

Note

Galaxea, Journal of Coral Reef Studies 27: 7–12 (2025)

Complete mitochondrial genomes of *Palauastrea ramosa* Yabe & Sugiyama, 1941 and *Stylocoeniella guentheri* (Bassett-Smith, 1890) reveal their molecular phylogenetic position

Yuki Yoshioka^{*1,†}, Megumi Kanaï^{2,†}, Takeru Tsuhako³, Noriyuki Satoh¹, and Tomofumi Nagata²¹ Marine Genomics Unit, Okinawa Institute of Science and Technology Graduate University, Onna, Okinawa 904-0495, Japan² Incorporated Foundation Okinawa Environment Science Center, Urasoe, Okinawa 901-2111, Japan³ Sensui Annai Okinawa, Uruma, Okinawa 904-1101, Japan[†] These authors equally contributed to this work* Corresponding author: Y. Yoshioka E-mail: y.yoshioka@oist.jp

Communicated by Frederic Sinniger (Associate Editor-in-Chief)

Received: 28 October 2024, Accepted: 17 February 2025

Published online: 9 April 2025

Abstract The scleractinian family Astrocoeniidae includes three genera. *Palauastrea* and *Stylocoeniella* are widely distributed throughout the Indo-Pacific region, while species of the genus *Stephanocoenia* (star corals) are found only in the Atlantic. Although Astrocoeniidae is considered to be phylogenetically most closely related to Pocilloporidae, their molecular phylogenetic position remains unclear. Here we report complete mitochondrial genomes of *Palauastrea ramosa* and *Stylocoeniella guentheri*. We also compared mitochondrial genomic structure between Astrocoeniidae (*P. ramosa* and *S. guentheri*) and Pocilloporidae. This study provides evidence of the phylogenetic structure and taxonomic relationships within Astrocoeniidae.

Keywords *Palauastrea*, Finger corals, *Stylocoeniella*, Thorn corals, Mitochondrial genome, Molecular phylogeny

Introduction

The family Astrocoeniidae Koby, 1890 is composed of three genera: *Palauastrea* Yabe & Sugiyama, 1941 (finger corals), *Stephanocoenia* Milne Edwards & Haime, 1848 (star corals), and *Stylocoeniella* Yabe & Sugiyama, 1935 (thorn corals). Finger corals and thorn corals are distributed in Indo-Pacific, while extant star corals are Atlantic species (Löser 2020). *P. ramosa* and three of the five extant species of *Stylocoeniella* have been classified to “Least Concern” (Johnson et al. 2024; Shlesinger et al. 2024) and *Stephanocoenia intersepta* has been classified to “Near Threatened” (Kitahara et al. 2022) in International Union for Conservation of Nature (IUCN) Red List.

The genus *Palauastrea* is monotypic (*P. ramosa* Yabe & Sugiyama, 1941). *P. ramosa* forms slender, arborescent clumps with a diameter of about 10 mm at the branch base, sometimes exceeding 30 cm in height, and espe-

cially when large, the colony shape can be hemispherical (Kajiwara 2024). Calices are round and very shallow, measuring 1–1.5 mm in diameter. They are particularly crowded at the tips of young branches, more spaced apart on older portion, but never as wide as the diameter of the calices themselves, occasionally displaying a tendency for serial arrangement (Yabe and Sugiyama, 1941; Kajiwara 2024). Currently, only one sequence (partial *cox1*) is available from *Palauastrea* sp. and close phylogenetic relationship with *Stylocoeniella* sp. has been suggested (Chuang et al. 2017).

