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ORIGINAL ARTICLE

On the phylogenetic position of Pseudophilomedinae within Sarsielloidea (Ostracoda, Myodocopida), with a description of one new *Harbansus* from Ningaloo Reef and redescription of *H. paucichelatus* from Yucatan

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Abstract Previous studies have suggested incongruence between current systematics and molecular phylogenies of Sarsielloidea, with a possible polyphyly of the family Philomedidae. Here, we provide molecular phylogenetic analyses based on 18S rDNA and 28S rDNA. The former includes five new sequences and 12 from the GenBank, and the latter two new and six sequences from the GenBank. We use three methods, maximum likelihood, maximum parsimony, and neighbor joining, and all reconstructed phylogenies support previously suggested polyphyly, indicating a closer relationship of the subfamily Pseudophilomedinae with one subfamily of Sarsiellidae than with the nominotypical subfamily of Philomedidae. Morphological characters that may be key indicators of the phylogenetic relationships between three Sarsielloidea families are discussed. We also describe the 21st representative of the Pseudophilomedinae genus, *Harbansus* Kornicker, (Smith Contrib Zool 260:75, 1978), *Harbansus ningalooi* n. sp., from the Ningaloo Reef, Western Australia. This is the first *Harbansus* reported from the Australian west coast and the second from the Australian coral reef systems. It differs from all other congeners by peculiar claw-like processes on the posterior infold. Most *Harbansus* species have relatively restricted distributions, except *Harbansus paucichelatus* (Kornicker, in Inst Mar Sci 5:195–300, 1958), which has also been postulated to represent a species complex. We present a detailed morphological redescription of this species, based on the freshly collected material from the Yucatan Peninsula, as well as four mitochondrial COI sequences. These become the first COI sequences of the entire superfamily Sarsielloidea available on the GenBank. To facilitate future identification, we include a key to species of *Harbansus*.

Keywords 18S · 28S · Australia · COI · Mexico · Philomedidae · Polyphyly

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Introduction

The superfamily Sarsielloidea contains three families: Philomedidae, Rutidermatidae, and Sarsiellidae. Recent molecular studies (Oakley 2005; Oakley and Cunningham 2002; Oakley et al. 2012) show that the phylogenetic relationships between the three Sarsielloidea families are not resolved, because some Sarsiellidae cluster with some Philomedidae, while other Philomedidae cluster with Rutidermatidae. However, these authors did not discuss morphological characters that may support these phylogenies, but instead call for better taxon sampling.

The family Philomedidae is divided in two subfamilies, Philomedinae and Pseudophilomedinae. Philomedinae contains eight genera: *Anarthron* Kornicker, 1975; *Euphilomedes* Poulsen, 1962; *Igene* Kornicker, 1975; *Paraphilomedes* Poulsen, 1962; *Philomedes* Ljiljborg, 1853; *Pleoschisma* Poulsen, 1962; *Scleroconcha* Skogsberg,

1920; and *Zeugophilomedes* Kornicker, 1986. Pseudophilomedinae has six genera: *Angulorostrum* Kornicker, 1981; *Harbansus* Kornicker, 1978; *Paramekodon* Brady and Norman, 1896; *Pseudophilomedes* Müller, 1893; *Streptoleberis* Brady, 1890; and *Tetragonodon* Brady and Norman, 1896. *Streptoleberis* is very similar to *Harbansus*, and Kornicker (1978) even speculated that they may be synonyms. Unfortunately none of the species have been newly reported, and this question remains unresolved. The two initial synapomorphies of Pseudophilomedinae (characteristic saber-like tooth on one of the basal endites of the fifth limb and a small terminal segment on the endopod of maxillula) were discarded by Kornicker (1978) who proposed several new synapomorphic characters but noticed that all of them are homoplastic on some other systematic level, and some even within the sister subfamily Philomedinae.

Harbansus Kornicker, 1978 is a widely distributed marine genus, recorded from all world oceans, with the exception of the Southern and Northern Oceans, and at depths ranging from 1 to 1,015 m (Kornicker 1978, 1992). At the moment, it contains 20 described species, and two in the open nomenclature (Kornicker 1978). Its most widely distributed representative, *Harbansus paucichelatus* (Kornicker 1958) was collected during a study of ostracods from the Yucatan Peninsula, and here, we use this material and some Philomedidae collected recently from the Sea of Japan to amplify 18S rDNA and 28S rDNA regions and further test the relationships within Sarsielloidea. We also amplified the mitochondrial COI sequence of *H. paucichelatus*, and these became the first such sequences for the entire superfamily Sarsielloidea. In fact, at the moment, the GenBank contains only four Myodocopida COI sequences (one unidentified Myodocopida and three of the family Cypridinidae), while the other 50 belong to Halocyprida.

Coral reefs around the world are critically endangered ecosystems. A recent global initiative named CReef (Census of Coral Reef Ecosystems) led by several world institutions from 2004 to 2010, significantly increased global knowledge of reefs' biodiversity. Ningaloo Reef is located on the west coast of Western Australia. The reef is 260 km long and is Australia's largest fringing coral reef and the only large reef positioned very close to a landmass. In 2011, the reef became a world heritage site, listed by the United Nations. Ningaloo Reef lies within a region identified as a marine biodiversity hotspot and is considered to be one of the 18 richest multi-taxon centers of endemism vulnerable to extinction worldwide (Roberts et al. 2002, Heyward et al. 2010). One new *Harbansus* species that was collected during a survey of Ningaloo Reef in Western Australia is described here. Myodocopid ostracods from the Ningaloo Reef are poorly known, and so far only two Sarsiellidae have been described (Karanovic 2012). The Great Barrier Reef in Queensland, which is Australia's and world's largest reef structure, is only slightly better studied, and the myodocopid fauna is mostly represented by Sarsiellidae, while only four Rutidermatidae and one species of Philomedidae (*Harbansus slatteryi* Kornicker, 1983) are described so far (Kornicker 1982, 1983, 1996).

Materials and methods

Collecting methods

Shallow coral reef habitats, from where the samples were taken, can be broadly divided into two zones: inter-tidal reef flat and sub-tidal outer reef to about 30 m. Both zones are sampled by taking dead coral substrate (including fossil or compacted reef, eroded and dead coral heads; coral rubble is particularly productive) in a 20–25-L plastic bucket. These samples were broken up in the laboratory, and the water was washed with alcohol (laced with 5–10 drops of concentrated formaldehyde solution if no subsequent DNA extraction was carried out) and left to stand for 5–30 min. Small samples were collected in 25- or 350-µL mesh bags and processed into a bucket the same way. The sample was then rinsed using a seawater hose with the washings passed through a wet sieve or fine-mesh net and either sorted immediately under a microscope or fixed in ethanol (or formaldehyde solution) for later sorting. Sampling on the Ningaloo Reef was carried out under the GBRMPA permit G08-27858.1 and General Fisheries permit (QLD DPI) 95152.

Samples from the Yucatan Peninsula were taken by hydropneumatic suction device for compressed air pulse operated by SCUBA diving, according to Holme and McIntyre (1984) modified as follow: The device consisted of a PVC tube of 127 cm long and 9 cm in diameter, where the 20.3 cm of the lower end of the tube was connected to a diving regulator, which remained attached to a SCUBA tank that supplied compressed air and puts pressure to push the sediment toward the opposite end of the tube which was connected to a bag of 1 m length with a mesh size of 500-µm, where the organisms were retained. The sediment (approximately 200 g) was poured in sample pots, and the fauna was anesthetized with 15 % $MgCl_2$ and fixed with 98 % alcohol if subsequent DNA extraction was carried or with a solution of 10 % formaldehyde if not. The depth was recorded by HONDEX Portable Depth Sounder. For subjacent water of the sediment, the transparence was taken by Secchi disk, temperature by HOBO H8 Data Logger, salinity by a salinometer, pH and dissolved oxygen by a digital potentiometer and oximeter, respectively. For sediment, the organic matter was analyzed using the technique proposed by Buchanan (1984) and the grain size by Bale

Table 1 List of species used in our molecular analyses, with corresponding sequences, and their GenBank accession numbers

Species	Locality	GenBank Accession Numbers					References
		18S	28S ddff	28S eemm	28S vvxx	COI	
Euphilomedes cacharodonta	Unknown	L81941.1	–	–	–	–	Spears and Abele (1997)
Euphilomedes sordida 1	Unknown	AF363299.1	AF363315.1	AF363330.1	AF363349.1	–	Oakley and Cunningham (2002)
Euphilomedes sordida 2	Central Japan (33°42′2″N 135°15′30″E)	AB076656.1	–	–	–	–	Yamaguchi and Endo (2003)
Euphilomedes sp. 1	Japan	AF363302.1	AF363314.1	AF363335.1	AF363345.1	–	Oakley and Cunningham (2002)
Euphilomedes sp. 2	South Korea (38°10′10″N, 128°36′29″E)	KM875567	KM875572	KM875574	KM875576	–	Present paper
Eusarsiella sp.	Belize	AF363305.1	AF363312.1	AF363338.1	AF363343.1	–	Oakley and Cunningham (2002)
Harbansus paucichelatus	unknown	AF363303.1	AF363311.1	AF363329.1	AF363356.1	–	Oakley and Cunningham (2002)
Harbansus paucichelatus M1	Yucatan (21°17′66″N, 89°36′34″W)	KM875570	KM875573	KM875575	KM875577	KM875580	Present paper
Harbansus paucichelatus M2	Yucatan (21°17′66″N, 89°36′34″W)	KM875571	–	–	–	KM875581	Present paper
Harbansus paucichelatus F1	Yucatan (21°17′66″N, 89°36′34″W)	KM875568	–	–	–	KM875578	Present paper
Harbansus paucichelatus F2	Yucatan (21°17′66″N, 89°36′34″W)	KM875569	–	–	–	KM875579	Present paper
Metavargula japonica	unknown	AF363300.1	–	–	–	–	Oakley and Cunningham (2002)
Philomedes sp.	East China Sea	DQ531747.1	–	–	–	–	unpublished (Yu and Chen)
Rutiderma apex	unknown	AF363308.1	AF363313.1	AF363336.1	AF363360.1	–	Oakley and Cunningham (2002)
Sarsiella misakiensis	Central Japan (33°42′12″N 135°17′54″E)	AB076655.1	–	–	–	–	Yamaguchi and Endo (2003)
Vargula hilgendorfii	unknown	AF363301.1	AF363317.1	AF363332.1	AF363357.1	–	Oakley and Cunningham (2002)
Vargula tsujii	unknown	HM009310.1	–	–	–	–	unpublished (Pasha et al.)

M male, *F* female

and Kenny (2005). The samples were kept in ice for later analysis in the laboratory, where the samples were sieved through a mesh size of 250-μm to separate sediment organisms. Ostracods were picked from the sieved samples under a stereomicroscope Leica Zoom 2000.

Taxonomic methods

Specimens were dissected and mounted on microscope glass slides in Faure's medium, which was prepared following the procedure of Stock and Von Vaupel Klein (1996). The dissected appendages were then covered with a coverslip, and the valves of each specimen were transferred to a micropalaeontological slide or scanning electron microscope stub. The species were observed under a Leica L2 stereomicroscope and Leica DM 2500 compound microscope with N-plan objectives, and the drawings were made using the drawing tube attachment to the latter microscope. Scanning Electron Micrographs (SEM) were taken with Hitachi S-4700 scanning electron microscope at Eulji University (Seoul) and FESEM JSM-7600F JEOL at Cinvestav in Merida, Yucatan.

