e-ISSN 1734-9168
https://www.isez.pan.krakow.pl/en/folia-biologica

Folia Biologica (Kraków), vol. 74 (2026), No 2
https://doi.org/10.3409/fb_74-2.04

First record of *Paramecium pentaurelia* (Ciliophora) from Japan

Sou Kato, Konoha Tezuka, Yuto Shimada, and Masaaki Iwamoto

Accepted July 09, 2026 *Published online July 31, 2026* *Issue online July 31, 2026*

Original article

Kato S., Tezuka K., Shimada Y., Iwamoto M. 2026. First record of *Paramecium pentaurelia* (Ciliophora) from Japan. Folia Biologica (Kraków) **74**: 32-40.

Two new *Paramecium* strains were isolated from an artificial pond on a university campus located in Tokyo, Japan. Morphological observations and molecular phylogenetic analyses based on partial sequences of heat shock protein 70 and cytochrome b gene sequences identified both strains as *Paramecium pentaurelia*, one of the sibling species within the *Paramecium aurelia* species complex. This represents the first confirmed record of *P. pentaurelia* in Japan. Although this species has rarely been reported on a worldwide basis, the phylogenetic analyses showed that the Japanese strains shared identical or nearly identical sequences with multiple *P. pentaurelia* strains from geographically distant regions, suggesting that this species may have a wider, possibly cosmopolitan, distribution than has currently been recognised. The limited number of records may reflect the infrequent occurrence of this species in environmental samples, which may hinder its detection.

Key words: *Paramecium aurelia* species complex, ciliates, sibling species, biogeography, molecular phylogeny

Sou Kato, Konoha Tezuka, Yuto Shimada, Masaaki Iwamoto✉, *Department of Biosciences, College of Humanities and Sciences, Nihon University, 3-25-40 Sakurajosui, Setagaya, Tokyo 156-8550, Japan.*

E-mail: iwamoto.masaaki@nihon-u.ac.jp

The genus *Paramecium* is a group of aquatic, free-living unicellular eukaryotes within the phylum Ciliophora that includes at least seventeen morphospecies, as summarised by Fokin (2010). Among these, the *Paramecium aurelia* (Ehrenberg, 1838), once regarded as a single morphospecies, is now recognised as a species complex consisting of more than a dozen closely-related sibling species. These species share a suite of common morphological and physiological traits, including a cell length of 80-200 µm, the presence of two vacuolar-type micronuclei and the ability to undergo autogamy. With a few exceptions, they are difficult to distinguish solely on morphological grounds (Sonneborn 1975).

Each sibling species is reproductively isolated and, with rare exceptions, mating reactions do not occur between different sibling species. Since these sibling species are morphologically similar but genetically distinct, molecular approaches have become important tools for their identification and evolutionary studies (Long *et al.* 2023). Historically, they were formerly regarded as fourteen mating groups, termed

syngens (1-14), within a single morphospecies, *P. aurelia*. Subsequent studies have demonstrated that each syngen could be identified by distinct isozyme patterns (Tait 1970; Allen *et al.* 1973). Accordingly, the fourteen syngens were elevated to the species rank and assigned Linnaean binomials: *P. primaurelia* (formerly Syngen 1), *P. biaurelia* (formerly Syngen 2), *P. triaurelia* (formerly Syngen 3), … and *P. quadecaurelia* (formerly Syngen 14) (Sonneborn 1975). In addition, *P. sonneborni*, previously described as a distinct morphospecies, was later recognised as an additional sibling species within the *P. aurelia* complex (Aufderheide *et al.* 1983), bringing the number of confirmed sibling species to fifteen. More recently, a novel strain potentially representing another sibling species was discovered in Mexico (Potekhin & Mayén-Estrada 2020), indicating that the diversity of the *P. aurelia* complex may be greater than is currently recognised.

The sibling species of the *P. aurelia* complex differ markedly in terms of their geographic distributions. For example, *P. primaurelia*, *P. biaurelia*, *P. tetraurelia* and *P. sexaurelia* have frequently been reported from a broad range of localities throughout the world (Przyboś & Fokin 2000). By contrast, the distributions of *P. undecaurelia* and *P. sonneborni* are geographically restricted (Przyboś & Surmacz 2010; Przyboś *et al.* 2014). Furthermore, several species, including *P. sexaurelia*, *P. octaurelia*, *P. tredecaurelia* and *P. quadecaurelia*, are considered to preferentially occur in warm regions, suggesting that the occurrence of individual species within the *P. aurelia* complex may be constrained by specific temperature ranges (Sonneborn 1975; Przyboś & Prajer 2015).