The genus *Stylocoeniella* is one of the widely distributed scleractinians in Indo-Pacific reefs. Colony morphology is massive, columnar, or encrusting, although they are not conspicuous because of small size, encrusting colonies, and often in crevices (Wells 1964). Corallites are plocoid, cylindrical, 0.5–1 mm apart, with granulated spines on the coenosteum surface, occasionally becoming

cerioid with fused walls in crowded areas (Yabe and Sugiyama 1935; Wells 1966). Currently, the genus *Stylocoeniella* includes five extant species (Hoeksema and Cairns 2024). Based on partial mitochondrial and nuclear genes, *Stylocoeniella* is most closely related to the family Pocilloporidae (Fukami et al. 2008, Kitahara et al. 2010). However, the phylogenetic position of *Stylocoeniella* varies depending on the gene sets used. For instance, *Stylocoeniella guentheri* appeared as a sister to Pocilloporidae in the mitochondrial gene (*cox1* and *cob*) -based topology, while in the partial nuclear gene (ribosomal DNA including ITS region) -based topology, *S. guentheri* clustered within Pocilloporidae, placing *Madracis myriaster* (Milne Edwards & Haime, 1850) outside the *S. guentheri* and Pocilloporidae cluster (Fukami et al. 2008).

Since the advent of next-generation sequencing, which provide higher resolution and coverage, it has become significantly faster and more cost-effective to obtain complete mitochondrial genomes (Metzker 2010; Smith 2016). As mitochondrial genome contains informative marker genes, it sometimes helps to resolve relationships among animals (Juszkiewicz et al. 2022). Recently, phylogenetic analysis using mitochondrial genome revealed that the genus *Madracis* occupied an early branching position within Pocilloporidae (Tucker et al. 2023); however, no mitochondrial genomes of any Astrocoeniidae was included in the analysis, indicating that phylogenetic position of Astrocoeniidae still remains unclear. In this study, we sequenced mitochondrial genomes of *P. ramosa* and *S. guentheri* and performed molecular phylogenetic analysis to confirm their molecular phylogenetic position. In addition, to characterize mitochondrial genome structure of *P. ramosa* and *S. guentheri*, we also compared mitochondrial genome organization between Astrocoeniidae and Pocilloporidae.

Materials and methods

We collected *Palauastrea ramosa* at Katsuren-Haebaru, Okinawa, Japan, on 1st October 2024 (Supplementary Fig. S5), and *Stylocoeniella guentheri* at Henza Island, Okinawa, Japan, on 27th May 2024 (Supplementary Fig. S6) under permit for coral collection provided by the Okinawa Prefectural Government (Permits 6–30). Speci-

mens were identified using morphological characters from taxonomic works (Veron and Pichon 1976; Veron 1986; Nishihira and Veron 1995; Veron 2000; Veron et al. 2016). For *P. ramosa*, we sampled a branch (approximately 5 cm in diameter) from a colony around 30–40 cm tall, located at a depth 5 meters on the reef moat environment. The colony featured immersed and circular corallites with a blunt style-like columella and two unequal cycles of septa (six thick septa for each cycle) that do not fuse the columella. Branch tips were round shape and fringed with fine spines irregularly. For *S. guentheri*, a small, rounded knob (approximately 2.5 cm in diameter) was collected from a massive, tuberculate coral colony at a depth of 5 meters on the reef moat environment. The colony had corallites widely spaced and coenosteum covered small tuberculae and distinctive spinule. Calice were less than 1 mm in diameter and indistinct septa of corallite are in two unequal cycles. The voucher specimens are stored at Ryukyu University Museum, Fujukan (RUMF-ZG-05480 for *P. ramosa*; RUMF-ZG-05481 for *S. guentheri*). Fragments were preserved in 99.5% ethanol until use. We extracted genomic DNA from a small part (0.5 cm in diameter) of preserved fragments using a Maxwell RSC Blood DNA Kit (Promega). Sequence libraries were constructed with a KAPA Hyper Prep Kit (KAPA Biosystems) according to the manufacturer's protocol. Sequencing libraries were sequenced on a MiSeq or NextSeq 1000, with 300-bp paired-ends. Mitochondrial assembly and annotation were performed as in Yoshioka et al. (2024). Complete mitochondrial genomes with gene annotation were visualized with OrganelleGenomeDRAW (Greiner et al. 2019). Gene order was visualized with Clinker (Gilchrist and Chooi 2021). Raw genomic DNA sequences used in this study were deposited in the DDBJ Sequence Read Archive under accession number DRR586685 and DRR613159 (BioProject ID: PRJDB17204). Mitochondrial genome assemblies have been deposited at DDBJ/EMBL/GenBank under accession number LC836041 and LC849181.

