IMPACT OF LIFE HISTORY AND ECOLOGY ON RATE OF DIVERSIFICATION AND SPECIATION, AS EXEMPLIFIED BY THORACOTREME CRABS ALONG THE WESTERN TROPICAL ATLANTIC AND ON BOTH SIDES OF THE ISTHMUS OF PANAMA

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ABSTRACT OF THE DISSERTATION

Impact of life history and ecology on rate of diversification and speciation, as exemplified by thoracotreme crabs along the western tropical Atlantic and on both sides of the Isthmus of Panama

by

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The progressive formation of the Isthmus of Panama during the Miocene, and its final closure during the Pliocene, was the preeminent geological event that affected the western tropical Atlantic and eastern Pacific oceans. As consequence of this closure, marine populations were isolated on both sides of the isthmus and initiated independent genetic divergence, affected by different environmental conditions. So far, phylogeographic patterns of species inhabiting western tropical Atlantic or eastern tropical Pacific oceans received only limited focus. The species used as model in this study correspond to American representatives of thoracotreme crabs, selected based on two major ecological characters that possibly affected their evolutionary histories: dispersal abilities and habitat. This should allow comparing the effect of these characters. The goals of this study are: 1) to investigate the consequences of the closure of the Isthmus of Panama, and to determine which of the two models proposed to explain the formation of the isthmus is better supported by the transisthmian divergence of marine taxa; 2) to explore the phylogeographic patterns in the western tropical Atlantic, especially the relationships between Caribbean and Brazilian marine faunas, and 3) to assess the phylogenetic relationships of the American sesarmid genera *Aratus*, *Armases*, *Metopaulias* and *Sesarma*. Our results support the final closure of the Isthmus of Panama to have occurred during the Pliocene rather than during the Miocene. Mangrove sister species present a smaller transisthmian divergence than rocky shore sister species, supporting the assumption of mangroves as last habitat to allow genetic exchanges between Atlantic and

Pacific oceans at the final closure of the isthmus. Populations of *Aratus pisonii* on both sides of the isthmus are morphologically and genetically distinct. As consequence, *Aratus pacificus* **n. sp.**, is presently described as the sister-species of *Aratus pisonii*. Along the western tropical Atlantic, unexpectedly contrasting differences between related taxa inhabiting the same biogeographic area could be found, ranging from apparent panmixis to deeply divergent lineages. Three species exhibit clear signs of past or recent changes in their population size. It reveals the importance of the ecological characteristics of the studied species, and their consequences on sympatric taxa. It also highlights the importance of individual histories of each species. Phylogenetic relationships in the American Sesarmidae reflect a rapid radiation that started 10.5 mya, when this family established on the American continent. The genus *Aratus* represents a deep branch in the genus *Armases* rather than its sister taxa, making *Armases* paraphyletic. The exploration of genetic patterns in American thoracotreme crabs at different levels shed light on how both environmental changes and ecological characteristics shaped the biodiversity of this region since the Miocene.

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To conclude these acknowledgements, I need to narrate a small anecdote that occurred in March 2013. At this period, I went with my colleagues to the 16th CrustTag in Greifswald (north Germany). This meeting was the place of a surrealistic discussion I had with few German colleagues. When I think about this anecdote, I realize that it could only have occurred in the scientific environment, but also wonder if I was fated to study Crustaceans.
Oliver Coleman (Museum für Naturkunde, Berlin) presented a poster about a new software for taxonomists. As I had to describe a new species (see Chapter 2 of this dissertation), we engaged a discussion about this software, to see if it could be useful for my project. We were soon followed in the discussion by Christiana Anagnostou (Kiel Universität), Sarah Hayer (bachelor student in my lab at this period) and Peter Dworschak (NHM Vienna). With the beer, the discussion switched quickly to other subjects, as Linux, new technologies, and finally to the tablet of Oliver.
He explained us how useful was this tablet, especially to read ebooks in travels, like the one he just recently downloaded. It was an ebook about an old Polish scientist living more or less a century ago, that had been recently translated from Polish to German by the students of the Polish studies at the universities of Bamberg, Dresden, and Cologne.

As Oliver's work is focused on amphipods, I immediately understood who was this Polish scientist. Peter also understood, and was nearly laughing by looking at me. Both Christiana and Sarah were wondering why, and Oliver did not had time to explain who was this Polish scientist that I finished his sentence before him !

The explanation is totally surrealistic by its improbability (or not, when we consider the profile of each protagonist). The Polish scientist mentioned before was just my grand-grand-grand cousin Benedykt Dybowski (the cousin of Jean Dybowski, grand-father of my grand-mother)! Oliver, Christiana and Sarah were extremely surprised by such explanation, as nobody could have expected it (except Peter who knew it, as we had an exchange few weeks before by email about Benedykt, explaining the large smile in his pepper-and-salt beard).

This work is dedicated to my mother, Michèle (1955-2006).

TABLE OF CONTENTS

General introduction & Material 1

1. A changing region: the Isthmus of Panama and the western Tropical Atlantic 2

2. The transisthmian vicariance and the problematic 'species' definition 5

3. The biological model: the American thoracotreme crabs 8

1. Ecological characteristics 8

2. Taxonomy of the American thoracotreme crabs 11

4. Objectives 12

Chapter 1. Comparative phylogeography of thoracotreme crabs in the western tropical Atlantic and the importance of different life histories 14

Introduction 14

Material and Methods 16

Results 23

Discussion 41

Conclusions 49

Chapter 2. Transisthmian differentiation in the tree-climbing mangrove crab *Aratus* H. Milne Edwards, 1853 (Crustacea, Brachyura, Sesarmidae), with description of a new species from the tropical eastern Pacific 50

Abstract 50

Introduction 51

Materials and Methods 52

Genetic results 55

Taxonomy 56

Aratus pisonii (H. Milne Edwards, 1837) 56

Aratus pacificus n. sp. 62

Discussion 68

Acknowledgements 69

Chapter 3. The age of the Isthmus of Panama: a marine crustacean perspective 71

Abstract 71

Introduction 72

Material and Methods 73

Results 78

Discussion 87

Conclusions 95

Acknowledgements 95

Chapter 4. Phylogenetic relationships of the American Sesarmidae (Decapoda, Brachyura, Thoracotremata): paraphyly, convergent evolution and rapid adaptations 96

Abstract 96

Introduction 97

Material and Methods 100

Results 104

Discussion 108

Conclusions 120

Acknowledgements 121

General discussion and conclusions 122

The Isthmus of Panama: a barrier between two oceans 122

Patterns along the tropical American coastlines 124

References 125

Supplementary material 149

Chapter 1 149

Chapter 3 150

Chapter 4 156

GENERAL INTRODUCTION & MATERIAL

In 1987, John C. Avise *et al.* introduced phylogeography as a new discipline. This field of study results from the combination of biogeography (i.e. the distribution of species and taxa on Earth and processes involved in their distribution) with the exploration of historical intraspecific patterns at the molecular level. Such exploration became possible with the 'PCR revolution' corresponding to development of new genetic tools in 1983 by Mullis, especially the polymerase chain reaction (PCR) and universal primers that enabled the possibility to amplify fragments from large amount of species (Avise *et al.* 1987, Avise 2000). It is thus the combination of both time (mutations) and space (geography). Phylogeography stands at the junction between microevolutionary (population) and macroevolutionary (interspecific) processes (Fig. I.1, Avise 2009). Such characteristics make of the phylogeography a key discipline to determine the respective importance of dispersal and vicariance in differentiation processes, but also to explore the biodiversity, including its cryptic component. The phylogeography is by consequence the most accurate discipline to explore the processes involved in the formation of the biota present along the Isthmus of Panama and the western Tropical Atlantic.

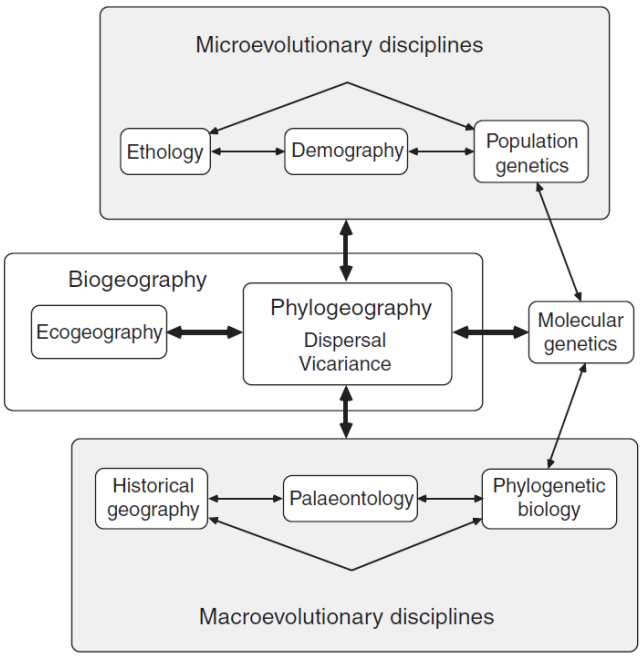

Figure I.1. Phylogeography at the junction between microevolutionary and macroevolutionary processes (based on Avise 2009, modified from Avise 2000).

1. *A changing region: the Isthmus of Panama and the western Tropical Atlantic*

The Tropical Atlantic corresponds to one of the twelve marine biogeographic realms used to describe the biodiversity patterns of the coastal seas and continental shelves (Spalding *et al.* 2007). This realm covers both sides of the Atlantic Ocean, and for its western part, ranges from the southern Florida and Gulf of Mexico (while its northern part is temperate), through the Caribbean and covers the South American coastline up to the Cape Frio near Rio de Janeiro in southern Brazil, representing nearly 10 000 km between its extremities. At the north and south of this realm, two biogeographic provinces (the Warm Temperate Northwest Atlantic and Warm Temperate Southwestern Atlantic) represent progressive biogeographic transitions between tropical and temperate habitats and faunas. The eastern part of the Tropical Atlantic realm covers the African coastline from Mauritania to Angola (Fig. I.2A).

Contrary to the Indian and Pacific oceans that are interconnected through the Coral Triangle (or Indonesian archipelago), the Tropical Atlantic realm is isolated to the north and south from other tropical regions by the presence of colder temperate waters (Fig. I.2B), preventing any recent genetic exchange with other tropical marine fauna. Nevertheless, this physical isolation is geologically recent, and is the direct consequence of the closure of the Central American Seaway by the rise of the Isthmus of Panama approximately 3 mya and the establishment of the Benguela upwelling approximately 2 mya (Rocha *et al.* 2005).

The closure of the Isthmus of Panama is a geological event that started during Late Cretaceous (~70 mya) to Early Tertiary (Eocene, ~50 mya). The subduction of the Farallon Plate (whose extant relicts are the Nazca and Cocos plates) under the Caribbean Plate lead to the formation of a submarine volcanic arc on the western side of the Caribbean Plate, at the west of the South America (Coates *et al.* 2004, Iturralde-Vinent 2006). This event ended in the formation of a land bridge between the North American continent and the South American island-continent during the Pliocene (Coates & Obando 1996, Coates *et al.* 2004). At the formation of this land bridge, the South America had been geologically isolated from other landmasses since at least 30 million years, when the connection with Antarctica broke up, final remnant of the fragmentation of the Gondwana supercontinent. South America had also been completely separated from Africa since approximately 100 million years (McLoughlin 2001), and had been possibly briefly connected to North America through a shallow chain of islands (corresponding nowadays to the Lesser Antilles Volcanic Arc) during the Upper

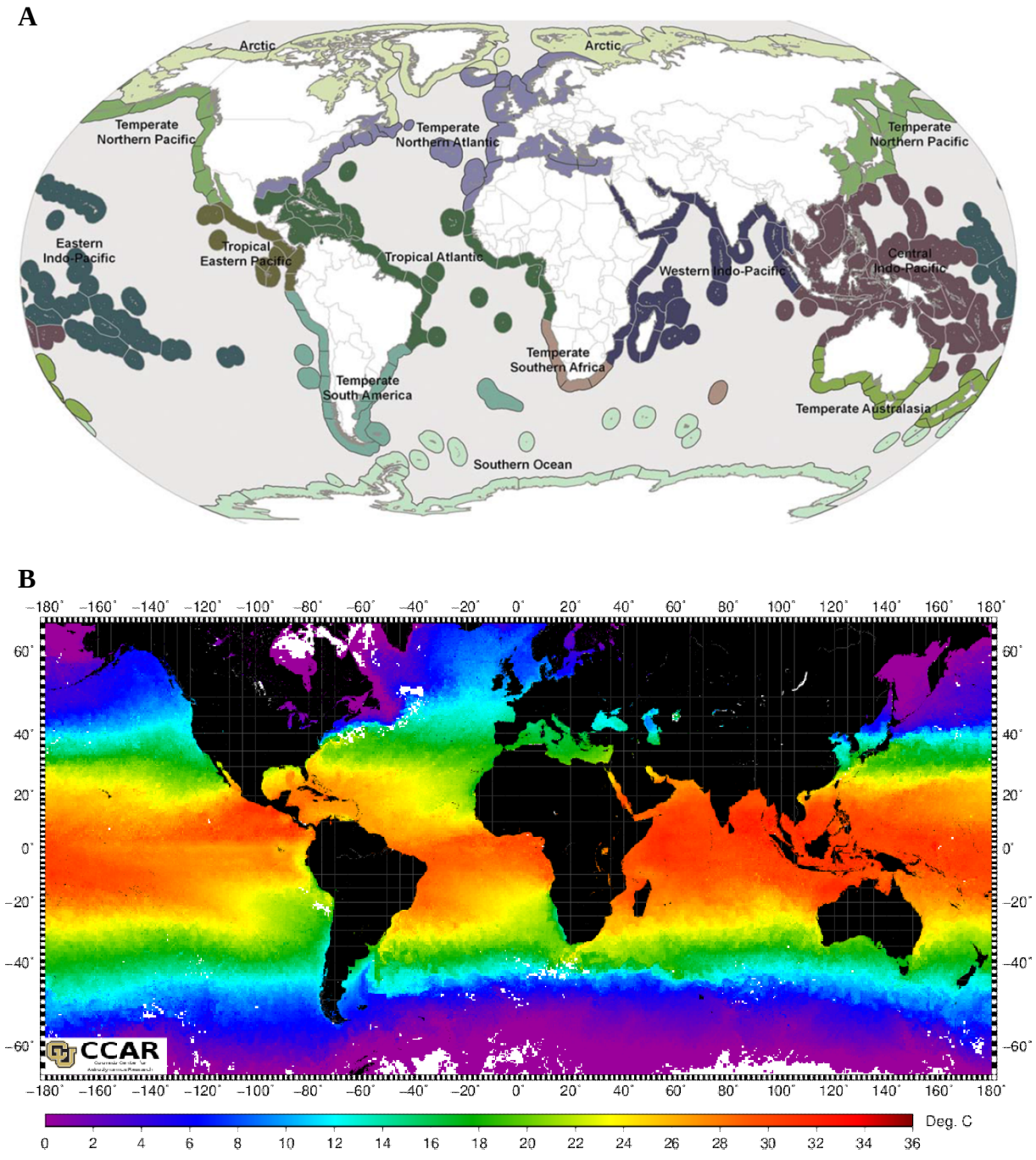

Figure I.2. A. Marine coastal realms and their subdivisions (figure from Spalding *et al.* 2007). **B. Map of the sea surface temperatures.** Average of 15 days of April-May 2014, based on the Moderate-resolution Imaging Spectroradiometer (MODIS) data from the satellite Aqua with a resolution of 4km (image generated by the Colorado Center for Astrodynamics Research Global MODIS SST Viewer). A clear correlation can be observed between the sea surface temperatures and the distribution of biogeographic realms.

Cretaceous, approximately 70 mya (Iturralde-Vinent 2006). As consequence, the South American biota was composed of taxa remnants of the Gondwanan fragmentation and of taxa arrived by oversea dispersal (De Queiroz 2005, 2014).

The westward displacement of the Caribbean Plate brought the submarine volcanic arc to the current position of the Isthmus, in a pre-collision setting. This volcanic arc became emergent in its northern part (corresponding to the Costa Rica) around 16 mya. At this time, the Central American Seaway, marine connection between the Atlantic and Pacific oceans, was a strait with abyssal waters, letting a large gap between the Central and South America (Coates & Obando 1996, Coates *et al.* 2004). During the early steps of the collision of the Caribbean Plate with the South America Plate, in the Late Miocene (around 12 mya), the seaway shallowed to a depth of 1000m in its southern part (Atrato Basin, nowadays Colombia, Duque-Caro 1990). During the collision process, the different basins shallowed to of depth of less than 200m around 6 mya (Duque-Caro 1990), and the proto-Isthmus formed an extended archipelago separated by narrow channels. These channels were filled by sediments, thereby closing the Central American Seaway in a range from 2.8 to 3.5 mya (Coates & Obando 1996, Coates *et al.* 1992, 2004, 2005).

The rise of the Isthmus of Panama and progressive closure of the Central American Seaway represents for marine taxa the most remarkable recent vicariant event (i.e. isolation of populations as consequence of habitat fragmentation by geological event). This vicariant event and its impact on marine taxa have been reviewed by Lessios (2008). As subject this doctorate project, this event sees its importance and new developments detailed in the different chapters composing this thesis, especially in the Chapter 3. This barrier for marine species is apparently robust but is not absolute. Indeed, recent genetic data revealed that the isthmus has been crossed at least twice by mangrove snails, a dispersal across the barrier possible by transport by migratory birds (Miura *et al.* 2012). Moreover, the robustness of this barrier might be affected by the completion of the Panama Canal, freshwater anthropogenic connection between the two sides of the Isthmus, built to ease sea transport between the two coasts of the American continent (Abele & Kim 1989). On another perspective, this barrier for marine species connected previously isolated continents and terrestrial faunas and allowed large scale dispersal of this terrestrial fauna in a biogeographic event called the Great American Biotic Interchange (see review of Leigh *et al.* 2014).

Before the completion of the isthmus, the Tropical Atlantic realm was physically connected to the tropical Eastern Pacific realm, which ranges from Baja California to northern Peru, over nearly 5000 km (Spalding *et al.* 2007), and explains the strong relationships

between the faunas of these two realms (Lessios 2008). The Tropical Eastern Pacific realm is partially connected to the Eastern Indo-Pacific realm through a semi-permeable barrier, the Easter Pacific Barrier, 5000km of abyssal waters without islands (Lessios & Robertson 2006).

The western tropical Atlantic is impacted by the presence of two of the most important rivers in terms of freshwater discharge in the ocean on Earth, the Amazon (forming a double estuary with the Tocantins River) and Orinoco rivers. The freshwater discharge of these two rivers strongly decrease the salinity and increase the turbidity of the Atlantic Ocean, with their effect traced up to the Caribbean Sea, as their waters are pushed northward by the Guiana, North Brazil, North Equatorial and Caribbean currents (Froelich *et al.* 1978, Hellweger & Gordon 2002). This decrease in salinity and increase in turbidity by the Amazon River massively impacts the habitats and species in this area and its continental shelf, from Piauí State (Brazil) to Trinidad. This corresponds to the North Brazil Shelf province, a region directly impacted by the waters of the Amazon and Orinoco. This province is biologically characterized by an absence of coral reefs. As consequence, the Amazon (and possibly the Orinoco) acts as semi-permeable barrier for species, especially reef fishes (Rocha 2003, Spalding *et al.* 2007). Another possible barrier in the western tropical Atlantic is proposed by Briggs (1974), corresponding to the Gulf Stream, that might isolate the Florida and the northern Gulf of Mexico from southern Caribbean populations.

In the changing region that is the Isthmus of Panama and the western Tropical Atlantic, the long-standing debate about dispersal versus vicariance (reviewed by De Queiroz 2014) is not useful anymore to determine which was process responsible of the present distribution of organisms, or the respective importance of both processes. Today we are more interested how the combination of these two mechanisms gave birth to the actual biodiversity and shaped the observed biogeographic patterns. This is especially true when considering that vicariant events will affect the different taxa from a geographic area, and that dispersal mostly corresponds to unique and infrequent events linked to the individual histories of the species.

2. *The transisthmian vicariance and the problematic 'species' definition*

One of the major consequences of the Isthmus closure for marine taxa was the interruption of the gene flow between the Atlantic and Pacific oceans, and the resulting vicariance, which led to the formation of couples of sister species on both sides of this barrier. However, as visible in Lessios (2008) and highlighted in the Chapter 2 of this dissertation,

several species (e.g. the shrimp *Alpheus floridanus* or the fish *Gerres cinereus*) are still considered to have a transisthmian distribution, i.e. being recognized as the same species on both sides of the Isthmus.

Such pattern is the direct consequence of inconsistencies between the different definitions of the 'species', most used notion in biology. Despite its importance, the definition of 'species' remains problematic, as scientists were looking "*for a concept-definition that is biologically relevant and meaningful, one that is easily applied, and one that encompasses natural biodiversity*" (Mayden 1997), meaning that the ideal concept should apply for organisms as different from each other as unicellular organisms (e.g. bacteria), multicellular animals or plants. As illustration to this problem, Mayden (1997) listed 24 definitions previously proposed for the concept of *species*, and analyzed the range of application of these definitions, to determine whether these definitions represent primary or secondary (i.e. operational) concepts. Only the Evolutionary Species Concept (ESC), defined as "*a lineage* (*an ancestral-descendant sequence of populations*) *evolving separately from others and with its own unitary evolutionary role and tendencies*" by Simpson (1961), was considered to be a primary concept. All the other concepts were determined to be secondary concepts, i.e. concepts more restricted in their definition, and by consequence, not applicable to the whole range of cases encountered in the life on Earth for the entities assumed to be species. However, Mayden (1997) recognized that the flaw of the ESC, by being the most theoretical concept, is the inoperability of this concept by scientists to identify species. As a result, the secondary concepts have to be used as operational tools to explore the variance in the diversity of living organisms and to identify units that can be considered as being *species*. Within these secondary concepts, three of them appear to be historically of major importance by their use as operational tools to identify species.

Historically, the oldest concept corresponds (or can be assimilated) to the Morphological Species Concept (MSC), based on the use of morphological characters to differentiate species from each other. This concept is used since Aristotle (4th century BC), first during pre-evolutionary times, with the 'species' as a fixed unit. With the theory of evolution, the 'species' was placed in a temporal frame, unit that perpetually evolves as result of natural selection. It was only robustly defined by Regan (1926), as "*a community, or a number of related communities, whose distinctive morphological characters are, in the opinion of a competent systematist, sufficiently definite to entitle it, or them, to a specific name*". Even if it is the easiest concept to understand, handle and the most commonly used for to define a *species*, this definition suffers from the arbitrary level of morphological divergence

needed to distinguish species (defined by the relative opinion of a *competent systematist*), but also from the bias of its definition mostly based on human vision (Bickford *et al.* 2006), when many species use chemical recognition to recognize conspecifics (Howard & Blomquist 2005).

Disagreeing with the importance of morphology to define a *species*, and based on his experience as ornithologist, Mayr (1940, 1942) proposed the Biological Species Concept (BSC) defined as "*groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups*". In this case, the *species* becomes an isolated gene pool, and speciation occurs when reproductive isolation is achieved. Such isolation can appear in a limited number of generations (Hendry *et al.* 2000). This definition also implies the presence of mechanisms (or barriers) limiting or preventing intergroup and favoring intragroup breeding. These mechanisms can be pre- or post-zygotic. Palumbi (1994) lists mechanisms used to identify reproductive isolation in marine taxa, as mate preference, spanning asynchrony or habitat specialization. However, as for the MSC, Mayden (1997) detailed the flaws of the BSC, with possibly the most problematic being that such definition excludes non-reproductive organisms, necessarily rejecting it as primary concept for the species.

The third major species concept, the Phylogenetic Species Concept (PSC), results from the massive increase of studies based on genetic data. This concept was defined by Cracraft (1983) as "*the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent*". This definition identifies the species as the smallest diagnosable monophyletic unit, and assumes the reproductive isolation by the accumulation of mutations between clusters. The exploration of the phylogenetic relationships highlighted the presence of incongruence in the topologies recovered from different gene trees, and let to consider the species tree as a pool of gene trees (Maddison 1997), but also highlighted the frequent paraphyly and polyphyly at the species level (Funk & Omland 2003).

The rapid development of molecular tools used for phylogeographic studies highlighted regularly incongruence between the MSC and the PSC (and at a lower level, the BSC). In addition, the presence of several divergent genetic lineages in what was considered to be a single species led to the concept of *cryptic species*, defined by Bickford *et al.* (2006) as: "*we consider two or more species to be 'cryptic' if they are, or have been, classified as a single nominal species because they are at least superficially morphologically indistinguishable*". Large cryptic diversity has been detected in marine habitats (Knowlton 1993, Knowlton 2000). More than 50% of the newly described species originated from cryptic

species complexes and probably represent a large part of the missing biodiversity (Ceballos & Ehrlich 2009, Scheffers *et al.* 2012). One of the main future challenges for both taxonomy and biodiversity will be to find criteria (morphological or not) able to distinguish these cryptic species to bring them out of their crypsis.

3. *The biological model: the American thoracotreme crabs*

1. Ecological characteristics

The species used as model in this thesis correspond to American representatives of the subsection Thoracotremata, and formerly called 'grapsoid crabs', i.e. species belonging to the superfamily Grapsoidea as defined by Ng *et al.* (2008). These species currently correspond to three families, the Sesarmidae (genera *Aratus*, *Armases*, *Metopaulias* and *Sesarma*), Grapsidae (genera *Geograpsus*, *Goniopsis*, *Grapsus* and *Pachygrapsus*) and the Varunidae (*Cyclograpsus*).

These species were selected based on two major ecological characters that possibly affected their evolutionary histories. This should allow comparing the effect of these characters: dispersal abilities and habitat. Both American Sesarmidae and Varunidae present relatively short larval development (from two to five larval stages, Anger 1995, Schubart & Cuesta 1998), whereas Grapsidae have longer larval development, up to eight larval stages (Cuesta *et al.* 2011), resulting in higher dispersal potential for the Grapsidae than the two other families.

Figure I.3 (next page). American thoracotreme crab genera and species used as models in this dissertation. Pictures used with the courtesy of their respective authors. ***Aratus*** *pisonii* (H. Milne Edwards, 1837), Florida (David Munroe, Flickr); ***Armases*** *angustum* (Smith, 1870). Costa Rica (Sergio Quesada, Flickr); ***Cyclograpsus*** *integer* H. Milne Edwards, 1837. Guadeloupe (Expedition KARUBENTHOS, MNHN 2012, J. Poupin, L. Corbari); ***Geograpsus*** *lividus* (H. Milne Edwards, 1837). Guadeloupe (Expedition KARUBENTHOS, MNHN 2012, J. Poupin, L. Corbari); ***Goniopsis*** *cruentata* (Latreille, 1803). Brazil, Bahia (Arthur Anker, Flickr); ***Grapsus*** *grapsus* (Linnaeus, 1758). Guadeloupe (Expedition KARUBENTHOS, MNHN 2012, J. Poupin, L. Corbari); ***Metopaulias*** *depressus* Rathbun, 1896. Jamaica (endemic species, Vogt 2013 based on Diesel & Schubart 2001); ***Pachygrapsus*** *gracilis* (Saussure, 1858). Guadeloupe (Expedition KARUBENTHOS, MNHN 2012, J. Poupin, L. Corbari); ***Pachygrapsus*** *transversus* (Gibbes, 1850). Brazil, São Paulo State (own work); ***Sesarma*** rectum Randall, 1840. Brazil, Paraná (own work).

Larval development / Habitat	Less than 4 stages	4 to 6 stages	8 stages
Freshwater and Mangroves	 *Metopaulias depressus*	 *Aratus sp.*	 *Goniopsis sp.*
	 *Sesarma sp.*	 *Armases sp.*	 *Pachygrapsus gracilis*
Rocky shores		 *Cyclograpsus integer*	 *Geograpsus lividus*
			 *Grapsus grapsus*
			 *Pachygrapsus transversus*

Figure I.4. Examples of sampled habitats in the tropical and subtropical Atlantic, mangroves and rocky shores. A. Frontline of the mangrove patch close to the Amazon river (Brazil, Pará, Marudá). B. Small channel in a large brackish bay (Brazil, Paraná, Baía de Guaratuba). C. Rocky plateau in front of Ilhéus (Brazil, Bahia). D. Rocky area in Matinhos (Brazil, Paraná).

On the other hand, these species or genera are representative inhabitants of two distinct coastal habitats: mangrove (*Aratus*, *Armases*, *Goniopsis*, *Pachygrapsus gracilis* and *Sesarma*, Figs. I.3 & I.4) and rocky shores (*Cyclograpsus*, *Geograpsus*, *Grapsus* and *Pachygrapsus transversus* / *socius*, Figs. I.3 & I.4). Knowlton & Weigt (1998) noticed that mangroves species of the pistol shrimp genus *Alpheus* exhibit lower genetic differentiation than other species, and concluded that mangroves were probably the last habitat to allow genetic exchanges between Atlantic and Pacific oceans populations, during the final steps of closure of the Isthmus of Panama, around 3.1 mya.

2. Taxonomy of the American thoracotreme crabs

The subsection Thoracotremata Guinot, 1977 represents one of the three clades of the Brachyura with the Heterotremata and Podotremata Guinot, 1977, and includes more than 1100 species, representing approximately 17% of the species of 'crabs' (Ng *et al.* 2008, De Grave *et al.* 2009). This clade, resulting from a massive taxonomical reorganization of the Brachyura based on morphological data (Guinot 1977), includes four superfamilies previously described during the 19th century: Cryptochiroidea Paul'son, 1875 ('gall crabs'), Grapsoidea MacLeay, 1838 ('square crabs'), Ocypodoidea Rafinesque, 1815 ('ghost crabs' and 'fiddler crabs') and Pinnotheroidea De Haan, 1833 ('pea crabs'). However molecular studies have challenged this traditional taxonomy, highlighting the polyphyly of two of the superfamilies (Grapsoidea and Ocypodoidea) in their current composition (Schubart *et al.* 2006, Tsang *et al.* 2014), and called for further studies to resolve the evolutionary histories in the Thoracotremata.

As result of the impact of these molecular studies on the taxonomy of the Thoracotremata, the Sesarmidae became the most speciose family in this subsection, with more than 250 species (De Grave *et al.* 2009). This family has a pan-tropical and subtropical distribution, with few temperate species. Sesarmid species typically inhabit mangroves, freshwater and terrestrial environments, representing an important part of the corresponding biodiversity (Schubart *et al.* 2000), being less common in rocky shores (see Ng & Liu 1999, Schubart *et al.* 2009). A summary of the complex taxonomic history of the Sesarmidae is illustrated in Figure I.5, and specifically the taxonomic history of the American endemic Sesarmidae genera can be found in the chapter 4 of this dissertation.

The Grapsidae on the other hand, constitute a family of 40 species in eight genera, present in subtropical and tropical regions, where they typically inhabit rocky shores, but few species are also found in mangroves (genera *Goniopsis* and *Metopograpsus*) and are even hyponeustonic (genus *Planes*). Two recent molecular phylogenies confirmed the monophyly of this family (Schubart 2011, Ip *et al.* 2015). These phylogenies also highlighted the polyphyly of the genus *Pachygrapsus*, which was erected on a morphological basis (Poupin *et al.* 2005).

The family Varunidae also belongs to the superfamily Grapsoidea, and apparently represents a monophyletic group of approximately 150 species (Schubart *et al.* 2006, De Grave *et al.* 2009).

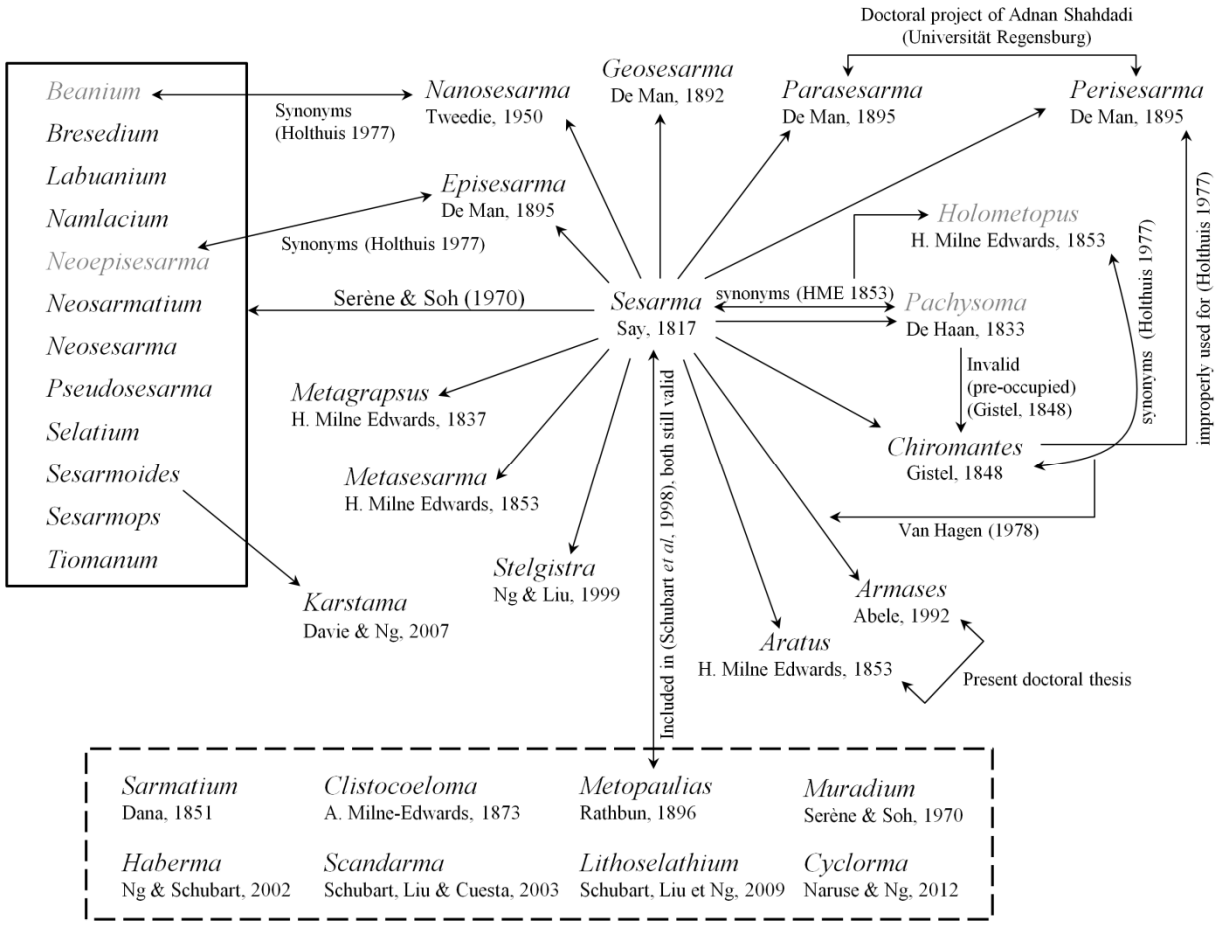

Figure I.5. Historical relationships between sesarmid genera. Vectors indicate in which genus the type-species of another genus was initially placed. Double-arrow indicates synonymy or confusion. The dashed box corresponds to genera with type-species originally described in their genus. The full box corresponds to the genera described in the massive reorganization of Southeast Asian Sesarmidae by Serène & Soh (1970). Black: valid genera; Grey: currently invalid genera.

4. *Objectives*

The objectives of this dissertation are to investigate the consequences of the closures of the Isthmus of Panama and the Central American Seaway, especially the impact of the genetic isolation of coastal populations affected by this closure. This investigation followed a range from intraspecific to intrageneric comparisons. At all the levels, a special emphasis was given to determine, if shared characteristics could be observed between species with similar ecological characteristics.

At the **intraspecific level**, the large distribution in the Atlantic of coastal crabs, especially several species affected by the closure of the isthmus (*Aratus pisonii* and *Pachygrapsus transversus*), called for a multispecies phylogeographic comparison. So far, only a limited number of studies explored the relationships between the Caribbean and Brazilian marine fauna. I investigated if these species are genetically homogeneous or present genetic breaks. In case of heterogeneity, could the observed genetic breaks be associated with geographic barriers? How strong is the genetic heterogeneity along a coastline compared to the genetic divergence resulting from the closure of isthmus? How do dispersal and vicariance affect populations and shape the population structure of studied species?

At the **transition between the intra and interspecific levels**, I wanted to determine, if the transisthmian populations of *Aratus pisonii* really belong to the same species, as both populations have been postulated to be morphologically not distinguishable. The absence of marked genetic differentiation between these populations would indicate the presence of gene flow between the two Central American coastlines and confirm that *Aratus pisonii* is present on both sides of the isthmus. In contrast, if a genetic break between the Atlantic and Pacific populations is revealed, it would indicate the presence of cryptic or overlooked species of what is called *Aratus pisonii*. If a genetic break is observed, can morphological criteria be identified to distinguish the Atlantic and Pacific lineages?

At the **interspecific level**, an alternative model and timing were proposed in 2012 to explain the closure of this isthmus (Montes *et al.* 2012a, 2012b). I tested this model in comparison with the traditional model using transisthmian sister species of crabs, to determine which model is supported by the transisthmian genetic divergence.

At the **intergeneric level**, I assessed the phylogenetic relationships of the American sesarmid genera *Aratus*, *Armases*, *Metopaulias* and *Sesarma*, to determine if the genetic relationships are concomitant with the ones previously assumed on morphological and ecological bases. One important question was, if *Aratus* is the sister taxa to *Armases* or did *Aratus* evolved within Armases?

CHAPTER 1

Comparative phylogeography of thoracotreme crabs in the western tropical Atlantic and the importance of different life histories

Introduction

The Caribbean is a major marine tropical hotspot in the Atlantic Ocean (Roberts *et al.* 2002, Bowen *et al.* 2013). The tropical Atlantic ranges from Florida (Cape Canaveral) and southern Gulf of Mexico, through the Caribbean to South American coastline up to southern Brazil, representing nearly 10 000 km between its limits. The eastern tropical Atlantic covers the African coastline from Mauritania to Angola (Spalding *et al.* 2007). The tropical Atlantic is isolated to the north and south from other tropical regions by the presence of colder temperate waters, preventing any recent genetic exchange with other tropical marine fauna. Nevertheless, this physical isolation is geologically recent. In the East Atlantic, it results from the establishment of the Benguela upwelling approximately 2 mya (Rocha *et al.* 2005). In the West Atlantic, the isolation is the direct consequence of the closure of the Central American Seaway by the rise of the Isthmus of Panama approximately 3.1 mya (Coates & Obando 1996, Coates *et al.* 2004).

The closure of the Isthmus of Panama was an important geological event, corresponding to the collision between Caribbean and the South American plates, at the area of interaction between the Caribbean, Cocos, Nazca and South American tectonic plates. This event was progressive, starting during the Miocene, and progressively narrowed and shallowed the Central American Seaway, ending during the Pliocene by the formation of a land-bridge between the previously isolated North and South American continents (Coates *et al.* 2004). At this point, marine populations became physically isolated on both sides of the Isthmus. As consequence, these populations initiated independent genetic divergence in different oceanographic environments. The consequences of the closure Central American Seaway on marine populations have been largely reviewed by Lessios (2008).

Approximately at the same period, the northern part of the South American continent was affected by the Andean uplift. This uplift reorganized the drainage systems of the

continent, as the Amazonian megawetlands disappeared approximately 7 mya; the Orinoco, orientated northward changed its course eastward, and both Orinoco and Amazon rivers outflows increased (Hoorn *et al.* 1995, 2010). The establishment of the Orinoco and Amazon rivers in their current configuration affected the biota, and created potential freshwater barriers between the Caribbean and Brazilian coastlines. These barriers are responsible for the present faunistic differences between these two coastlines, including their respective endemicity. However this barrier also appears to be permeable, allowing exchanges between the provinces it separates (Briggs 1995, Joyeux *et al.* 2001, Rocha 2003, Floeter *et al.* 2008, Luiz *et al.* 2012, Bowen *et al.* 2013, Luiz *et al.* 2013).

So far, the western tropical Atlantic has been mostly studied under a biogeographic approach, comparing both compositions and distributions of species it hosts. These studies remain largely focused on reef species, as corals and fishes, and only limited information is available concerning other taxa which can be limited to the Caribbean or Brazilian provinces (e.g. Oliveira-Neto *et al.* 2007, Kool *et al.* 2010). Among coastal species, the brachyurans are typical inhabitants of intertidal rocky shores, sandy beaches and mangroves, where they even represent the dominant macrofauna (Nagelkerken *et al.* 2008). The mangrove habitat is dynamic, being at the transition between marine and freshwater. Its distribution was strongly affected by Quaternary climatic oscillations, with changes in the sea surface temperatures and sea-level, resulting in a constriction of its distribution, affecting as consequence its biota (Woodroffe & Grindrod 1991, Hewitt 2004, Nettel & Dodd 2007). For the brachyurans, the relationship between Caribbean and Brazilian provinces have been only investigated by Schubart *et al.* (2005) and Laurenzano *et al.* (2013), describing different scenarios. Schubart *et al.* (2005) observed an absence of differentiation between the two provinces, when on the opposite, Laurenzano *et al.* (2013) identified the Orinoco River as gene flow barrier between them.

The present study is designed to fill this knowledge gap, by exploring the relationships between populations along the western tropical Atlantic in four species of thoracotreme crabs living on rocky shores (*Cyclograpsus integer* H. Milne Edwards, 1837 & *Pachygrapsus transversus* (Gibbes, 1850)) and in mangroves (*Aratus pisonii* (H. Milne Edwards, 1837) & *Pachygrapsus gracilis* (Saussure, 1858)), and characterized by differences in their dispersal abilities (relatively short larval development for *A. pisonii* and *C. integer*, long larval development for both species of *Pachygrapsus*, Anger 1995, Cuesta *et al.* 2011). A special emphasis is given to the relationships between Caribbean and Brazilian provinces to determine, if the Amazon – Orinoco freshwater Plume (AOP) acts as a barrier to gene flow in

these species, and taking into account the ecological differences among the species. As two species are living in mangroves, we wanted to determine the recent impact of Quaternary climatic oscillations on them, in comparison to the rocky shore species. Finally, the presence of sister species in the eastern tropical Pacific as consequence of the closure of the Isthmus of Panama for both *Aratus pisonii* and *Pachygrapsus transversus* brings an additional element of comparison to determine the actual impact of this much older event on the species inhabiting this region.

Material and Methods

Specimen collection

Specimens from four thoracotreme brachyuran species or species complexes, *Aratus pisonii* / *pacificus* (*n* = 194), *Cyclograpsus integer* (*n* = 71), *Pachygrapsus gracilis* (*n* = 48) and *Pachygrapsus transversus* / *socius* (*n* = 88) respectively, were collected between 1993 and 2014 along the western tropical Atlantic coastline, and along the eastern tropical Pacific coastline, covering the known distribution ranges along the American continent (Table 1.1). *Aratus sp.* and *P. gracilis* were collected in mangrove habitats, *C. integer* and *P. transversus / socius* on rocky shores. Specimens were preserved in ethanol >70%.

Extraction, sequencing and alignment

Genomic DNA was extracted from muscular leg tissue using the Puregene kit (Qiagen). Fragments of approximately 1000 basepairs (bp) of the mitochondrial cytochrome oxydase I gene (Cox1) were amplified with the Thoracotremata-specific primers COL8 and COH16 (Schubart 2009) with the following PCR profile: initial step 4 min at 94°C, 40 cycles with 45s at 95°C - 45s at 50°C - 75s at 72°C for denaturing, annealing and extension respectively, final extension step 5 min at 72°C. Previously, specimens of *Pachygrapsus transversus* / *socius* had been amplified with the shorter combination COL1b and COH1b (Schubart 2009) under similar parameters, rendering a fragment length of ~600bp. PCR products were outsourced for sequencing to LGC Genomics Gmbh or Macrogen, or sequenced on an Abi Prism 310.

Table 1.1. Species, sampling locality, number of specimens and site color coding in haplotype networks.

Species	Origin	*n*	Site
Aratus pisonii (H. Milne Edwards, 1837) – Atlantic Ocean			
	USA. Florida, Bonita Beach	2	
	USA. Florida, Boca Raton	2	
	USA. Florida, Long Key	2	
	USA. Florida, Everglades City	7	
	USA. Florida, Naples, Marco Island	5	
	USA. Florida, Cedar Key	4	
	USA. Florida, Sebastian Inlet	1	
	USA. Florida, Tampa, Alafia River	1	
	Mexico. Veracruz, Laguna San Angustin	2	
	Mexico. Veracruz, Laguna Buenpais	3	
	Mexico. Tabasco, Puerto Ceiba	1	
	Mexico. Taumalipas, Barra del Tordo	1	
	Jamaica. St. Ann Parish	15	
	Jamaica. St. James Parish, Montego Bay	7	
	Jamaica.	1	
	Dominican Republic. Bahía de Las Calderas, Las Salinas	1	
	Dominican Republic. Bahía de Luperón	12	
	Puerto Rico. Culebra Island	1	
	Honduras. Utila, Iron Bound	1	
	Belize. Twin Cays	6	
	Costa Rica. Limón, Puerto Viejo de Talamanca, Punta Uva	1	
	Venezuela. Falcón, Peninsula Paraguaná, Bahia de Yaima	4	
	Venezuela. Falcón, Cayo Sombrero	11	
	France. Martinique	1	
	Trinidad. Caroni swamp	2	
	Brazil. Pará, Marapanim, Marudá	18	
	Brazil. Bahia, Itacaré, Rio de Contas	17	
	Brazil. São Paulo, Bertioga, Rio Itapanhau	11	
Aratus pacificus Thiercelin & Schubart, 2014 – Pacific Ocean			
	Mexico. Baja California Sur, Bahia Magdalena, Puerto San Carlos	6	
	Costa Rica. Puntarenas, Punta Morales	11	
	Costa Rica. Puntarenas, Mata de Limón	18	
	Costa Rica. Golfo Dulce	4	
	Ecuador. Puerto Morro	16	

Cyclograpsus integer H. Milne Edwards, 1837 – Atlantic Ocean

USA. Florida, Fort Pierce	1
Cuba. Pinar del Rio, Puerto Esperanza	2
Cuba. Playa Larga	2
Jamaica.	1
Jamaica. St. James Parish, Montego Bay	18
Costa Rica. Limón, Cahuita	11
Panama. Maria Chiquita, Portobelo	3
Brazil. Bahia, Itacaré, Praia da Concha	21
Brazil. São Paulo, Sao Vincente, Haquitanduva	12

Pachygrapsus gracilis (Saussure, 1858) – Atlantic Ocean

Jamaica. St. Ann Parish, Priory, New Seville	7
Jamaica. St. James Parish, Montego Bay	3
Panama. Maria Chiquita, Portobelo	11
Costa Rica. Limón, Cahuita	2
Brazil. Pará, Marapanim, Marudá	15
Brazil. São Paulo, Praia Grande, Portinho	10

Pachygrapsus transversus (Gibbes, 1850) – Atlantic Ocean

USA. Florida, Boca Raton	12
Cuba. Artemisa, Playa Baracoa, Playa del Salado	7
Cuba. Pinar del Rio, Puerto Esperanza	3
Jamaica. St. Ann Parish, Priory	5
Jamaica. St. James Parish, Montego Bay	5
Costa Rica. Limón, Puerto Viejo de Talamanca, Manzanillo	10
Panama. Maria Chiquita	1
Brazil. Bahia, Ilhéus	10
Brazil. São Paulo, Sao Sebastião, Praia do Segredo	11
Brazil. São Paulo, Ubatuba	1
Brazil. Santa Catarina	1

Pachygrapsus socius Stimpson, 1871 – Pacific Ocean

Mexico. Baja California Sur, Playa Santispac	2
Mexico. Baja California Sur, Mulegé	1
Costa Rica. Puntarenas, Punta Morales	1
Costa Rica. Puntarenas, Mata de Limón	1
Costa Rica. Golfo Dulce, Golfito	15
Panama. Naõs	1
Peru. Lima, Callao	1

DNA sequences were proofread with Chromas 2.23 and aligned with ClustalW (Thompson *et al.* 1994) as implemented in BioEdit 7.0.5 (Hall 1999). To limit the risk of pseudogenes (highlighted by Williams & Knowlton 2001 for crustaceans), the absence of stop-codons, potential indicators of pseudogenes, was assessed with Artemis 14 (Rutherford *et al.* 2000) using the invertebrate mitochondrial genetic code (NCBI Table 5). Sequences have been submitted to EMBL and available at ENA/GenBank under accession number (pending).

Gene genealogies

Statistical parsimony haplotype networks were constructed with **TCS** 1.21 (Clement *et al.* 2000). As our older sequences of *Pachygrapsus transversus* were shorter than newer ones, the missing data were initially filled with question marks in the datasets. The impact of missing data in haplotype networks has been analyzed and reviewed by Joly *et al.* (2007). They highlight that missing data can give misleading networks due to the method of network reconstruction by TCS and the other haplotype network reconstruction software as Network or Arlequin. This was confirmed by tests conducted on our datasets, especially the strong underestimation of missing haplotypes between genetic clusters when sequences with missing data are present, even when following the recommendations made by Joly *et al.* (2007), as placing these sequences in the end of the dataset. None of these software packages has been since updated to improve the use of sequences with missing data. As a result, a trade-off between specimen exclusion and dataset shortening has been conducted to maximize the information. For *P. transversus* / *socius*, as early sequences were shorter (less than 600bp), haplotype networks built with a longer fragments but less individuals (60 individuals / 955bp) is included as Supplementary Material as element of comparison.

Population structure in Bayesian framework

In order to determine the genetic clustering of the different species and the distribution of these clusters in each ocean, the R-based computer package **GENELAND** 4.0.4 (Guillot *et al.* 2005) was used. Using genetic data of georeferenced populations and Markov chain Monte Carlo (MCMC), this software infers the most likely number of clusters and their geographic distribution under a Bayesian approach, based on an assumption of unknown number of clusters and their equal likelihood. Considering a maximum number of clusters K = 5 (*a priori* based on haplotype networks), a maximum rate of Poisson process equal to the number

of individuals in the dataset, a maximum number of nuclei equal to 3 times the maximum rate of Poisson process (as suggested by Guillot *et al.* 2005), we ran 5 000 000 MCMC iterations sampled each 1000, and the first 500 samples were discarded. For the Atlantic populations of *Aratus*, all the specimens were included in a first run (with K maximum = 10), and only *Aratus pisonii* sensu stricto was considered during the second run (with K = 5).

Genetic diversity and population structure

Standard genetic indices were calculated to determine the genetic diversity in the different species along their distribution range for each population with at least 9 individuals. The haplotype diversity (*Hd*), nucleotide diversity (π), number of polymorphic sites (*S*), number of haplotypes (*k*) were calculated with DnaSP 5.1 (Librado & Rozas, 2009). The neutrality tests Tajima's *D* and Fu's *Fs* were calculated with Arlequin 3.5 (Excoffier & Lischer 2010) with 1000 bootstraps to determine possible deviation from the neutral model, as excess of low frequency mutations indicating recent population expansions. Mismatch distributions of pairwise differences in Caribbean and Brazilian populations, and in other genetic lineages were calculated with DnaSP. The obtained frequencies were compared to expected frequencies under a constant population size model.

The partitioning of the populations within each ocean was assessed by Analyses of MOlecular VAriance (AMOVA) using Arlequin 3.5 and statistically assessed with 1000 bootstraps. For the Atlantic, two groups were defined and correspond respectively to the Caribbean Sea and the Brazilian coastlines, to determine if a disjunction is observed as a result of the Amazon - Orinoco freshwater outflow (Luiz *et al.* 2012). Pairwise population relationships (Φ_{ST}) were calculated with the same software (significance assessed with 1000 bootstraps). These pairwise comparisons allow to identify the possible presence of significant gene flow limitation among populations.

Table 1.2. Intra- and inter-clade uncorrected p-distances in *Aratus sp.*. Average ± standard deviation in percent.

		Aratus pisonii			*A. pisonii* Gulf of Mexico	*Aratus pacificus*		*A. pacificus* Baja Cal - CRica	*Aratus pacificus*
		Florida	Caribbean	Brazil		Costa Rica	Ecuador		
Aratus pisonii	Florida	0.05 ± 0.08							
	Caribbean	0.59 ± 0.13	0.16 ± 0.15						
	Brazil	1.04 ± 0.10	1.10 ± 0.13	0.09 ± 0.10					
Aratus pisonii GoM		4.62 ± 0.06	4.36 ± 0.08	5.12 ± 0.08	0.25				
Aratus pacificus	Costa Rica	4.52 ± 0.07	4.78 ± 0.13	5.26 ± 0.09	5.24 ± 0.04	0.03 ± 0.05			
	Ecuador	5.01 ± 0.07	5.26 ± 0.09	5.75 ± 0.09	4.98 ± 0.03	1.76 ± 0.05	0.02 ± 0.04		
A. pacificus Baja-CR		6.63 ± 0.08	6.62 ± 0.10	7.37 ± 0.10	5.41 ± 0.12	3.14 ± 0.07	2.90 ± 0.06	0.14 ± 0.17	
Aratus pacificus		7.97 ± 0.05	7.97 ± 0.09	8.32 ± 0.08	7.21	6.97 ± 0.03	6.96 ± 0.03	6.61 ± 0.06	

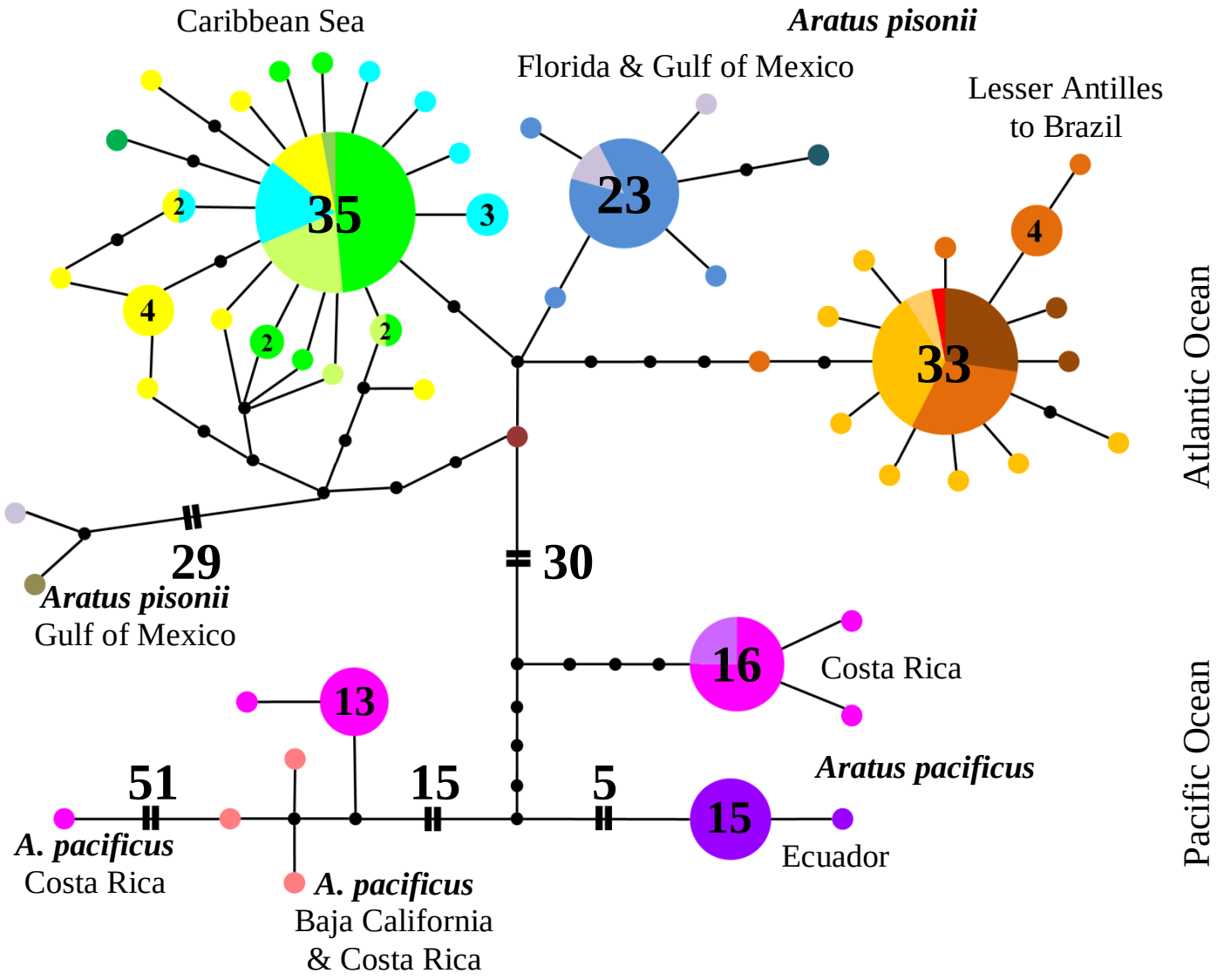

Figure 1.1. Maximum parsimony spanning network of *Aratus sp.* reconstructed with TCS based on 194 mtDNA Cox1 sequences (804bp). Each line represents a substitution, dots represent single missing haplotype, double bars with values indicates multiple missing haplotypes; size of the haplotypes proportional to the number of individuals with number of specimens indicated if superior at one. Color coding in Table 1.1.

