Molecular phylogeny, phylogeography and taxonomic revision of species of the genus *Perisesarma* De Man, 1895 (Crustacea: Decapoda: Brachyura: Sesarmidae)

Universität Regensburg

DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES DER NATURWISSENSCHAFTEN (DR. RER. NAT.) DER FAKULTÄT FÜR BIOLOGIE UND VORKLINISCHE MEDIZIN DER UNIVERSITÄT REGENSBURG

vorgelegt von
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Unterschrift:

This work is cordially dedicated to:

My family;

My parents, who devoted their life to their children,

My patient wife and my lovely son for making a sweet home,

My brothers and my sisters for their continuous countless supports

Abstract of the Dissertation

Molecular phylogeny, phylogeography and taxonomic revision of species of the genus *Perisesarma* De Man, 1895 (Crustacea: Decapoda: Brachyura: Sesarmidae)

by

Adnan Shahdadi

University of Regensburg

The present work forms part of an ongoing revision of sesarmid genera. The conspicuous and speciose genus *Perisesarma* De Man, 1895 is among the most taxonomically complex and problematic taxa of the family. This genus was suspected to be a polyphyletic taxon, comprising morphologically heterogeneous species. Moreover, some members of the genus have a close phylogenetic relationship with the sister genus *Parasesarma* De Man, 1895 and show many morphological similarities, except for the existence of an epibranchial tooth in *Perisesarma*. Therefore, the present study intends to evaluate the monophyly of *Perisesarma* and reconstruct phylogenetic relationships in comparison to *Parasesarma* by examining a variety of morphological characters and comparing them with results from molecular markers. Up to four genetic marker with different evolutionary mutation rates were used for different parts of this study. These include the mitochondrial genes Cox1, 16S, ND1 and the nuclear gene NaK. A comparative morphological analysis reveals that there is no unequivocal separation between species of *Parasesarma* and *Perisesarma*, because of intermediate conformations of the epibranchial tooth. In our molecular analysis, most species of *Perisesarma* cluster solidly with species of *Parasesarma*, but without being reciprocally monophyletic. Morphology and genetics also indicate that the West African species of *Perisesarma* and *P. fasciatum* are markedly different from all other species of the genus. Therefore, we here suggest with robust double support, a new classification, transferring most species of *Perisesarma* to *Parasesarma* and the

three West African representatives and *P. fasciatum* into two new genera, thus restricting the genus *Perisesarma* to the type species *P. dusumieri*.

This study also uncovers some un-described forms of the former genus *Perisesarma* and presents new insight in to the phylogeography of some phylogenetic clades. *Perisesarma* n. sp1. is described from mangroves of southern Vietnam, differing most significantly from congeners by the tuberculation pattern of the chelar dactylus and its unique G1 morphology. Genetically, *Perisesarma* n. sp1. is markedly divergent from other congeneric species, both in mitochondrial and nuclear DNA. This study helps to reveal marine biogeographic barriers with restricted gene flow, among them a putative barrier between the northern Australian coastline and adjacent areas of South East Asia and the South Pacific. In agreement with these findings, we provide evidence for genetic uniqueness of representatives of the mangrove crab genus *Parasesarma* from northern Australian mangroves, based on three mitochondrial and one nuclear DNA marker. This distinct taxon is here described as a new species. Morphologically the new species is very similar to *P. lividum* from the southwest Pacific and *P. samawati* from East Africa. Genetically, however, it is significantly distinct from all other congeners. The same genetic disjunction is found between the two very closely related species *P. semperi* from S.E Asia and P. *longicristatum* from Australia.

P. indiarum, was originally described from Ambon, Indonesia, and is assumed to be distributed all over S. E. Asian Island. But here presented genetic and morphological evidences reveal a distinct separation of Malay Peninsular populations from the types and therefore these are herein described as a new species.

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According to INTERNATIONAL CODE OF ZOOLOGICAL NOMENCLATURE

(ICZN), Article 8.3, here I state that:

"This work is not issued for the purpose of zoological nomenclature"

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Chapter 1

General Introduction

Discovering, nominating and describing species along with establishing accurate and natural classifications of these organisms according to their phylogeny are the fundamental aims of systematics (Winston, 1999; Wiens 2007; Glaubrecht, 2011; Camargo & Sites 2013; Ruggiero et al., 2015). Because of the complexity to interpret animal characters and difficulties in understanding their evolutionary backgrounds and identities, it has always been a challenging dilemma for systematists to distinguish phylogenetic divergence from phenotypic plasticity, to separate plesiomorphy from apomorphy, and to differentiate homology from homoplasy as important principles for classifications (Avise, 1993; Lemy et al., 2009; Baum & Smith, 2012). Therefore, new taxonomic approaches, methods, algorithms and tools have been established during the history of systematics (Avise, 1993, Carroll et al., 2005; Chu et al., 2009; Baum & Smith, 2012; Hedin et al., 2015; Hedin, 2015). In parallel, the numbers of described species in different taxa is constantly increasing (Winston, 1999; Roskov et al., 2014; Ruggiero et al., 2015). Consequently, many taxonomic groups have experienced rearrangement(s) since the start of systematic classification (according to Ruggiero et al., 2015).

Following the study by Sarich & Wilson (1967), molecular techniques were introduced to the world of phylogenetic studies in the late 1960ies (Avise, 1993; Page & Holmes, 1998). Even if not a panacea, these techniques are indeed a powerful tool helping taxonomic classifications (Avise, 1993; Ng et al., 2008). In recent years, developing phylogenetic algorithms along with computational advances resulted in dramatic increase of applying genetic markers as a promising approach in taxonomy (Rodriguez et al., 1990; Knowlton, 2000; Huelsenbeck & Ronquist, 2001; Hebert et al., 2003; Hajibabaei et al., 2007; Silvestro & Michalak, 2012; Posada & Buckley, 2004). Using multiple molecular markers with different evolutionary speed (e.g. mitochondrial protein coding genes like NADH dehydrogenase subunit 1 (ND1), cytochrome oxidase subunit 1 (Cox1), mitochondrial genes encoding the rRNA of the ribosomal subunits (12S and 16S), the nuclear protein-coding gene sodium-potassium ATPase alpha-subunit (NaK)) seems to effectively facilitate taxonomy at different hierarchical levels (Avis, 1993; Lynch & Jarrell, 1993; Schubart et al., 2006; Tsang et al., 2008; Chu et al., 2009; Baum & Smith, 2012). Their

mostly neutral identities also make these markers useful indicators to blindly evaluate phylogenetic usefulness of morphological characters (Avis, 1993; Hebert et al., 2003).

Among all living plant and animal groups, crustaceans seem to exhibit the highest range of morphological diversity (Martin & Davis, 2001). Consisting of more than 14,500 extant species (according to De Grave et al., 2009), the order Decapoda Latreille, 1802, is the most diverse group of crustacean alive today (Ng et al., 2008). The infraorder Brachyura Linnaeus, 1758, is the most successful of all decapod groups both in terms of taxonomic diversity (nearly 7200 valid species) and in the variety of their lifestyles (Davie et al., 2015a), colonizing almost all marine and terrestrial habitats (Ng et al., 2008). Their origin goes back to the Early Jurassic, radiating into multiple families by the Middle Jurassic (Davie et al., 2015b).

The brachyuran crab family Sesarmidae Dana, 1851, with 32 genera (listed in table 1.1) and over 250 species (De Grave et al., 2009; Naruse & Ng, 2012; Brösing et al., 2014), is the most speciose family in the subsection Thoracotremata Guinot, 1977. Ecologically, most sesarmid crabs are important faunal components of the world mangroves (Figs. 1.1 & 1.2) (Davie, 1994b; Tan & Ng, 1994; Lee, 1998; Ragionieri et al., 2009). Their generally herbivorous diet is responsible for an annual recycling of large amounts of primary products (mangrove leaves) (Robertson & Daniel, 1989) and make sesarmid crabs keystone species of mangrove communities (Fig. 1.3) (Smith et al., 1991; Lee, 1998).

Table 1.1 The current taxonomic position and genera of the family Sesarmidae (according to Martin & Davis, 2001; Ng et al., 2008; De Grave et al., 2009; Naruse & Ng, 2012; Brösing et al., 2014).

Subphylum	Crustacea Brünnich, 1772
Class	Malacostraca Latreille, 1802
Subclass	Eumalacostraca Grobben, 1892
Superorder	Eucarida Calman, 1904
Order	Decapoda Latreille, 1802
Suborder	Pleocyemata Burkenroad, 1963
Infraorder	Brachyura Linnaeus, 1758
Section	Eubrachyura de Saint Laurent, 1980
Subsection	Thoracotremata Guinot, 1977
Superfamily	Grapsoidea MacLeay, 1838
Family	**Sesarmidae Dana, 1851**
Genus	*Aratus* H. Milne Edwards, 1853
	Armases Abele, 1992
	Bresedium Serène & Soh, 1970
	Chiromantes Gistel, 1848
	Clistocoeloma A. Milne-Edwards, 1873
	Cyclorma Naruse & Ng, 2012
	Eneosesarma Brösing et al., 2014
	Episesarma De Man, 1895
	Geosesarma De Man, 1892
	Haberma Ng & Schubart, 2002
	Karstarma Davie & Ng, 2007
	Labuanium Serène & Soh, 1970
	Lithoselatium Schubart, Liu & Ng, 2009
	Metagrapsus H. Milne Edwards, 1853
	Metasesarma H. Milne Edwards, 1853
	Metopaulias Rathbun, 1896
	Muradium Serène & Soh, 1970
	Namlacium Serène & Soh, 1970
	Nanosesarma Tweedie, 1951
	Neosarmatium Serène & Soh, 1970
	Neosesarma Serène & Soh, 1970
	Parasesarma De Man, 1895b
	Perisesarma De Man, 1895b
	Pseudosesarma Serène & Soh, 1970
	Scandarma Schubart, Liu & Cuesta, 2003

Sarmatium Dana, 1851b
Selatium Serène & Soh, 1970
Sesarma Say, 1817
Sesarmoides Serène & Soh, 1970
Sesarmops Serène & Soh, 1970
Stelgistra Ng & Liu, 1999
Tiomanum Serène & Soh, 1970

Fig. 1.1. Mangrove swamp in Stradbroke Island, Queensland, Australia.

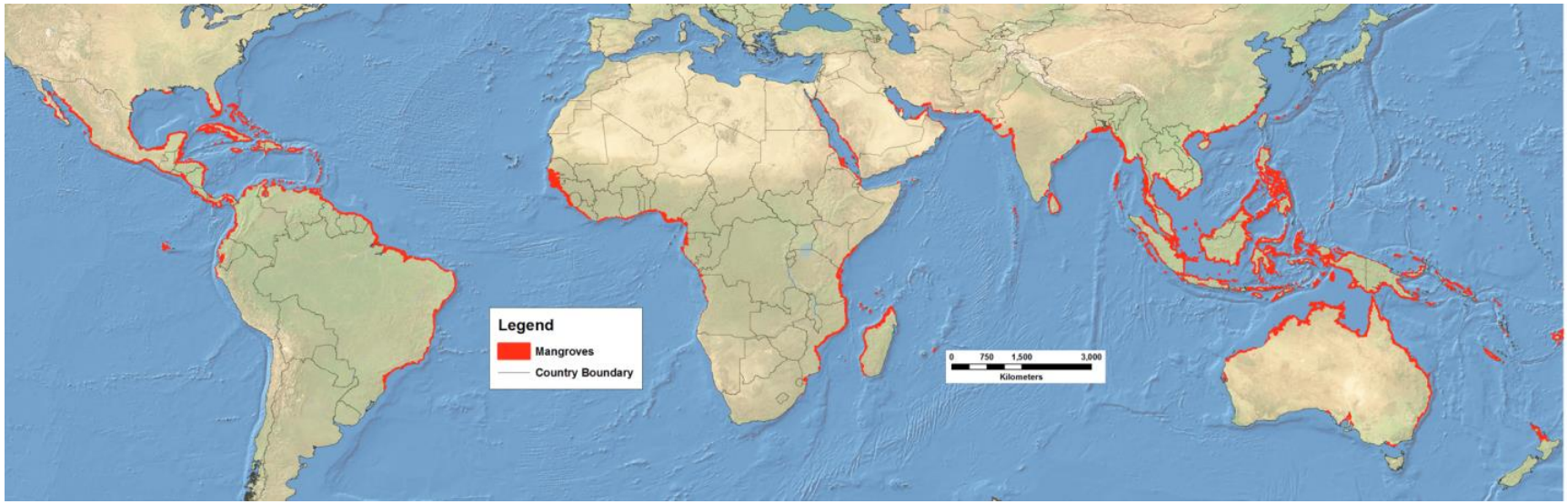

Fig. 1.2. Global mangrove forests distribution (after Giri et al., 2011). Map redrawn by UNEP/DEWA (https://na.unep.net).

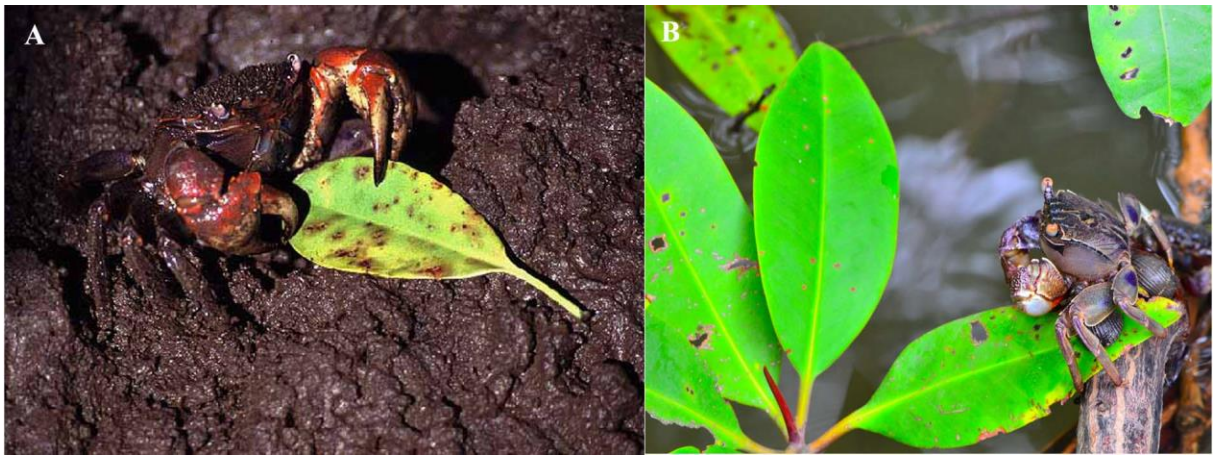

Fig. 1.3. Sesarmid mangrove crabs in the field, A. *Neosarmatium meinerti* from Seychelles (photo from http://www.oocities.org), B. *Episesarma* sp. from southern Thailand (photo from http://fxgallery.com).

Although the family by itself nowadays represents a quite stable monophyletic taxon (Fig. 1.4) (see Schubart et al., 2006), it nevertheless experienced a complex taxonomic history (Guerao et al., 2004). For a long time, most of the sesarmids were included in the genus *Sesarma* Say, 1817 *sensu lato*. Later on, this genus was divided into more genera/subgenera by De Man (1892; 1895), Tesch (1917), Tweedie (1950) and Dana (1951). Serène & Soh (1970) introduced a large number of new genera, and established a taxonomic system that is mostly still accepted today (see Ng et al., 2008). Several other genera were recognized by more recent studies (see the list of sesarmid genera in table 1.1). The historic changes and rearrangements of the sesarmid genera are nicely summarized and illustrated by Thiercelin (2015) (Fig. 1.5). Even after several recent revisionary studies (e.g., Davie, 1992; 1994a; Schubart et al., 2009; Davie, 2012), there are still many ambiguities and taxonomic problems at different levels within the family, calling for further studies (see notes on Sesarmidae, Ng et al., 2008).

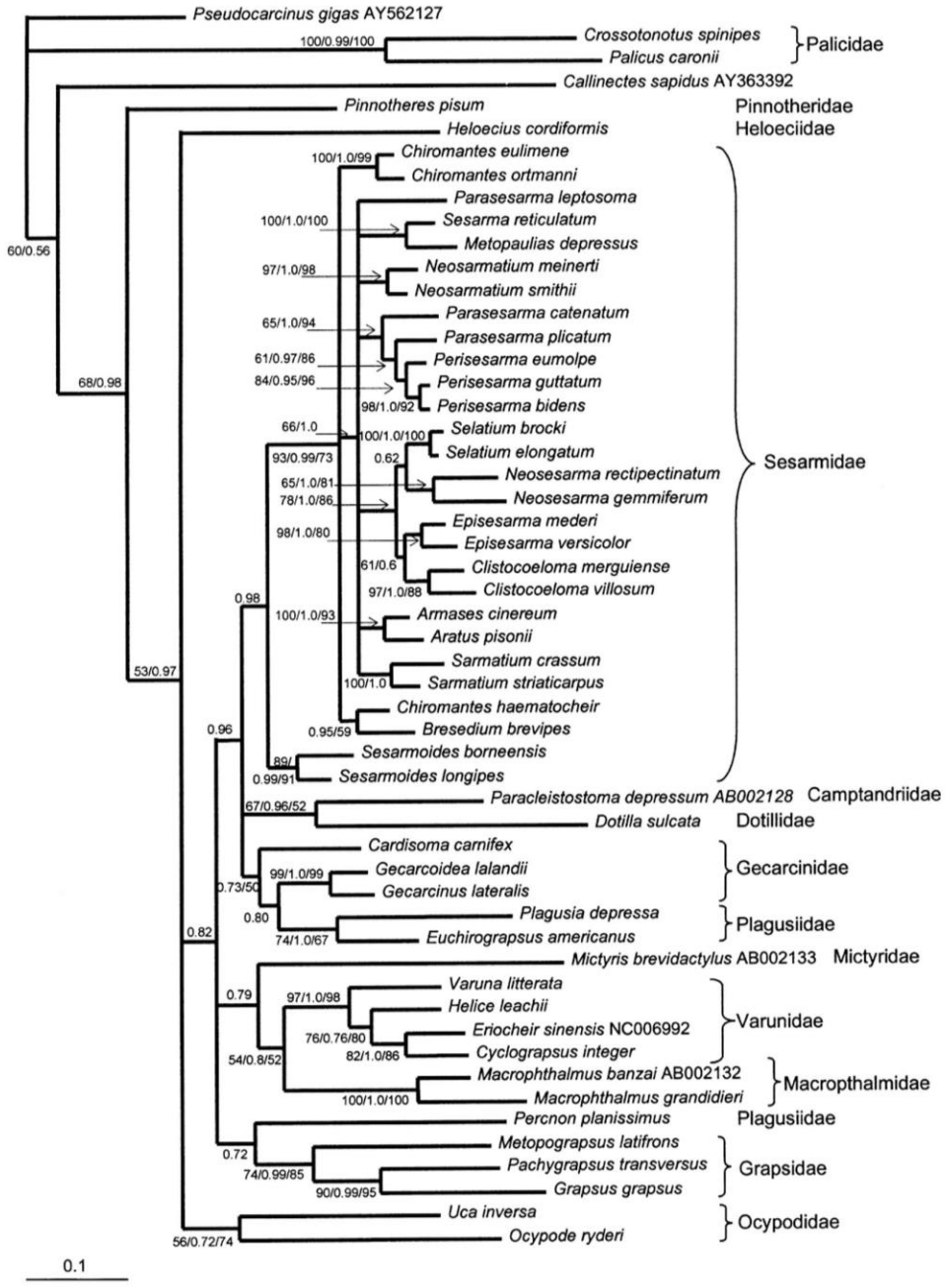

Fig. 1.4. Phylogenetic consensus tree of 54 brachyuran crabs (50 Thoracotremata) constructed with the maximum parsimony (MP), Bayesian inference (BI) and neighbour-joining (NJ) methods. Significance values are listed in the order MP (bootstrap values), BI (posterior probabilities), NJ (bootstrap values). Two thousand bootstrap pseudoreplicates were carried out with a heuristic search and random addition (after Schubart et al., 2006).

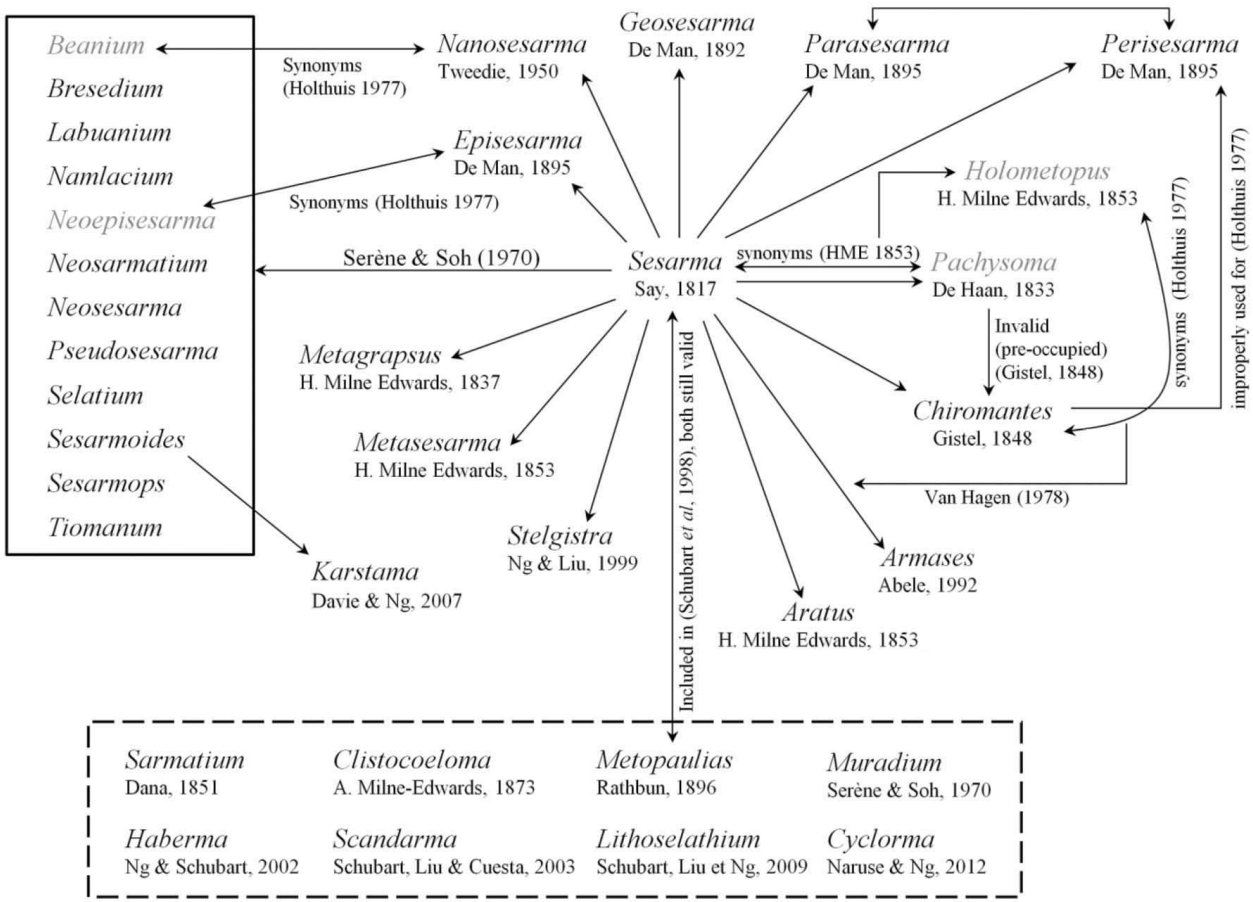

Figure 1.5. Historical relationships among sesarmid genera. Vectors indicate in which genus the type species of another genus was initially placed. Double arrows indicates synonymy or confusion. The dashed box corresponds to genera with type species originally described in their genus. The full box corresponds to the genera described in the massive reorganization of Southeast Asian Sesarmidae by Serène & Soh (1970). Black: valid genera; Grey: currently invalid genera (after Thiercelin, 2015).

The two conspicuous and speciose genera, *Parasesarma* and *Perisesarma* are among the most taxonomically complex and problematic taxa of the family (Guerao et al., 2004, Rahayu & Ng, 2010; Davie, 2010). Assumingly, these two genera also have a close mutual association, showing high morphological similarity (Guerao et al., 2004). They were described by De Man (1895) as two new subgenera, sharing the same type of tuberculation and pectinated crests on male chelipeds (Fig. 1.6). The only difference between these taxa is that *Perisesarma* is characterized by an anterolateral (= epibranchial) tooth (Fig. 1.7). However, this anterolateral tooth turns out to be a controversial character (see Abele, 1975; von Hagen, 1978) and its phylogenetic usefulness needs to be confirmed. Preliminary genetic studies (Fratini et al., 2005; Schubart et al., 2006) also confirm their tight phylogenetic connection under a supported monophyletic group, but not as reciprocally separate units. These studies revealed that some species of *Perisesarma* are phylogenetically nearer to *Parasesarma*, and that the latter genus is paraphyletic (Fig. 1.4).

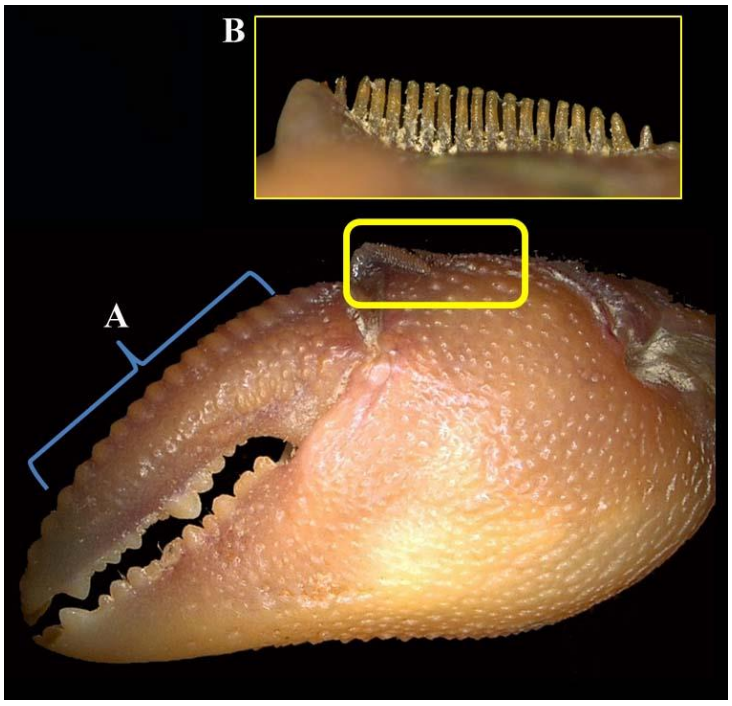

Fig. 1.6. Left chela of *Perisesarma bengalense* (specimen code S118 in Table 3.1) showing A. dactylar tubercles, B. primary pectinated crest.

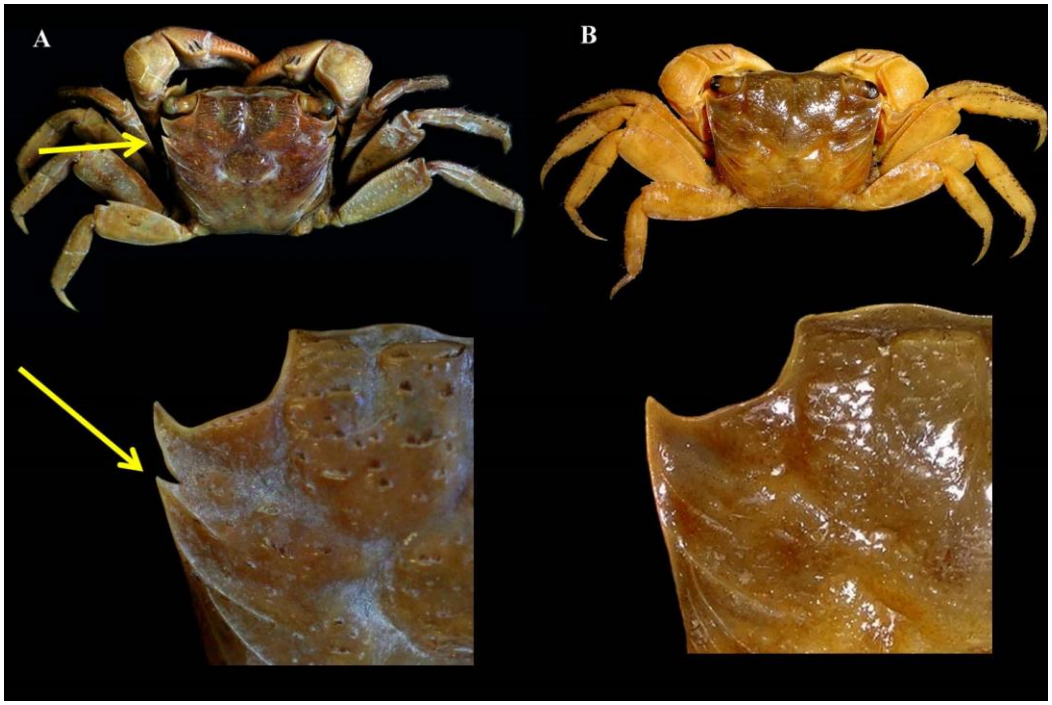

Fig. 1.7. Presence or absence of antero-lateral (epibranchial) tooth in A. *Perisesarma* (here *P. indiarum*), B. *Parasesarma* (here *P. plicatum*).

There is no general agreement on the number of species of the genus *Perisesarma*, to some extent because of the character which distinguishes its species from *Parasesarma* (see Introduction in Chapters 2 & 4). Ng et al. (2008) provided the most recent and widely accepted compilation of genera and species of brachyuran crabs and listed 23 species of *Perisesarma*. *P. holthuisi* was described later by Davie (2010). The 24 species (up to date) of *Perisesarma* are listed in table 1.2 alphabetically.

Table 1.2. The 24 species (up to date) of *Perisesarma* listed alphabetically.

Perisesarma alberti (Rathbun, 1921)
Perisesarma bengalense Davie, 2003
Perisesarma bidens (De Haan, 1835)
Perisesarma brevicristatum (Campbell, 1967)
Perisesarma cricotum Rahayu & Davie, 2002
Perisesarma darwinense (Campbell, 1967)
Perisesarma dusumieri (H. Milne-Edwards, 1853)
Perisesarma eumolpe (De Man, 1895)
Perisesarma fasciatum (Lanchester, 1900)
Perisesarma foresti Rahayu & Davie, 2002
Perisesarma guttatum (A. Milne-Edwards, 1869)
Perisesarma haswelli (De Man, 1887)
Perisesarma holthuisi Davie, 2010
Perisesarma huzardi (Desmarest, 1825)
Perisesarma indiarum (Tweedie, 1940)
Perisesarma kamermani (De Man, 1883)
Perisesarma lanchesteri (Tweedie, 1936)
Perisesarma lividum (A. Milne-Edwards, 1869)
Perisesarma longicristatum (Campbell, 1967)
Perisesarma maipoense (Soh, 1978)
Perisesarma messa (Campbell, 1967)
Perisesarma onychophorum (De Man, 1895)
Perisesarma samawati Gillikin & Schubart, 2004
Perisesarma semperi (Bürger, 1893)

Species of *Perisesarma* are distributed all over the Indo-West Pacific from the East African coasts (i.e., *P. guttatum*; *P. samawati*) to the South Pacific Islands (i.e., *P. lividum* in New Caledonia), with most species being distributed in South East Asia and Australia. There are also three West African species of *Perisesarma* that occupy Eastern Atlantic mangroves (Fig. 1. 8).

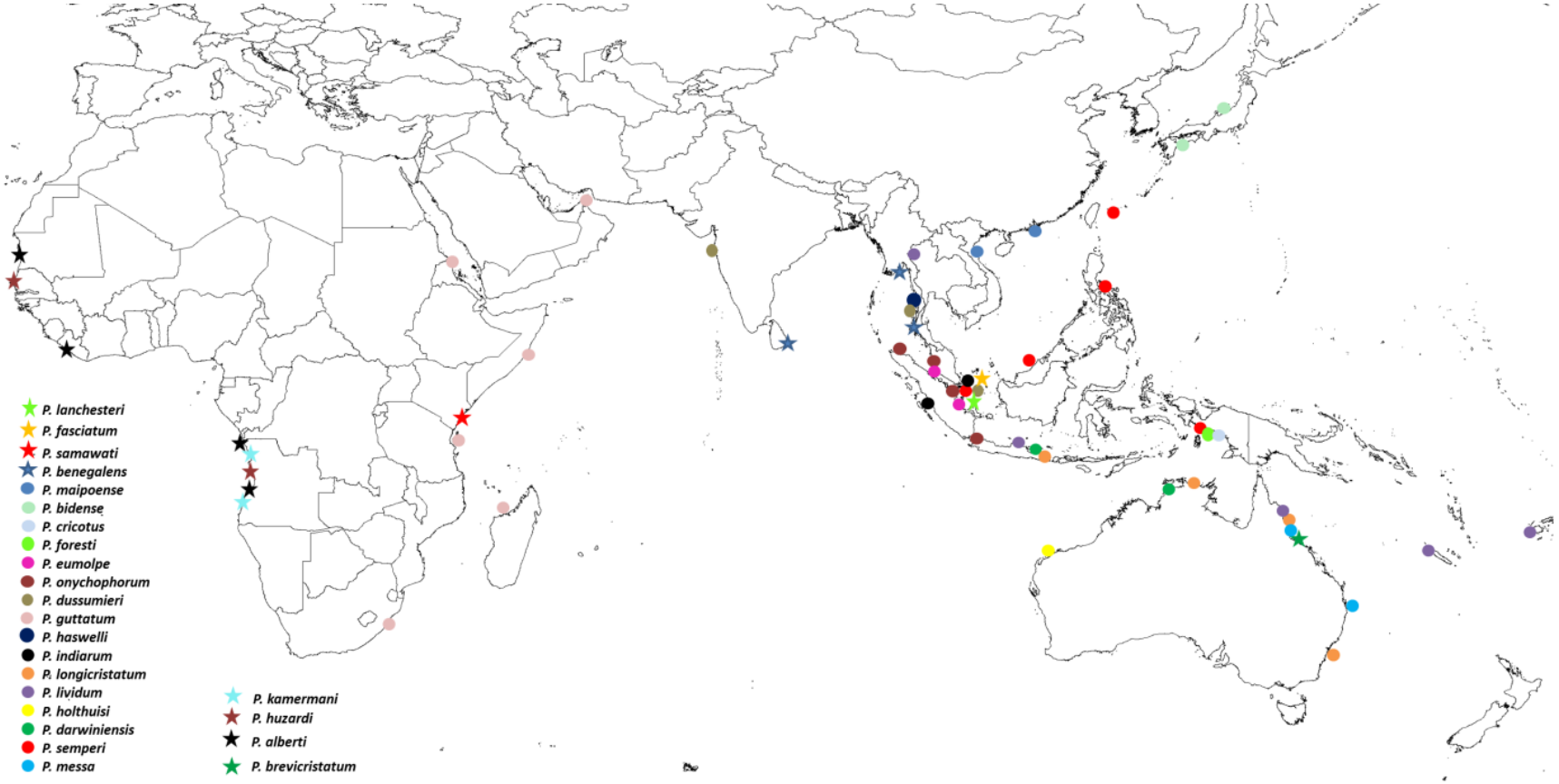

Fig. 1.8. Distribution map of the species of *Perisesarma.*

Species of *Perisesarma* are mostly active burrowers in muddy substrates of mangrove forests (Boon et al., 2009) (Fig. 1.9) and seem to depend on mangrove leaves for their diet (Boon et al., 2008). This burrowing activity and herbivorous habit make them important components of their community in soil nutrient dynamics and forest productivity (Smith et al., 1991). Ethologically, they perform interesting stridulatory behaviour that was initially recorded by Tweedie (1954) and later detailed by Boon et al. (2009) and Chen et al. (2014). According to Boon et al. (2009), male crabs produce acoustic signals and ground vibrations by rubbing chelar dactylar tubercles (pars stridens) against pectinated crests (plectrum) on the opposite chela (Fig. 1.6) (see also Davie et al., 2015a, pp. 86–92). These studies also revealed that differences in the morphology of the tubercles in *P. eumolpe* (De Man, 1895) and *P. indiarum* (Tweedie, 1940) result in different sound production.

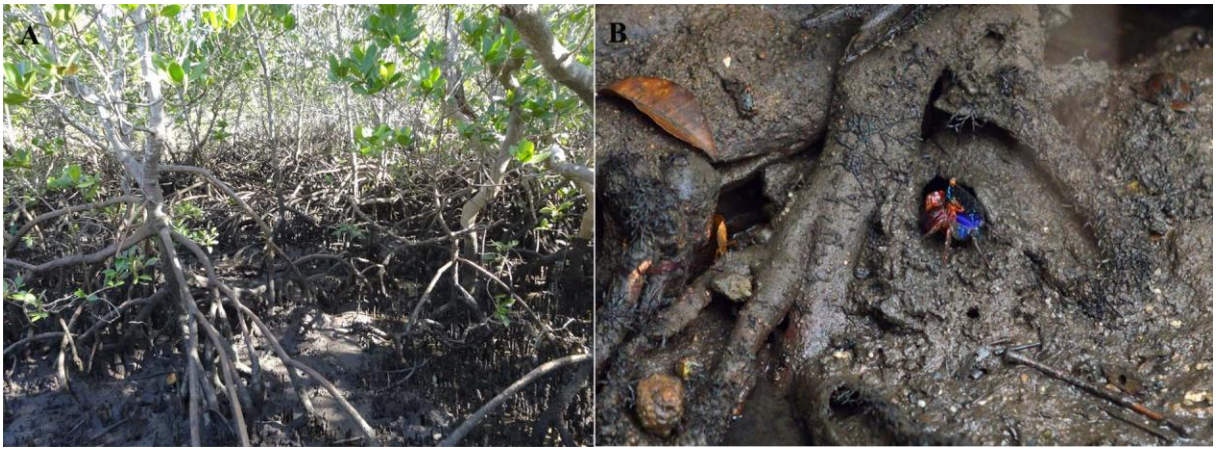

Fig. 1.9. Muddy substrates of mangrove forests, A. Stradbroke Island, Queensland, Australia, habitat for *Perisesarma messa*, B. a specimen of *P. eumolpe* close to the burrow in Singapore (photo B. from http://www.panzerwelten.de).

The taxonomic history of *Perisesarma* is characterized by several rearrangements and type species desigations and therefore gives a confused impression (Campbell, 1967; Holthuis, 1977; Davie, 2010; von Wyschetzki, 2012). The genus was originally defined by De Man (1895) as a subgenus of *Sesarma* for those species which are characterized by two to three rows of pectinated crests on the chelar palm and an epibranchial carapace tooth. De Man (1895) assigned *P. dusumieri* (H. Milne-Edwards, 1853) to this new subgenus and additionally described two new species for the subgenus: *P. eumolpe* and *P. onychophorum*. But not all the species with these morphological characters were included in the group at that time (e.g., *P. bidens* described by De Haan (1835) as *Grapsus* (*Pachysoma*) *bidens* from Japan).

De Haan (1833) defined the subgenus *Grapsus* (*Pachysoma*) and listed two species: *G.* (*P.*) *haematocheir* (De Haan, 1833) (now *Chriromantes haematocheir*, see Ng et al. (2008)) and *G.* (*P.*) *quadratus* (now as *Chriromantes dehaani* (H. Milne Edwards, 1853) (see Hothuis (1977) and Ng et al. (2008)). Later, De Haan (1835) described and assigned some new species in the subgenus *Pachysoma* [i.e., *Grapsus* (*Pachysoma*) *bidens* (De Haan, 1835) (now *Perisesarma bidens*), *G.* (*P.*) *intermedius* (De Haan, 1835) (now *Sesarmops intermedius*); *G.* (*P.*) *pictus* (De Haan, 1835) (now *Parasesarma pictum*)]. As *Pachysoma* De Haan, 1833 was a preoccupied name (the coleopteran *Pachysoma* MacLeay, 1821a), Gistel (1848) replaced it and used *Chiromantes* for this group. Rathbun (1909), applied the name *Chiromantes* Gistel, 1848 to De Man's *Perisesarma* by mistake, probably because De Haan (1835) started the list of *Pachysoma* (later *Chiromantes*) with *Grapsus* (*Pachysoma*) *bidens* (De Haan, 1835), but in this case *Pachysoma* (=*Chiromantes*) had priority to De Man's *Perisesarma*. But *Grapsus* (*Pachysoma*) *bidens* (De Haan, 1835) was not included in the original assignment of the subgenus *Pachysoma* (De Hann, 1833). Tesch (1917) followed Rathbun's system and placed all the species with the characters that De Man (1895) defined for *Perisesarma* (chelar palm with pectinated crests and carapace anterolateral tooth) into the subgenus *Chiromantes* and transferred the two original species of *Chiromantes* into the subgenus *Holometopus* together with many other species. Rathbun (1918) selected *Grapsus* (*Pachysoma*) *bidens* (De Haan, 1835) as type species for *Chiromantes*. Campbell (1967) described four new species of this group from Australia and established a key identification for all species of the subgenus to that date. Campbell (1967) also subsequently designed *Sesarma dusumieri* (H. Milne Edwards, 1853) as type species, because

Grapsus (*Pachysoma*) *bidens* (De Haan, 1835) had not been included in the subgenus by De Man (1895). Serène & Soh (1970) moved *Chiromantes* to full generic level. Holthuis (1977) discovered the mistake by Rathbun and re-validated the name *Perisesarma* (as subgenus) for the species previously included in the subgenus *Sesarma* (*Chiromantes*) sensu Tesch (1917) and sensu Campbell (1967). Conversely, *Chiromantes* Gistel, 1848, was the correct available name for the group previously referred to as *Holometopus* H. Milne Edwards, 1853. Holthuis (1977) also subsequently designated *P. eumolpe* as type species, but this is not accepted, because the action of Campbell has priority.

Therefore the genus *Perisesarma* with the type species *Sesarma dusumieri* has become a full ranked genus, now including 21 Indo-West Pacific and three West African species.

Currently, there are many taxonomic ambiguities related to species of *Perisesarma* (explained in more details in Chapter 2, Introduction) and their phylogenetic relationships are poorly known. Moreover, the phylogenetic relationship between the genera *Perisesarma* and *Parasesarma* need to be re-assessed. In the present study some of them have been addressed.

In Chapter 2, the consistency and taxonomic reliability of the more commonly used morphological characters (chelar dactylar tubercles and pectinated crests) for the identification of species of *Perisesarma* are evaluated.

In Chapter 3, a new species of *Perisesarma* from Vietnam is described based on its unique morphology and phylogenetic position.

In Chapter 4, the monophyly of the genus *Perisesarma* and phylogenetic relationship among the constituent species are investigated. Furthermore, the taxonomic reliability of the carapace epibranchial tooth and the phylogenetic positions of species of *Perisesarma* in the family are addressed.

In Chapter 5, an example of a cryptic species of *Perisesarma* from northern Australian mangroves is described, presenting new molecular evidences.

In Chapter 6, the taxonomy, phylogeny, and phylogeography of the Australasian mangrove crabs *Perisesarma semperi*, *P. longicristatum* and related species are examined based on morphological and molecular results.

In Chapter 7, a new species of *Perisesarma* from the Malay Peninsula is described according to morphological and phylogenetic evidence, previously it was referred to as *Perisesarma indiarum*, which originally was described from Ambon and ternate Islands, Indonesia.

The present work thereby contributes to an ongoing revisionary study of the family Sesarmidae and its constituent genera. Some taxonomic problems and phylogenetic ambiguities related to the species of *Perisesarma* De Man, 1895, are addressed here.

Chapter 2

Evaluating the consistency and taxonomic importance of cheliped and other morphological characters that potentially allow identification of species of the genus *Perisesarma* De Man, 1895 (Brachyura: Sesarmidae)

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Abstract

Recent studies suggest that the family Sesarmidae presents a monophyletic taxon, but within-family taxonomy and phylogenetic relationships need to be resolved. One of the most speciose and taxonomically complex genera of this family is *Perisesarma*. Only few characters allow discriminating species of this genus from each other. Among them, the number and shape of male dactylar tubercles and number of teeth of the pectinated crests are the main diagnostic features. A review of these key characters indicates some intraspecific variability which sometimes renders identification difficult. The present study shows that male dactylar tubercles and pectinated crests are in principle good diagnostic characters at species level in the genus *Perisesarma*, but in several cases show some overlap among species, due to intraspecific variability. New morphological characters should be considered, but in this study we show that the morphology of gonopods and the gastric mill are not informative enough for identification.

Introduction

The family Sesarmidae Dana, 1851 is one of the most diverse taxa and an important faunal component of mangrove communities (Davie, 1994; Guerao et al., 2004; Ragionieri et al., 2009). After the studies by Schubart & Cuesta (1998), Schubart et al. (2000, 2002), and Kitaura et al. (2002), the family nowadays presents a stable monophyletic taxon, although, within-family taxonomy and phylogenetic relationships need some rearrangements and further studies to be resolved (see notes on Sesarmidae in Ng et al., 2008; Schubart et al., 2009). One of the most conspicuous, speciose and also taxonomically complex genera of this family is *Perisesarma* De Man, 1895, distributed in African, Asian, and Australian mangroves (Rahayu & Davie, 2002; Guerao et al., 2004). The type species is *P. dussumieri* (H. Milne-Edwards, 1853) and was designated by Campbell (1967). The history of this genus is characterized by several rearrangements and now this genus consists of species previously attributed to the subgenus *Sesarma* (*Chiromantes*) sensu Tesch (1917) which later was considered a junior subjective synonym of *Perisesarma* (see Campbell, 1967; Holthuis, 1977). The genus was described together with *Parasesarma* by De Man, 1895 as two new subgenera, sharing the same type of tuberculation and pectinated crests on male chelipeds. The only difference between these taxa is that *Perisesarma* is characterized by an anterolateral (= epibranchial) tooth. However, this anterolateral tooth turns out to be a controversial character (see Abele, 1975; von Hagen, 1978; Guerao et al., 2004; Schubart et al., 2006) and its phylogenetic usefulness needs to be confirmed. Today, the genus *Perisesarma* is defined by having a squarish carapace with an anterolateral tooth, two rows of transverse pectinated crests on the dorsal part of the male chelar carpus, and dactylar tubercles on the upper border of the dacytlus (Campbell 1967; Guerao et al., 2004; Naderloo & Schubart, 2010).

The number of species within the genus is a matter of discussion and disagreement, as we will see in continuation to some extent, because of the characters which exclude members of this genus from others in the family. One of the controversial members of the genus is *P. fasciatum* (Lanchester, 1900), which was excluded from the genus by Campbell (1967), because of some incongruence with typical characters of *Perisesarma* characters, but included by Ng. et al. (2008). Later its belonging to the genus was questioned again by Davie (2010). Another ambiguous member of the genus is *P. lanchesteri* (Tweedie, 1936) which was included in the genus by Ng et al. (2008), but excluded by Davie (2010). Also the belonging of three West

African species, *P. alberti* (Rathbun, 1921), *P. huzardi* (Desmarest, 1825) and *P. kamermani* (De Man, 1883) to the genus is in doubt, because of sharing some unique synapomorphies (Ng et al. 2008; Davie, 2010). Similarly, the validity of *P. foresti* Rahayu and Davie, 2003 has been questioned, as it may be a junior synonym of *P. indiarum* (Tweedie, 1940) according to Davie (2010). Three species, *P. indiarum*, *P. lanchesteri* and *P. longicristatum* (Campbell, 1967), were originally described as subspecies (Table 2.1), but now they are ranked as full species without further explanation (e. g. in Ng et al. 2008; Davie, 2010). Moreover, some species of the genus are named and introduced with what today we would consider inadequate descriptions, comparisons, and illustrations (e. g. see descriptions of *P. haswelli* (De Man, 1887), *P. alberti*, *P. dussumieri*, *P. fasciatum*, *P. huzardi*, *P. kamermani*). So deciding on the number of species within this genus needs further studies and revision.

Unfortunately there is a limited number of diagnostic characters to distinguish and discriminate species of the genus *Perisesarma* from each other (see the description of species according to the references given in Table 2.1). Among them, the number and shape of dactylar tubercles and number of pectinated crests are the main diagnostic features which have been commonly most emphasized and employed (see the keys of Tesch, 1917; Campbell, 1967; Davie, 2010). This broad use and potentially species-specific shape of dactilar tubercles may be mainly because of the stridulatory application which was mentioned for the first time by Tweedie (1954). However, a review of these key characters indicates some intraspecific diversity and wide overlap among the species. These similarities sometimes make the identification difficult which has been recognized in previous studies (Davie, 2003; Davie, 2010).

The present study intends to review these key morphological characters separating species of *Perisesarma* in order to evaluate the consistency and reliability of these features for the identification of the constituent species.

Materials and Methods

To address the aims of this study we used available descriptions for species from previous studies (the references are given in Table 2.1). Additionally, material of all species of the genus, were re-examined morphologically, especially concerning the number and shape of dactylar tubercles and number and shape of teeth comprising the pectinated crests. Some of the material was collected from type localities and some was borrowed from or examined at different

museums, including the Australian Museum (AM) Sydney, the Natural History Museum (NHM) London, UK, the Naturalis Museum (RMNH), Leiden, Netherlands; the Queensland Museum (QM), Brisbane, Australia; Ryukyu University Museum, Fujukan (RUMF), Okinawa, Japan; the Senckenberg Museum (SMF), Frankfurt a.M., Germany; the Zoological Reference Collection, Raffles Museum (ZRC), Singapore. As there are sometime more than one name version for some species, the present names are all based on Ng et al. (2008) in order to avoid confusion.

Material examined. (The measurements always refer to carapace width × carapace length in mm) *Perisesarma alberti*, 1 male (14.04×12.21), Cameroon, Tiko, coll. C. H. Otto, 9.01.1934; 1 male (20.78×18.06), Ghana, Ada Foah, coll. C. D. Schubart, 14.07.2001; *Perisesarma bengalense*, 2 males (19.44×16.40; 14.77×12.70), Thailand, Phuket, coll. C. D. Schubart & J. Chai, 24.08.1999; *Perisesarma bidens*, 1 male (25.20/21.73) type (RMNH.CRUS.D.145), Japan, coll. V. Siebold; 1 male (22.17×18.68), China, Hainan, coll. C. D. Schubart, P. Koller & K. von. Wyschetzki, 30.06.2010; 1 male (18.89×15.45), Japan, Iriomote Island, coll. T. Naruse, 22.03.2010; 1 male (20.24×16.70) (RUMF-ZC-1337), Japan, Nagasaki, coll. T. Naruse, 22.11.2010; *Perisesarma brevicristatum*, 1 male (19.83/16.58) paratype (QM-W2459), Australia, Queensland, Innisfail, coll. B. Campbell, 29.10.1963; 1 male (19.49/15.37) paratype (AM-P.15347), Australia, Queensland, Townsville, coll. W. Macnae, 13.03.1962; 1 male (16.37×13.39) (QM-W8270), Australia, Queensland, coll. R. Timmins, 07.05.1978; *Perisesarma cricotum*, 2 males (17.10×14.46; 15.23×12.59) (ZRC 2000.1890), Indonesia, Irian Jaya, Ajkwa River, coll. G. Setyadi, 18.08.1999; 2 males (16.03×13.83; 15.96×13.44) (ZRC 2003.0480), Indonesia, Irian Jaya, Ajkwa River, coll. D. L. Rahayu, 11.01.2000; 3 males (12.51×10.89; 10.45×8.83; 10.44×8.28), Indonesia, Irian Jaya, Ajkwa River, unknown collector, 25.05.2007; 4 males (15.10×13.37; 14.34×12.16; 11.40×9.83; 9.62×8.38), Indonesia, Irian Jaya, Kamora River, unknown collector, 10.04.2008; *P*. cf. *cricotum*, 1 male (13.28×11.17) (QM-W16894), Australia, Queensland, Gulf of Carpentaria, coll. P. Davie, 30.11.1990; 1 male (22.65×18.94) Indonesia, Sulawesi, coll. C. D. Schubart, 19.01.2000; *Perisesarma darwinense*, 1 male (13.31/10.60) paratype (QM-W2443), Australia, Northern Territory, Darwin Island, coll. W. Macnea; 3 males (9.53×7.85; 8.49×6.80; 7.67×6.15), Australia, Northern Territory, Darwin Island, coll. E. Roux, 13.07.2001; *P*. cf *darwinense*, 1 male (12.24×10.24), Australia, Northern Territory, Darwin Island, coll. E. Roux, 13.07.2001; *P*. cf *darwinense*, 1 male (20.65/17.84) (AM-P68307), Australia, Northern Territory, Nungbalgarri Creek, unknown collector, 27.07.1976; *Perisesarma*

dussumieri, 1 male (22.40×19.62), Sri Lanka, Galle, coll. F. Dahdouh-Guebas, 14.11.2002; 3 males (25,21×21.81; 24.50×21.21; 24.26×21.25), Thailand, Phuket, coll. C. D. Schubart, 24.08.1999; *Perisesarma eumolpe*, 3 males (24.00×20.65; 23.48×19.43; 16.83×13.92), China, Hainan, coll. C. D. Schubart, P. Koller & K. von. Wyschetzki, 30.06.2010; 1 male (23.35×20.00), Thailand, collection data unknown; *Perisesarma fasciatum*, 1 male (11.96×10.35) (ZRC 2012.0273), Singapore, coll. B. Y. Lee, 06.08.2011; 1 male (11.22×9.64), Singapore, coll. Z. C. Kong, 01.2002; *Perisesarma foresti*, 2 males (20.41×16.63; 16.16×14.11), Indonesia, Irian Jaya, Ajkwa River, coll. D. L. Rahayu, 31.03.2000; 4 males (17.98×15.49; 17.89×15.58; 17.88×14.81; 16.97×14.64) (ZRC 2000.1889), Indonesia, Irian Jaya, Ajkwa River, coll. D. L. Rahayu, 31.03.2000; 2 males (17.95×15; 16.71×14.50) (ZRC 2000.1818), Indonesia, Irian Jaya, Kamora River, coll. D. L. Rahayu 03.04.2000; *P* cf. *foresti*, 2 males (18.50×15.68; 17.2×14.2), Indonesia, Irian Jaya, Kamora River, unknown collector, 04.04.2002; *Perisesarma guttatum*, 1 male (15.96×13.46), Kenya, Gazi, coll. D. P. Gillikin, 05.2005; *Perisesarma haswelli* (De Man, 1887), 1 male (9.80/7.95) (NHM. 1886.52) purchased from Calcutta museum by Dr. Anderson; *Perisesarma holthuisi*, 1 male (19.16×16.16) paratype (QM-W20314), Australia, Western Australia, Ashburton River, coll. P. Davie, 14.02.2009; *Perisesarma huzardi*, 2 males (43.69×38.49; 18.10×15.90), Ghana, Elmina, coll. C. D. Schubart & K. Duffner, 04.07.2001; *Perisesarma indiarum*, 2 males (28.96×24.76; 22.75×19.24) from syntype (RMNH. CRUS. D. 1415; D. 1910), Indonesia, Moluccas, Ambon, Ludeking collection, 1864; *Perisesarma kamermani*, 1 male (29.50×26.00) holotype (RMNH. CRUS. D. 166), Angola, Muserra, coll. P. Kamerman, 1879; 2 males (29.41/24.10; 22.62/19.3) (RMNH. CRUS. D. 27386; D. 27387), Angola, Luanda, coll. G. Hartmann, 17.06.1967; *Perisesarma lanchesteri*, 1 male (20.81/16.21) type (NHM. 1947.11.18.24) Singapore, coll. M. W. F. Tweedie, 04.1934; 1 male (19.92×15.86) (SMF 7142), unknown locality and collector, 05.07.1914; *Perisesarma lividum*, 1 male (21.44×18.06), Fiji, unknown collector, 30.11.1997; 2 males (28.08×23.92; 27.51×23.34) (RMNH. CRUS. D. 38587; D.1204), Indonesia, Amboina, Ludeking collection, 1863; 2 males (23.94×20.27; 16.90×15.06) (QM-W24243), New Caledonia, coll. P. Davie, 10.04.1992; *Perisesarma longicristatum*, 2 males (18.50/14.94; 14.31/11.58) paratype (QM, W2464), Australia, Queensland, Port Alma, coll. B. Campbell, 6.12.1961; 1 male (17.04×13.72) (QM-W6657), Australia, Northern Territory, Darbilla Creek, coll. Grace & Cooper, 30.08.1975; 1 male (17.57×14.61) (QM-W19924), Australia, South East Queensland, coll. P. Davie,

07.05.1994; 2 males (14.35×12.39; 12.82×11.15) (QM-W20314), Australia, Western Australia, Admiral Island, coll. P. Davie, 30.11.1994; 1 male (13.58/11.02) (QM, W20219), Australia, Western Australia, Mermaid Island, coll. J. W. Short, 18.11.1994 ; *Perisesarma maipoense*, 1 male (27.74/20.99) holotype (NHM. 1976.106) Hong Kong, Mai Po, coll. C. L. Soh, 15.06.1975; 1 male (25.27×19.57) (ZRC 2009.0800), Vietnam, Red River, coll. N. K. Hoang, 01.2008; *Perisesarma messa*, 3 males (18.80×16.05; 17.61×14.38; 12.81×10.48) paratype (QM-W2452), Australia, Queensland, Flying Fish Point, coll. B. M. Campbell, 29.10.1963;1 male (19.79/16.94) paratype (AM-P15349), Australia, Queensland, Flying Fish Point, coll. W. Macnae, 03.03.1962; 1 male (18.52/16.63) paratype (QM-W2446), Australia, Queensland Townsville, coll. W. Macnae, 2.1962; 1 male (21.82×19.69) (QM-W12046), Australia, Queensland, Peel Island, coll. S. Cook, 12.12.1974; 1 male (16.20×13.90) (QM-W18749), Australia, Queensland, Starcke, coll. P. Davie & J. Short, 10.11.1992; 1 male (17.16×14.42) (QM W19223), Australia, Queensland, Townsville, coll. P. Davie, J. Shore & A. Humpherys, 28.10.1993; *P*. cf *messa*, 1 male (19.36×16.74) (ZRC 1999.0650), Australia, Queensland, Brisbane, coll. P. K. L. Ng, 23,01,1997; *Perisesarma onychophorum*, 1 male (22.38×18.70), Malaysia, Pulau Pinang, coll. Y. Sivasothi, 23.04.2000; 1 male (23.93×19.29) (ZRC 2000.1490), Singapore, coll. C. D. Schubart & Y. Sivasothi, 25.04.2004; *Perisesarma samawati*, 1 male (23.70×20.54) holotype (SMF 29333), Kenya, Watamu, coll. D. P. Gillikin, 09-11.1998 ; 1 male (28.57×23.88) paratype (SMF 29334), Kenya, Watamu , coll. D. P. Gillikin, 09-11.1998; 1 male (23.91×19.12), Kenya, Watamu, coll. D. P. Gillikini, 11.1998; *Perisesarma semperi*, 1 male (16.23/12.97) (QM, W23454), Indonesia, Borneo, South Kalimantan, coll. R. & J. Powell, 07.12.1997; 2 males (14.82×12.71; 14.50×11.83), Indonesia, Irian Jaya, Tipoeka, unknown collector, 21.12.1999; 3 males (13.08/1054; 12.23/9.65; 11.72/9.58), Indonesia, Irian Jaya, Ajkwa, coll. A. Darmawan, 18.06.2011; 1 male (11.72/9.58) (QM, W27532), Indonesia, Irian Jaya, Ajkwa, coll. J. Volosin, 21.06.2000; 1 male (13.87/11.16) (NHM.1951.2.15.14), Malaysia, Labuan, coll. G. Nunorg, 1938.

