Dissertation

The Emergence of the Isthmus of Panama
– a biological perspective –

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Cover: Panama and the transisthmian sister species pair *Sesarma curacaoense* De Man, 1892 (western Atlantic) and *Sesarma rhizophorae* Rathbun, 1906 (eastern Pacific).

If there is one thing the history of evolution has

taught us it's that life will not be contained.

Life breaks free, it expands to new territories, and

crashes through barriers, painfully, maybe even dangerously,

but, uh...well, there it is.

Ian Malcolm **(Jurassic Park)**

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Part I

Synopsis

1 Summary

Several million years ago, a land bridge between the two American continents started to emerge. The appearance and final closure of this Isthmus resulted in a terrestrial connection of North- and South America and the separation of the western Atlantic and eastern Pacific oceans. The emergence of the Isthmus had considerable consequences on oceanographic, environmental, and faunistic conditions on a global as well as on a regional scale. Recently conducted studies challenge the widely accepted assumption that the rise of the Isthmus and its final closure occurred in Late Pliocene time (i.e. around 3–4 million years ago (Ma) with potential breaching of the Isthmus until about 1.8 Ma; 'common Pliocene model') and allocate this event much earlier, at around 15 Ma ('new Miocene model'). Due to the emergence and closure of the Isthmus, transisthmian sister species (TSS) originated. TSS are defined as species that have diverged due to the closure of the Isthmus and are each other's closest relatives on opposite sides of the barrier. However, the TSS concept (i.e. the definition of the term TSS and the fulfillment of five criteria regarding biogeographic distributions, morphological similarities, and molecular characteristics) is often inconsistently used in biogeographical research. Consequently, some studies suffer from an ambiguous and confusing TSS terminology, as well as misidentified TSS pairs. However, TSS pairs and the controversially discussed closure of the Isthmus of Panama play, among others, a key role in molecular clock calibrations. The inconsistency of the TSS concept, the complex and long lasting geological history of the Isthmus itself, as well as difficulties in molecular clock approaches may be the reasons why previously estimated divergence times for TSS pairs are not conclusive as to the time of final Isthmus closure.

Thus, it is important to develop an accurate and applicable TSS concept, which offers a precise and unambiguous terminology regarding TSS as well as suitable criteria to identify TSS. This might help preventing misleading assumptions regarding TSS and it may provide a robust terminology for future studies. However, divergence time estimations of correctly identified TSS pairs can provide crucial evidence regarding the timing of the Isthmus closure from a biological point of view. Therefore, this thesis aims at:

(i) providing a background to (a) the chronological emergence and final closure of the Isthmus of Panama, (b) the ecological consequences of the Isthmus emergence, (c) the evolution of transisthmian sister species, and (d) the molecular clock approach;
(ii) establishing a consistent and unambiguous terminology regarding TSS in respect to operative criteria, e.g., the time of TSS divergence or their arrangement in phylogenies;
(iii) identifying and analyzing TSS pairs and -complexes for the present study with respect to the applicability of the five TSS criteria proposed;
(iv) inferring divergence times for the studied species relative to the two models proposed for the final closure of the Isthmus; and
(v) assessing problems associated with divergence time estimations of TSS.

To achieve these aims, this thesis combines four in-depth reviews (i) and case studies (ii-v). Due to the complex interactions of the various subjects, the reviews should provide the background and associated difficulties of each subject. These difficulties are then addressed in the second part utilizing practical examples ('Case Studies'). The development of a suitable TSS concept is based on a comprehensive literature search and a thorough analysis of previously applied TSS terminologies. Additionally, phylogenetically identified TSS pairs and -complexes of four different decapod genera (*Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus*) are used to evaluate the five proposed operational criteria for TSS. Subsequent divergence time estimations are based on TSS pairs and -complexes identified before as well as an external molecular clock crustacean rate. The obtained divergence times should provide new biological perspectives regarding the time of final Isthmus closure.

The comprehensive literature search revealed 60 terms and derivatives relative to TSS in the context of the emergence and closure of the Isthmus of Panama. Although they are often used synonymously, from a strict semantic perspective, only a fraction of them can be considered as *true* synonyms. Based on this literature survey, three principles are suggested for terms implying a TSS status. Based on these three principles, 13 terms and derivatives could be identified. For reasons of comparability, only these terms are recommended to be employed in any study concerned with TSS. The criteria-analysis regarding TSS indicated that never all of the five operational criteria were fulfilled by the here studied TSS pairs and -complexes. Evidently clear confined criteria are difficult to develop, because the complex interrelations within biological systems restrain the establishment of certain categories or concepts. Thus, additional and/or modified criteria are suggested with respect to their practicability in non-theoretical frameworks. However, the development of a TSS identification key with precise characteristics is not possible due to the taxonomic and ecological diversity of TSS pairs. The results of the subsequently conducted divergence time estimations do not present conclusive evidence in favor of either the Miocene or the Pliocene model. In fact, the TSS pair of *Pachygrapsus* shows an early divergence age close to the Miocene model, whereas the TSS complexes of *Sesarma* and the TSS pair A of *Eurytium* rather point toward the Pliocene model. Moreover, TSS complex A of *Sesarma* and TSS pair A of *Eurytium* also show evidence for potential re-openings and -closures of the Isthmus after 3 Ma. These differences may be due to, for example, the complex and long lasting geological emergence of the Isthmus of Panama, missing species or sequences, species misidentifications, or the influence of various molecular parameters.

In conclusion, the major implications of this thesis are (i) to highlight potential lacks of knowledge, inconsistencies, and challenges regarding the Isthmus formation, TSS, and the molecular clock approach in general ('State of the Art'), and (ii) to study these difficulties with new case studies based on four decapod genera as model organisms in particular ('Case Studies').

2 Zusammenfassung

Vor mehreren Millionen Jahren begann sich, eine Landbrücke zwischen den beiden amerikanischen Kontinenten zu erheben. Die Entstehung und finale Schließung dieses Isthmus resultierte in einer Verbindung zwischen dem nord- und südamerikanischen Festland, sowie in der Teilung des Meeres in den westatlantischen und ostpazifischen Ozean. Die Entstehung des Isthmus hatte erhebliche Konsequenzen für die ozeanografischen, ökologischen und faunistischen Gegebenheiten sowohl auf globaler, als auch auf regionaler Ebene. Aktuelle Studien stellen die allgemeine Hypothese einer pliozänen Isthmusschließung (d.h. vor ungefähr 3–4 Millionen Jahren (Mio), mit möglichen Brüchen des Isthmus bis 1,8 Mio; 'gegenwärtiges Pliozän Modell') in Frage und datieren dieses Ereignis stattdessen auf etwa 15 Mio ('neues Miozän Modell'). Die Erhebung und Schließung des Isthmus hatte die Evolution von transisthmischen Schwesterarten (TSS) in den nun voneinander getrennten Ozeanen zur Folge. Unter TSS versteht man ursprünglich identische Arten, die nach der Schließung des Isthmus getrennt voneinander evolvierten und heute gegenseitig ihre nächsten Verwandten auf beiden Seiten der Landbrücke darstellen. Die Definition des Begriffs TSS und die Erfüllung von fünf Kriterien bezüglich der biogeografischen Verbreitung, der morphologischen Ähnlichkeiten und der molekularen Merkmale von TSS (d.h. TSS Konzept) wird in biogeografischen Studien oftmals inkonsistent verwendet. Dementsprechend mangelt es einigen Studien an einer deutlichen und eindeutigen TSS Terminologie, sowie einer korrekten Bestimmung von TSS-Paaren. Dennoch spielen TSS-Paare und die kontrovers diskutierte zeitliche Schließung des Isthmus von Panama eine Schlüsselrolle in sog. molekularen Uhr-Analysen. Die Unbeständigkeit des TSS Konzeptes, die komplexe und lang andauernde geologische Entstehung des Isthmus, sowie Schwierigkeiten in molekularen Uhr-Ansätzen könnten der Grund dafür sein, dass bisherige Divergenz-Zeiten von TSS- Paaren oftmals nicht mit der zeitlichen Schließung des Isthmus übereinstimmen.

Folglich ist es von Bedeutung, ein akkurates und anwendbares TSS Konzept zu entwickeln, welches eine präzise und eindeutige TSS Terminologie, sowie passende Kriterien zur Identifizierung von TSS bietet. Des Weiteren können errechnete Divergenz-Zeiten von richtig bestimmten TSS-Paaren entscheidende Hinweise bezüglich der zeitlichen Schließung des Isthmus von einem biologischen Standpunkt aus geben. Deshalb ist das Ziel dieser Arbeit:

(i) einen wissenschaftlichen Hintergrund zu (a) der chronologischen Entstehung und finalen Schließung des Isthmus von Panama, (b) den ökologischen Konsequenzen der Isthmus- Entstehung, (c) der Evolution von transisthmischen Schwesterarten und (d) dem molekularen Uhr-Ansatz zu geben;

(ii) eine konsistente und eindeutige TSS Terminologie, sowie anwendbare TSS-Kriterien zu etablieren;

(iii) TSS-Paare und -Komplexe in dieser Studie zu identifizieren und ihre Anwendbarkeit auf die fünf postulierten Kriterien zu prüfen;

(iv) den Zeitpunkt von Divergenz-Ereignissen von TSS zu ermitteln und mit den zwei postulierten Modellen der Isthmus-Schließung zu vergleichen; und

(v) Probleme von TSS Divergenz-Berechnungen herauszustellen und zu analysieren.

Um diese Ziele zu erreichen, vereint diese Arbeit vier tiefgreifende Reviews (i) mit Fallstudien (ii-v). Durch die weitreichenden und komplexen Zusammenhänge der verschiedenen Themen, sollen die Reviews den wissenschaftlichen Hintergrund liefern und etwaige Probleme der unterschiedlichen Themenbereiche herausstellen. Diese Probleme werden dann im zweiten Teil der Arbeit ('Case Studies') näher beleuchtet und an praktischen Beispielen analysiert. Die Entwicklung eines passenden TSS Konzeptes basiert auf einer umfangreichen Literaturstudie und auf der Analyse von bisher verwendeten TSS Terminologien. Zudem werden in dieser Arbeit phylogenetisch identifizierte TSS-Paare und -Komplexe vierer Dekapoden-Gattungen (*Sesarma*, *Panopeus*, *Eurytium* und *Pachygrapsus*) verwendet, um die fünf postulierten TSS Kriterien zu evaluieren. Die anschließenden Berechnungen von Divergenz-Zeiten basieren auf den zuvor identifizierten TSS-Paaren und -Komplexen, sowie auf einer externen molekularen Crustacea-Rate. Die erzielten Divergenz-Zeiten sollen einen neuen Hinweis auf die zeitlich finale Schließung des Isthmus aus biologischer Sicht erbringen.

Die umfangreiche Literaturanalyse offenbarte 60 TSS Begriffe und deren Derivate in Bezug auf die Entstehung und Schließung des Isthmus von Panama. Obwohl diese Begriffe oftmals synonym verwendet werden, können nur wenige im engeren semantischen Sinne als wahre Synonyme angesehen werden. Aufgrund dieser Literaturstudie können drei Annahmen einer eindeutigen TSS Definition gemacht werden. Basierend auf diesen drei Prinzipien konnten 13 Begriffe und Derivate identifiziert werden, die als wahre Synonyme bezeichnet werden können. Die TSS Kriterien-Analyse zeigte, dass immer nur ein Teil der fünf Kriterien von den analysierten TSS-Paaren und -Komplexen dieser Arbeit erfüllt wurde. Augenscheinlich sind strukturierte und eindeutige Kriterien schwer zu entwickeln. Der Grund sind die komplexen Beziehungen innerhalb biologischer Systeme. So werden zusätzliche bzw. modifizierte, anwendbare Kriterien vorgeschlagen. Dennoch ist aufgrund der taxonomischen und ökologischen Diversität von TSS-Paaren die Ausarbeitung eines TSS-Identifikationsschlüssels mit konkreten Merkmalen nicht möglich. Die Ergebnisse der anschließenden Divergenz-Berechnungen ergaben keine schlüssigen Hinweise in Bezug auf eines der beiden Modelle. Vielmehr ergab sich für das *Pachygrapsus* TSS-Paar eine frühe Trennung, die nah am Miozän Modell lag. Im Gegensatz dazu lagen die Divergenz-Zeiten der *Sesarma* TSS-Komplexe und des *Eurytium* TSS-Paar A näher am Pliozän Modell. Außerdem zeigten die Divergenz-Zeiten des *Sesarma* TSS-Paars A und des *Eurytium* TSS-Paars A Hinweise auf potentielle Wieder-Öffnungen und -Schließungen des Isthmus nach 3 Mio. Diese unterschiedlichen Divergenz-Ereignisse könnten durch die komplexe und lang andauernde Entstehung des Isthmus von Panama, fehlende Arten oder Sequenzen in den Berechnungen, oder Einflüsse verschiedenster Parameter in der molekularen Analyse bedingt sein.

Die Hauptanliegen dieser Arbeit sind (i) potentielle Wissenslücken, Ungenauigkeiten und Widersprüche bezüglich der Isthmusschließung, der TSS und des molekularen Uhr-Ansatzes im Generellen zu beleuchten ('State of the Art') und (ii) diese Schwierigkeiten in neuen Fallstudien anhand von vier Dekapoden-Gattungen als Modellorganismen zu analysieren ('Case Studies').

3 Motivation and Research Objectives

The emergence of the Isthmus of Panama (i.e. the formation of a land bridge between the two American continents) and its final closure (i.e. final interruption of the Atlantic and Pacific connection) is one of the best studied vicariance events in evolutionary biology. Its emergence and final closure have substantial consequences to ocean circulations, global climatic patterns, biogeography, ecology, and consequently the evolution of both terrestrial and marine biota. The geological and environmental isthmian characteristics observed today are the result of a complex and extended process that started several million years ago (Ma). Based on recently conducted studies, two models regarding the time of Isthmus closure are discussed: The common Pliocene model (i.e. Isthmus closure around 3 Ma) versus the new Miocene model (i.e. Isthmus closure around 15 Ma). This vicariance event initiated the evolution of transisthmian sister species (TSS; species on opposite sides of the barrier that were separated due to the closure of the Isthmus and are each other's closest relatives). However, the TSS concept (i.e. the definition of the term TSS and the fulfillment of five operational criteria to classify species as true TSS) is not consistently used in biogeographical studies, resulting in an ambiguous and partly confusing terminology as well as in controversial assignments of TSS pairs. The Isthmus debate as well as the neglect of the TSS concept is problematic, because they play an essential role in molecular clock approaches and divergence time estimations. Therefore, a comprehensive and precise understanding of TSS as well as of the chronological Isthmus formation (in particular of the timing of the Isthmus closure) is of crucial importance. To study these subjects, the thesis is divided into the parts 'State of the Art' (Part II) and 'Case Studies' (Part III). The part 'State of the Art' is concerned with the scientific background. Four composed reviews provide an introduction and highlight difficulties regarding the Isthmus emergence, the evolution of TSS, and the molecular clock approach, which are then addressed in empirical 'Case Studies'. These case studies are concerned with the neglect of the TSS concept in general and the suitability of TSS in molecular clock approaches and divergence time estimations in particular. Moreover, by taking up the ongoing Isthmus debate, the 'Case Studies' aim to investigate the temporal closure of the Isthmus from a biological perspective.

State of the Art

This part of the thesis presents the background to the subsequent 'Case studies' in four comprehensive reviews uniting the different topics of this thesis:

1. The emergence and closure of the Isthmus of Panama (Chapter 4).
2. The ecological consequences of the Isthmus formation (Chapter 5).
3. The evolution of transisthmian sister species (Chapter 6).
4. The molecular clock approach (Chapter 7).

Case Studies

–A Critical View at the Transisthmian Sister Species Concepts–

Toward an unified definition of transisthmian sister species

This chapter critically reviews the current literature with respect to the term *transisthmian sister species* (TSS). Terms referring to potential TSS are often used ambiguously and no consistent terminology is apparent. In particular, in a strict semantic way, only a fraction of the used terms are indeed synonyms in respect to the definition of TSS. The use of imprecise terms may lead to erroneous synonyms, redundancy, and eventually to confusing and misleading assumptions. Therefore, this part of the thesis aims to clarify the partly confusing and misleading terminology regarding TSS to reduce ambiguities and facilitate consistency. In particular, this part:

1. creates a list of synonyms referring to TSS, based on a comprehensive literature review (Subchapter 8.1) and
2. critically discusses the findings of the terminological survey, and presents recommendations for well defined, unambiguous terms (Subchapter 8.2).

Criteria of TSS pairs and -complexes

Populations of various marine species were separated by the Isthmus emergence and its final closure, and some experienced extinction or speciation events on either side of the Isthmus. During these processes, these initially genetically and phenotypically similar populations experienced divergent selection in different environments and subsequently evolved into TSS. Five assumptions regarding biogeographic distributions, morphological similarities, and molecular characteristics were defined to classify species as true TSS. This chapter is concerned with the arrangement of the identified TSS pairs and -complexes of this thesis in respect to these operative criteria. Therefore, the following questions are addressed:

1. Do the studied TSS pairs and -complexes of this study meet all five TSS criteria (Subchapter 9.1)?
2. Are the current criteria sufficient to identify TSS (Subchapter 9.2)?
3. What additional/new set of criteria can be suggested to identify TSS (Subchapter 9.4)?

–Divergence Time Estimations of Transisthmian Sister Species–

The emergence and final closure of the Isthmus of Panama was a complex and long-lasting vicariance event. However, the time of final Isthmus closure remains controversially discussed (see the common Pliocene and new Miocene models mentioned above). In this part of the thesis, divergence time estimations for TSS pairs and -complexes of four different decapod genera were performed. The objectives of these analyses were:

1. The molecular studies should point out the problems of divergence time estimations of TSS (Chapter 10).
2. The obtained divergence times of the study are then discussed relative to the two models proposed for the final closure of the Isthmus (Chapter 10)

Part II

State of the Art

4 Geological Evolution and Biological Evidences – The Formation of the Isthmus of Panama and its Closure

The emergence of the Isthmus of Panama is the most common vicariance event studied in a wide range of scientific fields. Thus, a comprehensive and precisely understanding of the chronological Isthmus formation (in particular of the timing of final Isthmus closure) is of importance, particularly in evolutionary studies. In this context, the time of Isthmus closure is commonly used as calibration point in divergence time estimations of transisthmian sister species (TSS; see Chapters 6 and 7). Therefore, this chapter presents a brief summary of the isthmian geography and environmental conditions, followed by a review of the two proposed models of the Isthmus formation and its time of assumed final closure ('new Miocene model' vs. 'common Pliocene model'). Subsequently, two major events will be briefly discussed in respect to the different chronological assumptions of the Isthmus formation, based on paleoceanographic, terrestrial, and marine biogeographic evidences. All data were adjusted to the geological timescale (Walker *et al.* 2012).

4.1 What is the Isthmus of Panama?

Definition:

[Synonyms for the term *Isthmus of Panama* found in the literature: *Isthmus of Darien* (Figure 4-1); *Central American Isthmus* (CAI); *American Isthmus*]

In his book *The Isthmus of Panamá*, Bidwell (1865) defined the Isthmus as "[…] a narrow neck of land which unites the continents of North and South America […]" (p. 7).

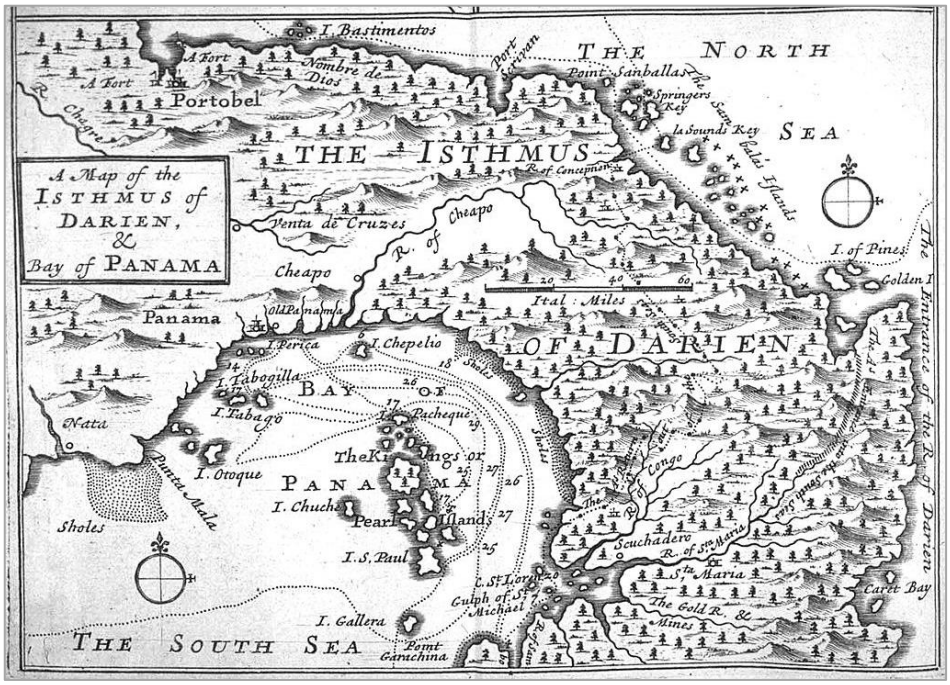

Figure 4-1: 'A New Voyage and Description of the Isthmus of America', Lionel Wafer, 1697. Historical map of the Isthmus of Darien.

Indeed, the Isthmus of Panama forms the southern part of a large land bridge (Isthmus), uniting North and South America. In this study, the isthmian range of interest is focused on Panama (roughly 07°00'N, 82°00'W) ranging from the border of Costa Rica in the west to the border of Colombia in the east, including nearby islands (e.g., Bocas del Toro, Coiba, Pearl Islands). In the north, the Isthmus is bordered by the western Atlantic (i.e. Caribbean) and in the south by the eastern Pacific oceans (Figure 5-2). The Isthmus of Panama is pronounced by diverse vegetation zones (from montane habitats to tropical and dry forests, mangroves, estuaries, savannas, and grasslands; Marshall 2007). The marine habitats differ considerably between the oceans. The around 1 295 km western Atlantic coastline of Panama (Miloslavich *et al.* 2010) is characterized by large coral reefs, calcareous beach sands and shelf sediments, and covers of seagrass beds. In contrast, along the around 1 450 km eastern Pacific coast (Palka 2005 and reference therein) mangroves and calm sand beaches are prevalent (but see Chapter 5 for details; Figure 5-2).

The geological and environmental isthmian shapes as we see them today are the result of a complex and extended process started several million years ago (Ma). This event had substantial consequences to ocean circulation, global climatic patterns, biogeography, ecology, and consequently the evolution of both the terrestrial and marine biota (e.g., Coates & Obando 1996 and references therein). Joseph Cushman (1929) was the first person who found evidence for a marine seaway that once connected the western Atlantic and eastern Pacific oceans based on foraminiferal assemblages from Venezuela and Ecuador (see Collins 2003).

Today, various geological (e.g., cores), oceanographical (e.g., marine sedimentary depositions), paleontological (e.g., fossils), and biological (e.g., divergence events) proxies are employed in numerous studies to investigate the history of Isthmus formation and final seaway closure in particular (e.g., Coates & Obando 1996; O'Dea & Collins 2013; and references therein). Recent speculations about a complete isolation of the eastern Pacific and western Atlantic around 15 Ma (Farris *et al.* 2011; Montes *et al.* 2012a; b) have led to an intense debate about the temporal uplift of the Isthmus of Panama (O'Dea & Collins 2013 and references therein). In fact, new geological, geochemical, and geophysical studies challenge the widely accepted opinion that the emergence of the Isthmus and its final closure occurred during the Late Pliocene (i.e. approximately 3 Ma and described here as the 'common Pliocene model'; e.g., Jackson *et al.* 1996a) and allocate this event much earlier, around 15 Ma (described here as the 'new Miocene model'; e.g., Farris *et al.* 2011; Montes *et al.* 2012a; b; for a chronological summary of the events see Table 4-2).

4.2 Chronology of events – The Miocene model

The emergence and closure of the Isthmus of Panama was not a steady and uniform event, rather it consisted of re-openings and -closures spanning over several million years. First evidences for an uplift of the Central American arc are dated back to the Late Cretaceous (Montes *et al.* 2012b). Due to the rotation of tectonic blocks between 38–28 Ma, the magmatic Campanian-Eocene belt was deformed, and achieved its final formation in the Late Oligocene

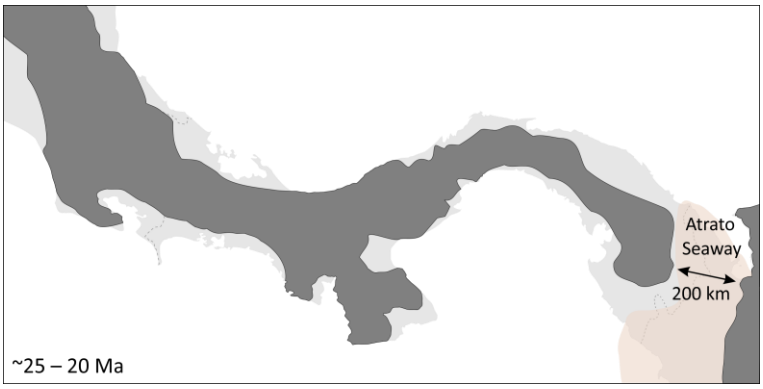

Figure 4-2: The emergence of the Isthmus of Panama – 'new Miocene model' (based on the proposed 'Peninsula model', see text for details; Figure 4-4), during the Late Oligocene – Early Miocene (~25–20 Ma). A 200 km wide abyssal gap (Atrato Seaway) still connects the western Atlantic and eastern Pacific oceans; light gray: shapes of Colombia, Panama, Costa Rica, and Nicaragua as seen today; dark gray: emergent land; dashed line: borders of Panama; red area: abyssal to bathyal depths; Ma = million years ago (after Bagley & Johnson 2014; Montes *et al.* 2012b).

(for tectonic details see Montes *et al.* 2012a, Fig. 9a-d). Estimated divergence ages of palms based on molecular studies support an emergence of land masses during that time (Bacon *et al.* 2013). During the Late Oligocene (28.1–23.0 Ma), a collision between the southern tip of Central America and South America occurred. However, a 200 km wide connection between the oceans remained (Farris *et al.* 2011; Montes *et al.* 2012b; Figure 4-2). Age estimates of terrestrial vertebrate fossils (Kirby & MacFadden 2005) and migration events of salamanders from Central to South America (Elmer *et al.* 2013) pointing toward an increase land uplift around 23 Ma. Evidences for a persistent and complete land connection between the continents are based on biological studies of saltwater-intolerant frogs (Weigt *et al.* 2005), freshwater fish (Bermingham & Martin 1998), plants (Cody *et al.* 2010), ash deposits of large terrestrial vertebrates (Campbell *et al.* 2010), and fossils (e.g., Marshall 1985, 1988; Webb 1985) indicating migration and spreading events between 16–5 Ma. Geological data support these biological evidences: Keller & Barron (1983) argued that a gradual shoaling started around 15 Ma and Montes *et al.* (2012a; b) suggested that 15 Ma the volcanic arc was in such a formation that the water connection between the eastern Pacific and western Atlantic was entirely interrupted and the closure of the Isthmus completed.

4.3 Chronology of events – The Pliocene model

The first collision of Central America with South America occurred in the Late Oligocene (around 25 Ma), but a major seaway still connected the Atlantic and Pacific oceans (Coates & Stallard 2013). In contrast, Coates *et al.* (2004) dated the collision at 14–12 Ma, based on sediment analyses. During the Early Miocene (around 17 Ma), the volcanic arc (today's southern part of Central America) was formed (Coates *et al.* 1992, 2003, 2004). In the Middle Miocene, tectonic disturbances triggered the initial uplift of the Panama sill (i.e. deep passage with local highs; Figure 4-2), which resulted in major changes of the oceanic conditions (Duque-Caro 1990). During this time, the bathyal zone was around 2000 m deep. Extensive collision between the Central American arc and South America led to a further shallowing of the oceans resulting in a

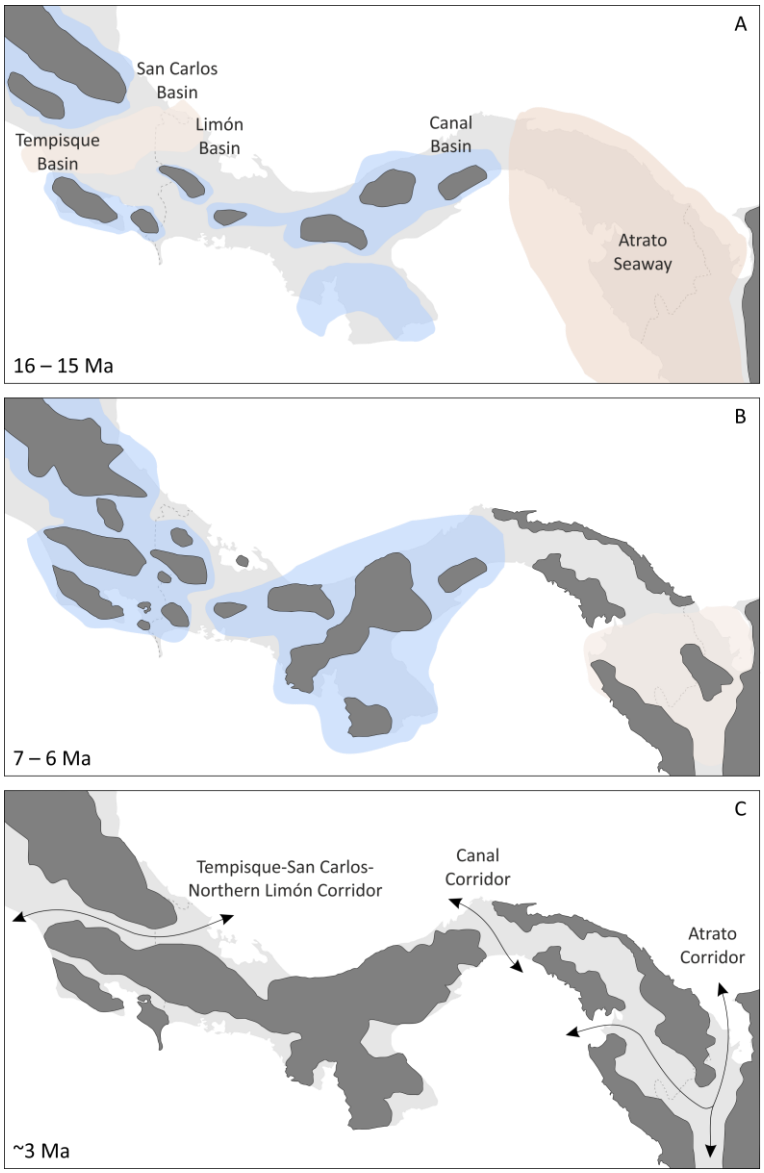

Figure 4-3: The emergence of the Isthmus of Panama – 'common Pliocene model' (based on the proposed 'Island model', see text for details; Figure 4-4), during A) the Middle Miocene (16–15 Ma), B) the Late Miocene (7–6 Ma), C) the Late Pliocene (~3 Ma); light gray: shapes of Colombia, Panama, Costa Rica, and Nicaragua as seen today; dark gray: emergent land; dashed line: borders of Panama; red area: abyssal to bathyal depths; blue area: neritic depths; arrows: marine corridors before Isthmus completion; Ma = million years ago (after Bagley & Johnson 2014; Coates & Obando 1996; Coates et al. 2004, 2005).

first interruption of deep- and intermediate-water connections between the western Atlantic and the eastern Pacific (Coates *et al.* 2004; Coates & Stallard 2013; Duque-Caro 1990; Wright *et al.* 1991; Figure 4-3 B). The bathyal depths during this time range from 1000–500 m, and decreased to inner neritic water depth (~150 m) during the Late Miocene (Duque-Caro 1990; Schmidt 2007). Furthermore, Roth *et al.* (2000) and Coates *et al.* (2003, 2004) provide stratigraphic evidences for an intermittent closure of the shallow-water connections and thus of a short-lived near-complete Isthmus approximately 11–9 Ma. This hypothesis is in concordance with reports of the first terrestrial interchange of raccoons from North to South America (Webb 1985). Between 9–6 Ma several species of mammals succeeded in crossing the emerging Isthmus

in both directions (Marshall 1985, 1988; Morgan 2002; Webb 1985), which then consisted of closely spaced islands (Molnar 2008). However, the paleogeographic structure of the emerging Isthmus is discussed controversial and either proposed as an 'Island model' or a 'Peninsula model' (Coates & Obando 1996; Kirby & MacFadden 2005; Molnar 2008; Figure 4-4). A further reduction of water exchange between the oceans occurred at around 7 Ma (Keigwin 1982a; Keller *et al.* 1989). Around 6 Ma almost all deep water passages had ceased (Coates & Obando 1996; Figure 4-3). In fact, Kirby *et al.* (2008) provide evidence for a short-lived strait across the Panama Canal Basin during that time. Between 6–4 Ma the oceanic conditions like temperature, salinity, and habitats on both sides of the emerging Isthmus changed substantially (e.g., Chaisson & Ravelo 2000; Haug *et al.* 2001; Keigwin 1982; Lear *et al.* 2003; see Chapter 5 for more details).

A low water level period between 4.6–3.1 Ma enhanced the further shallowing of the Isthmus (Haq *et al.* 1987). Based on biostratigraphic analyses and correlated divergence time estimations of mollusk fossils dating back 3.5 Ma, Coates *et al.* (1992) assumed that an almost complete barrier was formed around 3.7 Ma. However, the exact time of final Isthmus closure is discussed controversially (Table 4-1). Divergence times of tropical forest birds between 4–3 Ma (Weir *et al.* 2009), changes in salinity, temperature, upwelling, and productivity of both oceans (e.g., Jackson & O'Dea 2013; Leigh *et al.* 2014; and references therein), and the Great American Biotic Interchange of vertebrates at about 2.7 Ma (e.g., Coates *et al.* 1992; Marshall 1988; Webb 2006) are pointing toward a final Isthmus closure between 4–3 Ma. Summarizing geological processes and biological aspects, Collins (2003) dated the closure back to 4 Ma whereas Coates & Obando (1996) assumed an Isthmus closure between 3.1–2.8 Ma. However, they noted that temporary breaches of the Isthmus may have occurred. In fact, evidences for several short-lived re-openings during the Pliocene (3.8 Ma and 3.4–3.3 Ma; Haug & Tiedemann 1998), a shallow water connection between the eastern Pacific and Caribbean beyond 3 Ma (Bowen *et al.* 1998; Coates & Obando 1996), a breach of the Isthmus around 2 Ma (Cronin & Dowsett 1996), and remaining littoral-neritic breaks until around 1.8 Ma (Keller *et al.* 1989) are pointing toward a *final* Isthmus closure between 2.5–1.8 Ma (Table 4-1).

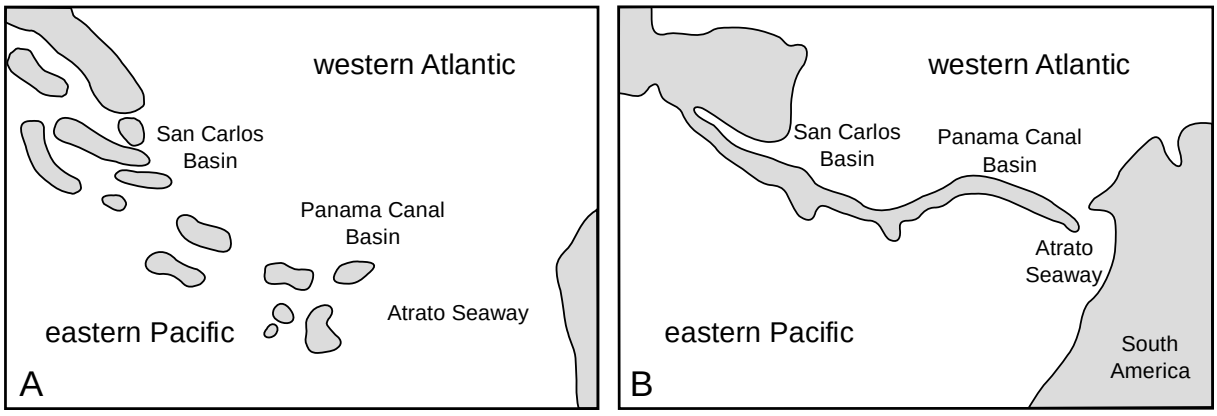

Figure 4-4: The two proposed models of Isthmus emergence during the Middle Miocene. A) The Island model. B) The Peninsula model (modified after Schmidt 2007). Land masses are highlighted in gray.

Table 4-1: Chronological order of isthmian re-openings and -closures.

Proposed Closure (Ma)	Event	Reference
15.0	**Final** Isthmus closure	Montes *et al.* (2012a; b)
11.0–9.0	Short-lived near-complete Isthmus	Coates *et al.* (2003, 2004); Roth *et al.* (2000)
9.0–6.0	Several mammal species crossed the Isthmus, which consisted of closely spaced islands	Marshall (1985, 1988); Molnar (2008); Morgan (2002); Webb (1985)
4.0	**Final** Isthmus closure	Collins (2003)
4.0–3.0	**Final** Isthmus closure	Jackson & O'Dea (2013); Weir *et al.* (2009)
3.8	Short-lived re-opening	Haug & Tiedemann (1998)
3.7	Almost complete Isthmus	Coates *et al.* (1992)
3.5–3.1	First complete closure	Coates & Obando (1996); Duque-Caro (1990); Keigwin (1978, 1982)
3.4–3.3	Short-lived re-opening	Haug & Tiedemann (1998)
3.1–2.8	Isthmus closure	Coates & Obando (1996)
3.0–2.8	Near closure to surface water	Cronin & Dowsett (1996)
beyond 3.0	Shallow water connection	Bowen *et al.* (1998); Coates & Obando (1996)
2.7	Great American Biotic Interchange of vertebrates	Coates *et al.* (1992); Marshall (1988); Webb (2006)
2.0	Breach of the Isthmus	Cronin & Dowsett (1996)
2.5–1.9	**Final** Isthmus closure	Cronin & Dowsett (1996)
2.4–1.8	**Final** Isthmus closure	Keller *et al.* (1989)

Proposed final Isthmus closures are marked in bold. Ma = million years ago.

4.4 Discrepancies between the models

4.4.1 Time of collision and Isthmus closure

There is a large time discrepancy regarding the collision of Central- and South America between the two models. The assumption of a collision 25–23 Ma and a closure of the seaway at around 15 Ma (i.e. Miocene model; Farris *et al.* 2011; Montes *et al.* 2012a; b) substantially predates the hypothesis of Coates *et al.* (2004), who suggested a collision at 14–12 Ma and a final Isthmus closure around 3.5 Ma (Coates *et al.* 1992; O'Dea *et al.* 2007) or rather 1.8 Ma (Keller *et al.* 1989), considering re-openings and -closures (Pliocene model).

Possible explanations

Montes *et al.* (2012a) discussed in particular the deep water passage between the oceans. Jackson & O'Dea (2013) concluded that this assumption would be consistent with earlier studies by the Panama Paleontology Project (Coates *et al.* 2004; Coates & Obando 1996). However, they argue that "geological data cannot possibly resolve paleogeographic landscapes and seaways on the scale of the few 10s of kilometers" (p. 793), in particular when the geological rock record suffers from incompleteness.

4.4.2 Migration- and divergence times of species

Both models differ considerably in respect to migration events between both the continents and oceans. Terrestrial lineages of e.g., palms (Bacon *et al.* 2013), salamanders (Elmer *et al.* 2013), and the fossil record of vertebrates (Kirby & MacFadden 2005) indicate a biotic exchange between North and Central- /South America 31–16 Ma. However, the vast majority of species migrated 5–2 Ma, pointing to a stable and constant land bridge (e.g., Pinto-Sánchez *et al.* 2012; Webb 2006). Numerous lineages of marine taxa including Foraminifera, mollusks, bryozoans, crustaceans, and fishes began to diverge as early as 20–10 Ma, but there are also numerous well-documented examples of biological exchange between the oceans as recently as the Pliocene (Lessios 2008, and references therein).

Possible explanations

These time differences may result due to the complex geological history of the closure of the Isthmus itself (Figure 4-3). Coates & Stallard (2013) pointed out, that there are no indications of a stable land connection during the Early Oligocene to Early Miocene, where terrestrial species could have migrated from North to South America and vice versa. Furthermore they argued that no definite terrestrial vertebrate fossils with South American affinities have been found in the current Panama Canal excavations. Jackson & O'Dea (2013) summarized several evidences that gene flow between marine species may have persisted long before or even after the final closure (i.e. 3 Ma), due to dispersal via e.g., birds (Miura *et al.* 2012), rafting (De Queiroz 2005), or plate movement through the nascent Isthmus region (details see Graham 2003). They also argued that yet marginal and narrow water connections are sufficient for the exchange of marine biota (Jackson & O'Dea 2013).

Several authors (Cronin & Dowsett 1996; Keller *et al.* 1989; Savin & Douglas 1985; Schmidt 2007) mentioned also potential breaching events of the Isthmus, which may have caused possible marine exchanges between the oceans. In contrast, Collins (1996a) argued that these breaching events would have had little effect on divergences of eastern Pacific and western Atlantic marine faunas. Coates & Obando (1996) assumed that differences in divergence times may also correlate with the specific habitat of the respective organism. Deep water species, for example, should have been affected first by the rising Isthmus than shallow water species, which may have crossed the Isthmus until just prior to its closure (Frey 2010; Knowlton & Weigt 1998; Miura *et al.* 2012; Schubart *et al.* 1998).

4.5 Summary

Various paleoceanographic, terrestrial, and marine biogeographic data demonstrate precisely the evolution of the Isthmus of Panama for *both* models. However, two key events occurred within both models, yet in different time ranges and thus, reflect the uncertainties regarding the timing of events of the Isthmus formation. The following table (Table 4-2) presents a summary of significant events in relation to the temporal closure of the Isthmus of Panama.

Table 4-2: Summary of significant events, which are related to the closure of the Panama Isthmus.

Age (Ma)	Event & Interpretation	Reference
Early Mesozoic or Earlier	Crustal fragments in southern Mexico and northern Central America consolidated; Southern regions (incl. Nicaragua, Costa Rica and Panama) initially as part of a volcanic arc.	Coates *et al.* (1992, 2003, 2004); Coates & Obando (1996); Mann & Kolarsky (1995)
* Late Cretaceous to Middle Eocene	Magmatic belt, reached about 200 km off South America; Cooling events as proxies for a continuous emergence.	Montes *et al.* (2012b)
* 38–28	Segmentation/deformation of the arc started; Almost completed in the Late Oligocene (~25 Ma).	Montes *et al.* (2012a)
* 31–16	Molecular studies of palms (*Copernicia* and *Pritchardia*) support an early divergence age.	Bacon *et al.* (2013)
* 25–23	Geologic collision of Central America with South America; Major seaway (200 km wide) between the eastern Pacific and western Atlantic remained; (Assumption predates the argument of Coates *et al.* (2004) of a geological collision at 14–12 Ma; see below).	Coates & Stallard (2013); Farris *et al.* (2011); Montes *et al.* (2012b)
* 23.6	Salamander (*Bolitoglossa*) migrations from Central- to South America.	Elmer *et al.* (2013)
* 23	Fossils of terrestrial vertebrates indicate that the arc formed a peninsula that was connected to North America (note: no definite terrestrial vertebrates of this age, with South American affinities, have been exhumed in the current Panama Canal excavations; Coates & Stallard 2013).	Kirby & MacFadden (2005)
* 19–16	Mammalian fossils suggest a continuous connection between Panama and North America.	Kirby & MacFadden (2005)
~17	Formation of the volcanic arc (forms today the southern part of Central America).	Coates *et al.* (1992, 2003, 2004)
~16	Deep, open oceanic conditions and free water circulation occur along the steep continental margins of NW South America.	Duque-Caro (1990)
16.1–15.1	Changes in bottom water circulation and sedimentation occurred due to tectonic disturbances that triggered the initial uplift of the Panama sill. Changes in organic nutrients sea surface temperature, sea level rise, and bathyal depths (2000 m).	Duque-Caro (1990)
* 16–5	Molecular studies of e.g. saltwater-intolerant frogs, freshwater fish, and plants show early migration times.	Bermingham & Martin (1998); Cody *et al.* (2010); Weigt *et al.* (2005)

Age (Ma)	Event & Interpretation	Reference
* between 15.4–14.7	Begin of gradual shoaling (based on deep sea records).	Keller & Barron (1983)
* 15	Configuration of the volcanic arc hampers seawater exchange between the eastern Pacific and western Atlantic. → (almost)complete closure (note: Coates & Stallard (2013) argued that "[…]none of the used proxies [in the study used by Montes *et al.* 2012a] can establish sea level or whether marine gaps in the Isthmus were present or not." p. 804).	Montes *et al.* (2012a; b)
15–12	Widespread shallowing of the Isthmus had created a paleogeography arc; Few narrow and deep marine passages maintain a marine connection between the oceans.	Coates & Stallard (2013)
14–12	Geological collision between South America and the Central American arc; Widespread shallowing of the sea around the Central American arc (compare 25–23 Ma above).	Coates *et al.* (2004)
13.45–13	Uplift of the sill to middle bathyal depths (1000–500 m); First restrictions of deep and intermediate water connections (based on fossils of benthic fauna in the Atrato Basin).	Duque-Caro (1990)
13	First phase of deep-water blockage of the Central American Seaway (CAS; based on the beginning of North Atlantic Deep Water (NADW) production).	Wright *et al.* (1991)
12.9–11.8	Abrupt foraminiferal paleobathymetric change from lower to middle bathyal depths indicates an uplift of the Panama sill to about 1000 m.	Duque-Caro (1990)
12.8–7.1	Shallowing of the CAS from bathyal to inner neritic depth (based on sedimentological evidence).	Duque-Caro (1990)
+ 12–7.5	"Carbonate Crash", Carbonate dissolution event in the eastern Pacific and Caribbean (in the Caribbean, this event was terminated 10 Ma); Subsequent shoaling of the CAS prevents inflow of less carbonate corrosive Atlantic/Caribbean intermediate and deep water into the Pacific.	Lyle *et al.* (1995); Roth *et al.* (2000)
10.7–9.4	Intermittent closure of shallow-water connections and formation of a short-lived near-complete land bridge.	Coates *et al.* (2003, 2004); Roth *et al.* (2000)
10.4–9.9	Increased abundances of foraminiferal assemblages (*Uvigerina*, *Valvulineria*) indicate another shallowing step, pointing toward an upper bathyal depth.	Duque-Caro (1990)
* 10.1–9.1	Earliest terrestrial interchange (racoons) from North to South America (dispersal is assumed to have happened along an Island arc system).	Webb (1985)
+ 9.3–4	Further steps in the diversification of benthic foraminiferal fauna between the eastern Pacific and Caribbean; 9.3–7.8 Ma: Shoaling of the sill to upper bathyal depths. Shallow-water connection was open. 7.8–6.9 Ma: Shoaling of the CAS to 150 m water depth. Pacific-Caribbean shallow-water connection was restricted. 6.9–4.0 Ma: The sill shoaled to less than 50 m water depth.	Duque-Caro (1990)

Age (Ma)	Event & Interpretation	Reference
* 9.3; 7.5–5.5	Raccoons and their allies crossed to South America.	Marshall (1985); Webb (1985)
* 9	Ground sloths (Megalonychidae) crossing to North America.	Morgan (2002)
	Ash deposits of proboscideans, tapirs, camelids, and peccaries from Peru pointing toward a migration to South America.	Campbell *et al.* (2010)
* 9–6	Exchange of few strong swimmers (mammals) between North and South America → Close spacing of Islands ("Island model").	Molnar (2008)
* 8.2	Two genera of South American sloths crossed northward.	Marshall (1985); Marshall *et al.* (1982); Webb (1985)
+ 8–5	Changes in the neodymium (Nd) and lead (Pb) isotopic composition of hydrogenous ferromanganese crusts in the Atlantic (Gulf Stream); Diminished supply of eastern Pacific water into the Atlantic (850 m water depth).	Frank *et al.* (1999)
* 7.5	Raccoons (Procyonidae) crossed to North America.	Marshall (1988)
7	Shallow water connections > 150–100 m started to become restricted.	Duque-Caro (1990)
+ 6.8–6.6	Increasing difference in benthic foraminiferal $\delta^{13}C$ values between eastern Pacific and Caribbean; Termination of deep- to intermediate-water exchange through the ocean gateway.	Keigwin (1982a)
	Planktonic foraminiferal assemblages indicate that significant upwelling began in the western Caribbean basin; Indication of restricted intermediate water flow through the ocean gateway.	Keller *et al.* (1989)
7–6.3	Water surface circulation between the eastern Pacific and Caribbean was re-established.	Duque-Caro (1990)
7–6	Deep water passages between the eastern Pacific and Caribbean had vanished.	Coates & Obando (1996)
6	Evidence for a short-lived strait across the Panama Canal Basin.	Kirby *et al.* (2008)
	High energy currents or tidal waves passed from the eastern Pacific to the Caribbean.	Collins (1996a)
	Sill depth had decreased to 150 m.	Schmidt (2007)
+ 6–5	Changes in the physical characteristics of proto-NADW (became saltier and warmer as indicated by benthic foraminiferal $\delta^{18}O$ and Mg/Ca); Subsequent restriction of the CAS, first enhancement of heat- and salt transport to high northern latitudes.	Lear *et al.* (2003)
+ 5–4	Development of an "east-west temperature gradient" in the tropical Pacific upper water column; Shoaling of the thermocline in the eastern Pacific was linked to the shoaling of the CAS and indicates changes in the tropical wind field (and/or changes in the amount of NADW-formation that lead to a global adjustment of the thermocline; Huang *et al.* 2000).	Chaisson & Ravelo (2000)
	Eolian grain size records indicate a decrease in the trade wind strength over the tropical eastern Pacific; These changes are attributed to the shoaling of the CAS.	Hovan (1995)

Age (Ma)	Event & Interpretation	Reference
5–3	Nineteen terrestrial families of southern mammals crossed the Isthmus to the north and 17 placental mammals to the south.	Leigh *et al.* (2014); Marshall (1988); Webb (1985, 2006)
+ 4.6	Gradual increase of benthic $\delta^{13}C$ values at deep Caribbean Site 999; Enhancement of NADW-formation in the North Atlantic, stronger supply of good ventilated water masses into the Caribbean.	Haug & Tiedemann (1998)
	Distinct increase in the Carbonate preservation at Ceara Rise, equatorial western Atlantic; Deepening of the lysocline due to enhancement of NADW-formation.	Tiedemann & Franz (1997)
+ 4.7–4.2	Caribbean surface salinity increased with respect to the eastern Pacific, based on the $\delta^{18}O$ enrichment of Caribbean planktonic foraminifers. Changes in the planktonic foraminiferal fauna (higher contents of *G. sacculifer*) also indicate higher salinity in the Caribbean; Restriction of surface water exchange between the eastern Pacific and Caribbean; Diminished inflow of low-salinity Pacific surface waters; Shoaling of the seaway to < 100 m water depth.	Haug *et al.* (2001); Keigwin (1982a); Keller *et al.* (1989)
+ 4.6–4.2	Shoaling of the thermocline in the eastern Pacific as indicated by multispecies planktonic $\delta^{18}O$ records; Interpreted to reflect changes in the tropical wind field.	Cannariato & Ravelo (1997)
4.6–3.1	Significant sea-level low-stand period enhanced the shallowing of the Isthmus.	Haq *et al.* (1987)
4.5	Caribbean foraminiferal fauna indicates an increase in salinity. Increase in endemism and decrease in diversity due to adaptations to new environmental conditions.	Chaisson & Ravelo (2000); Keller *et al.* (1989)
+ 4.4	The locus of maximum opal accumulation in the eastern Pacific abruptly shifted eastward; Reorganization of eastern Pacific surface circulation.	Farrell *et al.* (1995)
+ 4.4–4.3	Decrease in planktonic $\delta^{18}O$ values at Ceara Rise (Caribbean) was interpreted to reflect a southward shift of the Intertropical Convergence Zone; Changes in the atmospheric circulation and/or pole-to equator-temperature gradients were related to the shoaling of the CAS.	Billups *et al.* (1999); Chaisson & Ravelo (1997)
+ 4.4–2.6	The divergence and provinciality of near-shore and open-ocean faunas increased significantly. Initiation of the "Great American Interchange" of vertebrates over the Central American Isthmus at about 2.7 Ma; First indications of a final Isthmus closure.	e.g., Coates *et al.* (1992); Keigwin (1978, 1982b); Lundelius *et al.* (1987); Marshall (1988); Saito (1976)
+ 4.2	Cooling of Southern Ocean surface waters, based on diatom assemblages; Increased heat piracy (trans-equatorial heat transport into the North Atlantic) *via* an enhanced Gulf Stream; Stronger thermohaline circulation.	Whitehead & Bohaty (2003)
4–3	Earliest estimates of divergence events for antbirds and woodcreepers, which are restricted to tropical forest environments.	Weir *et al.* (2009)
3.8; 3.4–3.3	Short-lasting re-openings.	Haug & Tiedemann (1998)

Age (Ma)	Event & Interpretation	Reference
3.8–3.6	Closure almost completed, though a shallow water connection continued beyond 3 Ma most likely until about 2.5 Ma (Coates & Obando 1996).	Coates *et al.* (1992)
3.7	Shallow water mollusks indicate a complete closure. The occurrence of similar pairs of Late Pliocene gastropods (2.6–1.8 Ma) on both sides of the Isthmus suggests some interchange may still have been possible.	Coates *et al.* (1992)
3.5	Few shallow gaps.	Coates & Obando (1996)
	Complete seaway closure (assumption based on data showing seasonal variations in seawater temperatures recorded within the skeletons of bryozoans).	O'Dea *et al.* (2007)
3.5–2.5	Restriction of the CAS; Water depth too shallow for nearshore and inshore organisms to cross (assumption based on genetic distances between Kemp's ridley sea turtle and olive ridley turtle).	Bowen *et al.* (1998)
3.2	Major reorganization of the ocean-climate system (northern hemisphere glaciations and large-scale Arctic sea ice appeared).	Bartoli *et al.* (2005)
3.1	Divergence events in sea urchins from both sides of the Isthmus point toward the restriction of larval exchange.	Lessios *et al.* (2001)
3–2.6	Major exchange of mammals between North and South America.	Marshall (1985); Webb (1997, 2006)
Early-Middle Pleistocene	Still some marine connections existed between the Caribbean and eastern Pacific (based on gastropod occurrences).	Beu (2001)
~3	Possible breach of the Isthmus (trend of decreasing salinity in the western Atlantic); Evidence that eastern pacific waters may have spilled over the Isthmus during high sea level stand (evidence from planktonic foraminifers).	Cronin & Dowsett (1996)
2.8–2.5	Trend of increasing salinity (evidence for Isthmus re-closure at 2.8 Ma)	Cronin & Dowsett (1996)
[+] 2.5–1.9	Permanent divergence of eastern Pacific and Caribbean faunas and floras; End of sustained surface current flow through the gateway.	Crouch & Poag (1979); Gartner *et al.* (1987); Keller *et al.* (1989)
2	Possibly another breach of the Isthmus (indications from gross trends in salinity and from Atlantic Coastal Plains)	Cronin & Dowsett (1996)
[+] 1.8	Maximum divergence of faunal provinces began; "[…] littoral-neritic leakage" (p. 73; Keller *et al.* 1989) between the oceans until 1.8 Ma.	Keller *et al.* (1989)

Table from Steph (2005) modified and supplemented. For tectonic processes see Montes *et al.* (2012a) Fig. 9a-d; * = chronological events of the Miocene Isthmus closure; + = cited from Steph (2005), p. 1-17; Ma = million years ago.

5 Ecological Consequences of the Isthmus Formation

The geological history of the Isthmus of Panama had an immense impact on the environments of the divided oceans. These environmental changes influenced the evolution of the biotic fauna and flora substantially. Today the western Atlantic and the eastern Pacific environments differ notably in several physical and ecological characteristics (e.g., Jackson & Budd 1996; Keigwin 1982; Lawrence *et al.* 2006; Maier-Reimer *et al.* 1990; Rubinoff 1968). Species distribution patterns as we see them today can often be explained by extinction events and species origination and adaptation processes, which were driven by the changing ecological conditions. The understanding of this relationship (environmental changes due to the Isthmus formation – species distribution patterns) is important when studying the evolutionary history of species, which were separated by the Isthmus of Panama. This chapter is concerned with these changing oceanographic and environmental conditions during the Isthmus formation and describes the ecological patterns (based on abiotic factors) in both oceans we can observe today. In the second part of this chapter the occurrence and distribution of selected species groups in relation to the former described environmental conditions on both sides of the Isthmus (biotic differences) are summarized.

5.1 Abiotic changes during the isthmian uplift and patterns today

The emergence of the Isthmus of Panama and subsequent isolation of the eastern Pacific and western Atlantic was a long process, which began in the Middle Miocene (Coates *et al.* 2005, but see Chapter 4). The Isthmus emergence is considered to be the largest and most important geological event of the Cenozoic with wide effects on environmental and oceanographic conditions on a global (Kameo & Sato 2000 and references therein; Ravelo *et al.* 2004) and on a regional scale (Collins 1996a; Cronin & Dowsett 1996). Prior to emergence of the Panama Isthmus, the eastern Pacific and western Atlantic were connected and the westward flowing warm Equatorial Atlantic Current (EAC) passed unimpeded into the eastern Pacific (Maier-Reimer *et al.* 1990; for details see Chapter 4). Differences in environmental conditions of both oceans during the isthmian uplift were marginal (Jones & Hasson 1985; Keigwin 1982a). While the shoaling of the Isthmus proceeded, the marine connections between the oceans became narrower (Figure 4-3). In the western Atlantic, the EAC was diverted northward and the flow of the Gulf Stream was intensified (Berggren & Hollister 1974; Burton *et al.* 1997). By the end of the Pliocene, the oceanographic and environmental conditions between the western Atlantic and the eastern Pacific became more developed (Coates & Obando 1996; Teranes *et al.* 1996). Today, several of these conditions differ significantly between the marine systems of the divided oceans (e.g., Fuglister 1960; Glynn 1972; Jackson & D'Croz 1997; Wyrtki 1981; see Table 5-3), as well as on a regional scale. Therefore, O'Dea *et al.* (2004) divided the coasts of Panama into four ecoregions: The Bocas del Toro and San Blas regions on the Caribbean side, and the Gulf of Chiriquí and the Bay of Panama on the Pacific side (Figure 5-1). The authors defined the range of the regions as follows: "In the Caribbean, the Bocas del Toro region ranges from the Archipelago de Bocas del Toro in north-western Panama along the Golfo de Mosquitos to the exit of the

Panama Canal, whereas the San Blas region extends from the Canal eastwards along the Costa Arriba to the San Blas Province. On the Pacific side, the Gulf of Chiriquí extends from the border of Costa Rica to the central edge of the Azuero Peninsula, whereas the Bay of Panama ranges from the southeastern tip of the Azuero peninsula to the Darien" (p. 150, O'Dea *et al.* 2004, citation slightly modified; Figure 5-1).

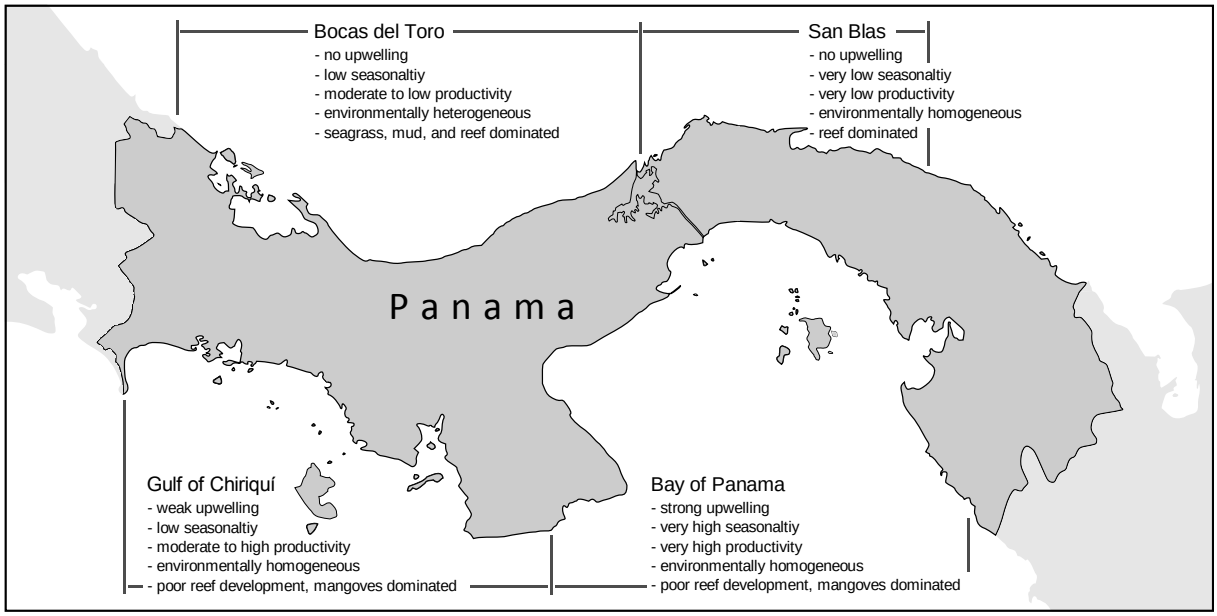

Figure 5-1: Four ecoregions along the Caribbean and eastern Pacific coasts of Panama. The Bocas del Toro and San Blas regions are on the Caribbean side. The Gulf of Chiriquí and the Bay of Panama are on the Pacific side (modified after O'Dea *et al.* 2004).

5.2 Climate and temperature

The changing environmental and oceanographic conditions due to the isthmian closure had a strong impact on the global climate. In the Early to Mid-Pliocene it was characterized by global surface temperatures, which were around 3.5 °C warmer than today (Sloan *et al.* 1996), and a stronger thermohaline circulation (Ravelo & Andreasen 2000). During the Late Pliocene the global temperature gradually decreased (Ravelo *et al.* 2004). The main causes for this event are still part of debate. Based on ostracods and planktonic foraminiferal studies, Cronin & Dowsett (1996) verified that around 3 million years ago (Ma) the oceanic heat flux of the North Atlantic increased in northward direction, which in turn, could have essentially influenced the global climate (Rind & Chandler 1991). Several studies postulate that the northeastern shift in western Atlantic currents (Bartoli *et al.* 2005; Haug *et al.* 2001; Haug & Tiedemann 1998) and accompanied redirection of warm, saline water to high latitudes (Berggren 1972; Berggren & Hollister 1974) had played a fundamental role in the onset of Plio-/Pleistocene glaciation (also known as the 'Panama hypothesis', Keigwin 1982a). Support for the Panama hypothesis is also given by Lunt *et al.* (2008). Based on an ocean-atmosphere circulation- and an ice sheet model they concluded that the Isthmus closure played a role in the onset of Northern Hemisphere Glaciation (NHG), although it was not a primary factor. They proposed that a decreasing level of

atmospheric CO_2 played a more fundamental role of NHG, as it was discussed by Berger *et al.* (1999). However, Klocker *et al.* (2005) challenged the assumption that the Isthmus closure and subsequent northward heat transport triggered in particular NHG. They argue that the heat transport resulted in higher air-temperatures of the (Sub-) Arctic with subsequent retreat of perennial snow cover. This assumption is also supported by an earlier study of Berger & Wefer (1996) who proposed that the increased heat transport rather postponed the formation of ice sheets in the Northern Hemisphere. However, in spite of the temporal accordance of Isthmus closure (i.e. Pliocene model) and the intensification of NHG, it is not clear whether the closure droved (Berggren & Hollister 1974), delayed (Berger & Wefer 1996) or preconditioned (Driscoll & Haug 1998; Haug & Tiedemann 1998) NHG.

Today, both sides of the Isthmus show substantial seasonal differences in their climate on a large, as well as on a regional scale (Figure 5-1). On a large scale, the western Atlantic shores experience generally stronger winds, rainfall, and more seasonal variation in cloud cover than the eastern Pacific (Glynn 1972). Usually, the wet season starts in May and reaches its climax in October or November (Glynn 1972) with high temperatures (Abele 1974). The dry season receives its peak from January to April with low temperatures and the occurrence of pronounced northeast trade winds (winds of high velocity; Abele 1974; D'Croz & O'Dea 2007; Glynn 1972).

Changes in sea surface temperatures occurred constantly during the Isthmus formation. In general, the western Atlantic was warmer than the eastern Pacific, which reflects modern conditions (Groeneveld *et al.* 2014). The temperature increased in the western North Atlantic around 3.5–2.8 Ma by 2–3 °C (Bartoli *et al.* 2005; Cronin & Dowsett 1996) and again between 2.4–2.0 Ma, possibly due to a re-closure of the Isthmus (Table 5-1; Cronin & Dowsett 1996). Based on foraminiferal Mg/Ca and $\delta^{18}O$ measurements, Groeneveld *et al.* (2014) studied sea surface temperatures for glacial-interglacial cycles after the intensification of NHG around 2.5 Ma. They found that sea surface temperatures varied between 21.1–25.3 °C in the eastern Pacific, and between 22.8–27.6 °C in the western Atlantic. The maximum temperatures in the eastern Pacific occurred during the interglacial, while minimum temperatures appeared during glacial periods. In contrast, maximum temperatures in the western Atlantic occurred during both peaks of glacial and interglacial times, while minima were observable during the glacial-interglacial transition (Groeneveld *et al.* 2014). Thermocline temperatures varied between 18.3–21.1 °C, were more stable, and warmer during the transition in the eastern Pacific, whereas they were more variable in the western Atlantic (17.3–22.8 °C) and in average 2–3 °C warmer in the late glacial periods (Groeneveld *et al.* 2014).

Today, water temperatures are pronounced by seasonal changes and differ on a regional scale, as well as between both oceans. In general, the sea surface temperatures of the western Atlantic are 2–3 °C warmer than in the eastern Pacific and characterized by only little variation during the year (Locarnini *et al.* 2006; O'Dea *et al.* 2004).

The year mean temperature on the Caribbean side is 28.2 °C (Glynn 1972). On a more regional scale, the mean temperature at Bocas del Toro (western Atlantic) is between 26.5–28.7 °C (Key *et al.* 2013) and for the Bay of Panama 26.6 °C (Glynn 1972). The water temperatures of the

Panama Canal entrances on both sides differ around two degrees. However, the Bay of Panama is generally affected by upwelling events that may decrease the water temperature within 24 hours to 15 °C (Glynn 1972). In contrast, during low tides, the water temperature of high tide pools can reach maxima of 43 °C.

The two divided regions of the Pacific coast of Panama, the Bay of Panama (shelf area 27 175 km^2) and the Gulf of Chiriquí (shelf area 13 119 km^2, D'Croz & O'Dea 2007), show considerably differences in their hydrology. The Bay of Panama is characterized by wind-driven upwelling during the dry season. In contrast, the Gulf of Chiriquí shows no evidence for a similar process (e.g., D'Croz & O'Dea 2007). However, the Gulf of Chiriquí has a higher freshwater input (due to higher rainfall and river discharge) than the Bay of Panama (D'Croz & O'Dea 2007). In 2007, the mean annual rainfall was 3415.12 mm in the Gulf of Chiriquí and 2158.33 mm in the Bay of Panama (D'Croz & O'Dea 2007).

The open surface water in both regions is pronounced by strong seasonal winds, whereas wind rates for the Bay of Panama are generally three times as strong as for the Gulf of Chiriquí during the dry season. While northerly winds are intense during the winter in the Bay of Panama (Xie *et al.* 2005), the high mountains in the west of Panama extenuate these winds and the Gulf of Chiriquí is less affected (D'Croz & O'Dea 2007). The Gulf Chiriquí is enclosed by land to the north and east (and only semi-enclosed to the west). Based on studies regarding surface circulations of the eastern Pacific (Kessler 2006), and their own observations, D'Croz & O'Dea (2007) assumed that in the Gulf of Chiriquí the surface water enters from the west and replaces wind displaced south moving water. In contrast, the Bay of Panama is only open to the south, hence, displace of surface water by northerly winds is much more effective. During the wet season, both regions are influenced by either southern or as well as northern winds (D'Croz & O'Dea 2007).