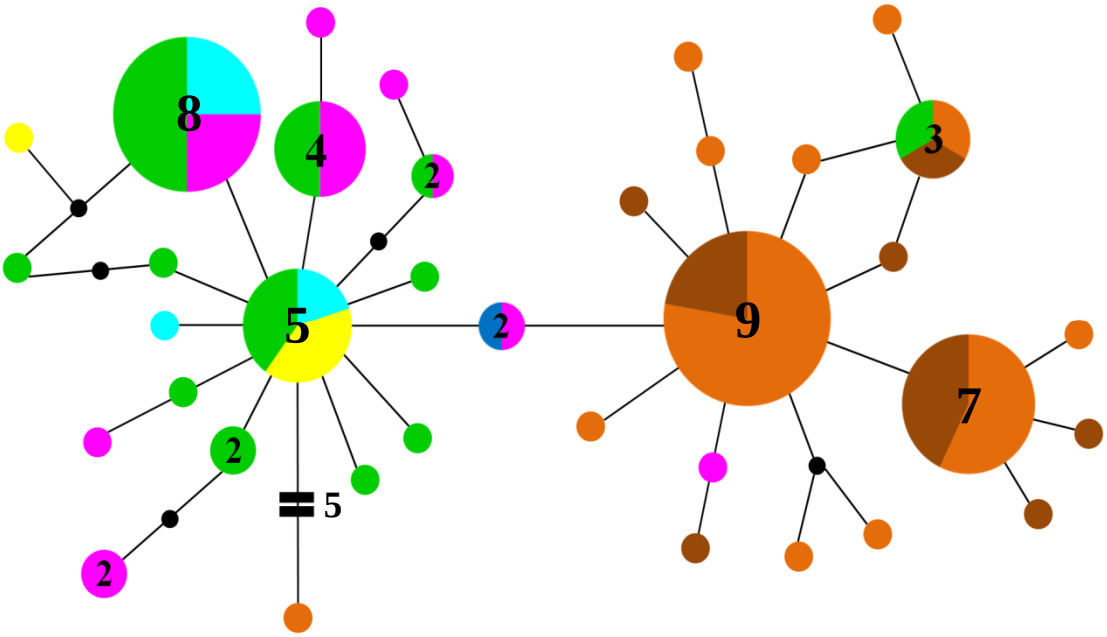

Figure 1.2. Maximum parsimony spanning network of *Cyclograpsus integer* reconstructed with TCS based on 70 mtDNA Cox1 sequences (807bp). Each line represents a substitution, dots represent single missing haplotype, double bars with values indicates multiple missing haplotypes; size of the haplotypes proportional to the number of individuals with number of specimens indicated if superior at one. Dark blue: Florida; turquoise: Cuba; green: Jamaica; pink: Costa Rica; yellow: Panama; orange: Brazil – Bahia; brown: Brazil – São Paulo.

Results

Gene genealogies

After alignment, datasets of 579bp and 955bp for *P. transversus* / *socius*, 804bp for *Aratus sp.*, 807bp for *C. integer* and 937bp for *P. gracilis* were obtained. A stop-codon (TAA) was detected in a specimen from *C. integer* (Bahia). As it is not possible to determine if this single mutation (resulting in the stop codon) represents a sequencing error or the amplification of a pseudogene (see Williams & Knowlton 2001), this specimen has been excluded from further analyses.

The haplotype networks depict the extremely different patterns among the Atlantic species. Differences in the number of clusters are observed, ranging from one genetic cluster to up to two clades and three clusters (Figs. 1.1 - 1.4). For the Pacific sister species,

differences are here also observed between the two species of our dataset. The most simple network recovered corresponds to the sister species *Pachygrapsus transversus* / *P. socius*. A p-distance of 11.71 ± 0.13% is found between the two species and only a single clusters are found per ocean. Each cluster includes specimens from the most distant sites collected (from Florida to South Brazil in *P. transversus*, from Baja California to Peru in *P. socius*), and both clusters present a star-like topology, with a central haplotype including most of the individuals surrounded by peripheral haplotypes with a distance of one or two mutations (Figs. 1.4 and S1.1). *Pachygrapsus gracilis* also presents a single clade in the Atlantic, but other than *P. transversus*, the network is strongly scattered, with up to 11 mutations between specimens from the same site (Panama). However, no geographic association can be observed in the distribution of the haplotypes, as some haplotypes are shared by specimens collected in Brazil and in Jamaica (Fig. 1.3). *Cyclograpsus integer* presents two clearly distinct clusters

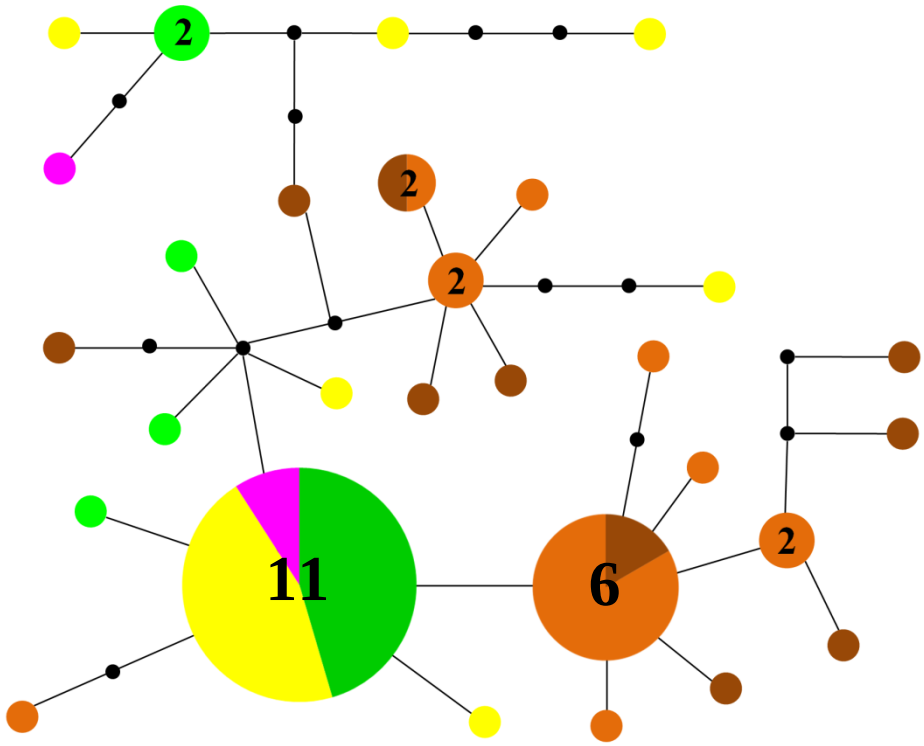

Figure 1.3. Maximum parsimony spanning network of *Pachygrapsus gracilis* reconstructed with TCS based on 48 mtDNA Cox1 sequences (937bp). Each line represents a substitution, dots represent single missing haplotype, double bars with values indicates multiple missing haplotypes; size of the haplotypes proportional to the number of individuals with number of specimens indicated if superior at one. Green: Jamaica; pink: Costa Rica; yellow: Panama; orange: Brazil – Pará; brown: Brazil – São Paulo.

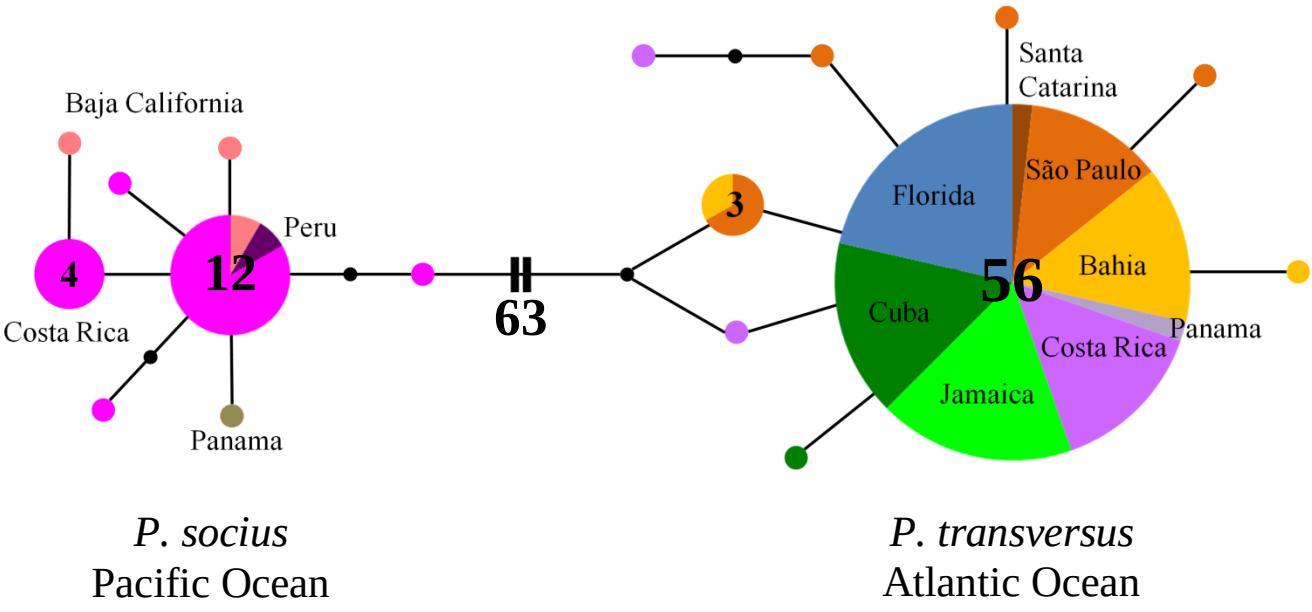

Figure 1.4. Maximum parsimony spanning network of the sister species *Pachygrapsus transversus* (Atlantic Ocean) and *P. socius* (Pacific Ocean) reconstructed with TCS based on 88 mtDNA Cox1 sequences (579bp). Each line represents a substitution, dots represent single missing haplotype, double bars with values indicates multiple missing haplotypes; size of the haplotypes proportional to the number of individuals with number of specimens indicated if superior at one.

that can be associated to the Caribbean and Brazilian coastlines respectively. Only a single mutation separates these clusters (p-distance: 0.53 ± 0.18%). Two specimens collected in the Caribbean (Jamaica and Costa Rica) are recovered in the Brazilian cluster, while on the other hand, a specimen from Bahia (Brazil) appears to be closer to the Caribbean than to the Brazilian cluster. Both clusters present a central haplotype surrounded by peripheral haplotypes, but the topology is more scattered than in *P. transversus* (Fig. 1.2). The most complex network is recovered for *Aratus sp.*, as several lineages and clusters are found in each ocean. The intra- and inter-clade p-distances are summarized in the Table 1.2. In the Atlantic Ocean, two sympatric clades are found: *Aratus pisonii* sensu stricto and an undescribed lineage found in the Gulf of Mexico. In *Aratus pisonii* sensu stricto, three geographically associated genetic clusters are observed (Fig 1.1). The first cluster corresponds to specimens from the Brazilian coast up to the Lesser Antilles (presently Trinidad and a single specimen of Martinique). The second one corresponds to specimens from the Caribbean Sea including the mainland and the Greater Antilles (from Cuba to Venezuela). The last cluster is represented by specimens from Florida and the Gulf of Mexico. In the

Pacific Ocean, four genetic clades are observed: one first clade with Ecuadorian specimens and another clade with exclusively Costa Rican specimens correspond to *Aratus pacificus* sensu stricto. The third clade gathers specimens from Baja California and Costa Rica, and the last clade is represented by a single specimen collected in Costa Rica. Individuals from the three clades sampled in Costa Rica are found in sympatry. Nearly all the clades in both Atlantic and Pacific present strongly marked star-like topologies. This remains unclear for the Baja California clade as a result of the small sample size (Fig. 1.1).

The two neutrality tests and the mismatch distributions also confirm the results observed with the haplotype networks, with a range among the species in the significance level. All species are postulated to be in population expansion, when all sequences are considered, but contrasting results are observed at the population level (Tables 1.3-1.6). *P. gracilis* is the only species which has populations at the equilibrium (Tajima's *D* & Fu's *Fs* close from 0 and non significant), except for the population of São Paulo for which a strongly negative and significant *Fs* is recovered. In this species, the ragged mismatch distribution of the Caribbean Sea population is congruent with a constant population size, but the unimodal distribution observed in Brazilian population suggests past population expansion (Fig. 1.14). For *Aratus pacificus* and *Pachygrapsus transversus*, the non-significance of the neutrality tests can be associated with the low haplotype diversity, probably resulting from bottlenecks in these species (Figs. 1.12 & 1.15). In contrast, both *Aratus pisonii* and *Cyclograpsus integer* present strongly negative and significant *Fs* and *D*, both indicating recent population expansions. This result is also recovered in the mismatch analyses with the unimodal distributions observed (Figs. 1.12 & 1.13).

Table 1.3. Genetic diversity indices and neutrality tests in *Aratus sp.*. In grey: Pacific sites or lineages. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *n*: number of individuals; *h*: number of haplotypes; *S*: number of polymorphic sites; *Hd*: haplotype diversity; π: nucleotide diversity.

Population	***n***	***h***	***S***	***Hd***	**π**	**Tajima's *D***	**Fu's *Fs***
Florida	23	4	3	0.249	0.00032	-1.73*	-2.96**
Dominican Republic	13	6	5	0.769	0.00124	-1.35	-3.13**
Jamaica	23	6	5	0.458	0.00064	-1.82*	-4.26***
Central America	11	4	4	0.491	0.0009	-1.71*	-1.41*
Venezuela	15	9	11	0.886	0.00291	-1.19	-3.80**
Amazon	18	8	8	0.641	0.00111	-2.15**	-5.93***
Bahia	17	5	5	0.625	0.00113	-1.24	-1.57
São Paulo	11	3	2	0.345	0.00045	-1.43*	-1.25*
Costa Rica Morales	11	2	1	0.182	0.00023	-1.13	-0.41
Costa Rica Mata de Limón	18	5	67	0.549	0.01642	-1.34[1]	9.44[1]
Ecuador	16	2	1	0.125	0.00016	-1.16	-0.70
Aratus pisonii	140	41	48	0.857	0.00648	-1.23	-18.91***
Caribbean Sea	92	28	38	0.795	0.00351	-1.95**	-17.48***
Brazil	48	14	15	0.555	0.00096	-2.38**	-14.60***
A. pisonii Gulf of Mexico	2	2	2	1	0.00249	-	0.69
Aratus pacificus	34	5	17	0.599	0.00916	2.58	9.10
A. pacificus Baja Cal. - CRica	17	5	6	0.426	0.00135	-1.30	-1.12

[1]When only *Aratus pacificus* sensu stricto is considered on this site (*n* = 14), the Tajima's *D* is -1.48 and the Fu's *Fs* is -1.48*.

Table 1.4. Genetic diversity indices and neutrality tests for *Cyclograpsus integer*. *P < 0.05, **P < 0.01, ***P < 0.001. *n*: number of individuals; *h*: number of haplotypes; *S*: number of polymorphic sites; *Hd*: haplotype diversity; π: nucleotide diversity.

Population	***n***	***h***	***S***	***Hd***	**π**	**Tajima's *D***	**Fu's *Fs***
Jamaica	19	13	16	0.947	0.00304	-1.74*	-8.60***
Costa Rica & Panama	14	10	15	0.956	0.0038	-1.43	-4.49**
Bahia	20	12	16	0.874	0.00314	-1.63*	-6.21**
São Paulo	12	8	8	0.894	0.00229	-1.21	-4.33**
Cyclograpsus integer	70	36	34	0.955	0.00407	-1.72*	-26.28***
Caribbean Sea	38	20	23	0.932	0.00311	-1.83*	-14.64***
Brazil	32	17	19	0.883	0.00285	-1.75*	-11.80***

Table 1.5. Genetic diversity indices and neutrality tests for *Pachygrapsus gracilis*. *P < 0.05, **P < 0.01, ***P < 0.001. *n*: number of individuals; *h*: number of haplotypes; *S*: number of polymorphic sites; *Hd*: haplotype diversity; π: nucleotide diversity.

Population	***n***	***h***	***S***	***Hd***	**π**	**Tajima's *D***	**Fu's *Fs***
Jamaica	10	5	7	0.756	0.00235	-0.47	-0.39
Caribbean coast	13	8	18	0.808	0.0043	-1.30	-1.32
Amazon	15	9	13	0.886	0.00325	-0.93	-2.76
São Paulo	10	10	17	1	0.00548	-0.68	-5.81***
Pachygrapsus gracilis	48	29	40	0.934	0.00409	-1.97**	-22.45***
Caribbean Sea	23	12	21	0.779	0.00339	-1.63*	-4.09*
Brazil	25	17	24	0.94	0.00411	-1.45	-9.38*

Table 1.6. Genetic diversity indices and neutrality tests for *Pachygrapsus transversus* / *socius*. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. In grey: Pacific lineage. *n*: number of individuals; *h*: number of haplotypes; *S*: number of polymorphic sites; *Hd*: haplotype diversity; π: nucleotide diversity.

Population	***n***	***h***	***S***	***Hd***	**π**	**Tajima's *D***	**Fu's *Fs***
P. transversus Florida	12	1	0	0	0	-	-
Cuba	10	2	1	0.2	0.00035	-1.11	-0.34
Jamaica	10	1	0	0	0	-	-
Costa Rica	10	3	4	0.378	0.00138	-1.67*	0.06
Bahia	10	3	2	0.378	0.00069	-1.40	-1.16*
São Paulo	13	5	4	0.628	0.00129	-1.44	-2.53**
P. socius Costa Rica	17	5	6	0.625	0.00168	-1.52*	-1.41
P. transversus all sequ.	66	9	9	0.281	0.00062	-2.16***	-9.52***
Caribbean Sea	43	4	5	0.136	0.0004	-2.00**	-2.73*
Brazil	23	6	5	0.514	0.00101	-1.67*	-3.79***
P. socius all sequences	22	8	9	0.688	0.00189	-1.86*	-4.33***

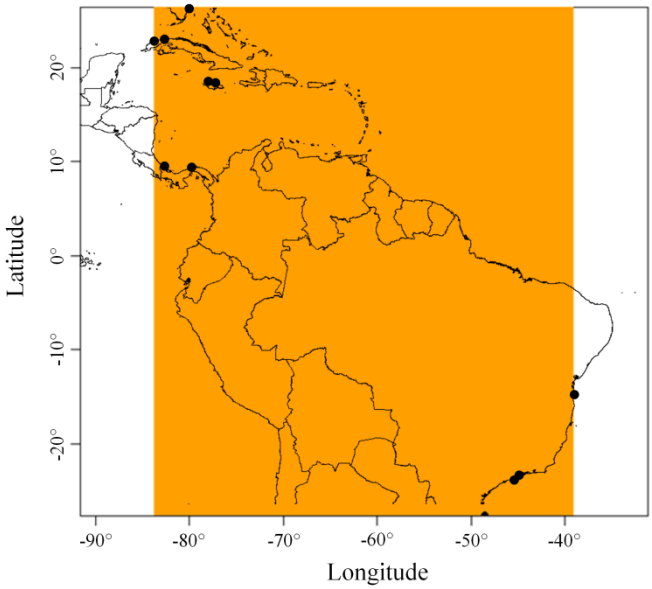

Figure 1.5. Map of posterior probability of cluster membership generated by Geneland based on 66 individuals of *Pachygrapsus transversus*. Black dots indicate the relative position of the sampled sites.

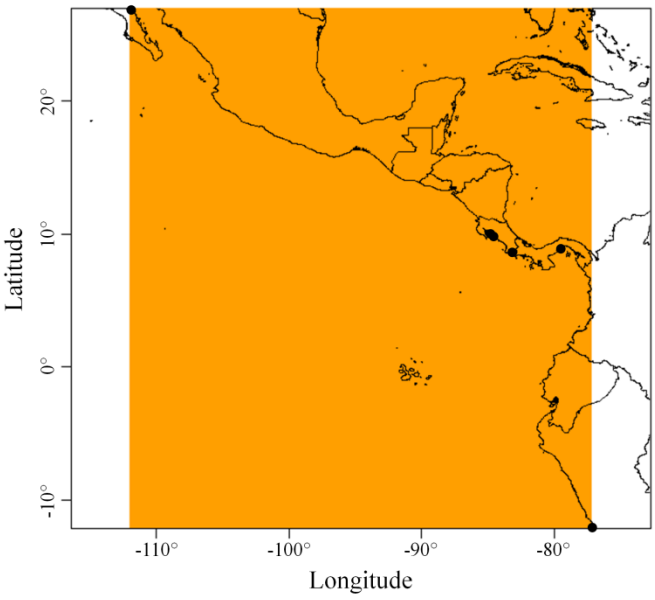

Figure 1.6. Map of posterior probability of cluster membership generated by Geneland based on 22 individuals of *Pachygrapsus socius*. Black dots indicate the relative position of the sampled sites.

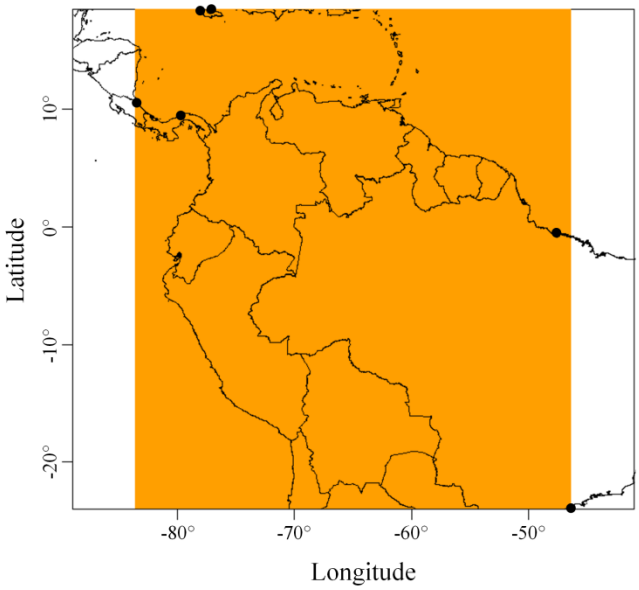

Figure 1.7. Map of posterior probability of cluster membership generated by Geneland based on 48 individuals of *Pachygrapsus gracilis*. Black dots indicate the relative position of the sampled sites.

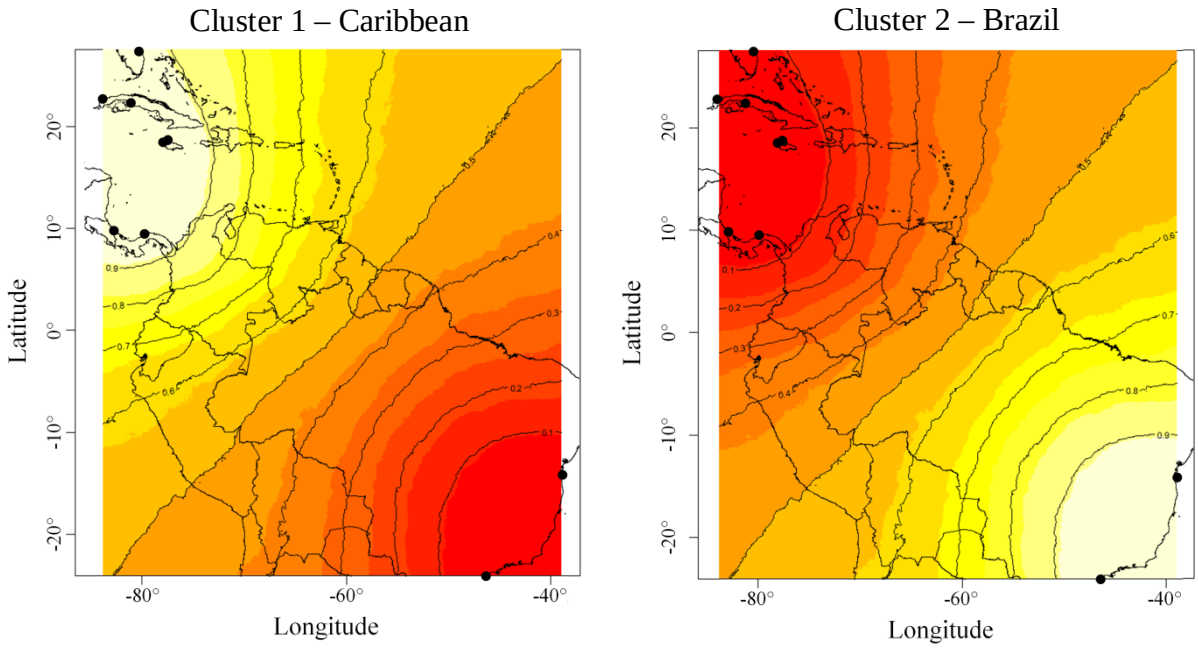

Figure 1.8. Maps of posterior probability of cluster membership generated by Geneland based on 48 individuals of *Cyclograpsus integer*. Black dots indicate the relative position of the sampled sites. Coloration proportional to the posterior probability of population membership to the cluster, with lighter color indicating higher probability, with isolines of probability.

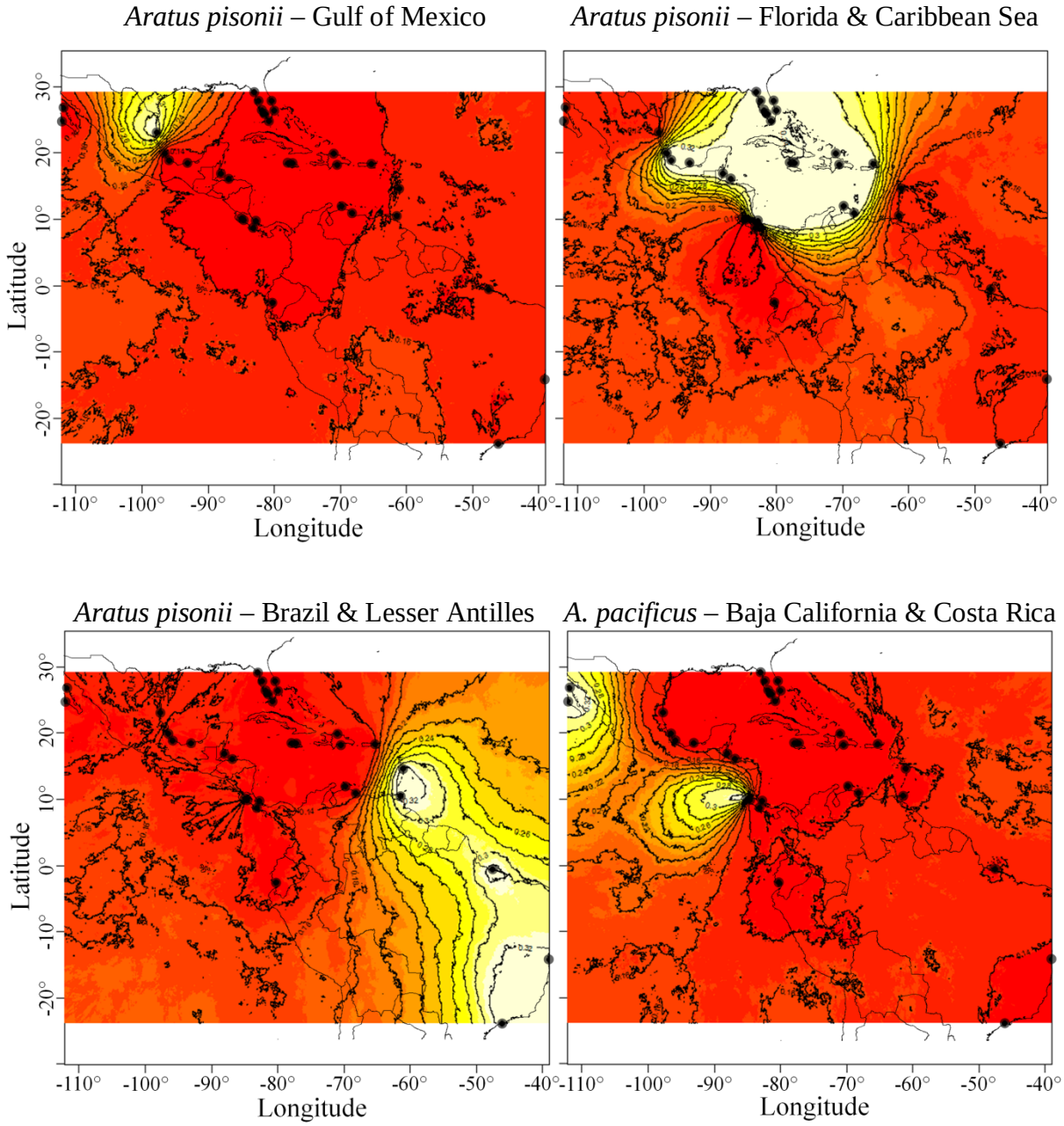

Figure 1.9. Maps of posterior probability of cluster membership generated by Geneland based on 194 individuals of *Aratus sp*. (deviant haplotype unresolved). Black dots indicate the relative position of the sampled sites. Coloration proportional to the posterior probability of population membership to the cluster, with lighter color indicating higher probability, with isolines of probability.

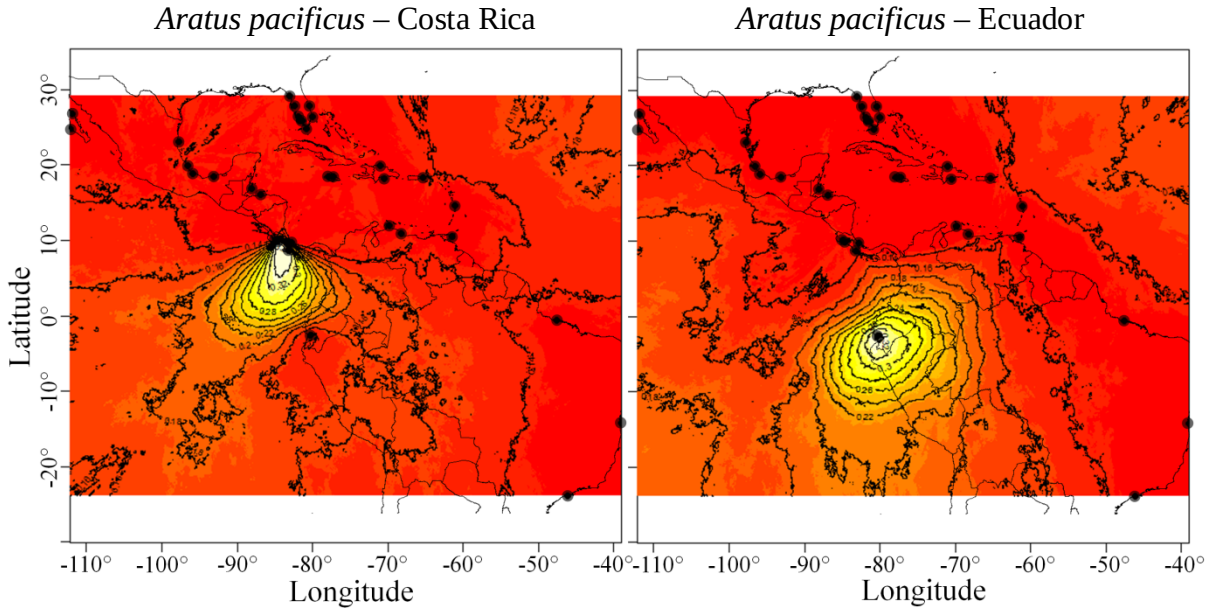

Figure 1.9. *Continued.*

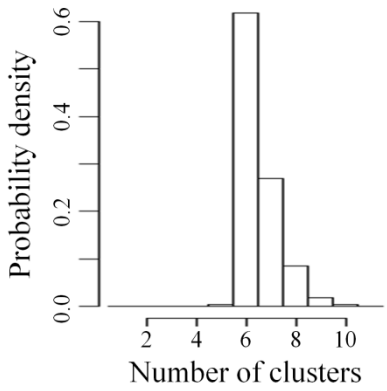

Figure 1.10. Number of supported clusters in *Aratus sp.*

Bayesian genetic clustering

With Geneland, a gradient between the species is observed considering the number of distinct lineages. Only a single cluster per ocean (K = 1) is recovered in the species of *Pachygrapsus* (*gracilis*, *socius*, *transversus*), supporting genetic exchanges between the populations (Figs. 1.5-1.7). Geneland supports two clusters (K = 2) in *Cyclograpsus*, in accordance with the results from the haplotype network. The first cluster corresponds to the Caribbean populations, whereas the second cluster corresponds to the Brazilian ones. But the

probability of cluster membership presents a strong gradient between the populations of these two clusters, as no population was included from the northern part of the South American continent. It does not allow Geneland to precisely determine the boundaries of each cluster and the sharpness of the transition between these clusters (Fig. 1.8). In the genus *Aratus*, the result depends on how many individuals are considered. When the whole dataset is considered (both oceans included), Geneland supports 6 distinct genetic clusters (K = 6, Fig. 1.10), corresponding respectively to *Aratus pisonii* – Caribbean populations (Florida included), *A. pisonii* – South American coastline up to Lesser Antilles, *Aratus sp.* – Gulf of Mexico, *Aratus pacificus* – Ecuador, *A. pacificus* – Costa Rican populations and *Aratus sp.* – Baja California and Costa Rica. The single specimen of *Aratus* from Costa Rica is not determined in this analysis (Fig. 1.9). When only *Aratus pisonii* is considered, Geneland supports three clusters: Florida and Yucatan; Caribbean; South American and Lesser Antilles. The cluster of the specimen of Puerto Rico is unclear, as the mean probability of belonging to the Caribbean cluster is only 55% (Fig. 1.11).

Genetic diversity and population structure

The genetic diversity greatly varies among the species having similar distribution ranges in the Atlantic Ocean (Tables 1.3-1.6), with a haplotype diversity ranging from 0.281 (*P. transversus*) to 0.955 (in *C. integer*), but also within the species among clusters (*Hd* ranging from 0.249 to 0.886 in *Aratus pisonii* and from 0 to 0.628 in *P. transversus*). In *P. transversus*, despite an overall low haplotype diversity, it is increasing along a southward gradient (Table 1.6). At a different scale, a similar result is observed for *P. gracilis* (Table 1.5). In contrast, in *Aratus pisonii*, the haplotype diversity is lower at the extremities of the distribution (Florida and São Paulo), and increases towards the center of the distribution area (Venezuela, Dominican Republic and Amazon, Table 1.3). The extremely high number of segregating sites found in *Aratus sp.* on the Pacific Costa Rican site of Mata de Limon is the consequence of co-occurring sympatric lineages.

As for the diversity indices, the pairwise φ_{ST} values show a range in gene flow among species, with *Aratus pisonii* and *Pachygrapsus transversus* as extremes (Tables 1.7-1.10). In *Aratus pisonii*, pairwise φ_{ST} values clearly confirm the strong isolation of the three genetic clusters, but also highlight significant gene flow reduction in the Caribbean. Indeed, the values

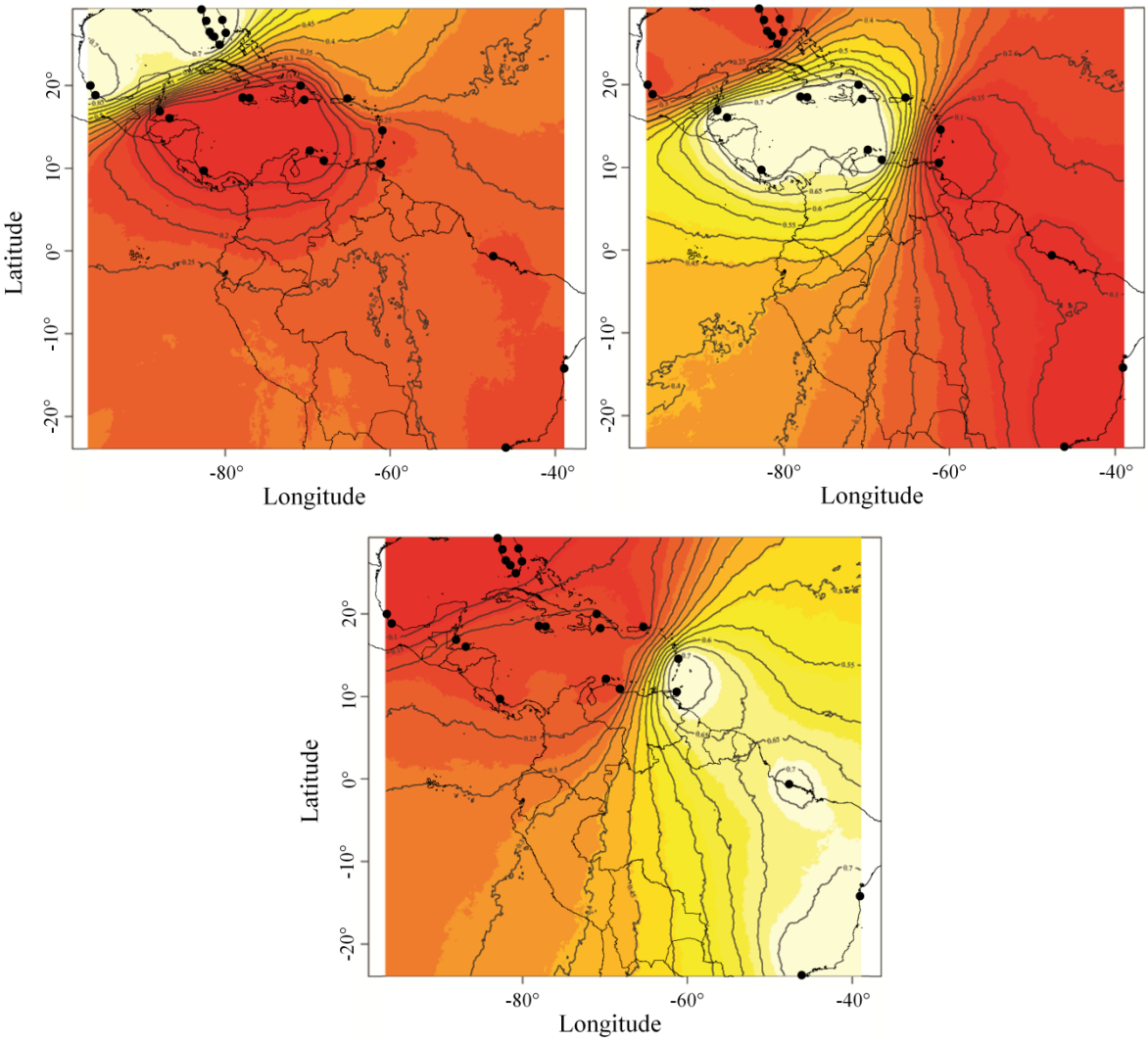

Figure 1.11. Maps of posterior probability of cluster membership generated by Geneland based on 139 individuals of *Aratus pisonii*. Black dots indicate the relative position of the sampled sites. Coloration proportional to the posterior probability of population membership to the cluster, with lighter color indicating higher probability, with isolines of probability.

strongly support an isolation of the Venezuelan population with regards to the three other Caribbean populations (Dominican Republic, Jamaica and the Central American coastline), and reduced but significant gene flow limitation is supported between the Dominican and Jamaican populations (φ_{ST}: 0.0690, $P < 0.05$). In the three populations from the Brazilian cluster, only a small differentiation is observed between north and central Brazil (φ_{ST}: 0.0756, $P < 0.05$), but is not observed between north and south or central and south Brazil (Table 1.7). In both *Cyclograpsus integer* and *Pachygrapsus gracilis*, gene flow reduction between Caribbean and Brazilian populations is supported, but this reduction is limited in *P. gracilis*

Table 1.7. Pairwise φ_{ST} values among populations of *Aratus pisonii*. Significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

	Florida	Dominican Rep.	Jamaica	Central America	Venezuela	Amazon	Bahia
Dominican Rep.	0.8837***						
Jamaica	0.9100***	0.0690*					
Central America	0.9061***	0.0400	0.0022				
Venezuela	0.7982***	0.1508**	0.2046***	0.1360*			
Amazon	0.9367***	0.8958***	0.9220***	0.9060***	0.8431***		
Bahia	0.9346***	0.8910***	0.9190***	0.9018***	0.8355***	0.0756*	
São Paulo	0.9635***	0.9170***	0.9437***	0.9348***	0.8420***	-0.0141	0.0841

Table 1.8. Pairwise φ_{ST} values among populations of *Cyclograpsus interger*. Significance: ***P* < 0.01.

	Jamaica	Costa Rica – Panama	Bahia
Costa Rica – Panama	-0.0196		
Bahia	0.3967**	0.3480**	
São Paulo	0.4825**	0.4316**	0.0160

Table 1.9. Pairwise φ_{ST} values among populations of *Pachygrapsus gracilis*. Significance: **P* < 0.05, ***P* < 0.01.

	Jamaica	Caribbean coast	Amazon
Caribbean coast	-0.0408		
Amazon	0.1763**	0.1542**	
São Paulo	0.1186*	0.0894*	-0.0054

Table 1.10. Pairwise φ_{ST} values among populations of *Pachygrapsus transversus*. Significance: **P* < 0.05.

	Florida	Cuba	Jamaica	Costa Rica	Bahia
Cuba	0.0191				
Jamaica	0.0000	-0.0000			
Costa Rica	0.0191	0.0000	-0.0000		
Bahia	0.0191	-0.0000	-0.0000	-0.0000	
São Paulo	0.0259	0.0121	0.0089	-0.0025	-0.0385

Table 1.11. Analyses of molecular variance (AMOVA) of *Aratus pisonii*. The analysis subdivides the populations from the Caribbean Sea (Florida, Dominican Republic, Jamaica, Central America and Venezuela) and the one from the Brazilian populations (Amazon River, Bahia, São Paulo). Significance: **P* < 0.05, ****P* < 0.001, n.s. = non significant.

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among regions	1	210.88	3.31911	74.30	Φ_{CT} = 0.74298*
Among populations within regions	6	73.138	0.73173	16.38	Φ_{SC} = 0.63728***
Within populations	123	51.226	0.41647	9.32	Φ_{ST} = 0.90677***
Total	130	335.244	4.46731		

Table 1.12. Analyses of molecular variance (AMOVA) of *Cyclograpsus integer*. The analysis subdivides the Caribbean Sea (Florida, Cuba, Jamaica, Costa Rica and Panama) from the ones of Brazilian populations (Bahia and São Paulo). Significance: ***$P < 0.001$, n.s. = non significant.

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among regions	1	31.182	0.86979	42.00	Φ_{CT} = 0.41997 n.s.
Among populations within regions	3	3.061	-0.01566	-0.76	Φ_{SC} = -0.01304 n.s.
Within populations	65	81.143	1.21693	58.76	Φ_{ST} = 0.41241***
Total	69	113.343	2.07106		

Table 1.13. Analyses of molecular variance (AMOVA) of *Pachygrapsus gracilis*. The analysis subdivides the Caribbean Sea (Jamaica, Costa Rica and Panama) from the ones of Brazilian populations (Amazon and São Paulo). Significance: **$P < 0.01$, n.s. = non significant.

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among regions	1	8.866	0.31454	15.26	Φ_{CT} = 0.15258 n.s.
Among populations within regions	2	2.709	-0.03683	-1.79	Φ_{SC} = -0.02108 n.s.
Within populations	44	78.487	1.78380	86.53	Φ_{ST} = 0.13471**
Total	47	90.062	2.06151		

Table 1.14. Analyses of molecular variance (AMOVA) of *Pachygrapsus transversus*. The analysis subdivides the Caribbean Sea (Florida, Cuba, Jamaica and Costa Rica) from the ones of Brazilian populations (Bahia and São Paulo). Significance: n.s. = non significant

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among regions	1	0.377	0.00809	4.34	Φ_{CT} = 0.04342 n.s.
Among populations within regions	4	0.554	-0.00410	-2.20	Φ_{SC} = -0.02302 n.s.
Within populations	59	10.762	0.18240	97.86	Φ_{ST} = 0.02140 n.s.
Total	64	11.692	0.18639		

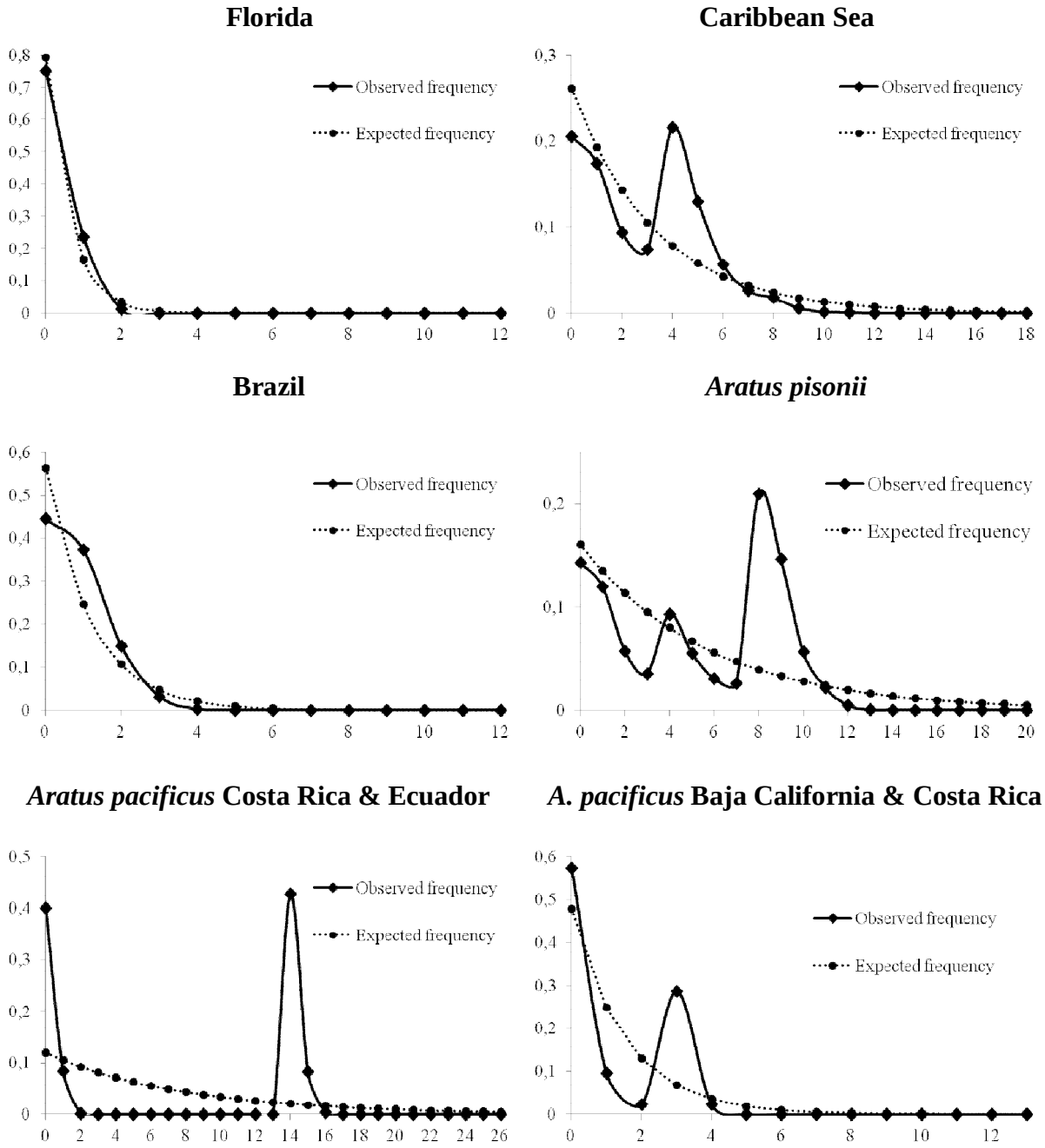

Figure 1.12. Mismatch distributions of pairwise differences in geographic clusters and genetic lineages of *Aratus sp.*, with expected frequencies under a constant population size model.

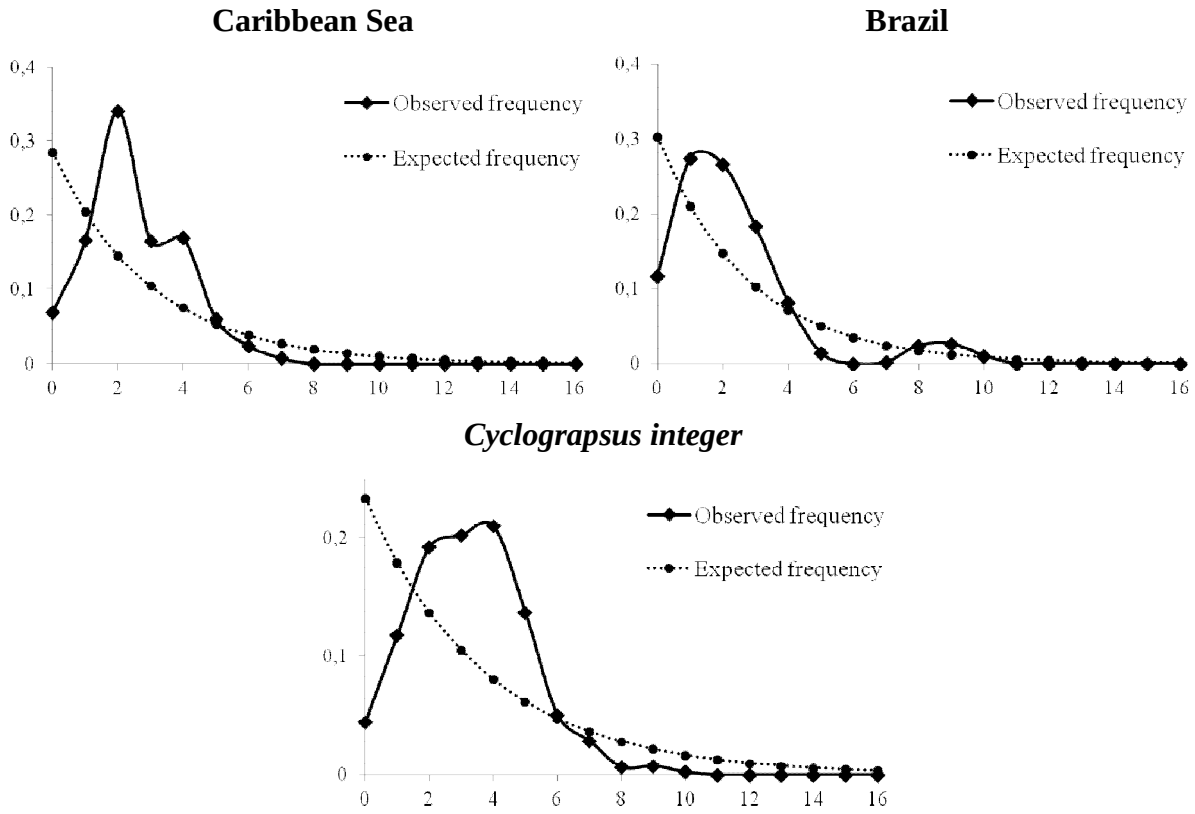

Figure 1.13. Mismatch distributions of pairwise differences in Caribbean Sea and Brazilian populations of *Cyclograpsus integer*, with expected frequencies under a constant population size model.

(Table 1.9), while it is relatively strong in *C. integer* (Table 1.8). In *Pachygrapsus transversus*, panmixis between the populations is supported, as no gene flow reduction can be observed among any of the populations (Table 1.10).

The AMOVA analyses also reveal large differences between the species (Tables 1.11-1.14). The percentage of variation observed in *Aratus pisonii* follows a gradient from regions to populations, and gene flow restriction is observed at each level (Table 1.11). In *C. integer*, most of the variation is observed between regions (percentage of variation: 42.00%) and at the population level (percentage of variation: 58.76%, Table 1.12), which is significant (Φ_{ST}: 0.41241, $P < 0.001$), but the populations of the same regions are homogeneous (Φ_{SC} = -0.01304). In *P. gracilis*, the AMOVA clearly rejects differentiation between the two regions and within regions, and only differences between all the populations is supported and significant (Φ_{ST} = 0.13471, $P < 0.01$, Table 1.13). Finally, in *P. transversus*, nearly no variance is observed at any scale, and the AMOVA rejects differentiation even among the populations (Table 1.14).