Results and Discussion

In Table 2.1, the 24 species which are currently assigned to the genus *Perisesarma* De Man, 1895 are listed with a summary of their main key characters (pectinated crests and dactylar tubercles). The presence of the double transverse pectinated crests is exclusive for species of the genera *Parasesarma* De Man, 1895 and *Perisesarma*, as a possible synapomorphy which would support the monophyly of the two genera. Ethological importance of these crests as stridulatory organs in inter- and intraspecific communication was highlighted in recent studies (Boon et al., 2009; Chen et al., 2014). But in addition to considerable intraspecific variability (see Table 2.1), the two chelipeds of one individual can show different numbers of teeth. Therefore we hypothesize that small differences in the number of the teeth comprising the crests do not cause a fundamental change in the crabs' behaviour. A general comparison of this character shows that there are three different types of pectination. Most species have two rows of transverse chitinous crests which are pectinated with long teeth (e.g. *P. bengalense* Davie, 2003), but some species have only one longitudinal row, which is pectinated with proportionally short teeth (e.g. *P. alberti* (Rathbun, 1921)) or *P. fasciatum* (Lanchester, 1900) has two longitudinal rows of chitinous butons. But nonetheless, a broad overlap in the number of the comprising teeth of the crests among the species and large intraspecific variability puts into question this character as a reliable diagnostic feature at species level. For example, this character was used to separate *P. longicristatum* (Campbell, 1967) from *P. semperi* (Bürger, 1893) and *P. bidens* (De Haan, 1835) from *P. foresti* Rahayu & Davie, 2002 (according to Davie, 2010), but present data show that the differences are not consistent.

Another taxonomically important feature at species level in the genus *Perisesarma* is the number and shape of tubercles which are situated on the dorsal surface of the dactyli of each cheliped. Ecological significance of this feature as stridulatory organ together with the pectinated crests was first reported and described by Tweedie (1954) and later detailed by Boon et al. (2009) and Chen et al. (2014). Boon et al. (2009) revealed that differences in the morphology of the tubercles in *P. eumolpe* (De Man, 1895) and *P. indiarum* (Tweedie, 1940) results in different sound production which can be a substantial component in their social behaviour, especially during male agonistic interactions. Later Chen et al. (2014) discovered that stridulation in *P. eumolpe* is a victory display which is exclusively used for announcing the triumph, and probably in its consequence to attract females. Nevertheless, we still do not fully understand the

importance of this behaviour in reproductive isolation and species divergence. It is also unknown, how the numbers of the tubercles are acoustically and ethologically important when they are similar in morphology.

Examining the morphology of dactylar tubercles in different species of *Perisesarma* throughout the present study, we observed several cases in which proximal tubercle(s) were very small, sometimes along with a few prominences (e.g. Fig. 2.1A) and found many specimens with distal very low and indistinct tubercle(s) (e.g. Fig. 2.1B). This most likely is also influenced by the time and abrasion since the last moult. So, determining the exact number of tubercles including the proximal and/or distal ones in these specimens is difficult, and consequently enumerating the dactylar tubercles can result in deviations among researchers.

As Table 2.1 indicates, there can be wide overlaps in the number of dactylar tubercles among the species. This can be the reason, why in newer species descriptions and identification keys (Campbell, 1967; Davie, 2010), the described species have been compared accurately with other species of the genus with the same or overlapping number of tubercles, concerning the morphology of dactylar tubercles and pattern of tuberculation. For example, Serène (1975) identified some samples of *Perisesarma* from Sri Lanka erroneously as *P. darwinense* (Campbell, 1967), because having similar numbers of dactylar tubercles. But later, Davie (2003) separated these samples from *P. darwinense* and described them as a new species, *P. bengalense*, having some morphological differences including the shape of the tubercles. Also Rahayu & Davie (2002) described *P. cricotum* as new species with 11-12 dactylar tubercles. They separated this new species from *P. brevicristatum* (Campbell, 1967) (10-11 tubercles) and *P. indiarum* (11-12 tubercles), based on the shape of the tubercles.

Examining more material from each species of the genus during the present study also revealed more intraspecific variation in the number of dactylar tubercles of e.g. *P. cricotum* (10-14), *P. darwinense* (13-16), *P. foresti* (10-13), *P. messa* (Campbell, 1967) (13-16) and *P. semperi* (6-9), (see Table 2.1). Considering this range of intraspecific diversity and possible inaccuracy in counting the number of the tubercles, it becomes necessary to compare all species to those with even slightly different number of the tubercles as well. For example, it is safer to compare *P. foresti* with 10-13 tubercles to *P. messa* with 13-16 tubercles as well, or *P. holthuisi* Davie, 2010 with 12-13 tubercles to *P. darwinense* with 14-16 tubercles, which was not done previously, because of relying on the different numbers of tubercles.

Aiming to find other morphologically diagnostic characters at species level within *Perisesarma*, we also examined the morphology of the median tooth plate of the gastric mill from different species (Fig. 2.2), a character recently introduced by Naderloo & Schubart (2010) as a diagnostic character separating *Parasesarma persicum* Naderloo & Schubart, 2010 from other closely allied species. In addition to this feature, we also compared the shape of the first gonopod from different species (Fig. 2.3), but had to realize that both characters are highly conserved in several species of this genus, suggesting an overall young age or morphological stasis among the constituent members.

In recent years, molecular markers have been increasingly used along with morphological data to address the taxonomy and phylogeny of several brachyuran groups, including the Sesarmidae (Gillikin & Schubart, 2004; Schubart et al., 2006). Concerning the taxonomic complexity of taxa like *Perisesarma* and *Parasesarma* with limited numbers of morphological diagnostic characters at the species level, genetic approaches provide additional tools to facilitate establishing a stable taxonomy (von Wyschetzki et al., 2010; Shahdadi & Schubart, 2014, in preparation). Furthermore, body coloration in live animal, which is not adequately covered in most taxonomic studies, may also be a useful tool in the taxonomy of these taxa. For example Gillikin & Schubart (2004) revealed that *P. samawati* has different coloration compared to the sympatric and congeneric species *P. guttatum* (A. Milne-Edwards, 1869). Likewise, a recent study by Huang et al. (2008) revealed significant differences in colour of facial bands between the sympatric species *P. indiarum* and *P. eumolpe*. Colour differences are also evident between sexes, postulating that these colour differences play an important role in intraspecific sexual recognition (Huang et al., 2008).

Conclusion

The present study shows that it is unclear, how many species should be considered to belong to the genus *Perisesarma* De Man, 1895, as species delimitations are sometimes vague. Even if the number of dactylar tubercles and of teeth of the pectinated crests are generally good diagnostic characters at species level in the genus *Perisesarma*, it is of crucial importance to compare the morphology of the tubercles and the pattern of tuberculation. The lack of a set of consistent diagnostic morphological characters for this taxon, makes the use of molecular markers recommendable as an additional tool for an upcoming revision of the genus. Such a revision is

important to address the taxonomic ambiguities within the genus and to reconstruct the evolutionary history of the corresponding speciation processes.

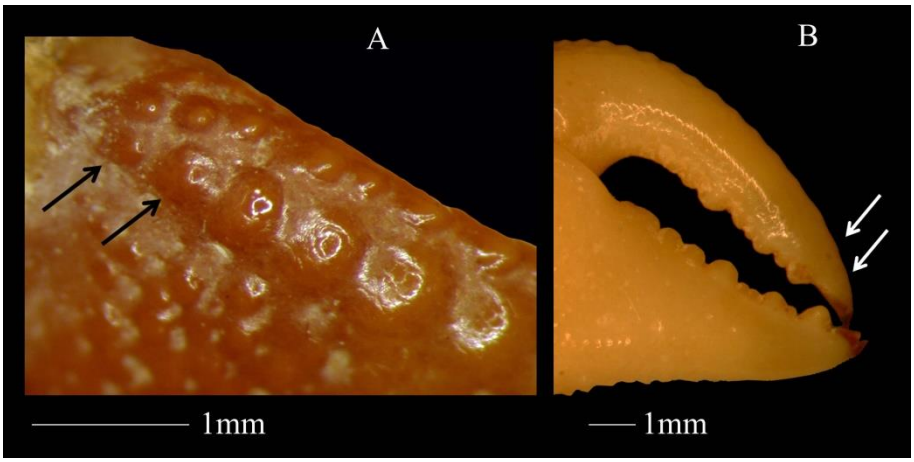

Fig. 2.1. Closeup view of right chela with dactylar tubercles. A. Dorsal view in *P.* cf *messa*, male (ZRC 1999.0650), Australia, Queensland, Brisbane, the arrows indicate proximal tubercles; B. Lateral view in *P. messa*, paratype, male (QM-W2452), Australia, Queensland, Flying Fish Point, The arrow indicate distal tubercles.

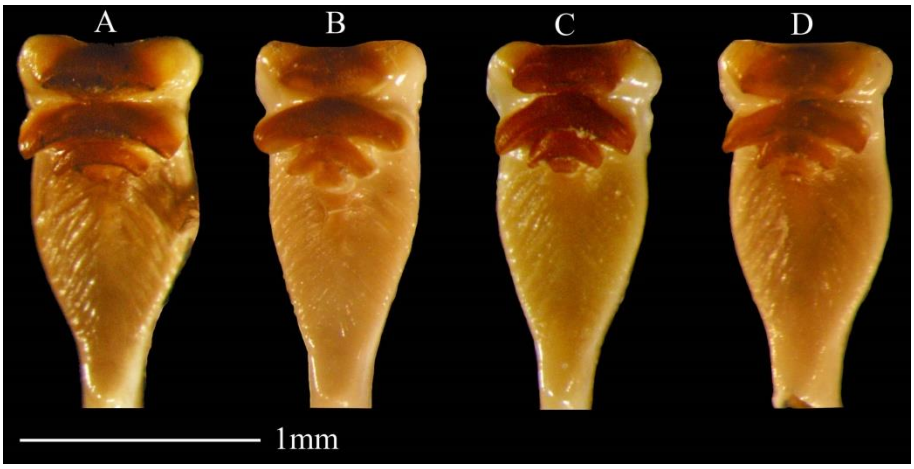

Fig. 2.2. Median tooth plate of the gastric mill. A. *P. bidens*, China, Hainan; B. *P. huzardi*, Ghana, Elmina; C. *P.* cf *cricotum*, Indonesia, Sulawesi; D. *P. eumolpe*, China, Hainan.

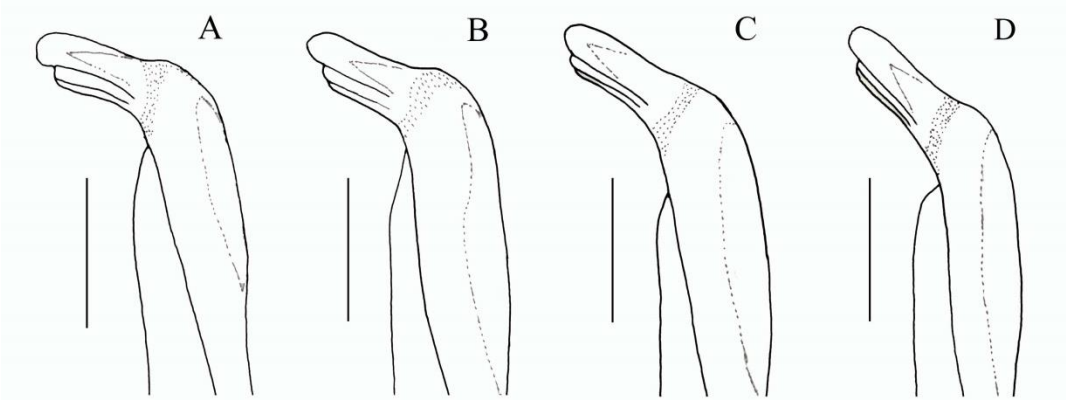

Fig. 2.3. Tips of male left first gonopods denuded of setae, A. *P. cricotum*, Indonesia, Irian Jaya, Kamora River; B. *P. bengalense*, Thailand, Phuket; C. *P. bidens*, Japan, Nagasaki (RUMF-ZC-1337); D. *P. samawati*, Kenya, Watamu. (Scale: 1mm)

Table 2.1. List of 24 species assigned to the genus *Perisesarma* De Man, 1895 with a summary of their main key characters (numbers and shape of dactylar tubercles and numbers and shape of teeth in distal pectinated crests) based on previous studies and present data respectively.

Species and original classification	**Dactylar tubercles**	**Distal pectinated crest teeth**	**References**
P. alberti* (Rathbun, 1921) *Sesarma (Chiromanthes)	31-33, significantly elongated transversely	19-20, nearly longitudinal, short teeth, proximally followed by small tubercles with chitinous tip	Present study
***P. bengalense* Davie, 2003**	16 - 18 closely spaced and transversely broadened proximally	14–18	Davie, 2003
	15-19	16-20	Present study
P. bidens* (De Haan, 1835) *Grapsus (Pachysoma)	12 - 13 oval, clearly distinct	19-22	Davie, 2011
	10 – 14	12-27	von Wyschetzki, 2012 (unpublished data); present study
P. brevicristatum* (Campbell, 1967) *Sesarma (Chiromanthes)	10 - 11 large, symmetrical, subcircular & dome-shaped	13-19	Campbell, 1967; Davie, 2011
	9-10	13-20	Present study
***P. cricotum* Rahayu & Davie, 2002**	11 - 12 prominent, subcircular & ringed with fine circular lines	14-22	Rahayu & Davie, 2002

	10 – 14	15-20	Present study
P. darwinense* (Campbell, 1967) *Sesarma (Chiromanthes)	15 - 16 distinct with median striated stripe	15-20	Campbell, 1967; Davie, 2011
	13 -16	14-22	Present study
P. dussumieri* (H. Milne- Edwards, 1853) *Sesarma	11 - 13, most tipped with chitinous granules		Davie, 2011
	11 – 13	13-18, not transvers and not longitudinal	Present study
P. eumolpe* (De Man 1895) *Sesarma (Perisesarma)	19 – 26		De Man, 1895; Gillikin & Schubart, 2004; Davie, 2011
	21 – 23	17-23	Present study
***P. fasciatum* (Lanchester, 1900) *Sesarma* = *Sesarma (Chiromantes) siamense Rathbun*, 1909**	5 - 6 low and spinose	nearly longitudinal	Lanchester, 1900; Davie, 2011
	6-7 low and spinose with chitinous tip	nearly longitudinal wrinkle with 11-20 chitinous buttons.	Present study
***P. foresti* Rahayu & Davie, 2002**	11 - 12 low, first 3 small and oval, followed by more rounded tubercles, larger and well separated	11-17	Rahayu & Davie, 2002
	10 – 13	11-17	Present study
P. guttatum* (A. Milne-Edwards, 1869) *Sesarma	11 - 13, proximal half oval, with median striated stripe	20	Gillikin &Schubart, 2004; Davie, 2011
	12 - 13	18-19	Present study
P. haswelli* (De Man, 1887) *Sesarma	16 - 19 asymmetrical		Davie, 2011

	17 oval, asymetrical	16	Present study
***P. holthuisi* Davie, 2011**	12 - 13 prominent, well separated & transversely broadened	18-21	Davie, 2011
	13	17	Present study
***P. huzardi* (Desmarest, 1825) *Grapsus* = *Sesarma africana* H. Milne Edwards, 1837**	13-18, rounded or oval, moderately low, proximal and distal one indistinct, some with chitinous tip.	16-18 nearly longitudinal, short teeth, proximally followed by small tubercles with chitinous tip	Present study
P. indiarum* (Tweedie, 1940), as *Sesarma (Perisesarma) bidens indiarum*, De Man, 1902 as *Sesarma bidens* var. *indica	11 - 12 broadly oval and low	11-17	Davie, 2011
	11-12	11-13	Present study
P . kamermani* (De Man, 1883) *Sesarma (Chiromanthes)	7-9 small and very low, distally followed by 16-17 chitinous granules.	17 tubercles, nearly longitudinal, the proximal ones with chitinous tip.	Present study
P. lanchesteri* (Tweedie, 1936) as *Sesarma (Parasesarma) calypso lanchesteri	8-9		Tweedie, 1936; 1950
	8-9 rounded and dome-shaped	18-21	Present study
P. lividum* (A. Milne-Edwards, 1869) *Sesarma	11 - 13, proximal 7 - 8 distinct, becoming less prominent distally, irregular shapes	15-17	Campbell, 1967; Davie, 2011
	10-12	10-18	Present study

P. longicristatum* (Campbell, 1967) as *Sesarma (Chiromanthes) semperi longicristatum	7 - 9 asymmetrical, the third the largest, gradually decreasing in size distally	25	Campbell, 1967; Davie, 2011
	7 – 9 mostly asymmetrical	18-28	Present study
P. maipoense* (Soh, 1978) *Chiromanthes	5 - 8 flattened & indistinct tubercles	15-16	Ng et al., 2010
	6 - 7, irregular in shape	12-14	Present study
P. messa* (Campbell,1967) *Sesarma (Chiromanthes)	14 - 16 low & indistinct	14-19	Campbell, 1967 & Davie, 2011
	13 – 16	12-17	Present study
P. onychophorum* (De Man, 1895) *Sesarma (Perisesarma)	9 - 10 circular or oval, distinct	11-12	Present study
***P. samawati* Gillikin & Schubart, 2004**	7 - 9 rounded & blunt	12–18	Gillikin & Schubart, 2004
	8 rounded or oval	15-16	Present study
P. semperi* (Bürger, 1893) *Sesarma	6 - 8 large, circular, prominent & well-spaced	13-20	Rahayu & Davie, 2002; Komai et al., 2004; Davie, 2011
	7 – 8 mostly symmetrical	18-27	Present study

Chapter 3

Perisesarma n. sp1., a new species of mangrove crab from Vietnam (Decapoda, Brachyura, Sesarmidae), with an assessment of its phylogenetic relationships

Submitted to Crustaceana (accepted for publication).

Abstract

A new species of *Perisesarma* is described from mangroves of Tan Thoi Island, southern Vietnam. Morphologically, the new species differs most significantly from congeners by the tuberculation pattern of the chelar dactylus, its unique G1 morphology, an unusually large maximum body size, and relatively short and broad ambulatory legs. Genetically, *Perisesarma* n. sp1. is markedly divergent from other congeneric species, both in mitochondrial and nuclear DNA. This is the Third species of *Perisesarma* reported from Vietnam.

Introduction

Brachyuran crabs of the family Sesarmidae Dana, 1851 are among the most important faunal components of world mangroves (Davie, 1994; Cannicci et al., 2008; Ragionieri et al., 2009). The taxonomy of the constituent genera of this family has been unstable and has undergone several rearrangements (Serène & Soh, 1970; Holthuis, 1977; Davie, 2010). Ng et al. (2008) have provided the most recent widely accepted compilation of genera and species. There are however a number of genera that are still likely to be polyphyletic, and the composition of *Perisesarma* is under particular scrutiny (see Shahdadi & Schubart, 2015) and ongoing studies are likely to cause some significant changes to the generic definition and delimitation. Nevertheless, the current concept of *Perisesarma* contains 24 species distributed across the Indo-West Pacific region and along the Atlantic coast of southern West Africa (Shahdadi & Schubart, 2015).

The mangrove crab fauna of Vietnam is still relatively poorly studied, and until now, *Perisesarma maipoense* Soh, 1978 and *P. eumolpe* (De Man 1895) are the only species of this genus previously recorded from there (Ng et al., 2010; Diele et al., 2013). But considering the fact that sesarmid mangrove crabs have marine planktonic larvae (see Guerao et al., 2004) and assuming connectivity of mangrove forests of Vietnam with adjacent areas (Giri et al., 2011), it seems likely that targeted collecting will reveal additional species.

Here we describe a new species of *Perisesarma* from the Mekong River Estuary, south of Ho Chi Minh City in southern Vietnam. The phylogenetic position of the newly described species among other related species and their genetic relationship are also presented here.

Material and Methods

Measurements (in millimeters) given in the text and Table 3.1 are of carapace width (cw) followed by carapace length (cl). Abbreviations: G1 = male first gonopod; NHM = Natural History Museum, London, United Kingdom; QM = Queensland Museum, Brisbane, Australia; RMNH = Naturalis Museum Leiden, The Netherlands; SMF = the Forschungsinstitut und Museum Senckenberg, Frankfurt a.M., Germany; ZRC = Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore.

Molecular analyses

Genetic analyses and comparisons were undertaken of putatively related morphologically similar species of *Perisesarma*as as well as other species of *Perisesarma* from neighbouring areas in South East Asia. The type species of the genus, *P. dussumieri*, was also included to examine the phylogenetic relationship with the new species. In addition, previously published sequences from other species of *Perisesarma* were also included (a total of 20 of the 24 species currently included in the genus) (Table 3.1).

Genomic DNA was isolated from leg muscle tissue using a modified Puregene method (Gentra Systems, Minneapolis) and a Mollusc DNA kit (Omega D3373-02) using manufacturers' protocols. Three genes, including the mitochondrial protein-coding gene cytochrome oxidase subunit 1 (Cox1), the mitochondrial gene encoding the rRNA of the large 16S ribosomal subunit (16S), and for a subset of species, the nuclear protein-coding gene sodium-potassium ATPase alpha-subunit (NaK) were partially amplified. Nuclear DNA was only included for those species with closer phylogenetic relationship to the new species in our Cox1 and 16S trees. Polymerase chain reactions (PCR) were carried out with different primer compositions (i.e., COL6/COH6; 16L29/16H11; NaK for-b2/NaK rev3, see Table 3.2) and the following profile: initial step 4 min at 94°C; 40 cycles with 45s at 95°C for denaturing, 60s at 48°C (Cox1), 50°C (16S) and 58°C (NaK) for annealing, 60s at 72°C for extension; and final extension with 5 min at 72°C.

The primers NaK for-b2 (forward) and NaK rev3 (reverse) amplify a segment of about 604 basepairs (including the primer regions) of the NaK gene, the crab-specific primer combination COL6/COH6 amplifies a segment of 709 basepairs of Cox1 (658 without primers), corresponding to the generally used barcoding region (Hebert et al., 2003), and the combination 16L29/16H11 amplifies a segment of approximately 584 basepairs of the 16S rDNA (Table 3.2). PCR products were outsourced for sequencing to Macrogen Europe. Sequences were proofread using Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia), primer regions were removed and the remaining sequences aligned automatically with ClustalW (Thompson et al., 1994) implemented in BioEdit 7.0.5 (Hall, 1999). In order to construct phylogenetic dendrogram, the sequences of Cox1 and 16s genes were then concatenated to create a single alignment. The concatenated alignment and NaK data set were converted with FaBox (Villesen, 2007) to Nexus files for phylogenetic analyses. The best evolutionary model describing our data

was determined with the aid of jModelTest (v. 2.1.4) (Guindon & Gascuel, 2003; Darriba et al., 2012) and selected with the Akaike information criterion (AIC) (Posada & Buckley, 2004). Because of uncertainties in the phylogenetic relationships among members of the genus *Perisesarma* and its sister genus *Parasesarma* (see Schubart et al., 2006), we did not want to force any of the constituent species into the outgroup and rather use unrooted dendrogram for presenting the phylogenetic relationships. These phylogenetic dendrogram was reconstructed with a Maximum Likelihood (ML) algorithm using the software raxmlGUI (v. 1.3) (Silvestro & Michalak, 2012). A maximum parsimony haplotype network (Tempelton et al., 1992) for NaK gene was constructed via Popart (http://popart.otago.ac.nz). Maximum Likelihood (ML) dendrograms were obtained with 2000 bootstrap pseudoreplicates. Pairwise genetic distances (Kimura 2-parameter = K2P) based on the Cox1 sequences among the new species and other related species were calculated with the software Mega version 5.2.2 (Tamura et al., 2011). The new sequences were submitted to GenBank (NCBI), and accession numbers are presented in Table 3.1.

Table 3.1. Material examined for genetic comparisons in this study, with size (cw×cl), sex (M= male, F= female), locality, museum catalogue number and Gen-Bank accession numbers for Cox1, 16S and NaK genes (see main text for museum abbreviations).

Species	Size, Sex	Locality	Catalogue no.	Cox1	16S	NaK
***P. bengalense* Davie, 2003**	14.77×12.70, M	Thailand, Phuket	SMF 49919	KX400901	KX423809	
***P. bidens* (De Haan, 1835)**		South China sea		KM605220*		
P. bidens		Japan, Wakayama			AJ621183*	
***P. berivicristatum* (Campbell, 1967)**	16.37×13.39, M	Australia, Queensland, Murray River	QM-W8270	KX400906	KX423800	
***P. cricotum* Rahayu & Davie, 2002**	14.34×12.16, F	Indonesia, Irian Jaya, Kamora	ZRC2016.0522	KX400897	KX423796	KX394811
***P. darwinense* (Campbell, 1967)**	12.24×10.24, M	Australia, Northern Territory, Darwin Island	SMF 49921	KX400904	KX423798	
***P. dussumieri* (H. Milne Edwards, 1853)**	25.21×21.81, M	Thailand, Phuket	ZRC2016.0525	KX400916	KX423814	
***P. eumolpe* (De Man 1895)**	16.83×13.92, M	China, Hainan, Wen Chang	SMF 49922	KX400891		KX394816
P. eumolpe		Singapore, Lim Chu Kang mangrove			AJ621184*	
***P. foresti* Rahayu & Davie, 2002**	14.25×11.85, F	Indonesia, Irian Jaya, Ajkwa	ZRC2016.0523	KX431204	KX423794	
***P. guttatum* (A. Milne-Edwards, 1869)**		East Africa		KX374922*		
P. guttatum		Kenya, Gazi Bay			AJ621185*	
***P. holthuisi* Davie, 2010**	19.16×16.16, M	Western Australia, Ashburton	QM-W28880, Paratype	KX400907	KX423806	KX394827
***P. indiarum* (Tweedie, 1940)**	28.96×24.76, M	Indonesia, Ambon	RMNH. CRUS. D.141, Lectotype	KX761164	KX761171	
***P. lanchesteri* (Tweedie, 1936)**	24.76×18.78, F	Singapore, Simpang Mak Wai River	ZRC1967.11.8.3	KX761168	KX761174	
***P. lividum* (A. Milne-Edwards, 1869)**	17.76×15.19, M	New Caledonia, Nanmea mangroves	QM-W24243	KX400893	KX423802	KX394812
***P. longicristatum* (Campbell, 1967)**	18.50×14.94, M	Australia, Queensland, Port Alma	QM-W2464, Paratype	KY198240	KY198245	KY198249
***P. maipoense* Soh, 1978**	25.27×19.57, M	Vietnam, mouth of the Red River	ZRC2009, 0800	KX400931		
***P. messa* (Campbell, 1967)**	19.36×16.74, M	Australia, Queensland, Brisbane	ZRC 1999.0650	KX431205	KX423795	KX394835
***P. onychophorum* (De Man, 1895)**	23.93×19.29, M	Singapore, Mandai mangroves	ZRC2000.1490	KX400913	KX423812	
***P. samawati* Gillikin & Schubart, 2004**		Kenya, Mida Creek			AJ621186*	
***P. semperi* (Bürger, 1893)**	11.72×9.58, M	Indonesia, Irian Jaya Ajkwa	QM-W27532	KX400910	KX423804	
***Perisesarma* n. sp1.**	28.23×24.00, M	Vietnam, Tan Thoi Island, Cua Tieu River	QM-W28348 Holotype	KY198241	KY198246	KY198248
***Perisesarma* n. sp1.**	25.27×21.20, F	Vietnam, Tan Thoi Island, Cua Tieu River	QM-W27002 Paratype	KY198242	KY198247	
***Perisesarma* n. sp1.**	23.46×20.30, M	Vietnam, Tan Thoi Island, Cua Tieu River	QM-W27002 Paratype	KY198243		
***Perisesarma* n. sp1.**	23.24×20.46, F	Vietnam, Tan Thoi Island, Cua Tieu River	QM-W27002 Paratype	KY198244		

*previously published sequences from Gen-Bank (https:\\www.ncbi.nlm.nih.gov\).

Table 3.2. Primers used in present study with (5′-3′ DNA sequence) and the corresponding references.

Gene	Primer	Sequence	Reference
Cox1	COL6	TYTCHACAAAYCATAAAGAYATYGG	Schubart, 2009
	COH6	TADACTTCDGGRTGDCCAAARAAYCA	Schubart & Huber, 2006
16S rRNA	16L29	YGCCTGTTTATCAAAAACAT	Schubart et al., 2001 (as 16L2)
	16H11	AGATAGAAACCRACCTGG	Schubart, 2009
NaK	NaK for-b2	ATGACAGTCGCYCAYATGTGGTT	Modified from NaK for-b (Tsang et al., 2008)
	NaK rev3	GGAGGRTCAATCATRGACAT	Tsang et al. 2014

Results

Taxonomy

Family Sesarmidae Dana, 1851

Perisesarma De Man, 1895

Perisesarma n. sp1.

Figs 3.1, 3.2, 3.3, 3.4, 3.7A and 3.8A.

Material examined. Holotype male (28.2×24.0 mm) (QM-W28348), Vietnam, Tan Thoi Island, Cua Tieu River (Mekong River estuary), ca. 70 km south of Ho Chi Minh City, 106°42'E, 10°17'N, coll. Nguyen van Xuan, Feb. 2002. Paratypes: 1 male (23.5×20.3), 2 females (25.3×21.2; 23.2×20.5) (QM-W27002), same data as for holotype.

Comparative material. *P. eumolpe* (De Man 1895): 1 male (16.8×13.9) (SMF 49922) China, Hainan, Wen Chang. *P. maipoense* (Soh, 1978): 1 male (27.7×21.0) holotype (NHM1976.106), Hong Kong, Mai Po marshes; 1 male (25.2×19.6) (ZRC2009, 0800), Vietnam, mouth of the Red River.

Diagnosis. Medium sized sesarmid crab (maximum size of studied specimens 28.2×24.0 mm); carapace rectangular, slightly broader than long; front moderately deflexed, with broad median emargination, postfrontal lobes prominent, median lobes slightly broader than lateral ones, separated by deep median furrow; dorsal carapace regions discernible, anterolateral margin with sharp exorbital angle and well developed epibranchial tooth. Chelipeds homochelous, large; upper surface of palm with 2 transverse pectinated crests, dorsal surface of dactylus with 22–24

low, but distinct, transversely broad tubercles. Ambulatory legs relatively short (ca. 1.5 times as long as carapace width) and broad (merus of third pair ca. 1.9 as long as wide. Male pleon triangular; telson slightly longer than basal width. G1 relatively stout, straight, apical corneous process apically truncated, bent at angle of about 35° to vertical axis, arched in cross section, aperture terminal.

Description. Medium sized sesarmid crab (maximum size of studied specimens 28.2×24.0 mm), carapace subrectangular, slightly broader than long, greatest width between exorbital angles (cw/cl 1.13-1.19, N=4), body relatively vaulted (body height/cw ca. 0.57). Carapace surface smooth, shining, punctate, with numerous short, transverse to slightly oblique crests edged with rows of short setae, sometimes forming low tufts. Front ca. 0.53 times carapace width, moderately deflexed, with broad, relatively deep, median concavity. Post-frontal lobes prominent, median lobes slightly broader than lateral ones, separated by deep furrow. Dorsal carapace regions moderately well indicated; gastric region most strongly demarcated; cardiac region separated from intestinal region, lateral branchial ridges prominent, upper orbital border smooth, lower orbital border finely granulate, anterolateral margin with sharp exorbital angle, well developed epibranchial tooth (carapace width between epibrancial teeth equal to, or only slightly less than, width between exorbital angles), no indication of second epibranchial tooth, lateral margin slightly concave, edged with row of short setae. Eyes pigmented, cornea slightly wider than eyestalk (Figs. 3.1A, 3.2A, C).

Chelipeds homochelous. Chela large (palm length ca. 0.77 × carapace width), robust (length ca. 1.74 × width) (Fig. 3.1). Merus with granulate dorsal border and distinct subdistal spine; ventral border granulate; anterior border granulate, with distinct subdistal spine; inner face smooth with 2 longitudinal rows of setae, ventral row more prominent, continuous, with longer setae, dorsal row interrupted, setae extending to dorsal border. Carpus with inner angle not produced; inner margin granular; outer surface striated, granular. Upper surface of palm with 2 transverse pectinated crests (Fig. 3.2D); distal (primary) crest composed of 18–21 tall, broad teeth (18 and 21 on opposite claws of holotype) (Fig. 3.2G); secondary crest well developed, with 13–16 teeth (13 and 16 on holotype); both crests terminate at inner end in short swollen, tubercular ridge and several blunt granules, 1 or 2 row(s) of coarse granules (some with chitinous cap) proximal to second crest. Upper margin of palm with strong tubercles (some with chitinous tip). Outer surface of palm without setae, coarsely granular except for smooth, punctate fixed finger, lacking

any indication of median longitudinal ridge (Fig. 3.2E); inner surface of palm coarsely granular except area facing carpus (Fig. 3.2F); ventral border of chela almost straight, length of cutting margin of fixed finger ca. 0.35 times length of entire propodus. Dactylus straight, stout, ca. 0.58 times propodus length; dorsal surface bearing 22–24 low, but distinct, transversely broadened tubercles, distinct to tip, each tubercle has series of fine longitudinal lines that give a finely wrinkled appearance, tubercles evenly rounded, lacking transverse sulcus (Figs. 3.2D, E, 6.8A). Row of about 11-17 rounded tubercles on proximal two-thirds of inner edge of dorsal surface. Fingers with crossing chitinous tips, adult males with or without a narrow gape when fingers closed. Cutting edge of both fingers with a series of variably sized teeth (Figs. 3.2E, F).

Ambulatory legs relatively short, broad; third pair longest (Figs. 3.1A, B, 6.4A, B), total length ca. 1.5 times carapace width, merus with anterior margin crenulated, ca. 1.9 times as long as wide, carpus twice as long as wide, propodus ca. 2.5 times as long as wide, dactylus ca. 0.6 times length of propodus; propodus of first ambulatory leg relatively wide (ca. 1.9 times as long as wide) and both margins covered with dense long setae (Fig. 3.2H).

Male pleon triangular, with telson slightly longer than basal width (length ca. 1.1 times width), slightly shorter than somite 6 (ca. 0.9 times length of somite 6); somite 6 longer than others, ca. 1.8 times wider than long; somite 5 trapezoidal, width ca. 2.41 times length; somite 4 also trapezoidal, width ca. 3.16 times length; somite 3 widest, laterally convex; somite 2 medially longer than lateral edges (Figs. 3.1B, 3.2B).

G1 relatively stout, straight (Fig. 3.3A, D); stem triangular with blunt angles in cross section; apical corneous process truncated, bent at an angle of about 35° to vertical axis (Fig. 3.3B), arched in cross section, aperture terminal (Fig. 6.3C). Few scattered short setae along most of gonopod, apical end covered by longer setae, almost completely obscuring corneous tip (Fig. 3.3D); 2 kinds of setae observed, long simple setae (Fig. 3.3E), and some plumose setae (Fig. 3.3F) restricted to outer side of subapical part. G2 short, curved, width of tip about one-third of width of base (Fig. 3.3G). Penis short, stout, curved (Fig. 3.3H).

Female with smaller chelipeds (Fig. 3.4A, B), ratio of palm length to carapace width ca. 0.63; distal dactylar pectinate crest well-developed, prominent as in male, but proximal crest reduced to row of granules with short chitinous tip. Pleon broad, evenly rounded, broadest at somite 4, fringed with long setae (Fig. 3.4D). In adult female specimens, pleon touches coxae of ambulatory legs, telson ca. 1.2 times wider than long, inserted into somite 6 more than half

length (Fig. 3.4B, D). Vulva in depression on anterior edge of sternite 5, somewhat covered by posterior margin of sternite 4; operculum in inner part (Fig. 3.4C).

Colour. The colour in life was not recorded, but the preserved material still shows that adult males appear to have a characteristic chela colour and pattern, with the outer and upper surfaces of the male palm and fixed finger dark reddish brown, while the movable finger is a pale yellowish cream (except for a narrow proximal reddish patch on the upper face adjacent to the articulation) (Fig. 3.2D, E).

Etymology. Warmly dedicated to the memory of Prof. Dr. Michael Türkay, a decapod crustacean specialist who made a significant contribution to our taxonomic understanding of Brachyura of the Asian region.

Habitat and distribution. Full ecological information is lacking for this species, but Davie & Nguyen (2003), in describing their new varunid species *Metaplax gocongensis*, reported that it cohabited with both a species of *Parasesarma* and a species of *Perisesarma*. The latter is the species here described as *Perisesarma* n. sp1. Therefore we know that *Perisesarma* n. sp1can at least tolerate the same conditions as *Metaplax gocongensis,* viz., the intertidal silty-mud zone of the banks of rivers, or ricefields subjected to tidal influence, where salinity ranges from 0–15 p.p.t. During the rainy season, the water may be completely fresh for up to six months, while in the dry months salinity will reach 10–15 p.p.t. on high tides. As an estimate of relative abundances, a shrimp pond belonging to the My Loi community (Vam Co River system, Tiền Giang Province) was drained for harvesting, and all available borrows in the four dike walls surrounding the pond were excavated. The dike walls covered a total area of 2815 m^2. Of 142 individuals collected, 16 were *Perisesarma* n. sp1., nine were unidentified *Parasesarma* species, and the remaining 117 belonged to *Metaplax gocongensis* (Davie & Nguyen, 2003). *Perisesarma* n. sp1. is still only known with certainty from the Vam Co River system, ca. 40 km south of Ho Chi Minh City, Tiền Giang Province, and the Cua Tieu River (Mekong River estuary), ca. 70 km south of Ho Chi Minh City, Vietnam, It can be expected to be more widespread through the region.

Fig. 3.1. *Perisesarmai* n. sp1., holotype, male (28.2×24.0 mm) (QL-W28348) A. Overall dorsal view, B. Ventral view (scale bar 1 cm).

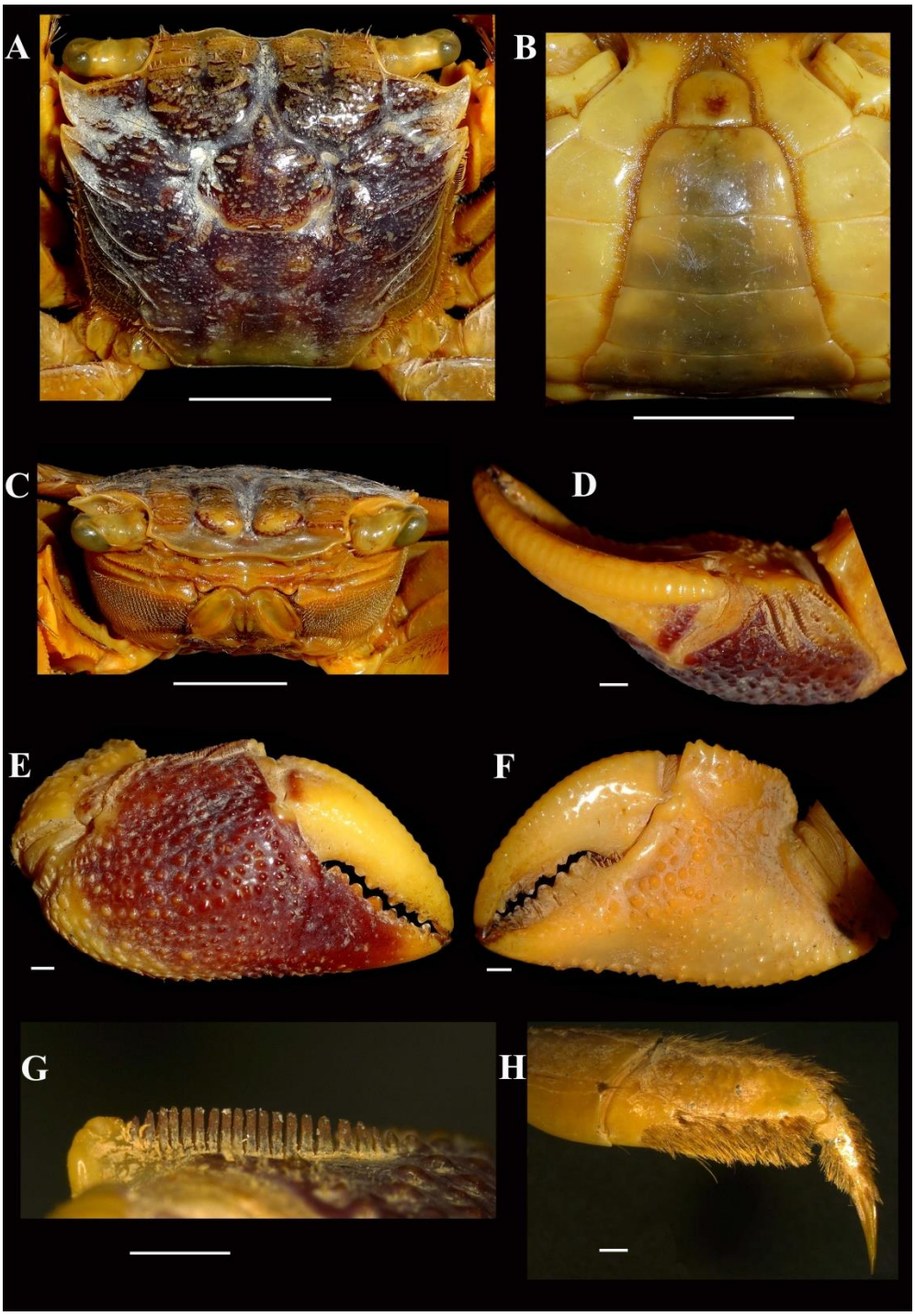

Fig. 3.2. ***Perisesarma*** **n. sp1., holotype male (28.2×24.0 mm) (QL-W28348) A. Carapace dorsal view, B. Pleon, C. Frontal view of carapace, D. Dorsal view of left chela, E. Outer view of right chela, F. Inner view of right chela, G. Frontal view of primary pectinated**

crests, H. Inner side of propodus and dactylus of second pereiopod (scale bar A-C 1 cm, D-H 1 mm).

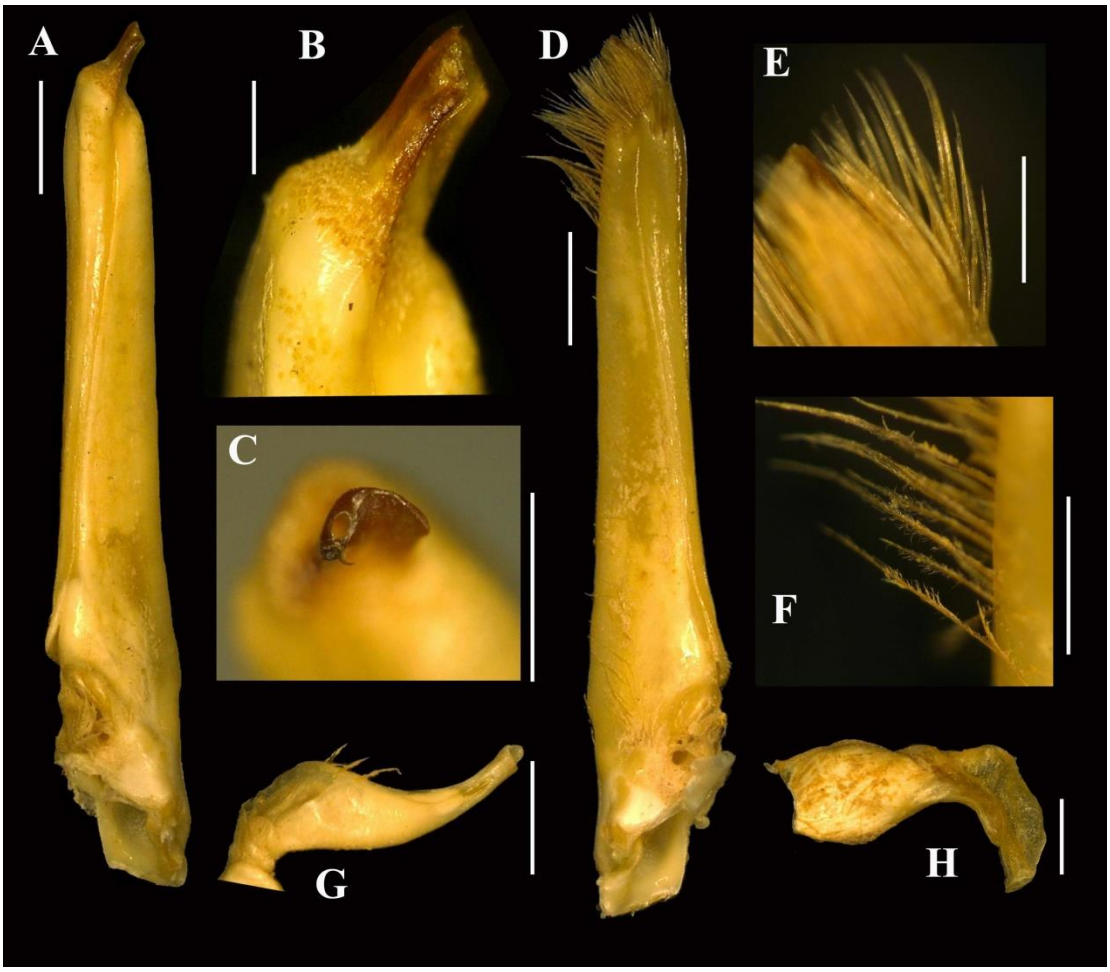

Fig. 3.3. *Perisesarma* n. sp1., holotype male (28.2×24.0 mm) (QM-W28348), A. Dorsal view of denuded right G1, B. Apical process of right G1, C. Cross view of apical corneous process and aperture, D. Left G1 with setae, E. Simple setae on apical process of left G1, F. Plumose setae on apical process of left G1, G. Second gonopod, H. Penis (scale bar A&D 1 mm, B, C, E–H 0.5 mm).

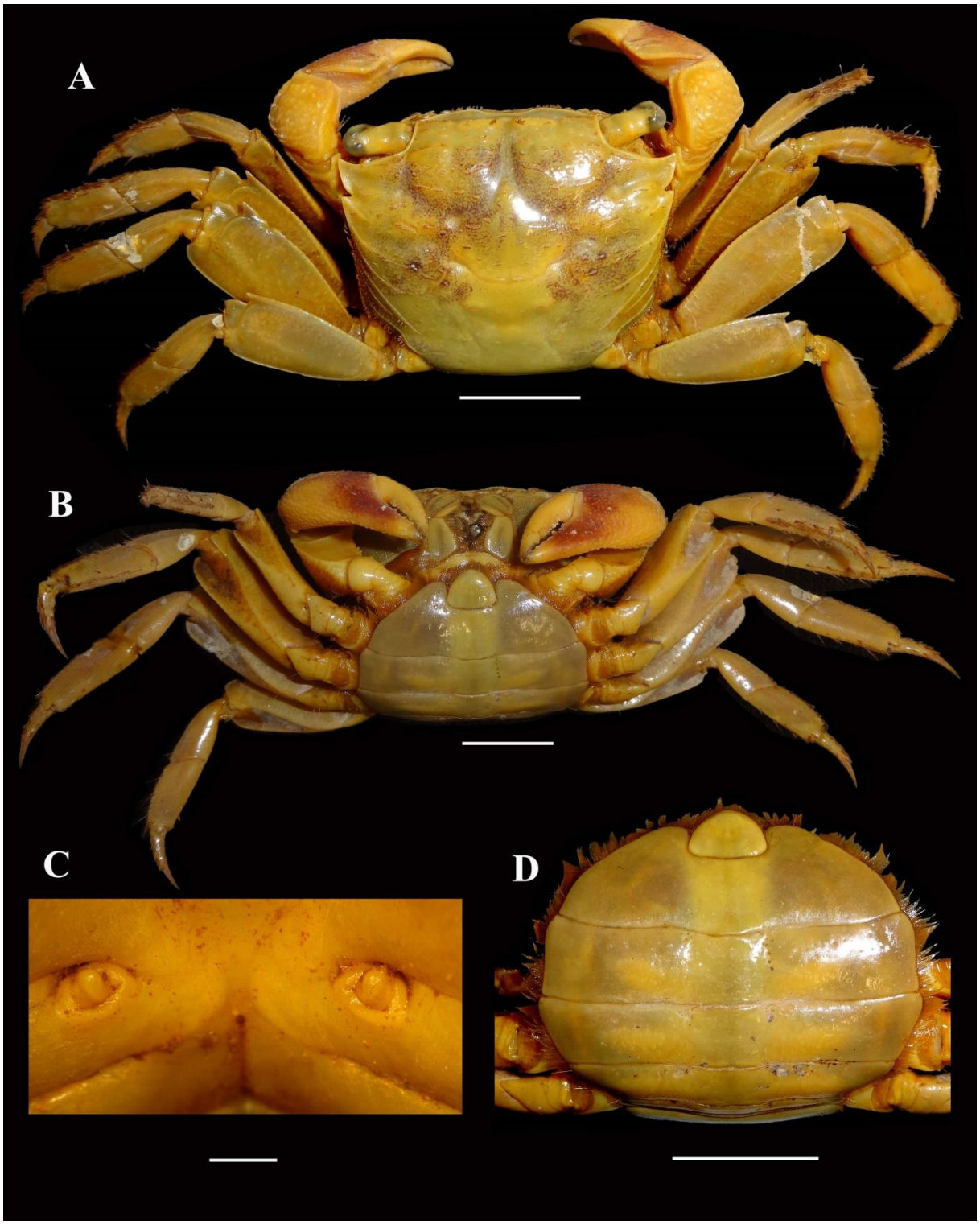
A
B
C
D

Fig. 3.4. *Perisesarma* n. sp1., paratype female (25.27×21.20 mm) (QM-W27002), A. Overall dorsal view, B. Ventral view, C. Vulva, D. Pleon (scale bar A, B, D 1 cm, C 1mm).

Molecular phylogeny

The cropped alignments of concatenated data set (Cox1 and 16S) and NaK consist of 1181 and 501 basepairs (bp) respectively, after removal of the primer sequence and adjacent regions. The alignments of the protein coding genes contained no stop codons, which would have indicated that the gene is not translated to a functional protein, and thus the presence of a pseudogene (see Schubart, 2009). The best evolutionary model explaining the data was the most complex, the General Time Reversible model plus Gamma (GTR+G, Rodriguez et al., 1990).

A phylogenetic dendrogram from the concatenated alignment (Cox1 and 16S genes) was constructed using Maximum Likelihood (ML) analyses (Fig. 3.5). The result shows that *P. tuerkay* n. sp. forms its own distinct clade and is well-separated from other species-groupings (Fig. 3.5).

In terms of Cox1 genetic distances, *P. tuerkay* is again clearly distant from other related species, with the closer being *P. lanchesteri* (K2P = 0.069), *P. foresti* and *P. eumolpe* (both with K2P = 0.071) (Table 3.3). This result is also reflected in the nuclear NaK gene network (Fig. 3.6) which confirms that *P. tuerkay* n. sp. is separated from other potentially related species *P. eumolpe, P. holthuisi* and *P.lividum*, by at least five mutational steps.

The dendrogram shows that the type species, *Perisesarma dussumieri* holds a relatively distant position from the other species of the genus. Also *P. maipoense*, *P. onychophorum* and *P. lanchesteri* hold isolated positions and do not group together with other examined species. In contrast, our analyses reveal some highly supported monophyletic clades among species of *Perisesarma*: *P. eumolpe*, *P. foresti*, *P. indiarum* and *P. messa* are genetically very close to each other and cluster together. The same is true for *P. longicristatum* and *P. semperi*. Another monophyletic clade is the one containing *P. bengalense, P. guttatum, P. bidens, P. cricotum, P. brevicristatum, P. darwinense* and *P. holthuisi*. Of these, the three species *P. brevicristatum, P. darwinense* and *P. holthuisi* appear to be especially closely related. The same can be observed for *P. bidens* / *P. cricotum* and *P. bengalense* / *P. guttatum*. The dendrogram also shows sister species relationship of *P. lividum* and *P. samawati* (Fig. 3.5).

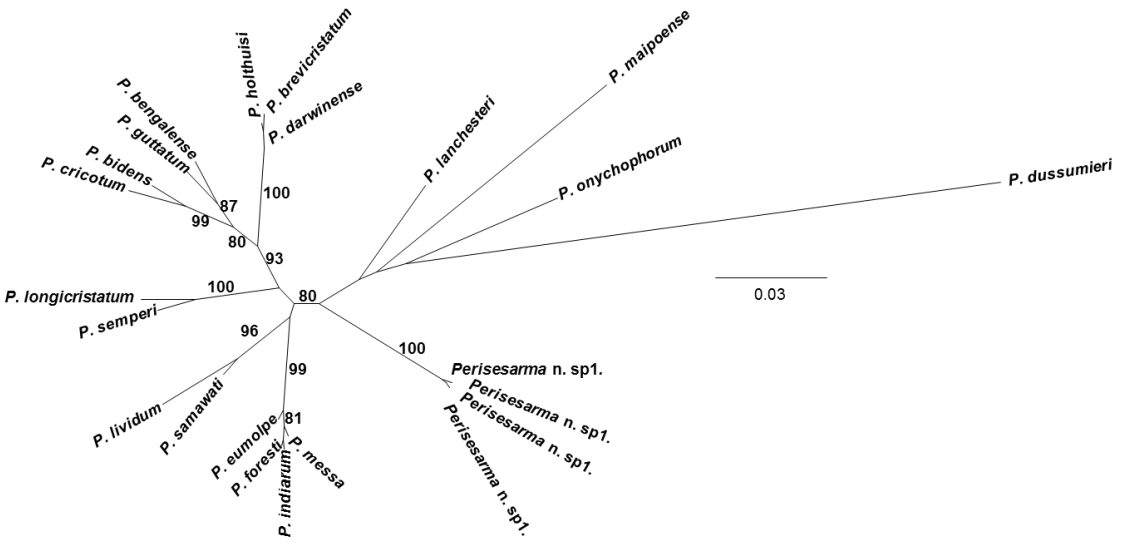

Fig. 3.5. Phylogenetic consensus dendrogram constructed with Maximum Likelihood (ML) (using the software raxmlGUI) of selected species of *Perisesarma,* including *Perisesarma* n. sp1. from a concatenated data set of Cox1 and 16S genes. Only confidence values (bootstrap values) higher than 50% are shown in the dendrogram.

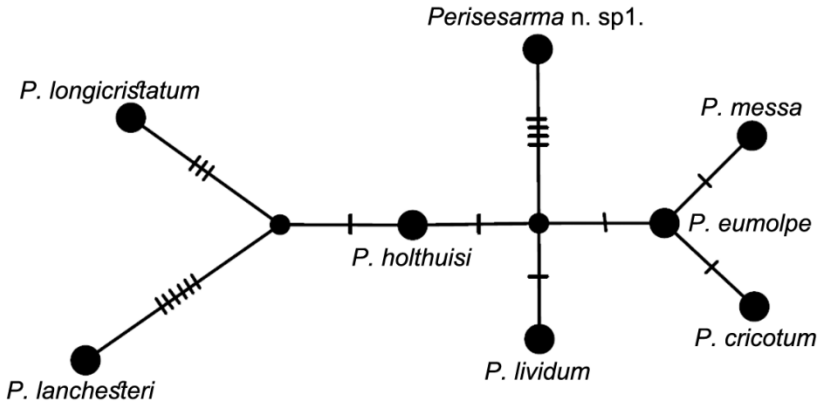

Fig. 3.6. Maximum parsimony genotype network for NaK gene for a subset of *Perisesarma* species, constructed via Popart.

