

***Pseudosiderastrea formosa* sp. nov. (Cnidaria: Anthozoa: Scleractinia) a New Coral Species Endemic to Taiwan**

Michel Pichon¹, Yao-Yang Chuang^{2,3}, and Chaolun Allen Chen^{2,3,4,*}

¹Museum of Tropical Queensland, 70-102 Flinders Street, Townsville 4810, Australia

²Biodiversity Research Center, Academia Sinica, Nangang, Taipei 115, Taiwan

³Institute of Oceanography, National Taiwan Univ., Taipei 106, Taiwan

⁴Institute of Life Science, National Taitung Univ., Taitung 904, Taiwan

(Accepted September 1, 2011)

Michel Pichon, Yao-Yang Chuang, and Chaolun Allen Chen (2012) *Pseudosiderastrea formosa* sp. nov. (Cnidaria: Anthozoa: Scleractinia) a new coral species endemic to Taiwan. *Zoological Studies* 51(1): 93-98. *Pseudosiderastrea formosa* sp. nov. is a new siderastreid scleractinian coral collected in several localities in Taiwan. It lives on rocky substrates where it forms encrusting colonies. Results of morphological observations and molecular genetic analyses are presented. The new species is described and compared to *P. tayamai* and *Siderastrea savignyana*, and its morphological and phylogenetic affinities are discussed.
<http://zoolstud.sinica.edu.tw/Journals/51.1/93.pdf>

Key words: *Pseudosiderastrea formosa* sp. nov., New species, Scleractinia, Siderastreid, Western Pacific Ocean.

A siderastreid scleractinian coral was collected from several localities around Taiwan and nearby islands, where it is relatively rare. The specimens present some morphological similarities with *Pseudosiderastrea tayamai* Yabe and Sugiyama, 1935, the only species hitherto known from that genus, and with *Siderastrea savignyana* Milne Edwards and Haime, 1849, which is the sole representative in the Indian Ocean of the genus *Siderastrea* de Blainville, 1830. In order to ascertain its taxonomic position, morphological observations were carried out on a suite of 33 specimens at the Museum of Tropical Queensland, Townsville, Australia and at the Biodiversity Research Center, Academia Sinica, Taipei, Taiwan. Molecular phylogenetic analyses were also conducted at the Biodiversity Research Center. The results presented below indicate that these specimens belong to a new species of

Pseudosiderastrea, described as *P. formosa* sp. nov.

MATERIAL AND METHODS

Specimens were collected by scuba diving at Wanlitung (21°59'48"N, 120°42'10"E) and the outlet of the 3rd nuclear power plant (21°55'51.38"N, 120°44'46.82"E) on the southeastern coast of Taiwan in Kenting National Park, Chi-Fai (23°7'0.59"N, 121°23'49.58"E) in Taitung County, and at Yeyu (22°3'1"E, 121°30'35") at Orchid I. (*Lanyu* in Chinese). Specimens for morphological studies were bleached to remove soft tissues by dipping them in household bleach (sodium hypochlorite) for 24 h. They were then rinsed with fresh water and thoroughly dried. Morphological observations were carried out using a Leica

*To whom correspondence and reprint requests should be addressed. E-mail: michel.pichon@bigpond.com; cac@gate.sinica.edu.tw

MX8 stereomicroscope, equipped with an ocular graticule. Scanning electron microscopy (SEM) was performed at James Cook Univ., Townsville, Australia on a JEOL 5410LV instrument.

Samples for the molecular phylogenetic analyses were collected from Bocas del Toro, Panama (*S. radians* and *S. siderea*); Isla Uraba, Panama (*S. glynni*); Brazil (*S. stellata*); Kiunga, Kenya (*S. savignyana*); and Kaohsiung (*P. tayamai*) and Wanlitung, Taiwan (*P. formosa* sp. nov.). For each sample, a piece of about 5 × 5 cm was stored in a modified guanidine solution or 75% ethanol. DNA extraction methods were as specified by Fukami et al. (2004). Cytochrome (Cyt) *b* sequences were amplified by a polymerase chain reaction (PCR) with the primer set, AcCytbF (5'-GCCGTCTCCTTCAAATATAAG-3') and AcCytbR (5'-AAAAGGCTCTTCTACAAC-3') (Fukami et al. 2008), with the following PCR conditions: 94°C for 2 min, followed by 35 cycles at 94°C for 45 s, 50°C for 30 s, and 72°C for 60 s, and ending with a final phase at 72°C for 10 min. PCR products were directly sequenced.

