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A new species of *Pachycerianthus* (Cnidaria, Anthozoa, Ceriantharia) from Tropical Southwestern Atlantic

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Abstract

A new species of *Pachycerianthus* (Cnidaria: Ceriantharia) is described from the Brazilian coast of the southwestern Atlantic Ocean. *Pachycerianthus schlenzae* **sp. nov.** is found in fine sand or mud in shallow waters of Abrolhos and Royal Charlotte Bank. The new species is only known from this area and is most notably different from other species of the genus *Pachycerianthus* in mesentery arrangement and cnidome. In addition to the description, we provide some biological data collected from individuals cultivated under laboratory conditions.

Key words: Systematics, DNA Barcoding, Morphology, Cerianthidae

Introduction

The Abrolhos Bank is an approximately 46,000 km^2 extension of the eastern Brazilian continental shelf in the south of Bahia State, Brazil. The best known area is the Abrolhos Archipelago, which was established as the first National Marine Park of Brazil and which comprises the largest and richest coral reefs of the South Atlantic, with at least 20 species of stony corals, including six endemic to Brazil (Leão *et al.*, 2003). The dynamic interaction of the reef biota and a local geochemical profile of both siliciclastic and carbonate deposition generates a coral reef system with an active terrigenous sedimentation area (Leão & Kikuchi, 1999). Waters of the Brazil Current flow over the Abrolhos Bank in a generally north to south direction (Meyerhöfer & Marone, 1996; Leão & Kikuchi, 1999).

This habitat is part of the Tropical Southwestern Atlantic biogeographic province (Spalding *et al.*, 2007), which is biodiverse with high rates of endemicity (*e.g.* for fish species, Moura & Sazima, 2000). The anthozoan fauna was studied in a broad sense (Castro & Pires, 2001; Castro *et al.*, 2010), but the diversity of Ceriantharia and other minor groups of Anthozoa (Zoanthidae and Corallimorpharia) has been neglected.

The family Cerianthidae Milne-Edwards & Haime, 1852 is defined by lacking specialized nematocyst-bearing internal structure called acontioids or cnidorages (den Hartog, 1977). It is composed of four genera known from adults and larvae (*Ceriantheomorphe* Carlgren, 1931*, Ceriantheopsis* Carlgren, 1912*, Cerianthus* Delle Chiaje, 1841 and *Pachycerianthus* Roule, 1904), and several "genera" known from larvae only (Tiffon 1987, Daly *et al.*, 2007). The genus *Pachycerianthus* Roule, 1904 currently comprises 16 valid species (Carter, 1995; Fautin, 2013). Torelli (1961) considered *Pachycerianthus* to be a synonym of *Cerianthus* because morphological characters hardly distinguish the genera. However, Arai (1965) refuted this synonymy because apparently it was only based on a confusion of literature terms. This genus has been little studied using modern tools and taxonomic concepts (*eg.* Carter, 1995), and very little is known about the biology and intraspecific variation of taxonomic characters (morphological and molecular). Here we address the lack of information about members of *Pachycerianthus* by describing a new species from the tropical Southwestern Atlantic Ocean and comparing it with other species of the genus from the Atlantic Ocean.

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Material and methods

Specimens were collected by SCUBA diving from Abrolhos Bank and Royal Charlotte Bank, Espírito Santo and Bahia states coast (Brazil). Individual polyps were collected and maintained alive for laboratory observations. Subsequently, specimens were anesthetized with magnesium chloride and preserved in 4% seawater-buffered formaldehyde solution for morphological studies; additional samples (mostly tentacles) were preserved in 90% ethanol for molecular studies. Additional specimens were recorded based on photographs during regular SCUBA diving operations and through the discussion weblist named DIVE-NET (http://br.dir.groups.yahoo.com/group/dive-net/); these observations helped to delimit the distribution. In addition some specimens were observed in Museu Nacional da Universidade Federal do Rio de Janeiro (MNRJ) and Naturalis, Leiden (formerly Rijks Museum van Natuurlijke Historie, NATURALIS). The holotype and one paratype were deposited in Museu de Zoologia da Universidade de São Paulo (MZUSP). The literature search was partially performed on "Hexacorallians of the World database" (Fautin, 2013).

Morphological study. The anatomical study of the polyps and cnidome were based on criteria defined by several authors (van Beneden, 1897; Carlgren, 1912; den Hartog, 1977; Tiffon, 1987; Spier *et al.*, 2012). Five whole animals were cut through the ventral side (opposite of siphonoglyph) using carbon steel surgical scalpels; each was opened and pinned using acupuncture needles. The holotype was observed alive and later preserved.

The classification of cnidae followed den Hartog (1977) and England (1991), but nomenclature was based solely on England (1991). Thirty measurements (undischarged capsules) were made for each type of cnida from each body region of three specimens (holotype and paratypes MNRJ 2852 and MZUSP 1949-50). The cnidae were analyzed under a Nikon Eclipse 80i microscope under phase contrast. All parts of the body were analyzed separately so that any contamination would be avoided. The two parts of mesenterial filaments (cnidoglandular tract and ciliated tract) were analyzed together using 30 measurements from each part. The paratype series of *P. curacaoensis* (NATURALIS 11358) and three specimens of *P. magnus* from Penghu, Taiwan, China (MZUSP1951) were also studied for comparative purposes. The distinctiveness of the cnidae in *P. schlenzae* **sp. nov.** versus those in *P. curacaoensis* was tested via Mann-Whitney test.