To ensure taxonomic identification, we compared mitochondrial sequences with publicly available sequences (Supplementary Fig. S1–4). For comparison, we downloaded *S. guentheri* (partial *cox1* [AB441225.1] and partial *cob* [AB441310.1]) and *Palauastrea* sp.

(partial *cox1* [KY887486.1]). Since *P. ramosa* is often confused with *Porites cylindrica*, we also included a mitochondrial sequence of *Porites cylindrica* (OZ037789.1) in the comparison. We extracted the 13 protein-coding genes, two rRNA genes, and a tRNA gene (*trnM*) for all samples and performed molecular phylogenetic analyses following Yoshioka et al. (2024).

Results and discussion

We obtained complete mitochondrial genome assemblies for *Palauastrea ramosa* and *Stylocoeniella guentheri*, with 393x and 387x mean sequencing depth estimated by the GetOrganelle pipeline, respectively (Fig. 1). The mitochondrial assembly size of *P. ramosa* and *S. guentheri* was 18,463 bp and 18,612 bp, which was ~1,000 bp larger than that of Pocilloporidae, since the largest one among Pocilloporidae was 17,425 bp of *Pocillopora damicornis* (Supplementary Table S1). To ensure taxonomic identification, we compared the obtained sequences with publicly available sequences. The partial *cox1* sequences of *S. guentheri* and *P. ramosa* were identical with the publicly available sequences

(Supplementary Fig. S1–3). However, some mismatches were observed between the partial *cob* sequence of *S. guentheri* (AB441310.1) and that of *S. guentheri* generated in this study, whereas the sequences were conserved between *S. guentheri* and *P. ramosa* (Supplementary Fig. S4). To determine whether such mismatches represent interspecific variation, further investigation will be required in the future. As with other scleractinian corals, *P. ramosa* and *S. guentheri* possessed 13 protein-coding genes (*nad1–6*, *nad4L*, *cox1–3*, *atp6*, *atp8*, and *cob*), two rRNA genes (*rrnL* and *rrnS*), and two tRNA genes (*trnM* and *trnW*) in the same order (Fig. 1; Supplementary Table S1). Although gene rearrangements such as duplication of *trnW* in *Seriatopora* and *Stylophora* (Chen et al. 2008b) and a gene order change in *Madreopora* (Lin et al. 2012) have been reported in phylogenetically related species, such case was not observed in *P. ramosa* and *S. guentheri* (Fig. 2), indicating gene order type SII (Lin et al. 2014).

As *trnW* is duplicated in some genera in Pocilloporidae, we used single-copy genes (13 protein-coding genes, two rRNA genes, and *trnM* gene) for molecular phylogenetic analysis. As Fukami et al. (2008) suggested, *P. ramosa*