Sequences were aligned using codons, and their genetic distances were calculated using MEGA5 (Tamura et al. 2011). Cyt *b*, a highly variable mitochondrial coding region in *Siderastrea*, was selected to analyze the divergence of *Pseudosiderastrea* specimens and their evolutionary status with the closely related genus, *Siderastrea* (Fukami et al. 2008). Using the final dataset, which contained 16 Cyt *b* sequences of 771 base pairs (bp), the best fitting evolutionary models were determined by the Akaike information criterion (AIC) test in ModelTest (Posada et al. 1998). A phylogenetic analysis was performed using PhyML 3.0 (Guindon et al. 2010) for maximum-likelihood (ML) and MrBayes (Ronquist et al. 2003) for Bayesian inference (BI), under the GTR+I model of DNA evolution. The ML was performed using Shimodaira and Hasegawa (SH-like) branch support with 1000 bootstrap replicates. Sequences obtained from this study were submitted to GenBank with accession nos. JN600483-98.

For the BI, 6 runs with 5 × 10⁶ generations each were calculated, while topologies were saved every 100 generations. One-fifth of the 50,000 topologies were discarded as burn-in, and the remaining ones were used to calculate posterior probabilities.

RESULTS

Family Siderastreidae Vaughan and Wells, 1943 Genus *Pseudosiderastrea* Yabe and Sugiyama, 1935

Pseudosiderastrea formosa sp. nov.

Synonymy: *Siderastrea savignyana* Dai & Horng 2008, p. 165.

Material examined: Holotype: Museum of Tropical Queensland G 64378 Taiwan, Wanlitung 21°59.85'N, 120°42.22'E. Depth 3 m, Coll. A. Chen, 20 Nov. 2009. Paratypes: Museum of Tropical Queensland. G 64374-7 Taiwan, Wanlitung 21°59.85'N, 120°42.22'E. Depth 3 m, Coll. A. Chen, 20 Nov. 2009.

Other material: Museum of Tropical Queensland. G 64352-64, Taiwan, Lanyu (Orchid I.); G 64365-73 Taiwan, Kenting. Biodiversity Research Center Museum. ASIZC0000958-9, Taiwan, Chi-Fai.

Description: Colony small, thin, and tightly encrusting substratum. Most specimens examined being fragments of colonies up to 5 cm in maximum dimension. Holotypic colony (fragment) 47 × 22 mm (Fig. 1). Growing margins very thin, often showing incomplete calcification of corallite structures. Corallites cerioid and uniform in shape and size within each specimen examined. Corallite shape possibly varying from subcircular to polygonal and even squarish in some specimens. In latter, arrangement of corallites tending to be in linear rows. Corallite size range 1.8-4.4 mm in calicular diameter (mean maximum diameter, 2.8 ± 0.2 mm). Septa wedge-shaped (Fig. 2), and hexamerally arranged in 3 cycles, sometimes part of 4th (S1 and S2 > S3 and S4). Smaller corallites with only 18 septa, whereas larger corallites with up to 34 septa. S3 and S4 tending to curve and flanking S1 and S2, sometimes deeply fused in fossa. Such a fusion pattern never involving more than 3 septa, and rarely present more than twice in any given corallite. A number of corallites completely lacking any septal fusion. Septa slightly exserted, continuous, and convex over corallite edge. Near corallite edge, septa only moderately inclined towards calicular center, and then sloping more steeply towards columellar pit. Septal axial edges bearing conspicuous ornamentation (Fig. 2) composed of 7-10 granules, sometimes flattened transversally (Fig. 3). Septal faces entire and ornamented with small, pointed granules (Fig. 3). Fossa up to 2 mm deep, containing a well-developed, convex, massive columella. Columella

circular and up to 1 mm in diameter (average diameter, 0.8 mm) sometimes reaching up to 1/3 of corallite diameter (Fig. 4). However, some corallites with a slightly elongate columella, composed of 2-4 smooth elements. Columella often visible below oral disc in live specimens. Wall solid and similar in thickness to septal outer edge (0.3 mm). Synapticular ring absent within corallite wall. However, a few synapticulae possibly present in some corallites, and in such specimens, some synapticulae also present on thin growing margin of corallum in a few incompletely developed peripheral corallites. Corallum white to light beige. Living colonies grayish-green, beige, or pink.

