

Carlgren's hesitation allayed: redescription and systematics of *Heteranthus verruculatus* Klunzinger, 1877 (Cnidaria, Actiniaria), with a redefinition of Heteranthidae Carlgren, 1900

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Abstract

Species boundaries delineating tropical sea anemones (Cnidaria, Actiniaria) of the zooxanthellate genus, *Heteranthus* Klunzinger, 1877, are unclear. There are currently two valid *Heteranthus* species: type species *Heteranthus verruculatus* Klunzinger, 1877, first reported from Koseir, Egypt, and *H. insignis* Carlgren, 1943, from Poulo Condore, Vietnam. In describing the latter from a single, poorly preserved specimen, zoologist Oskar Carlgren expressed apprehension with traits he had used to establish this species. Carlgren's doubts persisted later in writing when he found a similar-looking sea anemone from the Great Barrier Reef. Crucial details to positively identify either species have since remained limited. Here, we re-diagnosed *Heteranthus* and re-described its type species based on observations of specimens we have obtained from Singapore and Pulau Ambon (Indonesia), and of museum material collected elsewhere across the Indo-West Pacific region ($n > 180$). Supported by molecular phylogenetic evidence, the family Heteranthidae Carlgren, 1900 was reinstated and re-diagnosed. *Heteranthus verruculatus* is encountered in the lower intertidal region amongst seagrass, in rocky crevices, or coral rubble. It occurs as solitary individuals or in clonal clusters, well-camouflaged against the substratum. Individuals were observed to frequently propagate by longitudinal fission, resulting in a varied appearance. Type material of *H. verruculatus* and *H. insignis* were re-examined and as we found no differences between them, the two were synonymised. We inferred that Carlgren probably misinterpreted cnidae and histological data in defining *H. insignis* as a distinct species. This revision clarifies the taxonomy and geographic range of *H. verruculatus*, an Indo-West Pacific species that is found from the Red Sea to subtropical Australia and Hawaii.

Keywords

Actinioidea – Indo-West Pacific – longitudinal fission – *Phymanthus* – taxonomy – zooxanthellate

Introduction

As prolific as Swedish zoologist Oskar Carlgren was in describing hundreds of sea anemones (Cnidaria, Actiniaria) during his lifetime, uncertainties pervaded him as he struggled to distinguish between two species of the zooxanthellate genus *Heteranthus* Klunzinger, 1877. Currently, this genus comprises two valid species: *Heteranthus verruculatus* Klunzinger, 1877 and *H. insignis* Carlgren, 1943, the former

being the type species of *Heteranthus* by monotypy (Daly & Fautin, 2020).

When Carlgren (1943: 32) first described *H. insignis* from a single, poorly preserved specimen collected from Poulo Condore [= Côn Sơn Island, Vung Tau] Vietnam, he stated, “I have with some hesitation referred the specimen to a new species, not to *verriculatus* [*sic*; = *H. verruculatus*].” Later, when he applied the name *H. verruculatus* to a specimen obtained from the Great Barrier Reef, his doubts further

lingered as he admitted (Carlgren, 1950: 440), “I have referred the single specimen with some hesitation to *verruculatus* ...”. This hesitation was also noted by Fautin et al. (2008: 52) in their entry of *H. verruculatus* from Moreton Bay, Australia. Clearly, Carlgren was doubtful of traits he had used to define species of *Heteranthus*, in particular, its type species *H. verruculatus*.

Despite Carlgren's doubts concerning the two *Heteranthus* species, he was confident that they were closely related to *Phymanthus* Milne Edwards & Haime, 1851 of the family Phymanthidae Andres, 1883. Originally, Carlgren had established Heteranthidae Carlgren, 1900 to accommodate *Heteranthus* but later synonymised it and classified the genus in Phymanthidae (see Carlgren, 1943). We inferred that he did so on the basis of morphological similarities; *Heteranthus* and *Phymanthus* species all possess two distinct tentacle types: discal and marginal (Carlgren, 1949; Yap et al., 2019). Currently, Carlgren's designation of these two genera within Phymanthidae still stands (see Fautin, 2016). However, recent molecular studies (i.e., González-Muñoz et al., 2015; Brulger et al., 2018) have suggested that Phymanthidae is polyphyletic and *Heteranthus* is nested within Actiniidae Rafinesque, 1815.

Phymanthidae species are known to be difficult to identify with certainty. Apart from *Phymanthus crucifer* (Le Sueur, 1817), which is well-studied (e.g., Duerden, 1900; González-Muñoz et al., 2012, etc.), species boundaries among other phymanthids remain unclear. This is primarily because original descriptions detailing appearances of these sea anemones were brief and vague, and their diagnoses poorly defined (e.g., Klunzinger, 1877; Haddon & Shackleton, 1893; Pax, 1924). Only recently, *P. pinnulatus* Martens in Klunzinger, 1877 was re-described after more than a century (see Yap et al.,

2019). Regarding *H. verruculatus*, the recent accounts by both Cutress (1977) and Fautin et al. (2008), unlike others (e.g., den Hartog, 1997: 358; González-Muñoz et al., 2015), provided more detailed observations since Klunzinger's (1877) original description. However, their reports were likewise brief; both made no attempts to redefine or detail traits for distinguishing *H. verruculatus* from *H. insignis*, and were simply checklists of Hawaiian and Australian sea anemones. Like *Phymanthus*, a taxonomic revision is long overdue for *Heteranthus*.

Heteranthus verruculatus is a sea anemone that is infrequently sighted because it is well camouflaged against the substratum it lives in, in addition to its relative small size (oral disc diameter <25 mm). We encountered individuals at the lower intertidal zone, each with its pedal disc attached to loose coral rubble or roots of seagrass buried underneath the sand, or sometimes within crevices of rocks. Populations of these sea anemones occur as randomly distributed solitary individuals or in densely packed, clonal clusters, consisting of tens of individuals. We have also observed that individuals frequently reproduce asexually by longitudinal fission, which appear to induce irregular symmetry and a variable appearance in resulting clones. In the aquarium trade, *H. verruculatus* are marketed and sold as ‘sand anemones,’ a name used by hobbyists that also encompasses species of *Phymanthus* (Fosså & Nilsen, 1998).

The objective of this study is to provide an updated and detailed characterization of *H. verruculatus*. This species is re-described, based on new data collected from its type material and more than 180 individuals assigned to this species. We also synonymise *H. insignis* with *H. verruculatus* after the holotype of the former was re-examined thoroughly, and since we found that evidence used to distinguish it as a new species agrees

with what we obtained from the *H. verruculatus* type material (i.e., cnidae data and musculature; see Carlgren, 1943). Furthermore, we re-establish the family Heteranthidae solely for *Heteranthus*, based on molecular data from this study and others (Gonzalez-Muñoz et al., 2015; Brugler et al., 2018), which supports the polyphyly of Phymanthidae (*Phymanthus* + *Heteranthus*). As with many other sea anemone species throughout the world, data concerning *H. verruculatus*' biogeography and ecology are lacking. A detailed redescription of this widespread and common Indo-West Pacific sea anemone resulting from the present study allows further research on a firmer footing for a wide range of disciplines.

Materials and methods

Specimen collection and processing

All living materials were collected from Singapore by hand, mainly by either digging individuals out of the substratum or extracting them from rock crevices using a chisel. Some individuals were observed and photographed in situ. Field sampling was also carried out between 2016 and 2019 in the Red Sea at Hurghada (Egypt), Eilat (Israel) and Thuwal (Saudi Arabia), these three sites being in the vicinity of *H. verruculatus*' type locality at Koseir (Egypt). Sea anemone surveys were further conducted at Zanzibar Island (Tanzania, Western Indian Ocean) and Pulau Langkawi (Malaysia, Eastern Indian Ocean). At all these localities apart from Singapore, no *Heteranthus* specimens were encountered (map in supplementary fig. S1).

Collected animals were brought back to the laboratory and kept alive for a week for further observation. For each sea anemone, details of its living appearance and any behaviour were noted. Tissues of tentacles or pedal discs of ten individuals were sampled and preserved

in 100% molecular-grade ethanol for DNA extraction. Afterwards, all sea anemone specimens were relaxed in 7.5% magnesium chloride in seawater and fixed in 10% formalin.

Morphological observations

To study the musculature of the sea anemone, serial transverse and longitudinal sections each 8 µm thick, were made from formalin-fixed specimens and stained with haematoxylin and eosin (Humason, 1967). Other specimens were dissected so that the internal morphology could be observed.

Unfired cnidae capsules were viewed and measured at 1000x magnification. These were extracted from tissues of the marginal and discal tentacle tips, marginal projections, mid-section column, actinopharynx, and mesenterial filaments. Conventionally, only measurements of unfired cnidae capsules are used in characterising the cnidom of a sea anemone species (Dunn, 1981). However, to verify Carlgren's (1943: 31) measurements of fired basitrich capsules from the holotype of *H. insignis*, we also measured these. Furthermore, we used fired capsules obtained from live individuals to confirm the identities of other cnidae types encountered in the tissues of the materials examined (see Yap et al., 2014). For some preserved specimens (including type material), we found it difficult to isolate and measure cnidae from the tissue sampled due to the state, age, and rigidity of the preserved material. In many instances the cnidae capsules visualized from these appeared degraded; we were unable to accurately identify or measure them. All identities of good cnidae capsules, which could be seen clearly and measured, were assigned following Mariscal (1974).

Original descriptions in German (e.g., Klunzinger, 1877; Carlgren, 1900) were translated into English using Google Translate (Google, 2019). Specialized German zoological

terms that failed to be translated suitably (e.g., ‘flimmersteifen’) were interpreted following Stachowitsch (1992).

To redescribe *H. verruculatus*, we examined two known syntypes that were listed in Fautin et al. (2008: 52) and Fautin (2016: 416), kept at the Museum für Naturkunde Berlin (ZMB) and Naturhistoriska Riksmuseet, Stockholm, Sweden (NRS). Because Carlgren (1943, 1950) was not confident of characters he had used to define *H. insignis*, we studied its holotype, kept also at NRS.

