Citizen science meets integrated taxonomy to uncover the diversity and distribution of Corallimorpharia in Singapore

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Abstract. Corallimorpharia is an order of benthic cnidarians with a known global richness of 51 species, most of which are tropical and found in shallow-water habitats. These solitary organisms, which may occur in aggregations, are often overlooked compared to its more well-known sister group, Scleractinia (stony corals), as well as the closely-related Actiniaria (sea anemones) with which they are usually confused. Data on corallimorpharians in Singapore are sparse, though there are anecdotal photographic reports of various morphotypes at intertidal localities collated by citizen scientists. Combining these citizen-science observations and field collections, this study used both morphological and DNA sequencing approaches to establish the diversity and distribution of Corallimorpharia in Singapore. Based on a collection of 31 specimens and observational data from 23 coastal sites, examination of field photographs and gross morphology yielded nine distinct morphotypes. To infer their identities based on molecular data, three DNA loci were sequenced—mitochondrial intergenic region (IGR) between the cytochrome c oxidase subunits 1 (COX1) and 3 (COX3), part of the ATP synthase F0 subunit 6 gene (ATP6), and nuclear internal transcribed spacers (ITS). Phylogenetic analysis, supported by detailed examination of the internal and external morphology, recovered six morphospecies placed within four genus-level clades, including two species from Corynactis and Discosoma that are possibly new to science. The molecular phylogeny is nearly concordant with the morphological taxonomy and distinction between the Rhodactis species is equivocal, likely because of recent divergence and the limited number of molecular markers used. This study represents the first comprehensive inventory and phylogenetic analysis of corallimorpharians in Singapore that would facilitate further research on this understudied order.

Key words. Anthozoa, DNA sequencing, morphology, mushroom anemones, phylogenetics, tropical coastal habitats

INTRODUCTION

Globally, there are 51 valid species of corallimorpharians (Cnidaria: Anthozoa: Corallimorpharia) (WoRMS, 2018). Commonly known as ‘mushroom anemones’, they are highly sought after by the aquarium trade (Fossà & Nilsen, 1998; Rhyne et al., 2009). Most species are distributed across the reef zones of subtidal and intertidal reefs in the Indo-Pacific and Caribbean seas (Chadwick & Adams, 1991; Waheed & Hoeksema, 2012; Work et al., 2018), but there are also a handful of deep-sea species (Fautin et al., 2002; Fautin, 2016). Polyps are solitary and often found attached to coral rock by their pedal disc or body column (Wickel et al., 2017).

Superficially, corallimorpharians resemble sea anemones (Actiniaria) (Carlsgren, 1949), with numerous tentacles of variable shape and size, either scattered or radially distributed on the oral disc whilst the mouth is located in the middle (den Hartog, 1980). Some corallimorphian species also contain tentacles with nematocysts of similar structure to those in sea anemones (Allman, 1846). They can also mimic corals hence their name (Waheed & Hoeksema, 2012).

Corallimorpharians have paired identical mesenteries in a radial arrangement (den Hartog, 1980; Daly et al., 2003). This arrangement is similarly observed in sea anemones and stony corals (Scleractinia) (Daly et al., 2003). However, Corallimorpharia is distinct from Actiniaria and Scleractinia as its species lack a calcium carbonate skeleton (Stanley & Fautin, 2001) and do not have siphonoglyphs (den Hartog, 1980).

Corallimorpharia appears to be a relatively species-poor order compared to Actiniaria and Scleractinia. Despite inhabiting similar environments, the former remains severely understudied. In particular, taxonomic research on Corallimorpharia has traditionally been conducted by taxonomists specialising in Actiniaria (e.g., Carlsgren, 1949; den Hartog et al., 1993; Fautin, 2016), hence descriptions
often utilise morphological characters observed in Actiniaria (Carlgren, 1949; Fautin & Daly, 2009). This overlap has led to misclassifications such as the initial grouping of Corallimorpharia within Actiniaria (Fishelson, 1970). Similarities in polyp anatomy between Scleractinia and Corallimorpharia and an early phylogenetic analysis (Medina et al., 2006) have also suggested that corallimorpharians are a subgroup of stony corals; hence, the ability to secrete a calcium carbonate skeleton could be an ephemeral trait in Scleractinia (Hand, 1966; Stanley, 2003; Medina et al., 2006). Subsequent analyses using mitochondrial genomes (Barbeitos, 2007; Brugler & France, 2007; Kitahara et al., 2010), nuclear markers (Fukami et al., 2008) and transcriptomes (Lin et al., 2016, 2017) reject this ‘naked-coral hypothesis’ by re-establishing Corallimorpharia and Scleractinia as reciprocally monophyletic sister groups. Both cases highlight the importance of utilising multiple molecular markers along with rigorous phylogenetic analysis to clarify evolutionary relationships within Hexacorallia, a subclass of Anthozoa (den Hartog, 1980; Berntson et al., 1999; Medina et al., 2006; Kitahara et al., 2014). More broadly, as the sister group to Scleractinia, corallimorpharians play a key role in understanding the evolutionary origins and diversification of stony corals (Lin et al., 2017).

