Biodiversity, phylogeny and biogeography of the family Sphaeriidae (Mollusca: Bivalvia) in Morocco

Biodiversität, Phylogenie und Biogeographie der Familie Sphaeriidae (Mollusca: Bivalvia) in Marokko

This is a translation of the original thesis in French entitled: Biodiversité, phylogénie et biogéographie de la famille Sphaeriidae (Mollusca: Bivalvia) au Maroc

Dissertation for the degree of Doctor rerum naturalium

Hanane Rassam

Justus Liebig University – Faculty of Biology and Chemisty (Giessen, Germany)

&

Université Cadi Ayyad – Faculté des Sciences Semlalia (Marrakech, Morocco)

Supervisors:

Prof. Dr. Thomas Wilke

Prof. Dr. Mohamed Ghamizi

Deans:

Prof. Thomas Wilke (JLU-Giessen)

Prof. El Hassan El Mouden (UCA-Marrakech)

Abstract

The family Sphaeriidae Deshayes, 1855 is widely distributed across the globe, except for Antarctica, with a high species richness of up to 200 species. In Morocco, freshwater bivalves are receiving more and more attention in recent years and data on the families of this group are no longer a mystery for most of them. However, many uncertainties surround the current diversity and distribution of the family Sphaeriidae. This information, including taxonomy, phylogeography and conservation status, is essential for further studies to improve knowledge of this family in Morocco and represents the first step towards the development of a national conservation plan for all freshwater bivalves.

In the present work, several investigations were carried out to evaluate the diversity and distribution of Sphaeriidae in the different basins of Morocco, covering different types of habitats (lakes, springs, rivers and small ponds) and microhabitats (mud, aquatic plants, etc). The identification of specimens and their morpho-ecological characteristics was based on morphological and morphometric analyses carefully processed to obtain maximum precision. Several studies have shown, however, the influence that environmental factors can have on the morphology of species, especially for species with a high morphological plasticity that can distort their identification. A case study was proposed for the species *Pisidium casertanum*, evaluating the intraspecific morphological variability according to the habitats of the species by the application of Fourier Elliptic Analysis, a geometric morphometric method analyzing the contour of the specimens. To complete the morpho-geometric analyses of the species and to confirm their identification, molecular analyses were carried out by integrating the sequences resulting from the amplification of four genes, mitochondrial (COI and 16S rRNA) and nuclear (H3 and 28S rRNA). These Moroccan sequences, complemented with other *Pisidium* sequences, were integrated to infer, for the first time, their phylogeographic history in a larger framework: the Western Palearctic.

Shell morphology and morphometric analyses combined with molecular data revealed the existence of five species belonging to the genus *Pisidium* [*P. casertanum* (Poli, 1791), *P.* (cf.) *personatum* Malm, 1855, *P. subtruncatum* Malm, 1855, *P. amnicum* (O. F. Müller, 1774) and *Pisidium* sp.and one species belonging to the genus *Musculium*: *M. lacustre* (O. F. Müller, 1774). Sphaeriidae have been collected in all Moroccan basins, except for the Bouregreg and Sakia El Hamra-Oued Eddahab basins. The conservation status of Sphaeriidae in Morocco was evaluated for the first time and allowed updating the statuses previously assigned by the IUCN. *Pisidium casertanum* and *P.* (cf.) *personatum* are both classified as "Least Concern" while *P. subtruncatum* has been proposed as "Vulnerable", as the number of sites where these species are present is now more important. The status of "Regionally Extinct" is suggested for *P. amnicum* and *M. lacustre* as only empty shells have been found for both species.

The molecular clock used for the phylogeographic analysis allowed determining the dates and processes of divergence that have shaped the present diversity and distribution of the genus *Pisidium* in the Western Palearctic. These results showed that the presence of the genus in the Western Palearctic is the consequence of a dispersal from a locality of Eurasian origin during the early Oligocene period. This dispersal would have taken place via two routes: a northern route after the closure of the Turgai Strait connecting Asia to Europe; and a southern route when the Paratethys Sea separated from the Tethys Ocean giving rise to the Anatolian-Balkan-Iranian land mass. The radiation of *Pisidium* species in the Western Palearctic was maximal during the late Miocene and continued until the Pliocene. These radiations are mostly related to speciation that resulted from isolations in glacial refuges at the Mediterranean peninsulas. However, no clear geographic pattern has been identified between Moroccan and other populations, which is probably due to the dispersive nature of members of the family Sphaeriidae that can be transported long distances by birds and fish. All the information received through this work constitute basic elements to improve the knowledge on this family and to invest them in future projects of work on their bio-ecology, conservation and implication in ecotoxicological studies.

Keywords: Systematics, distribution, phylogeography, bivalves, freshwater, 16S rRNA, COI, 28S rRNA, H3, Morocco.

Zusammenfassung

Die Familie Sphaeriidae Deshayes, 1855 ist mit Ausnahme der Antarktis weltweit verbreitet und zeigt einen einen hohen Artenreichtum von bis zu 200 Arten. In Marokko erhalten Süßwassermuscheln in den letzten Jahren immer mehr Aufmerksamkeit und Daten über die Familien dieser Gruppe liegen für die meisten von ihnen vor. Viele Unsicherheiten existieren jedoch für den derzeitigen Artenreichtum und die Verbreitung der Familie Sphaeriidae. Diese Informationen, einschließlich Taxonomie, Phylogeographie und Naturschutz-Status, sind für weitere Studien unerlässlich, um das Wissen über diese Familie in Marokko zu verbessern, und stellen den ersten Schritt zur Entwicklung eines nationalen Erhaltungsplans für alle Süßwassermuscheln dar. In der vorliegenden Arbeit wurden mehrere Untersuchungen durchgeführt, um die Vielfalt und Verbreitung von Sphaeriidae in den verschiedenen Einzugsgebieten Marokkos zu bewerten, in den verschiedenen Typen von Lebensräumen (Seen, Quellen, Flüsse und kleine Standgewässer) und Mikrolebensräume (Schlamm, Wasserpflanzen usw.). Die Identifizierung der Exemplare und ihrer morpho-ökologischen Eigenschaften basierte auf morphologischen und morphometrischen Analysen, die sorgfältig verarbeitet wurden, um maximale Präzision zu erreichen. Mehrere Studien haben jedoch den Einfluss gezeigt, den Umweltfaktoren auf die Morphologie von Arten haben können, insbesondere bei Arten mit einer hohen morphologischen Plastizität, welche ihre Identifizierung verfälschen können. Für die Art *Pisidium* casertanum wurde eine Fallstudie vorgelegt, in der die intraspezifische morphologische Variabilität gemäß den Lebensräumen der Art durch die Anwendung der elliptischen Fourier-analyse, einer geometrischmorphometrischen Methode zur Analyse der Kontur der Exemplare, bewertet wurde. Um die morphogeometrischen Analysen der Arten zu vervollständigen und ihre Identifizierung zu bestätigen, wurden molekulare Analysen durchgeführt. Hierbei wurden Sequenzen integriert wurden, die sich aus der Amplifikation von vier Genen, mitochondrialen (COI und 16S rRNA) und nukleären (H3 und 28S rRNA) ergaben. Diese marokkanischen Sequenzen, ergänzt durch andere *Pisidium*-Sequenzen, wurden integriert, um zum ersten Mal auf ihre Phylogeographie in einem größeren Rahmen, der Westpaläarktis, zu schließen. Schalenmorphologie und morphometrische Analysen in Kombination mit molekularen Daten zeigten die Existenz von fünf Arten in Marokko, die zur Gattung *Pisidium* gehören [*P. casertanum* (Poli, 1791), *P.* (cf.) *personatum* Malm, 1855, *P. subtruncatum* Malm, 1855, *P. amnicum* (O. F. Müller, 1774) und *Pisidium* sp. und eine zur Gattung *Musculium* gehörende Art: *M. lacustre* (O. F. Müller, 1774). Sphaeriidae wurden in allen marokkanischen Einzugsgebieten gesammelt, mit Ausnahme der Becken von Bouregreg und Sakia El Hamra-Oued Eddahab. Der Naturschutz-Status von Sphaeriidae in Marokko wurde zum ersten Mal bewertet und ermöglichte die Aktualisierung der zuvor von der IUCN zugewiesenen Kategorien. *Pisidium casertanum* und *P.* (cf.) *personatum* werden beide als „nicht gefährdet“ eingestuft, während *P. subtruncatum* als regional „gefährdet“ vorgeschlagen wurde, da die Anzahl der Standorte, an denen diese Arten vorkommen, heute bedeutsamer sind. Der Status “regional ausgestorben”; wird für *P. amnicum* und *M. lacustre* vorgeschlagen, da für beide Arten nur leere Schalen gefunden wurden.

Die für die phylogeographische Analyse verwendete molekulare Uhr ermöglichte die Datierung und Bestimmung der Divergenzprozesse, die die gegenwärtige Vielfalt und Verbreitung der Gattung *Pisidium* in der Westpaläarktis geprägt haben. Diese Ergebnisse zeigten, dass das Vorkommen der Gattung in der Westpaläarktis die Folge einer Ausbreitung von einem Ort eurasischen Ursprungs während des frühen Oligozäns ist. Diese Ausbreitung hätte über zwei Routen stattfinden können: eine nördliche Route nach der Schließung der Straße von Turgai, die Asien mit Europa verbindet; und eine südliche Route, als sich das Paratethys-Meer vom Tethys-Ozean trennte und die anatolisch-balkanisch-iranische Landmasse entstand. Die Radiation von *Pisidium*-Arten in der Westpaläarktis war während des späten Miozäns maximal und hielt bis zum Pliozän an. Diese Radiation hängt hauptsächlich mit Speziationen zusammen, die aus Isolationen in Glazialrefugien auf den Mittelmeerhalbinseln resultierte. Es wurde jedoch kein klares geografisches Muster zwischen marokkanischen und anderen Populationen identifiziert, was wahrscheinlich auf die hohe Dispersalkapazität von Mitgliedern der Familie Sphaeriidae zurückzuführen ist, die von Vögeln und Fischen über große Entfernungen transportiert werden können. Alle durch diese Arbeit erhaltenen Informationen stellen grundlegende Elemente dar, um das Wissen über diese Familie zu verbessern und sie in zukünftige Projekte zu ihrer Ökologie, Erhaltung und Implikation in ökotoxikologischen Studien zu investieren.

Keywords: Systematik, Verbreitung, Phylogeographie, Muscheln, Süßwasser, 16S rRNA, COI, 28S rRNA, H3, Marokko.

Résumé

La famille des Sphaeriidae Deshayes, 1855 est largement répartie à travers le globe, à l'exception de l'Antarctique, avec une grande richesse spécifique allant jusqu'à plus de 200 espèces. Au Maroc, les bivalves des eaux douces reçoivent de plus en plus d'attention ces dernières années et les données sur les familles de ce groupe ne sont plus un mystère pour la majorité d'entre elles. Toutefois, de nombreuses incertitudes entourent la diversité et la distribution actuelles de la famille des Sphaeriidae. Ces informations, y compris la taxonomie, la phylogéographie et le statut de conservation, sont essentielles pour des études ultérieures visant à améliorer la connaissance de cette famille au Maroc et représente la première étape vers le développement d'un plan national de conservation pour tous les bivalves d'eau douce.

Dans le présent travail, plusieurs investigations ont été menées pour évaluer la diversité et la distribution des Sphaeriidae dans les différents bassins du Maroc, couvrant différents types d'habitats (lacs, sources, rivières et petits étangs) et microhabitats (vase, plantes aquatiques, etc). L'identification des spécimens et de leurs caractéristiques morpho-écologiques a été basée sur des analyses morphologiques et morphométriques soigneusement traités pour obtenir un maximum de précision. Plusieurs études ont montré, cependant, l'influence que peuvent avoir les facteurs environnementaux sur la morphologie des espèces en particulier pour les espèces ayant une grande plasticité morphologique qui peut fausser leur identification. Une étude de cas a été proposée pour l'espèce *Pisidium casertanum*, en évaluant la variabilité morphologique intraspécifique en fonction des habitats de l'espèce par l'application de l'Analyse Elliptique de Fourier, une méthode morphométrique géométrique analysant le contour des spécimens. Pour compléter les analyses morpho-géométriques des espèces et confirmer leur identification, des analyses moléculaires ont été menées en intégrant les séquences résultantes de l'amplification de quatre gènes, mitochondriaux (COI et ARNr 16S) et nucléaires (H3 et ARNr 28S). Ces séquences marocaines, complémentées avec d'autres séquences de *Pisidium* ont été intégrées pour inférer, pour la première fois, leur histoire phylogéographique dans un cadre plus élargi : le Paléarctique occidental.

La morphologie de la coquille et les analyses morphométriques combinés aux données moléculaires ont révélé l'existence de cinq espèces appartenant au genre *Pisidium* [*P. casertanum* (Poli, 1791), *P.* (cf.) *personatum* Malm, 1855, *P. subtruncatum* Malm, 1855, *P. amnicum* (O. F. Müller, 1774) et *Pisidium* sp.] et une espèce au genre *Musculium* [*M. lacustre* (O. F. Müller, 1774)]. Les Sphaeriidae ont été collectés dans tous les bassins marocains, à l'exception des bassins Bouregreg et Sakia El Hamra-Oued Eddahab. L'état de conservation des Sphaeriidae au Maroc a été évalué pour la première fois et a permis d'actualiser les statuts préalablement attribués par l'UICN. *Pisidium casertanum* et *P.* (cf.) *personatum* sont toutes les deux classées comme "Préoccupation mineure" alors que *P. subtruncatum* a été proposée comme espèce "Vulnérable", vu le nombre de localités où ces espèces sont présentes est aujourd'hui plus important. Le statut de "Eteint à l'échelle régionale" est suggéré pour *P. amnicum* et *M. lacustre* vu qu'uniquement des coquilles vides ont été retrouvées pour les deux espèces.

L'horloge moléculaire utilisée pour l'analyse phylogéographique a permis de déterminer les dates et processus de divergence qui ont forgé la présente diversité et distribution du genre Pisidium au Paléarctique occidental. Ces résultats ont montré que la présence du genre dans le Paléarctique occidental est la conséquence d'une dispersion depuis une localité d'origine Eurasienne pendant le début de l'époque Oligocène. Cette dispersion aurait eu lieu via deux voies : une voie nord après la clôture du détroit de Turgai connectant ainsi l'Asie à l'Europe ; et une voie sud quand la mer Paratéthys s'est séparée de l'océan Téthys donnant naissance à la masse terrestre Balkano-Anatol-Iranienne. Les radiations des espèces de *Pisidium* au Paléarctique occidental était maximale pendant la fin du Miocène et se poursuit jusqu'au Pliocène. Ces radiations sont liées majoritairement aux spéciations qui ont résulté des isolements dans les refuges glaciaires au niveau des péninsules Méditerranéennes. Cependant, aucun modèle géographique clair n'a été identifié entre les populations marocaines et celles des autres populations, ceci est probablement dû au caractère dispersif des membres de la famille Sphaeriidae qui peuvent être transporté à de longues distances par les oiseaux et les poissons.

Toutes les informations recueillies à travers ce travail constituent des éléments de base pour améliorer les connaissances sur cette famille et les investir dans des projets de travaux futurs sur leur bio-écologie, conservation et implication dans les études écotoxicologiques.

Mots clés : Systématique, répartition, phylogéographie, bivalves, eaux douces, ARNr 16S, COI, ARNr 28S, H3, Maroc.

Arabic abstract here , I will insert it when in the printing

In memory of the great researcher Ulrich Boessneck,

I owe him at least this and much more

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Dedications

I dedicate this work to my family that I love and that I will never

know how to thank as it should be,

In particular, to my parents M'hamed Rassam and Khadija

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of day without their support, encouragement and caring. I

would like to take this opportunity to express my gratitude

for their unconditional love,

their unceasing efforts to provide me with a dignified education that has

made me what I am today.

To my lovely sisters Maria and Selma, who always bring joy and

happiness to the whole family, may God give you happiness, health,

courage and success.

To my husband Hassan, who has spared no effort to help me, support me and never stopped advising and encouraging me throughout my thesis work...

I love you all

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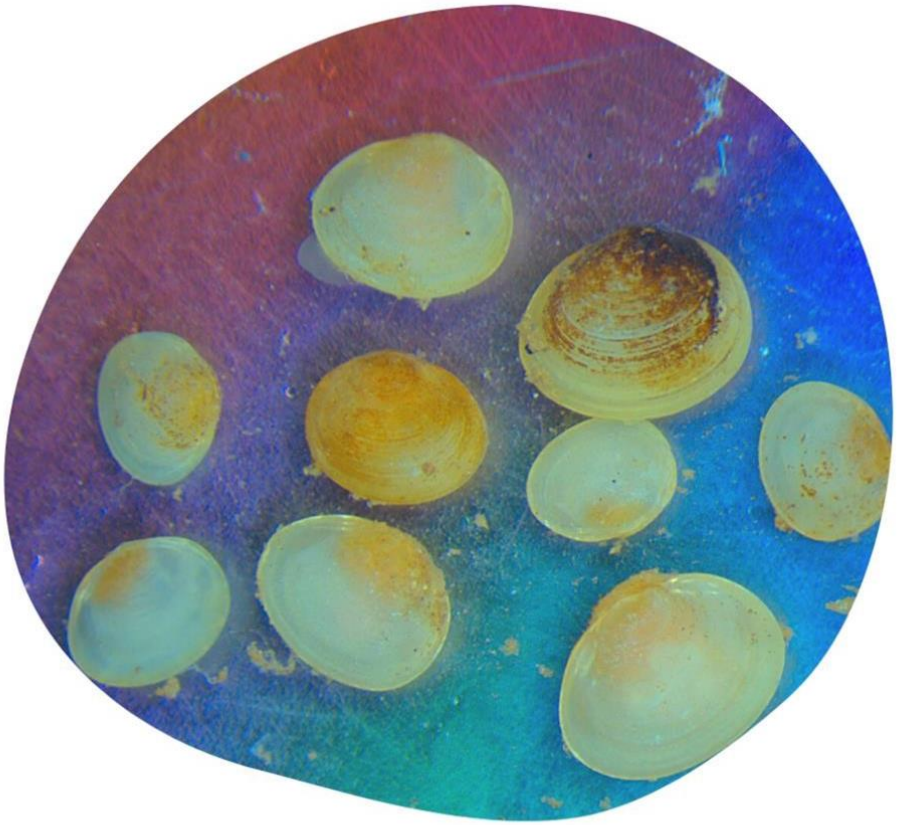


General Introduction

Natural freshwater ecosystems represent the terrestrial phases of the global hydrological cycle and include rivers, streams, lakes, ponds, wetlands and groundwater. While these ecosystems comprise only about 0.01% of the water on Earth and make up less than one-tenth of the world's land surface, they are home to more than 10% of all species recorded to date, including about 30% of all vertebrates (Reid et al., 2020). The frequency of endemism and speciation is high due to the natural properties of these ecosystems, which facilitate isolation and limit long distance dispersal and the resulting reduction in connectivity (Strayer and Dudgeon, 2010). However, these same properties of freshwater biodiversity make it highly vulnerable and sensitive to human activities (Dudgeon et al., 2006, Strayer and Dudgeon, 2010, Carpenter et al., 2011). In addition, freshwater ecosystems provide a wide range of essential resources for humans, such as supporting (e.g., water and nutrient cycles), provisioning (e.g., water), regulating (e.g., pollination), and cultural (e.g., ecotourism) services (Carpenter et al., 2011). The increasing need for these resources, particularly water, has led to increased pressure on freshwater biodiversity, which is now in decline (Dudgeon et al., 2006, Strayer and Dudgeon et al., 2010, Carpenter et al., 2011). This biodiversity is threatened in a variety of ways, some of which are internally generated (habitat alteration, fragmentation, overexploitation) while others are external (invasive non-native species, climate change, atmospheric pollution). In fact, almost all freshwater ecosystems in the world have already been altered (Leveque and Balion 2005).

In this context, the decline of freshwater biodiversity has attracted the interest of the scientific community and, more recently, many faunal groups such as invertebrates have received greater attention (e.g. Lydeard et al., 2004, Strayer et al., 2004, Clausnitzer et al., 2009). Invertebrates account for 99% of all animal diversity (Ponder and Lunney, 1999) and generally dominate the abundance and biomass of aquatic and terrestrial ecosystems (Cardoso et al., 2011). In addition, they are responsible for a wide variety of ecosystem functions and services including pollination, bioturbation, nutrient cycling, water purification among others (Hooper et al., 2005, Cardinale et al., 2012, Mace et al., 2012). However, for many decades, invertebrates were ignored in the majority of conservation studies, which focused almost exclusively on vertebrates, particularly mammals and birds (Lydeard et al 2004, Cardoso et al 2011). Nonetheless, more recently, invertebrates have been the subject of increasing scientific and media interest.

The North African region is globally identified as a territory with significant marine and continental biodiversity due to its geomorphological variability and the diversity of its ecosystems and landscapes (Figure 1). Among them, freshwater ecosystems are thought to be particularly fragile and several native species are threatened and at risk of extinction due to habitat loss and degradation induced by human pressure, pollution and climate change

(Carpenter et al., 2011; Barrios et al., 2014). Furthermore, the North African region is considered the poorest in terms of water resources at the African scale. The few scattered rivers in the Maghreb are restricted to Morocco (FAO, 2003). The latter has the largest river network in North Africa (Di Piazza, 2006). Rainfall in the high mountain ranges of the Rif, Middle Atlas, High Atlas, and Anti-Atlas feeds rivers that generally flow northwest to the Atlantic or southeast to the Sahara. The Moulouya River is the main exception, running from the Middle Atlas to the Mediterranean Sea. Also in Morocco, a number of mountain lakes are located above the 1800 m level, with large hydroelectric reservoirs and coastal brackish marshes mainly along the Atlantic coast (Barrios et al., 2014).

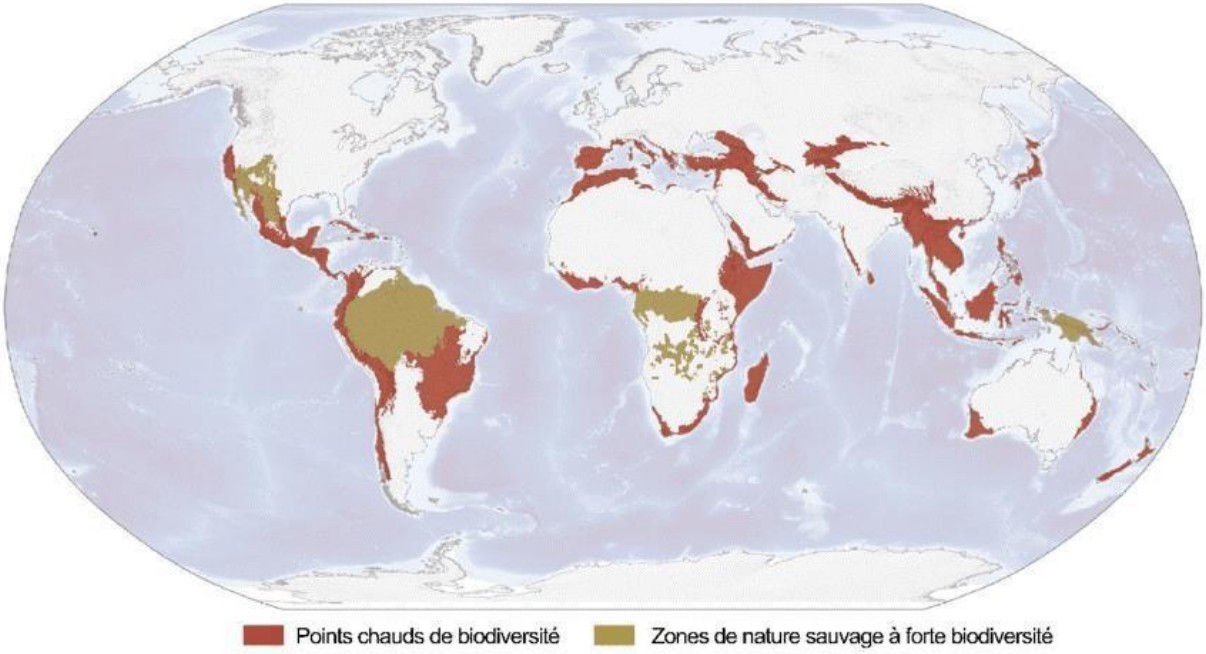


Figure 1. Global distribution of biodiversity hotspots and high biodiversity wilderness areas sources : modified from Mittermeier et al. (2002, 2004); Williams et al. (2011) (Bertzky et al., 2019).

One of the freshwater taxa present in these rivers is the family Sphaeriidae, comprising the world's smallest freshwater bivalves with lengths ranging from 2 to 25 mm (Frankiewicz, 2018) and has a global distribution, with the exception of Antarctica (Thorp and Covich, 2010, Williams et al., 2014). Prior to the present thesis work, knowledge of the species diversity and distribution of the family in Morocco was poor and no recently maintained studies have highlighted this family. Therefore, in-depth studies on the family, in all aspects, are lacking and a void is apparent in Morocco with respect to the data available on the Sphaeriidae in comparison to other freshwater molluscs, especially on their origin and evolutionary and biogeographic history in the region.

Phylogeography is the field of study concerned with the principles and processes that govern the geographic distribution of genealogical lineages, particularly those within and between

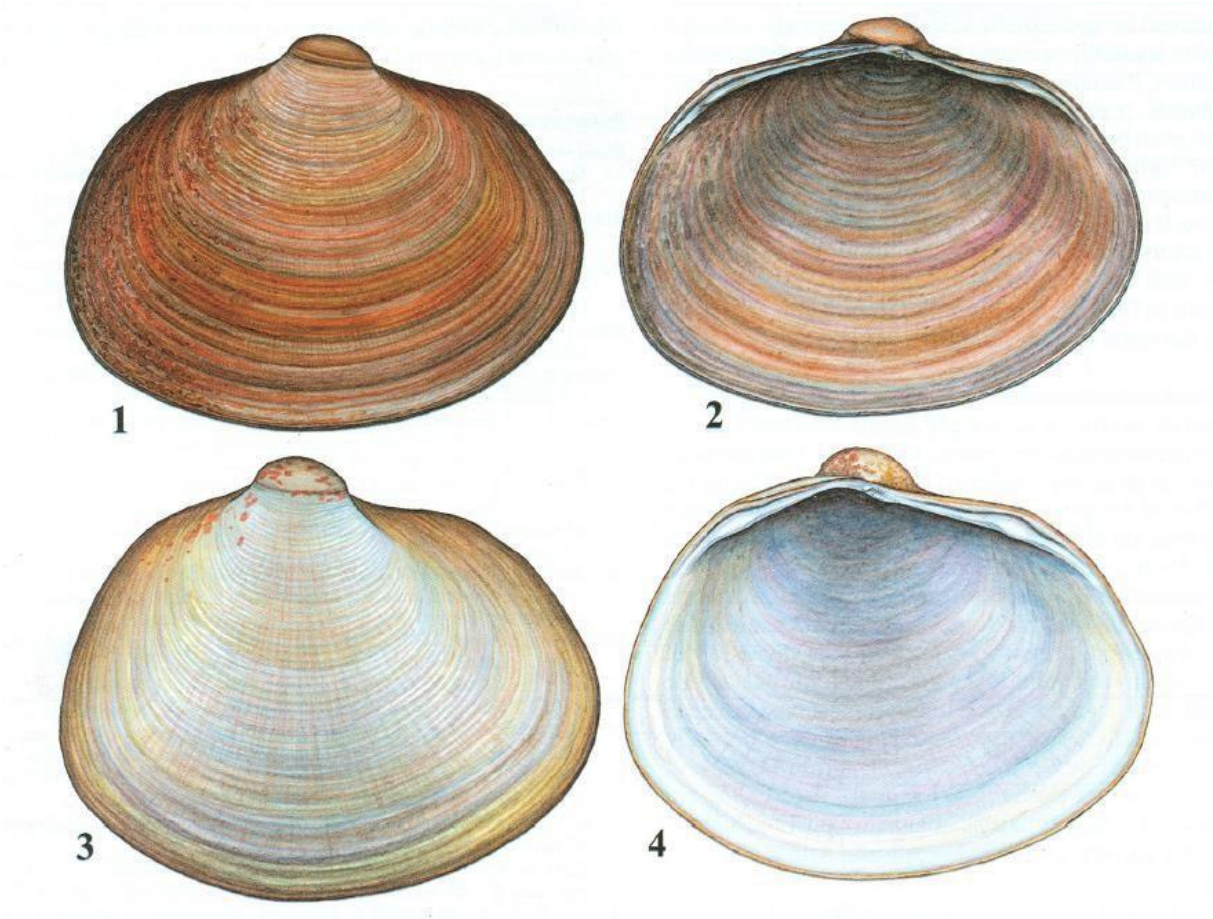
closely related species. As the name implies, phylogeography deals with the historical and phylogenetic components of the spatial distribution of genetic lineages (Avise, 2000). The phylogeographic study relies primarily on one of two main components: the fossil record and the tools of phylogenetic and molecular clocks (Wilke et al., 2009). For the case of the Sphaeriidae, phylogenetic inference by the molecular clock method remains the most appropriate methodology given the lack of sufficient fossil records of the family. Morocco being part of the western zone of the Palearctic biogeographic area, the phylogeographic study of the Sphaeriidae in Morocco should therefore be done taking into account the species of the family present in this region in order to study the potential affinities with them while considering the past events that may have shaped the present distribution.

The Western Palearctic was strongly affected by Pleistocene glaciations. Several works (Hewitt, 1996; Taberlet et al., 1998; Hewitt, 1999; Hewitt, 2000; Hewitt, 2004) have emphasized the influence of Pleistocene glaciations on the current phylogeography of taxa. Glacial-interglacial cycles resulted in large altitudinal and latitudinal shifts in taxa and ecosystems, so it was essential to move, adapt, or become extinct. It has long been established that the geographic ranges of species have periodically expanded and contracted in response to Quaternary climate change (Hewitt 1996; Provan & Bennett 2008; Stewart et al., 2010). Refugia, located at lower elevations, had suitable climatic conditions and then played a crucial role in the survival of temperate and thermophilic plant and animal species. In the early discussions of shifting species distributions in the Western Palearctic area, scientists agreed on the presence of three major refugia located in the southern European peninsulas: Iberia, Italy and the Balkans (Bennett et al. 1991, Hewitt, 1996, Hewitt, 1999; Taberlet et al. 1998). Their identification has been based on paleoecological and phylogeographic evidence (Huntley and Birks, 1983; Hewitt, 1996; 1999; 2000). De Lattin (1967) complemented this statement and described nine secondary refugial sub-centers in the Mediterranean region, namely the Atlanto-Mediterranean, Adriatic-Mediterranean, Ponto-Mediterranean, Tyrrhenian, Canary, Mauritanian, Cyrenian, Cretan and Cypriot refugia, emphasizing the importance of the North African region, which comprises two distinct sub-centers. In the presence of these claims by scientists on the role of North Africa as a refuge area for taxa during harsh glacial conditions, verifying the contribution of these refuges in building the current diversity and distribution of Western Palearctic organisms remains an intriguing avenue to explore.

