

Do Morphological Similarities and human-induced dispersal explain the non-native occurrence of Serpulidae (Annelida) in Southwest Atlantic? Taxonomic detailing is the key

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Abstract. Species of Serpulidae are common on benthic and biofouling communities, occurring attached on both natural and artificial substrates. In this paper, Serpulids were collected from intertidal to subtidal areas, on rocky shore and suspended artificial plates in port areas. Herein we report three new records of serpulid species to the coast of Rio de Janeiro, Brazil. We also discuss the misidentification of *Spirobranchus tetracerus*, which was previously reported to the north of Rio de Janeiro.

Key-Words. *Spirobranchus*; *Hydroides*; *Protula*; Introduced species; Tropical West Atlantic.

INTRODUCTION

The Serpulidae is a family of filter-feeding tubicolous polychaetes with broad global distributions, occurring in virtually all marine environments throughout an extensive bathymetric gradient (Fitzhugh, 1989; Bastida-Zavala & ten Hove, 2003; Sun *et al.*, 2015). Their bodies are divided into a branchial crown, a thorax, and an abdomen (ten Hove & Kupriyanova, 2009). Several species present distinct opercula that can vary considerably in shape, representing a useful morphological feature for differentiating species. Similarly, the different shapes and structures of their typically calcareous tubes have been used to diagnose species among distinctive genera (Bastida-Zavala & Salazar-Vallejo, 2000; ten Hove & Kupriyanova, 2009).

These polychaetes are encrusting organisms that colonise both natural (*e.g.*, algae, shells, and corals) and artificial substrates (*e.g.*, boat hulls, piers, floats, pillars, and solid residues) (Kießling *et al.*, 2015; Bastida-Zavala *et al.*, 2017; Gündoğdu *et al.*, 2017; Gracia *et al.*, 2018). Their notable ability to attach themselves to many types of substrates and different surfaces, even moving objects such

as ship hulls and floats, contributes to their passive transportation from one locality to another, with this colonisation ability perhaps responsible for their global invasiveness. Increasing polychaete abundances in different environments and on diverse substrates has prompted the need for expert identification of these species but, unfortunately, the lack of experienced taxonomists has resulted in ever increasing misidentifications (Rocha *et al.*, 2013; Wong *et al.*, 2014).

According to Çinar (2013), the Serpulidae accounts for ~15% of all reported alien species worldwide, with the most speciose genus *Hydroides* among them. For example, *Hydroides elegans* (Haswell, 1883) has been reported as invasive in many areas across the globe (Schawn *et al.*, 2016; Sun *et al.*, 2018), as is *Ficopomatus uschakovi* (Pillai, 1960) that forms large reef-like aggregates (Assis *et al.*, 2008; Bastida-Zavala & Garcia-Madrigal, 2012). These two species have become established as exotics in Brazil, being frequently found along the coast and usually occurring in high densities on hard substrates.

Rates of exotic species introductions in different oceans have been increasing over the last three decades, probably reflecting the greater

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intensity of anthropogenic activities such as shipping and aquaculture (Carlton, 1989; Ruiz *et al.*, 2000; Neves & Rocha, 2008). The use of sheltered harbors worldwide has increased connectivity among biogeographical regions (Clarke *et al.*, 2004; Lopes, 2009; Castro *et al.*, 2017) and greatly contributes to colonisation by several non-native species. Once biogeographical barriers have been overcome, regional or local transportation is considered crucial for further spread of exotic species (Kauano *et al.*, 2017; Pelletier-Rousseau *et al.*, 2019). Only recently has this topic received attention from local legislatures and in international treaties to control the spread of marine species (Clarke *et al.*, 2004; Lopes, 2009; Rocha *et al.*, 2013; Tricarico *et al.*, 2016; Castro *et al.*, 2017).

A first step in controlling the spread of marine species worldwide and increasing our knowledge of this threat was the 2000-2004 GloBallast Programme (Leal-Neto & Jablonski, 2004; Tricarico *et al.*, 2016), in which Brazil was a partner country. This program prompted the International Maritime Organization (IMO) to establish ballast water controls to prevent species transfer among regions (Clarke *et al.*, 2004; Tricarico *et al.*, 2016). Lopes (2009) listed for the first time the known invasive species in Brazilian marine waters. Rocha *et al.* (2013) highlighted the importance of taxonomic experts in elaborating lists of introduced species. A recent paper on exotic species identification in Brazil promotes collaboration between ecologists and taxonomists (Oricchio *et al.*, 2019).

Here, we discuss the distributions of certain polychaete species occurring in the densest area of ports along the central coast of Brazil and its nearby regions, based on accurate taxonomic identifications and descriptions of these species. We confirm the establishment of *Spirobranchus tetraceros* (Schmarda, 1861) in Brazil. This species was first reported in Brazil by Skinner *et al.* (2012), but those authors misidentified it as *S. giganteus* (Pallas, 1766), a mistake previously identified by Perry *et al.* (2017). We also report new occurrences of another two non-native species, *i.e.*, *Hydroides dianthus* (Verrill, 1873) and *Protula balboensis* Monro, 1933. We discuss the global distributions of these species and their possible invasiveness along the Brazilian coast and in the Southwest Atlantic.

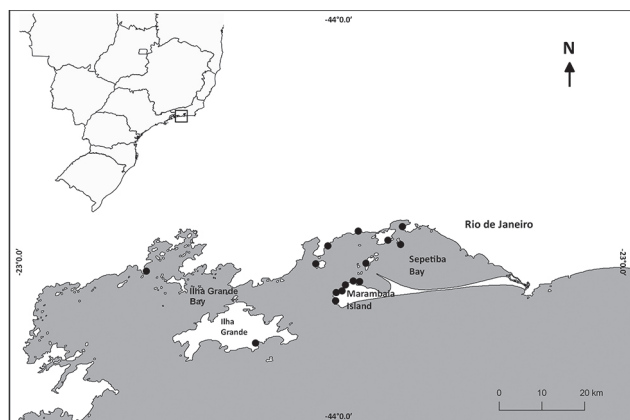


Figure 1. Distribution of the sampling locations at Sepetiba Bay, Ilha Grande and Marambaia Island in the Rio de Janeiro coast.

MATERIAL AND METHODS

We sampled the intertidal and subtidal region in several localities of Sepetiba Bay (22°54'-23°04'S, 43°34'-44°10'W) and in the subtidal area at Ilha Grande (22°50'-23°20'S, 44°00'-44°45'W) totalizing 15 different locations as indicated in Fig. 1. In this area there are five large harbors and one shipyard, all of them dealing with ships coming from different areas of the globe (Clarke *et al.*, 2004; Lopes, 2009; Castro *et al.*, 2017). This region has also the highest number of marinas and recreational boats on South America (Skinner *et al.*, 2016) that helps to create suitable environments for fouling species, and to help its spread.

The polychaetes were collected actively from natural substrates and also, from artificial substrates made of plastic, PVC and granite plates. After being removed alive from the substrate with their tubes, they were anesthetized and euthanized in seawater with Menthol, then fixed in 10% formalin and transferred for permanent preservation in 70% ethanol. All specimens resulted from these surveys are deposited in the Polychaeta collection at Museu Nacional, Rio de Janeiro, MNRJ (Universidade Federal do Rio de Janeiro, Brazil).

The identification of the specimens was performed using the relevant literature (ten Hove, 1970; ten Hove & Kupriyanova, 2009; Bastida-Zavala, 2009). The generic determination was based mainly on the morphology of both the operculum and tubes. For species determination, permanent slides of chaetae were prepared using Hoyer's mounting media (Krantz, 1978). The specimens were photographed and measured using Leica M205C binocular microscope, using LAS software to perform multi-focus images. The measurements of the individuals were obtained dorsally and followed: total length (distance from the anterior end of operculum to the end of pygidium), thoracic length, thoracic width (4th thoracic chaetigerous between the two parapodia), abdominal length, peduncle length (peduncle length plus basal plate of the operculum), operculum width (lateral axis), diameter of the operculum (dorsal-ventral axis). In all individuals, the number of radioles by crown lobe was counted, as well as the abdominal segments. Measurements were expressed in millimeters.