Within Corallimorpharia, classification at the genus and family levels is subject to debate (Fautin, 2011; Ocaña et al., 2017). For example, the status of *Pseudocorynactis* with respect to *Corynactis* remains unclear as morphological characters (e.g., cnidae and tentacle structure) describing the genus *Corynactis* differ from *Pseudocorynactis* (Ocaña et al., 2017). Compounded by the lack of taxonomic expertise, species-level taxonomy for Corallimorpharia is even less well-defined. Recent studies have shown that molecular markers can be effective in establishing relationships within Hexacorallia (Barbeitos, 2007; Brugler & France, 2007; Fukami, 2008; Fukami et al., 2008; Sinninger et al., 2008; Kitahara et al., 2010; Lin et al., 2016, 2017). A number of these have been applied within Corallimorpharia to complement taxonomic classification and identification (Chen et al., 1996; Cha, 2007; Lin et al., 2014).

In Singapore, although corallimorpharians have not been targeted for detailed taxonomic work, a substantial amount of observational data have been amassed through citizen scientists in the last two decades. Local sightings of corallimorpharians have been photographed and reported by non-scientists who visit the seashores almost monthly to document marine life (see WildSingapore Fact Sheets: http://www.wildsingapore.com/wildfacts/). While voluminous data on Singapore’s Corallimorpharia have been collated by citizen scientists, they have yet to be verified formally and analysed systematically. To address this knowledge gap, our study aims to estimate the diversity and distribution of Corallimorpharia among intertidal and subtidal sites in Singapore by leveraging the current citizen science database to infer species distributions, as well as using both morphology and DNA sequence data to delineate species. Results obtained will strengthen our understanding and knowledge of regional marine biodiversity as well as the evolutionary history of cnidarians.

**MATERIAL & METHODS**

**Citizen science data.** Corallimorpharian distribution data between 2000 and 2018 were retrieved for analyses from the WildSingapore Fact Sheets (http://www.wildsingapore.com/wildfacts/), a citizen-science platform with imaged sightings of marine intertidal flora and fauna across 23 intertidal localities in Singapore. Based on external appearance and colours, corallimorpharians found were grouped into nine morphotypes by the non-scientists (see http://www.wildsingapore.com/wildfacts/anemonef4.htm). Additional field collections utilised the distribution and morphotype images shared by citizen scientists for more targeted collections at sites where corallimorpharians have been recorded.

**Specimen collection.** Five reef sites were selected for collecting corallimorpharians. Twenty-one specimens were hand-collected from various intertidal sites: Pulau [Island] Ubin (1°24′07.8″N, 103°58′16.0″E), Sisters’ Islands (1°12′57.8″N, 103°49′52.5″E), Pulau Hantu (1°13′38.4″N, 103°44′56.7″E), St John’s Island (1°13′21.0″N, 103°50′47.0″E) and Raffles Lighthouse (1°09′35.1″N, 103°44′26.7″E) (Fig. 1). Ten specimens were collected at subtidal depths by SCUBA from Pulau Hantu, Raffles Lighthouse and Sisters’ Islands. Corallimorpharians were first identified in the field by eye. Intertidal specimens were photographed in situ and subtidal specimens photographed ex situ. All specimens were photographed with a ruler for scale bar calibration. At least three polyps for each morphotype were collected. About 0.4 cm² of tissue was sub-sampled from one polyp for preservation in 100% molecular grade ethanol and stored at −80°C. The remaining whole specimens were fixed in 10% buffered formalin and stored at room temperature. All voucher specimens were deposited in the
Fig. 1. Map of Singapore indicating the localities where corallimorpharians have been recorded, including sightings from the citizen science database, WildSingapore Fact Sheets (www.wildsingapore.com/wildfacts). PGL – Punggol; PRP – Pasir Ris Park; PU – Pulau Ubin; CJ – Chek Jawa; SKD – Pulau Sekudu; CHG – Changi; BB – Beting Bronok; TM – Tanah Merah; SNT – Sentosa; LAB – Labrador and Belayar Creek; CYR – Cyrene; KUS – Kusu Island; LAZ – Lazarus; SJI – St John’s Island; SIS – Sisters’ Islands; TKR – Pulau Tekukor; JNG – Pulau Jong; TSM – submerged reefs off Pulau Semakau comprising Terumbu Semakau, Terumbu Raya, Beting Bemban Besar, Terumbu Bemban; SMK – Pulau Semakau; HNT – Pulau Hantu; TPG – submerged reefs comprising Terumbu Pempang Darat, Tengah, Laut; LFA – live firing areas comprising Pulau Salu, Sudong, Pawai, Senang, Biola and submerged reefs in the area; RFL – Raffles Lighthouse. Coloured squares represent Corallimorpharia species recorded at the respective localities.

Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum, National University of Singapore.

**Species identification based on morphology.** Each specimen was first recognised as one of nine morphotypes as described on WildSingapore. External morphology was more useful as a species-level character than internal morphology (Fosså & Nilsen, 1998; Cha, 2007), so the external morphology of all specimens collected was examined first. Morphological characters utilised in this study include tentacle and body-column shape, tentacle distribution, discal shape, tentacle and body colour. We found these characters largely sufficient for species identification. Type specimens of *Discosoma nummiforme*, *Rhodactis inchoata*, and *R. indosinensis* kept at the Naturhistoriska Riksmuseet, Stockholm, Sweden (NRS) were studied to confirm species identities. Because the holotype of *Platyzoanthus mussoides* was missing (see Fautin, 2016: 37), we followed the description of Saville-Kent (1893).

Internal morphological characters in dissected specimens, such as cnidae and distribution of mesenteries were examined to complement species descriptions. Retractor muscles of the animals were observed from 8-µm thick histological sections stained with Mayer’s hematoxylin and eosin (Humason, 1962). Where necessary, unfired cnidae capsules were examined from tentacles and mesenteries of the animal and compared with examined syntypes to further confirm their identities. Cnidae were measured at 1000× and identification followed that of Mariscal (1974).

**DNA primer design.** Twelve corallimorpharian mitochondrial genomes published by Lin et al. (2014) were retrieved from GenBank and assembled in Geneious v.3.31. Pairwise comparisons of genes among the genomes showed highest variability in the intergenic region (IGR) between mitochondrial cytochrome c oxidase subunits 1 (COX1) and 3 (COX3), and in the ATP synthase F0 subunit 6 (ATP6) gene. Three primer pairs were designed at the flanking regions of these loci for DNA amplification (Table 1). We also used the 1S + 2SS primer pair (Chen et al., 1996) to amplify and sequence the nuclear internal transcribed spacers (ITS) (Table 1).

**DNA extraction, polymerase chain reaction (PCR), and Sanger sequencing.** Genomic DNA was extracted using the
Table 1. Primers designed and used in this study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Annealing temperature (°C)</th>
<th>Expected length (bp)</th>
<th>Target region</th>
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<tbody>
<tr>
<td>CO1F</td>
<td>GCG AAT AGT TCT CTA GAT GTT GTT T</td>
<td>53.1</td>
<td>863</td>
<td>Intergenic region between COX1 and COX3</td>
</tr>
<tr>
<td>CO1R</td>
<td>CTC TAA TAA CAT CTT GCC ACC AAA C</td>
<td>54.2</td>
<td></td>
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<tr>
<td>CO12F</td>
<td>TAC TGG RTA TTG TTA TAA TGA ATT</td>
<td>46.8</td>
<td>756</td>
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<tr>
<td>CO12R</td>
<td>ACT AAA TGA TAR GGG TGA CAT A</td>
<td>49.5</td>
<td></td>
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<tr>
<td>ATP6F</td>
<td>GTA GTC AAT TGG GGT WTA TGG TGG T</td>
<td>56.7</td>
<td>1070</td>
<td>ATP synthase F0 subunit 6</td>
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<tr>
<td>ATP6R</td>
<td>CCA CAT CTT TAG CTT TGA AGG CTA A</td>
<td>55.4</td>
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<td>1S</td>
<td>GGT ACC CTI TGT ACA CAC CGC CCG TCG CT</td>
<td>68.3</td>
<td>1000</td>
<td>ITS1, 5.8S and ITS2</td>
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<tr>
<td>2SS</td>
<td>GCT TTG GGC GGC AGT CCC AAG CAA CCC CCG CT</td>
<td>71.2</td>
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</table>

