Colonial Entoproct Epibiotic on a Sea Spider

Authors: Kakui, Keiichi, and Sekiguchi, Shogo

Source: Zoological Science, 41(6)

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zs240070

ZOOLOGICAL SCIENCE **41**:

Colonial Entoproct Epibiotic on a Sea Spider

Keiichi Kakui[1]* and Shogo Sekiguchi[2]

[1] *Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan*
[2] *Department of Natural History Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

We report an entoproct epibiotic on the surface of a sea spider (Pycnogonida). The pycnogonid was identified as *Nymphon* sp. (Nymphonidae). The entoproct was colonial, with three zooids, and was identified both morphologically and by a molecular phylogeny as *Barentsia* sp. (Barentsiidae). The largest zooid had eight tentacles and was about 0.7 mm long, smaller than for most colonial entoproct species. We determined partial sequences for the 18S rRNA and 28S rRNA genes from the entoproct. In an 18S-based maximum likelihood tree (1507 characters), the entoproct was the sister taxon to *Barentsia gracilis*.

Key words: benthos, epibiont, Kamptozoa, phylogeny, Pycnogonida, sessile

---

INTRODUCTION

Entoprocta is a phylum of small-sized, sessile or slow-moving invertebrates. About 200 species have been reported worldwide, 150 of which are solitary and the rest colonial (Borisanova and Schepetov, 2023). Although most entoprocts are marine, a few species inhabit fresh or brackish water. The majority of solitary entoprocts are commensals on other invertebrates. They can depart from the substratum, and a few may also be able to swim (Wasson, 2002). Colonial species are sessile, attaching to various abiotic or biotic substrata (e.g., stones, algae, mollusk shells, arthropods), and are generally believed not to show selectivity in their substratum preference (Borisanova, 2018).

Pycnogonids, or sea spiders, are almost exclusively marine invertebrates (cf. Kakui and Fujita, 2023). Except for some commensal or parasitic species, most of ca. 1400 species are free living (Arnaud and Bamber, 1987; Bamber et al., 2024). As they are slow-moving epibenthic animals with a hard exoskeleton, they provide attachment or egg-laying sites for many other organisms, including algae, diatoms, foraminiferans, ciliophorans, sponges, hydrozoans, bryozoans, brachiopods, polychaetes, leeches, gastropods, barnacles, and tunicates (Khan and Paul, 1995; Wambreuse et al., 2021). Some mobile epibionts (nemerteans, nematodes, mites, and isopods) have also been reported associated with them (Wambreuse et al., 2021).

In 2024, we found an entoproct colony on the surface of a sea spider in Japan. To our knowledge, there are only a few reports of entoprocts epibiotic on sea spiders (e.g., Marfenin and Belorustseva, 2006: p. 240). Here we describe the gross morphology of the entoproct, present partial nucleotide sequences for its nuclear 18S ribosomal RNA (18S) and 28S ribosomal RNA (28S) genes, and infer the species' phylogenetic position in Entoprocta based on 18S data.

MATERIALS AND METHODS

The sea spider with the attached entoproct was collected from a brackish estuary, Akkeshi-ko (43.047654° 144.857062°), at a depth of about 8 m on 23 June 2024, by means of a scallop dredge launched from the R/V *Misago-maru* (Hokkaido University, Japan). The sea spider was photographed alive on 25 June and fixed in 80% ethanol on 28 June. The entoproct colony comprising three zooids was detached from the fixed sea spider. One zooid was preserved in 99% ethanol; the others were observed with an Olympus BX53 light microscope and then preserved in 99% ethanol.

DNA was extracted from the three zooids by using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Germany). For the 18S gene, primers SR1 and SR12 (Nakayama et al., 1996) were used for amplification, and primers 18S-b3F, 18S-a4R, 18S-b5F, 18S-a6R, and 18S-b8F (Kakui et al., 2011, 2021; Kakui and Shimada, 2017; Kakui and Hiruta, 2022) for cycle sequencing. For the 28S gene, primers U178 and L1642 (Lockyer et al., 2003) were used for amplification, and primers U178, 300F, 300R, 900F, U1148 (Lockyer et al., 2003) for cycle sequencing. PCR amplification conditions for 18S and 28S with KOD ONE PCR Master Mix (Toyobo, Japan) were 45 cycles of 98°C for 10 s, 60°C for 5 s, and 68°C for 10 s. All nucleotide sequences were determined with a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated by using MEGA7 (Kumar et al., 2016). The sequences we determined were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan under accession numbers LC830819 (18S) and LC830820 (28S).