In Japan, the *P. aurelia* complex has been collected from various localities, with *P. primaurelia*, *P. biaurelia*, *P. tetraurelia*, *P. sexaurelia*, *P. decaurelia* and *P. dodecaurelia* being identified (Sonneborn 1950; Kościuszko & Koizumi 1984; Przyboś & Fokin 2001; Przyboś *et al.* 2003; Przyboś & Surmacz 2010). However, no other sibling species of the complex have been recorded to date.

In the present study, we collected two *Paramecium* strains presumed as belonging to the *P. aurelia* complex from an artificial pond located on the campus of Nihon University in Setagaya, Tokyo. Analyses of the nucleotide sequences of the cytosolic hsp70 and mitochondrial cytochrome b genes, followed by a comparison with those of the sibling species, revealed that the newly-obtained strains were *P. pentaurelia*. This species is considered to be rare, with a low frequency of its occurrence worldwide. To date, it has been reported from North America, Europe, the Russian Far East, India and Australia (Sonneborn 1975; Przyboś & Fokin 2000; Przyboś & Prajer 2015), but there have been no previous records from Japan. Therefore, the present finding extends the known geographic range of *P. pentaurelia* and provides new insight into the biogeography of the *P. aurelia* complex, contributing to a broader understanding of *Paramecium*'s diversity (Serra *et al.* 2021).

Materials and Methods

Materials

All chemicals and reagents were obtained from Nacalai Tesque (Tokyo, Japan), unless otherwise stated.

Culture medium for *Paramecium*

The grass infusion was prepared by adding 50 g of young barley grass powder (Yamamoto Kanpoh, Aichi, Japan) to 1 l of deionised water. The mixture was autoclaved and then centrifuged at a low speed to remove the grass powder residue. The resulting supernatant was subsequently filtered through cotton. This infusion was diluted 50-fold with a phosphate buffer (final concentrations: 1.4 mM Na_2HPO_4, 0.6 mM KH_2PO_4) and autoclaved. A 0.4% β-sitosterol stock solution in ethanol was then added at 1/5000 of the total volume to give a final concentration of 0.8 μg/ml. *Klebsiella pneumoniae* was inoculated into the grass infusion medium one day prior to the introduction of *Paramecium*. The culture was maintained at 26 ± 1°C, and a portion was transferred to a fresh medium once a week.

Isolation of single paramecia from pond water

Water from the pond was collected primarily from the surfaces of stones and walls using a plastic dropper. Paramecia were identified under a stereomicroscope (SZX7, Olympus, Tokyo, Japan), and single individuals were isolated using a micropipette and transferred individually to wells of a triple-well blood typing slide filled with a bacteria-free culture medium. Each cell was washed by successive transfers to different wells and subsequently transferred to a culture medium inoculated with bacteria to establish a clonal culture.

Measurement of the cell size

Paramecium cells in the early stationary phase of the culture were fixed with formalin at a final concentration of 2% for 30 min at room temperature.

After the fixation, the cells were washed three times with phosphate-buffered saline (PBS) and microscope slides were prepared. To prevent a compression of the specimens under the coverslip, thin strips of Scotch tape were attached to the slide to serve as spacers between the slide and the coverslip. The preparations were observed under bright-field illumination using a biological microscope (OPTIPHOT, Nikon, Tokyo, Japan) with a 40× objective lens. Images of the cells were captured using a digital camera (Nikon DSLR), and the lengths of the major and minor axes of 100 cells were measured using the Straight Line tool in ImageJ to calculate the average cell dimensions.

Observation of the nuclear morphology

Paramecia were fixed with formalin at a final concentration of 10%. After three washes with 5 mM Tris-HCl (pH 7.4), the DNA was stained with 0.5 µg/ml 4′,6-diamidino-2-phenylindole (DAPI).

To observe autogamic cells, logarithmically growing cells were transferred in 5 mM Tris-HCl (pH 7.4) to induce starvation and were fixed after 5 h.

The fixed and stained cells were observed using a Nikon A1 confocal fluorescence microscope equipped with a PlanApo 20×/NA 0.75 objective lens (Nikon). The pinhole was set to 1 AU. Fluorescence images were generated from multiple z-stack projections at 0.3 µm intervals.

Polymerase chain reaction

The PCR was performed using Ex Premier DNA polymerase (Takara, Kyoto, Japan), according to the manufacturer's instructions. The reaction mixture contained 25 µl of 2× master mix, 0.6 µM of each primer and 1 µl of *Paramecium* culture briefly concentrated by centrifugation, containing several cells, as the template, in a total reaction volume of 50 µl.

