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Morphological Description of Three Anaerobic Ciliates Unrecorded in Korea

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ABSTRACT

During the surveys of ciliates from hypoxic habitats, three marine anaerobic species were found: *Metopus spiculatus*, *M. vestitus*, and *Muranothrix felix*. These species have not been previously recorded in South Korea and belong to the taxonomic classes Armophorea and Muranotrichea. The morphology of these species was examined by both microscopic observations of live specimens, and stained cells using protargol impregnations. *Metopus spiculatus* has the following characteristics: body size 80–110×25–35 μm *in vivo*, beak-like structure at the end of preoral dome, ectosymbiotic bacteria covering cell surface, intracytoplasmic needle-shaped structures and the conspicuous tail end. *Metopus vestitus* has the following distinguishing characteristics: body size 95–130×25–45 μm *in vivo*, a cone-shaped body, a covering of ectosymbiotic bacteria on its cell surface, intracytoplasmic needle-shaped structures, somatic kineties arranged in 26–28 longitudinal rows, and a posterior part tapered into a tail. *Muranothrix felix* has the following characteristics: body size 100–130×20–30 μm *in vivo*, elongated body with twisted neck region, bristle-like cilia protruding perpendicular to the cell margin, ectosymbiotic bacteria covering the cell surface, about 10 macronuclear nodules, and a long, stiffened caudal cilium.

Keywords: Anaerobic ciliates, ectosymbionts, marine, *Metopus*, *Muranothrix*

INTRODUCTION

In recent years, there has been a surge in research on the 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., 2023). It is noteworthy that the genus *Metopus* Claparède and Lachmann, 1858 has received increased attention. So far, more than 70 nominal species have been recorded within this genus (Omar et al., 2017; Li et al., 2021; Zhuang et al., 2022). Discoveries in diverse habitats like soil, freshwater, and marine environments have rapidly expanded the number of anaerobic species (Omar et al., 2017; Rotterová et al., 2018; Bourland et al., 2020; Li et al., 2021; Zhuang et al., 2022). This exploration extends beyond the well-studied class Armophorea (Lynn, 2004) with the discovery of new classes or families. For example, the establishment of the class Muranotrichea (Rotterová et al., 2020) significantly contributed to our understanding of the growing diversity of anaerobic ciliates. The class Muranotrichea was established based on the basics of transcriptomic data, therefore the morphological features are limited. Briefly, members of the class Muranotrichea are mostly found in marine sediments, characterized by the elongated body shape with the twisted neck, associated with ectosymbiotic bacteria (Rotterová et al., 2020). These recently established taxa included a single order and family Muranotrichidae. Among them, the genus *Muranothrix* is composed of two nominal species so far, identified as *Muranothrix gubernata* and *M. felix* (Rotterová et al., 2020; Méndez-Sánchez et al., 2023).

Despite increased research worldwide, studies on Korean anaerobic ciliates remain scarce. For example, although many species have been described, only three *Metopus* species have been documented in Korea to date (He and Choi, 2015; Omar and Jung, 2021, 2022), and no species of the genus *Muranothrix* have been recorded in Korea so far. To fill this gap and enrich the ciliate diversity documented in Korea, this study describes two *Metopus* species, and one *Muranothrix* species collected from marine hypoxic habitats in Korea.

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MATERIALS AND METHODS

Metopus spiculatus Zhuang et al., 2022 was collected in the intertidal zone in Jeju Island, South Korea (33°51′18″N, 126° 89′83″E) in October 2022, salinity (18‰). *Metopus vestitus* Kahl, 1932 was discovered in the water way near the Mokpo Maritime University, Mokpo, South Korea (34°79′86″N, 126°36′31″E) in June 2022, salinity (18‰). *Muranothrix felix* Méndez-Sánchez et al., 2023 was collected from the water way near the fishing area in Jeju Island, South Korea (33°55′12″N, 126°64′65″E) in October 2022, salinity (20‰). Samples were collected with sediments and organic matters into one litter plastic jar, the water fills up the jar to keep the anaerobic condition. Samples were transferred and maintained at room temperature. Some wheat or rice grains were put to generate the bacteria which serve as a food source, the lid is always closed to maintain the anaerobic condition. The morphology of ciliate was investigated using both microscopic observations of live specimens and protargol impregnation staining of fixed cells (Wilbert, 1975). The living and impregnated specimens were observed under light microscope (Axio Imaget A1; Carl Zeiss, Oberkochen, Germany) with differential interference contrast optics. Images were captured using a CCD camera (AxioCam MRc; Carl Zeiss). Measurements and counts were performed under magnifications ranging from 100×–1,000×. Terminologies primarily follow Foissner and Agatha (1999), Lynn (2008), and Bourland et al. (2014). Classification scheme adheres to Jankowski (2007) and Rotterová et al. (2020).

