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Morphology and Molecular Phylogeny of Two Newly Recorded Plagiopylean Ciliate Species from South Korea

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ABSTRACT

Two brackish estuarine plagiopylean ciliate species, *Parasonderia vestita* and *Plagiopyla ovata*, were identified during survey of ciliates in low-oxygen habitats. These species have not been previously recorded in Korea. We examined their morphology by microscopic observations of live and protargol-impregnated specimens and analyzed the small subunit ribosomal DNA (SSU rDNA) sequence to infer their phylogeny. *Parasonderia vestita* is diagnosed by the following characteristics: body size of 50−78 × 28−44 μm *in vivo*; oval mouth opening sub-apically located; four circle kineties with 13−14 ventral and 11−14 dorsal somatic kineties; oral ciliatures composed of 14−17 prebuccal, 6−7 parabuccal, 12−14 postbuccal, and 10−13 intrabuccal polykineties. *Plagiopyla ovata* is diagnosed by followings: body size of 64−100 × 34−50 μm *in vivo*; tube-like buccal cavity covers 72−88% of body width *in vivo*; 55−80 somatic kineties; spherical micronucleus, 3−5 μm in diameter; distance from anterior cell end to the upper oral lip covers 25% of body length; dense ciliary kinetids arrangement above oral region. The SSU rDNA phylogeny confirms the morphological identifications. The Korean population of *Parasonderia vestita* is clustered together with two Chinese populations with full support (100/1.0, maximum likelihood [ML]/Bayesian inference [BI]), with 99.8% and 100% nucleotide similarity, respectively. Similarly, the Korean population of *Plagiopyla ovata* is firmly clustered with a Chinese population and ten congeners of *Plagiopyla* with full support (100/1.0, ML/BI). The Korean and Chinese populations of *P. ovata* share 99.8% nucleotide similarity.

Keywords: anaerobic ciliates, brackish estuarine, *Parasonderia*, *Plagiopyla*, SSU rDNA

INTRODUCTION

Anaerobic ciliates are a diverse group of single-celled organisms that thrive in oxygen-deprived environments. They have captured the attention of scientists and researchers around the world due to their unique adaptations and evolutionary history (Modeo et al., 2013; Bourland and Wendell, 2014; Bourland et al., 2014, 2017a, 2017b, 2018a, 2018b, 2020, 2024; Foissner, 2016a, 2016b; Vd'ačný and Foissner, 2017a, 2017b, 2019; Rotterová et al., 2018, 2020; Campello-Nunes et al., 2020; Zhuang et al., 2021; Chen et al., 2022; Fokin and Serra, 2022; Nguyen et al., 2024). The studies of class Plagiopylea were also significantly contributed to the development of ciliate systematics over the past decade (Modeo et al., 2013; Xu et al., 2013; Nitla et al., 2019; Li et al., 2021, 2022, 2023a, 2023b, 2024; Omar and Jung, 2022). Despite the widespread distribution of Plagiopylea members, their cryptic lifestyle, limited ability to be cultivated, and a few distinct taxonomic characteristics make species-level identification extremely difficult (Nitla et al., 2019). According to Lynn (2008), the class Plagiopylea Small and Lynn, 1985 comprises an order Plagiopylida Jankowski, 1978, which includes three families: Sonderiidae Small and Lynn, 1985; Plagiopylidae Schewiakoff, 1896; and Trimyemidae Kahl, 1933. The family Sonderiidae has five genera, including the genus *Parasonderia* Jankowski, 2007, which is known for its elliptical to circular oral opening, shallow buccal cavity, circular kineties, and oral ciliature (prebuccal, postbuccal, parabuccal, and intrabuccal polykineties) (Xu et al., 2013; Li et al., 2024). The family Plagiopylidae comprises four genera: *Plagiopyla* Stein, 1860; *Lechriopyla* Lynch, 1971; *Pseudoplagiopyla* Small and Lynn, 1985; and *Paraplagiopyla* Thurston and Grain, 1971. Members of the

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genus *Plagiopyla* are typically found in freshwater and marine anaerobic environments and are characterized by an ovoid body, a slit-like buccal aperture, a single macronucleus, and cytoplasmic extrusomes (Nitla et al., 2019; Li et al., 2021). To date, only three species of Plagiopylea have been identified in Korea: *Trimyema koreanum*, *Plagiopyla frontata*, and *Plagiopyla nasuta*, highlighting the limited but significant presence of plagiopylean species in Korea (Cho et al., 2008; Omar and Jung, 2022). To expand the known diversity of ciliates in Korea, we have documented the discovery and description of two previously unrecorded plagiopylean species inhabiting coastal regions.

MATERIALS AND METHODS

Sampling and morphology observation

Parasonderia vestita (Kahl, 1928) Xu et al., 2013 was collected from a drainage channel near Mokpo Maritime University, Jukgyo-dong, Mokpo-si, Jeollanam-do, Korea (34°47′56.0″N, 126°21′48.2″E), in October 2021, salinity 18‰. *Plagiopyla ovata* Kahl, 1931 was collected from a pond in saltmarsh, Sinjang-ri, Aphae-eup, Sinan-gun, Jeollanam-do, Korea (34°49′09.8″N, 126°21′29.4″E), in October 2021, salinity 20‰. Sediments containing organic matter and water were sampled in a one-liter plastic bottle. These samples were then transferred and stored at room temperature. A few grains of wheat or rice were added as a food source to increase bacterial colonies. The morphology of ciliates was studied in the laboratory by microscopic examination of live specimens and by protargol impregnation of fixed cells (Wilbert, 1975). The live and protargol-impregnated specimens were examined under a light microscope (Axio Image A1; Carl Zeiss, Oberkochen, Germany). Measurements and counts were performed at magnifications ranging from 100× to 1,000×. Terminology and classification scheme are based on Jankowski (2007), Lynn (2008), and Xu et al. (2013).