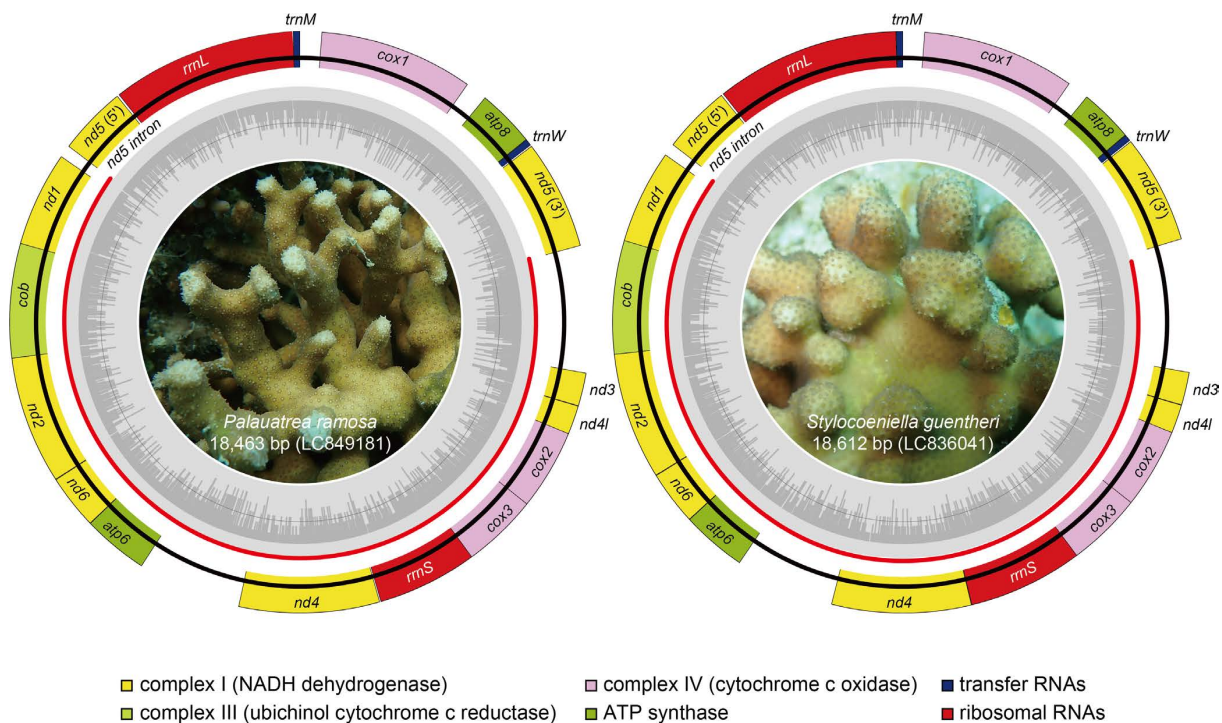


Fig. 1 Complete mitochondrial genome of *P. ramosa* and *S. guentheri*. Inner circles (grey) indicate GC contents (%). Inner red line indicates intron for *nd5*. NADH dehydrogenase (yellow), ubiquinol cytochrome c reductase (light green), cytochrome c oxidase (pink), ATP synthase (green), tRNAs (blue), and rRNAs (red). Photos taken by Megumi Kanai.