Table 3.3. Genetic K2P distances for the Cox1 gene among examined species of *Perisesarma*, including the new species *Perisesarma* n. sp1.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Perisesarma n. sp1.*																		
2	*P. lanchesteri*	0.069																	
3	*P. foresti*	0.071	0.075																
4	*P. eumolpe*	0.071	0.069	0.015															
5	*P. indiarum*	0.073	0.077	0.005	0.013														
6	*P. lividum*	0.075	0.073	0.065	0.059	0.067													
7	*P. longicristatum*	0.075	0.083	0.066	0.064	0.068	0.082												
8	*P. messa*	0.075	0.075	0.010	0.012	0.008	0.063	0.070											
9	*P. brevicristatum*	0.078	0.081	0.080	0.070	0.082	0.079	0.069	0.076										
10	*P. darwinense*	0.078	0.079	0.076	0.068	0.080	0.078	0.074	0.074	0.012									
11	*P. semperi*	0.080	0.079	0.072	0.070	0.074	0.084	0.031	0.076	0.066	0.070								
12	*P. holthuisi*	0.082	0.079	0.080	0.072	0.085	0.082	0.074	0.079	0.008	0.007	0.070							
13	*P. cricotum*	0.084	0.081	0.068	0.060	0.073	0.078	0.087	0.066	0.069	0.055	0.083	0.063						
14	*P. bidens*	0.088	0.083	0.078	0.074	0.083	0.078	0.088	0.082	0.078	0.063	0.089	0.072	0.037					
15	*P. guttatum*	0.093	0.088	0.077	0.074	0.083	0.076	0.078	0.085	0.076	0.064	0.079	0.072	0.052	0.050				
16	*P. bengalense*	0.093	0.088	0.077	0.066	0.079	0.068	0.085	0.077	0.078	0.068	0.088	0.076	0.050	0.056	0.031			
17	*P. onychophurum*	0.102	0.081	0.107	0.098	0.111	0.095	0.096	0.109	0.096	0.091	0.098	0.091	0.111	0.104	0.100	0.112		
18	*P. maipoense*	0.110	0.089	0.106	0.101	0.104	0.112	0.122	0.099	0.113	0.111	0.117	0.111	0.134	0.127	0.129	0.125	0.099	
19	*P. dussumieri*	0.127	0.121	0.146	0.134	0.149	0.139	0.144	0.146	0.137	0.127	0.139	0.132	0.142	0.142	0.152	0.152	0.135	0.149

Discussion

According to traditional generic/subgeneric definitions (De Man, 1895; Campbell, 1967), the new species belongs to *Perisesarma* by having two transverse pectinated crests on the upper face of the palm (used as a plectrum for stridulatory sound production, see Davie et al., 2015), and a distinct anterolateral epibranchial tooth. *Perisesarma* is indeed very close morphologically to *Parasesarma* with the only significant diagnostic character between them being the presence or absence of a well-defined epibranchial tooth. However, the importance of this tooth is now considered of doubtful value (see Abele, 1975; von Hagen, 1978; Guerao et al., 2004) and its phylogenetic usefulness needs to be confirmed. It seems likely that a number of species of *Parasesarma* may be much more closely related to species of *Perisesarma* than previously thought (Schubart et al., 2006), and the differences, if any, between these two genera will have to be reevaluated.

Perisearma maipoense has been previously reported from Vietnamese mangroves (Ng et al., 2010). This species can, however, be easily separated morphologically from *Perisesarmai* n. sp1.. *Perisesarma* n. sp1. has a relatively straight cheliped dactylus, with more than 20 distinct dorsal tubercles, while *P. maipoense* has a curved dactylus with 5–8 flattened and indistinct tubercles (compare Figs. 3.2D–F and 3.8A in present study with Plate 1d in Soh, 1978 and Figs 1–3 in Ng et al., 2010). There are also clear differences in the G1 morphology between these two species: G1 in *P. maipoense* has narrower apical corneous part, more bended (to create an angle of about 62 °C with vertical axis vs 35 °C in *Perisesarma* n. sp1.) and subterminal aperture while this is terminal in *Perisesarma* n. sp1. (Fig. 3.7E, C).

With regards to other species of *Perisesarma*, only *P. eumolpe* (which also has been previously reported from Vietnam (Diele et al., 2013)) has a similar large number of dactylar tubercles (19–26) (Davie, 2010). But this species is different from *Perisesarma* n. sp1.in tubercle morphology and pattern, bearing obvious transverse sulci across the middle of each tubercle (Fig. 3.8). *P. eumolpe* is also very different from *Perisesarma* n. sp1.in G1 morphology, having a long corneous process with round tip and subterminal aperture while corneous process in *Perisesarma* n. sp1.is rather short, truncated with terminal aperture (Fig. 3.7A, B).

Phylogenetic dendrogram based on mtDNA (Cox1 & 16S) and the NaK genotype network (Figs. 3.5, 3.6) also show that *Perisesarma* n. sp1. is clearly distinct from known congeners. The K2P

distances also confirm significant genetic isolation of the new species from other *Perisesarma* (Table 3.3).

Our present phylogenetic result confirms that in most cases, genetic relationships are in agreement with morphological similarities and also patterns of geographic distributions. For example, the two morphologically similar species (former subspecies), *P. longicristatum* and *P. semperi* are also genetic sister species. This is also the case for *P. foresti* and *P. indiarum*. The three indigenous Australian species, *P. brevicristatum*, *P. darwinense* and *P. holthuisi* are geographically allopatric (east coast, north coast and west coast respectively) but show a close genetic association suggesting evolutionary separation from a common ancestor isolated on that land mass. In contrast, *P. eumolpe* groups tightly with three species (*P. foresti*, *P. indiarum* and *P. messa*) which are morphologically strikingly different. Also two geographically distant species, the East African *P. samawati* and the western Pacific *P. lividum* show a close phylogenetic affinity. Further studies are needed to understand the relationships between biogeographic patterns, speciation mechanisms, and morphological divergences.

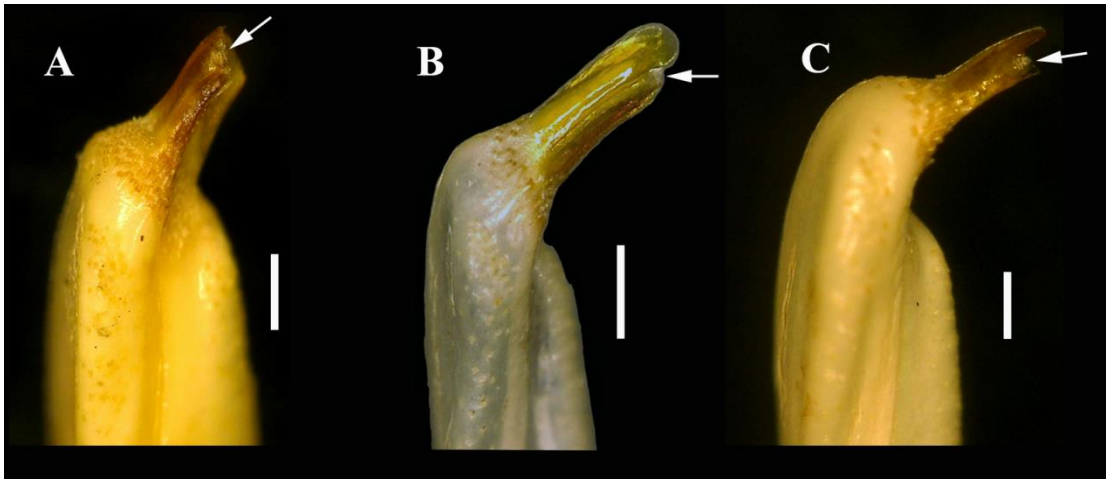

Fig. 3.7. Comparison of the apical processes of G1 (dorsal view) in selected species of *Perisesarma*, A. *Perisesarma* n. sp1., holotype, male (28.2×24.0 mm) (QM- W27002), B. *P. eumolpe*, male (16.83/13.92 mm) (SMF 49922) China, Hainan, Wen Chang, C. *P. maipoense* male (25.27/19.57 mm) (ZRC 2009.0800)Vietnam, mouth of the Red River, the arrows indicate position of aperture (scale bar 0.25 mm).

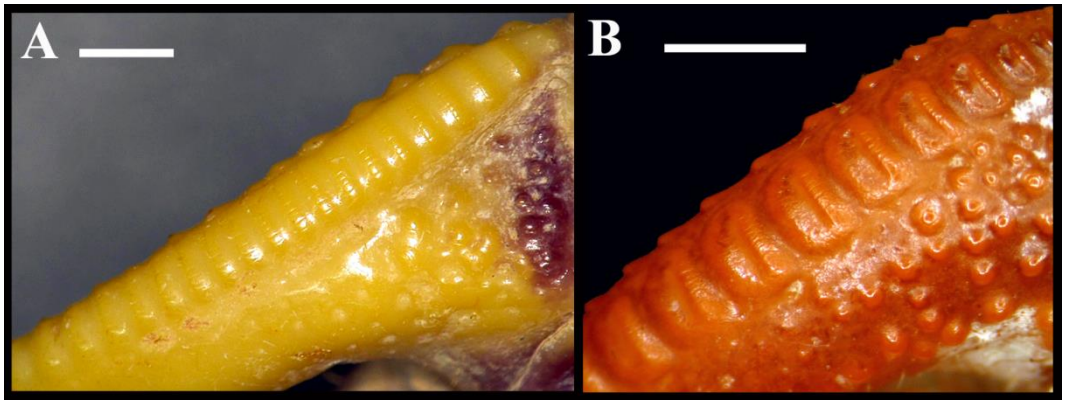

Fig. 3.8. Proximal dactylar tubercles in A. *Perisesarma* n. sp1., holotype, male (28.2×24.0 mm) (QM- W27002), B. *P. eumolpe*, male (16.83/13.92 mm) (SMF 49922) China, Hainan, Wen Chang, (scale bar 1mm).

Chapter 4

Taxonomic review of *Perisesarma* (Decapoda, Brachyura, Sesarmidae) and closely related genera based on morphology and molecular phylogenetics: new classification, two new genera, and the questionable phylogenetic value of the epibranchial tooth

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Abstract

The crab genus *Perisesarma* De Man, 1895 has been shown to be a polyphyletic taxon comprising morphologically heterogeneous species. Some members of the genus have a close phylogenetic relationship with the sister genus *Parasesarma* De Man, 1895, with which it shares many morphological similarities, except for the existence of an epibranchial tooth in *Perisesarma*. This study uses morphological and molecular approaches to reconstruct phylogenetic relationships of *Perisesarma* and related genera and to evaluate the phylogenetic importance and taxonomic usefulness of the epibranchial tooth. As genetic markers we use two mitochondrial and one nuclear gene. A comparative morphological analysis reveals that a clean separation between species of *Parasesarma* and *Perisesarma* is not possible, because of intermediate conformations of the epibranchial tooth. In our molecular analysis, most species of *Perisesarma* cluster solidly together with species of *Parasesarma*, but without being reciprocally monophyletic. Morphology and genetics also indicate that the West African species of *Perisesarma* and the Indo-Pacific *P. fasciatum* are markedly different from all other species of the genus. Therefore, we here suggest with robust double support, a new classification, transferring most species of *Perisesarma* to *Parasesarma* and the three West African representatives and *P. fasciatum* into new genera, thereby restricting the genus *Perisesarma* to the type species *P. dussumieri*.

Additional keywords: Anterolateral tooth – Crustacea – Multimarker DNA phylogeny – *Parasesarma* – Thoracotremata – Generic classification.

Introduction

The brachyuran crab family Sesarmidae Dana, 1851, with 32 genera and over 252 species (De Grave et al., 2009; Naruse & Ng, 2012; Brösing et al., 2014), is among the most diverse taxa and important faunal components of the world mangals (Davie, 1994; Tan & Ng, 1994; Lee, 1998; Guerao et al., 2004; Ragionieri et al., 2009). As a result of the studies by Schubart & Cuesta (1998), Schubart et al. (2000), Schubart, Cuesta & Felder (2002) and Kitaura, Wada & Nishida (2002), the former subfamily was raised to family and reorganized to an apparently monophyletic taxon (adopted in Ng, Guinot & Davie, 2008; Davie, Guinot & Ng, 2015). But within the family, taxonomy and phylogenetic relationships are far from being resolved (see Schubart et al., 2006; Ng et al., 2008).

The most conspicuous and speciose genera of this family are *Geosesarma* De Man, 1892 with 57 species (see De Grave et al., 2009; Schubart & Ng, 2014; Ng, Schubart & Lukhaup, 2015; Manuel-Santos et al., 2016), *Parasesarma* De Man, 1895 with 38 species (see Ng et al, 2008; Davie & Pabriks, 2010; Naderloo & Schubart, 2010; Rahayu & Li, 2013; Ng, Davie & Li, 2016) and *Perisesarma* De Man, 1895 with 24 species (see Shahdadi & Schubart, 2015). The number of species within *Parasesarma* and *Perisesarma* is a matter of discussion and disagreement, mainly because of the characters which delimits members of these genera (Koller, Liu & Schubart, 2010; Shahdadi & Schubart, 2015). The history of these genera is characterized by several re-arrangements and confused interpretations of type species (see Tesch, 1917; Campbell, 1967; Holthuis, 1977; Rahayu & Davie, 2002; Davie, 2010). *Parasesarma plicatum* (Latreille, 1803) and *Perisesarma dussumieri* (H. Milne Edwards, 1853) are the type species of the two genera, designated by Rathbun (1918) and Campbell (1967), respectively. The original description of the genera goes back to De Man (1895), who considered them as two subgenera, defined by the presence of dactylar tubercles and transverse pectinated (comb-shaped) crests on the male chelae. In addition, *Perisesarma* is characterized by an epibranchial (= anterolateral) tooth that is lacking in *Parasesarma*. The definition of the two genera has remained unchanged (Campbell, 1967), despite the fact that the presence/absence of the epibranchial tooth has gradually been recognized to be an unreliable character (Shen, 1932; Tweedie, 1940; von Hagen, 1978). Abele (1975) criticized the use of the epibranchial tooth/teeth for genus delimitations, because of inconsistencies of this feature in some American sesarmids: The genus *Sesarma* Say,

1817 includes species without the tooth (e.g., *Sesarma rectum* Randall, 1840; *S. rubinofforum* Abele, 1973), with a blunt tooth (e.g. *Sesarma verleyi* Rathbun, 1914) or with a distinct large tooth (e.g. *Sesarma rhizophorae* Rathbun, 1906) (see Abele, 1992). Moreover, Guerao et al. (2004) noted that this epibranchial tooth is not always clearly developed and assumed a close phylogenetic relationship of the two genera *Parasesarma* and *Perisesarma*, since otherwise they share most morphological characters in adult and larval morphology. *Perisesarma fasciatum* (Lanchester, 1900) and *Perisesarma lanchesteri* (Tweedie, 1936) are examples of species with ambiguous generic assignments, as both only have a small epibranchial protuberance (see Campbell, 1967; Ng. et al., 2008; Davie, 2010). The recently described *Parasesarma hartogi* Davie & Pabriks, 2010, also shows a small epibranchial tooth in some individuals, resembling some species of *Perisesarma*. Preliminary molecular studies by Fratini et al. (2005) and Schubart et al. (2006) suggest a highly supported monophyletic relationship of these two genera and noted that some members of *Perisesarma* (with an epibranchial tooth) are phylogenetically placed within *Parasesarma* (without an epibranchial tooth). This means that the two genera are not reciprocally monophyletic and that the phylogenetic value of the tooth is questionable.

According to De Man (1985) and Serène & Soh (1970), the taxonomically important morphological character that unites *Parasesarma* and *Perisesarma* and distinguishes them from all other genera of the family is the presence of one or two transverse rows of pectinated crest(s) on the dorsal face of the male chela palm. The ethological significance of these crests as stridulatory organs in inter- and intraspecific communication was recently reported for *Perisesarma indiarum* (Tweedie, 1940) and *Perisesarma eumolpe* (De Man, 1895) by Boon, Yeo & Todd (2009) and Chen, Carrasco & Ng (2014). Davie (2010), in his key of the Indo-West Pacific species of *Perisesarma*, categorized these crests into two types, longitudinal crests, as in *P. fasciatum*, and transverse ones in all other species. Shahdadi & Schubart (2015) indicated that *P. fasciatum* has two longitudinal rows of chitinous tubercles instead of the typical pectinated crests; and in West African species of *Perisesarma* (i.e., *Perisesarma alberti* (Rathbun, 1921); *Perisesarma huzardi* (Desmarest, 1825); *Perisesarma kamermani* (De Man, 1883)) these crests are more longitudinal and the teeth are proportionally short. So, even if the presence of pectinated crests could be a possible synapomorphy supporting the monophyly of *Parasesarma* and *Perisesarma*, the heterogeneity in pectination patterns and tooth type put in doubt their equivalent function. This diversity may be the result of independent evolutionary pathways,

which in this case would question monophyly and thus the current taxonomy. Interestingly, some other genera of the family, including *Clistocoeloma* A. Milne-Edwards, 1873, *Episesarma* De Man, 1895, *Lithoselatium* Schubart, Liu & Ng, 2009, *Metagrapsus* H. Milne Edwards, 1837, *Neosesarma* Serène & Soh, 1970, and *Selatium* Serène & Soh, 1970, also have a pectinated crest on the chelar palm, but in this case clearly shaped as single longitudinal or oblique row (Serène & Soh, 1970; Schubart et al., 2009; Davie, 2012).

Davie (2010) noted that in the type species of *Perisesarma*, *P. dussumieri* (H. Milne Edwards, 1853), somite 6 of the male pleon is unusually elongated, a character which separates this species from its congeners. Moreover, Ng et al. (2008) and Davie (2010) mentioned that the West African species of *Perisesarma* share some unique apomorphies and need to be transferred to a separate genus. In cases like this, when similar taxa present a limited number of morphological diagnostic characters among their constituent species, genetic comparisons are a promising approach to reconstruct phylogenetic relationships and to propose a new and stable taxonomy.

Concerning all the listed taxonomic open questions, the present study intends to reconstruct phylogenetic relationships within the genus *Perisesarma* and related genera by examining a variety of morphological characters and comparing them with results from molecular markers. This should shed some light on the usefulness and consistency of the epibranchial tooth and other characters for the taxonomic classification of the genera *Perisesarma* and *Parasesarma*. After questioning the monophyly of the genus *Perisesarma*, in this study we try to give answers based on morphological data and genetic approaches. The taxonomic position of species like *P. lanchesteri*, *P. fasciatum* and the West African representatives of *Perisesarma* shall thereby be clarified.

Material and Methods

Material examined

To examine monophyly and homogeneity of the genus *Perisesarma*, all species of *Perisesarma* were studied morphologically and genetically (with the exception of *P. haswelli* (De Man, 1887) for which unfortunately, no DNA data could be obtained so far). To evaluate the phylogenetic

usefulness and consistency of the epibranchial tooth for the taxonomic classification of *Parasesarma* and *Perisesarma*, five selected species of the sister genus *Parasesarma*, including the type species, *Parasesarma plicatum* (Latreille, 1803), were examined (see Table 1). *Parasesarma ungulatum* (H. Milne Edwards, 1853) and *P. plicatum* were also included, because of a presumed close phylogenetic relation to members of *Perisesarma* (according to the phylogeny in Naderloo & Schubart, 2010). Selected species from other genera with pectinated crests (i.e., *Episesarma versicolor* (Tweedie, 1940), *Lithoselatium kusu* Schubart, Liu & Ng, 2009, *Metagrapsus curvatus* (H. Milne Edwards, 1837), *Neosesarma gemmiferum* (De Man, 1887), *Selatium brockii* (De Man, 1887)) were morphologically studied in order to compare the diversity and variability in the shape of the crests in different genera of the family. For establishing the phylogenetic position of members of *Parasesarma* and *Perisesarma* within the Sesarmidae, some representatives of other genera (i.e. *Sesarma reticulatum* (Say, 1817), *Selatium brockii*, *Clistocoeloma villosum* (A. Milne-Edwards, 1869), and *Neosarmatium africanum* Ragionieri, Fratini & Schubart, 2012) were selected from different phylogenetic clades of this family, as established in previous phylogenetic work by Schubart et al. (2006). The inclusion of *M. curvatus* appeared important, because of its sympatric distribution with the West African species of *Perisesarma*. *Sesarmoides longipes* (Krauss, 1843) was used as outgroup for phylogenetic analyses, because of the basal position of the genus within the family (see Schubart et al., 2006).

If possible, material was collected or used from the respective type localities, preserved in ethanol 70% and transferred to the laboratory of the University of Regensburg for morphological and molecular examinations. Specimens were borrowed from different museums, including the Queensland Museum (QM), Brisbane, Australia; the Forschungsinstitut und Museum Senckenberg (SMF), Frankfurt a.M., Germany (including material which was incorporated from the Zoologisches Museum der Universität Göttingen (ZMG)); Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore, Singapore; Museo Zoologico Università di Firenze (MZUF), Florence, Italy; Naturhistorisches Museum (NHMW), Vienna, Austria; University of Louisiana Zoological Collection (ULLZ), Lafayette, USA; Ryukyu University Museum, Fujukan (RUMF), Okinawa, Japan; Naturalis Museum (RMNH), Leiden, Netherlands; Western Australian Museum (WAM), Perth, Australia. Some specimens were also examined morphologically at the Naturalis Museum (RMNH),

Leiden, Netherlands and Muséum National d'Histoire Naturelle (MNHN), Paris, France. Additional material was studied at the Natural History Museum (NHM) London, UK (see Table 1 for details on all the material examined).

As sometimes more than one name version exists for some species, the present names are based on Ng et al. (2008). Moreover, for convenience and to avoid any confusion in referring to specific individuals in the text, phylogenetic trees and figure captions, each specimen is coded by a number in the Table 1. Results of this study strongly support a new generic classification for some species. Therefore, we present their new taxonomic placements at the end of this manuscript (see Systematic Account) and in Tables 1 & 4. In the figure captions, species are named based on their new generic composition. The size of each specimen in the figure captions (in parenthesis) is the maximum carapace width/carapace length in milimeter.

Morphological analysis

Animals and body parts were examined and described using a stereomicroscope (Leica S4E & Leica Wild Heerbrugg M8) and a digital microscope (Keyence VHX500F). Photographs of whole animals, carapace and the male pleon of larger specimens were taken with a digital camera (Sony Corp. DSC–WX300), whereas photographs from other body parts, such as chelae, pectinated crests (plectrum), chela dactylar tubercles (rasp), male pleon locking mechanism (press button), male first gonopod (G1), the female genital openings (gonopores = vulvae), and the male pleon of smaller animals were taken with two digital microscopes (Zeiss V20 & Keyence VHX500F) in different magnifications. The photos were edited in Adobe Photoshop CS5 (version 3.0.0.400). G1 were removed from the base, dried and denuded from setae with a small razor blade before examinations. Drawings were prepared initially with the aid of a camera lucida attached to a stereomicroscope (Wild M7A) and edited in Adobe Photoshop. The measurements were carried out using a digital caliper with 0.01 mm accuracy. The general morphological terminologies are based on Davie et al. (2015) and the terminologies concerning female genital openings are based on Guinot, Tavares & Castro (2013), for the sake of consistency.

Table 1. Material examined for this study (in alphabetic order of old generic placements) with new generic composition by present suggestion (if changed), code number, size (carapace width/length in millimeters), sex (M = male, F = female), locality of collection, and museum catalogue number (see main text for museum abbreviations).

Species	New generic placement	Code	Size, Sex	Locality	Catalogue no.
Clistocoeloma villosum (A. Milne-Edwards, 1869)		S181	13.3/11.1, M	Kenya, Mida Creek	MZUF 2500
Episesarma versicolor (Tweedie, 1940)		S235	35.7/33.6, M	Singapore	RMNH.CRUS.D.23348
Lithoselatium kusu Schubart, Liu & Ng, 2009		S257	20.1/18.1, M	Singapore, St. John Island	SMF 49915
Metagrapsus curvatus (H. Milne Edwards, 1837)		S220	29.2/16.6, M	Nigeria, Odimodi	RMNH.CRUS.D.31013
		S254	21.5/17.7, M	Ghana, Ada Foah	SMF 49916
		S280	23.6/18.7, F		SMF 49916
Neosarmatium africanum Ragionieri, Fratini & Schubart, 2012		S185	45.5/40.1, M	South Africa	MNHN-B31275
Neosesarma gemmiferum (Tweedie, 1936)		S225	19.7/17.5, M	Angola, Muserra	RMNH.CRUS.D.40849
Parasesarma ungulatum (H. Milne Edwards, 1853)		S210	16.6/13.0, M	Indonesia, Irian Jaya, Ajkwa River	ZRC 2008.0815
		S650	12.1/8.4, M	Sulawesi	MNHN-B3694 (lectotype)
Parasesarma asperum (Heller, 1865)		S207	16.0/13.0, M	India, Tamil Nadu	ZRC 2001.0851
		S431	18.7/15.5, M	Nicobar	NHMW10408 (syntype)
Parasesarma dumacense (Rathbun, 1914)		S208	20.4/17.0, M	Philippines, Cebu, Kawasan Waterfall	ZRC 2008.0833
		S299	18.1/14.5, F		ZRC 2008.0833
Parasesarma hartogi Davie & Pabriks, 2010		S205	28.7/22.7, M	Australia, Western Australia, Onslow	QM-W28879 (paratype)
		S404	29.4/22.7, M	Australia, Western Australia, Camarvon	WAM C59485

Parasesarma plicatum (Latreille, 1803)		S160	20.4/17.0, M	Thailand, Phuket	ZRC 2000.1913
		S206	22.4/17.8, M		SMF 49918
		S288	17.4/13.8, F		SMF 49927
Perisesarma alberti (Rathbun, 1921)	*Guinearma* **gen. nov.**	S135	20.8/18.1, M	Ghana, Ada Foah	SMF 49911
		S279	24.9/22.1, F		SMF 49911
		S136	14.0/12.2, M	Cameroon, Tiko	SMF 49912
		S242	23.6/20.8, M	Nigeria, Port Harcourt	RMNH.CRUS.D. 15536
Perisesarma bengalense Davie, 2003	*Parasesarma*	S115	14.8/12.7, M	Thailand, Phuket	SMF 49919
		S118	19.4/16.4, M		SMF 49919
		S284	19.7/16.5, F		SMF 49919
Perisesarma bidens (De Haan, 1835)	*Parasesarma*	S140	18.8/15.5, F	Japan, Iriomote, Ryukyu	RUMF-ZC-1232
		S114	20.2/16.7, M	Japan, Nagasaki	RUMF-ZC-1337
		S236	25.2/21.7, M	Japan	RMNH.CRUS.D.145 (holotype)
Perisesarma brevicristatum (Campbell, 1967)	*Parasesarma*	S67	16.4/13.4, M	Australia, Queensland, Cardwell	QM-W8270
		S343	19.8/16.6, M	Australia, Queensland, Innisfail	QM-W2459 (paratype)
Perisesarma cricotum Rahayu & Davie, 2002	*Parasesarma*	S16	15.1/13.4, M	Indonesia, Irian Jaya, Kamora River	SMF 49920
		S38	14.3/12.2, F		ZRC 2016.0522
		S40	14.5/12.3, M		ZRC 2016.0522
		S286	12.6/10.6, F		SMF 49920
Perisesarma darwinense (Campbell, 1967)	*Parasesarma*	S57	12.2/10.2, M	Australia, Northern Territory, Darwin Island	SMF 49921

		S58	9.0/7.5, M		SMF 49921
		S341	13.3/10.6, M		QM-W2443 (paratype)
Perisesarma dussumieri (H. Milne Edwards, 1853)		S123	24.5/21.2, M	Thailand, Phuket	SMF 49930
		S124	25.2/21.8, M		ZRC 2016.0525
		S125	24.3/21.2, M		ZRC 2016.0525
		S214	22.8/20.4, M		SMF 49930
		S281	21.9/19.4, F		SMF 49930
		S221	18.8/16.8, M	Malaysia, Malacca	RMNH.CRUS.D.15285
		S624	31.6/26.2, M	India, Bombay	MNHN IU 2000-10963 (holotype)
		S628	34.5/30.4, M	India, Kerala, Kandankaly	ZRC 2016.0376
Perisesarma eumolpe (De Man, 1895)	*Parasesarma*	S119	16.8/13.9, M	China, Hainan, Wenchang	SMF 49922
		S145	24.0/20.6, F		SMF 49922
		S651	17.0/13.7, M	Malaysia, Chukai	MNHN-B.21854
Perisesarma fasciatum (Lanchester, 1900)	*Fasciarma* **gen. nov.**	S291	9.1/8.1, M	Singapore	NHM1900.10.22.274 (lectotype) (present designation)
		S127	11.2/9.6, M	Singapore, Lim Chu Kang	SMF 49906
		S172	11.9/10.3, M		ZRC 2012.0273
		S597	11.4/9.9, F		ZRC 2012.0273
		S282	13.6/11.5, F		ZRC 2012.0273
		S292	11.2/9.6, M		NHM1947.11.18.17
		S598	11.2/9.8, F		ZRC 2000.1953

		S599	10.5/8.8, F		ZRC 2000.1953
		S600	10.2/8.1, F		ZRC 2000.1953
		S601	10.1/8.8, F		ZRC 2000.1953
		S605	11.2/9.5, M		SMF 49908
		S606	11.3/9.8, M		SMF 49909
		S190	9.0/7.6, M	Singapore, Mandai Kechil	SMF 49907
		S602	9.5/8.2, F		SMF 49907
		S603	10.4/9.2, M	Singapore, Labrador	SMF 49910
		S604	8.5/7.6, M		SMF 49910
		S614	10.7/9.0, M	Hong Kong	QM-W28488
		S615	7.4/6.4, M		QM-W52525
		S648	12.0/10.0, M	Malaysia, Chukai,	MNHN-B.22159
		S649	12.3/10.6, F		MNHN-B.22159
Perisesarma foresti Rahayu & Davie, 2002	*Parasesarma*	S90	17.1/14.5, M	Indonesia, Irian Jaya, Ajkwa River	SMF 49923
		S3	14.2/11.5, F	Indonesia, Irian Jaya, Kamora	ZRC 2016.0523
		S96	14.2/11.8, F		SMF 49923
		S97	17.3/14.9, M		ZRC 2000.1818
		S612	16.6/14.5, M		ZRC 2003.0481 (paratype)
Perisesarma guttatum (A. Milne-Edwards, 1869)	*Parasesarma*	S132	16.2/12.9, M	Kenya, Gazi	SMF 49924
		S283	18.7/15.0, F		SMF 49924
		S652	27.0/22.0, M	Zanzibar	MNHN-B3949 (holotype)

Perisesarma haswelli (De Man, 1887)	*Parasesarma*	S289	9.8/7.9, M	Myanmar, Mergui Archipelago	NHM1886.52 (syntype)
Perisesarma holthuisi (Davie, 2010)	*Parasesarma*	S85	19.2/16.2, M	Australia, Western Australia, Ashburton River	QM-W28880 (paratype)
		S405	17.0/13.8, F	Australia, Western Australia, Onslow	WAM C42674 (paratype)
Perisesarma huzardi (Desmarest, 1825)	*Guinearma* **gen. nov.**	S137	15.0/13.3, M	Ghana, Elmina	SMF 49913
		S193	14.8/12.9, M		SMF 49913
		S187	37.6/35.0, M	Cameroon, Kribi	SMF 49914
		S188	26.2/23.6, M		SMF 49914
		S278	27.4/23.9, F		SMF 49914
		S215	39.0/35.0, M	Guinea Boutry coast	RMNH.CRUS.D.132
		S216	36.5/33.0, M	Nigeria, Movida	RMNH.CRUS.D.30867
		S638	20.2/17.4, F	Senegal	MNHN-B3643
		S639	30.0/27.2, M		MNHN-B16255
		S640	22.6/20.2, M		MNHN-B16255
		S641	30.0/27.2, M		MNHN-B16255
		S642	25.3/22.8, M		MNHN-B16255
		S643	20.6/18.1, M		MNHN-B16255
		S644	22.0/19.2, M		MNHN-B16255
		S645	19.1/16.3, M		MNHN-B16255
		S646	22.7/20.0, F		MNHN-B16255
		S647	21.9/19.1, F		MNHN-B16255

Perisesarma indiarum (Tweedie, 1940)	*Parasesarma*	S146	28.9/24.7, M	Indonesia, Ambon	RMNH. CRUS. D.141 (lectotype) (present designation)
		S502	27.9/23.9, M		RMNH. CRUS. D.19 (paralectotype)
Perisesarma kamermani (De Man, 1883)	*Guinearma* **gen. nov.**	S213	29.5026.0, M	Angola, Mussera	RMNH.CRUS.D.166 (holotype)
		S219	29.4/24.1, M	Angola, Luanda	RMNH.CRUS.D.27386
		S217	22.3/18.5, M		RMNH.CRUS.D.27387
Perisesarma lanchesteri (Tweedie, 1936)	*Parasesarma*	S175	19.9/15.8, M	Unknown locality	SMF 7142
		S176	12.8/10.5, M	Belok Besan	SMF 7139
		S426	24.8/18.8, F	Singapore	ZRC 1967.11.8.3
		S595	20.8/16.2, M		NHM 1947.11.18.24 (holotype)
Perisesarma lividum (A. Milne-Edwards, 1869)	*Parasesarma*	S68	17.2/14.2, F	New Caledonia	QM-W24243
		S69	17.8/15.2, M		QM-W24243
		S76	23.9/20.3, M		QM-W24243
		S148	27.5/23.3, M	Indonesia, Ambon	RMNH.CRUS. D.38587
		S637	26.4/22.2, M	New Caledonia	MNHN-B3634
Perisesarma longicristatum (Campbell, 1967)	*Parasesarma*	S66	20.8/16.9, F	Australia, South East Queensland, Moreton Bay	QM-W19924
		S70	17.5/14.6, M		QM-W19924
		S335	18.5/14.9, M	Australia, Queensland, Port Alma	QM-W2464 (paratype)
Perisesarma maipoense (Soh, 1978)	*Parasesarma*	S128	25.3/19.6, M	Vietnam, Red River	ZRC 2009.0800
		S596	27.7/21.0, M	Mai Po marshes, Hong Kong	NHM 1976.106 (holotype)
Perisesarma messa (Campbell, 1967)	*Parasesarma*	S10	18.8/16.0, M	Australia, Queensland, Flying Fish Point	QM-W2452 (paratype)

		S11	17.6/14.4, M		QM-W2452 (paratype)
		S7	19.4/16.7, M	Queensland, Brisbane, Redland,	ZRC 1999.0650
Perisesarma onychophorum (De Man, 1895)	*Parasesarma*	S120	23.9/19.3, M	Singapore	ZRC 2000.1490
		S121	22.4/18.7, M	Malaysia, Pulau Penang	SMF 49925
Perisesarma samawati (Gillikin & Schubart, 2004)	*Parasesarma*	S154	23.7/20.5, M	Kenya, Watamu	SMF 29333 (holotype)
		S174	28.6/23.9, M		SMF 29334 (paratype)
		S155	N.A	Seychelles	N.A
Perisesarma semperi (Bürger, 1893)	*Parasesarma*	S74	13.7/11.4, F	Indonesia, Irian Jaya, Tipoeka	ZRC 2016.0524
		S75	14.4/11.7, F		ZRC 2016.0524
		S448	17.1/13.9, M	Philippines, Bohol	ZMG 625 (lectotype) (present designation)
Selatium brockii (De Man, 1887)		S182	N.A	Kenya, Mida Creek	MZUF 2546
		S255	21.0/19.2, M	Malay Peninsula	SMF 49931
Sesarma reticulatum (Say, 1817)		S180	N.A	USA, Delaware, Woodland Beach	ULLZ 3835
Sesarmoides longipes (Krauss, 1843)		S183	13.0/16.9, M	Kenya, Mida Creek	MZUF 2505

* Not availabl

Molecular analysis

Genomic DNA was isolated using a modified Puregene method (Gentra Systems, Minneapolis) from muscular leg tissue. Fragments of three genes, including the mitochondrial protein-coding gene cytochrome oxidase subunit 1 (Cox1), the mitochondrial gene encoding the rRNA of the large ribosomal subunit (16S), and for a subset of specimens (one specimen selected from each phylogenetic clade of the Cox1 and 16S trees) the nuclear protein-coding gene sodium-potassium ATPase alpha-subunit (NaK), were amplified. Polymerase chain reactions (PCR) were carried out with different primers (see Table 2) and the following profile: initial denaturation step for 4 min at 94°C; 40 cycles with 45s at 95°C for denaturing; 60s at 48°C (Cox1), 50°C (16S) and 58°C (NaK) for annealing, 60s at 72°C for extension; and 5 min at 72°C as a final extension step. To amplify a segment of about 604 basepairs (including the primer regions) of the NaK gene, the primers NaK for-b2 (forward) and NaK rev3 (reverse) were used (Table 3). In case of Cox1 and 16S, different primer combinations were applied (detailed in Table 3), because of different quality and quantity of extracted DNA. For example, the primer combination COL6/COH6 was used to amplify a segment of 709 basepairs of Cox1 (658 without primers). But this combination did not work for old museum specimens (in some cases almost 50 years old), because of strong DNA degradation and fragmentation. Instead, two shorter fragments (about 350 to 400 basepairs) with newly designed taxon-specific primers allowed amplifying the same segment using the primer combinations COL6/COH7P and COL7/COH6 (for details see Tables 2 & 3). In case of the 16S gene, the longer combination was 16L2/16H11 (amplifying a segment up to 585 basepairs, depending on the number of indels), but shorter fragments were amplified for specimens with degraded DNA (detailed in Table 3). PCR products were outsourced for sequencing to Macrogen Europe.

Sequences were proofread using Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia), primer regions were removed and the remaining sequences aligned automatically with ClustalW (Thompson, Higgins & Gibson, 1994) implemented in BioEdit 7.0.5 (Hall, 1999). The best evolutionary model describing our data was determined with the aid of jModelTest (v. 2.1.4) (Darriba et al., 2012) and selected with the Akaike information criterion (AIC) (Posada & Buckley, 2004). Four alignments, three from the individual genes and one for the concatenated dataset, were converted with FaBox (Villesen, 2007) to Nexus files as input for phylogenetic analyses. Two methods of phylogenetic inference were applied to our dataset: Maximum

Likelihood (ML) using the software raxmlGUI (v. 1.3) (Silvestro & Michalak, 2012) and Bayesian Inference (BI) as implemented in Mr.Bayes (v. 3.2) (Huelsenbeck & Ronquist, 2001). The data of the concatenated alignment was first partitioned by genes as the markers have different mutation characteristics. Maximum Likelihood (ML) trees were obtained for each alignment with 1000 fast bootstrap repeats. For the Mr.Bayes analysis, we used 3 million generations with 4 chains (one heated) and a sample frequency of one tree per 1,000 generations. Sequences were submitted to NCBI and are available from GenBank under the accession numbers given in the phylogenetic trees (i.e., for Cox1: KX400885–KX400938, KX431204, KX431205, KX761164– KX76168; for 16S: KX423794–KX423841, KX761171– KX761174; for NaK: KX394810–KX394835, KX761169, KX761170).

Table 2. Primers used in present study with the corresponding DNA sequences (5′-3′) and references.

Gene	Primer	Sequence	Reference
Cox1	COL6	TYTCHACAAAYCATAAAGAYATYGG	Schubart, 2009
	COH7P	GRAGAGAAAAAATACCTA	new
	COL7	GGTGTKGGMACMGGATGAACTGT	Schubart, 2009
	COH6	TADACTTCDGGRTGDCCAAARAAYCA	Schubart & Huber, 2006
16S rRNA	16L2	TGCCTGTTTATCAAAAACAT	Schubart et al., 2001
	16L7	CYTGTTTAWCAAAAACATGTCT	new
	16H7	CCGGTCTGAACTCAAATCATGT	Schubart, 2009
	16H11	AGATAGAAACCRACCTGG	Schubart, 2009
NaK	NaK for-b2	ATGACAGTCGCYCAYATGTGGTT	Modified from NaK for-b (Tsang et al., 2008)
	NaK rev3	GGAGGRTCAATCATRGACAT	Tsang et al., 2014

Table 3. Used primer combinations with the corresponding alignment (for 16S) lengths in base pairs (including and excluding primer regions respectively) for each combination used in the present study.

Primer combination		Fragment length
Forward	Reverse	
COL6	COH6	708, 658
COL6	COH7P	429, 386
COL7	COH6	370, 321
16L2	16H11	584, 546
16L2	16H7	560, 518
16L7	16H7	558, 514
NaK for-b2	NaK rev3	604, 563

Results

Morphology

Comparative morphology of the epibranchial tooth in different species of *Parasesarma* and *Perisesarma* indicates that there is no consistent separation among species of the two genera and several species show a gradient concerning this character (Figs. 1 & 2). There are species with no sign of a tooth, prominence or notch (e.g., *Parasesarma plicatum*, see Figs. 1A & 2A) and species with a distinct tooth (e.g., *Perisesarma lividum*, see Figs. 1I & 2I), although with noticeable variability in the size and shape of the tooth (Figs 1F–I & 2F–I). However, there are also species with intermediate tooth shapes (i.e., in *Parasesarma dumacense* with a slight notch at the position of the epibranchial tooth (Figs. 1B & 2B), in *Parasesarma asperum* with a small prominence present at the same position (Figs. 1C & 2C), in *Parasesarma hartogi* this prominence is distinct (Figs. 1D & 2D), and in *Perisesarma lanchesteri* this prominence can be described as a small tooth (Figs. 1E & 2E)).

In the present study, different types of pectinated crests (on the dorsal face of chela palm) were recognized among the selected species of the family Sesarmidae (Figs. 3 & 4). Males of most species of *Parasesarma* and *Perisesarma* have two rows of transverse crests with elevated teeth, each row (crest) framed by a high and large tubercle on the inner side (e.g., *Parasesarma dumacense* (Figs. 3A & 4A), *Parasesarma plicatum* (Figs. 3B & 4B), *Perisesarma bengalense* (Figs. 3C & 4C) and *Perisesarma cricotum* (Figs. 3D & 4D)). *Perisesarma dussumieri* bears two oblique rows of crests with elevated teeth, followed by 2–4 small tubercles with chitinous tip on the inner side (Figs. 3E & 4E). *Perisesarma fasciatum* shows two low and nearly longitudinal to oblique ridges or wrinkles instead of true pectinated crests (Fig. 3F). In this species, each ridge bears a row of small chitinous caps (Fig. 4F). The outer ridge is positioned on the distal third of the upper surface of the palm, starting from the angle between inner and distal margin (Fig. 3F). The inner ridge takes its beginning from the same angle and ended to proximal rim, with chitinous caps only on the distal half (Fig. 3F). The West African species *Perisesarma alberti* (Figs. 3G & 4G), *Perisesarma huzardi* (Figs. 3H & 4H) and *Perisesarma kamermani* (Figs. 3I & 4I) have one oblique crest with short teeth on the distal half of the upper surface of the palm, the crest starting from the angle between inner and distal margin. The teeth are thicker and stouter distally and are proximally followed by a line of small granules. On the inner part of the upper

surface of the palm, adjacent to the pectinated crests, one to several short row(s) of granules, sometimes with chitinous tip, are present. A similar morphology of the crest (but without granules rows) can also be observed in *Metagrapsus curvatus* (Figs. 3J & 4J). In contrast, *Clistocoeloma villosum*, *Episesarma versicolor*, *Lithoselatium kusu*, *Neosesarma gemmiferum* and *Selatium brockii* have one longitudinal crest, with long teeth from stem to stern on the dorsal face of chela palm (Figs. 3K & 4K).

Morphological comparisons of the male pleon (= abdomen of Decapoda, see also Davie et al., 2015) in different species of *Parasesarma* and *Perisesarma* also revealed some heterogeneity (Figs. 5 & 6; Table 4). The pleon somites 5 and 6 as well as the telson in *Perisesarma dussumieri* (Figs. 5E & 6E), the West African species of *Perisesarma* and *Metagrapsus curvatus* (Figs. 5G–J & 6G–J) are proportionally longer than in other species of the compared genera. For example, the ratio of width to length for the somite 6 in *Perisesarma dussumieri* is ca. 1.45, while in other Indo-West Pacific species, it is at least 1.90 (see Table 4). This ratio for the somite 5 in the West African group is less than 2 (1.60–1.98), whereas it is at least 2.27 in other species (2.27–3.30, see Table 4). Among all examined species, *Perisesarma fasciatum* has significantly wider pleonal somites (Figs. 5F & 6F; Table 4). As an example, the ratio of width to length for the somite 6 in this species is $\geq$ 2.42, whereas it is $\leq$ 2.28 in the other species (see Table 4). Moreover, the presence of a shallow concavity between somite 5 and 6 in *Perisesarma dussumieri* distinguishes this species from other species of *Parasesarma* and *Perisesarma* with regard to this character (see the arrow in Fig. 6E).

We also examined the absence or presence of the press button of the male pleon locking mechanism on thoracic sternite 5 in some species of *Parasesarma* and *Perisesarma* (Fig. 7). In most species, a press button is absent (e.g., *Parasesarma dumacense* (Fig. 7A), *Perisesarma bengalense* (Fig. 7C), *Perisesarma cricotum* (Fig. 7D) and *Perisesarma fasciatum* (Fig. 7F)) or indistinct (e.g., *Parasesarma plicatum* (Fig. 7B)). The West African species of *Perisesarma* and *M. curvatus* have a small but distinct press button (Fig. 7G–J), whereas *Perisesarma dussumieri* bears a proportionally large press button (Fig. 7E).

Examining the morphology of G1 revealed some heterogeneity in the studied species. G1 in most species of the genera *Parasesarma* and *Perisesarma* are long and slender with long corneous apical processes (e.g., *Parasesarma dumacense*; *Parasesarma plicatum*; *Perisesarma*

bengalense; *Perisesarma cricotum* (Fig. 8A–D). In *Perisesarma dussumieri*, however, the G1 is proportionally long with a rather short corneous apical process (Fig. 8E). In *Perisesarma fasciatum*, it is short and stout with a long corneous apical process (Fig 8F). In contrast, West African species of *Perisesarma* have relatively long G1, with considerably short corneous apical processes notched in the middle (Fig. 8G–I). In *M. curvatus* the G1 is very slender and differs from others (Fig. 8J).

Morphological comparisons of the female pleon shows that in most species of *Parasesarma* and *Perisesarma*, the telsons are wider than long and almost more than half of their length is inserted into pleonal somite 6 (Fig. 9A–D). In *Perisesarma dussumieri* (Fig. 9E) and *Perisesarma fasciatum* (Fig. 9F), however, the telson is also wider than long, but less clearly inserted into somite 6. In West African *Perisesarma* and *M. curvatus* (Fig. 9G – H) the telson is longer than wide or equal and inserted into somite 6 by less than half of its length.

The female vulvae also reveal some interesting diversity. In most species of *Parasesarma* and *Perisesarma*, the vulvae are positioned on sternite 5, but the operculum (which is located anteriorly) reaches the line between sternite 4 and 5 (Figs. 10A–D). In *Perisesarma dussumieri* (Fig. 10E), however, the vulva is positioned completely in sternite 5, rimmed posteriorly and the operculum located on the inner side. The presence of a large sternal cover on the anterior side of the operculum further discriminates this species from others. The vulvae in *Perisesarma fasciatum* (Fig. 10F) have an elongated operculum that is rimmed perpendicular to sternal sutures and entirely located in sternite 5. The vulvae in West African *Perisesarma* (Fig. 10G & H) are more complex and have an elongated operculum almost parallel to sternal sutures that are located in sternite 5, the inner-most part reaching the sternal suture between sternite 4 and 5. Besides this, West African species of *Perisesarma* have a small sternal cover on the anterolateral corner of the operculum (Fig. 10G & H). *M. curvatus* is different from others by having vulvae with an elongated operculum that is rimmed parallel to sternal sutures and entirely located in sternite 5.

A comparison of overall carapace morphology among the West African species reveals that *M. curvatus* is very distinct from the others (i.e., *Perisesarma alberti*, *Perisesarma huzardi* and *Perisesarma kamermani*) by having a relatively transverse ovate carapace while it is almost rectangular in the other three species (Fig. 11).

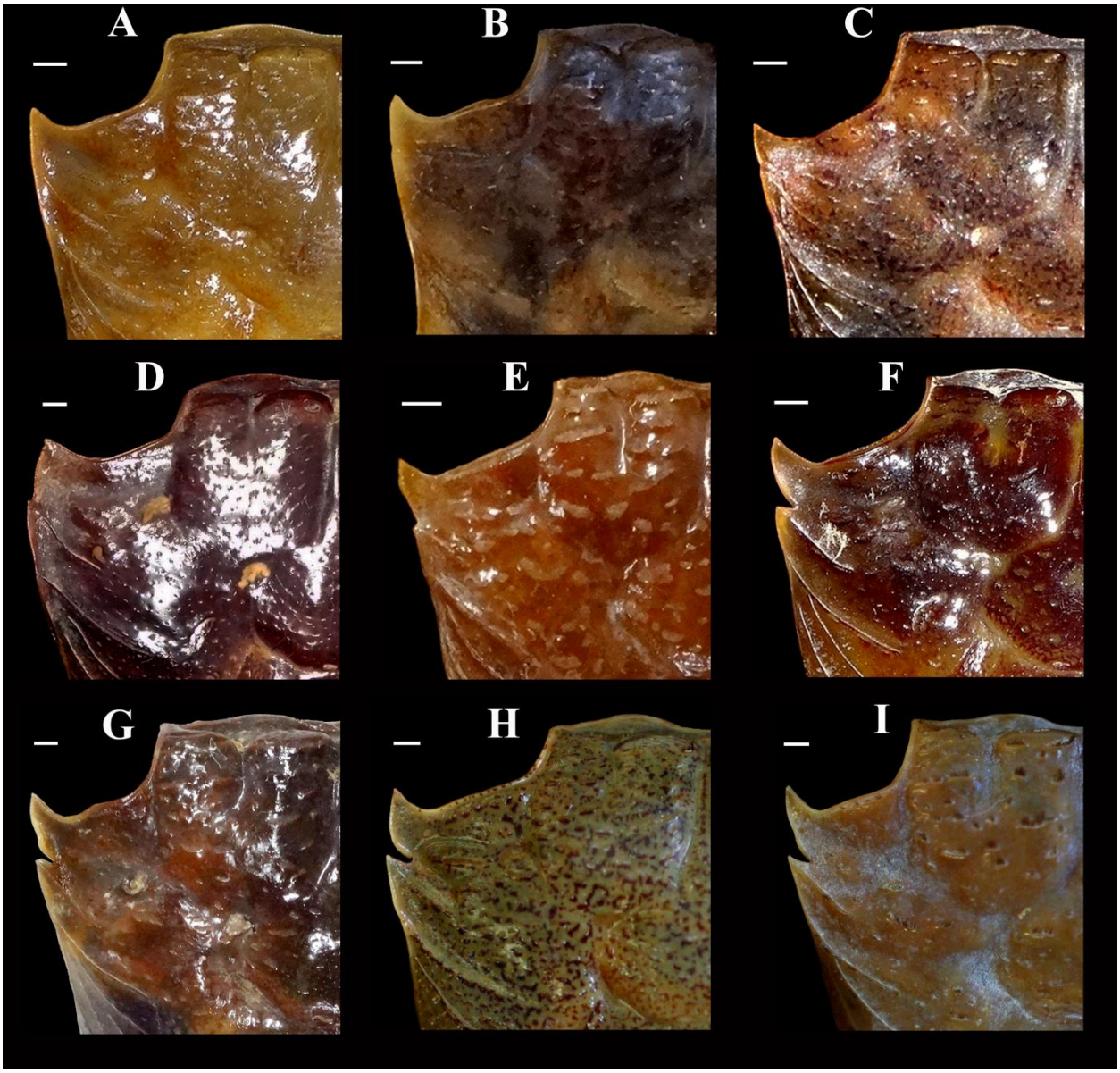


Figure 1. Dorsal view of left frontal part of carapace in male specimens for comparison of epibranchial tooth in selected species of *Parasesarma*: A, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); B, *Parasesarma dumacense* (20.4/17.0, ZRC 2008.0833 = S208); C, *Parasesarma asperum* (16.0/13.0, ZRC 2001.0851 = S207); D, *Parasesarma hartogi* (28.7/22.7, QM-W28879 = S205); E, *Parasesarma lanchesteri* comb. nov. (19.9/15.8, SMF 7142 = S175); F, *Parasesarma onychophorum* comb. nov. (23.9/19.3, ZRC 2000.1490 = S120); G, *Parasesarma samawati* comb. nov. (28.6/23.9, SMF 29334 = S174); H, *Parasesarma maipoense* comb. nov. (25.3/19.6, ZRC 2009.0800 = S128); I, *Parasesarma lividum* comb. nov. (27.5/23.3, RMNH.CRUS. D.38587 = S148); scale bar: 1.0 mm.

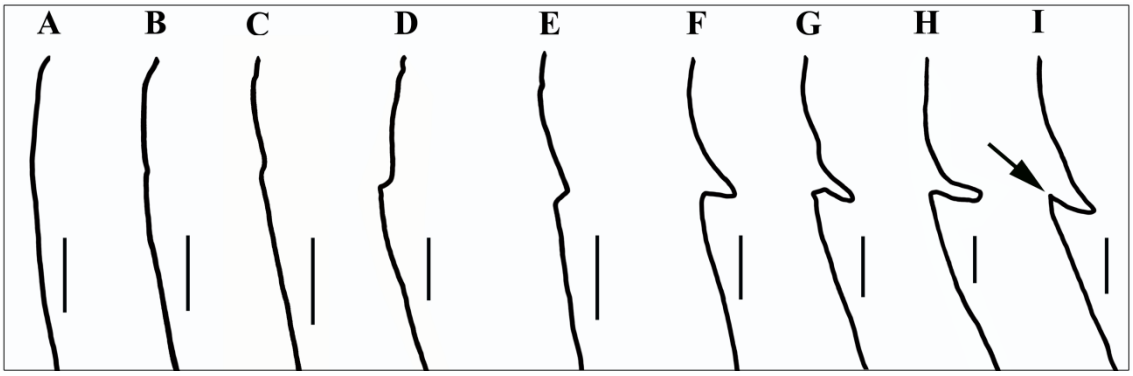


Figure 2. Line drawings of left epibranchial angle in male specimens (for comparison of epibranchial tooth, see arrow) in selected species of *Parasesarma*: A, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); B, *Parasesarma dumacense* (20.4/17.0, ZRC 2008.0833 = S208); C, *Parasesarma asperum* (16.0/13.0, ZRC 2001.0851 = S207); D, *Parasesarma hartogi* (28.7/22.7, QM-W28879 = S205); E, *Parasesarma lanchesteri* comb. nov. (19.9/15.8, SMF 7142 = S175); F, *Parasesarma onychophorum* comb. nov. (23.9/19.3, ZRC 2000.1490 = S120); G, *Parasesarma samawati* comb. nov. (28.6/23.9, SMF 29334 = S174); H, *Parasesarma maipoense* comb. nov. (25.3/19.6, ZRC 2009.0800 = S128); I, *Parasesarma lividum* comb. nov. (27.5/23.3, RMNH.CRUS. D.38587 = S148); scale bar: 1.0 mm.

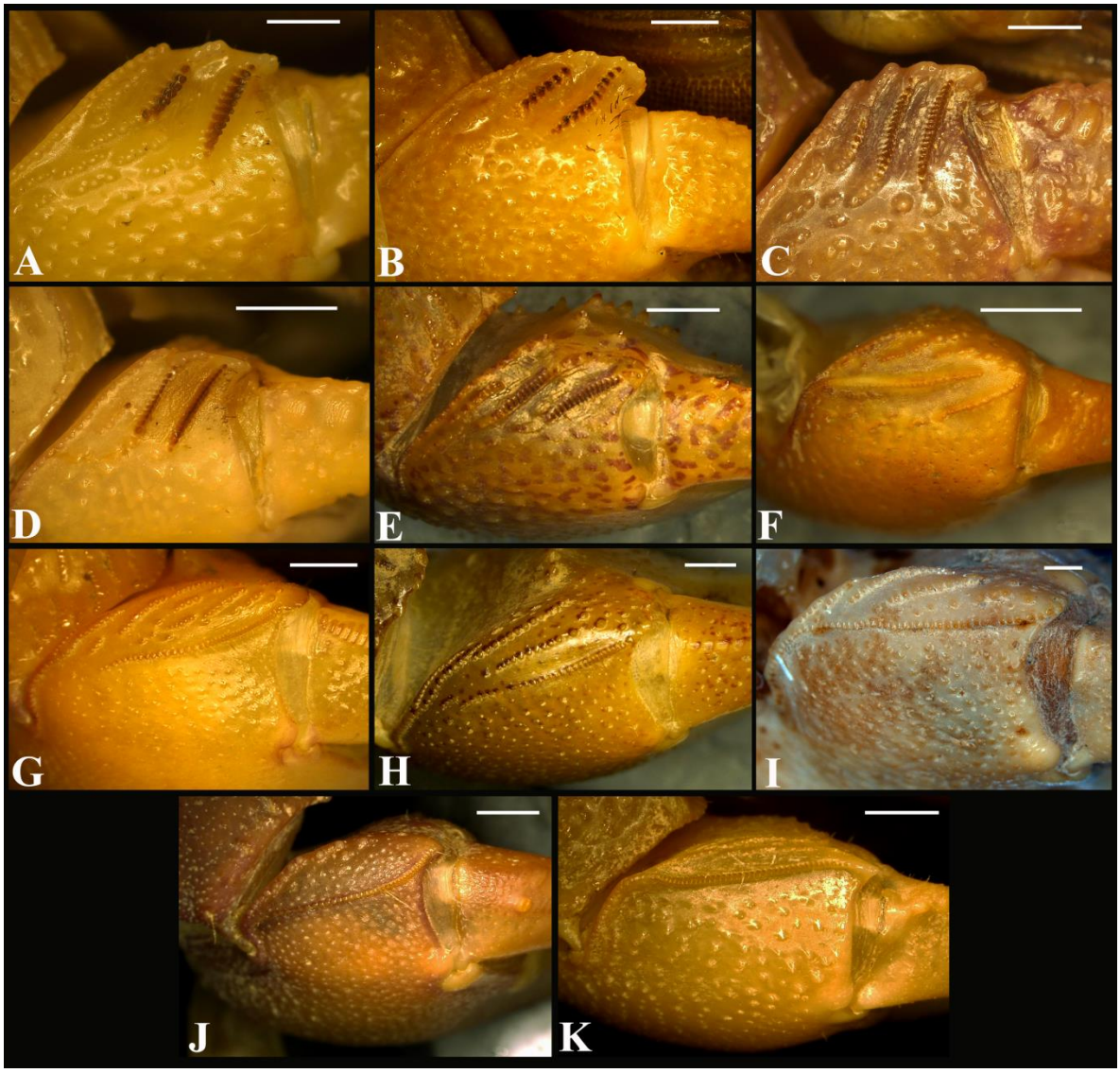


Figure 3. Upper view of pectinated crests on right palm in male specimens of selected species of *Parasesarma, Perisesarma, Metagrapsus* and *Selatium*: A, *Parasesarma dumacense* (20.4/17.0, ZRC 2008.0833 = S208); B, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); C, *Parasesarma bengalense* comb. nov. (19.4/16.4, SMF 49919 = S118); D, *Parasesarma cricotum* comb. nov. (15.1/13.4, SMF 49920 = S16); E, *Perisesarma dussumieri* (24.5/21.2, SMF 49930 = S123); F, *Fasciarma fasciatum* comb. nov. (12.0/10.3, ZRC 2012.0273 = S172); G, *Guinearma alberti* comb. nov. (20.8/18.1, SMF 49911 = S135); H, *Guinearma huzardi* comb. nov. (26.2/23.6, SMF 49914 = S188); I, *Guinearma kamermani* comb. nov. (29.5/26.0, RMNH.CRUS.D.166 = S213); J, *Metagrapsus curvatus* (21.5/17.7, SMF 49916 = S254); K, *Selatium brockii* (21.0/19.2, SMF 49931 = S255); scale bar: 1.0 mm.

