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

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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-Priët 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 Actiniinae, and placed in this group Paractis Milne Edwards, 1851; 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
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 Actinostolidae 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 (1978a) and Fautin and Hessler (1989) changed the definition of the family and reconsidered some features used to differentiate its members. Riemann-Zürneck (1978a) revised the mesosomal family Actinocyphiidae. 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 Actinocyphiidae based on its cnidom (Sanamyan and Sanamyan 2007). This redescription of Cyananthea highlights the confusion that remains about the circumscription of Actinostolidae and Actinocyphiidae. 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 Actinocyphiidae, 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

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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 Mason’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. Cnidarian 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 Epicnidae Verrill, 1869 and the acontarians Bathypellia Carlgren, 1932; Hormathia Gosse, 1859 and Kadosactis Daniellsen, 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 1978a; 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 Daniellsen, 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 (1978b) synonymised Paractinostola Carlgren, 1928 with Anthosactis, 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: Marianactis bythisos 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 pearseeae 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) 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 subfamilies 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 (1978a), 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 janmayeri Daniellsen, 1890; Cnidanthus Carlgren, 1927; Hormosoma Stephenson, 1918; Ophiodiscus, Paractinostola, Paracynosis Carlgren, 1921; Pycnanthus McMurrich, 1893; Sicyonis Hertwig, 1882; Stomphia, Synsyconis Carlgren, 1921; and Tealidium; this roughly corresponds to Carlgren’s subfamily Actinostolinidae. Henceforth, we refer to this clade as ‘Actinostolina’.

The other main clade includes the remaining genera previously attributed to Actinostolidae, Actinoscyphia, and the outgroups Bathypellia, 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, Pucmanactis López-González, Rodriguez & 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; Antholectta; Anthosactis; Antiparactis Verrill, 1899; Bathydactylus; Cnidaetha Carlgren, 1956; Cnidanthus; Hadalanthus; Hormosoma; Ophiodiscus; Paranthus; Parasicyonia; Pseudoparactis Stephenson, 1920; Pycnanthus; Sicyonia; Stomphia; Synsicyonia 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-cnenes. 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, 1893 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**


**Remarks**

Riemann-Zürneck (1978a) resurrected Actinosecyphiidae primarily based on Schmidt’s (1969, 1972) classification of types of cnidae. Thus, Riemann-Zürneck (1978a) characterises Actinosecyphiidae 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 the 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 phylogenetic 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 actinarians, 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 2B, D; 4D, 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 2A, 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. 4E). Mesoglea of distal column with small, darkly-staining inclusions; these are especially abundant near marginal sphincter.
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. 2A). 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 2A, 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. 2B). 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.

**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. 4C). 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 4B, G).

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

**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 undclinable 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

![Figure 5](image)

*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. 4C, 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 (Stephenson 1921). 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 *h*-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 pemmon on the parietobasilar muscles. *Marianactis* also has microbasic amastigophores rather than microbasic *p*-mastigophores in the tentacles, but this

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**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. $\bar{X}$ is the average size of a capsule, and ‘s.d.’ the standard deviation of the measured samples; values from pooled samples

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample</th>
<th>n</th>
<th>F</th>
<th>Range of length and width of capsules (μm)</th>
<th>$\bar{X}$ ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedal disc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basitrichs</td>
<td>4:4</td>
<td>64</td>
<td>++</td>
<td>(17.6–29.7) $\times$ (1.0–3.3)</td>
<td>22.9 ± 3.0 $\times$ 2.2 ± 0.4</td>
</tr>
<tr>
<td>Scapus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basitrichs</td>
<td>4:4</td>
<td>60</td>
<td>+++</td>
<td>(19.2–29.5) $\times$ (1.5–3.2)</td>
<td>23.85 ± 2.3 $\times$ 2.4 ± 0.4</td>
</tr>
<tr>
<td>Microbasic <em>p</em>-mastigophores</td>
<td>not seen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basitrichs</td>
<td>4:4</td>
<td>60</td>
<td>+++</td>
<td>(18–31.6) $\times$ (1.6–3.1)</td>
<td>25.5 ± 2.7 $\times$ 2.5 ± 0.4</td>
</tr>
<tr>
<td>Microbasic <em>p</em>-mastigophores</td>
<td>4:4</td>
<td>40</td>
<td>+/+</td>
<td>(24.6–37.6) $\times$ (3.5–6.1)</td>
<td>30.1 ± 2.7 $\times$ 4.6 ± 0.6</td>
</tr>
<tr>
<td>Holotrichs</td>
<td>2:4</td>
<td>6</td>
<td>–/+</td>
<td>(18.6–25.4) $\times$ (3.0–3.7)</td>
<td>21.5 ± 2.8 $\times$ 3.2 ± 0.3*</td>
</tr>
<tr>
<td>Outer tentacle base</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robust spirocysts</td>
<td>4:4</td>
<td>60</td>
<td>++</td>
<td>(18.7–47.4) $\times$ (2.3–7.2)</td>
<td>28.6 ± 6.8 $\times$ 4.5 ± 1.2</td>
</tr>
<tr>
<td>Basitrichs</td>
<td>4:4</td>
<td>61</td>
<td>+++</td>
<td>(16.4–35.6) $\times$ (1.2–3.2)</td>
<td>28.5 ± 3.3 $\times$ 2.3 ± 0.5</td>
</tr>
<tr>
<td>Holotrichs</td>
<td>not seen</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tentacle tip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robust spirocysts</td>
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<td>80</td>
<td>++++</td>
<td>(16.1–59.5) $\times$ (2.2–7.8)</td>
<td>32.2 ± 9.9 $\times$ 3.9 ± 1.1</td>
</tr>
<tr>
<td>Basitrichs</td>
<td>4:4</td>
<td>110</td>
<td>+++</td>
<td>(13.9–38.6) $\times$ (1.3–3.4)</td>
<td>30.7 ± 5.6 $\times$ 2.4 ± 0.5</td>
</tr>
<tr>
<td>Holotrichs</td>
<td>3:4</td>
<td>26</td>
<td>–/+</td>
<td>(21.4–38.4) $\times$ (4.5–8.2)</td>
<td>30.8 ± 4.5 $\times$ 6.1 ± 0.8*</td>
</tr>
<tr>
<td>Actinopharynx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basitrichs</td>
<td>3:3</td>
<td>23</td>
<td>–/+</td>
<td>(17.2–37.2) $\times$ (1.1–3.4)</td>
<td>30.3 ± 3.9 $\times$ 2.4 ± 0.6*</td>
</tr>
<tr>
<td>Microbasic <em>p</em>-mastigophores</td>
<td>3:3</td>
<td>60</td>
<td>+++</td>
<td>(27.3–39.4) $\times$ (3.5–5.8)</td>
<td>34.4 ± 2.2 $\times$ 4.7 ± 0.6</td>
</tr>
<tr>
<td>Filaments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basitrichs</td>
<td>3:3</td>
<td>44</td>
<td>+++</td>
<td>(13.2–33.3) $\times$ (1.2–4.1)</td>
<td>21.4 ± 4.9 $\times$ 2.2 ± 0.5</td>
</tr>
<tr>
<td>Microbasic <em>p</em>-mastigophores</td>
<td>3:3</td>
<td>60</td>
<td>+++</td>
<td>(28.0–39.4) $\times$ (3.0–5.8)</td>
<td>32.9 ± 2.5 $\times$ 4.5 ± 0.5</td>
</tr>
</tbody>
</table>