In both Pacific regions warm surface water lies on top of cool deep water. Assuming a thermocline by the position of the 20 °C isotherm (D'Croz & O'Dea 2007), the Bay of Panama shows a sharp rising thermocline almost to the surface during the dry season, which has cooling effects of the surface water. In contrast, the thermocline in the Gulf of Chiriquí rises to about 30 m and hence, there are no significant cooling effects for the surface water. D'Croz & O'Dea (2007) pointed out a significant correlation between surface water temperatures and the northern winds, but only in the Bay of Panama. However, during the year the Bay of Panama is characterized by changes in the sea surface temperature and a shallow thermocline, whereas the Gulf of Chiriquí shows stable surface temperatures but a deep thermocline (D'Croz & O'Dea 2007).

5.3 Salinity

The affection of the deep water circulation around 4–5 Ma due to the continuing closing of the Panama Isthmus, resulted in increasing surface salinities of the western Atlantic (Dowsett & Cronin 1990; Haug & Tiedemann 1998; Keigwin 1982a). While salinity concentrations decreased substantially in the western Atlantic about 3.1–2.8 Ma they increased again between 2.8–2.4

Ma, which hint toward a breaching and re-closure of the Isthmus (Cronin & Dowsett 1996; Table 5-1; see also Chapter 4).

Today, the western Atlantic and eastern Pacific differ in their salinity concentrations by around 1–2‰ (35.7–36.5‰ and 34.5–35‰ respectively; Antonov *et al.* 2006). This difference is mainly caused by evaporation in the western Atlantic, the transport of water-vapor over the Isthmus of Panama by the trade winds, and subsequent rainfall over the eastern Pacific (Groeneveld *et al.* 2014; Mestas-Nuñez *et al.* 2007). Near the Panama Canal entrances of both oceans, river runoffs influence the surface salinity additionally. During the wet season minimum salinity values attain 18–22‰ in these regions. Moreover, the intertidal zone especially on the Caribbean coast of Panama shows strong salinity fluctuations. During the 5-month wet-period, 330 cm of rain can fall and changes salinity concentrations in exposed pools by 20‰ in only few hours (Abele 1974).

In general, during the dry season (January-April) the surface waters around the Panama coasts are characterized by high salinity values, whereas low salinities occur during the wet season (Abele 1974). D'Croz & O'Dea (2007) measured regional variation in salinity along the Pacific coast and salinity concentrations in both regions are influenced by rainfall and river runoffs, especially during the wet season. The Gulf of Chiriquí is usually characterized by warm surface water, which is low in salinity throughout the year. In contrast, salinity values in the Bay of Panama are low only in the wet season. During the wet season, surface water salinity concentrations in both regions were 30‰ or less and increase to > 33‰ in deeper regions (D'Croz & O'Dea 2007). The Bay of Panama, however, showed more pronounced salinity fluctuations in the beginning of the wet season than the Gulf of Chiriquí, with concentrations below 29‰. During the dry season, surface water salinity concentrations rise in both regions and reach about 32‰ in the Gulf of Chiriquí and above 33‰ in the Bay of Panama, due to the displace of the warm and nutrient poor surface water by the transisthmian winds and the subsequent upwelling of deep, saline waters (D'Croz & O'Dea 2007).

Table 5-1: Historical temperature, salinity and sea level changes of the western Atlantic (WA) related to re-openings and -closures of the Panama Isthmus between 4–2 Ma.

Time Interval (Ma)	Panama Isthmus*	Southwestern North Atlantic Temperatures	WA Salinity	Eustatic Sea Level
2.4–2.0	closed?	warm	low	high
2.8–2.4	closing	cool	low → normal	low
3.1–2.8	open	warm	decreasing	high
3.5–3.1	closing	cool → warm	normal	high
4.2–3.5	open	cool	low?	high

*Closing and opening events of the Panama Isthmus refers to surface water (data and table from Cronin & Dowsett 1996, table p. 96). Ma = million years ago.

5.4 Hydrodynamic forcing

The western Atlantic and eastern Pacific differ considerably in upwelling events. In general, upwelling events bring deep nutrient-rich waters to the surface, reduce sea surface water temperatures (extreme local temperature variations from 27–15 °C within 24 hours) and increase primary production (Glynn 1972; Teranes *et al.* 1996 and references therein).

About 3.65 Ma when the eastern Pacific was no longer influenced by the northward diverted EAC (Kameo & Sato 2000) strong seasonal upwelling developed (Wellington & Robertson 2001). Upwelling events have stayed constant or increased during time in the low-latitudes of the eastern Pacific (Ibaraki 1997; Teranes *et al.* 1996) and did not change after the closure of the Isthmus of Panama (Maier-Reimer *et al.* 1990). Today, along the Pacific coast these upwelling events occur during the dry season from January to April, which show regional variations (O'Dea *et al.* 2004; Teranes *et al.* 1996; Figure 5-1). Especially the Bay of Panama experiences strong upwelling because the low-lying coast is affected by the northerly winds and surface water is moved offshore (Glynn 1972; O'Dea *et al.* 2004; Xie *et al.* 2005). In contrast, weak upwelling occurs along the coasts of Venezuela and Colombia during the wet season (D'Croz *et al.* 1991; Glynn 1972; Jackson & D'Croz 1997). In general, no upwelling events are observable along the western Atlantic coasts of the Isthmus (Jackson & D'Croz 1997; O'Dea & Collins 2013; Figure 5-1). However, several evidences (e.g., areas of cold temperatures in a general warm Late Pliocene, Cronin & Dowsett 1996; faunal indicators of cold waters, Allmon 1993; Allmon *et al.* 1996; evidence for high productivity, Keller & Barron 1983; Carbon and oxygen isotopics, Allmon *et al.* 1996; Jones & Allmon 1995) show that the low-latitude of the western Atlantic was pronounced by local upwelling events until about 3 Ma before declined (Allmon 2001). In contrast to the western Atlantic, no upwelling events occur along the Panama coasts of the eastern Pacific during the wet season (D'Croz & O'Dea 2007) and the Bay of Panama and the Gulf of Chiriquí show similar hydrological patterns (warm surface waters, intense thermocline, stratification of the water column).

The tides of both sides of the Panama Isthmus show differences in their time-intervals, forecasts, and amplitudes. The Bay of Panama is characterized by highly predictable semi-diurnal tides of a maximum daily range amplitude of 6 m, whereas the western Atlantic tides are mixed (i.e. semi-diurnal as well as diurnal), less predictable and characterized by only a slight amplitude (< 2 m). Moreover, western Atlantic tides are significantly influenced by local climatic conditions like onshore winds during the dry season, which results in unusual high water levels, turbidity and an increase of suspended sediments (Abele 1974; Chesher 1972; Glynn 1972; Palka 2005).

Sea level change is another parameter, which changed substantially in the western Atlantic about 3.1–2.8 Ma (high sea level) and again between 2.8–2.4 Ma (low sea level) and supports the assumption of a breaching and re-closure of the Isthmus (Cronin & Dowsett 1996; Table 5-1; see also Chapter 4). Today, in general the water level of the eastern Pacific along the American coast is around 50 cm higher than of the western Atlantic coast (Reid 1961). Around the Panama Canal entrances the average sea level on the eastern Pacific side is more than ¼ meter higher

than on the western Atlantic (Reid 1961). However, the water level on both oceans is fluctuating in response to wind and upwelling events (D'Croz & O'Dea 2007).

5.5 Nutrients and productivity

When studying nutrient compositions and productivity patterns in marine systems, the so called Redfield ratio (Redfield 1934) is an important tool to estimate primary and secondary productivity (Thangaradjou *et al.* 2014). More precisely, the Redfield ratio reflects the nitrogen–phosphorus relation in a ratio of 16:1, which is considered to be suitable for the growth of phytoplankton (D'Croz & O'Dea 2007; Redfield 1958). Additionally, chlorophyll a concentrations can be used as an index of marine phytoplankton abundances and productivity patterns (Thangaradjou *et al.* 2014). However, estimations of marine nutrient ratios and their effect on chlorophyll distribution can provide evidences for possible growth limitations in phytoplankton (Paul *et al.* 2008). This correlation is important because phytoplankton constitutes the food source for herbivorous consumers, which in turn, present the source for different levels of carnivorous consumers and, in the end, of top predators.

In general, high productivity rates are associated with upwelling events. Because of local upwelling in the western Atlantic until around 3 Ma (as mentioned above), productivity values were high. Several other evidences support this assumption. For example, Allmon *et al.* (1996) studied vertebrate and invertebrate fossils of the eastern Gulf of Mexico. Based on isotopic analyses they assumed that during the Pliocene the productivity in the western Atlantic was higher than today. Due to the isthmian closure, the environmental and oceanographic conditions especially in the Caribbean changed dramatically and the productivity broke down (Allmon 2001).

Today, the western Atlantic and the eastern Pacific show differences in their seasonal dynamics between nutrients and upwelling processes (D'Croz & O'Dea 2007). Variability occurs also on a regional scale. In the eastern Pacific the Gulf of Chiriquí and the Bay of Panama show seasonal differences (D'Croz & O'Dea 2007). Generally, during the wet season, both regions show low nutrient concentrations near the surface, which increase with depth. During the dry season upwelling, nutrient rich water rises from about 40 m to the surface in the Bay of Panama and nutrient concentrations increase (see above). Because enriched waters only move upward to a level of about 30 m in the Gulf of Chiriquí, nutrient concentrations stay low (D'Croz & O'Dea 2007). Along the western Atlantic coast, the Bocas del Toro archipelago and the San Blas region differ in their nutrient dynamics as well. The Chiriquí Lagoon of the Bocas del Toro archipelago has a large nutrient input resulting from freshwater runoffs and high human impact. Additionally, half of the lagoon is enclosed by land resulting in "long residence times of the water" (p. 423, D'Croz *et al.* 2005). In contrast, the San Blas region is more oligotroph due to low human impact and only small freshwater input. Moreover, this region is pronounced by "open coastal zones" (p. 423), thus nutrients are more easily washed away (D'Croz *et al.* 2005).

In respect to nitrogen–phosphorus ratios (N:P ratios) both regions show generally values (< 5:1) below the Redfield ratio (16:1) near the surface with increasing values by depth. This pattern

was observable in both regions during the wet season (no upwelling) down to 50 m depth (D'Croz & O'Dea 2007) and nutrients may become depleted by freshwater input due to maximum rainfall (Fiedler *et al.* 1991). However, in the Bay of Panama the Redfield ratio increases and shows maximum N:P ratios during the mid-dry season at about 40 m (D'Croz & O'Dea 2007).

Chlorophyll a concentrations are also different in both regions. During upwelling events, high chlorophyll a values are observable in the Bay of Panama, while low values occur in both regions during the wet no upwelling season (D'Croz *et al.* 1991; D'Croz & O'Dea 2007). D'Croz & O'Dea (2007) assumed that the general low rates of chlorophyll in the upper water level is related to limited nitrogen during the entire year in the Gulf of Chiriquí and during the wet season in the Bay of Panama. During upwelling events, nutrients and, in turn, chlorophyll concentration increased in the Bay of Panama.

5.6 Summary

In the southern Caribbean the water temperature is on average 2 °C warmer and the western Atlantic waters are around 1‰ more saline than those of the eastern Pacific (Haug *et al.* 2001; Teranes *et al.* 1996). Reasons for these temperature and salinity differences are higher rates of evaporation in the western Atlantic and transport of the moisture-rich air to the eastern Pacific. Moreover, the western Atlantic water is more influenced by freshwater due to estuaries and poor on nutrients, whereas the eastern Pacific water is influenced by lagoons and rich on nutrients (O'Dea *et al.* 2007). The eastern Pacific coast is pronounced by "interannual and seasonal variations in temperature and productivity associated with El Niño events and upwelling are great, planktonic productivity is high, corals and seagrasses are rare to absent, and suspension feeders overwhelmingly dominate benthic communities. In contrast, the Caribbean coast experiences no upwelling, much smaller interannual and seasonal variability, and lower planktonic productivity" (p. 5501, O'Dea *et al.* 2007 and references therein; Figure 5-1). Based on these described environmental characteristics of both oceans, the coasts of the western Atlantic are considered as more heterogeneous than the eastern Pacific coasts (O'Dea *et al.* 2007). Based on these criteria, the coasts of Panama can be split into four different regions: the Bocas del Toro and San Blas regions in the western Atlantic, the Gulf of Chiriquí and the Bay of Panama in the eastern Pacific (Figure 5-1; O'Dea *et al.* 2004). O'Dea *et al.* (2004) generalized the three main environmental differences between the western Atlantic and eastern Pacific. The authors argued that these differences do not only occur along the entire coast of each ocean, but also on a regional scale, i.e. between regions along the same coast (p. 148):

1) The coastal waters in the eastern Pacific are substantial more productive than in the western Atlantic.
2) The western Atlantic coast is environmentally more stable than the coast of the eastern Pacific. Environmental changes on the Pacific coast occur in different intervals: temperature and nutrient levels are influenced by upwelling events on a seasonal time-

scale. El Niño events intermittently interrupt the normal seasonal cycles on an inter-annual timescale, which can lead to attenuate upwelling.

3) The western Atlantic shows a wider range of habitats than the eastern Pacific and is ecologically more complex.

5.7 Biotic differences of the two oceans

The above described oceanographic changes have a significant impact on habitat structures (Heinze & Crowley 1997), which in turn, have an immense influence on faunal composition (see below). Cronin & Dowsett (1996) showed that the intervals of Isthmus re-openings and -closures led to shallower oceanic thermal gradients than those today, which resulted in wider ranges of suitable habitats for marine species. For example, ostracod species were able to disperse to middle and high latitudes due to these wider ranges of fitting refuges (Cronin & Dowsett 1996). Yet, in the Late Pliocene/Early Pleistocene, differences in faunal patterns existed only on a small scale, which were often pronounced due to sedimentation patterns, for example between the Bocas del Toro and Limón Basin (Collins *et al.* 1995). These observations were also noticed by Teranes *et al.* (1996) using $\delta^{18}O$ analyses of fossil bivalve shells. In another study, Schneider & Schmittner (2006) argue that the emergence of the Isthmus and the accompanying change in ocean circulation shifted marine biological productivity patterns in both oceans. The reduced flow-through of rich nutrient Pacific surface water led to a decrease of productivity in the Atlantic, while the productivity in the eastern Pacific increased.

Today, the western Atlantic is characterized by sympatric occurring coral reefs, seagrass beds, and mangroves. In contrast, the habitat structure in the eastern Pacific differs considerably (Figure 5-2). Coral reefs and mangroves occur separately from each other and seagrass beds are widely absent (Jackson & D'Croz 1997). The authors pointed out that "in the absence of reefs, mangroves and seagrasses are restricted to bays and estuaries, where they are protected from the full force of the sea. When reefs are present, mangroves and seagrasses may occur behind them anywhere along the coast" (p. 47, Jackson & D'Croz 1997).

As a result of the different environmental conditions described above, species occurrences and abundances along the eastern Pacific and western Atlantic coasts of Panama differ considerably (O'Dea *et al.* 2004). Several paleontological and stratigraphical studies reveal the patterns of faunal occurrence, which we can observe today (reviews in Allmon 2001; Budd 2000; Collins & Coates 1999; Jackson *et al.* 1996a). For example, cupuladriids (O'Dea *et al.* 2004), encrusting bryozoans (Cheetham & Jackson 2000), corals (Glynn 1982), sponges (van Soest 1994) and benthic foraminiferans (Collins 1999) are more diverse in the western Atlantic than the eastern Pacific (list from O'Dea *et al.* 2004). On the other hand, for example echinoderms, mollusks and crustaceans are slightly more diverse in the eastern Pacific (e.g., Abele 1972, 1976; Chesher 1972; Vermeij 1996).

The geographic distribution of mollusks and crustaceans in the western Atlantic and eastern Pacific is generally controlled by habitat types and, hence, by environmental conditions. In the 70s, the number of Panama associated mollusks (Bivalvia and Gastropoda) on the eastern Pacific

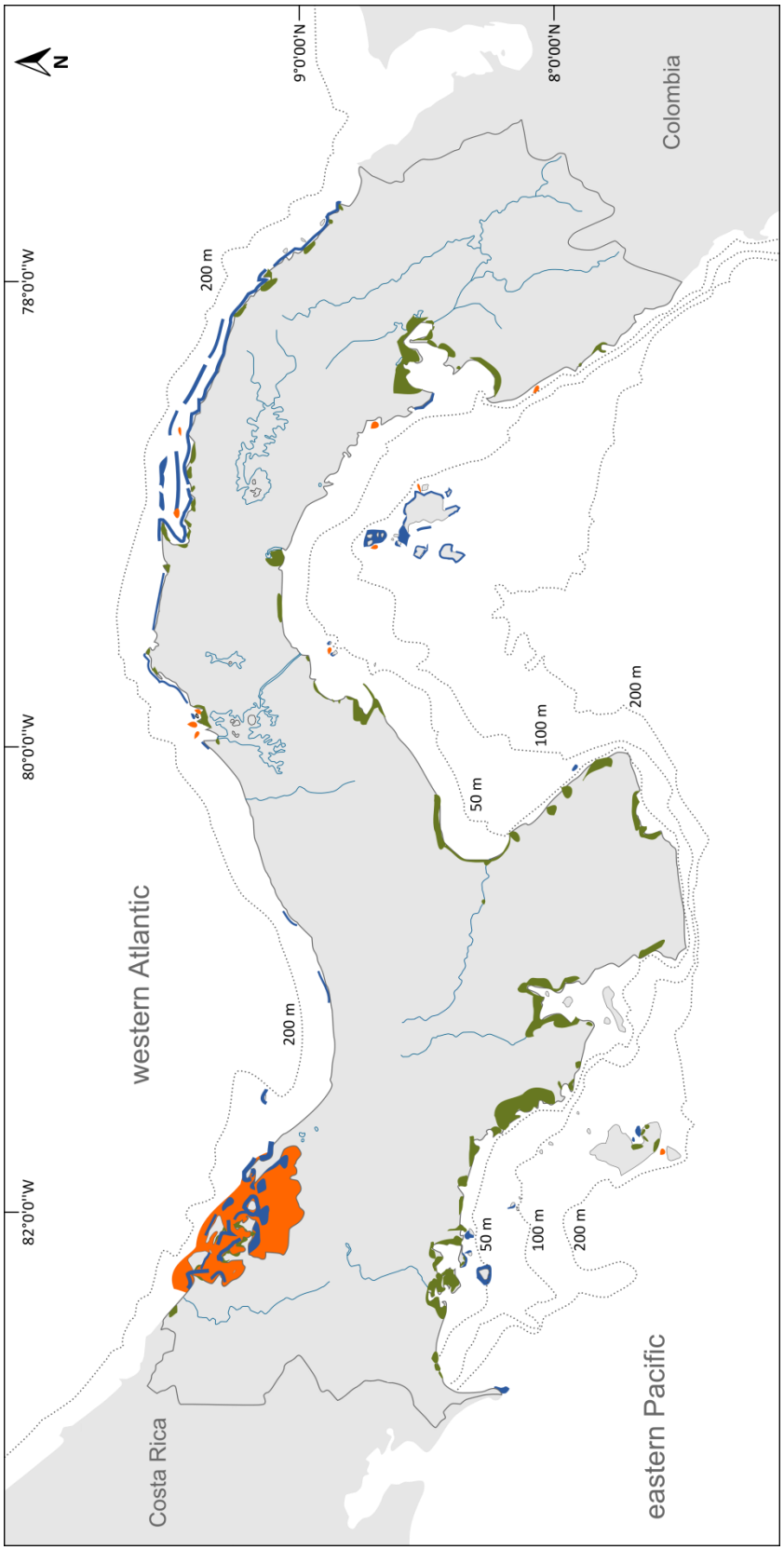

Figure 5-2: Panama – Distribution of mangroves, coral reefs and seagrasses. Gray shades: land masses, gray line: Panamian border to Colombia (right) and Costa Rica (left), blue lines: rivers, dashed lines show isobaths, green shades: mangrove distribution, blue shades: distribution of coral reefs, orange shades: distribution of seagrasses. Map modified after D'Croz & O'Dea 2007 (p. 326). For references of the different habitat distributions see 'Ocean Data Viewer A-C' (Chapter 13).

side was estimated by around 4500 species. At that time, the approximately number of mollusk species along the western Atlantic coast of Panama was unknown (Olsson 1972). Based on a metadata analysis in 2010, Miloslavich and colleagues estimated the mollusks diversity of the Caribbean and counted a total of 3032 species. Approximately 587 of these species inhabit the western Atlantic coast of Panama, which Miloslavich *et al.* (2010) described as intermediate molluskan richness (less than 1000 species). However, mollusks present the most diverse group in the Caribbean. Endemism is around 26% and many endemic species are found among Cypraeidae, Marginellidae, Olividae and Columbellidae (Díaz 1995; Miloslavich *et al.* 2010 and references therein).

Diversity and distribution patterns, as well as extinction and origination events of the marine biota on both sides of the barrier are associated with changing environmental and oceanographic conditions during the emergence of the Isthmus of Panama (Allmon 2001). Intense extinction- and origination events occurred in particular among mollusks in the western Atlantic during the Miocene and Pliocene (Allmon 2001; Todd *et al.* 2002; Woodring 1966), which is also observable in the fossil record (O'Dea *et al.* 2007). For example, from 27 species of the genus *Strombina* in the early Pliocene, only 3 recent species occur in the western Atlantic but are abundant in the eastern Pacific (Jackson *et al.* 1996b). For these strombinids the change of temperature due to the Isthmus closure seems a partial explanation for their extinction and origination events (Jackson *et al.* 1996b).

The change of nutrient availability and productivity, as well as changes in temperature during the Isthmus emergence (see above), influenced the occurrence and distribution of mollusk species in both oceans and forced morphological changes, for example in suspension- and non-suspension feeders (e.g., Turritellidae, Marginellidae, Columbellidae; Allmon *et al.* 1995). There are many examples of variation in diversity patterns among molluskan species between both oceans today. For example, Vermeij (1996) pointed out that the number of muricid mollusks in comparable habitats between the two oceans is slightly higher in the eastern Pacific. More precisely, the Pacific coast of Panama contains 61 muricid species, whereas 58 species occur in the western Atlantic around Florida, followed by 46 species from the coasts of Venezuela. Bivalves show similar patterns, whether lower rates than gastropods, of origination and extinction events during the Late Pliocene. For example, chionine bivalves of the family Veneridae experienced extinction rates of 82.6% in the western Atlantic and 38.5% in the eastern Pacific during the Pliocene (Roopnarine 1996). Due to high origination rates during the Pleistocene, the eastern Pacific chionine fauna is more diverse than the western Atlantic fauna today. Analog to the strombinid mollusks (Jackson *et al.* 1996b), morphological patterns of chionine bivalves are associated with a decrease in productivity in the Caribbean, i.e. Pacific species are larger than their Caribbean relatives (Roopnarine 1996). The same pattern is shown among corbulid bivalves (family: Corbulidae). Eastern Pacific species increased in size during the Late Neogene, whereas western Atlantic species decreased (Anderson 2001). The author argued that these size developments based on nutrient changes, especially a reduction of nutrient availability in the western Atlantic, during the emergence of the Isthmus (Anderson 2001; see above). Along the eastern Pacific shores dense populations of oysters can be found at mid- and

high-tidal levels, which in turn, provide microhabitats for a rich microbiota (Glynn 1972). In contrast, the western Atlantic shores at comparable tide-levels harbor only few sessile shelled species, though in most regions they are absent (Glynn 1972).

Crustaceans are the second diverse phylum within the western Atlantic counting approximately 2916 species (mollusks = 3032 species; Miloslavich *et al.* 2010). Environmental conditions, in particular the abundance of different substrates, influence the composition and richness of species in the respective habitats substantially (Abele 1974, 1976; Kinne 1963). For example, the feeding grounds and -times of decapods in the intertidal zone are affected by tides. At low tide, more feeding grounds are available, which is also reflected in high species abundances (Abele 1974). Other examples are the mangrove species *Panopeus herbstii* and *Eurytium limosum*. Abele (1976) pointed out that these widely distributed and mangrove associated species inhabit marshes, when mangroves are absent.

In 1972, Abele studied the decapod fauna of different ecotypes (sandy beach, mangrove, and rocky intertidal among others) and compared the decapod diversity between the eastern Pacific and western Atlantic in the respective habitat (Table 5-2). A total of 25 decapod species were collected in the sandy beach habitat of the eastern Pacific (17 species) and western Atlantic (8 species) coasts of Panama (Abele 1972). The most abundant species on the eastern Pacific coast was the mole crab *Emerita rathbunae* and accounted for over 50% of all collected individuals. On the western Atlantic coast, the most common species was the mole crab *Hippa testudinaria* (50% of all collected individuals; Abele 1972). The faunal assemblage and diversity is related to the substrate structure. The sand beaches of the eastern Pacific are quartz based and stabilized by mud. This is reflected by burrow-inhabiting species of e.g., *Callianassa*, *Pinnixa*, and *Ambidexter*. In contrast, the western Atlantic sand beaches are calcareous and affected by shifting due to strong winds and irregular tides during the dry season. Burrow-inhabiting species are absent (Abele 1972).

The red mangrove *Rhizophora mangle* is predominantly occurring on both coasts of Panama. The number of sampled mangrove associated species varies only slightly between the oceans (eastern Pacific –20 species, western Atlantic –17 species). The most abundant decapod genera on the Pacific side were *Petrolisthes* and *Sesarma*, in particular the species *Petrolisthes zacae*, *Eurytium tristani*, and *Sesarma rhizophorae*. Abele (1972) mentioned that *S. rhizophorae* was also found in the western Atlantic mangroves, despite the assumption that this species is restricted to the eastern Pacific. On the western Atlantic side, the most common decapod species were *Panopeus herbstii*, *Merguia rhizophorae*, *Uca rapax*, and *Sesarma curacaoense*.

The rocky intertidal comprises a diverse fauna of decapod species. Abele (1972) found 78 species in the eastern Pacific and 67 species on the western Atlantic coast of Panama. The most common species along the eastern Pacific rocky intertidal were the hermit crabs *Clibanarius albidigitus* and *Calcinus obscurus*, as well as *Xanthodius sternberghii* and *Petrolisthes armatus*. In contrast, on the western Atlantic coast, the most abundant decapod species were *Calcinus tibicen*, *Paraliomera dispar*, *Clibanarius antillensis*, *C. tricolor*, *Cataleptodius floridanus*, and *Pachygrapsus transversus*.

Abele (1972) summarized that the eastern Pacific coast is slightly richer in decapod species than the western Atlantic if same habitats are compared. Additionally, Abele (1972) realized that an increase of (closely related) species is related to an increase of the complexity of the habitat (Table 5-2). In his study, Abele (1976) estimated that around 6% of the observed decapods are identical between the two sides of the barrier, whereas 45% "have undergone slight morphological modifications resulting in the recognition of species-pairs termed geminate or analogous species" (p. 263; Table 5-2).

Table 5-2: Comparison of Panamanian crustacean communities between different habitat types.

Community	Number of Species		Number of Closely Related Species	Similarity Index (%)
	Eastern Pacific	Western Atlantic		
Sandy Beach	17	8	3 pairs	26
Mangrove	20	17	10 pairs	54
Rocky Intertidal	78	67	27 pairs	37

Table and data adapted from Abele 1972 (p. 130) and 1976 (p. 266).

Based on the abiotic conditions, the fauna of sandy beaches along the western Atlantic coast is more restricted in comparison to the eastern Pacific side. Glynn (1972 and references therein, but see Dexter 1972) pointed out that "a Pacific beach contained approximately three times as many species (n = 41), six times the density of individuals (1434/m^2) and nine times the biomass (9.13 gm/m^2) of an Atlantic beach community" (p. 23). In contrast, species of western Atlantic beaches are more uniform distributed.

Coral reefs shape the widely distributed calcareous sand beaches of the western Atlantic coasts of Panama (Glynn 1972). The extensive lava coasts and rarity of reefs in the eastern Pacific offer a striking biological and physical contrast to the limestone-coral coast of the Caribbean side. These patterns of reef growth and coral distribution is reflected in the more unstable environments of the eastern Pacific (see above; Glynn & Colgan 1992 and references therein; Porter 1972; Figure 5-2). In contrast to western Atlantic coral reefs, the reefs of the eastern Pacific are commonly small, isolated, and characterized by monospecies of *Pocillopora*, *Porites* or *Pavona* (Glynn & Colgan 1992). Porter (1972) pointed out that only six hermatypic scleractinian coral genera (out of 100) occur in both oceans, and on species level both oceans have probably only one species in common (out of 800). In respect to ahermatypic scleractinian coral genera the western Atlantic and eastern Pacific have around 20 genera in common (out of 150), and an undefined number of species. In general, the western Atlantic coasts of Panama are known as the richest coral region within the Caribbean, inhabits around 49 hermatypic and 16 ahermatypic scleractinian corals (Porter 1972). In contrast, the coasts of the eastern Pacific harbor around 16 hermatypic and one ahermatypic scleractinian corals (Porter 1972).

Earle (1972) reported a total of 195 marine plant species from both sides of the Panama Isthmus. Along the western Atlantic coast, 125 species were found and 90 species occurred along the

eastern Pacific coasts of Panama. A total of 20 species were common to both oceans and were often widely distributed or cosmopolitan. The well-developed seagrass beds in the western Atlantic coast of Panama are pronounced by *Thalassia testudinum* and *Halodule wrightii* (intertidal distribution), as well as *Syringodium filiforme* and *Halophila baillonis* (subtidal distribution; Earle 1972). In general, the western Atlantic and eastern Pacific differ also in the occurrence of algae groups. In the western Atlantic, fleshy algae are predominant within the low-tidal level, whereas filamentous algae are more common in the eastern Pacific (Glynn 1972). Around 15 species (15%) of green algae occur on both sides of the Isthmus of Panama (Wysor 2004). However, environmental differences between both oceans are reflected in the diversity of certain algae species, for example of the macroalgae family Udoteaceae. The western Atlantic inhabits around 34 species of this family, whereas only three species are present in the eastern Pacific. Wysor (2004) explained this pattern with “the lack of sandy habitats on the Pacific coast” (p. 227).

5.8 Summary

Beside the considerably oceanographic differences between the western Atlantic and eastern Pacific (see Subchapter 5.1) habitat and biotic differences between the oceans are also well pronounced (Figure 5-2). Cronin and Dowsett (1996) pointed out that the closure of the Isthmus presents a key event for the biotic evolution and occurrence of species on both sides of the Isthmus and for the evolution of *transisthmian sister species* (TSS) in particular (for a detailed discussion about TSS see Chapters 6 and 8). Thus, the following patterns of habitat structure and species occurrence can be observed between the western Atlantic and eastern Pacific:

- Coral reefs, seagrass beds, and mangroves occur sympatrically along the western Atlantic coasts, whereas coral reefs and mangroves occur separately from each other and seagrass beds are widely absent in the eastern Pacific (Table 5-3; Figure 5-2).
- Species distribution depends on habitat structure and environmental conditions. Thus, e.g., encrusting bryozoans, cupuladriids, corals, sponges, and benthic foraminiferans are more abundant in the western Atlantic, whereas mollusks, crustaceans, and echinoderms are slightly more diverse in the eastern Pacific (Table 5-3).

Table 5-3: Comparison of abiotic and biotic factors between the western Atlantic and eastern Pacific oceans.

	During the Emergence of the Isthmus of Panama		Today	
	Western Atlantic	**Eastern Pacific**	**Western Atlantic**	**Eastern Pacific**
Abiotic Factors				
Climate & Temperature	- Global surface temperatures around 3.5 °C warmer than today; - Stronger thermohaline circulation; - Decrease of global temperature; - Constantly changes in sea surface temperatures.		- Seasonal differences in their climate on a large, as well as on a regional scale; - Water temperatures of the Panama Canal entrances differ around 2 °C.	
	- 22.8–27.6 °C (sea surface temperatures); - 17.3–22.8 °C (thermocline temperatures).	- 21.1–25.3 °C (sea surface temperatures); - 18.3–21.1 °C (thermocline temperatures).	- Stronger winds, rainfall, and more seasonal variation in cloud cover; - Average sea surface temperatures (~ 28.2 °C), in general 2–3 °C warmer than in the EP; - Bocas del Toro: 26.5–28.7 °C.	- Average sea surface temperature Bay of Panama: 26.6 °C; - Upwelling events.
Salinity	–		- Differences in salinity concentrations 1–2‰ due to evaporation in the WA, transport of water-vapor and subsequent rainfall over the EP; - Salinity concentrations near the Panama Canal entrances low: 18–22‰ (wet season); - Dry season: high salinity values; wet season: low salinity values.	
	- Periodic changes in surface salinities (in relation to re-openings and -closures).	–	- Salinity concentration 35.7–36.5‰	- Salinity concentration 34.5–35‰
Hydrodynamic Forcing	- Low-latitude of the WA pronounced by local upwelling until about 3 Ma before declined; - Periodic changes in sea level.	- Development of strong seasonal upwelling.	- Weak upwelling during wet season in the South WA; - Less predictable semi-diurnal as well as diurnal tides with low amplitude; - Tides are influenced by local climatic conditions.	- Upwelling events during dry season with regional variations; - No upwelling during wet season, rather warm surface waters, intense thermocline, and stratification of the water column; - Highly predictable semi-diurnal tides with high amplitude; - Water level ~50 cm higher;

Nutrients & Productivity	–		- Seasonal differences in nutrient concentrations between the oceans and on a regional scale (see text for details).	
	- High productivity values.	–	- Poor in nutrients.	- Rich in nutrients.
Biotic Factors				
Habitat Structure and Species Abundances	- Development of various habitats; - Differences in faunal patterns only on a small scale.		–	–
	–	–	- Sympatrically occurring coral reefs, mangroves, and seagrass beds; - Limestone-coral coasts; - Diverse occurrence of cupuladriids, encrusting bryozoans, corals, sponges, and benthic foraminiferans; - More uniform distribution of species.	- Separated occurrence of coral reefs and mangroves, seagrass beds are widely absent; - lava coasts; - Slightly more diverse occurrence of echinoderms, mollusks and crustaceans; - Beach: three times as many species (n = 41), six times the density of individuals (1434/m^2), and nine times the biomass (9.13 gm/m^2) of a WA beach community.
Molluska	- Change of species occurrence and distribution; - Increase of morphological changes.		–	
	- Intense extinction- and origination events.	–	- Around 3032 species; - Less diverse; - Few sessile shelled species.	- Around 4500 species; - More diverse; - Dense populations of sessile shelled species.
Crustacea	–	–	Panama coast: - 8 decapod species in the sandy beach habitat; - 17 decapod species in the mangroves; - 67 decapod species in the rocky intertidal.	Panama coast: - 17 decapod species in the sandy beach habitat; - 20 decapod species in the mangroves; - 78 decapod species in the rocky intertidal.

Coral Reefs	–	–	- Richest coral region within the Caribbean (coast of Panama); - 49 hermatypic corals; - 16 ahermatypic corals.	- Rare, small isolated, monospecies; - 16 hermatypic corals; - 1 ahermatypic coral.
Plants and Algae	–		- 20 species of plants on both sides; - 15 species of green algae on both sides.	
	–	–	- 125 plant species; - Well developed seagrass beds; - Fleshy algae are common; - 34 species of the macroalgae family Udoteaceae.	- 90 plant species; - Rare occurrence of seagrass beds; - Filamentous algae are common; - 3 species of the macroalgae family Udoteaceae.

Summary of biotic and abiotic parameters during the emergence of the Isthmus of Panama and today; WA = western Atlantic, EP = eastern Pacific.

6 Transisthmian Sister Species

Terminological terms regarding transisthmian sister species (TSS) are clarified in this chapter and the theoretical criteria for determining TSS are outlined. In form of a review this chapter builds a theoretical framework for the later 'Case Studies'. Therefore, the first part of this chapter introduces the term TSS, presents a brief overview about the evolution of TSS, and summarizes the criteria, which have to be met to attain the status of a TSS. In the second part, these criteria will be reviewed and critically discussed in detail.

6.1 What are transisthmian sister species?

Note: The term transisthmian sister species (TSS) in not clearly defined and numerous synonyms and derivatives referring to TSS are casually used throughout the literature. In Chapter 8 this problematic and confusing determination will be discussed in detail. However, the following paragraph will briefly summarize and explain the most important terms regarding TSS used in this thesis

Definition:
[Terms referring to *transisthmian sister species* and used as *false* synonyms in this study: *geminate* species; *sibling* species; *sister* species; *twin* species; see Chapter 8 for details].

In 1908, David S. Jordan (Jordan 1908) coined the term *geminate species* for "twin species – each one representing the other on opposite sides of some form of barrier" (p. 75). Phylogenetically the term *sister species* refers to species, which are each other's closest relatives and whose evolutionary branches coalesce to a most recent common ancestor (MRCA, Figure 6-1). In the isthmian context, these sister species are also known as *transisthmian sister species* and refer to taxa that have diverged as a result of the emergence of the Isthmus of Panama. In almost complete isolation, each member of a pair evolved independently on either side of the Isthmus.

For a clear and unambiguous understanding of the following (sub-) chapters it is crucial to define and discriminate between different terms derived from TSS (see Chapter 8 for more details; Figure 6-1):

- *Transisthmian sister species pair* (**TSS pair**) – This term refers to TSS as one entity (i.e. one representative of the western Atlantic forms a pair with its sibling of the eastern Pacific, *directly linked* to the closure of the Isthmus of Panama).
- *Transisthmian sister species complex* (**TSS complex**) – This term refers to TSS where several species on one side of the Panama Isthmus, can represent the putative sister to the species on the other side (Collins 1996b).
- *Transisthmian pseudo sister species* (**pseudo-TSS**) – This term refers to TSS originated from speciation events, which are not linked to the Isthmus of Panama formation and occurred before or even after the final closure (e.g., due to dispersal or extinction events; Hurt *et al.* 2009; Miura *et al.* 2012).

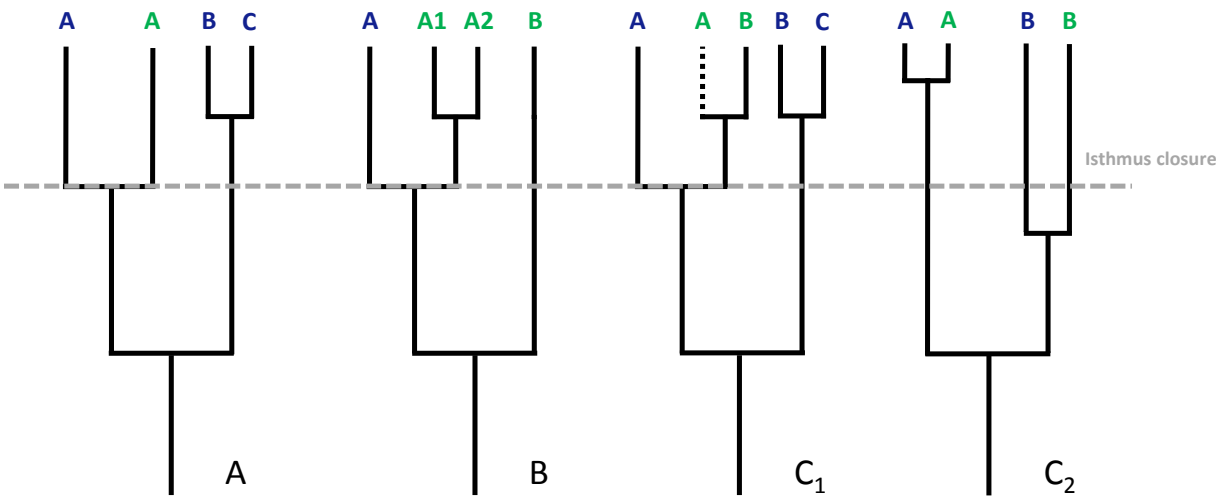

Figure 6-1: Definition of the different terms referring to transisthmian sister species (TSS). green = species of the western Atlantic, blue = species of the eastern Pacific; black dashed line = extinction event, gray dashed line = Isthmus closure; A: TSS pair; B: TSS complex; C_1-C_2: Pseudo-TSS – due to extinction (C_1), origination after (TSS pair A of C_2) and before the Isthmus closure (TSS pair B of C_2).

6.2 The evolution of transisthmian sister species

The closure of the Isthmus of Panama had a direct effect on oceanographic and environmental conditions of both oceans and the American continents, as well as on the diversity of the marine and terrestrial biota (see Chapter 5). In the marine context, populations of diverse marine taxa were separated and experienced one of three outcomes, which are traceable in phylogenetic analyses (Figure 6-2): (i) populations of the same species survived on both sides of the Isthmus (i.e. TSS evolved), (ii) the population survived on only one side of the barrier (i.e. no evolution of TSS), or (iii) the populations on both sides became extinct. Even though most populations only survived on one side or their paths ended in extinction numerous TSS evolved. These origination events were primarily driven by allopatric divergence, i.e. due to the emerging Isthmus and subsequent reproductive isolation between the populations (Lessios 1998; Wiens 2004). Diverged populations with initially similar genomes experienced separate evolution in different environments. These populations represent a natural experimental setting to study speciation processes in general and the mechanisms of genetic drift and natural selection in particular in a defined time frame (Lessios 2008; Palumbi 1994; Wiens 2004).

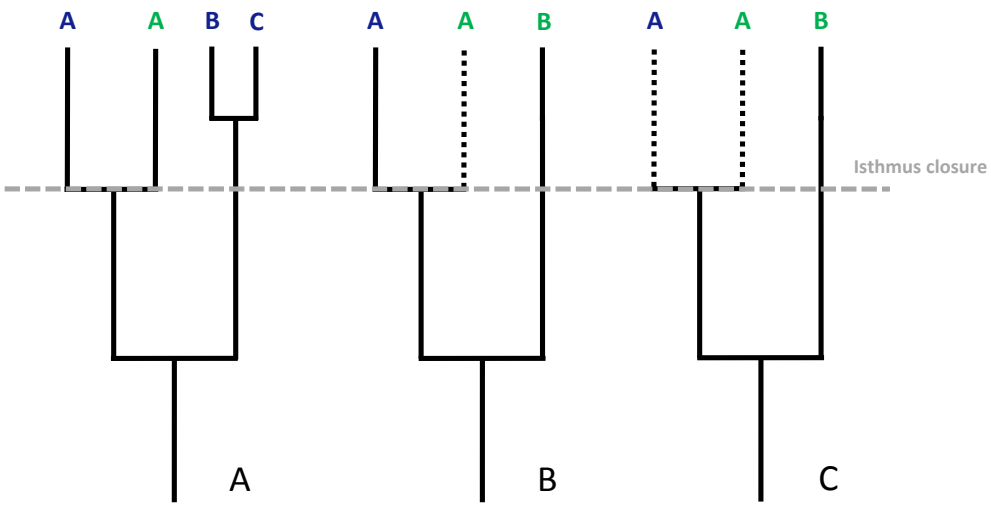

Figure 6-2: The evolution of TSS. Due to the closure of the Isthmus of Panama, populations of the same species A: survived on both sides of the Isthmus (TSS evolved), B: survived on only one side of the barrier (no evolution of TSS), or C: became extinct on both sides (black dashed line).

Today, species distributions and abundances are determined by the different physical environments of both oceans (Figures 5-1 and 5-2; for details see Chapter 5). The eastern Pacific is characterized by more productive coastal waters and an environmentally unstable coast, whereas the western Atlantic is formed by diverse habitats. These differences are reflected by the various occurrence of e.g., sponges (van Soest 1994), encrusting bryozoans (Cheetham & Jackson 2000), cupuladriids (O'Dea *et al.* 2004), and benthic foraminiferans (Collins 1999) in the western Atlantic (list from O'Dea *et al.* 2004). Coral reefs show quite different species distribution patterns between the western Atlantic and eastern Pacific. These highly diverse ecosystems cover large areas in the western Atlantic, whereas coral reefs in the eastern Pacific are patchy, less diverse and younger (Budd 2000; Cortés 1997; Glynn 1982; Figure 5-2). These characteristics of eastern Pacific coral reefs are the result of extreme temperature, salinity and nutrient conditions enduring since the isthmian closure (Cortés 1997; Chapter 5). Another example of different biodiversity patterns between the two oceans is represented by seagrass beds. Brasier (1975) postulated that the marine seagrass genus *Thalassia* inhabited frequently the bays of the eastern Pacific, but disappeared after final Isthmus closure. Today, seagrass beds are widely absent in the eastern Pacific, but very common in the western Atlantic (Jackson & D'Croz 1997). On the other hand, mollusks (Roy *et al.* 2000), crustaceans (Jones & Hasson 1985), and echinoderms (Chesher 1972) appear to be diverse in both oceans with a slightly higher abundance in the eastern Pacific (e.g., Abele 1972, 1976; O'Dea *et al.* 2004; Vermeij 1996).

6.3 The criteria to be a transisthmian sister species pair

When exactly do species achieve the status of a TSS pair? General assumptions as well as specific criteria regarding to biogeographic distribution, morphological similarities, and molecular characteristics were defined to classify species as geminates (see Collins 1996b). Specifically these criteria are:

1) Geographic isolation drives speciation processes
   (e.g., Eldredge & Gould 1972; Mayr 1954).
2) The barrier and the consequentially isolated taxa are of the same age
   (Humphries & Parenti 1999).
3) TSS distribution ranges are close to the Isthmus
   (Collins 1996b).
4) Related taxa on either side of the barrier show similarities in their morphology
   (Collins 1996b).
5) TSS pairs and -complexes within a genus show similar divergence ages
   (Collins 1996b).

These criteria are discussed in more detail in the following subchapters and resulting problems will be highlighted.

6.3.1 Geographic isolation drives speciation processes

Geographic isolation is the prevalent cause that drives speciation processes in organisms. The Isthmus of Panama, where sporadic gene flow between organisms exists until today, presents an outstanding example to study evolutionary processes in a natural environment in general and to provide a basis for intensive research on diverse topics on e.g., speciation (Cronin 1985; Jordan 1908; Mayr 1954), isolating processes (Lessios & Cunningham 1990), and biogeographic patterns (Ekman 1967; Vermeij 1978) in particular. Lessios (1998, p. 187) pointed out four reasons why the Isthmus of Panama and its resulting TSS is an ideal model to study first stages of geographic speciation:

a) The aspect of time.
   The time of Isthmus emergence and its final closure is very well defined (assuming an Isthmus closure around 3 million years ago) and sufficient for species to accumulate measurable differentiations.

b) The efficiency of the barrier.
   The Isthmus of Panama almost completely impeded genetic contact between the marine species on both sides of the barrier, likely more effective than any other comparable barrier.

c) Collective separation.
   The simultaneous separation of numerous different species (i.e. species with for example different life histories and dispersal abilities) provides comprehensive possibilities to test essential factors, which may play a key role in differentiation and speciation processes.

d) Different environmental conditions.
   The western Atlantic and the eastern Pacific environments differ markedly in several ecological parameters (e.g., temperature, salinity, upwelling events; see Chapter 5 for details) and have been in place for over 3 million years (My; Cronin & Dowsett 1996; D'Croz *et al.* 1991; Glynn 1972). Thus, the different environmental conditions of both oceans provide a useful setting to study adaptation processes of TSS.

In summary, the closure of the Isthmus of Panama and the evolution of TSS present an ideal setup to study first stages of speciation, even if the observed patterns are not always easy to interpret (Lessios 1998). Vermeij (1993) outlined that “Tropical America will continue to be perhaps the finest laboratory in which to answer the big questions about what controls biological diversity" (p. 1604).

6.3.2 The barrier and the consequentially isolated taxa are of the same age

Humphries & Parenti (1999) pointed out that the age of a barrier (like the Isthmus of Panama) and the age of the thereby isolated species has to be the same. Indeed, studies that concentrated on the evolution of TSS usually assumed that the youngest TSS pair of a phylogeny diverged as a direct consequence to the final Isthmus closure (note the discussion about possible re-openings and -closures of the Isthmus in Chapter 4) and, therefore, should be of the same age as the barrier itself (Collins 1996b). However, there are numerous examples of assumed TSS pairs showing dispersed divergence ages (e.g., reviewed in Lessios 2008). These findings result from speciation events, which are not directly linked to the Isthmus closure but indicate that species have separated long before the final closure or even afterwards. Several studies showed that the occupied habitat may have an influence of the species divergence ages. Deep water species, for example, should have been affected earlier by the rising Isthmus. Shallow water species, on the other hand, may have crossed the Isthmus until just prior to its final closure (Ekman 1967; Frey 2010; Knowlton & Weigt 1998). This assumption is in accordance with studies on crustacean species. Among crustaceans, mangrove and shallow water species show young divergence ages (conform to the Isthmus closure at 3 Ma) compared to those which occupy deeper habitats (e.g., Knowlton & Weigt 1998; Schubart *et al.* 1998). Also changing marine physical conditions accompanied by the rising Isthmus may have played a role regarding the time of divergent events. Species, with low tolerances to changing oceanographic conditions (e.g., salinity, temperature, current patterns) might have migrated before the last periods of Isthmus emergence (Duque-Caro 1990; Hurt *et al.* 2009). However, there are also descriptions of pseudo-TSS (note that the definition of pseudo-TSS in this study differs from Hurt *et al.* 2009). In their context, these TSS are defined to have originated from speciation events before the final Isthmus closure accompanied by extinction events. These pseudo-TSS exhibited larger genetic distances and therefore an older divergence age, than expected in true geminates (Hurt *et al.* 2009).

Extinction events and an incomplete sampling present other sources of questionable TSS pairs. For example, if one member of a TSS pair had gone extinct (e.g., the western Atlantic member), the phylogeny would prune down and identifies the next most related species to the extinct one as the *true* sibling (Lessios 1998; Figure 6-1 C1). This, in turn, would result in an apparent TSS pair with older divergence age (Marko & Jackson 2001). Knowlton & Weigt (1998) pointed out that such extinction events and hence delusive TSS pair relations are common and explained it with the oceanographic differences between the two oceans (see chapter 5). An example for such a scenario are mollusks, which show low numbers of recent TSS pairs, probably due to severe extinctions in the Pleistocene (e.g., Marko 2002; Williams & Reid 2004; Woodring 1966). Although the fossil record of marine organisms is often poor and contains gaps, it supports the

assumption that TSS status was, however, not enforced by the rising Isthmus alone but also driven by extinction events. Bivalves, gastropods (Stanley 1986; Todd *et al.* 2002) and corals (Budd 2000; Budd *et al.* 1996) present such organisms, which show pronounced pulses of extinction.

On the other hand, there are several examples of species, which show much younger divergence ages than the age of the Isthmus, pointing toward speciation events subsequent to the Isthmus closure (Lessios 2008). These observations may base on dispersal (Miura *et al.* 2012; see below), continued gene flow between geminate species after the final Isthmus closure, interrupted gene flow of different TSS pairs at different times (Lessios 1998), or an additional speciation event of one twin member after spatial isolation, which results in the appearance of TSS complexes (e.g., Collins 1996b and references therein).

In summary, the criterion that the age of a barrier is concordant to the age of the isolated species may result in misleading assumptions. Different sources of error account for wrong determined TSS pairs and divergence ages:

- Gene flow may have occurred before or after the *final* completion of the Panama Isthmus.
- Species' ability and thresholds to adapt to changing environmental conditions.
- Extinction events may have occurred or the sampling was incomplete.

Furthermore, it is misleading to assume a simultaneous splitting in all TSS pairs: "Species on either side of the Isthmus may have been separated by the same barrier, but the final interruption of gene flow may not have occurred at the same time" (p. 188, Lessios 1998). The fossil record may give critical evidence for questionable TSS pair relations (De Queiroz 2005) as it provides detailed information regarding divergence processes and past- and modern-day patterns of (sister) species occurrence (Budd 2000; Jackson *et al.* 1993).

6.3.3 TSS distribution ranges are close to the Isthmus

At the beginning of the 20th century Jordan & Kellogg (1907) noticed the phenomenon that related species are closely distributed to the separating barrier. Based on this observation they proposed the *law of distribution* for closely related species: "Given any species in any region, the nearest related species is not likely to be found in the same region nor in a remote region, but in a neighboring district separated from the first by a barrier of some sort, or at least by a belt of country, the breadth of which gives the effect of a barrier" (p. 120). However, there are also several examples where TSS show a widespread distribution, which is not directly linked to the barrier (e.g., crustaceans, Williams *et al.* 2001; echinoderms, Lessios *et al.* 2001; mollusks, Marko 2002). Such distributions can be explained by extinction (Cunningham & Collins 1994; Keppel *et al.* 2009) and various forms of dispersal- and migration events. Because distribution patterns are an important factor to study the evolution and occurrence of TSS, the following paragraph reviews this topic in more detail.

6.3.3.1 Gene flow in spite of a closed Isthmus

Transport and migration patterns of aquatic organisms (and in this context of TSS in particular), are important factors to understand species distribution, the colonization of new habitats, gene flow and, in the end, potential speciation processes (Walther *et al.* 2015). Movements of organisms take place constantly and the occupation of new regions depends on the availability of suitable habitats. The following paragraph will focus on migration and dispersal forms more closely, in particular on (i) oceano- and geographic modes, (ii) active and passive dispersal, and (iii) geological conditions.

(i) Several studies indicate that after the Isthmus closure, low regions of the Isthmus breached (several times) due to salt water incursions, which were enforced by sea level fluctuations (Cronin & Dowsett 1996; Haq *et al.* 1987). Lessios (1998) pointed out that such breaches persisted only for short time intervals. For example, foraminiferal assemblages point toward an "incipient littoral-neritic leakage" (p. 73, Keller *et al.* 1989) across the Isthmus between 2.4–1.8 Ma (Crouch & Poag 1979; Keller *et al.* 1989). However, re-openings and -closures of the Isthmus allowed species to migrate between the oceans beyond ~3 Ma, which is reflected in young divergence times of TSS pairs (e.g., Lessios 2008, see Chapters 4 and 10).

(ii) The movement of individuals to new regions is known as dispersal. Thereby two modes of transport can be differentiated: active and passive dispersal. Active dispersal involves self-generated movements of individuals to new sides, while passive dispersal implies transport by external vectors (currents, other organisms, wind, anthropogenic) and is accepted as the more common form (Bilton *et al.* 2001). Active movements are shown in a variety of aquatic species like meiobenthic copepods (Fleeger *et al.* 1995), soft-sediment invertebrates (Butman 1987), and reef fish larvae (Montgomery *et al.* 2001). The covered distances for active dispersal range from small scales (centimeters to meters in soft-sediment invertebrates) up to 100 kilometers in reef fish larvae (Butman 1987; Montgomery *et al.* 2001, respectively).

As mentioned before, passive dispersal is the more common form and can occur due to different vectors and on different scales:

Currents – they provide opportunities for long-distance dispersal in which dispersal distances vary widely and depend on oceanographic conditions, species life history strategies and larval behavior (Shanks 2009).

Biotic vectors – eggs, larvae, juveniles and even adult specimens of different invertebrate groups (mainly mollusks and crustaceans) are transported by migratory birds (in their guts and subsequent dispersion, attached to feathers, bills or legs; Figure 6-3; e.g., Green & Figuerola 2005; Sousa 1993; Wesselingh *et al.* 1999) and other animals (Bilton *et al.* 2001). A study by Miura *et al.* (2012) showed that closely related snails of *Cerithideopsis* spp. were only recently dispersed across the isthmian barrier by migrating shorebirds in both directions. Their molecular clock analyses (see Chapter 7) indicated dispersal events from the Pacific to the Atlantic about 750 000 years ago and from the Atlantic to the Pacific around 72 000 years ago.

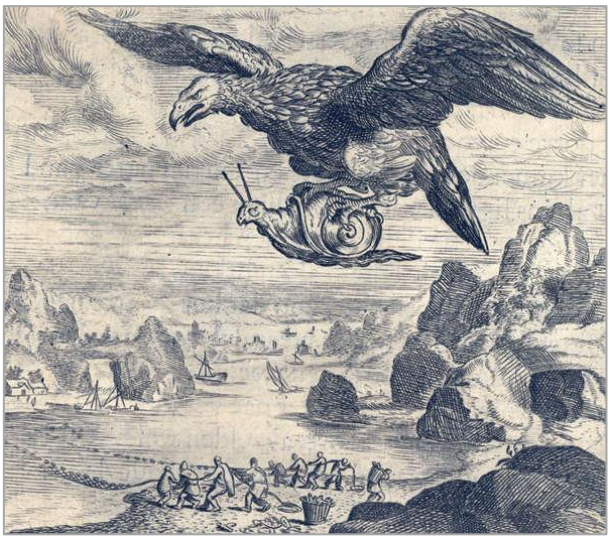

Figure 6-3: 'Pride comes before the fall'. Etching from Marcus Gheeraerts, 1567.

Wind – Vanschoenwinkel *et al.* (2008) demonstrated that wind is often an underestimated dispersal vector and plays an important role in distribution patterns of (freshwater) invertebrates, especially where other dispersal vectors are scarce or absent.

Anthropogenic – this factor has become common on (aquatic) species distribution. Bilton *et al.* (2001) summarized several examples of "human-mediated dispersal" (p. 173) due to e.g., introduction of species into a new environment through escapes or releases of species from aquariums, culturing farms, and bait fishing, and the development of artificial water channels – most prominently, the Panama Canal. This freshwater connection between the Caribbean and eastern Pacific constitutes a direct migration route between the oceans for several fish species (McCosker & Dawson 1975) since its opening in 1914. Yet, only one fish species is known to have successfully colonized the other side (Rubinoff & Rubinoff 1968). Furthermore, planktonic and fouling organisms have successfully crossed the Isthmus in ballast water of ships or attached to their hulls (Chesher 1968; Muirhead *et al.* 2015; Roy & Sponer 2002). The introduction of (nonnative) species into a new environment due to anthropogenic transport can result in critical invasions of the introduced species, even to the point of extinction of the native fauna (Clavero & García-Berthou 2005) and with dramatic effects on ecology and economy (Hebert *et al.* 1989; Pimentel *et al.* 2005). In any case, dispersal by humans implies rather recent events (within the last 3500 years; Keppel *et al.* 2009).

(iii) Beside the classic dispersal patterns discussed above, various marine connections could offer alternative ways for genetic exchange between taxa of the western Atlantic and the eastern Pacific even after the final Isthmus closure: in the north the Bering Strait (e.g., Gladenkov *et al.* 2002) and in the south the Drake Passage (Lessios 2008). However, both water ways were presumably insurmountable for tropical organisms, since water temperatures around the Bering Strait region decreased during the Pliocene due to Northern Hemisphere Glaciation (Maslin *et al.* 1998) and also the Drake Passage cooled down (Hodell & Warnke 1991).

Several studies give evidences for genetic contact between several taxa within the last 3 Ma through circumglobal migration patterns (e.g., trumpetfishes (*Aulostomus*) and sea urchins (*Diadema*); Bowen *et al.* 2001; Lessios *et al.* 2001, respectively). Thus, potential sister species with a member showing a cosmopolitan distribution may not have evolved as a result of the emerging Isthmus but rather due to long distance migration routes around the world (Lessios 1998).

In summary, the assumption that TSS distribution ranges are close to the Isthmus is not met by all TSS. Several authors pointed out that many taxa show broad geographical distributions (see above), which are based on different dispersal vectors, oceano- and geographic factors, as well as geological conditions. Additionally, extinction events play a significant role in distribution patterns and are crucial for biogeographical interpretations (Keppel *et al.* 2009).

6.3.4 Morphological similarity between TSS

Günther (1868) was the first to recognize morphological similarities between fish taxa from both sides of the Isthmus. Thus, the assumption arose that TSS in general are very similar in their morphology to each other. In fact, the literature is rich of TSS whose status was based merely on similar morphological characteristics, e.g., brittle stars and sea urchins (Chesher 1972; Roy & Sponer 2002), gastropods, bivalves, and cephalopods (e.g., Aronowsky & Angielczyk 2003; Marko & Jackson 2001; Vermeij 1978; Voight 1988), stomatopods and isopods (e.g., Manning 1969; Weinberg & Starczak 1989), and fishes (Jordan 1908; Lessios *et al.* 1995; Rubinoff 1963). However, the designation of sister species as geminates, which bases on morphological similarity alone is potentially problematic (Collins 1996b). For example, Losos (2009) showed that the degree of morphological similarity is not an unambiguous characteristic to determine the degree of relatedness in Caribbean lizards. Also Knowlton (1986) and Felder & Staton (1994) showed that morphological characters alone are often insufficient to distinguish closely related decapod species (Figure 6-4). These observations fit into the general problem of the morphological species concept. In their position paper, Meier & Willmann (2000) summarized the main objections regarding the morphological species concept (p. 34). They conclude that the classification of species, if based on morphological characters alone will result in random and unstructured taxonomy.

TSS complexes, on the other hand, constitute another source of error, because true TSS relationships can be easily obscured. Based on molecular analysis, Marko & Moran (2009) studied the bivalve genus *Acar* and found a high cryptic diversity within, which obscured the number of postulated TSS pairs. Their results are in accordance with the general paleontological perspective that high rates of species diversification occurred after final Isthmus closure. This example shows that molecular genetics may uncover patterns in certain studies, which were not detectable due to morphological methods, and that phylogenetic relationships between potential TSS can be revealed (Figure 6-4 A). On the other hand, if species are too diverse in their morphological characteristics, potential TSS pairs will stay undetected. This can happen if taxa have diverged long before the completion of the Isthmus (see above) and morphological characteristics have become various (Collins 1996b).

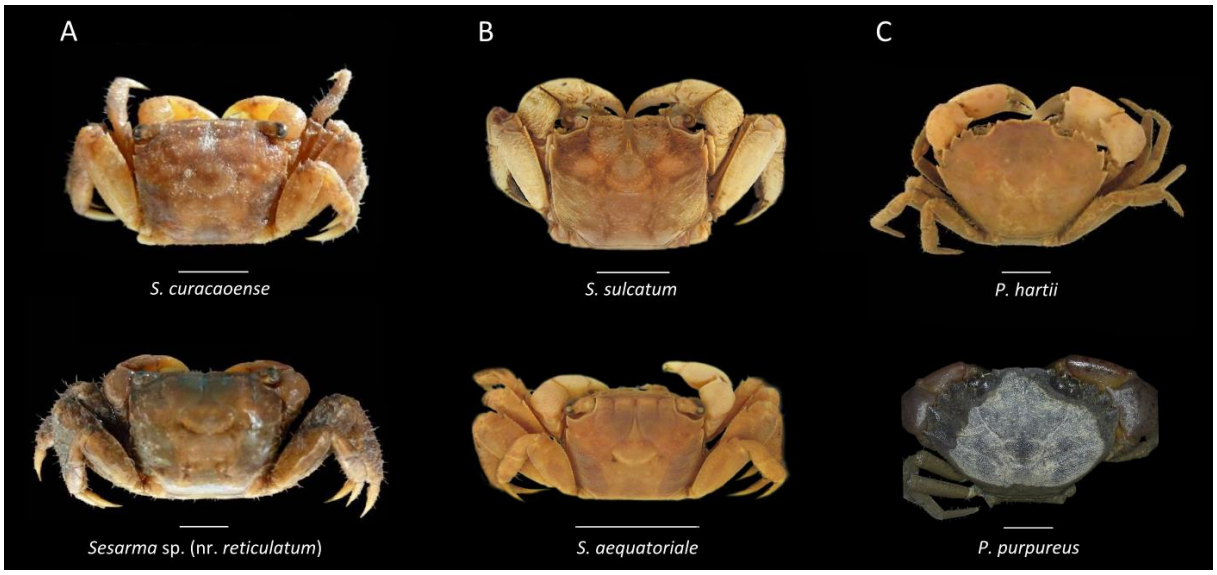

Figure 6-4: TSS determination based on morphological characters alone may result in misleading and false assumptions regarding TSS relationships. A) The species *Sesarma* sp. (nr. *reticulatum*) was assumed to be closely related to *S. reticulatum*. Molecular analysis revealed that the species is, in fact, *S. curacaoense*. B) The eastern Pacific species *S. sulcatum* and *S. aequatoriale* both belong to the *Sesarma* TSS complex B of this study. Based on a similar morphology, they are easily to confuse. C) The species *P. hartii* and *P. purpureus* (*Panopeus* TSS pair) show a very similar morphology. Additional photos of the species are shown in the Appendix (A3).

In summary, to study TSS on morphological characteristics alone is often problematic. TSS complexes or high morphological variation between species can lead to misinterpretations and finally to wrong TSS pair designations. Lessios (1998) summarized this problem: "[…] lacking a *morphological clock,* we have no means of estimating the amount of morphological change expected to occur in 3 million years" (p. 188). Thus, TSS analyses should not be based on morphological comparisons alone rather a combination of different methods should be applied to study the relationships between TSS pairs.

6.3.5 Similar divergence ages between TSS pairs and -complexes

Molecular analyses are a useful tool to reveal taxonomic relationships and to identify TSS pairs, especially where traditional methods like morphological comparisons are less clear (Collins 1996b; see above). The discovery of a correlation between the elapsed time since species separated from their MRCA and the amount of accumulated molecular differences (e.g., Gillespie 1991; Nei 1987), has been a huge step forward in molecular biology in general and in evolutionary studies in particular. Since the mid-1970s, various methods were developed to estimate genetic differences in species, e.g., through protein studies (e.g., Gorman & Kim 1977; Nei 1972; Vawter *et al.* 1980) or DNA comparisons (e.g., Kimura 1980; Knowlton *et al.* 1993). The general assumption is that evolutionary rates of the same protein/gene are similar among closely related TSS pairs and thus, divergence times can be compared with each other. This allows the identification of simultaneous divergence events between TSS pairs, which in turn, can be correlated to respective geological events (e.g., the closure of the Isthmus of Panama; Lessios 2008). This so-called 'molecular clock approach' offers a possibility to estimate divergence times, particularly when other information is unavailable (e.g., due to a poor fossil

record; see Chapter 7 for details). Based on similar genetic distances, several authors demonstrated simultaneous diversification processes of TSS within the same genus (e.g., Bermingham & Lessios 1993). However, many studies have also shown that rates vary considerably within taxa and between genes (e.g., due to occupation of different habitats; but see Chapter 7 for details). In 2008, Lessios published a review about the emergence of the Isthmus of Panama and resulting TSS pairs. In this review, Lessios reports a total of 38 DNA fragments, which have been sequenced in numerous different clades (n) of echinoids (n=9), crustaceans (n=38), fishes (n=42), and mollusks (n=26). After Lessios (2008) uniformly reanalyzed the previous studies he concluded that 34 of the published clades "are likely to have been separated at the final stages of Isthmus completion, 73 split earlier and 8 maintained post-closure genetic contact" (p. 63) due to the high genetic distance observed. For example, TSS pairs within bivalves, with the smallest genetic distance, have been separated from each other before the Isthmus closure (Marko 2002; but see Lessios 2008, table 4). The reason for the rarity of recent true TSS pairs might be related to, amongst others, extinctions of closer related lineages in the Pleistocene (e.g., Lessios 2008; Marko 2002; Williams & Reid 2004) or wrong assumptions regarding the abruptness and effectiveness of barriers, which geographically and genetically isolate species (Collins 1996b).

In summary, the molecular clock approach allows the dating of divergence events of closely related species, assuming relative evolutionary rate constancy through time. These divergence events can be correlated to vicariance events, such as the closure of the Isthmus of Panama. The prediction that TSS pairs show similar divergence times and rates, which correspond to the emergence of the Isthmus of Panama, is questionable for most of the predicted TSS pairs (Knowlton & Weigt 1998; Lessios 2008). In the majority of cases, TSS became isolated from their MRCA during different time intervals, independent of the Isthmus emergence (Lessios 2008, see discussion above).

6.4 Summary

The comprehensive review of the five specified criteria revealed that a general classification of TSS pairs into the five criteria is not practicable. The fulfillment of such criteria strictly depends on the analyzed TSS pair, in particular on their life histories, dispersal and adaptation abilities, tolerance thresholds, and preference of habitats. Therefore, only criterion 'a' (i.e. Geographic isolation drives speciation processes) is naturally met by any TSS pair. However, in Chapter 9, these criteria will be analyzed and discussed in respect to the studied TSS pairs and -complexes of this study.

