

# Taxonomy and Molecular Phylogeny of the Sea Anemone *Macrodactyla* (Haddon, 1898) (Cnidaria, Actiniaria), with a Description of a New Species from Singapore

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Received 12 August 2022 / Accepted 28 April 2023 / Published 6 July 2023  
Communicated by James D. Reimer

Sea anemones (Cnidaria, Actiniaria) are a successful group of marine invertebrates found in a diverse range of environments globally. In spite of their ubiquity, identities for many sea anemones remain unverified, especially those from the Indo-West Pacific region. Here, we clarify the taxonomy of the poorly known *Macrodactyla aspera*, a shallow-water species first described from the Torres Straits in northern Australia. We re-describe *M. aspera* based on new morphological and molecular data gathered from the type specimen, other museum vouchers, and from fresh material collected from Singapore. We tested the monophyly of *Macrodactyla* using three mitochondrial (12S, 16S and *cox3*) and one nuclear (28S) marker based on three congeners, recovering this genus to be polyphyletic. As a consequence, we transferred *M. dorensis* to the genus *Heteractis*, and describe a new species, *Macrodactyla fautinae* sp. nov. While both *M. aspera* and *M. fautinae* sp. nov. share the same arrangement and number of complete mesenteries, a similar distribution of cnidae, and are not symbiotically associated with any other biota, *M. fautinae* sp. nov. has perforated, lobe-like verrucae on its column, and lacks nematocyst batteries on its tentacles, unlike *M. aspera*. These two species also occur in similar habitats in Singapore. Finally, because *M. aspera* strongly resembles *Dofleinia armata*, the latter species flagged as a danger to public health due to its ability to inflict painful stings, we tested the relationship between these species and found them not to be closely related. However, tentacles of *M. aspera*, like *D. armata*, are densely covered with nematocyst batteries and harbour large nematocysts; we infer that *M. aspera* may also be capable of delivering stings that endanger public health. This study builds upon a growing number of studies that aim to ascertain identities and systematics of sea anemones historically reported from the Indo-West Pacific. Our findings will facilitate accurate species identification, which is crucial for advancing research, formulating conservation measures, and protecting public health.

**Key words:** Actinioidea, Anthozoa, Intertidal, Integrative taxonomy, Southeast Asia.

## BACKGROUND

Sea anemones (Cnidaria, Actiniaria) are a cosmopolitan group of marine invertebrates, occupying a diverse range of habitats globally, extending from the tropical shallow seas to deep oceanic trenches (Gomes et al. 2016). These animals are engaged in important marine ecological processes, including symbiotic relationships with micro- and macro-organisms (e.g., fishes; crustaceans, and dinoflagellates, etc.; see: Dunn 1981, Fransen 1989; Sunagawa et al. 2009, respectively). Sea anemones are also sought after in the marine aquarium trade, and in some cultures, these animals are consumed as food (Dunn 1981; Scott et al. 2014). Some are also feared by humans because of their ability to inflict painful stings upon contact. Like all other cnidarians, sea anemones manufacture intracellular stinging capsules (known as nematocysts) containing venom (Fautin 1988 2009; Mitchell et al. 2020). Stings of some tropical shallow-water species from the Indo-West Pacific region may leave severe, long-lasting scars on their human victims, or in some instances, cause death (Erhardt and Knop 2005). Amongst species from the region that harbour potent stings, and are a threat to public health, four are well-known: *Dofleinia armata* Wassilief, 1908, *Phyllodiscus semoni* Kwietniewski, 1897, and members of the family Actinodendridae Haddon, 1898 such as *Actinodendrum arboreum* (Quoy and Gaimard, 1833) and *Actinostephanus haeckeli* Kwietniewski, 1897 (see: Coleman 1999; Hoeksema and Crowther 2011; Mizuno et al. 2007; Rowlett 2020).

Despite their diversity, commercial popularity, and the possible threats they may impose on human health, identities for many sea anemone species remain poorly verified. This is especially so for those that occur within the Indo-West Pacific region (den Hartog 1997; Fautin et al. 2009; Yap et al. 2019). This taxonomic inadequacy is a result of these invertebrates being notoriously difficult to identify accurately with confidence. Sea anemones are skeleton-less and among species, they have a similar, uniform body-plan and an apparent “dearth of features” to distinguish them (Fautin 1988: 497). Many morphological characters that were historically used to delimit species boundaries are still poorly defined, leading to taxonomic confusion (see Daly 2003; Daly et al. 2017). Furthermore, emerging molecular evidence has revealed that many distantly related species may exhibit a great degree of morphological convergence among them (Rodríguez et al. 2014; Daly et al. 2017; Titus et al. 2019). Apart from these aforementioned challenges, many species originally described from the Indo-Pacific region have not been verified, due to a lack of taxonomic detail in their original descriptions (Dunn 1974; Fautin et al. 2009; Yap et al. 2019 2021). This

greatly impedes further research on these animals.

Here, we focused on clarifying the taxonomy of one Indo-West Pacific species: *Macrodactyla aspera* (Haddon and Shackleton, 1893). This species is of particular interest as it bears a strong resemblance to *D. armata*, and occurs in similar, sandy and silty habitats (Erhardt and Knop 2005; Rowlett 2020). Like *D. armata*, *M. aspera* also bears tentacles that are densely covered with visible batteries that are packed with nematocysts (i.e., nematocyst batteries) which may pose a threat to public health.

*Macrodactyla aspera* was first described from northern Australia by Haddon and Shackleton (1893) as *Condylactis aspera* (it is also the type species for the genus *Macrodactyla*; see Carlgren 1949; Fautin 2016). Since then, little taxonomic work has been conducted to confirm its identity. Presently, only two published descriptions have dealt directly with the taxonomy of this species: i) Haddon and Shackleton (1893), and ii) Haddon (1898). By contemporary standards, these two taxonomic publications lacked crucial taxonomic details to accurately identify the animal (e.g., absence of cnidom data). This species was most recently referred to in an annotated checklist of littoral biota from the Gulf of Aqaba (Fishelson 1970). A half-tone photograph of the living animal with its tentacles extended accompanied this checklist (see Fishelson 1970: 100, Fig. 9), but no taxonomic description was given. Much of the animal remained obscured in this half-tone photograph – it is unclear what *M. aspera* truly looks like when alive. With the exception of Fishelson’s (1970) account, it has been more than a century since the species boundaries of *M. aspera* have been evaluated and tested; this warrants an investigation of the diagnostic features that were previously reported to define this species.

Other taxonomic works referring to *M. aspera* were dedicated to revising the original diagnosis of the genus *Macrodactyla* Haddon, 1898 (i.e., Stephenson 1921 1922; Carlgren 1949; Dunn 1981). This genus was established by Haddon (1898: 431) to accommodate *C. aspera*, as he recognised that it was different from known members of *Condylactis*, although he was not explicit in stating his reasons for transferring this species to *Macrodactyla*. While Haddon and Shackleton (1893) placed this species in Actiniidae Rafinesque, 1815 – a taxonomic decision retained in Haddon (1898) and thereafter – the diagnosis of *Macrodactyla* by Haddon (1898) contradicted accepted diagnoses of Actiniidae during his time. Haddon (1898) had erroneously reported that all mesenteries present in *C. aspera* were ‘imperfect’ (i.e., incomplete). Stephenson (1922: 263) subsequently flagged Haddon’s account as an ‘ambiguity’, since members of Actiniidae were

accepted at that time to have more than six complete mesenteries (e.g., Hertwig 1882; Carlgren 1893; McMurrich 1889; see: Haddon 1898: 414). After examining the type specimen of *C. aspera*, Stephenson (1921 1922) corrected Haddon's (1898) diagnosis for *Macrodactyla*, confirming the presence of six pairs of complete mesenteries in the type material. However, because Stephenson (1922) had considered actiniids to possess more than six complete pairs of mesenteries, he moved *Macrodactyla* to a new family Myonanthidae, whose members strictly possessed only six complete pairs. While Carlgren's (1949) monograph that followed retained much of Stephenson's (1921 1922) revised diagnosis, he returned *Macrodactyla* to Actiniidae, as he posited that actiniids have "...Perfect [= complete] pairs of mesenteries rarely six, as a rule more than six," (Carlgren 1949: 47; additions in square parentheses, our own).

Present perspectives on the systematics of *Macrodactyla* stem from Dunn's (1981) [= DG Fautin] expanded definition of the genus. This genus had remained monotypic since its publication until Dunn's (1981) inclusion of a congener: *Macrodactyla doreensis* (Quoy and Gaimard, 1833). This congener's name was a new combination that Dunn (1981) had created, to apply to a number of sea anemones she had previously synonymised into one species. Among those synonymised were *Actinia doreensis* Quoy and Gaimard, 1833 and *Condylactis gelam* Haddon and Shackleton, 1893 (see Dunn 1981: 29, for the full synonymy list). While Dunn (1981) was at first uncertain in finding an appropriate genus to classify these synonymised species, she eventually settled on *Macrodactyla*, rationalizing that they were (Dunn 1981: 29), "... no doubt closely related [witness Haddon and Shackleton's (1893) original description of both as members of *Condylactis*]...". However, Dunn (1981: 35) also noted that name-bearing specimens of these synonymised species (e.g., syntypes of *C. gelam*), and the type specimen of the type species for *Macrodactyla* (i.e., *C. aspera*) were morphologically different in the "...distribution and size of cnidae, character of mesenterial retractor muscles... and tentacle number and form," casting doubt on her own decision. At present, it is unclear if *M. aspera* and *M. doreensis* are congeneric. Recent phylogenetic analyses using only *M. doreensis* samples have repeatedly recovered this species to be nested within *Heteractis* Milne Edwards, 1857 (Titus et al. 2019; Yap et al. 2021). Prior to this study, phylogenetic interpretation using *M. aspera* have not been conducted, and the monophyly of *Macrodactyla* remains untested.

Apart from these systematic problems, Fautin (2016) also highlighted a nomenclatural issue relating

to the use of the name *Macrodactyla*. This genus name was reported to be a junior homonym to a genus of a beetle described in Hitchcock (1833) (see: Neave 1940; Fautin 2016). Following Neave's (1940) listing and strict stipulations outlined by the International Commission on Zoological Nomenclature (i.e., Article 23.3.5; the "Code", henceforth), Fautin (2016) returned both species, presently placed in *Macrodactyla*, to *Condylactis* (therefore, as *C. aspera* and *C. doreensis*, respectively). Despite her nomenclatural actions, Fautin (2016: 105) also conceded that she had not consulted the original publication where the senior homonym had appeared (i.e., Hitchcock 1833), stating, "... not seen: "Rep. Geo. Min. Zool. Massach., 575..." (emphasis in bold, our own). Subsequent publications after Fautin (2016) have retained the use of *Macrodactyla* when referring to both *M. aspera* and *M. doreensis* (e.g., Titus et al. 2019; Yap et al. 2021), because their morphology was not congruent with *Condylactis* (e.g., presence of a sphincter muscle; see: González-Muñoz et al. 2012). The nomenclatural status for the genus name *Macrodactyla* needs to be resolved; Neave's (1940) assertions and Fautin's (2016) resultant nomenclatural actions must be verified for the unambiguous usage of this genus name for sea anemones.

In this study, we re-described *M. aspera* based on new data gathered from its type specimen, accompanied by fresh material collected from Singapore, and from vouchers kept in natural history museums worldwide. Nomenclatural issues relating to the use of the genus name *Macrodactyla*, were resolved to support its continued usage for sea anemones. We also tested the phylogeny of the two presently accepted species in *Macrodactyla* (i.e., *M. aspera* and *M. doreensis*) and recovered them as polyphyletic. Considering this new evidence, we revised the definition of *Macrodactyla*, and classified *M. doreensis* in *Heteractis* as *Heteractis doreensis* (Quoy and Gaimard, 1833) comb. nov. Additionally, we tested the relationship between *M. aspera* and *D. armata*, due to the strong morphological resemblance between these two species. We found them to be distantly related, with *D. armata* being recovered with Actinodendridae Haddon, 1898, its members well-known to inflict painful stings.

During field surveys conducted for this study in Singapore, we encountered another species of sea anemone that co-occurs with *M. aspera*. While this second species has been photographed and is known by local citizen scientists for many years, being nicknamed as the 'Purple-lip Sand Anemone' and 'Tiger Anemone' in both printed and web-based biodiversity guides (e.g., Rowlett 2020: 323; WildSingapore Fact Sheets: <http://www.wildsingapore.com/wildfacts/>, respectively), its identity has remained undetermined until now. This

second species is new to science, which we formally described as *Macroactyla fautinae* sp. nov., and is thus far only known from Singapore. In our phylogenetic analyses, we recovered this new species and *M. aspera* as a clade. *Macroactyla fautinae* sp. nov. differs from *M. aspera* in bearing adhesive and extensible, perforated lobe-like verrucae, and a lack of visible nematocyst batteries on its tentacles.

Information from this study builds on efforts to accurately identify sea anemones from the Indo-West Pacific region (e.g., England 1987; Fautin et al. 2009; Yap et al. 2019). This is necessary to prime future research involving these marine invertebrates from the region as so little is known about their ecology (e.g., reproductive biology, see Yong et al. 2021). Moreover, given that sea anemones can impact humans due to their ability to deliver harmful stings, precise identification is key to constructive management and mitigative efforts in safeguarding public health.

## MATERIALS AND METHODS

### Sea anemone collection and processing

Fresh material examined in this study were collected from intertidal habitats along Singapore's coastlines and offshore islands (Fig. S1). Sea anemones were observed and photographed *in situ* prior to removing individuals (*M. aspera*: N = 12; *M. fautinae* sp. nov.: N = 29) from the soft substratum using a hand-trowel. In the laboratory, tissue was sampled from the animal's pedal disc, column and tentacles, and preserved in 100% molecular-grade ethanol for further molecular analyses (see below). The animals were subsequently relaxed in 7.5% magnesium chloride and then fixed in 10% formalin. Identities of other sea anemone species, for which their tissues were used for the molecular analyses in this study, were verified using evidence published in primary scientific literature (e.g., Fautin et al. 2009 2015; Fautin and Tan 2016).

