



Description of *Calliactis tigris* sp. nov.: reconciling taxonomy and phylogeny in hermit-crab symbiotic anemones (Cnidaria: Actiniaria: Hormathiidae)

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Abstract

The symbiosis between sea anemones and hermit crabs is ubiquitous in the marine environment (except in the poles), occurring from shallow to deep waters; it involves one or more anemones living on a shell inhabited by a hermit crab. The anemone-crab partnership is a mutualism in which hermit crabs provide a hard substrate, increased access to oxygenated waters and food supply, in exchange for defense by the anemone. The vast majority of the sea anemone partners belong to three genera in family Hormathiidae: *Adamsia*, *Calliactis*, *Paracalliactis*. Given the remarkable nature of the symbiosis, hormathiid partners have been hypothesized to represent a monophyletic group. This has been rejected by Gusmão and Daly et al. (2010) and confirmed by our phylogenetic analysis using molecular markers (12S, 16S, 18S, 28S, COIII). We expand the results of Gusmão and Daly et al. (2010) by finding a monophyletic *Paracalliactis*, which was left untested in their analyses. Thus, characters of taxonomic significance associated to the symbiotic habit are interpreted as functional rather than phylogenetic. We reconcile taxonomy and the present evolutionary framework to avoid defining taxonomic groups based on characters prone to convergence. We formalize the synonymy of *Adamsia* and *Calliactis* and provide updated diagnoses for the valid genera *Calliactis* and *Paracalliactis* to bring more stability to the group. Under this new framework, we describe *Calliactis tigris* sp. nov. from Australia based on 21 specimens collected off the coast of New South Wales and Queensland and differentiate it from congeners and other hermit crab symbionts recorded in the Pacific Ocean.

Keywords Symbiosis · Mutualism · Convergence · *Adamsia* · *Paracalliactis* · Australia

Introduction

The symbiosis between sea anemones and hermit crabs is ubiquitous in the marine environment (except in the poles), occurring from shallow to deep waters; it involves one or more anemones living on a gastropod shell inhabited by a

hermit crab (Gusmão and Daly 2010). The anemone-crab partnership is a mutualism (Brooks and Gwaltney 1993; Mainardi and Rossi 1969) in which hermit crabs provide a hard substrate that prevents the anemone's burial and increase access to positive conditions, including oxygenated waters and food supply (Williams and McDermott 2004). In exchange, the anemones defend the crab from predators using their nematocysts (Ross 1971, 1974a; Ross and Boletzky 1979; Christidis et al. 1997). The presence of predators encourages acquisition of anemones by the crab in the laboratory (Balasch and Mengual 1973; Bach and Herrnkind 1980; Ross and Boletzky 1979) and reinforces the operational trigger of this association in nature with crabs carrying one or more anemones more likely to survive encounters with predators (Ross and Boletzky 1979; Brooks 1989). Some anemones may also protect the crab by producing a living “cloak” (i.e., carcinoecium) that expands the living space of the crab bypassing the necessity to change to bigger shells as they grow (Ross 1974a; Fautin 1992; Daly et al. 2004).

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Most sea anemone partners belong to three genera within family Hormathiidae Carlgren 1932: *Adamsia* Forbes 1840, *Calliactis* Verrill 1869, and *Paracalliactis* Carlgren 1928a. These genera have been hypothesized to represent a single monophyletic group given the remarkable nature of the symbiotic behavior exhibited by the partners (e.g., Ross 1974b; Ates 1997). This idea was supported by the related morphological adaptations seen in the hormathiid partners that form a morphological complex whose members are distinguished by a mosaic of characters (Hand 1975; Daly et al. 2004). Attributes of the symbiosis, such as the adaptation of the pedal disc to life on a shell and secretion of a carcinoecium, unite members of *Adamsia* and *Paracalliactis* and distinguish them from those in *Calliactis*. On the other hand, both *Calliactis* and *Adamsia* differ from *Paracalliactis* in having cinclides in the column. Carlgren (1928b) described the genus *Paracalliactis* as biological intermediate between *Adamsia* and *Calliactis*. Because no support for the monophyly of hormathiid hermit-crab symbiotic anemones was recovered in the multilocus analysis of Gusmão and Daly et al. (2010), similarities in morphology and behavior among these symbiotic anemones have to be interpreted as related to the ways in which a symbiotic habit arises and is maintained, not to shared evolutionary history.

Here, we reconcile the taxonomy of hormathiid hermit-crab symbionts with the present evolutionary framework for the group provided by the phylogenetic analyses of five nuclear and mitochondrial markers (12S, 16S, 18S, 28S, COIII). We formalize the findings of Gusmão and Daly et al. (2010) confirmed in our analysis, particularly the synonymy of genera *Adamsia* and *Calliactis*. We also expand the results of Gusmão and Daly et al. (2010) by finding a monophyletic *Paracalliactis*, which was left untested in their analyses. Updated diagnoses for the valid symbiotic hormathiid genera *Calliactis* and *Paracalliactis* are provided to help with taxonomic identification and bring more stability to this widespread group of sea anemones. Finally, we describe *Calliactis tigris* sp. nov. from Australia based on 21 specimens collected off the coast of New South Wales and Queensland and differentiate it from congeners as well as other hermit crab symbionts recorded in the Pacific Ocean.

Material and methods

Morphological study

The description of *Calliactis tigris* sp. nov. was based on 21 specimens collected in eastern Australia (Fig. 1) deposited in the Australian Museum (AM G.15586, AM G.16897, AM G.16907, AM G.17482-17486, AM G.17489, AM G.17491). External and internal morphology is described based on formalin-fixed specimens that were examined whole, dissected, and in serial sections. For microanatomical

examination, longitudinal and cross-sectional serial sections 10 μm thick were made using standard paraffin techniques and stained with Heidenhain's Azan Stain (Presnell and Schreiber 1997). Undischarged cnidae capsules were identified and measured in squash preparations of tissue from column, tentacles, actinopharynx, filaments, and acontia of five preserved specimens using differential interference microscopy (DIC) at $\times 1000$ magnification. Except for the rarer types, at least 20 capsules were measured. The presence of each type of cnidae in each tissue was confirmed in histological slides. We follow a nematocyst terminology that combines the classification of Weill (1934) modified by Carlgren (1940), thus differentiating "basitrichs" from "*b*-mastigophores" with that of Schmidt (1969, 1972, 1974) which captures the underlying variation seen in "rhabdoids" (see Gusmão et al. 2018 for more details). We include photographs of each type of nematocyst for reliable comparison across terminologies and taxa (see Fautin 1988). Higher-level classification for Actiniaria follows Rodríguez et al. (2014).

Molecular data collection and analysis

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

Newly generated sequences have been deposited in GenBank and combined into a dataset of sequences included in Rodríguez et al. (2014), Larson and Daly (2016), and Daly et al. (2017) with selected taxa within the suborder Anenthemonae Rodríguez and Daly, 2014 used as outgroups (Online Resource). The concatenated dataset has been



Fig. 1 Collection sites for *Calliactis tigris* sp. nov. off the coast of Australia, including the type locality off the coast of New South Wales (star) and additional localities off the coast of New South Wales (triangles) and Queensland (circle)

deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S24279>). Sequences for each marker were aligned separately in MAFFT v.7.0 online at <http://mafft.cbrc.jp/alignment/server/> (Katoh and Standley 2013; Katoh et al. 2017) using the following settings: strategy L-INS-I; scoring matrix for nucleotide sequences, 200 PAM/k = 2; gap open penalty, 1.53; offset value, 0.05. Alignments for each marker were analyzed separately and as a concatenated dataset.

Maximum likelihood (ML) analyses were performed for each marker separately (data not shown) and as a concatenated dataset on RAxML-NG v0.6.0 (Kozlov et al. 2018) using models of nucleotide substitution established for each gene partition using the Akaike information criterion (AIC) in JModeltest2 implemented on the Cipres Portal (Miller et al. 2010). The Majority Rule Criterion implemented in RAxML (-autoMRE) was used to assess clade support. Tree searches under maximum parsimony (MP) were conducted in TNT v1.1 (Goloboff et al. 2008) using random and constrained sectorial searches, tree drifting, and 100 rounds of tree fusing. Trees of minimum length were found at least ten times. The concatenated dataset was subjected to 1000 bootstrap rounds to assess clade support on the obtained strict consensus tree. Gaps were treated as missing data in all analyses.

Micro-CT scanning

In order to evaluate the growth pattern of mesenteries and their number along the column, one specimen of *Calliactis tricolor*

(Le Sueur 1817) (AMNH-IZ-3549) fixed and preserved in 10% formalin was stained with 1% osmium tetroxide for micro-CT scanning. For details about the staining protocol used here, see Gusmão et al. (2018). The material was scanned in 100% ethanol on a phoenix v|tome|x s240 GE (General Electric, Fairfield, CT, USA) at 60 kV and 70 μ A with a molybdenum target, for a final resolution of 25 μ m/voxel; exposure time for detector was 750 ms. The software Phoenix datos|x (General Electric, Wunstorf, Germany) was used to reconstruct the raw data and the resulting images were processed and edited using the software 3D slicer (Fedorov et al. 2012). Full micro-CT scan data are deposited in the Morphobase (www.morphosource.org) under project AMNH-IZ-3549.

Results

Taxonomic description

Order Actiniaria Hertwig 1882

Suborder Enthemonae Rodríguez and Daly in Rodríguez et al. 2014

Superfamily Metridioidea Carlgren 1893

Family Hormathiidae Carlgren 1932

Diagnosis (modified from Carlgren 1949; Daly et al. 2004; Rodríguez et al. 2012; additions in italics).

Enthemonae with distinct pedal disc with basilar muscles and strong mesogleal marginal sphincter. Mesenteries not

divisible into macro and microcnemes. Usually six pairs of perfect mesenteries, sometimes more, but never numerous. Perfect mesenteries rarely fertile. Acontia with basitrichs *or with basitrichs and p-mastigophores* B1. Spirocysts usually dimorphic: gracile spirocysts with narrower tubule and smaller capsule than robust spirocysts. Cnidom: robust and gracile spirocysts, basitrichs, *p-mastigophores* B1.

Included genera. *Actinauge* Verrill 1883; *Allantactis* Danielssen 1890; *Calliactis*; *Cataphellia* Stephenson 1929; *Chondrophellia* Carlgren 1925; *Haddonactis* Fautin 2016; *Handactis* Fautin 2016; *Hormathianthus* Carlgren 1943; *Gliactis* Gravier 1918; *Monactis* Riemann-Zürneck 1986; *Paracalliactis*; *Paraphelliactis* Carlgren 1928b; *Phelliactis* Simon 1892; *Stephanauge* Verrill, 1899 (according to Fautin 2016; except for *Amphianthus* Hertwig 1882 and *Stephanauge* Verrill, 1899 which were transferred to Amphianthidae Hertwig, 1882 by Rodríguez et al. 2012).

Type genus. *Hormathia*

Remarks. We made minor modifications to the familial diagnosis to reflect recent changes in higher-level classification of Actiniaria (i.e., Rodríguez et al. 2012, 2014) and the terminology used to classify nematocysts in this study (see Gusmão et al. 2018). These modifications are made in diagnoses throughout this study which have also been rearranged so that they are in parallel. We added the possibility of having *p-mastigophores* B1 in addition to basitrichs in the acontia of members of the family based on mounting evidence of long and narrow *p-mastigophores* B1 in the acontia of *Hormathia pectinata* (Hertwig 1882) in the Southwestern Atlantic (Riemann-Zürneck 1973; LCG personal observation).

Genus *Calliactis* Verrill 1869

Diagnosis (modified from Carlgren 1949, England 1971, Daly et al. 2004; additions in italics).