Molecular study. The molecular study was based on the methods described in Stampar *et al.* (2012) and Stampar *et al.* (2014). DNA was extracted using InstaGene (Bio-Rad) from single tentacles removed from the specimens. Target regions (16S, ITS1 and ITS2) were amplified using PCR; PCR products were purified with AMPure® kit (Agencourt®). The PCR primers CB1/ CB2 (Cunninghan & Buss, 1993) and jfITS-5f / CAS28sB1d (Ji *et al.*, 2003) were used to amplify part of the 16S gene and the target fragment of the nuclear ribosomal unit, including the complete Internal Transcriber Spacer 1, the 5.8S Ribosomal Subunit and the Internal Transcriber Spacer 2, respectively. Sequences were obtained from specimens of *Pachycerianthus fimbriatus* (Monterey Bay, California, USA, non-lethal sample from one specimen from Monterey Bay Aquarium); *P. magnus* (Penghu, Taiwan, China, three specimens, MZUSP 1951); *P. multiplicatus* (Gullmarsfjorden, Sweden, GNHM-Anthozoa 1206, one specimen) for comparison. GenBank Accession numbers for all samples studied are listed in Table 1. The alignment for each molecular marker was made using MUSCLE in default parameters (Edgar, 2004). Sequences of the Internal Transcribed Spacer (ITS1 and ITS2) and of 16S of some *Pachycerianthus* species and *P. schlenzae* **sp. nov.** were compared based on Kimura 2-parameter distances (Kimura, 1980) in MEGA5 (Tamura *et al.*, 2011).

TABLE 1. Species included in this study and GenBank accession numbers of each locus.

SPECIES	GENBANK NUMBERS		
	COI	16S	ITS1/ITS2
Ceriantheomorphe brasiliensis	JF15195	JF915193	JX138232
Pachycerianthus borealis	-	U40288	-
Pachycerianthus fimbriatus	AB859842	-	-
Pachycerianthus magnus	AB859841	AB859836	KJ872673
Pachycerianthus multiplicatus	-	-	KJ872674
***Pachycerianthus schlenzae* sp. nov.**	AB859840	AB859835	KJ872672

Results and discussion

Systematics

Class Anthozoa Ehrenberg, 1834

Subclass Ceriantharia Perrier, 1883 (see details in Stampar *et al.* 2014)

Suborder Spirularia den Hartog, 1977

Family Cerianthidae Milne Edwards & Haime, 1852

Genus *Pachycerianthus* Roule, 1904

Pachycerianthus aestuarii (Torrey and Kleeberger, 1909)
Pachycerianthus benedeni Roule, 1904
Pachycerianthus borealis (Verrill, 1873)
Pachycerianthus curacaoensis den Hartog, 1977
Pachycerianthus delwynae Carter, 1995
Pachycerianthus dohrni (Beneden, 1924)
Pachycerianthus fimbriatus (Kwietniewski, 1898)
Pachycerianthus insignis Carlgren, 1951
Pachycerianthus johnsoni (Torrey and Kleeberger, 1909)
Pachycerianthus longistriatus Carter, 1995
Pachycerianthus magnus (Nakamoto, 1919)
Pachycerianthus maua (Carlgren, 1900)
Pachycerianthus monostichus McMurrich, 1910
Pachycerianthus multiplicatus Carlgren, 1912
Pachycerianthus nobilis (Haddon and Shackleton, 1893)
Pachycerianthus schlenzae **sp. nov.**
Pachycerianthus solitarius (Rapp, 1829)

Type species. *P. multiplicatus* Carlgren, 1912 (see details in Kelly & Keegan, 2000)

Distribution: This genus is distributed worldwide, except Antarctica.

Diagnosis (*sensu* Arai, 1965): Cerianthidae with second couple of protomesenteries short and sterile. Arrangement of metamesenteries in each quartette M,B,m,b (1,3,2,4), more or less distinct.

Pachycerianthus schlenzae **sp. nov.**
Figs 1–3

Material examined (5 specimens). **Holotype:** MZUSP 1949, adult specimen (15 cm long), 5 m depth, Guaibura Beach (20°43'39.2"S 40°31'17.7"W), Guarapari, Espírito Santo state, Brazil, S.N. Stampar coll. (10/12/2008) (**I**). **Paratypes:** MNRJ 2852, adult specimen (13 cm long), 6 m depth, Viçosa Reef (17°58'59.4"S 39°16'34.2"W), Nova Viçosa, Bahia state, Brazil, C.C. Ratto & D.O. Pires coll. (02/04/1996) (**II**). MNRJ 2016, adult specimen (12 cm long), Spin Reef (17°05'45"S 39°09'47"W), Cumuruxatiba, Bahia state, Brazil, F.B. Pitombo coll. (01/30/1991) (**III**). MNRJ 4286, young specimen (8 cm long), Abrolhos (17°21'01.6"S 38°57'26.5"W), Bahia state, Brazil, P.C. Paiva coll. (02/27/2000) (**IV**). MZUSP 1950, adult specimen (15 cm long), 6 m depth, Baixa de Carapebús (20°14'47.55"S 40°12'0.617"W), Vitória, Espírito Santo state, Brazil, M.S.C da Hora coll. (10/20/2011) (**V**).

Diagnosis. Average length cerianthid (150 mm long). Marginal tentacles 60–85, to 120 mm long in living animals, 25–30 mm in preserved specimens, brown or purple with purple tips; 9–20 white dots along each tentacle

(Fig. 1), arrangement *4132.3132.4132.3132*, with more than 20 pores per each tentacle. Labial tentacles 60–80, to 40 mm long in living animals, 10–20 mm in preserved specimens, pale brown or whitish, arrangement *(2)213.2132.1321.2132*, unpaired labial tentacle present. Stomodeum long, extending 1/3 to 1/4 of total body length, hyposulcus 2 mm long, hemisulci distinct; siphonoglyph narrow, connected to first three mesenterial couples. Free part of sterile directive mesenteries same length to twice length of siphonoglyph. Second protomesenteries sterile, longer than directives but short and with few craspedonemes. Third protomesenteries sterile, shorter than directives; M and m-metamesenteries long, fertile; M_1 reaching aboral pore, with bundle of craspedonemes; B and b-mesenteries sterile; see Fig. 2 for schematic arrangement of mesenteries.

