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First Records of Two *Bothrostoma* Species (Ciliophora: Armophorea: Metopidae) from Korea

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ABSTRACT

This study focuses on the diversity of ciliates inhabiting the oxygen-depleted conditions in freshwater ponds in Korea. We identified two species, *Bothrostoma nasutum* (Cunha, 1915) Jankowski, 1964 and *B. undulans* Stokes, 1887, for the first time in Korea. Their morphological characteristics were determined based on observations of both live and protargol-impregnated specimens. *Bothrostoma nasutum* is characterized by body size 70−85 × 25−35 μm *in vivo*, body shape elongated ellipsoidal, anterior end extended as a proboscis; total 22−29 somatic kineties (including 7−8 postoral kineties), cortical granules arranged in 5 or 6 short, oblique rows; and 19−23 adoral membranelles. While *B. undulans* has the following features: body size 70−120 × 20−45 μm *in vivo*, body shape elongated ellipsoidal, anterior end terminated by an acute snout; total 18−22 somatic kineties (including 5−7 postoral kineties); 17−22 adoral membranelles; and cortical granules obliquely arranged in 6 or 7 short rows. Recently, the increasing interest in research on anaerobic ciliates has contributed to the understanding of the diversity of ciliates in Korea.

Keywords: anaerobic ciliates, *Bothrostoma*, cortical granules, freshwater, proboscis

INTRODUCTION

Recent research on anaerobic ciliates has received considerable attention. This has led to the extensive discoveries that expand our understanding of the vast diversity of anaerobic ciliates (Bourland and Wendell, 2014; Bourland et al., 2014, 2017a, 2017b, 2018a, 2018b, 2020; Foissner, 2016a, 2016b; Vd'ačný and Foissner, 2017a, 2017b, 2019; Rotterová et al., 2018; Li et al., 2021; Feng et al., 2022; Méndez-Sánchez et al., 2022; Nguyen et al., 2024). In particular, family Metopidae Kahl, 1927, one of the best studied groups, has been shown that its diversity is indeed more complex than previously understood. Within a short period of time, many genera have been explored and the members of this family reach up to 14 genera, including *Atopospira* Jankowski, 1964; *Brachonella* Jankowski, 1964; *Bothrostoma* Stokes, 1887; *Castula* Bourland et al., 2020; *Eometopus* Small and Lynn, 1985; *Heterometopus* Foissner, 2016; *Idiometopus* Bourland et al., 2018; *Lepidometopus* Vd'ačný and Foissner, 2017; *Longitenia* Bourland et al., 2020; *Metopus* Claré-pede and Lachmann, 1858; *Parametopidium* Aescht, 1980; *Planometopus* Rotterová et al., 2018; *Pidimetopus* Pomahač et al., 2024; and *Tenospira* Jankowski, 1964 (Stokes, 1887; Jankowski, 1964; Lee et al., 2000; Foissner, 2016a; Vd'ačný and Foissner, 2017b; Bourland et al., 2018b, 2020; Rotterová et al., 2018; Pomahač et al., 2024). Among them, genus *Bothrostoma* Stokes, 1887 is characterized by the lack of anterior axial torsion of the cell and possesses a flattened preoral dome (Jankowski, 1964; Méndez-Sánchez et al., 2022) compared to the other metopid groups. The genus *Bothrostoma* consists of nine morphospecies that have been studied so far (Méndez-Sánchez et al., 2022).

Recently, the diversity of anaerobic ciliates from Korea has started to receive more attention (Cho et al., 2008; He and Choi, 2015; Omar and Jung, 2021, 2022; Nguyen et al., 2024). However, compared to the huge diversity of anaerobic ciliates worldwide, their diversity in Korea remains scarce (Nguyen et al., 2024). To explore the diversity of freshwater anaerobic ciliates, we examined the morphological characteristics of two *Bothrostoma* species first recorded from freshwater ponds in Korea.