DNeasy Blood and Tissue Kit (Qiagen, Singapore) following the manufacturer’s protocol. Target loci were amplified using combinations of forward and reverse primers designed above (Table 1). The CO12 primer set and ATP6 primer set were used for all PCR reactions, except Corynactis sp. (amplified with CO12F + CO1R primer set) due to variation from the canonical corallimorpharian gene arrangement described in Lin et al. (2014). The 1S + 2SS primer set was used for eight Rhodactis samples to attempt to resolve species-level relationships within the genus. PCR reaction mixtures were prepared as follows: 2.0 μl DNA, 12.5 μl GoTaq DNA polymerase (Promega), 1.0 μl forward primer, 1.0 μl reverse primer, 8.5 μl water. PCR cycle reactions for the mitochondrial gene amplifications were as follows: 94°C for 180 seconds, 35 cycles of 94°C for 30 seconds, annealing (Table 1) for 30 seconds and 72°C for 75 seconds, ending with 72°C for 300 seconds. PCR cycle reactions using the 1S + 2SS primer set followed the protocol described in Chen et al. (1996). All DNA amplicons were purified using SureClean Plus (Bioline, Singapore), following the manufacturer’s protocol, before sequencing with an ABI 3130 XL DNA Analyzer (ThermoFisher Scientific).

Editing and de novo assembly of sequenced strands were done in Geneious under default parameters, and data were deposited at GenBank (accession numbers: MK621776 - MK621806). Sequences were 400–650 base pairs (bp) long for the amplification using CO12F + CO12R, 400–500 bp for CO12F + CO1R, 900–950 bp for ATP6, and 850–950 bp for 1S + 2SS. Sequences were searched against GenBank using BLAST to obtain preliminary species identities.

Phylogenetic analyses. Thirty-one IGR, 22 ATP6 and eight ITS sequences obtained were used for phylogenetic analyses. Orbicella annularis (GenBank accession number: AP008973) and Acropora muricata (GenBank accession number: KF448529), both belonging to sister group Scleractinia, were included as outgroups. The 12 corallimorpharian genomes represented by mitochondrial genomic data were also included (Lin et al., 2014). For each locus, Mesquite v.3.5 was used to consolidate all sequences into a data matrix before alignment with MAFFT v.7.313 under default parameters. All sequence alignments and tree files are available at Zenodo (http://dx.doi.org/10.5281/zenodo.1693772).

Analyses based on maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) were conducted for the IGR dataset as it provided the greatest phylogenetic resolution. The MP tree was inferred using TNT v.1.5 (Goloboff & Catalano, 2016) with 10,000 random addition sequence replicates, each with 100 cycles of sectorial searches, ratcheting, drifting, and tree fusing (gaps as missing data). Bootstrap support was evaluated using 10,000 bootstrap replicates. The ML tree was inferred using RAxML (Stamatakis, 2014), based on 50 random starting trees under the default GTRGAMMA model, with 1,000 bootstrap replicates. The more taxonomically focused datasets ATP6 and ITS were also analysed using ML. MrBayes v3.2.6 (Ronquist et al., 2012) was used to carry out BI using GTR + I as the best-fitting model—determined by jModelTest 2 (Darriba et al., 2012) using the Akaike information criterion. Two Markov chain Monte Carlo runs with 12 million generations each were performed, sampling every 100th tree. The first 20,000 trees were discarded as burn-in after run convergence was ascertained with Tracer v1.6.0 (Rambaut et al., 2014).

RESULTS

Morphological identification. Based on external morphology, all corallimorpharians that were observed and collected in Singapore could be separated into nine morphotypes concordant with the citizen science data. Detailed examination of the internal and external morphology
led to the placement of all specimens in four known genera (Corynactis, Discosoma, Platyzoanthus, and Rhodactis) and the recognition of six morphospecies (Corynactis sp., Discosoma sp., Discosoma nummiforme, Discosoma mussoides, Rhodactis inchoata, and Rhodactis indosinensis) (see species descriptions below). These included two morphotypes whose common names are derived from external anatomical features — ‘striped’ (Discosoma sp.) and ‘ball-tip’ (Corynactis sp.) — and which have not been formally described.

Molecular phylogeny. The IGR tree topologies inferred based on MP, ML and BI optimality criteria were concordant at the genus level (Fig. 2; Appendix 1), with all corallimorpharians sampled here grouped into five clades. MP and BI trees did not resolve most species-level relationships. Sequences from Rhodactis formed a polytomy on the IGR MP (strict consensus) and BI trees, suggesting that MP and BI were insufficient in distinguishing between Rhodactis species. Overall, the ML tree topology supported the six morphospecies—Corynactis sp., Platyzoanthus mussoides, Discosoma nummiforme, Discosoma sp., Rhodactis indosinensis, and R. inchoata—all moderately to strongly supported except for both Rhodactis species (Fig. 2).

