NEW SPECIES OF CALIPHYLLA (GASTROPODA: HERMAEIDAE) FROM TROPICAL SOUTHWESTERN ATLANTIC

JESSIKA ALVES, MAYARA LIMA, RODRIGO JOHNSON & ELIZABETH G. NEVES

LABIMAR: Laboratório de Invertebrados Marinhos: Crustacea, Cnidaria e Fauna Associada. Instituto de Biologia/UFBA. Rua Barão de Jeremoabo, no. 147. Campus Ondina. Salvador, BA. CEP: 40170–290

Abstract  Caliphylla mediterranea A. Costa, 1867, described from the warm and temperate Mediterranean Sea, is the type species of a cosmopolitan genus hitherto considered monospecific. Several notable variations are noted in the records around the world, but gaps in the knowledge of the sea slug anatomy have negatively reflected on the Caliphylla taxonomy. Thus, in line with modern integrative taxonomical studies, the morphology and anatomy of the Caliphylla from Northeastern Brazil (11°–13°S) were investigated. As a result, we describe a new species to the Tropical Southwestern Atlantic, being the second species of the genus reported worldwide.

Key words  Bahia State, Sacoglossa, Taxonomy, Anatomy, Brazil.

INTRODUCTION

Caliphylla has long been considered a monospecific genus, widespread throughout the Atlantic Ocean, and the Indo-Pacific. It is uniquely represented by Caliphylla mediterranea A. Costa, 1867, a mimetic and small sea slug usually associated with the filamentous green algae Bryopsis J. V. Lamouroux, 1809.

Algae are known to provide refuge, and/or a food resource to a myriad of small invertebrates (snails, polychaetes, copepods). And, similarly to the Bryopsis, Caliphylla also has a greenish colour pattern and the body is marked by flattened leaf-like cerata containing vein-like tubules (very thin extensions) of the digestive gland (Gascoigne, 1979; Jensen, 1997; Valdés et al., 2006; Matthews-Cascon et al., 2011). Indeed, these mimetic characteristics are likely to enhance Caliphylla survivorship against predators.

Originally described from the warm and temperate Mediterranean Sea, C. mediterranea became widespread through the Atlantic Ocean and the Indo-Pacific. Even exhibiting divergences in the morphology, the species was firstly described from Italy and later recorded from France, Canary Islands, Senegal, Morocco, Japan, Indonesia, Hawaii, Florida, Virgin Islands, Caribbean and Brazil (Costa, 1867; Trinchese, 1870; Engel, 1927; Pruvot-Fol, 1953, 1954; Marcus, 1958, 1977; Gascoigne, 1979; Ortea, 1981; Jensen & Clark, 1983; Cervera et al., 2006; Gosliner et al. 2008; Trowbridge et al., 2011; García et al., 2002; Padula et al., 2012; Delgado, 2015; Jensen, 2014).

Caliphylla systematics have changed over the last years and according to Bouchet, et al. (2017) it belongs to the order Sacoglossa, composed of three distinct clades Oxynoidea Stoliczka, 1868, the Platyhedyloidea Salvini-Plawen, 1973, and the shell-less Plakobranchoidea Gray, 1840 – the latter, a monophyletic group supported by morphological and genetic phylogenies (Jensen, 1996; Maeda et al., 2010). Following the most recent classification of the gastropods attested by Bouchet et al. (2017), Caliphylla should be included into the Hermaeidae H. Adams & A. Adams, 1854.

The wide range of its distribution, and the monospecificity of the genus have been interpreted as suspicious. The existence of other Caliphylla species has been suggested, although none of the studies referring to the ‘Caliphylla mediterranea complex’ have provided any definitive answer (Valdés et al., 2006, Goodheart et al., 2016; Medrano et al., 2019). Through a robust analysis, based on morphological, anatomical, histological and ecological data, a new species of Caliphylla is described to the Southwestern Atlantic, being the first record of the genus to the Bahia State (Northeastern Brazil), and the second species known to the world.