In the present paper, the terminology for the most posterior appendage on the body, so-called uropodal lamellae, follows Meisch (2007), while the maxillula and the fifth limb were labeled according to Kornicker (2001, 2002a, b). The material is deposited in the crustacean collection of the Western Australian Museum (WAM) and in the "Colección de Invertebrados Bentónicos de Yucatán" (CYMX).

Abbreviations used in text and figures: A1, antennula; A2, antenna; BO, Bellonci organ; Md, mandibula, Mxl, maxillula; L5, L6, L7, limbs; UL, uropodal lamellae.

PCR amplification methods

Specimens for molecular analysis were examined under a compound microscope (objective 63× dry) in propylene glycol for identification to morpho-species with only shell removed. The whole soft body was used for the DNA extraction, except in the case of *H. paucichelatus* M1, from which only one A1 and A2 were used. List of species used in the molecular analysis, gene sequences and the GenBank accession numbers are listed in Table 1. DNA was extracted using LaboPass Tissue Mini extraction kit, following the manufacturer protocol. Fragments of nuclear 18S rDNA (on average 1,800 bp) were amplified using the primer pair F1/R9 from Yamaguchi and Endo (2003), fragments of 28S rDNA were amplified using the primer pares dd/ff, ee/mm, and vv/xx from Hills and Dixon (1991), and for mitochondrial COI sequences, we used standard Folmer primers (Folmer et al. 1994). For all gene fragments, the PCR was done in a TaKaRa PCR Thermal Cycler Dice in 25 μL volume containing: 5 μL of the DNA template, 2.5 μL, 10× ExTaq Buffer, 0.25 μL of TaKaRa Ex Taq (5 U/μL), 2 μL of dNDTP Mixture (2.5 mM each), 1 μL each primer, and 13.25 μL distilled H_2O. The PCR protocol for 18S consisted of 5 min initial denaturation at 94 °C, followed by 35 cycles consisting of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 1 min, and the final extension at 72 °C lasted for 5 min. For all three 28S fragments, the settings were almost the same, with the exception that we used 40 cycles (denaturation/annealing/extension) and that annealing was done at 50 °C for 1 min. The protocol for COI segment consisted of initial denaturation for 5 min at 94 °C, 40 cycles of denaturation for 1 min at 94 °C, annealing for 2 min at 46 °C, and extension for 3 min on 72 °C. Final extension was on 72 °C for 10 min. The PCR products were electrophoresed on the 1 % agarose gels, and, if DNA was present, the products were purified for sequencing reactions, using the LaboPass PCR Purification Kit following the guidelines provided with the kit. DNA was sequenced on ABI automatic capillary sequencer (Macrogene, Seoul, South Korea) using the same set of primers.

Phylogenetic methods

All obtained sequences were visualized using FinchTV v 1.4 BLAST (Altschul et al. 1990). Analyses of GenBank revealed that the obtained sequences are ostracod in origin and not contaminants. Each sequence was checked for quality and sites with possible low resolution and corrected by comparing forward and reverse strands. Sequences were imported in MEGA 5.2.2 (Tamura et al. 2011), and all further analyses, including the addition of highly homologous sequences from the GenBank, were done using this software. In the analysis of 18S, we have chosen three outgroup species, *Vargula hilgendorfii* Müller, 1986, *V. tsujii* Kornicker and Baker, 1977, and *Melavargula japonica* Poulsen, 1962, and only the first one in the molecular analysis of 28S. They belong to the superfamily Cypridinoidea, which has been shown in previous molecular analysis (Oakley 2005; Oakley and Cunningham 2002; Oakley et al. 2012) to be the sister taxon of Sarsielloidea. Sequences for all three outgroup species, and another 30 highly similar sequences, all belonging to the superfamily

Table 2 Average pairwise distances (K-2 model) among mitochondrial COI sequences of four specimens of *Harbansus paucichelatus* Kornicker, 1958 from Yucatan

Harbansus paucichelatus F1			
Harbansus paucichelatus F2	0.02		
Harbansus paucichelatus M1	0.01	0.02	
Harbansus paucichelatus M2	0.00	0.02	0.00

For more details see Table 1

Table 3 Average pairwise distances (K2 + G model) among 18S rDNA sequences of 14 Sarsielloidea specimens and three outgroup species

Euphilomedes sordida 2																
Euphilomedes sordida 1	0.03															
Euphilomedes sp. 1	0.03	0.00														
Euphilomedes sp. 2	0.02	0.00	0.00													
Eusarsiella sp.	0.04	0.04	0.04	0.04												
Euphilomedes cacharodonta	0.03	0.02	0.02	0.02	0.04											
Harbansus paucichelatus F1	0.04	0.04	0.04	0.05	0.03	0.05										
Harbansus paucichelatus	0.05	0.05	0.05	0.05	0.04	0.05	0.01									
Harbansus paucichelatus F2	0.04	0.05	0.04	0.05	0.03	0.05	0.00	0.01								
Harbansus paucichelatus M1	0.04	0.04	0.04	0.05	0.03	0.05	0.00	0.02	0.00							
Harbansus paucichelatus M2	0.04	0.04	0.04	0.05	0.03	0.04	0.00	0.01	0.00	0.00						
Melavargula japonica	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08					
Philomedes sp.	0.04	0.04	0.04	0.04	0.02	0.04	0.04	0.04	0.04	0.04	0.04	0.08				
Rutiderma apex	0.03	0.03	0.03	0.03	0.04	0.04	0.05	0.05	0.05	0.04	0.05	0.07	0.04			
Sarsiella misakiensis	0.04	0.04	0.04	0.04	0.01	0.04	0.04	0.04	0.04	0.04	0.04	0.07	0.01	0.04		
Vargula hilgendorfii	0.06	0.06	0.06	0.07	0.06	0.07	0.07	0.07	0.07	0.06	0.07	0.04	0.07	0.07	0.07	
Vargula tsujii	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.08	0.07	0.08	0.03	0.07	0.07	0.07	0.02

For more details see Table 1

Sarsielloidea, were downloaded from the GenBank. Majority of species were only identified to the genus category, with the exception of *E. cacharodonta* Smith, 1952, *Euphilomedes sordida* Müller, 1890, *M. japonica* Poulsen, 1962, *R. apex* Kornicker and Harrison-Nelson, 1997, *Sarsiella misakiensis* Kajijama, 1912 (see Table 1). The 18S sequences were approximately 1,800 bp, while those of three different regions of 28S and COI were about 700 bp long. They were aligned with ClustalW (Thompson et al. 1994) with MEGA default parameters. The K2 + G+I was calculated to be the best nucleotide substitution model for the 18S data set, K2 + G for the 28S, and T92 for the COI. In distance calculations the gaps (missing data) were treated with pairwise deletion. The distance matrices are presented in Tables 2, 3, and 4. We performed three analyses: maximum likelihood (ML), neighbor joining (NJ), and maximum parsimony (MP) using MEGA. In all three analyses, the bootstrap values (Felsenstein 1985) were calculated with 1000 replicates. In the ML method, we used partial deletion (95 %), nearest-neighbor-interchange (NNI) as the heuristic search method, and the initial tree was created automatically (Default-NJ/BioNJ), while for MP, the SPR (Subtree-Pruning-Regrafting) was the tree searching method. Three pre-aligned sequences of 28S were combined before performing the analysis, while the 18S sequence was analyzed independently.

Table 4 Average pairwise distances (K2 + G model) among 28S rDNA sequences of seven Sarsielloidea specimens and one outgroup

Euphilomedes sordida 1							
Euphilomedes sp. 1	0.01						
Euphilomedes sp. 2	0.01	0.01					
Eusarsiella sp.	0.10	0.10	0.11				
Harbansus paucichelatus	0.11	0.11	0.11	0.09			
Harbansus paucichelatus M1	0.11	0.11	0.11	0.09	0.02		
Rutiderma apex	0.08	0.08	0.08	0.11	0.12	0.11	
Vargula hilgendorfii	0.18	0.18	0.18	0.17	0.19	0.17	0.18

For more details see Table 1

Results

Taxonomic description

Class Ostracoda Latreille, 1802
Subclass Myodocopa Sars, 1866
Order Myodocopida Sars, 1866
Suborder Myodocopina Sars, 1866
Family Phylomedidae Müller, 1906
Subfamily Pseudophilomedinae Kornicker, 1967
Genus *Harbansus* Kornicker, 1978
Harbansus ningalooi n. sp. (Figs. 1, 2, 3, 4, 5)

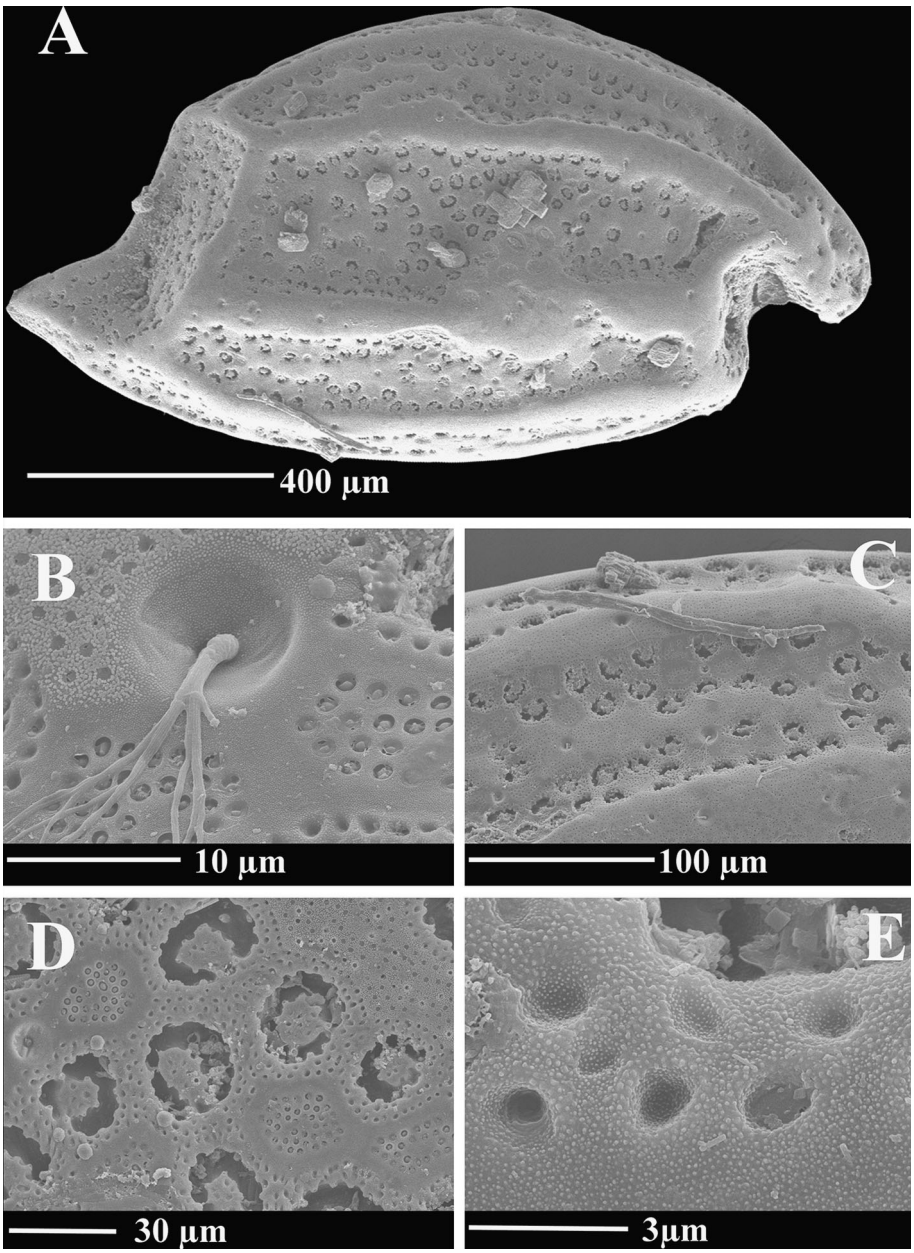

Fig. 1 *Harbansus ningalooi* n. sp. SEM, Holotype: **a** RV, lateral view from the outside; **b** branching surface seta; **c** detail of the surface showing non-branching setae; **d** details of the fossae structure; **e** pores around fossae

Type material

Holotype (Female), dissected on one slide, valves on SEM stub (WAM C57053); 2 paratypes (subadult females) kept in 70 % ethyl alcohol (WAM C57054).