7 The Molecular Clock

The so called *molecular clock approach* is concerned with the dating of divergence events between two sister species. If a molecular clock for a particular gene exists and its rate of evolution can be obtained, then the unknown divergence times between two species can be estimated. On the other hand, the assignation of time to a particular divergence process allows inferring rates of molecular evolution. These rates are based on the assumption that divergence events of closely related species occurred nearly at the same time. In this context, the closure of the Isthmus of Panama constitutes an important calibration point and is the most widely used geological event for calibrating the molecular clock.

This chapter presents a brief overview of the early history of the molecular clock, and the advantages and pitfalls of this method are critically reviewed. The last part of this review focuses on uncertainties of molecular clock calibrations and the use of the Isthmus of Panama as calibration point in particular.

7.1 The molecular clock — The discovery of the constant ticking

During the past decades, biologists have used a range of methods to study diversification processes of species from a common ancestor. One widely applied method is the *molecular clock approach*. The general concept of this inventive method is to relate the divergence times of taxa to the number of fixed mutations (i.e. substitutions) in nucleotide or amino acid sequences (e.g., Wilke *et al.* 2009, p. 25).

The basic idea of the molecular clock dates back to the sixties, when the scientists Emile Zuckerkandl and Linus Pauling (1962) observed that amino acid differences were accumulated in an amount, which was roughly proportional to divergence times of the species, estimated from fossil evidence. In 1965, Zuckerkandl & Pauling mentioned the molecular clock for the first time. They noted that, if their assumptions regarding a molecular clock were true, then "the changes in amino acid sequence will, however, be limited almost exclusively to the functionally nearly neutral changes" (p. 149, Zuckerkandl & Pauling 1965). This idea was taken by several scientists who suggested that neutral changes in sequences and the observed rate constancy can be useful tools to estimate divergence times (e.g., Kimura 1968; Sarich & Wilson 1967; Wilson & Sarich 1969). Based on these observations, the neutral theory of molecular evolution was proposed (Kimura 1968). This theory initially predicted that the number of neutral substitutions was constant (i.e. substitution rate) and equal to the rate of evolutionary change, regardless of the effective population size (N_e).

However, in 1971, Kimura & Ohta observed heterogeneity patterns in substitution rates of amino acids. This led to a modification of the neutral theory of molecular evolution to the *nearly* neutral theory of molecular evolution, where N_e and slightly deleterious substitutions were taken into account (Ohta 1972a; b, 1973). Thus, N_e scales inversely with the substitution rate, i.e. "it is possible for a large number of alleles with neutral and slightly deleterious mutations to

remain transient in a large population for relatively long periods of time before slowly being fixed or removed by genetic drift or purifying selection" (p. 707, Crandall *et al.* 2012).

7.2 The molecular clock — A controversial debate

The applicability, in particular the accuracy, of the molecular clock is discussed controversially. Numerous publications address problems regarding, for example, different evolution rates of genes, rate heterogeneity among different taxonomic groups, body size effects, or the time dependency of molecular clocks (e.g., Britten 1986; Bromham & Penny 2003; Ho & Larson 2006; Kimura & Ohta 1971; Takahata 2007; Thomas *et al.* 2006). Moreover, the careless and uncritical use of such a powerful tool for divergence time estimations leads additionally to unrealistic estimates and brings uneasiness and refusal against the molecular clock within the scientific community (Howell *et al.* 2008; Pulquério & Nichols 2007). Wilke *et al.* (2009) pointed out that this concern (i.e. use of the molecular clock as statistical tool) is often based on the complex mathematical background, inflexible software packages, confusing terminology, and a lack of credible calibration points/bounds or rates. Despite all the concerns and difficulties regarding the molecular clock approach, Takahata (2007; in reference to Bromham & Penny 2003) declared that:

"[…] the molecular clock can put a new timescale on the history of life, thereby allowing exploration of the mechanisms and processes of organismal evolution. Similarly, a molecular clock is an irreplaceable source of information in evolutionary biology and it would be foolish to abandon it altogether […]. Despite inherent fluctuations and various interpretations, the molecular clock has become a most useful tool—perhaps *the* most useful—for studying molecular evolution" (p. 4, Takahata 2007).

This statement seems to be prospective since the molecular clock is a powerful tool in evolutionary biology, as long as it is applied with caution and methodicalness. It should be noted that the use of the molecular clock does not necessarily implies an exact clock, but an approximation of the clock (i.e. estimation of significant errors which affect the clock rate), which still can be very useful (Takahata 2007). Nevertheless, this approximation in particular presents inconveniences (e.g., Ayala 1997; Ho & Phillips 2009; Pulquério & Nichols 2007).

7.3 Calibrating the clock — A difficult endeavor

In general, two main approaches are distinguishable in molecular clock analyses: the relaxed molecular clock and the strict molecular clock. On the one hand, there are several theoretical and practical studies published demonstrating that molecular clock rates can vary along branches of a phylogenetic tree (e.g., Ayala 1997; Bromham & Penny 2003; Drake *et al.* 1998; Takahata 2007; Thomas *et al.* 2006). These variations are reflected by relaxed molecular clocks (i.e. rates are allowed to vary based on several substitution rates along the branches of a phylogeny; Wilke *et al.* 2009). On the other hand, it has been shown that, under certain circumstances, variation within a group of species can be constrained (see table 3 in Wilke *et al.*

2009) and thus, a strict molecular clock (i.e. only one substitution rate for all branches of a phylogenetic tree) can be applied (Wilke *et al.* 2009).

However, some issues should be considered when using the molecular clock approach, such as rate heterogeneity (i.e. mutations occur at different rates along the branches of a phylogenetic tree), the persistence of ancestral polymorphism (i.e. the amount of heterogeneity that is present in an ancestral population before the divergence event), substitutional saturation of the data set, the effect of purifying selection (i.e. high short-term mutation rates vs. low long-term substitution rates), or the sensible estimation of the prior conditions of the clock values (list from Wilke *et al.* 2009). Moreover, the use of single sequences (hence the possibility of species misidentifications), inaccuracy in molecular analyses (amplification or use of different gene regions and, perhaps of different species), the inclusion of pseudogenes (see Lessios 2008), or algorithms that are not convenient for the data set often complicate molecular clock analyses (Bandelt 2008). These problems are widely discussed in the literature and sometimes considered and optimized in molecular clock studies (e.g., Hickerson *et al.* 2003; Wilke *et al.* 2009). Another important issue is that many studies do not address the data together with their model selections, resulting in ambiguous conclusions and, in the end, in unreproducible divergence times/rates. In fact, several authors pointed out (e.g., Ho *et al.* 2005; Pulquério & Nichols 2007; Wilke 2004, but see Wilke *et al.* 2009) that difficulties with data sets and misinterpretation of the yielded results can affect molecular clock estimates in the same taxon by over 1000%. Other issues are biological factors, which may influence molecular clock rates and hinder clock calibrations. These biological factors are discussed below in more detail.

7.4 Biological factors

In order to understand how rates can be constant, it is important to note that substitution rates can be affected by biological variables. Ayala (1999) specified five biological factors that may influence the substitution rate within and between lineages (list modified from Wilke *et al.* 2009, p. 34):

1) **Generation time** — shorter generation times *accelerate* the clock because the time for new mutations to be fixed is shortened. This assumption is especially important, if the major source of mutations is DNA replication-dependent errors; also see Takahata 2007.
2) **Effective population size** — larger population sizes will *slow* the clock because the time for new mutations to be fixed is increased; also see Woolfit & Bromham 2005.
3) **Species-specific differences in properties that affect DNA replication** — different species may have various properties, which in turn, lead to diverse mutation rates; also see Bromham & Penny 2003.
4) **Changes in the function of a protein as evolutionary time proceeds** — for example in the case of gene duplications, which may result in an *acceleration* of the mutation rate, and
5) **Natural selection** — adapting patterns and processes differ for every organism and therefore, making a precise prediction regarding evolutionary rates unfeasible.

Beyond these factors, Wilke *et al.* (2009) mentioned further variables:

6) **Body size effect** — smaller species lean to have *faster* rates of molecular evolution; Gillooly *et al.* 2005; Martin & Palumbi 1993, but see Thomas *et al.* 2006.
7) **Body temperature** — organisms differ in their body temperature, which affects metabolic rates. Higher metabolic rates *increase* the production of oxygen radicals causing DNA damage, which in turn, leads to mutations; Bleiweiss 1998; Gillooly *et al.* 2005, but see Lanfear *et al.* 2007, and
8) **Life history traits** — mutation rates of mitochondrial and nuclear genes are supposable higher in hermaphrodites than those in gonochorists; Davison 2006; Foltz *et al.* 2004.

Most of these predictions are highly hypothetical and several of these variables are controversially discussed, disproved or not even been tested in the context of the molecular clock (Wilke *et al.* 2009). As described above, the influence of these variables on substitution rates might not be uniform, but gene and taxon specific (e.g., Lanfear *et al.* 2007; Wilke *et al.* 2009). Several studies could not find a relationship between substitution rates and one of these variables. For example, Thomas *et al.* (2006) found no correlation between substitution rate and body size in invertebrates, and Kumar & Subramanian (2002) found no significant correlation between generation time and substitution rates among diverse groups of mammals. In fact, many scientists believe that taxon specific differences are the result of the uneven process of natural selection (e.g., Takahata 2007). However, in diverse groups, for example invertebrates, a universal molecular clock may be untenable (Thomas *et al.* 2006), but acts effectively and valid in smaller groups, like birds, where biological and life history variation is more constrained (Weir & Schluter 2008, see Wilke *et al.* 2009). In fact, the development of gene-specific molecular clock rates (i.e. *trait-specific*), which can be used for groups of species with similar biological requirements, would be a great advantage in the field of evolutionary biology. Therefore, it is important to know which variables affect the clock rate to correct for errors a priori (Wilke *et al.* 2009). Such potential studies on molecular clock rates are rare. However, an example is given by Wilke *et al.* (2009), who developed a trait-specific clock in invertebrates (i.e. *Protostomia clock*).

7.5 Set the clock — Calibration points/bounds and external molecular clock rates

In general, divergence times are estimated from node depths within a given phylogeny. To perform these estimations calibration point(s) or bound(s), or a suitable external molecular clock rate (i.e. universal clock rate or taxon- or gene-specific clock rates) are required (Table 7-1). The selection of adequate calibration points/bounds or external clock rates, both with high accuracy and validity, is not a simple decision, mainly due to a lack of reliable data that can be used to calibrate the clock (Andújar *et al.* 2014; Wilke *et al.* 2009). In fact, the procedure of clock calibration seems to be one of the most sensitive operations to yield conclusive estimations of divergence time (Bromham *et al.* 1999; Bromham & Penny 2003; Ho & Phillips 2009; Pulquério & Nichols 2007, see Wilke *et al.* 2009).

As discussed above, external molecular clock rates (i.e. one clock rate for all species of a wider taxonomic group or specific gene) are rare and affected by numerous factors. However, two

examples of external molecular clock rates are the trait-specific Protostomia clock estimated by Wilke *et al.* (2009) and the avian clock (Weir & Schluter 2008; Table 7-1). In this study, a 'crustacean clock' is applied to the data set to estimate divergence times of TSS pairs and -complexes, which were separated by the emergence of the Isthmus of Panama. This external clock was estimated by Marino *et al.* (2011) and the substitution rate was obtained from divergence time estimations of green crabs, which were separated during the Mediterranean Salinity Crisis (MSC).

Typically, calibration points/bounds are inferred from fossil or biogeographical data (see table 2 in Wilke *et al.* 2009). However, these calibration points include (time-dependent) uncertainties (Table 7-2). For example, fossils can only provide minimum ages because they are naturally younger than the divergence event (Benton & Donoghue 2007; Gandolfo *et al.* 2008; Hedges & Kumar 2004; Ho 2007; Inoue *et al.* 2009; Marshall 1990, see Wilke *et al.* 2009). Moreover, the problem of potential missing fossil taxa and the difficulties of reliable identification is present (Cutler 2000; Doyle & Donoghue 1993). Thus, if fossil data are utilized in molecular clock analyses, it is advisable to use them as lower constraints (Heads 2005).

Table 7-1: Pros and cons of different divergence time estimations.

	Calibration Point/Bounds from Externally Derived Dates	**External Molecular Clock Rate (e.g., Universal, Taxon-or Gene-Specific Clock Rates)**
Pros	- Locus-independent approach; - Multi-locus analyses possible.	- No calibration points required.
Cons	- Reliable calibration points rarely available; - Accuracy of the calibration point often difficult to assess; - Often only calibration bounds but no points available.	- Only applicable to data sets with strict molecular clock behavior; - Typically locus-specific and thus only applicable to certain genes; - Relatively few external clock rates available.
Examples	- Closure of the Panama Isthmus; - Mediterranean Salinity Crisis.	- Avian clock (Weir & Schluter 2008); - Trait-specific Protostomia clock (Wilke *et al.* 2009).

Advantages and disadvantages of estimating divergence times from calibration points/bounds (usually biogeographical events or fossils) or from external molecular clock rates (table adapted from Wilke *et al.* 2009, p. 29).

On the other hand, age estimates based on biogeographical data may be imprecisely, because divergence processes may be not necessarily associated with particular geographical events (Wilke *et al.* 2009; see Subchapter 6.2 for a discussion about the separation of transisthmian sister species (TSS) pairs of the eastern Pacific/western Atlantic as a possible result of the closure of the Isthmus of Panama). But even considering that a divergence event and a geological/environmental event are linked to each other, further problems may arise (Wilke *et al.* 2009). One of them is related to know the exact age of the employed event (Table 7-2). Whereas few geological episodes are dated with high accuracy (e.g., the period of the MSC; Krijgsman *et al.* 1999), others are discussed controversially. That is especially the case for the

opening of the Bering Strait (Gladenkov *et al.* 2002; Marincovich & Gladenkov 1999, 2001) or the time of the final closure of the Isthmus of Panama (see Chapter 4 for details; Table 7-2). In fact, the closure of the Isthmus of Panama is considered as an important calibration point and a widely used geological phenomenon for calibrating the molecular clock of TSS. However, the sequence of events leading to the final closure is complex and requires careful consideration (see Chapter 4). Two main hypotheses regarding the time of Isthmus closure are discussed controversially. The only recently arose 'new Miocene model' suggests a final Isthmus closure around 15 Ma (e.g., Montes *et al.* 2012a; b; Subchapter 4.2). In contrast, the 'common Pliocene model' assumes an Isthmus closure around 2.4–4.0 Ma (Collins 2003; Jackson & O'Dea 2013; Keller *et al.* 1989; Weir *et al.* 2009; Subchapter 4.3). However, in molecular clock calibrations usually an average time of around 3 Ma is used as calibration point. In this already imprecise time estimate, possible re-openings and -closures of the Isthmus until about 1.8 Ma (Keller *et al.* 1989) are not considered. Though these geological patterns are important, because species could have migrated between the western Atlantic and eastern Pacific until the *final* closure (i.e. until the last re-closure around 1.8 Ma). These time discrepancies can result in over- and underestimations of divergence ages on a large (Miocene vs. Pliocene model) and on a smaller, yet inaccurate (4 Ma vs. 1.8 Ma) extent. Ho (2007) pointed out that "In both cases [fossil and biogeographical data], the resulting estimates of divergence times and substitution rates will be artificially precise, which has a considerable impact on hypothesis testing" (p. 409). Thus, several approaches can be used to obtain more realistic divergence time estimations when using imprecisely calibration events. However, the common use of, for example, calibration bounds (i.e. time intervals) or multiple calibration points is discussed critically and should be applied with consideration (see Ho & Phillips 2009; Andújar *et al.* 2014 for details, respectively).

7.6 Summary

The molecular clock approach is a powerful method to estimate divergence times between species. However, the calibration of the clock is a complex and difficult challenge, influenced by several factors, e.g., rate heterogeneity, the persistence of ancestral polymorphism, saturation of the data set, the effect of purifying selection, used prior conditions of the clock values, an insufficient data set, or biological factors (see above). Additionally, difficulties to find accurate calibration points/bounds or external clock rates influence divergence time estimations. Although controversially discussed, the closure of the Isthmus of Panama is a frequently used vicariance event in molecular clock calibration. However, the use of this complex and long lasting event with potential re-openings and -closures (between 1.8–4.0 Ma) and the recently arose discussion about a potential Miocene closure (around 15 Ma) highlight the uncertainties regarding its accuracy.

Table 7-2: Selection of biogeographical events, which may be convenient for divergence time estimations.

Event	Time (Ma)	References (Selection)
Connection between **Baltic Sea** and **North Sea**	8 000 years ago	Björck (1995)
Significant flooding of the **Sunda Shelf**	19 600–14 600 years ago	Crandall *et al.* (2012)
Aldabra Island	125 000 years ago	Warren *et al.* (2003)
Grande Comore Island	500 000 years ago	Warren *et al.* (2003)
Lake Victoria **Almost completely dried out	750 000 years ago **14 700–15 600 years ago	Verheyen *et al.* (2003) and references therein
Lake Malawi Begin of rifting *Deep water conditions acquired **Almost completely dried out ***New regression	 8.6 *4.5 **1.6–1 ***0.42–0.25	 Delvaux (1995)
Lake Ohrid	1.2 (minimum)	Wagner *et al.* (2014)
Final closure of the **Isthmus of Panama** 'Common Pliocene model' *'New Miocene model'	 3–4 (1.8 including re-openings and -closures) *15	 Coates & Obando (1996); Cronin & Dowsett (1996); Keller *et al.* (1989) *Farris *et al.* (2011) ;Montes *et al.* (2012a; b)
Galapagos Archipelago **(Española Island)**	~3	Hall (1983)
Final disconnection of **Japan** from mainland *Initial disconnection of Japan from mainland	3.5 *15	Andújar *et al.* (2014)
Gulf of California	4	Moore & Buffington (1968)
Opening of the **Bering Strait** *New* age **Old* age	 5.5–5.4 *4.1–3.1	 Gladenkov *et al.* (2002); *Repenning & Brouwers (1992); White *et al.* (1997)
Isthmus of Kra Seaway (dissected the Thai-Malay Peninsula)	5.5–4.5	de Bruyn *et al.* (2005)
Emergence of the **Hawaiian Islands**: southwestern Islands: Hawaii (youngest), Kauai-(oldest); northwestern Islands: Nihao (youngest), Kure-(oldest)	southwestern Islands: 5.1–0.43 northwestern Islands: 28–7	Clague & Dalrymple (1987) Carson & Clague (1995); Fleischer *et al.* (1998)
Strait of Gibraltar (opening)	5.33	Andújar *et al.* (2014); Busack (1986)
Mediterranean Salinity Crisis (MSC)	~6 5.98 (begin); 5.33 (end)	Krijgsman *et al.* (1999)

Event	Time (Ma)	References (Selection)
Shallowing and closure of the **Indonesian Seaway**	9.9–7.5	Kennett *et al.* (1985); Linthout *et al.* (1997)
Mid-Aegean trench (separation of the western and eastern Aegean archipelago)	12–9	Papadopoulou *et al.* (2010)
Max. geological age of **Lake Tanganyika**	12	Cohen *et al.* (1993, 1997); Schön & Martens (2012)
Volcanic emergence of **Gran Canaria**	14.5	Andújar *et al.* (2014)
Final closure of the **Tethys Seaway**	20	Dercourt *et al.* (1986); Hrbek & Meyer (2003)
Separation of **Sardinia** from Corsica	20 (begin) – 9 (end)	Ketmaier *et al.* (2003)
Opening of **Drake Passage**	23.5	Barker & Burrell (1977); Beu *et al.* (1997)
Establishment of the **Antarctic Polar Front**	25–22	Bargelloni *et al.* (2000); Kennett (1982)
Age of **Lake Baikal**	30–20	Karabanov *et al.* (2004) and references therein
Disruption of **Farallon-Pacific Ridge** by subduction under the N-American Plate	28.5	Chevaldonné *et al.* (2002)
split of **Corsica–Sardinia** from the Pyrenees/ Iberic Peninsula	29	Ketmaier *et al.* (2003)
Separation of **New Zealand** from Australia-Antarctica	85	Andújar *et al.* (2014)
Gondwanan fragmentation events: *separation of East and West Gondwana **separation of Africa and South America ***separation of Madagascar and India	 *165–121 **101–86 ***88–63	Vences *et al.* (2001)

Selection of possible biogeographical events that can be used as calibration points/bounds in divergence time estimations. Events are mixed for terrestrial and aquatic taxa and ordered by time. References are only a selection. Asterisks indicate additional events or times in relation to the main event. Ma = million years ago.

Part III

Case Studies

–A Critical View at the Transisthmian Sister Species Concepts–

8 Toward an Unified Definition of Transisthmian Sister Species

This chapter of the thesis is a comprehensive study on transisthmian sister species (TSS) and focuses on the confusing terminology regarding TSS. The first part of this chapter presents the results of the terminological survey regarding derivative and synonymous terms focusing on TSS. The second part discusses in detail the results and recommends well defined, unambiguous terms and its synonyms. Note that the discussion about TSS is only referring to species, which were separated by the emergence of the Isthmus of Panama.

8.1 Terminological survey

The term *transisthmian sister species* and its derivatives are widely used throughout the literature, however, no consistent terminology is apparent (Table 8-1). In fact, within one single study up to 15 different terms referring to TSS, in context to their evolution due to the emergence of the Isthmus of Panama, are used (Knowlton & Weigt 1998; Table 8-1). A comprehensive literature search revealed a total of 60 terms and derivatives, which are listed in Table 8-1. All of these selected terms are strictly linked to the emergence and closure of the Isthmus of Panama. However, often clear definitions of the used terms are missing.

Table 8-1: List of terms and derivatives referring to TSS.

Terms and Derivatives for *Transisthmian Sister Species**	**Definition**	**References (Selection)**
-geminate species -twin species	"[…] –twin species–each one representing the other on opposite sides of some form of barrier." (p. 75).	Jordan (1908)
-geminate complex	"[…] species that were separated have continued to evolve independently and diverged from each other with time […] These pairs of population, with an obvious common origin, have been called *geminate*." (p. 88).	Rubinoff & Rubinoff (1971)
-geminate transisthmian Pacific sister taxa -Panamian transisthmian sister lineages -Panamian [*genus name*] transisthmian sister lineage -transisthmian Panamian [*genus name*] geminates	"[…] the relative importance of vicariance events in shaping the genetic structuring of populations is potentially obscured by […] and by the prevalence of sibling species complexes […]." (p. 3527).	Lee & Ó Foighil (2004)
-transisthmian geminate species	"This geologic event [emergence of the Isthmus of Panama] putatively produced several transisthmian geminate species in *Centropomus* […]." (p. 194).	Tringali *et al.* (1999)
-transisthmian geminates -transisthmian geminate species pairs	Not defined.	Lin & Hastings (2011)

Terms and Derivatives for *Transisthmian Sister Species**	**Definition**	**References (Selection)**
-transisthmian geminate clades -transisthmian geminate species complexes -geminate groups	"The rise of the Central American Isthmus separated many populations of marine organisms, with the final closure of the Isthmus of Panama producing geminate pairs of similar-looking species (Jordan, 1908)." (p. 456).	Alva-Campbell *et al.* (2010)
-twin species pair	"[…] uplift of the Panama Isthmus is generally recognized as the prime vicariance event responsible for these East Pacific - West Atlantic sister group relationships […]." (p. 272).	de Weerdt & Glynn (1991) and references therein
-transisthmian sister species groups	Not defined.	Schubart *et al.* (1998)
-daughter species -sibling species group/complex -species complex	"[…] daughter species accumulate genetic differences without accompanying morphological divergence. […]. Such cases are known as 'sibling species' groups or complexes […]." (p. 1427).	Mathews *et al.* (2002)
-amphi-American species -species pairs	Only summary of terms.	Rubinoff (1968) and references therein
-sister species -sister species pair -true sister species -transisthmian species -transisthmian sister species -transisthmian pair -transisthmian [*species name*] -transisthmian taxa -transisthmian sister taxa -transisthmian relatives -sibling species -sibling species pair -conspecifics -closest transisthmian relatives -pairs of sister species	"[…] most transisthmian sister-species pairs were separated at roughly the same time by final closure of the connection between the Caribbean and the eastern Pacific (Collins 1996) […]." (p. 2257).	Knowlton & Weigt (1998)
-transisthmian pair of sister -transisthmian pairs of sister taxa -pairs of marine sister taxa	"[…] specifically and unambiguously described as each other's closest relatives on the basis of morphological criteria." (p. 1629).	Knowlton *et al.* (1993) and references therein
-geminates -geminate pairs -geminate clades -sister clades -true geminate clades -transisthmian pairs of species -[species names] pair -pairs of congeneric species -pairs of geminate clades -congeneric counterparts	"[…] ranges of marine species were being sundered by an uninterrupted barrier that neither larvae nor adults could cross, starting them on a path of independent evolutionary trajectories […] Geminate species represent initially similar genomes placed into separate environments and constitute a natural experiment that can tell us much about evolutionary divergence and its causes." (p. 64).	Lessios (2008)

Terms and Derivatives for *Transisthmian Sister Species**	Definition	References (Selection)
-congeneric species -congeneric populations	"The rise of the isthmus split previously continuous ranges of many marine taxa and has resulted in pairs of closely related marine species, one on each side of Central America." (p. 2734).	Bermingham & Lessios (1993)
-(molluscan) cousins	"[…] Pacific and Atlantic members of a species pair whose ancestor existed before final closure of the Central American Seaway." (p. 1603).	Vermeij (1993)
-analogous species	"[…] disjunct populations have continued to evolve independently and have diverged to varying degrees with time." (p. 263).	Abele (1976)
-most closest pair -closely related congeneric species -closely related congeneric transisthmian geminate species -congeneric Panamic transisthmian geminate species -pair of geminate species	"[…] population disjunction may be triggered by vicariant events, such as the rise of the Isthmus of Panama, […], and resulted in large numbers of geminate species (Jordan 1908). In the absence of a land barrier, gene flow between disjunct marine populations may be limited by oceanographic features […] combined with reduced dispersal capabilities […]." (p. 4085).	Bernardi & Lape (2005) and references therein
-pair of Pacific/Caribbean geminate species -pairs of geminate sister species	"The separation of a pair of Pacific/Caribbean geminate species, for example, may be the result of the closure of the Isthmus of Panama some 3.0-2.5 million years ago (Mya)." (p. 30).	Wilke *et al.* (2009)
-paciphile and caribphiles	"[…] taxa that formerly lived in the western Atlantic part, but now are extinct there and survive in the eastern Pacific part […]." (p. 426). "Taxa that formerly lived in the eastern Pacific part of the Tertiary province, but now are extinct there and survive in the present Caribbean province […]." (p. 426).	Woodring (1966)

List of terms and derivatives referring to the term *transisthmian sister species*, used in the literature. All expressions are strictly linked to the emergence and closure of the Isthmus of Panama; *Note: Terms and derivatives are listed only once, i.e. identical terms used in different studies are not considered.

Terms and derivatives referring to TSS are often used as synonyms (e.g., Knowlton & Weigt 1998; Lessios 2008). However, in a strict semantic way only a fraction of the 60 terms and derivatives are *true* synonyms in respect to the definition of TSS (Table 8-2). Based on the here presented results, I suggest the three following criteria, which have to be met to consider terms referring to *transisthmian sister species* as true synonyms:

A) Term must imply the connection to the emergence and closure of the Isthmus of Panama.

B) Term must clear define that species of interest originated from a common ancestor.

C) Term must include that species of interest occur on opposite sides of the Isthmus of Panama.

Table 8-2: List of true synonyms referring to the term TSS.

True Synonyms for *Transisthmian Sister Species* (TSS)	
– One TSS Pair –	**– Several TSS Pairs –**
- Transisthmian geminates	- Transisthmian geminate species pairs
- Transisthmian geminate species	- Transisthmian geminate species complex
- Transisthmian geminate species pair	- Transisthmian geminate clade
- Transisthmian sister taxa	- Transisthmian sister species group
- Transisthmian pair	- Transisthmian pairs of sister taxa
- Transisthmian pair of sister	
- Transisthmian pair of sister taxa	
- Transisthmian Panamian [*genus name*] geminates	
- Geminate transisthmian Pacific sister taxa	
- Pair of Pacific/Caribbean geminate species	
- Closely related congeneric transisthmian geminate species	
- Congeneric Panamic transisthmian geminate species	
- Amphi-American species	
Total # of true synonyms: 13	Total # of true synonyms: 5

Term definitions

Transisthmian: *trans*– going across; *isthmian*– relating to an Isthmus (here: Isthmus of Panama);
(i.e. on both sides (western Atlantic and eastern Pacific) of the Isthmus of Panama).

Geminates: "-twin species- each one representing the other on opposite sides of *some* form of barrier." (p. 75, Jordan 1908).

Species: refers here to the lowest taxonomic rank. It can contain one or more individuals of the same or of different organisms.

Species group: "[…] encompasses all nominal taxa at the ranks of species and subspecies" (Article 45.1, ICZN 1999).

Pair: two individuals forming a unit.

Species complex: "[…] a cluster of closely related isolates whose individual members may represent more than one species" (p. 449, Fegan & Prior 2005).

Clade: "[…] ancestor (an organism, population, or species) and all of its descendants" (p. 28, Cantino & De Queiroz 2010).

Taxa (singular: taxon): "taxonomic units of extant or extinct animals" (Article 1.1, ICZN 1999).

Congeneric: "Congeneric species, that is species belonging to the same genus, can be regarded as a special case of closely related organisms for which the phylogenetic distance is based on traditional morphological characters and is the lowest that can be measured between clearly distinguishable organisms. […] different congeneric pairs are *not expected* to have the same divergence time neither the same evolutionary rate and pattern but their evolutionary divergence may be considered as minimized […]" (p. 309, Gissi *et al.* 2008).

Amphi-American: *amphi*– on both sides; *American*– of the American continent (incl. Central- and South America); i.e. occurrence in both, western Atlantic and eastern Pacific, oceans.

For references of the respective synonyms, see Table 8-1.

Based on the three suggested criteria and the general term definitions (see above and Table 8-2), 13 (regarding *one* TSS pair) and 5 (regarding *several* TSS pairs) derivatives of the term *transisthmian sister species* can be considered as true synonyms.

8.2 Discussion

8.2.1 Terminology of transisthmian sister species

Irrespective of any scientific field the use of a precise and unambiguous terminology is crucial, in particular when dealing with complex and challenging relationships (e.g., Envall 2008; Lourenço *et al.* 2014; Nelsen *et al.* 2014). However, often terms are not used adequately and applied as synonyms, even though most of them "are far from being [true] synonyms" (p. 2, Lourenço *et al.* 2014). The term *transisthmian sister species* is a considerable example to highlight this problem. Numerous terms and derivatives with ambiguous meanings originated during the last decades by increasing numbers of studies focusing on the evolution of TSS. As presented in Table 8-1, remarkable 60 terms and derivatives synonymising TSS were found in the literature. However, many terms imply a broad and unspecific meaning, e.g., *twin* species, *sister* species or *geminate* species, which can cause problems since those terms are not *per se* linked to specific assumptions or conditions, e.g., as to the Isthmus of Panama (Tables 8-1 and 8-2). However, these terms are used as synonyms by most authors, although only a fraction (13 regarding *one* TSS pair and 5 regarding *several* TSS pairs) of the 60 terms and derivatives can be considered as true synonyms (Table 8-2). Only these synonyms match the three suggested criteria highlighted above. In contrast, using several undefined and non-synonymous terms throughout a study can result in confusion. For example, Knowlton & Weigt (1998) used 15 different terms in their publication referring to TSS, but only one definition was given: "[…] most transisthmian sister species pairs were separated at roughly the same time by final closure of the connection between the Caribbean and the eastern Pacific […]" (p. 2257, see Table 8-1). However, in general their definition is clear and does not raise any questions. In contrast, terms like *conspecifics* or *sibling species* are, in a strict semantic way, imprecise and should be avoided, even though the reader could figure out their meanings due to the stories background. Thus, if several terms are used in a study they should be selected in relation to the respective context. In his publication "The law of geminate species", the Ichthyologist D. S. Jordan (1908) defined the term *geminate species* for sister species, which originated from a common ancestor. Although Jordan did not define the term exclusively for the Isthmus of Panama, he is often cited in studies dealing with species on both sides of the Isthmus (e.g., Rubinoff & Rubinoff 1971). In contrast, other studies do not mention the Isthmus in their definition at all (e.g., Abele 1976; Mathews *et al.* 2002; Table 8-1). Lourenço *et al.* (2014) pointed out that complex stories and "large numbers of studies has led to considerable variation in their context and how terms have been applied, but also to the introduction of additional terms by some authors [e.g., *analogous* species by Abele 1976; (molluscan) *cousins* by Vermeij 1993; *congeneric counterparts* by Lessios 2008]" (p. 2). Another strategy is to avoid the term TSS and its derivatives, as done by McCartney *et al.* (2000). They basically used the phrases Caribbean-, Atlantic- or Pacific species to describe relationships among TSS, which results in a clear and unambiguous description. However, this practice is not

suitable for all cases and can easily lead to intricateness, especially within complex stories. Note that the word *transisthmian* is a general term for 'across an Isthmus' and can be linked to *any* kind of Isthmus (e.g., Isthmus of Kra – Thailand, Isthmus of Tehuantepec – Mexico, Isthmus of Chignecto – Canada). Thus, it should be defined to *which* Isthmus *transisthmian* sister species refer to.

8.2.2. Two special cases focusing on confusing terms

8.2.2.1 Phylogenetic conditions

Often, TSS relationships are not clear dissolved within a phylogenetic tree and are represented as polytomies. This pattern might be due to an insufficient or incomplete dataset, resulting in low node supports and, hence, in a poor resolution of the evolutionary branches (i.e. *soft* polytomy). On the other hand, species can originate simultaneously from a common ancestor, resulting in true polytomies within the phylogenetic tree (i.e. *hard* polytomy; Lin *et al.* 2011 and references therein). These cladogenetic events (hard polytomies) are represented by often more than one potential twin. In any case, these circumstances have to be considered and an accurate term defined. In this study, the phylogeny of the divergence time estimation of *Panopeus* (Figure 10-7) represents a soft polytomy, presumably due to an insufficient dataset (see Subchapter 10.4.1). However, the TSS relationship within that phylogenetic tree is described as *TSS complex*, pointing out that several species on one side of the Isthmus, can represent the putative sister to the species on the other side (see definition in Subchapter 6.1). Other reasons for the occurrence of TSS complexes are speciation events on one side of the Isthmus, subsequent to divergence events due to the Isthmus closure (Collins 1996b; see below). Such speciation of one twin member results in the appearance of so called *cryptic species complexes* (e.g., Lessios 1979, 1981; Lessios & Cunningham 1990; Rubinoff & Rubinoff 1971; Vawter *et al.* 1980; Weinberg & Starczak 1989). However, in a strict sense the expression *cryptic* is incorrect, because by definition it means that very similar looking but yet distinct species are concealed under the same species name. Also important is to distinguish between transisthmian sister species *pairs* and *groups*. It makes a difference if two species (one on each side of the Isthmus) or a group of species (more than one species on the other side of the Isthmus) are their closest relatives.

8.2.2.2 Time of Isthmus closure and species distribution

Due to the complexity of the Isthmus emergence until its final closure it is essential to be very precise when using terms referring to TSS. The evolution of TSS and, hence this term, is commonly associated with the Isthmus closure (i.e. Isthmus of Panama). However, several studies have shown that many TSS pairs diverged long before or even after the final Isthmus closure (e.g., Hurt *et al.* 2009; Lessios 2008; Miura *et al.* 2012; but see Subchapter 6.3.2 for detailed references). The results of the divergence time estimations in this study present different divergence ages among and even within the four decapod genera (but see Chapter 10 for details). It may be an idea to differentiate between several times of divergences, i.e. well expressed terms, which refer to the species' separation long *before*, *during* or even *after* the final Isthmus closure. For example, Hurt *et al.* (2009) applied the term *pseudo*-TSS in their study

to point out that divergence events may have taken place before and unrelated to the Isthmus closure accompanied with species extinctions. Collins (1996b) already recognized that: "[…] definition does not necessarily assist in recognition. For any particular pair of species it is difficult to imagine how it could be proven that the divergence is the result of the emergence of the Isthmus" (p. 311). He suggested that a "combination of a phylogenetic hypothesis and the biogeographic distributions of the species in questions should clarify the geminate relationships" (p. 313). In fact, divergence times can depend on the species occupied habitat (Frey 2010; Knowlton & Weigt 1998; but see Chapter 6 for more details) or changing environmental and abiotic conditions (Duque-Caro 1990; Hurt *et al.* 2009). However, to define TSS in view of their habitat preference or tolerances to changing conditions can be a solution, but probably not always convenient.

8.3 Summary

The use of only few terms, but as many as necessary, can be a feasible way to avoid confusion and ambiguities in a study, especially when using true synonyms. Moreover, the applied terms should be clear defined in the beginning of each study. Some terms may be more useful (e.g., referring to the time of divergence) than others (e.g., referring to physiological tolerance thresholds).

In this thesis, terms and derivatives that refer to *transisthmian sister species* were well defined in the beginning and used consistently throughout the chapters (see Chapter 6). Moreover, used terms referring to TSS, which were used as *false* synonyms (*geminate* species; *sibling* species; *sister* species; *twin* species) were declared as those, also in the beginning of this thesis. However, due to the estimated divergence times in this study (Chapter 10), terms and derivatives have to be renewed:

- ***Transisthmian sister species pair*** (**TSS pair**) – refers to TSS representing one unity (i.e. one representative of the western Atlantic forms a pair with its sibling of the eastern Pacific).

  This term implies TSS origination due to the Isthmus emergence and closure, but without any time assumptions. The Isthmus closure is assumed only as a *reason* for TSS origination regardless of species habitat occupations, distribution patterns or tolerance thresholds. Based on the divergence time estimations in this study, it would be more accurate to distinguish between divergence events *before*, *during*, and *after* the final Isthmus closure. Based on the complex and long lasting emergence of the Panama Isthmus, specific time thresholds for the respective terms are not confined and should be determined in each study separately. Thus, the following terms are suggested:

  - *Pre-transisthmian sister species pair* (Pre-TSS pair)
  i.e. pre– pre- (Latin): before (the Isthmus closure)
  The term *pre* points out that TSS diverged *before* the closure of the Isthmus.

- Inter-transisthmian sister species pair (Inter-TSS pair)

i.e. inter– interval: a period of time between events.

The word *interval* implies a time-component, which appropriate describes the emergence and closure of the Panama Isthmus as a complex and long lasting event with several re-openings and -closures (e.g., Cronin & Dowsett 1996; Haug & Tiedemann 1998). Because the time of Isthmus emergence and its final closure is still under debate (Bacon *et al.* 2015 and references therein), a specific time-interval is not proposed.

- Post-transisthmian sister species pair (Post-TSS pair)

i.e. post – posterior: later

The term *posterior* points out that TSS diverged *after* the closure of the Isthmus.

Note that these terms also do not include any specific factor like habitat occupations, distribution patterns or tolerance thresholds of the TSS. These factors should be used as parameters to *explain* the different divergence times. It would be confusing, if terms are defined relative to those multifaceted factors. Moreover, the new defined terms cannot be precisely distinguished from each other, because they overlap in the temporal aspect. However, these new defined and combined terms provide more clarity in reference to time.

- ***Transisthmian sister species complex*** (**TSS complex**) – refers to TSS where several species on one side of the Isthmus can represent the putative sister to the species on the other side (Collins 1996b).

  This term does not imply time assumption. It may be useful to add the above supposed adjectives *pre-*, *inter-*, and *post-* to integrate time. Derivatives of this term (e.g., transisthmian geminate species complexes, geminate complex, or sibling species complex; see Table 8-1) can be find in the literature. However, the term *transisthmian sister species complex* was not used before. But especially this new composition implies every important aspect, which is missing within the other forms (for single word definitions see Table 8-2): (i) transisthmian geminate species complexes – redundant information that species are separated by a barrier and missing information about species relationship, (ii) geminate complex – does not imply sister species relationship, (iii) species complex – information regarding the Isthmus and relationship status are missing.

- ***Transisthmian pseudo sister species*** (**pseudo-TSS**) – refers to TSS originated from speciation events, which are not linked to the Isthmus formation. This term can be used as a true synonym for *Post-TSS pair* (see above).

  This term implies TSS origination independent of the Isthmus formation and without any time assumptions. The sister species occur on both sides of the Isthmus and may originated due to a variety of events e.g., extinction (Lessios 1998), dispersal (Miura *et al.* 2012), habitat occupations and distribution patterns (Frey 2010; Knowlton & Weigt 1998), tolerance thresholds (Hurt *et al.* 2009), or repeated speciation event of one sibling

(Collins 1996b; see Chapter 6 for details). As mentioned above, Hurt *et al.* (2009) already used this term in their study to refer to divergence events that may have taken place before and unrelated to the Isthmus closure. However, this study expands the term and suggests a more general meaning.

9 Criteria of Transisthmian Sister Species Pairs and -Complexes

In this chapter, the here studied TSS pairs and -complexes are analyzed in respect to the five suggested criteria, which evaluate species as true TSS (see Subchapter 6.3). These five criteria refer to biogeographic distribution, morphological similarities, and molecular characteristics. Each TSS pair or -complex of this study is analyzed according to these criteria (Table 9-1). Note, due to the thematic broad overlap of the different chapters, the following paragraphs contain already results, which will be discussed in principle in Chapter 10. For a detailed list of the here studied TSS pairs and -complexes see Subchapter 10.1.

9.1 The TSS pair and -complex evaluation

9.1.1 Geographic isolation drives speciation processes

This first assumption is fulfilled by all TSS pairs and -complexes (Table 9-1). As pointed out in Subchapter 6.3.1, geographic isolation is the prevalent cause that drives speciation processes in organisms. Thus, this criterion is naturally met by all TSS pairs and -complexes.

9.1.2 The barrier and the consequentially isolated taxa are of the same age

For the second criterion, the TSS pairs and -complexes were evaluated in respect to three assumptions: (i) an Isthmus closure around 15 Ma (Miocene model), (ii) an Isthmus closure around 3 Ma (Pliocene model), and (iii) re-openings and -closures until about 1.8 Ma (Table 9-1).

The upper range of the 95% high posterior density interval (HPD; interval which contains 95% of the age distribution of all trees) of the *Pachygrapsus* TSS pair shows a divergence time close to the Miocene model (13.54 Ma). However, the mean value of the MRCA is 9.56 Ma (Table 10-6). These observations are still valid, if corrections for ancestral polymorphism are taken into account (13.29 Ma and 9.31 Ma, respectively; Table 10-6). Assumption (ii) is met by both *Sesarma* complexes and TSS pair A of *Eurytium*. While *Sesarma* complex A and *Eurytium* TSS pair A match the 3 Ma assumption quite well (3.03 Ma and 2.91 Ma, respectively), the mean divergence time of *Sesarma* complex B slightly exceeds the assumption of a Pliocene closure (4.36 Ma). If corrected for ancestral polymorphism (i.e. 4.11 Ma), the divergence age is close to the upper bound of the Pliocene model (i.e. 4 Ma). However, the lower range of the 95% HPD is within the range of assumption (ii) (3.27 Ma; Table 10-6). The lower ranges of the 95% HPDs of *Sesarma* complex A (2.37 Ma) and *Eurytium* TSS pair A (1.95 Ma) are within the range of assumption (iii) (Table 10-6), even if corrected for ancestral polymorphism (2.12 Ma and 1.70 Ma, respectively). The remaining TSS pair B of *Eurytium* shows a young divergence time (mean value 0.63 Ma) and thus, none of the three time assumptions applies to it (Table 10-6).

Table 9-1: Identified TSS pairs and -complexes compared to the five criteria.

		Sesarmidae		Panopeidae			Grapsidae
		Sesarma		*Panopeus*	*Eurytium*		*Pachygrapsus*
Criteria		Complex A Srh–Scu* Srh–Sret	Complex B Scra–Ssul Scra–Saeq	TSS pair Pha–Psp Pha–Ppu	TSS pair A Eli–Psp	TSS pair B Etr–Poc	TSS pair Pso–Ptrans
1	**Geographically Isolated**	✓	✓	✓	✓	✓	✓
2	**TSS and Barrier are of the same Age** – **15 Ma Model**	X	X	X	X	X	✓[1]
	TSS and Barrier are of the same Age – **3 Ma Model**	✓	✓[2]	X	✓	X	X
	TSS and Barrier are of the same Age – **Re-openings and -closures (until 1.8 Ma)**	✓[3]	X	X	✓[3]	X	X
3	**Close Distribution to the Barrier**	X	X	X	X	X	X
4	**Similar Morphology**	✓[4]	✓[5]	✓[6]	X	X	✓
5	**Similar Divergence Ages between TSS within the same Genus**	✓[7]	✓[7]	n/a	X	X	n/a

Overview of the identified TSS pairs and -complexes in respect to the suggested geminate criteria; check mark = fulfilled, X = unfulfilled, n/a = none information available, Ma = million years ago, [1]upper range of the 95% high posterior density intervals (HPD; interval which contains 95% of the age distribution of all trees; 13.54 Ma) close to the supposed closure of the Miocene model (i.e. around 15 Ma). [2]95% HPD within the range of assumed age of Isthmus closure (3.27–5.56 Ma). [3]Considering re-openings and -closures, the lower ranges of the 95% HPD of *Sesarma* complex A and *Eurytium* TSS pair A are within this time range (2.37 Ma and 1.95 Ma, respectively), [4]Srh and Sret show similarities, [5]all specimens are very similar to each other. Note that observations for Scra base on a drawing alone, [6]all specimens are very similar to each other. Note that from a morphological point of view Psp looks identical to Ppu, [7]overlapping 95% HPD (see text for details), *incl. *Sesarma* sp. (nr. *reticulatum*), Srh (*Sesarma rhizophorae*), Scu (*Sesarma curacaoense*), Sret (*Sesarma reticulatum*), Scra (*Sesarma crassipes*), Saeq (*Sesarma aequatoriale*), Ssul (*Sesarma sulcatum*), Eli (*Eurytium limosum*), Etr (*Eurytium tristani*), Psp (*Panopeus* sp.), Poc (*Panopeus occidentalis*), Pha (*Panopeus hartii*), Ppu (*Panopeus purpureus*), Pso (*Pachygrapsus socius*), Ptrans (*Pachygrapsus transversus*).

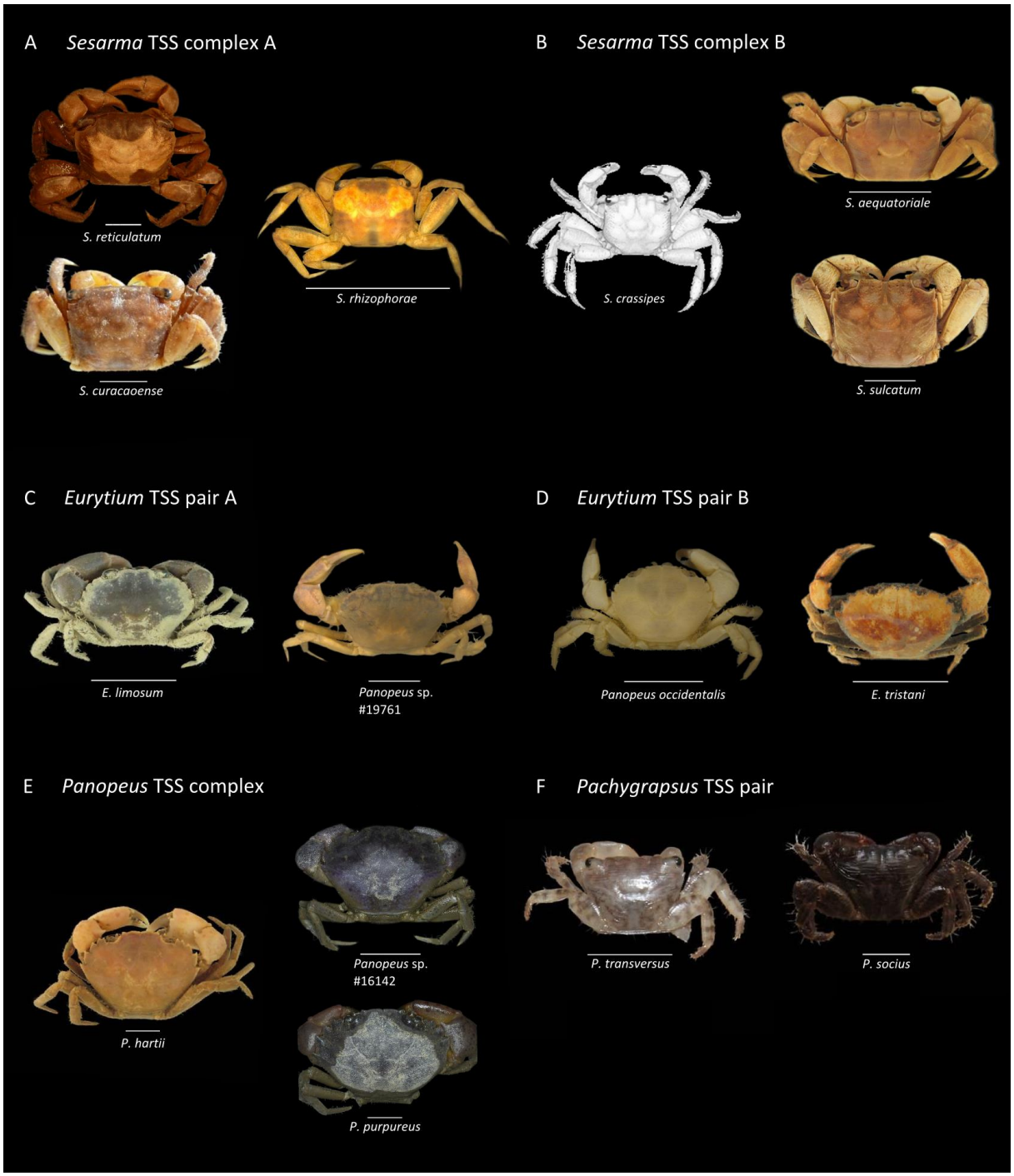

Figure 9-1: Comparisons of morphological similarities between the species of the identified TSS pairs and -complexes. A) *Sesarma* TSS complex A; B) *Sesarma* TSS complex B; C) *Eurytium* TSS pair A; D) *Eurytium* TSS pair B; E) *Panopeus* TSS complex; F) *Pachygrapsus* TSS pair. Left arranged species are associated with the western Atlantic; right arranged species are associated with the eastern Pacific. The specimens are shown in dorsal view. The scale bar represents 1 cm. Note that the drawing of *S. crassipes* is adapted from Abele (1992). Additional photos of the species are shown in the Appendix (A3). Photos of *P. transversus* and *P. socius* are by courtesy of C. D. Schubart.

9.1.3 TSS distribution ranges are close to the Isthmus

The validation of this third criterion is difficult. However, all species are distributed along the western Atlantic and eastern Pacific coasts, yet they are not exclusively occur within the Isthmus

and bordering countries (Tables 10-1 – 10-3). The southernmost species of the western Atlantic (*Pachygrapsus transversus*) inhabits the coasts of southern Brazil and the northernmost distributed species (*Sesarma reticulatum*) the intertidal of Massachusetts. In the eastern Pacific the southernmost species (*Panopeus purpureus*, *Eurytium tristani*, and *Pachygrapsus socius*) occur along the coast of Peru and the northernmost species (*P. socius*) in the Gulf of California. In fact, none of the studied TSS pairs and -complexes show an exclusive distribution close to the barrier. Therefore, all species of the TSS pairs and -complexes are evaluated as *not closely distributed* (Table 9-1).

9.1.4 Morphological similarity between TSS

The aspect of morphological similarity is fulfilled by almost all studied TSS pairs and -complexes. The species *S. reticulatum* and *S. rhizophorae* of the *Sesarma* TSS complex A are very similar to each other (Figure 9-1 A). Based on only this characteristic alone, they would be certainly classified as a true TSS pair. Although the western Atlantic species *S. crassipes* is only pictured as a drawing (Abele 1992), this specimen looks very similar to its potential eastern Pacific geminates (Figure 9-1 B). All species of the *Panopeus* complex are very similar to each other. Note that from a morphological point of view the unidentified eastern Pacific species *Panopeus* sp. seems identical to *P. purpureus* (Figure 9-1 E). In contrast, the species of both TSS pairs of *Eurytium* show no similarities among each other. This is not surprising, since the potential twin species of both TSS pairs belong to the genus *Panopeus* (see Chapter 10 for detailed discussion; Figure 9-1 C, D). Although *P. socius* and *P. transversus* (*Pachygrapsus* TSS pair) show differences in their color pattern, their general morphology is very similar (Figure 9-1 F).

9.1.5 Similar divergence ages between TSS pairs and -complexes

Only the TSS pairs and -complexes in the genera *Sesarma* and *Eurytium* are comparable. The two identified TSS complexes of the genus *Sesarma* show different mean divergence ages (2.88 Ma compare to 4.21 Ma; Table 10-6). Though, the 95% HPDs show low range of overlap (2.37–3.71 Ma compare to 3.27–5.56 Ma; Table 10-6). In contrast, TSS pairs A and B of the genus *Eurytium* show different divergence ages. The average difference between both pairs is more than fourfold (2.91 Ma compare to 0.63 Ma; Table 10-6).

9.2 Discussion

In this chapter TSS pairs and -complexes were analyzed in respect to the five proposed criteria, which have to be fulfilled to determine species as true TSS (see Subchapter 6.3). The aim of this analysis was to clarify the following questions:

1. Do the studied TSS pairs and -complexes of this study meet all five TSS criteria?
2. Are the current criteria sufficient to identify TSS?
3. What additional/new set of criteria can be suggested to identify TSS?

9.2.1 Geographic isolation drives speciation processes

This assumption is met by all TSS pairs and -complexes used in this study (Table 9-1). Mayr (1954) and other early on naturalists pointed out that geographic isolation is the predominant factor driving speciation processes in organisms. The Isthmus of Panama acts as an ideal model to study these processes in TSS. In particular the reasons are: (i) assuming an Isthmus closure around 3 Ma or earlier, enough time has passed to accumulate differentiations between the species, (ii) gene flow between the species is almost complete suppressed, and (iii) several species where separated roughly at the same time but differ in their life histories, dispersal abilities or preferred habitats, which all play an important role in speciation processes (see Chapter 6 and Lessios 1998 for details). If species are assumed to be TSS they originated from a common ancestor and occurred in habitats on either side of the Isthmus. Hence, they evolved separately and experience patterns of speciation, based on the reasons mentioned above.

9.2.2 The barrier and the consequentially isolated taxa are of the same age

The fulfillment of the second criterion depends on the respective assumption: (i) an Isthmus closure around 15 Ma (Miocene model), (ii) an Isthmus closure around 3 Ma (Pliocene model), and (iii) re-openings and -closures until about 1.8 Ma. Only the upper range of the 95% HPD of the *Pachygrapsus* TSS pair (*P. socius*/*P. transversus*) is close to the determined age of the Miocene model (13.54 Ma). Thus only one TSS pair (out of six TSS pairs and -complexes) shows a suitable divergence time in respect to the second criterion under assumption (i). The second assumption (i.e. Pliocene model) is met by three (out of six) TSS pairs and -complexes (both *Sesarma* complexes and TSS pair A of *Eurytium*; Table 9-1). Assuming several Isthmus re-openings and -closures as proposed by Cronin & Dowsett (1996) and Keller *et al.* (1989), the *final* closure is to set around 1.8–1.9 Ma (assumption (iii)). Two (out of six) TSS pairs and -complexes are within this range (lower range of the 95% HPD of *Sesarma* complex A (2.37 Ma) and the average divergence time of the *Eurytium* TSS pair A (1.95 Ma); Table 10-6). Thus, *Sesarma* complex A and *Eurytium* TSS pair A both met assumptions (ii) and (iii) (Table 9-1). However, as discussed above, the emergence and closure of the Panama Isthmus was a complex event, taking place over several million years. It would be inaccurate to determine a particular time of closure, and therefore it would be more conscientious to assume ranges (Lessios 1979), as shown for *Sesarma* complex A and *Eurytium* TSS pair A. On the other hand, even if TSS and the Isthmus have the same age (e.g., *Sesarma* complex A and *Eurytium* TSS pair A), it can be a coincident and species have diverged due to other factors, independent of the Isthmus emergence, e.g., due to dispersal (Miura *et al.* 2012) or extinction events (e.g., Williams & Reid 2004; but see Chapter 6). A similar but yet different factor is pointed out by Lessios (1998): “Species on either side of the Isthmus may have been separated by the same barrier, but the final interruption of gene flow may not have occurred at the same time” (p. 188), for example due to “differences in mode of dispersal, habitat preferences, physiological tolerances, and vagility of adults and larvae“ (p. 189, Lessios 1998).

9.2.3 TSS distribution ranges are close to the Isthmus

Criterion three is difficult to assess – what does apply as close? Collins (1996b) defined *close* as "adjacent to the barrier" (p. 312). In this respect the studied TSS pairs and -complexes cannot be evaluated as closely distributed (Tables 10-1 – 10-3). The here studied species are not cosmopolitan, but their distribution ranges extend within the entire western Atlantic, yet up the eastern U.S. coast (*P. occidentalis, S. reticulatum*) and down to the coasts of Brazil (*P. transversus, E. limosum, P. hartii, S. crassipes, S. curacaoense*). Similar patterns are apparent along the eastern Pacific coast. The species distribution show ranges from north (Bay of California; *P. socius*) to south (Peru; *P. socius, E. tristani, P. purpureus*). Thereby, this pattern is not restricted to specific genera, but rather one species of each TSS pair or -complex shows such a wide distribution. This distribution pattern is not an exception to the here studied species, but also appears in several TSS of other studies e.g., marine fishes, gastropods, bivalves, and other crustacean genera (Banford *et al.* 2004; Hastings 2000; Duda & Kohn 2005; Marko 2002; Marko & Moran 2009; Thiercelin & Schubart 2014, respectively). Species which have a planktonic larvae stage in their reproduction cycle, show high dispersal ability. This might be one reason for large distribution ranges (Marko & Moran 2009). Moreover, birds or ships are also possible disperser for larvae (see Subchapter 6.3.3), which are then offered new open niches to occupy. Collins (1996b) pointed out that cosmopolitan species are not necessarily separated by the Isthmus closure, and that distribution via a circumglobal route might be another factor (Lessios 2008). However, a variety of other dispersal- and migration factors, oceano- and geographic conditions, as well as extinction events can play a role in large distribution patterns of TSS (but see chapter 6 for a detailed review). In general, Collins (1996b) argued that enough time has passed since an assumed Isthmus closure around 3 Ma for species to disperse. Thus, it would be peculiar if distributional ranges of recognized TSS are generally restricted to the isthmian barrier.

9.2.4 Morphological similarity between TSS

Most of the here studied TSS pairs and -complexes show a similar morphology. In general it is supposed, that closely related decapod species are not easily to distinguish based on morphological characters alone (Cuesta & Schubart 1998). In reference to the closure of the Isthmus of Panama and subsequent evolution of TSS, Abele (1976) pointed out that about 6% of decapods are morphologically identical, whereas around 45% show "slight morphological modifications" (p. 263). He linked this observation to environmental adaptations of the species. For example, Schubart *et al.* (2001) showed that specimens of the crab genus *Brachynotus*, which were genetically not distinguishable but lived in different depths, represented "different ecophenotypes" (p. 45). Similar results were also found by Spivak & Schubart (2003) in the crab genus *Cyrtograpsus*. Hence, if TSS inhabit niches on each side of the Isthmus that are similar in their structure, it might be that these species develop similar morphological shapes, which "[…] may reflect phenotypic plasticity or convergence and not genetic similarity" (p. 864, Reuschel & Schubart 2006). In general, morphological studies in crustaceans are predominantly done on larvae (Anger 2001), whereas morphological studies in adult specimens are more rare (Schubart & Cuesta 1998) and need to be intensified.

Morphological similarities within Panopeidae: The Panopeidae are merged into the superfamily Xanthoidea and thus, share a significant characteristic – their "extreme morphological similarity" (p. 182, Martin & Abele 1986; Schubart *et al.* 2000a; Thoma *et al.* 2014; Figures A3-14 – A3-36). This trait is not observable within the *Eurytium* TSS pair A (*E. limosum*/*Panopeus* sp.) and TSS pair B (*E. tristani*/*P. occidentalis*). This is not surprising because one twin of each TSS pair belongs to the genus *Panopeus* (see Chapter 10 for detailed discussion). In contrast, the specimens of the TSS pair/complex of *Panopeus* show a high degree of morphological similarity (Figure 9-1). However, as in most species groups, consistent and common characteristics are missing for decent discriminations (Martin & Abele 1986), also in larval morphology (e.g., Rodríguez & Paula 1993). Within the Panopeidae, species identifications based on either molecular or morphological characters differ considerably and result in disparate sister species relationships (Schubart *et al.* 2000a). The authors argue that these similarities between closely related sister species may base on independent adaptations to similar environments or cessation of morphological evolution in key characters (Schubart *et al.* 2000a).

Morphological similarities within *Pachygrapsus*: The family Grapsidae is recognized as "morphologically homogenous" (p. 472) in both, adult and larvae specimens (Schubart 2011 and references therein). However, differences in the color pattern are observable between the studied TSS *P. transversus* and *P. socius* (Figures 9-1, A3-37, A3-38). The previously assumption that *P. transversus* is distributed along the coasts of the western Atlantic as well as eastern Pacific (Poupin *et al.* 2005; Rathbun 1918) was disproved by Schubart *et al.* (2005). In 1998, Cuesta & Schubart showed distinct differences in morphological characteristics (coloration of the outer face of the chela in adult specimens) as well as evidences obtained from molecular data between eastern Pacific and western Atlantic populations. In 2005, Schubart and colleagues officially changed the name of all representatives of eastern Pacific *P. transver*sus to *P. socius* based on a comprehensive analysis of morphological and molecular data.

Morphological similarities within *Sesarma*: Within the studied *Sesarma* TSS complex A (*S. rhizophorae*/*S. curacaoense, S. reticulatum*) and TSS complex B (*S. crassipes*/*S. sulcatum*, *S. aequatoriale*) morphological similarities are apparent (Figures 9-1, A3-4 – A3-12). The endemic Jamaican populations of *Sesarma* are morphologically well analyzed (e.g., Schubart & Koller 2005). These species show a great variety in their morphology and can be distinguished on morphological characters alone, specifically due to their shape of the carapace, and the male's chelae and pleon (Reimer *et al.* 1998; Schubart & Koller 2005 and references therein; Türkay & Diesel 1994). A recently conducted study by Thiercelin & Schubart (2014) showed that closely related species within the Sesarmidae can also be distinguished due to morphological differences in their gonopods.

9.2.5 Similar divergence ages between TSS pairs and -complexes

Several studies have shown that in the majority of cases, TSS became isolated from their MRCA during different time intervals and often uncorrelated to the Isthmus closure (e.g., Lessios 2008 and references therein; see Chapter 6 and discussion above). Assuming a constant substitution rate within the same gene among related species groups, in his comprehensive comparison of

numerous TSS pairs Lessios (2008) pointed out that most of the species were separated from each other "[…] during different time intervals, even in cases in which morphological divergence would suggest otherwise" (p. 74). This pattern is not only well pronounced in TSS pairs and -complexes among different genera, but also within the same genus. Focusing on the TSS pairs and -complexes in this study, only the two *Sesarma* TSS complexes show overlaps in their divergence time ranges (2.37–3.71 Ma compare to 3.27–5.56 Ma; Table 10-6), and thus, similar divergence times can be presumed. In contrast, divergence times of the two *Eurytium* TSS pairs differ considerably (Table 10-6). These differentiations can base on various factors (e.g., complex history of the Isthmus emergence and closure with potential re-openings and -closures, high dispersal capability of the species, or simply an incomplete dataset), which were discussed in detail in the previous sections. However, which of these factors accord to the studied TSS pairs in this study remains uncertain. Assuming the unlikely fact that the specimens of the *Eurytium* TSS pair B are *true* geminates (see discussion above), their very young divergence ages point toward possible dispersal events. In their study, Miura *et al.* (2012) showed that two dispersal events (via birds) occurred across the isthmian barrier successfully long time after the Isthmus completion (750 000 and 72 000 years ago). Although this example refers to marine gastropods, similar scenarios can be supposed for crustaceans as well (e.g., Green & Figuerola 2005 and references therein). A recently developed Bayesian computation method by Hickerson *et al.* (2006) that "tests for simultaneous divergence" (p. 2435) can be a useful tool to analyze obtained divergence times of TSS pairs. However, due to a small intraspecific dataset, this approach was not applicable in this study.

9.3 Summary

Even though none of the here studied TSS pairs and -complexes fulfills all five criteria (Table 9-1), the TSS are, however, suitable for divergence time estimations. First of all, the time of Isthmus closure is not considered in the analyses. The divergence time estimations base on an external crustacean rate (Marino *et al.* 2011; see Chapter 10) which in turn, was estimated according to the Mediterranean Salinity Crisis. This geological event is well dated and, in contrast to the Isthmus closure, there is no doubt about the chronological progression. Moreover, the studied crab genera are distributed within the littoral zone (mangroves, rocky shores and shallow water) along the coasts of both oceans. It is the general assumption that species of those habitats reflect the time of Isthmus closure best (e.g., Knowlton & Weigt 1998). Another important advantage is the occurrence of several TSS pairs and -complexes and a comprehensive dataset for most of the genera (Table 10-4). Thus, the divergence times of the studied TSS pairs and -complexes can be compared among each other, independent of a defined time of Isthmus closure and disregarded of possible re-openings and -closures of the Isthmus. In any case, the here presented results draw conclusions on the time of Isthmus closure, in respect to the debated Miocene- and Pliocene models.

9.4 Criteria revised

Based on the TSS pair and -complex evaluation in this chapter, it is advisable to refine and replace the five criteria in respect to their practicability in non-theoretical frameworks. Thereby it should be noted that clear structured and confined criteria are difficult to establish. More precisely, the analyses showed that based on complex and active interrelations within biological systems, TSS can hardly be forced into specific categories or concepts. Thus, it is not possible to develop a TSS identification key for scientists. However, different factors should be considered in the process of TSS identification. Therefore, the following improvements are suggested:

1) The barrier and the evolved TSS pairs and -complexes do not necessarily have to have the same age. However, well-defined and unambiguous terms should be used, which refer to the respective time of species isolation (before, during or after Isthmus completion, see Chapter 8). Therefore it is necessary to define the time of presumed Isthmus closure (Pliocene vs. Miocene model, consideration of possible re-openings and -closures).
2) The distributional ranges of TSS pairs and -complexes should be within the Atlantic and Pacific oceans, with a focus on the western Atlantic and eastern Pacific. Note that, however, some species show cosmopolitan distribution. Especially in these cases, other separation events than the Isthmus closure (e.g., migration via a circumglobal route) should be considered.
3) Morphological characteristics can be a useful tool to obtain evidences regarding possible TSS pairs. However, such method should never be used alone, but can be a strong approach combined with molecular analyses.
4) TSS pairs and -complexes within a genus can show different divergence ages. In these cases, possible reasons should be pointed out.

Case Studies

– Divergence Time Estimations of Transisthmian Sister Species–

10 Divergence Time Estimations of Transisthmian Sister Species

This chapter is concerned with a brief summary of the studied genera and identified transisthmian sister species (TSS) pairs and -complexes of the four decapod genera *Sesarma* Say, 1827 (family Sesarmidae), *Panopeus* H. Milne Edwards, 1834, *Eurytium* Stimpson, 1859 (both family Panopeidae), and *Pachygrapsus* Randall, 1840 (family Grapsidae). Moreover, it focuses on the results of the phylogenetic analyses and subsequent divergence time estimations of the studied TSS pairs and -complexes.

Note: For detailed descriptions regarding the applied methods for the molecular analyses as well as species and locality information see Appendices A1, A2 and A3, respectively.