DNA extraction and sequencing

Three cells from each population were selected using a glass pipette under a stereomicroscope, washed three times, and each transferred to an EP tube with a minimum volume of water. DNA was extracted from each cell using the RED Extract-N-Amp Tissue PCR Kit (Sigma Aldrich, St. Louis, MO, USA) according to manufacturer's protocol. The 18S rDNA was amplified using the EukA (5′-AAC CTG GTT GAT CCT GCC AG-3′) and D1D2-R2 (5′-ACG ATC GAT TTG CAC GTC AG-3′) primer combinations (Medlin et al., 1988; Sonnenberg et al., 2007) for both species and the PCR cycling conditions were based protocol described by Chen and Song (2001), using the Q5 Hot Start High-Fidelity DNA polymerase kit. The amplified 18S rDNA was sequenced in both directions using five primers (EukA, EukB, 528F, 900F, and 900R) at the sequencing company (Cosmo Genetech, Seoul, Korea). The contigs were assembled using Geneious Prime ver. 2019.0.4 (https://www.geneious.com).

Phylogenetic analyses

Maximum likelihood (ML) and Bayesian inference (BI) methods were used to reconstruct the phylogenetic tree for the two species, using a total of 83 small subunit ribosomal DNA (SSU rDNA) sequences from plagiopylean members and eight prostomatean species as an outgroup. DNA sequences were aligned online using the MUSCLE package on the European Bioinformatics Institute web server (http://www.ebi.ac.uk/Tools/msa/muscle/). The alignment result, which was manually modified using MEGA7 to trim both ends (Kumar et al., 2016). The final dataset used for phylogenetic analyses comprised 1,786 sites. ML analysis with 1,000 ultrafast bootstrap replicates was performed using IQTREE ver. 2.3.6 under GTR + I + G model, which was selected by Corrected Akaike Information Criterion (Minh et al., 2020). BI analysis was performed with MrBayes ver. 3.2 using the GTR + I + G model (Huelsenbeck and Ronquist, 2001), which was selected by Corrected Akaike Information Criterion in Mrmodeltest ver. 2 (Nylander, 2004). Markov chain Monte Carlo simulations were run for 1,000,000 generations, sampling every 100 generations. The first 25% of trees were discarded as burn-in. The tree topology was visualized using FigTree ver. 1.4.3 (Rambaut, 2010).

RESULTS

Class Plagiopylea Small and Lynn, 1985
Order Plagiopylida Jankowski, 1978
[1]*Family Sonderiidae Small and Lynn, 1985
[2]*Genus *Parasonderia* Jankowski, 2007

[3]****Parasonderia vestita*** **(Kahl, 1928) Xu et al., 2013 (Table 1, Figs. 1, 2)**

Plagiopyla vestita Kahl, 1928: 91, fig. 19(b).
Sonderia vestita Kahl, 1931: 270, fig. 456.
Plagiopyla vestita Carey, 1992: 103, fig. 355.
Sonderia vestita Jankowski, 2007: 790.
Parasonderia vestita (Kahl, 1928), Xu et al., 2013: 107, figs. 1–3.
Parasonderia vestita (Kahl, 1928), Li et al., 2024: 126087, 6–10, fig. 5.

Korean name: [1]*가로등근입섬모충과, [2]*복합구강섬모충속, [3]*등근핵복합구강섬모충

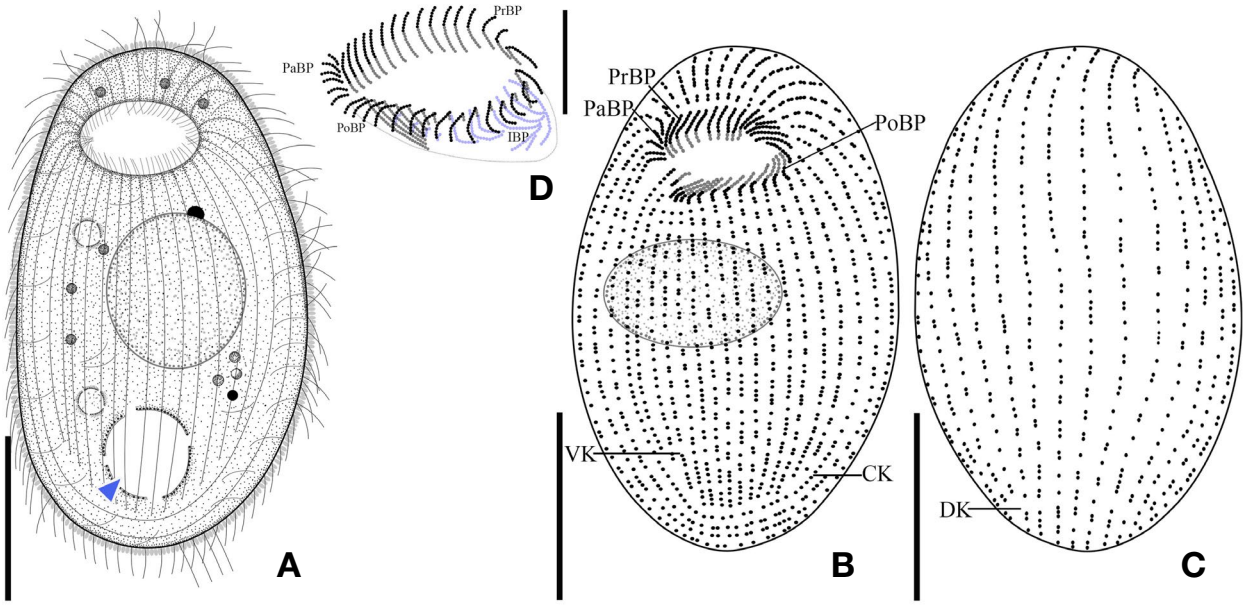

Fig. 1. Line drawings of *Parasonderia vestita* from live (A) and after protargol impregnation (B–D); A, Ventral view of a representative individual, blue arrowhead marks the contractile vacuole; B, C, Ventral (B) and dorsal (C) views of the same specimen showing the somatic ciliary pattern; D, Details of oral polykineties. CK, circle kineties; DK, dorsal somatic kineties; IBP, intrabuccal polykineties; PaBP, parabuccal polykineties; PoBP, postbuccal polykineties; PrBP, prebuccal polykineties; VK, ventral somatic kineties. Scale bars: A–C = 20 μm, D = 10 μm.

