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Two new deep-sea species of burrowing anemones (Cnidaria: Actiniaria: Edwardsiidae) from Whittard Canyon off the southwestern coast of Ireland

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ABSTRACT

Burrowing sea anemones have a simple morphology with an elongate body and a round aboral end that anchors the animal into mud, sand, or gravel, leaving only the tentacle crown exposed. Edwardsiids are easily differentiated from other burrowing sea anemones by their distinctive mesentery arrangement of eight unpaired macrocnemes at midcolumn with microcnemes restricted to the distal column at the base of the tentacles. Though edwardsiids may be frequently collected in biodiversity surveys, oceanographic expeditions, and ecological monitoring projects, their identification is particularly hampered by their small size, the need for histology, the high number of undescribed species, and the few specialists able to identify them. *Scolanthus* belongs to the subfamily Edwardsiinae, which is characterized by nemathybomes; it is differentiated from other members of the subfamily by having nemathybomes with basitrichs and periderm in the proximal end, at least eight microcnemes, and 16 or more tentacles in adults. The 14 valid species of *Scolanthus* are distributed worldwide, but only four species have been recorded from waters deeper than 100 m (*S. ingolfi*, 1461 m; *S. nidarosiensis*, 125–150 m; *S. intermedius*, 223 m; *S. triangulus,* 71–271 m). Here we describe *Scolanthus shrimp*, sp. nov., and *S. celticus*, sp. nov., the first two sea anemones recorded from the deep-sea Whittard Canyon off the coast of Ireland. We provide detailed morphological descriptions of the new species, including micro-CT scanning of

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S. celticus, and differentiate them from other species in the genus. We also generate a phylogeny using five molecular markers (12S, 16S, 18S, 28S, CO3) to establish the phylogenetic position of the new species. Based on our results, we discuss the relationship of *Scolanthus* to other edwardsiid genera and implications for the morphology and evolution of the group.

INTRODUCTION

Burrowing sea anemones are characterized by an elongate body and round proximal end that anchors the animal into mud, sand, or gravel, leaving only the tentacle crown exposed (England, 1987; Daly et al., 2002; Daly and Ljubenkov, 2008). Edwardsiids are easily distinguishable from other burrowing anemones by their distinctive mesentery arrangement of eight unpaired macrocnemes at midcolumn with microcnemes located only distally at the base of tentacles (Carlgren, 1949). Edwardsiidae Andres, 1881, is among the most speciose taxon in the Order Actiniaria with 10 genera and approximately 95 valid species (Fautin, 2016; our data) distributed worldwide from polar to tropical zones, including hypersaline environments (Daly et al., 2012) and Antarctic ice (Daly et al., 2013; Sanamyan et al., 2015), from 0–3550 m (Sanamyan and Sanamyan, 2013). Though edwardsiids may be frequently collected in biodiversity surveys, oceanographic expeditions, and ecological monitoring projects, their identification is difficult due to the high number of undescribed species, their small size and need for histological examination for specific identification, and the small number of specialists able to identify them (Daly and Ljubenkov, 2008; Gusmão et al., 2016).

Scolanthus Gosse, 1853, *Edwardsia* de Quatrefages, 1842, and *Edwardsianthus* England, 1987, belong to the subfamily Edwardsiinae Carlgren, 1921, which is characterized by having nemathybomes—hollow, epidermis-lined, nematocyst-dense pockets sunk into the mesoglea of the column (Daly, 2002). Species of *Scolanthus* are differentiated from other members of the subfamily by having nemathybomes with basitrichs and periderm not only on the scapus but also the proximal end (not a true physa), at least eight microcnemes at the base of tentacles, and 16 or more tentacles in adults (see Brandão et al., 2019). Half of the diversity of *Scolanthus* has been described in the last 10 years, with most species recorded from the Pacific Ocean (*S. ignotus* (Carlgren, 1922), *S. armatus* (Carlgren, 1931), *S. triangulus* Daly and Ljubenkov, 2008, *S. scamiti* Daly and Ljubenkov, 2008, *S. ena* Izumi and Fujita, 2018, *S. isei* Izumi and Fujita, 2018, *S. kopepe* Izumi and Fujita, 2018). The remaining species are known from the Mediterranean (*S. mediterraneus* Ocaña and Çinar, 2018) and Caribbean seas (*S. curacaoensis* (Pax, 1924), the southwestern Atlantic (*S. crypticus* Brandão, Gusmão, and Gomes, 2019), and the North Atlantic (*S. callimorphus* Gosse, 1853; *S. ingolfi* (Carlgren, 1921), *S. nidarosiensis* (Carlgren, 1942)). *Scolanthus intermedius* (McMurrich, 1893) is the only species recorded in more than one ocean; it is found in the Atlantic portion of the Southern Ocean and Chilean Pacific. Most *Scolanthus* spp. have been recorded from shallow waters, with only four species known from waters deeper than 100 m: *S. ingolfi* (1461 m), *S. nidarosiensis* (125–150 m), *S. intermedius* (223 m), and *S. triangulus* (71–271 m).

Here we describe *Scolanthus shrimp*, sp. nov., and *Scolanthus celticus*, sp. nov., from the North Atlantic off the coast of Ireland (Whittard Canyon) and contribute to the trend of a

steady increase of species described for the group, which has doubled in the past 10 years, even in one of the best sampled marine regions in the world. We provide a detailed morphological description for both new species, including micro-CT scans of *S. celticus*. In addition, we differentiate the new species from the remaining species in the genus and generate an actiniarian phylogeny using three mitochondrial (12S, 16S, CO3) and two nuclear markers (18S, 28S) to establish their phylogenetic position based on molecular data. Based on our morphological and phylogenetic analyses, we discuss the relationship between *Scolanthus* and other edwardsiid genera and the evolution of characters diagnostic for the group.

MATERIAL AND METHODS

Anatomy, Microanatomy, and Cnidae Methodology

The morphological description of *Scolanthus shrimp* (AMNH_IZC 331456) and *S. celticus* (AMNH_IZC 331453–55) is based on specimens deposited in the American Museum of Natural History (AMNH). Specimens were collected by the R/V *Celtic Explorer* during the Ecosystem Functioning and Biodiscovery Expedition at Whittard Canyon Expedition (CE14009), a multibranch system of submarine canyons some 400 km southwest of Cork, Ireland. Ethanol-fixed specimens were examined whole, in dissection, and as serial sections. Longitudinal and cross-sectional serial sections 10 μm thick were made from one specimen of *S. shrimp* and two specimens of *S. celticus* using standard paraffin techniques and stained with Heidenhain Azan stain (Presnell and Schreibman, 1997). Small pieces of tissue from each region of the body (actinopharynx, column, mesenterial filaments, nemathybomes, tentacles) were macerated on a slide and undischarged cnida capsules were examined using differential interference contrast (DIC) microscopy at 1000× magnification. Except for the rarer types, at least 20 capsules were measured from each tissue to calculate length and width range. The presence of the types of cnidae in each tissue was confirmed on histological slides. Mean and standard deviation are given to provide information about variability in capsule size, though these are not statistically significant (see Sanamyan and Sanamyan, 2013). We follow a nematocyst terminology that combines the classification of Weill (1934) and Carlgren (1940), thus differentiating "basitrichs" from "*b*-mastigophores" with that of Schmidt (1969, 1972, 1974), which captures the underlying variation seen in "rhabdoids" (see Gusmão et al., 2018, for more details). We include photographs of each type of nematocyst for reliable comparison across terminologies and taxa (see Fautin, 1988). Higher-level classification for Actiniaria follows Rodríguez et al. (2014).

Micro-CT Scanning of *Scolanthus celticus*

One specimen of *Scolanthus celticus* (AMNH_IZC331455), fixed and preserved in 96% ethanol, was stained using 1% osmium tetroxide. Because only one specimen of *S. shrimp* was available for study, it could not be fixed in osmium tetroxide for CT-scanning as it would render the specimen unavailable for morphological and histological slides. For

details on the staining protocol used here see Gusmão et al. (2018). Once stained and washed, the specimen was transferred to and scanned on a 50 ml polyethylene tube filled with 100% ethanol on a phoenix v|tome|x s240 GE (General Electric, Fairfield, CT) at 60 kV and 70 μA with a molybdenum target at the Microscopy and Imaging Facility (MIF) at AMNH. The exposure time for the detector was 400.145 msec for a final resolution of 7.57 microns/voxel. We used the software Phoenix datos|x (General Electric, Wunstorf, Germany) to reconstruct the raw data; the resulting files and images were processed and edited on 3D slicer (Fedorov et al., 2012). Full micro-CT scan data is deposited in the Morphobase (www.morphosource.org) under project AMNH_IZC331455.

Molecular Data Collection and Phylogenetic Analyses

We isolated genomic DNA from approximately 25 mg of tissue from the column of *Scolanthus shrimp* and *S. celticus* using the Qiagen DNeasy kit. We amplified whole genomic DNA for three mitochondrial markers (12S, 16S, CO3) and nuclear markers (18S, 28S) using published primers (e.g., Geller and Walton, 2001; Daly et al., 2008; Lauretta et al., 2014). PCR products were cleaned using Thermo Scientific Fermentas clean-up protocol using Exonuclease I and FastAP thermosensitive alkaline phosphatase per manufacturer's specifications (shrimp alkaline phosphatase replaced by FastAP™). A total of 5 μL of cleaned PCR product, at a concentration of 25 ng of product for every 200 base pairs (bp) of marker length, was cycle-sequenced in an ABI BigDye® Terminator v3.1 (Applied Biosystems) reaction following the manufacturer's protocols using PCR amplification primers. We cleaned cycle-sequencing products using Centri-Sept columns (Princeton Separations; following the manufacturer's protocol) containing DNA-grade Sephadex (G-50 fine; GE Healthcare) and sequenced them on an ABI 3770x at the in-house facilities of the AMNH. We assembled and edited forward and reverse sequences in Geneious v.10.0.9 (Kearse et al., 2012) and blasted the assembled sequences against the nucleotide database of GenBank to confirm amplification of target marker/organism.

