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new taxa paper

Description of a new species of *Marionia* (Gastropoda: Heterobranchia: Tritoniidae) from the Gulf of Guinea (eastern Atlantic Ocean)

Felipe De Vasconcelos Silva [1 *]
Aketza Herrero-Barrencua [2]
Marta Pola [3,4]
Juan Lucas Cervera [1, 5]

[1] *Departamento de Biología, Facultad de Ciencias del Mar y Ambientales; Campus de Excelencia Internacional del Mar (CEI·MAR), Av. República Saharaui, s/n, Ap. 40, 11510 Puerto Real (Cádiz), Spain.*

[2] *BIOCON Research Group, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria; Islas Canarias, Spain.*

[3] *Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid; Campus de Excelencia Internacional UAM + CSIC, C/ Darwin, 2, 28049 Madrid, Spain.*

[4] *Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM); Campus de Excelencia Internacional UAM + CSIC C/Darwin 2, 28049 Madrid, Spain.*

[5] *Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI·MAR), Universidad de Cádiz, Av. República Saharaui, s/n, Ap. 40 11510 Puerto Real (Cádiz), Spain.*

***** *Corresponding author email: <felipe.devasconcelos@uca.es>.*

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ABSTRACT.—A new species of dendronotid nudibranch, *Marionia abrahamorum* sp. nov., is described here. This species was found on Príncipe Island (Gulf of Guinea, eastern Atlantic Ocean), and its description is based on its morphological characteristics as well as molecular data from two mitochondrial (cytochrome c oxidase subunit I and 16S rRNA) markers and one nuclear (histone-3) marker. This species differs from all known Tritoniidae in terms of its size, color pattern, notum pattern, number of velar processes, number of gills, presence of stomach plates, and radular formula. The phylogenetic results support the results of the morphological analysis, confirming its placement within the genus *Marionia*.

Sea slugs from the family Tritoniidae (Nudibranchia, Cladobranchia, Dendronotidae) span a variety of sizes and colors, but they feed exclusively on octocorals (McDonald and Nybakken 1999). The eight currently valid genera are *Tritonia*, *Tritoniopsis*, *Tritoniella*, *Marionia*, *Marioniopsis*, *Marianina*, *Paratritonia*, and *Tochuina* (Pola and Gosliner 2010, Bertsch 2014, Hulett et al. 2015), with a recent inclusion (Bertsch et al. 2009) and subsequent exclusion (Bertsch 2014) of the genus *Trivettea*. Among the accepted genera, *Marionia* is the second-most speciose, with 28 accepted species, including most of the largest species in the family. On his

last revision of this family, Odhner (1963) rediagnosed *Marionia* as having stomach plates, a digestive gland divided into two masses, jaws with three to six rows of fine denticles, a radula possessing tricuspid central teeth with differentiated first lateral teeth, gills branched and of uniform size, and compound velar papillae. Since most of these characters require a thorough internal morphological study, identifications performed in situ or based only on external characters can easily misdirect researchers to other genera in Tritoniidae. Furthermore, the differences among the genera included in the family have become blurred since Odhner (1963), with descriptions ranging from those lacking information on important details of the internal anatomy, i.e., *Tritonia taliartensis* Ortea and Moro, 2009 and *Marionia ghanensis* Edmunds and Carmona, 2017, to those in which characters create doubt about which genus the species should be assigned to, such as the unique presence of stomach plates in *Tritonia odhneri* Er. Marcus, 1959, among the *Tritonia*, or the unicuspid rachidian in *Tritonia khaleesi* Silva, Azevedo and Matthews-Cascon, 2014.

Most species of *Marionia* have been recorded in the Indo-Pacific region (Smith and Gosliner 2007, Gosliner et al. 2008, García and Bertsch 2009), with only nine found in the Atlantic Ocean as follows: *Marionia blainvillea* Risso, 1818, *Marionia cucullata* (Couthouy, 1852), *Marionia cabindae* White, 1955, *Marionia vanira* Ev. Marcus and Er. Marcus, 1966, *Marionia pusa* Ev. Marcus and Er. Marcus, 1968, *Marionia tedi* Ev. Marcus, 1983, *Marionia limceana* Silva, Meirelles and Matthews-Cascon, 2013, *Marionia ghanensis* Edmunds and Carmona, 2017, and *Marionia gemmi* Almón, Pérez and Caballer, 2018 (*see* MolluscaBase 2018).

From October to November of 2016, field sampling was performed along the coasts of Príncipe Island (declared by UNESCO as Reserve of Biosphere in 2012) to create a map of the marine habitats, as well as to examine the poorly known marine flora and fauna of Príncipe Island in detail. The resulting collection included specimens of an unknown reddish tritoniid whose internal anatomy revealed that they belong to the genus *Marionia*. To confirm the taxonomic status of these specimens, we performed molecular phylogenetic and species delimitation analyses based on two mitochondrial [cytochrome c oxidase subunit (COI) and 16S rRNA (16S)] markers and one nuclear [histone-3 (H3)] marker, as well as a complete and detailed anatomical study. The new species is compared with other congeneric Atlantic species and with one reddish species from the Indian-Pacific region.

Material and Methods

Samples for Morphological Studies.—Four specimens of an undescribed species of *Marionia* were collected by scuba diving on the shallow called Pedra Mitade (01°32′N, 7°25′E) off Príncipe Island, São Tomé and Príncipe. These specimens were observed and photographed under an optical microscope, and the results were compared with data from the literature on other species in this genus. Three specimens were dissected for the study of the internal organs. Their internal features were examined and drawn using a dissecting microscope and camera lucida. Special attention was paid to the morphology of the digestive and reproductive systems. The buccal mass was removed and dissolved in 10% sodium hydroxide until the radula and the jaws were isolated from the surrounding tissue. The radula and jaws were then rinsed in water, dried, and mounted for examination with a LEO 1430VP scanning electron microscopy. The specimens are deposited at the Museu Nacional de História e da Ciência de

Lisboa (MB) under voucher numbers MB28-005053, MB28-005054, MB28-005055, and MB28-005056.