10.1 Studied species

Crustaceans that inhabit littoral environments are promising candidates for divergence time estimations to receive evidences for the time of seaway closure between North- and South-America. Several studies show that organisms of shallow coastal water habitats reflect the final time of the Isthmus closure best (e.g., Knowlton & Weigt 1998; Lessios 2008 and references therein). These species are assumed to be the last that were able to cross the Isthmus before all salt-water connections were restricted. Based on transisthmian sister species (TSS) pairs and -complexes of four decapod genera, the controversially discussed time of Isthmus closure is studied.

10.1.1 *Sesarma* Say, 1817

Systematic classification

Class: Malacostraca
Order: Decapoda
Infraorder: Brachyura
Superfamily: Grapsoidea
Family: Sesarmidae
Genus: *Sesarma*

The genus *Sesarma* consists of 18 well-described (Ng *et al.* 2008; Schubart & Santl 2014) and additionally one undescribed species, which is assumed to be related to *S. reticulatum* and hereafter referred to as *Sesarma* sp. (nr. *reticulatum*) (Figure 10-1; Zimmerman & Felder 1991). Ten out of 19 species are endemic to the Caribbean island Jamaica (Diesel *et al.* 2000; Diesel & Schubart 2000; Schubart & Santl 2014), whereas the others are well distributed along the eastern Pacific (EP) and western Atlantic (WA) coasts of North-, Central- and South-America (Tables 10-1 and A2-1). *Sesarma* species are typical inhabitants of soft-sediment littoral environments like mangroves and marshes (Schubart & Koller 2005; (Tables 10-1 and A2-1), although endemic Jamaican species are distributed in creeks and streams, as well as in caves and even terrestrial habitats (Schubart *et al.* 1998; Schubart & Santl 2014; Table A2-1).

Figure 10-1: Morphological observation of *Sesarma* sp. (nr. *reticulatum*). The species (middle) is assumed to be related to *S*. *reticulatum* (left). However, morphological observations as well as molecular analysis point toward a relation to *S*. *curacaoense* (left). Note that the dark color of *S*. *reticulatum* bases on light conditions. The scale bar represents 1 cm. Additional photos of the species are shown in the Appendix (A3).

Within the genus, two TSS complexes are postulated (Schubart *et al.* 1998; Table 10-1): Complex A: *S. rhizophorae* from the EP is either the sister of *S*. *reticulatum*, *S*. *curacaoense* or *Sesarma* sp. (nr. *reticulatum*), all from the WA (Table 10-1; Figure 10-2); Complex B: *S*. *crassipes* from the WA is either the sister of *S*. *sulcatum* or *S*. *aequatoriale*, both from the EP (Table 10-1; Figure 10-2). Tables 10-4 and 10-5 present an overview of the missing taxa, the total number of species, which occur in the WA and EP, and the number of WA and EP taxa, which were used in this study.

The following characteristics emphasize *Sesarma* for a good case study taxon:
– two defined TSS complexes
– well studied ecology of the genus
– complete and comprehensive sampling of all American representatives, which are known to inhabit the coasts of the EP and WA (Tables 10-4 and 10-5).

Note: The recently new described species *Sesarma abeokuta* n. sp. (Schubart & Santl 2014) was not included in the phylogenetic analyses of this study, since sequences are only available for the partial ND1 gene for NADH1 dehydrogenase subunit 1 (EMBL molecular database HF678402-HF678413; Schubart & Santl 2014). However, this species radiated from the endemic Jamaican *S*. *dolphinum* and, therefore, would have been clustered within the endemic Jamaican species complex (Schubart & Santl 2014).

Table 10-1: TSS complexes of the genus *Sesarma* Say 1817.

TSS Species Complex; Author[1]; Ocean	UGSB/Prep. #	Habitat	Occurrence
Species Complex A *Sesarma*			
S. rhizophorae Rathbun, 1906; EP	9716, 11988 / 18149, 19530	[2]common in burrows in mangrove swamps, mud, salinity range 20-27 ppt.	[2]Costa Rica, Panama (common along the Pacific coast)
S. reticulatum (Say, 1817); WA	11975 / 19519; [6]EU329170	[2]eulittoral region of *Spartina* marshes, burrows in red mangrove swamps, in wood holes near mud flats, intertidal areas along stream banks and well-drained salt-marshes, estuarine habitats of low salinity (salinity range: 2-35 ppt; prefers 16 ppt).	[2]Massachusetts, North Carolina, Florida
S. curacaoense de Man, 1892; WA	11969, 11970 / 19514, 19515	[2]brackish, estuaries, common in mangrove swamps and among clumps of oysters and rocks on a mud substrate.	[2]Florida, Cuba, Puerto Rico, Jamaica, Curaçao, Trinidad, Panama, Brazil
[4]*Sesarma* sp. (nr. *reticulatum*); WA	11983-11986 / 19525-19528	[3]coastal habitats, estuaries, in burrows, fresh and salt marshes, prefers salinities under 12 ppt (salinity range: 1-15 ppt), but also found in hypersaline habitats.	[4]Florida
Species Complex B *Sesarma*			
S. crassipes Cano, 1889; WA	[5]AJ225859	[2]river mouths and estuaries, sometimes with surface salinity of 0 ppt.	[2]Costa Rica, Brazil
S. sulcatum Smith, 1870; EP	12108 / 19618; [5]AJ225880	[2]mangrove swamps, estuaries, burrows above the banks of brackish water rivers, prefers salinities around 22 ppt (occurred although in very low salinity water of 4-6 ppt).	[2]El Salvador, Mexico, Nicaragua, Costa Rica, Panama, Colombia
S. aequatoriale Ortmann, 1894; EP	11636 / 19300; [5]AJ225883	[2]semiterrestrial, brackish water streams, rivers, mangroves, under rocks and debris, common around lower salinities (salinity range: 0-22 ppt).	[2]Costa Rica, Mexico, Panama, Ecuador

Assumed TSS complexes of the genus *Sesarma* Say, 1817 with habitat description and occurrences; EP = eastern Pacific, WA = western Atlantic, UGSB = University Giessen Systematics and Biodiversity collection, Prep. # = DNA isolation number; [1]Ng *et al.* (2008); [2]Abele (1992) and references therein; [3]Zimmerman & Felder (1991) and references therein; [4]from the field collection of C. D. Schubart (coll.: C.D. Schubart, D.I. Felder, R.B. Landstorfer 8th April 2008; see Table A2-1 for details); [5](Schubart *et al.* 1998); [6]Mahon & Neigel (2008).

10.1.2 *Panopeus* H. Milne Edwards, 1834 / *Eurytium* Stimpson, 1859

Systematic classification

Class: Malacostraca

Order: Decapoda

Infraorder: Brachyura

Superfamily: Xanthoidea

Family: Panopeidae

Genera: *Panopeus* / *Eurytium*

The genera *Panopeus* and *Eurytium* both belong to the genus-rich family Panopeidae. Species of these genera are regarded to be highly abundant along the tropical, subtropical and temperate coasts of North-, Central- and South-America (Felder & Martin 2003; de Souza *et al.* 2013; Tables 10-2, A2-2, A2-3). The species inhabit the marine intertidal and shallow subtidal, as well as oligohaline and freshwater estuarine environments (Schubart *et al.* 2000a). The genus *Panopeus* consists of 16 well-described species (note, Ng *et al.* (2008) assigned 17 species to the genus *Panopeus*. However, since 2009/2010 the status of *P. turgidus* is invalid and is now allocated to the genus *Eurypanopeus*; Ng & Davie 2015). The genus *Eurytium* is composed of only four species (Ng *et al.* 2008). Proposed TSS pairs and -complexes of *Panopeus* and *Eurytium* are based on the comprehensive phylogenetic analyses of this study.

Within the genus *Panopeus*, one TSS pair is assumed (Table 10-2; Figure 10-7): *P. hartii* from the WA is the sister to the unidentified species *Panopeus* sp. (#16142) from the EP. On the other hand, low node supports (Figure 10-7) support also the assumption of a TSS relationship between *P. purpureus* (EP) and *P. hartii*.

In contrast to recently conducted studies (Thoma *et al.* 2014), the following two TSS pairs of the genus *Eurytium* are assumed here (Table 10-2; Figure 10-8): TSS pair A: The WA species *E. limosum* is the sister to the unidentified EP species named here as *Panopeus* spp. (#19756-57, #19760-63; Table 10-2; Figure 10-8). TSS pair B: *E. tristani* from the EP is the sister to *P. occidentalis* (#19871) from the WA (note, the identification of the species *P. occidentalis* (#19871) is probably correct, based on morphological observations (Figure A3-32 D). The other individuals of *P. occidentalis* cluster within the remaining *Panopeus* species, though unevenly distributed among the phylogeny; Figure 10-4). Tables 10-4 and 10-5 present an overview of the missing taxa, the total number of species, which occur in the WA and EP, and the number of WA and EP taxa, which were used in this study.

The following characteristics emphasize *Panopeus* as well as *Eurytium* as good case study taxa:

– several assumed TSS pairs
– comprehensive sampling of almost all representatives (Tables A2-2 and A2-3).

Table 10-2: TSS pairs and -complex of the Panopeidae *Panopeus* H. Milne Edwards, 1834 and *Eurytium* Stimpson, 1859.

TSS Pair; Author[1]; Ocean	UGSB/Prep. #	Habitat	Occurrence
TSS Pair/Complex *Panopeus*			
P. hartii Smith, 1869; WA	11599 / 19631	[10]large boulders, intertidal pool, subtidal rocky shore	[2]Florida to Brazil
Panopeus sp.; EP	7794 / 16142	-	[11]Costa Rica
P. purpureus Lockington, 1877; EP	7787, 9691-9694 / 16137, 18125-18128	[3]coastal lagoon and estuaries, [10]mangroves	[2]Mexico to Peru
TSS Pair A *Eurytium*			
E. limosum (Say, 1818); WA	9697-9704, 11586 / 18131-18138, 19743; [9]ULLZ4012	[4]estuaries and mangroves, [7]when mangroves are absent in marshes (temperate USA)	[4,7,9]from eastern US coast to Brazil
Panopeus spp.; EP	12258, 12259, 12263, 12264, 12266, 12267/ 19756-19757, 19760-19763	[11]soft mud river bank, soft ground in general	[11]Ecuador
TSS Pair B *Eurytium*			
E. tristani Rathbun, 1906; EP	9695 / 19862	[5]mangroves	[5]Panama, [8]El Salvador, Peru, Colombia, Ecuador, [9]Nicaragua
P. occidentalis Saussure, 1857; WA	11620 / 19871	[8]intertidal of sandy beaches, mangroves, [2]under rock and rubble	[6,2]North Carolina to Florida, Southern Gulf of Mexico, Central America, The West Indies, northern South America, Guianas, Brazil; Bermuda

Assumed transisthmian sister species (TSS) pairs and -complex of the genus *Panopeus* H. Milne Edwards, 1834 and *Eurytium* Stimpson, 1859, with habitat description and occurrences; EP = eastern Pacific, WA = western Atlantic, UGSB = University Giessen Systematics and Biodiversity collection, Prep. # = DNA isolation number; [1]Ng *et al.* (2008); [2]Plotnick *et al.* (1988); [3]Hendrickx (1996); [4]Guimarães & Negreiros-Fransozo (2002) and references therein; [5]NMNH (catalogue numbers USNM 155281, 155282); note: the holotype of *E. tristani* (catalogue number USNM 32366) was found by Rathbun (1906) on the *western Atlantic* coast of Costa Rica); [6]Bertini *et al.* (2004); [7]Abele (1976); [8]gbif.org; [9]Thoma *et al.* (2014); [10]NMNH (catalogue numbers USNM 221947, 256590); [11]from the field collection of C. D. Schubart (coll.: T. Poettinger, 29th April 2011; see Tables A2-2 and A2-3 for details).

10.1.3 *Pachygrapsus* Randall, 1840

Systematic classification

Class: Malacostraca
Order: Decapoda
Infraorder: Brachyura
Superfamily: Grapsoidea
Family: Grapsidae
Genus: *Pachygrapsus*

The genus *Pachygrapsus* is a worldwide distributed decapod genus and can be found along tropical, subtropical and temperate coasts (Cannicci *et al.* 1999; Flores & Negreiros-Fransozo 1999; Tables 10-3 and A2-4). *Pachygrapsus* consists of 14 well-described species (Ng *et al.* 2008) and inhabits caves and cervices of rocky intertidal shores as well as mangroves (Abele *et al.* 1986; Cuesta & Schubart 1998; Tables 10-3 and A2-4). Within the genus, one TSS pair is proposed (Schubart 2011; Schubart *et al.* 2005; Table 10-3; Figure 10-10): *P. transversus* from the western Atlantic (WA) is the sister to *P. socius* from the eastern Pacific (EP). Based on the unavailability of cytochrome c oxidase subunit I (COI) sequences, the results from Ip *et al.* (2015) are employed as template for the species arrangement in the phylogenetic tree (Figure 10-9). Tables 10-4 and 10-5 present an overview of the missing taxa, the total number of species, which occur in the WA and EP, and the number of WA and EP taxa, which were used in this study.

The following characteristics emphasize *Pachygrapsus* for a good case study taxon:
– one clear defined TSS pair
– well studied ecology of the TSS
– complete sampling of almost all WA and EP representatives (Tables 10-4 and A2-4).

Note: The species *Pachygrapsus transversus* is well studied. *P. transversus* was supposed to occur along the eastern Pacific coast, on both sides of the Atlantic, as well as in the Mediterranean Sea (Crocetta *et al.* 2011; Schubart *et al.* 2005). However, detailed studies by Schubart *et al.* (2005) revealed that the eastern Pacific representatives of *P. transversus* represent a separate taxon, namely *P. socius* (which was formerly regarded as a junior synonym of *P. transversus*, Ng *et al.* 2008). Therefore, *P. transversus* and *P. socius* are considered as TSS pair.

Table 10-3: TSS pair of the genus *Pachygrapsus* Randall, 1840.

TSS Pair; Author[1]; Ocean	UGSB/Prep. #	Habitat	Occurrence[3]
TSS Pair *Pachygrapsus*			
P. socius Stimpson, 1871; EP	9125, 9126, 9131 / 17515, 17516, 17521	[4]intertidal, on wharfs and sea walls, red mangrove swamps, in holes and crevices	from Gulf of California to Galápagos and Peru
P. transversus (Gibbes, 1850); WA	9134-9136 / 17524-17526	[2]intertidal, (sub-) tropical rocky shores (for adults); [5]sabellariid worm reefs and mytilid mussel beds (for juveniles)	from Florida to southern Brazil, from Israel (Mediterranean) to the mouth of the Congo River (eastern Atlantic)

Assumed transisthmian sister species (TSS) pair of the genus *Pachygrapsus* Randall, 1840, with habitat description and occurrences; EP = eastern Pacific, WA = western Atlantic, UGSB = University Giessen Systematics and Biodiversity collection, Prep. # = DNA isolation number; [1]Ng *et al.* (2008); [2]Cuesta & Schubart (1998) and references therein; [3]Schubart *et al.* (2005) and references therein; [4]Abele *et al.* (1986) and references therein; [5]Flores & Negreiros-Fransozo (1999).

Although species of each genus are well sampled, few taxa are missing in the datasets (Table 10-4). Within the genus *Sesarma*, the species *S. abeokuta* n. sp. (Schubart & Santl 2014) is not included in the analyses. As mentioned above (Subchapter 10.1.1) this species is endemic to Jamaica and radiated from the endemic Jamaican *S. dolphinum*. Thus, the deficiency of this taxon should have no significant influence on the results of the divergence time estimations. The dataset of the genus *Panopeus* is missing three taxa: *P. boekei*, *P. convexus,* and *P. diversus*. Whereas the former species occurs in the WA (Plotnick *et al.* 1988), the latter two are common along the EP coasts of Chile (Plotnick *et al.* 1988) and the Gulf of California (Garth 1960), respectively. The deficiency of these species has to be considered in respect to divergence time estimations (see Subchapter 10.4). Within the genus *Pachygrapsus*, three species are not included in the dataset, of Ip *et al.* (2015): *P. corrugatus*, *P. loveridgei*, and *P. propinquus*. The occurrence of all three species is not limited to either the EP or the WA coasts. In fact, *P. corrugatus* occurs from the Caribbean to the South Atlantic (Fransen 2015a). *P. loveridgei* inhabits the coasts of St. Helena and Ascensión (both are oceanic islands in the South Atlantic; Fransen 2015b) and *P. propinquus* occurs at the east coast of India (Sahoo *et al.* 2008). Additionally, in the comprehensive Grapsidae phylogeny of Schubart (2011) *P. corrugatus* is the sister species to *P. plicatus* and nested with distance to the species of interest. Thus, the results of the divergence time estimations should not be essentially influenced by the deficiency of these species.

Table 10-4: Missing species in the phylogenetic studies.

Sesarmidae	**Panopeidae**	**Grapsidae**
Sesarma	***Panopeus***	***Pachygrapsus***
S. abeokuta n. sp. (Jamaica; Schubart & Santl 2014)	*P. boekei* (Caribbean, Lesser Antilles; Plotnick *et al.* 1988)	*P. corrugatus* (Bahamas, Cuba, St. Paul (UK), Ascensión, Puerto Rico, Virgin Island; Fransen 2015a)
	P. convexus (Chile; Plotnick *et al.* 1988)	*P. loveridgei* (St. Helena, Ascensión; Fransen 2015b)
	P. diversus (Gulf of California; Garth 1960)	*P. propinquus* (east coast of India; Sahoo *et al.* 2008)

Missing taxa in the phylogenetic studies of the crab genera *Sesarma*, *Panopeus*, and *Pachygrapsus*. Localities of occurrence and the respective references are in brackets.

10.2 Phylogenetic analyses and divergence time estimations

The phylogenetic studies of this thesis are twofold. First, a phylogenetic analysis of each genus was conducted to identify and confirm TSS pairs and -complexes. Second, divergence time estimations of the supposed TSS pairs and -complexes were performed. Based on molecular clock analyses the time of species separation was determined and analyzed in respect to the chronologically emergence and closure of the Isthmus of Panama. For detailed information regarding the conducted phylogenetic analyses and divergence time estimations see Materials

and Methods (Appendix A1). An overview of the used dataset for phylogenetic analyses and divergence time estimations is given below (Table 10-5).

Table 10-5: Species used in phylogenetic analyses and divergence time estimations.

	Sesarmidae	**Panopeidae**		**Grapsidae**
	Sesarma	***Panopeus***	***Eurytium***	***Pachygrapsus***[3]
Phylogenetic Analysis				
Total # Species within Genus[1]	19*	16	4	14
Total # Species Used in this Study	18*	13**	4	11[2]
Total # Specimens Used in this Study	32	78***	14	11[2]
# Western Atlantic Species Used in this Study (Total # of WA Species)	14 (15)	11*** (12***)	1 (1)	2[2] (3)
# Eastern Pacific Species Used in this Study (Total # of EP Species)	4 (4)	3*** (5***)	3 (3)	2[2] (2)
# Species not Associated with WA/EP Used in this Study (Total #)	-	1 (1)	-	7[2] (9)
Length of Alignment COI / 16S (bp)	1 136 / 642	1 184 / 638		2 247[2]
Divergence Time Estimation				
# of Species	18*	3	6	4
# of Specimens	32	10	22	8
# Postulated TSS Pairs / Complexes	2 complexes	1 pair	2 pairs	1 pair
Length of Alignment COI / 16S (bp)	1 136 / 642	1 184 / 638	555 / 638	687 / -

Overview of the number (#) of species used in phylogenetic analyses and divergence time estimations of the genera *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus*; WA = western Atlantic; EP = eastern Pacific; TSS = transisthmian sister species; [1]Ng *et al.* (2008); [2]Phylogenetic topology adapted from Ip *et al.* (2015), based on five genes; [3]due to few available sequences, only divergence time estimations based on COI sequences were conducted; *****incl. *Sesarma* sp. (nr. *reticulatum*); **excl. *Panopeus* spp. from the WA and EP; ***incl. *Panopeus* spp.; note: species of other genera are not included in this overview.

10.3 Results

This subchapter is divided according to the four studied genera: *Sesarma*, *Panopeus*, *Eurytium* and *Pachygrapsus*. It highlights the results of the phylogenetic analyses and divergence time estimations. An overview of the obtained results of the divergence time estimations is given in Table 10-6.

10.3.1 Phylogenetic studies of the genus *Sesarma*

The topology of the phylogenetic analysis of the genus *Sesarma* (COI (TrN+I+G) + 16S (TrN+G); relaxed clock; Yule process; ngen: 50 million; log: 1000; burnin: 5000) is identical to the topology of the divergence time estimation (COI (HKY+G) + 16S (HKY+G); strict clock; Yule process; ngen: 20 million; log: 1000; burnin: 2000). Thus, only the divergence time tree is shown below (Figure 10-2). The divergence time tree represents a maximum clade credibility tree with node ages, branch supports, and the 95% highest posterior density (HPD) for specific nodes (i.e. interval which contains 95% of the age distribution of all trees; Table 10-6). For detailed information about the molecular analyses see Appendices A1.2.7 and A1.2.8).

For divergence time estimation the COI substitution rate (i.e. 0.98% per million years; My^{-1}) according to Marino *et al.* (2011) was established. The substitution rate for 16S rRNA (inferred from the COI rate) was 0.58% My^{-1} (Table A1-4). The phylogenetic tree (Figure 10-2), including all taxa of the genus *Sesarma*, which are known to occur in the eastern Pacific (EP) and western Atlantic (WA) includes three well-supported monophyletic lineages (clades A-C). The taxonomic arrangement coincides with previous findings by Schubart *et al.* (1998). The molecular analysis revealed that the undescribed species *Sesarma* sp. (nr. *reticulatum*) is identical to the species *S. curacaoense* (clade B) and not to *S. reticulatum* as previously assumed due to morphological observation (see above; Figures 10-1 and 10-2). Clade A contains only endemic Jamaican crabs, which are supposed to separate from their marine ancestors at around 4.5 million years ago (Ma; +/- 0.42; Schubart *et al.* 1998). The most recent common ancestor (MRCA) of the Jamaican clade in this study originated around 5.13 Ma (95% HPD: 4.27–6.02 Ma) and therefore, shows a slightly higher mean divergence age as suggested by Schubart *et al.* (1998). The second clade (clade B) includes the TSS complex *S. rhizophorae* (EP) and its WA sister species *S. reticulatum* and *S. curacaoense* (species complex A; Table 10-1; Figure 10-2). Their MRCA occurred 3.03 Ma (95% HPD: 2.37–3.71 Ma; silhouette A). Clade C includes also a TSS complex: *S. crassipes* (WA) and its EP sister species *S. sulcatum* and *S. aequatoriale* (species complex B; Table 10-1; Figure 10-2). Their MRCA occurred 4.36 Ma (95% HPD: 3.27–5.56 Ma; silhouette B). Mean divergence times corrected for ancestral polymorphism are 2.78 Ma (species complex A) and 4.11 Ma (species complex B; Table 10-6).

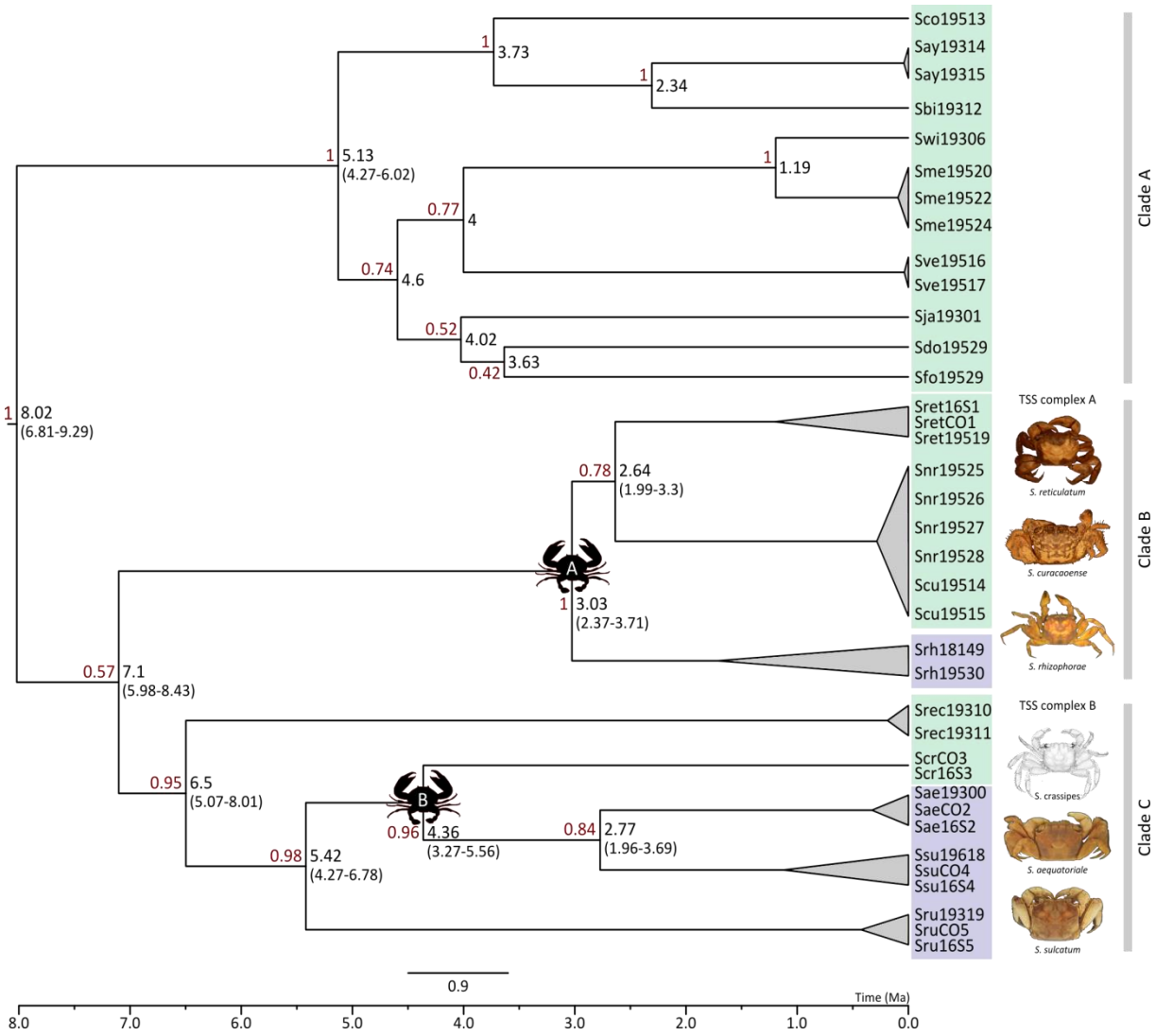

Figure 10-2: Divergence time tree of the genus *Sesarma*, based on a combined data set (COI, 16S). Maximum clade credibility tree: (COI (HKY+G) + 16S (HKY+G); strict clock; Yule process; ngen: 20 million; log: 1000; burnin: 2000); branch supports (red), node ages (black), numbers in brackets present the 95% high posterior density intervals (HPD; interval which contains 95% of the age distribution of all trees). Crustacean silhouettes (A and B) present the time of species divergence of the *Sesarma* TSS complex A (silhouette A) and the *Sesarma* TSS complex B (silhouette B); A: 3.03 Ma (95% HPD: 2.37–3.71 Ma), B: 4.36 Ma (95% HPD: 3.27–5.56 Ma). Species framed in green are associated with the western Atlantic and species framed in blue are associated with the eastern Pacific. Ma = million years ago; Note that the drawing of *S. crassipes* is adapted from Abele (1992). For information about the species code see Subchapter A1.2.6.

10.3.2 Phylogenetic studies of the family Panopeidae

The topology of the phylogenetic analysis of the family Panopeidae represents a Maximum-likelihood tree (COI (GTR+G) + 16S (GTR+G); relaxed clock; 1 000 bootstrap runs; Figure 10-4). The composition of the here analyzed dataset bases on the comprehensive Xanthoidea phylogeny of Thoma *et al.* 2014 (p. 92; Figure 10-3). However, the dataset was complemented with numerous additional species and sequences (Tables A2-2, A2-3, A2-5). Note that based on the tree dimension, Figure 10-4 shows only the contour of the phylogenetic tree. Detailed information regarding the relationships of *Panopeus* and *Eurytium* are represented in Figures 10-5 and 10-6.

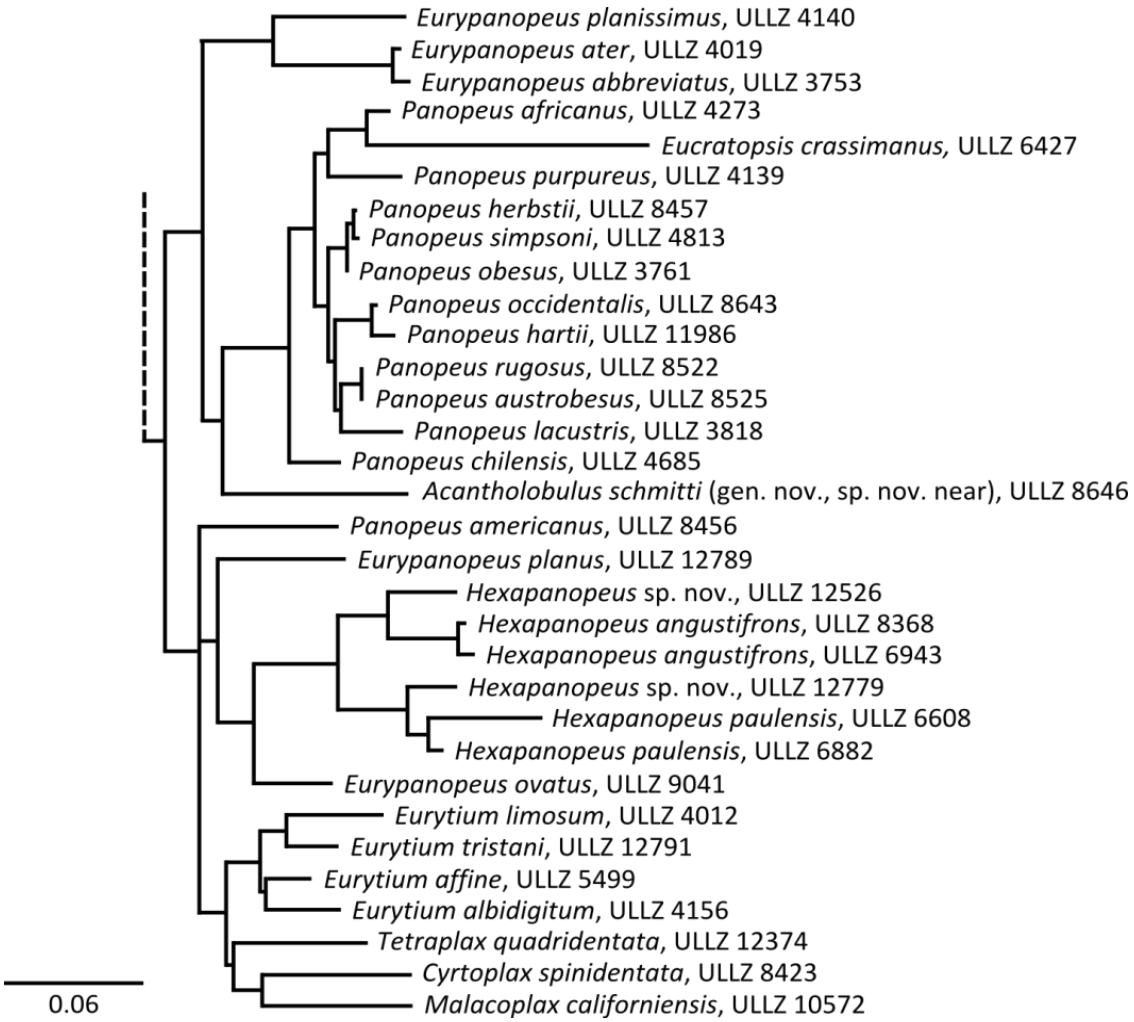

Figure 10-3: Subset of interest of the comprehensive Xanthoidea phylogeny of Thoma *et al.* 2014 (p. 92). This phylogenetic section was used as template and complemented with own species and sequences for the conducted molecular analysis (Figure 10-4). The Maximum-likelihood tree bases on a combined data set of six genes (1 000 bootstrap runs).

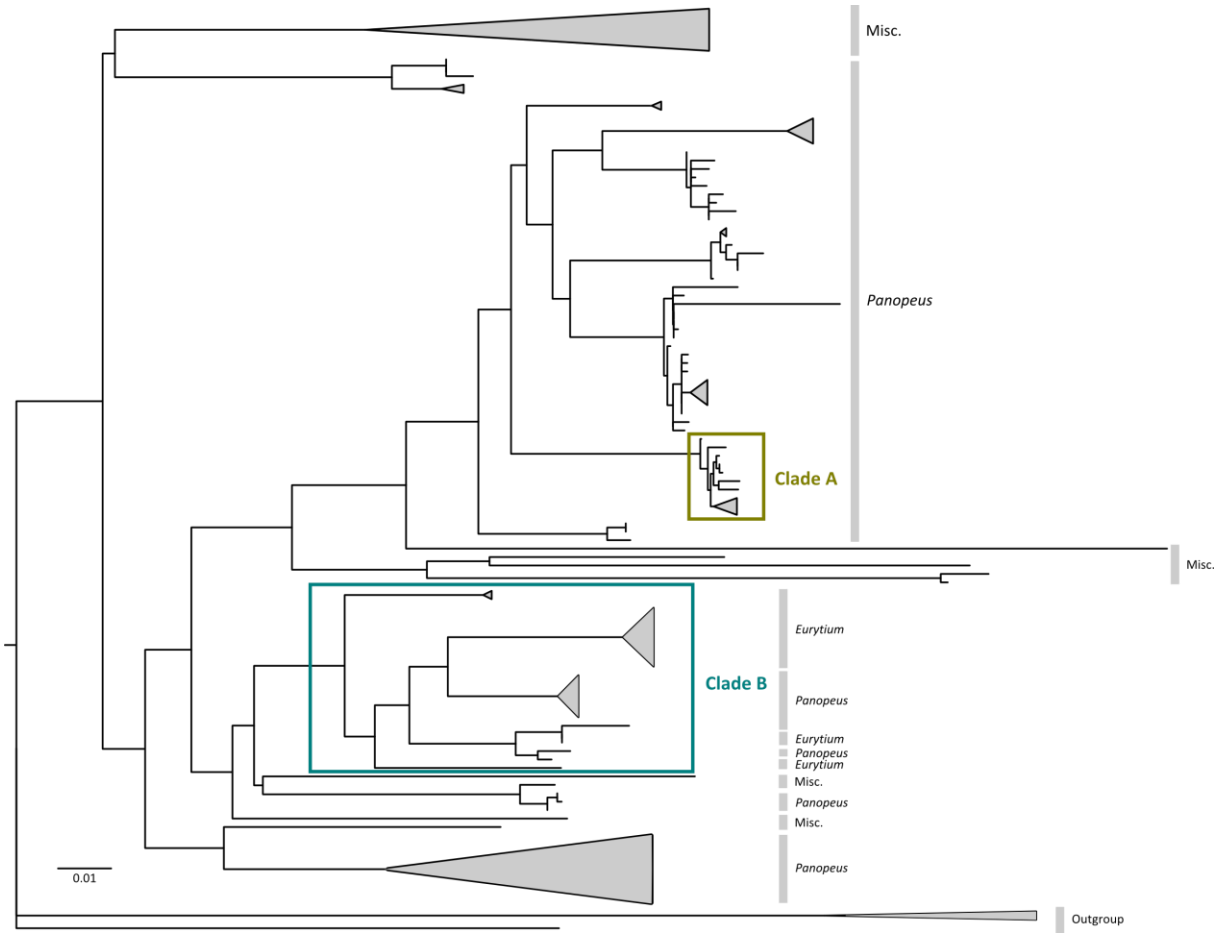

Figure 10-4: Overview of the phylogenetic Panopeidae tree, based on the comprehensive Xanthoidea phylogeny of Thoma *et al.* 2014 (p. 92) and complemented with numerous additional species and sequences. Detailed descriptions of the phylogenetic relationships of *Panopeus* and *Eurytium* are represented in Figures 10-5 and 10-6. Clade A (green) represents the subset for the divergence time estimation of *Panopeus*. Clade B (blue) represents the subset for the divergence time estimation of *Eurytium*. Species of other genera than *Panopeus* and *Eurytium* are named Misc. ('miscellaneous') within this phylogeny.

Figure 10-4 represents an overview of the phylogenetic Panopeidae tree. The tree includes 13 species (out of 16) of the genus *Panopeus* and all 4 representatives of the genus *Eurytium*. Figures 10-5 and 10-6 are detailed subsets of the Panopeidae tree. In general, the node supports within the tree are weak (green and purple stars, supports < 0.5 are not shown). The genera *Panopeus* and *Eurytium* are not as well separated as in the phylogeny of Thoma *et al.* (2014). Assuming correct species identifications both genera are paraphyletic (note, monophyly of *Eurytium* in Thoma *et al.* 2014). Clade A (Figure 10-5) consists of only *Panopeus* species and contains the TSS pair *P. hartii* (WA) and *Panopeus* sp. (EP; Table 10-2). Detailed information about this relationship and divergence time estimations are outlined below (Subchapter 10.3.2.1). In this study species of clade A are closer related to *Eurypanopeus* and *Eucratopsis* as to species of clade B (Figure 10-5). However, node supports for the species arrangement are low. Clade B is paraphyletic and contains all species of *Eurytium* and several *Panopeus* taxa (Figure 10-6). Two TSS pairs can be identified: TSS pair A consisting of *E. limosum* (WA) and *Panopeus* spp. (EP) and TSS pair B consisting of *E. tristani* (EP) and *P. occidentalis* (WA; Table 10-2).

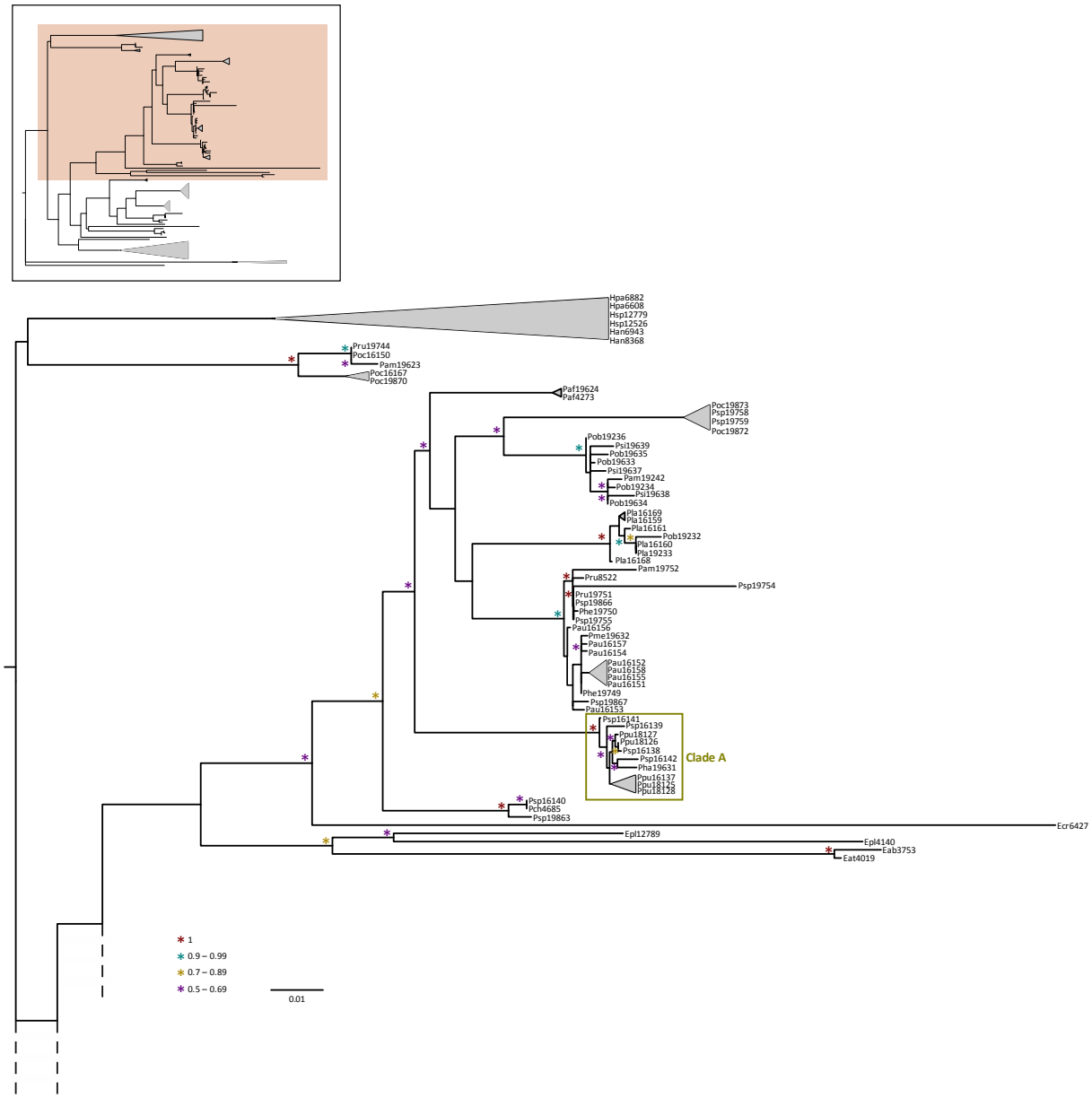

Figure 10-5: The upper subset of the Panopeidae tree represents a Maximum-likelihood tree (COI (GTR+G) + 16S (GTR+G); relaxed clock; 1 000 bootstrap runs, best scoring tree), based on a combined data set (COI, 16S). Clade A (green frame) is monophyletic and contains the TSS pair *P. hartii* (WA) and *Panopeus* sp. (EP). Node supports are labeled by colored stars (purple: 0.5-0.69, green: 0.7-0.89, blue: 0.9-0.99, red: 1). Node supports lower than 0.5 are not shown. For information about the species code see Appendix A1.2.6.

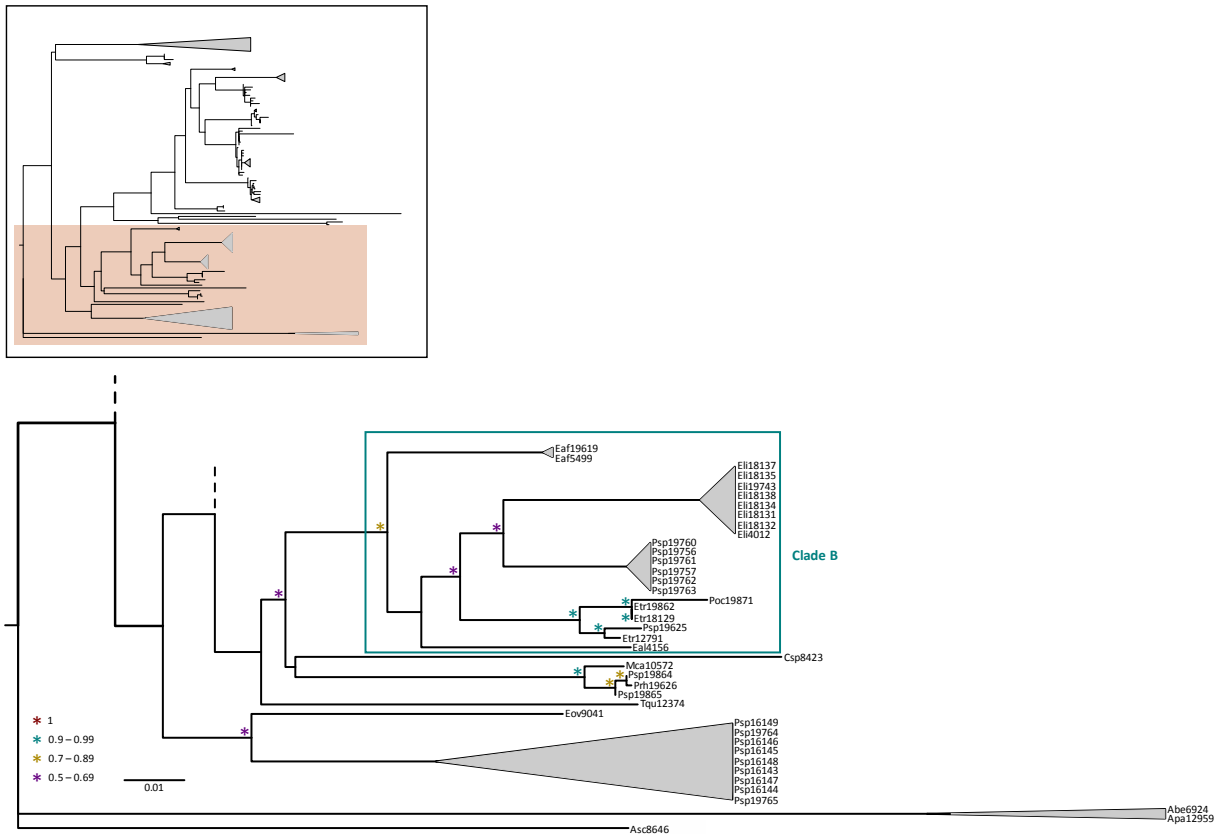

Figure 10-6: The lower subset of the Panopeidae tree represents a Maximum-likelihood tree (COI (GTR+G) + 16S (GTR+G); relaxed clock; 1 000 bootstrap runs, best scoring tree), based on a combined data set (COI, 16S). Clade B (blue frame) is paraphyletic and contains all species of *Eurytium* and several *Panopeus* taxa. Clade B includes the *Eurytium* TSS pair A (*E. limosum*, WA and *Panopeus* spp., EP) and *Eurytium* TSS pair B (*E. tristani*, EP and *Panopeus occidentalis*, WA). Node supports are labeled by colored stars (purple: 0.5-0.69, green: 0.7-0.89, blue: 0.9-0.99, red: 1). Node supports lower than 0.5 are not shown. For information about the species code see Appendix A1.2.6.

10.3.2.1 Divergence time estimation of the genus *Panopeus*

For the divergence time estimation of the genus *Panopeus*, a strict clock, the HKY+G model of evolution, and the COI substitution rate of Marino *et al.* (2011) (0.98% My^{-1}) were employed. The substitution rate for 16S rRNA (estimated by the program BEAST) is 0.66% My^{-1} (Table A1-4). The dataset of the tree (Figure 10-7) bases on clade A of the phylogenetic Panopeidae tree (Figure 10-5).

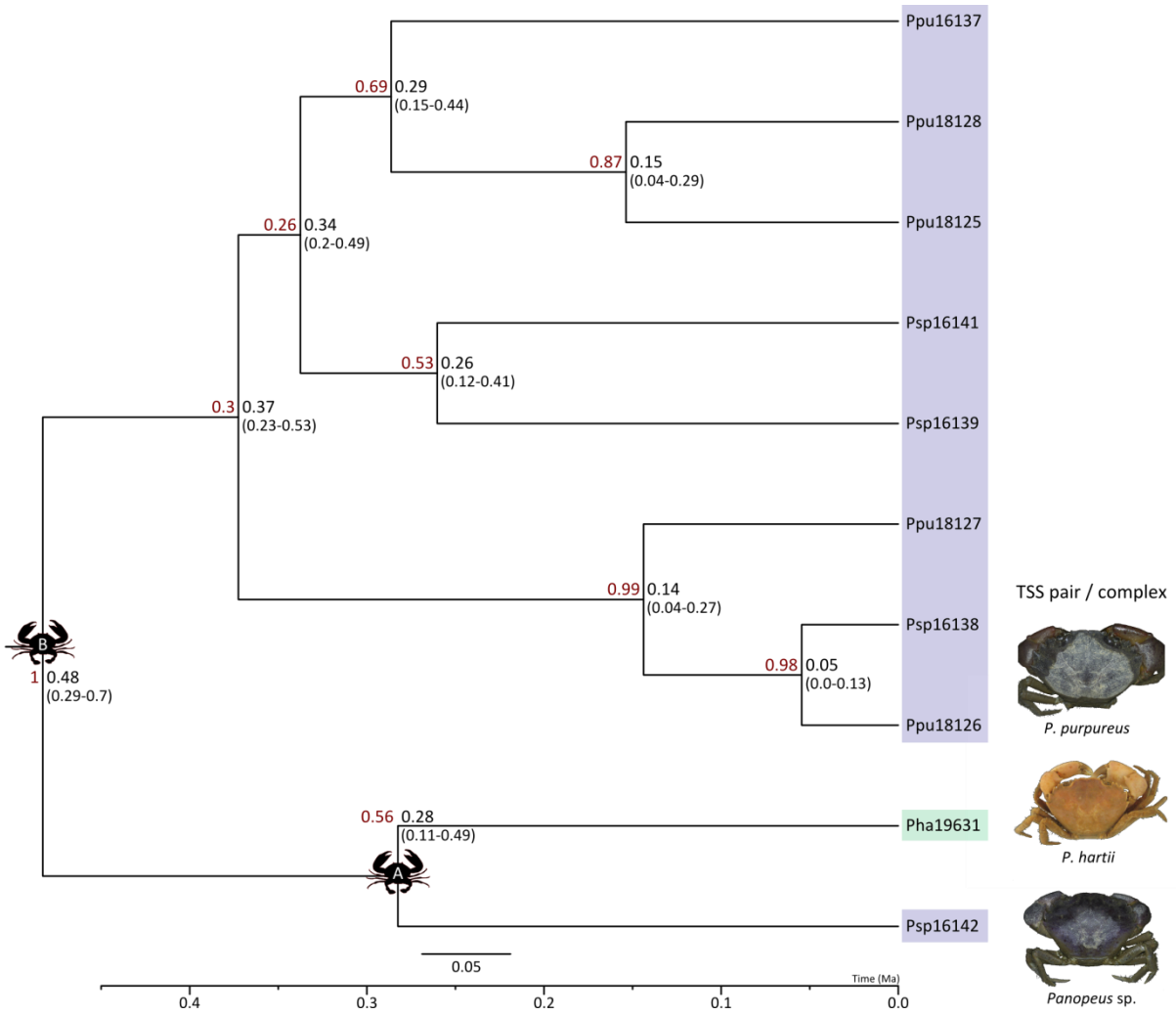

Figure 10-7: Divergence time tree of the genus *Panopeus*, based on a combined data set (COI, 16S). Maximum clade credibility tree: (COI (HKY+G) + 16S (HKY+G); strict clock; Yule process; ngen: 20 million; log: 1000; burnin: 2000); branch supports (red), node ages (black), numbers in brackets present the 95% high posterior density intervals (HPD; interval which contains 95% of the age distribution of all trees). Crustacean silhouettes (A and B) present the time of species divergence of the *Panopeus* TSS pair (silhouette A) or rather the *Panopeus* TSS complex (silhouette B), see text for details; A: 0.28 Ma (95% HPD: 0.11–0.49 Ma), B: 0.48 Ma (95% HPD: 0.29–0.7 Ma). Species framed in green are associated with the western Atlantic and species framed in blue are associated with the eastern Pacific. Ma = million years ago; for information about the species code see Appendix A1.2.6.

The divergence time tree shows very low node supports especially for the deeper nodes and thus, a polytomy of the tree may be accepted (Figure 10-7). The taxonomic arrangement is disordered: Species of *P. purpureus* are arranged with unidentified *Panopeus* species (*Panopeus* spp.). Two possible divergence ages are described here: First, *P. hartii* and *Panopeus* sp. separated 0.28 Ma (95% HPD: 0.11–0.49 Ma; Figure 10-7, silhouette A). In respect to the assumed polytomy, the resulting TSS composition is a complex, consisting of *P. hartii* and *P. purpureus*/*Panopeus* spp. The MRCA of this arrangement occurred around 0.48 Ma (95% HPD: 0.29–0.7 Ma; Figure 10-7, silhouette B). Due to young divergence times, corrections for ancestral polymorphism were negligible (Table 10-6). Note that according to Thoma *et al.* (2014), *P. hartii* is far related to *P. purpureus*.

10.3.2.2 Divergence time estimation of the genus *Eurytium*

For the divergence time estimation of the genus *Eurytium*, a strict clock, the HKY+G model of evolution, and the COI substitution rate of Marino *et al.* (2011) (0.98% My^{-1}) were employed. The substitution rate for 16S rRNA (estimated by the program BEAST) is 0.51% My^{-1} (Table A1-4). The dataset of the tree (Figure 10-8) bases on clade B of the phylogenetic Panopeidae tree (Figure 10-6).

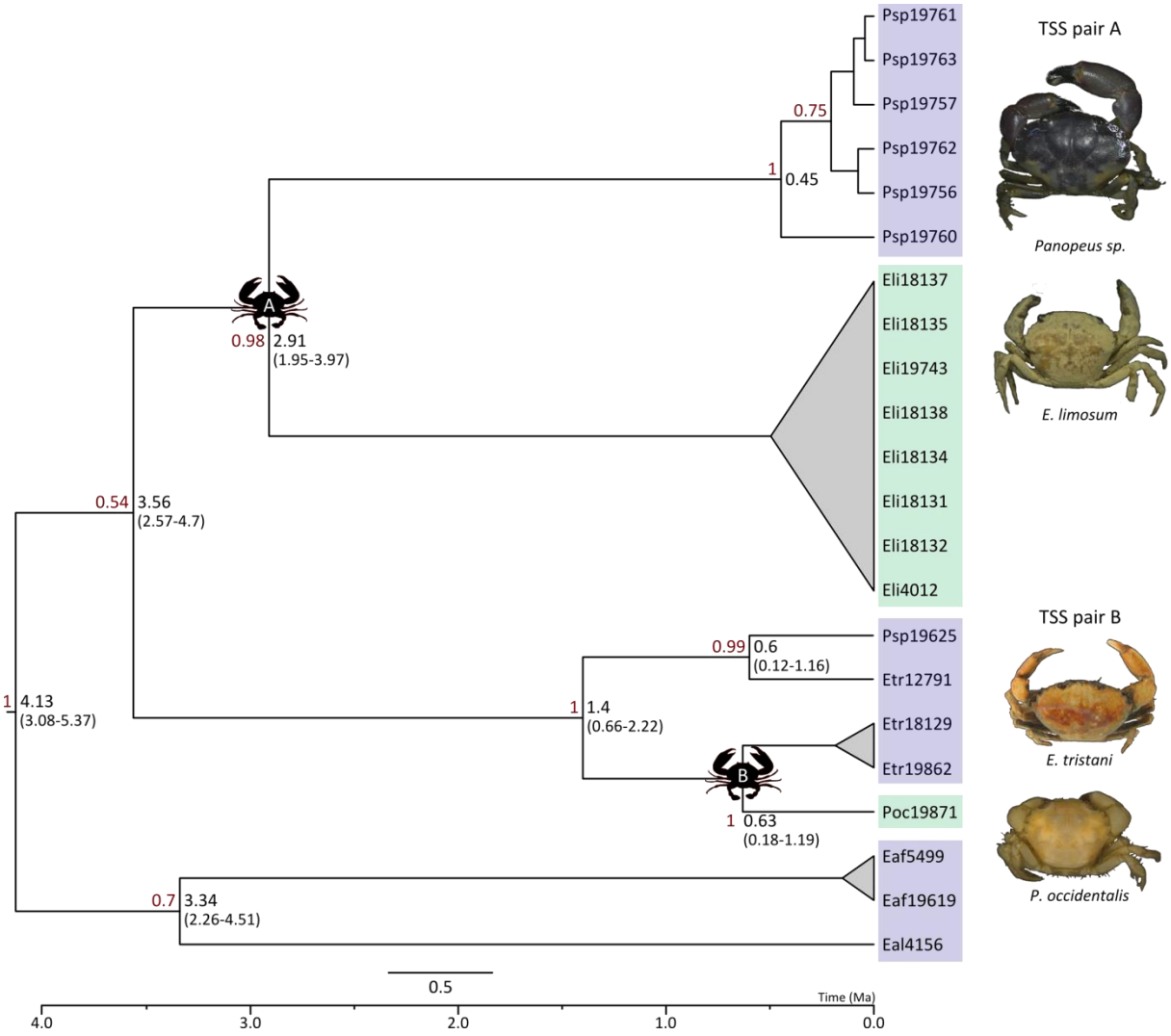

Figure 10-8: Divergence time tree of the genus *Eurytium*, based on a combined data set (COI, 16S). Maximum clade credibility tree: (COI (HKY+G) + 16S (HKY+G); strict clock; Yule process; ngen: 20 million; log: 1000; burnin: 2000); branch supports (red), node ages (black), numbers in brackets present the 95% high posterior density intervals (HPD; interval which contains 95% of the age distribution of all trees). Crustacean silhouettes (A and B) present the time of species divergence of the *Eurytium* TSS pair A (silhouette A) and the *Eurytium* TSS pair B (silhouette B); A: 2.91 Ma (95% HPD: 1.95–3.97 Ma), B: 0.63 Ma (95% HPD: 0.18–1.19 Ma). Species framed in green are associated with the western Atlantic and species framed in blue are associated with the eastern Pacific. Ma = million years ago; for information about the species code see Appendix A1.2.6.

The divergence time tree shows high node supports for all the important branches (branches, which resolve TSS relationships; Figure 10-8). The tree is paraphyletic and includes all species of the genus *Eurytium*, as well as species of the genus *Panopeus* (Figure 10-8). The two TSS pairs (TSS pair A and B) identified above (Figure 10-6) are well-supported. TSS pair A consists of *E. limosum* (WA) and its EP sister species *Panopeus* spp. (#19756-67, 19760-63; Table 10-2; Figure 10-8). Their MRCA occurred 2.91 Ma (95% HPD: 1.95–3.97 Ma; silhouette A). TSS pair B consists of *E. tristani* (EP) and its WA sister species *P. occidentalis* (# 19871; Table 10-2; Figure 10-8). They were separated from each other around 0.63 Ma (95% HPD: 0.18–1.19 Ma; silhouette B). Mean divergence times corrected for ancestral polymorphism are 2.66 Ma (TSS pair A) and 0.38 Ma (TSS pair B; Table 10-6).

10.3.3 Phylogenetic studies of the genus *Pachygrapsus*

The phylogenetic topology of the genus *Pachygrapsus* is adapted from the comprehensive Grapsidae phylogeny of Ip *et al.* (2015) (Figure 10-9). Their tree represents a Bayesian consensus topology, composed of five genes (total length of alignment is 2 247bp; Table A1-4). Eleven out of 14 *Pachygrapsus* species are included in their analysis. *Pachygrapsus* represents a polyphyletic group within the Grapsidae (Ip *et al.* 2015). However, all studied WA and EP representatives form an independent group (including the Japanese species *P. minutus*) with high node supports (Ip *et al.* 2015; Figure 10-9). The phylogenetic analysis revealed one TSS pair: *P. socius* from the EP and *P. transversus* from the WA (Figure 10-9, red frame).

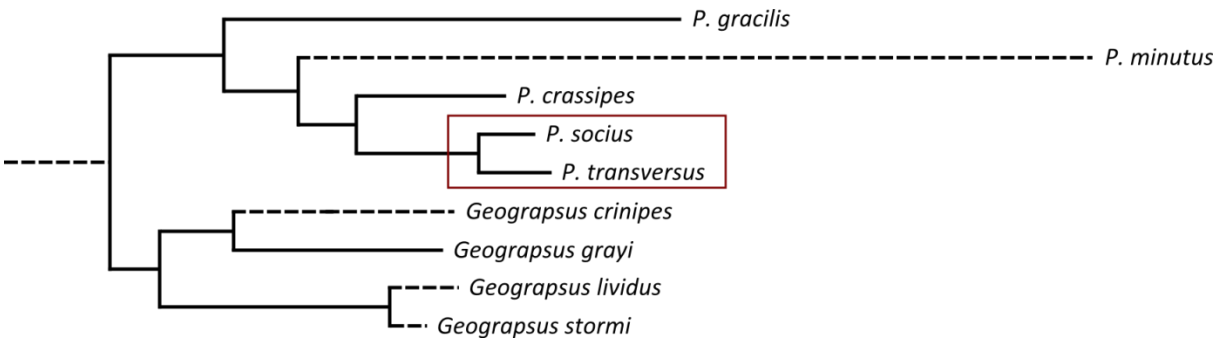

Figure 10-9: Subset of the Bayesian consensus Grapsidae phylogeny of Ip *et al*. 2015 (p. 6). This phylogenetic section was used as template and complemented with own species and sequences for the divergence time estimation (Figure 10-10). Dashed branches indicate that these species were excluded from the subsequent divergence time estimation. The supposed TSS pair *P. socius* (EP) and *P. transversus* (WA) is framed in red.

For the divergence time estimation of the genus *Pachygrapsus*, a strict clock, the HKY+G model of evolution, and the COI substitution rate of Marino *et al.* (2011) (0.98% My^{-1}) were employed. Due to missing 16S sequences, the analysis bases on COI sequences alone (Tables 10-5, A1-4, A2-4). Species with dashed branches were excluded from the subsequent divergence time estimation (Figure 10-9).

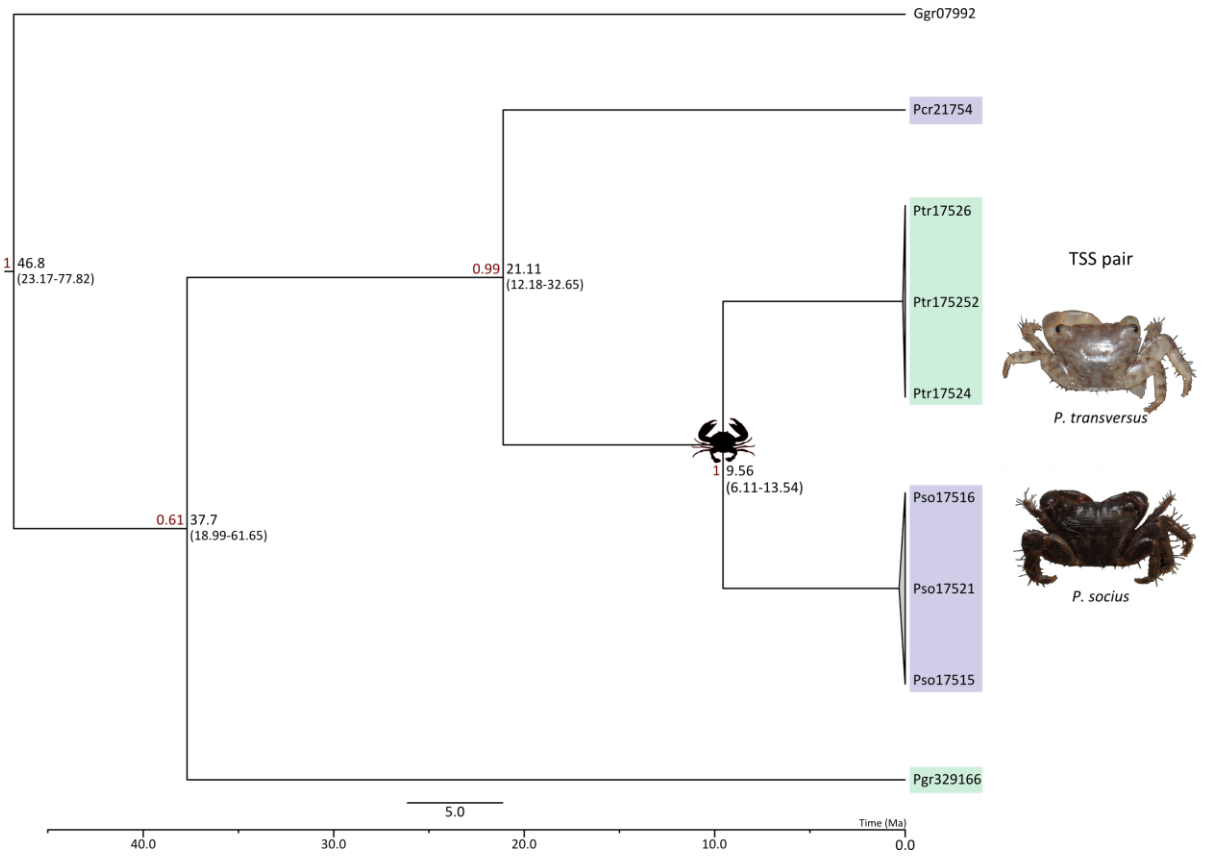

Figure 10-10: Divergence time tree of the genus *Pachygrapsus*, based on COI. Maximum clade credibility tree: (COI (HKY+G); strict clock; Yule process; ngen: 20 million; log: 1000; burnin: 2000); branch supports (red), node ages (black), numbers in brackets present the 95% high posterior density intervals (HPD; interval which contains 95% of the age distribution of all trees). Crustacean silhouette presents the time of species divergence of the *Pachygrapsus* TSS pair: 9.56 Ma (95% HPD: 6.11–13.54 Ma). Species framed in green are associated with the western Atlantic and species framed in blue are associated with the eastern Pacific. Ma = million years ago; for information about the species code see Appendix A1.2.6.

The divergence time tree shows high node supports for all branches (Figure 10-10). The TSS pair consists of *P. socius* (EP) and its WA sister *P. transversus* (Table 10-3; Figure 10-10). Their MRCA originated 9.56 Ma (95% HPD: 6.11–13.54 Ma). The mean divergence time corrected for ancestral polymorphism is 9.31 Ma (Table 10-6).

Table 10-6: Overview of the estimated divergence times for the genera *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus*.

	Sesarmidae		**Panopeidae**			**Grapsidae**
	Sesarma		***Panopeus***	***Eurytium***		***Pachygrapsus***
	Complex A Srh–Scu*/ Sret	Complex B Scra–Ssul / Saeq	TSS pair/complex Pha–Psp Pha–Ppu	TSS pair A Eli–Psp	TSS pair B Etr–Poc	TSS pair Pso–Ptrans
Divergence Times[1] (Ma; 95% HPD)	3.03 (2.37–3.71)	4.36 (3.27–5.56)	0.28 (0.11–0.49) 0.48 (0.29–0.7)	2.91 (1.95–3.97)	0.63 (0.18–1.19)	9.56 (6.11–13.54)
Corrected for Ancestral Polymorphism[2] (Ma; Mean Values)	2.78 (2.12–3.46)	4.11 (3.02–5.31)	–	2.66 (1.70–3.72)	0.38 (0–0.94)	9.31 (5.86–13.29)
Ecology	Soft-sediment littoral environments like mangroves and marshes.		Marine intertidal and shallow subtidal, as well as oligohaline and freshwater estuarine environments.			Caves and cervices of rocky intertidal shores as well as mangroves.

Overview of divergence times (including corrections for ancestral polymorphism) for the four genera *Sesarma*, *Eurytium*, *Panopeus*, and *Pachygrapsus*; TSS = transisthmian sister species, [1]using the program BEAST, 95% HPD (high posterior density interval, which contains 95% of the age distribution of all trees), [2]assumption for ancestral polymorphism 250 000 years, *incl. *Sesarma* sp. (nr. *reticulatum*), Srh (*Sesarma rhizophorae*), Scu (*Sesarma curacaoense*), Sret (*Sesarma reticulatum*), Scra (*Sesarma crassipes*), Saeq (*Sesarma aequatoriale*), Ssul (*Sesarma sulcatum*), Eli (*Eurytium limosum*), Etr (*Eurytium tristani*), Psp (*Panopeus* sp.), Poc (*Panopeus occidentalis*), Pha (*Panopeus hartii*), Ppu (*Panopeus purpureus*), Pso (*Pachygrapsus socius*), Ptrans (*Pachygrapsus transversus*).