The cnidome (Fig. 4, Table 2) is composed of spirocysts, atrichs (I–II), microbasic b-mastigophores (I–V), and ptychocysts.

TABLE 2. Comparison of the cnidome of tropical shallow species of *Pachycerianthus* occurring on the Atlantic Ocean. Measurements expressed as length and width of 30 undischarged capsules (n = 3 specimens), data appear as the mean (minimum–maximum) values.

		Pachycerianthus schlenzae sp. nov.	*Pachycerianthus curacaoensis*
		length x width (in µm)	length x width (in µm)
Column			
	Pytchocysts	55.19 (46.8–62.92) x 17.47 (15.2–19.76)	53.74 (49.4–63.44) x 20.12 (16.64–23.4)
	Atrichs II	51.27 (47.84–54.6) x 15.52 (13.52–18.2)	49.71 (41.6–61.36) x 18.77 (18.2–20.8)
	b-mastigophores I	40.32 (33.8–44.72) x 7.59 (6.24–9.36)	40.56 (37.44–42.64) x 7.59 (6.76–8.32)
	b-mastigophores II	26 (23.4–30.16) x 4.99 (4.16 x 5.72)	30.1 (26–33.28) x 5.77 (5.2–6.76)
Marginal Tentancles			
	b-mastigophores I	35.46 (34.84–37.44) x 7.8 (6.76–8.32)	29.45 (28.6–30.68) x 5.12 (4.68–5.2)
	b-mastigophores III #	29.12 (27.04–30.68) x 5.14 (4.68–5.2)	20.04 (16.64–21.84) x 2.7 (2.6–3.12)
Labial Tentacles			
	b-mastigophores I #	40.48 (38.48–42.64) x 7.46 (6.76–7.8)	29.56 (27–31.2) x 4.6 (4.16–5.2)
	b-mastigophores III #	27.4 (26–28.6) x 3.27 (2.6–4.1)	20.8 (18.2–21.84) x 2.57 (2.08–3.12)
	Atrichs #	35.93 (29.1–41.08) x 7.33 (6.24–8.32)	27.11 (24.9–30.1) x 5.17 (4.6–5.2)
Stomodeum			
	b-mastigophores I	29.56 (22.56–31.35) x 6.55 (5.89–7.35)	22.99 (20.8–24.7) x 4.12 (3.9–4.42)
	b-mastigophores III	26.1 (24.2–27.8) x 3.15 (2.6–3.9)	25.89 (23.92–28.08) x 7.43 (6.76–7.8)
	Atrichs	36.82 (32.1–42.35) x 7.52 (6.8–8.5)	32.24 (28.6–35.36) x 6.26 (5.72–6.76)
Mesenteries Type B			
	b-mastigophores I #	48.88 (42.64–53.06) x 8.24 (7.8–8.84)	33.17 (28.6–36.4) x 5.94 (5.2–6.76)
	b-mastigophores III #	27.3 (24.96–31.2) x 6.5 (5.2–7.28)	20.17 (17.16–23.4) x 5.22 (5.2–5.72)
	Atrichs	31.9 (28.6–35.36) x 7.51 (6.2–8.32)	30.68 (26–37.44) x 5.66 (5.2–6.24)
Mesenteries Type M			
	b-mastigophores IV	25.97 (21.84–28.08) x 5.46 (4.68–6.24)	29.36 (27–31.2) x 4.8 (4.2–5.4)
	b-mastigophores V #	16.51 (13.5–18.72) x 4.83 (4.16–5.2)	21.8 (18.2–21.84) x 2.72 (2.23–3.24)
	Atrichs	--	27.21 (24.3–31.1) x 5.17 (4.6–5.2)

Distribution. Brazil, from Bahia to Espírito Santo states (Abrolhos Bank and Royal Charlotte Bank) (Fig. 3). The southernmost record is from Guarapari, Espírito Santo state (20°43'39"S 40°31'17"W) and the northernmost record is from Camamu Bay, Bahia state (13°53'19"S 38°57'38"W).

