ACTA
PROTOZOOLOGICA

Acta Protozool. (2019) 58: 43–51
www.ejournals.eu/Acta-Protozoologica
doi:10.4467/16890027AP.19.005.10836

# Morphology and Molecular Analyses of a New Marine Ciliate, *Arcuseries minima* sp. nov. (Ciliophora: Urostylidae)

**ZooBank:** urn:lsid:zoobank.org:pub:97942DC9-41A9-431A-96FC-1D2F48A2CA67

**Kang-San KIM[1,2], Kyu-Seok CHAE[1], Gi-Sik MIN[1]**

**[1] Department of Biological Sciences, Inha University, 100 Inha-ro, Nam-gu, Incheon 22212, South Korea**
**[2] Restoration Assessment Team, Research Center for Endangered Species, National Institute of Ecology, Gomal-gil 23, Yernygong-gon, Gegornagsangbark-do 36531, Korea**

**Abstract.** A new marine urostylid ciliate, *Arcuseries minima* sp. nov., was discovered in South Korea. Morphological observations and molecular phylogenetic analyses based on small subunit ribosomal DNA (18S rDNA) sequences were used to describe the new species. *Arcuseries minima* is most similar to *A. scutellum* among all *Arcuseries* species, but differs in the following main characters: number of adoral membranelles (13–16 vs. 17 or 18), cortical granules (yellowish, clustered around cirri and dorsal bristles vs. colorless, irregularly scattered), number of macronuclear nodules (20–27 vs. 42–90), number of midventral cirri (5–10 vs. 12–14), and number of transverse cirri (5 or 6 vs. 8). The new species and *A. scutellum* differ from *A. petzi* and *A. warreni* in having smaller body size (≤80 μm vs. ≥80 μm) and fewer cirri: left marginal (≤ 17 vs. ≥ 18) and transverse (≤ 8 vs. ≥8) cirri. This relationship was supported by the phylogenetic tree, where these two groups were separated into two branches.

**Key words:** Marine ciliate, *Arcuseries*, phylogenetic analysis, urostylid, 18S rDNA sequence.

## INTRODUCTION

The genus *Arcuseries* Huang *et al*., 2014 is characterized by the following combination of featues: marine habitat; non-dorsomarginalian hypotrichs; roughly U-shaped-arranged transverse cirri; midventral complex composed of cirral pairs only; three frontal cirri; buccal cirrus, frontoterminal cirri, and pretransverse ventral cirri present; one right and one left marginal row; three bipolar dorsal kineties; caudal cirri lacking; undulating membranes roughly straight and more or less arranged in parallel; many macronuclear nodules (Berger 2006, Huang *et al*. 2014). In addition, *Arcuseries* is monophyletic and is supported by phylogenetic analyses (Huang *et al*. 2014). To date, three *Arcuseries* species, namely *A. scutellum* (Cohn, 1866) Huang *et al*., 2014, *A. petzi* (Shao *et al*., 2011) Huang *et al*., 2014 (type species), and *A. warreni* (Song and Wilbert, 1997) Huang *et al.*, 2014, have been reported with morphological descriptions and molecular information (Berger 2006, Chen *et al*. 2010, Shao *et al*. 2011). Of the three *Arcuseries* species, two, *A. petzi* and *A. warreni*, have been recorded in Korean coastal waters (Kim *et al*. 2013, Kim and Min 2018). In the present study, we provide morphological descriptions and phylogenetic analyses of a new *Arcuseries* species.

Address for correspondence: Gi-Sik Min, Department of Biological Sciences, Inha University, 100 Inha-ro, Michuhol-gu, Incheon 22212, South Korea; Tel: +82-32-860-7692; Fax: +82-32-874-6737; Cell phone: +82-10-6219-0752; E-mail: gisikmin@gmail.com

## MATERIALS AND METHODS

### Sample collection and identification

Specimens of *Arcuseries minima* were isolated from coastal waters in Jeju province, South Korea (salinity, 33‰; water temperature, 27.1°C) (33°14'9.21"N; 126°35'55.38"E) in August 2017. Seawater filtered with a 200 μm nylon mesh was transferred to Petri dishes, and rice grains were added to enrich the growth of bacteria and bacteriovorous flagellates at room temperature (18–22°C). Six months later, the new species was observed by chance. At that time, the salinity of the culture was over 50‰ (exact salinity could not be measured). Also, no ciliates were present in the raw culture used for the present study, except for *A. minima.*

Living and protargol-stained specimens were observed under a stereo microscope (SZH10; Olympus, Tokyo, Japan) and a light microscope (DM2500; Leica, Wetzlar, Germany) at magnifications ranging from × 100 to × 1,000. Cell staining was performed according to Foissner (2014) (Procedure A). Line drawings of the specimens were conducted at magnification of × 400 and × 1,000, before being digitally drawn using Adobe Illustrator CS6 (Adobe, San Jose, CA, USA). In general, classification and terminology follow Berger (2006).

