

Induced Differentiation of Tentaculozooids in the Polymorphic Hydroid *Stylactaria misakiensis* (Hydractiniidae) by Competition with *Leuckartiara* sp. (Pandeidae) under Laboratory Conditions

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Abstract Zooid polymorphism in the hydrozoan species *Stylactaria misakiensis* (Hydractiniidae) includes tentaculozooids, in addition to gastrozooids and gonozooids. Laboratory experiments demonstrated that differentiation of such tentaculozooids was induced in response to stolon contact between the former and *Leuckartiara* sp. (Pandeidae) during interspecific competition for living space.

Key words: tentaculozooids, differentiation, stolon contact, *Stylactaria misakiensis*, *Leuckartiara* sp., laboratory experiments.

Introduction

A significant number of colonial hydrozoan species exhibit zooid polymorphism, comprising gastrozooids for feeding, gonozooids for sexual reproduction, and dactylozooids that are believed to have a defensive function (Bouillon *et al.*, 2006). The research interest in such polymorphism is due to its importance when considering the evolution of hydrozoan coloniality (Petersen, 1979; Cartwright and Nawrocki, 2010, and so on).

Tentaculozooids, a specialized form of dactylozooid observed in a polymorphic hydroid of *Stylactaria conchicola* (Hydractiniidae), was found to serve a defensive function during interactions with certain epifaunal space competitors (Namikawa *et al.*, 1992). Such tentaculozooids were observed engaging in defensive behavior along the border of the colony, attacking neighboring sessile animals in efforts to defend the territory of the former. Nevertheless, the differentiation mechanism of tentaculozooids in *S. conchicola* has remained elusive, largely due to

difficulty in laboratory rearing of specimens. However, during an early laboratory rearing experiment on spatial interspecific interactions between the hydroids *Stylactaria* sp. (Hydractiniidae) and *Leuckartiara* sp. (Pandeidae), the former had been observed differentiating tentaculozooids at the contact area of stolons of the two species. Approximately four decades later, those results have remained unpublished. However, the author was recently able to differentiate tentaculozooids in reproductive experiments involving a hydroid species identified as *Stylactaria misakiensis*, during studies conducted as part of a National Museum of Nature and Science project, entitled “Adaptive Trends, Evolution, and Modeling of Organisms to Respond to Natural and Artificial Environments.” *Stylactaria misakiensis* is known to possess tentaculozooids in addition to gastrozooids and gonozooids (Hirohito, 1988). Accordingly, the environmental factors that differentiate tentaculozooids in *S. misakiensis* (and also *Stylactaria* sp.) are described here, being a preliminary step towards elucidating the mechanism of differentiation of tentaculozooids, including gene expression.

Materials and Methods

The experimental studies were conducted in 1986–1987 (Study A) and 2022–2024 (Study B), focusing on the reproductive experiments conducted in Study A). The rearing conditions in these studies are shown in Table 1. In both studies, each clonal strain of *Stylactaria* sp. and *S. misakiensis*, and *Leuckartiara* sp. was treated as an original colony and reared in laboratory containers. The original colonies were maintained throughout the transplantation of newly differentiated zooids (i.e., daughter zooids) onto new glass dishes or styrol containers, at intervals of three to six months, thereby facilitating the formation of successive colonies. For both *Stylactaria* sp. (Study A) and *S. misakiensis* (Study B), both gastrozooids and gonozooids were observed to differentiate in their original colonies. However, tentaculozooids were not apparent. The hydroid considered in Study A (and regarded as *Stylactaria* sp.) is now most likely to have been *S. misakiensis*, due to the presence of an eumedusoid gonophore, being a sexual reproductive structure. However, because that identification remains tentative due to the lack of supporting background data, including the nature of the substrata utilized in the field, the Study A hydroid remains classified as *Stylactaria* sp., and reference to the combined hydroids of the two studies as *Stylactaria* spp. The opponent hydrozoans were identified as *Leuckartiara* sp., based on polyp morphology and that of newly liberated medusae.