Species boundaries of *H. verruculatus* were further established through examining voucher specimens kept at Natural History Museum in London (NHM), Reference Collection of Lembaga Ilmu Pengetahuan Indonesia, Pulau Ambon Field Station (RCLA), Museum of Tropical Queensland, Queensland, Australia (MTQ), and Museum of Natural History, University of Florida, Gainesville, Florida, USA (UFM). While examining voucher material at the Museum Nationale d'Histoire Naturelle, Paris, France (MNHN) and the Western Australian Museum, Perth, Australia (WAM), the first author also examined specimens closely resembling *Heteranthus*, some of which were identified as the species by D.G. Fautin but not published.

For all nomenclatural matters, we act as First Revisers as explained in Article 24.2.1 of the International Code of Zoological Nomenclature (the “Code” henceforth) (International Commission on Zoological Nomenclature, 1999).

Molecular analyses

Genomic DNA was isolated from column or pedal disc tissues of each sea anemone by standard CTAB extraction (see Rodríguez et al., 2014, and references therein). Four molecular markers comprising three mitochondrial (i.e., partial 12S rDNA, 16S rDNA and *cox3*) and one nuclear (i.e. partial 28S rDNA) loci were targeted with

polymerase chain reaction (PCR) using published cycling profiles (see Lauretta et al., 2013; Rodríguez et al., 2014 and references therein). We purified all PCR products with SureClean Plus (Bioline, Singapore). Cycle sequencing was carried out with BigDye Terminator v3.1 (Applied Biosystems, Foster City, California) following the manufacturer’s protocol. Amplicons were sequenced on the ABI 3130 XL Genetic Analyzer (Thermo Scientific).

All new sequences obtained in this study were assembled in Geneious v11.1.3 (Invitrogen Corporation) using default parameters. Contigs were searched against those available on GenBank via BLASTn, on the basis of sequence similarity, to affirm that they were of sea anemones. To determine the phylogenetic position of *Heteranthus*, the single published 12S sequence of *Heteranthus* sp. was included in our analyses (i.e., accession number: KC812147; see González-Muñoz et al., 2015), together with newly obtained and published sequences of taxa from both Actiniidae and Stichodactylidae Andres, 1883 (i.e., those published in Daly et al., 2017; Titus et al., 2019), and our own collections (supplementary table S1). To further clarify the relationship of *Heteranthus* and the family Phymanthidae, new sequences of *Phymanthus loligo* (Hemprich & Ehrenberg in Ehrenberg, 1834) collected recently from the Red Sea were also included (supplementary table S1).

Sequences for each marker were aligned using MAFFT v.7.313 under *--auto* setting. Aligned sequences of all mitochondrial and nuclear markers were also concatenated into a single matrix. We conducted separate analyses for alignments of each marker, and for the concatenated dataset. The concatenated dataset comprised 149 specimens with 6229 sites that were used for phylogeny reconstruction. Maximum likelihood (ML) analyses were conducted in RAxML v.8.2.11 (Stamatakis, 2014) with 100 random starting trees and 1000

bootstrap pseudo-replicates, under the GTR-GAMMA model for both individual markers and the concatenated matrix. Pairwise genetic distances between samples for each molecular marker were calculated using Geneious v11.1.3.

Bayesian inference was carried out on the concatenated matrix using MrBayes v3.2.6 (Ronquist et al., 2012). Based on the Akaike information criterion (AIC) implemented in jModelTest2 (Darriba et al., 2012), we selected GTR + I + G as the best-fit model for *cox3*, 12S, and 28S, whereas HKY + G was selected for 16S. We performed four Markov chain Monte Carlo (MCMC) runs for 12 million generations and sampled every 100th tree. To determine if the runs converged, we used Tracer v1.7 (Rambaut et al., 2018), and discarded the first 20,000 trees as burn-in. Overall, our four MCMC runs did not converge, as with observations from previous studies (see Rodríguez et al., 2014; Titus et al., 2019). However, Bayesian inference (BI) probabilities were still included in our final results, on branches that were also recovered in the ML analyses.

All new *H. verruculatus* voucher specimens collected from Singapore were deposited in the Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum, National University of Singapore. All new sequences were deposited in GenBank (supplementary table S1). Sequence alignment for each molecular marker and pairwise genetic distances calculated were deposited in Zenodo (<https://doi.org/10.5281/zenodo.4034666>).

Results

Phylogeny reconstruction

Topologies inferred from ML analyses of the concatenated dataset and those of the molecular markers independently were similar at the genus level, but not fully concordant (fig. 1; supplementary figs. S2–S6). In all ML

trees, the family Phymanthidae was recovered as polyphyletic as *Heteranthus verruculatus*, *Phymanthus crucifer*, and *P. loligo* together did not form a monophyletic group. Overall, all *H. verruculatus* specimens formed a group (= Heteranthidae clade; fig. 1) that included some species of *Anthopleura* Duchassaing & Michelotti, 1860 and *Gyractis* Boveri, 1893 (fig. 1, supplementary fig. S2). This clade was sister to another that included other species of *Anthopleura*, species of *Heteractis* Milne Edwards & Haime, 1851 (excluding *H. magnifica* (Quoy & Gaimard, 1833)), *Macrodactyla doreensis* (Quoy & Gaimard, 1833) and *P. loligo* (i.e., AHMPL clade; fig. 1). However, relationships among these two clades were not well-supported, with bootstrap values being <50 at the most inclusive nodes (fig. 1, supplementary fig. S2). Samples of *H. verruculatus* collected from the same population (e.g., ZRC. CNI.1230; table S1) also differentiated into a number of branches with low bootstrap support (<50) at diverging nodes (i.e., 16S tree; supplementary fig. S4). *Phymanthus crucifer* was recovered to be nested within Actiniidae. Within the AHMPL clade, *P. loligo* was recovered as a monophyletic group that was sister to *Heteractis*/*Macrodactyla*, a relationship that was strongly supported (bootstrap = 86; fig. 1).

Pairwise genetic distances between Heteranthidae and its sister-AHMPL clade ranged from 0% to 26.3%, dependent on the molecular marker examined (table 1). Among members within the Heteranthidae clade, the greatest variation was observed for the 16S molecular marker, which ranged from 0% to 3.7%, whereas those of *cox 3*, 12S and 28S markers were from 0% to 2.7% (table 1). Likewise, among *H. verruculatus* individuals, the greatest pairwise genetic distance was also observed for the 16S marker (i.e., 3.7%), whereas those of the remaining three markers were low (e.g., 28S: ≤0.5%; see: <https://doi.org/10.5281/zenodo.4034666>).

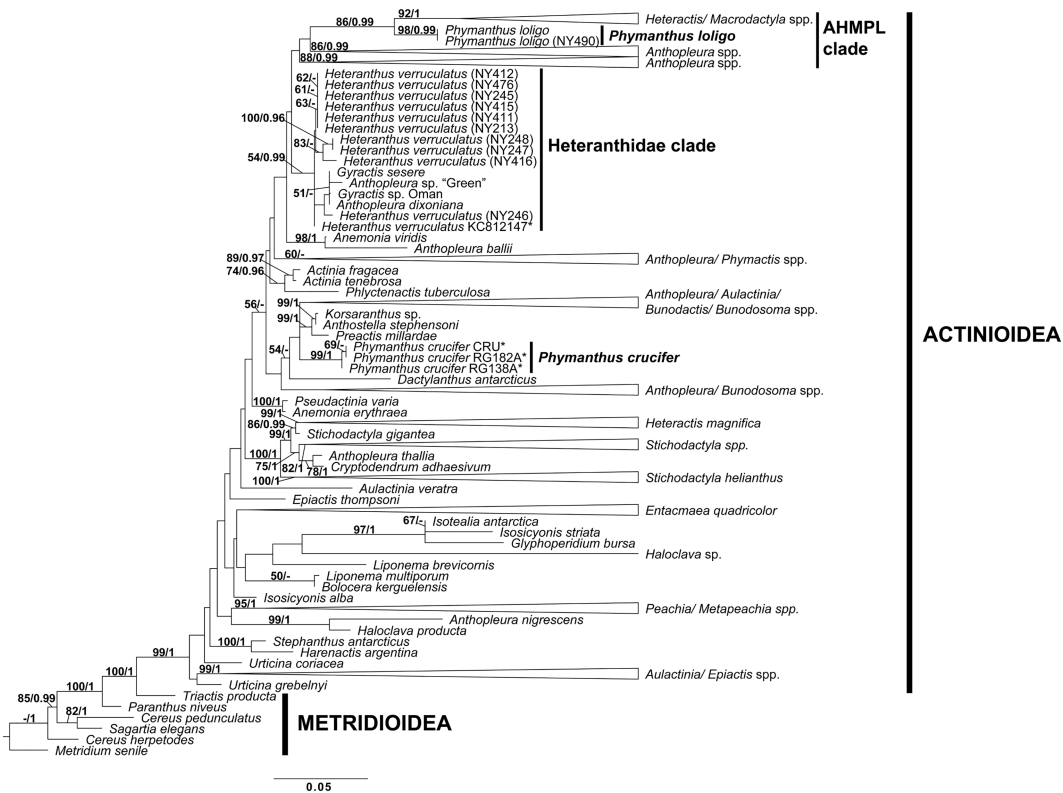


FIGURE 1 Maximum likelihood phylogram of sea anemones closely related to *Heteranthus verruculatus*. Topology presented consists of a concatenated dataset (i.e., *cox3*, 12S, 16S, 28S). Bootstrap resampling values under ML, and posterior probability values of Bayesian Inference (BI), are indicated at the branches as ML/BI. Only bootstrap values ≥ 50 and posterior probability ≥ 0.8 are shown; values < 50 and < 0.8 are indicated by (-). Text in parentheses are catalogue numbers for tissue samples of new sequences in this study (see supplementary table S1); (*) denotes *H. verruculatus* sample collected from Australia, previously published in González-Muñoz et al. (2015). For full tree, refer to supplementary fig. S2.

TABLE 1 Pairwise genetic distance range among the taxa for the four molecular markers targeted in this study

Pairwise genetic distance range			
Molecular markers used		Between members of Heteranthidae and AHMPL clade	Among members recovered within Heteranthidae clade
Mitochondrial	<i>cox 3</i>	1.8% to 10.7%	0.0% to 2.0%
	12S	0.1% to 1.2%	0.0% to 1.4%
	16S	0.0% to 4.1%	0.0% to 3.7%
Nuclear	28S	0.5% to 26.3%	0.0% to 2.7%

Systematic account

Order Actiniaria Hertwig, 1882

Suborder Enthemonae Rodríguez & Daly, 2014 in Rodríguez et al., 2014

Superfamily Actinioidea Rafinesque, 1815

Family Heteranthidae Carlgren, 1900

We revised Carlgren's (1900: 92) diagnosis written in German. Key changes interpreted from recent studies (e.g., González-Muñoz et al., 2015; Brugler et al., 2018) and are here indicated in **bold**; all minor additions (i.e., of word order, updated terminology, adjectives for clarity, etc.) are in *italics*.

