Bull. Natl. Mus. Nat. Sci., Ser. A, 49(1), pp. 1–6, February 22, 2023
DOI: 10.50826/bnmnszool.49.1_1

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

Hiroshi Namikawa[1]*, Yuich Kameda[2] and Ryuta Yoshida[3]

[1] Department of Zoology, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan
[2] Department of Anthropology, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan
[3] Tateyama Marine Laboratory, Institute for Marine and Coastal Research, Ochanomizu University
Kou-yatsu 11, Tateyama, Chiba 294–0301, Japan
*E-mail: namikawa@kahaku.go.jp

(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* (Cytaeididae) are recognized as epizoic 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. uchidae*), 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

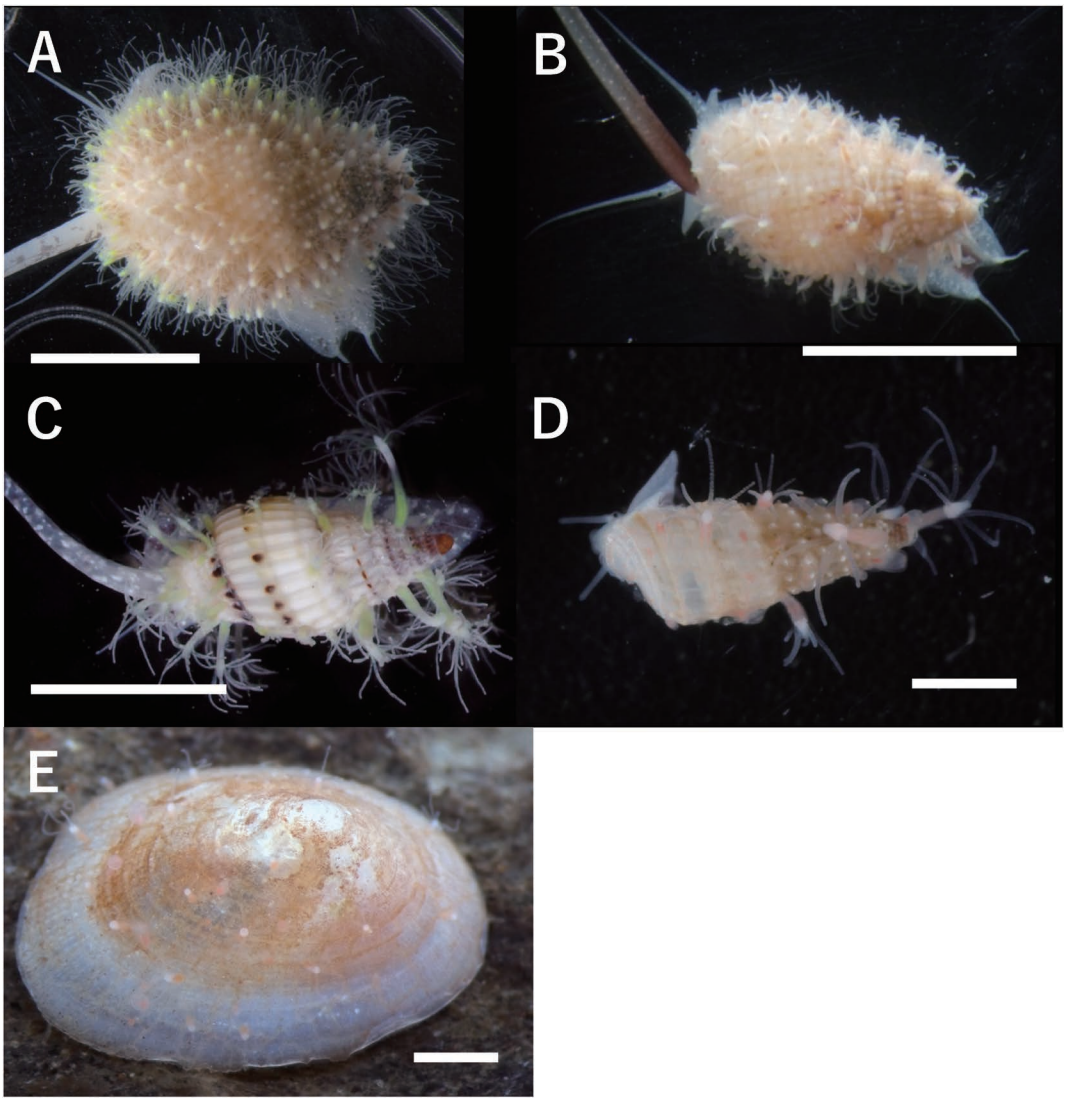

Fig. 1. Newly discovered five gastropod hosts of Japanese *Cytaeis* species. A. *Nassarius albescens*, B. *Nassarius splendidulus*, C. *Vexillum exasperatum*, D. *Argyropeza izekiana*, E. *Sagamilepeta sagamiensis*. Scale = 5 mm (A–C), 1 mm (D–E).