Newly generated sequences were combined with sequences from previous phylogenetic studies of sea anemones (e.g., Rodríguez et al., 2014; Daly et al., 2017; Titus et al., 2019) downloaded from Genbank (see supplementary data, doi.org/10.5531/sd.sp.37). Sequences were combined in two datasets: a broad Actiniaria-level dataset and a more focused dataset of members of suborder Anenthemonae Rodríguez and Daly, 2014, particularly of family Edwardsiidae, including members for which only 18S sequences were available (see supplementary data, doi.org/10.5531/sd.sp.37). Alignments used in our analyses are available from Treebase (http://purl.org/phylo/treebase/phylows/study/TB2:S24884). Sequences were aligned separately for each marker using MAFFT v.7.0 online (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013; Katoh et al., 2017) and the following settings: Strategy L-INS-I; Scoring matrix for nucleotide sequences, 200 PAM/k = 2; Gap open penalty, 1.53; offset value, 0.05. We chose the best model of nucleotide substitution for each marker using Akaike information criterion (AIC) on jModeltest2 (Guindon and Gascuel, 2003; Darriba et al., 2012) implemented on the

FIG. 1. Map of type localities for *Scolanthus shrimp*, sp. nov. (triangle), and *S. celticus*, sp. nov. (star), at the Whittard Canyon in the North Atlantic.

CIPRES Portal (Miller et al., 2010). Maximum likelihood (ML) analyses were performed on RAxML-NG v0.6.0 (Kozlov et al., 2018) for each marker separately and as a concatenated dataset (mitochondrial, nuclear, concatenated dataset) using the appropriate model of nucleotide substitution for each marker. We used the majority rule criterion to access clade support and allowed automatic hault (-autoMRE). An additional ML analysis was conducted on PhyML v.3.0 online (http://www.atgc-montpellier.fr/phyml/) (Guindon et al., 2010) allowing automatic model selection using the AIC criterion (Lefort et al., 2017) and 1000 rounds of bootstrap for each marker separately and in combination. We also conducted maximum parsimony (MP) analyses in TNT v1.1 (Goloboff et al., 2008) using random and constrained sectorial searches, tree drifting, and 100 rounds of tree fusing; trees of minimum length were found at least 10 times. Clade support on the obtained strict consensus tree was accessed after 1000 bootstrap rounds for each marker separately and as a concatenated dataset. Gaps were treated as missing data in all analyses.

RESULTS

Taxonomic Description

Order Actiniaria Hertwig, 1882

Suborder Enthemonae Rodríguez and Daly, 2014, in Rodríguez et al. (2014)

Superfamily Edwardsioidea Andres, 1881

Family Edwardsiidae Andres, 1881

Diagnosis: (from Daly and Ljubenkov, 2008) Anenthemonae with elongate, vermiform body usually divisible into two or more regions: between long scapus provided with periderm and short capitulum may be short scapulus that lacks periderm and epidermal specializations. Proximal end rounded, without basilar muscles, may be differentiated into a physa. Single weak siphonoglyph. No sphincter muscle or acontia. Mesenteries divisible into macro- and microcnemes; always eight macrocnemes and at least four microcnemes. Macrocnemes comprise two pairs of directives and four lateral mesenteries, two on each side, whose retractors face sulcar (= ventral) directives. Retractors restricted, diffuse to strongly circumscribed; parietal muscles always distinct. Cnidom: spirocysts, basitrichs, *b*-mastigophores, *p*-mastigophores A, pterotrichs, t-mastigophores.

Type genus: *Edwardsia.*

Valid genera: *Drillactis* Verrill, 1922; *Edwardsia*; *Edwardsianthus*; *Edwardsiella* Andres, 1883; *Halcampogeton* Carlgren, 1937; *Isoscolanthus* Brandão, Gusmão and Gomes, 2019; *Nematostella* Stephenson, 1935; *Paraedwardsia* Carlgren in Nordgaard, 1905; *Scolanthus; Synhalcampella* Carlgren, 1921.

Genus *Scolanthus*

Diagnosis: (from Brandão et al., 2019) Edwardsiidae with body divisible into scapus and scapulus. Proximal region of body rounded, with nemathybomes and periderm; nemathybomes scattered or forming several longitudinal rows on scapus. Nemathybomes with basitrichs. At least eight microcnemes. Tentacles at least 16 in adults, arranged octamerously; inner ones shorter than outer ones. Retractor muscles relatively large, well developed, diffuse to circumscribed; parietal muscles distinct, symmetrical, well developed. Cnidom: spirocysts, basitrichs, *b*-mastigophores, and *p*-mastigophores A.

Type species: *Scolanthus callimorphus* Gosse, 1853 by monotypy.

Valid species: *Scolanthus armatus*; *S. callimorphus*; *S. crypticus*; *S. curacaoensis*; *S. ena*; *S. ignotus*; *S. ingolfi*; *S. intermedius*; *S. isei*; *S. kopepe*; *S. mediterraneus*; *S. nidarosiensis; S. scamiti*; *S. triangulus*.

Remarks: We agree with the circumscription of Brandão et al. (2019) for the genus *Scolanthus*, considering the former genus *Isoedwardsia* Carlgren, 1921, a junior synonym of *Scolanthus* (Manuel, 1981a) and, thus, including *S. ignotus* and *S. ingolfi* within *Scolanthus*.

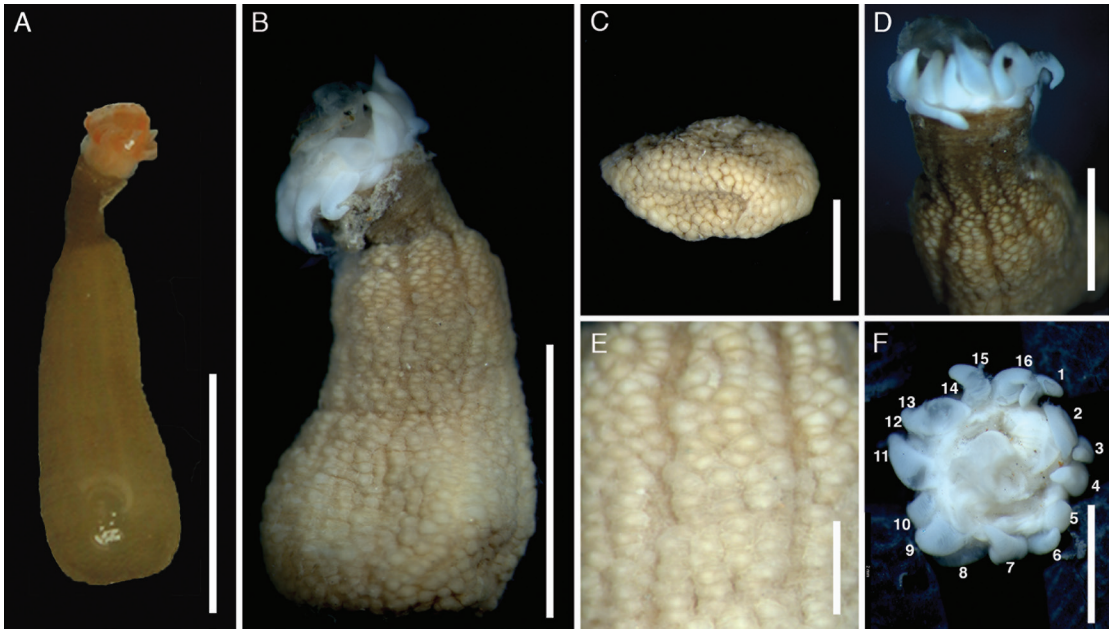

FIG. 2. External anatomy of *Scolanthus shrimp*. **A,** lateral view of live holotype; **B,** lateral view of preserved holotype showing nemathybomes arranged in rows on scapus; **C,** aboral view of the holotype showing nemathybomes on proximal end; **D,** detail of distal scapus with nemathybomes scarcely present distributed in rows; **E,** detail of nemathybomes on column; **F,** oral view of holotype with oral disc and 16 tentacles. Scale bars: **A, E,** 1 mm; **B,** 5 mm; **C,** 3.5 mm; **D,** 2.5 mm; **F,** 2 mm.

***Scolanthus shrimp*,** sp. nov.

Figures 2–4, table 1

Material: Holotype AMNH_IZC 331456; Locality: Ireland, Whittard Canyon, 48°29.238′ N, 10°23.802′ W, collected 17 June 2014, by R/V *Celtic Explorer*, CE14009, event 31, gear ROV (1774 m). *Material examined for comparison: Isoscolanthus janainae* Brandão, Gusmão, Gomes, 2019, MZUSP 2729 (10 specimens; paratypes); Locality: Brazil, Rio de Janeiro, 21°31′ S, 40°08′ W, collected 31 May 1987, by TAAF, Sta. 56, CB96 (295–300 m). *Edwardsianthus gilbertensis* (Carlgren, 1931) AMNH 3614 (one specimen); Locality: Guam, NW Pacific, det. ca. 2001 by M. Daly. AMNH 3614A (two slides); Locality: Guam, NW Pacific, det. ca. 2001 M. Daly. *Edwardsia tuberculata* AMNH 3616A (two slides); Locality: Bergen, Norway, det. ca. 2001 by M. Daly.

Diagnosis: *Scolanthus* with large, single nemathybomes that protrude into epidermis. Basitrichs of nemathybomes 40.0–52.2 × 3.0–4.2 µm. Thin, tightly adherent periderm. Retractor and parietal muscles well developed and of similar size. Retractor circumscribed, with easily recognizable pennon; parietal muscle large, trianguloid, relatively broad.