Etymology: The species name *formosa* (Latin *formosus*: beautiful, elegant) refers to the regular and neat aspect of the corallum. It is also reminiscent of the old name for Taiwan, where this species is thought to be endemic.

Distribution: Known only from Taiwan and

nearby islands, incrusting bare rocky outcrops at < 10 m deep (Fig. 5), where it may co-occur with *P. tayamai*.

Remarks: Overall, skeletal characters display

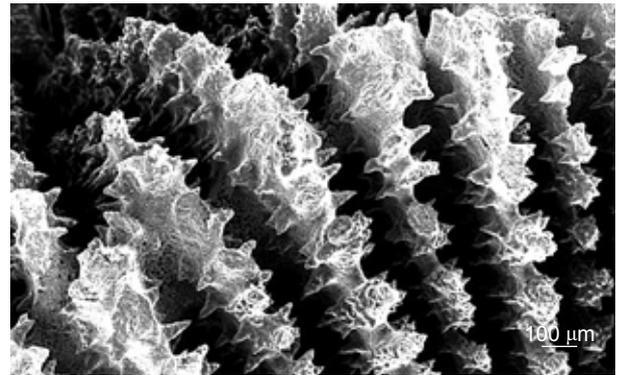


Fig. 3. *P. formosa* sp. nov. MTQ G 64373. Scanning electron microscopic image. Note the laterally flattened septal dentations and conical ornamentation on the septal sides.



Fig. 1. *P. formosa* sp. nov., holotype MTQ G 64378. Wanlitung, Taiwan.

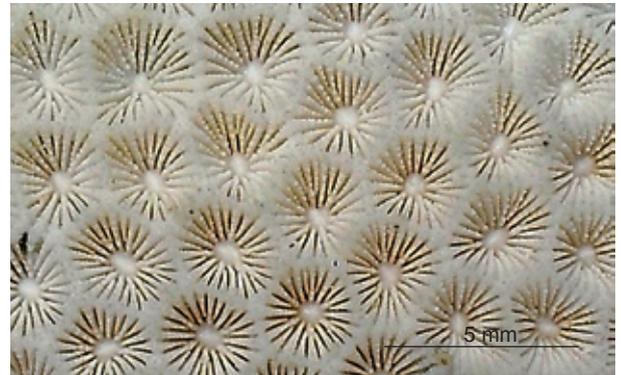


Fig. 4. *P. formosa* sp. nov., holotype. Note the non-exserted septa, solid wall, and well-developed columella.



Fig. 2. *P. formosa* sp. nov. MTQ G 64365. Scanning electron microscopic image. Note the exserted septa and well-developed septal ornamentation.



Fig. 5. *P. formosa* sp. nov. from Wanlitung. A small colony living on rocky substrate.

little variation among specimens, and only minor differences were observed. They principally concern the size of the corallites and number of septa, development of the septal margin ornamentation, more or less exsert character of the septa above the common wall, and the size of the columella. The series of specimens examined; however, is rather homogeneous, and no significant variations among the 3 geographic locations where the specimens were collected were noted.

Molecular phylogenetic analyses

Fifty-two variable sites containing 50 parsimoniously informative sites were found in 16 sequences of the *Siderastrea-Pseudosiderastrea* group examined. Pairwise genetic distances were calculated under the setting of the Kimura 2-parameter model, and averaged 0.03 between the *Pseudosiderastrea* and *Siderastrea* groups. The overall distance within *Siderastrea* was 0.012, while that within *Pseudosiderastrea* was only 0.003. Most species of *Siderastrea* occur in the Atlantic Ocean (Caribbean and Brazil) (Budd et al. 1994), and their pairwise genetic distance was smaller than that found in the single Indo-Pacific species *S. savignyana* (Atlantic group: 0.00037; *S. savignyana*: 0.00086). Within the genus *Pseudosiderastrea*, the genetic distance

between *P. tayamai* and *P. formosa* sp. nov. was 0.004, which is much higher than that of species comparisons among Atlantic species of *Siderastrea*.