Type locality

Western Australia, Ningaloo Reef, AU VI, 1489, ARMS-1, 17/05/2010, rock, $d = 13$ m, CReef, 22°46′8.832″S 113°42′16.488″E.

Diagnosis

Surface with shallow pits, two dorsal and two ventral longitudinal ridges, one anterior and one posterior. Beside large shallow pith, smaller pores also present. Shell elliptical with prominent pointed caudal process. Clear bulge present antero-ventrally. Antero-ventral infold striate. Posterior infold with several thick setae and claw-like extensions.

Description of adult female

Carapace sub-rectangular with prominent rostrum, and projecting (relatively pointed) caudal process. Dorsal

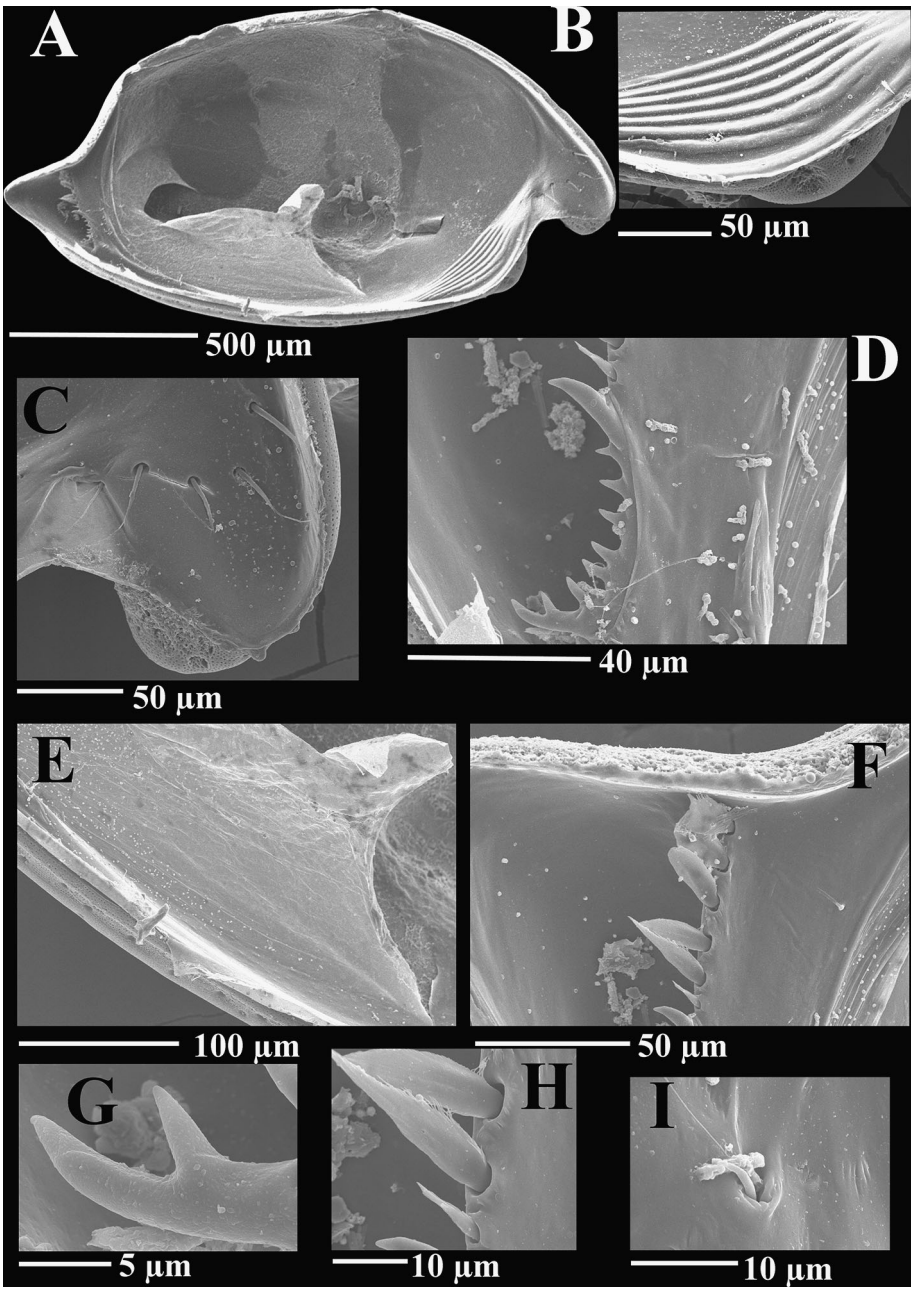

Fig. 2 *Harbansus ningalooi* n. sp. SEM, Holotype: **a** LV, lateral view from inside; **b** details of the antero-ventral infold; **c** anterior infold showing setae; **d** posterior infold showing claw-like structures; **e** detail of the postero-ventral margin; **f** setae on the posterior infold; **g** detail of the largest claw on the posterior infold; **h** detail of setae on the posterior infold; **i** setae on the ventral margin

margin almost evenly rounded, with the greatest high before middle L, and gently sloping towards posterior end. Ventral margin rounded (Figs. 1a, 2a). Surface of carapace with four longitudinal ridges: two running dorsally and two ventrally. Two vertical ridges also present: one running posteriorly, other anteriorly; vertical ridges connecting horizontal ones. Surface covered with large, shallow pits (Fig. 1c, d), in between which numerous small pores also present (Fig. 1d, e). Some surface setae branched and long (Fig. 1b), some short and not branched. Two clear rounded bulging structures present: one situated antero-ventrally (Fig. 2b), other on rostrum (Fig. 2c). These bulging structures best observed from the inside, lateral view (Fig. 1a). Selvage very narrow all around carapace (Fig. 2e). Rostral infold with five setae (Fig. 2c), antero-ventral infold with seven ribs (striation). Posterior infold with six short and broad papappose setae situated dorsally (Fig. 2f, h), and eight claw-like structures: dorsal most and ventral most claws being largest, and ventral most additionally forked distally (Fig. 2d, g). Several short setae also present along posterior and ventral infold (Fig. 2i). Carapace 1.4 mm long.

A1 (Fig. 3b, c). First segment bare. Second segment with one serrulate dorsal seta situated bellow middle L of

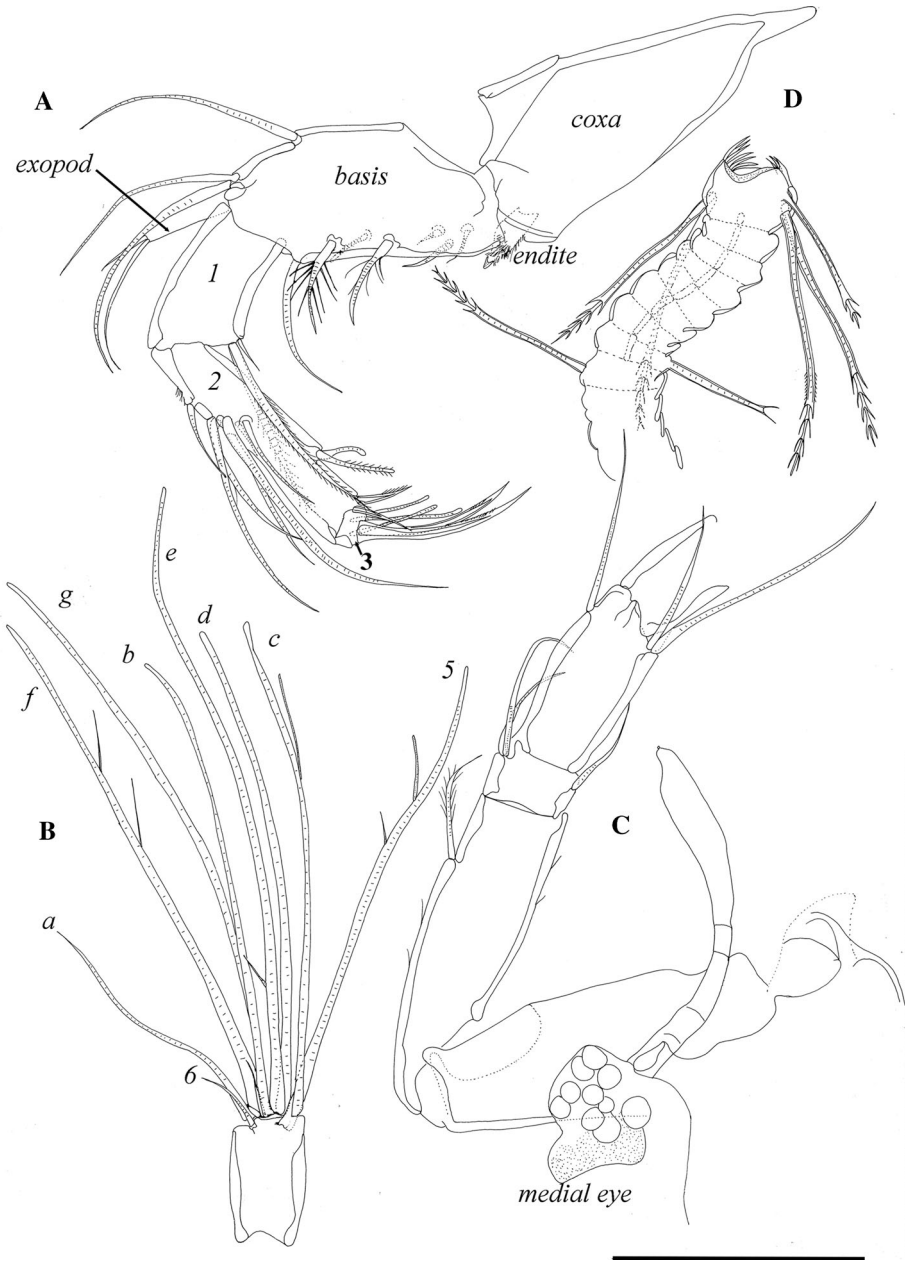

Fig. 3 *Harbansus ningalooi* n. sp. Holotype: **a** Md (*1*, *2*, *3*—three segments of the endopod); **b** distal segments of A1; **c** proximal segments on A1 with Bellonci organ and medial eye; **d** L7. *Scale* 0.1 mm

segment and exceeding distal margin of the following segment. Third segment short and with one ventral annulated, bare seta; and two dorsal bare setae; none of these setae exceeds distal end of the fourth segment. Fourth segment with one dorsal, bare and annulated seta exceeding distal end of the seventh segment. Same segment with two ventral annulated bare setae, one longer than other. Ventral seta of the fifth segment with two distal marginal filaments. Sixth segment fused with the fifth and with very short medial bare seta. Seventh segment with a-seta much longer than seta on the sixth segment; c-seta with one long filament; b- and d-setae subequally long and about same L as seta on the fifth segment. Eight segment with setae g- and e- (g-seta bare, e-seta with one filament) almost subequally long, f-seta with two filaments. One very short additional seta present (most probably) on the eight segment.