### DNA extraction, PCR amplification, and sequencing

Cells were washed several times with distilled water to isolate single cells from the raw culture. Genomic DNA was then extracted using a REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich Co., St. Louis, MO, USA) based on the manufacturer's protocol. Optimized PCR conditions were as follows: initial denaturation at 94°C for 2 min 30 s, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 3 min, and a final extension at 65°C for 7 min. The EukA (forward; 5′-AAC CTG GTT GAT CCT GCC AGT-3′) and the EukB (reverse; 5′-TGA TCC TGC AGG TTC ACC TAC-3′) primers (Medlin *et al*. 1988) were used to amplify almost the entire 18S rDNA sequence. Sequencing was performed using the ABI 3700 platform (Applied Biosystems, Foster city, CA, USA) using two internal primers, 18S-300 (5′-CAT GGT AGT CCA ATA CAC TAC-3′) and 18S+810 (5′-GCC GGA ATA CAT TAG CAT GG-3′).

### Phylogenetic analyses

18S rDNA sequences determined in this study and those retrieved from GenBank were aligned using BioEdit (Hall 1999). jModelTest 2.1.7 (Darriba *et al*. 2012) was used to determine the appropriate DNA substitution model for maximum likelihood (ML) and Bayesian inference (BI) analyses and the TIM2 + I (0.5490) + G (0.4830) model was selected. For ML and BI analyses, the FASTA file was converted to NEXUS and PHYLIP files using MEGA 5.0 (Tamura *et al*. 2011). The ML analysis was conducted using PhyML version 3.1 and 1,000 bootstrap replicates (Guindon *et al*. 2010). BI assessment was conducted on MrBayes 3.2.2 (Ronquist *et al.* 2012) with a chain length of 1,000,000 generations. Trees were sampled every 100 generations with a prior burn-in of 30%. Sequence dissimilarity (absolute distance) was calculated using MEGA 5.0 (Tamura *et al*. 2011) using the pairwise distance option. Phylogenetic trees were visualized using FigTree v1.4.1 (http://tree.bio.ed.ac.uk/spftware/figtree/, written by A. Rambaut). The pairwise distance and the number of nucleotides differences were calculated using MEGA 5.0 (Tamura *et al*. 2011).

## RESULTS

### *Arcuseries minima* sp. nov.

**ZooBank:** urn:lsid:zoobank.org:act:F17B7069-8B86-4A70-9654-42F85CDA0AC7

**Diagnosis.** Size *in vivo* 40–55 × 20–30 μm; elliptical in shape, highly flexible, and contractile; 20–27 macronuclear nodules and 1–3 micronuclei; contractile vacuole lacking. Three type of cortical granules: (1) colorless, spherical shape, scattered, ca. 3 μm; (2) yellowish, clustered around cirri and dorsal bristles, ca. 1 μm; (3) colorless, scattered, ca. 0.5 μm. About 14 adoral membranelles; 3 frontal, 1 buccal, 2 frontoterminal, 5–10 midventral, 2 pretransverse, and 5 or 6 transverse cirri; 1 left (9–13 cirri) and 1 right (9–14 cirri) marginal row; 3 dorsal kineties.

**Type locality.** Seawater (salinity, 33‰; water temperature, 27.1°C) taken from Jeju province, South Korea (33°14'9.21"N; 126°35'55.38"E) in August 2017.

**Type specimens.** The slide (NIBRPR0000109740) containing the holotype specimen and two slides (NIBRPR0000109741, NIBRPR0000109742) including protargol-stained specimens have been deposited in the National Institute of Biological Resources (NIBR), Incheon, South Korea.

**Etymology.** The species-group name, *minima*, is the feminine version of the Latin adjective *minimus* (smallest) to indicate the small body size of the new species.

**Description:** Size *in vivo* 40–55 × 20–30 μm (n=15), about 34 × 11 μm in protargol preparations; body highly flexible, contractile, oval to elongated elliptical, left and right cell margins slightly convex, both ends rounded, and cell color yellowish to grayish at low magnification (Figs. 1A, F, 2A–C, H). Macronuclear nodules size about 3.3 × 1.9 μm in protargol impregnation, 20–27 in number; one to three micronuclei of size about 1.6 × 1.4 μm in protargol impregnation (Figs. 1G, 2J). Contractile vacuole lacking. Three types of cortical granules: type I (large-sized), colorless, spherical, about 3 μm in size, irregularly distributed on dorsal surfaces; type II (medium-sized), yellowish, spherical, about 1 μm in diameter, clustered around cirri and dorsal bristles; type III (small-sized), colorless, irregularly distributed on dorsal surfaces, less than 0.5 μm in diameter (Figs.