Diagnosis. **Actinioidea** with a well-developed *pedal* disc. Sphincter *diffuse* to circumscribed, *weak to strong*. **Number of siphonoglyphs variable**. *Distalmost margin with marginal projections*, fosse *generally shallow*. **Distal column with verrucae**. **Number of mesenteries variable**, *retractor muscles strong*, typically *diffuse circumscribed*. Tentacles *without protuberances nor globose in appearance*. **Zooxanthellate**. **Cnidom: spirocysts, basitrichs, microbasic amastigophores, microbasic *p*-mastigophores**.

Type genus. *Heteranthus* Klunzinger, 1877

Other valid genera. None.

Remarks. Based on morphological similarity, Carlgren (1943) synonymised Heteranthidae and Phymanthidae, and classed *Heteranthus* in the latter. We resurrect Heteranthidae for *Heteranthus* as molecular phylogenetic results from this and previous studies (see González-Muñoz et al., 2015; Brugler et al., 2018) do not support Carlgren's (1943) conclusion. Following this line of evidence, we also revised the mention of higher-level classification in the original diagnosis. Among characters absent from Carlgren's (1900) diagnosis are cnidom and arrangement of the internal musculature. We included these here as they are a necessary part of contemporary sea anemone taxonomy.

Genus *Heteranthus* Klunzinger, 1877

Diagnosis modified after Carlgren (1949) and Fautin et al. (2008: 52). Substantial changes from our research are indicated in **bold**; all minor additions are in *italics*.

Diagnosis. Pedal disc well-developed. Column, apart from most proximal part, with verrucae, which are smaller and more numerous at the margin, and overhang the fosse, *resembling papillae*. Endodermal marginal sphincter muscle, **restricted to circumscribed in form, may form** a few folds. **Tentacles as marginal and discal tentacles**. Marginal tentacles conical, simple, **without ramified protuberances**, arranged in cycles, **innermost cycle longer than outermost**; discal tentacles shorter and papilliform, *arranged as dense or sparse radial rows*. *Siphonoglyphs well-developed, when present*. *Number of directives pairs, and pairs of complete and incomplete mesenteries variable*, gametogenic tissue may be present **on both**; retractor muscles *diffuse to diffuse circumscribed*, well developed. Parietobasilar muscles weak to fairly strong. **Frequently propagates by longitudinal fission**. Cnidom: spirocysts, basitrichs, **microbasic amastigophores, microbasic *p*-mastigophores**

Gender. Masculine

Type species. *Heteranthus verruculatus* Klunzinger, 1877, by monotypy (see Fautin, 2016)

Remarks. Carlgren (1949: 75) stated that the column is "provided with large verrucae," but we found this character to be variable in size and visibility, among preserved specimens. We adhere to the rewording by Fautin et al. (2008) in not specifying size of verrucae to allow for this variation. On marginal tentacles, Klunzinger (1877) reported that these were of equal length, but Fautin et al. (2008) found otherwise in studying live specimens. Likewise, we observed that the innermost cycle of marginal tentacles was longer than those at the outermost

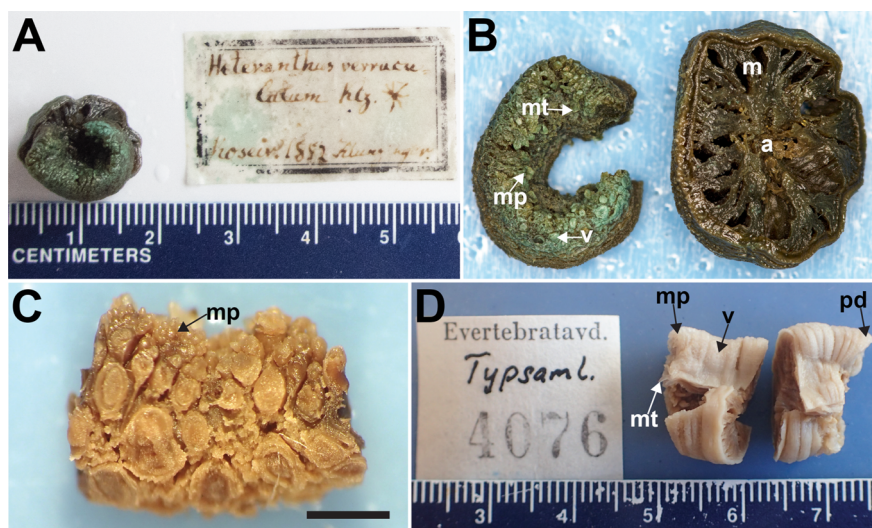


FIGURE 2 Name-bearing type materials of *Heteranthus verruculatus* and *Heteranthus insignis*. A. dried lectotype of *H. verruculatus* (ZMB 1852), top view. B. close-up of ZMB 1852 in two pieces, showing details of distalmost end, and mesenteries, top view. Note that a slice of the distalmost end is missing. C. the piece of distalmost end from lectotype ZMB 1852, now at Naturhistoriska Riksmuseet (NRS 4861), Sweden, side view. Note details of conspicuous verrucae and marginal projections with papillae-like bumps. D. holotype of *H. insignis*, an entire specimen (NRS 4076), side view. Abbreviations: a, actinopharynx; m, mesenteries; mp, marginal projection; mt, marginal tentacles; pd, pedal disc; v, verrucae. Scale bar = 1 mm. Photographs by NWL Yap.

in living individuals. Note that in many preserved specimens, this feature may be less-pronounced and tentacles may contract. Specification of the number of siphonoglyphs present was removed as this varied among individuals or be absent entirely. Similarly, the number of directive pairs, and pairs of complete and incomplete mesenteries also varies among individuals. Like both Carlgren (1949) and Fautin et al. (2008), we too failed to find any gametogenic tissues in all but those specimens from Western Australia. Moreover, we observed that living polyps readily undergo longitudinal fission. The diagnosis is modified to indicate the presence of gametogenic tissue in some individuals and its reproductive mode.

We confirm the presence of microbasic amastigophores in live materials examined for cnidae; this cnidae type has not been reported in *Heteranthus*. Because unfired capsules of

microbasic amastigophores strongly resemble those of microbasic *p*-mastigophores in preserved specimens, they may have been mistaken for the latter and were not reported (see Östman, 2000; Yap et al., 2014).

Heteranthus verruculatus Klunzinger, 1877
figs. 2–10

Heteranthus verruculatus Klunzinger, 1877: 84 (original description); Carlgren, 1900: 92; Stephenson, 1922: 290; Carlgren, 1943: 31; Carlgren, 1949: 75; Cutress, 1977: 138; den Hartog, 1997: 358; Fautin et al., 2008: 52; Fautin, 2016: 416

Actinothrix verruculata: Andres, 1883: 509

Heteranthus verriculatus [no author]: Carlgren, 1943: 32

Heteranthus insignis Carlgren, 1943: 30 (original description); Carlgren, 1950: 440; Fautin, 2016: 275 NEW SYNONYMY

Heteranthus sp.: González-Muñoz et al., 2015: 3; Brugler et al., 2018: 10

Material examined (*- observed alive by the first author)(table S2). Indian Ocean. – Oman, Bar Al Hikan Peninsula (UFM 4220 x2); Western Australia, Perth, Rottnest Island: Radar Reef (WAM Z33600 x1; WAM Z33604 x6), Cape Vlamingh (WAM Z33601 x3).

Western Pacific Ocean. – Singapore: Cyrene Reef (ZRC.CNI.0496 x2; ZRC.CNI.0505 x8; ZRC.CNI.0506 x3; ZRC.CNI.0507 x17; ZRC.CNI.0583 x3; ZRC.CNI.1207 x8*; ZRC.CNI.1230 x>50*; ZRC.CNI.1367 x18*), Pulau Jong (ZRC.CNI.1368 x>35*), Pulau Tekukor (photograph only), Raffles Lighthouse (photograph only); Indonesia (Pulau Ambon): Latuhalat (RCLA.Cni.004 x3*), Tial (RCLA.Cni.003 x4*, RCLA.Cni.008 x2*); Eastern Australia: Low Isle on Snapper Island, Great Barrier Reef (NHM 1954.6.28.23 x1), Moreton Bay (MTQ-G59967 x2; MTQ-G59968 x3; MTQ-G59391 x21; MTQ-G59392 x1), Mystic Sands, Townsville (MTQ-G59393 x5).

South Pacific Ocean. – French Polynesian Islands, Mangareva, Rikitea (MNHN-IK-2019-8 x3).

Nomenclatural considerations and type material examined. Both Fautin et al. (2008: 52) and Fautin (2016: 416) listed two syntypes of *H. verruculatus*: the first is a nearly complete polyp that is kept in Berlin (ZMB 1852), while the second is a small fragment of a syntype, kept in Stockholm (NRS 4861). Regarding the latter, it has a recent label indicating that the sample was collected by ‘Stuhlmann’ between ‘1888–1889,’ from ‘East-Africa, Sansibar, Kokotoni, S of Insel Puopo. Rock.’ This recent label is almost certainly an error. Despite these two syntype lots being kept in separate museums, they are not of different individuals; the fragment present in Stockholm is from the same individual present in Berlin, therefore only one syntype specimen exists instead of two.

While Carlgren’s (1900) publication dealt with material collected by Stuhlmann from East Africa, it also presented diagnoses of Heteranthidae and *Heteranthus*. Carlgren

(1900: 92) did not report any species of the genus collected from that locality and explicitly states that, “... *Heteranthus* KLUNZ. mit nur einer Spezies, *H. verruculatus*. Diese Spezies ist nicht in der STUHLMANN’schen Sammlung enthalten.”. In other words, Carlgren did not find *Heteranthus* individuals from East Africa in Stuhlmann’s collection. We infer that Carlgren’s (1900) inclusion of the diagnoses served to facilitate discussion regarding East African *Phymanthus* specimens, which he described in detail in his publication.

