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Zooxanthellate, Sclerite-Free, and Pseudopinnuled Octocoral *Hadaka nudidomus* gen. nov. et sp. nov. (Anthozoa, Octocorallia) from Mesophotic Reefs of the Southern Ryukyus Islands

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Abstract: Shallow water coral reefs are the most diverse marine ecosystems, but there is an immense gap in knowledge when it comes to understanding the diversity of the vast majority of marine biota in these ecosystems. This is especially true when it comes to understudied small and cryptic coral reef taxa in understudied ecosystems, such as mesophotic coral reef ecosystems (MCEs). MCEs were reported in Japan almost fifty years ago, although only in recent years has there been an increase in research concerning the diversity of these reefs. In this study we describe the first stoloniferous octocoral from MCEs, *Hadaka nudidomus* gen. nov. et sp. nov., from Iriomote and Okinawa Islands in the southern Ryukyus Islands. The species is zooxanthellate; both specimens host *Cladocopium* LaJeunesse & H.J.Jeong, 2018 (formerly *Symbiodinium* 'Clade C') and were collected from depths of ~33 to 40 m. Additionally, *H. nudidomus* gen. nov. et sp. nov. is both sclerite-free and lacks free pinnules, and both of these characteristics are typically diagnostic for octocorals. The discovery and morphology of *H. nudidomus* gen. nov. et sp. nov. indicate that we still know very little about stoloniferous octocoral diversity in MCEs, their genetic relationships with shallower reef species, and octocoral–symbiont associations. Continued research on these subjects will improve our understanding of octocoral diversity in both shallow and deeper reefs.

Keywords: *Cladocopium*; cryptofauna; marine biodiversity; mesophotic coral reef environments (MCEs); Octocorallia; stoloniferous octocorals; Symbiodiniaceae; taxonomy

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1. Introduction

Coral reefs make up only 0.2% of the earth's ocean but are estimated to harbor a quarter of all marine species [1,2] and are the most diverse marine ecosystems on the planet. Unfortunately, these diverse marine communities are also one of the most threatened [3–6]. The 'hotspot' concept, a term used to mark a relatively restricted geographic area accommodating exceptionally high concentrations of biodiversity and endemism [7–9] has highlighted the wealth of species that are at risk and how localized such areas of richness can be [10]. However, there are vast gaps in knowledge concerning the majority of marine biota [11,12], making the recognition of biodiversity geographic patterns and hotspots questionable [13,14], as priorities identified for one taxon may not reflect the diversity of other taxa [14,15]. This is especially true for understudied localities and environments, such as understudied coral reef ecosystems.

Mesophotic coral reef ecosystems (MCEs) occur at depths below 30–40 m to 100 m or deeper in tropical and sub-tropical regions [16–19]. MCEs are considered understudied, as their depths make them difficult to access via normal SCUBA technology, yet too shallow for most submersibles [19,20]. However, research regarding MCEs has increased in recent years, along with calls for increased awareness and protection of these ecosystems [21]. Additionally, studies have demonstrated that MCEs can accommodate high levels of endemism [19,22] and harbor distinct geographical communities [19].

The coral reefs of southern Japan are at the top of the list in terms of global marine conservation priority, when considering the region's high levels of multi-taxon endemism and the high risk of biodiversity loss due to overexploitation and coastal development [23]. The Ryukyus Islands (RYS), i.e., Ryukyu Archipelago, encompass the southernmost region of Japan and include islands of different geological formations, ages, and sizes [24,25]. The surrounding waters and coral reefs fringing the islands are strongly influenced by the warm water brought from tropical areas around the Philippine islands by the Kuroshio Current, which flows towards the north along the west side of the island chain [24–26], extending warm water conditions northerly. As such, the RYS experience higher sea temperatures compared to other areas at similar latitudes, such as eastern Australia [27,28], thus creating unique coral reef conditions. Serious taxonomic and geographic biases are present in marine biodiversity research in the RYS. Most work in the RYS has been conducted on the phyla Pisces, Crustacea, and Cnidaria, with the majority of research on hermatypic hard corals (Scleractinia) and, surprisingly, far less work on other commercially important groups such as Echinodermata and Mollusca, as well as on other understudied small and cryptic coral reef taxa [25].

One such understudied small and cryptic group are octocorals belonging to the subordinal group, Stolonifera. Stoloniferan octocorals are characterized by having relatively simple colony growth forms, where the polyps are united basally by ribbon-like stolons, instead of being embedded side by side within a common coenenchymal mass [29–31]. There are seven families that are considered to belong to Stolonifera: Acrossotidae Bourne, 1914; Arulidae McFadden & Van Ofwegen, 2012; Clavulariidae Hickson, 1894; Coelogorgiidae Bourne, 1900; Cornulariidae Dana, 1846; Pseudogorgiidae Utinomi & Harada, 1973; and Tubiporidae Ehrenberg, 1828. The most speciose as well as the most studied family is Clavulariidae, which comprises approximately 30 genera and over 60 species. Until recently, all other families are all either monospecific or monogeneric, with no more than a few described species; recent studies have additionally introduced new genera and species for Arulidae [32,33], which is the most recently erected family.

