ZOOLOGIA
An International Journal for Zoology

XV International Symposium on Flatworm Biology
CEBIMar, BRAZIL, 2023

ZOOLOGIA 41: e23080
ISSN 1984-4689 (online)

scielo.br/zool

RESEARCH ARTICLE
PROCEEDINGS OF THE XV ISFB

Proseriate turbellarians (Platyhelminthes: Rhabditophora) in the egg mass of the commercially important horsehair crab, *Erimacrus isenbeckii* (Crustacea: Decapoda), from Hokkaido, Japan

Robert K. Okazaki[1], **Hiroshi Kajihara**[2]

[1]*Department of Zoology, Weber State University. 1415 Edvalson Street, Dept. 2505, Ogden, UT 84408-2505, USA.*
[2]*Department of Natural History Sciences, Graduate School of Science, Hokkaido University, N10 W8, Sapporo 060-0810, Japan.*
Corresponding author: Hiroshi Kajihara (kajihara@eis.hokudai.ac.jp)

https://zoobank.org/BEBECC95-F810-49BA-81ED-77343C8F96DC

ABSTRACT. We report a complex community of ectosymbionts in the egg mass of the horsehair crab *Erimacrus isenbeckii* (Brandt, 1848) from Hokkaido, Japan. We examined eight individual crab pleopods containing eggs and 56 pleopods without eggs. The following ectosymbionts were found: 207 turbellarians, 22 copepods, two nematodes, two ciliates, and one nemertean. Our preliminary morphological observation and molecular phylogenetic analysis based on partial sequences of the 28S rDNA suggested that the turbellarians would belong to the genus *Ectocotyla* Hyman, 1944, which was nested within Monocelidinae in a maximum-likelihood phylogenetic tree along with *Minona* Marcus, 1946, *Monocelis* Ehrenberg, 1831, and *Pseudomonocelis* Meixner, 1943, members of which commonly possess the simplex type of male copulatory bulbs. Further studies on the feeding biology of this monocelidid may lead to its possible impact on the crab egg mass community and/or the host crab's population dynamics. This study also reveals the crustacean egg mass as a unique habitat for turbellarians.

KEY WORDS. Lithophora, marine invertebrates, Monocelididae, Pacific, Proseriata, Rhabditophora.

INTRODUCTION

Turbellarians are generally considered free-living and found in many different habitats from aquatic to terrestrial (Angus 2007, Schockaert et al. 2008, Sluys 2019). One unique habitat in which turbellarians have been found is crustaceans. Fleming and Burt (1978a, 1978b) observed three species of monocelidid proseriates, *Ectocotyla hirudo* (Levinsen, 1879), *E. multitesticulata* Fleming & Burt, 1987, and *Peraclistus oofagus* (Friedmann, 1924), on the snow crab *Chionoecetes opilio* (Fabricius, 1788) and the spider crab *Hyas araneus* (Linnaeus, 1758). Fleming et al. (1981) observed that turbellarians associated with crabs have long and closer relationships with their hosts in contrast to turbellarians found on mussels and clams. McDermott et al. (2010), however, discovered *Ec. hirudo* to be a facultative symbiont of hermit crabs, *Pagurus pubescens* Krøyer, 1838 and *Parapagurus pilosimanus* Smith, 1879, perhaps due to short life histories of the host crabs.

The horsehair crab *Erimacrus isenbeckii* (Brandt, 1848) is a commercially important fishery in Hokkaido, Japan (Abe 1992). Nagao et al. (1999) reported that in the lab, embryogenesis of horsehair crab was observed to be about 12 months and estimated even longer durations in the field where water temperatures are lower. Ovigerous female *Er. isenbeckii* brood their eggs for approximately 18 months in the field (Abe 1992). We hypothesized that the long brooding durations would be conducive to the existence of a complex community of ectosymbionts. To test our hypothesis, we performed a survey, which yielded a number of ectosymbionts including turbellarians. Our aims in this paper are i) to report the composition of the *Er. isenbeckii* egg-mass symbionts and ii) to identify the obtained turbellarians to the lowest possible taxonomic rank.

MATERIAL AND METHODS

Eight ovigerous horsehair crabs were collected by the Hokkaido Mariculture Fisheries Research Institute in Muroran, Hokkaido. When we started our survey on 11 March, 2016, eggs from seven crabs were unexpectedly hatching. Using a Nikon SMZ-10 dissecting microscope, we examined one ovigerous female with eight pleopods with eggs. The eight pleopods without eggs of the remaining seven crabs that experienced host eclosion (hatching) were then observed systematically for ectosymbionts. Photographs of the ectosymbionts were taken with a Nikon D5200 digital camera.

Two individuals of the ectosymbiontic turbellarians were examined under an Olympus BH-2 compound microscope and photographed with the same digital camera; these were anesthetized in a $MgCl_2$ solution isotonic to seawater, slightly pressed under a coverslip, destroyed after examination, and thus not preserved. The rest of the ectosymbiontic turbellarians were fixed in either 10% formalin seawater or 99% ethanol.