The present thesis has as a general objective to shed light on the family Sphaeriidae, which has been neglected for a long time, by studying its biodiversity and distribution in Morocco, its phylogeny and its biogeography in relation to the other members of the family throughout the Western Palearctic area. To achieve this objective, the following two specific axes were studied:

- The presentation of a first assessment of the diversity of Sphaeriidae in Morocco, including species richness and composition, based on intra- and interspecific morphometric and geometric analysis, as well as the evaluation of the distribution pattern of Sphaeriidae in Moroccan freshwater basins and the assignment of a regional conservation status to Moroccan species of Sphaeriidae.
- Determining the diversity of the genus *Pisidium* in the Western Palearctic, as well as reconstructing and inferring phylogenetic relationships among *Pisidium* representatives in the Western Palearctic through time and analyzing the biogeographic affinities that led to the current distribution pattern in the region.

Chapter I: Presentation of the family: Sphaeriidae (Deshayes, 1855)



Sphaeriidae: *Musculium goshaitanensis* (1&2) & *Musculium indicum* (3&4)
(Plate I, Nesemann et Sharma, 2005)

1. Background

In Morocco, studies on freshwater bivalves have received more attention in recent years. However, these studies have been mainly devoted to the family Unionidae (Benaissa et al, 2019; Gomes-dos-Santos et al, 2019) and Margaritiferidae (Benaissa et al., 2021; Sousa et al., 2016; Sousa et al., 2018; Sousa et al., 2019). Regarding other groups of freshwater bivalves such as the family Sphaeriidae, work is very limited to faunistic studies of other aquatic invertebrate groups (Talami, 1998) and records of occurrences in some areas of Morocco (Kuiper, 1972; Rassam et al, 2020). Key information, including taxonomy, diversity and distribution of this family is still lacking.

The first collections of the family Sphaeriidae from Morocco are deposited at the Scientific Institute of Rabat, being collected by Kuiper in 1972 and include more than 500 specimens. In literature, the first publication referring to the family Sphaeriidae from Morocco belongs to Pallary (1915) who mentioned the species *Pisidium atlasicum*, a synonym of *P. casertanum* (Poli, 1791) (Kuiper, 1964). Subsequently, a second publication by Pallary in 1921 had referred to the species *P. rotundatum*, which was renamed by the same author in 1936 to *P. marocanum*, a synonym of the species *P. casertanum* (Kuiper, 1964). The first specific study of a genus of the family Sphaeriidae in Morocco was carried out by Kuiper (1972) during a biological mission to Morocco organized by the Belgian University Ghent. At the end of this mission, seven species of the genus *Pisidium* were recorded, including five new ones for Morocco. Since this publication, no work has been devoted specifically to the family Sphaeriidae in Morocco.

I. Taxonomy and systematics

The nomenclature within the family Sphaeriidae (Order: Sphaeriida, Figure 2) has been discussed many times and the genera included in the family Sphaeriidae are not yet definitively concluded (Dreher Mansur and Meier-Brook, 1992, 2000; Herrington, 1962; Kuiper, 1983; Korniushin, 2000; Korniushin & Glaubrecht, 2002; Lee & Ó Foighil, 2003), however, both *Sphaerium* and *Pisidium* are clearly marked genera and are considered to be the most diverse in the family.

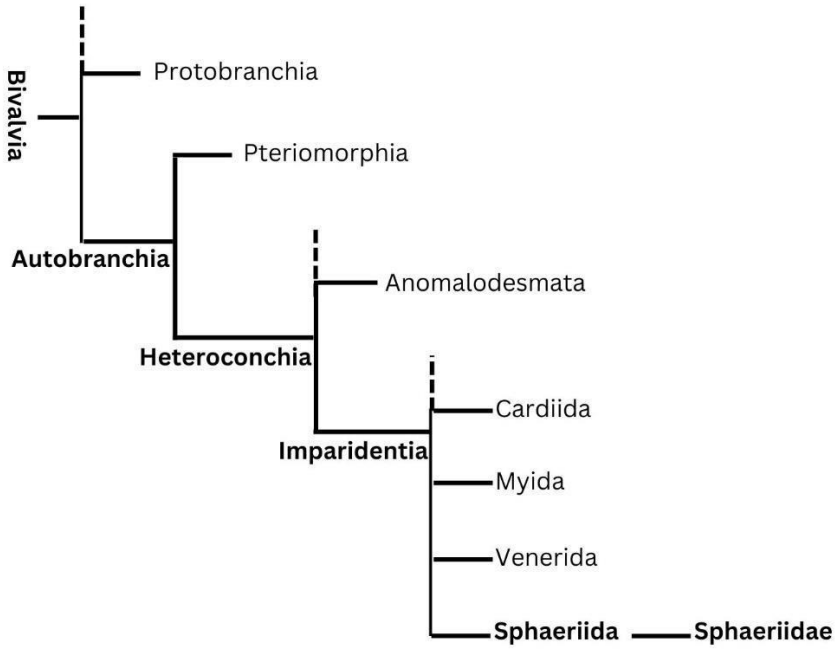


Figure 2. Phylogeny of bivalve molluscs according to Gombosch et al. (2017) & MolluscaBase.

Systematics of the Sphaeriidae is a complex topic because of the lack of agreement regarding the delineation of genera and subgenera. Recent phylogenetic studies (Dreher-Mansur and Meier-Brook, 2000; Korniushin and Glaubrecht, 2002; Lee and Ó Foighil, 2003) distinguish two main subgroups: Euperinae and Sphaeriinae.

The subfamily Euperinae consists of the genera *Byssanodonta* d'Orbigny, 1846, and *Eupera* Bourguignat, 1854, and has a restricted geographic distribution. The genus *Byssanodonta* is represented by a single species, *B. paranensis*, endemic to the Paraná River, Argentina (Dreher-Mansur and Ituarte, 1999). It occurs in Central and South America and Africa, although one species has been introduced into the southern United States (Heard, 1965), and its range has recently expanded into the upper Mississippi River basin (Sneen et al., 2009). The genus *Eupera*, on the other hand, shows greater richness. The subfamily Sphaeriinae has traditionally been represented by three cosmopolitan genera, *Sphaerium* Scopoli, 1777, *Musculium* Link, 1807, and *Pisidium* Pfeiffer, 1821.

Heard (1977) considers *Musculium* as a valid genus, separate from *Sphaerium*, for several reasons; 1- more species of *Musculium* are able to live in temporary pools than *Sphaerium* species; 2- the extra marsupial larvae do not carry eggs or sperm as was the case for most *Sphaerium* species; 4- the 2 siphons in *Musculium* are fused together at their basal halves only, whereas they are fused along their entire length for *Sphaerium*. However, a series of molecular studies (Lee and Ó Foighil, 2003; Schultheiss et al., 2008; Clewing et al., 2013) have, systematically, considered *Musculium* as a subgenus included in *Sphaerium*, and the former subgeneric assemblage of *Pisidium* (*Afropisidium* Kuiper, 1962, *Odhneripisidium*

Kuiper, 1962, *Euglesa* Jenyns, 1832, and *Pisidium* s. str.) to be generic-level clades. Although the relative branching order is unstable, a common feature of all gene trees is that *Afropisidium* is sister to the rest of the Sphaeriidae, either alone or in combination with *Odhneripisidium*.

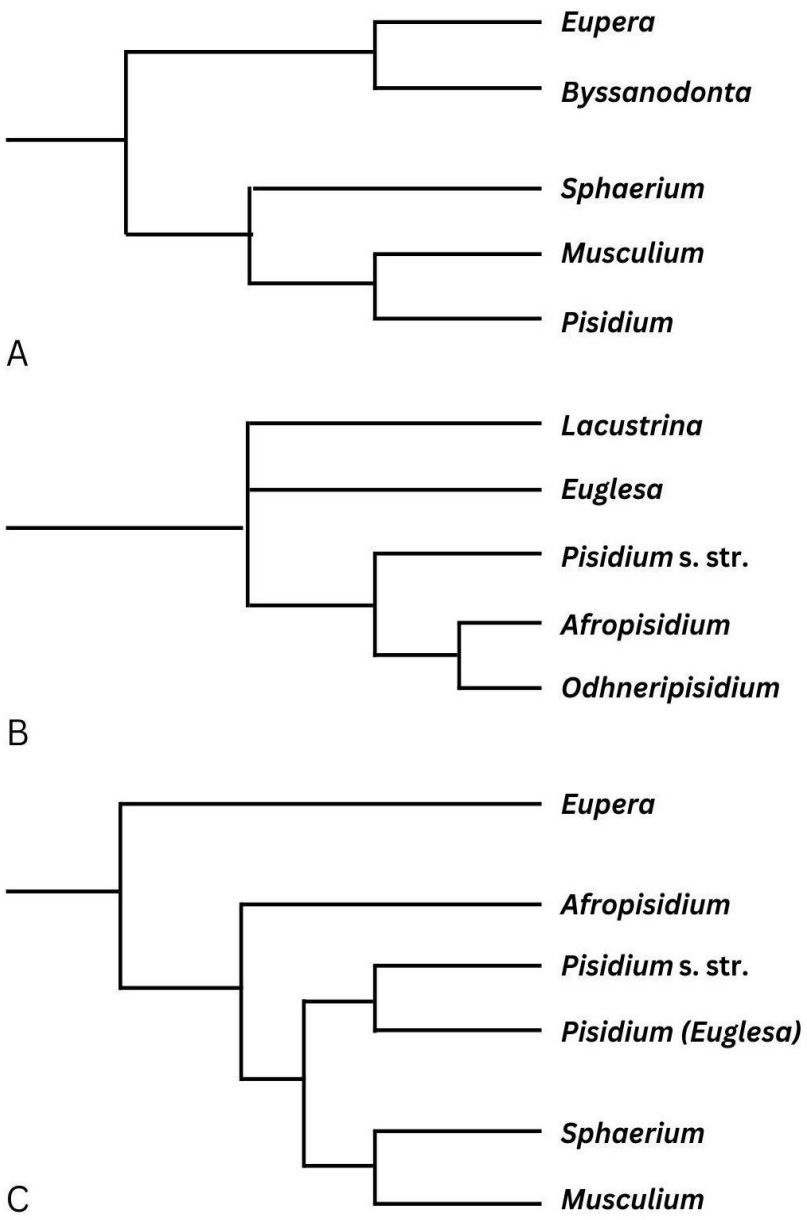


Figure 3. Suggested phylogenetic reconstructions for the family Sphaeriidae. A- Phylogenetic relationships between genera according to Dreher Mansur and Meier-Brook (1992, 2000). B-C- Phylogenetic relationships between subgenera of *Pisidium* according to Korniushin (1998).

It is currently unknown whether Sphaeriidae have congenital marine outgrowths. Taxonomists (Keen and Caseay, 1969) have often grouped the Sphaeriidae with the Cyrenidae (= Corbiculidae), which have not only freshwater but also brackish/marine representatives, in the superfamily Corbiculoidea, based on common shell and life history characteristics. However, the affinity between these two families has been rejected by morphological (Dreher-Mansur and Meier-Brook, 2000) and molecular (Park and Ó Foighil, 2000; Taylor et al., 2007) studies

(Figure 3). Similar brooding traits observed in freshwater Sphaeriidae and Cyrenidae would represent convergent adaptations to freshwater habitats (Park and Ó Foighil, 2000). Recent phylogenetic analyses (Taylor et al., 2007; Bieler et al., 2014) suggest that the Sphaeriidae form a basal lineage within the Neoheterodontei, unclassified with various marine bivalve groups, such as Myidae, Mactroidae, Cyamiidae, Gaimardiidae, Ungulinidae, and Veneroida.

Taxonomy of the Sphaeriidae has been hampered by the rarity and plasticity of shell characters that have contributed to a dramatic overestimation of species diversity (Lee, 2004). Kuiper (1983), for example, recorded over 1000 published nominal species for *Pisidium* s. lat. but estimated that only about 80 species could actually be diagnosed. Herrington's (1962) revision of the family Sphaeriidae in North America presented the first nomenclature of over 330 names, whereas Burch (1975) listed only 37 species for North America. The systematics of the Sphaeriinae has also been confused with the wide geographic ranges of many taxa. For example, *Pisidium casertanum* occurs on every continent except Antarctica (Burch, 1975; Kuiper, 1983) and nearly half of the North American Sphaeriidae occur in Europe and Asia (Burch, 1975). Passive dispersal by insects, birds, and fish (Rees, 1965; Brown, 2009) coupled with the ability to self-replicate (Meier-Brook, 1970) may explain their wide distribution. Another problem has been the use of a remarkably divergent classification system by the Russian taxonomic school (Korniushin, 1998). In general, Russian specialists assigned significant weight to all levels of morphological variation and established elaborate taxonomic rankings; Western taxonomists tended to recognize larger, more inclusive taxa. For example, nearly 200-500 species of Sphaeriidae have been extrapolated for the former Soviet Union alone (Scarlato, 1981), whereas fewer than 60 species are recognized in North America and Europe combined (Bowden and Heppell, 1968; Burch, 1975).

Graf (2013) reports that a total of 227 species of Sphaeriidae are recognized on all continents except Antarctica up to the time of publication of his work, although there is evidence that this may be an underestimation. Extensive sampling efforts in mountainous areas and ancient lakes have recovered multiple genetically and morphologically distinct cryptic lineages (Guralnick, 2005; Schultheiss et al., 2008; Clewing et al., 2013), and new species have recently been added to the list, the most recent of which is *P. boessnecki* (Clewing et al., 2022).

II. Biology and ecology

1. Biology

The Sphaeriidae exhibit complex reproductive and developmental characteristics, some of which may be associated with the evolutionary colonization of freshwater habitats. All Sphaeriidae studied to date are simultaneous hermaphrodites, with each individual simultaneously producing eggs and sperm (Heard, 1965; Ituarte, 1997). It is thought that

Sphaeriidae generally reproduce by cross-fertilization but are, optionally, capable of self-fertilization (Heard, 1965, 1977; Mackie et al., 1974; Ituarte, 1997).

The planktonic veliger larval stage, which is phylogenetically widespread among marine bivalves, has been completely lost (Dreher-Mansur and Meier-Brook, 2000), as in freshwater cyrenids and unionids (Cummings and Graf, 2009). Sphaeriidae taxa, however, exhibit varying degrees of complexity in the details of how brooding is accomplished. The simplest form, synchronous brooding, is found in the subfamily Euperinae, where all embryos in a brood reproduce at the same time and develop into a single cohort (Heard, 1965; Dreher-Mansur and Ituarte, 1999; Dreher-Mansur and Meier-Brook, 2000). *Pisidium* s. lat. species are also synchronous brooders.

As opposed to the genera Euperinae (*Byssanodonta* and *Eupera*), embryos of *Pisidium* s. lat. develop in a separate brood sac, which is bound to the parental tissues (Heard, 1965, 1977; Mackie et al., 1974) (Figure 4). In contrast, *Sphaerium* and *Musculium* species are sequential brooders, where multiple embryos resulting from different oviposition events are simultaneously present in separate brood sacs (Heard, 1977; Mackie, 1978). Molecular phylogenetic studies (Cooley and Ó Foighil, 2000; Lee and Ó Foighil, 2003) suggest the evolutionary elaboration of the complexity of parental care, from relatively simple synchronous brooding, to the origin of marsupial sacs and parental embryonic feeding, and finally to sequential brooding.

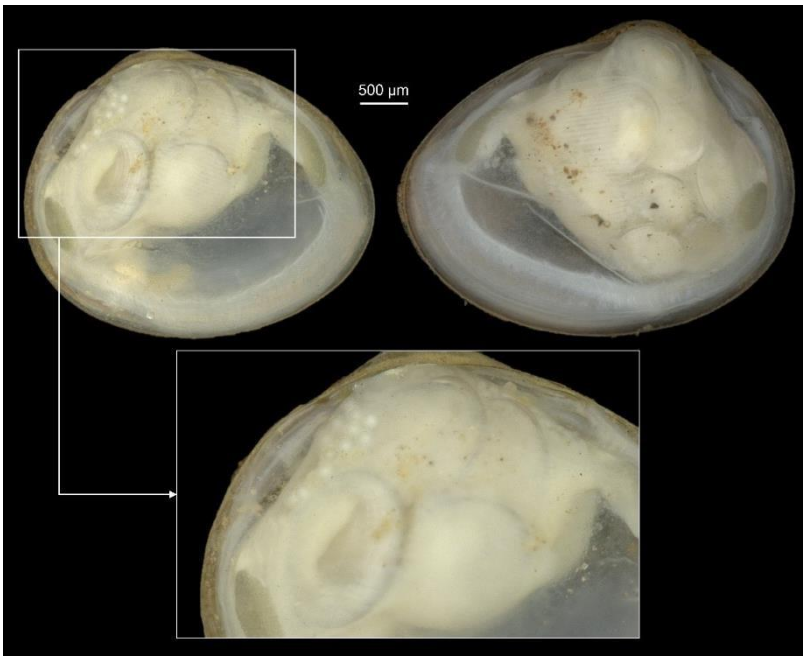


Figure 4. Brooding of embryos in the gills in *Pisidium* (©Rassam, 2020).

Cytogenetic studies (Lee, 1999; Kořínková and Moravkoa, 2010) of various species belonging to the subfamily Sphaeriinae have added a genomic dimension to inferences about

reproduction, cladogenesis, and evolution in the family. These authors revealed a striking degree of polyploidization, extending from 90 to 247 mitotic chromosomes. Of the ≈25 species observed, only 4 species-3 of which are closely related in Europe (*Sphaerium* corneum: 2n=30 or 36, *S. nucleus*: 2n=30, and *S. solidum*: 2n=30)-and 1 in North America (*S. rhomboideum*: 2n=44)-had diploid chromosomal complements (Petkevičiūtė et al., 2007; Kořínková and Kral, 2011). All other taxa had well over 100 chromosomes, far more than the normal range known for Bivalves. Not only is genome amplification significant, but a substantial variation in ploidy levels (2n-13n) occurs in the subfamily. Although polyploidy is rare in animals, especially in bivalve mollusks, it has been recorded in multiple asexual lineages of freshwater *Corbicula* (Komaru et al., 1998) and marine *Lasaea* (O Foighil and Thiriot-Quiévreux, 1991).

Marked polyploidy is frequently associated with asexuality (Mogie, 1986), and the similarities, such as polyploidy, hermaphroditism, and extended parental care, observed in the Sphaeriinae and the asexuals *Corbicula* and *Lasaea* are remarkable. Molecular evidence (Lee and Ó Foighil, 2002) suggests that ancestral patterns of polyploidization may account for much of the current diversity of this freshwater bivalve radiation, although the evolutionary origins of genome duplication are not yet clear.

2. Ecology

Sphaeriidae is a family of bivalves with a cosmopolitan distribution in a variety of freshwater habitats, including rivers, streams, ponds, lakes and even small mayflies (Burch, 1975; Kuiper, 1983). Many species of freshwater bivalves live in stagnant as well as flowing waters, but some Sphaeriidae may replace freshwater mussels as dominant bivalves in headwater streams.

The general ecology of these animals has been discussed by McMahon (1991). The distribution of Sphaeriidae is affected by sediment particle size and water depth (Green 1971; Kilgour and Mackie 1988). These factors are often most optimal in nearshore sites.

Sphaeriidae prefer circumneutral to slightly basic pH environments (Green 1971). Some Sphaeriidae live in the mountains at 3000-3600 m above sea level, while others have been collected in the Arctic Circle. Most species of *Sphaerium* inhabit permanent water bodies, while *Musculium* also occurs in temporary water sites (Heard 1977).

III. Morphology

In species of the genera *Sphaerium* and *Musculium*, the umbo (upper part of the shell) is located in the center or on the anterior side of the center. The animal has separate branchial (lower) and anal (upper) siphons; water enters the branchial siphon and exits through the anal siphon (Figure 5). The grooved muscular foot, which is used for digging and crawling, extends from the anterior (shorter) end of the shell and the siphons from the posterior end. *Musculium*

species often have a calycle, meaning that the umbo is separated from the rest of the shell by being clearly raised.

Sphaeriidae other than *Musculium* are generally not calyculate. The cardinal teeth of *Musculium* are small compared to those of *Sphaerium*, and their shells are thinner. Burch (1975) and Smith (1995) stated that in *Sphaerium*, the siphons are fused only at their base, whereas in *Musculium* they are fused along much of their length; Heard (1977) and Mackie et al. (1980) stated the opposite.

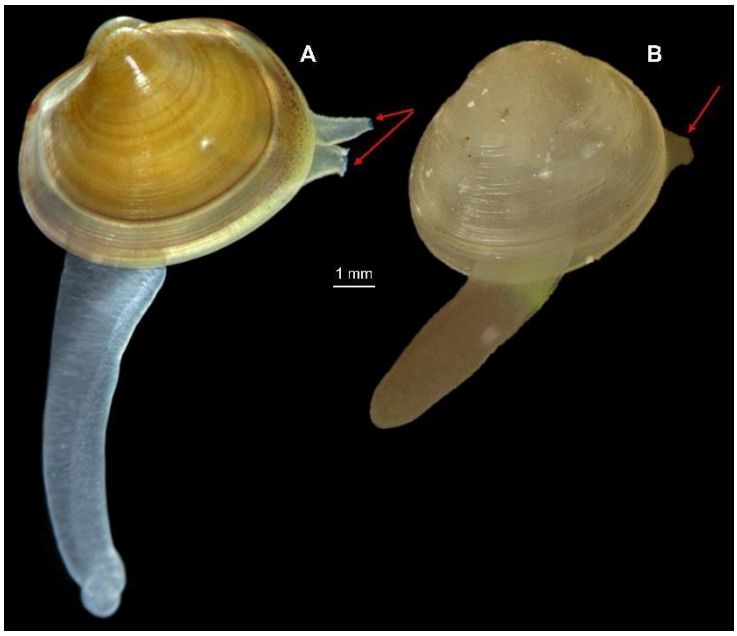


Figure 5. Position and number of siphons in the genera *Musculium* (A) and *Pisidium* (B) (©Gardiner & David Jones, 2008).

In species of the genus *Pisidium*, the shell umbo is on the posterior side of the center, the anterior end of the shell is longer than the posterior end, the muscular foot is large in proportion to the size of the animal, and only the anal siphon is developed, with the gill siphon reduced to a mantle slit (Figure 5). The teeth of *Pisidium* are stronger and more robust in proportion to shell size than in *Sphaerium* and *Musculium*. The ligament that holds the two valves together is short and poorly developed.

The pallial line, formed where the mantle (the organ that secretes the shell) attaches, is indistinct, as are the scars of the adductor muscles whose contraction holds the valves together. The cardinal teeth are so named because of their proximity to the heart of the animal (from the Greek kardia means "heart"). There are lateral teeth on both sides of the cardinals (this is the typical dentition of true clams, whether marine or freshwater). Usually, there are two cardinal teeth (C2, C4) in the left valve and one (C3) in the right, while there are two simple lateral teeth (A2, P2) in the left valve and two pairs of lateral teeth (Al, A3; P1, P3) in the right.

The soft parts of the animal also play an important role in the identification and classification of Sphaeriidae (Figure 6). The following characters are usually considered: the structure of the siphons or siphonic openings, the structure of the gills and kidneys.

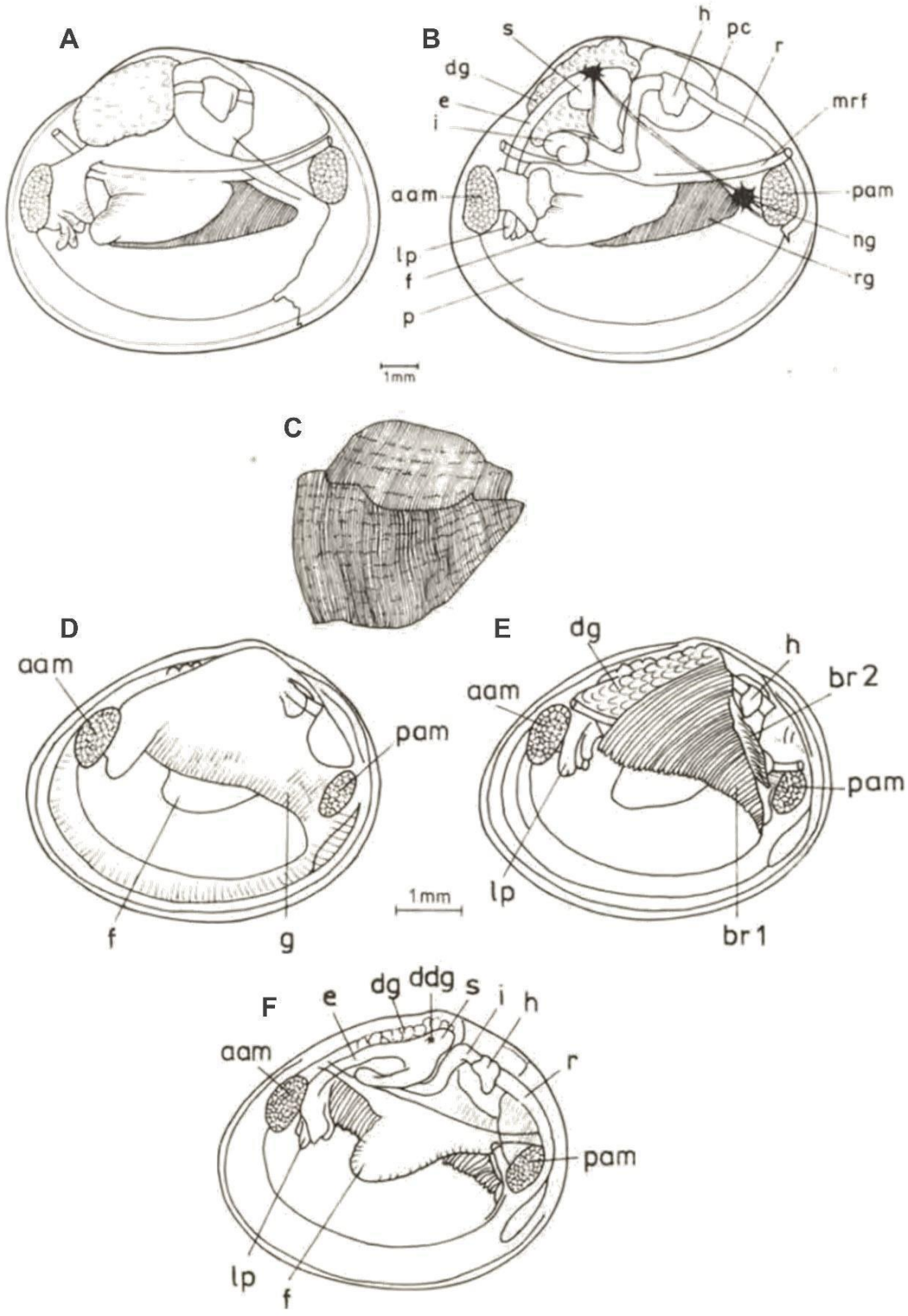


Figure 6. Morphology and anatomy of Sphaeriidae (A, B, C: *S. corneum*; D, E, F: *P. amnicum*). A- After removal of the left valve and the left half of the mantle lobe. B- Animal with soft parts partially removed to show some internal organs. C- Isolated gills. D- After removal of the left valve. F- Animal with the left half of the mantle lobe removed to show the gills. F- Animal with soft parts partially removed to show some internal organs. aam: anterior adductor muscle; dg: digestive gland; e: esophagus; f: foot; h: heart; i: intestine; lp: labial palps; mrf: foot retractor muscle; ng: nerve ganglion; p: mantle; pam: posterior adductor muscle; pc: pericardium; r: rectum; rg: right gill; s: stomach; br1: internal gill; br2: external gill; ddg: digestive gland duct (Piechocki, 1989).

IV. Roles in the ecosystem

The family Sphaeriidae is considered the second most species-rich family of freshwater bivalves (Graf, 2013). Species in this family are dominant in temporary habitats, ponds, and deeper parts of lakes (Herrington, 1962) and can be of great importance in the ecosystem, such as their importance to nutrient cycling, especially phosphorus (Kasprzak, 1986; Cummings and Graf, 2009) and their role as food for fish (Baker, 1982). The tiny, thin-shelled Sphaeriidae are regularly consumed by a number of waterfowl and bottom-feeding fish (Gale 1973). They are therefore important in wildlife productivity and management studies. They are also preyed upon by larvae of marsh flies (Sciomyzidae) (Foote 1976). Decaying bodies of Sphaeriidae that are not eaten by predators serve as food for saprophytes in the aquatic ecosystem, as they are short-lived (typically 1-4 years) and often present in large numbers. Sphaeriidae serve as intermediate hosts for trematode rediae that infest primarily fish, but also beetles, crayfish, and amphibians (Mackie 1976).

Fossil Sphaeriidae provide useful information to the paleontologist attempting to date a geologic deposit or reconstruct an ancient habitat (Herrington 1962; Bickel 1973). Fossils of Sphaeriidae were collected from marl deposits in Aroostook County, Maine, USA (Nylander 1941, 1943); these fossils were virtually identical to recent forms. In 1891, the remains of a mastodon were discovered in Randolph County (North Carolina, USA), and a number of shells were collected from the associated mud. Not only were these Sphaeriidae contemporary with the mastodon, but the modern forms of this post-Pleistocene clam fauna were essentially unchanged in morphology (Martin, 1998).

Some species of Sphaeriidae are useful as indicators of lake trophic stages. Thus, *Sphaerium nitidum* and *Pisidium conventus* are indicators of oligotrophic lakes, *Sphaerium striatinum* of mesotrophic lakes, and *S. simile* and *Pisidium rotundatum* of eutrophic lakes (Clarke 1979). Sphaeriidae concentrate heavy metals in both their shells and soft parts and are therefore potentially useful as markers of metal and organic pollution (Wurtz 1955; Anderson 1977; Gadzała-Kopciuch et al., 2004; Alhejoj et al., 2017). The utility of Sphaeriidae as indicators of environmental disturbance, particularly diking and eutrophication conditions, was discussed by Fuller (1974).

Chapters II, III, IV present the work of two published and one ongoing papers:

Published:

Rassam, H., Albrecht, C., Sousa, R., Lopes-Lima, M., Benaissa, H., Ghamizi, M. 2021. Intraspecific variation in the common pea clam *Pisidium casertanum* (Poli, 1791) (Bivalvia: Sphaeriidae): A geometric morphometric analysis. *Malacologia*, 63, 183-194.

Rassam, H., Ghamizi, M., Benaissa, H., Clewing, C., Albrecht, C. 2021. The fingernail clams (Bivalvia: Veneroida: Sphaeriidae) of Morocco: Diversity, distribution and conservation status. Biodiversity Data Journal 9: e73346.

In preparation:

Rassam, H., et al. **in preparation**. Testing for Western Palearctic freshwater refugia in North Africa: evidence from bivalves (Sphaeriidae, *Pisidium*).

Chapter II: Material & Methods

I. Sampling and field surveys

The present thesis work took place in the nine Moroccan river basins (Moulouya, Loukkos, Sebou, Bouregreg, Oum Er Rabia, Tensift, Drâa-Ziz-Rhéris, Souss-Massa, Sakia El Hamra-Oued Eddahab) (Figure 8), between 2016 and 2019 at a monthly frequency, independent of seasonal calendars. Samples collection covered all types of freshwater habitats ranging from large rivers and lakes to small dayas and ephemeral ponds (e.g. Figure 7). Coverage of all aquatic environments, where Sphaeriidae may be present, was however limited by vehicle accessibility to them.

Figure 7. Examples of surveyed stations (lakes, rivers, springs, etc). 1- Tiferguine stream (Tensift), 2- Ait Allal spring (Oum Er Rabia), 3- Oued Iriri (Drâa), 4- Ait M'hamed cave (Oum Er Rabia), 5- Seguia in the region of Tighdouine (Tensift), 6- Tislit lake (Oum Er Rabia) (©Rassam, between 2017 and 2019).