Samples for Molecular Studies.—The specimens MB28-005053, MB28-005054, and MB28-005055 were used for the molecular analyses. We also included one specimen of an unidentified *Marionia* sp., which is henceforth known as "*Marionia* sp. 1," which was collected from the Gulf of Cadiz, southwest Iberian Peninsula (06°28.7′W, 36°16.3′N), to provide better taxon sampling. This specimen was deposited at the Museu Nacional de História e da Ciência de Lisboa (MB) under voucher number MB28-005057.

DNA Extraction, Amplification, and Sequencing.—DNA extractions were performed using a Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions, with some minor changes (a 100-µL final elution instead of 200 µL). Partial sequences of COI, 16S, and H3 were amplified by polymerase chain reaction (PCR) using the universal primers LCO1490 and HCO2198 for COI (Folmer et al. 1994), 16S ar-L and 16S br-H for 16S (Palumbi et al. 1991), and H3AD5′3′ and H3BD5′3′ for H3 (Colgan et al. 1998). The master mix for the PCR was based on 25-µl volume reactions. Each PCR contained 2.5 µl of Qiagen buffer, 3 µl of DNA, 2.5 µl of dNTP (2 mM), 5 µl of Q-solution (Qiagen), 1.5-3.5 µM magnesium chloride, 1.5 µl of each forward and reverse primer (10 µM), 0.25 µl of DNA polymerase (250 units μ^{-1}), and nuclease-free water. Successful PCR products were purified and sequenced by Macrogen, Inc. (Netherlands). All the resulting new sequences were deposited in GenBank. COI amplification was performed with an initial denaturation for 3 min at 94–95 °C, followed by 39–40 cycles of 30–45 s at 94 °C (denaturation), 30–45 s at 42 °C (annealing temperature), and 1–2 min at 72 °C (extension) with a final extension of 5 min at 72 °C. A 16S amplification was performed with an initial denaturation for 3 min at 94–95 °C, followed by 39 cycles of 39–45 s at 94 °C, 30–50 s at 45 °C (annealing temperature), and 2 min at 72 °C, with a final extension of 5–10 min at 72 °C. H3 amplification was performed with an initial denaturation for 3 min at 95 °C, followed by 40 cycles of 45–60 s at 94–95 °C, 45 s at 50 °C (annealing temperature), and 2 min at 72 °C, with a final extension of 10 min at 72 °C.

Phylogenetic Analyses.—We successfully obtained seven new sequences from our undescribed *Marionia* on Príncipe Island; the sequences of COI, 16S, and H3 were obtained from MB28-005053, but COI amplifications for MB28-005054 and MB28-005055 were not successful, resulting in a total of one sequence of COI, three of 16S, and three of H3. Additionally, we obtained COI, 16S, and H3 sequences for *Marionia* sp. 1 (MB28-005057). In addition to the 10 new sequences obtained in this study, 99 additional sequences from different Tritoniidae species were retrieved from GenBank, along with nine sequences from three species in the families Arminidae, Hancockiidae, and Pleurobranchidae as follows: *Armina semperi* (Bergh, 1866), *Hancockia californica* MacFarland, 1923, and *Pleurobranchus areolatus* Mörch, 1863, respectively, which were included as outgroups following recent molecular studies of the family (Pola and Gosliner 2010, Hulett et al. 2015, Goodheart 2017). All the species and sequences used in this study, including the sequences retrieved from GenBank, are listed in Table 1.

Table 1. Specimens used for molecular analyses, vouchers, and GenBank accession numbers. New sequences obtained in this study are in bold.

Species	Voucher	16S	COI	H3
Pleurobranchus areolatus	CPIC00829	KM521755	KM521756	KM521617
Armina semperi	CASIZ177534	HM162606	HM162696	HM162512
Hancockia californica	CASIZ175722	HM162621	HM162702	HM162527
Marianina rosea	CASIZ175746	HM162656	HM162733	HM162565
***Marionia abrahamorum* sp. nov.**	**MB28005053**	**MH892386**	**MH892390**	**MH892392**
***Marionia abrahamorum* sp. nov.**	**MB28005054**	**MH892387**	**-**	**MH892393**
***Marionia abrahamorum* sp. nov.**	**MB28005055**	**MH892388**	**-**	**MH892394**
Marionia arborescens	CASIZ177735	KP226859	KP226855	KP226857
Marionia blainvillea	CASIZ176812	HM162645	HM162721	HM162553
Marionia blainvillea	-	KY629594	KY629603	KY629612
Marionia blainvillea	-	KY629593	KY629604	KY629613
Marionia distincta	CASIZ110364	KP226860	KP226856	KP226858
Marionia distincta	CASIZ173317	HM162648	HM162725	HM162557
***Marionia* sp. 1**	**MB28005057**	**MH892389**	**MH892391**	**MH892395**
Marionia levis	CASIZ173454	HM162654	HM162731	HM162563
Marionia levis	CASIZ192357A	HM162655	HM162732	HM162564
Marionia levis	CASIZ192357B	KP153268	KP153301	KP153334
Marionia sp. 2	CASIZ166891	KP153267	KP153300	KP153333
Tritonia antarctica	CASIZ171177	KP153269	KP153302	KP153335
Tritonia festiva	CASIZ186478	HM162653	HM162730	HM162562
Tritonia hamnenorum	CASIZ181095	KP153259	KP153292	KP153325
Tritonia hamnenorum	CASIZ181090	KP153260	KP153293	KP153326
Tritonia manicata	KY629602	KP153276	KP153309	KP153342
Tritonia nilsodhneri	CASIZ176218A	KP153272	KP153305	KP153338
Tritonia nilsodhneri	CASIZ176218B	KP153275	KP153308	KP153341
Tritonia nilsodhneri	CASIZ176218C	KP153263	KP153296	KP153329
Tritonia nilsodhneri	CASIZ176219	HM162641	HM162716	HM162548
Tritonia nilsodhneri	CASIZ176222	KP871702	KP871653	KP871677
Tritonia pickensi	CASIZ175718	HM162642	HM162717	HM162549
Tritonia striata	BAU2695	LT596542	LT596540	LT615407
Tritonia striata	BAU2696	LT596543	LT596541	LT615408
Tritoniidae sp. 1	CASIZ 189262A	KP153276	KP153309	KP153342
Tritoniidae sp. 2	CASIZ 189311A	KP153272	KP153305	KP153338
Tritoniidae sp. 3	CASIZ189311B	KP153273	KP153306	KP153339
Tritoniidae sp. 4	CASIZ189311C	KP153274	KP153307	KP153340
Tritoniidae sp. 5	CASIZ189419	KP153277	KP153310	KP153343
Tritoniidae sp. 6	CAS189459	KP153275	KP153308	KP153341
Tritoniopsis alba	CASIZ69928	KP153281	KP153314	KP153347
Tritoniopsis alba	CASIZ69980	KP153282	KP153315	KP153348
Tritoniopsis frydis	CASIZ181156	KP153278	KP153311	KP153344