The 18S dataset for phylogenetic analysis included the one sequence we determined, and 20 entoproct sequences and an outgroup sequence from the INSD (Mackey et al., 1996; Littlewood et al., 1998; Fuchs et al., 2009, 2010; Rundell and Leander, 2012; Hartikainen et al., 2013; Kajihara et al., 2015; Borisanova et al., 2015, 2018; Borisanova and Schepetov, 2023). The sequences were aligned by using the online version of MAFFT ver. 7 (Katoh and Standley, 2013; Katoh et al., 2019) with the "Auto" strategy ("L-INS-i" selected; Katoh et al., 2005) and then trimmed with MEGA7 to match the shortest length among them. Alignment-ambiguous sites were removed with Gblocks ver. 0.91b (Castresana, 2000) in NGPhylogeny.fr (Lemoine et al., 2019) and the "relaxed"

---

* Corresponding author. E-mail: kakui@eis.hokudai.ac.jp
doi:10.2108/zs240070

parameters described in Talavera and Castresana (2007). The aligned dataset contained 1507 characters. Methods for selecting the optimal substitution model (GTR+F+I+G4), the maximum likelihood (ML) analysis, estimation of clade support (analyses of 1000 pseudoreplicates for both Shimodaira–Hasegawa-like approximate likelihood ratio tests [SH-aLRT] and ultrafast bootstraps [UFBoot]), and drawing the tree were as described by Shimada et al. (2023).

RESULTS AND DISCUSSION

The sea spider bearing the entoproct (Fig. 1A) had an ocular tubercle posterior to the neck, uniarticulate chelifores, denticulate chelae, 5-articulate palps, and ovigers with a terminal claw, and four paired legs, and lacked cement gland pores. We thus identified it as a female of *Nymphon* sp. (Nymphonidae). Its trunk length (from the base of the chelifore to the posterior end of the fourth lateral process) was 3.62 mm. Along with the entoproct, several tunicates, hydroids, and ciliophorans were attached to the sea spider.

One entoproct colony with three zooids was found on the dorsal surface of trunk segment 3 of the sea spider (Fig. 1). As each zooid sprouted from a creeping stolon and had a stalk with a thick muscular node and a thin, stiff peduncle (*Barentsia*-type zooid), and the colony lacked *Pedicellina*-type zooids, we identified the entoproct as *Barentsia* sp. (Barentsiidae) (Borisanova and Potanina, 2016). We could not rule out that larger colonies might also contain *Pedicellina*-type zooids, i.e., that the ectoproct was a species of *Pseudopedicellina*. The largest zooid in the colony had eight tentacles and was about 0.7 mm long (Fig. 2), smaller than for the majority of colonial entoproct species (average lengths around several millimeters; Borisanova, 2018). In an 18S tree (Fig. 3), *Barentsia* sp. was the sister taxon to *B. gracilis*, with high nodal support (SH-aLRT/UFBoot, 97.5%/96%). The p-distance in the aligned region between *Barentsia* sp. and *B. gracilis* was 0.2%.

Fig. 1. Female *Nymphon* sp. with the entoproct *Barentsia* sp. attached; living animals photographed on 25 June 2024. **(A)** Habitus of *Nymphon* sp., dorsal view. **(B)** Posterior portion of *Nymphon* sp., dorsal view. Abbreviations: abd, abdomen; llp3, 4, left lateral processes 3, 4; rlp3, 4, right lateral processes 3, 4; st, sterile segment; ts3, 4, trunk segments 3, 4; zo, zooid.

Fig. 2. Two zooids of *Barentsia* sp. arising from a stolon (one zooid lacking its calyx; the loss occurred sometime between 25 June and 28 June); ethanol fixed specimen (fixed on 28 June 2024).

Colonial entoprocts are generally thought to lack substrate preference (Borisanova, 2018). *Loxokalypus socialis*, however, is epibiotic on a certain polychaetous annelid species, and some other species are found more frequently on certain substrata (e.g., "*B.* [*Barentsia*] *conferta* is found on algae more often than other barentsiids are."; Wasson, 1997: p. 30). Because colonial entoprocts are sessile, the type of substratum and site of attachment can potentially affect their survival rate. This raises the question whether single entoproct species reported from various substrata might represent several substrate-specific cryptic species. It is unclear whether the sea spider is the sole substrate utilized by our entoproct species. If it is restricted to one species of sea spider, or sea spiders in general, our entoproct is likely undescribed. Among about 50 colonial species, 18S sequences are currently publicly available for only