Sampling was carried out in areas likely to shelter freshwater bivalves, particularly Sphaeriidae, called micro-habitats (muddy or sandy-silty sediments with slow currents, dead leaves and macrophytes), using a sapmling net or a household strainer with a mesh size not exceeding 200 µm in diameter.

The sampling effort was the same and the collected individuals (empty shell or living animal), were immediately and delicately, with an entomology forceps, put in tubes of 2 ml containing alcohol diluted to 80% of concentration with a label that contains the necessary information such as the date and place of collection. The sampling also included the collection of a quantity of sediment likely to hide the smallest individuals. The contents of the tube were well homogenized. The soft body of the animal is fragile and decomposes rapidly, and poor

homogenization will result in rapid decomposition of the individuals remaining at the bottom of the tube. Details of the localities where Sphaeriidae individuals were collected are shown in Table 6 in the Appendix. The distribution of each species is represented on chorological maps and the diversity per basin was mapped by grid analysis with an area of 50 x 50 km per grid.

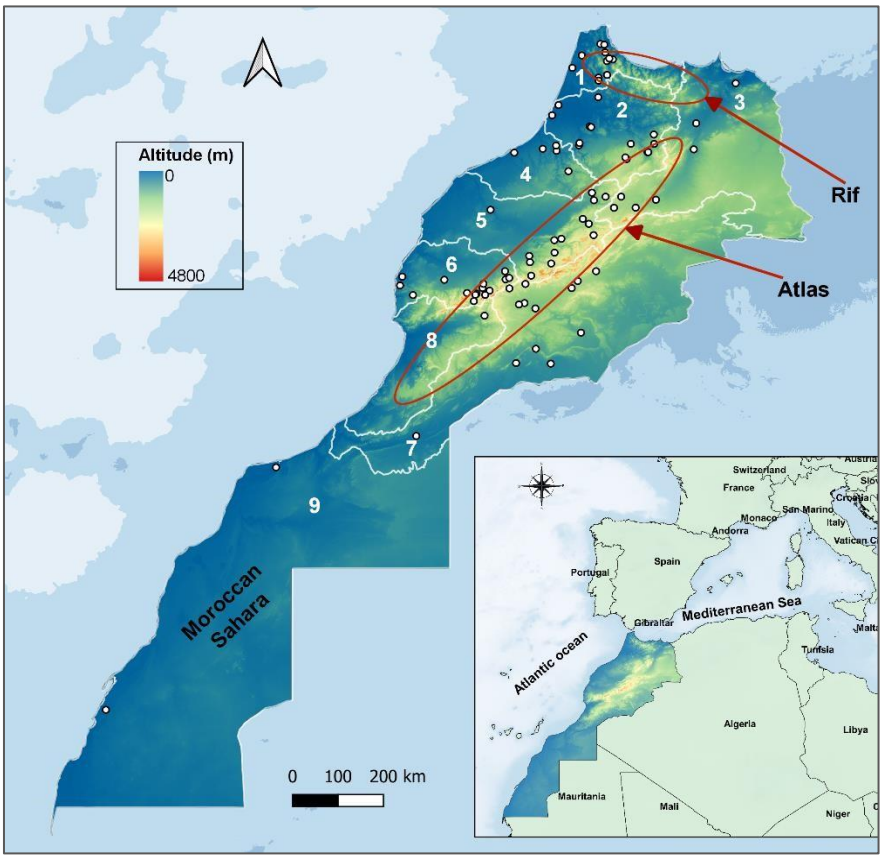


Figure 8. Map of Morocco with sampling stations marked with white dots and delineation of the nine surveyed watersheds. The watersheds are the following: 1- Loukkos, 2- Sebou, 3- Moulouya, 4- Bouregreg, 5- Oum Er Rabia, 6- Tensift, 7- Drâa-ZizRhéris, 8- Souss-Massa, 9- Sakia El Hamra-Oued Eddahab.

The measurements of the physico-chemical parameters were not carried out on all of the stations surveyed for technical reasons. Therefore, the results will be presented as an indication only and will not be taken into account in the analysis of the results. The parameters measured are: pH, conductivity, temperature and dissolved oxygen, using a multi-parameter probe (portable probe Hanna HI98194) in situ in the water column.

Figure 9. Distribution of *Pisidium* samples across the Western Palearctic. Red squares mark the successfully sequenced samples while the white squares are the samples that were not sequenced. Yellow squares represent the distribution of GenBank sequences.

For a phylogeographical study, the genus *Pisidium* was chosen as a taxonomic model because of its taxonomic richness and wide distribution throughout the Western Palearctic region. In addition to samples collected from Morocco, pre-collected material sent by other researchers or deposited in the UGSB collection (University of Giessen Systematics and Biodiversity) (Diehl et al., 2018) was used for this study. Although it was difficult to acquire *Pisidium* samples from localities throughout the Western Palearctic, especially in North Africa (e.g., sampling in Tunisia was not successful), the main bio-regions were covered (Figure 9).

II. In the laboratory

In the laboratory, the jars of sediment collected are sorted using brushes and flexible forceps under a binocular magnifying glass. The individuals of Sphaeriidae found are then put back in the tubes corresponding to the localities of origin or in new tubes in the case where the direct collection on site did not allow to find individuals.

For subsequent analyses, 10 individuals as a maximum number of random shapes and sizes are photographed from each locality using a binocular loupe connected to the computer (Leica M80 and Keyence VHX-2000). The soft part extracted from the shell of the photographed individuals is preserved in alcohol at -4°C for later molecular analysis.

III. Voucher specimens

In order to keep a verifiable record of the research data and the organisms studied, voucher specimens have been labeled with the following information: date, locality, collector(s) and possible comments. The vouchers (shells, DNA or images) are deposited in the Natural History Museum of Marrakech at Cadi Ayyad University and in the collection of the Department of Systematics and Biodiversity (UGSB; Diehl et al., 2018) to which identifying numbers have been assigned.

IV. Morpho-geometric analyses

2. General

Morphology is a critical source of information in the natural sciences, materials science and engineering. At the level of these three fields, the appearance of an object is characterized by two primary components: size and shape. Size is the physical scale of an object, often determined by comparing one or more of its spatial dimensions (e.g., length, width, diameter, depth, perimeter, volume) to a reference that serves as a basis for measurement. Shape is also often conceptualized by comparison of some reference (e.g. circle, triangle, average configuration point, average distance) and is operationally defined as the aspect component of the object that remains after differences in size, position, and rotation are eliminated (Kendall, 1977). The Chinese philosopher Mozi (470-390 B.C.) is the author of the earliest known description of a mathematical point, which he defined as the part of a line that cannot be divided into smaller parts (Needham, 1959). These concepts of point and separation are fundamental to morphometry where the locations of points and the ends of line segments are often called "landmarks" and their separations "distances" respectively.

Morphometric studies as a science have attracted many students and researchers with wide and diverse interests for the urgent need that quantification and visualization of shape gave to solve many problems related to different scientific research. It was in the early 70's (1971) that Blackith and Reyment published their book on multivariate morphometry, this subject was still new and its applications to solve biological problems were limited. The second edition of the book (co-authored with N.A. Campbell) appeared in 1984. Since 1984, the growth of interest and publications on applications of morphometric methods has been exponential (Adams et al., 2004, 2013).

Generally, two approaches are used for a morphometric analysis:

- The analysis of linear morphometry based on distances between landmarks.
- The analysis of geometric morphometry based on the landmarks themselves.

3. Linear morphometry analysis

Shells of random shapes and sizes (including right and left valves) were scanned with a Keyence VHX-2000 microscope connected to a computer (Figure 10) and used to perform linear morphometric analyses, with a maximum of 10 individuals (when possible) per species from each station. Shells were photographed closed (with the animal) and open (animal removed) to show internal characters. Photos were taken using a fine black sand background that served as a support for the shells and created a perfect contrast so that the individuals were clear. Eight linear morphometric distances of the shell were measured with tpsDig2 v. 2.27 software (Rohlf, 2017) following the method of Korniushin (2000) (Table 1, Figure 11-A).

Table 1. Measured linear morphometric variables.

Distance	Significance
L	Length
H	Height
LA	Length of the anterior part
LP	Length of the posterior part
LL	Length of ligament
LU	Length of the umbo
LH	Length of the hinge
HH	Height of the hinge
W	Width

These extracted distances were used to derive the following ratios: H/L, LA/LP, LL/L, LH/L and HH/H. A t-test for equality of means was performed to test the significance of the difference between these ratios across species using PAST v.3.23 software (Hammer, 2001).

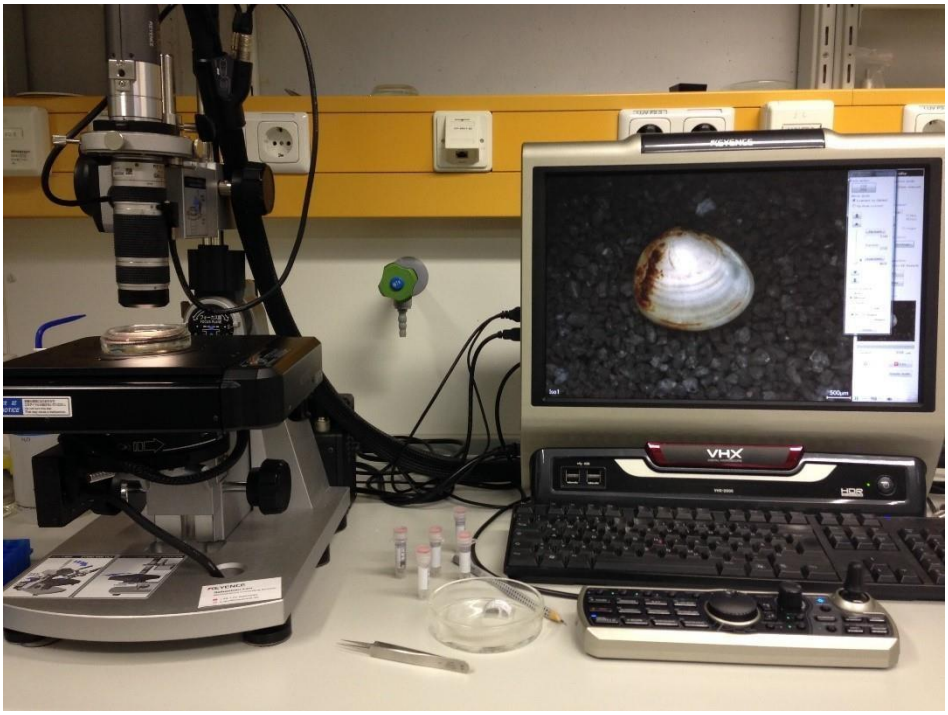


Figure 10. Taking pictures of the specimens with Keyence VHX-2000.

4. Analysis of geometric morphometry: the outline

To reduce any unpredictable source of variability, only the straight valve of each sample was used. For the outline analysis and in the absence of homologous "landmarks", 60 "semilandmarks" were digitized on the shell outline with tpsDig2 v. 2.27 (Rohlf, 2017) to correctly define the shape of the valves (Figure 11-B). These semi landmarks were located using a virtual grid that was overlaid on each image. The grid position was defined based on the homologous structure of the specimens; in this case the most extreme point of the umbo. The semi landmarks were therefore applied to the points corresponding to the intersection between the grid and the shell.

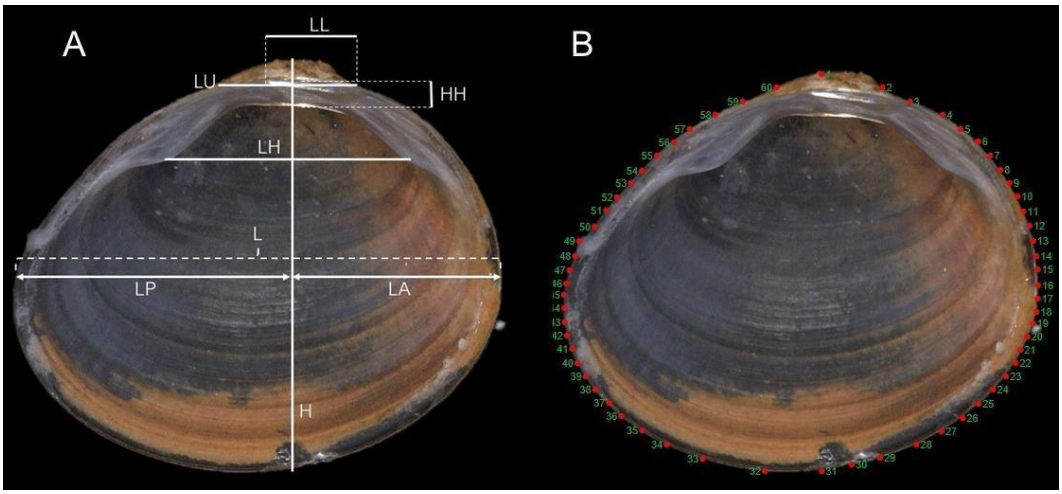


Figure 11. Morpho-geometric variables used from the shell of the species. A- Linear morphometric measurements of the different parameters. B- Outline analysis.

Locating the semi landmarks on the shells with tpsDig2 yielded a series of X and Y Cartesian coordinates that contained the shape and size information of each specimen. Elliptic Fourier Analysis (EFA) was performed using the 60 contour coordinates. The coordinates were normalized with overlay by the Procrustes generalized analysis to remove variations related to size, position, and orientation of individuals. This eliminated any variation not directly related to shape and generated a new set of residual coordinates that were used in the analysis. Significant differences between the contours of different species were tested with a Multivariate Analysis of Variance (MANOVA). Principal Component Analysis (PCA) was applied to the EFA coefficients to visualize differences in shell contours. All contour analyses were performed with PAST software. Differences in mean shell contour lines between specimens were shown using the tpsRelw v. 1.70 consensus method (Rohlf, 2019) to obtain a mean species shape. This analysis was not applied to *P. amnicum* and *M. lacustre* species as the number of individuals was far below the minimum required.

V. Diversity data processing

To identify the spatial distribution of Sphaeriidae in the Moroccan basins, the georeferenced records were projected on the map using QGIS software v. 3.4.1 (2018). “Between basins” comparison of the species composition was implemented using Jaccard’s Similarity Index, working with presence-absence data using the following expression:

$$Cj= a/(a+b+c),$$

where a is the number of species shared between the two compared sites, b and c are the number of species exclusive to site 1 and site 2, respectively. The index value goes from 0 (no similarity) to 1 (identical). A dendrogram was plotted using PAST v.4.06 (Hammer et al. 2001), showing the relationship between the different basins, based on the similarity of species.

VI. Genetic analysis

1. Deoxyribonucleic acid (DNA) extraction

Soft body DNA from 1-3 individuals per site was isolated and preserved using the CTAB cetyl trimethylammonium bromide protocol (Wilke et al., 2006). The protocol is described as follows:

o The first step

Specimens are dried from the alcohol where they were kept with a paper towel and moved to 0.5 ml tubes (one specimen per tube). 300 µl of a cold buffer solution is added to each tube in which the specimens remain trumpeted for 3 to 5 minutes on a frozen stand. This operation allows the elimination of the alcohol in which they were previously preserved. After 5 minutes,

the specimens are removed and dried and placed in new labeled tubes containing 200 µl of cell lysis buffer and 3 µl of Proteinase K (20 µg/µl) for protein digestion. The tubes are placed in a water bath at a temperature between 55 and 60 °C for at least 3 h (preferably overnight).

o The second step

The tubes are recovered from the water bath and centrifuged rapidly (8 s). To each tube are added 35 µl of 5M NaCl and 35 µl of a 5% CTAB/NaCl solution for cell lysis and polysaccharide removal. The tubes are then mixed gently and centrifuged rapidly and placed in a fume hood where 270 µl of chloroform is added to each tube before mixing gently for about 3 minutes and then spun in the centrifuge at 9000 rpm for 5 minutes to separate the two phases. The upper aqueous phase contains the extracted DNA while the lower phase contains the cellular debris and tissue.

The aqueous phase is collected into new tubes and 270 µl of the CTAB precipitation buffer is added to it. The tubes are mixed well and left to stand for 45 min at room temperature.

Subsequently, the tubes are put in the centrifuge and spun for 10 min at 12,000 rpm before being emptied of the liquid to keep only the precipitated pellet at the bottom. The pellet is redisolved with a solution of 1M NaCl/TE (Tris-EDTA) (100µl/tube) and RNase (100mg/ml) (1µl/tube) to remove any RNA contamination. Tubes are mixed and incubated for 5-10 minutes at 65 °C (Figure 12). 250 µl of cold 100% ethanol is added per tube. The tubes are mixed gently and allowed to precipitate for 3 h (preferably overnight) at -20 °C.

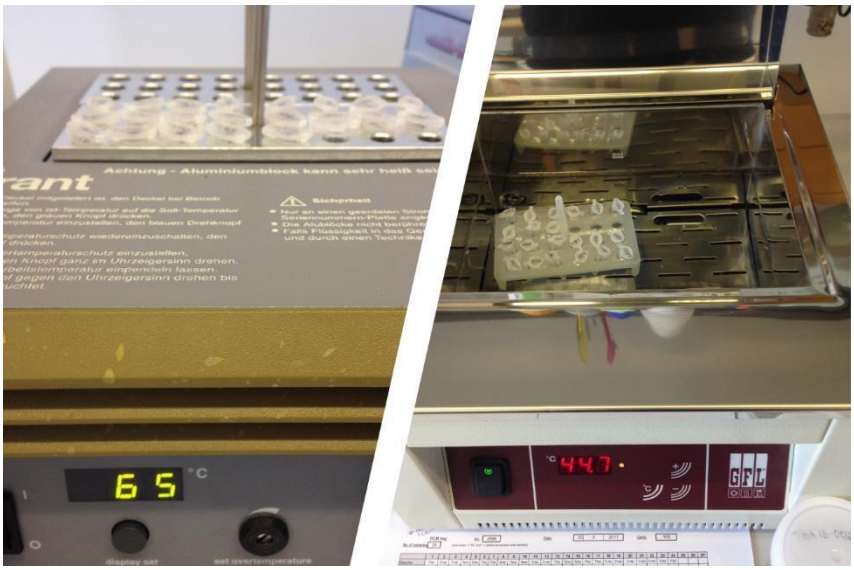


Figure 12. DNA extraction in the laboratory. Right: Preparation of samples for water bath; left: Incubation of samples at 65°C.

o The 3rd and final step

The tubes are put for centrifugation for 15 min at 12,000 rpm before emptying their contents. The pellet remaining at the bottom of the tubes, consisting of the extracted DNA, is cleaned from the previously added solutions by adding 300 µl per tube of cold 70% ethanol and the tubes are mixed for 10 seconds and put back for centrifugation for 5 min at a speed of 12,000 rpm, then the solution is poured and the same operation is repeated. After pouring the solution, the tubes are centrifuged again at 8000 rpm to collect the remaining droplets of the solution for removal by pipetting. The tubes are finally left to dry open for 5-10 minutes and the resulting DNA pellet is re-dissolved with double distilled water (25-100 µl/tube).

Using the Thermo Scientific NANODROP 2000 spectrophotometer connected to the computer, the concentration of DNA in each tube in 1 µl is measured. The results are automatically saved in Excel format in the computer in ng/µl under a wavelength of 260/280. The DNA is diluted in each tube with 40 µl of distilled water and 1-3 µl are pipetted to new PCR tubes.

2. Gene amplification by Polymerase Chain Reaction (PCR)

o *Principle*

Sometimes referred to as "molecular photocopying", Polymerase Chain Reaction (PCR) is a rapid and inexpensive technique used to "amplify" (copy) small segments of DNA. Because large quantities of a DNA sample are required for molecular and genetic analyses, studies of single pieces of DNA are almost impossible without PCR amplification. Often touted as one of the most important scientific advances in molecular biology, PCR has revolutionized the study of DNA to the point that its creator, Kary B. Mullis, was awarded the Nobel Prize in Chemistry in 1993.

To amplify a segment of DNA using PCR, the sample is first heated so that the DNA denatures, or splits into two pieces of single-stranded DNA. Then an enzyme called "Taq Polymerase" synthesizes two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Each of these strands can then be used to create two new copies, and so on. The cycle of denaturing and synthesizing new DNA is repeated up to 30 or 40 times, resulting in over a billion exact copies of the original DNA segment.

The entire PCR cycling process is automated and can be completed in just a few hours. It is driven by a machine called a thermal cycler, which is programmed to change the temperature of the reaction every few minutes to allow denaturation and DNA synthesis.

o *PCR process*

The thermal cycler used in the present work is the Eppendorf Mastercycler proS. In an ice-cold holder, the following mixture is prepared for a volume of 20 µl per tube:

▪ 2 µl 10 x Buffer (Thermopol Buffer): provides a suitable chemical environment for DNA polymerase activity.

▪ 1.4 µl of the dNTPs (each 2.5 mM): (deoxyribose nucleotide triphosphate) they serve as "building blocks" from which the DNA polymerase can synthesize new strands.

▪ 1.4 µl of the direct primer (10 mM)

▪ 1.4 µl of the reverse primer (10 mM)

▪ 11 µl of double distilled water

▪ 1.4 µl MgCl2 (50 mM): an essential cofactor that enhances DNA Taq Polymerase activity.

▪ 0.2 µl TMAC (0.5 M): (Tetramethyl Ammonium Chloride) increases the specificity of hybridization and raises the melting temperature.

▪ 1.2 µl BSA (10 mg/ml): (Bovine Serum Albumin) it is useful to control contaminants such as phenolic compounds. It also prevents reaction components from adhering to tube walls.

▪ 0.4 µl of Taq Polymerase (5 U/ml)

Approximately 18 µl of the mixture is added to each tube along with a negative control tube without DNA and the tubes then go into the thermal cycler. The polymerization chains proceed in three steps:

▪ **Denaturation**: or separation, of the two strands of the DNA molecule by the effect of temperature. This is done by heating the starting material to temperatures of about 95°C. Each strand is a template on which a new strand is built.

▪ **Annealing**: the temperature is reduced to about 50-60 °C so that the primers can hybridize to their complementary sequences on the template.

▪ **Elongation**: the temperature is raised to about 72 °C, and the DNA polymerase begins to add nucleotides to the ends of the hybridized primers, synthesizing new DNA strands.

At the end of the cycle, which lasts about five minutes, the temperature is raised and the process begins again. The PCR conditions are presented in the table below.

Table 2. PCR conditions for the amplification of 16S, COI, H3 and 28S genes.

Step	Times	Temperature (°C)/Duration	Phase
1	1x	95 (1 mm)	Denaturation
2	34x	95 (30 s)	

3	1x	52 (30 s)	Annealing
4	1x	72 (3 mm)	Elongation

o Electrophoretic revelation

The quality of the fragments resulting from the PCR amplification were tested by electrophoresis on 1% agarose gel. 1 µl of each sample is mixed with 1 µl of loading dye (Bromophenol blue based). The dye will allow the sinking of the sample in the well and the visualization of the migration by fluorescence. Each well on the gel then contained the samples and the negative control mixed with the dye and one well contained only the dye to serve as a fragment length scale. Successful samples show fluorescence under UV light in contrast to unamplified samples.

o Genes selection

The molecular analysis in this thesis was based on the amplification of four genes; two mitochondrial genes (16S and Cytochrome c Oxidase subunit I COI) and two nucleic genes (28S and Histone 3 H3). The 16S rRNA gene encodes the ribosomal RNA molecules of the small subunit of ribosomes, responsible for the essential process of converting genetic messages into functional cellular components via translation of mRNA into proteins. As such, ribosomal RNA is a component of all self-replicating systems; it is easily isolated and its sequence changes slowly over time, allowing the detection of relatedness between widely separated species. Sequence analysis of the 16S rRNA gene and structural modeling of 16S rRNA have revealed that the gene sequence contains multiple conserved and variable regions (Woese and Fox, 1977; Byrne et al., 2018). Cytochrome c oxidase is a mitochondrial protein, located in the inner mitochondrial membrane, and is a key enzyme in the electron transport chain. Therefore, it plays a central role in the metabolism of eukaryotic aerobic organisms. It is composed of several subunits, and the cytochrome c oxidase catalytic subunit I is encoded in the mitochondrial genome. COI has a higher rate of evolution than nucleus-encoded genes and should be better suited for discrimination between closely related taxa (Hebert et al., 2003). COI, however, is one of the most conserved mitochondrial protein-coding genes in animals (Mueller, 2006), and thus has a better phylogenetic signal (Hebert et al., 2003).

In contrast, the use of nucleic genes such as histone 3 H3 (one of the five major histone proteins located in the nucleus of eukaryotic cells and involved in the formation of the basic structure of chromatin) and 28S (28S ribosomal RNA being the major rRNA constituting the large 60S subunit of eukaryotic ribosomes), is less popular for identification at a lower scale (species, genus) because of their conserved character which allows identification at a wider taxonomic scale (Dabert et al. , 2011; Hu et al., 2019). However, these two genes have been

widely used in genetic work on the family Sphaeriidae (Park and O Foighil, 2000; Taylor et al., 2007; Clewing et al., 2013; Bößneck et al., 2016; Clewing et al., 2022). The availability of sequences for these genes in the GenBank database also accentuated the choice.

The list of primers used for each gene with their sequences and references is presented below:

Table 3. List of genes used with the corresponding sequences for each primer.

Genes	Primers	Sequences	References
16S	16SF (F)	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
	16SR (R)	CCGGTCTGAACTCAGATCACGT	
COI	COIF14 (F)	TTGTTCAACAAAAATATAAAGA	Folmer et al. (1994)
	COI722B (R)	TAAACTTCAGGGTGACCAAAAAATYA	Wilke et Davis (2000)
	LCO (F)	GCTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
	HCO (R)	TAAACTTCAGGGTGACCAAAAAATCA	
28S	28SD23F (F)	GAGAGTTCAAGAGTACGTG	Park & O' Foighil (2000)
	28S D6R (R)	CCAGCTATCCTGAGGGAAACTTCG	
H3	H3F (F)	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (2000)
	H3R (R)	ATATCCTTRGGCATRATRGTGAC	

3. DNA sequencing

The DNA from the samples that gave clear bands in electrophoresis and therefore was well amplified, was sent to an external company (LGC Ltd, Berlin, Germany) for sequencing with the ABI 3730 XL sequencer with the Big Dye Terminator kit (Life Technologies). The sequencing was performed using the Sanger method.

DNA sequencing is the process of determining the sequence of nucleotide bases (As, Ts, Cs and Gs) in a piece of DNA. The Sanger method was developed by the British biochemist Fred Sanger and his colleagues in 1977. The components needed for Sanger sequencing are: a DNA polymerase enzyme; a primer; the four DNA nucleotides (dNTPs); the template DNA to be sequenced; and dideoxy versions of the four nucleotides (ddNTPs), each labeled with a different dye color.

Dideoxy nucleotides are similar to regular, or deoxy, nucleotides with one difference: they do not have a hydroxyl group on the 3' carbon of the ribose ring. In an ordinary nucleotide, the

hydroxyl group at 3' acts as a "hook", allowing a new nucleotide to be added to an existing chain (Figure 13).

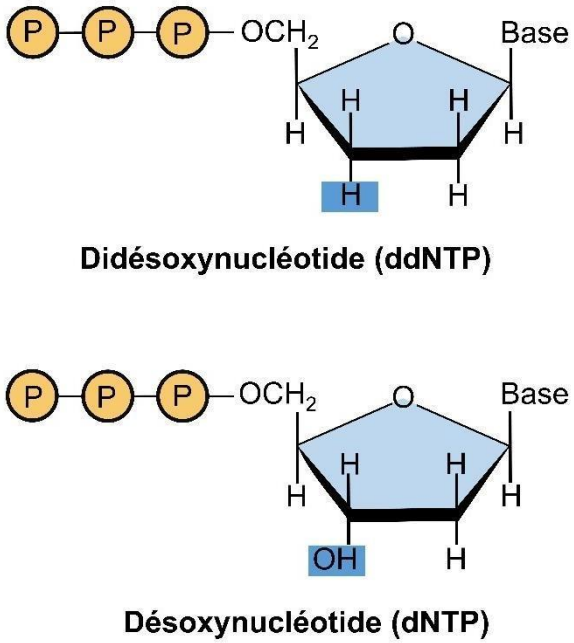


Figure 13. Structural difference between dNTPs and ddNTPs.

The DNA sample to be sequenced is combined in a tube with a primer, DNA polymerase and dNTPs. The four dye-labeled ddNTPs are also added but in much smaller amounts than regular nucleotides.

The mixture is first heated to denature the template DNA, then cooled so that the primer can bind to the single-stranded template. Once the primer has bound, the temperature is raised again, allowing the DNA polymerase to synthesize new DNA from the primer. The DNA polymerase continues to add nucleotides to the chain until it adds a dideoxy nucleotide. At this point, no more nucleotides can be added, so the strand ends with the dideoxy nucleotide. This process is repeated in a number of cycles.

Once the reaction is complete, the fragments are passed through a long, thin tube containing a gel matrix for capillary gel electrophoresis. As each fragment crosses the "finish line" at the end of the tube, it is illuminated by a laser, allowing detection of the dye attached to it. Thus, from the dye colors recorded one after the other on the detector, the sequence of the original piece of DNA can be reconstructed nucleotide by nucleotide. The data recorded by the detector consists of a series of fluorescence intensity peaks. The DNA sequence is read from the peaks of the chromatogram.

4. Sequence processing

o Sequence editing

The analysis of the chromatograms and the editing of the resulting sequences were performed on BioEdit v. 7.2.5 (Hall, 1999). This essential step consists of removing the primers and correcting any errors at the nucleotide level based on the chromatograms obtained from both direct and reverse primers. The sequencing result is presented as two DNA sequences of the same sample with the two primers used. The first step is the creation of a consensus sequence. Errors at the nucleotide level may occur as a result of the combination of the two sequences and the correction of these errors relies on the identification of the real identity of the nucleotide to be corrected. This is deduced by comparing the peaks in the chromatograms to the position of the nucleotide in question and where the peak is well presented, the correct nucleotide is identified (Figure 14).

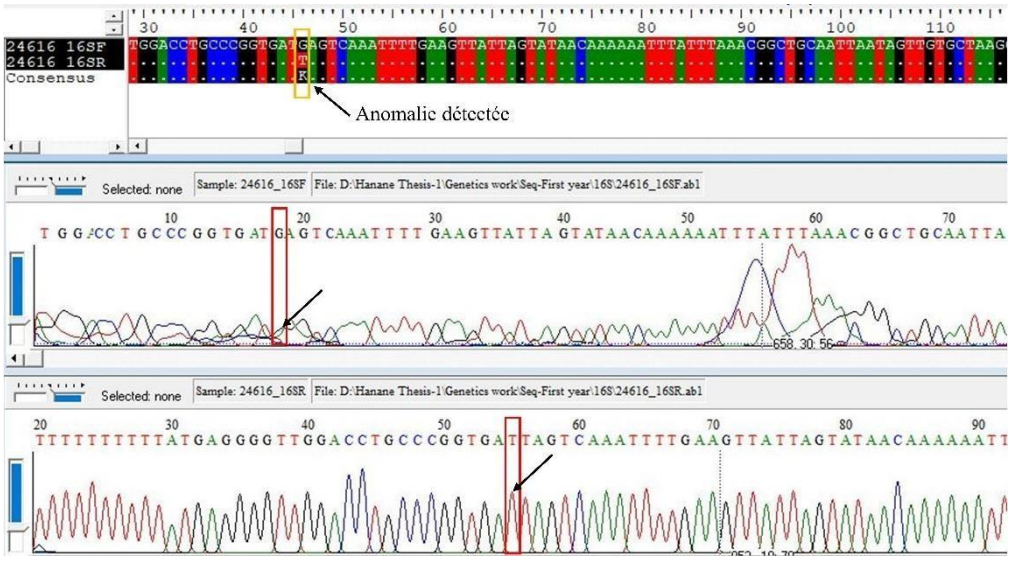


Figure 14. Sequence editing (combination of direct and reverse sequences) on BioEdit. The red rectangle refers to an erroneous nucleotide and in this case we refer to the peak on the chromatogram.