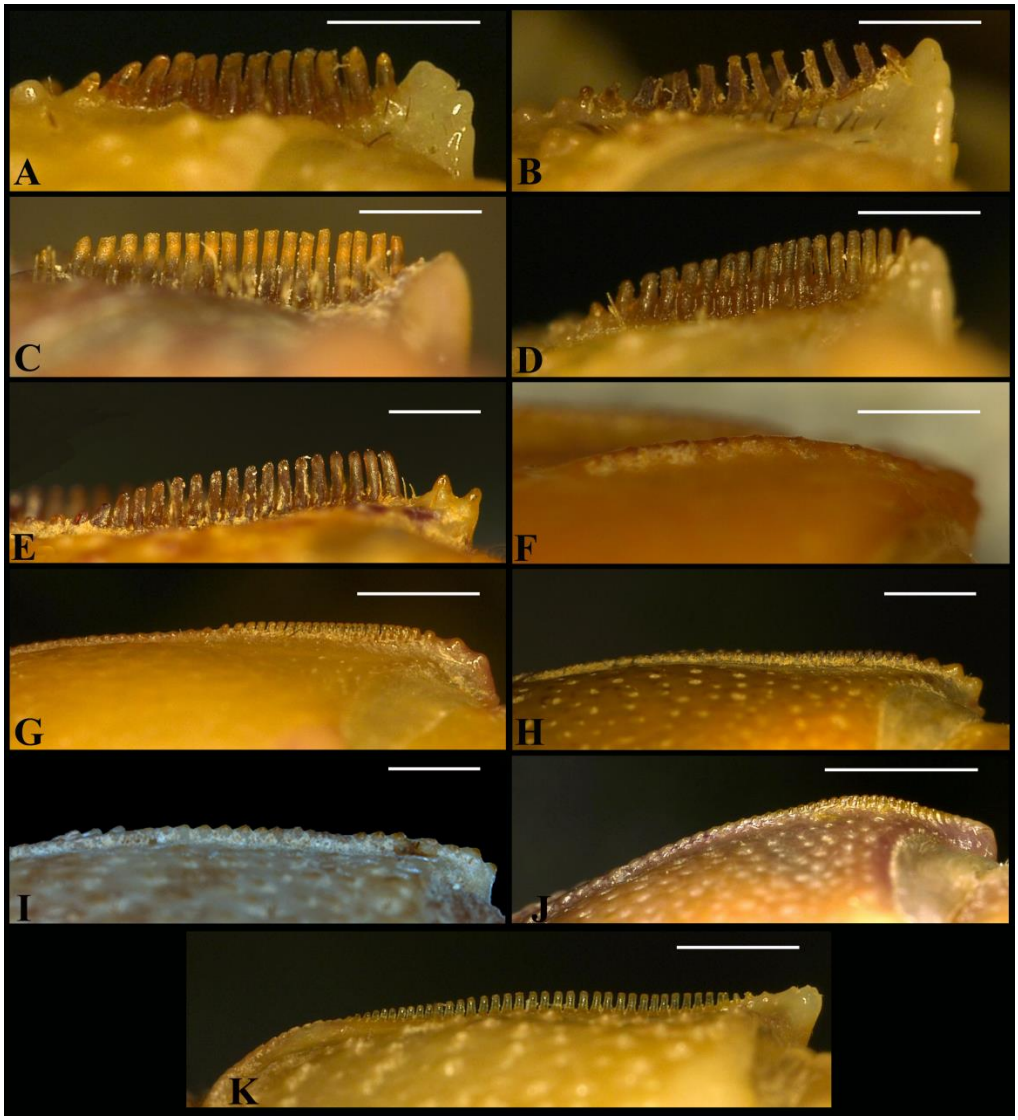


Figure 4. Frontal view of distal pectinated crests on left palm in selected male specimens of *Parasesarma*, *Perisesarma*, *Metagrapsus* and *Selatium*: A, *Parasesarma dumacense* (20.4/17.0, ZRC 2008.0833 = S208); B, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); C, *Parasesarma bengalense* comb. nov. (19.4/16.4, SMF 49919 = S118); D, *Parasesarma cricotum* comb. nov. (15.1/13.4, SMF 49920 = S16); E, *Perisesarma dussumieri* (24.5/21.2, SMF 49930 = S123); F, *Fasciarma fasciatum* comb. nov. (12.0/10.3, ZRC 2012.0273 = S172); G, *Guinearma alberti* comb. nov. (20.8/18.1, SMF 49911 = S135), H, *Guinearma huzardi* comb. nov. (26.2/23.6, SMF 49914 = S188); I, *Guinearma kamermani* comb. nov. (29.5/26.0, RMNH.CRUS.D.166 = S213); J, *Metagrapsus curvatus* (21.5/17.7, SMF 49916 = S254); K, *Selatium brockii* (21.0/19.2, SMF 49931 = S255); scale bar: A–F 0.5 mm, G–K 1.0 mm.

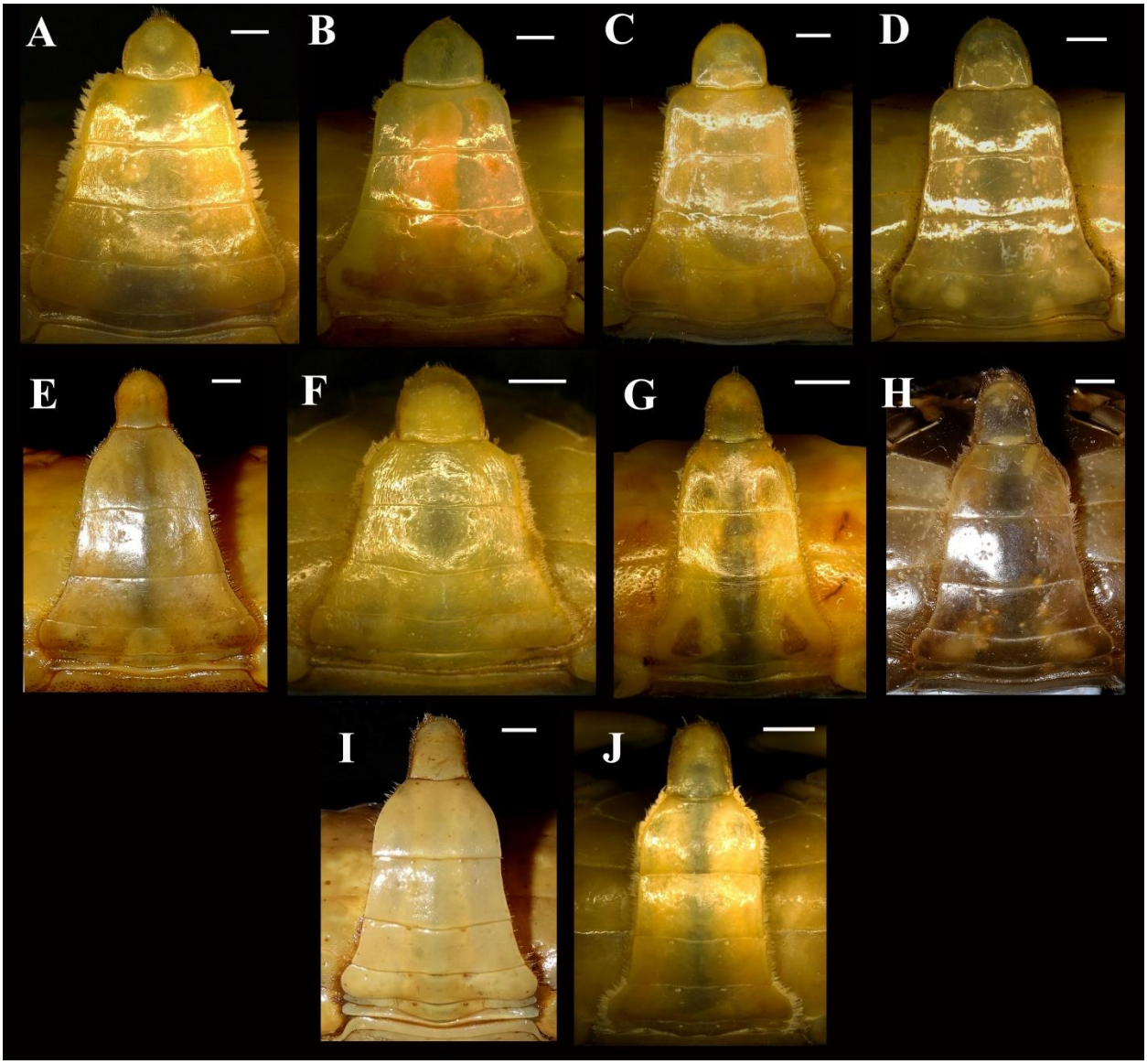


Figure 5. Male pleon in selected species of *Parasesarma*, *Perisesarma*, and *Metagrapsus*: A, *Parasesarma dumacense* (20.4/17.0, ZRC 2008.0833 = S208); B, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); C, *Parasesarma bengalense* comb. nov. (19.4/16.4, SMF 49919 = S118); D, *Parasesarma cricotum* comb. nov. (15.1/13.4, SMF 49920 = S16); E, *Perisesarma dussumieri* (24.5/21.2, SMF 49930 = S123); F, *Fasciarma fasciatum* comb. nov. (12.1/10.3, ZRC 2012.0273 = S172); G, *Guinearma alberti* comb. nov. (20.8/18.1, SMF 49911 = S135); H, *Guinearma huzardi* comb. nov. (26.2/23.6, SMF 49914 = S188); I, *Guinearma kamermani* comb. nov. (29.5/26.0, RMNH.CRUS.D.166 = S213); J, *Metagrapsus curvatus* (21.5/17.7, SMF 49916 = S254); scale bar: 1.0 mm.

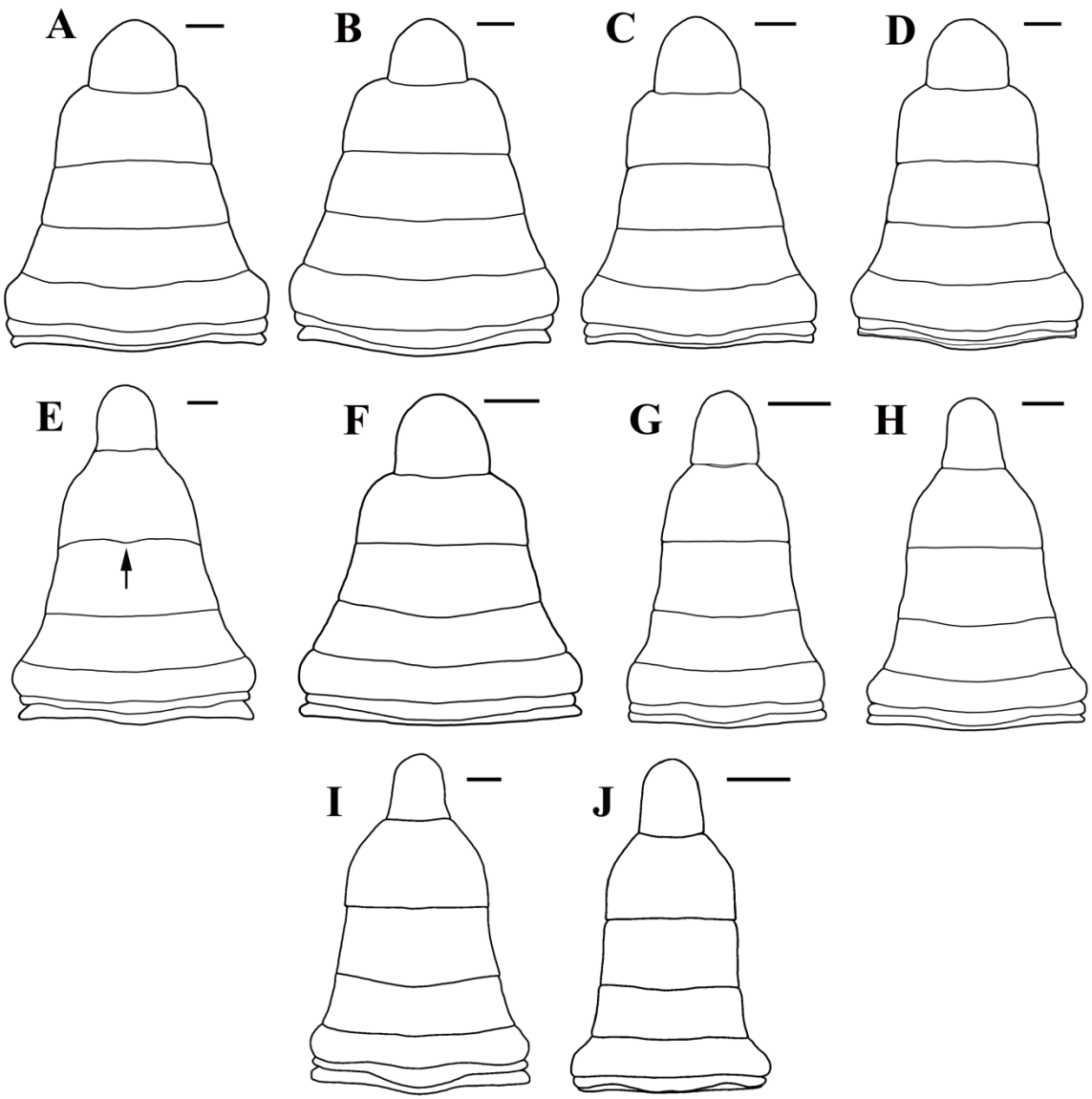


Figure 6. Line drawings of male pleon in selected species of *Parasesarma*, *Perisesarma*, and *Metagrapsus*: A, *Parasesarma dumacense* (20.37/17.03, ZRC 2008.0833 = S208); B, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); C, *Parasesarma bengalense* comb. nov. (19.4/16.4, SMF 49919 = S118); D, *Parasesarma cricotum* comb. nov. (15.1/13.4, SMF 49920 = S16); E, *Perisesarma dussumieri* (24.5/21.2, SMF 49930 = S123); F, *Fasciarma fasciatum* comb. nov. (12.0/10.3, ZRC 2012.0273 = S172); G, *Guinearma alberti* comb. nov. (20.8/18.1, SMF 49911 = S135); H, *Guinearma huzardi* comb. nov. (26.2/23.6, SMF 49914 = S188); I, *Guinearma kamermani* comb. nov. (29.5/26.0, RMNH.CRUS.D.166 = S213); J, *Metagrapsus curvatus* (21.5/17.7, SMF 49916 = S254); scale bar: A–F 1.0 mm, G–J 2mm.

Table 4. The ratio of width to length for the pleon somites 5, 6 and telson in selected species (single individuals) of *Parasesarma* and *Perisesarma* examined in the present study (new generic placements by present suggestion also included).

Species	New generic placement	5th	6th	Telson
Parasesarma dumacense (S208)		2.88	2.28	1.16
Parasesarma plicatum (S206)		2.73	1.92	1.23
Parasesarma plicatum (S265)		2.70	2.15	1.10
Perisesarma bengalense (S115)	*Parasesarma*	2.37	1.92	1.07
Perisesarma bengalense (S118)	*Parasesarma*	2.50	1.90	1.16
Perisesarma cricotum (S16)	*Parasesarma*	2.58	1.93	1.11
Perisesarma cricotum (S40)	*Parasesarma*	2.55	1.98	1.10
Perisesarma dussumieri (S122)		2.30	1.46	0.90
Perisesarma dussumieri (S124)		2.27	1.44	0.80
Perisesarma fasciatum (S127)	*Fasciarma* **gen. nov.**	3.30	2.83	1.16
Perisesarma fasciatum (S172)	*Fasciarma* **gen. nov.**	3.00	2.42	1.15
Perisesarma alberti (S135)	*Guinearma* **gen. nov.**	1.88	1.72	0.80
Perisesarma alberti (S136)	*Guinearma* **gen. nov.**	1.80	1.56	0.78
Perisesarma huzardi (S187)	*Guinearma* **gen. nov.**	1.98	1.89	0.89
Perisesarma huzardi (S193)	*Guinearma* **gen. nov.**	1.60	1.61	0.97
Perisesarma kamermani (S213)	*Guinearma* **gen. nov.**	1.86	1.60	0.61

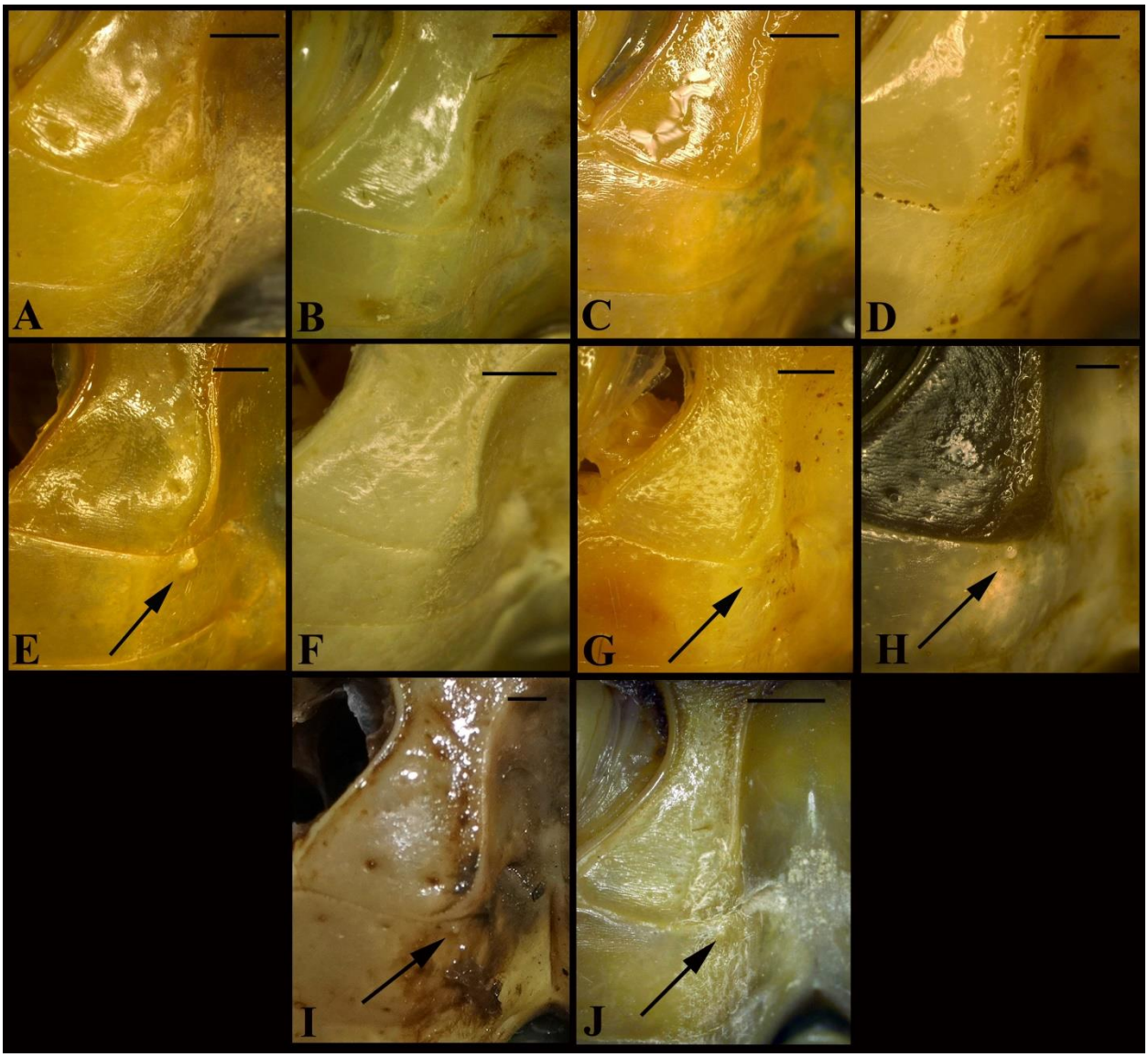


Figure 7. Male press button in selected species of *Parasesarma*, *Perisesarma*, and *Metagrapsus*: A, *Parasesarma dumacense* (20.4/17.0, ZRC 2008.0833 = S208); B, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); C, *Parasesarma bengalense* comb. nov. (19.4/16.4, SMF 49919 = S118); D, *Parasesarma cricotum* comb. nov. (15.1/13.4, SMF 49920 = S16); E, *Perisesarma dussumieri* (24.5/21.2, SMF 49930 = S123); F, *Fasciarma fasciatum* comb. nov. (12.0/10.3, ZRC 2012.0273 = S172); G, *Guinearma alberti* comb. nov. (20.8/18.1, SMF 49911 = S135), H, *Guinearma huzardi* comb. nov. (26.2/23.6, SMF 49914 = S188); I, *Guinearma kamermani* comb. nov. (29.5/26.0, RMNH.CRUS.D.166 = S213); J, *Metagrapsus curvatus* (21.5/17.7, SMF 49916 = S254); scale bar: 1.0 mm.

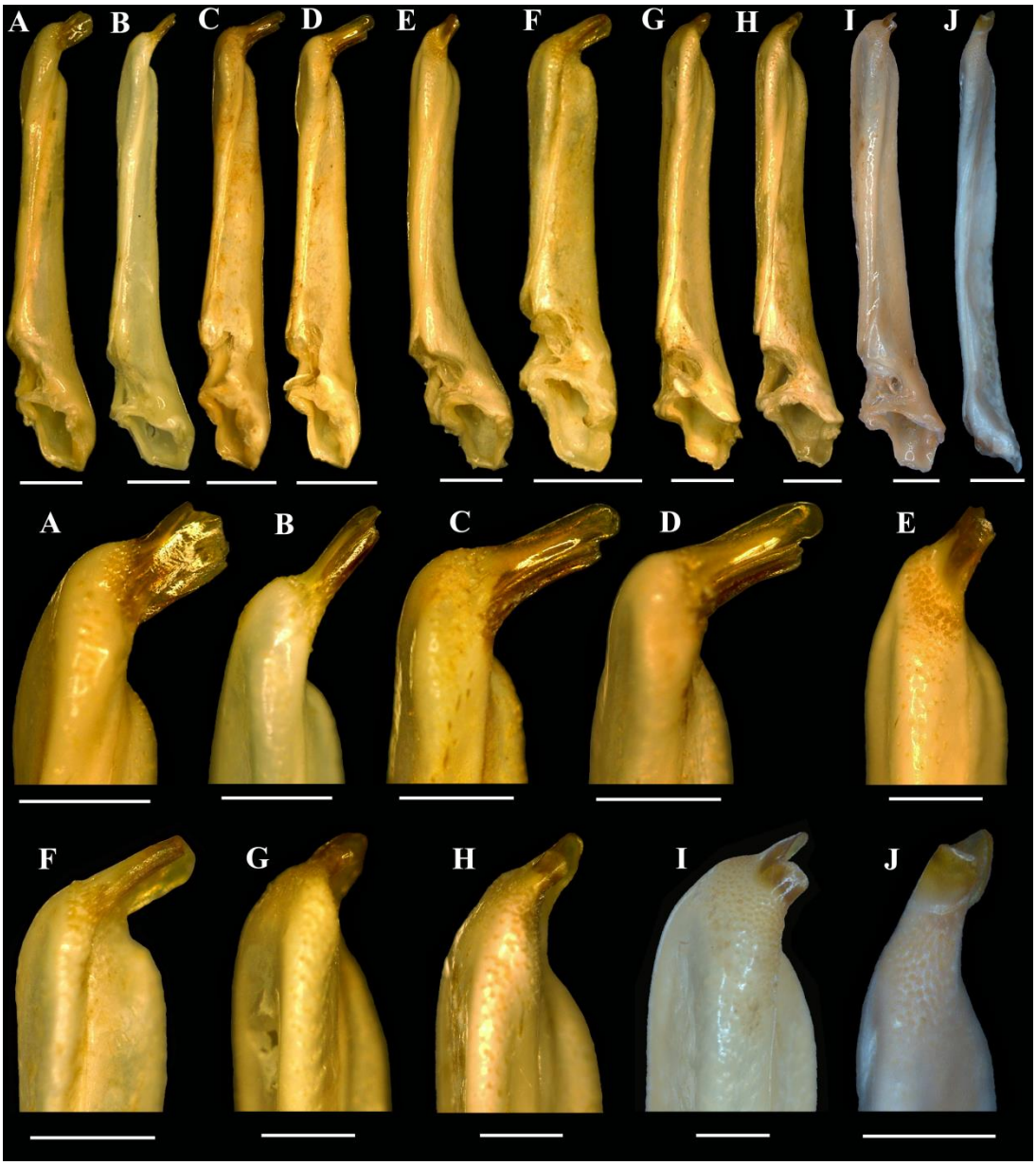


Figure 8. Denuded G1. In: A, *Parasesarma dumacense* (20.4/17.0, ZRC 2008.0833 = S208); B, *Parasesarma plicatum* (22.4/17.8, SMF 49918 = S206); C, *Parasesarma bengalense* comb. nov. (19.4/16.4, SMF 49919 = S118); D, *Parasesarma cricotum* comb. nov. (15.1/13.4, SMF 49920 = S16); E, *Perisesarma dussumieri* (22.8/20.4, SMF 49930 = S214); F, *Fasciarma fasciatum* comb. nov. (12.0/10.3, ZRC 2012.0273 = S172); G, *Guinearma alberti* comb. nov. (20.8/18.1, SMF 49911 = S135), H, *Guinearma huzardi* comb. nov. (26.2/23.6, SMF 49914 = S188); I, *Guinearma kamermani* comb. nov. (29.5/26.0, RMNH.CRUS.D.166 = S213); J, *Metagrapsus curvatus* (29.2/16.6, RMNH.CRUS.D.31013 = S220); scale bar: 1.0 mm for whole length, 0.5 mm for apical part.

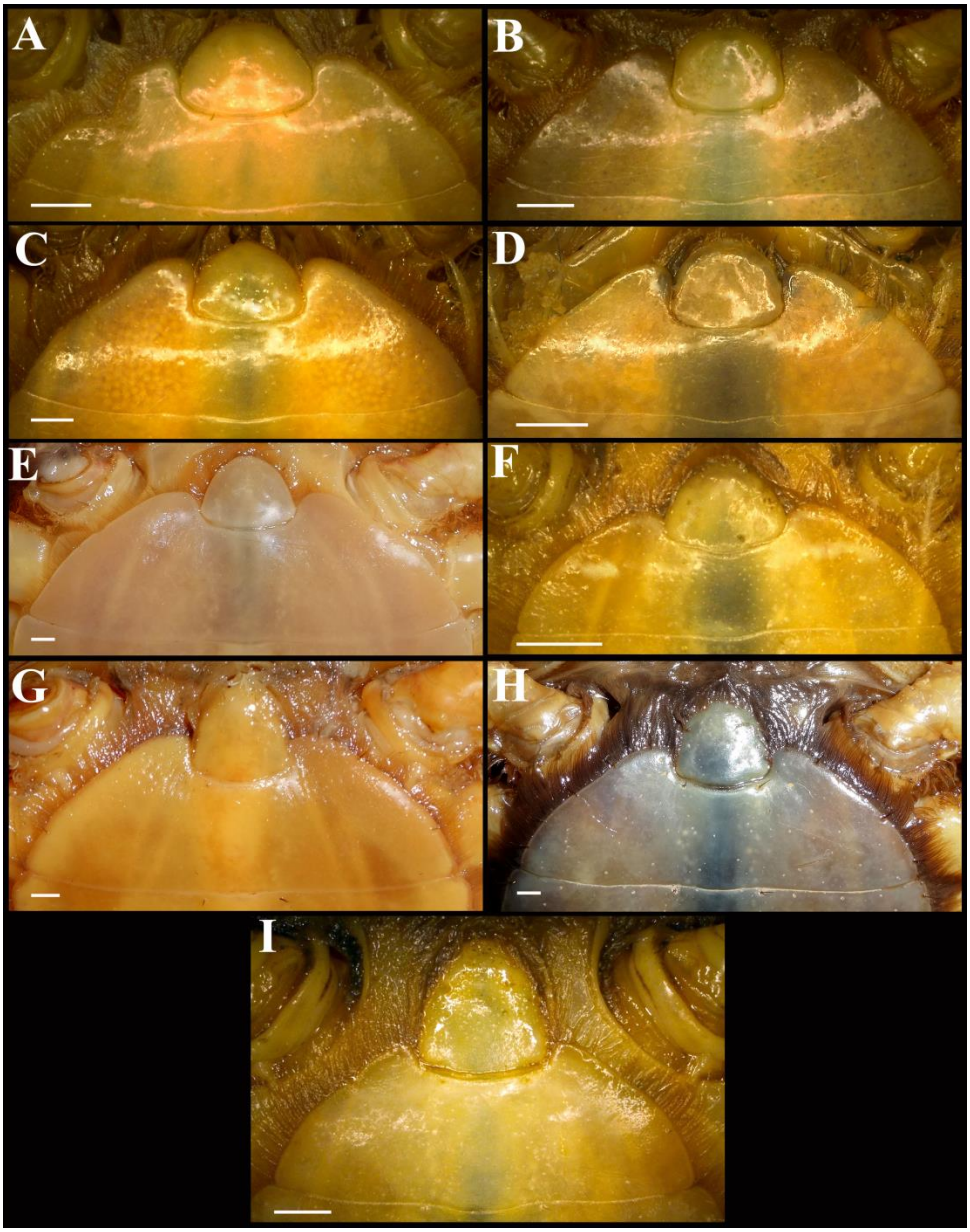


Figure 9. Female pleonal somite 6 and telson in selected species of *Parasesarma*, *Perisesarma*, and *Metagrapsus*: A, *Parasesarma dumacense* (18.1/14.5, ZRC 2008.0833 = S299); B, *Parasesarma plicatum* (17.4/13.8, SMF 49927 = S288); C, *Parasesarma bengalense* comb. nov. (19.7/16.5, SMF 49919 = S284); D, *Parasesarma cricotum* comb. nov. (12.6/10.6, SMF 49920 = S286); E, *Perisesarma dussumieri* (22.0/19.4, SMF 49930 = S281); F, *Fasciarma fasciatum* comb. nov. (13.6/11.5, ZRC 2012.0273 = S282); G, *Guinearma alberti* comb. nov. (24.9/22.1, SMF 49911 = S279), H, *Guinearma huzardi* comb. nov. (27.4/23.9, SMF 49914 = S278); I, *Metagrapsus curvatus* (23.6/18.7, SMF 49916 = S280); scale bar: 1.0 mm.

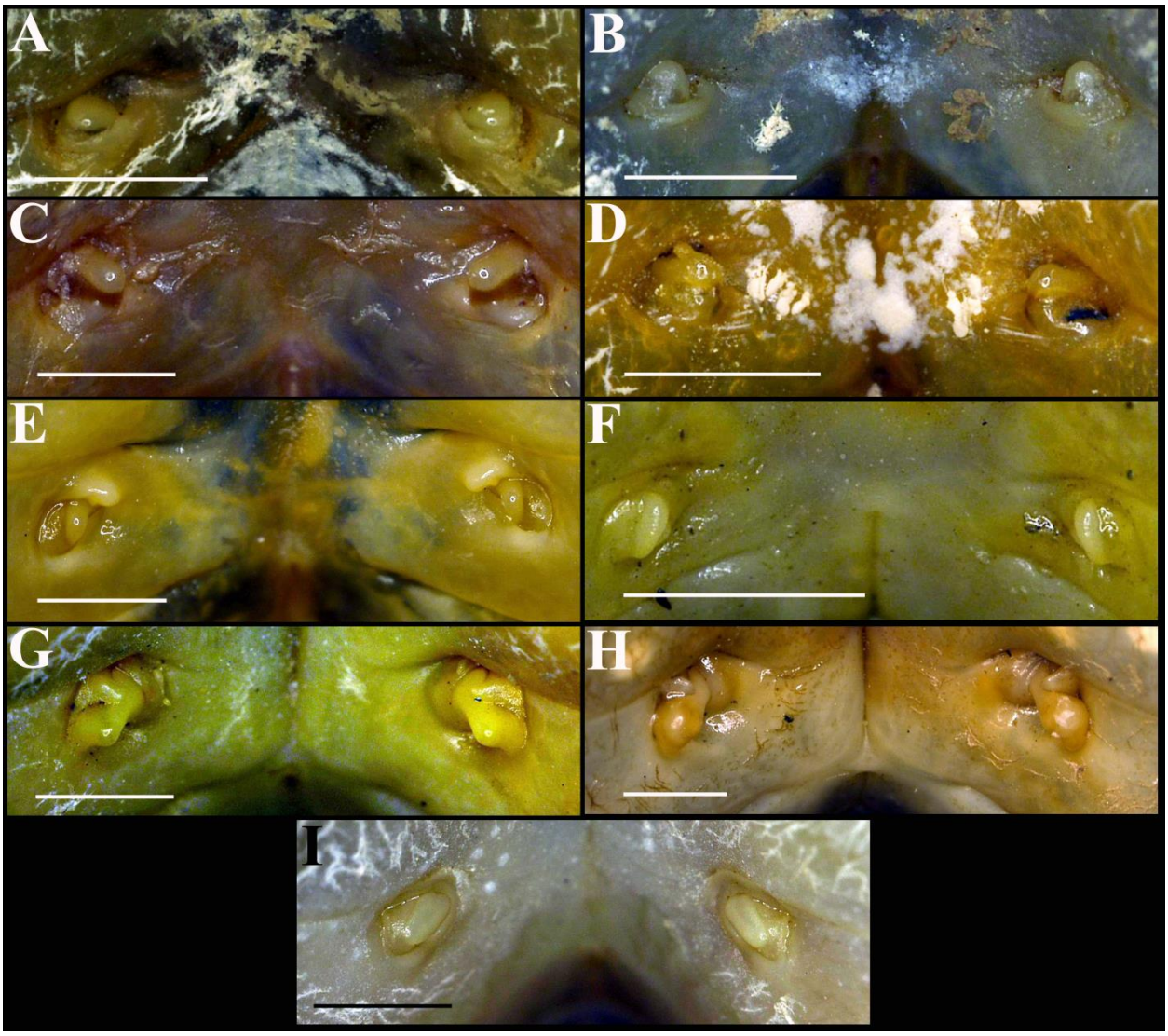


Figure 10. Female vulvae in selected species of *Parasesarma*, *Perisesarma*, and *Metagrapsus*: A, *Parasesarma dumacense* (18.1/14.5, ZRC 2008.0833 = S299); B, *Parasesarma plicatum* (17.4/13.8, SMF 49927 = S288); C, *Parasesarma bengalense* comb. nov. (19.7/16.5, SMF 49919 = S284); D, *Parasesarma cricotum* comb. nov. (12.6/10.6, SMF 49920 = S286); E, *Perisesarma dussumieri* (22.0/19.4, SMF 49930 = S281); F, *Fasciarma fasciatum* comb. nov. (13.6/11.5, ZRC 2012.0273 = S282); G, *Guinearma alberti* comb. nov. (24.9/22.1, SMF 49911 = S279), H, *Guinearma huzardi* comb. nov. (27.4/23.9, SMF 49914 = S278); I, *Metagrapsus curvatus* (23.6/18.7, SMF 49916 = S280); scale bar: 1.0 mm.

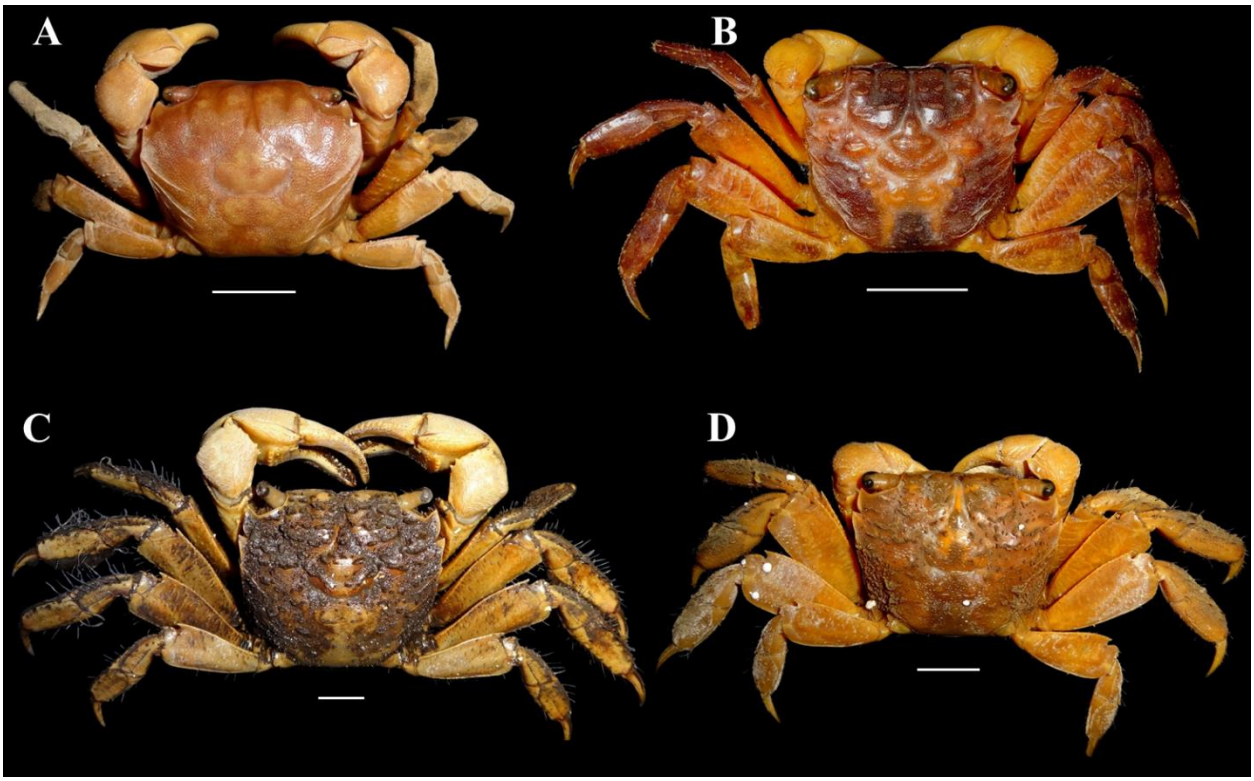


Figure 11. Dorsal view of the whole animal in A, *Metagrapsus curvatus* (male, 29.2/16.6, RMNH.CRUS.D.31013 = S220); B, *Guinearma alberti* comb. nov. (male, 20.8/18.1, SMF 49911 = S135); C, *Guinearma huzardi* comb. nov. (male, 39.0/35.0, RMNH.CRUS.D.132 = S215); D, *Guinearma kamermani* comb. nov. (male, 29.4/24.1, RMNH.CRUS.D.27386 = S219); scale bar: 1 cm.

Molecular data

The cropped alignments of Cox1, 16S, NaK and the concatenated file consist of 645, 564, 528 and 1736 basepairs (bp) respectively, after removal of the primer sequence and adjacent regions. The alignments of the protein coding genes contained no stop codons, which would have indicated the presence of pseudogenes. The best evolutionary model for further genetic analysis obtained with the aid of jModelTest (v. 2.1.4) (Guindon & Gascuel, 2003; Darriba et al., 2012), was the General Time reversible plus Gamma (GTR+G, Rodriguez et al., 1990).

Eight phylogenetic trees from the four alignments (two trees for each alignment) were obtained using Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. As the trees from ML and BI had highly similar topologies, here we present only ML trees including bootstrap values and posterior probabilities for ML and BI analyses respectively (Figs. 12 & 13). The standard deviation of split frequencies at the end of the Bayesian analyses were 0.003906, 0.004664, 0.007890 and 0.004767 for Cox1, 16S, NaK and the concatenated file, respectively.

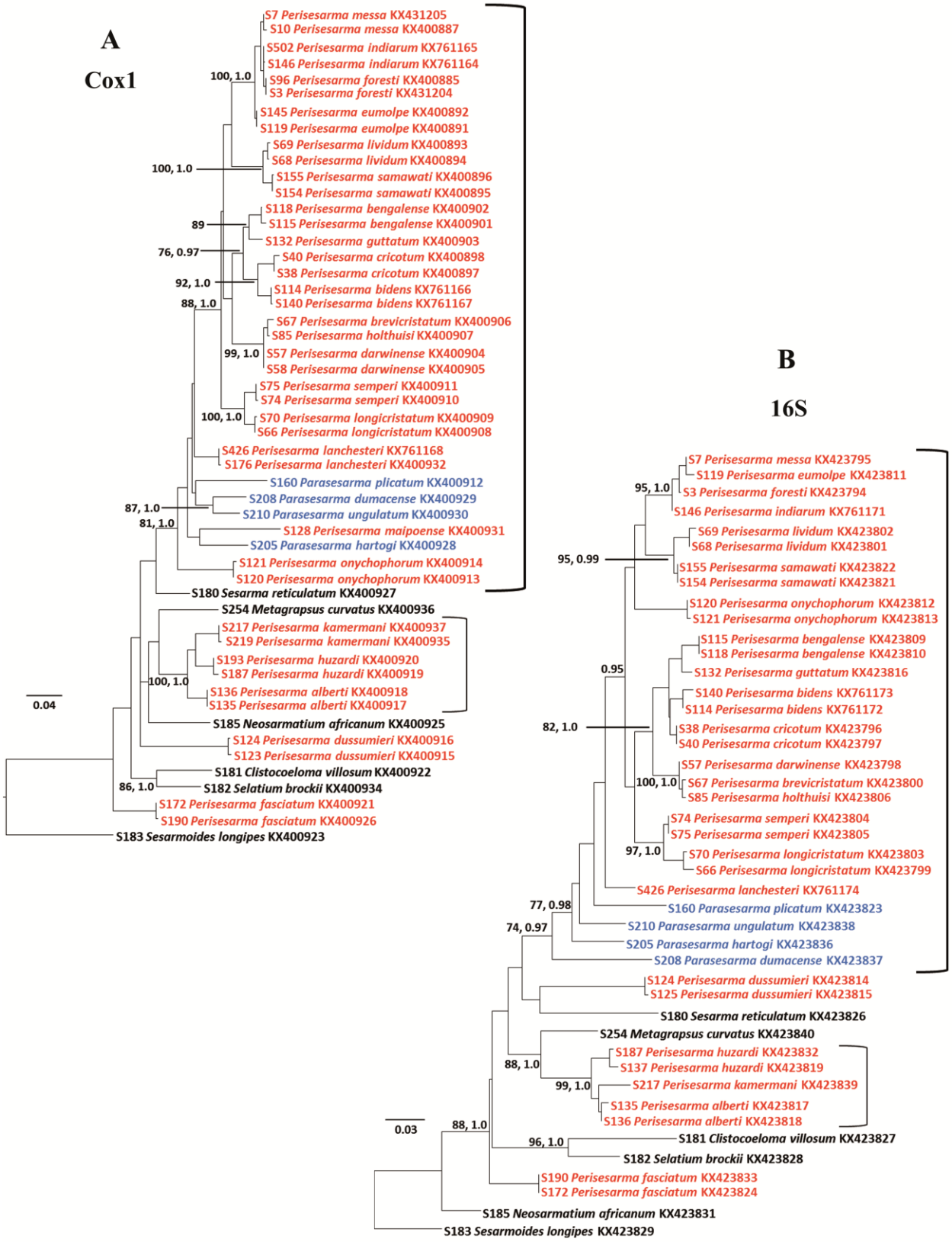
None of the resulting phylogenetic trees recovers either *Parasesarma* (blue labels) or *Perisesarma* (red labels) as previously defined as a monophyletic taxon (Figs. 12 & 13). Instead, *Perisesarma* always turns out to be a heterogeneous polyphyletic group and *Parasesarma* at least paraphyletic. Some representatives of *Perisesarma*, including *P. dussumieri, P. fasciatum* and the West African species (i.e., *P. alberti*; *P. huzardi; P.kamermani*) (thin brackets in Figs. 12 & 13) are distantly separated from other species of *Perisesarma* and *Parasesarma*. *Perisesarma dussumieri* and *Perisesarma fasciatum* constitute two separate clades and were not grouped stably with any other genus included in this study. West African *Perisesarma* are grouped together with high support in all cases (thin brackets in Figs. 12 & 13), far from *Perisesarma* s. str., but in close association with *Metagrapsus curvatus*.

When excluding *P. dussumieri, P. fasciatum* and the West African species (thin brackets), most members of *Perisesarma*, cluster together with species of *Parasesarma* and always form a solid monophyletic clade with high support values in both analyses (bold brackets in Figs. 12 & 13). Within this clade, most species of *Perisesarma* group together and form a well-supported monophyletic subclade, except for *Perisesarma maipoense* (Fig. 12 A) and *Perisesarma lanchesteri* which stay outside of this subclade. *Perisesarma onychophorum* also is found outside of this subclade in more cases (Cox1, NaK & concatenated), except in the 16S tree (Fig. 12B). In

16S tree, *Parasesarma* appears as a paraphylum within the supported *Parasesarma-Perisesarma* clade (bold bracket in Fig. 12B). In the other three trees (Cox1, NaK and concatenated alignment), members of these genera are more mixed and are not separated corresponding to their original generic classification (Figs. 12 & 13).

Generally, species of *Parasesarma* hold more ancestral positions in the tree than members of *Perisesarma*. In the 16S tree, *Parasesarma dumacense* and *Parasesarma hartogi* appear as ancestral within the monophyletic *Parasesarma-Perisesarma* clade (Fig. 12B), while in other trees *Perisesarma onychophorum* represents the first split (Figs. 12A, 13A & B).

The more variable mitochondrial markers, Cox1 and 16S, also reveal some phylogenetic affinities among the studied species. For example, *P. eumolpe*, *P. foresti*, *P. indiarum* and *P. messa* are genetically very close to each other and cluster firmly together in both Cox1 and 16S trees (Fig. 12). The tree of the concatenated dataset, which is constructed for a subset of species, also recovers this cluster (Fig. 13B). The same is true for the group including *P. brevicristatum*, *P. darwinense* and *P. holthuisi* and the group containing *P. longicristatum* and *P. semperi* (Fig. 12, both in Cox1 and 16S). The mitochondrial trees also reveal a sister species relationship of *P. lividum* and *P. samawati* (Fig. 12). The Cox1 tree (Fig. 12A) furthermore recovers another supported clade including *P. bengalense*, *P. guttatum*, *P. bidens* and *P. cricotum*. Of these, *P. bidens* and *P. cricotum* appear to be especially closely related. The same can be observed for *P. bengalense* and *P. guttatum*. The nuclear NaK gene is clearly much conserved at this level (Fig. 13A).


A
Cox1
S7 Perisesarma messa KX431205
S10 Perisesarma messa KX400887
S502 Perisesarma indiarum KX761165
S146 Perisesarma indiarum KX761164
S96 Perisesarma foresti KX400885
S3 Perisesarma foresti KX431204
S145 Perisesarma eumolpe KX400892
S119 Perisesarma eumolpe KX400891
S69 Perisesarma lividum KX400893
S68 Perisesarma lividum KX400894
S155 Perisesarma samawati KX400896
S154 Perisesarma samawati KX400895
S118 Perisesarma bengalense KX400902
S115 Perisesarma bengalense KX400901
S132 Perisesarma guttatum KX400903
S40 Perisesarma cricotum KX400898
S38 Perisesarma cricotum KX400897
S114 Perisesarma bidens KX761166
S140 Perisesarma bidens KX761167
S67 Perisesarma brevicristatum KX400906
S85 Perisesarma holthuisi KX400907
S57 Perisesarma darwinense KX400904
S58 Perisesarma darwinense KX400905
S75 Perisesarma semperi KX400911
S74 Perisesarma semperi KX400910
S70 Perisesarma longicristatum KX400909
S66 Perisesarma longicristatum KX400908
S426 Perisesarma lanchesteri KX761168
S176 Perisesarma lanchesteri KX400932
S160 Parasesarma plicatum KX400912
S208 Parasesarma dumacense KX400929
S210 Parasesarma ungulatum KX400930
S128 Perisesarma maipoense KX400931
S205 Parasesarma hartogi KX400928
S121 Perisesarma onychophorum KX400914
S120 Perisesarma onychophorum KX400913
S180 Sesarma reticulatum KX400927
S254 Metagrapsus curvatus KX400936
S217 Perisesarma kamermani KX400937
S219 Perisesarma kamermani KX400935
S193 Perisesarma huzardi KX400920
S187 Perisesarma huzardi KX400919
S136 Perisesarma alberti KX400918
S135 Perisesarma alberti KX400917
S185 Neosarmatium africanum KX400925
S124 Perisesarma dussumieri KX400916
S123 Perisesarma dussumieri KX400915
S181 Clistocoeloma villosum KX400922
S182 Selatium brockii KX400934
S172 Perisesarma fasciatum KX400921
S190 Perisesarma fasciatum KX400926
S183 Sesarmoides longipes KX400923
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88, 1.0
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87, 1.0
81, 1.0
100, 1.0
86, 1.0
0.04
B
16S
S7 Perisesarma messa KX423795
S119 Perisesarma eumolpe KX423811
S3 Perisesarma foresti KX423794
S146 Perisesarma indiarum KX761171
S69 Perisesarma lividum KX423802
S68 Perisesarma lividum KX423801
S155 Perisesarma samawati KX423822
S154 Perisesarma samawati KX423821
S120 Perisesarma onychophorum KX423812
S121 Perisesarma onychophorum KX423813
S115 Perisesarma bengalense KX423809
S118 Perisesarma bengalense KX423810
S132 Perisesarma guttatum KX423816
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S180 Sesarma reticulatum KX423826
S254 Metagrapsus curvatus KX423840
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S217 Perisesarma kamermani KX423839
S135 Perisesarma alberti KX423817
S136 Perisesarma alberti KX423818
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S182 Selatium brockii KX423828
S190 Perisesarma fasciatum KX423833
S172 Perisesarma fasciatum KX423824
S185 Neosarmatium africanum KX423831
S183 Sesarmoides longipes KX423829
95, 1.0
95, 0.99
0.95
82, 1.0
100, 1.0
97, 1.0
77, 0.98
74, 0.97
88, 1.0
99, 1.0
88, 1.0
96, 1.0
0.03

Figure 12. Phylogenetic consensus trees constructed with Maximum Likelihood (ML) and Bayesian Inference (BI) of selected sesarmid crabs (with focus on *Perisesarma*). A, Cox1; B, 16S. Nodal support values are listed in the order ML (bootstrap values) and BI (posterior probabilities). *Sesarmoides longipes* was used as outgroup. Only support values higher than 70% and 95% respectively for ML and BI are shown in the trees. The numbers in front of each individual refer to the GenBank accession number. Color labels highlight generic placements of species (prior to this study) (i.e., blue = *Parasesarma*; red = *Perisesarma*; black = other genera). The thin brackets indicate the position of the West African members of *Perisesarma* (now *Guinearma* gen. nov.) and the bold brackets represent the monophyletic clade of *Parasesarma-Perisesarma* complex (now = *Parasesarma* comb. nov.)

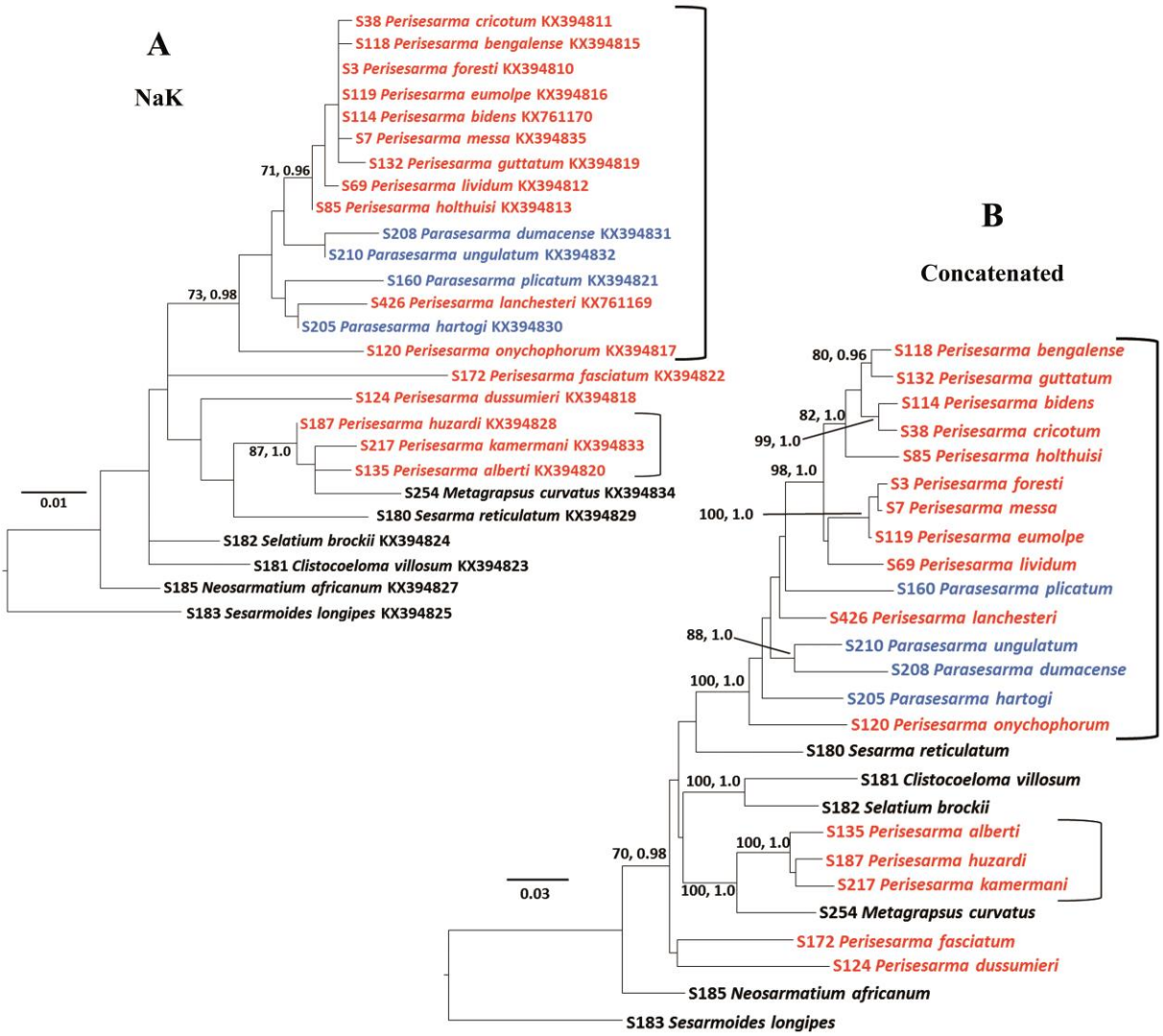

Figure 13. Phylogenetic consensus trees constructed with Maximum Likelihood (ML) and Bayesian Inference (BI) of selected sesarmid crabs (focusing on *Perisesarma*). A, NaK; B, concatenated data set (Cox1, 16S & NaK). Nodal support values are listed in the order ML (bootstrap values) and BI (posterior probabilities). *Sesarmoides longipes* was used as outgroup. Only support values higher than 70% and 95% respectively for ML and BI are shown in the trees. The numbers in front of each individual refer to the GenBank accession numbers. Color labels highlight generic placements of species (prior to this study) (i.e., blue = *Parasesarma*; red = *Perisesarma*; black = other genera). The thin brackets indicate position of the West African *Perisesarma* (now = *Guinearma* gen. nov.) and the bold bracket represent the monophyletic clade of *Parasesarma-Perisesarma* complex (now = *Parasesarma* comb. nov.)