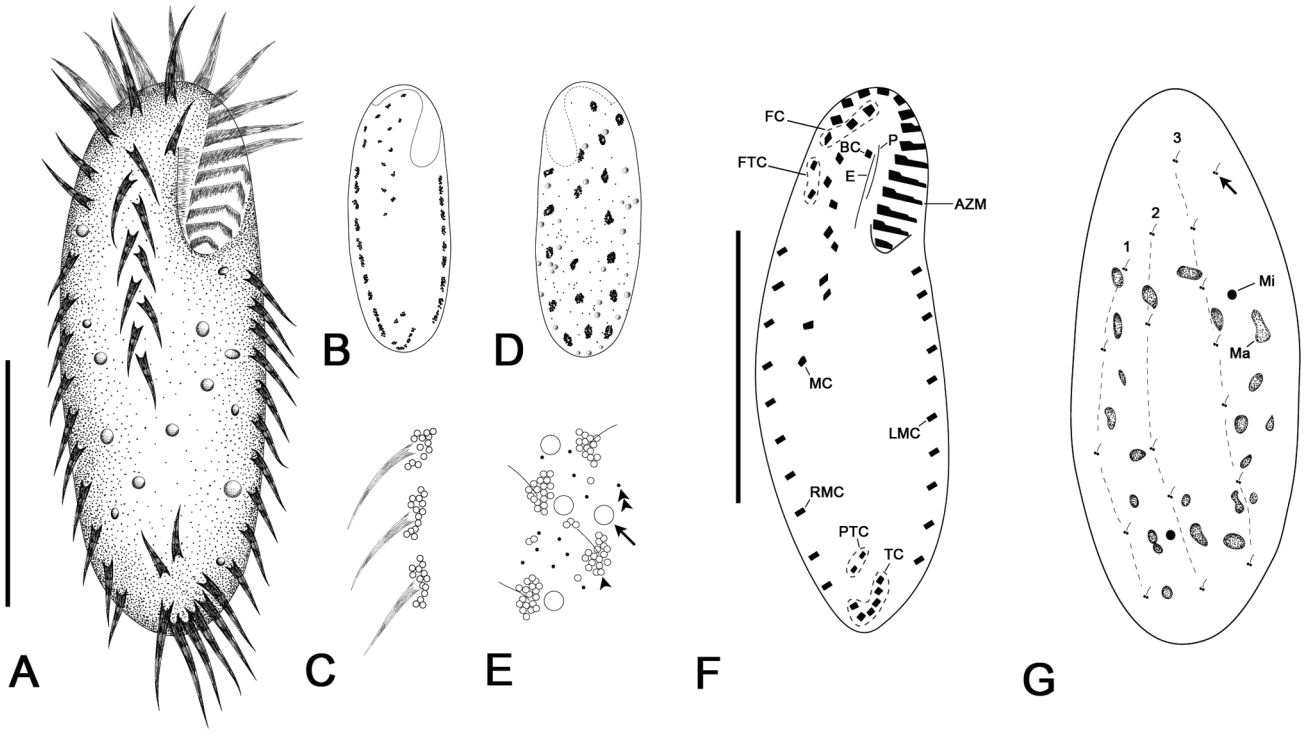


**Fig. 1.** *Arcuseries minima* sp. nov. *in vivo* (A–E) and after protargol impregnation (F, G). (A) Ventral view of a representative specimen. (B, C) Cortical granulation in ventral surface. (D, E) Cortical granulation in dorsal surface, three types of cortical granules: the large (arrow), medium-sized (arrowhead), small (double arrowhead). (F) Ventral view of holotype specimen. (G) Dorsal view of a paratype specimen, arrow indicates a basal body. AZM = adoral zone of membranelles; BC = buccal cirrus; E = endoral; FC = frontal cirri; FTC = frontoterminal cirri; LMC = left marginal cirri; Ma = macronuclear nodules; MC = midventral cirri; Mi = micronuclei; P = paroral; PTC = pretransverse cirri; RMC = right marginal cirri; TC = transverse cirri; 1–3 = dorsal kineties 1–3. Scale bars: 20 μm.

1B–E, 2D–G). Cytoplasm colorless, with 3–5 μm sized food vacuoles. Feeds on bacteria.

Adoral zone of membranelles occupies about 31.5% of cell length in protargol preparations, base of the largest membranelles is about 3.5 μm long, composed of 13–16 membranelles. Paroral and endoral membranes side by side, endoral membrane longer than paroral membrane (Figs. 1F, 2H, I). All cirri are relatively fine, generally 6–8 μm long *in vivo* except frontal and transverse cirri. Three slightly enlarged frontal cirri about 10 μm in length, the rightmost cirrus located behind distal end of the adoral zone of membranelles. Two frontoterminal cirri behind the rightmost frontal cirrus, buccal cirrus on the anterior of endoral membrane, and two pretransverse cirri located ahead the transverse cirri. Five or six slightly enlarged transverse cirri, 10–12 μm in length. Midventral complex consists of two to five midventral pairs (five to ten cirri), arranged in a zigzag pattern, commencing near buccal cirrus and terminating on or above midbody. Two marginal rows, one left (9–13 cirri) and one right (9–14 cirri) commencing behind the posterior end of the buccal field, and both rows non-confluent posteriorly (Figs. 1A, F, 2H, I).

Invariably three dorsal kineties, cilia about 3 μm long *in vivo*: kineties 1 and 2 composed of four to six bristles, anteriorly shortened; almost bipolar kinety 3 composed of five to seven bristles. One pair of basal bodies located ahead of the right marginal cirral row (Figs. 1G, 2K).