Hormathiidae with well-developed pedal disc, equal to slightly larger in diameter than oral disc, *or broad and bilobed*; may secrete a thin cuticle *or a carcinoecium*. Column smooth, rarely with tubercles; divisible into scapus and *short* scapulus; scapus with numerous epidermal pits and a thin, deciduous cuticle. Cinclides always present in proximal column, *sometimes inconspicuous*. Column with basitrichs *or basitrichs and p-mastigophores* B1. Tentacles in several cycles, their longitudinal muscles ectodermal. Strong, mesogleal marginal sphincter muscle present. Mesenteries hexamerously arranged, more numerous distally; six pairs of perfect and sterile mesenteries, including two pairs of directives each associated to a siphonoglyph. Retractor and parietobasilar muscles weak. Species often live attached to gastropod shells inhabited by hermit crabs with oral disc directed away from the aperture of the shell *or directly below the aperture*.

Type species. *Actinia decorata* Couthouy in Dana 1846 by original designation.

Included species. *Calliactis algoensis* Carlgren 1938; *C. androgyna* Riemann-Zürneck 1975; *C. annulata* Carlgren

1922, *C. argentacolorata* Pei 1996; *C. brevicornis* (Studer 1879); *C. conchiola* Parry 1952; *C. japonica* Carlgren 1928a, *C. parasitica* (Couch 1842); *C. polypores* Pei 1996; *C. polypus* (Forsskål 1775); *C. reticulata* Stephenson 1918; *C. tricolor*; *C. variegata* Verrill 1869; *C. xishaensis* Pei 1996; *C. palliata* (Müller 1776) comb. nov., *C. sociabilis* comb. nov. (Verrill 1882).

Remarks. Because *Calliactis palliata* comb. nov. nested within a clade of *Calliactis* with high support in our phylogenetic analyses and previous studies (e.g., Gusmão and Daly 2010; Rodríguez et al. 2012, 2014). We transfer two former valid species of *Adamsia* (*A. palliata*, *A. sociabilis*) to *Calliactis* (*A. obvolva* is transferred to *Paracalliactis*: see “Discussion”). We modify the diagnosis of *Calliactis* to reflect this decision. These modifications include the possibility of having a bi-lobed pedal disc, oral disc positioned directly below the aperture of the shell, and secretion of a carcinoecium which are considered specific to *C. sociabilis* comb. nov. and *C. palliata* comb. nov. (see “Discussion” for more details). After examination of many specimens of *C. palliata* comb. nov., we consider that the observation of 12 pairs of perfect mesenteries is an artifact of cross-sections taken very high up the column before the second cycle of mesenteries.

According to the Principle of Priority (Art. 23, ICZN 1999), *Adamsia* is the senior subjective synonym and thus must be used over the junior synonym, *Calliactis*. Although the genus name *Calliactis* currently has a broader use than *Adamsia* (particularly in non-taxonomic works: e.g., Spagnuolo et al. 1994; Williamson et al. 2000; Wiedenmann et al. 2004; Stewart et al. 2017; including having a toxin named after it—calitoxin: Cariello et al. 1989), this name does not fulfill the requirements for a reversal of precedence of a junior synonym—i.e., Art. 23.9.1 of the Code—ICZN 1999). *Calliactis* (15 species, including *C. tigris* sp. nov.), however, is more speciose than *Adamsia* (two species) and generally encompasses species living in shallower habitats that are more accessible. In our opinion, the use of the senior synonym (*Adamsia*) will cause confusion and threaten stability, thus we wish to maintain the use of the junior synonym (*Calliactis*). Following Art. 23.9.3 of the Code (ICZN 1999), the matter has been referred to the ICZN (Rodríguez and Gusmão submitted: Case 3805) and is awaiting resolution. While the case is under consideration, the junior name is to be maintained (ICZN 1999); thus, here we use *Calliactis* for species formerly included in genus *Adamsia*.

We also included in the diagnosis the presence of tubercles in the column of *Calliactis* given that some species do not have a fully smooth column and some tubercles are visible in live or freshly collected specimens (e.g., *C. japonica*, *C. tricolor*, *C. polypus*: Gusmão 2010). We replaced the more general term “ectodermal invagination” to describe the invaginations of the epidermis of the column into the mesoglea with “epidermal pit,” which was used by Hand (1975; as

ectodermal pit), but not incorporated to the diagnosis of *Calliactis* in his study. We corrected the information regarding the proportion of tentacles to mesenteries proximally based on our finding of an extra cycle of mesenteries proximally in *C. tigris* sp. nov. and confirmed in micro-CT scans of *C. tricolor* (see “Discussion”). We also added the possibility of having *p*-mastigophores B1 in addition to basitrichs in the column based on mounting evidence for the presence of these nematocysts in the column of *Calliactis* species (e.g., *C. tigris* sp. nov., *C. conchiola*, *C. polypus*). The lack of *p*-mastigophores B1 in the column of other *Calliactis* species is likely due to the general scarcity of nematocysts in the column compared to other types of tissue (e.g., actinopharynx or filaments).

Calliactis tigris sp. nov.

Material. Holotype: AM G.17486 (P.77572), F.R.V. Kapala Cruise, Sta. K85-21-06, north east of Long Reef, New South Wales, Australia, 33°42' 00" S, 151° 54' 00" E, 466 m depth, 19 December 1985. Paratypes: AM G.16907 (1 specimen), northeast of Long Reef, New South Wales, Australia, 33° 42' 00" S, 151° 54' 00" E, 450 m; AM G.17489 (4 specimens), F.R.V. Kapala, between Sydney and Port Stephens, New South Wales, Australia, 33° 49' 60.00" S, 151° 13' 00" E, 365 m depth, July 1972; AM G.17482 (2 specimens), F.R.V. Kapala, Sta. K71-10-02, east of Bulli, New South Wales, Australia, 34° 15' 00" S, 151° 25' 00" E, 275 m depth, 28 June 1971.

Additional material. AM G.15586 (2 specimens), east of Mooloolaba, Queensland, Australia, 26° 52' 44" S, 153° 35' 20" E, 350 m depth; AM G.16897 (2 specimens), east of Mooloolaba, Queensland, Australia, 26° 52' 44" S, 153° 35' 20" E, 300 m depth; AM G.17484 (2 specimens), Sta. SEAS QLD 1119, east of Mooloolaba, Queensland, Australia, 26° 52' 44" S, 153° 35' 20" E, 160 m depth, 3 August 1994, collected by J.K. Lowry, K. Dempsey & J. McIlwain; AM G.17491 (2 specimens), east of Mooloolaba, Queensland, Australia, 26° 52' 44" S, 153° 35' 20" E, 312 m depth, 3 August 1994, collected by J.K. Lowry & K. Dempsey; AM G.17483 (2 specimens), Sta. SEAS QLD 1119, east of Mooloolaba, Queensland, Australia, 26° 52' 44" S, 153° 35' 20" E, trawl, 160 m depth, 3 August 1994, collected by J.K. Lowry, K. Dempsey & J. McIlwain; AM G.17485 (P.77571) (3 specimens), Sta. SEAS QLD 1119, east of Mooloolaba, Queensland, Australia, 26° 52' 44" S, 153° 35' 20" E, 160 m, 3 August 1994, collected by J.K. Lowry, K. Dempsey & J. McIlwain.

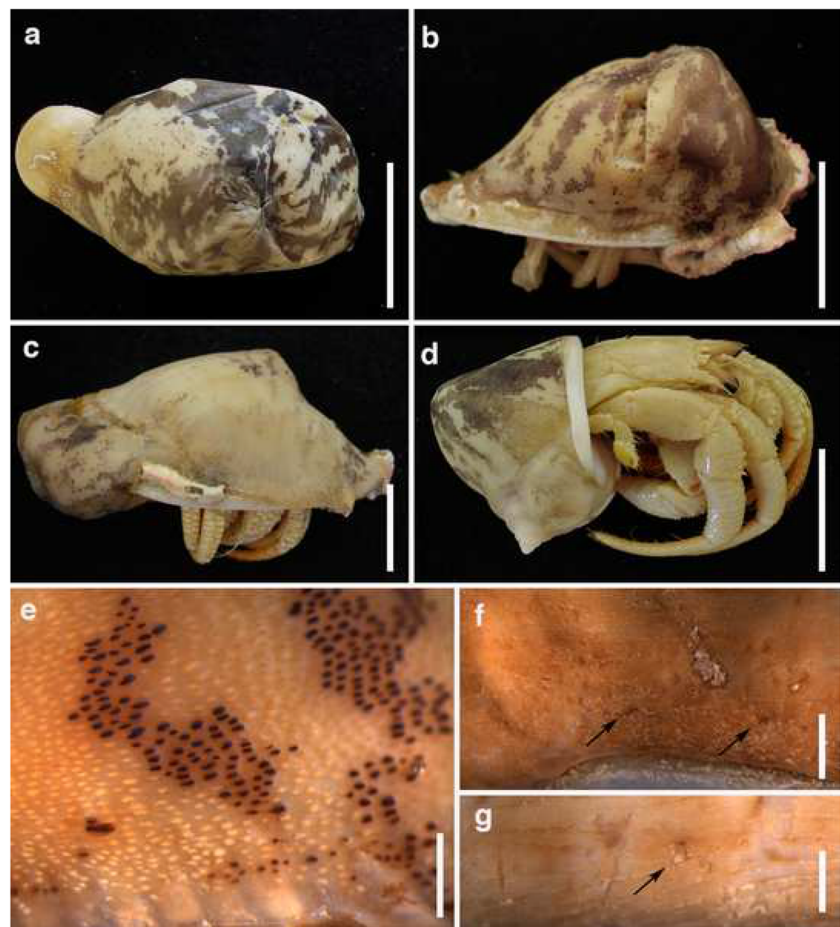
Material examined for comparison. *Calliactis japonica* UUZM 688a (1 specimen, syntype), Gote Island, Misaki, Kanagawa-ken, Sagami Bay, Honshu, Japan, 34° 00' 00" N, 132° 36' 00" E, 165 m depth; UUZM 688b (1 specimen, syntype), Gote Island, Misaki, Kanagawa-ken, Sagami Bay, Honshu, Japan, 34° 00' 00" N, 132° 36' 00" E, 110 m depth; USNM 20290 (4 specimens), Suruga Bay, Honshu Island,

Shizuoka, Japan, 35° 01' 58.8" N, 138° 46' 1.2" E, 110–128 m depth; USNM 50291 (2 specimens), Hong Kong, China, 21° 30' 00" N, 116° 31' 58.8" E, 256 m depth. *Calliactis polypus* USNM 50481 (3 specimens), 2 miles South of Agat Bay, Guam, North Pacific Ocean, August 1947, collected by G.S. Mansfield; USNM 50475 (3 specimens), Carolina Islands, Micronesia, North Pacific Ocean, 9° 32' 31.45" N, 138° 3' 21.60" E, Sta. Y-258, collected by R.W. Hiatt, ID by C.E. Cutress; AM G.17480 (4 specimens), Sta. NSW 1901, south of Smoky Cape, south side of Fish Rock, New South Wales, Australia, 30° 56' 27" S, 153° 05' 58" E, 20 m depth, 14 February 2002, hand collected on SCUBA by R.T. Springthorpe. *Calliactis tricolor* AMNH 3549 (12 specimens), North Beach, St. Catherines Island, Georgia, collected by C. Boyko & W. Sage III, 21 May 1994. *Adamsia palliata* YPM IZ-7145 (4 specimens), off Port Vendres, France, Mediterranean Sea, Atlantic Ocean, collected by Staff of the Laboratoire Arago, ID by Willard D. Hartman, 60–100 m, 25 April 1958. YPM IZ-7146 (4 specimens), off Port Vendres, France, Mediterranean Sea, Atlantic Ocean, collected by Staff of the Laboratoire Arago, ID by Willard D. Hartman, 60–100 m, 25 April 1958.

