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Botruanthus mexicanus (Cnidaria: Ceriantharia), a new species of tube-dwelling anemone from the Gulf of Mexico

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Abstract A second tube-dwelling anemone species of the genus *Botruanthus* (Cnidaria: Ceriantharia), *B. mexicanus* sp. nov., was observed in the Veracruz Reef System (1 m depth), Veracruz, Gulf of Mexico, and is described herein. To date, the recorded range of *Botruanthus* has been restricted to the Pacific Ocean. This new species is the first of this genus reported from the Atlantic Ocean. The number of tentacles, the number of mesenteries attached to the siphonoglyph, and the size of P2 mesentery permit a decisive distinction from *Botruanthus benedeni*. Some comments are provided, nonetheless, on the difference between *Botrucnidifer* and *Botruanthus*, the only two genera of the family Botrucnidiferidae.

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Introduction

Scientific knowledge of the subclass Ceriantharia (Cnidaria: Anthozoa) dates back to the late 1700s, and still presents a number of gaps (Stampar et al. 2016). One of these concerns the family Botrucnidiferidae Carlgren, 1912 (Molodtsova 2001), which comprises only three benthic species, organized in two genera: *Botruanthus* McMurrich, 1910, and *Botrucnidifer* Carlgren, 1912. However, a large number of larval morphotypes have been described for the family as well (Molodtsova 2001; Stampar et al. 2015a).

The single species of the genus *Botruanthus* was originally described as belonging to the genus *Cerianthus* Delle Chiaje, 1841, *C. benedeni* Torrey and Keeberger, 1909. McMurrich (1910) reported, however, that this species could not be part of the genus *Cerianthus*: based on the subdivision proposed by van Beneden (1897; the suborders Acontiferae and Botrucnidiferae), the presence of botrucnid (a bunch of "cnidorhagae" mounted on a stalk) on the mesenteries remitted this species to the family Botrucnidiferidae. Thus, the author described a new genus, *Botruanthus*, to include this species. This new genus was part of the family Botrucnidiferidae, which together with the family Cerianthidae Milne-Edwards and Haime, 1852, constitutes the order Spirularia den Hartog, 1977 (den Hartog 1977). To date, the genus *Botruanthus* is monospecific. However, Carlgren (1912) suggested that some larval forms of the "genera" *Cerianthula* van Beneden, 1897—*Hensenanthula* van Beneden, 1897, and *Calpanthula* van Beneden, 1897—are juvenile forms of different undescribed species of *Botruanthus*. Yet no studies on the development of *Botruanthus* have allowed this kind of association, and the

genus is represented only by the species described from the Pacific coast of the USA.

The present study focuses on the description of the second species of this genus, the first known from the Atlantic Ocean. The study is based on the morphology and anatomy of specimens from Veracruz, Mexico.

Material and methods

The cerianthid specimens were collected by hand using a small shovel, in La Gallega reef, one of the 25 coral reef formations of the Veracruz Reef System (Tunnel 2007). Collected polyps were preserved in a 4 % seawater-buffered formaldehyde solution for morphological studies. In addition, the holotype of *Botruanthus benedeni* (Torrey and Keeberger, 1909) was observed in the National Museum of Natural History (NMNH), and several specimens of *Botrucnidifer norvegicus* Carlgren, 1912, were observed, which were on loan from the Norwegian University of Science and Technology, Museum of Natural History and Archaeology (NTNU). The holotype and one paratype of the new species were deposited in the cnidarian collection of Museu de Zoologia da Universidade de São Paulo (MZUSP); one additional paratype was deposited in the collection of Cnidarians of the Gulf of Mexico and Mexican Caribbean Sea of the Unidad Multidisciplinaria de Docencia e Investigación en Sisal (UMDI-Sisal) at the Universidad Nacional Autónoma de México (UNAM).

Morphological study

The anatomical study of the polyps and cnidome were based on criteria defined by several authors (Carlgren 1912; Arai 1965; den Hartog 1977; Stampar et al. 2012, 2015b). Three whole specimens were cut through the ventral side (opposite to the siphonoglyph) using a carbon steel surgical scalpel. Dissected polyps were mounted using acupuncture needles.

The classification of cnidae followed England (1991) and Stampar et al. (2014b). Thirty measurements (undischarged capsules) were taken from each cnida type in each body region of two specimens (the holotype MZUSP002757 and the paratype MZUSP002758). The cnidome was analyzed under a Nikon Eclipse 80i microscope with phase contrast. All body parts were analyzed separately to avoid contamination. The two parts of mesenterial filaments (cnidoglandular tract and botrucnids) were analyzed separately using 30 measurements from each part.

Histology

Longitudinal and transverse histological sections (8 μm thick) were made from two additional specimens, and stained with hematoxylin and eosin (Estrada-Flores et al. 1982). Histological sections were examined under a Nikon Eclipse 80i microscope.

