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Research paper

A new species of the genus *Parasesarma* De Man 1895 from East African mangroves and evidence for mitochondrial introgression in sesarmid crabs

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A B S T R A C T

The Sesarmidae (Decapoda; Brachyura: Thoracotremata) is the most speciose family of crabs occurring in the mangroves of East Africa, accounting for 12 species belonging to seven genera. Among these, the genus *Parasesarma* accounts for a total of four species. Here we describe a new species, *Parasesarma gazi* sp. nov., based on specimens collected in mangrove forests of Kenya and Tanzania. The phylogenetic position of this new species within the family Sesarmidae was reconstructed, based on three mitochondrial and one nuclear marker. While nuclear data genetically resolve the systematic relationships, mitochondrial data reveal a surprising similarity of *Parasesarma gazi* sp. nov. and its sister species *P. leptosoma*. This result may reflect a short history of reproductive isolation or recent mitochondrial introgression between these two species. This is the first time that such an evolutionary event is reported for the family Sesarmidae and for mangrove crabs, in general.

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1. Introduction

The mangrove forests of East Africa are inhabited by a consistently homogeneous assemblage of brachyuran crab species, distributed from southern Somalia to the Eastern Cape Province in South Africa. A long tradition of studies carried out at various localities along the whole latitudinal gradient (Macnae, 1968; Hartnoll, 1975; Icely and Jones, 1978; Vannini and Valmori, 1981) revealed that these forests harbour about 35 species of obligate and facultative mangrove crabs, mainly belonging to the families Sesarmidae and Ocypodidae. Within East African mangroves, the family Sesarmidae appears to be the richest in terms of diversity, accounting for 12 species belonging to seven genera. This predominant mangrove taxon also shows a high degree of regional endemism, since eight of the recorded species are known from this geographic region only (Hartnoll, 1975; Vannini and Valmori, 1981), which can be explained by their mode of development, as discussed in Schubart et al. (2000). Sesarmid crabs evolved peculiar adaptations for living in mangrove forests, such as tree climbing behaviour (Warner, 1967; von Hagen, 1977; Vannini and Ruwa, 1994; Cannicci et al., 1996; Cannicci et al., 1999; Fratini et al., 2005) and a diet mainly based on mangrove leaf litter (Lee, 1993; Díaz and Conde, 1988; Dahdouh-Guebas et al., 1997; Dahdouh-Guebas et al., 1999; Skov and Hartnoll, 2002). They are also known to be keystone species in these coastal forests, playing a fundamental role in ecological functions (Lee, 1998), such as carbon sink (Bouillon et al.,

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2008; Andreetta et al., 2014) and enrichment of organic production (Cannicci et al., 2008).

The genus *Parasaserma* (*sensu* Shahdadi and Schubart, 2017) is the second largest genus in terms of species numbers within the Sesarmidae and is currently represented in East Africa by four species, two of which, *P. guttatum* (A. Milne-Edwards, 1869) and *P. samawati* (Gillikin and Schubart, 2004), both characterised by well-developed epibranchial teeth, were only recently moved into this genus (Shahdadi and Schubart, 2017). The other two East African species of *Parasesarma* have no epibranchial teeth and can be distinguished as follows: *P. leptosoma* (Hilgendorf, 1869) has a flat body, adapted to climb mangrove trees and feeding on the canopy (Vannini and Ruwa, 1994; Cannicci et al., 1996). It is most abundant in the equatorial areas and far less frequent in South Africa (Emmerson et al., 2003). *P. catenatum* (Ortmann, 1897), on the other hand, has a temperate-subtropical distribution, being the most abundant crab of South African estuarine marshes (Taylor and Allanson, 1993) and present, in low densities, in the mangroves of southern Mozambique (Cannicci et al., 2009).

There is general consensus on the fact that the systematic of the very diverse Sesarmidae is in need of revision (Schubart et al., 2006; Ng et al., 2008). Only recently, DNA-based techniques were used in support of the traditional morphological approach for species descriptions and classification. The exclusive use of mitochondrial DNA (mtDNA) markers supporting species descriptions (see for example Gillikin and Schubart, 2004; Naderloo and Schubart, 2010) is nowadays often complemented with nuclear markers (see Thiercelin and Schubart, 2014). In brachyuran crabs, these genetic markers successfully allow the reconstruction of phylogenetic relationships among species (see Kitaura et al., 2002; Schubart et al., 2006; Shih et al., 2009; Tsang et al., 2014; Ip et al., 2015), the understanding of the origin of species and the dating of speciation events (see Schubart et al., 1998; Tsang et al., 2014), as well as the evolution of adaptive characters (see Schubart et al., 1998; Fratini et al., 2005; Tsang et al., 2014). For what concerns East Africa, in the last 15 years, the integrated use of molecular and morphological techniques led to the description of two new sesarmid species belonging to the genera *Perisesarma* (now moved to *Parasesarma*) and *Neosarmatium* (see Gillikin and Schubart, 2004; Ragionieri et al., 2009, 2012, respectively).

In this paper, we describe a new species of mangrove crab belonging to the genus *Parasesarma*, *P. gazi* sp. nov., collected in Tanzanian and Kenyan mangroves. We provide a morphological description of the new species and compare it with *P. leptosoma*, the only congeneric species without epibranchial teeth present in sympatry. Using genetic data corresponding to mitochondrial and nuclear DNA markers, we also reconstruct the systematic relationships of the new species within the family Sesarmidae, sharing new light upon possible speciation pathways within this family. Specimens examined are deposited in the Natural History Museum of the University of Florence (MZUF), the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum of the National University of Singapore, the National Museum of Natural History of Paris (MNHN) or used to belong to the Zoological Museum of Amsterdam (ZMA), now integrated in the Naturalis Museum in Leiden.

2. Material and methods

2.1. Morphological analysis

Eight specimens (5 males and 3 females) of the undescribed species were collected during different surveys performed in the mangroves of Ras Dege and Mtoni Creek (Tanzania) and Gazi Bay (Kenya). All specimens were collected by hand and immediately preserved in 75% ethanol, except for single legs that were placed in absolute ethanol for further genetic analyses.

Various parts of the crabs were drawn using a camera lucida fitted on a stereo microscope. The type specimens are deposited in the Zoology Section of the Natural History Museum of the University of Florence (MZUF) and in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum (ZRC) of the National University of Singapore. The carapace width and length in millimeters are provided with the description of the deposited material. The following abbreviations are used in the text: cw, maximum carapace width; cl, carapace length; iw, interorbital width; lcp, ventral length of the chelar propodus; hcp, height of the chelar propodus, 4prl, dorsal length of the fourth pereiopod; 4prp, propodus length of fourth pereiopod; 4prd, dactylus length of fourth pereiopod; tell, telson length; telw, telson width; 6abl, length of sixth abdominal segment; 6abw, width of sixth abdominal segment.

2.2. Genetic analyses

Four type specimens of the new species were selected for genetic analyses and compared with genetic data from 14 other specimens of sesarmid crabs, belonging to *Parasesarma leptosoma*; *P. catenatum*; *P. melissa* (De Man, 1887); *P. guttatum* (A. Milne-Edwards, 1869); *P. samawati* (Gillikin and Schubart, 2004); *Chiromantes ortmanni* (Crosnier, 1965); *C. eulimene* (De Man, 1895): *Sarmatium crassum* Dana, 1851; *Selatium brockii* (De Man, 1887); *Neosarmatium africanum* Ragionieri, Fratini and Schubart, 2012; *Sesarmoides longipes* (Krauss, 1893) (details on the samples are reported in Table 1).

All samples were collected by the authors in East African mangroves, except *P. melissa*, collected by N. Sivasothi, C. Barelli and L. Morino in Singapore (Table 1). Three mitochondrial genes (the cytocrome oxidase subunit I, COI, and the large and small rRNA subunits, 16S and 12S respectively) and one nuclear gene (the large rRNA subunit 28S) were partially sequenced. Voucher specimens are archived and catalogued at the Natural History Museum of the University of Florence (Table 1). Total genomic DNA extraction was performed from muscle tissue using the Qiagen QIAmp tissue kit. Dried DNA was resuspended in sterilised distilled water and preserved at $-20\,^{\circ}$C for long-term storage.