Stoloniferous octocorals often have inconspicuous small colonies and polyps, which makes them hard to detect [32–34]. There are critical gaps that remain in the understanding of the functional and ecological significance of octocoral–zooxanthellae symbioses [35]. To date, only a handful of data are available on stoloniferous octocoral–symbiont relationships, which all concern members of the speciose genus *Clavularia* Blainville, 1830. *Clavularia* spp. from Australia all hosted *Durusdinium* LaJeunesse, 2018 [36,37]. On one other occasion, a single *Clavularia* sp. specimen from the Caribbean was found to host *Durusdinium* [38].

Obligate mutualistic symbioses play important roles in extending available energy resources and thus potentially influence biodiversity on reefs [36,39]; however, stoloniferous octocorals and their host–symbiont associations are a relatively underexamined fauna in the RYS, particularly from within MCEs. In this study we formally describe the zooxanthellate, sclerite-free, and pseudopinnuled octocoral *Hadaka nudidomus* gen. nov. et sp. nov. from MCEs around Okinawa and Iriomote Islands.

2. Materials and Methods

2.1. Specimen Collection and Morphological Examinations

One specimen was collected from one location each around Okinawa (August 2017; 26.856412 N, 128.245093 E) and Iriomote (December 2016; 24.370413 N, 123.736428 E) Islands (Figure 1). The specimens were found at depths of 33 and 40 m, respectively, by means of SCUBA (atmospheric air) and were preserved in 70–90% ethanol and subsamples in 95% ethanol. The current study is part of an ongoing survey of mesophotic and deep reef work. Vouchers and type material were deposited at the National Museum of Nature and Science (NSMT), Tokyo, Japan (Table 1). Both specimens were examined for the presence of sclerites by dissolving entire polyps and stolons in 4% hypochlorite (household bleach). Additionally, to visualize polyp tentacles and pseudopinnules, polyps were fixed in 20% formalin and embedded in methylene blue (1%).

2.2. DNA Extraction, Amplification, and Sequencing

DNA was extracted from polyps using a DNeasy Blood and Tissue kit (Qiagen, Tokyo, Japan). PCR amplification and sequencing were performed for four markers, of which three were mitochondrial (cytochrome c oxidase subunit I (COI), the MSH homologue mtMutS, and subunit ND6) and the fourth was the nuclear ribosomal marker (28S rDNA). Additionally, for Symbiodiniaceae, the nuclear internal transcribed spacer (ITS) region of ribosomal DNA was amplified. Protocols in [34] were followed and PCR products were treated with Exonuclease I and alkaline phosphate (shrimp) and sent for bidirectional sequencing on an ABI 3730XL (Fasmac, Kanagawa, Japan). Sequences were assembled and edited using Geneious R11 [40] and BioEdit [41]. COI, mtMutS, and ND6 were checked for introns, exons, and stop-codons in AliView [42].

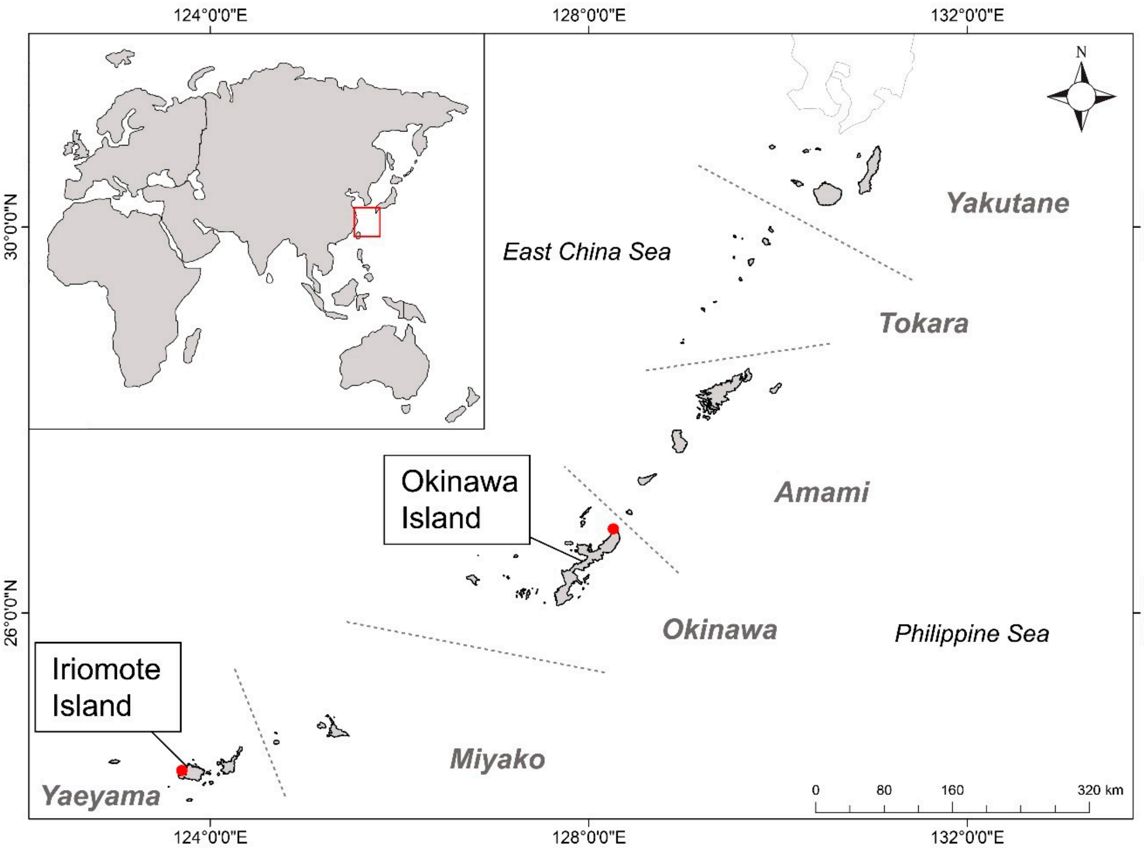

Figure 1. Map of the Ryukyus Islands (RYS), with the six island group divisions (grey dotted lines) and the two dive locations where *Hadaka nudidomus* gen. nov. et sp. nov. specimens were found (red dots) at Iriomote (NSMT-Co 1681, holotype) and Okinawa (NSMT-Co 1682, paratype) Islands.