Lateral eyes absent, medial eye (Fig. 3c) present and pigmented, with about 10 ommatidia. Bellonci organ with five septae, distally with very small tip.

A2 (Fig. 4a). Protopod without any seta. Endopod 3-segmented: first segment basally with two short setae, second segment with one long sub-distal seta, and third segment with pointed tip but without any seta. Exopod 9-segmented. First segment bare; segments from 2 to 8 with one strong, seta with thick spines proximally and thin setulae distally. Ninth segment with two seta, one long, but

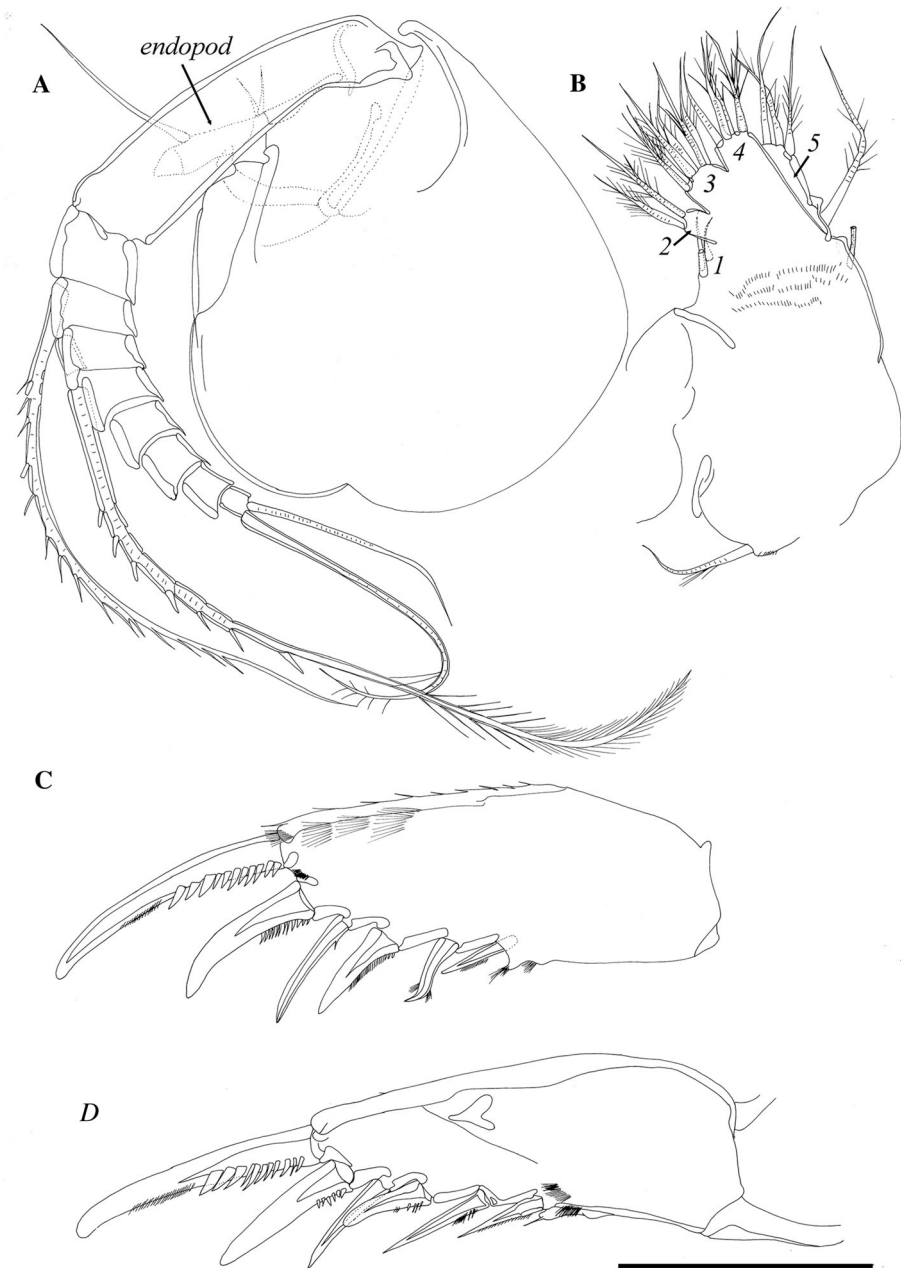

Fig. 4 *Harbansus ningalooi* n. sp. Holotype: **a** A2; **b** L6 (*1*, *2*, *3*, *4*, *5*—endites); **c**, **d** UL. *Scale* 0.1 mm

almost bare seta (except for thin setulae distally), and one shorter bare seta.

Md (Fig. 3a). Coxa endite spinous with distal tip strongly sclerified. Basis with three subequally long, bare and annulated dorsal setae. Ventrally basis with proximal group of three small bare setae, followed by two serrulate longer setae, in between which another small and bare seta situated; one additional longer, bare annulated seta present on the distal margin; this seta longer than any other ventral setae. Exopod 1-segmented and segment not reaching distal margin of the first endopodal segment. Exopod distally with two thin, bare setae, one two times longer than the other. First endopodal segment with three setae ventro-distally: two long, serrulate and annulated setae exceeding distal end of the following segment, and one very short and bare seta. Second segment of endopod dorsally with a total of seven setae, some short, some long, but all bare and annulated; same segment medio-ventrally with two setae: one serrulate, other bare and with rounded tip; two more spine-like setae present ventro-distally. Terminal segment with two strong (but finely serrated) claws and three fine, bare setae, all with rounded tips.

Mxl (Fig. 5a). Precoxa with five spine-like setae, each with long and stiff setulae. Coxa with one dorsal serrulate seta and two endites with five and six claw-like setae, some

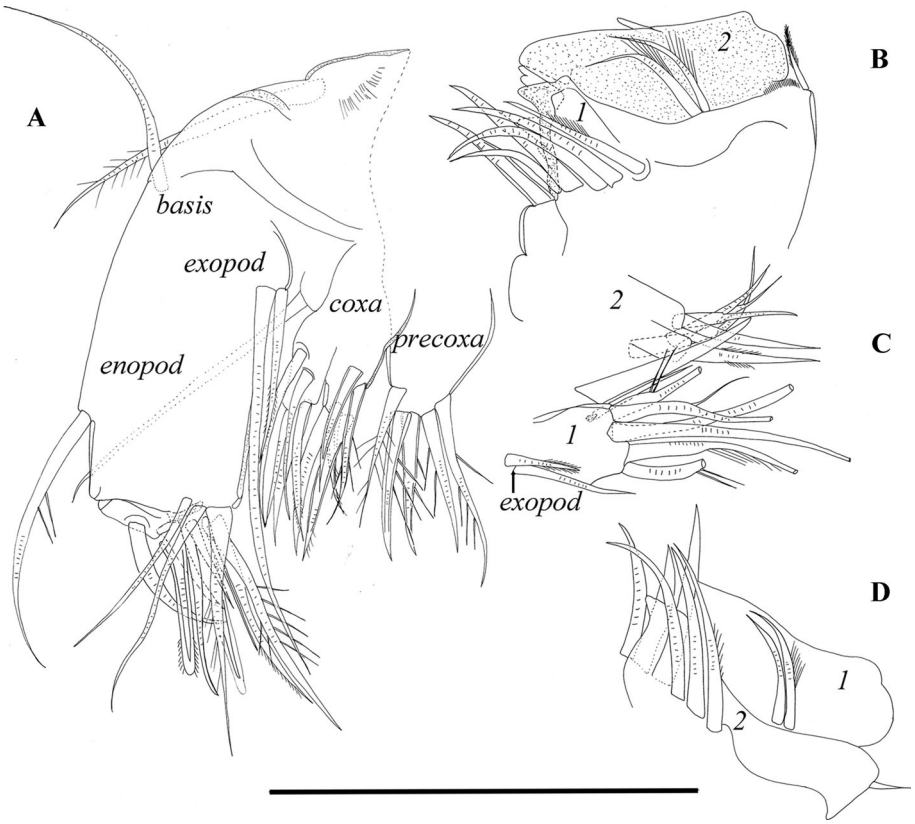

Fig. 5 *Harbansus ningalooi* n. sp. Holotype: **a** Mxl; **b** square tooth on basis second endite L5 (*1* and *2*—basis endites); **c** exopod, and endopodal segments (*1* and *2*) of L5; **d** part of basis and some of the setae (*1* and *2*—basis endites). *Scale* 0.1 mm

serrulate, some plumose. Basis with one annulated, bare dorsal seta and one bare, long ventral seta. Exopod with two setae: longer one bare, shorter serrulate. Endopod first segment with dorsal seta carrying few setulae. Total of 12 setae and/or claws present distally, some belonging to the first and some to the second segment. Exact position of each seta very hard to observe.

L5 (Fig. 5b–d). Basis endite one with bluntly serrated teeth, two medial pappose setae and one short pappose setae distally. Basis endite two consisting of a large tooth with medial spine-like extension. Three coxal endites hard to distinguish between each other, but third one carrying six setae. Exopod with two setae, endopod with two endites: one with seven setae, followed by one claw, and other with six setae.

L6 (Fig. 4b). With one short epipodite seta. First endite with three short and smooth setae; second with two pappose, annulated setae; third endite with six pappose annulated setae; fourth with four pappose annulated setae; end segment with three distal annulated pappose setae and one longer seta (also pappose and annulated).

L7 (Fig. 3d). Two setae in proximal group, one on each side and six setae in distal group (three on each side). Six curved teeth present on the comb, and three on the peg side.

UL (Fig. 4c, d). Each lamella with six claws followed by small posterior extension. Claws 1, 2, and 4 primary; claw 3, 5, and 6 secondary. Only the first claw with strong teeth on posterior margin, other claws with weaker teeth, and secondary claws with very fine serration.

Males: Not known.

Etymology: The species is named after the type locality.

