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Morphological phylogeny of the family Actinostolidae (Anthozoa : Actiniaria) with description of a new genus and species of hydrothermal vent sea anemone redefining the family Actinoscyphiidae

Estefanía Rodríguez[A,B], *Christopher N. Castorani*[A] *and Marymegan Daly*[A]

[A]Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, OH 43212, USA.

[B]Corresponding author. Email: fani@us.es

Abstract. We describe a new genus and species of hydrothermal vent sea anemone from the eastern north Pacific Ocean. The combination of characters in *Alvinactis reu*, gen. et sp. nov. is unique among currently known genera of the actiniarian superfamily Mesomyaria; most notable among its external features is a belt of verrucae and cinclides in the distal column. We assess the placement of *Alvinactis*, gen. nov. and evaluate taxonomic features used to distinguish groups within Actinostolidae Carlgren, 1893 and Actinoscyphiidae Stephenson, 1920 with a cladistic analysis of morphological characters. Phylogenetic analysis reveals that *Alvinactis*, gen. nov. and several genera previously ascribed to Actinostolidae belong in Actinoscyphiidae. Morphological evidence fails to support monophyly of Actinostolidae, but does support monophyly of the previously proposed subfamily Actinostolinae.

Additional keywords: Actinoscyphiidae, Cnidaria, deep-sea diversity, hydrothermal vents, Pacific Ocean.

Introduction

Sea anemones attributed to the family Actinostolidae Carlgren, 1893 dominate in the deep sea and polar waters (Carlgren 1949; Fautin and Barber 1999) and at hydrothermal vents (López-González and Segonzac 2006). The majority of the genera currently placed in it are monotypic (Fautin 2007), suggesting that the taxonomic characters traditionally used to differentiate genera need to be re-assessed. The descriptions of several new monotypic genera in recent decades (Doumenc and Van-Präet 1988; Fautin and Hessler 1989; Fautin and Barber 1999; López-González *et al*. 2003, 2005) demonstrate the difficulty of accommodating new taxa in narrowly defined existing groups, and further argue for a re-evaluation of the family. Furthermore, a synthetic, phylogenetic assessment of Actinostolidae would clarify the relationship between the monotypic genera and large, heterogeneous groups such as the type genus, *Actinostola* Verrill, 1883. However, such an assessment is difficult because the family is likely to comprise a paraphyletic grade or a polyphyletic assemblage rather than a monophyletic group.

Actinostolidae has a long and complex taxonomic history (Table 1). Several members of Actinostolidae were first grouped together by Hertwig (1882: p. 41) in the family Paractidae, which he defined as comprising 'Hexactiniae with numerous perfect septa and with very contractile moderately long tentacles, which can be completely covered; circular muscle very strong, mesodermal'. In this family, he included *Antholoba* Hertwig, 1882; *Dysactis* Milne Edwards, 1857; *Ophiodiscus* Hertwig, 1882; *Tealidium* Hertwig, 1882 and taxa no longer considered valid, such as its type genus *Paractis* Milne Edwards & Haime, 1851. Andres (1883) used the name Paractidae for a subfamily of his Actininae, and placed in this group *Paranthus* Andres, 1883; *Paractinia* Andres, 1883 and *Paractis*. Hertwig's (1882) use of the name has priority.

Carlgren (1893) redefined Paractidae and transferred its previous diagnosis to a new family, Actinostolidae, into which he placed *Actinostola* and *Stomphia* Gosse, 1859. Carlgren (1893: p. 64) defined Actinostolidae as: 'Actiniaria with pedal disc, with very contractile and moderate long tentacles and usually numerous perfect mesenteries. Pairs of mesenteries of the last cycles (third and forth cycles) irregularly developed, so the mesentery, which retractor muscles are facing the next cycle, is more developed than the other. Radial muscles of oral disc and longitudinal tentacle muscles generally mesogleal. Sphincter mesogleal usually well developed. No acontia or cinclides'. Carlgren (1893: p. 137) also provided a new diagnosis for Paractidae: 'Actiniaria with pedal disc, with moderate long tentacles and usually numerous perfect mesenteries. Mesenteries of the same pair regularly developed. Radial muscles of oral disc and longitudinal tentacle muscles generally mesogleal. Sphincter mesogleal usually well developed. No acontia or cinclides'. His distinction between the two was based on the development of pairs of mesenteries: in Actinostolidae, the two members of a pair are not identical in size and morphology; in Paractidae, the two members of a pair are identical. Carlgren (1899) subsequently reclassified

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Actinostolidae and Paractidae as subfamilies of the family Paractidae, later adding a third subfamily, Polysiphoniinae Carlgren, 1918. Polysiphoniinae was later removed from Paractidae and reclassified as Exocoelactidae Carlgren, 1925.

Although he used Carlgren's subfamilies, Stephenson (1921) was not sure that the distinctions between them were clear, and did not think that any of them merited the rank of family. In particular, Stephenson (1921) considered Actinostolinae and Paractinae a single, difficult to subdivide group. Carlgren (1927) was unable to determine a valid diagnosis for the type genus *Paractis*, and later (Carlgren 1932) resurrected the family name Actinostolidae for some members of Paractidae.

Recent works by Riemann-Zürneck (1978*a*) and Fautin and Hessler (1989) changed the definition of the family and reconsidered some features used to differentiate its members. Riemann-Zürneck (1978*a*) revised the mesomyarian family Actinoscyphiidae Stephenson, 1920, clarifying the distinctions between this group and Actinostolidae. Fautin and Hessler (1989) amended Carlgren's (1949) key to the genera of Actinostolidae, correcting his errors and incorporating new species. In their revised key, Fautin and Hessler (1989) omitted *Cyananthea* Doumenc & Van Praët, 1988 because the sole account of its type species was too fragmentary to evaluate many of the critical features. This genus has been recently redescribed and placed in the family Actinoscyphiidae based on its cnidom (Sanamyan and Sanamyan 2007). This redescription of *Cyananthea* highlights the confusion that remains about the circumscription of Actinoscyphiidae and Actinostolidae: Sanamyan and Sanamyan (2007) point out that additional genera, which had been described in Actinostolidae, are likely to be more appropriately placed in Actinoscyphiidae, but they failed fully to address this issue or formally re-assign the genera.

We describe *Alvinactis reu*, gen. et sp. nov. from the East Pacific Rise of the north Pacific Ocean. This new genus has a mesogleal sphincter and lacks acontia, and thus belongs to Mesomyaria. To assess the distinctiveness of *Alvinactis*, gen. nov. and to evaluate whether it belongs to Actinostolidae or Actinoscyphiidae, we generated a data matrix of morphological features of genera of Actinostolidae and Actinoscyphiidae. Although morphological attributes may be subject to convergence, preservation artefacts, or other sources of systematic error, these are the only data available for many of these taxa, because most are known only from formalin-fixed museum material. Phylogenetic analysis of this matrix is used to explore the consistency and information content of various taxonomic features used in classification of Actinostolidae and Actinoscyphiidae, to test the monophyly of each family, and to identify potentially monophyletic groups within Actinostolidae. This is the first cladistic analysis for members of the actiniarian superfamily Mesomyaria.

Materials and methods

Specimens were collected during a cruise of the Woods Hole Oceanographic Institution research vessel 'Atlantis' using the Deep Submergence Vessel 'Alvin'. All specimens came from one

Table 1. Synthesis of the taxonomic history of the families Actinostolidae and Actinoscyphiidae

Names are written as given in the original publication, with current valid names given in brackets. First use of suprageneric names in bold

Year	Author	Family	Subfamilies	Genera (when given)
1882	Hertwig	**Paractidae**		*Antholoba*, *Dysactis*, *Ophiodiscus*, *Paractis*, *Tealidium*
1883	Andres	Actininae	Paractidae	*Paranthus*, *Paractis*, *Paractinia*
1893	McMurrich	Paractidae		*Actinernus* [*Actinoscyphia*], *Actinostola*, *Antholoba*, *Cymbactis* [*Paractinostola*], *Ophiodiscus*, *Paractis*, *Paranthus*, *Paractinia*, *Pycnanthus*, *Tealidium*
1893	Carlgren	Paractidae		*Antholoba*, *Kadosactis*, *Kyathactis* [*Actinostola*], *Ophiodiscus*, *Paractinia*, *Paractis*, *Paranthus*, *Tealidium*
		Actinostolidae		*Actinostola*, *Stomphia*
1899	Carlgren	Paractidae	**Actinostolinae**	*Actinostola*, *Stomphia*
			Paractinae	*Antholoba*, *Ophiodiscus*, *Paractis*, *Paranthus*, *Tealidium*
1918	Carlgren	Paractidae	Paractinae	
			Actinostolinae	
			Polysiphoniidae (Exocoelactiidae)	*Polysiphonia* [*Exocoelactis*]
1920	Stephenson	**Actinoscyphiidae**		*Actinoscyphia*, *Paranthus*, *Isoparactis* [*Acraspedanthus*], *Lilliella* [*Hormathia*]?
1921	Stephenson	Paractidae	Paractinae, Actinostolinae, Polysiphoniinae	*Actinostola*, *Antholoba*, *Anthosactis*, *Hormosoma*, *Exocoelactis*, *Ophiodiscus*, *Paractis*, [*Cnidanthus*, *Paractinostola*, *Parasycionis*, *Pycnanthus*, *Stomphia*, *Sycionis*], *Paranthus*, *Tealidium*
1932	Carlgren	Actinostolidae		*Actinostola*, *Anthosactis*, *Pycnanthus*, *Stomphia*
1949	Carlgren	Actinostolidae		*Actinostola*, *Actinoscyphia*, *Antholoba*, *Anthosactis*, *Antiparactis*, *Bathydactylus*, *Cnidanthus*, *Isoparactis*, *Epiparactis*, *Hormosoma*, *Ophiodiscus*, *Paractinostola*, *Paranthus*, *Parasicyonis*, *Pseudoparactis*, *Pycnanthus*, *Sicyonis*, *Stomphia*, *Synsicyonis*, *Tealidium*
1978	Riemann-Zürneck	Actinoscyphiidae		*Actinoscyphia*, *Epiparactis*
2007	Sanamyan and Sanamyan	Actinoscyphiidae		*Actinoscyphia*, *Cyananthea*, *Epiparactis*, *Marianactis*?, *Pacmanactis*?

collection during dive 3941, on 26 November 2003, in the north Pacific Ocean: East Pacific Rise, 12°42.680′N, 103°54.462′W, depth 2600 m. Specimens were collected using Alvin's manipulator arm; at the surface, specimens were placed in chilled water and allowed to relax before being anaesthetised with isotonic magnesium chloride. Pieces of some specimens were fixed immediately in 95% ethanol. The remaining specimens were fixed in 10% seawater formalin and later transferred to 70% ethanol for long term storage. All specimens were deposited at the Field Museum of Natural History (FMNH).