Amplification of the cytosolic hsp70 gene (*HSP70*) was carried out using the forward primer 5'-atggtaccctcaaaggctgttatcacag-3' (HSP70F_KpnI) and the reverse primer 5'-atgagctcgttaagatagcagcttagac-3' (HSP70R_SacI). These primers were designed to amplify a larger region of the *HSP70* coding sequence than the region analysed by Hori *et al.* (2006). We initially tested the primers reported by Hori *et al*. (2006); however, an efficient amplification of the target region was not achieved using our strains. This reaction yielded a 686 bp fragment corresponding to a partial region of the *HSP70* coding sequence, including the 378 bp region analysed by Hori *et al.* (2006).

To specifically amplify the mitochondrial cytochrome b gene (*CYTB*) region analysed by Barth *et al.* (2008), a primer pair was designed using the *CYTB* sequence of *P. pentaurelia* stock 87 obtained from the *Paramecium* Database. Because our preliminary *HSP70* analysis suggested that the Japanese isolates belonged to *P. pentaurelia*, we selected a *P. pentaurelia CYTB* sequence as a reference for the primer design. The forward primer 5'-atggtaccatgttggcctttagcct-3' (PpenCytF_KpnI) and the reverse primer 5'-cgtctagaatgcaataagtcaagccatg-3' (PpenCytR_XbaI) were used to amplify a 618 bp fragment corresponding to a partial sequence of the *CYTB* gene.

The PCR cycling conditions for the amplification of both genes consisted of 30 cycles of 98°C for 10 s, 55°C for 15 s and 68°C for 30 s.

Cloning and sequencing of the PCR products

Each PCR product was purified using the NucleoSpin Gel and PCR Clean-up kit (MACHEREY-NAGEL, Düren, Germany), digested at both ends with the appropriate restriction enzymes (New England Biolabs, Beverly, MA), and cloned into the multiple cloning site of pBluescript SK(+). Plasmid DNA was isolated and purified from transformed *E. coli* cells (strain C2984H, New England Biolabs) and subjected to Sanger sequencing by Eurofins Genomics (Tokyo, Japan), using either the M13-20 or M13 Reverse primer.

Molecular phylogenetic analysis

To identify the species of the isolated strains, molecular phylogenetic analyses were performed using partial sequences of *HSP70* and *CYTB*. For the *HSP70* analysis, sequences of the *P. aurelia* sibling species reported by Hori *et al.* (2006) were used. For the *CYTB* analysis, sequences of the *P. pentaurelia* strains reported by Przyboś *et al.* (2011a) and the related *P. aurelia* sibling species reported by Barth *et al.* (2008), Przyboś *et al.* (2011b) and Przyboś *et al.* (2015) were used. All of the reference sequences were retrieved from GenBank.

The sequences of strains NU23A1 and NU23B1, together with the reference sequences, were aligned separately for each gene using ClustalW (Thompson *et al.* 1994) and converted into the Phylip format. Maximum-likelihood (ML) phylogenetic analyses were performed using ATGC (Guindon *et al.* 2010) with 1,000 bootstrap replicates. The best-fit nucleotide substitution model was selected based on the Bayesian information criterion (BIC). *Paramecium multimicronucleatum* and *Paramecium schewiakoffi*

were used as outgroups for the *HSP70* and *CYTB* analyses, respectively. *P. schewiakoffi* was selected for the *CYTB* analysis based on previous phylogenetic analyses of the *P. aurelia* complex (Barth *et al.* 2008). The resulting phylogenetic trees were visualised using FigTree v1.4.4.

The accession numbers of the reference sequences used for the phylogenetic analyses are provided in Supplementary Table 1 (SM.01).

Results and Discussion

In June 2023, two ciliate strains of the genus *Paramecium*, designated NU23A1 and NU23B1, were isolated from an artificial pond located on the campus of Nihon University, College of Humanities and Sciences, Setagaya, Tokyo (Fig. 1). The pond is a completely closed water body with no inflow from external sources, while the water is maintained by a filtration-circulation system. Strain NU23A1 was collected at coordinates (35.663220° N, 139.634763° E), and NU23B1 was collected at (35.663228° N, 139.634667° E), approximately 8 m apart. To assign the morphospecies of NU23A1 and NU23B1, cytological observations were conducted for both strains.

At the early stationary phase of the culture, the cell dimensions were measured, and a nuclear morphology was examined after DAPI staining. The cell dimensions (mean ± SD, n = 100) of strain NU23A1 were 132.2 ± 13.9 µm in length and 39.2 ± 4.97 µm in width, while those of strain NU23B1 were 140.4 ± 11.9 µm in length and 40.6 ± 10.8 µm in width. No significant differences in the cell dimensions were detected between the two strains.