Discussion

The reliability of the epibranchial tooth for describing and delimiting genera in the family Sesarmidae has been questioned for a long time, because of its inconsistency, unclear definition and phylogenetic incongruence (Guerao et al., 2004; Shen, 1932; Tweedie, 1940; von Hagen, 1978; Abele, 1975, 1992; Schubart et al., 2006). Consequently, it caused some disagreements and controversies in the taxonomy of *Parasesarma* and *Perisesarma* (see Campbell, 1976; Ng. et al., 2008; Davie, 2010). Consistent with previous criticisms, morphological data of the present study show that the shape and size of the tooth can delimit species and help species identification, but it is not a discriminative character to separate all representatives of *Parasesarma* and *Perisesarma*. Mainly because of the transitions or intermediate conformations, it is difficult to draw a line between the genera according to this character (Figs. 1 & 2). The combination of morphology and genetic data also show that the taxa with (*Perisesarma*) and without (*Parasesarma*) epibranchial tooth are phylogenetically mixed and not reciprocally isolated as separate clades and monophyletic taxa (see blue and red labels in Figs. 12 & 13). To facilitate the understanding of evolutionary relationships within the complex, and to establish an accurate and natural taxonomy in agreement with phylogenetic relationships, here we suggest merging members of the two current genera to a large unified genus, at the same time excluding the species which are morphologically and genetically heterogeneous (explained in continuation). The monophyly of this large assemblage and close phylogenetic relationships of the constituent members are well supported by morphological synapomorphies and molecular evidence. In our phylogenetic analysis, such a *Parasesarma-Perisesarma* unified group (bold brackets) always forms a stable monophyletic clade, always supported with high confidence values (i.e., in both ML and BI analyses for all the three genes) (Figs. 12 & 13). Morphologically, all of the members of this unified taxon have one, two or three (mostly two in males and one in females) transverse pectinated crest(s) with elevated teeth on the dorsal face of the chela palm, flanked by a big tubercle on the proximal side (Figs. 3A–D & 4A–D). Considering that these features are ethologically important as part of a stridulatory system for inter-intraspecific communication (Boon et al., 2009; Chen et al., 2014; Davie et al., 2015), it highlights their evolutionary significance and possible kinship of the carriers. Here we hypothesize that the large tubercle on the inner side of the pectinated crests (Figs. 3A–D & 4A–D) can function as a stabilizer for the stridulatory mechanism as described by Boon et al. (2009).

The members of this unified genus also show similarity in the synapomorphic shape of the first gonopods (G1) (Fig. 8A–D).

Recent work on different taxonomic groups of the family Sesarmidae has uncovered several undescribed forms. The genera *Haberma* Ng & Schubart, 2002, *Scandarma* Schubart, Liu & Cuesta, 2003, *Karstarma* Davie & Ng, 2007, *Lithoselatium*, Schubart, Liu & Ng, 2009, *Cyclorma* Naruse & Ng, 2012 and *Eneosesarma* Brösing et al., 2014 were established, because of unique morphological features. The present analyses also indicate some heterogeneity (morphological and genetic) among species of *Perisesarma*. *Perisesarma dussumieri* is overall similar in morphology to representatives of the unified *Parasesarma-Perisesarma* complex by having two rows of pectinated crests with elevated teeth, but shows important morphological differences compared to all other representatives of this complex. In this species, the rows of pectinated crests are rather oblique and not flanked by large tubercles on the inner side (Figs. 3E & 4E). Also the male and female pleons, especially the somite 6 in males, is different from other species of the two genera (Figs. 5E, 6E & 10E). Moreover, having a large male press button (Fig.7E), the distinct morphology of male G1 (Fig. 8E) and female vulvae (Fig. 10E) differentiates this species from all others. Phylogenetically, *Perisesarma dussumieri* is distantly separated from the main *Parasesarma-Perisesarma* cluster (bold brackets in Figs. 12 & 13) as well as from other groups of the present study, and always forms a distinct clade (Figs. 12 & 13). As this is the type species of *Perisesarma* (designated by Campbell, 1976) and possesses several unique morphological characters and an isolated phylogenetic position, we thus suggest restricting the genus *Perisesarma* to only this species. Other species of the genus will eventually be transferred to either newly defined genera, or become part of an enlarged and redefined genus *Parasesarma* (explained in continuation).

Present morphological and molecular evidences reveal that *Perisesarma fasciatum* does not belong to the *Parasesarma* complex or *Perisesarma*, but represent a distinct taxon. Having two ridges with chitinous caps (on the dorsal surface of chela palm) instead of pectinated crests (Figs. 3F & 4F), makes this species morphologically unique in the family, which has already been discussed in the past (Campbell, 1967; Ng et al., 2008; Davie, 2010; Shahdadi & Schubart, 2015). Interestingly, Guerao et al. (2004) also reported a specific larval morphology (i.e., presence of only five setae on the exopod of the uropod in megalopae) that also distinguishes this

species within the family. Along with morphological uniqueness, its separate position in the molecular phylogenies (Figs. 12 & 13) convinced us to classify this species as separate genus within Sesarmidae (see Systematic Account below).

Confirming previous discussions (e.g., Ng et al., 2008; Davie, 2010), here we also found some exclusive characters in West African members of the genus *Perisesarma*. These species (i.e., *P. alberti*, *P. huzardi* and *P. kamermani*) bear a single oblique row of pectinated crest with short teeth on the distal half of upper surface of palm (detailed in Results and Figs. 3G–I & 4G–I), which is different from other types of pectinated crests in the species of *Parasesarma* and *Perisesarma*, but resembles to some extent the shape of the ones in *Metagrapsus curvatus* (Figs. 3J & 4J). These species are also different from the genera *Selatium*, *Lithoselatium*, *Episesarma* and *Neosesarma* which have one longitudinal pectinated crest with elevated teeth from stem to stern (Serène & Soh, 1970; Schubart et al., 2009; Davie, 2012; present study (Figs. 3K & 4K)). Interestingly, the aforementioned genera along with *Clistocoeloma* form a solid monophyletic clade (Schubart et al., 2006; *Lithoselatium* not included as it was described later in Schubart et al., 2009). Our molecular data also indicate that the three species of West African *Perisesarma* are closely related (thin brackets in Figs 12 & 13), but distantly separated from the main *Parasesarma-Perisesarma* clade (bold brackets in Fig. 12 & 13). Among the studied species, they show most phylogenetic affinity to *M. curvatus* (Figs. 12 & 13). Nevertheless, these three species remain distinct and form their own monophyletic group. Also morphologically they show some similarity to *M. curvatus* in pectinated crests (Figs. 3G–J & 4G–J) and male pleon (Figs. 5G–J & 6G–J), but they differ markedly in many other aspects, e.g., in morphology of male G1 (Fig. 8G–J), female vulvae (Fig. 10G–J), and carapace (Fig. 11). Therefore, on the basis of several morphological synapomorphies and genetic relatedness between the West African *Perisesarma*, we also transfer them to a new genus (see Systematic Account below).

The present paper provides unambiguous morphological (briefly discussed above and diagnosed in the following sections for each group) and molecular data to 1) merge most species previously referred to *Parasesarma* and *Perisesarma* within a larger monophyletic assemblage under the name of *Parasesarma*, restricting *Perisesarma* to a monotypic genus comprising exclusively *Perisesarma dussumieri*, 2) to describe *Perisesarma fasciatum* as a new genus, and 3) also to

transfer the three West African species (i.e., *Perisesarma alberti*, *Perisesarma huzardi* and *Perisesarma kamermani*) to a new genus.

Morphological and molecular heterogeneity as here described for *Perisesarma* is also detectable in members of *Parasesarma* (e.g., see the phylogenetic position of *Parasesarma leptosoma* in Schubart et al., 2006) which calls for a future revision of all the members of this now enlarged genus (ongoing studies by the authors and colleagues).

Systematic Account

Sesarmidae Dana, 1851

***Parasesarma* De Man, 1895**

Type species: *Cancer quadratus* Fabricius, 1798, subsequent designation by Rathbun, 1918; gender neuter (for historical rearrangements and detail description of the type species see Ng. et al., 2008; Rahayu & Ng, 2010).

This genus is herewith enlarged and now contains 56 nominal species which are listed here in alphabetic order. The synonyms (after =) and the original combinations (in square brackets) are adopted from Ng et al. (2008).

Parasesarma affine (De Haan, 1837) [*Grapsus* (*Pachysoma*)]
Parasesarma anambas Yeo, Rahayu & Ng, 2004
Parasesarma asperum (Heller, 1865) [*Sesarma*]
Parasesarma batavianum (De Man, 1890) [*Sesarma*]
Parasesarma bengalense (Davie, 2003), **comb. nov.** [*Perisesarma*]
Parasesarma bidens (De Haan, 1835), **comb. nov.** [*Grapsus* (*Pachysoma*)]
Parasesarma brevicristatum (Campbell, 1967), **comb. nov.** [*Sesarma*]
Parasesarma calypso (De Man, 1895) [*Sesarma* (*Parasesarma*)]
Parasesarma carolinense (Rathbun, 1907) [*Sesarma* (*Parasesarma*)]
Parasesarma catenatum (Ortmann, 1897) [*Sesarma*]
Parasesarma charis Rahayu & Ng, 2005
Parasesarma cognatum Rahayu & Li, 2013
Parasesarma corallicum Ng, Davie & Li, 2016

Parasesarma cricotum (Rahayu & Davie, 2002), **comb. nov.** [*Perisesarma*]

Parasesarma darwinense (Campbell, 1967), **comb. nov.** [*Sesarma* (*Chiromantes*)]

Parasesarma dumacense (Rathbun, 1914) [*Sesarma* (*Parasesarma*)]

Parasesarma ellenae (Pretzmann, 1968) [*Sesarma* (*Parasesarma*)]

Parasesarma erythodactyla (Hess, 1865) [*Sesarma*]

Parasesarma eumolpe (De Man, 1895), **comb. nov.** [*Sesarma* (*Perisesarma*)]

Parasesarma exquisitum Dai & Song, 1986

Parasesarma foresti (Rahayu & Davie, 2002), **comb. nov.** [*Perisesarma*]

Parasesarma guttatum (A. Milne-Edwards, 1869), **comb. nov.** [*Sesarma*]

Parasesarma hartogi Davie & Pabriks, 2010

Parasesarma haswelli (De Man, 1887), **comb. nov.** [*Sesarma*]

Parasesarma holthuisi (Davie, 2010), **comb. nov.** [*Perisesarma*]

Parasesarma indiarum (Tweedie, 1940), **comb. nov.** [*Sesarma* (*Perisesarma*)]

= *Sesarma* (*Perisesarma*) *indica* De Man, 1902 (pre-occupied name)

Parasesarma jamelense (Rathbun, 1914) [*Sesarma* (*Parasesarma*)]

Parasesarma kuekenthali (De Man, 1902) [*Sesarma* (*Parasesarma*)]

Parasesarma lanchesteri (Tweedie, 1936), **comb. nov.** [*Sesarma*]

Parasesarma lenzii (De Man, 1895) [*Sesarma* (*Parasesarma*)]

Parasesarma lepidum (Tweedie, 1950) [*Sesarma*]

Parasesarma leptosoma (Hilgendorf, 1869) [*Sesarma*]

= *Sesarma* (*Holometopus*) *limbense* Rathbun, 1914

Parasesarma liho Koller, Liu & Schubart, 2010

Parasesarma lividum (A. Milne-Edwards, 1869), **comb. nov.** [*Sesarma*]

Parasesarma longicristatum (Campbell, 1967), **comb. nov.** [*Sesarma*]

Parasesarma luomi Serène, 1982

Parasesarma maipoense (Soh, 1978), **comb. nov.** [*Chiromanthes*]

Parasesarma melissa (De Man, 1887) [*Sesarma*]

Parasesarma messa (Campbell, 1967), **comb. nov.** [*Sesarma*]

Parasesarma moluccense (De Man, 1892) [*Sesarma* (*Parasesarma*)]

Parasesarma obliquifrons (Rathbun, 1924) [*Sesarma* (*Parasesarma*)]

Parasesarma onychophorum (De Man, 1895), **comb. nov.** [*Sesarma* (*Perisesarma*)]

Parasesarma palauense (Takeda, 1971) [*Sesarma* (*Parasesarma*)]

Parasesarma pangauranense (Rathbun, 1914) [*Sesarma* (*Parasesarma*)]

Parasesarma paucitorum Rahayu & Ng, 2009

Parasesarma persicum Naderloo & Schubart, 2010

Parasesarma pictum (De Haan, 1835) [*Grapsus* (*Pachysoma*)]

= *Sesarma rupicola* Stimpson, 1858

Parasesarma plicatum (Latreille, 1803) [*Ocypode*]

= *Alpheus quadratus* Weber, 1795 (nomen nudum)

= *Cancer quadratus* Fabricius, 1798 (pre-occupied name)

Parasesarma prashadi (Chopra & Das, 1937) [*Sesarma* (*Parasesarma*)]

Parasesarma raouli Rahayu & Ng, 2009

Parasesarma rutilimanum (Tweedie, 1936) [*Sesarma*]

Parasesarma samawati (Gillikin & Schubart, 2004), **comb. nov.** [*Perisesarma*]

Parasesarma semperi (Bürger, 1893), **comb. nov.** [*Sesarma*]

Parasesarma sigillatum (Tweedie, 1950) [*Sesarma*]

Parasesarma tripectinis (Shen, 1940) [*Sesarma*]

= *Parasesarma acis* Davie, 1993

Paraesarma ungulatum (H. Milne Edwards, 1853) [*Sesarma*]

Diagnosis: Small to medium sized crabs (usually < 2.5 cm, up to about 4 cm carapace width). Carapace squarish (slightly broader than long), carapace regions well defined, frontal region 4-lobed, median groove extending to gastric region, gastric region rather well defined, lateral margins of the carapace either without tooth, with a shallow indentation, or an epibranchial tooth of varying size. Male chelipeds robust, with 1–3 (usually 2) transverse pectinated crests on the upper surface of the palms, flanked by a large tubercle on the inner side; the crests consist of tall chitinous teeth, chelar dactylus dorsally tuberculated. Pleon of male relatively short, triangular, somite 3 widest, somite 6 slightly longer than somite 5, telson small, slightly wider than long in most species. Male press button absent or indistinct. Female vulvae are positioned on sternite 5, the operculum reaches the line between sternite 4 and 5 in most species.

Remarks: The genus now includes most of those species previously attributed to *Perisesarma* in addition to the previously recognized ones. Some of which are poorly known and need

confirmation and re-description (see Rahayu & Ng, 2010; Rahayu & Li, 2013). Moreover, as in the present study only some key species of *Parasesarma* were studied, the species allocations of the remaining representatives are based on the literature. Therefore, they are conditional and pending further revisions. Previous studies (e.g., Schubart et al., 2006) and own unpublished data predict that, even after its current enlargement, this genus remains polyphyletic and some species will have to be removed and placed into separate genera.

PERISESARMA DE MAN, 1895

Type species: *Sesarma dussumieri* H. Milne Edwards, 1853, subsequent designation by Campbell, 1967; gender neuter (see Ng et al., 2008).

Diagnosis: Small to medium sized crab (up to about 3.4 cm carapace width in studied material). Carapace squarish (slightly broader than long), carapace regions distinguishable, not well marked, lateral margins of carapace with a small epibranchial tooth. Male chelipeds robust, with 2 oblique rows of pectinated crests on upper surface of palms, followed by 2–4 small tubercles with chitinous peak on inner sides, the crests composed of tall chitinous teeth, chela dactylus with dorsal tuberculation. Walking legs relatively flat. Pleon of male relatively long, triangular, somite 6 considerably elongated, telson small and narrow, longer than wide. Male press button is proportionally large. Female vulvae are positioned completely in sternite 5 with operculum located on inner side and accompanied by a large sternal cover on the anterior side.

Remarks: This genus is now restricted to a single species. Here we take the opportunity to briefly re-describe *P. dussumieri* concerning its key characters, as it was not adequately illustrated before.

***Perisesarma dusumieri* (H. Milne Edwards, 1853)**

(Figs. 3E, 4E, 5E, 6E, 7E, 8E, 9E, 10E, 11E, 14)

Sesarma dusumieri (sic) H. Milne Edwards, 1853: 185.

Sesarma dussumieri – Targioni-Tozzetti, 1877: 145; De Man, 1888: 177; Campbell, 1967: 4 (in key).

Sesarma bidens – Kingsley, 1880: 215 (see label in Fig. 14F in the present study).

Sesarma (*Perisesarma*) *dussumieri* – De Man, 1895: 208.

Sesarma (*Chiromantes*) *dussumieri* – Tesch, 1917: 146; Tweedie, 1936: 66.

Chiromantes dussumieri – Tan & Ng, 1994: 82 (in list)

Perisesarma dussumieri – Ng et al., 2008: 222 (in list); Davie, 2010: 204 (in key); Shahdadi & Schubart, 2015: 1082 (in Table 1).

Material examined: The examined material is listed in Table 1. A single dry male specimen (carapace width = 31.60 cm) in the Muséum National d'Histoire Naturelle, Paris, France (MNHN IU 2000-10963) from India, Bombay is considered to be the holotype (Table 1, S624). If it turns out to be part of a type series, it will be designated as lectotype (D. Guinot, personal information).

Re-description: A small to medium sized crab (up to about 3.4 cm carapace width in studied material). Carapace squarish, greatest width between exorbital angles, slightly broader than long (about 1.15 times broader than long), front ca. 0.51 times carapace width, deflexed, with shallow median invagination, carapace regions distinguishable, postfrontal region 4-lobed with almost equal width, separated by shallow furrows, gastric region distinct, lateral surface lined with oblique striae, anterolateral margin with small epibranchial tooth, lateral margin slightly concave, lined with row of short setae (Fig. 14A, B & F).

Chelipeds subequal. Merus with posterior border granulate and small subdistal spine, lower border granulate, anterior border granulate with distinct subdistal spine. Carpus with inner and outer margins granular and dorsally rough. Chelae proportionally large and robust, palms with 2 oblique pectinated crests on the upper surface, followed by 2–4 small tubercles (with chitinous peak) on inner side, proximal crest with 11–14 tall teeth, shorter than distal ones with 13–17 tall teeth (Figs. 3E & 4E), outer surface of palm coarsely granulated (Fig. 14C), inner surface of palm with several granules bearing chitinous peaks (Fig. 14D), ventral surface with coarse granules with chitinous tip over entire length (Fig. 14D), length of cutting edge 0.35 times length of propodus. Chela dactylus with 11–13 broadly oval, low but distinct tubercles on dorsal surface (Fig 14C), most tubercles have transverse row of chitinous granules (3–6 granules), enlarged chitinous granules on inner side of dorsal surface of dactylus (Fig. 14E). Fingers with tips

chitinous, cutting edges with small and large teeth, leaving no gap when closed in adult males and with tufts of long, coarse setae on inner side (Fig. 14C & D).

Walking legs (Fig. 14A & F) relatively long. Third pair longer than others (about 1.9 times carapace width), merus flattened, relatively broad (about 2.2 times as long as wide), propodus relatively broad (about 2.3 times as long as wide), dorsal and ventral margins with dense brush-like stiff setae.

Male pleon relatively long, triangular, somite 3 widest, with lateral margins strongly convex, somite 4 more trapezoidal than somite 5, somite 6 considerably elongated and more hexagonal than trapezoidal, with a shallow concavity between somites 5 and 6, telson small, longer than basal width, somites 1 and 2 very narrow (Figs. 5E & 6E) (Table 6). The male press button is proportionally large (Fig. 7E).

G1 (Fig. 8E) relatively stout, with thick base, apical process short, truncated, slightly bent to form an angle about 50° with vertical axis, arched in cross section with syncline toward lateral surface, aperture terminal. G2 short, narrow, basal part wider (3 times wider than apical part), bent to form angle of about 140°.

Females with proportionally smaller chelipeds, smaller and less prominent dactylar tubercles. Pectinated crests vestigial or absent. Walking legs less setose compared to males. Pleon broadened, telson triangular, wider than long, inserted into somite 6 with less than half of its length (Fig. 9E). The vulvae are completely positioned on sternite 5, with operculum in inner part, accompanied by a large sternal cover anteriorly (Fig. 10E).

Distribution: Bombay, India (type locality) (H. Milne Edwards, 1853); Mergui Archipelago (Myanmar), mainland Myanmar and Penang (Malaysia) (De Man, 1888; 1895); Singapore (Tweedie, 1936; Tan and Ng 1994); Malacca (Malaysia), Phuket (Thailand) and Kerala, India (present study).

Remarks: According to the wide distribution range of this species from Bombay to Singapore, a morphological and genetic comparative study, examining the material from all over its geographic distribution, can significantly improve our understanding of the diversity and phylogeography of the taxon.

Figure 14. ***Perisesarma dusumieri*** **(H. Milne Edwards, 1853). A, Whole animal dorsal view (male, 34.5/30.4, ZRC2016.0376 = S628); B, Frontal view (S628); C, Right chela, outer face**

(S628); D, Right chela, inner face (S628); E, Dorsal view of right chela dactylus and dactylar tubercles (male, 22.8/20.4, SMF 49930 = S214); F, Holotype (male, 31.6/26.2, MNHN IU 2000-10963 = S624) whole animal dorsal view; scale bar: A–D & F 1.0 cm, E 1.0 mm. Figs. A–D taken by Jose Christopher E. Mendoza from the National University of Singapore and Fig. F taken by Noemy Mollaret from the Muséum National d'Histoire Naturelle, Paris.

***Fasciarma* gen. nov.**

Type species: *Sesarma fasciata* Lanchester, 1900, by present designation.

Diagnosis: Relatively small sized (up to 1.36 cm carapace width among studied material). Carapace squarish (slightly broader than long), carapace regions distinguishable, but not well defined, lateral margins of carapace with indentation. Male chelipeds relatively robust, chela palm with 2 oblique to nearly longitudinal ridges on the dorsal surface, each with chitinous caps, dorsal surface of chela dactylus bears low tubercles with chitinous peaks. Walking legs relatively long, slender, meri narrow. Pleon of male relatively wide, triangular, telson slightly wider than long. Male press button absent. Female vulvae entirely located in sternite 5 with an elongated operculum, rimmed perpendicular to sternal sutures.

Etymology: The name *Fasciarma* is composed of two parts: *fascia*, is derived from the name of the type species *fasciatum*, and *arma* is derived from the last part of its former genus *Perisesarma*, as well as from the type genus of the family, *Sesarma*. Gender neuter.

Remarks: This genus is restricted to a single species, *F. fasciatum*. We briefly re-describe this species with regards to its key characters, as it was not adequately illustrated before. The synonym (after =) is adopted from Ng et al. (2008).

***Fasciarma fasciatum* (Lanchester, 1900), comb. nov.**

(Figs. 3F, 4F, 5F, 6F, 7F, 8F, 9F, 10F, 11F, 15)

Sesarma fasciata Lanchester, 1900: 758, pl. 47 fig. 12.

=*Sesarma (Chiromantes) siamens* Rathbun, 1909: 109.

Sesarma (Chiromantes) siamensis – Tesch, 1917: 199.

Sesarma (Parasesarma) fasciata – Tesch, 1917: 153.

Sesarma (Chiromantes) fasciata –Tweedie, 1936: 66, pl. 15 fig. 3; Dai & Yang, 1991: 539, pl. 69 fig. 5.

Sesarma fasciata –Tweedie, 1950

Chiromantes fasciatus – Tan & Ng, 1994: 82 (in list).

Perisesarma fasciatum – Guerao et al., 2004; Ng et al., 2008: 222 (in list); Davie, 2010: 204 (in key); Shahdadi & Schubart, 2015: 1083 (in Table 1).

Material examined: The examined material is listed in Table 1. The male animal (carapace width = 9.15) from the syntype series was selected as lectotype (NHM1900.10.22.274) (S291 in Table 1) (Fig. 15F & G) (present designation).

Re-description: A small sized crab species (up to 1.36 cm carapace width among studied material, N=19). Carapace squarish, greatest width between epibranchial prominences, slightly broader than long, about 1.15±0.03 times as broad as long (N=17), front ca. 0.52±0.02 times carapace width (N=14), deflexed, with shallow median invagination. Carapace considerably punctuated (covered with coarse pits), regions not well defined, postfrontal region 4-lobed, not well marked, separated by shallow furrows, median lobes broader than lateral ones, gastric region distinguishable, but not well marked, lateral surface lined with oblique striae made by rows of fine granules, antero-lateral margins with an indentation (a distinct prominence) (in larger specimens also second prominence is visible), lateral margin straight, edged with row of short setae (Figs. 15A & B).

Chelipeds equal to subequal. Merus with borders finely granulated, with small distal and subdistal spines. Carpus with rows of fine granules dorsally. Chelae large (palm length/carapace width in males = 0.71±0.08, N=7, in female = 0.57±0.03 N=7), quite robust (palm width/length in males = 0.57±0.05, N=7, in female = 0.55±0.03, N=7), palm smooth externally, with some granules internally, with 2 oblique to nearly longitudinal ridges or wrinkles dorsally, each one bearing chitinous caps, outer ridge shorter, positioned on distal third of upper surface of the palm, initiated from the angle between inner and distal margin, bearing 10–16 chitinous caps (Fig. 3F), inner ridge initiated almost from the same angle and ending at proximal rim, with

chitinous caps mostly on the distal half (17–25 caps), 2 or 3 short rows of granules behind the inner ridge (Fig. 3F). Fingers with chitinous tips, cutting edges with small and large sharp teeth (movable finger with 4 large, 5–6 small teeth, fixed finger with 3 large and 6–8 small teeth) and leaving no gap when closed (Fig. 15C) (in animals with mature cuticle fingers without chitinous tips, blunt teeth, leaving a gap when closed (Fig. 15D)), length of palm cutting edge 0.43±0.03 times length of propodus (N=14), upper surface of the chela dactylus bears 5–8 low and smooth tubercles with chitinous peak (Figs. 15C–E).

Walking legs proportionally long, second and third pairs almost equal and longer than others. Third pair = 1.72±0.09 times carapace width (N=13), relatively narrow (e.g. merus = 2.88±0.14 times as long as wide, N=13), carpus, propodus, and dactylus with tufts of thick and long setae on both margins (Fig. 15B).

Male pleon relatively wide, triangular, somite 1 and 2 very narrow, somite 2 medially longer than lateral edges, somite 3 widest with lateral margins slightly convex, somites 4 and 5 trapezoidal, telson small, slightly wider than long (Figs. 5F & 6F). Male press button absent (Fig. 7F).

G1 (Fig. 8F) short, stout, apical process rather long and bent to form an angle of about 65° with vertical axis, tip truncated, aperture terminal. G2 short, bow-shaped, fairly stout, basal part wider (2 times wider than apical part).

Females with proportionally smaller chelipeds. Pleon broadened, telson wider than long, inserted into somite 6 less than half of its length (Fig. 9F). Vulvae completely positioned on sternite 5, with elongated operculum on inner part rimmed perpendicular to sternal sutures (Fig. 10E).

Distribution: Singapore (type locality) (Lanchester, 1900; Tweedie, 1936), Selangore, Malaysia (Tweedie, 1936), Hainan Island and Fujian, China (Dai & Yang, 1991), Ko Kut, Thailand, Gulf of Siam (Rathbun, 1909), Labuan, Malaysia (Tweedie, 1950) and Hong Kong (present study).

***Guinearma* gen. nov.**

Type species: *Grapsus huzardi* Desmarest, 1825, by present designation.

Diagnosis: Medium to large sized crabs (up to 5 cm carapace width among the studied material). Carapace squarish (slightly broader than long), carapace regions well defined, frontal region four-lobed, median groove extending to gastric region, gastric region defined (meso- and metagastric region distinguishable), cardiac and intestinal regions marked, lateral margins of the carapace with two teeth or indentations, the posterior one being smaller. Male chelipeds robust, palm with one oblique or curved row of pectinated crest on the dorsal surface, the crest consisting of short and thick teeth, followed by a line of small granules proximally, dorsal surface of the chelar dactylus with low tubercles. Walking legs relatively flat. Pleon of males relatively long, triangular, somite 3 widest; telson small, longer than wide. Male press button small, but distinct. Female vulvae are located in stenite 5 with elongated opercula almost parallel to sternal sutures, the inner-most part reaches the sternal suture between sternite 4 and 5, accompanied by a small sternal cover on the anterolateral corner.

Etymology: The name *Guinearma* is composed of two parts: *Guinea* is derived from the Gulf of Guinea, the main distribution area of the genus and *-arma* has become a typical ending for new genera in the family Sesarmidae, making reference to the type genus *Sesarma*. Gender neuter.

This genus includes three species as follows (the synonym (after =) and original combinations [in square brackets] are adopted from Ng et al., 2008):
Guinearma alberti (Rathbun, 1921), **comb. nov.** [*Sesarma* (*Chiromantes*)]
Guinearma huzardi (Desmarest, 1825), **comb. nov.** [*Grapsus*]
= *Sesarma africana* H. Milne Edwards, 1837
Guinearma kamermani (De Man, 1883), **comb. nov.** [*Sesarma* (*Chiromantes*]

Remarks: *Grapsus huzardi* was selected as type species of the genus, because it is the first described species among its congeners, as well as the more common and more frequently reported species (see Monod, 1956; Manning & Holthuis, 1981). These three species were previously considered members of *Perisesarma* (see Ng et al., 2008). Among them, *G. kamermani* is a poorly known species and was originally described based on one male specimen and without further reports. As we have examined more material, a comparative description of the three *Guinearma* species is now in preparation to facilitate their identifications and will be published elsewhere.

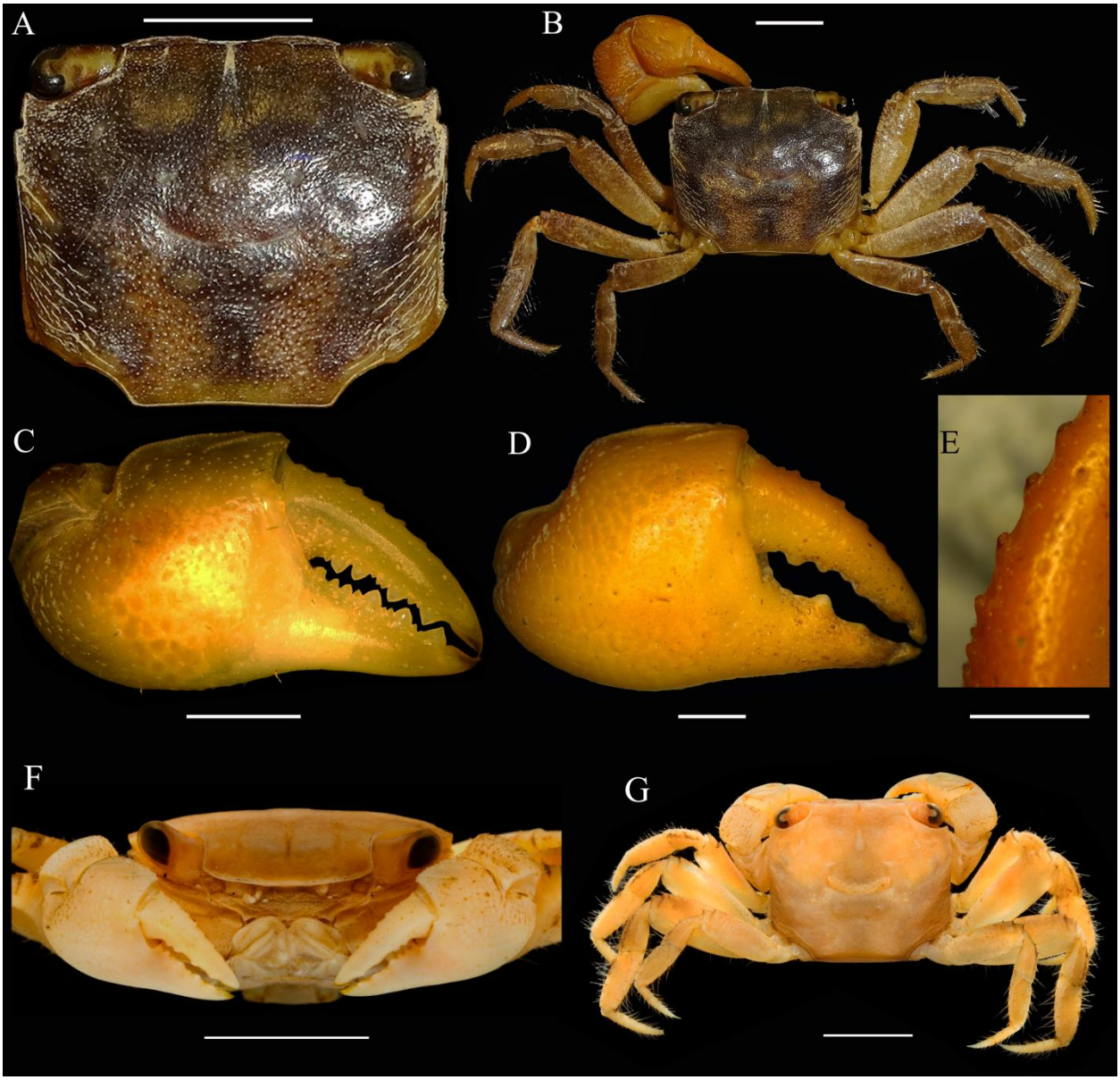

Figure 15. ***Fasciarma fasciatum*** **(Lanchester, 1900). A, Carapace (male, 12.0/10.3, ZRC 2012.0273 = S172); B, Whole animal dorsal view (S172); C, Right chela, outer face in an animal with new cuticle (male, 9.0/7.6, SMF 49907 = S190); D, Right chela, outer face in an animal with old cuticle (S172); E, Right chela dactylus, close up view of dactylar tubercles with chitinous tip (S172); F, Lectotype, frontal view (male, 9.1/8.1, NHM1900.10.22.274 = S291); G, Lectotype, whole animal dorsal view (S291); scale bar: A, B, F & G 5.0 mm, C–E 1.0 mm. Figs. F & G taken by Harry Taylor, NHM Photo Unit.**

Chapter 5

Molecular evidence for a cryptic species of the genus *Parasesarma* (Decapoda: Brachyura: Sesarmidae) from northern Australian mangroves

Abstract

During the last decade, molecular taxonomic studies have uncovered many cryptic species, even among marine representatives with plankton-dispersed larvae. These studies helped to reveal marine biogeographic barriers with restricted gene flow, among them a putative barrier between the northern Australian coastline and adjacent areas of South East Asia and the South Pacific. In agreement with these findings, we provide evidence for genetic uniqueness of representatives of the mangrove crab genus *Parasesarma* from northern Australian mangroves based on three mitochondrial and one nuclear DNA marker. This distinct taxon is here described as a new species. Morphologically the new species is very similar to *P. lividum* from the southwest Pacific and *P. samawati* from East Africa. Genetically, however, it is significantly distinct from all other congeners.

Introduction

It has been a long-standing assumption for the marine environment that genetic connectivity is maintained over large distances by ocean currents transporting the planktonic larvae (see Becker et al., 2007). However, recent molecular studies uncovered several cryptic species among marine coastal animals (Bickford et al., 2007), including different crustacean taxa (e.g., Lai et al., 2010 in portunid crabs; Tsang et al., 2012 in chthamalid barnacles; Tsoi et al., 2005 in penaeid shrimp). The phylogeographic disjunctions in oceanic realms can be attributed to more or less obvious marine barriers that delimit larval dispersal (Knowlton, 2000; Tsang et al., 2012; Wang et al., 2015).

Also among sesarmid crabs that are typical and fundamental components of mangrove ecosystems (Lee, 1998; Smith et al., 1991), cryptic or pseudocryptic diversity has been demonstrated: Ragionieri et al. (2009) discovered significant phylogeographic structure among different Indo-West Pacific populations of the apparently widely distributed species *Neosarmatium meinerti* (De Man, 1887). These findings resulted in separation of *N. australiense*, limited to the northern Australian coastline, from *N. asiaticum*, distributed in the Southeast Asian islands (Ragionieri et al., 2012). Silva et al. (2010) discovered a cryptic divergence based on mtDNA between southern and northern populations of *Parasesarma guttatum* (A. Milne-Edwards, 1869) in Mozambique, which is hypothesized to be mediated by the Agulhas Current. Like many other sesarmid crabs, members of the genus *Parasesarma* have marine larvae (see Flores et al., 2002; Guerao et al., 2004; Lago, 1993) and thus potential high dispersal ability.

In recent years, DNA barcoding has become a powerful method in alpha taxonomy by recognizing new species and delimiting existing ones (Hajibabaei et al., 2007; Hebert et al., 2003). This approach is being increasingly used for marine taxa (Bucklin et al., 2011; Schander & Willassen, 2005; Zemlak et al., 2009), among them also for the Crustacea (Costa et al., 2007; Lefébure et al., 2006; Matzen da Silva et al., 2011). Considering the underlying high morphological similarity and limited diagnostic characters among mangrove crab species of the genus *Parasesarma*, Shahdadi & Schubart (2015) also proposed to use of genetic approaches as additional tools to clarify their taxonomy.

In the present study we give evidence for and describe a cryptic species of *Parasesarma* from northern Australian mangroves using three different mitochondrial and one nuclear DNA marker.

The new species is genetically closest to *P. lividum* (A. Milne-Edwards, 1869) from the southwest Pacific and *P. samawati* Gillikin & Schubart, 2004, from East Africa, which is in agreement with striking morphological similarities among those species. Nevertheless, the northern Australian representatives hold a distinct phylogenetic position, providing evidence for a long independent evolutionary history and justifying recognition as a separate species.

Material and methods

Molecular analyses

Genomic DNA was isolated using a modified Puregene method (Gentra Systems, Minneapolis) and Mollusc DNA kit (Omega D3373–02) from muscular leg tissue using the manufacturers' protocol. In the cases of very old specimens or those preserved in formalin, the muscular tissues were soaked in GTE buffer overnight prior to the DNA extraction (following Shedlock et al., 1997). Four genes, including two mitochondrial protein-coding genes, cytochrome oxidase subunit 1 (Cox1) and NADH dehydrogenase 1 (ND1), the mitochondrial gene encoding the rRNA of the large ribosomal subunit (16S), and the nuclear protein-coding gene sodium-potassium ATPase alpha-subunit (NaK) were partially amplified using different primer combinations (Table 5.1). For the NaK gene, only two specimens of the three related species (i.e., the new northern Australian species, *P. lividum* and *P. samawati*) were examined. The primer combination COL6/COH6 was used to amplify a segment of 709 basepairs (bp) of Cox1 (658 bp without primers), corresponding to the generally used barcoding region (Hebert et al., 2003). For the 16S gene, the longest primer combination was 16L29/16H11 (amplifying a segment of approximately 584 bp depending on the amount of indels), but in cases with degraded DNA, shorter fragments were amplified using the combination 16L29/16H37. To amplify 478 bp (including primer regions) of the ND1 gene, the primer combination NDL5/NDH8 was used. The primer combination NaK for-b/NaK rev3 was used to amplify a segment of 604 basepairs (including the primer regions) of the NaK gene. Polymerase chain reactions (PCR) were carried out with the following profile: initial step 4 min at 94°C; 40 cycles with 45s at 95°C for denaturing; 60s at 48°C for annealing (58°C in case of NaK); 60s at 72°C for extension; and 5 min at 72°C for a final extension step. PCR products were outsourced for sequencing to Macrogen Europe.

Sequences were proofread using Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia), primer regions were removed and the remaining sequences aligned automatically with ClustalW (Thompson et al., 1994) implemented in BioEdit 7.0.5 (Hall, 1999). Three alignments were obtained, one from the Cox1 gene, one from the NaK gene, and one with a concatenated dataset of the three genes (Cox1, 16S and ND1). The alignments were converted with FaBox (Villesen, 2007) to nexus files. To construct phylogenetic trees, the best evolutionary model describing our data was determined with the aid of jModelTest (v. 2.1.4) (Darriba et al., 2012) and selected with the Akaike information criterion (AIC) (Posada & Buckley, 2004). The nexus file of the Cox1 datasets were furthermore used to construct a Neighbor-Net network using the software Split Tree version 4.14.2 (Huson & Bryant, 2006). Two methods of phylogenetic inference were applied to our dataset: Maximum Likelihood (ML) with the software raxmlGUI (v. 1.3) (Silvestro & Michalak 2012) and Bayesian Inference (BI) as implemented in Mr.Bayes (v. 3.2) (Huelsenbeck & Ronquist, 2001). Two ML and also two BI trees were constructed from the Cox1 file and the concatenated dataset. *P. messa* and *P. lanchesteri* were selected as best outgroups for constructing trees based on the Cox1 and the concatenated alignments according to previous preliminary analyses. Maximum Likelihood (ML) trees were obtained with 2000 bootstrap pseudoreplicates. For the Mr.Bayes runs, we used 2 million generations with four chains and a sample frequency of 1,000 generations. Genetic distances (Kimura 2-parameter = K2P) based on the Cox1 sequences among the new species and other related species were calculated with the software Mega version 5.2.2 (Tamura et al., 2011). New sequences were submitted to Gen-Bank (NCBI), and accession numbers are given in Table 5.2.

Material examined

Specimens were borrowed from or studied in different museums, including the Australian Museum (AM), Sydney, Australia; the Queensland Museum (QM), Brisbane, Australia; the Forschungsinstitut und Museum Senckenberg (SMF), Frankfurt a.M., Germany (including material which was formerly transferred from the Zoologisches Museum der Universität Göttingen (ZMG)); Zoological Reference Collection (ZRC), of the Lee Kong Chian Natural History Museum, National University of Singapore and the Natural History Museum (NHM) London, UK. Additional material originated from own collections and was preserved in ethanol 70% and transferred to the University of Regensburg for morphological and molecular examination (see Table 5.2 for details on all the material examined). For genetic comparisons,

representatives of possibly related clades were included (i.e., *P. cricotum* Rahayu & Davie 2002; *P. holthuisi* Davie 2010; *P. lanchesteri* (Tweedie 1936); *P. lividum* (Milne-Edwards 1869); *P. longicristatum* (Campbell 1967); *P. messa* (Campbell 1967); *P. samawati* Gillikin & Schubart, 2004). The species *P. lanchesteri*, *P. lividum*, *P. longicristatum, P. samawati* and *P. semperi* (Bürger 1893) were also included in morphological comparisons. For convenience, and to avoid any confusion when referring to specific specimens, every examined individual is coded with a specific number in the Table 5.2.

Table 5.1. Primers used in present study with corresponding's DNA sequences (5′-3′) and the corresponding references.

Gene	Primer	Sequence	Reference
COI	COL6	TYTCHACAAAYCATAAAGAYATYGG	Schubart, 2009
	COH6	TADACTTCDGGRTGDCCAAARAAYCA	Schubart & Huber, 2006
16S rRNA	16L29	YGCCTGTTTATCAAAAACAT	Schubart, 2009
	16H37	CCGGTYTGAACTCAAATCATGT	Klaus et al., 2006
	16H11	AGATAGAAACCRACCTGG	Schubart, 2009
ND1	NDL5	TTGCTGGWTGRTCTTCWAATTG	New
	NDH8	AYCTTTTYCAWGCTAAATA	New
NaK	NaK for-b	ATGACAGTCGCYCAYATGTGGTT	Tsang et al., 2008
	NaK rev3	GGAGGRTCAATCATRGACAT	Tsang et al., 2014

Table 5.2. Material examined in this study with code numbers, sex (M= male, F= femal), size (carapace with× length in millimeter), locality, catalogue number and GenBank accession numbers (Cox1, 16S, ND1 and NaK).

Species	Code	Sex and size	Locality	Catalogue No.	GenBank accession No. (pending)			
					Cox1	16S	ND1	NaK
P. cricotum Rahayu & Davie, 2002	(S38)	F, 14.34×12.16	New Guinea, Kamora	ZRC2016.0522	KX400897	KX423796		
Parasesarma n. sp2	(S302)	M, 22.17×18.04	Australia, Northern Territory, Arrla Ck.	AM, P.99000 holotype	Pending	Pending	Pending	
	(S304)	F, 19.81×14.28	Australia, Northern Territory, Arrla Ck.	AM, P.99001 paratype	Pending			
	(S308)	M, 18.55×14.8	Australia, Northern Territory, King River	AM, P99002 paratype	Pending			
	(S300)	M, 17.15×13.12	Australia, Northern Territory, Arrla Ck.	AM, P.99003 paratype	Pending	Pending	Pending	
	(S301)	F, 19.79×16.07	Australia, Northern Territory, Arrla Ck.	AM, P.99003 paratype				
	(S460)	F, 16.98×13.86	Australia, Northern Territory, Arrla Ck.	AM, P.99003 paratype				
	(S344)	M, 16.63×13.36	Western Australia, Kimberly coast	QM, W20159 paratype	Pending			
	(S345)	F, 21.10×17.04	Western Australia, Kimberly coast	QM, W20159 paratype	Pending	Pending	Pending	
	(S374)	M, 24.49×20.48	Australia, Northern Territory, Allnight Ck.	QM, W6651 paratype				
	(S375)	F, 26.70×22.05	Australia, Northern Territory, Allnight Ck.	QM, W6651 paratype	Pending			
P. holthuisi Davie, 2010	(S85)	M, 19.16×16.16	Western Australia, Ashburton	QM, W28880 Paratype	KX400907	KX423806		
P. lanchesteri. (Tweedie, 1936)	(S426)	F, 24.76×18.78	Singapore, Simpang Mak Wai River	ZRC1967.11.8.3	KX761168	KX761174		
P. lanchesteri	(S175)	M, 19.92×15.86	Unknown locality	SMF 7142				
P. lividum (A. Milne-Edwards, 1869)	(S372)	M, 28.70×25.28	Australia, Queensland, Innisfail	QM, W2558				
	(S5)	M, 28.48×23.49	Australia, Queensland, Harmer Ck.	QM, W16331	Pending			
	(S76)	M, 23.94×20.27	New Caledonia	QM, W24243	Pending			
	(S371)	M, 22.71×18.44	Australia, Queensland, Lizard Island	AM, P88290	Pending			
	(S77)	M, 21.44×18.06	Fiji, Suva	Pending	Pending			
	(S370)	M, 20.80×16.53	Australia, Queensland, Lizard Island	QM, W19924	Pending			
	(S69)	M, 17.76×15.19	New Caledonia, Noumea	QM, W24243	KX400893	KX423802	Pending	KX394812
	(S375)	F, 26.70×22.05	New Caledonia	QM, W24243				
	(S6)	F, 23.11×18.73	Australia, Queensland, Harmer creek	QM, W16331				

Species	Code	Sex and size	Locality	Catalogue No.	GenBank accession No. (pending)			
					Cox1	16S	ND1	NaK
	(S68)	F, 17.24×14.19	New Caledonia, Noumea	QM, W24243	KX400894	KX423801		
	(S78)	F, 14.07×11.22	Fiji, Suva	Pending	Pending	Pending	Pending	
P. longicristatum (Campbell, 1967)	(S335)	M, 18.50×14.94	Australia, Queensland , Port Alma	QM, W2464 Paratype	KY198240	KY198245		
P. samawati Gillikin & Schubart, 2004	(S174)	M, 28.57×23.88	Kenya, Watamu	SMF 29334, paratype	Pending	Pending	Pending	
	(S273)	M, 25.46×21.14	Aldabra atoll	NHM2006.1806-1808				
	(S154)	M, 23.70×20.54	Kenya, Watamu	SMF 29333, holotype	KX400895			
	(S129)	M, 23.49×19.56	Kenya, Watamu	Pending	Pending			
	(S453)	M, 18.47×15.3	Seychelles	Pending	Pending	Pending	Pending	
	(S376)	F, 23.96×19.93	Kenya, Watamu	SMF 29334, paratype	Pending			
	(S454)	F, 19.05×15.13	Seychelles	Pending	Pending			
	(S155)	N.A	Seychelles	N.A	KX400896			
P. messa (Campbell, 1967)	(S7)	M, 19.36×16.74	Australia, Queensland, Brisbane	ZRC 1999.0650	KX431205	KX423795		
P. semperi (Bürger, 1893)	(S150)	M, 17.78×14.80	Philippines, Bohol	ZMG 625 lectotype				

Results and discussion

The cropped alignments after removal of the primer sequence and adjacent regions were as follows: Cox1=631 bp; 16S=532 bp; ND1=444 bp; NaK=526 bp, with a concatenation of the three mitochondrial genes (Cox1, 16S and ND1) of 1607 bp. The alignments of the protein coding genes contained no stop codons, which may have indicated the presence of pseudogenes. The best evolutionary model obtained with jModelTest (v. 2.1.4) (Darriba et al., 2012; Guindon & Gascuel, 2003) was the General Time reversible plus Gamma (GTR+G, Rodriguez et al., 1990).

A Neighbor-Net network (Fig. 5.1A) and two phylogenetic trees (ML and BI) (here only ML tree is shown) (Fig. 5.1B) were obtained from Cox1 gene, focusing on the new species and the two closest related species *P. lividum* and *P. samawati*. Phylogenetic trees were also constructed from the concatenated dataset of the three mitochondrial genes (only ML tree is shown here) (Fig. 5.1C).

The genetic data show that *P. lividum*, from the South Pacific, and *P. samawati*, from East Africa, are phylogenetically closest to the new species from northern Australia (Fig. 5.1C). But these data (the network and the trees) also give evidence for the distinct separation of the new species, which forms a unique and distinct phylogenetic group. The mean K2P distance between the new species (north Australian group) and the two other related species (*P. lividum* and *P. samawati*) amounts to 4%, while it is only 2% between these recognized species. The two specimens of the new species share the same genotype of the nuclear NaK gene, whereas four specimens of *P. lividum* and *P. samawati* differ from the new species by one to two mutation step(s).

Surprisingly, these analyses reveal that the two geographically very distant species, *P. lividum* (from the South Pacific) and *P. samawati* (from East Africa) have a very close phylogenetic relationship and are sister species. Occurrences of *P. lividum* in isolated Pacific islands as Lizard Is., Fiji and New Caledonia give evidence for a high capability of long distance distribution during a potentially extended marine larval phase. In addition to those islands, *P. lividum* has also been reported from South East Asian areas like Ambon and the Gulf of Thailand (Tesch, 1917). Despite their apparent capability of long distance dispersion, *P. lividum* s.str. did not maintain regular gene flow with northern Australian mangroves and may

thus have allowed populations of these areas to achieve phylogenetic isolation. Similarly, Ragionieri et al. (2009, 2011) discovered phylogeographic structure between *N. australiense* from northern Australian coasts and *N. asiaticum* from South East Asian islands. Lai et al. (2010) reported a similar phylogeographic disjunction among populations of the *Portunus pelagicus* (Linnaeus, 1758) species complex. Our new species thus provides additional evidence for a strong physical marine barrier isolating the northern coast of Australia from adjacent areas. This disconnection has been partly assigned for a coral reef fish to the Arlindo Current (Ovenden et al., 2002).

The shape and number of cheliped dactylar tubercles and the pattern of tuberculation are among the main diagnostic and important morphological key characters for species delimitation in the genus *Parasesarma* (Campbell, 1967; Davie, 2010; Shahdadi & Schubart, 2015). This importance can be attributed to the broad phenotypic range of this character among the species of this genus. Shahdadi & Schubart (2015) confirmed that this character is a useful feature for species identification; but considering the underlying intraspecific diversity and overlap among species they argue that it should be accompanied by additional morphological characters and molecular markers for taxonomic clarification. In this study we show that the two species *P. lividum* and *P. samawati* are genetically closely related sister species, but distinct according to their number and shape of dactylar tubercles: *P. lividum* has 10–13 small transversely elongated tubercles, while *P. samawati* has 7–8 round tubercles (this comparison was not included in the original description of *P. samawati* by Gillikin & Schubart (2004)). This contrasts with the high similarity between *P. samawati* and the new north Australian species (i.e., both having 7–8 round tubercles), while they are genetically more distant (Fig. 5.1).

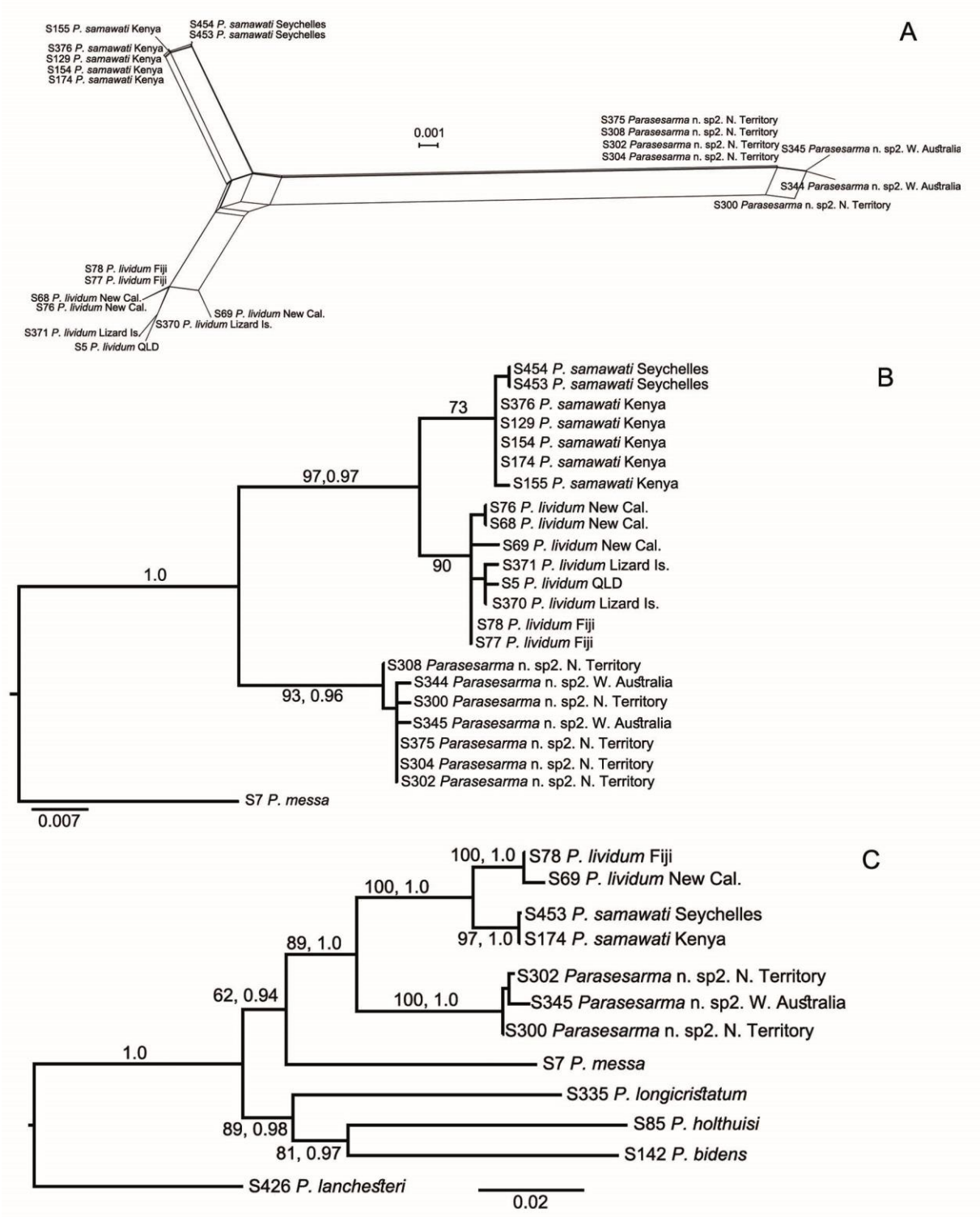
A
S155 P. samawati Kenya
S454 P. samawati Seychelles
S453 P. samawati Seychelles
S376 P. samawati Kenya
S129 P. samawati Kenya
S154 P. samawati Kenya
S174 P. samawati Kenya
0.001
S375 Parasesarma n. sp2. N. Territory
S308 Parasesarma n. sp2. N. Territory
S302 Parasesarma n. sp2. N. Territory
S304 Parasesarma n. sp2. N. Territory
S345 Parasesarma n. sp2. W. Australia
S344 Parasesarma n. sp2. W. Australia
S300 Parasesarma n. sp2. N. Territory
S78 P. lividum Fiji
S77 P. lividum Fiji
S68 P. lividum New Cal.
S76 P. lividum New Cal.
S69 P. lividum New Cal.
S370 P. lividum Lizard Is.
S371 P. lividum Lizard Is.
S5 P. lividum QLD
B
S454 P. samawati Seychelles
S453 P. samawati Seychelles
73
S376 P. samawati Kenya
S129 P. samawati Kenya
S154 P. samawati Kenya
S174 P. samawati Kenya
97,0.97
S155 P. samawati Kenya
S76 P. lividum New Cal.
S68 P. lividum New Cal.
S69 P. lividum New Cal.
90
S371 P. lividum Lizard Is.
1.0
S5 P. lividum QLD
S370 P. lividum Lizard Is.
S78 P. lividum Fiji
S77 P. lividum Fiji
S308 Parasesarma n. sp2. N. Territory
S344 Parasesarma n. sp2. W. Australia
93, 0.96
S300 Parasesarma n. sp2. N. Territory
S345 Parasesarma n. sp2. W. Australia
S375 Parasesarma n. sp2. N. Territory
S304 Parasesarma n. sp2. N. Territory
S302 Parasesarma n. sp2. N. Territory
S7 P. messa
0.007
C
100, 1.0
S78 P. lividum Fiji
S69 P. lividum New Cal.
100, 1.0
S453 P. samawati Seychelles
97, 1.0
S174 P. samawati Kenya
89, 1.0
S302 Parasesarma n. sp2. N. Territory
62, 0.94
100, 1.0
S345 Parasesarma n. sp2. W. Australia
S300 Parasesarma n. sp2. N. Territory
1.0
S7 P. messa
S335 P. longicristatum
89, 0.98
S85 P. holthuisi
81, 0.97
S142 P. bidens
S426 P. lanchesteri
0.02

Fig. 5.1. (A) A Neighbor-Net consensus network of Cox1 gene using the software Split Tree, focusing on the new species (*Parasesarma* n. sp2.), *P. lividum* and *P. samawati*. (B) Phylogenetic consensus tree constructed with Maximum Likelihood (ML) and Bayesian Inference (BI) of Cox1 gene, focusing on the new species, *P. lividum* and *P. samawati, P. messa* was selected as outgroup. (C) Phylogenetic consensus trees constructed with Maximum Likelihood (ML) and Bayesian Inference (BI) of the concatenated data set of the three genes Cox1, ND1 and 16S, from selected species of *Parasesarma, P. lanchesteri* was used as outgroup. Only confidence values higher than 50% and 90% respectively for ML (bootstrap value) and BI (posterior probabilities) are shown in the trees.