The DNA sequences were assembled, edited, and aligned using Geneious 10.2.2 (Kearse et al. 2012). All the sequences were checked for contamination with BLAST (Altschul et al. 1990) as implemented in the GenBank database. The protein-coding sequences were translated into amino acids to confirm the alignments. Pairwise uncorrected *p*-distances were calculated in MEGA 7.0 (Tamura et al. 2011). The

levels of substitution saturation in the individual gene sequence alignments were investigated using the test developed by Xia et al. (2003) and Xia and Lemey (2009) in DAMBE (Xia and Xie 2001). The convergence of phylogenetic trees generated in MrBayes was confirmed by eye using the Trace function in Tracer 1.5 (Rambaut et al. 2014). The most variable regions from the 16S rRNA alignment were removed in the first analyses, using both the default settings and the standard options for stringent and less stringent selections in Gblocks based on studies in which the elimination of ambiguous data in the non-protein-coding gene leads to significantly better phylogenetic trees depending on the size of the alignment (Jeffroy et al. 2006, Talavera and Castresana 2007). However, when these regions were excluded from the analyses, the combined phylogenetic tree showed low node support. Therefore, final analyses were performed by including all the bases.

We conducted phylogenetic analyses for each data set of COI (658 bp), H3 (328 bp), and 16S (462 bp) separately, in addition to two concatenated combinations (COI+16S and COI+H3+16S). All the taxa were included for the concatenated analysis of the three markers (total of 118 sequences). The COI+16S data set included four partitions (COI-1st, COI-2nd, COI-3rd, and 16S). The COI+H3+16S data set included seven partitions (COI-1st, COI-2nd, COI-3rd, H3-1st, H3-2nd, H3-3rd, and 16S). The best-fit evolution models for each data set were determined using the Akaike information criterion (Akaike 1974) as implemented in PartitionFinder 2 (Lanfear et al. 2016), resulting in TRN+G for COI-1st, TRN+I for COI-2nd, TVM+I for COI-3rd, TIM+I for H3-1st, K81+I for H3-2nd, HKY+G for H3-3rd, and TVM+I+G for 16S. Maximum likelihood (ML) analyses were performed using RAxML software v8.2.4 (Stamatakis 2006), and the node support was assessed with a non-parametric bootstrap (BS) with 5000 replicates, random starting trees, and parameters estimated from each data set under the model selected for the original data set. Bayesian Inference (BI) analyses were conducted using MrBayes v3.1.2b (Ronquist and Huelsenbeck 2003) for five million generations with two independent runs and a sampling frequency of 1000. The implemented models were the ones that were estimated with PartitionFinder 2. BI and ML phylogenetic analyses were performed on the 280-core "PhyloCluster" hosted at the Center for Comparative Genomics, California Academy of Sciences. Only the nodes supported by PP ≥ 0.95 and BS ≥ 75 were considered as resolved. The BI and ML trees were visualized, collapsed (PP ≥ 0.95, BS ≥ 75), and edited in TreeGraph v2.7.1 (Stöver and Müller 2010). The final graphical editing of the trees was completed in Photoshop CS6.

An automatic barcode gap discovery (ABGD) (Puillandre et al. 2012) was performed twice, with the COI alignment (35 specimens, excluding the outgroup) and with the 16S alignment (37 specimens, excluding the outgroup), using the online version of the software. ABGD was run using the following parameters: Jukes-Cantor and Kimura (80) Pmin = 0.001, Pmax = 0.1, NB = 20, Steps = 10, and relative gap width = 1.

Results

The results of the substitution saturation test for each gene alignment showed significant *P*-values (≤0.5) for all the gene alignments and index of substitution saturation (Iss) values that were less than the Iss.c (critical index of substitution saturation) in all cases, indicating little saturation (Table 2). The combined data set (COI+H3+16S)

Table 2. Results of substitution saturation tests for each gene alignment. Alignments with a significant *P*-value (<0.05) and Iss (index of substitution saturation) < Iss.c (critical index of substitution saturation) show little saturation. Saturation tests were conducted for fully resolved sites only, in the program DAMBE. OTU= operational taxonomic units; df = degrees of freedom. Results were interpreted for symmetrical trees.