External anatomy (Fig. 2). Pedal disc broad, often circular, adherent, irregularly shaped, wider than column diameter, never bi-lobed or covering the shell completely (Fig. 2a–d); two specimens may coexist in the same shell (Fig. 2c). Pedal disc diameter 17.0–80.0 mm in preserved specimens; carcinoecium absent, but a thin cuticle may be present. Column cylindrical, usually narrower than diameter of pedal disc, with characteristic pattern in preserved specimens: column white or beige with numerous purple spots not forming longitudinal stripes as in other *Calliactis* species, but irregular patches (Fig. 2a–d) formed by purple colored epidermal pits visible to the naked eye on scapus (Fig. 2e). Column firm, with thick mesoglea, divided into scapus and scapulus with differing epidermal thickness and cnidae content; scapus with thick epidermis and longer basitrichs; scapulus with thin epidermis and short basitrichs. Scapus with thin, deciduous cuticle sometimes present. One row of inconspicuous cinclides on limbus close to pedal disc; these communicate to endocoel of first or second cycle mesenteries; in some individuals cinclides hardly visible unless acontia are present (Fig. 2f, g). Column 15.0–59.0 mm height and 19.0–75.0 mm diameter in preserved specimens. Oral disc always located opposite to gastropod shell aperture (Fig. 2a–d); when relaxed, oral disc circular and wide, slightly wider than pedal disc, diameter 2.0–20.0 mm in contracted specimens. Up to 110 tentacles restricted to outer half of oral disc, hexamerously arranged in 5 cycles (6 + 6 + 12 + 24 + 48 + n); tentacles short, conical, inner longer than outer ones; white/beige with a longitudinal darker line along its length.

Internal anatomy and microanatomy (Fig. 3). Body wall with thick mesoglea 2690–3102 μm and gastrodermis 46–

Fig. 2 External anatomy of preserved specimens of *Calliactis tigris* sp. nov. **a** Oral view of a specimen with characteristic column coloration pattern. **b** Lateral view of a specimen attached to a shell; note remains of cuticle produced by pedal disc. **c** Two specimens attached to a single shell, one of which has lost part of its coloration pattern. **d** Lateral view of a specimen attached to a shell with hermit crab visible. **e** Detail of epidermal pits on proximal column; note white and purple epidermal pits. **f** Cinclides on proximal column (arrows). **g** Detail of a cinclide (arrow). Scale bars, **a** 50 mm, **b** 30 mm, **c** 21 mm, **d** 30 mm, **e** 1.6 mm, **f** 4 mm, **g** 2 mm



59 μm (Fig. 3a); epidermis thinnest in scapulus (5–9 μm) (Fig. 3b), thickest at distal scapus (27–80 μm) with intermediate thickness at mid-scapus (33–47 μm). Numerous epidermal pits (i.e. invaginations of the epidermis of the column into the mesoglea) on scapus (Fig. 3c); these are mostly single (Fig. 3d), rarely fused together (Fig. 3e), always open to the exterior (Fig. 3c–e); diameter of major axis 117–318 μm . Marginal sphincter muscle mesogleal, well-developed (Fig. 3a), occupies most of mesoglea close to base of tentacles (Fig. 3a, b) decreasing proximally at distal scapus, always closer to epidermis than gastrodermis (Fig. 3a); alveolar with tendency to horizontal stratification distally (Fig. 3f), less so proximally (Fig. 3g). Tentacle longitudinal muscle ectodermal (Fig. 3h); tentacles without basal mesogleal thickening (Fig. 3i). Forty-eight pairs of mesenteries, hexamerously distributed in four cycles (6 + 6 + 12 + 24) distally (Fig. 3j) and at mid-column (Fig. 3k); incomplete fifth cycle of mesenteries only proximally very close to the base (32 pairs; 80 pairs of mesenteries total). Mesenteries of first cycle perfect, sterile, including two directive pairs (Fig. 3j) each associated to a well-developed siphonoglyph; second and third cycles imperfect, fertile (Fig. 3l–n); fourth cycle imperfect, sterile (Fig. 3l, o); mesenteries of fifth cycle poorly developed, found only very proximally. Mesenteries more developed proximally (Fig. 3k).

Mesenterial filaments and acontia restricted to first, second and third cycles at mid-scapus (Fig. 3l); mesenteries of first to fourth cycles with filament and acontia proximally. Retractor muscle diffuses in cycles 1–4 (Fig. 3l); absent from mesenteries of fifth cycle. Parietobasilar muscle weak in mesenteries of first to third cycles (Fig. 3l–n); stronger in mesenteries of fourth cycle, but with no mesogleal flap (Fig. 3o). Basilar muscle strong (not shown). Species gonochoric: major axis of oocytes 23–58 μm in diameter; major axis of spermatid cysts 34–93 μm in diameter.

Cnidom. Gracile and robust spirocysts, basitrichs, and *p*-mastigophores B1 (Fig. 4). For size and distribution, see Table 1.

Natural history and distribution. Usually only one specimen is found per shell, rarely two individuals cohabit a single shell (Fig. 2c). *Calliactis tigris* sp. nov. is known only from the western Pacific Ocean, off the coast of New South Wales and Queensland in Australia. The new species has been collected between 166 and 508 m depth.

Etymology. The species epithet “*tigris*” refers to the characteristic feline-like coloration pattern of the column of species of *Calliactis*, more specifically the tiger-like coloration pattern of *C. tigris* sp. nov.

Genus *Paracalliactis* Carlgren 1928a

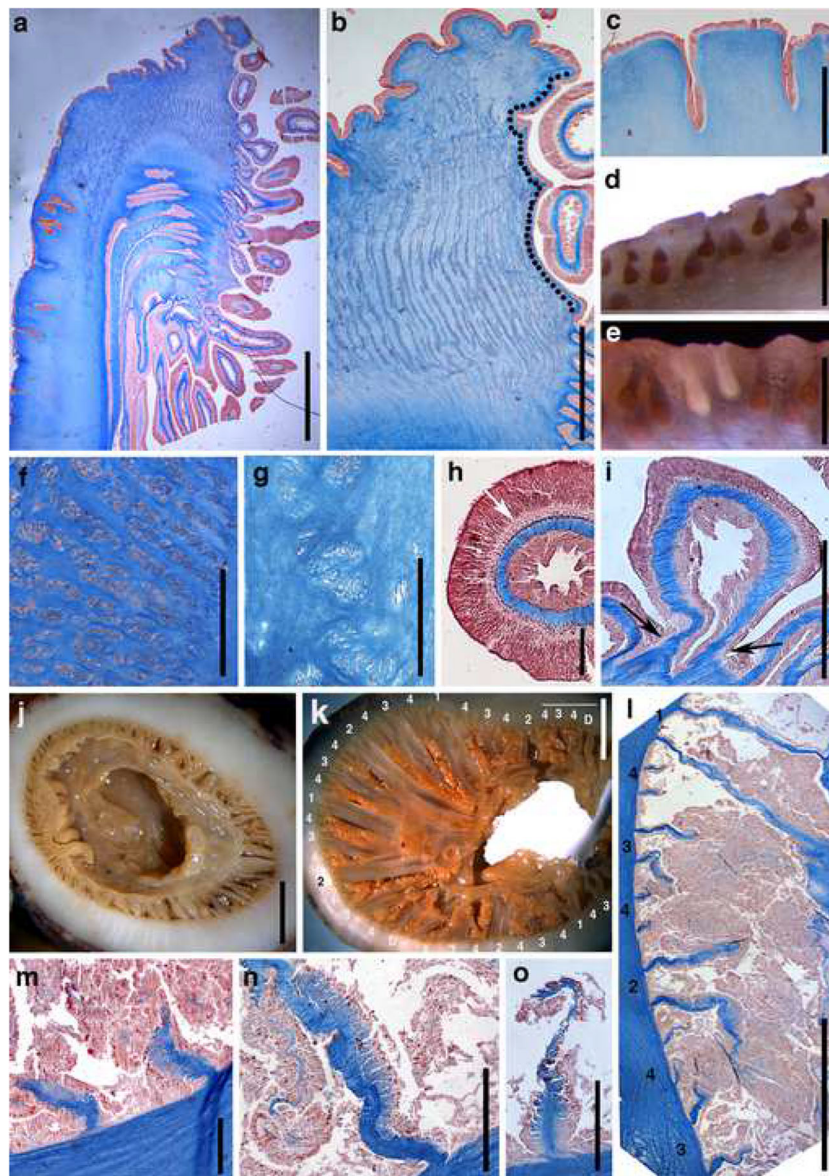


Fig. 3 Internal anatomy and microanatomy of *Calliactis tigris* sp. nov. **a** Longitudinal histological section through distal column showing marginal mesogleal sphincter muscle. **b** Detail of longitudinal histological section through distal column showing short capitulum (dotted line). **c** Longitudinal histological section through distal scapus showing two epidermal pits. **d** Epidermal pits on scapus under cross-section ($0.8\times$). **e** Detail of epidermal pits under higher magnification ($2.25\times$). **f** Detail of alveolar arrangement of distal marginal mesogleal sphincter muscle; note tendency for horizontal stratification. **g** Detail of alveolar arrangement of proximal marginal mesogleal sphincter muscle. **h** Histological cross section of a tentacle showing longitudinal ectodermal muscle (arrow). **i** Histological longitudinal section through tentacle

without basal thickening (arrows). **j** Cross section through distal column showing 48 pairs of mesenteries attached to oral disc. **k** Cross section through proximal column showing pairs of mesenteries distributed in four cycles (cycles indicated by numbers). **l** Histological cross-section through mid-scapus showing four cycles of mesenteries (indicated by numbers); note a pair of first cycle of mesenteries still attached to siphonoglyph. **m** Detail of mesenteries of third cycle; note diffuse retractor and weak parietobasilar muscles. **n** Detail of mesentery of second cycle; note diffuse retractor muscle. **o** Detail of mesentery of fourth cycle; note weak retractor and well-developed parietobasilar muscle. *D* directive pair of mesenteries. Scale bars, **a, j–l** 4 mm, **b** 2 mm, **c** 0.4 mm, **e, l, n** 1 mm, **f–h** 0.35 mm, **i** 0.03 mm, **m** 0.5 mm, **o** 0.2 mm

Diagnosis (modified from Carlgren 1949, Hand 1975, Daly et al. 2004; additions in italics).

