Check List the journal of biodiversity data
NOTES ON GEOGRAPHIC DISTRIBUTION

First record of *Prostoma clepsinoides* Dugès, 1828 (Nemertea, Eumonostilifera) from Hachijo-jima Island, Japan

Masae Kyoku[1], **Hiroki Akimoto**[1], **Masaki Hoso**[2]

1 Department of Integrative Bioscience and Biomedical Engineering, Graduate School of Advanced Science and Engineering, Waseda University, Shinjuku, Tokyo, Japan
2 Faculty of Education and Integrated Arts and Sciences, School of Education, Waseda University, Shinjuku, Tokyo, Japan

Corresponding author: Masaki Hoso (MasakiHoso@gmail.com)

Abstract. *Prostoma clepsinoides* Dugès, 1828 is reported from Hachijo-jima Island, Tokyo, Japan, based on a mitochondrial COI barcode sequence from one freshwater specimen. The specimen was collected from a spring-fed stream and grouped with published sequences currently assigned to *P. clepsinoides*. To our knowledge, this is the first published record of *Prostoma* from Hachijo-jima Island. No morphological voucher specimen was retained because the entire specimen was used for DNA extraction.

Keywords. Cosmopolitan species, DNA barcoding, freshwater ribbon worm, molecular phylogeny, Prostomatidae

Kyoku M, Akimoto H, Hoso M (2026) First record of *Prostoma clepsinoides* Dugès, 1828 (Nemertea, Eumonostilifera) from Hachijo-jima Island, Japan. Check List 22 (5): 1043–1049. https://doi.org/10.15560/22.5.1043

Introduction

Nemerteans are predominantly marine or estuarine, and only a few species occur in freshwater habitats (Gibson and Moore 1976; Sundberg and Gibson 2008). The freshwater genus *Prostoma* Dugès, 1828 has a complicated taxonomic history, as many nominal species have been described and their taxonomic status has been repeatedly revised (Gibson and Moore 1976; Kajihara 2025). Kajihara (2025) recently revised the genus *Prostoma* based on morphological observations, COI-based species delimitation, and a literature review. In that revision, two valid species were recognised: the epigean *P. clepsinoides* Dugès, 1828 and the stygobiont *P. puteale* Beauchamp, 1932. Under this taxonomic treatment, many previously recognised nominal species were synonymised with *P. clepsinoides*, and records from many regions worldwide are now attributable to this species. Kajihara (2025) also detected three genetically divergent COI lineages within *P. clepsinoides*, suggesting that additional molecular data from more localities are needed to better understand the species boundaries and phylogeographic structure of *P. clepsinoides*.

In Japan, the earliest record currently attributable to *P. clepsinoides* was published as *Stichostemma grandis* from Hiroshima Prefecture (Ikeda 1913). Subsequent Japanese records under several different names were summarised by Kajihara (2007). Among Japanese island localities, *Prostoma* has been recorded from Sugashima, Mie Prefecture, as *P. graecense* (Böhmig, 1892) (Sudzuki 1953). To our knowledge, *Prostoma* has not previously been recorded from Hachijo-jima Island. Here, we report *P. clepsinoides* from Hachijo-jima Island, Japan, based on COI DNA barcoding, representing the first published record of the genus *Prostoma* from Hachijo-jima Island (Figure 1).

OPEN ACCESS

Academic editor: Fernando Carbayo
Received: 25 May 2026
Accepted: 17 August 2026
Published: 21 October 2026

Copyright © The authors.
This is an open-access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0)

Methods

The specimen was collected from a spring-fed stream on Hachijo-jima Island, Tokyo, Japan, on 18 December 2025. The entire specimen was used for DNA extraction, and no morphological voucher specimen was retained. Total DNA was extracted from the whole specimen using the Maxwell RSC Genomic DNA Kit (Promega, Madison, WI, USA). A partial mitochondrial COI fragment (658 bp) was amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAAC TTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). The 50 µL PCR mixture contained 1.0 µL each of