RESULTS

Family SERPULIDAE Rafinesque, 1815 Genus *Spirobranchus* Blainville, 1817 *Spirobranchus tetraceros* (Schmarda, 1861) (Figs. 2-4)

Pomatoceros tetraceros Schmarda, 1861: p. 30; Taf. XXI, fig. 179.

Spirobranchus tetraceros: ten Hove, 1970: p. 3-14; figs. 1-6; 7-14; 15-22; 23-27. Ben-Eliahu & ten Hove, 2011: p. 88-94; fig. 33A-E; table 5. Kupriyanova *et al.*, 2015: p. 337-339; fig. 30C-D.

Examined material: 199 specimens. Rio de Janeiro State, Mangaratiba Municipality: Ilha de Itacuruçá: 22°56'58.6"S, 43°53'13.4"W, MNRJP2184 (105 specimens). Ilha dos Martins: 22°57'17.0"S, 43°51'39.2"W (3 specimens). Ilha de Jaguanum: 23°00'08.8"S, 43°56'14.4"W (6 specimens). Ilha Guaíba: 23°00'13.1"S, 44°03'07.9"W (1 specimen). Mangaratiba: 22°58'57.1"S, 44°03'04.7"W (2 specimens). Praia de Muriqui: 22°55'43.4"S, 43°57'17.9"W (10 specimens). Ibicuí: 22°57'45.3"S, 44°01'28.5"W (4 specimens). Ilha da Marambaia: Praia de João Emanuel: 23°02'35.4"S, 43°57'42.0"W (7 specimens). Praia da Armação: 23°02'39.9"S, 43°57'06.6"W (10 specimens). Praia Suja: 23°03'57.2"S, 43°59'31.3"W (21 specimens). Praia Grande: 23°03'57.5"S, 43°59'32.2"W (4 specimens). Praia da Cutuca: 23°04'03.7"S, 43°59'42.2"W (10 specimens). Rio de Janeiro State, Itaguaí Municipality: Ilha da Madeira: 22°55'06.3"S, 43°51'14.3"W (16 specimens). Rio de Janeiro State, Angra dos Reis municipality: Piraquara: 23°01'14.0"S, 44°26'24.5"W (8 specimens). Dois Rios: 23°11'08.0"S, 44°11'24.4"W (2 specimens).

Description (based on 10 individuals collected from Itacuruçá Island and Marambaia Island, Brazil)

Tube: Externally white/light pink and sometimes violet in smaller tubes, inside light pink (Fig. 2A). Sub-triangular in cross-section, with a single prominent wavy median and longitudinal ridge, laterally with fine transverse growth markings, lacking alveoli and peristomes (Fig. 2A). Anterior end with a fine tooth extending over the opening (Fig. 2A). Attached to rocky shores, *Perna perna* (Linnaeus, 1758) mussels and artificial substrates, such as PVC plates.

Radiolar crown: Radioles arranged in a circle on each side, with 18 radioles per lobe. Inter-radiolar membrane extending to about $\frac{1}{3}$ of radiolar length, smooth and bearing external rounded processes (Fig. 2B). Internally,

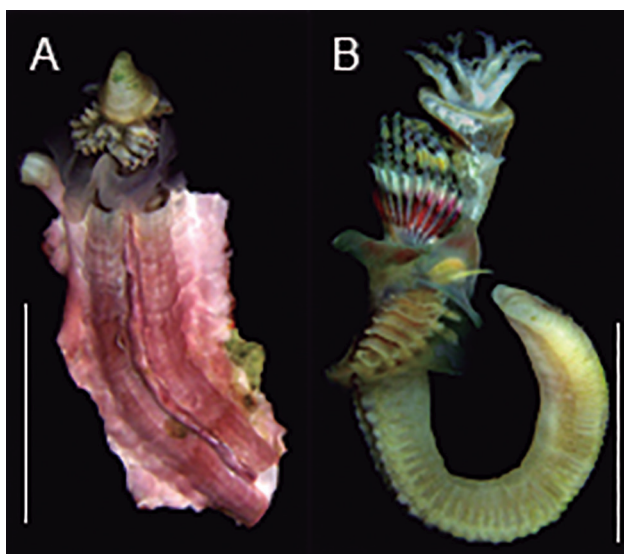


Figure 2. *Spirobranchus tetracerus*. (A) Tube with longitudinal ridge and projection in the anterior part of the tube; (B) Complete body, lateral view. Scale bars: A-B: 2 mm.

radioles with two rows of pinnules of the same length. Terminal filament without pinnules. Stylodes absent. Eyespots absent. In live specimens, the bases of radioles (including the inter-radiolar membrane) exhibit a mix of black/pink/yellow/white pigments (Figs. 2B, 3A, C-D). In fixed specimens, the radiolar crown has blue pigments (Fig. 3B).

Peduncle: Smooth, inserted left of the radiolar crown, near the medial line; dark pigments with irregular darker stripes on the dorsal side (Fig. 3); fixed specimens with blue pigments. Proximal area smooth and with narrow stem; distal area flattened and with a broad stem below the opercular plate (Fig. 3). Peduncle color extends into lateral wings. Pair of peduncular wings fringed with digitate processes (Fig. 3). Length: mean = 1.12 (SD = 0.38; n = 10).

Operculum: Four distinct morphotypes, all with a circular calcareous opercular plate (Fig. 3). Type A: distal end of operculum conical and with concentric striations, without spines (Fig. 3A). Type B: initial bifurcation of short non-ramified spines that share the same base and lack spinules, with two spines lying dorso-lateral and a third ventral to the top of the opercular plate (Fig. 3B). Type C: four spines, but a group of three shares the same base; two of which are dorso-lateral, larger and forked once, a third is ventral, smaller and also forked once; spines with spinules at their tips and not divided to the opercular plate (Fig. 3C). Type D: similar to type C, but the spines are developed totally and exhibit more ramifications at their tips than other types, spinules present. Dorso-lateral spines ramified three or two times, ventral spine ramified twice (Fig. 3D). Spines white. Diameter: mean = 0.7 (SD = 0.16; n = 10); Width: mean = 0.71 (SD = 0.18; n = 10).

Collar and thoracic membranes: Collar well-developed (Figs. 2B, 3C), covering $\frac{1}{2}$ of the radiolar crown, divided into one ventral and two lateral lobes of the same size. Ventral lobe triangular. Tonguelet present, dark in colour (black) between ventral and latero-dorsal lobes. Latero-dorsal lobes extend to thoracic membranes, producing a short ventral apron. Two types of collar chaetae: 1) limbate or 2) bayonet-like with many small teeth at the base of the 'blade' (Fig. 4A). Collar and thoracic membranes light brown in colour (Fig. 3C).

Thorax: Seven chaetigers, six of which are uncinigerous (Fig. 2B); collar fascicle without row of uncini. Thoracic chaetae limbate and of two different sizes (Fig. 4B). Uncini saw-shaped, with 9-10 teeth and including an anterior-most gouge-shaped peg tooth (Fig. 4D).

Abdomen: Number of abdominal chaetigers varies from 32 to 71 (mean = 49.9; SD = 15.93; n = 10). Chaetae trumpet-shaped (Fig. 4C). Uncini saw-shaped, with 10-12 teeth and including gouge-shaped peg tooth (Fig. 4E).

Measurements: Total length: mean = 5.4 (SD = 2.0; n = 10); Thoracic length: mean = 1.24 (SD = 0.36;

n = 10); Thoracic width: mean = 0.64 (SD = 0.27; n = 10); Abdominal length: mean = 2.6 (SD = 1.35; n = 10).

Remarks: Two species of *Spirobranchus* have been reported for the Brazilian coast (Amaral et al., 2013), *Spirobranchus giganteus* (Pallas, 1766) and *Spirobranchus minutus* (Rioja, 1941). Both of these species are readily discernible from our collected specimens, identified herein as *S. tetraceros*.

The radioles of the radiolar crown in *S. giganteus* are spirally arranged, and the wings of the penduncle form a smooth inverted triangle (ten Hove, 1970). In contrast, the radioles of the radiolar crowns of our specimens are circularly arranged and the wings of the penduncle are fringed. The opercula of our specimens also differ from

those of *S. giganteus*. The opercular spines of *S. giganteus* (two large ones, as well as other smaller ones) are ramified. The opercula of our specimens present four basic shapes (Fig. 3), varying from conical to a flat disc, and with three spines (one ventral and two lateral) sharing the same base (Fig. 3D).