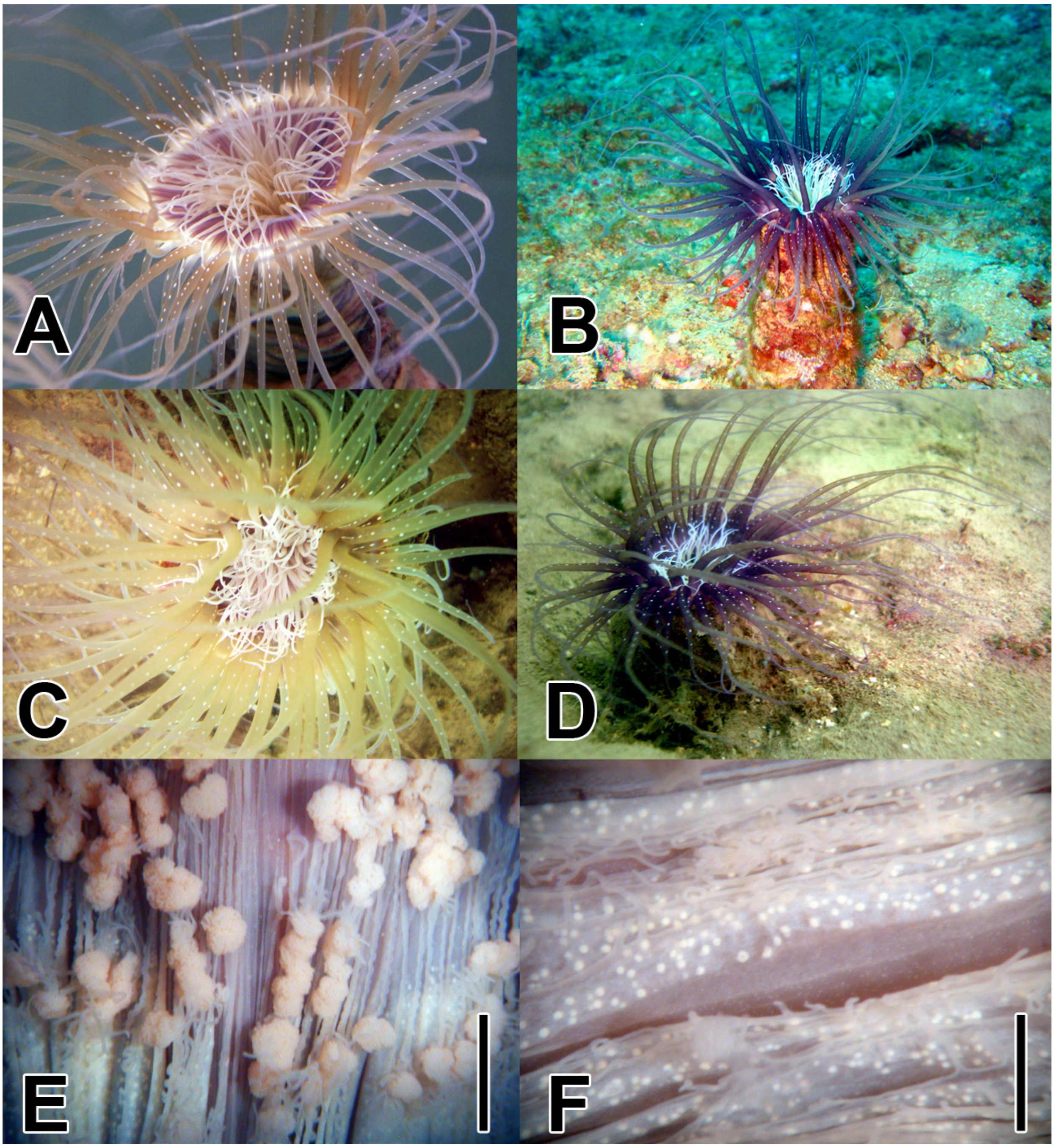

FIGURE 1. *Pachycerianthus schlenzae* **sp. nov.** : A—Live specimen from Guarapari-ES (Holotype—MZUSP1949), B—Live specimens at Camamu Bay-BA, Image only (Image: Claudio Sampaio), C and D—Live specimen at Porto Seguro-BA, Image only (Images: Rodrigo Maia-Nogueira), E—General view of the mesentery just below the stomodeum (scale 1 cm) (Holotype—MZUSP1949), F—Detail of fertile mesenteries with many oocytes (scale 6 mm) (Holotype—MZUSP1949).

Etymology. The specific name "*schlenzae*" honors Dr. Erika Schlenz (retired researcher from the Zoology Department, University of São Paulo, Brazil). Dr. Schlenz is a well-known actiniarian specialist, and she supervised many researchers working with cnidarians in Brazil.

Color variation. The color of the marginal tentacles varies from yellowish brown to deep purple, but tentacles always have white spots. The labial tentacles are always very light from beige to deep white.

Symbiosis. None of the tubes of *Pachycerianthus schlenzae* had Phoronida associated with them as had been observed by Stampar *et al.* (2010) for *Cerianthemorphe*. Some tubes bore colonies of a bryozoan, *Fenestrulina commensalis* also firstly described from the same area (Vieira & Stampar, 2014).

