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Preliminary examination of zooxanthellate zoanthid (Hexacorallia, Zoantharia) and associated zooxanthellae (Symbiodinium spp.) diversity in Singapore

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species diversity depended on small-sized trees in the forest of Okinawa Island, whereas it depended on large-sized trees in the forest of Ishigaki Island. The forest of Okinawa Island (H'=4.83 bit) showed higher woody species diversity than the forest of Ishigaki Island (H' = 4.36 bit). According to successively decreasing height of layers from the top downward, the value of H' increased continuously from the top layer downward in the forest of Okinawa Island. This increasing trend was different from the forest of Ishigaki Island, where the value of H' increased up to the second layer and then decreased downward. In the forest of Okinawa Island, the expected number of species increased continuously from the top toward the bottom layer, i.e. the bottom layer contained the highest potential number of species (65). However, in the forest of Ishigaki Island, it increased from the top to the fourth layer, and then decreased to the bottom layer, i.e. the fourth layer contained the highest potential number of species (90). The species composition in the forest of Okinawa Island was different from that in the forest of Ishigaki Island, though approximately half of the species were common between the forests. The highest degree of similarity in species composition was between the second and third layers in the forest of Okinawa Island, whereas it was between the third and bottom layers in the forest of Ishigaki Island. The degree of similarity in species composition between the top and the lower three layers was high in the forest of Okinawa Island, whereas it was very low between the top and the lower four layers in the forest of Ishigaki Island. Except the top and the bottom layer respectively for the forests of Okinawa Island and Ishigaki Island, the spatial distribution of trees was random in each layer. The degree of overlapping in the spatial distribution of trees among layers in the forests suggested that light can not penetrate easily to the lower layers. For both of the forests, mean tree weight of each layer decreased from the top toward the bottom layer, whereas the corresponding tree density increased from the top downward. This trend resembled the mean weight-density trajectory of self-thinning plant populations.

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The order Zoantharia (=Zoanthidea, Zoanthiniaria) remains one of the most taxonomically neglected and least examined orders of Cnidaria despite a worldwide distribution in marine environments. In particular, zooxanthellate zoanthids from the genera Palythoa (family Sphenopidae) and Zoanthus (Zoanthidae) are very common in tropical and subtropical shallow waters. Confusion surrounding the taxonomy and species diversity in these two families is largely attributable to the morphological plasticity (i.e. polyp shape and size, oral disk color, etc.) within species, the lack of accurate morphological markers to properly discern species, and the paucity of accurate species descriptions in past literature. However, recent examinations utilizing mitochondrial 16S ribosomal DNA (mt 16S rDNA), mitochondrial cytochrome oxidase subunit I (COI) and the internal transcribed spacer of ribosomal DNA (ITS-rDNA) data from Zoanthus and Palythoa spp. specimens from Japan combined with more traditional morphological methods have begun to bring taxonomic order to these two genera. Other studies utilizing ITS-rDNA from symbiotic zooxanthellae (Symbiodinium spp.) from these two genera have shown varying levels of symbiont specificity and flexibility between zoanthid species. Here, we utilize the "combined" molecular and morphological methodology to examine the species diversity of these two genera in the waters of Singapore. 44 zooxanthellate zoanthid specimens representing a wide range of morphotypes were collected from three locations (Raffles Lighthouse, Lazarus Island, Kusu Island) in November-December 2006. Upon collection, specimens were assigned tentative species identifications based solely on morphology. Further examinations of mt 16S rDNA, COI, as well as ITS-rDNA from Symbiodinium, allowed us to 1) identify specimens to the species level, and 2) examine the accuracy of morphological identifications. Our results show collected specimens represent five species of zooxanthellate zoanthids; Zoanthus sansibaricus (n=11), Zoanthus vietnamensis (n=17), Palythoa tuberculosa (n=13), Palythoa mutuki (n=2), and a potentially undescribed Palythoa species (n=1) closely related to Palythoa heliodiscus. Species identifications are somewhat tentative due to the almost complete lack of previous reports of zoanthids in Singapore. Based on morphology alone, we were able to identify

only 35 of 44 specimens (=80%) correctly. Acquired *Symbiodinium* ITS-rDNA sequences reflect previously seen patterns of association in *Zoanthus*, with *Zoanthus sansibaricus* (*Symbiodinium* subclade C1z) and *Zoanthus vietnamensis* (C15-related) both showing identical zooxanthellae types as seen in Japan and other Indo-Pacific locations. Unexpectedly, the majority of *Palythoa tuberculosa* (n=9) associated with *Symbiodinium* clade E, not previously seen in zoanthids, and only a few specimens (n=4) associated with clade C1/C3 as observed in most previous studies. The results of this study highlight the need for further sampling and examination of zooxanthellate zoanthids from other locations to help complete the global picture of zoanthid and associated zooxanthellae distribution patterns and diversity.



L to R; potential new Palythoa species, Zoanthus vietnamensis, and Palythoa tuberculosa.