MATERIALS AND METHODS

Bothrostoma nasutum (Cunha, 1915) Jankowski, 1964 was collected along the stream in the Samnak Ecological Park,

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Busan, Korea (35°09′22.3″N, 128°58′04.8″E) in February 2024. *Bothrostoma undulans* Stokes, 1887 was found in the pond adjacent to the National Youth Ecology Center on the Eulsuk-do Island in Busan, Korea (35°06′51.1″N, 128°56′53.8″E) in October 2023. Specimens were collected with sediments, organic materials and mud in a litter jar with water filling the jars to maintain the anaerobic condition. After sampling, the samples were transferred to the laboratory and kept at room temperature, some rice grains were added to the jars to provide a food source. Ciliates morphology was examined both by microscopic observation of live specimens and by protargol impregnation of fixed cells (Wilbert, 1975). The live and impregnated specimens were observed under a light microscope (Axio Imager A1; Carl Zeiss, Oberkochen, Germany) with differential interference contrast optics. Images were captured with a CCD camera (AxioCam MRc; Carl Zeiss). Measurements and counts were performed at magnifications ranging from 100× to 1,000×. Drawings were based on the observations of live specimens and protargol-impregnated specimens. Terminology follows Bourland et al. (2014), and Méndez-Sánchez et al. (2022). Classification mainly follows Jankowski (2007), and Méndez-Sánchez et al. (2022). Species belonging to genus *Bothrostoma* are mostly characterized by a narrow to broadly elliptical cell shape, anterior end terminating in a "proboscis" or "snout" of varying shape and length, and a truncate to broadly rounded posterior end, the preoral dome flattened as opposed to convex or vaulted. The perizonal ciliary stripe is invariably five-rowed with two basic arrangements; "even"-type with all rows closely located and regularly spaced, while "4+1"-type the row 5 separated from regularly-spaced rows 1–4 by a larger gap; perizonal stripe dikinetids not forming into "false-kineties". Oral cavity is large and shallow; very prominent sail-like paroral membrane; and an adoral zone of distinctive, very narrow, curved membranelles confined to a wide, non-spiraling peristome on the ventral side (Jankowski, 1964; Méndez-Sánchez et al., 2022).

RESULTS

Classification
Class Armophorea Lynn, 2004
Order Metopida Jankowski, 1980
Family Metopidae Kahl, 1927
Genus [1]*Bothrostoma* Stokes, 1887

1. [2]*Bothrostoma nasutum* (Cunha, 1915) Jankowski, 1964 (Table 1, Fig. 1)

Metopus nasutus Cunha, 1915: 129; 1916: 69, fig. 12; Kahl, 1932: 406, fig. 697; Kreutz and Foissner, 2006: 137, figs. 1, 2.
Metopus vexilliger Penard, 1922: 196, fig. 194.
Bothrostoma nasutum (Cunha, 1915) Jankowski, 1964: 207; Méndez-Sánchez et al., 2022: 5, table 3, figs. 3, 4.

Material examined. Sample collected from the stream in the Samnak Ecological Park, Samnak-dong, Sasang-gu, Busan, Korea (35°09′22.3″N, 128°58′04.8″E) in February 2024.

Voucher specimens. One slide of protargol-impregnated voucher specimens (registration number: UBL20241001001) is deposited at the Biodiversity Laboratory, University of Ulsan.

Improve diagnosis. Shape elongated ellipsoidal; size 70–150×22–44 μm. Long flexible proboscis (18–50 μm in length). 22–29 somatic kineties, including 7–9 postoral kineties; 21–29 adoral zone membranelles. Cortical granules, 5–6 discontinuous interkinetal rows.

Description. Body size 70–85×25–35 μm *in vivo*, with a ratio of body length to body width 2.6: 1, size after protargol impregnation 85×25 on average. Body shape elongated ellipsoidal, widest at the cell equator; posterior end truncated or broadly rounded (Fig. 1A–C, G, L, M); at the left side of an anterior end extended as a proboscis (Fig. 1A–C, G, J, O), length 20–40 μm *in vivo*, weakly impregnated with protargol (Fig. 1O). Macronucleus located in the anterior half of body, usually ellipsoidal in shape, sometimes bean-shaped, size after protargol impregnation about 20×10 μm (Fig. 1B, H, M–O). One micronucleus attached to the middle of the macronucleus, rounded shape, average size about 3.5 μm across (Fig. 1H, M). Cortex flexible; cytoplasm transparent, some circular pigments distributed throughout the cytoplasm, rod-shaped endosymbiotic bacteria scattered throughout the cytoplasm and especially around the macronucleus; cortical granules arranged in short oblique lines between the somatic kineties, five or six rows (Fig. 1I). Contractile vacuole located at the posterior end of the cell, conical in shape (Fig. 1A, C). Food vacuoles contained bacteria and algae. Swimming by rotation about main body axis, proboscis moving rapidly during swimming.