Hormathiidae with well-developed, broad, and asymmetric pedal disc *but not bi-lobed as in some Calliactis species*. Pedal disc slightly wider than oral disc; secretes carcinoecium that may or may not project beyond shell aperture. Column

divisible into scapus and scapulus; smooth or with distal tubercles that may form a complete corona; may have a thin, easily deciduous cuticle. No cinclides. Column with basitrichs and *p*-mastigophores B1. Tentacles hexamerously arranged, about the same number as mesenteries proximally, their longitudinal muscles ectodermal. Strong, mesogleal marginal

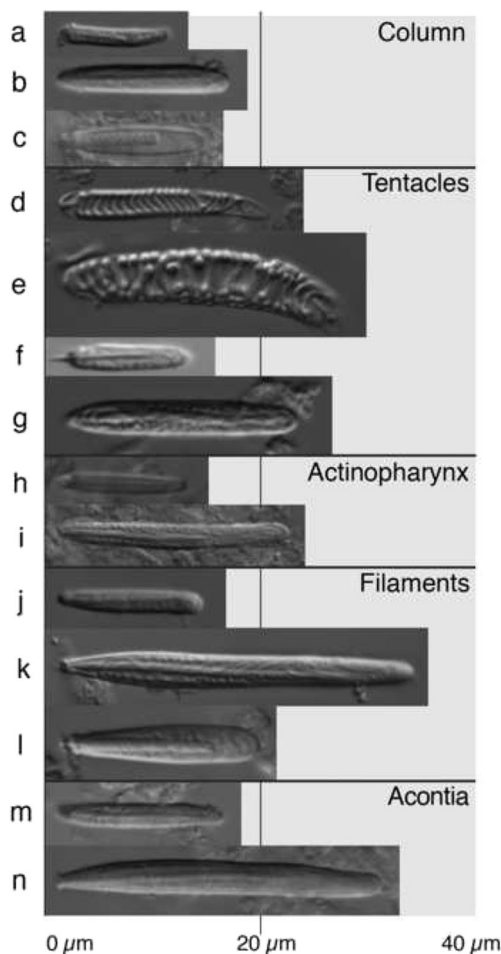


Fig. 4 Cnidom of *Calliactis tigris* nov. sp. **a, b, f–k, m, n** Basitrich. **c, l** *P*-mastigophore B1. **d** Gracile spirocyst. **e** Robust spirocyst

sphincter muscle present. About the same number of mesenteries proximally and distally; six pairs of perfect and sterile mesenteries, including two pairs of directives each associated to a siphonoglyph. Retractor and parietobasilar muscles weak. Species live attached to gastropod shells inhabited by hermit crabs with oral disc positioned directed away from the aperture of the shell (dorsally) or below the aperture of the shell (ventrally).

Type species. *Paracalliactis valdiviae* Carlgren 1928a by original designation.

Included species. *Paracalliactis azorica* Doumenc 1975; *P. consors* (Verrill 1882); *P. mediterranea* Ross and Zamponi 1982; *P. michaelsarsi* Carlgren 1928a; *P. obvolva* comb. nov. (Daly et al. 2004); *P. rosea* Hand 1975; *P. sinica* Pei 1982; *P. stephensoni* Carlgren 1928a; *P. valdiviae* Carlgren 1928a.

Remarks. We maintain the original intention of Carlgren (1928a) for the genus but modify the diagnosis of the genus to differentiate the asymmetry of the pedal disc of *Paracalliactis* species from the bi-lobed shape of those seen in some *Calliactis* species formerly belonging to *Adamsia* (*C. sociabilis* comb. nov., *C. palliata* comb. nov.). Although

the position of the anemone on the shell has been shown to vary within hermit-crab genera and not carry phylogenetic signal (see “**Discussion**”), we included this information in the diagnosis given the uniqueness of this feature within the order Actiniaria which may aid in its generic placement. After examination of the holotype (KUNHM 01595) and paratypes (KUNHM 01591, USNM 1004627, USNM 1004630) of *P. obvolva* comb. nov., we found that the cinclides described by Daly et al. (2004) for the species are likely an artifact and result from observation of longitudinal ridges characteristic of species of *Paracalliactis* that may become indented both longitudinally and transversely (Doumenc 1975; Hand 1975; personal observation) and give the impression of small perforations in the column, similar to cinclides. The location of the cinclides described for *P. obvolva* comb. nov. (i.e., mid-column) also indicates they might not be true cinclides as these are restricted to the proximal part of the column close to the pedal disc as in all species of *Calliactis* (after adjusted diagnosis). Under close inspection, the structures observed by Daly et al. (2014) are not cinclides, which precludes the inclusion of *P. obvolva* comb. nov. within *Calliactis*. In addition, because *P. obvolva* comb. nov. presents other features characteristic of *Paracalliactis* (e.g., asymmetrical pedal disc), this species is transferred to *Paracalliactis* (*P. obvolva* comb. nov.). In addition, we agree with Hand (1975) and do not consider *P. lacazei* Dechancé and Dufaure 1959 a valid species as it possesses cinclides in the proximal column. Whether *P. lacazei* should be transferred to *Cataphellia* as suggested by Hand (1975) is uncertain, pending a detailed examination of this species. Unfortunately, no specimens of *P. lacazei* were available for study.

Micro-CT scanning

Micro-CT scans of *Calliactis tricolor* (Fig. 5) showed an almost complete fifth cycle of mesenteries proximally (i.e., more mesenteries proximally; Fig. 5f–i), but we did not observe corresponding mesenteries of a fifth cycle distally close to the margin (Fig. 5a, b). A similar pattern was observed in dissected specimens of *C. tigris* sp. nov. and *C. tricolor*, suggesting that in these species mesenteries develop in the proximal end of the body and grow upwards towards the oral disc but might not reach it (as opposed to growing simultaneously at the proximal and distal end of the body; see Carlgren 1927).

Phylogenetic relationships

The concatenated dataset of five markers comprising the newly generated sequences and those downloaded from GenBank resulted in a dataset of 191 taxa and 8237 sites. Trends/patterns in marker variability followed those previously reported for Actiniaria (e.g., Daly et al. 2010; Gusmão and Daly et al. 2010; Rodríguez et al. 2012, 2014; Lauretta et al. 2014). In

Table 1 Size ranges of the cnidae of *Calliactis tigris* sp. nov. X, mean; SD, standard deviation; S, proportion of specimens in which each cnidae was found; N, total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; *, rare

Categories	Range of length and width of capsules (μm)	X \pm SD	S	N	F	<i>Calliactis japonica</i> (Gusmão 2010)	<i>Calliactis conchiola</i> (Hand 1975a)
Scapus							
Basitrichs (a, b)	07.5–18.1 \times 1.3–2.5	15.1 \pm 1.9 \times 1.8 \pm 0.2	219	5/5	++	10.2–17.7 \times 1.1–1.8	10.0–13.0 \times 1.0–1.5
Basitrichs	–	–	–	–	–	–	14.0–20.0 \times 2.0–3.0
<i>P</i> -mastigophores A (c)	15.9–17.2 \times 2.7–3.1	16.7 \pm 0.6 \times 3.0 \pm 0.2	48	3/5	+	–	15.0–20.0 \times 2.5–3.0*
Tentacles							
Spirocysts (d, e)	12.4–37.1 \times 2.0–6.6	25.1 \pm 5.1 \times 3.8 \pm 0.1	234	5/5	+++	16.9–32.6 \times 1.9–3.9	15.0–30.0 \times 2.0–3.5
Basitrichs (f)	14.4–22.7 \times 1.6–3.5	18.7 \pm 1.7 \times 2.1 \pm 0.5	211	5/5	+++	18.3–25.0 \times 1.8–2.4	10.0–13.0 \times 1.0–1.5*
Basitrichs	–	–	–	–	–	–	20.0–25.0 \times 2.0–3.0
Pharynx							
Basitrichs	–	–	–	–	–	–	11.0–14.0 \times 1.0–1.5
Basitrichs II (i)	18.1–24.4 \times 1.8–2.4	21.5 \pm 1.7 \times 2.0 \pm 0.2	175	5/5	+++	22.7–33.2 \times 2.2–3.9	22.0–31.0 \times 2.5–3.5
<i>P</i> -mastigophores A	–	–	–	–	–	17.6–23.8 \times 2.3–3.6	17.0–20.0 \times 4.0–5.0*
Filament							
Basitrichs I (k)	10.5–15.4 \times 1.9–2.3	12.5 \pm 1.3 \times 2.1 \pm 0.1	79	4/5	++	–	11.0–14.0 \times 1.0–1.5
Basitrichs II (l)	19.3–42.5 \times 2.2–4.1	32.6 \pm 6.4 \times 3.5 \pm 0.4	20	4/5	+	18.2–26.8 \times 2.3–3.5	–
<i>P</i> -mastigophores A (m)	14.2–17.9 \times 2.4–3.4	16.4 \pm 0.8 \times 3.0 \pm 0.3	254	5/5	+++	24.9–35.8 \times 2.6–3.9	18.0–25.0 \times 2.5–3.5
ACONTIA							
Basitrichs I (n)	12.0–19.7 \times 1.6–2.1	16.1 \pm 2.4 \times 1.9 \pm 0.1	61	5/5	+	–	10.0–14.0 \times 1.0–1.5
Basitrichs II (o)	26.1–34.9 \times 1.7–3.2	31.5 \pm 2.2 \times 2.5 \pm 0.4	325	5/5	+++	35.7–42.3 \times 2.3–3.2	22.0–34.0 \times 3.0–4.0

general, the model-based topology showed greater support than the most parsimony trees, but both ML and MP phylogenetic analyses of the concatenated dataset agreed in basic relationships (at different resolution levels). The topology resulting from ML analysis of the concatenated dataset is provided with bootstrap values for MP and ML analyses (Fig. 6: only support values > 50% are shown). The monophyly of the two suborders and five actiniarian superfamilies were recovered in both ML and MP analyses, though those within Enthemonae are weakly supported compared to those in Anenthemonae as in most previous phylogenetic analyses of Actiniaria to date (e.g., Daly et al. 2008; Rodríguez et al. 2012, 2014).

The superfamily Metridioidea is comprised of clades Cuticulata and Acuticulata of Rodríguez et al. (2014), except for *Sagartia elegans* Dalyell 1848 and a clade containing *Cereus pedunculatus* (Pennant 1777), *Neoaiptasia* Parulekar 1969, *Actinothoe* Fischer 1889, and *Anthothoe* Carlgren 1938, which are resolved outside of these clades as found by previous studies (e.g. Rodríguez et al. 2014; Grajales and Rodríguez 2016; Gusmão et al. 2018). Within the metridioidean clade Cuticulata of Rodríguez et al. (2014), we recovered a monophyletic *Calliactis* (sensu this contribution) in both MP and ML analyses, though with low bootstrap support (< 50%). A sister relationship between *C. tigris* sp. nov. and *C. japonica* is recovered with high bootstrap support (100%) in both analyses. The two species of *Paracalliactis* included are recovered as each other's closest relative with