Spirobranchus minutus has three longitudinal ridges and alveoli at the base of the tube (Rioja, 1941), unlike *S. tetraceros*, that has serrated ridges along the tube forming a tip without alveoli. Moreover, *S. minutus* has a triangular opercular penduncle with narrow and pointed wings (Zibrowius, 1970), whereas that of *S. tetraceros* is fringed with digitate wings. The operculum of *S. minutus* is globose, almost transparent, extends over a calcified plate (Zibrowius, 1970), and lacks spines.

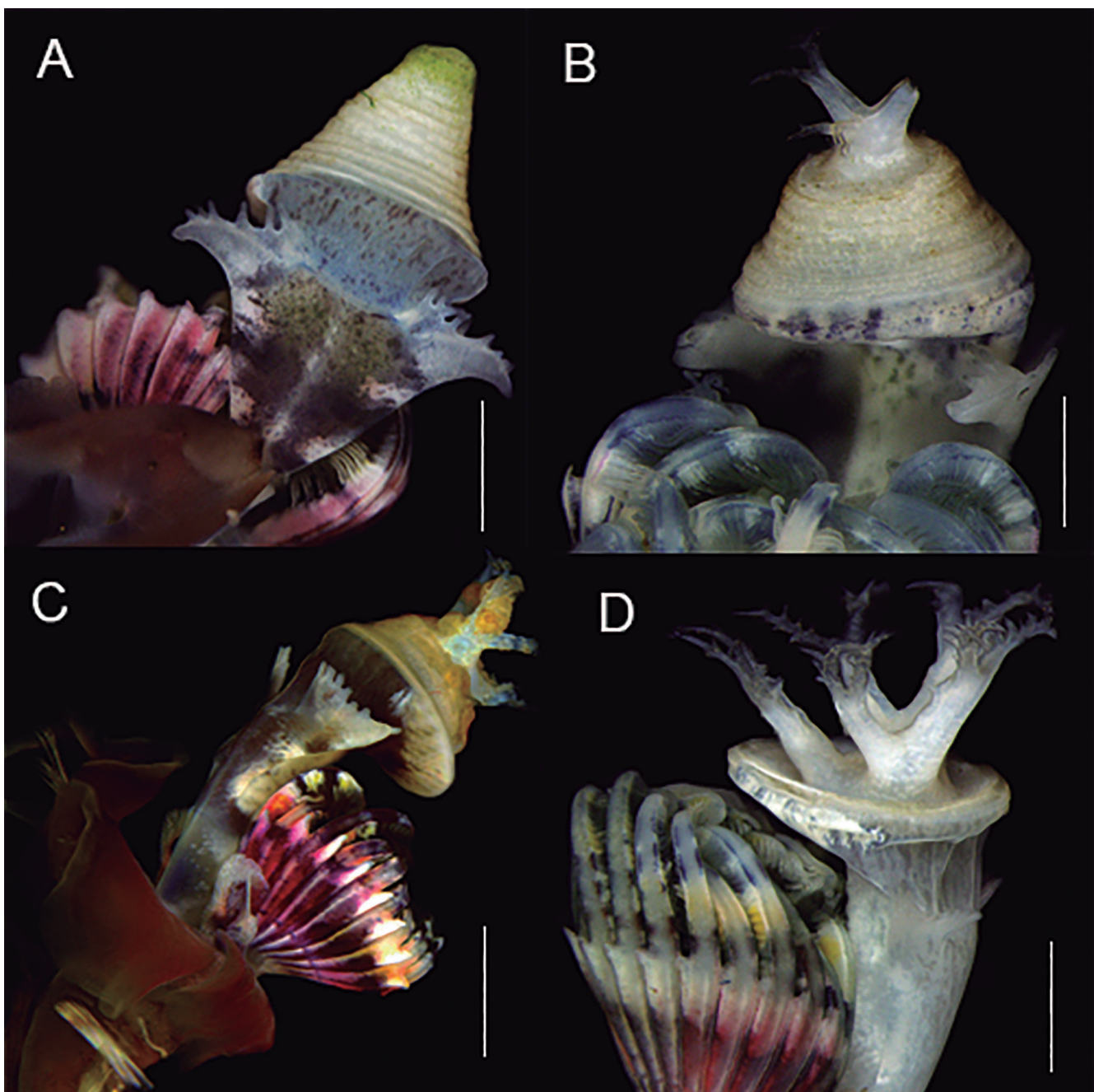


Figure 3. *Spirobranchus tetraceros*, operculum morphotypes. (A) Conical operculum, latero-dorsal view; (B) Initial bifurcation of operculum, dorsal view; (C) Bi-horned operculum, latero-dorsal view; (D) Bi-horned operculum, lateral view. Scale bars: A-D: 500 µm.

The species of *Spirobranchus* that occur in Curaçao (Caribbean Sea) were reviewed by ten Hove (1970), resulting in 22 taxa synonymized under the name *S. tetraceros*. However, Bastida-Zavala & Salazar-Vallejo (2000) re-elevated *Spirobranchus dendropoma* (Mörch, 1863) to the species level based on their Mexican Caribbean material, distinguishing it from *S. tetraceros* based on morphological and biogeographical differences (Perry *et al.*, 2018). In their analyses of the material collected from the Mexican Caribbean, Bastida-Zavala & Salazar-Vallejo (2000) observed that their specimens were morphologically more similar to *S. dendropoma* than to *S. tetraceros*, and also considered biogeographic information in identifying the species. Herein, we consider morphological characters for our specimens identifications, including

the position of processes extending from the radiolar membrane. These processes occur at the bases of the radioles in *S. dendropoma* (Benedict, 1887; ten Hove, 1970). In our examined specimens of *S. tetraceros*, the interradiole membrane processes occur between radioles and present a rounded shape.

Spirobranchus tetraceros was originally described from New South Wales in Australia (Schmarda, 1861), but it has also been recorded in the Red Sea (Perry *et al.*, 2017), Mediterranean Sea, Hong Kong (Sun *et al.*, 2012), Egypt, the Persian Gulf, and Curaçao (ten Hove, 1970). This species was first recorded in Brazil at Arraial do Cabo (Skinner *et al.*, 2012), and herein we expand its distribution to Sepetiba Bay and Ilha Grande, both of which are in the southeast of the country.