Results

Systematics

Class Anthozoa Ehrenberg, 1834

Subclass Ceriantharia Perrier, 1883 (*sensu* Stampar et al. 2014a)

Suborder Spirularia den Hartog, 1977

Family Botrucnidiferidae Carlgren, 1912

Genus *Botruanthus* McMurrich, 1910

= *Botryanthus* McMurrich, 1910 (typo)

Type species *Botruanthus benedeni* (Torrey and Kleeberger, 1909)

Distribution. Restricted to the Northeast Pacific Ocean (northwestern coast of the USA), but see data of new species.

Diagnosis

Botrucnidiferidae with cnidorages and/or botrucnid dispersed irregularly over the craspedonemes and bunches. Cnidoglandular tract region on protomesenteries 2 and metamesenteries M and m. Protomesenteries 2 more or less short, sterile. Arrangement of metamesenteries in quartets *M, b, m, B* fairly distinct (after Carlgren 1912).

Botruanthus mexicanus sp. nov.

Figures 1, 2 and 3

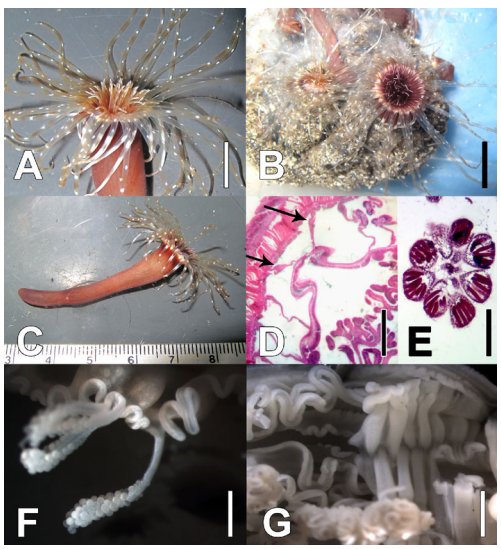

Fig. 1 *Botruanthus mexicanus* sp. nov. **a**–**c** Live specimen (A scale: 1 cm; B scale: 8 mm). **d** Transverse section at stomodeum with detail on directive mesenteries (*arrows*; scale 2 mm). **e** Transverse section of cnidorage (scale 300 μm). **f** Detail of botrucnid/cnidorage (scale 3 mm; Holotype MZUSP002757). **g** Detail of stomodeum region (scale: 3 mm)

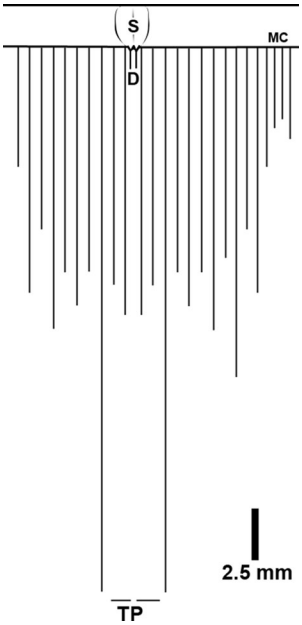

Fig. 2 Graphical representation of the arrangement of mesenteries of *Botruanthus mexicanus* sp. nov. Abbreviations: *M.C.*, Multiplication chamber; *D*, Directives; *T.P.*, Terminal pore; *S*, Siphonoglyph

Material examined (3 specimens)

Holotype: MZUSP002757, adult specimen (28 mm long), 1 m depth, La Gallega, Veracruz Reef System, (19°13′19.91″ N 96°7′38.74″W), Veracruz, Mexico, R. González-Muñoz coll. (08/v/2010).

Paratypes: MZUSP002758, adult specimen (26 mm long), 1 m depth, La Gallega, Veracruz Reef System, (19°13′ 19.91″N, 96°7′38.74″W), Veracruz, Mexico, R. González-Muñoz coll. (8 May 2010); YUC-CC-254-11, adult specimen (27 mm long), 1 m depth, La Gallega, Veracruz Reef System (19°13′19.91″N, 96°7′38.74″W), Veracruz, Mexico, R. González-Muñoz coll. (8 May 2010).