### Morphological observations

All fixed specimens were dissected so that the internal morphology could be examined. Histological sections, 8 µm thick, were prepared from these materials so that mesentery arrangements and musculature of the animal could be observed. All sections were stained with haematoxylin and counterstained with eosin (Humason 1967).

Cnidae present in the formalin-fixed tissues of the sea anemone's tentacle tip, marginal projections, mid-column, actinopharynx and mesenterial filaments,

were viewed at 1000x magnification under a light microscope. Only intact capsules (i.e., undischarged capsules) were measured using conventions presented in Dunn (1981) and Yap et al. (2021). Cnidae taxonomy followed Mariscal (1974).

Translations of original taxonomic descriptions written in languages other than English (e.g., Wassilieff 1908) were carried out using Google Translate (Google Translate: <https://translate.google.ca>). For specialised scientific terms that could not be translated suitably, we followed definitions listed in Stachowitsch (1992).

To redefine the species boundaries of *M. aspera*, syntypes of *C. aspera* housed at both University Museum of Zoology, Cambridge, United Kingdom (MZC), and Museum of Zoology, Lund University, Sweden (MZL), were examined (for type material listing, see: Fautin 2016). Furthermore, we located and examined museum voucher material that closely resembled *M. aspera*, or those that were identified as *D. armata* on their labels. These vouchers originated from Australia, New Caledonia, Singapore and Israel (Fig. S2), in the collections of Muséum National d'Histoire Naturelle, Paris, France (MNHN), Steinhardt Museum of Natural History, Tel Aviv, Israel (SMNH-TAU), and Western Australian Museum, Perth, Australia (WAM).

All fresh material collected from Singapore for this study were deposited into the Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum, National University of Singapore.

### Molecular analyses

Genomic DNA from ethanol preserved tissues were isolated via standard CTAB extraction (see Rodríguez et al. 2014, and references therein). We utilised four published molecular markers in this study: three mitochondrial (i.e., partial 12S rDNA: Chen et al. 2002; 16S rDNA and *cox3*: Geller and Walton 2001) and one nuclear (i.e., partial 28S rDNA: Chen and Yu 2000). Loci for these markers were amplified with polymerase chain reaction (PCR), following published cycling profiles (i.e., Lauretta et al. 2013; Rodríguez et al. 2014 and references therein). All PCR products were purified with SureClean Plus (Bioline, Singapore). Thereafter cycle sequencing was performed using BigDye Terminator v3.1 (Applied Biosystems, Foster City, California) following the manufacturer's protocol. All amplicons obtained were sequenced on an ABI 3130 XL Genetic Analyzer (Thermo Scientific).

New sequences acquired in this study were assembled in Geneious v11.1.3 (Invitrogen Corporation) using default parameters. All contigs were searched against GenBank sequences via BLASTn; this was to affirm that nucleotide sequences we had obtained

and successfully amplified were only sea anemones. Selected sequences previously published were also used in our analyses (*i.e.*, Rodríguez et al. 2014; Daly et al. 2017; Titus et al. 2019; Yap et al. 2021). Gene sequences were aligned separately using MAFFT v.7.313 under *--auto* setting. These aligned sequences were then concatenated into a combined matrix. In all, separate analyses for alignments were conducted over the five resultant datasets (*i.e.*, 12S, 16S, *cox3*, 28S and concatenated matrix).

Maximum likelihood (ML) analyses were conducted in RAxML-NG v0.8.1 (Stamatakis 2014; Kozlov et al. 2019) using 50 random and 50 parsimonious starting trees, with 1000 bootstrap pseudo-replicates. The best model of DNA evolution was determined using ModelTestNG (Darriba et al. 2020) and specified for all datasets in both ML analyses and Bayesian inference (BI) described below. Uncorrected pairwise distances, for each alignment, was calculated using MEGA X (Kumar et al. 2018).

Bayesian inference was carried out only on the concatenated matrix using MrBayes v3.2.6 (Ronquist et al. 2012). Four Markov Chain Monte Carlo (MCMC) runs were carried out for 12 million generations and sampled at every 100th tree. To determine if the runs converged, Tracer v1.7 (Rambaut et al. 2018) was used to visualise the posteriors. Convergence was achieved after discarding the first 60,001 trees as burn-in (average standard deviation of split frequencies = 0.0322, ESS<sub>LnL</sub> = 1152, ESS<sub>LnPr</sub> = 2860).

All new sequences obtained in this study are deposited in GenBank (Table S1).

## RESULTS

### Phylogeny reconstruction

Overall, 198 taxa were present in the concatenated dataset, with 6,195 sites used for phylogeny reconstruction. Broadly, while trees inferred from both ML and BI analyses of the concatenated dataset and four individual molecular markers were not fully concordant, topologies of phylogenetic relationships inferred across all trees remained similar at genus level (Fig. 1; Figs. S3–S7).

In all inferred trees, *Macroactyla* was found to be polyphyletic, with *M. aspera* and *M. doreensis* failing to form a clade. Instead, *M. doreensis* was nested among most members of *Heteractis*, with strong support. *Macroactyla aspera* was recovered to be a sister to *M. fautinae* sp. nov., forming a distinct clade (Fig. 1; Table 1). This relationship is well-supported, with bootstrap values being > 50 at inclusive nodes (Fig. 1). Despite

their similarity in appearance, *M. aspera* and *D. armata* were not recovered as a clade. The latter was recovered in a clade consisting of members of Actinodendridae (*i.e.*, *A. arborerum* and *Ac. haeckeli*, with strong support (*i.e.*, bootstrap > 80) (Fig. 1).

Pairwise genetic distances between congeners *M. aspera* and *M. doreensis* ranged from 2.4% to 16.1%; dependent on the molecular marker examined (Table 1). Between *M. aspera* and *M. fautinae* sp. nov., these genetic distances were smaller among the mitochondrial markers (*i.e.*, *cox3*, 12S and 16S: 0.8% to 2.0%). In contrast, genetic distances were larger in nuclear markers (*i.e.*, 28S: 6.4% to 10.1%; Table 1). While *M. aspera* may superficially resemble *D. armata*, pairwise differences of the mitochondrial markers between these two species were larger than between members of *Macroactyla*, ranging between 3.0% and 3.8% (Table 1). *Dofleinia armata* was recovered as a sister to the actinodendrids (Fig. 1), and this finding is further supported here by the small pairwise genetic differences (Table 1).

## TAXONOMY

**Order Actiniaria Hertwig, 1882**  
**Suborder Enthemonae Rodríguez and Daly,**  
**2014 in Rodríguez et al. 2014**  
**Superfamily Actinioidea Rafinesque, 1815**  
**Family Actiniidae Rafinesque, 1815**  
**Genus *Macroactyla* Haddon, 1898**

*Condylactis* [pro parte] – Haddon and Shackleton 1893: 123; Fautin 2016: 105.

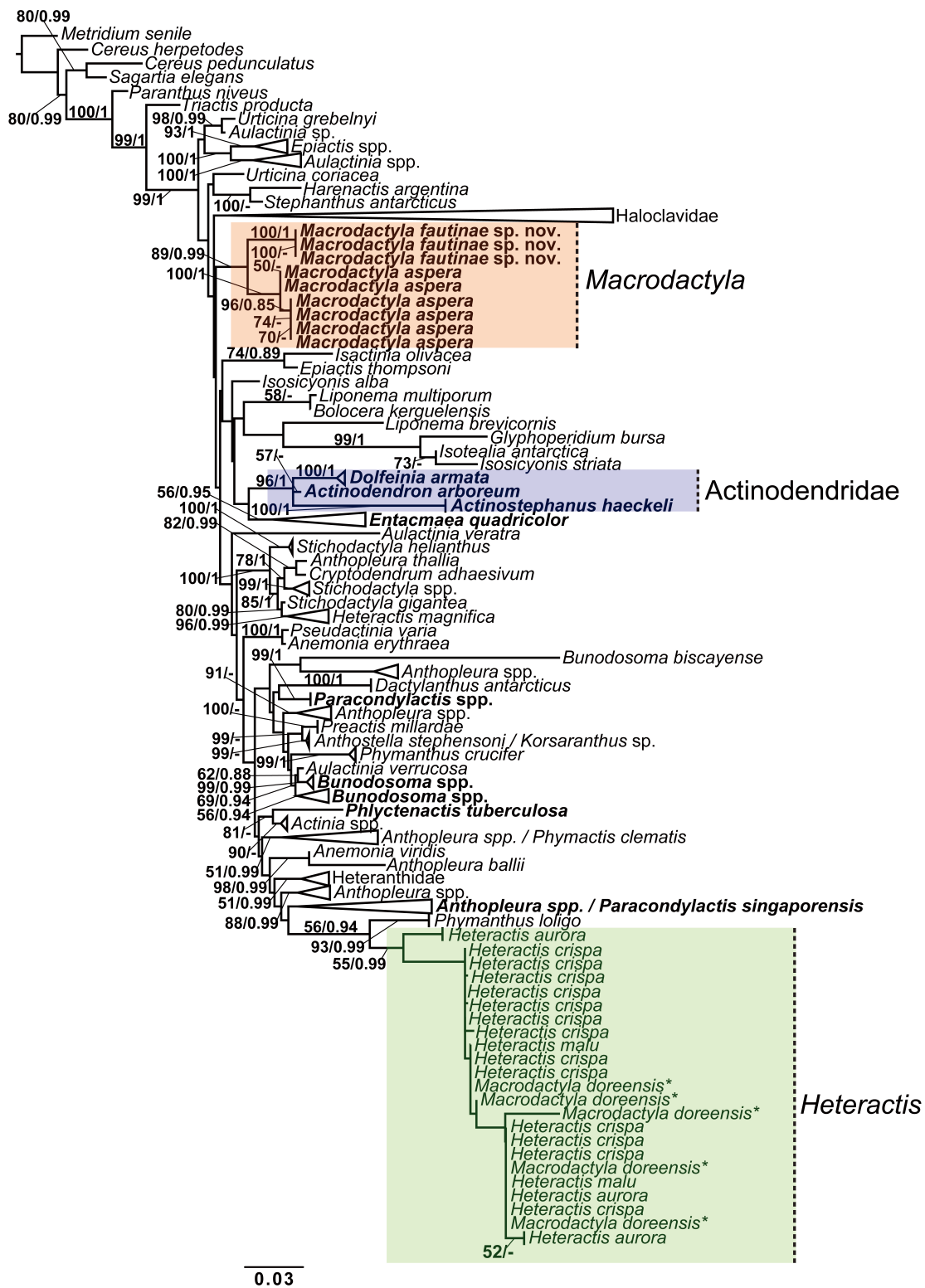
*Macroactyla* [pro parte] – Haddon 1898: 431 [original description]; Stephenson 1921: 524, 531, 545; Stephenson 1922: 263, 265; Carlgren 1949: 63; Dunn 1981: 28, 35; Fautin et al. 2007: 213; Fautin et al. 2008: 49; Fautin 2016: 104, 105.

*Etymology gender*: Feminine.

*Type species*: *Condylactis aspera* Haddon and Shackleton, 1893, by monotypy, in agreement with Dunn (1981), Fautin et al. (2007) and Fautin (2016).

Definition of genus modified after Dunn (1981) and Fautin et al. (2008). Substantial changes from our research are indicated in **bold**; all minor additions are underlined.

*Diagnosis*: Pedal disc distinct, circular **to oval in outline, as wide as oral disc**. Column **elongated, marginal projections present**. Adhesive verrucae present on distal end, often **with foreign materials often attached, may be lobed and perforated**. Fosse **very shallow**. Tentacles long and stout, **covered with nematocyst batteries or entirely smooth**; inner tentacles considerably longer than the outer.



**Fig. 1.** Phylogeny reconstruction of *Macrodactyla aspera* and *Macrodactyla fautinae* sp. nov. among actiniid sea anemones. Tree topology a result of maximum likelihood (ML) analysis, using a concatenated dataset (i.e., *cox3*, 12S, 16S, 28S). At the branches, bootstrap ML resampling values, and Bayesian inference (BI) posterior probability values, are presented as ML/BI. Only bootstrap values > 50, and posterior probability > 0.8 are indicated here; those less than 50 and 0.8 are denoted by (-). Taxa indicated in **bold** denotes the presence of new sequences obtained for this study. Indicated by an asterisk (\*), we retain the name *Macrodactyla doreensis* in this figure to aid with the discussion within the main-text. For sequences used and the full tree, refer to table S1 and figure S3, respectively.

Actinopharynx **often everted when alive**, with a pair of diametric siphonoglyphs. **Symmetrically-arrayed mesenteries; always 12 pairs complete**, all remainder incomplete. All fertile. Retractor muscles band-like, diffuse-circumscribed. Endodermal marginal sphincter muscle conspicuous and restricted. Parietobasilar muscles well-developed, with an extended pennon present for lower order mesenteries. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

*Nomenclatural considerations:* Neave (1940) reports that the *Macroactyla*, as a name for a sea anemone genus, is a junior homonym to a beetle genus described by TW Harris in Hitchcock (1833). As mentioned at the outset, Fautin (2016) had returned both *Macroactyla* species (*i.e.*, *M. aspera* and *M. doreensis*) to the next oldest available generic name applied to them: *Condylactis*.

We consulted Harris' contribution in Hitchcock (1833: 575) and found the name *Macroactyla*, confirming its appearance in the publication. This listed name is further accompanied by a species epithet, a letter that denotes its taxonomic authority of the species (*i.e.*, 'F' = JC Fabricius), and a common name – all of which we render here exactly as (including the way it was stylised; see Hitchcock 1833: 575): (*Macroactyla*) *subspinosa*, F. Rose-Bug.

Harris' entry in Hitchcock (1833) was meant simply to be part of an inventory of the insect biota of Massachusetts, and it lacks an accompanying description to *Macroactyla*.

No beetle species is known by the name listed by Harris in Hitchcock (1833). The closest match is the species *Macroactylus subspinus* (Fabricius, 1775), which also occurs in Massachusetts, and is known by the common names, 'Rose-bug' and 'Rose-chafer' (see: Blatchely 1910: 953; Schoolmeesters 2021). Originally described by Fabricius (1775) as a member of the genus *Melolontha* Fabricius, 1775, this species was later placed in a new genus, *Macroactylus*, created by

Latreille (1825: 372) (Schoolmeesters 2021).