Remarks and affinities

Harbansus ningalooi stands apart from other congeners by the presence of peculiar teeth-like structures on the posterior infold and only one (instead of two) long seta on the posterior extension of the L6. It belongs to the group of species with longitudinal ridges on the carapace, together with *H. bradmyersi* Kornicker, 1978; *H. ferox* Kornicker, 1992; *H. flax* Kornicker, 1998; *H. magnus* Kornicker, 1984; *H. paucichelatus, H. slatteryi* Kornicker, 1983; *H. thrix* Kornicker, 1992; *H. vatrax* Kornicker, 1995; *H. vix* Kornicker, 1991; and *H. vortex* Kornicker, 1995. *Harbansus ningalooi* is morphologically most similar to *H. slatteryi*, described from the Lizard Island, Queensland (Kornicker 1983). The two species have a very similar shape and ornamentation of carapace, and they both have two long claws on the terminal segment of the Md endopod. The new species and *H. magnus* are the only two in this group that have seven dorsal bristles on the second endopodal segment of Md. However, *H. magnus* is the largest species in the genus (more than 2 mm long) and has more than six claws on the UL. All other species clearly differ from *H.*

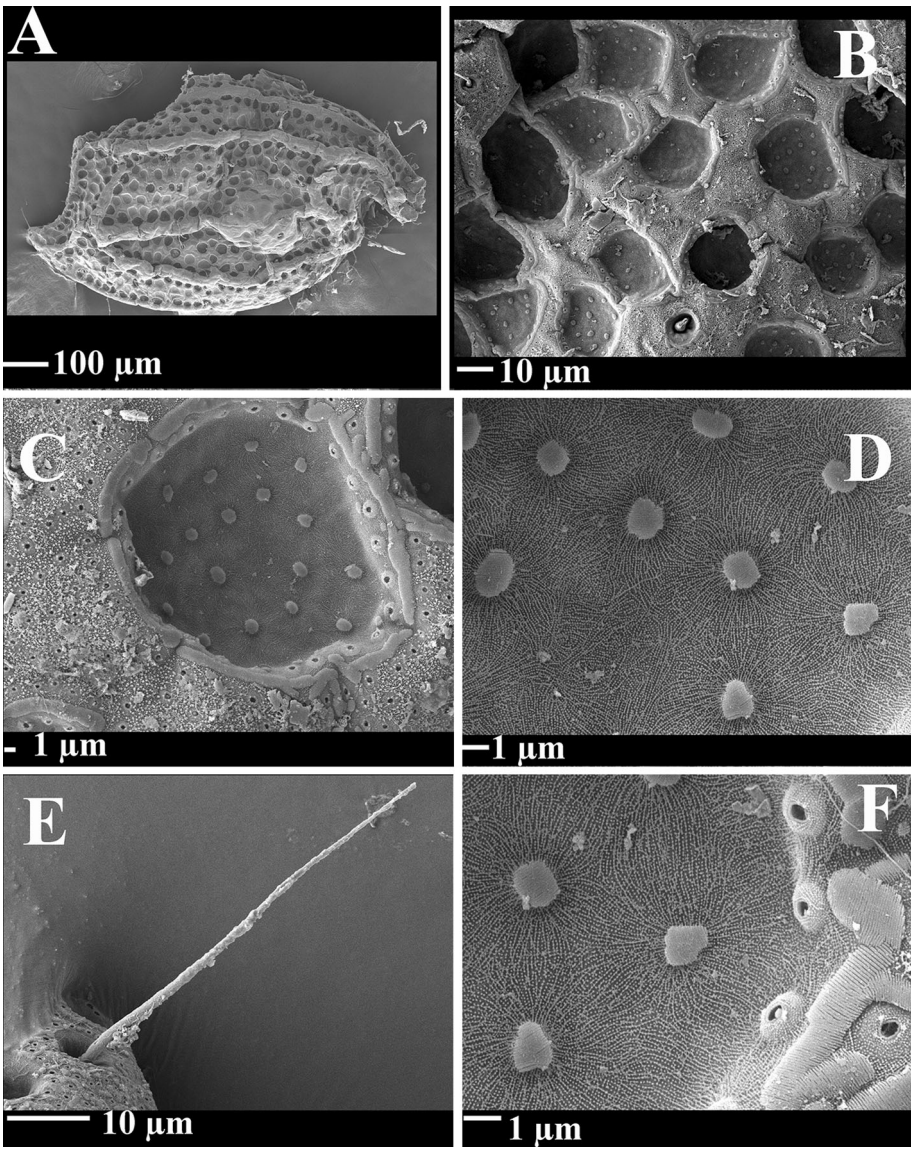

Fig. 6 *Harbansus paucichelatus* (Kornicker, 1958) SEM, female: **a** RV, lateral view from the outside; **b** fossae; **c** details of fossae; **d** small nodules inside fossae; **e** seta on the surface; **f** nodules inside fossae and pores on the edges. *Scale* 0.1 mm

ningalooi by the carapace shape, chaetotaxy of the Md, and other details of their morphology.

The adult females of the new species have swimming bristles on the A2 exopod which, according to Kornicker (1978), means that they are swimmers, not crawlers. We have not found males, and the presence of the lateral eyes in this sex is therefore not certain. As for the females, it seems that they only have the medial eye.

Harbansus paucichelatus (Kornicker, 1958) (Figs. 6, 7, 8, 9).

Synonymy

Philomedes paucichelata new species—Kornicker, 1958: p. 233, Figs. 46, 4A–B, 54, A–E, 55, A–C, 87, B, E, H.

Harbansus paucichelatus Kornicker, 1958 n. comb.—Kornicker, 1978: p. 16, Figs. 5, 6, 7, 8, 9, plates 1, 2.

Harbansus paucichelatus Kornicker, 1958—Kornicker, 1984: p. 59, Figs. 32–37; Kornicker et al., 2002: p. 35, Figs. 23–31; Kornicker et al. 2007b: p. 87.

Material examined

1. Three female dissected on three slides and one male dissected on one from: Mexico, Yucatan Peninsula, Between Chabihau and Santa Clara, 21°22′4.33″N, 89°04′13.66″W, 08/05/2005, d = 1.4 m, transparence (water) = 0.9 m, T (water) = 29.8 °C, salinity = 41, pH = 7.96, Dissolved oxygen = 7.59 mg L^{-1}, organic matter = 1.15 %, grain size (sediment) = 1.6 Ø. One female mounted on SEM stub from: Mexico,

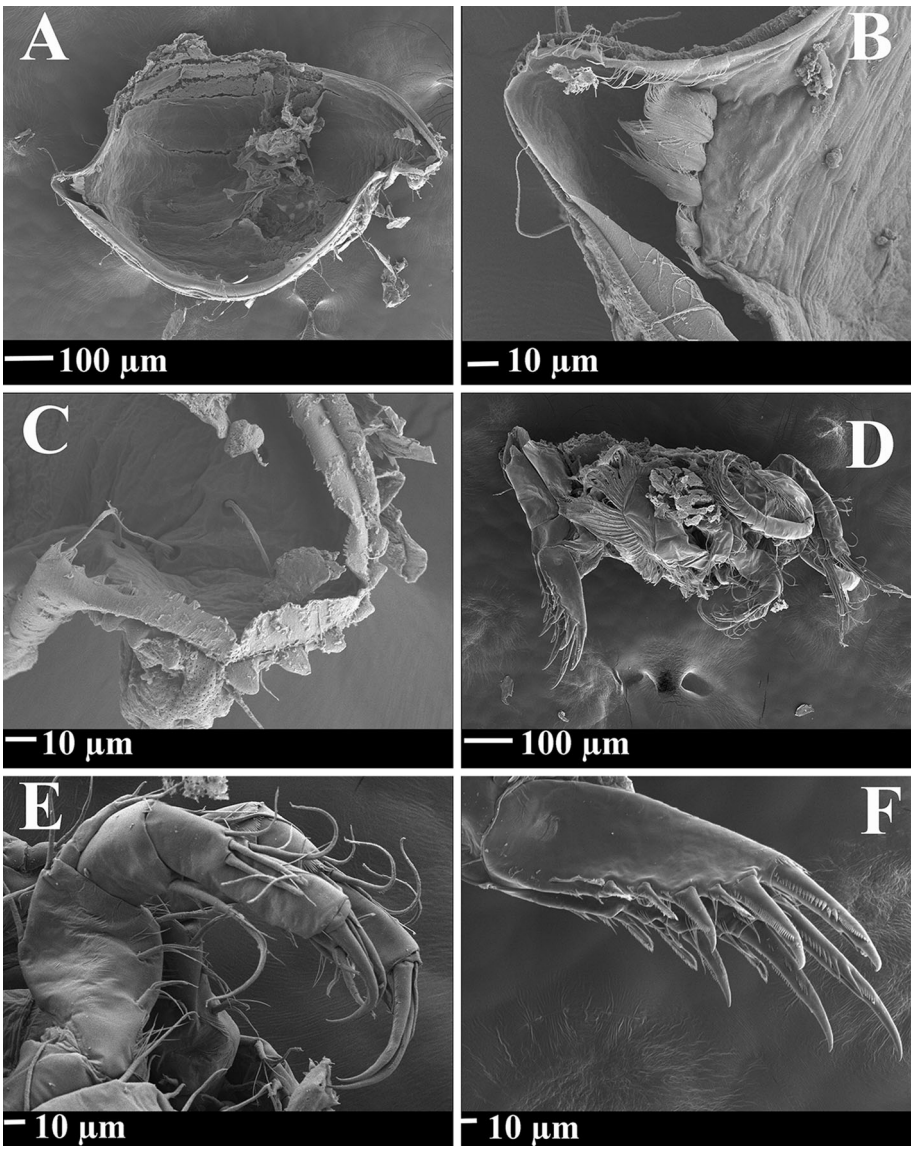

Fig. 7 *Harbansus paucichelatus* (Kornicker, 1958) SEM, female: **a** LV, lateral view from the inside; **b** detail of the posterior infold setae; **c** detail of the rostral infold; **d** soft body; **e** Md; **f** UL. *Scales* 0.1 mm

Yucatan Peninsula, Between Chabihau and Santa Clara, 21°22′06.28″N, 89°04′14.59″W, 08/05/2005, $d = 1.1$ m, transparence (water) = 0.7 m, T (water) = 21.8 °C, salinity = 30, pH = 7.74, dissolved oxygen = 5.8 mg L^{-1}, organic matter = 0.46 %, grain size (sediment) = 1.2 Ø. 47. Specimens are kept in alcohol (CYMX-1601).

2. One male dissected on one slide, shell on micropaleontological slide and one antenna and antennula used for DNA extraction (WAM C57055), soft parts of one male and two females used for DNA extraction, their shell kept on micropaleontological slides (in the first author collection) from: Mexico, Yucatan Peninsula, Between Progreso and Telchac, 21°17′66″N, 89°36′34″W, 29/11/2013.

Diagnosis

Surface with deep ridges: two dorsal and two ventral longitudinal, one anterior, and one posterior ridge. Beside large pits, smaller pores also present. Shell sub rectangular, with prominent, pointed caudal process. Posterior infold with several thick setae.