Table 1. Morphometric data of the Korean population of *Parasonderia vestita*

Characters	Mean	Median	SD	CV	Min	Max	n
Body, length (μm)	50.8	49.0	8.2	16.2	41.0	64.0	13
Body, width (μm)	34.1	33.0	5.9	17.4	26.0	44.0	13
Ratio of body length: width	1.5	1.5	0.1	9.1	1.4	1.8	13
Anterior end of body to anterior end of prebuccal polykinetids, distance (μm)	5.0	5.0	1.5	30.6	3.0	8.0	13
Oral apparatus, length (μm)	12.1	12.0	2.0	16.4	8.0	15.0	13
Anterior end of body to macronucleus, distance (μm)	18.2	19.0	3.3	18.4	14.0	23.0	13
Pre-buccal polykineties, number	15.8	15.0	1.1	7.2	14.0	17.0	13
Post-buccal polykineties, number	13.4	14.0	0.8	5.7	12.0	14.0	13
Para-buccal polykineties, number	6.2	6.0	0.4	7.0	6.0	7.0	13
Intra-buccal polykineties, number	11.4	12.0	1.1	9.5	10.0	13.0	13
Ventral somatic kineties, number	13.8	14.0	0.4	2.7	13.0	14.0	13
Dorsal somatic kineties, number	12.3	12.0	1.0	8.4	11.0	14.0	13
Circle kineties, number	4.0	4.0	0.0	0.0	4.0	4.0	13
Macronucleus, length (μm)	19.0	18.0	3.1	16.2	15.0	26.0	13
Macronucleus, width (μm)	13.7	13.0	2.4	17.5	9.0	18.0	13

All data based on protargol-impregnated specimens.
Mean, arithmetic mean; SD, standard deviation of the arithmetic mean; CV, coefficient of variation in (%); Min, minimum value; Max, maximum value; n, number of indoviduals investigated.

Material examined. Sediments collected from a drainage channel near Mokpo Maritime University, Jukgyo-dong, Mokpo-si, Jeollanam-do, Korea (34°47′56.0″N, 126°21′48.2″E) in October 2021.

Voucher specimens. Two slides of protargol-stained voucher specimens (registration number: HNIBRPR5, HNIBRPR6) are deposited in the Honam Institute of Biological Resources.

Descriptions. Body size *in vivo* 50−78 × 28−44 μm (n = 12). Body shaped ovoid with body length: width ratio about 1.9 : 1 *in vivo*; dorsoventrally flattened (Figs. 1A, 2A−F). Buccal