Data set	NumOTU	Iss	Iss.c	T	df	*P*
COI	32	0.282	0.695	12.581	342	<0.0001
16S	32	0.310	0.781	7.831	91	<0.0001
H3	32	0.122	0.702	17.286	126	<0.0001

yielded a sequence alignment of 1448 positions and provided better resolution than the COI, 16S, H3 (Online Figs. S1–S6) or COI+16S (1120 bp) separately (Online Figs. S7–S8). Figure 1 shows the phylogenetic hypothesis based on the combined data set (COI+H3+16S) constructed by BI. The topology of the ML tree (Online Fig. S9) was very similar to the one obtained by BI. The ML bootstrap values are shown in Figure 1 below their respective branches.

The monophyly of the Tritoniidae family was not recovered either by BI or ML due to the lack of resolution at the basal node (Fig. 1). The Tritoniidae species included in this analysis clustered in seven different clades without a relationship between them. Our new species from Príncipe Island appeared in a well-supported clade (PP = 1, BS = 99) including *Marionia* sp. nov., *M. blainvillea, Marionia arborescens* Bergh, 1890, *Marionia distincta* Bergh, 1905, *Marionia levis* Eliot, 1904, *Marionia* sp. 1, *Marionia*

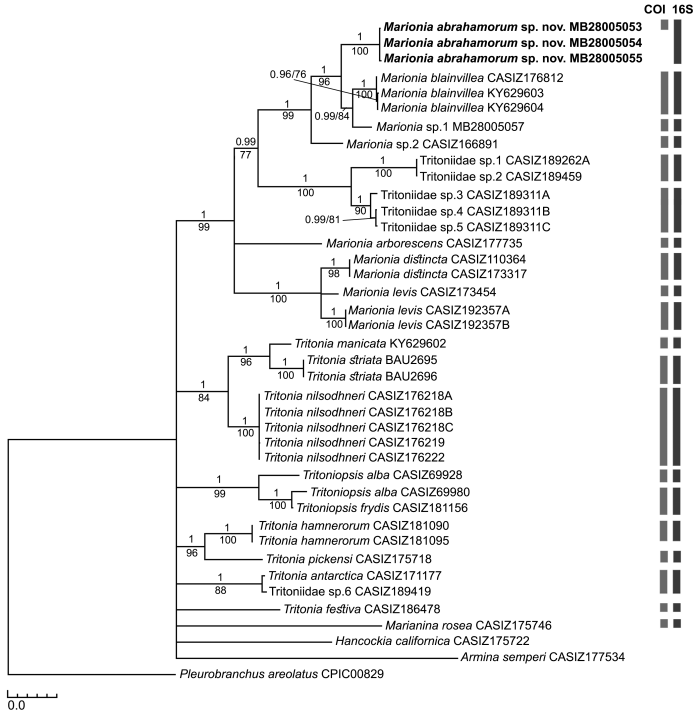

Figure 1. Bayesian phylogenetic tree based on the concatenated molecular data (COI+H3+16S). Bayesian posterior probabilities are shown above branches and maximum likelihood bootstrap values are shown below branches. Unsupported branches are not labelled. Rectangles are automatic barcode gap discovery groups (Jukes-Cantor and Kimura parameter) for the COI and 16S data set.

Table 3. Maximum and minimum COI gene pairwise uncorrected *p*-distance between *Marionia abrahamorum* sp. nov. and other species of Tritoniidae.

Species	COI genetic distances (%)
Marionia blainvillea	12
Marionia sp. 1	13
Marionia sp. 2	15
Tritonia pickensi	16
Tritonia hamnerorum	17
Marionia distincta	18
Marionia levis	18
Tritonia antarctica	18
Tritonia festiva	18
Tritonia manicata	18
Tritoniidae sp. 3	18
Tritoniidae sp. 5	18
Marianina rosea	19
Tritonia nilsodhneri	19
Tritoniidae sp. 1	19
Tritoniidae sp. 6	19
Tritoniopsis alba	19
Marionia arborescens	20
Tritoniopsis frydis	20
Tritonia striata	21

sp. 2, and five undetermined species of Tritoniidae. In this clade, *M. blainvillea* was the sister taxon to *Marionia* sp. 1 (PP = 0.99, BS = 84), and these two species clustered into a well-supported clade (PP = 1, BS = 96) with *M.* sp. nov.

Within the clade that included *M.* sp. nov., the maximum interspecific pairwise uncorrected *p*-distance for COI was 20% between *M.* sp. nov. and *M. arborescens*, while the minimum was 12% between *M.* sp. nov. and *M. blainvillea* (Table 3). The maximum interspecific pairwise uncorrected *p*-distance for the COI found between *M.* sp. nov. and the remaining taxa was 21% with *T. striata*, while the minimum was 16% with *T. pickensi* Ev. Marcus and Er. Marcus, 1967.

The ABGD species delimitation analysis recovered nine partitions with prior maximal distances from 0.001 to 0.059 using the COI alignment, and seven partitions with prior maximal distances from 0.001 to 0.021 using the 16S alignment, with both the Jukes-Cantor (JC69) and Kimura (K80) parameters. All the partitions recovered with both data sets had 20 groups (Fig. 1), with the exception of Partition 1 (prior maximal distance, $P = 0.001$) as recovered with the COI dataset, which had 21 groups. The phylogenetic analyses, the pairwise uncorrected *p*-distance and the species delimitation analyses all support the idea that our new specimens from Príncipe Island are in a distinct species belonging to the genus *Marionia* as described below.