In both strains, two micronuclei with a 'vesicular' appearance were observed adjacent to the macronucleus (Fig. 2A; see Fokin 2010). In the cultures examined shortly after the onset of starvation, many non-pairing individuals exhibiting an increased number of micronuclei, due to meiotic divisions during autogamy, were observed (Fig. 2B, C). Although the micronuclei appeared 'vesicular', the overall morphology and the ability to undergo autogamy were consistent with members of either the *Paramecium aurelia* species complex or the closely-related *P. jenningsi* species complex, both of which share similar morphological characteristics (Diller & Earl 1958; Przyboś & Tarcz 2019).

Fig. 1. Sampling site of *Paramecium* strains NU23A1 and NU23B1. Photograph of the artificial pond in the Setagaya campus of Nihon University in Tokyo, where the strains were collected. Yellow arrows indicate the sampling locations of strains NU23A1 and NU23B1.

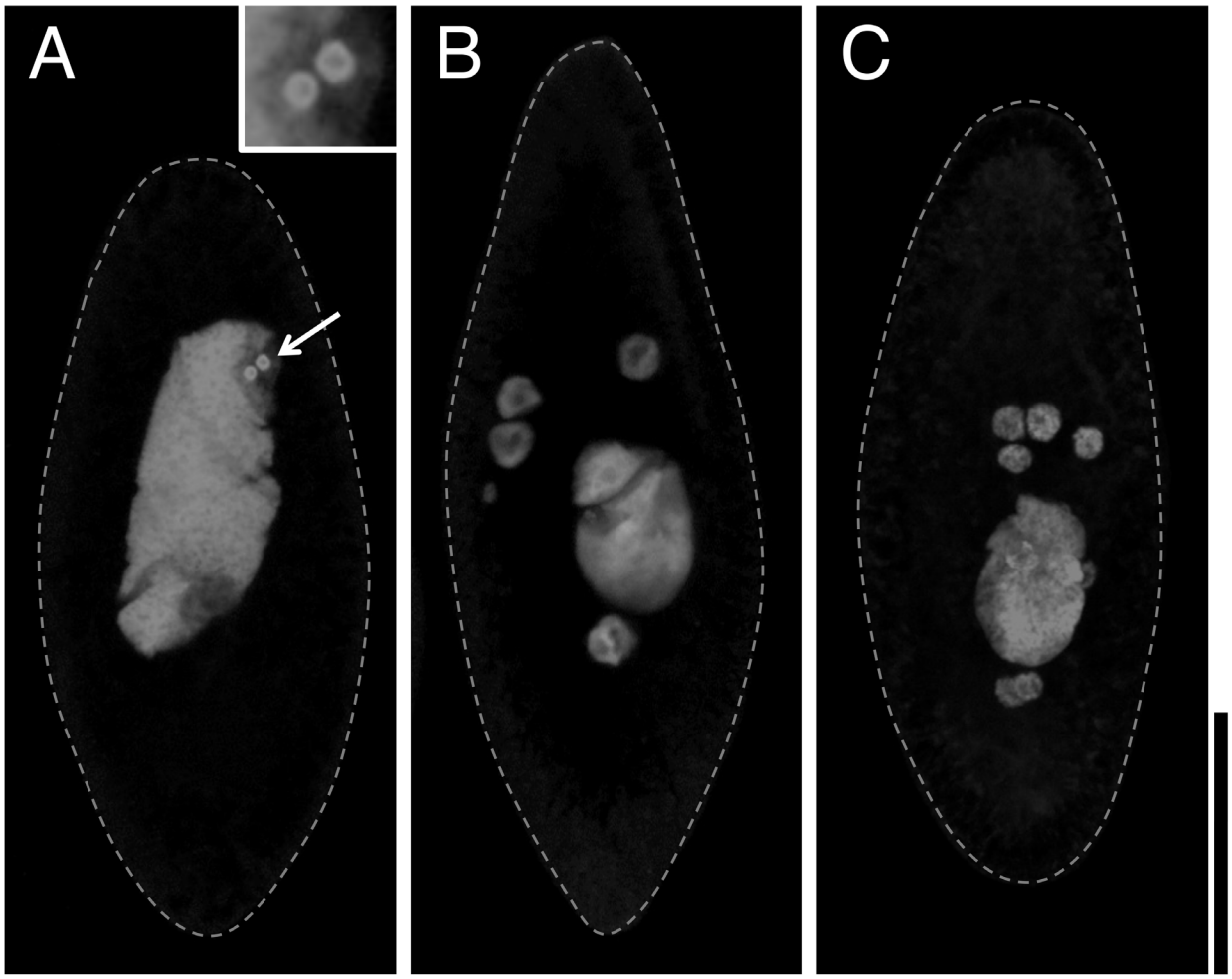

Fig. 2. Cell morphology of *Paramecium* NU strains stained with DAPI. A – An NU23B1 cell in the early stationary phase of culture. An arrow indicates two adjacent micronuclei. The inset shows a magnified view of the 'vesicular' appearance of the micronuclei. B, C – NU23B1 cells undergoing autogamy. These cells contain multiple enlarged micronuclei produced by meiosis, presumably representing an early stage of autogamy. The cell shown in B – contains four micronuclei; whereas the cell shown in C – contains eight, two of which overlap with the macronuclear staining. The NU23A1 cells are not shown because they exhibited essentially the same morphology. Broken lines indicate the outlines of the cells. Scale bar: 50 μm.