RESULTS

Class Armophorea Lynn, 2004
Order Metopida Jankowski, 1980
Family Metopidae Kahl, 1927
Genus [1*]*Metopus* Claparède and Lachmann, 1858

1. [2*]*Metopus spiculatus* Zhuang et al., 2022 (Table 1, Fig. 1)

Metopus spiculatus Zhuang et al., 2022: 6, table 2, figs. 4, 5.

Material examined. Sediments were collected from Yongmokgae wadang reservoir, Hado-ri, Gujwa-eup, Jeju-si, Jeju, Korea (33°51′18″N, 126°89′83″E) in Oct 2022, salinity (18‰).
Voucher specimens. One slide of protargol-stained voucher specimens (registration number: UBL20240729001) is deposited in Biodiversity lab, University of Ulsan.
Description. Body size 80–110×25–35 μm *in vivo* with a body length to width ratio approximately (3.3 : 1), and 75–110×20–30 μm after staining, with a body length to width ratio (3.4 : 1). Body shape elongated to oblong, sometimes appearing stout; posterior end tapered into a tail, approximately 25 μm long (Fig. 1A, C, E, F); preoral dome highly compressed, overhanging the left margin and slightly twisted, tapered to a beak-like structure at the tip (Fig. 1A, B, F, H). Macronucleus located at anterior half, usually elongated or somewhat circular in shape, size after staining 25×12 μm on average (Fig. 1E–H). Micronucleus spherical in shape, located adjacent to the macronucleus, size about 3 μm across (Fig. 1F). Cortex flexible; ectosymbiotic bacteria shaped like rods, approximately 2 μm long, covered cell surface perpendicularly, except for the dome region and the tail (Fig. 1B–D). Cytoplasm transparent and contained scattered reddish granules (possibly sulfur-like) and needle-shaped structures concentrated at preoral dome, with some scattered throughout the cytoplasm, reaching about 10 μm in length (Fig. 1B, C). Contractile vacuole about 10 μm in diameter, located at cell terminal end (Fig. 1A–C). Swimming moderate.

Ordinary somatic cilia about 10 μm in length, somatic kineties arranged regularly from beneath the adoral zone to the posterior end of the ventral side, and from the anterior to posterior end of the dorsal side, extending to the tail, 19–24 rows, composed of dikinetids (Fig. 1D–F, H). Perizonal stripes contained cilia longer than somatic cilia, reaching up to 20 μm in length, consisted of five invariable rows, forming into “false kineties” (Fig. 1I). The adoral zone consisted of 17–23 membranelles, occupying about 30% of the body length (Fig. 1A, E, J). Paroral membrane consisted of two files of basal bodies (PM1 and PM2) (Fig. 1G).
Distribution. China (Qingdao), Korea (present study).

2. [3*]*Metopus vestitus* Kahl, 1932 (Table 1, Fig. 2)

Metopus vestitus Kahl, 1932: 416, figs. S.415, 37; Tucolesco, 1962: 23, figs. 38; Fenchel, 1969: 91, fig. 45; Esteban et al., 1995: 150, figs. 33–37.

Material examined. Sediments were collected from the water way near the Mokpo Maritime University, 91 Haeyangdaehak-ro, Mokpo-si, Jeollanam-do, Korea (34°79′86″N, 126° 36′31″E) in Jun 2022, salinity (18‰).
Voucher specimens. Two slides of protargol-stained voucher specimens (registration number: HNIBRPR3, HNIBRPR4) are deposited in Honam National Institute of Biological Resources.
Description. Body size 95–130×25–45 μm *in vivo*, with a body length to body width ratio approximately (3.15 : 1), and 95–135×25–35 μm after staining, with a body length to body

Korean name: [1*]고개돌린섬모충속 (신칭), [2*]부리고개돌린섬모충 (신칭), [3*]덮힌고개돌린섬모충 (신칭)

Table 1. Morphometric data of *Metopus spiculatus* (spi.), *M. vestitus* (ves.)

