and to 175 m during MIS 5 (47 m b.l.f.). In MIS 3 (20 m b.l.f.), the minimum water depth increased to 250 m, returning to a level similar to that observed in the lower half of the record. Note that this method for estimating water depth contains several sources of uncertainties. The actual water depth during each period may have been much higher, as individual reflectors may continue to shallower water depths or even above the present lake level but cannot be mapped due to missing data coverage in shallow water depth, or reflectors may have been eroded during a following period of a lower lake level. Ongoing subsidence might also have affected the shape of the individual reflectors and potentially increased the maximum depth difference of individual reflectors. Nonetheless, the data suggest a general trend from deeper waters from prior to MIS 16 through to MIS 13/12, followed by decreasing water depths with a minimum in MIS 5 and a subsequent deepening to present-day lake level. As a result, the deepening of the Lake Ohrid basin was apparently not a continuous and gradational process; we assume that short- or mid-term changes reflect changes in water budgets while subsidence is a much slower process. However, already at or shortly after the end of the MPT at 700 ka (Clark et al., 2006), the lake showed similar or even higher water depths compared to present lake level. The seismic data do indicate periods of very low lake levels or even a completely desiccated lake since that time.

Mapping of the hydro-acoustic reflectors indicates that the shape of the Ohrid basin slightly altered over time. Based on the isopleths, the deeper part of the basin changed from a more elongated shape to a roundish shape during the last ca. 700 kyr, with a formation of a secondary basin in the north-western part of the lake after the MIS 13/12 boundary at 478 ka (Fig. 2). This also reflects the extension of the lake basin.

4.3 Tephrostratigraphic and environmental history

4.3.1 Tephrostratigraphy

The DEEP site sequence drilled in 2013 provides the most complete tephrostratigraphic record obtained from Lake Ohrid. A total of 39 tephra layers have been identified in the upper 247.8 mcd so far (Fig. 3; Leicher et al., 2016, and unpublished data). Major element analyses (SEM-EDS/WDS; see Leicher et al., 2016, for details) on juvenile glass fragments suggest an origin exclusively from Italian volcanic provinces. Of these tephra layers (OH-DP-0027 to OH-DP-2060), 13 could be identified and correlated with known and dated widespread eruptions (Leicher et al., 2016, and references therein). They include the Mercato tephra (OH-DP-0027, 8.43–8.63 cal ka BP) from Somma–Vesuvius, the Y-3 (OH-DP-0115, 26.68–29.42 cal ka BP), the Campanian Ignimbrite/Y-5 (OH-DP-0169, 39.6 ± 1.6 ka) and the X-6 (OH-DP-0404, 109 ± 2 ka) from the Campanian volcanoes, the P-11 (OH-DP-0499, 129 ± 6 ka) from Pantelleria, the Vico B (OH-DP-0617, 162 ± 6 ka) from the Vico volcano,

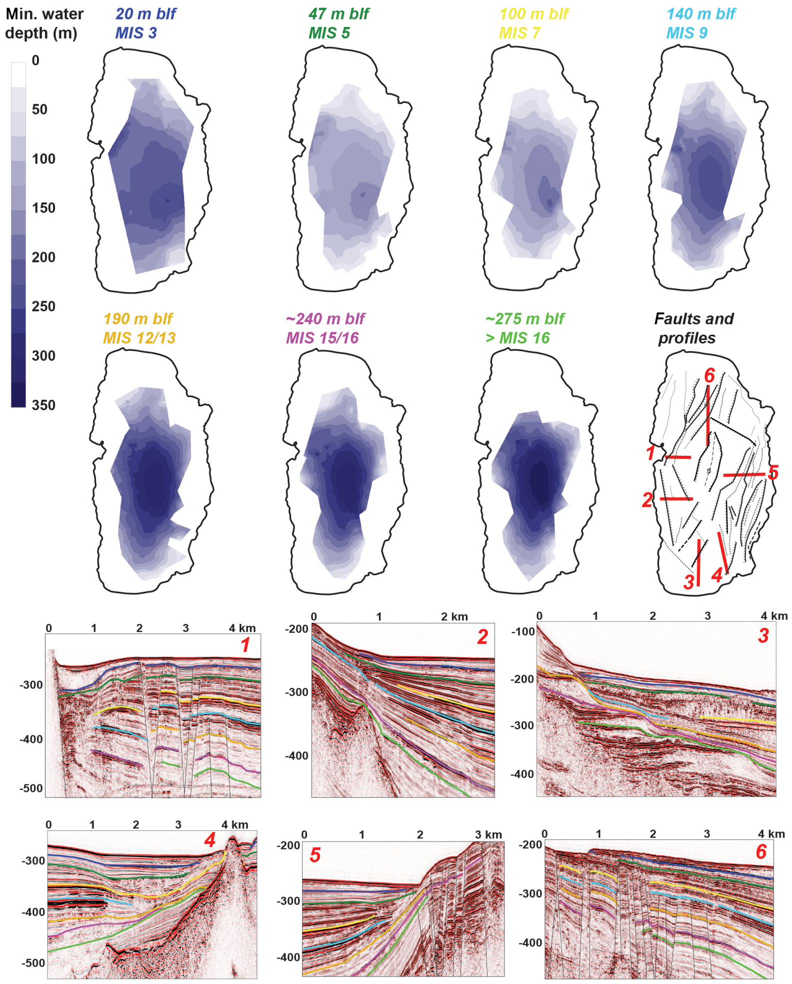

Figure 2. Selected seismic profiles and calculated water depths at different times (see text for details). The arrow of the reflector at 140 m b.l.f. (MIS 8/9) indicates the existence of a secondary basin in the north-western part of the lake. Please note that the lake was probably larger for most periods but individual reflectors cannot be traced to the shallower water depth due to faults. This also explains why the estimated water depth is not zero at the edges of the shown lake coverage.

the Pozzolane Rosse (OH-DP-1817, 457 ± 2 ka) and the Tufo di Bagni Albule (OH-DP-2060, 527 ± 2 ka) from the Colli Albani volcanic district, and the Fall A (OH-DP-2010, 496 ± 3 ka) from the Sabatini volcanic field. Furthermore, a comparison of the Ohrid record with tephrostratigraphic records of mid-distal Mediterranean archives enabled the identification of less-well-known tephra layers, such as the TM24-a/POP2 (OH-DP-0404, 101.8 ka; Regattieri et al., 2015) from Lago Grande di Monticchio and the Sulmona basin, the SC5 (OH-DP-1955, 493.1 ± 10.9 ka) from the Mercure basin, and the A11/12 (OH-DP-2017, 511 ± 6 ka) from the Acerno basin, whose specific volcanic sources are still poorly constrained. OH-DP-0624 was tentatively correlated with the CF-V5/PRAD3225 layers from the Campo Felice basin/Adriatic Sea and thus to the Pitigliano Tuff from the Vulsini volcanic field (ca. 163 ka; Leicher et al., 2016). However, recent tephrochronological results including ^{40}Ar / ^{39}Ar of a tephra from the Fucino basin, central Italy, suggest that these tephras correspond to an un-known eruption from the Neapolitan volcanic area at 158.8 ± 3.0 ka (Giaccio et al., 2017). In order to obtain a consistent set of ages all ^{40}Ar / ^{39}Ar were calculated by using the same flux standard (1.194 Ma for Alder Creek sanidine, which corresponds to Fish Canyon sanidine at 28.02 Ma). The chronological information of 11 of the well-identified tephras from Lake Ohrid was used as first-order tie points for the age–depth model of the composite core, and complemented by tuning of sediment proxies to orbital parameters, such as summer insolation and winter season length (Francke et al., 2016).