**Molecular analyses:** The 18S rDNA sequence of *Arcuseries minima* sp. nov. is 1,598 bp in length and has a GC content of 46.9%. The sequence of *A. minima* was deposited in GenBank with accession number MK889350. BI and ML analyses produced similar topologies; thus, the ML tree was presented as our phylogenetic tree. In the gene tree, *A. minima* clusters with *A. scutellum*, and is a sister species to *A. petzi* and *A. warreni* (Fig. 3). In addition, *A. minima* shows a shorter pairwise distance with *A. scutellum* (0.009, 14 or 15 differences in 1,598 nt) than with *A*. sp. (0.018, 29 differences in 1,598 nt),

**Table 1.** Morphometric data of *Arcuseries minima* sp. nov.

| Characteristic[a] | Mean | M | SD | SE | CV | Min | Max | *N* |
|---|---|---|---|---|---|---|---|---|
| Body length | 34.0 | 33.0 | 4.8 | 1.0 | 14.2 | 28.0 | 40.0 | 22 |
| Body width | 11.0 | 10.0 | 2.1 | 0.5 | 19.4 | 8.0 | 15.0 | 22 |
| Length/width body portion | 3.1 | 3.0 | 0.5 | 0.1 | 16.1 | 2.5 | 4.1 | 22 |
| Adoral zone of membranelles length | 11.7 | 12.0 | 1.0 | 0.2 | 8.8 | 10.0 | 14.0 | 22 |
| Adoral membranelles, number | 14.2 | 14 | 1.0 | 0.2 | 7.1 | 13 | 16 | 22 |
| Frontal cirri, number | 3.0 | 3 | 0.0 | 0.0 | 0.0 | 3 | 3 | 22 |
| Buccal cirri, number | 1.0 | 1 | 0.0 | 0.0 | 0.0 | 1 | 1 | 22 |
| Frontoterminal cirri, number | 2.0 | 2 | 0.0 | 0.0 | 0.0 | 2 | 2 | 22 |
| Midventral cirri, number | 7.8 | 8 | 1.4 | 0.3 | 18.6 | 5 | 10 | 22 |
| Pretransverse ventral cirri, number | 2.0 | 2 | 0.0 | 0.0 | 0.0 | 2 | 2 | 22 |
| Transverse cirri, number | 5.6 | 6 | 0.5 | 0.1 | 8.7 | 5 | 6 | 22 |
| Left marginal cirri, number | 11.2 | 11 | 1.2 | 0.3 | 11.0 | 9 | 13 | 22 |
| Right marginal cirri, number | 11.2 | 11 | 1.3 | 0.3 | 11.6 | 9 | 14 | 22 |
| Dorsal kineties, number | 3.0 | 3 | 0.0 | 0.0 | 0.0 | 3 | 3 | 11 |
| Dikinetids on dorsal kinety 1, number | 4.5 | 4 | 0.7 | 0.2 | 15.4 | 4 | 6 | 11 |
| Dikinetids on dorsal kinety 2, number | 5.2 | 5 | 0.6 | 0.2 | 11.6 | 4 | 6 | 11 |
| Dikinetids on dorsal kinety 3, number | 6.6 | 7 | 0.7 | 0.2 | 10.2 | 5 | 7 | 11 |
| Macronuclear nodules length | 3.3 | 3.0 | 1.2 | 0.3 | 37.9 | 1.5 | 5.0 | 22 |
| Macronuclear nodules width | 1.9 | 1.5 | 1.2 | 0.2 | 60.0 | 1.0 | 4.5 | 22 |
| Macronuclear nodules, number | 23.0 | 22.5 | 2.4 | 0.5 | 10.6 | 20 | 27 | 22 |
| Micronucleus length | 1.6 | 1.5 | 0.5 | 0.1 | 28.6 | 1.0 | 2.5 | 22 |
| Micronucleus width | 1.4 | 1.0 | 0.5 | 0.1 | 34.3 | 1.0 | 2.5 | 22 |
| Micronucleus, number | 2.0 | 2 | 0.8 | 0.2 | 40.2 | 1 | 3 | 22 |

All data based on randomly selected, protargol-stained specimens. CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; *N*, number of specimens examined; SD, standard deviation; SE, standard error of the arithmetic mean.
[a] All data are based on protargol-stained specimens, measurements in μm.

*A. petzi* (0.024–0.027, 38–43 differences in 1,600 nt), and *A. warreni* (0.026, 42 differences in 1,600 nt) (Table 3).

## DISCUSSION

**Comparison with congeners:** In the genus *Arcuseries*, to date, three species, namely *A. scutellum*, *A. petzi,* and *A. warreni*, have been described (Huang *et al.* 2014). In addition, *Holosticha* sp., described by Wilbert and Song (2005), has similar morphological features to *Arcuseries* species in terms of body shape, size, nuclear apparatus, and ciliary structure; thus, in this section, we compare the new species to these four populations (Table 2).