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

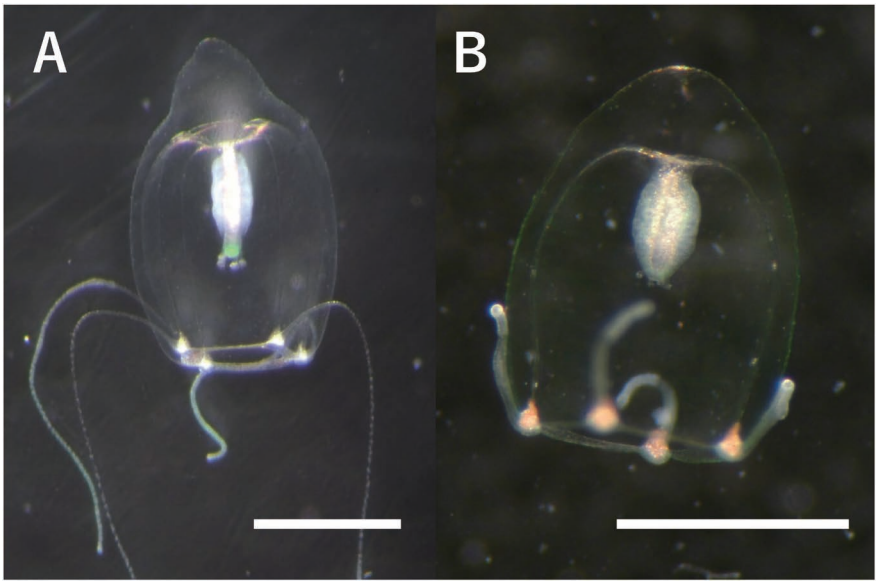

Fig. 2. Matured medusae of two *Cytaeis* species obtained in the laboratory. A. female medusa of *Cytaeis kakinumae* on *N. splendidulus* shell from Kakeroma-jima; B. female medusa of *Cytaeis* sp. 2 on *N. albescens* shell from Tokashiki-jima. Scale = 1 mm.

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 AAAAACATA-3′) (Cunningham and Buss, 1993). DNA extraction, polymerase chain reac-