We posit that Harris' entry in Hitchcock (1833) was a misspelling of the coleopteran genus name *Macroactylus*. Nomenclatural information we have found on *Ma. subspinus* corroborated with much of those present in Harris' entry (*i.e.*, taxonomic authority, species epithet, common name, etc.), as with this species' geographical occurrence. Moreover, Harris' revised checklist in Hitchcock's (1835: 565) latter work strongly supports our assertion: in this updated listing, Harris attributed the taxonomic authority of the misspelled beetle genus name (still listed as *Macroactyla*) to PA Latreille. No works of Latreille prior to Harris' entry in Hitchcock (1833) had mentioned *Macroactyla*, only the name *Macroactylus* was ever used in Latreille's (1825) publication.

The name *Macroactyla* in Hitchcock (1833) is thus not a senior homonym to the genus name that Haddon (1898) had used for the sea anemones; Harris' entry is an incorrect spelling of the coleopteran genus name *Macroactylus*, and therefore cannot enter homonymy (Article 54.3, the Code). As First Revisers, we retain the name *Macroactyla* as a genus name for sea anemones; it is valid and available for use.

*Taxonomic considerations for the genus Macroactyla:* Historically, sea anemone systematists have considered Haddon's (1898) report of mesentery number and its arrangement in *Macroactyla* to be ambiguous. Based only on the type species, *C. aspera*, Haddon defined *Macroactyla* to have "six pairs of imperfect [= incomplete] mesenteries, and a second and third cycle of imperfect mesenteries" (Haddon 1898: 431; additions in square parentheses, our own). Stephenson (1921) later revised this feature to denote the presence of only six complete mesenteries, elaborating further that Haddon (1898) may be mistaken on this feature (Stephenson 1922: 263). Later definitions of *Macroactyla* retained Stephenson's assertions (*e.g.*, Carlgren 1949). By including a second

**Table 1.** Pairwise genetic distances among *Macroactyla*, *Dofleinia*, and members of family Actinodendridae (*i.e.*, *Actinodendron* and *Actinostephanus*) for the four molecular markers targeted in this study. Dashes indicate that no comparison were made, as we failed to obtain 28S sequences from *D. armata* (see Table S1)

		Pairwise genetic distance range			
Molecular markers		<i>M. aspera</i>	<i>M. aspera</i>	<i>M. aspera</i>	<i>D. armata</i>
		and <i>M. doreensis</i>	and <i>M. fautinae</i> sp. nov.	and <i>D. armata</i>	and <i>actinodendriids</i>
Mitochondrial	<i>cox3</i>	5.3–6.5%	1.7–2.0%	3.0–3.6%	1.0–1.5%
	12S	2.4–2.8%	0.8–0.9%	2.5–2.8%	0.3–3.01%
	16S	2.4–3.0%	0.9–1.09%	3.3–3.8%	1.3–1.5%
Nuclear	28S	10.8–16.1%	6.4–10.1%	-	-

species to the genus, *M. doreensis*, Dunn (1981: 28) had amended its definition to include, "... six or more pairs of mesenteries perfect,..." because this second species had more than six complete pairs of mesenteries. However, in Dunn's [as Fautin] later publication of this genus, its diagnosis was changed, indicating that only six pairs of complete mesenteries were present in *Macrodactyla* (see Fautin et al. 2008: 49). In studying the type specimen of the type species for *Macrodactyla* (i.e., *C. aspera*; MZC.I.33665), we found that it has 12 pairs of complete mesenteries instead of six, a feature that is consistent across all voucher and fresh material. Here, we have revised the definition of the genus to reflect this.

No data concerning the cnidom for the type species of *Macrodactyla* (i.e., *C. aspera*) have ever been reported, it being absent in earlier descriptions of the genus (i.e., Haddon and Shackleton 1893; Haddon 1898; Stephenson 1921 1922). In recent definitions of *Macrodactyla*, which had included *M. doreensis*, Fautin et al. (2008: 49) simply reported the types of cnidae present in sea anemones of the genus, stating the presence of, "spirocysts, basitrichs, microbasic *p*-mastigophores". We confirmed that these cnidae were present in the name-bearing type specimen of *C. aspera*.

We deemed *Macrodactyla* to be the most appropriate genus to place the new species, *M. fautinae* sp. nov., in the light of both morphological, ecological and genetic evidence gathered from this study. Both *M. fautinae* sp. nov. and *M. aspera* share the same number and arrangement of complete mesenteries. Both species also have visible adhesive verrucae on their column, and harbour the same types of cnidae in their tissues (see below, in their respective species description). Like *M. aspera*, we did not find any symbiotic micro/macro-organisms associated with *M. fautinae* sp. nov. Our placement of this new species in *Macrodactyla* is further supported by genetic evidence we have gathered: we had repeatedly recovered both *M. fautinae* sp. nov. and *M. aspera* to be sister to each other, within a clade, with strong support (bootstrap > 80; Fig. 1). Our revised diagnosis of *Macrodactyla* now includes traits of this second species, thereby expanding on its definition.

We failed to recover *M. doreensis* and *M. aspera* as a monophyletic clade (Fig. 1). Genetic evidence from this study, as with observations from recent studies, have repeatedly recovered *M. doreensis* to be nested among clownfish and dinoflagellate-associated sea anemones of the genus *Heteractis* (Titus et al. 2019; Yap et al. 2021; Fig. 1). Apart from genetic evidence, both *M. aspera* and *M. doreensis* also do not share many morphological and ecological traits that would characterise them to be classified in the same genus. While both have verrucae on the distal end, those of

*M. aspera* are always adhesive, whereas verrucae of *M. doreensis* are not (Dunn 1981; Fautin et al. 2008). Mesentery number and arrangement between them also differ; those in *M. aspera* are arranged in up to three cycles while those of *M. doreensis* may go up to seven (Dunn 1981). Furthermore, we found microcnemes to be present in *M. doreensis*, consistent to its depiction in Dunn (1981: Fig. 13), a trait that is absent in *M. aspera*. Ecologically, *M. doreensis* is a host to photosynthetic dinoflagellates and macro-fauna such as clownfishes (Dunn 1981; Fautin and Allen 1992). In contrast, we have not encountered nor observed *M. aspera* to be involved in any symbiotic relationships with other micro/macro-organisms.

Given the genetic, morphological and ecological evidence presented, we place *M. doreensis* in the genus *Heteractis*, under a new combination: *Heteractis doreensis* (Quoy and Gaimard, 1833). In revising the diagnosis of *Macrodactyla*, we removed all characters that had extended to *M. doreensis* (e.g., presence of deep fosse).

***Macrodactyla aspera* (Haddon and Shackleton, 1893)**  
(Figs. 2–6)

*Condylactis aspera* – Haddon and Shackleton, 1893: 117, 124–125 [original description]; Dunn 1981: 29; Fautin 2016: 77, 105, 164.  
*Macrodactyla aspera* – Haddon 1898: 398, 415, 431–432; Stephenson 1922: 263; Carlgren 1949: 63; Fishelson 1970: 109, 110; Stephenson 1922: 263; Dunn 1981: 35.  
*Dofleinia armata* – Rowlett 2020: 292, 293.

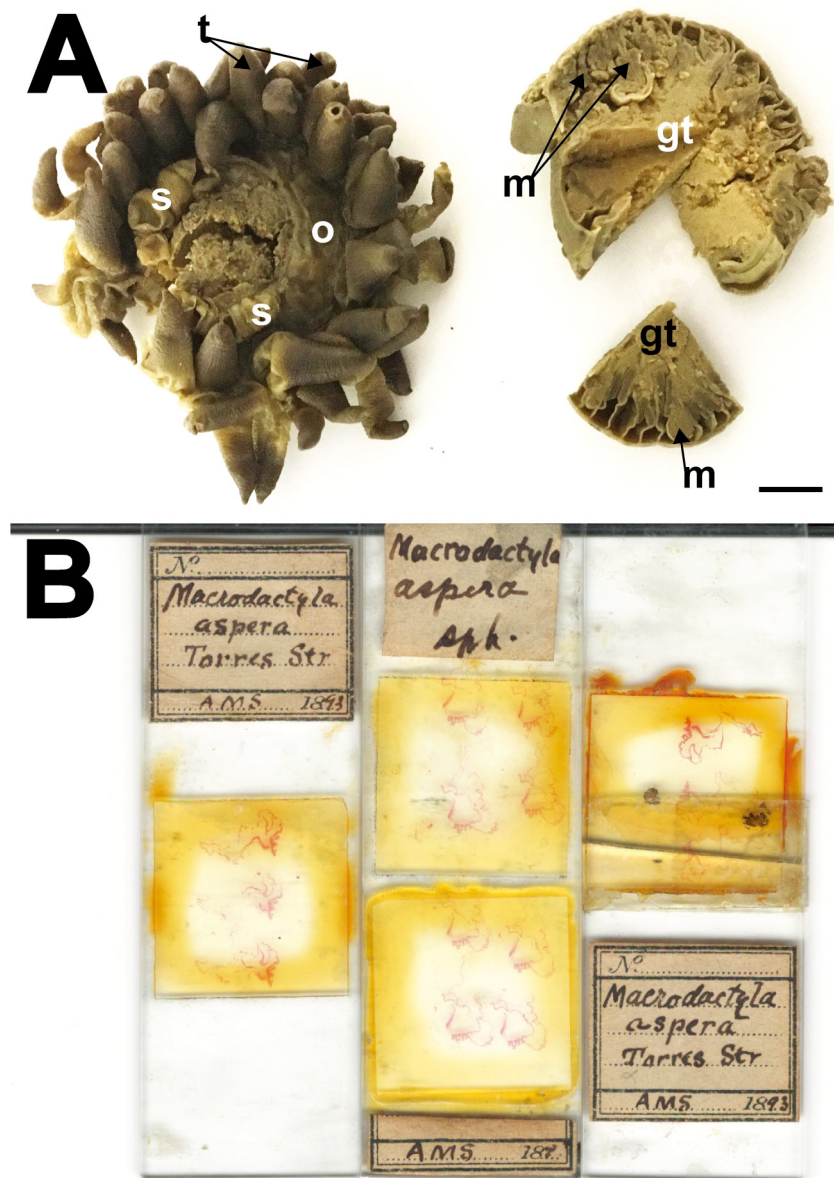
*Nomenclature considerations and type material examined:* Two lots of the syntypes of *C. aspera* that was listed in Fautin (2016), were of sea anemones collected from Murray Islands in the Torres Strait, Australia: i) a nearly complete specimen kept at the Cambridge, UK (MZC I.33665, previously without a catalogue number assigned) (Fig. 2A), and ii) three microscope slides comprising of histological sections kept in Lund, Sweden (MZL, no catalogue number) (Fig. 2B). We confirmed Fautin's (2016) listing of these specimens; the labels present on these slides indicated that they were prepared from the material collected by Haddon and Shackleton (1893) (Fig. 2B). Among these three microscope slides, only histological sections of one closely corresponded to Haddon's depiction of the musculature, while those present on the other two slides did not agree to his remaining illustrations (see: Haddon 1898: Plate 28, Figs. 3, 4 and 5). Despite this, sections on these slides agreed with the shape of pieces that were missing from the syntype which we had studied at the MZC. These other histological sections from the syntype were likely prepared by O Carlgren, who then



kept them at the MZL where he had worked, a practice which he also carried out on other sea anemone species (Fautin 2016: 13; Yap et al. 2021). Pertaining to the original histological sections that were prepared by Haddon and used for his illustrations, we were unable to locate it at the museums we have visited.

To further provide nomenclatural objectivity to the name *Condylactis aspera*, we hereby designate MZC.I.3365 and the three MZL slides as the lectotype, as the latter slides were prepared from the specimen at MZC. While there was no mention of the number of individuals that were collected and examined in the

original description for the species, the account of the species' tentacle arrangement alludes to more than one individual being examined, as Haddon and Shackleton (1893: 124) stated, "... large tentacles in three or four cycles..." [underlined emphasis, our own]. This observation of a variation is only possible if more than one individual was examined. In this study, NWL Yap attempted to locate other syntypes in other museums where they may be possibly held (e.g., NHM, etc), but failed to find any. Should these other materials be found, they are the paralectotypes of *C. aspera* (Article 74.1.3, the Code).



**Fig. 2.** Type material of *Condylactis aspera* Haddon and Shackleton, 1893. A, lectotype of *C. aspera* (MZC I.33665), in three pieces, top view. Note the gastro-cavity of specimen is filled densely with gametogenic tissue. B, Paralectotypes of *C. aspera*, as three histological slides at MZL (no catalogue number). Abbreviations: gt, gametogenic tissue; m, mesenteries; o, oral disc; s, siphonoglyph; t, tentacle. Scale bar = 10 mm.

*Lectotype: Condylactis aspera* (MZC I.3365), collected by Haddon and Shackleton from the Torres Straits (Fig. 2A). A single specimen cut into three pieces. Two pieces already removed prior to being examined by the first author: i) a wedge of the oral disc, and ii) a quarter section of the base. Column diameter about 5 mm. Entire specimen greyish, with a dark brown cast (Fig. 2A). Gastro-cavity of specimen choked with gametogenic tissue.

*Lectotype:* MZL (no catalogue number), three microscope slides prepared from MZC.I.3365 (Fig. 2B). One labelled as “*Macroductyla aspera* Torres Str AMS 1893, consists of three transverse sections of the distal most end of the column and tentacle. Second slide, with two labels. The first wider label bears the words, “*Macroductyla aspera* sph”, the second slimmer label has “AMS 187” written on it. The slide bears eight sections, all being the distal most end of the specimen. The third slide has a single label with the following printed on it: “*Macroductyla aspera* Torres Str, AMS 1893”. This slide also bears three histological sections of the distal most end of the animal. All sections present on these slides are too faded to infer any useful details from them.

*Material examined from Singapore* (Fig. S1): (\*-observed alive): Beting Bronok (ZRC.CNI.1015 x3), Changi beach (ZRC.CNI.1055 x1; ZRC.CNI.1099 x1\*; ZRC.CNI.1217 x2\*), Changi East beach (ZRC.CNI.1090 x1\*), Cyrene Reef (ZRC.CNI.1080 x1; Photographed but not collected), Pulau Sekudu (ZRC.CNI.1222 x3\*).