*Arcuseries petzi* was recorded in Chinese and Korean coastal waters, and both populations are well described. However, because there is no significant difference between the two populations (Kim *et al.* 2013, Shao *et al*. 2011), the Chinese population (original) was compared with the new species. The Chinese population of *A. petzi* differs from *A. minima* sp. nov. in the following features: body length *in vivo* (85–105 μm vs. 35–55 μm); number of macronuclear nodules (55–115 vs. 20–27); number of adoral membranelles (20–30 vs. 13–16); numbers of midventral (20–32 vs. 5–10), left marginal (18–32 vs. 9–13), right marginal (24–42 vs. 9–14), and transverse cirri (8–11 vs. five or six); dorsal bristles in kinety 1 (12, counted from illustration vs. four to six). These two species have three types of cortical granules; of these, the medium-sized granules differ

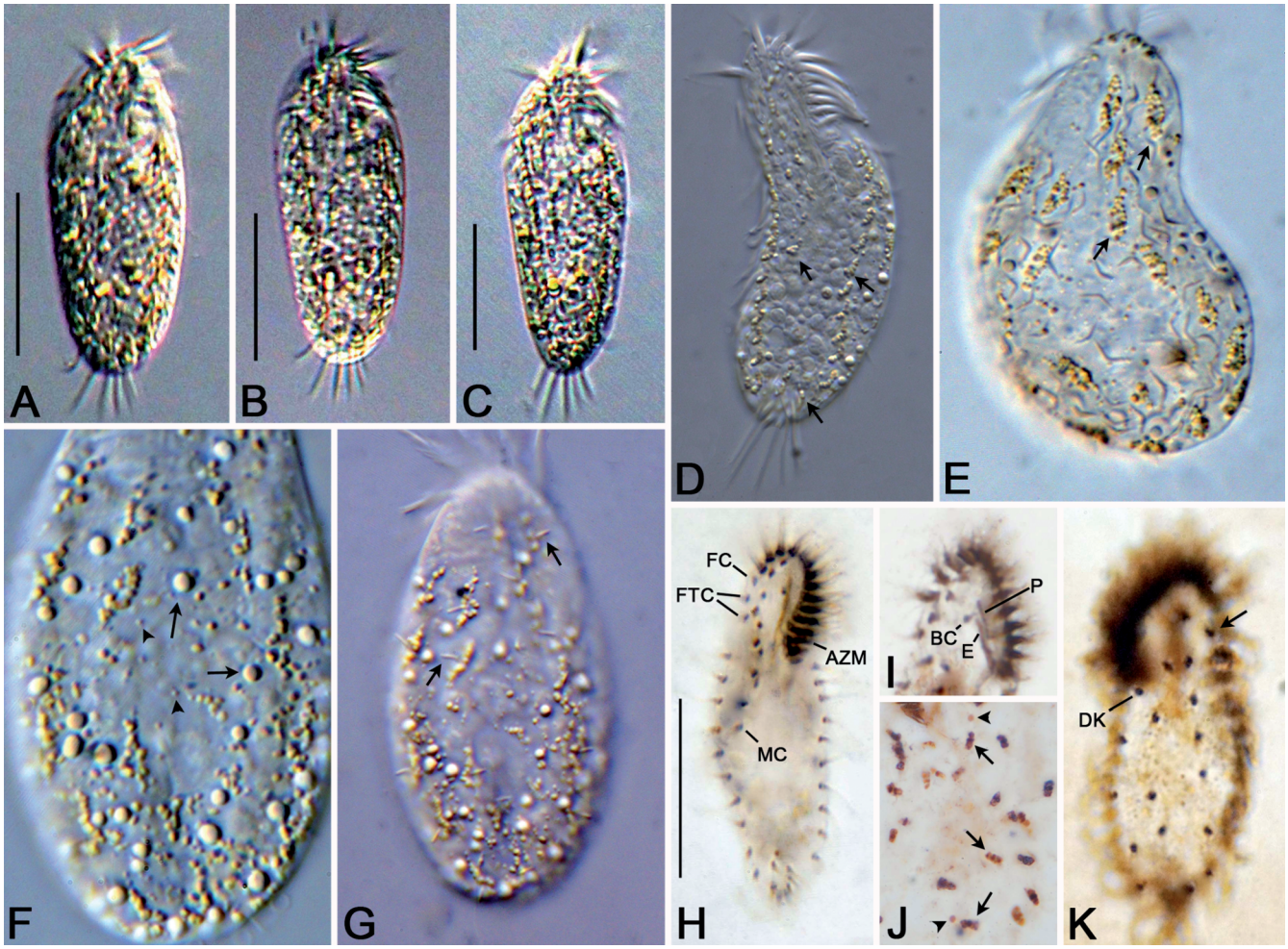


**Fig. 2.** *Arcuseries minima* sp. nov. *in vivo*. (A–G) and after protargol impregnation (H–K). (A–C) Ventral views showing body outline. (D) Cortical granulation in ventral surface, arrows indicate medium-sized cortical granules. (E) Cortical granulation in dorsal surface, arrows mark medium-sized cortical granules. (F) Arrows mark large cortical granules, arrowheads indicate small cortical granules. (G) Arrows indicate dorsal cilia. (H) Ventral view of the holotype specimen. (I) Ventral view of buccal field. (J) Arrows indicate macronuclear nodules, arrowheads mark micronuclei. (K) Dorsal view of a specimen, arrow indicates basal body pairs. AZM = adoral zone of membranelles; BC = buccal cirrus; DK = dorsal kineties; E = endoral; FC = frontal cirri; FTC = frontoterminal cirri; MC = midventral cirri; P = paroral. Scale bars: 20 μm.

in color between species (dark in *A. petzi* vs. yellowish in *A. minima*) (Shao *et al*. 2011).

