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PS-3 **DNA barcoding and molecular phylogeny in the photosymbiotic *Diplosoma* from the Ryukyus (Ascidiacea: Didemnidae).**

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Colonial animals tend to have simple zooids, and hence they have less morphological features for species identification. This is one of the crucial reason why the taxonomy of colonial animals is more difficult and sometimes less reliable than the taxonomy of solitary ones. On the other hand, recent advances of the use of DNA sequences as taxonomical tags may improve our recognition of species and verify the phylogenetic significance of each morphological feature.

The species of the genus *Diplosoma* (Ascidiacea, Didemnidae) are always colonial and their zooids are very small, a few millimeters or less in length. Some *Diplosoma* species inhabiting in tropical and subtropical waters harbor photosymbiotic cyanophytes, *i.e.*, *Prochloron*, in the common cloacal cavity. To date, five photosymbiotic *Diplosoma* have been recorded from subtropical waters in Japan, and three of them were recently described as new species from the Ryukyu Archipelago, Japan. Moreover, we have recognized three undescribed species in this area, indicating that taxonomical studies on this taxon is insufficient in this area. On the other hand, pohotosymbiotic ascidians are hopeful source of bioactive compounds, and some chemists often request specific identification of their specimens that are usually poorly preserved.

In the present study, we determined the partial sequences of COI gene of some photosymbiotic *Diplosoma* species to develop the tool for the specific identification of the photosymbiotic *Diplosoma*.

A total of ten haplotypes were obtained by sequencing the target partial COI gene fragment (401bp) of eight *Diplosoma* species including three undescribed ones. Monophyly of the genus *Diplosoma* was supported by high bootstrap value. Two haplotypes were obtained from each of *D. variostigmatum* and *Diplosoma* sp. A, and three haplotypes were obtained from *Diplosoma* sp. B. Monophyly of each *Diplosoma* species was supported by high bootstrap value.

The stigma numbers are invariable in each photosymbiotic *Diplosoma* species, except *D. variostigmatum* (see Hirose and Oka, 2008). On the other hand, some species have the same pattern of stigma numbers, but can be discriminated by the emerging point of the retractor muscle from the zooids, which is from the underside of the thorax (T-type) or halfway down the esophageal neck (N-type) (see Kott, 2001). The ten *Diplosoma* mtCOI sequences from this study fell into two groups. One group consisted four haplotypes from three N-type species and the other consisted six haplotypes from five T-type species. Monophyly of each group was supported by moderate bootstrap values.

In the present study, we determined the partial sequences of COI gene to infer the phylogeny of the Japanese photosymbiotic *Diplosoma* species, and the phylogenetic tree supported the phylogenetic significance of the emerging point of the retractor muscle.