Character	Species	Mean	Median	Min	Max	SD	CV	n
Body length (A)	spi.	89.9	87.5	75.0	110.0	9.9	11.0	16
	ves.	111.3	107.5	96.0	133.0	12.9	11.6	10
Body width (B)	spi.	26.7	26.0	21.0	32.0	3.0	11.2	16
	ves.	30.2	30.5	24.0	36.0	3.6	11.9	10
Ratio (A : B)	spi.	3.4	3.4	2.8	4.0	0.3	10.3	16
	ves.	3.7	3.7	3.1	4.0	0.3	7.8	10
Perizonal cilia rows, number	spi.	5.0	5.0	5.0	5.0	0.0	0.0	12
	ves.	5.0	5.0	5.0	5.0	0.0	0.0	10
Anterior to proximal end of perizonal stripes, length (C)	spi.	33.5	33.0	22.0	45.0	6.5	19.3	15
	ves.	39.1	40.0	30.0	47.0	4.8	12.4	10
Ratio (C : A) (%)	spi.	34.8	37.4	0.0	48.4	10.5	30.2	16
	ves.	35.3	34.6	29.8	41.0	4.1	11.5	10
Anterior to proximal end of adoral zone, length (D)	spi.	33.5	32.5	28.0	45.0	5.3	15.9	16
	ves.	39.4	40.5	31.0	46.0	4.5	11.3	10
Ratio (D : A) (%)	spi.	37.4	37.6	27.7	48.4	5.0	13.4	16
	ves.	35.6	34.6	30.7	42.0	3.8	10.6	10
AM, number	spi.	20.3	20.0	17.0	23.0	1.7	8.3	14
	ves.	19.2	19.0	17.0	20.0	0.9	4.8	10
PM1, length	spi.	17.1	17.0	13.0	23.0	2.7	15.9	15
	ves.	20.8	21.0	18.0	23.0	2.0	9.6	10
PM2, length	spi.	9.3	9.0	8.0	11.0	0.9	9.3	9
	ves.							
Macronucleus, length (E)	spi.	26.5	27.5	9.0	36.0	8.1	30.7	16
	ves.	36.9	36.5	26.0	48.0	6.2	16.8	10
Macronucleus, width	spi.	12.5	7.0	4.3	88.0	21.0	167.6	15
	ves.	9.2	9.0	6.0	13.0	1.9	21.0	10
Micronucleus, size	spi.	3.3	3.1	2.8	4.0	0.4	13.7	10
	ves.	3.7	4.0	3.0	4.0	0.5	12.5	11
Anterior end to the end of macronucleus, length	spi.	38.4	39.0	22.0	50.0	6.7	17.5	16
	ves.	49.0	50.5	35.0	57.0	7.0	14.3	10
Ratio (E : A) (%)	spi.	29.6	32.8	9.8	39.5	8.7	29.4	16
	ves.	33.2	34.6	26.0	37.0	4.1	12.3	10
Somatic kineties, number	spi.	21.6	22.0	19.0	24.0	1.8	8.5	15
	ves.	26.8	26.0	26.0	28.0	1.0	3.9	10

All measurements of size, length, and width in μm. All data are based on protargol-impregnated specimens.
AM, adoral membranelles; CV, coefficient of variation (%); Max, maximum value; Mean, arithmetic mean; Min, minimum value; n, number of individuals investigated; PM1, 2; paroral membrane 1, 2; SD, standard deviation of the arithmetic mean.

width ratio (3.7 : 1). Cell slender, widest at dome region and narrowly to posterior like corn-shaped, dorso-ventrally flattened; posterior end tapered into a tail, length about 30 μm on average (Fig. 2A, D, E); preoral dome highly compressed twisted anterior from the right to left side (Fig. 2A, F). Macronucleus posited at anterior half, oval to elongated shape, size about 35 × 10 μm (Fig. 2B–D). Micronucleus attached to the macronucleus, circular shape, size about 4 μm in diameter (Fig. 2C). Cortex flexible; on the cell surface, a layer of ectosymbiotic bacteria covered perpendicularly, each bar-shaped length about 2 μm, covered most of the cell except for the dome region and the tail (Fig. 2B, C). Cells yellowish at low magnifications, cytoplasm containing of many granules, bar-shaped intracytoplasmic structures scattered whole body and accumulated densely at the anterior pole, length about 13 μm (Fig. 2A–C). Contractile vacuole located terminally (Fig. 2A). Swimming rotated following to the body axis at a moderate pace.

Somatic kineties composed of dikinetids arranged into 26–28 longitudinal rows, the ordinary cilia length about 12 μm (Fig. 2D, E). Perizonal stripes arranged into five rows, composed of dikinetids, forming "false kineties" (Fig. 2F). The adoral zone consisted of 17–20 membranelles, occupying about 30% of the body length (Fig. 2A, D, F, G). The paroral