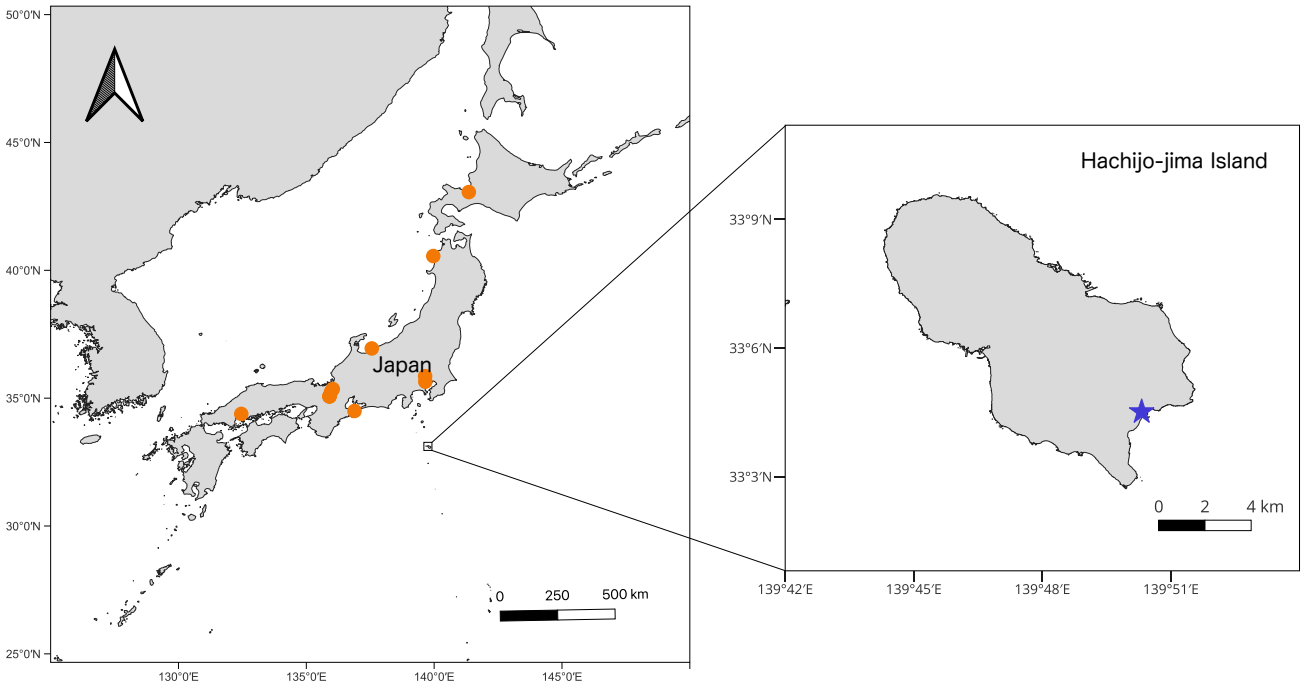

Figure 1. Japanese records of *Prostoma*. Blue star = present record of *P. clepsinoides* from Hachijo-jima Island; orange circles = previous records. Their positions are approximate or representative, based on published locality information, and do not necessarily indicate exact collection sites. For previous Japanese records, see Kajihara (2007, 2025) and Ohtaka and Kimura (2026).

forward and reverse primers (10 µM), 10 µL of 2 mM dNTPs (TOYOBO, Osaka, Japan), 25 µL of 2× PCR Buffer for KOD FX Neo (TOYOBO), 1.0 µL of KOD FX Neo (1 U/µL; TOYOBO), 2.7 µL of autoclaved distilled water, and 9.3 µL of DNA extract. PCR amplification was performed at 94 °C for 2 min; 35 cycles of 98 °C for 10 s, 53 °C for 30 s, and 68 °C for 20 s; and a final extension at 68 °C for 7 min. PCR products were checked by electrophoresis on an E-Gel and quantified using the QuantiFluor ONE dsDNA System (Promega) according to the manufacturer's instructions.