10.4 Discussion

The emergence and final closure of the Isthmus of Panama was a complex and long-lasting geographical event, which only recently came into focus of an international and interdisciplinary debate (Hoorn & Flantua 2015; Montes *et al.* 2015; O'Dea & Collins 2013). The main issue of this dispute focuses on the time of Isthmus closure. Two models are predicted: the 'new Miocene model' (i.e. Isthmus closure around 15 million years ago; Ma) and the 'common Pliocene model' (i.e. Isthmus closure around 3 Ma; see Chapter 4 for details). In general, independent of the model and based on the complex process of the Isthmus emergence, the time of the *final* closure cannot be precisely determined. Indeed, several studies found evidence of several re-openings and -closures (e.g., Cronin & Dowsett 1996; Haug & Tiedemann 1998), pointing toward a *final* closure around 1.9–1.8 Ma (Cronin & Dowsett 1996; Keller *et al.* 1989). In this study, divergence time estimations of transisthmian sister species (TSS) pairs and -complexes were performed in four different decapod genera. The aims of these analyses were twofold:

1. The molecular studies should point out the problems of divergence time estimations of TSS.
2. The obtained divergence times of the study are then discussed relative to the two models proposed for the final closure of the Isthmus.

10.4.1 Problems of divergence time estimations of TSS

In general, divergence time estimations of TSS can be influenced by a number of molecular and biological/geological factors (see Chapters 6 and 7 for details). In respect to molecular parameters these are, for example, rate heterogeneity, the persistence of ancestral polymorphism, saturation of the data set, inclusion of pseudogenes, used prior conditions of the clock values, inaccurate calibration points/bounds or external clock rates, or an insufficient data set. Divergence time estimations may also be influenced by bio- and geological factors like, for example, the complex history of the Isthmus emergence, the possibility of re-openings and -closures, and the dispersal capability of species (Lessios 2008). To avoid these factors and reduce the uncertainties in divergence time estimations as much as possible, different approaches should be considered.

10.4.1.1 (External-) Substitution rates

Among others, the protein coding cytochrome c oxidase subunit I (COI) gene and the non-coding ribosomal subunit 16S rRNA gene (16S) are widely used in phylogenetic analyses and divergence time estimations in particular (Schubart 2009; Schubart *et al.* 2000b). However, to calibrate the molecular clock, the time of closure of the Isthmus of Panama is frequently used as calibration point. For that matter, an Isthmus closure at around 3 Ma is generally assumed (see Chapters 4 and 7). Based on several uncertainties regarding this assumption (see discussion below and Chapter 4 for details), an external COI molecular clock crustacean rate (0.98% My^{-1}), which was estimated by Marino *et al.* (2011) was applied. This rate bases on the Mediterranean Salinity Crisis (MSC) around 6 Ma ago (Krijgsman *et al.* 1999; Table 7-2), and thus, it is independent of

the time of Isthmus closure. In turn, this rate can be used to estimate divergence times of TSS pairs and -complexes of crustaceans and may gain evidences on the time of Isthmus closure.

The external COI substitution rate of Marino *et al.* (2011; 0.98% My^{-1}) is comparable with other COI crustacean rates (note that most of them base on an Isthmus calibration) in the literature. For *Alpheus* two very similar substitution rates are found: 1.1-1.3% My^{-1} using 3.0–3.5 Ma as isthmian calibration bound (Knowlton *et al.* 1993; K2P), and 0.7% My^{-1} using 3 Ma as isthmian calibration point (Knowlton & Weigt 1998; K2P). In previous studies of the crab genera *Sesarma*, the COI substitution rate was 0.83-1.17% My^{-1} using 3.1 Ma as isthmian calibration point (Schubart *et al.* 1998; K2P).

The estimated substitution rates for 16S rRNA of the genera *Sesarma*, *Panopeus*, and *Eurytium* were 0.58%, 0.66%, and 0.51% My^{-1}, respectively. These estimates are slightly higher if compared to rates obtained for other crab genera: 0.45% My^{-1} using 3.0–3.5 Ma as isthmian calibration bound for *Uca* (Sturmbauer *et al.* 1996; K2P), and 0.27-0.68% My^{-1} using 3 Ma as isthmian calibration point for the species *Petrolisthes armatus* (Stillman & Reeb 2001; HKY). Schubart *et al.* (1998) estimated a substitution rate for *Sesarma* as well and obtained a rate of 0.33-0.44% My^{-1}, based on the Isthmus of Panama as calibration point (3.1 Ma; K2P).

The applied external COI substitution rate of Marino *et al.* (2011) is comparable to those of other studies (see above). In contrast, the estimated 16S substitution rates of this study are slightly higher compared to those found in the literature (see above). The observed differences among the 16S substitution rates may be explained by the chosen substitution model. Models with gamma distribution and invariable sites show an increased substitution rate (Wilke *at al.* 2009; see Subchapter 10.4.1.3). Estimated 16S substitution rates in this study base on the applied HKY+G model and are compared to substitution rates, which base on the K2P model (see above). This might be a reason for the slightly higher 16S substitution rates obtained in this study. Moreover, Schubart *et al.* (2000b) pointed out that comparisons of 16S substitution rates, which were estimated from different lengths and locations of the gene, have to be handle with caution because the “highly conserved and more variable regions” (p. 826) can have an influence of the rate. In fact, the sequences within the 16S alignments of this study are not uniform, but differ in length and completeness (Appendix A2). This may “result in an upwardly biased estimate of divergence” (p. 826, Schubart *et al.* 2000b) because informative regions may be missing. In contrast, this observation could not be confirmed for the COI gene (Lessios 2008). Knowlton *et al.* (1993) showed that their substation rate for COI is comparable to other COI rates, although the analyzed DNA regions differed among the studies. However, it should be noted that the sequences within the COI alignments in this study varied partially in their lengths (Appendix A2). This can cause problems in tree topologies (i.e. wrong species arrangements or very low node supports) and hence, in divergence time estimates). However, Lessios (2008) pointed out that certain variations within substitution rates are unproblematic. Indeed, varying 16S rates in (non transisthmian) species has also been shown by Hiller *et al.* (2006) in porcellanids and Wares (2001) in barnacles. Based on this assumption, the range of variation of the obtained 16S substitution rates in this study might be considered comparable to rates in the literature.

The similarity of different COI substitution rates inferred from the closure of the Isthmus of Panama (i.e. based on the assumption of the Pliocene model) compared to the crustacean rate inferred from the MSC indicates that the Isthmus of Panama is not *per se* an ineligible calibration point. However, several factors make the Isthmus inappropriate for divergence time estimations of TSS: (i) since the time of final Isthmus closure is unknown, the assumption of a 3 Ma Isthmus closure is inherently problematic and includes a source of error, (ii) often the youngest TSS pair is equalized to the assumed age of Isthmus closure. In turn, divergence times of all other nodes in the tree are then based on this assumption, which may result in wrong divergence times. Moreover, it is defective to assume that the youngest TSS pair in the tree has diverged due to the Isthmus closure. There are several other factors, which can play a role in species separation events (see Chapter 6), (iii) the defined calibration point of 3 Ma in many studies is too inflexible. The emergence and closure of the Isthmus of Panama was not a uniform event, but a complex and long lasting process. To account for this condition it is more precisely to use (at least) calibration bounds with a defined time interval, and (iv) the application of the Isthmus as calibration point includes *per se* a circular argument, when testing for the time of Isthmus closure. However, if the Isthmus of Panama *has to be used* as calibration point (i.e. alternative calibration events, fossils or external clocks are not available), it would be more accurate to assume an upper and lower bound than to apply calibration points (i.e. application of a time interval instead of a fixed time; see Wilke *et al.* 2009 and references therein for detailed discussion; Chapters 4 and 7).

As mentioned above, the external COI substitution rate of Marino *et al.* (2011) is comparable to those of other studies. However, Ho (2007) criticized that external molecular clock rates (e.g., the avian clock rate of 2% My^{-1}), are widely applied without the awareness of the corresponding uncertainties. However, I am aware that the applied external crustacean rate may account for certain errors in the divergence time estimations of this study. For example, the clock calibration by Marino *et al.* (2011) bases on a relaxed clock assumption and the calibration point of 5.59 Ma. To obtain a single substitution rate, Marino *et al.* (2011) used the average of all yielded substation rates inferred from the relaxed clock analyses. Unfortunately, they did not specify the confidence interval and thus, the included error cannot be considered here. Additionally, their calibration point reflects the earliest evidence of Atlantic–Mediterranean isolation (Krijgsman *et al.* 1999). As discussed above a calibration bound, which corrects for temporal uncertainties, would have been more appropriate.

10.4.1.2 Dataset

For the accuracy of molecular clock calibrations, several sequences of each species should be used to include a large range of haplotypes, because intraspecific variation can influence molecular clock estimations (Schubart *et al.* 2000b). Unfortunately, few datasets of this study suffer from low specimen/sequence numbers (Tables A2-1 – A2.4) and/or missing taxa (Table 10-4), which may influence species relationships and, in the end, divergence times of TSS pairs (Andújar *et al.* 2014; Craig *et al.* 2004). For example, the datasets of the genera *Panopeus* and *Pachygrapsus* suffer from missing taxa, which may be relevant in respect to phylogenetic

relationships and species arrangements. Within the genus *Panopeus*, one western Atlantic species (*P. boekei*) and two eastern Pacific species (*P. convexus* and *P. diversus*) are missing within the phylogenetic study (Table 10-4). Unfortunately, there is none phylogeny available in the literature, which could reveal the phylogenetic relationship of the three missing taxa to the other species of this genus. Thus, the obtained phylogenetic relationships within this genus and, hence, assumed TSS pairs and their divergence times, have to be analyzed with consideration. Within the genus *Pachygrapsus*, one essential species is missing in the here performed phylogenetic analysis (*P. corrugatus*; Table 10-4). This species is widely distributed within the western Atlantic and was also found along the coast of the United Kingdom. However, in the phylogeny of Schubart (2011), *P. corrugatus* appears to be the sister to *P. plicatus*, a cosmopolitan species with a main distribution in the tropical Indo Pacific. Both species are not closely clustered to the proposed TSS pair. The other two missing species of *Pachygrapsus* (Table 10-4) are distributed along the east coast of India and the southern Atlantic Ocean. Thus, it is reasonable to assume, that the missing species would not have significantly influence the divergence time estimation of the identified TSS pair (*P. transversus*/*P. socius*) in this study.

10.4.1.3 Substitution model

The influence of the selected substitution model on divergence times is low, resulting in similar average clock rates (table 3 in Wilke *et al.* 2009). However, Wilke *et al.* (2009) pointed out that substitution models with gamma distribution and invariable sites (Γ+I) show an increased substitution rate. This is important to consider, if external substitution rates are applied in own analyses (i.e. and potentially other substitution models are used), or if divergence time estimations from different models are compared with each other (see above). Lessios (2008) criticized the common approach to compare transisthmian species by their genetic distance and to equal the smallest genetic distance with the closure of the Isthmus. He argued that this method will possibly result in questionable divergence times, as shown for mollusks (Lessios 2008 and references therein). In this study, the HKY+G substitution model was used in the divergence time estimations. This substitution model was also applied by Marino *et al.* (2011). Hence, the introduce error rate should be negligible.

10.4.1.4 Ancestral polymorphism

Another source of error includes the aspect of ancestral polymorphism. Ancestral polymorphism plays a significant role in divergence time estimations of species. In mollusks, Wilke *et al.* (2009) found that ancestral polymorphism results in overestimations of divergence times of 10-70% in young (around 1 Ma) and 2-9% in old (around 5 Ma) events. However, the influence of ancestral polymorphism is widely ignored in divergence time studies (Edwards & Beerli 2000; Hickerson *et al.* 2003, 2006). Corrections for ancestral polymorphism in this study are problematic. The population datasets of the analyzed genera are insufficient to calculate the value of ancestral polymorphism correctly (see Appendix A2). However, based on the estimations of ancestral polymorphism in mollusks by Wilke *et al.* (2009), an average value of 5% per million years was chosen for all decapod genera in this study. Thus the average value for ancestral polymorphism for the studied genera is about 250 000 years (Table 10-6).

10.4.1.5 Other parameters

As mentioned above, several additional parameters may influence divergence times of TSS: (i) Saturation of the data set can be excluded for all genera, based on the test for substitutional saturation (Xia 2013; see Appendix A1.2.7), (ii) pseudogenes can be excluded for all datasets in this study, and (iii) the used prior conditions (Appendices A1.2.7 and A1.2.8; Table A1-4) were suitable for the conducted phylogenetic analyses and divergence time estimations. Obtained ESS values in Tracer were well above 200 for each dataset (Rambaut & Drummond 2009). Additional parameters like dispersal capability of the studied species, missing sequences, and species misidentifications, which may have an influence of the results of the divergence time estimations, are discussed below. Although various statistical models are developed for different approaches of divergence time estimations to yield most realistic estimates (e.g., Hickerson *et al.* 2003, 2006; Huelsenbeck *et al.* 2000; Tavaré *et al.* 2002), none of these models could have been applied here, because of insufficient datasets.

10.4.2 Evidences for the time of Isthmus closure

10.4.2.1 Phylogenetic studies of the genus *Sesarma*

The phylogenetic analysis of the genus *Sesarma* revealed three monophyletic clades (clades A-C; Figure 10-2). Clade A consists of only (semi-)terrestrial Jamaican crabs, which originated from a marine ancestor around 5.13 Ma (95% HPD: 4.27–6.02 Ma). Schubart *et al.* (1998) dated their divergence time at around 4.5 Ma (+/- 0.42). This slight discrepancy may base on the different approaches, which were used to estimate the divergence times. Schubart *et al.* (1998) estimated the divergence times based on sequence divergences between the species and assumed an isthmian closure at 3.1 Ma. In contrast, divergence time estimations in this study base on an external substitution rate, which was inferred from the MSC, under the HKY+G substitution model. However, note that the 95% HPD falls within the estimated divergence time of Schubart *et al.* (1998). If the here estimated divergence time is corrected for ancestral polymorphism (i.e. 4.88 Ma, 95% HPD: 4.02–5.77 Ma; Table 10-6) divergence times of both studies are similar.

Divergence time estimations were performed for two *Sesarma* TSS complexes (complex A and complex B; Figure 10-2), which were identified due to the former conducted phylogenetic analysis. Complex A consists of *S. rhizophorae* (EP) and the potential TSS *S. curacaoense* and *S. reticulatum* (WA). Complex B consists of *S. crassipes* (WA) and the potential TSS *S. sulcatum* and *S. aequatoriale* (EP). Both TSS complexes were also found by Schubart *et al.* (1998).

The development of TSS complexes (i.e. several species on one side of the Isthmus can represent the putative sister to the species on the other side) may base on additional separation events followed by incomplete lineage sorting on one side of the barrier, as pointed out by Reuschel & Schubart (2006). The divergence times of both complexes fall within the time range of the Pliocene model. The average divergence time of 3.03 Ma (TSS complex A) matches exactly the Pliocene assumption (slightly younger if corrected for ancestral polymorphism). Although, the average divergence time of TSS complex B (4.36 Ma; 4.11 Ma if corrected for ancestral polymorphism) is slightly older than 3 Ma, the proposed upper bound of the Isthmus closure is

around 4 Ma (Collins 2003; Jackson & O'Dea 2013; Weir *et al.* 2009; Table 4-1). Thus, the estimated divergence time fits well into the time range of a Pliocene Isthmus closure. Anyway, it has to be noticed that different parameters (e.g., imprecise corrections for ancestral polymorphism, or an incomplete dataset) may account for overestimations of the divergence times (see above). However, in general the obtained divergence times of both *Sesarma* TSS complexes reflect the time of Isthmus closure of the 'common Pliocene model'. Assuming re-openings and -closures of the Isthmus until around 1.8 Ma (Keller *et al.* 1989), the 95% HPD of TSS complex A (2.37–3.71 Ma, 2.12–3.46 Ma if corrected for ancestral polymorphism) indicates that TSS may have been separated from each other during this time. However, as mentioned before the emergence and closure of the Isthmus of Panama was a complex and long lasting event and thus, precise times of the final closure cannot be determined.

10.4.2.2 Phylogenetic studies of the family Panopeidae

The phylogenetic analysis of the Panopeidae based on a subset of the comprehensive Xanthoidea phylogeny by Thoma *et al.* (2014) (Figures 10-3 and 10-4). The taxonomic arrangement in this study differs to the species arrangement of Thoma *et al.* (2014) considerably. The different species arrangement may occur due to the complement of the dataset with numerous of own species and sequences. Differences on an intra-specific level (i.e. species are not clustered together but are widely distributed within the phylogenetic tree, e.g., *P*. *americanus*, *P*. *rugosus*, *P*. *occidentalis*) may be due to misidentifications or, more likely, due to an incomplete dataset. Particularly for the species in question either COI or 16S sequences are missing and thus, genetic information is only available for one of the genes (Tables A2-2 and A2-3). Andújar *et al.* (2014) pointed out that missing sequences can result in wrong species arrangements. On a genus level, the genera *Panopeus* and *Eurytium* are also not well separated. Whereas *Eurytium* is monophyletic in Thoma *et al.* (2014), here both genera are paraphyletic (Figures 10-5 and 10-6).

In respect to the studied TSS pairs and -complex, species arrangements differ between both studies (i.e. Thoma *et al.* 2014 vs. this study). In the phylogenetic analysis of this study, one *Panopeus* TSS pair (*P*. *hartii*/*Panopeus* sp.) can be identified within clade A (Figure 10-5). However, node supports are low, which are even more pronounced in the divergence time tree (Figure 10-7). Thus, assuming a polytomy of this clade the TSS pair A changes to a TSS complex consisting of *P*. *hartii* (WA) and *Panopeus* spp./*P*. *purpureus* (EP). The reason for this TSS complex is probably the inadequate dataset of *P*. *hartii* (missing 16S sequence and only short COI sequence; Table A2-3). However, the MRCA of this here presented complex occurred at around 480 000 years ago (95% HPD: 0.29–0.7 Ma). For this young age, corrections for ancestral polymorphism are negligible. Two explanations for such a young age can be supposed. First, the species arrangement is wrong (i.e. evidences are given by low node supports and an incomplete dataset of *P*. *hartii*; see discussion above). Moreover, in the study of Thoma *et al.* (2014) *P*. *hartii* and *P*. *purpureus* are far arranged from each other. Second, assuming an accurate species arrangement, recent dispersal events may have occurred. Recent dispersal events (< 1.8 Ma) between both sides of the Isthmus have been shown by Miura *et al.* (2012) in mollusks,

McCartney *et al.* (2000) in sea urchins, and by Lessios (2008, and references therein) in different species groups. Additional species of *P. hartii* and a completion of the dataset could bring light into the here presented pattern.

The genus *Eurytium* occurs to be paraphyletic in the here presented phylogeny and contains two TSS pairs (Figure 10-6, clade B). The species *E. limosum* (WA) and *E. tristani* (EP) are not closely related to each other in this study, as postulated by Thoma *et al.* (2014). In fact, TSS pair A consists of *E. limosum* (WA) and the species *Panopeus* spp. (EP). *Panopeus* spp. (#19756-67, 19760-63) are unidentified species. It remains uncertain, if *Panopeus* spp. can be considered as *Panopeus*, or if they may be undescribed species of *Eurytium*. However, TSS pair A shows a divergence time of 2.91 Ma (95% HPD: 1.95–3.97 Ma) and thus, it matches the time frame of the assumed Pliocene model (i.e. Isthmus closure around 3 Ma). However, the lower range of the 95% HPD interval of TSS pair A reflects also the time of possible isthmian re-openings and -closures (Cronin & Dowsett 1996; Keller *et al.* 1989). TSS pair B consists of *E. tristani* (EP) and *P. occidentalis* (#19871; WA; Table 10-2; Figure 10-8). They were separated from each other around 0.63 Ma (95% HPD: 0.18–1.19 Ma). It is surprising that *P. occidentalis* (#19871) occurs to be the sister to *E. tristani*. The reason may be that the missing COI sequence of *P. occidentalis* (#19871) results in a misleading species arrangement (Andújar *et al.* 2014; Table A2-3). Comparing the datasets of all specimens of *P. occidentalis* in this study it becomes apparent that *P. occidentalis* (#19872) shows a missing 16S sequence and the specimens *P. occidentalis* (#19870, #19873) show missing COI sequences (Table A2-3). The remaining specimens of *P. occidentalis* (#16150, #16167) show a complete dataset. Thus, it is surprising that *P. occidentalis* (#16150) clusters to *P. rugosus* and *P. americanus* (which themselves have incomplete datasets, see Table A2-3), and that *P. occidentalis* (#16167, #19870) as well as *P. occidentalis* (#19872, #19873) each clustering together (Figure 10-8). The low divergence age of TSS pair B supports this assumption. Furthermore, no differences were observable in the morphological comparisons between all specimens of *P. occidentalis* (Figures A3-32 – A3-33).

Based on only morphological comparisons, Martin & Abele (1986) pointed out: “Systematically, the genus *Panopeus* H. Milne Edwards has a long-standing reputation as a problem group” (p. 183). However, the young age of the MRCA of the here presented TSS pair B (0.63 Ma) may have three reasons. First, *P. occidentalis* (#19871) is misidentified. However, morphological comparisons do not support such an assumption (Figures A3-32 – A3-33). Second, the species arrangement is wrong of the above discussed reasons (incomplete dataset). Third, assuming an accurate species arrangement, recent dispersal events may have occurred. Similar to the discussion of *P. hartii* (see above), a completion of the dataset could bring light to the here observed pattern.

10.4.2.3 Phylogenetic studies of the genus *Pachygrapsus*

The phylogenetic analysis of the genus *Pachygrapsus* based on a subset of the comprehensive Grapsidae phylogeny by Ip *et al.* (2015) (Figure 10-9). Their species arrangements are in well accordance with the topology of the phylogenetic analysis of Schubart (2011).

Pachygrapsus represents a polyphyletic group within the Grapsidae (Ip *et al.* 2015; Schubart 2011). However, all studied WA and EP representatives form a monophyletic group with high node supports (Figure 10-10). Due to missing 16S sequences of the supposed TSS pair (*P. socius*, EP and *P. transversus*, WA), the divergence time analysis bases on COI sequences alone (Table A2-4). *P. corrugatus* is the only missing WA species of this genus (see above; Table 10-4). Because of the unavailability of COI sequences for *P. corrugatus* (as well as the Japanese species *P. minutus*), these species could have not been included in the divergence time estimation of this study. Anyway, in the comprehensive 16S Grapsidae phylogeny of Schubart (2011), *P. corrugatus* appears to be the sister species to *P. plicatus*, which in turn, is arranged far apart from the studied TSS pair (see also Ip *et al.* 2015). Thus, it is most likely that *P. corrugatus* would not have had influenced the result of the divergence time estimation. The MRCA of the studied TSS pair *P. socius* (EP) and *P. transversus* (WA) occurred 9.56 Ma (95% HPD: 6.11–13.54 Ma; Table 10-6; Figure 10-10). This time of divergence matches the proposed time range of a temporary near-complete Isthmus around 11–9 Ma (Coates *et al.* 2003, 2004; Roth *et al.* 2000; see Chapter 4; Table 4-2). In fact, the estimated divergence time of the here studied TSS pair is reasonable, if set into context to different studies. In contrast to Montes *et al.* (2012a; b), who postulated an Isthmus closure around 15 Ma, Keller & Barron (1983) as well as Coates & Stallard (2013) proposed a gradual shoaling of the Isthmus between 15–12 Ma. Coates & Stallard (2013) pointed out that a complete interruption of water exchange around 15 Ma is unlikely, rather narrow marine connections persist. Moreover, several studies indicate migration events of terrestrial and freshwater species between North- and South America during 5–16 Ma (Bermingham & Martin 1998; Cody *et al.* 2010; Marshall 1985, 1988; Morgan 2002; Webb 1985; Weigt *et al.* 2005). The estimated divergence time and the 95% HPD of the TSS pair indicates a much earlier separation of the species, compared to the TSS pairs and -complexes of the other studied genera. In contrast, Schubart (2011) assumed a divergence age of *P. socius*/*P. transversus* around 3 Ma. In his study, Schubart (2011) equalizes the habitat preference of *Pachygrapsus* (upper intertidal) with the general assumption that species of shallow water environments were the last, which have crossed the Isthmus before the final closure (i.e. 3 Ma; Knowlton & Weigt 1998; see Chapter 4). However, Schubart (2011) also pointed out that "changes in current regimes and water temperature already took place a few million years earlier […] and some species may thus have diverged before 3 Mya, depending on their ecology […]" (p. 477, Schubart 2011).

10.4.2.4 Incongruence of achieved divergence times

The obtained divergence times of the here studied TSS pairs and -complexes are not concordant to each other and thus, do not point unambiguously toward a Miocene or a Pliocene closure of the Isthmus. In fact, the yielded results reflect the general pattern, which is observable in the literature. Several identified TSS pairs of aquatic as well as terrestrial species show divergence times much older than 3 Ma (e.g., Anker *et al.* 2007; Knowlton & Weigt 1998; Lessios 2008 and references therein; Marko 2002; see Chapters 4 – 6). In contrast, divergence time estimations of several TSS pairs in various studies resulted in young divergence ages, pointing toward re-

openings and -closures of the Isthmus or dispersal events (e.g., Lessios 2008 and references therein; Miura *et al.* 2012; Stillman & Reeb 2001). Even though the Isthmus of Panama was avoided as calibration point in the here performed divergence time estimations, fossils would have been theoretically another possibility to calibrate the molecular clock, although they include specific uncertainties (see Chapter 7). In general, the fossil record of crustaceans is highly influenced by environmental conditions and the former species way of life (Plotnick *et al.* 1988). For example, "[…] preservational models developed in cold, nutrient rich areas may not be applicable to the low organic matter, carbonate environments of epeiric seas" (p. 40, Plotnick *et al.* 1988). On the other hand, calcified exoskeletons, and (semi-) infaunal or widely distributed species, like the genus *Panopeus*, should provide good fossil records. In fact, Plotnick *et al.* (1988) pointed out that xanthid crabs show a large fossil record that reaches back to the Palaeocene. However, fossils for the here studied genera were not available to this study.

10.5 Summary

The here studied TSS pairs and -complexes of the four decapod genera *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus* do not present clear evidence for either the Miocene or the Pliocene model. In fact, the TSS pair of *Pachygrapsus* shows an early divergence age close to the Miocene model, whereas the TSS complexes of *Sesarma* and the TSS pair A of *Eurytium* rather point toward the Pliocene model. Moreover, TSS complex A of *Sesarma* and TSS pair A of *Eurytium* also show evidence of potential re-openings and -closures of the Isthmus after 3 Ma (Table 10-6). The TSS pair of *Panopeus* shows such a young divergence age that recently dispersal events should be considered. However, based on several factors that may have influenced the TSS arrangement and divergence time estimations (e.g., missing species or sequences, and misidentifications), the obtained results should be interpreted with care, in particular for the TSS pair of *Panopeus* and the TSS pair B of *Eurytium* (see discussion above).

11 Conclusion

11.1 A critical view at the transisthmian sister species concepts

11.1.1 Toward an unified definition of transisthmian sister species

1. The comprehensive study of the term *transisthmian sister species* (TSS) resulted in several conclusions regarding the responsible and structured use of this term and its synonyms. It should be considered that terms used in a study, should be clear and unambiguous defined in the beginning. To avoid confusion a minimal number of different terms should be used. Moreover, common terms should be applied and general (e.g., sister species, species pairs), complex (e.g., transisthmian pairs of sister taxa, closest transisthmian relatives), and unusual terms (e.g., daughter species, congeneric counterparts, analogous species) avoided. Hard (cladogenetic event) and soft polytomies within the phylogenetic tree can provide evidence for true or misleading TSS relationships. In this context, habitat preferences and distribution ranges of the studied TSS pairs and -complexes may provide further evidence for TSS relationships.
2. In this study, three terms (and their synonyms) were defined in the beginning of this thesis, based on the species composition:

   A) *Transisthmian sister species pair* (**TSS pair**) – term that refers to TSS representing one unity.

   B) *Transisthmian sister species complex* (**TSS complex**) – term that points out an unresolved TSS relationship, containing several species.

   C) *Transisthmian pseudo sister species* (**pseudo-TSS**) – term that implies that separation events occurred independently of the Isthmus formation.

   After the here presented divergence time estimations, the term *TSS pair* had to be revised and divided into more precise terms in respect to the time of assumed Isthmus emergence. Thus, the following three new terms were proposed to distinguish between TSS divergence events *before*, *during* and *after* assumed Isthmus completion, respectively:

   - *Pre- transisthmian sister species pair* (**Pre-TSS pair**)
   - *Inter- transisthmian sister species pair* (**Inter-TSS pair**)
   - *Post- transisthmian sister species pair* (**Post-TSS pair**)

   Thus, these overall five terms should be sufficient to describe any time-depending pattern of TSS in respect to the Isthmus closure, without developing new and additional terminology that may increase confusion.

3. Only a part of the used TSS synonyms in the literature is believed to comprise the true meaning of the term *transisthmian sister species*. Thus, the three following criteria have to be met to consider terms as true synonyms:

A) Term must imply the connection to the emergence and closure of the Isthmus of Panama.

B) Term must clear define that species of interest originated from a common ancestor.

C) Term must include that species of interest occur on opposite sides of the Isthmus of Panama.

4. The terminology discussion and the introduction of new terms were important for several reasons. To decrease ambiguity and facilitate consistency in biological studies, which are concerned with TSS, it is necessary to avoid redundant terms. Moreover, the proper use of terms referring to the respective context between TSS and the Isthmus of Panama facilitate the understanding of a study. We should be aware that many terms, which are used as synonyms for TSS, are semantically distinct and should not be misused.

5. Perhaps, some readers disagree with the emphasized need to reduce the usage of terms regarding TSS and evaluate this discussion as excessive. However, I agree with Nelsen *et al.* (2014), who believed that improved and classified definitions "should make (these) terms more accessible to and better understood by both researchers and the general public" (p. 461).

11.1.2. Criteria of TSS pairs and -complexes

1. The five proposed criteria, which categorize species into TSS are only partly fulfilled by each studied TSS pair or -complex in this study:

   1) All TSS pairs and -complexes experienced speciation processes through geographic isolation, independent if the closure of the Isthmus itself was the driving force or not.

   2) Some of the studied TSS pairs and -complexes show overlaps in their estimated divergence times compare to the Isthmus of Panama. This might be a coincidence because several factors can cause or influence species divergences. The fact that the divergence time estimations show similar ages can result in misleading conclusions.

   3) Distributional ranges of recognized TSS are generally not restricted to the Isthmus region and its bordered countries. In fact, most TSS show a wide distributional pattern.

   4) Several studies show that morphological similarity can base on environmental adaptations and are forced by similar habitat structures. Therefore, a TSS determination which only bases on morphological characteristics is insufficient and may result in misleading TSS pair classifications. However, the representatives of the TSS pairs and -complexes in this study (except for TSS pair A and B of *Eurytium*) are morphologically similar.

   5) It is common that TSS pairs and -complexes show different divergence times. Different factors can play a role, e.g., the complex history of the Isthmus emergence and closure, or high dispersal capability of the species.

2. As discussed above, only a part of the five proposed criteria is fulfilled by the studied TSS pairs and -complexes. Nevertheless, they are suitable to conduct divergence time estimations because of several reasons. An external substation rate (Marino *et al.* 2011) is available for the studied TSS pairs and -complexes, which was estimated independent of the Isthmus closure. Moreover, the species are inhabitants of mangroves and the shallow water, which reflect the time of final closure best. Furthermore, several TSS pairs and -complexes could have been identified in most genera and a comprehensive dataset is available.
3. If studying TSS in context to the emergence and closure of the Isthmus of Panama, several factors may be helpful to choose suitable TSS pairs. Species with high dispersal ability (e.g., species with strong shell, operculum, sticky eggs, and high tolerance to freshwater) should be avoided. Likewise, species with confined distribution ranges should be preferred in contrast to cosmopolitan species. Furthermore, shallow water species should be used (e.g., mangrove associates, high intertidal species), because they reflect the time of final Isthmus closure best.
4. The five criteria, which define species as TSS have to be reconsidered. However, the establishment of a clear confined TSS concept is difficult to assess and it is not possible to develop a significant TSS identification key. Nevertheless, different factors should be considered and taken into account when identifying TSS:
    1) The barrier and the evolved TSS pairs and -complexes can be of different age.
    2) The distributional ranges of TSS pairs and -complexes are within the Atlantic and Pacific oceans, with a focus in the western Atlantic and eastern Pacific.
    3) Morphological characteristics should be used combined with molecular analyses.
    4) TSS pairs and -complexes within a genus can show different divergence ages.

11.2 Divergence time estimations of TSS

1. The following TSS pairs and -complexes of the four studied decapod genera *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus* have been identified due to phylogenetic analyses:

    - *Sesarma*: Two TSS complexes

    - TSS complex A: *S. rhizophorae* (EP) / *S. curacaoense* and *S. reticulatum* (WA).
    - TSS complex B: *S. crassipes* (WA) / *S. sulcatum* and *S. aequatoriale* (EP).

    - *Panopeus*: One TSS pair, or rather -complex (if polytomy of the tree is accepted)

    - TSS pair: *P. hartii* (WA) / *Panopeus* sp. (#16142; EP).
    - TSS complex: *P. hartii* (WA) / *Panopeus* spp. and *P. purpureus* (EP).

    Note that within the genus *Panopeus*, one western Atlantic species (*P. boekei*) and two eastern Pacific species (*P. convexus* and *P. diversus*) are missing in the phylogenetic study. Thus, TSS relationships may be different (and hence divergence time estimations) if missing species would have been included.

- *Eurytium*: Two TSS pairs

- TSS pair A: *E. limosum* (WA) / *Panopeus* spp. (EP).
- TSS pair B: *E. tristani* (EP) / *P. occidentalis* (WA).

Note that *Panopeus* spp. is unidentified, thus it may also belong to another, yet undescribed species of *Eurytium*. Due to a missing COI sequence of *P. occidentalis* (#19871) the species arrangement might be wrong and thus, *P. occidentalis* is probably not the true TSS of *E. tristani*.

- *Pachygrapsus*: One TSS pair

- TSS pair: *P. socius* (EP) / *P. transversus* (WA).

2. Divergence time estimations of the identified TSS pairs and -complexes resulted in none clear trend for either the Miocene or the Pliocene model. The Miocene model was supported by the upper bound of the 95% HPD interval of *Pachygrapsus* (13.54 Ma). The Pliocene model was supported by both TSS complexes of *Sesarma* (2.37–3.71 Ma; 3.27–5.56 Ma) and the TSS pair A of *Eurytium* (1.95–3.97 Ma). Re-openings and -closures (< 2.5 Ma) were supported by the lower bounds of TSS complex A of *Sesarma* (2.37 Ma) and TSS pair A of *Eurytium* (1.95 Ma). The TSS complex of *Panopeus* shows a young divergence time (0.29–0.7 Ma), which may point toward a recent dispersal event.
3. Divergence time estimations of TSS pairs and -complexes in this study are influenced by a number of parameters, which have to be considered when interpreting the results. For example, the datasets of this study suffer particularly from missing sequences. This may influence species relationships and, in the end, divergence times of TSS pairs and -complexes. Thus, achieved results should be interpreted with care, in particular for the TSS pair of *Panopeus* and the TSS pair B of *Eurytium* (see above). However, divergence times are also influenced by ancestral polymorphism. Due to an insufficient dataset, ancestral polymorphism for the specific genera could not have been estimated and thus, an average value is used, which was taken from the literature. Unfortunately, fossils for the here studied species were not available. They could have given additional evidence for the time of species divergence. Although an external molecular clock crustacean rate was applied, which bases on the Mediterranean Salinity Crisis, the substitution rate includes uncertainties a priori (e.g., imprecise calibration point, use of a relaxed clock).
4. When studying the geological processes of the Isthmus of Panama, including divergence time estimations of species, it is crucial to remember few factors. For example, the time of *final* Isthmus closure is not resolved and thus, the use of the Isthmus as calibration point comprises additional uncertainties for divergence time estimations. Therefore, the use of an external substitution rate, which is estimated independent of the time of Isthmus closure, should be favoured if possible. For divergence time estimations, a time interval (i.e. no defined time of closure, better upper and lower bounds) or a relaxed molecular clock should be chosen, if an external substitution rate is not available. Moreover, the emergence and closure of the Isthmus of Panama was a complex and long lasting geological event with probably several re-openings and -closures.

12 Outlook

The first part of this study focused on the transisthmian sister species (TSS) concept. The definition of the term *transisthmian sister species* is comprehensively discussed and the importance for a general and consistent terminology expressed. Whereas future studies may follow the suggested recommendations, which are mainly based on the time of TSS divergence, additional or more specific terms may be proposed with respect to, e.g., ecological or species-specific life history parameters. Additionally, future studies may focus on the five proposed operative criteria to classify species as TSS, which were not applicable for the here studied decapod species, and may develop practical principles for other species groups.

The second part of this study was concerned with divergence time estimations of the identified TSS pairs and -complexes of four decapod genera in order to highlight challenges of divergence time estimations of TSS. Additionally, the obtained divergence times were evaluated with respect to the controversially discussed 'common Pliocene model' and the 'new Miocene model'. This thesis pinpoints several factors that can influence the species arrangement of a phylogenetic tree and thus the divergence time estimations of TSS. Future studies may complement the dataset of this thesis with new fragments and additional sequences or species. Based on a comprehensive dataset statistical models can then be employed, which, e.g., relax the clock in divergence time estimations (Huelsenbeck *et al.* 2000), or estimate and compare ancestral TSS population sizes (Hickerson *et al.* 2003).

The divergence time estimations in this study were based on an external substitution rate, which was estimated from the Mediterranean Salinity Crisis, and do not conclusively reject either model of the Isthmus closure. However, the obtained divergence times and 16S substitution rates in this study correspond to the results found in the literature. In a next step, external substitution rates, which are inferred from other geological events (see Table 7-2), can be tested for their suitability to estimate divergence times of TSS. Moreover, this thesis was only concerned with mangrove and intertidal TSS pairs and -complexes of the four decapod genera. By using TSS pairs of additional taxa such as mollusks or fishes, which differ in e.g., their habitat preference (e.g., inhabitants of deep water or benthic organisms) the here presented results could be complemented and compared with respect to the inferred divergence times, in order to find further evidence for the temporal emergence and closure of the Isthmus of Panama.

13 Bibliography

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Part IV

Appendix

A1 Materials and Methods

A1.1 Materials

A1.1.1 Sources of animal tissues

This study is based on in alcohol-preserved voucher specimens of the four decapod genera *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus*, which are on loan with courtesy of the Senckenberg Museum Frankfurt, Germany, from the collections of PD Dr. Schubart from the University of Regensburg as well as on sequences, obtained from the National Center for Biotechnology Information (NCBI). Photos, a detailed description, and information about the obtained sequences are listed for most analyzed specimens in Appendices A2 and A3.

Although specimens of the phylum Mollusca were collected on a field trip to the western Atlantic and eastern Pacific coasts of Panama (permit: Resolución DGOMI-PEFC N°17), only the voucher decapod specimens were analyzed in this study. However, the field trip took place in March 2012 in cooperation with Dr. Aaron O'Dea from the Smithsonian Tropical Research Institute (STRI). Field trips were undertaken to mangroves, shallow coastal waters, and flat coral reefs near Veracruz and the Naos Marine Laboratory (STRI), (eastern Pacific coast), as well as to the Galeta Marine Laboratory (STRI) and Colón at the western Atlantic coast. Mollusk samples were conserved in 100% ethanol (EtOH), exported to Germany, and stored at the University Giessen Systematics and Biodiversity collection (UGSB).

A1.1.2 Chemicals

Chemicals used in this study. Manufacturer, location of principal office, and country are in brackets.

- **Agarose** (Lonza, Kaiserslautern, GER)
- **Bovine Serum Albumin (BSA)** (New England Biolabs GmbH, Frankfurt a.M., GER)
- **Cetyl Trimethyl Ammonium Bromide (CTAB)** (Carl Roth GmbH + Co. KG, Karlsruhe, GER)
- **Chloroform** (Carl Roth GmbH + Co. KG, Karlsruhe, GER)
- **Double distilled water (ddH_2O)** (Carl Roth GmbH + Co. KG, Karlsruhe, GER)
- **Ethylene-diamine-tetraacetic acid (EDTA)** (Carl Roth GmbH + Co. KG, Karlsruhe, GER)
- **Ethanol (EtOH)** (Carl Roth GmbH + Co. KG, Karlsruhe, GER)
- **Magnesium chloride** 2.5 mM **($MgCl_2$)** (Carl Roth GmbH + Co. KG, Karlsruhe, GER)

A1.1.3 Solutions

All solutions were prepared using ddH_2O and stored in gas-tight bottles or screw cap tubes. Stock solutions were diluted according manufactures instructions or protocols. When solutions were not self-prepared manufacturers names are add in brackets.

- **Agarose gel**, 1%
  - 1 g Agarose
  - 100 ml 0.5x TBE buffer
  - 10 µl 0.5 µg/µl GelRed™

- **CTAB precipitation buffer** (Cetyl Trimethyl Ammonium Bromide)
  - 1% CTAB
  - 0.05 M Tris base
  - 0. 01 M EDTA
    add ddH_2O up to 100 ml
- **CTAB/NaCl solution**, 5%
  - 5% CTAB
  - 0. 5 M NaCl
    add ddH_2O up to 100 ml
- **Exchange buffer**, pH 8.0
  - 20 mM Tris base
  - 0. 1 M EDTA
    add ddH_2O up to 100 ml
- **DNA Marker Phi X 174 DNA – HaeIII Digest**, 1 µg/ml (New England Biolabs GmbH)
- **dNTP solution** 20 mM (Promega)
  - 200 µl 100 mM dATP (20 mM)
  - 200 µl 100 mM dCTP (20 mM)
  - 200 µl 100 mM dGTP (20 mM)
  - 200 µl 100 mM dTTP (20 mM)
    add 9.2 ml ddH_20
- **EDTA** (Ethylene-diamine-tetraacetic acid), 0.2 M (Carl Roth GmbH + Co. KG)
  - 7.44 g Diaminoethane-tetraacetic acid
    add ddH_2O up to 100 ml
- **GelRed™** (Biotium Inc.)
- **Loading Dye**
  - 95 ml 95% Formamid deion
  - 3.72 g 10 mM EDTA
  - 0.01 g Bromphenolblue
- **NaCl solution**, 5 M
  - 29.22 g 5 M NaCl
  - 100 ml ddH_2O
- **NaCl in 1xTE**, 1 M
  - 20 ml 5 M NaCl
  - 10 ml 10x TE
    add 70 ml ddH_2O up to 100 ml
- **TE buffer**, 10x
  - 10 ml 1 M Tris pH 8.0
  - 5 ml 0.2 M EDTA
    add 85 ml ddH_2O up to 100 ml
- **TMAC** (Tetramethylammonium chloride), 0.5 M (Carl Roth GmbH + Co. KG)
  - 0. 548 g TMAC
  - 10 ml ddH_2O

- **TBE buffer**, 5x
  - 54 g Tris base
  - 27 g boracic acid
  - 3.73 g 0.2 M EDTA pH 8.3
    add ddH_2O up to 1000 ml
- **Thermopol reaction buffer,** 10x (New England Biolabs GmbH)
  - 200 mM Tris-HCl
  - 100 mM $(NH_4)_2SO_4$
  - 100 mM KCl
  - 20 mM $MgSO_4$
  - 1% Triton X-100
  - pH 8.8
- **Turner lyses buffer**, pH 8.0
  - 20 mM Tris base (TRIZMA)
  - 0.1 M EDTA
  - 0.5% SDA (Sarkosyl)
    add ddH_2O up to 1000 ml

A1.1.4 Enzymes

Enzymes used in this study are listed below. Manufacturer, city, and country are given in brackets. For more information about buffers and sources of the enzymes see manufacturer protocols.

- **Proteinase K** (New England Biolabs GmbH, Frankfurt a.M., GER)
- **Taq DNA polymerase** (New England Biolabs GmbH, Frankfurt a.M., GER)

A1.1.5 Consumable material

The consumable material used in this study is listed below. The manufacturer, city, and country are given in brackets.

- **Collecting tubes**
  - 2 ml microtubes (Sarstedt, Nümbrecht, GER)
  - 7 ml tube with white cap (Sarstedt, Adelaide, AUS)
  - 30 ml Nalgene wide-mouth jars, translucent (MAGV GmbH, Rabenau-Londorf, GER)
  - 50 ml centrifuge tubes (VWR International GmbH, Darmstadt, GER)
  - 60 ml Nalgene polypropylene wide-mouth jars, translucent (MAGV GmbH, Rabenau-Londorf, GER)
  - 250 ml Nalgene polypropylene wide-mouth jars, translucent (MAGV GmbH, Rabenau-Londorf, GER)
- **Gloves**
  - Nitrile (Meditrade, Kiefersfelden, GER)
  - Latex (Meditrade, Kiefersfelden, GER)
- **Modeling clay**, black (to adjust specimens under the digital microscope)
- **Parafilm®** (Carl Roth GmbH + Co. KG, Karlsruhe, GER)

- **Pipet tips** (Starlab, Hamburg, GER)
  - 0.1 – 10 µl
  - 0.1 – 20 µl
  - 10 – 100 µl
  - 50 – 200 µl
  - 1000 µl
- **Reaction tubes**
  - 0.2 ml PCR reaction tubes (Peqlab Biotechnologie GmbH, Erlangen, GER)
  - 0.5 ml reaction tubes (MAGV GmbH, Rabenau-Londorf,)
  - 0.5 ml safe lock reaction tubes (MAGV GmbH, Rabenau-Londorf,)
  - 1.5 ml reaction tubes (MAGV GmbH, Rabenau-Londorf,)
- **Sand**, black (to adjust specimens under the digital microscope)

A1.1.6 Lab equipment

The following lab equipment was used to conduct molecular analyses. The manufacturer, city, and country are given in brackets.

- **Centrifuges**
  - Biofuge pico (Thermo Fisher Scientific Inc., Waltham, USA)
  - Eppendorf centrifuge 5415 D (Eppendorf AG, Hamburg, GER)
- **Digital Camara** (Canon Deutschland GmbH, Krefeld, GER)
  - Powershot A70
- **Gel chamber and slides** (Life Technologies, Grand Island, NY, USA)
  - Horizon 58, Gibco BRL Horizontal Gel Electrophoresis Apparatus
- **Keyence** (Keyence Deutschland GmbH, Neu-Isenburg, GER)
  - Digital microscope VHX 2000
- **NanoDrop™** (Thermo Fisher Scientific Inc., Waltham, USA)
  - 2000 UV-Vis spectrophotometer
- **Pipettes** – Reference, Multipette plus (Eppendorf AG, Hamburg, GER)
  - 0.1 – 2.5 µl (Reference)
  - 0.5 – 10 µl (Multipette plus)
  - 0.5 – 10 µl (Reference)
  - 2 – 20 µl (Reference)
  - 10 – 100 µl (Reference)
  - 50 – 200 µl (Reference)
  - 100 – 1000 µl (Reference)
- **Power supplies** (Consort Group, Ottawa, USA)
  - Electrophoresis Power Supply E 835 / E802
- **Scale** (Kern & Sohn GmbH, Balingen-Frommern, GER)
  - Kern ABS
- **Shaker and heating block** (Eppendorf AG, Hamburg, GER)
  - Thermomixer comfort

- **Thermocycler** (Eppendorf AG, Hamburg, GER)
  - Mastercyler pro S
- **UV Illuminator** (Biometra, Göttingen, GER)
  - TI 1
- **Vortex** (IKA® –Werke GmbH & Co. KG, Staufen, GER)
  - MS2 Minishaker

A1.1.7 Oligonucleotides

Oligonucleotides (primers) and their target genes used in this study are listed in Table A1-1. All primers were synthesized by Metabion GmbH (Planegg/Steinkirchen, GER). Slightly modifications of the COI primers COL6, COR722b and COH1b are based on the oligonucleotide sequences LCO1490 and HCO2198 published by Folmer *et al.* (1994), and the primer COIa from Palumbi *et al.* (1991), respectively. The primer sequences of COL6, COH1b, COL8, and COH16 for the COI gene were all modified by Schubart (2009) in respect to molecular analyses of crustaceans. The used primer COR722b for the COI gene was modified by Wilke & Davis (2000). The specific primer pair 16L2/16HLeu for the 16S gene in crustacean analyses was designed by Schubart *et al.* (2002) and by Schubart (2009), respectively.

Table A1-1: Oligonucleotides used for molecular analysis.

Primer Pair	Direction	Sequence 5' → 3'	Gene	Attached Primer Position	Expected Fragment Length (bp)	T_M (°C)
COL6	forward	TYTCHACAAAYCATAAAGAYATYGG	COI	17	658	60
COR722b	reverse	TAAACTTCAGGGTGACCAAAAAATYA		700		61
COL6	forward	TYTCHACAAAYCATAAAGAYATYGG	COI	17	1276	60
COH1b	reverse	TGTATARGCRTCTGGRTARTC		1318		57
COL8	forward	GAYCAAATACCTTTATTTGT	COI	529	955	49
COH16	reverse	CATYWTTCTGCCATTTTAGA		1504		51
16L2	forward	TGCCTGTTTATCAAAAACAT	16S	638	650	50
16HLeu	reverse	CATATTATCTGCCAAAATAG		1308		50

Abridgment of the IUB code for mixed base sites: N = G, A, T, C; H = A, T, C; W = A, T; R = A, G; Y = C, T.

A1.1.8 Computer programs

Programs used for data-, graphic-, sequence- and phylogenetic-analyses are listed below in alphabetic order. The function of the respective program is given in brackets.

- **Bio Edit** v7.1.3.0, Hall (1999) (sequence alignment)
- **BEAST** v1.7.5, Drummond *et al.* (2012) (Bayesian evolutionary analysis)
  - BEAUti (creating BEAST input files)
  - StarBEAST (*Beast), Heled & Drummond (2010) (phylogenetic analysis)
  - TreeAnnotator (summarizing the information in a sample of trees)
- **DAMBE** v5.3.64, Xia (2013) (test for substitutional saturation)
- **FigTree** v1.3.1, Rambaut (2009) (viewing trees and sum up information produced by TreeAnnotator)
- **Gimp** v2.8.4, 2014 (image processing program)
- **Inkscape** v2, 1991 (image processing program)
- **jModeltest** v0.1.1, Posada (2008) (estimating evolution models)
- **Keyence Software** (Digital Imaging Processing System)
- **MEGA** v5.1, Tamura *et al.* (2011) (sequence alignment)
- **Microsoft Office Excel,** 2007 (spreadsheet program, data sorting and handling)
- **NanoDrop 2000 Software** (concentration measurement program)
- **RAxML** v7.7.1 Stamatakis *et al.* (2008) (Maximum-likelihood analysis)
- **Remote Capture** (Digital Imaging Processing System)
- **Tracer** v1.5, Rambaut & Drummond (2009) (analyzing results from Bayesian analyses)

A1.2 Methods of molecular biology

A1.2.1 DNA extraction from crustacean tissue

The basic principle of a molecular phylogenetic analysis is the efficient extraction of DNA. DNA extraction followed a slightly modified protocol for DNA isolation of mollusks (Wilke *et al.* 2006), including a CTAB-based purification step as established by Doyle & Doyle (1987). CTAB (Cetyl trimethylammonium bromide) acts as a detergent, separating the DNA from remaining proteins and polysaccharides, which would inhibit the subsequent enzyme reactions. CTAB binds to the DNA and forms a CTAB/nucleic acid complex under low-salt conditions (< 0.6 M NaCl) but interact with proteins and polysaccharides when salt conditions are high (> 0.7 M NaCl) facilitating DNA isolation. Muscle tissue of a walking leg (pereiopod) of ethanol-preserved decapod specimens was carefully removed with a scalpel and tweezers (Figure A1-1). In this step it was important to avoid contamination due to pieces of the crustacean's exoskeleton, which could inhibit the isolation, DNA amplification or sequencing procedures.

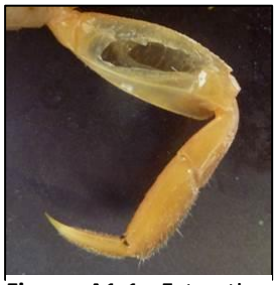


Figure A1-1: Extraction of muscle tissue from a walking leg.

The DNA isolation contains of the following steps:

Tissue preparation (extraction of the alcohol)

1. Drop extracted muscle tissue in 0.5 ml reaction tube, filled with 300 µl exchange buffer. Soak for 5-10 minutes.

Denature of proteins

2. Transfer tissue into fresh 0.5 µl reaction tube, filled with 200 µl Turner lysis buffer + 3 µl Proteinase K (20 µg/µl).
3. Incubate in water bath at 55 °C for at least 3 hours.

Dissolving the proteins

4. Spin up to 8000 rounds per minute (rpm), stop immediately.
5. Add 35 µl 5 M NaCl + 35 µl 5% CTAB/NaCl solution. Mix gently by hand.
6. Add 270 µl chloroform (under the flue; chloroform denatures the contained proteins). Mix gently by hand for 2-3 minutes.
7. Spin for 5 minutes at 9000 rpm (phase separation).
8. Transfer aqueous phase (upper phase) into new reaction tube.
9. Add 270 µl CTAB precipitation buffer. Mix gently by hand for 1 minute.
10. Precipitate at room temperature for 45 minutes.

Precipitation and washing of the DNA

11. Spin for 15 minutes at 12000 rpm. Discard supernatant.
12. Re-suspend pellet in 100 µl 1 M NaCl/TE + 1 µl RNase (10 mg/ml).
13. Incubate at 65 °C for 5-10 minutes.
14. *Ethanol precipitation*: add 250 µl ice-cold 100% EtOH. Mix gently by hand.
15. Precipitate at -20 °C for at least 3 hours.
16. *Washing of the DNA*: Spin for 5 minutes at 12000 rpm. Discard supernatant.
17. Re-suspend pellet in 300 µl ice-cold 70% EtOH.
18. Spin for 5 minutes at 12000 rpm. Discard supernatant.
19. Repeat the washing treatment (steps 17 and 18). Discard remaining ethanol with a pipette.
20. Air dry DNA pellet for ~10 minutes at room temperature.
21. Re-suspend DNA pellet in 30 µl ddH_2O.

DNA concentrations were measured with a NanoDrop™ 2000 (Subchapter A1.2.2). The DNA was stored at -20 °C for further applications.

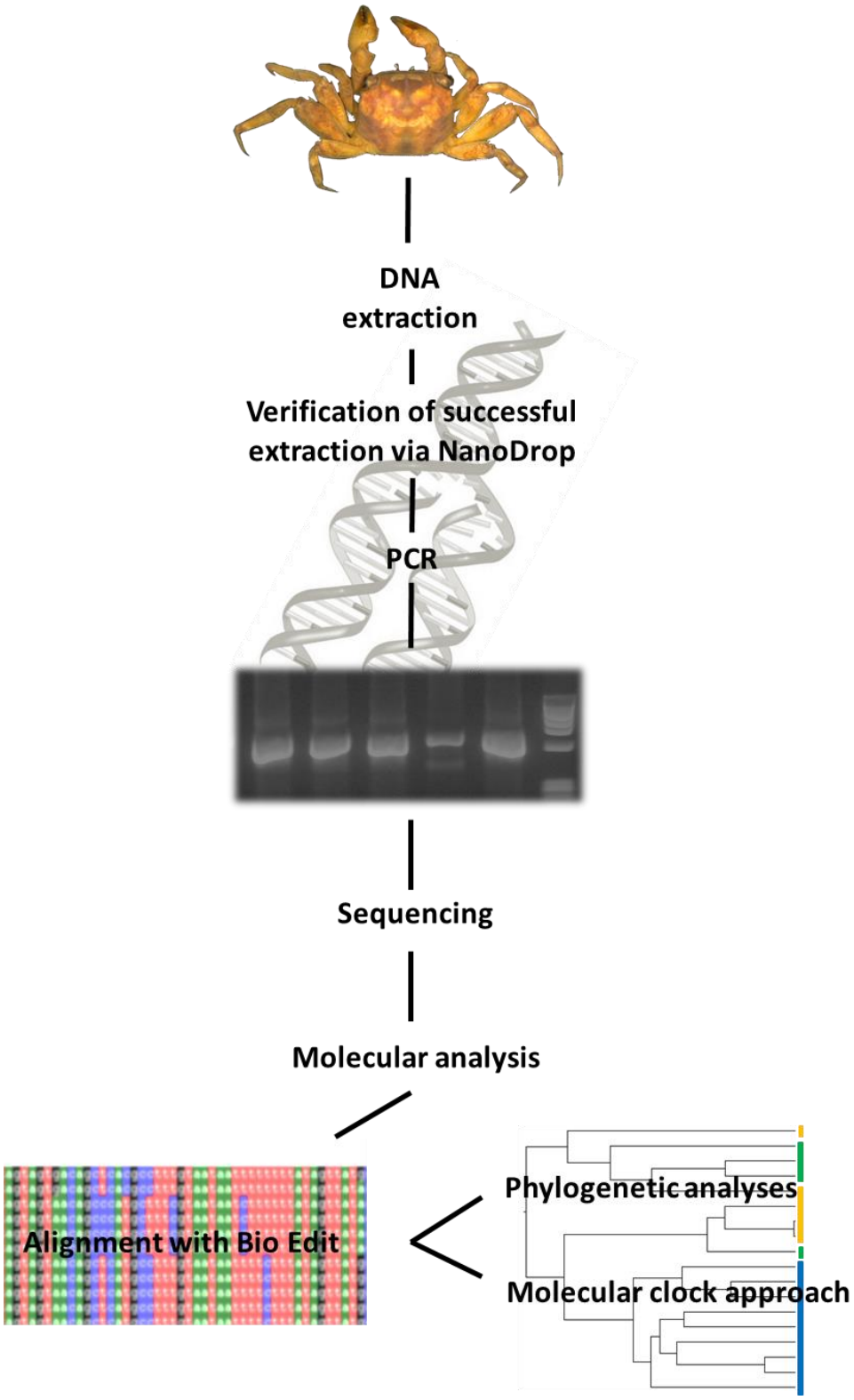


Figure A1-2: Overview of methods used in this study. DNA was extracted from a walking leg of crustacean specimen and the yielded DNA concentration was measured with a NanoDropTM 2000. Certain gene fragments were amplified via polymerase chain reaction, checked by agarose gel electrophoresis and directly sequenced (see text for detailed description of the methods).

A1.2.2 NanoDrop™ 2000

The NanoDrop™ 2000 UV-Vis spectrophotometer (in the following refers to *NanoDrop*) is a common tool in molecular biology to quantify the purity of isolated DNA by measuring the ratio of ultraviolet light (UV) absorbance of nucleic acids at 260 nm. The ratio of the absorbance at 260 and 280 nm ($A_{260/280}$) is then used as an indicator for the purity of the nucleic acid sample (Gallagher & Desjardins 2007). For highly purified DNA, the $A_{260/280}$ is around 1.8. However, lower $A_{260/280}$ ratios can indicate that the DNA is contaminated by proteins or other substances from the isolation procedure, which absorb light at 280 nm. Insufficient attention during the measurement process (e.g. not properly cleaned up surfaces of the NanoDrop) can also affect the sample accuracy and purity.

Based on the Lambert-Beer Law $A = \varepsilon c l$ (this law relates the amount of absorbed light to the concentration of the substance through which the light is moving), where A is the absorbance at a particular wavelength, ε is the extinction coefficient, c is the concentration of the DNA, and l is the path length (i.e. the gap between two optical surfaces; Gallagher & Desjardins 2007) the NanoDrop software automatically calculates the DNA concentration of the measured sample:

$$\mathrm{c}\ (\mu\mathrm{g/ml}) = \frac{\mathrm{A}_{260}}{0.020}$$

The above equation assumes 1 mm and 0.2 mm paths. The extinction coefficient ε represents here the specific absorption coefficient at a wavelength of 260 nm and has units of $(\mu g/ml)^{-1}cm^{-1}$. The value of ε for double-strand DNA (dsDNA) is 0.020 $(\mu g/ml)^{-1}cm^{-1}$. Thus, using these variables, an A260 of 1.0 indicates 50 µg/ml dsDNA (Gallagher and Desjardins 2007). In this study the NanoDrop measurement was used to verify the success of the performed DNA isolation and to determine the adequate volume of DNA template used in the subsequent polymerase chain reactions (Subchapter A1.2.3). Following the manufactures protocol:

Measurement preparation

1. Open the NanoDrop software and select the “nucleic acids” module.

Measurement adjustment

2. Perform a blank measurement. Load 1 µl of the substance in which sample is dissolved (in this study: ddH_2O) onto the pedestal, close the arm and select the “blank” button on the screen (the result should be zero).
3. Clean both optical surfaces with a common laboratory paper.

Measurement of sample

4. Pipette 1 µl of the sample onto the pedestal.
5. Close the arm of the NanoDrop and select the “measure” button on the screen.
6. The pedestal automatically adjusts the sample at both a 0.2 mm and 1 mm path length.
7. Clean both optical surfaces with a common laboratory paper.
8. Measurements and calculations are automatically transferred into an excel-sheet.

A1.2.3 Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is a commonly used method to manifold a short, well-defined part of a DNA strand (i.e. amplification of a specific gene fragment) in vitro (Saiki *et al.* 1988). This technique was performed to amplify fragments of two mitochondrial markers (mtDNA), cytochrome c oxidase subunit I (COI) and the 16S rRNA (16S). These two mtDNA fragments have high mutation rates and are extensively used in evolutionary studies on freshwater and marine crabs (e.g., Harrison 2004; Knowlton & Weigt 1998; Lessios 2008; Morrison *et al.* 2004; Schubart *et al.* 1998). The basic principle of a PCR is based on a recurring cycle of three steps – denaturation, annealing, and elongation (Table A1-3):

Denaturation: The PCR-solution is heated up to 95 °C during the main cycles. Thus, the complementary strands of the template DNA denature into two single strands (ssDNA).

Annealing: Due to a primer-depending temperature reduction, the primers bond complementary to the DNA. This is the initial location where the polymerase starts to attach further complementary nucleotides.

Elongation: The temperature increases to 72 °C (or accordant to the temperature optimum of the used polymerase). The polymerase attaches further nucleotides to the respective ssDNA until an exact double-strand replica of the template DNA is formed. Regions of nucleotides and DNA, which are not entirely complementary break off.

Due to the replication of these three steps, the number of copied DNA-molecules increases exponentially. The cycle (denaturation, annealing, and elongation) is repeated between 30 and 40 times. The duration of the single steps depends on the length of the expected gene fragment.
For all enzymatic reactions in this study the *Thermus aquaticus* DNA-polymerase (taq-polymerase) was used. The taq-polymerase was first isolated from a heat stable bacteria-strain, inhabiting 70 °C hot springs (Chien *et al.* 1976). This DNA-polymerase shows an optimal enzymatic activity at 72 °C and a synthesis rate (i.e. extension rate; the DNA polymerase assembles the four deoxynucleotides, dATP, dCTP, dGTP, and dTTP, into a complementary polynucleotide chain in 5’ to 3’ direction) of about 60 nucleotides/second (Innis *et al.* 1988; Saiki *et al.* 1988). Despite missing a 3’ to 5’ proofreading exonuclease, the taq-polymerase performs highly accurate DNA synthesis in vitro. During a single cycle of DNA replication, the error rate for base substitutions is 1/9 000 polymerized nucleotides and frameshift errors occur at a frequency of 1/41 000 (Tindall & Kunkel 1988). In general, the fidelity of DNA synthesis depends on the used dNTPs and $MgCl_2$ concentration as well as the pH (Eckert & Kunkel 1990). To yield synthesized DNA of sufficient quality, the authors suggest utilizing low concentrations of dNTPs and $MgCl_2$.

For all simultaneous PCR reactions a standard *mastermix* was prepared on ice, including all required reagents (except for the DNA template). Amongst others, tetramethylammonium chloride (TMAC) is a salt, which acts as a catalyzer for the PCR reaction, and the availability of bovine serum albumin (BSA) leads to stabilization and higher activity patterns of the enzymes. Table A1-2 shows the reagents and their respective volumes of the mastermix, calculated for one PCR reaction tube with a total volume of 20 µl.

Table A1-2: Reagents of a standard mastermix for a 20 µl PCR.

Reagent	**Volume (µl)**
10x Thermopol buffer	2
$MgCl_2$	1.4
dNTPs	1.4
Primer 1 forward	1.4
Primer 2 reverse	1.4
ddH_2O	10.6
TMAC (0.5 M)	0.2
BSA (10 mg/ml)	1.2
Taq-polymerase	0.4
Total reaction volume	20.0

Always 20 µl of the mastermix were pipetted into a 2 µl PCR reaction tube (conducted on ice). In general, 3 µl of the respective DNA template was added to each PCR reaction tube. To control for contamination, a negative control was prepared for every conducted PCR. Therefore, 3 µl ddH_20 was added instead of the DNA template. The PCR settings base on the length of the expected fragment, the optimum temperature of the polymerase, and the melting temperature of the used oligonucleotides. An overview of the used PCR conditions is listed in Table A1-3. To verify the success of the PCR, a small volume of each PCR sample was pipetted on a 1% Agarose gel (Subchapter A1.2.4).

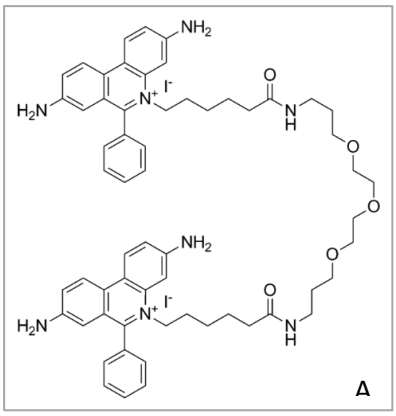
Table A1-3: PCR programs used in this study for the COI and 16S gene fragments. Identical initial step and final cycle were performed for all PCR reactions.

Initial Step	**Denaturation** 94 °C for 240 s				
Main Cycles					
Primer Pair	**Fragment Length (bp)**	**Gene**	**Denaturation**	**Annealing**	**Elongation**
			Temperature and Duration		
COL6 COR722b	658	COI	95 °C for 45 s	50 °C for 45 s	72 °C for 75 s
COL6 COH1b	1276	COI	95 °C for 45 s	50 °C for 45 s	72 °C for 75 s
COL8 COH16	955	COI	95 °C for 45 s	50 °C for 45 s	72 °C for 75 s
16L2 16HLeu	650	16S	95 °C for 45 s	48.0 °C for 60 s	72 °C for 60 s
Replication of 40 cycles for each PCR reaction					
Final step	**Elongation** 72 °C for 300 s				
Holding temperature at 10 °C					

PCR programs used in this study for the COI and 16S gene fragments. Identical initial step and final cycle were performed for all PCR reactions; s = seconds.

A1.2.4 Agarose gel electrophoresis

The agarose gel electrophoresis is a method to separate nucleic acids by size (Aaij & Borst 1972). The separation is performed by using an agarose matrix, consisting of pores in different sizes. These pores act like a filter and determine the migration of the negatively charged nucleic acid molecules through the agarose matrix via an electric field. The negative charged DNA moves from the cathode (-) to the anode (+). Thereby, the migration of the DNA molecules is reciprocally proportional to the logarithm of their fragment length (i.e. short DNA molecules move faster and migrate further than large ones; Helling *et al.* 1974). The quality of fragment separation is based on their length and the concentration of the agarose. Because the pore size (and hence the density of the agarose matrix) correlates to the agarose concentration, higher concentrations will lead to a more efficient separation of small molecules.



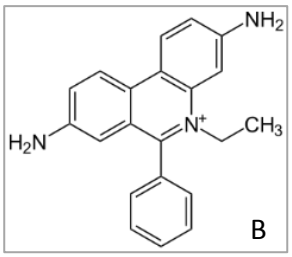


Figure A1-3: GelRed (A) is structurally closely related to the highly controversially discussed ethidium bromide (B). It consists of two ethidium subunits, bridged by a linear spacer. Both reagents interact with the nucleic acids and fluorescent when exposed to UV light.