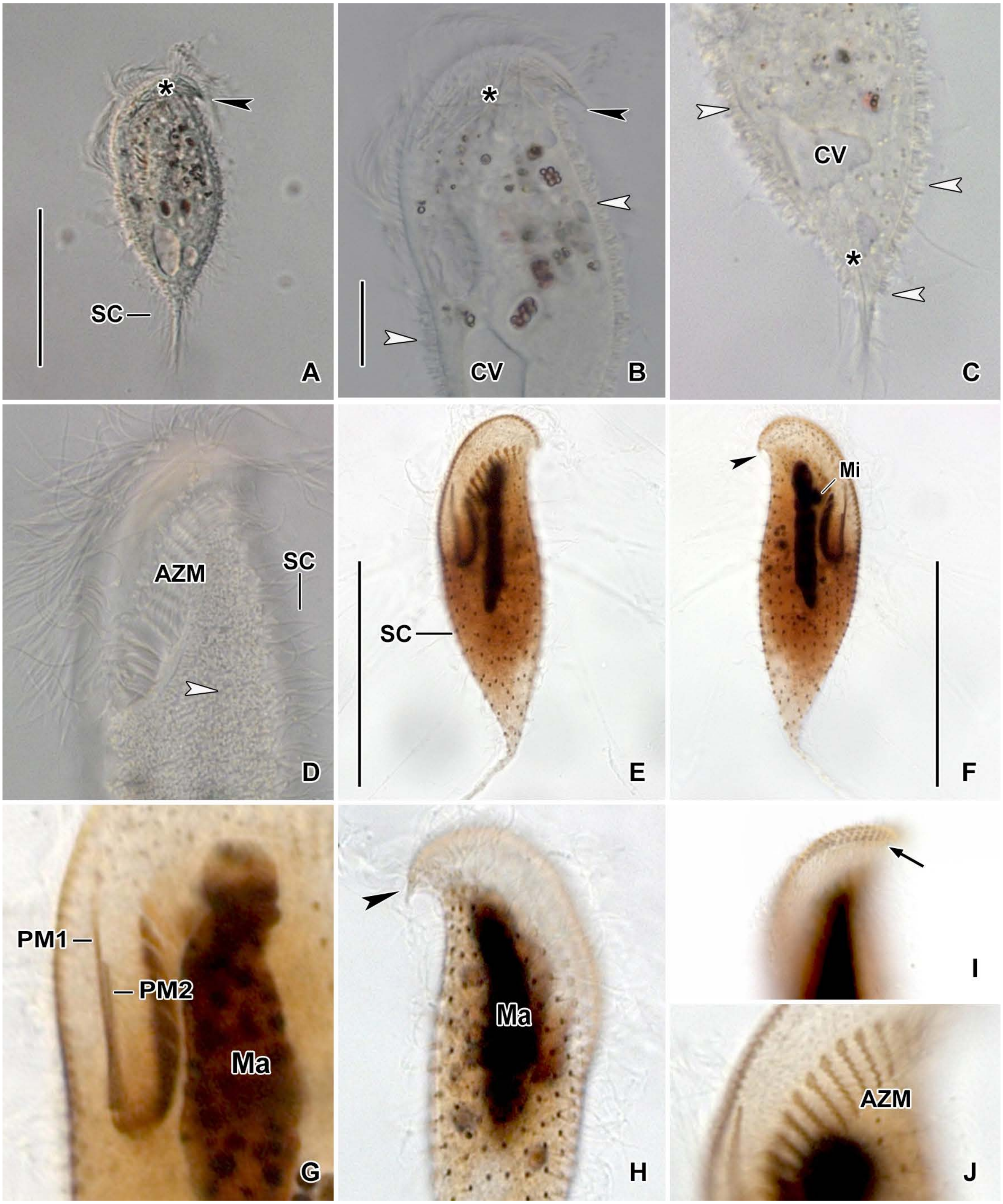

Fig. 1. *Metopus spiculatus* from live (A–D), after protargol impregnation (E–J). A–C, Ventral view of a representative specimen, black arrowheads indicating the beak-like structure at the end of the preoral dome (A, B), white arrowheads pointing to the ectosymbiotic bacteria covering the cell surface (B, C), asterisks denoting intracytoplasmic needle-shaped structures scattered throughout the cytoplasm and near the tail (C); D, Another view focusing on the surface showing the densely packed ectosymbiotic bacteria (white arrowhead); E, F, Ventral and dorsal views of the stained specimen, demonstrating the somatic ciliatures, nuclear apparatus, paroral membranes, and the beak-like structure denoted by a black arrowhead (F); G, Paroral membranes composed of two files of basal bodies (PM1 and PM2); H, Dorsal view showing the beak-like structure (black arrowhead); I, Black arrow indicating the perizonal stripes arranged into false kineties; J, Details of adoral zone of membranelles. AZM, adoral zone of membranelles; CV, contractile vacuole; Ma, macronucleus; Mi, micronucleus; PM1, 2, paroral membrane 1, 2; SC, somatic cilia. Scale bars: A, E, F = 50 μm, B = 10 μm.