Contact author : elizabeth.neves@gmail.com
**Material and Methods**

*Specimen Collection*
Specimens were collected at Farol da Barra Beach, Todos-os-Santos Bay (TSB), Brazil (13°0'37''S 38°31'58''W), between 2014 and 2016, along the intertidal zones (Fig. 1). All sea slugs were collected indirectly associated to the filamentous green algae *Bryopsis plumosa* (Hudson) C. Agardh 1823, also commonly found in the type locality. In the laboratory, *Caliphylla* specimens were sorted, selected and kept alive and monitored in a germination chamber, under controlled temperature (24°C), and photoperiod (12-hour cycles). Live individuals were photographed, and then anesthetized with menthol crystals. All samples were fixed in formalin 4%, (to harden the tissue, facilitating dissection), and afterwards transferred into ethanol 96%. The holotype and paratypes were deposited in the Mollusca Collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP 153879, MZUSP 153880. Curator: Dr. Luiz R. L. de Simone)

*Radular examinations*
Specimens, previously typed by external morphology and internal anatomy, had the pharynx dissected, and placed in 10% NaOH for 1 hour to remove all soft tissues. Prior to mounting for SEM images (ZEISS, model LEO 1430 VP Scanning Electron Microscope) radula was first washed in distilled water, then placed in ethanol and dried.

*Anatomy Study*
The specimens were dissected and illustrated under stereomicroscope with camera lucida (NIKON, model SMZ 800). Digestive, reproductive and nervous systems were separated for description and illustration.
Results
SUPERORDER SACOGLOSSA von Ihernig, 1876
SUPERFAMILY PLAKOBANCHOIDEA Gray, 1840
FAMILY HERMAEIDAE H. Adams & A. Adams, 1854

Genus *Caliphylla* A. Costa, 1867
Type species *Caliphylla mediterranea* A. Costa, 1867

*Caliphylla yemanjae* new species Alves, Lima Johnsson & Neves (Figs 2–6)

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Holotype Adult specimen (dissected and drawn), 20.0mm in length, Farol da Barra Beach, Todos-os-Santos Bay (13°0′37″S/ 38°31′58″W), associated with *Bryopsis plumosa* (Hudson) C. Agardh 1823, collected in ebb tide. MZUSP 153879.

Paratypes 10 adult specimens (10.0–20.0mm), Farol da Barra Beach, Todos-os-Santos Bay (13°0′37″S/ 38°31′58″W), associated with *Bryopsis plumosa* (Hudson) C. Agardh 1823, collected in ebb tide. MZUSP 153880.

Type Locality Praia do Farol da Barra (13°0′37″S/ 38°31′58″ W), Salvador City, Todos-os-Santos Bay, Bahia State.


Description External morphology: Elongated and cylindrical body varying from 10 to 20mm in total length (Fig. 2). Living specimens show dark green color, with little light green papillae. Hermaphrodite ampulla is not easily visible through the foot. A translucent smooth tegument covers the dorsal surface of the body, this tegument is intercepted by a row of green canals, that corresponds to the digestive gland branches. These canals reach cerata, head, tail and rhinophores. Head is rounded, subtly distinct from the body. Two bifid rhinophores are present, without grooves. Eyes externally visible, dark and rounded, located immediately behind each rhinophore basis.

About 30–40 cerata distributed into four longitudinal rows along each side of the body are present, the first and fourth rows show reduced cerata – about two times longer than wide, and the second and third rows have the longest ones – about 3 times longer than wide. In all rows, posterior cerata are longer than anterior ones. Dorsal midline without cerata, medial line with light green papillae that sometimes appear in dorsal line.

On anterior region of the body there is a ventral mouth with subtriangular oral lobes and small tentacular processes. Oral lobes separated from
the head and foot by two grooves. On the medial portion of dorsum there is a rounded and inflated area, in which is located the pericardium. Renal pore is located immediately after pericardium on the right side. Penial opening on the right side of body, posteriorly to the eyes, followed by the anus. Between the anus and penial opening there is a female opening. Foot is muscular, smooth, narrow and long. Tail is short, and posteriorly narrowed and rounded.

Digestive system: Radula uniseriate, composed by 26 elements fixed on the chitinous radular ribbon; a cavity separates the radula in two sets of elements: the superior set formed by 6 elements, and the inferior with 20 elements, forming a lancet (Fig. 4). Well-developed, elongated pharyngeal pouch, 2.0mm in size, surrounded by septal lines separating the buccal wall in several compartments (Figs 3B, C). A large and long diverticulum, 2.0mm in size, is located posteriorly to pharynx, showing two globules surrounded by an internal large muscle layer (Figs 3B, C). Oral glands paired surrounding the anterior region of pharynx. Muscular and tubular pharynx (Figs 3B, C). Esophagus cylindrical posteriorly connected to the stomach lumen cavity. Examined specimens show uniform grey esophagus (Fig. 3B). Near the esophagus end there is a large esophageal pouch with conical protrusions (Fig. 3B). Stomach is large, about two times wider than long, with vertical folds all along its surface. Intestine arises from anterior dorsal surface of the stomach and proceeds to the right side, where is inflated forming a rectal ampulla. Passing through the body skin there is the anterior anus (Fig. 3B).