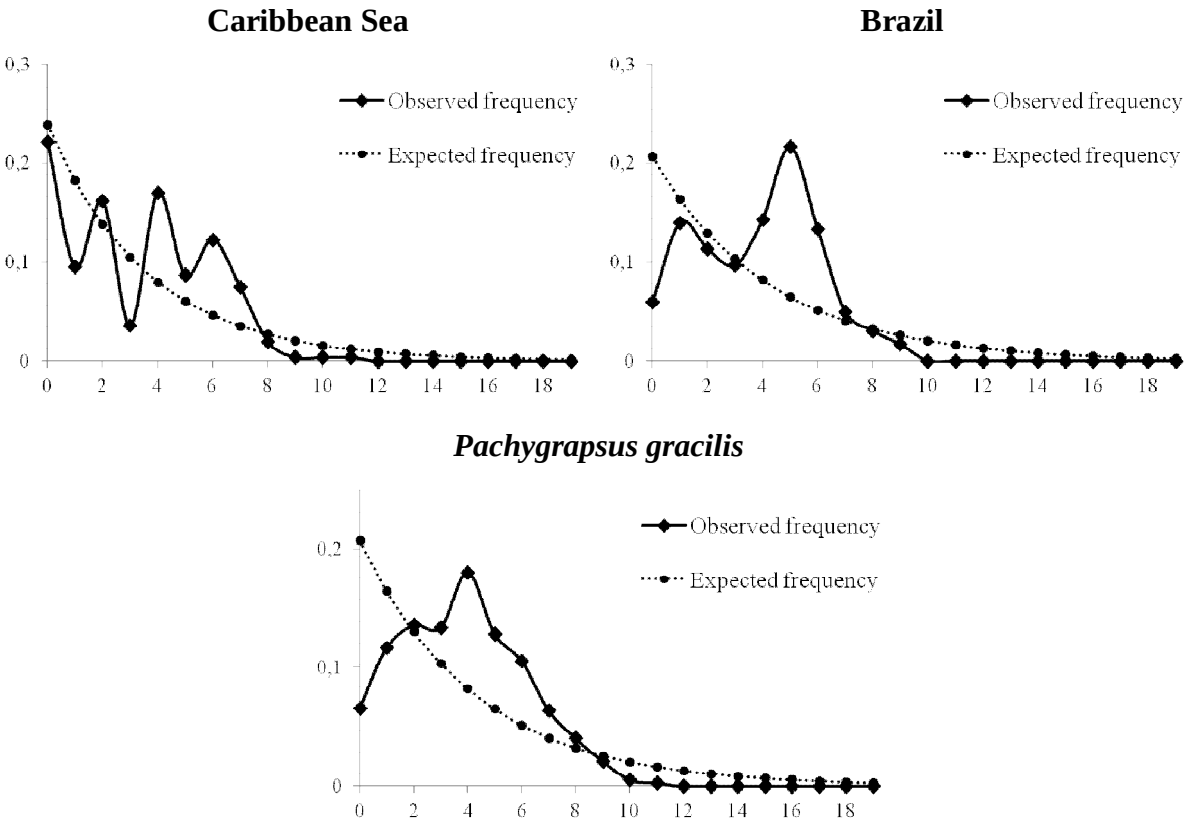

Figure 1.14. Mismatch distributions of pairwise differences in Caribbean Sea and Brazilian populations of *Pachygrapsus gracilis*, with expected frequencies under a constant population size model.

Discussion

Even if the tropical Atlantic is considered to be the second major tropical marine biodiversity hotspot after the Coral Triangle (Bowen *et al.* 2013), surprisingly only a limited set of studies investigated the phylogeographic patterns in its constituent marine taxa. This is of special interest for the relationships between Caribbean and Brazilian populations, as these populations are separated by the massive outflows of the Amazon and Orinoco rivers. So far, biogeographic investigations exploring the Atlantic marine tropical biodiversity mostly focus coral reef fishes (Bowen *et al.* 2013). Other taxa as crustaceans remain largely unexplored.

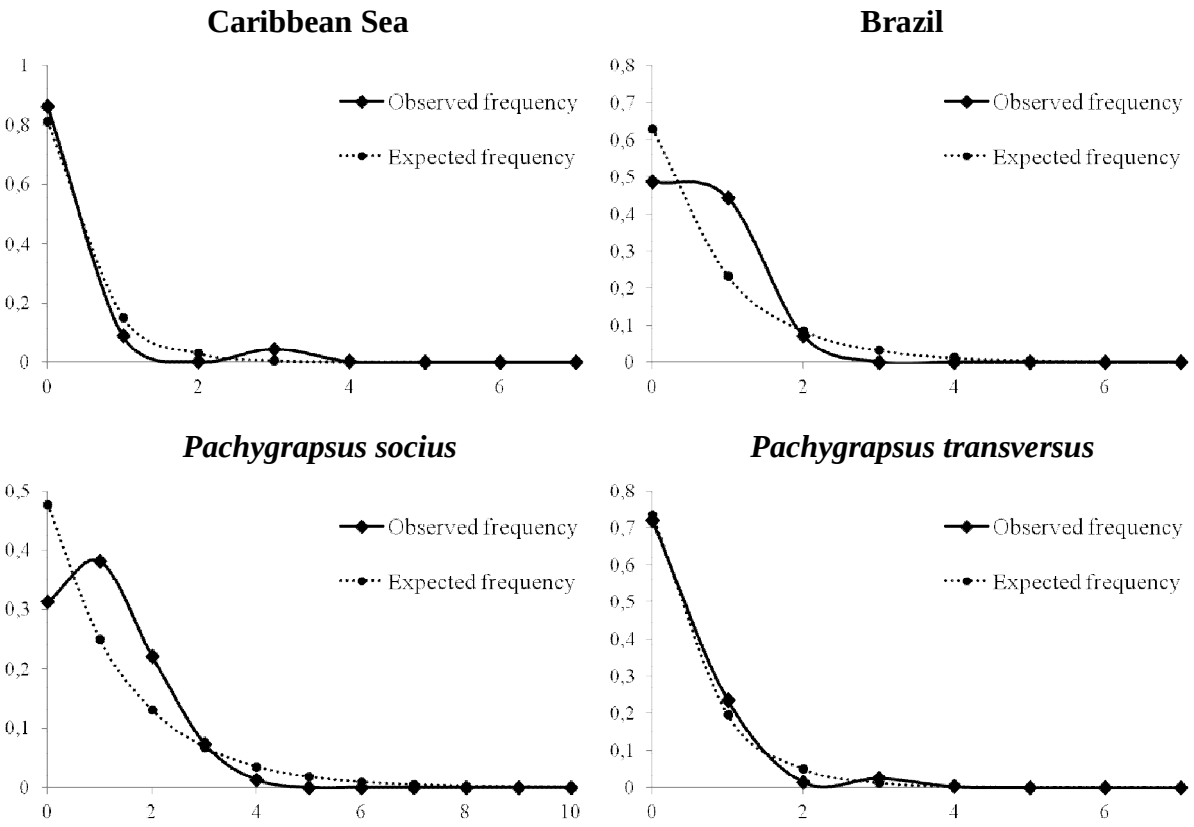

Figure 1.15. Mismatch distributions of pairwise differences in Caribbean Sea and Brazilian populations of *Pachygrapsus transversus*, and in *Pachygrapsus transversus* and *P. socius*, with expected frequencies under a constant population size model.

Contrasting patterns in closely related taxa

The comparison of phylogeographic patterns recovered in this study reveals unexpectedly contrasting differences between related taxa inhabiting the same biogeographic area, ranging from apparent panmixis to deeply divergent lineages, similar to what was observed in surgeonfishes (Rocha *et al.* 2002). This contrast is also reinforced by the unique results obtained for each species.

The two species of *Pachygrapsus* present no or limited genetic differentiation along their distribution range in the Atlantic. *Pachygrapsus transversus* exhibits a central haplotype that covers 85% of the individuals of this species and distributes over ~10 000km between Florida and Santa Catarina (Brazil). An equivalent result is obtained for its sister taxon *P. socius*, with a central haplotype present on the most distant sites (Baja California and Peru), separated by ~5 000km (Fig. 1.4, Tables 1.10 & 1.14), as observed by Schubart *et al.* (2005). *Pachygrapsus gracilis* also presents no clear geographic pattern, as no causal link can be

observed between haplotype and geographic distances. However, the absence of haplotypes shared by the two regions nuances this conclusion (Fig. 1.3, Tables 1.9 & 1.13). Despite this reduced differentiation in *P. gracilis*, the Bayesian analysis clearly rejects more than a single genetic cluster along the tropical American coastlines for each of the three species of *Pachygrapsus* (Fig. 1.5-1.7). This conclusion is congruent with the absence or limited differentiation between northern and southern tropical Atlantic populations observed in reef fishes species, as *Acanthurus chirurgus* (see Rocha *et al.* 2002) or *Chromis multilineata* (see Rocha *et al.* 2008), but also in sponges (Lazoski *et al.* 2001), sea urchins (Zigler & Lessios 2004), and ascidians (Nóbrega *et al.* 2004). For each of these studies, the authors concluded that this absence of differentiation reflects current or recent gene flow between Caribbean (or Bermuda) and Brazilian coastlines, and by consequence reflects the high dispersal abilities of these species. As grapsid crabs have long larval development, up to eight larval stages (Cuesta *et al.* 2011), the high gene flow between distant populations may be the consequence of the dispersal potential of the three species of *Pachygrapsus*.

On the opposite, a clear geographic clustering between Caribbean and Brazilian populations is recovered for both *Cyclograpsus* and *Aratus*, and confirmed by the Bayesian approach (Figs. 1.1-1.2, 1.8-1.9, 1.11). These two species are characterized by shorter larval development, and potentially larval retention mechanisms (Anger 1995). In *Cyclograpsus integer*, this clustering is associated with marked gene flow reduction between the Caribbean and Brazilian populations, but the difference between regions is surprisingly non significant in AMOVA (Tables 1.8 & 1.12). An equivalent result is observed in the fiddler crab *Uca rapax*, as Caribbean and South American clusters are separated by a single mutation step, but do not share any haplotype (Laurenzano *et al.* 2013).

Aratus presents a more complex pattern than any other species in this study. In the Atlantic Ocean, the two lineages are found in sympatry in the Gulf of Mexico. The population endemic to the Gulf of Mexico might represent another example of endemic brachyuran in this region where the endemicity for this taxon is approximately of 10% (Powers 1977). In the Pacific Ocean, *Aratus pacificus* may include two cryptic lineages inhabiting sympatrically the Costa Rican coastline (Fig. 1.1). Even if they are sympatrically distributed in Costa Rica, these lineages present distinct distributions, as one inhabits the southern part of the tropical East Pacific, from Costa Rica to Ecuador (based on the specimens of this study, with Ecuador being the type-locality of the species), whereas the second lineage is present from Baja California to Costa Rica. The northern part of the tropical East Pacific (Fig. 1.9), and the restricted overlapping distribution of these two lineages suggests an allopatric speciation

followed by secondary contact in Costa Rica (see also Quenouille *et al.* 2011). All the genetic lineages are deeply divergent, and the p-distance among them may suggest inter-specific level, using the divergence between *Aratus pisonii* and *A. pacificus* as reference (Table 1.2). When compared to the transisthmian divergence, the lineages from the Atlantic and Pacific present smaller genetic differentiations, indicating divergences initiated after the final closure of the isthmus, when the Atlantic and Pacific oceans were already isolated from each other. On the other hand, a single specimen of *Aratus pacificus* appears to be the most divergent lineage, and its divergence apparently preceded the final closure of the isthmus. If the presence of this lineage is confirmed with independent markers, it indicates that the genetic divergence in *Aratus* occurred in multiple steps, before, during and after the final closure of the Isthmus of Panama. As in the genus *Aratus*, the development of molecular phylogeographic studies revealed sympatric cryptic lineages in the tropical Atlantic or tropical East Pacific in a wide range of taxa, as fishes (e.g. *Albula*, Colborn *et al.* 2001), crustaceans (e.g. *Alpheus*, Mathews & Anker 2009), bivalves (e.g. *Acar*, Marko & Moran 2009), polychaetes (Barroso *et al.* 2010), and sea urchins (e.g. *Mellita*, Coppard *et al.* 2013), and correspond to divergence preceding or following the closure of the Isthmus. Two of the lineages in the genus *Aratus* are so far known from a reduced number of specimens and sites. By consequence, the knowledge concerning their distribution is extremely limited. It is not possible to determine, if they represent largely distributed cryptic lineages or, if they are strongly endemic, as observed for other species closely related to *Aratus* (*Armases gorei*, *A. magdalenense* and *Sesarma crassipes*), only known from a single locality (Abele 1992).

In addition to these cryptic lineages, *Aratus pisonii* and *A. pacificus* are both genetically clustered in geographic units. These clusters present clear allopatric distribution and do not share haplotypes, suggesting the impact of gene flow barriers, possibly reinforced by the larval retention mechanisms in Sesarmidae (Anger 1995, Schubart *et al.* 2000). The three clusters subdividing the main lineage of *A. pisonii* correspond respectively to the Florida and the Gulf of Mexico, to the Caribbean Sea except the Lesser Antilles, and to the Brazilian coastline, Trinidad and Martinique. The main lineage of *A. pacificus* is subdivided in two clusters, corresponding to Costa Rican and Ecuadorian individuals respectively, confirming the result of Thiercelin & Schubart (2014) based on the 16S rRNA gene (Figs. 1.1 & 1.9). Their divergence is approximately one third of the transisthmian divergence between *A. pisonii* and *A. pacificus*, despite an absence of morphological differences observed between specimens from these two sites (Thiercelin & Schubart 2014).

Unique population genetic histories in sympatric taxa

When compared, the species do not only show different geographic structuring, but also tell different histories within their populations. Indeed, the mangrove species *Pachygrapsus gracilis* is the only species in this study to present indications of overall constant population size, with the exception of its São Paulo population that exhibit a population expansion. This stable population size is highlighted by a strongly structured haplotype network, the ragged mismatch distributions, the non-significant neutrality tests and the high genetic diversity. This rejects the hypothesis of population reduction along the distribution area of this species.

In contrast, the other three species exhibit clear signs of past or recent changes in their population size. Indeed, these species present star-like haplotype patterns, in which high numbers of single haplotypes circle around a central and preeminent haplotype. This pattern is typically associated with bottlenecks followed by population expansion. The idiosyncrasies of these species may indicate that these populations's changes are not the consequence of a unique event with different impact. Contrary to *P. gracilis*, *Aratus sp.* living in the same habitat present clear differences between populations and clusters. The cluster of the Caribbean Sea is the most structured and presents the highest number of unique haplotypes. But is characterized by a clear distinction between the Dominican Republic / Venezuela and Jamaica / Central America. Dominican Republic and Venezuela have high haplotypic diversities ($Hd > 0.75$), whereas Jamaica and Central America have moderate haplotypic diversities ($Hd < 0.50$). Moreover both Dominican Republic and Venezuelan populations have non-significant Tajima's *D*, while this neutrality test is significant and clearly negative for the Jamaican and Central American populations. These results indicate that the two eastern sites (Dominican Republic & Venezuela) were less affected in intensity or time by the population constriction than the two western sites (Jamaica & Central America). The Venezuelan population appears to be partially isolated from other Caribbean populations, as significant gene flow restriction is observed (Table 1.7). The Caribbean has been shown to represent an heterogeneous region, expressed in the network as interconnected independent populations. Marked gene flow restriction has been observed between its populations, even in species with long larval development, suggesting in this case active retention mechanisms (Taylor & Hellberg 2003, Baums *et al.* 2006, Kool *et al.* 2010, Laurenzano *et al.* 2013, Robertson & Cramer 2014). Along the Brazilian coastline, populations present a gradient from north to south in term of genetic diversity, and the Amazon population has the most

negative values for both neutrality tests. The Floridan cluster presents the lowest genetic diversity. The peak of genetic diversity in *Aratus pisonii* is observed along the northern coastline of South America for both Caribbean and Brazilian clusters, whereas peripheral populations are genetically depauperate (Table 1.3). The northern coastline of South America represented a glacial refugium for *A. pisonii*, possibly as consequence from the distribution changes of mangrove habitats. Especially a contraction of their distribution during the Quaternary, as consequence of climatic oscillations during this period, resulted in lower sea-level and colder sea surface temperatures (Woodroffe & Grindrod 1991, Hewitt 2004, Nettel & Dodd 2007). It was probably followed by a recolonization in higher latitudes along the South American coastline. Other studies focused on mangroves inhabitants, the red mangrove tree *Rhizophora mangle* L., 1753 and the mangrove crabs *Ucides cordatus* (Linnaeus, 1763) and *Eurytium limosum* (Say, 1818), also highlighted post-glacial population expansion in southward direction along the South American continent, based on the higher genetic diversity in northern sites compared to southern populations. The lower genetic diversity of the southernmost populations is assumed to reflect a relatively young age of this populations and the consequence of founder effects (Oliveira-Neto *et al.* 2007, Pil *et al.* 2011, Wawrschin & Schubart, unpublished data). Given the strong association between *Aratus pisonii* and *Rhizophora mangle*, the post-glacial recolonization dynamics of *Aratus sp.* was probably concomitant with the dynamics of the red mangrove. *A. pisonii* might even have shortly predated the mangrove expansion, as this species is able to expand its distribution faster than the mangrove trees, by switching habitat (from mangrove to salt marsh) during the range expansion process (Riley *et al.* 2014). The species is nowadays present in the northernmost (~30°N) and southernmost (~28°S, pers. observation) mangrove patches along the eastern American coastlines, and presents a recent northward expansion of its distribution up to the Georgia State (USA) salt marshes (Riley *et al.* 2014). Despite strong differences observed between the two mangrove-inhabiting species *A. pisonii* and *P. gracilis*, especially the absence of genetic clusters in *P. gracilis*, the southernmost population in this species is congruent with a post-glacial recolonization pattern. But dispersal abilities of *P. gracilis* probably made this species less sensitive to the Quaternary climatic changes.

Cyclograpsus integer was affected by the oldest event of population changes, reflected by the extremely high genetic diversity (over 0.88 for all the populations, 0.957 overall, Table 1.4), strongly significant neutrality tests and unimodal distributions of the mismatch distributions associated to star-like topology of the haplotype network. The differences observed between the two neutrality tests (stronger significance for Fu's test than for the

Tajima's test) are explained by the higher sensitivity of the Fu's *Fs* to population changes (Fu 1997). Both regions (Caribbean and Brazilian coastlines) present equivalent patterns of past bottlenecks, indicating that the distribution range of *C. integer* was affected at large scale. But contrary to *Aratus pisonii* the species seems to have not been affected by the most recent glacial events, especially the shift in sea surface temperatures and sea level changes.

Pachygrapsus transversus was affected by a more recent bottleneck than *C. integer*, as four out of five populations exhibit extremely low genetic diversity (Hd < 0.4). This genetic diversity is increasing along a north - south gradient. This is confirmed by the Fu's *Fs*, presenting more negative values for southern sites (Brazil) than northern sites (Caribbean, Table 1.6). The concordance between these different results indicates that the glacial refugium of *P. transversus* was not located around the equator, as observed for other tropical taxa (Hewitt 2004), but rather along the southern Brazilian coastline, later followed by a northward population expansion along the whole tropical Atlantic. This surprising result is also recovered in the fiddler crab *Uca leptodactylus* (seev Laurenzano & Schubart, unpublished data). This population expansion or recolonization is possible by the large dispersal abilities of the Grapsidae as consequence of their long larval development (Anger 1995, Cuesta *et al.* 2011) and is facilitated by the water circulation in the tropical Atlantic. Indeed, along the northern South American coastline, both North Brazil and Guyana currents powerfully transport surface waters up to the Caribbean Sea, where the Caribbean Current deffects these surface waters to Florida (Mariano & Ryan 2015). Future phylogeographic studies will help to determine, if this pattern is more frequent than expected, or if it remains an exception.

Barriers in the western tropical Atlantic

Several biogeographic studies dealt with the importance of the Amazon and Orinoco rivers as barriers along the Western Atlantic. Indeed, the massive freshwater discharge of these rivers decreases the salinity and increases the turbidity of the Atlantic Ocean, impacting the whole coastline between their mouths to the Caribbean Sea (Froelich *et al.* 1978, Hellweger & Gordon 2002, Spalding 2007). It makes this region unsuitable for coral reefs, highlighted by the approximate stretch of 2500 km without reefs (Collette & Rützler 1977). The strong endemicity of reef fishes in the Brazilian province (18–20%, Joyeux *et al.* 2001), but also the exchanges between this province and the Caribbean Sea, identified the Amazon – Orinoco Plume (AOP) as soft barrier or filter, of which the intensity depends of the respective larval duration, sea-level fluctuations and ability to use different habitats as the sponge-

corridor (Collette & Rützler 1977, Briggs 1995, Joyeux *et al.* 2001, Rocha 2003, Floeter *et al.* 2008, Luiz *et al.* 2012). Nettel & Dodd (2007) in mangrove trees, and Laurenzano *et al.* (2013) in fiddler crabs, noticed an absence of genetic differentiation between populations on the two sides of the Amazon River, indicating that this river does not act as barrier to dispersal for these species. In contrast, a clear genetic break is observed between populations of mangrove trees and fiddler crabs on both sides of the Orinoco River (Nettel & Dodd 2007, Laurenzano *et al.* 2013), which seems to represent the most important component of the AOP barrier.

The present study recovers congruent results with the AOP as barrier to gene flow, as one out of four species present genetic differentiations between the Caribbean and Brazilian populations. But it cannot be stated, which of the two rivers act as barrier. As observed in reef fishes, the species presenting genetic differentiation are the ones with the shortest larval development in this study (Anger 1995). However, this has to be nuanced. Indeed, for *Cyclograpsus*, the absence of sampling sites between central Brazil (Bahia State) and Jamaica limits the resolution of this conclusion. For *Aratus pisonii*, the presence of the Brazilian haplotypes up to Martinique, in the Lesser Antilles may suggest no effect of the AOP as barrier. However, this explanation necessarily implies an isolation of the Lesser Antilles from both Venezuela and Puerto Rico (Fig. 1.11). This pattern would not fit with any of the clusters recovered by Robertson & Cramer (2014) for shorefishes. On the other hand, the genetic divergence between the central haplotype of the Brazilian and Caribbean / Florida clusters in *A. pisonii* (8 mutation steps, ~1.1%, Table 1.2, Fig. 1.1) indicates divergence estimated at 380 000 years (95% highest posterior density interval: 200-590ky, Thiercelin & Schubart, unpublished data). The genetic divergence and divergence time are roughly equivalent to the ones observed between the sister species the reef fishes *Halichoeres sazimai* (southwestern Atlantic) and *H. bathyphilus* (northwestern Atlantic), estimated to have diverged ~300 000 years ago (Luiz *et al.* 2009). Moreover, the distribution pattern found in the Brazilian genetic cluster of *A. pisonii* is strikingly congruent with the infrequent distribution pattern of '*south-western Atlantic endemic reef fishes with limited Caribbean distribution*' (Joyeux *et al.* 2001:833-834), which reflects the occasional crossing of the then semi-permeable AOP barrier. The crossing of this barrier is more frequent in the direction Brazil towards Caribbean than the other way, favored by the direction of the North Brazil Current (Luiz *et al.* 2013). For *A. pisonii*, it might have been facilitated by the relative freshwater tolerance of this species thriving in mangroves. Lastly, the genetic isolation between Caribbean and Florida populations in *A. pisonii* (Fig. 1.11) is congruent with the Florida Current as biogeographic

barrier as proposed by Briggs (1995). The phylogeographic patterns in *A. pisonii* head toward the biodiversity feedback model proposed to explain the tropical marine biodiversity, where biodiversity hotspots export species to peripheral habitats, followed by subsequent dispersal of novel taxa to the hotspot (Rocha *et al.* 2008, Bowen *et al.* 2013).

Conclusions

The comparative approach in this study highlights how in the western tropical Atlantic closely related brachyurans were affected by processes at different scales, as for example the closure of the Isthmus of Panama and the Quaternary climatic and environmental fluctuations. It reflects the importance of the ecological characteristics of the studied species, and their consequences on sympatric taxa. It also allows to identify, how marked can be the similarities and differences with other organisms from the same biogeographic realm. Some of the recovered patterns do not fit with initial assumptions, especially the recent bottleneck in the rocky shore species *P. transversus* and relatively stable population size in the mangrove inhabitant *P. gracilis*. When comparative biogeographic studies reveal the patterns of distributions, comparative phylogeographic studies also reveal the histories and mechanisms behind these distributions and genetic breaks in these distribution, unveiling and highlighting the importance of individual histories of each species (Arbogast & Kenagy 2001, Patarnello *et al.* 2007, Maggs *et al.* 2008).

CHAPTER 2

Transisthmian differentiation in the tree-climbing mangrove crab *Aratus* H. Milne Edwards, 1853 (Crustacea, Brachyura, Sesarmidae), with description of a new species from the tropical eastern Pacific

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Abstract

The tree-climbing mangrove crab *Aratus pisonii* (H. Milne Edwards, 1837) (Brachyura, Sesarmidae) is considered to have a transisthmian distribution, due to its presence in mangroves of the Western Atlantic as well as the Eastern Pacific. We here present evidence, based on the morphologies of male gonopods and on genetic data that populations from these two coastlines are morphologically and genetically distinct and call for the description of a new species, *Aratus pacificus* **n. sp.**, as the sister-species of *Aratus pisonii*. The corresponding speciation event can be regarded as the outcome of differentiation following the closure of the Isthmus of Panama. As these coastal brackish species were probably among the last ones to become separated, the speciation can thus be dated to a time frame of no more than 3.1 million years.

Introduction

Several publications over the last two decades have discussed the biological consequences of the tectonic closure of the Isthmus of Panama and stimulated further research on the relationships between populations or sister species affected by this important geological event (reviewed by Lessios 2008). From this work, has emerged a better understanding of the possible speciation steps this phenomenon induced in marine species. Perhaps contrary to expectation, Knowlton & Weigt (1998) and Williams & Knowlton (2001) showed that probably most of the transisthmian sister species in the snapping shrimp genus *Alpheus* began to genetically diverge not at the final closure of the Isthmus around 2.8-3.1 Mya (see Coates & Obando 1996), but much earlier (up to 18 Mya), depending on the ecology of the corresponding species. Indeed, Lessios (2008) stated that approximately two thirds of the transisthmian sister species that have been studied separated before the final stages of the closure. He also remarked that several species were still considered to have a transisthmian distribution, i.e. being recognized as the same species on both sides of the Isthmus, despite the long separation time, and suggested that they might include cryptic species.

Among the species considered to have a transisthmian distribution is the tree-climbing mangrove crab *Aratus pisonii* (H. Milne Edwards, 1837) (Decapoda, Brachyura: Sesarmidae). This species is restricted to mangroves, as it lives in close association with aerial roots and leaves of the red mangrove *Rhizophora mangle* and *R. samoensis* (see Woodroffe & Grindrod 1991, Duke & Allen 2006). Compared with other brachyuran crabs from the neotropics (Melo 1996), *A. pisonii* has a surprisingly wide distribution. It is reported to range in the tropical Western Atlantic from the southern tip of Florida, the southwestern Gulf of Mexico and the Caribbean to southern Brazil (São Paulo state), and in the tropical Eastern Pacific from central Baja California to northern Peru (Beever *et al.* 1979, Melo 1996).

Aratus pisonii has been considered to have a transisthmian distribution for almost two centuries. However, sister species of several fishes, molluscs, echinoderms, and many crustaceans on each sides of the Isthmus have been identified as distinct species since the end of the nineteenth century (see Lessios 2008: tables 1–4), but attempts to separate *Aratus pisonii* into two distinct species have failed until now. In this context, Kingsley (1879: 402) stated: "I am unable to separate specimens from the west coast of Nicaragua (McNeil) from the east coast forms" (specimens listed in Smith 1871).

Aratus pisonii (H. Milne Edwards, 1837) was initially described as a member of the genus *Sesarma*. H. Milne Edwards later recognised that it did not belong in *Sesarma* sensu stricto and assigned it to the monotypic genus *Aratus* H. Milne Edwards, 1853. The family Sesarmidae includes a large number of species (over 253 according to De Grave *et al.* 2009), with a significant number of genera and species described within the last 20 years (Ng *et al*. 2008). This family is restricted to tropical and subtropical areas and contains many species with restricted ranges, probably as a result of an evolutionary trend towards abbreviated or direct development (Schubart *et al.* 2000), an adaptation to help them maintain proximity to their specialised estuarine, intertidal or semiterrestrial habitats. The family is most diverse in the Indo-West Pacific region (Serène 1968), with only four genera in the neotropics (Schubart *et al.* 2000; see Schubart & Cuesta 1998, Schubart *et al.* 2002 for recent history of the family).

The apparent transisthmian distribution makes *Aratus pisonii* a noteworthy target-species to determine, if the populations of the two oceans could be considered as distinct species, consistent with the arguments detailed by Lessios (2008), or if this species was able to maintain occasional gene flow across the wetlands of the Isthmus of Panama over extended periods of time, and thus still represents a single species along the two American coastlines. We address this question on the basis of morphological and genetic evidences.

Materials and methods

Specimen collection

Specimens of *Aratus pisonii* were collected in mangrove habitats from the Western Atlantic and Eastern Pacific (Fig. 2.1) between 1998 and 2011, preserved in ethanol > 90% and stored in ethanol 70%. The specimens have been deposited at the following museums: Museo de Zoologia, Universidad de Costa Rica (UCR), San José, Costa Rica; Museo de Zoologia, Universidade de São Paulo (MZUSP), São Paulo, Brazil; Muséum National d'Histoire Naturelle (MNHN), Paris, France; Naturalis Museum (RMHN), Leiden, The Netherlands; Naturhistorisches Museum Wien (NHMW), Vienna, Austria; Raffles Museum of Biodiversity Research, National University of Singapore (ZRC), Republic of Singapore; Senckenberg Museum und Forschungsanlage (SMF), Frankfurt am Main, Germany;

Zoologische Staatssammlung München (ZSM), Munich, Germany. Measurements of the carapace of specimens examined are given as carapace width × carapace length.

Genetic analyses

Genomic DNA was extracted from muscular leg tissue using the Puregene kit (Qiagen) from the following specimens: RMNH.CRUS.D.55078 (Jamaica), MNHN-IU-2009-3094 (Dominican Republic), SMF43533 (Brazil), RMNH.CRUS.D.55083 (Ecuador) and MZUCR 3173-01 (18.11 × 17.82mm, Costa Rica).

A fragment of approximately 620 basepairs (bp) of the mitochondrial DNA encoding the rRNA of the large (16S) ribosomal subunit gene was amplified with decapod specific primers 16L2 (5'-TGCCTGTTTATCAAAAACAT-3') and 16HLeu (5'-CATATTATCTGCCAAAATAG-3') (Schubart 2009) with the following profile: initial step 4 min at 94°C, 40 cycles with 45s at 95°C - 60s at 48°C - 60s at 72°C for denaturing, annealing and extension respectively, final extension step 5 min at 72°C. A fragment of approximately 940bp of the nuclear 28S ribosome subunit gene was amplified with a touchdown PCR using the unpublished primers 28D2L (5'-TACCGTGAGGGAAAGYTGAAA-3') and 28H2 (5'-CGATTTGCACGTCAGAATTGCT-3') with the following profile: initial step 3 min at 94°C, 10 cycles touchdown with 45s at 97°C - 45s at 65°C to 60°C (-0.5°C per cycle) - 75s at 72°C, 30 cycles with 45s at 97°C - 45s at 60°C - 75s at 72°C for denaturing, annealing and extension respectively, and a final extension step of 5 min at 72°C. PCR products were outsourced for sequencing to LGC Genomics Gmbh, Berlin. Sequences were aligned automatically with ClustalW (Thompson *et al.* 1994) implemented in BioEdit 7.0.5 (Hall 1999) and proofread with Chromas 2.23. Primer regions were removed. Two maximum parsimony spanning networks were calculated with TCS 1.21 (Clement *et al.* 2000) corresponding to the nuclear and mitochondrial genetic relationships. Kimura-2-parameter distances (K2P, Kimura 1980) between the Atlantic and Pacific populations were calculated with Mega 5.2.1 (Tamura *et al.* 2011). Sequences were submitted to EMBL and are available from GenBank under the reference numbers HG939507 to HG939516.

Morphological analyses

Boone (1930: 207–209) provided an extensive description of *Aratus pisonii*. All the characters described by Boone (1930) for the species were re-examined for Atlantic and Pacific specimens, and although we undertook exhaustive morphological examinations, the

only significant differences we observed were in regard to the male first gonopods (G1). The shape of the G1 was shown to be species-specific in Sesarmidae of Central America by Abele (1992). The left first gonopod of each specimen was removed, air-dried, coated with gold-palladium and studied by means of a Zeiss DSM 950 scanning electron microscope (SEM). The setae were removed and the gonopods observed and photographed under different standardized angles for better examination of the underlying structure.

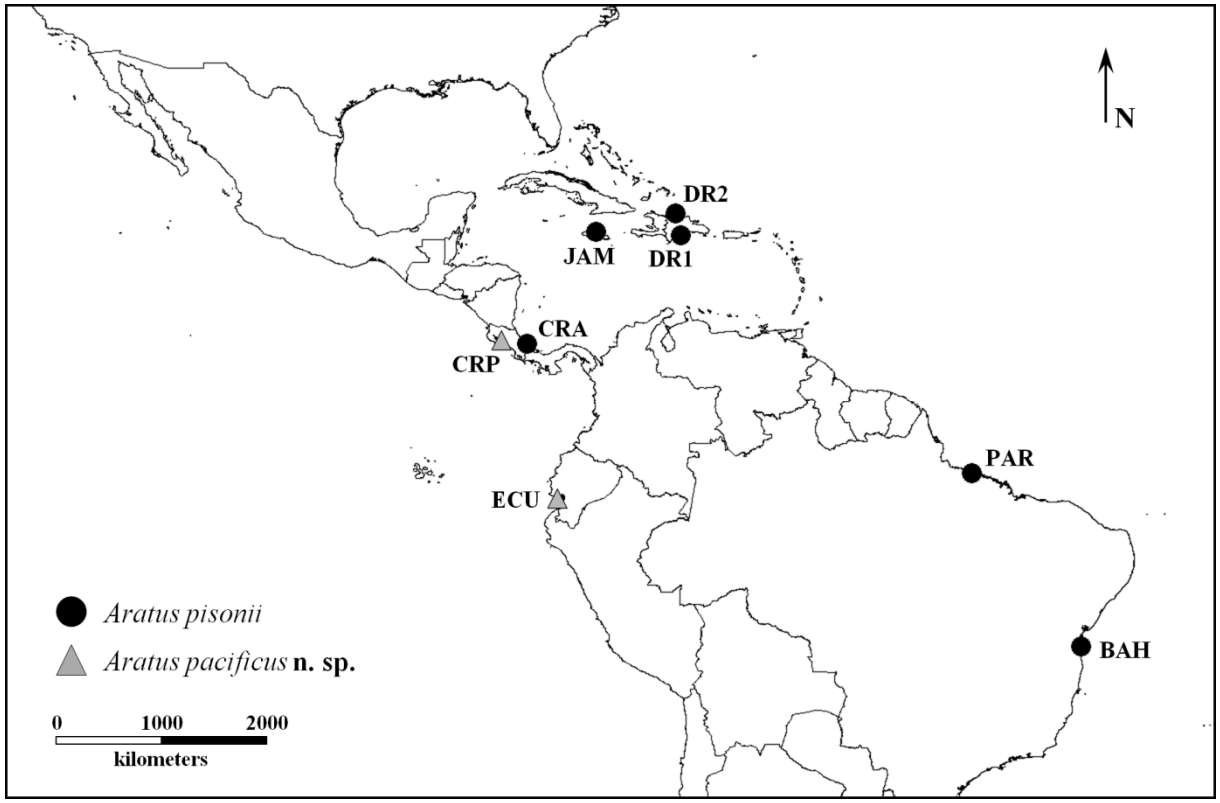

Figure 2.1. Sampling locations. BAH: Itacaré, Bahia, Brazil; CRA: Punta Uva, Costa Rica; CRP: Mata de Limón, Costa Rica; DR1: Las Salinas, Dominican Republic; DR2: Laguna Luperón, Dominican Republic; ECU: Puerto Morro, Ecuador (type-locality); JAM: Priory, Jamaica; PAR: Maruda, Pará, Brazil.

Genetic results

Both genetic networks indicate a clear separation between Pacific and Atlantic representatives of *Aratus pisonii*, which from now on we consider distinct species. At least 19 mutations separate these two species for the 16S mitochondrial gene. Both species present heterogeneity, as each specimen is represented by a distinct haplotype, with two mutations between the most distant haplotypes (Jamaica and Brazil) in Atlantic *Aratus*, while Ecuador and Costa Rica are separated by four mutations in Pacific *Aratus* (Fig. 2.2A). As expected, the resolution provided by the 28S nuclear gene is limited, as only two mutations separate the two species. In this gene, the specimen MNHN-IU-2009-3094 (Dominican Republic) appeared to be heterozygote, whereas all the other ones are homozygote. The Dominican specimen clusters with both RMNH.CRUS.D.55078 (Jamaica) and SMF43533 (Brazil) specimens, and the most distant allele is shown in the genetic network. Contrary to the 16S gene, only a single allele is found in Pacific *Aratus* for the 28S gene (Fig. 2.2B).

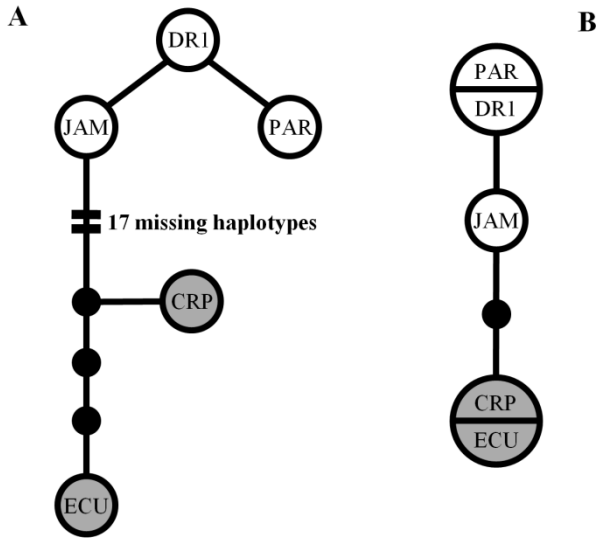

Figure 2.2. Maximum parsimony spanning networks of 16S constructed with TCS of *Aratus pisonii* (white circles) and *Aratus pacificus* **n. sp.** (grey circles) of 16S ribosomal mitochondrial gene (A, 624bp) and 28S ribosomal nuclear gene (B, 940bp). Each line represents a substitution and dots represent missing haplotypes. CRP: Mata de Limón, Costa Rica; DR1: Las Salinas, Dominican Republic; ECU: Puerto Morro, Ecuador (type-locality of *Aratus pacificus* **n. sp.**); JAM: Priory, Jamaica; PAR: Maruda, Pará, Brazil.

The K2P genetic distance between the two species is in average 3.28 % (ranging from 2.97 to 3.47%) for the 16S mitochondrial gene. The intraspecific differentiation for the 16S gene is 0.21% in *Aratus pisonii* and 0.4% in *A. pacificus* **n. sp.**, implying an interspecific differentiation approximately 10 times higher than the intra-specific differentiation. For the 28S nuclear gene, the K2P genetic distance between the two species is 0.21-0.32%.

Taxonomy

Aratus pisonii (H. Milne Edwards, 1837)

(Figs. 2.3A, B, E, F, I, J, M, N; 2.4A, B, E, F, I, J; 2.6A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X; 2.7A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R; 2.8A, B, G, H, M, N, S, T; 2.9A, B, G, H, M, N)

Aratu pinima Piso & Marcgrave 1648: 185
Sesarma pisonii H. Milne Edwards, 1837: 76, pl. 19, figs. 4, 5
Aratus pisoni H. Milne Edwards 1853: 187
Aratus pisonii Rathbun 1918: 322, pl. 96, figs. 1, 2. – Boone 1930: 207, pl. 70. – Warner 1967: 321. ; 1968: 249, figs. 1–6. – Beever *et al*. 1979: 317. – Díaz & Conde 1989: 148. – Schubart *et al*. 2000: 179. – Leme 2002: 553. – Fratini *et al*. 2005: 219. – Schubart *et al*. 2006: 193.

Type material

Two dry specimens (male, 19.6 × 20.6mm, female, 19.9 × 20.6mm) from the type series in the Muséum National d'Histoire Naturelle, Paris (MNHN B3341, photographs) were recognised as syntypes by N.G. Ng, T. Naruse, P.A. Rodriguez Moreno and D. Guinot (pers. comm., work in progress), of which one will be designated as the lectotype. Type locality: Martinique.

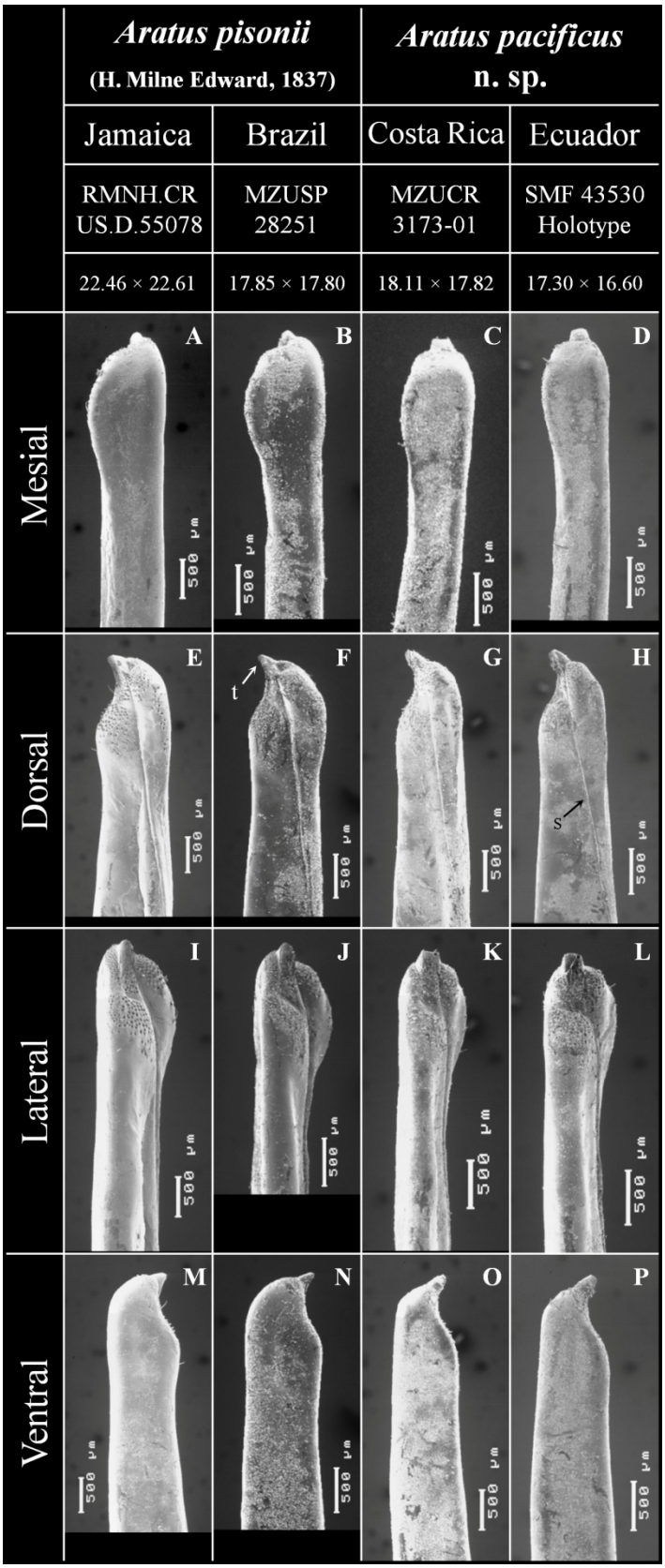

Figure 2.3. First male left gonopods of *Aratus pisonii* and *Aratus pacificus* **n. sp.** observed in scanning electron microscopy (SEM) in longitudinal mesial (A–D), dorsal (E–H), lateral (I–L) and ventral (M–P) views. CW × CL in mm. Abbreviations: s, suture; t, chitinous tip.

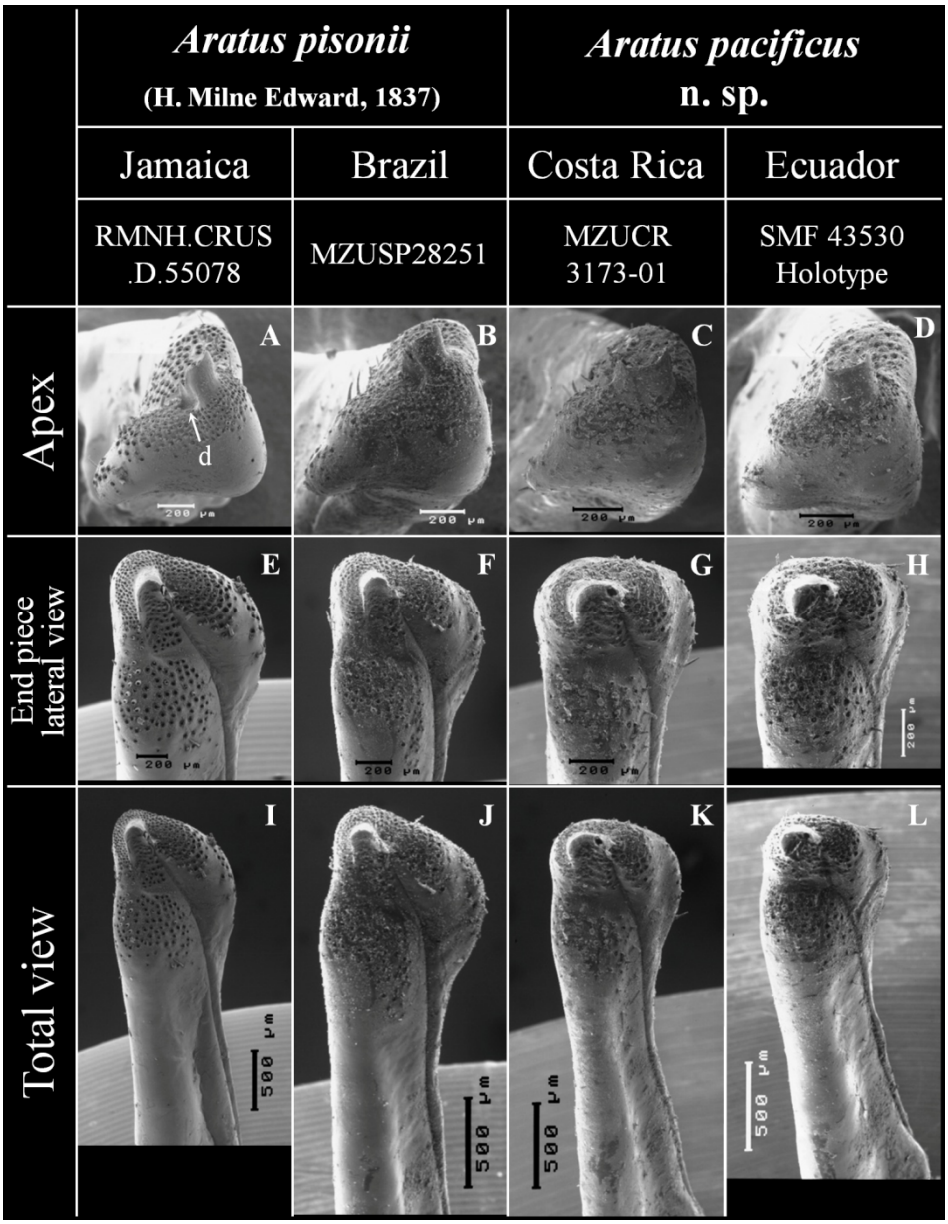

Figure 2.4. First male left gonopods of *Aratus pisonii* and *Aratus pacificus* **n. sp.** observed in scanning electron microscopy (SEM) in apical view (A–D), detail of the endpiece in lateral view (E–H) and total view (I–L). Abbreviation: d, depression.

Material examined

Jamaica. St. Ann Parish, Priory, 18°27.1'N-77°13.5'W, 1 ♂ (22.46 × 22.61mm) (RMNH.CRUS.D.55078), coll. C.D. Schubart *et al.*, 8 Mar.2011; St. Ann Parish, Priory, New Seville, 18°26.756'N-77°12.644'W, 1 ♂ (26.5 × 24.8mm) (NHMW 25475), coll. C.D. Schubart & P. Koller, 1 Mar.2011. *Dominican Republic.* Bahía de Las Calderas, Las Salinas, 18°12.75'N-70°32.46'W, 1 ♂ (18.45 × 17.65mm) (MNHN-IU-2009-3094), coll. C.D. Schubart, R. Landstorfer, N. Rivera, 1 Nov.2006 ; Bahia de Luperón, 19°53.76'N-

70°57.56'W, 2 ♂ (18.65 × 17.3, 20.2 × 19.35mm) (SMF 43534), coll. C.D. Schubart, R. Landstorfer, N. Rivera, 25 Oct.2006. *Costa Rica*. Limón, Puerto Viejo de Talamanca, Punta Uva, 9°37.930'N-82°40.200'W, 1 ♂ (20.35 × 20.10mm) (MNHN-IU-2009-5103), coll. C.D. Schubart, 28 Jan.2006. *Brazil*. Pará, Marapanim, Marudá, 0°37.023'S-47°37.946'W, 1 ♂ (16.65 × 16.25mm) (SMF 43533), coll. C.D. Schubart & N. Thiercelin, 15 Nov. 2010; Bahia, Itacaré, Rio de Contas, 14°16.494'S-38°59.869'W, 2 ♂ (17.85 × 17.8, 17.05 × 16.55 mm) (MZUSP28251 & MZUSP28255), coll. C.D. Schubart & N. Thiercelin, 11 Nov.2010.

Description

First male gonopod (Figs. 2.3A, B, E, F, I, J, M, N; 2.4A, B, E, F, I, J; 2.6A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X; 2.7A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R; 2.8A, B, G, H, M, N, S, T; 2.9A, B, G, H, M, N) elongated. Stem triangular in cross section; basal diameter wider than medial diameter. Mesial, ventral faces flat; slanted suture (sperm channel) clearly marked on dorsal face with terminal section in strongly marked depression; endpiece larger than medial part, easily noticeable in mesial, lateral views, endpiece bulging in lateral view; dorsal part almost spoon-shaped in mesial view. Mesial endpiece of ventral part slightly higher than dorsal part (angle approx. 25° in mesial view); short, chitinous, tip strongly curved (U-shaped) in ventral position, with pore on dorsal margin; suture aligned with chitinous edge of tip on apex of gonopod, with mesial face of chitinous tip/dorsal suture angle of approx. 115°; mesial face of chitinous tip/mesial face of approx. 110°; small denticles on lateral face of chitinous process. Small depression in medial position near tip. Large number of long setae on apex around chitinous tip, as well as on lateral, dorsal sides of endpiece lateral bulge. One line of setae on endpiece at edge between mesial, dorsal faces. Few single short setae on gonopod. Presence of few plumo-denticulate setae on dorsal part of endpiece bulge.

Biology

There is an extensive body of scientific literature on *Aratus pisonii* sensu stricto, as almost all the studies concerning this genus were conducted in the Atlantic Ocean. A detailed summary of this literature was given by Cuesta *et al.* (2006), including life history aspects (Warner 1967, Díaz & Conde 1989, Leme 2002) and larval description (Warner 1968).

Geographical distribution

Aratus pisonii sensu stricto is now restricted to the Atlantic Ocean, from Florida to south Brazil (Santa Catarina) including the Caribbean Sea and the Gulf of Mexico (Beever *et al.* 1979, Melo 1996).

Remarks

The species was named in honour of Willem Piso, who made the first description and drawing of this species in 1648 (Piso & Marcgrave 1648). Piso, however, seems to have confused *A. pisonii* with *Goniopsis cruentata* (Latreille, 1803), a grapsid crab living in the same habitat and also able to climb on mangrove roots and stems, as the colour pattern described in the text does not correspond to the one observed in *Aratus*.

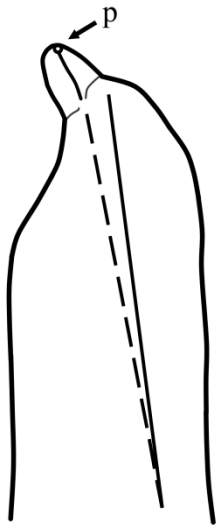

Figure 2.5. Schematic drawing of a left first male gonopod in dorsal view showing the alignment differences of the suture compared to the chitinous tip in *Aratus pisonii* (full line) and *Aratus pacificus* **n. sp.** (dashed line). Abbreviation: p, pore.

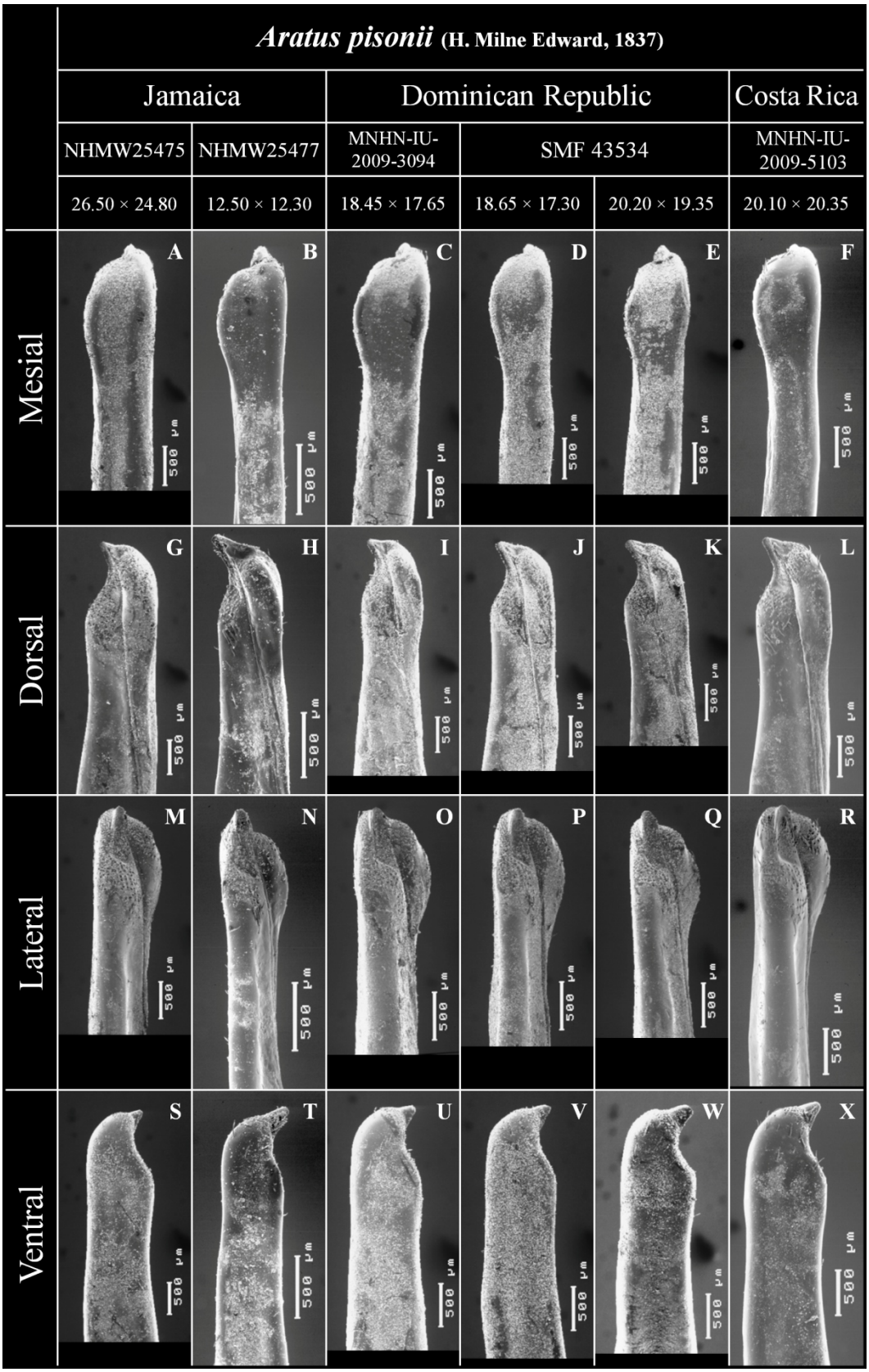

Figure 2.6. First male left gonopods of *Aratus pisonii* from the Caribbean Sea as observed in scanning electron microscopy (SEM) in longitudinal mesial (A–F), dorsal (G–L), lateral (M–R) and ventral (S–X) views. CW × CL in mm.