Table 1. Overview of information on octocoral specimens collected from mesophotic coral reef ecosystems (MCEs) at Iriomote and Okinawa Islands, Okinawa Prefecture, Japan, including GenBank accession numbers and locality. Catalogue number: NSMT = National Museum of Nature and Science, Tokyo, Japan; n.a. = not available.

Family	**Species**	**Catalogue Number**	**Locality/GPS (DMS)**	**Symbiodiniaceae Genus**	**GenBank Accession Numbers**				
					28S rDNA	**COI**	**mtMutS**	**ND6**	**ITS**
Clavulariidae	*Hadaka nudidomus* gen. nov. et sp. nov.	NSMT-Co 1681 (holotype)	NE Uchibanare, Iriomote Isl./24.370413 N, 123.736428 E	*Cladocopium*	MN488601	MN488603	MN488605	n.a.	MN488607
	Hadaka nudidomus gen. nov. et sp. nov.	NSMT-Co 1682 (paratype)	Entrance Hedo Dome, Cape Hedo, Okinawa Isl./26.856412 N, 128.245093 E	*Cladocopium*	MN488602	MN488604	n.a.	MN488606	MN488608

2.3. Molecular Phylogenetic Analyses

Multiple sequence alignments were performed using MAFFT 7 [43] and coding markers were aligned using MACSE [44] under default parameters. The phylogenetic position of the collected specimens ($n = 2$) was determined by aligning the consensus sequences for markers 28S rDNA, COI, and mtMutS to a reference dataset of 124 octocoral genera, including *Cornularia pabloi* and *Cornularia cornucopiae* as outgroup (total $n = 144$), as used in Lau and Reimer [33]. This resulted in alignments of 887 bp for 28S rDNA, 717 bp for COI, and 714 bp for mtMutS, and a total concatenated three-marker dataset of 2318 bp. The separate markers were run in ML analyses, to check for contamination and congruency (Supplementary Materials Figures S1–S3).

A separate phylogenetic analysis was made to examine the lower level phylogenetic relationships of the collected mesophotic specimens, using a concatenated four-marker dataset. The concatenated four-marker dataset resulted in an alignment of 2670 bp (total $n = 12$). A total of seven reference species were included in the analysis, which clustered in nearby clades with the specimens in the three-marker dataset, including *Rhodelinda* sp. and *Telesto* sp. as outgroup. The four separate markers (28S rDNA, 787 bp; COI, 708 bp; mtMutS, 734 bp; ND6, 441 bp) were also run in ML analyses, to check for contamination and congruency (Supplementary Materials Figures S4–S7).

Additionally, ITS sequences from the two specimens were aligned with a total of 25 reference sequences (*Cladocopium* spp. and *Durusdinium* spp.), including *Gerakladium* sp. as outgroup. The resulting dataset comprised 641 bp and a total of 27 sequences and was run in ML analyses (Supplementary Materials Figure S8).

Alignments of the separate markers were concatenated using SequenceMatrix 1.8 [45]. ML analyses were run with RAX-ML 8 [46], using the GTRCAT model. The best ML tree was calculated using the –D parameter. A multi-parametric bootstrap search was performed, which automatically stopped based on the extended majority rule criterion. The Bayesian inference was performed with ExaBayes 1.5 [47] using the GTR substitution model. Four independent runs were run for 10,000,000 generations during which convergence (with a standard deviation of split frequencies < 2%) was reached. Bootstrap supports and posterior probabilities were depicted on the branches of the best ML tree using P4 [48]. The resulting trees were visualized in FigTree 1.4.2 [49]. Additionally, average distance estimations within species and within genera were computed using MEGA X [50] by analyzing pairwise measures of genetic distances (uncorrected *P*) among sequences (Supplementary Materials Tables S1–S3).

3. Systematic Account

Class Anthozoa
Subclass Octocorallia Ehrenberg, 1831
Order Alcyonacea Lamouroux, 1812
Family Clavulariidae Hickson, 1894

3.1. Genus *Hadaka* gen. nov.

Type species: *Hadaka nudidomus* sp. nov. by original designation and monotype.

Diagnosis: Colony with polyps connected through flattened ribbon-like stolons, which are loosely attached to a hard substrate. Polyps retract fully into the calyx, which is cylindrical to conical in shape, narrowing at the base and does not retract fully into the stolon. Tentacles have a wide rachis with a protruding ridge and pseudopinnules of different lengths arranged on either side, giving the polyps feather shaped tentacles. No sclerites. Zooxanthellate.

