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Morphology and Molecular Phylogeny of *Pseudocyrtohymenides lacunae* nov. gen., nov. spec. (Ciliophora: Oxytrichidae) from South Korea

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Abstract. We collected an 18-cirri oxytrichid ciliate from the brackish lagoon Songjiho, South Korea, in March 2012. Based on analyses of morphological and molecular attributes, we conclude that it is new genus and species. *Pseudocyrtohymenides lacunae* nov. gen., nov. spec. has similar morphological attributes to the genus *Pseudocyrtohymena*, however, the former species lacks caudal cirri. The sequence similarity of the nuclear small subunit ribosomal RNA (SSU rRNA) gene was 99.4% (10 nt difference) between *Pseudocyrtohymenides lacunae* and *Pseudocyrtohymena koreana* (type species).

Key words: New genus, new species, Korea, SSU rRNA gene, protargol impregnation, Sporadotrichida

INTRODUCTION

Oxytrichids with 18 frontal-ventral-transverse (FVT) cirri are a diverse group of ciliates (Berger 1999). The type genus *Oxytricha* Bory de Saint-Vincent in Lamouroux *et al.*, 1824 has the following morphological diagnosis: usually 18 FVT cirri; one left and one right marginal cirral row; dorsal kinety 3 fragmented (*Oxytricha* pattern) or non-fragmented (*Urosomoida* pattern); caudal cirri present; and undulating membranes in *Oxytricha* pattern (Berger 1999). Although they have limited variation in traits such as 18 FVT cirri, new taxa are constantly being discovered, even at the genus level (Kumar *et al.* 2015, Foissner 2016, Jung *et al.* 2016a). In the Oxytrichidae Ehrenberg, 1838, it is generally found that combinations of morphological attributes (see above) define new genera, rather than single characteristics (e.g., *Aponotohymena* Foissner, 2016, *Architricha* Gupta *et al.*, 2006, *Monomicrocaryon* Foissner, 2016, *Pseudocyrtohymena* Jung *et al.*, 2015, *Quadristicha* Foissner, 2016).

Of the recently established genera in Oxytrichidae, our new species has similar morphological attributes to the genus *Pseudocyrtohymena* that consists of the sole species *P. koreana* (monotypy) and mainly differs from the genus *Oxytricha* by its undulating membranes (*Cyrtohymena* pattern vs. *Oxytricha* pattern) (Jung *et*

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al. 2015). *Pseudocyrtohymena* has a non-fragmented dorsal kinety 3 (*Urosomoida* pattern) and reduced caudal cirri (Jung *et al.* 2015).

During an investigation of ciliate diversity in Korea, we collected a *Pseudocyrtohymena*-like sp. from a brackish lagoon and identified it based on live observation, protargol impregnation, and the small subunit (SSU) rRNA gene. From our results, we conclude it is a new genus and species. Here, we report the morphological description and phylogenetic relationships of this new species.

MATERIALS AND METHODS

Sample collection and identification

We collected surface waters from the brackish lagoon Songjiho (38°20'09"N, 128°30'57"E), South Korea, in March 2012. The surface waters (5.3 psu, 9.9°C) were obtained from eastern side, near inflow of saline water, on the lagoon. Clonal cultures were maintained in Petri dishes and also in 50-mL tissue culture flasks (Greiner Bio-One, Frickenhausen, Germany) at room temperature (ca. 18°C). Rice grains were added to the cultures to enrich bacteria as a food resource for protozoa (flagellates) including ciliates. Living specimens were observed under a light microscope (Leica DM2500, Wetzlar, Germany) at magnifications ranging from 50 × to 1000 ×. Protargol impregnation was performed to observe infraciliatures and nuclear apparatus (Foissner 1991).

Terminology and classification are according to Berger (1999), Lynn (2008), and Jung *et al.* (2015).