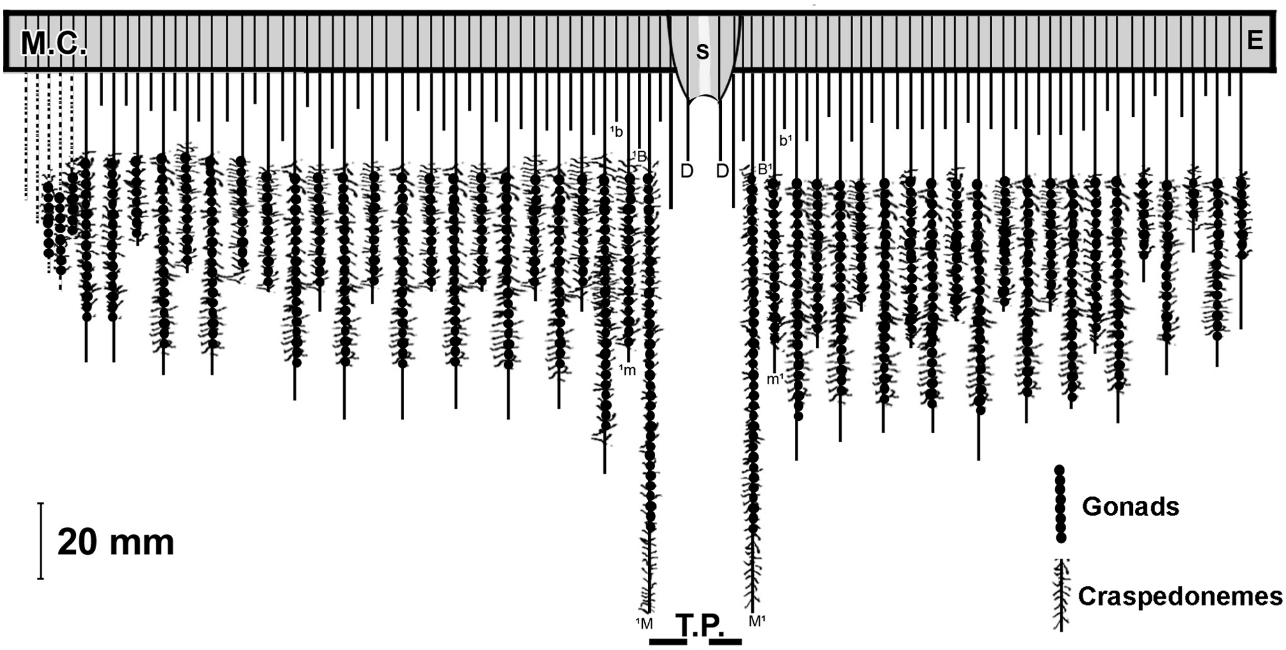

FIGURE 2. Graphical representation of the arrangement of mesenteries of *Pachycerianthus schlenzae* **sp. nov.** Abbreviations: B—B-mesenteries, b—b-mesenteries, D—Directives, E—Stomodeum, M—M-mesenteries, m—m-mesenteries, M.C.—Multiplication chamber, S—Siphonoglyph, T.P.—Terminal pore.

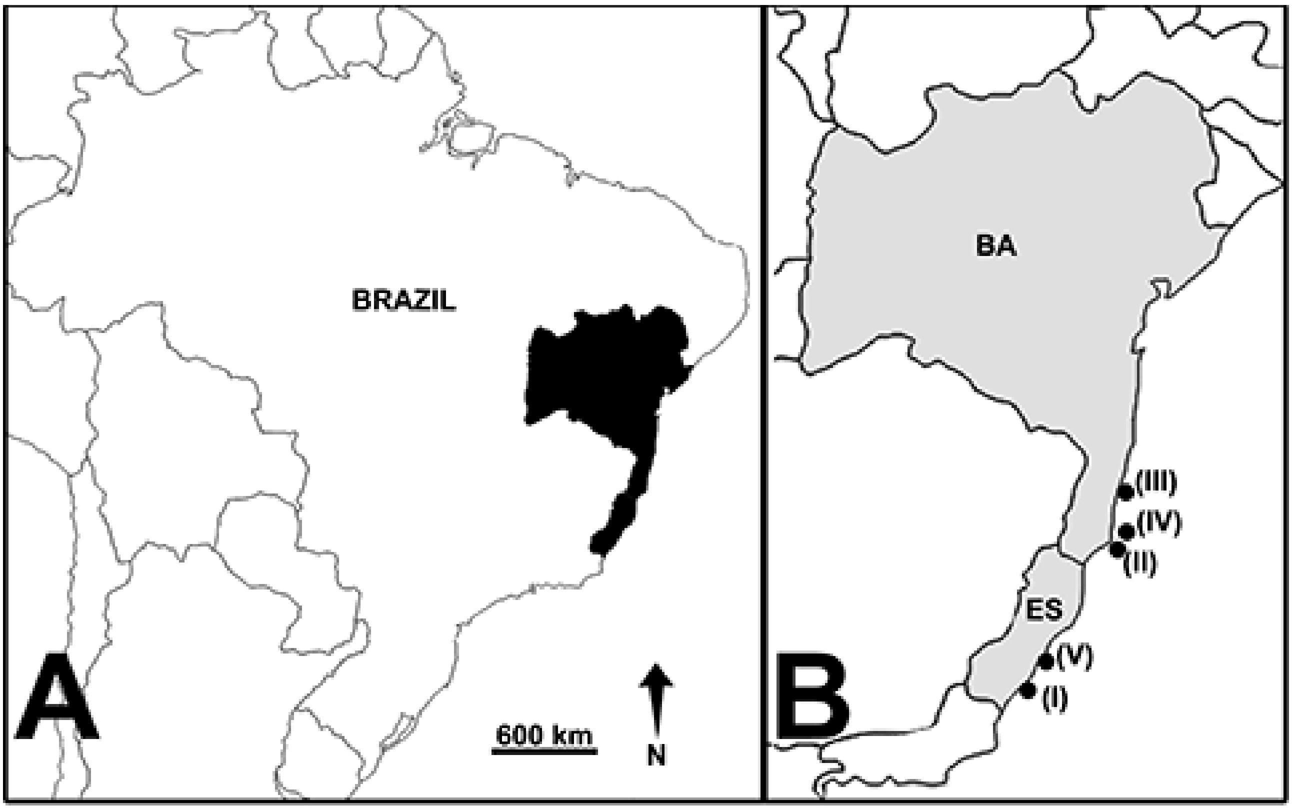

FIGURE 3. Distribution of *Pachycerianthus schlenzae* **sp. nov.** along the Brazilian coast. **A—**Bahia and Espirito Santo States in South American Map; **B—**Detail of Bahia (BA) and Espirito Santo (ES) States, the black circles correspond to specimens analyzed with the corresponding numbers presented in the "Material examined".