Systematic account

The abbreviations are: cw = maximum carapace width; cl = maximum carapace length; G = male first gonopod. The measurements in the material examined are cw×cl in millimeter. The measurements in the description are only for adult male animals.

Family Sesarmidae Dana, 1851

Genus *Parasesarma* De Man, 1895

***Parasesarma* n. sp2.**

(Figs. 5.2, 5.3, 5.4D & H)

Material examined

Holotype: a male (22.17×18.04) (AM, P.99000) from Australia, Northern Territory, Arrla Creek. Paratypes: a male (18.55×14.8) (AM, P99002), Australia, Northern Territory, King River; a male (17.15×13.12) and two females (19.79×16.07; 16.98×13.86) (AM, P.99003) from Australia, Northern Territory, Arrla Creek; a female (19.81×14.28) (AM, P.99001) from Australia, Northern Territory, Arrla Creek; a male (24.49×20.48) and a female (26.70×22.05) (QM, W6651) from Australia, Northern Territory, Allnight Creek; a male (16.63×13.36) and a female (21.10×17.04) (QM, W20159) from Western Australia, Kimberly coast.

Comparative Material: For the comparative materials see Table 5.2 and the section 2.2. Material examined.

Diagnosis.

Small to medium sized crab, carapace subrectangular, slightly broader than long, post-frontal lobes prominent, median and laterals equal in width, dorsal carapace regions moderately well indicated, anterolateral margin with a distinct epibranchial tooth, chelipeds subequal, chelae large, with short fingers, upper surface of palm with 2 transverse pectinated crests, dorsal surface of dactyl bearing 7–8 distinct and rounded tubercles, each one with transvers line, walking legs relatively short and broad, male pleon triangular with telson slightly shorter than basal width, G1 slender, straight, apical corneous process long with tip rounded and subterminal aperture.

Description.

Small to medium sized crab (Maximum cw in our collection = 24.49 mm, see 4.1. Mterial examined). Carapace subrectangular, slightly broader than long, greatest width between exorbital angles (cl/cw = 1.25±0.04, N=5), carapace surface smooth, shining, with transverse rows or tufts of setae. Front = 0.59±0.01 times carapace width (N=3), moderately deflexed, with broad median emargination. Post-frontal lobes prominent, median and laterals equal in width, median lobs separated by a deep furrow. Dorsal carapace regions moderately well indicated, with gastric region demarcated, cardiac region separated from intestinal region, lateral branchial ridges prominent, upper orbital border smooth, lower orbital border finely granulate, anterolateral margin with sharp exorbital angle and distinct epibranchial tooth, lateral edged slightly concave with row of short setae (Fig. 5.2A & B).

Chelipeds subequal, chela large, (palm length = 0.65±0.05 times carapace width, N=3), robust (the ratio of the length/width = 1.81±0.06, N=3) (Figs. 5.2C, 5.3A). Merus with granulate dorsal border and distinct subdistal spine, ventral border granulate, anterior border granulate, with distinct subdistal spine, inner face smooth with two longitudinal rows of setae, ventral row more prominent, continuous, with longer setae, dorsal row interrupted, setae extending to dorsal border, ventral face smooth, outer face with rows of fine granules. Upper surface of palm with 2 transverse pectinated crests (Fig. 5.3G), distal (primary) crest composed of 13–17 tall, broad teeth, second crest well developed, with 12–14 teeth, (in holotype, left chela show sign of third crest), primary and secondary pectinated crests end up with a big hump on the inner side and followed by several blunt tubercles on the outer side, one row of coarse granules proximal to second. Outer surface of palm without setae, coarsely granular except

outer surface of fixed finger which becomes punctate, ventral border of the chela almost straight and the granules becomes fine and crate short lines, inner surface of palm coarsely granular except area facing carpus, length of cutting edge of fixed finger = 0.38±0.01 times of the maximum length of propodus (N=3). Dactylus quiet strait and stout, slightly curves in dorsal view, its length is = 0.57±0.01 times of the propodus maximum length (N=3), dorsal surface of dactyl bearing 8 distinct and rounded tubercles, each one with transvers line (Fig. 5.3A & B), row of about 10–12, rounded tubercles on proximal two thirds of inner edge of dorsal surface. Fingers with chitinous tips, intermeshing and without gape left when fingers closed. Cutting edge of both fingers with a series of variably sized teeth (Fig. 5.3A).

Walking legs relatively short and broad, flattened, third pair longer than others, in length = 1.62 times carapace width in the holotype, merus of second leg 1.92±0.03 as long as wide (N=3), propodus of second leg = 2.31±0.12 times as long as wide (N=3) (Fig. 5.2A & C).

Male pleon triangular with telson slightly shorter than basal width (the width = 1.11±0.04 time than the length, N=3), virtually pentagonal, slightly longer than somite 6 (1.14±0.03 time as the length of somite 6, N=3). Somite 6, 2.16±0.27 times wider than long (N=3). Somite 5 and 4 are trapezoidal. Somite 3 is the widest one, convexed laterally. Somite 2 medially slightly longer than lateral edges (Fig. 5.3D).

G1 slender, straight, apical corneous process long with tip rounded and slightly bent to create an angle about 48° with vertical axis, aperture subterminal (Fig. 5.3E).

Female with smaller chelipeds, the ratio of palm length to carapace width ca. 0.59 in a female paratype (S301). Distal dactylar pectinated crest well developed and prominent as in male, proximal crest reduced to a row of tubercles sometime with chitinuos tip. Pleon fringed with long setae, broadened, evenly rounded, pleon touches coxae of walking legs, telson ca. 1.10 times wider than long in a female paratype (S301) (inserted to somite 6 about half of its length) (Fig. 5.3C). Vulva in depression on anterior edge of sternite 5, somewhat embraced by posterior margin of sternite 4, the operculum in the inner part (Fig. 5.3F).

Distribution and habitat.

Mangroves of Western Australia and Northern Territory, Australia.

Remarks.

Morphologically, the new species is similar to *P. longicristatum* and *P. semperi* in having 7–8 rounded, distinct and well developed chelar dactylar tubercles. But the new species is different from *P. semperi* and *P.longicristatum* in many morphological aspects. The new species has shorter fingers (i. e length of cutting edge ca. 0.38 times length of propodus while this is ca. 0.44 in *P. semperi* and *P. longicristatum*, and also the ratio of the length of dactylus to length of propodus in the new species is ca. 0.58 while it is at least ca. 60 in the other two species) (see Fig. 5.4). Moreover the new species is also different from the other two having well marked dorsal carapace regions and frontal lobes. The genetic data also indicate that the new species is very different from these two sister species (Fig. 5.1C).

The new species is also similar to *P. lanchesteri* in having 7–8 rounded, distinct and well developed chelar dactylar tubercles (Shahdadi & Schubart, 2015). But *P. lanchesteri* is different from the new species having dactylar tubercles with transvers lines on proximal slope. They are also different in G1 morphology (i.e., in *P. lanchesteri* the apical corneous part strongly bent to create an angle about 80° with the vertical axis while this is ca. 50° in the new species). The genetic data also indicate that the new species is quiet distant from the abovementioned species.

Corresponding to the genetic connection, specimens of the new species show high morphological similarities to *P. lividum* and *P. samawati*. They are undistinguishable in carapace regions, frontal lobes, walking legs, palm morphology and having short chelar fixed fingers (i.e., the ratio of length of cutting edge/length of propodus in *P. lividum* = 0.38, N=5; in *P. samawati* = 0.39, N=5; in the new species = 0.38, N=3). They also have short chela dactyli (i.e., the ratio of length of dactylus/length of propodus in *P. lividum* = 0.58, N=5; in *P. samawati* = 0.57, N=5; in the new species = 0.57, N=3) (Fig. 5.4). Contrasting with the carapace similarity, the morphology of dactylar tubercles shows obvious differences between the new species and *P. lividum*. Specimens of *P. lividum* have 10–13 small, low and transversely elongated dactylar tubercles, while the new species has 7–8 round tubercles distinct to the end (Fig. 5.4). Despite being morphologically similar to *P. samawati*, the new

species is genetically clearly distinct from this species, as well as from *P. lividum* (Fig. 5.1). The minimum interspecific mitochondrial Cox1 distance between the new species and other related species of *Parasesarma* (K2P = 4%) is large enough to support its long independent evolutionary history and divergence. This value is larger than the minimum interspecific distances of other brachyuran crabs which have closely related species pairs. e.g., in *Parasesarma*: 2% between *P. samawati* & *P. lividum* (present study); in *Neosarmatium*: 2.8% between *N. africanum* Ragionieri, Fratini & Schubart, 2012, and *N. meinerti* (De Man, 1887) (according to Ragionieri et al., 2009 & 2012); in *Portunus*: 1.8% between *P. armatus* (A. Milne-Edwards, 1861) & *P. reticulatum* (Herbst, 1799), 3.5% between *P. segnis* (Forskål, 1775) & *P. pelagicus* (Linnaeus, 1758) (see Lai et al., 2010); in *Geothelphusa*: 2.83% between *G. candidiensis* Bott, 1967 and *G. olea* Shy, Ng & Yu, 1994 (according to Do et al., 2016).

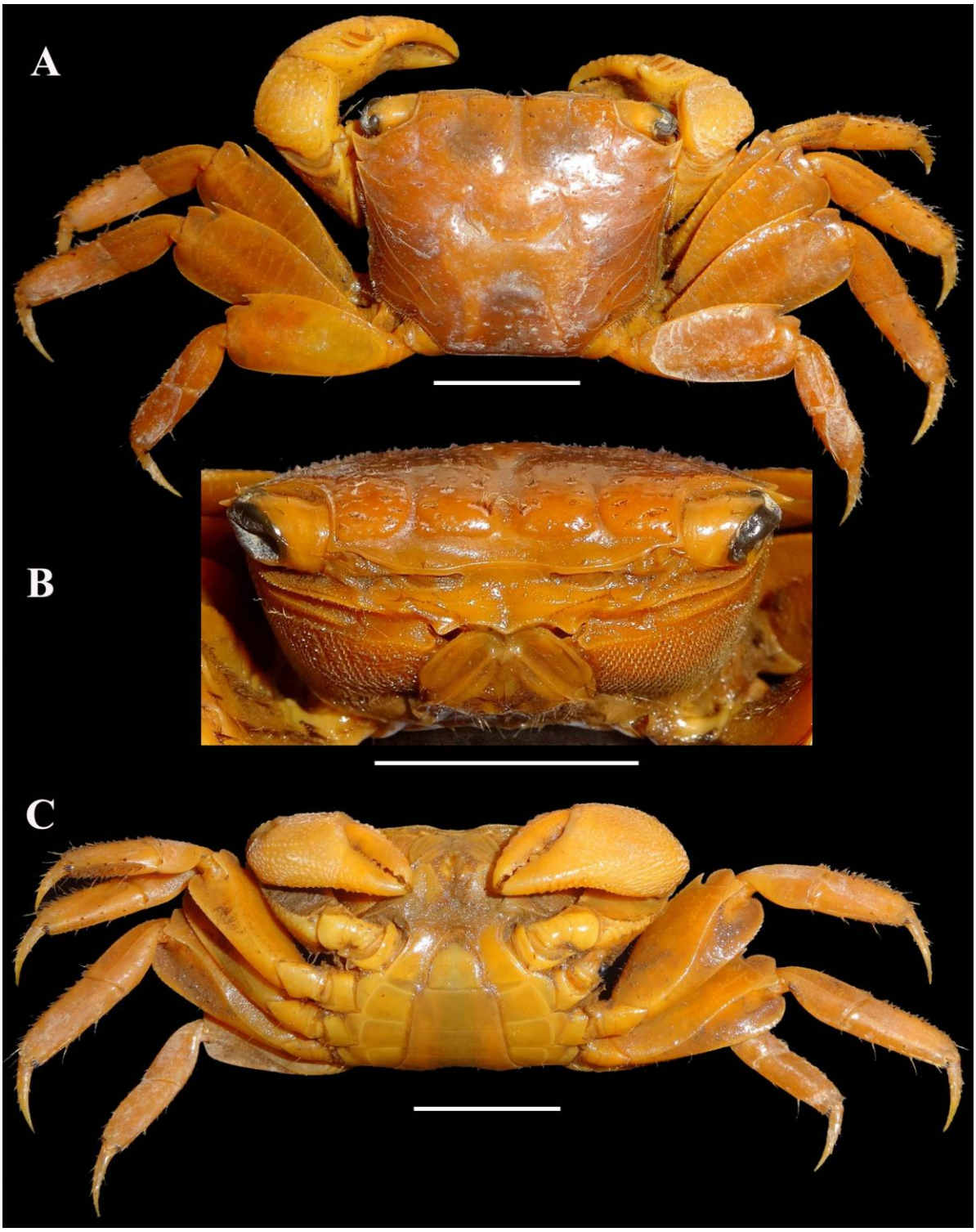

Fig. 5.2. ***Parasesarma*** **n. sp2. holotype (AM, P.99000) (S302). (A) Dorsal view of whole animal. (B) Frontal view of carapace. (C) Ventral view of whole animal. Scale bars = 1cm.**

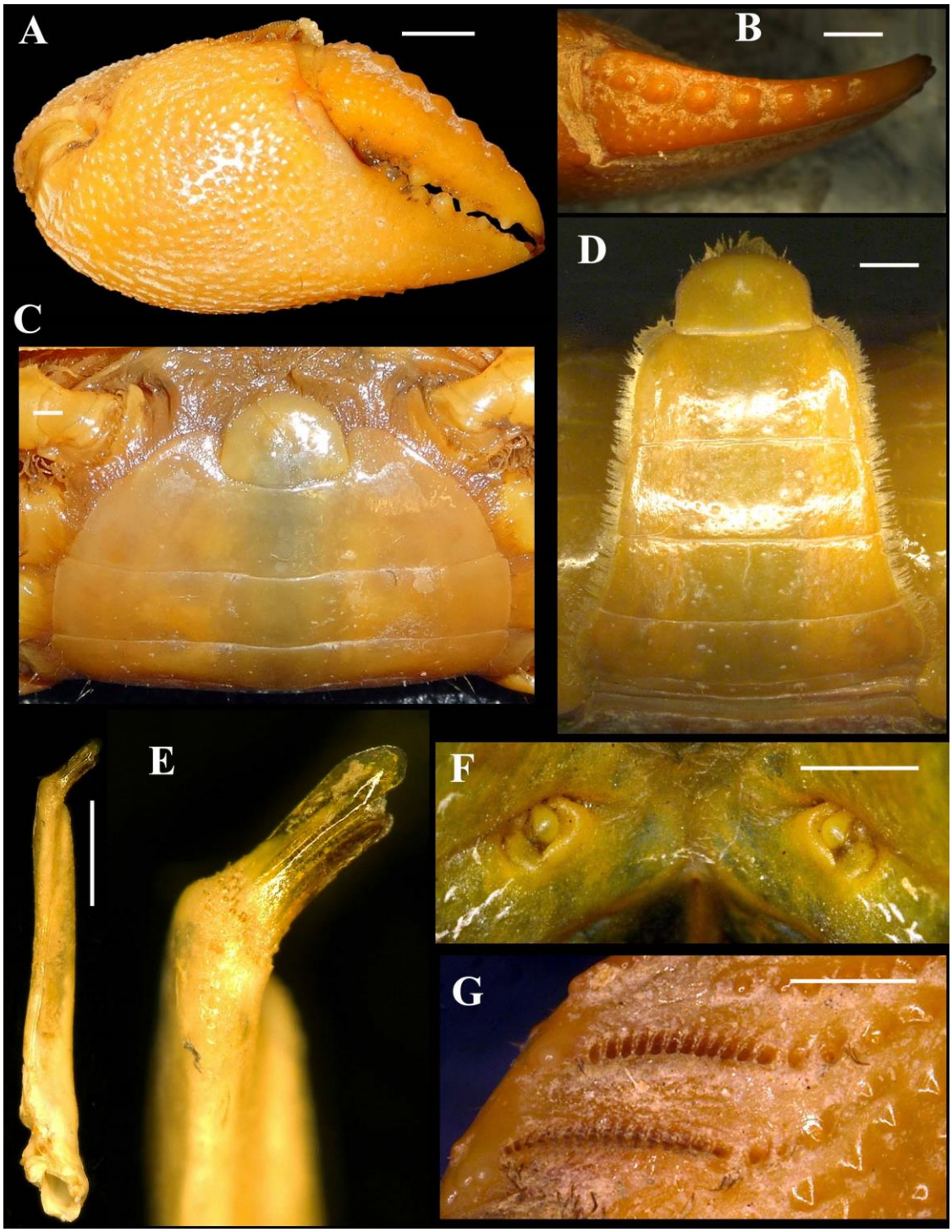

Fig. 5.3. ***Parasesarma*** **n. sp2., A, B, D, E & G = holotype (AM, P.99000) (S302), C & F = a female paratype (AM, P.99003) (S301). (A) Male chela, outer view. (B) Male chela, dactylar tubercles, dorsal view. (C) Female pleon. (D) Male pleon. (E) G1. (F) Vulva. (G) Male pectinated crests, dorsal view. Scale bars = 1 mm.**

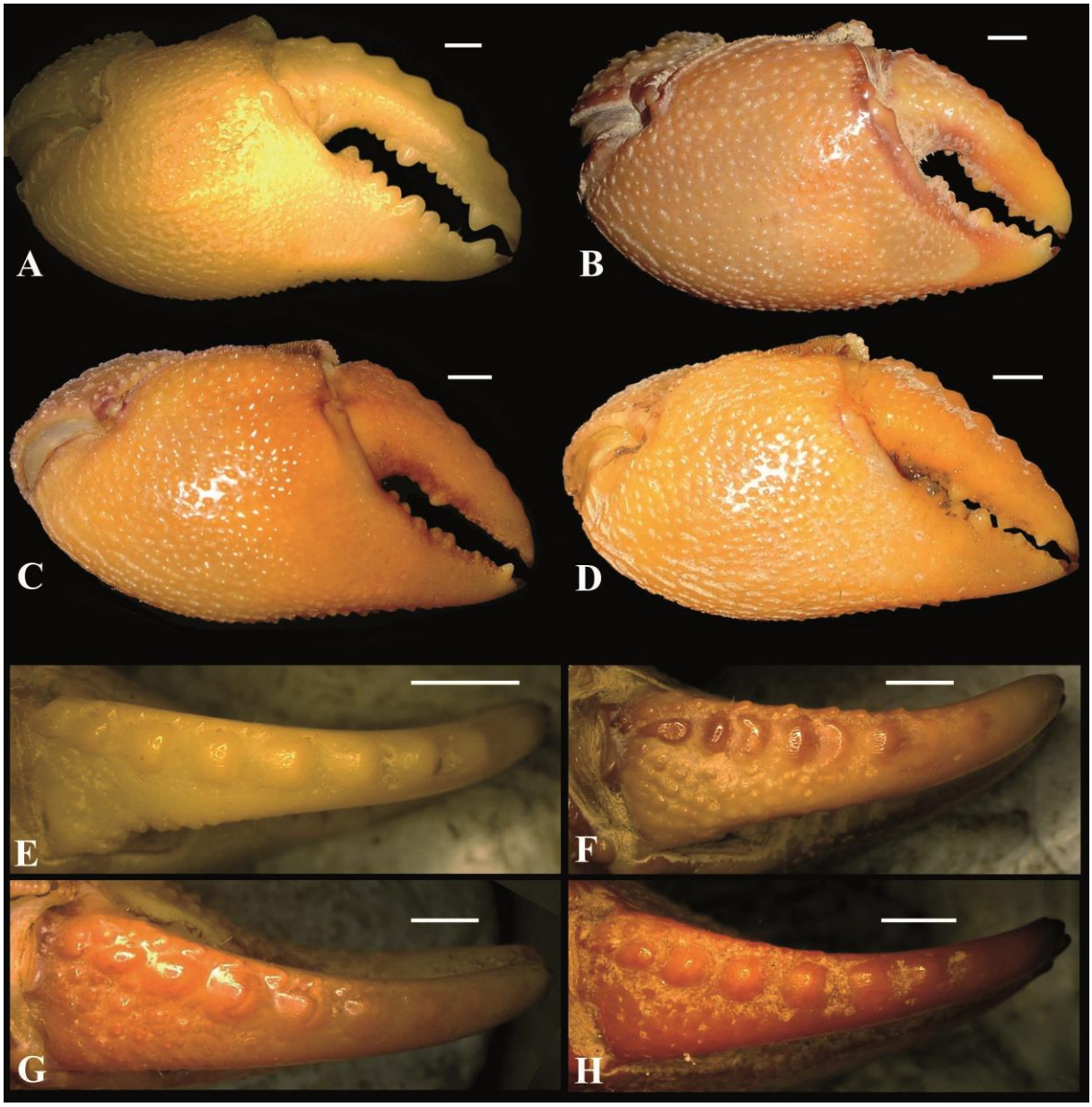

Fig. 5.4. A–D Cheliped outer view. E–H Cheliped Dorsal view. (A & E) *P. longicristatum* (S335). (B & F) *P. lividum* (S5). (C & G) *P. samawati* (S174). (D & H) *Parasesarma* n. sp2. (S302). Scale bars = 1 mm.

Chapter 6

Systematics and phylogeography of the Australasian mangrove crabs *Parasesarma semperi* and *P. longicristatum* (Decapoda: Brachyura: Sesarmidae) based on morphological and molecular data

Submited to the journal "Invertebrate Sysytematics"

Abstract

Parasesarma semperi (Bürger, 1893) was first described from Bohol in the Philippines and is considered to be widely distributed in Southeast Asia. *P. longicristatum* (Campbell, 1967) was originally described as a subspecies of *P. semperi* from Queensland, Australia and later recognized as a full species. In this study, we re-examine specimens of the two species from across their entire geographic range using genetic markers, a morphometric analysis, and traditional morphological characters. Previous taxonomic species diagnoses were found to be unreliable, but morphometric Principle Component Analyses consistently separate the two species, with the length to width ratio of the propodus of the fourth pereiopod being of particular importance. Genetic data corresponding to the mitochondrial genes Cox1, ND1, 16S confirmed a close sister relationship between the two species, forming reciprocally monophyletic groups. Both species have high haplotype diversities and high intraspecific gene flow.

Keywords: Gene flow, morphometrics, insular species, re-description, Thoracotremata.

Introduction

Sesarmid crabs are considered essential to the healthy functioning of mangrove communities through their role in a number of key ecosystem services (Smith et al. 1991; Lee 1998). Species of the newly defined genus *Parasesarma* are the most common, speciose, and ubiquitous of the Indo-Pacific sesarmids, and are widely distributed. There are now 57 described species of this genus, following the recent inclusion of the majority of the species of *Perisesarma* (see Shahdadi and Schubart, 2017). The separation of *Parasesarma* and *Perisesarma* had been based exclusively on the presence or absence of a distinct epibranchial tooth, and although the phenotypic variability of this has long been suspected, it was finally shown to be phylogenetically meaningless by this recent study. *Parasesarma* is also one of the taxonomically more difficult genera, largely because the constituent species are relatively uniform in appearance, with few morphological characters to distinguish them. For this reason, the use of genetic markers has been suggested as an important tool to help resolve taxonomic uncertainties and reveal potentially cryptic species (Shahdadi and Schubart 2015). Ongoing studies involving the taxonomy and phylogeny of *Parasesarma* by the present authors have revealed a particular difficulty in reliably distinguishing between *P. semperi* (Bürger, 1893) and *P. longicristatum* (Campbell, 1967), the former being widespread in Southeast Asia, while the latter, originally described as a subspecies of *P. semperi*, is restricted to Australia (Davie, 2002).

Campbell (1967) stated that *P. semperi longicristatum* differs from typical *P. semperi* by: (l) the presence of 8–9 asymmetrical dactylar tubercles (7–8 symmetrical tubercles in *P. semperi semperi*); (2) less distinct anterolateral margins of the mesogastric region; (3) lower and anteriorly less abruptly marked median postfrontal lobes; (4) the dorsal face of the chelar palm having longer pectinated crests (c. 25 teeth in distal crest, while *P. semperi semperi* has c. 20 teeth). However, Campbell (1967) had only limited comparative material of *P. semperi semperi* from Singapore. Shahdadi and Schubart (2015), after examining more samples from across the distributional range, found that both the number of teeth in the pectinated crests and the number of dactylar tubercles varied more than previously thought, and that the two species had overlapping counts in both characters. They also found few specimens of *P. semperi* with asymmetrical dactylar tubercles. Considering these ambiguities, together with the fact that the distinctiveness of the mesogastric region and the prominence of the frontal lobes can also vary

due to size, age, and/or time since moulting, we considered it necessary to re-examine whether the two nominal species are indeed valid. We did this by using genetic markers, and by searching for the presence of more reliable morphological characters. We further used the genetic analyses to study gene flow, phylogeography, and underlying speciation mechanisms.

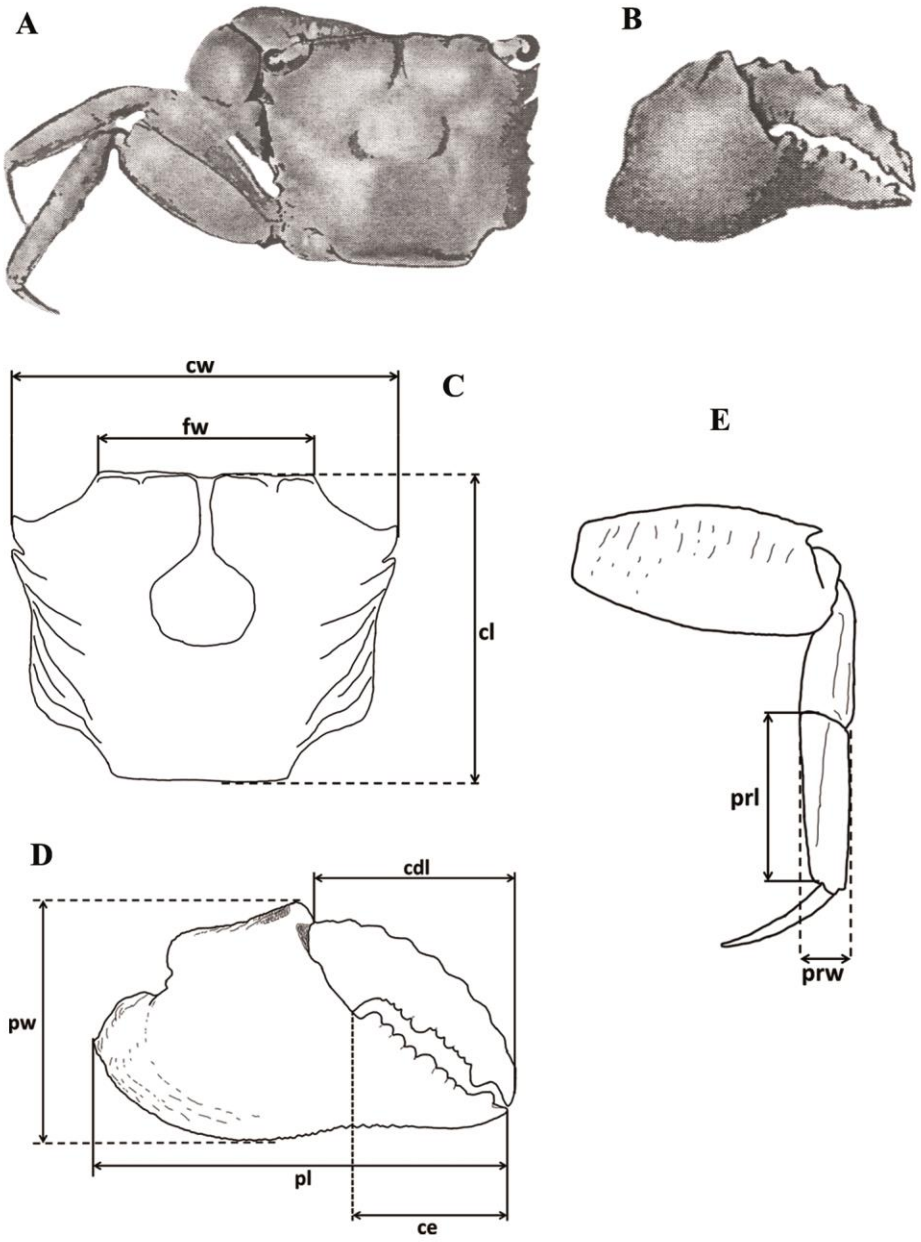

Fig. 6.1 A & B, Original figures of *Parasesarma semperi* from Bürger, 1893. A, whole animal dorsal view; B, right chela; C–E, morphological diagram indicating the

morphometric characters that were used in PCA analyses. C, carapace; D, chela; E, third ambulatory leg. Abbreviations are: cw = Maximum carapace width; cl = maximum carapace length; fw = front width; pl = maximum palm length; pw = maximum palm width; ce = palm cutting edge; cdl = cheliped dactylus length; prl = propodus length of third ambulatory leg; prw = propodus width of third ambulatory leg.

Material and Methods

Material examined

Specimens of the two species were examined from different localities across their geographic range, and are held in the collections of the Australian Museum, Sydney, Australia (AM); the Queensland Museum, Brisbane, Australia (QM); the Forschungsinstitut und Museum Senckenberg, Frankfurt a.M., Germany (SMF); the Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore (ZRC); the Natural History Museum, London (NHM); and the Natural History Museum and Institute Chiba, Japan (CBM). Freshly collected material of *P. semperi* from Irian Jaya (Indonesia) was donated by Dwi Listyo Rahayu.

The holotype and paratype material of *P. longicristatum* in the Queensland and Australian museums was examined. The type material of *P. semperi* was originally deposited in the Göttingen Museum (Germany), but the Göttingen collection has since been transferred to the Senckenberg Museum in Frankfurt. A search of the Senckenberg collection uncovered three damaged male specimens of *P. semperi* from Bohol (The Philippines), labeled as syntypes. In total, 37 specimens from Australia (24 males; 13 females) and 36 specimens from Southeast Asia (24 males; 12 females) were examined (see Table 1).

Because of the apparent overlap in the diagnostic characters, we initially separated and labelled the samples of the two species based on their geographic localities, e.g. all Australian specimens were considered to belong to *P. longicristatum*, while the Southeast Asian specimens were all considered to be *P. semperi*. This split was later supported by our genetic results.

For genetic comparisons, representatives of sympatric and morphologically similar congeneric species were also included (i.e., *Parasesama cricotum* (Rahayu & Davie, 2002); *Parasesarma darwinense* (Campbell, 1967); *Parasesarma lanchesteri* (Tweedie, 1936);

Parasesarma lividum (A. Milne-Edwards, 1869); *Parasesarma messa* (Campbell, 1967)). Specific details of the material examined and localities are presented in Table 1 and Fig. 4. To avoid any confusion when referring to specific specimens, every individual listed in Table 1 has its own code number.

Table 6.1. Material examined in this study (ordered by code and size) with locality, catalogue number, GenBank accession number (Cox1, ND1 and 16S respectively), and chelar characteristics of male *Parasesarma semperi* (Bürger, 1893) and *P. longicristatum* (Campbell, 1967). Size (carapace width× carapace length in mm), sex (M = male, F = female), number of teeth on distal pectinated crest (PC) (left and right chela respectively if different), and number of dactylar tubercles (DT) (left and right chela respectively if different).

Species	Code	Sex & Size	locality	PC	DT	Catalogue number	Accession No.		
							Cox1	ND1	16S
P. longicristatum	I1	M, 19.1× 15.1	Australia, Queensland, Weipa, Andoom Creek	27, 23	8	QM-W16895	MF173018	MF173031	MF173000
	I2	M, 18.5× 14.9	Australia, Queensland , Port Alma	25, 23	9, 8	QM-W2464 Paratype	KY198240	MF173032	KY198245
	I3	M, 17.6× 14.6	Australia, Queensland, Toorbul, Elimbah Creek	19, 21	8	QM-W19924	KX400909	MF173030	KX423803
	I4	M, 17.5× 16.7	Australia, Queensland , Burnett Heads	22	8	QM-W2462			
	I5	M, 17.5× 13.8	Australia, Queensland , Hervey Bay	24	9	QM-W5328			
	I6	M, 17.0× 13.7	Australia, Northern territory, Darbilla Creek	26, 25	7, 8	ZRC-1995.953			
	I7	M, 16.9× 13.7	Australia, Queensland , Cato River	25	8	QM-W6659	MF173012		
	I8	M, 16.9× 13.7	Australia, Northern Territory, Slippery Creek			QM-W6650	MF173006		
	I9	M, 16.4× 13.4	Australia, Northern Territory, Nungbalgarri Creek	22	8	AM-P68357			
	I10	M, 14.7× 11.7	Australia, Northern Territory, Slippery Creek			QM-W6650	MF173016	MF173029	MF172999
	I11	M, 14.5× 11.5	Australia, Northern Territory, Kakadu	24	8	QM-W28687			
	I12	M, 14.3× 12.4	Western Australia, Kimberley, Admiral Island	23, 19	8	QM-W20314			
	I13	M, 14.3× 11.4	Australia, Queensland, Thompson's Point, Rockhampton	26	8	QM-W2461 Paratype			
	I14	M, 13.6× 11.0	Western Australia, Kimberley, Mermaid Island	29, 23	8	QM-W20219	MF173014	MF173033	MF172996
	I15	M, 13.4× 10.3	Australia, Northern Territory, Arrla Creek			AM-P68302	MF173005		

I16	M, 12.9× 10.5	Australia, Northern Territory, Arrla Creek	18, 20	7	AM-P68302	MF173013		
I17	M, 12.8× 11.1	Western Australia, Kimberley, Admiral Island	23	9	QM-W20314	MF173002		
I18	M, 12.8× 10.2	Australia, Queensland, Laradeenya Creek	20	8	QM-W16718			
I19	M, 12.6× 10.8	Australia, Queensland, Burnett Heads			QM-W2462			
I20	M, 12.6× 10.3	Australia, Northern Territory, Arrla Creek	22	7	AM-P68302			
I21	M, 12.6× 9.9	Australia, Northern Territory, Andranangoo Creek	24	8	AM-P.68407	MF173008	MF173034	MF172998
I22	M, 12.3× 10.0	Australia, Queensland, Boyne River, Gladstone	20	8	AM-P15346 Paratype			
I23	M, 11.1× 8.7	Australia, Northern Territory, Andranangoo Creek	21	8	AM-P68407			
I24	M, 10.7× 8.3	Western Australia, Kimberley, Myrmidon Ledge			QM-W20989			
I25	F, 20.8× 16.9	Australia, Queensland, Moreton Bay			QM-W19924	KX400908		KX423799
I26	F, 16.4× 13.1	Australia, Northern Territory, Darbilla Creek			ZRC1995.953	MF173003		
I27	F, 16.4× 13.0	Australia, Queensland, Hervey Bay			QM-W5328			
I28	F, 15.6× 12.6	Australia, Northern Territory, Andranangoo Creek			AM-P68407	MF173009		
I29	F, 15.5× 12.5	Australia, Northern Territory, Nungbalgarri Creek			AM-P68357			
I30	F, 15.5× 12.1	Australia, Northern Territory, King River			AM-P.68355	MF173007	MF173035	MF172997
I31	F, 15.2× 11.1	Western Australia, Kimberly, Myrmidon Ledge			QM-W20989			
I32	F, 14.3× 11.6	Australia, Queensland, Murray River			QM-W9010	MF173011		
I33	F, 14.3× 11.6	Australia, Queensland , Port Alma			QM-W2464 Paratype	MF173004		
I34	F, 13.3× 10.6	Australia, Queensland, Thompsons Point, Rockhampton			QM-W2461 Paratype	MF173010		
I35	F, 13.3× 11.0	Australia, Queensland, Andoom Creek			QM-W16895	MF173017		

	I36	F, 12.2× 10.0	Australia, Queensland , Burnett Heads			QM-W2462			
	I37	F, 9.0× 7.5	Australia, Northern Territory, Arrla Creek			AM-P68302	MF173015		
P. semperi	I38	M, 17.8× 14.8	Philippines, Bohol	17	7	ZMG 1560 Paralectotype	MF173019		
	I39	M, 17.1× 13.9	Philippines, Bohol	20	7	ZMG 625 Lectotype	MF173021		
	I40	M, 16.2× 13.0	Indonesia, Borneo, South Kalimantan, Pulau Sebuku,	17	8	QM-W23454	MF173023	MF173040	MF172993
	I41	M, 16.0× 13.7	New Guinea, Kamora	20	8	ZRC2003.0485			
	I42	M, 15.2× 12.4	New Guinea, Kamora	26	8	ZRC 2000.1891	MF173020		
	I43	M, 14.8× 12.7	New Guinea, Tipoeka	13	8	ZRC2016.0524	MF173027		
	I44	M, 14.5× 11.8	New Guinea, Tipoeka	12, 14	8	ZRC2016.0524	MF173022		
	I45	M, 13.9× 11.2	Malaysia, Labuan	20	8	NHM.1951.2.15.14	MF173025		
	I46	M, 13.1× 10.5	New Guinea, Ajkwa	27, 25	8, 7	SMF 49929			
	I47	M, 13.0× 10.9	New Guinea, Kamora	22	8	ZRC2003.0485			
	I48	M, 12.2× 9.6	New Guinea, Ajkwa	22, 23	7, 8	SMF 49928	MF173024	MF173039	MF172994
	I49	M, 12.1× 9.9	Malaysia, Labuan	16	7	ZRC1969.9.8.4			
	I50	M, 11.7× 9.6	New Guinea, Ajkwa	17, 21	8	QM-W27532	MF173026	MF173038	MF172995
	I51	M, 11.3× 8.8	New Guinea, Kamora	19	7	ZRC2003.0485			
	I52	M, 11.2× 9.7	New Guinea, Ajkwa	19	8	QM-W27532			
	I53	M, 11.2× 9.2	New Guinea, Kamora	18	7	ZRC2003.0485			
	I54	M, 10.6× 8.4	Malaysia, Labuan	16	7	ZRC1969.9.8.4			
	I55	M, 10.4× 8.6	New Guinea, Kamora	20	7	ZRC2003.0485			
	I56	M, 10.1× 8.0	New Guinea, Kamora	23	7	ZRC2003.0485			
	I57	M, 9.9× 8.3	New Guinea, Kamora	22	8	ZRC2003.0485			
	I58	M, 9.7× 7.7	New Guinea, Kamora	17	7	ZRC2003.0485			
	I59	M, 9.7× 7.6	New Guinea, Kamora	19	7	ZRC2003.0485			
	I60	M, 8.8× 7.3	New Guinea, Kamora	23	8	ZRC2003.0485			
	I61	M, soft carapace	Philippines, Bohol	8	8	ZMG1560 Paralectotype			
	I62	F, 16.3× 13.5	New Guinea, Kamora			ZRC2003.0485			
	I63	F, 14.8× 11.8	New Guinea, Kamora			ZRC2003.0485			
	I64	F, 14.× 11.6	New Guinea, Kamora			ZRC2003.0485			
	I65	F, 14.4× 11.7	New Guinea, Tipoeka			ZRC2016.0524	KX400911	MF173037	KX423805
	I66	F, 13.7× 10.8	Indonesia, Borneo, South Kalimantan, Pulau Sebuku			QM-W23454			

	I67	F, 13.7× 11.4	New Guinea, Tipoeka	ZRC2016.0524	KX400910	MF173036	KX423804
	I68	F, 13.5× 11.7	New Guinea, Kamora	ZRC2003.0485			
	I69	F, 13.3× 10.6	New Guinea, Kamora	ZRC2003.0485			
	I70	F, 12.6× 10.8	New Guinea, Ajkwa	SMF 49928			
	I71	F, 12.0× 10.0	New Guinea, Ajkwa	QM-W27532			
	I72	F, 11.4× 10.6	New Guinea, Ajkwa	SMF 49928			
	I73	F, 9.1× 7.5	New Guinea, Kamora	ZRC2003.0485			
Parasesarma sp.	I74	M, 27.6× 22.9	Japan, Iriomote Island	CBM ZC7192	MF173028	MF173045	MF173001
P. cricotum Rahayu & Davie, 2002	I77	F, 14.3× 12.2	New Guinea, Kamora	ZRC2016.0522	KX400897	MF173042	KX423796
P. darwinense (Campbell, 1967)	I78	M, 12.2× 10.2	Australia, Northern Territory, Darwin	SMF 49921	KX400904	MF173043	KX423798
P. lanchesteri (Tweedie, 1936)	I82	M, 19.9× 15.9	Locality unknown	SMF 7142			
P. lanchesteri (Tweedie, 1936)	I83	F, 24.8× 18.8	Singapore, Simpang Mak Wai River	ZRC1967.11.8.3	KX761168	MF173046	KX761174
P. lividum (A. Milne-Edwards, 1869)	I84	M, 17.8× 15.2	New Caledonia	QM-W24243	KX400893	MF173044	KX423802
P. messa (Campbell, 1967)	I85	M, 19.4× 16.7	Australia, Queensland, Brisbane, Cleveland	ZRC1999.0650	KX431205	MF173041	KX423795

Molecular analyses

Genomic DNA was isolated using a modified Puregene method (Gentra Systems, Minneapolis) or Mollusc DNA kit (Omega D3373–02) from muscular leg tissue using the manufacturers' protocols. In the cases of very old animals or those preserved in formalin, the muscle tissues were soaked in GTE buffer overnight prior to the DNA extraction process (according to Shedlock *et al*. 1997).

Three mitochondrial genes were partially amplified using different primer combinations: the protein-coding genes cytochrome oxidase subunit 1 (Cox1) and NADH dehydrogenase 1 (ND1), and the gene encoding the rRNA of the large ribosomal subunit (16S) (Table 2). The primer combination COL6/COH6 was used to amplify a segment of 709 basepairs (bp) of Cox1 (658 without primers). For older specimens, two shorter and partly overlapping fragments (about 350 to 400 bp) allowed amplifying the same segment using the primer combinations COL6/COH7P and COL7P/COH6. In case of the 16S gene, the longest primer combination was 16L29/16H11 (amplifying a segment of approximately 584 bp depending on the indels), but in cases with degraded DNA, shorter fragments were amplified using the combination 16L29/16H37 (approx. 550 bp). To amplify 478 bp (including primer regions) of the ND1 gene, the primer combination NDL5/NDH8 was used (Table 2). Polymerase chain reactions (PCR) were carried out with the following profile: initial step 4 min at 94°C; 40 cycles with 45s at 95°C for denaturing; 60s at 48°C for annealing, 60s at 72°C for extension; and 5 min at 72°C for final extension. PCR products were outsourced for sequencing to Macrogen Europe. For the Cox1 gene, 11 sequences were obtained from *P. semperi* and 17 from *P. longicristatum*, including two of the type specimens of each species (Table 1). Sequences of the two other mitochondrial genes (ND1 and 16S) were obtained for a subset of specimens (Table 1).

Sequences were proofread using Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia), primer regions were removed and the remaining sequences aligned automatically with ClustalW (Thompson *et al*. 1994) implemented in BioEdit 7.0.5 (Hall 1999). Four alignments were constructed, three with individual genes and one with the concatenated dataset of the three genes. The alignments were converted with FaBox (Villesen 2007) to nexus files. The files of the individual genes were then used to construct statistical parsimony networks with the algorithm outlined in Tempelton *et al.* (1992) and the software Popart (http://popart.otago.ac.nz). The best evolutionary model describing our data was determined with the aid of jModelTest (v. 2.1.4)

(Darriba *et al*. 2012) and selected with the Akaike information criterion (AIC) (Posada and Buckley 2004). According to their phylogenetic position determined in Shahdadi and Schubart (2017), *Parasesarma lanchesteri* was selected as outgroup for tree construction from the concatenated dataset. Two methods of phylogenetic inference were applied to our tree constructions: Maximum Likelihood (ML) using the software raxmlGUI (v. 1.3) (Silvestro and Michalak 2012) and Bayesian Inference (BI) as implemented in MrBayes (v. 3.2) (Huelsenbeck and Ronquist 2001). ML trees were obtained with 2000 bootstrap pseudoreplicates. For the MrBayes runs, we used 2 million generations with 4 chains and a sample frequency of 1,000 generations. Sequences were submitted to NCBI and are available from GenBank under the accession numbers given in Table 1.

Standard genetic indices were calculated to determine the genetic diversity within the two species based on Cox1 sequences. The haplotype diversity (*Hd*), nucleotide diversity (π), number of polymorphic sites (*S*), and number of haplotypes (*k*) were calculated with DnaSP 5.1 (Librado and Rozas 2009).

Morphological analyses

Preserved material was examined and described using a stereo microscope (Leica S4E) and a digital microscope (Keyence VHX500F). Photographs were taken with either a digital camera (Sony Corp. DSC–WX300) or the digital microscope. Male first gonopods (G1) were carefully removed at their base, dried, and setae removed with a small blade. Measurements of carapace width (cw) and carapace length (cl) were made using a digital caliper to an accuracy of 0.1 mm.

Male specimens with a fully formed, mature G1 were assumed to be adult, and only these were used for the primary morphological statistical comparisons. A Principal Component Analysis (PCA) was undertaken to compare *Parasesarma semperi* and *P. longiristatum*, using seven morphometric ratios (see Fig. 1C–E): 1, maximum carapace width (between the exorbital angles) to maximum carapace length (cw/cl); 2, front width to carapace width (fw/cw); 3, maximum palm length to carapace width (pl/cw)4, maximum palm width to palm length (pw/pl); 5, palm cutting edge to palm length (ce/pl); 6, cheliped dactylus length to palm length (cdl/pl); 7, the ratio of length to width of propodus of the third ambulatory leg (prl/prw). The measurements were obtained using the program tpsDig 2.10 (Rohlf 2006) from digital photographs of the

carapaces, chelipeds and legs. Because of missing appendages, only 19 specimens of *P. semperi* and 17 of *P. longicristatum* were included in this analysis (5 missing for prl/prw).

The two species were then compared with a Man-Whitney U-Test based on the morphometric ratios that had proved most useful for species discrimination (1st component of the PCA), with the number of teeth of the distal pectinated crests (dpc) added as a meristic character.

To examine sexual dimorphism in *P. semperi*, three morphometric characters (i.e., cw/cl, pl/cw and pl/pw) were compared between adult males and adult females (with broad, well formed pleon, and well developed pleopods) again using the Man-Whitney U-Test (considered significant at $P \leq 0.05$). Data analyses and graphs were performed using IBM SPSS Statistic 21.

Table 6.2. Primers used in present study with corresponding's DNA sequences (5′-3′) and the corresponding references.

Gene	Primer	Sequence	Reference
COI	COL6	TYTCHACAAAYCATAAAGAYATYGG	Schubart 2009
	COH7P	GRAGAGAAAAAATACCTA	New
	COL7P	GGTGTKGGMACMGGATGAACTGT	New
	COH6	TADACTTCDGGRTGDCCAAARAAYCA	Schubart & Huber, 2006
16S rRNA	16L29	YGCCTGTTTATCAAAAACAT	Schubart , 2009
	16H37	CCGGTYTGAACTCAAATCATGT	Klaus et al. 2006
	16H11	AGATAGAAACCRACCTGG	Schubart 2009
ND1	NDL5	TTGCTGGWTGRTCTTCWAATTG	New
	NDH8	AYCTTTTYCAWGCTAAATA	New

Results

Genetics

The cropped alignments of Cox1, ND1, 16S, and the concatenated file, consist of 645, 444, 532 and 1619 bp respectively, after removal of the primer sequence and adjacent regions. The best evolutionary model obtained with jModelTest (v. 2.1.4) (Guindon and Gascuel 2003; Darriba *et al*. 2012) was the General Time reversible plus Gamma (GTR+G, Rodriguez et al.,

1990). Two phylogenetic trees (ML and BI) from the Cox1 sequences, two from the concatenated file, and three haplotype networks from individual genes were obtained (Figs 2, 3).

Our genetic results show that *P. semperi* and *P. longicristatum* cluster closely together, but are still separable along geographic lines (Fig 2A). Both are widely separated from other similar species of *Parasesarma* that could potentially occur sympatrically (Fig. 2B). Nevertheless, they represent reciprocally monophyletic taxa with high support values (Fig. 2A). This is similarly reflected in the two distinct groups evident from the haplotype networks (Fig. 3). The Australian east coast specimens from the Pacific Ocean (including paratypes of *P. longicristatum* from central Queensland) cluster with the Indian Ocean specimens from the Northern Territory and northern Western Australia, in a single clade (Figs 2, 3, 4). Alternately, all Southeast Asian specimens analyzed from Malaysia (Labuan) to New Guinea, including paratypes of *P. semperi* from Bohol in the Philippines, cluster tightly together forming a sister group to the Australian *P. longicristatum* (Figs 2, 3, 4). The specimen from Iriomote Island, Japan (CBM ZC7192) that was thought to belong to the *P. semperi*, is genetically much closer to *P. lividum* as shown by the phylogenetic trees (Fig. 2B), and therefore has not been included as part of the network (Fig. 3) and will be treated elsewhere.

The standard indices show that the genetic diversities in both species are high (i.e., in *P. longicristatum*: *Hd*=0.867; π=0.00551; *S*=12; *k*=9; in *P. semperi*: *Hd*=0.800; π=0.00355; *S*=7; *k*=6).

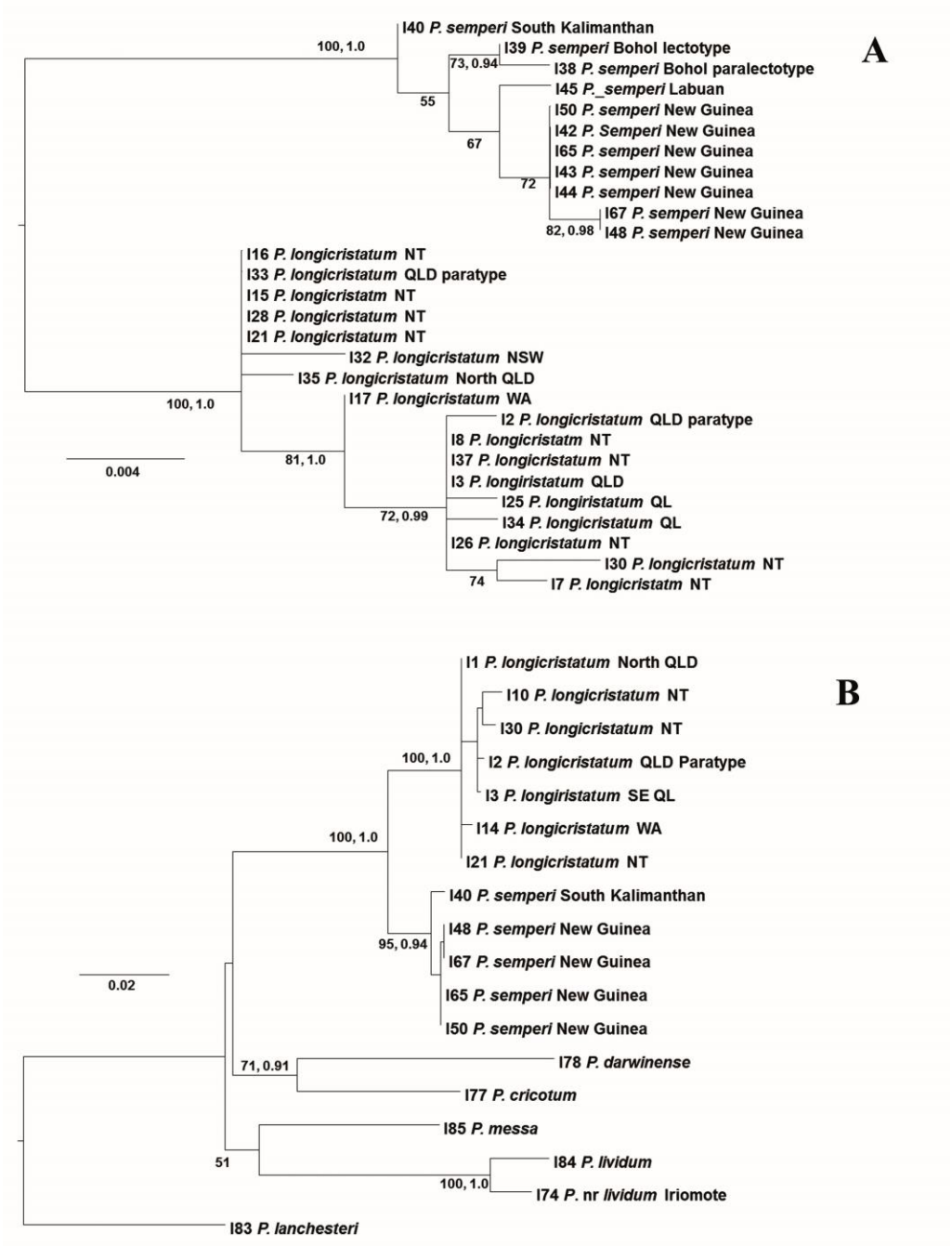

Fig. 6.2. Phylogenetic consensus trees constructed with Maximum Likelihood (ML) and Bayesian Inference (BI) of A, Unrooted Cox1 tree from *Parasesarma semperi* and *P. longicristatum;* B, the concatenated dataset (Cox1, ND1 and 16S genes) from selected species of *Parasesarma,* focusing on *P. semperi* and *P. longicristatum*. *P. lanchesteri* was used as outgroup. Only support values higher than 50% and 90% respectively for ML and BI are shown in the trees.

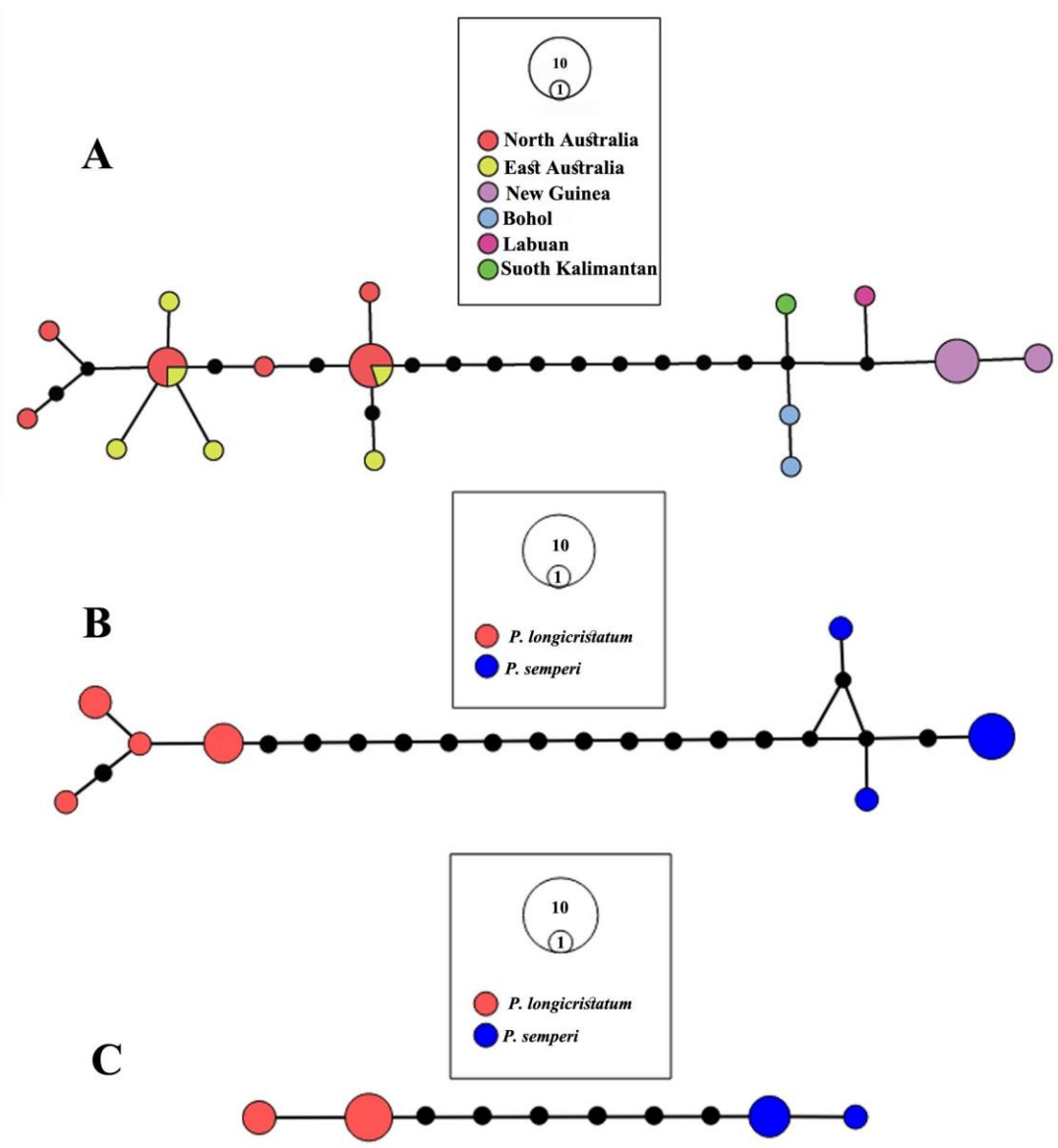

Fig. 6.3. Maximum parsimony haplotype network for specimens of *Parasesarma semperi* and *P. longicristatum*, constructed via Popart. A, Cox1; B, ND1; C, 16S.