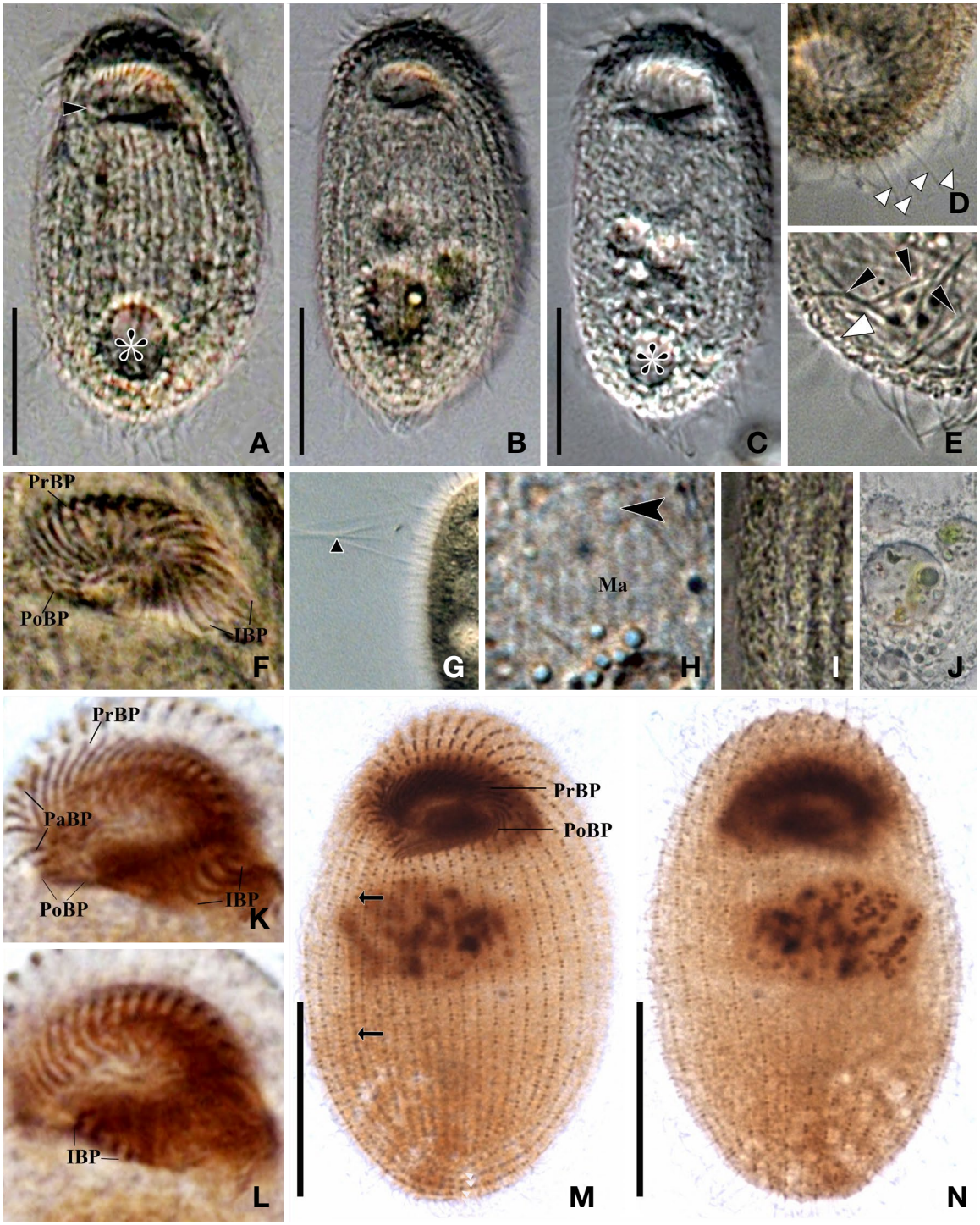

Fig. 2. *Parasonderia vestita* from live (A–J) and after protargol impregnation (K–N); A–C, Ventral view of representative individuals showing circular oral opening (black arrowhead) and the posteriorly located contractile vacuole (asterisk), and showing the variation of body shapes; D, The caudal cilia in the posterior region (white arrowheads); E, I, Ectosymbiotic bacteria covered the cell surface, the white arrowhead marks bacterial layer, and the black arrowhead denotes extrusomes; F, Oral ciliary pattern in the ventral view; G, Extruded extrusomes shown in black arrowhead; H, Details of the nuclear apparatus, single macronucleus and spherical micronucleus (black arrowhead); J, Bacteria or cyanobacteria in food vacuoles; K, L, Details of oral ciliary pattern, showing prebuccal, parabuccal, postbuccal, and intrabuccal polykineties; M, N, Ventral (M) and dorsal (N) views of the same individual, showing the somatic kineties and macronucleus, the first inner circle kinety is indicated by black arrows. IBP, intrabuccal polykineties; Ma, macronucleus; PaBP, parabuccal polykineties; PoBP, postbuccal polykineties; PrBP, prebuccal polykineties. Scale bars: A–C, M, N = 20 µm.

cavity with an oval mouth opening, distinct, subapical (Figs. 1A, 2A–F). Single ellipsoidal macronucleus, size 15–26×9–18 μm, located below the buccal cavity, with a spherical micronucleus, observed only in live specimens (Fig. 2H, M, N). Cytoplasm transparent and colorless, with several food vacuoles containing bacteria and cyanobacteria (Fig. 2B, C, J). Two types of extrusomes, straight and curved, needle-like, 8–15 μm long, dispersed in the cytoplasm and extruded on stimulation (Fig. 2E, G). Numerous refractile granules, approximately 2 μm in length, located mainly in posterior region of the body (Fig. 2E, H). Cell surface completely covered by dense curved ectosymbiotic bacteria, 2–3 μm long (Fig. 2E, I). Single contractile vacuole, 6–10 μm in diameter, located in posterior region of cell (Fig. 2A–D). Swims clockwise around the main body axis.

Ordinary somatic cilia 7–10 μm long (Fig. 2A, B), while caudal cilia 12–14 μm long, approximately four caudal cilia at the posterior end region (Figs. 1A, 2D). Somatic kineties consisted of four circle kineties, and 13–14 ventral and 11–14 dorsal somatic kineties, both adjacent to circle kineties; their kinetids composed mainly of di-kinetids and some mono- and tri-kinetids (Figs. 1B, C, 2M, N). Usually, four circle kineties originated anteriorly either left or right of the mouth opening, extended posteriorly along the cell margin, and converged at posterior end of cell; the first inner circle kinety consistently comprised of monokinetids (Figs. 1A, B, 2M). Oral ciliature consisted of four types of polykineties: 14–17 prebuccal, 6–7 parabuccal, 12–14 postbuccal, and 10–13 intrabuccal polykineties (Figs. 1B, D, 2F, K, L).

Distribution. Germany (Oldesloe), China (Qingdao), Korea (present study).

Family Plagiopylidae Schewiakoff, 1896

[1]*Genus *Plagiopyla* Stein, 1860

[2]****Plagiopyla ovata*** **Kahl, 1931 (Table 2, Figs. 3, 4)**

Plagiopyla ovata Ozaki and Yagiu, 1941: 159–180.

Plagiopyla ovata Burkovsky, 1970: 47–65.

Plagiopyla ovata Dragesco and Dragesco-Kernéis, 1986: 1–559.

Plagiopyla ovata Alekperov and Asadullayeva, 1996: 763–769.

Plagiopyla ovata Alekperov, 2005: 74.

Plagiopyla ovata Li et al., 2021: 004936.

Material examined. Sediments collected from the pond in

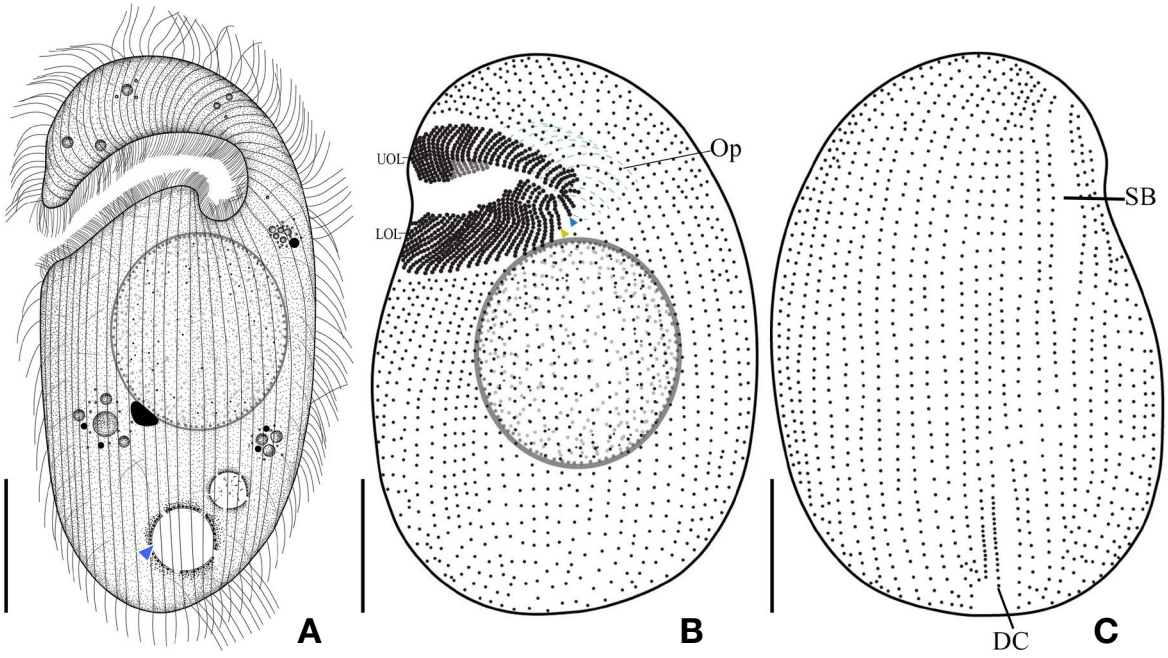

Fig. 3. Line drawings of *Plagiopyla ovata* from live (A) and after protargol impregnation (B, C); A, Ventral view of a representative individual, blue arrowhead indicates contractile vacuole; B, C, Ventral (B) and dorsal (C) views of the same specimen showing the somatic and oral ciliatures, proximal end of upper oral lip (blue arrowhead) and lower oral lip (yellow arrowhead). DC, dense cilia; LOL, lower oral lip; Op, oral opening; UOL, upper oral lip; SB, striated band. Scale bars: A–C=20 μm.