DNA was also extracted from a syntype of *Parasesarma lenzii* (De Man, 1894) collected at Atjeh, Sumatra, Indonesia, and now deposited at the Naturalis Museum in Leiden, of which 585 base pairs of the COI gene could be successfully recovered. Due to the DNA's degradation, we were unable to amplify the other three genetic markers.

Polymerase Chain Reaction (PCR) was used for amplification of the four DNA-fragments analysed in the present study. A 658 base pair (bp) fragment of the COI, approximately 600 bp of 16S and 540 bp of 12S (excluding the primer regions) were obtained by the following PCR protocol: 40 cycles: 45 s 94 °C/1 min 48–50 °C/1 min 72 °C denaturing/annealing/extension temperatures. The following primer combinations were used: for the COI fragment HCOI2198 and LCOI1490 (both Folmer et al., 1994); for the 16S region 16sar (Simon et al., 1994) and 16H10 (Schubart, 2009); and for the 12S fragment 12Sb (Simon et al., 1994) and 12L4 (Schubart et al., 2006). For the amplification of a 632 bp long segment of the nuclear rDNA 28S, the primers 28L4 and 28H4 were used (Ragionieri et al., 2009) with the following PCR conditions: 40 cycles: 65 s 97 °C/1 min 50 °C/1 min 72 °C denaturing/annealing/extension temperatures.

PCR products were purified with Sure Clean (Bioline), resuspended in water, and then sequenced with the ABI BigDye terminator mix followed by electrophoresis in an ABI Prism 310 Genetic Analyzer (Applied Biosystem, Foster City, USA). New sequences were submitted to molecular databases (accession numbers in Table 1). The sequences were corrected manually with the

Table 1
Specimens of sesarmid crabs used for genetic analyses.

#	Species	Museum collection number	Collection site	COI	16S	12S	28S
1	*Parasesarma gazi*[a]	MZUF 3677	Mtoni, Tanzania	MF564006	MF564029	MF564023	MF554644
2	*Parasesarma gazi*	MZUF 3670	Gazi Bay, Kenya	MF564005	MF564029	MF564023	MF554644
3	*Parasesarma gazi*	MZUF 3621	Gazi Bay, Kenya	MF564007	MF564029	MF564023	MF554644
4	*Parasesarma gazi*	MZUF 3603	Gazi Bay, Kenya	MF564008	MF564029	MF564023	MF554644
5	*Parasesarma leptosoma*	MZUF 2547	Mida Creek, Kenya	MF564010	AJ784024	AJ784300	MF554645
6	*Parasesarma leptosoma*	MZUF 3616	Mida Creek, Kenya	MF564009	MF564030	MF564024	MF554645
7	*Parasesarma leptosoma*	MZUF 3676	Mida Creek, Kenya	MF564011	MF564030	MF564024	MF554645
8	*Parasesarma leptosoma*[a]	MZUF 3680	Gazi Bay, Kenya	MF564012	MF564030	MF564024	MF554647
9	*Parasesarma melissa*	MZUF 2597	Singapore	MF564015	MF564031	MF564025	MF554648
10	*Parasesarma catenatum*	MZUF 2509	Mgazana, S. Africa	MF564013	AJ784025	AJ74299	MF554649
11	*Parasesarma guttatum*	MZUF 3848	Ras Dege, Tanzania	MF564016	MF564032	MF564026	MF554650
12	*Parasesarma samawati*	MZUF 3682	Mida Creek, Kenya	MF564017	MF564033	MF564027	MF554651
13	*Chiromantes ortmanni*	MZUF 2523	Gazi Bay, Kenya	MF564018	AJ784016	AJ784292	MF554653
14	*Chiromantes eulimene*	MZUF 2501	Mida Creek, Kenya	MF564019	AJ784017	AJ784291	MF554652
15	*Sarmatium crassum*	MZUF 2545	Mida Creek, Kenya	MF564021	AJ784015	AJ784302	FN392199
16	*Selatium brockii*	MZUF 2546	Mida Creek, Kenya	MF564020	AJ784022	AJ784303	MF554654
17	*Neosarmatium africanum*	MZUF 3767	Mida Creek, Kenya	FN392140	FN392170	MF564028	FN392199
18	*Sesarmoides longipes*	MZUF 2505	Mida Creek, Kenya	MF564022	AJ784026	AJ784305	MF554654
19	*Parasesarma lenzii*	ZMA CRUS. D. 102653	Sumatra, Indonesia	MF564014	na	na	na

For each, the collection number (MZUF, Natural History Museum of the University of Florence: ZMA, Zoological Museum of Amsterdam), the collection site, and the Genebank accession numbers are reported. In case of species for which more specimens have been sequences,
[a] Indicates the specimens included in phylogenetic inference analysis.

program CHROMAS v. 1.55 (Technelysium Pty Ltd, Queensville, Australia) and aligned by eye with BioEdit v. 7.2.5 (Hall, 1999).

We calculated the mean genetic p-distance among taxa with MEGA 6.0.6 (Kumar et al., 2004) with a simple nucleotide model (number of substitutions), with homogeneous patterns among lineages and a uniform rate among sites. The standard errors for the overall mean p-distance values as well as the p-distances between groups and within groups (in case more than one specimen per species was sequenced) were calculated by 500 bootstrap replicates.

MODELTEST v. 3.6 (Posada and Crandall, 1998) was used for determining the best fitting model of sequence evolution. The best model and the likelihood parameters were calculated for each gene separately and for the concatenated alignment of the three mtDNA genes, applying the Akaike Information Criterion (AIC).

Phylogenetic congruence among COI, 16S and 12S data partitions was tested using the incongruence length difference (ILD) test (Farris et al., 1995) implemented in PAUP version 4.0 (Swofford, 2002) as the partition-homogeneity test (Swofford, 2002). For this test, we used random taxon addition, TBR branch swapping, and a heuristic search with 1000 randomisations of the data.

Two methods of phylogenetic inference were applied to the combined COI + 16S + 12S dataset: Maximum likelihood (ML) using PAUP (Swofford, 2002) and Bayesian Inference (BI) as implemented in MrBayes v. 3.2.6 (Huelsenbeck and Ronquist, 2001). Due to its lower mutation rate and resolution, the 28S dataset was analysed using BI only. For ML, the parameters of the suggested model of evolution were implemented in PAUP. The ML starting tree was obtained via stepwise addition and replicated ten times, with each replicate starting with a random input order of sequences. Clade support was obtained from 500 bootstrap replicates (Felsenstein, 1985). BI analyses were calculated with four MCMC chains for 2,000,000 generations, saving a tree every 500 generation (with a corresponding output of 4000 trees). The -lnL converged on a stable value after 8000 generations. The first 8000 generations were thus discarded from the analysis and the posterior probability was determined by constructing a 50% majority rule consensus of the remaining trees. *Sesarmoides longipes* was used as outgroup in inference phylogenetic analyses, in accordance to Schubart et al. (2006). All phylogenetic trees were reconstructed including one specimen per species. These phylogenetic analyses were run excluding *P. lenzii*. This sample was instead included in a separate BI analysis (run as described above) based on the COI alignment only.

Finally, a minimum spanning network was built with TCS version 1.21 (Clement et al., 2000), based on the combined mtDNA dataset of the four specimens of *Parasesarma gazi* sp. nov. and *P. leptosoma*. The network was calculated using a total of 1591 bp of mtDNA, since for this analysis all the sequences need to be of equal length. In the network, every line between two points indicates a nucleotide substitution, the connection limit was fixed at 95%, and the proposed ancestral haplotype is represented by a rectangle. No gaps were present in this dataset.