The sequences of the direct and reverse primers must be eliminated to keep only the sequence of the sample. To do this, the previously known sequences of the primers are identified towards the beginning (direct primer) and the end of the sequence (reverse primer). The reverse primer must be searched in reverse nucleotide order.

The resulting sequences were then analyzed with the BLAST tool in the GenBank database of the US Center for Biotechnology Information "NCBI" to check the most similar sequences available and confirm the species identification. Subsequently, the edited sequences were pooled in MEGA7 software (Tamura et al. 2013) for alignment.

o *Alignment*

Sequence alignment refers to the correspondence between its nucleotides. It allows the comparison of nucleotides that are actually comparable, this step allows the highlighting of possible substitution, insertion and/or deletion phenomena in the sequences. In the case of

this thesis, the alignment was done using a heuristic algorithm that favors the stable areas of the sequences and allows to obtain a reasonable first alignment (ClustalW implemented in MEGA7 software as well as the online alignment tool MAFTT v. 7.402 (Katoh and Standley, 2013) for the alignment of the 16S non-coding gene fragment), then the alignment was refined with some manual modifications (Figure 15). The final alignments had the following length: 608 bp for COI, 516 bp for 16S, 743 bp for 28S, and 328 bp for H3, Phylogenetic relationships were concatenated and estimated for the four genes used.

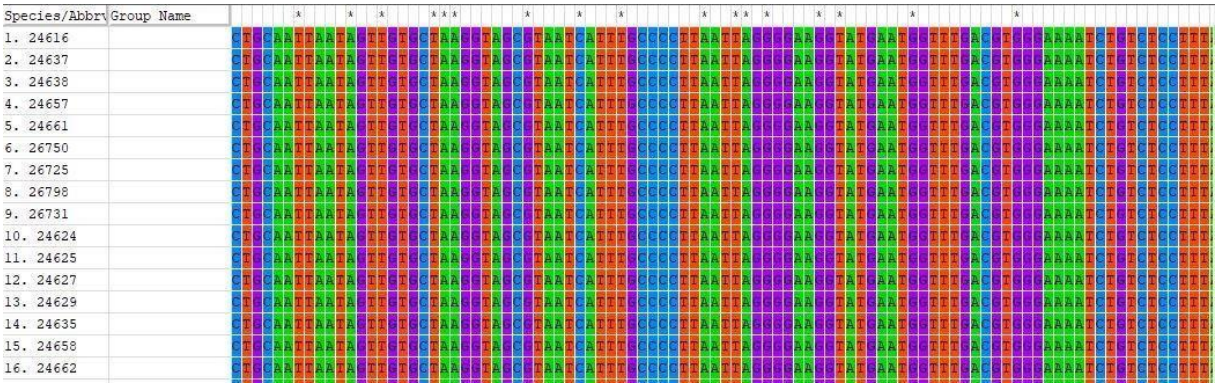


Figure 15. Sequence alignment of the samples with the MEGA7 software.

Additional sequences from the family Sphaeriidae of the four genes used (when available) were added to the analysis from either the GenBank database or from DNA of old or newly isolated samples from the Sphaeriidae collection of the Department of Systematics and Biodiversity of the University of Giessen. The list of samples with details of their origins is detailed in Table 6 in the Appendix.

o *Genetic inference and estimation of temporal divergence*

A crucial step precedes the genetic inference; the choice of the correct surrogate model. The program jModelTest v2.1.4 (Darriba et al., 2012) was used to identify the best-fitting sequence evolution model based on the corrected Akaike information criterion (AICc) to test model quality. For our dataset, the GTR (general time-reversible model) +I (Invariant Sites) model was selected for the 16S gene while HKY (model described by Hasegawa, Kishino, and Yano (1985)) +I was chosen for the remaining three genes.

Bayesian inference (BI), developed on MrBayes v3.2.2 (Ronquist et al., 2012), was performed for the reconstruction of phylogenetic relationships with four independent simulations using the standard "Markov Chain Monte Carlo" (MCMC) algorithm, searching for 100,000 generations, with sampling every 100 generations and a burn-in of 25%. The final result of the topologies is visualized with FigTree v. 1.3.1 (Rambaut, 2010).

Divergence times were estimated using BEAST V1.8.4 (Drummond et al., 2012) on the CIPRES Science Gateway web portal (Miller et al., 2010),with two fossil calibration points

implemented. Three criteria were considered for the selection of sphaeriid fossils; i) at least three genetic markers represented the respective species; ii) monophyly of the respective species; and iii) the sister-group relationship was well supported. The published phylogenies of Clewing et al. (2013) and Bößneck et al. (2016) were used for the latter two criteria. The two selected fossils are the following: 1) *M. lacustre* was used to calibrate the most recent common ancestor (MRCA) of *M. lacustre* and *M. kashmirensis*, with an age estimated between 3.0 and 3.2 Ma (Nordsieck, 1982) (record from Kuiper (1972)); 2) *P. amnicum* was used to calibrate the MRCA of *P. amnicum* and *P. dilatatum*, with an estimated age of 11.5 – 12.7 Ma (Schneider and Prieto, 2011). Each analysis was performed in four replicates with the settings as follows: substitution and clock models unlinked, monophyly of ingroup monophyly, speciation = birth–death process; calibration point 1 (split of *M. lacustre* and *M. kashmirensis*) with a gamma distribution prior: offset = 3.0, scale = 0.07; calibration point 2 (split of *P. amnicum* and *P. dilatatum*) with a gamma distribution prior: offset = 11.5, scale = 0.4; uniform prior for substitution rates; number of MCMC generations = 40,000,000; sample frequency = 2,000. Two analyses were performed using either the strict-clock model or the uncorrelated lognormal relaxed-clock model. Log and tree files were combined with LogCombiner v. 1.8.4 and summarized in TreeAnnotator v. 1.8.4. Tracer v. 1.5 (Rambaut and Drummond, 2007) was used to display the Effective Sample Size (ESS) values for the combined log files.

o Analysis of the specific delimitation

Specific delimitation was analyzed by the non-parametric method with the "Poisson Tree Process" (PTP) model, which identifies the taxonomic status of species based on the distribution of branch lengths in a phylogenetic tree (Zhang et al., 2013).

PTP uses a rooted non-ultrametric tree so that it does not depend on the molecular clock, which can be severely impaired for widely separated species. The approach implicitly assumes reciprocal monophyly in the gene tree and a perfect match between the gene tree and the species tree. Thus, this method should be most effective in identifying species separated by long intervals between speciation events and with small populations.

The number of species within the genus *Pisidium* is not precisely known. Identification based on morphological features is useful for species determination but still not accurate due to the high intraspecific variability and the cryptic aspect of certain species. Therefore, several preliminary analyses were carried out in order to identify Molecular Operational Taxonomic Units (MOTUs) that represent *Pisidium* species-level taxa. Further, we applied two tree topology based species delimitation methods to delineate molecular operational taxonomic units (MOTUs), the Bayesian Generalized Mixed Yule Coalescent (bGMYC) model (Reid and Carstens, 2012) and the Bayesian Poisson Tree Process (bPTP: PTP-ML and PTP-BI) model

(Zhang et al., 2013). All analyses were done on the species delimitation web server of the Exelixis Lab (https://species.h-its.org). For both analyses, the BEAST tree was used as input file, and a bPTP analysis was run using the following settings: tree = rooted; number of generations = 100,000; thinning = 100; burnin = 10%. The program TCS v. 1.21 (Clement et al., 2000) was used to perform a statistical parsimony network analysis for *P. personatum,* for which several operational taxonomic units (OTUs) were identified, based on 16S dataset with a connection limit of 95%.

VII. Evaluation of the conservation status

1. Overview

Many factors are used to assess the conservation status of a species, including: the number of individuals remaining, overall population increase or decrease over time, reproductive success rates, and known threats.

There are various global systems for recognizing conservation status, the most widely applied of which is the IUCN Red List system. To date, more than 93,500 species have been assessed for the IUCN Red List. Created in 1964, the IUCN Red List of Threatened Species has become the world's most comprehensive source of information on the conservation status of animal, plant and fungal species.

It is a global conservation status listing and ranking system. It divides species into nine categories, of which the official term "threatened" is a subset of three categories: Critically Endangered, Endangered, and Vulnerable:

- Extinct (EX)
- Extinct in the Wild (EW)
- Critically Endangered (CR)
- Endangered (EN)
- Vulnerable (VU)
- Near Threatened (NT)
- Least Concern (LC)
- Data Deficient (DD)
- Not Evaluated (NE)

Evaluation by the IUCN system relies on the analysis of certain ranking criteria to decide the status to be assigned to the evaluated taxa.

2. Conservation Status Assessment

The geographic range assessment (criterion B) was used to evaluate the Red List category of Sphaeriidae in Morocco based on the IUCN regional guidelines (IUCN, 2012). Extent of occurrence (EOO) and area of occupancy (AOO) are respectively defined according to IUCN (2001, 2012) as "the area contained within the shortest continuous imaginary boundary that can be drawn to encompass all known sites, inferred or projected from the current occurrence of a taxon, excluding cases of vagrancy," while AOO is "the area within its 'range of occurrence' that is occupied by a taxon, excluding cases of vagrancy." EOO and AOO were calculated for all species based on occurrence points, using the ConR package on R (Dauby et al., 2017), with a 2 km grid as the IUCN default parameters. AOO was obtained based on a 100 m buffer along the sample sites. The AOO and EOO values were then used, in addition to the number of locations the continued decline and/or extreme fluctuations in the sub-criteria to assess the conservation status of each Sphaeriidae species in Morocco.

Chapter III: Results

I. Species diversity

During the work period (2016-2019), 164 sites were sampled throughout Morocco. Of the 164, 56 sites contained species of the family Sphaeriidae. The study of the collected individuals and their identification based on morphological characters and genetic analyses revealed the presence of a total of six species of Sphaeriidae living in freshwater basins of Morocco, represented by two genera: *Pisidium* with five species (*P. casertanum*, *P.* (cf.) *personatum*, *P. subtruncatum*, *P. amnicum*, and *Pisidium* sp.) and *Musculium* with a single species (*M. lacustre*).

Pisidium (cf.) *personatum* refers to specimens that are very similar to *P. personatum*, but need to be confirmed by further analysis. They both share the same morphological characters; however, they formed distinct, though closely related, genetic clades. *Pisidium* sp. refers, on the other hand, to specimens that showed clear genetic differences from the other species and, therefore, cannot be given a specific name. Genetic data (Rassam et al., submitted) revealed that this clade differs from all other species described for Morocco, suggesting that it may be a different and potentially new species.

Species identification was based primarily on morphological characters observed under magnifying glass; these observations were compared to identification keys presented by Adam (1960), Korniushin et al. (2000) and Killeen et al. (2004). The identification was subsequently confirmed by the results of the genetic sequences which were compared to the sequences available in the Genbank database with the BLAST tool.

The most frequently collected species, and thus the most abundant, was *P. casertanum* (62%) followed by *P.* (cf.) *personatum* (23%), then *P. subtruncatum* (13%). In contrast, *P. amnicum* was the rarest species of the genus *Pisidium*, similar to the species *Musculium lacustre*, the only representative of the genus in Morocco. Local diversity at each station is low with at most 2 species of Sphaeriidae co-existing at the same locality.

II. Distribution and spatial diversity

1. Altitudinal gradient

Across Morocco, individuals of the family Sphaeriidae were found in sites of medium to high altitude (between 462 and 3137 m). The species *P. casertanum* showed the broadest altitudinal spectrum (ranging from the lower to the upper limit of occurrence of the Sphaeriidae) and the highest altitude populations, compared to *P.* (cf.) *personatum* and *P. subtruncatum*. *Pisidium amnicum* and *M. lacustre*, on the other hand, are present in only one locality with the same altitude (1663 m). A montane chorotype was deduced from the altitudinal distribution of

the species, extending along the Moroccan Atlas range with a few points in the Rifan mountains chain.

2. Distribution patterns

Running water ecosystems (rivers, canals and springs) were the richest in terms of individuals harboring the greatest number of specimens, followed by lakes and marshes where the number of individuals is minimal (2 individuals for the case of *M. lacustre*) (see Table 4). Specifically, riparian habitats represented the ecosystems most frequently inhabited by Sphaeriidae (35% of total habitats), followed by springs with 30%. Marshes were the least represented with only 2% (Figure 16).

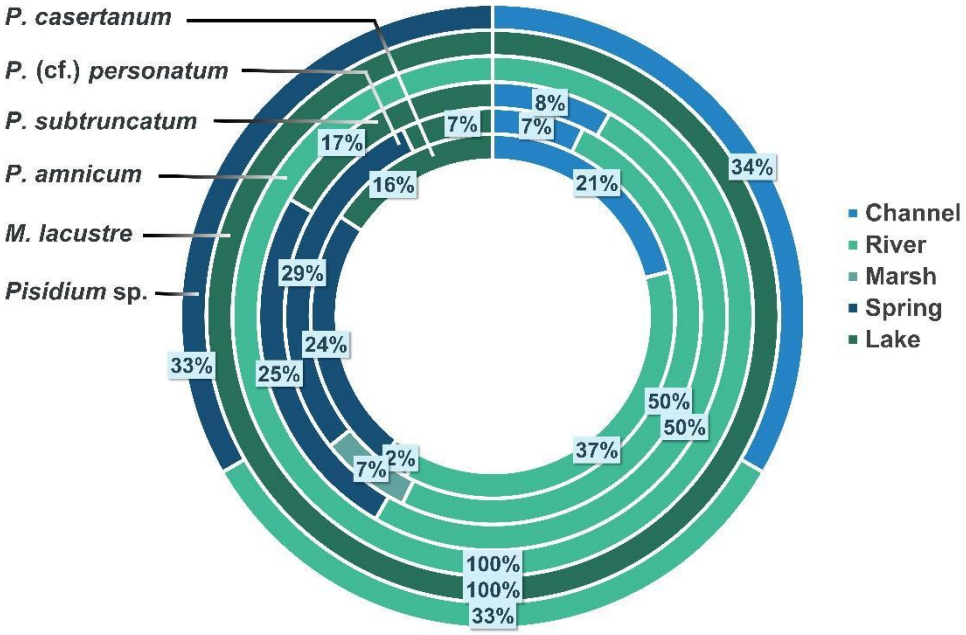


Figure 16. Frequency of species of Sphaeriidae by habitat type in Morocco (n = 56 localities).

The species diversity of the family Sphaeriidae showed a strong correlation with species distribution. *Pisidium casertanum* was the most present species in all habitat types, followed by *P.* (cf.) *personatum* and *P. subtruncatum*. *Pisidium amnicum* and *M. lacustre* ranked last, appearing in only one locality.

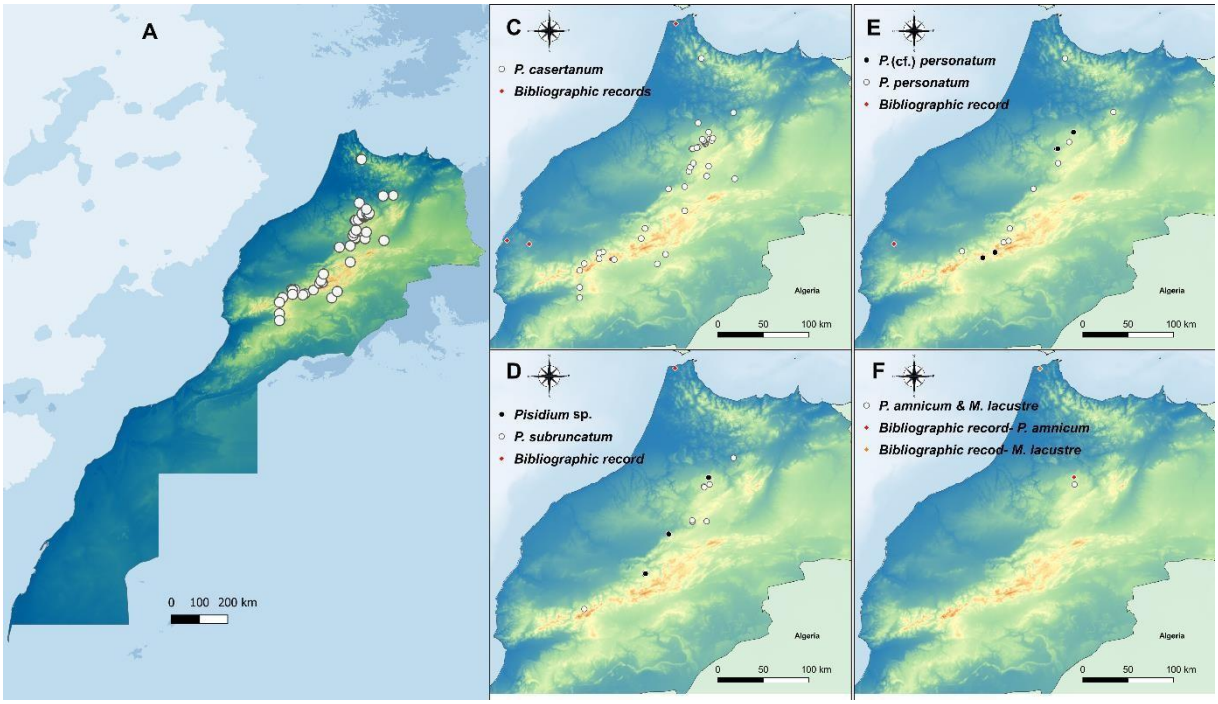


Figure 17. Distribution of Sphaeriidae in Morocco (A). C -F Distribution maps of all five species (*M. lacustre* and *P. amnicum* occur in the same locality) across the country with their respective bibliographic records (marked with rhombi), white circles represent the sampling sites with no specimens found.

At the watershed level, the distribution of species was uneven (Figure 17). The Sebou basin was the richest basin in terms of species with 32% or all six species found in Morocco, followed by the Oum Er Rabia and Tensift basins with 18% of species, while Souss-Massa and Moulouya had the lowest number of species with only one species found. The Bouregreg and Sakia El Hamra-Oued Eddahab basins were exceptional since no species of Sphaeriidae were found during the field surveys. *Pisidium casertanum* was found in all basins except Bouregreg and Sakia El Hamra- Oued Eddahab. *Pisidium* (cf.) *personatum* was collected in five basins: Loukkos, Tensift, Sebou, Souss-Massa and Oum Er Rabia. *Pisidium subtruncatum* was recorded in four basins: Tensift, Sebou, Moulouya and Oum Er Rabia. *Pisidium* sp. was present in both the Oum Er Rabia and Sebou basins, while *P. amnicum* and *M. lacustre* were only present in the Sebou basin (Figure 18).

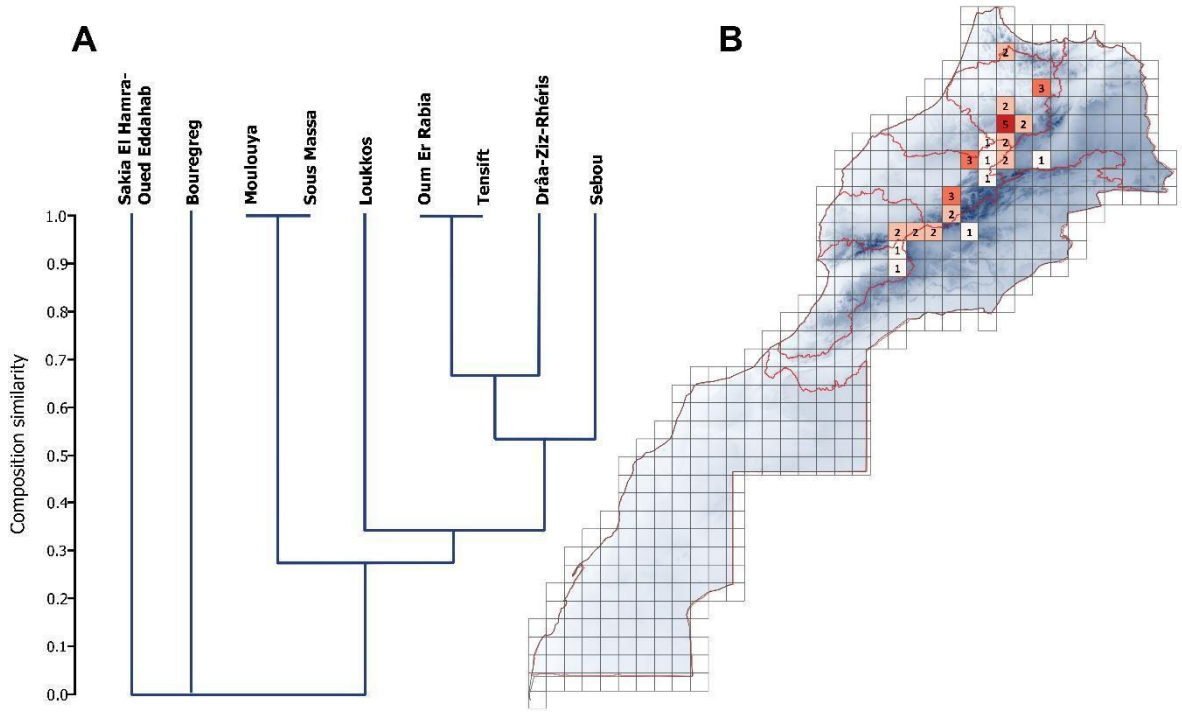


Figure 18. Species richness by basin. A- Dendrogram of species composition by basin using Jaccard's Similarity Index. B- Grid map 50 x 50 km showing the geographical distribution of species by basin.

Grid analysis of the distribution of Sphaeriidae in Morocco showed that only about 5% of the total grid cells contain points of occurrence of Sphaeriidae (i.e. 21). Of these 21 cells, 33% contain at least one species, 38% contain 2 species, and 9.5% contain 3 to 4 species, while 4.7% (one cell) contain five species combined.

Table 4. Summary of the α, β and ɣ diversity of the family Sphaeriidae in Morocco.

	River	**Lake**	**Spring**	**Marsh**	**Channel**
P. casertanum	x	x	x	x	x
P. subtruncatum	x	x	x		x
P. personatum	x	x	x	x	x
P. amnicum	x				
M. lacustre		x			
α diversity	4	4	3	2	3
β diversity	Rv/Lk: 2 Rv/Sp: 1	Lk/Sp: 1 Lk/Mr: 2	Sp/Mr: 1 Sp/Ch: 0	Mr/Ch: 1 Mr/Rv: 2	Ch/Rv: 1 Ch/Lk: 1
ɣ diversity			6		

III. Morpho-geometry of Sphaeriidae

Morphological diagnosis and morphometric analysis of the sampled specimens were of great help, as they allowed the identification and discrimination of species based on the different morphometric ratios calculated as shown in Figure 19. The result of the PCA showed two distinct morpho-groups; the first including the species *P. subtruncatum* and the second including *P.* (cf.) *personatum* and *P. casertanum*. A clear difference was revealed between *P. subtruncatum* and the other two species.

The most discriminating ratios that allowed a clear separation of *P. subtruncatum* were LL/L and LA/LP (defining, respectively, the size of the ligament pit and the overall shape of the shell), whereas *P. casertanum* and *P.* (cf.) *personatum* are somewhat confused (Fig. 19-A) and no ratio allowed a discrimination between the two species. The 61 plotted semi-locations generated an overall shell contour for all three species. The differences between the average contours of the three species are shown in Figure 19-B. The greatest differences observed are in the umbo and anterior ventral margin, i.e., *P.* (cf.) *personatum* has a rounded shape and flattened umbo, whereas *P. subtruncatum* has a posteriorly oriented umbo and a relatively elongated anterior ventral margin.

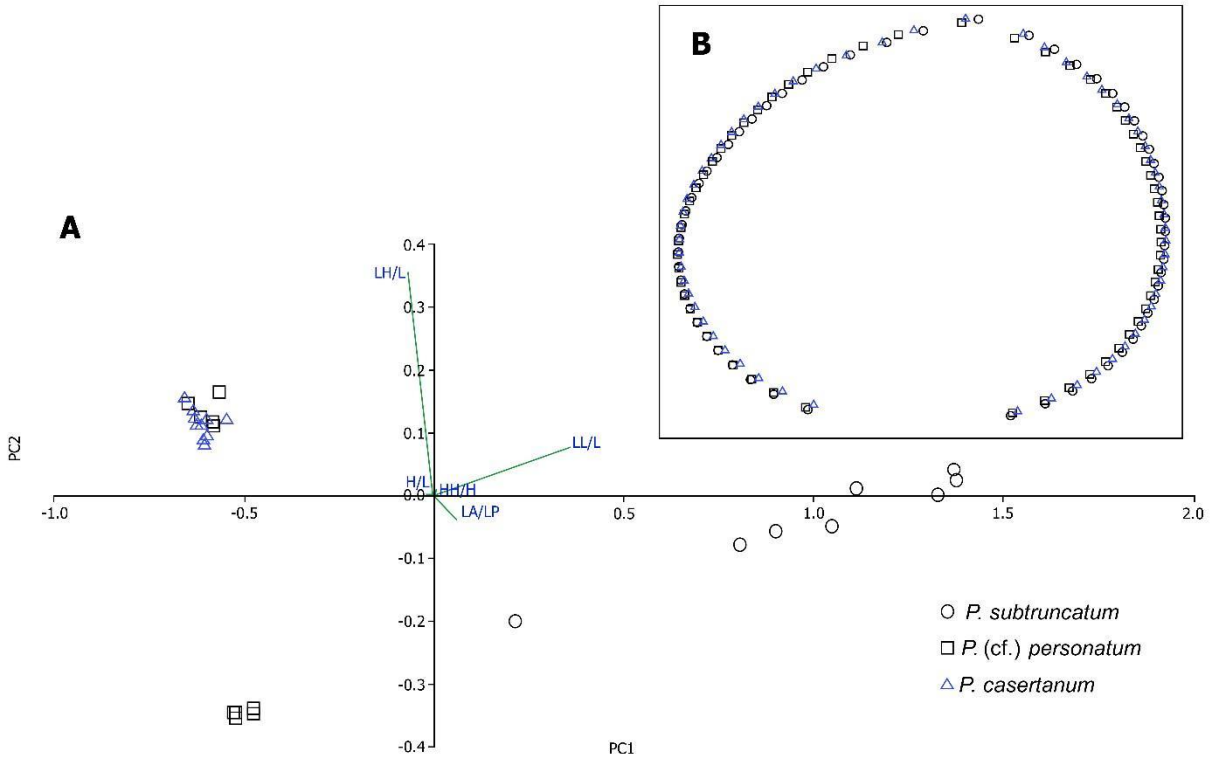


Figure 19. Results of the morphometric analysis of three species of Sphaeriidae from Morocco. A- Principal Component Analysis (PCA) with variables that contributed most to the PCA analysis, based on the measured ratios. Length L (maximum distance on the anterior – posterior axis), length of anterior part LA, length of posterior part LP, height H (maximum distance on the dorsal-ventral axis), length of umbo LU, length of the ligament of the left valve LL, length of the hinge LH,height of the hinge of the left valve HH; B- Mean outline of semi-landmarks of the three species (species symbols as in A).

IV. Intra-specific variation: case of *P. casertanum* from the Sebou basin

The shells of *P. casertanum* collected at the different sites in the Sebou basin varied from 1.57 mm to 5.76 mm in length and from 1.35 mm to 5.08 mm in height. The H/L ratio, describing the overall shape of the shell, shows a higher value in specimens from lakes than in those from streams and springs, which had more elongated shells. The hinge plate is wider and larger in lakes but higher in spring specimens. These differences were confirmed by the t-test for equality of means which showed a significant difference between variables in each habitat type where $p < 0.01$ with t-test values for H/L, HH/H, LL/L, LH/L and LA/LP are equivalent to 357.63; 68.13; 72.879; 133.72 and 132.2, respectively.

The morphometric relationships obtained between the measured length and height variables of *P. casertanum* from the three different habitat types were determined. A linear correlation between length and height for all populations sampled from lakes, streams, and springs was observed, exhibiting a negative allometric growth pattern with a slope equal to 0.981; 0.999 and 0.967, respectively.

PCA of Fourier coefficients showed better separation along PC1 (57.431%) and PC2 (21.998%). As with PC1, the separation between individuals is based more on shell length while the variation in PC2 is more related to shell height. Thus, differences between shell shapes in the three habitats studied were detected: Stream shells tend to be more elongated than spring and lake shells; the latter are taller and shorter, resulting in a rounded outline (Figure 20).

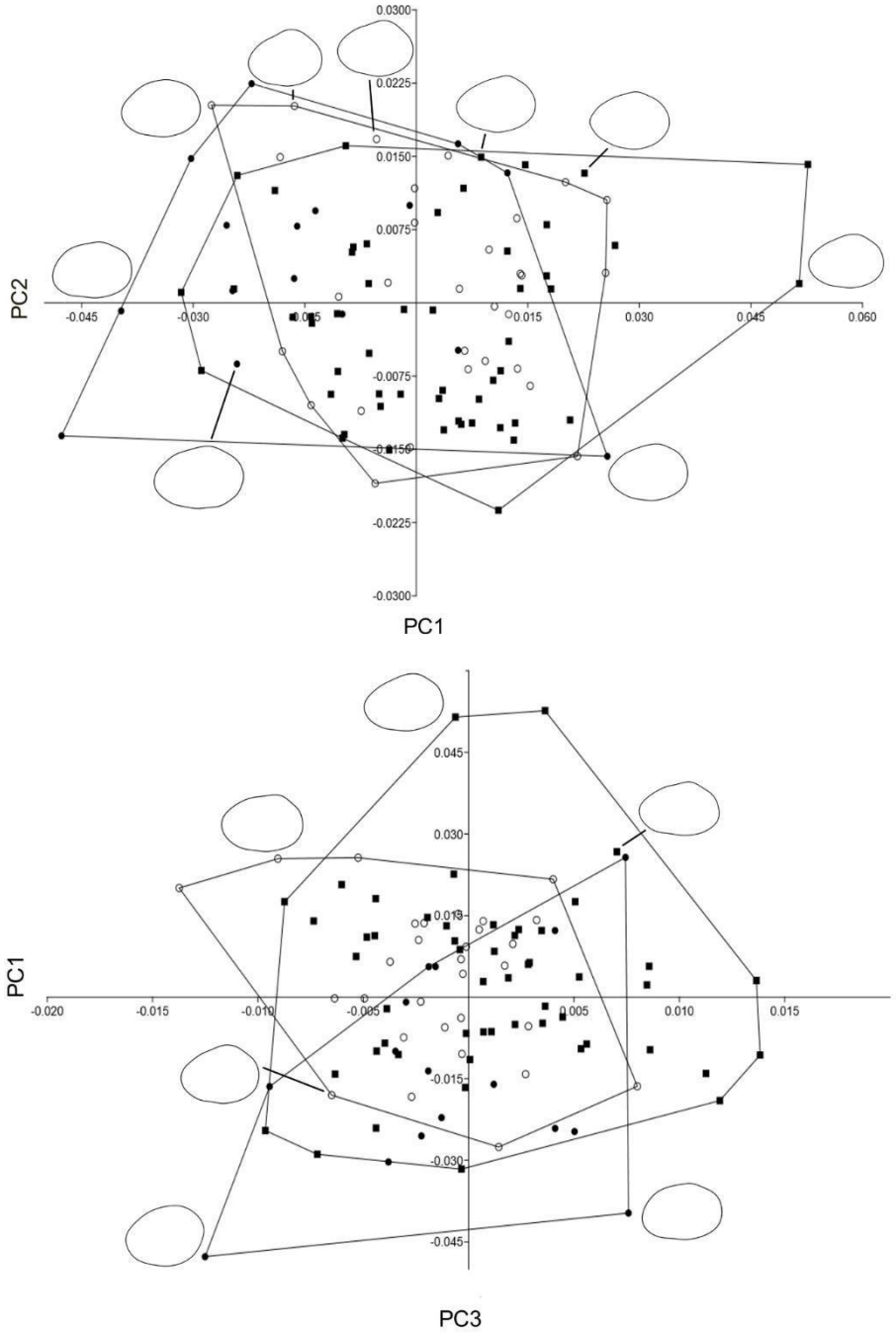


Figure 20. Plots of the first three components of the PCA showing the differences between shell shapes of *Pisidium casertanum* from the three habitat types studied: streams, lakes and springs.