Fifteen additional tephra horizons have been identified within the lower hemipelagic section of the DEEP

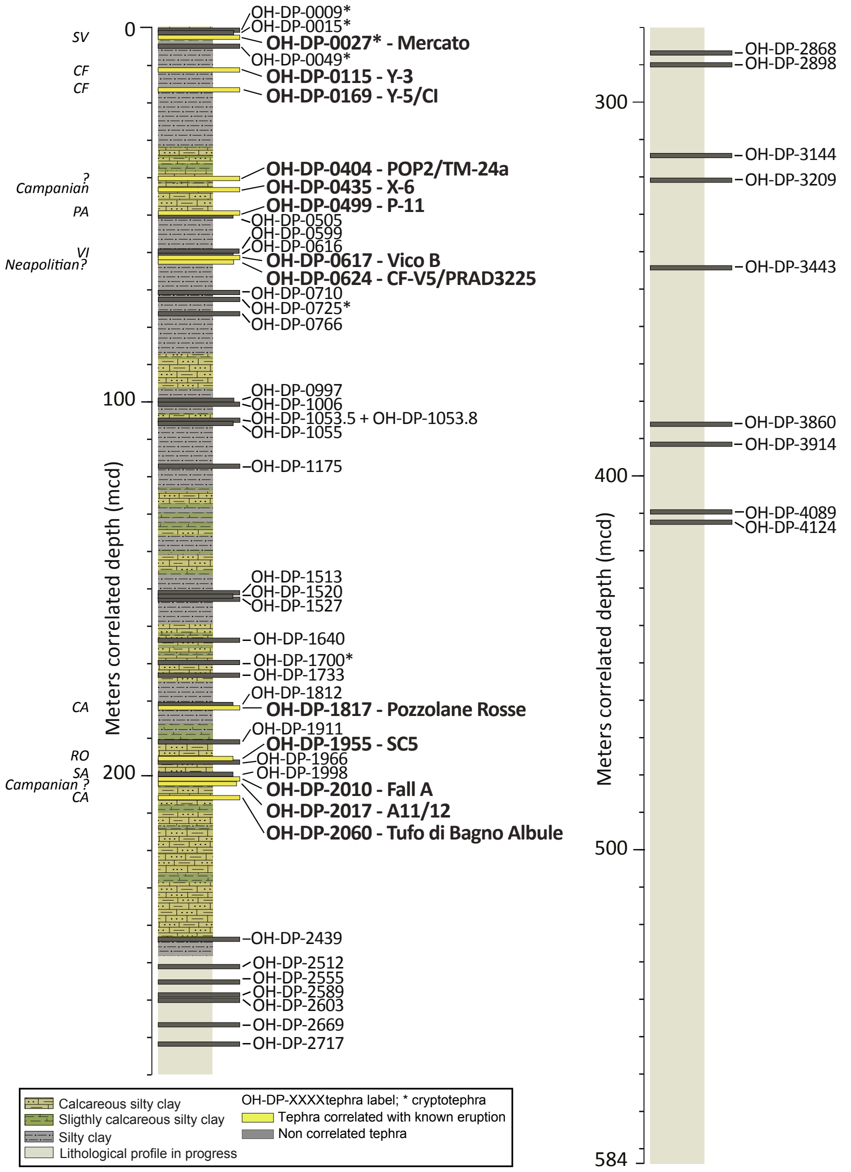

Figure 3. Lithostratigraphy of the upper 247.8 mcd and tephra and cryptotephra horizons in the DEEP sediment sequence. For nomenclature and details see Leicher et al. (2016). Tephra in bold was used as tie points for the age–depth model for the upper 247.8 mcd spanning the last 637 kyr (Francke et al., 2016; Leicher et al., 2016). Tephrostratigraphic work on tephra from below 247.8 mcd is ongoing.

site sequence between 248 and 450 mcd (Fig. 3) and are the subject of ongoing work. Although knowledge of tephrostratigraphy for the period > 637 ka is restricted, a combination of tephrochronological with palaeomagnetic information should provide a robust chronology for this part of the sequence.

With a total of at least 54 tephra layers intercalated in a continuous sediment succession of > 1.3 Ma, the tephrostratigraphic record from Lake Ohrid is a strong candidate to become the template for central Mediterranean tephrostratigraphy, especially for the poorly known and explored Lower and Middle Pleistocene periods. The tephrostratigraphic record may also help to allow re-evaluation and improvement of the chronology of dated and undated tephra layers from other key sites, such as the age of the Fall A tephra (Leicher et al., 2016). Moreover, the tephras constitute valuable independent tie points that resolve leads and lags between changes in different components of the climate system and allow a synchronisation of the Lake Ohrid record with other regional records (Zanchetta et al., 2016).

4.3.2 Environmental history

The examination of the environmental history of Lake Ohrid over the last 637 kyr focuses both on long-term changes over several glacial–interglacial periods and short-term changes on the sub-orbital scale.