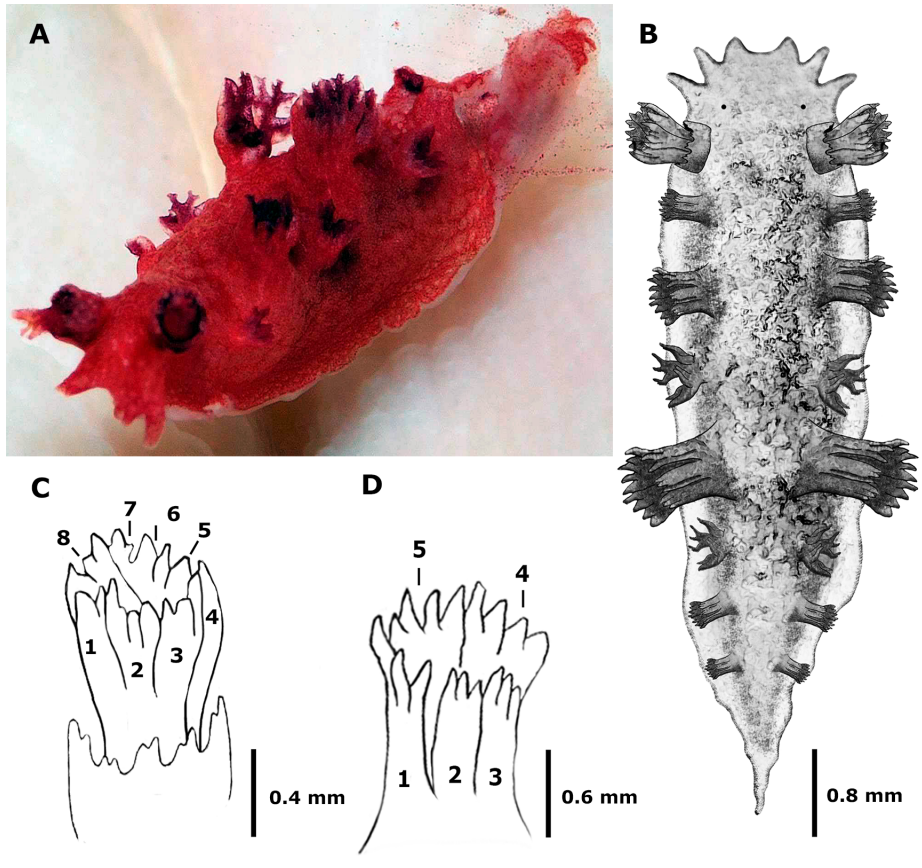

Figure 2. *Marionia abrahamorum* sp. nov. MB28-005053. (A) Digital photograph, mature adult, 12 mm in length; (B) line drawing of dorsal view; (C) line drawing, rhinophore with numbered plumes; (D) line drawing, gill with numbered plumes.

Systematics

Family TRITONIIDAE Lamarck, 1809

Genus *Marionia* Vayssière, 1877

Marionia abrahamorum new species

urn:lsid:zoobank.org:act:B5DAB4C8-6846-4DA1-A338-9E0A429781B0

(Figs. 2–6)

Material Examined.—MB28-005053, holotype, dissected and sequenced (COI, 16S and H3), 12 mm in length preserved. Paratypes: MB28-005054, dissected and sequenced (16S and H3), 10 mm in length preserved; MB28-005055, dissected and sequenced (16S and H3), 7 mm in length preserved; MB28-005056, 6 mm in length preserved. All the specimens were collected at a depth of 16 m from Pedra Mitade, Príncipe Island, São Tomé and Príncipe, Gulf of Guinea (01°32′N, 7°25′E), by A Herrero on November 27, 2016.

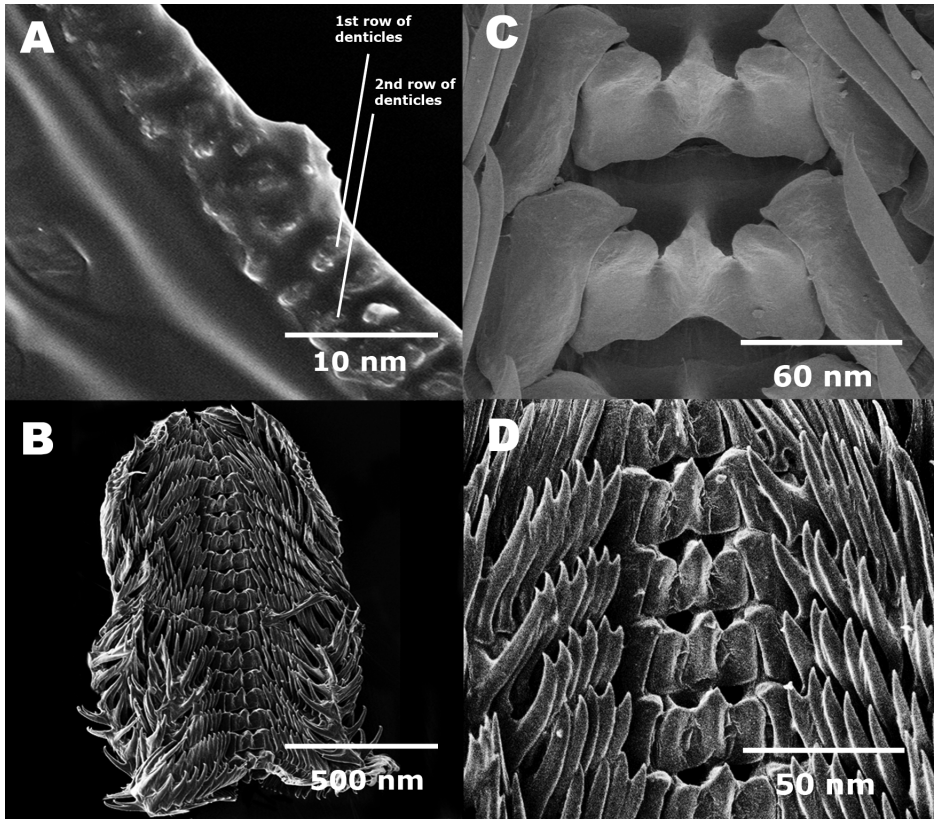

Figure 3. *Marionia abrahamorum* sp. nov. scanning electron micrographs. (A) Denticle rows from masticatory border of the jaws (MB28-005053); (B) overview of radula (MB28-005053); (C) rachidian tooth and first lateral tooth (MB28-005054); (D) central view of radula, with rachidian and lateral teeth (MB28-005053).