The Chinese population of *Arcuseries warreni* differs from *A. minima* as follows: body length *in vivo* (80–120 μm vs. 35–55 μm); number of macronuclear nodules (ca. 50 vs. 20–27); number of adoral membranelles (26–31 vs. 13–16); numbers of midventral (20–32 vs. 5–10), left marginal (22–27 vs. 9–13), right marginal (21–26 vs. 9–14), and transverse cirri (10–12 vs. five or six); dorsal bristles in kinety 1 (19, counted from illustration vs. four to six); erythrocyte-shaped granules and extrusomes (present vs. absent) (Song and Wilbert 1997). The Korean population of *A. warreni* can be distinguished from *A. minima* by the following features: body length *in vivo* (70–100 μm vs. 35–55 μm); number of macronuclear nodules (43–60 vs. 20–27); number of adoral membranelles (28–30 vs. 13–16); numbers of midventral pair (seven to nine vs. two to five); left marginal (20–25 vs. 9–13), right marginal (20–27 vs. 9–14), and transverse cirri (10–12 vs. five or six); dorsal bristles in kinety 1 (14, counted from illustration vs. four to six). In addition, color of

**Table 2.** Morphological comparison of *Arcuseries minima* sp. nov. with four similar species.

| Characteristic[a] | *A. scutellum* | *A. petzi* | *A. warreni* | *A. warreni* | *Holosticha* sp. | *A. minima* |
|---|---|---|---|---|---|---|
| Body length in vivo | 50–80 μm | 85–105 μm | 80–120 μm | 70–100 μm | – | 35–55 μm |
| Body length | ca. 55 μm | ca. 102 μm | ca. 82.6 μm | ca. 75 μm | ca. 65 μm | ca. 34 μm |
| Cortical granules in vivo | Colorless, scattered, ca. 1 μm | Type I: colorless, mitochondria-like, ca. 3 μm; Type II: dark, clustered around cirri and dorsal bristles, ca. 1 μm; Type III: colorless, scattered, ca. 0.5 μm | Colorless, erythrocytes in shape, arranged dorsally in three irregular rows, ca. 2 μm | Type I: Colorless, erythrocytes in shape, arranged dorsally in three irregular rows, ca. 2 μm; Type II: colorless, clustered around cirri and dorsal bristles, ca. 1 μm | Colorless, ellipsoidal to spherical shape, ca. 3 μm | Type I: colorless, spherical shape, ca. 3 μm; Type II: yellowish, clustered around cirri and dorsal bristles, ca. 1 μm; Type III: colorless, scattered, ca. 0.5 μm |
| Macronuclear nodules, number | ca. 62 | ca. 80 | ca. 50 | ca. 52 | ca. 30 | ca. 23 |
| Adoral membranelles, number | ca. 18 (17 or 18) | ca. 25 (20–30) | ca. 28 (26–31) | ca. 29 (28–30) | ca. 17 | ca. 14 (13–16) |
| Midventral pairs, number | 6 or 7 | 10–16 | 7–9 | 7–9 | 3 or 4 | 2–5 |
| Left marginal cirri, number | ca. 14 (11–17) | ca. 25 (18–32) | ca. 23 (22–27) | ca. 22 (20–25) | ca. 14 | ca. 11 (9–13) |
| Right marginal cirri, number | ca. 15 (10–18) | ca. 30 (24–42) | ca. 23 (21–26) | ca. 23 (20–27) | ca. 17 | ca. 11 (9–14) |
| Transverse cirri, number | 8 | 8–11 | 10–12 | 10–12 | 6 or 7 | 5 or 6 |
| Dorsal kineties, number | 3 | 3 | 3 | 3 | 4 | 3 |
| Dikinetids on dorsal kinety 1, number[b] | 8 | 12 | 19 | 14 | 6 | 4–6 |
| Reference | Chen *et al.* (2010) | Shao *et al.* (2011) | Song and Wilbert (1997) | Kim and Min (2018) | Wilbert and Song (2005) | This study |

[a] All data based on protargol-stained specimens.
[b] Counted from illustrations.