Preserved specimens were examined whole, in dissection, and as serial sections. Serial sections were prepared using standard paraffin techniques. Histological slides were stained in Masson's trichrome (Presnell and Schreibman 1997). Small pieces of tissue from tentacles, column, pedal disc, mesenterial filaments, and actinopharynx were smeared on a slide; nematocysts in these smears were examined using DIC at 1000× magnification. Cnidae terminology follows Mariscal (1974).

The phylogenetic analysis of genera of Actinostolidae is based on a matrix of characters scored from direct observation or descriptions of type species. The characters are those traditionally used to recognise taxa within Actinostolidae, including those features identified by Carlgren (1949) in his key to the family. Some of these features (e.g. bathymetric range, habitat) are not strictly morphological, but can be interpreted as proxies for physiological attributes. All characters are treated as unordered and weighted equally. Outgroups include four genera classified in more distant groups: the endomyarian *Epiactis* Verrill, 1869 and the acontiarians *Bathyphellia* Carlgren, 1932; *Hormathia* Gosse, 1859 and *Kadosactis* Danielssen, 1890. These species span the diversity of Actiniaria and thus provide a strong test of monophyly of Actinostolidae. We include the mesomyarian *Actinoscyphia* Stephenson, 1920 because it was once included in Actinostolidae (Table 1), and because several taxa originally assigned to Actinostolidae have been hypothesised to be closely related to this genus (Riemann-Zürneck 1978*a*; Sanamyan and Sanamyan 2007). The character states attributed to the generic exemplars in the analysis were evaluated from direct observation or literature reports of type species, except in the case of *Bathydactylus* Carlgren, 1928. We considered *Bathydactylus krogni* Carlgren, 1956, rather than *Bathydactylus valdiviae* Carlgren, 1928, because the type species of the genus is known only from a single, poorly-preserved specimen. We included three species of *Anthosactis* Danielssen, 1890 because the great heterogeneity of the genus (White *et al.* 1999; Daly and Gusmão 2007) raises concern that the group is not monophyletic. Riemann-Zürneck (1978*b*) synonymised *Paractinostola* Carlgren, 1928 with *Actinostola*, but recognised that the latter was likely to be a paraphyletic group. We have included the type species of the former *Paractinostola*, *Paractinostola bulbosa* Carlgren, 1928 in recognition of the heterogeneity in *Actinostola*. The initial assessment of nematocyst types in the tentacles of *Paranthosactis* was equivocal (López-González *et al.* 2003). Upon reconsideration of their material and photographs, we find that the nematocysts called microbasic *b*-mastigophores by López-González *et al.* (2003) are holotrichs similar in size and morphology to those seen in the tentacles of *Alvinactis*, gen. nov. Other comparative material examined includes: *Marianatis bythios* Fautin & Hessler, 1989, deposited at the US National Museum of Natural History (USNM 84401, 84402); *Bathydactylus krogni* and *Epiparactis dubia* Carlgren, 1928, deposited at Zoological Museum in Copenhagen, and *Anthosactis pearseae* Daly & Gusmão, 2007, deposited at the California Academy of Sciences (CAS 174323–174325) and the US National Museum of Natural History (USNM 1096705, 1096706).

The final matrix of 41 characters (Appendix 1 and 2) was analysed in NONA (Goloboff 1999), using Winclada (Nixon 1999) to initiate 50 rounds of TBR branch swapping. Further rounds of swapping were not recommended by the results of the initial searches. We present the strict consensus of the equally parsimonious trees with Bremer support (Bremer 1994) calculated for all clades appearing in the consensus. The character optimisations discussed are those features that can be placed unambiguously at a particular node. Numbers in the text, on Fig. 1, and in Appendix 2 refer to the characters of Appendix 1.

Carlgren (1949) used the ranks 'tribe' and 'subtribe' to refer to groups between suborders and families. We have corrected this misapplication of ranks in our treatment of the taxonomy of *Alvinactis reu*, gen. et sp. nov. We have based our diagnoses of higher taxa on those of Carlgren (1949) and Riemann-Zürneck (1978*a*), altering them to be parallel and telegraphic; more substantive changes are indicated in italics.

Results

Phylogenetic analysis recovered 22 trees of length (L) = 166 (*CI* = 0.30, *RI* = 0.59). The strict consensus of these (Fig. 1) includes two main clades. One of these is a large clade that includes *Actinostola*, *Antholoba*, *Anthosactis janmayeni* Danielssen, 1890; *Cnidanthus* Carlgren, 1927; *Hormosoma* Stephenson, 1918; *Ophiodiscus*, *Paractinostola*, *Parasicyonis* Carlgren, 1921; *Pycnanthus* McMurrich, 1893; *Sicyonis* Hertwig, 1882; *Stomphia*, *Synsicyonis* Carlgren, 1921; and *Tealidium*; this roughly corresponds to Carlgren's subfamily Actinostolinae. Henceforth, we refer to this clade as 'Actinostolina'.

The other main clade includes the remaining genera previously attributed to Actinostolidae, *Actinoscyphia*, and the outgroups *Bathyphellia*, *Hormathia* and *Kadosactis*, which nest among members of Actinostolidae. This clade comprises two smaller clades: one includes the acontiate outgroups together with *Bathydactylus* and *Hadalanthus* Carlgren, 1956; the second includes *Actinoscyphia*, *Epiparactis* Carlgren, 1921, and the taxa from chemosynthetic habitats (Fig. 1). The membership of this second clade corresponds closely to Actinoscyphiidae *sensu* Sanamyan and Sanamyan (2007); we refer these taxa to this family. All taxa from hydrothermal vents and cold seeps (*Alvinactis*, gen. nov., *Cyananthea*, *Maractis*, *Marianactis*, *Pacmanactis* López-González, Rodríguez & Segonzac, 2005 and *Paranthosactis*) form a clade without consistent internal resolution. This chemosynthetic habitat clade, hereafter called Chemosynthina, is strongly supported by six morphological characters (#s 5, 6, 9, 17, 26, 31) and two additional ones referring to the habitat (#s 38, 39). The three species of *Anthosactis* do not group together.

Taxonomy

Order **ACTINIARIA** Hertwig, 1882

Suborder **NYNANTHEAE** Carlgren, 1899

Superfamily **MESOMYARIA** Stephenson, 1921

Family **ACTINOSTOLIDAE** Carlgren, 1893

Included genera

Actinostola; *Antholoba*; *Anthosactis*; *Antiparactis* Verrill, 1899; *Bathydactylus*; *Cnidanthea* Carlgren, 1956; *Cnidanthus*; *Hadalanthus*; *Hormosoma*; *Ophiodiscus*; *Paranthus*; *Parasicyonis*; *Pseudoparactis* Stephenson, 1920; *Pycnanthus*; *Sicyonis*; *Stomphia*; *Synsicyonis* and *Tealidium.*

Diagnosis

Nynantheae with basilar muscles and mesogleal marginal sphincter; column commonly smooth, rarely tuberculate or with papillae. Tentacles regularly arranged; their aboral sides sometimes with nematocysts batteries, sometimes thickened. Mesenteries not divisible into macro- and micro-cnemes. Younger mesenteries not bilaterally arranged. Retractor muscles diffuse, rarely circumscribed. No acontia. Cnidom: *Gracile spirocysts*, *basitrichs*, *and microbasic b- and p-mastigophores*. (Modified from Carlgren 1949.)

Remarks

Carlgren (1949) listed the authorship of Actinostolidae as Carlgren, 1932 but the family was erected by him in 1893 (Table 1).

Family **ACTINOSCYPHIIDAE** Stephenson, 1920

Included genera

Actinoscyphia; *Alvinactis*, gen. nov.; *Cyananthea*; *Epiparactis*; *Maractis* Fautin & Barber, 1999; *Marianactis* Fautin & Hessler, 1989; *Pacmanactis* and *Paranthosactis*.