Korean name: [1]*경사섬모충속, [2]*난형경사섬모충

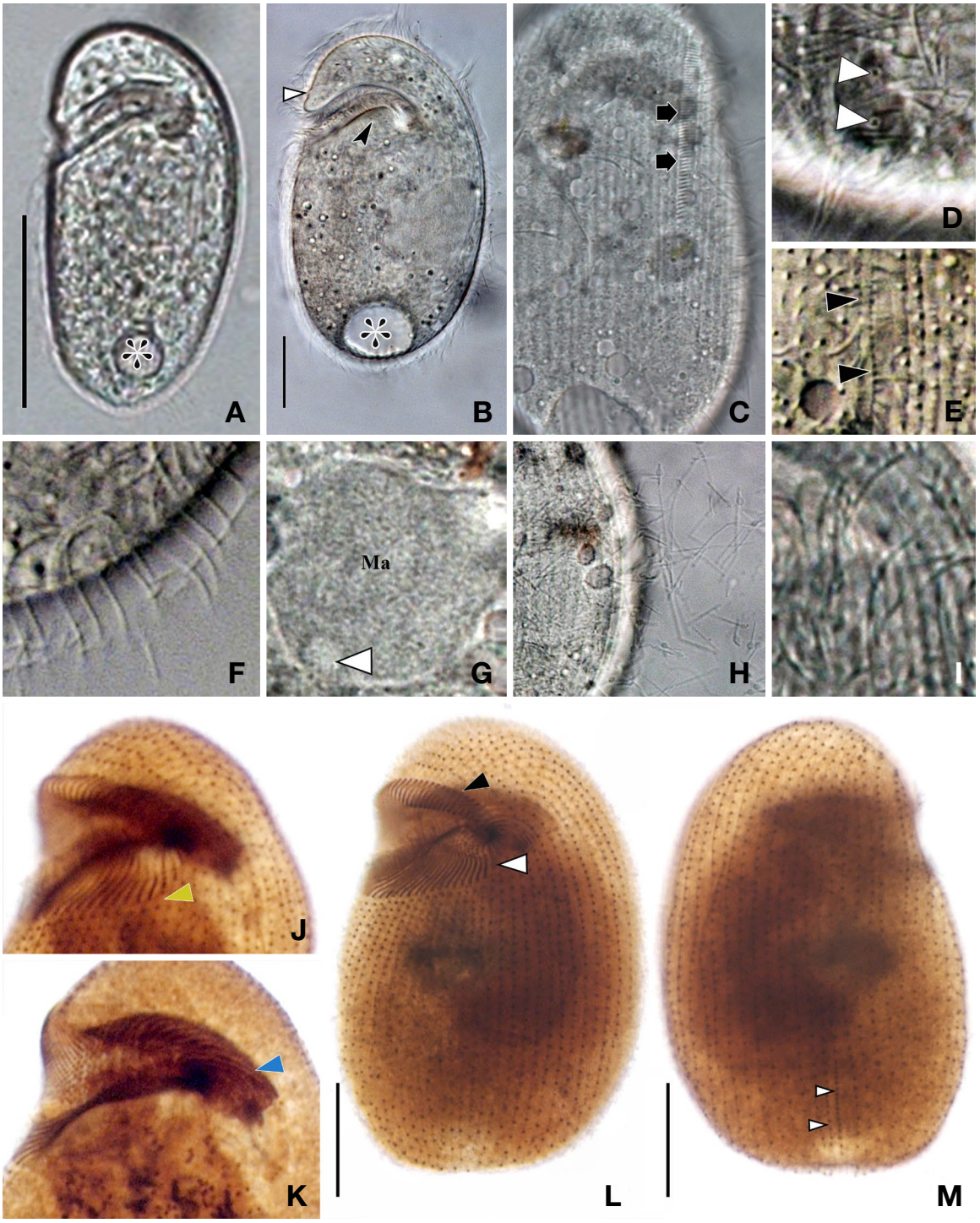

Fig. 4. *Plagiopyla ovata* from live (A–I) and after protargol impregnation (J–M); A, B, Ventral view of representative of an individual with contractile vacuole in posterior part (asterisk), the black arrowhead indicates the mid-portion of buccal cavity where it curves upward, white arrowhead denotes the prominent nose-like structure; C, Dorsal view of the cell, striated band indicated by black arrows; D, Contractile vacuolar pores indicated by two white arrowheads; E, Posterior region, dense ciliary rows indicated by two black arrowheads; F, Caudal cilia in posterior end region; G, Macronucleus and micronucleus (white arrowhead); H, Extruded extrusomes; I, Showing extrusomes (curved and straight) under the cortex in the cell margin; J, K, Narrow gap between oral lip and somatic kineties (yellow arrowhead), and oral opening (blue arrowhead); L, M, Ventral (L) and dorsal (M) views of the same individual, showing the somatic ciliature and nuclear apparatus, details of dense oral lip kineties: upper (black arrowhead) and lower (white arrowhead). Ma, macronucleus. Scale bars: A, L, M = 40 μm, B = 20 μm.