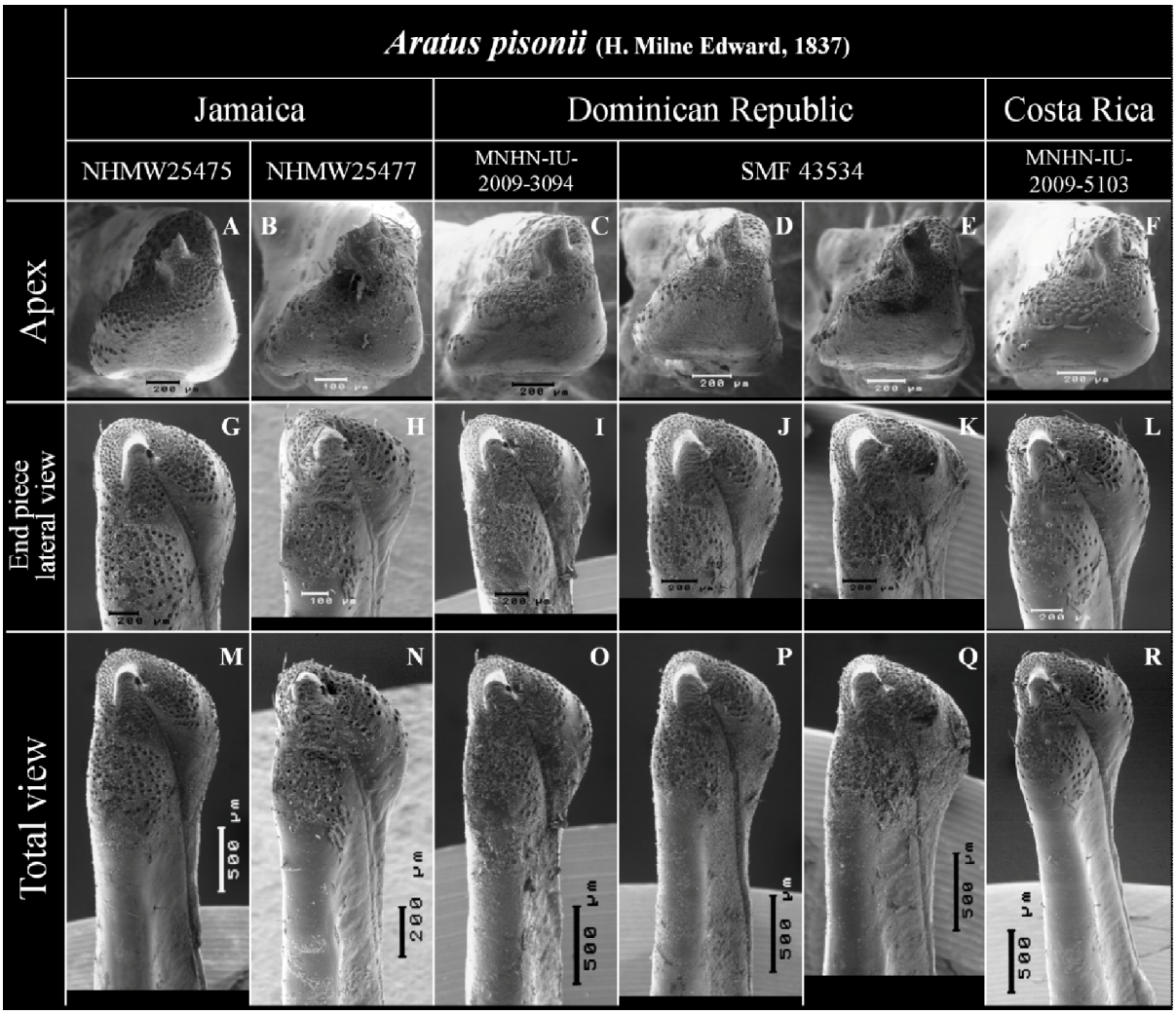

Figure 2.7. First male left gonopods of *Aratus pisonii* from the Caribbean Sea as observed in scanning electron microscopy (SEM) in apical view (A–F), detail of the endpiece in lateral view (G–L) and total view (M–R).

Aratus pacificus n. sp.

(Figs. 2.3C, D, G, H, K, L, O, P; 2.4C, D, G, H, K, L; 2.8C, D, E, F, I, J, K, L, O, P, Q, R, U, V, W, X; 2.9C, D, E, F, I, J, K, L,O, P, Q, R)

Aratus pisonii Smith 1871: 92. – Kingsley 1879: 402. – Rathbun 1918: 322-325, pl. 96, fig. 1 & 2. – Crane 1947: 86. – Cuesta *et al.* 2006: 175-196, fig. 1-7.

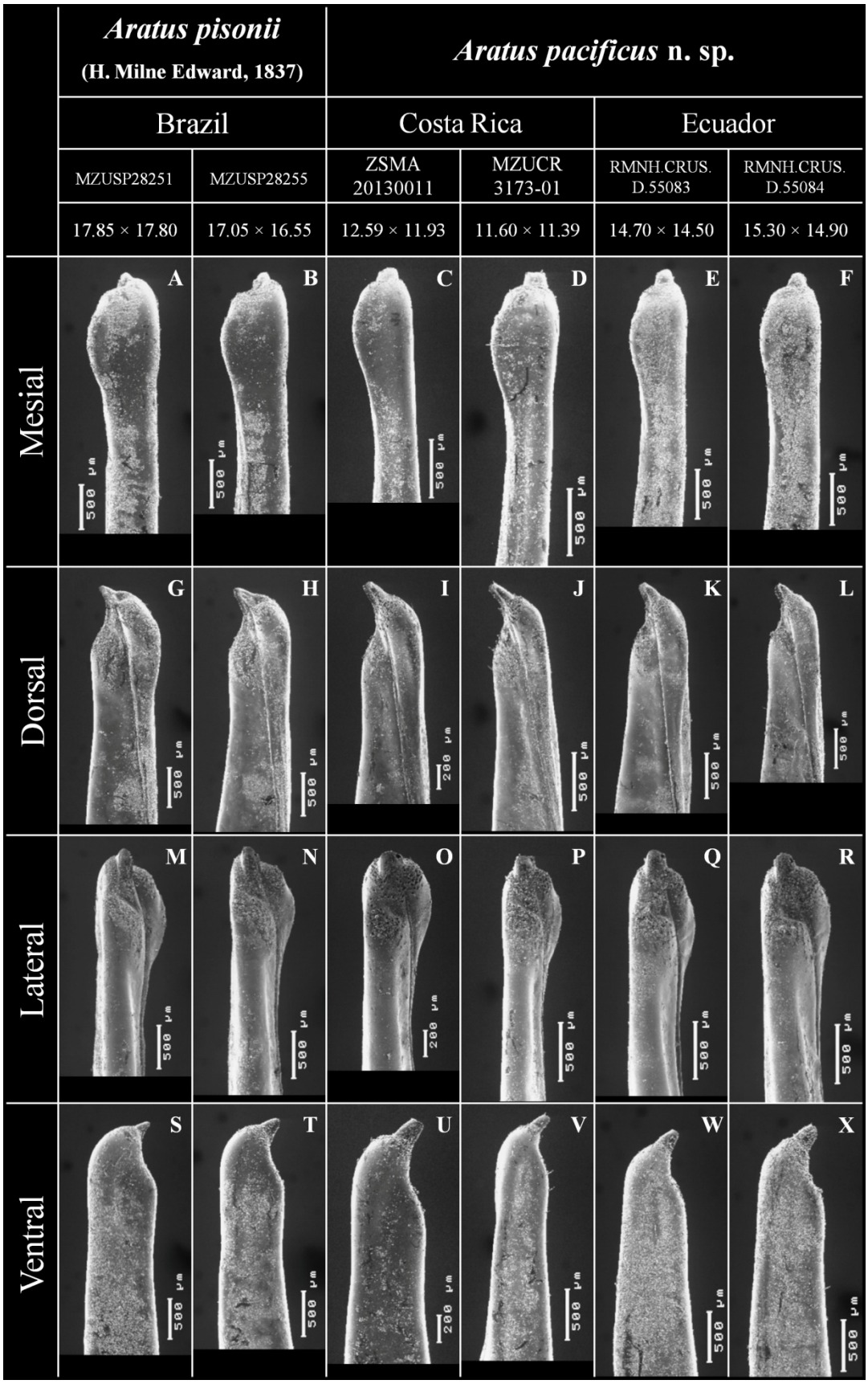

Figure 2.8. First male left gonopods of *Aratus pisonii* (Brazil) and *Aratus pacificus* **n. sp.** as observed in scanning electron microscopy (SEM) in longitudinal mesial (A–F), dorsal (G–L), lateral (M–R) and ventral (S–X) views. CW × CL in mm.

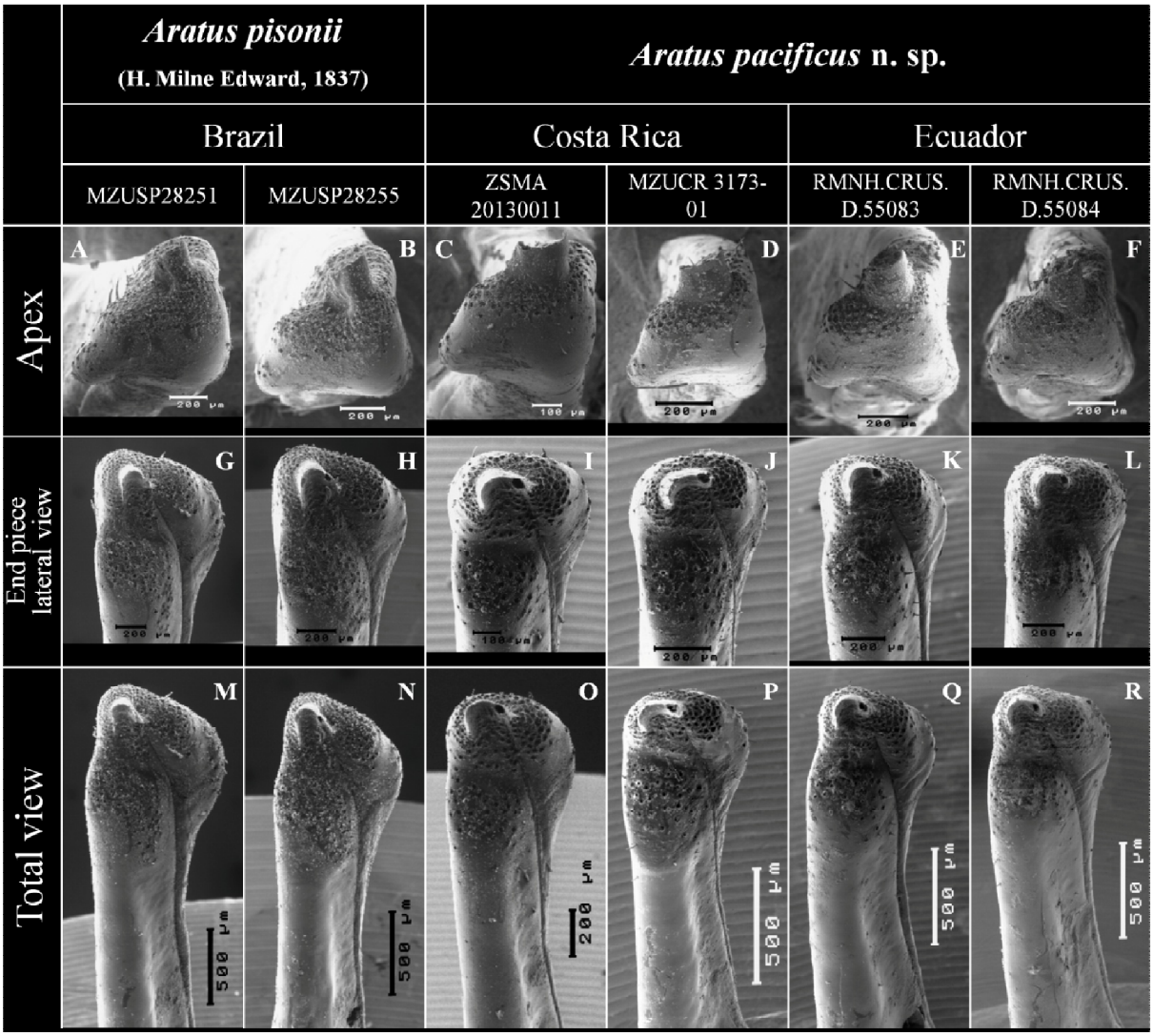

Figure 2.9. First male left gonopods of *Aratus pisonii* (Brazil) and *Aratus pacificus* **n. sp.** as observed in scanning electron microscopy (SEM) in apical view (A–F), detail of the endpiece in lateral view (G–L) and total view (M–R).

Type material

Holotype male (17.3 × 16.6mm) (SMF 43530), Ecuador, Puerto Morro, 2°36.498'S-80°18.245'W, coll. C.D. Schubart & R. Landstorfer, 10 Oct.2007.

Paratypes

Ecuador. Puerto Morro, 2°36.498'S-80°18.245'W, 2 ♂ 1 ♀ (14.7 × 14.5, 15.3 × 14.9, 14.6 × 14.5mm) (RMNH.CRUS.D.55082-55084) - 2 ♂ 1 ♀ (13.45 × 13.0, 13.4 × 13.35, 15.05 × 14.9mm) (MNHN-IU-2009-3091, MNHN-IU-2009-3092, MNHN-IU-2009-3093) - 1 ♂ 2 ♀ (12.35 × 12.25, 12.55 × 12.45, 13.25 × 13.25mm) (NHMW 25472-25474) - 1 ♂ 1 ♀ (12.9 ×

12.55, 11.05 × 10.7mm) (ZRC.2013.0452) - 2 ♂ 2 ♀ (10.7 × 10.55, 8.9 × 8.9, 9.75 × 9.65, 9.25 × 9.5) (SMF 43531), coll. C.D. Schubart & R. Landstorfer, 10 Oct.2007. *Costa Rica*. Puntarenas, Mata de Limón, 9°55.422'N-84°42.759'W, 1 ♂ 2 ♀ (12.59 × 11.93, 14.48 × 14.4, 12.39 × 12.18mm) (ZSMA 20130010-20130012) - 3 ♂ 2 ♀ (18.11 × 17.82, 11.6 × 11.39, 11.55 × 11.55, 13.52 × 13.44, 11.26 × 10.70mm) (MZUCR 3173-01) - 3 ♂ 3 ♀ (15.49 × 15.38, 9.62 × 9.48, 6.36 × 6.19, 13.48 × 13.21, 13.06 × 12.98, 6.10 × 6.10mm) (SMF 43532), coll. N. Thiercelin & T. Poettinger, 29 Apr. & 2 May 2011.

Description

Carapace trapezoidal, frontal margin conspicuously wide, orbits extending to anterolateral margin; front vertical, four lobes, about 4 times as long as deep; lateral margins subparallel, upper margin with row of granules, clear median groove separating wider outer lobes from inner lobes. Lower margin divided by median incision. Postorbital angles acute; lateral margins carinate, convergent, posterior margin slightly rounded. Presence of 5 or 6 subparallel carinae running obliquely along posterior half of lateral margin in direction to postlateral angle; carapace regions sharply delineated; granular frontal region, gastric lobes, sides finely punctate, remaining upper surface smooth, shiny. Pterygostomian region finely, regularly beaded. Seven-segmented male abdomen, subcircular, except for last somite abruptly narrowed with distal margin rounded. Transversely folded antennules under frontal margin. Small antennae excluded from orbit by broad, rounded lobe. Short eyestalk; large cornea, terminal, well developed. Well separated external third maxillipeds, with distance between them near equal to width of one maxilliped. Unequally suboval ischium, inner side more convex than outer side; distinct median channel on ischium of outer face; oblong oval merus, scarcely one-sixth longer than ischium, with distinct groove subparallel to outer margin, deeper groove obliquely channelling median region; narrow palp elongate, setiferous; maxillipeds inner lateral margins fringed with long, stiff setae.

Chelipeds sexually dimorphic. Small cheliped with sharp subdistal tooth on upper ischial margin; three-sided merus, granular on lower surface margins; convex carpus, granular. Greatly dilated male propodus, convex, granular, furnished with clusters of stiff bristles; fingers nearly as long as palm, gaping widely in males, tips meeting, inner edges weakly dentate; bristles present on proximal half of both fingers. Ambulatories long, slender, decreasing in length posteriorly; meral joint oblong, suboval, one-third as wide as long, anterior lateral margin with acute subdistal tooth; upper surface roughly granular; elongated

carpus, narrowed basally, dilated distally with 2 longitudinal carinae; distally narrowed propodus about twice carpus length; short dactyli, stout, less than one-third of propodi length, conspicuously acuminate.

First male gonopod (Figs. 2.3C, D, G, H, K, L, O, P; 2.4C, D, G, H, K, L; 2.8C, D, E, F, I, J, K, L, O, P, Q, R, U, V, W, X; 2.9C, D, E, F, I, J, K, L,O, P, Q, R) elongated, slender. Stem triangular in cross section; basal diameter wider than medial diameter. Mesial, ventral faces flat; slanted suture (sperm channel) clearly marked on dorsal face with terminal section in strongly marked depression; endpiece larger than medial part, easily noticeable in mesial, lateral views, endpiece bulging in lateral part; dorsal part almost spoon-shaped in mesial view. Mesial endpiece ventral, dorsal parts at same level (horizontal in mesial view); tip short, chitinous and slightly curved (approx. 90°) in medial position of apex, with pore on dorsal margin; suture aligned with pore on chitinous tip; mesial face of chitinous tip/mesial face approx. 130°; small denticles on lateral face of chitinous process. Large number of long setae on apex around chitinous tip, lateral, dorsal sides of endpiece lateral bulge. Row of setae on endpiece at edge between mesial, dorsal faces. Few short setae on gonopod.

Etymology

The epithet *pacificus* refers to the geographical distribution of the new species, which is distributed in the eastern Pacific Ocean, in contrast to *Aratus pisonii*, which is restricted to the western Atlantic Ocean.

Biology

Found in mangroves from estuaries and lagoons, in saline to hypersaline (e.g. SMF 43530, RMNH.CRUS.D.55083-55084) or brackish waters (e.g. MZUCR 3173-01, ZSMA20130011). Rebolledo-Navarro & Wehrtmann (2012) and Wehrtmann & Rebolledo-Navarro (2012) compared the ecology of *A. pisonii* and *A. pacificus* **n. sp.** in Costa Rica. They observed 1) smaller egg volume in *A. pacificus* **n. sp.** than in *A. pisonii*, and 2) differences in the population structure of the two species, with females found in an intermediate size class in *A. pacificus* **n. sp.**, whereas in *A. pisonii* females are found in larger size classes.

Coloration

Wehrtmann & Rebolledo-Navarro (2012) pointed out differences between the coloration of Atlantic and Pacific populations in Costa Rica, with the Atlantic population having reddish

chelae, whereas the Pacific populations have red-orange chelae. Similar patterns were observed in Panama (R. Lasley and C.S. McKeon, personal communication). We remain cautious concerning the reliability of the live coloration, however, as variability was observed in the field between specimens from north and central Brazil. Photographs of Florida specimens even exhibit a different coloration with purple coloration on the external side and reddish on the inner side of the chelae, which differs from the Brazilian populations which present only reddish pattern on both sides of the chelae. It remains difficult to use the color pattern to distinguish between the two species, as long as the variability is not clearly established between the different populations of *Aratus pisonii* sensu stricto along its entire distribution range. The carpi of the ambulatory legs of alcohol-preserved specimens often exhibit stripes in *Aratus pacificus* **n. sp.**, whereas they are of a homogeneous colour in *Aratus pisonii*.

Geographical distribution

Tropical eastern Pacific coastline, from Baja California to Peru (Beever *et al.* 1979), although only specimens between Costa Rica and Ecuador have had their identity confirmed in the present study.

Remarks

Aratus pacificus **n. sp.** can be distinguished from *A. pisonii* by their respective male first gonopods. The gonopod of *A. pacificus* **n. sp.** is slightly more slender than the gonopod of *A. pisonii*. In *A. pisonii* the ventral mesial part of the terminal end is higher than the dorsal part (angle approx. 25° in mesial view), whereas both parts are at the same level in *A. pacificus* **n. sp.** (Figs. 2.3A–D, 2.6A–F and 2.8A–F). The chitinous tip is located ventrally and is U-shaped in *A. pisonii* (Figs. 2.4A, B, E, F, 2.7A–L, 2.9A, B, G, H), whereas the tip has a central position on the apex and is curved not more than 90° in *A. pacificus* **n. sp.** (Figs. 2.4C, D, G, H, 2.9C–F and 2.9I–L). A small depression at the base of tip is present in *A. pisonii*, but absent in *A. pacificus* **n. sp.** (Figs. 2.4A–D, 2.7A–F and 2.9A–F). In *A. pisonii*, the suture is aligned with the edge of the chitinous tip on the apex (Figs. 2.3E–F, 2.5, 2.6G–L and 2.8G, H). In *A. pacificus* **n. sp.**, in contrast, the suture is aligned with the pore on the chitinous tip (Fig. 2.3G, H, 2.5 and 2.8I–L). This also implies that it is possible to measure the angle in dorsal view between the mesial face of the chitinous tip and the suture in *A. pisonii* (approx. 115°), which is not possible in *A. pacificus* **n. sp.** (Fig. 2.5). The angle between the mesial

side of the gonopod and the mesial side of the chitinous tip is approx. 110° in dorsal view in *A. pisonii* and approx. 130° in *A. pacificus* **n. sp.** (Figs. 2.3E–H, 2.6G–L and 2.8G–L). Allometric changes in the gonopod shape can be observed between the smallest and largest specimens in both species (as in specimens NHMW25475 and NHMW25477 for *A. pisonii;* MZUCR 3173-01 (18.11 × 17.82mm) and MZUCR 3173-01 (11.60 × 11.39mm) for *A. pacificus* **n. sp.**). These changes are more marked in dorsal and ventral views, where the gonopods appear more slender for the smallest specimens in both species, and remains clearly visible in preserved specimens whatever their size.

Discussion

We provide evidence of morphological and genetic differentiation between transisthmian populations of *Aratus pisonii* and place the respective populations from the Atlantic and Pacific oceans in two distinct species, one of which is new to science. No consistent morphological differences and limited genetic differentiation were found among the studied populations from the same ocean (Jamaica, Hispaniola, Brazil on one hand, Costa Rica and Ecuador on the other).

The morphology of the male gonopods, as used here to differentiate between the two transisthmian species, has been often illustrated in sesarmid crabs (e.g. Abele 1992, Niem 1996, Schubart & Koller 2005, Naderloo 2011), and is used in all modern descriptions of new brachyuran species, if male specimens are available. Most of these gonopod descriptions often lack detail. Here we confirm that differences in gonopod morphology can be used to distinguish between sister species in Sesarmidae. These differences remain constant within species, even between geographically distant populations as Jamaica and Brazil (approx. 6400 km). This observation reinforces the importance of detailed descriptions of gonopods in the description of species, if possible with SEM illustrations which are often more precise than drawings.

The genetic data present in this study indicate a DNA sequence divergence of 3.0–3.5% for the mitochondrial 16S ribosomal gene and of 0.2–0.3% for the 28S ribosomal nuclear gene between the transisthmian populations. These data confirm the genetic separation between *Aratus pisonii* and *A. pacificus* **n. sp.**, as observed for other couples of

transisthmian sister-species (Lessios 2008), and will be reinforced by the results of a separate phylogeographic study on the *A. pisonii* species complex (Thiercelin & Schubart, in preparation), indicating a DNA sequence divergence of 4.5–5.7% for the mitochondrial cytochrome oxydase I gene. The arguments detailed by Lessios (2008) for undescribed taxa in species with transisthmian distribution, thus appear to be confirmed in *Aratus*.

The recognition of distinct species on opposite sides of the Isthmus of Panama has been increasing over the years, with the description of new eastern tropical Pacific species of barnacles (Laguna 1987) and shrimps (Anker *et al.* 2009) and the revalidation of synonymised species in crabs (Schubart *et al.* 2005) that had been considered to have a transisthmian distribution. Additional cases of undescribed cryptic species were discovered with the development of molecular tools in polychaete worms (Barroso *et al.* 2010), molluscs (Lee & Foighil 2005), sea urchins and shrimps (see Lessios, 2008). At the same time, genetic divergence across the Isthmus of Panama has also been documented for the remaining three coastal crab species, which were considered to have a transisthmian distribution (*Pachygrapsus transversus/Pachygrapsus socius*; Schubart et al. 2005) and for which old names can also be revalidated (*Geograpsus lividus*, Guerao *et al.* 2003; *Grapsus grapsus*, Schubart 2011).

Efforts now concentrate on covering more populations of *Aratus* from throughout their distribution ranges, with special emphasis on populations within partly isolated water bodies such as the Gulf of Mexico and the Gulf of California in order to understand the full potential of morphological and genetic differentiation within this highly specialized genus.

Acknowledgments

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CHAPTER 3

The age of the Isthmus of Panama: a marine crustacean perspective

Abstract

Recently, a new model for the closure of the Isthmus of Panama was put forward suggesting a Middle Miocene continuous land bridge between both Americas, about 12 million years earlier than the previous Standard model with a final closure at roughly 3 Ma. If the Miocene model would be confirmed, this would have a severe impact on studies relying on the closure of the Isthmus of Panama to calibrate molecular clocks, and subsequent studies that used these rates to date phylogenies. Also the apparent Pliocene fossil evidence for the Great American Interchange of mammal faunas would be in need of careful re-investigation. Here we investigate both temporal models for the closure of the Isthmus of Panama from a marine perspective based on the transisthmian divergence of six sister species of coastal brachyuran crabs. In detail, we looked at sesarmid crabs of the genera *Aratus* and *Armases*, and the grapsid genera *Grapsus*, *Geograpsus*, *Goniopsis* and *Pachygrapsus*. We confirm that these species most likely diverged during two distinct temporal instances, associated to their respective habitat (mangroves and rocky shores), but are based on a common mechanism for the allopatry in the respective sister species pairs.

We tested both the standard and the early model of Isthmus formation in a Bayesian framework against a general arthropod mitochondrial substitution rate of 1.77% per my (mean of lognormal distribution with an 2.5–97.5% quantiles of 0.205–11.89% per my) based on studies that did not calibrate with the Isthmus of Panama. It showed that in 10 of 16 cases the Standard model fits better to this independent rate of sequence evolution, whereas the early model would result in strongly decelerated rates of mitochondrial evolution compared to other known rates. We interpret these results in favor of the Standard model and a final closure of the Isthmus of Panama during the Pliocene.

Introduction

The rise of the Isthmus of Panama (IoP) is one of the most prominent biogeographic events used for the calibration of molecular clocks, especially considering of the amount of taxa it isolated and the claimed precise geological dating of its final closure (Knowlton & Weigt 1998, Lessios 2008).

The closure of the IoP started during the Eocene and ended by the formation of a land-bridge between the North and South American continents, as a consequence of the subduction of the Farallon Plate (with its extant relics, the Nazca and Cocos plates) under the Caribbean Plate, first leading to a volcanic arc, followed by collision with the South American Plate thereby closing the Central American Seaway, which functioned as a marine connection between the Atlantic and Pacific oceans (Coates & Obando 1996). This closure resulted in the physical isolation of Atlantic and Pacific faunas, and subsequent appearance of couples of "geminate species" across the IoP, as already noted by early researchers (Jordan 1908).

This Standard model for the geological chronology of the final IoP closure was based on the analysis of stratigraphic sequences from sedimentary basins, and dates this geological event at 3.1 mya (Coates & Obando 1996, Coates *et al.* 2004, Bartoli *et al.* 2005), with a range from 2.8 mya (Coates *et al.* 2005) to 3.5 mya (Coates *et al.* 1992). A possible breach is considered at 2 Mya by Cronin & Dowsett (1996) when salinity decreased temporarily in the Atlantic.

This timing was recently challenged by several studies that propose final closure of the IoP at a much earlier time, i.e., during the Miocene, starting with the narrowing of the last seaway about 25 mya and completed closure about 15 mya. This Miocene model is based on radiometric dating (uranium-lead dating), on thermochronological analyses in zircon (U-Th/He dating), on paleomagnetic reconstructions and on geochemical changes, and interprets the cooling of granitoid rocks and the geochemical changes observed during the Miocene as evidence for the uplift of the IoP (Farris *et al.* 2011, Montes *et al.* 2012a, 2012b).

This Miocene model of IoP closure elicited a controversy, with Coates & Stallard (2013) and Jackson & O'Dea (2013) reviewing the already existing geological and biological data in support of the Standard model. They provided explanations for the strongly distinct timeframes of the two models for the same geological event based on analogy to the ongoing convergence of Sunda and Sahul shelves in Southeast Asia, arguing that even small seaways

can prevent the complete admixture of terrestrial faunas and floras, as well as allow for gene flow between marine populations on both sides of such seaways.

However, as these two studies only reviewed already available results, a rigorous assessment of the biological evidence for either of the two models is still missing. This appears to be especially urgent when we consider that the final closure of the IoP has been used as reference value to infer rates of molecular evolution and calibrate molecular clocks for a large number of studies (Lessios 2008).

Here we use six transisthmian sister species pairs of brachyuran crustacean occurring along the eastern and western Central American coastlines as model to test the two proposed closing times of the IoP from a marine perspective, by comparing the fit of both models to the observed genetic data under the assumption of independently estimated arthropod mitochondrial substitution rates. Furthermore, we test the hypothesis that the exact timing for the cessation of gene flow between the two oceans depends on the species' habitat, with mangrove-dwelling species separating later than subtidal or rocky shore species during the closure of the IoP (Knowlton & Weigt 1998, Williams *et al.* 2001, Marko 2002, Lessios 2008, Miura *et al.* 2010).

Material and Methods

Collection of specimens

Specimens from six thoracotreme brachyuran crustacean genera where collected at both Atlantic and Pacific Central American coastlines (see Table S3.1 in Supplementary Material), and preserved in 70-96% ethanol. The sampling scheme was designed to cover the whole distribution range of the species to reflect the genetic polymorphism (including ancestral polymorphisms) in the target genes of the species. Two genera belong to the family Sesarmidae (*Aratus* and *Armases*) and four to the Grapsidae (*Geograpsus, Goniopsis, Grapsus* and *Pachygrapsus*).

DNA extraction and sequencing

Genomic DNA was extracted from muscular leg tissue using the Puregene kit (Qiagen). Fragments of at least 650bp of the mitochondrial cytochrome oxydase I gene

(Cox1) were amplified with the Thoracotremata-specific primers COL1b, COL8, COH1b and COH16 (Schubart 2009) with the following PCR profile: initial step 4 min at 94°C, 40 cycles with 45s at 95°C - 45s at 50°C - 75s at 72°C for denaturing, annealing and extension respectively, final extension step 5 min at 72°C.

A fragment of ~615bp of the mitochondrial 16S rRNA gene was amplified with the Thoracotremata-specific primers 16L2 and 16HLeu (Schubart 2009) with the following PCR profile: initial step 4 min at 94°C, 40 cycles with 45s at 95°C - 60s at 48°C - 60s at 72°C for denaturing, annealing and extension respectively, and final extension step 5 min at 72°C. PCR products were outsourced for sequencing to LGC Genomics GmbH or Macrogen Europe.

The sequences were proofread with Chromas 2.23 and aligned with ClustalW (Thompson *et al.* 1994) as implemented in BioEdit 7.0.5 (Hall 1999). To limit the risk of pseudogenes in the Cox1 sequences, the absence of stop-codons, as potential indicators of pseudogenes, was assessed with Artemis 14 (Rutherford *et al.* 2000) using the invertebrate mitochondrial genetic code (NCBI Table 5). Sequences were submitted to EMBL and are available at ENA/GenBank under the accession numbers (pending) for the Cox1 and (pending) for the 16S.

Evolutionary rates resulting from Standard and Miocene models of IoP closure

Transisthmian molecular divergence (pairwise differences) for various marine taxa (including Crustacea) assumed to have initiated their divergence at the closure of the isthmus, range from 4 to 9% for the Cox1 and 2 to 7% for the 16S rRNA mitochondrial genes based on the Kimura-2-parameter distance (Lessios 2008). To obtain results that are comparable with this review and confirm the assumptions of Knowlton & Weigt (1998) that within the mangrove dwelling taxa (in our case *Aratus, Armases* and *Goniopsis*) divergence was initiated during the final steps of the IoP closure, while within rocky shore taxa (*Grapsus, Geograpsus* and *Pachygrapsus*) divergence occurred before the final closure, we calculated the interspecific Kimura-2-parameter corrected genetic distances for the transisthmian sister species pairs in Mega 6.1 (Tamura *et al.* 2013).

The rates of molecular evolution for the mangrove species were calculated for both models of the IoP closure, with a divergence initiated at 3.1 mya for the Standard model and at 15 mya for the Miocene model. Rates were obtained with BEAST 1.8.0 (Drummond *et al.* 2012) using as priors the best model of nucleotide evolution recovered with jModelTest 2.1.4 (Darriba *et al.* 2012) and uniform rates (0-20% per mya for the Cox1, 0-2% per mya for the 16S rRNA), with 50 to 100 million generations (until an effective sample size of sampled

parameters >100 was achieved); the first 25% samples were discarded. The same method was applied for the dataset of Miura *et al.* (2012) based on transisthmian mangrove snails, for both Cox1 and 16S rRNA genes, as element of comparison.

Simultaneous divergence across the IoP

In order to verify with our dataset the hypothesis from Knowlton & Weigt (1998) that couples of transisthmian species / populations did not all initiated their divergence during the final steps of the Isthmus closure, we used the hierarchical approximate Bayesian computation approach implemented in the MTML-msBayes pipeline (Hickerson *et al.* 2006, 2007, Huang *et al.* 2011) to estimate the number of co-divergence events (Ψ) in our six selected pairs, this method assuming a unique rate of molecular evolution between the taxa. The so-called ABC method runs in a three step process (Hickerson *et al.* 2007): the first step corresponds to the calculation of an observed summary statistic vector based on the observed dataset. The second step simulates random datasets with the same sequence length and the same number of specimens to the observed dataset and based on prior parameters. The third step calculates acceptance / rejection and hyper-posterior parameters (number of divergence events Ψ, mean population divergence E(τ) and dispersion index of the divergence time Ω) based on a sampling of the simulated datasets. One million simulated datasets were calculated (setting file available in Supplementary Material), and the 1000 closest simulated draws (proportion: 0.001) were sampled as recommended in the software instructions. The transition / transversion ratios were estimated for the HKY model, inferred as best fit model of sequence evolution in jModelTest 2.1.4 (Darriba *et al.* 2012). msBayes also allows the fixation of the number of divergence events to determine the number of taxon pairs in each assumed divergence event, and then assumes a normal distribution of the prior probability for 1 to n-1 couples in each divergence event (meaning a lower prior probability for the most extreme numbers of divergence events, and a higher prior probability for intermediate numbers of divergence events). The number of divergence events Ψ was thus fixed to two to test the hypothesis of an early and a late divergence events.

We also sorted according to their habitat preference as supposed by Knowlton & Weigt (1998). Two subsets were defined, one for the mangrove dwelling crabs, (the transisthmian sister species within *Aratus*, *Armases* and *Goniopsis*) that are assumed to have diverged in the final steps of the closure of the Isthmus, when marine connections between the two oceans where only corresponding to mangrove swamps; and a second subset for rocky

shore dwellers (the genera *Geograpsus*, *Grapsus* and *Pachygrapsus*). The latter have potentially initiated their divergence before the mangrove species. The two subsets were tested with the same parameters as the whole dataset.

Divergence times of both habitat subsets were determined based on the following equation: $t = E(\tau) \times T \times \theta_{ave}/\mu$ (Hickerson et al. 2007), with T being the generation time of the species (one year per generation based on the life history of these species; Conde & Díaz 1989, Díaz & Conde 1989, Flores & Negreiros-Fransozo 1998, Leme & Negreiros-Fransozo 1998, Lira *et al.* 2013), θ_{ave} being half of the upper bound of the θ prior (upper bound fixed as prior), μ corresponds to the divergence rate per lineage, and $E(\tau)$ is the mean divergence time in units of μ (per gene per generation). We used the arthropod mitochondrial substitution rate of 1.77% per my proposed by Papadopoulou *et al.* (2010), independent of the closure of the IoP, to determine the divergence time of the difference couples.

Model testing

We analyzed the fit of the Standard and the Miocene model for the closure of the IoP to genetic data under the assumption of external arthropod substitution rates that have not been calibrated at the IoP. Thereby we constrained independently the divergence of transisthmian species pairs according to the two models and compared the resulting marginal likelihoods of the models using the Bayes factor.

Ho & Phillips (2009) discussed about the effect of calibration uncertainty in phylogenetic estimation of divergence times and recommended to consider the uncertainty of the calibration points by representing the calibration timing as a parametric distribution of probability density. In this study, it implies to consider not only the divergence time but also the uncertainty going with it.

The standard model of closure of the Isthmus of Panama, proposed by Coates & Obando (1996) considers a final closure 3.1 mya. To take into account different geological estimates on the closing time, ranging from 3.5 mya (Coates *et al.* 1992) to 2.8 mya (Coates *et al.* 2005), a normal distribution with a mean value of 3.1 mya (Coates & Obando, 1996) was used. The normal distribution is symmetric with the probability density centered around this central value of 3.1 mya, with this type of distribution considered to be suitable for biogeographic calibration (Ho & Phillips 2009). The standard deviation was set to 0.153 resulting in a 2.5–97.5% quantile of 2.8–3.4 mya.

The Miocene model proposes a final closure 15 mya (Montes *et al.* 2012a, 2012b). Montes *et al.* (2012b:23) stated that the Central American Seaway disappeared 'about' 15 mya. Thus, to take into account potential temporal uncertainty, we applied a normal distribution centered at 15 mya with a standard deviation of 0.51, resulting in a 2.5–97.5% quantile of 14–16 mya. It implies that the probability of closure was maximal at the timing indicated by Montes *et al*. (2012b), but still considers a degree of uncertainty (Ho & Phillips 2009).

The null hypothesis was also tested using only the rate of molecular evolution and without temporal constraint, to verify the significance of the results obtained under the two geological models and to investigate the effect of calibration on divergence times.

Analyses were run for each family separately, Sesarmidae (*Aratus* and *Armases*) and Grapsidae (*Geograpsus*, *Goniopsis*, *Grapsus* and *Pachygrapsus*). Based on the results of the K2P genetic divergence and on the test of simultaneous divergence, the divergence time was constrained for the mangrove genera *Aratus* and *Goniopsis* respectively. Indeed, *Goniopsis* is the only mangrove genus in the Grapsidae dataset, and thus the only one to be assumed to have initiated its divergence at the IoP closure. *Armases* presents a higher tolerance to reduced salinity than *Aratus*, the latter being restricted to mangrove habitats whereas *Armases americanum* and *A. angustum* can be found on banks of freshwater streams. Therefore *Aratus* represents probably better the interruption of marine exchanges between the two sides of the IoP (Abele 1992, Thiercelin & Schubart 2014).

The input files for each analysis were generated with Beauti 1.8.0 (part of the BEAST package; Drummond *et al.* 2012). Marginal likelihoods were estimated using path (Lartillot & Philippe 2006) and stepping stone sampling (Xie *et al.* 2011) approaches, considered as being currently the two most efficient model estimators (Baele *et al.* 2012, 2013) (code for marginal likelihood estimation available at http://beast.bio.ed.ac.uk/Model_selection). The analyses were performed for both Cox1 and 16S rRNA genes with BEAST 1.8.0 (Drummond *et al.* 2012) on the CIPRES Science Gateway (Miller *et al.* 2010), and four replicate runs were performed using the "Clone" function of CIPRES.

The rates of molecular evolution used as prior in the analyses are based on arthropod rates independent from the closure of the IoP. For the Cox1 gene, we used a lognormal distribution of the substitution rate (i.e., rate per lineage or half the pairwise divergence rate). This distribution allows to have a near-normal distribution for its central part, but also allows to consider higher arthropod rates that resulted from calibration with recent divergence events (Craft *et al.* 2008, Papadopoulou *et al.* 2010, Crandall *et al.* 2011).

The median was fixed to 1.77% per my based on the insect rate proposed by Papadopoulou *et al.* (2010) in their revision of the insect mitochondrial molecular clock using a calibration based on the opening of the mid-Aegean trench. The lognormal standard deviation of 1.06 resulted in an upper bound of the distribution's 2.5–97.5% quantile of 11.89% per my corresponding to the upper bound of the Cox1 rate recovered by Crandall *et al.* (2011) in the mantis shrimp *Haptosquilla pulchella*, based on the expansion of Sunda Shelf populations following the Last Glacial Maximum. A hard upper bound was fixed at 20% per my, the rate recovered in Hawaiian shrimps of the genus *Halocaridina* (see Craft *et al.* 2008). A hard lower bound was fixed to 0.205% per my, corresponding to the fossil calibrated rate in the *Limnoporus* water striders (Sperling *et al.* 1997, and see Table S1 of Papadopoulou *et al.* 2010). For the 16S rRNA gene, we used a normal distribution to model the substitution rate of a mean and standard deviation based on a fossil calibrated phylogeny of Old World freshwater crabs by Klaus *et al.* (2010) (mean: 1.02% per mya; SD: 0.194%; 2.5–97.5% quantile 0.64-1.42% per my).

We excluded the widely used arthropod rate of Brower (1994; 1.15% per my) as this rate includes divergence of transisthmian *Alpheus* shrimps and merges protein-coding and ribosomal markers which most likely have extremely distinct rates, as noticed by Papadopoulou *et al.* (2010).

Each run was conducted four times with different random seeds. The best model of closure of the Isthmus was determined based on the 2 × log Bayes Factor following the recommendation of Kass & Raftery (1995).

Results

Genetic differentiation

For the Cox1 mitochondrial gene, the three mangrove species exhibit the smallest K2P values, whereas the three rocky shore species present the highest values (Table 3.1). For *Aratus*, the divergence between Atlantic and Pacific oceans range from 4.6 to 7.8 %. For *Armases*, the divergence ranges from 5.2 to 6.3 %. Thus, despite an average divergence higher for *Aratus* in regards with *Armases*, this genus exhibits the smallest transisthmian divergence of our dataset. For *Goniopsis*, it ranges from 7.6 to 9.3 %. For *Grapsus*, only a single

haplotype is found in the Atlantic, and the range is limited by the inclusion of a single Pacific specimen in the dataset. As a result a divergence of 9.49% is obtained between Atlantic and Pacific haplotypes. For *Geograpsus*, it ranges from 10.9 to 11.9 %. For *Pachygrapsus*, the divergence ranges from 13.7 to 14.5 %.

Table 3.1. Mean Cox1 Kimura-2-parameter (K2P) percent difference between Atlantic and Pacific clades. N_A: number of Atlantic specimens ; N_P: number of Pacific specimens.

Pacific clade	**N_P**	**Atlantic clade**	**N_A**	**Mean K2P (%) Atlantic / Pacific**
Armases angustum	3	*Armases americanum*	20	5.751
Aratus pacificus	11	*Aratus pisonii*	10	6.072
Goniopsis pulchra	8	*Goniopsis cruentata*	6	8.394
Grapsus grapsus	1	*Grapsus grapsus*	6	9.493
Geograpsus lividus	10	*Geograpsus lividus*	11	11.348
Pachygrapsus socius	16	*Pachygrapsus transversus*	16	14.235

Table 3.2. Mean 16S Kimura-2-parameter (K2P) percent difference between Atlantic and Pacific clades. N_A: number of Atlantic specimens ; N_P: number of Pacific specimens.

Pacific clade	**N_P**	**Atlantic clade**	**N_A**	**Mean K2P (%) Atlantic / Pacific**
Armases angustum	3	*Armases americanum*	10	0.660
Aratus pacificus	11	*Aratus pisonii*	10	3.503
Goniopsis pulchra	8	*Goniopsis cruentata*	3	3.256
Grapsus grapsus	1	*Grapsus grapsus*	6	4.400
Geograpsus lividus	10	*Geograpsus lividus*	9	3.955
Pachygrapsus socius	8	*Pachygrapsus transversus*	10	4.198

As for the Cox1 gene, for the 16S rRNA gene, mangrove species present smaller genetic differentiation than rocky shore species (Table 3.2). *Armases* presents the smallest divergence of the dataset, ranging from 0.7 to 0.8%. For *Goniopsis*, it ranges from 2.8 to 3.9%. For *Aratus*, it ranges from 3.0 to 4.1%. For *Geograpsus*, it ranges from 3.7 to 4.3%. For *Grapsus*, the differentiation is ranging from 4.2 to 4.6%. For *Pachygrapsus*, the transisthmian divergence ranges from 4.1 to 4.2%.

For the Cox1 gene, the average substitution rate obtained for the mangrove species is 1.5% per my (95% HPD: 0.69–2.57% per my, Table 3.3) under the Standard model, and from 0.32% per my (95% HPD: 0.13–0.57% per my) under the Miocene model. For the 16S rRNA gene, an average substitution rate of 1.16% per my is recovered for the Standard model (95% HPD: 0.51–1.94% per my), and 0.4% per my for the Miocene model (95% HPD: 0.07–1.08% per my). For the dataset of Miura *et al.* (2012), an average substitution rate of 2.78% per my (95% HPD: 1.67–4.30% per my, Table 3.4) is recovered for the Cox1 under the Standard model, and of 0.57% per my (95% HPD: 0.33–0.90% per my) under the Miocene model. For the 16S rRNA gene, an average substitution rate of 0.68% per my (95% HPD: 0.26–1.16% per my) is obtained with the Standard model and of 0.16% per my (95% HPD: 0.05–0.25% per my) under the Miocene model.

Table 3.3. Average substitution rate (in percent per my) and 95% HPD interval of mangrove species for both Cox1 and 16S rRNA mitochondrial genes under the Standard (3.1 mya) and Miocene (15mya) models of closure of the Isthmus.

	Cox1		**16S rRNA**	
	3.1 mya	**15 mya**	**3.1 mya**	**15 mya**
Mean	1.50	0.32	1.16	0.40
95% HPD	0.69–2.57	0.13–0.57	0.51–1.94	0.07–1.08

Table 3.4. Average substitution rate (in percent per my) and 95% HPD interval of transisthmian mangrove snails (dataset of Miura *et al.* 2012) for both Cox1 and 16S rRNA mitochondrial genes under the Standard (3.1 mya) and Miocene (15mya) models of closure of the Isthmus.

	Cox1		**16S rRNA**	
	3.1 mya	**15 mya**	**3.1 mya**	**15 mya**
Mean	2.78	0.57	0.68	0.16
95% HPD	1.67–4.30	0.33–0.90	0.26–1.16	0.05–0.25

Model selection

The model selection based on the Bayes Factor supports for both Cox1 and 16S rRNA genes a final closure of the Isthmus around 3 mya rather than 15 mya (Table 3.5). When using the 2logBF threshold, the Byes Factor calculated based on run average and only on the best run supports positively (2logBF > 2) the Standard model of closure and reject the Miocene model in all comparisons. In 10 of 16 comparisons, the support for the Standard model of closure is very strong (2logBF > 10). In two cases (Grapsidae / Cox1 gene / only best run considered) the result is not significant (2logBF = 1.1 and 1.4). The constrained runs performed significantly better than the unconstrained null hypothesis for both genes and both families. Even if the run convergence appears to be suboptimal, the different runs appear to vary in a same range for both models (Table S3.2 in Supplementary Material), and both path and stepping stone sampling approaches give similar results.

msBayes

The hierarchical approximate Bayesian computation approach, used to test for simultaneous divergence of co-distributed couples of sister-species or populations, rejects the null hypothesis of simultaneous divergence of the six transisthmian pairs. However, it remains unclear concerning the number of divergence events (Ψ), highlighted by the close probability of the other hypotheses (posterior probability ranging from 0.15 to 0.24, Fig. 3.1-A). The highest posterior probability is observed for three divergence events (posterior probability: 0.24). The value of the dispersion index Ω (mean: 0.31; 95% HPD: 0.00–0.61) also supports heterogeneity in the divergence time when all the pairs are considered (Fig. 3.1-B).

Table 3.5. Model comparison for both Cox1 and 16S rRNA genes. For each model, the mean log marginal likelihood (based on four runs per model, two for the null hypothesis), the Bayes factor between the Standard and Miocene models based on the mean and the best of the four runs are indicated, and between the Standard model and the null hypothesis based on the best run, with both path and stepping stone sampling methods.

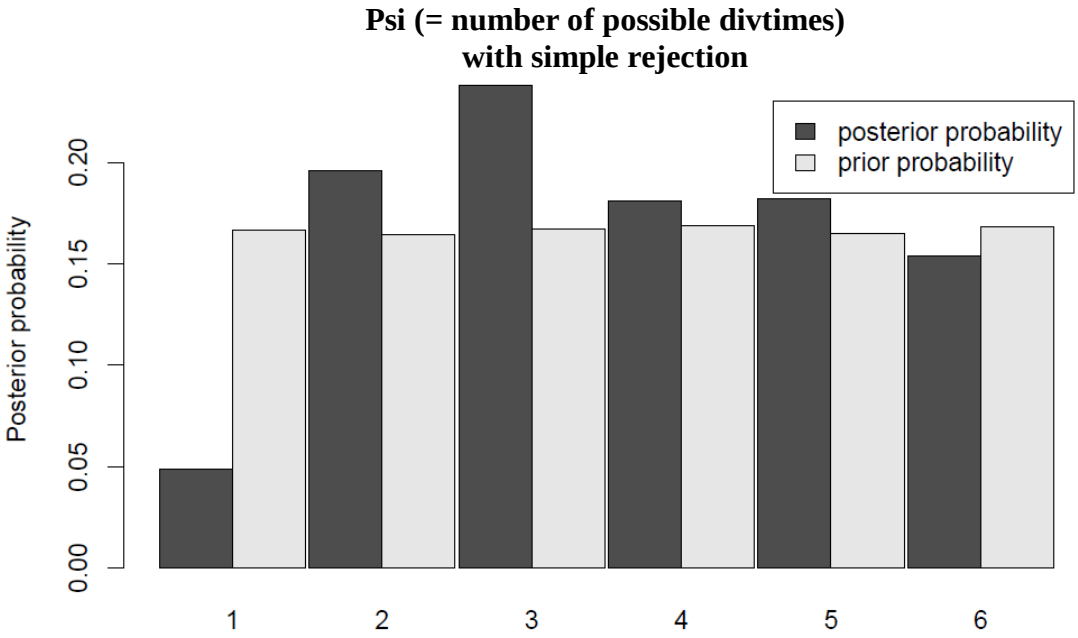
Cox1		**Path Sampling**			**Stepping stone Sampling**		
	Model	**Mean**	**2lnBF**	**Best 2lbBF**	**Mean**	**2lnBF**	**Best 2lbBF**
Sesarmidae	**3 mya**	-2758.7	11.6	19.6	-2752.5	17.1	34.6
	15 mya	-2764.5			-2761.1		
	Null	-2835.7		181.3	-2835.9		208.3
Grapsidae	**3 mya**	-4301.2	3.7	1.4	-4300.7	3.7	1.1
	15 mya	-4303.0			-4302.5		
	Null	-4370.2		138.1	-4370.5		140.4

16S rRNA		**Path Sampling**			**Stepping stone Sampling**		
	Model	**Mean**	**2lnBF**	**Best 2lbBF**	**Mean**	**2lnBF**	**Best 2lbBF**
Sesarmidae	**3 mya**	-1191.6	25.9	14.8	-1185.2	28.0	10.2
	15 mya	-1204.6			-1199.2		
	Null	-1264.9		161.8	-1262.5		168.8
Grapsidae	**3 mya**	-2286.0	4.3	12.5	-2285.6	4.6	14.8
	15 mya	-2288.2			-2287.9		
	Null	-2341.7		118.9	-2341.9		121.9

However, when Ψ is fixed to 2, three taxon pairs are found in each of the divergence event (Fig. 3.2). Moreover, when the dataset is split in two subsets associated with the species habitat (the mangrove subset including the genera *Aratus* / *Armases* / *Goniopsis*, the rocky shore subset the genera *Geograpsus* / *Grapsus* / *Pachygrapsus*), the peak of posterior probability supports a unique divergence event within each subset (mode mangrove: 1; mode rocky shore: 1). Indeed, for the mangrove habitat, the highest posterior probability is obtained for the hypothesis of a single divergence event (posterior probability: 0.45, Fig. 3.3-A), and similar result is obtained for the rocky shore habitat (posterior probability: 0.45, Fig. 3.4-A). The dispersion index (Ω) is extremely low and indicates homogeneity within the subset for both mangrove (mean: 0.16; 95% HPD: 0.00–0.58, Fig. 3.3-B) and rocky shore (mean: 0.08; 95% HPD: 0.00–0.39, Fig. 3.4-B).

The calibration by the external rate of each divergence event indicates that the rocky shore subset diverged earlier (*t*: 2.5 mya ; 95% HPD: 1.5–3.5 mya) than the mangrove one (*t*: 0.5mya ; 95% HPD: 0–1.3 mya).

A.



B.

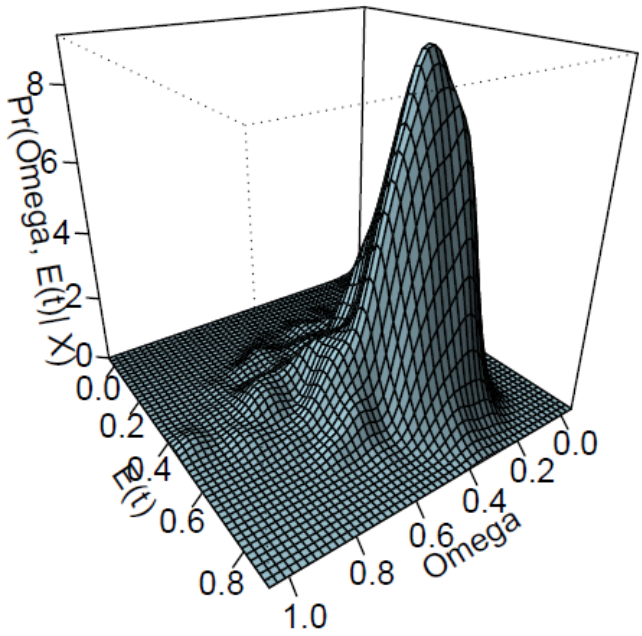
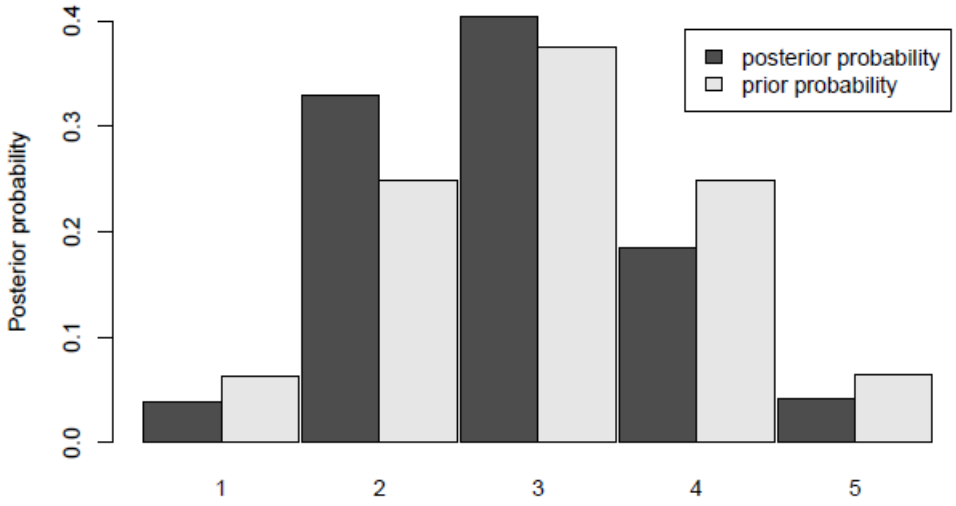


Figure 3.1. **A.** Prior and posterior probabilities of the number of divergence events (Ψ) with the six transisthmian taxon pairs. **B.** Joint posterior probability densities for E(τ) and Ω with the six taxon pairs.