Older labels within lot NRS 4861 provided further support that this fragment is part of the syntype kept in Berlin. Though faded, one label has the number “1852,” written on it, which corresponds to the catalogue number present on the old label of the Berlin specimen (fig. 2A). Other older labels, likely written by Carlgren himself, indicate that the fragment was from “Röda havet” [= Red Sea], and “Klunz origl”. In addition, the NRS fragment corresponds to the position of the missing slice of ZMB 1852, though slightly smaller in width, with the oral disc and actinopharynx absent; presumably Carlgren had prepared histological slides from the NRS material (see Carlgren, 1950). This confirms that the *H. verruculatus* fragment numbered NRS 4861 is part of the syntype ZMB 1852.

We hereby designate both ZMB 1852 and NRS 4861, previously considered as separate syntypes but now found to be of the same syntype specimen, as the lectotype for the name *Heteranthus verruculatus* Klunzinger, 1877 (Code Article 74), to enhance its nomenclatural objectivity. While C.B. Klunzinger described the species from more than one individual that he had collected from Egypt (Klunzinger, 1877; Fautin, 2016), the first author was not able to locate any other syntypes at museums where Klunzinger’s specimens are now kept, or in those that O. Carlgren have visited and possibly transferred some. Should these other syntypes be found, they are the paralectotypes

of *H. verruculatus* (Code Article 74.1.3). In this publication, all three conditions stipulating the designation of a lectotype after 1999 have been met (Code Article 74.7).

Heteranthus verruculatus – Lectotype. ZMB 1852 (fig. 2A, B), collected by C.B. Klunzinger, no later than 1877 from the Red Sea (Koseir). Two pieces of a single specimen, rigid and brittle, cut transversely across at mid-column (fig. 2B). Distal-most column slit longitudinally, a slice of distal-most margin missing (fig. 2B). An oily scum layer encrusts oral disc. Both pieces dark-greenish or grey. Total length 14 mm, mid-column 13 mm wide, distal-most and proximal end 15 mm wide, respectively; NRS 4861 (fig. 2C) a single fragment originating from ZMB 1852. A piece of distal-most end of column, also includes part of the mid-column, dark sandy-brown in colour, in good condition. Fragment 4 mm wide.

Heteranthus insignis – Holotype, NRS 4076, collected by D.C. Dawydoff from Vietnam and given to O. Carlgren (fig. 2D). A single specimen, 17 mm in length, cut transversely into two pieces at mid-column, with a longitudinal cut along each. A slice of distal-most margin missing. Both pieces firm, in good condition. Both pieces cream-coloured in preservative.

Natural history. Encountered during low spring tides along shallow tropical coasts, with oral disc and marginal tentacles expanded (fig. 3A). Sand grains and shell fragments adhere to verrucae at distal-most end (fig. 3B). May occur as solitary or as clonal, clustering individuals (fig. 3C, D), inhabiting narrow rocky crevices. Clustering type abundant in sandy seagrass meadows of *Halophila ovalis*, with pedal end attached to the roots of plant or coral rubble underneath. Asexual propagation via longitudinal fission frequently observed (fig. 4A–F; supplementary video S1). Zooxanthellate.

Oral disc. When fully expanded, margin may be slightly undulate in solitary individuals; in smaller clustering ones, flat (fig. 5A, B). Outline

oval to round; elongated oval during onset of longitudinal fission (fig. 4A). In life, light to dark-brown, may be speckled with white patches. Thin-walled, mesenterial insertions seen as radial dark lines extending from mouth to marginal tentacles in living animals, as white lines in preserved materials. Discal tentacles present, papilliform (fig. 5C, D). Area immediately around mouth without discal tentacles (fig. 5D), arranged in radial rows from mid-way extending towards marginal tentacles. Radial rows of discal tentacle both endocoelic and exocoelic, numerous. In solitary individuals, discal tentacles conspicuous and densely arranged, clustering type less conspicuous and less dense (fig. 5D), becoming obscure when preserved. Central mouth flat, edges tinged brown, white or pink in life, cream-white in preserved specimens.

Marginal tentacles. Numerous, numbers variable; in solitary individuals that exhibit a regular symmetry (i.e., mesenteries arranged symmetrically) typically 96, arranged in five cycles (e.g., RCLA.Cni.008). One per endo/exocoel; innermost cycles endocoelic, outermost exocoelic. Innermost cycle longer than outermost, longest length approximately $\frac{3}{4}$ radius of oral disc. Simple, conical, without ramified protuberances in life, some individuals slightly inflated at mid-tentacle (figs. 3A, C, 5A). Tip blunt, without perforation in live individuals; after preservation may appear perforated due to tentacle retraction (e.g., NHM 1954.6.28.23; fig. 5C). Wide at base, narrow toward tip. Colour in life greenish-brown, some with light horizontal cross bands along oral face (fig. 5A, D). Base coloured white to dark brown, tip with white specks in life, cream-white in preserved specimens.

Column. Distal-most end flared slightly outwards when expanded. Distal margin with marginal projections, both endo/exocoelic. Larger marginal projections, endocoelic; smaller: exocoelic. In solitary individuals,

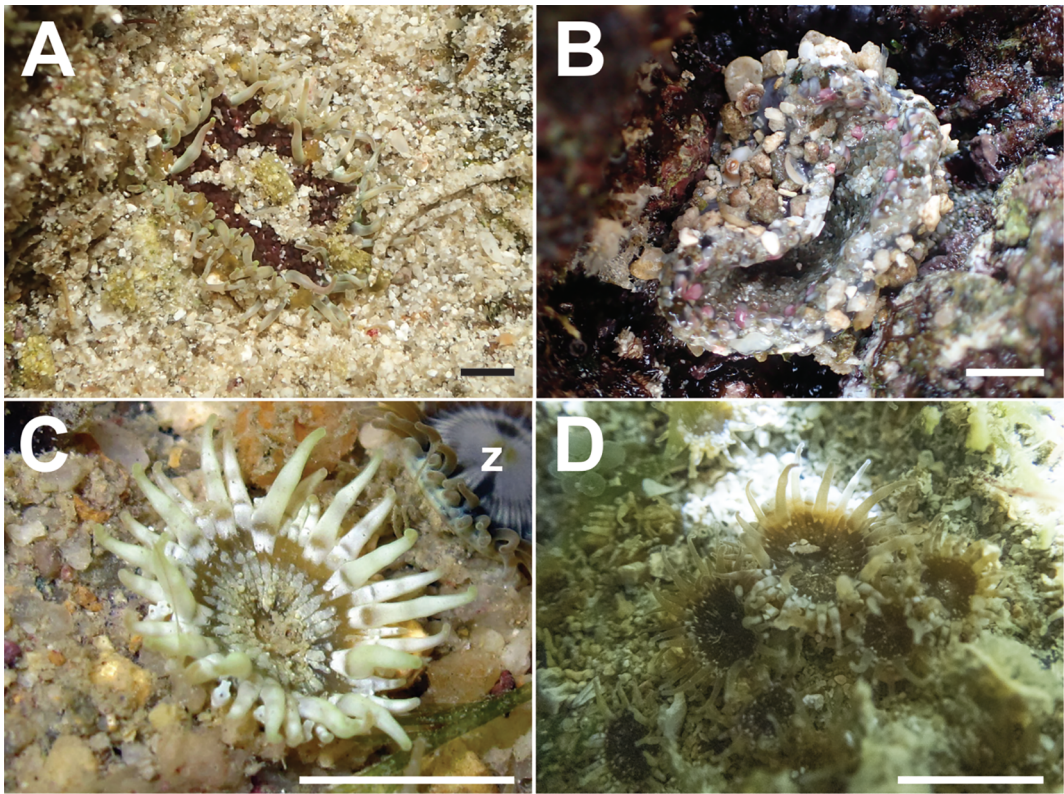


FIGURE 3 Living specimens of *Heteranthus verruculatus*. A. an expanded individual (RCLA.Cni.003), camouflaged with the sand that it is buried in, Tial, Pulau Ambon, Indonesia. Photograph by NWL Yap. B. a partially contracted individual (RCLA.Cni.003) extending from a rocky crevice, with sand grains and shell fragments still densely attached to distalmost end, at Tial, Pulau Ambon, Indonesia. Photograph by NWL Yap. C. an expanded smaller individual with tentacles extended, encountered in a bed of *Zoanthus* at Pulau Tekukor, Singapore, in situ. Photograph by KS Loh, with permission. D. a population of densely packed clustering individuals (ZRC.CNI.1207), encountered in a *Halophila ovalis* meadow at Cyrene Reef, Singapore. Photograph by NWL Yap. Abbreviation: z, *Zoanthus* sp. Scale bar = 5 mm.

marginal projections densely covered with conspicuous, papillae-like verrucae (fig. 6A–C) that may be less dense or inconspicuous in clustering individuals (e.g., ZRC.CNI.1207, MTQG59393; fig. 6D). Longitudinal rows of round verrucae extend proximally from marginal projections, smaller near distalmost end, increasing in size proximally towards mid-column. Verrucae very conspicuous in solitary individuals, edges thickened and slightly raised, middle depressed (fig. 6E); less conspicuous in clustering polyps (fig. 6F). Longitudinal verrucae rows both endo/exocoelic. Endocoelic rows longer, up to

20 verrucae per row; exocoelic: shorter, up to four in each row; shell fragments, coral rubble and/or sand grains may adhere to the verrucae. Column wall thin, mesenterial insertions can be seen through as faint light lines extending from distal to proximal end. From mid-column to proximal end of column smooth, without cinclides. Fosse present, shallow. In life, light-brown with greenish-tinge to cream-white with reddish orange/pink splotches, distalmost end dark grey (fig. 6E–F). In preserved specimens, column entirely cream-white; distalmost end grey in recently preserved materials.

