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Stylactaria misakiensis (Hydrozoa, Hydractiniidae) Having Hydrorhizae Changed in Morphology by Host Replacement

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Abstract The continuous morphological variation of hydrorhizae from *Stylactaria misakiensis* to *S. inabai* by host replacement was ascertained based on the examination of newly-collected specimens and by host replacement experiments. *Stylactaria inabai* had been distinguished from *S. misakiensis* by the differences in the morphology of hydrorhizae and their host animals. Recently, DNA analysis, however, revealed *S. inabai* is a synonym of *S. misakiensis*. This study confirmed the accuracy of the results of the DNA analysis.
Key words: *Stylactaria misakiensis*, *Stylactaria inabai*, Hydractiniidae, hydrorhiza, host.

Introduction

Species belonging to the genus *Stylactaria* (Hydractiniidae) are mainly epizoic hydrozoans growing on living gastropod shells, gastropod shells inhabited by hermit crabs, or on other benthic organisms (His Majesty the Showa Emperor Hirohito, 1988). Some species of this genus are host-specific and, in this case, the species specificity to their host animals are occasionally accepted as the discriminate character to distinguish them from closely related species (Namikawa, 1991 and 2001; Namikawa *et al.*, 1993).

Stylactaria inabai (Hirohito, 1988), growing on gastropod shells inhabited by hermit crabs, has gastropod-shell-type hydrorhizae extending from its core host shell (Fig. 1C, D). This species was distinguished from the closely related species, *S. misakiensis* (Iwasa, 1934), growing on the shell of living gastropods (Fig. 1A, B), by differences in the hydrorhizal shapes and in the kind of host animals (His Majesty the Showa Emperor Hirohito, 1988).

Recently, Miglietta *et al.* (2009) revealed that *S. inabai* is conspecific with *S. misakiensis* by DNA analysis. They supposed that the difference in hydrorhizal shape between *S. inabai* and *S. misakiensis* is caused by host replacement from the living gastropods to the hermit crabs (Miglietta *et al.*, 2009). The gap in hydrorhizal shapes between the museum specimens of the two nominal species, however, has not yet been bridged with respect to morphology (Fig. 1). This paper aims to confirm the continuous process of extension of hydrorhizae due to the replacement of host animals, based on morphological observations of newly-collected material. The experiments on host replacement were performed to obtain additional solid evidence.

Materials and Methods

Morphological observations

The morphological variation of hydrorhizae of specimens identified as *Stylactaria inabai* and *S. misakiensis* were examined based on materials newly obtained from Nabeta Bay, Izu Peninsula, and Tateyama Bay, Boso Peninsula, Japan. Specimens collected from Nabeta Bay in 2001 were kindly received from Dr. Yayoi Hirano.

