

Newly Discovered Gastropod Hosts of Japanese *Cytaeis* Species (Cnidaria, Hydrozoa)

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(Received 7 December 2022; accepted 21 December 2022)

Abstract Colonial hydrozoans were newly found on the shells of five living gastropod species (*Nassarius albescens*, *N. splendidulus*, *Vexillum exasperatum*, *Argyropeza izekiana* and *Sagamilepeta sagamiensis*), previously unknown as hydrozoan hosts in Japan. The hydrozoans included four *Cytaeis* species, identified by a molecular analysis of mitochondrial 16S ribosomal DNA sequences, as follows: *C. kakinumae* on *N. splendidulus*, *Cytaeis* sp. 1 on *A. izekiana* and *S. sagamiensis*, *Cytaeis* sp. 2 on *N. albescens*, and *Cytaeis* sp. 3 on *V. exasperatum*. The findings showed that *Cytaeis* species can exploit shells belonging to different gastropod families, although demonstrating strong host specificity.

Key words: Japanese *Cytaeis*, host gastropods, 16S DNA.

Introduction

All anthoathecate hydrozoans belong to the genus *Cytaeis* (Cytaeidae) are recognized as epizoid species, growing exclusively on the shells of living gastropods, mainly nassariid species (Bouillon *et al.*, 2006; Prudkovsky *et al.*, 2016). These include five previously documented Japanese *Cytaeis* species (viz. *C. capitata*, *C. imperialis*, *C. kakinumae*, *C. nuda* and *C. uchidaei*), all growing on the shells of different gastropod species (Rees, 1962; Uchida, 1964; Hirohito, 1988; Namikawa and Deguchi, 2013; Namikawa, 2014; Namikawa and Kameda, 2019). Accordingly, *Cytaeis* species are considered as significant for elucidating the speciation process by host selection in hydrozoa. Therefore, it is important to list all *Cytaeis* species and their host gastropods as a basis for clarifying their

phylogenetic relationships.

Anthoathecate hydrozoans were newly found on the shells of five living gastropod species, previously unknown as hydrozoan hosts in Japan, in our several research to collect marine invertebrates between 2015 and 2021 (Fig. 1, Table 1). The hydrozoan specimens collected were identified as *Cytaeis* species, following a molecular analysis of mitochondrial 16S ribosomal DNA sequences. Their phylogenetic relationships are briefly discussed.

Materials and Methods

Hydrozoan specimens growing on the shells of five living gastropod species (*Nassarius splendidulus* (Dunker, 1846), *Nassarius albescens* (Dunker, 1846), *Vexillum exasperatum* (Gmelin, 1791), *Argyropeza izekiana* Kuroda, 1949 and *Sagamilepeta sagamiensis* (Kuroda and Habe, 1971)), were collected from several localities