Description of adult female

Carapace sub-rectangular with prominent rostrum and relatively pointed caudal process. Dorsal margin almost evenly rounded, with greatest high around middle L, and gently sloping towards posterior and anterior ends. Ventral margin rounded (Figs. 6a, 7a, 8a). Surface of carapace with

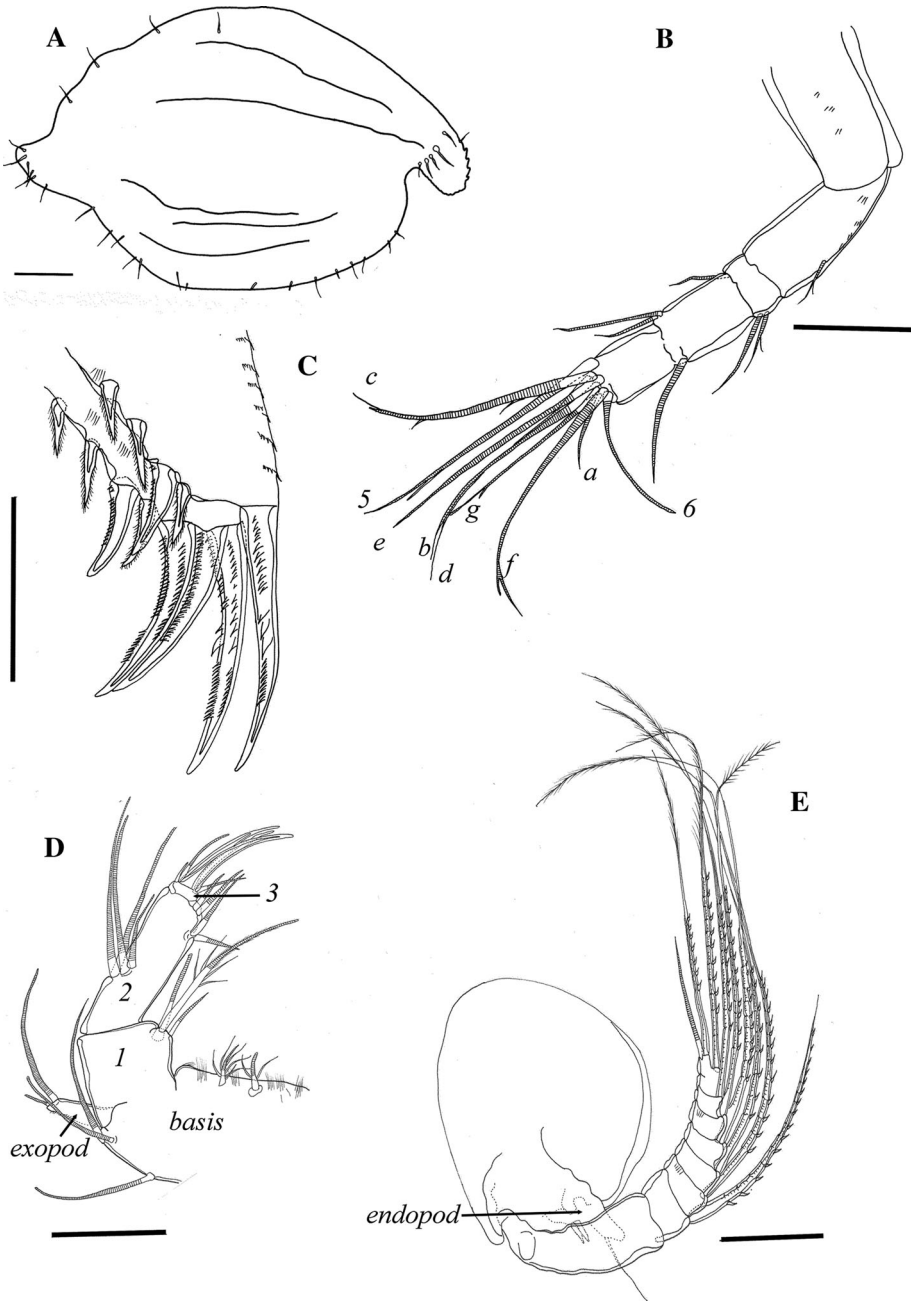

Fig. 8 *Harbansus paucichelatus* (Kornicker, 1958) *a*, *b*, *e*, female, slide 2; *c*, *d*, female, slide 1: **a** RV, lateral view from the outside; **b** A1; **c** UL; **d** Md (*1*, 2, 3—endopod segments); **e** A2. *Scales* 0.1 mm

four longitudinal ridges: two running dorsally and two ventrally. Two vertical ridges also present one running posteriorly, other anteriorly; vertical ridges connecting horizontal ones. Surface covered with large, deep pits (Fig. 6b), in between which numerous small pores also present, as well as around rims of large pits. Inside these pits peculiar structure consisting of nodules connected with radial networks of small, linearly distributed small dots (Fig. 6c, d, f). Some surface setae not branched (Fig. 6e). Selvage relatively narrow all around carapace (Fig. 7a–c) and with fringe of setulae. Rostral infold with five setae, rostrum also marginally serrated. Posterior infold with five short and broad pappose setae situated dorsally (Fig. 7b). Carapace approximately 0.8 mm long.

A1 (Fig. 8b). First segment bare. Second segment with one serrulate dorsal seta situated below middle L of segment and not reaching distal margin of same segment. Third segment short and with one ventral annulated, bare seta; and two dorsal bare setae; none of these setae exceeds distal end of fourth segment. Fourth segment with one dorsal, bare and annulated seta exceeding distal end of fifth segment. Same segment with two ventral annulated bare setae, one longer than other. Ventral seta of fifth segment with one distal marginal filament. Sixth segment fused with

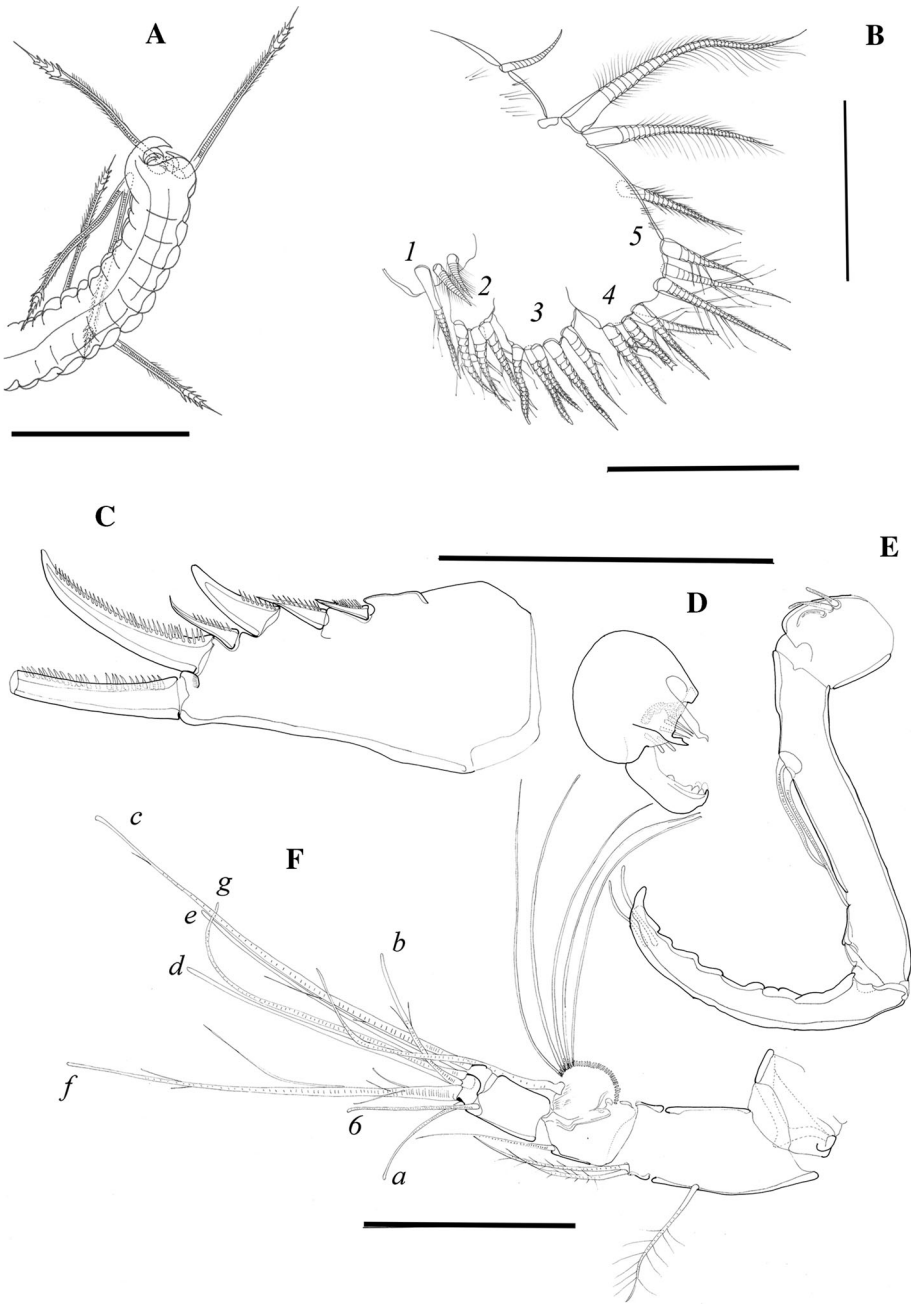

Fig. 9 *Harbansus paucichelatus* (Kornicker, 1958). **a** Female, slide 1; **b** female slide 3; **c–f** male: **a** L7; **b** L6 (*1*, *2*, *3*, *4*, *5*—endites); **c** UL; **d** hemipenis; **e** endopod A2; **f** A1. *Scales* 0.1 mm

fifth and with bare seta. Seventh segment with a-seta much shorter than seta on sixth segment. b- and d-seta subequally long and without filaments; c-seta three filaments; e-seta bare, g-seta slightly shorter and with one filament, f-seta with one filament. Lateral eyes absent, medial eye small and pigmented.

A2 (Fig. 8e). Protopod without any seta. Endopod 2-segmented: first segment basally with two short setae, second segment with one long sub-distal seta, this segment with rounded tip. Exopod 9-segmented. First segment bare; segments from 2 to 8 with one strong seta with thick spines proximally and thin setulae distally. Ninth segment with two seta, one long, and similar to other setae, and one shorter bare seta.

Md (Figs. 7e, 8d). Basis with three subequally long, bare and annulated dorsal setae. Ventrally basis with two short pappose setae. Exopod 1-segmented and segment not reaching distal margin of first endopodal segment. Exopod distally with two thin, bare setae, one about five times longer than the other. First endopodal segment with three setae ventro-distally: one long, pappose and annulated setae exceeding distal end of following segment, and two

short and bare seta. Second segment of endopod dorsally with total of four setae; same segment ventro-distally with four short bare setae. Terminal segment with three claws and three fine, bare setae.

L6 (Fig. 9b). First endite with three setae: two short pappose and one longer seta; second with three pappose, annulated setae; third endite with five pappose annulated setae; fourth with five pappose annulated setae, end segment with three distal annulated pappose setae and three longer seta (also pappose and annulated).

L7 (Fig. 9a). Two setae in proximal group, one on each side and four setae in distal group (two on each side). Three curved teeth present on the comb, and three on the peg side. Each seta with four bells at the tip.

UL (Figs. 7f, 8c). Each lamella with six claws followed. Claws 1, 2, and 4 primary; claw 3, 5, and 6 secondary. All primarily claws strongly serrated.