Diagnosis

Small ceriantharian, up to 40 mm long, 5–7 mm width; 48–68 brown to translucent marginal tentacles (40–45 mm in preserved specimens), 10–15 white small transverse lines or spots over each tentacle length (Fig. 1a–c), arrangement *(1)324.1342.1342…*, with at least 10 pores per tentacle, unpaired marginal tentacle present; 40–60 brown to purple labial tentacles (up to 10 mm long in preserved specimens), arrangement *(2)314.2314.2314…,* unpaired labial tentacle present; short pleated stomodeum extending over one-ninth to one-eighth of total body length, hyposulcus 2 mm long, hemisulci indistinct to very short; siphonoglyph narrow, connected only to directives (Fig. 1d); total length of sterile directive mesenteries slightly longer than siphonoglyph, free part very short with small portion with simple mesenterial filaments. Second protomesenteries short, reaching almost half of column length, sterile, bearing ciliated tract with small bundle of craspedonemes, followed by very short cnido-glandular tract and long (~70 % of the mesentery) craspedion tract with botrucnid. Third protomesenteries sterile, shorter than second protomesenteries with craspedonemes, without botrucnid. M and m-metamesenteries long, fertile with botrucnid; M_1 reach seven-eighths of total body length and M_2 reach one-half to three-fifths of total body length, with bundle of craspedonemes and botrucnid/cnidorages; B and b-mesenteries, sterile, B-mesenteries 10–15 % longer than b-mesenteries without botrucnid; see Fig. 2 for schematic arrangement of mesenteries. The cnidome of the species (Fig. 3) is composed of spirocysts, holotrichs, microbasic *b*-mastigophores (four types), and ptychocysts (Table 1).

Distribution. Recorded only at Veracruz, Mexico (Veracruz Reef System).

Etymology. The specific name *mexicanus* refers to Mexico, where this species was found.

Color variation. The color of the marginal tentacles is fairly variable, from translucent to brown, but always with white transverse lines or spots. The labial tentacles are brown to purple with a whitish end. Preserved material is whitish brown.

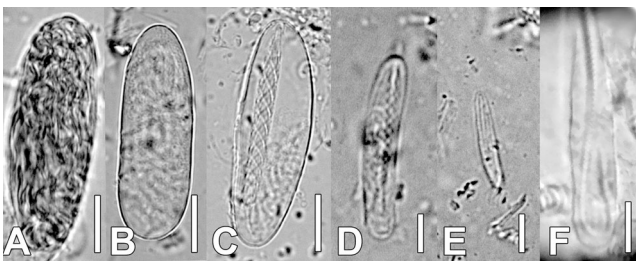

Fig. 3 Cnidome of *Botruanthus mexicanus* sp. nov. **a** ptychocysts I. **b** holotrichs. **c** microbasic *b*-mastigophores I. **d** microbasic *b*-mastigophores II. **e** microbasic *b*-mastigophores III. **f** microbasic *b*-mastigophores VI. Scale: 20 μm

Description of holotype (MZUSP002757)

Small polyp, 28 mm long, 5 mm in diameter just below the marginal tentacles, and 2 mm close to the aboral end. Marginal tentacles 64 in number, arranged in four pseudocycles, 12 mm long and 2 mm in diameter close to the base. Labial tentacles 52 in number, about 3 mm long, brown with a white apical tip, directive labial present, arrangement of marginal tentacles *(1)324.1342.1342…* and labial tentacles *(2)314.2314.2314….* Oral disc 0.6 mm wide, stomodeum 12 mm long, light brown, siphonoglyph narrow and elongate with two mesenteries attached, hyposulcus 2 mm long, hemisulci 0.5 mm long. Free part of directive mesenteries 3 mm, with small portions of simple mesenterial filament. Second protomesenteries reach almost half of column length, sterile, bearing ciliated tract with bundle of craspedonemes. Third protomesenteries sterile,

Table 1 Cnidome of *Botruanthus mexicanus* sp. nov. based on two specimens

		Length (μm)	Width (μm)
Column			
	Ptychocysts	36.8 (26.5–48.4)	11.0 (7.7–15.4)
	Holotrichs	35.3 (29.1–41.6)	10.5 (8.1–12.1)
	b-mastigophores I	15.1 (13.1–18.3)	3.1 (2.6–3.6)
	b-mastigophores II	21.0 (19.2–27.2)	6.4 (4.5–8.1)
	b-mastigophores III	53.1 (48.9–56.7)	12.2 (10.9–14.2)
Marginal tentacles			
	b-mastigophores I	17.3 (14.1–21.8)	3.2 (2.7–3.9)
	b-mastigophores II	23.3 (21.2–25.0)	5.3 (4.6–6.3)
	b-mastigophores III	51.8 (49.0–57.7)	15.3 (12.0–16.0)
Labial tentacles			
	b-mastigophores I	16.9 (14.4–20.1)	3.0 (2.7–3.6)
	b-mastigophores II	27.9 (25.8–30.6)	5.9 (4.9–6.4)
Stomodeum			
	b-mastigophores I	16.1 (13.6–18.0)	2.9 (2.4–3.5)
	b-mastigophores II	25.5 (23.5–28.6)	5.6 (4.8–6.2)
Mesenteries type B			
	b-mastigophores I	18.5 (16.8–21.0)	3.4 (3.0–3.6)
	b-mastigophores II	24.7 (23.4–27.0)	6.6 (6.0–8.4)
Mesenteries yype M			
	b-mastigophores I	18.8 (16.8–20.4)	3.9 (3.6–4.8)
Botrucnid			
	b-mastigophores III	57.6 (54.0–61.2)	38.5 (36.6–41.4)
	b-mastigophores IV	16.5 (15.0–17.4)	6.9 (5.4–9.0)

shorter than second protomesenteries with craspedonemes, without botrucnid. M_1 reach seven-eighths of total body length and M_2 reach three-fifths of total body length, with bundle of craspedonemes and botrucnid/cnidorages; B and b-mesenteries, sterile, B-mesenteries 10–15 % longer than b-mesenteries without botrucnid.