Diagnosis

Nynantheae with basilar muscles and mesogleal marginal sphincter. Pedal disc *flat*, sometimes small, grasping. Column commonly smooth, *often with distal row of cinclides and sometimes verrucae*. Tentacles usually marginal on wide oral disc, their aboral sides sometimes thickened. Oral disc sometimes lobed. *Mesenteries not divisible into macro- and micro-cnemes*. At least six pairs of perfect and fertile mesenteries. Retractor muscles diffuse and weak. Longitudinal muscles of the tentacles ectodermal. No acontia. Cnidom: *Robust and gracile* spirocysts, basitrichs, *holotrichs* and microbasic *p*-mastigophores. (Modified from Riemann-Zürneck 1978*a*.)

Remarks

Riemann-Zürneck (1978*a*) resurrected Actinoscyphiidae primarily based on Schmidt's (1969, 1972) classification of types of cnidae. Thus, Riemann-Zürneck (1978*a*) characterises Actinoscyphiidae as having '*p*-rhabdoids B' and lacking '*p*-rhabdoids A'. Schmidt's (1972) distinction between the categories '*p*-rhabdoids A/*p*-rhabdoids B' roughly corresponds with Mariscal's (1974) distinction between 'microbasic *p*-mastigophores/amastigophores'. Nomenclature issues aside, although these types are certainly different in ultrastructure (shaft and tubule spination), accurate recognition of their distinctiveness requires observing them in a discharged state under scanning electron microscopy (SEM). To use all of Schmidt's subdivisions of *p*-mastigophores it is necessary to observe the fine details of spine length, density and angle of attachment that are important characters in this system (England 1991; Östman 2000). Using the ultrastructure of *p*-mastigophores, Schmidt (1972, 1974) grouped mesomyarian families into 'Early' and 'Late' Mesomyaria. However, his distinction was based on examination of relatively few species; these types of nematocysts have not been distinguished for most of the genera. Furthermore, many actiniarian families are polyphyletic (Daly *et al.* 2008), making combining them into groups especially problematic. The phylogenetic interpretation of morphological differences among nematocyst types is not clear. Because molecular evidence does not support Schmidt's (1972, 1974) distinction between 'Early' and 'Late' Mesomyaria (Daly *et al.* 2008), attributing high phylogentic significance to the distinction between A or B *p*-mastigophores may be unwarranted. Given the current lack of clarity about the generality and applicability of this character to many taxa, and its dubious value as a phylogenetic feature, we prefer not to include these differences in the definition of the families.

Genus ***Alvinactis***, gen. nov.

Types species

Alvinactis reu, sp. nov.

Diagnosis

Pedal disc well developed. Column smooth, not divisible into scapus and scapulus, with distal row of verrucae and cinclides. Distal margin of column distinctly marked as marginal ring. Tentacles of uniform thickness along entire length, those of inner cycle longer than those of outer cycle. Longitudinal muscles of tentacles ectodermal, equally developed. Mesenteries arranged in four cycles, only first cycle perfect. Same number of mesenteries proximally and distally. All mesenteries except those of youngest cycle fertile. Two well developed siphonoglyphs each attached to pair of directives. Retractor muscles diffuse; parietobasilar muscles not differentiated. Cnidom: robust and gracile spirocysts, basitrichs, holotrichs, microbasic *p*-mastigophores.

Etymology

The name *Alvinactis* combines the name of the submersible 'Alvin' and '-actis' a common suffix for actiniarians, referring to their rayed or star-like external morphology.

Alvinactis reu, sp. nov.

(Figs 2–5, Table 2)

Material examined

Holotype. North Pacific Ocean: East Pacific Rise, 12°42.680′N, 103°54.462′W, 2600 m, 'Alvin' dive 3941, 26.xi.2003, J. Voight (FMNH 13976).

Paratypes. 3, data as for holotype (FMNH 11504).

Diagnosis

Column of preserved specimens cylindrical, not divisible into scapus and scapulus, with more or less distinct marginal ring. Column smooth except for distal belt of small, round, perforate verrucae. Mesenteries hexamerously arranged in four cycles, all larger ones fertile, only those of first cycle perfect. Tentacles with numerous spirocysts and basitrichs; holotrichs in tips of tentacles of most specimens. Pedal disc diameter 14–59 mm, column height 6–34 mm (contracted and preserved specimens).

Description

Base and column

Column stout, of approximately equal diameter throughout in preserved specimens, encircled by distal belt of 24 small, hollow outgrowths of all three layers of column, perforate verrucae (Figs 2*B*, *D*; 4*D*, *E*). Verrucae inside crease beneath sphincter, associated with endocoelic spaces of stronger mesenteries, likely adherent. No fosse, although distal edge of column may extend over base of tentacles in contracted specimens (Figs 2*A*, *B*). Column of preserved specimens uniform brownish-pink. In life, column trumpet-shaped, flaring slightly from base (Fig. 3); column, tentacles and oral disc of living specimens uniform translucent grayish-green. Strong columnar circular musculature and mesogleal sphincter; sphincter spans distal quarter of column, reticulated, lies closer to epidermis than gastrodermis, tapers more distally than proximally (Fig. 4*E*). Mesoglea of distal column with small, darkly-staining inclusions; these are especially abundant near marginal sphincter.

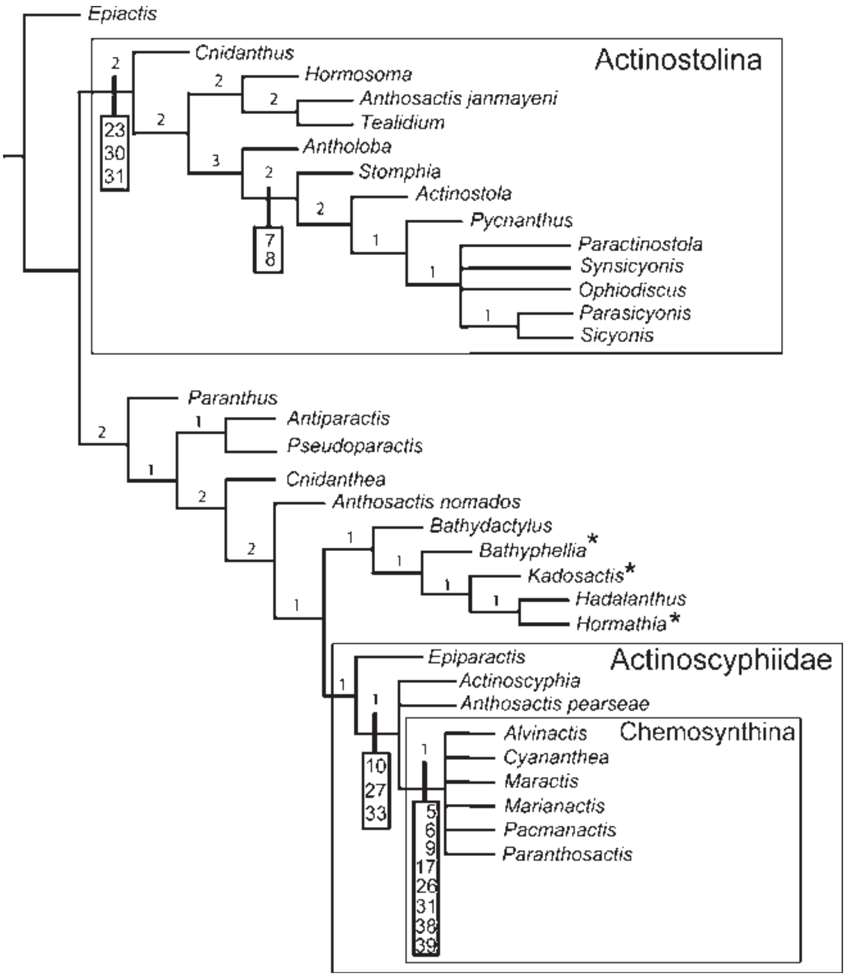

Fig. 1. Strict consensus of 22 equally parsimonious trees (L = 167, *CI* = 0.29, *RI* = 0.59) recovered from analysis of morphological data (Appendix 2). Numbers above the branches are Bremer support values. Characters supporting Actinostolina, Actinoscyphiidae and Chemosynthina are indicated; numbers refer to Appendix 1.

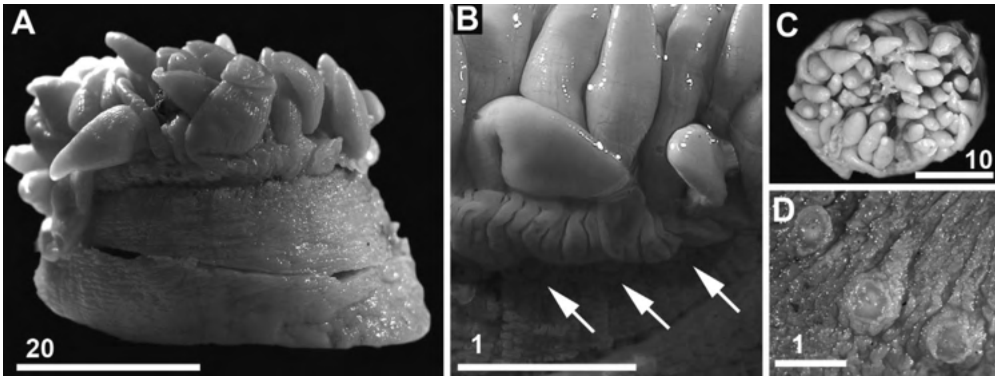

Fig. 2. External anatomy, preserved specimens *Alvinactis reu*, gen. et sp. nov. Scale in mm. *A*, lateral view; *B*, close-up of column margin (note verrucae inside crease in distal column (arrows)); *C*, top view; *D*, perforate verrucae.