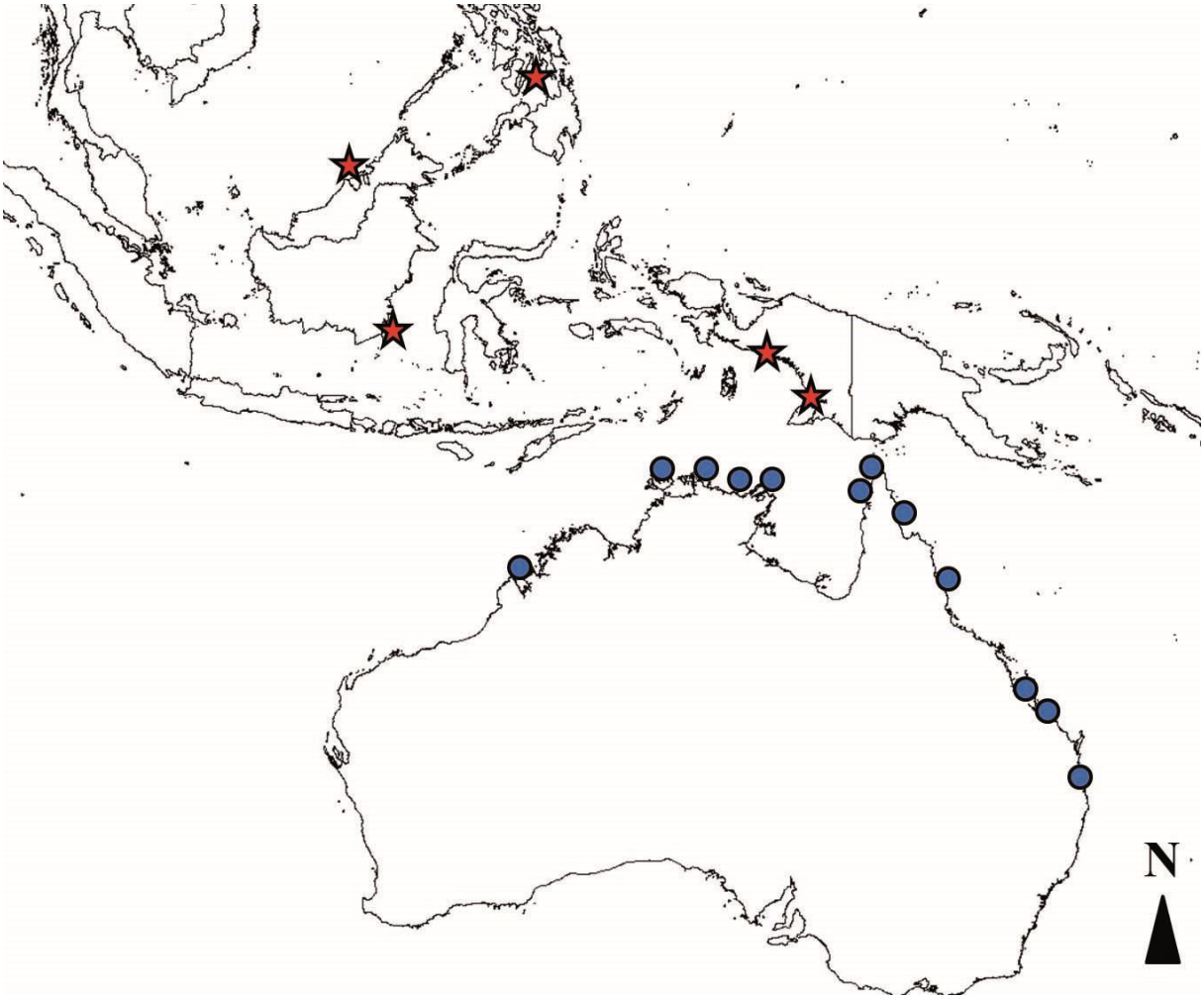

Fig. 6.4. Map of the localities of specimens examined in the present study. Stars: *Parasesarma semperi*, circles: *P. longicristatum*.

Morphology

Normally, *Parasesarma longicristatum* and *P. semperi* both have 7–8 chela dactylar tubercles (Table 1). *P. longicristatum* typically has asymmetrical tubercles, but in a few samples the 3–4 median tubercles are symmetrical (Fig. 11). In contrast, *P. semperi* always has symmetrical median tubercles, but the proximal and the distal ones can be asymmetrical (Fig. 10) (see descriptions for more detail).

The number of teeth on the distal pectinated crest on the dorsal surface of the chelar palm show overlapping counts between the two species (Table 1), but there is nevertheless, a statistically significant difference (Man-Whitney U =163.500, *P. longicristatum* n=20, *P. semperi* n=24, $p < 0.001$ two-tailed) (Fig. 8A).

Our comparison of carapace morphologies show that individuals of both species present slight differences in the prominence of their mesogastric region, and also in the prominence of the frontal lobes, but no clear and consistent difference was detected at the species level (Fig. 5). The G1 morphology is very similar for the two species, but the apical corneous tip of *P. longicristatum* is more rounded than in *P. semperi*, which is more pointed (Fig. 6).

The PCA analysis (*P. semperi* N=19; *P. longicristatum* N=17) based on the seven morphometric characters, indicated the presence of two distinct groups (Fig. 7). The ratio of the length versus width of the third ambulatory legs (prl/prw) was the best single character to discriminate the two species (Man-Whitney U = 0.00, *n P. longicristatum* = 17, *n P. semperi* = 14, $p < 0.001$ two-tailed) (Fig. 8B).

Morphometric comparisons within *P. semperi* showed that males and females differ significantly in chelae characters (i.e. pl/cw: Man-Whitney U =14.500, *n male* = 19, *n female* = 10, $p < 0.001$ two-tailed; pl/pw: Man-Whitney U =20.500, *n male* = 19, *n female* = 10, $p = 0.001$ two-tailed), but not in carapace shape (cw/cl).

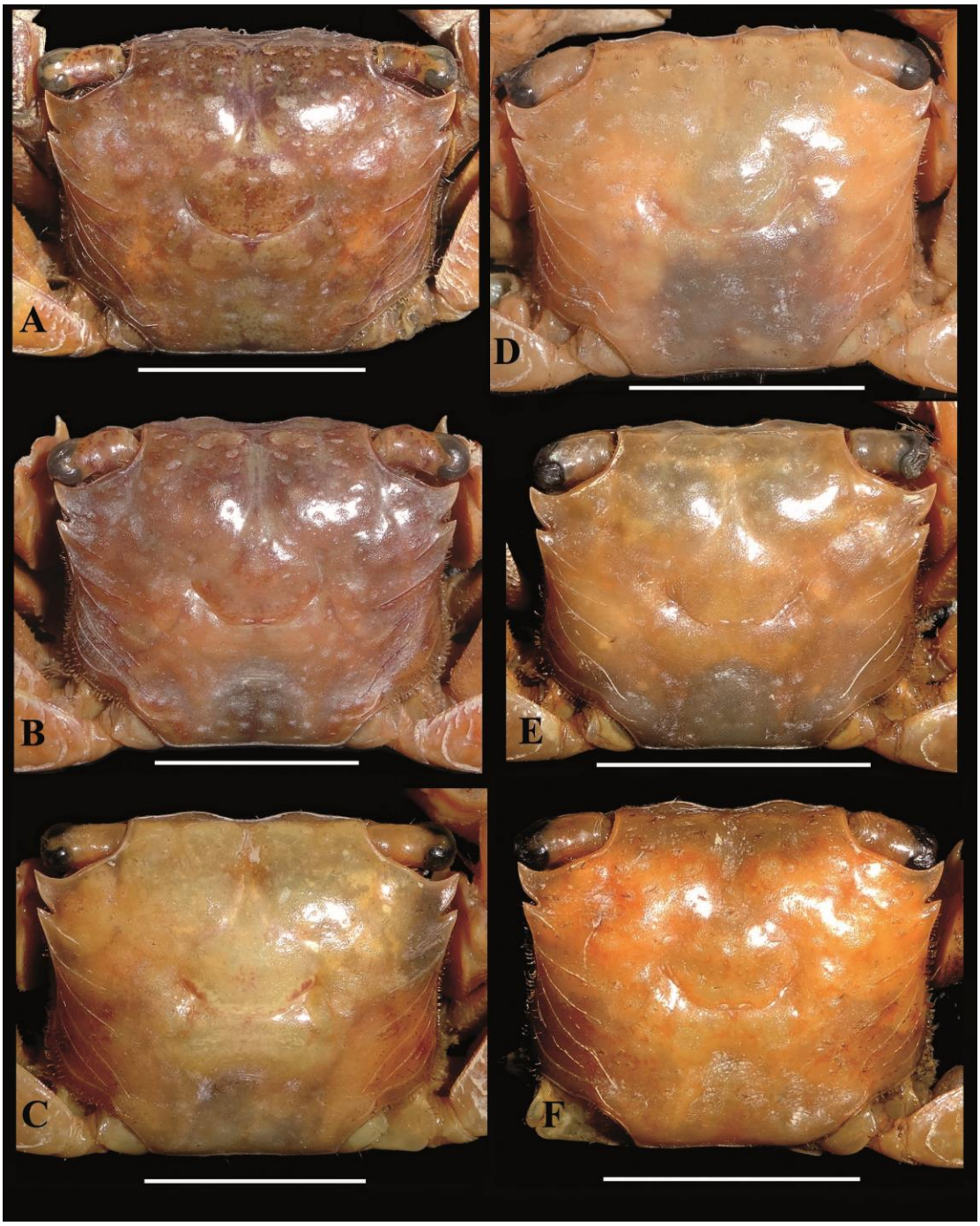

Fig. 6.5. Comparison of carapace morphology, dorsal view in A–C. *Parasesarma longicristatum*, D–F. *P. semperi*. A, female from Queensland, Australia (QM, W19924) (I25); B, male from Queensland, Australia (QM, W19924) (I3); C, male from the Northern Territory, Australia, (ZRC1995.953) (I6); D, male from Tipoeka, New Guinea (ZRC2016.0524) (I44); E, male from Tipoeka, New Guinea (ZRC2016.0524) (I43); F, male from Ajkwa, New Guinea, (SMF 49929) (I46). Scale bar = 1cm.

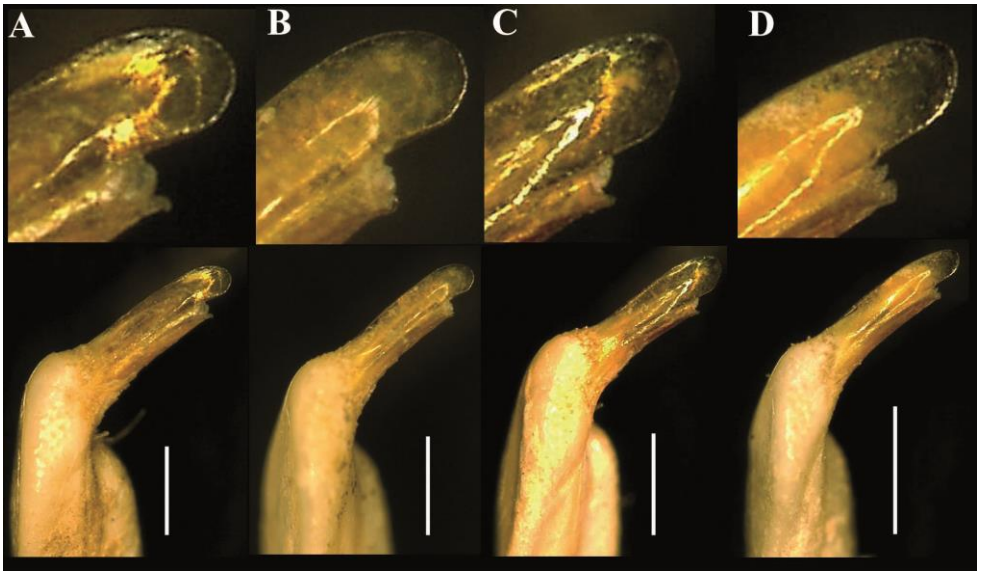

Fig. 6.6. The apical part of right G1: A, *Parasesarma longicristatum* from Queensland, Australia (QM-W19924) (I3); B, *P. longicristatum* paratype from Queensland Australia (QM-W2464) (I2); C, *P. semperi* lectotype from Bohol Philippines (ZMG 625) (I38); D, *P. semperi* from Ajkwa, New Guinea (SMF 49928) (I48). Scale bar 1mm.

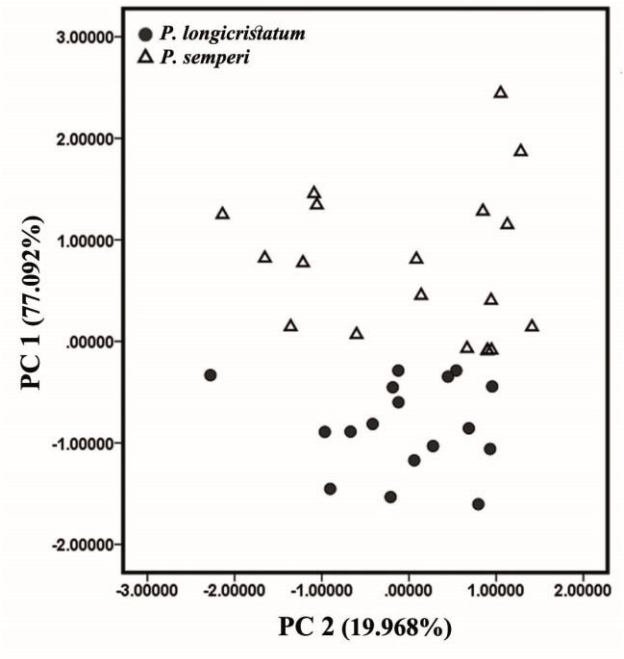

Fig. 6.7. Plot of principal components analysis (PCA) of the two species *Parasesarma semperi* and *P. longicristatum*. The first PC explains 77.092% of the variation; the second PC 19.968% of the variation.

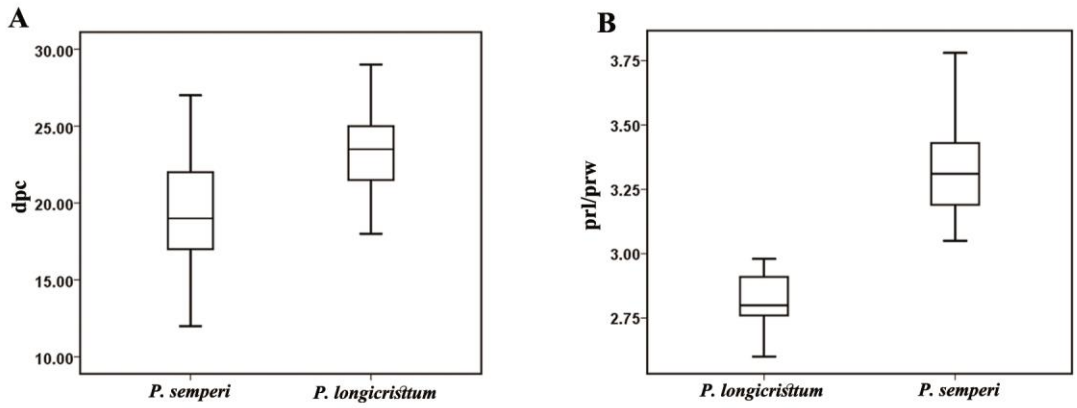

Fig. 6.8. Boxplots of morphological comparisons between *Parasesarma semperi* and *P. longicristatum*. A, the number of teeth on the distal pectinated crests (dpc); B, ratios between length to width of propodus of third ambulatory leg (prl/prw).

Discussion

Parasesarma semperi and *P. longicristatum* are morphologically very similar. Character states that had previously been considered diagnostic, e.g., the number of dactylar tubercles, and number of teeth on the distal pectinated crest (Campbell 1967; Davie 2010; see Table 1, Fig. 8A), are here shown to overlap. The finding of symmetrical dactylar tubercles in some specimens of *P. longicristatum* (Fig. 11) also casts doubt on the reliability of this character. Differences in the morphology of the dorsal carapace regions, also proposed as helpful in distinguishing the two species, were also inconsistent (Fig. 5). The only character to show small but consistent differences among the examined material, is the shape of the apical portion of the G1, with *P. longicristatum* having a more rounded corneous tip (Fig. 6).

Despite the lack of marked and consistent differences between the species, a Principle Component Analysis (PCA) based on morphometric characters consistently separates individuals of the two species (Fig. 7). The PCA showed that the most important character was the ratio of length to width of the propodus of the third ambulatory leg (fourth pereiopods), with specimens of *P. semperi* having slightly longer propodi than those of *P. longicristatum* (see Results, Fig. 8B). The pairwise comparison also showed that, even if the distal pectinated crest of the two species have overlapping tooth counts, *P. longicristatum* does indeed, on average, have significantly more teeth (and thus longer pectinated crests), than *P. semperi* (see Results, Fig. 8A), a fact that reinforces the usefulness of the specific name given by Campbell (1967).

A morphometric comparison of male and female *P. semperi* shows that the male chelipeds are relatively larger (the ratio of palm to carapace width), and more robust (the ratio of palm width to length) compared to females (see Results). Such sexual dimorphism results from increased allometric growth of the male chelae after adulthood (see also Flores et al., 2002). There is no obvious sexual dimorphism in carapace width (see Results), as has been shown in some grapsid crabs (Flores and Negreiros-Fransozo 1999).

While our genetic analysis shows that *P. semperi* and *P. longicristatum* represent reciprocally monophyletic clades, it also shows that they are sister taxa compared to other species of *Parasesarma* (Figs 2, 3). Such a close phylogenetic relationship with low genetic divergence, together with very similar morphologies, suggests a recent origin of the two lineages. The detected morphometric divergence, on its own, could be attributable to phenotypic plasticity as a response to possible environmental differences (see Smith 2004; Silva *et al*. 2010; Schubart et al. 2010). However the habitats of the two species seem outwardly similar, and the genetic analysis indicates that a fundamental genetic difference is more likely to be the cause.

A full understanding of population genetics and gene flow for each species would require more sampling sites and larger sample sizes, but the haplotype networks presented here (Fig. 3) show that, even though the haplotype diversity in *P. longicristatum* is high, the Australian Pacific and Indian Ocean populations are not separated (Fig. 4) and connected by gene flow. Davie (1985) recorded 55 species of intertidal crab species endemic to Australia. Of these 45% (25) are restricted to eastern Australia, 34% (19) to north-western Australia, and 21% (12) have a broader tropical/subtropical distribution. Davie (1985) pointed out that although the eastern Pacific coast has clearly had a lengthy period of isolation to allow such a large endemic fauna to evolve, at least 12 species have nevertheless been able to establish a distributional interchange between the distinctive eastern and western faunas. Our knowledge of the Australian crab fauna is now much improved (e.g., Davie 2002), but the underlying tenets of these intertidal distribution patterns remain the same. Davie (1985) believed that *Parasesarma semperi semperi* was the northwestern form of the species and had invaded southwards from SE Asia, whereas *P. semperi longicristatum* occurred only along the eastern coast. At that time, he had not had a chance to examine "true" *P. semperi*, so that this interpretation can now be corrected, demonstrating that *P. longicristatum* occurs all across tropical Australia. This distribution is

consistent with mangrove plant connectivity throughout the geographic range of *P. longicristatum* (Giri *et al*. 2011).

The patterns of tidal and current flow through the Torres Strait are complex (see Wolanski *et al.* 2013, fig. 9), but models developed by Wolanski *et al.* (2013) show that a prevalence of highly energetic tidal flows around shoals, reefs, islands and reef passages ensure a small net westward flow. Thus, far-northern tropical east coast crab species could be swept via larvae or adults into the Gulf of Carpentaria and further westwards onto Australia's northern coasts. The larval stages and life cycle of other species of *Parasesarma* (see Guerao *et al*. 2004) can easily enable such long distance dispersal. While oceanic currents are clearly important in promoting long distance gene flow of littoral animals via larval dispersal, current systems have also been shown to act as barriers for *Parasesarma* populations. For example, Silva *et al*. (2010) showed that southern and northern populations of *P. guttatum* have a genetic disjunction, and they attributed this to complex differences in hydrographic systems between the north and south of the island.

Parasesarma semperi also shows high haplotype diversity (see genetic indices in Results), throughout Southeast Asia (Fig. 3), but interestingly can occur in areas closely adjacent to *P. longicristatum*. In particular, *P. semperi* occurs along the southeastern coast of New Guinea on the northern side of the Arafura Sea, while *P. longicristatum* occurs along the northern Australian coastline in the southern Arafura Sea (Fig. 4). Nevertheless, the genetic gap between the two species remains distinct. It seems a paradox that these two closely related sister species should have such an abruptly disjunct distribution when they both clearly have high dispersal abilities. Davie (1985) discussed the biogeography of mangrove crabs in northern Australia with a range of factors that could have led to present day distributions, and concluded that no single explanation can be invoked to explain the variety of different patterns and relationships. Nevertheless, the one thing that is clear, is that the paleogeography of the Australian continent has led to a large number of endemic marine species, some of which have restricted distributions, and some that are more wide-ranging. Historical and physical factors have also been recognized as contributing to the phylogeographic structure of coastal marine animals in other parts of the world (e.g., Dawson 2001).

Ragionieri *et al*. (2009, 2012) reported similar phylogeographic separation to that reported here, within the *Neosarmatium meinerti* (De Man 1887) species-complex. They separated *N.*

australiense for the north Australian populations, and *N. asiaticum* for those of Southeast Asia, based on both genetic sequence differences and morphology.

Conclusion

The present study confirms that *Parasesarma semperi* and *P. longicristatum* are phylogenetic sister species, in accordance with their previously recognized morphological similarity (Campbell 1967). Our morphological analyses and comparisons (morphometric PCA, pectinated crest and G1 morphology) concur with our genetic results and confirm the existence of two distinct phylogeographic lineages (each monophyletic) that we continue to consider full species following recent taxonomic interpretations (Davie 2002: 224), viz., the Australian *P. longicristatum* and the Southeast Asian *P. semperi*.

Taxonomy

Parasesarma semperi (Bürger 1893)
(Figs 1, 5A–C, 6C, D, 9, 10)

Sesarma semperi Bürger 1893: 630, pl. 21, fig. 1; Tweedie 1950: 342, fig. 1e.
Sesarma (*Perisesarma*) *semperi*: De Man 1902: 542.
Sesarma (*Chiromantes*) *semperi*: Tesch 1917: 198.
Sesarma (*Chiromantes*) *semperi semperi*: Campbell 1967: 4 (in key).
Chiromantes semperi: Tan & Ng 1994: 82 (in list).
Perisesarma semperi semperi: Nakasone & Irei, 2003: 272 (key), 275.
Perisesarma semperi: Rahayu & Davie 2002: 605 ; Ng *et al.* 2008: 222 (in list); Rahayu & Setyadi 2009: 55 (with colour picture); Davie, 2010: 204 (in key); Shahdadi & Schubart, 2015: 1085 (in Table 1).
Parasesarma semperi: Shahdadi & Schubart, 2017: XX.
Not *Perisesarma semperi*: Komai *et al.*, 2004: 48-52, figs. 1E, 6, 7A, C (see Remarks).

Material examined

See Table 1. One male syntype from Bohol, Philippines, was selected by Shahdadi and Schubart (2017) as the lectotype (ZMG 625, cw/cl=17.10/13.90, Code No. 139 in Table 1); the other two examined male syntype (ZMG 1560) is a thus a paralectotype (see Table 1).

Redescription

A moderate-sized *Parasesarma* (maximum cw in the present study 17. 8 mm) (Table 1). Carapace quadrate, slightly broader than long (cw/cl=1.23±0.04, N=23) (Table 1). Front 0.56±0.02 (N=19) times carapace width, deflexed, with median emargination; post-frontal lobes low but distinct, median lobes broader than lateral, separated by shallow furrow. Dorsal carapace regions distinguishable but not well marked. Anterolateral margin with sharp exorbital angle; epibranchial tooth well-developed, pointed anterolaterally (Figs 1A, 5A-C, 9A, B).

Chelipeds equal to subequal, large (pl/cw= 0.68±0.09, N=19); ratio of pl/pw 1.84±0.16 (N=19); cutting edge 0.44±0.02 (N=19) times pl. Upper surface of palm with two transverse pectinated crests, distal crest bearing 12–27 tall teeth (19.29±3.58, N=24) (Table 1; Fig 10B, D, E). Dactylus 0.66±0.01 (N=19) times pl; dorsal surface bearing 7–8 distinct, large, well-spaced, dome-shaped tubercles (Table 1); first and second proximal tubercles (in a few specimens also third tubercle) and last three distal tubercles usually asymmetrical; medial tubercles 3–5 (proximal to distal) symmetrical (Fig. 10A–C).

Ambulatory legs relatively short, third pair longest (1.80 time carapace width in lectotype), propodus of third ambulatory leg 3.34±0.22 (N=16) times longer than wide (Fig. 9A).

Male pleon (Fig. 9C): telson 1.11 times wider than long (in lectotype), slightly longer than 6th somite; 6th somite 2.09 times wider than long (in lectotype), 5th and 4th somites trapezoidal, 3rd somite broadest; medial length of 2nd somite equal to, or slightly longer than, length at lateral edges. G1 long, apical corneous process long, bent at about 54° to vertical axis; tip rounded, aperture subterminal (Figs 10F, 6C, D).

Females with smaller chelipeds (pl/cw= 0.54±0.02, N=10); but chelae more elongated (pl/pw =2.08±0.08, N=10). Palm with distal pectinated crest well-developed, prominent, but proximal crest in some individuals reduced to row of granules. Dactylus with low, asymmetrical tubercles. Pleon broad, evenly rounded, broadest at 4th somite, fringed with long setae. Vulva in depression on anterior edge of sternite 5, embraced by posterior margin of sternite 4, rimmed at inner side; operculum placed anteriorly to the gonopore.

Distribution

Southeast Asia: Philippines (Bürger 1893), Labuan, Malaysia, and New Guinea (Rahayu and Davie 2002), Singapore (Tan and Ng 1994), and Java, Indonesia (Nordhaus *et al*. 2011).

Remarks

Parasesarma semperi was originally described with the following diagnostic characters: carapace strongly arched with large epibranchial tooth bent upward, frontal lobes strongly bent downwards, inner lobes wider than outer and with deep median furrow (Fig. 1A) and bearing 6–7 large, dome shaped or conical dactylar tubercles (Fig. 1B). This was sufficient to separate it from its nearest relatives at that time, but is now no longer concise enough to separate it from the sister species *P. longicristatum* (Campbell, 1967). Thus the new diagnosis provided here is necessary to make reliable separation of the two species possible.

The specimen from Iriomote Island (Japan), (CBM ZC7192) recorded as *P. semperi* by Komai *et al*. (2004), is genetically distant from our *P. semperi* clade and holds a position close to *P. lividum* (Fig. 3). The short fixed chelar finger, and the well-marked frontal lobes also make this specimen morphologically more concordant with *P. lividum*.

Specimens of *Parasesarma longicristatum* identified from Java by Nordhaus *et al*. (2011) are most likely to be *P. semperi*, especially since *P. semperi* is already known from Indonesia (Rahayu and Davie 2002) and nowhere else have we found the two species occurring sympatrically. Considering the overlap in morphological characters between the two species, such a misidentification is easily understandable.

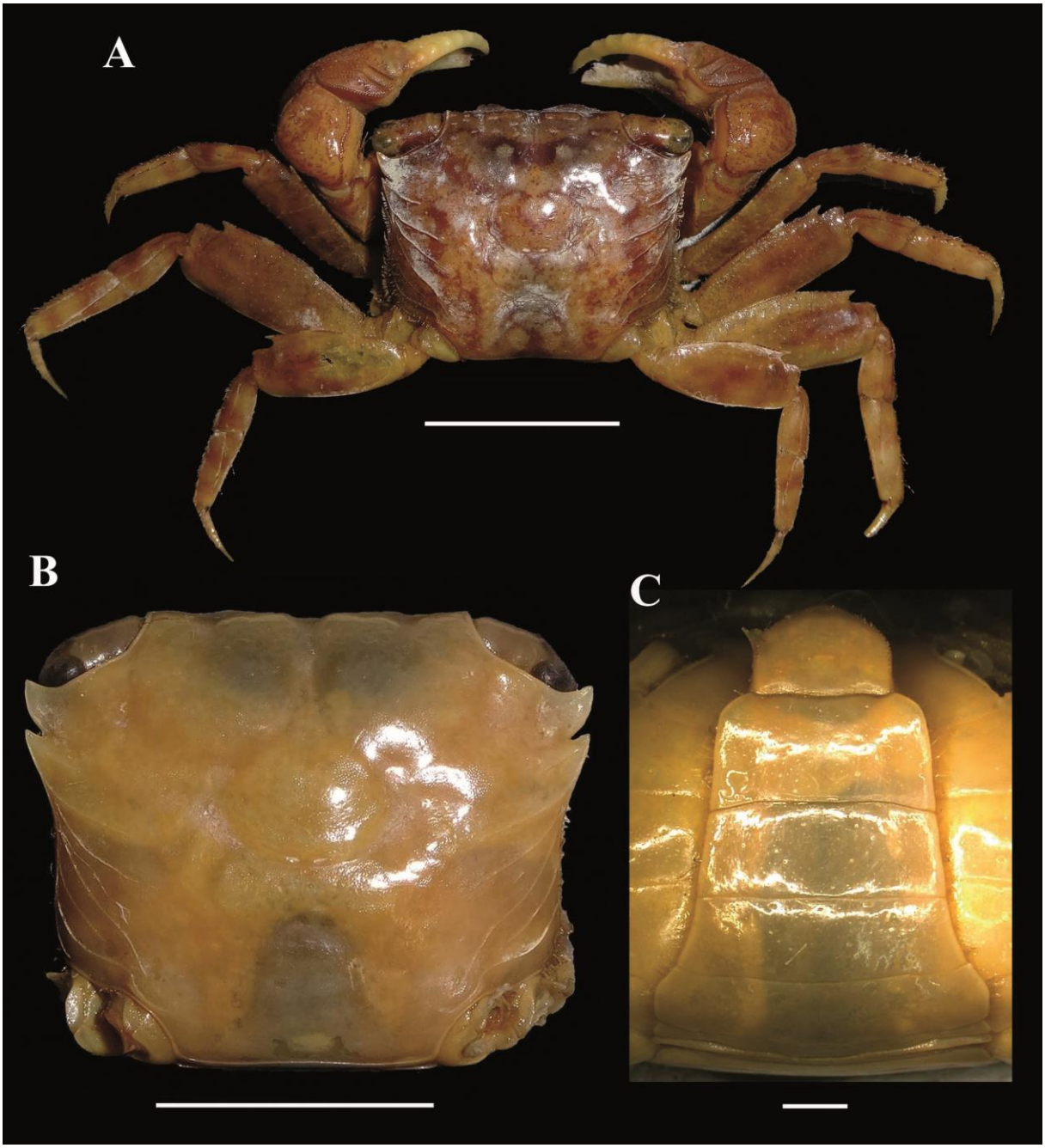

Fig. 6.9. ***Parasesarma semperi.*** **A, dorsal view of a whole male animal from Kamora, New Guinea (ZRC 2000.1891) (I42); B, dorsal view of carapace of the male lectotype (ZMG 625) (I39), from Bohol, The Philippines; C, pleon of the lectotype (I39). Scale bars: A, B = 1cm; C = 1mm.**

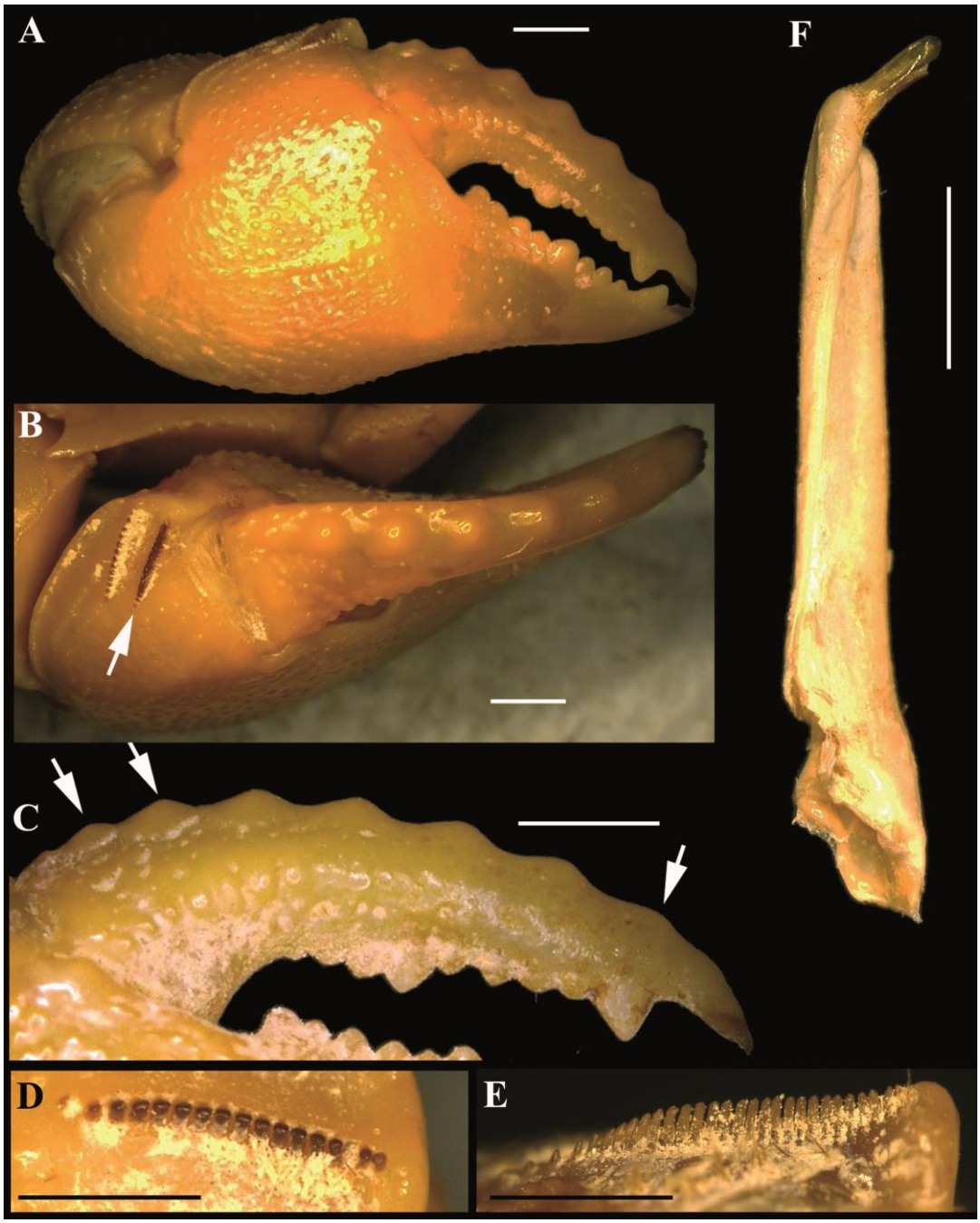

Fig. *Parasesarma semperi*. A, outer view of right chela of the lectotype (I39); B, dorsal view of the same chela (the arrow indicates distal pectinated crest); C, outer view of right cheliped dactylus of a male from Kamora, New Guinea (I43), the arrows indicate the asymmetrical tubercles; D, dorsal view of the distal pectinated crest of the lectotype male (ZMG 625) (I38); E, frontal view of the distal pectinated crest of a male (ZRC 2000.1891) (I42) from Kamora, New Guinea; F, right G1 of the male lectotype (ZMG 625) (I38). Scale bar = 1mm.

Parasesarma longicristatum (Campbell, 1967)
(Figs 5D–F, 6A, B, 11, 12)

Sesarma (Chiromantes) semperi longicristatum: Campbell, 1967: 14, Figs 1E, 2E pl.5.
Perisesarma longicristatum: Davie, 2002: 224; Poore, 2004: 509, fig. 163b; Ng *et al*., 2008: 222; Davie, 2010: 197; Shahdadi & Schubart, 2015: 1085 (in Table 1).
Parasesarma longicristatum: Shahdadi & Schubart, 2017: XX.

Material examined

Examined material is listed in Table 1. Type data: holotype male, QM-W2460, Rose Bay, Townsville, Queensland; paratypes, QM-W2461–2465, AM-P15346, AM-P7915.

Redescription

A moderate-sized *Parasesarma* (maximum cw in present study =20.8, N=37) (Table 1). Carapace quadrate, slightly broader than long (mean cw/cl=1.23±0.05, N=25) (Table 1). Front 0.56±0.02 (N=17) times cw, deflexed, with median emargination; post-frontal lobes low but distinct, median lobes broader than lateral, separated by shallow furrow. Dorsal carapace regions distinguishable but not well marked. Anterolateral margin with sharp exorbital angle; epibranchial tooth well-developed, pointing anterolaterally (Figs 11A, 5D–F).

Chelipeds equal to subequal, large (pl/cw= 0.72±0.10, N=10); ratio of pl/pw 1.76±0.11 (N=17), cutting edge of fixed finger 0.42±0.02 (N=17) times pl. Upper surface of palm with two transverse pectinated crests, distal crest bearing 18–29 tall teeth (Fig. 11F–G) (mean 23.25±2.81, N=20) (Table 1). Dactylus 0.63±0.01 (N=17) times pl; dorsal surface bearing 7–9 distinct, large, dome-shaped, well-spaced tubercles (Table 1; tubercles typically asymmetrical (Fig. 11C), but sometimes median tubercles may be symmetrical (Fig. 11E) (e.g. specimens I6, I16 and I23 in Table 1).

Ambulatory legs relatively short, third pair longest (1.64 times carapace width in male paratype (cw/cl=18.5/14.94, QM, W2464, I2 in Table 1), propodus of third ambulatory leg 2.85±0.18 (N=17) times longer than wide (Fig. 11A).

Male pleon (Fig. 11D): telson 1.17 times wider than long (in I2), virtually pentagonal, almost as long as 6th somite; 6th somite 2.04 times wider than long (in I2), 5th and 4th somites

trapezoidal, 3rd somite broader than others; medial length of 2nd somite equal to, or slightly longer than, length at lateral edges. G1 long, corneous apical process long, bent at about 54° to vertical axis; tip rounded, aperture subterminal (Figs 11B, 6A & B).

Females with smaller chelipeds, but more elongated chelae. Dactylar tubercles less prominent, distinctly asymmetrical (Fig. 12A). Distal pectinate crest on upper palm well developed, prominent; proximal crest in some individuals reduced to row of granules. Pleon broad, evenly rounded, widest at 4th somite, fringed with long setae (Fig. 12B). Vulva in depression on anterior edge of sternite 5, embraced by posterior margin of sternite 4; rimmed at inner side; operculum placed anteriorly to the gonopore (Fig. 12C).

Distribution

Tropical and subtropical Australia: from SE Queensland to the Northern Territory and northern Western Australia.

Remarks

Bürger (1893) compared his new species, *P. semperi*, with *P. lividum* because this was the only other known species at that time to have a similar number of obvious dactylar tubercles. *P. lividum* in fact has 11–13 tubercles but only the proximal 7–8 are distinct. *P. lividum* is different from *P. semperi* and *P.longicristatum* in many other morphological aspects. *P. lividum* has shorter chelae fingers, length of the cutting edge is approximately 0.37 times the entire length of propodus (Campbell 1967; Davie 2010), while it is 0.44 and 0.42 in *P. semperi* and *P. longicristatum* respectively (see Results). Moreover, dactylar tubercles are lower and transversely elongated in *P. lividum*, while the other two species have seven rounded dome shaped tubercles. *P. lividum* is also different from the other two species by having well marked dorsal carapace regions and frontal lobes.

P. lanchesteri is also similar to *P. semperi* and *P. longicristatum* in having 7 round dactylar tubercles. But *P. lanchesteri* differs from the two latter species in having a small anterolateral tooth (strongly developed in the other two species), well-marked dorsal carapace regions, and dactylar tubercles with transverse lines on the proximal slope. *P. lanchesteri* also has the apical corneous part portion of the G1 strongly bent at an angle of about 80° to the vertical axis, while it is noticeably less bent (about 54°) in both *P. semperi* and *P. longicristatum*. Genetic data also

confirm that the *P. semperi/P. longicristatum* clade is distant from both *P. lividum* and *P. lanchesteri* (Fig. 2).

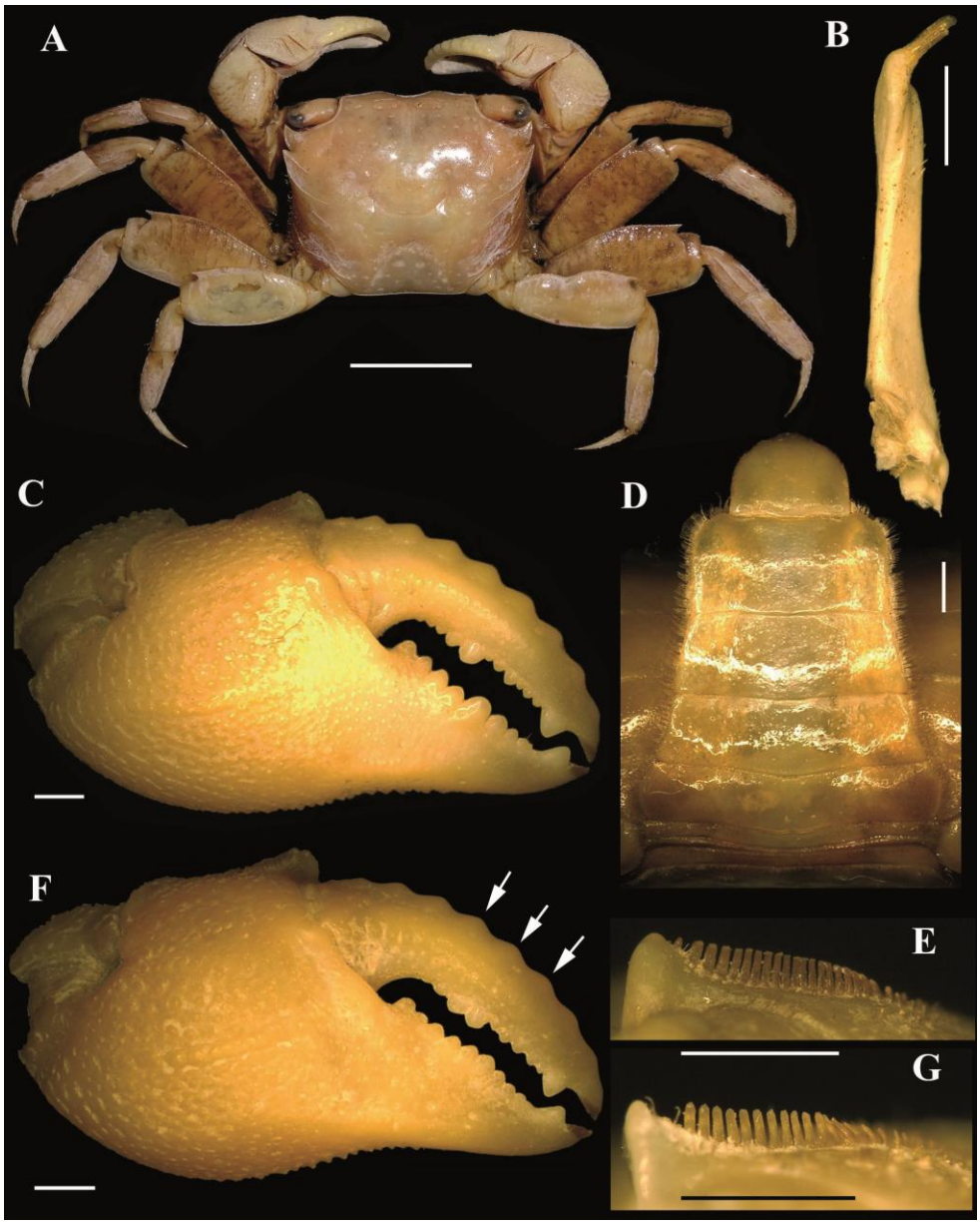

Fig. 6.11. ***11. Parasesarma longicristatum.*** **A–E, male paratype (QM-W2464) (I2): A, dorsal view of whole animal; B, right G1; C, right chela, outer view; D, pleon; E, frontal view of distal pectinated crest; F, outer view of right chela of a male from the Northern Territory, Australia, (ZRC-1995.953) (SI6), arrows indicate symmetrical tubercles; G, frontal view of distal pectinated crest on a male from Western Australia (QM-W20314) (I12). Scale bars: A = 1cm; B–G = 1mm.**

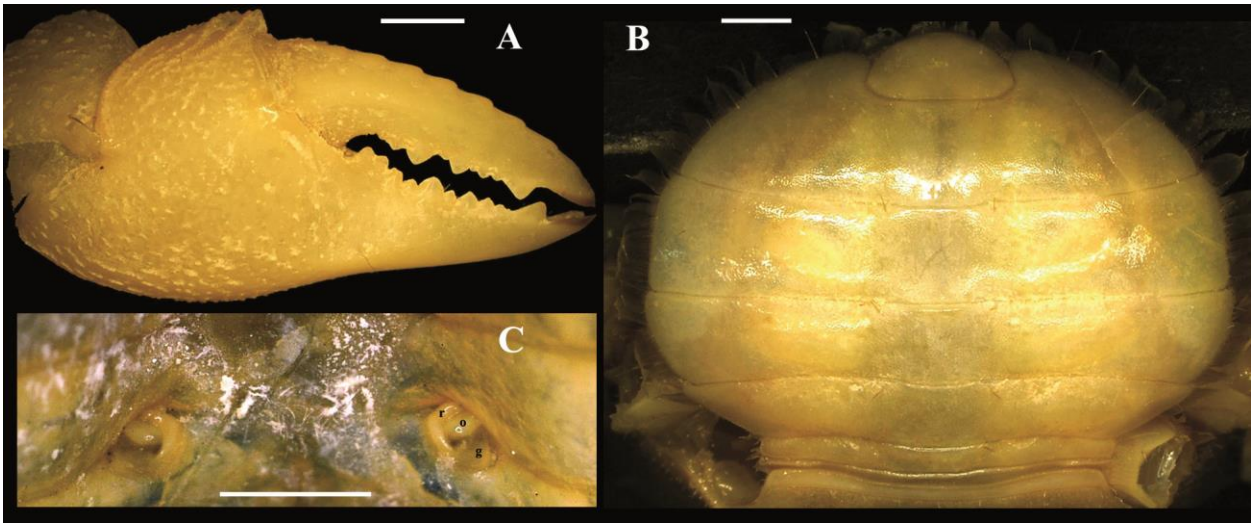

Fig. 6.12. ***Parasesarma longicristatum,*** **female (I26). A, right chela, outer view; B, pleon; C, vulvae (r = rim; o = operculum; g = gonopore). Scale bar = 1mm.**

Chapter 7

Morphological and phylogenetic evidence for a new species of *Parasesarma* De Man, 1895 (Decapoda, Brachyura, Sesarmidae) from the Malay Peninsula, previously referred to as *Perisesarma indiarum*

Abstract

Mangroves from the Malay Peninsula host a great abundant of sesarmid species. One of the most common and prominent species is *Parasesarma* n. sp3. which previously referred to as *P. indiarum*, firstly recorded from Singapore by Tweedie, (1940). *P. indiarum* originally was described by De Man, 1902 from Ambon, Indonesia and up to now assumed to be distributed all over S. E. Asian Island. The phylogenetic position and taxonomy of the populations from the Malay Peninsula have not been evaluated after their first record. The present study compares the material of these populations to the type material from Ambon, by using morphological characters as well as a genetic marker. Morphology and molecular evidences support monophyly of the populations from the Malay Peninsula as a separate unit, but they also show distinctness from the type material. We therefore describe the Malay Peninsular populations as a separate new species. The new species is also compared morphologically and genetically to other related species.

Introduction

The genus *Parasesarma* de Man, 1895 was recently enlarged after incorporating most species previously classified within the sister genus *Perisesarma* De Man, 1895 (Shahdadi & Schubart, in press). It now consists of 57 species, thus becoming one of the most speciose taxa of thoracotreme crabs, distributed all over Indo-West Pacific (De Grave et al., 2009; Shahdadi & Schubart, in press). Considering that 16 species have been described within the last 15 years, an even higher biodiversity of this group must be expected. According to the limited number of diagnostic features (see Shahdadi & Schubart, 2015), molecular approaches play an increasingly important role to uncover the underlying diversity of such taxa. These crabs are of relatively small size (Shahdadi & Schubart, in press) and mostly live burrowed in the soft-sediment muddy shores of marshlands and mangrove forests (Tan & Ng, 1994; Lee, 1998; Guerao et al., 2004), which are additional reasons that the actual biodiversity of this genus has been underestimated. Despite recent species descriptions (Davie & Pabriks, 2010; Koller et al., 2010, Rahayu & Ng, 2010; Rahayu & Li, 2013), the taxonomy of this genus still needs further attention to befully resolved, especially as it harbors several poorly known species.

Mangroves from the Malay Peninsula host a great abundant of brachyuran species, of which Sesarmidae Dana, 1851 is the most diverse family (Tan & Ng, 1994). Up to date, 15 species of *Parasesarma* (sensu Shahdadi & Schubart, in press) are known from this area and surrounding islands: Tan & Ng (1994) reported 10 species in their list (i.e., *P. batavianum* (De Man, 1890); *P. calypso* de Man, 1895; *P. eumolpe* (De Man, 1895); *P. indiarum* (Tweedie, 1940); *P. lanchesteri* (Tweedie, 1936); *P. melissa* (De Man, 1887); *P. onychophorus* (De Man, 1895); *P. plicatum* (Latreille, 1803); *P. rutilimanum* (Tweedie, 1936); *P. semperi* (Bürger, 1893)). Later, *P. raouli* Rahayu & Ng, 2009 was described from Johor Strait and *P. ungulatum* (H. Milne Edwards, 1853) was recorded from the area by Rahayu & Ng (2010). According to their original descriptions, three other species also inhabit adjacent islands (i.e., *P. lenzii* (De Man, 1894) from Penang Island, Malaysia; *P. prashadi* (Chopra & Das, 1937) from Mergui Island, Myanmar; *P. anambas*, Yeo, Rahayu & Ng, 2004 from Anambas, Indonesia).

From the abovementioned species, *P. indiarum* is one of the most common and prominent species in mangroves of Peninsular Malaysia and Singapore (Boon et al., 2008). This species was originally described by De Man (1902) as *Sesarma bidens* var. *indica* from Ambon and

Ternate, Indonesia. Later, Tweedie (1940) recorded this species from Singapore and changed its name to *Sesarma bidens indiarum*, as the original name was occupied for another species (*Tiomanum indicum* (H. Milne-Edwards, 1837) = originally *Sesarma indica*). Campbell (1967) treated it as full species for first time. Since the first record from Singapore, many biological and ecological aspects of this species have been investigated and it has become a model sesarmid species of the region (e.g., Boon et al., 2008; 2009; Huang et al., 2008). However, the phylogenetic position and taxonomy of the populations from the Malay Peninsula have not been evaluated after their first record. Therefore, the present study compares the material of these populations to the type material (deposited in the Naturalis Museum, Leiden Netherland) from Ambon that De Man (1902) used for his original description, by using morphological characters as well as a genetic marker. Morphology and molecular evidences support monophyly of the populations from the Malay Peninsula as a separate unit, but they also show distinctness from the type material. We therefore describe the Malay Peninsular populations as a separate species. The new species is also compared morphologically and genetically to other related species.

Material and Method

Material examined.

If possible, material was collected from throughout the geographic distribution area of the studied species, preserved in ethanol 70%, and transferred to the laboratory of the University of Regensburg for morphological and molecular examinations. Most specimens and types were borrowed from or studied in natural history museums, including the Queensland Museum (QM), Brisbane, Australia; the Forschungsinstitut und Museum Senckenberg (SMF), Frankfurt a.M., Germany; the Zoological Reference Collection (ZRC), of the Lee Kong Chian Natural History Museum, National University of Singapore; and the Naturalis Museum (RMNH), Leiden, Netherlands.

Besides the types of *P. indiarum*, material of *P. eumolpe*, *P. foresti* and *P. messa* was included for genetic and morphologic comparisons, according to their phylogenetic association and morphological similarity with *P. indiarum* (see Shahdadi & Schubart, 2015; Shahdadi & Schubart, in press). Among those species, *P. eumolpe* has a sympatric distribution and also the

closest genetic relationship with the new species (prelimminary results), so that we included several specimens of this species from several localities within its distribution range for more detailed comparisons. Representatives of other related clades of the genus were also included, i.e., *P. cricotum* (Rahayu & Davie, 2002), *P. darwinense* (Campbell 1967), *P. lanchesteri* (Tweedie 1936), *P. lividum* (A. Milne-Edwards 1869), and *P. longicristatum* (Campbell 1967) (see Table 7.1).

Molecular analyses.

Genetic analyses and comparisons were undertaken with several specimens of the species covered in this study (Table 7.1).

Genomic DNA was isolated from leg muscle tissue using a modified Puregene method (Gentra Systems, Minneapolis) and a Mollusc DNA kit (Omega D3373-02) using the manufacturers' protocols. 709 basepairs (including primer regions) of the mitochondrial protein-coding gene cytochrome oxidase subunit 1 (Cox1), corresponding to the generally used barcoding region (Hebert et al., 2003), were amplified by polymerase chain reactions (PCR) with the primer combination COL6 (5′-TYTCHACAAAYCATAAAGAYATYGG-3′) (Schubart, 2009) as forward primer and COH6 (5′-TADACTTCDGGRTGDCCAAARAAYCA-3′) (Schubart & Huber, 2006) as reverse primer, using the following PCR profile: initial step 4 min at 94°C; 40 cycles with 45s at 95°C for denaturing, 60s at 48°C for annealing, 60s at 72°C for extension; and final extension with 5 min at 72°C.

PCR products were outsourced for sequencing to Macrogen Europe. Sequences were proofread using Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia), primer regions were removed and the remaining sequences aligned automatically with ClustalW (Thompson et al., 1994) implemented in BioEdit 7.0.5 (Hall, 1999). Two alignments were prepared, one for constructing phylogenetic trees and one to create haplotype network focusing on *P. eumolpe* and the new species. The alignments were then converted with FaBox (Villesen, 2007) to Nexus files. To construct phylogenetic trees the best evolutionary model describing our data was determined with the aid of jModelTest (v. 2.1.4) (Darriba et al., 2012) and selected with the Akaike information criterion (AIC) (Posada & Buckley, 2004). According to the phylogenetic position (preliminary data), *P. lanchesteri* was selected as outgroup for tree construction. Two

methods of phylogenetic inference were applied to our concatenated dataset: Maximum Likelihood (ML) using the software raxmlGUI (v. 1.3) (Silvestro & Michalak 2012) and Bayesian Inference (BI) as implemented in MrBayes (v. 3.2) (Huelsenbeck & Ronquist 2001). Maximum Likelihood (ML) trees were obtained with 2000 bootstrap pseudoreplicates. For the MrBayes runs, we used 2 million generations with four chains and a sample frequency of 1,000 generations. To illustrate genetic relationship between the two more related species, *P. eumolpe* and the new species at a higher resolution, a maximum parsimony haplotype network (Tempelton et al., 1992) was constructed with Popart (http://popart.otago.ac.nz). Pairwise genetic distances (Kimura 2-parameter = K2P) based on the Cox1 sequences between the new species and *P. eumolpe* was calculated with the software Mega version 5.2.2 (Tamura et al., 2011). The new sequences were submitted to GenBank (NCBI), and accession numbers are presented in Table 7.1.

Table 7.1. Material examined in this study, with specimen code, size (carapace width×carapace length in millimeter), sex (M= male, F=female), locality, catalogue number and Gen-Bank accession number (Cox1).