After quantification, the PCR product was purified with ExoSAP-IT Express (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed by Azenta Japan Corp. (Tokyo, Japan). After trimming, a 616-bp COI sequence was obtained. To confirm species identity and phylogenetic placement, COI sequences from GenBank were used for comparison (Table 1). The sequence obtained in this study has been deposited in DDBJ/EMBL/GenBank under accession number LC919661.

All COI sequences were aligned using MEGA v. 12.1 (Kumar et al. 2024). A maximum-likelihood tree was inferred using IQ-TREE v. 3.0.1 (Wong et al. 2026) under the GTR+G model. Branch support was evaluated using 1,000 replicates of the SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010) and 1,000 ultrafast bootstrap replicates (Hoang et al. 2018). *Cyanophthalma obscura* (Schultze, 1851) and *Emplectonema gracile* (Johnston, 1837) were used as outgroups. The tree was visualised in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and the haplotype network was estimated using PopART v. 1.7 (Leigh and Bryant 2015) with the TCS algorithm (Clement et al. 2000). For the haplotype network analysis, sequences were trimmed to a common length of 429 bp.

Results

***Prostoma clepsinoides* Dugès, 1828**

Figure 2

New records. JAPAN — Tokyo Metropolis • Hachijo-jima Island; 33°04′30.6″N, 139°50′19.1″E; elev. 3 m; 18.XII.2025; M. Kyoku leg.; spring-fed stream; 1 specimen, no voucher retained; GenBank: LC919661.

Identification. Species identity was inferred from DNA barcoding. The 616-bp COI sequence obtained in this study was grouped with sequences assigned to *P. clepsinoides* under the taxonomic treatment of Kajihara (2025), specifically within haplogroup 1 (Figure 3). In the haplotype network, the Hachijo-jima sequence shared a haplotype with a sequence from Massachusetts, USA (Figure 3B). Because

Table 1. COI sequences of *Prostoma* and selected outgroup taxa used in the phylogenetic analysis, including DDBJ/EMBL/GenBank accession numbers, database taxon labels, localities, and sources.