Psi.1 (= number of taxon pairs that divergence at corresponding tau) with simple rejection



Psi.2 (= number of taxon pairs that divergence at corresponding tau) with simple rejection

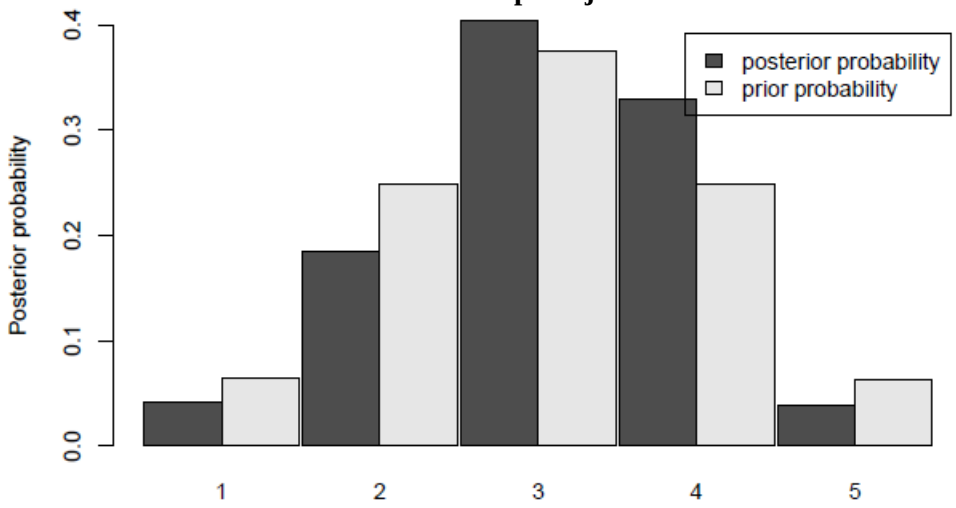
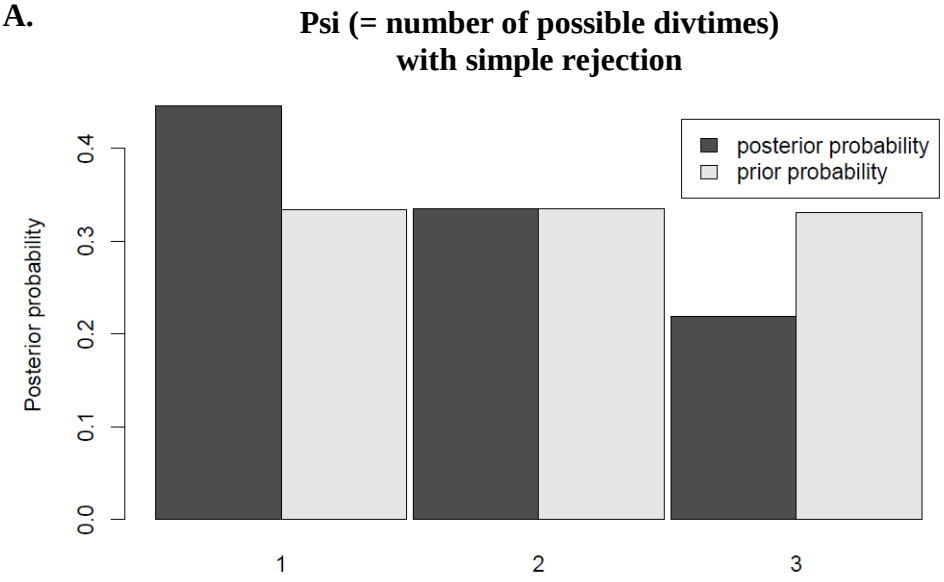


Figure 3.2. Prior and posterior probabilities for the number of taxon pairs in each divergence event for Ψ fixed to 2.

A.



B.

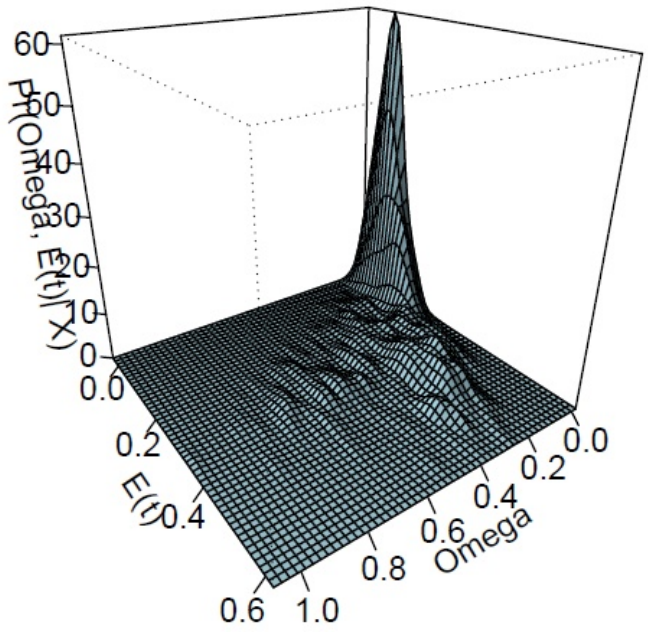
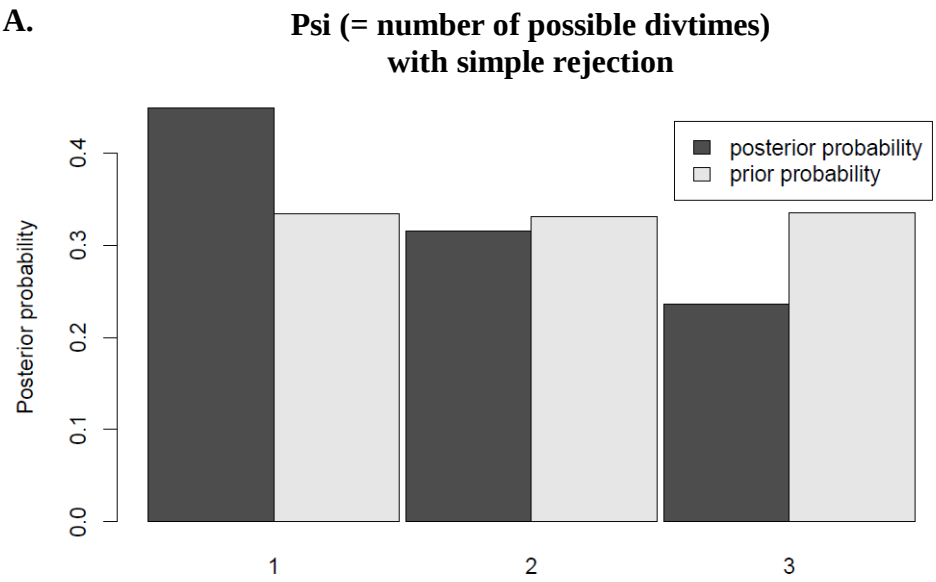


Figure 3.3. A. Prior and posterior probabilities of the number of divergence events (Ψ) for the mangrove habitat. **B.** Joint posterior probability densities for E(τ) and Ω for the mangrove habitat.

A.



B.

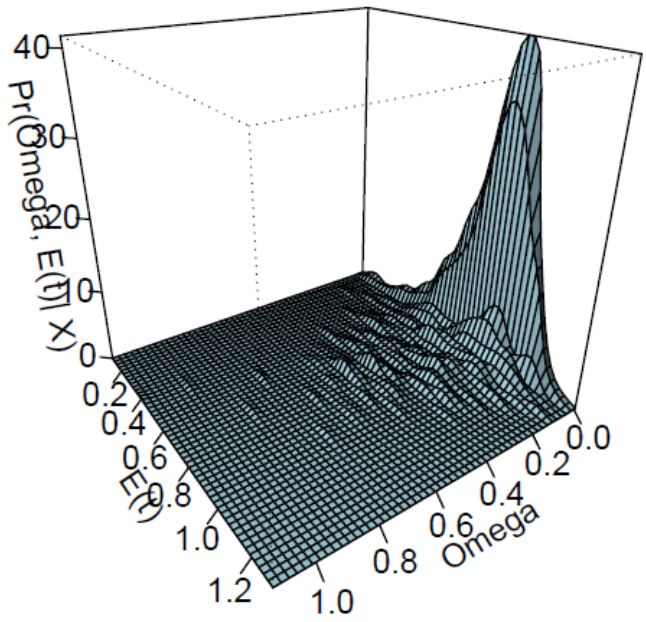


Figure 3.4. A. Prior and posterior probabilities of the number of divergence events (Ψ) for the rocky shore habitat. **B.** Joint posterior probability densities for E(τ) and Ω for the rocky shore habitat.

Discussion

Coates & Stallard (2013) and Jackson & O'Dea (2013) attempted to reconcile the Standard and Miocene models for the closure of the IoP, considering them as being in reality the two distinct faces of a same process, the progressive closure of the Central American Seaway and rise of the Panama Isthmus. In this case the Miocene model would correspond to the geological collision of those microplates that gave rise to the IoP, whereas the Standard model describes the subsequent paleogeographic changes during this progressive closure (Coates & Stallard 2013:801-802). However, they mostly reviewed the arguments in favor of the Standard model and argued that these arguments are not incompatible with the results of Montes *et al.* (2012a, 2012b).

Among their arguments, the question of the genetic divergence of the latest diverging marine species and their estimate for the timing of separation is considered by both Coates & Stallard (2013) and Jackson & O'Dea (2013) as supporting the Standard model. We caution against their conclusion, at it appears to be a recursive approach. Indeed, most of the molecular studies that include transisthmian species pairs, including Knowlton & Weigt (1998) and Lessios (2008), cited by both reviews as references to justify the molecular support to the Standard model, used the Pliocene timing of the closure to determine the divergence time of species pairs separated by this barrier. Therefore, the biological support of the two models has previously not been tested independently since the beginning of the controversy.

The threshold used for the Bayes Factor in our study strongly supports an interruption of the gene flow between Atlantic and Pacific marine brachyuran crabs during the Pliocene, as considered by the Standard model, and strongly rejects the hypothesis of isolation of these populations during the Miocene, as assumed by the Miocene model of closure of the Isthmus (Table 3.5). Moreover, the null hypothesis (no constraint) is rejected, minimizing the risk of a false positive. The current results strengthen the Standard model, and on the other side, give the first biological evidence against a final isolation of Pacific and Atlantic oceans during the Miocene.

The divergence calibration by msBayes also assumes a recent divergence time of the taxon pairs, and does not support the Miocene model. Indeed, the values recovered by this analysis are for the rocky shore species close to the timing of closure assumed by the Standard model (95% HPD: 1.5 - 3.5 mya), but are younger than the closure of the Isthmus for the

mangrove species (0 - 1.3 mya). Such timing probably reflects a subobtimal rate of molecular evolution used for the calibration (1.77% per million year, Papadopoulou *et al.* 2010), that might be too fast in regards with the intrinsic rates in our species (M. Hickerson, pers. communication).

Additionally, by considering the divergence of the less divergent couples as corresponding to the final closure (respectively 3.1 or 15 mya depending on the model), a strict molecular clock estimates a divergence time of the Grapsidae pairs around 30 mya and more than 40 mya (corresponding to the Middle Eocene) for the most divergent sister species *Pachygrapsus transversus* / *P. socius* under the assumption of the Miocene model. The divergence times based on this model are in relative contradiction with brachyuran fossil records and estimated age for the family based on phylogenetic reconstructions. Indeed, Brösing (2008) considers the family Grapsidae to appear at least in the Middle Eocene based on the oldest fossil record of grapsid crab. On the other side, Tsang *et al.* (2014) estimated the separation of *Metopograpsus* in regards with the other Grapsidae during the Paleocene around 64 mya (*Metopograpsus* was strongly supported to hold a basal position within the Grapsidae family according to Schubart 2011). An Eocene transisthmian divergence for the genera *Geograpsus, Grapsus* and *Pachygrapsus* appears thus strongly unlikely in regards with the age estimations of the whole family, even more so when considering that *Geograpsus lividus*, *Grapsus grapsus* and *Pachygrapsus transversus* were or are still considered as being the same species on both side of the Isthmus and having a transisthmian distribution (Guerao *et al.* 2003, Schubart *et al.* 2006, Schubart 2011). Only an extremely limited number of other studies investigated transisthmian divergence with calibration based on fossil records (Marko 2002, Marko & Moran 2009), mostly as a consequence of the absence of adequate fossil records for transisthmian taxa. These studies found their less divergent transisthmian couples to diverge at a timeframe congruent with the closure of the Central American Seaway under the Standard model.

For both Cox1 and 16S rRNA genes, the rates obtained under the Miocene model appear to be dramatically low in regards with arthropod rates calibrated elsewhere (respectively Papadopoulou *et al.* 2010 for the Cox1 and Klaus *et al.* 2010 for the 16S rRNA, with Cox1: 1.77% per my; 16S rRNA: 1.02 % per my). Indeed, compared to the rate priors used in the model testing, the rates for both genes resulting from the Miocene model without external rate are under 0.40% per my, thus lower than the 5% quantile bound of the 16S rRNA substitution rate prior (0.64% per my) and close to the hard lower bound for the Cox1 rate prior (0.205% per my). The 16S rRNA Miocene rates recovered are especially

problematic when compared to the 16S rRNA rate of Klaus *et al.* (2010). Indeed, this rate was built based on the fossil calibration of freshwater crabs, and relies on the accuracy of both fossil dating and phylogenetic reconstruction. The Miocene rates would be difficult to explain from a biological perspective in regards with the Southeast Asian freshwater crabs. A similarly low 16S rRNA rate is recovered in the transisthmian mangrove snails of the genus *Cerithideopsis* calibrated according to the Miocene model (Miura *et al.* 2012) compared to the rate of Cretan land snails *Albinaria*. A molecular rate of 1.0–1.2% per my was found in *Albinaria* for the 16S rRNA mitochondrial gene (Douris *et al.* 1998), based on the divergence between subspecies of *Albinaria hippolyti*, divergence resulting from a vicariant event by the fragmentation of the Crete during the Miocene (8-10 mya). Based on the dataset of Miura *et al.* (2012), we obtain an average rate of 0.68% per my (Standard model calibration) or 0.16% per my (Miocene model calibration), highlighting again the extremely low rates for trans-isthmian species compared to other relatives under the assumptions of the Miocene model (Table 3.4). Similar results are obtained for the Cox1 gene. Wilke (2003) recovered for this gene a substitution rate of 1.83% per my in the genus *Salenthydrobia* based on a calibration by the Messinian Salinity Crisis. This value fits within the 95% HPD interval obtained under the Standard model, but is clearly outside of the interval obtained based on the Miocene model (Table 3.4).

If we consider the Miocene rates as correct (implying a complete isolation of the Atlantic and Pacific oceans around 15 mya), it would also imply that most of the rates of molecular evolution cannot be trust, as they could range from one to five within the infraorder Brachyura or the class Gastropoda. Moreover, these result would call for re-analysis of most of the publications using already published transisthmian rates.

On the other side, the rates recovered based on the Standard model are relatively close from the calibration rates for both genes, and range within their 2.5–97.5% quantiles. A similar conclusion concerning the rate is obtained for the *Cerithideopsis* snails with the Standard model. The large differences between the rates under the Standard and Miocene models and their comparison with rates independent of the Isthmus closure tend to a strong congruence with the Standard model. In contrast, strikingly low rates are obtained with the Miocene model for different groups, and these low rates recovered in different taxa that cannot find biological explanation in regards with non-isthmian relatives.

In addition to our results based on molecular data, Jackson and O'Dea (2013) highlighted in their review other biological arguments strongly supporting a completion of the

Isthmus around 3 mya, arguments hardly explained when a final closure of the Isthmus during the Miocene is assumed.

Our results are backed by the timing of the Great American Biotic Interchange (GABI), i.e., the interchange of previously isolated North and South American biota at the completion of the isthmus, at approximately 3 mya. This remarkable event was first recognized by Wallace (1876: 153) based on the paleontological record of mammals and was elaborated with the increase of the fossils record, accuracy of sediment dating as well as additional evidence from extant taxa (Marshall *et al.* 1982, Stehli & Webb 1985, Marshall 1988, Leigh *et al.* 2014). The comprehension of this interchange is mostly based on mammals and flightless bird fossils, but recent studies contributed to refine this comprehension, especially the different waves of exchange (MacFadden 2006, Woodburne 2010, Leigh *et al.* 2014), but also the asynchrony between mammals and plant in this interchange linked to the earlier crossing of the seaway by plants allowed by long-distance dispersal (Cody *et al.* 2010). Pre-landbridge biotic interchange at the IoP became recently a controversial aspect with several studies arguing for an earlier IoP closure based on phylogenetic reconstructions recovering Miocene invasion of the South American continent, and considering the saltwater intolerant physiology of the studied species (amphibians, freshwater fish) or their low-dispersal abilities (palm trees) (Bermingham & Martin 1998, Pinto-Sánchez *et al.* 2012, Bacon *et al.* 2013, Elmer *et al.* 2013, Ornelas *et al.* 2014).

However, taking the extant biotic interchange between Oriental and Australian fauna as a comparable scenario (Coates & Stallard 2013, Jackson & O'Dea 2013), with multiple crossing of the Wallace Line by saltwater intolerant organisms (freshwater crabs, Klaus *et al.* 2013; amphibians, Li *et al.* 2013), these events are likely to be part of pre-landbridge faunal dynamics by oceanic dispersal across the Central American Seaway (Marshall *et al.* 1982, Jackson and O'Dea 2013, Leigh *et al.* 2014), as observed in fossil records (ground sloths, Marshall *et al.* 1979; procyonids, Koepfli *et al.* 2007; boine snakes, Head *et al.* 2012). Additionally, the argument of saltwater intolerant physiology to argue about the impossibility of long distance oceanic dispersal in amphibians has been dismissed by Measey *et al.* (2007) based on the phylogenetic relationships of São Tomé and Príncipe endemic amphibians, showing clear long distance dispersal in this taxa.

These interpretations are in contradiction with our molecular results supporting the last genetic exchanges between marine population on both sides of the Isthmus up the Pliocene. In addition, these studies are based on terrestrial species, and did not considered for their interpretations the impact of an early closure on marine species and the genetic

inconsistencies resulting from the Miocene model (with the exception of Bacon *et al.* 2013 mentioning the low mitochondrial rates resulting from this model, but could not bring any explanation to these rates). For terrestrial species, these early invasions most probably result from oceanic dispersal and do not require the presence of a completed isthmus during the Miocene, when the marine genetic data necessarily imply the presence of a long duration marine connection between both oceans, especially when overseas dispersals are well documented in organisms assumed to be saltwater intolerant (Vences *et al.* 2003). Moreover, oceanic dispersal has been for long time underestimated, but this process appears nowadays to be an important biogeographic mechanism as highlighted by the recent review of De Queiroz (2005). On the other side, the crossing of physical barriers like a completed isthmus by marine species, in the absence of breaches or straits, appears strongly improbable, but it is not totally impossible as highlighted by the marine snails *Cerithideopsis* which crossed twice the Isthmus about 750 000 and 72 000 years by bird hitchhiking (Miura *et al.* 2012). Moreover, the inconsistency of the extremely long temporal stasis of 10 my between the hypothesized Miocene completion of the IoP and the intensification of terrestrial interchange by land mammals (at around 3mya) remains difficult to explain and rather supports the Standard model of the IoP closure. Several studies mentioned the possible role of the climate on the GABI and the presence of savanna-like habitats during the Pliocene which might have facilitated mammal exchanges during this period (Webb 1991, Woodburne 2010, Bacon *et al.* 2013), and could contribute to partially explain the temporal stasis, but even in this condition, the timescale required by such temporal stasis remains difficult to explain and relatively improbable in regards to the increasing knowledge about dispersal and biotic exchanges. Moreover, the importance of the savanna corridor on the GABI is emphasized by Woodburne (2010) which reminds the large range of ecological diversity from the northward immigrant species, making the climate hypothesis fragile in regards to the timing of the GABI. The increased temporal congruence of the GABI with the Standard model, and the inconsistencies generated by the Miocene model, especially the problematic temporal stasis it necessarily requires, make more probable a final closure during the Pliocene rather than during the Miocene. Coates & Stallard (2013) and Jackson & O'Dea (2013) indicate strong geological and biological similarities between the isthmus region during the last steps of the closure and the Indonesian Volcanic Arc, and highlight that even narrow marine connections can nearly completely isolate terrestrial faunas, while maintaining gene flow between both coastlines. They consider that the Central American Seaway probably played a role equivalent to the present Wallace and Lyddeker lines in Southeast Asia. This interpretation fits perfectly with

the pattern preceding the complete closure of the isthmus and resulted in the Great American Biotic Interchange for terrestrial species and to the great American schism for marine species.

Another strong argument for the Standard model of IoP closure is based on the distribution and age of fossil benthic foraminiferans from the Panama Canal basin. Collins *et al.* (1996) report that in the Late Miocene Caribbean Chagres formation (8.6–5.3 mya) 55% of the benthic Foraminifera have a strong Pacific affinity, with the species composition indicating that this formation was deposited at depths of 200–500 m. Similar results are recovered by Duque-Caro (1990) for the Atrato Basin in Colombia, with the presence of a Late Miocene marine strait depth of 1000 m. Even if both studies agree for a temporary barrier between Pacific and Caribbean surface water around 8 mya, these two studies strongly argue for the presence of water exchanges between both oceans at least until 5 mya through narrow straits.

Paleooceanographic evidences supporting the Standard model and a closure of the Central American Seaway around 3 mya were deeply reviewed and explained by Jackson & O'Dea (2013). These arguments present similar issues in regards with the Miocene model: either clear evidences of surface water exchanges between the Pacific and Caribbean up to the Pliocene are recovered, or a large temporal delay is observed between an assumed closure at 15 mya and the effect of this closure between 3 to 5 mya. These evidences are based on variations in stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) and carbonate-sand fraction (Keigwin 1982, Haug & Tiedemann 1998, Bartoli *et al.* 2005), but also on morphological changes in bryozoans fossils linked to changes in environmental conditions (O'Dea *et al.* 2007).

Influence of the habitat on marine species' divergence time at the IoP

The early assumption concerning transisthmian sister-species was that most of the couples diverged during the final steps of closure of the Central American Seaway, i.e. the marine connection between the Atlantic and Pacific Oceans (Martin *et al.* 1992, Bermingham & Lessios 1993). As a result, the timing of this closure was used to calibrate the rate of molecular evolution of all species pairs. This assumption was dismissed by Knowlton & Weigt (1998), who noticed that mangrove species of the pistol shrimps *Alpheus* present smaller transisthmian genetic divergence than shallow-water species. They interpreted this result as the indication that mangroves were most likely the last habitats allowing genetic exchange between the two oceans, and considered that only the less divergent transisthmian couples could be assumed to have diverged at the final closure of the Isthmus. These results

also highlighted the probable overestimation of previously estimated rates of molecular evolution by these earlier studies, as their species most probably diverged earlier (up to 18 mya for the pistol shrimps, Knowlton & Weigt 1998). Since then, similar results have been observed in other organisms from the Central America, as *Alpheus* and *Synalpheus* shrimps (Morrison *et al.* 2004), anomurans (Hiller *et al.* 2006), barnacles (Wares 2001) or snails (Miura *et al.* 2010). The mangrove species, with low transisthmian divergence with respect to their close relatives, are assumed to have been the last ones affected by the rise of the isthmus and the resulting interruption of gene flow.

The divergence time estimation in msBayes also results in a recent divergence time for the investigated brachyuran taxon pairs and does not support the Miocene model, in line with the model testing approach. The values recovered by this analysis for the rocky shore species (95% HPD 3.5–1.5 mya) are congruent with the Standard model of IoP closure, but are older than the divergence estimate for the mangrove species (1.3–0.0 mya). Thus, our results support the assumption made by Knowlton & Weigt (1998) that mangrove dwelling species generally exhibit younger transisthmian divergence than species from other habitats for both genes used in this study (Tables 3.1 & 3.2). Compared to the values present in the review of Lessios (2008), the genus *Aratus* presents the second smallest transisthmian divergence observed in crustaceans for the Cox1 mitochondrial gene, but this divergence almost double between the most distant populations of the Isthmus (Brazil and Baja California respectively), close from the upper K2P values for species assumed to have diverged at the final closure. Despite having a higher transisthmian divergence than the genera *Aratus* and *Armases* for the Cox1, the genus *Goniopsis* also clusters with the species assumed by Lessios (2008) to have initiated their divergence at the final closure of the Isthmus. The higher K2P value observed in this species pair with respect to the two other mangrove species pairs possibly results from the sampled population in our dataset (Brazil), as no data was available for Caribbean sites in this study. However, *Goniopsis* exhibits a smaller genetic differentiation than *Aratus* for the 16S gene, highlighting differences between the individual gene histories in our set of species.
In contrast, all the rocky shore species present higher transisthmian divergence than mangrove species for both Cox1 and 16S genes, with values congruent with the hypothesis of a divergence preceding the final steps of the closure for both genes. This supposedly reflects the progressive shift of habitats from subtidal through mangrove to complete terrestrial during the closure of the IoP, as assumed by Knowlton & Weigt (1998). For the 16S rRNA gene, the investigated rocky shore crabs show a lower genetic transisthmian differentiation compared to other species that supposedly diverged before the final IoP closure (Lessios 2008). Possibly,

this reflects the intertidal habitat of the rocky shore crabs, contrary to crustaceans that inhabit the subtidal environment (Morrison *et al.* 2004).

The test for simultaneous divergence confirms the assumption of Knowlton & Weigt (1998) of sequential divergence of species pairs depending on the habitat, since then recovered by several studies (Marko 2002, Hickerson *et al.* 2006, Hurt *et al.* 2009, Miura *et al.* 2010). The exact number of simultaneous divergence events remains unclear when all species are considered, reflected by the close posterior probabilities of the different hypotheses (Fig. 3.1). When the test is performed for a single habitat, however, simultaneous divergence is supported within each habitat (Figs. 3.3A & 3.4A).

Usefulness of the Panama Isthmus for molecular clocking

The congruence between the transisthmian K2P values observed within the Decapoda between the species that diverged at the final closure of the isthmus suggests that most of the species of this order exhibit similar rates of molecular evolution for the mitochondrial cytochrome oxydase I and 16S ribosomal genes. For molecular clocks, this congruence between K2P values implies a reduced risk of error, when using these rates to calibrate divergence time of relatively distant taxa, and can be exemplified by the wide range of studies focused on Crustacean using the rates proposed by Knowlton & Weigt (1998) or Schubart *et al.* (1998).

For non-decapod crustacean species, the mangrove transisthmian geminate species of the barnacles *Euraphia*, represents the only species pair of the dataset by Wares (2001) separated at the final closure of the Isthmus. It presents an average K2P distance of 9.8%, clearly higher than any other crustacean species pair assumed to have been separated at the final closure. Based on the assumption that these transisthmian geminate species of barnacle diverged at the final closure of the Isthmus, it implies for the Cirripedia a higher rate of molecular evolution than in Decapods, meaning that the calibration of molecular clocks using rates based on Decapoda may overestimate the correct divergence time for other crustecean species. It reminds to be careful in the use of rates of molecular evolution from distant groups, which can led to incorrect conclusions concerning the timing of divergence, and to use rates based on close taxa, when available, or to use a wide range of rates to take account of the uncertainty.

Conclusions

Several studies contributed to better refine our knowledge of the closure of the Panama Isthmus from a marine perspective. Knowlton & Weigt (1998), by highlighting that all the transisthmian couples did not diverge at the final closure, obliged to reconsider which species should be used to calibrate genetic divergence and allowed a more precise calibration of genetic divergence. Montes *et al.* (2012a, 2012b) proposed a Miocene model for the closure of the Isthmus and generated a controversy that made necessary to reconsider the different arguments and their robustness towards the two proposed models. More recently, Coates & Stallard (2013) and Jackson & O'Dea (2013) discussed strongly the arguments toward the Standard model, but did not test these two models in regards with each other by an independent approach. Our study, using such approach, is the first one to confront the same transisthmian couples in regards with the two hypotheses and confirm the interruption of marine exchanges during the Pliocene. As a result, our study also provide new rates for crustaceans that will provide additional information concerning the range of these rates for the calibration of future studies. Precise timing of geological events is necessary for precise calibration of molecular rates, but on another side, biological data can provide precious hints to determine the respective probability of competing geological models.

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CHAPTER 4

Phylogenetic relationships of the American Sesarmidae (Decapoda, Brachyura, Thoracotremata): paraphyly, convergent evolution and rapid adaptations

Abstract

The family Sesarmidae corresponds to one of the most speciose family (> 250 species) of the Thoracotremata, one of the two major brachyuran clades. Within this family mostly found in the Indo-Pacific, the American Sesarmidae represent a small subset of 34 species in four different genera (*Aratus*, *Armases*, *Metopaulias* and *Sesarma*) inhabiting mangroves and freshwater habitats along the American and Caribbean coastlines and islands. Previous investigations focused only on two of these genera (*Sesarma* and *Metopaulias*) and highlighted conflicts between morphology and genetic data by the paraphyly of *Sesarma*. In this study, we analyzed the phylogenetic relationships of all the 34 American species using Bayesian inference and maximum likelihood methods based on fragments of two mitochondrial (Cox1 and 16S rRNA) and one nuclear (28S rRNA) genes. Our results reflect a rapid radiation in the American Sesarmidae that started 10.5 mya, when this family established on the American continent, rapid radiation reinforced by the high number of cryptic lineages revealed in this study. Our results highlight that the genus *Aratus* represents a deep branch in the genus *Armases* rather than its sister taxa, making *Armases* paraphyletic, in a similar situation to *Sesarma* and *Metopaulias*. Previously assumed relationships between the species *Aratus pisonii*, *Armases elegans* and *Armases rubripes* based on morphological data are not recovered with genetic data and morphological observation of the gonopod 1, and by consequent result from convergent evolution to arboreal life. Finally, divergence time estimates between transisthmian taxa are all congruent with divergences as consequence of the final closure of the Isthmus of Panama.

Introduction

The brachyuran family Sesarmidae is composed of at least 254 species in 29 genera. Within them, the American Sesarmidae correspond to a subset of 34 species in four genera, *Aratus* H. Milne Edwards, 1853, *Armases* Abele, 1992, *Metopaulias* Rathbun, 1856 and *Sesarma* Say, 1817 (De Grave *et al.* 2009). These four genera are all endemic to the Neotropics, with the exception of *Armases elegans* found along the Tropical East Atlantic coastline (Serène & Soh 1970, Abele 1992). They are distributed in tropical and subtropical environments, and salt marshes, mangroves, freshwater and terrestrial habitats (Abele 1992).

While the genera *Aratus*, *Metopaulias* and *Sesarma* were described during the nineteenth century, the genus *Armases* finds its origin within the complex and contradicting taxonomic debate about the genera and subgenera in the Sesarmidae (considered a subfamily until 2002, see Schubart *et al.* 2002). Among the taxonomic confusion was the question of the composition and validity of the genera *Holometopus* H. Milne Edwards, 1853, *Pachysoma* De Haan, 1833 and *Chiromantes* Gistel, 1848. The first two have the species *Grapsus (Pachysoma) haematocheir* De Haan, 1833 as type species. *Chiromantes* was proposed by Gistel (1848:x) as replacement for *Pachysoma* by being a pre-occupied name. Holthuis (1977) reminded the invalidity of the genus *Pachysoma*, highlighted the synonymy of *Chiromantes* and *Holometopus*, and concluded that only the genus *Chiromantes* was valid.

Apparently not aware of Holthuis (1977) and its conclusions, von Hagen (1978) discussed the position of *Sesarma* (*Holometopus*) *rectum* regarding the subgenera *Sesarma* sensu stricto and *Holometopus* H. Milne Edwards, 1853, placing *Sesarma rectum* in the subgenus *Sesarma* instead of *Holometopus*. *Holometopus* was described based on the absence of antero-lateral carapace teeth posterior to the external orbital angle (Rathbun 1897, 1918, Tesch 1917), but this character was questioned and considered as non-reliable (Tweedie 1940, Abele 1975, von Hagen 1978). However, after a proper reassignment of the species in the subgenera *Sesarma* and *Holometopus*, von Hagen (1978) noticed clear differences between the two subgenera based on the cheliped tuberculation, epistome, ambulatory legs, margin of the carapace and male pleon. At that point, *Sesarma* (*Holometopus*) included the type species of *Holometopus*, *S.* (*H.*) *haematocheir* as well as Neotropical, African and Indo-Pacific species. Von Hagen (1978) mentions that *Sesarma* (*Holometopus*) *haematocheir* is clearly morphologically distinct from the Neotropical species of this subgenus and raises the question, if they belong to the same genus. In his review of the genus *Sesarma*, Abele (1992)

agrees with von Hagen (1978) and separates the American species into two distinct genera, but excluding *Chiromantes haematocheir*, thus describing the new genus *Armases* Abele, 1992 with *Armases cinereum* (Bosc, 1802) as type-species. He also included the African *Sesarma elegans* within the genus *Armases*. All the other West African species of Sesarmidae are closer related to the Indo-Pacific species and placed in the subgenera *Chiromantes* and *Perisesarma* (see Manning & Holthuis 1981).

Almost simultaneously, Niem (1993:193) discussed the position of the American species *Metagrapsus rubripes* (Rathbun, 1897), compared to other American species from the genera *Sesarma* and *Aratus*, and considered a common origin for the species *Metagrapsus rubripes*, *Sesarma* (*Chiromantes*) *elegans* (Herklots, 1851) and *Aratus pisonii* (H. Milne Edwards, 1837), due to the presence of *coxae of last three walking legs with hairy process antero-dorsally* as a synapomorphy. As consequence, Niem (1993) transferred *Metasesarma rubripes* to the genus *Sesarma*, whereas the *status quo* of *Aratus* as a monotypic genus stayed due to the high number of derived characters with regards to *Sesarma*. The phylogenetic relationships within the genus *Armases* were examined by Niem (1996) based on morphological data, who considered it to be a subgenus, different from Abele (1992). Niem (1996) noticed the presence of three groups (*Aratus*-, *roberti*- and *cinereum*- groups) within *Armases* and *Aratus* based on shared apomorphies between the species. *Armases benedicti* was considered as having a basal position in *Armases* due to some characters (lateral margin of the carapace, shape of male pleon) being closer to *Sesarma* than to other species of *Armases* (see Von Hagen 1978, Niem 1996). The *Aratus*-group clusters *Aratus pisonii*, *Armases elegans* (these two being sister species, as suggested by Greene 1986) and *Sesarma* (*Armases*) *rubripes* mainly due to the characteristics of the coxal processes of the walking legs, as mentioned by Niem (1993). This grouping also suggested *Aratus pisonii* as part of the *Armases* lineage, despite Niem (1993) considering the monotypic genus *Aratus* to be valid with regards to its specific adaptation to tree-climbing. The *roberti*-group clusters *Armases roberti*, *A. angustum* and *A. americanum* as result of the distal insertion of the setae on the male gonopod, with *Armases roberti* and *A. angustum* assumed to be sister species based on the similar gonopod shape. The *cinereum*-group clusters *Armases cinereum*, *A. angustipes*, *A. miersii* and *A. ricordi* due to the specific epistome shape of these species, with proposed sister species relationships between *Armases cinereum* / *A. ricordi* and *A.angustipes* / *A. miersii*. Niem (1996) could not determine the position of the Pacific species *Armases occidentale*, *A. magdalense* and *A. gorei*, but kept them within *Armases*, due to the configuration of the carapace margins.

A more recent taxonomic change among these genera was carried out by Števčić (2005) in his massive reclassification of the Brachyura based on morphological data, by placing the genus *Aratus* in a distinct tribe than *Sesarma*, without considering the possible close relationships between these two genera with *Armases* and *Metopaulias*, as highlighted by Niem (1993, 1996). Recently, Thiercelin & Schubart (2014) highlighted that populations of *Aratus pisonii* on both sides of the Isthmus of Panama were genetically and morphologically distinct, and described consequently *Aratus pacificus*, sister species of *A. pisonii* in the Pacific ocean.

The first phylogenetic relationships of the Sesarmidae based on genetic data were presented for *Sesarma* (sensu stricto) by Schubart *et al.* (1998). The most preeminent result was the fast radiation with adaptation to terrestrial life observed in the Jamaican endemic species following the invasion of the island ca. 4.5 mya, and the clear rejection of the hypothesis by Serène & Soh (1970) that *Sesarma jarvisi* and *S. verleyi* should be included in the southeast Asian genus *Sesarmoides*. Moreover it confirmed the hypothesis of Hartnoll (1964) and Niem (1993) that *Metopaulias depressus* derived from within the Jamaican endemic *Sesarma*. Additionally, the two species of *Armases* included in the phylogeny clustered outside of the genus *Sesarma*. Schubart *et al.* (2000, 2002) reclassified some of the genera that were previously considered to belong to the Sesarminae. In America, this affected the placement of *Cyclograpsus* and *Chasmagnathus* (today *Neohelice* according to Sakai *et al.* 2006), which were transferred to the Varuninae. At the same time, they gave full family status to the former grapsid subfamilies, thus for the first time considering the Sesarmidae as a family. Schubart *et al.* (2002, 2006), in their phylogenies of the grapsoid crabs, recovered *Chiromantes haematocheir* to be in basal position compared to the American species, confirming the observations of von Hagen (1978). Fratini *et al.* (2005), studying the convergent evolution in tree-climbing mangrove crabs, observed that *Armases elegans* is genetically closer to *Armases cinereum* than to *Aratus pisonii*, which is opposed to the results of Niem (1993, 1996) and Greene (1986) and considered that the two genera should remain distinct.

In the present study, we focus on the American Sesarmidae to determine with genetic tools the phylogenetic relationships among the four genera currently recognized, *Aratus*, *Armases*, *Metopaulias* and *Sesarma*, and for the first time among the species of the genus *Armases*. These relationships will help to determine 1) the processes that shaped this progressive radiation, 2) the impact of the major geological events that effected the American marine biota as the rise of the Isthmus of Panama (IoP, Lessios, 2008) and 3) have a better

comprehension of the biogeographic distribution patterns according to the phylogenetic relationships.

Material and Methods

Specimen collection

67 specimens were collected manually and preserved in 70-95% ethanol, or loaned from museum collections, from all the known American Sesarmidae. It corresponds to a total of 18 species in the genus *Sesarma*, 13 species in the genus *Armases*, two species of the genus *Aratus* and the three populations of the monotypic genus *Metopaulias* were included in the analyses. For some species, specimens from different parts of the distribution range were used. The southeastern Asian species *Chiromantes haematocheir* was used as outgroup in the analyses as it appeared in basal position and distinct from the American Sesarmidae (Schubart *et al.* 2002, 2006). Details on the specimens, including locality, museum number when available, and GenBank accession numbers on can be found in Table S4.1 in Supplementary Material.

For the species *Aratus pisonii*, *Armases americanum*, *A. angustipes*, *A. benedicti*, *A. elegans*, *A. miersii*, *A. occidentale*, *A. roberti* and *A. nr. rubripes*, the left first gonopod of each specimen was analysed in endpiece lateral view on a Zeiss DSM 950 scanning electron microscope (SEM).

DNA extraction, amplification and sequencing

For a majority of species from the genera *Sesarma* and *Metopaulias*, we used the same specimens that were used by Schubart *et al.* (1998), adding the newly described *S. meridies* Schubart & Koller, 2005 and *S. abeokuta* Schubart & Santl, 2014, and adding additional populations. As this study already published mitochondrial (16S subunit rRNA and cytochrome oxydase I) sequences, we used the 16S rRNA sequences available on GenBank. Since the cytochrome oxydase I (Cox1) sequences published in this earlier study were only 551bp long, we acquired a longer fragment for the specimens, if possible. For the specimens for which re-amplification was not successful, the already published Cox1 sequences from GenBank were used. Nuclear sequences from these specimens were newly acquired.

Genomic DNA was extracted from muscular leg tissue using the Puregene kit (Qiagen), and PCR were used to amplify two mitochondrial genes (Cox1 and 16S rRNA) and one nuclear gene (28S subunit rRNA). For the mitochondrial Cox1, a fragment was amplified with the specific primers for Thoracotremata COL8 and COH16 (Schubart 2009) with the following profile: initial step 4 min at 94°C, 40 cycles with 45s at 95°C – 45s at 50°C – 75s at 72°C for denaturing, annealing and extension respectively, and final extension step 5 min at 72°C. For the mitochondrial 16S subunit rRNA gene, a fragment was amplified with the primers 16L2 and 16HLeu (Schubart 2009) with the following profile: initial step 4 min at 94°C, 40 cycles with 45s at 95°C – 60s at 48°C – 60s at 72°C for denaturing, annealing and extension respectively, and final extension step 5 min at 72°C. For the nuclear 28S subunit rRNA gene, a fragment was amplified using the primers 28D2L (5'-TACCGTGAGGGAAAGYTGAAA-3') and 28H2 (5'-CGATTTGCACGTCAGAATTGCT-3') (Thiercelin & Schubart 2014) with the following profile: initial step 3 min at 94°C, 40 cycles with 45s at 97°C – 45s at 48°C – 75s at 72°C for denaturing, annealing and extension respectively, and a final extension step of 5 min at 72°C. For the species where multiple amplification bands were obtained, the fragment was amplified with a touchdown PCR to increase the specificity under the following profile: initial step 3 min at 94°C, 20 cycles with 45s at 97°C – 45s at 70 to 60°C (-0.5°C per cycle) – 75s at 72°C for denaturing, annealing and extension, 20 cycles with 45s 97°C – 45s 60°C– 75s 72°C for denaturing, annealing and extension respectively and final extension step 5 min at 72°C. PCR products were outsourced for sequencing to LGC Genomics Gmbh or Macrogen, or sequenced on a Abi Prism 3100.

Sequence alignment and phylogenetic analyses

The sequences were proofread with Chromas 2.23 and aligned with ClustalW (Thompson *et al.* 1994) implemented in BioEdit 7.0.5 (Hall 1999). Missing information was filled by question marks. After alignment, a concatenated dataset of 2530bp corresponding to 958bp of the COI gene, 628bp of the 16S gene and 944bp of the 28S gene was obtained. The absence of stop-codons in the COI sequences was assessed with Artemis 14 (Rutherford *et al.* 2000) using Invertebrate mitochondrial code (NCBI Table 5). New sequences will submitted to EMBL and are available from GenBank.

The best evolutionary models of DNA substitution for the different genes were determined using MrModelTest 2.3 (Nylander 2004) using the MrMTGUI interface (Nuin, 2005) respectively, and with jModelTest 2.1.4 (Darriba *et al.* 2012), depending of the method

used (MrModelTest for MrBayes, jModelTest for BEAST and raxMLGUI). The best model was selected in both case based on the Akaike information criterion (Posada & Buckley 2004). We analyzed and reconstructed the phylogenetic histories using both Bayesian Inference (BI) and Maximum Likelihood (ML). The analyses were conducted first for each of the genes, and later used a combined dataset. The ML analyses were conducted with raxMLGUI1.3 (Silvestro & Michalak 2012), and the node robustness was assessed by 1000 rapid bootstraps. The BI analyses were conducted first with MrBayes 3.2 (Ronquist *et al.* 2012) using standard parameters, 4 Markov Chain Monte Carlo (MCMC, 3 cold chains, one heated chain) chains, with 3 000 000 generations sampled each 1000 generation and a burn-in of 25% was applied to discard the non-stationary phase. For the tree using concatenated sequences, we created an input block for MrBayes which defines the different gene components of the alignment and assigned the corresponding evolutionary model to each gene. Individual gene trees were also calculated with the same methods and are present in Supplementary Material.

A second Bayesian approach with BEAST 1.8.0 (Drummond *et al.* 2012) on the CIPRES Science Gateway (Miller *et al.* 2010), and used the concatenated dataset to determine the timing of divergence within the American Sesarmidae. The American species were defined as monophyletic under tree prior, resulting in *Chiromantes haematocheir* as outgroup. Each gene had independent site and uncorrelated lognormal relaxed clock models, with a Yule process tree prior applied to the dataset. Independent runs of 100 millions generations and sampled each 10000 generations. The log files resulting from these runs were analyzed with Tracer 1.5 (http://beast.bio.ed.ac.uk/Tracer) to assess a proper Effective Sample Size (ESS at least superior to 200), which was reached with 8 independant runs, and combined with a 10% burn-in of each run in LogCombiner 1.8.0 (http://beast.bio.ed.ac.uk/LogCombiner), resulting in a total of 72 000 trees. We used the separation between the Atlantic and Pacific lineages of *Aratus*, resulting of the final closure of the Central American Seaway and the rise of the Isthmus of Panama, as calibration point for the tree.

The final closure of the Isthmus of Panama has been traditionally assumed to range between 2.8 to 3.5 mya (Standard model, Coates *et al.* 1992, 2005, Coates & Obando 1996), with a mean value of *circa* 3.1 mya. A controversy concerning the timing of closure rose recently with the publication of the Miocene model of closure (Montes *et al.* 2012a, 2012b) that supports a final closure of the Isthmus during the Miocene, approximately 15 mya. As this controversy is still currently open, and as no molecular data based on marine species is

currently available considering this new scenario, we decided in the present study to follow the Standard model of closure. It implies for the analyses a mean closure time of the Isthmus of Panama at 3.1 mya with a standard deviation of 0.153 corresponding to the values of 2.8 and 3.4 mya for the 2.5 and 97.5 quantiles respectively, based on the range proposed by Coates *et al.* (1992, 2005). In the genus *Aratus*, the lineage called '*strange*' in this study was excluded of the calibration set, as this lineage found in the Pacific Ocean appeared to have diverged from the other lineages within this genus prior to the final closure of the isthmus. In addition to this calibration node, as BEAST requires prior information for the rates of molecular evolution, we decided to use relaxed rates from literature data independent from a calibration by the Isthmus of Panama closure to limit the impact of the controversy concerning the closure timing.

For the Cox1 gene, we used a lognormal distribution of the substitution rate (= rate per lineage, or pairwise divergence rate / 2). This distribution allows to have a near-normal distribution for its central part, but also allows to consider higher rates, often associated with recent speciation or divergence events (Craft *et al.* 2008, Papadopoulou *et al.* 2010, Crandall *et al.* 2012). The median value was fixed to 1.77% per my based on the Insecta rate proposed by Papadopoulou *et al.* (2010) in their revision of the insect mitochondrial molecular clock using a calibration based on the mid-Aegean trench biogeographic barrier. The upper bound of the 2.5–97.5% quantile was fixed to 11.89% per my corresponding to the upper bound recovered by Crandall *et al.* (2012) in the mantis shrimp *Haptosquilla pulchella*, based on Sunda Shelf populations following the Last Glacial Maximum. A hard upper bound was fixed at 20% per my, rate recovered in the Hawaiian shrimps *Halocaridina* that speciated 100 kya (Craft *et al.* 2008). A hard lower bound was fixed to 0.205% per my, corresponding to the fossil calibrated rate in the *Limnoporus* water striders (Sperling *et al.* 1997, and see Table S1 of Papadopoulou *et al.* 2010). We excluded the widely used arthropod rate of Brower (1994) as this rate is built based on 1) distinct taxa including transisthmian couples of *Alpheus* shrimps, and 2) protein-coding and ribosomal markers that might have extremely distinct rates, as noticed by Papadopoulou *et al.* (2010).

For the mitochondrial 16S rRNA gene, we used as prior the lineage rate resulting from Klaus *et al.* (2010) under a normal distribution (mean: 1.02% per my ; 2.5–97.5% quantile: 0.64–1.42% per my). This rate is based on a southeast Asian freshwater crabs phylogeny calibrated by fossil records. For the 28S nuclear gene, no crustacean specific rate was available, but Klaus *et al.* (2010) indicated that the nuclear gene histone H3 rate was approximately 5-6 times slower than the 16S mitochondrial rate. Based on it, we decided

arbitrary to use as prior a strongly relaxed rate with a central value 10 times smaller than the 16S rate (0.102% per my ranging from 0 to 0.6% per my). This value also allows to include the 28S lineage rate recovered of Papadopoulou *et al.* (2010) of 0.06% per my.

Results

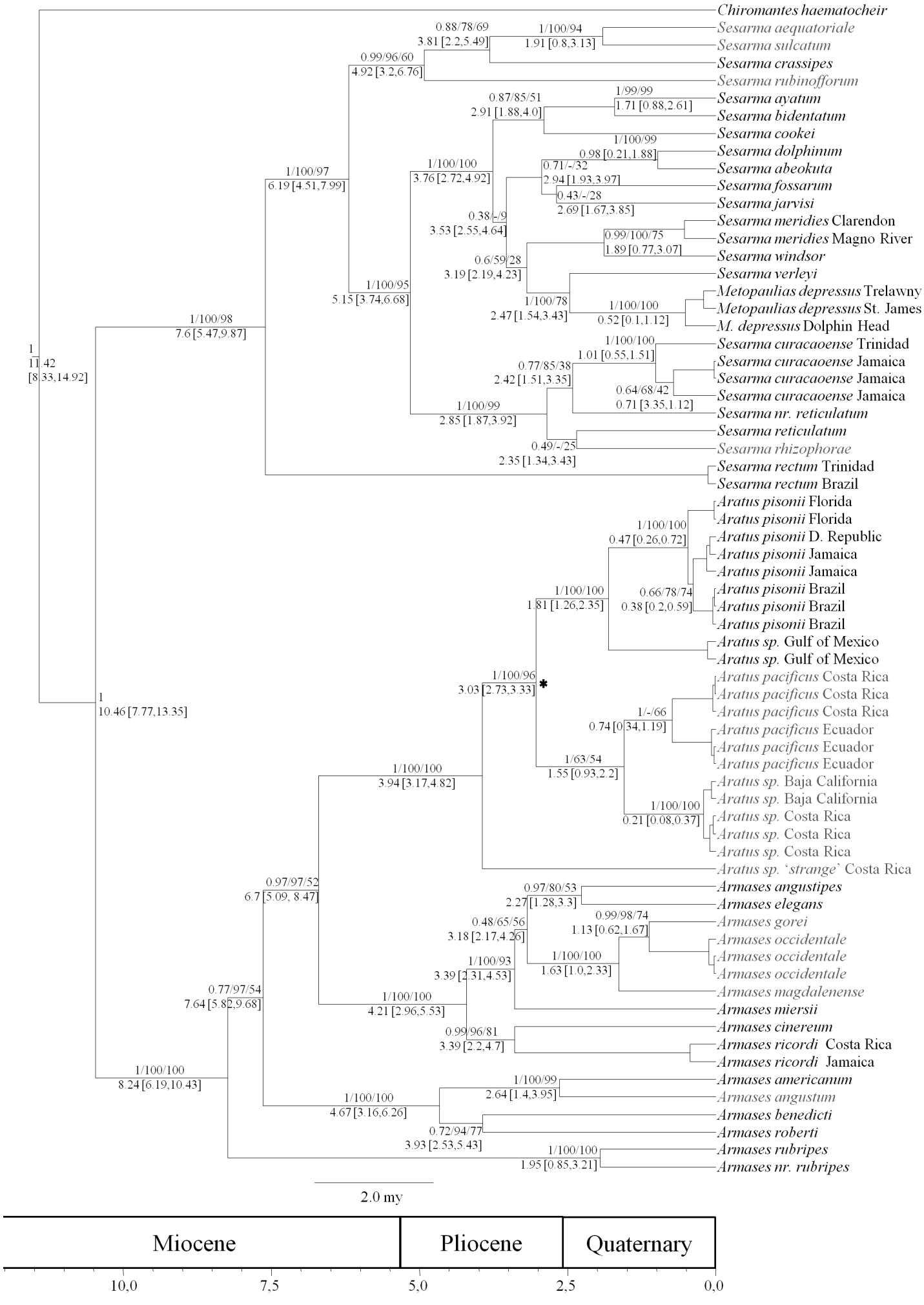
Nucleotide model selection

MrModelTest resulted in the General Time-Reversible model (GTR, Tavaré 1986) with gamma distribution shape parameter (G) and invariant sites (I) to be the best evolution model for the COI (G = 1.1871; I = 0.5839), the 16S (G = 0.5342; I = 0.5278) and the 28S (G = 1.0281; I = 0.7872). jModelTest also suggested the General Time-Reversible model with gamma distribution shape parameter (G) and invariant sites (I) to be the best evolution model for the COI (G = 1.1930; I = 0.5830), for the 16S (G = 0.5400; I = 0.5430) and for the 28S gene (G = 1.1490; I = 0.7940).

Phylogenetic relationships

The phylogenic tree obtained from the two Bayesian Inference (BI, MrBayes and BEAST) and from the Maximum Likelihood (ML) approaches are nearly identical and only minor differences in the topology of nodes distinguish them. The topology in Figure 4.1 corresponds to the one obtained by BEAST with molecular clock calibration; the two additional trees can be found in Supplementary Material (Figs. S4.1 & S4.2) for comparison. Clades supported by ≥50% bootstrap replicates (bs) and ≥0.90 (or 90%) Bayesian posterior probability (pp with ppm for MrBayes and ppb for BEAST) are considered as significant and clades with ≥70% bs and ≥0.95 (95%) pp are considered as robust.

Figure 4.1 (next page). Calibrated phylogeny of American Sesarmidae inferred from a multigene (mitochondrial Cox1 and 16S rRNA, nuclear 28S rRNA) Bayesian analysis recovered with BEAST. Upper values at nodes indicate BEAST Bayesian posterior probability / MrBayes Bayesian posterior probability (in percent) / RAxML likelihood bootstrap support of this node. Lower values at nodes indicate mean divergence time in my and associated 95% HPD. Black: Atlantic Ocean, grey: Pacific Ocean. Star indicates the calibration point.