V. Conservation status

Extent of Occurrence and Area of Occupancy differed significantly between the regional populations of the species. EOO ranged from 15,219 km^2 to 50,915 km^2, while AOO ranged between 40 km^2 and 104 km^2. Table 1 gives the detailed results of the assessment with the assigned Red List categories to each species. *Pisidium casertanum* had the highest EOO and AOO across the country among the different Sphaeriidae species, followed by *P.* (cf.) *personatum* while *P. subtruncatum* is the least present species among the three assessed

species and is therefore the most vulnerable one (Table 1). *Pisidium amnicum* and *M. lacustre* were listed as critically endangered species. However, only empty shells of these two species were included in the analysis; thus, the results for their conservation status cannot be determined accurately.

Table 5. Results of the regional conservation status assessment for three species of Sphaeriidae in Morocco. EOO: Extent of Occurrence, AOO: Area of Occupancy; no. unique occ.: number of unique occurrences; no. subPop.: number of subpopulations; no. loc: number of locations; LC: Least Concern; VU: Vulnerable; NA: Not applicable.

	P. casertanum	*P. personatum*	*P. subtruncatum*
EOO (km^2)	50915	45741	15219
AOO (km^2)	104	52	40
Nb_unique_occ.	27	13	11
Nb_subPop	16	13	8
Nb_loc	20	13	8
Category_CriteriaB	LC	LC	VU
Category_Code	LC B1a+B2a	LC B1a+B2a	VU B1a+B2a

VI. Taxonomic account

VII. Genus *Pisidium* Pfeiffer, 1821

Pisidium casertanum (Poli, 1791)

Species status: Native

Distribution: Cosmopolitan. More widespread in the northern hemisphere than in the southern hemisphere, where it is more limited to high-altitude localities (Kuiper 1983). Found throughout Morocco (Figure 21).

Shell dimensions: Average length = 4.09 ± 0.7 mm; average height = 3.42 ± 0.59 mm; average width = 2.52 ± 0.76 mm.

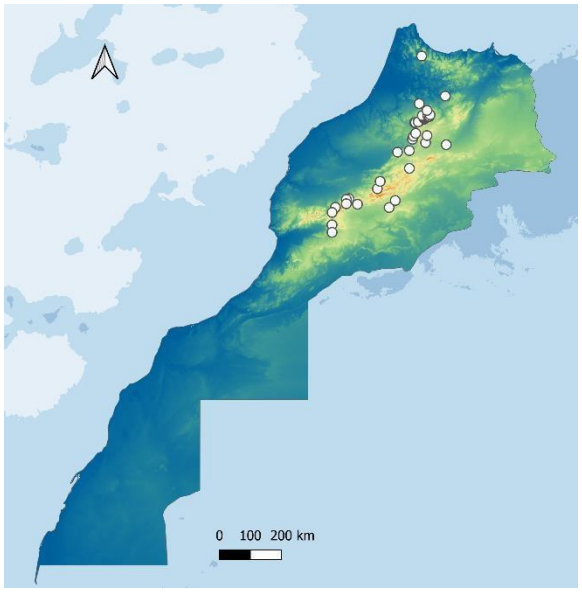


Figure 21. Distribution of *P. casertanum* in Morocco.

Main characteristics: Extremely variable, but can be distinguished from other species by its flattened umbo and shell. Silky periostracum covered with ferruginous deposits. The dorsal

margin is relatively long. Cardinal teeth: C2 arched, C4 straight and short, C3 strongly curved, sometimes bifurcated posteriorly.

External characteristics: Small, laterally compressed shell. Oval to subtriangular outline. Rounded anterior and posterior ends. Large and rounded but not prominent umbo, located posterior to the central point. The shell exhibits very weak concentric striations, giving it a rather smooth appearance. Dull to silky, non-glossy periostracum. Color ranges from cream-white to grayish-brown, often covered with ferruginous deposits.

Internal characteristics: Moderately long, thickened, and strongly curved hinge plate. Prominent cardinal teeth, with C2 triangular and C4 slightly curved, obliquely positioned to the rear, and C3 strongly curved with a thickened, grooved, or bifurcated posterior end. Well-developed lateral teeth, especially A1 and P1 in the right valve, with both teeth narrow and A2 stronger than P2, and parallel teeth in the right valve. Long and moderately wide ligament pit.

Habitat: The species inhabits all types of freshwater habitats, ranging from large rivers (e.g., M'goun River) to ephemeral ponds.

Note: The species is known to be highly variable morphologically (Rassam et al. 2021). The different ecological forms that the species can take are related to the effect of habitat conditions (Piechocki 1989).

Figure 22. Shells of *P. casertanum* with a view on the hinge plate (Ouzioua River, Ain Aghbal spring & Tizirt spring).

Pisidium subtruncatum Malm, 1855

Species status: Native.

Distribution: Holarctic, found in Europe, North Africa, Siberia up to Lake Baikal, and North America (Ellis 1978).

Shell dimensions: Average length = 2.92 ± 1.23 mm; average height = 2.45 ± 1.08 mm; average width = 1.74 ± 1.37 mm.

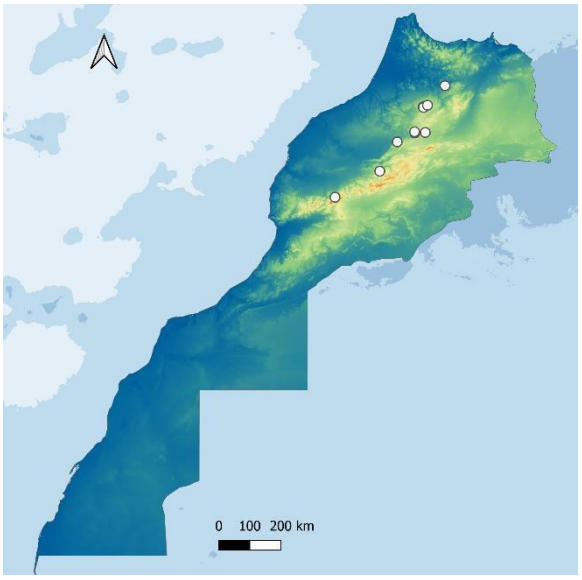


Figure 23: Distribution of *P. subtruncatum* in Morocco.

Main characteristics: Sub-triangular shape with a striated shell, opisthogyrous umbo. The posterior end is truncated, and the anterior part is elongated. The hinge plate is relatively thick and arched. The left valve has 2 long, parallel cardinal teeth (C2, C4). C3 is relatively straight and slightly curved, while the lateral teeth are well-developed.

External characteristics: Small inflated shell. Highly oblique contour. Prominent and rounded umbilicus, inclined backward (opisthogyrous), located near the posterior end. Truncated posterior end, elongated anterior part with rounded ends. Shell with fine, slightly irregular concentric striations. Silky and slightly glossy to matte periostracum. Grayish-white color. The shell is often entirely or partially covered with a thick layer of ferruginous mud.

Internal characteristics: Thick and arched hinge plate. Cardinal teeth located closer to the anterior lateral teeth. Left valve with 2 long and parallel cardinal teeth, with the higher one (C4) usually covering the lower one (C2) at each end. C3 in the right valve is relatively long, straight, or slightly curved. The lateral teeth are moderately well-developed. Long ligament pit, umbo at the posterior end.

Habitat: The species is more commonly found in running waters (rivers, small streams, canals) than in stagnant waters (lakes).

Figure 24. Shells of *P. subtruncatum* with a view on the hinge plate (Ifri N'Touya cave & Tiferguine stream).

Pisidium personatum Malm, 1855

Species status: Native

Distribution: Europe, Asia, and North Africa (Kuiper 1983).

Shell dimensions: Average length = 2.92 ± 0.7 mm; average height = 2.45 ± 0.6 mm; average width = 1.74 ± 0.88 mm.

Main characteristics: Rounded shape, umbo located at the center but not prominent. The presence of a raised callus in the hinge is a specific characteristic of the species, located between the lateral teeth and the ligament pit. The callus is primarily present in the right valve and may be absent in the left valve.

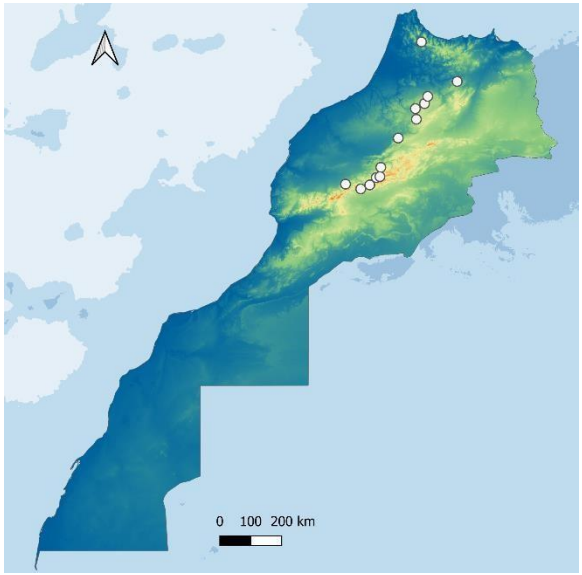


Figure 25. Distribution of *P. personatum* in Morocco.

External characteristics: Small, rounded and oval shell, often with angles between the anterior and posterior dorsal margins. Large and rounded umbilicus, but not prominent, placed more or less at the center. Shell with very fine and irregular concentric striations, and with numerous pores sometimes visible on the surface. Satiny periostracum (dull to silky). Grayish color but often covered with a thick layer of reddish-brown or black ferruginous deposits, with the shell speckled with ferruginous deposits.

Internal characteristics: The most distinctive and reliable element for confirmation is the presence of a raised callus on the hinge plate, located between the lateral teeth and the ligament pit. The callus is more strongly developed and prominent in the right valve, while it is less developed or absent in the left valve. Cardinal teeth, C3 curved and thickened posteriorly, C2 curved with C4 straight obliquely at the posterior part. Long and moderately wide ligament pit.

Remarks: *Pisidium personatum* can be confused with *P. casertanum* because both species share similar morphological characteristics. However, *P. personatum* can be easily distinguished from other Pisidium species by the presence of a callus in the hinge plate between the ligament pit and the lateral teeth on both valves, although it is less pronounced on the left valve.

Habitat: The species is present in habitats of low quality. *Pisidium personatum* inhabits various types of habitats with a widespread presence in rivers and springs.

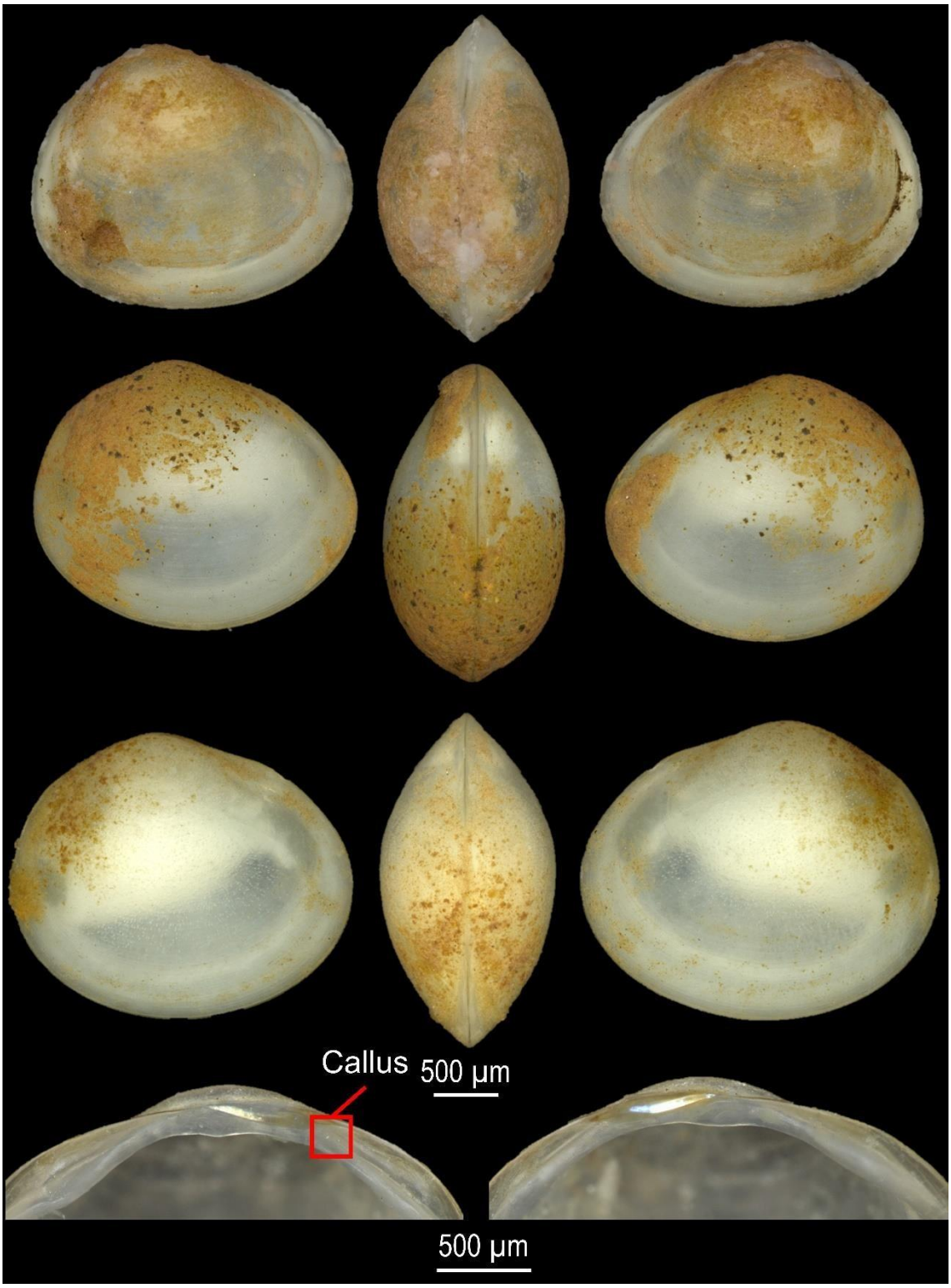


Figure 26. Shells of *P. personatum* with a view on the hinge plate showing the appearance of the callus (Tizguit River & Tessaout River).

Pisidium amnicum (O. F. Müller, 1774)

Species status: Native

Distribution: Palearctic. More common in northern Europe than in the south. In Morocco, it was only reported in the present study at the outlet of a reservoir (Figure 28).

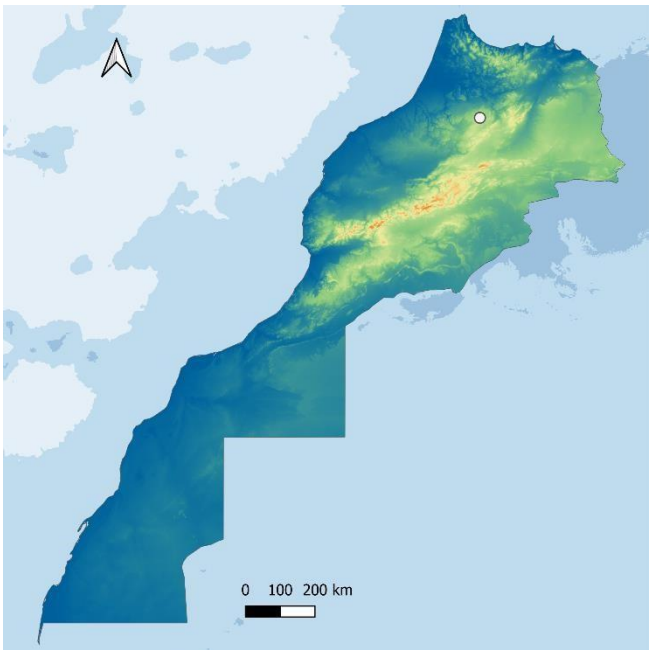


Figure 27. Distribution of *P. amnicum* in Morocco.

Shell dimensions: Average length = 8.5 ± 1.5 mm; average height = 6.96 ± 1.24 mm.

Main characteristics: Large size (up to 10 mm) and clearly marked striations on the shell, irregularly spaced and denser near the umbo. The umbo is not prominent, located posteriorly. The hinge plate is thick. C2 is triangular with C4 shorter, C3 is triangular and often bifurcated.

External characteristics: Wide and oval shell. Anterior and posterior ends rounded. Large and rounded umbo, but not prominent, at the back of the central point. Smooth area around the umbo or with very fine concentric lines. Shell with generally prominent and irregularly spaced concentric ridges. Glossy periostracum.

Internal characteristics: Thick hinge plate with well-developed lateral and cardinal teeth. C2 is generally triangular with C4 located diagonally posteriorly to the hinge, C3 is also triangular and usually notched or bifurcated towards the inner edge. Lateral teeth are very prominent, particularly in the right valve, with parallel anterior A1 and A3 and posterior P1 and P3. Long and wide ligament pit, particularly towards the posterior end.

Habitat: The species was found at a single locality corresponding to the outlet of a small dam with moderate flow.

Remarks: *Pisidium amnicum* remains the largest representative of *Pisidium*, making it easy to distinguish.

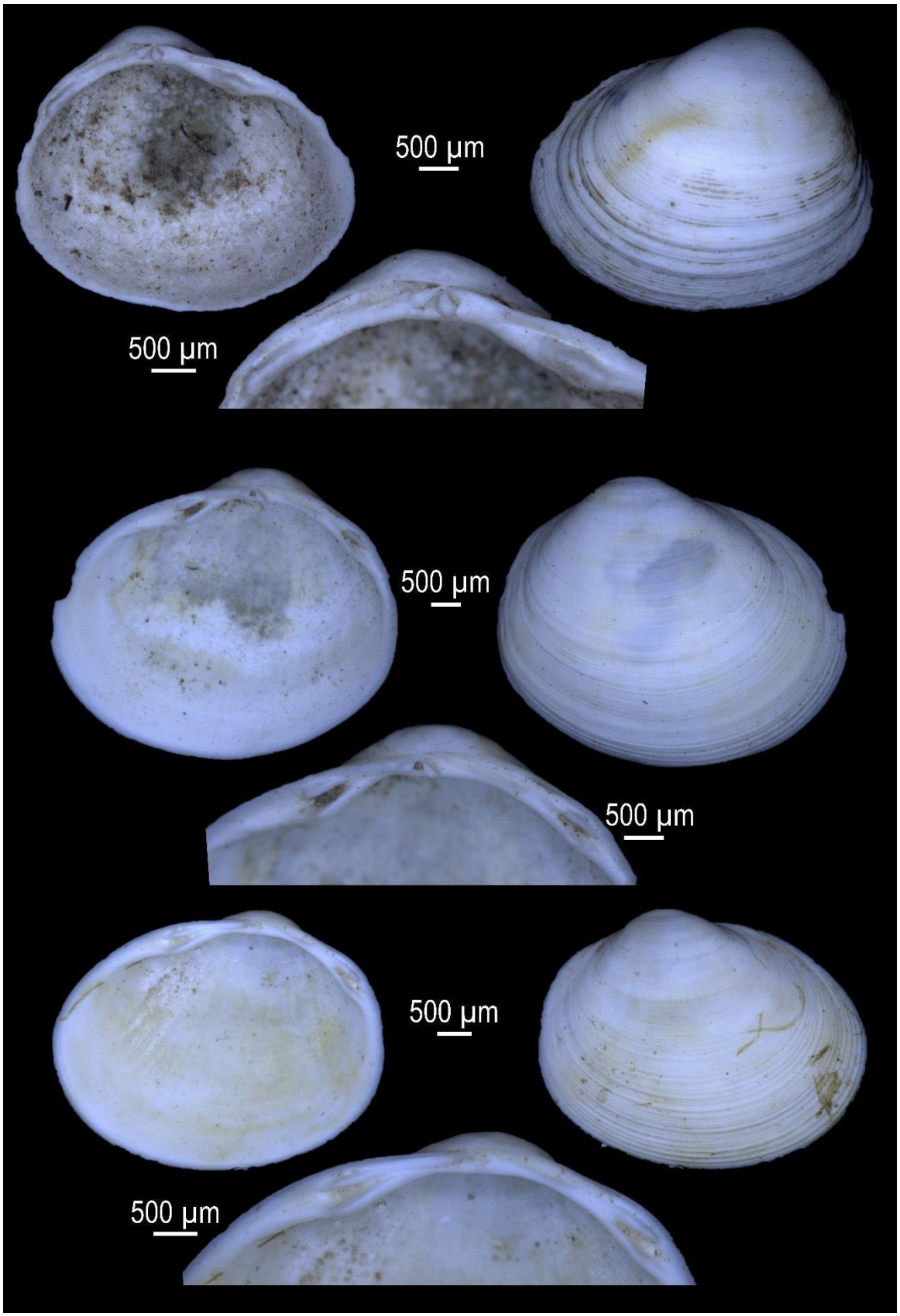


Figure 28. Subfossil shells of *P. amnicum* with a view on the hinge plate (Hachlaf dam outlet).

***Pisidium* sp.**

Notes: Overall, no external distinguishing morphological features. However, the shell outline and hinge plate are closer to *P. casertanum*.

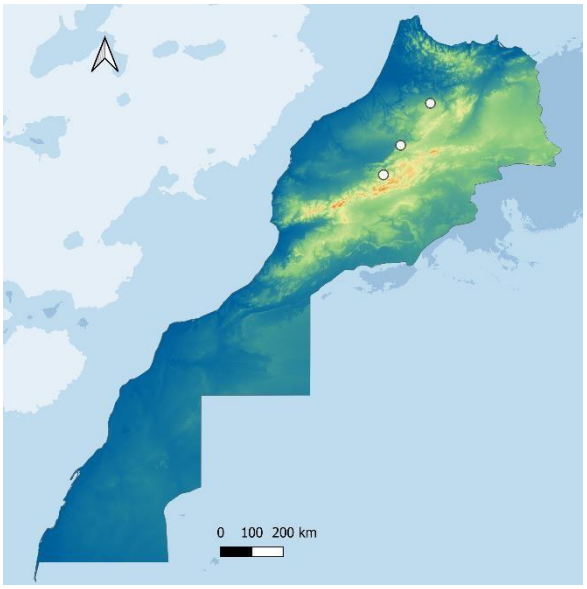


Figure 29. Distribution of *Pisidium* sp. in Morocco.

Genus *Musculium* Link, 1807

Musculium lacustre (O. F. Müller, 1774)

Species status: Native

Distribution: Holarctic

Shell dimensions: Length = 5.91-5.41 mm; Height = 4.71-4.45 mm.

Main characteristics: Quadrangular shell with a hood-shaped umbo placed in the center. The shell is very thin, fragile, and smooth. The hinge plate is very narrow, and the cardinal teeth are very small.

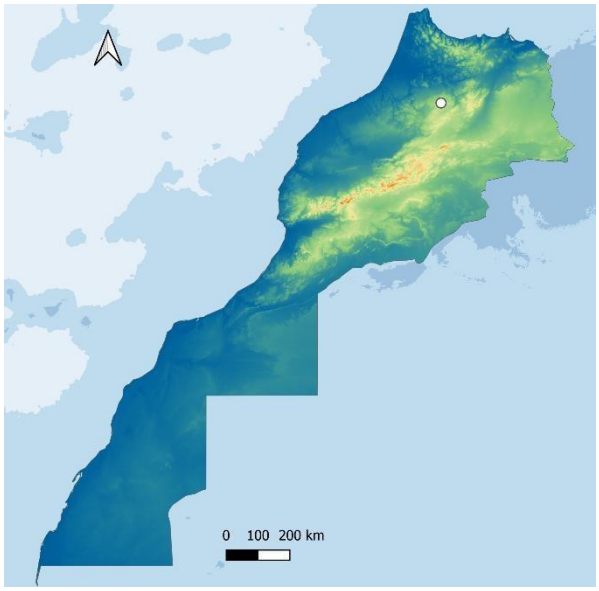


Figure 30. Distribution of *M. lacustre* in Morocco (only shell record).

External characteristics: Very thin and fragile shell. Oval to quadrangular in shape, with the posterior end wide and obliquely truncated, and the anterior end inclined and somewhat angular. Dorsal margin rather straight and inclined upward at the back and downward at the front. Umbo located more or less at the center, clearly raised, narrow, angular towards the anterior end, and covered by the juvenile shell. Sculpture of very fine concentric striae, appearing almost smooth. Ligament short, very narrow, and not visible externally. Glossy periostracum. Gray or slightly creamy in color.

Internal characteristics: Hinge plate very narrow and much shorter in the anterior part than in the posterior part. Tiny cardinal teeth, C3 curved and thickened posteriorly, C2 and C4 short and rather straight. Long and slender lateral teeth. Long and narrow ligament pit.

Habitat: In Morocco, the shell of M. lacustre has only been found in one locality: the shores of a small dam lake.

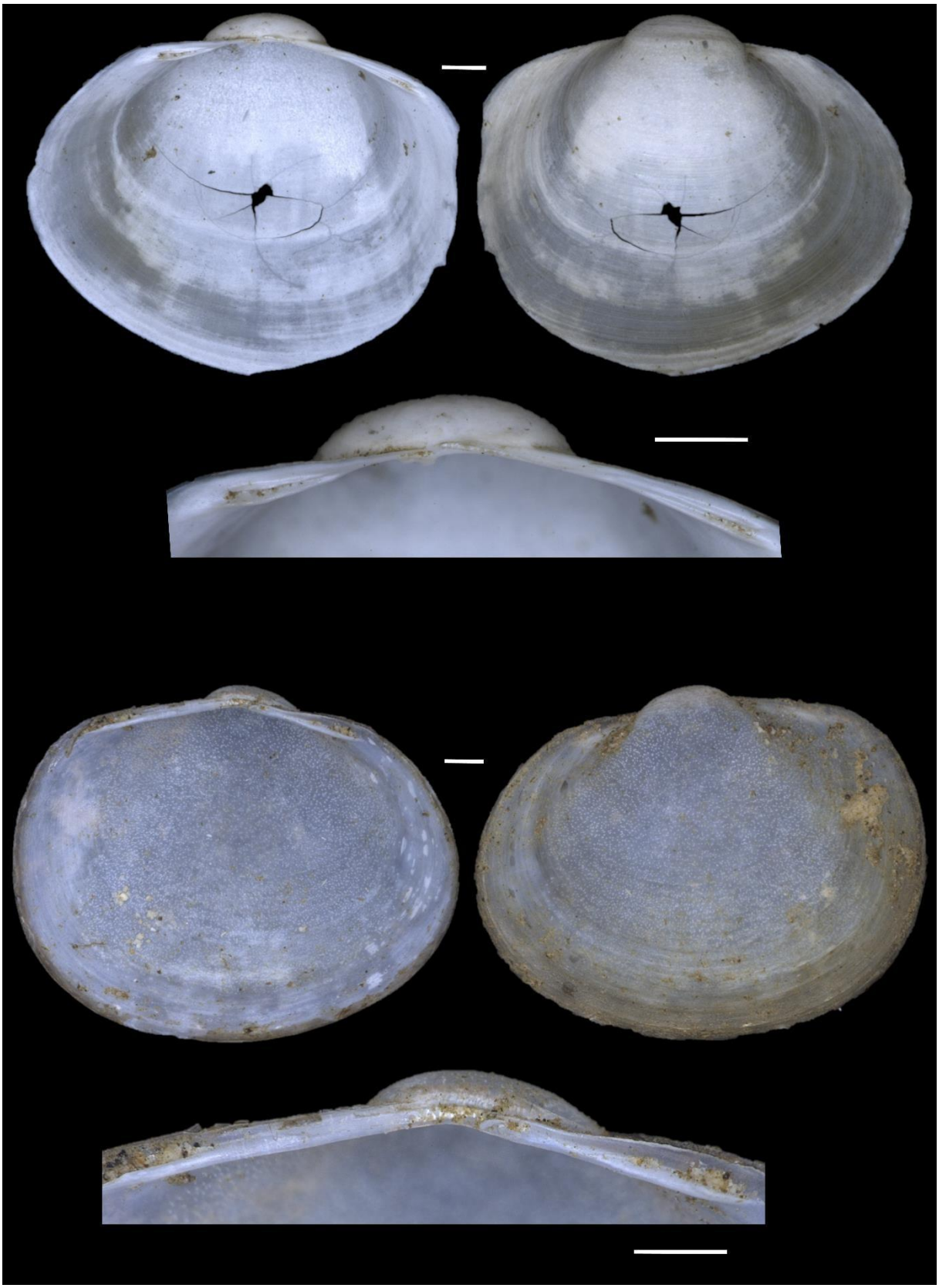


Figure 31. Subfossil shells of *M. lacustre* with a view on the hinge plate (Hachlaf Lake).

VIII. Phylogenetic analysis

The phylogenetic reconstruction with MrBayes provided a strongly consistent and supported phylogeny and the analyses resulted in the construction of four clades of WP *Pisidium* specimens (C1-C4) (Figure 32). C4 forms a separate and well-supported clade including the species *P. lillgerbojii* from Norway and Russia as sister species to *P. henslowanum* from Macedonia and *P. supinum* from France and Germany. The C4 is a sister clade to the three remaining clades. C3 forms a less-supported clade (BPP = 0.73) and includes mainly specimens belonging to *P. personatum* from Spain, Morocco, Germany, Turkey, Iran and Georgia. The Moroccan *P. personatum* is not monophyletic according to our phylogeny and clusters within SC1.3, SC2.3 and SC4.3. In addition to the Moroccan specimens, SC1 includes Spanish, German, Turkish, Iranian, and Georgian specimens, while SC2 consists of only Moroccan specimens clustering with a specimen from Lebanon. SC3 is a highly supported subclade and includes specimens of *P. casertanum* from Iberia (Spain and Portuguese Azores Island 27650). SC4 does not show a high resolution, including exclusively Moroccan specimens. SC5 comprises the species *P. obtusale* from Germany, *P. interstitialis* from Germany and specimens from Spain. SC6 is a highly- supported subclade and includes *P. personatum* from Germany.