Reproductive system: Androdiaulic. Hermaphrodite gland covers stomach and esophagus on ventral region, occupying about 80% of posterior half of body cavity. Hermaphrodite gland shows a tubular shape, about four times longer than wide, composed of several acinose follicles grouped in a unique dilation. Each follicle resembles a bunch of grapes, being connected by ducts to the central larger hermaphrodite duct. The hermaphrodite duct proceeds anteriorly, showing a slight turn to the left, where is located a globose and curved hermaphrodite ampulla, which shows a “s” shape and is intensely pigmented (Fig. 3A). Anteriorly to hermaphrodite ampulla, the hermaphrodite duct is bifurcated into oviduct and deferent duct.

Deferent duct is thin, proceeding to the prostate. Prostate is a large and acinose gland that shows an ovoid shape. Distal deferent duct with a strong retracting muscle and takes an undulating course towards the right and tapers in diameter before entering a conical penis. Penis about 0.8mm long, retracted in the long and large penial sheath. Penile papilla conical, broad and armed with cuticle (Figs 5A, B). Flagellum short and thin, stylet present (Figs 5A, B). Stylet with some roughness, showing an elongate and curved cusp, with a longitudinal slot (Figs 5C, D).

Oviduct long and extended for the fertilization region. The union between the fertilization region and the capsule gland resembles a U-shaped globose curve. The posterior side of the curve corresponds to the fertilization region (Fig. 3A – indicated by number 1) and the anterior side corresponds to the capsule gland (Fig. 3A – indicated by number 2). The foreign sperm duct emerges from anterior region of oviduct and proceeds to a region between two ducts: albumen duct and bursal duct. Bursal duct thin and short, ends in a rounded bursa copulatrix at the distal division of oviduct, inserted into the long and thin vaginal duct. Albumen duct is long and ends in a tuberose albumen gland about 0.2mm long. Mucus gland enlarged, lobed and expanded, with a long peduncle covering the hermaphrodite ampulla. Genital receptacle spherical, about 1.5 times wider than long and 3 times wider than bursa copulatrix. Particularities of the female duct: (1) an expansion present in the fertilization region; (2) a modification to form the capsule gland and the mucus gland; (3) a final narrowing forming the female aperture.

Nervous system: Central nerve system composed of nine ganglia and nerves. Anteriorly, there are two buccal ganglia, located near the junction between pharynx and esophagus. Buccal ganglia rounded showing about 1/3 of the cerebral ganglia width, connected by a double commissure. Buccal ganglia are connected with cerebral ganglia by a long and thick cerebrobuccal connective, which emerges from anterior surface towards cerebral ganglia, above esophagus (Fig. 3D).

Immediately posterior to the pharynx, surrounding the esophagus there is the nerve ring. This ring is composed of four ganglia: two cerebro-pleural ganglia and two pedal ganglia, cerebral ganglia rounded, slightly expanded to
lateral margin, connected by an apparent commissure. Cerebro-pleural ganglia show two main nerves: the optic nerve and the rhinophores nerve. Pedal ganglia slightly larger than cerebro-pleural ganglia, rounded, located below the esophagus on both sides. Pedal ganglia are connected with cerebro-pleural ganglia by the double cerebropedal connective, completing the nerve ring (Fig. 3D).

Posteriorly, there is a short visceral loop connecting three ganglia to the cerebral ganglia: the sub-intestinal ganglion, the abdominal ganglion and the supra-intestinal ganglion. Abdominal ganglion and supra-intestinal ganglion rounded and similar in size. Sub-intestinal ganglion smaller than the supra-intestinal ganglion, squeezed between right brain ganglion and abdominal ganglion (Fig. 3D).

Reproductive behaviour
Caliphylla yemanjae sp. nov. is a simultaneous hermaphrodite species. In the laboratory, eight
Figure 5  SEM micrographs of the internal reproductive anatomy of *Caliphylla yemanjæ* sp. nov. A, B SEM of the penis. C, D Stylet of the same specimen. Arrow indicating tiny protuberances. Scale bars: 100.0µm (A, B), 4.0µm (C, D). Legends: fl- flagellum; md- male duct; pe- penis; st- stylet.