External anatomy (fig. 2): Specimen elongate, wider proximally than distally, 2.3–5.1 mm in diameter and 15 mm in height. Column dark beige with eight mesenterial insertion visible in live specimens; preserved specimen yellow with mesenterial insertion visible. Proximal end rounded, not externally differentiated from rest of column (fig. 2A–C); body divided

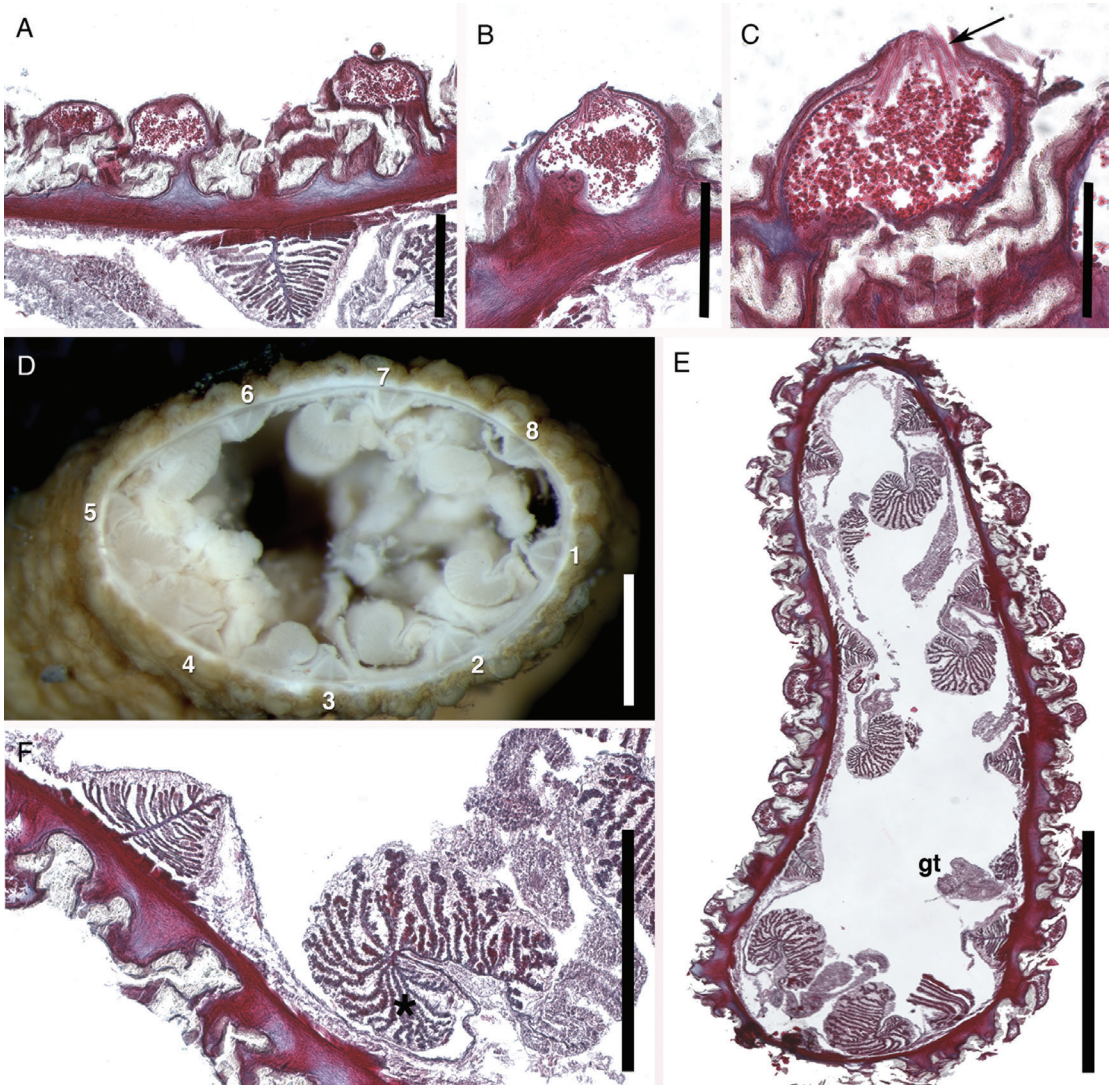

FIG. 3. Internal anatomy and microanatomy of *Scolanthus shrimp*. **A,** detail of periderm and nemathybomes on scapus; **B,** Nemathybome on scapus; **C**, detail of nemathybome with basitrichs inside (arrow); **D**, cross section through midcolumn showing mesenterial arrangement of eight macrocnemes (indicated by numbers); **E**, histological cross section through midcolumn showing mesenterial arrangement; **F**, detail of macrocneme in cross section showing circumscribed retractor with pennon (indicated by asterisk) and strong parietal muscle. Abbreviations: gt, gametogenic tissue. Scale bars: **A,** 0.2 mm**; B,** 0.4 mm **C, D**, 0.1 mm; **E**, 2 mm; **F**, 0.5 mm.

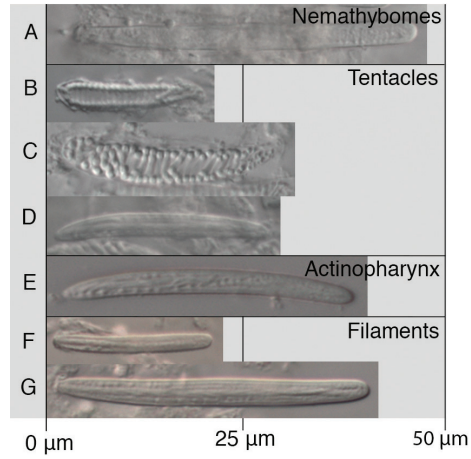

FIG. 4. Cnidom of *Scolanthus shrimp*. **A, D–G,** basitrich; **B,** gracile spirocyst; **C,** robust spirocyst.

in aboral end, scapus, scapulus, and capitulum (fig. 2A, B, D). Periderm thin, tightly adherent, not deciduous, covering column and nemathybomes from distal scapus to proximal end (fig. 2A–C). Nemathybomes single, conspicuous, irregularly scattered on entire body (fig. 2B, E), but forming eight rows more visible in distal column perhaps due to contraction (fig. 2D). Sixteen small tentacles, arranged in two cycles, presumably all of same size (fig. 2F); tentacle length to 1.5 mm; light orange in live specimen, white in preserved state.

Internal anatomy and histology (fig. 3): Epidermis of entire body covered by thin periderm and nemathybomes (fig. 3A). Nemathybomes simple, large, to 213.6 × 181.0 µm, into mesoglea, but always protruding into epidermis (fig. 3B, C); basitrichs visible on top of nemathybome (fig. 3C). Longitudinal muscles of the tentacles ectodermal. Physa without terminal pore. Actinopharynx short, occupying less than one-third of column length, highly folded. No differentiated siphonoglyph.

Mesenterial arrangement as typical for edwardsiids: eight macrocnemes span length of body, from distal column to midcolumn (fig. 3D, E); eight microcnemes only in distalmost column, at bases of tentacles (not shown). Retractor and parietal muscles both well developed, strong (fig. 3E, F). Retractor muscle of macrocnemes strong, circumscribed, with easily recognizable pennon (fig. 3F). Retractor with relatively numerous processes (12–16), tightly spaced, variable in height and degree of ramification, more branched at extremities, including large pennon (fig. 3F). Parietal muscle large, trianguloid, relatively broad, with longer, branched lamellae closer to body wall; central lamella same thickness as side branches (fig. 3F). Specimen with oocytes, length 69.6–85.4 µm (fig. 3E); species inferred gonochoric.

Cnidom (fig. 4): Spirocysts, basitrichs, *p*-mastigophores A. See figure 4 and table 1 for size and distribution.

Distribution and natural history: *Scolanthus shrimp*, sp. nov., is known only from its type locality in the Whittard Canyon approximately 400 km off the coast of Ireland in the Celtic Sea, North Atlantic, at 1774 m depth.

TABLE 1. Cnidom of *Scolanthus shrimp*, sp. nov., and *Scolanthus celticus*, sp. nov.
P-mast, *p*-mastigophores; S, proportion of specimens in which each cnidae was found; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; *, rare.

	Scolanthus shrimp, sp. nov.						*Scolanthus celticus*, sp. nov.					
Tissue/categories	fig. 4	Range of length and width of capsules (μm)	± SD	N	S	F	fig. 7	Range of length and width of capsules (μm)	± SD	N	S	F
NEMATHYBOMES												
Basitrichs	(A)	41.7–58.4x 3.3–4.6	51.8±3.9 x 3.9±0.3	48	1/1	++	(A)	42.0–58.4 x 3.8–5.6	48.3±3.5 x 4.6±0.4	36	2/2	++
TENTACLES												
Spirocysts	(B–C)	14.4–32.8 x 2.8–7.6	24.1±4.1 x 4.6±0.9	108	1/1	+++	(B–C)	15.6–42.2 x 3.1–8.3	29.6±5.5 x 5.0 ±1.1	177	2/2	+++
Basitrichs	(D)	22.4–37.3 x 2.9–4.2	27.4±2.5 x 3.5±0.3	58	1/1	++	(D)	27.2–35.6 x 2.2–4.2	31.0±1.7 x 3.5±0.3	100	2/2	+++
PHARYNX												
Basitrichs I		—	—				(E)	14.2 x 3.2	—	1	1/2	*
Basitrichs II	(E)	32.4–40.1 x 2.8–3.9	35.2±1.0 x 3.3±0.4	51	1/1	++	(F)	28.3–34.1 x 3.0–3.9	30.8±1.9 x 3.5±0.3	42	2/2	+++
FILAMENT												
Basitrichs I	(F)	17.6–25.8 x 2.0–2.9	21.9±2.2 x 2.4±0.2	59	1/1	++	(G)	19.2–30.9 x 1.9–3.5	23.6±2.3 x 2.5±0.3.	78	2/2	++
Basitrichs II	(G)	39.0–51.9 x 3.2–4.7	44.6±3.0 x 3.8±0.3	47	1/1	+++	(H)	38.8–54.1 x 2.1–4.7	48.0±2.9 x 3.9±0.4	110	2/2	+++
P-mast A		—	—				(I)	25.0–32.0 x 5.0–6.5	28.9±1.8 x 5.8±0.4	26	2/2	+

Etymology: The specific name "shrimp" honors the Science Research Mentoring Program (SRMP) at the AMNH and their high school students known informally as srmpers. The SRMP supported C.Q., S.L.B., and L.C.G. The species epithet is a noun in apposition.

Remarks: Though the description of *Scolanthus shrimp* is based on a single specimen, the specimen was well preserved and all external and internal characters were easily described.

Scolanthus celticus, sp. nov.

Figures 5–7, table 1

Material: Holotype AMNH_IZC 331453; Locality: Ireland, Whittard Canyon, 48°42.534′ N, 10°33.642′ W, collected 11 June 2014, by R/V *Celtic Explorer*, CE14009, event 9, gear ROV (1130 m). Paratypes AMNH_IZC 331454 (2 specimens), sampled at the same site of the holotype. Additional material. AMNH_IZC 331455 (1 specimen), sampled at the same site of the holotype. *Material examined for comparison: Scolanthus crypticus* Brandão, Gusmão, Gomes, 2019, MNRJ 8685 (8 specimens; paratypes); Locality: Brazil, São Paulo, offshore Ubatuba, 23°34′ S, 44°48′ W, collected 22 October 1986 (40 m).