Description of adult male

A1 (Fig. 9f). Second segment with one pappose dorsal seta. Third segment short with two pappose dorsal setae, no ventral setae. Fourth segment with one dorsal seta, no ventral setae. Fifth segment wedged ventrally between the fourth and the sixth segments; sensory seta with bulbous proximal part with abundant filaments, and stem with three filaments near middle and two spines at tip. Sixth segment medial seta with short. Seventh segment: a-seta same L as seta on the sixth segment; b-seta one-third longer than a-bristle, with 2 distal filaments; c-seta longer than sensory seta on the fifth segment. Eight segment: d- and e-setae shorter than c-bristle, bare with blunt tips, f-seta as long c-seta, with marginal filaments; g-bristle shorter than c-bristle. Both lateral and medial eyes present.

A2 (Fig. 9e). Endopodite 3-segmented, first segment short with three short anterior setae; second segment elongated, with two long proximal setae; third segment elongated, reflexed, with two short setae near sclerotized beak-like tip.

Hemipenis (Fig. 9d). With rounded basal part carrying five seta-like structures and two hooks. An elongated process attached to the round base, curved distally and with seven teeth.

UL (Fig. 9c). Similar to female, except that the fourth claw being broader.

Remarks

Harbansus paucichelatus is the most widespread species of the genus. Kornicker (1984) noticed that it is very variable, so that it may represent a species-complex, and he considered the following features to be variable: primary claws on the UL can be stout or slender; shell may have many, few or no spines on the surface; exopod of A2 may bare long or short swimming setulae; tip of A2 endopod in female with or without terminal spine. Although Kornicker (1978) provided SEM photographs of the shell surface, it is impossible to discern on them peculiar structures detected in our specimens, i.e., small nodules within fossae interconnected with radial lines of dots. Our specimens all suffered from decalcification (due to improper sample fixation with non-buffered formaldehyde solution), as well as the ones that Kornicker (1978) illustrated. The Mexican specimens did not have any spines on the surface of the shell; the UL had stout claws; and the female A2 endopod lack terminal spine. We have also found that the average pairwise distances between 18S rDNA sequences of the new material and the one deposited on the GenBank is about 1 %, and between 28S rDNA sequences 2 %, which may also indicate a separate species (see Appendix 1), but this needs further studies as we cannot confirm identification of previously available GenBank sequences. Due to the high similarity in carapace shape and the morphology of other appendages, including A1 and hemipenis of our males and the ones reported by Kornicker (1984), we identified our specimens from Yucatan as *H. paucichelatus*. By providing mitochondrial COI sequence, we open a possibility for future molecular comparisons between our population and other populations of *H. paucichelatus* from its wide area of distribution.

Key to species of *Harbansus*

1. Posterior infold with claw-like structures … *H. ningalooi* n. sp.
   - Posterior infold without spine-like structures… 2
2. Shell more than 2 mm long … *H. magnus* Kornicker, 1984
   - Shell around 1.5 mm or less … 3
3. Carapace not ornamented with fossae … 4
   - Carapace ornamented with fossae … 6
4. Eight to 10 setae on the rostral infold … *H. tenax* Kornicker, 1995
   - Four or 5 setae on the rostral infold … 5
5. Five setae dorsally on the second endopod segment Md … *H. ferox* Kornicker, 1992
   - Two setae dorsally on the second endopod segment Md … *H. vatrax* Kornicker, 1995
6. Carapace with longitudinal ribs … 7
   - Carapace without longitudinal ribs … 14

7. Distal seta on the second endopod segment A2 absent … 8
   - Distal seta on the second endopod segment A2 present … 9
8. Five setae dorsally on second endopod segment Md and 6 setae on third endite L6 … *H. slatteryi* Kornicker, 1983
   - Six setae dorsally on second endopod segment Md and 5 setae on third endite L6 … *H. thrix* Kornicker, 1992
9. More than six setae on rostral infold and three setae on the basal segment of endopod A2 … *H. vortex* Kornicker, 1995
   - Less than 6 setae on rostral infold and 2 setae on the basal segment of endopod A2 … 10
10. Six setae on the rostral infold … 11
    - Five or 4 on the rostral infold … 12
11. Seven setae on the second endopod segment Md and 7 setae on third endite L6 … *H. schornikovi* Kornicker and Caraion, 1977
    - Five setae on the second endopod segment Md and 5 setae on third endite L6 … *H. bradmyersi* Kornicker, 1978
12. Three setae on each side of L7 terminus … *H. vix* Kornicker, 1991
    - Two setae on each side of L7 terminus … 13
13. Three teeth on the peg side and 1 on the comb side of L7 terminus … *H. flax* Kornicker, 1998
    - Six spines on peg side and 2-3 on the comb side of L7 terminus … *H. paucichelatus* Kornicker, 1958
14. UL with more than 6 claws … *H. hapax* Kornicker, 1995
    - UL with 6 claws … 15
15. Terminal segment of endopod A2 without setae or any other small extensions … 16
    - Terminal segment of endopod A2 with seta … 18
16. Two setae on each side of terminus L7 … *H. felix* Kornicker, 1995
    - Three setae on each side of terminus L7 … 17
17. Only one seta on the basal segment of A2 endopod and two setae on endite one L6 … *H. bowenae* Kornicker, 1978
    - Two setae on the basal segment of A2 endopod and 3 setae on endite one L6 … *H. dayi* Kornicker, 1978
18. Three setae on each side of terminus L7 … 19
    - Two setae on each side of terminus L7 … 20
19. Three setae on the basal segment of endopod A2 and 7 setae on the second endopod segment Md … *H. barnardi* Kornicker, 1978
    - Two setae on the basal segment of endopod A2 and 8 setae on the second endopod segment Md … *H. hox* Kornicker, Harrison-Nelson and Coles, 2007.
20. Four setae on the rostral infold … *H. rhabdion* Kornicker, 1970
    - Eight setae on the rostral infold … *H. mayeri* Kornicker, 1978.

Distribution of *Harbansus*

Most of the species have been reported only from their type localities, or nearby areas. The only exception is *H. paucichelatus* (Fig. 10) which has been collected from several localities in the Atlantic Ocean, from North Carolina to Belize (Kornicker 1978, 1984, Kornicker et al. 2002), at depths of 15 cm to 135 m. The present finding is from Mexico (Yucatan Peninsula), at 1.1 and 1.4 m. The new species is so far the only representative of the genus known from the coral reefs on the west coast of Australia, while another species, *H. satteryi*, was described from the Great Barrier Reef, Queensland (Kornicker 1983). The following species have been described from coral reefs around the world: *H. hox* from French Frigate Shoals (Kornicker et al. 2007a); *H. trix* and *H. ferox* from Mayotte coral reef area (Kornicker 1992); *H. vix* from Enewetak (Kornicker 1991); and *H. flax* from the Tuléar Reef (Kornicker and Thomassin 1998). The other 14 species are known from continental shelves and slopes, with *H. rhabdion* collected from deepest waters, i.e., 1,015 m at Peru–Chile trench (Kornicker 1970, 1978).

Molecular results

Pairwise distances of the COI sequences between four specimens of *H. paucichelatus* from the Yucatan Peninsula varied between 0 and 2 % (Table 2), which is indicative of intraspecific variation when compared to other crustacean groups (Lefébure et al. 2006). Since there are no other COI sequences belonging to this genus, or any species of the family Philomedidae, we did not provide any cladistic tree.

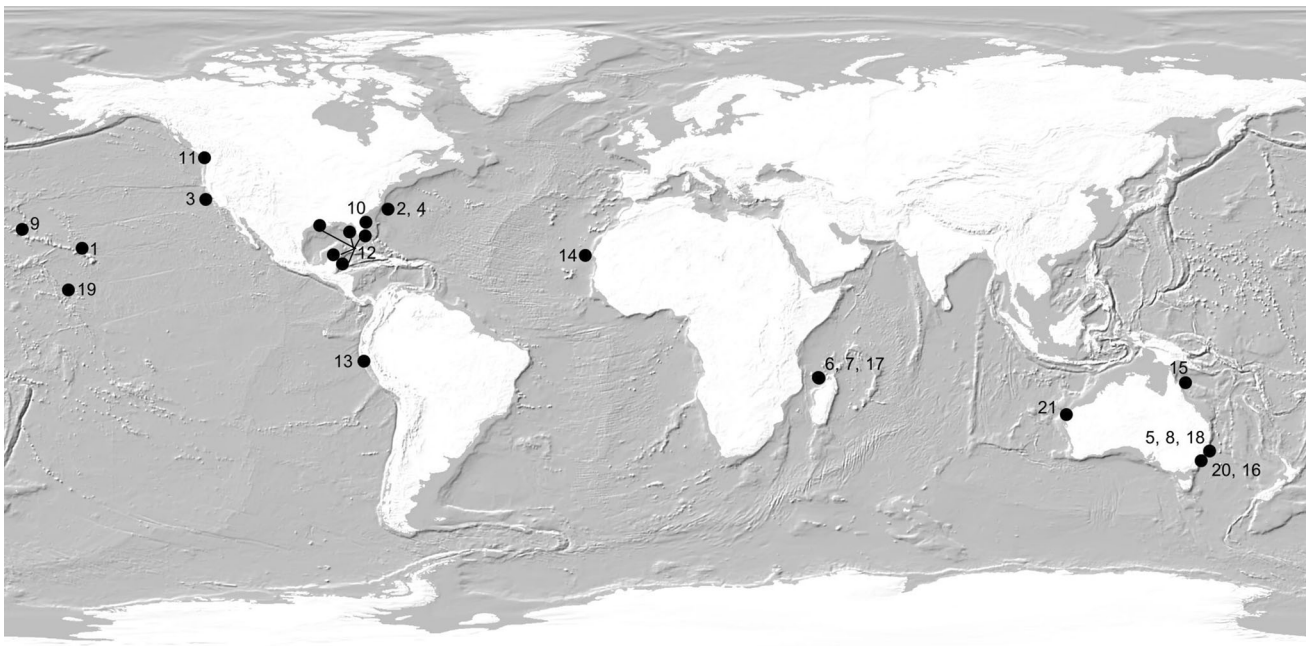

Fig. 10 Map of distribution of *Harbansus* Kornicker, 1978: *1 H. barnardi* Kornicker, 1978; *2 H. bowenae* Kornicker, 1978; *3 H. bradmyersi* Kornicker, 1978; *4 H. dayi* Kornicker,1978; *5 H. felix* Kornicker, 1995; *6 H. ferox* Kornicker, 1992; *7 H. flax* Kornicker, 1998; *8 H. hapax* Kornicker, 1995; *9 H. hox* Kornicker, Harrison-Nelson, Coles, 2007; *10 H. magnus* Kornicker, 1984; *11 H. mayeri* Kornicker, 1978; *12 H. paucichelatus* (Kornicker, 1958); *13 H. rhabdion* (Kornicker, 1970); *14 H. schornikovi* (Kornicker and Caraion, 1977); *15 H. slatteryi* Kornicker, 1983; *16 H. tenax* Kornicker, 1995; *17 H. thrix* Kornicker, 1992; *18 H. vatrax* Kornicker, 1995; *19 H. vix* Kornicker, 1991; *20 H. vortex* Kornicker, 1995; *21 H. ningalooi* n. sp

The aligned sequence data set of 18S rDNA contained 1930 bp, of which 246 bp was variable, while 172 bp was parsimony informative. Distances between 18S rDNA sequences of the 17 studied species varied between 0 and 8 % (Table 3), and those between previously available sequence of *H. paucichelatus* (AF363303.1) and our four specimens of the same species (*H. paucichelatus* F1, F2, M1, M2) was 1 %. The lowest pairwise distance was between some species of the genus *Euphilomedes* and between the Mexican specimens of *H. paucichelatus*. The greatest distance (8 %) was between the ingroup (Sarsielloidea) and outgroup taxa (Cypridinoidea). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Fig. 11). The ML, NJ and MP trees did not share the same topology. The NJ supports the position of *Rutiderma apex* as basal to all other *Euphilomedes* (bootstrap value 87 %) and *E. sordida* 2 as basal to the rest of *Euphilomedes* (bootstrap value 78 %). Maximum parsimony places *R. apex* as basal to the entire ingroup, while *E. sordida* 2 represents a basal branch within the ingroup (bootstrap support 71 %). For the MP tree, bootstrap values for the consensus (50 % cutoff) of seven equally parsimonious trees are shown. The consensus tree had a length of 159 steps, the consistency index 0.786164, and the retention index 0.841121.