*Materials examined from elsewhere* (Fig. S2): Israel (Eilat): Gulf of Aqaba (SMNHTAU-Co.7813 x1; SMNHTAU-Co.12133 x1). Australia (Perth): North Fremantle (WAM Z99100 x1), Cockburn Sound (WAM Z50023 x1); Rowley Shoals (WAM Z50026 x2). New Caledonia: Noumea (MNHN 1321 x1); Unspecific location (MNHN 1571 x1).

*Description: Natural history:* Usually solitary, at the middle intertidal of soft silty sand, sandy areas, and among seagrasses (Fig. 3A–B). Distal end of animal exposed during low spring tides, when encountered during the early morning or dusk. Animal partially buried in substratum (Fig. 3A–B). Tentacles extended and oral disc expanded, flat against substratum (Fig. 3A). Often encountered with actinopharynx everted outwards; if everted partially, animal may appear to have luscious lips (Fig. 3B). Small shell pieces, coral rubble, sand and/or small stones adhere to exposed verrucae of distal most column end (Fig. 3C–D). When disturbed, animal does not retract completely, with tentacles and/or its tips remaining partially exposed (Fig. 3D). Cells of symbiotic dinoflagellates not observed in tissue.

*Tentacles:* 48. Arranged in four cycles, one per endo-/exocoel. Outermost exocoelic; innermost cycles: endocoelic. Innermost cycle longer, greater or equal to length of oral disc radius; outermost shortest, 1/3 length of innermost (Fig. 3A). Covered entirely with visible nematocyst batteries (Figs. 3A–D, 4A, 4B). Tapering in life; may appear conical in fixed specimens due to contraction by fixative (Fig. 4B). Wide at base, narrows to point. Tip pointed-blunt, not perforated. In life, cream-colour with dark-brown splotches on oral side, batteries white or brown (Figs. 3A–D, 4A). Sticky; readily adheres to surfaces of containers. Does not abscise readily when disturbed. Preserved specimens coloured grey, brown to mauve, with intense brown (or dark mauve) cast near the tip (Fig. 4B).

*Oral disc:* Outline round, flat when fully expanded (Fig. 3A). Diameter up to 45 mm. Nematocyst batteries of tentacles may extend across disc, in some specimens all the way to mouth. Thin-walled; mesenterial insertions as radial lines seen through disc, extending from mouth to tentacles, in life these appear as faint brown lines (Fig. 3A, B), in preserved specimens as white lines. Oval central-mouth, flat, may be slightly raised in some. Actinopharynx often everted (Fig. 3B), sometimes obscuring entire animal. When alive, edge of mouth typically white in colour, with thick brown splotches radiating outwards, translucent when expanded. In preserved materials, edge of mouth with a brown cast.

*Column:* Thin-walled. Fosse present, very shallow in vivo; in preserved specimens fosse not distinct. When expanded, distal end flared outwards, overhangs slightly. Distal end with marginal projections, endocoelic (Fig. 3C, D). Longitudinal rows of conspicuous verrucae present, extending from distal end to mid-column, 6 to 13 per row; endocoelic (Fig. 3C, D). Verrucae oval, edges slightly raised and thickened, middle thin and translucent; in life, verrucae cream-white with a pink dot in the middle (Fig. 4C), colour is lost in preserved specimens (Fig. 4D). Verrucae adhesive, with small shell fragments, sand, small stones, and/or coral rubble often attached (Fig. 3C, D) even after preservation (Fig. 4D). Mesenterial insertions seen as light lines extending from the distal to proximal end. Remainder of column smooth, no other structures present (e.g., cinclides). In life, distal end brown, region from mid-column to proximal end bright orange; once preserved, specimen may be entirely cream-white, dark brown or grey.

*Pedal disc:* Overall oval in outline, flat; thin-walled. Mesenterial insertions seen through as white lines. Limbus slightly scalloped. In life, orange with splotches; once preserved entirely cream-coloured or greyish, with a dark-brown cast.

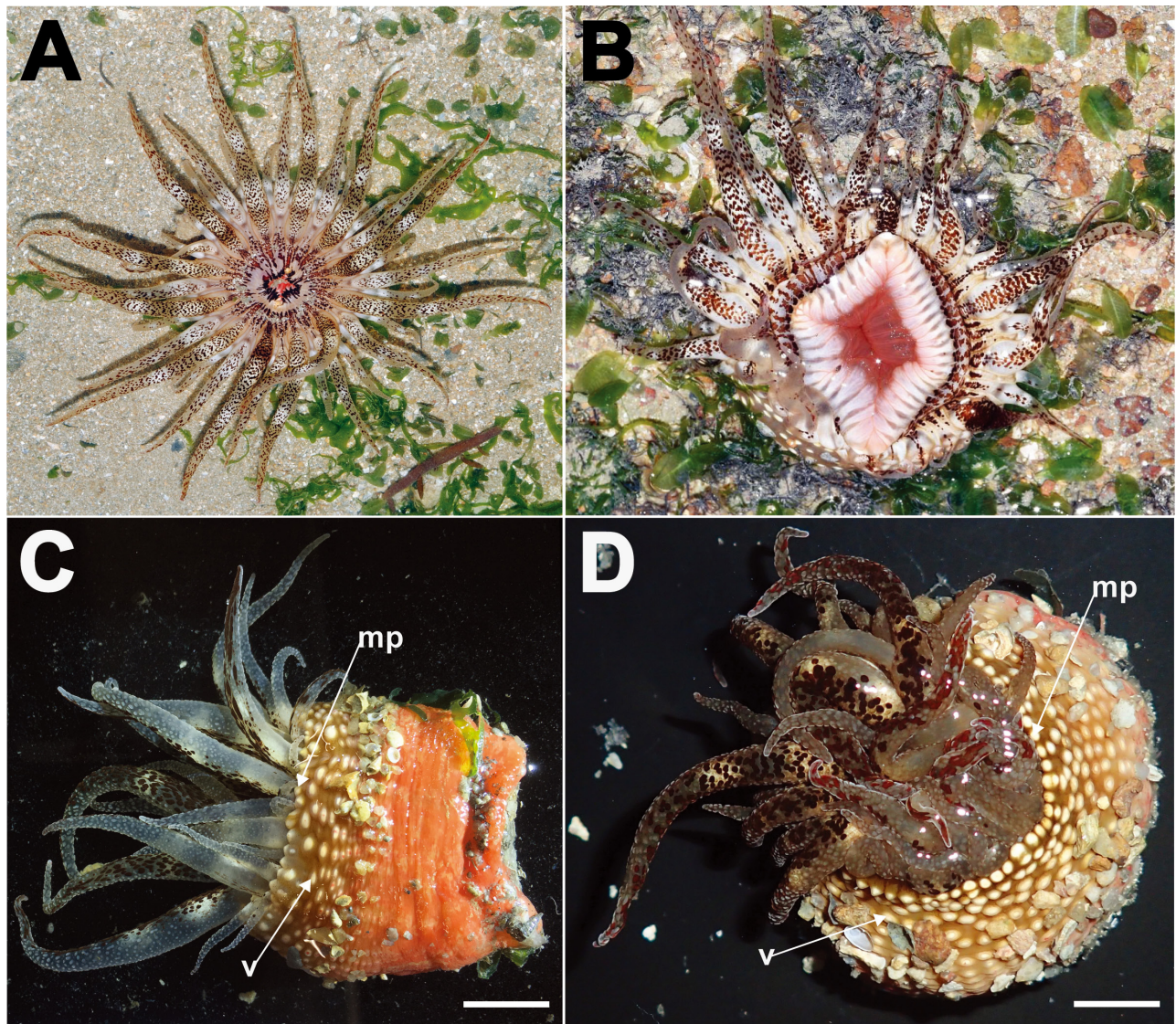
*Internal morphology:* 24 pairs of mesenteries

regularly arrayed in three cycles (6 + 6 + 12), with two pairs of directives symmetrically opposite each other, each pair attached to a siphonoglyph. Mesenteries in primary and secondary cycles complete (*i.e.*, 6 + 6 pairs); those in third cycle, incomplete (*i.e.*, 12 pairs). All mesenteries extended through entire length of column. Mesenterial retractor muscles diffuse-circumscribed, well-developed (Fig. 5A). Parietobasilar muscles of first two cycles well-developed, with pennon (Fig. 5A). Marginal sphincter muscle conspicuous, diffuse-circumscribed. Oral and marginal stomata present (Fig. 5B), but in some specimens may be

difficult to view (*e.g.*, SMNHTAU-Co.12133). Parietobasilar muscle present but not easily discernible. Actinopharynx extends proximally slightly past mid-column, longitudinally pleated, pinkish translucent in live, cream-white when preserved (Fig. 5C).

*Cnidom*: Spirocysts, basitrichs, and microbasic *p*-mastigophores. Sizes and distribution are shown in table 2; illustrations in figure 6.

*Distribution (Fig. S2)*: *Type locality*: Australia, Torres Straits, Mabuig Reef (Haddon and Shackleton 1893; Haddon 1898). *Published records*: Israel, Red Sea, Gulf of Aqaba, Eilat (Fishelson 1970). *New*

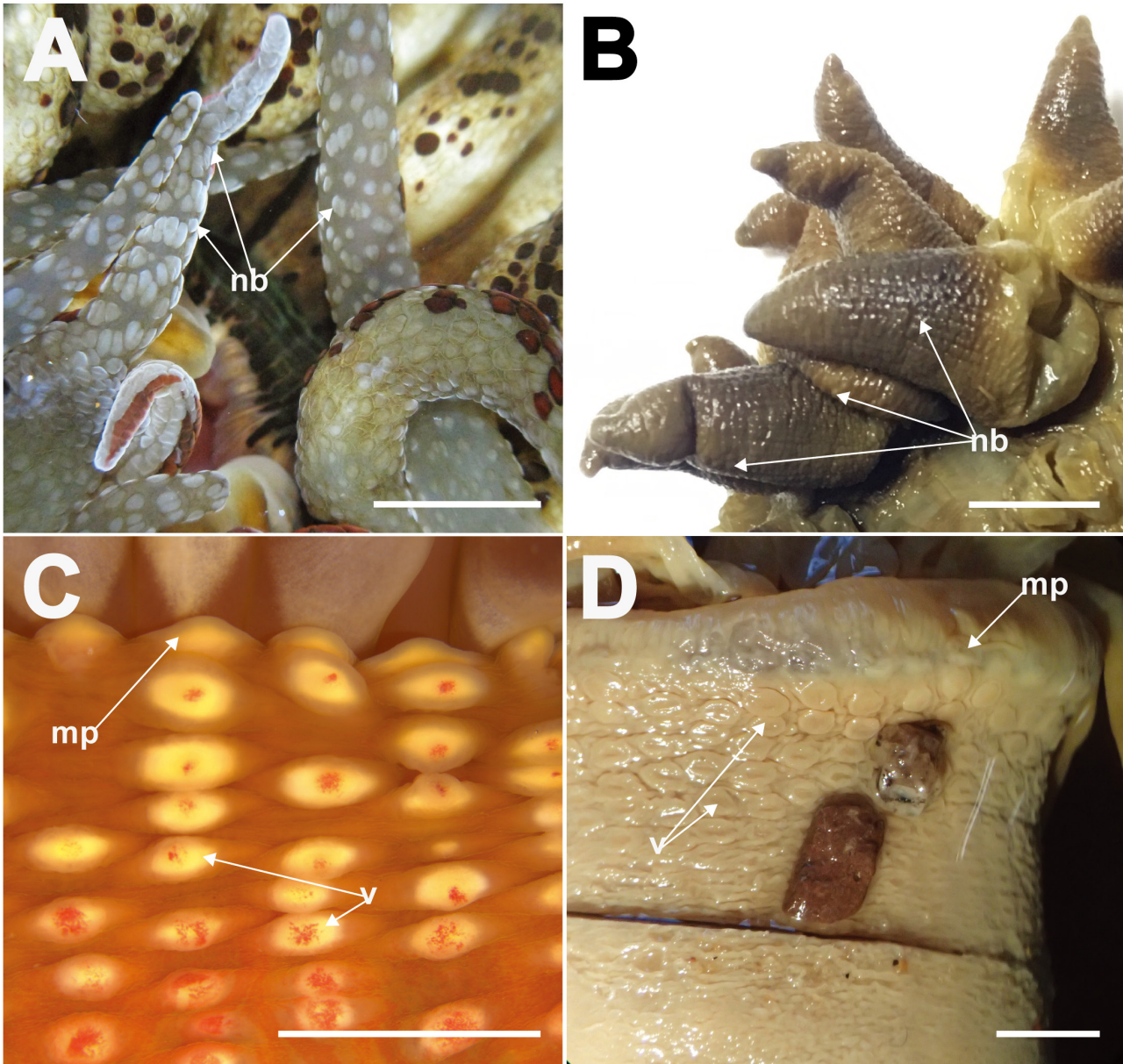


**Fig. 3.** Living individuals of *Macrodactyla aspera*, external morphology. A, B, expanded individuals extending from the substratum, *in situ*, top view. Note splotchy brown patterns on oral disc and tentacles in both A and B, and partially everted actinopharynx resembling thick lips in B. Photographs by KS Loh. C, a live collected specimen (ZRC.CNI.1099), side view. Note reddish-orange column and adhesive, conspicuous verrucae, with grains of substratum attached to it. D, a partially retracted specimen (ZRC.CNI.1099), top view. Note that individual does not completely retract its tentacles; note also the presence of marginal projections. Abbreviations: mp, marginal projections; v, verrucae. Scale bars = 10 mm.