Diagnosis: *Scolanthus* with nemathybomes single or clustered, largely sunken into mesoglea protruding only a little in epidermis. Periderm very thick, rusty brown, deciduous. Basitrichs of nemathybomes 42.0–58.4 × 3.8–5.6 μm. Retractor strong, elongated, diffuse-circumscribed, with numerous processes (23–33) and easily recognizable pennon. Parietal muscle small, ovoid, and much smaller than retractor muscle. With *p*-mastigophores A in filament.

External anatomy (fig. 5): All specimens elongate but stout, robust, wider proximally than distally, 5–10 mm in diameter, and 14–24 mm in height. Proximal end rounded, externally differentiated from rest of column but not true physa (fig. 5A, B); body divided in aboral end, scapus, scapulus, and capitulum. All preserved specimens with distal column and oral disc retracted, including part of scapus, scapulus, and capitulum; tentacles not visible (fig. 5A). Periderm thick, rusty brown, tightly adherent, deciduous, covering column and nemathybomes from distal scapus to proximal end (fig. 5A, B). Nemathybomes conspicuous**,** irregularly scattered on body (fig. 5B, C), single or compound, more aggregated on proximal end (fig. 5B) due to contraction of body. Sixteen small tentacles, arranged in two cycles, presumably all of same size; tentacle length to 3 mm.

Internal anatomy and histology (fig. 6): Epidermis of entire body covered by thick periderm and nemathybomes (fig. 6A, E). Nemathybomes large, to 247.0 × 146.6 μm, simple or compound, sunken into mesoglea, but protruding into epidermis (fig. 6B, C). Longitudinal muscles of tentacles ectodermal (fig. 6D). Physa not histologically differentiated from scapus; without terminal pore. Actinopharynx short, up to 1.5 mm, occupying less than one-third of body length, highly folded. No differentiated siphonoglyph.

Mesenterial arrangement as typical for edwardsiids: eight macrocnemes span length of body, from distal column (fig. 6E) to midcolumn (fig. 6F, G); eight microcnemes only in distalmost column, at bases of tentacles (not shown). Retractor and parietal muscles both well developed, strong (fig. 6F, H). Retractor muscle of macrocnemes strong, circumscribed to somewhat diffuse, with easily recognizable pennon (fig. 6H). Retractor with numerous processes (23–33), tightly spaced,

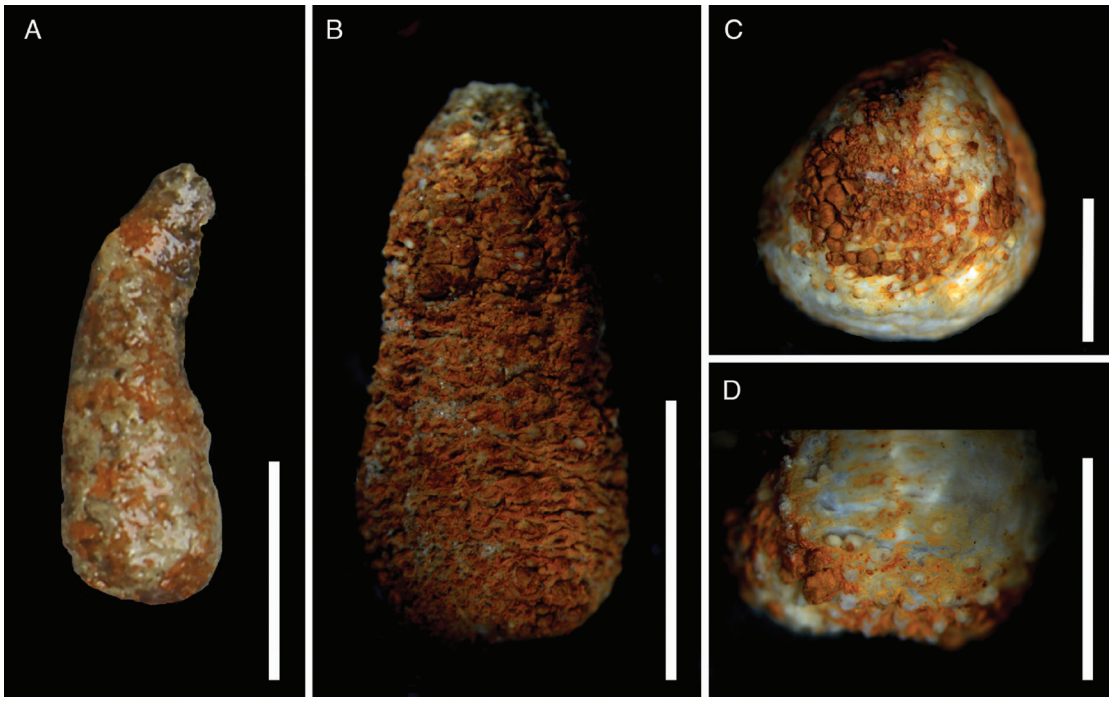

FIG. 5. External anatomy of *Scolanthus celticus*. **A,** lateral view of live holotype; **B,** lateral view of preserved holotype showing thick periderm on scapus; **C,** Oral view of proximal end with periderm and nemathybomes; **D,** Detail of nemathybomes on proximal scapus. Scale bars: **A,** 10 mm; **B**, 6 mm; **C–D**, 3.8 mm.

variable in height and degree of ramification, more branched at the pennon (fig. 6H). Parietal muscle small, weak, trianguloid, with longer, branched lamellae closer to body wall; central lamella thicker than side branches (fig. 6I). Specimens examined (holotype and paratype AMNH_IZC 331454) with spermatic cysts (fig. 6J), length 45.2–114.3 μm; species inferred to be gonochoric.

Cnidom (fig. 7): Spirocysts, basitrichs, *p*-mastigophores A. See figure 7 and table 1 for size and distribution.

Distribution and natural history: The four specimens were collected in a single collection site in the Whittard Canyon approximately 450 Km from the SW coast of Ireland, North Atlantic, at 1130 m depth. *Scolanthus celticus* was collected less than 30 km from the type locality of *S. shrimp*, suggesting a pattern of sympatry common among edwardsiids (Daly and Ljubenkov, 2008).

Etymology: The specific name *celticus* honors the *Celtic Explorer*, a multipurpose research vessel operated by the Marine Institute in Galway, Ireland, which collected specimens of the new species.

Micro-CT Scanning of *Scolanthus celticus*

The single specimen of *Scolanthus celticus* was successfully stained with osmium tetroxide, and micro-CT scanning resulted in high-contrast in which external characters traditionally used in the taxonomy of edwardsiids were readily observed, including the presence of periderm

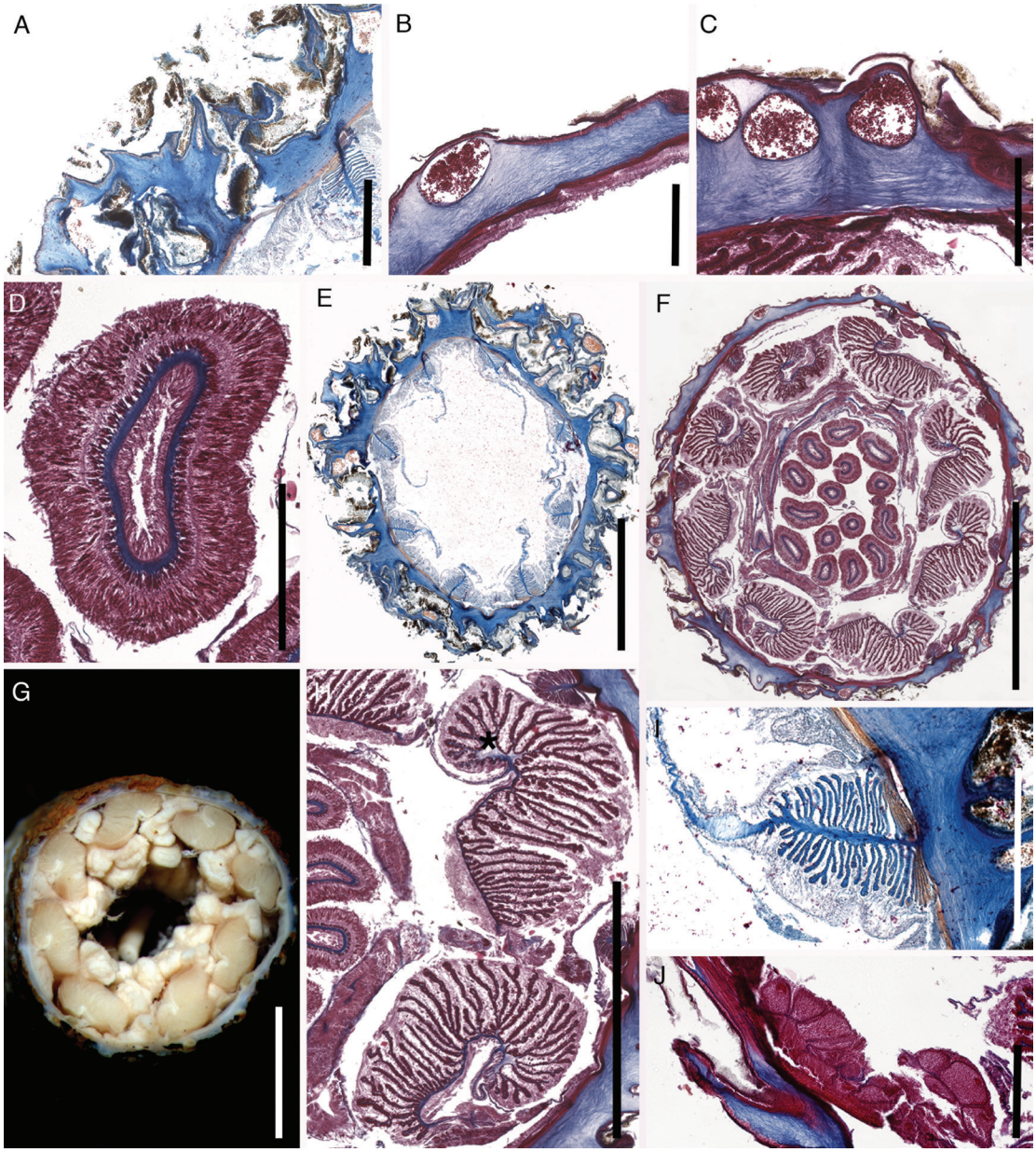

FIG. 6. Internal anatomy and microanatomy of *Scolanthus celticus*. **A,** detail of periderm on distal column; **B,** Simple nemathybome on midcolumn; **C,** detail of three nemathybomes protruding into epidermis; **D,** longitudinal muscle of tentacles ectodermal; **E,** eight parietal muscles indicating eight macrocnemes on distal column (retractors missing); **F,** cross-section through distal column showing eight macrocnemes; note 16 tentacles in cross-section; **G,** cross section through midcolumn below actinopharynx showing eight macrocnemes; **H,** detail of retractor muscle; pennon indicated by asterisk; **I,** detail of parietal muscle on distal column; **J,** spermatic cysts. Scale bar: **A**, 0.35 mm**; B–D**,**F, J,** 0.25 mm; **E**, 1.3 mm; **G**, 3.2 mm; **H**, 1.35 mm; **I**, 0.28 mm.