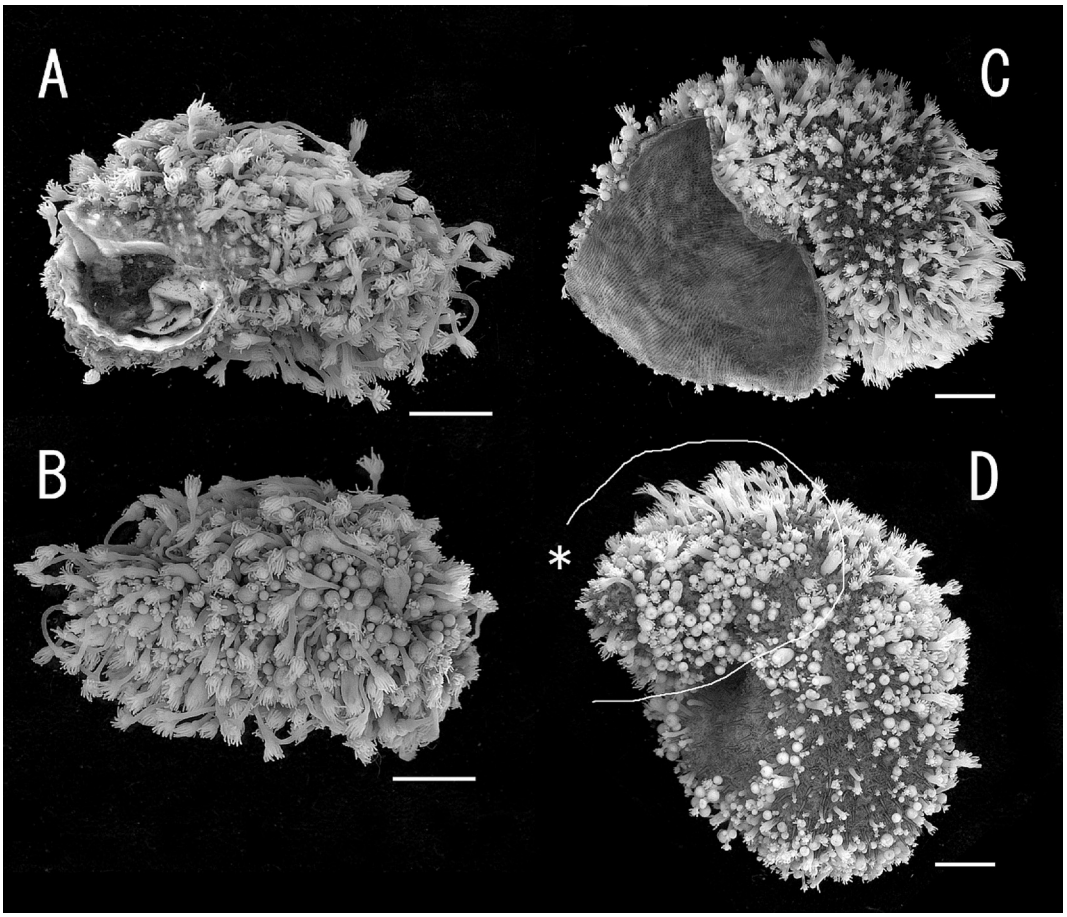

Fig. 1. Museum specimens labeled as *Stylactaria misakiensis* and *S. inabai*. A: a specimen of *S. misakiensis* (NSMT-Hy R3024), B: other side of A, C: holotype of *S. inabai* (NSMT-Hy R3888-I), D: other side of C. * in photo of D, the left part partitioned by a white line is the shell which gastropod-like type hydrorhiza originated in. Scale = 2 mm. 'NSMT-Hy R' is the abbreviation of the lot number applied in the hydroid collection deposited in the Showa Memorial Institute, the National Museum of Nature and Science.

Twelve specimens growing on gastropod shells inhabited by hermit crabs and eighteen on the shells of living gastropods were collected together by a trawl from 5–10 m depth in Nabeta Bay on 24 May 2001. Five specimens growing on gastropod shells inhabited by hermit crabs and ten on the shells of living gastropods were collected together by a dredge from 10–12 m depth in Tateyama Bay on 22 March 2008. The shells of living gastropods and those inhabited by hermit crabs were identified as *Reticunassa japonica* A. Adams, 1852 with reference to Tsuchiya (2001). The hermit crabs collected from Nabeta Bay were identified as *Pagurus minutus* Hess, 1865 by Dr. Masayuki Osawa and those collected from Tateyama Bay were identified as *Pagurus quinquelineatus* Komai, 2003 by Dr. Tomoyuki Komai.

Host replacement experiments

More than 50 colonies of *Stylactaria misakiensis*, growing on the shells of living *R. japonica*, were collected by traps baited with fish meat in Tateyama Bay from 2001 to 2006. They were kept in the laboratory by feeding the hydrozoans with *Artemia* (just after hatching) and the host