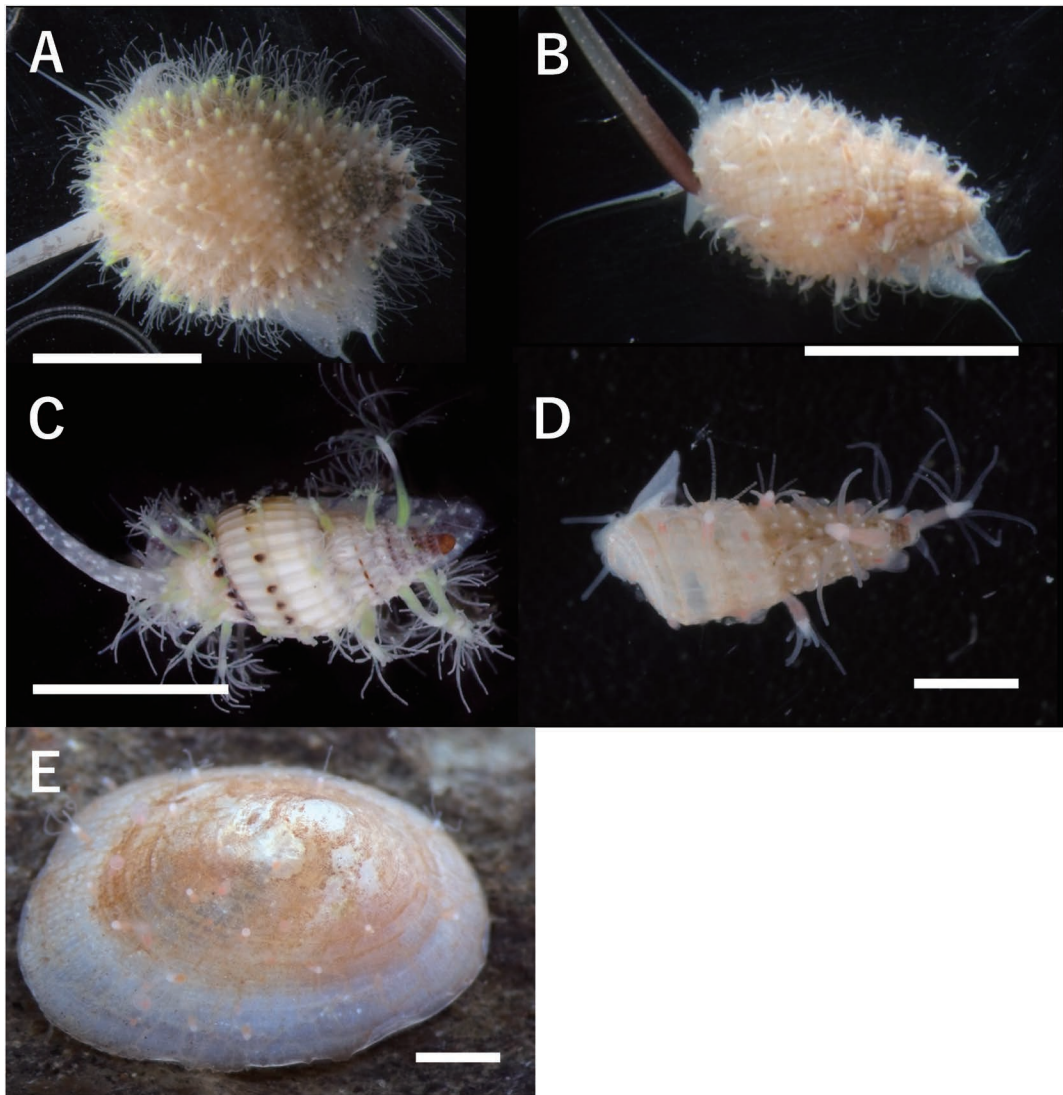


Fig. 1. Newly discovered five gastropod hosts of Japanese *Cytaeis* species. A. *Nassarius albescens*, B. *Nassarius splendidulus*, C. *Vexillum exasperatum*, D. *Argyropeza izekiana*, E. *Sagamilepeta sagamiensis*. Scale = 5 mm (A–C), 1 mm (D–E).

around Japan during 2015–2021 (for detailed sampling data see Table 1). The hydrozoan specimens on the shells were transported to and maintained alive in the laboratory in the National Museum of Nature and Science, Tsukuba, in order to obtain matured medusae which bore important characteristics for distinguishing between species. Culturing of the hydrozoan specimens and their host gastropods was performed following Namikawa and Kameda (2019), although fully matured medusae

were obtained only from hydrozoan specimens growing on the shells of *Nassarius splendidulus* and *N. albescens* (Fig. 2). Newly liberated medusae of hydrozoans on the remaining three gastropod hosts failed to grow, their very small sizes making them unable to eat the nutrients provided (nauplius of *Artemia* sp.). Therefore, identifications of these specimens were determined from sequences of a partial region of mitochondrial 16S ribosomal DNA being generally used for DNA