Taxon name for database entry	Accession number	Locality	Source
Cyanophthalma obscura	EF208980	Sweden	Sundberg et al. 2007
Cyanophthalma obscura	PZ245896	Gulf of Finland, Russia	Starunova and Demidova 2026, unpublished
Emplectonema gracile	KU839995	Sweden	Sundberg et al. 2016
Emplectonema gracile	KU839994	Sweden	Sundberg et al. 2016
Prostoma clepsinoides	LC919661	Hachijo-jima Island, Japan	present study
Prostoma cf. *eilhardi*	MK047682	Massachusetts, USA	Zattara et al. 2019
Prostoma cf. *eilhardi*	MK047681	Indiana, USA	Zattara et al. 2019
Prostoma graecense	EF208981	Sweden	Sundberg et al. 2007
Prostoma graecense	LC763728	Japan: Shiga, Lake Biwa	Takamura and Kondo 2023, unpublished
Prostoma graecense	KY523851	Mexico	Lagunas-Calvo et al. 2018
Prostoma graecense	MF314117	Mexico	Lagunas-Calvo et al. 2018
Prostoma graecense	MF314116	Mexico	Lagunas-Calvo et al. 2018
Prostoma graecense	LC651415	Hedared, Borås, Västra Götaland, Sweden	Kajihara 2025
Prostoma graecense	LC651414	Hedared, Borås, Västra Götaland, Sweden	Kajihara 2025
Prostoma graecense	LC651413	Femsjøen Lake, Halden, Østfold, Norway	Kajihara 2025
Prostoma graecense	LC651160	Japan (Lake Biwa/Nanko, Shiga)	Kajihara 2025
Prostoma sp.	HQ939311	California, USA	Stein et al. 2014
Prostoma sp.	HQ938796	California, USA	Stein et al. 2014
Prostoma sp.	MN199038	UK	Quigg et al. 2020
Prostoma sp.	MN199039	UK	Quigg et al. 2020
Prostoma sp.	MN199040	UK	Quigg et al. 2020
Prostoma sp.	MN199041	UK	Quigg et al. 2020
Prostoma sp.	MN199042	UK	Quigg et al. 2020
Prostoma sp.	MN199043	UK	Quigg et al. 2020
Prostoma sp.	MN199044	UK	Quigg et al. 2020
Prostoma sp.	MN199045	UK	Quigg et al. 2020
Prostoma sp.	MN199046	UK	Quigg et al. 2020
Prostoma sp.	MN199047	UK	Quigg et al. 2020
Prostoma sp.	MN199048	UK	Quigg et al. 2020
Prostoma sp.	MN199049	UK	Quigg et al. 2020
Prostoma sp.	MN199050	UK	Quigg et al. 2020
Prostoma sp.	MN199051	UK	Quigg et al. 2020
Prostoma sp.	MN199052	UK	Quigg et al. 2020
Prostoma sp.	MN199053	UK	Quigg et al. 2020
Prostoma sp.	MN199054	UK	Quigg et al. 2020
Prostoma sp.	MN199055	UK	Quigg et al. 2020
Prostoma sp.	MN199056	UK	Quigg et al. 2020
Prostoma sp.	MN199057	UK	Quigg et al. 2020
Prostoma sp.	MN199058	UK	Quigg et al. 2020
Prostoma sp.	MN199059	UK	Quigg et al. 2020
Prostoma sp.	MN199060	UK	Quigg et al. 2020
Prostoma sp.	MN199061	UK	Quigg et al. 2020
Prostoma sp.	MN199062	UK	Quigg et al. 2020
Prostoma sp.	MN199063	UK	Quigg et al. 2020
Prostoma sp.	MN199064	UK	Quigg et al. 2020
Prostoma sp.	MN199065	UK	Quigg et al. 2020
Prostoma sp.	MN199066	UK	Quigg et al. 2020
Prostoma sp.	MN199067	UK	Quigg et al. 2020
Prostoma sp.	MN199068	UK	Quigg et al. 2020
Prostoma sp.	MN199069	UK	Quigg et al. 2020
Prostoma sp.	MN199070	UK	Quigg et al. 2020
Prostoma sp.	MN199071	UK	Quigg et al. 2020
Prostoma sp.	MN199072	UK	Quigg et al. 2020
Prostoma sp.	MN199073	UK	Quigg et al. 2020
Prostoma sp.	MN199074	UK	Quigg et al. 2020
Prostoma sp.	MN199075	UK	Quigg et al. 2020
Prostoma sp.	MN199076	UK	Quigg et al. 2020
Prostoma sp.	MN199077	UK	Quigg et al. 2020
Prostoma sp.	MN199078	UK	Quigg et al. 2020
Prostoma sp.	MN199079	UK	Quigg et al. 2020
Prostoma sp.	MN199080	UK	Quigg et al. 2020
Prostoma sp.	MN199081	UK	Quigg et al. 2020
Prostoma sp.	MN199082	UK	Quigg et al. 2020
Prostoma sp.	MN199083	UK	Quigg et al. 2020
Prostoma sp.	MN199084	UK	Quigg et al. 2020
Prostoma sp.	MN199085	UK	Quigg et al. 2020
Prostoma sp.	MN199086	UK	Quigg et al. 2020
Prostoma sp.	MN199087	UK	Quigg et al. 2020

Figure 2. Habitat and live specimen of *Prostoma clepsinoides* from Hachijo-jima Island, Tokyo, Japan. **A.** Sampling site, a spring-fed stream. **B.** Live specimen. Scale bar = 1 mm.

the entire specimen was used for DNA extraction, no voucher specimen is available for morphological re-examination.

