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A new genus of Asterocheridae (Copepoda: Siphonostomatoida) ectoassociate of the ascidian *Eudistoma vannamei* Millar, 1977 (Polycitoridae) from Brazil

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Abstract

Asterocheres Boeck, 1860 is the largest genus of the siphonostomatoid copepod family Asterocheridae, containing 63 valid species. The genus is known for its symbiotic relationships with many marine invertebrate taxa, especially sponges, cnidarians, bryozoans, and echinoderms. Recent studies have restricted the diagnosis of this genus. Consequently, many species are now considered as *species inquirendae*. The present paper describes a new species living externally on the tunic of *Eudistoma vannamei* Millar, 1977, an endemic ascidian from Brazil. As the new species does not fit *Asterocheres* in the strict sense, a new genus is erected to accommodate it. *Setacheres* gen. nov. is characterized by its possession of two distal setae on the third endopodal segment of P3, thus differing from the distal seta and spine pattern that is deemed as diagnostic of *Asterocheres.* A revision and comparison of *Asterocheres´ species inquirendae* revealed eight species sharing the same generic characteristics and were thus reallocated as members of the new genus.

Key words: copepod symbiosis, associated fauna, symbiotic fauna, Ascidiacea

Introduction

Copepods of the order Siphonostomatoida are, with rare exceptions, symbiotic organisms that live as commensals or parasites (Ho 2001) of a wide range of marine metazoans, including sponges, cnidarians, molluscs, bryozoans, polychaetes, echinoderms, ascidians and vertebrates (Huys & Boxshall 1991; Ivanenko & Smurov 1997; Boxshall & Halsey 2004; Johnsson & Neves 2012). The siphonostomatoid family Asterocheridae Giesbrecht is the largest family living in association with invertebrates, containing at least 56 genera and 294 species (Ahyong *et al.* 2011). The most speciose genus of this family is *Asterocheres* Boeck, 1860 whose representatives display a cyclopiform body shape and somewhat resemble planktonic copepods. Members of this genus generally exhibit less restricted associations and utilize a wide variety of hosts.

The symbiotic relationships between *Asterocheres* species and various invertebrate host groups such as bryozoans, corals, echinoderms and sponges have been well documented (Ivanenko & Smurov 1997; Kim 2010; Bahia *et al.* 2012; Johnsson & Neves 2012). In contrast, only few species of this genus have been recorded as associates of ascidians (Johnsson *et al.* 2001, Giesbrecht 1897, 1899, Sewell 1949, Brady 1880). According to Brady (1880) Thorell´s specimens of *A. boeckii,* were obtained from the ascidian *Ascidia* (= *Corella*) *parallelogramma*" (Müller, 1776). Sewell (1949) recorded one male specimen of *A. ovalis* Sewell, 1949 from an ascidian. *Asterocheres unicus* Johnsson, 2001 was reported from an unidentified ascidian, but only four specimens (three females and one male) were obtained (Johnsson *et al.* 2001); the same species was also recorded in slightly larger numbers (four females and six males) from other hosts, including sea urchins.

The present study is focused in the ascidian genus *Eudistoma* Caullery, 1909 (family Polycitoridae) which contains around 126 species and is widely distributed in the world's oceans, being more diverse in tropical seas than in temperate ones (Lotufo 2002). *Eudistoma vannamei* Millar, 1977 is a colonial tunicate, composed of zooids of approximately 20 mm. It is endemic to the northeastern coast of Brazil where it has been found in the intertidal

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zone (Millar 1977, Lotufo 2002). This study describes a new asterocherid genus related to *Asterocheres* found in association with *E. vannamei*, being the first copepod known as a symbiont of this host.

Material and methods

The host ascidian, *E. vannamei*, was hand-collected on 10 December 2012 in the intertidal zone during low tide, at depths ranging from 3–6 m, in Porto da Barra Beach (13°0'14.01"S, 38°32'3.14"W), Salvador city, Bahia State, Brazil (Fig. 1). Ten small colonies were collected and immediately put in individual plastic bags. In the laboratory, ethanol was gradually added to the sea water until a final concentration of 5% was reached. Despite the addition of alcohol the specimens did not detach from the host, which were then examined under the dissection microscope. Therefore the asterocherid specimens were found attached to the external tunic of two colonies of *E. vannamei* and then obtained from the hosts with tweezers and fixed in 70% ethanol.