Origins of colonies used for experiments

In Study A, one clonal colony of each hydrozoan species, *Stylactaria* sp. and *Leuckartiara*

sp., collected from the seacoast of Kagoshima City and subsequently maintained in the laboratory, was reared in glass dishes in the laboratory of the Department of Biology, Faculty of Science, Kagoshima University, Kagoshima Prefecture. These were treated as the original colonies for subsequent zooid transplantation. Unfortunately, the original colonies of both species used in Study A had degenerated by 1988.

The original colony of *Stylactaria misakiensis* used in Study B was derived from a specimen living on the shell of the gastropod *Reticunassa japonica* (Nassariidae), collected from the sandy-mud bottom of Nabeta Bay, Shimoda City, Shizuoka Prefecture, on June 16, 2001. The original colony of *Leuckartiara* sp. was derived from a specimen encrusting the shell of *Reticunassa japonica*, collected from the sandy-mud bottom off the coast of Futtsu City, Chiba Prefecture, on May 6, 2015. Zooids of both hydrozoans (equivalent to gastrozooids in *S. misakiensis*) were removed from the colonies living on each host shell and transplanted into styrol containers in a laboratory at the National Museum of Nature and Science, Tsukuba, so as to create new colonies under artificial conditions.

Interspecific competition observed between two hydroid species in the laboratory

Contact experiments to observe interspecific competition between the two hydroid species were conducted in which one-to-two week old daughter zooids of two hydrozoans (gastrozooid in *Stylactaria* spp.) were each transplanted from their original colony to the base of glass dishes (Study A) or a cover glass (30 × 39 mm in size) in styrol containers (Study B). The distance between the two zooids transplanted to each con-

Table 1. Comparison of experimental conditions between Studies A (1986–1987) and B (2022–2024).

	Study A	Study B
Experimental container	Glass dishes (φ9 cm × H6 cm)	Styrol containers (φ6 cm × H3 cm)
Rearing water	Distild natural sea water from Kagoshima Bay	Artificial seawater (Marine Art SF-1: Osakayakken. Co. Ltd, Osaka)
Temperature	20–25 °C	20–23 °C
Feeding	<i>Artemia</i> nauplii, once every 2 days	<i>Artemia</i> nauplii, once every 7 days
Water change	Water renewed daily	Water renewed after 3 and 24 hours from feeding

tainer was maintained at over 5 mm so as to prevent direct contact between their respective tentacles. The colonies were subsequently reared (following settlement of the transplanted zooids) under the conditions shown in Table 1. Colony growth and the distribution of tentaculozooids within them were observed under binocular microscope in three cases in Study A and 12 cases in Study B. The size of nematocysts of *Stylactaria misakiensis* was measured using a phase-contrast microscope.

Results and Discussion

Morphology and functions of tentaculozooids

The tentacle-like structures observed in this study, present in both *Stylactaria* sp. and *S. misakiensis*, exhibited a highly elastic filamentous morphology and were armed with nematocysts (microbasic eurytele type) at their tips (Fig. 1). Such nematocysts comprised only a large form, distributed in the gastrozooid and gonozooid hypostomes in *S. misakiensis* (Table 2). Moreover, the zooids of *Leuckartiara* sp. degenerated following contact with the tentacle-like structures of the two *Stylactaria* species under investigation (Figs. 2B, 3). These observations indicated that the tentacle-like structures of *Stylactaria* sp. and *S. misakiensis* served in a defensive role against the zooids of *Leuckartiara* sp., the former therefore being identified as true tentaculozooids, equivalent to those previously observed in a colony of *S. conchicola* (Nami-kawa *et al.*, 1992).