In this study, the lengths of the amplified fragments are between 490bp and 1300bp (see Chapter A2). To verify the success of the PCR, 1% agarose gels were prepared to separate the amplified PCR fragments. A DNA marker was applied as size reference. Commonly, nucleic acids are enriched with ethidium bromide (EtBr) to detect the fragments in the agarose matrix under ultraviolet (UV) light. Instead of EtBr, which is discussed controversial regarding its toxicity (e.g., Karib *et al.* 1954; Murilla *et al.* 2002; Quillardet & Hofnung 1988; Saeidnia & Abdollahi 2013), the agarose gel in this study was enriched with the intercalating fluorescent nucleic acid agent *GelRed*, which also binds to the nucleic acids (Figure A1-3). When exposed to UV light, it will fluoresce with a red-orange color. The DNA in the agarose gels was stained by adding 10 µl GelRed per 100 ml agarose gel. The gel chamber was filled with an adequate volume of 5x TE buffer. 0.5 µl running buffer (6x loading dye solution) was added to each 3 µl DNA sample (as well as to the negative control). Each template solution and 3 µl of DNA marker (0.5 µg/µl, DNA ladder, 100bp) were loaded into a separate well of the gel. Electrical current (~120 mV) was applied for approximately 30 minutes. The gel was exposed to UV light, pictures were taken with a digital camera and analyzed.

A1.2.5 DNA sequencing

The aim of DNA sequencing is to determine the nucleotide order of a given DNA fragment. In this study, DNA sequencing is based on the chain-termination method ('Sanger sequencing'), which was developed by Sanger *et al.* in 1977. This technique is based on sequence extensions of ssDNA templates and forced chain termination by specific nucleotides. These dideoxynucleotides (ddNTPs) are modified deoxynucleotides (dNTPs) which lack a 3'–OH group (marked in red in Figure A1-4). The general idea of Sanger sequencing is based on this missing hydroxyl group (–OH), which is essential for the phosphodiester bond between two nucleotides in DNA elongation. Due to this missing link, the DNA-polymerase aborts the DNA synthesis when a ddNTP is embedded.

DNA sequencing is performed in four independent reactions, which only differ in their dNTP/ddNTP composition. Thus, for each reaction DNA-polymerase, primers (which are the same as in the PCR reaction), and three of the four 'normal' dNTPs (dATP, dCTP, dGTP, and dTTP) are mixed. Additionally, the respective missing nucleotide is added in form of a fluorescently labeled ddNTP (ddATP, ddCTP, ddGTP, or ddTTP). All four reaction samples are simultaneously

electrophoresed in parallel lanes and the DNA fragments are separated by their size. The DNA sequence can then, for example, be directly read of the autoradiogram of the sequencing gel.

DNA sequencing was performed on an ABI 3730xl DNA analyzer (Life Technologies) using the Big Dye Terminator Kit (Life Technologies) by the company LGC Genomics GmbH (Berlin, Germany). Each fragment was sequenced in forward and reverse direction. DNA samples, the according primer pairs and the ordering form had to be prepared in advance. Therefore, around 20 µl of each DNA sample was pipetted into a well of a 96-well plate and 10 µl of the relevant primer's stock solution (100 µM/µl) was provided. The online order was filled out following the companies' instruction. DNA purification with EXO/SAP was carried out by LGC Genomics. The samples were picked up by a delivery service at Giessen University. The generated sequences were downloaded from the company's online order system (*www.lgcgenomics.com*) and saved for further analyses.

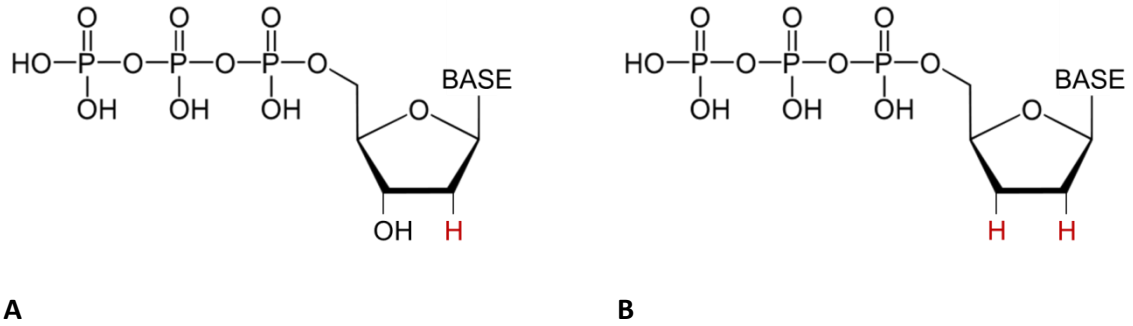


Figure A1-4: A) Deoxynucleotide (dNTP) and B) Dideoxynucleotide (ddNTP) used in Sanger sequencing. Due to the missing 3'-hydroxyl group (–OH) in the dideoxynucleotide (marked in red), the DNA-polymerase ceases the DNA synthesis when a ddNTP is incorporated in the growing polynucleotide chain.

A1.2.6 DNA alignments

Forward and reverse sequences of each specimen were aligned using the *Clustal W* algorithm implemented in the program Bio Edit (Hall 1999), chromatograms and alignments were checked and corrected by eye and saved as consensus sequence. One single alignment (consisting of the consensus sequences) for each gene fragment and genus was purpose-built and saved. The files were edited by hand in MEGA (Tamura *et al.* 2011) to create a uniform matrix block (i.e. all sequences have to have the same length) for the subsequent Bayesian analysis. The abbreviations used for the sequence names based on the first letter of the genus name and the first two letters of the species name, followed by the UGSB (collection number) or the prep. # (preparation number) code, e.g., Srh11660 = *Sesarma rhizophorae*, UGSB collection number: 11660; Srh19318 = *Sesarma rhizophorae*, preparation number: 19318.

A1.2.7 Phylogenetic analysis

For phylogenetic analyses, a data set of 32 specimens (1-6 representatives) of 18 species of the mangrove crab genus *Sesarma* from both sides of the Isthmus of Panama was analyzed (Tables 10-5 and A2-1). A single analysis of the genera *Panopeus* and *Eurytium* was performed, which based on 78 specimens (1-8 representatives) of 14 species of the mud crab genus *Panopeus* and

14 specimens (1-8 representatives) of 4 species of the mud crab genus *Eurytium* (Tables 10-5, A2.2, A2.3). The Panopeidae dataset composition in this study bases on the comprehensive phylogenetic analysis of Xanthoidea by Thoma *et al.* (2014) and was complemented with own species and sequences (Tables A2.2 and A2.3; Figures 10-3 and 10-4). Because of missing sequences, the phylogenetic topology of the crab genus *Pachygrapsus* was taken from Ip *et al.* (2015). Their analysis included 11 specimens (1 representative each) of 11 species (Table 10-5).

Final sequence lengths of the COI and 16S alignments were 1 136bp/642bp for *Sesarma* and 1 184bp/638bp for *Panopeus*/*Eurytium* (Table 10-5). A 12bp region (position 365-376) of the 16S *Sesarma* alignment was excluded prior to the phylogenetic analyses due to alignments ambiguities. This procedure is commonly performed in phylogenetic analysis for short ambiguous regions of the 16S gene (e.g., Harrison 2004; Schubart *et al.* 2000b; Stillman & Reeb 2001). No regions of ambiguity in the COI alignment and no occurrence of pseudogenes (translocated copies of the 16S gene in the nuclear genome; Schubart *et al.* 2000b) were observed in any of the studied datasets. The topology of *Pachygrapsus* was taken from the phylogenetic analysis of Ip *et al.* (2015). Their analysis bases on five genes with a total alignment length of 2 247bp. The optimal model of DNA sequence evolution was selected under the Akaike Information Criterion (AIC; Posada & Crandall 1998) implemented in the program jModeltest v0.1.1 (Posada 2008; COI: TrN+I+G, GTR+G, GTR+G; 16S: TrN+G, GTR+G, GTR+G for the species *Sesarma*, *Panopeus*, and *Eurytium*, respectively). The subsequent test for substitutional saturation (Xia *et al.* 2003) performed for all COI datasets using the program DAMBE v5.3.64 (Xia 2013), revealed no substantial saturation.

Phylogenetic analyses were performed differently for each family. For *Sesarma*, a Bayesian Inference (BI) phylogenetic analysis in BEAST v1.7.5 (uncorrelated lognormal relaxed clock; speciation yule process; 50 million generations (Ngen), log every 1000 tree (log)) was performed (Table A1-4). Maximum-likelihood (ML) analysis for the Panopeidae dataset was carried out using RAxML 7.7.1 (Stamatakis *et al.* 2008; 1000 bootstrap runs; Table A1-4). The best scoring tree was selected. Convergence of parameters and their effective sample size (ESS > 200) were confirmed in Tracer v1.5 (Rambaut & Drummond 2009) for each analysis. For the *Sesarma* dataset, 5000 trees were discarded as burn-in and a maximum clade credibility tree was computed with TreeAnnotator v1.7.5. (Drummond *et al.* 2012). All phylogenetic trees were visualized with FigTree v1.3.1 (Rambaut 2009). For *Pachygrapsus*, Ip *et al.* (2015) conducted ML (RAxML) and BI (MrBayes) analyses of the grapsid crabs based on five genes. Both of their analyses yielded the same topology, which is shown in Figure 10-9.

A1.2.8 Divergence time estimations

Divergence time estimations based on TSS pairs and -complexes identified from the phylogenetic analyses, which were performed before (see Subchapter A1.2.7). Data sets for divergence time estimations contain 32 specimens of 18 species of the mangrove crab genus *Sesarma*, 10 specimens of 3 relevant species of the mud crab genus *Panopeus,* 22 specimens of 6 relevant species of the genus *Eurytium*, and 8 specimens of 4 relevant species of the crab genus *Pachygrapsus* (Table 10-5). Final sequence lengths of the COI and 16S alignments were

1 136bp/642bp for *Sesarma*, 1 184bp/638bp for *Panopeus*, 555bp/638bp for *Eurytium*, and 687bp of the COI fragment for *Pachygrapsus* (Table 10-5). To avoid rates that involve calibrations based on the Isthmus closure, the marine crustacean rate for COI (0.98% My^{-1}) of Marino *et al.* (2011) was applied. This substitution rate bases on the Mediterranean Salinity Crises (5.59 Ma, upper bound where the isolation from the Atlantic was established; Krijgsman *et al.* 1999), under the HKY+G model. The substitution rate for 16S rRNA was independently calculated by the used program.

The optimal model of DNA sequence evolution was selected under the Akaike Information Criterion (AIC; Posada & Crandall 1998) as well as under the Bayesian Information Criterion (BIC; Schwarz 1978) implemented in the program jModeltest v0.1.1 (Posada 2008; COI: HKY+G; 16S: HKY+G, for all species). In order to decide whether the use of a strict clock is appropriate for the data, the COI coefficient of variation (COV) was analyzed. COV values close to zero denote a clock-like evolution among lineages, whereas larger values indicate a higher variation of rates among branches. The COV values of the uncorrelated lognormal relaxed clock were slightly different than zero for *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus* (COV = 0.0592, 95% HPD: 0.0374-0.0809; 0.194, 0.0-0.5271; 0.21, 0.0-0.6069; 1.548, 0.537-2.3942; respectively). Alternatively, to validate the support for a strict clock a Bayes factor (BF) analysis (log10 Bayes factors) using tree likelihoods of both strict and relaxed lognormal clock analyses in Tracer v1.5 (1000 bootstrap replicates; log10 Bayes factor strict vs. relaxed) was also conducted for each genus. Kass & Raftery (1995) suggested thresholds for deciding in favor of or against a strict clock: 0-3 (positive support), 3-6 (strong support), and > 6 (decisive support). The BF values for *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus* were 0.4, 0.063, 0.1, and 1.85, respectively. Thus, both clock tests suggest that a strict clock may be appropriate for all to analyze COI datasets.

Divergence time estimations were performed slightly different for each dataset (Table A1-4). For the genus *Sesarma*, the software StarBEAST (*BEAST; Heled & Drummond 2010) was used to obtain an ultrametric species tree. For the Panopeidae and *Pachygrapsus* datasets, BI phylogenetic analyses in BEAST v1.7.5 were performed. The input files of all datasets were prepared using the interface BEAUti v1.7.5 (Drummond *et al.* 2012; Table A1-4) with two separate alignment partitions (COI and 16S; note that only COI was analyzed for *Pachygrapsus*), whose substitution models and clocks were unlinked (strict clock; speciation yule process; Ngen 20, log 1000). Convergence of parameters and their effective sample size (ESS > 200) were confirmed in Tracer v1.5 (Rambaut & Drummond 2009). Accordingly, 2000 trees of each run were discarded as burn-in. A maximum clade credibility tree was computed with TreeAnnotator v1.7.5 and visualized with FigTree v1.3.1 (Rambaut 2009) for each analysis.

Table A1-4: Used parameters for phylogenetic analyses and divergence time estimations.

	Sesarma	***Panopeus***	***Eurytium***	***Pachygrapsus***
Phylogenetic Analysis				
Program (Analysis)	Beast (Bayesian analysis)	[2]RAxML (Maximum-likelihood analysis)		
Model of Evolution (COI/16S)	TrN+I+G/TrN+G	GTR+G/GTR+G		
Clock Model	Uncorrelated lognormal relaxed clock	Relaxed clock		
Tree Prior	Speciation Yule process	-		Phylogenetic topology adapted from Ip *et al.* 2015
Ngen (Mio)	50	1 000 bootstrap runs		
Log	1 000	-		
Burnin	5 000	-		
ESS Values	All > 200	-		
Remark	-	Best scoring tree was selected		
Divergence time estimations				
Program	*Beast	Beast	Beast	Beast
Model of Evolution (COI/16S)	HKY+G/HKY+G	HKY+G/HKY+G	HKY+G/HKY+G	HKY+G / -
Clock Model	Strict clock	Strict clock	Strict clock	Strict clock
Substitution Rate $[\%]^{-1}$ (COI)	0.98[1]	0.98[1]	0.98[1]	0.98[1]
Substitution Rate $[\%]^{-1}$ (16S)	0.58	0.66	0.51	-
Tree Prior	Speciation Yule process	Speciation Yule process	Speciation Yule process	Speciation Yule process
Ngen (Mio)	20	20	20	20
Log	1 000	1 000	1 000	1 000
Burnin	2 000	2 000	2 000	2 000
ESS Values	All > 200	All > 200	All > 200	All > 200

Overview of the parameters used for the phylogenetic analyses and divergence time estimations for the genera *Sesarma*, *Panopeus*, *Eurytium*, and *Pachygrapsus*; [1]Marino *et al.* 2011; [2]Stamatakis *et al.* 2008.

A2 Analyzed Species

A2.1 Genus *Sesarma* Say, 1817

Table A2-1: Species used for phylogenetic studies. Family Sesarmidae; Genus *Sesarma*.

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-1 A	*S. aequatoriale,* Ortmann, 1894	11633	19298	18.03.1996	Costa Rica, Rincón	EP	1	25971	-	672
A3-1 B	*S. aequatoriale,* Ortmann, 1894	11635	19299	10.03.1996	Panama, Gulf of Chiriquí, Boca Chica close to David, in tunnels	EP	1	23306	699	-
A3-1 C	*S. aequatoriale,* Ortmann, 1894	11636	19300	10.03.1996	Panama, Gulf of Chiriquí, Boca Chica close to David, in tunnels	EP	1	23306	1143	668
-	*S. aequatoriale,* Ortmann, 1894	-	CO2*	-	Panama, Miraflores Locks	EP	3	AJ225883	551	-
-	*S. aequatoriale,* Ortmann, 1894	-	16S2*	-	Panama, Miraflores Locks	EP	3	AJ225874	-	503
A3-1 D	*S. ayatum,* Schubart, Reimer & Diesel, 1998	11656	19314	25.04.1994	Jamaica, Portland, John Crown Mountains, inflow to the Drivers River	WA	1	23716	1134	650
A3-2 A	*S. ayatum,* Schubart, Reimer & Diesel, 1998	11657	19315	25.04.1994	Jamaica, Portland, John Crown Mountains, inflow to the Drivers River	WA	1	23716	1186	650
A3-2 B	*S. ayatum,* Schubart, Reimer & Diesel, 1998	11658	19316	25.04.1994	Jamaica, Portland, John Crown Mountains, inflow to the Drivers River	WA	1	23716	693	650
A3-2 C	*S. ayatum,* Schubart, Reimer & Diesel, 1998	11659	19317	25.04.1994	Jamaica, Portland, John Crown Mountains, inflow to the Drivers River	WA	1	23716	635	649
A3-2 D	*S. bidentatum,* Benedict, 1892	11653	19312	20.06.1987	Jamaica, Middlesex, St. Mary, Lucky Hill farm cave, from a creek in the cave	WA	1	19536, JAM-620	1188	642
A3-3 A	*S. bidentatum,* Benedict, 1892	11654	19313	20.06.1987	Jamaica, Middlesex, St. Mary, Lucky Hill farm cave, from a creek in the cave	WA	1	19536, JAM-620	688	641

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-3 B	*S. buettikoferi,* De Man, 1883	11639	19302	16.02.1980	Cameroon, S-Kribi, Rocheur la Loup, beach	-	1	SMF-11712	604	668
A3-3 C	*S. buettikoferi,* De Man, 1883	11640	19303	16.02.1980	Cameroon, S-Kribi, Rocheur la Loup, beach	-	1	SMF-11712	-	662
A3-3 D	*S. buettikoferi,* De Man, 1883	11641	19304	16.02.1980	Cameroon, S-Kribi, Rocheur la Loup, beach	-	1	SMF-11712	558	660
A3-4 A	*S. cookie,* Hartnoll, 1971	11967	19513	1996	Jamaica, Sherwood Forest	WA	2	R-346b	1190	664
-	*S. crassipes*, Cano, 1889	-	CO3*	-	Costa Rica, Rio Tortuguero	WA	3	AJ225859	551	-
-	*S. crassipes*, Cano, 1889	-	16S3*	-	Costa Rica, Rio Tortuguero	WA	3	AJ225869	-	504
A3-4 B	*S. curacaoense,* De Man, 1892	11966	19512	03.11.2006	Dominican Republic, Sánchez	WA	2	-	1182	666
A3-4 C	*S. curacaoense,* De Man, 1892	11969	19514	10.02.2004	USA, Florida, Bovista Beach, Lovers Beach State Park	WA	2	-	1191	676
A3-4 D	*S. curacaoense,* De Man, 1892	11970	19515	10.02.2004	USA, Florida, Bovista Beach, Lovers Beach State Park	WA	2	-	1210	664
A3-5 A	*S. dolphinum,* Reimer, Schubart & Diesel, 1998	11987	19529	27.02.2011	Jamaica, Orange River	WA	2	-	1192	662
A3-5 B	*S. fossarum,* Schubart, Reimer, Diesel & Türkay, 1997	11646	19307	15.03.1993	Jamaica, Cornwall, Trelawny	WA	1	23274	1195	650
A3-5 C	*S. fossarum,* Schubart, Reimer, Diesel & Türkay, 1997	11647	19308	15.03.1993	Jamaica, Cornwall, Trelawny	WA	1	23274	686	666
A3-5 D	*S. jarvisi,* Rathbun, 1914	11637	19301	24.05.1998	Jamaica, St. Ann, Mt. Diablo; between Moneague and Ewerton; forest floor, in a gastropod shell	WA	1	24565	1152	647
A3-6 A	*S. meridies,* Schubart & Koller, 2005	11977	19520	14.03.2003	Jamaica, Clarendon: Grantham, Rio Minho Tributary	WA	2	-	1188	666

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-6 B	*S. meridies,* Schubart & Koller, 2005	11978	19521	14.03.2003	Jamaica, Clarendon: Grantham, Rio Minho Tributary	WA	2	-	1225	664
A3-6 C	*S. meridies,* Schubart & Koller, 2005	11979	19522	14.03.2003	Jamaica, Clarendon: Grantham, Rio Minho Tributary	WA	2	-	1200	665
A3-6 D	*S. meridies,* Schubart & Koller, 2005	11980	19523	14.03.2003	Jamaica, Clarendon: Grantham, Rio Minho Tributary	WA	2	-	1189	665
A3-7 A	*S. meridies,* Schubart & Koller, 2005	11981	19524	14.03.2003	Jamaica, Clarendon: Grantham, Rio Minho Tributary	WA	2	-	1187	664
A3-7 B	*S. rectum,* Randall, 1840	11649	19309	15.08.1994	Grenada, Island: Fort Jeudy, Calivigny Harbour, mangrove	WA	1	23248	638	-
A3-7 C	*S. rectum,* Randall, 1840	11650	19310	15.08.1994	Grenada, Island: Fort Jeudy, Calivigny Harbour, mangrove	WA	1	23248	1189	673
A3-7 D	*S. rectum,* Randall, 1840	11651	19311	15.08.1994	Grenada, Island: Fort Jeudy, Calivigny Harbour, mangrove	WA	1	23248	1141	673
A3-8 A	*Sesarma* sp. (nr. *reticulatum*)	11983	19525	08.04.2008	USA, Florida, Enconfinca R., Mossy Hammock Rd.	WA	2	-	1196	666
A3-8 B	*Sesarma* sp. (nr. *reticulatum*)	11984	19526	08.04.2008	USA, Florida, Enconfinca R., Mossy Hammock Rd.	WA	2	-	1195	664
A3-8 C	*Sesarma* sp. (nr. *reticulatum*)	11985	19527	08.04.2008	USA, Florida, Enconfinca R., Mossy Hammock Rd.	WA	2	-	1196	665
A3-8 D	*Sesarma* sp. (nr. *reticulatum*)	11986	19528	08.04.2008	USA, Florida, Enconfinca R., Mossy Hammock Rd.	WA	2	-	1153	679
A3-9 A	*S. reticulatum,* (Say, 1817)	11975	19519	22.08.2000	USA, North Carolina, Wilmington (UNCW)	WA	2	-	669	644
-	*S. reticulatum,* (Say, 1817)	-	CO1*	-	USA, Delaware, Woodland Beach	WA	3	AJ225885	551	-
-	*S. reticulatum,* (Say, 1817)	-	16S1*	-	USA, Delaware, Woodland Beach	WA	3	AJ225867	-	647

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-9 B	*S. rhizophorae,* Rathbun, 1906	11988	19530	11.10.2007	Ecuador, Guayas, Rio Morro	EP	2	R156-8	1185	663
A3-9 C	*S. rhizophorae,* Rathbun, 1906	9718	18151	21.01.2006	Ecuador, Puerto Morro	EP	2	-	708	665
A3-9 D	*S. rhizophorae,* Rathbun, 1906	11660	19318	17.03.1996	Costa Rica, Puerto Jiménez	EP	1	25975	1109	677
A3-10 A	*S. rhizophorae,* Rathbun, 1906	9709	18142	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	1188	-
A3-10 B	*S. rhizophorae,* Rathbun, 1906	9711	18144	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	924**	668
A3-10 C	*S. rhizophorae,* Rathbun, 1906	9712	18145	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	-	678
A3-10 D	*S. rhizophorae,* Rathbun, 1906	9713	18146	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	986**	677
A3-11 A	*S. rhizophorae,* Rathbun, 1906	9714	18147	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	990**	677
A3-11 B	*S. rhizophorae,* Rathbun, 1906	9715	18148	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	1196	665
A3-11 C	*S. rhizophorae,* Rathbun, 1906	9716	18149	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	1185	665
A3-11 D	*S. rhizophorae,* Rathbun, 1906	9717	18150	21.01.2006	Costa Rica, Coco Rocas	EP	2	-	-	683
A3-12 A	*S. rubinofforum,* Abele, 1973	11661	19319	17.03.1996	Costa Rica, Puerto Jiménez	EP	1	25974	491	675
-	*S. rubinofforum,* Abele, 1973	-	CO5*	-	Panama, Rio Chorcha	EP	3	AJ225882	551	-
-	*S. rubinofforum,* Abele, 1973	-	16S5*	-	Panama, Rio Chorcha	EP	3	AJ225852	-	496

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-12 B	*S. sulcatum,* Smith, 1870	11632	19297, 19641	18.12.1951	El Salvador, Rio Cosmagna	EP	1	2078	-	-
-	*S. sulcatum,* Smith, 1870	12108	19618	04.06.1999	Mexico, Pto. San Carlos, burrowed in sand	EP	2	VSLZ 4125	688	-
-	*S. sulcatum,* Smith, 1870	-	CO4*	-	Mexico, near Isla Tiburon	EP	3	AJ225880	551	-
-	*S. sulcatum,* Smith, 1870	-	16S4*	-	Mexico, near Isla Tiburon	EP	3	AJ225853	-	505
A3-12 C	*S. verleyi,* Rathbun, 1914	11972	19516	-	Jamaica, Trelawny, Windsor cave, glade entrance	WA	2	R150	1300	666
A3-12 D	*S. verleyi,* Rathbun, 1914	11973	19517	-	Jamaica, Trelawny, Windsor cave, glade entrance	WA	2	R150	1189	664
A3-13 A	*S. verleyi,* Rathbun, 1914	11974	19518	-	Jamaica, Trelawny, Windsor cave, glade entrance	WA	2	R150	1142	-
A3-13 B	*S. windsor,* Türkay & Diesel, 1994	11643	19305	09.03.1995	Jamaica, Cornwall, Trelawny, Printed Circuit Cave, near Rock Spring, from a cave-river	WA	1	23271	626	662
A3-13 C	*S. windsor,* Türkay & Diesel, 1994	11644	19306	09.03.1995	Jamaica, Cornwall, Trelawny, Printed Circuit Cave, near Rock Spring, from a cave-river	WA	1	23271	944	665

Table showing figure numbers of the respective species ('Plate'), species names including author ('Species'), University of Giessen Systematic and Biodiversity collection number ('UGSB'), Species' preparation number ('Prep. #'), sampling date, information about the collection locality ('Locality Information'), species associated Ocean (i.e. wester Atlantic (WA) or eastern Pacific (EP), 'Ocean'), loaner or loan institution ('Loan') and the respective collection number (including GenBank accession numbers; 'Previous Loan #'), and the obtained sequence lengths of the cytochrome c oxidase subunit I and the 16S rRNA fragments in base pairs ('COI bp' and '16S bp', respectively). [1]Senckenberg museum Frankfurt, [2]Christoph D. Schubart (collection at University Regensburg), [3]NCBI. *species code shown in the phylogenetic tree, sequence taken from GenBank, **back part of COI fragment (COL8/COH16). Note that some species yielded no usable sequences, however, they might be pictured (Appendix A3) for informative reasons.

A2.2 Genus *Eurytium* Stimpson, 1859

Table A2-2: Species used for phylogenetic studies. Family Panopeidae; Genus *Eurytium*.

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-14 A	*E. affine*, (Streets & Kingsley, 1877)	11608	19748	1957	Ecuador, Galapagos, Academie Bay	EP	1	2539	-	-
-	*E. affine*, (Streets & Kingsley, 1877)	12109	19619	03.06.1999	Mexico, Pto. San Carlos, at the beach, under stones and tires	EP	2	ULLZ 4117	695	655
-	*E. affine*, (Streets & Kingsley, 1877)	-	5499*	-	Mexico, Baja California Sur, Puerto San Carlos, mangrove	EP	3, 4	ULLZ 5499, KF682757 (COI) KF682963 (16S)	524	521
-	*E. albidigitum*, Rathbun, 1933	-	4156*	-	Mexico, Baja California Norte, Bahia de los Angeles, Salicornia back beach	EP	3, 4	ULZZ 4156, KF682826 (COI) KF682958 (16S)	524	521
A3-14 B	*E. limosum*, (Say, 1818)	9697	18131	26.11.2009	Brazil, SP, Portinho	WA	2	-	-	653
A3-14 C	*E. limosum*, (Say, 1818)	9698	18132	26.11.2009	Brazil, SP, Portinho	WA	2	-	1071	652
A3-14 D	*E. limosum*, (Say, 1818)	9700	18134	25.11.2011	Brazil, SP, Bertioga	WA	2	-	-	635
A3-14 E	*E. limosum*, (Say, 1818)	9701	18135	12.11.2010	Brazil, Bahia, Acuipe	WA	2	-	-	638
A3-14 F	*E. limosum*, (Say, 1818)	9703	18137	12.11.2010	Brazil, Bahia, Acuipe	WA	2	-	-	656
A3-14 G	*E. limosum*, (Say, 1818)	9704	18138	12.11.2010	Brazil, Bahia, Acuipe	WA	2	-	-	656
A3-15 A	*E. limosum*, (Say, 1818)	11586	19743	08.01.2008	USA, Florida, Marsh near Cedar Key	WA	2	-	-	635

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
-	*E. limosum,* (Say, 1818)	-	4012*	-	Mexico, Veracruz, Laguna S. Agustin, one mile N. of Sta. Ana, closed lagoon	WA	3, 4	ULLZ 4012 (isolate BT 086201), KF682755 (COI) GU144455 (16S)	524	496
A3-15 B	*E. tristani*, Rathbun, 1906	9695	19862	11.10.2007	Ecuador, Guapas, Puerto Morro	EP	2	-	-	635
A3-15 C	*E. tristani*, Rathbun, 1906	11618	19241, 19640	01.08.1984	Peru, Puerto Pizzaro, mangrove	EP	1	13169	- -	- -
-	*E. tristani*, Rathbun, 1906	-	12791*	-	Nicaragua, Paso de Caballos	EP	3, 4	ULZZ 12791, KF682756 (COI) KF682953 (16S)	524	521

Table showing figure numbers of the respective species ('Plate'), species names including author ('Species'), University of Giessen Systematic and Biodiversity collection number ('UGSB'), Species' preparation number ('Prep. #'), sampling date, information about the collection locality ('Locality Information'), species associated Ocean (i.e. wester Atlantic (WA) or eastern Pacific (EP), 'Ocean'), loaner or loan institution ('Loan') and the respective collection number (including GenBank accession numbers; 'Previous Loan #'), and the obtained sequence lengths of the cytochrome c oxidase subunit I and the 16S rRNA fragments in base pairs ('COI bp' and '16S bp', respectively). [1]Senckenberg museum Frankfurt, [2]Christoph D. Schubart (collection at University Regensburg), [3]NCBI, [4]Thoma *et al.* 2014. *species code shown in the phylogenetic tree, sequence taken from GenBank, Note that some species yielded no usable sequences, however, they might be pictured (Appendix A3) for informative reasons.

A2.3 Genus *Panopeus* H. Milne Edwards, 1834

Table A2-3: Species used for phylogenetic studies. Family Panopeidae; Genus *Panopeus*.

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-16 A	*Panopeus* sp.	7789	16138	02.05.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1062	635
A3-16 B	*Panopeus* sp.	7790	16139	02.05.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1144	602
A3-16 C	*Panopeus* sp.	7791	16140	02.05.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	-	675
A3-16 D	*Panopeus* sp.	7793	16141	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1182	635
A3-17 A	*Panopeus* sp.	7794	16142	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1231	635
A3-17 B	*Panopeus* sp.	7795	16143	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1195	635
A3-17 C	*Panopeus* sp.	7796	16144	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1183	635
A3-17 D	*Panopeus* sp.	7797	16145	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1198	635
A3-18 A	*Panopeus* sp.	7798	16146	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1186	635
A3-18 B	*Panopeus* sp.	7799	16147	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1195	635
A3-18 C	*Panopeus* sp.	7800	16148	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	1195	635
A3-18 D	*Panopeus* sp.	7801	16149	29.04.2011	Costa Rica, Mata de Limon, Gulf of Nicoya	EP	2	-	631	635
A3-19 A	*Panopeus* sp.	12115	19625	10.10.2007	Ecuador, Puerto Morro, mud flat	EP	2	-	-	654
A3-19 B	*Panopeus* sp.	12256	19754	11.11.2007	Brazil, Praia Dura, mangrove, estuarine	WA	2	-	498	635
A3-19 C	*Panopeus* sp.	12257	19755	11.10.2007	Brazil, Praia Dura, mangrove, estuarine	WA	2	-	639	635
A3-19 D	*Panopeus* sp.	12258	19756	10.11.2007	Ecuador, Guayas, Puerto Morro, soft mud river bank	EP	2	-	-	635
A3-20 A	*Panopeus* sp.	12259	19757	10.11.2007	Ecuador, Guayas, Puerto Morro, soft mud river bank	EP	2	-	-	635
A3-20 B	*Panopeus* sp.	12260	19758	11.10.2007	Brazil, Praia do Segredo	WA	2	-	635	635
A3-20 C	*Panopeus* sp.	12261	19759	11.10.2007	Brazil, Praia do Segredo	WA	2	-	-	635

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-21 A	*Panopeus* sp.	12264	19761	10.11.2007	Ecuador, Guayas, Puerto Morro	EP	2	-	633	635
A3-21 B	*Panopeus* sp.	12266	19762	10.11.2007	Ecuador, Guayas, Puerto Morro, soft ground	EP	2	-	-	635
A3-21 C	*Panopeus* sp.	12267	19763	10.11.2007	Ecuador, Guayas, Puerto Morro, soft ground	EP	2	-	637	635
A3-21 D	*Panopeus* sp.	12269	19764	20.12.2006	Costa Rica, Punta Morales, mangrove	EP	2	-	543	635
A3-22 A	*Panopeus* sp.	12270	19765	20.12.2006	Costa Rica, Punta Morales, mangrove	EP	2	-	641	635
A3-22 B	*Panopeus* sp.	12273	19863	27.02.2008	Costa Rica, Golfito, mud flat	EP	2	-	1074	635
A3-22 C	*Panopeus* sp.	12275	19864	10.10.2007	Ecuador, Guayas, Puerto Morro, soft mud river bank	EP	2	-	1052	635
A3-22 D	*Panopeus* sp.	12276	19865	10.10.2007	Ecuador, Guayas, Puerto Morro, soft mud river bank	EP	2	-	633	635
A3-23 A	*Panopeus* sp.	12278	19866	11.11.2007	Brazil, Rio Maranduba or Praia Dura	WA	2	-	-	635
A3-23 B	*Panopeus* sp.	12279	19867	11.11.2007	Brazil, Rio Maranduba or Praia Dura	WA	2	-	-	635
A3-23 C	*P. africanus*, A. Milne-Edwards,1867	12114	19624	20.04.2004	Spain, Cadiz, Corrales de Rota	-	2	-	553	-
-	*P. africanus*, A. Milne-Edwards, 1867	-	4273*	-	Spain, Cadiz	-	3, 4	ULLZ 4273, KF682774 (COI) EU863370 (16S)	524	521
A3-23 D	*P. americanus*, De Saussure, 1857	11624	19242	1904	USA, North Carolina, Beufort	WA	1	1732	646	-
A3-24 A	*P. americanus*, De Saussure, 1857	11625	19752	1904	USA, North Carolina, Beufort	WA	1	1732	-	635
A3-24 B	*P. americanus*, De Saussure, 1857	11626	19753	1904	USA, North Carolina, Beufort	WA	1	1732	-	-
A3-24 C	*P. americanus*, De Saussure, 1857	12113	19623	10.11.2007	Brazil, Praia de Segredo	WA	2	-	996	654
A3-24 D	*P. austrobesus*, Williams, 1983	7804	16151	15.11.2010	Brazil, Marudá	WA	2	-	1193	636

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-25 A	*P. austrobesus*, Williams, 1983	7805	16152	15.11.2010	Brazil, Marudá	WA	2	-	1184	636
A3-25 B	*P. austrobesus*, Williams, 1983	7806	16153	15.11.2010	Brazil, Marudá	WA	2	-	1193	636
A3-25 C	*P. austrobesus*, Williams, 1983	7807	16154	15.11.2010	Brazil, Marudá	WA	2	-	1193	636
A3-25 D	*P. austrobesus*, Williams, 1983	7808	16155	15.11.2010	Brazil, Marudá	WA	2	-	1193	636
A3-26 A	*P. austrobesus*, Williams, 1983	7809	16156	15.11.2010	Brazil, Marudá	WA	2	-	1193	636
A3-26 B	*P. austrobesus*, Williams, 1983	7810	16157	15.11.2010	Brazil, Marudá	WA	2	-	1193	636
A3-26 C	*P. austrobesus*, Williams, 1983	7811	16158	15.11.2010	Brazil, Marudá	WA	2	-	1193	-
A3-26 D	*P. bermudensis*, Benedict & Rathbun, 1891	11601	19745	28.05.2010	Brazil, Santa Catarina, Penha, Praia de Itapocoroy-Cultivo de Vieras	WA	1	40193	-	654
A3-27 A	*P. bermudensis*, Benedict & Rathbun, 1891	11602	19746	28.05.2010	Brazil, Santa Catarina, Penha, Praia de Itapocoroy-Cultivo de Vieras	WA	1	40193	-	642
A3-27 B	*P. bermudensis*, Benedict & Rathbun, 1891	11603	19747	28.05.2010	Brazil, Santa Catarina, Penha, Praia de Itapocoroy-Cultivo de Vieras	WA	1	40193	743	654
A3-27 C	*P. chilensis*, H. Milne Edwards & Lucas, 1843	11617	19246, 19627	06.05.1950	Peru, Mancora, marine sandy beach with rocks	EP	1	2284	-	-
-	*P. chilensis*, H. Milne Edwards & Lucas, 1843	-	4685*	-	Nicaragua, Chinandega, El Estero de Aserradores, south of Aposentillo	EP	3, 4	ULZZ 4685, KF682734 (COI) KF682955 (16S)	524	519
A3-27 D	*P. convexus*, A. Milne-Edwards, 1880	11607	19240, 19630	1952	El Salvador, El Triunfo	EP	1	2094a	-	-
A3-28 A	*P. hartii*, Smith, 1869	11599	19631	1969	Colombia, Isla de Salamanca	WA	1	7034	637	664

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-28 B	*P. herbstii*, H. Milne Edwards, 1834	11611	19749	19.01.1979	Brazil, Sao Paulo, Iolade Sao, Sebastiao, Praia de Araca	WA	1	10017	567	636
A3-28 C	*P. herbstii*, H. Milne Edwards, 1834	11612	19750	19.01.1979	Brazil, Sao Paulo, Iolade Sao, Sebastiao, Praia de Araca	WA	1	10017	635	636
A3-28 D	*P. lacustris*, Desbonne, in Desbonne & Schramm, 1867	7813	16159	28.02.2011	Jamaica, St. Anne, Priory	WA	2	-	1193	636
A3-29 A	*P. lacustris*, Desbonne, in Desbonne & Schramm, 1867	7814	16160	28.02.2011	Jamaica, St. Anne, Priory	WA	2	-	1193	636
A3-29 B	*P. lacustris*, Desbonne, in Desbonne & Schramm, 1867	7815	16161	28.02.2011	Jamaica, St. Anne, Priory	WA	2	-	1192	636
A3-29 C	*P. lacustris*, Desbonne, in Desbonne & Schramm, 1867	7824	16168	28.02.2011	Jamaica, St. Anne, Priory	WA	2	-	1193	636
A3-29 D	*P. lacustris*, Desbonne, in Desbonne & Schramm, 1867	7825	16169	28.02.2011	Jamaica, St. Anne, Priory	WA	2	-	1146	635
A3-30 A	*P. lacustris*, Desbonne, in Desbonne & Schramm, 1867	11591	19233	08.02.2003	Cuba, Pinar del Rio, Puerto Esperanza, Rocks near Beach	WA	2	-	1186	636
A3-30 B	*P. meridionalis*, Williams, 1983	11609	19632	15.06.2010	Brazil, Santa Catarina, Praia de Porto Belo	WA	1	40172	630	655
A3-30 C	*P. obesus*, Smith, 1869	11588	19633	04.03.2001	USA, Louisiana, Grand Isle	WA	2	-	633	655
A3-30 D	*P. obesus*, Smith, 1869	11589	19634	04.03.2001	USA, Louisiana, Grand Isle	WA	2	-	631	653
A3-31 A	*P. obesus*, Smith, 1869	11590	19232	04.03.2001	USA, Louisiana, Grand Isle	WA	2	-	696	-
A3-31 B	*P. obesus*, Smith, 1869	11593	19234	08.01.2008	USA, Florida, Marsh near Cedar Key	WA	2	-	1128	654
A3-31 C	*P. obesus*, Smith, 1869	11594	19635	08.01.2008	USA, Florida, Marsh near Cedar Key	WA	2	-	637	635

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-31 D	*P. obesus*, Smith, 1869	11595	19236	08.01.2008	USA, Florida, Marsh near Cedar Key	WA	2	-	1180	635
A3-32 A	*P. occidentalis*, De Saussure, 1857	7802	16150	11.11.2010	Brazil, Itacaré, Pnaia da Capcha (?)	WA	2	-	686	635
A3-32 B	*P. occidentalis*, De Saussure, 1857	7822	16167	28.02.2011	Jamaica, St. Anne, Priory	WA	2	-	691	635
A3-32 C	*P. occidentalis*, De Saussure, 1857	11619	19870	05.11.1988	Colombia, Magdalena, Burukuka, 5-10 m depth	WA	1	-	-	591
A3-32 D	*P. occidentalis*, De Saussure, 1857	11620	19871	05.11.1988	Colombia, Magdalena, Burukuka, 5-10 m depth	WA	1	-	-	580
A3-33 A	*P. occidentalis*, De Saussure, 1857	11621	19872	-	Brazil, Santa Catarina, Porto Belo, Prai de Lopes	WA	1	-	532	-
A3-33 B	*P. occidentalis*, De Saussure, 1857	11622	19873	-	Brazil, Santa Catarina, Porto Belo, Prai de Lopes	WA	1	-	-	635
A3-33 C	*P. purpureus*, Lockington, 1877	7787	16137	29.04.2011	Costa Rica, Matá de Limon, Gulf of Nicoya	EP	2	-	1196	635
A3-34 A	*P. purpureus*, Lockington, 1877	9691	18125	10.10.2007	Ecuador, Puerto Morro	EP	2	-	1104	-
A3-34 B	*P. purpureus*, Lockington, 1877	9692	18126	10.10.2007	Ecuador, Puerto Morro	EP	2	-	867	635
A3-34 C	*P. purpureus*, Lockington, 1877	9693	18127	10.10.2007	Ecuador, Puerto Morro	EP	2	-	1183	635
A3-34 D	*P. purpureus*, Lockington, 1877	9694	18128	10.10.2007	Ecuador, Puerto Morro	EP	2	-	1192	635
A3-35 A	*Panopeus* sp.	12116	19626	10.10.2007	Ecuador, Puerto Morro, sand flat	EP	2	-	925	654
A3-35 B	*P. rugosus*, A. Milne-Edwards, 1880	11614	19751	17.05.2010	Brazil, Santa Catarina, Penha, Praia Itapocoroy-Costao	WA	1	40211	-	635

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
A3-35 C	*P. rugosus*, A. Milne-Edwards, 1880	11615	19744	17.05.2010	Brazil, Santa Catarina, Penha, Praia Itapocoroy-Costao	WA	1	40211	-	654
-	*P. rugosus*, A. Milne-Edwards, 1880	-	8522*	-	Brazil, Sao Paulo, Sao Vicente, Paranopus beach	WA	3, 4	ULZZ 8522, KF682773 (COI) KF682969 (16S)	524	522
A3-36 A	*P. simpsoni*, Rathbun, 1930	11581	19637	08.01.2008	USA, Florida, Cedar Key, beach with rocks	WA	2	-	-	659
A3-36 B	*P. simpsoni*, Rathbun, 1930	11582	19638	08.01.2008	USA, Florida, Cedar Key, beach with rocks	WA	2	-	595	655
A3-36 C	*P. simpsoni*, Rathbun, 1930	11583	19639	08.01.2008	USA, Florida, Cedar Key, beach with rocks	WA	2	-	1127	653

Table showing figure numbers of the respective species ('Plate'), species names including author ('Species'), University of Giessen Systematic and Biodiversity collection number ('UGSB'), Species' preparation number ('Prep. #'), sampling date, information about the collection locality ('Locality Information'), species associated Ocean (i.e. wester Atlantic (WA) or eastern Pacific (EP), 'Ocean'), loaner or loan institution ('Loan') and the respective collection number (including GenBank accession numbers; 'Previous Loan #'), and the obtained sequence lengths of the cytochrome c oxidase subunit I and the 16S rRNA fragments in base pairs ('COI bp' and '16S bp', respectively). [1]Senckenberg museum Frankfurt, [2]Christoph D. Schubart (collection at University Regensburg), [3]NCBI, [4]Thoma *et al.* 2014. *species code shown in the phylogenetic tree, sequence taken from GenBank, Note that some species yielded no usable sequences, however, they might be pictured (Appendix A3) for informative reasons.

A2.4 Genus *Pachygrapsus* Randall, 1840

Table A2-4: Species used for phylogenetic studies. Family Grapsidae; Genus *Pachygrapsus.*

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
-	*P. crassipes*, Randall, 1840	-	21754*	-	-	EP	3, 4	NC_021754	1534	1312
-	*P. gracilis*, (De Saussure, 1858)	-	329166*	March 2004	Panama: Playa Bluff, Bocas del Toro	WA	3, 4	EU329166	531	-
A3-37 A	*P. socius,* Stimpson, 1871	9123	17513	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58214	685	-
A3-37 B	*P. socius,* Stimpson, 1871	9124	17514	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58215	686	-
A3-37 C	*P. socius,* Stimpson, 1871	9125	17515	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58216	687	-
-	*P. socius,* Stimpson, 1871	9126	17516	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58217	687	-
-	*P. socius,* Stimpson, 1871	9127	17517	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58218	686	-
-	*P. socius,* Stimpson, 1871	9128	17518	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58219	686	-
A3-37 D	*P. socius,* Stimpson, 1871	9129	17519	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58220	686	-
-	*P. socius,* Stimpson, 1871	9130	17520	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58221	687	-
A3-37 E	*P. socius,* Stimpson, 1871	9131	17521	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58222	688	-
-	*P. socius,* Stimpson, 1871	9132	17522	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58223	686	-
A3-37 F	*P. socius,*	9133	17523	29.04.2011	Costa Rica, Mata de Limon, human built wave breaker	EP	2	R58224	686	-

Plate	Species	UGSB	Prep. #	Sampling Date	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
-	*P. transversus,* (Gibbes, 1850)	9134	17524	06.05.2011	Costa Rica, Manzanillo	WA	2	R5921	695	-
-	*P. transversus,* (Gibbes, 1850)	9135	17525	06.05.2011	Costa Rica, Manzanillo	WA	2	R5922	692	-
-	*P. transversus,* (Gibbes, 1850)	9136	17526	06.05.2011	Costa Rica, Manzanillo	WA	2	R5923	696	-
A3-38 A	*P. transversus,* (Gibbes, 1850)	9137	17527	06.05.2011	Costa Rica, Manzanillo	WA	2	R5924	687	-
A3-38 B	*P. transversus,* (Gibbes, 1850)	9138	17528	06.05.2011	Costa Rica, Manzanillo	WA	2	R5925	694	-
A3-38 C	*P. transversus,* (Gibbes, 1850)	9140	17530	06.05.2011	Costa Rica, Manzanillo	WA	2	R5927	702	-
-	*P. transversus,* (Gibbes, 1850)	9142	17532	06.05.2011	Costa Rica, Manzanillo	WA	2	R5929	672	-

Table showing figure numbers of the respective species ('Plate'), species names including author ('Species'), University of Giessen Systematic and Biodiversity collection number ('UGSB'), Species' preparation number ('Prep. #'), sampling date, information about the collection locality ('Locality Information'), species associated Ocean (i.e. wester Atlantic (WA) or eastern Pacific (EP), 'Ocean'), loaner or loan institution ('Loan') and the respective collection number (including GenBank accession numbers; 'Previous Loan #'), and the obtained sequence lengths of the cytochrome c oxidase subunit I and the 16S rRNA fragments in base pairs ('COI bp' and '16S bp', respectively). [2]Christoph D. Schubart (collection at University Regensburg), [3]NCBI, [4]Thoma *et al.* 2014. *species code shown in the phylogenetic tree, sequence taken from GenBank. Photos of *P. transversus* and P. socius courtesy of C .D. Schubart (University of Regensburg).

A2.5 Additional genera

Table A2-5: Species of additional genera used for phylogenetic studies.

Species	*Prep. #	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
Acantholobulus bermudensis, (Benedict & Rathbun, 1891)	6924	USA, Florida, Ft. Pierce, on rope at old SMS docks	WA	3, 4	ULLZ 6924, KF682779 (COI) EU863372 (16S)	524	519
Acantholobulus pacificus, (Edmondson, 1931)	12959	USA, Hawaii, Oahu Island Honolulu, Waikiki Marina Channel near Ala Moana Beach	-	3, 4	ULLZ 12959, KF682778 (COI) KF682977 (16S)	524	520
Acantholobulus schmitti (gen. nov., sp. nov. near); *Hexapanopeus* sp.	8646	USA, Texas, South Padre Island Jetty	WA	3, 4	ULLZ 8646, KF682761 (COI) EU863361 (16S)	524	521
Cyrtoplax nr. *Spinidentata,* (Benedict, 1892)	8423	USA, Florida, Ft. Pierce, Coon Island small channel in red mangrove	WA	3, 4	ULLZ 8423, KF682759 (COI) EU863369 (16S)	524	521
Eucratopsis crassimanus, (Dana, 1851)	6427	USA, Florida, Ft. Pierce, N. of S. A1A Causeway	WA	3, 4	ULLZ 6427, KF682799 (COI) EU863392 (16S)	524	519
Eurypanopeus abbreviates, (Stimpson, 1860)	3753	USA, Florida, Ft. Pierce, jetty on beach under boulders	WA	3, 4	ULLZ 3753, KF682823 (COI) EU863388 (16S)	524	521
Eurypanopeus ater, Rathbun, 1930	4019	Mexico, Veracruz, Punta Delgada, south of Santa Ana	WA	3, 4	ULLZ 4019, KF682824 (COI) KF682965 (16S)	524	521
Eurypanopeus ovatus, (Benedict & Rathbun, 1891)	9041	Mexico, Baja California Sur, Gulf of California, Playa Santispac, Bahía Concepción	EP	3, 4	ULLZ 9041, KF682733 (COI) KF682960 (16S)	524	521
Eurypanopeus planissimus, (Stimpson, 1860)	4140	Mexico, Baja California Sur, Playa Santispac, nr Mulege	EP	3, 4	ULLZ 4140, KF682765 (COI) EU863386 (16S)	524	522
Geograpsus grayi,	07992	French Polynesia	-	4	KC706770	310	-

Species	*Prep. #	Locality Information	Ocean	Loan	Previous Loan #	COI (bp)	16S (bp)
Eurypanopeus planus, (Smith, 1869)	12789	Nicaragua, El Estero de Aserradores, south of Aposentillo	EP	3, 4	ULLZ 12789, KF682760 (COI) KF682954 (16S)	524	522
Hexapanopeus angustifrons, (Benedict & Rathbun, 1891)	6943	USA, Florida, Capron Shoal	WA	3, 4	ULLZ 6943, KF682832 (COI) EU863343 (16S)	524	520
Hexapanopeus angustifrons, (Benedict & Rathbun, 1891)	8368	USA, Florida, off St. Petersburg	WA	3, 4	ULLZ 8368, KF682833 (COI) EU863380 (16S)	524	517
Hexapanopeus sp. nov.	12779	Costa Rica, Gulf of Nicoya, off Playa Hermosa	EP	3, 4	ULLZ 12779, KF682993 (16S)	-	521
Hexapanopeus sp. nov.	12526	Belize, South Water Cay	WA	3, 4	ULLZ 12526, KF682772 (COI) KF682952 (16S)	524	521
Hexapanopeus paulensis, Rathbun, 1930	6608	Brazil, Sao Paulo	WA	3, 4	ULLZ 6608, KF682831 (COI) EU863373 (16S)	524	520
Hexapanopeus paulensis, Rathbun, 1930	6682	USA, Gulf of Mexico, off Texas	WA	3, 4	ULLZ 6882, KF682829 (COI) EU863375 (16S)	524	521
Malacoplax californiensis, (Lockington, 1877)	10572	Mexico, Baja California Sur, Gulf side	EP	3, 4	ULLZ 10572 (isolate BT 088903), KF682732 (COI) GU144460 (16S)	524	521
Tetraplax quadridentata, (Rathbun, 1898)	12374	Panama, off Bocas del Toro	WA	3, 4	ULLZ 12374, KF682754 (COI) KF682959 (16S)	524	522

Table showing species names including author ('Species'), Species' preparation number ('Prep. #'), information about the collection locality ('Locality Information'), species associated Ocean (i.e. wester Atlantic (WA) or eastern Pacific (EP), 'Ocean'), loaner or loan institution ('Loan') and the respective collection number (including GenBank accession numbers; 'Previous Loan #'), and the obtained sequence lengths of the cytochrome c oxidase subunit I and the 16S rRNA fragments in base pairs ('COI bp' and '16S bp', respectively). [3]NCBI, [4]Thoma *et al.* 2014. *species code shown in the phylogenetic tree, sequence taken from GenBank.

A3 Photo Tables of the Specimens

A3.1 Genus *Sesarma* Say, 1817

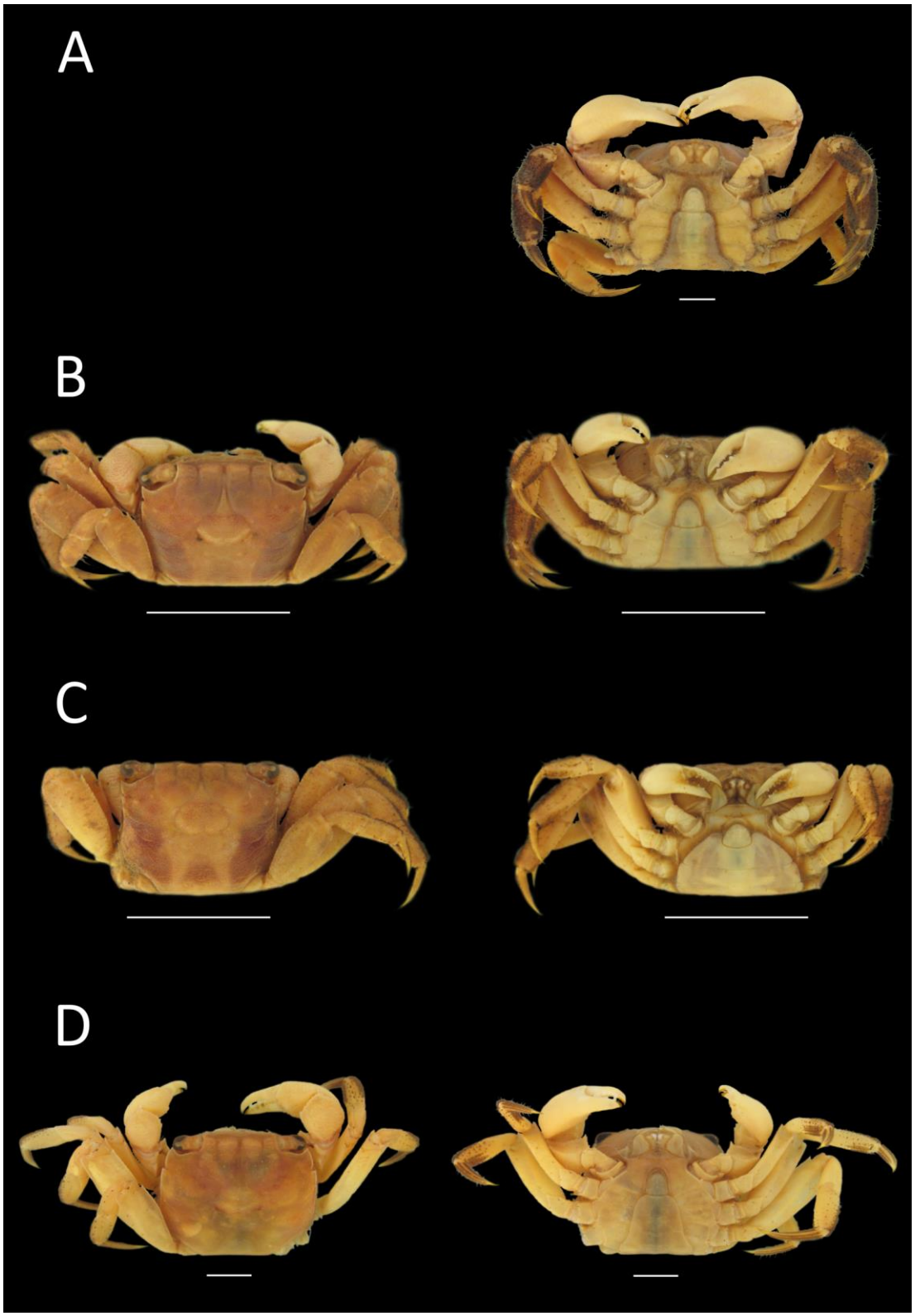


Figure A3-1: Three specimens of *Sesarma aequatoriale* Ortmann, 1894. A, Prep. #19298; B, Prep. #19299; C, Prep. #19300. Specimen of *Sesarma ayatum* Schubart, Reimer & Diesel, 1998. D, Prep. #19314. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

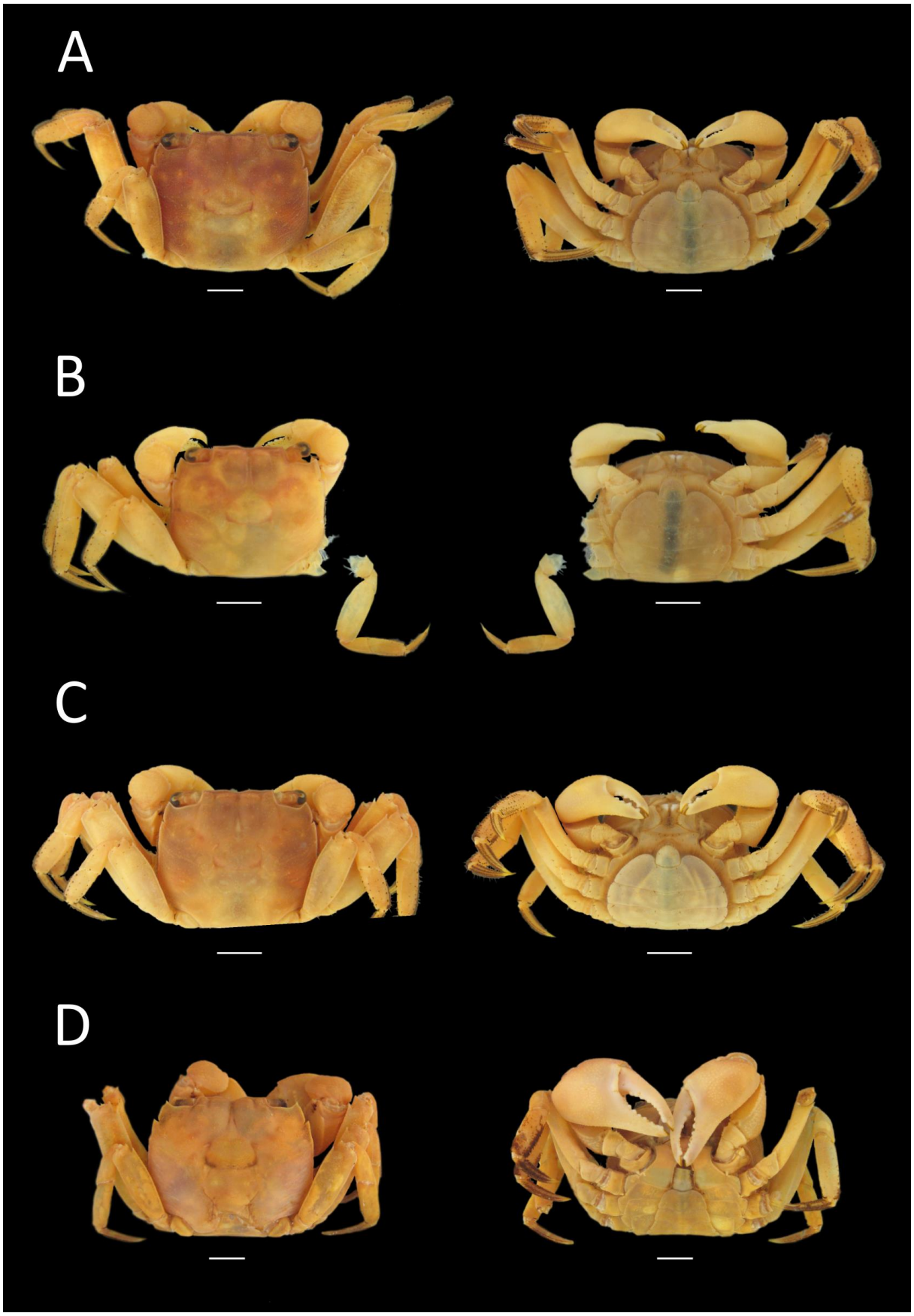


Figure A3-2: Three specimens of *Sesarma ayatum* Schubart, Reimer & Diesel, 1998. A, Prep. #19315; B, Prep. #19316; C, Prep. #19317. Specimen of *Sesarma bidentatum* Benedict, 1892. D, Prep. #19312. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

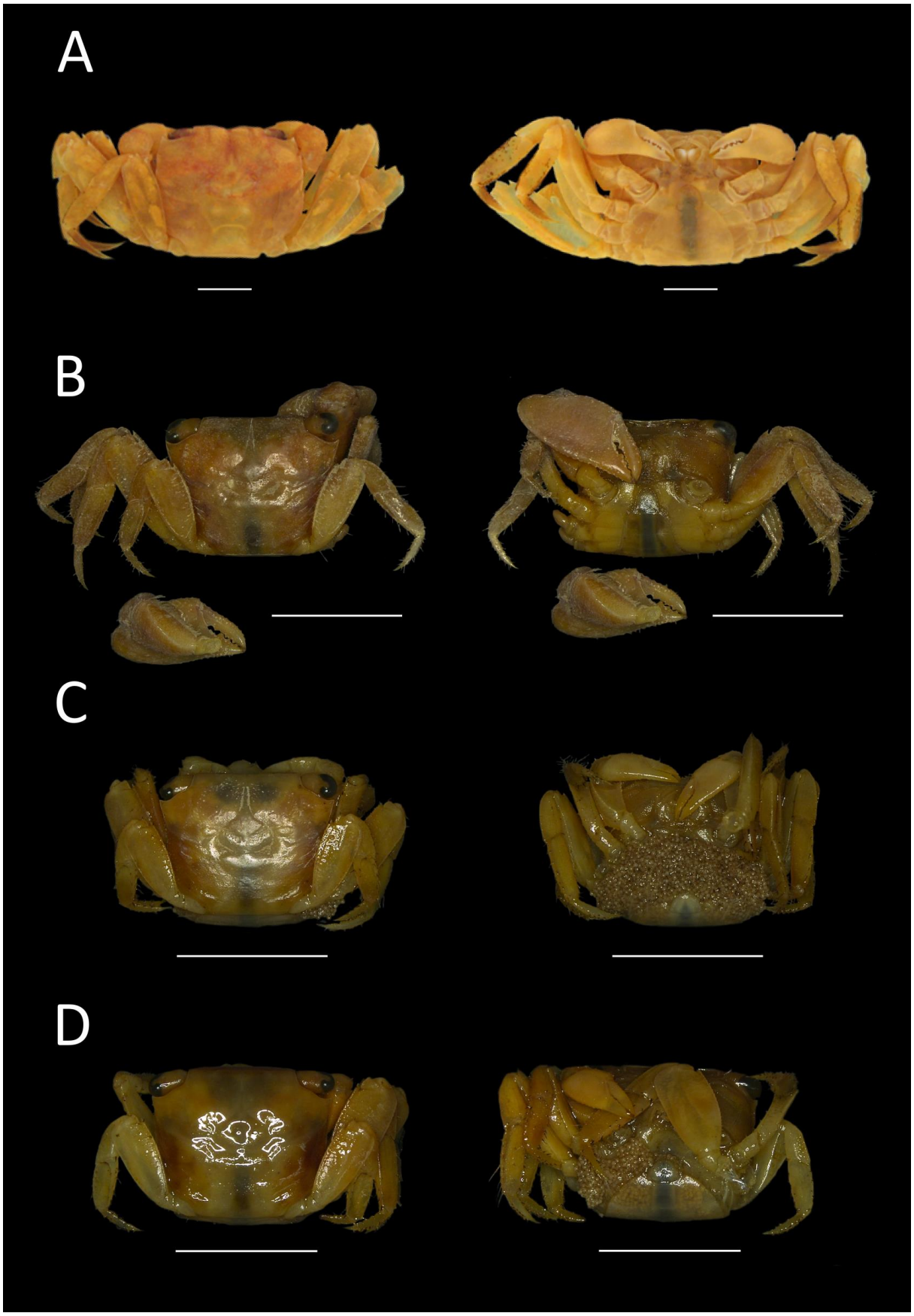


Figure A3-3: Specimen of *Sesarma bidentatum* Benedict, 1892. A, Prep. #19313. Three specimens of *Sesarma buettikoferi* De Man, 1883. B, Prep. #19302; C, Prep. #19303; D, Prep. #19304. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

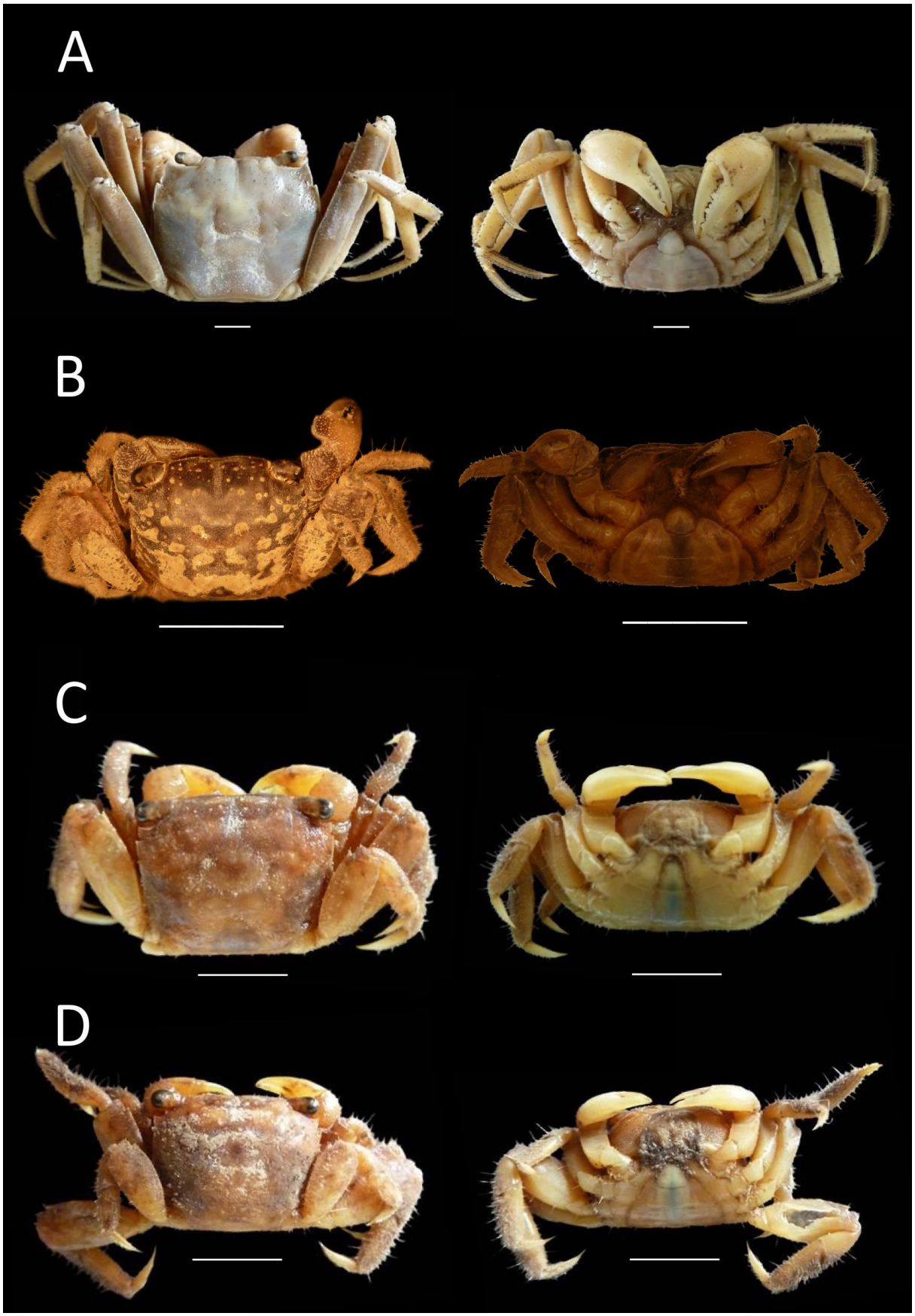


Figure A3-4: Specimen of *Sesarma cookie* Hartnoll, 1971. A, Prep. #19513. Three specimens of *Sesarma curacaoense* De Man, 1892. B, Prep. #19512; C, Prep. #19514; D, Prep. #19515. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