The copepods were examined and measured in temporary glycerol mounts. After clearing in lactic acid and staining in Black Chlorazol E, the paratype was dissected and mounted on a permanent slide with CMC-9 (Huys & Boxshall 1991). The drawings were made with an Olympus CH30 compound microscope equipped with a drawing tube.

In the armature formula of legs 1−4 Roman numerals represent spines and Arabic numerals indicate setae. Antennulary segments are denoted by Roman numerals indicating ancestral segments following Huys & Boxshall (1991). Type specimens were deposited in the Museu de Zoologia of the Universidade Federal da Bahia (UFBA).

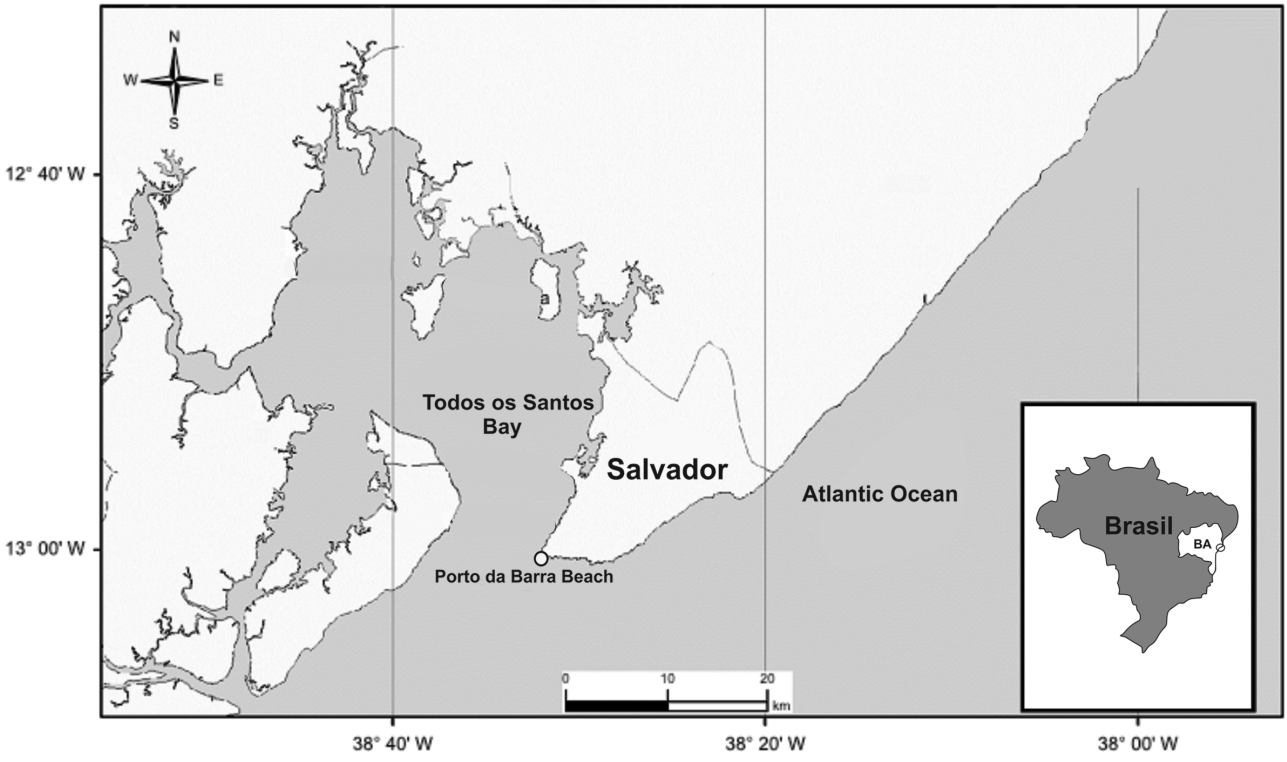

FIGURE 1. Sampling site in Porto da Barra Beach, Todos-os-Santos Bay, Salvador City, Bahia, Brazil.

