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Preliminary Notes on the Life History of a Pycnogonid, *Ammothella biunguiculata* (Pycnogonida, Ammotheidae): Comparison between the Populations of Izu and Kii Peninsulas*1,*2

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Ammothella biunguiculata is a pycnogonid species belonging to the family Ammotheidae and inhabiting shallow water in Sagami Bay and southward in Japan. They have been recorded also in the Mediterranean Sea (original description), the Pacific coast of North America, Hawaii, Korea, Australia and Madagascar, and considered to be pantemperate (Child, 1987). Miyazaki (2002a, 2002b) reported the endoparasitic larval forms of *A. biunguiculata* in an actinian, *Entacmaea actinostoloides* (synonymized to *E. quadricolor* after Dunn (1981) and Yanagi (2006)) in the population of Shimoda, Izu Peninsula. In the population of Shirahama, Kii Peninsula, however, the larvae were found as free-living (Miyazaki, 2002b). In the present study, we conducted the monthly samplings in Shimoda and Shirahama to compare the life history between the two populations.

For the population of Izu Peninsula (Izu population), specimens of *E. quadricolor* were collected monthly (n = 10–20) from tide pools at the tip of Noroshi-Zaki point near the Shimoda Marine Research Center, University of Tsukuba from May 2012 to June 2013 excepting October, December, and March due to a typhoon or a stormy weather. The parasitic pycnogonids were extracted from the dissected actinians, and the developmental stages, the body length and the number of pycnogonids per host were recorded. We also collected free-living individuals under the boulders of the same location in July, August, September, December 2012 and January 2013. For the population in Kii Peninsula (Kii population), the monthly collection of free-living *A. biunguiculata* (n = about 100) was conducted from intertidal zone near the Seto Marine Biological Laboratory, Kyoto University, from May 2012 to July 2013. Collected specimens were observed in the same way as those of the Izu population. The ratio of ovigerous males (adult males with fertilized eggs or embryos on their ovigers) and of adult females with mature oocytes in their pedal ovaries was also recorded. Most specimens of the Kii population were released to their habitats after examination. We collected several specimens of *Entacmaea* actinians from the same locality of the Kii population, but no pycnogonids could be found. Some fixed specimens of both populations were stained with a chitin-specific fluorescent dye, Uvitex 2B (Polysciences) followed by Yoshinaga *et al.* (2010) to enhance the segmentation pattern on trunk and legs which was essential to decide the postembryonic stages.

From the Izu population, we sampled 629 pycnogonid specimens from 182 actinians in total. We could divide 11 postembryonic stages from protonymphon (hatching larvae) to adult in this population (Mochizuki and Miyazaki, in press). There were parasitic larvae from second to tenth (subadult) instar on host actinians, but no adults. The first instar was collected only from the free-living ovigerous males. From July to August, the number of parasitic larvae per host was very low, and in September, no larvae were found. The number had a peak at January, and then decreased again in the next spring season. The second instar occurred only in November and January, which showed the period of recruitment of parasitic larvae to the host actinians. These newly larvae should have been fertilized in summer season. Free-living adults as well as ninth and tenth instars were collected from July to January. Ovigerous males were recorded only in December and January. Considering the period of the occurrence of ovigerous males and of the parasitic larval recruitment, there are two possibilities about the reproductive period of the Izu population. One is that the breeding period continues from summer to winter, and the other is that it occurs twice a year, summer and winter. As the sampling duration of the free-living individuals was limited, further discussion is impracticable.

From the Kii population, we sampled 1511 specimens in total. Protonymphon larvae (first instar) clinging the ovigers of adult males and free-living individuals from sixth instar to

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*2 This article, which was accepted in 2013 and should have been published in 2014, was printed in 2017 being much delayed due to various circumstances.

adult were collected, but no larvae from second to fifth instars could be found from this population. We found some specimens in the postembryonic stage apparently between the eighth and ninth of the Izu population, and thus the Kii population shows at least 12 instars. The youngest (= sixth) instar first appeared in January. Adults occurred throughout the year, and predominated from September to December. Ovigerous males appeared between October and May, which showed the reproductive period. The ratio of their number had a peak in December exceeding 80%. The fluctuation of the ratio of adult females with mature oocytes showed a similar pattern to that of the ovigerous males. Considering these fluctuation patterns and the reproductive period, the Kii population apparently has an annual life cycle. Collecting of larvae before sixth instar is necessary to complete the life history in this population.

There are some remarkable differences on life history between the Izu and the Kii populations of *A. biunguiculata* such as the mode of larval life (endoparasitism or free-living), the presumed number of postembryonic stages, and the reproductive period. In addition, we have found a distinct difference in the spinning spines at larval chelifores (Mochizuki and Miyazaki, unpublished data). These differences suggest that the two populations represent two different species though we could not detect any differences in adult morphology. Another possibility is that the difference in the larval spinning spines is a case of adaptive phenotypic plasticity reflecting their modes of life. Further phylogenetic study is required to resolve the problem.

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