3. Results

3.1. Morphology and systematic account

FAMILY SESARMIDAE DANA, 1851
GENUS PARASESARMA, DE MAN, 1895
PARASESARMA GAZI Cannicci, Innocenti and Fratini sp. nov.
Sesarma (Parasesarma) lenzii De Man 1894. – as used by Crosnier (1965): 66–67, figs. 98–101.

3.2. Material examined

Holotype: male (16.9 × 13.8) (MZUF 3670), Kenya: Gazi Bay, mangroves near the fishermen landing site (4°25’ S 39°30’E), Oct. 2007, coll. S. Cannicci, (DNA sequences). Paratypes: 1 male (15.7 × 12.9 mm) (MZUF 3671), 1 male (15.15 × 12.9 mm) (ZRC 2017.0467), 2 females (12.4 × 10.0 mm; 12.0 × 10.0 mm; (MZUF 3673; 3674), 1 female (12.65 × 9.9 mm) (ZRC 2017. 0468), same data as holotype; 1 male (14.65 × 12.3 mm) (MZUF 3677), Tanzania: Dar es Salaam, Mtoni Creek mangroves (6°53’S 39°18’E), Oct. 2005, coll. S. Cannicci and J. Paula; 1 male (14.2 × 11.7 mm) (MZUF 3678), Tanzania: Ras Dege mangroves (6°52’S 39°27’E), Oct. 2005, coll. S. Cannicci and J. Paula.

Comparative material. *Parasesarma leptosoma*: male (15.5 × 12.7 mm) (MZUF 3603), Kenya, Gazi Bay, (4°25’ S 39°30’E), Mar. 2007, coll. L. Ragionieri and M. Fusi, female (18.7 × 17.0 mm) (MZUF 3679), Kenya, Mida Creek, (3°20′S 40°00′E), Nov. 1997, coll. S. Fratini, male (19.3 × 16.5 mm) (MZUF 4869), Kenya, Mida Creek, (3°20′S 40°00′E), Oct. 1990, coll. M. Vannini, female (17.6 × 15.0 mm) (MZUF 4870), *Parasesarma lenzii* (De Man, 1895), syntype male (13.3 × 11.3 mm) (ZMA CRUS. D. 102653) (now in

Naturalis Biodiversity Center, Leiden, The Netherlands), Indonesia, Sumatra, Atjeh.

3.3. Description

A medium-sized crab species (largest male encountered measures 17.01 mm cw) with trapezoidal carapace shape (Fig. 1A, B, H, 2A): Anterior margin of carapace wider than posterior (ratio = 1.14 ± 0.02, N = 8). Carapace broader than long (cl/cw = 0.082 ± 0.01, N = 8). Carapace regions well defined (Figs. 1 A, B, H, 2 A). Carapace relatively smooth, with very sparse rows of granules exclusively on the frontal lobes. Front broader than half of carapace width (iw/cw = 0.60 ± 0.01, N = 8), strongly deflexed, with broad median indentation. Interorbital region subdivided into four prominent, smooth frontal lobes, median lobes slightly broader than lateral ones (Figs. 1 B and D, 2 A). Anterolateral margin with acute and outward angle, no epibranchial tooth. Posterior lateral margins sub-parallel, slightly concave. Pterygostome and branchiostegites with dense cover of geniculate setae. Eyestalks short, pigmented cornea large and wider than eyestalks. Suborbital ridge granular and setose. Epistome granular and glabrous; ventral border of epistome lined by row of tubercles (endostomial crista). Third maxillipeds granular, merus broad (3mxl/3mxw = 0.77 ± 0.01, N = 8).

Chelipeds sexually dimorphic; in males subequal (lcp left cheliped/lcp right cheliped = 1.1, hcp left cheliped/hcp right cheliped = 1.37, for the holotype), while equal in females (Fig. 1H). Male chelae larger than female ones (hpc/cw = 0.36 ± 0.01, N = 5, and hpc/cw = 0.22 ± 0.01, N = 3, respectively). Outer face of cheliped merus ample and covered with granules, ventral face with scattered granules; inner face of carpus smooth, lined with rows of small tubercles, inner border lined with minute granules; external margin of the carpus smooth, dorsal face trapezoidal, with transverse rows of small granules. Upper surface of palm in males with three transverse crests, of which the two distal ones are more conspicuous and pectinated, whereas the proximal one is still discernible as a row of granules (Figs. 1 F and 2 G); the three crests end gradually to a row of granules that bend and fuse in proximal part of the face of the palma; the crests are surrounded by granules that are also present on the inner face of the palm; in females, all crests are less conspicuous and granulated. In both sexes the outer and the inner faces of the palm are covered with granules; the ventral border between these two faces consists of a row of tubercles that are less conspicuous distally and end at the beginning of the fixed finger; palm otherwise glabrous. External face of both fingers almost smooth, with very sparse, small granules; dorsal surface of dactylus bearing 10–12 blunt, transversely elongated tubercles, becoming smaller towards the distal part (Figs. 1 F and 2 G); in females, the tubercles on the dactylus are less prominent. The cutting edge of the dactylus bears 7–8 subequal teeth, evenly distributed, while the fixed fingers has 5–6 small teeth along the proximal part of the cutting edge, and 5–6 large ones towards the tip. Finger tips chitinous.

Pereiopods 2–5 relatively long and slender (Fig. 1A, B, H), fourth pair longest (4prl/cw = 1.66 ± 0.05, N = 8); dactylus medium sized (4prd/4prp = 0.16 ± 0.01, N = 8), slightly bent and ending in a sharp tip. Walking legs glabrous, but bearing scattered long setae on both ventral and dorsal borders, becoming more abundant on the propodus and dactylus; dorsal face of meri and propodi granulated; dorsal margin of meri with a subdistal, pointed spine.

Male abdomen with telson slightly shorter than wide at base (tell/telw = 0.87 ± 0.05, N = 5, Fig. 2B), rounded and approximately as long as the sixth abdominal segment (tell/6abl = 0.98 ± 0.02, N = 5); sixth segment nearly half as long as wide (6abl/6abw = 0.46 ± 0.02, N = 5). In females, the abdomen is evenly rounded, broadest at the fifth segment, and fringed with long setae; telson wider than long (tell/telw = 0.79 ± 0.04, N = 3) and slightly shorter than the sixth abdominal segment (tell/6abl = 0.93 ± 0.04, N = 3). Thoracic sternites in both sexes smooth and almost glabrous.

First male gonopods slender, almost straight (Fig. 2C); apical process bent by approximately 15°, with corneous tip (Fig. 2D, E); very few and scattered short setae along most of the gonopod, apical end covered by longer setae, completely covering corneous tip (Fig. 2D, E). Female gonopores prominent, located between the 6th and 5th sternite, near the bottom of sternal cavity (Fig. 1I).

3.3.1. Colouration

In live animals, the carapace of *Parasesarma gazi* sp. nov. is characterized by a dark brown colour, consistently mottled with numerous light brown-greenish patches (Fig. 1A). The mottled pattern extends to the ocular peduncles, while the corneas are brown black. Front and epistome bear the same mottled pattern, pterygostomial region pale grey, becoming darker on branchiostegal region. Suborbital region brown. Merus of cheliped brown, with small pale grey spots on both faces; carpus brown; palm and dactylus brown in the proximal parts, but becoming pale grey in the distal part. Only the dorsal face of the dactylus maintains the brown colouration, but the tip of the more prominent dactylar tubercles becomes pale grey. Ambulatory legs brown, with meri and carpi bearing small pale grey spots, which are present, but not abundant on the propodi.

3.3.2. Ecology

At all three sites, *Parasesarma gazi* sp. nov. was found in the *Rhizophora mucronata* Lamk, 1804 dominated belt of the mangrove forest, on the muddy floor. Only the specimens collected at Gazi Bay were collected on the lower part of *R. mucronata* roots, and there was no evidence of climbing behavior, such as in *P. leptosoma*. In all cases, they were found in areas where the by far dominant crab species was *P. guttatum*, with which they were mixing and totally overlapping.