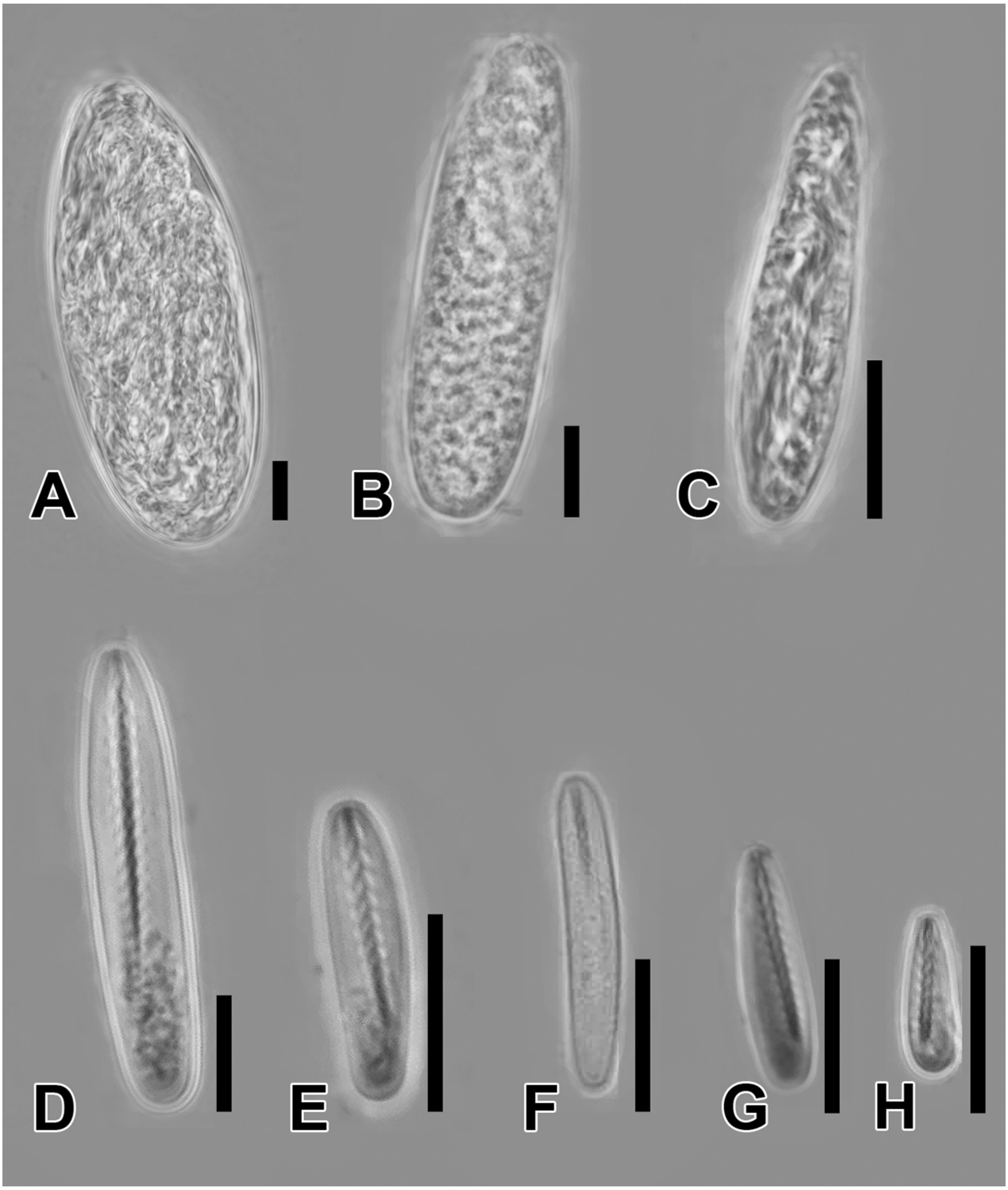

FIGURE 4. Cnidome of *Pachycerianthus schlenzae* **sp. nov.** A—ptychocysts, B—atrichs type II, C—atrichs type I/ ptychocysts, D—microbasic b-mastigophores I, E—microbasic b-mastigophores II, F—microbasic b-mastigophores III, G—microbasic b-mastigophores IV and H—microbasic b-mastigophores V. Scale 10µm.

Description of holotype (MZUSP 1949). Polyp elongate, length 14 cm, diameter 25 mm just below marginal tentacles and 20 mm near aboral end. Marginal tentacles 82, arranged in 4 pseudocycles, 50–40 mm long and 6–5 mm in diameter near base, brownish-yellowish at base, purple at tips. Space between cycles of marginal and labial tentacles dark red. Labial tentacles 72, about 30 mm long, bright white with white longitudinal line, directive labial present. Arrangement of marginal tentacles *4132.3132.4132.3132…* and labial tentacles *(2)213.2132.1321.2132…* . Oral disk 2 cm wide, stomodeum 42 mm long, light brown, siphonoglyph narrow and elongate with 6 mesenteries attached, hyposulcus 7.5 mm long with short (3 mm) hemisulci. Free part of directive mesenteries 40 mm long, without mesenterial filament. Second protomesenteries (p_2) relatively short with developed mesenterial filament: 39 mm with fairly developed craspedonemes. Third protomesenteries short (p_3) about 19 mm with developed craspedonemes and cnidoglandular tract. Metamesenteries in quartets: MBmb, metamesenteries of first cycle (M_1) reach aboral pore, have craspedonemes up to aboral region; remaining type M mesenteries exhibit same basic morphology, reaching no less than half of column, with numerous craspedonemes uniformly arranged until 4/5 of the mesentery length; mesenteries type m same as type M only shorter; metamesenteries type B and b, sterile, short, each with bunch of craspedonemes.

Morphological remarks. The specimens observed varied much more in the coloration of tentacles than in internal anatomy. The mesenteries have the same size ratio in relation to column length as the described specimen. The arrangement of the tentacles was also constant in all observed specimens. The craspedonemes range in length from 1- 4 mm and are always arranged in the upper region of the mesentery. The number of craspedonemes varies greatly between mesenteries of the same type in the same specimen, however craspedonemes are always present.

Comparison with other species of *Pachycerianthus*. Morphological aspects. *Pachycerianthus* comprises 16 species in addition to the one described here. All species of the genus were compared with the new species and were compared based on the analysis of type materials or on the original species description.