PCR amplification and sequencing

Specimens were washed several times with distilled water in order to isolate a single cell. Genomic DNA was extracted using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA) according to the manufacturer's protocol. A modified New EukA (5'-CTG GTT GAT YCT GCC AGT-3') forward primer (Jung *et al.* 2012) and LSU rev3 (Sonnenberg *et al.* 2007) reverse primer were used for PCR amplification of the nearly complete nuclear SSU rRNA gene. The optimized PCR conditions were as follows: denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 4 min, and a final extension step at 72°C for 7 min. The PCR products were purified using a QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany). The following two internal primers were used for sequencing: 18S+810 and 18S–300 (Jung *et al.* 2011). DNA sequencing was performed using an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

Nucleotide sequences were assembled using Geneious v6.1.6 (Biomatters Ltd, Auckland). Pairwise sequence similarity was calculated using MEGA 5.2 (Tamura *et al.* 2011). For analyses of phylogenetic relationships, we retrieved the sequences of 42 hypotrichs from GenBank. The sequences were aligned using Clustal X 1.81 (Jeanmougin *et al.* 1998) implemented in BioEdit (Hall 1999). We evaluated phylogenetic relationships by using maximum likelihood (ML) and Bayesian inference (BI). The maximum likelihood analysis was conducted using PhyML 3.1 (Guindon *et al.* 2010). Confidence in the analyses was assessed using the bootstrap procedure, with 1000 replications for ML. BI assessment was performed using MrBayes 3.2.6 (Ronquist *et al.* 2012), simulating a Markov chain Monte Carlo (MCMC) for 1,000,000 generations, 300,000 of which were discarded as the burn-in. Furthermore, to determine the appropriate DNA substitution model for ML and BI analyses, we used the Akaike information criterion to identify the best-fit model according to the jModelTest 2.1.10 (Darriba *et al.* 2012). The model selected was GTR + I (0.7180) + G (0.4770). The bootstrap values and posterior probablities, above 70% and 0.95, respectively, were statistically interpreted as sufficient evidences, given the bootstrap values of ≥ 70% likely corresponds to ≥ 95% accuracy (Alfaro *et al.* 2003; Guindon *et al.* 2010).

RESULTS

Pseudocyrtohymenides nov. gen.

Diagnosis: Oxytrichidae with undulating membranes in *Australocirrus–Cyrtohymena* pattern; body flexible, colorless cytoplasm; cortical granules present; non-fragmented dorsal kinety 3 and one or more dorsomarginal kineties (*Urosomoida* pattern); caudal cirri lacking.

Type species: *Pseudocyrtohymenides lacunae* nov. spec.

Etymology: Composite of the generic name *Pseudocyrtohymena* and the Greek suffix *-ides* (similar), meaning a ciliate similar to *Pseudocyrtohymena*; masculine gender.

Pseudocyrtohymenides lacunae nov. spec.

Diagnosis: Size *in vivo* 120–165 μm × 25–50 μm; body flexible and slightly contractile, slender to ellipsoidal in shape, grayish to slightly yellowish under low magnification; two macronuclear nodules with approximately two micronuclei; contractile vacuole at left mid-body; cortical granules spherical, yellowish, approximately 1 μm in diameter, irregularly distributed on cortex; on average, 37 adoral membranelles, 18 FVT cirri, and 23 left and 27 right marginal cirri; undulating membranes in *Australocirrus–Cyrtohymena* pattern; four or five dorsal kineties composed of three dorsal and one or two dorsomarginal rows (*Urosomoida* pattern); dorsal kinety 3 non-fragmented; caudal cirri absent.

Type locality: Songjiho lagoon (38°20'09"N, 128°30'57"E), South Korea near the East Sea; salinity of 5.3 psu.

Type materials: The holotype (NIBRPR0000104 263) and three paratype (NIBRPR0000104264–NIBR

PR0000104266) slides with protargol-impregnated specimens were deposited in the National Institute of Biological Resources (NIBR), South Korea. The other three paratype slides (MABIK PR00042645–00042647) have been deposited in the National Marine Biodiversity Institute of Korea (MABIK), South Korea. The holotype and other relevant specimens were marked using circles on the bottoms of the slides.