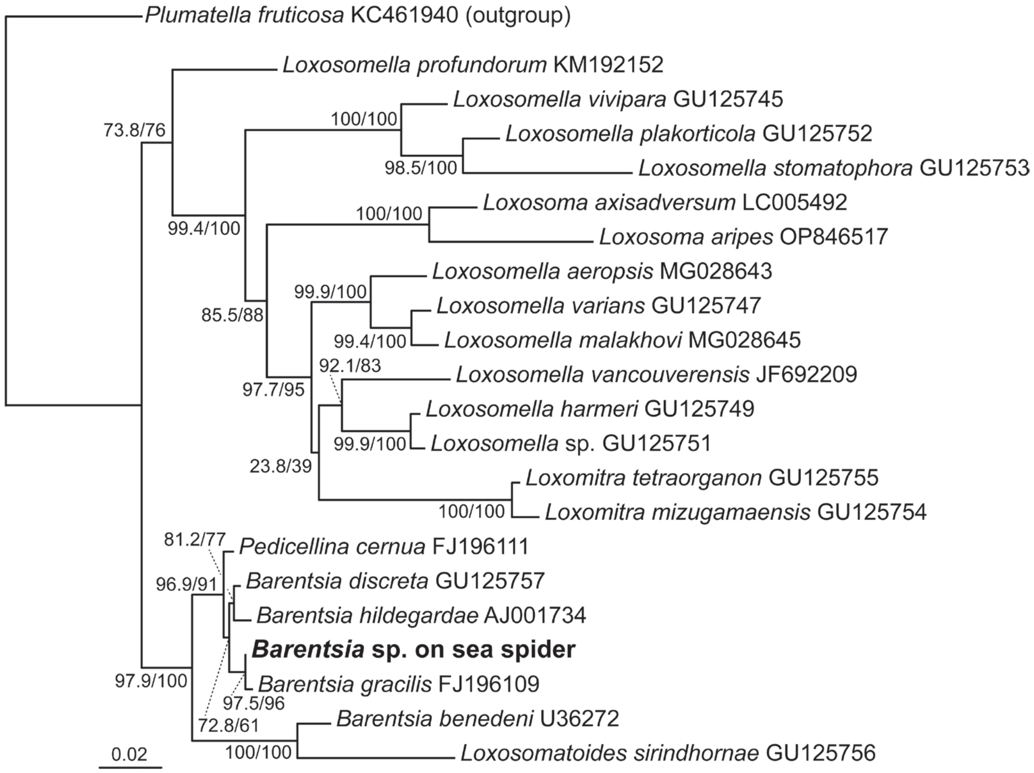

Fig. 3. ML tree for entoprocts based on 18S sequences (1507 characters). Numbers near nodes are SH-aLRT/UFBoot values as percentages; the scale below the tree indicates branch length in substitutions per site.

six. Additional molecular data from colonial entoprocts on various substrate types may reveal higher species diversity and unexpected relationships between entoprocts and their substrata.

ACKNOWLEDGMENTS

We thank Shoichi Hamano, Hidenori Katsuragawa, Hiroshi Kajihara, Shoki Shiraki, Yoshinobu Matsushima, and participants in the Laboratory Course in Marine Biology I for help in sampling; the staff of the Akkeshi Marine Station, Hokkaido University, for providing laboratory facilities; Yasunori Kano for help in surveying the literature; and Matthew H. Dick for reviewing the manuscript and editing the English.

COMPETING INTERESTS

We declare no competing interests.

AUTHOR CONTRIBUTIONS

KK conceived and designed the study, observed the morphology of the entoproct, and conducted the molecular analysis. SS observed the morphology of the sea spider. KK and SS collected the sea spider, wrote the manuscript, and read and approved the final draft.

REFERENCES

Arnaud F, Bamber RN (1987) The biology of Pycnogonida. Adv Mar Biol 24: 1–96

Bamber RN, El Nagar A, Arango CP (2024) Pycnobase: World Pycnogonida Database. URL: https://www.marinespecies.org/pycnobase Accessed 15 July 2024

Borisanova AO (2018) Entoprocta (Kamptozoa). In: “Miscellaneous Invertebrates” Ed by A Schmidt-Rhaesa, De Guyter, Berlin, pp 111–161

Borisanova AO, Potanina DM (2016) A new species of *Coriella*, *Coriella chernyshevi* n. sp. (Entoprocta, Barentsiidae), with comments on the genera *Coriella* and *Pedicellinopsis*. Zootaxa 4184: 376–382

Borisanova A, Schepetov D (2023) Clarification of the diagnosis of the genus *Loxosoma* s.l. (Entoprocta; Loxosomatidae) based on morphological and molecular data. Zootaxa 5325: 342–358

Borisanova AO, Chernyshev AV, Neretina TV, Stupnikova AN (2015) Description and phylogenetic position of the first abyssal solitary kamptozoan species from the Kuril-Kamchatka Trench area: *Loxosomella profundorum* sp. nov. (Kamptozoa: Loxosomatidae). Deep Sea Res II 111: 351–356

Borisanova AO, Chernyshev AV, Ekimova IA (2018) Deep-sea Entoprocta from the Sea of Okhotsk and the adjacent open Pacific abyssal area: new species and new taxa of host animals. Deep Sea Res II 154: 87–98