Long-term changes

The study of the long-term environmental history of Lake Ohrid and its surrounding area includes the reconstruction of minimum lake levels based on hydro-acoustic information, by vegetation changes in the catchment, and by internal lake proxies. According to the established age model (Francke et al., 2016), hydro-acoustic data (Lindhorst et al., 2015), and borehole logging data (Baumgarten et al., 2015), the sediments deposited at 637 ka are now located ∼ 240 m b.l.f. at the DEEP site. If the altitude of the Lake Ohrid outlet or the bedrock gap used by the river Crni Drim would have been the same as it is today (693.5 m a.s.l.), the water depth of Lake Ohrid at 637 ka would have been more than 480 m. There is no evidence in the seismic or sedimentological data for such a great water depth at that time, which implies that subsidence or other tectonic activity affected the sediment succession in the lake basin or the altitude of the outlet. Nevertheless, the hydro-acoustic data suggest a fairly deep lake at the end of the MPT, with a water depth similar or even deeper than today (Figs. 2 and 4). Shallower minimum water depths are tentatively indicated between MIS 9 and MIS 3, with an absolute minimum during MIS 6 or MIS 5. Tectonic activity and the relative altitude of the outlet are probably the most significant contributors to water-depth variations in Lake Ohrid. A comparison of the minimum water-depth data with pollen data shows some differences but suggests that climate change may also have triggered water-depth fluctuations. Although the Lake Ohrid watershed was a refugial area for both temperate and montane trees during the glacial periods of the last 500 kyr, high amounts of herbs (grasses, chenopods, Cichorioideae, and Cyperaceae) are found in the earlier glacials MIS 12, MIS 10, and MIS 8 and indicate the presence of open formations and grassland (Sadori et al., 2016). Such vegetation requires relatively humid conditions, whereas steppe vegetation with unexpected high amounts of *Artemisia* and pioneer taxa typical of dry conditions dominated during MIS 6, MIS 4, and MIS 2 (Fig. 4; Sadori et al., 2016). The pollen data suggest that in addition to glacials, a drying trend is also observed in interglacials. This is mainly based on the reduction of montane trees, particularly *Abies* and *Picea* values in MIS 5 and the Holocene (Sadori et al., 2016), which may indicate a rearrangement of vegetation in altitudinal belts. The overall progressive change from cooler and wetter conditions recorded during both interglacial and glacial periods prior to 288 ka to subsequently warmer and drier interglacials and glacials (Sadori et al., 2016) broadly matches with the generally shallower minimum water levels reconstructed by tracing hydro-acoustic reflectors throughout the basin. Moreover, driest conditions and a maximum in steppe vegetation between 160 and 129 ka (Sadori et al., 2016) correspond to a prominent lake-level lowstand and the formation of a subaquatic terrace ∼ 60 m below the present lake level in the north-eastern Ohrid basin (Fig. 4; Lindhorst et al., 2010). This lowstand was reconstructed based on hydro-acoustic studies and tephrochronological information from two short sediment cores. Two tephras deposited on the terrace were previously correlated with MIS 5 tephras C-20 (ca. 80 ka) and X5 (105 ± 2 ka) (Sulpizio et al., 2010), and it was supposed that the formation of this terrace took place during MIS 6 (Lindhorst et al., 2010). However, new tephrostratigraphic results suggest that the two tephras instead correspond with Vico B (OH-DP-0617, 162 ± 6 ka) and CF-V5/PRAD3225 (OH-DP-0624, ca. 163 ka; Leicher et al., 2016). This constrains the formation of this terrace to the earlier part of MIS 6 and the subsequent lake-level increase to late MIS 6 or early MIS 5, with a secondary lowstand around 100 ka (Fig. 4). The lake-level curve from north-eastern Lake Ohrid is only partly in phase with the minimum lake-level curve based on the new hydro-acoustic reconstruction (Figs. 2 and 4). Whereas the terraces in the north-eastern basin provide relatively precise water depths, the reconstruction based on hydro-acoustic information (Fig. 2) can give only minimum water depths and is certainly biased by subsidence.

Internal lake proxies support the long-term trend seen in pollen from generally wetter and cooler interglacial and glacial periods between 637 ka and ca. 300 ka to drier and warmer stages between 300 ka and the present. The oxygen isotope composition of lake water ($\delta^{18}O_{\text{lakewater}}$), calculated from $\delta^{18}O$ of endogenic calcite, shows only moderate variability between interglacial periods with a relatively stable climate from MIS 15 to MIS 13, progressively wetter conditions during MIS 11 and MIS 9, and increasingly evaporated, drier conditions in more recent interglacials (Fig. 4; Lacey et al., 2016). In particular, higher $\delta^{18}O_{\text{lakewater}}$ through MIS 5 and the Holocene indicates higher evaporation due to dry and warm conditions prevailing under a Mediterranean-type climate. During glacials calcite is typically absent, but $\delta^{18}O_{\text{lakewater}}$ reconstructed from early diagenetic siderite shows a more pronounced long-term shift, with values being consistent with the adjacent interglacials during MIS 14, MIS 12, and MIS 10, a transition to lower values through MIS 8, and very low $\delta^{18}O_{\text{lakewater}}$ during MIS 6, MIS 4, and MIS 2 (Fig. 4). The similarity between interglacial and glacial lake water prior to ca. 300 ka suggests that Lake Ohrid may have experienced regular and complete mixing, as calcite and siderite form in different environments – calcite in surface waters during summer months and siderite as a product of early diagenesis in the surface sediments. Lower average $\delta^{18}O_{\text{lakewater}}$ before ca. 300 ka indicates moderate summer temperatures (reduced seasonality). It may also suggest higher activity of the karst system due to more pre-