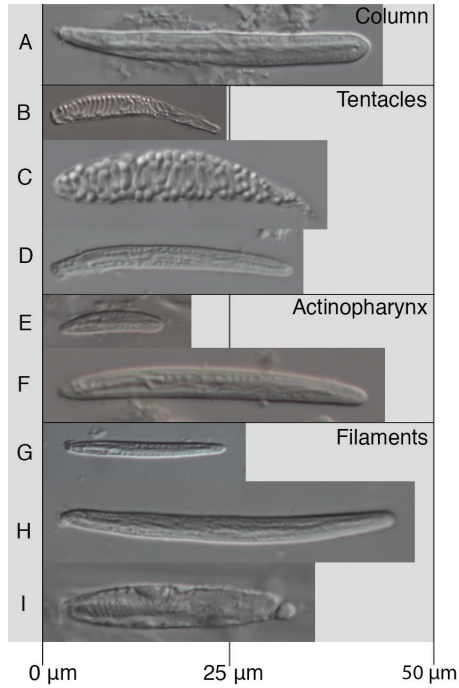

FIG. 7. Cnidom of *Scolanthus celticus*. **A, D–H,** basitrich; **B,** gracile spirocyst; **C,** robust spirocyst; **I,** *p*-mastigophore A.

and nemathybomes in entire body (fig. 8A, B), column morphology and its division into scapus, capitulum, and tentacles (fig. 8B). The internal anatomy and arrangement of mesenteries along the body was also well established, from proximal end of the body (fig. 8C, D), to mid-column (fig. 8E) and distal part of the scapus (fig. 8F), and capitulum (fig. 8G, H). Muscle shape and morphology of macrocnemes were accurately established from 2D scans (e.g., fig. 8E, F), but the resolution of the images obtained was not sufficient to visualize details of musculature (e.g., number and branching pattern in retractors or parietal).

Molecular Phylogenetic Analyses

Similar sequence lengths were obtained for *Scolanthus shrimp* and *S. celticus*: approximately 750 bp were sequenced for 12S, 470 bp were sequenced for 16S, and 691 bp for CO3 mitochondrial rDNA; approximately 1800 bp were obtained for 18S nuclear rDNA and 3200 bp for 28S nuclear rDNA. The concatenated dataset (12S, 16S, CO3, 18S, 28S) had a total of 213 taxa and 8292 sites. Trends in marker variability for both broad and focused datasets followed those previously reported for Actiniaria (e.g., Daly et al., 2008, 2010; Gusmão and Daly, 2010; Rodríguez et al., 2012, 2014; Lauretta et al., 2014; Gusmão et al., 2018). All phylogenetic reconstructions (MP, ML) using the concatenated data for the broad ordinal dataset agreed basically with the topology depicted in figure 9: two major clades corresponding to the two actiniarian suborders and five superfamilies were recovered, including superfamily Edwardsioidea/family Edwardsiidae with high bootstrap support (98% ML/100% MP). In general, the model-based

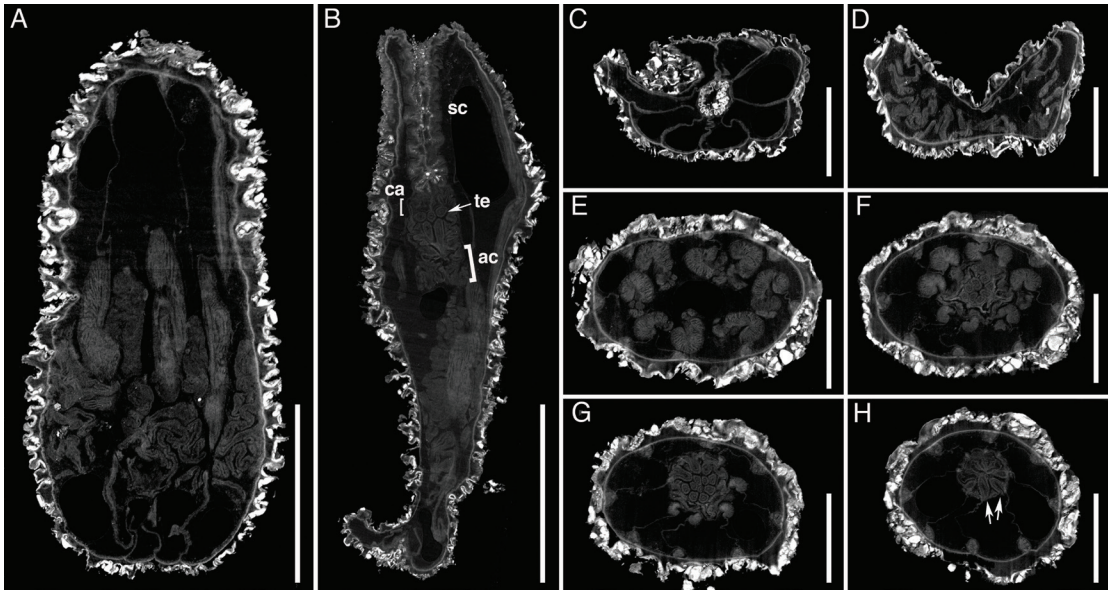

FIG. 8. Micro-CT scans of *Scolanthus celticus*. **A,** longitudinal section (XY plane) through whole specimen showing periderm and nemathybomes on scapus and proximal end; **B,** longitudinal section (XZ plane) through specimen showing introverted scapulus, capitulum, tentacles, actinopharynx, and gastrovascular cavity; **C,** cross section through proximal end of specimen showing eight macrocnemes with well weak retractor and parietal muscles; **D,** cross section through proximal scapus showing eight macrocnemes with diffuse retractor; **E,** cross section through midcolumn showing eight macrocnemes with circumscribed retractors and strong parietal muscles; **F,** cross section through actinopharynx showing eight macrocnemes with small circumscribed retractors and strong parietal muscles; note tentacles inside actinopharynx; **G,** cross section through upper scapulus showing eight macrocnemes: six with small retractor muscles and two without retractors; **H,** cross section through scapulus showing eight macrocnemes without retractors but with parietal muscles; note two tentacles in a macrocele (arrows). Abbreviations: **ac**, actinopharynx ; **ca,** capitulum; **sc,** scapulus; **te,** tentacles; **ac,** actinopharynx. Scale bars: **A–B**, 5 mm; **C–H,** 2.5 mm.

analyses provided greater support than the parsimony ones; *S. shrimp* and *S. celticus* were resolved together with high bootstrap support (100% ML/100% MP) as sister to *Edwardsianthus gilbertensis* (Carlgren, 1931), albeit there is low support for this relationship in the analyses (ML: 66%; MP: 58%). The inclusion of *Scolanthus* spp. and *E. gilbertensis* rendered *Edwardsia* paraphyletic; but the three genera are recovered within a clade corresponding to the subfamily Edwardsiinae with high bootstrap support (94% ML/95% MP).

The analysis of the more focused dataset including members of suborder Anenthemonae resulted in the topology seen in figure 10: Anenthemonae is monophyletic, though with low bootstrap support, with a highly supported monophyletic Edwardsioidea/ Edwardsiidae (93%). The focused dataset recovered similar results to the broad analysis, including a highly supported relationship between *Scolanthus shrimp* and *S. celticus* (92%), a sister-group relationship between the two new species and the two individuals of *Edwardsianthus gilbertensis* (78%), and a paraphyletic *Edwardsia*. We also recovered a monophyletic Edwardsiinae, but the position of *Edwardsiella loveni* (Carlgren, 1892) rendered genus *Edwardsiella* and subfamily Milneedwardsiinae paraphyletic.