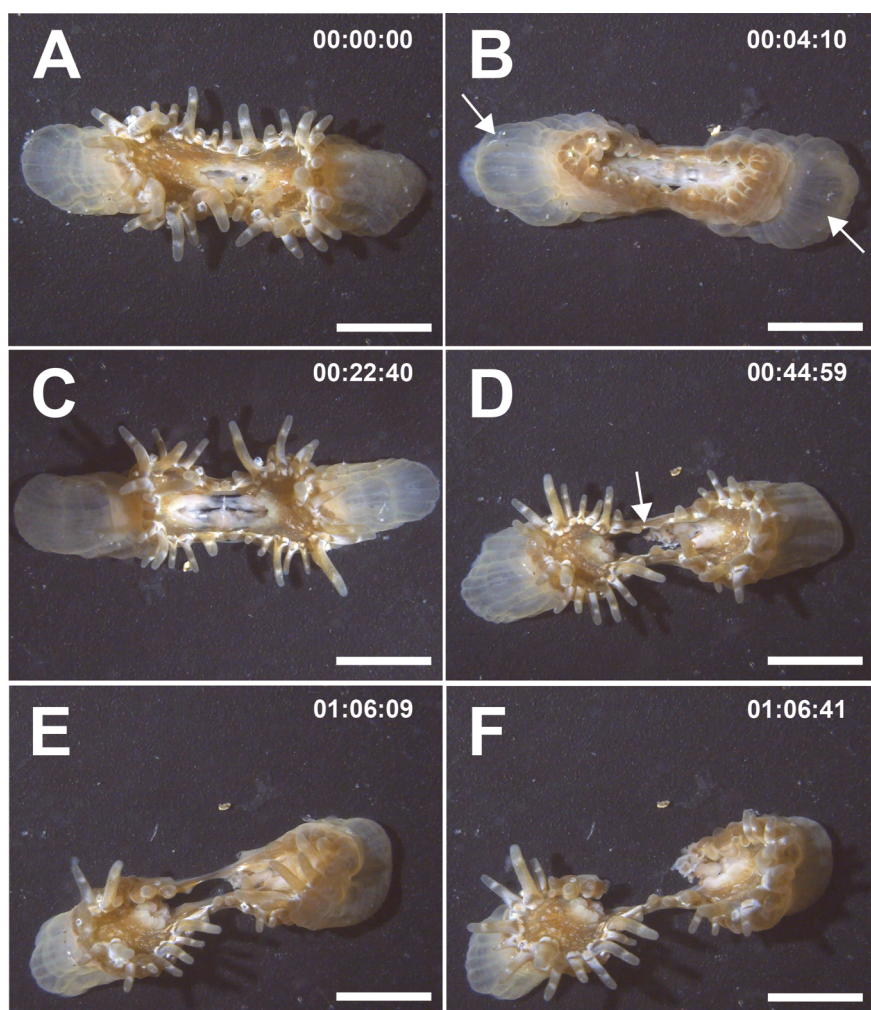


FIGURE 4 A clustering *Heteranthus verruculatus* (ZRC.CNI.1230) undergoing longitudinal fission. A, B. lateral stretching, with pedal-end extending the widest. Both column and pedal-ends inflate and deflate (arrowed) as the sea anemone pulls itself in opposite directions. C, D. column cleaved; continued stretching of actinopharynx that eventually splits, with distalmost end remaining (arrowed). E, F. pulling and splitting of the distalmost end of column where sphincter muscles are. Duration of observation is denoted at the top right-hand corner of each panel, time rendered in hours, minutes, and seconds (i.e., hh:mm:ss). Scale bar = 2 mm. Video screen capture by NWL Yap. For full video, refer to supplementary video S1.

Pedal disc. Adheres readily to surfaces when animal is alive. Outline: oval, may be flat or inflated. Limbus slightly scalloped. Thin-walled, mesenterial insertions seen as light lines. Diameter may be equal or greater than oral disc.

Internal anatomy. Anatomy typically arranged regularly in solitary individuals (e.g., RCLA.Cni.008); irregular in clustering

individuals (e.g., NRS 4076, WAM Z33601). Actinopharynx pleated longitudinally, extends proximally to mid-column; white in life, cream-colored in preserved specimens. Marginal and oral stomata present. Usually two siphonoglyphs in solitary individuals, symmetrical; clustering individuals with supernumerary siphonoglyphs (i.e., three

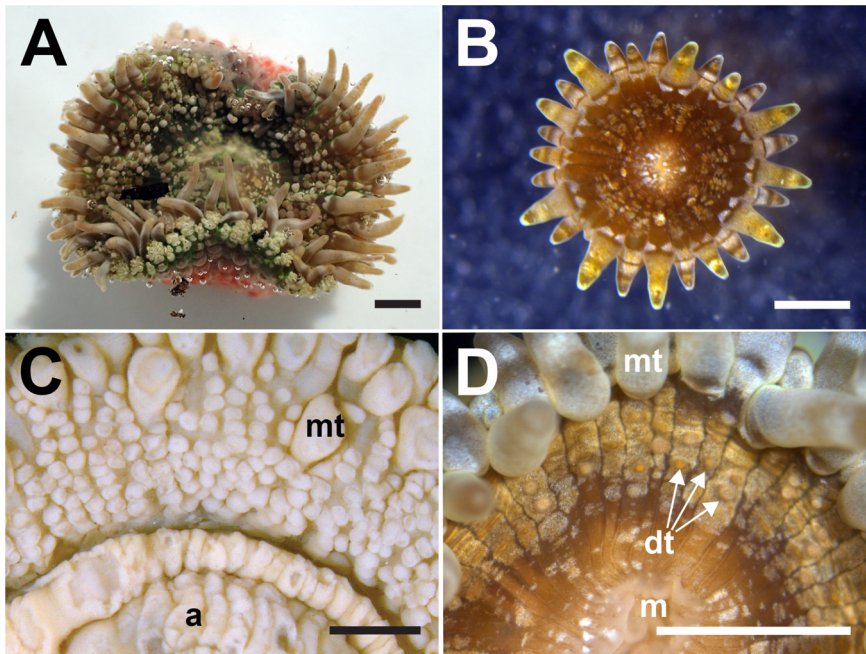


FIGURE 5 Oral disc appearance of *Heteranthus verruculatus*. A. an expanded solitary individual (RCLA.Cni.008) with a crenulated, oval oral disc. B. an expanded clustering polyp (ZRC.CNI.1207) with a round, flat oral disc. In both A and B, innermost cycle of marginal tentacles is longer than outermost. C. dense arrangement of discal tentacles of a solitary individual from the Great Barrier Reef (NHM 1954.6.28.23). D. discal tentacles of a clustering polyp (ZRC.CNI.1230) with a sparse distribution of tentacles (arrowed). Abbreviations: a, actinopharynx; dt, discal tentacles; m, mouth; mt, marginal tentacles. Scale bar = 2 mm. Photographs by NWL Yap.

siphonoglyphs in WAM Z33604) or may be entirely absent, asymmetrical. Mesenteries numerous, number of pairs variable in clustering individuals (fig. 7A, B). In solitary individuals, mesenteries typically arranged in up to four cycles (i.e., $6 + 6 + 12 + 24$ pairs), symmetrical. Mesentery pairs in first two cycles complete, those of third cycle incomplete, all with filaments and gametogenic tissue, if present (fig. 7C). Mesentery pairs in fourth cycle incomplete, without retractor muscle or filaments. Retractor muscles strong, diffuse, always humped along its edge but less pronounced in clustering individuals. Parietobasilar muscles poorly developed, retractor pennon reduced or absent; if present, extends away from mesentery (fig. 7A–C). Directives attached to a siphonoglyph each, if present. Sphincter muscle below fosse, form

variable among specimens. Typically strong, conspicuous and circumscribed, or restricted or with a few folds (fig. 8A–C).

Cnidom Spirocysts, basitrichs, microbasic amastigophores, and microbasic *p*-mastigophores (table 2; fig. 9).

Distribution (fig. 10). **Type localities:** Egypt, Koseir (of *H. verruculatus*; Klunzinger, 1877); Vietnam, Poulo Condore [= Côn Sơn Island, Vung Tau] (of *H. insignis*; Carlgren, 1943). **Published records:** Eastern Australia, Great Barrier Reef (Carlgren, 1950), Moreton Bay and Townsville (Fautin et al., 2008); Indonesia (den Hartog, 1997: locality not specified); Hawaii, Oahu, Kaneohe Bay (Cutress, 1977); Western Australia (Fautin et al., 2008: locality not specified). **New records:** French Polynesian Islands, Mangareva, Rikitea; Oman, Bar Al Hikman Peninsula; Singapore.