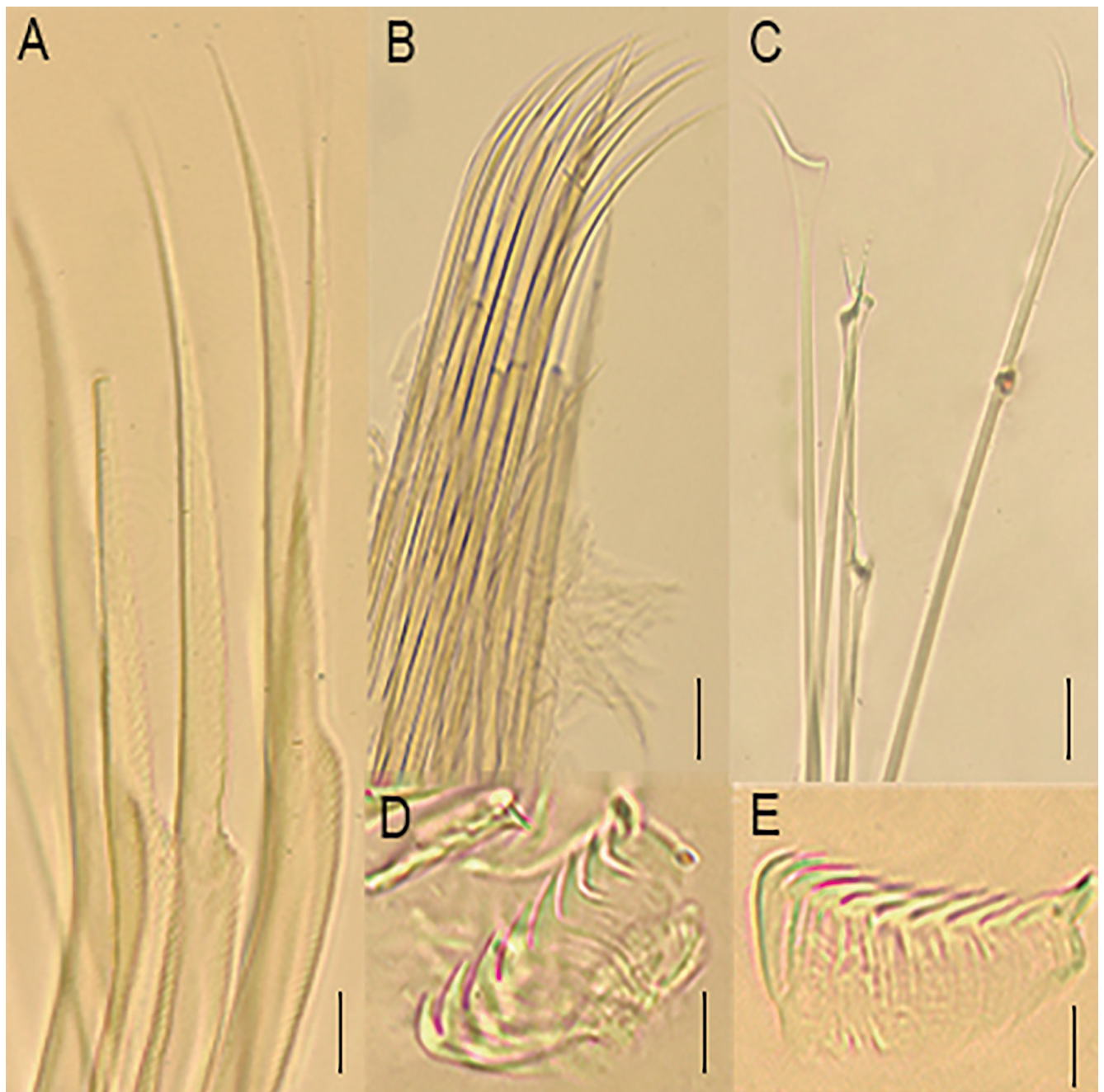


Figure 4. *Spirobranchus tetraceros*, types of chaetae. (A) Collar chaetae; bayonet chaetae, with tip processes; (B) Thoracic chaetae; limbate; (C) Abdominal chaetae; chaetae trumpet-shaped; (D) Uncini thoracic with 8 teeth; (E) Uncini abdominal with 11 teeth. Scale bars: A-E: 500 µm.

Habitat: The species were found attached to rocky shores, *Perna perna* mussels and artificial substrates, such as PVC plates in subtidal areas.

Type-locality: New South Wales, Australia.

Distribution: West Pacific Ocean: Philippines; Port Jackson; Pandanon; Ubay; West Indian Ocean: Tanzania; Mozambique; Madagascar; Central Indian Ocean: Pearl Banks of Ceylon; Sri Lanka; Red Sea: Gulf of Aqaba; Red Sea; Djiboutian part of the Gulf of Aden; Persian Gulf; Mediterranean Sea; Lebanese part of the Mediterranean Sea; Greece; West Atlantic Ocean: Gulf of Mexico; Colombia (Read, 2018b); Brazil, at Arraial do Cabo, misidentified as *S. giganteus*, see Perry et al., 2017, Sepetiba Bay and Ilha Grande Bay (current work).

Genus *Hydroides* Gunnerus, 1768
***Hydroides dianthus* (Verrill, 1873)**
(Figs. 5 and 6)

Serpula dianthus Verrill, 1873: p. 620.

Serpula dianthus var. *citrine*: Verrill, 1873: p. 620-621.

Hydroides dianthus: Bastida-Zavala & ten Hove, 2002: p. 143-147; figs. 23A-M, 24A-K, 28; Bastida-Zavala et al., 2017: p. 25-28; figs. 4C, 5.

Examined material: 70 specimens. Rio de Janeiro State, Mangaratiba Municipality: Praia de Ibicuí: 22°57'45.3"S, 44°01'28.5"W, MNRJP2186 (1 specimen). Ilha de Itacuruçá: 22°56'58.6"S, 43°53'13.4"W, MNRJP2185 (18 specimens). Praia de Muriqui: 22°55'43.4"S, 43°57'17.9"W (9 specimens). Ilha dos Martins: 22°57'17.0"S, 43°51'39.2"W (11 specimens). Ilha de Jaguanum: 23°00'08.8"S, 43°56'14.4"W (1 specimen). Rio de Janeiro State, Itaguaí Municipality: Ilha da Madeira: 22°55'06.3"S, 43°51'14.3"W (31 specimens).

Description (based on 10 individuals collected from Madeira Island and Muriqui)

Tube: Tube white both internally and externally (Fig. 5A), and circular in cross-section. Without longitudinal ridge, laterally with fine transverse growth markings (Fig. 5A); alveoli and peristomes absent. Attached to rocky shores, mollusc shells and artificial substrates such as PVC plates.

Radiolar crown: Radioles arranged in semi-circles, with 14-16 radioles per lobe (Fig. 5C). Inter-radiolar membrane absent. External side of radioles smooth, internal side with two rows of pinnules of the same length (Fig. 5C). Terminal filament without pinnules. Stylodes absent. Eyespots absent. Base of radiolar crown exhibits a mixture of orange and yellow pigments, together with alternating bands of dark brown and white (Fig. 5C). In fixed animals, color is light yellow.

Peduncle: Cylindrical, but with a constriction ill-defined at the distal end (Fig. 5B); proximal part a smooth nar-

row stem. Peduncle inserted left side. Pseudopericulum present, smaller, rudimentary and inserted right side. Length: mean = 1.2 (SD = 0.26; n = 10).

Operculum: Bioperculated (Fig. 5B); funnel-shaped base with 30-33 radii and a pointed tip; verticil with 10 amber-coloured spines, four dorsal spines curving towards internal face of verticil; ventral spines smaller and with spinules on their external faces (Fig. 5B), without central tooth; without internal and lateral spinules. Diameter: mean = 0.33 (SD = 0.08; n = 10); Width: mean = 0.33 (SD = 0.06; n = 10).

Collar and thoracic membranes: Tri-lobed, subdivided into one medio-ventral and two lateral lobes (Fig. 5C). Tonguelet absent. Latero-dorsal lobes extend to thoracic membranes with a short ventral apron. Two types of chaetae: limbate or bayonet-like with two blunt elongate teeth, distal blade smooth (Fig. 6A).

Thorax: Seven chaetigers, six of which are uncinigerous; collar fascicle without row of uncini. Chaetae limbate (Fig. 6B), with two different sizes; uncini saw-shaped with 8-10 teeth including a gouge-shaped peg tooth (Fig. 6C).

Abdomen: Total number of chaetigers varies from 31 to 83 (mean = 56; SD = 16.9; n = 10). Chaetae of anterior and mid-abdominal chaetigers trumpet-shaped; uncini saw-shaped, with 6-8 teeth including a gouge-shaped peg tooth (Fig. 6D). Posterior chaetigers with capillary chaetae.

Measurements: Total length: mean = 6.2 (SD = 1.35; n = 10); Thoracic length: mean = 1.3 (SD = 0.34; n = 10); Thoracic width: mean = 0.57 (SD = 0.14; n = 10); Abdominal length: mean = 3.8 (SD = 1.2; n = 10).

Remarks: There are 92 described species for the genus *Hydroides* (Sun et al., 2015), 13 of which have already been reported the Brazilian coast: *H. brachyacantha* Rioja, 1941; *H. cruciger* Mörch, 1863; *H. dianthus*; *H. dirampha* Mörch, 1863; *H. elegans*; *H. gairacensis* Augener, 1934; *H. norvegica* Gunnerus, 1768; *H. parvus* (Treadwell, 1902); *H. plateni* (Kinberg, 1867); *H. sanctaegrucis* Krøyer in Mörch, 1863; *H. similoides* Bastida-Zavala & ten Hove, 2002; *H. lambecki* Bastida-Zavala & ten Hove, 2002; and *H. uncinata* (Philippi, 1844) (Zibrowius, 1970; Amaral et al., 2013; Schawn et al., 2016).

Among those, only *H. sanctaegrucis* could possibly be confused with *H. dianthus*, by the presence of the ventrally curved spines at verticil. However, external spinules are present on the spines of *H. sanctaegrucis*, which are lacking in *H. dianthus* (Bastida-Zavala & ten Hove, 2002). All 10 verticil spines of *H. dianthus* are smooth, with four larger dorsal spines, and six smaller ventrally (Bastida-Zavala & ten Hove, 2002; Lewis et al., 2006).