Remarks: *Hadaka* gen. nov. et sp. nov. shows gross resemblance to *Hanabira* Lau, Stokvis, Imahara & Reimer, 2019 in having a similar polyp shape with feather or petal shaped tentacles and fused pinnules, which can still be distinguished by shallow furrows. *Hadaka* gen. nov. et sp. nov. differs from *Hanabira* in having no sclerites in any part of the colony and having a protruding ridge on the upper side of the tentacle. Genetically, *Hadaka* gen. nov. is well-supported and positioned in a different phylogenetic clade from *Hanabira*. The closest sister taxa of *Hadaka* gen. nov. is *Acrossota* Bourne, 1914, which is also sclerite-free, but morphologically very different; *Acrossota* lacks pinnules completely.

Etymology: From the Japanese word *hadaka* (裸), meaning naked, bare, nude; denoting the absence of two characteristic features of octocorals, sclerites, and free pinnules. Gender: feminine.

http://zoobank.org/39430672-5ADA-4EFF-9F5A-B4076B6B90C0

3.2. Hadaka nudidomus sp. nov.

See Figure 2.

Material examined: All specimens were collected from Okinawa Prefecture, Japan. *Holotype*: NSMT-Co 1681, northeast Uchibanare, Iriomote Island (24.370413 N,123.736428 E), ~40 m depth, 19 December 2016, coll. D. Uyeno. GenBank accession numbers: 28S rDNA, MN488601; COI, MN488603; mtMutS, MN488605. *Paratype*: NSMT-Co 1682, entrance to Hedo Dome, Cape Hedo, Okinawa Island (26.856412 N, 128.245093 E), 33 m depth, 18 August 2017, coll. J.D. Reimer. GenBank accession numbers: 28S rDNA, MN488602; COI, MN488604; ND6, MN488606.

Description: Holotype colony consists of 15 polyps with flattened ribbon-like stolons encrusting a sponge. Polyps can be seen individually or clustered in groups and are spaced apart irregularly, 3 mm to 2 cm in between polyps and clusters. Stolons are 0.5 mm at their narrowest and 1 mm at their widest point. Polyps retract fully into the calyx (~1.8 mm wide and ~3.55 mm in length), which is cylindrical to conical shaped, narrowing at the base, and does not retract fully into the stolon. Expanded polyps are ~4–5 mm diameter in life. Tentacles have a wide rachis with a protruding ridge on the upper side and long pseudopinnules arranged on either side (~24–26 pseudo-pairs), giving the polyps feather shaped tentacles. When stained with methylene blue, the outline of the tentacles can be observed. Structures of the pinnule axis are visible; however, the notches that distinguish the pseudopinnules are not observed in the contour of the tentacle (Figure 2d). No sclerites were found in any parts of the specimens. Polyps are brown in life and yellowish-white in ethanol (Figure 2c). Zooxanthellate.

Morphological variation: There is a difference in color between the polyps of the holotype (NSMT-Co 1681) and paratype (NSMT-Co 1682); the polyps of the holotype are brown with a white oral disc and base of the tentacles and the polyps of the paratype are whitish yellow with a bright blue oral disc (Figure 2a,b).

Distribution: Southwestern Japan, southern Ryukyus Islands, around northern Okinawa Island, and inside the bay of western Iriomote Island in the East China Sea. Specimens were collected from depths of ~33–40 m.

Remarks: The polyps of paratype NSMT-Co 1682 were all used for DNA extraction and sclerite examination, as they were initially thought to be a *Hanabira yukibana* specimen; three fragments of rock with stolon remain. The holotype colony (NSMT-Co 1681) was attached to sponge tissue, but this epibiont is not obligate, as the paratype was attached to rock.

Habitat: The holotype (NSMT-Co 1681) was found attached to sponge on a large piece of coral rubble (>15 cm) lying on a mixed small rubble/soft sediment bottom. The paratype (NSMT-Co 1682) was found on consolidated hard carbonate bottom. Both colonies were on the upward-facing side of the bottom.

Etymology: From Latin *nudus*, meaning naked or bare, and *domus*, meaning home or house; denoting the 'naked' host habitat in which the zooxanthellae reside, as the species is sclerite-free.

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4. Molecular Results

This study added a total of six sequences of *Hadaka nudidomus* gen. nov. et sp. nov. to the public reference database GenBank and no barcodes were available before. For the family Symbiodiniaceae, two *Cladocopium* spp. sequences were added. The phylogenies resulting from the ML analyses of the separate markers (COI, 28S rDNA, mtMutS, ND6) were highly congruent with those from the concatenated alignments for both the three- and four-marker datasets (Supplementary Materials Figures S1–S7). ML and Bayesian analyses for the concatenated datasets yielded almost identical tree phylogenies (Supplementary Materials Figure S9). Sequences of *Hadaka nudidomus* gen. nov. et sp. nov. collected from Okinawa and Iriomote Islands formed a completely-supported clade, containing sclerite-free species only: species of clavulariid genus *Phenganax* Alderslade & McFadden, 2011 and monospecific acrossotid genus *Acrossota* Bourne, 1914 in both the three- and four-marker analyses (Figures 3 and 4).