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

Our phylogenetic analysis of morphological data highlights problems with the taxonomy and organisation of Actinostolidae sensu Carlgen (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 actinarians, 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 actinarian families. The remaining diagnostic features are absences: lack of the nematocyst-dense threads called aconitia and of microcnemic mesenteries. The lack of resolution and the inclusion of Actinoscyphia and the outgroups Bathypellia, Hormathia and Kadosactis among the ingroup taxa suggests that some members of Actinostolidae are not closely related to one another; Bathypellia, Hormathia and Kadosactis belong to the superfamily Acontiaria. Molecular evidence suggests that all Acontiaria belong to a monophyletic group, although this clade also includes taxa without aconitia (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 Carlgen (1899) included in his original description of the subfamily Actinostolinae, including the type genus Actinostola. Two synapomorphies for Actinostolina are characteristics Carlgen (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 (Carlgen 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 (#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).

Carlgen (1949) used the dissimorphic morphology of mesenteries of a pair (#7, 8) to divide the actinostolids (groups I and II, see Carlgen 1949). These features are a synapomorphy for a clade within Actinostolina that encompasses most of the taxa Carlgen (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 Carlgen (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. janmayeni, 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 Tealidium. The two other species of Anthosactis, A. nomados and A. pearseae, are in the other main clade: A. pearseae is the sister-group to Chemosynthia, and A. nomados is the sister-group to the clade composed of Actinoscyphiidae and its sister clade.

In the consensus tree, Epiparactis, Actinoscyphia and Anthosactis pearseae are sister to the clade we call Chemosynthia, and this clade is the sister to a group composed of Hadalanthus, Bathydactylus and the acontiarian 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 (1978a) 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 (1978a) further hypothesised that members of Actinoscyphiidae had lost aconitia. 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 acontiarian genera Hormathia and Acraspedanthus Carlgen, 1924 respectively. Sanamyan and Sanamyan (2007) considered Cyananthea and Epiparactis within Actinoscyphiidae, following the diagnosis given by Riemann-Zürneck (1978a). They also noted that the ring of cinclides in the distal column of Cyananthea strongly recalls Kadosactis, thereby relating Cyananthea to acontiates. 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 cnidome of Marianactis and Cyananthea. Based on these comparisons, 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 acontarian outgroups plus Hadalanthus and Bathycyclus. Thus, this analysis suggests a close relationship between Actinoscyphiidae and some Acontiaria.

Within Actinoscyphiidae is Chemosyntina, the clade containing the genera reported from hydrothermal vents and cold seeps. Anthosactis pearseae, known from whalefalls, is the sister-group to Chemosyntina in some but not all primary trees. Monophyly of Chemosyntina 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 Anthosacida, 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. jannamayenii, lies within Actinostolidae pending a comprehensive species-level revision of Anthosactis, we leave it in Actinoscyphiidae.

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 cryptic 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 Chemosyntina and its allies.

Acknowledgements

Specimens were collected by Janet Voight, with the assistance of the scientific party of cruise AT 11–03, the crew of the R/V Atlantis and the crew of the DSV Atlantis. J. Gerber of the FMNH helped in the accession and loan of the specimens. Collection of specimens was funded through NSF DEB-0072695 to J. Voight; their description was funded through NSF EF-0531763 to MD and a OSU CBS Dean’s Undergraduate Research award to CNC. J. Voight of the FMNH, S. Cairns of the USNM, R. Van Syoc of the CAS, and O. Tendal of the ZMUC provided comparative material. J. Wenzel gave advice on coding characters and identifying networks within the tree. A. Reif is thanked for her helpful advice and comments on cnidae.

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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
(6) 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

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Characters</th>
</tr>
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<tr>
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<td><strong>Anthosactis janmayeni</strong></td>
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</tr>
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<td><strong>Anthosactis nomados</strong></td>
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</tr>
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