Hydroides dianthus was originally described for the eastern coast of the United States and from Cape Cod (Connecticut). The species occurs in eastern USA, northern Gulf of Mexico, Curaçao, along the Atlantic coasts of

Europe and western Africa, Mediterranean Sea (Bastida-Zavala & ten Hove, 2002), and at Cabo Frio (Rio de Janeiro State) in Brazil (Sun *et al.*, 2017). Here we include the occurrence of *H. dianthus* in Sepetiba Bay (Rio de Janeiro State), with specimens collected in the intertidal zone, growing on rocks, gastropods, and piers, and co-occurring with other species of the same genus.

Habitat: The species were found attached to rocky shores, mollusc shells and artificial substrates such as PVC plates.

Type-locality: Connecticut (Great Egg Harbor to New Haven and Cape Cod), USA.

Distribution: West Pacific Ocean: Japan (Otani & Yamanishi, 2010; Link *et al.*, 2009); North Atlantic Ocean: Spain (Zibrowius, 1983); United Kingdom (Otani & Yamanishi, 2010; Link *et al.*, 2009); France (Dauvin *et al.*, 2003); Belgium; Mediterranean: Mediterranean (Zenetos *et al.*, 2005; Zenetos *et al.*, 2010); Adriatic Sea; Italy (Marchini *et al.*, 2015; Corriero *et al.*, 2016); Turkey (Trott, 2004); Greece (Otani & Yamanishi 2010; Link

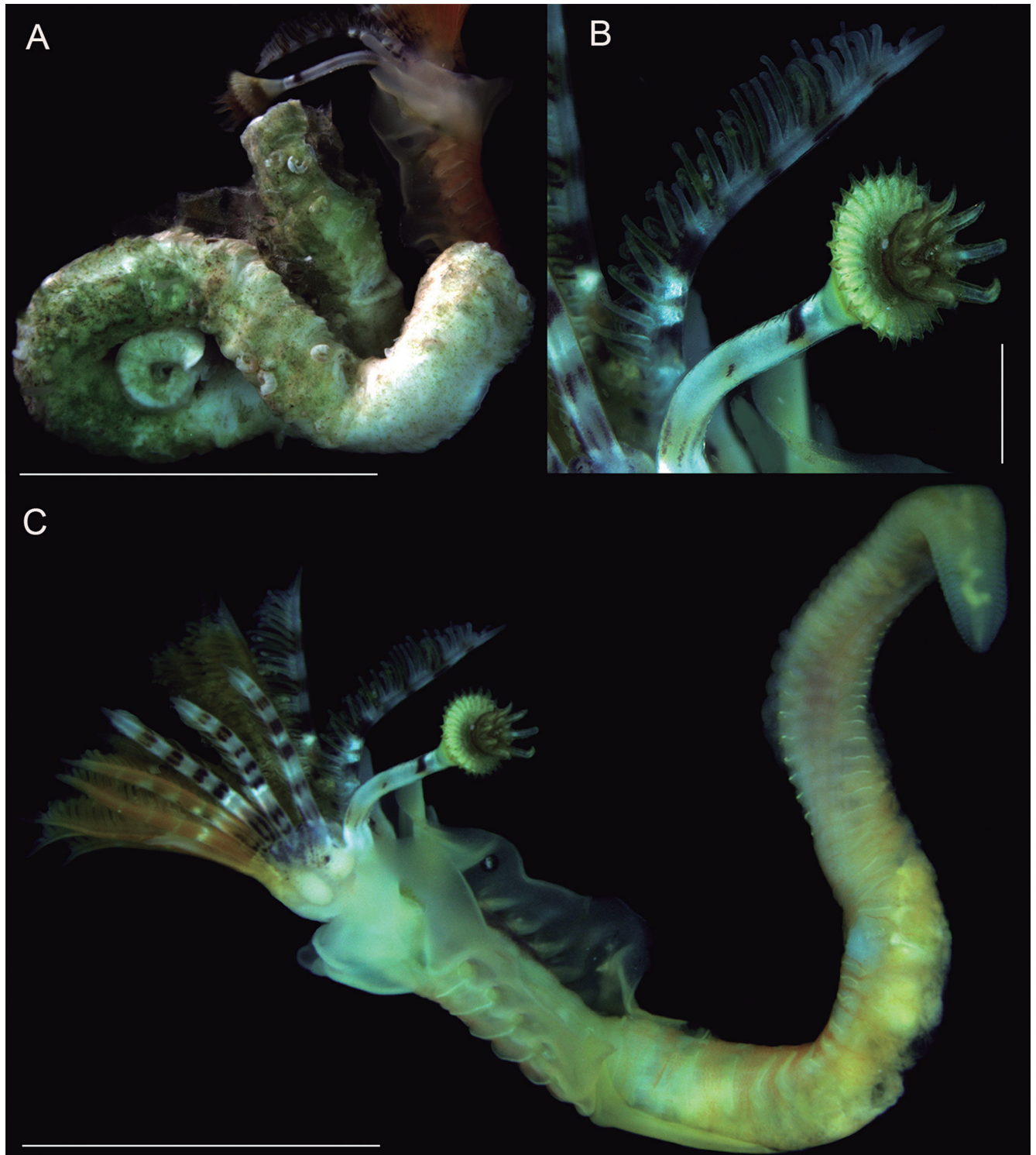


Figure 5. *Hydroides dianthus*. (A) Tube; (B) Operculum detail; (C) Complete body, dorso-lateral view. Scale bars: A and C: 2 mm; B: 500 μ m.

et al., 2009); East Atlantic Ocean: Gulf of Guinea (Dauvin *et al.*, 2003; Read, 2018a); West Atlantic Ocean, United States: Cobscook Bay (Trott, 2004); Gulf of Maine (Otani & Yamanishi, 2010; Link *et al.*, 2009); Massachusetts (Marchini *et al.*, 2015; Corriero *et al.*, 2016); Narragansett Bay, Rhode Island; Chesapeake Bay, Virginia; Charleston, South Carolina; Jacksonville, Indian River, Biscayne Bay, Tampa Bay and Pensacola Bay, Florida; and Galveston Bay and Corpus Christi, Texas (Bastida-Zavala *et al.*, 2017); Mexican Caribbean (Read, 2018a); West Atlantic Ocean: Cabo Frio, Brazil (Sun *et al.*, 2017), Sepetiba Bay (current work).

Genus *Protula* Risso, 1826
***Protula balboensis* Monro, 1933**
(Figs. 7 and 8)

Protula tubularia balboensis Monro, 1933: p. 1088-1090; text-figure 30, A-D.

Protula balboensis: Zibrowius, 1970: p. 17-18; pl. 4; fig. 9; Bastida-Zavala, 2008: p. 37; fig. 8H; Bastida-Zavala *et al.*, 2017: p. 42-43; figs. 7B-E, 8.

Examined material: 28 specimens. Rio de Janeiro State, Mangaratiba Municipality: Ilha da Madeira: Praia do Sino: 23°05'01.7"S, 44°00'27.9"W, MNRJP2187 (22 specimens). Praia de Ibicuí: 22°57'45.3"S, 44°01'28.5"W (3 specimens). Ilha de Itacuruçá: 22°56'58.6"S, 43°53'13.4"W

(2 specimens). Ilha Guaíba: 23°00'13.1"S, 44°03'07.9"W (1 specimen).

Description (based on 10 individuals collected from Marambaia Island)

Tube: Externally, white and rugose, inside white (Fig. 7C); circular in cross-section. Without longitudinal ridge, alveoli and peristomes. Attached to rocky shores of intertidal zone.

Radiolar crown: Radioles arranged in semi-circles with 18-20 radioles per lobe (Fig. 7A). External side smooth, internal side with two rows of pinnules. Terminal filament without pinnules. Inter-radiolar membrane $\frac{1}{3}$ of radioles length, with rounded processes at end of membrane (Fig. 7B). Stylodes absent. Eyespots present, pigmented with red or yellow colours. Colour base in live animals a mixture of orange/yellow pigments, in fixed animals a light yellow.

Peduncle: Absent.

Operculum: Absent.