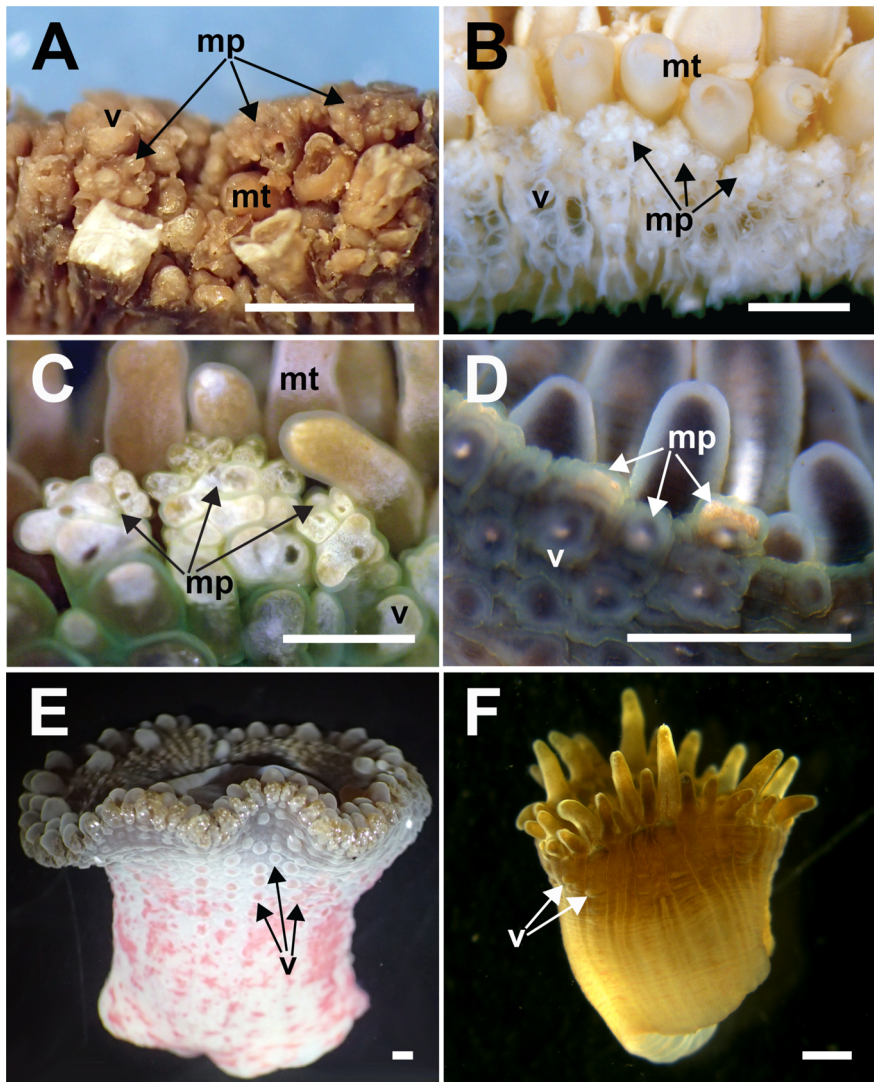


FIGURE 6 Appearance of the column of *Heteranthus verruculatus*, external view. A. marginal projections on lectotype (NRS 4861), with numerous papillae-like verrucae, top view. B. marginal projections of *H. insignis* [= *H. verruculatus*], holotype (NRS 4076) with papillae-like verrucae, side view. C. marginal projections of a solitary individual (RCLA.Cni.008) covered densely with papillae-like verrucae. D. variation in appearance on marginal projection appearance on a clustering one (ZRC.CNI.1207). E. conspicuous verrucae of a solitary individual (RCLA.Cni. 004). F. inconspicuous verrucae of a clustering polyp (ZRC.CNI.1207). Abbreviations: mp, marginal projections; mt, marginal tentacles; v, verrucae. Scale bar = 1 mm. Photographs by NWL Yap.

Discussion

Comparisons of type and voucher specimens. The animals from Singapore and Pulau Ambon, and of museum vouchers collected elsewhere (e.g., Oman and Australia), closely

resemble *H. verruculatus* which Klunzinger had described and depicted (see Klunzinger, 1877: pl. 5, fig. 9). They also agree in habitats where Klunzinger collected his animals from (i.e., seagrass, rock clefts, small stones; see Klunzinger, 1877: 84). Despite some variation

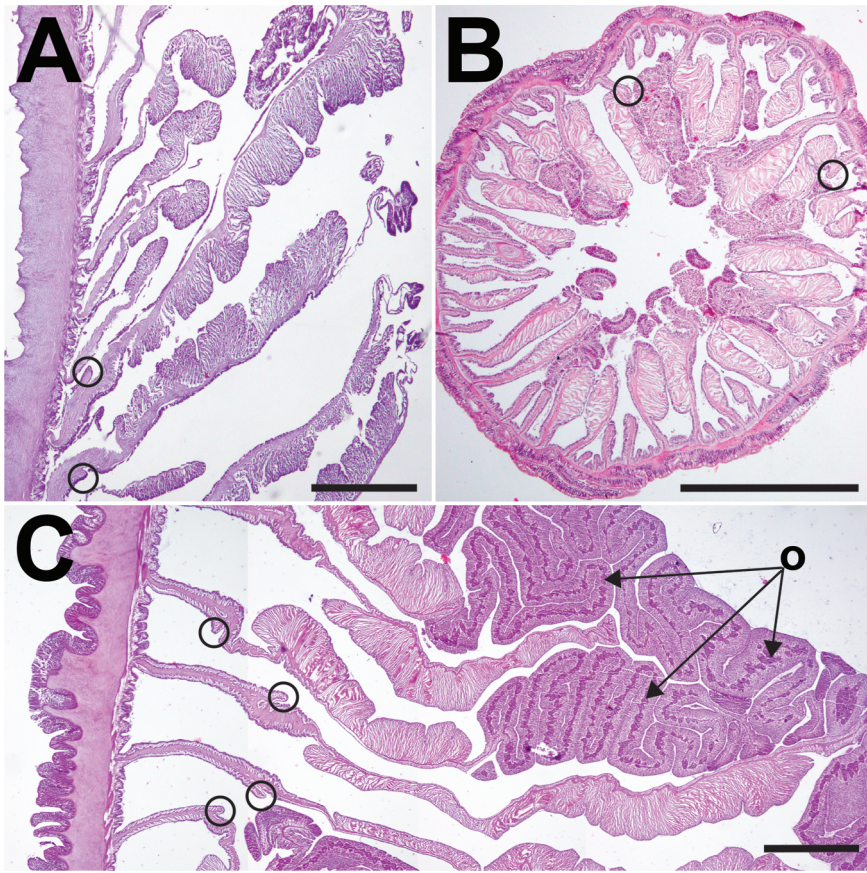


FIGURE 7 Cross sections of *Heteranthus verruculatus*, through the level below actinopharynx, internal morphology. A. mesenteries of holotype *Heteranthus insignis* (NRS 4076) [= *H. verruculatus*]. Note complete mesenteries have humps along its edges. B. asymmetrical arrangement of mesenteries in a clustering individual (ZRC.CNI.1207), directives are absent. C. gametogenic tissues with oocytes present on both complete and incomplete mesenteries of an individual (WAMZ33604). Circles indicate the presence of the parietobasilar muscles as pennons. Abbreviation: o, oocytes. Scale = 2 mm. Photographs by NWL Yap.

in external morphologies among many of them (e.g., MTQG59391, ZRC.CNI.1368), all are clearly *H. verruculatus*. The animal's frequent propagation through longitudinal fission is responsible for differences observed between those reported by Klunzinger (1877) and what we have found.

Klunzinger's (1877) original description did not include data on internal musculature form or cnidom, common in many sea anemone taxonomic descriptions published at that time. Overall, our data on the retractor and sphincter musculature of vouchers agree

with Carlgren's (1943, 1950) observations of the *H. verruculatus* type material. While the sphincter muscle was circumscribed in most individuals (e.g., NHM 1954.6.28.23), in others we found that it may vary in shape and form; it can be restricted (e.g., MTQ-G59391) or multilobed (fig. 8C, WAM Z33600). In comparing new cnidae measurements made from the lectotype fragment of *H. verruculatus* (NRS 4861), and voucher materials we studied, the overlap is nearly complete. Our measurements also agreed with those made by Carlgren (1943).

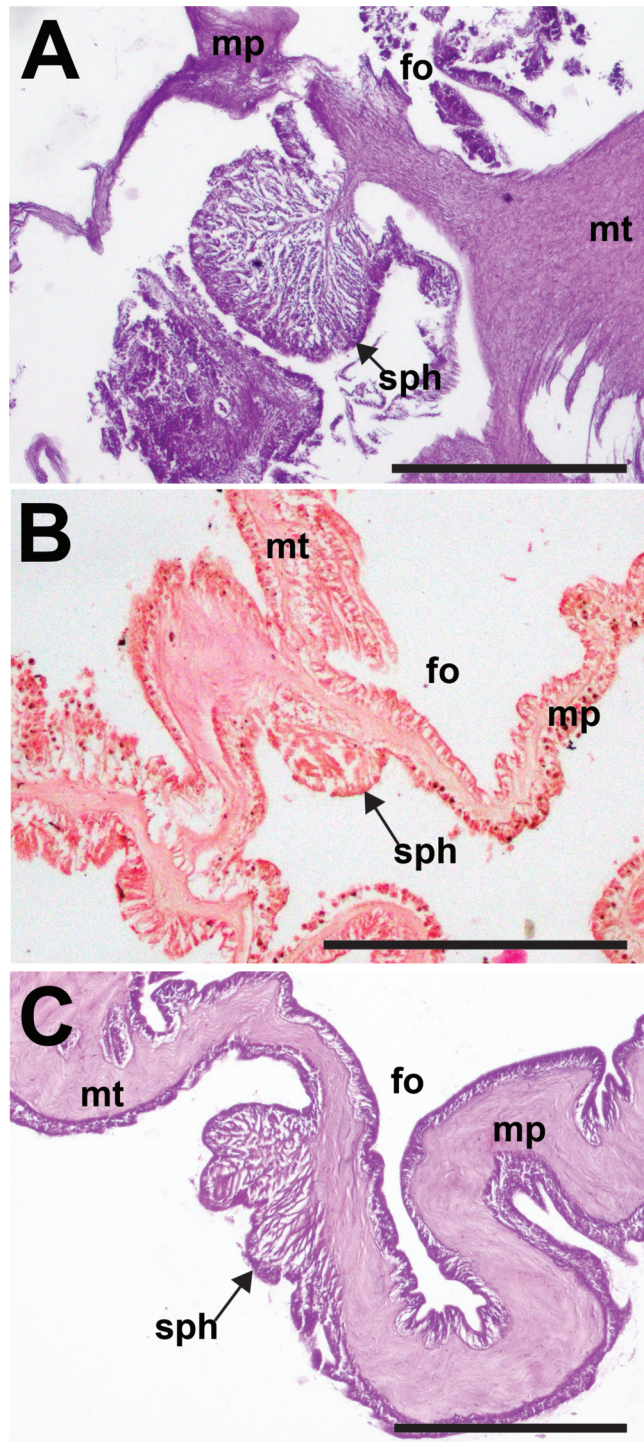


FIGURE 8 Longitudinal sections of *Heteranthus verruculatus* at distalmost end illustrating varying sphincter muscle forms, internal morphology. A. conspicuous and circumscribed (NHM 1954.6.28.23). B. restricted and inconspicuous (MTG59392). C. conspicuous with many folds (WAM Z33604). Abbreviations: fo, fosse; mp, marginal projection; mt, marginal tentacle; sph, sphincter muscle. Scale = 1 mm. Photographs by NWL Yap.