Taxonomy

Order Siphonostomatoida Thorell, 1859

Family Asterocheridae Giesbrecht, 1899

***Setacheres* gen. nov.**

Diagnosis. Asterocheridae. Body with ovoid or discoid prosome. Urosome 4-segmented in female, 5-segmented in male. Antennule of female with ancestral segments IX-XII fused, 19-21-segmented, with large aesthetasc on 18th segment and 1 to 3 distal segments, respectively. A fusion on the ancestral segments IX to XIII may be observed and the antennule will show 18 to 20 segments, with aesthetasc on 17th segment and 1 to 3 distal segments, respectively. Antenna with 1-segmented exopod and 3-segmented endopod bearing distal claw or spine. Oral cone short or elongate, siphon-like. Mandible consisting of apically pointed stylet and 1- or 2-segmented palp bearing 2 distal setae. Maxillule bilobed. Maxilla 2-segmented; distal segment forming claw. Legs 1-4 with 3-segmented exopod and endopod. Basis of leg 1 with 2 elements, one on each outer and inner margins. Female armature formula of third exopodal segment III,2,2 for leg 1, III,I,4 or III,1,4 for legs 2-4. Female armature formula of third endopodal segment 1,2,3 for legs 1 to 3 or 1,1+I,2 for leg 4. Second endopodal segment of legs 1-4 with 2 inner setae. Free segment of leg 5 with 2 or 3 setae.

Remarks. The females of *Setacheres* gen. nov. have a 4-segmented urosome, a 2-segmented mandibular palp armed with 2 distal setae, legs 1-4 with both rami 3-segmented. The third exopodal segment of leg 1 has 7 elements and that of legs 2-4 8 elements. The third endopodal segment of legs 1-3 has 6 elements and leg 4 5 elements. These characters are shared with other asterocherid genera like *Phyllocheres* Humes 1996, *Chelacheres* Stock & Humes 1995, *Asterocheres* Boeck 1860, *Stockmyzon* Bandera & Huys 2008 and *Hetairosynella* Kim, 2010 (Kim 2010, Bandera & Huys 2008, Stock & Humes 1995, Humes 1996). In *Chelacheres* the autapomorphy is the setation of the third endopodal segment of the antenna, with two robust opposed setae forming a chela-like structure (Stock & Humes 1995). *Phyllocheres* shows a broad prosome, leaf-like, with the tergite of the third pedigerous somite expanded posteriorly over the rest of the prosome (Humes 1996, Boxshall & Halsey 2004). *Stockmyzon* has a distinctly annulated stylet on the antennule.

Despite the strong resemblance between *Setacheres* gen. nov. and *Asterocheres*, the latter genus shows the third endopodal segment of leg 3 armed with 3,1+I,1 and the former shows 3,2,1, as in legs 1 and 2. Such pattern on the leg 3 endopodal was relevant for Kim (2010) to use it as criteria to transfer some species originally placed in *Asterocheres*, to *species inquirendae*. *Cheramomyzon* Humes, 1989 is the only genus within the Asterocheridae that shows the third endopodal segment of leg 3 armed with 3,2,1. However Johnsson & Neves (2004), when redescribing *C. abyssale* Humes, 1989, concluded that *Cheramomyzon* has many characters which are observed either in Asterocheridae or in Artotrogidae, but never on both genera, and therefore the genus should be placed within its own family. Another relevant characteristic of *Setacheres* gen. nov. is the presence of a distal seta instead of a spine on the third exopodal segment of legs 2 to 4 of almost all species of the new genus has not been reported in any other genus of Asterocheridae.

In the revision of *Asterocheres* by Kim (2010), the generic diagnosis focused mainly on the female antennule and leg setation pattern. The antennule bears an aesthetasc on the 18th segment and the distal section has 1–3 segments resulting from fusions for a total of 19–21 segments. The leg setation pattern is strict, with no variations. Consequently, 12 *Asterocheres* species are considered by Kim (2010) *species inquirendae.* Among these species, 8 of them show the third endopodal segment of leg 3 armed with two setae: *Asterocheres abrolhensis* Johnsson, 1998, *A. aplysinus* Johnsson, 2002, *A. lunatus* Johnsson, 1998, *A. paraboecki* Johnsson, 1998, *A. picinguabensis* Johnsson, 2001, *A. spinopaulus* Johnsson, 1998, *A. unicus* Johnsson, 2001, *A. ventricosus* (Brian, 1927) (Johnsson 1998, 2002, Johnsson *et al.* 2001, Brian 1927) and therefore should be transferred to the new genus.

Etymology. The generic name *Setacheres* is a combination of the Latin *seta* (bristle) and *cheres*, apparently derived from the Greek *achtheros* meaning distressing or troublesome to.