Results among the three molecular markers used in this study were similar but not fully concordant. Rhodactis inchoata and R. indosinensis mainly formed two clades on the IGR ML tree but bootstrap values on the diverging nodes were <50, indicating weak support for their lineages (Fig. 2). The ATP6 and ITS regions were analysed to try to improve the resolution between these two species. However, the ATP6 tree was poorly resolved and did not differentiate genera and morphospecies as well as the IGR trees. The ITS tree (Appendix 2) fared better in separating R. indosinensis and R. inchoata with a topology that was similar to the IGR tree (Fig. 2). Nevertheless, conflict between morphological identification and phylogenetic placement was apparent for some Rhodactis specimens (REL179, REL233, and REL350).

Diversity and distribution of Corallimorpharia in Singapore. Integration of the present collection data and intertidal citizen science data from WildSingapore Fact Sheets, collected over 18 years, gave a more comprehensive picture of species distributions (Fig. 1; Table 2). Corynactis sp. (ball-tip morphotype) was the most widely distributed species in Singapore, being recorded at 16 of the 23 localities surveyed. Discosoma sp., represented by the ‘striped’
Table 2. Species distributions of Corallimorpharia in Singapore, arranged according to their common names. × – Intertidal specimen collected. Δ – Subtidal specimen collected. √ – Recorded sighting from WildSingapore.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Corynactis sp.</th>
<th>Discosoma nummiforme</th>
<th>Discosoma nummiforme</th>
<th>Discosoma sp.</th>
<th>Platyzoanthus mussoides</th>
<th>Platyzoanthus mussoides</th>
<th>Rhodactis inchoata</th>
<th>Rhodactis indosinensis</th>
<th>Rhodactis indosinensis</th>
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<td></td>
<td>Ball-tip</td>
<td>Beaded</td>
<td>Stubby</td>
<td>Striped</td>
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<td>Ridged</td>
<td>Frilled</td>
<td>Carpet</td>
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<td>Submerged reefs off Semakau**</td>
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<td>8</td>
<td>14</td>
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* Terumbu Pempang Darat, Terumbu Pempang Tengah and Terumbu Pempang Laut.
** Terumbu Raya, Beting Bemban Besar, Terumbu Bemban and Terumbu Semakau.
*** Salu, Sudong, Pawai, Senang, Biola and submerged reefs in the area.
morphotype, was the least frequently sighted species with only two reported intertidal localities (Pulau Hantu and the submerged reefs of Pulau Semakau). All corallimorpharian species were distributed across the southern shores of Singapore. Only the *Corynactis* sp. was recorded at both northern and southern shores. The northern shores are more nutrient-rich and have restricted water flow due to the Johor-Singapore Causeway linking Singapore and Malaysia (Chou et al., 2019). These factors could influence species distribution patterns though more studies have to be conducted to ascertain this.

**SYSTEMATICS**

Order Corallimorpharia Carlgren, 1940

Family Discosomidae Verrill, 1869

Genus *Discosoma* Rüppell & Leuckart, 1828

*Discosoma nummiforme* Rüppell & Leuckart, 1828

(Fig. 3)

**Material examined.** 1 specimen (syntype), NRS1156x1, Red Sea; 4 specimens, ZRC.CNI.1353 (REL342, REL345), Pulau Hantu, Singapore, coll. R.M. Oh, November 2017 – March 2018; 3 specimens, ZRC.CNI.1354 (REL390), St John’s Island, Singapore, coll. R. M. Oh, November – March 2018.

**Description.** Body disc-shaped. Oral disc with several irregular grooves extending from the centre to the margin. Colour variable, greenish to reddish brown. Outline of mouth round to oval. Diameter up to 20 mm. Column very short, both distal and proximal ends spread outwards. Thin-walled, mesenterial insertions seen as orange lines extending the entire length of column. Discal tentacles poorly developed, appearing as bumps or warts, arranged in radiating lines from the center to the disc margin. Marginal tentacles delicate, closely arranged and bulbous. Mesenteries complete. Cnidom atrichs, holotrichs, microbasic *b*-mastigophores, microbasic *p*-mastigophores, spirocysts (see Carlgren, 1943). Found in groups of at least two or three animals.