Etymology: The species-group name "*lacunae*" is derived from the Latin noun *lacuna* (lagoon) in the singular genitive case, denoting where the specimens were discovered.

Description: Size *in vivo* 120–165 μm × 25–50 μm (~ 3.2:1, *n* = 5; Figs 1A; 2A–D), on average 105.1 μm × 36.6 μm in protargol preparations (Table 1; Figs 1C, D; 2F, G). Body flexible and slightly contractile; cell colour grayish to slightly yellowish under low magnification. Invariably two macronuclear nodules, elliptical to elongated oval, 14.0–19.5 μm × 4.5–8.5 μm (stained); one to four micronuclei, spherical, 1.5–3.0 μm × 1.0–2.5 μm (stained). Contractile vacuole on left side of mid-body, approximately 10 μm in diameter without conspicuous collecting canals (Figs 1A; 2A). Cortical granules irregularly distributed on cortex, yellowish, spherical, approximately 1 μm in diameter (Figs 1B; 2C, E). Cell inclusions with food vacuoles (bacteria, small protists) and granular inclusions. Crawling moderately fast on bottom of Petri dish.

Table 1. Morphometric data of *Pseudocyrtohymenides lacunae* nov. gen., nov. spec.

Characteristics (protargol-impregnated specimens)	Min	Max	Mean	M	SD	CV	*n*
Body, length	92	122	105.1	103.6	8.6	8.2	21
Body, width	26	52	36.6	35.3	6.4	17.4	21
Adoral zone of membranelles, length	35	46	39.7	39.8	2.7	6.9	21
Percentage (%) of body length occupied by adoral zone	33.3	41.0	37.8	37.9	2.3	6.1	21
Longest adoral membranelles, length	4.5	6.0	5.3	5.2	0.4	8.4	21
Adoral membranelles, number	31	44	37.3	37	3.3	8.8	21
Frontal cirri, number	3	3	3	3	0	0	21
Frontoventral cirri, number	4	4	4	4	0	0	21
Buccal cirrus, number	1	1	1	1	0	0	21
Ventral cirri, number	5	9	5.4	5	1.0	19.0	21
Transverse cirri, number	3	6	5	5	0.6	11.7	21
Posterior end of cell to rearmost transverse cirrus, distance	9	15	12.6	12.8	1.6	12.5	21
Left marginal cirri, number	19	27	22.6	22	2.3	10.1	21
Right marginal cirri, number	23	33	27.1	27	2.8	10.3	21
Dorsal kineties, number	4	5	5	5	0.2	4.4	21
Bristles in dorsal kinety 1, number	13	22	17.6	17	2.7	15.2	21
Bristles in dorsal kinety 2, number	17	25	21.5	21	2.2	10.2	21
Bristles in dorsal kinety 3, number	17	26	21.6	22	2.4	11.2	21
Bristles in dorsomarginal row 1, number	12	19	16.2	17	2.1	12.8	21
Bristles in dorsomarginal row 2, number	1	3	1.5	1	0.6	41.7	20
Total number of bristles	62	92	78.3	79	7.1	9.1	21
Macronucleus nodules, number	2	2	2	2	0	0	21
Macronucleus nodules, length[a]	14.0	19.5	16	15.4	1.6	9.7	21
Macronucleus nodules, width[a]	4.5	8.5	6.8	6.8	1.1	15.7	21
Micronuclei, number	1	4	1.7	1	0.9	52.6	21
Micronuclei, length[a]	1.5	3.0	2.2	2.3	0.4	17.4	21
Micronuclei, with[a]	1.0	2.5	1.8	1.9	0.3	16.3	21

All measurements in μm.

[a] A macro- or micronucleus was randomly chosen in each cell.

CV, coefficient of variation (%); M, median; Max, maximum; Min, minimum; *n*, number of specimens investigated; SD, standard deviation.