Type species. *Setacheres eudistomus* sp. nov.

Other species:

S. abrolhensis (Johnsson, 1998) comb. nov.
S. aplysinus (Johnsson, 2002) comb. nov.
S. lunatus (Johnsson, 1998) comb. nov.
S. paraboecki (Johnsson, 1998) comb. nov.
S. picinguabensis (Johnsson, 2001) comb. nov.
S. spinopaulus (Johnsson, 1998) comb. nov.

S. unicus (Johnsson, 2001) comb. nov.
S. ventricosus (Brian, 1927) comb. nov.

***Setacheres eudistomus* sp. nov.**
(Figs. 2–4)

Material examined. Holotype female (UFBA 1722) and two paratype females (UFBA 1723); (UFBA 1724), Porto da Barra Beach (13°0'14.01"S, 38°32'3.14"W), Salvador city, Bahia State, Brazil, collected by C. Bahia and V. Queiroz on 10 December 2012. All specimens were found attached externally on the tunic of *Eudistoma vannamei*. Paratype (UFBA 1723) dissected and mounted on slide. Remaining types preserved in alcohol.

Description of female. Mean body length (excluding caudal setae) 740 µm (736–744 µm) and mean body width 431 µm (428–434 µm) (n = 3). Body (Fig. 2A) cyclopiform, dorsoventrally flattened, prosome longer than wide, and urosome cylindrical. Pedigerous somite 1 completely fused to cephalosome to form cephalothorax. Pedigerous somites 2–4 with lateral posterior margins rounded. Pedigerous somite 3 more curved, longer than pedigerous somite 2 and partly covering pedigerous somite 4. Pedigerous somite 4 smaller than third somite and slightly larger than fifth somite.

Prosome 511 µm long and 431 µm width. Length: width ratio = 1.2:1. Urosome (Fig. 2B) 4-segmented. Fifth pedigerous somite 43 µm long and 113 µm width. Genital double-somite 86 µm long, maximum width 102 µm, length: width ratio = 0.8:1. Vestigial leg 6 mid-laterally located, close to spinule; row of setules along posterolateral margins and two tooth-like projections close to genital openings. Two postgenital somites, both wider than long (36 × 59, 36 × 54 µm, respectively), lateral margins naked; epimera postero-laterally pointed. Ventral surface of anal somite covered by spinules. Prosome: urosome ratio = 2.5:1. Caudal rami (Fig. 2B) slightly longer than wide, 27 × 25 µm with spinules along inner margin, with six setae (seta I absent); setae VI and VII naked and setae IV and V plumose. Posterior margin with rounded projection between setae III and IV. Length of setae II–VII 45, 125, 186, 245, 80 and 42 µm, respectively.

Rostrum (Fig. 2C) large, wider than long (234 × 145 µm), triangular with rounded apex. Antennule (Fig. 2D) 300 µm long (not including setae), 21-segmented. Length of segments: 34, 17, 8, 8, 11, 5, 11, 7, 11, 4, 13, 13, 19, 20, 17, 20, 24, 27, 12, 13 and 6 µm, respectively. Segmental homologies and armature as follows: 1(I)-2; 2(II)-2; 3(III)-2; 4(IV)-2; 5(V)-2; 6(VI)-2; 7(VII)-2; 8(VIII)-2; 9(IX–XII)-6; 10(XIII)-2; 11(XIV)-2; 12(XV)-2; 13(XVI)-2; 14(XVII)-2; 15(XVIII)-2; 16(XIX)-2; 17(XX)-2; 18(XXI)-2 + aesthetasc; 19(XXII–XXIII)-2; 20(XXIV–XXV)-2; 21(XXVI–XXVIII)-6. Aesthetasc 64 µm long. First segment with both setae plumose at distal half. Segments 9-11 with distal tooth-like projection.

Antenna (Fig. 3A) 211 µm long (including distal claw); coxa 25 µm long, with small seta along outer margin; basis 54 µm long, with sparse spinules along inner margin and setules along outer margin. Exopod 1-segmented, 11 µm long, with two apical setae and small lateral seta; all setae naked. Endopod 3-segmented; first segment 59 µm long, unarmed; second segment 9 µm long, armed with naked seta; third segment 12 µm long, armed with two distal setae, small seta proximally and distal claw (52 µm long).