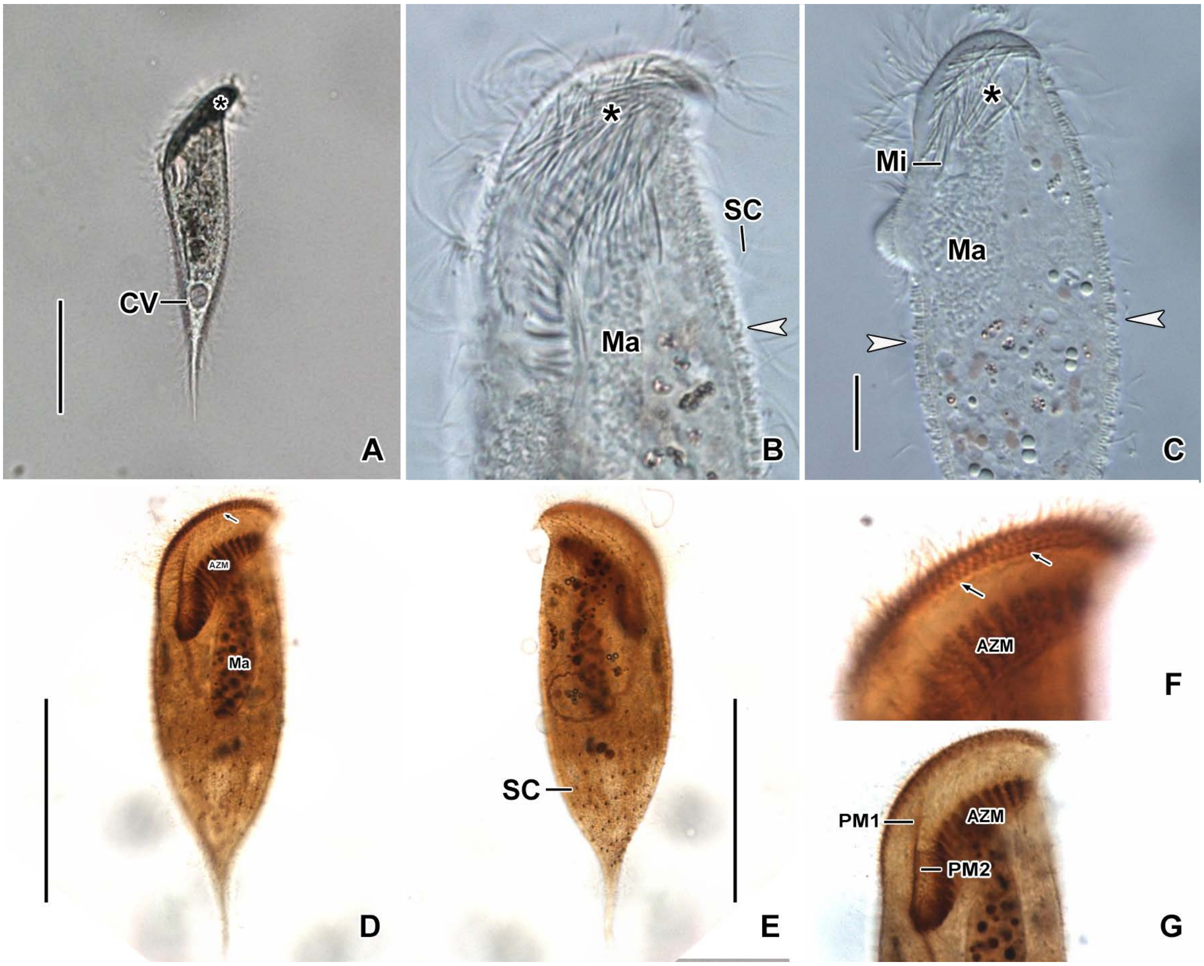

Fig. 2. *Metopus vestitus* from live (A–C), after protargol impregnation (D–G). A, Ventral view of a representative specimen showing contractile vacuole located sub-terminally; B, C, Ventral view showing the ectosymbiotic bacteria covering the cell surface perpendicularly (white arrowheads), nuclear apparatus, intracytoplasmic needle-shaped structures (asterisks), and somatic cilia; D, E, Ventral and dorsal views of a stained specimen revealing the ciliatures, nuclear apparatus, adoral zone of membranelles, black arrow indicating the false kineties; F, The perizonal stripes forming false kineties (black arrows); G, Paroral membranes composed of two files of basal bodies (PM1 and PM2). AZM, adoral zone of membranelles; CV, contractile vacuole; Ma, macronucleus; Mi, micronucleus; PM1, 2, paroral membrane 1, 2; SC, somatic cilia. Scale bars: A, D, E = 50 μm, C = 10 μm.

membrane consisted of two files of basal bodies (PM1 and PM2) (Fig. 2G).

Distribution. Germany (Kieler Förde), Black Sea, Denmark (Øresund, Helsingør), Korea (present study).

Class Muranotrichea Rotterová et al., 2020

The class Muranotrichea is identified by the elongated body shape with the adoral zone spiraling rightward around the distinctly neck-like anterior body part; somatic kineties composed of dikinetids and spiraling leftward; invariably with prokaryotic ectosymbionts and cytoplasmic endosymbionts; mostly found in the saline water (Rotterová et al., 2020).