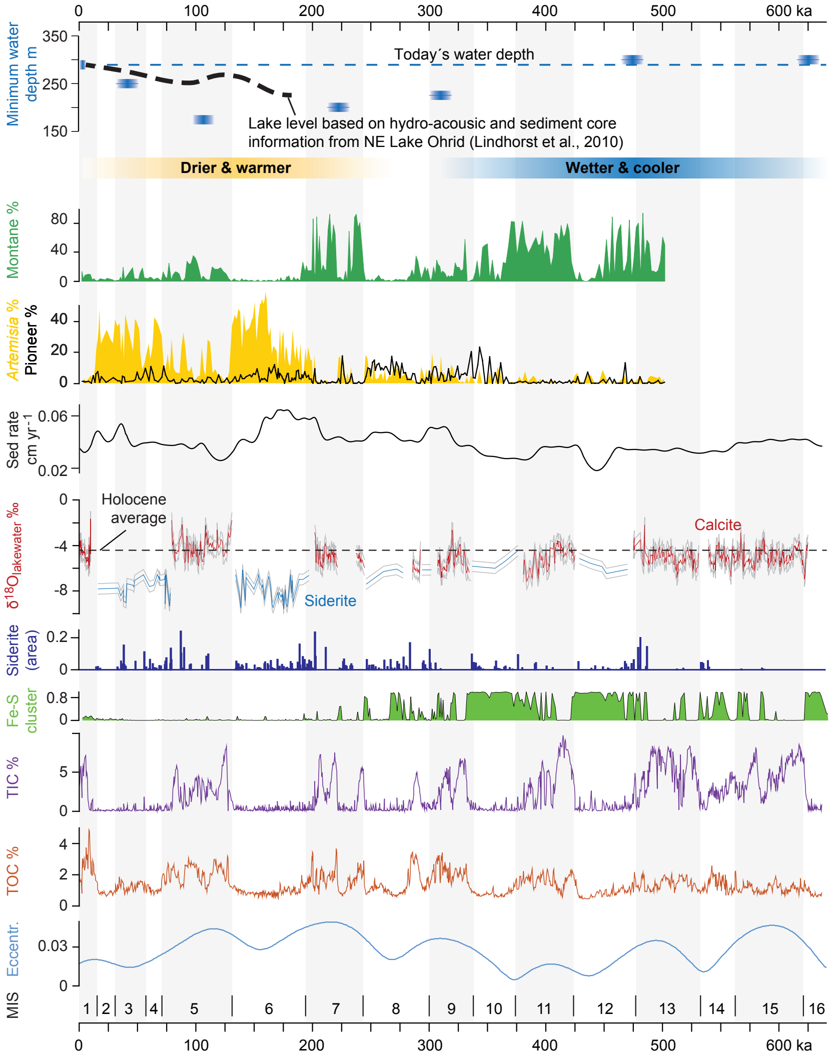

Figure 4. Lake-level reconstructions (modified from Lindhorst et al., 2010; for details see Sect. 4.3.2 of this study), pollen (Sadori et al., 2016), sedimentological, and geochemical data over the last 637 kyr (Francke et al., 2016; Just et al., 2016; Lacey et al., 2016) indicate a long-term shift from cooler and wetter to drier and warmer glacial and interglacial periods around 300 ka. Pollen curves have been corrected with respect to those reported in Sadori et al. (2016). MIS boundaries are according to Lisiecki and Raymo (2005).

cipitation and/or a higher lake level of neighbouring Lake Prespa. Subsequently, a trend to higher $\delta^{18}O_{lakewater}$ during interglacials indicates stronger rates of summer evaporation and drier conditions, and lower $\delta^{18}O_{lakewater}$ in glacial periods suggests isotopically fresh conditions most likely due to low evaporation. Increasing summer aridity towards present is also backed by the gradual increase of Mediterranean taxa pollen percentages.

A transition from generally wetter and cooler to drier and warmer conditions is also indicated by a shift from relatively invariant and low total organic carbon (TOC) prior to ca. 300 ka towards more fluctuating and higher TOC, particularly during the more recent interglacials (Fig. 4; Francke et al., 2016). Wetter and cooler conditions after the MPT drive a high activity of the karst system and intense mixing of the water column, thus promoting decomposition of organic matter. This would, in turn, increase the supply of sulfur to the sediments and allow for the formation of greigite (Fig. 4; Just et al., 2016). A greater activity of the karst system and associated high ion (Ca^{2+}, HCO_3^-) input is further supported by the relatively high TIC during MIS 15, MIS 14, and MIS 13 (Fig. 4; Francke et al., 2016). Pollen data suggest moderate summer temperatures, i.e. conditions that would have favoured mixing and, hence, increased organic matter degradation. Conversely, drier and warmer conditions after ca. 320 ka likely reduced mixing of the water column during the interglacials, which would lead to anoxic bottom waters and a better preservation of organic matter. Just et al. (2016) proposed a decrease in sulfide availability, either by lower sulfate concentration in lake water or ceased upward migrating fluids, changing the geochemical regime in Lake Ohrid. Such conditions are indicated by a shift from predominant glacial formation of Fe sulfides to siderite around 320 ka, when higher Fe concentrations and limited sulfur content of sediments may have prevented the formation of greigite (Fig. 4; Just et al., 2016).

The maximum sedimentation rate during early MIS 6 (Francke et al., 2016) correlates well with the formation of the subaquatic terrace located at 60 m below the present lake level (Fig. 4; Lindhorst et al., 2010). The lower lake level during early MIS 6 led to exposure and erosion of formerly shallow parts of the lake and a lower distance from inlets to the central part of the lake. However, there is no indication, e.g. in isotope or redox sensitive data, for an endorheic lake at that time or any other time during the last 637 kyr. It thus seems that the outlet was active most of the time and climate driven lake-level change may have existed only for relatively short periods or has been compensated at least partly by tectonic activity. Significant variations in TOC and isotope data during early MIS 6 imply a higher variability of the climate compared to the latter period of MIS 6 (Fig. 4). These observations correspond well with palynological studies from the Ioannina basin, where distinct vegetation changes between 185 and 155 ka indicate a high climate variability, whereas a greater abundance of steppe taxa and other herbaceous elements, combined with lower tree pollen percentages, during the latter MIS 6 after 155 ka indicates that the landscape was predominantly open in character and more stable (Roucoux et al., 2011).

Sub-orbital changes

On a sub-orbital scale, prominent environmental changes in the Northern Hemisphere that potentially affected Lake Ohrid include Dansgaard–Oeschger (D/O) and Heinrich events (HE) (e.g. Bond et al., 1992, 1993; Dansgaard et al., 1993; Raymo et al., 1998; McManus et al., 1999). D/O events are a pervasive feature of the last glacial (e.g. Wolff et al., 2010) and also of older glacial periods (Stein et al., 2009; Naafs et al., 2014). They are likely related to variations in the Atlantic Meridional Overturning Circulation (AMOC) and are recorded as climatic perturbations in many marine and terrestrial records (e.g. Genty et al., 2003; Rohling et al., 2003; Margari et al., 2009; Fletcher et al., 2013; Naafs et al., 2014; Seierstad et al., 2014; Stockhecke et al., 2016). In the eastern Mediterranean, D/O events may have influenced regional hydrology and led to large-scale droughts during the past four glacial cycles (Stockhecke et al., 2016). HE are distinctively represented by deposition of ice-rafted debris (IRD) in North Atlantic marine cores (e.g. Hemming, 2004) and are also well documented to have had an imprint on marine and terrestrial records for the last glacial and beyond (e.g. Shackleton et al., 2000; Roucoux et al., 2001, 2011; Sanchez-Goni et al., 2002; Martrat et al., 2004; Margari et al., 2010; Naafs et al., 2013). At the IODP drill site U1308 in the North Atlantic, HE are first indicated during MIS 16 and are represented by IRD layers that are rich in detrital carbonate and poor in biogenic carbonate (Hodell et al., 2008). It has been speculated that ice volume and the duration of glacial conditions surpassed a critical threshold during MIS 16 and activated the dynamic processes responsible for Laurentide Ice Sheet instability in the region of Hudson Strait, which led to increased iceberg discharge and weakening of thermohaline circulation in the North Atlantic (Hodell et al., 2008).

MIS 12 is considered to be one of the most severe glacials during the Quaternary, with the lowest summer sea surface temperatures (SSTs) recorded across multiple records (e.g. Shackleton, 1987; Naafs et al., 2013, 2014; Rohling et al., 2014). Abrupt sea surface warming events of 3–6 °C in the midlatitude North Atlantic during MIS 12 likely reflect the imprint of D/O events and probably had a substantial impact on global climate (Naafs et al., 2014). In contrast to the observations from MIS 16, a temporal lag between the occurrence of IRD and surface water cooling during MIS 12 implies that HE were not the cause for a weakening of the thermohaline circulation in the North Atlantic at this time (Naafs et al., 2014).