Somatic kineties arranged longitudinally, total 22–29 rows (including 7–8 postoral kineties) with preoral sutures, and postoral sutures (Fig. 1B, C, L, M), somatic cilia about 11 μm long (Fig. 1G); caudal cilia about 35 μm long (Fig. 1A, G). Perizonal stripes invariably consisted of five rows, evenly distributed, row 1 of perizonal stripes shorter than the others (Fig. 1B, L, N), row 4–5 continuously extending

Korean name: [1]돗대입섬모충속, [2]긴코돗대입섬모충

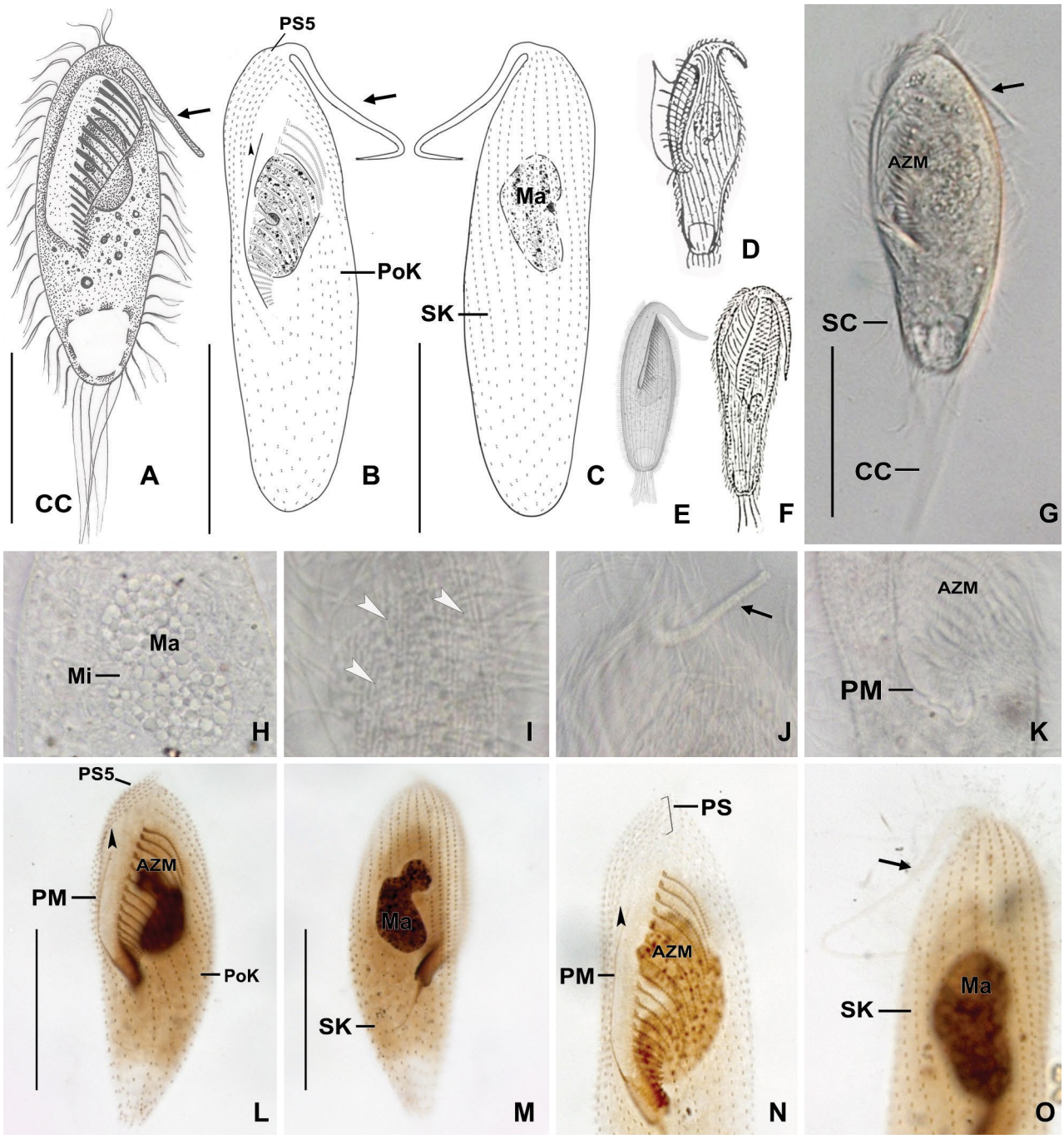

Fig. 1. *Bothrostoma nasutum* from life (A, D–K) and after protargol impregnation (L–O). A, G, Representative specimens showing the general characteristics, the arrow indicates the proboscis at the anterior end, caudal cilia distributed at the posterior cell end; B, C, Ventral-, dorsal views showing the somatic kineties, the nuclear apparatus, adoral zone of membranelles, the arrowhead denoting the end of perizonal stripes 1; D, *Bothrostoma nasutum* after Penard, 1922; E, *Bothrostoma nasutum* after Cunha, 1916; F, *Bothrostoma nasutum* after Kahl, 1932; H, Macronucleus and micronucleus from live observation; I, Cortical granules arranged in short, oblique rows between the somatic kineties (white arrowheads); J, Arrow denoting the proboscis glamours, and some small cortical granules-like along the proboscis; K, Posterior end of the adoral zone, and the paroral membrane; L–N, Stained specimens showing the ciliatures, nuclear apparatus, paroral membrane, black arrowhead indicating the end of perizonal stripe 1 (L, N); O, Arrow denoting the proboscis weakly impregnated with protargol. AZM, adoral zone of membranelles; CC, caudal cilia; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane; PoK, postoral kineties; SC, somatic cilia; SK, somatic kineties. Scale bars = 40 μm.

Table 1. Morphometric data of *Bothrostoma nasutum* (nas.), *B. undulans* (uns.)

Characters	Species	Mean	Median	Min	Max	SD	CV	n
Body, length (A)	nas.	82.9	85.0	58.0	105.0	13.9	16.8	15
	uns.	81.1	80.0	70.0	100.0	9.5	11.7	14
Body, width (B)	nas.	24.9	25.0	18.0	34.0	4.5	18.2	15
	uns.	22.4	22.0	17.0	29.0	3.4	15.3	14