Comparison with other family members: *Botruanthus benedeni* and *Botrucnidifer norvegicus*

The genera *Botruanthus* and *Botrucnidifer* can easily be distinguished by simple observation of the internal anatomy.

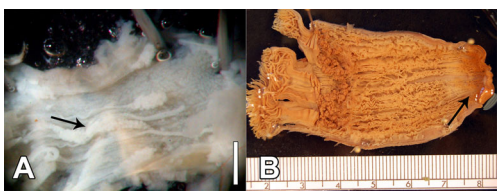

Fig. 4 **a** Detail of the cnidorage of *Botrucnidifer norvegicus* (*arrow*; scale: 4 mm). **b** Overview of *Botruanthus benedeni* with detail of the mesenteries reaching the aboral region (*arrow*)

Specimens of the genus *Botrucnidifer* have botrucnids located on the mesentery (Fig. 4a), while in the genus *Botruanthus* the botrucnids are organized in clusters on the mesenteric filament expansions (Fig. 1e). Another persistent trait of *Botrucnidifer* is its occurrence at depths below 50 m (Carlgren 1912; Molodtsova 2001, 2004), which differs from the species described in this study, as *Botruanthus mexicanus* nov. sp. occurs in the intertidal zone.

The most recent study about the genus *Botruanthus* was performed by Carlgren (1912), focusing on the species *B. benedeni* (at that time the only species known for the genus). *B. benedeni* was described and recorded only from the Pacific coast of California, USA (Torrey and Kleeberger 1909), Mexico (Baja California; Carlgren 1951) and Ecuador (Galapagos Islands; Fautin et al. 2007). Until now, the new species *B. mexicanus* described in this paper was recorded only from the Gulf of Mexico, Atlantic Ocean.

In addition to the difference in distribution, distinctive morphological features are present (Table 2). The most obvious difference is the overall size and the number of marginal tentacles: *B. mexicanus* sp. nov. is smaller, with fewer marginal tentacles than *B. benedeni*. The arrangement of the insertion of the labial tentacles also differs between the two species.

Table 2 Comparison of anatomical features of species of *Botruanthus*

	B. benedeni	*B. mexicanus* sp. nov.
Marginal tentacles	Up to 90–100	Up to 40–60
Directive labial tentacle	Present	Present
Arrangement of labial tentacles	(1)321.3213.3213	(2)314.2314.2314.2314
Actinopharynx	1/4–1/3 of gastric cavity	1/5–1/4 of gastric cavity
Oral disc	1.1–1.3 cm	0.5–0.7 cm
Siphonoglyph	Broad, 8 mesenteries attached	Narrow, 2 mesenteries attached
Directive mesenteries	> Actinopharynx (= size of Actinopharynx)	> Actinopharynx
P2	Long, almost to aboral pole (>2/3 of gastric cavity)	Short (<1/3 of gastric cavity)
P3	Short (1/3 of P2)	Short (~P2)
M1	To aboral pore	Almost to aboral pore
M3	Almost to aboral pore	Short, 1/2 of M1
Cnido-glandular tract at fertile mesenteries of first quartets	Present	Present
Craspedion tract at fertile mesenteries	5/7–8/9	8/9
Cnido-glandular tract at B	<1/2	3/4
Craspedonemes of craspedion at fertile mesenteries	Sometimes present	Sometimes present
Botrucnidae	Rare in m and B, absent in M and b mesenteries	Very abundant (4–5 groups) in M and m, absent in B and b mesenteries

Regarding the internal anatomy, the two species differ in the number of the mesenteries attached to the siphonoglyph, the organization of botrucnid, and the distribution of mesenteries in the gastric cavity.

Conclusion

The species described in this study, *Botruanthus mexicanus* sp. nov., is distinguishable by a number of features (Table 2); however, the number of tentacles, the number of mesenteries attached to the siphonoglyph, and the size of P2 mesentery enable a rapid discernment from *Botruanthus benedeni*. The relation of the new species with larval morphotypes is totally unknown, but studies such as the one by Stampar et al. (2014a), which combines traditional morphology with new molecular bar code approaches, may be the solution to the still unstable classification system in Ceriantharia.

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