Collar and thoracic membranes: Trilobed, well-developed, reddish thoracic membrane in live, subdivided into one mid-ventral and two lateral lobes, the same size (Fig. 7A). Tonguelet absent. Latero-dorsal lobes continuing in thoracic membranes well-developed producing a

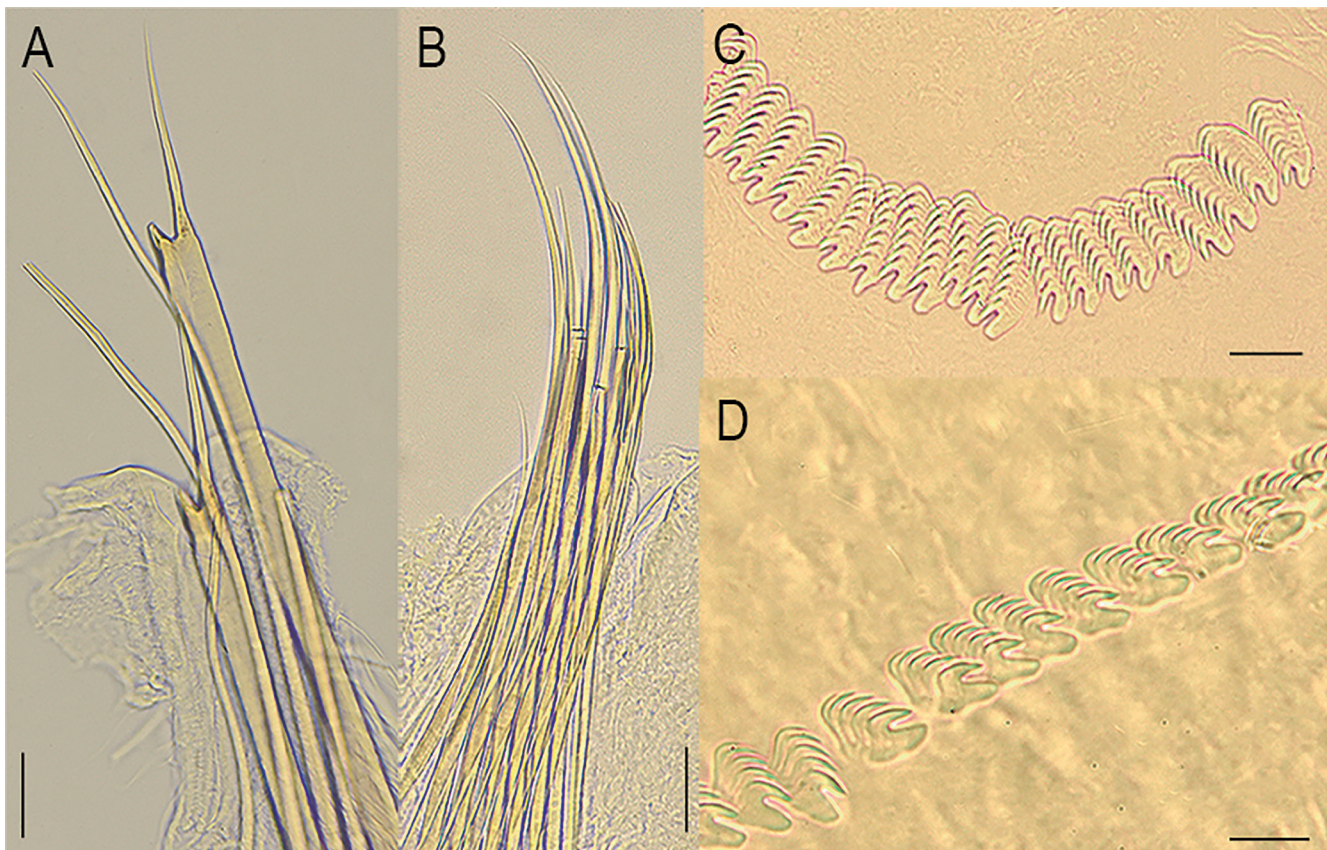


Figure 6. *Hydroides dianthus*, types of chaetae. (A) Collar chaetae; bayonet chaetae with rounded process; (B) Thoracic chaetae; limbate; (C) Uncini thoracic with 8 teeth; (D) Uncini abdominal with 6 teeth. Scale bars: A-D: 500 μ m.

short ventral apron. Collar chaetae of only one type: limbate (Fig. 8A).

Thorax: Seven chaetigers, including five uncinigerous; collar fascicle without row of uncini. Second chaetiger with chaetae limbate, without uncini. Chaetae limbate

of 2 sizes (Fig. 8B). Uncini rasp-shaped teeth including peg; peg (anterior-most tooth) gouge shaped. *Apomatus* chaetae absent.

Abdomen: Total number of chaetigers varies from 26 to 42 (mean = 37; SD = 5.7; n = 10). Anterior and mid-ab-

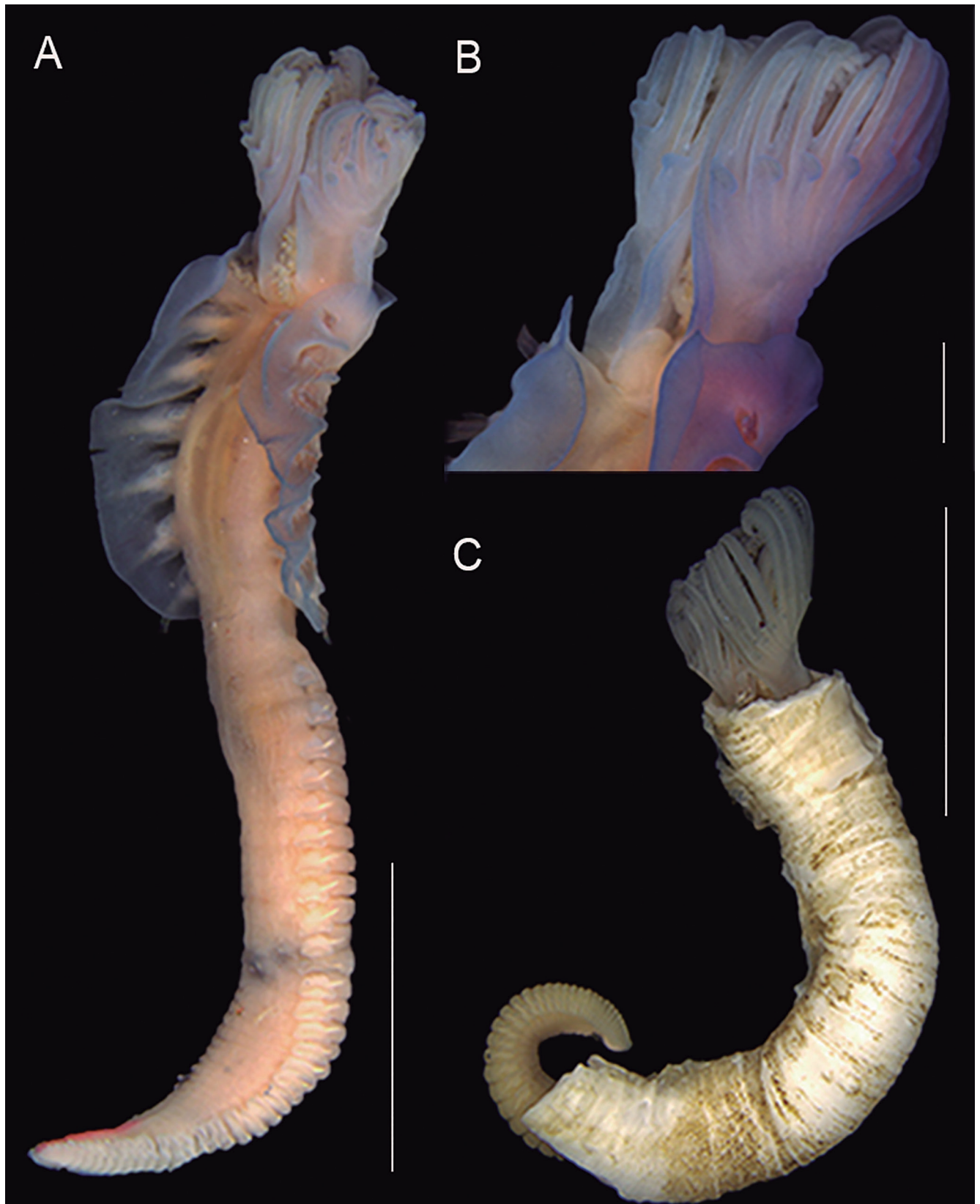


Figure 7. *Protula balboensis*. (A) Complete body, lateral view; (B) Branchial crown; (C) Tube with a worm inside. Scale bars: A and C: 2 mm; B: 500 μ m.

dominal chaetigers, chaetae geniculate (Fig. 8C); abdominal uncini similar to thoracic uncini. Posterior chaetigers with capillary chaetae.