To accurately identify the species of these strains, we subsequently conducted molecular phylogenetic analyses. Partial coding sequences of the cytosolic heat shock protein 70 gene (*HSP70*) and the mitochondrial cytochrome b gene (*CYTB*) were used as molecular markers, as these genes have been used to investigate phylogenetic relationships and genetic diversity among *P. aurelia* sibling species in previous studies. The use of both a nuclear and a mitochondrial marker allowed us to evaluate the phylogenetic placement of these strains using information from different genomic compartments. The corresponding regions were amplified by PCR from each strain, and their nucleotide sequences were used for the phylogenetic analyses, together with reference sequences obtained from GenBank (listed in Supplementary Table 1, SM.01).

For the *HSP70* analysis, a 378-bp region within the PCR-amplified partial sequence of *HSP70* was used, corresponding to the region analysed by Hori *et al.* (2006). The PCR products were sequenced, and the obtained partial *HSP70* sequences (686 bp excluding the primer sequences) from NU23A1 and NU23B1 were found to be identical to each other and were deposited in GenBank under Accession Numbers LC939786 and LC939787, respectively. In the maximum-likelihood phylogenetic analysis, NU23A1 and NU23B1 clustered with the reference sequences of *P. pentaurelia* (Fig. 3A). The analysed *HSP70* region showed a 100% identity to that of *P. pentaurelia* Ep-9 (collected in Hungary; Accession No. AB232950) and differed by only one nucleotide from that of 87 (collected in Pennsylvania; AB100848).