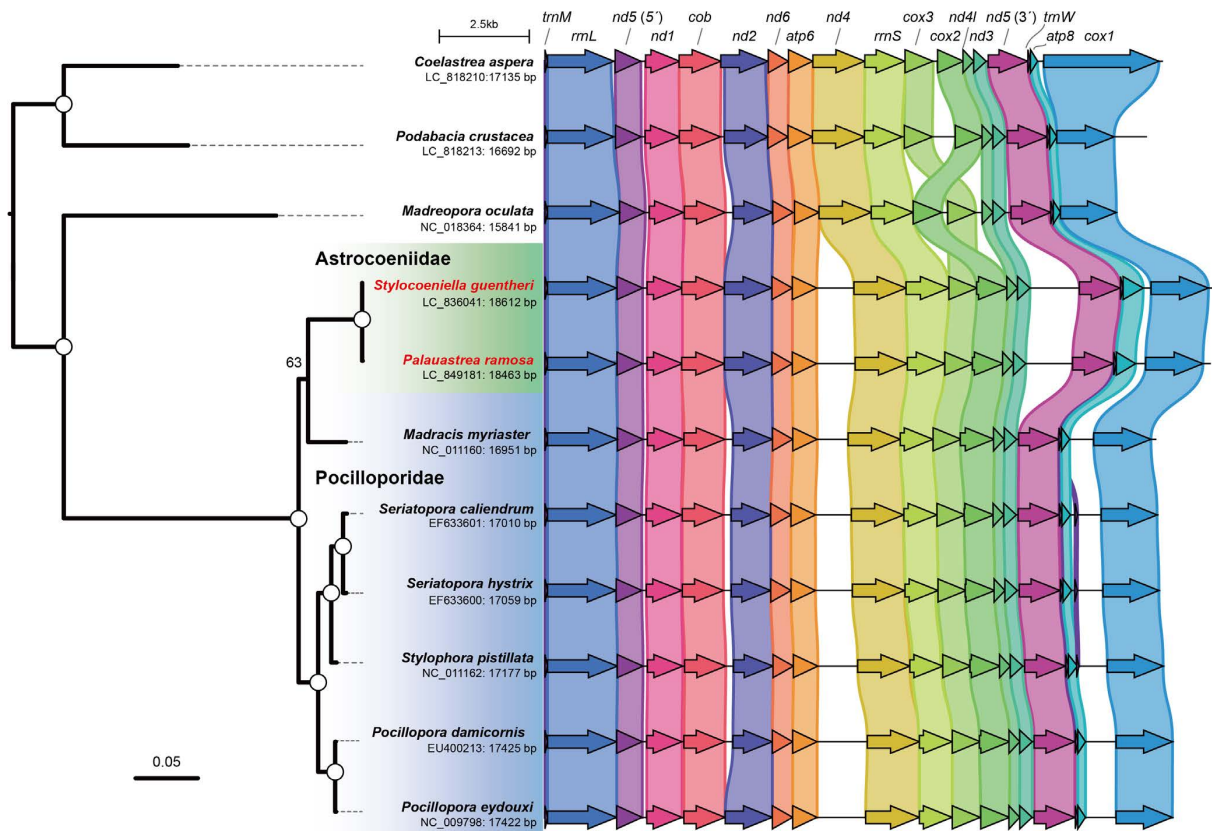


Fig. 2 Comparison of mitochondrial genome organization. Molecular phylogenetic tree based on mitochondrial genome (15,592 positions) (left). The bar indicates expected substitutions per site in aligned regions. Nodes with open circle have 100% bootstrap support unless otherwise noted. Species sequenced in this study are shown in red. Accession numbers and mitochondrial genome size are given on the next line of binomials. Astrocoeniidae and Pocilloporidae are shaded by green and blue, respectively. Comparison of mitochondrial genomic organization (right). Each gene is colored and orthologs are connected. The bar indicates 2.5 Kbp.

and *S. guentheri* was clustered with Pocilloporidae (bootstrap value=100). However, this analysis resulted in two clades: (i) *P. ramosa*, *S. guentheri* and *Madracis mirabilis* and (ii) other Pocilloporidae (Fig. 2). Namely, the result clearly indicates that the phylogenetic position of *P. ramosa* and *S. guentheri* is sister to Pocilloporidae, as with *Madracis* as previously suggested by Fukami et al. (2008) and Kitahara et al. (2010). Fukami et al. (2008) suggested the phylogenetic placement of the genus *Madracis* is variable depending on gene sets used. Its bootstrap support for the clade of *Madracis* with *P. ramosa* and *S. guentheri* was 64% in this study, suggesting that further validation is needed to determine the phylogenetic position of the genus *Madracis*.

Palauastrea ramosa and *Stylocoeniella guentheri* possessed five intergenic regions: between *nd5* (5') and *nd1*, *atp6* and *nd4*, *nd3* and *nd5* (3'), *atp8* and *cox1*,

and *cox1* and *trnM* (Fig. 1). All of these intergenic regions are known as mitochondrial genomic features of Pocilloporidae (Chen et al. 2008a), suggesting that they originated from a common ancestor shared by Astrocoeniidae and Pocilloporidae. Comparison of mitochondrial genome organization revealed that the intergenic region between *nd3* and *nd5* (3') was ~1,000 bp larger than that in Pocilloporidae (Fig. 2), suggesting that this expansion may have occurred after the divergence of Astrocoeniidae/Pocilloporidae and contributed to the larger mitochondrial genomic size in *P. ramosa* and *S. guentheri*.

In conclusion, we sequenced the complete mitochondrial genomes of *Palauastrea ramosa* and *Stylocoeniella guentheri*, identifying a longer intergenic region between *nd3* and *nd5* (3'), a feature of mitochondria in *Palauastrea* and *Stylocoeniella*. Molecular phylogenetic analysis

using mitochondrial genome clearly indicated that *P. ramosa* and *S. guentheri* are sister group to Pocilloporidae. At the same time, the analysis raised a question about the validity of the classification of the genus *Madracis*. Currently, *Madracis* is classified to Pocilloporidae (Hoeksema & Cairns 2024). For further discussion to untangle the taxonomic placement, replication and integrative taxonomic approach are required to understand the taxonomic placement of this genus. Recently, mitochondrial genes have been used not only for evolutionary analyses but also as a database in metabarcoding analyses of environmental DNA (eDNA) of scleractinians (Shinzato et al. 2021; Satoh et al. 2025), although species-level identification needs to be resolved in the future. Thus, the mitochondrial sequence information presented here not only provides evidence for the phylogenetic placement of *P. ramosa* and *S. guentheri* but is also expected to contribute to ecological studies utilizing eDNA.