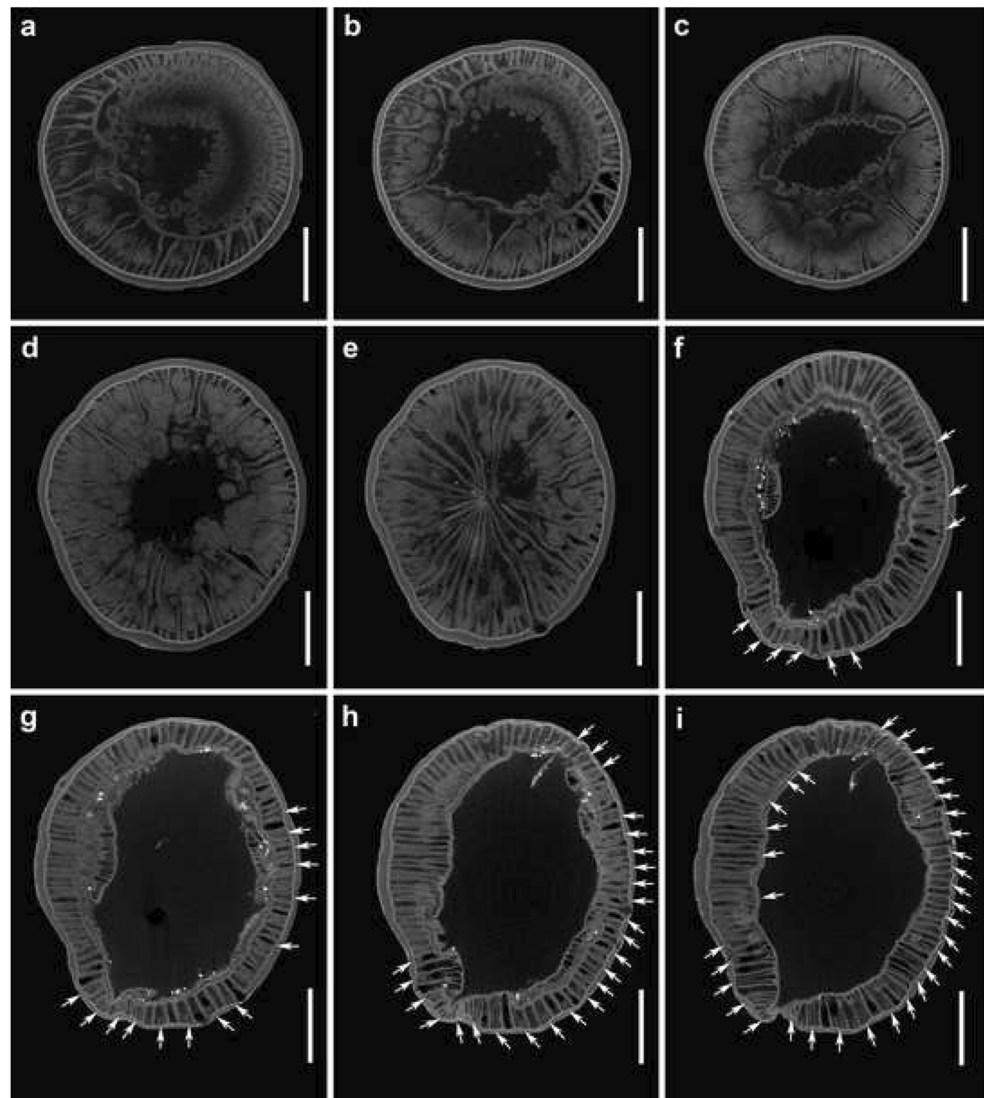
high support only in the ML analysis (80%; 29% in MP but found in all 31 most parsimonious trees). Both of these species are recovered within Hormathiidae but outside of the clade containing *Calliactis* species. Instead, *Paracalliactis* is recovered as sister to *Paraphelliactis* (57% ML) or unresolved within a clade with *Paraphelliactis* and *Chondrophellia* in the MP analysis.

Discussion

Taxonomic placement and differentiation of *Calliactis tigris* sp. nov.

Having a mesogleal marginal sphincter muscle and only basitrichs in the acontia unequivocally places *Calliactis tigris* sp. nov. within the family Hormathiidae. Recent findings of Hormathiidae paraphyly (e.g., Gusmão and Daly et al. 2010; Rodríguez et al. 2012, 2014; Grajales and Rodríguez 2016; Gusmão et al. 2018) and evidence for the presence of other types of nematocysts besides basitrichs in the acontia (e.g., *Hormathia pectinata*: Riemann-Zürneck 1973; LCG personal observation), suggest that our current definition of the family (i.e., sensu Carlgren 1949) is inadequate. This results from a reliance on few morphological features to diagnose the family (e.g., cnidom of acontia, dimorphic mesenteries, basilar muscle) whose distribution is not thoroughly understood.

Fig. 5 Micro-CT scans of *Calliactis tricolor* (Le Sueur 1817) showing pairs of mesenteries in cross-section throughout length of column. **a** Cross section through distal column showing 48 pairs of mesenteries (distributed in 4 cycles); most mesenteries still connected to oral disc. **b** Cross section through distal column showing 48 pairs of mesenteries; note mesenteries connected to oral disc and actinopharynx. **c** Cross section through distal column showing 48 pairs of mesenteries at actinopharynx level; note mesenteries of first cycle partially connected to actinopharynx. **d** Cross section through mid-column showing 48 pairs of mesenteries. **e** Cross section through proximal column showing 48 pairs of mesenteries. **f** Cross section through proximal column showing 57 pairs of mesenteries: 48 pairs plus nine pairs of 5th cycle (arrows). **g** Cross section through proximal column showing 62 pairs of mesenteries: 48 pairs plus 14 pairs of 5th cycle (arrows). **h** Cross section through proximal column showing 73 pairs of mesenteries: 48 pairs plus 25 pairs of 5th cycle (arrows). **i** Cross section through proximal column showing 82 pairs of mesenteries: 48 pairs of mesenteries plus 34 pairs of 5th cycle (arrow). Scale bars, **a–i** 5 mm



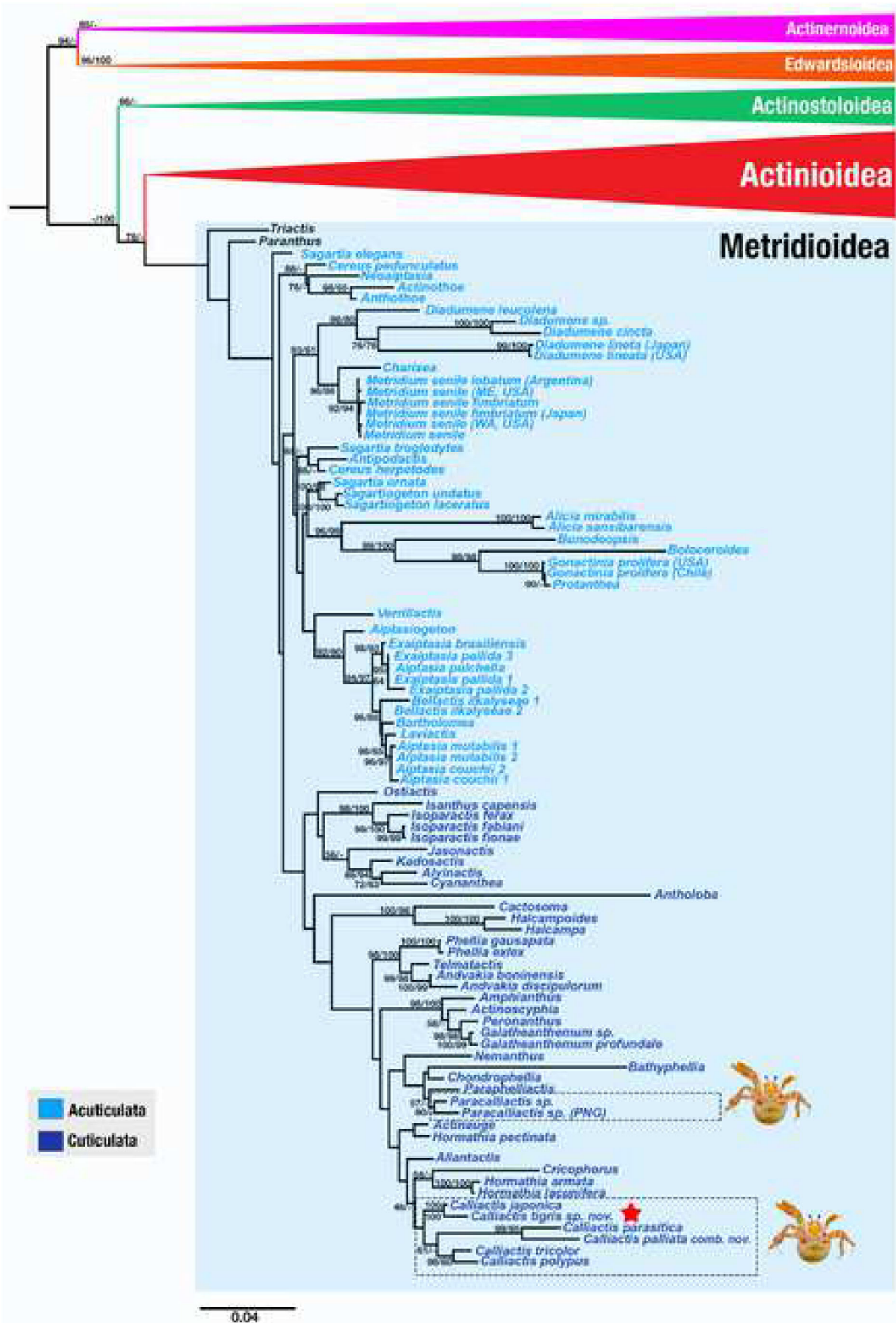
Regardless, *C. tigris* sp. nov. carries a combination of characters that prevents its placement in any other metridioidean family and is firmly nested within a clade containing most hormathiid genera in the phylogenetic analysis, including the type genus *Hormathia* (Fig. 6).

Because *Calliactis tigris* sp. nov. is always found attached to shells inhabited by a hermit crab, has cinclides in the proximal column, six pairs of perfect sterile mesenteries, and weak retractor and parietobasilar muscles, it is unambiguously placed within the genus *Calliactis*. The taxonomic classification of *C. tigris* sp. nov. based on morphology is further supported by its position in the tree from the phylogenetic analyses (see Fig. 6).

Calliactis tigris sp. nov. is the second valid species of the genus recorded off the coast of Australia where *C. polypus* is also found. Both species have been collected off the coast of New South Wales and Queensland but are easily differentiated from each other by external and internal anatomy and cnidae.

Externally, *C. tigris* sp. nov. is much bigger than *C. polypus*, has a very distinct coloration pattern in the column with irregular purple patches that resemble a tiger pattern (vs. clear longitudinal stripes in *C. polypus*), and has small, inconspicuous cinclides (vs. conspicuous cinclides positioned on tubercles with distinct color in the column of *C. polypus*). In addition, *C. tigris* sp. nov. has 48 pairs of mesenteries at mid-column (vs. 96 pairs in *C. polypus*) and around 96 tentacles (vs. up to 240 tentacles in *C. polypus*). Differences in the size

Fig. 6 Phylogenetic reconstruction of the concatenated dataset (12S, 16S, 18S, 28S, COIII) from maximum likelihood (ML) analysis. Metridioidean species corresponding to clades Acuticulata and Cuticulata of Rodríguez et al. (2014) differentiated by shades of blue; other actiniarian superfamilies collapsed; species epithets given only for genera represented by more than one species. Hermit-crab symbiotic genera indicated by dashed rectangles; *Calliactis tigris* sp. nov. indicated by red star. Bootstrap resampling values indicated above branches space permitting (ML/MP); only support values > 50% are shown



range of basitrichs in the column, actinopharynx, and filament further differentiates these two species (see comparison in Table 1). Although both species are found in the same geographic area, they show disjunct bathymetry: *C. tigris* sp. nov. is found from 160 to 508 m depth whereas *C. polypus* is found at shallower depths, between 0 and 45 m.