Oral cone (Fig. 3B) 162 × 61 µm (long × width), reaching to point between bases of maxillipeds and leg 1. Mandible (Fig. 3C) with 2-segmented palp; both segments slender and naked, measuring 18 and 13 µm long, respectively; second segment with two distal setae, smallest seta spinulated and half the length of longer one which is unilaterally spinulated. Mandibular stylet 122 µm long, proximally stout, tapering distally with ten subterminal teeth. Maxillule (Fig. 3D) bilobed; inner lobe 36 µm long, with five distal setae, one of them smaller and more slender than the others; three of the remaining setae unilaterally plumose; inner margin of inner lobe with setules proximally and bunch of setules distally; outer margin with set of spinules. Outer lobe 13 µm long, armed with four distal setae, two being plumose; outer margin with few spinules. Maxilla (Fig. 3E) 250 µm long, consisting of syncoxa (100 µm long, with tubular extension of maxillary gland opening) and strongly distally curved claw with naked seta near inner subdistal margin.

Maxilliped (Fig. 2F) 5-segmented, 267 µm long; syncoxa 82 µm long, with setules along outer margin; basis 84 µm long, with row of setules and few setules along inner and outer margins, respectively. Endopod 3-segmented, segments measuring 8, 11 and 21 µm, respectively; first segment naked; second one with distal tooth-like projection and two naked setae; third segment with serrated spine and distal curved claw-like element, 61 µm long.

Legs 1–4 (Fig. 4A−D) biramous, with 3-segmented rami. Armature formula as follows:

	coxa	basis	exopod	endopod
Leg 1	0–1	1–1	I–0; I–1; III,4	0–1; 0–2; 1,2,3
Leg 2	0–1	1–0	I–1; I–1; III,I,4	0–1; 0–2; 1,2,3
Leg 3	0–1	1–0	I–1; I–1; III,I,4	0–1; 0–2; 1,2,3
Leg 4	0–1	1–0	I–1; I–1; III,I,4	0-1; 0-2; 1,1-I,2