Total DNA was extracted from one of the ethanol-fixed ectosymbiontic turbellarians using DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. A partial region of the 28S rDNA was PCR amplified using the primer pair LSU5 and LSU3 (Littlewood 1994) and sequenced with the same primer pair, in addition to the internal primers D2F (Littlewood 1994) and 28z (Hillis and Dixon 1991); the thermal cycling condition followed that of Kajihara et al. (2011). The 1053-bp sequence has been deposited in DDBJ/EMBL/GenBank.

As a MegaBLAST search (Morgulis et al. 2008) with the 1053-bp partial 28S rDNA sequence at the National Center for Biotechnology Information website (https://blast.ncbi.nlm.nih.gov/) suggested an affiliation of the species in question to the Monocelididae (Proseriata: Lithophora), a maximum-likelihood (ML) analysis was performed using IQ-TREE ver. 2.2.2.7 (Minh et al. 2020) to see its phylogenetic position within this family. For this purpose, a dataset of 54 monocelidid 28S rDNA sequences available in GenBank was compiled, represented by 10 monocelidid genera (Appendix 1); two archimonocelidid species were chosen as the outgroup, since Archimonocelididae and Monocelididae have been shown to be sister taxa in recent molecular phylogenetic works (e.g., Curini-Galletti et al. 2010, Scarpa et al. 2017). After the newly determined one was added, the sequences were aligned with MAFFT ver. 7 online version employing the 'E-INS-i' iterative refinement method (Katoh et al. 2019). Uncertainly aligned sites were removed with ClipKIT (Steenwyk et al. 2020), resulting in a 1694-bp dataset, of which 758 were parsimony-informative. The best-fit model was assessed with ModelFinder (Kalyaanamoorthy et al. 2017) (by the '-m MFP' option), which suggested the GTR + F + R5 model according to Bayesian Information Criterion. Branch supports were assessed by UFBoot with 1000 replicates (Hoang et al. 2018) using the '-bnni' option to reduce the risk of overestimating nodal supports.

RESULTS

The following five groups of ectosymbionts were found: turbellarians, copepods, nematodes, ciliates, and nemertean (Table 1). The highest number of ectosymbionts were the 207 turbellarians found generally at the base of the pleopods while only a single nemertean was observed.

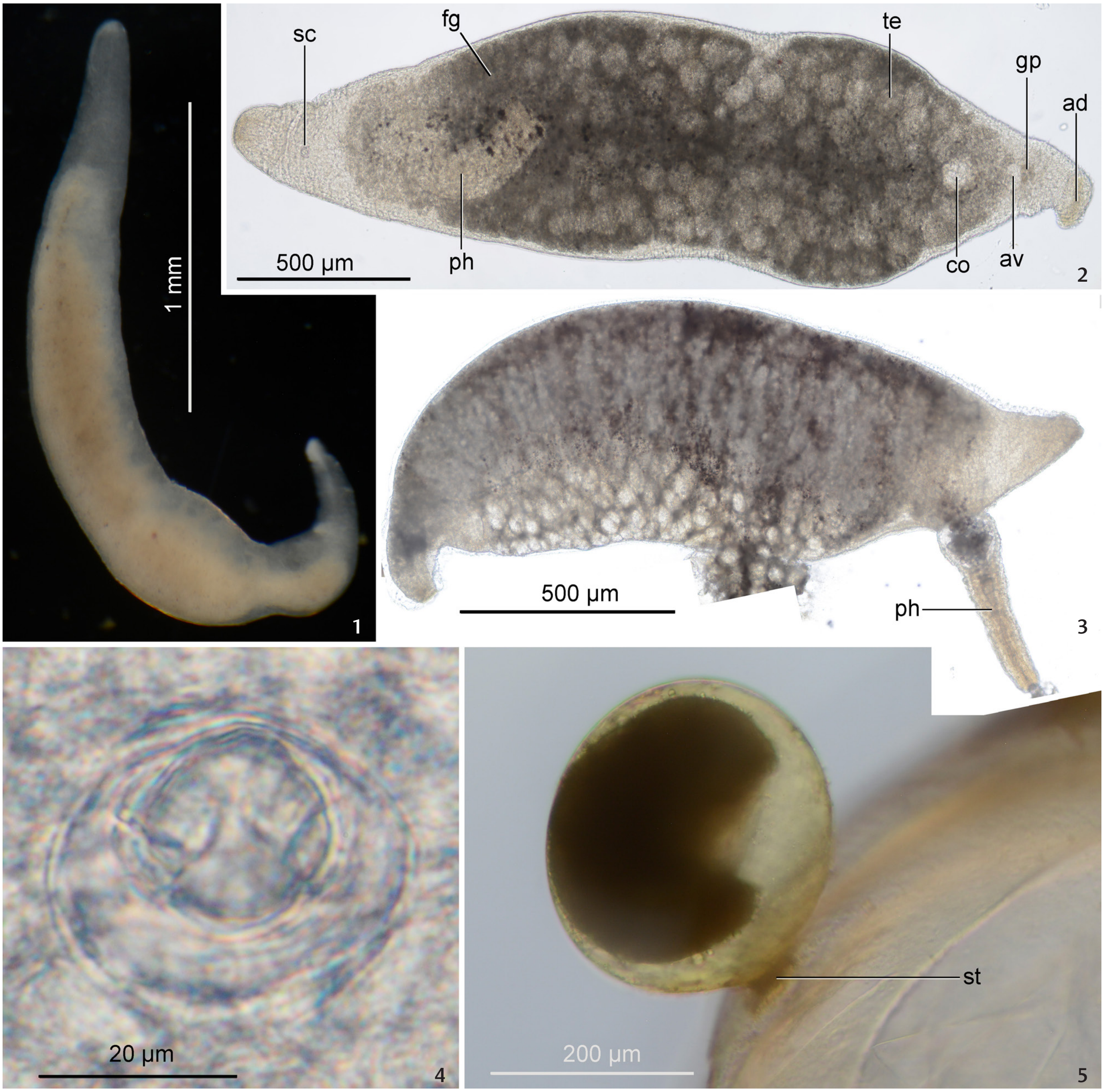
Table 1. Numbers of ectosymbionts in the egg mass and pleopods of eight individuals of *Erimacrus isenbeckii*.