Chiromantes haematocheir
Sesarma aequatoriale
Sesarma sulcatum
Sesarma crassipes
Sesarma rubinofforum
Sesarma ayatum
Sesarma bidentatum
Sesarma cookei
Sesarma dolphinum
Sesarma abeokuta
Sesarma fossarum
Sesarma jarvisi
Sesarma meridies Clarendon
Sesarma meridies Magno River
Sesarma windsor
Sesarma verleyi
Metopaulias depressus Trelawny
Metopaulias depressus St. James
M. depressus Dolphin Head
Sesarma curacaoense Trinidad
Sesarma curacaoense Jamaica
Sesarma curacaoense Jamaica
Sesarma curacaoense Jamaica
Sesarma nr. reticulatum
Sesarma reticulatum
Sesarma rhizophorae
Sesarma rectum Trinidad
Sesarma rectum Brazil
Aratus pisonii Florida
Aratus pisonii Florida
Aratus pisonii D. Republic
Aratus pisonii Jamaica
Aratus pisonii Jamaica
Aratus pisonii Brazil
Aratus pisonii Brazil
Aratus pisonii Brazil
Aratus sp. Gulf of Mexico
Aratus sp. Gulf of Mexico
Aratus pacificus Costa Rica
Aratus pacificus Costa Rica
Aratus pacificus Costa Rica
Aratus pacificus Ecuador
Aratus pacificus Ecuador
Aratus pacificus Ecuador
Aratus sp. Baja California
Aratus sp. Baja California
Aratus sp. Costa Rica
Aratus sp. Costa Rica
Aratus sp. Costa Rica
Aratus sp. 'strange' Costa Rica
Armases angustipes
Armases elegans
Armases gorei
Armases occidentale
Armases occidentale
Armases occidentale
Armases magdalenense
Armases miersii
Armases cinereum
Armases ricordi Costa Rica
Armases ricordi Jamaica
Armases americanum
Armases angustum
Armases benedicti
Armases roberti
Armases rubripes
Armases nr. rubripes
1 11.42 [8.33,14.92]
1 10.46 [7.77,13.35]
1/100/98 7.6 [5.47,9.87]
1/100/97 6.19 [4.51,7.99]
0.99/96/60 4.92 [3.2,6.76]
0.88/78/69 3.81 [2.2,5.49]
1/100/94 1.91 [0.8,3.13]
1/100/95 5.15 [3.74,6.68]
1/100/100 3.76 [2.72,4.92]
0.87/85/51 2.91 [1.88,4.0]
1/99/99 1.71 [0.88,2.61]
1/100/99 0.98 [0.21,1.88]
0.71/-/32 2.94 [1.93,3.97]
0.43/-/28 2.69 [1.67,3.85]
0.38/-/9 3.53 [2.55,4.64]
0.99/100/75 1.89 [0.77,3.07]
0.6/59/28 3.19 [2.19,4.23]
1/100/78 2.47 [1.54,3.43]
1/100/100 0.52 [0.1,1.12]
1/100/100 1.01 [0.55,1.51]
0.77/85/38 2.42 [1.51,3.35]
0.64/68/42 0.71 [3.35,1.12]
1/100/99 2.85 [1.87,3.92]
0.49/-/25 2.35 [1.34,3.43]
1/100/100 0.47 [0.26,0.72]
1/100/100 1.81 [1.26,2.35]
0.66/78/74 0.38 [0.2,0.59]
1/100/96 3.03 [2.73,3.33] *
1/-/66 0.74 [0.34,1.19]
1/63/54 1.55 [0.93,2.2]
1/100/100 3.94 [3.17,4.82]
1/100/100 0.21 [0.08,0.37]
0.97/97/52 6.7 [5.09, 8.47]
0.97/80/53 2.27 [1.28,3.3]
0.48/65/56 3.18 [2.17,4.26]
0.99/98/74 1.13 [0.62,1.67]
1/100/100 1.63 [1.0,2.33]
1/100/93 3.39 [2.31,4.53]
0.77/97/54 7.64 [5.82,9.68]
1/100/100 4.21 [2.96,5.53]
0.99/96/81 3.39 [2.2,4.7]
1/100/100 8.24 [6.19,10.43]
1/100/99 2.64 [1.4,3.95]
1/100/100 4.67 [3.16,6.26]
0.72/94/77 3.93 [2.53,5.43]
1/100/100 1.95 [0.85,3.21]
2.0 my
Miocene
Pliocene
Quaternary
10,0
7,5
5,0
2,5
0,0

Within the American Sesarmidae, two strongly supported clades are observed. The first one corresponds to the genera *Sesarma* and *Metopaulias* (ppb = 1.0, ppm = 100%, bs = 100%; Fig. 4.1), as highlighted by Schubart *et al.* (1998), from whom a part of the dataset was re-used in the present study). The second clade clusters the genera *Armases* and *Aratus* (ppb = 1.0, ppm = 100%, bs = 100%). Similar to the position of *Metopaulias* within *Sesarma*, the genus *Aratus* is located within the genus *Armases* (ppb = 0.97, ppm = 97%, bs = 52%), implying *Armases* as well as *Sesarma* to be paraphyletic.

The species groups identified within *Sesarma* by Schubart *et al.* (1998) are also recovered in the present study (Fig. 4.1). *Sesarma rectum* is strongly supported as basal species of the genus *Sesarma* (ppb = 1.0, ppm = 100%, bs = 100%). The *sulcatum*-group (*Sesarma aequatoriale*, *S. crassipes*, *S. rubinofforum* and *S. sulcatum*) is strongly supported (ppb = 0.99, ppm = 96%, bs = 60%) and shows that the Pacific *Sesarma aequatoriale* and *S. sulcatum* are sister species (ppb = 1.0, ppm = 100%, bs = 94%). With the exception of *Sesarma crassipes* found in the Caribbean Sea, this group is only composed of species from the Pacific coast of the American continent. The *sulcatum*-group is the sister clade to the ensemble formed by the *reticulatum*-group and the Jamaican endemic species. The *reticulatum*-group is strongly supported (ppb = 1.0, ppm = 100%, bs = 99%) and its link with the Jamaican endemic clade is significant (ppb = 1.0, ppm = 100%, bs = 95%) but the relationships inside this clade are poorly supported and contradictory between the analyses, letting unresolved the position of the Pacific endemic *Sesarma rhizophorae*. A similar pattern is observed for the Jamaican endemic group, strongly supported (ppb = 1.0, ppm = 100%, bs = 100%), but where most species relationships remain unresolved, excepted the strongly supported sister species relationship for the couples *Sesarma ayatum* / *S. bidentatum* (ppb = 1.0, ppm = 99%, bs = 99%), *S. dolphinum* / *S. abeokuta* (ppb = 1.0, ppm = 100%, bs = 99%), *S. verleyi* / *Metopaulias depressus* (ppb = 1.0, ppm = 100%, bs = 78%) and *S. meridies* / *S. windsor* (ppb = 0.99, ppm = 100%, bs = 75%).

Within the highly-supported *Aratus-Armases* clade (ppb = 1.0, ppm = 100%, bs = 100%), four groups can be recognized, using the names given by Niem (1996): the *Armases rubripes*-complex, the *roberti*-group, the *cinereum*-group and the *Aratus*-complex (Fig. 4.1). The most basal split corresponds to *Armases rubripes* and an undescribed cryptic lineage referred as *Armases nr. rubripes* in the present study, implying *Armases rubripes* could be a species complex. The second group, referred as *roberti*-group, see its grouping with the two last clades being less robust (ppb = 0.77, ppm = 97%, bs = 54%), but is itself extremely strongly supported (ppb = 1.0, ppm = 100%, bs = 100%). This group presents two sister

species relationships, *Armases americanum* / *A. angustum* (ppb = 1.0, ppm = 100%, bs = 99%) and *A. roberti* / *A. benedicti* (ppb = 0.72, ppm = 94%, bs = 77%). The *cinereum*-group appears to be the sister clade to the genus *Aratus* (ppb = 0.97, ppm = 97%, bs = 52%), with most of the relationships of its species well-supported. Strong sister species relationships can be noticed: *Armases cinereum* / *A. ricordi* (ppb = 0.99, ppm = 96%, bs = 81%), *A. occidentale* / *A. gorei* (ppb = 0.99, ppm = 98%, bs = 74%), and between *Armases angustipes* and the African species *A. elegans* (ppb = 0.97, ppm = 80%, bs = 53%). The last clade corresponds to the genus *Aratus*, fully supported (ppb = 1.0, ppm = 100%, bs = 100%), composed of five well supported branches corresponding to the two currently described species and to three cryptic lineages. *Aratus pisonii* and a first undescribed lineage from the Gulf of Mexico are found in the Atlantic (ppb = 1.0, ppm = 100%, bs = 100%). *Aratus pacificus* and the two last cryptic lineages, referred as *Aratus sp.* 'Baja' and *Aratus sp.* 'strange', are found in the Pacific. *Aratus sp.* 'strange' corresponds to a basal lineage in *Aratus*, and a sister-taxa relationship between *A. pacificus* and *Aratus sp.* 'Baja' (ppb = 1.0, ppm = 63%, bs = 54%).

Divergence time estimates

The origin of the American Sesarmidae is herewith dated to the Late Miocene (~10.46 mya) with the separation between the *Aratus - Armases* and *Sesarma - Metopaulias* clades. At the end of the Miocene (5.33 mya), all the major groups in both clades have evolved (Fig. 4.1). The origin of the Jamaican endemic species (including *Metopaulias depressus*), estimated first by Schubart *et al.* (1998), is here dated at 5.15 mya (95% HPD: 3.74–6.68 mya) and the first speciation event within this group occurs at 3.76 Mya (95% HPD: 2.72–4.92 mya). The genus *Aratus* separated from the other species of *Armases* at 6.7 mya (95% HPD: 5.09–8.47 mya). Almost all the divergence events leading to extant species happened in the range from 1.5 to 4.5 mya, with a similar pattern in the two major clades of the study. The most recent speciation event (separation *Armases gorei* / *A. occidentale*) is aged at 1.13 mya (95% HPD: 0.62–1.67 mya), even if the status of more recent lineages within different species (as *Sesarma curacaoense*) has to be determined.

Four robust separations (and one unclear) can be associated with the closure of the Central American Seaway and the rise of the Isthmus of Panama 3.1 mya (Fig. 4.1). The separation between *Sesarma crassipes* (Atlantic) and the other closely related Pacific species is dated to 3.81 mya, before the complete closure of the Isthmus. The separation between the Atlantic and Pacific lineages in the mangrove genus *Aratus* was used as calibration point in

the analyses, corresponding to the end of marine water exchanges between the Atlantic and Pacific oceans and is thus defined at 3.03 mya. The earlier Pacific cryptic lineage in the genus *Aratus* separated from the other ones at 3.93 mya (95% HPD: 3.17–4.82 mya), before the closure of the Isthmus. Two speciation events in *Aratus*, one in each ocean, are post-Isthmus closure by being dated respectively at 1.81 mya (Atlantic) and 1.55 mya (Pacific). The separation between the Atlantic couple *A. angustipes* / *A. elegans* and the Pacific species *A. gorei / A. magdalenense / A. occidentale* is estimated at 3.18 mya, congruent with the timing of the Isthmus final closure. One last trans-isthmian speciation event is dated as more recent than the final closure of the Isthmus, at 2.64 mya for the couple *Armases americanum* / *A. angustum*, but the 95% HPD ranges from 1.4 to 3.95 mya, thus being congruent with the timing of the closure of the Isthmus. The last but unclear transisthmian divergence corresponds to *S. rhizophorae* (Pacific) compared to the other species of the *reticulatum*-group. The basal node of this group is fully supported by all the methods, but not the relationships within. The divergence time of this node is 2.85 mya (95% HPD: 1.87–3.92 mya), value congruent with a divergence in the final step of the Isthmus closure, if *S. rhizophorae* is the basal species of this group.

As *Armases elegans* corresponds to the single African species of the study, the sister species relationship between *Armases angustipes* and *A. elegans* corresponds to an amphi-Atlantic speciation event. This event is estimated at 2.27 mya (95% HPD: 1.28–3.3 mya), posterior (or concomitant based on the 95% HPD) to the closure of the Isthmus of Panama (Fig. 4.1).

Discussion

Until recently, the morphological approach has been the traditional and only approach to resolve the relationships within the family Sesarmidae. However, it resulted in disagreements between authors, as exemplified by the splittings and lumpings in the southeast Asian Sesarmidae (see Serène & Soh 1970, Holthuis 1977).

Schubart *et al.* (1998) was the first genetic study determined to resolved relationships at the species level, and the only one focused on the American Sesarmidae before the present one, with a special emphasis on the genus *Sesarma* and the origin of the Jamaican endemic

species. It identified these species as resulting from a single adaptive radiation by a marine ancestor. However, it did not go into depth concerning the validity of the genus *Armases* that was separated from *Sesarma* by Abele (1992), and the position of *Aratus pisonii* with regards to these two genera. Other recent genetics studies considering Sesarmidae focused either on the genus level (Schubart *et al.* 2002, 2006, Fratini *et al.* 2005) or on Indo-Pacific species (Naderloo & Schubart 2009, 2010, Ragionieri *et al.* 2009).

Following the results highlighted by Schubart *et al.* (1998) and the groupings hypothesized by Niem (1993, 1996), the phylogenetic relationships recovered in this study considered all the currently known species in the four genera endemic from the American continent, in addition to *Armases elegans* from West Africa. The present study thus corresponds to the first attempt to clarify all relationships between the different American genera of the Sesarmidae using molecular phylogenetic methods. Both Bayesian and likelihood approaches strongly support two clades within the American Sesarmidae (Fig. 4.1). The groups observed by Schubart *et al.* (1998) in the genus *Sesarma* are recovered here, linked to the partial re-use of their dataset. On the other hand, all the species from the genus *Armases* genetically group together, in congruence with the morphological groupings proposed by Abele (1992). Nevertheless, the presence of the genus *Aratus* within *Armases* makes the latter genus paraphyletic, in an equivalent situation to *Metopaulias* and *Sesarma* (see Schubart *et al.* 1998, and assumed by Hartnoll 1964 and Niem 1993 based on morphological data). Similar examples from literature are recovered between the shrimp genera *Exhippolysmata* and *Lysmata* (Baeza *et al.* 2009, Baeza 2010, Baeza & Fuentes 2013, De Grave *et al.* 2014), *Cryphiops* and *Macrobrachium* (Pileggi & Mantelatto 2010), *Palaemon* (De Grave *et al.* 2013) or in the crab genus *Portunus* (Mantelatto *et al.* 2009).

Species relationships in the American Sesarmidae

1) *Sesarma - Metopaulias*

Except the common ancestry of the Jamaican endemic *Sesarma* assumed since Hartnoll (1964), the relationships within the restricted genus after Serène & Soh (1970) have never been considered by studies based on morphological data. The first indications concerning the genetic relationships in this genus came from the phylogenetic reconstruction by Schubart *et al.* (1998). Compared to this study, few differences are obtained in the present one. *Sesarma rectum* is recovered and strongly supported as the most basal species in the

genus and no significant differentiation could be observed between two sites distant of ~4700 km, covering 70% of the distribution area of the species (Fig. 4.1). With regards to the previous phylogeny of *Sesarma*, similar relationships in the *sulcatum*-group are reported here (and strongly supported by both BI and ML), with the Pacific species *S. aequatoriale* and *S. sulcatum* assumed to be sister species, with their ancestral lineage diverging from *S. crassipes* as result of the Isthmus closure. In this group, *S. rubinofforum* is recovered at a basal position (Fig. 4.1). Abele (1973, 1992) considered *S. rubinofforum* as being the Pacific analogue to *S. rectum* due to the morphological and ecological similarities of the two species. A sister species relationship of these two species can be refuted according to our data. It implies that these similarities might either be convergence to equivalent trophic niches on both sides of the American continent, or either are plesiomorphic (i.e. inherited from a shared ancestor).

In the *reticulatum*-group, the relationships between the species remain unclear, contradicted and unsupported by the different analyses (Fig. 4.1). The BI with BEAST and ML presents a dichotomous relationship between the couples *S. curacaoense* - *S. nr. reticulatum* and *S. rhizophorae* - *S. reticulatum*. It rejects the assumed sister species relationship between *S. reticulatum* and the cryptic species *S. nr. reticulatum*, and instead suggests a transisthmian relationship between *S. rhizophorae* and *S. reticulatum*. However, the sister species relationships between *S. rhizophorae* - *S. reticulatum* is not supported by these two analyses. The BI with MrBayes does not solve this node and let the possibility of an initial separation of the Pacific species *S. rhizophorae* and the speciation of the Atlantic species *S. curacaoense*, *S. reticulatum* and *S. nr. reticulatum* after the closure of the Isthmus. In both cases, these relationships might be artifacts resulting from their methods, which both require dichotomy, contrary to the BI with MrBayes indicating the non-resolution of this node as a rake. In this study, the long branches within of *S. curacaoense* indicate the presence of a possible species complex, with distinct genetic lineages in Trinidad, and two in Jamaica.

In the Jamaican endemics, the position of *Metopaulias depressus* was previously unresolved, while our study strongly supports a sister species relationship to *Sesarma verleyi* (Fig. 4.1), both species presenting strongly specialized ecologies and morphologies. *Sesarma windsor*, that appeared to be the sister species of *S. verleyi* in Schubart *et al.* (1998), is the newly described sister species of *S. meridies* Schubart & Koller, 2005. The second recently described species, *S. abeokuta*, is recovered as sister species of *S. dolphinum* as identified by Schubart & Santl (2014). The grouping of *S. ayatum* (referred as *Sesarma sp.* in Schubart *et al.* 1998) with *S. bidentatum* is recovered here, in addition to their clustering with *S. cookei*. However the sister species between *S. fossarum* and *S. jarvisi* is not supported, as well as the

grouping of *S. abeokuta, S. dolphinum, S. fossarum* and *S. jarvisi*. This low support is related to incongruences between the gene trees in this group (Figs. S4.3-S4.8). Such incongruence has been previously explained in other taxa by incomplete lineage sorting associated or not with past hybridization and introgression, especially in the case of rapid radiations (Takahashi *et al.* 2001, Leaché & McGuire 2006). Examples of hybridizations with introgression were recently highlighted in southeast Asian freshwater crabs (Schubart & Ng 2008, Schubart *et al.* 2008). Another explanation could be the low mutation rate of the 28S nuclear gene used in this study that provide only limited information in closely related species (e.g. Landstorfer & Schubart, 2010).

2) *Aratus - Armases*

The position of *Aratus* regarding *Armases* recovered in this study contradicts both Niem (1993, 1996) and Števčić (2005) based on morphological data (Fig. 4.1). Indeed, Niem (1993, 1996) supports a grouping of *Aratus pisonii*, *Armases elegans* and *Armases rubripes* in an *Aratus*-group, supported by apomorphies of the walking legs' coxae, of the carpus of the chelipeds and on the inner surface of cheliped merus. Within the *Aratus*-group, a sister species relationship was assumed between *Aratus pisonii* and *Armases elegans* based on the merus of walking legs, on the dactylus length of walking legs and on the number of transversal ridge of the gastric mill median tooth (Niem, 1996). In contrast, our results indicate no close relationships between these three species, as *Armases rubripes* is located in a basal position within the genus *Armases*, the *Aratus pisonii* species complex is placed in the middle of the genus *Armases*, and finally *Armases elegans* is partially supported to be the sister species of *A. angustipes*, with both belonging to the *cinereum*-group as defined by Niem (1996). The contradiction of our genetic results with the morphological apomorphies considered by Niem (1993, 1996), imply these apparent apomorphies to be evolutionary convergences rather than synapomorphies inherited from a common ancestor. As these three species present a clear trend toward arboreal lifestyle, these convergent characters, in addition to the trapezoidal and flat carapace, long propodi and short dactyli observed, also present in the Southeast Asian species *Parasesarma leptosoma*, might represent adaptive steps under selection pressure imposed by an arboreal lifestyle. That has been claimed to be convergent evolution and observed at larger scale in the Sesarmidae and Grapsidae (Fratini *et al.* 2005).

Additionally, our results genetically confirm the classification of *Armases rubripes* within the genus *Armases*. This species has originally been described from East Asian genus *Metasesarma* H. Milne Edwards, 1853 based on the position of its antenna (Rathbun 1918).

Niem (1993, 1996) refuted this conclusion and highlighted the clear affinity of this species with the American *Sesarma*, justifying its transfer to this genus, and restricting *Metasesarma* to the Indo-Pacific. Our results, by placing *Armases rubripes* in the American Sesarmidae, comfort the argument of Niem (1993) of the non robustness of the criteria used by Rathbun (1918) and the affinity of this species with other American species rather than with Indo-Pacific species.

However, no morphological character is presently known to support the recovered basal position of *A. rubripes* in *Armases* (Fig. 4.1). Further studies will be necessary to determine what characters can be used to morphologically support its basal position (Schubart et al., in prep.). The *Aratus pisonii* species complex appears to harbor more than the two currently recognized species, *Aratus pisonii* (H. Milne Edwards, 1837) and *A. pacificus* Thiercelin & Schubart 2014. Indeed, five deeply divergent and solidly supported lineages are observed, with two of them in the Atlantic and three in the Pacific. All the Pacific cryptic species were found in Costa Rica and future work will also determine if morphological characters can support or not a species level for these lineages (Thiercelin & Schubart, in prep., Fig. S4.9).

Contrary to Niem (1993, 1996) suggesting close relationships between *Aratus* and some *Armases* species based on morphological apomorphies, Števčić (2005) considered the morphological unique characteristics of the genus *Aratus* (as the black bristles on the outer part of the chelae) sufficient to place it in its own tribe –*Aratini*– with regards to all the other sesarmid species placed in the *Sesarmini* tribe. However, the reduced number of species in the *Aratini* (a single species before the description of *Aratus pacificus* by Thiercelin & Schubart 2014) allowed a precise description of this tribe, contrary to the description of the *Sesarmini* obliged to fit with the more variable shapes observed in this large tribe (over 250 species based on De Grave *et al.* 2009). Our results contradict this conclusion of Števčić (2005), as the genetic relationships recovered in this study place *Aratus* not only within the American Sesarmidae, but inside the genus *Armases*, and thus invalidate the *Aratini* tribe described by Števčić (2005) (Fig. 4.1). We therefore synonymize the tribe *Aratini* with the family Sesarmidae in its current composition. It highlights that the unique characters and adaptations of *Aratus* are derived compared to the ones observed in the other species from the *Aratus-Armases* clade and linked to the deep ecological adaptations of this species. Similar morphological habitat-related adaptations have been observed in several Jamaican endemic sesarmid species, e.g. *Metopaulias depressus* and *Sesarma verleyi* (recovered as sister species in this study). The bromeliad-associated *Metopaulias depressus* has been identified by

Hartnoll (1964) and Niem (1993) as being from *Sesarma* ancestry, strongly modified by the requirements of its habitat, result later confirmed by the phylogenetic study of Schubart *et al.* (1998). The cave species *Sesarma verleyi* exhibits typical adaptations to underground life, with reduced eyes and elongated legs, adaptations also observed in the Southeast Asian cavernicolous sesarmids *Karstarma*, especially with *Karstarma jacobsoni* exhibiting the strongest morphological adaptations to cave life (Ng 2002).

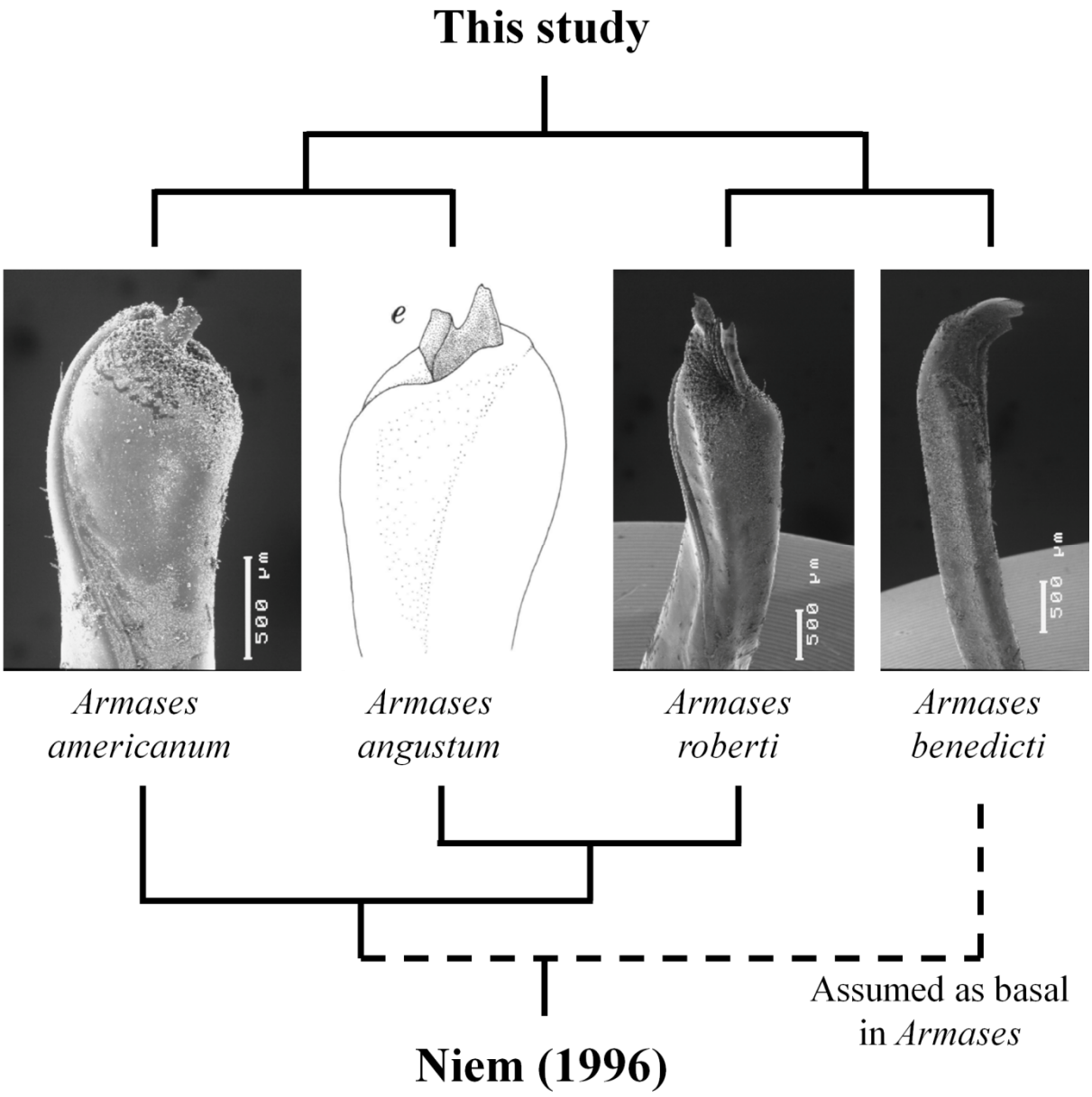


Figure 4.2. Gonopod 1 of *Armases americanum*, *A. angustum*, *A. benedicti* and *A. roberti*, with assumed relationships by Niem (1996) and this study. *A. angustum* from Abele (1992).

In addition to the position and affinities of *Aratus pisonii*, two other groups are recovered in the *Aratus* - *Armases* cluster and robustly supported by both BI and ML. The first one corresponds to the *roberti*-group, initially defined by Niem (1996) in his morphological review of the (sub)genus *Armases*, and supported by the distal insertion of setae on the male gonopod, distinct from all the other species in the genus. However, the sister species relationships presently recovered (respectively *A. americanum* / *A. angustum* and *A. benedicti* / *A. roberti*, Fig. 4.1) do not support the relationships assumed based on morphology and their synapomorphies (Niem 1996). This author assumed a sister species relationship between *A. roberti and A. angustum* in relation with a closer similarity in the shape of their gonopods regarding *A. americanum,* and *A. benedicti* holding a basal position in *Armases*, by assuming its characters (almost reduced epistome vaults and gonopod shape) to be ancestral. From its phylogenetic position in the *roberti*-group, we now deduce that these characters are not ancestral but derived. Especially, we interpret the specific gonopod shape of this species to correspond to a secondary torsion based on the shape observed in the three other species from this group. Moreover, *A. roberti* and *A. benedicti* present relatively slender gonopods, while *A. americanum* and *A. angustum* present bulgy endpieces and speciated as result of the closure of the Isthmus of Panama (Fig. 4.2).

The last group postulated by Niem (1996) corresponds to the *cinereum*-group. He claimed sister species relationships between *Armases cinereum* / *A. ricordi* and *A. angustipes* / *A. miersii* based on limited criteria, but could not determine the position of the three Pacific species *A. gorei*, *A. magdalenense* and *A. occidentale* by lack of material. The complex taxonomy of *A. angustipes* has been summarized by Abele (1992), and this species has repeatedly been confused by Rathbun (1897, 1918) with *A. miersii*, but is presently confirmed as distinct species based on genetic data. Our results (BI, ML) strongly support the *cinereum*-group with the inclusion of the three East Pacific species *A. gorei*, *A. magdalenense*, *A. occidentale* and the West African species *A. elegans* (Fig. 4.1). They also support the *cinereum*-group as the sister group of *Aratus*. As assumed by Niem (1996), *Armases cinereum* and *A. ricordi* appear as sister species, but our results surprisingly reject a close relationship between for *A. angustipes* and *A. miersii* and instead support *A. elegans* to be the closest relative of *A. angustipes*. The gonopod of *A. elegans*, illustrated for the first time in this publication (Fig. 4.3) exhibits clear morphological similarity to the one of *A. angustipes* and *A. miersii*, strongly supporting the close relationships between these three species. It also confirms that the morphology of *A. elegans* is derived and probably results from selective

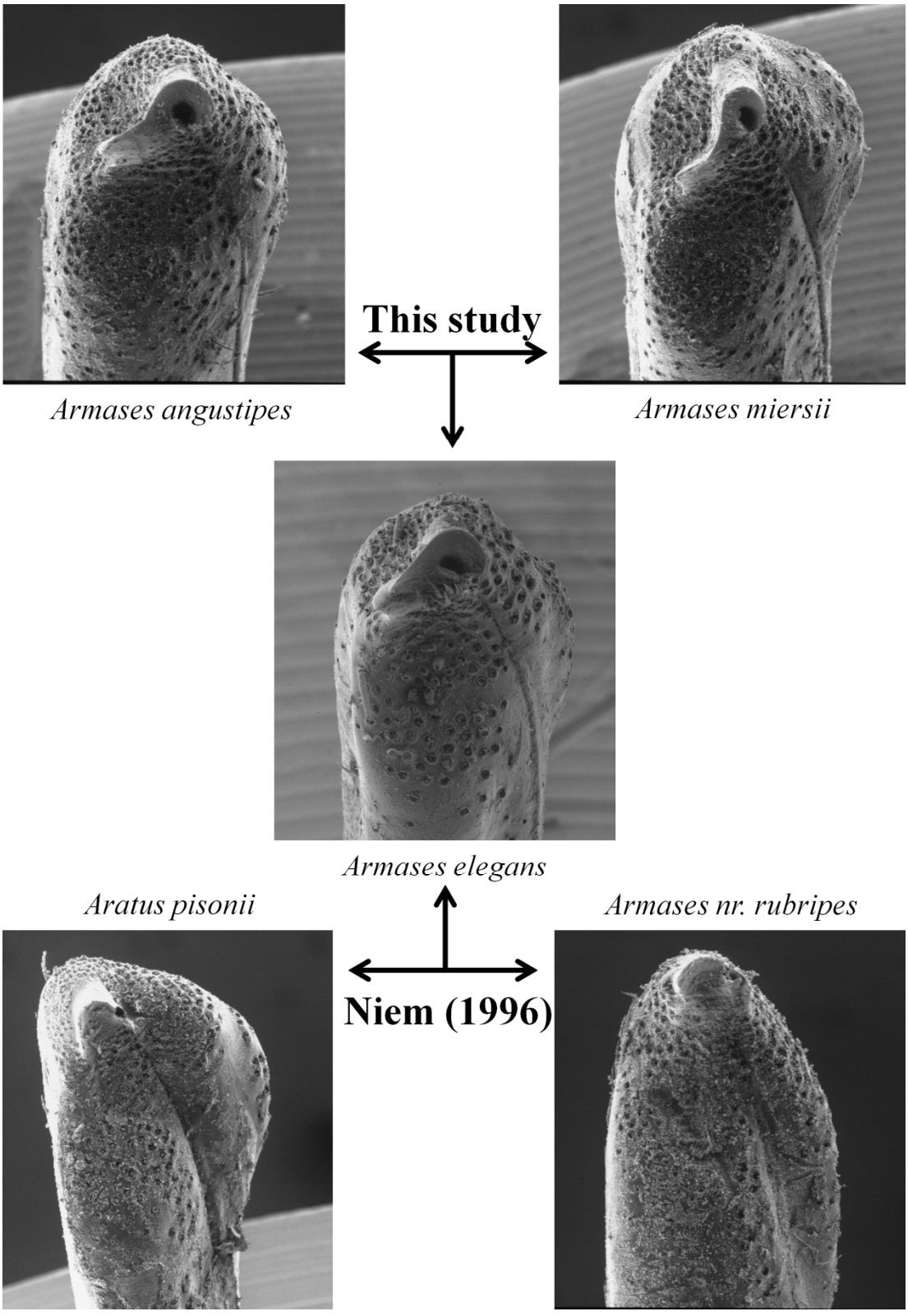


Figure 4.3. Gonopod 1 in frontal view of *Aratus pisonii*, *Armases angustipes*, *A. elegans*, *A. miersii* and *A. nr. rubripes*, with assumed relationships by Niem (1996) and this study.

adaptations related to its arboreal ecology compared to its two relatives, in a similar way to *Aratus*. The three east Pacific species *A. gorei*, *A. magdalenense*, *A. occidentale* originate from a single Pacific ancestor, with *A. gorei* and *A. occidentale* unexpectedly forming a well-supported sister species relationship. The gonopod shape of these three species, illustrated by Abele (1992) in his review of the genus, reinforces the genetic results obtained here. Indeed the gonopods of *A. gorei* and *A. occidentale* appear closer to each other than to *A. magdalenense*, while the gonopod of *A. occidentale* species presents shape similarities with the one of *A. miersii* (Abele 1992:figs. 37 & 39, Fig. 4.4).

The gonopod shape, illustrated by Abele (1992) for the American sesarmid species, appears to be a strongly reliable morphological criterion to identify close relationships between species. Niem (1996) properly used it to identify the *roberti*-group, but did not consider it for the other species of *Armases*. The gonopod shape might be an interesting complement to genetic data in the recovery of the relationships between species and genera in thoracotreme crabs, and more generally in Brachyura.

Figure 4.4. Gonopod 1 in frontal view of *Armases occidentale*.

Timing of speciation and geographical distribution

The origin of the American sesarmid is estimated to have occurred between 11.42 and 10.46 mya during the Late Miocene, but the radiation in this group accelerated after the Miocene - Pliocene transition 5.3 mya (Fig. 4.1). Several divergence events are found congruent with the closure of the Isthmus of Panama, and the youngest species pair, *Armases gorei* - *A. occidentale*, has an approximate age of 1.13 mya. Younger lineages are found, especially in *Sesarma curacaoense* and the Jamaican endemics, but their status will require

further investigation. The timing and area of radiation of the American Sesarmidae is extremely concomitant to the ones of the snapping shrimp *Alpheus armatillus* species complex, estimated to have initiated its divergence ~10 mya and represented today by 19 divergent lineages in the Atlantic and Pacific oceans (Mathews & Anker 2009). The timing of radiation (< 4 mya) of the Jamaican endemic *Sesarma* is similar to the timing initially estimated by Schubart *et al.* (1998), despite distinct calibration points and molecular rates used by both studies. It strengthens the hypothesis of radiation in this genus much later than the Tertiary emergence of Jamaica.

None of the American sesarmid species presents a transisthmian distribution. *Aratus pisonii*, the last species considered to be present along both east Pacific and west Atlantic coastlines, has been separated in two distinct species, with *Aratus pisonii* restricted to the Atlantic and *A. pacificus* restricted to the Pacific (Thiercelin & Schubart 2014). In total, five allopatric speciation events can be associated with the closure of the Great American Seaway and the rise of the Isthmus (Lessios, 2008) based on their distribution and timing of divergence: *Aratus sp.* (Atlantic = *A. pisonii* + *A. sp* 'GoM') / *Aratus sp.* (Pacific = *A. pacificus* + *A sp.* 'Baja') (3.03 mya, 95% HPD: 2.3–3.33 mya); (*A. angustipes* - *A. elegans*) / (*Armases gorei* - *A. magdalenense* - *A. occidentale*) (3.18 mya, 95% HPD: 2.17–4.26 mya); *A. americanum* / *A. angustum* (2.64 mya, 95% HPD: 1.4–3.95 mya); *Sesarma rhizophorae* / Atlantic *reticulatum*-group (2.85 mya, 95% HPD: 1.87–3.92 mya) and *S. crassipes* / (*S. aequatoriale* - *S. sulcatum*) (3.81 mya, 95% HPD: 2.2–5.49 mya). Based on molecular clock calibration, all these divergence events are concomitant with the final steps of the closure of the Isthmus, estimated at 3.1 mya by Coastes & Obando (1996), and resulted in the presence of sister clades on both sides of the American continent. This result is congruent with the results of Knowlton & Weigt (1998) and the review of Lessios (2008), that highlighted the species from marine ancestry living in mangrove swamp or in habitats with reduced salinities to be the last ones that diverged before the interruption of the marine connection between the east Pacific and west Atlantic. As a result of such conclusions, the genus *Aratus*, strongly associated to the red mangrove *Rhizophora* (see Beever *et al.*, 1979), was used in this study as calibration point, and the separation between the Atlantic and Pacific lineages of this genus is assumed to correspond to the last marine connection between the two oceans.

However, the couple *Armases americanum* / *A. angustum* is found with a mean divergence time of 2.64 mya, younger than the transisthmian divergence of *Aratus* assumed to correspond to the last marine water exchange between the two oceans. Its 95% HPD (1.4–3.95 mya) is congruent with a divergence at the final closure of the Isthmus, but this result

can also be interpreted by two other ways. First it can imply a final closure during the Late Pliocene, as suggested by Coates *et al.* (2005) rather than 3.1 mya. Second, it can indicate that this couple diverged after the last marine connection. Indeed, *A. americanum* is described by Abele (1992) as living along freshwater streams, and collected from 150m up to 5km from river mouth (possibly up to 32km, based on a specimen collected at the border Honduras-Belize mentioned in Abele 1992), and was also identified in the freshwater section of the Panama Canal (Abele & Kim 1989). On the other hand, *A. angustum* was collected up to 1km from the sea (Gray & Christy 2000). This higher tolerance to freshwater than *Aratus* possibly allowed this couple to maintain temporary genetic exchanges across the early complete isthmus, and comforts the observation that most freshwater tolerant species were the last ones to diverge during the Great American Schism (Lessios, 2008).

In the *roberti*-group, the relationships and distribution of the species also highlight a complete absence of overlap of their distributions (see Abele 1992, Melo 1996, Fig S4.10). Indeed, in the Atlantic, *A. americanum* is only found along the continental Caribbean coastline, when *A. roberti*, only present along West Indies coastline and totally absent from the continent with the exception of Venezuela. Finally, *A. benedicti* is found along the South American coastline starting from the Orinoco Delta. The initial speciation event in this group, that occurred during the early Pliocene (4.67 mya, 95% HPD: 3.16–6.26 mya), separated the western populations (nowadays *A. americanum* & *A. angustum*) from the eastern ones (*A. benedicti* & *A. roberti*). A later divergence event separated the populations of the West Indies from the South American populations (3.93 mya, 95% HPD: 2.53–5.43 mya). The edge of distribution of the three Atlantic species corresponds to northern part of the South American continent, between Venezuela and north Brazil. This region has been affected during the Miocene and Pliocene by the Andean uplift, resulting in major changes in the drainage systems, as both Orinoco and Amazon rivers changed their courses and the Amazonian megawetlands disappeared (Hoorn *et al.* 1995, 2010). This tectonic event and its effects on both landscape and climate was a driver on the Neogene diversification of the South American biota, widely documented for terrestrial and freshwater faunas (Hoorn *et al.* 2010, Rull 2011). It also potentially explains the speciation events observed in marine taxa as suggested for penaeid shrimps (Gusmão *et al.* 2000) or seahorses (Boehm et al. 2013). However, these speciation events do not explain the current absence of contact zone or secondary sympatric distribution for the three Atlantic species of this group, especially as these species present pelagic larvae, corresponding to the dispersal stage in marine organisms (Pineda *et al.* 2007, Cowen & Sponaugle 2009). Two mechanisms might explain such pattern.

First, larval retention might be limiting the effective dispersion range, mechanism already known in American Sesarmidae (Anger 1995). Larval retention has also been highlighted in Caribbean reef fishes with long larval duration (Taylor & Hellberg 2003), allowing the maintain of color morphs and genetic differentiation at regional scale. Second, it might represent an example of competitive exclusion between closely related species, which can reduce the frequency of co-existence when traits are conserved in these closely related species (Webb *et al.* 2002, Mayfield & Levine 2010, Violle *et al.* 2011).

An opposite pattern is observed in the *sulcatum*-group, where three closely related species (*S. aequatoriale*, *S. rubinofforum* and *S. sulcatum*) are present in sympatry on a large part of their distribution in the Pacific ocean (Fig. S4.11). However, these species present differences in their salinity preferences along a gradient, and probably inhabit different niches, as *S. aequatoriale* is semi-terrestrial and prefers low salinity in the range 0–22.4‰, *S. rubinofforum* lives in salinities ranging from 16 to 24‰ and *S. sulcatum* is more common in salinities of 22‰ than in low salinity (Abele, 1992). Coexistence of closely related species is possible when the niche difference exceeds the competitive ability differences of these species, and such mechanism probably explain the observed distribution (Mayfield & Levine 2010).

A single amphi-Atlantic speciation event is known in the Sesarmidae (Fig. 4.1), and is dated as posterior to the final closure of the Isthmus (2.27 mya, 95% HPD: 1.28–3.3 mya). Indeed, *Armases elegans* was identified by Abele (1992) to be from American ancestry rather than from Southeast Asian one as all the other African Sesarmidae. Indications of amphi-Atlantic gene flow posterior to transisthmian speciation have been previously observed for the grapsid genera *Geograpsus*, *Goniopsis*, *Grapsus* and *Pachygrapsus* (see Schubart *et al.* 2006, Schubart 2011). Grapsidae possess relatively long larval development, with up to eight larval stages (Cuesta *et al.* 2011). As the dispersal distance of a species is usually linked to the pelagic larval duration (Shanks *et al.* 2003), their long larval duration is assumed to be responsible of the genetic exchange between both sides of the Atlantic Ocean after the closure of the Isthmus. On the other hand, sesarmids (including *Armases angustipes*) have shorter larval development than grapsids (usually four larval stages), and several species, as *A. miersii* or the Jamaican endemic *Sesarma*, even present abbreviated development (Cuesta *et al.* 1999, Cuesta & Anger 2001). For this reason, the amphi-Atlantic speciation of the couple *A. angustipes* - *A. elegans* represents a new example of peripatric speciation by colonization from America across the Atlantic by oceanic dispersal rather than a vicariant event. The importance of oceanic dispersal in biogeography was recently revised by De Queiroz (2005),

and has been assumed to explain post-Gondwana divergence in terrestrial taxa present on both sides of the Atlantic ocean, as exemplified by geckos (Carranza *et al.* 2000), monkeys (Schrago & Russo 2003), or plants (Givnish *et al.* 2000, Renner *et al.* 2001, Cronn & Wendel 2003). In absence of land-bridge or islands allowing stepping stone dispersal (as the presence of large islands in the South Atlantic is considered up to the Eocene by Ezcurra & Agnolin 2012), rafting has been the preeminent scenario explaining these long distance oceanic dispersals for both marine and terrestrial taxa (Jokiel 1990, Schubart *et al.* 2001, De Queiroz 2005, Ali & Huber 2010). In the case of *A. elegans*, rafting can possibly be associated by the tree-climbing behavior observed in this species and its sister taxa.

Conclusions

This phylogenetic study on the American Sesarmidae is the first to completely disentangle the relationships between the different genera of this family. We highlight with how much care morphological data should be considered in a taxonomic and phylogenetic framework. Two opposite evolutionary forces are presently observed. On one side, numerous morphological characters considered by taxonomists to be ancestral appear in reality to be derived and result from rapid and repeated adaptations to specific ecological niches. On the other side, several convergent events let incorrectly assume sister-species relationships, blurring the real taxonomic history. As American species represent only fifteen percent of the whole family, future studies on Sesarmidae will focus on the southeast Asia species, diversity hotspot of the family, and on their African relatives, to explore the differences and similarities with regards to the American radiation. Moreover, at an upper level, relationships at the species, genus and family level need to be resolved in the Thoracotremata (see Tsang *et al.* 2014, Ip *et al.* 2015), as the same evolutionary mechanisms resulted in polyphyletic genera highlighted in recent molecular studies.

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GENERAL DISCUSSION AND CONCLUSIONS

The Isthmus of Panama: a barrier between two oceans

The progressive formation of the Isthmus of Panama during the Miocene, and its final closure during the Pliocene, was the preeminent geological event that affected the western tropical Atlantic and eastern Pacific oceans (Lessios 2008), and current patterns in the Atlantic and Pacific. The present dissertation is a step towards the understanding of the genetic histories of coastal brachyurans along the tropical American coastlines, including the Panama Isthmus. It also allows a better comprehension of both geological processes and ecological characteristics affecting and shaping the biodiversity at different scales.

As consequence of the physical isolation between the Atlantic and Pacific oceans, called the Great American Schism, independent genetic divergences occurred in the populations of these two oceans, reviewed by Lessios (2008). Different species of thoracotreme crabs strongly follow this biogeographic pattern. Indeed, up to 2006, four species were considered to present transisthmian distribution (i.e. to be present on both side of the isthmus), *Aratus pisonii*, *Geograpsus lividus*, *Grapsus grapsus* and *Pachygrapsus transversus* respectively. Schubart *et al.* (2006) pointed out that the populations of *Pachygrapsus transversus* on both sides of the isthmus are genetically and morphologically distinct, and therefore rehabilitated *Pachygrapsus socius* Stimpson, 1870 as valid species. The genetic divergence between these two species of *Pachygrapsus* was revisited in this dissertation using an additional mitochondrial gene, fully supporting their distinction (Chapters 1 & 3). But the present dissertation also confirms that the three remaining thoracotreme species with transisthmian distribution present deep genetic breaks between their Atlantic and Pacific populations, as observed by Schubart (2011). This result implies that these species did not maintain gene flow across the isthmus after its completion, and as consequence, Atlantic and Pacific lineages might represent distinct species, as the genetic distances are equivalent to the one observed between transisthmian sister species (Lessios 2008). For *Aratus*, the presence of robust morphological differences between male first gonopod of transisthmian populations supports the description of the Pacific population as *Aratus pacificus* Thiercelin & Schubart, 2014 (Chapter 2). For *Geograpsus* and *Grapsus*, further studies are required to identify morphological criteria supporting the description or

rehabilitation of distinct species for the Pacific lineage, as suggested by Guerao *et al.* (2003) for *Geograpsus*.

The Isthmus of Panama has always been of major importance for phylogenetic studies, and has been used in two distinct ways. Directly, as calibration point for species that diverged as consequence of the isthmian vicariance (Martin *et al.* 1992, Bermingham & Lessios 1993, Miura *et al.* 2010), or indirectly, by the use of molecular clock, calibrated with the rates of molecular divergence between transisthmian sister species, as the ones of Knowlton & Weigt (1998) or Schubart *et al.* (1998). As consequence, the usefulness of calibrations based on the Isthmus depends of two factors: 1) the accuracy and reliability of time estimations of geological events and 2) the correct causal link assumed between genetic divergence and these time estimates.

These accuracies of time estimates depend on the methods employed to reconstruct the different steps of geological events. In phylogenetic reconstructions, the methods taking into account the timing uncertainty were reviewed by Ho & Phillips (2009). For the Isthmus of Panama, it implies to take into account of the uncertainty concerning the timing of closure under the Standard model, ranging from 2.8 to 3.5 mya (Coates & Obando 1996, Coates *et al.* 1992, 2004, 2005). The proposition of an alternative model of closure of the Isthmus (Montes *et al.* 2012a, 2012b), indicating an extremely different timing dating back to the Miocene (~15mya), obliged to examine the reliability of the geological (Coates & Stallard 2013) and biological (Jackson & O'Dea 2013, Chapter 3) arguments supporting each model.

On the other hand, the question of the causal link between genetic divergence and timing of the geological events was raised by Knowlton & Weigt (1998). They highlighted that all the species did not diverge during the final closure of the isthmus, but on the opposite diverged progressively, as mangroves species appear to be less divergent than rocky intertidal or subtidal species. They concluded that mangrove inhabitants were the last species that diverged during the final steps of closure of the Isthmus of Panama, while other habitats probably diverged earlier. It revealed the large importance of the ecological characteristics of the different species on this vicariant event. This dissertation supports this conclusion, as transisthmian couples inhabiting mangrove and freshwater habitats (genera *Aratus, Armases, Goniopsis* and *Sesarma*) are less divergent than couples inhabiting other intertidal habitats as rocky shores (genera *Geograpsus, Grapsus* and *Pachygrapsus*). As consequence, for accurate calibration of phylogenetic studies using the transisthmian vicariance, with the final closure of the Isthmus as calibration point, the use of non-brackish couples should be avoided. Finally,

Craig *et al.* (2004) called for carefulness in the assumption of sister species relationships based on morphological basis, that can lead to incorrect conclusions.

Patterns along the tropical American coastlines

As highlighted in Chapter 1, phylogeographic patterns of brachyurans inhabiting western tropical Atlantic and / or eastern tropical Pacific oceans received only limited focus so far, as only Schubart *et al.* (2006), Oliveira-Neto *et al.* (2007) and Laurenzano *et al.* (2012, 2013) explored these intraspecific patterns, all observing an homogeneity of the Brazilian coastline. However, Schubart *et al.* (2006) and Laurenzano *et al.* (2013) observed contradicting results concerning the impact of the Amazon-Orinoco freshwater plume on the connectivity between populations along the western tropical Atlantic. The reanalysis of one of these species and the addition of three other species in this dissertation indicates that the differences observed between these species are the results of individual evolutionary histories, congruent to what has been observed for other biogeographic barriers (Patarnello *et al.* 2007), and revealed by multispecies comparative phylogeographic studies.

At higher taxonomic level, phylogenetic studies of marine taxa inhabiting this region revealed the presence of Miocene to Pliocene radiations including cryptic lineages (Schubart *et al.* 1998, Williams *et al.* 2001, Morrison *et al.* 2004, Mathews & Anker 2009, Coppard *et al.* 2013). This Miocene to Pliocene radiation is recovered in the American Sesarmidae (Chapter 3), which also includes the presence of previously unknown deeply divergent genetic lineages in several species (*Aratus pacificus, A. pisonii*, *Armases rubripes* and *Sesarma curacaoense*) whose status as distinct species remains to determine or can be already considered (Fig. S4.9).

To conclude, American thoracotreme crabs are typical representatives of coastal marine fauna inhabiting both tropical American coastlines. The exploration of genetic patterns in these species at different levels shed light on how both environmental changes and ecological characteristics shaped the biodiversity of this region since the Miocene. It also calls for further studies on these species, especially those with large distribution ranges in order to reveal intraspecific differentiation processes.

REFERENCES

A

Abele LG (1973) A new species of *Sesarma*, *S.* (*Holometopus*) *rubinofforum*, from the Pacific coast of Panamá (Crustacea, Decapoda, Grapsidae). *Proceedings of the Biological Society of Washington*, **86**, 333–338.

Abele LG (1975) The identity of *Sesarma hanseni* Rathbun, 1897, a supposedly West Indian species, with *S. dehaani* H. Milne Edwards, 1853, from the West Pacific (Decapoda, Grapsidae). *Crustaceana*, **28**, 48–52.

Abele LG (1992) A review of the grapsid crab genus *Sesarma* (Crustacea: Decapoda: Grapsidae) in America, with the description of a new genus. *Smithsonian Contributions to Zoology*, **527**, 1–60.

Abele LG, Kim W (1989) The decapod crustaceans of the Panama Canal. *Smithsonian Contributions to Zoology*, **482**, 1–50.

Ali JR, Huber M (2010) Mammalian biodiversity on Madagascar controlled by ocean currents. *Nature*, **463**, 653–656.

Anger K (1995) The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics. *Journal of Experimental Marine Biology and Ecology*, **193**, 119–145.

Anker A, De Grave S (2008) *Zuzalpheus* Ríos and Duffy, 2007: a junior synonym of *Synalpheus* Bate, 1888 (Decapoda: Alpheidae). *Journal of Crustacean Biology*, **28**, 735–740.

Anker A, Hurt C, Knowlton N (2009) Description of cryptic taxa within the *Alpheus bouvieri* A. Milne-Edwards, 1878 and *A. hebes* Kim and Abele, 1988 species complexes (Crustacea: Decapoda: Alpheidae). *Zootaxa*, **2153**, 1–23.

Arbogast BS, Kenagy GJ (2001) Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*, 819–825.

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge (Massachusetts), 447 pp.

Avise JC (2009) Phylogeography: retrospect and prospect. *Journal of Biogeography*, **36**, 3–15.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between

population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.

B

Bacon C, Mora A, Wagner W, Jaramillo C (2013) Testing geological models of evolution of the Isthmus of Panama in a phylogenetic framework. *Botanical Journal of the Linnean Society*, **171**, 287–300.

Baele G, Lemey P, Bedford T, Rambaut A, Suchard MA & Alekseyenko AV (2012) Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, **29**, 2157–2167.

Baele G, Li WLS, Drummond AJ, Suchard MA & Lemey P (2013) Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. *Molecular Biology and Evolution*, **30**, 239–243.

Baeza JA (2010) Molecular systematics of peppermint and cleaner shrimps: phylogeny and taxonomy of the genera *Lysmata* and *Exhippolysmata* (Crustacea: Caridea: Hippolytidae). *Zoological Journal of the Linnean Society*, **160**, 254–265.

Baeza JA, Fuentes MS (2013) Exploring phylogenetic informativeness and nuclear copies of mitochondrial DNA (numts) in three commonly used mitochondrial genes: mitochondrial phylogeny of peppermint, cleaner, and semi-terrestrial shrimps (Caridea: *Lysmata*, *Exhippolysmata*, and *Merguia*). *Zoological Journal of the Linnean Society*, **168**, 699–722.

Baeza JA, Schubart CD, Zillner P, Fuentes S, Bauer RT (2009) Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Hippolytidae): the evolutionary origins of protandric simultaneous hermaphroditism and social monogamy. *Biological Journal of the Linnean Society*, **96**, 415–424.

Barroso R, Klautau M, Solé-Cava A, Paiva P (2010) *Eurythoe complanata* (Polychaeta: Amphinomidae), the “cosmopolitan” fireworm, consists of at least three cryptic species. *Marine Biology*, **157**, 69–80.

Bartoli G, Sarnthein M, Weinelt M, Erlenkeuser H, Garbe-Schönberg D, Lea DW (2005) Final closure of Panama and the onset of northern hemisphere glaciation. *Earth and Planetary Science Letters*, **237**, 33–44.

Baums IB, Paris CB, Chérubin LM (2006) A bio-oceanographic filter to larval dispersal in a reef-building coral. *Limnology and Oceanography*, **51**, 1969–1981.

Beever JW, Simberloff D, King LL (1979) Herbivory and predation by the mangrove tree crab *Aratus pisonii*. *Oecologia*, **43**, 317–329.

Bermingham E, Lessios HA (1993) Rate variation of protein and mitochondrial DNA evolution as revealed by sea urchins separated by the Isthmus of Panama. *Proceedings of the National Academy of Sciences*, **90**, 2734–2738.

Bermingham E, Martin AP (1998) Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology*, **7**, 499–517.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2006) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, **22**, 148–155.

Boehm JT, Woodall L, Teske PR, Lourie SA, Baldwin C, Waldman J, Hickerson M (2013) Marine dispersal and barriers drive Atlantic seahorse diversification. *Journal of Biogeography*, **40**, 1839–1849.

Boone L (1930) Crustacea: Stomatopoda and Brachyura. In: Scientific results of the cruises of the yachts "Eagle" and "Ara", 1921–1928, William K. Vanderbilt commanding. *Bulletin of the Vanderbilt Oceanographic Marine Museum*, **2**, 5–228.

Bowen BW, Rocha LA, Toonen RJ, Karl SA (2013) The origins of tropical marine biodiversity. *Trends in Ecology & Evolution*, **28**, 359–366.

Bracken HD, Toon A, Felder DL, Martin JW, Finley M, Rasmussen J, Palero F, Crandall KA (2009) The Decapod tree of life: compiling the data and moving toward a consensus of decapod evolution. *Arthropod Systematics & Phylogeny*, **67**, 99–116.

Briggs JC (1974) Marine Zoogeography. McGraw-Hill, New York, 475 pp.

Briggs JC (1995) *Global Biogeography*. Elsevier, New York. 451 pp.

Bromham L, Penny D (2003) The modern molecular clock. *Nature Reviews: Genetics*, **4**, 216–224.