The species of *Pachycerianthus* can be split into two groups based on the relative length of the directive mesenteries and the stomodeum. The directive mesenteries are longer than the stomodeum in *Pachycerianthus schlenzae* **sp. nov.**, whereas in *Pachycerianthus benedeni*, *P. insignis, P. johnsoni*, *P. maua, P. multiplicatus,* and *P. solitarius*, the directive mesenteries are shorter than the stomodeum. A second attribute that can be used to discriminate species is the length of M1 mesenteries: in *Pachycerianthus schlenzae* **sp. nov.**, M1 reach the aboral pore; in *P. delwynae, P. dohrni, P. fimbriatus* and *P. magnus*, M1 mesenteries are shorter and do not reach the pore. Another differentiating characteristic is the number of mesenteries attached to the siphonoglyph region: *Pachycerianthus schlenzae* **sp. nov.** has 6 mesenteries attached, whereas *P. curacaoensis*, *P. borealis*, *P. monotichus,* and *P. aestuari* have 4, 8, 8 and 16, respectively. Finally it is possible to distinguish the remaining species in relation to *Pachycerianthus schlenzae* **sp. nov.** by the number of tentacles: *Pachycerianthus schlenzae* **sp. nov.** has up to 85 tentacles, wheras *P. longistriatus* and *P. nobilis* have at least 140 in adult specimens.

Species from related biogeographical area. Only three members of *Pachycerianthus* have been recorded from Atlantic waters: *Pachycerianthus borealis* (Danielssen, 1860) from the coast of Canada and northern USA (1–50 meters); *Pachycerianthus multiplicatus* Carlgren, 1912 from North Sea (10–130 meters); and *Pachycerianthus curacaoensis* den Hartog, 1977 from Curaçao (Caribbean) (65–75 meters). These differ from one another and from *P. schlenzae* **sp. nov.** in the number of marginal tentacles: *P. borealis* (140–160), *P. multiplicatus* (160–170), *P. curacaoensis* (74–105), and *P. schlenzae* **sp. nov.** (60–85). Another obvious difference are the white spots in the marginal tentacles in *P. schlenzae* **sp. nov.** and *P. curacaoensis*, which are absent from the other two species; however this feature is not visible some time after preservation. Features of internal anatomy also differentiate the Atlantic species. The length of stomodeum in *P. borealis* is about 1/6 of the total length of the column; this ratio increases to around 1/3 in *P. schlenzae* **sp. nov.** At least five couples of M-mesenteries reach the region of the aboral pore in *P. borealis*, whereas in *P. schlenzae* **sp. nov.** only one couple of M-mesenteries reaches the aboral pore. Specimens of *P. multiplicatus* have at least five couples of M-mesenteries that reach the aboral pore.

The Caribbean species *P. curacaoensis* and the Brazilian *P. schlenzae* **sp. nov.** differ in subtle ways. The external morphology (number of tentacles and color pattern) is similar in both species. However, the stomodeum in *P. curacaoensis* extends about 1/8 to 1/12 of the total length of the body; in contrast, the stomodeum in *P. schlenzae* **sp. nov.** is about 1/3 to 1/4 of the total length of the body. The number of mesenteries attached to the syphonoglyph is also different: 4 in *P. curacaoensis* and 6 in *P. schlenzae* **sp. nov.** In *P. curacaoensis*, only the couple M_1 exceeds half of the column, whereas in *P. schlenzae* **sp. nov.** at least 8 pairs of M-mesenteries (M_1–M_8) exceed half of the column length. The two species differ in the size of cnidae (see Tables 2, 3). Comparative anatomical characters for all known species of *Pachycerianthus* are presented in Table 4.

Molecular aspects. Molecular markers have been used as a tool for the taxonomy of Ceriantharia (Rodriguez *et al.*, 2012; Stampar *et al.*, 2012; Stampar & Morandini, 2014). The results (Table 5) showed that the discrepancies between the molecular markers of the species of *Pachycerianthus* are considerable, as was also shown by Stampar *et al.* (2012) for species of *Isarachnanthus* .

TABLE 3. Mann-Whitney test comparing the length of cnidae in *Pachycerianthus curacaoensis* and *P. schlenzae* **sp. nov.** (only comparisons with statistical significance). See Table 2 for measurements. Note that p-values smaller than 0.05 indicate that that samples are different.

Cnidae	Body part	P-value
b-mastigophore I	Labial tentacle	<0.0001
b-mastigophore I	Mes. type B	<0.0001
b-mastigophore III	Mes. type B	<0.0001
b-mastigophore II	Marginal tentacle	<0.0001
b-mastigophore III	Labial tentacle	<0.0001
b-mastigophore V	Mes. type M	<0.0001
Atrichs	Labial tentacle	<0.0001

Remarks on general taxonomy. The species described in this study is distinguishable by a number of features. However, to describe it and differentiate it from other species of the genus required careful re-examination of the genus *Pachycerianthus* as well as the entire family Cerianthidae, both using morphology to refine the descriptions of certain species and collecting molecular data from a several species. At the same time it is necessary to understand the morphological variation within each species and always associate it with genetic variation. Some studies have addressed morphological variation within some morphotypes (*e.g.* Carlgren, 1912; van Beneden, 1924), unfortunately there is no confidence that these specimens belong in fact to the same species; maybe there are cryptic species within. The issue of cryptic species was only addressed for Ceriantharia recently (Stampar *et al.*, 2012), but it is widely recognized on other groups (*e.g.* Zoanthidae—Reimer *et al.*, 2006). This type of study may not provide relevant results for species with a very restricted distribution, such as *Pachycerianthus schlenzae* sp. nov. However, some ceriantharian species (*eg. Ceriantheomorphe brasiliensis*) have very wide distribution and these species may present interesting results with respect to discrepancies between morphological and molecular differences.