Environmental Factors responsible for differentiation of tentaculozooids

The present study demonstrated that tentaculozooids appeared in the colonies of *Stylactaria* sp. and *S. misakiensis* that were in contact with *Leuckartiara* sp. colonies. Closer observation showed that the tentaculozooids of *Stylactaria* sp. and *S. misakiensis* exhibited a tendency to differentiate around only those areas where their stolons encountered those of *Leuckartiara* sp. (Fig. 2, Table 3). Moreover, in *S. misakiensis*, the

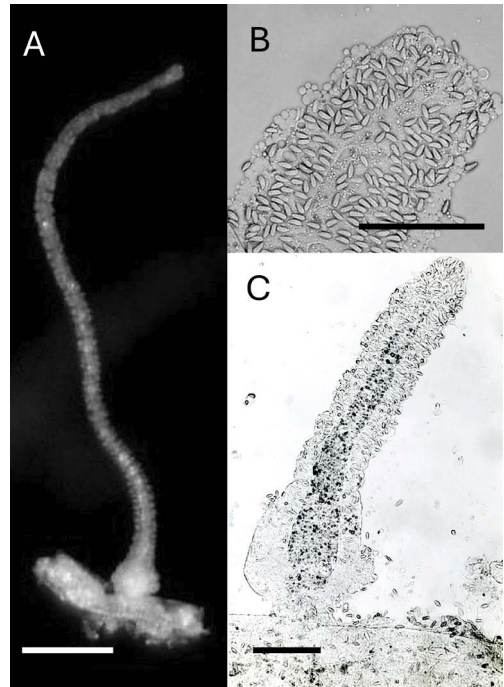


Fig. 1. Tentaculozooids detached from colonies of *Stylactaria* spp. A. An elongated tentaculozooid of *Stylactaria misakiensis*, B. The apical end of the tentaculozooid with densely packed nematocysts of large microbasic euryteles in *Stylactaria misakiensis*, C. A contracted tentaculozooid of *Stylactaria* sp. Scales = 0.5 mm (A), 0.05 mm (B), 0.1 mm (C).

zooids exhibited a tendency to differentiate in close proximity to daughter gastrozooids, which were close to the stolon contact points of the two species (Fig. 2B). Distances separating tentaculozooids from the closest gastrozooids were ca. $0.40 \text{ mm} \pm 0.22$ (0.1–0.9 mm) ($n = 43$ zooids/12 colonies). The appearance of tentaculozooids occurred approximately two days after stolon contact in *Stylactaria* sp. and seven days in *S. misakiensis*, i.e., differentiation of the tentaculozooids of *Stylactaria* sp. and *S. misakiensis* was induced by the contact stimuli of stolons of *Leuckartiara* sp. to stolons of the former, although minor differences existed in the experimental conditions between Studies A and B. All tentaculozooids persisted for 2 to 3 weeks after differentiation, before degeneration.

Table 2. Nematocyst composition of gastrozooids and tentaculozooids in *Stylactaria misakiensis*.

	n	Length (μm)	Width (μm)
Gastrozooids			
Tentacles			
desmonemes	30	3.0 ± 0.1 (2.9–3.1)	1.4 ± 0.05 (1.3–1.5)
microbasic euryteles	30	4.0 ± 0.4 (3.9–4.0)	1.4 ± 0.8 (1.3–1.5)
Hypostomes			
microbasic euryteles	30	5.0 ± 0.1 (4.8–5.1)	1.9 ± 0.1 (1.7–2.1)
Tentaculozooids			
microbasic euryteles	30	5.0 ± 0.1 (4.8–5.1)	2.0 ± 0.1 (1.8–2.1)