Order Muranotrichida Rotterová et al., 2020

The order Muranotrichida with the characteristics of the class contains a single family Muranotrichidae.

Family Muranotrichidae Rotterová et al., 2020

Family Muranotrichidae is characterized as the order definition. It consists of two genera.

Genus [1]**Muranothrix* Rotterová et al., 2020

Medium-sized, markedly contractile, thus postoral body part

Korean name: [1]*무라노섬모충속 (신칭)

narrowly to broadly ellipsoidal; long "bristle-like" somatic cilia interspersed with "ordinary" somatic cilia; several long cohesive caudal cilia upon which shorter posterior somatic cilia converge in an inverted cone arrangement; paroral membrane tripartite, extends around proximal buccal vertex; intrabuccal lip on left wall of buccal cavity (Rotterová et al., 2020).

3. [1*]*Muranothrix felix* Méndez-Sánchez et al., 2023 (Table 2, Fig. 3)

Muranothrix felix Méndez-Sánchez et al., 2023: 7, table 3, figs. 4, 5.

Material examined. Sediments were collected in the water way near the entrance of Sinheung fishing park, 711-1 Sinheung-ri, Jocheon-eup, Jeju-si, Jeju, Korea (33°55′12″N, 126°64′65″E) in Oct 2022, salinity (20‰).

Voucher specimens. One slide of protargol-stained voucher specimens (registration number: UBL20240729002) is deposited in Biodiversity lab, University of Ulsan.

Diagnosis. Size 80–140×14–30 μm; anterior neck-like region spirals rightward about 360° around long axis; 6–14 macronuclear nodules grouped in mid-body; peristome about 1/2 of body length; 26–46 adoral membranelles; perizonal stripe comprises about 12 false kineties; long caudal cilium present.

Description. Body size 100–135×20–30 μm *in vivo*, with a body length to body width ratio approximately (4.6 : 1), and 95–140×20–35 μm after staining, a body length to body width ratio (4.6 : 1). Elongated body shape, wider in the posterior half; anterior half slender forming neck region, twisted rightwards (Fig. 3A, C, H, I). Nuclear apparatus consisted of multiple macronuclei, on average 10 nodules, with various sizes and shapes, from ellipsoid to round shaped, size after protargol preparations about 6×5 μm (Fig. 3K). Single micronucleus located around the macronuclei, usually spherical shape, size about 3.5 μm (Fig. 3K). Cortex flexible; cell surface covered with a layer of ectosymbiotic bacteria, thickness about 1.5 μm (Fig. 3D–F). Cytoplasm transparent, contained of some granules, and droplets with various sizes (Fig. 3A–C). Contractile vacuole located terminally (Fig. 3B, C). Swimming moderate.

The somatic structure consisted of dikinetids, ordinary cilia length about 10 μm (Fig. 3B), bristle-like somatic cilia about 11 μm interspersed along with the somatic cilia, perpendicularly oriented to the cell margin (Fig. 3B, C). Somatic kineties arranged into 10–12 rows, slightly twisted left (Fig. 3H, I). Long caudal cilium located in the middle of the posterior end, 35–60 μm long, surrounded by somatic cilia forming a cone-shaped tuft (Fig. 3A, C). Adoral zone torsion following the neck region, which occupied about 55% of the body length,

Table 2. Morphometric data of *Muranothrix felix*

Characters	Mean	Median	Min	Max	SD	CV	n
Body length (A)	115.4	116	96	138	11.68	10.12	18
Body width (B)	25.8	26	18	36	5.20	20.16	18
Ratio (A : B)	4.64	4	3.0	7.4	1.01	21.8	18
Anterior to proximal end of AZM, length (C)	61.9	63	53	68	4.15	6.71	16
Anterior to distal end of AZM, length (D)	5.1	5	4	6.8	0.82	16.01	11
Ratio (C : A) (%)	53.8	54	44.2	65.6	5.41	10.06	16
Ratio (D : A) (%)	4.3	4	3.2	7.0	1.07	24.82	11
Adoral zone, length (E)	58.4	59	48.2	65	5.05	8.64	16
Ratio (E : A) (%)	50.8	51	40.2	65.6	6.71	13.21	16
Adoral membranelles, number	42.5	42	40	46	2.17	5.11	10
Somatic kineties, number	10.9	11	10	12	0.80	7.33	18
Macronucleus, nodules	10.0	10	8	12	1.41	14.14	6
Macronucleus, length	6.4	6	5	8	1.28	19.97	6
Macronucleus, width	5.6	6	4.5	7	0.92	16.43	6
Micronucleus, number	1.0	1	1	1	0.00	0.00	12
Micronucleus, size	3.4	3	2	5	0.92	27.46	12
Paroral membrane, length (F)	55.7	55	50	64	5.47	9.82	6
Ratio (F : E) (%)	94.5	95	86.4	98.1	4.72	4.99	5