Base flat or slightly withdrawn inside column. Pedal disc adherent, muscular, same colour as column in preserved material, approximately equal or slightly wider in diameter than oral disc in preserved specimens (Fig. 2*A*). Pedal disc circular in smaller specimens; oval in largest specimen.

Oral disc and tentacles

Tentacles marginal, ~100 in five cycles; those of outer cycle markedly shorter; those of inner cycles obscure oral disc in contracted specimens (Figs 2*A*, *B*). Specimens with more than 96 tentacles do not have additional mesenteries, suggesting tentacle regeneration rather than additional cycles of mesenteries at distal column. Tips of tentacles perforated. Inner tentacles moderate in length, to 21 mm long, longitudinally sulcated in preserved specimens (Fig. 2*B*). In life, tentacles conical, approximately equal in length or longer than column. Oral disc flat, mouth oval; two prominent siphonoglyphs. Tentacles, oral disc, lips, actinopharynx and siphonoglyphs same colour as column.

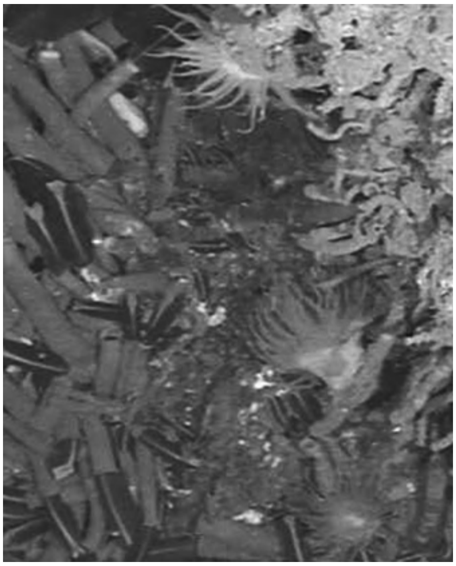

Fig. 3. External anatomy and habitat, living *Alvinactis reu*, gen. et sp. nov.

Mesenteries and internal anatomy

Mesenteries arranged hexamerously in four cycles, those of first cycle perfect; two pairs of directives, each attached to a well developed siphonoglyph. All mesenteries of first, second and third cycles (including directives) bear filaments and gametogenic tissue; those of fourth cycle weak, lacking filaments and gametogenic tissue (Fig. 4*C*). Species gonochoric; all specimens collected in late November sexually mature, with either oocytes or spermatic vesicles (48–234 µm and 31–120 µm in diameter, respectively; Figs 4*B*, *G*).

Longitudinal muscles of mesenteries diffuse (Figs 4*G*, *H*). Pennon of parietobasilar muscles not differentiated (Fig. 4*H*). Basilar muscles present, equally developed (Fig. 4*A*).

Cnidom

Robust and gracile spirocysts, basitrichs, holotrichs, microbasic *p*-mastigophores (Fig. 5). See Table 2 for size and distribution.

Habitat and biology

All specimens living on and among oxidised clumps of the tubeworm *Tevnia* (Fig. 3). Multiple individuals co-occur on single clump, but specimens typically not close enough to touch one another.

Etymology

The specific name ‘*reu*’ honors the NSF Research Experience for Undergraduates program, which supported CNC’s participation in this project. The species epithet should be considered an undeclinable Latin noun.

Discussion

Comparison of Alvinactis reu with other genera

As is true of other sea anemones described from chemosynthetic environments (e.g. Fautin and Hessler 1989; Fautin and Barber 1999; López-González *et al.* 2003, 2005), *Alvinactis* presents an unusual combination of characters that make it incompatible with the diagnoses of other genera. It has four cycles of mesenteries; although only those of the first cycle are perfect, all of the larger mesenteries are fertile. It has a belt of perforate verrucae encircling the distal column, and a strong circumferential marginal ring.

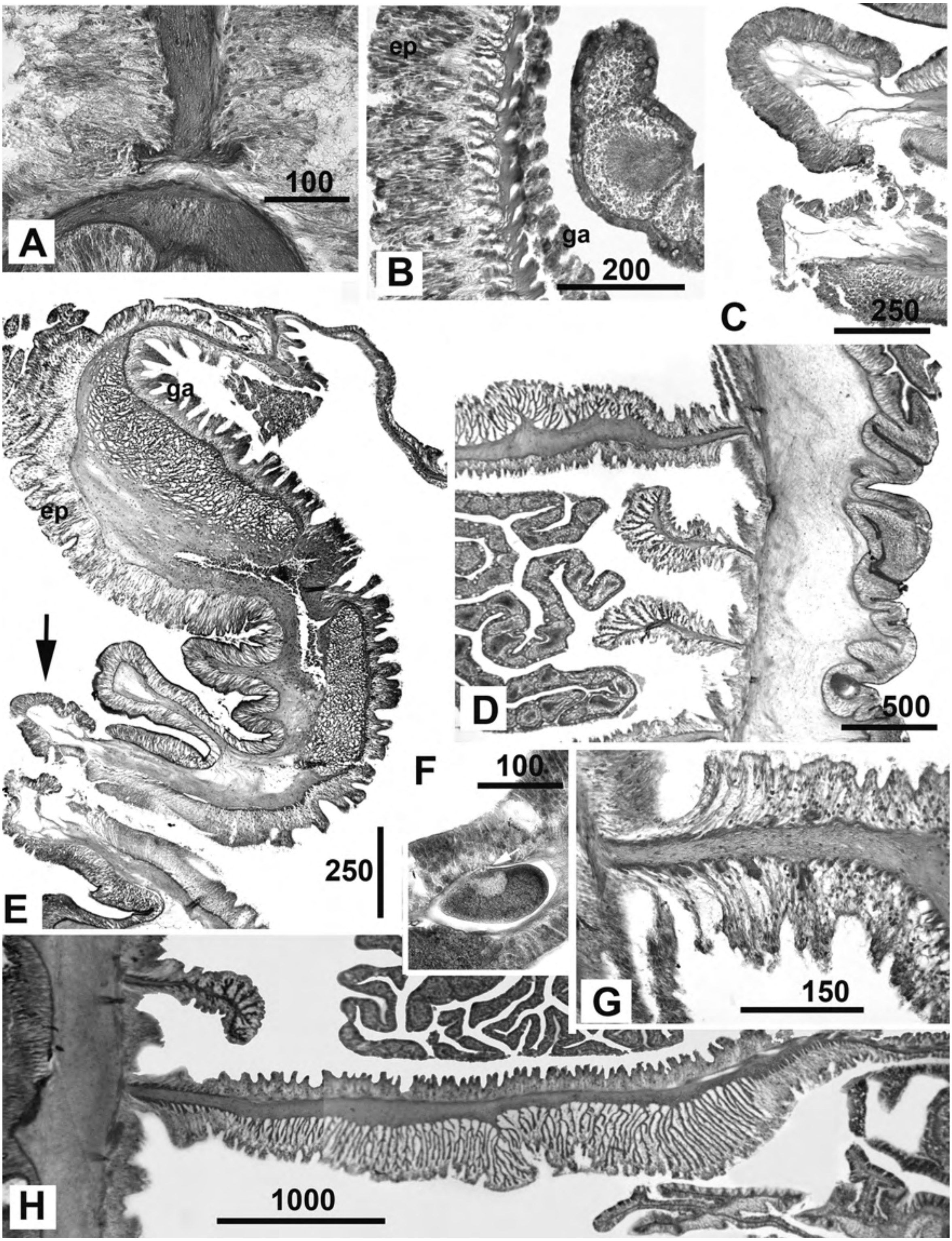

Fig. 4. Internal anatomy and histology, *Alvinactis reu*, gen. et sp. nov. Scale in µm. *A*, basilar muscles; *B*, cross-section through a tentacle of a contracted individual (note gametogenic tissue in coelenteric space of tentacle); *C*, longitudinal section through verrucae in distal column; *D*, cross-section through mesenteries below actinopharynx, showing size dimorphism of mesenteries of the first and third cycles; *E*, longitudinal section through distal column, showing mesogleal sphincter and verruca (arrow) (the space separating the distal and proximal portions of the sphincter is not present in all specimens or all sections from a single specimen); *F*, maturing oocyte with trophonema (arrow); *G*, cross-section through parietobasilar muscle of larger mesentery; *H*, cross-section through mesenteries below actinopharynx, showing diffuse retractor musculature. Abbreviations: ep, epidermis; ga, gastrodermis.