High-resolution records from the Mediterranean region, which can be used to test a larger regional or even global impact of D/O and HE during MIS 16 or MIS 12, are scarce (e.g. Hughes et al., 2006; Tzedakis et al., 2006; Girone et al., 2013; Capotondi et al., 2016). A multi-proxy record with lithological, geochemical, and isotope data from the Sulmona basin in central Italy covering MIS 12 shows pronounced hydrological variability at orbital and millennial timescales, which replicates North Atlantic and western Mediterranean SST fluctuations (Fig. 5; Regattieri et al., 2016). Several short-term fluctuations in the MIS 12 Sulmona record most likely reflect sub-orbital-scale hydrological variations and are apparently related to reduced precipitation sourcing from the North Atlantic due to episodes of iceberg melting and IRD deposition at the west Iberian margin (Regattieri et al., 2016, and references therein). However, as the timing of these IRD events at the western Iberian margin was used to improve the chronology of the Sulmona record, the correlation of hydrological variations in central Italy and IRD deposition in the North Atlantic is not fully independent.

At Lake Ohrid and further to the east, the arboreal pollen concentration in the Tenaghi Philippon record from Greece correlates well with the general pattern of the SSTs in the North Atlantic during MIS 12 (Fig. 5; Tzedakis et al., 2006). The resolution of the existing record is too low yet to allow a clear identification of D/O or HE related climate change. The high-resolution record from Lake Van in eastern Turkey also cannot be used for testing the climatic impact of D/O or HE on the eastern Mediterranean, as the sediments of MIS 12

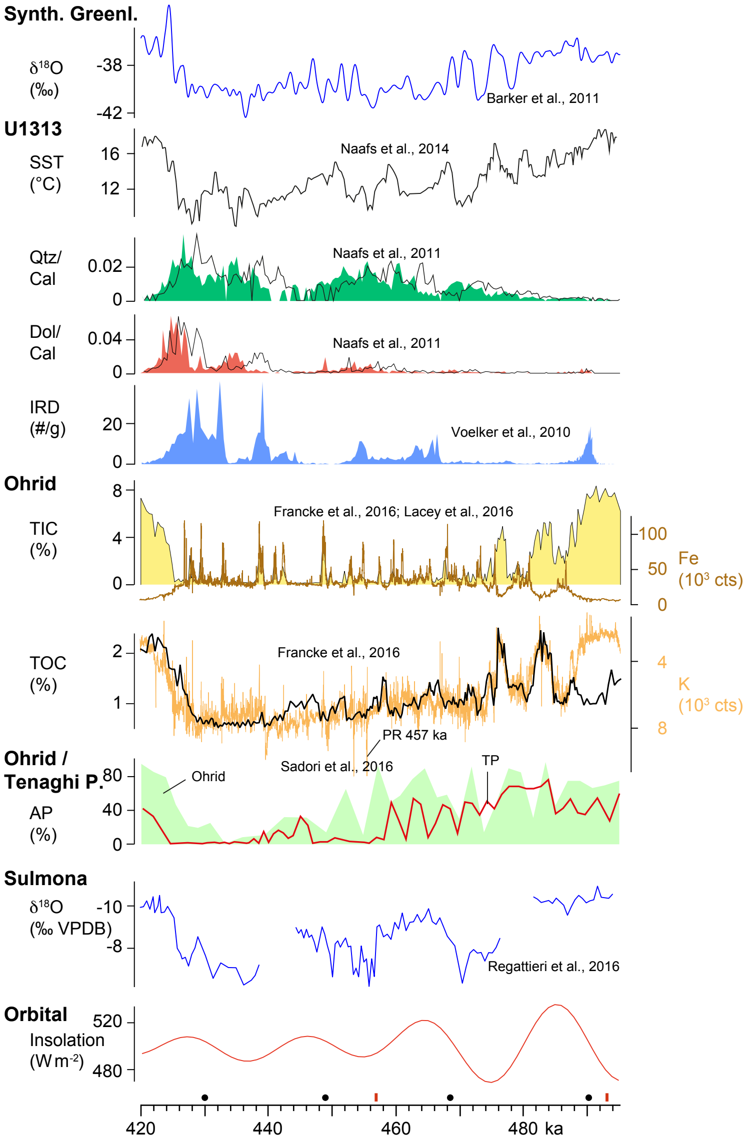

Figure 5. Geochemical data from the DEEP site sequence with sub-orbital changes during MIS 12 in comparison with other records from a similar latitude (for location of North Atlantic core U1313, the pollen record from Tenaghi Philippon, and the isotope record from Sulmona basin see Fig. 1). Arboreal pollen (AP) records are excluded of *Pinus*, *Juniperus*, and *Betula* (Sadori et al., 2016); the record from Tenaghi Philippon is based on pollen data from Wijmstra (1969) and Wijmstra and Smit (1976) and the age model from Tzedakis et al. (2006) (see also Sadori et al., 2016). Red bars and black dots at the bottom age axis indicate tephrochronological tie points and tuning points used for the age model of the DEEP site sequence (Francke et al., 2016).

and the onset of MIS 11 are disturbed and lack independent age control (Stockhecke et al., 2014).

The new high-resolution record from the DEEP site in Lake Ohrid now offers the possibility to assess the impact of D/O or HE during MIS 12 on a broader regional scale, particularly as it provides two absolute tephra age control points with ages centred at 493.1 ± 10.9 and 457 ± 2 ka (Fig. 5; Francke et al., 2016; Leicher et al., 2016). During MIS 12, potassium (K) shows a long-term increase, which supports the overall trend towards colder temperatures, such as can be inferred from other marine, terrestrial, or synthetic climate records (Fig. 5). K represents the proportion of clastic, terrigenous matter relative to the content of carbonate (reflected by TIC) and organic matter (reflected by TOC and bSi). TOC was used to infer the severity of glacials at Lake Ohrid (Francke et al., 2016) and shows a remarkable saw tooth pattern during MIS 12, which resembles fluctuations in SST related to D/O variability from the North Atlantic marine record U1313 (Fig. 5; Naafs et al., 2014). Higher TOC is favoured by both increased overall productivity (on land and in the water column) as well as increased organic matter preservation, with the latter resulting from oxygen depletion of the bottom water due to enhanced thermal stratification, decreased mixing, and higher temperatures. These higher temperatures at Lake Ohrid likely correlate with higher SSTs in the North Atlantic. The TOC record from Lake Ohrid thus would be the first terrestrial record to indicate D/O cycle-related teleconnections between the North Atlantic thermohaline circulation and the climate in the north-eastern Mediterranean region during MIS 12. Interestingly, the dominant *Pinus* pollen abundance in the vegetation record indicates a regular ∼ 8 kyr variability during MIS 12 and 10, for which a high-resolution analysis is now being performed (Fig. 2 in Sadori et al., 2016).