Ratio of body length : body with (A : B)	nas.	3.4	3.3	2.7	4.1	0.4	12.6	15
	uns.	3.7	3.7	2.8	4.8	0.6	15.7	14
Anterior end of the cell to posterior end of adoral zone (C)	nas.	49.5	50.0	28.0	60.0	8.7	17.5	15
	uns.	45.7	47.5	37.0	55.0	6.1	13.2	14
Ratio (C : A) %	nas.	59.8	61.3	48.3	66.7	5.1	8.6	15
	uns.	56.4	54.8	51.4	66.7	4.9	8.7	14
Anterior end of the cell to anterior end of adoral zone (D)	nas.	11.2	11.5	7.0	15.0	2.3	20.7	14
	uns.	10.9	10.5	7.0	15.0	2.2	20.4	14
Ratio (D : A) %	nas.	13.3	13.6	8.3	16.9	2.1	15.7	14
	uns.	13.4	13.1	8.6	17.3	2.5	18.5	14
Anterior end of the cell to posterior end of perizonal stripe 1 (E)	nas.	19.5	20.0	13.0	24.0	3.3	17.0	10
	uns.	16.0	15.0	11.0	23.0	3.2	19.8	9
Ratio (E : A) %	nas.	22.5	22.7	20.2	25.6	1.4	6.4	10
	uns.	19.8	18.1	15.0	29.1	4.6	23.0	9
Anterior end of the end cell to posterior end of macronucleus	nas.	40.7	42.0	25.0	54.0	7.3	18.0	15
	uns.	39.7	40.0	30.0	46.0	5.4	13.5	13
Macronucleus, length (F)	nas.	21.7	21.0	13.0	32.0	4.3	19.8	15
	uns.	16.3	17.0	11.0	22.0	3.1	19.1	13
Macronucleus, width	nas.	10.5	10.0	7.0	14.0	2.2	21.2	15
	uns.	10.4	10.0	7.0	16.0	2.7	25.6	13
Ratio (F : A) %	nas.	26.2	25.7	21.5	30.5	2.9	11.0	15
	uns.	19.9	20.0	15.1	25.7	3.2	16.2	13
Micronucleus, size	nas.	3.3	3.1	2.8	3.8	0.4	11.6	11
	uns.	3.4	3.3	3.0	4.0	0.5	14.2	4
Adoral zone of membranelles, length (G)	nas.	39.7	39.0	30.0	48.0	5.4	13.7	14
	uns.	37.3	38.0	28.0	50.0	6.4	17.2	14
Ratio (G : A) %	nas.	47.1	47.7	40.0	53.3	4.0	8.5	14
	uns.	45.9	46.2	37.8	55.6	5.6	12.2	14
Adoral membranelles, number	nas.	21.5	22.0	19.0	23.0	1.3	6.0	14
	uns.	18.8	18.0	17.0	22.0	1.7	8.9	13
Paroral membrane, length (H)	nas.	35.2	35.0	25.0	42.0	5.3	14.9	12
	uns.	30.9	30.5	25.0	36.0	4.5	14.5	14
Ratio (H : A) %	nas.	40.7	40.5	37.2	44.4	2.1	5.3	12
	uns.	38.0	36.9	32.5	43.4	3.5	9.3	14
Ratio (H : G) %	nas.	87.8	87.0	80.4	97.6	4.8	5.5	12
	uns.	83.8	86.9	62.0	100.0	10.8	12.9	14
Postoral kineties, number	nas.	7.7	8.0	7.0	8.0	0.5	6.4	15
	uns.	6.5	7.0	5.0	7.0	0.7	10.1	13
Somatic kineties, total number	nas.	25.1	25.0	22.0	29.0	2.1	8.5	15
	uns.	19.8	19.5	18.0	22.0	1.6	8.0	14
Perizonal stripes, number	nas.	5.0	5.0	5.0	5.0	0	0	15
	uns.	5.0	5.0	5.0	5.0	0	0	14