Table 1. Data of newly collected hydrozoan specimens sequenced in this study

DDBJ accession	Host gastropods	Locality	Depth	Date	Sampling methods
LC744778	<i>Nassarius splendidulus</i>	Off Kou-yatsu, Tateyama, Chiba, Japan	15–20 m	7 July, 2021	Dredge by R/V Seastar*
LC744779	<i>Nassarius splendidulus</i>	Off Saneku, Kakeroma-jima, Kagoshima, Japan	12–19 m	8 November, 2017	Scuba**
LC744780	<i>Nassarius albescens</i>	Rocky shore in vicinity of Tokashiki harbor, Tokashiki-jima, Okinawa, Japan	0 m	17 April, 2018	Baited trap**
LC744781	<i>Nassarius albescens</i>	Rocky shore in vicinity of Tokashiki harbor, Tokashiki-jima, Okinawa, Japan	0 m	17 April, 2018	Baited trap**
LC744782	<i>Vexillum exasperatum</i>	Southeast of Hatoma-jima, Okinawa, Japan	12–15 m	13 April, 2019	Scuba***
LC744783	<i>Vexillum exasperatum</i>	Southeast of Hatoma-jima, Okinawa, Japan	12–15 m	13 April, 2019	Scuba***
LC744784	<i>Argyropeza izekiana</i>	Off Minami-Izu, Shizuoka, Japan	100–110 m	12 November, 2015	Dredge by R/V Tsukuba II****
LC744785	<i>Argyropeza izekiana</i>	Off Minami-Izu, Shizuoka, Japan	100–110 m	12 November, 2015	Dredge by R/V Tsukuba II****
LC744786	<i>Sagamilepeta sagamiensis</i>	Off Jogashima, Miura, Kanagawa, Japan	90–100 m	20 January, 2015	Dredge by R/V Rinkaimaru****
LC744787	<i>Sagamilepeta sagamiensis</i>	Off Jogashima, Miura, Kanagawa, Japan	90–100 m	20 January, 2015	Dredge by R/V Rinkaimaru****

*: Survey by third author ("Seastar", research vessel of Ochanomizu University).

** : Surveys undertaken by KUROSHIO project of the National Museum of Nature and Science, Tsukuba.

***: Survey by third author and T. Naruse (Iriomote Station, the University of the Ryukyus).

****: Surveys performed by JAMBIO (the Japan Association for Marine Biology conducted by the University of Tsukuba and the University of Tokyo). ("Tsukuba II", research vessel of Shimoda Marine Research Center, the University of Tsukuba; "Rinkaimaru", research vessel of Misaki Marine Biological Station, the University of Tokyo).

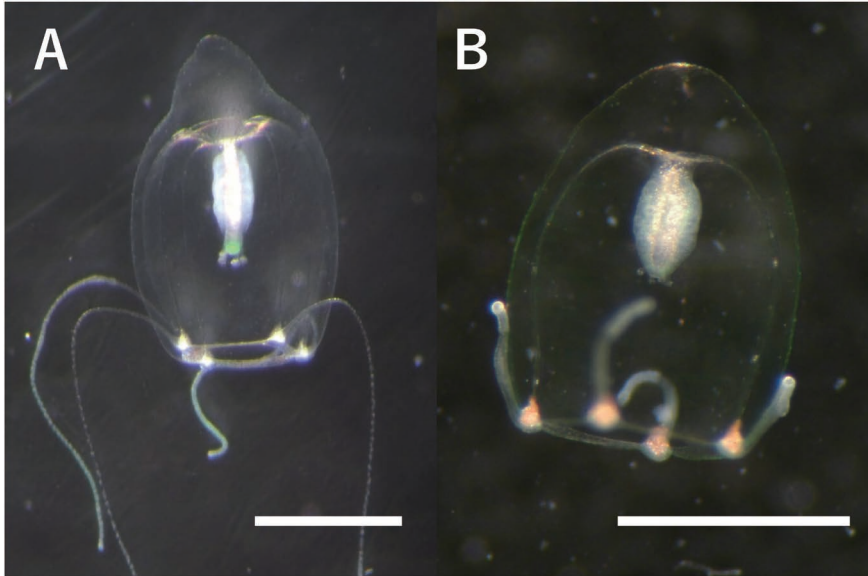


Fig. 2. Matured medusae of two *Cytaeis* species obtained in the laboratory. A. female medusa of *Cytaeis kakinumae* on *N. splendidulus* shell from Kakeroma-jima; B. female medusa of *Cytaeis* sp. 2 on *N. albescens* shell from Tokashiki-jima. Scale = 1 mm.

barcoding in hydrozoans (Schuchert, 2018).

Medusae just after liberation were used for the molecular analysis. A partial region of mitochondrial 16S ribosomal DNA was amplified using

the primers SHA (5'-ACGGAATGAACTCAAA TCATGT-3') and SHB (5'-TCGACTGTTTACC AAAACATA-3') (Cunningham and Buss, 1993). DNA extraction, polymerase chain reac-