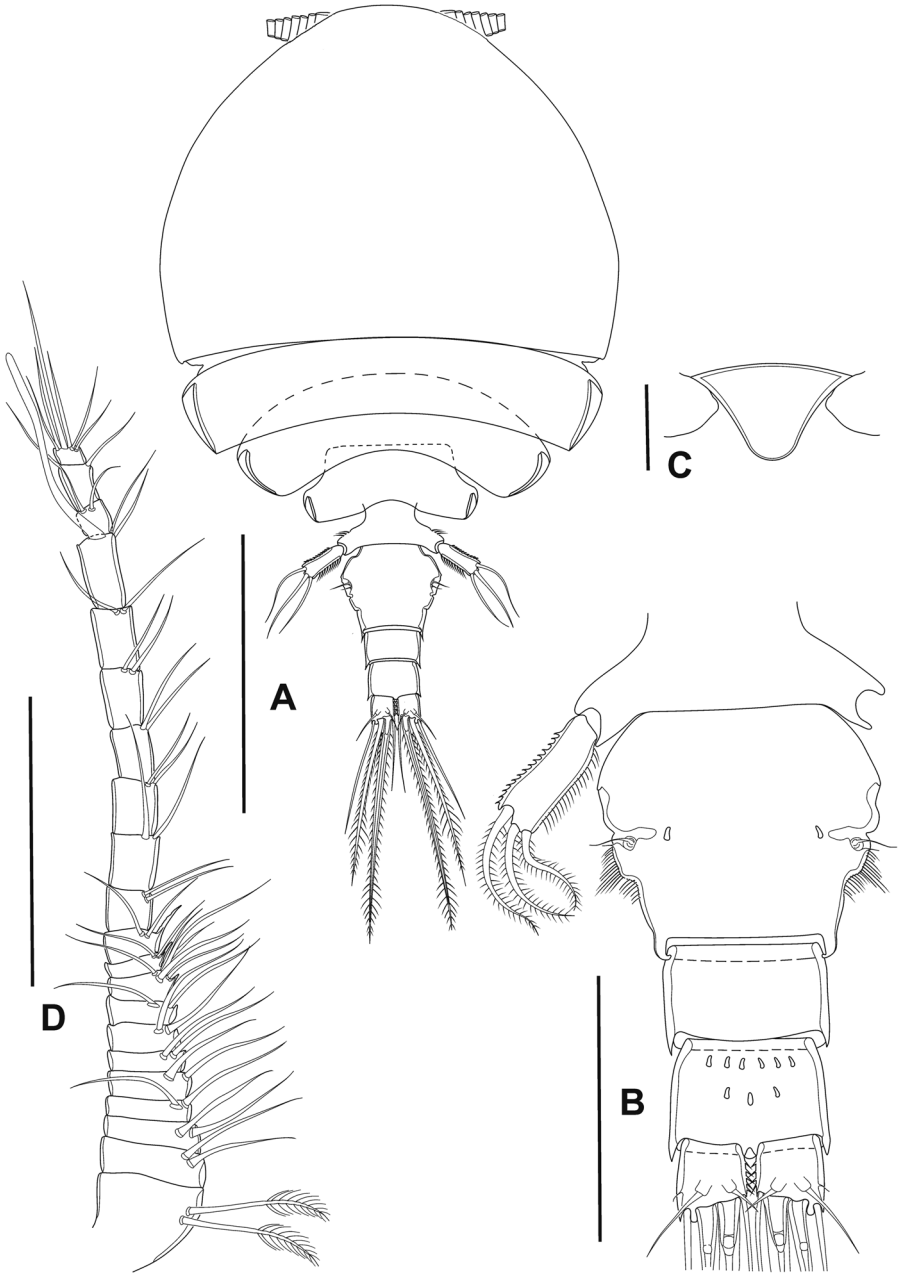

FIGURE 2. *Setacheres eudistomus* **gen. et sp. nov.** female (paratype: UFBA 1723). A, body , dorsal view; B, urosome; C, antennule; D, rostrum. Scale bars: A = 300 µm; B = 250 µm; C = 100 µm; D = 50 µm.

Leg 1 with setules along outer margin of exopod and endopod; proximal exopod segment with large outer spine and without inner seta; middle exopod segment with small outer spine. Legs 2–4 with spinules and setules along outer margins of exopods and endopods, respectively. Leg 5 (Fig. 4E) with three distal setae, with spinules and setules along outer and inner margins, respectively. Intercoxal plate of leg 1 covered with setules, intercoxal plates of remaining legs totally naked.