Porites porites, *Dendrophyllia* sp., and *Stephanocoenia michelinii* were used as outgroups in the phylogenetic analysis. The resulting ML and BI topologies were similar for the *Siderastrea* and *Pseudosiderastrea* groups (Patristic distance correlation = 0.95) (Fourment et al. 2006) (Fig. 6), and consisted of 4 clades: clade I included *Siderastrea* species from the Atlantic Ocean group (Forsman et al. 2005); clade II included all specimens of *S. savignyana* collected from the Indian Ocean; and clades III and IV contained all specimens of *Pseudosiderastrea*. All clades had strong statistical support ($\geq 75\%$) in both the ML (bootstrap) and BI (posterior probability) analyses.

DISCUSSION

The genus *Pseudosiderastrea* was established by Yabe and Sugiyama (1935) for the species *P. tayamai* from Aru Is., but was subsequently treated as a subgenus of *Anomastrea* (Vaughan and Wells 1943, Wells 1956). However, more recently it was again treated as a genus in its full right (Veron and Pichon 1979). In the original description, Yabe

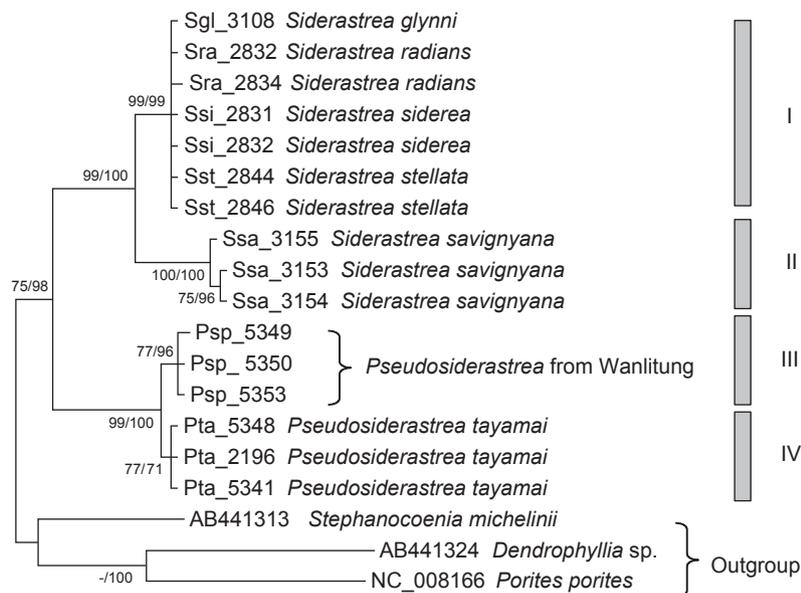


Fig. 6. Phylogenetic analyses based on Bayesian inference and maximum likelihood of the partial mitochondrial cytochrome (Cyt) *b* gene. Ten *Siderastrea* and 6 *Pseudosiderastrea* specimens were included and separated into 4 clades, including clades I and II for *Siderastrea* and clades III and IV for *Pseudosiderastrea*. *Stephanocoenia*, *Dendrophyllia*, and *Porites* were chosen as outgroups.

and Sugiyama (1935) remarked that *P. tayamai* was close to the Atlantic *S. radians* and *S. siderea*, which were the only *Siderastrea* species available for them to compare. According to Yabe and Sugiyama (1935), the major morphological differences between these 2 genera were the absence of septal perforations and the reduced development of synapticulae in *Pseudosiderastrea*. They also remarked that *Pseudosiderastrea* has similar features to *Anomastrea*, the latter differing by the presence of perforated septa and septal dentation increasing in size towards the center of the calice, with a tendency to form pali-like structures.

The relative regularity of the corallite shape within each colony of *P. formosa* sp. nov., in the material examined, is reminiscent of *S. savignyana* Milne Edwards & Haime (1849), which is particularly widespread in the western Indian Ocean (Fig. 7). By comparison, *Pseudosiderastrea* specimens most often display a more-irregular corallite shape, although occasionally some regularly shaped corallites were noted (see Veron and Pichon 1979, fig. 145). However, the solid walls and septa, and the almost total absence of synapticulae and synapticular rings leave no doubt as to the generic position of our specimens, which clearly belong to the genus *Pseudosiderastrea*, for which they represent a new species. *Pseudosiderastrea formosa* sp. nov. differs from *P. tayamai* (Fig. 8) in having more-regularly-shaped corallites, a smaller number of septa which are slightly wedge-shaped and seldom fused at their inner margin, coarser septal ornamentation, and a very developed, highly conspicuous columella.