**Remarks.** Description follows Rüppell & Leuckart (1828), Haddon (1898), and Carlgren (1943). Globally, species have been recorded in the Red Sea, Tanzania, Indian Ocean, the Ryukyus Archipelago and Cambodia (Fautin, 2009, 2016). This species is found only along the southern shores of Singapore and is commonly known as the ‘stubby’ or ‘beaded’ corallimorpharian (Table 2). The animal adheres
Discosoma sp. (Fig. 4)

Material examined. 4 specimens, ZRC.CNI.1355 (IP444), Sisters’ Islands, Singapore, coll. Y. C. A. Ip, January 2016.

Description. Body column smooth, uniform in colour. Discal tentacles short, radially extending towards the disc margin. Marginal tentacles smaller, more delicate. Both discal and marginal tentacles of similar colour to the disc. Distinct white ridges present on disc extending from the center to the disc margin. Larger tentacles appear to be present on these ridges. Specimens typically found clustered in groups of three or more.

Remarks. This may represent an undescribed species, but more extensive collections in the region will help ascertain its status in relation to other nominal species. Locally, it is commonly known as the ‘striped’ corallimorpharian and have only been found on Singapore’s southern shores (Table 2). No intertidal specimens were observed during our collection work. All specimens examined are of subtidal origin.

Genus Rhodactis Milne Edwards & Haime, 1851

Rhodactis inchoata Carlgren, 1943

(Fig. 5)

Material examined. 1 specimen (syntype), NRS4022x5, Lien Chien Tourane, Vietnam; 1 specimen (syntype), NRS4021x2, Macclesfield Bank, South China Sea; >3 specimens, ZRC.CNI.1356 (REL344, REL375, REL389), Pulau Hantu, Singapore, coll. R.M. Oh, August 2017 – March 2018; 2 specimens, ZRC.CNI.1349 (REL347), Sisters’ Islands, Singapore, coll. R.M. Oh, August 2017 – March 2018; 1 specimen, ZRC.CNI.1350 (REL372, REL373, REL374), St John’s Island, Singapore, coll. R.M. Oh, August 2017 – March 2018.

Description. Body oval. Pedal and oral disc very wide. Column reddish-brown to dark purple. Some specimens with longitudinal lines extending from the entire column, of similar colour to discal tentacle tips. Oral disc light green to brown, outline irregular oval, overall flat, with radial ridges along its margin. Discal tentacles arranged in circles of increasing radius, innermost are low star-shaped bumps as depicted in Carlgren (1943), light brown to green. Marginal tentacles simple and more developed. Colours include pale yellow with green tips. No clear space separating marginal from discal tentacles. Central mouth, overall oval and slightly raised. Mesenteries complete. Cnidom atrichs, holotrichs, microbasic b-mastigophores, microbasic p-mastigophores (see Carlgren, 1943).

Remarks. Description follows Carlgren (1943). This species has been recorded in Vietnam and the South China Sea (Fautin, 2009, 2016). Locally, the species is known as the ‘frilled’ corallimorpharian. Specimens only found on the southern shores of Singapore (Table 2). Both intertidal and subtidal specimens are usually attached to coral rock. On the intertidal they are often observed close to one another in dense groups, sometimes with 10 or more individuals. Morphological identification is incongruent with molecular phylogenetic placement for two specimens collected, REL179 and REL344, which have distinct innermost discal tentacles as star-shaped bumps but are placed within the Rhodactis indosinensis clade.

Rhodactis indosinensis Carlgren, 1943

(Fig. 6)


Description. Oral and pedal discs outline oval and wide. Column low, with longitudinal ridges extending the entire column. Column colour from purple to brown. Marginal tentacles well developed. Discal tentacles increasingly tightly to coral rock and is difficult to dislodge without damaging the column.
branched and closely arranged near the oral disc margin. Discal tentacles may cover the entire disc surface, appearing robust despite possessing thin walls. Mesenterial filaments may spill out through discal tentacles. Marginal and discal tentacles brown, usually similar in colour to oral disc. Tips of tentacles often a lighter shade or different in colour (e.g., green). Central mouth elevated, oval, with simple discal tentacles on the mound. Light radial lines corresponding to the mesenterial insertions are seen, extending from the mouth, to the disc margin. Marginal tentacles connected to these radial lines. Mesenteries complete. Cnidom atrichs, holotrichs, microbasic $h$-mastigophores, microbasic $p$-mastigophores (see Carlgren, 1943).