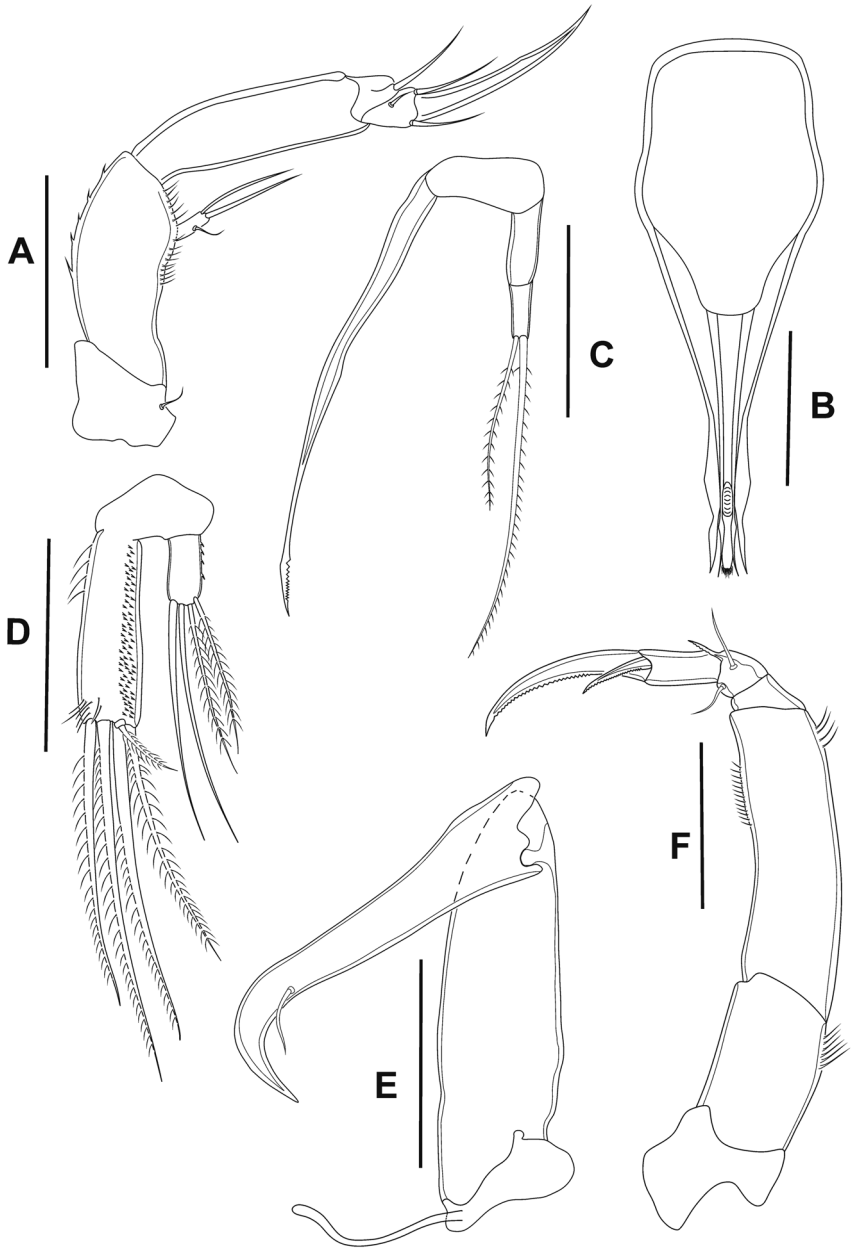

FIGURE 3. *Setacheres eudistomus* **gen. et sp. nov.** female (paratype: UFBA 1723). A, antenna; B, oral cone; C, mandible; D, maxillule; E, maxilla; F, maxilliped. Scale bars: A–F = 50 μm.

Male. Unknown.

Type locality. Porto da Barra Beach (13°0'14.01"S, 38°32'3.14"W), Salvador city, Bahia State, Brazil.

Etymology. The specific name "*eudistomus*" refers to the ascidian host of the new species.

Remarks. Among the species belonging to the new genus *Setacheres* it is recognizable the presence of one to three segments posteriorly to the ancestral antennulary segment XXI indicating their degree of fusion (Johnsson 1998, Brian 1927, Johnsson *et al.* 2001, Johnsson 2002). *Setacheres eudistomus* sp. nov. shows, as observed in *S. lunatus* and *S. paraboecki,* 3 segments posterior to the ancestral XXI (Johnsson 1998). However, in *S. paraboecki* the ancestral segments IX-XIII are fused and therefore show a total of 20 segments while *S. lunatus* and the new species share the fusion of articles IX-XII resulting in a 21-segmented antennule. *Setacheres eudistomus* sp. nov. differs from *S. lunatus* in its having of 5 setae on the inner maxillulary lobe, instead of 4 in the latter species and a 5-segmented maxilliped vs. a 6-segmented one in *S. lunatus* (Johnsson 1998). The new species shows the first exopodal segment of leg 1 without an inner seta (I-0) while all other species of the new genus shows the regular setation pattern with an inner seta (I-1).