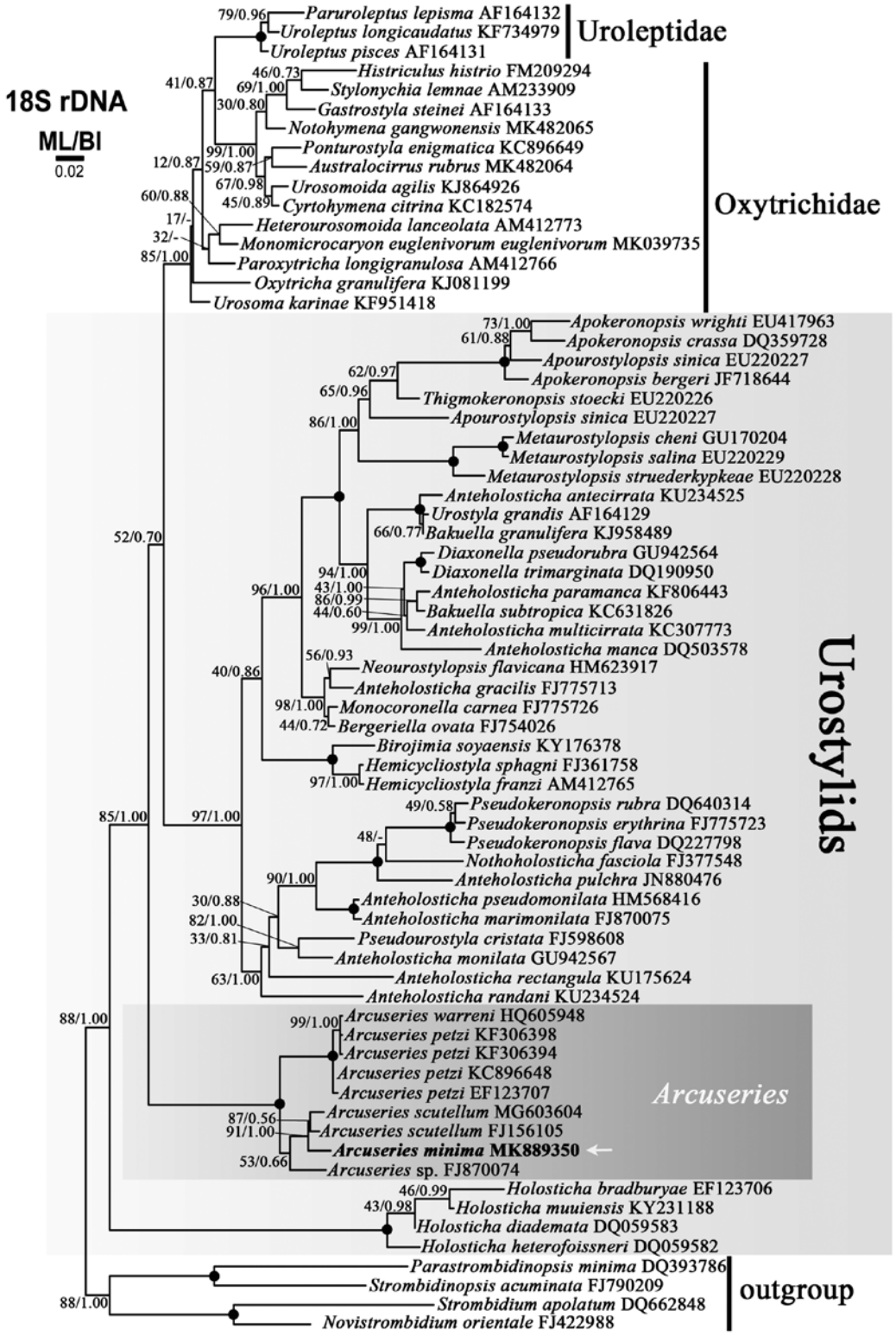


**Fig. 3.** Maximum likelihood and Bayesian inference analyses based on 18S rDNA sequences. The new sequence provided in the present work is indicated in bold and by a white arrow. Numbers at nodes indicate the bootstrap values of ML out of 1,000 replicates and the posterior probability of BI. Fully supported (100/1.00) branches are marked with solid circles. The scale bar corresponds to 2 substitutions per 100 nucleotide positions.

**Table 3.** Absolute distance (above the diagonal) and pairwise distance (below the diagonal) among the *Arcuseries* species.

| 18S rDNA sequences | *A. minima* | FJ156105 | MG603604 | FJ870074 | KC896648 | EF123707 | KF306398 | HQ605948 |
|---|---|---|---|---|---|---|---|---|
| *A. minima* This study | | 14 | 15 | 29 | 38 | 40 | 43 | 42 |
| *A. scutellum* FJ156105 | 0.009 | | 10 | 25 | 33 | 35 | 38 | 37 |
| *A. scutellum* MG603604 | 0.009 | 0.006 | | 23 | 37 | 39 | 42 | 41 |
| *A.* sp. FJ870074 | 0.018 | 0.016 | 0.014 | | 37 | 39 | 42 | 41 |
| *A. petzi* KC896648 | 0.024 | 0.021 | 0.023 | 0.023 | | 2 | 5 | 4 |
| *A. petzi* EF123707 | 0.025 | 0.022 | 0.024 | 0.024 | 0.001 | | 7 | 6 |
| *A. petzi* KF306398 | 0.027 | 0.024 | 0.026 | 0.026 | 0.003 | 0.004 | | 3 |
| *A. warreni* HQ605948 | 0.026 | 0.023 | 0.026 | 0.026 | 0.003 | 0.004 | 0.002 | |

cortical granules (medium-sized) were different (colorless in *A. warreni* vs. yellowish in *A. minima*) (Kim and Min 2018).

Unlike the two species described above, *Arcuseries scutellum* has a small body size (50–80 μm long *in vivo*) and is similar to *A. minima*. But *A. scutellum* differs from *A. minima* in number of macronuclear nodules (41–90 vs. 20–27); number of adoral membranelles (17 or 18 vs. 13–16); number of midventral pairs (six or seven vs. two to five); number of transverse cirri (eight vs. five or six); dorsal bristles in kinety 1 (eight, counted from illustration vs. four to six); distribution of medium-sized cortical granules (irregularly scattered vs. clustered around cirri and dorsal bristles) (Chen *et al*. 2010).