Remarks. Description follows Carlgren (1943). This species has been recorded in Cambodia, Vietnam, and Japan (Fautin, 2009, 2016). In Singapore, it is known as the ‘carpet’ or ‘white-tip’ corallimorpharian, depending on tentacle colour. Specimens are only found along the southern shores, attached to coral rock in both subtidal and intertidal habitats (Table 2). Morphological identification is incongruent with molecular phylogenetic placement for the specimen REL350, which has well-developed discal tentacles with branched tips but is placed in the Rhodactis inchoata clade.

Genus Platyzoanthus Saville-Kent, 1893

Platyzoanthus mussoides Saville-Kent, 1893
(Fig. 7)


Description. Body column low, disc shape highly irregular. Individuals may have more than one oral centre. Tentacles usually exceptionally short, slightly lobate, covering most of the oral disc. Colour of the disc light to reddish brown. Tentacles similar colour as oral disc. Species grows in clusters of overlapping individuals, usually spread across the surface of coral rock.

Remarks. Description follows Saville-Kent (1893). This species was recorded in Queensland, Australia (Fautin, 2009, 2016). Locally, one variant is known as the ‘ridged’ corallimorpharian and previously found on the southern shores (Table 2). The other variant with invaginations on the disc has never been observed prior to this study. Specimens collected in this study are of subtidal origin, and no intertidal specimens have been encountered.
Family Corallimorphidae Hertwig, 1882

Genus Corynactis Allman, 1846

Corynactis sp.

(Fig. 8)


Description. Body smooth, with visible longitudinal white lines spanning the entire column. Body column uniform in colour: purple, rust red, yellow, orange or blue. Body column short. Mouth outline round, elevated at the disc centre. Tentacles arranged in three or more circles of increasing radius around mouth (den Hartog et al., 1993). All tentacles translucent, ending in rounded, beige-coloured acrospheres (Acuña & Garese, 2009). Diameter of tips ranges from 15 to 20 mm. Mesenteries can be complete or incomplete. Often found in groups of three or more individuals.

Remarks. These animals may represent an undescribed species, but more extensive collections in the region will help ascertain its status in relation to other nominal species. Corynactis sp. most closely resembles Corynactis caribbeorum but is differentiated by a uniformly coloured, highly mutable body column lacking a distinct scapulus. Furthermore, Corynactis caribbeorum is found in the Caribbean and Atlantic Ocean near north Africa (Fautin, 2016).

Locally, Corynactis sp. is commonly known as the ‘ball-tip’ corallimorpharian from its distinct acrosphere shape, and is widespread in the intertidal habitats of both northern and southern shores in Singapore (Table 2). During low tide, its body column envelops the tentacles and oral disc. Once submerged the body column retracts and tentacles are fully extended. Individuals are identified in the field by its uniformly coloured, mutable body form adhering to common substrata including coral rock and granite pillars, and could be easily removed from the substrata.

DISCUSSION

The integrated use of both morphological and molecular methods has been useful in identifying the corallimorpharians of Singapore. A few uncertainties remain as the phylogenetic results based on the mitochondrial intergenic region (IGR), supported by nuclear ITS, show minor incongruences for the morphospecies in Rhodactis. Nevertheless, from the
initial nine morphotypes identified by citizen scientists, both methods recover four genera represented by six species in this study. We expect that our inferred tree topology would provide insights for ongoing work on taxonomic classification within Corallimorpharia. We also emphasise that this citizen science dataset has been useful in establishing the distribution of corallimorpharians within our short study, thus the viability of using such data in future studies will be discussed.

Combining the distribution data obtained from WildSingapore Fact Sheets (citizen science database) and field surveys, we generated a comprehensive inventory of Corallimorpharia diversity (Fig. 1; Table 2). Individual records from WildSingapore were sorted using images of their external morphology. Sorting was done only by co-author Ria Tan to minimise bias. Even though nine morphotypes were initially described, we found that some morphotypes belong to the same species after a detailed examination of both internal and external morphologies. Most importantly, the descriptions accompanying the image records from WildSingapore aligned with published taxonomic descriptions (Rüppell & Leuckart, 1828; Allman, 1846; Saville-Kent, 1893; Carlgren, 1943). Overall, we found the citizen science database to be a useful introduction to morphotypes present in Singapore, thus accelerating our species identification process, which relied on published taxonomic work and molecular phylogenetic analysis. The integrated inventory of morphotype distributions also can serve as a useful reference for future work; for example more strategic field collections particularly at localities where specific morphotypes have been sighted. More broadly, results from this study underscore the value of robust citizen science data and their relevance to taxonomic and biodiversity research (Cohn, 2008; Theobald et al., 2015).