Acknowledgments

We thank members of the Sequencing Section of OIST for conducting genome sequencing and members of the Scientific Computing and Data Analysis section of OIST for computing resources. We also thank Mayuki Suwa (Marine Genomics Unit, OIST) for preparing sequencing libraries and Masayuki Tamae (Marine Space) for helping sample collection. This study was supported in part by the OIST Coral Project, Okinawa Prefecture Innovation/Ecosystem Joint Research Promotion Program, and grants from the Japan Society for the Promotion of Science (JP23KJ2129 to YY).

Compliance/Conflict of interest declaration

We declare no competing interests.

References

- Chen C, Chiou CY, Dai CF, Chen CA (2008a) Unique mitogenomic features in the scleractinian family Pocilloporidae (Scleractinia: Astrocoeniina). *Mar Biotechnol* 10: 538–553
- Chen C, Dai CF, Plathong S, Chiou CY, Chen CA (2008b) The complete mitochondrial genomes of needle corals, *Seriatopora* spp. (Scleractinia: Pocilloporidae): an idiosyncratic *atp8*, duplicated *trnW* gene, and hypervariable regions used to determine species phylogenies and recently diverged populations. *Mol Phylogenet Evol* 46: 19–33
- Chuang Y, Kitahara M, Fukami H, Tracey D, Miller DJ, Chen CA (2017) Loss and gain of group I introns in the mitochondrial *cox1* gene of the Scleractinia (Cnidaria; Anthozoa). *Zool Stud* 56: 9
- Fukami H, Chen CA, Budd AF, Collins A, Wallace C, Chuang YY, Chen C, Dai CF, Iwao K, Sheppard C, Knowlton N (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
- Gilchrist CLM, Chooi YH (2021) Clinker & clustermap.js: Automatic generation of gene cluster comparison figures. *Bioinformatics* 37: 2473–2475
- Greiner S, Lehwerk P, Bock R (2019) OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res* 47: W59–W64
- Hoeksema BW, Cairns S (2024). World List of Scleractinia. Pocilloporidae Gray, 1840. Accessed at: World Register of Marine Species at: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=135079> on 2024-10-24
- Johnson J, Luzon K, Dellisanti W, Cowburn B, Lewis C (2024) *Palauastrea ramosa*. The IUCN Red List of Threatened Species 2024: e.T133075A165707827. Accessed on 2024-12-25
- Juszkiewicz DJ, White NE, Stolarski J, Benzoni F, Arrigoni R, Hoeksema BW, Wilson NG, Bunce M, Richards ZT (2022). Phylogeography of recent Plesiastrea (Scleractinia: Plesiastreidae) based on an integrated taxonomic approach. *Mol Phylogenet Evol* 172: 107469
- Kajiwara K (2024) *Palauastrea ramosa* Yabe & Sugiyama, 1941. In: Japanese Society for Coral Taxonomy (ed) Online Monograph of Zooxanthellate Corals of Japan. Accessed at: https://coralmonogr.jpn.org/Palauastrea_ramosa.html on 2024-10-23
- Kitahara MV, Cairns SD, Stolarski J, Blair D, Miller DJ (2010) A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data. *PLoS One* 5: e11490
- Kitahara MV, Sweet M, Vermeij M (2022) *Stephanocoenia intersepta*. The IUCN Red List of Threatened Species 2022: e.T133521A165931015. Accessed on 27 December