Our phylogenetic analysis demonstrates that *Alvinactis* is clearly part of the Actinoscyphiidae, and lies within the Chemosynthina, but its relationship to other genera is unclear. In some of the primary trees, *Alvinactis* and *Paranthosactis* together are the sister clade to a clade of *Cyananthea* and *Pacmanactis* because all have a marginal ring (#26). The marginal ring optimises elsewhere on the tree, as a synapomorphy for *Hormosoma*, *Anthosactis janmayeni* and *Tealidium*, and in *Bathydactylus* and in the outgroup *Kadosactis*. In other primary trees, *Alvinactis* and

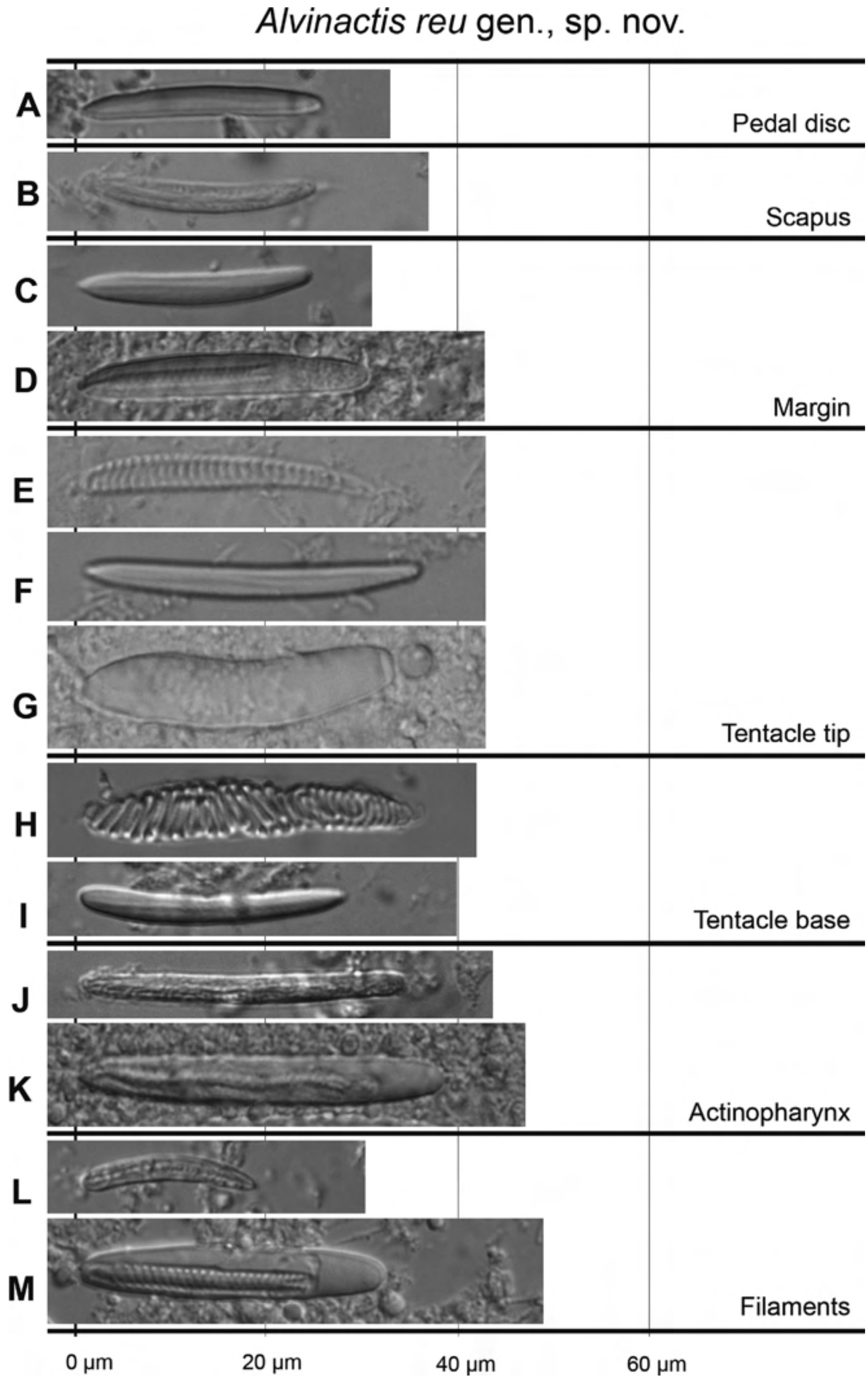

Fig. 5. Cnidae of *Alvinactis reu*, gen. et sp. nov. *A*, basitrich; *B*, basitrich; *C*, basitrich; *D*, microbasic *p*-mastigophore; *E*, gracile spirocyst; *F*, basitrich; *G*, holotrich; *H*, robust spirocyst; *I*, basitrich; *J*, basitrich; *K*, microbasic *p*-mastigophore; *L*, basitrich; *M*, microbasic *p*-mastigophore.

Paranthosactis are together (but not resolved) as the sister to *Maractis* and *Marianactis*, based on an imperfect second cycle of mesenteries (#12). *Alvinactis* is also associated with *Maractis* and *Pacmanactis* individually: as sister to *Maractis* because both lack microbasic *p*-mastigophores in the tentacles (#31), or as sister to *Pacmanactis* because both have a distal row of verrucae (#4).

The primary anatomical difference between *Alvinactis* and *Maractis*, *Marianactis* and *Paranthosactis* is the distal belt of verrucae in *Alvinactis*. Verrucae are hollow outgrowths of all three layers of the column; the ectodermal musculature and epidermis of verrucae differ from that of the surrounding column (Stephenson 1928; den Hartog 1987). These are most commonly seen in endomyarian sea anemones, in members of the family Actiniidae in particular (Stephenson 1928; Carlgren 1949; den Hartog 1987). The columnar outgrowths of acontiarian and mesomyarian anemones are typically called ‘suckers’ or ‘tenaculi’; these structures are solid rather than hollow (Stephenson 1921). As the columnar structures of *Alvinactis* are hollow (Fig. 4*C*, *E*), and are identical in form to verrucae of actiniid anemones (see, e.g. den Hartog 1987; Daly 2004), we consider them verrucae rather than suckers. Although there is no material adhering to the verrucae of *Alvinactis reu*, this is frequently the case in preserved specimens of species known to bear verrucae (MD, personal observation).

Alvinactis further differs from *Maractis* because the latter lacks a marginal ring, and from *Paranthosactis* because *Alvinactis* lacks microbasic *p*-mastigophores in the tentacles. In other actiniarians (e.g. Actiniidae, Edwardsiidae, Isanthidae, etc.), having specialisations like verrucae, tenaculi, or vesicles on the column is of generic significance (Carlgren 1949). It is possible that it is of lesser significance among mesomyarians, and that *Alvinactis*, *Maractis* and *Paranthosactis* belong in the same genus. However, because cladistic analysis of morphological data (including all the aforementioned similarities) did not consistently recover sister-group relationships among these taxa, we have no objective basis for synonymising them.

Alvinactis, *Cyananthea*, *Pacmanactis* and *Marianactis* are all known from chemosynthetically active habitats in the Pacific Ocean, but these three genera are clearly distinct. Like *Pacmanactis*, *Alvinactis* has distal perforate verrucae, although the distal structures are not histologically defined in *Pacmanactis* (López-González *et al*. 2005); they differ in that *Alvinactis* lacks microbasic *p*-mastigophores in the tentacles, and lacks microbasic *b*-mastigophores in the column margin and in the tentacles. Both *Pacmanactis* and *Cyananthea* have two cycles of perfect mesenteries, whereas *Alvinactis* has only one perfect cycle. Furthermore, *Cyananthea* has a distal belt of cinclides in the distal column but not verrucae. *Marianactis* has a distal belt of cinclides in the column and only one cycle of perfect mesenteries—characteristics seen in *Alvinactis*. Nevertheless, *Marianactis* lacks both verrucae and a marginal ridge, lacks holotrichs in the distal column or tentacles, and has a differentiated pennon on the parietobasilar muscles. *Marianactis* also has microbasic amastigophores rather than microbasic *p*-mastigophores in the tentacles, but this

Table 2. Summary of size ranges of cnidae of *Alvinactis reu*, gen. et sp. nov.

‘Sample’ indicates the number of specimens in which each cnidae was found compared with the number of specimens examined; ‘*n*’ indicates the total number of capsules measured; ‘*F*’ is the relative frequency of each type of capsule in that tissue: +++, very common, ++, common, +, less common, –, sporadic $\overline{X}$. is the average size of a capsule, and ‘s.d.’ the standard deviation of the measured samples; values from pooled samples

Category	Sample	*n*	*F*	Range of length and width of capsules (μm)	$\overline{X}$ ± s.d.
Pedal disc					
Basitrichs	4:4	64	++	(17.6–29.7) × (1.0–3.3)	22.9 ± 3.0 × 2.2 ± 0.4
Scapus					
Basitrichs	4:4	60	+++	(19.2–29.5) × (1.5–3.2)	23.85 ± 2.3 × 2.4 ± 0.4
Microbasic *p*-mastigophores				not seen	
Margin					
Basitrichs	4:4	60	+++	(18.8–31.6) × (1.6–3.1)	25.5 ± 2.7 × 2.5 ± 0.4
Microbasic *p*-mastigophores	4:4	40	+/++	(24.6–37.6) × (3.5–6.1)	30.1 ± 2.7 × 4.6 ± 0.6
Holotrichs	2:4	6	–/+	(18.6–25.4) × (3.0–3.7)	21.5 ± 2.8 × 3.2 ± 0.3*
Outer tentacle base					
Robust spirocysts	4:4	60	++	(18.7–47.4) × (2.3–7.2)	28.6 ± 6.8 × 4.5 ± 1.2
Basitrichs	4:4	61	+++	(16.4–35.6) × (1.2–3.2)	28.5 ± 3.3 × 2.3 ± 0.5
Holotrichs				not seen	
Tentacle tip					
Robust spirocysts	4:4	80	++/+++	(16.1–59.5) × (2.2–7.8)	32.2 ± 9.9 × 3.9 ± 1.1
Basitrichs	4:4	110	+++	(13.9–38.6) × (1.3–3.4)	30.7 ± 5.6 × 2.4 ± 0.5
Holotrichs	3:4	26	–/+	(21.4–38.4) × (4.5–8.2)	30.8 ± 4.5 × 6.1 ± 0.8*
Actinopharynx					
Basitrichs	3:3	23	–/+	(17.2–37.2) × (1.1–3.4)	30.3 ± 3.9 × 2.4 ± 0.6*
Microbasic *p*-mastigophores	3:3	60	+++	(27.3–39.4) × (3.5–5.8)	34.4 ± 2.2 × 4.7 ± 0.6
Filaments					
Basitrichs	3:3	44	+/++	(13.2–33.3) × (1.2–4.1)	21.4 ± 4.9 × 2.2 ± 0.5
Microbasic *p*-mastigophores	3:3	60	+++	(28.0–39.4) × (3.0–5.8)	32.9 ± 2.5 × 4.5 ± 0.5

(*) Average based on fewer than 40 capsules; the measurement of at least 40 capsules is the minimum sufficient for statistical significance (Williams 1998, 2000).

distinction is of less value because of the difficulty of distinguishing between these nematocysts when undischarged (Östman 2000).