Measurements: Total length: mean = 4.2 (SD = 1.4; n = 10); Thoracic length: mean = 1.4 (SD = 0.45 [n = 10]); Thoracic width: mean = 0.54 (SD = 0.16; n = 10). Abdominal length: mean = 2.2 (SD = 0.87; n = 10).

Remarks: Twenty-three species of *Protula* are known from across the globe (ten Hove & Kupriyanova, 2009), of which only three have been recorded from the Brazilian coast: *P. tubularia* (Montagu, 1803), *P. submedia* Augener, 1906, and *P. balboensis* (Amaral et al., 2013; Pagliosa et al., 2014).

Protula tubularia and *P. balboensis* are similar in having limbate thoracic chaetae and geniculate abdominal

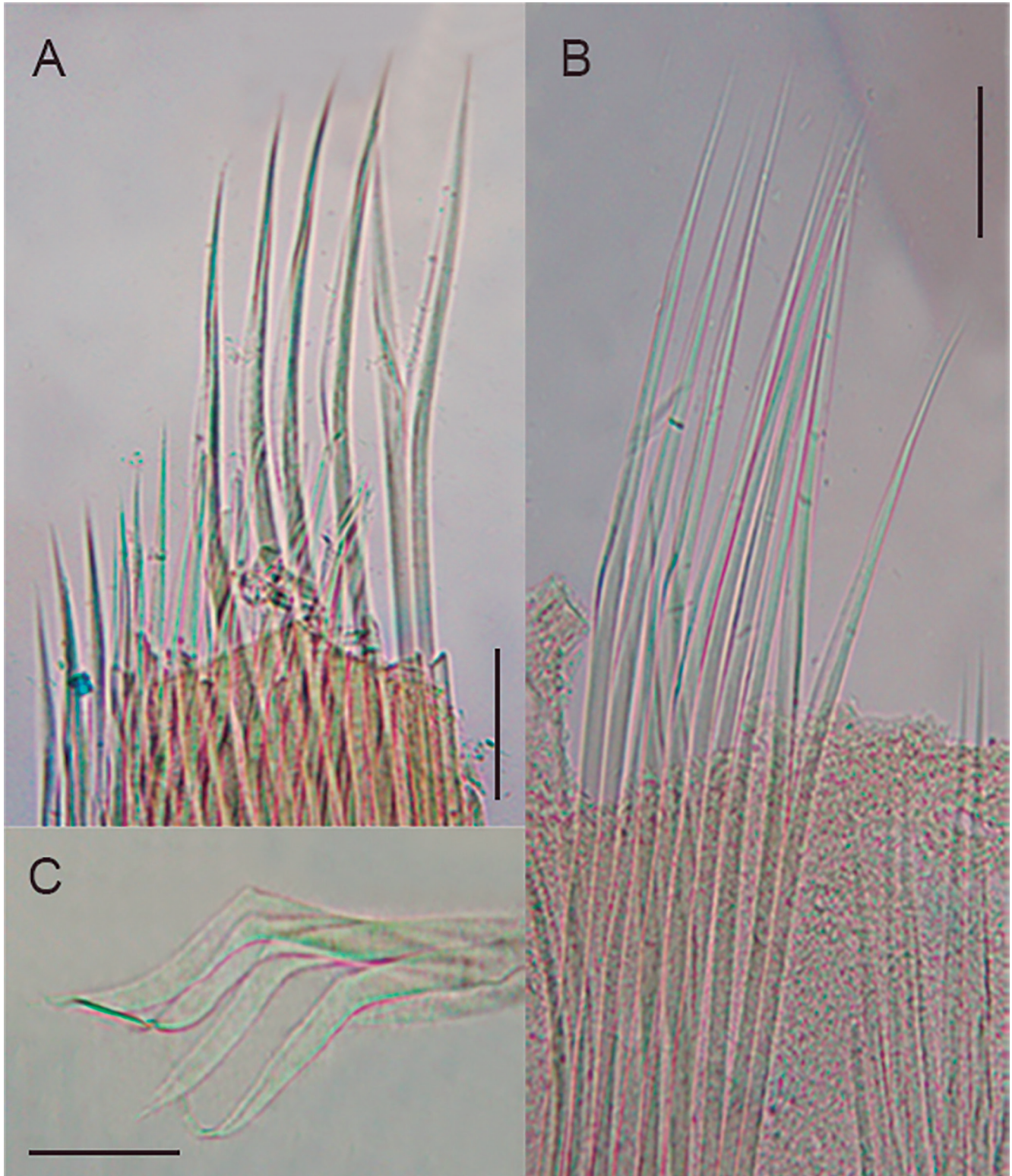


Figure 8. *Protula balboensis*, types of chaetae. (A) Collar chaetae; limbate chaetae; (B) Thoracic chaetae; limbate chaetae; (C) Abdominal chaetae; geniculate chaetae. Scale bars: A-C: 500 µm.

chaetae. *Protula balboensis* has interradiolar processes on the external face of the radiolar membrane and a rugose tube, whereas *P. tubularia* lacks interradiolar processes and its tube is smooth (*i.e.*, without ridges, peristomes or alveoli) (Monro, 1933; Zibrowius, 1970).

Protula submedia and *P. balboensis* are quite distinct. *Protula submedia* produces a tube showing growth lines with peristomes in the anterior section, the number of radioles varies between 25-50, and their abdominal chaetae are straight (Zibrowius, 1970). In contrast, *P. balboensis* produces a rugose tube (without ridges, peristomes or alveoli), has from 18 to 20 radioles per lobe in large individuals, and its abdominal chaetae are geniculate.

Our specimens have rugose tubes, lacking ridges, peristomes or alveoli, membrane processes are present, the collar and thoracic chaetae are limbate but the abdominal chaetae are geniculate/sickle-shaped. *Protula balboensis* was described from Balboa on the Pacific coast of Panama. In Brazil, the species was recorded previously at Recife da Lixa, Bahia State, Brazil and, here, we present the first record of this species from Rio de Janeiro State, Sepetiba Bay, in southeastern Brazil.

Habitat: The specimens were found attached to rocks of the intertidal zone and attached under *Phragmatopoma* sp. aggregate.

Type-locality: Balboa, Pacific Panama.

Distribution: West Pacific Ocean: Japan (Uchida, 1978); West Atlantic Ocean, United States: Jacksonville; Florida; Biscayne Bay, Florida; and Corpus Christi, Texas (Bastida-Zavala *et al.*, 2017); Gulf of California (Bastida-Zavala, 2008); Pacific Ocean: Mexico (Bastida-Zavala *et al.*, 2016), Acapulco (Bastida-Zavala, 2008), Taboga Island, Panama and Gorgona Island (Bastida-Zavala *et al.*, 2016); Colombia (Monro, 1933); Atlantic Ocean: Bocas Del Toro (Bastida-Zavala *et al.*, 2016), Atlantic Panama (Bastida-Zavala *et al.*, 2016); West Atlantic Ocean, Brazil: Bahia (Zibrowius, 1970) and Rio de Janeiro, Sepetiba Bay (current work).