Discussion

Our identification follows Kajihara's (2025) provisional treatment of epigean *Prostoma* as *P. clepsinoides*. Kajihara (2025) recovered three genetically distinct COI haplogroups and noted that this treatment possibly represents over-lumping of this species. The present record adds a new locality to this framework, but because the record is based on a single specimen and COI sequence data alone, further sampling from the Izu Islands and adjacent regions, together with multilocus data, will be needed to evaluate the origin of the population and species boundaries within *P. clepsinoides*.

The present record represents the first published record of *Prostoma* from Hachijo-jima Island. Hachijo-jima is a volcanic island in the Izu Islands (Figure 1), isolated from the freshwater systems of mainland Japan. The occurrence of a freshwater nemertean there is therefore biogeographically notable. Under the current taxonomic treatment, *P. clepsinoides* is regarded as broadly distributed and probably cosmopolitan (Kajihara 2025). This broad distribution raises the question of how freshwater *Prostoma* disperses among isolated freshwater habitats. However, how *P. clepsinoides* reached Hachijo-jima Island remains unknown. The present record alone cannot distinguish between natural long-distance dispersal and human-mediated transport. Previous records suggest that freshwater *Prostoma* may be associated with aquatic vegetation and other freshwater-associated materials. In Tasmania, a record now attributable to *P. clepsinoides* was from decaying leaves and stems of reeds and rushes, suggesting the possibility of introduction with freshwater plants (Gibson and Moore 1971). In Mexico, specimens were collected from roots of the introduced Water Hyacinth, *Eichhornia crassipes* Mart. (Lagunas-Calvo et al. 2018). Further sampling and genetic data are needed to clarify the origin and dispersal history of the Hachijo-jima population.

Acknowledgements

We are grateful to Dr. Hiroshi Kajihara (Faculty of Science, Hokkaido University) and Dr. Natsumi Hookabe (Institute of Biogeosciences, JAMSTEC) for valuable advice on the distribution of *P. clepsinoides*. We also thank the academic editor and reviewers for constructive comments, which helped improve the manuscript.

Additional information

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical statement

No ethical statement is reported.