3.3.3. Etymology

The name of this new species, *Parasesarma gazi*, is derived from Gazi Bay (also named Maftaha Bay) and the village of Gazi, on the South coast of Kenya. In Arab, *gazi* or *ghazi* is the active participle of the verb *gaza* 'to strive for' or also 'the one who struggles'. The new species is dedicated to the people of Gazi village and to the staff of the Gazi Mangrove Research Station of KMFRI, since without their help the largest known population of this new species would never have been found. We also believe that 'the one who struggles' correspondingly applies to mangrove crabs and their challenging adaptations to intertidal life, and that they are 'key' in mangrove ecosystems. The specific name is used as a noun in apposition.

3.3.4. Remarks

From a morphological point of view, the above described specimens of *Parasesarma gazi* sp. nov. definitely appears identical to the specimens described as *Sesarma (Parasesarma) lenzii* De Man 1894, by Crosnier (1965). The general morphology of carapace, cheliped, abdomen, crests of the upper palm, dactylar tubercles and gonopods (Crosnier, 1965: Figs. 98-101, Fig. 1C) leaves no doubt about the fact that the specimens described here and by Crosnier (1965) belong to the same species. We could not perform any genetic analysis on the original specimen collected by Crosnier, but the morphological comparisons between the specimen described in Crosnier (1965) and the syntype of *Parasesarma lenzii* De Man 1894 from Indonesia clearly shows that these are two different species (see below).

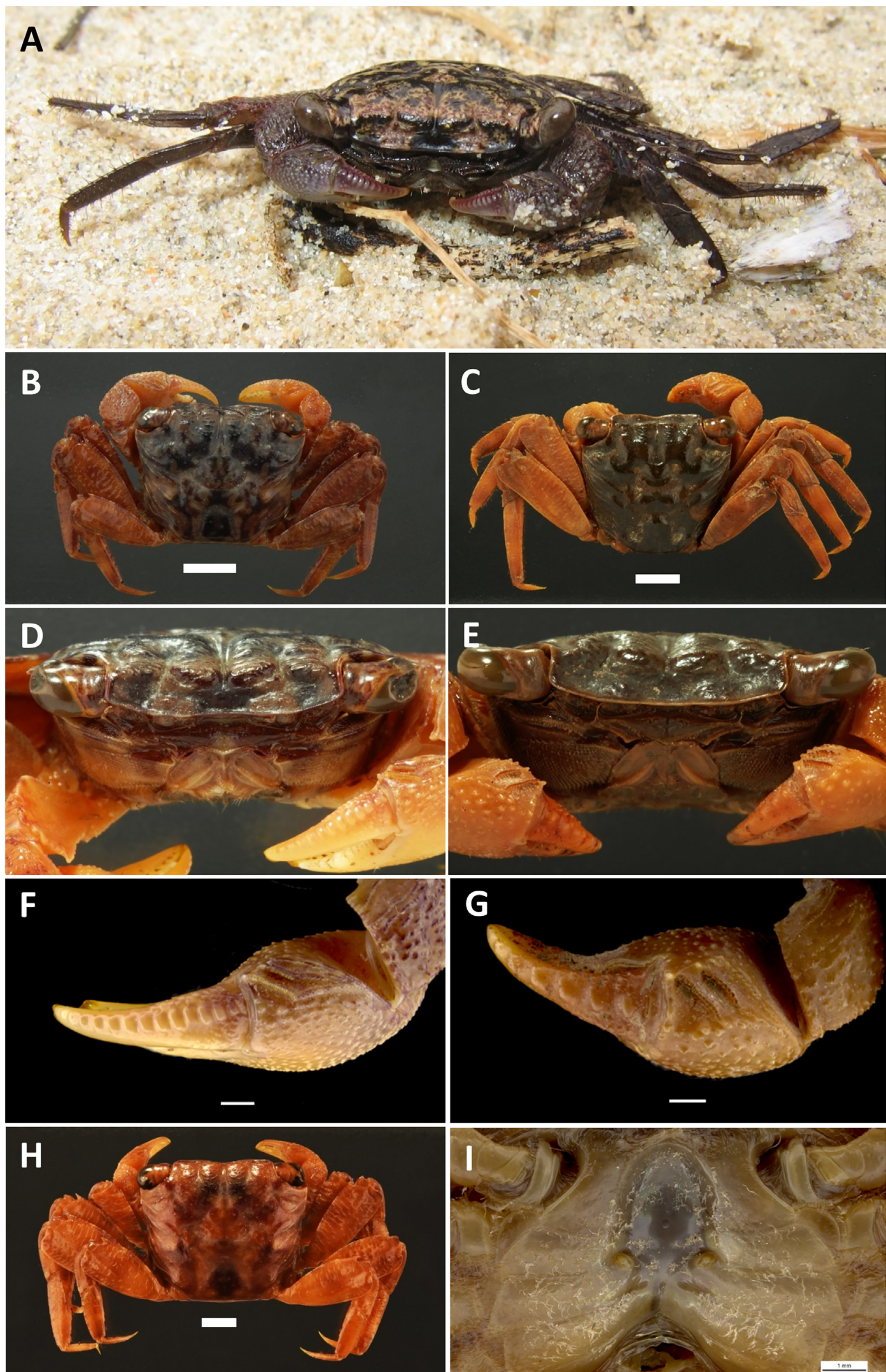

Fig. 1. *Parasesarma gazi* sp. nov. life colours (A), paratype male (14.2 × 11.7 mm) (MZUF 3678); upper view (B), frontal view of cephalothorax (D) and upper view of left chela of a male (F), holotype male (16.9 × 13.8 mm) (MZUF 3670). *Parasesarma leptosoma* (Hilgendorf, 1869), upper view (C), frontal view of cephalothorax (E) and upper view of right chela of a male (G) (15.5 × 12.7 mm) (MZUF 3603). *P. gazi* sp. nov., upper view (H) and view of gonopores (I) in a female paratype specimen (MZUF 3674). Scale bars = 5 mm.

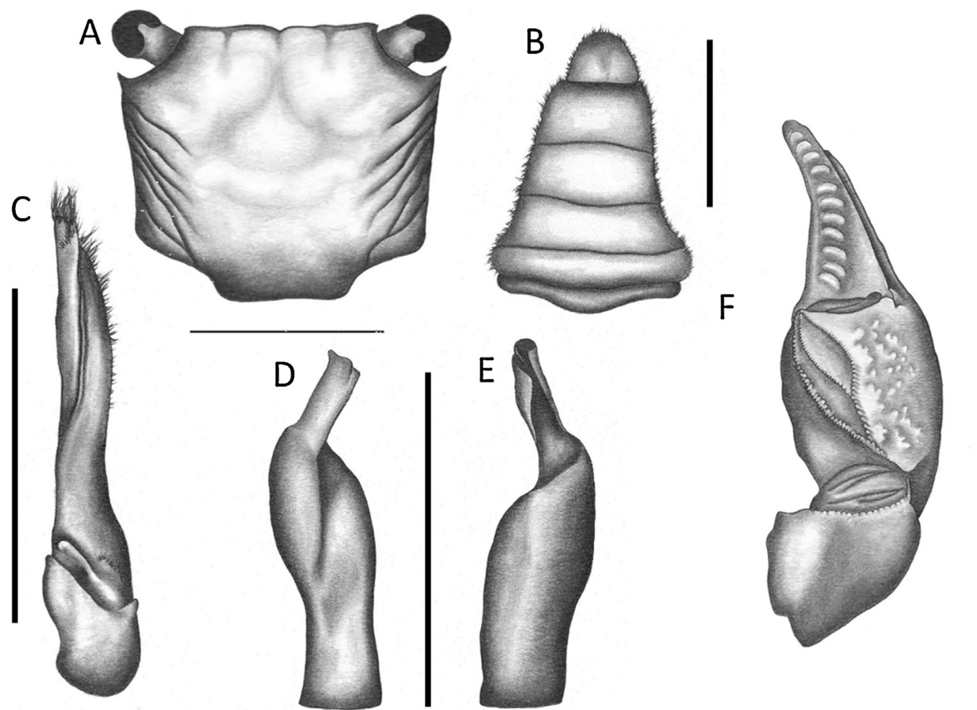

Fig. 2. *Parasesarma gazi* sp. nov. dorsal view of carapace (A), pleon (B), left G1 (dorsal view, C), distal part of left G1 (dorsal view, D), distal part of left G1 (ventral view, E), upper view of the right chela of a male (F), holotype male (16.9 × 13.8 mm) (MZUF 3670). Scale bars: A, F = 10 mm; B, C, D E = 5 mm.