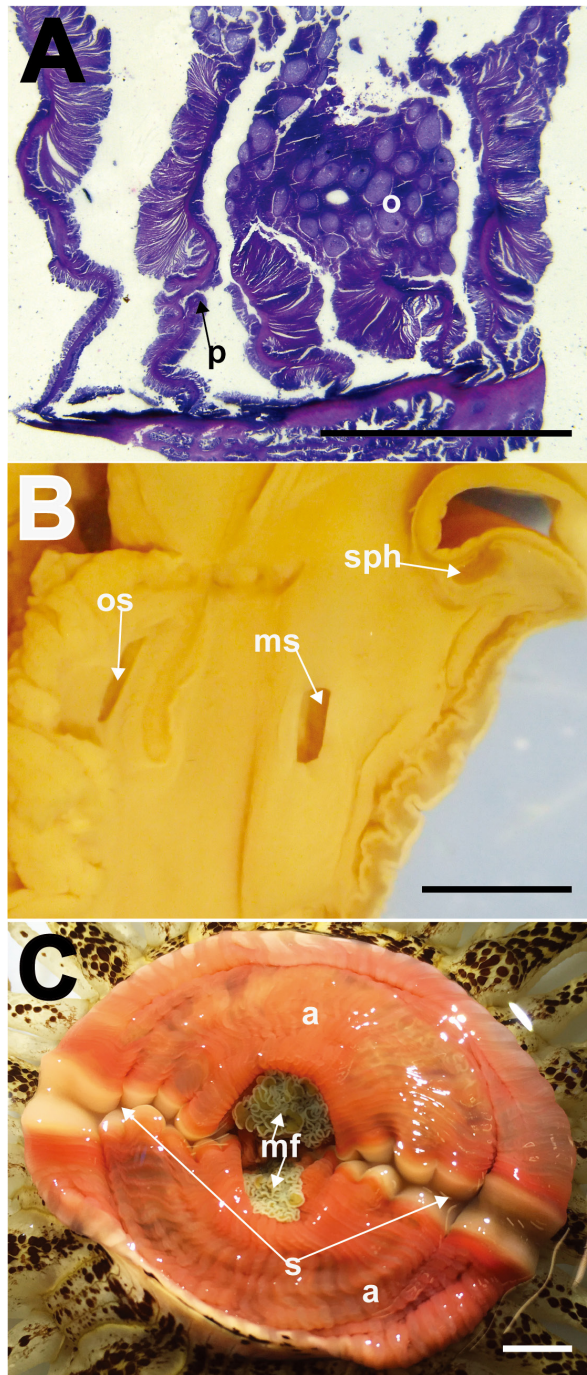
records: Australia, Indian Ocean, Perth (*i.e.*, Cockburn Sound, North Fremantle, and Rowley Shoals); New Caledonia, Coral Sea, Noumea; Singapore, in the Straits of Johor (*i.e.*, Beting Bronok, Changi region beaches, Pulau Sekudu) and Singapore Strait (*i.e.*, Cyrene Reef).

**Comparisons with other similar sea anemones:** In having nematocyst batteries densely covering its tentacles, other discerning taxonomic features (*e.g.*,

adhesive verrucae) hidden in the sand, and in sharing a similar habitat, *M. aspera* have been misidentified as *D. armata* (*e.g.*, WAM Z99100), or assumed to be a different morphotype of the latter (*i.e.*, Rowlett 2020: 292, 293). However, the presence of longitudinal, adhesive verrucae rows is the obvious feature that distinguishes these two species. These rows are only present on the distal end of *M. aspera*, while they



**Fig. 4.** Close-up appearance of tentacles and distal most column of *Macrodactyla aspera*, external view. A, extended tentacles of a live specimen (ZRC.CNI.1080), covered densely with nematocyst batteries. B, tentacles of a fixed specimen (*i.e.*, lectotype, MZC.I.33665). Note the overall stout, conical shape of tentacles once fixed, and the bumpy texture for each of them, indicating the presence of nematocyst batteries. C, marginal projections and adhesive verrucae of a live individual (ZRC.CNI.1080). D, marginal projections and adhesive verrucae of a fixed specimen (SMNHTAU-Co.7813). Note how the shallow fosse had inflated. Also note how the grains of substratum remained attached to the specimen, when it was collected by L Fishelson in 1968. Abbreviations: f, fosse; mp, marginal projections; nb, nematocyst batteries; v, verrucae. Scale bars = 10 mm.



**Fig. 5.** *Macrodactyla aspera*, internal morphology. A, mesenteries of the lectotype (MZC.I.3365), cross section at mid-column. Note the diffuse circumscribed appearance of the retractor muscles, and presence of the retractor pennon. B, transverse section of the distal most end of column (SMNHTAU-Co.7813). Note the presence of a conspicuous, restricted marginal sphincter muscle and the presence of both oral and marginal stomata. C, everted actinopharynx of a live specimen (ZRC.CNI.1090). Note its pinkish appearance, and the presence of a diametric pair of siphonoglyphs. Abbreviations: a, actinopharynx; mf, mesenterial filaments; ms, marginal stomata; o, oocytes; os, oral stomata; p, pennon; s, siphonoglyph; sph, marginal sphincter muscle. Scale bars = 5 mm.

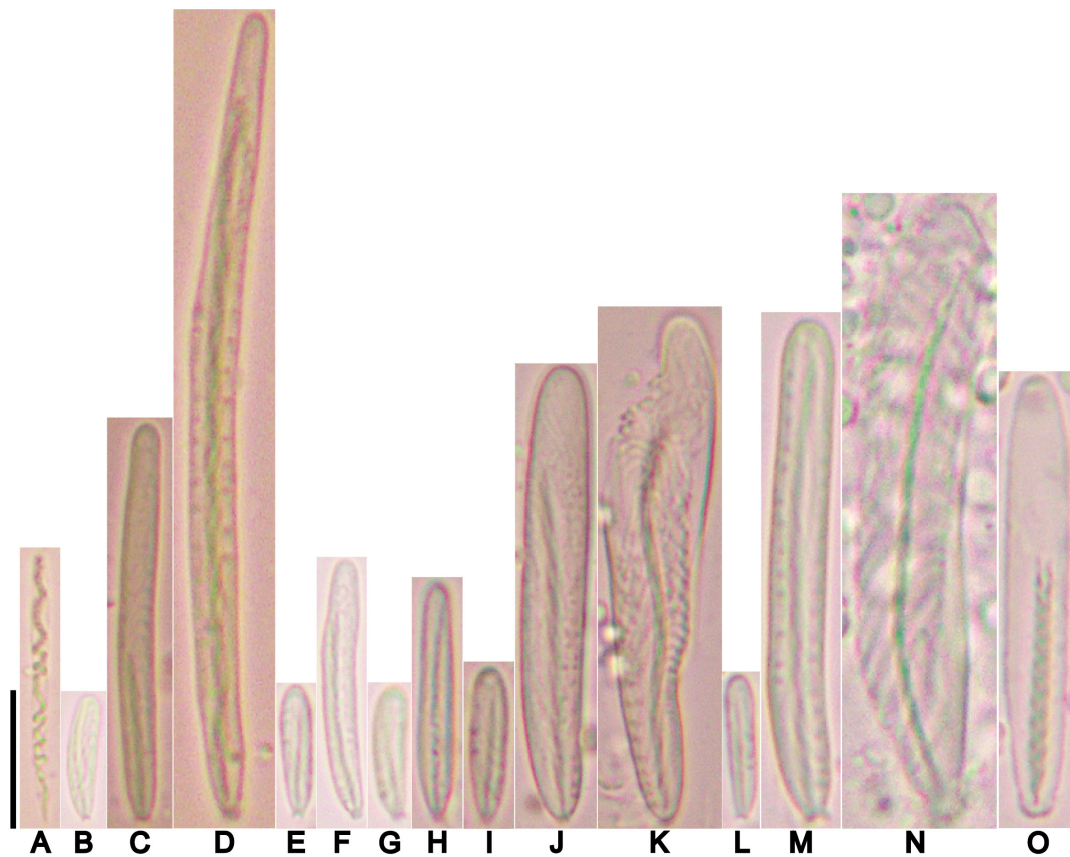
are absent in *D. armata*; the absence of verrucae is consistent with taxonomic accounts of *D. armata* and its holotype (Wassilieff 1908; Carlgren 1949). When alive, another trait that distinguishes *D. armata* from *M. aspera* is that the former readily autotomises its tentacles when disturbed, but those of the latter will remain intact.

**Remarks:** The animal that we have found closely matches the species and its type specimen that was collected and described by Haddon and Shackleton (1893). In all these specimens studied, we found that: i) their tentacles were densely covered with nematocyst batteries, ii) visible, adhesive verrucae were present at the distal end of the column, iii) cnidom data agreed between these specimens, and iv) as stated in our revised definition of the genus, the mesentery arrangement and number were also consistent.

Although the distinct tentacles of *M. aspera* (i.e., it being densely covered by nematocyst batteries) were not mentioned in its Haddon and Shackleton’s (1893) original description of the species, nor in the main text of Haddon’s (1898) expanded account, they were illustrated in the latter (see: Haddon 1898: Plate 22, Figs. 10 and 11). His depiction also provided a close-up view of a studded tentacle, clearly showing the nematocyst batteries. In examining the lectotype, Dunn (1981: 35) also found this feature to be obvious. The presence of nematocyst batteries densely covering each tentacle was also observed in fresh material collected, and in museum voucher specimens we have found and studied. Because the tentacles of *M. aspera* strongly resemble those of *D. armata*, many museum vouchers of the former species were identified and labelled as the latter (e.g., MNHN 1321, WAM 67). We have also examined specimens that Fishelson (1970) had collected (i.e., SMNHTAU-Co.7813 and SMNHTAU-Co.12133), and verified them to be *M. aspera*.

Like Haddon and Shackleton (1893), we found large visible, adhesive verrucae present on the distal end of the anemone, with shell fragments and small rocks often attached to them. While Haddon and Shackleton (1893: 124) also mentioned that the column of the anemone was also “covered with small, very adhesive suckers,” we failed to find any structural indication of this feature in both the lectotype and the voucher specimens.

The few published accounts pertaining to this species and its lectotype provided no detailed measurements of cnidom data (e.g., Haddon 1898; Fishelson 1970; Dunn 1981). We report these here for the first time. Broadly, measurements made on undischarged capsules extracted from tissues of the voucher material fell within the range of those obtained from the lectotype (Table 2). We were unable to assess



**Fig. 6.** Cnidae of *Macrodactyla aspera*. A, spirocyst. B, small basitrich. C, basitrich. D, large basitrich. E, small basitrich. F, basitrich. G, small basitrich. H, basitrich. I, small basitrich. J, large basitrich. K, wide basitrich. L, small basitrich. M, large basitrich. N, wide basitrich. O, microbasic *p*-mastigophore. Scale bar = 10  $\mu$ m. Refer to table 2 for key to letters.

**Table 2.** Cnidom of *Macrodactyla aspera* (Haddon and Shackleton, 1893). Sizes of single capsules that fell out of the size range for the cnida type measured are indicated as numerical values in parentheses

Tissue	Cnidae	Lectotype MZC.I.33665		Vouchers		
		Range length $\times$ width ( $\mu$ m)	<i>n</i>	Range length $\times$ width ( $\mu$ m)	<i>n</i>	N
Tentacles	Spirocysts (A)	-	-	(17.0)20.0–30.3 $\times$ 1.5–2.5	40	4/4
	Small basitrichs (B)	5.0–11.0 $\times$ 2.0–3.0	10	8.0–12.0(15.0) $\times$ (1.5)2.0–3.0	30	3/4
	Basitrichs (C)	30.0–36.4 $\times$ 2.5–3.5	18	17.0–32.0(34.0) $\times$ 2.0–3.5	40	4/4
	Large basitrichs (D)	37.9–54.5(57.5) $\times$ (2.0)2.5–3.5	20	33.0–50.0 $\times$ 2.5–3.5	40	4/4
Marginal projections	Small basitrichs (E)	9.0–12.0(15.0) $\times$ 2.0–3.0	10	7.0–15.0 $\times$ 2.0–3.0(4.0)	40	4/4
	Basitrichs (F)	17.0–20.5 $\times$ 3.0–3.5	10	(17.7)18.0–22.2 $\times$ 2.5–3.0	40	4/4
Column	Small basitrichs (G)	9.0–11.0 $\times$ 2.0–3.0	19	6.0–12.0(15.7) $\times$ 2.0–3.5	40	4/4
	Basitrichs (H)	(15.0)18.0–22.0 $\times$ (2.0)2.5–3.0	20	(16.0)17.0–24.2 $\times$ 2.0–3.0	50	4/4
Actinopharynx	Small basitrichs (I)	-	-	(7.0)9.0–17.0 $\times$ (1.5)2.0–3.5	40	4/4
	Large basitrichs (J)	-	-	30.5–42.0 $\times$ 4.0–6.5	40	4/4
	Wide basitrichs (K)	-	-	34.0–41.0(43.0) $\times$ 7.0–14.0(18.0)	40	4/4
Mesenterial filaments	Small basitrichs (L)	9.5–14.0 $\times$ 2.0–3.0	10	7.0–15.5 $\times$ 2.0–3.0	40	4/4
	Large basitrichs (M)	(31.0)35.0–43.5 (46.0) $\times$ 3.0–4.0	10	32.0–47.0 $\times$ 3.0–5.0	40	4/4
	Wide basitrichs (N)	50.0–59.0 $\times$ (4.0)5.0–6.0(7.0)	10	(32.0)35.0–55.0 $\times$ 6.0–12.0	40	4/4
	Microbasic <i>p</i> -mastigophores (O)	(23.0)26.0–29.5 $\times$ (4.5)5.0–6.0	10	25.0–35.0 $\times$ 4.0–5.5(6.0)	40	4/4

Abbreviations: N, the number of specimens for which the type of cnida was present to the total number of specimens examined; *n*, the number of cnida capsules measured in this study. Refer to figure 6 for illustrations corresponding to the letters in parentheses.

cnidae of the lectotype's actinopharynx as we failed to obtain a piece of tissue for it. Pertaining to the tentacle spirocysts of the lectotype, we found these to be degraded in the tissue, hindering measurements. Despite these two caveats, cnidae measurements of the lectotype and voucher materials agreed well.