The environmental impact of HE or other short-term climate events has been studied in detail for the last glacial cycle in several records from the Balkans (e.g. Tzedakis et al., 2004; Müller et al., 2011). Based on pollen and diatom analyses from lakes Prespa and Ohrid, HE in the North Atlantic during MIS 4 to MIS 2 led to short spells of very dry and cold conditions superimposed on the glacial conditions (Panagiotopoulos et al., 2014; Cvetkoska et al., 2015). Moreover, there is an increased formation of Fe and Mn concretions in Lake Prespa sediments, most likely driven by a significant shift in the bottom water redox conditions (Wagner et al., 2010). According to diatom studies spanning the last 92 ka, Lake Prespa experienced significant regime shifts that are correlated with lake-level fluctuations and changes between (oligo-) meso- and eutrophic conditions (Cvetkoska et al., 2016). Lake Ohrid seems to be less sensitive to short-term climate change due to its higher volume-to-surface area ratio (e.g. Wagner et al., 2010; Leng et al., 2013). It does not indicate sub-orbital timescale lake-level changes and shifted between ultra oligo- and oligotrophic conditions during the last 92 kyr (Cvetkoska et al., 2016). However, the formation of Fe and Mn concretions and the occurrence of siderite indicate that Lake Ohrid is also sensitive to shifts in the bottom water redox conditions (Lacey et al., 2016). During MIS 12, Fe peaks in X-ray fluorescence data are positively correlated with TIC and indicate the formation of early-diagenetic siderite in response to a shift in bottom water redox conditions towards a more oxic environment (Fig. 5; Francke et al., 2016; Lacey et al., 2016). The Fe peaks during the coldest period of this glacial match particularly well with the number of IRD grains and with maxima in the quartzite–calcite or dolomite–calcite ratio in the U1313 record from the North Atlantic (Fig. 5). The latter are interpreted as millennial ice-rafting-driven events (Voelker et al., 2010; Naafs et al., 2011, 2013) and thus demonstrate that North Atlantic HE may have caused changes in internal lake conditions, such as bottom water redox conditions.

One of the HE, the H4 event at 40.4–38.4 ka, is superimposed by another short-term event: the eruption from the Campi Flegrei volcanoes (39.6 ± 1.6 ka). This eruption is one of the most severe volcanic eruptions during the Pleistocene and left a 15 cm thick tephra known as Campanian Ignimbrite or Y-5 marine tephra layer in the records from lakes Ohrid and Prespa (e.g. Wagner et al., 2009; Vogel et al., 2010b; Damaschke et al., 2013). High-resolution studies of diatoms in both lake sediment records indicate little evidence for a response of the diatom community related to the H4 event, but they do show a clear and rapid change following tephra deposition (Jovanovska et al., 2016). This strong change is likely due to fertilisation and the availability of nutrients, particularly silica, such as it was shown in laboratory studies and leaching experiments of tephra with Lake Ohrid water (D'Addabbo et al., 2015). After the initial response, diatom community compositions in lakes Ohrid and Prespa returned to their quasi pre-disturbance state. In Lake Ohrid, the recovery time was ca. 1100 years vs. ca. 4000 years in Lake Prespa (Jovanovska et al., 2016). Although both lakes are resilient to short-term environmental change, it seems that Lake Ohrid is even more resilient than Lake Prespa, likely due to differences in geology, lake age, limnology, and intrinsic parameters of the diatom proxies (Jovanovska et al., 2016).

4.4 Drivers of biodiversity change

One of the major interdisciplinary goals of the SCOPSCO project is to infer the drivers of the extraordinary endemic biodiversity in Lake Ohrid, in general, and to evaluate the influence of major environmental events on evolutionary processes, in particular. Lake Ohrid thus serves as a model system to address questions that have puzzled evolutionary biologists for decades. These questions include the problem whether the high number of endemic species is mainly a result of an accumulation of relict species ("reservoir function") and/or of a high rate of intralacustrine speciation ("cradle function"). Moreover, if intralacustrine speciation plays a significant role, is it primarily driven by geographic or environmental gradients during periods of relatively constant environmental conditions, possibly supported by a high ecosystem resilience of the lake, or does ongoing environmental change lead to an increase (or decrease) in rates of species diversification? Finally, what role do potentially "catastrophic" environmental fluctuations play, such as lake-level change or significant changes in the trophic state?

4.4.1 Reservoir vs. cradle function of Lake Ohrid

As discussed in Föller et al. (2015), ancient lakes have often been considered to serve as evolutionary or geographic refugia, either harbouring old and distinct lineages or enabling the accumulation of species from extralimital areas during periods of adverse environmental changes, respectively (reservoir function). However, previous evolutionary studies in Lake Ohrid on selected animal taxa could not demonstrate the existence of such relict species (sensu Grandcolas et al., 2014) because either ancestral distribution ranges are largely unknown (e.g. Schultheiß et al., 2008) or the native species are not extraordinarily old (e.g. Albrecht et al., 2008; Hauswald et al., 2008). Instead, intralacustrine speciation after immigration events prevails. Most endemic animal species in Lake Ohrid are considerably younger than the lake itself and form monophyletic clades (also see Sect. 4.1.2). This suggests that the high endemic species richness in Lake Ohrid invertebrates is predominantly a result of intralacustrine diversification (cradle function; e.g. Albrecht et al., 2006, 2008; Wilke et al., 2007; Schultheiß et al., 2008; Wysocka et al., 2014; Föller et al., 2015).

Interestingly, the situation is different for plant species inhabiting the surrounding of Lake Ohrid. For example, the existing pollen record from the DEEP site sequence, which covers the last 500 kyr, indicates that the Lake Ohrid catchment has indeed been a refugial area for both temperate and montane trees during glacial periods (Sadori et al., 2016), comparable to the Lake Ioannina catchment (Tzedakis et al., 2002).

4.4.2 Impact of environmental change on species diversification

Ancient lakes are often considered to be comparatively stable systems, potentially resulting in constant diversification rates (i.e. speciation minus extinction rates) over time. Nonetheless, several factors, often related to environmental, geological, or climatic changes, and depending on the genetic features of the species, have been suggested to affect the tempo of diversification in ancient lake species flocks. Accordingly, phases of rapid environmental fluctuations may lead to net evolutionary change. Diversification rates may be higher in the initial phase of lake colonisation and may decline once niche space is increasingly occupied. Alternately, there might be a pronounced lag phase between the colonisation of a lake and the onset of subsequent diversification (reviewed in Föller et al., 2015).

Although high-resolution sediment-core analyses, covering the last 637 kyr, indicate that Lake Ohrid experienced several environmental changes, phylogenetic studies on a microgastropod group using lineage-through-time plots and diversification-rate analyses did not reveal significant changes in this rate over time (Föller et al., 2015). Moreover, diatom community analyses conducted from the DEEP sediment cores could not show extinction events due to major environmental events such as tephra deposition (Jovanovska et al., 2016; for details see Sect. 4.3.2) and climate change over the last 92 kyr (Cvetkoska et al., 2016). However, the potential for a regime shift increases with recent human impact on the diatom flora of both lakes Ohrid (Zhang et al., 2016) and Prespa (Cvetkoska et al., 2015) although, again, Ohrid appears to be more well-buffered from eutrophication than Prespa.