External Morphology (Fig. 2).—The body is slender, elongated, and red, with spots of lighter and darker shades along the notum, rhinophores, and gills (Fig. 2A). The notum is covered by small tubercles concentrated on the area anterior to the cardiac region. The mantle is wide, with an undulated margin. The foot is broad, rounded at the anterior region, and whitish along the margins. The mouth is located ventrally and anterior to the foot. The veil is slightly bilobed, containing six short digitiform appendages (Fig. 2B). The outermost anterior appendages are the largest, and they folded over themselves. The rhinophore sheaths have a crenulated margin divided into pairs of digital extensions. The retractable rhinophoral club has eight pinnate plumes, which are divided into three branches (Fig. 2C). All the specimens have seven gills on either side. The 1st, 3rd, 6th, and 7th pairs of gills are small, while the 2nd and 4th are the largest. The gills have five pinnate branches, which are divided into one group of two and another of three (Fig. 2D), and they are usually organized in a cylindrical shape. The genital opening is large and located laterally between the 2nd and 3rd gills, while the anus is located laterally between the 3rd and 4th gills on the right side.

Anatomy (Figs. 3, 4, 5).—The jaws are broad, yellowish, with two rows of denticles on the inner edge (Fig. 3A), and they are easily recognizable in preserved animals in

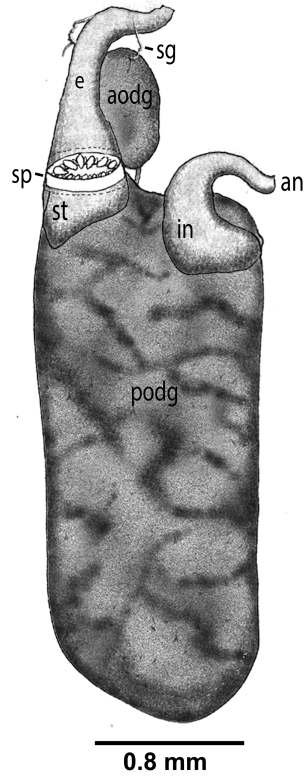

Figure 4. *Marionia abrahamorum* sp. nov. MB28-005053. Line drawing of the digestive system (dorsal view): an, anus; aodg, anterior part of the ovotestis-digestive gland complex; e, esophagus; in, intestine; podg, posterior part of the ovotestis-digestive gland complex; sg, salivary glands; sp, stomach plates; st, stomach.

external view. The radular formulae of two of the specimens are 20 × 5-14.1.1.1.5-14 (MB28-005054) and 25 × 10-16.1.1.1.10-16 (MB28-005053) (Fig. 3B). The rachidian tooth (Fig. 3C) is tricuspid with a long and sharp triangular central cusp, and blunt lateral cusps. The central cusp is worn in some cases. The first lateral teeth are short and broad, each with a pointed cusp curved towards the rachidian tooth. The remaining lateral teeth are long, slightly curved, and narrow (Fig. 3D). A pair of small and elongate salivary glands is visible on the outer wall of the proximal end of the esophagus and opens into the pharyngeal cavity (Fig. 4). The stomach is a large ovoid sac with a girdle of 36 triangular and yellowish stomach plates (MB28-005053 and MB28-005054); these detachable plates alternate in size, with a small one following a large one. Posterior to the girdle is an opening to the posterior ovotestis-digestive gland complex. The ovotestis-digestive gland complex is divided into two parts; the larger posterior part fills a large portion of the posterior region of the animal and is primarily formed by the ovotestis in mature specimens. The digestive gland lies at the core of both parts of the complex and is surrounded by the ovotestis. The anterior part of the complex is much smaller and is adjacent to the esophagus and the reproductive mass. A flat duct connects the two parts of the ovotestis-digestive gland complex (Fig. 4).

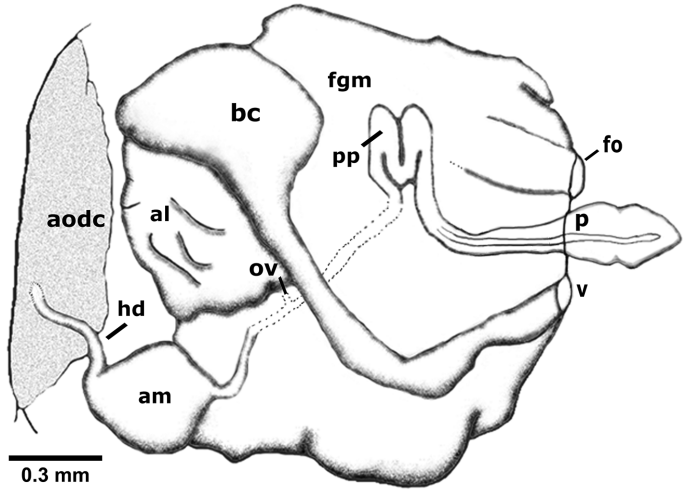

Figure 5. *Marionia abrahamorum* sp. nov. MB28-005053. Line drawing of the reproductive system, dorsal view: am, ampulla; al, albumen gland; aodc, anterior part of the ovotestis digestive gland complex; bc, bursa copulatrix; fgm, female gland mass; fo, female opening; hd, hermaphroditic duct; ov, oviduct; p, penis; pp, prostatic part of the deferent duct; v: vagina.