C2 included three subclades SC1, SC2 and SC3 where SC1 was formed of *P. casertanum* from Georgia, Turkey and Spain along with a group of specimens from Morocco. SC2 is a sister subclade to SC2 and is highly resolved (BPP=1). It includes the *P. subtruncatum* group from Macedonia, Georgia, Italy, Morocco and Ireland. SC3, which is also a well-supported subclade (BPP= 0.94), consists of *P. maasseni* clustered with *P. edlaueri* from Macedonia and *P. nitidum* from Germany and Turkey. Those three species formed a sister group with *P. milium* from Germany.

C1 groups three subclades with SC1 and SC2 are sister groups and both are highly supported. SC1 includes a *P. casertanum* group from all over the Mediterranean (e.g. Lebanon, Albania, Greece, Morocco, France and Spain). SC2 also includes *P. casertanum* from Germany, Macedonia and France (Corsica) grouped with two Asian species (*P. atkinsonianum* and *Pisidium* sp.). SC3 comprises two northern species; *P. globulare* from Russia and Germany and *P. waldeni* from Norway.

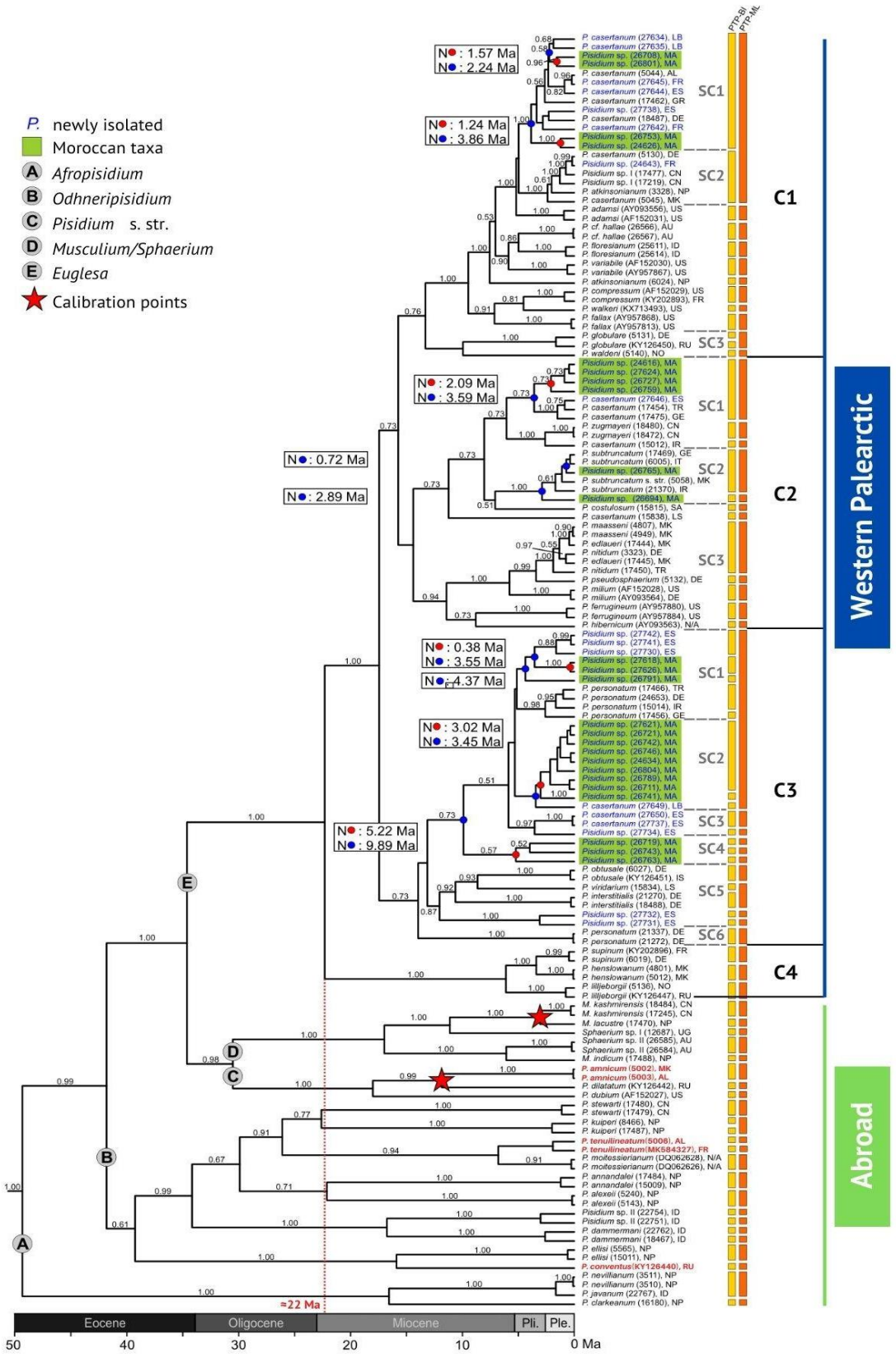


Figure 32. Dated phylogeny of the family Sphaeriidae including posterior probabilities based in four genes (16S, COI, 28S, H3). The yellow and orange bars refer to the species delimitation results with PTP for the BI and the ML, respectively. Numbers on branches represent the Bayesian Posterior Probability (BPP) values. Calibration points are marked with a red star (splits between *M. lacustre* and *M. kashmirensis* & *P. amnicum* and *P. dilatatum*). All newly sequenced specimens have a blue font color. Species from the WP and belong to *Pisidium* s. str. and *Odhneripisidium* are designated with a red font.

IX. Diversity and species delimitation

Our data-set comprises the presence of 20 species of *Pisidium* in the Western Palearctic area (Figure 32) with 16 species belonging to *Euglesa* subgenus, two species belonging to *Pisidium* s. str., and two species belonging to the subgenus *Odhneripisidium*. The amplification of both mitochondrial and nuclear genes was most successful for 16S gene with 122 newly sequenced specimens, followed by H3, COI, and 28S with 63, 48, and 44, respectively, newly sequenced individuals of *Pisidium*.

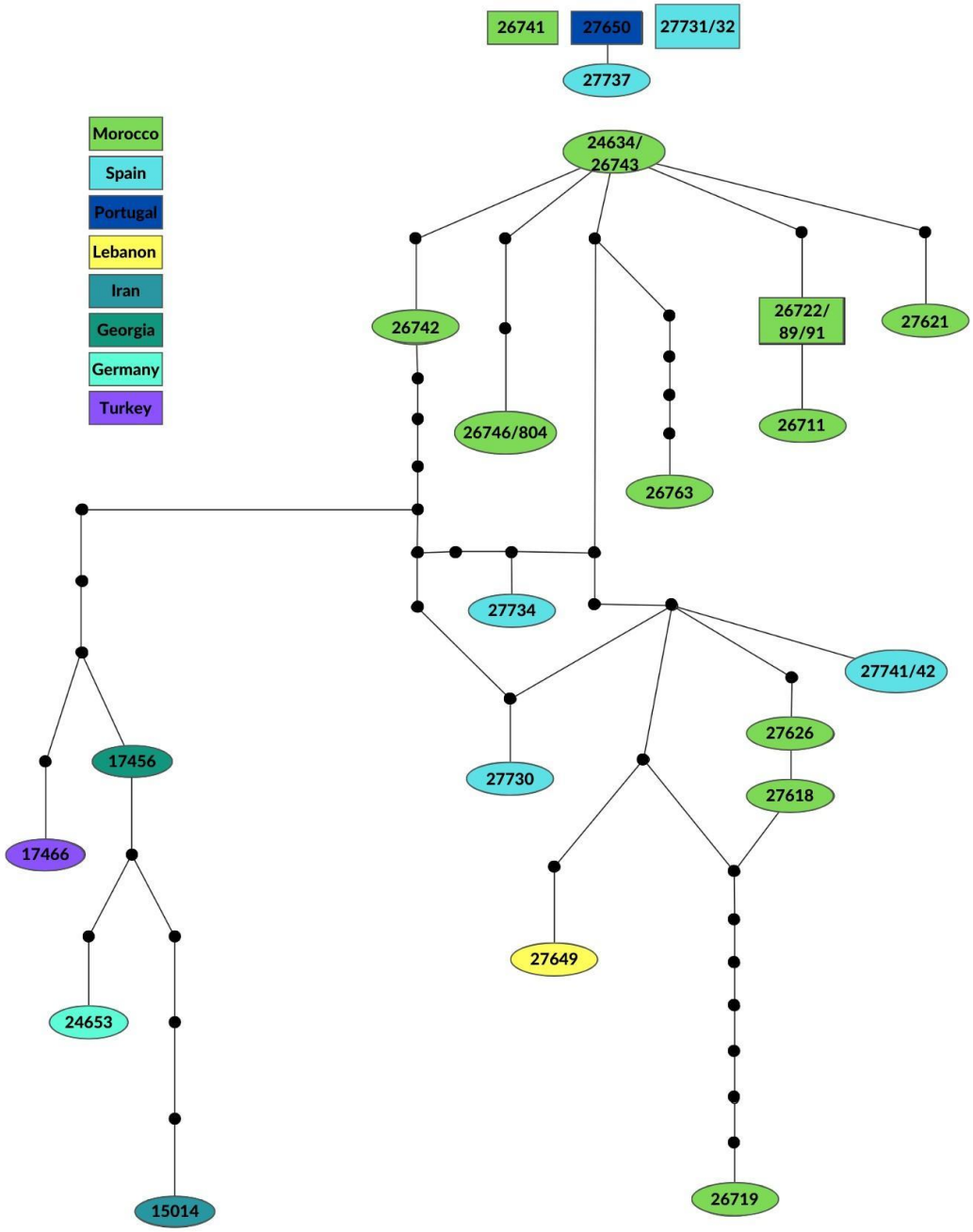


Figure 33. Statistical parsimony haplotype network applied to *P. personatum* based on 16S dataset. The connection limit is 95%. Colors refer to locations and black dots represent mutational steps. The size of elements is proportional to the number of unique haplotypes.

Both methods used for the delimitation of species boundaries for the concatenated dataset of COI and 16S revealed a different number of MOTUs (38 OTUs with the BI method and 30 with

the ML method). While the number of species inferred by PTP-BI and PTP-ML was the same for C2 and C4 (eight and three respectively), results of species delimitation in C1 and C3 were not congruent between both methods with the highest rate of discrepancy shown in C3 (BI 19 MOTUs, ML 11 MOTUs).

The differences occurring in C3 mostly concerned the Moroccan MOTUs. PTP-BI delimited seven MOTUs, while PTP-ML indicated four Moroccan MOTUs only. The specimens 27618/27626, 26791, 26741, 26719, 26743 and 26763 were identified as separate OTUs from the Moroccan remaining *P. personatum* specimens. The BI-based delimitation showed a high concordance with the results obtained from the "*Personatum*" haplotype network given the high number of mutations among these haplotypes (Figure 33).

X. Divergence time estimates

The Beast-generated phylogeny is highly congruent with the ML analysis. The monophyly of all major clades is recovered, except for three specimens (27650, 27737 and 26743) that clustered in the ML tree within clade C1.

The MRCA of the genus *Pisidium* was recovered to be around 50 Ma (early Eocene) with the *Afropisidium* lineage generating at this time. The remaining lineages originated in the midst Eocene around 42 Ma. The two species from the WP representing the subgenus *Odhneripisidium: P. conventus* and *P. tenuilineatum* originated chronologically around 15.9 Ma (middle Miocene) and 2 Ma (Pleistocene) respectively. *Euglesa* was the most diverse lineage including the majority of the WP species and it originated between the late Eocene and early Miocene (34 Ma – 22 Ma). The diversification of this subgenus began around 22 Ma. During the Miocene, three divergence events led to the formation of the four clades, and four further events formed the subclades except two of them (SC1 and SC2 (C1)) that were formed from one divergence event during early Pliocene. C1 and C2 evolved at the same time around 15.8 Ma after diverging from Clade C3 around 17.5 Ma.

The radiation of clade C3 was estimated to have started in the Miocene with the oldest split of the German species in SC6 dated between 14-8.8 Ma, whereas the split separating the European specimens from their Ibero-Moroccan conspecifics dated around 5.22 Ma during the Zanclean stage (SC1.C3).

The first split occurring in clade C1 dated of 13 Ma where the northern specimens diverged (SC3.C1). The WP subclade SC1 nested within this highly diverse clade originated in the Pliocene around 5 Ma. This suggests that there was a post-Messinian colonization of this area (Mediterranean). The subsequent divergence splits in the topology occurred during the Plio-

Pleistocene. Thus, the colonization of most of *Pisidium* species in the central and southern part of the WP was recent and started in the Pliocene.

Chapter IV: Discussion

I. Species diversity

The results of the present study confirmed the existence of at least six sphaeriid species occurring in different basins in Morocco. These findings have cleared up doubts about the existence of other species mentioned in the literature as synonyms or living species in Morocco.

All the seven species recorded by Kuiper (1972) co-existed in a single locality in the Middle Atlas (Sebou Basin) and some specimens were deposited in the Natural History Museum of the Scientific Institute in Rabat (Rassam et al. 2021), therefore, only four out of the seven species were found in the whole country. Given the overall comparatively low diversity of Sphaeriidae in Morocco, it is interesting that we discovered a new species for the fauna of the country. Moreover, it is interesting to see a species potentially new to science. New species of Sphaeriidae are rarely described in the Palaearctic (e.g. Groh et al. 2020). Detailed analyses must assess whether *Pisidium* sp. is indeed an endemic to Morocco.

The difference in the number of species mentioned for Morocco may have several possible explanations: the identification on which the authors relied at the time was based on morphological and anatomical features, however, the genus *Pisidium* is cryptic and its species show pronounced intra-specific variations, influenced by environmental conditions (Holopainen and Hanski 1986, Holopainen and Kuiper 1982, Funk and Reckendorfer, 2008, Rassam et al. 2021), thus, the identification may be confounded in the absence of clear morphological features which can be seen, however, with the combination of both morphological and geo-morphometric approaches. The species *P. tenuilineatum,* for example, is easily confused with *P. subtruncatum* by the outline shape of the shell (Piechocki 1989) and has never been reported from altitudes above 500 m a.s.l. (while Kuiper's record of the species is above 1,700 m), suggesting that this is potentially a misidentification.

The second possible explanation is the change in environmental conditions, a study by Økland and Kuiper (1982) showed the impact that water acidification can have on the disappearance of *Pisidium* species. *Musculium lacustre* was cited for the first time in Morocco as *Sphaerium maroccanum* Pallary, 1898 in the surroundings of Tangier, a city in the North West, thus it was thought to be restricted in Africa to the Algerian coast (Van Damme 1984). The high touristic activity that the region has experienced in recent years causing a low water quality (Figure 34) combined with the increasing eutrophication due to the nutrients drained through the basin from the surrounding agricultural fields, may probably be of high impact for the presence of the three other *Pisidium* species, this impact may be related to changed ecological factors which require more intense studies to test this hypothesis.

Figure 34. Ain Vitel spring (Sebou basin) with traces of pollution in its stream (plastic bottles and bags) (© Rassam, 2017).

II. Intraspecific variation of the species *P. casertanum*

This study represents the first population-level investigation on the common pea clam *P. casertanum* in Africa. Furthermore, new insights about intraspecific variation in three different habitats – lakes, springs and streams – are given. The linear morphometric measurements and the shell contour analysis results were coherent and complementary, revealing considerable differentiation between specimens from different habitats within the Sebou basin. Morphometric differences were obvious especially for the hinge plate, which is broader in shells of lakes and higher in shells of springs and rivers. The present lacustrine specimens are similar to previously described forms of *P. casertanum* from Lake Bourget in France that also showed a large hinge plate and sub-pentagonal form (Favre, 1940). The length of the hinge is related to the valves' opening ability, with longer hinge plates allowing for wider opening gaps (Ubukata, 2000). This suggests that the shells of *P. casertanum* with long and broad hinge plates in lakes need wider opening gaps to improve feeding and respiration in habitats with low water current.

As for the shell shape represented in the morphometric analysis by the H/L ratio, the shells of lakes and springs were shorter and higher than those from rivers, which showed more elongated forms. This was also confirmed by the contour analysis of shells with EFA. Besides shell morphology, the effects of habitats can also be seen in other biological features ranging from life history (Holopainen & Hanski, 1986; Hornbach & Cox, 1987) to secondary production

(Hamill, 1979), being influenced by biotic and abiotic factors related to habitat types. *Pisidium casertanum* is a cosmopolitan and taxonomically contentious species with its polymorphous and puzzling forms (Kuiper, 1983; Kuiper et al., 1989, Mouthon & Taïr- Abbaci, 2012). Many varieties of *P. casertanum* may represent ecological forms from the effect of habitat conditions (Piechocki, 1989). The shell morphometry of sphaeriid specimens from different localities showed that the highest shells of *P. casertanum* were those from lakes (Holopainen & Kuiper, 1982). Moreover, the paraphyly of the species was confirmed by Lee & Ó Foighil (2003), indicating that it incorporates a complex of cryptic species. These observations were confirmed by subsequent phylogenetic studies that included *P. casertanum* from more regions of the world (Clewing et al., 2013; Bößneck et al., 2016).

Two *P. casertanum* morphotypes were already observed in a previous study, one with a short and high shell and the other more elongated and lower shell (Mackie et al., 1980). These two morphotypes are both present in the present study; the first morphotype corresponds to lakes and springs specimens and the second to specimens from streams/rivers. The relationship between environmental conditions and the shell morphology of *Pisidium* species was the subject of several studies (e.g., Saunders & Rung, 1990; Funk & Reckendorfer, 2008; Myzyk, 2017). This study also showed a strong influence of physical-chemical factors in the shell shape of *P. casertanum*. For instance, the conductivity and dissolved water oxygen were highly correlated with the H/L and LH/L indexes. Dissolved oxygen was much higher in lakes than other sampled habitats and so was the H/L index and this is probably an adaptive explanation. The specimens of lakes were collected in the littoral zone, which is an area exposed to high water turbulence (thus, higher dissolved oxygen) caused by wind, narrow and short shells would be more likely stable and lodged in such conditions.

Hinch et al. (1989) also concluded the same explanation when studying the morphology of the unionid species *Elliptio camplanata*. In lakes and springs, conductivity was the highest and this correlated with the high mean value of shells' length and height from these two habitats which can potentially be related to the concentration of calcium ions present in the site, thus, further experimental studies need to confirm this statement. More studies are needed, therefore, to test and confirm this hypothesis, particularly since the species is benthic and physical-chemical factors are variant with vertical gradient notably DO which will be the target factor in future studies.

In the present study, and with the lack of detailed information regarding the spatial and temporal variation of the main physical-chemical parameters, it is difficult to understand the contribution of these parameters to the intraspecific variation of *P. casertanum.* However, from a hypothetical point of view, the dissolved oxygen and conductivity do not directly influence

the morphology of the specimens from the different habitat types. Rather, these parameters represent limnological, hydrological and edaphic circumstances of the overall aquatic system, not necessarily reflect conditions in the microhabitat settled by the pea clams. Therefore, further analyses based on more detailed data collected seasonally should be considered to answer these questions such as current velocity, calcium carbonate and food availability, which are known for their impact on bivalves shell morphology (Alunno-Bruscia et al., 2001; Preston & Roberts, 2007; Jacob et al., 2008; Caill-Milly et al., 2012; Zając et al., 2017). Knowing the way habitat type influences intraspecific variation of *P. casertanum*, may contribute to the correct identification of the species. Moreover, it shows abiotic and biotic factors of a given habitat are really important factors affecting shell morphology. Geometric morphometric tools remain a certain and efficient way to discern this. In a classic systematic description of a species, usually the diagnosis is given with the morphological characters necessary for species recognition along with its occurrence habitat description. However the morphological features may change considerably with the habitat type occupied which should be taken into account more thoroughly when conducting taxonomic work.

III. Distribution patterns and conservation status

Sphaeriids were collected in different habitat types, including lakes and reservoirs, springs, channels, marshes and rivers (streams, dam outlets and larger tributaries). River systems were the habitat with the greatest abundance of Sphaeriidae.

Pisidium casertanum is the most abundant species of Sphaeriids in all habitat types. This species is considered the most common member of the family and is therefore euryecious. It can be found in almost all habitat types ranging from temporal and ephemeral ponds to large rivers and lake bottoms (Clarke 1973, Saunders and Rung 1990). This species is also known to be tolerant in terms of environmental conditions such as low pH values and low calcium concentrations (Økland and Kuiper 1982, Horsák and Hájek 2003), consequently, its occurrence in all habitat types in this study is not surprising. *Pisidium personatum* is present in all habitats sampled but is more concentrated in springs and rivers. *Pisidium personatum* is a typical cold-stenoecious inhabitant of springs and wells (Dyduch-Falniowska 1982, Wagner et al. 2011) and seeks nutrient-rich sites (Horsák et al. 2007, Kubíková et al. 2011), which explains its abundance in marshy areas. *Pisidium amnicum* occurred in only one locality which was a small outlet of a dam reservoir in the Sebou basin, where only few empty shells were found. The species is not flexible in terms of water quality and prefers oligosaprobic and mesosaprobic waters (Piechocki 1989), moreover it is abundant especially in northern and Central Europe (Zettler 1996, Zettler and Daunys 2007) and its presence in Morocco may be an introduction but more research is necessary to test this hypothesis. These two

characteristics may explain its rarity in the samples. *Pisidium subtruncatum* occurs in all types of habitats surveyed, except marshes, with a low abundance in rivers (Figure 16). It is an euryecious species which ranks behind *P. casertanum* and *P. personatum* in the variety of its habitats. *Musculium lacustre* is the only representative of its genus in Morocco, its representativity is nevertheless not very pronounced, as only two old valves were found in a dam reservoir with submerged vegetation (Figure 16), the habitat record is in harmony with those of Swanson and Ormerod (2005) and Killeen et al. (2004), however the very small number of individuals collected indicates to resample in this same locality more intensively.

The distribution of Sphaeriidae species in Morocco is uneven across the nine basins and across the different altitudes. The highest diversity is recorded in the Middle Atlas which links the Sebou and Oum Er Rabia basins with the presence of all the 5 species in the 50x50 km square around the city of Ifrane. The Middle Atlas is known for its richness in aquatic resources mainly from snowmelt and heavy rainfall, resulting in a variety of ecological habitats (springs, lakes, tributaries, marshes and large rivers), which may explain the specific diversity that occurs in this region.

As for the elevation, the highest diversity of sphaeriids species in Morocco is found between 1,000 and 2,000 m a.s.l. in the Sebou basin. No species were recorded below 462 m. *Pisidium casertanum* is the species that showed a wide altitudinal spectrum ranging from 462 to 3,137 m a.s.l.. Being a lowland species (Piechocki 1989, Moorkens and Killeen 2009), *P. amnicum* was found in our study at a relatively high altitude (1663 m a.s.l.). In Europe, *P. subtruncatum* has been recorded at a maximum altitude of 2,300 m in the Pyrenees (Kuiper 1966b), in this work this limit is exceeded to 2,645 m a.s.l. in the Tensift basin. Globally, *P. subtruncatum* has the highest point of occurrence in the Tibetan Plateau (Clewing et al. 2013). *Pisidium personatum* has a narrower altitudinal range (Figure 35) with a maximum of 2,175 m a.s.l. which does not exceed that recorded by Kuiper (1974) in the Alps (2,500 m a.s.l.). The difference in distribution and species composition of Sphaeriidae between basins (Figure 18) may be explained by the fact that the Sebou basin, which is the richest in species, is one of the largest in Morocco (40,000 km^2), and therefore greater diversity is expected (Kallimanis et al. 2008). A second explanation for the altitudinal distribution may be the fact that the Sebou, Oum Er Rabia and Tensift are the highest basins of the country, the presence of mountain chains crossing them provides them a variety and diversity of habitats.

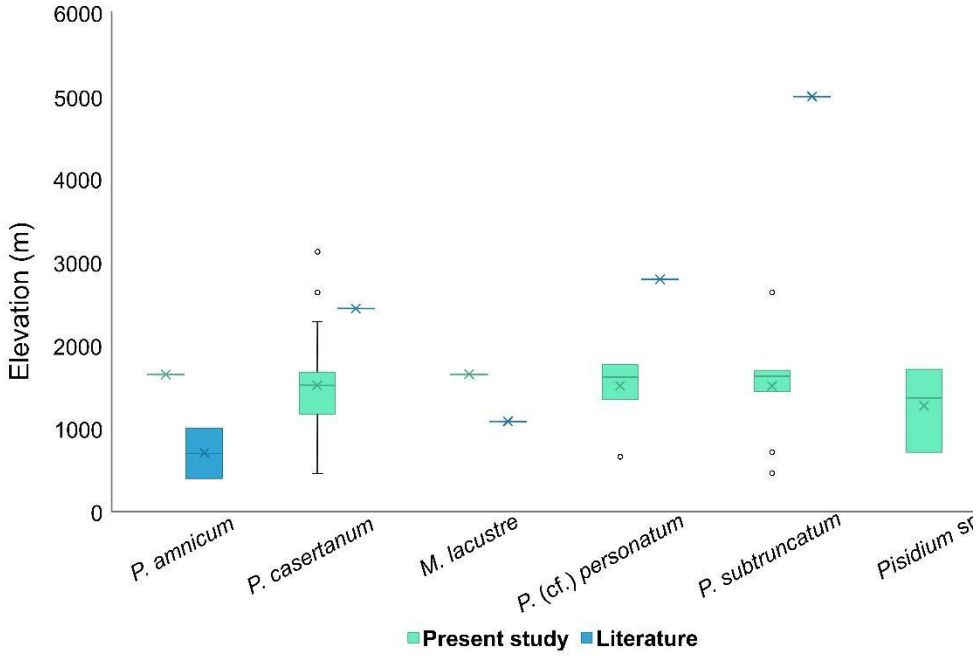


Figure 35. Altitudinal distribution range of species of Sphaeriidae: *P. amnicum* (Kuiper 1972, Hubenov 2007); *P. casertanum* (Mouthon 1983); *M. lacustre* (Piechocki 1989); *P. personatum* (Kuiper et al. 1989); *P. subtruncatum* (Clewing et al. 2013) (the crossmarks and the horizontal bars on the boxes refer to the mean and median, respectively).

The conservation status following the IUCN Red List guidelines was elaborated for the first time at a national level for all the species of Sphaeriidae recorded in Morocco. Out of the five species, *P. casertanum* and *P. personatum* have been assessed as “Least Concern” species in Morocco. *Pisidium personatum* was previously assessed by IUCN Red List at the North African scale as Vulnerable (Van Damme et al. 2010). The conservation status assessment of

P. subtruncatum classified the species as “Vulnerable” while at the North African scale it was assessed as “Critically Endangered”. The suggested status for *P. amnicum* and *M. lacustre* at the national level is "Regionally Extinct" while at the North African level the species is in the "Data Deficient" class as exhaustive sampling have failed to record any living animals, moreover, only empty shells of *M. lacustre* were collected in one locality which is nowadays completely dry.

IV. Phylogeography of the genus *Pisidium*

In the present work, the biodiversity of *Pisidium* in the WP has been displayed and grouped for the first time plainly. 38 OTUs resulted from the species delimitation methods used which is a quite high number compared to the known number of species from the studied area. In fact, 33 is the total number of species designated for all Sphaeriidae in the Palearctic realm (Graf, 2013). The genus *Pisidium* includes some cryptic species complexes (e.g. *P. casertanum* and *P. personatum*) which are morphologically similar, leading to a very probable

misidentification. In fact, the identification of *Pisidium* species mainly relies on the morphological shell characters, which can be misleading due to the intraspecific variability of the shell in response to environmental factors (Rassam et al., 2021a). Several studies have focused on the efficiency of the species delimitation concept to reveal the number of species from a cryptic species complex (Razkin et al., 2016; Mahulu et al., 2021; Wang et al., 2022). Likely, the number of species in the WP is even higher than the described due to the lack of samples from all over this sub-realm. However, future extensive studies are required regarding the taxonomy of the family to shed light on its serious taxonomic doubts, combining both molecular data and morphometric tools.

1. Dipsersal vs Vicariance: Which mechanism drove the present distribution?

There could be two hypothetical scenarios explaining the present distribution of WP *Pisidium* species: 1) dispersal, 2) multiple vicariance. Under the hypothesis of dispersal, it is believed that *Pisidium* specimens have crossed from Asia to Europe via some vectors such as freshwater fish and other animals. Studies have shown a similar distribution pattern for the colonization of Europe and North Africa by freshwater fish (Doadrio, 1990; Perea et al., 2010). Jump-dispersal of the genus *Pisidium* throughout the Western Palearctic can also be linked to waterfowl to which the species attaches itself either on its feathers or on legs in a phoresy interaction (Mackie, 1979). In fact, the order Anseriformes is practically the oldest of the waterfowls and the migration of these species follows the "east Atlantic pattern" which includes in one of its parts the western Palearctic area. Amphibians also contribute, in part, to the dispersal of Sphaeriidae, notably the genus *Pisidium*, although the scale of dispersal is rather local, as well as large insects such as diving beetles (Mackie, 1979; Kappes and Haas, 2012). Under the scenario of multiple vicariance, it is likely that an Asian distributed common ancestor species was fragmented into several isolated populations by the fragmentation of an ancestral joint distribution. These populations then evolved to genetically different species through time. A work published by Dyduch-Falniowska (1989) pointed to the presence of two geographically vicariant species of *Pisidium* (one with a Western Palearctic distribution and another with a South Asiatic distribution) with an overlapping area which is the Mediterranean region. These two hypotheses are by no means exclusive of each other and trying to treat them as such can lead to aberrant patterns that cannot be explained by either mechanism. A better assumption would integrate the mechanisms of vicariance and dispersal to explain the contemporary pattern of *Pisidium* species diversity and distribution. Hence, a better geographic coverage is needed considering more sampling where geographic gaps occur.

No clear geographical signal was found regarding the splits of the resulting clades in the phylogeny inferred as they all include representatives dispersed across the studied area. Nevertheless, at a finer scale, some "sister clades" have interesting allopatric disconnections.

As is the case in clade C3 where specimens from Morocco and Spain split from those from central Eastern Europe (Georgia, Turkey and Germany), both being separated by the Pyrenean mountains' barrier. The same was noticed in the clade of *P. subtruncatum* between the Moroccan and European specimens. The ancestral distributions and lineages connection pattern are being discussed below with a chronological framework.

2. Eocene-Oligocene boundary: The beginning

The *Euglesa* lineage arose between the late Eocene and the Oligocene periods as a result of a divergence event separating it from *Pisidium* s. str. and *Musculium/Sphaerium* lineages. Most of *Euglesa* specimens in our phylogeny belong to the WP zone. The sister relationship between *Euglesa* and both other lineages (consisting of mainly Eurasian specimens), can lead to the hypothesis that WP *Pisidium* specimens have a Eurasian common origin. The closure of the Turgai strait around 29 Ma (which formerly connected the Arctic Sea to the Paratethys and represented a considerable geographical barrier between the east and west of the Palearctic (Sanmartin et al., 2001)) around the Eocene-Oligocene boundary permitted a northdispersal model towards Northern Europe while Urals were forming (Rögl, 1999; Popov et al., 2004). This hypothesis matches the initial splitting of the basal C4 first, being a Northern distributed clade.