Table 2. Morphometric data of the Korean population of *Plagiopyla ovata*

Characters	Mean	Median	SD	CV	Min	Max	n
Body, length (μm)	80.4	78.0	11.2	13.9	68.0	102.0	16
Body, width (μm)	53.9	51.5	8.3	15.5	44.0	71.0	16
Ratio of body length: width	1.5	1.5	0.1	6.4	1.3	1.7	16
Anterior end of body to posterior end of prebuccal polykinetids, distance (μm)	30.6	30.6	4.5	14.6	24.7	40.8	15
Distance of anterior end of body to anterior end of prebuccal polykinetids: body length (%)	24.5	24.0	3.6	14.5	18.0	30.0	15
Pre-buccal kineties, number	33.4	34.0	3.5	10.4	26.0	39.0	15
Post-buccal kineties, number	27.4	27.0	2.3	8.5	24.0	32.0	15
Ventral kineties, number	33.4	32.0	4.5	13.6	28.0	43.0	16
Dorsal kineties, number	31.1	30.0	3.4	10.8	27.0	38.0	16
Somatic kineties, number	64.5	64.0	6.3	9.7	55.0	80.0	16
Macronucleus, length (μm)	37.6	33.5	8.8	23.4	27.0	53.0	16
Macronucleus, width (μm)	33.8	32.0	5.9	17.3	26.0	47.0	15
Micronucleus, diameter (μm)	4.3	4.5	0.9	20.9	3.0	5.0	8
Dense ciliary rows, number	2.0	2.0	0.0	0.0	2.0	2.0	16
Dense ciliary kinetids I, number	20.1	21.0	2.7	13.4	15.0	23.0	15
Dense ciliary kinetids II, number	16.2	15.0	3.7	23.0	12.0	28.0	15
Striated band, length (μm)	45.9	43.5	9.6	20.9	31.0	64.0	16
Striated band, width (μm)	3.9	4.0	0.7	18.5	3.0	5.0	16

All data based on protargol-impregnated specimens.
Mean, arithmetic mean; SD, standard deviation of the arithmetic mean; CV, coefficient of variation in (%); Min, minimum value; Max, maximum value; n, number of indoviduals investigated.

saltmarsh, Sinjang-ri, Aphae-eup, Sinan-gun, Jeollanam-do, Korea (34°49′09.8″N, 126°21′29.4″E) in October 2021.

Voucher specimens. Two slides of protargol-stained voucher specimens (registration number: HNIBRPR1, HNIBRPR2) are deposited in the Honam Institute of Biological Resources.

Descriptions. Body size *in vivo* 64–100 × 34–50 μm (n = 10). Body shape ovoid with body length: width ratio 1.9 : 1 *in vivo*; dorsoventrally flattened, with a prominent oral opening ventrally (Figs. 3A, 4A, B, K). Tube-like buccal cavity occupied 72–88% of body width *in vivo* (Figs. 3A, 4A, B). Single ovoid macronucleus, size 27–53 × 26–47 μm, located in the mid of body; a roundish compact micronucleus, 3–5 μm in diameter, located close to the macronucleus, observed both live and stained specimens (Fig. 4G, L, M). Cytoplasm colorless; numerous extrusomes, curved and straight, uniformly distributed under the cortex, extruded on stimulation, 9–12 μm long at rest (Figs. 3A, 4H, I). Single contractile vacuole in the posterior region of the cells, opening dorsally through two contractile vacuolar pores (Fig. 4A, D). Swims by rotating counter-clockwise with respect to the longitudinal axis of the body.

Ordinary somatic cilia 6–7 μm long (Fig. 4A, B); while caudal cilia 8–10 μm long, approximately ten caudal cilia at the posterior end (Fig. 4F). Somatic kineties consisted of 55–80 longitudinal rows of monokinetids, distributed in 28–43 and 27–38 rows on ventral and dorsal surfaces (Figs. 3B, C, 4L, M), ventral rows starting just below the oral region leaving a narrow empty space (Figs. 3A, 4J). All somatic kineties extended to the posterior end of the body, except for 3–5 rows on the dorsal surface that terminated before posterior end (Figs. 3C, 4E, L, M). Unciliated striated band, 31–64 μm long, 40–80% of body length, beginning above the oral region and extending to the dorsal surface of the cell (Figs. 3C, 4C, M). Two dense ciliary rows (I and II), row I (left) and II (right) consisted of 15–23 and 12–28 kinetids, respectively, located subequatorial on dorsal surface (Figs. 3C, 4E, M). The buccal field consisted of two lip-like structures (upper and lower lips), prominent nose-like structure on the right side of the upper oral lip (Fig. 4B). Oral lip kineties denser than somatic kineties, in total 50–71 oral lip kineties (Figs. 3B, 4J, L). The distance from the anterior end of cell to the anterior end of buccal cavity occupied approximately 25% of the body length (Fig. 4J, L).

Distribution. Germany (Oldesloe), Africa (Benin), Azerbaijan (Absheron peninsula), China (Qingdao), Japan (Hiroshima), Korea (present study).

Phylogenetic positions of the Korean populations of *Parasonderia vestita* and *Plagiopyla ovata*

The SSU rDNA sequences of the Korean populations of *Parasonderia vestita* (PV110190) and *Plagiopyla ovata* (PV110 191) have lengths of 1,772 bp and 1,761 bp, with GC contents

of 43.1% and 42.9%, respectively. As the ML and BI trees have similar topologies, only the ML tree is shown here. Plagiopylida comprises three families: Trimyemidae, Sonderiidae, and Plagiopylidae, which are distributed into two main clades: families Sonderiidae + Plagiopylidae together form a clade with full support (100/1.0, ML/BI), while Trimyemidae form a separate clade at the basal position of the class Plagiopylea with full support (100/1.0, ML/BI). The odontostomatid family Epalxellidae is positioned as a sister group to the clade containing the families Sonderiidae and Plagiopylidae with moderate support (82/0.54, ML/BI). The Korean population of *Parasonderia vestita* clades together with two Chinese populations (JN857941 and PP476945) with full nodal support (100/1.0, ML/BI), with 99.8% and 100% nucleotide similarity, respectively. Similarly, the Korean population of *Plagiopyla ovata* is clustered together with a Chinese population (MW762810) and ten congeners of *Plagiopyla*, with full nodal support (100/1.0, ML/BI), with the Korean and Chinese populations of *P. ovata* share 99.8% nucleotide similarity. The larger cluster, including Korean + Chinese populations of *P. ovata* and ten other *Plagiopyla* species, forms a clade with *P. rariseta* (OP114647) with high nodal support (92/0.99, ML/BI), with the SSU rDNA sequence of *P. rariseta* showing 98.7% nucleotide similarity to that of the Korean population of *P. ovata* and 98.5% similarity to that of the Chinese population of *P. ovata*.