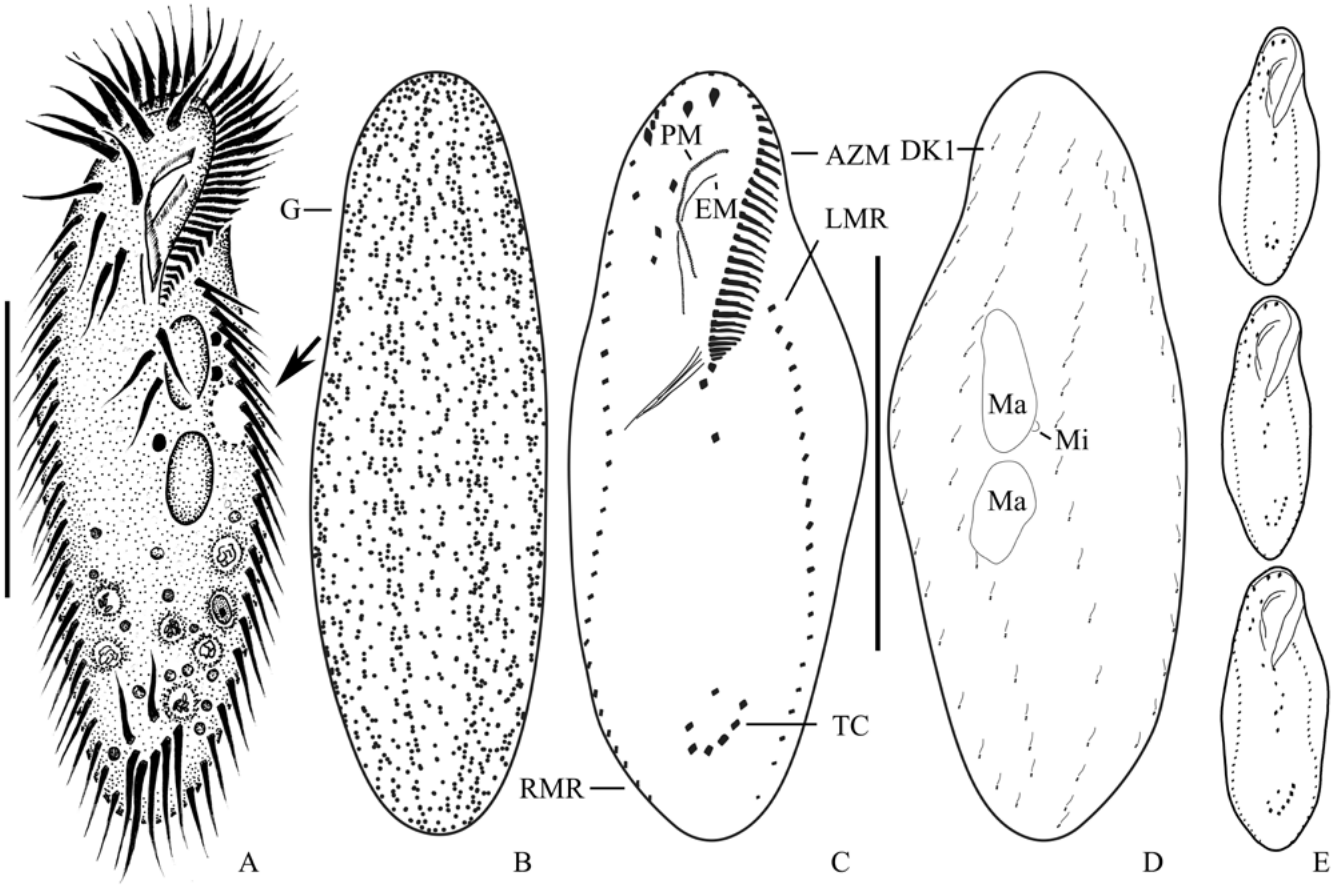

Fig. 1. *Pseudocyrtohymenides lacunae* nov. gen., nov. spec. (A, B), living specimens and (C–E), after protargol impregnation. (A, B), ventral (A) and dorsal (B) views of representative specimens; arrow denotes contractile vacuole. (C, D), ventral (C) and dorsal (D) views of the holotype specimen. (E), ventral views showing the variation of frontal-ventral-transverse cirri. AZM, adoral zone of membranelles; DK1, dorsal kinety 1; EM, endoral membrane; G, cortical granules; LMR, left marginal cirral row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; RMR, right marginal cirral row; TC, transverse cirri. Scale bars: 50 μm.