3.4. Morphological differences between Parasesarma gazi *sp. nov. and* P. leptosoma

The two species differ remarkably from a morphological point of view (Fig. 1A–G). Although the front of *Parasesarma gazi* sp. nov. is also deflexed, the one of *P. leptosoma* is much more so, forming a very acute angle (Fig. 1E). Another obvious difference concerns the ratio between the propodus and dactylus in walking legs, which is around 3 in *P. leptosoma* (see Vannini et al., 1997) and 1.77 ± 0.09 (N = 8) in *Parasesarma gazi* sp. nov. The number and the shape of the dactylar tubercles are also different: *P. leptosoma* bears 6–7 small tubercles, almost conical in shape, while *Parasesarma gazi* sp. nov. has 10–12 larger, transversely elongated tubercles, as described above (Fig. 1F–G). In live animals, the claws of *P. leptosoma* are orange, especially in the distal parts of both fingers, while the claws of *Parasesarma gazi* sp. nov. are pale grey to white.

3.5. Morphological differences between Parasesarma gazi *sp. nov. and* P. lenzii

The most striking difference between the syntype of *P. lenzii* and the males of *Parasesarma gazi* sp. nov. is the morphology of the first male gonopods (Fig. 3D). In *Parasesarma gazi* sp. nov. they are almost straight (Fig. 2C), with the apical process bent by approximately 15° (Fig. 2D, E), while in *P. lenzii* the apical process bends much more, by approximately 35° (Fig. 3D, E). In the upper surface of the chelar palm of the male syntype of *P. lenzii* the three transverse crests (Fig. 3C), of which the two distal ones are still more conspicuous and pectinated, do not end with visible rows of granules bending and fusing together, as in *Parasesarma gazi* sp. nov. males (Fig. 2G), although some granules are visible. *P. lenzii* dactylar tubercles are on average larger in number, 12–13, and smaller in shape, with respect to *Parasesarma gazi* sp. nov. (Figs. 1 F, 3 C). The carapace of *P. lenzii* is also smoother than the one of *Parasesarma gazi* sp. nov., lacking the rows of granules in the frontal region (Fig. 3A, B).

3.6. Genetic analysis

We obtained sequence alignments of a total of 1842 bp of three mitochondrial genes and 609 bp of the nuclear 28S. No variable regions were excluded from the alignment before the phylogenetic analysis. The nucleotide composition and variation (i.e. the number of variable and parsimony-informative sites, the mean number of pairwise transitions and transversions) for each analysed fragment and the combined mtDNA alignment are reported in Table 2, together with the selected evolutionary model. All the mitochondrial sequences are A-T rich (Table 2) in agreement with observation of an A-T bias for mitochondrial DNA of arthropods (Simon et al., 1994), while a G + C bias was recorded for the nuclear 28S (Table 2). The selected models of DNA substitution by AIC were the GTR model for all the genes and the combined mitochondrial dataset, plus G (except for 28S) and I (except for 16S) (Rodríguez et al., 1990) (Table 2).

Tables 3 and 4 report the pairwise p-distance matrices for the four analysed genes. The overall mean p-distance values are 7.5 ± 0.8 for the 12S mtDNA, 5.5 ± 0.6 for the 16S mtDNA, 8.7 ± 0.6 for the COI mtDNA, and 3.0 ± 0.4 for the nuclear 28S. The highest mutation rate is thus associated to the mitochondrial coding gene, as expected (see Avise, 2004), while, the nuclear ribosomal gene is the most conservative with a mutation rate 2-3 times lower than the average of the three mitochondrial genes.

The species pair *Parasesarma gazi* sp. nov. – *P. leptosoma* is characterised by very low p-distance values for all three mitochondrial genes (12S p-distance = 0.4 ± 0.3; 16S p-distance = 0.6 ± 0.3; COI p-distance = 0.7 ± 0.3) in comparison to all the other pairs (Tables 3 and 4). These values are 5–10 times lower with respect to other congeneric pairwise comparisons (see, for example *P. guttatum* - *P. samawati* and *C. eulimene* – *C. ortmanni* in Tables 3 and 4). For the COI gene, the average of all pairwise genetic distances involving *Parasesarma gazi* sp. nov. (*P. leptosoma* excluded) is 11.0 ± 0.7. Otherwise, the 28S pairwise *Parasesarma gazi* sp. nov. – *P. leptosoma* p-distance value is 0.8 ± 0.4, and thus higher than that recorded for

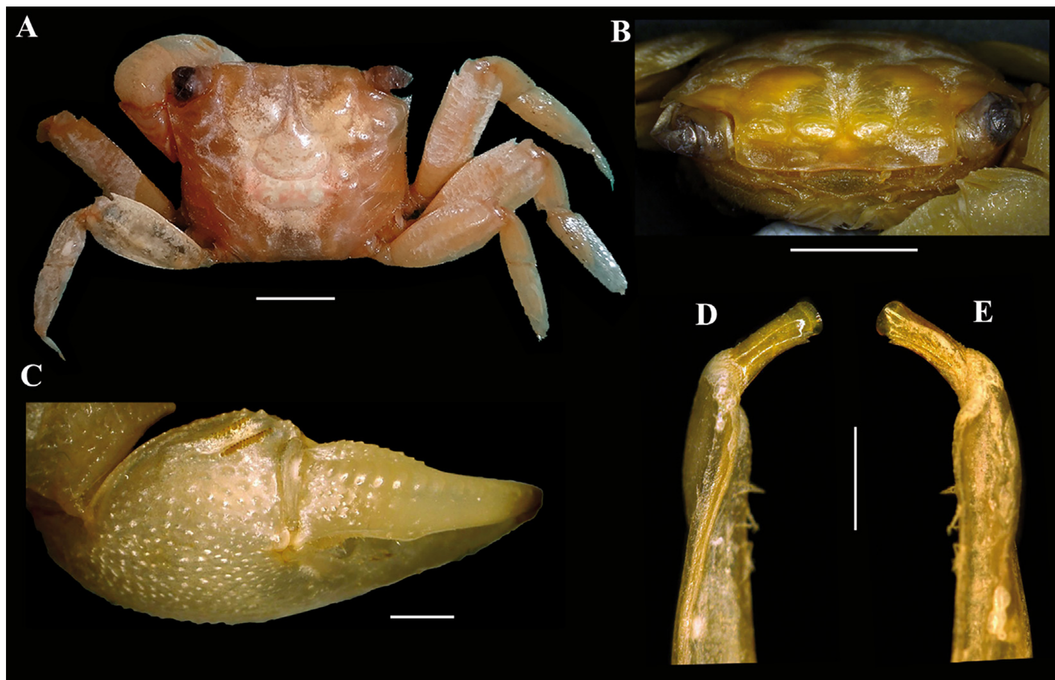

Fig. 3. *Parasesarma lenzii*, syntype, male, 27 × 11.28 mm, Leiden, ZAM CRUS. D. 102653. Dorsal view (A); frontal view (B); right chela, upper view (C); dorsal (D) and ventral view (E) of the distal part of left G1. Scale bars: A, B 5 mm, C 1 mm, D, E 0.5 mm.

Table 2
Information on mtDNA and nuclear gene fragments amplified for the present study as well as for the combined COI + 16S + 12S dataset.