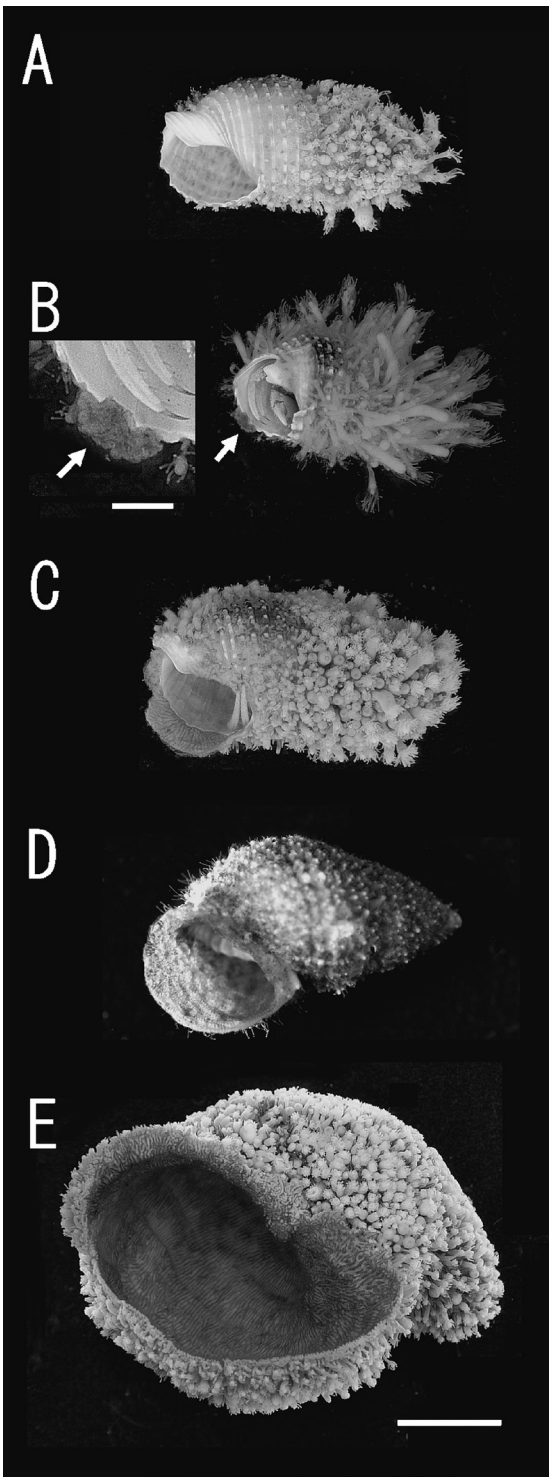

Fig. 2. Morphological variations of hydrorhizae growing on the shells of *Reticunassa japonica* inhabited by hermit crabs (*Pagurus minutus* (A–C), *P. quinquelineatus* (D–E)). Scale = 5 mm. Arrows in B indicate the hydrorhizae extending from edge of shell (Scale in the enlargement = 1 mm).