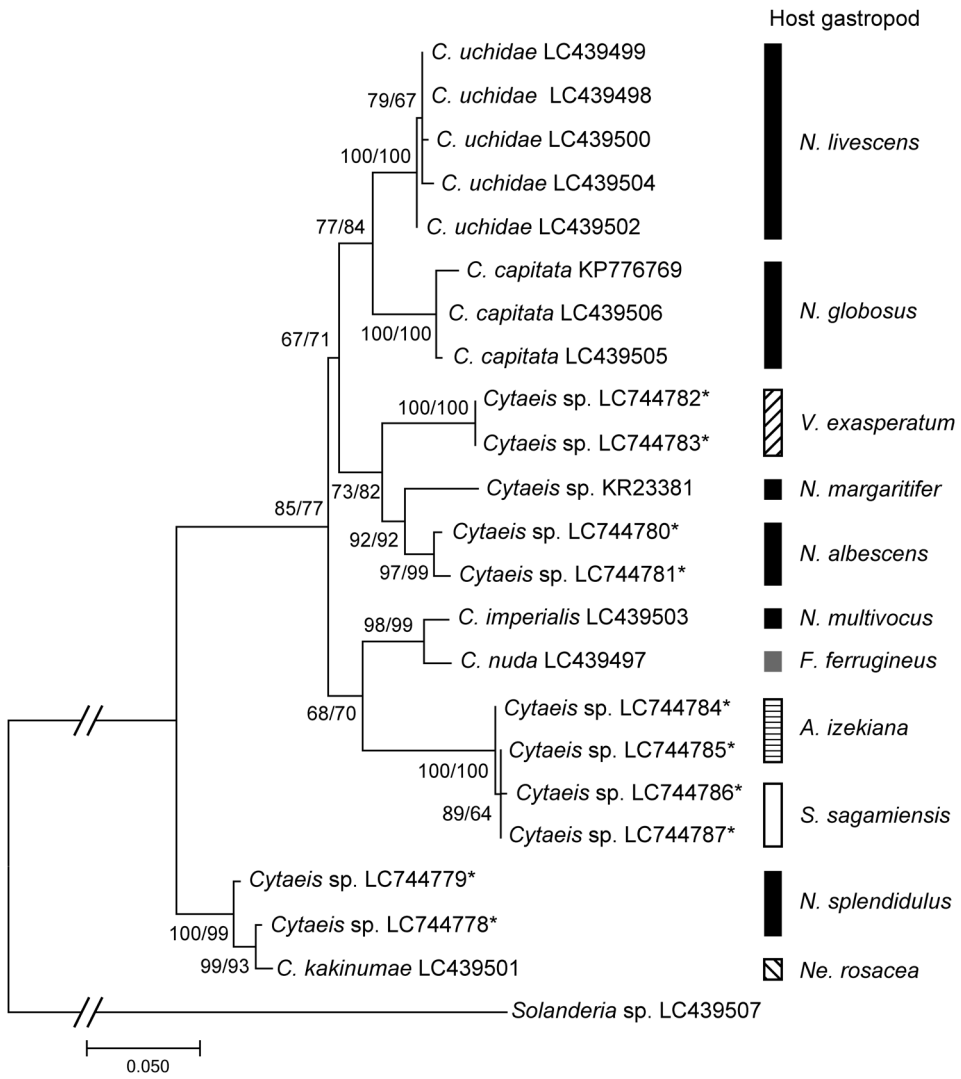

Fig. 3. Maximum likelihood tree showing phylogenetic relationships among *Cytaeis* species [tree based on 462 bp of mitochondrial 16S rDNA sequences (-ln likelihood = 1707.1514)]. Numbers on branches indicate bootstrap values for MP and ML analyses (shown only for higher nodes with values ＞50). *: Sequences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

tion, and sequencing followed Namikawa and Kameda (2019), resulting in 16S sequences of 462 bp 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 *Cytaies* species

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

References

Bouillon, J., C. Gravili, F. Pages, J.-M. Gili and F. Boero 2006. An introduction to Hydrozoa. Memoires du Museum national d'Histoire naturelle 194: 1–591.

Cunningham, C. W. and L. W. Buss 1993. Molecular evidence for multiple episodes of paedomorphosis in the family Hydractiniidae. Biochemical Systematics and Ecology 21: 57–69.

Hirohito, His Majesty the Showa Emperor 1988. The hydroids of Sagami Bay. Biological Laboratory, Imperial Household, Tokyo. 179 pp. (English text) + 110 pp (Japanese text).

Kumar, S., G. Stecher and K. Tamura 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.

Millard, N. A. H. 1975. Monograph on the Hydroida of southern Africa. Annals of the South African Museum 68: 1–513.

Namikawa, H. 2014. *Cytaeis nuda* Rees, 1962 (Cnidaria: Hydrozoa) from Toyama Bay, Japan. National Museum of Nature and Science Monographs 44: 23–27.

Namikawa, H. and R. Deguchi 2013. A new species of the genus *Cytaeis* (Cnidaria, Hydrozoa) from Tateyama Bay, Japan. Bulletin of the National Museum of Nature and Science, Series A 39: 63–67.

Namikawa, H. and Y. Kameda 2019. *Cytaeis capitata* Puce *et al.*, 2004 (Cnidaria, Hydrozoa) newly recorded from Amami-Oshima Island, Southwestern Japan. Bulletin of the National Museum of Nature and Science, Series A 45: 1–5.

Prudkovsky, A. A., V. N. Ivanenko, M. A. Nikitin, K. A. Lukyanov, A. Belousova, J. D. Reimer and M. L. Berumen 2016. Green fluorescence of *Cytaeis* hydroids living in association with *Nassarius* gastropods in the Red Sea. PLoS ONE 11: e0146861. doi:10.1371/journal.pone.0146861.

Puce, S., A. Arillo, C. Cerrano, R. Romagnoli and G. Bavestrello 2004. Description and ecology of *Cytaeis capitata* n. sp. (Hydrozoa, Cytaeididae) from Bunaken Marine Park (North Sulawesi, Indonesia). Hydrobiologia, 530/531: 503–511.

Rees, W. J. 1962. Hydroids of the family Cytaeidae L. Agassiz, 1862. Bulletin of the British Museum (Natural History) Zoology 8: 381–400.

Schuchert, P. 2018. DNA barcoding of some Pandeidae species (Cnidaria, Hydrozoa, Anthoathecata). Revue Suisse de Zoologie 125: 101–127.

Uchida, T. 1964. A new hydroid species of *Cytaeis*, with some remarks on the interrelationships in the Filifera. Publication of the Seto Marine Biological Laboratory 12: 133–144.