Crab	Pleopod	Turbellarian	Copepod	Nematode	Ciliate	Nemertean
#1	L1	5	–	–	–	–
#1	L2	129	19	2	2	1
#1	L3	1	–	–	–	–
#1	L4	1	–	–	–	–
#1	R1	27	2	–	–	–
#1	R2	–	1	–	–	–
#1	R3	5	–	–	–	–
#1	R4	1	–	–	–	–
#2	n.a.	2	–	–	–	–
#3	n.a.	–	–	–	–	–
#4	n.a.	17	–	–	–	–
#5	n.a.	17	–	–	–	–
#6	n.a.	2	–	–	–	–
#7	n.a.	–	–	–	–	–
Total		207	22	2	2	1

(L) Left, (R) Right, (n.a.) not available.

The turbellarians were represented by *Ectocotyla* sp. (see Discussion for genus identification). They were whitish in color, about 3 mm in length and 370 µm in width when fully stretched (Fig. 1). They were highly contractile and mostly attached themselves to a substrate with the posterior end. The pharynx is situated anterior to gonads (Fig. 2); when protruded by pressure under a cover slip, it was 430 µm long (Fig. 3). The testes (~90 in number) were 55–100 µm in longer diameter (average 77 µm, n = 10), measured from a squeezed preparation (Fig. 2). The statocyst, about 30–37 µm in diameter, contains a single statolith with a diameter of 20 µm (Fig. 4). In one of the two specimens examined

(Fig. 2), putative female gonads (either representing ovaries or vitellaries) were found anterior to the testes; the copulatory apparatuses (accessory vesicle, copulatory organ, and gonopore) were also found in this specimen. In the other specimen (Fig. 3), no trace of female gonad and copulatory apparatus was found. An egg capsule (~300 µm in diameter) containing a developing young was found attaching to an empty crab eggshell with a stalk (Fig. 5).



Figures 1–5. *Ectocotyla* sp.: (1) living worm; (2) composite photomicrograph of a squeezed live specimen, dorsal view, head to the left; (3) composite photomicrograph of a squeezed live specimen, lateral view, head to the right; the body is ventrally ruptured; (4) statocyst in the same individual as (2), anterior to the top; (5) egg capsule attaching to an empty crab eggshell with a stalk, containing a developing young. Abbreviations: (ad) adhesive disk, (av) accessory vesicle, (co) copulatory organ, (fg) putative female gonad, either an ovary or vitellary, (gp) gonopore, (ph) pharynx, (sc) statocyst, (st) stalk, (te) testis.

In the resulting ML tree (Fig. 6), *Ectocotyla* sp. was nested in Monocelidinae sensu Litvaitis et al. (1996) along with species in *Minona* Marcus, 1946, *Monocelis* Ehrenberg, 1831, and *Pseudomonocelis* Meixner, 1943, members with the simplex type of male copulatory bulbs. However, due to low support values especially in some basal branches within Monocelidinae, the sister-taxon relation of *Ectocotyla* sp. was not fully resolved in the present analysis (Fig. 6).

DISCUSSION

To our knowledge, this was the first survey of ectosymbionts associated with the eggs of horsehair crab, *Erimacrus isenbeckii*. This study confirmed our hypothesis of a complex community consisting of turbellarians, nematodes, copepods, ciliates, and a nemertean in the egg mass of the horsehair crab.

We identified the present ectosymbiotic turbellarians as belonging to *Ectocotyla* Hyman, 1944, chiefly based on i) the position of the pharynx situated anterior to the testes and ii) the presence of posterior adhesive disk, and somewhat also on iii) the ectosymbiotic nature on decapod crustaceans. Our identification must be regarded as tentative since further examination of the reproductive-organ morphology—which was not performed in our study—is necessary for preciseness (cf. Fleming and Burt 1978a). Still, the phylogenetic position of our taxon being nested within Monocelidinae sensu Litvaitis et al. (1996) (Fig. 6) would render some support to our genus identification, as taxonomic closeness between *Ectocotyla* and *Monocelis*/*Minona* has earlier been suggested based on morphology (e.g., Marcus 1949, Karling 1966, summarized in Fleming and Burt 1978a).