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

Korean name: [1*]긴꼬리털무라노섬모충 (신칭)

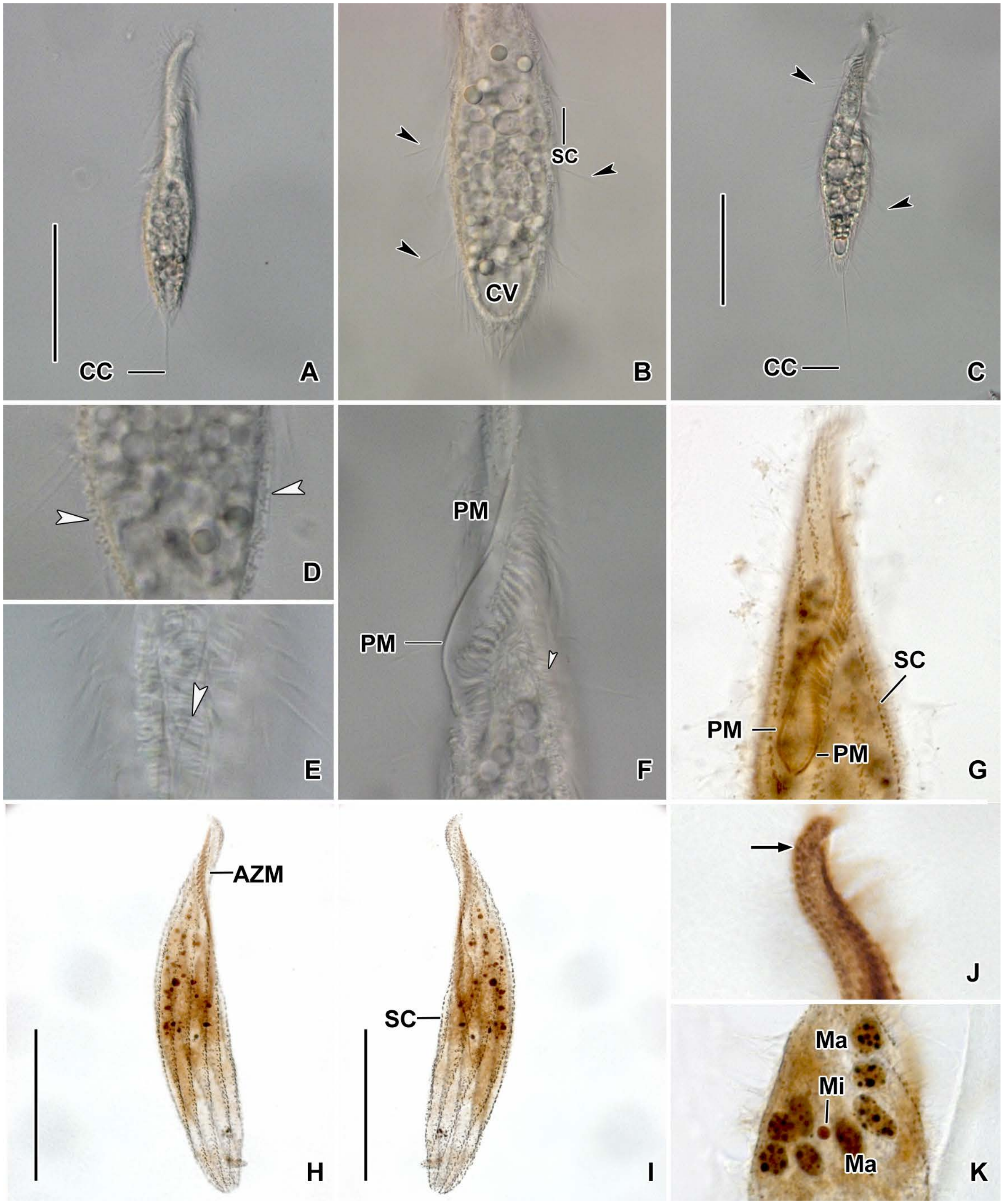

Fig. 3. *Muranothrix felix* from live (A–F), after protargol impregnation (G–K). A–C, Showing the general shape and long caudal cilia (A, C), black arrowheads indicating bristle-like cilia oriented slightly parallel to the cell margin and adjacent to the somatic cilia, the contractile vacuole located terminally; D, E, Ectosymbiotic bacteria covered the cell margin (white arrowheads), (D) lateral view of the bacteria, (E) cell surface showing the bacteria oriented transversely between the ciliary rows; F, G, The tripartite paroral membranes in live and stained specimens, and somatic cilia; H, I, Impregnated specimen revealing the somatic structures, adoral zone of membranelles; J, Black arrow indicates the perizonal stripes arranged into false kineties; K, Single micronucleus located around macronuclei. AZM, adoral zone of membranelles; CC, caudal cilia; CV, contractile vacuole; Ma, macronuclei; Mi, micronucleus; PM, paroral membrane; SC, somatic cilia. Scale bars: A, C, H, I=50 μm.

comprised 40–46 membranelles (Fig. 3A, C, G–I). Perizonal stripes forming to “false kineties” (Fig. 3J). Paroral membrane tripartite following the adoral zone (Fig. 3F, G).
Distribution. USA (Rhode Island), Korea (present study).