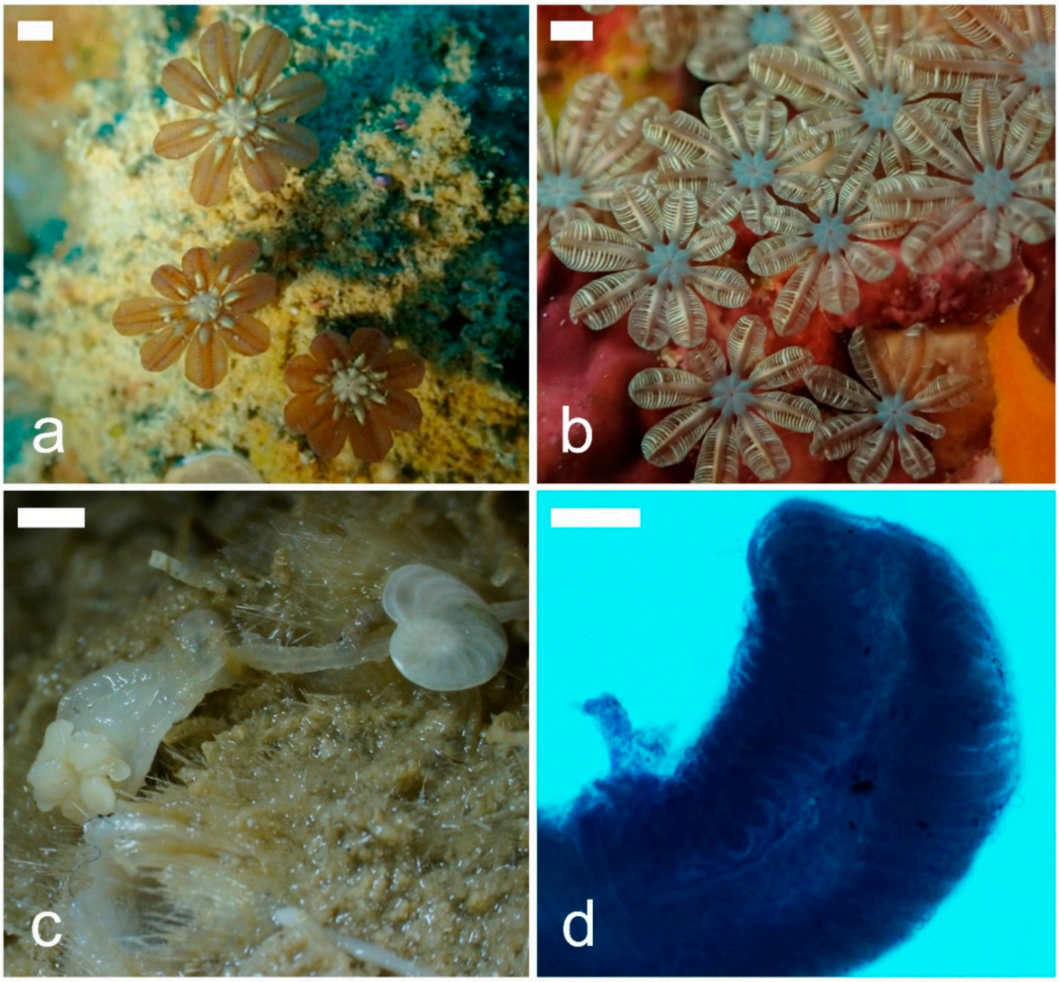

Figure 2. Photographs of *Hadaka nudidomus* gen. nov. et sp. nov.: (**a**) in situ holotype NSMT-Co 1681, scale bar approximately 1 mm; (**b**) in situ paratype NSMT-Co 1682, scale bar approximately 1 mm; (**c**) holotype in ethanol, scalebar 1 mm; (**d**) holotype in methylene blue staining, scale bar 0.1 mm.