The colonization of Europe was not restricted to a Northern dispersal event. A south Asian to central Europe dispersal is a plausible hypothesis given the results of our data, where Asian and southeastern European specimens are clustered together with Mediterranean and central Europe specimens in all of the clades. This dispersal event might have occurred during the early Oligocene when the Paratethys sea and Tethys Ocean were separated giving rise to the Balkanian-Anatolian-Iranian landmass (Rögl, 1998; 1999) (Figure 36), which was separated to an Archipelago and served as a migration route for several taxa westward (e.g. fish (Perea et al., 2010) and mammals (Markovic et al., 2018)). However, the land connection was created during early Burdiganian when the Slovenian corridor linking the Mediterranean to the Paratethys closed.

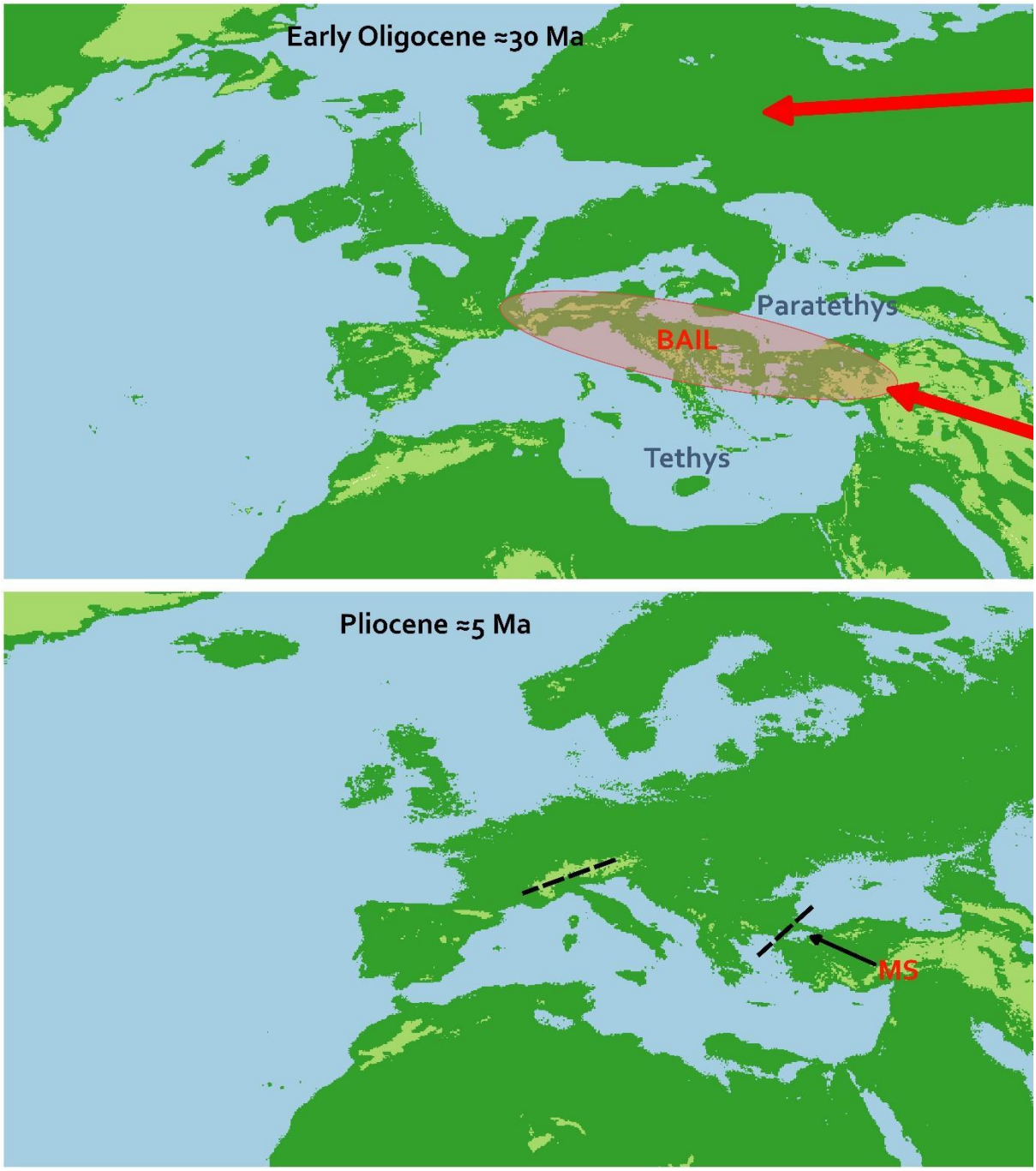


Figure 36. Map of the main paleogeographic events during the early Oligocene and Pliocene. Red arrows refer to the dispersal routes from Asia towards Europe. Dashed lines represent the geomorphological barriers during the Pliocene. BAIL: Balkanian-Anatolian-Iranian Landmass; MS: Marmara Sea. Map rasters from Scotese and Wright (2018).

3. The Miocene – Pliocene diversification

The radiation of the WP specimens of *Pisidium* occurred during the mid-Miocene and major speciation events that gave rise to the present southern and central WP species occurred largely starting from the Pliocene. Most of the splits between Moroccan and Iberian clades occurred between the late Miocene and early Pliocene.

During the Late Miocene, the Messinian Salinity Crisis (MSC) permitted a circumMediterranean connection from 5.6 to 5.3 Ma through freshwater courses allowing a faunal exchange between both sides of the Mediterranean basin (Froufe et al., 2016). Connections between adjacent areas of the Mediterranean remained possible also via brackish water that came from surrounding rivers draining the sea and the flood from the Parathetys Sea that permitted dispersal of specimens from eastern to Western Europe and Mediterranean countries through the “Lago Mare" between 5.5 and 5.3 Ma (Andreetto et al., 2021). However, the opening of the strait of Gibraltar afterwards led to the splitting between the Moroccan subclade and the European one (e.g. the Iberian subclade SC3.3 and the subclades SC1.3 and SC2.3), this same pattern was reported for mammals (Cosson et al., 2005), many reptiles (Pleguezuelos et al., 2008) and some invertebrates (Horn et al., 2006, Araujo et al. 2017).

The Pliocene has been postulated as the time of diversification of the Mediterranean *Pisidium*. The beginning of this period is characterized by high humidity in the Mediterranean region, which probably promoted the diversification of *Pisidium* in the area through the colonization of possible newly established freshwater ecosystems after the MSC. The Mio-Pliocene is also the period of final settling of geomorphological barriers across the Mediterranean (Italian Alps, Marmara Sea; Yaltirak et al., 2002), preventing direct dispersal and promoting the independent evolution of populations in the area, which explains the species radiation during this period. The affinity between Italian and Balkanian species could be a result of the exchange of freshwater taxa during glaciations that led to a drop in sea level and expansion of freshwater rivers from both sides of the Adriatic Sea during the lacustrine phase of the Mediterranean Sea (Bianco, 1990; Froufe et al., 2017).

4. The Late Pliocene to Pleistocene radiation

This period was characterized by the maximum of diversification rate of *Pisidium* throughout the W*P.* The climatic oscillations that marked the plio-pleistocene periods induced serious challenges (local environment changes and species interactions) for the organisms related to the colonization of new territories and facing new environments. These challenges caused the divergence and speciation of genomes (Hewit, 1996; 2000; Bibi and Kiessling, 2015).

The WP includes a number of ocean islands, which despite experiencing different climate conditions during Pliocene and Pleistocene, they were not affected by glaciations due to their isolation from the continent. This represents a triggering factor for the diversification of *Pisidium* during glaciations, especially in the Iberian Peninsulas (27650/27737). Starting from late Pliocene and extending to the Pleistocene, the WP *Pisidium* has known the highest radiation rate in the southern area (i.e. Mediterranean). This area has played an important role as

refuges during the Plio-Pleistocene glaciations. The clustering of some Northern and central European specimens within southern clades may be related to the interglacial phases where conditions were more favorable for the recolonization northwards. The Moroccan populations of *Pisidium* species showed a high genetic diversity alike many other taxa reported in different previous studies (Cosson et al., 2005; Perera and Harris, 2010; Sousa et al., 2010; Husemann et al., 2012). This diversity might be the result of topography across the area. Husemann et al. (2014) pointed the role that the Atlas Mountains played as orographic barriers for latitudinal shifts and, instead, acted as a refugium for species during drier and hotter interglacial phases. During our sampling, Moroccan specimens' distribution followed the Atlas and Rif Mountains direction (see Rassam et al., 2021b). A similar pattern was observed for taxa in the Alps and the Pyrenees (Schmitt, 2009; Mouret et al., 2011). Although, further investigations are needed to support and confirm these findings which represented a real pattern and not an artifact.

General Conclusion

In Morocco, the works related to the family of Sphaeriidae are limited to the few records of presence/absence which do not reach, in the majority of the cases, at the specific level. The diversity and distribution of the species of this family was therefore uncertain given the cryptic character of the species and the difficulty that their miniature size represents for the determination, in a precise way, of the outstanding features for their identification. In the present work, the presence of six species belonging to the family Sphaeriidae in Morocco was confirmed (five species of the genus *Pisidium* and only one species of the genus *Musculium*), which removes three species from the list previously provided by Kuiper*: P. milium*, *P. nitidum* and *P. tenuilineatum*. A detailed morphological description was presented for each species, highlighting the main characteristic features for their identification. Present mainly in riparian habitats, species of the family Sphaeriidae are unevenly distributed in the different Moroccan watersheds, the Sebou basin being the richest in terms of species, in contrast to the Bouregreg and Oued Eddahab-Sakia El Hamra basins where no species have been collected. A well-defined distribution pattern was observed in the Sphaeriidae, where the sites of occurrence of the species followed the Moroccan mountain ranges in an overlapping manner.

The identification of species of the genus *Pisidium*, in particular, may be compromised because of the high intraspecific morphological variability and, on the other hand, high interspecific morphological similarity, which could explain the number of species previously described by Kuiper. Morphometric geometric analysis has been of great importance in discerning morphological differences, often difficult to perceive with the naked eye. This analysis also allowed to focus on the intraspecific variability that results from environmental factors, the type of habitat in the case of the study of this thesis. Indeed, the type of habitat, with different geodynamic characters (current speed, nature of the sediment, etc), was revealed to have an influence on the morphology of the shell of the species *P. casertanum.*

A second possible reason for the absence of the three above-mentioned species from our species list could be the degradation of habitat quality. The increasing pressure on continental aquatic ecosystems (e.g. tourism, pumping, discharges) threatens all the fauna and flora that inhabit them. The review of the conservation status of species of the family Sphaeriidae in Morocco revealed that two species were already extinct at the regional scale, while another species is considered "vulnerable". The number of localities of occurrence, relatively large compared to what was previously known, has allowed the species (still present) to be moved to categories of lower danger according to the IUCN. Certainly, a management of ecosystems in a way that integrates the conservation of biodiversity is strongly necessary for the preservation of this diversity.

In this thesis, molecular and phylogenetic data on the genus *Pisidium* from Morocco were presented for the first time. These molecular analyses confirmed the identifications of the species with some reluctance as to the species *P.* (cf.) *personatum* and *Pisidium* sp., these two taxa showed morphological characteristics identical to the other corresponding species, however the inferred phylogeny showed some distinction between them and the other clades which is still to be confirmed by further analyses. The study of the phylogeography of *Pisidium* in the Western Palearctic, through the analysis of the calibrated molecular clock, has shown that the genus present in the studied area originated in Eurasia, from where it would have dispersed towards the beginning of the Oligocene epoch. This epoch has known various geological events that have led to the closure of the strait of Turgai that separated the actual Asia from Europe. This closure allowed the passage westward to colonize the habitats of northern and central Europe. At the same time, the Paratethys Sea was separated from the great Tethys Ocean by a land mass that favored the continental passage of taxa towards the Mediterranean part of Europe.

The majority of the divergence between the Moroccan and Iberian clades took place towards the end of the Miocene and the beginning of the Pliocene. This period is characterized during the Messinian stage by the drying of the Mediterranean followed by the immersion during the Zanclean, thus limiting the connections on both sides of the Mediterranean. The subsequent diversification of the species of the genus was, mainly, the results of dispersal and regression events from refuge areas during the Pleistocene glacial and interglacial cycles. It was concluded that Morocco also represented an additional refuge area to those described for the Mediterranean, through displacements in the altitudinal refuges that the Moroccan mountain ranges served. However, solid and well-supported conclusions on the phylogeography of *Pisidium*, especially in North Africa, imperatively require the inclusion of the Algerian and Tunisian specimens, in particular, in order to be able to draw a clearer picture taking into account the paleogeological events of the whole region. The family Sphaeriidae is an exceptional family because of its exclusive characteristics such as size, intra- and interspecific variation in its life history and the high rate of ploidy in the vast majority of species. All these characteristics deserve in-depth study to improve our understanding of the bio-ecology of this family. In addition, the ecotoxicological and bioindicator effects of the soft part and shell of the animals may also represent an avenue of great interest to explore.

Literature cited

Adam, W. 1960. Faune de Belgique : Mollusques terrestres et dulcicoles. *Institut Royal des Sciences Naturelles de Belgique, Bruxelles*, 402 pp.

Adams, D. C., Rohlf, F. J., Slice, D. E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, 71(1), 5–16.

Adams, D. C., Rohlf, F. J., Slice, D. E. 2013. A field comes of age: geometric morphometrics in the 21st century. *Hystrix*, 24(1), 7–14.

Alhejoj, A., Bandel, K., Salameh, E. M. 2017. Aquatic Mollusks: Occurrences, Identification and Their Use as Bioindicators of Environmental Conditions (Salinity, Trace Elements and Pollution Parameters) in Jordan. Water Ressources in Arid Areas: The Way Forward. *Springer Water*, 295–318 pp.

Alunno-Bruscia, M. M., Bourget, E., Frechette, M. 2001. Shell allometry and length-massdensity relationship for *Mytilus edulis* in an experimental food-regulated situation. *Marine Ecology Progress Series*, 219, 177–188.

Anderson, K. B. 1977. *Musculium transversum* in the Illinois River and an acute potassium bioassay method for the species. M.S. thesis, *Western Illinois University, Macomb*, 79 pp.

Andreetto, F., Krijgsman, W., Fortuin, A., Flecker, R., Beets, C. J. 2021. High Mediterranean water-level during the Lago-Mare phase of the Messinian Salinity Crisis: insights from the Sr isotope records of Spanish marginal basins (SE Spain). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 562, 1 - 19.

Araujo, R., Buckley, D., Nagel, K. O., García-Jiménez, R., Machordom, A. 2017. Species boundaries, geographic distribution and evolutionary history of the Western Palaearctic freshwater mussels *Unio* (Bivalvia: Unionidae). *Zoological Journal of the Linnean Society*, 20, 1–25.

Avise, J. C. 2000. Phylogeography: The History and Formation of Species. *Harvard University Press,* 447 pp.

Baker, F. C. 1982. The freshwater Mollusca of Wisconsin. Part II. Pelecypoda. Bull. N° 70, *Wisconian Geological Natural History Surveys, Madison*, 482 pp.

Balian, E. V., Lévêque, C., Segers, H., Martens, K. 2008. Freshwater animal diversity assessment. *Hydrobiologia*, 198, 1–637.

Barrios, V., Carrizosa, S., Darwall, W. R. T., Freyhof, J., Numa, C., Smith, K. 2014. Freshwater key biodiversity areas in the Mediterranean basin hotspot: informing species conservation and development planning in freshwater ecosystems. *IUCN*, 86 pp.

Benaissa, H., Teixeira, A., Lopes-Lima, M., Sousa, R., Varandas, S., Rassam, H., Ghamizi, M. 2019. Fish hosts of the freshwater mussel *Unio foucauldianus* Pallary, 1936. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29, 2176– 2184.

Benaissa, H., Ghamizi, M., Teixeira, A., Sousa, R., Rassam, H., Varandas, S., Lopes-Lima, M. 2021. Preliminary data on fish hosts and their conservation importance for the critically endangered *Pseudunio marocanus* (Pallary, 1918). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 32 (2), 229-238.

Bennett, S., Woods, T., Liyanage, W. and Smith, D. 1991. A Simplified General Method for Cluster Sample Surveys of Health in Developing Countries. *World Health Statist Quart*, 44, 98106.

Bertzky, B., Bertzky, M., Worboys, G. L., Hamilton, L. S. 2019. Earth's natural heritage. En G.L. Worboys, M. Lockwood, A.Kothari, S. Feary y I. Pulsford (eds.). Gobernanza y gestión deáreas protegidas, Bogotá: *Editorial Universidad ElBosque y ANU Press*, 43-80.

Bianco, P. G., Miller, P. J. 1990. Yugoslavian and other records of the Italian freshwater goby *Padogobius martensii* and a character polarisation in gobioid fishes. *Journal of Natural History*, 24, 1289-302.

Bibi, F., Kiessling, W. 2015. Continuous evolutionary change in Plio-Pleistocene mammals of eastern Africa. *Proceedings of the National Academy of Sciences U S A*, 112 (34), 10623-8.

Bickel, D. 1973. Non-marine mollusks and two new species of *Pisidium* from the Tongue River Formation (Paleocene), North Dakota. *Nautilus*, 87, 13-18.

Bieler, R., Mikkelsen, *P.* M., Collins, T. M., Glover, E. A., et al. 2014. Investigating the Bivalve Tree of Life—an exemplar-based approach combining molecular and novel morphological characters. *Invertebrate Systematics,* 28(1), 32–115.

Blackith, R. E., Reyment, R. A. 1971. Multivariate Morphometrics. *Academic Press: New York IX* +, 412 pp.

Bößneck, U., Clewing, C., Albrecht, C. 2016. Exploring high-mountain limnic faunas: discovery of a novel endemic bivalve species (Sphaeriidae : *Pisidium*) in the Nepal Himalayas. *Invertebrate Systematic*s, 30, 588–597

Bowden, J., Heppell, D. 1968. Revised list of British Mollusca. 2. Unionacea-Cardiacea. *Journal of Conchiliology. London*, 26, 237-272.

Brown, R .2009. Freshwater mollusks survive fish gut passage. *Arctic*, 60(2), 124-128.

Brusca, R. C., Brusca, G. J. 1990. Invertebrates. *Sunderland, MA: Sinauer Association*, 922 pp.

Burch, J. B .1975. Freshwater Sphaeriacean clams (Mollusca: Pelecypoda) of North America. *Malacological Publications, Hamburg, Michigan*, 96 pp.

Byrne, S. J., Butler, C. A., Reynolds, E. C., Dashper, S. G. 2018. Taxonomy of Oral Bacteria. *Methods in Microbiology*, 45, 171-201

Caill-Milly, N., Bru, N., Mahé, K., Borie, C., D'Amico, F. 2012, Shell shape analysis and spatial allometry patterns of Manila clam (*Ruditapes philippinarum*) in a mesotidal coastal lagoon. *Journal of Marine Sciences*, 1, 1–11

Cardinale, B. J., Duffy, J. E., Gonzalez, A., et al. 2012. Biodiversity loss and its impact on humanity. *Nature*, 486, 59-67

Cardoso, P., Erwin, T. L. et al. 2011. The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, 144, 2647-2655.

Carpenter, R. S., Stanley, E. E., Zanden, J. V. 2011. State of the World's Freshwater Ecosystems: Physical, Chemical and Biological Changes. *Annual Review of Environment and Resources*, 36 (1), 75-99.

Clarke, A. H .1979. Sphaeriidae as indicators of trophic lake stages. *Nautilus*, 94 (4), 178-184.

Clausnitzer, V., Kalkman, V. J., Ram, M., et al. 2009. Odonata enter the biodiversity crisis debate: the first global assessment of an insect group. *Biological Conservation*, 142, 1864–1869.

Clement, M., Posada, D., Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology, 9 (10), 1657-1660.

Clewing, C., Bössneck, U., Oheimb, *P.*, Albrecht, C .2013. Molecular phylogeny and biogeography of a high mountain bivalve fauna: The Sphaeriidae of the Tibetan Plateau. *Malacologia*, 56, 231-252.

Clewing, C., Geertz, T., Rassam, H., Woldekiros, T. H., Albrecht, C. 2022. Freshwater diversity at a biogeographical edge zone: the high-mountain pea-clams of Ethiopia. *Systematics and Biodiversity*, 20 (1), 1-15.

Colgan, D. J., Ponder, W. F., Eggler, P. E. 2000. Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta*, 29, 29–63.

Combosch, D. J., Collins, T. M., Glover, E. A., Graf, D. L., Harper, E. M., Healy, J. M., Kawauchi, G. Y., Lemer, S., McIntyre, E., Strong, E. E., Taylor, J. D., Zardus, J. D., Mikkelsen, P. M., Giribet, G., Bieler, R. 2017. A family-level Tree of Life for bivalves based on a Sanger sequencing approach. *Molecular Phylogenetics and Evolution*, 107:191-208.

Cooley, L. R., Ó Foighil, D. 2000. Phylogenetic analysis of the Sphaeriidae (Mollusca: Bivalvia) based on partial mitochondrial 16S rDNA gene sequences. *Invertebrate Biology*, 119, 299–308.

Cosson, J. F., Hutterer, R., Libois, R., Sarà, M., Taberlet, P., Vogel, P. 2005. Phylogeographical footprints of the Strait of Gibraltar and Quaternary climatic fluctuations in the western Mediterranean: a case study with the greater white-toothed shrew, *Crocidura russula* (Mammalia: Soricidae). *Molecular Ecology*, 14, 1151–1162.

Dabert, M., Bigos, A., Witalinski, W. 2011. DNA barcoding reveals andropolymorphism in *Aclerogamasus* species (Acari: Parasitidae). *Zootaxa*, 3015, 13–20.

Darriba, D., Taboada, G. L., Doallo, R., Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9 (8), 772.

Dauby, G., Stévart, T., Droissart, V., et al. 2017. ConR: An R package to assist largescale multispecies preliminary conservation assessments using distribution data. *Ecology and Evolution*, 7 (24), 1-12.

De Lattin, G. 1967. Grundriß der Zoogeographie. Verlag Gustav Fischer, Jena. Germany.

Di Piazza, F. D. 2006. Morocco in Pictures. *Twenty-First Century Books*, 80 pp.

Diehl, E., Jauker, B., Albrecht, C., Wilke, T., Wolters, V. 2018. GIEßEN: University Collections: Justus Liebig University Gießen. In: Beck L.A. (ed.) *Zoological Collections of Germany. The Animal Kingdom in its Amazing Plenty at Museums and Universities*, Springer, Cham., 373–381.

Doadrio, I. 1990. Phylogenetic relationships and classification of west Paleartic species of the genus *Barbus* (Osteichthyes, Cyprinidae). Aquatic Living Resources, 3, 265-282.

Dreher Mansur, M. C., Ituarte, C. F. 1999. Morphology of *Eupera elliptica* Ituarte & DreherMansur 1993, with comments on the status of the genera within the Euperinae (Bivalvia: Sphaeriidae). *Malacological Review*, Suppl. 8 (Freshwater Mollusca I): 59-68.

Dreher Mansur, M. C., Meier-Brook, C. 1992. Morphology of *Eupera* Bourguignat, 1854 and *Byssanodonta* Orbigny, 1846 and the phylogenetic affinities within the family Sphaeriidae (Bivalvia, Veneroida). In Giusti, F., Manganelli, G. (Eds) Abstracts of the 11th International Malacological Congress, Siena: Unitas Malacologica, 335–336 pp.

Dreher Mansur, M. C., Meier-Brook, C. 2000. Morphology of *Eupera* Bourguignat, 1854 and *Byssanodonta* Orbigny, 1846 with contributions to the phylogenetic systematics of Sphaeriidae and Corbiculidae (Bivalvia: Veneroida). *Archiv für Molluskenkunde*, 128, 1–59.

Drummond, A. J., Suchard, M. A., Xie, D., Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29(8), 1969-1973.

Dudgeon, D., Arthington, A. H., Gessner, M. O., et al. 2006 Freshwater Biodiversity: Importance, Threats, Status and Conservation Challenges. *Biological Reviews*, 81, 163-182.

Dyduch-Falniowska, A., Koziol, R. 1989. Anatomical and conchological characters in the systematics of the Uionidae of Poland. Malakologische Abhandlungen, *Staatliches Museum für Tierkunde Dresden*, 14(5), 35-52.

Dyduch-Falniowska, A.1982. Oscillations in density and diversity of *Pisidium* communities in two biotopes in southern Poland. *Hydrobiological Bulletin*, 16, 123-132.

Ellis, A. E. 1978. British freshwater bivalve Mollusca. *Linnean Society*: *Synopses of the British Fauna*, 11, 1-109.

Favre, J. 1940. La faune malacologique postglaciaire et actuelle du lac du Bourget. *Annales de L'Ecole Nationale des Eaux et Forêts et de la Station de Recherches et Expériences*, 7, 293–444.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Food and Agriculture Organization. 2003. Review of World Water Resources by Country. Water Reports, ISSN: 1020-1203.

Foote, B. A., 1976. Biology and larval feeding habits of three species of *Renocera* (Diptera: Sciomyzidae) that prey on fingernail clams (Mollusca: Sphaeriidae). *Annals of the Entomological Society of America*, 69, 121–133.

Frankiewicz, A. 2018. Guideline for field collecting and preserving Sphaeriidae clams, for DNA and taxonomic research. Detailed Sampling Procedures and Quality Control, Institute for Great Lakes Research.

Fuller, S. L. H. 1974, Clams and Mussels (Mollusca: Bivalvia), In Pollution Ecology of Freshwater Invertebrates, C.W., Hart and S.L.H., Fuller, Eds., *Academic press, New York,* 275-312 pp.

Funk, A., Reckendorfer, W. 2008. Environmental heterogeneity and morphological variability in *Pisidium subtruncatum* (Sphaeriidae, Bivalvia). *International Review of Hydrobiology*, 93(2), 188-199.

Gadzała-Kopciuch, R., Berecka, B., Bartoszewicz, J., Buszewski, B. 2004. Some considerations about bioindicators in environmental monitoring. *Polish Journal of Environmental Studies*, 13, 453–462.

Gale, W. 1973. Predation and parasitism as factors affecting *Sphaerium transversum* (Say) populations in Pool 19, Mississippi River. *Researches on Population Ecology*, 14(2), 169-187.

Gomes-dos-Santos, A., Froufe, E., Gonçalves, D. V., Sousa, R. et al. 2019. Freshwater conservation assessments in (semi) arid regions: testing river intermittence and buffer strategies using freshwater mussels (Bivalvia, Unionida) in Morocco. *Biological Conservation*, 236, 420-434.

Graf, D. L. 2013. Patterns of freshwater bivalve global diversity and the state of phylogenetic studies on the Unionoida, Sphaeriidae and Cyrenidae. *Amercian Malacological Bulletin*, 31 (1), 135-153.

Graf, D.L., Cummings, K.S. 2009. Actual and alleged freshwater mussels (Mollusca: Bivalvia: Unionoida) from Madagascar and the Mascarenes, with description of a new genus, *Germainaia*. *Proceedings of the Academy of Natural Sciences of Phladelphia,* 158, 221–238.

Green, R. H. 1971. A multivariate statistical approach to the Hutchinsonian niche: Bivalve molluscs of central Canada. *Ecology*, 32, 543-556.

Guralnick, R. P. 2005. Combined molecular and morphological approaches to documenting regional biodiversity and ecological patterns in problematic taxa: a case study in the bivalve group *Euglesa* (Sphaeriidae, Bivalvia) from western North America. *Zoologica Scripta*, 34 (5), 469–482.

Haas, F. 1969. Superfamilia Unionacea. In: R. Mertens, W. Hennig & H. Wermuth (eds.). *Das Tierreich. Eine Zusammenstellung und Kennzeichnung der rezenten Tierformen*. Lieferung 88: X + Berlin, 663 pp.

Hall, T. A. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hamill, S. E., Qadri, S. U., Mackie, G. L. 1979. Production and turnover of *Pisidium casertanum* (Pelecypoda; Sphaeriidae) in Ottawa River near Ottawa-Hull, Canada. *Hydrobiologia*, 62, 225– 230.

Hammer, O., Harper, D. A. T., Ryan, P. D. 2001. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4 (1), 9.

Heard, W. H. 1965. Comparative life histories of North American pill clams (Sphaeriidae: *Pisidium*). *Malacologia*, 2, 381–411.

Heard, W. H. 1977. Reproduction of fingernail clams (Sphaeriidae: *Sphaerium* and *Musculium*). *Malacologia*, 16, 421– 455.

Hebert, P. D. N., Ratnasingham, S., Dewaard, J. R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences*, 270, 1–4.

Herrington, H. B. 1962. A revision of the Sphaeriidae of North America (Mollusca: Pelecypoda). *Miscellaneous Publications, Museum of Zoology, University of Michigan*, 118, 1–74.

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, 68, 87-112.

Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. *Nature*, 405, 907-913.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359, 183-195.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society,* 58, 247-276.

Hinch, S. G., Kelly, L. J., Green, R. H. 1989. Morphological variation of *Elliptio complanata* (Bivalvia: Unionidae) in differing sediments of soft-water lakes exposed to acidic deposition. *Canadian Journal of Zoology*, 67, 1895–1899.

Holopainen, I. J., Hanski, I. 1986. Life history variation in *Pisidium* (Bivalvia: Pisidiidae). *Ecography*, 9 (2), 85-98.

Holopainen, I. J., Kuiper, J. 1982. Notes on the morphometry and anatomy of some *Pisidium* and *Sphaerium* species (Bivalvia, Sphaeriidae). *Annales Zoologici Fennici*, 19, 93-107.

Hooper, D. U., Chapin III, F. S., Ewel, J. J., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, 75, 3-35.

Horn, A. R., Kirsten, G. F., Kroon, S. M., et al. 2006. Phototherapy and exchange transfusion for neonatal hyperbilirubinemia: Neonatal academic hospitallas' consensus guidelines for South Africa hospitals and primary care facilities. *South African Medical Journal*, 96, 819-824.

Hornbach, D. J., Cox, C. 1987. Environmental influences on Life history Traits in *Pisidium casertanum* (Bivalvia, Pisidiidae) – field and laboratory experimentation. *American Malacological Bulletin*, 5, 49–64.

Horsák, M., Hájek, M. 2003. Composition and species richness of molluscan communities in relation to vegetation and water chemistry in the western Carpathian spring fens: The poor–rich gradient. *Journal of Molluscan Studies*, 69 (4), 349-357.

Horsák, M., Hájek, M., Tichý, L., Juřičková, L. 2007. Plant indicator values as a tool for land mollusc autecology assessment. *Acta Oecologica*, 32 (2), 161-171.

Hu, L., Zhao, Y. E., Yang, Y.J., Niu, D. L., Yang, R. 2019. LSU rDNA D5 region: the DNA barcode for molecular classification and identification of Demodex. *Genome*, 62, 295–304.

Hubendick, B. 1978. Systematics and comparative morphology of the Basommatophora. *In*: Fretter V., Peake, J. (Eds.) *Pulmonates*, 2A: *Academic Press, London*. 1–47 pp.

Huntley, B., Birks, H. J. B. 1983. An atlas of past and present pollen maps for Europe: 0-13 000 years ago. *Cambridge University Press, Cambridge-London-New York-New RochelleMelbourne-Sydney*, 667 pp.

Husemann, M., Schmitt, T., Stathi, I., Habel, J. C. 2012. Evolution and radiation in the scorpion *Buthus elmouatakili* (Scorpiones: Buthidae) at the foothills of the Atlas Mountains (North Africa). *Journal of Heredity*, 103, 221– 229.