All measurements of size, length, and width in μm. All data are based on protargol-impregnated specimens.
CV, coefficient of variation (%); Max, maximum value; Mean, arithmetic mean; Min, minimum value; n, number of individuals investigated; SD, standard deviation.

to the ordinary somatic cilia; perizonal stripes cilia slightly longer than the somatic cilia, about 15 μm long. Adoral zone located on the ventral surface, oral cavity large and shallow, occupying about 50% of the body length, composed of 19–23 membranelles (Fig. 1B, K, L, N). Paroral membrane long and prominent, about 35 μm (Fig. 1B, K, L, N).

Distribution. Brazil (Rio de Janeiro), Czech Republic (Žinkovy), Mexico (Lerma), Israel (Galilei), USA (Missouri,

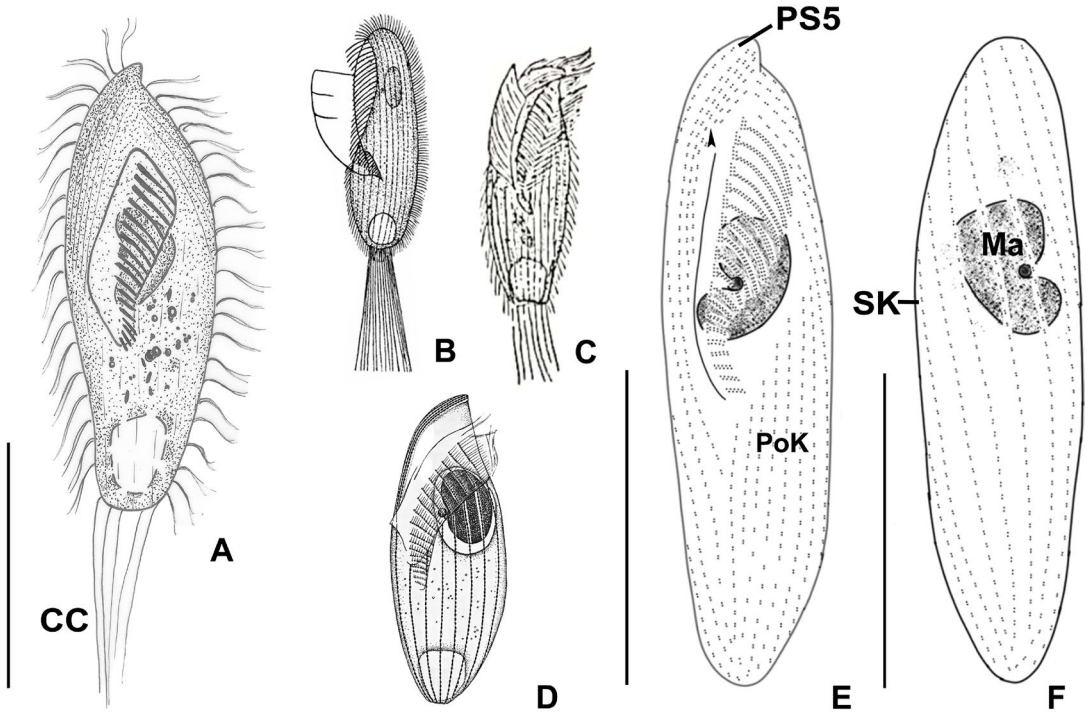

Fig. 2. *Bothrostoma undulans* from life (A–D), and after protargol impregnation (F, G); A, Ventral view of a typical specimen; B, *Bothrostoma undulans* after Stokes, 1887; C, *Bothrostoma undulans* after Kahl, 1932; D, *Bothrostoma undulans* after Jankowski, 1964; E, F, Ciliary pattern of ventral and dorsal view of the same specimen, the shortening of the perizonal stripe 1 (black arrowhead). CC, caudal cilia; Ma, macronucleus; PoK, postoral kineties; PS, perizonal stripes; SK, somatic kineties. Scale bars = 40 μm.

Wisconsin), and Korea (this study).

2. [1]*Bothrostoma undulans* Stokes, 1887 (Table 1, Figs. 2, 3)

Bothrostoma undulans Stokes, 1887: 248, fig. 9; Jankowski, 1964: 207, fig. 15; Foissner, 1980: 76, fig. 4; Wagener et al., 1986: 201, fig. 4a; Foissner et al., 1992: 401, figs. 72–78; Bourland et al., 2017b: 224, fig. 15; Méndez-Sánchez et al., 2022: 4, table 2, figs. 1, 2.

Blepharisma bothrostoma Mermod, 1914: 76, fig. 11.