Species	Code	Size, sex	Locality	Museum no.	Cox1
P. cricotum (Rahayu & Davie, 2002)	S38	14.34×12.16, F	Indonesia, Irian Jaya, Kamara	ZRC2016.0522	KX400897
P. darwinense (Campbell, 1967)	S57	12.24×10.24, M	Australia, Northern territories, Darwin Island	SMF 49921	KX400904
P. eumolpe (De Man, 1895)	S116	23.35×20, M	Thailand, Phuket, Chalong	Pending	Pending
	S119	16.83×13.92, M	China, Hainan, Wen Chang Chen	SMF 49922	KX400891
	S144	23.56×19.89, M	China, Hainan, Wen Chang Chen	SMF 49922	Pending
	S145	24.26×20.68, M	China, Hainan, Wen Chang Chen	SMF 49922	KX400892
	S616	23.76×20.72, M	China, Hainan, Wen Chang Chen	SMF 49922	
	S617	18.72×16.03, M	China, Hainan, Wen Chang Chen	SMF 49922	
	S618	20.82×17.99, M	China, Hainan, Wen Chang Chen	SMF 49922	
	S619	18.63×16.43, M	China, Hainan, Wen Chang Chen	SMF 49922	
	S620	17.33×14.70, M	China, Hainan, Wen Chang Chen	SMF 49922	
	S621	15.69×13.36, M	China, Hainan, Wen Chang Chen	SMF 49922	
	S622	19.07×15.84, F	China, Hainan, Wen Chang Chen	SMF 49922	
	S623	19.58×16.58, F	China, Hainan, Wen Chang Chen	SMF 49922	
	S529	14.99×15.75, F	Singapore	Pending	
	S572	21.47×17.96, M	Thailand, Koh Chang	Pending	
	S573	20.76×17.50, M	Thailand, Koh Chang	Pending	Pending
	S574	20.38×17.43, M	Thailand, Koh Chang	Pending	
	S575	13.55×11.59, F	Singapore, Mandai	Pending	
	S576	19.41×16.62, M	Singapore, Buloh	Pending	
	S398	20.60×18.37, M	Singapore, Lim Chu Kang	QM,W24182	Pending
	S577	21.55×18.50, M	Singapore, Lim Chu Kang	Pending	Pending
	S578	19.52×16.51, M	Singapore, Lim Chu Kang	Pending	
	S579	18.66×16.06, M	Singapore, Lim Chu Kang	Pending	
	S580	16.13×13.66, M	Singapore, Lim Chu Kang	Pending	
	S581	14.77×12.72, M	Singapore, Lim Chu Kang	Pending	
	S582	21.31×18.63, M	Singapore, Lim Chu Kang	Pending	
	S583	15.36×12.83, M	Singapore, Lim Chu Kang	Pending	
	S584	19.42×16.14, F	Singapore, Lim Chu Kang	Pending	
	S585	17.03×14.42, F	Singapore, Lim Chu Kang	Pending	
	S586	18.44×15.59, F	China Hainan, Dongzhai	Pending	
	S587	21.92×18.08, F	China Hainan, Dongzhai	Pending	
	S588	26.80×22.66, M	China Hainan, Dongzhai	Pending	
	S589	25.10×21.38, M	China Hainan, Dongzhai	Pending	
	S590	24.30×20.53, M	China Hainan, Dongzhai	Pending	
	S566	24.19×20.96, M	Singapore Mandai Besar	Pending	
	S399	14.33×11.91, F	Brunei	QM-W17212	
P. foresti (Rahayu & Davie, 2002)	S612	16.64×14.47, M	Indonesia, Irian Jaya, Kamora	ZRC 2003.0481 paratype	Pending

	S3	14.33×11.72, F	Indonesia Irian Jaya, Kamora	ZRC2016.0523	KX431204
P. indiarum (Tweedie, 1940)	S146	28.99×24.98, M	Indonesia, Ambon	RMNH. CRUS. D.141 lectotype	KX761164
	S147	22.79×19.38, M	Indonesia, Ambon	RMNH. CRUS. D.19 Paralectotype	Pending
	S450	22.25×18.48, F	Indonesia, Ambon	RMNH. CRUS. D.141 Paralectotype	
Parasesarma n. sp3.	S134	25.52×22.22, M	Singapore, Mandai mongroves	Pending Holotype	Pending
	S626	25.08×22.07, M	Singapore, Mandai mongroves	Pending	
	S627	23.52×19.8, M	Singapore, Mandai mongroves	Pending	Pending
	S518	17.12×14.21, F	Singapore, Mandai mongroves	Pending	Pending
	S195	24.37×20.57, M	Singapore, Besar mangroves	ZRC 2012.0260	KX400890
	S194	20.97×18.81, M	Singapore, Besar mangroves	ZRC2013.1551	KX400889
	S521	25.2×22.25, M	Singapore, Lim Chu Kang	Pending	
	S525	21.02×18.06, F	Singapore, Lim Chu Kang	Pending	
	S526	23.79×20.76, F	Thailand, Chumphon	Pending	Pending
	S527	23.35×20.23, F	Singapore	Pending	
	S528	19.99×17.04, F	Singapore	Pending	
	S530	12.74×10.76, M	Thailand, Thap Lamu	Pending	Pending
	S531	11.10×9.09, M	Singapore	ZRC 2000.1795	
	S532	10.17×8.38, M	Singapore	ZRC 2000.1795	
	S533	10.43×8.6, F	Singapore	ZRC 2000.1795	
	S534	12.88×10.69, F	Singapore	ZRC 2000.1795	
	S522	19.65×16.76, M	Indonesia, Riau Archipelago, Batam Island	ZRC 2000.2034	Pending
	S523	15.73×13.43, M	Indonesia, Riau Archipelago, Batam Island	ZRC 2000.2034	
	S524	13.27×11.23, F	Indonesia, Riau Archipelago, Batam Island	ZRC 2000.2034	
	S133	12.21×10.78, M	Indonesia, Riau Archipelago, Batam Island	ZRC 2000.2034	Pending
	S535	12.76×10.75, M	Indonesia, Riau Archipelago, Batam Island	ZRC 2000.2034	
	S536	10.87×9.04, M	Indonesia, Riau Archipelago, Batam Island	ZRC 2000.2034	
P. lanchesteri (Tweedie, 1936)	S426	24.76×18.78, F	Singapore, Simpang Mak wai river	ZRC1967.1183	KX761168
*P. lividum (*A. Milne-Edwards, 1869)	S69	17.76×15.19, M	New Caledonia , Nanmea mongroves	QM, W24243	KX400893
P. longicristatum (Campbell, 1967)	S335	18.50×14.94, M	Australia, Queensland, Port Alma	QM, W2464 paratype	KY198240
P. messa (Campbell, 1967)	S10	19.15×16.27, M	Australia, Queensland, Flying Fish Point	QM, W2452 paratype	KX400887
	S342	18.65×15.59, M	Australia, Queensland, Townsville	QM, W2446 paratype	
	S386	19.18×15.97, F	Australia, Queensland, Townsville	QM, W2446 paratype	Pending
Perisesarma n. sp1.	S382	28.23×24.00, M	Vietnam, Tan Thoi Island, Cua Tieu River	QM-W28348 Holotype	KY198241

Result & Discussion

Two alignments of the Cox1 gene were constructed. After removal of the primer sequence and adjacent regions both alignments consisted of 639 basepairs and did not contain any stop codons, which may have indicated the presence of pseudogenes. The best evolutionary model obtained with jModelTest (v. 2.1.4) (Guindon and Gascuel 2003; Darriba *et al*. 2012) was the General Time reversible plus Gamma (GTR+G, Rodriguez et al., 1990).

Two phylogenetic trees (ML & BI) and a haplotype network were constructed (Fig. 7.1). As the topologies of the trees showed high similarity, here we present only the ML trees with both bootstrap values and posterior probabilities for ML and BI respectively (Fig 7.1A). The phylogenetic analyses indicate that individuals previously referred to as *P. indiarum* from the Malay Peninsula represent a highly supported monophyletic clade, distinctly separated from other related species as well as from the type of *P. indiarum*. Among other examined species, this new species shows a significant phylogenetic association to the four species, *P. eumolpe, P. foresti, P. indiarum* and *P. messa* (Fig 7.1A). Of them, the three species *P. foresti, P. indiarum* and *P. messa* form a stable subclade. Although being very close, the haplotype network shows a stable and distinct genetic gap between the new species and *P. eumolpe* (Fig. 7.1B). The Average K2P distance between these two species was around 2.5%.

Despite being very similar in general morphology, the new species shows clear differences in the morphology of chela dactylar tubercles compared to other related species (Figs. 7.2 & 7.3). Similar to *P. indiarum* and *P. foresti*, the new species also has 10-12 low, oval tubercles. But in contrast to them, the tubercles of the new species are distinctly different being considerably elongated transversely, having a distinct transverse keel followed by a shallow transverse sulcus. The new species also differs from *P. messa* in the same way, besides that *P. messa* usually has more than 12 low and oval dactylar tubercles. The morphology of proximal tubercles in the new species resembles to some extent those in *P. eumolpe*, having the sulcus distal to the transvers keel. In contrast, the tuberculation pattern in *P. eumolpe* is markedly different by having 19-26 tubercles with very obvious sulci (Figs 7.2 & 7.3; see also Fig. 2 in Boon et al., 2009 for SEM photos of tuberculation patterns in these two species).

Because of morphological ambiguities and identification difficulties (Davie, 2010; Shahdadi & Schubart, 2015) and also the lack of phylogeographic studies, the geographic distribution of the abovementioned related species are not well documented. Up to know, *P. messa* is known as an Australian species (Davie, 2002) and *P. foresti* known only from its type locality, Irian Jaya (New Guinea) (Rahayu & Davie, 2002). *P. indiarum* was originally described from Ambon and Ternate Islands (Indonesia) (De Man, 1902) and later has been reported from New Guinea, south coast of Java (Tesch, 1917), Singapore, both coasts of Malay Peninsula, West Sumatra (Tweedie, 1936; 1940) and Labuan (Tweedie, 1950). In the present study we demonstrate that the specimens from the Malay Peninsula, which hitherto have been assigned to *P. indiarum*, actually belong to a separate new species, and no one of those samples clusters with *P. indiarum* and or *P. foresti*. Therefore, a targeted phylogeographic study, examining material from all aforementioned area like Java, Sumatra, Labuan and also other south East Asian Island is required to map distribution boundaries of these species. Considering high morphological similarities between the two species *P. foresti* and *P. indiarum* (Davie, 2010; Shahdadi & Schubart, 2015), their phylogenetic status (present data) and overlapping distributions, such a study also becomes necessary to evaluate their taxonomic position.

Among the closely related species, *P. eumolpe* co-occurs sympatrically with the new species in the same habitat (for more details see habitat and distribution of each species in the Taxonomic accounts in continuation), while keeping their own genetic and morphologic identities. Close genetic affinity (K2P = 2.5%) and morphological resemblance, sign a very recent divergence between these two species of crabs. Considering their overlapping distributions and habitats and their general similarity, this can highlights the importance of the morphology of chelar dactylar tubercles (dactylus tuberculation pattern) as stridulatory organs (Tweedie, 1954; Boon et al., 2009; Chen et al., 2014) and facial color band as optical discriminative signal (Huang et al., 2008) in intraspecific communication and interspecific isolation. Such a relatively quick divergence in ecologically/ethologically important characters could be a possible explanation for radiation of speciose taxa like *Parasesarma*.

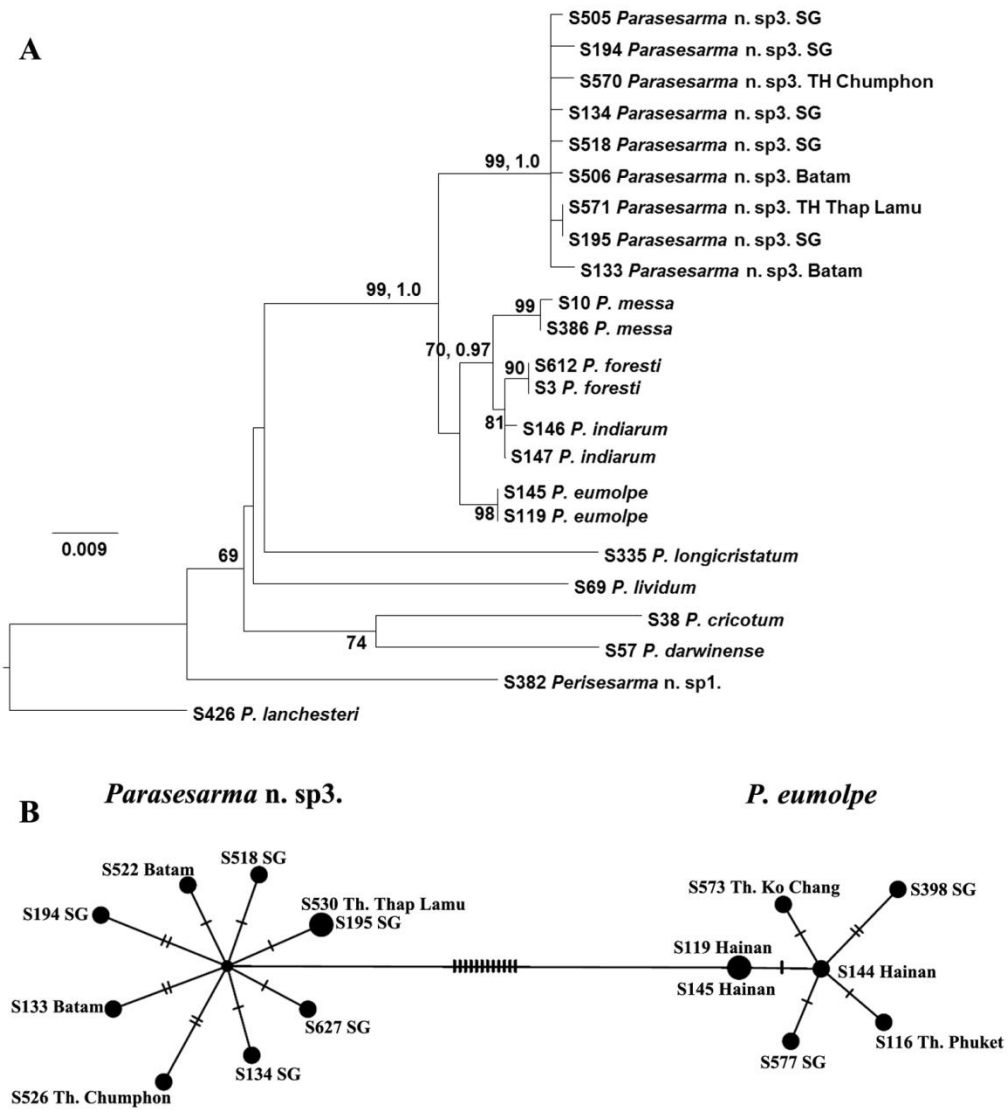

Fig. 7.1. A. Phylogenetic consensus trees of the Cox1 gene, constructed with Maximum Likelihood (ML) and Bayesian Inference (BI) from selected species of *Parasesarma,* focusing on *Parasesarma* n. sp3.; *P. lanchesteri* was used as outgroup, Only confidence values higher than 50% and 95% respectively for ML and BI are shown in the trees, B. Maximum parsimony haplotype network of Cox1 gene for specimens *Perisesarma* n. sp3. and *P. eumolpe* constructed with Popart.

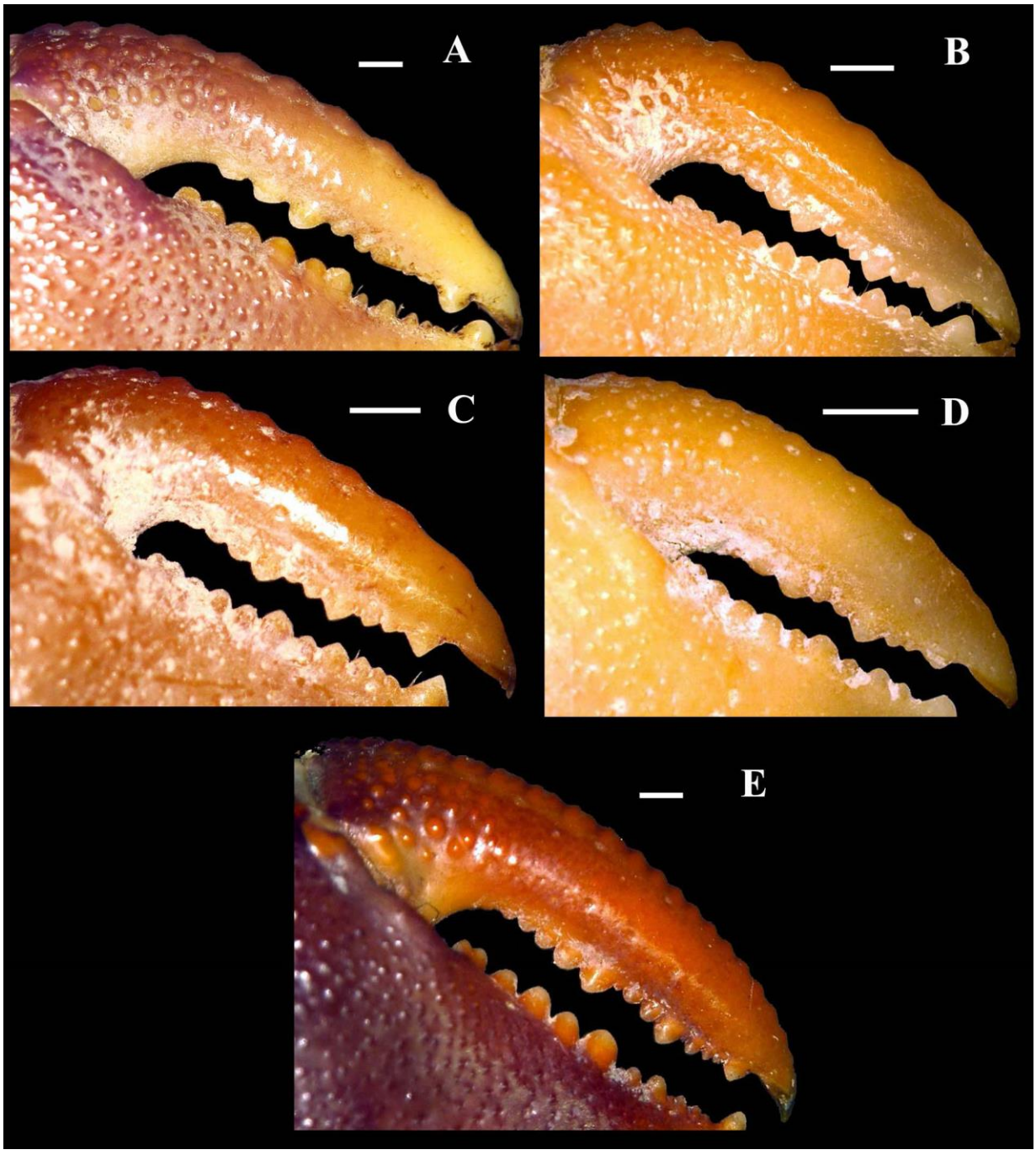

Fig. 7.2. Comparisons of chelar dactylus and tuberculation patterns, outer view in A. *Parasesarma* n. sp3. (holotype, S134), B. *P. indiarum* (paralectotype, S147), C. *P. foresti* (paratype, S612), D. *P. messa* (paratype, S342), E. *P. eumolpe* (S145) (scale bars 1mm).

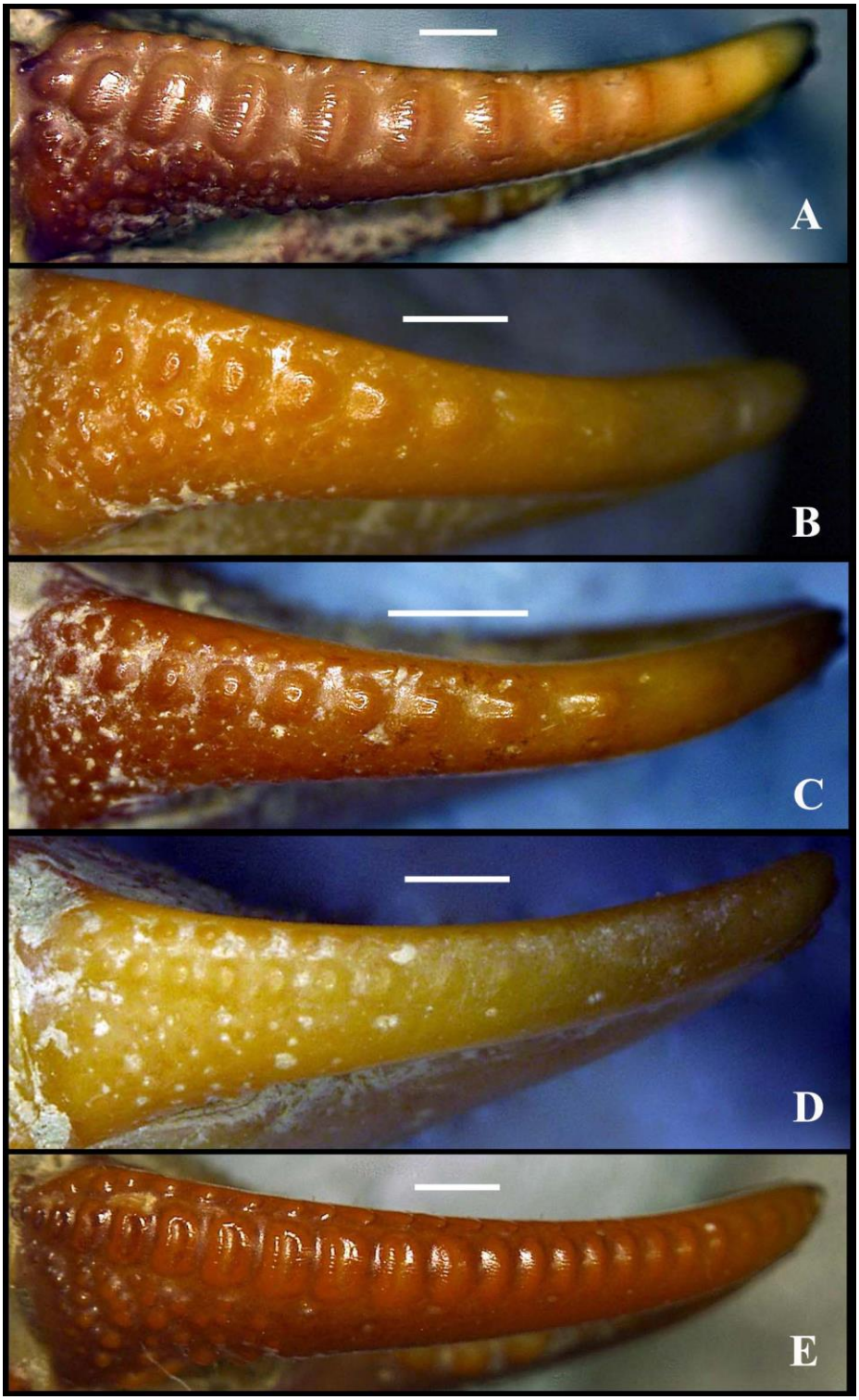

Fig. 7.3. Comparisons of chelar dactylus and tuberculation patterns, dorsal view in A. *Parasesarma* n. sp3. (holotype, S134), B. *P. indiarum* (paralectotype, S147), C. *P. foresti* (paratype, S612), D. P. messa (paratype, S342), E. P. eumolpe (S145) (scale bars 1mm).

Taxonomic account

Here we focused on the description and taxonomic accounts of the new species, *Parasesarma* n. sp3., and also updated the taxonomic account of its closely related sympatric species, *P. eumolpe*. The taxonomic issues of other three related specie, *P. foresti*, *P. indiarum* and *P. messa* and their phylogeography is being investigated by present authors and colleagues and will be discussed elsewhere.

To conduct this study, we located two slots containing type material of *P. indiarum* in Naturalis collection (Leiden, the Netherland) (RMNH. CRUS. D.141 & RMNH. CRUS. D.19). An adult male animal in relatively good preservation condition from Indonesia, Ambon (RMNH. CRUS. D.141, Carapace width×length = 28.99×24.98 mm) (= S146 in Table 7.1) was selected as a lectotype for *P. indiarum* from the type series, the remaining type individuals becoming paralectotypes. The lectotype, a male and a female from the type series were examined and included in this study (see Table 7.1).

Family Sesarmidae Dana, 1851

Genus *Parasesarma* De Man, 1895

***Parasesarma* n. sp3.**

(Figs. 7.2A, 7.3A, 7.4, 7.5, 7.6, 7.7 & 7.8)

Sesarma (*Chiromantes*) *bidens indica*: Tweedie, 1936: 66.

Sesarma bidens indiarum: Tweedie, 1940: 93.

Chiromantes indiarum: Tan & Ng 1994: 82 (in list)

Perisesarma indiarum: Boon et al., 2008; Huang et al., 2008; Boon et al., 2009.

Material examined. An adult male from Singapore, Mandai mangroves (Voucher number pending, carapace width×length = 25.52×22.22 mm) (= S134 in Table 7.1) was selected as holotype. The other examined material being paratypes (for more details about material examined see Table 7.1)

Comparative material. For the comparative materials see Table 7.1 and the material and methods.

Diagnosis. Small to medium sized sesarmid crab; carapace subrectangular, slightly broader than long. Front moderately deflexed, with broad, relatively deep, median concavity, postfrontal lobes prominent, median lobes broader than lateral ones, separated by a deep median furrow; dorsal carapace regions well indicated, anterolateral margin with sharp exorbital angle and well developed epibranchial tooth. Chelipeds homochelous, large; upper surface of palm with two transverse pectinated crests, dorsal surface of dactylus bearing 11–12 low,distinct, transversely broadened tubercles. Ambulatory legs relatively short and broad. Male pleon triangular with telson slightly longer than basal width. G1 relatively long and straight, apical corneous process long, bent at angle of about 54° to vertical axis, arched in cross section, aperture subterminal.

Description. Small to Medium sized sesarmid crab (maximum carapace width in studied specimens = 25.52 mm), carapace subrectangular, slightly broader than long, greatest width between exorbital angles (cw/cl 1.17±0.03, N=20), Carapace surface smooth, shining, punctate, with numerous short, transverse to slightly oblique crests edged with rows of short setae, sometimes forming low tufts. Front = 0.58±0.01 times carapace width (N=20), moderately deflexed, with broad, relatively deep, median concavity (Fig. 7.4A). Post-frontal lobes prominent, median lobes broader than lateral ones, separated by deep furrow (Fig. 7.5A & B). Dorsal carapace regions well indicated; gastric region most strongly demarcated; cardiac region separated from intestinal region, lateral branchial ridges prominent, upper orbital border smooth, lower orbital border finely granulate, anterolateral margin with sharp exorbital angle, well developed epibranchial tooth (carapace width between epibrancial teeth only slightly less than, width between exorbital angles), no indication of second epibranchial tooth, lateral margin slightly concave, edged with row of short setae. Eyes pigmented, cornea as wide as eyestalk (Fig. 7.4A).

Chelipeds homochelous (Figs. 7.4). Chela large (Fig. 7.5A) (ration of palm length/carapace width = 0.65±0.13, N=20) (because of allometric growth, the ration of palm length/carapace width in adult males is higher, for example in examined animals this ration ranges from 0.48 in a female juvenile to 0.86, in an adult male), chelae relatively robust (Fig. 7.5F) (width = 0.53±0.04 × length, N=19, ranges 0.45–0.60). Merus with granulate dorsal border and distinct subdistal spine, proximal 1/3rd decorated with long setae; ventral border granulate; anterior border granulate, with distinct subdistal spine; inner face smooth with two longitudinal rows of setae,

ventral row more prominent, continuous, with longer setae, dorsal row interrupted, setae extending to dorsal border, anterior face also smooth, outer faced with transvers lines of fine granules and scattered small chitinous spines. Upper surface of palm with two transverse pectinated crests (Fig. 7.5E); distal (primary) crest composed of 13–22 tall, broad teeth (19 on claws of holotype) (Fig. 7.5G); secondary crest well developed in males, with 7–17 teeth (11 and 12 on opposite chelae of holotype); both crests terminate at inner end in short swollen, tubercular ridge and several blunt granules at outer end, in some specimens (not all) one row of coarse granules proximal to second crest, scattered fine setae distal to primary crest. Upper margin of palm with granules. Outer surface of palm without setae, coarsely granular except for smooth, punctate fixed finger, lacking any indication of median longitudinal ridge (Fig. 7.5F); inner surface of palm coarsely granular except area facing carpus; ventral border of chela sinuous, with lines of fine granules on the proximal half, length of cutting margin of fixed finger = 0.41±0.02 times length of entire propodus (N=20). Dactylus = 0.60±0.02 times propodus length, gently arched downwards and moderately curved inwards distally (Figs. 7.2A, 7.3A, 7.5E & F), dorsal surface bearing 10–12 low, transversely broadened tubercles, distinct to tip ((Figs. 7.2A, 7.3A, 7.5E & F), each tubercle with transvers distict keel followed by a shallow sulcus (Figs. 3A & 5H), resembling to the dactylar tubercles in *P. eumolpe* (Fig. 7.3E), tubercles with chitonlike lines, except 1 or 2 proximal tubercle(s), the rests are slightly asymmetric and distal slope longer than proximal (dactylar tubercles in females (Fig. 7.7D) and juvenile males are not well differentiated: low, rounded to slightly oval tubercles without or with indistinct keel and sulci). Row of about 11–17 rounded tubercles on proximal two thirds of inner edge of dorsal surface. Fingers with intermeshing chitinous tips, adult males with a narrow gape when fingers closed. Cutting edge of both fingers with a series of variably sized teeth and bunches of ling setae at the inner side (Fig. 7.5F).

Ambulatory legs (Fig. 7.4) relatively short and broad; third pair longest, total length ca. 1.70 times carapace width in holotype, merus with anterior margin crenulated, dorsal faces with transvers lines of fine granules (exept for the fourth pair), third pair with merus = 2.23±0.17 times as long as wide (N=19), propodus = 3.20±0.37 times as long as wide, dactylus ca. 0.73±0.05 times length of propodus (N=17).

Male pleon (Figs. 7.4B & 7.5C) triangular, telson as long as basal width, virtually pentagonal, slightly longer than somite 6 (=1.14±0.08 times length of somite 6, N=13); somite 6 longer than others, ca. 2.04±0.13 times wider than long; somite 5 and 4 trapezoidal; somite 3 widest, laterally convex; somite 2 medially longer than lateral edges (Fig. 7.5D).

G1 (Fig. 6) relatively long, straight (Fig. 7.6A); stem triangular with blunt angles in cross section; apical corneous process long, bent at an angle of about 54° to vertical axis (Fig. 7.6B), arched in cross section, aperture subterminal positioned dorsally (Fig. 7.6C). Few scattered short setae along most of gonopod, apical end covered by longer setae, almost completely obscuring corneous tip; two kinds of setae recognizable, long simple setae, and some plumose setae restricted to outer side of subapical part (Fig. 7.6D).

Female (Fig. 7.7) with smaller chelipeds (Fig. 7.7A); distal dactylar pectinate crest well-developed, prominent as in male, but proximal crest reduced to row of granules; dactylar tubercles also well-developed, round, distinct to tip (Fig. 7.7D &F). Pleon broad, rounded, or even laterally slightly ovate, broadest at somite 4, fringed with long setae. In adult female specimens, pleon touches coxae of ambulatory legs, telson ca. 1.23±0.09 times wider than long (N=6), inserted into somite 6 more than half of its length (Fig. 7.7B). Vulva in depression on anterior edge of sternite 5, somewhat embraced by posterior margin of sternite 4; operculum rounded and positioned in anterior part (Fig. 7.7C).

Colour. Carapace almost black, chelae dark brownish red except for the distal half of dactyli which are red (Fig. 7.8). For the details about facial band see Huang et al. (2008).

Distribution. So far and based on present material, known from both coast of Malay Peninsula, western coast to Thailand, Thap Lamu, eastern side to Thailand Chumphon, southward to Singapore and Batam Island (Riau Archipelago, Indonesia).

Habitat. In its distribution range, *Perisesarma* n. sp3. is a common species in mangroves swamps that live sympatrically or allopatrically with *P. eumolpe* in dense aggregations on muddy substrates and they are active burrowers within mangrove tree aerial root systems (Boon et al., 2009; Hoang et al., 2008)

Remarks. As it is argued before (see Discussion) this species resembles four other phylogenetically related species (*P. foresti*, *P. eumolpe*, *P. indiarum* and *P. messa*) in general morphology. It is difficult to discriminate their females and juvenile animal from *P. foresti*, *P. indiarum* and *P. messa*: all have relatively rounded low chelae dactylar tubercles. But the morphology of these tubercles and tuberculation pattern in adult males distinguishes the new species from all others (Fig. 7.3). The genetic data also confirms monophyly of the new species and its divergence as separate unit (Fig. 7.1A). As here in this study, only the material from the Malay Peninsula were examined, the identities of other Southeast Asian populations need to be examined.

P. eumolpe (De Man 1895)

Sesarma (*Perisesarma*) *eumolpe*: De Man 1895: 208.

Sesarma (*Chiromantes*) *eumolpe*: Tesch 1917: 150; Tweedie 1936: 66; Campbell 1967: 5 (in key).

Sesarma eumolpe: Tweedie, 1950: 340; 1954.

Chiromantes eumolpe: Tan & Ng 1994: 82 (in list); Ng & Sivasoti, 1999: 66–67.

Perisesarma eumolpe: Ng *et al*. 2008: 222 (in list); Boon et al., 2008 & 2009; Davie 2010: 204 (in key); Chen et al., 2014; Shahdadi & Schubart, 2015: 1083 (in Table 1).

Material examined. Materials of this species from different populations through its distribution rage were examined (For more details about material examined see Table 1).

Remarks. As the species was described by De Man, (1895) (based on one male specimen, carapace width×length ca. 20.5×16.60 From Penang, Malaysia), we assumed that the type should be either in Naturalis museum (Leiden, the Netherlands) or Senckenberg collection (Frankfurt, Germany) (being transferred from Gottingen). In an attempt to locate the type in those collections, we were able to find only two dry samples of this species in Naturalis which are not corresponding to the type description. Therefore we can not address the type here in this study.

This species is a well-known sesarmid model species (Boon et al., 2008 & 2009; Chen et al., 2014), frequently reported (see the synonyms), described in detail and previously well illustrated (see De Man, 1902, pp. 209–213, fig. 38; Tweedie, 1954, fig. 2; Ng & Sivasothi, 1999: fig. in p.

67; Boon et al., 2009, fig. 1 & 2; Shahdadi & Schubart, 2015, fig. 2). Among species of *Parasesarma* with distinct carapace epibranchial tooth, this species has a unique tuberculation pattern on its chelar dactyli and is easily recognizable by having large number of these tubercles (19–26) with a marked sulcus on each tubercle (Fig.7.3).

Distribution. Penang, Malaysia (De Man, 1895); Java, Indonesia (Tesch, 1917); Singapore and neighboring Islands (Tweedie, 1936); Labuan, Malaysia (Tweedie, 1950); Phuket, Thailand; Koh Chang, Thailand; Hainan, China; Brunei (present study).

Fig. 7.4. *Parasesarma* n. sp3. holotype (S134), the whole animal, A. dorsal view, B. Ventral view (scale bars 1cm).

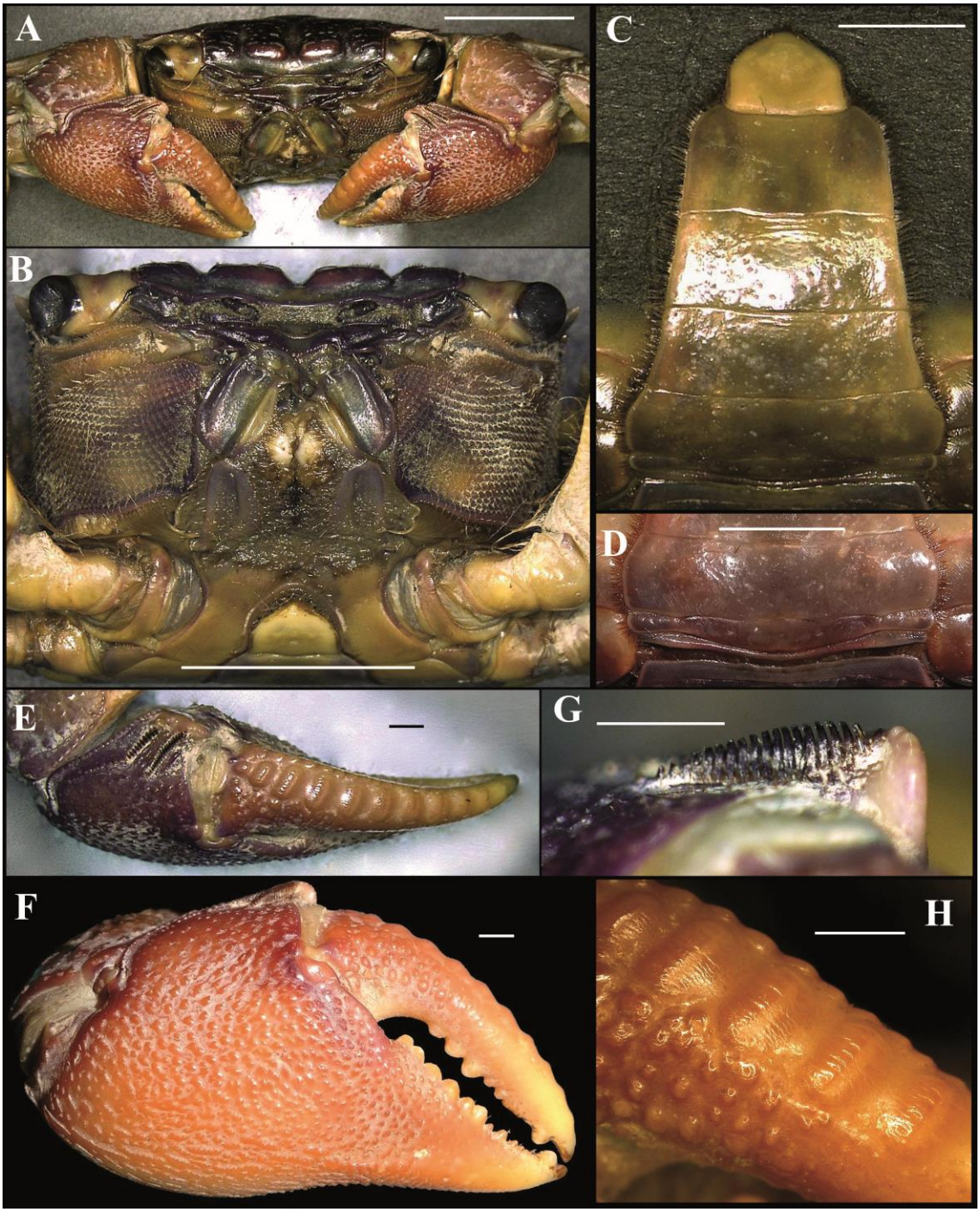

Fig. 7.5. ***Parasesarma*** **n. sp3. holotype (S134), A. Front view, B. Third maxillipeds and branchial region, C. Whole pleon, D. Pleonal somites 1–3, E. Right chela, dorsal view, F. Right chela, outer view, G. Distal pectinated crest, H. proximal chelae dactylar tubercles (scale bars A, B 1cm, C, D 5 mm, E–H 1mm).**

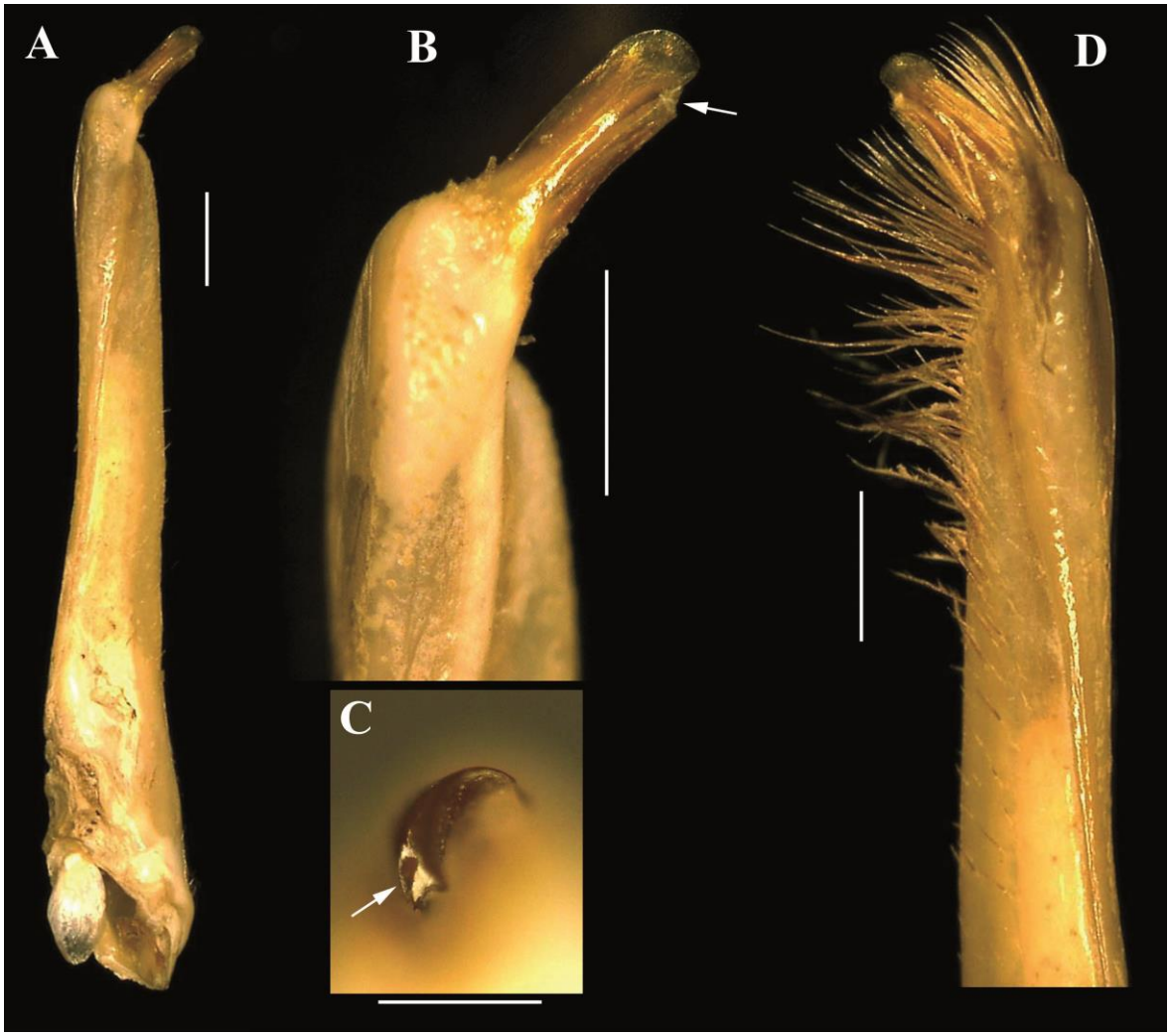

Fig. 7.6. ***Parasesarma*** **n. sp3. (paratype, S626) G1 morphology, A. Denuded right G1, dorsal view, B. Right G1, apical part, C. Cross view of apical corneous and aperture, D. Left G1 with setae (the arrows pointing to the aperture) (scale bars A, B, D 1mm, C 0.25 mm).**

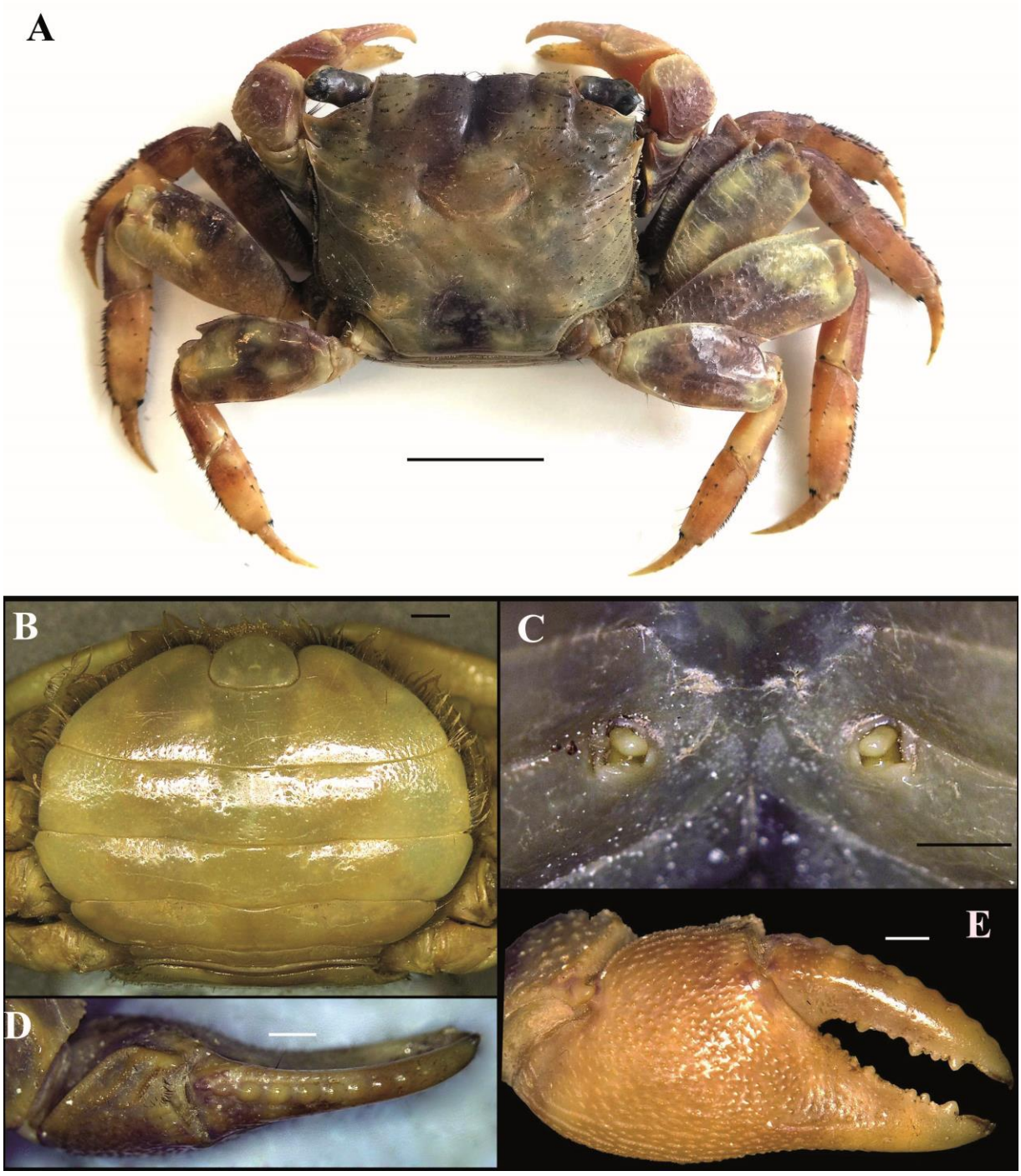

Fig. 7.7. *Parasesarma* n. sp3., a female animal (paratype, S527), A. Dorsal view of the whole animal, B. Pleon, C. Vulvae, D. Right chela, dorsal view, E. Right chela, outer view (scale bars A 1 cm, B–G 1mm).

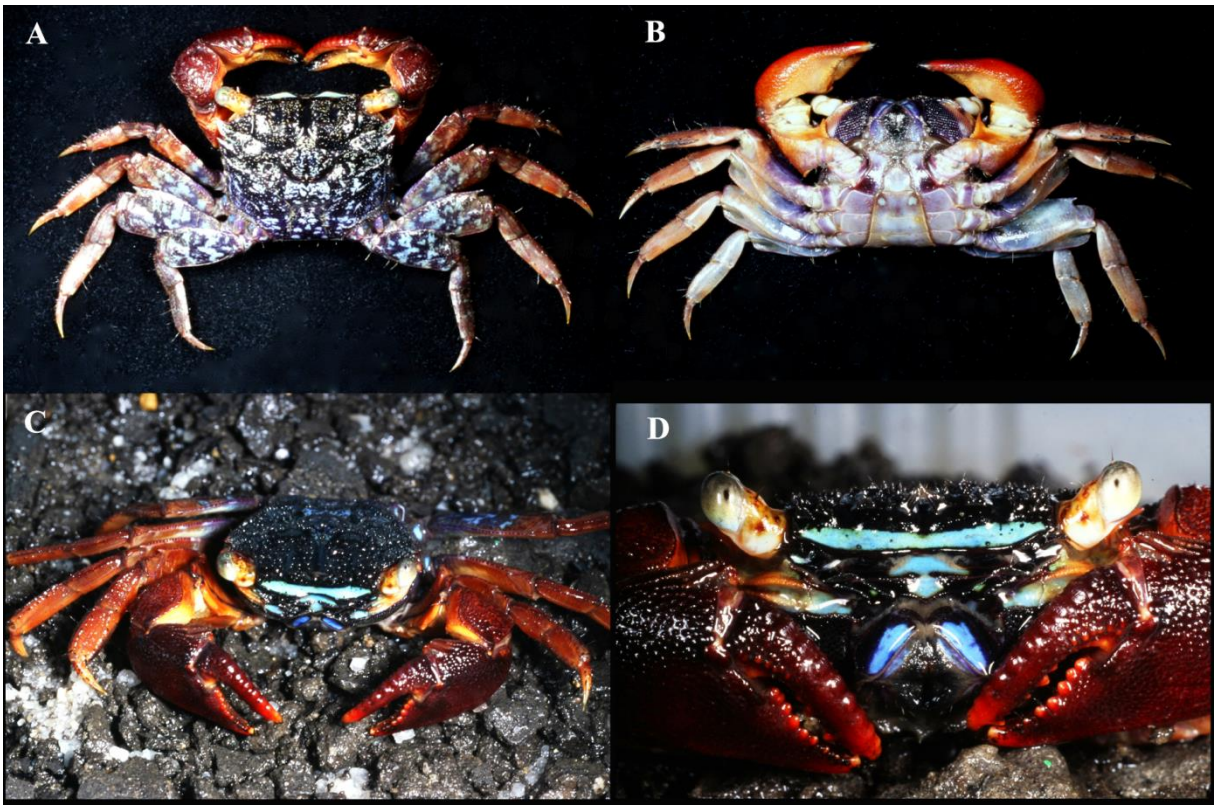

Fig. 7.8. ***Parasesarma*** **n. sp3., A&B, color photos of a freshly collected male animal from Singapore, Mandai mangrove, photographed by C. Schubart (specimen not available), A. Dorsal view, B. Ventral view; C&D, a live male crab with life color from Singapore, Palau Ubin, photographed by C. Schubart (specimen not available), C. The whole animal, D. close up photo indicating facial band and third maxiliped coloration.**

Chapter 8

General Discussion

Considering the limited number of known morphological characters to separate the species of *Parasesarma* and the uncertainty about their phylogenic usefulness (Chapter 2), this work uses genetic markers and establishes additional morphological features to address some taxonomic ambiguities. The following datasets of DNA sequences of *Perisesarma* were thereby applied to reconstruct the current phylogeny with molecular markers. A fragment of about 650 basepairs (bp) of the mitochondrial gen Cox1 (corresponding to the barcoding region = the so-called Folmer region) (Folmer et al., 1994, Schubart, 2009) and a segment of about 500 bp of the mitochondrial 16S rRNA gene for almost all extant species of *Perisesarma* (except for *P. haswelli*, for which no well-preserved specimen was available for DNA extraction, and except for 16S of *P. maipoense*) were sequenced and submitted to GenBank. A fragment of about 500 bp of the more conserved nuclear gene, NaK was also obtained for most species in order to improve the phylogeny at the generic level. Different morphological characters were also used to revise the taxonomy at both generic and species levels.

Maybe the most important contribution of this study is to put an end to the historic argument of the use of the carapace epibranchial tooth for generic delimitation to separate species of *Parasesarma* from *Perisesarma* (Chapter 4). For this purpose, this study includes some representatives of the genus *Parasesarma* besides all the species of *Perisesarma*. Moreover, representatives of other genera of the family reppresenting different phylogenetic clades (according to Schubart et al., 2006) were included to the study to evaluate the monophyly and reveal the phylogenetic positions of species of *Perisesarma* within the Sesarmidae (Chapter 4). The use of three different genetic markers with different evolutionary speed provided highly supported results and helped to clarify the phylogenetic positions of species of *Perisesarma* and also to evaluate the phylogenetic weight of some other morphological characters. The results prove that the carapace epibranchial tooth is not a phylogenetically reliable feature and species of the two mentioned genera, *Parasesarma* (without tooth) and *Perisresarma* (with tooth), which otherwise share lots of morphological similarities, are not reciprocally monophyletic groups, but phylogenetically mixed (Fig. 4.12, 4.13). Therefore, these results revealed that *Perisesarma* in its current composition is a polyphyletic group and needed to be divided into different genera.

Having different types of evidence, in conclusion we suggested a new taxonomic classification for the constituent species of *Perisesarma* by 1) transferring most species of *Perisesarma* to *Parasesarma*, 2) transferring *Perisesarma fasciatum* to a new genus and also transferring the three West African species (i.e., *Perisesarma alberti*, *Perisesarma huzardi* and *Perisesarma kamermani*) to another new genus (see taxonomic accounts in Chapter 4), so that finally *Perisesarma* is restricted to a monotypic genus comprising exclusively *Perisesarma dussumieri*. Interestingly, the morphology of the pectinated crests on the male chelar palm diagnoses the phylogenetic groups in their newly suggested generic classification, being different among these groups, but almost homogenous within each group, which highlights the evolutionary significance of this character. A more comprehensive ethological study, comparing stridulatory mechanisms and their signal production (vibration or vocal signals), similar to the study by Boon et al. (2009) for *P. indiarum* and *P. eumolpe*, among the taxa with different kinds of plectra, could evaluate better the evolutionary significance of this organ.

Three species of *Parasesarma* (according to the new generic composition) are here described as new to science. Among them, *Perisesarma* n. sp1. is a morphologically conspicuous and genetically very isolated species from southern Vietnam (Chapter 3). This shows that better sampling of areas that have not received enough taxonomic attention, will likely result in discovering more undescribed species.

Two other new species were distinguished in this study with the aid of genetic markers. *Parasesarma* n. sp3., a species from the Malay Peninsula, was assumed to belong to *P. indiarum* for a long time, because of high morphological similarities. Manydifferent biological aspects of this species have been studied since its first record from Singapore. But our molecular results showed its divergence from the types of *P. indiarum* and we found few morphological characteristics distinguishing the two species. The second example of an apparently cryptic species in this thesis is *Parasesarma* n. sp2., a northern Australian species very similar to *P. samawati* and *P. lividum*. But the genetic data show a significant divergence from both. Considering the very limited number of morphological diagnostic characters, these findings indicate that real biodiversity in this group seems to be underestimated and further studies will likely uncover more of these cases.

In contrast to hidden undescribed forms, some recognized species show very close genetic associations according to molecular results. For example the three species, *P. messa*, *P. indiarum* and *P. foresti*, have very close genetic relationships. Among them, *P. indiarum* and *P. foresti* are also very similar in morphology. *P. messa* appears to be different by having larger numbers of chelar dactylar tubercles. As discussed in chapter 2, only slight differences in this number may not be a very reliable diagnosis. Geographically, *P. messa* is known as an Australian species and the other two are Southeast Asian ones. Therefore, considering these issues, the validity of the abovementioned species is under investigation, examining more material of those species from their geographical distribution and using morphological and molecular markers. In this case, the real biodiversity may have been overestimated.

About 1.6 million extant animal species are identified on earth (Ruggiero et al., 2015). This number is increasing by describing new ones (either from remote area or cryptic forms) and according to a rough estimation, the real total species number might be in the range of 10–15 million (Hammond, 1992; Hawksworth & Kalin-Arroyo, 1995). This insight is occurring while at the same time sadly the taxonomic expertise is collapsing, and we do not have enough taxonomists to describe these diversities, even if many biological studies still depend upon species identification (Hebert, 2003). Because of this reason, and some other problems concerning traditional species identification (e.g. phenotypic plasticity, cryptic forms, morphological changes during life stages of a single species), Hebert et al. (2003) introduced the DNA-based identification as a promising substitute tool to overcome this predicament in the future. But biological identification through DNA barcode needs two precursors: first, a comprehensive DNA databank for the living species and second, accurate taxonomic classifications corresponding to genetically based phylogenies. The present study makes an important contribution to this approach by establishing DNA sequences for many species of *Parasesarma* (in most cases for first time) and also by suggesting a new taxonomic classification supported by genetic results.

Marine biogeographic research is a difficult task, because it deals with a three dimensional arena and because of difficulties in tracking samples in the field. Producing mostly large numbers of offspring and dispersing as microscopic planktonic larvae with mostly unknown biological capabilities, the corresponding species make this field of study even more intricate. Genetically

based phylogeography, however, appears to be a very helpful approach in this area of research, addressing dispersal patterns and population structures in different marine animals (see chapter 5, Introduction). For example, traditionally and for a long time it has been assumed that in marine animals, oceanic populations are interconnected by larval dispersal even in a vast geographic range (according to Becker et al., 2007). But recent molecular studies uncovered several cryptic species among marine coastal animals (Bickford et al., 2007). Phylogeographic structures of coastal animals with planktonic larvae are defined to some extent by their larval dispersal pattern. Biological capabilities/limitations (e.g., larval longevity), environmental physical factors (e.g., oceanic currents, either as barrier to dispersal or as a long distance fast carried) and ecological factors (e.g. habitat connectivity) are among the key factors that delimit the distribution of these animal.

Although the present work is a taxonomic revision and has a systematic objective, the side results give some pilot view of phylogeographic structures of several of the studied groups in Southeast Asia and Australia (Chapters 5 & 6). In chapter 5, the distribution of *P. lividum* in different South Pacific to Southeast Asian islands highlights the long-distance dispersal potential of this species. Interestingly, this study revealed that *P. samawati* from East Africa is the closest relative to *P. lividum*, among all other congeners. But the genetic results still indicate a distinct genetic disjunction between these two species and their northern Australian cousin, here described as a new species (Fig 5.1). The same pattern is found between the two close species *P. semperi* (Southeast Asia) and *P. longicristatum* (Australia) (Chapter 6): Despite their apparent capability of long distance dispersal, the Southeast Asian populations did not maintain regular gene flow with northern Australian mangrove inhabitants and may thus have caused populations of these areas to achieve genetic isolation. Confirming previous results (e.g., Ragionieri et al., 2009 for the *Neosarmatium meinerti* species complex), this study provides supporting evidences for a strong physical marine barrier isolating the northern coast of Australia from adjacent areas, driving allopatric speciation in different taxa.

Considering the newly reconstructed phylogenetic relationship among some species of *Parasesarma* (e.g., Figs. 3.5, 4.12) and their corresponding geographic distributions, it appears that allopatric speciation mediated by geographic isolation is not the only evolutionary process responsible for the current species diversity. High species diversity in *Parasesarma* (up to now

57 species), is possibly the consequence of a combination of different evolutionary mechanisms. But what is more prominent in this context, is the extreme diversities in the morphology of chelar dactylar tubercles and tuberculation patterns among the species of *Parasesarma* that otherwise show high similarity in general morphology. Ethological significance of this structure in intraspecific communication emphasizes its role in species radiation in *Parasesarma* (see Chapter 7, Discussion). Further studies need to confirm the importance of the stridulatory behavior in reproductive and communicative isolation among sympatric species of *Parasesarma*.

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