Husemann, M., Schmitt, T., Zachos, F. E., Ulrich, W., Habel, J.C. 2014. Palaearctic biogeography revisited: evidence for the existence of a North African refugium for Western Palaearctic biota. *Journal of Biogeography*, 41, 81–94.

Ituarte, C. F. 1997. The role of the follicular epithelium in the oosorption process in *Eupera platensis* Doello Jurado, 1921 (Bivalvia: Sphaeriidae): a light microscope approach. *Veliger*, 40, 47-54.

IUCN. 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, U.K.

IUCN. 2012. IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK.

Kallimanis, A., Mazaris, A., Tzanopoulos, J., Halley, J., Pantis, J., Sgardelis, S. 2008. How does habitat diversity affect the species–area relationship? *Global Ecology and Biogeography*, 17 (4), 532-538.

Kappes, H., Haase, P. 2012. Slow, but steady: dispersal of freshwater molluscs. *Aquatic Sciences*, 74, 1–14.

Kasprzak, K. 1986. Role of Unionidae and Sphaeriidae (Mollusca, Bivalvia) in the Eutrophic Lake Zbęchy and its Outflow. *Revue der gesamten Hydrobiologie und Hydrographie*, 71 (3), 315-334.

Katoh, K., Standley, D. M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30 (4), 772-780.

Keen, M., Casey, H. 1969. Superfamily Corbiculacea. In: Moore, RC (ed.) Treatise on Invertebrate Paleontology. Pt. N, vol. 2, Mollusca 6. Bivalvia. *The Geological Society of America, New York, and University of Kansas Press, Lawrence,* U.S.A. 665-669 pp.

Kendall, D. G. 1977. The diffusion of shape. *Advances in Applied Probability*, 9, 428–430.

Kilgour, B. W., Mackie, G. L. 1988. Factors affecting the distribution of Sphaeriid bivalves in Britannia Bay of the Ottawa River. *Nautilus*, 102, 73–77.

Killeen, I., Aldridge, D., Oliver, G. 2004. Freshwater bivalves of Britain and Ireland. *First edition. Field Studies Council*, 119 pp.

Komaru, A., Kawagishi, T., Konishi, K. 1998. Cytological evidence of spontaneous androgenesis in the freshwater clam *Corbicula leana* Prime. *Development Genes and Evolution,* 208, 46–50.

Kořínková, T., Král, J. 2011. Structure and meiotic behaviour of B chromosomes in *Sphaerium corneum*/*S. nucleus* complex (Bivalvia: Sphaeriidae). *Genetica*, 139 (2), 155-65.

Kořínková, T., Morávková, A. 2010. Does polyploidy occur in central European species of the family Sphaeriidae (Mollusca: Bivalvia)? *Central European Journal of Biology*, 5 (6), 777-784

Korniushin, A. V. 1998. On the identity of the anatomically distinct form of *Pisidium casertanum* (Poli) (=*P. roseum* sensu Korniushin 1995). *Heldia*, 2 (5/6), 133-135.

Korniushin, A. V. 2000. Review of the family Sphaeriidae (Mollusca: Bivalvia) of Australia, with the description of four new species. *Records of Australian Museum*, 52, 41-102.

Korniushin, A. V., Glaubrecht, M. 2002. Phylogenetic analysis based on the morphology of viviparous freshwater clams of the family Sphaeriidae (Mollusca, Bivalvia, Veneroida). *Zoologica Scripta*, 31 (5), 415–459.

Korniushin, A., Glaubrecht, M. 2001. Annotated catalogue of the type specimens of Sphaeriidae (Bivalvia, Heterodonta, Veneroidea) in the Mollusc collection of the Museum für

Naturkunde Berlin, with a review of their current taxonomic status. *Mitteilungen aus dem Museum für Naturkunde zu Berlin, Zoologische Reihe.*, 77 (1), 131-152.

Kubíková, L., Simon, O., Fricová, K. 2011. The occurrence of *Pisidium* species (Bivalvia: Sphaeriidae) in oligotrophic springs of the Blanice River catchment (Czech Republic) in relation to ecological conditions. *Biologia*, 66 (2), 299-307.

Kuiper, J. G. J. 1964. Kritische Uebersicht der in Nord-Afrika lebenden Arten des Genus *Pisidium*. *Archiv für Molluskenkunde*, 93, 127-137.

Kuiper, J. G. J. 1966. La distribution des espèces vivantes du genre *Pisidium* C.P.F. en France. *Journal de Conchyliologie*, 105, 181-215.

Kuiper, J. G. J. 1972. Une récolte de *Pisidium* dans le Moyen Atlas. *Basteria*, 36, 2-5.

Kuiper, J. G. J. 1972. Sphaeriidenfunde in den pliozänen Deckschichten der rheinischen Braunkohle. Arch. für Molluskenkd, 102, 125–130.

Kuiper, J. G. J. 1974. Die Pisidien der Hochalpengewässer. *Archiv für Molluskenkunde*, 104(1/3), 1-27.

Kuiper, J. G. J. 1983. The Sphaeriidae of Australia. *Basteria*, 47, 3–52.

Kuiper, J. G. J., Økland, K. A., Knudsen, J., Koli, L., Proschwitz, T., Valovirta, I. 1989. Geographical distribution of the small mussels (Sphaeriidae) in North Europe (Denmark, Faries, Finland, Iceland, Norway and Sweden). *Annales Zoologici Fennici*, 26, 73-101.

Lee, T. 1999. Polyploidy and meiosis in the freshwater clam *Sphaerium striatinum* (Lamarck) and chromosome numbers in the Sphaeriidae (Bivalvia, Veneroida). *Cytologia*, 64, 247– 252.

Lee, T. 2004. Morphology and phylogenetic relationships of genera of North American Sphaeriidae (Bivalvia, veneroida). *American Malacological Bulletin*, 19, 1–13.

Lee, T., Ó Foighil, D. 2002. 6-phosphogluconate dehydrogenase (PGD) allele phylogeny is incongruent with a recent origin of polyploidization in some North American Sphaeriidae (Mollusca, Bivalvia). *Molecular Phylogenetics and Evolution*, 25, 112–124.

Lee, T., Ó Foighil, D. 2003. Phylogenetic structure of the Sphaeriinae, a global clade of freshwater bivalve molluscs, inferred from nuclear (ITS-1) and mitochondrial (16S) ribosomal gene sequences. *Zoological Journal of the Linnean Society*, 137, 245-260.

Lévêque, C., Balian, E. V. 2005. Conservation of Freshwater Biodiversity Does the Real World Meet Scientific Dreams. *Hydrobiologia*, 542, 23-26.

Lopes-Lima, M., Teixeira, A., Froufe, E., Lopes, A., Varandas, S., Sousa, R. 2014. Biology and conservation of freshwater bivalves: past, present and future perspectives. *Hydrobiologia*, 735, 1–13.

Lydeard, C., Cowie, W. F., Ponder, R. H., Bogan, A. E., Bouchet, *P.*, Clark, S.A. 2004. The global decline of nonmarine mollusks. *Bioscience*, 54, 321-330.

Mace, G. M., Norris, K., Fitter, A. H. 2012. Biodiversity and ecosystem services: a multilayered relationshi*P. Trends in Ecology & Evolution*, 27 (1), 19-26.

Mackie, G. L. 1976. Trematode parasitism in the Sphaeriidae clams, and the effects in three Ottawa River species. *Nautilus*, 90, 36-41.

Mackie, G. L. 1978. Are sphaeriid clams ovoviviparous or viviparous? *Nautilus*, 92, 145-146.

Mackie, G. L. 1979. Dispersal mechanisms in Sphaeriidae (Mollusca: Bivalvia). *Bulletin of the American Malacological Union*, 45, 17-21

Mackie, G. L., Qadri, S. U., Clarke, A. H. 1974. Development of brood sacs in *Musculium secures*: Sphaeriidae. *Nautilus*, 88 (1), 109-111.

Mackie, G. L., White, D. S., Zbeda, T. W. 1980. A guide to freshwater mollusks of the Laurentian Great Lakes with special emphasis on the genus *Pisidium*. EPA Report 600/3-80068. Environmental Research Laboratory, Office of Research & Development, U. S. Environmental Protection Agency, Duluth, Minnesota, 144 pp.

Mahulu, A., Stelbrink, B., Bocxlaer, B. V., Riedel, F., Albrecht, C. 2021. Going with the flow? Diversification of gastropods reflects drainage evolution in Africa. *Journal of Biogeography*, 48(7), 1579-1593.

Marković, Z., Milivojević, M., De Bruijn, H., et al. 2018. Paleontological research on fossil small mammals from the open-pit coal mines of Bosnia and Herzegovina: Overview of results. *Bulletin of the Natural History Museum*, 11, 7-17.

Martin, S. M. 1998. Freshwater Fingernail and Pea Clams (Bivalvia: Veneroida: Sphaeriidae) of Maine. *Northeastern Naturalist*, 5 (1), 29-60.

McMahon, R. F. 1991. Mollusca: Bivalvia. In: Ecology and classification of North American freshwater invertebrates. Thorp JH & Covich AP, eds., *Academic Press, New York*. 315-399 pp.

Meier-Brook, C. 1970. Untersuchungen zur Biologie einiger *Pisidium*-Arten (Mollusca; Eulamellibranchiata; Sphaeriidae). *Archiv für Hydrobiologie*, 38, 73-150.

Mittermeier, R. A., Gil, P. R., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C. G., Lamoreux, J., daFonseca, G. A. B. 2004. Hotspots Revisited: Earth's biologically richest and most endangered terrestrial ecoregions, *CEMEX, Mexico City*, 431 pp.

Mittermeier, R. A., Mittermeier, C. G., Gil, P. R., Pilgrim, J., da Fonseca, G. A. B., Konstant, W. R., Brooks, T. 2002. Wilderness: Earth's last wild places, *CEMEX, Mexico City*, 576 pp.

Mogie, M. 1986. Automixis: its distribution and status. *Biological Journal of the Linnean Society*, 28 (3), 321-329.

Moorkens, E. A., Killeen, I. J. 2009. Database of association with habitat and environmental variables for non-shelled slugs and bivalves of Britain and Ireland. Irish Wildlife Manuals, 41. National Parks and Wildlife Service, Ireland.

Mouret, V., Guillaumer, A., Cheylan, M., Pottier, G., Ferchaud, A. L., Crochet, P. A. 2011. The legacy of ice ages in mountain species: post-glacial colonization of mountain tops rather than current range fragmentation determines mitochondrial genetic diversity in an endemic Pyrenean rock lizard. *Journal of Biogeography*, 38, 1717–1731.

Mouthon, J. 1983. Les Malacocénoses de quatre lacs haut-alpins. *Annales de Limnologie*, 19 (2), 101-106.

Mouthon, J., Taïr-Abbaci, K. 2012. The taxonomic confusion surrounding *Pisidium* (Bivalvia, Sphaeriidae): the possible birth of a new taxon. *Basteria*, 76 (4-6), 126-130.

Mueller, R. L. 2006. Evolutionary rates, divergence dates, and the performance of mitochondrial genes in Bayesian phylogenetic analysis. *Systematic Biology*, 55, 289–300.

Myzyk, S. 2017. On the reproduction of *Pisidium* C. Pfeiffer, 1821 (Bivalvia: Sphaeriidae) from Sąpolno (NW. Poland). *Folia Malacologia*, 25, 175–194.

Needham, J. 1959. Science and Civilization in China, Volume 3: Mathematics and the Sciences of the Heavens and Earth. *Cambridge University Press, Cambridge*, 877 pp.

Nesemann, H. F., Sharma, S. 2005. Illustrated checklist of pea clams (Mollusca: Sphaeriidae) from Nepal. *Himalayan Journal of Sciences*, 3 (5), 57-65.

Nordsieck, H. 1982. Zur Stratigraphie der neogenen Fundstellen der Clausiliidae und Triptychiidae Mittel- und Westeuropas (Stylommatophora, Gastropoda). Mitteilungen der Bayer. *Staatssammlung für Paläontologie und Hist. Geol*., 22, 137–155.

Nylander, O. 1941. Marl deposit in Houlton, Aroostook County, Maine. *Nautilus*, 54 (4), 143144.

Nylander, O. 1943. Marl deposits in Bonaventure, north of Bay Chaleur, Quebec, Canada, and in Houlton, Maine. *Nautilus*, 57 (2), 45-46.

Ó Foighil, D., Thiriot-Quievreux, C. 1991. Ploidy and pronuclear interaction in Northeastern Pacific Lasaea clones (Mollusca: Bivalvia). *The Biological Bulletin*, 181, 222-231.

Økland, K., Kuiper, J. G. J. 1982. Distribution of small mussels (Sphaeriidae) in Norway, with notes on their ecology. *Malacologia*, 22, 469-477.

Pallary, P. 1915. Description de quelques mollusques nouveaux du Grand Atlas. Bull. Mus. Nat. Hist. Nat. Paris 21, 21-28., 1921. Faune malacologique du Grand Atlas. *Journal de Conchyliologie*, 66, 89-185.

Pallary, P. 1921. Faune malacologique du Grand Atlas. *Journal de Conchyliologie*, 66, 89-185. , 1927. Complément à la faune malacologique de la Berbérie (suite). *Journal de Conchyliologie*, 71, 197-277.

Pallary, P. 1936. Deuxième complément à la faune malacologique de la Berbérie. *Journal de Conchyliologie*, 80, 5-65.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. 1991. *The Simple Fool's Guide to PCR. Version 2. Honolulu, Hawaii: The University of Hawaii*, 45 pp.

Park, J. K., Ó Foighil, D. 2000. Sphaeriid and Corbiculid Clams Represent Separate Heterodont Bivalve Radiations into Freshwater Environments. *Molecular Phylogenetics and Evolution*, 14 (1), 75-88.

Perea, S., Böhme, M., Zupančič, P., Freyhof, J., et al. 2010. Phylogenetic relationships and biogeographical patterns in Circum-Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. *BMC Ecology and Evolution*, 10, 265.

Perera, A., Harris, D. J. 2010. Genetic Variability in the Ocellated Lizard *Timon tangitanus* in Morocco. *African Zoology*, 45 (2), 321-329.

Petkevičiūtė, R., Stanevičiūtė, G., Stunžėnas, V., Lee, T., Ó Foighil, D. 2007. Pronounced karyological divergence of the North American congeners *Sphaerium rhomboideum* and *S. occidentale* (Bivalvia: Veneroida: Sphaeriidae), *Journal of Molluscan Studies*, 73, 4, 315–321.

Piechocki, A. 1989. The Sphaeriidae of Poland (Bivalvia, Eulamellibranchia). *Annales Zoologici*, 24, 249-320.

Pleguezuelos, J. M., Fahd, S., Carranza, S. 2008. El papel del Estrecho de Gibraltar en la formación de la actual fauna de anfibios y reptiles en el Mediterráneo Occidental. *Boletín de la Asociación Herpetológica Española*, 19, 2-17.

Poli, S. G. 1791. Testacea utriusque Siciliae eorumque historia et anatome tabulis aeneis illustrata, vol. 1. *Regio Typographeio, Parmae*, 341 pp.

Ponder, W. F., Lunney, D. 1999. The Other 99%: The Conservation and Biodiversity of Invertebrates. Royal Zoological Society of New South Wales, Mosman, Australia.

Popov, S.V., Rögl, F., Rozanov, A.Y., et al. 2004. Lithological-paleogeographic maps of Paratethys-10 maps late Eocene to Pliocene. *Courier Forschungsinstitut Senckenberg*, 250, 1-46.

Preston, S. J., Roberts, D. 2007. Variation in shell morphology of *Calliostoma zizyphinum* (Gastropoda: Trochidae). *Journal of Molluscan Studies*, 73, 101–104.

Provan, J., Bennett, K. D. 2008. Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution*, 23, 564–571.

Rambaut, A. 2010. FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

Rassam, H., Albrecht, C., Sousa, R., Lopes-Lima, M., Benaissa, H., Ghamizi, M. 2021a. Intraspecific variation in the common pea clam *Pisidium casertanum* (Poli, 1791) (Bivalvia: Sphaeriidae): A geometric morphometric analysis. *Malacologia*, 63, 183-194.

Rassam, H., Benaissa, H., Ghamizi, M. 2021c. Review of the reference collection of freshwater bivalves deposited in the Scientific Institute of Rabat. *Bulletin de l'Institut Scientifique*, 43, 1–7.

Rassam, H., Ghamizi, M., Benaissa, H., Clewing, C., Albrecht, C. 2021b. The fingernail clams (Bivalvia: Veneroida: Sphaeriidae) of Morocco: Diversity, distribution and conservation status. Biodiversity Data Journal 9: e73346.

Rassam, H., Moutaouakil, S., Benaissa, H., Albrecht, C., Ghamizi, M. 2020. First record of *Pisidium subtruncatum* Malm, 1855 (Bivalvia, Sphaeriidae) in an African cave. *Subterranean Biology*, 34, 99–108.

Razkin, O., Sonet, G., Breugelmans, K., Madeira, M. J., Gómez-Moliner, B. J., Backeljau, T. 2016. Species limits, interspecific hybridization and phylogeny in the cryptic land snail complex *Pyramidula*: The power of RADseq data. *Molecular Phylogenetics and Evolution*, 101, 267278.

Rees, W. J. 1965. The aerial dispersal of Mollusca. *Journal of Molluscan Studies*, 36 (5), 269–282.

Reid, A. J., Carison, A. K., Hanna D. E. L., Olden, J. D., Ormerod S. J., Cooke, S. J. 2020. Conservation challenges to freshwater ecosystems. In book: Reference Module in Earth Systems and Environmental Sciences. Encyclopedia of the World's Biomes.*Elsevier*, 270-278 pp.

Reid, N. M., Carstens, B. C. 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC Evolutionary Biology, 12, 196.

Rögl, F. 1998. Palaeogeographic Considerations for Mediterranean and Paratethys Seaways (Oligocene to Miocene). *Annalen des Naturhistorischen Museums in Wien*, 99, 279–310.

Rögl, F. 1999. Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography. *Geologica carpathica*, 50, 339-349.

Rohlf, F. J. 2017. tpsDig2 v.2.27. State University of New York at Stony Brook.

Rohlf, F. J. 2019. tpsRelw, relative warps analysis,. 1.53. Department of Ecology & Evolution, State University of New York at Stony Brook.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, et al. 2012. MrBayes 3.2 : efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61 (3), 539-42.

Sanmartin, I., Enghoff, H., Ronquist, F. 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biological Journal of the Linnean Society*, 73, 345-390.

Saunders, J., Rung, G. 1990. Species distributions and shell characteristics of *Pisidium* (Mollusca: Bivalvia) in the Colorado Front Range: the role of abiotic factors. *Freshwater Biology*, 24 (2), 275-285.

Scarlato, O. A. 1981. Research of the Soviet malacologists in the recent years. *Venus*, 40, 160- 176.

Schmitt, T. 2009. Biogeographical and evolutionary importance of the European high mountain systems. *Frontiers in Zoology*, 6, 9.

Schneider, S., Prieto, J. 2011. First record of an autochthonous community of fluviatile freshwater molluscs from the Middle/Late Miocene Upper Freshwater Molasse (southern Germany). Archiv für Molluskenkunde, 140, 1–18.

Schultheiß, R., Albrecht, C., Bößneck, U., Wilke, T. 2008. The neglected side of speciation in ancient lakes: phylogeography of an inconspicuous mollusc taxon in lakes Ohrid and Prespa. *Hydrobiologia*, 615 (1), 141-156.

Scotese, R. C., Wright, N. 2018. PALEOMAP Paleodigital Elevation Models (PaleoDEMS) for the Phanerozoic. PALEOMAP Project, Research School of Earth Sciences, Australian National University.

Smith, E. P. 1995. 'Design and Analysis of Multispecies Experiments', in Cairns Jr., J. and Niederlehner, B. R. (eds.), Ecological Toxicity Testing: Scale, Complexity, and Relevance, Lewis Publishers, London, 73-96 pp.

Sneen, M., Cummings, K. S., Minarik, T., Wasik, J. 2009. The Discovery of the Nonindigenous, Mottled Fingernail Clam, Eupera cubensis (Prime, 1865) (Bivalvia: Sphaeriidae) in the Chicago Sanitary and Ship Canal (Illinois River Drainage), Cook County, Illinois. 35 (4), 627-629.

Sousa P., Froufe, E., Alves, P. C., Harris, D. J. 2010. Genetic diversity within scorpions of the genus *Buthus* from the Iberian Peninsula: mitochondrial DNA sequence data indicate additional distinct cryptic lineages. *Journal of Arachnology*, 38, 206-211.

Sousa, R., Teixeira, A., Benaissa, H., Varandas, S., Ghamizi, M., Lopes-Lima, M. 2019. Refuge in the sāqya: Irrigation canals as habitat for one of the world's 100 most threatened species, *Biological Conservation*, 238, 108209.

Sousa, R., Teixeira, A., Santos, A., Benaissa, H., Varandas, S., Ghamizi, M. et al. 2018. Oued Bouhlou: A new hope for the Moroccan pearl mussel. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28 (1), 247-251.

Sousa, R., Varandas, S., Teixeira, A., Ghamizi, M., Froufe, E., Lopes-Lima, M. 2016. Pearl mussels (*Margaritifera marocana*) in Morocco: conservation status of the rarest bivalve in African fresh waters. *Science of the Total Environment*, 547, 405-412.

Stewart, J. R., Lister, A. M., Barnes, I., Dalén, L. 2010. Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B*, 277, 661–671

Strayer, D. L., Downing, J. A., Haag, W. R.. King, T. L., Layzer, J. B., Newton, T. J., Nichols, S. J. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience*, 54, 429–439.

Strayer, D. L., Dudgeon, D. 2010. Freshwater Biodiversity Conservation Recent Progress and Future Challenges. *Freshwater Science*, 29, 344-358.

Swanson, A. M., Ormerod, S. J. 2005. The distribution and conservation of threatened Sphaeriidae on British grazing marshland. *Biodiversity and Conservation*, 14 (9), 2207–2220.

Taberlet, P., Fumagalli, L., Wust-Saucy, A. G., Cosson, J. F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, 7, 453–464.

Talami, Z. 1998. Application des méthodes biologiques et chimiques pour la détermination de la qualité des eaux des rivières du Haut-Atlas marocain. Université Cadi Ayyad.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30 (12), 27252729.

Taylor, J. D., Williams, S. T., Glover, E. A., Dyal, P. 2007. A molecular phylogeny of heterodont bivalves (Mollusca: Bivalvia: Heterodonta): New analyses of 18S and 28S rRNA genes. *Zoologica Scripta*, 36 (6), 587–606.

Thorp, J. H., Covich, A. P. 2010. Ecology and Classification of North American Freshwater Invertebrates. *Academic Press*, 1021 pp.

Ubukata, T. 2000. Theoretical morphology of hinge and shell form in Bivalvia: geometric constraints derived from space conflict between umbones. *Paleobiology*, 26, 606–624.

Van Damme, D. 1984. The Freshwater Molluscs of Northern Africa: Distribution, Biogeography and Palaeoecology. *Springer Netherlands*, 164 pp.

Wagner, R., Marxsen, J., Zwick, P., Cox, E. J. 2011. Central European stream ecosystems: The long-term study of the Breitenbach. *John Wiley & Sons*, 69 pp.

Wang, J., Yu, X., Gong, W., Liu, X., et al. 2022. EZH2 noncanonically binds cMyc and p300 through a cryptic transactivation domain to mediate gene activation and promote oncogenesis. *Nature Cell Biology*, 24 (3), 384-399.

Wilke, T., Davis, G. M. 2000. Infraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissooidea: Gastropoda): do their different life histories affect biogeographic patterns and gene flow? *Biology Journal of the Linnean Society*, 70, 89–105.

Wilke, T., Davis, G. M., Qiu, D. C., Spear, R. C. 2006. Extreme mitochondrial sequence diversity in the intermediate schistosomiasis host *Oncomelania hupensis robertsoni* : another case of ancestral polymorphism? *Malacologia*, 48 (1–2), 143–157.

Wilke, T., Schultheiß, R., Albrecht, C. 2009. As time goes by: a simple fool's guide to molecular clock approaches in invertebrates. *American Malacological Bulletin*, 27, 25–45.

Williams, J. D., Butler, R. S., Warren, G.L, Johnson, N. A. 2014. Freshwater mussels of Florida. *University of Alabama Press*, 498 pp.

Williams, K. J., Ford, A., Rosauer, D. F., et al. 2011. Forests of east Australia: the35th biodiversity hotspot. In F. E. Zachos and J. C.Habel (eds.). Biodiversity Hotspots: Distribution and protection of conservation priority areas, *Springer, Berlin*, 295–310 pp.

Woese, C. R., Fox, G. E. 1977. Phylogenetic structure of prokaryotic domain—primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*, 74 (11), 5088–5090.

Wurtz, C. B. 1955. Stream biota and stream pollution. *Sewage and Industrial Wastes*, 27, 1270–1278.

Yaltırak, C. 2002. Tectonic evolution of the Marmara Sea and its surroundings. *Marine Geology*, 190, 493–529

Zając, K., Zając, T., Ćmiel, A. 2017. What can we infer from the shell dimensions of the thickshelled river mussel *Unio crassus*? *Hydrobiologia*, 810, 415–431.

Zettler, M. L. 1996. The aquatic malacofauna (Gastropoda et Bivalvia) in the catchment area of a North German Lowland river, the Warnow. *Limnologica*, 26, 327-337.

Zettler, M., Daunys, D. 2007. Long-term macrozoobenthos changes in a shallow boreal lagoon: Comparison of a recent biodiversity inventory with historical data. *Limnologica*, 37 (2), 170185.

Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29 (22), 2869-2876.

Declaration/Erklärung

“I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of an anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University Giessen „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“in carrying out the investigations described in the dissertation.”

…….

Hanane Rassam

Giessen, December 2022

5. Appendix

Table 6. Coordinates of the sites of occurrence of sphaeriidae in morocco with cross-presence of species by basin. DRA: Drâa-Ziz-Rhéris basin, OER: Oum Er Rabia basin, SEB: Sebou basin, TEN: Tensift basin, SM: Souss-Massa basin, ML: Moulouya basin, LK: Loukkos basin, Pca: *P. casertanum,* Ppe: *P.* (cf.) *personatum*, Psu: *P. subtruncatum,* Pam: *P. amnicum*, Mla: *M. lacustre.* The cross is marked in bold where *P*. (cf.) *personatum* was present.

Site	Longitude	Latitude	Bassin	Pca	Ppe	Psu	Pam	Mla
Télouet	31°16’54’’N	7°08’57.6"O	DRA	X				
Zaouit Cheikh	32°38'39.6"N	5°54'48.8"O	OER	X		X		
Sources O. Rbiaa	33°03'11.41"N	5°24'50.23"O	OER	X				
Ouiouane	33°07’48’’N	5°21’10.2’’O	OER	X	X			
Aguelmame Sidi Ali	33°04’25.92"N	5°00’11.28’’O	SEB	X				
O. Guigou	32°53'20.58"N	5° 2'58.56"O	SEB	X		X		
O. Mikkès	33°33’44.4’’N	5°07'25.8’’O	SEB	X				
Hachlaf (outlet)	33°34'50.1"N	4°58'44.1"O	SEB			X		X
Hachlaf (dam)	33°34'50.1"N	4°58'44.1"O	SEB	X			X	
Dayet Ifrah	33°33'31.00"N	4°55'47"O	SEB	X				
Imlil	31°03’54.6’’N	7°56’14.4’’O	TEN	X				
Tiferguine	31°11'48.6"N	7°50'03"O	TEN	X		X		
Amghass1	33°22'52.32"N	5°26'30.61"O	SEB	X	**X**			
Aguelmame Azegza	32°58'25.15"N	5°26'42.24"O	OER	X				
Oued Boulmane	31°11'46.00"N	6°10'7"O	DRA	X				
Oued Mgoune	31°22'25.00"N	5°59'29"O	DRA	X				

Zegmouzen	30°31'60.00"N	7°55'57"O	SM	X				

Grotte Ifri N'Touya (Ait M'hamed)	31°52'46.45"N	6°27'01.87"O	OER			X		
Grotte Ifri N'Touya	31°52'46.45"N	6°27'01.87"O	OER		X	X		
Ouzioua	30°44'11.00"N	7°55'57"O	SM	X				
Région Tighdouine	31°24'28.12"N	7°29'23.99"O	TEN	X				
Source Tizirt	31°17'38.11"N	7°29'33.38"O	TEN	X				
Oued Chbouka	32°52'34.43"N	5°22'10.21"O	OER			X		
Source Chbouka	32°52'48,34"N	5°22'04,73"O	OER			X		
Lac Miaami	32°54'13.17"N	5°22'45.30"O	OER			X		
Source, route Bouleman st.3	33°29’58.25’’N	5°04’42.06’’O	SEB	X		X		
Tizguit (st.4)	33°30’42.25’’N	5°05’16.72’’O	SEB	X				
Tizguit (st.5)	33°33’25.8”N	5°06’15.23’’O	SEB		X	X		
Tizguit, Termila	33°32’44.86”N	5°06’20.17”O	SEB	X				
Mikkès, route Zaouit Ifrane	33°32’31.08”N	5°06’54.73”O	SEB	X		X		
Ain Vitel, en aval de la source	33°32'50.27"N	5°06'44.99"O	SEB	X				
Ain Aghbal (Azrou)	33°26'23.73"N	5°14'47.65"O	SEB	X				

Oued Tassaout	31°24'57.91"N	6°47'20.73"O	OER		**X**			

Lac Isli	32°12'49.84"N	5°32'57.13"O	OER	X				
Dayet Iffer	33°36'23.04"N	4°54'28.33"O	SEB	X				
Source Ouayfirte	31°26'17.3"N	7°32'6.5"O	TEN		X			
Ait Bououli, rte Ait-Bouguemez	31°36'45.47"N	6°35'13.90"O	OER		X			
Ait Bouguemez	31°38'25,49"N	6°28'40.75"O	OER	X	X			
Rte entre Ighil et Tanmrt	31°41'29.85"N	6°32'14.71"O	OER	X				
Source Mlaeb	34° 3'40.92"N	4°9'51.64"O	SEB	X				
Source Kawan	34°6'37.49"N	4°19'13.61"O	SEB	X				
Tazlida	31°25'35"N	7°24'39"O	TEN	X				
Tizguit	33°35’8.376’’N	5°09’2.376’’O	SEB	X				
Route Chhida	33°24’19.44’’N	5°23’13.2’’O	SEB	X				
Rte Sidi Addi	33°23'58.71"N	5°19'33.05"O	SEB		**X**			
Oued Tigrigra	33°25’31.51’’N	5°16’23.88’’O	SEB	X				
Ain Sultan	33°43’15.6’’N	5°00’17.71’’O	SEB		**X**			
Ain Fendel	34°05’44.41’’N	4°26’24.36’’O	SEB	X		X		
Source Tamda	31°18'50.74"N	7°3'53.89"O	DRA		**X**			
Rte Aghbalou N'Serdane	32°41'00.1"N	5°32'55.3"O	SEB	X				
L'ksabi	32°50'06.88"N	4°24'37.26"O	MOL	X				

Oued Majjo	35°06'20.15"N	5°11'32.19"O	LK		X			
Grotte Izoura	34°05'41.00"N	4°05'55.72"O	SEB		X			
Séguia région Ain Taoujdat	33°53'35.29"N	5°14'30.06"O	SEB	X				
Séguia région Chefchaoun	35°6'10.164"N	5°10'12.954"O	LK	X				