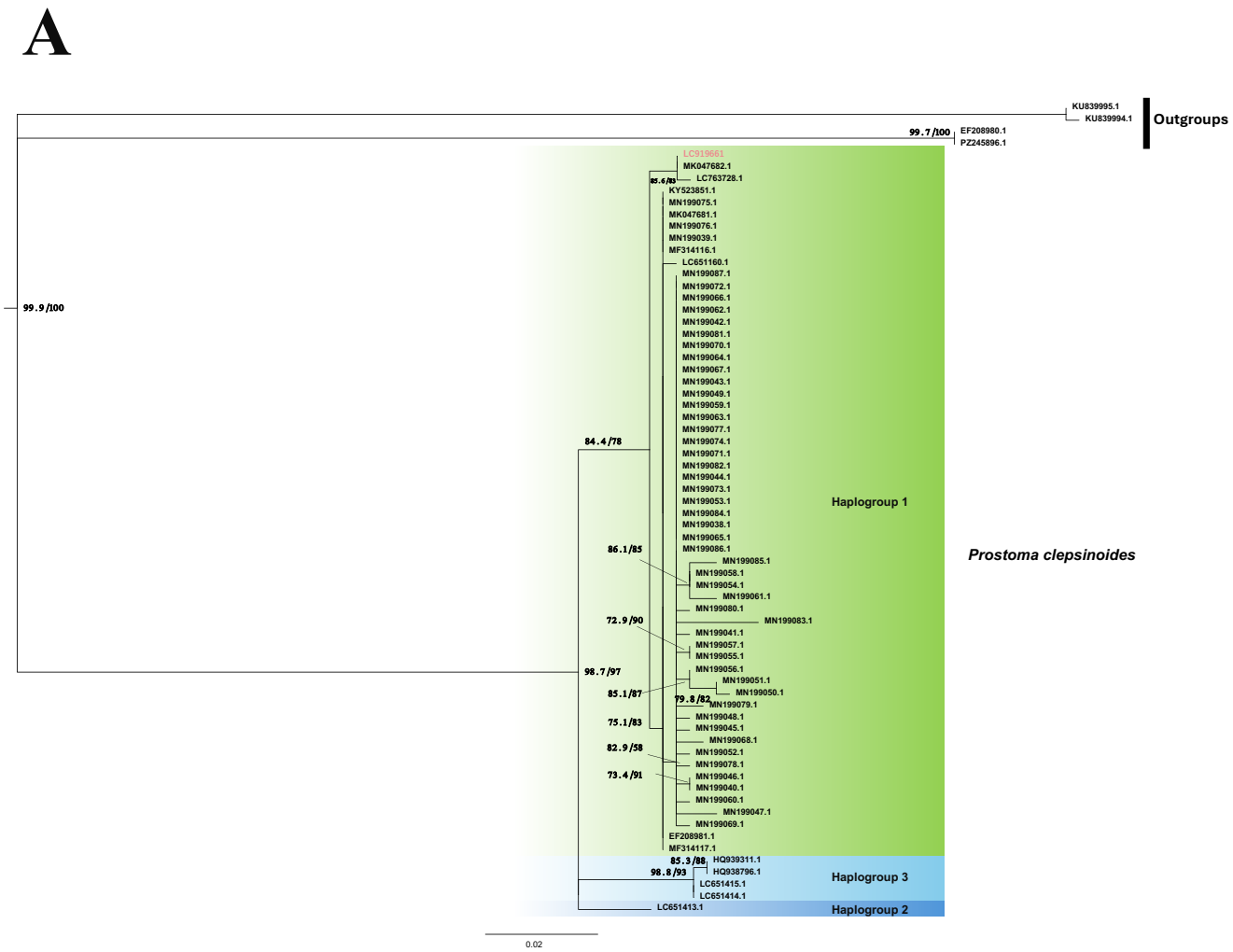

Figure 3. Phylogenetic tree and haplotype network of *Prostoma clepsinoides*. Haplogroups 1–3 in both panels follow the classification of Kajihara (2025). **A.** Maximum-likelihood phylogenetic tree based on mitochondrial COI of *P. clepsinoides*. Numbers on nodes correspond to SH-aLRT/UFBoot, respectively. The sequence from Hachijo-jima Island is shown in pink. The scale bar indicates 0.02 substitutions per site. **B.** Haplotype network based on COI (429 bp) of *P. clepsinoides*. Circles represent haplotypes, with size proportional to the number of sequences and color indicating geographic origin. Black nodes represent hypothetical haplotypes, and short bars indicate nucleotide substitutions. The arrowhead indicates the haplotype from Hachijo-jima Island.

Use of artificial intelligence (AI)

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI. ChatGPT (OpenAI) was used for language editing in some parts of the manuscript and not used to generate data or results. All AI-assisted revisions were reviewed by the authors.

Funding

This work was supported by Japan Society for the Promotion of Science KAKENHI grants 20KK0169, 23K23951, and 25K02348 to M.H. and 24KJ2104 to H.A.

Author contributions

Conceptualisation: MK, HA. Formal analysis: MK. Funding acquisition: HA, MH. Investigation: MK, HA. Writing – original draft: MK, HA. Writing – review and editing: MK, HA, MH.

Author ORCID iDs

Hiroki Akimoto https://orcid.org/0009-0006-2134-7654
Masaki Hoso https://orcid.org/0000-0002-5518-309X

Data availability

All data that support the findings of this study are available in the main text.

References

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Gibson R, Moore J (1971) Occurrence of the freshwater hoplonemertean *Prostoma graecense* (Böhmig) in Tasmania. Freshwater Biology 1: 193–195. https://doi.org/10.1111/j.1365-2427.1971.tb01556.x

Gibson R, Moore J (1976) Freshwater nemerteans. Zoological Journal of the Linnean Society 58: 177–218. https://doi.org/10.1111/j.1096-3642.1976.tb00828.x

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321. https://doi.org/10.1093/sysbio/syq010

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

Ikeda I (1913) A new fresh-water nemertine from Japan (Stichostemma grandis). Annotationes Zoologicae Japonenses 8: 239–256.