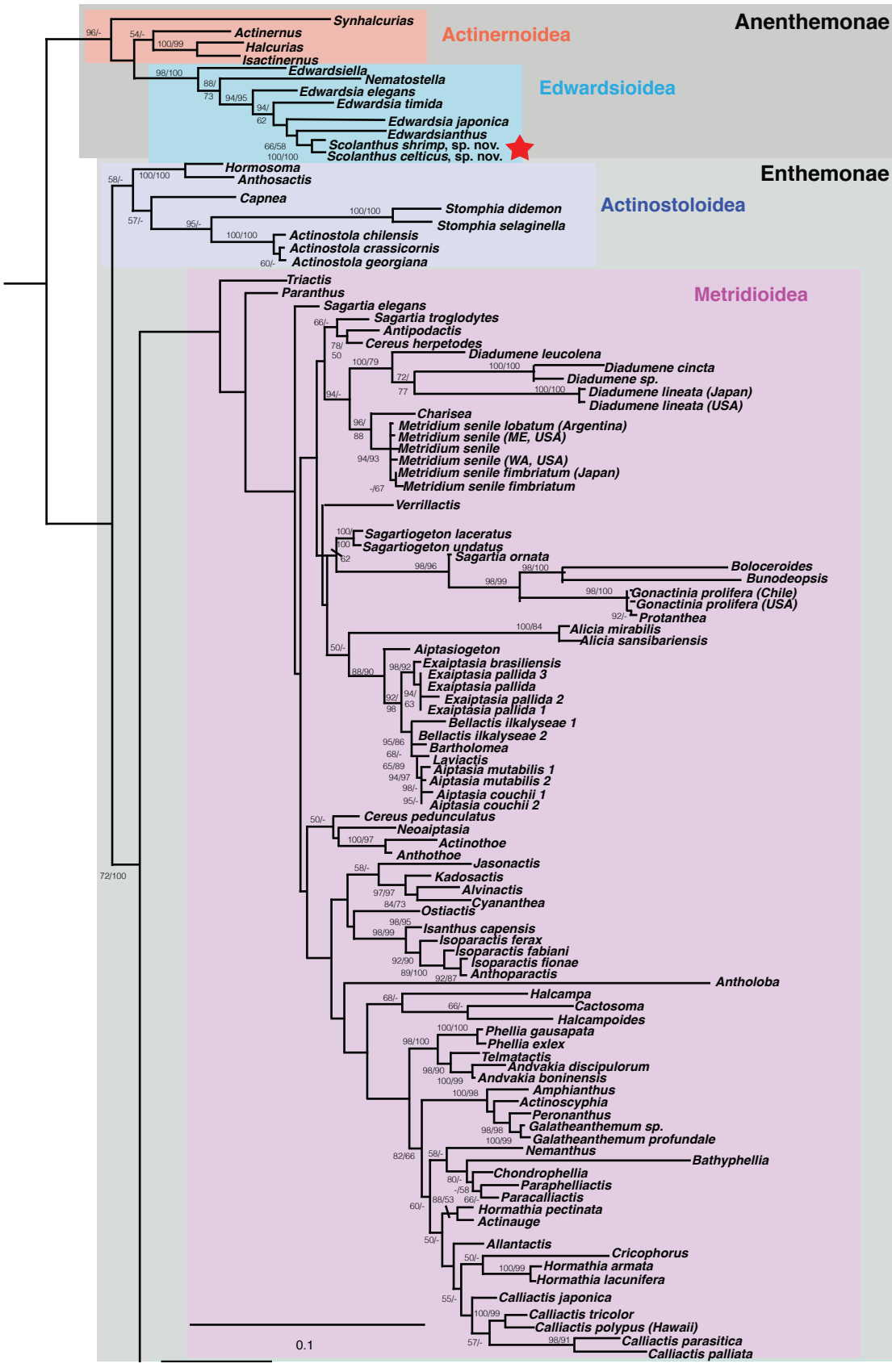
Anenthemonae
Actinernoidea
Edwardsioidea
Enthemonae
Actinostoloidea
Metridioidea
Synhalcurias
Actinernus
Halcurias
Isactinernus
Edwardsiella
Nematostella
Edwardsia elegans
Edwardsia timida
Edwardsia japonica
Edwardsianthus
Scolanthus shrimp, sp. nov.
Scolanthus celticus, sp. nov.
Hormosoma
Anthosactis
Capnea
Stomphia didemon
Stomphia selaginella
Actinostola chilensis
Actinostola crassicornis
Actinostola georgiana
Triactis
Paranthus
Sagartia elegans
Sagartia troglodytes
Antipodactis
Cereus herpetodes
Diadumene leucolena
Diadumene cincta
Diadumene sp.
Diadumene lineata (Japan)
Diadumene lineata (USA)
Charisea
Metridium senile lobatum (Argentina)
Metridium senile (ME, USA)
Metridium senile
Metridium senile (WA, USA)
Metridium senile fimbriatum (Japan)
Metridium senile fimbriatum
Verrillactis
Sagartiogeton laceratus
Sagartiogeton undatus
Sagartia ornata
Boloceroides
Bunodeopsis
Gonactinia prolifera (Chile)
Gonactinia prolifera (USA)
Protanthea
Alicia mirabilis
Alicia sansibariensis
Aiptasiogeton
Exaiptasia brasiliensis
Exaiptasia pallida 3
Exaiptasia pallida
Exaiptasia pallida 2
Exaiptasia pallida 1
Bellactis ilkalyseae 1
Bellactis ilkalyseae 2
Bartholomea
Laviactis
Aiptasia mutabilis 1
Aiptasia mutabilis 2
Aiptasia couchii 1
Aiptasia couchii 2
Cereus pedunculatus
Neoaiptasia
Actinothoe
Anthothoe
Jasonactis
Kadosactis
Alvinactis
Cyananthea
Ostiactis
Isanthus capensis
Isoparactis ferax
Isoparactis fabiani
Isoparactis fionae
Anthoparactis
Antholoba
Halcampa
Cactosoma
Halcampoides
Phellia gausapata
Phellia exlex
Telmatactis
Andvakia discipulorum
Andvakia boninensis
Amphianthus
Actinoscyphia
Peronanthus
Galatheanthemum sp.
Galatheanthemum profundale
Nemanthus
Bathyphellia
Chondrophellia
Paraphelliactis
Paracalliactis
Hormathia pectinata
Actinauge
Allantactis
Cricophorus
Hormathia armata
Hormathia lacunifera
Calliactis japonica
Calliactis tricolor
Calliactis polypus (Hawaii)
Calliactis parasitica
Calliactis palliata
0.1

FIG. 9. Phylogenetic reconstruction from maximum likelihood analyses using concatenated dataset of five markers (12S, 16S, 18S, 28S, CO3). Dashed boxes represent actiniarian suborders and colored boxes actiniarian superfamilies. *Scolanthus shrimp* and *S. celticus* indicated by red star. Bootstrap resampling values indicated above branches (ML/MP); only support values > 50% are shown.

DISCUSSION

Familial and Generic Placement Based on Morphological and Molecular Data

The vermiform body without marginal sphincter or basilar muscles and the mesenterial arrangement of eight macrocnemes spanning the body with microcnemes restricted to the base of tentacles in *Scolanthus shrimp* and *S. celticus* clearly place them within superfamily Edwardsioidea and family Edwardsiidae. Molecular data complement the morphological data: the taxonomic position of *S. shrimp* and *S. celticus* within Edwardsiidae is further supported by the phylogenetic analysis, which places both species within a highly supported Edwardsioidea (see fig. 9). In addition, both new species are recovered with high support within a clade of six species corresponding to the subfamily Edwardsiinae, suggesting that nemathybomes are a strong synapomorphy for the group as found by Daly (2002). Similar to Daly (2002), we found a close relationship between *Scolanthus* and *Edwardsianthus*, which is supported by three morphological features in her analyses (i.e., discontinuous ciliated tracts, ovoid parietal muscle, and mesoglea of the aboral end equal in thickness to that of the column). Although the limited taxon sampling and lack of resolution within Edwardsiidae in the analyses of our broad data set prevent us from further commenting on the relationships of *Edwardsia*, *Scolanthus*, and *Edwardsianthus* and of those and other edwardsiids, the analysis of the more focused data set helped clarify relationships within family Edwardsiidae. The nonmonophyly of *Edwardsiella* can be partly explained by its diagnosis based on the absence of characters (i.e., tenaculi, nemathybomes, and nematosomes) that characterize other edwardsiid genera. Daly (2002) suggested the use of three additional morphological features to help distinguish members of *Edwardsiella* from other edwardsiids (i.e., number of tentacles in the second cycle, microcneme arrangement in the dorsolateral compartment, and wide nematocysts in the tentacles). In addition, considering that *Edwardsia* is defined by a symplesiomorphy (i.e., nemathybomes on the scapus) and is comprised of species that cannot be assigned to other edwardsiid genera, the paraphyly recovered in our analysis is not surprising and has been previously recovered (e.g., Daly 2002). Because no clear pattern regarding relevant characters (e.g., arrangement of microcnemes and nemathybomes on scapus, types of nematocysts in nemathybomes) can be established based on relationships recovered within the few members of *Edwardsia* included in our analyses (five species of more than 50 nominal ones), the circumscription of the genus cannot be resolved by our data.

Scolanthus has a convoluted taxonomic history with species and generic reassignments (see Daly and Ljubenkov, 2008; Brandão et al., 2019), but the presence of nemathybomes and periderm on the proximal end, nemathybomes with only basitrichs, and at least eight microcnemes and, thus, 16 tentacles unequivocally place *S. shrimp* and *S. celticus* within the genus. The taxonomic position of *S. celticus* within *Scolanthus* is further supported by the confirmation of certain diagnostic features in micro-CT scans such as the presence of periderm and nemathybomes on the scapus and proximal end (fig. 8A, B). However, the resolution of 7.57 microns/voxel was not sufficient to observe the nemathybomes (e.g., fig. 8A) or the microcnemes at the base of tentacles in detail (fig. 8F–H). Similarly, though the general shape and morphology of retractors were easily

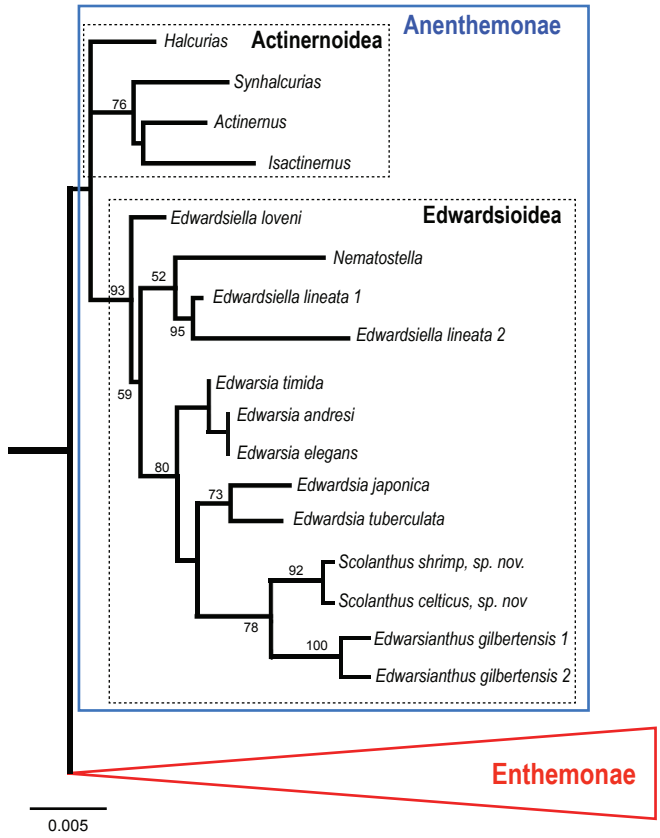

FIG. 10. Phylogenetic reconstruction from maximum likelihood analysis using focused dataset of 18S sequences for members of Suborder Anenthemonae. Colored lines represent actiniarian suborders; dashed boxes represent Anenthemonae superfamilies. Bootstrap resampling values indicated above branches; only support values >50% are shown.

established in the 2D scan images, it was not possible to determine the number and ramification of muscle processes in the retractor or parietal muscles. Although our results somewhat restrict the utility of micro-CT scanning for the study of edwardsiids as suggested by Gusmão et al. (2018), at least under the current resolution, we agree with Daly (2002) that a detailed exploration and description of morphological characters will likely help us resolve relationships among edwardsiid genera. As suggested by Daly (2002), this means that when possible, morphological features should be quantified and qualified in detail (e.g., muscle morphology, number and distribution of tentacles per cycle, number and distribution of microcnemes), and character complexes (e.g., presence and distribution of microcnemes on distal column) should be broken down with its constituents evaluated separately for phylogenetic informative potential. We should also strive for precise definition and application of terms that describe morphological features, especially among groups diagnosed by inaccurate and incomplete anatomical features, which affects most actiniarian genera. This would lead to a much-needed increase in the current insufficient number of diagnostic characters for most groups of sea anemones by clarifying the distribution,

homology, and phylogenetic informative potential of morphological features. This approach would benefit many actiniarian groups, but particularly those that are diverse and comprised of members distinguished by the absence or a mosaic of unique traits, such as Edwardsiidae.