Among Monocelididae, *Ectocotyla* and *Peraclistus* Steinböck, 1932 are known to include members that are symbiotic to decapod crustaceans (Fleming and Burt 1978a, 1978b). Members of *Ectocotyla* were previously found on the host decapods' body surfaces including the mouthparts, the gills, the gill chambers, and, in the case of anomuran hosts, in shells (e.g., Levinsen 1879, Hyman 1944, Fleming and Burt 1978a, Petrov 2000).

Our study might have represented the first finding of *Ectocotyla* flatworms among the decapod egg mass. Steinböck (1932: 328–329) reported the turbellarian "*Coleophora chionoecetis*" [nomen nudum] on the eggs of the spider crab *Chionoecetis opilio* and another turbellarian *Peraclistus oofagus* under the abdomen of the crab *Hyas araneus* (reviewed by Gordon 1966, Fleming and Burt 1978b, Kuris 1991). Fleming and Burt (1978b) suspected that "*Coleophora chionoecetis*" would be synonymous with *Peraclistus oofagus.*

In his comprehensible review of crustacean brood mortality due to egg predators, including turbellarians, Kuris (1991: 129) reported personal observations of egg contents of northern kelp crab, *Pugettia producta* (Randall, 1840) in the gut of "cf. *Monocelis*". Williams and McDermott (2004) also expressed the idea that these turbellarian symbionts may consume the host's eggs. Currently, however, we do not have any direct evidence of the nutrition of the present *Ectocotyla* sp. Further studies on the feeding biology of this monocelidid may lead to its possible impact on the crab egg mass community as well as the host crab's population dynamics.

Fleming and Burt (1978a) differentiated the two genera by the relative position of the pharynx, either anterior (*Ectocotyla*, as in our material) or posterior (*Peraclistus*) to the gonads. Two species are currently considered valid in *Ectocotyla*, *Ec. hirudo* (Levinsen, 1879) (with the type species *Ec. paguri* Hyman, 1944 being a junior synonym) and *Ec. multitesticulata* Fleming and Burt, 1978 (Fleming and Burt 1978a). The two species are distinguished by the body size (up to 2.0 mm long in *Ec. hirudo*; up to 8.2 mm long in *Ec. multitesticulata*), the testis size (49–67 × 33–49 µm in *Ec. hirudo*; 67–100 × 33–67 µm in *Ec. multitesticulata*), the number of the layers of the testes (one in *Ec. hirudo*; four in *E. multitesticulata*), and the egg size (320–490 µm in *Ec. hirudo*; 650–900 µm in *Ec. multitesticulata*). The present *Ectocotyla* sp. has a single statolith (Fig. 4), while Fleming and Burt (1978a) mentioned two statoliths in *Ec. hirudo* and *Ec. multitesticulata*. Proseriate turbellarians nominally have a single statolith. The number of statoliths found in the two *Ectocotyla* species reported by Fleming and Burt (1978a, 1978b) needs to be validated before further formal taxonomic description of our present species can proceed.

Members of *Ectocotyla* are known to brood juveniles within the gut (Fleming and Burt 1978a, Petrov 2000). On the other hand, no fertilized egg was found in the two specimens of *Ectocotyla* sp. in this study, suggesting that they were in the early stages of the reproductive cycle. The first specimen from this study showed putative female gonads and copulatory apparatuses (Fig. 2), and thus would correspond to the second or third of the five reproductive stages that Petrov (2000) recognized in *Ec. hirudo*. The second specimen showed no female gonad and copulatory apparatus (Fig. 3), suggesting that it would represent the first reproductive stage sensu Petrov (2000). Petrov (2000) argued that the ectocommensal lifestyle would facilitate the young to be able to attach themselves to the host's body just after