DISCUSSION

Comparison of the Korean population of *Parasonderia vestita* with its congeners

The Korean population was identified as *P. vestita* based on the body size, body shape, the oval mouth opening, the presence of extrusomes in the cytoplasm, the single ellipsoidal macronucleus in the mid-body, the ectosymbiotic bacteria covering the cell, and its brackish water habitat (18‰ salinity) (Kahl, 1928, 1931; Fauré-Fremiet, 1973; Jankowski, 2007). Xu et al. (2013) and Li et al. (2024) redescribed the Chinese populations of *P. vestita* from the same locality i.e., a sewage outfall in Qingdao, at different times. These populations correspond to the Korean population in all morphological characteristics, both *in vivo* and in protargol-impregnated specimens, however there is a slight difference in macronucleus size (12−17 × 8−14 vs. 15−26 × 9−18 μm) within the range of variation.

Four species of the genus *Parasonderia* have been described: *Parasonderia cyclostoma*, *P. elongata*, *P. kahli*, and *P. vestita*. All members of *Parasonderia* share morphological characteristics such as an oval or circular oral opening, which is subapically located, and the buccal cavity is depressed (Fauré-Fremiet, 1973; Xu et al., 2013). *Parasonderia vestita* closely resembles *P. cyclostoma* in terms of the body size, the body shape, and the ellipsoidal macronucleus. However, *P. vestita* can be distinguished from *P. cyclostoma* on the basis of mouth opening (oval vs. subcircular), the numbers of ventral somatic kineties (13−14 vs. 9−12), dorsal somatic kineties (11−14 vs. 8−11), prebuccal polykineties (14−17 vs. 12−14), postbuccal polykineties (PoBP) (12−14 vs. 8−10), and intrabuccal polykineties (4−6 vs. 9−11) (Kahl, 1931; Li et al., 2024). *Parasonderia vestita* is also morphologically close to *P. kahli* in terms of the body size, the body shape, and mouth lip opening, but differs from the latter in the shape of macronucleus (ellipsoidal vs. incompletely triangular blade) and the striated band (absent vs. present) (Kahl, 1928; Fauré-Fremiet, 1973; Jankowski, 2007). Finally, *P. vestita* resembles *P. elongata* only in body size. However, the body shape (oval vs. slender ellipsoidal), the number of total somatic kineties (28−32 vs. 33−41), and the organization of PoBP, i.e., several leftmost kineties on the cell surface progressively shortened, and the leftmost prebuccal polykinety starts above the leftmost PoBP (vs. several leftmost PoBP kineties of similar length, and the leftmost prebuccal polykinety starts to the left of the PoBP) distinguish *P. vestita* from *P. elongata* (Xu et al., 2013; Li et al., 2024).

Comparison of the Korean population of *Plagiopyla ovata* with its congeners

The Korean population of *Plagiopyla ovata* matches the redescription of the Chinese population in terms of the ovoid body shape, the placement of oral opening, the buccal cavity tube almost parallel to the anterior margin, approximately 10 inconspicuous caudal cilia, a contractile vacuole located in the posterior region, the ellipsoidal macronucleus and located in the mid-body, micronucleus size, tube-like buccal cavity occupies approximately 85% of body width *in vivo*, the average proportion of the anterior cell end to upper oral lip to body length is 25%, dense ciliary kinetids arrangement above oral opening, and its habitat in organic-rich marine sediment (Kahl, 1931, 1932; Li et al., 2021). The Korean population also resembles the African population of *P. ovata* in terms of body size, buccal kineties arrangement, the organization and number of somatic kineties, and the presence of 8−12 caudal cirri (Dragesco and Dragesco-Kernéis, 1986). We observed morphological variation in the number of dense ciliary rows on the dorsal surface of the cell. The Korean population consistently has two dense ciliary rows, while the Chinese population shows variability, with one or two rows, averaging a single dense ciliary row (Li et al., 2021).

Two species of *Plagiopyla* have been previously recorded in Korea: *P. frontata* and *P. nasuta* (Omar and Jung, 2022). Al-