Differential Diagnoses of *Scolanthus shrimp* and *S. celticus*

The current circumscription of *Scolanthus* is based on the morphological and molecular phylogenetic analysis of Daly (2002) and the revisionary work of Daly and Ljubenkov (2008), and it includes species formerly described in the genus *Isoedwardsia* (i.e., *S. curacaoensis*, *S. nidarosiensis*, *S. ingolfi*) and *Alfredus* Schmidt, 1979 (*Alfredus lucifugus* (Fischer, 1888), now a synonym of *S. callimorphus*) as well as some former *Edwardsia* species (*S. armatus*, *S. intermedius*). The presence of *p*-mastigophores A in *S. nidarosiensis* and *S. curacaoensis* raised doubts about whether these species belonged in *Scolanthus* (Manuel, 1981a, 1981b) as all *Scolanthus* spp. at the time lacked *p*-mastigophores A. Since then, five more species have been described with *p*-mastigophores A in their cnidom (e.g., *S. scamiti*: Daly and Ljubenkov, 2008; *S. ena* and *S. isei*: Izumi and Fujita, 2018; plus *S. shrimp* and *S. celticus*). Thus, from the current 16 species of *Scolanthus*, seven species have *p*-mastigophores A in their cnidae (no information is available for *S. intermedius*). Because *p*-mastigophores A were somewhat hard to observe in the two new species described here, its absence in other species might be due to challenges in observing rare cnidae in these small edwardsiids.

The combination of having 16 tentacles and four microcnemes of the first cycle (plus four of the second cycle) distinguishes *Scolanthus shrimp* and *S. celticus* from species with 20 tentacles and no microcnemes of the first cycle in the genus (i.e., *S. ignotus*, *S. ena*, *S. isei*). The size of basitrichs in the nemathybomes of both new species overlaps and distinguishes them from the other five species with 16 tentacles in the genus: *S. kopepe* (18.89–24.97 × 2.67–4.33 and 29.79–58.01 × 2.80–5.20: Izumi and Fujita, 2018), *S. nidarosiensis* (62.0–67.0 × 2.5–3.0: Carlgren, 1942), *S. curacaoensis* 38(43)–53 × 2.5(3): Carlgren, 1931), *S. triangulus* (63.8–89.8 × 4.0–5.4: Daly and Ljubenkov, 2008), *S. callimorphus* (60–90 × 3–5: Manuel, 1981a). Though the size of basitrichs in nemathybomes of *S. crypticus* overlaps in the lower range with those in both new species, additional differences include the nature of nemathybomes (small, inconspicuous, arranged in longitudinal rows) and small size and poor development of the retractor and parietal muscles in *S. crypticus*. Though no histological information was provided for the recently described *S. mediterraneus* (Ocaña and Çinar, 2018), the presence of retractors in only 6–7 macrocnemes was never observed in the new species; in addition, the lack of *p*-mastigophores A and presence of *b*-mastigophores in *S. mediterraneus* as well as its known distribution in shallow waters of the Sea of Marmara further differentiates this species from *S. shrimp* and *S. celticus*. The microanatomy of *S. armatus* with very diffuse retractors even at the actinopharynx level (see Izumi and Fujita, 2018) unequivocally differentiates this species from *S. shrimp* and *S. celticus*. In addition, details of cnidae (i.e., nonoverlapping size of basitrichs in tentacles and large basitrichs of actinopharynx) and geographic distribution (various localities in the Pacific Ocean) further differentiate this species from the two newly described ones. Differences in microanatomy (morphology and develop-

ment of retractor and parietal muscles) as well as cnidae (nonoverlapping size of *p*-mastigophores in filament) and geographic distribution (northeast Pacific) of *S. scamiti* further easily distinguishes this species from *S. shrimp* and *S. celticus*.

Scolanthus shrimp and *S. celticus* resemble *S. intermedius*, recorded from the Atlantic portion of the Southern Ocean (South Georgia), southeastern Pacific (Strait of Magellan, Chile), and Ushuaia (Tierra del Fuego, Argentina) (but see Williams, 1981, for a discussion about each population's specific identity), and *S. ingolfi* recorded from the North Atlantic (south of Iceland) in general anatomy and size of basitrichs in the nemathybomes. *Scolanthus shrimp* differs from *S. intermedius* and *S. ingolfi* in the nature of the periderm (thick in *S. ingolfi*; with foreign material attached in *S. intermedius*), the number of tentacles in *S. intermedius* (16–28 tentacles; 16 in *S. shrimp*), and the morphology of retractors (elongated, strong in both *S. ingolfi* and *S. intermedius*); moreover, the proportionally small, weak parietal of *S. intermedius* differs from the large, strong parietal of *S. shrimp*. Although the thick periderm of *S. celticus* is somewhat similar to that of *S. ingolfi* and *S. intermedius*, the rusty and adherent periderm on the former differs from the two other species in the Atlantic. In addition, the extremely diffuse nature of the retractor and the arrangement of nemathybomes in *S. intermedius* (single, never clustered) further differentiate it from *S. celticus*. The morphology of the retractor and parietal muscles in *S. ingolfi* plus details of the cnidae of this species (nonoverlapping size of basitrichs in actinopharynx, lack of *p*-mastigophores A) further differentiates it from *S. celticus*.

Scolanthus shrimp and *S. celticus* are easily differentiated from each other based on the morphology of nemathybomes, which are larger, always protruding into epidermis, and never clustered in the only available specimen of *S. shrimp* (fig. 3A-C) and smaller, predominantly sunken into mesoglea, protruding only slightly into epidermis in *S. celticus* (fig. 5B, C). In addition, microanatomical details, including the morphology of retractors (shorter, circumscribed, reniform, in *S. shrimp*: fig. 3F; elongated, stronger, diffuse-circumscribed in *S. celticus*: fig. 6H), cnidae differences (presence of *p*-mastigophores A only in *S. celticus*), and the nature of periderm (thin, adherent in *S. shrimp*.: fig. 2B, E; thick, deciduous with particles of sand/grains attached in *S. celticus*: fig. 5B) further differentiates the two new species.

The cooccurrence of *Scolanthus shrimp* and *S. celticus* in the Whittard Canyon is not uncommon and has been described for other pairs of edwardsiids such as *Scolanthus* and *Edwardsia* in western Ireland, northern England, and Singapore (Daly and Ljubenkov, 2008). Because molecular data are available for only two of the 16 valid species of *Scolanthus*, we cannot comment on the diversification of the group, but the distribution of taxa in several ocean basins suggests a complex diversification process as hypothesized for other edwardsiid genera (e.g., *Edwardsia*: Daly and Ljubenkov, 2008). *Scolanthus shrimp* and *S. celticus* represent the second and third species in the genus recorded for waters deeper than 1000 m, suggesting that the bathymetry of species in this genus is likely broader.

Though the North Atlantic is among the best-sampled ocean basins in the world (Lavender et al., 2005; Molodtsova et al., 2008) and one of the only for which we have a good estimation of distribution patterns for marine invertebrates (Fautin and Soberón, 2013), our description of two new species for the area indicate that the study of sea anemones, particularly burrowing anemo-

nes, might still reveal more diversity in the area. The study of marine ecosystems such as the Whittard Canyon, which covers over 5000 square kilometers, most of which is in depths below 1500 m, has revealed vulnerable cold-water scleractinians (Robert et al., 2014), deep-water oysters (Johnson et al., 2013) as well as remarkable diversity and abundance of rare black corals (Morris et al., 2013). Our study adds to this knowledge by describing burrowing sea anemone species that are usually undersampled due to their patchy distribution in sediment, which is not optimal for deep-sea sampling (Gusmão et al., 2019); similar to most sea anemone groups, burrowing anemones are also often underdescribed due to the necessity of histology for specific identification and backlog of new species descriptions, which makes their identification difficult for both specialists and nonspecialists.

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REFERENCES

Andres, A. 1881. Prodromus neapolitanae actinarium faunae addito generalis actiniarum bibliographiae catalogo. Mittheilungen aus der Zoologischen Station zu Neapel 2: 305–371.

Andres, A. 1883. Le Attinie. Atti dell'Accademia de Lincei 14: 211–673.

Brandão, R.A, L.C. Gusmão, and P.B. Gomes. 2019. Diversity of Edwardsiidae sea anemones (Cnidaria: Anthozoa: Actiniaria) from Brazil, with the description of a new genus and species. Journal of the Marine Biological Association of the United Kingdom: 1–12. [doi: 10.1017/S0025315419000109]

Carlgren, O. 1892. Beiträge zur Kenntnis der Edwardsien. Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar 1892: 451–461.

Carlgren, O. 1921. Actiniaria I. Danish Ingolf-Expedition 5: 1–241.

Carlgren, O. 1922 (for 1920). Actiniaria und Zoantharia von Juan Fernandez und der Osterinsel. *In* C. Skottsberg (editor), The Natural History of Juan Fernandez and Easter Island. Uppsala: Almquist & Wiksells Boktryckeri-A.-B.

Carlgren, O. 1931. Zur Kenntnis der Actiniaria Abasilaria. Arkiv für Zoologi 23: 1–48.

Carlgren, O. 1937. A new actinian. Smithsonian Miscellaneous Collections 91: 1–4.

Carlgren, O. 1940. A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. Kungliga Fysiografiska Sällskapets Handlingar 51: 1–62.

Carlgren, O. 1942. Actiniaria Part II. Danish Ingolf-Expedition 5: 1–92.

Carlgren, O. 1949. A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. Kungliga Svenska Vetenskapsakademiens Handlingar 1: 1–121.