TABLE 2 Cnidom of *Heteranthus verruculatus* Klunzinger, 1877, comprising type specimens (i.e., those of *H. verruculatus* and *H. insignis*), and voucher specimens. Regarding the latter, cnidae measurements were taken from both solitary and clustering polyps

Tissue	Cnidae	Type Specimens			Voucher Specimens			
		NRS 486i		NRS 4076	Solitary polyps		Clustering polyps	
		Range length × width	n	Range length × width	n	Range length × width	n	Range length × width
Marginal tentacles	Spirocysts (A)	(10.0)12.0–17.0 × 1.5–3.0	10	(9.6)10.8–18.0 × 2.4	10	(11.0)13.0–23.0 × 2.0–3.0	60	10.0–20.2 × (1.5)2.0–3.0
	Basitrichs (B)	(14.0)16.5–19.0 × 2.0–3.0	10	15.6–18.0 × 2.4–3.0(3.6)	10	14.0–18.7 × 2.0–3.0	60	(12.1)13.0–19.0 × 2.0–3.0
		14.8–18.3 × 2.0–2.5	?					
Discal tentacles	Spirocysts (C)	–	–	13.2–16.8 × 2.4–3.0	10	(8.0)10.0–19.0 × 2.0–3.5	60	(9.0)11.1–17.2 × 2.0–3.0(3.5)
	Basitrichs (D)	–	–	10.8–13.2 × 2.4	10	9.0–16.0 × 2.0–3.0	60	8.0–20.5(22.0) × (1.5)2.0–2.5(3.0)
								7.5–13.1 × 2.0–2.5
Marginal projections	Basitrichs (E)	8.0–10.0 × 1.5–2.0	10	12.0–18.6(24.0) × 2.4–3.0(3.6)	5	10.0–15.0 × 2.0–2.5	59	7.5–13.1 × 2.0–2.5
	Column	18.0–21.0 × 2.0–3.0	10	(12.0)15.0–25.2 × 2.0–3.0	23	11.0–19.2 × 2.0–3.0(3.5)	60	11.0–17.0 × (1.5)2.0–2.5
		15.5–20.6 × 2.0–2.5	?					
Actino-pharynx	Basitrichs (G)	–	–	(18.6)20.4–23.4 × 2.4–3.0	10	(15.0)16.0–25.3 × 2.0–4.0	60	16.0–23.5 × 2.0–3.0(3.5)
	Microbasic p-mastigophores (H)	–	–	15.6–18.6 × (2.4)3.6–4.8	10	15.0–25.3 × 3.0–6.0	60	15.0–25.5 × 3.0–7.0

Tissue	Cnidae	Type Specimens			Voucher Specimens			
		NRS 4861	NRS 4076		Solitary polyps		Clustering polyps	
			Range length × width	n	Range length × width	N	Range length × width	N
Mesenterial filaments	Small basitrichs (I)	–	(9.0)12.0–13.8 × 2.4	10	7.0–14.0 × (1.5)2.0–3.0	6/6	9.0–15.0 × 2.0–2.5	6/7
	Basitrichs (J)	–	13.0–20.0(24.0) × 2.0–2.5	12	12.5–23.2(25.3) × 2.0–3.0	6/6	(11.0)13.0–24.0 × 2.0–5.0	5/7
	Large basitrichs (K)	–	(26.0)27.5–37.2(38.4) × 2.0–3.0	20	23.0–36.0 × 2.0–5.0	6/6	21.0–41.0(45.0) × 2.0–5.1	6/7
	Microbasic <i>p</i> -mastigophore/amastigophores (L)	–	14.5–18.0 × 2.0–4.0(5.0)	14	11.0–24.0 × 2.0–5.0(6.0)	6/6	13.0–24.0 × 2.0–5.0	7/7

All measurements in μm . Those in bold are sizes of *H. verruculatus* reported in Carlgren (1943), for the type material (NRS 4861). Outliners of single capsules are indicated within parentheses; '?' denotes the unspecified number of capsules that Carlgren had measured. Abbreviations: N = number of specimens of which that type of cnida was found to total specimens examined; n = total number of undischarged capsules measured. Letters in parentheses following cnidae type refer to illustrations in fig. 9.

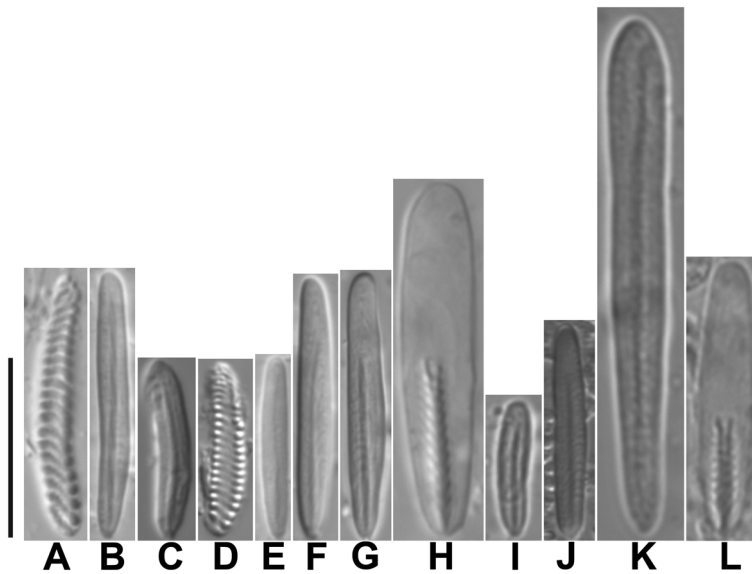
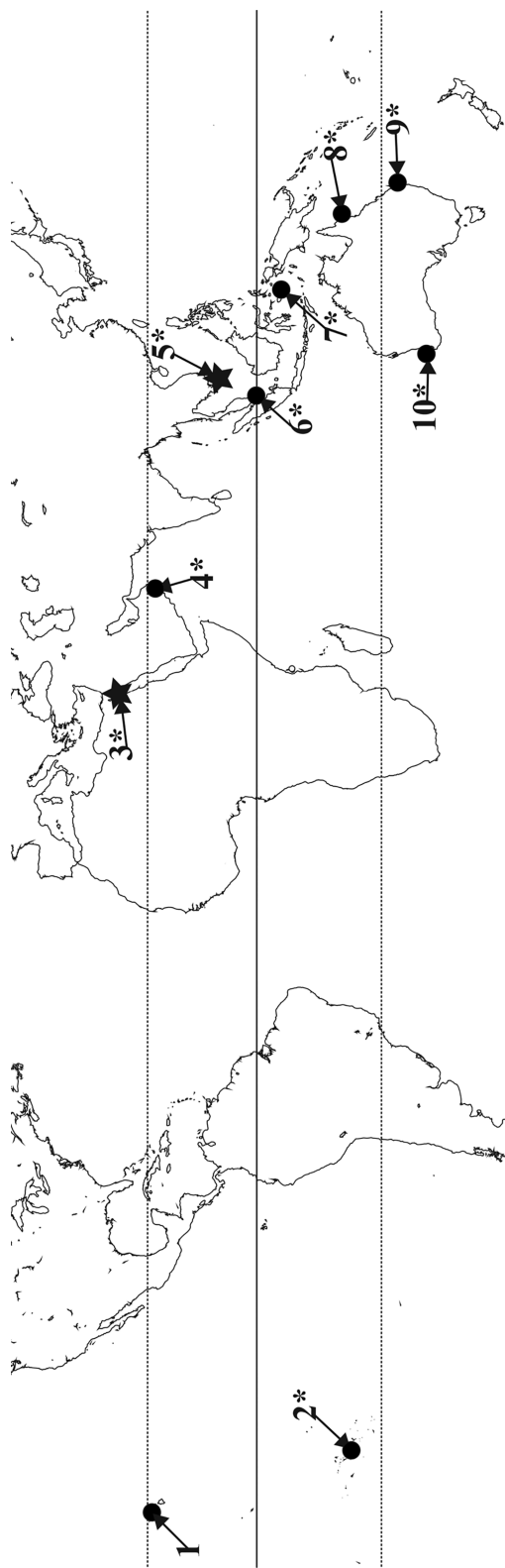


FIGURE 9 Ctenocytes of *Heteranthus verruculatus* Klunzinger, 1877. A. spirocyst. B. basitrich. C. basitrich. D. spirocyst. E. basitrich. F. basitrich. G. basitrich. H. microbasic *p*-mastigophore. I. small basitrich. J. basitrich. K. large basitrich. L. microbasic amastigophore/microbasic *p*-mastigophore. Scale bar = 10 μ m. Refer to table 2 for key to letters. Photographs by NWL Yap.

Carlgren (1950) failed to find gametogenic tissue in *H. verruculatus* specimens he had studied and did not mention them when he described *H. insignis* (see Carlgren, 1943). Because of this, he was unclear about its distribution of these reproductive tissues as evident in his diagnosis for *Heteranthus* (Carlgren, 1949: 75). Similarly, on the *H. verruculatus* specimen he had collected from the Great Barrier Reef, he surmised that, "... the arrangement of gonads [= gametogenic tissue] ... they are probably absent" (Carlgren, 1950: 440). Likewise, Fautin et al. (2008) did not find gametogenic tissue in specimens collected from the eastern coast of Australia. Like Carlgren (1950) and Fautin et al. (2008), the first author failed to find gametogenic tissue in the specimens examined by them and in all other vouchers studied (i.e., those from Singapore, Pulau Ambon). However, in the present study oocytes were found in the material collected from Rottnest Island in Western Australia (WAM Z33600), which were in the early phase of oogenesis,

attached to complete and incomplete mesenteries (fig. 7C).

As stated at the outset, Carlgren (1943: 32) described *H. insignis* based on a single poorly preserved specimen. In studying the type specimens, we could not find differences between the holotype of *H. insignis* and the lectotype of *H. verruculatus*. The length of basitrich capsules from the holotype's column was one of the characters Carlgren (1943) used to distinguish *H. insignis* as a new *Heteranthus* species. He reported finding discharged column basitrichs as $33.8\text{--}38.1 \times 2.5 \mu\text{m}$ from the *H. insignis* holotype, which are much larger than those present in the *H. verruculatus* syntype (Carlgren, 1943). Carlgren placed great emphasis on this character to differentiate between *Heteranthus* members, as evident from remarks in his later publication (see Carlgren, 1950: 440). Like him, we found large, discharged basitrichs in *H. insignis* holotype's column, and did not encounter any undischarged ones. However, we also found numerous smaller-sized, column basitrichs,



for which the size and distribution were consistent with those found in the *H. verruculatus* lectotype fragment (NRS4861), and all these we encountered were undischarged in both holotype and syntypes examined. These smaller column basitrichs were more transparent than the larger basitrichs in the *H. insignis* holotype and Carlgren likely may have missed them. All column basitrichs that Carlgren (1943) measured were discharged; we suspect that his data represent contamination from another tissue. In our experience, in fixed or preserved specimens, most cnidae capsules typically remain undischarged in the tissue from which it was produced. Carlgren's (1943) observations were probably based on contamination from the mesenterial filaments, as we found large undischarged basitrichs there, conforming in both size and form with Carlgren's (1943) column basitrichs.