Phylogenetic relationships of Actinostolidae and Actinoscyphiidae

Our phylogenetic analysis of morphological data highlights problems with the taxonomy and organisation of Actinostolidae *sensu* Carlgren (1949). Neither the strict consensus tree (Fig. 1) nor any of the primary trees from our data support monophyly of Actinostolidae, suggesting that it is a grade rather than a clade. Phylogenetic analysis of a more diverse assemblage of actiniarians, including representatives of *Actinostola*, *Actinoscyphia*, *Anthosactis*, *Hormosoma*, *Stomphia* and the taxa used here as outgroups recovers a pattern of relationships compatible with the morphological evidence of the present work (Daly *et al.* 2008).

The sole feature shared by all members of Actinostolidae is a mesogleal marginal sphincter, an attribute common to many other actiniarian families. The remaining diagnostic features are absences: lack of the nematocyst-dense threads called acontia and of microcnemic mesenteries. The lack of resolution and the inclusion of *Actinoscyphia* and the outgroups *Bathyphellia*, *Hormathia* and *Kadosactis* among the ingroup taxa suggests that some members of Actinostolidae are not closely related to one another; *Bathyphellia*, *Hormathia* and *Kadosactis* belong to the superfamily Acontiaria. Molecular evidence suggests that that all Acontiaria belong to a monophyletic group, although this clade also includes taxa without acontia (Daly *et al.* 2008). It is likely that at least some members of the family will need to be transferred to other families or placed in new families.

Nevertheless, our phylogenetic analysis indicates that some genera share uniquely derived attributes (Fig. 1). Our clade Actinostolina contains many of the taxa Carlgren (1899) included in his original description of the subfamily Actinostolinae, including the type genus *Actinostola*. Two synapomorphies for Actinostolina are characteristics Carlgren (1899) ascribed to the subfamily Actinostolinae, including mesogleal longitudinal muscles in tentacles (#23) and microbasic *b*-mastigophores in the tentacles (#30). The third feature, microbasic *p*-mastigophores in the tentacles (#31), has also been used to distinguish actinostolid genera (Carlgren 1949). Internal brooding of offspring (#35) is seen outside of Actinostolina only in *Anthosactis pearseae*; as *Anthosactis* is a polyphyletic assemblage, the interpretation of this character is unclear. Perfect mesenteries in the second and third cycles (#s 12, 13) characterise most members of the Actinostolina, except *A. janmayeni*, *Tealidium* and *Ophiodiscus*; in these three taxa, none of the mesenteries of the third cycle are perfect (#13).

Carlgren (1949) used the dissimilar morphology of mesenteries of a pair (#s 7, 8) to divide the actinostolids (groups I and II, see Carlgren 1949). These features are a synapomorphy for a clade within Actinostolina that encompasses most of the taxa Carlgren (1949) placed in group I (Fig. 1). However, at least two genera (*Antholoba* and *Pycnanthus*) with similar mesenteries group with the clade of taxa with dissimilar mesenteries (Fig. 1). The tree provides no support for the monophyly of the taxa Carlgren (1949) placed in group II, although this is not surprising, as having paired mesenteries of similar morphology is common to most Actiniaria.

The genus *Anthosactis* is very heterogeneous, and previous authors have suggested that it may be a polyphyletic assemblage rather than monophyletic clade (e.g. Riemann-Zürneck 1997; White *et al.* 1999; Daly and Gusmão 2007). Our results bolster this interpretation: the three species of *Anthosactis* in our analysis did not group together, being widely dispersed through the tree. The type species, *A. janmeyeni*, groups with *Hormosoma* and *Tealidium* as the sister clade to Actinostolina (Fig. 1). A close relationship between *Tealidium* and *Anthosactis* has been proposed previously (Riemann-Zürneck 1997). Batteries of microbasic *b*-mastigophores in the aboral bases of the tentacles (#29) and the sphincter forming a marginal ring (#26) group *Hormosoma* and the clade comprised of *A. janmayeni* and *Tealidim*. The two other species of *Anthosactis*, *A. nomados* and *A. pearseae*, are in the other main clade: *A. pearseae* is the sister-group to Chemosynthina, and *A. nomados* is the sister-group to the crown clade consisting of Actinoscyphiidae and its sister clade.

In the consensus tree, *Epiparactis*, *Actinoscyphia* and *Anthosactis pearseae* are sister to the clade we call Chemosynthina, and this clade is the sister to a group composed of *Hadalanthus*, *Bathydactylus* and the acontiate outgroups. Although the clustering of outgroup and ingroup taxa points to problems in the circumscription of these groups, some components of this tree have been advocated by other authors. In the discussion that accompanied her resurrection of family Actinoscyphiidae, Riemann-Zürneck (1978*a*) hypothesised a close relationship between *Epiparactis* and *Actinoscyphia*. Following Schmidt's (1972, 1974) subdivision of mesomyarians in 'Early' and 'Late' groups based on attributes of the cnidae, Riemann-Zürneck (1978*a*) further hypothesised that members of Actinoscyphiidae had lost acontia. Stephenson (1920) expressed a similar idea by including *Lilliella* Stephenson, 1918 and *Isoparactis* Stephenson, 1920 in Actinoscyphiidae; these genera have since been synonymised with the acontiate genera *Hormathia* and *Acraspedanthus* Carlgren, 1924 respectively. Sanamyan and Sanamyan (2007) considered *Cyananthea* and *Epiparactis* within Actinoscyphiidae, following the diagnosis given by Riemann-Zürneck (1978*a*). They also noted that the ring of cinclides in the distal column of *Cyananthea* strongly recalls *Kadosactis*, thereby relating *Cyananthea* to acontiarians. Finally, Sanamyan and Sanamyan (2007) pointed out the similarities between *Pacmanactis* and *Cyananthea* (both only differing in the presence of verrucae and the number of mesenteries distally and proximally), and highlighted the similarities in the cnidom of *Marianactis* and *Cyananthea*. Based on these comparions, they proposed *Pacmanactis* and *Marianactis* be transferred to Actinoscyphiidae but they did not make the change (see Sanamyan and Sanamyan 2007).

In our consensus tree, most of the taxa Sanamyan and Sanamyan (2007) included in Actinoscyphiidae group together (Fig. 1). *Epiparactis* is basal to the rest of the genera of this clade. It lacks holotrichs in the tentacles (#33), a feature shared by all other taxa except *Marianactis*, and has three rather than four complete cycles of mesenteries. Most members of

Actinoscyphiidae have a marginal sphincter situated closer to the epidermis (#27), and four cycles of mesenteries (#10). The Actinoscyphiidae is the sister to a clade that includes the acontiarian outgroups plus *Hadalanthus* and *Bathydactylus*. Thus, this analysis suggests a close relationship between Actinoscyphiidae and some Acontiaria.

Within Actinoscyphiidae is Chemosynthina, the clade containing the genera reported from hydrothermal vents and cold seeps. *Anthosactis pearseae*, known from whalefalls, is the sister-group to Chemosynthina in some but not all primary trees. Monophyly of Chemosynthina is supported by a mosaic of characters: the presence of cinclides (#5), relatively robust or thick column walls (#6, except *Pacmanactis* and *Marianactis*), equal numbers of mesenteries proximally and distally (#9, except *Cyananthea*), fertile mesenteries in the third cycle (#17, except *Pacmanactis*), a strong sphincter (#25, except *Pacmanactis*), a marginal ring (#26, absent in *Maractis* and *Marianactis*) and microbasic *p*-mastigophores in the tentacles (#31, except *Maractis* and *Alvinactis*). In our re-examination of the type material of *Marianactis*, we found a belt of small cinclides in the distal column; the cinclides are very small and are inconspicuous in preserved material, and are therefore easily overlooked. Their presence may also have been overlooked in *Maractis* and *Paranthosactis*.

Based on these results, we accept Sanamyan and Sanamyan's (2007) circumscription of Actinoscyphiidae, and add to it *Marianactis*, *Pacmanactis*, *Alvinactis*, *Maractis* and *Paranthosactis*. Actinostolidae as presented in this paper includes the genera in Actinostolina, plus an assemblage of taxa that are sister to Actinostolina or Actinoscyphiidae, including *Anthosactis*, *Antiparactis*, *Cnidanthea*, *Cnidanthus*, *Hormosoma*, *Paranthus*, *Pseudoparactis* and *Tealidium*. Actinostolidae in its new sense is not monophyletic. Reorganising it to reflect monophyly will require dense sampling across Actiniaria, and should include molecular as well as morphological data. *Anthosactis* is polyphyletic, with some members more closely related to genera in Actinoscyphiidae than to those in Actinostolidae. Because the type species, *A. janmayeni*, lies within Actinostolidae pending a comprehensive species-level revision of *Anthosactis*, we leave it in Actinostolidae.