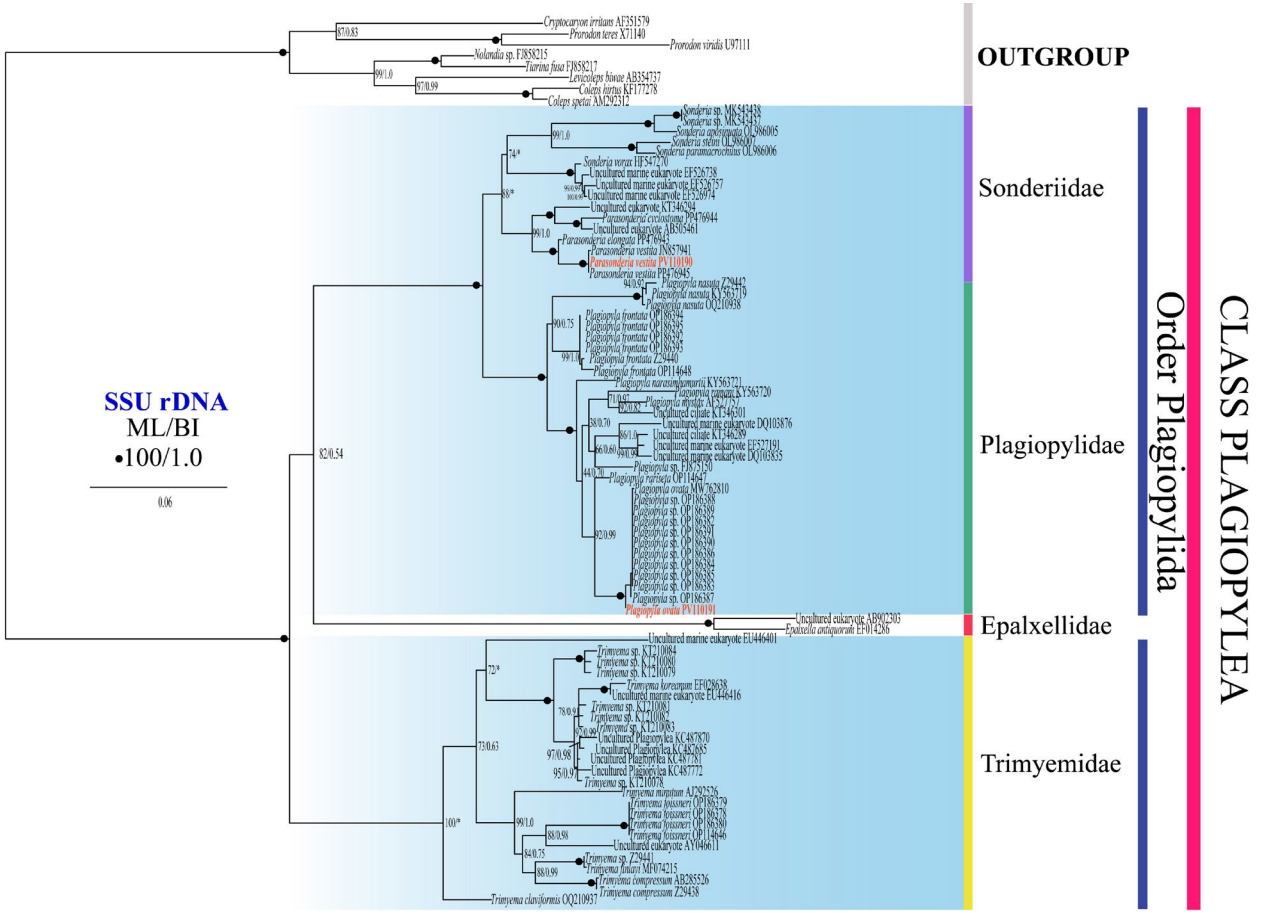

Fig. 5. Phylogenetic tree based on maximum likelihood (ML) and Bayesian inference (BI) analyses of small subunit ribosomal DNA sequences, showing the phylogenetic position of the Korean population of *Parasonderia vestita* and *Plagiopyla ovata* (red). Numbers at nodes represent ML bootstrap values and BI posterior probabilities (ML/BI), respectively. Asterisks (*) indicate discrepancies between the topologies of the ML and BI trees. The scale bar represents six substitutions per 100 nucleotide positions.

though *P. ovata* and *P. frontata* can be found in marine sediments, *P. ovata* differs from *P. frontata* in terms of oval body shape, an elongated frontal segment, an upwardly curved buccal cavity tube, and an upper oral lip that is not perpendicular to the buccal opening (Li et al., 2021; Omar and Jung, 2022). Furthermore, *P. ovata* differs from *P. nasuta* in terms of habitat (marine vs. freshwater), and type of extrusomes (curved and straight vs. straight only) (Nitla et al., 2019; Omar and Jung, 2022; Li et al., 2023b). *Plagiopyla ovata* is closely related to *P. rariseta* (OP114647) in terms of SSU rDNA phylogeny, but they differ morphologically in the following points: the prominent nose-like structure on the right side of the upper oral lip *in vivo* (present vs. absent), the average proportion of the region above the oral opening to the body length (25% vs. 30%), the micronucleus diameter (3–5 μm vs. 1.4 μm), and the arrangement of the ciliary kinetids in the region above the oral region (dense vs. loose) (Li et al., 2023a).

Phylogenetic analyses

In the present phylogenetic analyses, the Plagiopylida appears non-monophyletic; however, the constituent families: Plagiopylidae, Sonderiidae, and Trimyemidae are monophyletic, in agreement with previous studies (Modeo et al., 2013; Xu et al., 2013; Fernandes et al., 2018; Nitla et al., 2019; Li et al., 2022, 2023a, 2023b, 2024). Family Trimyemidae branches off basally within Plagiopylea with full support (Fig. 5), which is consistent with previous studies (Li et al., 2021, 2023a, 2023b, 2024). Despite the odontostomatid Epalxellidae exhibits distinct features from the members of the order Plagiopylida, such as a rigid armored cell with spines, a reduction of somatic cilia, the presence of prominent dorsal keels, and a frontal ciliary band (Kahl, 1932; Jankowski, 1964; Li et al., 2023b), SSU rDNA phylogenetic study supports the retention of the Epalxellidae within the Plagiopylea (Stoeck et al., 2007). However, given the cur-

rent state of knowledge, family Epalxellidae is still considered *incertae sedis*, according to Fernandes et al. (2018) and Lynn (2008).

All members of the Sonderiidae form two separate clades: *Sonderia* and *Parasonderia* (Fig. 5), supporting their morphological distinction in terms of somatic kineties organization (uniformly distributed vs. ventral and dorsal somatic kineties bordered by circle kineties) and oral buccal kineties organization (uniformly distributed vs. pre-, para-, post-, intra-buccal polykineties) (Kahl, 1932; Modeo et al., 2013; Li et al., 2022, 2024). The nucleotide differences between the three populations of *P. vestita* and *P. elongata* range from 17 to 18 nucleotides, consistent with a previous study (Li et al., 2024).

The two subclades within members of *Plagiopyla*: subclade I consists of *P. nasuta* and *P. frontata* and subclade II consists of *P. ovata*, *P. ramani*, *P. rariseta*, *P. mystax*, *P. narasimhamurtii*, one unidentified species (FJ875150), four uncultured marine eukaryote sequences (DQ103876, DQ103835, EF27 191, KT346289), a sequence of uncultured ciliate (KT346 301), and ten *Plagiopyla* species (Fig. 5), in agreement with previous studies (Li et al., 2021, 2023b). Li et al. (2023b) suggest that these subclades show distinct morphological differences in the organization of the buccal cavity: the buccal cavity of subclade I appears to be shorter, with the mid-region slightly or not curved upwards, whereas that of subclade II has a longer buccal cavity with a distinct curve in the mid-portion. The clade containing the Korean and Chinese populations of *P. ovata* forms a sister group to *P. rariseta* with a high support (92/0.99, ML/BI), in agreement with previous studies (Li et al., 2024).

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CONFLICTS OF INTEREST

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