Figure 6  *Caliphylla yemanjæ* sp. nov. A, B Living specimens (15.0mm) in laboratory; C Encapsulated eggs, forming curled masses; D Zoom on the eggs. Scale bars 1.0mm (C), 500µm (D). Legends: c- cerata, f- foot, ot- oral tentacles, r- rhinophores, v- velum.
Figure 7  Light micrography of Caliphylla yemanjae sp. nov development. A Zygote; B Morula 2-n stage; C Morula 3-n stage; D Morula 4-n stage; E Morula final stage; F Trochophore larva; G 3th day pre-veliger; H 5th day veliger.
A new species of Caliphylla were selected for monitoring the reproductive behaviour. In all cases observed, the specimens approached their heads, and the rhinophores started touching. After the apparent recognition of the reproductive partner, copula began and the penis of an animal was simultaneously inserted into the other, both remaining in this position for a few seconds. This mating behaviour occurred always when the sea slugs were kept together, with the same couple reproducing several times. However, mating chain has never been observed in the species, although copula among three or more individuals has been often described in the sacoglossans.

Mucus-embedded egg masses were usually deposited at the base of an algae fragment, or free into the aquarium, consisting of a transparent cylindrical tube, curled in flat turns, composed by approximately 500 eggs (Fig. 6). Eggs of C. yemanjae sp. nov. are white, not containing extracapsular yolk and are about 65µm of diameter, capsule of 155×90µm in size.

**Early Development**

The spawning occurred under laboratory conditions. Egg masses were observed up to the free-swimming larval stage, being kept at a temperature of 20°C in small Petri dishes, (exchange of seawater occurring at two-day intervals). Zygotes were firstly observed (Figs 6D–7A). Approximately 12 hours after the zygote formation, it was possible to distinguish a sequence of cleavages, including two cells, three cells and four cell stages (Figs 7B–D). Cleavages continued for 12 hours, when the morula stages were completed (Fig. 7E). In the second day, about 24 hours after the appearance of the final morula, some eggs showed the first trochophore larvae (Fig. 7F). In the 3rd day, first pre-veligers were detected (Fig. 7G). During 5th day after spawning, the final veliger stage of C. yemanjae was observed, veligers have thin, cup-shaped shell of 120µm in length and a transparent epidermis, velum and foot, which were kept retracted into the shell (Figs 7H, 8). In the 9th day, egg masses had disintegrated and some planktotrophic larvae were noted inside the algae bag. Larval metamorphosis was not observed.

**Etymology**

The specific name “yemanjae” is a reference to Yemanjá, known as goddess of the sea by common African religions in the type locality.

**Geographic range**

The new species is only known from the type locality.

**Remarks**

Caliphylla yemanjae sp. nov. differs from Caliphylla mediterranea in several aspects. As pointed out by Gascoigne (1979), Caliphylla mediterranea presents four rows of small fusiform cerata and a dorsal brown tissue covers the dorsal region of body. The new species shows four rows of long and leaf-shaped cerata, which are irregular in length. Cerata of second and third row are longer than the others. Moreover, a light green tissue covers the dorsal region of body in the new species. It also did not show red or brown spots along its body length – as observed in Caliphylla mediterranea by Costa (1869a, 1869b), Trinchese (1870), Bergh (1877) and Gascoigne (1979). According to Gascoigne (1979), Caliphylla mediterranea shows a head continuous with the body, and a long and acute tail, contrasting with the rounded head, distinct from the body, and the short and conical tail, as observed Caliphylla yemanjae sp. nov.

The new species shows some differences in digestive system in comparison with Caliphylla mediterranea. Caliphylla yemanjae sp. nov. has
Table 1  Data on the morphological variation in *Caliphylla* species across the globe