The reasons for the relatively constant diversification rate over time observed in microgastropods and the lack of diatom extinction events during the Late Pleistocene/Holocene remain largely unknown. However, the lack of environmentally induced extinction events in Lake Ohrid and/or the high resilience of its ecosystems may have played a role (Föller et al., 2015; Cvetkoska et al., 2016; Jovanovska et al., 2016). Local buffering from extreme environmental effects in a refugial area, such as Lake Ohrid, may have not only led to reduced extinction rates but also allowed divergence of lineages to proceed. Refugia thus may have acted both as "museums" for the conservation of diversity and as "cradles" for the production of new diversity (Tzedakis et al., 2002; Tzedakis, 2009). Nonetheless, though environmental changes may have had only a minor direct effect on diversification processes in endemic taxa of Lake Ohrid during the last 637 kyr, these changes potentially altered the abundance and community compositions of diatoms and ostracods (e.g. Belmecheri et al., 2010; Reed et al., 2010; Zhang et al., 2016), thus indirectly affecting speciation processes. In fact, the analysis of the gastropod community in Lake Ohrid implied the presence of both geographical and ecological speciation due to physical barriers and divergence across environmental or life history gradients, respectively (Hauffe et al., 2016).

Another aspect of environmental change is the impact of anthropogenic activity on species composition, diversity, and diversification. As previously suggested, Lake Ohrid is facing a "creeping biodiversity crisis", as increasing human impact in and around the lake already jeopardises endemic species (Kostoski et al., 2010). For example, the presence of globally invasive species has been recently demonstrated for Lake Ohrid (Albrecht et al., 2014). Moreover, human-mediated environmental change is also predicted to alter the trophic state of the lake (e.g. Matzinger et al., 2006). Given the small size of both the lake and its catchment, increasing negative effects on the endemic biodiversity of Lake Ohrid and the respective habitats are foreseeable and will likely foster extirpation. Only concerted and international conservation activities might help mitigating the human impact on the sensitive and highly biodiverse ecosystem of Lake Ohrid.

5 Conclusions and outlook

The SCOPSCO deep drilling project was initiated in 2004 and aimed at inferring (i) the age and origin of Lake Ohrid (Former Yugoslav Republic of Macedonia/Republic of Albania), (ii) its regional seismotectonic history, (iii) volcanic activity and climate change in the central northern Mediterranean region, and (iv) the influence of major geological events on the evolution of its endemic species. The project included phylogenetic and metacommunity analyses of living invertebrates and sampling from main modern terrestrial organic matter pools from the lake and its surroundings, seismic and hydro-acoustic surveys of the lake's internal sediment architecture, and the recovery of surface sediments and sediment cores. Within the framework of the ICDP a deep drilling in Lake Ohrid took place in spring 2013 and provided, among others, a 584 m long sediment sequence from the central part (DEEP site) of the lake. Initial results of the study of this sediment sequence in combination with the results of the biological and geophysical as well as former sedimentological studies reveal that the Ohrid basin formed during the Miocene and Pliocene. Lake Ohrid established between 1.9 and 1.3 Ma and provides a continuous record of distal tephra deposition and climatic and environmental change in the central northern Mediterranean region. With its geographical location, the Lake Ohrid record provides a unique opportunity to align marine records from the North Atlantic with long-term and independently dated terrestrial archives in the northern and eastern Mediterranean, such as the records from the Sulmona basin, Tenaghi Philippon, Lake Van, or Dead Sea. This is a major precondition to disentangle longitudinal climate gradients and investigate leads and lags circumventing age model uncertainties.

More detailed studies exist meanwhile on the upper 247.8 m of the DEEP site sediment sequence and indicate that this part represents the last 637 kyr. Over this period, Lake Ohrid experienced significant environmental change, which is related to orbital-scale climate forcing and regional geological events. These changes apparently did not cause major extinction events in Lake Ohrid, as evident from both the microgastropod phylogeny and the diatom fossil record. The potential high resilience of the ecosystem to past climatic and environmental changes together with relatively low extinction rates may explain the extraordinary degree of endemic biodiversity in the lake. Ongoing biological studies and more detailed analyses of the early stages of Lake Ohrid basin, based on the now accessible sediment records, will help to better understand the drivers of biological diversification and endemism. Lake Ohrid is thus a key site to further resolve the link between biological and geological evolution and should centre our attention on protecting the endemic community from a substantial biodiversity crisis due to the increasing anthropogenic impact.

Data availability. Data are stored at the Drilling Information System (DIS) of ICDP (www.icdp-online.org) and at the Pangaea database (www.pangaea.de).

Competing interests. The authors declare that they have no conflict of interest.

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Part III

Appendix

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Abstract

One of the major challenges of eco-evolutionary research is to understand how metacommunity structuring processes scale up over time and imprint evolutionary dynamics. Insular ecosystems are suitable systems to study the eco-evolutionary intersection due to ecological, evolutionary, and geological processes occurring on similar timescales. Island Biogeography Theory (IBT) postulates that available niche space varies over the life cycle of an insular ecosystem and regulates insular biodiversity through the feedback of available niche space on immigration, speciation, and extinction rates. Competitive interaction among species for ecological resources and/or geographical space occurs on short time scale and at the level of the metacommunity. In concert with dispersal limitation and the sorting of species along environmental gradients according to their ecological niche, it should therefore also shape metacommunity structure by affecting the assembly of local communities. Metacommunity structure in turn is pivotal for diversification where different metacommunity dynamics may scale up over time to distinct biodiversity trajectories.

However, there is limited empirical evidence on whether the processes shaping the co-existence of species in local communities in insular ecosystems imprint the species richness and endemism trajectories through time. In order to address this knowledge gap, this synthesis (i) summarized how individual level processes theoretically scale up to shape macroevolutionary dynamics, (ii) compiled confirmatory and contradictory evidence for such a mechanistic link from several insular ecosystems, putting specific emphasis on gastropods of the model system Lake Ohrid, and (iii) outlined future avenues for enhancing the understanding of the consequences of metacommunity processes on short timescales on evolutionary dynamics over long timescales.

Simulation experiments suggested a differential upscaling of metacommunity assembly processes over time, resulting in distinct macroevolutionary diversification dynamics. This offers the possibility to infer mechanisms of species co-existence from evolutionary dynamics and vice versa. Results of empirical studies across several taxa like cichlids, lizards, spiders, and plants of different insular ecosystems such as Lake Tanganyika and the Carribean and Hawaiian Islands, suggest a match between declining diversification rate and community assembly driven by biotic interactions. In contrast, the demonstrated diversification decline for Madagascan herpetofauna is not reflected by their metacommunity assembly processes.