Kajihara H (2007) A taxonomic catalogue of Japanese nemerteans (phylum Nemertea). Zoological Science 24: 287–326. https://doi.org/10.2108/zsj.24.287

Kajihara H (2025) A taxonomic revision of the freshwater monostiliferous hoplonemertean genus Prostoma Dugès, 1828 (Nemertea: Eumonostilifera): a radical solution or an over-lumping? Zootaxa 5646: 451–500. https://doi.org/10.11646/zootaxa.5646.4.1

Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K (2024) MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. Molecular Biology and Evolution 41: msae263. https://doi.org/10.1093/molbev/msae263

Lagunas-Calvo O, Hernández-Mena DI, Rivas G, Contreras- Mirón S, Mendoza-Garfias B, Echeverría-Martínez OM, Oceguera-Figueroa AF (2018) Rediscovery of the nemertean *Prostoma graecense* from Xochimilco, 75 years after its first and only record. Revista Mexicana de Biodiversidad 89: 39–45. https://doi.org/10.22201/ib.20078706e.2018.1.2094

Leigh JW, Bryant D (2015) Popart: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6: 1110–1116. https://doi.org/10.1111/2041-210x.12410

Ohtaka A, Kimura N (2026) Inventory and faunal characteristics of benthic animals in the Jūniko Lakes, Shirakami Mountains, northern Japan. Aquatic Animals 2026: AA2026-13. https://doi.org/10.34394/aquaticanimals.2026.0_aa2026-13

Quigg SM, Lowe CN, Butt KR, Mitcham T, Iyengar A (2020) A re-examination of the taxonomic status of Prostoma jenningsi-a Freshwater Nemertean. Zootaxa 4722: 175–184. https://doi.org/10.11646/zootaxa.4722.2.4

Stein ED, White BP, Mazor RD, Jackson JK, Battle JM, Miller PE, Pilgrim EM, Sweeney BW (2014) Does DNA barcoding improve performance of traditional stream bioassessment metrics? Freshwater Science 33: 302–311. https://doi.org/10.1086/674782

Sudzuki M (1953) On Japanese fresh-water nemerteans. Zoological Magazine 62: 212–219. https://dl.ndl.go.jp/pid/10837650

Sundberg P, Gibson R (2008) Global diversity of nemerteans (Nemertea) in freshwater. Hydrobiologia 595: 61–66. https://doi.org/10.1007/s10750-007-9004-6

Sundberg P, Gibson R, Strand M (2007) Swedish nemerteans (phylum Nemertea), with description of a new hoplonemertean genus and species. Journal of Natural History 41: 2287–2299. https://doi.org/10.1080/00222930701589939

Sundberg P, Kvist S, Strand M (2016) Evaluating the utility of single-locus DNA barcoding for the identification of ribbon worms (phylum Nemertea). PLoS ONE 11: e0155541. https://doi.org/10.1371/journal.pone.0155541

Wong TKF, Ly-Trong N, Ren H, Demotte P, Baños H, Roger AJ, Susko E, Bielow C, De Maio N, Goldman N, Hahn MW, Dos Reis M, Sy Vinh L, Huttley G, Lanfear R, Minh BQ (2026) IQ-TREE 3: phylogenomic inference software using complex evolutionary models. Molecular Biology and Evolution 43: msag117. https://doi.org/10.1093/molbev/msag117

Zattara EE, Fernández-Álvarez FA, Hiebert TC, Bely AE, Norenburg JL (2019) A phylum-wide survey reveals multiple independent gains of head regeneration in Nemertea. Proceedings of the Royal Society B: Biological Sciences 286: 20182524. https://doi.org/10.1098/rspb.2018.2524