The aligned sequence data set of three 28S rDNA segments (dd/ff, ee/mm, and vv/xx) contained 2,356 bp, of which 487 bp was variable, while 231 bp was parsimony informative. Distances between 28S rDNA sequences of eight species varied between 1 and 18 % (Table 4), and the ones between *H. paucichelatus* from the GenBank (AF363303.1) and one specimen of the same species collected from the Yucatan Peninsula (*H. paucichelatus* M1) was 2 %. The lowest pairwise distance was between species of the genus *Euphilomedes*, and the greatest between the ingroup (Sarsielloidea) and the outgroup taxon (Cypridinoidea). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Fig. 12), and the ML, NJ and MP trees shared the same topology. For the MP analysis, single most parsimonious tree had a length of 486 steps, the consistency index of 0.864198, and the retention index of 0.78.

Discussion

With the newly described species, the genus *Harbansus* contains 21 members. Among the five other genera of the subfamily Pseudophilomedinae, it is most closely related to *Angulorostrum, Streptoleberis*, and *Tetragonodon,* as they

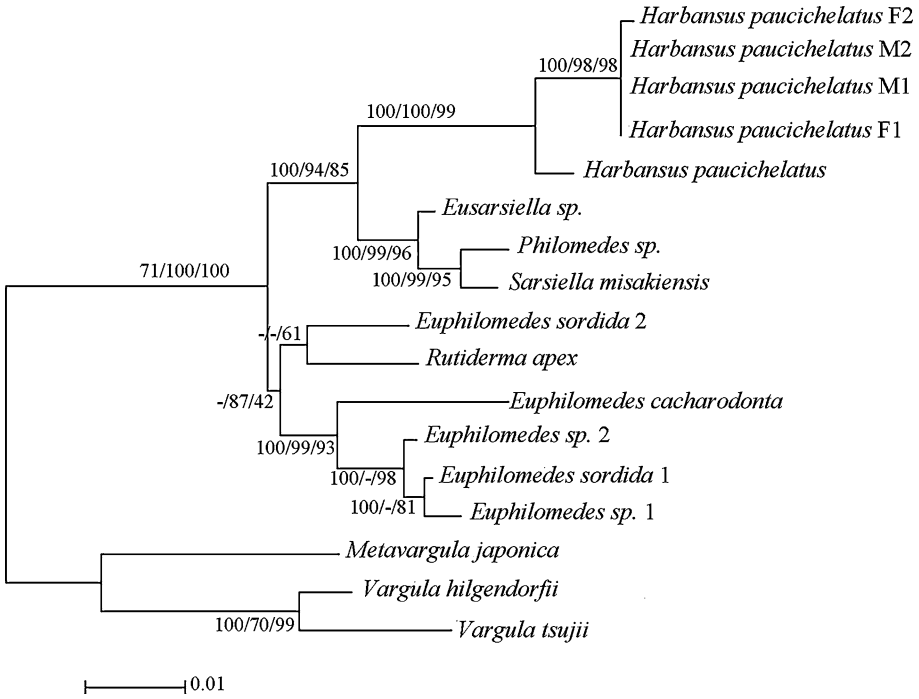

Fig. 11 The ML cladogram based on 18S rDNA sequences. Numbers above branches represent bootstrap values for MP/NJ/ML. The tree is rooted with the *Metavargula-Vargula* clade and drown to scale

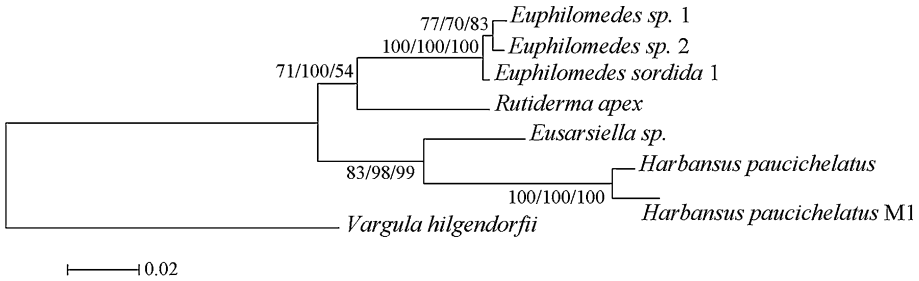

Fig. 12 The ML cladogram based on 28S rDNA sequences. Numbers above branches represent bootstrap values for MP/NJ/ML. The tree is rooted with *Vargula* clade, and drown to scale

all share a square-like tooth on the basis endite two of L5, and relatively elongated caudal and rostral processes. *Harbansus* differs from *Angulorostrum* and *Tetragonodon* by the morphology of the UL, i.e., the secondary claw (third one) is proceeded (second one) and followed (fourth one) by a primary claw. In *Angulorostrum* and *Tetragonodon* the size of the claws decreases along the UL. The morphology of the UL is unknown in *Streptoleberis*, and this genus may even turn out to be a senior synonym of *Harbansus* (see the "Introduction" section above). In the other two genera of Pseudophilomedinae, *Pseudophilomedes*, and *Paramekodon*, the second endite on the L5 bears a saber-like elongation. Although the saber-like tooth was considered to be an autapomorphy of Pseudophilomedinae (Kornicker 1967), this is probably not a phylogenetically informative character state, because several genera of Philomedinae also have similar kind of elongation, as noted by Kornicker (1978). The square-like tooth as in *Harbansus, Angulorostrum, Streptoleberis,* and *Tetragonodon* cannot be found in any other Philomedidae. Even in genera where a saber-like prolongation is not as large as in *Pseudophilomedes,* the large tooth is quite different from these four genera, i.e., it does not have a flat distal margin and often has at least slightly elongated anterior end of the basis second endite. On the other hand, characters that clearly separate Pseudophilomedinae from Philomedinae are the presence of peculiar setae on the posterior infold, morphology of the males sensory bristle on the A1, and (partly—see below) not elongated posterior part of the end segment of the L6.

The peculiarly looking setae on the posterior infold with broad base and thickly covered with setulae (see Fig. 2f, h) are not found in any of the Philomedinae genera, but it is common in almost all representatives of the family Sarsiellidae, in particular in the subfamily Dantyinae. Here, we want to point out that the species described here, *H. ningalooi*, has a peculiar claw-like structure ventral to these setae. A relatively similar structure was found in a sarsiellid species, *Spinacopia ningalooi* Karanovic, 2012, recently described from the Ningaloo Reef (Karanovic 2012). Although Myodocopa ostracods are common on the coral reefs around the world, especially family Sarsiellidae

(see Karanovic 2012), such peculiar structures have never been reported, and therefore, it would be hard to speculate that this may be an adaptation on coral reefs.

The male sensory bristle on the A1 in Pseudophilomedinae is also common in Sarsiellidae, but not in the subfamily Philomedinae, in which this seta has a more elongated base (see Kornicker 1978) and the setulae are not arranged circularly. Although not mentioned as a diagnostic feature of Philomedinae, we believe that the characteristic appearance of c- and f-setae on the male A1 may be a synapomorphy of this subfamily. Namely, males of this subfamily have these two setae with very strong base and turned upwards (see for example descriptions of several *Philomedes* species in Kornicker, 1984), while in Pseudophilomedinae they have the same direction as all the other setae on the appendage. A short end joint of the L6 is also common to Sarsiellidae and Rutidermatidae, but it is very rarely found in Philomedinae, such as, for example in the genus *Igene* (see Kornicker 1978).

The above mentioned morphological characters clearly indicate that Pseudophilomedinae are more closely related to Sarsiellidae than to its supposedly sister subfamily Philomedinae. However, there are numerous important morphological differences even between two members of Sarsiellidae, Dantyinae and Sarsiellinae. The former has a more elongated carapace shape with a prominent rostrum and caudal process (similar to *Harbansus* and its related genera). Although females of Sarsiellinae are globular, males are elongated. Mandibula of Dantyinae is more similar to Pseudophilomedinae in the distribution of setae and claws, but it is more similar to Sarsiellinae in the segments ratio (see Kornicker and Cohen 1978). All Sarsiellidae have a characteristic morphology of the Mxl endopod with a very short first segment and a typical distribution of claws on the terminal segment, but in Dantyinae, its morphology is somewhere between typical Sarsiellinae and Pseudophilomedinae. Finally, the Sarsiellinae females lack teeth on the L5, while females of Dantyinae have a square tooth similar to *Harbansus* and its related genera.

The results of our molecular phylogenetic analyses are in accordance with Oakley (2005), Oakley and Cunningham (2002), and Oakley et al. (2012) indicating polyphyly of Philomedidae (Figs. 11, 12). The bootstrap support for the clade *Harbansus* and Sarsiellidae (*Eusarsiella* sp. and *S. misakiensis*) on the 18S tree (Fig. 11) is 85 % for ML, 94 % for NJ, and 100 % for MP. The clade consisting of *Rutiderma apex* (Rutidermatidae) and other *Euphilomedes* species (Philomedinae) is supported in our ML (although with a very law bootstrap value of 42 %) and NJ (bootstrap value 87 %) analyses, but not in our MP analyses. These values are not enough to support close phylogenetic relationship between Philomedinae and Rutidermatidae. The sequence of *Philomedes* sp. clusters together with two Sarsiellidae and the branch has a high support, but this may be a misidentification, maybe belonging to a male of Sarsiellinae, since they are quite different from females and may easily be confused with Philomedidae. The tree based on 28S sequences (Fig. 12) has also a high support for the Sarsiellidae/Pseudophilomedinae clade, while the Rutidermatidae/Philomedinae clade is not strongly supported on the ML tree (bootstrap value of 54 %). The molecular results are therefore congruent with morphological data, i.e., that Pseudophilomedinae are more closely related to Sarsiellidae than to Philomedinae. Future systematics of Sarsielloidea could include subfamilies Sarsiellinae, Dantyinae and Pseudophilomedinae, but on the family rank. Both Sarsielloidea and Philomedidae will need revision, but when molecular phylogeny is concerned, it should be tested with representatives of more than one genus of Pseudophilomedinae and Philomedinae, and at least some representatives of Dantyinae.

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