For the gastropods of Lake Ohrid, a new approach of inferring metacommunity assembly processes revealed a high importance of dispersal limitation and environmental filtering, whereas competitive interaction played a minor role in structuring gastropod communities. Over long timescales, weak competitive interaction may be caused by plenty vacant niche space, which is a scenario meshing with the identified constant diversification, and that results on evolutionary timescales in a higher species richness than under strong competitive interaction. The identified assembly processes may therefore explain the evolution of the high endemic gastropod richness of Lake Ohrid. In order to improve our understanding of the reciprocal effect between metacommunity assembly processes and diversification dynamics, this synthesis presented new approaches for estimating time-varying diversification rates for

insular communities arising from multiple colonization events and outlines a way to infer the importance of community assembly processes needed for a mechanistical upscaling to the observed diversification dynamics over time.

The eco-evolutionary dynamic of mainly dispersal limited community assembly and constant geographic speciation resulted in the gradual build-up of Lake Ohrid's gastropod diversity through time. This and the suggested approaches to infer diversification dynamics and the way to link them to metacommunity assembly contribute to the aim of inferring the driving forces of biotic evolution in Lake Ohrid.

Zusammenfassung

Eine der größten Herausforderungen in ökologisch-evolutionärer Forschung ist zu verstehen wie Prozesse zur Strukturierung von Metaartengemeinschaften über die Zeit skalieren und evolutiven Dynamiken beeinflussen. Insuläre Ökosysteme sind geeignete Systeme um die sich ergebende ökologisch-evolutionäre Schnittmenge zu untersuchen, da die ökologischen, evolutionären und geologischen Prozessen auf ähnlichen Zeitskalen erfolgen. Die Inselbiogeographie-Theorie postuliert dass der verfügbare Nischenraum über den Lebenszyklus eines insulären Ökosystems variert und die insuläre Biodiversität durch die Rückkoppelung zwischen verfügbare Nischenraum und Immigrations-, Speziations- und Extinktionsraten reguliert. Kompetitive Interaktionen um ökologische und/oder räumliche Ressourcen zwischen Arten finden auf kurzen Zeitskalen und der Ebene der Metaartengemeinschaft statt. Zusammen mit der Ausbreitungsbeschränkung und der Anordnung von Arten entlang von Umweltgradienten entsprechend ihrer ökologischen Nische sollten sie daher die Struktur der Metaartengemeinschaft durch die Zusammensetzung lokaler Artengemeinschaften beinflussen. Die Struktur der Metaartengemeinschaft wiederrum ist ausschlaggebend für die Artendiversfizierung, wobei verschieden Dynamiken der Metaartengemeinschaften über die Zeit zu distinkten Biodiversitätverläufen hochskalieren.

Empirische Evidenzen dafür, ob die Prozesse welche die Koexistenz von Arten in lokalen Gemeinschaften in insulären Ökosystemen beeinflussen auch den zeitlichen Verlauf von Artenreichtum und Endemismus bedingen, sind rar. Um diese Wissenslücke zu schließen, hat diese Synthesis (i) zusammengetragen wie theoretisch Prozesse auf Individuenebene zeitlich skalieren um makroevolutionäre Dynamiken zu beeinflussen, (ii) bestätigende und widersprechende Evidenzen von verschiedenen insulären Ökosystemen für solch eine mechanistische Verbindung kompiliert, wobei den Gastropoden des Ohridsees als ein Modelsystems besondere Gewichtung entgegengebracht wird und (iii) zukunftige Wege umrissen um unser Verständniss der Konsequenzen von Zusammensetzungsprozessen der Metaartengemeinschaften über kurzen Zeitskalen, auf evolutionäre Dynamiken über lange Zeitskalen hinweg zu verbessern.

Simulationsexperimente deuten auf ein differentielles Hochskalieren von Zusammensetzungsprozessen der Metaartengemeinschaften über die Zeit hin, was zu distinkten makroevolutionären Diversifikationsdynamiken führt. Dies bietet die Möglichkeit, Mechanismen der Koexistenz von Arten aus der evolutionären Dynamik abzuleiten und umgekehrt. Ergebnisse empirischer Studien von verschiedensten Taxa, wie zum Beispiel Buntbarsche, Eidechsen, Spinnen und Pflanzen aus verschiedenen insulären Ökosystemen, wie dem Tanganjikasee, der karibischen und der hawaiianischen Inseln, legen eine Übereinstimmung nahe zwischen der Abnahme der Artendiversifikation und biotische Interaktionen welche deren Artengemeinschaften determinieren. Im Gegensatz dazu spiegelt sich der nachweisliche Rückgang der Diversifizierung der madagassischen Herpetofauna nicht in den Zusammensetzungsprozessen ihrer Metaartengemeinschaft wider.

Für die Gastropoden des Ohridsees zeigte ein neuer Ansatz zum Abschätzen der Zusammensetzungsprozesse von Metaartengemeinschaften eine hohe Bedeutung für Ausbreitungsbegrenzung und Anordnung entsprechend von Umweltbedingungen, wohingegen kompetitive

Interaktionen eine untergeordnete Rolle bei der Strukturierung von Gastropodengemeinschaften spielten. Über lange Zeiträume kann eine schwache kompetitive Interaktion durch zahlreiche freie Nischenräume bedingt warden, was ein Szenario darstellt welches mit der identifizierten konstanten Artendiversifizierung übereinstimmt und auf evolutionären Zeitskalen in einem höheren Artenreichtum resultiert als starke kompetitive Interaktionen. Die identifizierten Zusammensetzungsprozesse von Artengemeinschaften mögen daher die Evolution des hohen endemischen Gastropodenreichtums erklären. Um die Wechselwirkungen zwischen Zusammensetzungsprozessen von Metaartengemeinschaften und Diversifikationsdynamiken besser zu verstehen, wurden in dieser Synthese neue Ansätze zur Abschätzung zeitlich variierender Diversifizierungsraten für Inselgemeinschaften vorgestellt, welche aus mehreren unabhängigen Kolonisationsereignissen resultieren, und es wurde ein methodischer Weg skizziert um die Bedeutung von Zusammensetzungsprozessen für eine mechanistische Hochskalierung zu den beobachteten Diversifikationsdynamiken über die Zeit abzuschätzen.

Die ökologische-evolutionären Dynamiken der Zusammensetzung von Artengemeinschaften durch Ausbreitungsbeschränkung und die konstante geographische Artbildung resultieren in einen graduellen Aufbau der Gastropodendiversität im Ohridsee über die Zeit. Dies, als auch die vorgeschlagenen Ansätze um Diversifikationsdynamiken abzuschätzen und die Möglichkeit diese mit der Zusammensetzung von Metaartengemeinschaft zu vereinen, tragen zum Anliegen bei, die Triebkräfte der biotischen Evolution im Ohridsee zu identifizieren.

Declaration

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Gießen, 4. März 2019