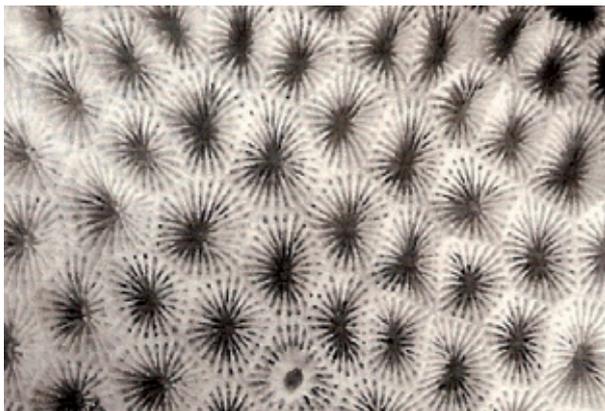


Fig. 7. *Siderastrea savignyana*. Specimen from Kuwait clearly showing the well-developed synapticular rings. (Photo: P. Harrison)

Molecular phylogenetic affinities

The *Pseudosiderastrea* spp. clade grouped as a sister group to *Siderastrea* spp. (Fig. 6), and as such, the monophyletic status of both genera is confirmed. Using cytochrome oxidase subunit 1 (COI) and Cyt *b*, Fukami et al. (2008) reexamined the familial and generic relationships of many scleractinian representatives, and found that the Pacific "*Siderastrea*" (samples collected from Wanlitung, Taiwan), which in fact belonged to *P. formosa* sp. nov., and the Atlantic *Siderastrea* specimens were a monophyletic group. The monophyletic origins of *Siderastrea* and *Pseudosiderastrea* were also confirmed by the COI phylogeny of scleractinian corals proposed by Kitahara et al. (2010). Those results clearly indicated that *Pseudosiderastrea* and *Siderastrea* have a very recent common ancestor.

Following morphological observations provided herein, the Cyt *b* phylogeny indicated that *P. formosa* sp. nov. and *P. tayamai* belong to the same genus based on monophyletic support of Cyt *b* phylogeny (clades III and IV, Fig. 6). The genetic distance of Cyt *b* between *P. formosa* sp. nov. and *P. tayamai* ($p = 0.004$) was relatively larger than the interspecific distance for species in the Atlantic clade (clade I), which contains the most recently diverged *Siderastrea* species from the Atlantic Ocean, *S. glynni* ($p = 0.0006$ for Cyt *b* in this study) (Forsman et al. 2005). The smaller distance we showed in *Pseudosiderastrea* is due to the slower evolution of mitochondrial DNA in anthozoans (Shearer et al. 2002). Comparing our results with others using the same marker, the



Fig. 8. *Pseudosiderastrea tayamai* (MTQ G 64630) from Kaohsiung, Taiwan, showing irregularly shaped and sized corallites and frequent fusion of the predominantly lamellar septa.

genetic distance between *P. formosa* sp. nov. and *P. tayamai* was equivalent to the interspecific distance of Cyt *b* found in *Acropora* ($p = 0.004$ between *P. formosa* sp. nov. and *P. tayamai*, $p = 0.0039$ in interspecific comparisons of *Acropora*; Chen et al. 2008). The genetic distance between *P. formosa* sp. nov. and *P. tayamai* implies that the genetic divergence of these 2 species is sufficiently large to support *P. formosa* sp. nov. being a different species from *P. tayamai*.

Acknowledgments: The authors acknowledge the assistance of Dr. P. Muir (S.E.M. and pictures) and Ms. B. Done (collection manager), both of the Museum of Tropical Queensland, Townsville, Australia. We are also grateful to Dr. H. Fukami and Dr. Z. Forsman for gifts of *Siderastrea* DNA samples from Panama, to Dr. D. Obura for providing material from Kenya, his input in the early stages of the project, and comments on the manuscript, to Dr. P. Harrison for permission to reproduce an illustration of *S. savignyana*, and to 2 anonymous reviewers, Dr. D. Miller, and members of the Coral Reef Evolutionary Ecology and Genetics (CREEG) laboratory, Biodiversity Research Center, Academia Sinica (BRCAS) for constructive comments. A collection permit was granted by the Kenting National Park, Ministry of the Interior, Pingtung, Taiwan. This study was made possible by grants from Academia Sinica and the National Science Council, Taiwan to C.A.C. This is the CREEG-BRCAS contribution no. 72.