The reproductive system (Fig. 5) is androdiaulic. The whitish ovotestis is visible and covers the surface of the ovotestis-digestive gland complex. A narrow but long hermaphroditic duct arises from the anterior ovotestis-digestive gland complex and connects the ovotestis to the ampulla. The ampulla is small, slightly spherical, with no convolutions, and its connection to the vas deferens is concealed inside the female gland mass. The vas deferens emerges from this mass and becomes the flat and slender prostatic part that curves twice before turning into a bulbous and unarmed penis, which is extroverted in all the examined specimens. The bursa copulatrix is slightly elliptical and bulky, and is connected to the gonopore through a long vaginal duct. The albumen gland is discernible among the female gland mass. The female gland mass, together with the penis and the vagina, open to the exterior through a common atrium below the third gill.

Biology.—The specimens were found on an unidentified gorgonian, camouflaged with the same reddish color and with rhinophores and gills mimicking the gorgonian's polyps. Away from the gorgonian, the specimens changed their appearance: the rhinophores and gills were distinctly more extended and did not resemble a gorgonian's polyps as much (Fig. 2A).

Egg masses were found on the gorgonians, close to the specimens. They are narrow, slightly reddish, and transparent, with a characteristic constriction repeated almost regularly every few millimeters along the entire spawn. The eggs are small but distinguishable by the naked eye (Fig. 6B).

Type Locality and Habitat.—Pedra Mitade, Príncipe Island, São Tomé and Príncipe, Gulf of Guinea (01°32´N, 7°25´E). All the *Marionia abrahamorum* sp. nov. specimens were collected on an unidentified red gorgonian from a depth of 16 m (Fig. 6A).

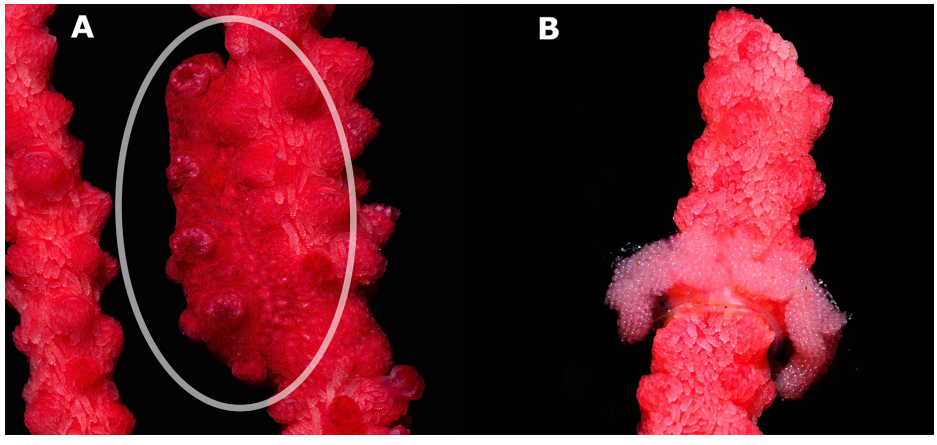

Figure 6. *Marionia abrahamorum* sp. nov., mature adult. (A) Specimen (circled) on left side of branch of unidentified gorgonian showing its cryptic coloration; (B) spawning mass.

Etymology.—This species is named *Marionia abrahamorum* in honor of Aketza Herrero's wife, Luz María Gonzalez Abraham, and his mother-in-law's family.

Geographical Distribution.—Marionia abrahamorum sp. nov. has only been reported from Príncipe Island.

Discussion

The small body size, alternating gill sizes, and simple velar papillae in the examined specimens are external morphological features that presumably characterize the genus *Tritonia* (Odhner 1963). However, hard stomach plates and a digestive gland divided into two lobes are features usually found in the genus *Marionia* (Odhner 1963, Marcus Ev. 1983). This confusion exemplifies the need to find better morphological characters as key features to distinguish the genera of Tritoniidae. To safely assign the new species to the genus *Marionia*, this description was based not only on internal morphological features, but also on results from molecular phylogenetic analyses. Table 4 compares morphological features among Atlantic species of *Marionia*.

When compared with *M. cabindae*, *M. vanira*, *M. pusa*, and *M. ghanensis*, the four *Marionia* species from the western coast of Africa, *M. abrahamorum* sp. nov. presents enough external and internal differences to be considered a different species. Among these species, *M. abrahamorum* sp. nov. has the fewest gills, fewer rows of denticles on the inner edge of the jaws, and the smallest radular formula (Table 4). Among the four species of *Marionia* known from the western African coast, color in life is only known for *M. ghanensis*, the only one described from a fresh specimen (Edmunds and Carmona 2017), and its purple color does not match the reddish color of *M. abrahamorum* sp. nov. Moreover, the notum of *M. abrahamorum* sp. nov. does not show any reticulation as described in *M. vanira*, nor the white rings found in *M. ghanensis* and *M. pusa*, being more similar to the "papillose notum" of *M. cabindae*. Although stomach plates were not noted in the latter species, these plates are easily distinguished in *M. abrahamorum* sp. nov. The lower numbers of veil appendages, gills, rows of denticles on the inner edge of the jaws, smaller radular formula, and

Table 4. Comparison among the Atlantic species of the genus *Marionia* using main morphological characters. *Information based on the description drawing. u. = under the pair of gills; b. = between the pair of gills; NEA: north eastern Atlantic; NWA: north western Atlantic; SWA: south western Atlantic.