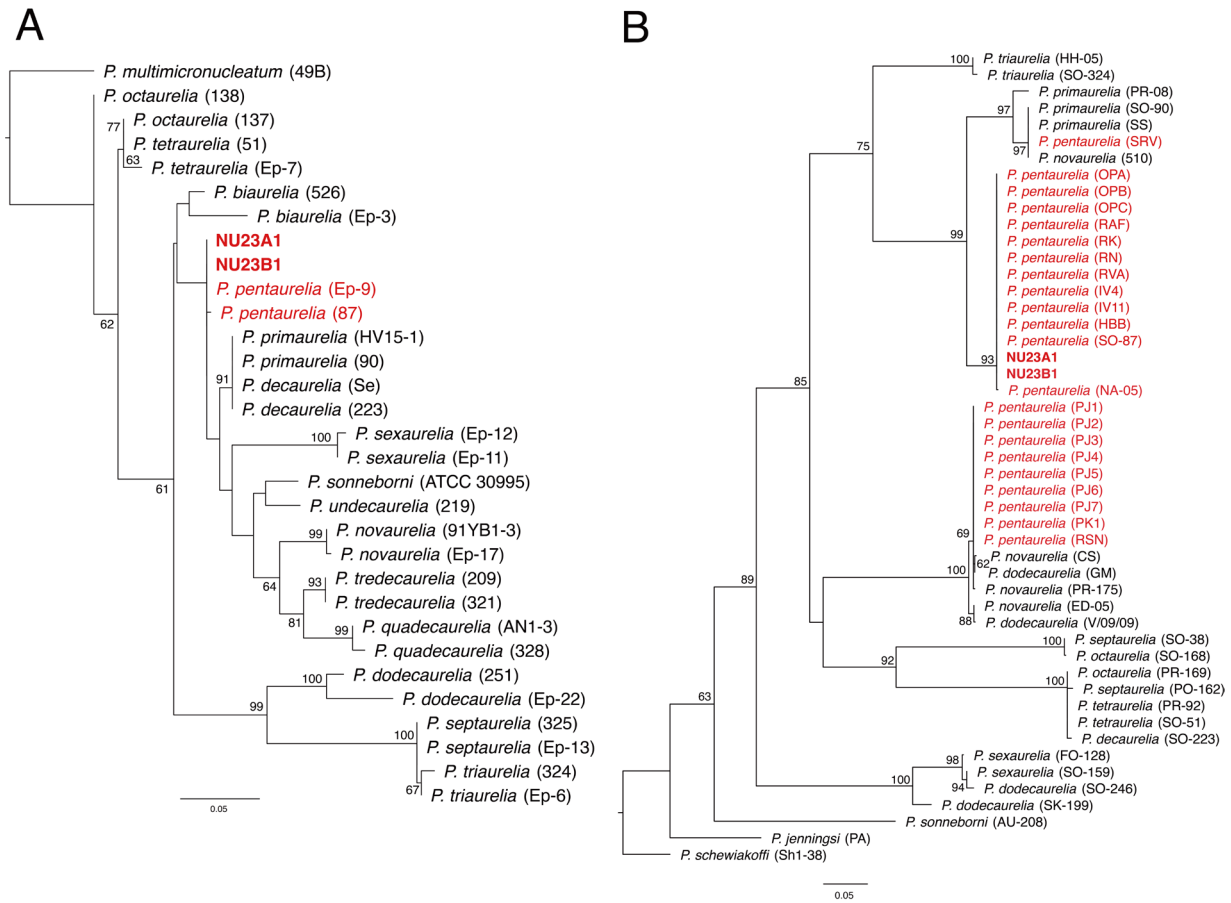

Fig. 3. Molecular phylogenetic analyses of NU23A1 and NU23B1 based on *HSP70* and *CYTB* sequences. Maximum-likelihood (ML) trees were constructed based on partial sequences of the cytosolic heat shock protein 70 gene (*HSP70*) (A) and the mitochondrial cytochrome b gene (*CYTB*) (B). The trees were generated using sequences obtained from strains NU23A1 and NU23B1, as well as reference sequences of *P. aurelia* sibling species and outgroup taxa retrieved from GenBank. Bootstrap values based on 1,000 replicates are shown at nodes when ≥ 50%. The scale bars indicate 0.05 substitutions per site. *P. pentaurelia* strains are indicated in red, while the strains isolated in this study (NU23A1 and NU23B1) are shown in bold.

Next, we analysed the *CYTB* gene. A 618-bp partial sequence of the coding region, as described by Barth *et al*. (2008) and Przyboś *et al.* (2011a), was used for the comparison. The obtained partial *CYTB* sequences of NU23A1 and NU23B1 were found to be identical to each other and were deposited in GenBank under Accession Numbers LC939788 and LC939789, respectively. In the *CYTB*-based maximum-likelihood phylogenetic analysis, NU23A1 and NU23B1 were included within a *P. pentaurelia* cluster (Fig. 3B). The *CYTB* sequences of NU23A1 and NU23B1 were found to be identical to those of several *P. pentaurelia* strains, including SO-87 (AM949782; same as 87), and differed by only one nucleotide from NA-05 (collected in Naples, Italy; AM949781).

Together, the *HSP70*- and *CYTB*-based phylogenetic analyses supported the identification of NU23A1 and NU23B1 as *P. pentaurelia*. The *CYTB* haplotype identical to that of the NU strains has been reported from geographically distant *P. pentaurelia* strains.

Polymorphisms have been reported among *P. pentaurelia* strains in the 618-bp region of the *CYTB* gene analysed in this study (Przyboś *et al.* 2011a). Among the strains examined in that study, the RAF strain collected in Altai, Russia (in the Central Asian region), showed a complete identity with NU23A1 and NU23B1, as did SO-87 and several strains collected from various regions of Europe. By contrast, the RSN strain collected in Novosibirsk, Russia (in the Central Asian region), exhibited only an 84.6% sequence identity. Strains with *CYTB* sequences identical to those of the NU strains have thus been identified in geographically distant regions across multiple continents, suggesting that there is no apparent correlation between *CYTB* polymorphisms and their geographic distribution.

In this broader context, the discovery of *P. pentaurelia*, a globally rare species, in Japan for the first time

suggests that the seemingly limited distribution of certain sibling species may simply reflect insufficient sampling across broad geographic areas, combined with their low natural abundance. With more extensive surveys, such species are likely to be discovered in a wider range of regions. Therefore, the currently recognised differences in the apparent distributions of these sibling species may not necessarily reflect their true natural ranges.

The route by which *P. pentaurelia* invaded the artificial pond on the university campus remains unknown. Large, free-living protists, such as *Paramecium* species, which do not form resting cysts or possess a protective shell, are unlikely to disperse easily over long distances. Because the water surface of the pond is located a few metres higher than the surrounding terrain, it is improbable that an overflow from nearby aquatic habitats has entered the pond. However, the spot-billed duck (*Anas zonorhyncha*), a waterfowl species known to act as a potential vector of aquatic microorganisms (Coughlan *et al.* 2017), is often observed at the site. In addition, pigeons and starlings are frequently seen bathing in the pond water, and these terrestrial birds may also carry microorganisms (Johansson *et al.* 2021). Insects are another possible vector (Maguire 1963; Beladjal & Mertens 2009), as aquatic insects such as water striders and dragonfly nymphs commonly inhabit the pond. However, frogs and their tadpoles, which could also serve as potential vectors (Sabagh *et al.* 2011; Cochak *et al.* 2021), are not present in the pond at all.