*Holosticha* sp., described by Wilbert and Song (2005), has only been studied based on stained specimens, and morphometric data were calculated based on average values. Thus, the available data is limited. *Holosticha* sp. differs from *Arcuseries minima* in the following features: body length in stained specimens (ca. 65 μm vs. ca. 34 μm, 28–40 μm); number of macronuclear nodules (ca. 30 vs. ca. 23, 20–27); number of adoral membranelles (ca. 17 vs. ca. 14, 13–16); number of left marginal (ca. 14 vs. ca. 11, 9–13), and right marginal (ca. 17 vs. ca. 11, 9–14) cirri; number of dorsal kineties (four vs. three). When comparing the average values, the values of the new species are lower than those of *Holosticha* sp. (Table 2). However, the range of values seems likely to overlap, except those for body length and number of dorsal kineties. In addition, the granules of *Holosticha* sp. are similar to the large-sized cortical granules of the new species. Thus, *Holosticha* sp. is considered to have a close relationship with *Arcuseries*, and molecular data from *Holosticha* sp. is needed to clarify this assumption.

**Phylogenetic analyses:** In molecular analyses based on 18S rDNA sequences, the genus *Arcuseries* is monophyletic (Huang *et al*. 2014, Zhao *et al*. 2015). *Arcuseries* is morphologically very close to *Anteholosticha* but differs in terms of the arrangement of transverse cirri (U-shaped vs. J-shaped), number of dorsal kineties (invariably three vs. more than three), and molecular differences (the *Arcuseries*-clade is clearly separated from *Anteholosticha* clades) (Huang *et al*. 2014). Our tree also clearly showed *Arcuseries* as being monophyletic as in previous studies (Fig. 3). The new species was included in the *Arcuseries* clade (Fig. 3) and has transverse cirri arranged in U-shape and three dorsal kineties. In the gene tree, the new species clustered with

*A. scutellum* (Fig. 3), and the difference in 18S rDNA sequences between these two species was the smallest of all the *Arcuseries* species (Table 3). As mentioned in the comparison subsection above, *A. minima* and *A. scutellum* displayed smaller body sizes when compared with *A. petzi* and *A. warreni*, and had fewer cirri. The gene tree also reflects these morphological differences by dividing these groups into two branches (Fig. 3).

**Acknowledgements.** This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201902204).

## REFERENCES

Berger H. (2006) Monograph of the Urostyloidea (Ciliophora, Hypotricha). *Monogr. Biol.* **85:** 1–1303, i–xv

Chen X. R., Gao S., Song W. B., Al-Rasheid K. A. S., Warren A., Gong J., et al. (2010) *Parabirojimia multinucleata* spec. nov and *Anteholosticha scutellum* (Cohn, 1866) Berger, 2003, marine ciliates (Ciliophora, Hypotrichida) from tropical waters in southern China, with notes on their small-subunit rRNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **60:** 234–243

Darriba D., Taboada G. L., Doallo R., Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9:** 772

Foissner W. (2014) An update of 'basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa'. *Int. J. Syst. Evol. Microbiol.* **64:** 271–292

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

Hall T. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41:** 95–98

Huang J., Chen Z. G., Song W. B., Berger H. (2014) Three-gene based phylogeny of the Urostyloidea (Protista, Ciliophora, Hypotricha), with notes on classification of some core taxa. *Mol. Phylogenet. Evol.* **70:** 337–347

Kim K. S., Jung J. H., Min G. S. (2013) New record of two marine ciliates (Ciliophora: Spirotrichea) in South Korea. *Anim. Syst. Evol. Divers.* **29:** 144–151

Kim K. S., Min G. S. (2018) Brief morphological description of stichotrichid ciliates (Ciliophora: Stichotrichia) from Korea. *J. Species Res.* **7:** 323–332

Medlin L., Elwood H. J., Stickel S., Sogin M. L. (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71:** 491–499

Ronquist F., Teslenko M., Mark P. v. d., Ayres D. L., Darling A., Höhna S., et al. (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61:** 539–542

Shao C., Gao F., Hu X., Al-Rasheid K. A., Warren A. (2011) Ontogenesis and molecular phylogeny of a new marine urostylid ciliate, *Anteholosticha petzi* n. sp. (Ciliophora, Urostylida). *J. Eukaryot. Microbiol.* **58:** 254–265

Song W. B., Wilbert N. (1997) Morphological studies on some free living ciliates (Ciliophora: Heterotrichida, Hypotrichida) from marine biotopes in Qingdao, China, with descriptions of three new species: *Holosticha warreni* nov. spec., *Tachysoma ovata* nov. spec. and *T. dragescoi* nov. spec. *Europ. J. Protistol.* **33:** 48–62

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28:** 2731–2739

Wilbert N., Song W. (2005) New contributions to the marine benthic ciliates from the Antarctic area, including description of seven new species (Protozoa, Ciliophora). *J. Nat. Hist.* **39:** 935–973

Zhao X., Gao S., Fan Y., Strueder-Kypke M., Huang J. (2015) Phylogenetic framework of the systematically confused *Anteholosticha-Holosticha* complex (Ciliophora, Hypotrichia) based on multigene analysis. *Mol. Phylogenet. Evol.* **91:** 238–247

Received on 17th April, 2019; revised on 17th May, 2019; accepted on 5th July, 2019