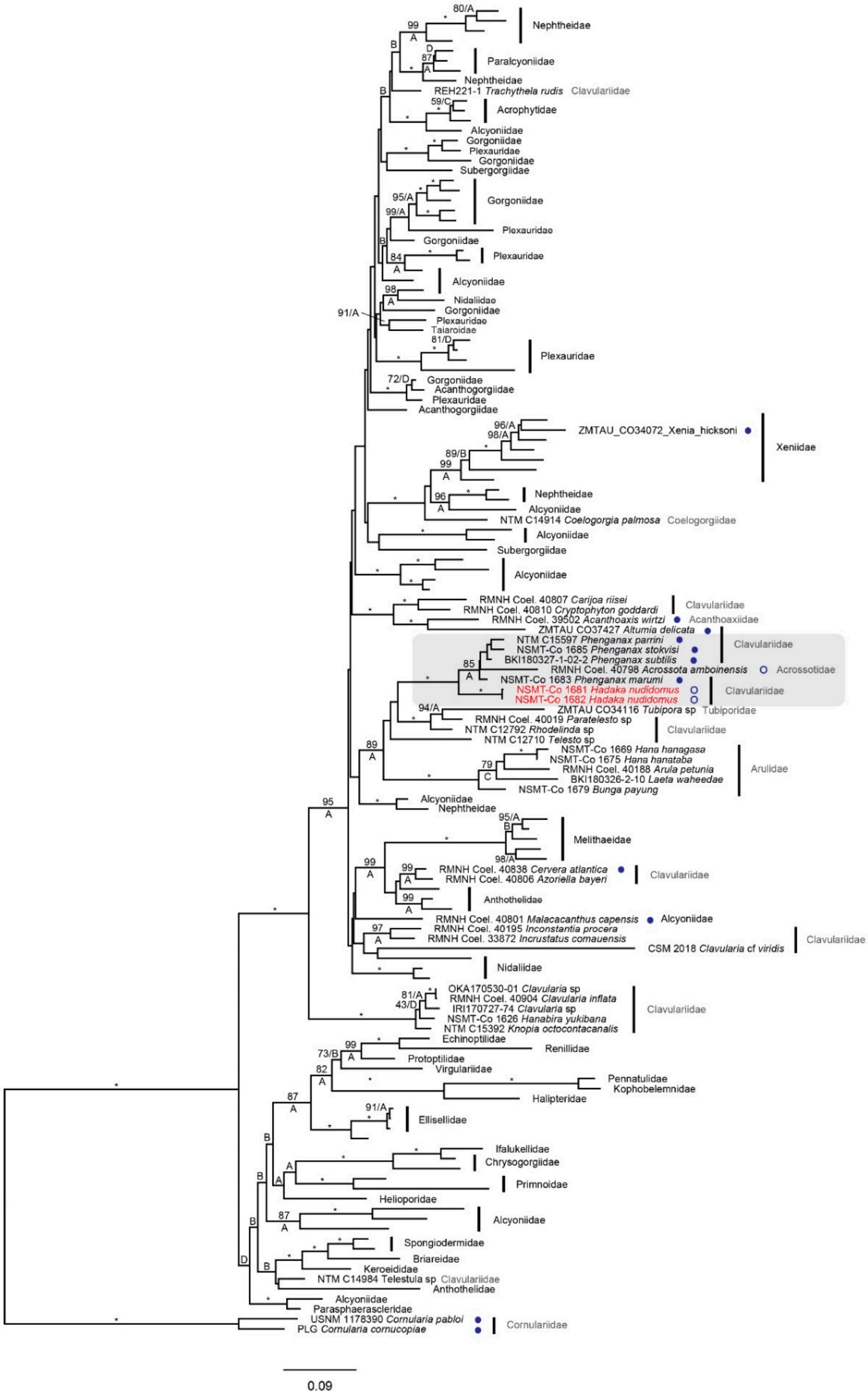

Figure 3. Phylogenetic relationships among 122 octocoral genera (total $n = 144$), including two species, *Hadaka nudidomus* gen. nov. et sp. nov. (highlighted red), collected at Iriomote and Okinawa Islands using the combined 28S rDNA + COI + mtMutS dataset. The best maximum likelihood tree is shown, with values at branches representing bootstrap probabilities (shown when >70%; top/left) and Bayesian posterior probabilities (shown when >0.80; bottom/right; A = 1.00, B = 0.95–0.99, C = 0.90–0.94, D = 0.80–0.89). * represents 100%/1.00 for both analyses. Non-stoloniferous families are shown with family classification only and stoloniferous families are highlighted in grey. Sclerite-free species are indicated with a blue dot. Species that are both sclerite-free and lack free pinnules are indicated with a blue circle. *Cornularia* spp. were used as outgroup.

Hadaka gen. nov. was sister to the remaining clade in the three-marker analyses; however, in the four-marker analyses, *Phenganax* was sister to *Hadaka* gen. nov. and *Acrossota*. Nonetheless, in both phylogenies, the two specimens of *Hadaka* gen. nov. formed a completely-supported clade.

Additionally, genetic distances gave further support to phylogenetic affinities and morphological features justifying the establishment of a new genus. Between-genus distances (*Hadaka* compared to *Acrossota* and *Phenganax*) for COI were 2.52–2.54% and 7.69–13.33% for mtMutS, which are well above the intergeneric range for octocorals [51]. Additional comparisons between *Hadaka nudidomus* sp. nov. specimens, *Acrossota amboinenesis* Burchardt, 1902 and *Phenganax* spp.; *Phenganax parrini* Alderslade & McFadden, 2011, *Phenganax marumi* Lau & Reimer, 2019, *Phenganax subtilis* Lau & Reimer, 2019, *Phenganax stokvisi* Lau & Reimer, 2019, also resulted in ranges (COI: 2.15–2.97%, MSH: 7.00–13.33%) that indicated that *Hadaka* gen. nov. specimens belong to a different genus (Supplementary Materials Tables S1–S3). There were no differences (0%) when comparing genetic distances within the two *Hadaka* specimens, indicating that the specimens are of the same species.

Hadaka nudidomus gen. nov. et sp. nov. specimens were analyzed for the presence of zooxanthellae, and identical sequences of Symbiodiniaceae were found. Both *Hadaka nudidomus* specimens collected from Okinawa and Iriomote Island hosted *Cladocopium* LaJeunesse & H.J.Jeong, 2018 (formerly *Symbiodinium* 'Clade C').