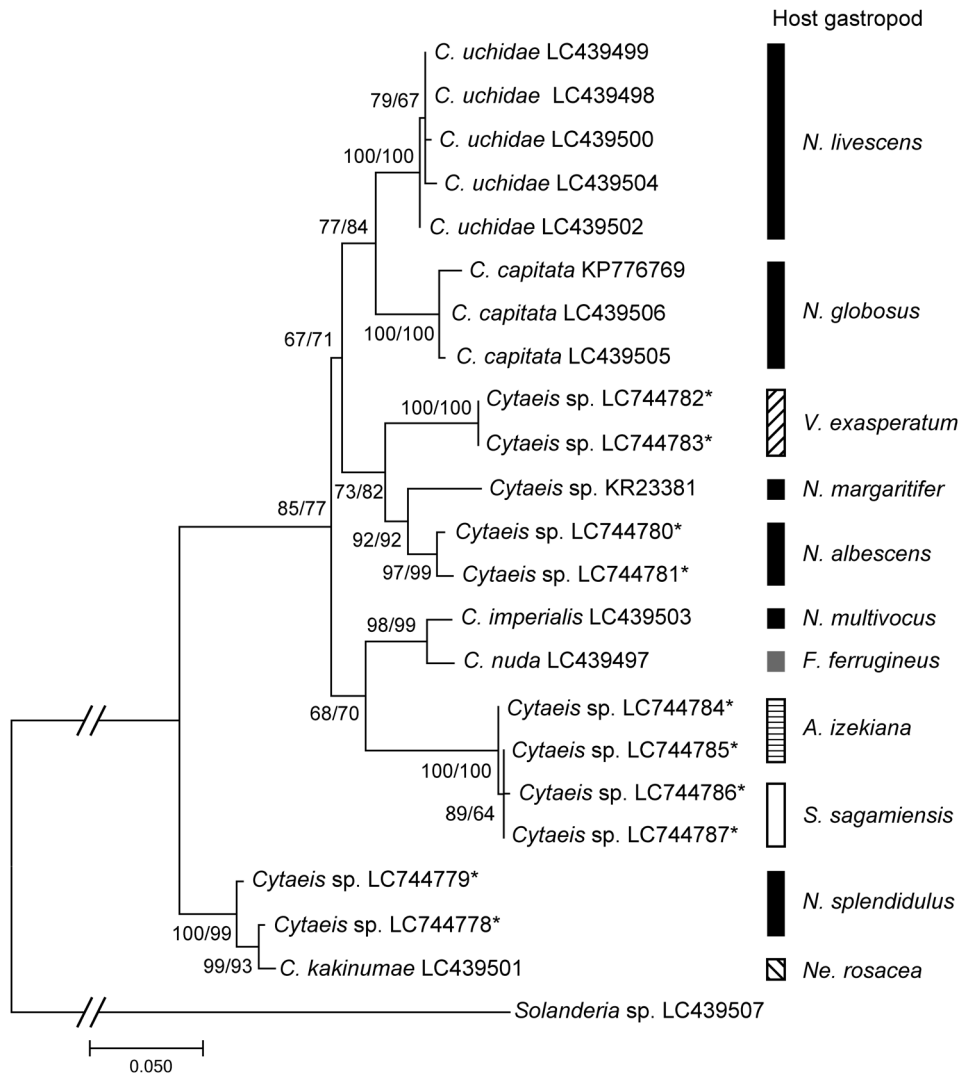


Fig. 3. Maximum likelihood tree showing phylogenetic relationships among *Cytaeis* species [tree based on 462bp of mitochondrial 16S rDNA sequences (-ln likelihood = 1707.1514)]. Numbers on branches indicate bootstrap values for MP and ML analyses (shown only for higher nodes with values >50). *: Sequences obtained in this study.

tion, and sequencing followed Namikawa and Kameda (2019), resulting in 16S sequences of 462bp being obtained. The nucleotide sequences have been submitted to the DNA Data Bank of Japan (DDBJ) under accession numbers LC744778–LC744787. The sequences obtained in this study and those previously known for *Cytaeis* species [Namikawa and Kameda (2019) and GenBank] were aligned using the Muscle method implemented in MEGA7 (Kumar *et al.*,

2016). Phylogenetic trees were obtained by maximum parsimony (MP) and maximum likelihood (ML) methods implemented in MEGA7. Based on selected model (GTR + I + G), MP and ML analyses were performed with subtree-pruning-regrafting-extensive branch swapping (SPR). Nodal support for the trees were assessed using bootstrap analyses with 1000 replications using MP and ML methods.