Calliactis conchiola is the only species of the genus described for New Zealand and one of the few described from temperate waters (Hand 1975). Although Carlgren (1927) hypothesized that the proportion of mesenteries proximally and distally was of generic significance and helpful in differentiating *Calliactis* from other hormathiids, this character remained controversial. Daly et al. (2004) diagnosed *Calliactis* as having tentacles as numerous as mesenteries proximally whereas Carlgren (1949) and Hand (1975) diagnosed *Calliactis* as having more tentacles than mesenteries at the base. England (1971) observed one or two extra cycles of mesenteries at the margin of *C. polypus*; he also found more mesenteries proximally than at mid-column. Nevertheless, England (1971) considered a higher number of mesenteries distally than proximally as a diagnostic feature for *Calliactis*. A higher number of tentacles than mesenteries at mid-column are used as a proxy for having mesenteries more numerous distally without appropriate examination of mesenteries proximally. In this study, we observed an almost complete fifth cycle of mesenteries proximally in dissected specimens of *C. tigris* sp. nov. but not distally. Likewise, a fifth cycle was observed proximally but not distally in dissections or micro-CT scans of *C. tricolor* (see Fig. 5). This suggests that in *C. tigris* sp. nov. and *C. tricolor*, mesenteries develop and remain restricted to the proximal most part of the column (as opposed to growing simultaneously at the proximal and distal end of the body; see Carlgren 1927). We suspect that closer examination of other *Calliactis* species will reveal a similar pattern of mesentery growth which is often difficult to observe given the small size and hidden position very close to the pedal disc (or margin) of the last cycle of mesenteries. *Calliactis conchiola*, unlike all other species in *Calliactis* has an equal number of tentacles and mesenteries with the latter running from margin to pedal disc. In the original description of *C. conchiola*, Parry (1952) noted a fifth cycle of mesenteries but Hand (1975) corroborated this for only the largest three of the dozens of specimens he examined. Hand (1975) noted that Parry's (1952) description may be based on individuals of both *C. conchiola* and *Paracalliactis rosea*. Because Parry did not deposit type material it is not possible to check the identity of her material, but Hand (1975) suggests that Parry's description applies best to *C. conchiola*. Despite this uncertainty, *C. tigris* sp. nov. can be differentiated from *C. conchiola* based on its 96 tentacles (up to 110 in *C. tigris* sp. nov.), same number of mesenteries distally and proximally (more mesenteries proximally in *C. tigris* sp. nov.), fertility of the fourth cycle of mesenteries (always sterile in *C. tigris* sp. nov.),

longer and stronger marginal mesogleal sphincter compared to the one seen in *C. tigris* sp. nov., ring of small protuberances in distal scapus (absent in *C. tigris* sp. nov.), as well as differences in nematocyst types and size in actinopharynx, filaments, and acontia (see Table 1).

Calliactis tigris sp. nov. and *C. japonica* are both found in the Pacific Ocean, though *C. japonica* has been collected exclusively from the North Pacific off the coast of Japan, and more recently, from the Frigate Shoals in Hawaii (LCG personal observation). *Calliactis tigris* sp. nov. can be differentiated from *C. japonica* by its tiger-like column coloration pattern (vs. red dots resembling a leopard pattern in *C. japonica*), the absence of tubercles in the column of *C. tigris* sp. nov. (scattered tubercles present in *C. japonica*), its 96 tentacles and 48 pairs of mesenteries at mid-column (up to 200 tentacles and always more than 48 pairs of mesenteries in *C. japonica*), and the absence of filaments and acontia in the fourth cycle of mesenteries (present in *C. japonica*). Details of cnidae further distinguish *C. tigris* sp. nov. from *C. japonica*: non-overlapping size range of basitrichs and *p*-mastigophores B1 in the filaments and presence of *p*-mastigophores B1 in the actinopharynx of *C. tigris* sp. nov. which are absent in *C. japonica*. While the bathymetric range of both species overlaps slightly, with *C. japonica* found between 0 and 210 m depth and *C. tigris* sp. nov. between 160 and 508 m, their geographic distribution does not overlap.

Besides having a non-overlapping geographic distribution, *Calliactis tigris* sp. nov. is distinguished from other species in the genus recorded from the Mediterranean, Atlantic, or Indian Ocean based on the higher number of mesenteries at mid-column (48 pairs in *C. tigris* sp. nov. vs. 82 pairs in *C. androgyna*; 96 pairs in *C. parasitica*) and tentacles (96 tentacles in *C. tigris* sp. nov. vs. 194 tentacles in *C. brevicornis*; 190 tentacles in *C. tricolor*), and aspects of external morphology and cnidae (*C. algoensis* and *C. annulata*). Although the three species of *Calliactis* described by Pei (1996) for the east and south China Sea (*C. argentacolorata*, *C. polypores*, *C. xishaensis*) are not described in much detail, *C. tigris* sp. nov. can be superficially distinguished from them based on the presence of tubercles with cinclides in *C. argentacolorata* and by coloration pattern, lack of horizontal stratification in the marginal sphincter, and nematocysts in *C. xishaensis*. Unfortunately, the loan of type material for *C. polypores*, *C. xishaensis*, and *C. argentacoloratus* was not possible and no other specimens from the type locality were available, preventing a more detailed comparison of *C. tigris* sp. nov. with these Chinese species of *Calliactis*.

Calliactis tigris sp. nov. is easily distinguished from other non-hormathiid hermit-crab symbiotic species recorded in the Indo-Pacific by having only basitrichs in the acontia (basitrichs and *p*-mastigophores B2a in acontia of *Carcinactis ichikawai* Uchida 1960 and *Verrillactis paguri* (Stimpson in Verrill

1869)) and by having acontia and mesogleal marginal sphincter muscle (acontia absent and endodermal marginal sphincter muscle in *Stylobates* (Dall 1903).

Systematics of former *Adamsia*, *Calliactis*, *Paracalliactis*

The position of former *Adamsia palliata* nested within a clade of *Calliactis* species and *Paracalliactis* spp. recovered in a different clade in our analysis (Fig. 6) confirm the results of Gusmão and Daly et al. (2010) of two independent origins for the hermit-crab symbiosis within Hormathiidae. These results provide a context for evaluating the mosaic of characters that have been traditionally used to delimit taxa in this group: characters related to a symbiotic habit (such as the shape and position of the pedal disc and the secretion of a carcinoecium) vary within and between monophyletic groups and are hypothesized to be convergent within Hormathiidae. The carcinoecium and complex behavioral and anatomical features associated with the symbiosis are interpreted as having evolved at least twice within Hormathiidae and seem to be phylogenetically labile (Gusmão and Daly et al. 2010). At the same time, the paraphyly of *Calliactis* due to the inclusion of former *Adamsia* demonstrates that anatomical and micro-anatomical characters, including the occurrence of cinclides, carry phylogenetic signal and should have a greater role in the taxonomy of the family. To reconcile taxonomy and the current phylogenetic hypothesis, we have formally modified the circumscription of *Calliactis* by broadening the definition of the genus to include characters present in the type species of former *Adamsia* (*C. palliata* comb. nov.) and the other species in the genus (see remarks in “Taxonomic description”).

Characters such as the position of the anemones on the shell included in the original diagnosis of *Calliactis* by Verrill (1869) to differentiate it from the former *Adamsia*, as well as the secretion of a carcinoecium and the shape of pedal disc, are now interpreted as particular to specific partnerships and not of generic significance within Hormathiidae. In fact, the position of the anemone on a shell may be the result of variability of shell-mounting behavior exhibited by the sea anemones and the activity of hermit crabs that associate with them (i.e., *Pagurus* Fabricius 1775, *Dardanus* Paulson 1875, and *Diogenes* Dana 1851 with *Calliactis*; *Parapagurus* Smith 1879, *Oncopagurus* Lemaitre 1996, and *Sympagurus* Smith 1883 with *Paracalliactis*; Ross and Sutton 1961; Ross 1975). The “extreme” morphology of *C. palliata* comb. nov. might be explained by the active behavior exhibited by its symbiont (*Pagurus prideauxi* Leach 1815) which is not active towards *C. parasitica* in some parts of the anemone’s distribution (Gusmão and Daly et al. 2010). Likewise, species of *Paracalliactis* also show variability in their position on the shell, with *P. michaelsarsi* being the only species to be positioned ventrally below the aperture of the shell whereas all

other species in the genus are placed dorsally with the oral disc directed away from the shell’s aperture. A parallel case of variation in the position of an individual on the shell is found among species of *Stylobates*: *S. aeneus* Dall 1903, *S. cancrisocia* (Carlgren 1928c), and *S. birtlesi* Crowther, Fautin and Wallace, 2011 are found with the oral disc oriented towards the substrate close to the shell aperture whereas *S. loisetteae* Fautin 1987 is found with its oral disc oriented away from the shell aperture (Dunn et al. 1980; Crowther et al. 2011). The difference in orientation seen in species of *Stylobates* on the shell might be related to the ways the hermit crab carries the anemone: *Sympagurus dofleini* (Bals 1912) (partner of *S. aeneus*) has long legs and probably can carry the anemone high above the floor. The displacement seen in *S. loisetteae* might be an adaptation to avoid injury as the anemone is dragged along the seafloor. Thus the precise orientation of the anemone on shell is not of generic significance in *Stylobates* but an adaptation to its partner (Fautin 1987).

Our molecular phylogenetic analysis suggests that anatomical and micro-anatomical characters shared between members of former *Adamsia* and *Calliactis* such as the presence of cinclides carry phylogenetic signal and should have a greater role in the taxonomy of Hormathiidae as emphasized by previous authors (Hand 1975; Daly et al. 2004). Although Carlgren (1928a) questions whether all species of *Calliactis* possess cinclides in the column, England’s (1971) and our own observations (LCG pers. obs.) confirm the diagnostic significance of this character for *Calliactis*. We expand the results of Gusmão and Daly et al. (2010) by finding a monophyletic *Paracalliactis*, which was left untested in their analyses. An exclusive relationship between *Paracalliactis*, *Chondrophellia*, and *Paraphelliactis* (all of which lack cinclides) further supports this hypothesis (see “Taxonomic description”). Our results also confirm that similarities shared between members of *Calliactis* and *Paracalliactis* in internal anatomy (e.g. number of perfect mesenteries, fertility) are not an indication of a close affinity between them but shared more broadly among members of Hormathiidae.

In addition, the mesenterial growth pattern reflected by the proportion of mesenteries close to the margin and to the pedal disc may also help distinguishing *Calliactis* (i.e., more mesenteries proximally) from *Paracalliactis* (same number of mesenteries proximally and distally) (Carlgren 1928a). Micro-CT scans of a specimen of *C. tricolor* (see Fig. 5) have confirmed extra pairs of mesenteries proximally thus we have corrected this information in the diagnosis given by Carlgren (1949) who considered a higher number of tentacles than mesenteries as of generic significance for *Calliactis* (see the taxonomic remarks). Moreover, we have confirmed that these extra pairs of mesenteries of fifth cycle are not present distally, suggesting that in *C. tigris* sp. nov. and *C. tricolor* and probably in all species of *Calliactis* mesenteries develop in the proximal end of the body and grow upwards towards the oral

disc but do not reach it (as opposed to growing simultaneously at the proximal and distal end of the body; see Carlgren 1927).

Small mesenteries of fifth cycle have been traditionally difficult to observe in dissections and histological sections, but they are easily observed in micro-CT scans provided an appropriate resolution is attained (see Fig. 5). Because a whole specimen can be scanned resulting in hundreds of images that can be stacked and easily surveyed, micro-CT scanning can augment the study of mesenterial growth pattern in sea anemones if combined with histology by facilitating the observation of small pairs of mesenteries very distally close to the margin or very proximally close to the pedal disc (see Fig. 5).

Our reconciliation of morphology and phylogeny of hermit-crab symbiotic anemones illustrates the difficulties of defining groups of sea anemones based on morphology alone: the modifications of the pedal disc to living on a shell inhabited by a hermit crab previously assumed to indicate a close relationship between *Calliactis* (as *Adamsia* at the time) and *Paracalliactis* is interpreted as functional rather than phylogenetic. Likewise, highly complex morphological features such as secretion of a carcinoecium are found to be phylogenetically labile within the order Actiniaria and inferred to be the result of convergent evolution. Perhaps due to the simplicity of their body plan, convergent evolution has been recovered as a major trend among sea anemones (Rodríguez et al. 2014). In addition, many of the relatively few morphological characters used to identify and classify anemones have been repeatedly lost among groups in the order (Rodríguez et al. 2014). Several lineages within Metridioidea, for example, have lost or modified features that define the group (Rodríguez et al. 2014): independently derived ectodermal muscles in bolocerozoans and multiple independent losses of acontia in the deep-sea groups (e.g., DeepSina, Chemosynthina) (Rodríguez et al. 2014). Similarly, burrowing is associated with the loss of structures (Daly et al. 2002) and the absence of basilar muscle among burrowers is interpreted as primitive in edwardsiids, but represent independent losses among burrowing anemones in lineages of Actinioidea (e.g., haloclavids *Peachia* Gosse 1855, *Stephanthus* Rodríguez and López-González 2003, *Harenactis* Torrey 1902) and Metridioidea (e.g., halcampids *Halcampa* Gosse 1858 and *Halcampoides* Danielssen 1890). Interpretations of morphological convergence among sea anemones, however, depend on detailed examination of features and interpretations of homology which is not always available or may be recognized only after phylogenetic analysis (Daly et al. 2017). For this reason, a combination of phylogenetic analyses based on molecular data and detailed morphological studies seem to be the only reliable way to define natural groups within Actiniaria.