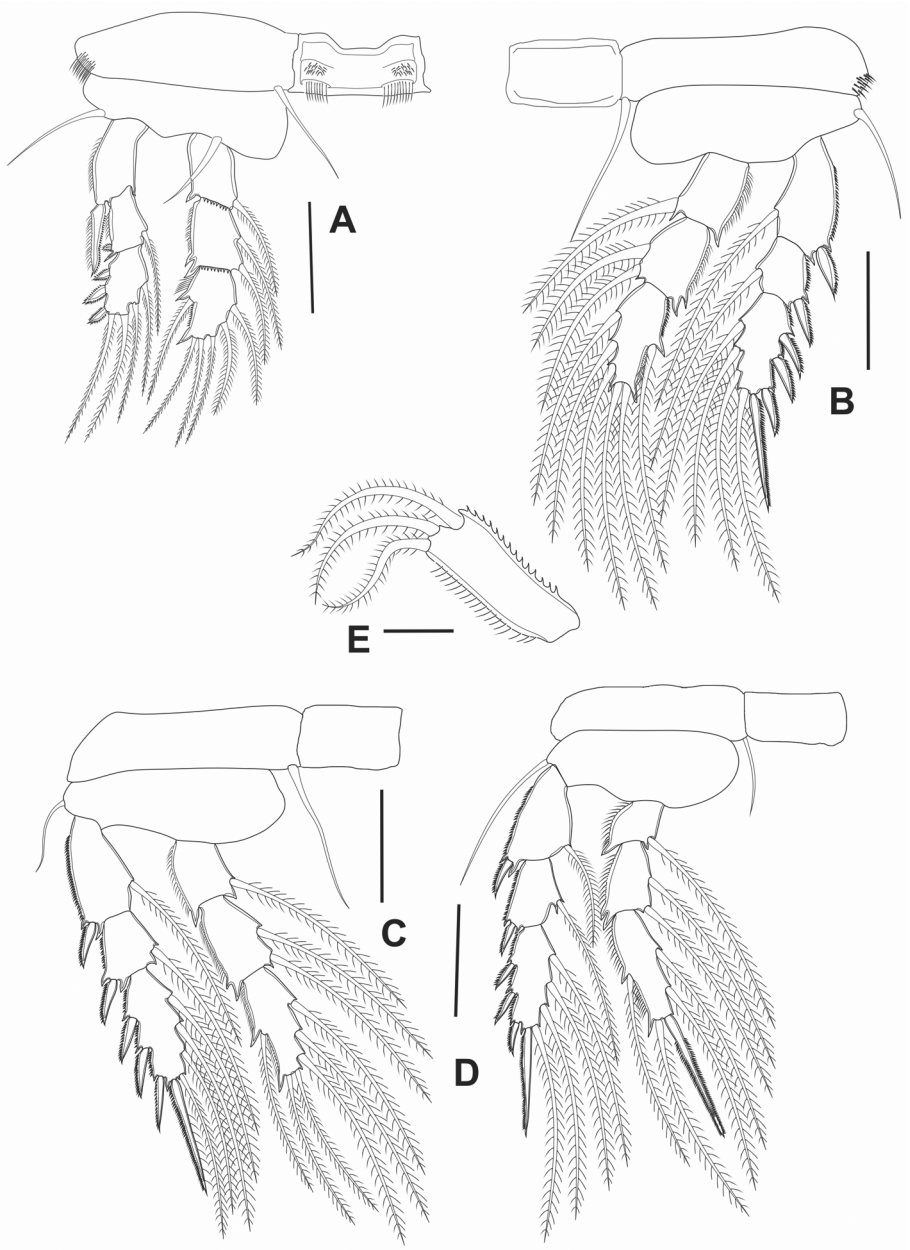

FIGURE 4. *Setacheres eudistomus* **gen. et sp. nov.** female (paratype: UFBA 1723). A, leg 1; B, leg 2; C, leg 3; D, leg 4; E, leg 5. Scale bars: A–D = 50 µm; E = 10 µm.

Distribution. The nine species of *Setacheres* gen. nov. are recorded exclusively from the Atlantic Ocean. *Setacheres ventricosus* is known from the Aegean Sea, an embayment of the Mediterranean Sea. The remaining eight species have been recorded exclusively from the western Atlantic; most of them are so far restricted to the Brazilian coast. *Setacheres picinguabensis* is known to occur in São Paulo State, Warm Temperate Southwestern Atlantic province (WTSA) and *S. abrolhensis* in Bahia State (Johnsson & Neves 2012), Tropical Southwestern Atlantic province (TSA) (Spalding *et al*. 2007). Three species, *S. lunatus, S. aplysinus* and *S. spinopaulus* are restricted to the northeastern coast, occurring in Bahia, Pernambuco and Alagoas States (Johnsson & Neves 2012), all in TSA (Spalding *et al.* 2007). *Setacheres paraboecki* and *S. unicus* are the only species recorded in Rio de Janeiro and Bahia States and São Paulo and Alagoas, respectively and therefore occur in both WTSA and TSA provinces. As *S. paraboecki* is also recorded from Cuba (Varela 2012), this species ranges in two distinct biogeographic realms, the Tropical Atlantic and the Temperate South America, thus suggesting a wide distributional range than that known in other species as *S. unicus*. So far, the remaining six species appear to be restricted to the TSA province. These data corroborate the distributional similarity found in other organisms as

stony corals, decapods, mollusks and polycladids (Neves *et al*. 2006, Neves *et al*. 2008, Neves *et al*. 2010, Queiroz *et al*. 2011, Queiroz *et al*. 2013, Sales *et al*. 2011).

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