Brösing A (2008) A reconstruction of an evolutionary scenario for the Brachyura (Decapoda) in the context of the Cretaceous-Tertiary boundary. *Crustaceana*, **81**, 271–287.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA Evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 6491–6495.

C

Carranza S, Arnold EN, Mateo JA, López-Jurado LF (2000) Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society B.* **267**, 637–649.

Ceballos G, Ehrlich PR (2009) Discoveries of new mammal species and their implications for conservation and ecosystem services. *Proceedings of the National Academy of Sciences*, **106**, 3841–3846.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Coates AG, Collins LS, Aubry MP, Berggren WA (2004) The geology of the Darien, Panama, and the late Miocene-Pliocene collision of the Panama arc with northwestern South America. *Geological Society of America Bulletin*, **116**, 1327–1344.

Coates AG, Jackson JBC, Collins LS, Cronin T, Dowsett H, Bybell L, Jung P, Obando J (1992) Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. *Geological Society of America Bulletin*, **104**, 814–828.

Coates AG, McNeill DF, Aubry MP, Berggren WA, Collins LS (2005) An introduction to the geology of the Bocas del Toro Archipelago, Panama. *Caribbean Journal of Science*, **41**, 374–391.

Coates AG, Obando JA (1996) The geologic evolution of the Central American isthmus. In: *Evolution and Environment in Tropical America* (eds Jackson JBC, Budd AF, Coates AG), pp. 21–56. University of Chicago Press, Chicago, Illinois.

Coates AG, Stallard RF (2013) How old is the Isthmus of Panama? *Bulletin of Marine Science*, **89**, 801–813.

Cody S, Richardson JE, Rull V, Ellis C, Pennington RT (2010) The great American biotic interchange revisited. *Ecography*, **33**, 326–332.

Colborn J, Crabtree RE, Shaklee JB, Pfeiler E, Bowen BW (2001) The evolutionary enigma of bonefishes (*Albula spp.*): cryptic species and ancient separations in a globally distributed shorefish. *Evolution*, **55**, 807–820.

Collette BB, Rützler K (1977) Reef fishes over sponge bottoms off the mouth of the Amazon River. *Proceedings of the 3rd International Coral Reef Symposium*, Miami, Florida, U.S.A. pp. 305–310.

Collins LS, Coates AG, Berggren WA, Aubry MP (1996) The late Miocene Panama isthmian strait. *Geology*, **24**, 687–690.

Conde JE, Díaz H (1989) The mangrove tree crab *Aratus pisonii* in a tropical estuarine coastal lagoon. *Estuarine, Coastal and Shelf Science*, **28**, 639–650.

Coppard S, Zigler K, Lessios H (2013) Phylogeography of the sand dollar genus *Mellita*: Cryptic speciation along the coasts of the Americas. *Molecular Phylogenetics and Evolution*, **69**, 1033–1042.

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, **1**, 443–466.

Cracraft J (1983) Species concepts and speciation analysis. In: *Current Ornithology* (ed. Johnson RF), pp. 159–187. Plenum Press, New York.

Craft JD, Russ AD, Yamamoto MN, Iwai TY, Hau S, Kahiapo J, Chong CT, Ziegler-Chong S, Muir C, Fujita Y, Polhemus DA, Kinzie III RA, Santos SR (2008) Islands under islands: The phylogeography and evolution of *Halocaridina rubra* Holthuis, 1963 (Crustacean: Decapoda: Atyidae) in the Hawaiian archipelago. *Limnology and Oceanography*, **53**, 675–689.

Craig MT, Hastings PA, Pondella II DJ (2004) Speciation in the Central American Seaway: the importance of taxon sampling in the identification of trans isthmian geminate pairs. *Journal of Biogeography*, **31**, 1085–1091.

Crandall ED, Sbrocco EJ, DeBoer TS, Barber PH, Carpenter KE (2011) Expansion dating: calibrating molecular clocks in marine species from expansions onto the Sunda Shelf following the Last Glacial Maximum. *Molecular Biology and Evolution*, **29**, 707–719.

Crane J (1947) Eastern Pacific expeditions of the New York Zoological Society: XXXVIII. Intertidal brachygnathous crabs from the west coast of tropical America with special reference to ecology. *Zoologica*, **32**, 69–95.

Cronin TM, Dowsett HJ (1996) Biotic and oceanographic response to the Pliocene closing of the Central American Isthmus. *Evolution and environment in tropical America. Univ. of Chicago Press, Chicago, IL*, 76–104.

Cronn R, Wendel JF (2003) Cryptic trysts, genomic mergers, and plant speciation. *New Phytologist*, **161**, 133–142.

Cuesta JA, Anger K (2001) Larval morphology of the sesarmid crab *Armases angustipes* Dana, 1852 (Decapoda, Brachyura, Grapsoidea). *Journal of Crustacean Biology*, **21**, 821–838.

Cuesta JA, García-Guerrero MU, Rodríguez A, Hendrickx ME (2006) Larval morphology of the sesarmid crab, *Aratus pisonii* (H. Milne Edwards, 1837) (Decapoda, Brachyura, Grapsoidea) from laboratory-reared material. *Crustaceana*, **79**, 175–196.

Cuesta JA, Guerao G, Schubart CD, Anger K (2011) Morphology and growth of the larval stages of *Geograpsus lividus* (Crustacea, Brachyura), with the descriptions of new larval characters for the Grapsidae and an undescribed setation pattern in extended developments. *Acta Zoologica*, **92**, 225–240.

Cuesta JA, Schuh M, Diesel R, Schubart CD (1999) Abbreviated development of *Armases miersii* (Grapsidae: Sesarminae), a crab that breeds in supralittoral rock pools. *Journal of Crustacean Biology*, **19**, 26–41.

D

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

De Grave S, Ashelby CW (2013) A re-appraisal of the systematic status of selected genera in Palaemoninae (Crustacea: Decapoda: Palaemonidae). *Zootaxa*, **3734**, 331–344.

De Grave S, Li CP, Tsang LM, Chu KH (2014) Unweaving hippolytoid systematics (Crustacea, Decapoda, Hippolytidae): resurrection of several families. *Zoologica Scripta*, **43**, 496–507.

De Grave S, Pentcheff ND, Ahyong ST, Chan T-Y, Crandall KA, Dworschak PC, Felder DL, Feldmann RM, Fransen CHJM, Goulding LYD, Lemaitre R, Low MEY, Martin JW, Ng PKL, Schweitzer CE, Tan SH, Tshudy D, Wetzer R (2009) A classification of living and fossil genera of decapod crustaceans. *Raffles Bulletin of Zoology*, **Supplement No. 21**,1–109.

De Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology & Evolution*, **20**, 68–73.

De Queiroz A (2014) *The monkey's voyage: how improbable journeys shaped the history of life*. Basic Books, New York, 360 pp.

Díaz H, Conde JE (1989) Population dynamics and life history of the mangrove crab *Aratus pisonii* (Brachyura, Grapsoidea) in a marine environment. *Bulletin of Marine Science*, **45**, 148–163.

Diesel R, Schubart CD (2000) Die außergewöhnliche Evolutionsgeschichte jamaikanischer Felsenkrabben. *Biologie in unserer Zeit*, **30**, 136–147.

Douris V, Cameron RA, Rodakis GC, Lecanidou R (1998) Mitochondrial phylogeography of the land snail *Albinaria* in Crete: long-term geological and short-term vicariance effects. *Evolution*, **52**, 116–125.

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

Duke NC, Allen JA (2006) *Rhizophora mangle*, *R. samoensis*, *R. racemosa*, *R.* × *harrisonii* (Atlantic–East Pacific red mangrove). *In: Traditional trees of Pacific Islands: Their Culture, Environment, and Use* (ed. Elevitch CR), pp. 623–640. Permanent Agriculture Resources (PAR), Holualoa, Hawaii.

Duque-Caro H (1990) Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South America and the evolution of the Panama Seaway. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **77**, 203–234.

E

Elmer K, Bonett R, Wake D, Lougheed S (2013) Early Miocene origin and cryptic diversification of South American salamanders. *BMC Evolutionary Biology*, **13**, 59.

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Ezcurra MD, Agnolín FL (2012) A new global palaeobiogeographical model for the late Mesozoic and early Tertiary. *Systematic Biology*, **61**, 553–566.

F

Farris DW, Jaramillo C, Bayona G, Restrepo-Moreno S, Montes C, Cardona A, Mora A, Speakman R, Glascock M, Valencia V (2011) Fracturing of the Panamanian Isthmus during initial collision with South America. *Geology*, **39**, 1007–1010.

Floeter S, Rocha L, Robertson D, Joyeux JC, Smith-Vaniz WF, Wirtz P, Edwards AJ, Barreiros JP, Ferreira CEL, Gasparini JL, Brito A, Falcón JM, Bowen BW, Bernardi G (2008) Atlantic reef fish biogeography and evolution. *Journal of Biogeography*, 35, 22–47.

Flores AA, Negreiros-Fransozo ML (1998) External factors determining seasonal breeding in a subtropical population of the shore crab *Pachygrapsus transversus* (Gibbes, 1850)(Brachyura, Grapsidae). *Invertebrate Reproduction & Development*, **34**, 149–155.

Fratini S, Vannini M, Cannicci S, Schubart CD (2005) Tree-climbing mangrove crabs, a case of convergent evolution. *Evolutionary Ecology Research*, **7**, 219–233.

Froelich P, Atwood D, Giese G (1978) Influence of Amazon River discharge on surface salinity and dissolved silicate concentration in the Caribbean Sea. *Deep Sea Research*, **25**, 735–744.

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 397–423.

G

Gistel JNFX (1848) *Naturgeschichte des Thierreichs für höhere Schulen bearbeitet*. Stuttgart, xvi+220 pp., 32 pls.

Givnish TJ, Evans TM, Zjhra ML, Patterson TB, Berry PE, Sytsma KJ (2000) Molecular evolution, adaptive radiation, and geographical diversification in the amphiatlantic family Rapateaceae: evidence from ndhF sequences and morphology. *Evolution*, **54**, 1915–1937.

Gray HM, Christy JH (2000) Predation by the grapsid crab, *Armases angustum* (Smith, 1870), on tadpoles of the green poison frog, *Dendrobates auratus* Girard, 1855. *Crustaceana*, **73**, 1023–1025.

Greene JL (1986) Biology of *Aratus elegans*. Doctoral dissertation, MSc thesis, University of North Wales.

Guerao G, Schubart CD, Cuesta JA (2003) The first zoeal stage of *Grapsus grapsus* L. and *Geograpsus lividus* (H. Milne Edwards) (Decapoda, Brachyura, Grapsidae) from the western Atlantic. *Nauplius*, **9**, 111–121.

Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

Guinot D (1977) Propositions pour une nouvelle classification des Crustacés Décapodes Brachyoures. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Serie D*, **285**, 1049–1052.

Gusmão J, Lazoski C, Solé-Cava AM (2000) A new species of *Penaeus* (Crustacea: Penaeidae) revealed by allozyme and cytochrome oxidase I analyses. *Marine Biology*, **137**, 435–446.

H

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hartnoll RG (1964) The freshwater grapsid crabs of Jamaica. *Proceedings of the Linnean Society of London*, **175**, 145–169.

Haug GH, Tiedemann R (1998) Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. *Nature*, **393**, 673–676.

Head J, Rincon A, Suarez C, Montes C, Jaramillo C (2012) Fossil evidence for earliest Neogene American faunal interchange: *Boa* (Serpentes, Boinae) from the early Miocene of Panama. *Journal of Vertebrate Paleontology*, **32**, 1328–1334.

Hellweger F, Gordon A (2002) Tracing Amazon River water into the Caribbean Sea. *Journal of Marine Research*, **60**, 537–549.

Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science*, **290**, 516–518.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **359**, 183–195.

Hickerson M, Stahl E, Lessios H (2006) Test for simultaneous divergence using approximate Bayesian computation. *Evolution*, **60**, 2435–2453.

Hickerson M, Stahl E, Takebayashi N (2007) msBayes: pipeline for testing comparative phylogeographic histories using hierarchical approximate Bayesian computation. *BMC Bioinformatics*, **8**, 268.

Hiller A, Kraus H, Almon M, Werding B (2006) The *Petrolisthes galathinus* complex: Species boundaries based on color pattern, morphology and molecules, and evolutionary interrelationships between this complex and other Porcellanidae (Crustacea: Decapoda: Anomura). *Molecular Phylogenetics and Evolution*, **40**, 547–569.

Ho S, Phillips M (2009) Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Systematic Biology*, **58**, 367–380.

Holthuis L (1977) The Grapsidae, Gecarcinidae and Palicidae (Crustacea: Decapoda: Brachyura) of the Red Sea. *Israel Journal of Zoology*, **26**, 141–192.

Hoorn C, Guerrero J, Sarmiento GA, Lorente MA (1995) Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology*, **23**, 237–240.

Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartin I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A (2010) Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, **330**, 927–931.

Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, **50**, 371–393.

Huang W, Takebayashi N, Qi Y, Hickerson M (2011) MTML-msBayes: approximate Bayesian comparative phylogeographic inference from multiple taxa and multiple loci with rate heterogeneity. *BMC Bioinformatics*, **12**, 1.

Hurt C, Anker A, Knowlton N (2009) A multilocus test of simultaneous divergence across the Isthmus of Panama using snapping shrimp in the genus *Alpheus*. *Evolution*, **63**, 514–530.

I

Ip BHY, Schubart CD, Tsang LM, Chu KH (2015) Phylogeny of the rocky shore family Grapsidae (Decapoda: Brachyura: Thoracotremata) based on a multilocus approach. *Zoological Journal of the Linnean Society*, in press. doi: 10.1111/zoj.12235.

Iturralde-Vinent MA (2006) Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *International Geology Review*, **48**, 791–827.

J

Jackson JB, O'Dea A (2013) Timing of the oceanographic and biological isolation of the Caribbean Sea from the Tropical Eastern Pacific Ocean. *Bulletin of Marine Science*, **89**, 779–800.

Jokiel PL (1990) Long-distance dispersal by rafting: reemergence of an old hypothesis. *Endeavour*, **14**, 66–73.

Joly S, Stevens MI, van Vuuren BJ (2007) Haplotype networks can be misleading in the presence of missing data. *Systematic Biology*, **56**, 857–862.

Jordan DS (1908) The law of geminate species. *American Naturalist*, **42**, 73–80.

Joyeux J-C, Floeter S, Ferreira C, Gasparini J (2001) Biogeography of tropical reef fishes: the South Atlantic puzzle. *Journal of Biogeography*, **28**, 831–841.

K

Kass RE, Raftery AE (1995) Bayes factors. *Journal of the American statistical association*, **90**, 773–795.

Keigwin L (1978) Pliocene closing of Isthmus of Panama based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores. *Geology*, **6**, 630–634.

Keigwin L (1982) Isotopic paleoceanography of the Caribbean and East Pacific: role of Panama uplift in late Neogene time. *Science*, **217**, 350–353.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.

Kingsley J (1879) On a collection of Crustacea from Virginia, North Carolina, and Florida, with a revision of the genera of Crangonidae and Palaemonidae. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **31**, 383–427.

Kitaura J, Wada K, Nishida M (2002) Molecular phylogeny of grapsoid and ocypodoid crabs with special reference to the genera *Metaplax* and *Macrophthalmus*. *Journal of Crustacean Biology*, **22**, 682–693.

Klaus S, Schubart CD, Streit B & Pfenninger M (2010) When Indian crabs were not yet Asian-biogeographic evidence for Eocene proximity of India and Southeast Asia. *BMC Evolutionary Biology*, **10**, 287.

Klaus S, Selvandran S, Goh JW, Wowor D, Brandis D, Koller P, Schubart CD, Streit B, Meier R, Ng PKL, Yeo DC (2013) Out of Borneo: Neogene diversification of Sundaic freshwater crabs (Crustacea: Brachyura: Gecarcinucidae: Parathelphusa). *Journal of Biogeography*, **40**, 63–74.

Knowlton N (1993) Sibling species in the sea. *Annual Review of Ecology and Systematics*, **24**, 189–216.

Knowlton N (2000) Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia*, **420**, 73–90.

Knowlton N, Weigt LA (1998) New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **265**, 2257–2263.

Koepfli K-PP, Gompper ME, Eizirik E, Ho C-C, Linden L, Maldonado JE, Wayne RK (2007) Phylogeny of the Procyonidae (Mammalia: Carnivora): Molecules, morphology and the Great American Interchange. *Molecular Phylogenetics and Evolution*, **43**, 1076–1095.

Kool JT, Paris CB, Andréfouët S, Cowen RK (2010) Complex migration and the development of genetic structure in subdivided populations: an example from Caribbean coral reef ecosystems. *Ecography*, **33**, 597–606.

L

Laguna JE (1987) *Euraphia eastropacensis* (Cirripedia, Chthamaloidea), a new species of barnacle from the tropical eastern Pacific: morphological and electrophoretic comparisons with *Euraphia rhizophorae* (de Oliveira) from the tropical eastern Atlantic and molecular evolutionary implications. *Pacific Science*, **41**, 132–140.

Landstorfer RB, Schubart CD (2010) A phylogeny of Pacific fiddler crabs of the subgenus *Minuca* (Crustacea, Brachyura, Ocypodidae: *Uca*) with the description of a new species from a tropical gulf in Pacific Costa Rica. *Journal of Zoological Systematics and Evolutionary Research*, **48**, 213–218.

Lartillot N, Philippe H (2006) Computing Bayes factors using thermodynamic integration. *Systematic Biology*, **55**, 195–207.

Laurenzano C, Farías NE, Schubart CD (2012). Mitochondrial genetic structure of two populations of *Uca urugayensis* fails to reveal an impact of the Rio de la Plata on gene flow. *Nauplius*, **20**, 15–25.

Laurenzano C, Mantelatto F, Schubart C (2013) South American homogeneity versus Caribbean heterogeneity: population genetic structure of the western Atlantic fiddler crab *Uca rapax* (Brachyura, Ocypodidae). *Journal of Experimental Marine Biology and Ecology*, **449**, 22–27.

Lazoski C, Solé-Cava A, Boury-Esnault N, Klautau M, Russo C (2001) Cryptic speciation in a high gene flow scenario in the oviparous marine sponge *Chondrosia reniformis*. *Marine Biology*, **139**, 421–429.

Leaché AD, McGuire JA (2006) Phylogenetic relationships of horned lizards (*Phrynosoma*) based on nuclear and mitochondrial data: Evidence for a misleading mitochondrial gene tree. *Molecular Phylogenetics and Evolution*, **39**, 628–644.

Lee T, Foighil DÓ (2005) Placing the Floridian marine genetic disjunction into a regional evolutionary context using the scorched mussel, *Brachidontes exustus*, species complex. *Evolution*, **59**, 2139–2158.

Leigh EG, O'Dea A, Vermeij GJ (2014) Historical biogeography of the Isthmus of Panama. *Biological Reviews*, **89**, 148–172.

Leme MHA, Negreiros-Fransozo ML (1998) Reproductive patterns of *Aratus pisonii* (Decapoda: Grapsidae) from an estuarine area of São Paulo northern coast, Brazil. *Revista de Biología Tropical*, **46**, 673–678.

Leme MHA (2002) A comparative analysis of the population biology of the mangrove crab *Aratus pisonii* and *Sesarma rectum* (Brachyura, Grapsidae) from the north coast of São Paulo State, Brazil. *Journal of Crustacean Biology*, **22**, 553–557.

Lessios HA (2008) The Great American Schism: Divergence of marine organisms after the rise of the Central American Isthmus. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 63–91.

Lessios HA, Robertson DR (2006) Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2201–2208.

Li JT, Li Y, Klaus S, Rao DQ, Hillis DM, Zhang YP (2013) Diversification of rhacophorid frogs provides evidence for accelerated faunal exchange between India and Eurasia during the Oligocene. *Proceedings of the National Academy of Sciences*, **110**, 3441–3446.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Lira PRJJ, dos Santos Calado TC, Araújo MSLC (2013) Breeding period in the mangrove crab *Goniopsis cruentata* (Decapoda: Grapsidae) in Northeast Brazil. *Revista de Biología Tropical*, **61**, 29–38.

Luiz OJ, Ferreira CE, Rocha LA (2009) *Halichoeres sazimai*, a new species of wrasse (Perciformes: Labridae) from the Western South Atlantic. *Zootaxa*, **2092**, 37–46.

Luiz OJ, Floeter SR, Rocha LA, Ferreira CE (2013) Perspectives for the lionfish invasion in the South Atlantic: are Brazilian reefs protected by the currents?. *Marine Ecology Progress Series*, **485**, 1–7.

Luiz OJ, Madin JS, Robertson DR, Rocha LA, Wirtz P, Floeter SR (2012) Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. *Proceedings of the Royal Society B: Biological Sciences*, rspb20111525.

Lunt DJ, Valdes PJ, Haywood A, Rutt IC (2007) Closure of the Panama Seaway during the Pliocene: implications for climate and Northern Hemisphere glaciation. *Climate Dynamics*, **30**, 1–18.

M

MacFadden BJ (2006) Extinct mammalian biodiversity of the ancient New World tropics. *Trends in Ecology & Evolution*, **21**, 157–165.

Maddison WP (1997) Gene trees in species trees. *Systematic Biology*, **46**, 523–536.

Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J, Olsen J, Perez KE, Stam W, Väinölä R, Viard F, Wares J (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology*, **89**, S108–S122.

Manning RB, Holthuis, LB (1981) West African brachyuran crabs (Crustacea: Decapoda). *Smithsonian Contributions to Zoology*, **306**, 1–379.

Mantelatto FL, Robles R, Schubart CD, Felder DL (2009) Molecular phylogeny of the genus *Cronius* Stimpson 1860, with reassignment of *C. tumidulus* and several American species of *Portunus* to the genus *Achelous* de Haan, 1833 (Brachyura: Portunidae). In: *Decapod Crustacean Phylogenetics* (eds Martin JW, Crandall KA, Felder DL), *Crustacean Issues*, **18**, 554–562.

Mariano AJ, Ryan EH (2015) Surface Currents in the Atlantic Ocean. http://oceancurrents.rsmas.miami.edu/atlantic/atlantic.html

Marko PB (2002) Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution*, **19**, 2005–2021.

Marko PB, Moran A (2009) Out of sight, out of mind: high cryptic diversity obscures the identities and histories of geminate species in the marine bivalve subgenus *Acar*. *Journal of Biogeography*, **36**, 1861–1880.

Marshall LG (1988) Land mammals and the great American interchange, *American Scientist*, **76**, 380–388.

Marshall LG, Butler RF, Drake RE, Curtis GH, Tedford RH (1979) Calibration of the great American interchange. *Science*, **204**, 272–279.

Marshall LG, Webb SD, Sepkoski JJ, Raup DM (1982) Mammalian evolution and the great American interchange. *Science*, **215**, 1351–1357.

Martin AP, Naylor GJP, Palumbi SR (1992). Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature*, **357**, 153.

Mathews LM, Anker A (2009) Molecular phylogeny reveals extensive ancient and ongoing radiations in a snapping shrimp species complex (Crustacea, Alpheidae, *Alpheus armillatus*). *Molecular Phylogenetics and Evolution*, **50**, 268–281.

Mayden RL (1997) A hierarchy of species concepts: the denouement in the saga of the species problem. In: *Species: the Units of Biodiversity* (eds Claridge MF *et al.*), pp. 381–424. Chapman & Hall, London.

Mayfield MM, Levine JM (2010) Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters*, **13**, 1085–1093.

Mayr E (1940) Speciation phenomena in birds. *The American Naturalist*, **74**, 249–278.

Mayr E (1942) *Systematics and the origin of species, from the viewpoint of a zoologist*. Harvard University Press, Cambridge (Massachusetts), 334 pp.

McLoughlin S (2001) The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany*, **49**, 271–300.

Measey GJ, Vences M, Drewes RC, Chiari Y, Melo M, Bourles B (2007) Freshwater paths across the ocean: molecular phylogeny of the frog *Ptychadena newtoni* gives insights into amphibian colonization of oceanic islands. *Journal of Biogeography*, **34**, 7–20.

Melo GAS (1996) *Manual de Identificação dos Brachyura (Caranguejos e Siris) dos Litoral Brasileiro*. Plêiade / FAPESP, São Paulo, Brazil, 604 pp.

Miller MA, Pfeiffer W, Schwartz T (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees". In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans, LA pp 1–8.

Milne Edwards H (1837) *Histoire Naturelle des Crustacés Comprenant l'Anatomie, la Physiologie et la Classification de ces Animaux*. Libraries Encyclopédique de Roret, Paris. vol. 2, 531 pp.

Milne Edwards H (1853) Mémoire sur la famille des Ocypodiens. *Annales des Sciences Naturelles*, **20**, 163–228.

Miura O, Torchin M, Bermingham E (2010) Molecular phylogenetics reveals differential divergence of coastal snails separated by the Isthmus of Panama. *Molecular Phylogenetics and Evolution*, **56**, 40–48.

Miura O, Torchin ME, Bermingham E, Jacobs DK, Hechinger RF (2012) Flying shells: historical dispersal of marine snails across Central America. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1061–1067.

Montes C, Bayona G, Cardona A, Buchs DM, Silva CA, Morón S, Hoyos N, Ramírez DA, Jaramillo CA, Valencia V (2012b) Arc-continent collision and orocline formation: Closing of the Central American seaway. *Journal of Geophysical Research*, **117**.

Montes C, Cardona A, McFadden R, Morón SE, Silva CA, Restrepo-Moreno S, Ramírez DA, Hoyos N, Wilson J, Farris D, Bayona GA, Jaramillo CA, Valencia V, Bryan J, Flores

JA (2012a) Evidence for middle Eocene and younger land emergence in central Panama: Implications for Isthmus closure. *Geological Society of America Bulletin*, **124**, 780–799.

Morrison CL, Rios R, Duffy JE (2004) Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (*Synalpheus*). *Molecular Phylogenetics and Evolution*, **30**, 563–581.

N

Naderloo R (2011) Grapsoid crabs (Decapoda: Brachyura: Thoracotremata) of the Persian Gulf and the Gulf of Oman. *Zootaxa*, **3048**, 1–43.

Naderloo R, Schubart CD (2009) Redescription and mitochondrial identification of *Chiromantes boulengeri* (Calman, 1920)(Decapoda: Brachyura: Sesarmidae) based on fresh material from the Persian Gulf, Iran. *Zootaxa*, **2128**, 61–68.

Naderloo R, Schubart CD (2010) Description of a new species of *Parasesarma* (Crustacea; Decapoda; Brachyura; Sesarmidae) from the Persian Gulf, based on morphological and genetic characteristics. *Zoologischer Anzeiger*, **249**, 33–43.

Nagelkerken I, Blaber SJM, Bouillon S, Green P, Haywood M, Kirton LG, Meynecke J-O, Pawlik J, Penrose HM, Sasekumar A, Somerfield PJ (2008) The habitat function of mangroves for terrestrial and marine fauna: a review. *Aquatic Botany*, **89**, 155–185.

Nettel A, Dodd RS (2007) Drifting propagules and receding swamps: Genetic footprints of mangrove recolonization and dispersal along tropical coasts. *Evolution*, **61**, 958–971.

Ng PK (2002) New species of cavernicolous crabs of the genus *Sesarmoides* from the western Pacific, with a key to the genus (Crustacea: Decapoda: Brachyura: Sesarmidae). *The Raffles Bulletin of Zoology*, **50**, 419–435.

Ng PK, Guinot D, Davie P (2008) Systema Brachyurorum: Part 1. An annotated checklist of extant brachyuran crabs of the world. *The Raffles Bulletin of Zoology*, **17**, 1–286.

Ng PK, Liu HC (1999) The taxonomy of *Sesarma tangi* Rathbun, 1931 and *S. stormi* De Man, 1895 (Crustacea: Decapoda: Brachyura: Grapsidae: Sesarminae), with establishment of a new genus for *S. stormi*. *Zoological Studies*, **38**, 228–237.

Niem VH (1993) Phylogenetic and systematic position of *Sesarma rubripes* Rathbun, 1897 (Brachyura: Grapsidae). *Zoologische Mededelingen*, **67**, 185–195.

Niem VH (1996) Phylogenetic relationships among American species of *Sesarma* (subgenus *Armases*)(Brachyura, Grapsidae). *Crustaceana*, **69**, 330–348.

Nóbrega R, Solé-Cava A, Russo C (2004) High genetic homogeneity of an intertidal marine invertebrate along 8000 km of the Atlantic coast of the Americas. *Journal of Experimental Marine Biology and Ecology*, **303**, 173–181.

Nuin P (2005) MrMTgui 1.0 (version 1.6). Program distributed by the author at http://www.genedrift.org/mtgui.php

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

O

O'Dea A, Jackson JB, Fortunato H, Smith JT, D'Croz L, Johnson KG, Todd JA (2007) Environmental change preceded Caribbean extinction by 2 million years. *Proceedings of the National Academy of Sciences*, **104**, 5501–5506.

Oliveira-Neto J, Pie M, Boeger W, Ostrensky A, Baggio R (2007) Population genetics and evolutionary demography of *Ucides cordatus* (Decapoda: Ocypodidae). *Marine Ecology*, **28**, 460–469.

Ornelas JF, González C, de los Monteros AE, Rodríguez-Gómez F, García-Feria LM (2014) In and out of Mesoamerica: temporal divergence of *Amazilia* hummingbirds pre-dates the orthodox account of the completion of the Isthmus of Panama. *Journal of Biogeography*, **41**, 168–181.

P

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, **25**, 547–572.

Papadopoulou A, Anastasiou I, Vogler AP (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution*, **27**, 1659–1672.

Patarnello T, Volckaert FA, Castilho R (2007) Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographical break?. *Molecular Ecology*, **16**, 4426–4444.

Pil MW, Boeger MR, Muschner VC et al. (2011) Postglacial north-south expansion of populations of *Rhizophora mangle* (Rhizophoraceae) along the Brazilian coast revealed by microsatellite analysis. *American Journal of Botany*, **98**, 1031–1039.

Pileggi LG, Mantelatto FL (2010). Molecular phylogeny of the freshwater prawn genus *Macrobrachium* (Decapoda, Palaemonidae), with emphasis on the relationships among selected American species. *Invertebrate Systematics*, **24**, 194–208.

Pineda J, Hare JA, Sponaugle S (2007) Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanography*, **20**, 22–39.

Pinto-Sánchez N, Ibáñez R, Madriñán S, Sanjur OI, Bermingham E, Crawford AJ (2012) The Great American Biotic Interchange in frogs: multiple and early colonization of Central America by the South American genus *Pristimantis* (Anura: Craugastoridae). *Molecular Phylogenetics and Evolution*, **62**, 954–972.

Piso W, Marcgrave G (1648) *Historia Naturalis Brasiliae*. 293 pp.

Posada D, Buckley T (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, **53**, 793–808.

Poupin J, Davie PJF, Cexus JC (2005) A revision of the genus *Pachygrapsus* Randall, 1840 (Crustacea: Decapoda: Brachyura, Grapsidae), with special reference to the Southwest Pacific species. *Zootaxa*, **1015**, 1–66.

Powers LW (1977) A catalogue and bibliography to the crabs (Brachyura) of the Gulf of Mexico. *Contributions to Marine Science*, **Supplement 20**, 1–190.

Q

Quenouille B, Hubert N, Bermingham E, Planes S (2011) Speciation in tropical seas: allopatry followed by range change. *Molecular Phylogenetics and Evolution*, **58**, 546–552.

R

Rathbun MJ (1897) Synopsis of the American Sesarmae, with description of a new species. *Proceedings of the Biological Society of Washington*, **11**, 89–92.

Rathbun MJ (1918) The grapsoid crabs of America. *Bulletin of the United States National Museum*, **97**, xxii + 461, pls. 1–161.

Rebolledo-Navarro A, Wehrtmann IS (2012) Population structure of the mangrove crab *Aratus pisonii* (Brachyura: Sesarmidae) from the Pacific coast of Costa Rica. *The Crustacean Society summer meeting*, June 2012, Athens, Greece, Poster presentation session C, 14.

Regan CT (1926) Organic Evolution. *Report of the British Association for the Advancement of Science*, **1925**, 75–86.

Renner SS, Clausing G, Meyer K (2001) Historical biogeography of Melastomataceae: the roles of Tertiary migration and long-distance dispersal. *American Journal of Botany*, **88**, 1290–1300.

Riley ME, Johnston CA, Feller IC, Griffen BD (2014) Range expansion of *Aratus pisonii* (mangrove tree crab) into novel vegetative habitats. *Southeastern Naturalist*, **13**, N43–N48.

Roberts CM, McClean CJ, Veron JE, Hawkins JP, Allen GR, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F, Vynne C, Werner TB (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, **295**, 1280–1284.

Robertson D, Cramer K (2014) Defining and dividing the Greater Caribbean: insights from the biogeography of shorefishes. *PLoS One*, **9**, e102918.

Rocha LA (2003) Patterns of distribution and processes of speciation in Brazilian reef fishes. *Journal of Biogeography*, **30**, 1161–1171.

Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Molecular Ecology*, **11**, 243–252.

Rocha LA, Rocha CR, Robertson DR, Bowen BW (2008) Comparative phylogeography of Atlantic reef fishes indicates both origin and accumulation of diversity in the Caribbean. *BMC Evolutionary Biology*, **8**, 157.

Rocha LA, Robertson DR, Rocha CR, Tassell JL, Craig MT, Bowen BW (2005) Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish. *Molecular Ecology*, **14**, 3921–3928.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard, MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

Rull V (2011) Neotropical biodiversity: timing and potential drivers. *Trends in Ecology & Evolution*, **26**, 508–513.

Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream M-A, Barrell B (2000) Artemis: sequence visualization and annotation. *Bioinformatics*, **16**, 944–945.

S

Sakai K, Türkay M, Yang S-L (2006) Revision of the *Helice*/*Chasmagnathus* complex (Crustacea: Decapoda: Brachyura). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **565**, 1–76.

Scheffers BR, Joppa LN, Pimm SL, Laurance WF (2012) What we know and don't know about Earth's missing biodiversity. *Trends in Ecology & Evolution*, **27**, 501–510.

Schrago CG, Russo CAM (2003) Timing the origin of New World monkeys. *Molecular Biology and Evolution*, **10**, 1620–1625.

Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: *Crustacean Issues*, **18***: Decapod Crustacean Phylogenetics* (eds Martin JW, Crandall KA, Felder DL), pp. 45–63. Taylor & Francis/CRC Press, Boca Raton, Florida.

Schubart CD (2011) Reconstruction of phylogenetic relationships within Grapsidae (Crustacea: Brachyura) and comparison of trans-isthmian versus amphi-atlantic gene flow based on mtDNA. *Zoologischer Anzeiger*, **250**, 472–478.

Schubart CD, Cannicci S, Vannini M, Fratini S (2006) Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current superfamily classification. *Journal of Zoological Systematics and Evolutionary Research*, **44**, 193–199.

Schubart CD, Cuesta JA (1998) First zoeal stages of four *Sesarma* species from Panama, with identification keys and remarks on the American Sesarminae (Crustacea: Brachyura: Grapsidae). *Journal of Plankton Research*, **20**, 61–84.

Schubart CD, Cuesta J, Diesel R, Felder DL (2000) Molecular phylogeny, taxonomy, and evolution of nonmarine lineages within the American grapsoid crabs (Crustacea: Brachyura). *Molecular Phylogenetics and Evolution*, **15**, 179–190.

Schubart CD, Cuesta JA, Felder DL (2002) Glyptograpsidae, a new brachyuran family from Central America: larval and adult morphology, and a molecular phylogeny of the Grapsoidea. *Journal of Crustacean Biology*, **22**, 28–44.

Schubart CD, Cuesta JA, Felder DL (2006) Phylogeography of *Pachygrapsus transversus* (Gibbes, 1850): The effect of the American continent and the Atlantic Ocean as gene flow barriers and recognition of *Pachygrapsus socius* Stimpson 1871 as a valid species. *Nauplius*, **13**, 99–113.

Schubart CD, Diesel R, Hedges S (1998) Rapid evolution to terrestrial life in Jamaican crabs. *Nature*, **393**, 363–365.

Schubart CD, González-Gordillo JI, Reyns NB, Liu HC, Cuesta JA (2001) Are Atlantic and Indo-Pacific populations of the rafting crab, *Plagusia depressa* (Fabricius), distinct? New evidence from larval morphology and mtDNA. *Raffles Bulletin of Zoology*, **49**, 301–310.

Schubart CD, Koller P (2005) Genetic diversity of freshwater crabs (Brachyura: Sesarmidae) from central Jamaica with description of a new species. *Journal of Natural History*, **39**, 469–481.

Schubart CD, Liu HC, Ng PK (2009) Revision of *Selatium* Serène & Soh, 1970 (Crustacea: Brachyura: Sesarmidae), with description of a new genus and two new species. *Zootaxa*, **2154**, 1–29.

Schubart CD, Ng PK (2008) A new molluscivore crab from Lake Poso confirms multiple colonization of ancient lakes in Sulawesi by freshwater crabs (Decapoda: Brachyura). *Zoological Journal of the Linnean Society*, **154**, 211–221.

Schubart CD, Santl T (2014) Differentiation within a river system: ecology or geography driven? Evolutionary significant units and new species in Jamaican freshwater crabs. In *Advances in Freshwater Decapod Systematics and Biology* (eds Yeo DCJ, Klaus S and Cumberlidge N), 173–193, University of Hull, Kingston upon Hull.

Schubart CD, Santl T, Koller P (2008) Mitochondrial patterns of intra- and interspecific differentiation among endemic freshwater crabs of ancient lakes in Sulawesi. *Contributions to Zoology*, **77**, 83–90.

Serène R (1968) The Brachyura of the Indo-West Pacific region. *In: Prodromus for a Check List of the Non-Planctonic Marine Fauna of South East Asia*, UNESCO, Singapore National Academy of Science, Special Publication 1, Fauna III Cc3, pp. 33–118.

Serène R, Soh C (1970) New Indo-Pacific genera allied to *Sesarma* Say 1817 (Brachyura, Decapoda, Crustacea). *Treubia*, **27**, 387–416.

Shanks AL, Grantham BA, Carr MH (2003) Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications*, **13 Supplement**, 159–169.

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution*, **12**, 35–37.

Simpson GG (1961) *Principles of Animal Taxonomy*. Columbia University Press, New York, NY and London, 247 pp.

Smith SI (1871) List of the Crustacea collected by J. A. McNiel in Central America. *Annual Report of the Peabody Academy of Science*, **1869**, 87–98.

Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, Jorge MA, Lombana A, Lourie SA, Martin KD, McManus E, Molnar J, Recchia CA, Robertson J (2007) Marine ecoregions of the world: a bioregionalization of coast and shelf areas. *BioScience*, **57**, 573–583.

Sperling FA, Spence JR, Andersen NM (1997) Mitochondrial DNA, allozymes, morphology, and hybrid compatibility in *Limnoporus* water striders (Heteroptera: Gerridae): do they all track species phytogenies?. *Annals of the Entomological Society of America*, **90**, 401–415.

Stehli F, Webb S (1985) The Great American Biotic Interchange. *Plenum Press*, New York.

Števčić Z (2005) The reclassification of brachyuran crabs (Crustacea: Decapoda: Brachyura). *Natura Croatica*, **14**, 1–159.

T

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.

Takahashi K, Terai Y, Nishida M, Okada N (2001) Phylogenetic relationships and ancient incomplete lineage sorting among cichlid fishes in Lake Tanganyika as revealed by analysis of the insertion of retroposons. *Molecular Biology and Evolution*, **18**, 2057–2066.

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. In: *Some Mathematical Questions in Biology — DNA Sequence Analysis* (ed. Miura RM), pp. 57–86, American Mathematical Society, Providence.

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science*, **299**, 107–109.

Tesch JJ (1917) Synopsis of the genera *Sesarma, Metasesarma, Sarmatium* and *Clistocoeloma*, with a key to the determination of the Indo-Pacific species. *Zoologische Mededelingen Leiden*, **3**, 127–260.

Thiercelin N, Schubart CD (2014) Transisthmian differentiation in the tree-climbing mangrove crab *Aratus* H. Milne Edwards, 1853 (Crustacea, Brachyura, Sesarmidae),

with description of a new species from the tropical eastern Pacific. *Zootaxa*, **3795**, 545–560.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

Tsang LM, Schubart CD, Ahyong ST, Lai JC, Au EY, Chan TY, Ng PKL, Chu KH (2014) Evolutionary history of true crabs (Crustacea: Decapoda: Brachyura) and the origin of freshwater crabs. *Molecular Biology and Evolution*, **31**, 1173–1187.

Tweedie M (1940) New and interesting Malaysian species of *Sesarma* and *Utica* (Crustacea Brachyura). *The Raffles Bulletin of Zoology*, **16**, 88–11.

V

Vences M, Vieites D, Glaw F, Brinkmann H, Kosuch J, Veith M, Meyer A (2003) Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 2435–2442.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. *Ecology Letters*, **14**, 782–787.

Vogt G (2013) Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. *Biological Reviews*, **88**, 81–116.

Von Hagen (1978) The systematic position of *Sesarma* (*Sesarma*) *rectum* Randall and a new definition of the subgenus *Sesarma* (Brachyura, Grapsidae). *Crustaceana*, **34**, 45–54.

W

Wallace AR (1876) The geographical distribution of animals; with a study of the relations of living and extinct faunas as elucidating the past changes of the Earth's surface. *Macmillan & Co*, London.

Wares J (2001) Patterns of speciation inferred from mitochondrial DNA in North American *Chthamalus* (Cirripedia: Balanomorpha: Chthamaloidea). *Molecular Phylogenetics and Evolution*, **18**, 104–116.

Warner GF (1967) The life history of the mangrove crab, *Aratus pisonii*. *Journal of the Zoological Society, London*, **153**, 321–335.

Warner GF (1968) The larval development of the mangrove tree crab, *Aratus pisonii* (H. Milne Edwards), reared in the laboratory (Brachyura, Grapsidae). *Crustaceana Supplement*, **2**, 249–258.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. *Annual Review of Ecology, Evolution, and Systematics*, **33**, 475–505.

Webb SD (1991) Ecogeography and the great American interchange. *Paleobiology*, **17**, 266 – 280.

Wehrtmann IS, Rebolledo-Navarro A (2012) Reproduction of a transisthmian crab species: comparing egg production of *Aratus pisonii* (Brachyura: Sesarmidae) from both coasts of Costa Rica. *The Crustacean Society summer meeting*, June 2012, Athens, Greece, oral presentation.

Wilke T (2003) *Salenthydrobia* gen. nov. (Rissooidea: Hydrobiidae): a potential relict of the Messinian salinity crisis. *Zoological Journal of the Linnean Society*, **137**, 319–336.

Williams ST, Knowlton N (2001) Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. *Molecular Biology and Evolution*, **18**, 1484–1493.

Williams ST, Knowlton N, Weigt LA, Jara JA (2001) Evidence for three major clades within the snapping shrimp genus *Alpheus* inferred from nuclear and mitochondrial gene sequence data. *Molecular Phylogenetics and Evolution*, **20**, 375–89.

Woodburne MO (2010) The Great American Biotic Interchange: dispersals, tectonics, climate, sea level and holding pens. *Journal of Mammalian Evolution*, **17**, 245–264.

Woodroffe C, Grindrod J (1991) Mangrove biogeography: the role of quaternary environmental and sea-level change. *Journal of Biogeography*, **18**, 479–492.

X

Xie W, Lewis PO, Fan Y, Kuo L & Chen MH (2010) Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology*, **60**, 150–160.

Z

Zigler KS, Lessios HA (2004) Speciation on the coasts of the new world: phylogeography and the evolution of bindin in the sea urchin genus *Lytechinus*. *Evolution*, **58**, 1225–1241.

SUPPLEMENTARY MATERIAL

Figure S1.1. Maximum parsimony spanning network of the sister species *Pachygrapsus transversus* (Atlantic Ocean) and *P. socius* (Costa Rica, Pacific Ocean) reconstructed with TCS based on 60 mtDNA Cox1 sequences (955bp). Each line represents a substitution; dots represent single missing haplotype, double bars with values indicates multiple missing haplotypes; size of the haplotypes proportional to the number of individuals with number of specimens indicated if superior at one.

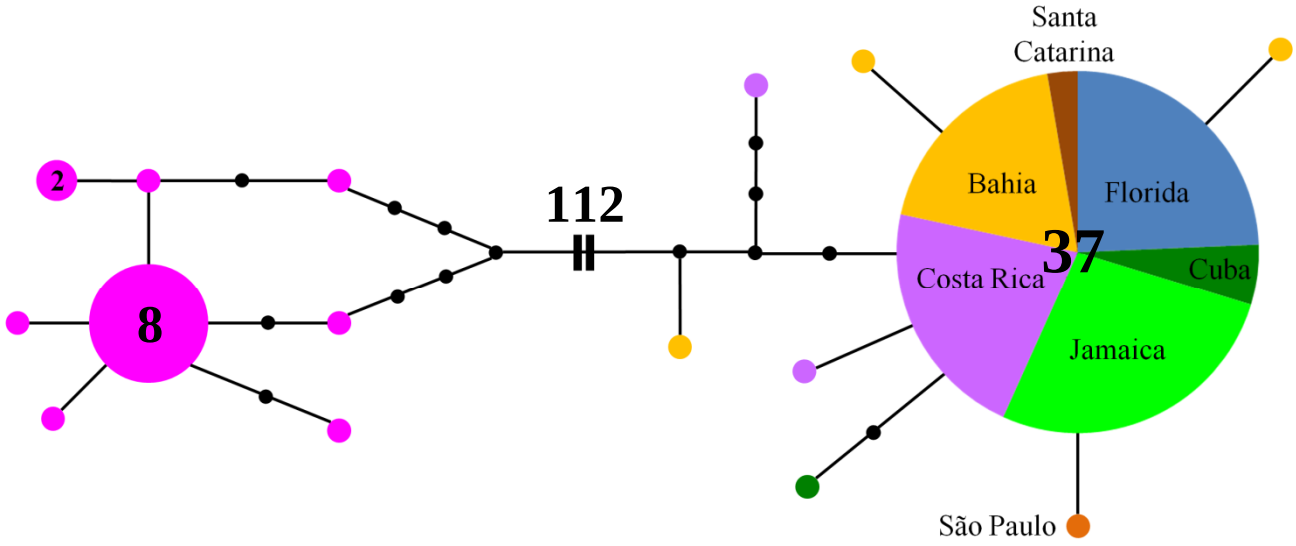


Table S3.1. Detail of brachyuran specimens, including habitat of the species, sampling locality (with coordinates when available), GenBank accession numbers and museum collection numbers. **A.** Cox1.

Species	Habitat	Ocean	Sampling locality	Coordinates	Specimens	GenBank	Collection number
Aratus							
Aratus pisonii	Mangrove	Atlantic	*USA.* Florida, Naples, Marco Island		488-14 -15	•	
			Dominican Republic. Bahía de Las Calderas, Las Salinas	18°12.75'N 70°32.46'W	487-1	•	MNHN-IU-2009-3094
			Jamaica. St. Ann Parish, Priory	18°27.1'N 77°13.5'W	568-1 -2	•	RMNH.CRUS.D.55078
			Brazil. Pará, Marapanim, Marudá	0°37.023'S 47°37.946'W	476-4 -12 -14	•	SMF 43533
Aratus sp. Gulf of Mexico			*Mexico.* Tamaulipas, Barra del Tordo		L15	•	ULLZ 3701
			Mexico. Veracruz, Laguna Buenpais (Laguna Alvarado)		653-7	•	ULLZ 4078
Aratus pacificus		Pacific	*Costa Rica.* Puntarenas, Mata de Limón	9°55.422'N 84°42.759'W	593-1 -3 -8	•	MZUCR 3173-01
			Ecuador. Puerto Morro	2°36.498'S 80°18.245'W	490-14 -15 -18	•	MNHN-IU-2009-3091 ; MNHN-IU-2009-3092 ; RMNH.CRUS.D.55083
Aratus sp. Pacific			*Mexico.* Baja California, Bahia Magdalena, Puerto San Carlos		653-9 -11	•	ULLZ 4110
			Costa Rica. Puntarenas, Mata de Limón	9°55.422'N 84°42.759'W	593-6 -11 -18	•	
Armases							
Armases americanum	Mangrove	Atlantic	*Costa Rica.* Limón, Cahuita	9°44'N 82°50'W	623-1	•	
			Costa Rica. Limón, Punta Uva	9°37.930'N 82°40.200'W	630-2 -3 -5 -7 -8 -9	•	
			Venezuela. Turiamo, Rio San Miguel	10°25.837'N 67°50.974'W	658-1 to -6 & -8 to -12	•	
Armases angustum		Pacific	*Costa Rica.* Golfo Dulce, Bahia Cana Blanca		637-1 -2 -3	•	
Geograpsus							
Geograpsus lividus	Rocky shore	Atlantic	*Puerto Rico.* Culebra Island	18°19.170'N 65°15.296'W 18°18.019'N 65°15.709'W	314-10 620-1 -2 -3	•	
			Puerto Rico. Maunabo	17°58.554'S 65°55.958'W	342-6 -7 -8 -9	•	
			Jamaica		620-4	•	

			Jamaica. St. Ann Parish, Priory, Hofstra Marine Lab		620-5 -6	•
Geograpsus lividus '*occidentale*'		Pacific	*Mexico.* Baja California, Mulege		597-1 to -5 620-8	•
			Panama. Naos		580-6 -7 -9 -12	•
Goniopsis						•
Goniopsis cruentata	Mangrove	Atlantic	*Brazil.* São Paulo State, Ubatuba, Maranduba, Rio Maranduba	23°32.955'S 45°13.953'W 23°32.962'S 45°13.952'W	298-1 -3 -4 -8 318-2 -3	•
Goniopsis pulchra		Pacific	*Costa Rica.* Puntarenas, Punta Morales		401-2 -4 -8	•
			Costa Rica. Puntarenas, Golfito		304-1 -2 -3 -5 -8	•
Grapsus						
Grapsus grapsus	Rocky shore	Atlantic	*Puerto Rico.* Bosque Estatal Guánica, Bahía Ballena	17°57.271'N 66°51.125'W	SR26-1 -2 -3 -4 -5	•
			Jamaica. Portland Parish, Christmas River		PA94-1	•
Grapsus grapsus		Pacific	*Panama.* Naos		PA96-5	•
Pachygrapsus						
Pachygrapsus transversus	Rocky shore	Atlantic	*USA.* Florida, Boca Raton, Boca Inlet		627-2	•
			Cuba. Pinar del Rio, Puerto Esperanta		314-7	•
			Jamaica. St. Ann Parish, Priory	18°27.208'N 77°13.578'W	576-1	•
			Costa Rica. Limón, Manzanillo	9°38.243'N 82°39.028'W	592-2 -3 -4 -5 -8 -9	•
			Brazil. Bahia, Ilhéus	between 14°48.676'S 39°01.448'W and 14°49.019'S 39°01.497'W	574-2 -3 -4 -5 -6 -11	•
			Brazil. São Paulo, Praia do Segundo	23°49.67'S 45°25.37'W	311-8	•
Pachygrapsus socius		Pacific	*Costa Rica.* Puntarenas, Mata de Limón	9°55.369'N 84°42.564'W	582-4	•
			Costa Rica. Puntarenas, Golfo Dulce, Golfito	8°38.578'N 83°10.354'W	455-1 to -15	•

Table S3.1 (continued). Detail of brachyuran specimens, including habitat of the species, sampling locality (with coordinates when available), GenBank accession numbers and museum collection numbers. **B.** 16S rRNA.

Species	Habitat	Ocean	Sampling locality	Coordinates	Specimens	GenBank	Museum number
Aratus							
Aratus pisonii	Mangrove	Atlantic	*USA.* Florida, Naples, Marco Island		488-14 -15	•	
			Dominican Republic. Bahía de Las Calderas, Las Salinas	18°12.75'N 70°32.46'W	487-1	•	MNHN-IU-2009-3094
			Jamaica. St. Ann Parish, Priory	18°27.1'N 77°13.5'W	568-1 -2	•	RMNH.CRUS.D.55078
			Brazil. Pará, Marapanim, Marudá	0°37.023'S 47°37.946'W	476-4 -12 -14	•	SMF 43533
Aratus sp. Gulf of Mexico			*Mexico.* Tamaulipas, Barra del Tordo		L15	•	ULLZ 3701
			Mexico. Veracruz, Laguna Buenpais (Laguna Alvarado)		653-7	•	ULLZ 4078
Aratus pacificus		Pacific	*Costa Rica.* Puntarenas, Mata de Limón	9°55.422'N 84°42.759'W	593-1 -3 -8	•	MZUCR 3173-01
			Ecuador. Puerto Morro	2°36.498'S 80°18.245'W	490-14 -15 -18	•	MNHN-IU-2009-3091 ; MNHN-IU-2009-3092 ; RMNH.CRUS.D.55083
Aratus sp. Pacific			*Mexico.* Baja California, Bahia Magdalena, Puerto San Carlos		653-9 -11	•	ULLZ 4110
			Costa Rica. Puntarenas, Mata de Limón	9°55.422'N 84°42.759'W	593-6 -11 -18	•	
Armases							
Armases americanum	Mangrove	Atlantic	*Costa Rica.* Limón, Cahuita	9°44'N 82°50'W	623-1	•	
			Costa Rica. Limón, Punta Uva	9°37.930'N 82°40.200'W	630-1 -2 -3 -4	•	
			Venezuela. Turiamo, Rio San Miguel	10°25.837'N 67°50.974'W	658-1 to -5	•	
Armases angustum		Pacific	*Costa Rica.* Golfo Dulce, Bahia Cana Blanca		637-1 -2 -3	•	

Geograpsus						
Geograpsus lividus	Rocky shore	Atlantic	*Puerto Rico.* Culebra Island	18°19.170'N 65°15.296'W + 18°18.019'N 65°15.709'W	620-1 -2 -3	•
			Puerto Rico. Maunabo	17°58.554'S 65°55.958'W	342-6 -7 -8	•
			Jamaica.		620-4	•
			Jamaica. St-Ann, Priory, Hofstra Marine Lab		620-5 -6	•
Geograpsus lividus '*occidentale*'		Pacific	*Mexico.* Baja California, Mulege		597-1 to -5 620-8	•
			Panama. Naos		580-6 -7 -9 -12	•
Goniopsis						
Goniopsis cruentata	Mangrove	Atlantic	*Brazil.* São Paulo State, Ubatuba, Maranduba, Rio Maranduba	23°32.955'S 45°13.953'W + 23°32.962'S 45°13.952'W	298-3 -4 -8	•
Goniopsis pulchra		Pacific	*Costa Rica.* Puntarenas, Punta Morales		401-2 -4 -8	•
			Costa Rica. Puntarenas, Golfito		304-1 -2 -3 -5	•
Grapsus						
Grapsus grapsus	Rocky shore	Atlantic	*Puerto Rico.* Bosque Estatal Guánica, Bahía Ballena	17°57.271'N 66°51.125'W	SR26-1 to -5	•
			Jamaica. Portland Parish, Christmas River		PA94-1	•
Grapsus grapsus		Pacific	*Panama.* Naos		PA96-5	•
Pachygrapsus						
Pachygrapsus transversus	Rocky shore	Atlantic	*Costa Rica.* Limón, Manzanillo	9°38.243'N 82°39.028'W	592-1 to -5	•
			Brazil. Bahia, Ilhéus	between 14°48.676'S 39°01.448'W and 14°49.019'S 39°01.497'W	574-2 to -6	•
Pachygrapsus socius		Pacific	*Costa Rica.* Puntarenas, Golfo Dulce, Golfito	8°38.578'N 83°10.354'W	455-1 -3 to -9	•

Document S3.1. Configuration file *batch.masterIn.fromIM* of msBayes.

```
# bounds for theta per site (guessed from observed pi within subpops)
upperTheta = 0.0180848
lowerTheta = 4e-11
# upper limit of tau (divergence time)
upperTau = 3.0
# number of tau classes (Psi): 0 means Psi are drawn from [1,#taxonPairs]
numTauClasses = 0
# upper bound of migration rate (0 disables migration)
upperMig = 0.0
upperRec = 0.0
# Ancestral theta multiplier:
#  product of this and upperTheta is the upper bound of ancestral theta
upperAncPopSize = 0.25
reps = 1000000
# Most users don't want to constrain the subparameters
constrain = 0
subParamConstrain = 111111111

# taxonName locusName   Ne_Scalar   Mut_Scalar  sampleSizeA sampleSizeB tstv  seqLen      Afreq Cfreq Gfreq
      fastaFileName
BEGIN SAMPLE_TBL
AratusCOI   CO1   0.25  20    10    11    9     926   0.295 0.177 0.148 fastaFromIM/AratusCOI_CO1.fasta
ArmasesCOI  CO1   0.25  20    20    3     6     960   0.290 0.158 0.147 fastaFromIM/ArmasesCOI_CO1.fasta
GeograpsusCOI     CO1   0.25  20    11    10    5     802   0.256 0.202 0.153 fastaFromIM/GeograpsusCOI_CO1.fasta
GrapsusCOI  CO1   0.25  20    6     1     8     579   0.273 0.223 0.147 fastaFromIM/GrapsusCOI_CO1.fasta
GoniopsisCOI      CO1   0.25  20    6     8     3     790   0.259 0.193 0.169 fastaFromIM/GoniopsisCOI_CO1.fasta
PachygrapsusCOI   CO1   0.25  20    16    16    4     953   0.226 0.266 0.165 fastaFromIM/PachygrapsusCOI_CO1.fasta
END SAMPLE_TBL

# Most users can ignore the following table
BEGIN CONSTRAIN
1.0   0.9   0.1   0.5   0.0   10.1  1.5   0.1   0.0
1.1   0.8   0.2   0.6   0.0   20.1  1.4   0.2   0.0
1.2   0.7   0.3   0.7   0.0   30.1  1.3   0.3   0.0
1.0   0.3   0.7   0.8   0.0   40.1  1.2   0.4   0.0
1.0   0.3   0.8   0.9   0.0   5.1   1.1   0.5   0.0
1.0   0.3   0.9   0.3   0.0   25.1  1.0   0.5   0.0
END CONSTRAIN
```

Table S3.2. Model comparison for both Cox1 and 16S rRNA genes with results of each cloned simulation. The Bayes factor between models is compared to the Standard model.