REFERENCES

- Blainville HM de. 1830. "Zoophytes". In *Dictionnaire des Sciences Naturelles*. Paris, 60, pp. 295-364.
- Budd AF, HM Guzman. 1994. *Siderastrea glynni*, a new species of scleractinian coral (Cnidaria, Anthozoa) from the eastern Pacific. *P. Biol. Soc. Wash.* **107**: 591-599.
- Chen IP, CY Tang, CY Chiou, JH Hsu, NV Wei, CC Wallace et al. 2008. Comparative analyses of coding and noncoding DNA regions indicate that *Acropora* (Anthozoa: Scleractinia) possesses a similar evolutionary tempo of nuclear vs. mitochondrial genomes as in plants. *Mar. Biotechnol. (NY)* **11**: 141-152.
- Dai C, S Horng. 2008. Scleractinia fauna of Taiwan. I. The complex group. Taipei, Taiwan: National Taiwan Univ., 172 pp.
- Forsman ZH, HM Guzman, CA Chen, GE Fox, GM Wellington. 2005. An ITS region phylogeny of *Siderastrea* (Cnidaria: Anthozoa): Is *Siderastrea glynni* endangered or introduced? *Coral Reefs* **24**: 343-347.
- Fourment M, MJ Gibbs. 2006. Patristic: a program for calculating patristic distances and graphically comparing the components of genetic change. *BMC Evol. Biol.* **6**: 1.
- Fukami H, A Budd, G Paulay, A Sole-Cava, C Chen, K Iwao, N Knowlton. 2004. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* **427**: 832-835.
- Fukami H, CA Chen, AF Budd, A Collins, C Wallace, YY Chuang et al. 2008. Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (order Scleractinia, class Anthozoa, phylum Cnidaria). *PLoS One* **3**: e3222.
- Guindon S, JF Dufayard, V Lefort, M Anisimova, W Hordijk, O Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of phylml 3.0. *Syst. Biol.* **59**: 307-321.
- Kitahara MV, SD Cairns, J Stolarski, D Blair, DJ Miller. 2010. A comprehensive phylogenetic analysis of the scleractinia (Cnidaria, Anthozoa) based on mitochondrial COI sequence data. *PLoS One* **5**: e11490.
- Milne Edwards H, J Haime. 1849. Recherches sur les polypiers. 4eme memoire: monographie des astréides (suite). *Ann. Sci. Nat. Paris*, **3**: 95-197.
- Posada D, KA Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.
- Ronquist F, JP Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.
- Shearer TL, MJH Van Oppen, SL Romano, G Worheide. 2002. Slow mitochondrial DNA sequence evolution in the anthozoa (Cnidaria). *Mol. Ecol.* **11**: 2475-2487.
- Tamura K, D Peterson, N Peterson, G Stecher, M Nei, S Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* (In press).
- Vaughan TW, JW Wells. 1943. Revision of the suborders, families and genera of the Scleractinia. New York: Geological Society of America Special Paper 44, 363 pp., 51 plates.
- Veron JEN, M Pichon. 1979. Scleractinia of eastern Australia. Part III Families Agariciidae, Siderastreidae, Fungiidae, Oculinidae, Merulinidae, Mussidae, Pectiniidae, Caryophylliidae, Dendrophylliidae. Australia: Australian Institute of Marine Science Monograph Series no. 4, 422 pp.
- Wells JW. 1956. "Scleractinia." In RC Moore, ed. *Treatise on invertebrate paleontology. Part F: Coelenterata*. Geological Society of America, Kansas: Univ. Kansas Press, pp. F328-F440.
- Yabe H, T Sugiyama. 1935. A new living coral, *Pseudosiderastrea tayamai*, from Dobo in Wamar, Aru Islands. *Proc. Imp. Acad. Tokyo* **11**: 373-375.