All cirri relatively fine, mostly 12–21 μm long *in vivo*; frontal and transverse cirri 18–21 μm long; other cirri 12 μm long (Figs 1A; 2D, G). Arrangement of FVT cirri as in other flexible 18-cirri hypotrichs; usually three frontal, four frontoventral, one buccal, five ventral, and five transverse cirri. A few cells show variation in the number of cirri, ranging from 16 to 23; frontal, frontoventral, buccal cirri invariably eight; ventral and transverse cirri 5–9 and 3–6 cirri, respectively; transverse cirri obliquely arranged, slightly J-shaped. One left and one right marginal row, composed of 19–27 cirri and 23–33 cirri, respectively; both posterior ends never connected, terminate at similar level. Usually five dorsal kineties, that is constantly three bipolar kineties and usually two (20 of 21 specimens analysed) dorsomarginal kineties; dorsal kinety 4 posteriorly slightly shortened (12–19 bristles); kinety 5 conspicuously posteriorly shortened, if present, composed of 1–3 bristles (Figs 1D; 2F); dorsal kinety 3 non-fragmented; dorsal cilia approximately 2.5–3.0 μm long (Fig. 2E). Caudal cirri absent.

Adoral zone of membranelles approximately 38% of body length in impregnated specimens; base of the largest membranelles approximately 5 μm long; cilia of membranelles approximately 13 μm long. Paroral and endoral membrane in *Australocirrus–Cyrtohymena* pattern, namely, anterior part of paroral membrane distinctly curved in leftward direction and recurved slightly distally; undulating membranes intersect in mid-buccal cavity (Figs 1C, E; 2H).

Phylogenetic analyses: The SSU rDNA sequence of *Pseudocyrtohymenides lacunae* was 1,576 bp in