Data set	T	C	A	G	bp	var	pi	Ts	Tv	R	Pinvar	α	Evolutionary model
COI	38.4	15.6	29.3	16.6	658	195	131	27.97	25.41	1.22	0.61	2.20	GTR + I + G
16S	38.8	9.0	36.7	15.6	619	148	90	17.19	17.86	1.08	0	0.15	GTR + G
12S	38.4	7.5	42.7	11.4	565	155	88	20.22	13.12	1.69	0.44	0.51	GTR + I + G
COI + 16S + 12S	38.5	11.1	35.6	14.8	1842	499	309	66.20	57.31	1.49	0.63	1.66	GTR + I + G
28S	17.7	32.5	13.9	35.9	609	68	38	11.56	4.94	2.40	0.77	0	GTR + I

Base frequencies, total number of base pairs (bp), number of variable (var) and parsimony-informative (pi) sites, mean number of pairwise transitions (Ts), transversions (Tv) and transition to transversion ratios (R), proportion of invariant sites (Pinvar), α value of gamma distribution, and the evolutionary model selected using the AIC (for more details see the text).

Table 3
Pairwise p-distance values for the nuclear 28S gene (below the diagonal) and the mtDNA COI gene (above the diagonal).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*P. gazi*	–	0.0	0.0	0.0	0.8	0.7	0.9	0.8	9.0	10.6	11.1	10.2	8.9	10.8	7.3	9.1	9.3	14.70
2	*P. gazi*	0.0	–	0.0	0.0	0.7	0.7	0.8	0.7	9.0	10.6	11.1	10.1	8.7	10.7	7.4	9.1	9.0	14.4
3	*P. gazi*	0.0	0.0	–	0.0	0.6	0.7	0.6	0.6	8.7	10.3	10.7	10.2	8.9	10.7	7.2	9.0	8.9	14.4
4	*P. gazi*	0.2	0.2	0.2	–	0.7	0.7	0.8	0.6	8.9	10.5	11.1	10.2	8.8	10.7	7.3	9.1	9.2	14.6
5	*P. leptosoma*	0.7	0.7	0.7	0.9	–	0.0	0.0	0.0	8.6	10.2	10.2	10.1	9.3	10.6	7.2	8.8	8.9	14.6
6	*P. leptosoma*	0.7	0.7	0.7	0.9	0.0	–	0.0	0.0	8.7	10.0	10.4	10.1	9.0	10.5	6.8	8.6	8.2	14.5
7	*P. leptosoma*	0.7	0.7	0.7	0.9	0.0	0.0	–	0.0	8.9	10.4	10.4	10.1	9.2	10.5	7.5	9.1	9.0	14.8
8	*P. leptosoma*	0.7	0.7	0.7	0.9	0.0	0.0	0.0	–	8.5	10.1	10.2	10.1	9.2	10.6	7.1	8.7	8.8	14.5
9	*P. melissa*	2.2	2.2	2.2	2.5	2.5	2.6	2.7	2.6	–	10.3	8.9	8.0	11.0	10.5	7.8	9.3	10.3	16.0
10	*P. catenatum*	2.4	2.4	2.4	2.5	2.7	2.7	2.7	2.7	0.8	–	12.1	12.4	12.1	14.1	8.8	10.3	11.4	15.5
11	*P. guttatum*	2.0	2.0	2.0	2.4	2.4	2.4	2.5	2.4	0.5	1.0	–	6.6	13.4	12.9	10.6	10.8	12.4	16.9
12	*P. samawati*	2.0	2.0	2.0	2.4	2.4	2.4	2.5	2.4	0.5	1.0	0.0	–	11.6	12.4	10.2	10.4	11.2	17.1
13	*C. ortmanni*	3.2	3.2	3.2	3.6	3.2	3.2	3.4	3.2	2.9	2.7	2.4	2.4	–	6.5	9.5	9.5	10.7	13.2
14	*C. eulimene*	3.4	3.4	3.4	3.8	3.4	3.4	3.6	3.4	3.0	2.9	2.5	2.5	0.2	–	10.0	9.7	10.6	14.6
15	*S. crassum*	4.9	4.9	4.9	5.5	4.9	5.0	5.3	5.0	5.6	5.4	5.1	5.1	5.3	5.4	–	7.7	7.8	12.8
16	*S. brockii*	3.4	3.4	3.4	3.4	3.7	3.8	3.6	3.6	4.9	4.7	4.4	4.4	5.2	5.4	4.8	–	9.1	15.5
17	*N. africanum*	4.0	4.0	4.0	4.2	4.2	4.2	4.2	4.2	4.7	4.5	4.2	4.2	4.5	4.7	5.1	4.3	–	14.9
18	*S. longipes*	4.1	4.1	4.1	4.4	4.5	4.5	4.5	4.5	4.5	4.1	3.9	3.9	4.3	4.5	4.9	5.2	3.7	–

Species codes as in Table 1.

P. melissa – *P. guttatum*, *P. melissa* – *P. samawati*, *C. eulimene* – *C. ortmanni* (Table 3), as well as for *P. guttatum* – *P. samawati* whose interspecific p-distance value is zero (Table 3).

As expected with degraded DNA template, the size of the PCR product obtained from the *P. lenzii* syntype was a little shorter than that from fresh materials (i.e. 585 bp long). Based on the genetic comparison of this COI fragment, we recorded a pairwise p-distance value between *Parasesarma gazi* sp. nov. and *P. lenzii* of 2.2 ± 0.2, i.e. a value that indicates a clear-cut genetic separation between these two species confused by Crosnier (1965). A similar value

Table 4
Pairwise p-distance values for the mtDNA 12S (below the diagonal) and 16S genes (above the diagonal).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*P. gazi*	–	0.2	0.2	0.2	0.8	0.9	0.9	0.7	9.5	8.0	7.7	8.2	9.2	8.6	8.6	5.6	7.0	10.1
2	*P. gazi*	0.0	–	0.0	0.2	0.5	0.7	0.7	0.5	9.6	8.0	7.7	8.3	8.9	8.4	8.5	5.4	6.8	9.8
3	*P. gazi*	0.0	0.0	–	0.2	0.5	0.7	0.7	0.5	9.6	8.0	7.7	8.3	8.9	8.4	8.5	5.4	6.8	9.8
4	*P. gazi*	0.0	0.0	0.0	–	0.7	0.7	0.7	0.7	9.7	8.3	8.0	8.5	9.3	8.7	8.7	5.6	7.1	9.9
5	*P. leptosoma*	0.4	0.4	0.4	0.4	–	0.0	0.0	0.0	9.1	7.7	7.3	8.1	8.9	8.5	8.1	5.4	7.6	10.1
6	*P. leptosoma*	0.4	0.4	0.4	0.4	0.0	–	0.0	0.0	8.2	7.3	7.0	7.5	8.6	8.0	7.4	5.4	6.7	10.3
7	*P. leptosoma*	0.4	0.4	0.4	0.4	0.0		–	0.0	8.2	7.3	7.0	7.5	8.6	8.0	7.4	5.4	6.7	10.3
8	*P. leptosoma*	0.4	0.4	0.4	0.4	0.0	0.0	0.0	–	9.1	7.4	7.1	7.8	8.9	8.5	8.1	5.4	7.6	10.1
9	*P. melissa*	9.5	9.5	9.5	9.5	9.8	9.2	9.2	9.2	–	8.3	6.5	6.1	11.5	10.2	8.6	10.3	9.2	12.5
10	*P. catenatum*	13.7	13.7	13.7	12.5	13.3	12.2	13.7	13.2	7.5	–	5.2	5.0	9.6	8.8	8.6	7.1	7.6	9.6
11	*P. guttatum*	12.4	12.4	12.4	10.8	12.0	10.9	12.8	12.1	7.9	7.6	–	2.6	10.6	9.4	7.6	7.5	8.0	10.2
12	*P. samawati*	10.8	10.8	10.8	10.8	11.7	11.1	11.2	11.2	7.6	9.2	5.6	–	10.4	9.3	8.2	7.9	8.0	11.0
13	*C. ortmanni*	9.9	9.9	9.9	9.9	10.8	10.1	10.1	10.1	10.4	8.2	7.8	8.6	–	3.8	8.5	9.4	8.5	10.3
14	*C. eulimene*	9.1	9.1	9.1	9.1	9.4	8.9	8.9	8.9	8.2	7.0	7.0	7.8	2.7	–	8.0	9.0	8.0	9.6
15	*S. crassum*	9.9	9.9	9.9	9.9	10.3	9.7	9.7	9.7	10.2	10.2	9.5	10.3	8.6	8.2	–	9.3	8.2	11.5
16	*S. brockii*	9.9	9.9	9.9	9.9	10.8	10.2	10.2	10.2	7.7	9.5	8.8	9.1	9.3	8.6	9.6	–	7.7	9.8
17	*N. africanum*	10.3	10.3	10.3	10.3	11.2	10.6	10.6	10.6	9.2	9.3	8.5	9.3	7.5	6.7	9.7	8.5	–	8.9
18	*S. longipes*	11.7	11.7	11.7	11.7	12.1	11.4	11.4	11.4	10.8	11.4	12.5	12.2	9.8	9.6	12.2	11.1	10.0	–