Metopus bothrostoma var. *longior* (Mermod, 1914) Kahl, 1927.

Metopus (*Bothrostoma*) *undulans* (Stokes, 1887) Kahl, 1932: 407, fig. 695.

Material examined. Sediment collected from the pond adjacent to the National Youth Ecology Center on the Eulsuk-do Island in Busan, Hadan-dong, Saha-gu, Busan, Korea (35°06′51.1″N, 128°56′53.8″E) in October 2023.

Voucher specimens. One slide of protargol-impregnated voucher specimens (registration number: UBL20241001002) is deposited in the Biodiversity Laboratory, University of Ulsan.

Improve diagnosis. Body shape elongated slender to ellipsoidal, size 60–180 × 20–45 μm *in vivo*, cell colorless to brownish. Conspicuous acute snout. 15–22 somatic kineties, including 4–7 postoral kineties; 15–22 adoral membranelles. Cortical granules, 5–7 discontinuous interkinetal rows.

Description. Body size 70–120 × 20–45 μm *in vivo*, with a ratio of body length to body width 2.9: 1, size after protargol impregnation 80 × 22 μm on average. Body shape elongated, widest at the cell equator, posterior end truncated, sometimes appeared narrowly ellipsoidal shape in impregnated specimens (Figs. 2A, E, F, 3A, E, F, H), anterior end terminating in a prominent acute snout (length about 4 μm) (Fig. 3A, B). Macronucleus located in the anterior half, varying in shape from rounded or ribbon-like to sausage-like, size after protargol impregnation average 16 × 10 μm (Fig. 2E, F, 3D). Micronucleus surrounded by macronucleus, usually 3–4 μm in diameter (Fig. 3D). Cortex flexible; cytoplasm transparent, some lipid droplets sparsely distributed throughout the cytoplasm; cortical granules arranged in 6–7 short oblique rows (Fig. 3C). Food vacuoles containing bacteria and dia-