DISCUSSION

Lopes (2009) reported eight invasive polychaete species from the Brazilian coast, none of which were serpulids. Later, Rocha *et al.* (2013) listed 44 non-native polychaete species in Brazil, 10 (22%) of which were serpulids: *F. uschakovi*, *H. brachyacantha*, *H. dianthus*, *H. dirampha*, *H. norvegica*, *Janua brasiliensis* (Grube, 1872), *Janua pagenstecheri* (Quatrefages, 1866), *S. minutus*, *Salmacina incrustans* Claparède, 1870 and *Salmacina huxleyi* (Ehlers, 1887), but *J. pagenstecheri* is unaccepted, now it named *J. heterostropha* (Montagu, 1803). From among those species, we also collected specimens of *H. brachyacantha*, *H. dianthus* and *H. dirampha*, thereby expanding their occurrence to northwestern Rio de Janeiro State. These species have also been reported previously in the states of Pernambuco, Bahia (both northeastern Brazil),

São Paulo, Santa Catarina, as well as southeastern Rio de Janeiro. Oricchio *et al.* (2019) reported two other polychaete species as introduced in Brazil, *Branchiomma luctuosum* (Grube, 1870) (Sabellidae) and *H. elegans* (Serpulidae). However, two other serpulid species could not be identified in that study, reinforcing the need for experts in taxonomy to assist in studies on introduced species.

One of the problems encountered in compiling lists of introduced species relates to a general lack of detailed knowledge of native species, especially in tropical regions (Junqueira, 2013). Most of the material that Brazilian researchers have access to has come from collection efforts financed by oil companies operating along the Brazilian coast to meet local environmental regulations. However, in order to effectively combat the threats of invasive species, accurate taxonomy is necessary to better define the local fauna (Trebitz *et al.*, 2017). Accurate taxonomic assignments are not always immediately available, especially because of a lack of specialists in taxonomically complex groups, such as the Serpulidae (Lewis *et al.*, 2006; Pyšek *et al.*, 2013; Schwan *et al.*, 2016). Molecular approaches can help to solve this issue, but formal species descriptions using morphological characters are still necessary and some research groups may not have access to expensive and technologically-intensive molecular techniques (ten Hove & Kupriyanova, 2009; Sun *et al.*, 2016, 2018; Perry *et al.*, 2018).

Bays and estuaries are considered hotspots for species introductions via fouling on ships and the dumping of ballast water (Rocha *et al.*, 2013; Bumbeer & Rocha, 2016; Ferrario *et al.*, 2017). Recent studies have indicated other possible routes of non-native species introductions. For example, Campbell *et al.* (2017) reported that floats and plastic ropes used in aquaculture installations can serve as artificial substrates for encrusting organisms, including serpulid polychaetes. Among the water-borne anthropogenic materials that have drawn attention in recent years, plastic is known to act as a vector for non-native species dispersal (Ruiz *et al.*, 1997; Bumbeer & Rocha, 2016; Campbell *et al.*, 2017).

Zibrowius (1970) pioneered the study of the Serpulidae in Brazil. Subsequent research greatly expanded our knowledge of the diversity and ecology of this group in the southern Atlantic (Nonato & Luna, 1970; ten Hove, 1975; Rullier & Amourex, 1979; Silva *et al.*, 1980; Knight-Jones & Knight-Jones, 1991; Paiva, 1993; Santa-Isabel *et al.*, 2000; Nogueira & ten Hove, 2000; Neves & Omena, 2003; Winston & Migotto, 2005; Assis *et al.*, 2008, 2009, 2012; Nogueira & Abbud, 2009; Skinner *et al.*, 2012; Amaral *et al.*, 2013; Schawn *et al.*, 2016; Vieira *et al.*, 2016). As a result of these efforts, the serpulid fauna in Brazil is known to number 43 species, of which only 14% are originally described for Brazil and the remaining 86% are considered exotic species (Amaral *et al.*, 2013; Pagliosa *et al.*, 2014).

Thirty-four species of the genus *Spirobranchus* are known to occur in subtropical and tropical waters (Pillai, 2009; Perry *et al.*, 2018). In Brazil, the genus is represented by two non-native species, *S. minutus* and *S. giganteus*.

Spirobranchus minutus was described from Acapulco in Mexico (Rioja, 1941) and the first Brazilian specimen was collected from São Sebastião (São Paulo State) and later from Ubatuba, in the same State (Zibrowius, 1970; Amaral et al., 2013). The first Brazilian specimen of *S. giganteus* collected was from Fernando de Noronha Island (Zibrowius, 1970), with subsequent specimens collected from the states of Bahia, Alagoas and Rio de Janeiro (Amaral et al., 2013). Skinner et al. (2012) reported new associations of *S. giganteus* with substrates other than corals at Arraial do Cabo, Rio de Janeiro, but among the photographs presented in that article, one specimen does not correspond to *S. giganteus* (Skinner et al., 2012: fig. 1D), but rather to *S. tetraceros*, a mistake previously identified by Perry et al. (2017) and confirmed in the present study.

Species of the genus *Spirobranchus* present differences in opercular morphology, which is the main identification character of the group. However, intraspecific variation in this character can result in misidentifications or incorrect assignment as new species. Based on our material, we consider four ontogenetic morphotypes, i.e., those with: i) a completely conical operculum with concentric striations; ii) an operculum with three small rudimentary spines; iii) an operculum with three more fully-developed and bifurcated spines; or iv) an operculum with two bifurcated lateral spines and a central well-developed spine (Fig. 3A-D). Such within-species morphological variability has been reported for other species. Szabó (2015) observed differences in operculum development among specimens of *S. lamarcki* (Quatrefages, 1866), but the present issue is not discussed based on operculum ontogeny (Rodrigues et al., in preparation). *Spirobranchus tetraceros* was originally identified from Australia, but today is broadly distributed from the Indo-Pacific region to the Caribbean. This species is considered invasive in some of these localities, e.g., Turkey, in the Mediterranean Sea (Pillai, 2009; Perry et al., 2017). In Brazil, Silva (2008) noted *S. tetraceros* among the species identified in her study and Skinner et al. (2012) recorded *S. tetraceros* at Arraial do Cabo. Here, we add the third record of this non-native species in Brazil.

Thirteen species of the genus *Hydroides* have been identified from along the Brazilian coast (Amaral et al., 2013; Pagliosa et al., 2014; Schawn et al., 2016; Ananias, 2017). Based on the results presented in Schwan et al. (2016) and Amaral et al. (2013), *H. elegans* and *H. dirampha* appear to be commonly found in Brazilian port zones. Dispersal of these species within or between localities primarily occurs through encrusting human infrastructure, shipping, plastic rafting, and marine litter (Mangano et al., 2018). Recently, *H. dianthus* was collected from Arraial do Cabo in Rio de Janeiro State by Sun et al. (2017), with this species also potentially being invasive in port zones. *Hydroides dianthus*, originally described from Connecticut (USA), has been introduced via anthropogenic transport to China, Europe, Japan, the West Indies and Brazil (Sun et al., 2017). Here, we add the second record of *H. dianthus* for the Rio de Janeiro State, Brazil.

Of the Serpulidae that do not have opercula, three genera have been recorded from along the Brazilian

coast: *Salmacina*, *Filogranella*, and *Protula* (ten Hove & Kupriyanova, 2009). *Protula balboensis* was originally described from Balboa (Panama), and it has been found on rocks, dock pillars and breakwaters during low tides at Taboga Island (Panama) and Gorgona Island (Colombia) (Monro, 1933), all in the Pacific Ocean. Thereafter, there was a long gap in reports of this species in any survey, until recently when Ananias (2017) recorded the species in the municipality of São Sebastião (Brazil) where it inhabited subtidal and intertidal zones. Our new record from the southern coast of the Rio de Janeiro State is near the locality explored previously by Ananias (2017) and helps to clarify the distribution of occurrences for this species along the Brazilian coast. However, additional surveys are necessary to fully establish the distributional extent of this species in Brazil.

Morphological similarities among some species of polychaetes, particularly with regard to features such as their calcareous tubes and opercula, as is the case for the Serpulidae, can result in errors of taxonomic identification if they are not analyzed by experts. Incorrect taxonomic assignment can result in specimens being elevated to the status of invasive species when in fact they may be undescribed native species or are simply exhibiting within-population variation.

Here, we present three new records of Serpulidae for the Rio de Janeiro State: *S. tetraceros*, *H. dianthus*, and *P. balboensis*. *Spirobranchus tetraceros* was initially recorded as being an invasive in Brazil from the southern coast of Rio de Janeiro, and we confirm its continued occurrence there, representing a good opportunity to follow and manage the spread of this species to nearby areas. Our study contributes to filling the knowledge gap regarding occurrences of these three species along the Brazilian coast and increases the number of non-native polychaete species (Rocha et al., 2013) reported in Brazil to 46. However, we strongly recommend further surveys to assess invasiveness in the Brazilian marine environment.

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