Although several animals may serve as potential vectors, *P. pentaurelia* has not yet been detected in ponds or streams around the campus. Its original source therefore remains unclear. To elucidate the natural habitat of *P. pentaurelia*, further surveys will be necessary to better understand the distribution of sibling species within the *P. aurelia* complex in the Tokyo area, including at sites around the university campus.

The strains NU23A1 and NU23B1 isolated in this study have been deposited in the National Bio-Resource Project (NBRP) '*Paramecium*' and are available from the project as strain numbers PA050003B and PA050004B, respectively (https://nbrp.jp/en/resource/paramecium-en/).

Acknowledgements

We thank the *Paramecium* Database (ParameciumDB) for providing the gene sequence information used in this study and the National BioResource Project '*Paramecium*' of the MEXT for accepting the deposition of the strains identified in this work.

Funding

This research was funded by a Research Grant of the Institute of Natural Sciences at Nihon University for 2024-2025.

Author Contributions

Research concept and design: M.I.; Collection and/or assembly of data: S.K., K.T., Y.S., M.I.; Data analysis and interpretation: Y.S., M.I.; Writing the article: M.I.; Final approval of the article: S.K., K.T., Y.S., M.I.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Materials

Supplementary Materials to this article can be found online at:
https://www.isez.pan.krakow.pl/en/folia-biologica
Supplementary files:
SM.01. Supplemental Table 1. Reference sequences obtained from GenBank used for the phylogenetic analysis.

References

Allen S.L., Farrow S.W., Golembiewski P.A. 1973. Esterase variations between the 14 syngens of *Paramecium aurelia* under axenic growth. Genetics **73**: 561-573. https://doi.org/10.1093/genetics/73.4.561

Aufderheide K.J., Daggett P.-M., Nerad T.A. 1983. *Paramecium sonneborni* n. sp., a new member of the *Paramecium aurelia* species complex. J. Protozool. **30**: 128-131. https://doi.org/10.1111/j.1550-7408.1983.tb01046.x

Barth D., Przyboś E., Fokin S.I., Schlegel M., Berendonk T.U. 2008. Cytochrome *b* sequence data suggest rapid speciation within the *Paramecium aurelia* species complex. Mol. Phylogenet. Evol. **49**: 669-673. https://doi.org/10.1016/j.ympev.2008.08.007

Beladjal L., Mertens J. 2009. Diaspore dispersal of Anostraca by flying insects. J. Crust. Biol. **29**: 266-268. https://doi.org/10.1651/08-3059R.1

Cochak C., de Oliveira F.R., Lansac-Tôha F.M., Meira B.R., Guimarães Durán C.L., Vitule J.R.S., Velho L.F.M. 2021. Relative contributions of disparate animal vectors to the development of freshwater ciliate communities. Hydrobiologia **848**: 1121-1135. https://doi.org/10.1007/s10750-021-04518-9

Coughlan N.E., Kelly T.C., Davenport J., Jansen M.A.K. 2017. Up, up and away: bird-mediated ectozoochorous dispersal between aquatic environments. Freshw. Biol. **62**: 631-648. https://doi.org/10.1111/fwb.12894

Diller W.F., Earl P.R. 1958. *Paramecium jenningsi*, n. sp. J. Protozool. **5**: 155-158. https://doi.org/10.1111/j.1550-7408.1958.tb02545.x

Fokin S.I. 2010. *Paramecium* genus: biodiversity, some morphological features and the key to the main morphospecies discrimination. Protistology **6**: 227-235.

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. Syst. Biol. **59**: 307-321. https://doi.org/10.1093/sysbio/syq010

Hori M., Tomikawa I., Przyboś E., Fujishima M. 2006. Comparison of the evolutionary distances among syngens and sibling species of *Paramecium*. Mol. Phylogenet. Evol. **38**: 697-704. https://doi.org/10.1016/j.ympev.2005.09.006

Johansson N.R., Kaasalainen U., Rikkinen J. 2021. Woodpeckers can act as dispersal vectors for fungi, plants, and microorganisms. Ecol. Evol. **11**: 7154-7163. https://doi.org/10.1002/ece3.7648

Kościuszko H., Koizumi S. 1984. Habitats of the *Paramecium aurelia* complex in Japan. Folia Biol. (Kraków) **32**: 57-62.