Biological aspects. Two specimens of *P. schlenzae* **sp. nov.** were maintained in aquarium for three years (2009–2011). These specimens were maintained on a closed aquarium system (5000L) under natural light regime and constant temperature (21°C). In early spring (Sep 2009), both specimens released many oocytes. This event was restricted to one day. After more than two months, few days before the beginning of summer (Dec 2009), one of the individuals released sperm, two or three times per day, over four days. In late summer (Feb 2010), a new release of gametes occurred, however this event was different since both sperm and oocytes were released simultaneously. Once again the liberation of oocytes lasted only one day and was continuous, and the release of sperm was episodic and took four days. The oocytes were yellow to brown color with a mean diameter of 190 µm (±30µm). Identical events were observed during the next seasons (2010–2011). In both cases and in late summer, we mixed up gametes of both sexes but fertilization was not observed. Uchida (1979) accomplished fertilization for *Pachycerianthus magnus* using the same methods.

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TABLE 4. Comparison of anatomical features of species of *Pachycerianthus* (? = no data or uncertain). See text for discussion.

Species	Directive mesenteries length	Directive labial tentacle	M-mesentery (M1) length	M-mesentery (M2) length	M-mesentery (m1) length	M-mesentery (m2) length	Mesenteries attached to siphonoglyph	Siphonoglyph shape	Number of marginal tentacles	Occurrence area	References
***Pachycerianthus schlenzae* sp. nov.**	> stomodeum	Present	Reach aboral pore	3/4 of M-1	1/2 of M-1	1/3 of M-1	6	Long and narrow	60-85	Brazil	This study
P. aestuari	> stomodeum	?	Reach aboral pore	≅ M-1	1/5 of M-1	= m-1	16	Wide	30-34	USA (Pacific Coast)	Torrey & Kleeberger, 1909
P. benedeni	< stomodeum	?	Reach aboral pore	?	?	?	6?	Wide?	~125	Japan	Role, 1904
P. borealis	> stomodeum	?	Reach aboral pore	= M-1	3/4 of M-1	~1/3 of M-1	8	Wide	139-155	Canada - USA (North Atlantic)	Kingsley, 1904
P. curacaoensis	> stomodeum	Absent	Reach aboral pore	1/2 of M-1	1/4 of M-1	2/3 of m-1	4	Short and narrow	74-105	Caribbean Sea (Curaçao)	den Hartog, 1977; this study
P. delwynae	> stomodeum	Present	Almost reach aboral pore	Larger than M-1	1/3 of M-1	1/2 of M-1	6	Narrow	89-114	Australia	Carter, 1995
P. dohrni	?		Half column	> M-1	?	?	?	?	~160	Mediterranean	Beneden, 1924
P. fimbriatus	> stomodeum	Present	Reach aboral pore	3/4 of M-1	1/3 of M-1	1/3 of M-1	8	Wide and long	<60	Pacific (Canada -USA/ Indonesia - Malaysia)	McMurrich, 1910; Arai, 1965
P. insignis	< stomodeum	Present	Almost reach aboral pore	≅M-1	≅ M-1	≅M-2	8	?	~100	Gulf of California - Mexico	Carlgren, 1951
P. johnsoni	< stomodeum	?	Reach aboral pore	≅3/4 of M-1	3/4 of M-1	1/2 of M-1	8	Wide	~108	USA (Pacific Coast)	Torrey & Kleeberger, 1909
P. longistriatus	> stomodeum	Present	Reach aboral pore	=M-1	1/3 of M-1	1/4 of M-1	6	Wide	138-140	Australia	Carter, 1995
P. magnus	> stomodeum	Present	Almost reach aboral pore	3/4 of M-1	1/3 of M-1	1/2 of M-1	6	Short and narrow	~120	Japan, China	Uchida, 1979; this study
P. maua	< stomodeum	Absent	Reach aboral pore	1/4 of M-1?	1/3 of M-1?	1/3 of M-1?	6	Narrow	~150	Red Sea, Gulf of Aden, Tanzania	Carlgren, 1900; 1912; Carter, 1995
P. monostichus	> stomodeum	Present	Reach aboral pore	≅M-1	1/2 of M-1	≅ M-1	8	Narrow and long	~47	Indonesia	McMurrich, 1910
P. multiplicatus	> stomodeum	Absent	Reach aboral pore	=M-1	1/3 of M-1	1/3 of M-1	6	Narrow	175	North Sea	Carlgren, 1912;
P. nobilis	?	?	?	?	?	?	?	?	160-170	Australia	Haddon & Shackleton, 1893
P. solitarius	> stomodeum	Present	Reach aboral pore	≅ M-1	1/4 of M-1	1/5 of M-1	6	Narrow	~64	Mediterranean, Black Sea, Aegean Sea, Atlantic.	Beneden, 1924

TABLE 5. Estimates of divergence from *P. schlenzae* in sequences of ITS1, ITS2 and 16S for a subset of species in *Pachycerianthus*. Analyses were conducted using the Kimura 2-parameter model (Kimura, 1980). "--" means that the sequence was not obtained for that species.

	In relation to *Pachycerianthus schlenzae* **sp. nov.**				
	P. multiplicatus	*P. fimbriatus*	*P. borealis*	*P. magnus*	*Ceriantheormorphe brasiliensis*
ITS1	14.3%	--	--	10.6%	30.4%
ITS2	6.4%	--	--	9.4%	21.9%
16S	--	--	10%	3.8%	8.4%
COI	--	2.6%	--	4.3%	14.6%

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