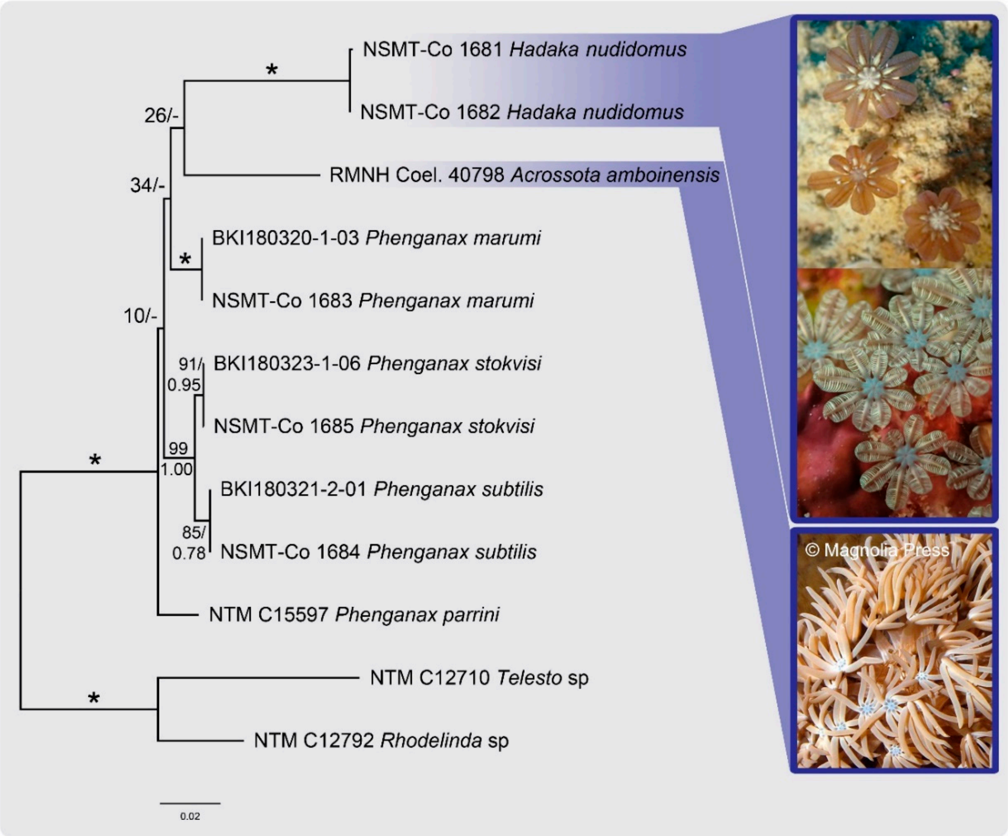

Figure 4. Phylogenetic reconstruction using a four-marker concatenated dataset (28S rDNA + COI + mtMutS + ND6) among *Hadaka nudidomus* gen. nov. et sp. nov., closest sister species *Phenganax* spp. and outgroup specimens *Rhodelinda* sp. and *Telesto* sp. (total $n = 12$). The best maximum likelihood tree is shown, with values at branches representing bootstrap probabilities in percentages (top/left) and Bayesian posterior probabilities (bottom/right). In situ photographs are shown for the two octocoral species that are sclerite-free and lack free pinnules, *Hadaka nudidomus* gen. nov. et sp. nov. and *Acrossota amboinensis*. Photograph credit: in situ image RMNH Coel. 40798 *Acrossota amboinensis*, by Daniel Knop (modified from [52]; reproduced with permission from copyright holder).

5. Discussion

In the three- and four-marker phylogenies, there was disparity in the position of *Hadaka nudidomus* gen. nov. et sp. nov. and *Acrossota amboinensis*. It remains unresolved how these genera and genus *Phenganax* are related to one another. A possible explanation could be that there is no sufficient signal in the sequences of both *Hadaka nudidomus* gen. nov. et sp. nov. and *Acrossota amboinenesis* due to the fact that the closest relatives for these genera are yet to be discovered.

Morphologically, there was a difference between the coloration of the polyps of the holotype and paratype found at Iriomote and Okinawa Islands, respectively. The differences in coloration suggested that perhaps the specimens hosted different members of Symbiodiniaceae. However, both specimens hosted genus *Cladocopium* and thus no biogeographical distinction in Symbiodiniaceae was observed. Members of *Cladocopium* spp. are known to be adapted to a wide range of temperatures and irradiances [53], which would be expected from MCEs, where irradiances are not only subject to seasonal variations but are already reduced.

Hadaka nudidomus gen. nov. et sp. nov is the first zooxanthellate stoloniferous octocoral described from mesophotic depths. Only one other zooxanthellate octocoral, an alcyoniid species, *Sinularia mesophotica* Benayahu, McFadden, Shoham & van Ofwegen, 2017, has been explicitly described from mesophotic depths [54]. However, it was not further specified which genus or species of Symbiodiniaceae was hosted by *S. mesophotica* and therefore, we cannot yet hypothesize differences of zooxanthellae hosted by octocorals from MCEs.

Nonetheless, another recent study has shown that there are geographical differences in the genera of Symbiodiniaceae in *Hanabira yukibana* Lau, Stokvis, Imahara & Reimer, 2019 from shallow coral reefs, as specimens found from Okinawa Island hosted *Cladocopium* while *Durusdinium* LaJeunesse, 2018 was hosted in specimens from Iriomote Island. However, in this previous study, similar to the current study, no consistently different patterns of polyp coloration related to symbiont associations were observed [34]. To this end, finer-scale examinations of Symbiodiniaceae using faster-evolving DNA markers [55] may reveal patterns yet unseen.

Hadaka nudidomus gen. nov. et sp. nov. is the second species within Octocorallia after *Acrossota amboinensis* that has no sclerites in any part of the colony and also no free pinnules; both species are taxonomically placed within family Clavulariidae. *Acrossota amboinensis* differs from *Hadaka nudidomus* gen. nov. et sp. nov. in colony form, polyp morphology, and habitat (Figure 4); *A. amboinensis* does not have pseudopinnules, but instead lacks pinnules completely and has, so far, not been found at mesophotic depths. When comparing *A. amboinensis* to *Phenganax* spp. there are also distinct morphological differences; all *Phenganax* species have free pinnules and have completely different polyp and tentacle shapes. Nonetheless, the genera *Acrossota* and *Phenganax* are phylogenetically closely related. In a recent study, the phylogenetic topology for these genera was different from that generated in the current study [33], in which *Acrossota* is placed basally to all *Phenganax* species. As a result of the unresolved phylogenetic location of *Acrossota* within Clavulariidae, while it is clear these three genera are distinct, it remains unclear how *Hadaka* and *Phenganax* are related to *Acrossota*.