<table>
<thead>
<tr>
<th>Geographic Distribution</th>
<th>Cerata</th>
<th>Radula</th>
<th>Penis</th>
<th>Penile stile</th>
<th>Hermaphrodite ampulla</th>
<th>Velum</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahia</td>
<td>Elongated and flattened</td>
<td>26 elements</td>
<td>Sub-triangular</td>
<td>Cuticular, elongated, cusped and curved with longitudinal groove and with small protuberances</td>
<td>Pigmented dark and convoluted</td>
<td>Triangular with short and discrete lateral tentacular processes</td>
<td>Acinose</td>
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<td>Present study</td>
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<td>(Padula <em>et al</em>., 2014)</td>
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<td>Italy (Gascoigne, 1979)</td>
<td>Ovoid and flattened</td>
<td>20–36 elements</td>
<td>Conical</td>
<td>Pigmented and elongated</td>
<td>Stretched corners in sharp lobes</td>
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<td>Fernando de Noronha</td>
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<td>Costa Rica (Caramacho-</td>
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<td>Azores (Jensen, 2014)</td>
<td>Flat</td>
<td>24 elements</td>
<td>Cusped</td>
<td>Cusped and curved</td>
<td>Cusped and curved</td>
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<td>Elongated and</td>
<td>20 elements</td>
<td>Conical and</td>
<td>Yellowish forming a large arch</td>
<td>Triangular tissue circulating</td>
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<td>1870)</td>
<td>pointed</td>
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<td>elongated</td>
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<td>the mouth</td>
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<td>Italy (Bergh, 1877)</td>
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<td>Bay of Santos (Marcus,</td>
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<td>Not described</td>
<td>Not described</td>
<td>Crescent shape with lateral</td>
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<td>1958)</td>
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<td>tentacular processes</td>
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<td>Geographic Distribution</td>
<td>Cerata</td>
<td>Radula</td>
<td>Peris</td>
<td>Penis</td>
<td>Pericardium N.</td>
<td>Hemiphractite</td>
<td>Ampulla</td>
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<td>Cabo Verde (Ortea e Moro, 2018)</td>
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<td>West India (Engel, 1927)</td>
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<td>Conical and globose.</td>
<td>Curved, with longitudinal groove and small denticles at the tip</td>
<td>Catylic, short</td>
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Table 1 (Continued)
vertical stomach grooves, while its congener shows horizontal stomach grooves. Following Gascoigne (1979), Caliphylla mediterranea shows about 30 to 36 radula elements connected to radular ribbon. The new species shows 26 radula elements. Caliphylla mediterranea has an esophagus that enlarges towards stomach and a dark color is present in anterior region, Caliphylla yemanjae sp. nov. shows a cylindrical shape and a uniform grey colour.

The new species also differs from Caliphylla mediterranea in reproductive system. Caliphylla mediterranea shows a triangular hermaphrodite gland, with a rounded and smaller mucus gland, while Caliphylla yemanjae sp. nov. shows a longer and tubular hermaphrodite gland with a lobulated and large mucus gland. The hermaphrodite ampulla observed in the new species is S-shaped and is inflated and clearly distinct from hermaphrodite duct. In Caliphylla mediterranea, hermaphrodite ampulla is ovoid and notably less inflated.

Caliphylla yemanjae sp. nov. and Caliphylla mediterranea share similar shape of penis. However, the new species is unique by showing a penial flagellum with a cuticular stylet cusp elongated and curved, with a longitudinal groove and small protuberances at the tip. According to Gascoigne (1979), Caliphylla mediterranea shows a penial flagellum about three times longer than penis, while in Caliphylla yemanjae sp. nov. the penial flagellum is only 1.3 times longer than penis.

Supported by the analyses developed in this study, Caliphylla yemanjae sp. nov. is clearly distinct from all of specimens hitherto recorded as Caliphylla mediterranea around the world (see Table 1). Florida specimens of C. mediterranea studied by Clark & Jensen (1981) shows different egg measures in comparison with C. yemanjae sp. nov., specimens from Florida show mean egg diameter with 89µm, while the new species shows 65µm. The capsule size is also different, specimens from Florida show 142×117µm of capsule size, whereas C. yemanjae sp. nov. shows 155×90µm of capsule size.

In other words, by integrating morphological and anatomical data, a reliable definition of the interspecific limits within the Caliphylla complex was obtained. Considering the congeners could have been possibly misidentified, resulting in wrong previous records, here we emphasize the importance of a multi-approach methodology for Sacoglossa taxonomy. Indeed, the Heterobranchia is a highly diverse group of snails and slugs, certainty relying on the standards of the modern integrative taxonomy to consolidate the global gastropod inventory.

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References

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GALVÃO FILHO HC, ARAÚJO AK, SILVA FV, AZEVEDO VM, MEIRELLES CA & MATTHEWS-CASCON H 2015 Sea slugs (Gastropoda: Heterobranchia) from the tropical northwestern Atlantic Marine Biodiversity Records 8 1–11.


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