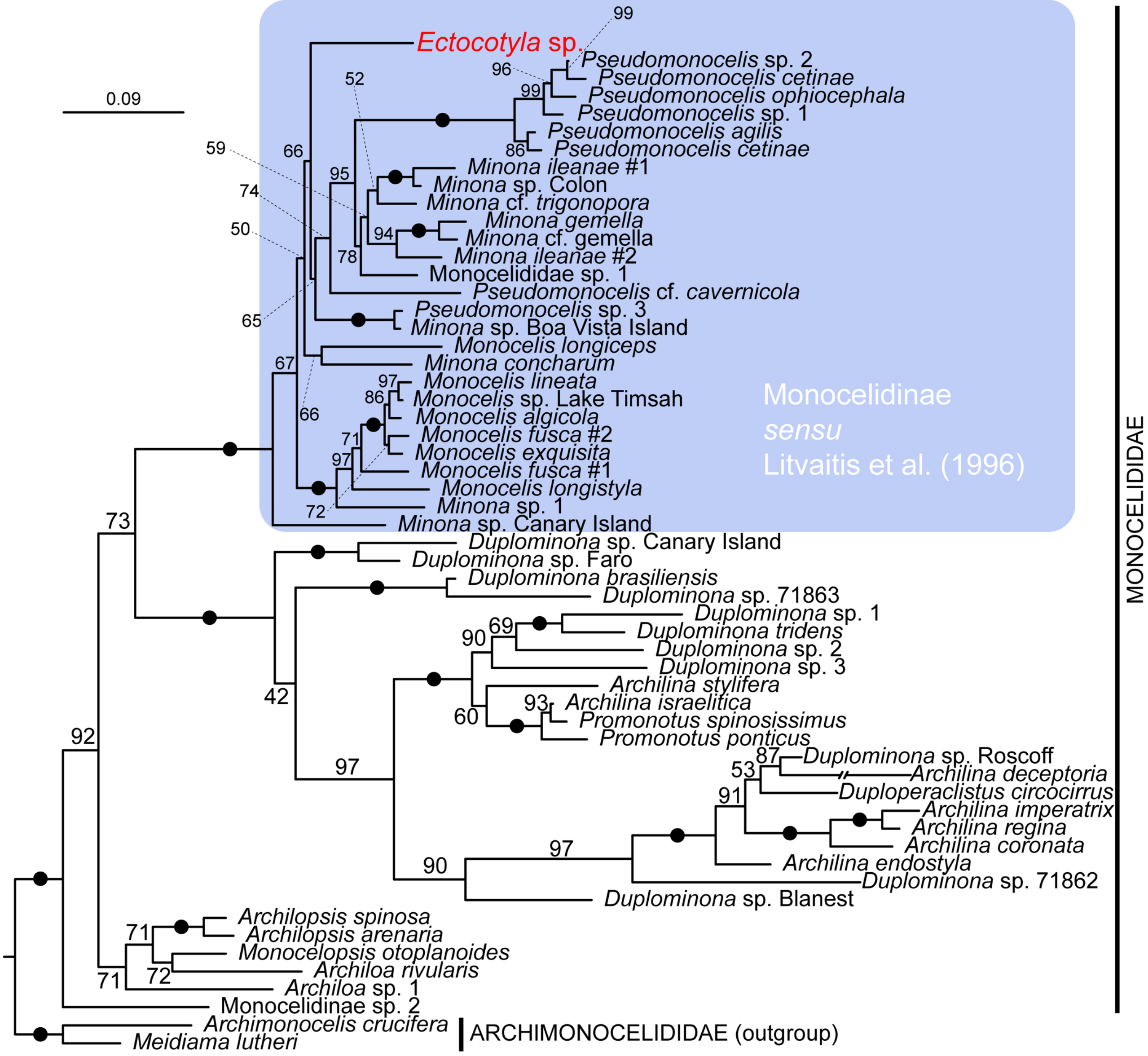


Figure 6. The consensus tree of 1000 ultrafast bootstrap (UFBoot) maximum-likelihood tree in IQ-TREE (Minh et al. 2020) based on aligned partial sequences (1694 bp) of the 28S rDNA, showing the present *Ectocotyla* sp. from the horsehair crab *Erimacrus isenbeckii* nested within Monocelidinae sensu Litvaitis et al. (1996). Numbers near branches indicate UFBoot values; fully supported branches are indicated with solid circles.

emerging from the egg, which would necessitate larger-sized hatchlings, for which the intra-gut-lumen brooding would be advantageous. Although we had no chance to observe any new hatchlings, the young in an egg capsule would be active if it had hatched, given Petrov's (2000) argument is correct.

Our study revealed that crab egg mass of *Er. isenbeckii* is a unique habitat for *Ectocotyla* sp. The most abundant numbers of *Ectocotyla* sp. were observed at the base of second and first pleopods. Shields et al. (1990) found high abundance, presence, and immigration of the decapod-egg-predatory nemertean *Carcinonemertes epialti* (Coe, 1902) at the base of the pleopods of the yellow rock crab *Cancer anthonyi* (Rathbun, 1897). Fleming and Burt (1978b) reported turbellarians were also found on other body parts of the host crabs, such as the abdomen and the gills. Further investigations are needed to determine whether turbellarians somehow prefer these innermost crab pleopods and to search the gills as well.

Abe (1992) reported that the king crab *Paralithodes camtschaticus* (Tilesius, 1815) and the hanasaki crab *Paralithodes brevipes* (Milne-Edwards and Lucas, 1841) are also important in the Hokkaido fisheries besides the horsehair crab. Further studies to survey ectosymbionts, specifically turbellarians, in the egg masses of these two decapod species would be of interest.

ACKNOWLEDGEMENTS

The Research, Scholarship and Professional Growth Committee, Department of Zoology and College of Science of Weber State University funded the research and travel support for RKO. Facilities, supplies, and logistical support were generously provided by the Faculty of Science, Hokkaido University. We would like to thank Takahiro Takashima at the Hokkaido Mariculture Fisheries Research Institute in Muroran for collecting the horsehair crabs. We are grateful for comments from the two anonymous reviewers who helped to greatly improve the manuscript.