DISCUSSION

Comparison of Korean populations of *Metopus spiculatus*, *M. vestitus* and closely related species

The Korean population of *Metopus spiculatus* almost coincides with the original description by Zhuang et al. (2022) in terms of body size and shape, the presence of a beak-like projection on the preoral dome, ectosymbiotic bacteria covering the cell surface, needle-shaped intracytoplasmic structures, the number of adoral membranelles (17–23 vs. 17–22), and the number of somatic kineties (19–24 vs. 19–25). These features strongly support the identification of the Korean population as *M. spiculatus*.

The Korean population of *M. vestitus* shares some similarities with the original description (Kahl, 1932) and the Danish population (Esteban et al., 1995) in terms of a cell outline tapering to a tail, a highly compressed preoral dome, the presence of ectosymbiotic bacteria on the cell surface, and inhabiting a marine environment. The Korean population has a larger body size compared to the original population and the Danish population (95–130 vs. 70–80 vs. 70 μm). This discrepancy could be caused by different environmental conditions, the Korean population was cultured in the laboratory with rich nutrients provided. Despite the difference in body size, the Korean population also agrees with the description of Esteban et al. (1995) in terms of the number of somatic ciliary rows (26–28 vs. 25), as well as the number of adoral membranelles (17–20 vs. 20), these characters are considered stable for species determination.

While both *Metopus spiculatus* and *M. vestitus* are closely related and share many features, the presence of the beak-like structure at the end of the preoral dome is unique to *M. spiculatus*. This single characteristic serves as a clear distinction between the two species (Figs. 1A, B, H, 2B, C, E, G). In addition, the Korean populations of *M. spiculatus* and *M. vestitus* differ from each other in terms of body shape (elongate to oblong vs. slender and conical), and the number of somatic kineties (19–24 vs. 26–28).

Another marine species *M. paravestitus* (Li et al., 2021), not recorded in Korea, which also harbors ectosymbionts on its cell surface, and possesses intracytoplasmic needle-shaped structures. However, *M. paravestitus* can be distinguished from both *M. spiculatus* and *M. vestitus* by the following characteristics: it lacks a distinct tail-like structure at the cell terminal, a beak-like structure present in *M. spiculatus*, and it has different number of somatic kineties (30–42 vs. 19–24 vs. 26–28), respectively.

Comparison of Korean population of *Muranothrix felix* with original description and congener

The Korean population of *Muranothrix* was identified as *M. felix* based on its close match to the original description by Méndez-Sánchez et al. (2023). This match includes several key characteristics: elongated body shape, body length (95–140 vs. 80–140 μm), a comparable number of macronuclear nodules (8–12 vs. 6–14), the somatic ciliary rows (10–12 in both), and the length of caudal cilia about 50 μm *in vivo* in both. However, the Korean population has a higher number of adoral membranelles compared to the original description (40–46 vs. 26–33).

The genus *Muranothrix* consists of two species that have been studied so far. Both species have a slender body and a twisted neck region. However, *Muranothrix felix* can be distinguished from *M. gubernata* Rotterová et al., 2020 by two key features: the number of macronuclear nodules (8–12 vs. 15–33), and body length (95–140 vs. 70–105 μm).

Key to identify five recorded *Metopus* from Korea

In general, genus *Metopus* mostly identified by the preoral dome more or less twist leftwards; band-like adoral zone membranelles situated in the anterior running obliquely around and down the body, terminating at the cytostome; invariably of five-rows perizonal stripes.

1a. Marine habitat ··· 2
1b. Fresh water habitat ··· 3
2a. Tail-like posterior region, ectosymbionts covered cell, with beak-like anterior region present ··· *Metopus spiculatus*
2b. Tail-like posterior region, ectosymbionts covered cell, with beak-like anterior region absent ··· *Metopus vestitus*
3a. Perizonal stripes not forming false kineties ··· *Metopus es*
3b. Perizonal stripes forming false kineties ··· 4
4a. Preoral dome broad, stiff and very long caudal cilia, dense somatic kineties (>20 rows) ··· *Metopus setosus*
4b. Preoral dome slender, flexible and long caudal cilia, loose somatic kineties (<20 rows) ··· *Metopus hasei*

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CONFLICTS OF INTEREST

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