***Macrodactyla fautinae*, sp. nov. Yap, Mitchell, Quek, and Huang**

(Figs. 7–11)

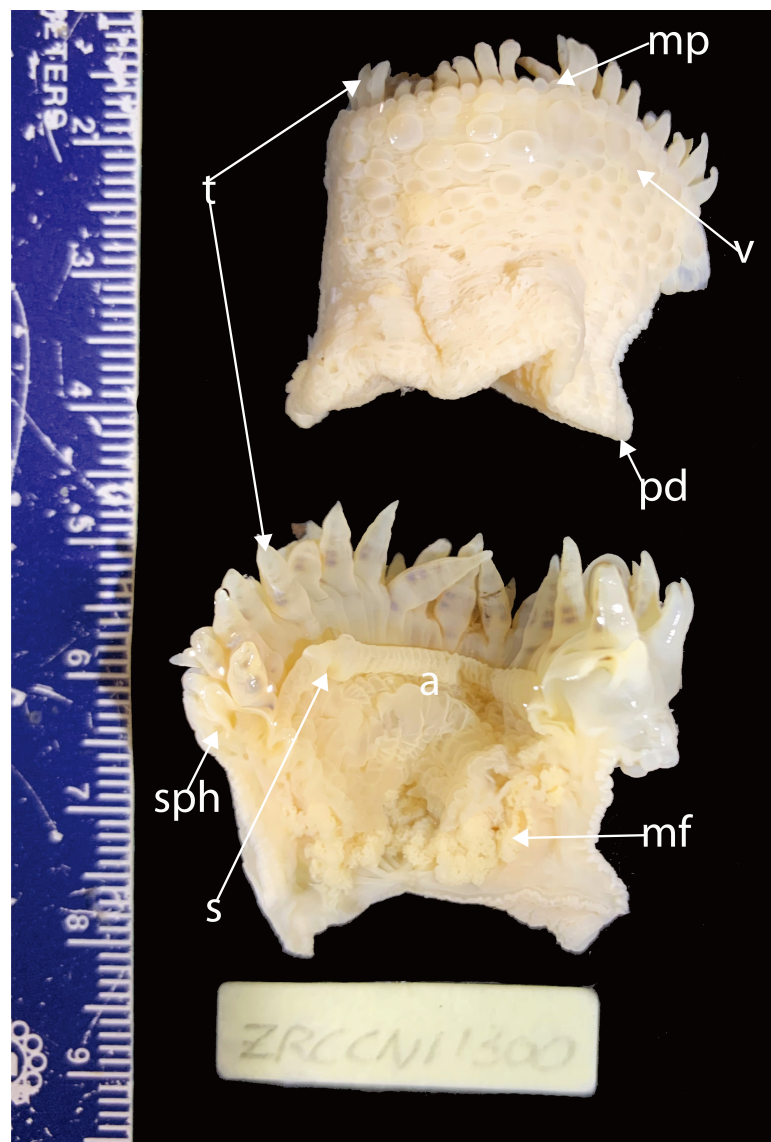
urn:lsid:zoobank.org:act:DC99CC5F-0956-47A9-B0E9-A0B3206F5ADE

*Material examined: Holotype:* ZRC.CNI.1300 (Fig. 7), collected from the beach off Changi Beach

(near Carpark 7), Singapore, on 5 July 2016 by the staff of LKCNHM. An individual of unknown gender, cut longitudinally into two equal pieces. Tissue from the pedal disc sampled for genetic work (Table S1).

*Paratypes:* ZRC.CNI.1156, collected from Changi Beach (near Carpark 4) (Fig. S1), Singapore, on 4 August 2016 by NWL Yap. Tissue sampled from its pedal disc for genetic work (Table S1). ZRC.CNI.0296, collected from Changi Point Beach on 22 July 2009 by DG Fautin et al. A dissected female from which 10 microscope slides with histological sections of the animal were prepared.

*Material examined from Singapore (Fig. S1) (\*- observed alive):* Changi Beach (ZRC.CNI.1020



**Fig. 7.** Holotype of *Macrodactyla fautinae* sp. nov. (ZRC.CNI.1300). Abbreviations: a, actinopharynx; m, mesenterial filaments; mp, marginal projections; pd, pedal disc; s, siphonoglyph; sph, sphincter muscle; t, tentacles; v, verrucae.

x5\*; ZRC.CNI.1156 x1\*; ZRC.CNI.1159 x1\*; ZRC.CNI.1300 x1\*), Changi Point beaches (ZRC.CNI.0022 x1; ZRC.CNI.0025 x1; ZRC.CNI.00296 x1; ZRC.CNI.00297 x1), Changi Point SAF Chalet beach (ZRC.CNI.0558 x1\*; ZRC.CNI.0559 x1\*; ZRC.CNI.0566 x1\*; ZRC.CNI.0598 x1\*; ZRC.CNI.0648 x3\*), Changi Point Ferry Terminal beach (ZRC.CNI.0023 x1; ZRC.CNI.0024 x1), Chek Jawa (ZRC.CNI.1370 x1\*), Pasir Ris (ZRC.CNI.00254 x1; ZRC.CNI.00255 x1), Pulau Ubin, OBS Camp beach (ZRC.CNI.0788 x1\*), Pulau Sekudu (ZRC.CNI.0026 x1).

*Etymology:* The name *Macroactyla fautinae* sp. nov. honours the late Emeritus Professor Daphne Gail Fautin. Throughout her career, she had worked tirelessly to advance the knowledge of sea anemones.

*Description: Natural history:* Occurs as solitary individuals, at the middle intertidal in soft silty, sandy areas, and also found in seagrass meadows (Fig. 8A–E). Animal partially buried in substratum, distal end of animal exposed during the low tides, with small shell pieces, sand and/or rocks adhering to verrucae (Fig. 8C). Oral disc and tentacles typically expanded, with actinopharynx often everted and inflated outwards, in many instances obscuring the animal (Fig. 8D). This species has also been observed to swallow its prey whole (Fig. 8E). When disturbed, animal does not retract completely, tips of tentacles remaining partially exposed (Fig. 8C). In the process of contraction, water may also be expelled from verrucae-like structures, akin to a watering can.

*Tentacles:* 96. Arranged in five cycles, one per endo-/exocoel. Outermost exocoelic; innermost cycles, endocoelic. Innermost cycles longer and of similar length, greater or equal to length of oral disc radius; outermost shortest,  $\frac{1}{2}$  length of innermost cycles (Fig. 8A, B). All alike in appearance; simple, smooth without nematocyst batteries, tapering to a blunt end in life; in preservative all appeared similarly sized, short, conical with a blunt end, possibly due to contraction in fixative. Colour in life translucent with horizontal bands of dark brown and white bands (Fig. 8A, B); in fixative, colours retained in freshly preserved specimens (*i.e.*, < two years) but are expected to be lost over time, to become translucent cream in colour.

*Oral disc:* Outline round, flat when expanded (Fig. 8A, B). Diameter up to 80 mm. Smooth and thin-walled, mesenterial insertions may be seen as dark radial lines extending from mouth to disc margin, more prominent in individuals with colourless disc or in fixed specimens (*e.g.*, Fig. 8A). In life, colour and pattern variable among individuals: may be completely colourless, greenish-grey, entirely dark-brown, or brown with white markings or vice-versa, sometimes accompanied by markings of cross bands being V- or W-shaped (seen

typically larger individuals; see also Fig. 8A, B); disc in fixative all appear translucent cream-coloured. Central mouth lipless and oval in outline, with actinopharynx everted to some extent (Fig. 8C, D). Two symmetric siphonoglyphs, each marked by bright pink dot in live individuals (Fig. 8A, B); pink dot may be visible in freshly preserved specimens but fades over time.

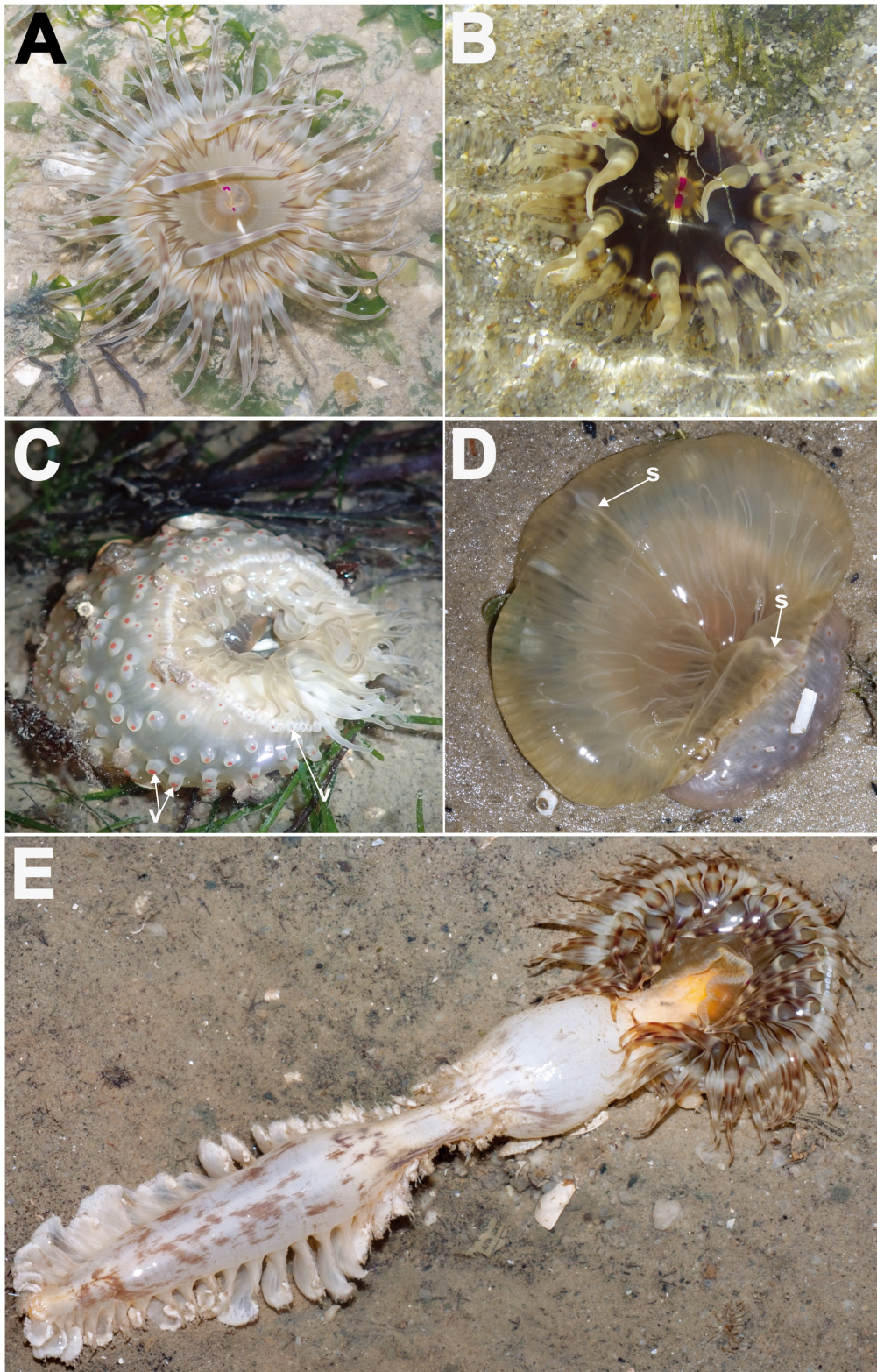
*Column:* In life, translucent cream-coloured, with reddish-orange splotches present on lower end (Fig. 9A); once fixed, colour may be retained in specimens, but fades over time. Fosse present, shallow to deep. Thin-walled, mesenterial insertion visible through wall as longitudinal light lines, extending from distal most to proximal end (Fig. 9B); visibility most prominent when animal is expanded and/or relaxed, may be obscured when animal is slightly contracted (Fig. 9A). Distal end with marginal projections, perforated, both endo-/exocoelic (Fig. 9C). Conspicuous adhesive, verrucae present, endocoelic, arranged in vertical rows that extend proximally from the distal end to mid-column, five to seven per row. Verrucae raised, outline round, with a pink dot in the middle (Fig. 9B, C), perforated. When extended, verrucae papillae-like (Fig. 9B). In fixed specimens, verrucae contracted but still prominent. From mid-column to proximal end, without any obvious structures (*e.g.*, cinclides, etc.); smooth.

*Pedal disc:* Outline irregular oval in outline, following contours of surface it clings onto. Thin-walled; mesenterial insertions visible through it as radiating white lines. Scattered reddish-orange splotches present on base. Adheres readily to surfaces.

*Internal morphology:* 48 pairs of regularly arrayed mesenteries arranged in four cycles (6 + 6 + 12 + 24); all extend the entire length of column. Two pairs of diametric directives, each pair attached to a siphonoglyph. Mesenteries in first two cycles complete, those in third and fourth incomplete. Gametogenic tissues on all mesenteries, if present. Sexes separate. Mesenterial retractor muscles well-developed, diffuse-circumscribed (Fig. 10A); mesenteries of lower orders more circumscribed than those higher. Parietobasilar well-developed; free pennon associated with mesenteries of lower orders (Fig. 10A). Oral and marginal stomata present, however former may be difficult to visualise in some individuals (*e.g.*, ZRC.CNI.0566). Marginal sphincter muscle conspicuous and circumscribed, close to fosse (Fig. 10B). Parietobasilar muscle present but not easily discernible. Actinopharynx longitudinally pleated and thin-walled, translucent-white in life, cream-coloured in preserved specimens; extends proximally towards pedal end, slightly pass mid-column.

*Cnidom:* Spirocysts, basitrichs, and microbasic *p*-mastigophores. Sizes and distribution are shown in table 3; illustrations in figure 11.





**Fig. 8.** Living individuals of *Macrodictyla fautinae* sp. nov., external morphology, *in situ*, top view. A, an expanded individual with a pale oral disc. B, another expanded colour morph with a dark-brown oral disc. Note that in both A and B, a pair of diametric bright pink dots marks the position of the siphonoglyphs. C, a contracted individual with adhesive, papillae-like verrucae at its distal end. Note that shell fragments are attached to the verrucae. D, an individual with much of its actinopharynx everted, obscuring the animal. E, a sea pen (*Pteroeides* sp.) being swallowed whole by a *M. fautinae* sp. nov. individual. Abbreviation: s, siphonoglyph; v, verrucae. Photographs by R Tan.