In addition to identifying potential synapomorphies for Actinoscyphiidae, Actinostolidae and their subgroups, our analysis highlights characters that seem to have little ability to group taxa. The number of distal and proximal mesenteries (#9) varies widely in the family, and has not been assessed for many taxa. Similarly, having the sphincter form a marginal ring (#26) occurs quite broadly across the tree, as do broad bathymetric ranges (#37). As with the number of mesenteries, these features may have been scored inconsistently by some authors, making them appear less informative than they actually are. The relative thickness of the column wall (#6) is often not recorded and is very subjective, varying with degree of contraction and preservation state.

The types of nematocysts in the tentacles have been used as a generic character in Actinostolidae (Carlgren 1949; Fautin and Hessler 1989). The presence of microbasic *b*-mastigophores is a potential synapomorphy of Actinostolina: their presence and arrangement in batteries distinguishes *Tealidium*, *Hormosoma* and *Anthosactis* (see Carlgren 1949). However, the use of these features as taxonomic characters has been challenged in recent studies (see López-González *et al*. 2003). Similarly, although microbasic *p*-mastigophores or amastigophores have been used to differentiate actinostolid genera, the phylogenetic value of these characters is far from clear because these types are difficult to distinguish with light microscopy (Östman 2000). Holotrichs in the tentacles are inducible in some species (e.g. Fautin 1988; Edmands and Fautin 1991), rendering them suspect as a taxonomic or phylogenetic feature. Nonetheless, holotrichs in the tentacles are phylogenetically useful in this analysis, grouping Chemosynthina and its allies.

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References

Andres, A. (1883). 'Le Attinie (Monografia)'. (Coi Tipi der Salviucci: Roma.)

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10**, 295–304.

Carlgren, O. (1893). Studien über Nordische Actinien. *Kungliga Svenska Vetenskaps – Akademiens Handlingar* **25**, 1–148.

Carlgren, O. (1899). Zoantharien. *Hamburger Magalhaensische Sammelreise* **4**(1), 1–48.

Carlgren, O. (1918). Die Mesenterienanordnung der Halcuriiden. *Kungliga Fysiografiska Sällskapets Handlingar* **29**(29), 1–37.

Carlgren, O. (1921). Actiniaria. I. *Danish Ingolf – Expedition* **5**(9), 1–241.

Carlgren, O. (1924). Actiniaria from New Zealand and its Subantarctic Islands. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening (Copenhagen)* **77**, 179–261.

Carlgren, O. (1925). Zur Kenntnis der Hexacorallen. *Zoologischer Anzeiger* **65**, 87–99.

Carlgren, O. (1927). Actiniaria and Zoantharia. *Further Zoological Results of the Swedish Antarctic Expedition 1901–1903* **2**(3), 1–102.

Carlgren, O. (1928). Actiniaria der Deutschen Tiefsee-Expedition. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899* **4**(22), 125–266.

Carlgren, O. (1932). Die Ceriantharien, Zoantharien und Actiniarien des arktischen Gebietes. In 'Eine Zusammenstellung der arktischen Tierformen mit besonderer Berücksichtigung des Spitzbergen-Gebietes auf Grund der Ergebnisse der Deutschen Expedition in das Nördliche Eismeer im Jahre 1898. Vol. 6'. (Eds F. Römer, F. Schaudinn, A. Brauer and W. Arndt.) pp. 255–266. (Gustav Fischer: Jena.)

Carlgren, O. (1949). A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskaps – Akademiens Handlingar* **1**, 1–121.

Carlgren, O. (1956). Actiniaria from depths exceeding 6000 meters. *Galathea Reports* **2**, 9–16.

Daly, M. (2004). Anatomy and taxonomy of three species of sea anemones (Cnidaria: Anthozoa: Actiniidae) from the Gulf of California, including *Isoaulactina hespervolita* Daly, n. sp. *Pacific Science* **58**, 377–390.

Daly, M., and Gusmão, L. (2007). The first sea anemone (Cnidaria, Anthozoa) from a whale fall. *Journal of Natural History* **41**, 1–11.

Daly, M., Chaudhuri, A., Gusmão, L., and Rodríguez, E. (2008). Phylogenetic Relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular Phylogenetics and Evolution* **48**, 292–301. doi: 10.1016/j.ympev.2008.02.022

Danielssen, D. C. (1890). Actinida. In 'Den Norske Nordhavs-Expedition 1876–1878. Zoology'. p. 184. (Grøndahl and Søn: Christiania.)

den Hartog, J. C. (1987). A redescription of the sea anemone *Bunodosoma biscayensis* (Fischer, 1874) (Actiniaria, Actiniidae). *Zoologische Mededeelingen* **61**, 533–559.

Doumenc, D., and Van-Präet, M. (1988). Actinies abyssales d'un site hydrothermal du Pacifique oriental. *Oceanologica Acta* **8**, 61–68.

Edmands, S., and Fautin, D. G. (1991). Redescription of *Aulactinia veratra* n. comb. (=*Cnidopus veratra*) (Coelenterata: Actiniaria) from Australia. *Records of the Western Australian Museum (Perth)* **15**(1), 59–68.

England, K. W. (1991). Nematocysts of sea anemones (Actiniaria, Ceriantharia, and Corallimorpharia: Cnidaria): nomenclature. *Hydrobiologia* **216/217**, 691–697.

Fautin, D. G. (1988). Importance of nematocysts to Actinian taxonomy. In 'The Biology of Nematocysts'. (Eds D. A. Hessinger and H. M. Lenhoff.) pp. 487–500. (Academic Press: San Diego.)

Fautin, D. G. (2007). 'Hexacorallians of the World.' Available online at http://hercules.kgs.ku.edu/hexacoral/anemone2/index.cfm [Accessed 18 December 2007].

Fautin, D. G., and Barber, B. R. (1999). *Maractis rimicarivora*, a new genus and species of sea anemone (Cnidaria: Anthozoa: Actiniaria: Actinostolidae) from an Atlantic hydrothermal vent. *Proceedings of the Biological Society of Washington* **112**, 624–631.

Fautin, D. G., and Hessler, R. R. (1989). *Marianactis bythios*, a new genus and species of actinostolid sea anemone (Coelenterata: Actiniaria) from the Mariana vents. *Proceedings of the Biological Society of Washington* **102**, 815–825.

Goloboff, P. A. (1999). NONA (software and documentation). Available at www.cladistics.com [Accessed 20 October 2007].

Gosse, P. H. (1859). Characters and descriptions of some new British sea anemones. *Annals and Magazine of Natural History* **3**(13), 46–50.

Hertwig, R. (1882). 'Die Actinien der Challenger expedition.' (Gustav Fischer: Jena.)

López-González, P. J., and Segonzac, M. (2006). Cnidaria: Anthozoa: Actiniaria: Actinostolidae. In 'Handbook of Deep-Sea Hydrothermal Vent Fauna'. (Eds D. Desbruyeres, M. Segonzac, and M. Bright.) pp. 544. (Denisa: Linz, Austria.)

López-González, P. J., Rodríguez, E., and Segonzac, M. (2005). A new species of sea anemone (Cnidaria: Anthozoa: Actiniaria) from Manus Basin Hydrothermal vents, South-western Pacific. *Marine Biology Research* **1**, 326–337.

López-González, P. J., Rodríguez, E., Gili, J. M., and Segonzac, M. (2003). New records on sea anemones (Anthozoa: Actiniaria) from hydrothermal vents and cold seeps. *Zoologische Verhandelingen Leiden* **345**, 215–243.

Mariscal, R. N. (1974). Nematocysts. In 'Coelenterate biology: reviews and new perspectives'. (Eds L. Muscatine and H. M. Lenhoff.) pp. 129–178. (Academic Press: New York.)

McMurrich, J. P. (1893). Report on the Actiniæ 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.

Milne Edwards, H. (1857). 'Histoire Naturelle des Coralliaires ou Polypes Proprement Dits. Vol. 1.' (Librairie Encyclopédique de Roret: Paris.)

Milne Edwards, H., and Haime, J. (1851). Reserches sur les polypiers; septième mémoire. Monographie des poritides. *Annales des Sciences Naturelles* **16**(3), 21–70.

Nixon, K. C. (1999). 'Winclada, version 0.9.9.' Available at www.cladistics.com [Accessed 20 October 2007].

Östman, C. (2000). A guideline to nematocyst nomenclature and classification, and some note on the systematic value of nematocysts. *Scientia Marina* **64**(1), 31–46.

Presnell, J. K., and Schreibman, M. P. (1997). 'Humason's animal tissue techniques.' pp. 572. (The Johns Hopkins University Press: Baltimore.)

Riemann-Zürneck, K. (1978*a*). Tiefsee-Aktinien der familie Actinoscyphiidae aus dem Nordatlantik (Actiniaria, Mesomyaria). *Zoologica Scripta* **7**, 145–153.

Riemann-Zürneck, K. (1978*b*). Actiniaria des Südwestatlantik IV. *Actinostola crassicornis* (Hertwig, 1882) mit einer Diskussion verwandter Arten. *Veröffentlichungen des Institutes für Meeresforschung Bremerhaven* **17**, 65–85.

Riemann-Zürneck, K. (1997). *Anthosactis janmayeni* Danielssen, 1890, a rare high-arctic sea anemone. *Polar Biology* **17**, 487–491.