Species codes as in Table 1.

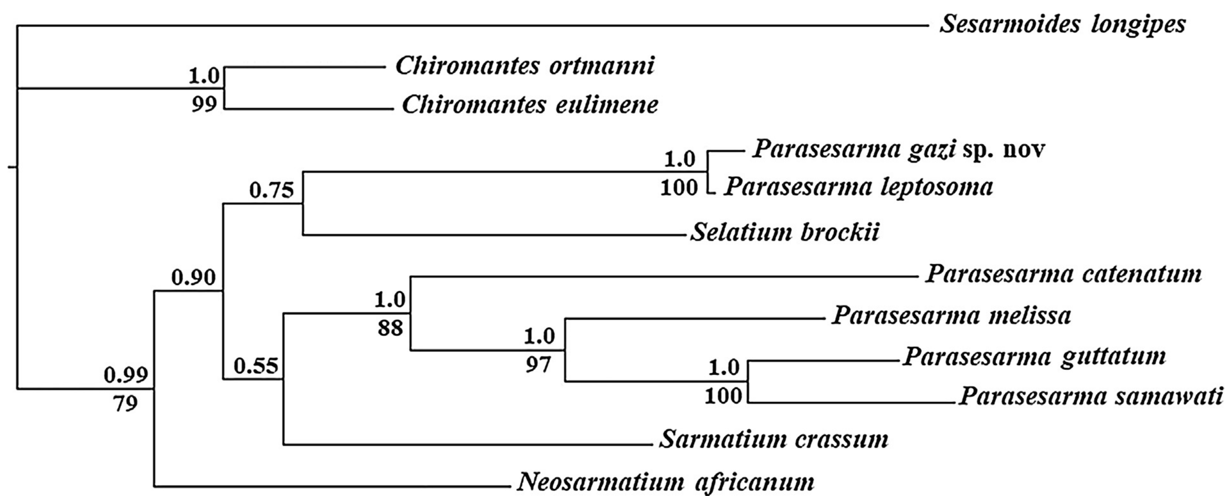

Fig. 4. Phylogenetic BI consensus tree of 12 Indo-Pacific sesarmid crabs based on 1842 bp of mtDNA, encoding the COI as well as the 16S and 12S rRNAs genes. Bayesian Inference (upper) and maximum likelihood (lower) confidence values are reported on each node. Only confidence values higher than 50% are reported on the tree.

comes from the *P. leptosoma*-*P. lenzii* comparison (2.5 ± 0.3), while it increases to 8.4 ± 1.1 and 10.1 ± 1.3 in the pairwise comparisons with other two analysed *Parasesarma* species (i.e. *P. melissa* and *P. catenatum*).

The ILD test indicated that character congruence among 12S, 16S and COI could not be rejected (P = 0.275), allowing us to use the combined dataset for all further phylogenetic inference analyses. Moreover, the test for homogeneity of base frequencies composition indicated that homogeneity could not be rejected across taxa both for the combined mitochondrial dataset (with outgroup, i.e. *Sesarmoides longipes*: $\chi^2 = 58.03$, d.f. = 54, P = 0.33; without outgroup: $\chi^2 = 43.92$, d.f. = 51, P = 0.75) and for the 28S dataset (with outgroup: $\chi^2 = 8.03$, d.f. = 54, P = 1; without outgroup: $\chi^2 = 7.76$, d.f. = 51, P = 1).

The phylogenetic analyses performed with 1842 bp of mtDNA indicated a sister species relationship between *P. leptosoma* and *Parasesarma gazi* sp. nov., while *P. catenatum*, *P. melissa*, *P. guttatum* and *P. samawati* grouped together (Fig. 4). Based on 28S, the sister relationship between *Parasesarma gazi* sp. nov. and *P. leptosoma* is confirmed with a very high consensus posterior probability value (Fig. 4). The 28S analysis also grouped together the four species *P. catenatum*, *P. guttatum*, *P. melissa* and *P. samawati* (Fig. 5).

The BI analysis run using the short fragment of the COI grouped the *P. lenzii* haplotype with *Parasesarma gazi* sp. nov. and *P. leptosoma* haplotypes (posterior probability value = 1.0: figure not shown), indicating a higher genetic similarity with these two species than with the other two analysed species of *Parasesarma*.

Resolution of the minimum spanning network is lower than expected, due to the very low genetic variation recorded at the three mtDNA genes (Fig. 6). *Parasesarma gazi* sp. nov. has two distinct haplotypes separated by a single mutation, one of which shared by 3 specimens. This appears to be the most ancestral haplotype, from which the other two haplotypes may have derived. The *P. leptosoma* haplotype is separated from the most common haplotype of *Parasesarma gazi* sp. nov. by eight point mutations (i.e. 0.5% sequence divergence) (6).

4. Discussion

In this study, we describe a new crab species belonging to the genus *Parasesarma* De Man 1895, *Parasesarma gazi* sp. nov., based on morphological and genetic (mtDNA and nuclear DNA) evidences. With *Parasesarma gazi* sp. nov., the number of species belonging to the genus *Parasesarma* increases to 57 (Ng et al., 2016; Shahdadi and Schubart, 2017), and it becomes the fifth species of the genus described from East Africa (De Grave et al., 2009). According to the morphological description by Crosnier (1965: 66–67: figs. 98–101), he erroneously identified the Malagasy specimens as *P. lenzii*. But,

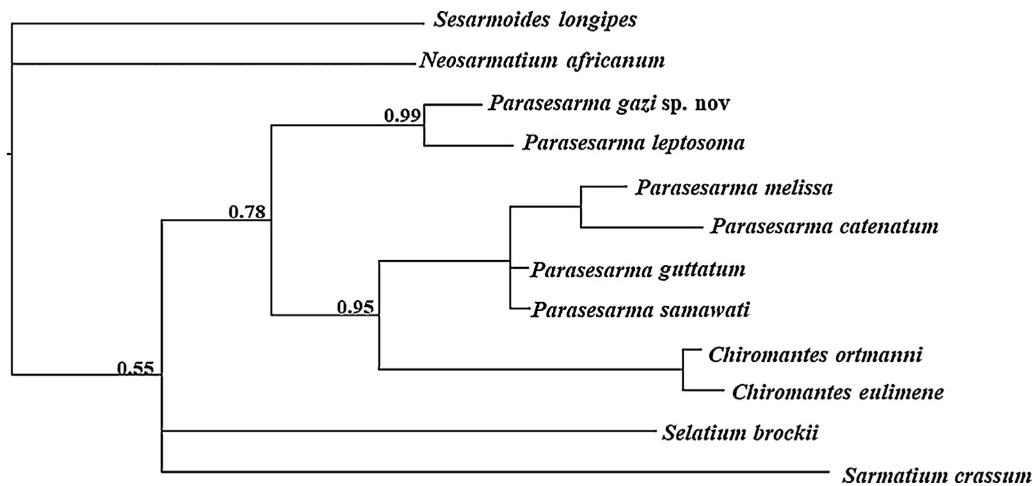

Fig. 5. Phylogenetic BI consensus tree of 12 Indo-Pacific sesarmid crabs based on 602 bp of nDNA coding for the 28S rRNAs gene. Bayesian Inference posterior probabilities are reported on each node. Only confidence values higher than 50% are reported on the tree.