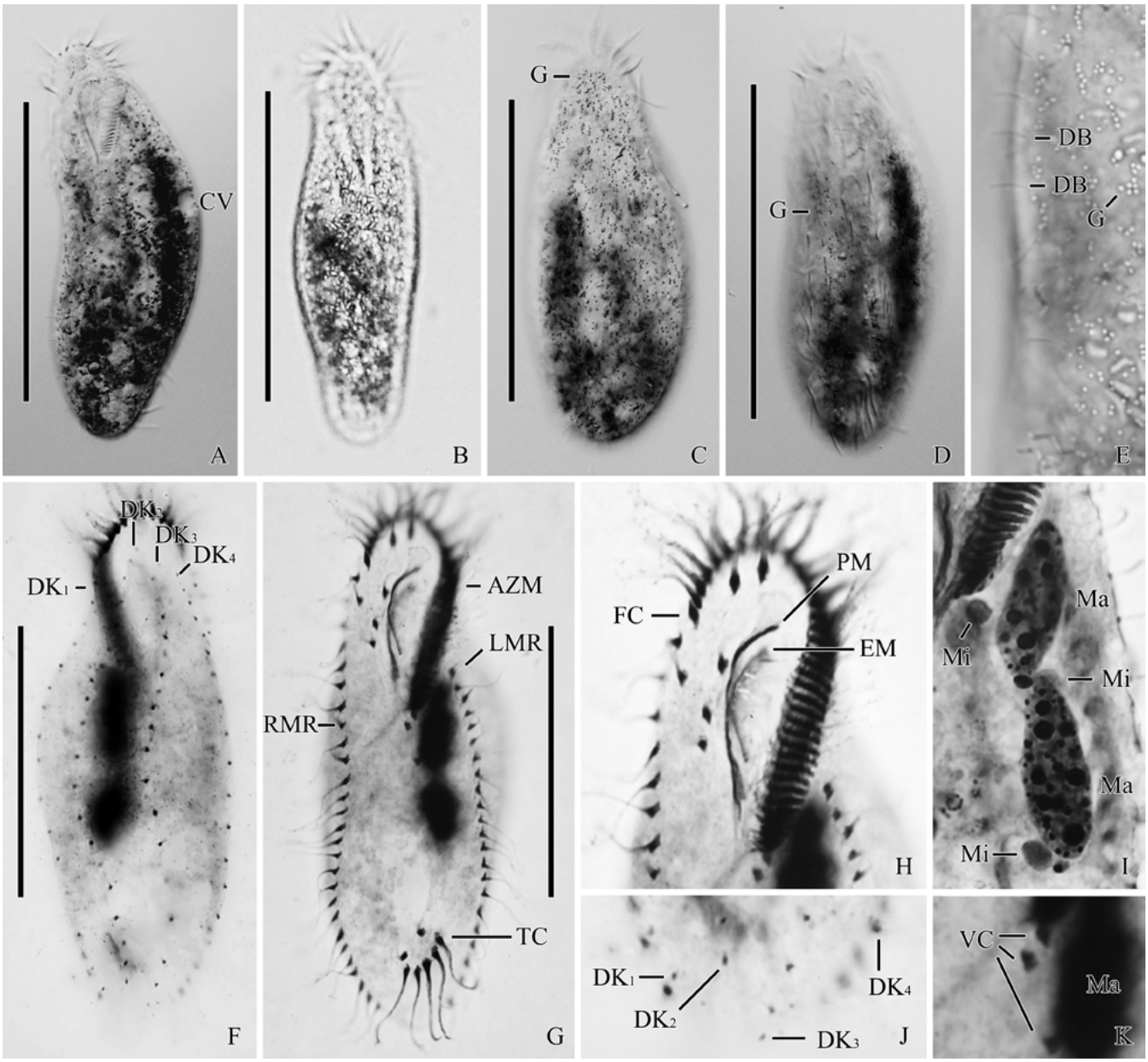

Fig. 2. *Pseudocyrtohymenides lacunae* nov. gen., nov. spec. (A–E), living specimens; (F–K), after protargol impregnation. (A, D), ventral views of representative specimens. (B, C, E), dorsal views showing cortical granules and dorsal bristles. (F–K), dorsal (F, J) and ventral (G–I, K) views showing infraciliature and nuclear apparatus. AZM, adoral zone of membranelles; CV, contractile vacuole; DB, dorsal bristle; DK_{1-4}, dorsal kineties 1–4; EM, endoral membrane; FC, frontal cirrus; G, cortical granules; LMR, left marginal cirral row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; RMR, right marginal cirral row; TC, transverse cirri; VC, ventral cirri. Scale bars: 100 μm in A–D and 50 μm in F–G.

length (GenBank accession number: MF319121). The sequence similarity between *Pseudocyrtohymena koreana* and *Pseudocyrtohymenides lacunae* was 99.4% (10 nt difference). In the phylogenetic tree (Fig. 3), although *Pseudocyrtohymenides* nov. gen. clustered with the genus *Pseudocyrtohymena*, the supporting values were not significant (posterior probability of 0.52, bootstrap value of < 50%). The clade *Pseudocyrtohymena–Pseudocyrtohymenides* showed a sister relationship with *Pseudogastrostyla flava–Rubrioxytricha haematoplasma–R. ferruginea–Ponturostyla enigmatica* (posterior probability of 0.85, bootstrap value of < 50%).

In addition, the clade *Pseudocyrtohymena–Pseudocyrtohymenides* did not cluster with *Cyrtohymena* species.