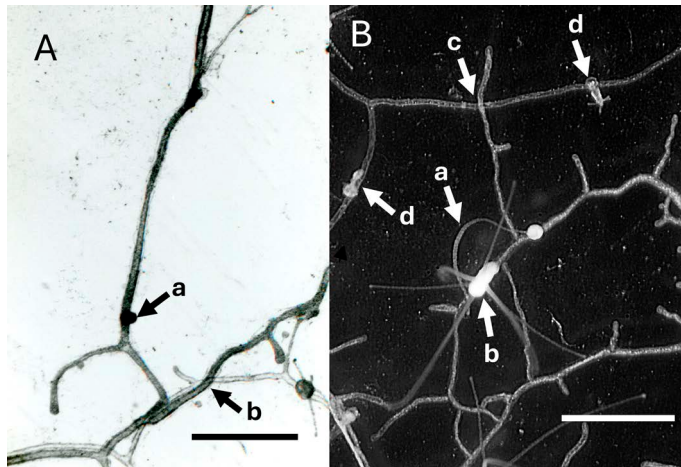


Fig. 2. Differentiated positions of tentaculozooids. A. A tentaculozooid bud (a) emerging in close proximity to the region of stolon contact (b) between *Stylactaria* sp. and *Leuckartiara* sp. in Study A. B. Fully developed tentaculozooids of *S. misakiensis* (a) situated in the vicinity of a daughter gastrozooid (b); stolon contact point (c) also indicated. *Leuckartiara* sp. zooids (d) degenerated after being attacked by this tentaculozooid. Scales = 0.5 mm.

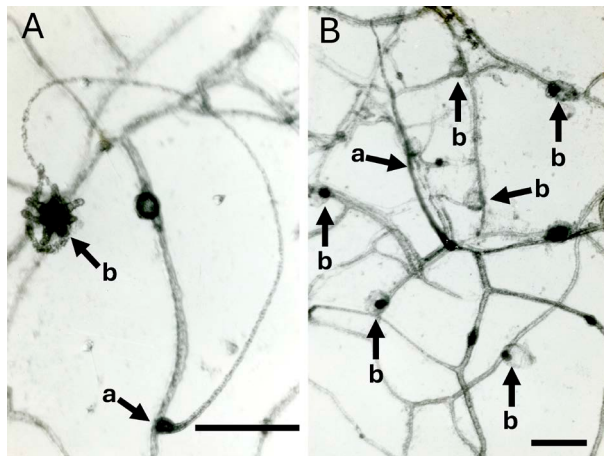


Fig. 3. The defensive function of tentaculozooids against *Leuckartiara* sp. zooids. A. A *Stylactaria* sp. tentaculozooid (a) attached to a *Leuckartiara* sp. zooid (b) in Study A. B. Degeneration of *Leuckartiara* sp. zooids (b) following attacks by a *Stylactaria* sp. tentaculozooid (a) in Study A. Scales = 0.5 mm.

Table 3. Distances separating tentaculozooids from closest contact points between *Stylactaria* spp. and *Leuckartiara* sp.

	n	Distances (mm)
<i>Stylactaria</i> sp.	15 zooids/3 colonies	0.33 $\pm$ 0.18 (0.1–0.8)
<i>S. misakiensis</i>	43 zooids/12 colonies	0.35 $\pm$ 0.21 (0.1–1.0)

In the family Hydractiniidae, intra- and inter-specific spatial competition has been the subject of extensive investigation in laboratory experiments using *Hydractinia symbiolongicarpus* (e.g., Buss *et al.*, 1984; McFadden, 1986). However, *H. symbiolongicarpus* has not been observed to produce tentaculozooids as a defense system against allogeneic colonies or colonies of the closely related *Podocoryna carnea* in laboratory experiments, although tentaculozooids have been documented in the field along the periphery of natural colonies of the former (Buss and Yund, 1989).

The above results indicated that hydractiniid hydrozoans may produce tentaculozooids as a defense system in competition with hydrozoans of different families, but not between closely related species, indicating that the defense system in hydractiniid species (dependent upon competitors) is of significant interest from an evolutionary perspective, particularly in regard to the evolution of coloniality in polymorphic hydrozoans. Further investigations of *S. misakiensis* are necessary for further clarification of the distinctive defense mechanisms utilized within Hydractiniidae.

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