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References

- Ates, R. M. L. (1997). Observations on the symbiosis between *Colus gracilis* (Da Costa, 1778) (Mollusca: Gastropoda) and *Hormathia digitata* (O. F. Muller, 1776) (Cnidaria: Actiniaria). *Zoologische Verhandlungen*, 323, 257–262.
- Bach, C. E., & Hernkind, W. F. (1980). Effects of predation pressure on the mutualistic interaction between the hermit crab, *Pagurus pollicaris* Say, 1817, and the sea anemone, *Calliactis tricolor* (LeSueur, 1817). *Crustaceana*, 38, 104–108.
- Balash, J., & Mengual, V. (1973). The behavior of *Dardanus arrosor* in association with *Calliactis parasitica* in artificial habitat. *Marine and Freshwater Behaviour and Physiology*, 2, 251–260.
- Balss, H. (1912). Paguriden. In C. Chun (Ed.), *Wiss. Ergebnisse der Deutschen Tiefsee-Exp. "Valdivia" 1898–1899* (pp. 85–124). Gustav Fischer: Jena.
- Brooks, W. R. (1989). Hermit crabs alter sea anemone placement patterns for shell balance and reduced predation. *Journal of Experimental Marine Biology and Ecology*, 132, 109–121.
- Brooks, W. R., & Gwaltney, C. L. (1993). Protection of symbiotic cnidarians by their hermit crab hosts: Evidence for mutualism. *Symbiosis*, 15, 1–13.
- Cariello, L., de Santis, A., Piccoli, R., Spagnuolo, A., Zanetti, L., & Parente, A. (1989). Calitoxin, a neurotoxic peptide from the sea anemone *Calliactis parasitica*: Amino acid sequence and electrophysiological properties. *Biochemistry*, 28, 2484–2489.
- Carlgren, O. (1893). Studien über nordische Actinien. *Kungliga Svenska Vetenskapsakademiens Handlingar*, 25, 1–148.
- Carlgren, O. (1922). Actiniaria and Zoantharia von Juan Fernandez und der osterinsel. In C. Skottsberg (Ed.), *The Natural History of Juan Fernandez and Easter Island* (pp. 145–160). Uppsala: Almqvist & Wiksells Botryckeri-A. -B.
- Carlgren, O. (1925). Zur kenntnis der Hexacorallen. *Zoologischer Anzeiger*, 65, 87–99.
- Carlgren, O. (1927). Actiniaria and Zoantharia. In T. Odhner (Ed.), *Further zoological results of the Swedish Antarctic expedition 1901–1903* (pp. 1–102). Stockholm: P.A. Norstedt & Söner.
- Carlgren, O. (1928a). Zur symbiose zwischen Actinien und Paguriden. *Zeitschrift für Morphologie und Ökologie der Tiere*, 12, 165–173.
- Carlgren, O. (1928b). Ceriantharier, Zoantharier och Actiniarier. *Meddelelser om Grönland*, 23(Suppl), 253–308.
- Carlgren, O. (1928c). Actiniaria der Deutschen Tiefsee-Expedition. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899*, 2, 125–266.
- Carlgren, O. (1932). Die Ceriantharier, Zoantharier und Actiniarier des arktischen Gebietes. In F. Romer, F. Schaudinn, A. Brauer, & W. Arndt (Eds.), *Eine Zusammenstellung der arktischen Tierformen mit*

- besonderer Berücksichtigung des Spitzbergen-Gebietes auf Grund der Ergebnisse der Deutschen Expedition in das Nördliche Eismeer im Jahre 1893 (pp. 255–266). Jena: Gustav Fischer.
- Carlgrén, O. (1938). South African Actiniaria and Zoantharia. *Kungliga Svenska Vetenskapsakademiens Handlingar*, 17, 1–148.
- Carlgrén, O. (1940). A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. *Kungliga Svenska Vetenskapsakademiens Handlingar*, 51, 1–62.
- Carlgrén, O. (1943). East-Asiatic Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskapsakademiens Handlingar*, 20, 1–43.
- Carlgrén, O. (1949). A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskapsakademiens Handlingar*, 1, 1–121.
- Christidis, J., Chintiroglou, C. H., & Culley, M. B. (1997). A study of the populations of *Calliactis parasitica* (Couch, 1842) in symbiosis with anomuran decapods in Thermaikos Gulf (N. Aegean Sea). *Crustaceana*, 70, 227–238.
- Couch, R. Q. (1842). An essay on the zoophytes of Cornwall. *Annual Report of the Royal Cornwall Polytechnic Society*, 9, 27–91.
- Crowther, A. L., Fautin, D. G., & Wallace, C. C. (2011). *Stylobates birtlesi* sp. n., a new species of carcinoecium-forming sea anemone (Cnidaria, Actiniaria, Actiniidae) from eastern Australia. *Zookeys*, 89, 33–28.
- Dall, W. H. (1903). A new genus of Trochidae. *Nautilus*, 17, 61–62.
- Daly, M., Lipscomb, D. L., & Allard, M. W. (2002). A simple test: Evaluating explanations for the relative simplicity of the Edwardsiidae (Cnidaria: Anthozoa). *Evolution*, 56, 502–510.
- Daly, M., Ardelean, A., Cha, H., Campbell, A. C., & Fautin, D. G. (2004). A new species, *Adamsia obvolva* (Cnidaria: Anthozoa: Actiniaria), from the Gulf of Mexico, and a discussion of the taxonomy of carcinoecium-forming sea anemones. *Bulletin of Marine Science*, 74, 385–399.
- Daly, M., Chaudhuri, A., Gusmão, L., & Rodríguez, E. (2008). Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular Phylogenetics and Evolution*, 48, 292–301.
- Daly, M., Gusmão, L. C., Reft, A. J., & Rodríguez, E. (2010). Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (Cnidaria, Actiniaria). *Integrative and Comparative Biology*, 50(3), 371–388.
- Daly, M., Crowley, L. M., Larson, P., Rodríguez, E., Saucier, E. H., & Fautin, D. G. (2017). *Anthopleura* and phylogeny of Actinioidea (Cnidaria: Anthozoa: Actiniaria). *Organisms, Diversity and Evolution*, 17, 545–564.
- Dalyell, J. G. (1848). *Rare and remarkable animals of Scotland*. London: John Van Voorst, Paternoster Row.
- Dana, J. D. (1846). *Zoophytes. Volume VII of the United States exploring expedition. During the years 1838, 1839, 1840, 1841, 1842. Under the command of Charles Wilkes, U.S.N.* Philadelphia: Lea and Blanchard.
- Dana, J. D. (1851). *Conspectus crustaceorum quae in orbis terrarum circumnavigatione, Carolo Wilkes e classe reipublicae foederatae duce, lexit et descripsit. Proceedings of the Academy of Natural Sciences of Philadelphia*, 5, 267–272.
- Danielssen, D. C. (1890). *Actinida*. Christiania: Den Norske Nordhavs-Expedition.
- Dechancé, M., & Dufaure, J. (1959). Une nouvelle association entre une actinie et un pagure. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, 249, 1566–1568.
- Doumenc, D. (1975). Actinies bathyales et abyssales de l'océan Atlantique nord familles des Hormathiidae (genres *Paracalliactis* et *Phelliactis*) et des Actinostolidae (genres *Actinoscyphia* et *Sicyonis*). *Bulletin du Muséum National d'Histoire Naturelle (Paris)*, 197(287), 157–204.
- Dunn, D. F., Devaney, D. M., & Roth, B. (1980). *Stylobates*: A shell-forming sea anemone (Coelenterata, Anthozoa, Actiniidae). *Pacific Science*, 34, 379–388.
- England, K. W. (1971). Actiniaria from Mururoa Atoll Tuamotu, Polynesia (Hormathiidae; *Calliactis polypus* Sagartiidae: *Verrillactis* n. gen. *paguri*). *Cahiers du Pacifique*, 15, 23–40.
- Fabricius, J. C. (1775). *Systema entomologiae, sistens insectorum classes, ordines, genera, species, adiectis, synonymis, locis, descriptionibus, observationibus*. Officina Libraria Kortii: Flensburgi et Lipsiae.
- Fautin, D. G. (1987). *Stylobates loisetteae*, a new species of shell-forming sea anemone (Coelenterata: Actiniidae) from western Australia. *Proceedings of the California Academy of Sciences*, 45(1), 1–7.
- Fautin, D. G. (1988). The importance of nematocysts to actiniarian taxonomy. In D. A. Hessinger & H. M. Lenhoff (Eds.), *The biology of nematocysts* (pp. 487–500). New York: Academic Press.
- Fautin, D. G. (1992). A shell with a new twist. *Natural History*, 4, 50–57.
- Fautin, D. G. (2016). Catalog to families, genera, and series of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). *Zootaxa*, 4145(1), 1–449.
- Fedorov, A., Beichel, R., Kalpathy-Cramer, J., Finet, J., Fillion-Robin, J.-C., Pujol, S., Bauer, C., Jennings, D., Fennessy, F., Sonka, M., Buatti, J., Aylward, S., Miller, J. V., Pieper, S., & Kikinis, R. (2012). 3D slicer as an image computing platform for the quantitative imaging network. *Magnetic Resonance Imaging*, 30(9), 1323–1341.
- Fischer, P. (1889). Nouvelle Contribution à l'Actinologie Française. *Première Partie. Actes de la Société Linnéenne de Bordeaux*, 43, 252–309.
- Forbes, E. (1840). On the British Actiniadae. *Annals and Magazine of Natural History*, 5, 180–184.
- Forsskål, P. (1775). *Descriptions Animalium Avium, Amphibiorum, Piscium, Insectorum, Vermium; Quae in Itinere Orientali Observavit*. Copenhagen: Molleri.
- Geller, J. B., & Walton, E. D. (2001). Breaking up and getting back together: Evolution of symbiosis and cloning in sea anemones (genus *Anthopleura*) inferred from a molecular phylogeny. *Evolution*, 55, 1781–1794.
- Goloboff, P. A., Farris, J. S., & Nixon, K. C. (2008). TNT, a free program for phylogenetic analysis. *Cladistics*, 24, 774–786.
- Gosse, P. H. (1855). Description of *Peachia hastata*, a new genus and species of the class Zoophyta; with observations on the family Actiniadae. *Transactions of the Linnean Society (London)*, 21, 267–276.
- Gosse, P. H. (1858). Synopsis of the families, genera, and species of the British Actiniae. *Journal of Natural History*, 1, 414–419.
- Grajales, A., & Rodríguez, E. (2016). Elucidating the evolutionary relationships of the Aiptasiidae, a widespread Cnidarian-dinoflagellate model system (Cnidaria: Anthozoa: Actiniaria: Metridioidea). *Molecular Phylogenetics and Evolution*, 94, 252–263.
- Gravier, C. (1918). Note préliminaire sur les hexactiniaires recueillis au cours des croisières de la Princesse-Alice et de l'Hirondelle de 1888 à 1913 inclusivement. *Bulletin de l'Institut Océanographique (Monaco)*, 346, 1–24.
- Gusmão, L. C. (2010). Systematics and evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. Dissertation. The Ohio State University. https://etd.ohiolink.edu/pg_10??:NO:10:P10_ACCESSION_NUM:osu1291150200. Accessed 20 February 2019.
- Gusmão, L. C., & Daly, M. (2010). Evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. *Molecular Phylogeny and Evolution*, 56, 868–877.
- Gusmão, L. C., Grajales, A., & Rodríguez, E. (2018). Sea anemones through X-rays: Visualization of two species of *Diadumene* (Cnidaria, Actiniaria) using micro-CT. *American Museum Novitates*, 3907, 1–45.