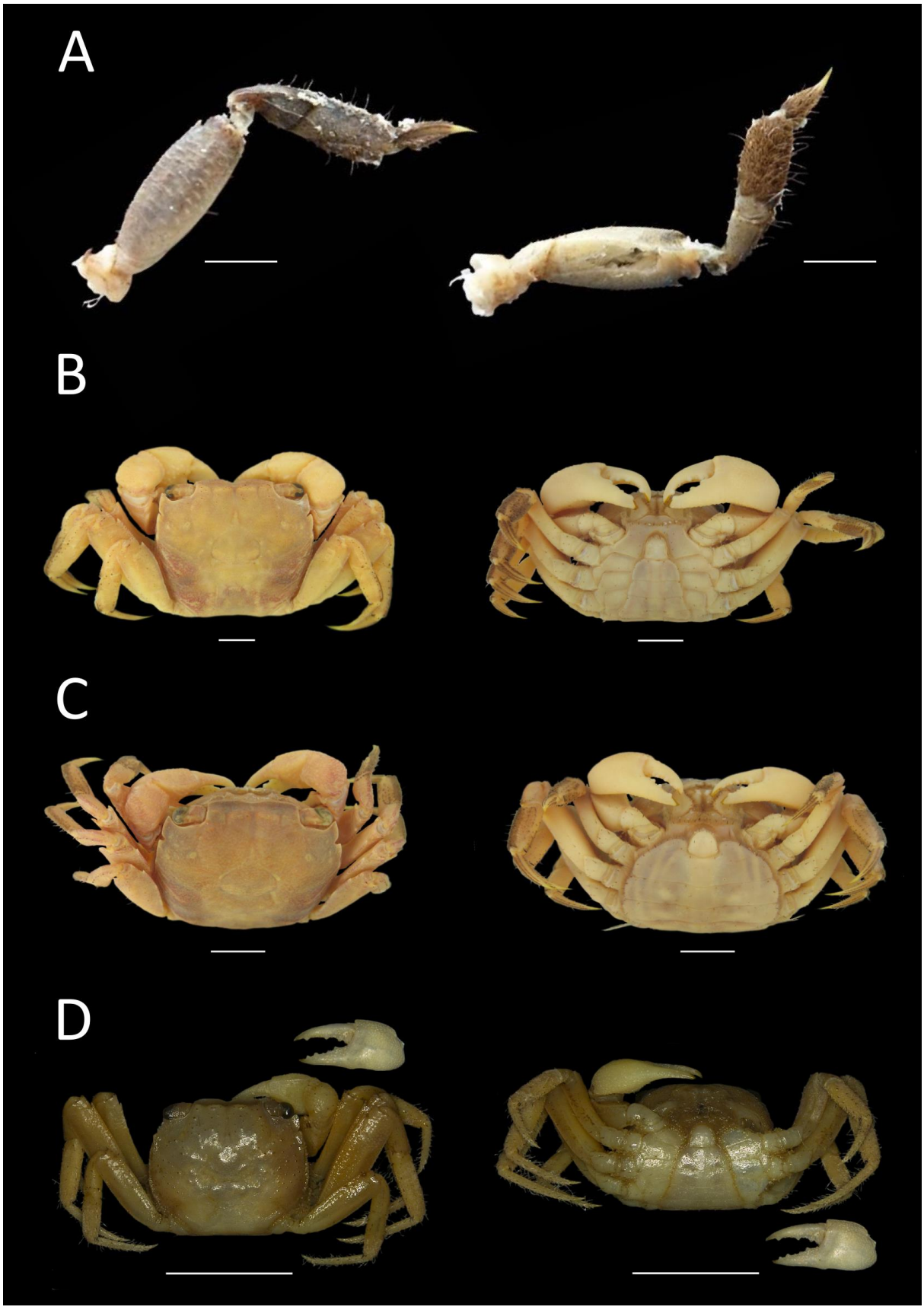


Figure A3-5: Specimen of *Sesarma dolphinum* Reimer, Schubart & Diesel, 1998. A, Prep. #19529. Two specimens of *Sesarma fossarum* Schubart, Reimer, Diesel & Türkay, 1997. B, Prep. #19307; C, Prep. #19308. Specimen of *Sesarma jarvisi* Rathbun, 1914. D, Prep. #19301. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

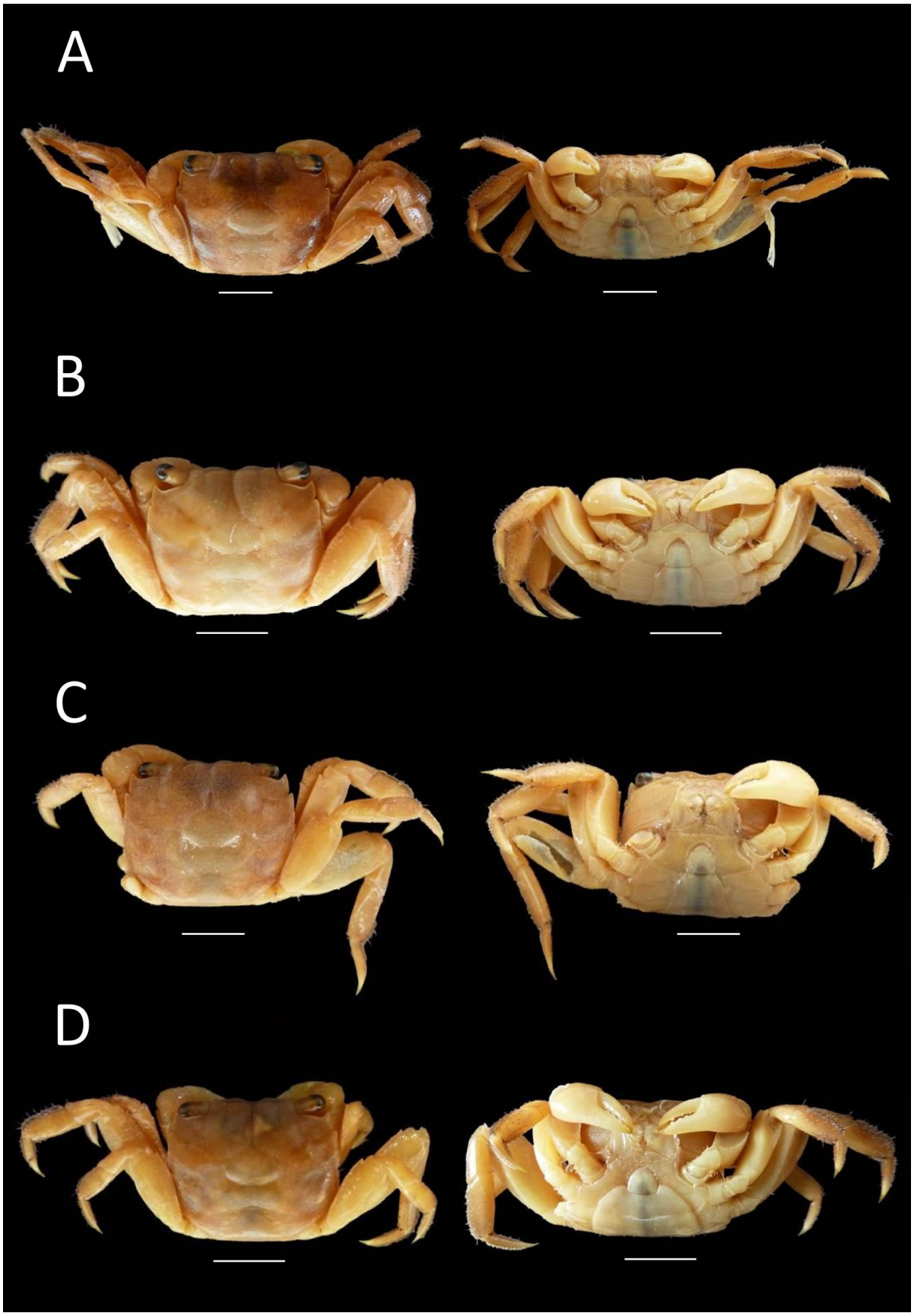


Figure A3-6: Specimens of *Sesarma meridies* Schubart & Koller, 2005. A, Prep. #19520; B, Prep. #19521; C, Prep. #19522; D, Prep. #19523. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

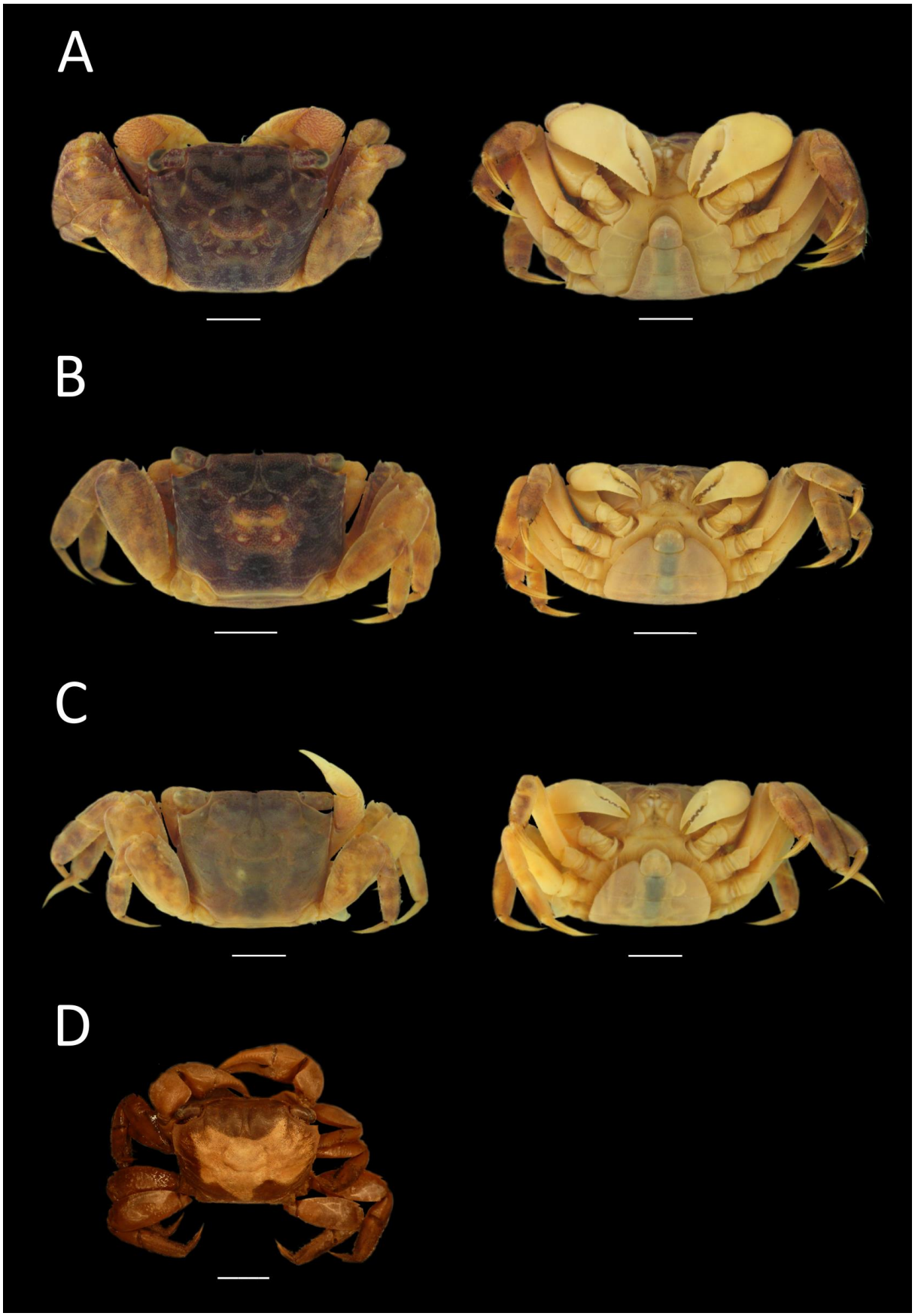


Figure A3-7: Specimen of *Sesarma meridies* Schubart & Koller, 2005. A, Prep. #19524. Three specimens of *Sesarma rectum* Randall, 1840. B, Prep. #19309; C, Prep. #19310; D, Prep. #19311. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

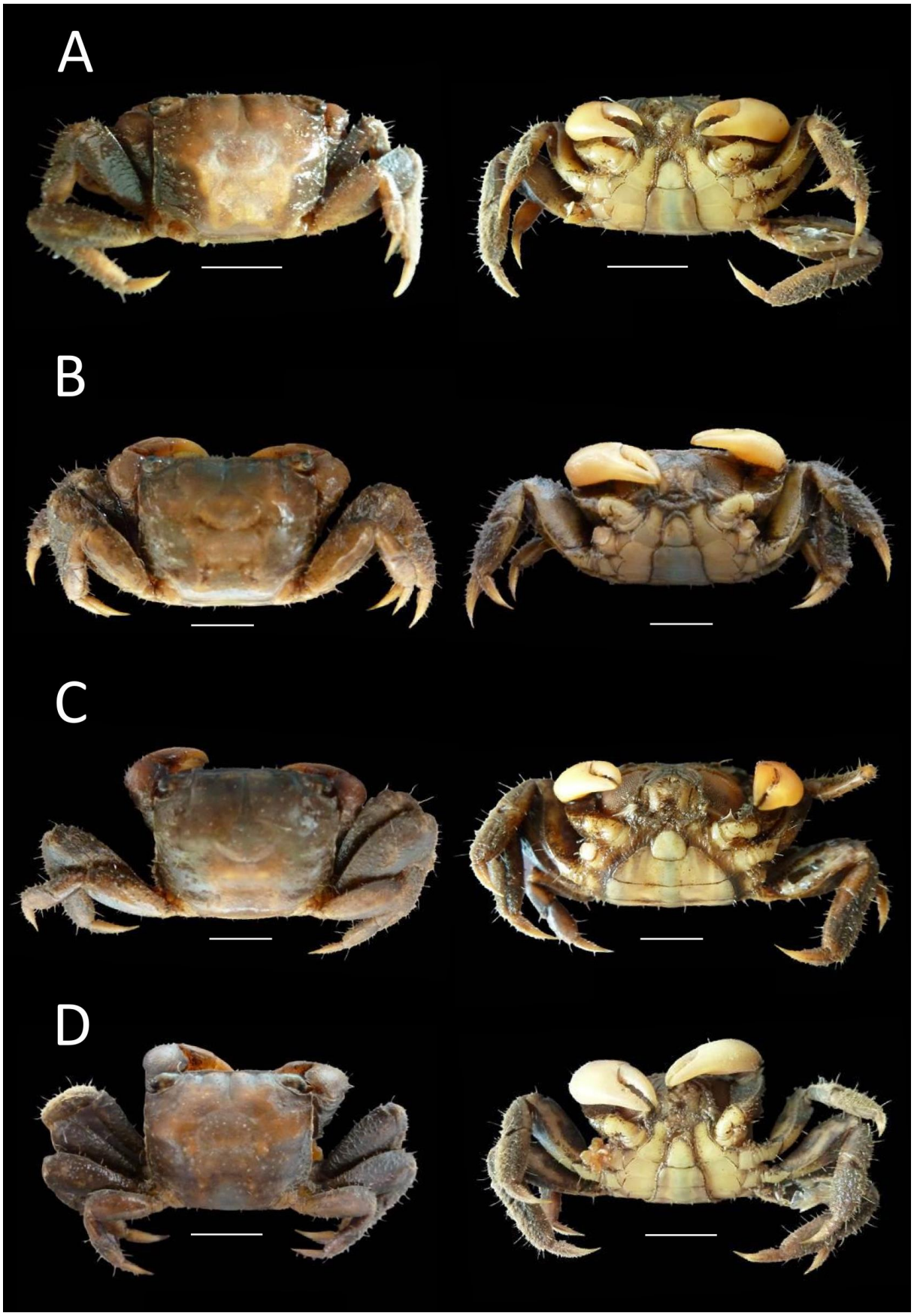


Figure A3-8: Specimens of *Sesarma* sp. (nr. *reticulatum*). A, Prep. #19525; B, Prep. #19526; C, Prep. #19527; D, Prep. #19528. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

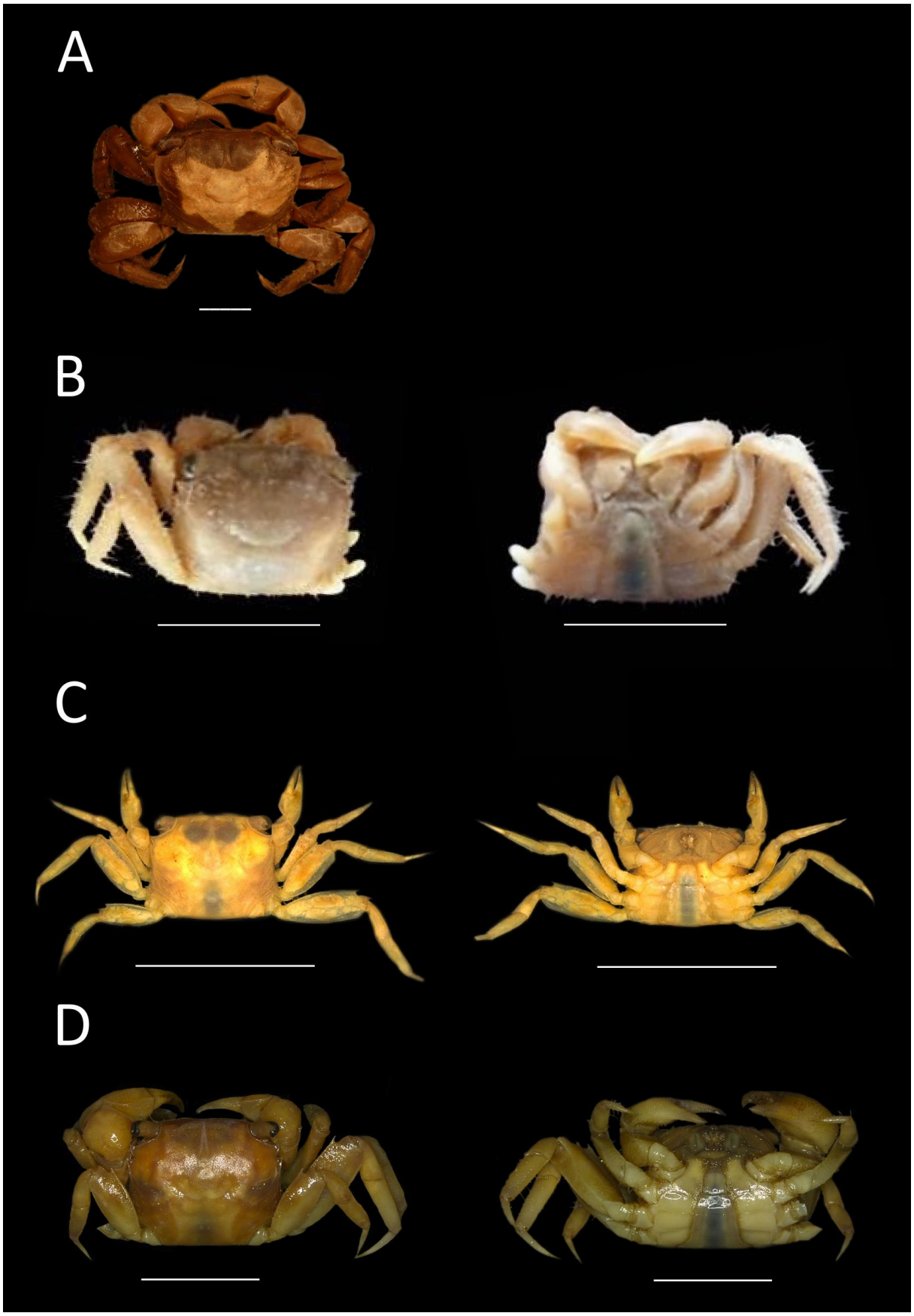


Figure A3-9: Specimen of *Sesarma reticulatum* (Say, 1817). A, Prep. #19519. Three specimens of *Sesarma rhizophorae* Rathbun, 1906. B, Prep. #19530; C, Prep. #18151; D, Prep. #19318. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

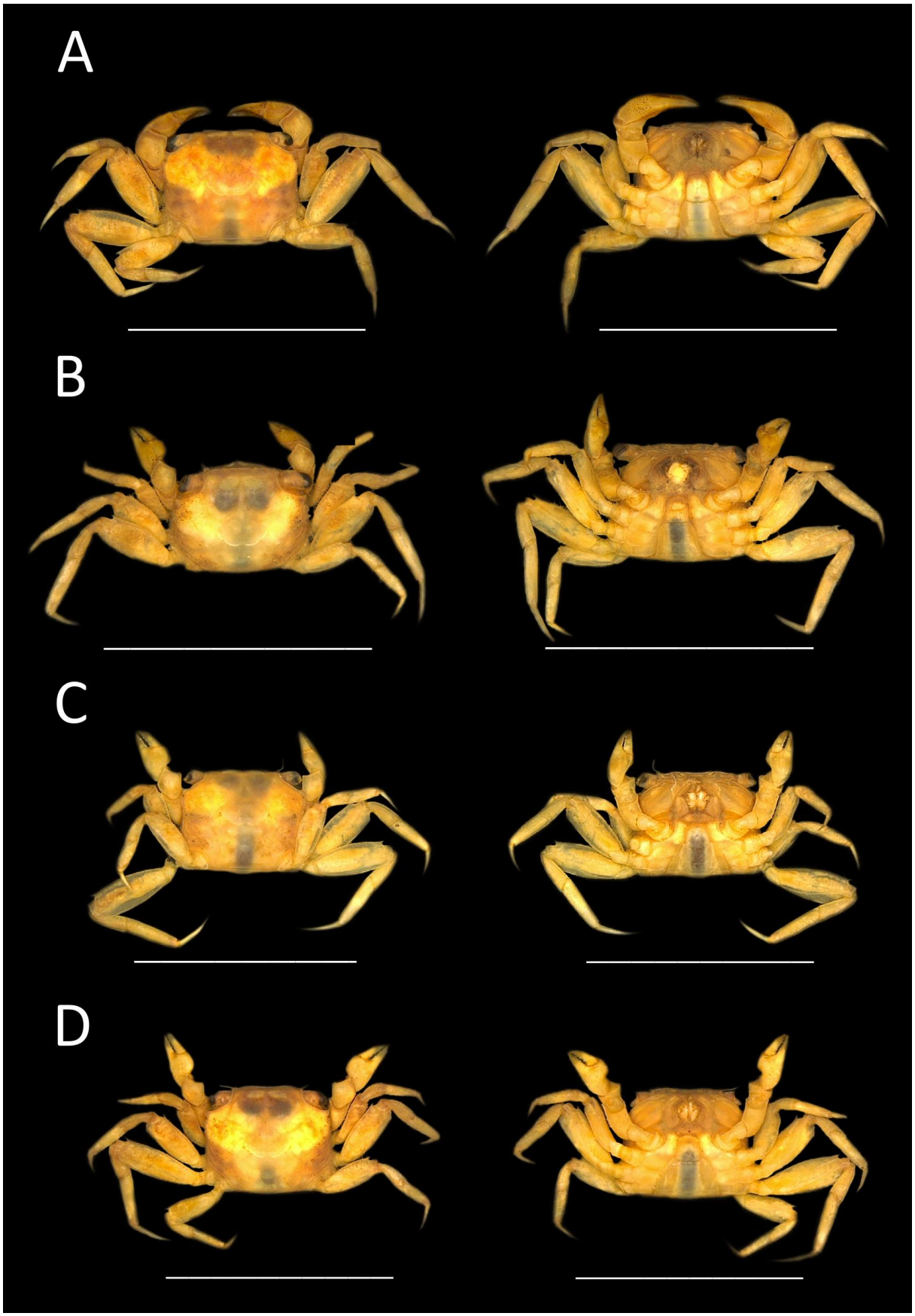


Figure A3-10: Specimens of *Sesarma rhizophorae* Rathbun, 1906. A, Prep. #18142; B, Prep. #18144; C, Prep. #18145; D, Prep. #18146. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

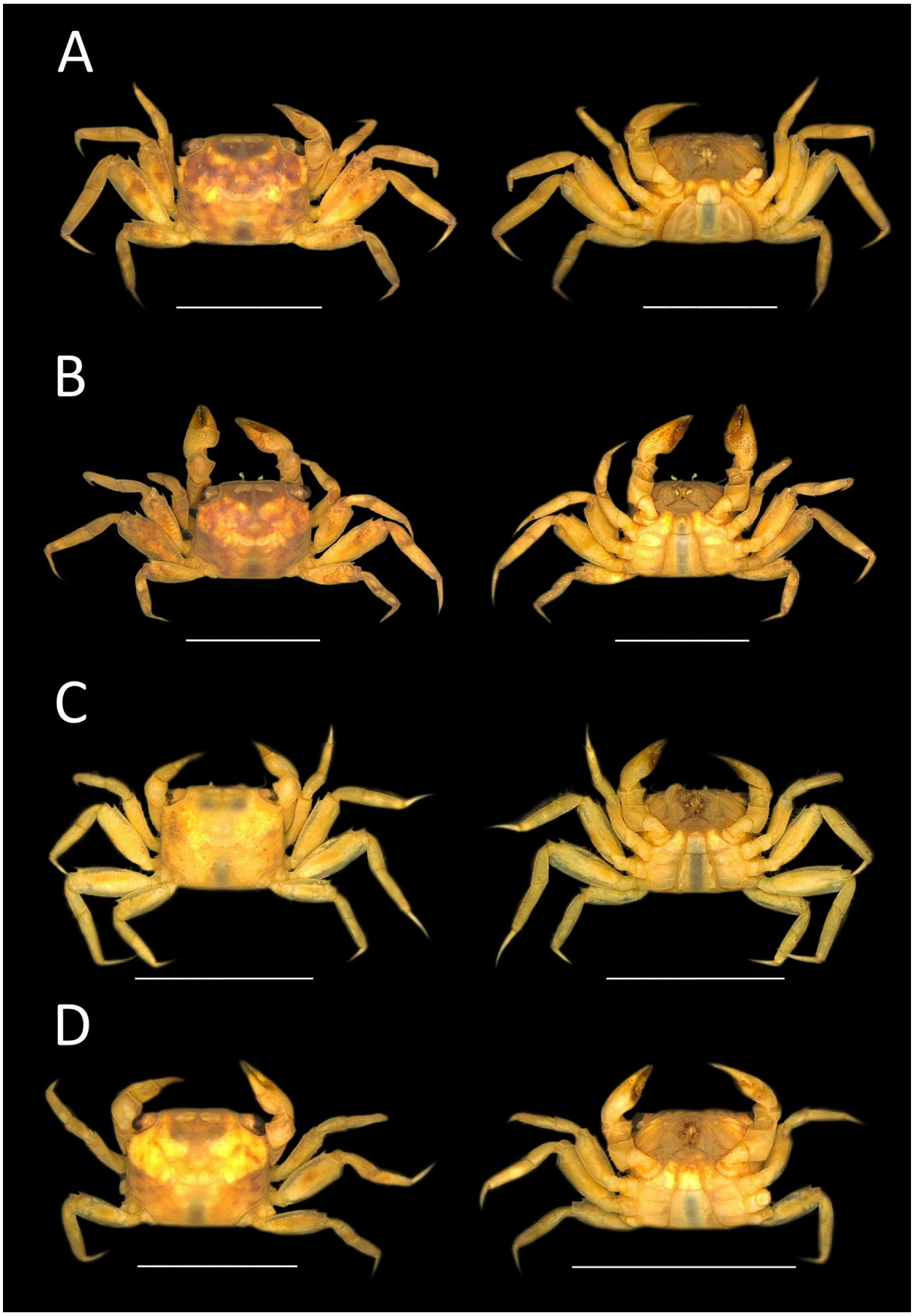


Figure A3-11: Specimens of *Sesarma rhizophorae* Rathbun, 1906. A, Prep. #18147; B, Prep. #18148; C, Prep. #18149; D, Prep. #18150. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

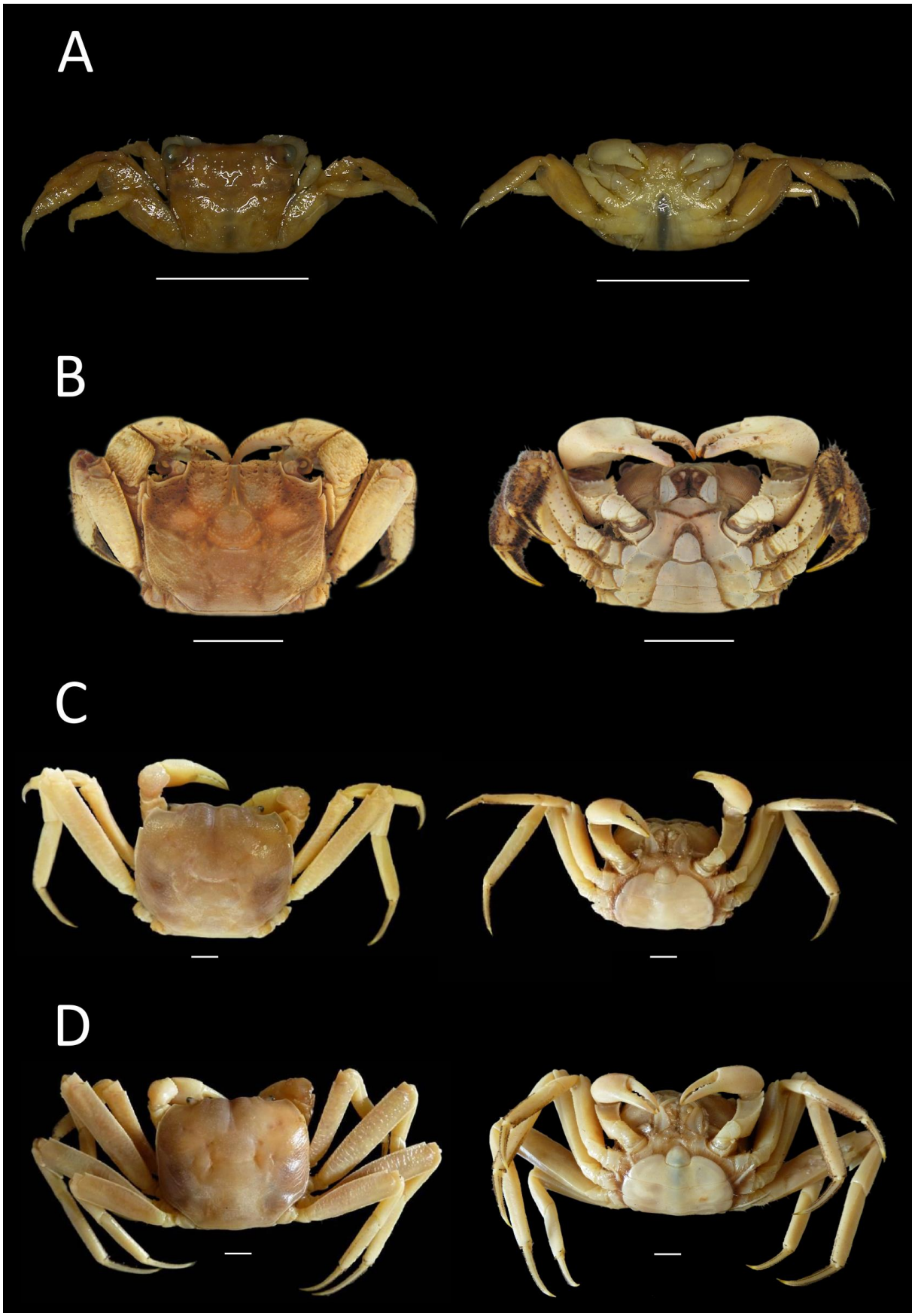


Figure A3-12: Specimen of *Sesarma rubinofforum* Abele, 1973. A, Prep. #19319. Specimen of *Sesarma sulcatum* Smith, 1870. B, Prep. #19297, #19641. Two specimens of *Sesarma verleyi* Rathbun, 1914. C, Prep. #19516; D, Prep. #19517. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

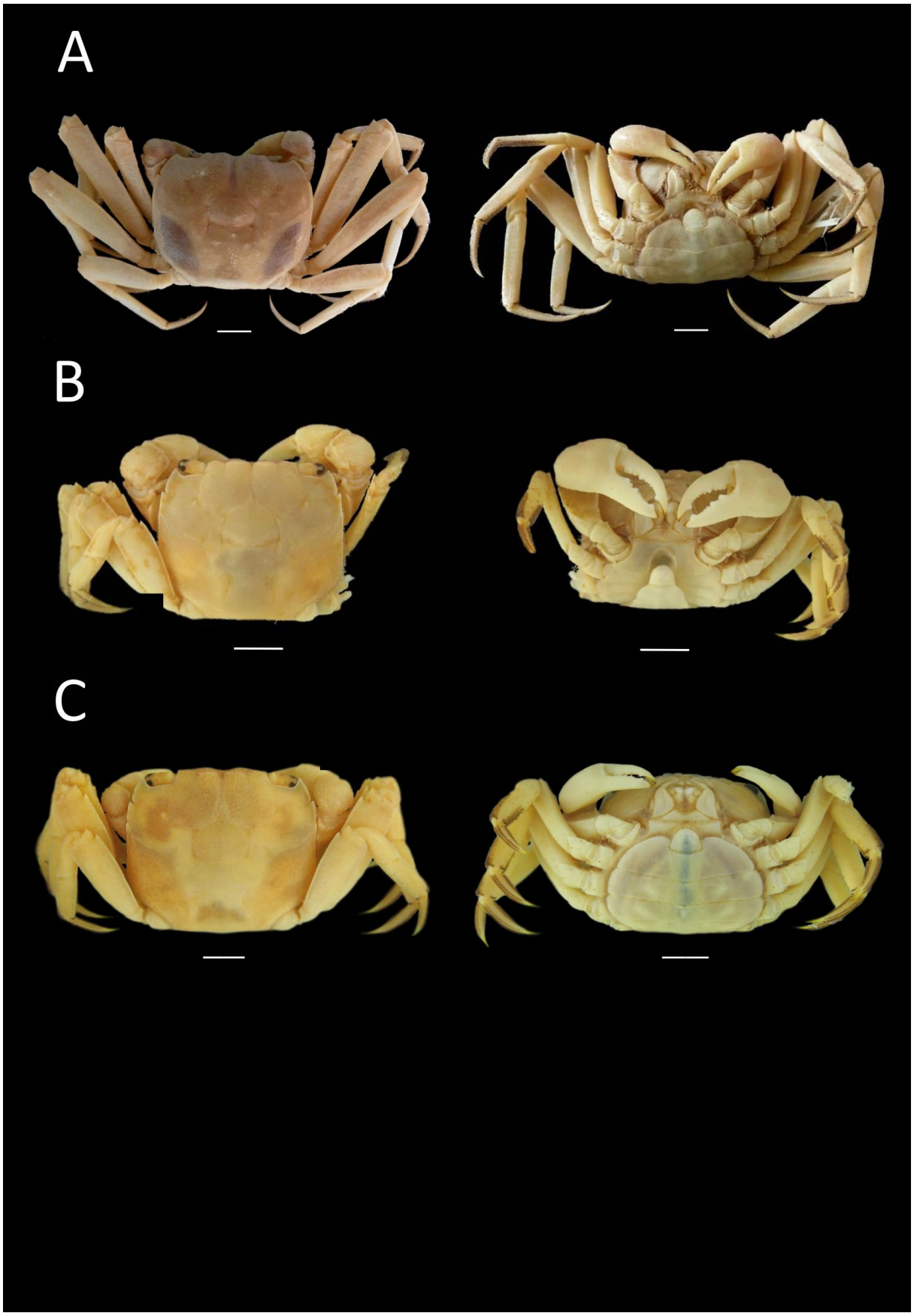


Figure A3-13: Specimen of *Sesarma verleyi* Rathbun, 1914. A, Prep. #19518. Two specimens of *Sesarma windsor* Türkay & Diesel, 1994. B, Prep. #19305; C, Prep. #19306. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

A3.2 Genus *Eurytium* Stimpson, 1859

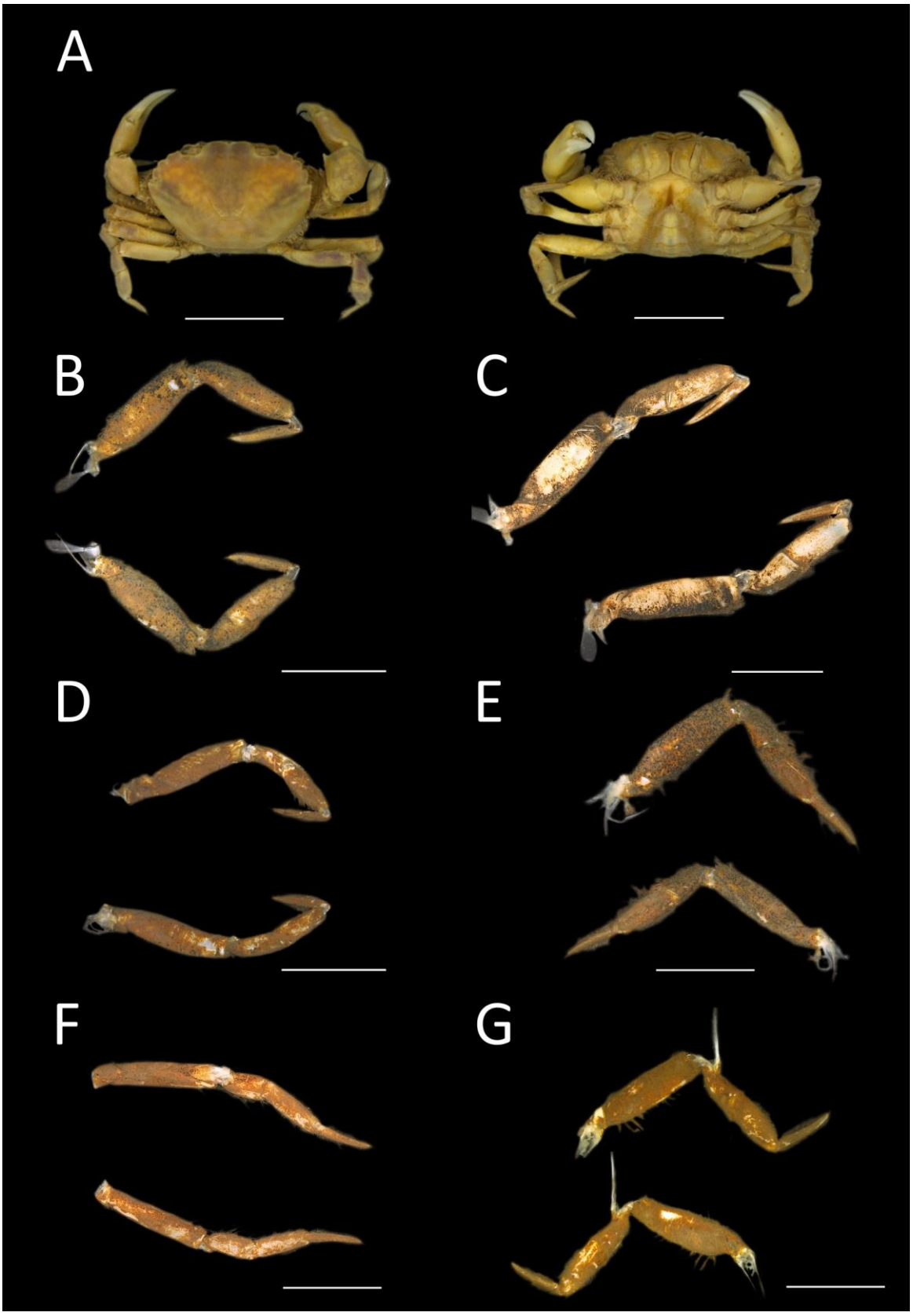


Figure A3-14: Specimen of *Eurytium affine* (Streets & Kingsley, 1877). A, Prep. #19748. Legs of *Eurytium limosum* (Say, 1818). B, Prep. #18131; C, Prep. #18132; D, Prep. #18134; E, Prep. #18135; F, Prep. #18137; G, Prep. #18138. The specimen and legs are shown in dorsal and ventral views. The scale bar represents 1 cm.

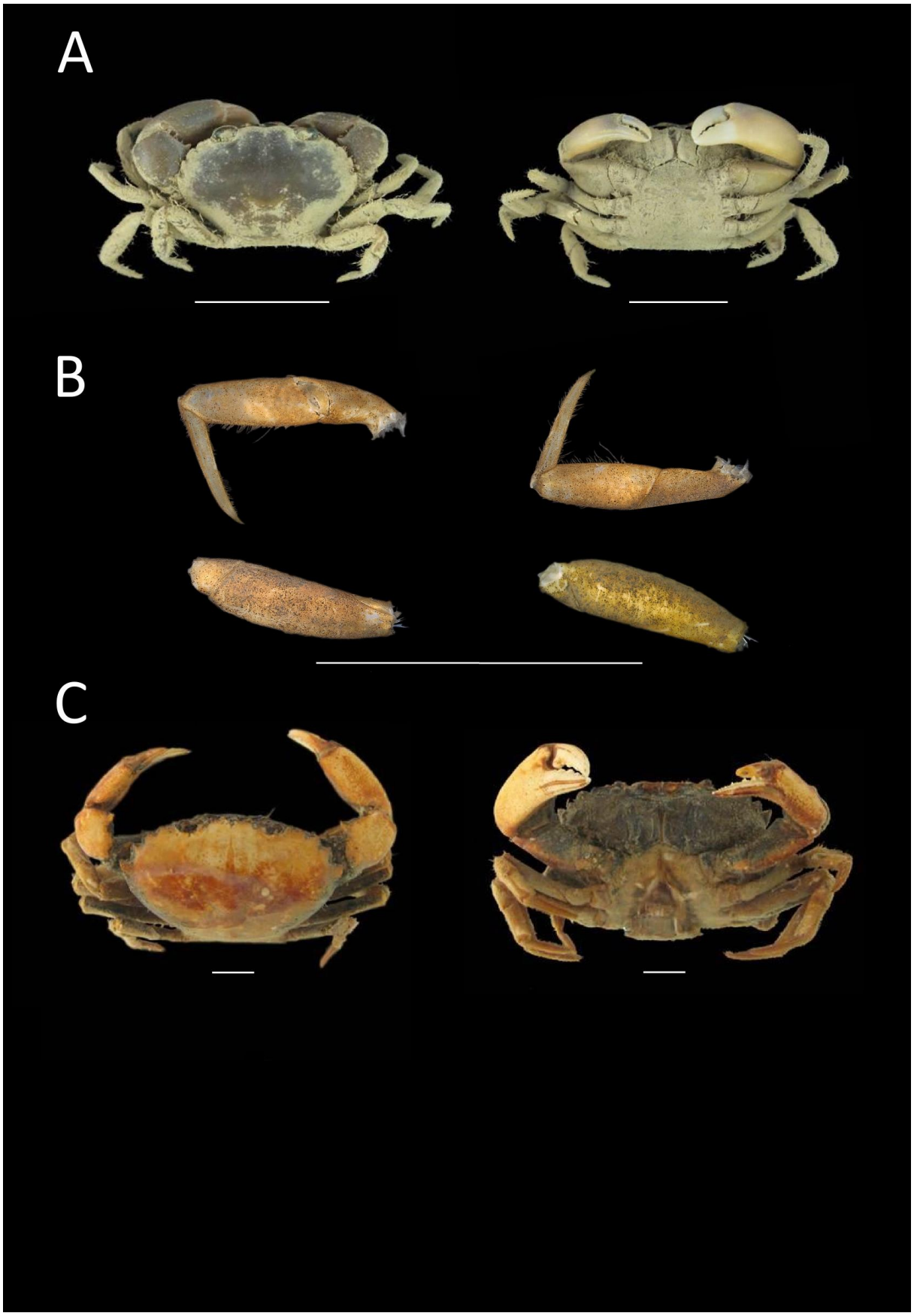


Figure A3-15: Specimen of *Eurytium limosum* (Say, 1818). A, Prep. #19743. Legs of *Eurytium tristani* Rathbun, 1906. B, Prep. #19862. Specimen of *Eurytium tristani* Rathbun, 1906. C, Prep. #11618. The specimens and legs are shown in dorsal and ventral views. The scale bar represents 1 cm.

A3.3 Genus *Panopeus* H. Milne Edwards, 1834

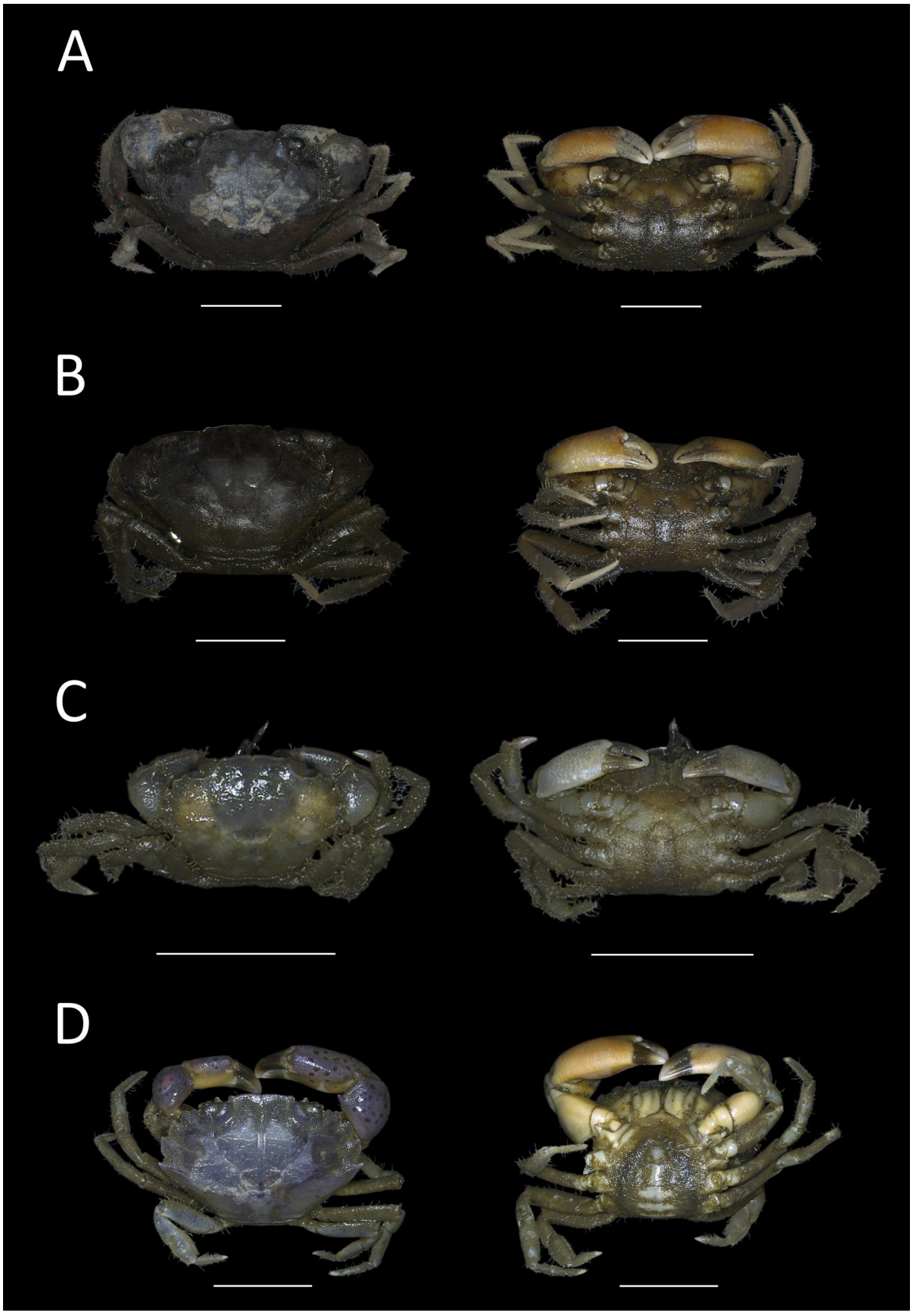


Figure A3-16: Specimens of *Panopeus* sp. A, Prep. #16138; B, Prep. #16139; C, Prep. #16140; D, Prep. #16141. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

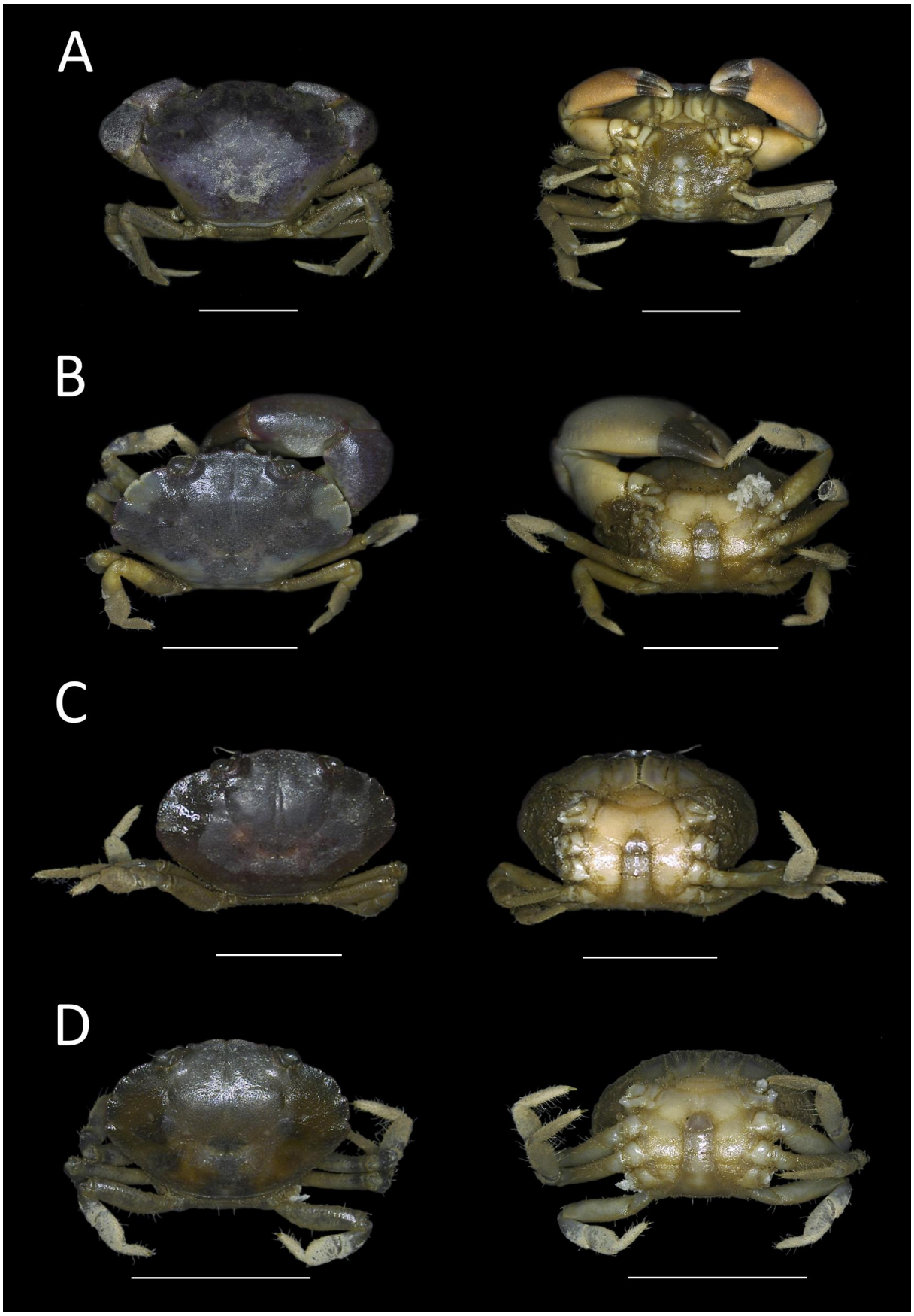


Figure A3-17: Specimens of *Panopeus* sp. A, Prep. #16142; B, Prep. #16143; C, Prep. #16144; D, Prep. #16145. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

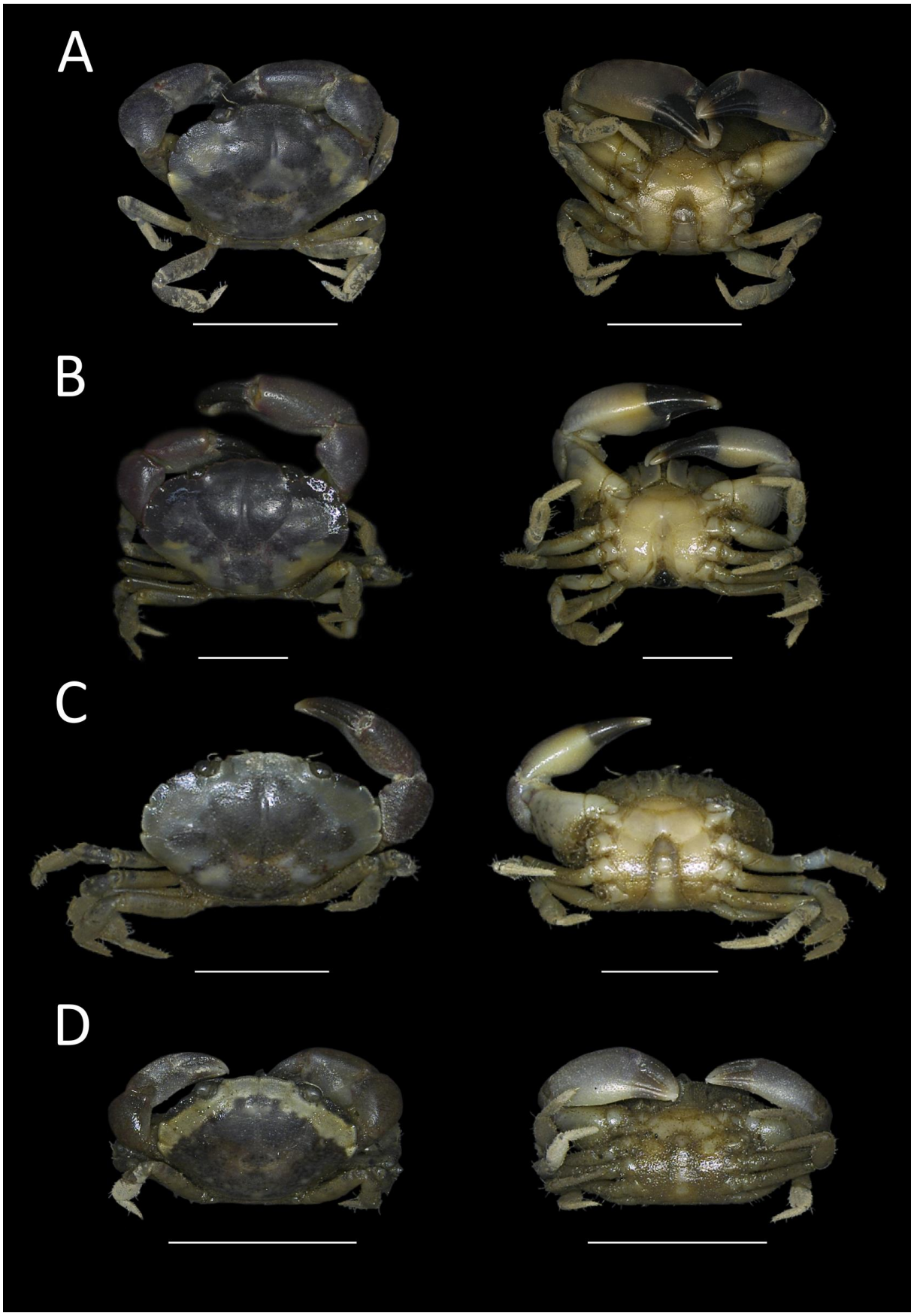


Figure A3-18: Specimens of *Panopeus* sp. A, Prep. #16146; B, Prep. #16147; C, Prep. #16148; D, Prep. #16149. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

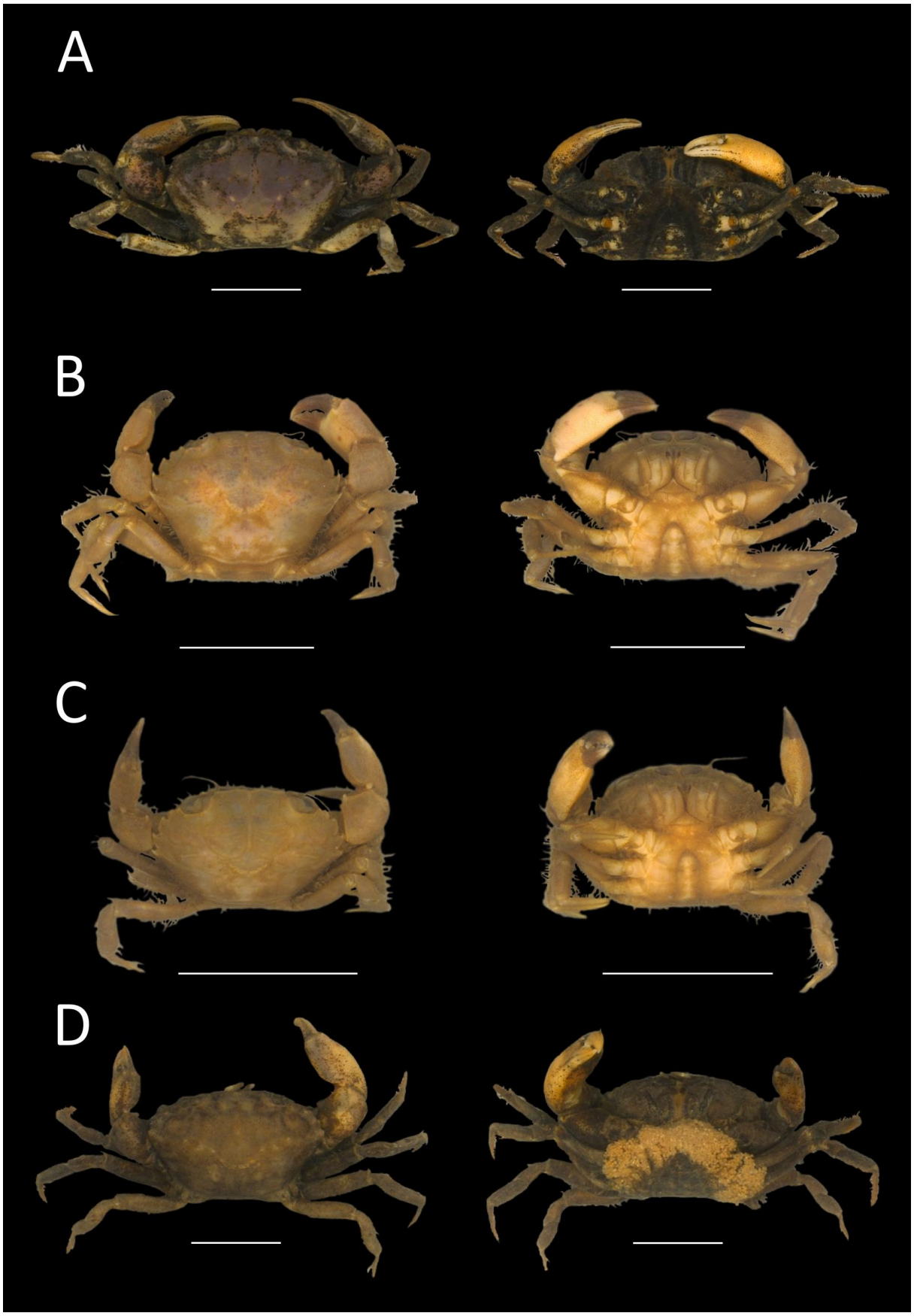


Figure A3-19: Specimens of *Panopeus* sp. A, Prep. #19625; B, Prep. #19754; C, Prep. #19755; D, Prep. #19756. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

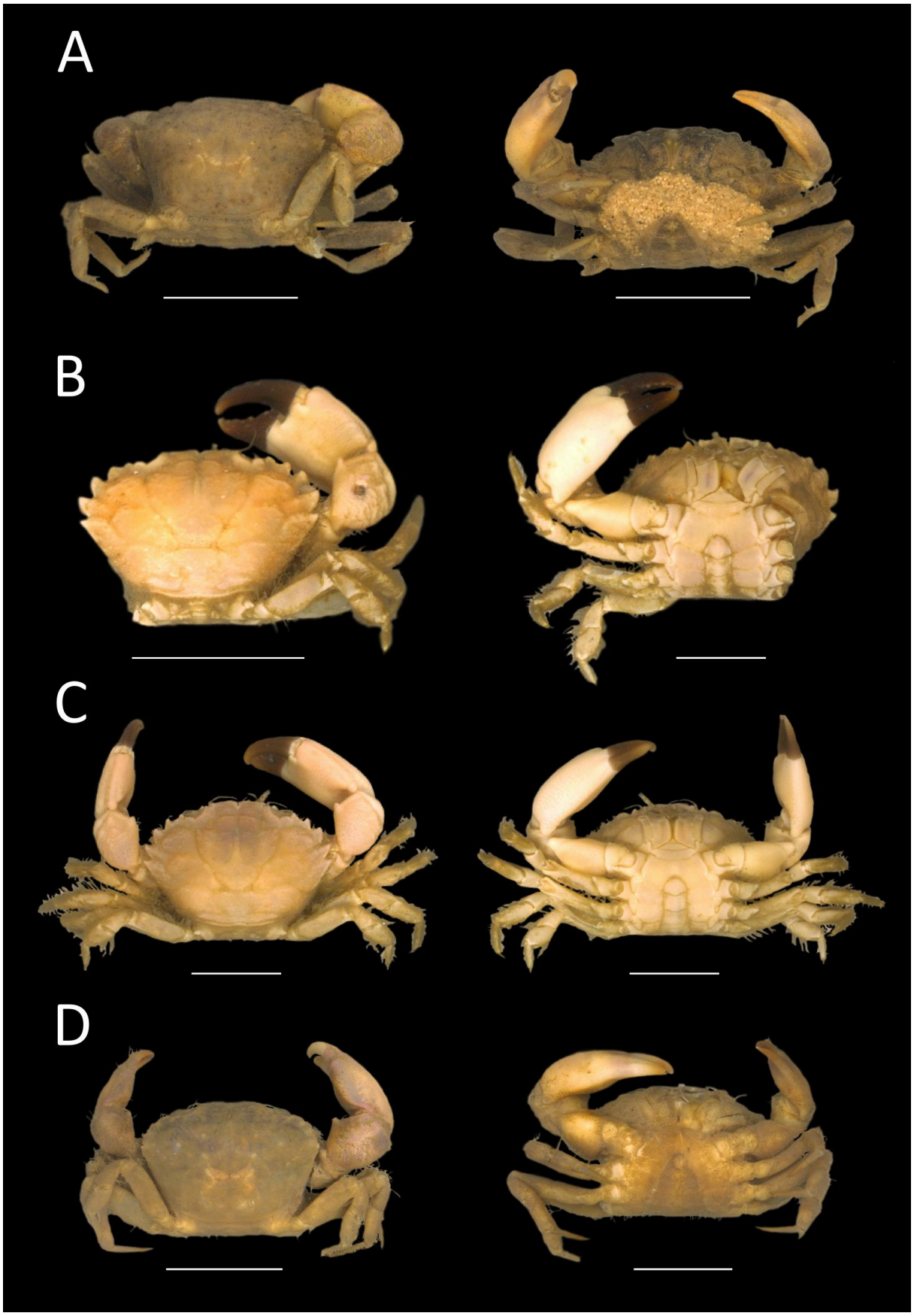


Figure A3-20: Specimens of *Panopeus* sp. A, Prep. #19757; B, Prep. #19758; C, Prep. #19759; D, Prep. #19760. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

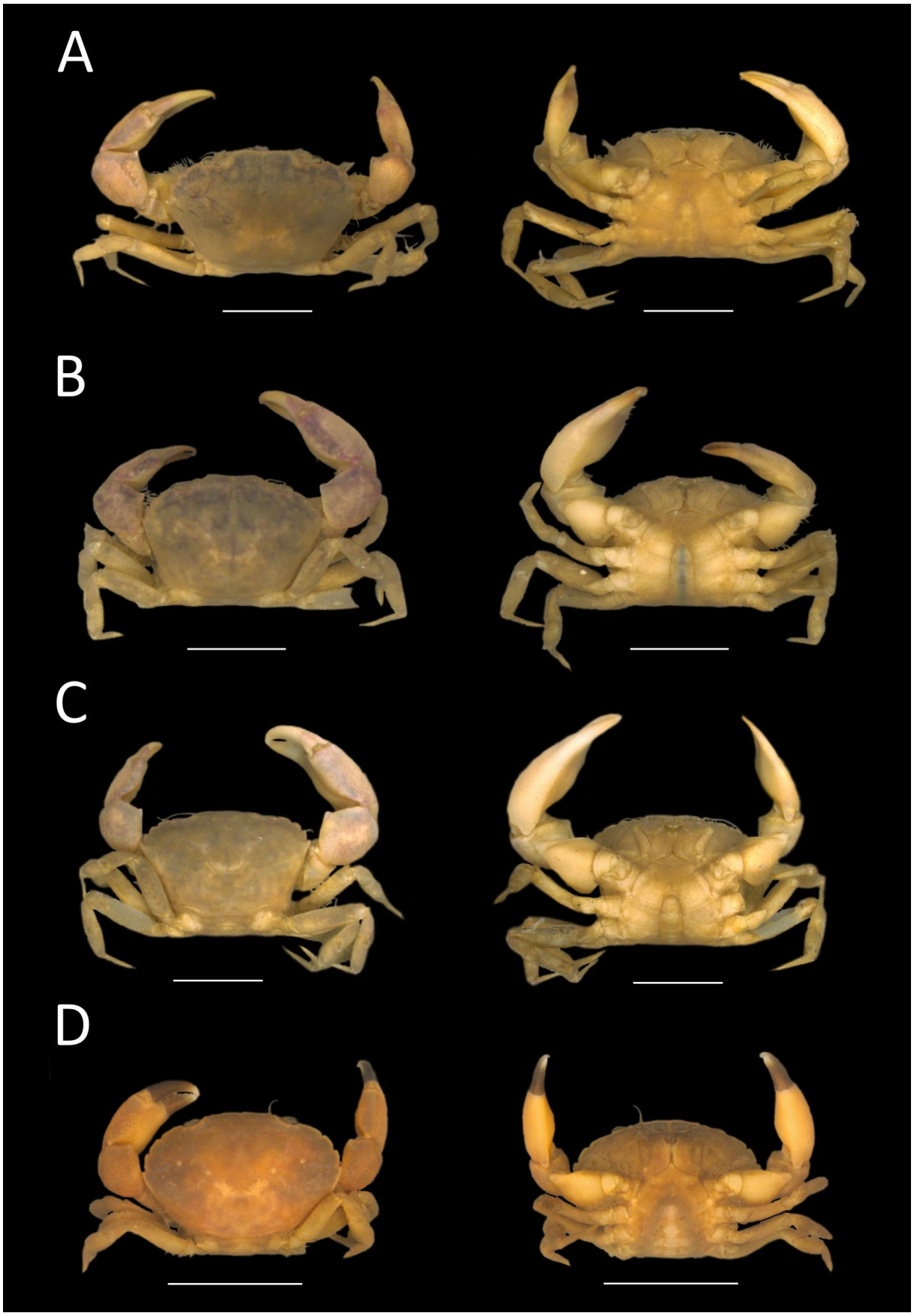


Figure A3-21: Specimens of *Panopeus* sp. A, Prep. #19761; B, Prep. #19762; C, Prep. #19763; D, Prep. #19764. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