Moreover, it can be concluded that several octocoral species lack both sclerites and free pinnules, and thus, that such features are not completely rare in octocorals, which raises important implications for the definition of subclass Octocorallia, as sclerite characterization and the presence of pinnated tentacles are two of the major diagnostic features of the group [52,56,57].

It is clear that more species diversity data from many marine regions are needed before we can state with certainty that the southern Ryukyus harbor high levels of stoloniferous octocoral diversity and endemism, but at least it can be said that this region potentially harbors many undiscovered species, not only in shallow coral reefs [33,34], but also among the many unexplored MCEs in this region.

Recent studies have shown that MCEs harbor distinct and independent biological communities when compared to shallower reefs [21]. MCEs are not only affected by anthropogenic and natural impacts as are shallow reefs but have seldom been the focus of specific conservation efforts [21,58].

Thus, researchers have only begun to scratch the surface of what we know about mesophotic marine life [21,58,59], including information on stoloniferous octocoral diversity and octocoral–zooxanthellae relationships in MCEs. The discovery of *Hadaka nudidomus* gen. nov. et sp. nov. and other recent discoveries [54,57] emphasize the need for continued studies on MCE octocoral diversity, as undescribed species may disappear before we have the opportunity to discover and study them [21].

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/11/10/176/s1, Figure S1: Maximum Likelihood phylogeny reconstruction of 28S rDNA gene region of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and octocoral references from 123 genera, including outgroup *Cornularia* spp., Figure S2: Maximum Likelihood phylogeny reconstruction of COI gene region of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and octocoral references from 123 genera, including outgroup *Cornularia* spp., Figure S3: Maximum Likelihood phylogeny reconstruction of mtMutS gene region of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and octocoral references from 123 genera, including outgroup *Cornularia* spp., Fiugre S4: Maximum Likelihood phylogeny reconstruction of 28S rDNA gene region of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and five octocoral references (*Phenganax* spp., *Acrossota amboinensis*), and outgroup (*Telesto* sp., *Rhodelinda* sp.)., Figure S5: Maximum Likelihood phylogeny reconstruction of COI gene region of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and five octocoral references (*Phenganax* spp., *Acrossota amboinensis*), and outgroup (*Rhodelinda* sp.)., Figure S6: Maximum Likelihood phylogeny reconstruction of mtMutS gene region of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and four octocoral references (*Phenganax* spp., *Acrossota amboinensis*), and outgroup (*Telesto* sp.)., Figure S7: Maximum Likelihood phylogeny reconstruction of ND6 gene region of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and two octocoral references (*Phenganax* spp.)., Figure S8: Maximum likelihood phylogenetic reconstruction of gene region ITS of Symbiodiniaceae hosted by *Hadaka nudidomus* gen. nov. et sp. nov. specimens from Okinawa and Iriomote Islands (Japan) and reference taxa *Durusdinium* sp. (= former *Symbiodinium* 'Clade D', n = 14) and *Cladocopium* sp. (former Symbiodinium 'Clade C', n = 10) and outgroup sister taxa, *Gerakladium* sp. (= former *Symbiodinium* 'Clade G') as used in Lau et al (2019)., Figure S9: Bayesian inference phylogeny reconstruction of the combined 28S rDNA+COI+mtMutS gene regions of *Hadaka nudidomus* gen. nov. et sp. nov. from Okinawa and Iriomote Islands (Japan) and octocoral references from 123 genera, including outgroup *Cornularia* spp., Table S1: Number of base differences per site from averaging over all sequence pairs between stoloniferous octocoral genera (*Hadaka* gen. nov., *Phenganax*, *Acrossota*) is shown (p expressed as percentage) for COI and mtMutS gene regions. Standard error estimates (S.E.) are shown above the diagonal. Analysis involved 9 and 6 nucleotide sequences for COI and mtMutS, respectively. All positions containing gaps and missing data were eliminated. There were totals of 708 and 734 positions in the final dataset for COI and mtMutS, respectively. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)., Table S2: Number of base differences per site from averaging over all sequence pairs between stoloniferous octocoral taxa (*Hadaka nudidomus* gen. nov. sp. nov., *Phenganax* spp., *Acrossota amboinensis*) is shown (p expressed as percentage) for gene regions COI and mtMutS. Standard error estimates (S.E.) are shown above the diagonal. Analysis involved 7 nucleotide sequences for both COI and mtMutS. All positions containing gaps and missing data were eliminated. There were totals of 717 and 881 positions in the final dataset for COI and mtMutS, respectively. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)., Table S3: Estimates of average evolutionary divergence over sequence pairs within stoloniferous octocoral genera (*Hadaka* gen. nov., *Phenganax*, *Acrossota*, *Rhodelinda*, *Telesto*) for gene regions COI and mtMutS. The numbers of base differences per site from averaging over all sequence pairs within each group (d) are shown (p expressed as percentage). Standard error estimates (S.E.) are shown in the second column and were obtained by a bootstrap procedure (1000 replicates). Analyses involved 9 and 6 nucleotide sequences for COI and mtMutS, respectively. All positions containing gaps and missing data were eliminated. There were totals of 708 and 734 positions in the final dataset for COI and mtMutS, respectively. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

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