- Hand, C. (1975). Descriptions of two New Zealand sea anemones (Actiniaria: Hormathiidae). *New Zealand Journal of Marine and Freshwater Research*, 9, 493–507.
- Hertwig, R. (1882). *Die Actinien der Challenger Expedition*. Jena: Gustav Fischer.
- International Commission on Zoological Nomenclature. (1999). *International code of zoological nomenclature* (Fourth ed.). London: International Trust for Zoological Nomenclature. <https://doi.org/10.5962/bhl.title.50608>.
- Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772–780.
- Katoh, K., Rozewicki, J., & Yamada, K. D. (2017). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief in Bioinformatics bbx108, <https://doi.org/10.1093/bib/bbx108>.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649.
- Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2018). RAXML-NG: A fast scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *bioRxiv*. <https://doi.org/10.1101/447110>.
- Larson, P., & Daly, M. (2016). Phylogenetic analysis reveals an evolutionary transition from internal to external brooding in *Epiactis* Verrill (Cnidaria: Anthozoa: Actiniaria) and rejects the validity of the genus *Cnidopus* Carlgren. *Molecular Phylogenetics and Evolution*, 94, 548–558.
- Lauretta, D., Häussermann, V., Brugler, M. R., & Rodríguez, E. (2014). *Isoparactis fionae* sp. n. (Cnidaria: Anthozoa: Actiniaria) from Southern Patagonia with a discussion of the family Isanthidae. *Organisms, Diversity and Evolution*, 14(1), 31–42.
- Le Sueur, C. A. (1817). Observations on several species of the genus *Actinia*; illustrated by figures. *Journal of the Academy of Sciences of Philadelphia*, 1, 159–189.
- Leach, W. E. (1815). *Malacostraca Podophthalmata Britanniae; or descriptions of such British species of the Linnean genus Cancer as have their eyes elevated on footstalks*. London: B. Meredith.
- Lemaitre, R. (1996). Hermit crabs of the family Parapaguridae (Crustacea: Decapoda: Anomura) from Australia: Species of *Strobopagurus* Lemaitre, 1989, *Sympagurus* Smith, 1883 and two new genera. *Records of the Australian Museum*, 48(2), 163–221.
- Mainardi, D., & Rossi, A. C. (1969). Relations between social status and activity toward the sea anemone *Calliactis parasitica* in the hermit crab *Dardanus arrosor*. *Accademia Nazionale dei Lincei, Rnma, Classe di Scienze Fisiche, Matematiche e Naturali*, 47, 116–121.
- Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 1–8.
- Müller, O. F. (1776). *Zoologiae Danicae Prodrömus, seu animalium danicae et Norvegiae indigenarum characteres, nomina, et synonyma imprimis popularium*. Havniae.
- Parry, G. (1952). The Actiniaria of New Zealand. A check-list of recorded and new species a review of the literature and a key to the commoner forms part 2. *Records of the Canterbury Museum*, 6, 121–141.
- Parulekar, A. (1969). *Neoaipiasia commensali*, gen. et sp. nov.: An actiniarian commensal of hermit crabs. *Journal of the Bombay Natural History Society*, 66(1), 57–62.
- Paulson, O. (1875). Izsledovaniya rakoobrazykh krasnago morya s zametkami otnositel'no rakoobrazykh drugikh morei: I–XIV, 1–144, pis. 1–21. (1961, English translation: 1–164, pis. 1–21).
- Pei, Z. (1982). A new species of the genus *Paracalliactis* (Hormathiidae: Actiniaria) from the East China Sea. *Studia Marina Sinica*, 19, 69–71.
- Pei, Z. (1996). Three new species of the genus *Calliactis*. *Studia Marina Sinica*, 37, 177–187.
- Pennant, T. (1777). *A British zoology* (4th ed.). London: Benj White.
- Presnell, J. K., & Schreibman, M. P. (1997). *Humason's animal tissue techniques*. Baltimore: Johns Hopkins University Press.
- Riemann-Zürneck, K. (1973). Actiniaria des Südwestatlantik I. Hormathiidae. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 25, 273–325.
- Riemann-Zürneck, K. (1975). Actiniaria des Südwestatlantik III. *Calliactis androgyna* sp. n. (Hormathiidae). *Veröffentlichungen des Institutes für Meeresforschung Bremerhaven*, 15, 387–395.
- Riemann-Zürneck, K. (1986). On some abyssal sea anemones of the North Atlantic (Actiniaria: Hormathiidae). *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut*, 83, 7–29.
- Rodríguez, E., & Gusmão, L. C. Case 3805: *Calliactis* Verrill, 1869 (Cnidaria, Anthozoa, Hexacorallia, Actiniaria): proposed precedence over *Adamsia* Forbes, 1840. *Bull. Zool. Nomencl.* (submitted).
- Rodríguez, E., & López-González, P. J. (2003). *Stephanthus antarcticus* a new genus and species of sea anemone (Actiniaria, Haloclavidae) from the South Shetland Islands, Antarctica. *Helgoland Marine Research*, 57, 54–62.
- Rodríguez, E., Barbeitos, M., Daly, M., Gusmão, L. C., & Häussermann, V. (2012). Toward a natural classification: Phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). *Cladistics*, 28, 375–392.
- Rodríguez, E., Barbeitos, M. S., Brugler, M. R., Crowley, L., Grajales, A., Gusmão, L. C., Häussermann, V., Reft, A., & Daly, M. (2014). Hidden among sea anemones: The first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of Hexacorals. *PLoS One*, 9(5), e96998.
- Ross, D. M. (1971). Protection of hermit crabs (*Dardanus* spp.) from octopus by commensal sea anemones (*Calliactis* spp.). *Nature*, 230, 401–402.
- Ross, D. M. (1974a). Behavior patterns in associations and interactions with other animals. In L. Muscatine & H. M. Lenhoff (Eds.), *Coelenterate biology: Reviews and new perspectives* (pp. 281–312). New York: Academic Press.
- Ross, D. M. (1974b). Evolutionary aspects of associations between crabs and sea anemones. In W. B. Vernberg (Ed.), *Symbiosis in the sea* (pp. 111–125). South Carolina: University of South Carolina Press.
- Ross, D. M. (1975). The behavior of pagurids in symbiotic associations with actinians in Japan. *Publications of the Seto Marine Biological Laboratory*, 22(1–4), 157–170.
- Ross, D. M., & Boletzky, S. (1979). The association between the pagurid *Dardanus arrosor* and the actinian *Calliactis parasitica*. Recovery of activity in “inactive” *D. arrosor* in the presence of cephalopods. *Marine Behaviour and Physiology*, 6, 175–184.
- Ross, D. M., & Sutton, L. (1961). The response of the sea anemone *Calliactis parasitica* to shells of the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society B*, 155, 266–281.
- Ross, D. M., & Zamponi, M. O. (1982). A symbiosis between *Paracalliactis mediterranea* n. sp. (Anthozoa-Actiniaria) and *Pagurus variables* A. Milne-Edwards and Bouvier. *Vie Millie*, 32, 175–181.
- Schmidt, H. (1969). Die nesselkapseln der Aktinien und ihre differentialdiagnostische Bedeutung. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 19, 284–317.
- Schmidt, H. (1972). Die Nesselkapseln der Anthozoen und ihre Bedeutung für die phylogenetische Systematik. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 23, 422–458.

- Schmidt, H. (1974). On evolution in the Anthozoa. *Proceedings 2nd International Coral Reef Symposium*, 1, 533–560.
- Simon, J. A. (1892). *Ein Beitrag zur Anatomie und Systematik der Hexactinien*. München: Val. Hvfiling.
- Smith, S. I. (1879). The stalked-eyed crustaceans of the Atlantic coast of North America north of Cape Cod. *Transactions. Connecticut Academy of Arts and Sciences*, 5, 27–136.
- Smith, S. I. (1883). Preliminary report on the Brachyura and Anomura dredged in deep water off the south coast of New England by the United States Fish Commission in 1880, 1881, and 1882. *Proceedings of the United States National Museum*, 6, 1–57.
- Spagnuolo, A., Zanetti, L., Cariello, L., & Piccoli, R. (1994). Isolation and characterization of two genes encoding calitoxins, neurotoxic peptides from *Calliactis parasitica* (Cnidaria). *Gene*, 138, 187–191.
- Stephenson, T. A. (1918). Coelenterata. Part I. Actiniaria. *Natural History Reports on British Antarctic ("Terra Nova") Expedition 1910*, 5, 1–68.
- Stephenson, T. A. (1929). A contribution to actinian morphology: The genera *Phellia* and *Sagartia*. *Transactions of the Royal Society of Edinburgh*, 56(6), 121–139.
- Stewart, Z. K., Pavasonic, A., Hock, D. H., & Prentis, P. J. (2017). Transcriptomic investigation of wound healing and regeneration in the cnidarian *Calliactis polyopus*. *Scientific Reports*, 7. <https://doi.org/10.1038/srep41458>.
- Studer, T. (1879). Zweite Abtheilung der Anthozoa polyactinia, welche während der Reise S. M. S. Corvette Gazelle um die Erde gesammelt wurden. Monatsberichte der königlich preussischen Akademie der Wissenschaften zu Berlin, 524–550.
- Torrey, H. B. (1902). Papers from the Harriman Alaska Expedition. XXX. Anemones, with discussion of variation in *Metridium*. *Proceedings of the Washington Academy of Sciences*, 4, 373–410.
- Uchida, T. (1960). *Carcinactis ichikawai*, n. gen.; n. sp., an actiniaria commensal with the crab *Dorippe granulata*. *Japanese Journal of Zoology*, 12(4), 595–601.
- Verrill, A. E. (1869). Review of the corals and polyps of the west coast of America. *Transactions. Connecticut Academy of Arts and Sciences*, 1(6), 377–567.
- Verrill, A. E. (1882). Notice of the remarkable marine fauna occupying the outer banks off the southern coast of New England, No. 4. *American Journal of Science*, 23, 216–225.
- 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, Harvard University*, 11, 1–72.
- Weill, R. (1934). *Contribution à l'étude des Cnidaires et de leurs nématocystes*. Paris: Les Presses Universitaires de France.
- Wiedenmann, J., Ivanchenko, S., Oswald, F., & Nienhaus, G. U. (2004). Identification of GFP-like protein in nonbioluminescent, azooxanthellate Anthozoa opens new perspectives for bioprospecting. *Marine Biotechnology*, 6(3), 270–277.
- Williams, J. D., & McDermott, J. J. (2004). Hermit crab biocoenoses: A worldwide review of the diversity and natural history of hermit crab associates. *Journal of Experimental Marine Biology and Ecology*, 305, 1–128.
- Williamson, M., Hauser, F., & Grimmelikhuijzen, C. J. P. (2000). Genomic organization and splicing variant of a peptidylglycine α -hydroxylating monooxygenase from sea anemones. *Biochemical and Biophysical Research Communications*, 277(1), 7–12.

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