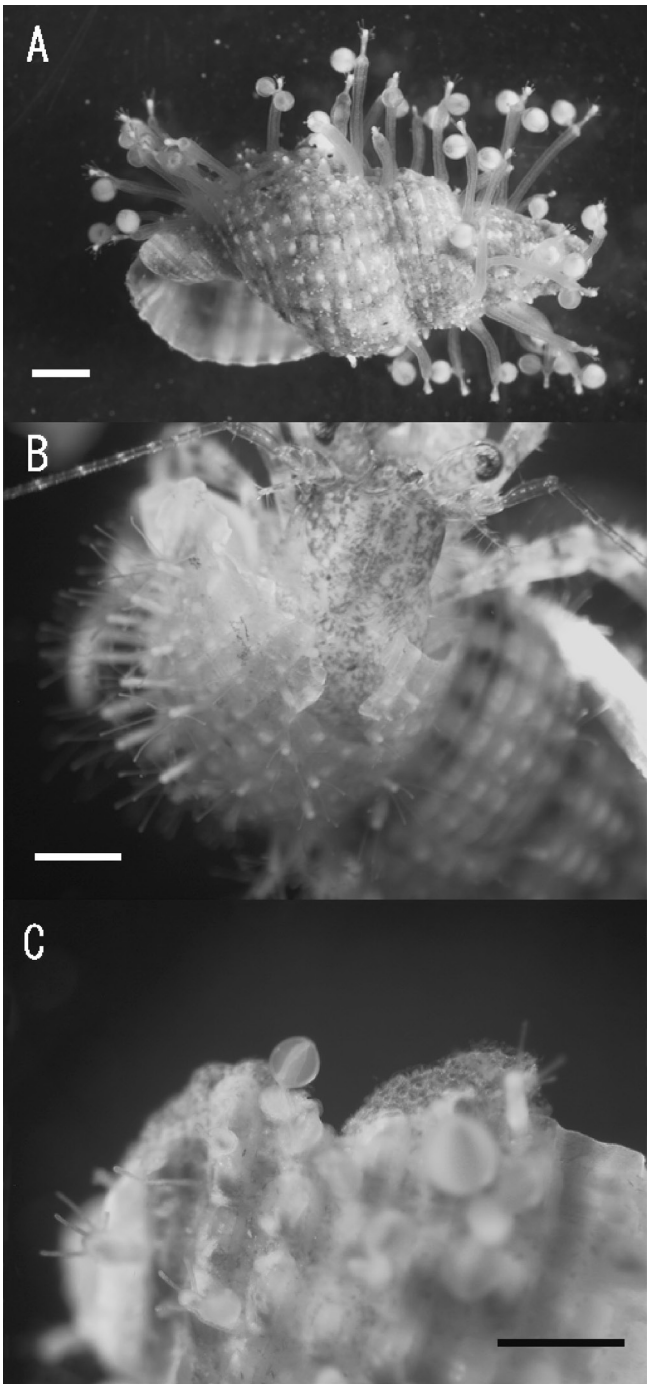

Fig. 3. An example of the host replacement examination. A: a shell of *Reticunassa japonica* with a hydroid colony on it after one month after the gastropod died. B: a shell of *R. japonica* with a hydroid colony on it just after insertion of *Pagurus minutus*. C: the extension of hydrorhizae of the hydroid colony over the edge of the shell after one month after insertion of *P. minutus* into the shell. Scale = 1 mm.

gastropods with solid food, TetraMin (Tetra Japan Corporation). During long-term culture in the laboratory, some host gastropods died accidentally while still having live hydrozoans on their shells and their soft body parts were scavenged by other gastropods (Fig. 3A). These empty shells of *R. japonica* with living hydrozoans were used for host replacement experiments. *Pagurus minutus* in the gastropod shells of *Batillaria multiformis* (with no hydroid colonies on them) were collected from the tidelands of Miura Peninsula, Japan and used as the new host in the host replacement experiments. Seventeen specimens of *P. minutus* were carefully fished out of the original gastropod shells and inserted into the empty shells with living hydrozoans during the period from 2003 to 2008. The morphological changes in the hydrorhizae were observed for a month while feeding *Artemia* to the hydrozoans and TetraMin to the hermit crabs. Ten hydrozoan colonies on empty shells were also observed from 2003 to 2008 without the insertion of hermit crabs for the same period while feeding *Artemia* as a control.

Results and Discussion

The newly-collected specimens of *Stylactaria* growing on gastropod shells inhabited by hermit crabs expressed various shapes of the extending hydrorhizae. These various shapes of hydrorhizae can be lined up to trace the formation process of hermit crab-shell-type to gastropod-shell-type hydrorhizae (Fig. 2).

Moreover, the host replacement experiments showed that 24% of hydrozoan colonies on the empty shells of *R. japonica* extended their hydrorhizae over the edge of their gastropod shell after inhabitation by *Pagurus minutus* for a month period, whereas they can not extend their hydrorhizae without the presence of *P. minutus* (Fig. 3 and Table 1). These results indicate that the gap in the hydrorhizal morphology of nominal *Stylactaria inabai* and *S. misakiensis* was due to a lack of material and corroborates the conclusion that *S. inabai* is synonym of *S. misakiensis*, as suggested by DNA analysis.

Associations between hydractiniid hydroids and paguriid hermit crabs are well-studied (summarized in Williamas and McDermott, 2004). Some hydractiniid species have tight symbiotic relationships with specific paguriid hermit crabs, such as *Hydractinia sodalis* (Stimpson) and *Pagurus constans* (Stimpson) (Rees, 1967). Rees (1967) indicated that larvae of *H. sodalis* can settle only on gastropod shells inhabited by young *P. constans* and metamorphose into polyps. On the other hand, the relationship between *Stylactaria misakiensis* and two host hermit crabs (*Pagurus minutus* and *P. quinquelineatus*) may not be so specific. In the field many individuals of these two *Pagurus* species have been observed utilizing the shells of various small gastropod species other than *R. japonica*. In these cases, the gastropod shells inhabited by the two *Pagurus* species have no hydrozoans on them, as was also the case with the shells of *Batillaria multiformis* inhabited by *P. minutus* that were used in the host replacement experiment. This indicates that, unlike for *H. sodalis*, the larvae of *S. misakiensis* can not settle on gastropod shells inhabited by the two *Pagurus* species. The host replacement experiments showed that either of the two *Pagurus* species has the ability to induce the her-

Table 1. Results of the host replacement experiments performed from 2003 to 2008.

Insertion of *Pagurus minutus* into empty shells of *Reticunassa japonica* with hydrozoans on them	Extension of hydrorhizae over the edge of gastropod shells*		Total
	+	—	
Yes	4	13	17
No	0	10	10

*This table shows the results after one month from the starts of each experiment.

mit crab-shell-type hydrorhizae in *Stylactaria misakiensis*, whenever they come across the empty shells of *R. japonica* with hydrozoans on them and inhabit the shells. In other words, *Stylactaria misakiensis* is the species originally growing on the shell of living *R. japonica* and its life is occasionally saved by inhabitation of either of the two *Pagurus* species after the shell has lost the living host gastropod.

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