LITERATURE CITED

Abe K (1992) Important crab resources inhabiting Hokkaido waters. Marine and Freshwater Behaviour and Physiology 21: 153–183. https://doi.org/10.1080/10236249209378825

Angus BM (2007) The study of turbellarians (Platyhelminthes) at the Queensland Museum. Memoirs of the Queensland Museum 53: 157–185.

Casu M, Cossu P, Sanna D, Lai T, Scarpa F, Curini-Galletti M (2011) A reappraisal of the monophyly of the genus *Pseudomonocelis* Meixner, 1943 (Platyhelminthes: Proseriata), with the description of a new species from the Mediterranean. Zootaxa 3011: 59–68. https://doi.org/10.11646/zootaxa.3011.1.6

Casu M, Scarpa F, Delogu V, Cossu P, Lai T, Sanna D, Curini-Galletti M (2014) Biodiversity patterns in interstitial marine microturbellaria: a case study within the genus *Parotoplana* (Platyhelminthes: Rhabditophora) with the description of four new species. Journal of Zoological Systematics and Evolutionary Research 52: 190–202. https://doi.org/10.1111/jzs.12058

Curini-Galletti M, Webster BL, Huyse T, Casu M, Schockaert ER, Artois T, Littlewood DTJ (2010) New insights on the phylogenetic relationships of the Proseriata (Platyhelminthes), with proposal of a new genus of the family Coelogynoporidae. Zootaxa 2537: 1–18.

Fleming LC, Burt MDB (1978a) A revision of the turbellarian genus *Ectocotyla* Hyman 1944 (Seriata, Monocelididae) associated with the crabs *Chionoecetes opilio* and *Hyas araneus* (Linnaeus). Journal of the Fisheries Research Board of Canada 35: 1223–1233.

Fleming LC, Burt MDB (1978b) On the genus *Peraclistus* (Turbellaria, Proseriata) with redescription of *P. oofagus*. (Friedman). Zoological Scripta 7: 81–84. https://doi.org/10.1111/j.1463-6409.1978.tb00590.x

Fleming LC, Burt MDB, Bacon GB (1981) On some commensal Turbellaria of the Canadian east coast. Hydrobiologia 84: 131–137. https://doi.org/10.1007/BF00026171

Gordon I (1966) Crustacea: general considerations. Mémoires de l'Institut Français d'Afrique Noire 77: 27–86.

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Quarterly Review of Biology 66: 411–453.

Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522. https://doi.org/10.1093/molbev/msx281

Hyman LH (1944) Marine Turbellaria from the Atlantic coast of North America. American Museum Novitates 1266: 1–15. http://hdl.handle.net/2246/3687

Kajihara H, Olympia M, Kobayashi N, Katoh T, Chen HX, Strand M, Sundberg P (2011) Systematics and phylogeny of the hoplonemertean genus *Diplomma* (Nemertea) based on molecular and morphological evidence. Zoological Journal of the Linnean Society 161: 695–722. https://doi.org/10.1111/j.1096-3642.2010.00650.x

Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Karling TG (1966) Marine Turbellaria from the Pacific coast of North America. IV. Coelogynoporidae and Monocelididae. Arkiv für Zoologi 18: 493–528.

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20: 1160–1166. https://doi.org/10.1093/bib/bbx108

Kuris AM (1991) A review of patterns and causes of crustacean brood mortality. In: Wenner A, Kuris AM (Eds) Crustacean egg production. Crustacean Issues 7. Balkema, Rotterdam, 117–141.

Laumer CE, Giribet G (2014) Inclusive taxon sampling suggests a single, stepwise origin of ectolecithality in Platyhelminthes. Biological Journal of the Linnean Society 111: 570–588. https://doi.org/10.1111/bij.12236

Levinsen GMR (1879) Bidrag til Kundskab om Grønlands Turbellariefauna. Videnskabelige Meddelelser fra den naturhistoriske Forening i København [41]: 165–204. https://www.biodiversitylibrary.org/item/110689#page/175/mode/1up

Littlewood DTJ (1994) Molecular phylogenetics of cupped oysters based on partial 28S rRNA gene sequences. Molecular Phylogenetics and Evolution 3: 221–229. https://doi.org/10.1006/mpev.1994.1024

Littlewood DTJ, Curini-Galletti M, Herniou EA (2000) The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. Molecular Phylogenetics and Evolution 16: 449–466. https://doi.org/10.1006/mpev.2000.0802

Litvaitis MK, Curini-Galletti MC, Martens PM, Kocher TD (1996) A reappraisal of the systematics of the Monocelididae (Platyhelminthes, Proseriata): inferences from rDNA sequences. Molecular Phylogenetics and Evolution 6: 150–156. https://doi.org/10.1006/mpev.1996.0067