Carlgren (1943) also stated that sizes of actinopharynx cnidae present in *H. insignis* holotype were different from those found in the *H. verruculatus* syntype, referring to his earlier work. He makes no mention of cnidae type present in this tissue, simply stating, "... the sizes of the nematocysts in the actinopharynx seem not to agree" (Carlgren, 1943: 32). We scoured the study (i.e., Carlgren, 1900) that Carlgren (1943) had cited but failed to find any *Heteranthus* cnidae data within it, even within its genus entry (see Carlgren, 1900: 92). We further reviewed all his pre-1943 publications (e.g. Carlgren, 1940) that might justify the claim but found none to support it. Presently, the well-preserved fragment of the *H. verruculatus* lectotype (NRS 4861) lacks an actinopharynx tissue, while the remaining material in Berlin (ZMB 1852) is dried up, and not in a condition to yield any useful data from it.

Nevertheless we suggest that Carlgren (1943) may have been measuring basitrichs from the actinopharynx, based on data from

his Great Barrier Reef specimen (i.e., NHM 1954.6.28.23). Basitrich sizes of the *H. insignis* holotype agree with these. Furthermore, Carlgren appears to abandon the use of this character to distinguish between the two *Heteranthus* species, as he made no reference to the difference of actinopharynx cnidae sizes between *H. insignis* and *H. verruculatus* in this latter publication (i.e., Carlgren, 1950). We also found microbasic *p*-mastigophores in the actinopharynx of the *H. insignis* holotype and NHM 1954.6.28.23, of which their size range overlaps well; Carlgren overlooked the presence of these cnidae in the actinopharynx as well.

Another character that Carlgren (1943) used to support his description of a new *Heteranthus* species was the form of the mesentery muscle. He reported that complete mesenteries of *H. insignis* "seem rarely to form humps," and that "the parietobasilar muscles form usually no distinct projection on the mesenteries; rarely is such a projection here ..." (Carlgren, 1943: 32). We prepared histological cross-sections from the holotype, at the level which Carlgren (1943) did, and found otherwise: numerous 'humps' were present along the edges of complete mesenteries, and parietobasilar muscles extending as a pennon at the base of each mesentery were fairly common (fig. 7A).

Although *H. verruculatus* and *H. insignis* were collected from two widely separated localities (the Red Sea and Vietnam, respectively), the overall morphology for both type specimens were virtually indistinguishable. In our re-examination of type material, we found that the characters used by Carlgren (1943) to separate the two species are questionable and inconsistent. All other characters (e.g., external morphology, sphincter muscle form, cnidom of remaining tissue, etc.) present in both type materials corresponded well with each other. We are therefore certain that the animal Carlgren (1943) had described as

H. insignis is *H. verruculatus* instead, and we thus synonymize the two species.

England (1987: 207) reportedly found *H. verruculatus* from the intertidal mud in Aden. In examining England's *Heteranthus* specimens from Aden (NHM 1972.5.25.15 x2), we found that they were not this species. Based on the overall appearance in having only one tentacle type which are tightly packed in wedges with spaces in between each group, an absence of papillae-covered marginal projections and its cnidae type and sizes, these are actually *Stichodactyla tapetum* (Hemprich & Ehrenberg in Ehrenberg, 1834), following evidence presented in Carlgren (1943, 1949, 1950), Dunn (1981) and Fautin et al. (2008, 2009).

Comparisons with other species. Clonal clusters of *H. verruculatus*, as densely packed populations of similarly sized polyps, may be mistaken for dense mats of *Zoanthus* spp. (Cnidaria, Zoantharia); both occupy the same habitat (fig. 3C). *Zoanthus* spp. are colonial invertebrates with their polyps interlinked by a network of connective tissue. *Heteranthus verruculatus* does not form colonies and exists as individual polyps. While in some instances *H. verruculatus* may appear as interconnecting polyps, this is temporary, and it is likely in the midst of longitudinal fission; the pair soon splits after.

In sharing the same habitat types and having longitudinal rows of conspicuous verrucae that extend distally from marginal projections, *H. verruculatus* superficially resembles species of *Anthopleura* or *Oulactis*. Furthermore, the distal margins of both *H. verruculatus* and *Oulactis* spp. appear frilly, due to the dense presence of overhanging verrucae. This is reflected in museum voucher specimens that were erroneously identified as either species of *Anthopleura* or *Oulactis* (e.g., MNHN-IK-2019-8, WAM Z33601). When expanded, *H. verruculatus* is easily distinguishable due to the presence of discal tentacles

arranged in radial rows on its oral disc; this feature is absent in the other two genera.

Stichodactyla tapetum (i.e., NHM 1972.5.25.15) is another species that *H. verruculatus* has been misidentified with. Individuals are similar in having an oral disc covered with tentacles, being small in size as well as being zooxanthellate. However, they are distinct from each other because:

- 1) Tentacles of *Heteranthus verruculatus* are clearly differentiated into two types: discal and marginal, whereas *Stichodactyla tapetum* only bears one tentacle type.
- 2) Tentacles present on the oral disc of *S. tapetum* are bulbous, may appear hexagonal in form, and are tightly arranged as dense radial wedges that extend from the mouth to the distal margin, while those of *H. verruculatus* are papillae-like and not arranged in dense radial wedges. Moreover, discal tentacles of *H. verruculatus* do not extend up until the distal margin, and are absent around the mouth.
- 3) *Stichodactyla tapetum* lacks both conspicuous verrucae on its column and a distal margin that overhangs with verrucae, whereas these are distinct and present on the column of *H. verruculatus*.
- 4) While both *H. verruculatus* and *S. tapetum* occur in similar habitats (e.g., rocky crevices, seagrass meadows), we have not encountered both species co-occurring in the field.

With the presence of both marginal and discal tentacles, *H. verruculatus* may be confused for species of *Phymanthus*. Some specimens have been identified as such (e.g., UFM 4220), and Carlgren (1943) had thought both are closely related. Yap et al. (2019: 15) provided a detailed discussion on features distinguishing *Heteranthus* and *Phymanthus*. Here we expand on that with the following

observation: *Heteranthus verruculatus* reproduces asexually frequently, forming aggregating clonal populations, whereas individuals of *Phymanthus* tend to occur singly; so far asexual reproduction has not been observed in *Phymanthus*.

Phylogeny of Heteranthidae. Our phylogeny reconstruction of *Heteranthus* showed that it overall formed a group that was distinct from *Phymanthus*, corroborating González-Muñoz's et al. (2015) findings. Moreover, we recovered *Phymanthus* as polyphyletic, in agreement with Brugler et al. (2018: fig. 3) and Titus et al. (2019: fig. 2). As of writing, a revision of the genus *Phymanthus* is ongoing and its relationship with *Heteranthus* will be discussed in a separate manuscript. While support for the Heteranthidae clade in ML analyses was weak (i.e., bootstrap = 54; fig. 1), those of Bayesian analyses showed otherwise (posterior probability >0.8). Nonetheless, both optimality criteria agreed in the overall topologies (i.e., *Heteranthus* forms a distinct clade) for three of the four molecular markers (16S excluded) that we used as well for the concatenated dataset.

Phylogenetic relationships for most sea anemones remain poorly resolved at the species level (Daly et al., 2017; Titus et al., 2019). This could be attributed to two reasons: 1) current genetic markers are of limited utility to infer relationships at species level, and 2) insufficient taxon and genetic sampling (e.g., Rodríguez et al., 2014; González-Muñoz et al., 2015; this study). On the latter, we have increased the sample size of *H. verruculatus* and the number of genetic markers. Among our three mitochondrial markers, 16S differentiated some *H. verruculatus* individuals into different branches, although they were collected from the same population (i.e., ZRC.CNI.1230, supplementary table S1; fig. 1, supplementary figs. S2–S6). Despite this, we are certain that these individuals are of

a single species (i.e., *H. verruculatus*); agreement in morphological features of these polyps and comparisons with others in the same population support this, despite the variation in some genetic markers used (i.e., 16S; <https://doi.org/10.5281/zenodo.4034666>). Nevertheless, the overall topologies of our ML and Bayesian trees are similar. We resurrected the family Heteranthidae because molecular data, including those obtained here, have repeatedly shown that *Heteranthus* and *Phymanthus* do not form a monophyletic group (see González-Muñoz et al., 2015; Brugler et al., 2018).

Summary and conclusion

In re-describing sea anemone species first reported in early sea anemone taxonomic works, we concur with Dunn's (1974: 177) view that the accompanying descriptions in those studies were, "... vague as to be virtually worthless for taxonomic purposes." Many species in those early works were mostly described from a single specimen, and species boundaries defined were unclear. We suspect that Carlgren's (1943, 1950) 'hesitation' was in part because he had only examined three *Heteranthus* individuals during his lifetime, of which two were in poor condition (i.e. type specimens of *H. verruculatus* and *H. insignis*). Furthermore, like many of his peers, he was probably not thorough in examining the syntype of *H. verruculatus*. Carlgren (1950: 440) laments this in his later publication that, "... unfortunately I have no opportunity of examining the nematocysts of *verruculatus* in further detail." The animal's variable appearance also likely exacerbated Carlgren's uncertainty. Perhaps if he had re-examined the *H. verruculatus* syntype again and studied more individuals of *H. verruculatus*, he would have his hesitation allayed.

Herein, we marshalled evidence from morphological, molecular, and historical data (i.e., specimen lot label details) from over 180 specimens collected across the Indo-West Pacific, to resolve the identity of *H. verruculatus* and define its species boundaries. For the first time since Klunzinger's (1877) description, we provide colour illustrations of *H. verruculatus*' living appearance, an updated cnidae dataset, an expanded description of the animal's internal musculature, and evidence of its reproductive behaviour. Moreover, in re-examining the holotype of *H. insignis*, we did not find any differences with the lectotype of *H. verruculatus*, and demonstrated that *H. insignis* is a junior synonym of *H. verruculatus*. As with another of Klunzinger's (1877) species that was re-described recently (Yap et al., 2019), we suspect that to resolve taxonomic confusion among many sea anemone species described in early accounts, this line of thorough treatment is required.

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Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.13055765>

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