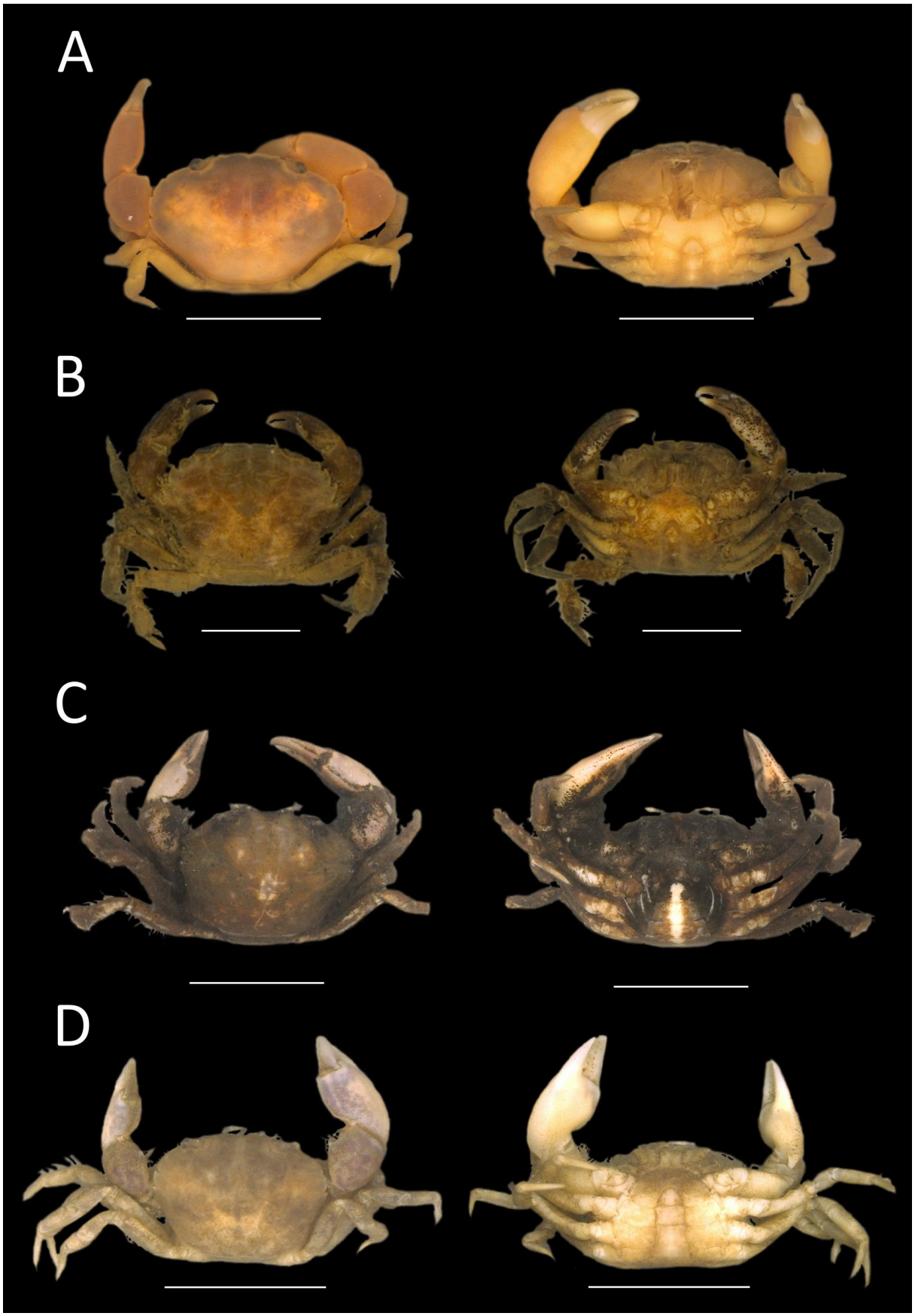


Figure A3-22: Specimens of *Panopeus* sp. A, Prep. #19765; B, Prep. #19863; C, Prep. #19864; D, Prep. #19865. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

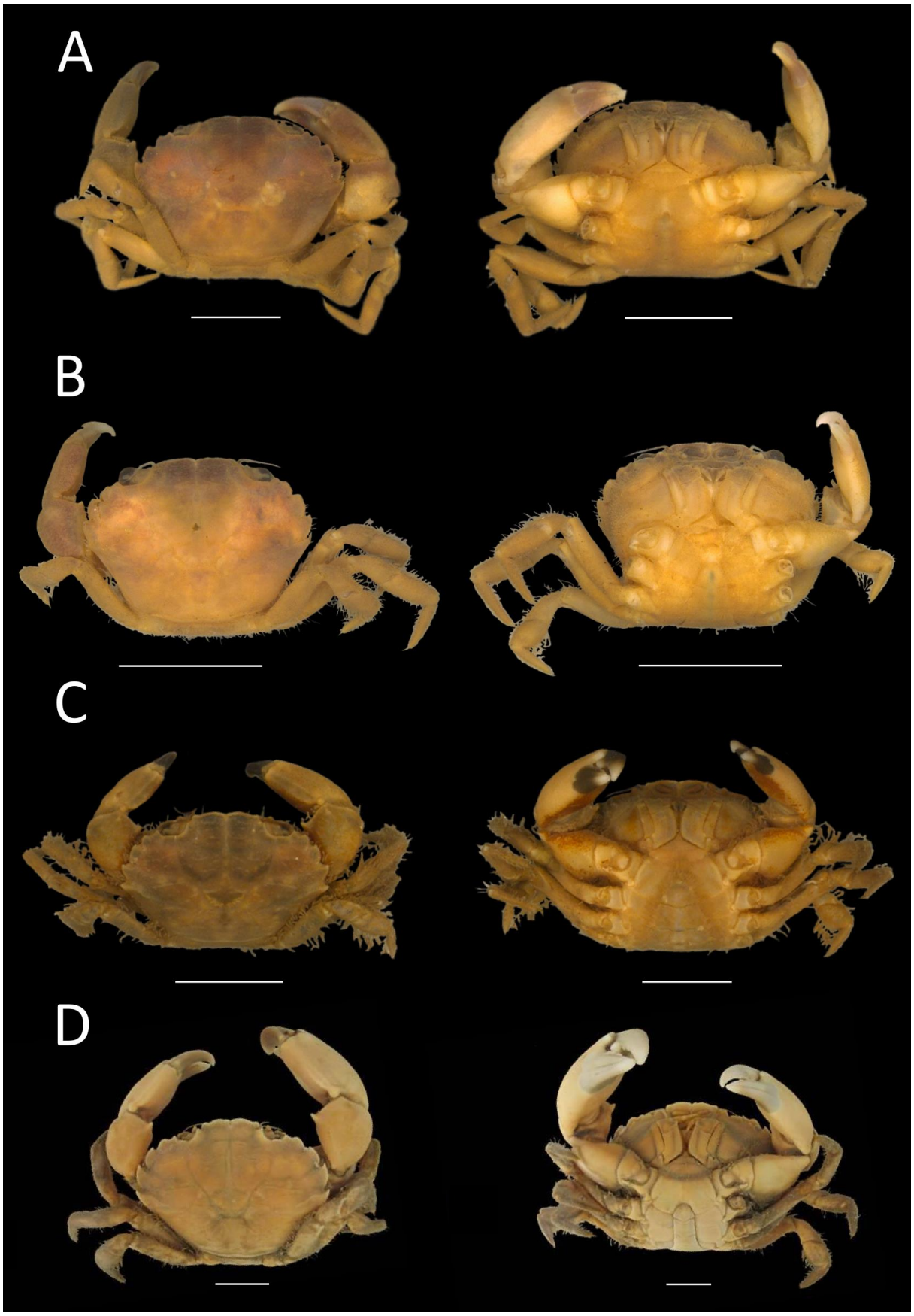


Figure A3-23: Two specimens of *Panopeus* sp. A, Prep. #19866; B, Prep. #19867. Specimen of *Panopeus africanus* A. Milne-Edwards, 1867. C, Prep. #19624. Specimen of *Panopeus americanus* De Saussure, 1857. D, Prep. #19242. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

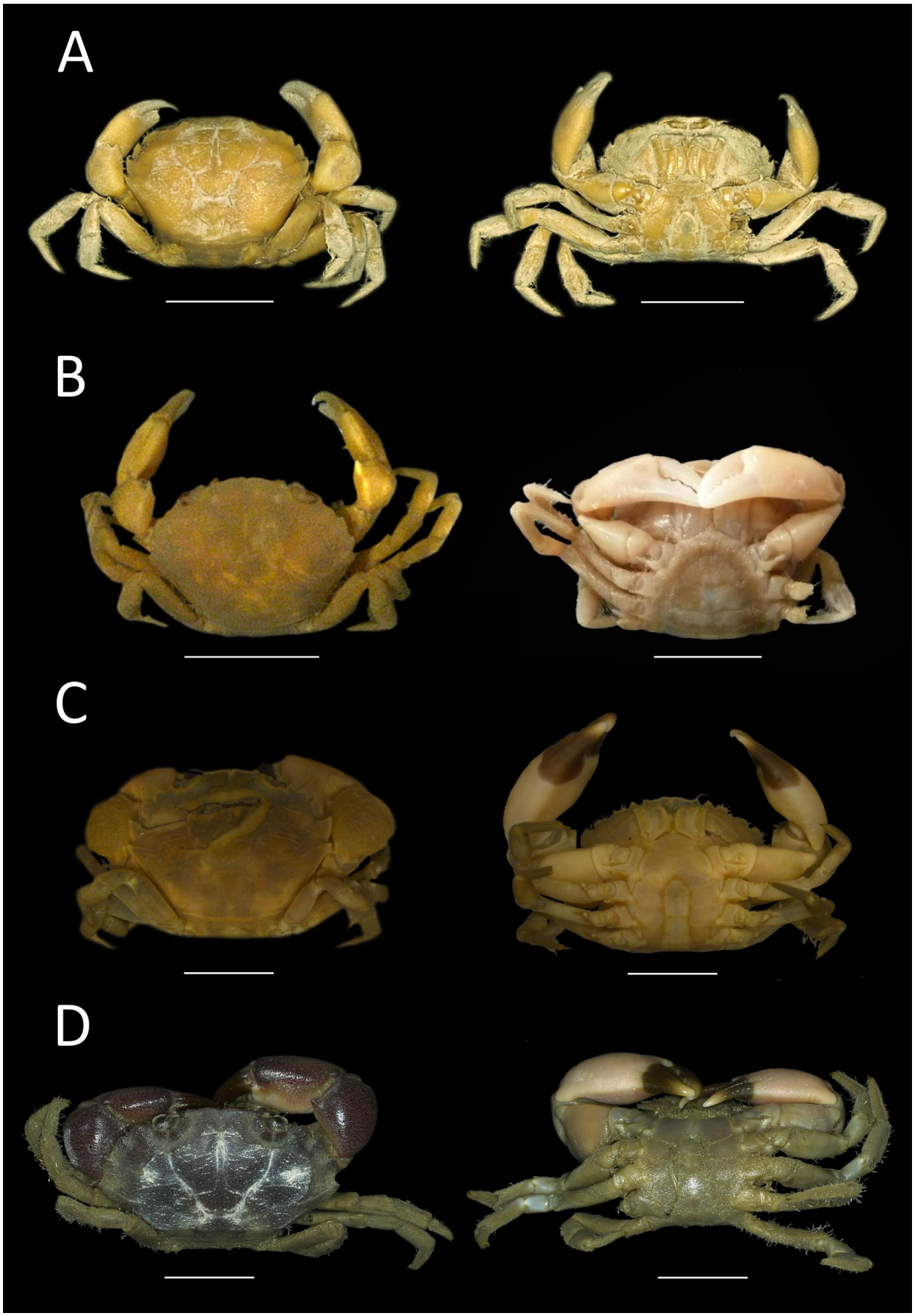


Figure A3-24: Three specimens of *Panopeus americanus* De Saussure, 1857. A, Prep. #19752; B, Prep. #19753; C, Prep. #19623. Specimen of *Panopeus austrobesus* Williams, 1983. D, Prep. #16151. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

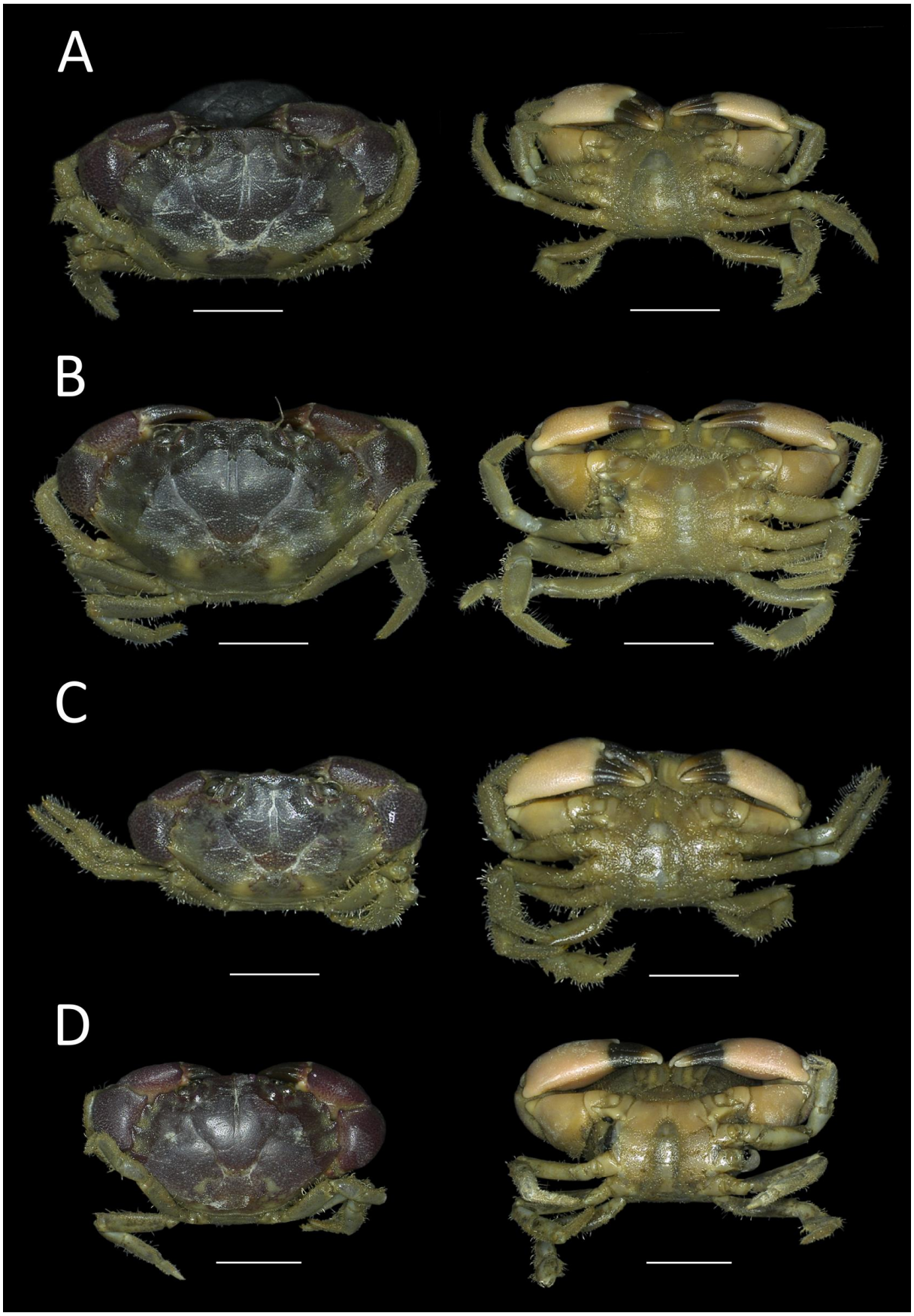


Figure A3-25: Specimens of *Panopeus austrobesus* Williams, 1983. A, Prep. #16152; B, Prep. #16153; C, Prep. #16154; D, Prep. #16155. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

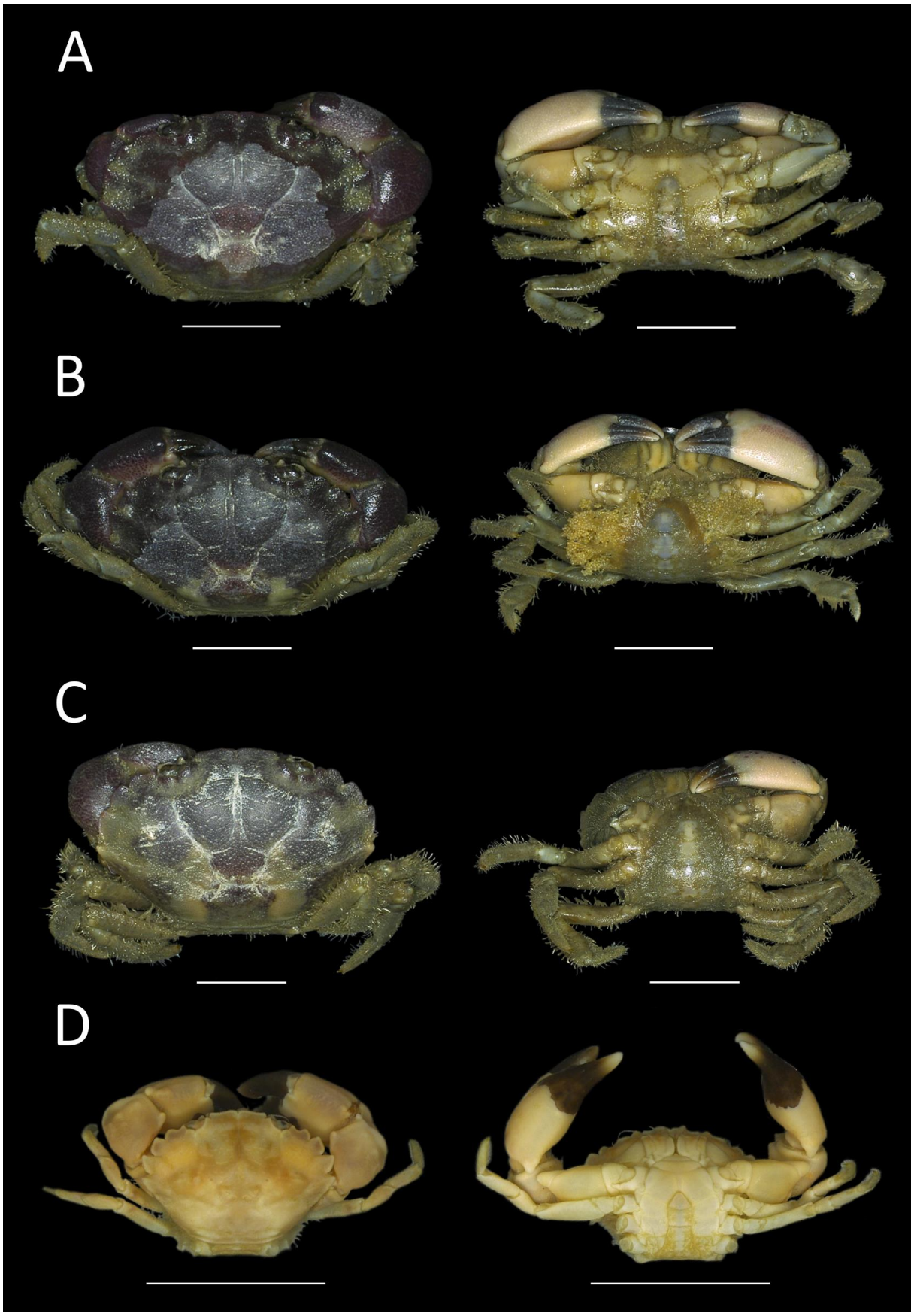


Figure A3-26: Three specimens of *Panopeus austrobesus* Williams, 1983. A, Prep. #16156; B, Prep. #16157; C, Prep. #16158. Specimen of *Panopeus bermudensis* Benedict & Rathbun, 1891. D, Prep. #19745. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

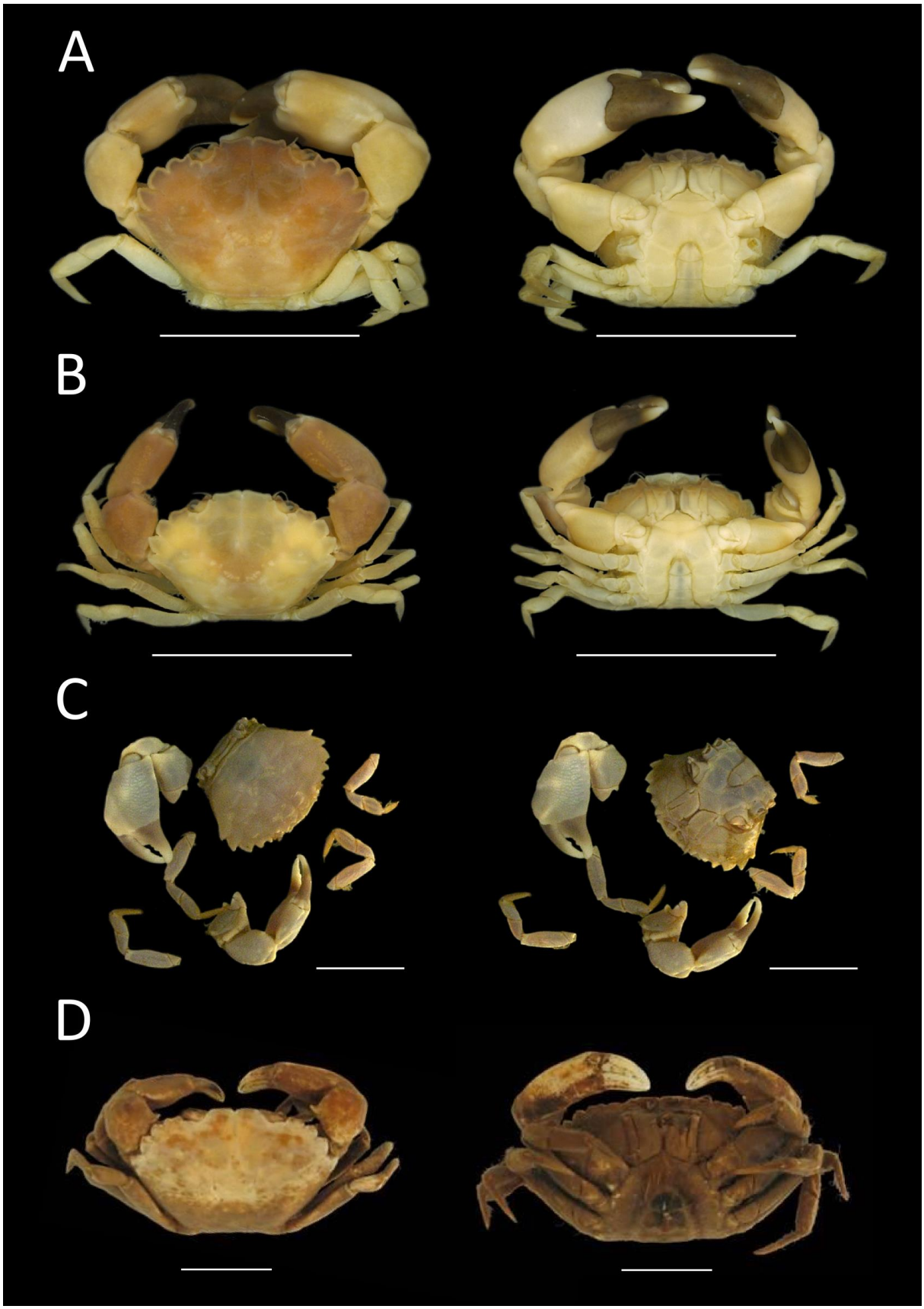


Figure A3-27: Two specimens of *Panopeus bermudensis* Benedict & Rathbun, 1891. A, Prep. #19746; B, Prep. #19747. Legs of *Panopeus chilensis* H. Milne Edwards & Lucas, 1843. C, Prep. #19246, #19627. Specimen of *Panopeus convexus* A. Milne-Edwards, 1880. D, Prep. #19240, #19630. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

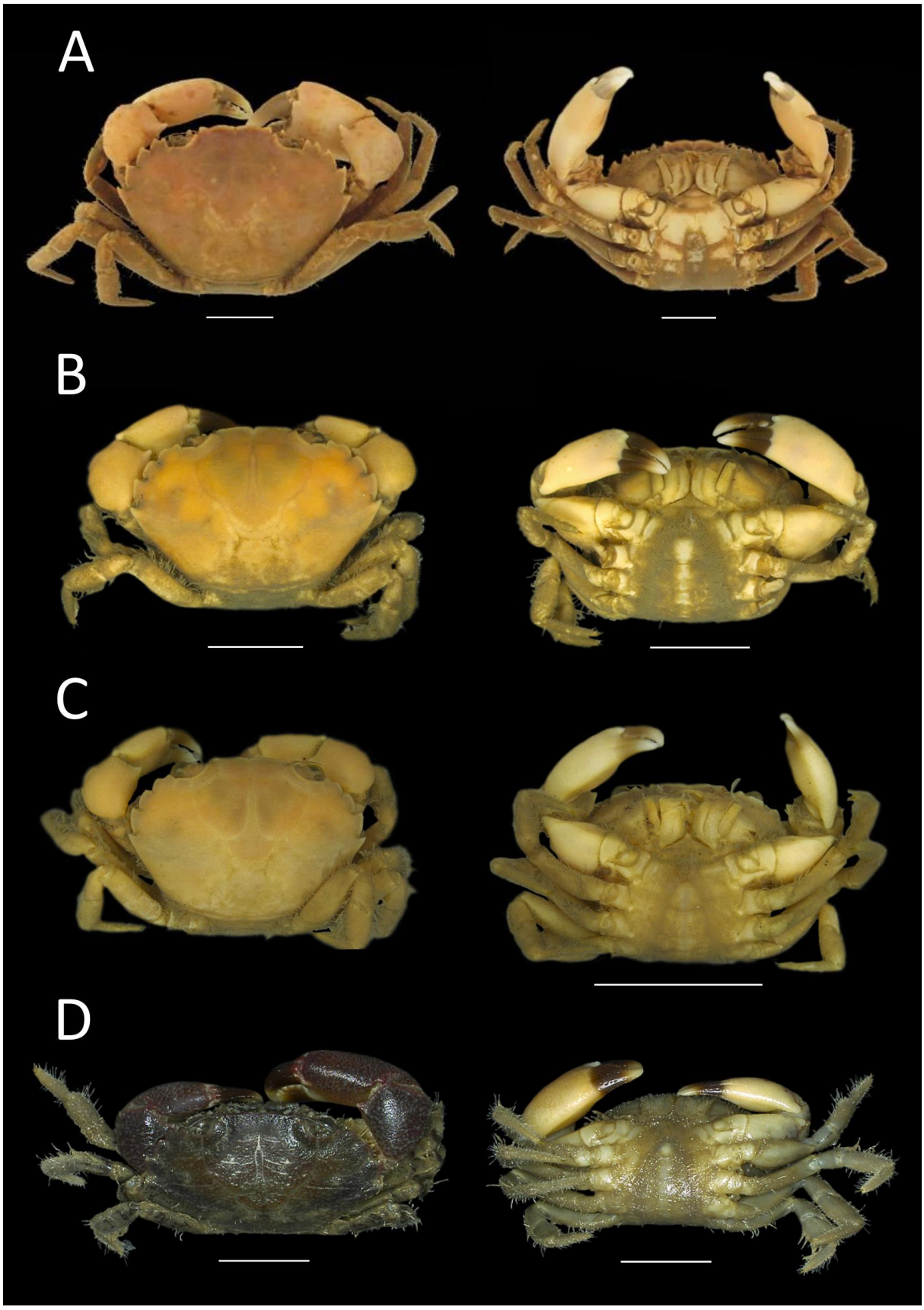


Figure A3-28: Specimen of *Panopeus hartii* Smith, 1869. A, Prep. #19631. Two specimens of *Panopeus herbstii* H. Milne Edwards, 1834. B, Prep. #19749; C, Prep. #19750. Specimen of *Panopeus lacustris* Desbonne, in Desbonne & Schramm, 1867. D, Prep. #16159. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

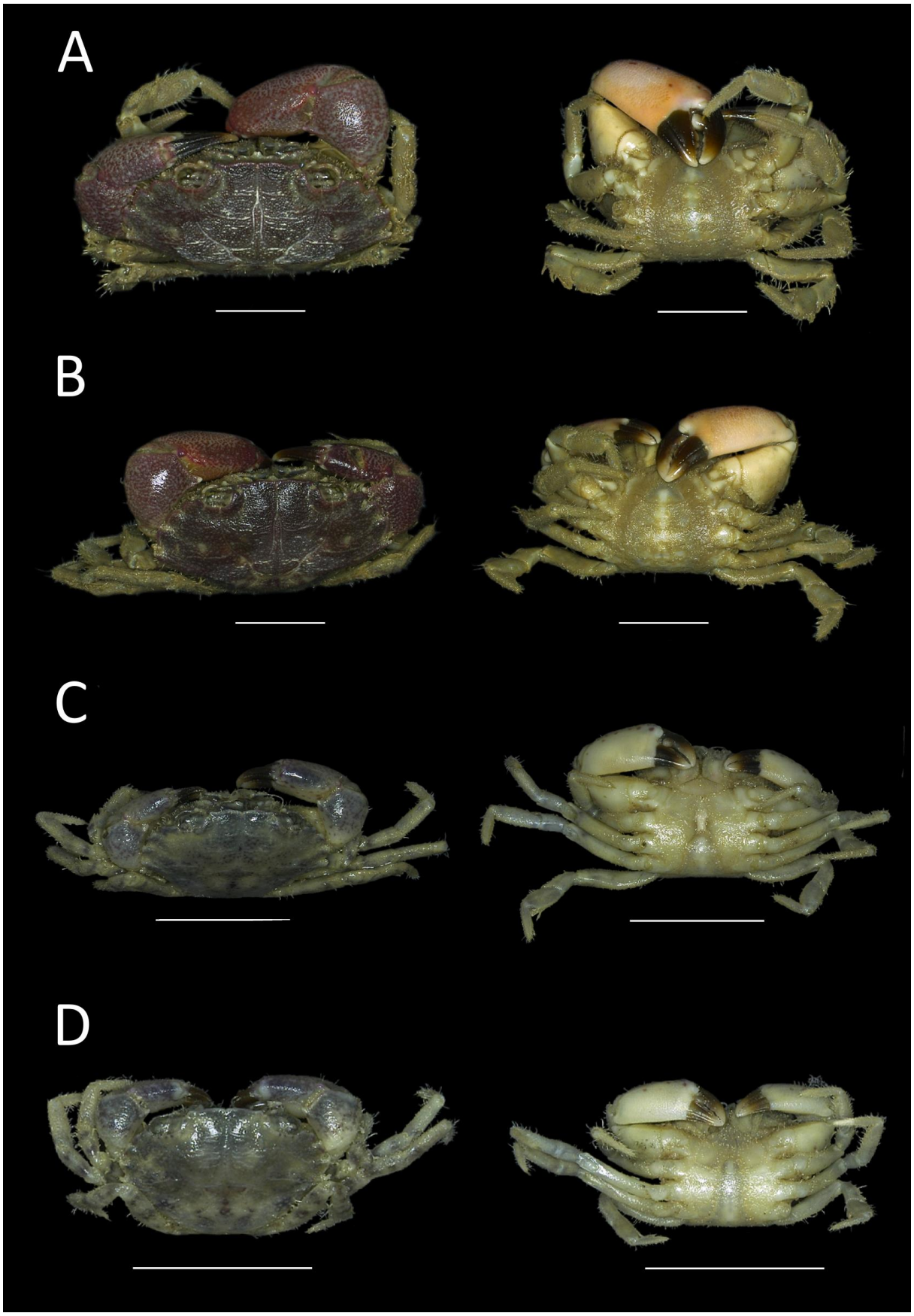


Figure A3-29: Specimens of *Panopeus lacustris* Desbonne, in Desbonne & Schramm, 1867. A, Prep. #16160; B, Prep. #16161; C, Prep. #16168; D, Prep. #16169. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

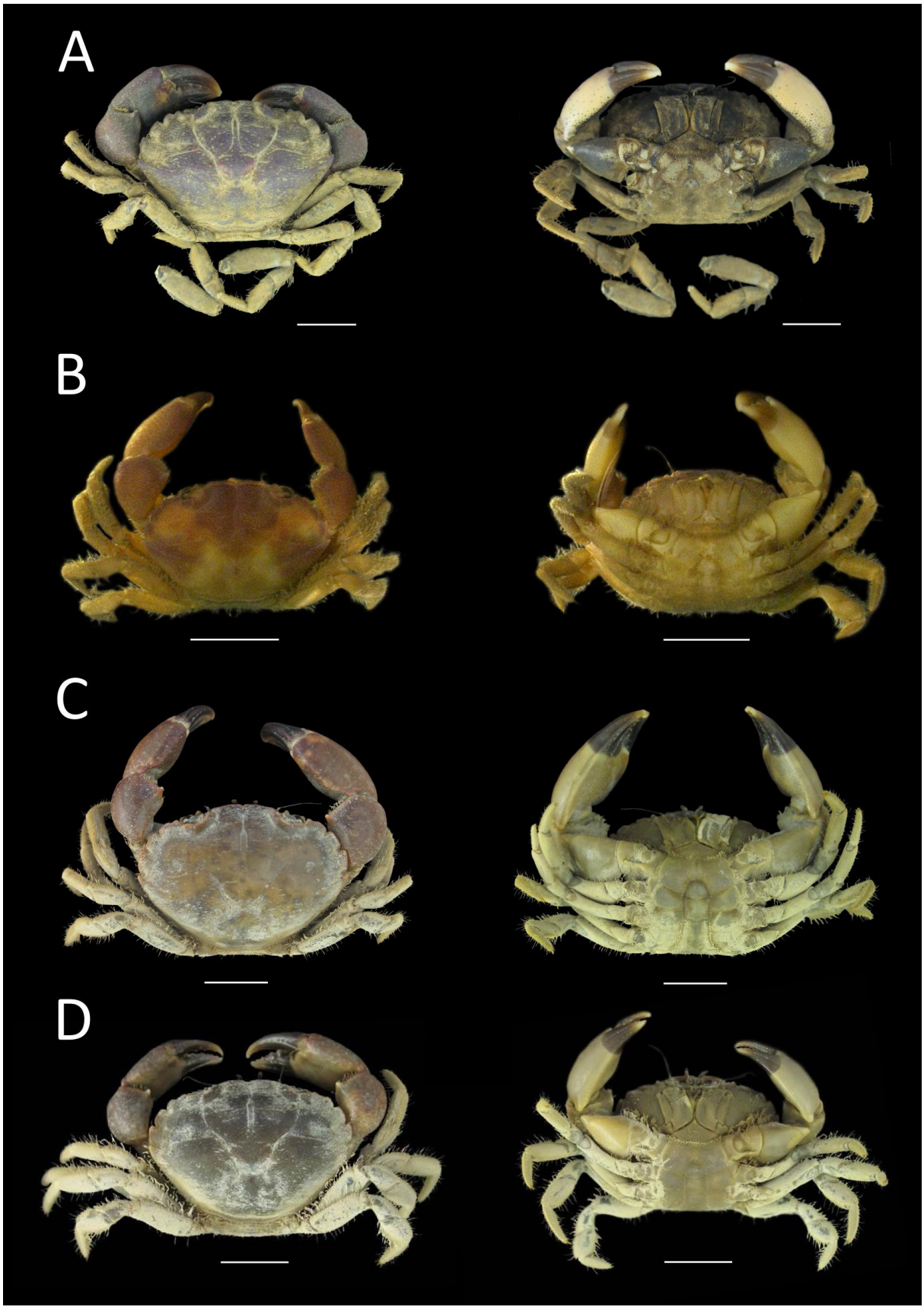


Figure A3-30: Specimen of *Panopeus lacustris* Desbonne, in Desbonne & Schramm, 1867. A, Prep. #19233. Specimen of *Panopeus meridionalis* Williams, 1983. B, Prep. #19632. Two specimens of *Panopeus obesus* Smith, 1869. C, Prep. #19633; D, Prep. #19634. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

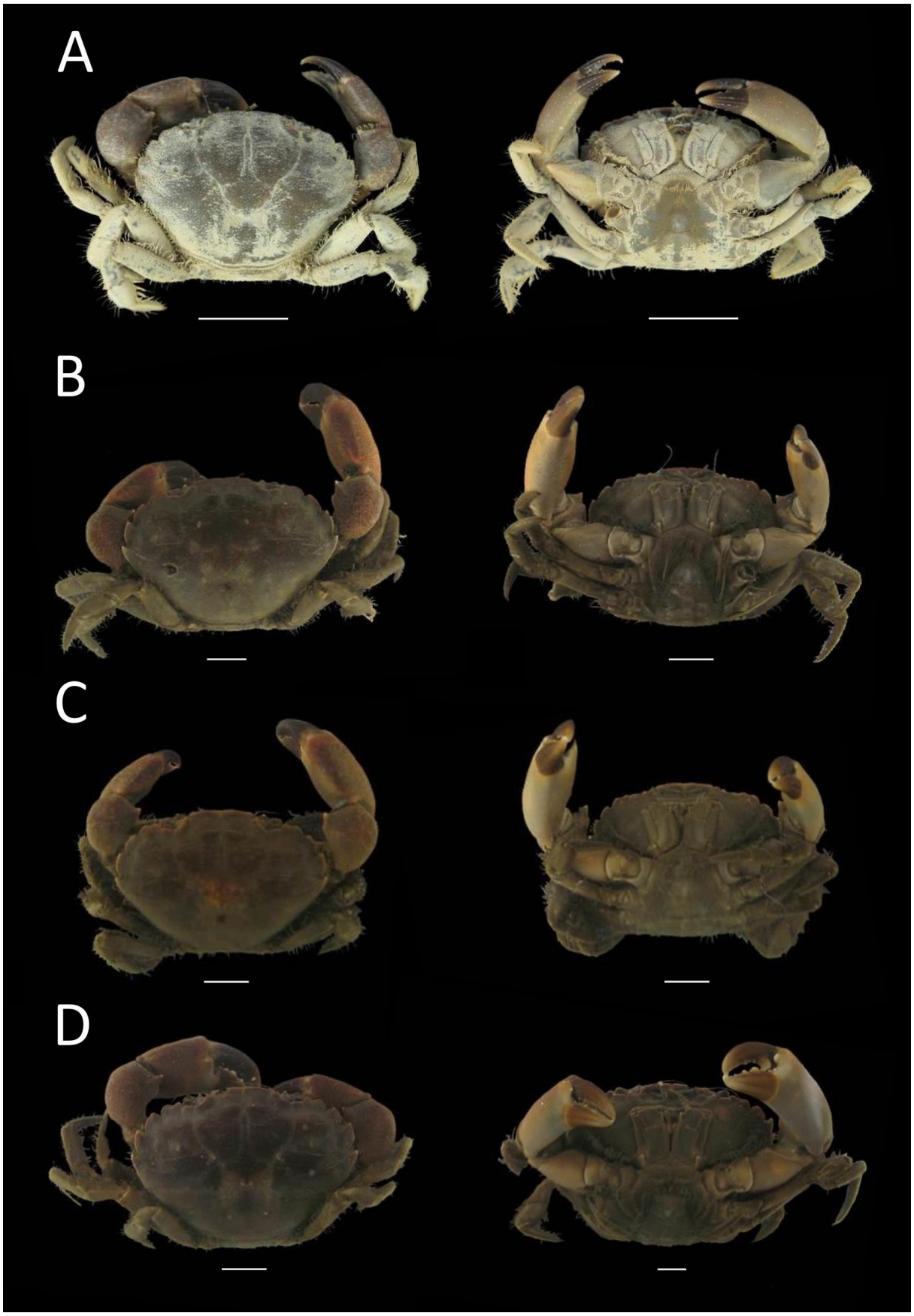


Figure A3-31: Specimens of *Panopeus obesus* Smith, 1869. A, Prep. #19232; B, Prep. #19234; C, Prep. #19635; D, Prep. #19236. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

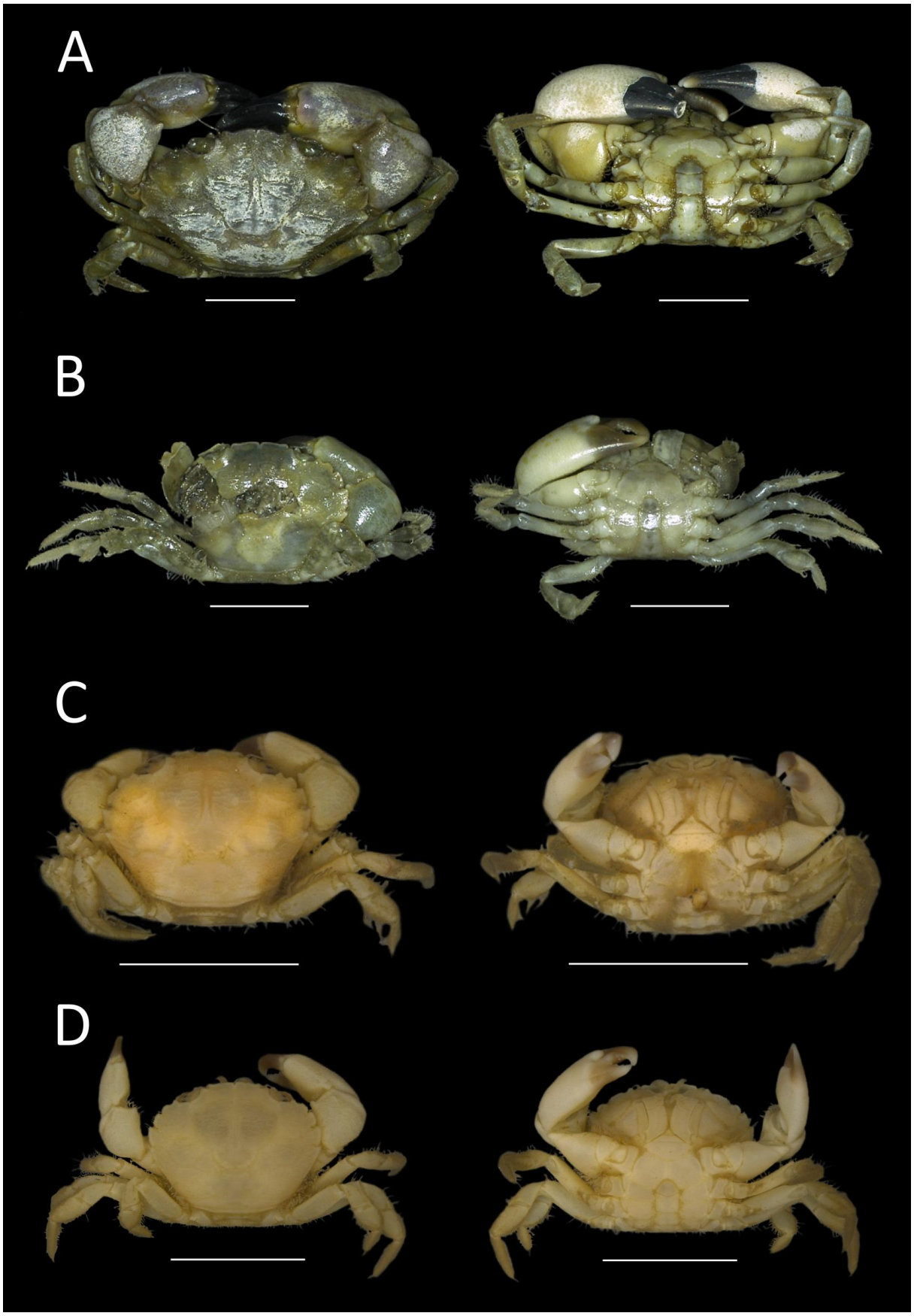


Figure A3-32: Specimens of *Panopeus occidentalis* De Saussure, 1857. A, Prep. #16150; B, Prep. #16167; C, Prep. #19870; D, Prep. #19871. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

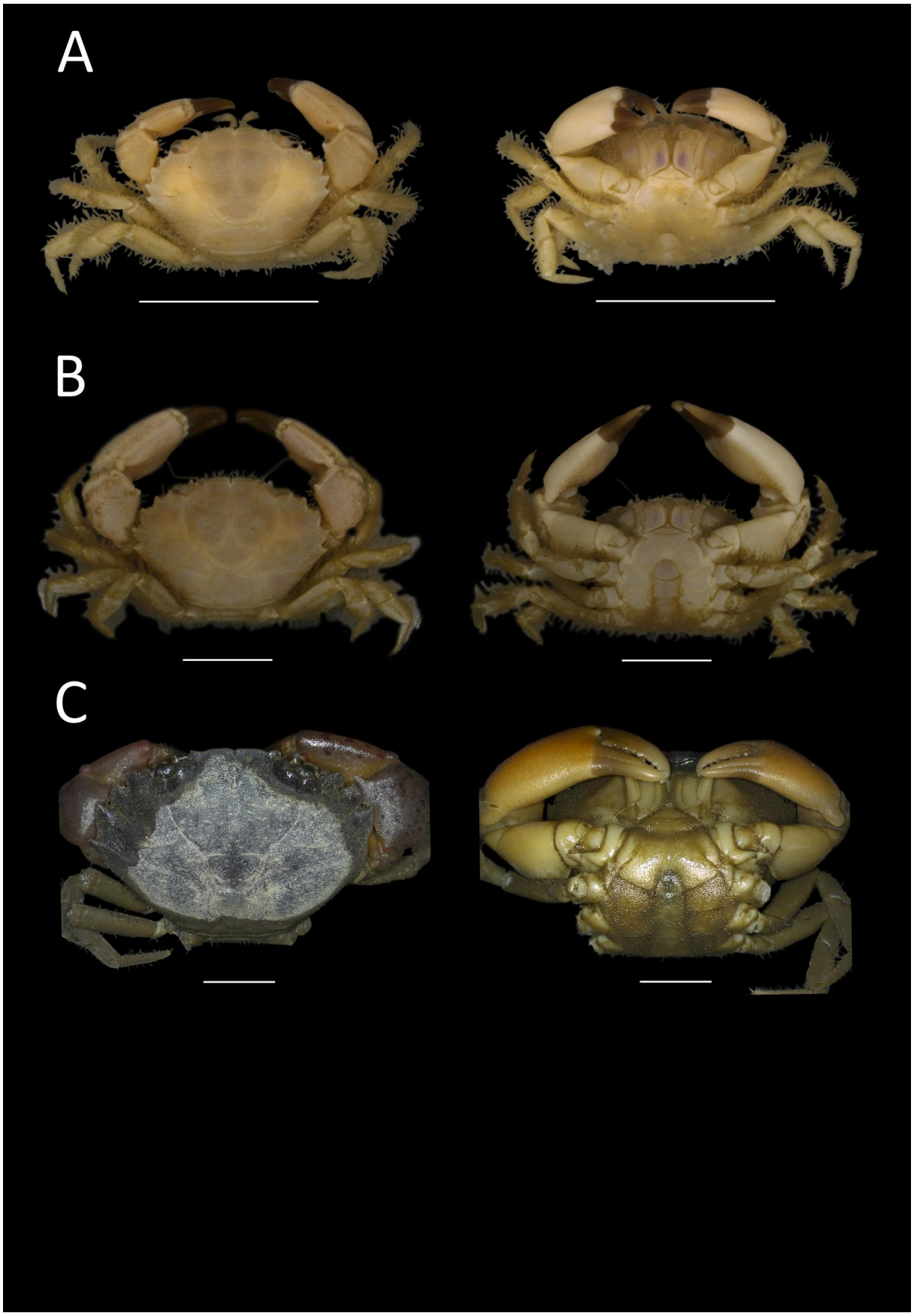


Figure A3-33: Two specimens of *Panopeus occidentalis* De Saussure, 1857. A, Prep. #19872; B, Prep. #19873. Specimen of *Panopeus purpureus* Lockington, 1877. C, Prep. #16137. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

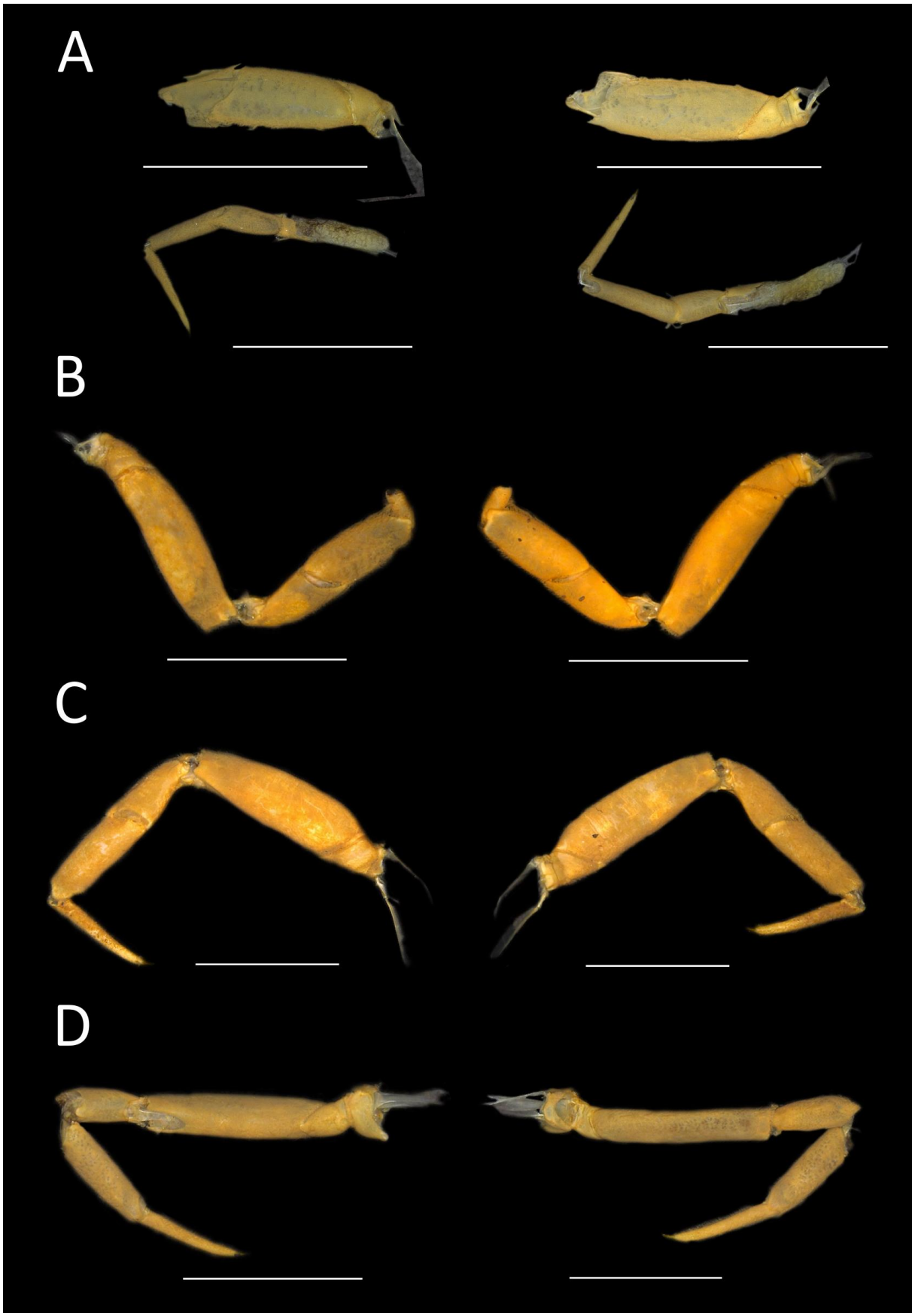


Figure A3-34: Legs of *Panopeus purpureus* Lockington, 1877. A, Prep. #18125; B, Prep. #18126; C, Prep. #18127; D, Prep. #18128. The legs are shown in dorsal and ventral views. The scale bar represents 1 cm.

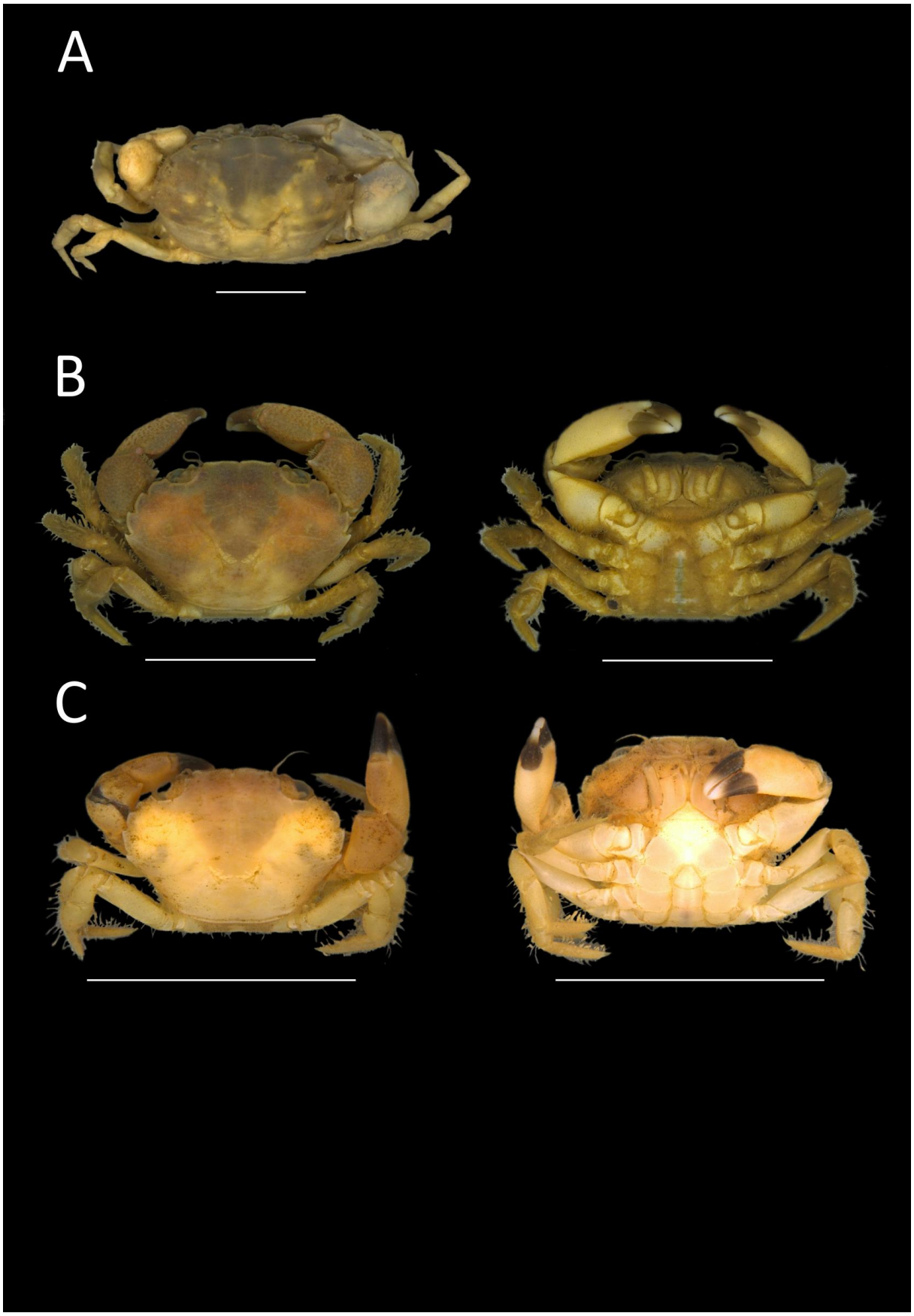


Figure A3-35: Specimen of *Panopeus* sp. A, Prep. #19626. Two specimens of *Panopeus rugosus* A. Milne-Edwards, 1880. B, Prep. #19751; C, Prep. #19744. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

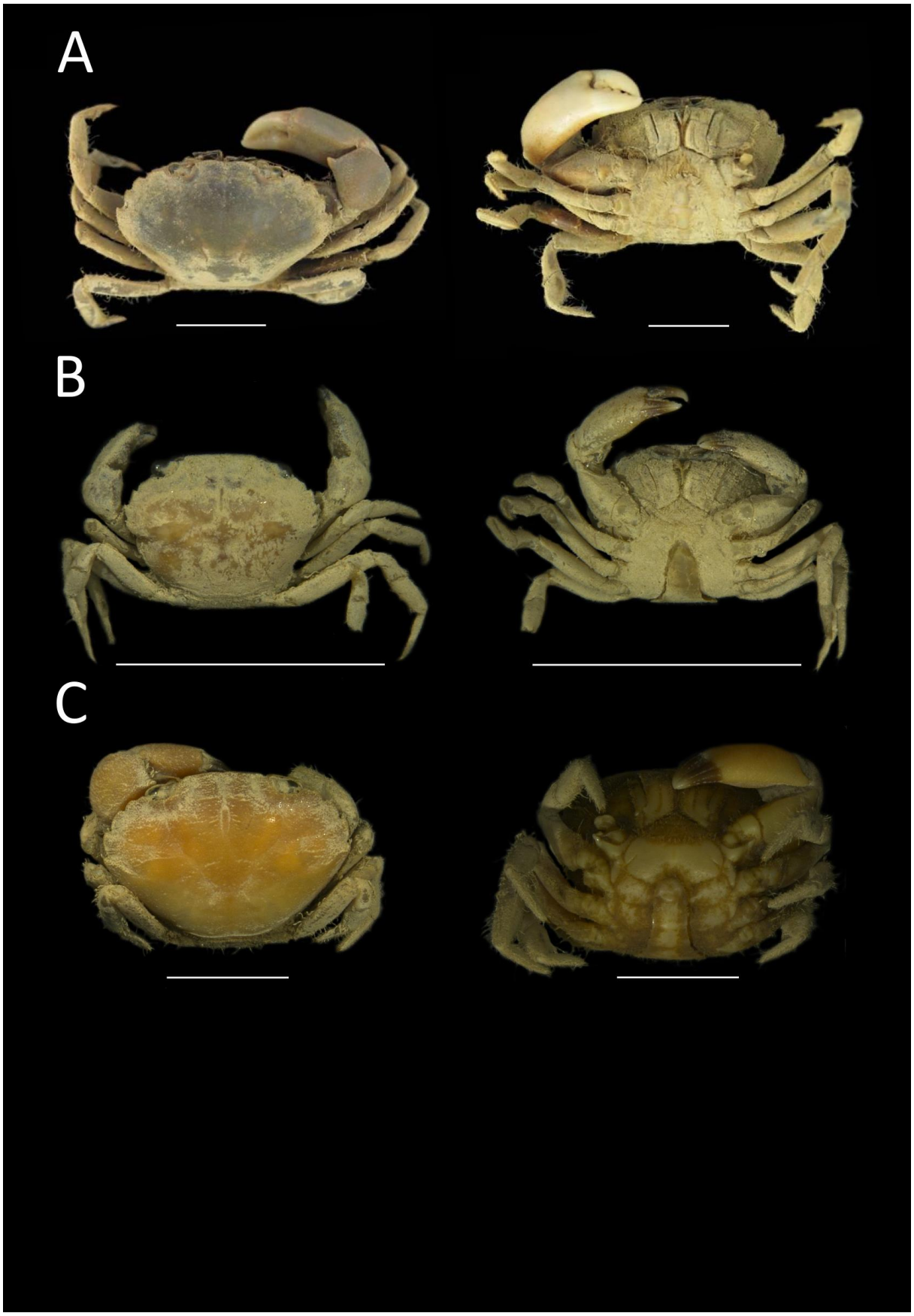


Figure A3-36: Specimens of *Panopeus simpsoni* Rathbun, 1930. A, Prep. #19637; B, Prep. #19638; C, Prep. #19639. The specimens are shown in dorsal and ventral views. The scale bar represents 1 cm.

A3.4 Genus *Pachygrapsus* Randall, 1840

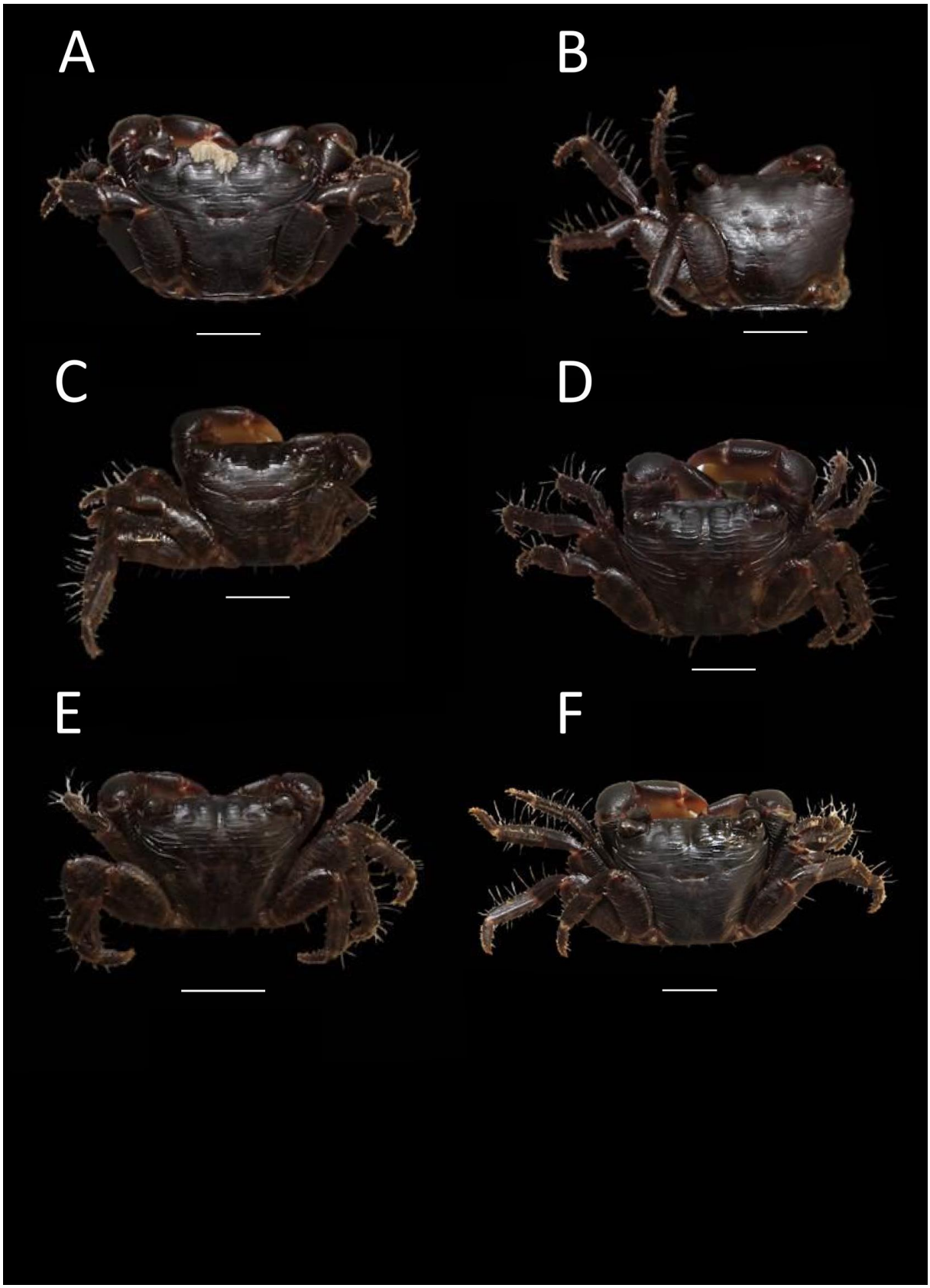


Figure A3-37: Specimens of *Pachygrapsus socius* Stimpson, 1871. A, Prep. #17513; B, Prep. #17514; C, Prep. #17515; D, Prep. #17519; E, Prep. #17521; F, Prep. #17523. The specimens are shown in dorsal view. The scale bar represents 1 cm. Photos of *P. socius* are by courtesy of C. D. Schubart (University of Regensburg).

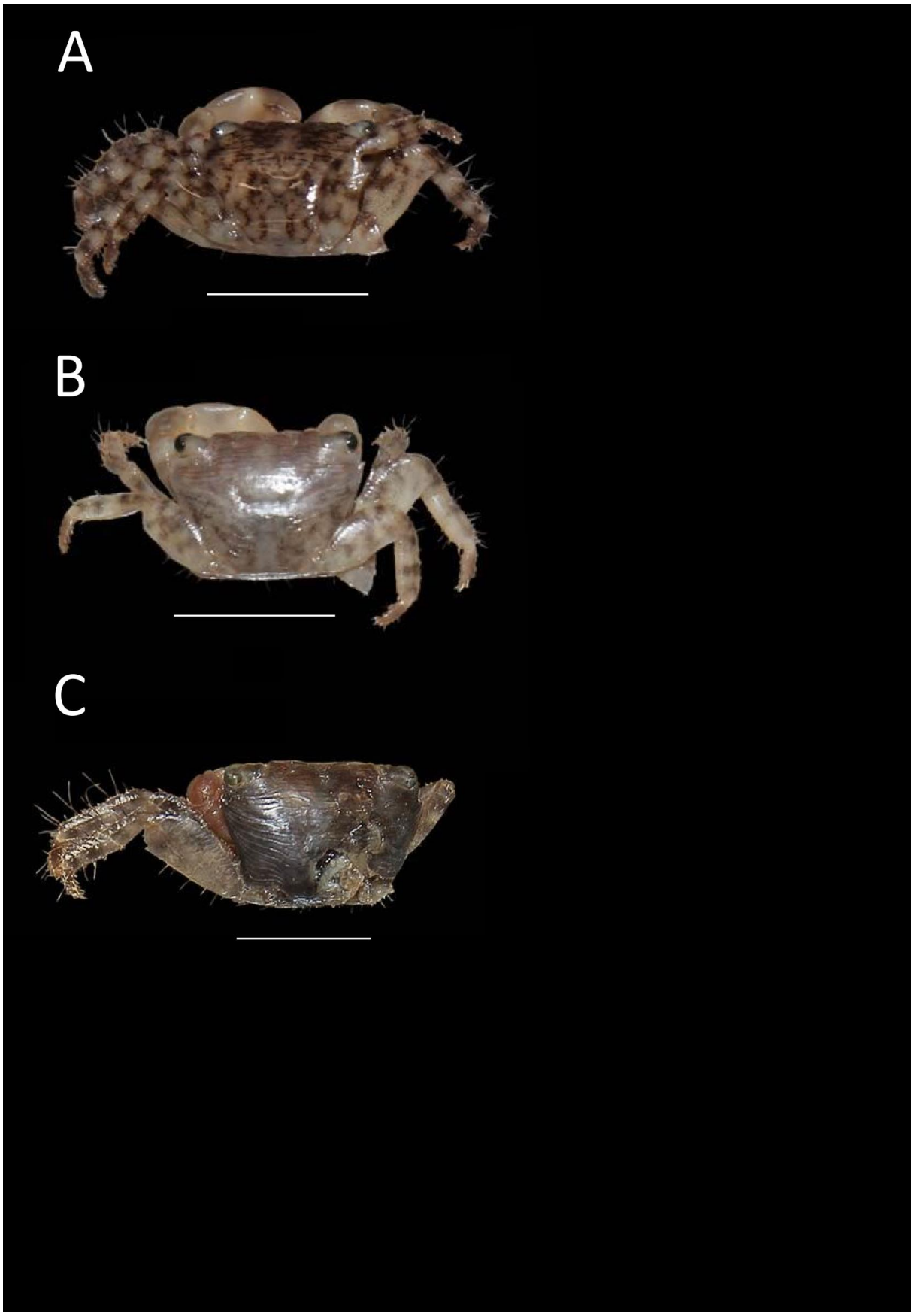


Figure A3-38: Specimens of *Pachygrapsus transversus* (Gibbes, 1850). A, Prep. #17527; B, Prep. #17528; C, Prep. #17530. The specimens are shown in dorsal view. The scale bar represents 1 cm. Photos of *P. transversus* are by courtesy of C. D. Schubart (University of Regensburg).

Erklärung

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Gießen, August 2015

Carina Marek