Our phylogenetic reconstruction shows that Corynactis forms a monophyletic group, distinct from Pseudocorynactis, thus supporting a recent morphology-based argument for the restoration of Pseudocorynactis as a standalone genus (Ocãna et al., 2017). We note that the Corynactis clade is not well-supported (bootstrap <50, posterior probability <0.5). IGR sequences of Corynactis obtained here are also very distinct from the previously sequenced Corynactis californica, as shown by the long branch length subtending the Singapore clade (Appendix 1). No published records of Corynactis species have been found in the Southeast Asian region (den Hartog, 1997; Fossà & Nilsen, 1998), and the nearest locality where Corynactis has been sighted (Corynactis globulifera) is Queensland, Australia (Fautin, 2009, 2016). We thus surmise that Corynactis sp. examined in this study could be distinct from previously known Corynactis species. If the weak grouping of Corynactis here is not supported by other genes and morphological data, the phylogenetic distinction observed for the Singapore clade

Fig. 7. Platyzoanthus mussoides. A, B, REL203 in situ; C, D, REL240 in situ. Scale bar = 1 cm.
of Corynactis sp. may warrant the establishment of a new genus. Nevertheless, the current sampling of Corynactis is insufficient for a precise understanding of the relationships among Corynactis, Pseudocorynactis, and other related taxa. Therefore, future studies using different molecular markers and incorporating a greater diversity of Corynactis species could help resolve the phylogenetic relationships among these putative genera.

Our analyses of the spatial distribution of species in Singapore reveals a distinct pattern between Corynactis sp. and other corallimorpharians—the former is found at the northern sites and extends to the southern shores, while the other species are not restricted to any particular sites in the south but do not extend to the northern shores (Table 2). Corynactis sp. is furthermore very abundant at sites where they are found, hence the finding that they form a distinct lineage emphasises our limited understanding of their systematics.

Genus Platyzoanthus consists of the single species P. mussoides. Interestingly, it is nested within Discosoma, and forms a well-supported sister group to Discosoma sp. and two previously sequenced Discosoma (excluding D. nummiforme). Discosoma nummiforme, Platyzoanthus mussoides, and Discosoma sp. are clustered together in a well-supported clade (bootstrap supports and posterior probability of 100/1/99). Taxonomically, these taxa together with Rhodactis spp. belong to the same family, Discosomidae (Verrill, 1869), thus corresponding with the observed tree topology. The nesting of Platyzoanthus within Discosoma suggests that Discosoma sp. from Singapore should be placed in a separate genus if Platyzoanthus were to be retained. However, the taxonomy of Discosoma species has been poorly resolved (den Hartog, 1980). Clearly, improved sampling of Discosoma is necessary to ascertain if and how the classification should be revised. Such increased sampling will also help determine the morphological relationships among described species and the yet-unidentified Discosoma sp.

Clades representing Rhodactis inchoata and R. indosinensis have weak bootstrap support and posterior probability values of <50 and <0.5 respectively. Furthermore, our phylogenetic results showed three specimens not recovered in their expected clades. REL350 was found within the R. inchoata clade on both IGR and ITS trees, despite observable, well-developed, branched discal tentacles—a distinguishing character separating R. indosinensis from R. inchoata. Conversely, REL179 and REL344 were in the R. indosinensis clade despite visible ‘star-shaped’ discal tentacles barely reaching above the surface of the disc characteristic of R. inchoata. A possible explanation is that both species are exhibiting morphological plasticity, although this has almost never been studied among corallimorpharians. The
culturing of these species from the juvenile stage in diverse environmental conditions or reciprocal transplant experiments may help uncover morphological shifts that account for the phylogenetic patterns found here.

In conclusion, morphological identification and phylogenetic analyses of nine morphotypes observed in Singapore have shed light on corallimorpharian species diversity and distribution. Data collected during this study combined with citizen science data yield an updated inventory of six distinct species, including two potentially undescribed taxa. Additional morphotypes and species may yet exist in both intertidal and subtidal habitats, considering the inconspicuousness of corallimorpharians. Finding and recognizing these obscure organisms to build a comprehensive and precise inventory of their diversity and distribution would require more sampling time and effort in the future.

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APPENDICES

Appendix 1. Maximum likelihood phylogram of mitochondrial intergenic region (IGR) sequences (see Fig. 2).
Appendix 2. Maximum likelihood phylogram of nuclear internal transcribed spacer (ITS) sequences. Bootstrap support values are indicated on branches.