Korean name: [1]파동돗대입섬모충

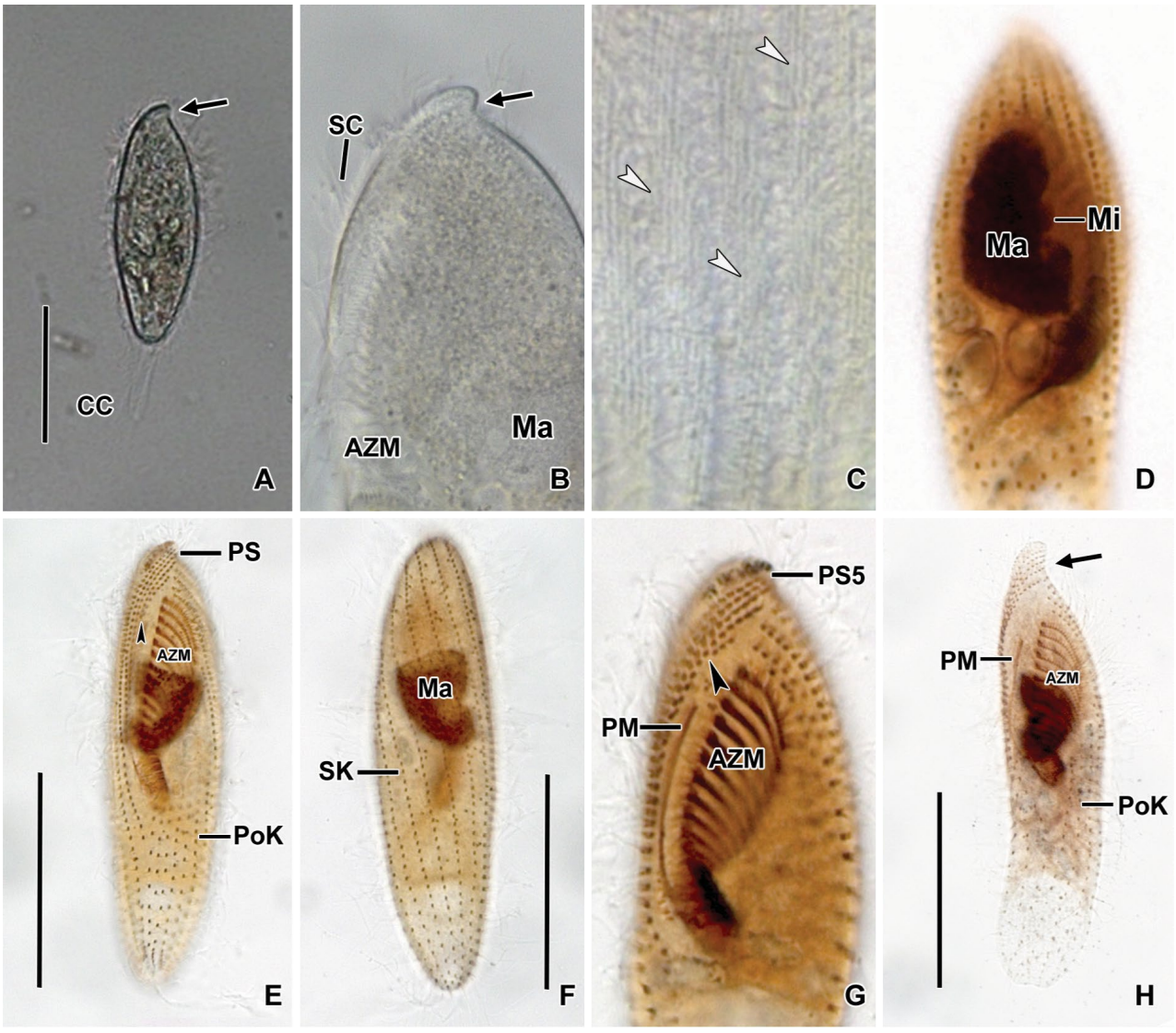

Fig. 3. *Bothrostoma undulans* from life (A–C), and after protargol impregnation (D–H). A, B, Live specimens showing the snout at the anterior end (black arrows), caudal cilia, macronucleus, somatic cilia; C, White arrowheads indicate the cortical granules distributed between the somatic cilia; D, Macronucleus and micronucleus after protargol impregnation; E–G, Ciliary pattern revealed after protargol impregnation, black arrowheads indicate the end of perizonal stripes 1 (E, G), somatic kineties, postoral kineties (E), evenly distributed perizonal stripes (G), adoral zone (E, G), paroral membrane (G); H, Different body shape, black arrow points the snout, the postoral kineties arranged below the adoral zone. AZM, adoral zone of membranelles; CC, caudal cilia; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane; PoK, postoral kineties; PS, perizonal stripes; SC, somatic cilia; SK, somatic kineties. Scale bars = 40 μm.

toms. Swimming moderate.