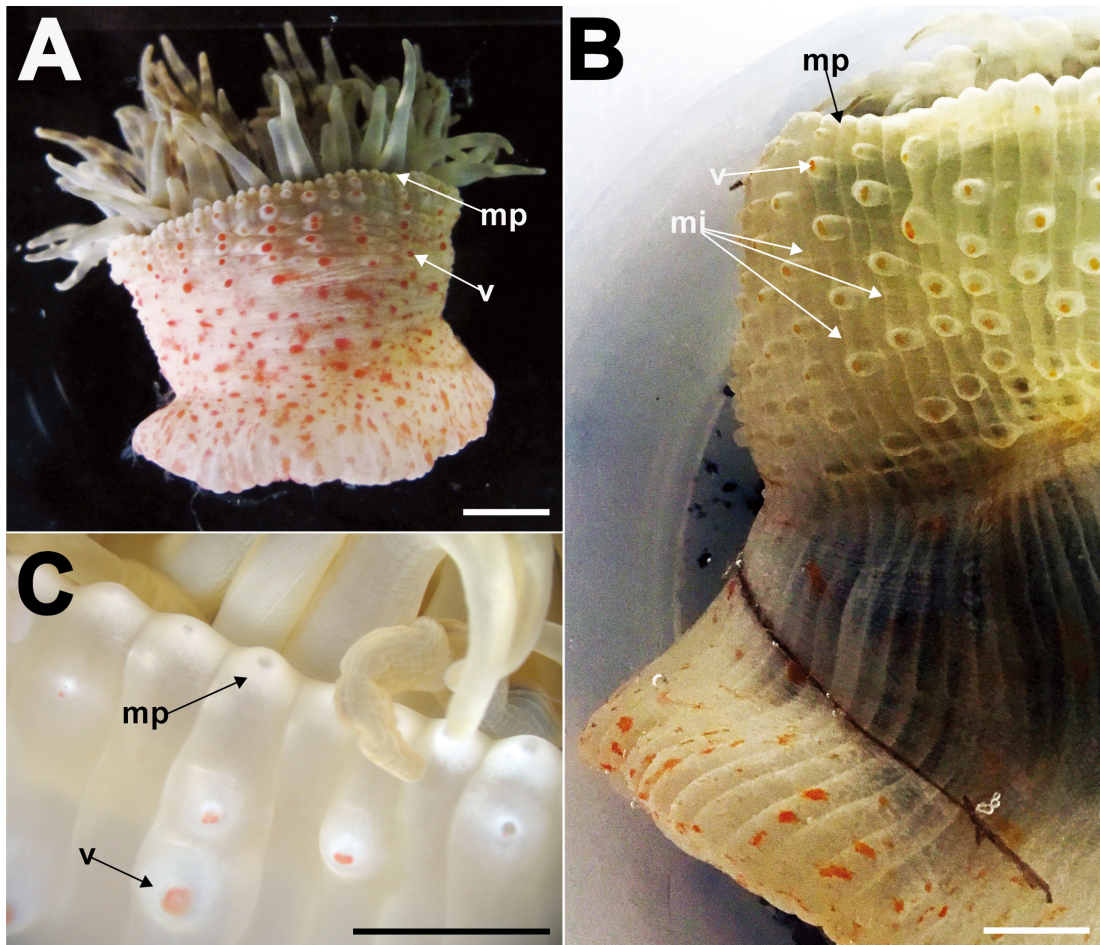
**Distribution:** This species has so far been encountered only along the northern shoreline of Singapore, in the Straits of Johor (Fig. S1).

**Comparisons with *M. aspera*:** The most obvious feature that distinguishes these two species is the texture of the tentacles of their type species. Those of *M. aspera* appear rough to the eye, as they are densely covered with nematocyst batteries, while the tentacles of *M. fautinae* sp. nov. are smooth. We have not encountered any individuals of *M. fautinae* sp. nov. that bear nematocyst batteries on their tentacles.

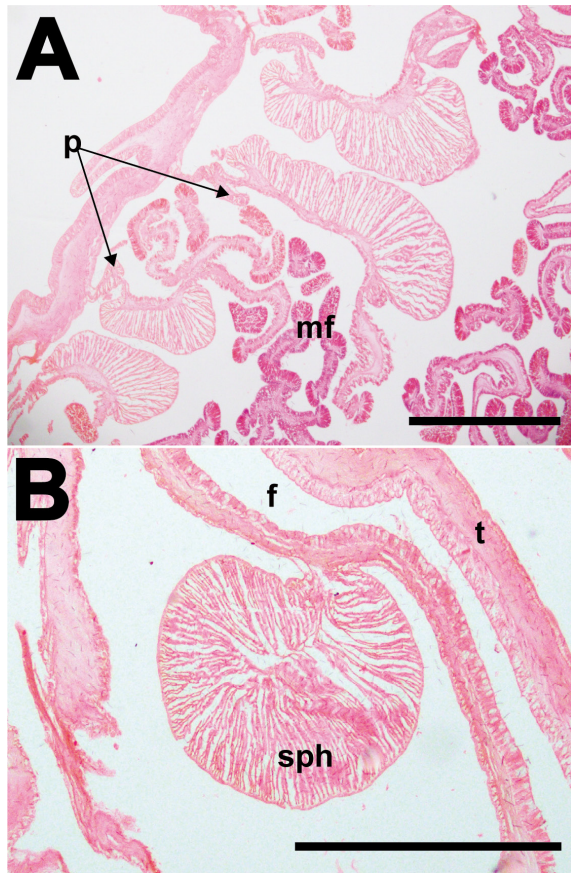
Oral disc appearance also differed between the two type species. As with the tentacles, nematocyst batteries are often on the oral disc of *M. aspera*, while *M. fautinae* sp. nov. lacks this feature. In many of the examined live *M. aspera* individuals, the oral disc is

often marked by dark brown splotches, whereas those of *M. fautinae* sp. nov. may vary in appearance, and markings – if present – are typically cross bands that are V- or W-shaped. Consistently in all individuals we have encountered and collected, bright pink dots demarcate the siphonoglyphs of *M. fautinae* sp. nov., a feature not observed on the oral disc of live *M. aspera* individuals examined (see Figs. 3A, B; 8A, B).

The appearance of the column also differed consistently between the two species. In general, colour may not be a useful feature when distinguishing between sea anemone species (Stephenson 1928: 69), however, we found this feature to be consistent as a whole in all individuals of both species studied. *Macrodactyla aspera* has a dark-coloured distal-end and the remainder of its column being bright orange,



**Fig. 9.** Column appearance of *Macrodactyla fautinae* sp. nov., external morphology, side view. A, a freshly collected individual that is slightly contracted (ZRC.CNI.0648). Note the pale pinkish column and the presence of splotchy dark pink patches from mid-column to the proximal end. Note also that the mesenterial insertions are not visible when the anemone is in a contracted state. B, a live individual (ZRC.CNI.1159) with a column that is expanded, with mesenterial insertions that extend the entire column visible as light lines. Note also the extended papillae-like verrucae. C, close-up of the marginal projections and verrucae at the distal end of a live individual (ZRC.CNI.1159). Note that marginal projections are perforated, and a pink dot marks the middle of each verruca. Abbreviations: mi, mesenterial insertions; mp, marginal projections; v, verrucae. Scale bars = 10 mm.



**Fig. 10.** *Macrodactyla fautinae* sp. nov., internal morphology. A, diffuse-circumscript mesenteries of an individual (ZRC.CNI.0296), cross section at mid-column. Note the presence of the retractor pennon. B, a conspicuous, circumscribed marginal sphincter muscle of another individual (ZRC.CNI.0255). Abbreviations: f, fosse; mf, mesenterial filaments; p, pennon; sph, marginal sphincter muscle; t, tentacles. Scale bars = 1 mm.

whereas that of *M. fautinae* sp. nov. is entirely cream-white, with pink splotches. Columnar structures also differ between both species. While verrucae found in these two species are adhesive, those of *M. fautinae* sp. nov. are lobe-like when extended, and expels jets of water when the animal contracts, a feature not observed in *M. aspera*.

**Ecological remarks:** Both *M. fautinae* sp. nov. and *M. aspera* are found to occur in similar habitats along Singapore’s northern coastline, in the Straits of Johor. However, while we have found the latter to also occur in intertidal areas of Singapore’s southern shoreline (i.e., in the Singapore Strait), we have only encountered *M. fautinae* sp. nov. on the northern shoreline and in no other southern locations that bear a similar habitat (Fig. S1). Apart from sharing similar habitat types, both species also exhibit a curious behaviour of everting an extensive part of the actinopharynx when exposed during the low tide, obscuring most of the animal. The exact rationale of this behaviour is unclear. Inferring from the presence of nematocysts present in the tissue of the actinopharynx and the extent of the eversion, we hypothesize that this behaviour aids in the capture of larger prey, as we have observed (Fig. 8E). To test this hypothesis, further experimental studies involving prey size and measuring the extent of actinopharynx eversion may be conducted.

**Similar species:** *Macrodactyla fautinae* sp. nov. may be confused with three other similar looking species that also occur at its type locality, Singapore: *Anthopleura buddemeieri* Fautin, 2005, *An. handi* Dunn, 1978, and *Paracondylactis singaporensis* (England 1987).

While both *M. fautinae* sp. nov. and *An. buddemeieri* have a column patterned with splotches

**Table 3.** Cnidom of *Macrodactyla fautinae* sp. nov. Sizes of single capsules that fell out of the size range for the cnida type measured are denoted as numerical values in parentheses

Tissue	Cnidae	Range length × width (µm)	n	N
Tentacles	Spirocysts (A)	15.0–27.5 × 2.0–3.0	53	5/5
	Large spirocysts (B)	30.0–41.0(43.0) × 2.5–5.0	47	5/5
	Basitrichs (C)	(9.0)10.0–20.0 × 2.0–3.0	51	5/5
	Large basitrichs (D)	27.5–33.0(34.0) × 2.0–3.0	50	5/5
Marginal projections	Basitrichs (E)	17.0–21.0 × 2.0–3.0(3.5)	50	5/5
Column	Basitrichs (F)	(10.5)15.0–20.0 × 2.0–3.0	52	5/5
Actinopharynx	Basitrichs (G)	(23.0)24.0–31.0 × 2.5–4.0	50	5/5
	Microbasic <i>p</i> -mastigophores (H)	15.0–23.0 × 4.0–6.5(7.0)	52	5/5
Mesenterial filaments	Small basitrichs (I)	10.0–13.0(14.0) × 2.0–2.5(3.0)	50	5/5
	Microbasic <i>p</i> -mastigophores (J)	17.0–22.0(23.0) × 4.0–7.0	50	5/5
	Large basitrichs (K)	27.0–34.0(36.0) × (3.0)4.0–6.0(7.0)	50	5/5

Abbreviations: N, the number of specimens for which the type of cnida was present to the total number of specimens examined; n, the number of cnida capsules measured in this study. Refer to figure 11 for illustrations corresponding to the letters in parentheses.

of red, with both species having adhesive verrucae on its distal most end, these two species occupy different habitats. *Macrodactyla fautinae* sp. nov. tends to occur in silty, sandy areas, among seagrasses meadows of the middle intertidal zones, while *An. buddemeieri* occurs underneath rocks in the upper intertidal zone (Fautin 2005; Fautin et al. 2009). While the size of a sea anemone may not be a good character to distinguish species (Stephenson 1922; Fautin et al. 2008), we have consistently found that adult individuals of *M. fautinae* sp. nov. are much larger than *An. buddemeieri* (e.g., oral disc diameter: 80 mm versus 7 mm, respectively; see Fautin (2005) for size of *An. buddemeieri*).

In occupying the same habitat types (i.e., silty and sandy substratum), and having translucent, patterned tentacles, *M. fautinae* sp. nov. may be mistaken for either *An. handi*, or *P. singaporensis* in the field. However, *M. fautinae* sp. nov. can be immediately distinguished from these other two species by using the following features: 1) a pair of conspicuous pink dots, each marking a siphonoglyph (Fig. 8A, B) – a feature that is absent in both *An. handi* and *P. singaporensis*; 2) a light coloured column with bright pink splotches, which differs from the consistently dull and darkly-coloured columns of *An. handi* and *P. singaporensis* (England 1987; Fautin et al. 2009; Fautin and Tan

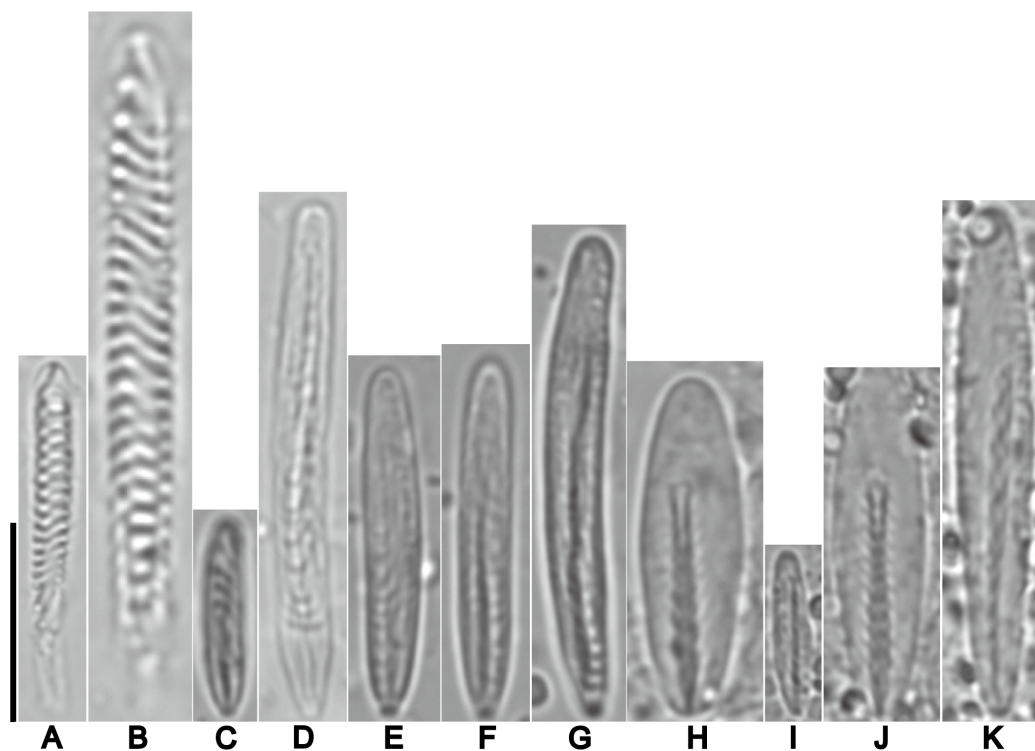
2016); and 3) differing ecology, as *M. fautinae* sp. nov. does not burrow deeply into the substratum with its column completely buried as *P. singaporensis* does (England 1987; Fautin and Tan 2016); nor, does it occur in dense clusters like *An. handi*, (Dunn 1978; Fautin et al. 2009) but rather often occurs singly. For these reasons, *M. fautinae* sp. nov. cannot be confused for the other two species.