Daly, M. 2002. A systematic revision of Edwardsiidae (Cnidaria: Anthozoa). Invertebrate Biology 121: 212–225.

Daly, M., and J.C. Ljubenkov. 2008. Edwardsiid sea anemones of California (Cnidaria: Actiniaria: Edwardsiidae), with descriptions of eight new species. Zootaxa 1860: 1–27.

Daly, M., D.L. Lipscomb, and M.W. Allard. 2002. A simple test: evaluating explanations for the relative simplicity of the Edwardsiidae (Cnidaria: Anthozoa). Evolution 56: 502–510.

Daly, M., A. Chaudhuri, L.C. Gusmão, and E. Rodríguez. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). Molecular Phylogenetics and Evolution 48: 292–301.

Daly, M., L.C. Gusmão, A.J. Reft, and E. Rodríguez. 2010. Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (Cnidaria, Actiniaria). Integrative and Comparative Biology 50: 371–388.

Daly, M, R. Perissinotto, M. Laird, D. Dyer, and A. Todaro. 2012. Description and ecology of a new species of *Edwardsia* de Quatrefages, 1842 (Anthozoa, Actiniaria) from the St Lucia Estuary, South Africa. Marine Biological Research 8: 233–245.

Daly, M., F. Rack, and R. Zook. 2013. *Edwardsiella andrillae*, a new species of sea anemone from Antarctic ice. PLoS ONE 8: 1–8.

Daly, M., et al. 2017. *Anthopleura* and phylogeny of Actinioidea (Cnidaria: Anthozoa: Actiniaria). Organisms. Diversity and Evolution 17: 545–564.

Darriba, D., G.L. Taboada, R. Diallo, and D. Posada. 2012. jModeltest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772.

England, K.W. 1987. Actiniaria from the Red Sea and tropical Indo-Pacific. Bulletin of the British Museum of Natural History 53: 205–292.

Fautin, D.G. 1988. The importance of nematocysts to actiniarian taxonomy. *In* D.A. Hessinger and H.M. Lenhoff (editors), The biology of nematocysts: 487–500. New York: Academic Press.

Fautin, D.G. 2016. Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). Zootaxa 4145: 1–449.

Fautin, D.G., and J. Soberón. 2013. Latitudinal diversity of sea anemones (Cnidaria: Actiniaria). Biological Bulletin 224, 89–98.

Fedorov, A., et al. 2012. 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magnetic Resonance Imaging 30: 1323–1341.

Fischer, P. 1888. Contribution à l'actinologie française. Archives de Zoologie Expérimentale et Générale 5: 381–442.

Geller, J.B., and E.D. Walton. 2001. Breaking up and getting together: evolution of symbiosis and cloning by fission in sea anemones (Genus *Anthopleura*). Evolution 55: 1781–1794.

Goloboff, P.A., J. Farris, and K. Nixon. 2008. TNT, a free program for phylogenetic analysis. Cladistics 24: 774–786.

Gosse, P.H. 1853. On new or little known marine animals (No. 2). Annals and Magazine of Natural History 12: 153–159.

Guindon, S., and O. Gascuel. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696–704.

Guindon, S, et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321.

Gusmão, L.C., and M. Daly. 2010. Evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. Molecular Phylogenetics and Evolution 56: 868–877.

Gusmão, L.C., R.A. Brandao, and M. Daly. 2016. *Edwardsia migottoi* sp. nov., the first sea anemone species of Edwardsia de Quatrefages 1842 (Anthozoa: Actiniaria: Edwardsiidae) from the Southwestern Atlantic. Marine Biodiversity 48: 1–11.

Gusmão, L.C., A. Grajales, and E. Rodríguez. 2018. Sea anemones through X-rays: visualization of two species of *Diadumene* (Cnidaria, Actiniaria) using micro-CT. American Museum Novitates 3907: 1–45.

Gusmão, L.C., L. Berniker, V. Van Deusen, and E. Rodríguez. 2019. Halcampulactidae (Actiniaria, Actinostoloidea), a new family of burrowing sea anemones with external brooding from Antarctica. Polar Biology 42 (7): 1271–1286. [doi.:10.1007/s00300-019-02516-1]

Hertwig, R. 1882. Die Actinien der *Challenger* Expedition. Jena, Germany: Gustav Fischer.

Izumi, T., and T. Fujita. 2018. Description of three new species of *Scolanthus* (Cnidaria, Anthozoa, Actiniaria, Edwardsiidae): first records of the genus from Japan. Zookeys 794: 1–21.

Johnson, M.P., et al. 2013. A vertical wall dominated by *Acesta excavata* and *Neopycnodonte zibrowii,* part of an undersampled group of deep-sea habitats. PLoS ONE 8: e79917.

Katoh, K., and D.M. Standley. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30: 772–780.

Katoh, K., J. Rozewicki, and K.D. Yamada. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief in Bioinformatics. [doi:10.1093/bib/bbx108]

Kearse, M., et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649.

Kozlov, A.M., D. Darriba, T. Flouri, B. Morel, and A. Stamatakis. 2018. RAxML-NG: a fast scalable, and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics: btz305. [doi:10.1101/447110]

Lauretta, D., V. Häussermann, M.R. Brugler, and E. Rodríguez. 2014. *Isoparactis fionae* sp. n. (Cnidaria: Anthozoa: Actiniaria) from Southern Patagonia with a discussion of the family Isanthidae. Organisms Diversity and Evolution 14: 31–42.

Lavender, K.L., W.B. Owens, and R.E. Davis. 2005. The mid-depth circulation of the subpolar North Atlantic Ocean as measured by subsurface floats. Deep Sea Research, part I: Oceanographic Research Papers 52: 767–785.

Lefort, V., J. Longueville, and O. Gascuel. 2017. SMS: Smart Model Selection in PhyML. Molecular Biology and Evolution 34: 24221–2424.

Manuel, R.L. 1981a. On the identity of the sea anemone *Scolanthus callimorphus* Gosse, 1853 (Actiniaria: Edwardsiidae). Journal of Natural History 15:265–276 .

Manuel, R.L. 1981b. British Anthozoa: Synopses of the British Anthozoa. London: Academic Press.

McMurrich, J.P. 1893. Report on the Actiniae collected by the United States Fish Commission Steamer Albatross during the winter of 1887–1888. Proceedings of the United States National Museum 16: 119–216.

Miller, M.A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE): 1–8.

Molodtsova, T.N., N.P. Sanamyan, and N.B. Keller. 2008. Anthozoa from the northern Mid-Atlantic Ridge and Charlie-Gibbs Fracture Zone. Marine Biology Research 4: 112–130.

Morris, K.J., R.A. Tyler, D.G. Masson, V.I.A. Huvenne, and A. Rogers. 2013. Distribution of cold water corals in the Whittard Canyon NE Atlantic. Deep Sea Research II 92: 136–144.

Nordgaard, O. 1905. Hydrographical and biological investigations in Norwegian fiords. Bergen: John Grieg.

Ocaña. O., and M.E. Çinar. 2018. Descriptions of two new genera, six new species and three new records of Anthozoa (Cnidaria) from the Sea of Marmara. Journal of Natural History 52: 2243–2282.

Pax, F. 1924. Anthozoen des Leidener Museums. Zoologische Mededelingen, Leiden 8: 1–17.

Presnell, J.K., and M.P. Schreibman. 1997. Humason's animal tissue techniques. Baltimore, MD: Johns Hopkins University Press.

Quatrefages, A. de. 1842. Memoire sur les Edwardsies. Annales des Sciences Naturelles, Zoologie 18: 65–109.

Robert, K., Jones, D.O.B., Tyler, P.A., Van Rooij, D., and V.A.I. Huvenne. 2014. Finding the hotspots within a biodiversity hotspot: fine-scale biological predictions within a submarine canyon using high-resolution acoustic mapping techniques. Marine Ecology 36: 1256–1276.

Rodríguez, E., M. Barbeitos, M. Daly, L.C. Gusmão, and V. Häussermann. 2012. Toward a natural classification: phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). Cladistics 1: 1–18.

Rodríguez, E., et al. 2014. Hidden among sea anemones: first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria: Anthozoa: Hexacorallia) reveals a novel group of hexacorals. PLoS ONE 9: 1–15.

Sanamyan, N., and K. Sanamyan. 2013. *Edwardsia sojabio* sp. n. (Cnidaria: Anthozoa: Actiniaria: Edwardsiidae), a new abyssal sea anemone from the Sea of Japan. Deep Sea Research II 86–87: 225–230.

Sanamyan, N.P., K.E. Sanamyan, and D. Schories. 2015. Shallow water Actiniaria and Corallimorpharia (Cnidaria: Anthozoa) from King George Island, Antarctica. Invertebrate Zoology 12: 1–51.

Schmidt, H. 1969. Die Nesselkapseln der Aktinien und ihre differentialdiagnostische Bedeutung. Helgoländer Wissenschaftliche Meeresuntersuchungen 19: 284–317.

Schmidt, H. 1972. Die Nesselkapseln der Anthozoen und ihre Bedeutung fur die phylogenetische Systematik. Helgoländer Wissenschaftliche Meeresuntersuchungen 23: 422–458.

Schmidt, H. 1974. On evolution in the Anthozoa. Proceedings of the Second International Coral Reef Symposium 1: 533–560.

Schmidt, H. 1979. Beitrage zur differentialdiagnose, morphologie und evolution der Edwardsiidae (Actiniaria, Anthozoa). Zeitschrift fur Zoologische Systematik und Evolutionsforschung 17: 211–220.

Stephenson, T.A. 1935. The British sea anemones. Vol. 2. London: Ray Society.

Titus, B.M., et al. 2019. Phylogenetic relationships among the clownfish-hosting sea anemones. Molecular Phylogenetics and Evolution 139: 106526. [10.1016/j.ympev.2019.106526]

Verrill, A.E. 1922. The Actiniaria of the Canadian Arctic Expeditions, with notes on interesting species from Hudson Bay and other Canadian localities. Report on the Canadian Arctic Expedition 1913–1918 8: 89–164.

Weill, R. 1934. Contribution à l'étude des cnidaires et de leurs nématocystes. Paris: Les Presses Universitaires de France.

Williams, R.B. 1981. A sea anemone, *Edwardsia meridionalis* sp. nov., from Antarctica and a preliminary revision of the genus *Edwardsia* de Quatrefages, 1841 (Coelenterata: Actiniaria). Records of the Australian Museum 33: 325–360.

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