Ordinary somatic cilia about 12 μm long, total somatic kineties 18–22 (including 5–7 postoral kineties) with preoral sutures, and postoral sutures (Figs. 2E, F, 3E, F, H). Caudal cilia distributed at posterior end, length approximately 35 μm (Figs. 2A, 3A). Perizonal stripes composed of five rows, evenly distributed, the first row shortened compared to the other rows, occupying 20% of the body length, row 4–5 continuously extending to the ordinary somatic cilia (Figs. 2E, 3E, G). The adoral zone, located on the ventral surface, occupying about 55% of the body length and composed of 17–22 membranelles (Figs. 2E, 3E, G). The paroral membrane averaged about 30 μm in length (Figs. 2E, 3E, G).

Distribution. Austria (Carinthia), Czech Republic (Karlovice), Germany, Mexico (Lerma), Netherlands (Nijmegen), Russia (Jankowski, 1964), Switzerland (Sainte-Croix), Tur-

key (Van), Ukraine (Sumy Oblast), USA, and Korea (this study).

DISCUSSION

Comparison of the Korean population of *Bothrostoma* with previous descriptions

Bothrostoma nasutum can be easily identified by the anterior end forming a proboscis (Fig. 1A–F). The original description by Cunha (1916) recognized this species as *Metopus nasutus* with an elongated body shape, more or less flattened dorsoventrally, and a proboscis length of 30–40 μm *in vivo*, consistent with the Korean population (Fig. 1A, E). However, the body size of the original description is larger than that of the Korean population (100 vs. 70–85 μm). Penard (1922) described this species under name of *Metopus vexilliger* (Fig. 1D). The description of Penard (1922) of *M. vexilliger* matches the morphology of *B. nasutum* by the elongated body shape, the truncated posterior end, the large oral cavity and the proboscis at the anterior end. Hence, *Metopus vexilliger* should be synonymized as *B. nasutum*, and this synonymization agreed by Kahl (1932), and Méndez-Sánchez et al. (2022). The Frech population (Penard, 1922) is approximately the same size as the Korean population (70–85 vs. 80–90 μm). Korean population of this species shows a smaller body size (70–85 vs. 100–130 μm) and a shorter proboscis length (20–40 vs. 30–50 μm) compared to those of Kahl's (1932) redescription (Fig. 1A, F). The Korean population of *B. nasutum* shares many characteristics with the description by Méndez-Sánchez et al. (2022) based on the strain LERMA7, such as the arrangement of cortical granules, perizonal stripes evenly distributed, the number of somatic kineties (22–29 vs. 25–33), and the number of adoral membranelles (19–23 vs. 21–26).

Bothrostoma undulans has been studied by many researchers (Stokes, 1887; Mermod, 1914; Kahl, 1927, 1932; Jankowski, 1964; Foissner, 1980; Wagener et al., 1986; Foissner et al., 1992; Senler and Yildiz, 2004; Babko et al., 2020; Méndez-Sánchez et al., 2022) (Fig. 2B–D). Compared to the previous studies, the closest population to the Korean population of *B. undulans* is the Mexican population (LERMA2 strain) studied by Méndez-Sánchez et al. (2022). Both populations are congruent in most of the following features: the acute snout, the number of adoral membranelles (17–22 vs. 16–20), the number of somatic kineties (18–22 vs. 16–20), and the arrangement of cortical granules. However, the body size of the Korean population is smaller than that of the Mexican population (70–120 vs. 60–180 μm). Jankowski (1964) reinvestigated the genus *Bothrostoma* and separated it from the genus *Metopus* based on the difference in body shape, the buccal cavity being larger in *Metopus* and the body having only a slight torsion anteriorly as first described by Stokes (1887) (Fig. 2D). The present population of *B. undulans* also agrees with the description by Jankowski (1964) in terms of body size (70–120 × 20–45 μm vs. 80–110 × 30–40 μm), the number of adoral membranelles (17–22 vs. 18), within the range of variation.

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CONFLICTS OF INTEREST

Mann Kyoon Shin, an executive editor of Animal Systematics, Evolution and Diversity, was not involved in the editorial evaluation or decision to publish this article. The remaining author has declared no conflicts of interest.

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