Species	*Marionia blainvillea*	*Marionia cucullata*	*Marionia cabindae*	*Marionia vanira*	*Marionia pusa*	*Marionia tedi*	*Marionia limceana*	*Marionia ghanensis*	*Marionia gemmi*	***Marionia abrahamorum* sp. nov.**
Size (mm)	40–50	45	60–65	52	70	40	31	70	200	6–12
Gills (number of pairs)	10–12	12–16	13	14	12	10	11–14	11–13/14	7–8	7
Veil appendages (number of pairs)	Bilobed, 7	Bilobed, 7-11	6/6-8	8–10, each one up to 8 tips	14–18	8*, each one up to 3 tips	8	Bilobed, numerous papillae	Bilobed, 9	Bilobed, 6
Jaws (denticle rows)	2–4	6	Several	6	Several	20	3–4	Unknown	Smooth	2
Radular formula	26 × 21.1.1.1.21	50 × 49.1.1.1.49	62 × 100.1.108	55 × 104.1.1.1.104	70 × 125.1.1.1.125	51 × 43.1.1.1.43	26 × 26-32.1.1.1.26-32	85 × 2-124.1.1.1.2-124	96 × 100-120.1.1.1.100–120	20–25 × 14-16.1.1.1.14-16
Stomach plates	40	30–40	Not present	50	Present; number unknown	Present; number unknown	18	Unknown	57	36
Genital pore (position)	u.3	u.3	u.3	u.3	?	u.2	u.3	u.3	u.3	u.3
Anal pore (position)	u.4	u.5	b.4-5/u.5	u.5	?	u.3	u.4	b.5-6	b.4-5	b.3-4
Rhinophore sheath	Cylindrical, irregularly scalloped	Chalice-shaped	Lobed sheath with four simple processes	Crenulated sheath	Lobated rim beset with short branched processes	Pointed sheath	Chalice-shaped	Undulating margin sheath, 4 bipinnated clubs	Almost cylindrical with the top slightly narrower	Bicrenulated
Penis shape	-	Conical, armed	Globular sheath	Conical, slightly flattened	Unknown	Tubular with blunt tip	Round and unarmed	Unknown	Conical, unarmed	Bulky, unarmed
Background color	Yellowish brown to greenish, or reddish with dark pigment	Olive color	Cream with few brown streaks	Green	Ivory, semi-transparent	Black	Whitish with small patches of silver	Reddish (juvenile), purple (adult)	Cream	Red, with spots of lighter and darker shades
Notum	Network made by dark pigment, with opaque white pigment	Tubercles	Papillose	Indistinctly reticulated and gibbous	Smooth, with white dorsal rings	Black	White stripe at the center, pattern of red polygons	Spots with white rim and reddish purple ring	Tubercles, with bright orange dorsal midline	Papillose
Geographic distribution	NEA (Eastern Europe, Mediterranean)	NWA (Caribbean), SWA (Brazil)	NEA (Africa)	NEA (Africa)	NEA (Africa)	NWA (Caribbean), SWA (Brazil)	SWA (Brazil)	NEA (Africa)	NEA (Eastern Europe)	NEA (Africa)
References	Risso (1818), Schmekel and Portmann (1982)	Couthouy (1852), Marcus Er. (1983)	White (1955)	Marcus Ev. and Marcus Er. (1966)	Marcus Ev. and Marcus Er. (1968)	Marcus Ev. (1983)	Silva et al. (2013)	Edmunds and Carmona (2017)	Almón et al. (2018)	Present study

background color also allow us to differentiate *M. abrahamorum* sp. nov. from the remaining *Marionia* species found in the Atlantic Ocean: the western *M. cucullata*, *M. tedi*, and *M. limceana*, and the giant European *M. gemmi* (Table 4).

However, the Atlantic *M. blainvillea* and the Indo-Pacific *Marionia bathycarolinensis* V.G. Smith and Gosliner, 2005 share a similar background color with *M. abrahamorum* sp. nov; the former can display yellowish to green as its background color and *M. bathycarolinensis* was described as "brick red." However, these two species may be easily differentiated from our new species by the color patterns on their notum; *M. blainvillea* has a network of dark reddish pigment with opaque white spots (Table 4), while *M. bathycarolinensis* does not display any pattern at all, only "patches of greenish tinge" (Smith and Gosliner 2005). Additionally, *M. bathycarolinensis* is a giant compared to *M. abrahamorum* sp. nov., with the preserved holotype of *M. bathycarolinensis* described as measuring 155 mm in length, with 25 or more rows of denticles on the inner edge of the jaws, and a radular formula of 72 × 142.1.1.142 (Smith and Gosliner 2005), whereas the largest specimen of *M. abrahamorum* sp. nov. is 12 mm long and has 2 rows of denticles on the inner edge of the jaws, and a radula formula of 25 × 10-16.1.1.1.10-16. Additionally, *M. abrahamorum* sp. nov., at up to 12 mm long, is also the smallest Atlantic *Marionia* species; the remaining species range from 31 mm (*M. limceana*) to 200 mm (*M. gemmi*). As a side note, the bursa copulatrix in most *Marionia* species is generally equal to or larger than the rest of the reproductive mass but is smaller in *M. abrahamorum* sp. nov. The shape of the ampulla is also not typical for the genus, being spherical and small when compared to the large, long, bulky, convoluted, or folded ampullae of the other described *Marionia* (Marcus Ev. and Marcus Er. 1968, Marcus Ev. 1983, Smith and Gosliner 2005, 2007, Silva et al. 2013, Almón et al. 2018).

Our study did not recover Tritoniidae as monophyletic, in agreement with the conclusions of other recent studies (Pola and Gosliner 2010, Hulett et al. 2015, Mahguib and Valdés 2015). Although the species of Tritoniidae included in our analysis were clustered into seven different clades with poorly supported basal nodes, one of the clades was well supported and nested five undescribed species of Tritoniidae and all the *Marionia* species, including *M. blainvillea*, a result similar to recent studies including this genus (Almón et al. 2018, Valdés et al. 2018). Nevertheless, unravelling the phylogeny of the Tritoniidae is beyond the scope of our study, which aimed to show that *M. abrahamorum* sp. nov. is a valid species in the *Marionia* genus. Given that *M. blainvillea* is the type species of the genus, it is safe to assume that our species belong to the genus *Marionia*, since they cluster together, supporting the morphological data presented above. The BI and ML analyses, *p*-distance, and ABGD species delimitation analysis also strongly support the hypothesis that *M. abrahamorum* sp. nov. is a new and valid species.

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