Sanamyan, N. P., and Sanamyan, K. E. (2007). Deep-water Actiniaria from East Pacific hydrothermal vents and cold seeps. *Invertebrate Zoology* **4**(1), 83–102.

Schmidt, H. (1969). Die Nesselkapseln der Aktinien und ihre differentialdiagnostiche 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. In 'Proceedings of the Second International Coral Reef Symposium, Vol. 1'. (Eds A. M. Cameron *et al.*) pp. 533–560. (Great Barrier Reef Committee: Brisbane, Australia.)

Stephenson, T. A. (1918). Coelenterata. I. Actiniaria. *Natural History Reports on British Antarctic ("Terra Nova") Expedition 1910* **5**(1), 1–68.

Stephenson, T. A. (1920). On the classification of Actiniaria. Part I. Forms with acontia and forms with a mesogleal sphincter. *The Quarterly Journal of Microscopical Science* **64**, 425–574.

Stephenson, T. A. (1921). On the classification of Actiniaria. Part II. Consideration of the whole group and its relationships, with special reference to forms not treated in Part I. *The Quarterly Journal of Microscopical Science* **65**, 493–576.

Stephenson, T. A. (1928). 'The British Sea Anemones. Vol. 1.' (The Ray Society: London.)

Verrill, A. E. (1869). Review of the corals and polyps of the west coast of America. *Transactions of the Connecticut Academy of Arts and Sciences* **1**(6), 377–558.

Verrill, A. E. (1883). Reports on the Anthozoa, and on some additional species dredged by the "Blake" in 1877–1879, and by the U. S. Fish Commission Steamer "Fish Hawk" in 1880–82. *Bulletin of the Museum of Comparative Zoology* **11**(1), 1–72. [Harvard University]

Verrill, A. E. (1899). Descriptions of imperfectly known and new Actinians, with critical notes on other species, IV. *American Journal of Science and Arts* **7**(4), 205–218.

White, T. R., Wakefield Pagels, A. K., and Fautin, D. G. (1999). Abyssal sea anemones (Cnidaria: Actiniaria) of the northeast Pacific symbiotic with molluscs: *Anthosactis nomados*, a new species, and *Monactis vestita* (Gravier, 1918). *Proceedings of the Biological Society of Washington* **112**(4), 637–651.

Williams, R. B. (1998). Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), II: further studies of differences amongst sample means and their taxonomic relevance. *Scientia Marina* **62**, 361–372.

Williams, R. B. (2000). Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), III: ranges and other measures of statistical dispersion, their interrelations and taxonomic relevance. *Scientia Marina* **64**, 49–68.

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Appendix 1. Morphological characters used in cladistic analysis of Actinostolidae

Characters in bold used in Carlgren's 1949 key to the actinostolid genera. Characters that do not manifest exclusive states in all members of a taxon have been broken into several binary characters (e.g. #s 22 and 23) rather than coded as single multistate characters. Characters applicable only to outgroup taxa indicated

External anatomy

(*0*) *Column regions*: absent (0); scapus/scapulus present (1).
(*1*) *Column surface*: smooth (0); mesogleal papillae present (1).
(*2*) *Column with cuticle*: absent (0); present (1).
(*3*) *Column with tenaculi*: absent (0); present (1).
(*4*) *Distal verrucae on column*: absent (0); present (1).
(*5*) *Distal cinclides on column*: absent (0); present (1).
(*6*) *Column mesoglea*: thin (0); thick (1).

Internal anatomy

(*7*) *Mesenteries of a pair equally developed*: yes (0); no (1).
(*8*) *Muscles of larger mesentery of an unequal pair* (*from 7*): facing the nearest mesentery of preceding cycle (0); facing both preceding and ante-preceding cycle (1).
(*9*) *Number of distal v. proximal mesenteries*: fewer (0); same (1); more (2).
(*10*) *Maximum number of cycles of mesenteries*: three cycles (0); four cycles (1); five cycles (2); six cycles (3); seven cycles (4).
(*11*) *Perfect mesenteries in first cycle*: absent (0); present (1).
(*12*) *Second cycle of mesenteries perfect*: none (0); some (1); all (2).
(*13*) *Third cycle of mesenteries perfect*: none mesenteries (0); some mesenteries (1); all mesenteries (2).
(*14*) *Forth cycle of mesenteries perfect*: none (0); some (1); all (2); non-applicable (-).
(*15*) *Fertile first mesentery cycle*: absent (0); present (1).
(*16*) *Fertile second mesentery cycle*: absent (0); present (1).
(*17*) *Fertile third mesentery cycle*: absent (0); present (1).
(*18*) *Smallest mesentery cycle fertile*: absent (0); present (1).
(*19*) *Dimorphic*, *filament-free fertile and filament-bearing sterile mesenteries*: absent (0); present (1).
(*20*) *Basal tentacle mesoglea*: not thickened (0); thickened (1).
(*21*) *Development of longitudinal tentacles muscles*: similar (0); more developed on the oral side (1).
(*22*) *Ectodermal longitudinal tentacle muscles*: absent (0); present (1).
(*23*) *Mesogleal longitudinal tentacle muscles*: absent (0); present (1).
(*24*) *Sphincter*: mesogleal (0); endodermal (1).
(*25*) *Sphincter development*: weak (0); strong (1).
(*26*) *Sphincter forming a marginal ring*: absent (0); present (1).
(*27*) *Sphincter position in mesoglea*: closer to gastrodermis (0); closer to epidermis (1); centred (2).
(*28*) *Parietobasilar muscles*: not distinctly marked nor differentiated as a separate lamella (0); distinctly marked but without forming a separate lamella (1); differentiated as a separate lamella (2).

Tentacle cnidae

(*29*) *Batteries of microbasic* b-*mastigophores on basal*, *aboral side of outer tentacles*: absent (0); present (1).
(*30*) *Microbasic* b-*mastigophores in the tentacles*: absent (0); present (1).
(*31*) *Microbasic* p-*mastigophores in the tentacles*: absent (0); present (1).
(*32*) *Basitrichs in the tentacles*: absent (0); present (1).
(*33*) *Holotrichs in the tentacles*: absent (0); present (1).
(*34*) *Robust spirocysts*: absent (0); present (1).

Ecology and life history

(*35*) *Internal brooding*: absent (0); present (1).
(*36*) *Deep sea*: absent (0); present (1).
(*37*) *Shallow*: absent (0); present (1).
(*38*) *Occurs in chemosynthetic habitats*: no (0); yes (1).
(*39*) *Type of chemosynthetic habitat*: vent (0); seeps (1); whale falls (2); non-applicable (-).

Character for outgroup genera

(*40*) *Acontia*: absent (0); present (1).

Appendix 2. Morphological character state distributions for outgroup and ingroup genera

Dash indicates that the corresponding state is unknown or inapplicable. Outgroup genera in bold. See Appendix 1 for character list

Taxa	Characters				
	0000000000	1111111111	2222222222	3333333333	4
	0123456789	0123456789	0123456789	0123456789	0
Actinoscyphia	00000010-1	2120011000	1011000000	001110100-	0
Bathyphellia	111100-0-0	0100-10000	0010010220	001010100-	1
Epiactis	01000000-0	2122011100	0010110-20	010001110-	0
Hormathia	11100010-1	1100001110	0010000010	001010110-	1
Kadosactis	11110110-1	0120-11000	1-10011010	011010100-	1
Actinostola	0000001100	4121000110	1001000000	111001110-	0
Alvinactis	00001100-1	1100011100	0010011000	0011101010	0
Antholoba	00000010-2	4122100010	0011000000	001001010-	0
Anthosactis janmayeni	00000010--	1110011110	-110011101	101000110-	0
Anthosactis nomados	00000000-0	0120-11000	0-100102--	101010100-	0
Anthosactis pearseae	00000010-0	1100011000	0010000000	0011111012	0
Antiparactis	00000000-0	1100001100	-010010200	----00010-	0
Bathydactylus	10000110-0	0100-11000	0010011-10	00100010--	0
Cnidanthea	010000-0-1	0100-11000	0010010200	0110-0010-	0
Cnidanthus	00000000-0	1121011100	0001010000	1010-1010-	0
Cyananthea	00000100-2	2120011100	0010011000	0111101010	0
Epiparactis	00000010-	21000----0	0010000200	001010100-	0
Hadalanthus	11110000-1	1100011--0	0010010000	0110-010-	0
Hormosoma	00000010-1	1122011110	0001011121	100001010-	0
Maractis	00000000-1	1100011110	0010010010	0011101010	0
Marianactis	00000110-1	1100011110	0010010020	0110101010	0
Ophiodiscus	000000110-	1120000011	1101000-0-	-----010--	0
Pacmanactis	00001110-1	1120011000	0010001101	1111101010	0
Paractinostola	0000001102	4122100110	1101000000	1010-0110-	0
Paranthosactis	00000-00-1	1100011100	0010011000	0111101010	0
Paranthus	000010-0-2	1120011000	--10000000	011000010-	0
Parasicyonis	0000001110	2122100010	0101010000	0010-0100-	0
Pseudoparactis	10000-0-0-	1----011-0	--1000020-	-----0010-	0
Pycnanthus	00000010-0	2121000110	1101010010	111001100-	0
Sicyonis	0000001110	3121000010	1101000010	001000110-	0
Stomphia	0000001100	1121000110	0011000001	101001110-	0
Synsicyonis	0000001112	1121000110	1101000---	-----0100-	0
Tealidium	11000010--	1110011110	1110011201	1010-0100-	0