## DISCUSSION

### Phylogeny of *Macrodactyla* and *D. armata*

In this study, we tested the relationship between *M. aspera* and *D. armata*, due to their strong resemblance to each other in having tentacles densely covered with visible nematocyst batteries. Our phylogeny reconstruction did not recover *M. aspera* and *D. armata* as a clade. Instead, both formed two distinct groups: *M. aspera* and the new species, *M. fautinae* sp. nov., were recovered as a clade, while *D. armata* was closely related to the actinodendrids (i.e., *A. arboreum* and *Ac. haeckeli*; Fig. 1).

Morphological and ecological evidence supported our findings that *Macrodactyla* species and *D. armata*



**Fig. 11.** Cnidae of *Macrodactyla fautinae* sp. nov. A, spirocyst. B, large spirocyst. C, basitrich. D, large basitrich. E, basitrich. F, basitrich. G, basitrich. H, microbasic *p*-mastigophore. I, small basitrich. J, microbasic *p*-mastigophore. K, large basitrich. Scale bar = 10  $\mu$ m. Refer to table 3 for key to letters.

belong to two distinct groups. Anatomically, *D. armata* lacks adhesive verrucae on its column, while these are present on *M. aspera* and *M. fautinae* sp. nov. In both *Macrodactyla* species, the sphincter muscle is circumscribed in form, while this musculature is diffused in *D. armata* (Carlgren 1949). Evidence supporting the close phylogenetic relationship of both macrodactylid species (*i.e.*, *M. aspera* and *M. fautinae* sp. nov.) have been discussed earlier in this study (see genus entry of *Macrodactyla*, above).

Together with our molecular findings and some evidence present in published taxonomic accounts of the Actinodendridae species targeted here, these strongly suggest that *D. armata* may be actually more closely related to actinodendrids than to Actiniidae, the family in which *Dofleinia* is currently placed. Our proposition here agrees with Rowlett (2020: 292), who also suggested the close relatedness of *Dofleinia* to actinodendrids, based on similarities in the ecology and morphology of these animals.

Ecologically, like *D. armata*, actinodendrids are also well-known to be able to inflict painful stings upon contact; they are popularly referred to as ‘Hell’s Fire Anemones’ (Ardelean and Fautin 2004; Erhardt and Knop 2005; Fautin et al. 2009). Both *D. armata* and actinodendrids are hosts to anemone shrimps (Bruce 1969 2005; Franssen 1989). Additionally, Rowlett (2020: 292) notes that these anemones exhibit, “burrowing behaviour.”

Broad morphological similarities are shared between *D. armata* and these actinodendrids, particularly: i) in having a smooth column without any visible structures (*e.g.*, adhesive verrucae, etc.) (Carlgren 1949), and ii) in bearing regions of dense nematocyst clusters along their tentacles (for a brief list of other morphological similarities, see Rowlett 2020: 292). Pertaining to i), while assuming *M. aspera* to be a morphological variant of *D. armata*, Rowlett (2020: 292) posited that the presence of verrucae is a diagnostic trait of *Dofleinia*, despite conceding that this trait was not found on the holotype of its type species (“*Dofleinia*... having... adhesive verrucae... strangely absent from many specimens (including the type)”). In light of our molecular findings, and the possibility of *Dofleinia* being related to actinodendrid anemones, we propose that the presence of adhesive verrucae is not a diagnostic trait of *Dofleinia*. Regarding ii), we note that this feature does differ in appearance between *D. armata* and the actinodendrids (for an example of acrospheres of an actinodendrid, see Ardelean and Fautin 2004: 192, Fig. 3). Rowlett (2020: 292) suggested that this feature may be homologous between these anemones. At present, the taxonomy of Actiniidae is in chaos and taxa within this family have been found to be polyphyletic

(Daly et al. 2017). To determine if *D. armata* is truly closely related or belongs to the family Actinodendridae will require further thorough taxonomic and genetic work that is beyond the scope of this present study.

### ***Macrodactyla aspera* as a potential threat to public safety**

In bearing visible nematocyst batteries, and having large nematocysts in its tentacles (*i.e.*, undischarged capsules of length > 50 µm), *M. aspera* may be capable of delivering a painful sting on contact. *Dofleinia armata* is another, similar example: it bears visible nematocyst batteries on its tentacles, and have basitrichs that are > 50 µm (*e.g.*, up to 76 µm; see Carlgren 1945). Other sea anemones that have been reported to be a threat to public health, such as *Phyllodiscus semoni* and members of Actinodendridae also bear specialised structures along their tentacles that harbour a dense reservoir of nematocysts (*e.g.*, nematocyst batteries) and cnidae larger in size (for sizes, see Carlgren 1945; Fautin et al. 2009; Hoeksema and Crowther 2011). Following this line of logic, we suggest that sea anemone species that may inflict painful stings are ones that usually bear specialised structures on their tentacles, and/or have large nematocysts; *M. aspera* may also be a potential threat to humans. This requires further investigation and comparisons with other species that are not deemed to be a threat but also bear large nematocysts and specialised structures on their tentacles (*e.g.*, *Haloclava hercules* Izumi, 2021).

### **Importance of re-examining name-bearing type specimens**

In this study, we re-described *M. aspera* using new morphological data (*i.e.*, living appearance, cnidom) gathered not only from fresh and voucher specimens, but also from the name-bearing specimen of *M. aspera* [= *C. aspera*]. This was necessary to ascertain that established diagnostic characters reported for *Macrodactyla* (*e.g.*, Stephenson 1921 1922; Carlgren 1949; Dunn 1981) were truly definitive of the genus. Here, we found that the complete mesentery numbers and arrangement present in the lectotype differ from previous accounts that were published. Our findings were not unexpected; long-accepted diagnostic characters of a taxon are often disputed when new observations arise from a careful re-examination of name-bearing types. Thorough treatment involving a comprehensive study of name-bearing types will be needed to resolve the taxonomic challenges for many groups of sea anemones. Members of the superfamily Actinioidea are taxonomically unresolved (Rodríguez et al. 2014; Titus et al. 2019) and will

benefit from this detailed approach. Recent efforts have demonstrated such an approach is feasible and fruitful (e.g., Fautin and Tan 2016; Yap et al. 2019 2021).

## CONCLUSIONS

In this study, morphological and molecular evidence were integrated to re-diagnose the genus *Macroactyla* and redescribe *M. aspera*. Furthermore, we provided historical, nomenclatural evidence to support the continued usage of the genus name. Since its first description by Haddon and Shackleton (1893), there have been very few published accounts of *M. aspera*, despite its wide geographical range across the Indo-West Pacific. The reason for this, we suspect, is that this species may have been misidentified as *D. armata*, due to its strong superficial resemblance. Museum vouchers we have examined support this assertion; some were indeed misidentified as *D. armata*. Herein, we provided molecular evidence to demonstrate that these two species are distantly related.

Findings from this study add to a growing number of sea anemone species recorded from Singapore. Previous studies have already documented 37 species to occur on the shores and islands of this city-state (e.g., England 1987; Fautin et al. 2009; Yap et al. 2021). Affirming the identities of these sea anemones primes new avenues where more Indo-West Pacific species may be included in future research.

**Acknowledgments:** This work and the new species name were registered with ZooBank under urn:lsid:zoobank.org:pub:560079AC-51E0-448F-8AAE-449058A9CBF8. We thank the efforts of Singapore's 'Anemone Army'; many of the specimens featured in this study were found or collected by these citizen scientists. Collections of sea anemones were also made during the Comprehensive Marine Biodiversity Survey (CMBS, 2010–2015) and associated workshops led by the National Parks Board Singapore (NParks) and National University of Singapore (NUS). The CMBS workshops were supported by generous contributions from Asia Pacific Breweries Singapore, Care-for-Nature Trust Fund, Keppel Care Foundation, Shell Companies in Singapore and The Air Liquide Group. We also thank the following institutions that have granted us permission to examine type material and voucher specimens: Lee Kong Chian Natural History Museum, National University of Singapore, Singapore (Singapore); Muséum National d'Histoire Naturelle, Paris (France); University Museum of Zoology, Cambridge University, Cambridge (UK); Museum of Zoology, Lund University, Lund (Sweden); Steinhardt

Museum of Natural History, Tel Aviv University, Tel Aviv (Israel); and Western Australian Museum, Perth (Australia). We are grateful to Mr Lok Kok Sheng for permission to use his photograph in this publication. We thank Professor Peter Ng Kee Lin for his advice on nomenclatural matters on the new species. We also thank both reviewers of this manuscript for providing constructive feedback and spotting typographical errors. Computational work for this article was partially performed on resources of the National Supercomputing Centre, Singapore (<https://www.nscg.sg>). The St John's Island National Marine Laboratory provided the facilities necessary for conducting this study. This Laboratory is a National Research Infrastructure under the National Research Foundation (NRF), Prime Minister's Office, Singapore. This research was also supported by the NRF, under its Marine Science R&D Programme (MSRDP-P03 and MSRDP-P38).

**Authors' contributions:** NWLY collected fresh specimens, performed morphological comparisons on museum types and vouchers, extracted, sequenced and analysed genetic data, drafted and formatted this manuscript. MLM examined additional WAM specimens, provided 28S sequences for the initial phylogeny analyses and drafted the manuscript. ZBRQ sequenced and analysed the genetic data and had drafted the manuscript. RT collected fresh specimens, photographed them and provided habitat data on these animals. KST had guided NWLY on this study, providing inputs to descriptions and nomenclature matters, and had drafted the manuscript. DH had guided NWLY on this study, analysed the genetic data and drafted the manuscript.

**Competing interests:** The authors declare that they have no conflict of interest.

**Availability of data and materials:** Specimens documented are kept in natural history museums as stated in the paper, genetic sequences and supplementary materials were deposited into GenBank and Zenodo respectively.

**Consent for publication:** Not applicable.

**Ethics approval consent to participate:** Not applicable.

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## Supplementary materials

**Table S1.** Sea anemones species included in our phylogenetic analyses. Information on voucher specimens and associated museum catalogue numbers are provided here, if available. Dashes denote that such information is absent. Accession numbers in **bold** denotes new sequences to this study. Abbreviations: AMNH, American Museum of Natural History, New York; CAS, California Academy of Sciences, San Francisco; Cat. nos., Catalogue numbers; KUNHM, University of Kansas Natural History Museum; ZRC, Zoological Records Collection, Lee Kong Chian Natural History Museum, Singapore. (download)

**Fig. S1.** Map of Singapore where specimens of both *Macrodactyla aspera* (Haddon & Shackleton, 1893) and *Macrodactyla fautinae* sp. nov. were collected for this study: 1, Pulau Ubin OBS Camp (1°25'07.7"N, 103°55'44.3"E); 2, Pasir Ris (1°22'56.45"N, 103°57'3.88"E); 3, Changi Region beaches [this area encompass the following localities that are in very close proximity to each other: Changi beach, Changi beach near Carpark 4; Changi Point beaches; Changi Point SAF Chalet beach; Changi Point Ferry Terminal beach] (1°23'33.34"N, 103°59'20.94"E); 4, Pulau Sekudu (1°24'19"N, 103°59'17"E); 5, Chek Jawa (1°24'25"N, 103°59'23"E); 6, Beting Bronok (1°26'13.00"N, 104°02'58.00"E); 7, Changi beach near Carpark 7 (1°22'26.03"N, 104°0'24.79"E); 8, Changi East next to Changi water Reclamation Plant (1°18'45"N, 104°00'31"E); 9, Cyrene Reef (Terumbu Pandan) (1°15'28"N, 103°45'19"E). White circles represent *M. aspera*, while stars (both black and white) denote the occurrences of *M. fautinae* sp. nov., with the single black star indicating the type locality of this new species. Numbers not accompanied by any symbols indicate the presence of both species occurring at the same site. See main-text for full details. (download)

**Fig. S2.** Distribution of *Macrodactyla aspera* (Haddon & Shackleton, 1893) and *Macrodactyla fautinae* sp. nov. 1. Murray Islands, Torres Straits. 2. Noumea, New Caledonia. 3. Perth, Western Australia. 4. Singapore. 5. Eilat, Israel. Symbols beside each number represent the occurrence of the sea anemones: circles being *M. aspera*, stars represents *M. fautinae*. Symbols that are coloured black denote the type locality of the species they represent. See main-text for full details. (download)

**Fig. S3.** Maximum likelihood phylogram (RAxML) of concatenated dataset (*i.e.*, 12S, 16S, 28S and *cox3*). 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 less than these limits are denoted by a dash (-). (download)

**Fig. S4.** Maximum likelihood phylogram (RAxML) of 12S sequences. Bootstrap values ( $\geq 50$ ) are indicated on branches. (download)

**Fig. S5.** Maximum likelihood phylogram (RAxML) of 16S sequences. Bootstrap values ( $\geq 50$ ) are indicated on branches. (download)

**Fig. S6.** Maximum likelihood phylogram (RAxML) of 28S sequences. Bootstrap values ( $\geq 50$ ) are indicated on branches. (download)

**Fig. S7.** Maximum likelihood phylogram (RAxML) of *cox3* sequences. Bootstrap values ( $\geq 50$ ) are indicated on branches. (download)