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17: 540–552

Fuchs J, Obst M, Sundberg P (2009) The first comprehensive molecular phylogeny of Bryozoa (Ectoprocta) based on combined analyses of nuclear and mitochondrial genes. Mol Phylogenet Evol 52: 225–233

Fuchs J, Iseto T, Hirose M, Sundberg P, Obst M (2010) The first internal molecular phylogeny of the animal phylum Entoprocta (Kamptozoa). Mol Phylogenet Evol 56: 370–379

Hartikainen H, Waeschenbach A, Wöss E, Wood T, Okamura B (2013) Divergence and species discrimination in freshwater bryozoans (Bryozoa: Phylactolaemata). Zool J Linn Soc 168: 61–80

Kajihara H, Tomioka S, Kakui K, Iseto T (2015) Phylogenetic position of the queer, backward-bent entoproct *Loxosoma axisadversum* (Entoprocta: Solitaria: Loxosomatidae). Species Divers 20: 83–88

Kakui K, Fujita Y (2023) New sea spider species (Pycnogonida: Austrodecidae) from a submarine cave in Japan. J Mar Biol Assoc U K 103: e44

Kakui K, Hiruta C (2022) Description of a new *Hamatipeda* species, with an 18S molecular phylogeny (Crustacea: Tanaidacea: Typhlotanaidae). Zool Sci 39: 140–146

Kakui K, Shimada D (2017) A new species of *Tanaopsis* (Crustacea: Tanaidacea) from Japan, with remarks on the functions of serial ridges and grooves on the appendages. Zootaxa 4282: 324–336

Kakui K, Katoh T, Hiruta SF, Kobayashi N, Kajihara H (2011) Molecular systematics of Tanaidacea (Crustacea: Peracarida) based on 18S sequence data, with an amendment of suborder/superfamily-level classification. Zool Sci 28: 749–757

Kakui K, Fukuchi J, Shimada D (2021) First report of marine horsehair worms (Nematomorpha: *Nectonema*) parasitic in isopod crustaceans. Parasitol Res 120: 2357–2362

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30: 772–780

Katoh K, Kuma K, Toh H, Myata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33: 511–518

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20: 1160–1166

Khan RA, Paul AJ (1995) Life cycle studies on Arcto-boreal leeches (Hirudinea). J Helminthol Soc Wash 62: 105–110

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874

Lemoine F, Correia D, Lefort V, Doppelt-Azeroual O, Mareuil F, Cohen-Boulakia S, et al. (2019) NGPhylogeny.fr: new generation phylogenetic services for non-specialists. Nucleic Acids Res 47: W260–W265

Littlewood DTJ, Telford MJ, Clough KA, Rohde K (1998) Gnathostomulida—an enigmatic metazoan phylum from both morphological and molecular perspectives. Mol Phylogenet Evol 9: 72–79

Lockyer AE, Olson PD, Littlewood DTJ (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biol J Linn Soc 78: 155–171

Mackey LY, Winnepenninckx B, De Wachter R, Backeljau T, Emschermann P, Garey JR (1996) 18S rRNA suggests that Entoprocta are protostomes, unrelated to Ectoprocta. J Mol Evol 42: 552–559

Marfenin NN, Belorustseva SA (2006) Illustrated Atlas of White Sea Invertebrates. KMK Publishing, Moscow (in Russian)

Nakayama T, Watanabe S, Mitsui K, Uchida H, Inouye I (1996) The phylogenetic relationship between the Chlamydomonadales and Chlorococcales inferred from 18SrDNA sequence data. Phycol Res 44: 47–55

Rundell RJ, Leander BS (2012) Description and phylogenetic position of the first sand-dwelling entoproct from the western coast of North America: *Loxosomella vancouverensis* sp. nov. Mar Biol Res 8: 284–291

Shimada D, Kakui K, Fujita Y (2023) A new species of free-living marine nematode, *Fotolaimus cavus* sp. nov. (Nematoda, Oncholaimida, Oncholaimidae), isolated from a submarine anchialine cave in the Ryukyu Islands, southwestern Japan. Zoosyst Evol 99: 519–533

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol 56: 564–577

Wambreuse N, Hamel J-F, Mercier A (2021) Epibiotic associations with the deep-sea pycnogonid *Nymphon hirtipes* Bell, 1855 as a source of biodiversity. Deep Sea Res I 178: 103660

Wasson K (1997) Systematic revision of colonial kamptozoans (entoprocts) of the Pacific coast of North America. Zool J Linn Soc 121: 1–63

Wasson K (2002) A review of the invertebrate phylum Kamptozoa (Entoprocta) and synopsis of kamptozoan diversity in Australia and New Zealand. Trans R Soc S Aust 126: 1–20

(Received July 19, 2024 / Accepted September 2, 2024 /
Published online November 21, 2024)