Table 2. List of host gastropods of *Cytaeis* species

Cytaeis species	Host gastropods	Localities	References
<i>C. capitata</i>	<i>Nassarius globosus</i>	Indonesia	Puce <i>et al.</i> , 2004
<i>C. imperialis</i>	<i>Nassarius multivocus</i>	Japan	Namikawa and Kameda, 2019
<i>C. kakinumae</i>	<i>Nebularia rosacea</i>	Japan	Uchida, 1964; Hirohito, 1988
	<i>Nassarius splendidulus</i>	Japan	Namikawa and Deguchi, 2013
<i>C. nassa</i>	<i>Nassarius albescens</i>	Red Sea	This study
	<i>Nassa arcularia</i>	Madagascar	Millard, 1975
	<i>Nassa coronata</i>	Mozambique	
	<i>Nassarius fenestratus</i>		
<i>C. niotha</i>	<i>Nassarius albescens</i>	Australia	Rees, 1962
<i>C. nuda</i>	<i>Fusinus ferrugineus</i>	Japan	Rees, 1962; Hirohito, 1988; Namikawa, 2014
<i>C. uchidae</i>	<i>Nassarius livescens</i>	Japan	Rees, 1962
<i>Cytaeis</i> sp. 1	<i>Argyropeza izekiana</i>	Japan	This study
	<i>Sagamilepeta sagamiensis</i>	Japan	
<i>Cytaeis</i> sp. 2	<i>Nassarius albescens</i>	Japan	This study
<i>Cytaeis</i> sp. 3	<i>Vexillum exasperatum</i>	Japan	This study
<i>Cytaeis</i> sp.	<i>Nassarius margaritifera</i>	Red Sea	Prudkovsky <i>et al.</i> , 2016

Results and Discussion

The hydrozoans growing on the shells of five newly collected living gastropod species included four species of *Cytaeis*, identified following partial sequencing of mitochondrial 16S ribosomal DNA. The analysis confirmed the identity of *Cytaeis kakinumae* Namikawa and Deguchi, 2013, found on *Nassarius splendidulus* and previously recorded on *Nebularia rosacea* in Japan (0.1%; Fig. 3). The identification was supported by the matured medusa morphology, characterized by a conical tip (Fig. 2A; fig. 2B–D in Namikawa and Deguchi, 2013). *Cytaeis* specimens on *Argyropeza izekiana* and *Sagamilepeta sagamiensis* showed very low genetic divergence (0.0%; Fig. 3) and are listed as *Cytaeis* sp. 1 in Table 2. In addition, a close relationship between *Cytaeis* spp. on *Nassarius albescens* (*Cytaeis* sp. 2) from Japan and *Nassarius margaritifera* (*Cytaeis* sp. in Prudkovsky *et al.*, 2016) from the Red Sea was also strongly supported by the genetic analysis (genetic divergence 0.4%; Fig. 3). *Cytaeis* sp. could not be further identified due to confusion of the morphological taxonomy of species from the Red Sea (viz. *Cytaeis nassa* and *C. tetrastyla*) (Prudkovsky *et al.*, 2016). Prudkovsky *et al.*, 2016 also indicated that reliable morphological characters of polyp features were too few for distinguishing between species of

Cytaeis. This was also the case for *Cytaeis* sp. 1, 2 and 3. in the present study. In particular, *Cytaeis* sp. 2 could not be further identified, even though matured medusae were obtained (Fig. 2B), due to two *Cytaeis* species, *C. nassa* (Millard, 1959) and *C. niotha* (Pennycuik, 1959), without information on their matured medusa morphology and sequence data, have been previously found on *N. albescens* collected from Madagascar (the former), and Australia (the latter) (Table 2).

Acknowledgements

We wish to express our sincere gratitude to Dr. G. S. Hardy (New Zealand) for his critical review of our manuscript. We are also grateful to Drs. H. Nakano (Univ. of Tsukuba), M. Kiyomoto (Ochanomizu Univ.), T. Akiyama (Okayama Univ.), T. Fujii (Kagoshima City Aquarium), T. Naruse (Univ. of the Ryukyus), and H. Saito and H. Komatsu (National Museum of Nature and Science), in addition to Staff of the Shimoda Marine Research Center of Univ. of Tsukuba, the Misaki Marine Biological Station, Univ. of Tokyo, the Tateyama Marine Laboratory, Institute for Marine and Coastal Research, Ochanomizu Univ., and the Tropical Biosphere Research Center, Iriomote Station, Univ. of the Ryukyus, S. Yokoyama (Amami-Oshima Island)

and JAMBIO personnel, for their generous help in collecting specimens.

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