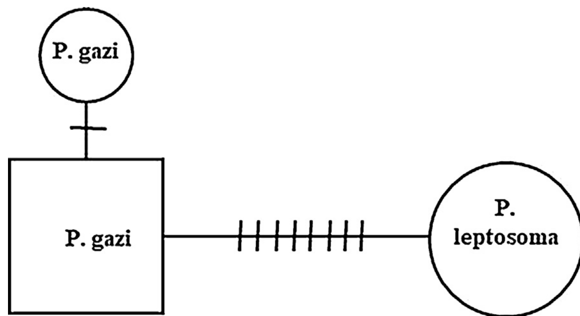

Fig. 6. Minimum parsimony spanning network of 1591 bp of the combined mtDNA dataset from *Parasesarma gazi* sp. nov. (n = 4) and *Parasesarma leptosoma* (n = 4). Each line represents one mutational step. The square represents the ancestral haplotype and the size of the circle is proportional to the frequency of the haplotypes.

in particular the male first gonopod and the upper part of the chelar palm of the specimen described by Crosnier (1965: 67: figs. 99 and 101) are identical to the one here described for *Parasesarma gazi* sp. nov., being very different to the gonopod of *P. lenzii*. It is thus much more reasonable to assume that the specimens described by Crosnier belong to the newly described East African *Parasesarma gazi* sp. nov. rather than to *P. lenzii*. Recently, other specimens attributed to *P. lenzii* were collected on Europa Island (Poupin et al., 2012), and it will be very interesting to compare those specimens with the holotype of *Parasesarma gazi* sp. nov. In any case, both the geographical distribution and ecological characteristics of *Parasesarma gazi* sp. nov. are still largely unknown. This species is definitely not very abundant in both Kenyan and Tanzanian mangrove forests.

Phylogenetic relationships reconstructed by means of genetic markers indicate that *Parasesarma gazi* sp. nov. is the sister species of *P. leptosoma* and both form a monophyletic clade with *P. lenzii* among the analyzed species. Conversely, these three species do not cluster with the other four species of *Parasesarma* analysed for this study (i.e. *P. catenatum*, *P. guttatum*, *P melissa*, *P. samawati*). This confirms the observations by Guerao et al. (2004), Fratini et al. (2005), Schubart et al. (2006), and Shahdadi and Schubart (2015, 2017) regarding the necessity of a redefinition of the genus *Parasesarma*. Fratini et al. (2005) and Schubart et al. (2006) also suggested the placement of *P. leptosoma* in a separate genus and, if this will be realized, it is likely that *Parasesarma gazi* sp. nov. and *P. lenzii* will have to be transferred along with it.

Overall, our findings confirm that the integration of multiple datasets facilitates correct taxonomic work and provides interesting information on the evolutionary history of the individual species, as recently underlined by Padial et al. (2010). These authors proposed a new taxonomic approach consisting in an "integrative taxonomy" that should be based on phenotypic (i.e. morphological, ecological and behavioural) data and genetic information from multiple and different markers. By using mitochondrial and nuclear DNA, the integrative taxonomy clearly takes a step ahead with respect to the DNA-barcoding approach, as proposed by Hebert et al. (2003a,b). Even if on one side the mtDNA allows to clearly recognize cryptic and pseudocryptic species (see Ragionieri et al., 2009), it is not always able to distinguish recently split sister species and to identify hybrids due to its evolutionary properties, such as maternal inheritance, reduced effective population size, low average mutation rate, recombination and heteroplasmy (for a review see Rubinoff et al., 2006 and Baker et al., 2009).

From the above point of view, the description of this new species is symptomatic, since it would not be possible using only a morphological or a mtDNA-based approach. At a first attempt, in fact, the morphological comparison of collected specimens with the available literature showed a morphological correspondence with the samples of Crosnier (1965), possibly leading to a misidentification with *P. lenzii*. However, the comparison between mtDNA COI sequences obtained from our specimens and a syntype of *P. lenzii*, definitely distinguished them as separate species, questioning its belonging to *P. lenzii* (De Man, 1895). However, relying exclusively on the DNA-barcoding gene COI (Hebert et al., 2003a), could also result in taxonomic confusion. Despite their evident morphological differences, *Parasesarma gazi* sp. nov. is not obviously distinguishable as separate species from *P. leptosoma* based on this method. The COI p-distance value between these two species of 0.7 ± 0.3 is in fact within the order of magnitude of intraspecific genetic variation as recorded in other mangrove crabs (see Table 5) and below the 2% postulated as the barcode threshold to distinguish species. In comparison to other congeneric pairwise comparisons as recorded in this study and reported by Hebert et al. (2003a), the distances are 5–10 times lower. In other congeneric species pairs, COI genetic divergence of less than 2% was interpreted as short history of reproductive isolation or mitochondrial introgression (Hebert et al., 2003a). The normally much more conserved nuclear gene 28S sequences also revealed p-distance values between 0.7 and 0.9 between *Parasesarma gazi* sp. nov. and *P. leptosoma*, which argues against a recent separation.

Low divergence values of mtDNA, coupled with consistent divergence values of the conservative nuclear rDNA 28S gene between *Parasesarma gazi* sp. nov. and *P. leptosoma*, may be an indi-

Table 5
Percentage of sequence divergence (expressed as overall mean p-distance ± standard error) of COI at intra-specific level for some species of mangrove crabs from East Africa.

Species	p-distance ± SE	Source
Scylla serrata	0.3 ± 0.1 (n = 190)	Fratini et al., 2010; Fratini et al., 2016
Cranuca inversa	0.3 ± 0.1 (n = 120)	Fratini et al., 2016
Gelasimus hesperiae	0.2 ± 0.1 (n = 125)	Fratini et al., 2016
Austruca occidentalis	0.0 ± 0.0 (n = 421)	Silva et al., 2010a; Fratini et al., 2016
Neosarmatium africanum	0.4 ± 0.1 (n = 147)	Ragionieri et al., 2010; Fratini et al., 2016
Cardisoma carnifex	0.1 ± 0.1 (n = 78)	Fratini et al., 2016
Parasesarma guttatum	0.4 ± 0.1 (n = 224)	Silva et al., 2010b; Fratini et al., 2016

The number of individuals analysed (n) and the original sources are reported.

cation for a introgression of mtDNA between the two species, likely due to an interspecific hybridization event. The role of hybridization in evolution is still debated among biologists. While botanists commonly recognise that hybridization plays a critical role in creating new diversity (see Barton 2001), zoologists traditionally view hybridization as a rare phenomenon (see Dowling and Secor 1997). However, recently an increasing number of studies, based on new and abundant molecular data, have discovered new examples for hybridization guiding speciation in animals (reviewed in Mallet, 2007) and introgression in decapod crustaceans, resulting in identity of mtDNA among different genera (Schubart et al., 2008).

Further genetic analyses using hypervariable nuclear markers are needed to confirm the recent hybridization hypothesis and to clarify how this may have occurred (i.e. which is the parental species and the hybrid one? Did the hybridization event occur secondarily after speciation or was it the speciation mechanism?). In any case, if confirmed, this is the first report of a hybridization event for sesarmid crabs. This opens new interesting questions on the evolutionary mechanisms occurring within the Sesarmidae and in particular for those living in mangrove forests.

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