Marcus E (1949) Turbellaria Brasileiros (7). Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Zoologia 14: 7–155. https://doi.org/10.11606/issn.2526-4877.bsffclzoologia.1949.129106

McDermott JJ, Williams JD, Boyko CB (2010) The unwanted guests of hermits: a global review of the diversity and natural history of hermit crab parasites. Journal of Experimental Marine Biology and Ecology 394: 2–44. https://doi.org/10.1016/j.jembe.2010.06.022

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution 37: 1530–1534. https://doi.org/10.1093/molbev/msaa015

Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R, Schaffer AA (2008) Database indexing for production MegaBLAST searches. Bioinformatics 15: 1757–1764. https://doi.org/10.1093/bioinformatics/btn322

Nagao J, Munehara H, Shimazaki K (1999) Embryonic development of the hair crab *Erimacrus isenbeckii*. Journal of Crustacean Biology 19: 77–83. https://doi.org/10.1163/193724099X00277

Petrov AA (2000) Morphological transformations of the reproductive system in the family Monocelididae (Turbellaria, Proseriata). Annual Reports of the Zoological Institute RAS. https://www.zin.ru/publications/sessions/annrep/2000/18.html [Accessed: 20/10/2023]

Scarpa F, Cossu P, Delogu V, Lai T, Sanna D, Leasi F, Norenburg JL, Curini-Galletti M, Casu M (2017) Molecular support for morphology-based family-rank taxa: the contrasting cases of two families of Proseriata (Platyhelminthes). Zoologica Scripta 46: 753–766. https://doi.org/10.1111/zsc.12251

Scarpa F, Cossu P, Lai T, Sanna D, Curini-Galletti M, Casu M (2016) Meiofaunal cryptic species challenge species delimitation: the case of the *Monocelis lineata* (Platyhelminthes: Proseriata) species complex. Contributions to Zoology 85: 123–145. https://doi.org/10.1163/18759866-08502001

Scarpa F, Cossu P, Sanna D, Lai T, Norenburg JL, Curini-Galletti M, Casu M (2015) An 18S and 28S-based clock calibration for marine Proseriata (Platyhelminthes). Journal of Experimental Marine Biology and Ecology 463: 22–31. https://doi.org/10.1016/j.jembe.2014.10.020

Scarpa F, Sanna D, Cossu P, Lai T, Casu M, Curini-Galletti M (2019) How to achieve internal fertilization without a vagina: the study case of the genus *Archilina* Ax, 1959 (Platyhelminthes, Proseriata) from Canary Islands. Marine Biodiversity 49: 2057–2073. https://doi.org/10.1007/s12526-018-0890-9

Scarpa F, Sanna D, Cossu P, Lai T, Curini-Galletti M, Casu M (2018) A molecular approach to the reconstruction of the pre-Lessepsian fauna of the Isthmus of Suez: the case of the interstitial flatworm *Monocelis lineata* sensu lato (Platyhelminthes: Proseriata). Journal of Experimental Marine Biology and Ecology 502: 174–181. https://doi.org/10.1016/j.jembe.2017.08.011

Schockaert ER, Hooge M, Sluys R, Schilling S, Tyler S, Artois T (2008) Global diversity of free living flatworms (Platyhelminthes, "Turbellaria") in freshwater. Hydrobiologia 595: 41–48. https://doi.org/10.1007/978-1-4020-8259-7_5

Shields JD, Okazaki RK, Kuris AM (1990) Brood mortality and egg predation by the nemertean, *Carcinonemertes epialti*, on the yellow rock crab, *Cancer anthonyi*, in southern California. Canadian Journal of Fisheries and Aquatic Sciences 47: 1275–1281. https://doi.org/10.1139/f90-146

Sluys R (2019) The evolutionary terrestrialization of planarian flatworms (Platyhelminthes, Tricladida, Geoplanidae): a review and research programme. Zoosystematics and Evolution 95: 543–556. https://doi.org/10.3897/zse.95.38727

Steenwyk JL, Buida TJ III, Li Y, Shen XX, Rokas A (2020) ClipKIT: a multiple sequence alignment trimming software for accurate phylogenomic inference. Plos Biology 18: e3001007. https://doi.org/10.1371/journal.pbio.3001007

Steinböck O (1932) Die Turbellarien des Arktischen Gebietes. Fauna Arctica 6: 295–342.

Williams JD, McDermott JJ (2004) Hermit crab biocoenoses: a worldwide review of the diversity and natural history of hermit crab associates. Journal of Experimental Marine Biology and Ecology 305: 1–128. https://doi.org/10.1016/j.jembe.2004.02.020

Submitted: November 1, 2023
Accepted: July 22, 2024
Editorial responsibility: Fernando Carbayo

Author Contributions

RKO: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. HK: Conceptualization, Formal Analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing.

Competing Interests

The authors have declared that no competing interests exist.

Funding

Research and travel support were funded by the Research, Scholarship and Professional Growth Committee, Department of Zoology and College of Science of Weber State University.

Data Resources

A partial sequence of the 28S rDNA newly generated in this study has been deposited in DDBJ/EMBL/GenBank.