Long H., Johri P., Gout J.F., Ni J., Hao Y., Licknack T., Wang Y., Pan J., Jiménez-Marín B., Lynch M. 2023. *Paramecium* Genetics, Genomics, and Evolution. Annu. Rev. Genet. **57**: 391-410. https://doi.org/10.1146/annurev-genet-071819-104035

Maguire B. Jr. 1963. The passive dispersal of small aquatic organisms and their colonization of isolated bodies of water. Ecol. Monogr. **33**: 161-185. https://doi.org/10.2307/1948560

Potekhin A., Mayén-Estrada R. 2020. *Paramecium* diversity and a new member of the *Paramecium aurelia* species complex described from Mexico. Diversity **12**: 197. https://doi.org/10.3390/d12050197

Przyboś E., Fokin S. 2000. Data on the occurrence of species of the *Paramecium aurelia* complex world-wide. Protistology **1**: 179-184.

Przyboś E., Fokin S. 2001. Species of the *Paramecium aurelia* complex in Japan. Folia Biol. (Kraków) **49**: 105-106.

Przyboś E., Fujishima M., Nakaoka Y. 2003. *Paramecium decaurelia* and *Paramecium dodecaurelia* from the *P. aurelia* spp. complex in Japan. Folia Biol. (Kraków) **51**: 223-224.

Przyboś E., Prajer M. 2015. New stands of species of the *Paramecium aurelia* complex; is the occurrence of particular species limited by temperature barriers? Folia Biol. (Kraków) **63**: 215-220. https://doi.org/10.3409/fb63_3.215

Przyboś E., Surmacz M. 2010. New, world-wide data on the distribution of species of the *Paramecium aurelia* complex (Ciliophora, Protozoa). Folia Biol. (Kraków) **58**: 185-188. https://doi.org/10.3409/fb58_3-4.185-188

Przyboś E., Tarcz S. 2019. Global molecular variation of *Paramecium jenningsi* complex (Ciliophora, Protista): a starting point for further, detailed biogeography surveys. Syst. Biodivers. **17**: 527-539. https://doi.org/10.1080/14772000.2019.1643424

Przyboś E., Tarcz S., Greczek-Stachura M., Surmacz M. 2011a. Polymorphism of *Paramecium pentaurelia* (Ciliophora, Oligohymenophorea) strains revealed by rDNA and mtDNA sequences. Eur. J. Protistol. **47**: 138-143. https://doi.org/10.1016/j.ejop.2010.11.001

Przyboś E., Tarcz S., Greczek-Stachura M., Surmacz M. 2011b. Seasonal and spatial variability of species occurrence of the *Paramecium aurelia* complex in a single natural pond (Protista, Ciliophora). Hydrobiologia **663**: 233-244. https://doi.org/10.1007/s10750-010-0575-2

Przyboś E., Tarcz S., Rautian M., Lebedeva N. 2014. The first European stand of *Paramecium sonneborni* (*P. aurelia* complex), a species known only from North America (Texas, USA). Eur. J. Protistol. **50**: 236-247. https://doi.org/10.1016/j.ejop.2014.03.001

Przyboś E., Tarcz S., Rautian M., Sawka N. 2015. Delimiting species boundaries within a paraphyletic species complex: insights from morphological, genetic, and molecular data on *Paramecium sonneborni* (*Paramecium aurelia* species complex, Ciliophora, Protozoa). Protist **166**: 438-456. https://doi.org/10.1016/j.protis.2015.07.001

Sabagh L.T., Dias R.J.P., Branco C.W.C., Rocha C.F.D. 2011. News records of phoresy and hyperphoresy among treefrogs, ostracods, and ciliates in bromeliad of Atlantic Forest. Biodivers. Conserv. **20**: 1837-1841. https://doi.org/10.1007/s10531-011-0050-z

Serra V., Fokin S.I., Gammuto L., Nitla V., Castelli M., Basuri C.K., Satyaveni A., Sandeep S.V., Modeo L., Petroni G. 2021. Phylogeny of *Neobursaridium* reshapes the systematics of *Paramecium* (Oligohymenophorea, Ciliophora). Zool. Scr. **50**: 241-268. https://doi.org/10.1111/zsc.12464

Sonneborn T.M. 1950. Methods in the general biology and genetics of *Paramecium aurelia*. J. Exp. Zool. **113**: 87-147. https://doi.org/10.1002/jez.1401130106

Sonneborn T.M. 1975. The *Paramecium aurelia* complex of fourteen sibling species. Trans. Am. Microsc. Soc. **94**: 155-178. https://doi.org/10.2307/3224977

Tait A. 1970. Enzyme variation between syngens in *Paramecium aurelia*. Biochem. Genet. **4**: 461-470. https://doi.org/10.1007/BF00486596

Thompson J.D., Higgins D.G., Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**: 4673-4680. https://doi.org/10.1093/nar/22.22.4673