Cox1		Path Sampling						
	Model	**clone 1**	**clone 2**	**clone 3**	**clone 4**	**Mean**	**2ln(Mean)**	**Best 2lbBF**
Sesarmidae	**3 mya**	-2775.0	-2743.0	-2774.2	-2742.5	-2758.7	11.6	19.6
	15 mya	-2773.2	-2775.0	-2752.3	-2757.3	-2764.5		
	Null	-2833.1	-2838.2			-2835.7	154.0	181.3
Grapsidae	**3 mya**	-4301.7	-4300.5	-4299.8	-4302.7	-4301.2	3.7	1.4
	15 mya	-4300.6	-4304.2	-4303.9	-4303.4	-4303.0		
	Null	-4371.5	-4368.9			-4370.2	138.1	138.1

		Stepping stone Sampling						
	Model	**clone 1**	**clone 2**	**clone 3**	**clone 4**	**Mean**	**2ln(Mean)**	**Best 2lbBF**
Sesarmidae	**3 mya**	-2775.0	-2729.2	-2774.2	-2731.6	-2752.5	17.1	34.6
	15 mya	-2772.0	-2774.7	-2746.5	-2751.1	-2761.1		
	Null	-2833.4	-2838.5			-2835.9	166.9	208.3
Grapsidae	**3 mya**	-4301.6	-4299.4	-4299.2	-4302.6	-4300.7	3.7	1.1
	15 mya	-4299.7	-4304.0	-4303.8	-4302.6	-4302.5		
	Null	-4371.7	-4369.4			-4370.5	139.7	140.4

16S rRNA		Path Sampling						
	Model	**clone 1**	**clone 2**	**clone 3**	**clone 4**	**Mean**	**2ln(Mean)**	**Best 2lbBF**
Sesarmidae	**3 mya**	-1197.8	-1213.9	-1176.4	-1178.6	-1191.6	25.9	14.8
	15 mya	-1205.5	-1215.2	-1213.8	-1183.8	-1204.6		
	Null	-1257.3	-1272.6			-1264.9	146.5	161.8
Grapsidae	**3 mya**	-2288.1	-2281.6	-2288.0	-2286.3	-2286.0	4.3	12.5
	15 mya	-2287.9	-2288.3	-2288.6	-2287.9	-2288.2		
	Null	-2341.1	-2342.2			-2341.7	111.3	118.9

		Stepping stone Sampling						
	Model	**clone 1**	**clone 2**	**clone 3**	**clone 4**	**Mean**	**2ln(Mean)**	**Best 2lbBF**
Sesarmidae	**3 mya**	-1192.1	-1209.6	-1168.2	-1170.7	-1185.2	28.0	10.2
	15 mya	-1200.3	-1213.3	-1209.7	-1173.3	-1199.2		
	Null	-1252.6	-1272.4			-1262.5	154.7	168.8
Grapsidae	**3 mya**	-2288.0	-2280.3	-2287.9	-2286.2	-2285.6	4.6	14.8
	15 mya	-2287.7	-2287.7	-2288.5	-2287.7	-2287.9		
	Null	-2341.2	-2342.5			-2341.9	112.6	121.9

Table S4.1. Table detailing the species identification, sampling locality, catalog number and GenBank accession number for the Cox1, 16S rRNA and 28S rRNA genes. Bullets refers to sequences that will be submitted to GenBank; dashes indicate missing sequences.

Species	Locality	Catalog number	Cox1	16S rRNA	28S rRNA
Armases					
americanum	*Costa Rica.* Limón, Cahuita		•	•	•
angustipes	*Brazil.* Bahia, Acuipe, Rio Acuipe		•	•	•
angustum	*Costa Rica.* Cana Blanca		•	•	•
benedicti	*Brazil.* Pará, Belém, University campus		•	•	•
cinereum	*Mexico.* Tamaulipas, la Pesca		•	•	•
elegans	*Ghana.* Ada Foah		•	•	•
gorei	*Ecuador*. Guayas, Puerto Morro		•	•	•
magdalenense	*Mexico.* Baja California, Bocana del Rio, Mulege		•	•	–
miersii	*Jamaica.* Trelawny Parish, Glistening Water		•	•	•
occidentale	*Costa Rica.* Rincon		•	•	•
occidentale	*Panama.* Miraflores Locks		•	•	–
occidentale	*Panama.* Farfán		AJ225881	AJ225856	–
ricordi	*Costa Rica.* Limón, Manzanillo		•	•	•
ricordi	*Jamaica.* Trelawny Parish, Glistening Water		AJ225887	AJ225876	-
roberti	*Jamaica.* Mintpiece River		•	•	•
rubripes	*Brazil.* São Paulo, Bertioga, Rio Itapanhau		•	•	•
nr. rubripes	*Venezuela.* Tacarigua		•	•	–
Aratus					
pisonii	*USA.* Florida, Naples, Marco Island		•	•	•
pisonii	*USA.* Florida, Naples, Marco Island		•	•	•
pisonii	*Jamaica.* St. Ann Parish, Priory	RMNH.CRUS.D.55078	•	•	•
pisonii	*Jamaica.* St. Ann Parish, Priory	RMNH.CRUS.D.55078	•	•	•
pisonii	*Dominican Republic.* Bahía de Las Calderas, Las Salinas	MNHN-IU-2009-3094	•	•	•
pisonii	*Brazil.* Pará, Marapanim, Marudá	SMF 43533	•	•	•
pisonii	*Brazil.* Pará, Marapanim, Marudá	SMF 43533	•	•	•

pisonii	*Brazil.* Pará, Marapanim, Marudá		•	•	•
sp. 'Gulf of Mexico'	*Mexico.* Tamaulipas, Barra del Tordo	ULLZ 3701	•	•	•
sp. 'Gulf of Mexico'	*Mexico.* Veracruz, Laguna Buenpais	ULLZ 4078	•	•	•
pacificus	*Costa Rica.* Puntarenas, Mata de Limón	MZUCR 3173-01	•	•	•
pacificus	*Costa Rica.* Puntarenas, Mata de Limón	ZSMA20130010	•	•	–
pacificus	*Costa Rica.* Puntarenas, Mata de Limón	MZUCR 3173-01	•	•	–
pacificus	*Ecuador*. Guayas, Puerto Morro	MNHN-IU-2009-3091	•	•	•
pacificus	*Ecuador*. Guayas, Puerto Morro	MNHN-IU-2009-3092	•	•	•
pacificus	*Ecuador*. Guayas, Puerto Morro	RMNH.CRUS.D.55083	•	•	•
sp. 'Baja'	*Costa Rica.* Puntarenas, Mata de Limón		•	•	–
sp. 'Baja'	*Costa Rica.* Puntarenas, Mata de Limón		•	•	-
sp. 'Baja'	*Costa Rica.* Puntarenas, Mata de Limón		•	•	•
sp. 'Baja'	*Mexico.* Baja California, Bahia Magdalena, Puerto San Carlos	ULLZ 4110	•	•	–
sp. 'Baja'	*Mexico.* Baja California, Bahia Magdalena, Puerto San Carlos	ULLZ 4110	•	•	•
sp. 'strange'	*Costa Rica.* Puntarenas, Mata de Limón		•	•	•
Sesarma					
abeokuta	*Jamaica*. Galloway.		•	•	•
aequatoriale	*Panama.* Miraflores Locks		•	AJ225874	•
ayatum	*Jamaica.* Portland Parish, Ecclesdown		AJ225889	AJ225891	•
bidentatum	*Jamaica.* St. Mary Parish, Wag Water		•	AJ225890	•
cookei	*Jamaica.* Portland Parish, Ecclesdown		AJ225857	AJ225854	•
crassipes	*Costa Rica.* Limón, Rio Tortuguero		AJ225859	AJ225869	–
curacaoense	*Trinidad.* Caroni Swamps		•	AJ225870	•
curacaoense	*Jamaica.* St. Elizabeth Parish, nr. Blade River, Broad River bridge		•	•	•
curacaoense	*Jamaica.* St. Elizabeth Parish, nr. Blade River, Broad River bridge		•	•	•
curacaoense	*Jamaica.* St. Elizabeth Parish, nr. Blade River, Broad River bridge		•	•	–
dolphinum	*Jamaica.* Hanover Parish, Askenish		•	AJ225872	•
fossarum	*Jamaica.* Trelawny Parish, Windsor		AJ225865	AJ225873	•
jarvisi	*Jamaica.* Trelawny Parish, Windsor		AJ225878	AJ225868	•
meridies	*Jamaica.* Clarendon Parish		–	AJ621820	•
meridies	*Jamaica.* St. Catherine Parish, Magno River		–	AJ621821	•

nr. reticulatum	*USA.* Texas, Sabine Pass		•	AJ225871	•
rectum	*Trinidad.* Caroni Swamps		•	AJ225866	•
rectum	*Brazil.* Bahia, Acuipe, Rio Acuipe		•	•	•
reticulatum	*USA.* Delaware, Woodland Beach		AJ225885	AJ225867	•
rhizophorae	*Panama.* Pacific coast, Diablo Heights		•	AJ225851	•
rubinofforum	*Panama.* Pacific coast, Rio Chorcha		•	AJ225852	•
sulcatum	*Mexico.* Sonora, near Isla Tiburón		AJ225880	AJ225853	–
verleyi	*Jamaica.* Trelawny Parish, Printed Circuit Cave		AJ225858	AJ225850	•
windsor	*Jamaica.* Trelawny Parish, Printed Circuit Cave		AJ225886	AJ225849	•
Metopaulias					
depressus	*Jamaica.* Dolphin Head		–	AJ250636	•
depressus	*Jamaica.* Trelawny Parish, Windsor		AJ225861	AJ225875	•
depressus	*Jamaica.* St. James Parish, nr. Wakefield, Spring Vale		•	–	•
Chiromantes					
haematocheir	*Japan.* Wakayama Pref., Samusaura	SMF 25989	•	AJ308414	•

Figure S4.1. Phylogeny of American Sesarmidae inferred from a multigene (mitochondrial Cox1 and 16S rRNA, nuclear 28S rRNA) maximum likelihood analysis with RAxML. Values next to nodes indicate likelihood bootstrap support of this node.

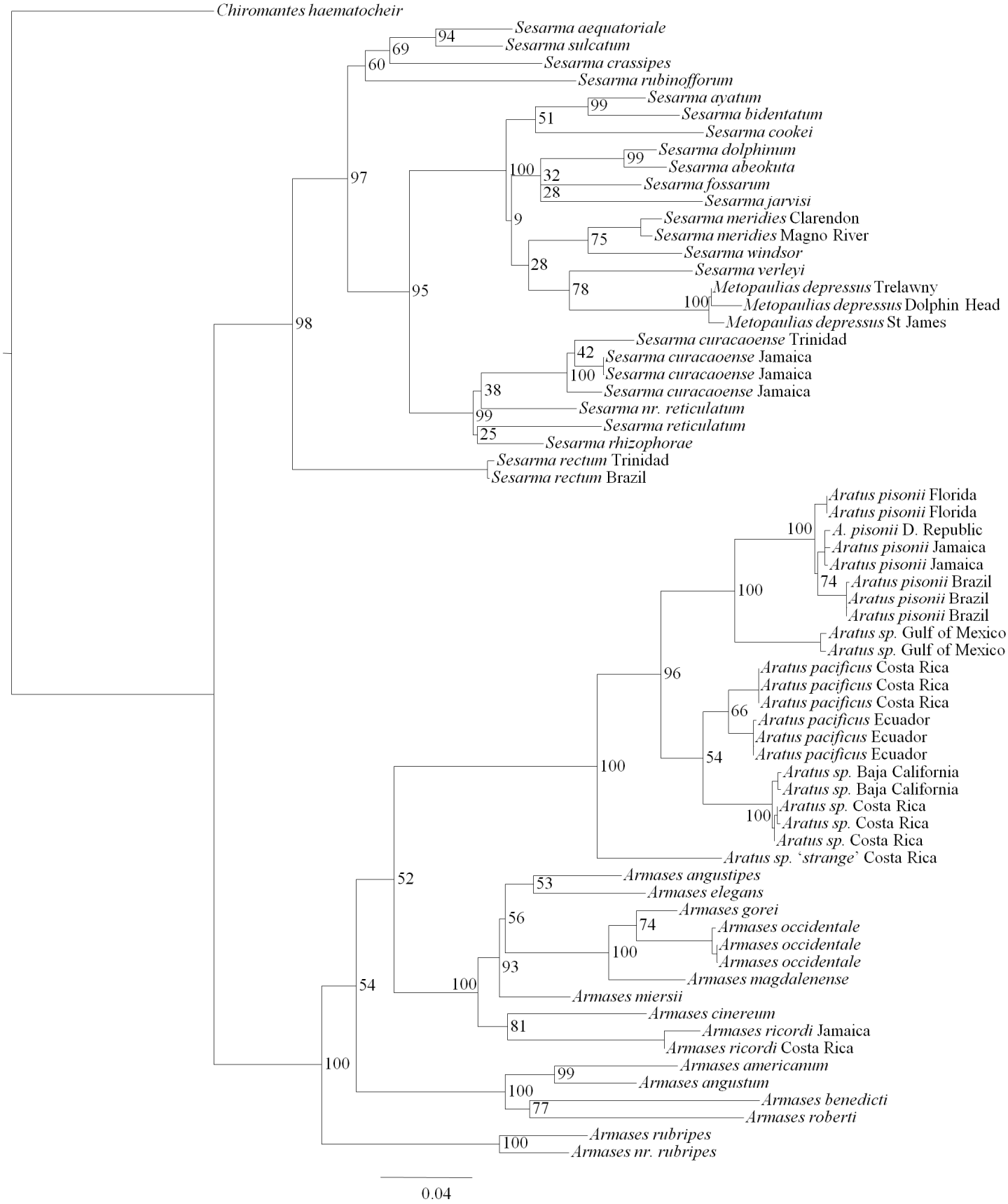


Figure S4.2. Phylogeny of American Sesarmidae inferred from a multigene (mitochondrial Cox1 and 16S rRNA, nuclear 28S rRNA) Bayesian analysis with MrBayes. Values next to nodes indicate MrBayes Bayesian posterior probability (in percent) of this node.

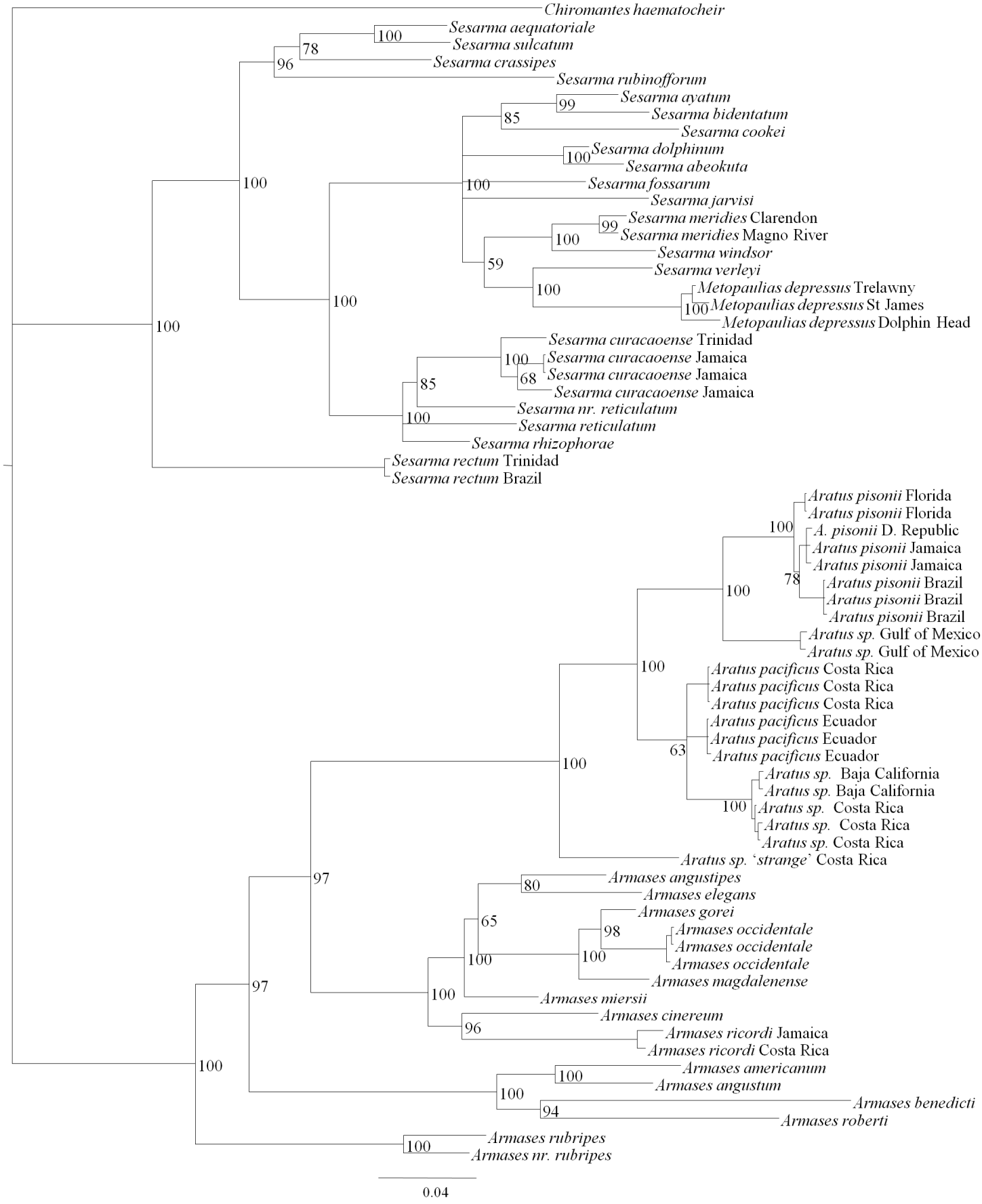


Figure S4.3. Phylogeny of American Sesarmidae inferred from a mitochondrial Cox1 maximum likelihood analysis with RAxML. Values next to nodes indicate likelihood bootstrap support of this node.

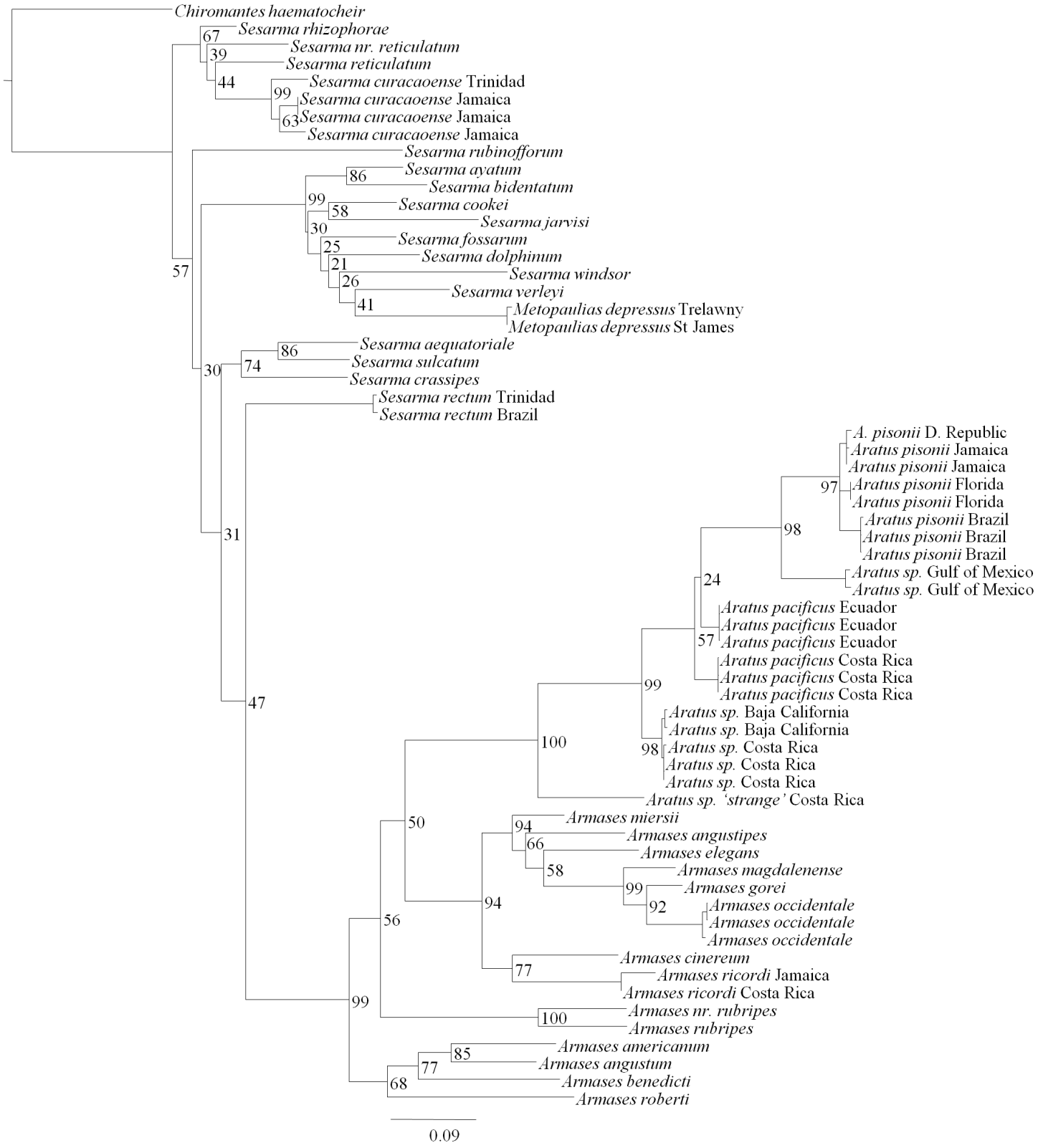


Figure S4.4. Phylogeny of American Sesarmidae inferred from a mitochondrial 16S rRNA maximum likelihood analysis with RAxML. Values next to nodes indicate likelihood bootstrap support of this node.

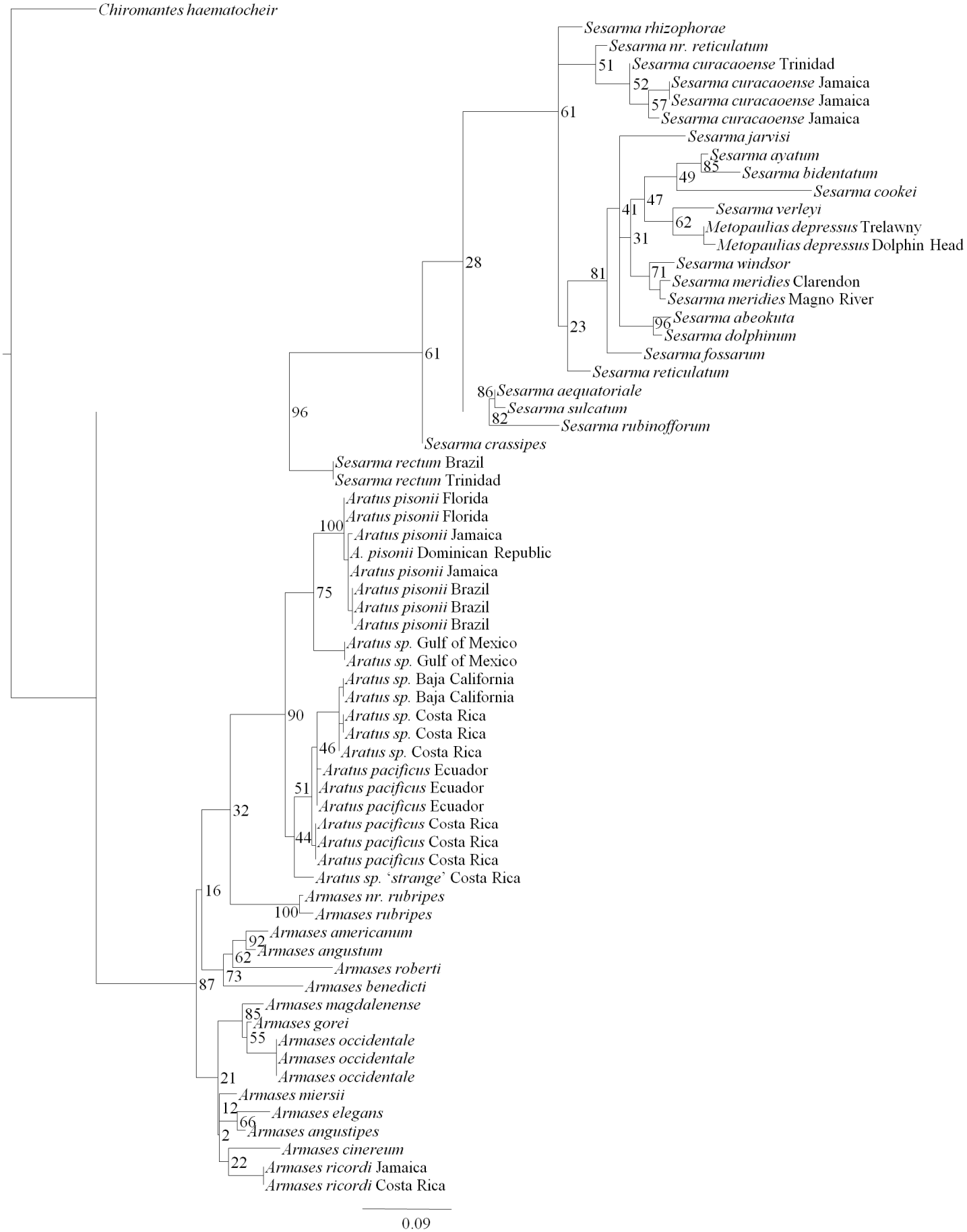


Figure S4.5. Phylogeny of American Sesarmidae inferred from a nuclear 28S rRNA maximum likelihood analysis with RAxML. Values next to nodes indicate likelihood bootstrap support of this node.

Figure S4.6. Phylogeny of American Sesarmidae inferred from a mitochondrial Cox1 Bayesian analysis with MrBayes. Values next to nodes indicate MrBayes Bayesian posterior probability (in percent) of this node.

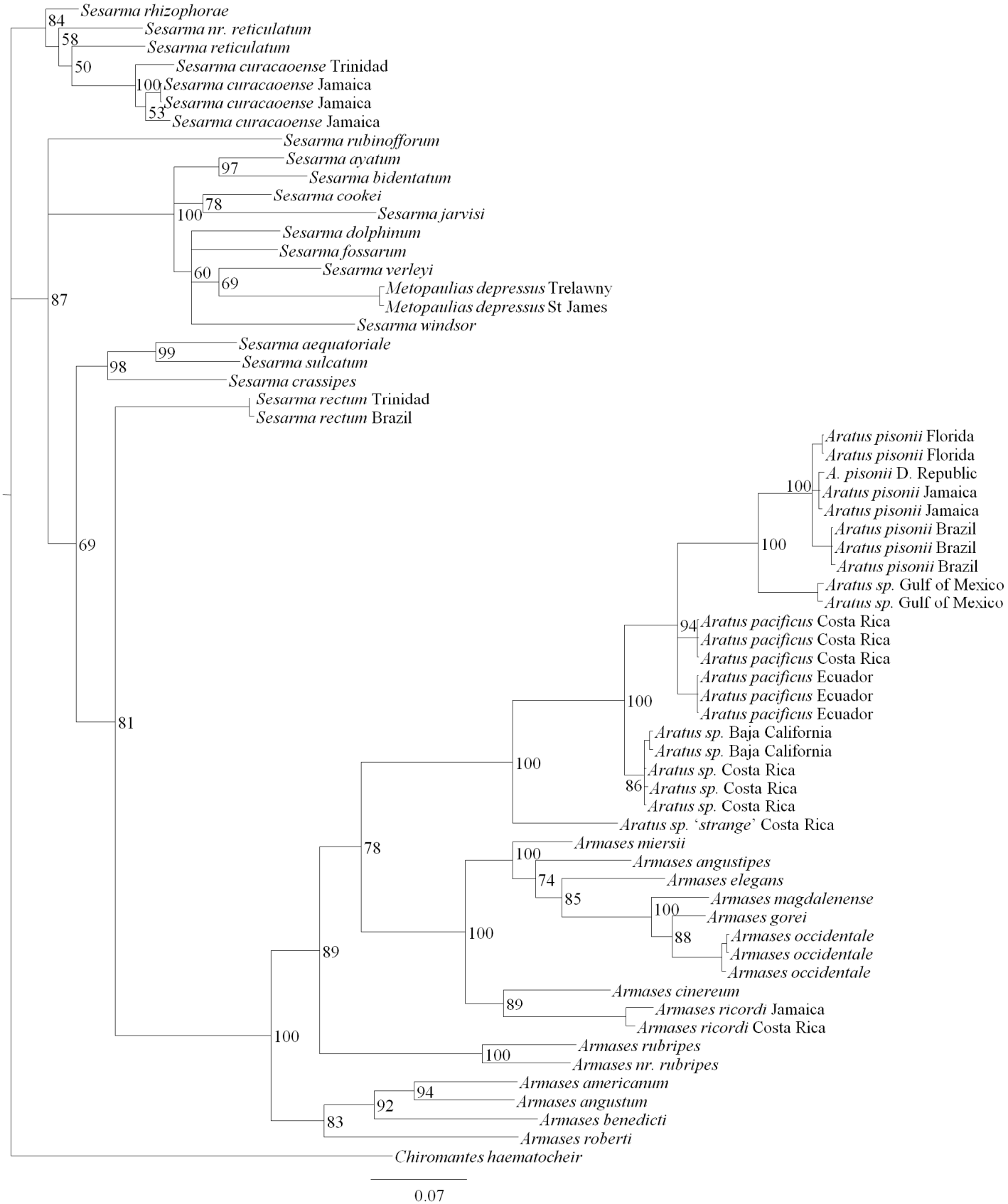


Figure S4.7. Phylogeny of American Sesarmidae inferred from a mitochondrial 16S rRNA Bayesian analysis with MrBayes. Values next to nodes indicate MrBayes Bayesian posterior probability (in percent) of this node.

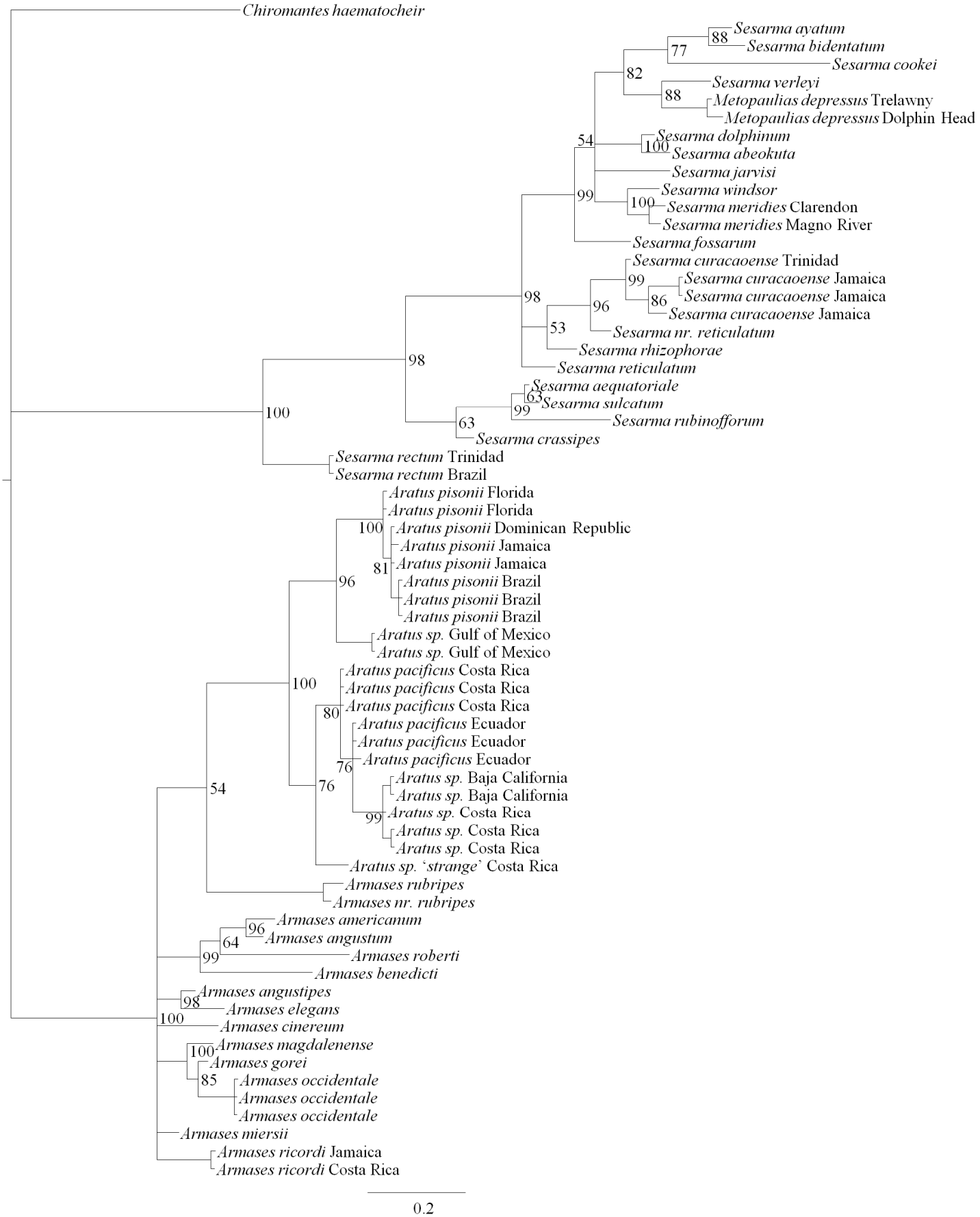


Figure S4.8. Phylogeny of American Sesarmidae inferred from a nuclear 28S rRNA Bayesian analysis with MrBayes. Values next to nodes indicate MrBayes Bayesian posterior probability (in percent) of this node.

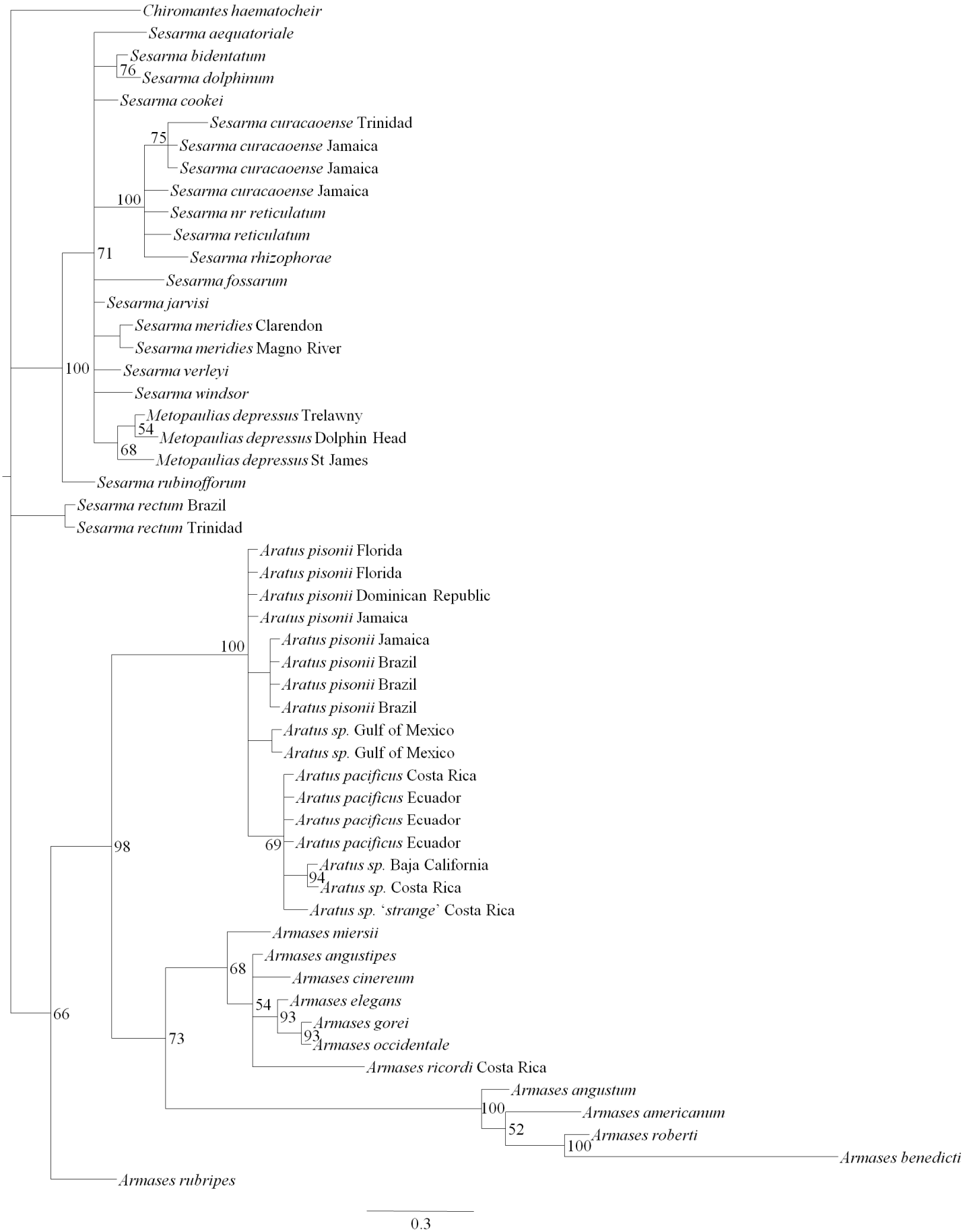


Figure S4.9. First male left gonopods of *Aratus pisonii* (A-B; Jamaica; RMNH.CRUS.D.55078), *Aratus sp.* 'Gulf of Mexico' (C-D; Mexico, Taumalipas; ULLZ 3701), *Aratus pacificus* (E-F; Ecuador; SMF 43530, holotype) and *Aratus sp.* 'Baja California – Costa Rica' (G-H; Mexico, Baja California; ULLZ 4110) observed in scanning electron microscopy. Dorsal (A, C, E & G) and total views (B, D, F & H).

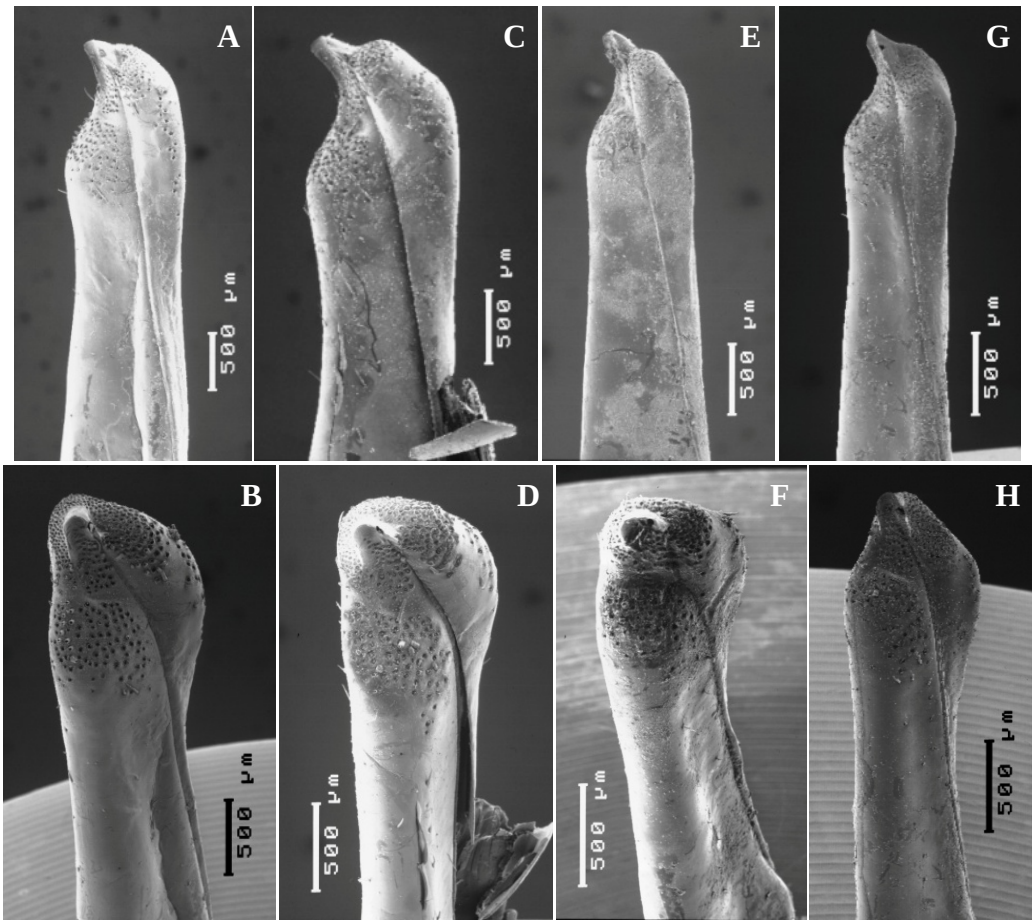


Figure S4.10. Distribution map of the *americanum*-group based on Abele (1992), Melo (1996) and specimens collected in this study. Green: *Armases angustum*; orange: *A. americanum*; yellow: *A. roberti*; blue: *A. benedicti*.

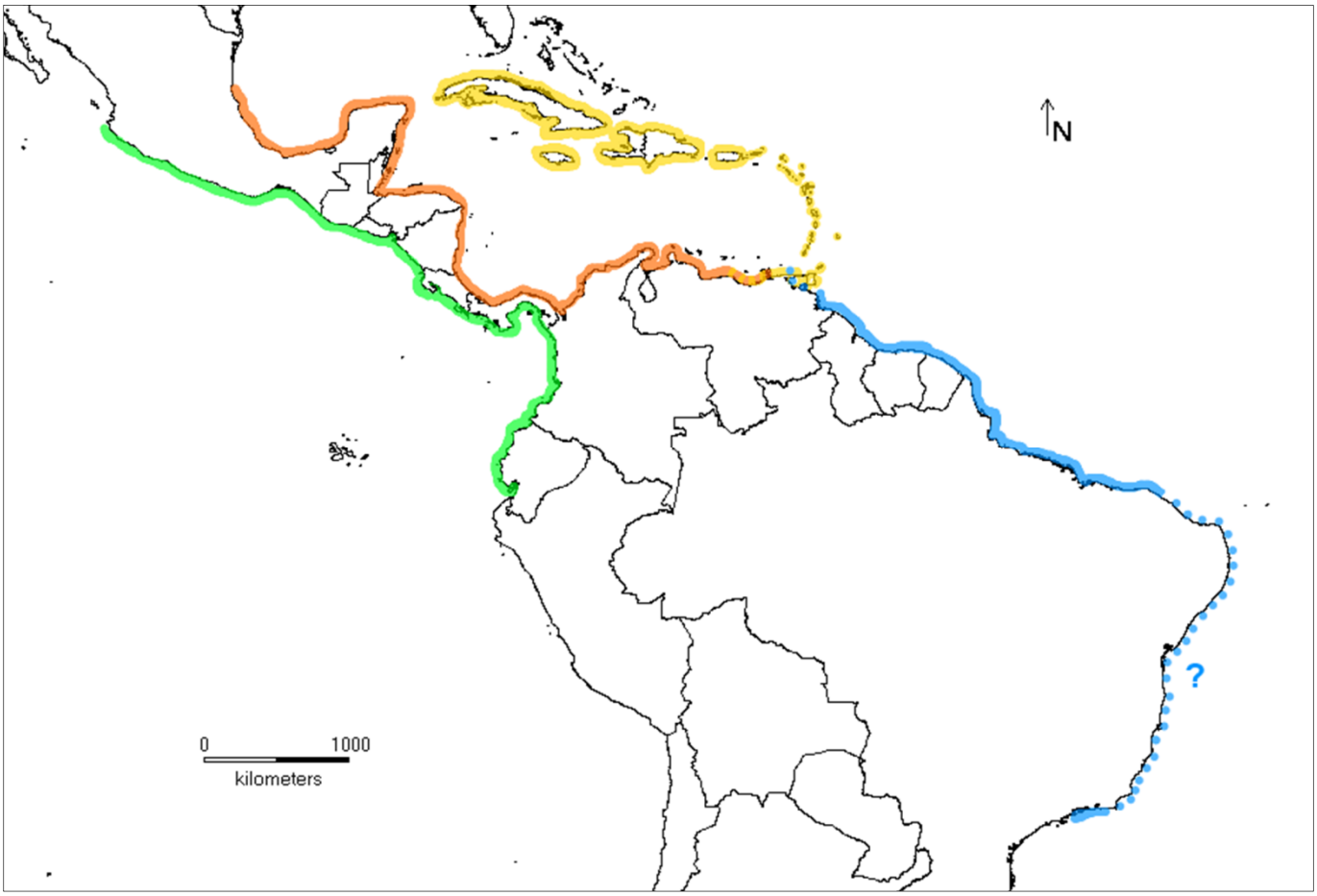
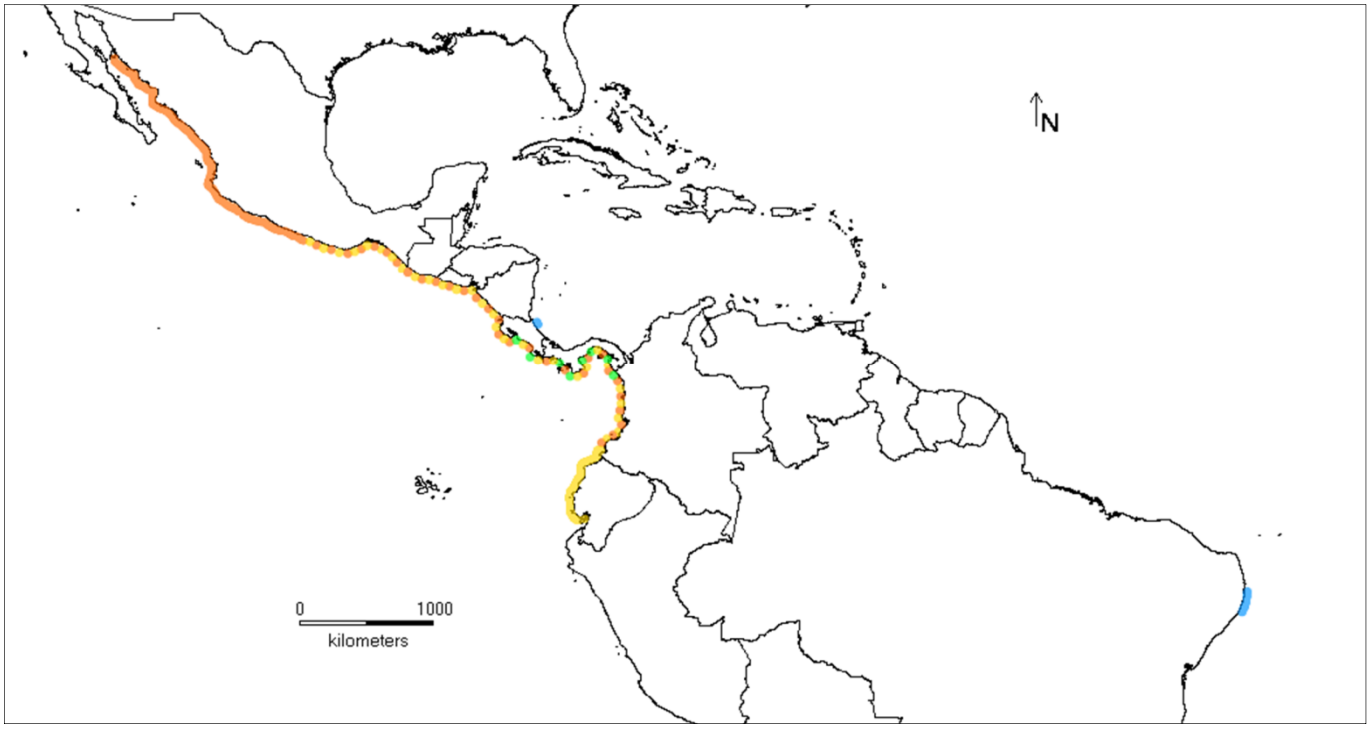


Figure S4.11. Distribution map of the *sulcatum*-group based on Abele (1992), Melo (1996) and specimens collected in this study. Yellow: *Sesarma aequatoriale*; blue: *S. crassipes*; green: *S. rubinofforum*; orange: *S. sulcatum*. Brazilian population of *S. crassipes* considered as doubtful by Abele (1992).



NOTES

Two additional references have been published during the weeks following the submission of this thesis, and appear to be relevant in the debate concerning the timing of closure of the Isthmus of Panama (Chapter 3), considering their results and the ones present in this thesis:

Bacon CD, Silvestro D, Jaramillo C, Smith BT, Chakrabarty P, Antonelli A (2015) Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences*, **112**, 6110–6115.

Montes C, Cardona A, Jaramillo C, Pardo A, Silva JC, Valencia V, Ayala C, Pérez-Angel LC, Rodriguez-Parra LA, Ramirez V, Niño H (2015) Middle Miocene closure of the Central American Seaway. *Science*, **348**, 226–229.

ABSTRACT

Die fortschreitende Bildung des Isthmus von Panama während des Miozäns und seine vollständige Schließung im Pliozän waren die bedeutendsten geologischen Vorgänge in der westlichen tropischen Atlantik und dem östlichen Pazifik. In Folge der vollständigen Schließung des Isthmus wurden marine Populationen auf beiden Seiten voneinader isoliert. Dadurch gab es eine unabhängige genetische Entwicklung, beeinflusst von unterschiedlichen Umgebungsbedingungen. Bisher erhielten die phylogeographischen Muster der Arten die den westlichen Atlantik oder den östlichen Pazifik bewohnen nur geringe Aufmerksamkeit. Die Tiere, die in dieser Arbeit als Modellorganismen benutzt wurden gehören zu amerikanischen Vertretern der thoracotremen Krabben. Sie wurden auf Grund von zwei bedeutenden ökologischen Eigenschaften ausgewählt, die vermutlich Einfluss auf ihre jeweilige Evolutionsgeschichte hatten: Verbreitungspotential und Habitat. So sollte es möglich sein den Einfluss dieser zwei Eigenschaften miteinander zu vergleichen. Die Ziele dieser Arbeit sind: 1) die Auswirkungen der Schließung des Isthmus von Panama zu ergründen und zu testen welches der beiden Modelle der zeitlichen Entwicklung des Isthmus die genetischen Unterschiede zwischen den transisthmischen Schwesterarten besser wiederspiegelt; 2) die phylogeographischen Muster im westlichen tropischen Atlantik und insbesondere die Beziehung zwischen der karibischen und der brasilianischen marinen Fauna zu erforschen; 3) die phylogenetische Beziehung der amerikanischen gattungen der Sesarmidae *Aratus, Armases, Metopaulias* und *Sesarma* ab zu schätzen. Unsere Ergebnisse deuten auf eine vollständige Schließung des Isthmus im Pliozän und nicht im Miozän hin. Transisthmische Schwesterarten aus den Mangroven besitzen eine geringere genetische Differenz als Schwesterarten aus den Felsküsten. Das deutet darauf hin, dass Mangroven die letzten Lebensräume waren, die noch genetischen Austausch zwischen Atlantik und Pazifik während der endgültigen Schließung des Isthmus ermöglicht haben. Populationen von *Aratus pisonii* auf beiden Seiten des Isthmus sind sowohl morphologisch, als auch genetisch unterschiedlich voneinander. Als Folge daraus wird gerade *Aratus pacificus* **n. sp.** als Schwesterart von *Aratus pisonii* beschrieben. Entlang der tropischen Atlantikküste fanden sich unerwartet deutliche Unterschiede zwischen nahverwandten Taxa innerhalb der gleichen biogeographischen Region. Diese reichten von Panmixis bis zu deutlich voneinander abgegrenzten Stammlinien. Drei der untersuchten Arten zeigen deutliche Hinweise auf frühere Veränderungen ihrer Populationsgrößen. Dies zeigt die Bedeutung der ökologischen Eigenschaften der untersuchten Arten und ihre Konsequenzen für sympatrische Arten. Die phylogentischen Beziehungen innerhalb der amerikanischen Sesarmidae spiegeln eine schnelle Radiation wieder, die vor 10.5 mya begann, als ihre Vorfahren sich auf dem amerikanischen Kontinent etablierten. Die Gattung *Aratus* repräsentiert dabei einen Ast tief innerhalb der Gattung *Armases*, was die Gattung insgesamt paraphyletisch macht und nicht wie bisher angenommen eine Schwestergattung. Die Erforschung genetischer Muster in amerikanischen thoracotremen Krabben auf verschiedenen Niveaus wirft ein Licht darauf wie sowohl Veränderungen der Umwelt als auch der ökologischen Eigenschaften die Biodiversität dieser Region seit dem Miozän geformt haben.