How to cite this article

Okazaki RK, Kajihara H (2024) Proseriate turbellarians (Platyhelminthes: Rhabditophora) in the egg mass of the commercially important horsehair crab, *Erimacrus isenbeckii* (Crustacea: Decapoda), from Hokkaido, Japan. Zoologia 41: e23080. https://doi.org/10.1590/S1984-4689.v41.e23080

Published by

Sociedade Brasileira de Zoologia at Scientific Electronic Library Online (https://www.scielo.br/zool)

Appendix 1. List of monocelidids included in the phylogenetic analysis with their GenBank accession numbers for 28S rDNA sequences.

Taxon	Accession Number	Reference
Monocelididae		
Archilina coronata	MG778596	Scarpa et al. (2019)
Archilina deceptoria	MG778604	Scarpa et al. (2019)
Archilina endostyla	MG778606	Scarpa et al. (2019)
Archilina imperatrix	MG778578	Scarpa et al. (2019)
Archilina israelitica	U39927	Litvaitis et al. (1996)
Archilina regina	MG778589	Scarpa et al. (2019)
Archilina stylifera	MG778601	Scarpa et al. (2019)
Archiloa rivularis	U40049	Litvaitis et al. (1996)
Archiloa sp. 1	MG778602	Scarpa et al. (2019)
Archilopsis arenaria	KJ682443	Scarpa et al. (2015)
Archilopsis spinosa	KJ682442	Scarpa et al. (2015)
Duplominona brasiliensis	KJ682429	Scarpa et al. (2015)
Duplominona tridens	KJ682433	Scarpa et al. (2015)
Duplominona sp. Blanes	KJ682441	Scarpa et al. (2015)
Duplominona sp. Canary Island	KJ682426	Scarpa et al. (2015)
Duplominona sp. Faro	KJ682427	Scarpa et al. (2015)
Duplominona sp. Roscoff	KJ682438	Scarpa et al. (2015)
Duplominona sp. 1	KJ682430	Scarpa et al. (2015)
Duplominona sp. 2	KJ682435	Scarpa et al. (2015)
Duplominona sp. 3	KJ682436	Scarpa et al. (2015)
Duplominona sp. 71862	U41375	Litvaitis et al. (1996)

Continues

Taxon	Accession Number	Reference
Duplominona sp. 71863	U41377	Litvaitis et al. (1996)
Duploperaclistus circocirrus	KJ682439	Scarpa et al. (2015)
Ectocotyla sp.	LC782820	present study
Minona concharum	U42002	Litvaitis et al. (1996)
Minona gemella	KJ682409	Scarpa et al. (2015)
Minona cf. *gemella*	KJ682412	Scarpa et al. (2015)
Minona ileanae #1	KJ682405	Scarpa et al. (2015)
Minona ileanae #2	U42000	Litvaitis et al. (1996)
Minona cf. *trigonopora*	KJ682408	Scarpa et al. (2015)
Minona sp. Boa Vista Island	KJ682415	Scarpa et al. (2015)
Minona sp. Canary Island	KJ682423	Scarpa et al. (2015)
Minona sp. Colon	KJ682406	Scarpa et al. (2015)
Minona sp. 1	KJ682421	Scarpa et al. (2015)
Monocelididae sp. 1	U42004	Litvaitis et al. (1996)
Monocelidinae sp. 2	HM026560	Curini-Galletti et al. (2010)
Monocelis algicola	KR364691	Scarpa et al. (2016)
Monocelis exquisita	KR364686	Scarpa et al. (2016)
Monocelis fusca #1	KC869867	Laumer and Giribet (2014)
Monocelis fusca #2	KR364659	Scarpa et al. (2016)
Monocelis lineata	JN224911	Casu et al. (2011)
Monocelis longiceps	KC971087	Casu et al. (2014)
Monocelis longistyla	KC971088	Casu et al. (2014)
Monocelis sp. Lake Timsah	KY205366	Scarpa et al. (2018)
Monocelopsis otoplanoides	KY320146	Scarpa et al. (2017)
Promonotus ponticus	U40199	Litvaitis et al. (1996)
Promonotus spinosissimus	U40201	Litvaitis et al. (1996)
Pseudomonocelis cetinae	JN224913	Casu et al. (2011)
Pseudomonocelis ophiocephala	U40197	Litvaitis et al. (1996)
Pseudomonocelis sp. 1	JN224909	Casu et al. (2011)
Pseudomonocelis sp. 2	JN224908	Casu et al. (2011)
Pseudomonocelis agilis	JN224912	Casu et al. (2011)
Pseudomonocelis cetinae	U40051	Litvaitis et al. (1996)
Pseudomonocelis cf. *cavernicola*	JN224914	Casu et al. (2011)
Pseudomonocelis sp. 3	JN224915	Casu et al. (2011)
Archimonocelididae (outgroups)		
Archimonocelis crucifera	AJ270163	Littlewood et al. (2000)
Meidiama lutheri	KY320135	Scarpa et al. (2017)