- 2024
- Lin MF, Kitahara MV, Luo H, Tracey D, Geller J, Fukami H, Miller DJ, Chen CA (2014) Mitochondrial genome rearrangements in the Scleractinia/Corallimorpharia complex: implications for coral phylogeny. *Genome Biol Evol* 6(5): 1086–1095
- Lin MF, Kitahara MV, Tachikawa H, Fukami H, Miller DJ, Chen CA (2012) Novel organization of the mitochondrial genome in the deep-sea coral, *Madrepora oculata* (Hexacorallia, Scleractinia, Oculinidae) and its taxonomic implications. *Mol Phylogenet Evol* 65: 323–328
- Löser H (2020) Regional persistence of the extant coral genus *Stephanocoenia* since the Early Cretaceous in the Western Atlantic. *PalZ* 94: 17–39
- Metzker ML (2010) Sequencing technologies - the next generation. *Nat Rev Genet* 11: 31–46
- Nishihira M, Veron JEN (1995) *Hermatypic corals of Japan*. Kaiyusha, Tokyo, Japan
- Satoh N, Sinniger F, Narisoko H, Hagahama S, Okada N, Shimizu Y, Yoshioka Y, Hisata K, Harii S (2025) Using underwater mini-ROV for coral eDNA survey: a case study in Okinawan mesophotic ecosystems. *Coral Reefs* 44: 209–219
- Shinzato C, Narisoko H, Nishitsuji K, Nagata T, Satoh N, Inoue J (2021) Novel mitochondrial DNA markers for scleractinian corals and generic-level environmental DNA metabarcoding. *Front Mar Sci* 8: 758207
- Shlesinger T, Johnson J, Luzon K, Dellisanti W, Cowburn B, Lewis C (2024) *Stylocoeniella guentheri*. The IUCN Red List of Threatened Species 2024: e.T133049A165694719. Accessed on 2024-12-25
- Smith DR (2016) The past, present and future of mitochondrial genomics: have we sequenced enough mtDNAs? *Brief Funct Genomics* 15(1): 47–54
- Tucker J, Barrios LM, Preziosi R, Baeza JA (2023) A first genomic portrait of the deep-water azooxanthellate reef-building coral *Madracis myriaster*: genome size, repetitive elements, nuclear RNA gene operon, mitochondrial genome, and phylogenetic placement in the family Pocilloporidae. *Coral Reefs* 42: 1241–1256
- Veron JEN (1986) *Corals of Australia and the Indo-Pacific*. Angus & Robertson, Sydney, Australia
- Veron JEN (2000) *Corals of the world*. Australian Institute of Marine Science, Townsville, Australia.
- Veron JEN, Pichon M (1976) *Scleractinia of Eastern Australia*. Part I. Families Thamnasteriidae, Astroceoniidae, Pocilloporidae. Australian Institute of Marine Science Monograph Series. 1: 1–86
- Veron JEN, Stanford-Smith MG, Turak E, DeVantier LM (2016) *Corals of the World*. Accessed at https://www.coralsofttheworld.org/species_factsheets/species_factsheet_summary/stylocoeniella-guentheri/ on 2024-12-19
- Wells JW (1964) Fossil corals from Eniwetok Atoll. United States Geological Survey Professional Paper, 260-DD: 1101–1111
- Wells JW (1966) Notes on Indo-Pacific Scleractinian Corals, Part 4: A Second Species of *Stylocoeniella*. *Pac Sci*, 20 (2): 203–205
- Yabe H, Sugiyama T (1935) *Stylocoeniella*, a new coral genus allied to *Stylocoenia* and *Astrocoenia*. *Jpn J Geol Geogr* 12: 103–105, pl. 15 <https://gbank.gsj.jp/ld/resource/geolis/88807688.html>
- Yabe H, Sugiyama T (1941) Recent reef-building corals from Japan and the South Sea Islands under the Japanese mandate. II. The science reports of the Tohoku Imperial University. Second series, Geology, Special volume: 67–91
- Yoshioka Y, Nagata F, Nonaka M, Satoh N (2024) Molecular phylogenetic position of the family Fungiidae (Cnidaria: Anthozoa) based on complete mitochondrial genome sequences. *Galaxea, J Coral Reef Stud* 26(1): 43–47

Electronic supplementary material

ESM Figs. S1-S6 and ESM Table 1 can be downloaded from the J-STAGE

website: <https://doi.org/10.3755/galaxea.G27-4>

©Japanese Coral Reef Society