DISCUSSION

Morphological comparison to related species

The genus *Pseudocyrtohymena* Jung *et al.*, 2015 was established as monotypic (type species: *P. koreana*). The type species conspicuously differs from *Pseudocyrtohymenides lacunae* nov. spec. by caudal cirri (1–3 vs. 0; Jung *et al.* 2015). The type species has the caudal cirri only at the end of dorsal kinety 3, while the dorsal kineties 1 and 2 do not form the cirri at the ends during the morphogenesis (Jung *et al.* 2015). As reported by Jung *et al.* (2015), *Pseudocyrtohymena* does not produce the fragmentation of dorsal kinety anlage 3 (*Urosomoida* pattern), that is, dorsal kinety 3 could be considered as kinety 4 in the typical oxytrichids, which splits into two kineties (single fragmentation) (Berger 1999). In terms of the undulating membranes, the two species have similar type (*Australocirrus–Cyrtohymena* pattern). Their paroral membranes are slightly distally recurved that place them intermediate position between *Australocirrus* and *Cyrtohymena* pattern (Kumar and Foissner 2015).

Of the members in Oxytrichidae, *Rubrioxytricha indica* Naqvi *et al.* 2006, as previously compared by Jung *et al.* (2015), shares similar morphological characteristics to *Pseudocyrtohymena* and *Pseudocyrtohymenides* (see Table 2; Naqvi *et al.* 2006). However, *R. indica* can be separated from *P. lacunae* by body size (on average of stained cells, 69 × 27 μm vs. 105 × 37 μm), contractile vacuole (3 vs. 1), cortical granules (dark greenish vs. yellowish), adoral membranelles (27–31 vs. 31–44), and caudal cirri (1 vs. 0) (Naqvi *et al.* 2006). The colourless cytoplasm and the reduced caudal cirrus of *R. indica* suggests this species could be considered as a congener to *Pseudocyrtohymena*. However, until the genetic material of *R. indica* is obtained, we maintain its current classification.

Molecular phylogeny of *Pseudocyrtohymenides lacunae*

In our phylogenetic tree (Fig. 3), *P. lacunae* clustered with the species *P. koreana.* However, the supporting values on the clade *Pseudocyrtohymena–Pseudocyrtohymenides* were not significant enough to convince us that these two species are congeners. Unfortunately, the low values of oxytrichids on the nodes for these analyses have been easily found before in oxytrichids (e.g., Chen *et al.* 2015, Fan *et al.* 2015, Jung *et al.* 2016a). Although the urostylids, one of several large groups in the hypotrichs, have been analysed using the same gene, their supporting values are higher than the oxytrichids (e.g., Jo *et al.* 2015, Jung *et al.* 2016b, Pan *et al.* 2016). To improve the resolution of phylogenetic relationships in the oxytrichids, more sampling of other oxytrichids or expanding the genes targeted for the analyses is required (for instance, see Zhao *et al.* 2012, Gao *et al.* 2014).

The clade *Pseudocyrtohymena–Pseudocyrtohymenides* clustered with the group *Pseudogastrostyla flava–Rubrioxytricha haematoplasma–R. ferruginea–*

Table 2. Comparison of morphological features of *Pseudocyrtohymenides lacunae* nov. spec. to closely related species.

Characteristics[a]	*Pseudocyrtohymenides lacunae* nov. spec.	*Pseudocyrtohymena koreana* (type species)	*Rubrioxytricha indica*
Body, size	105 × 37	112 × 47	69 × 27
Cytoplasm, colour	colourless	colourless	colourless
Cortical granules, colour	yellowish	yellowish	dark greenish
Contractile vacuole, number	1	1	3
Adoral membranelles, number	31–44	38–53	27–31
Caudal cirri, number (in each dorsal kinety 1–3)	0 (0, 0, 0)	1–3 (0, 0, 1–3)	1 (0, 0, 1)
Dorsal kineties, number[b]	4 or 5 (usually 4)	5	5
Fragmentation of dorsal kinety 3	absent	absent	absent
Data source	this study	Jung *et al.* (2015)	Naqvi *et al.* (2006)

All measurements in μm.
[a]Data based on protargol-impregnated specimens.
[b]Including dorsomarginal rows.

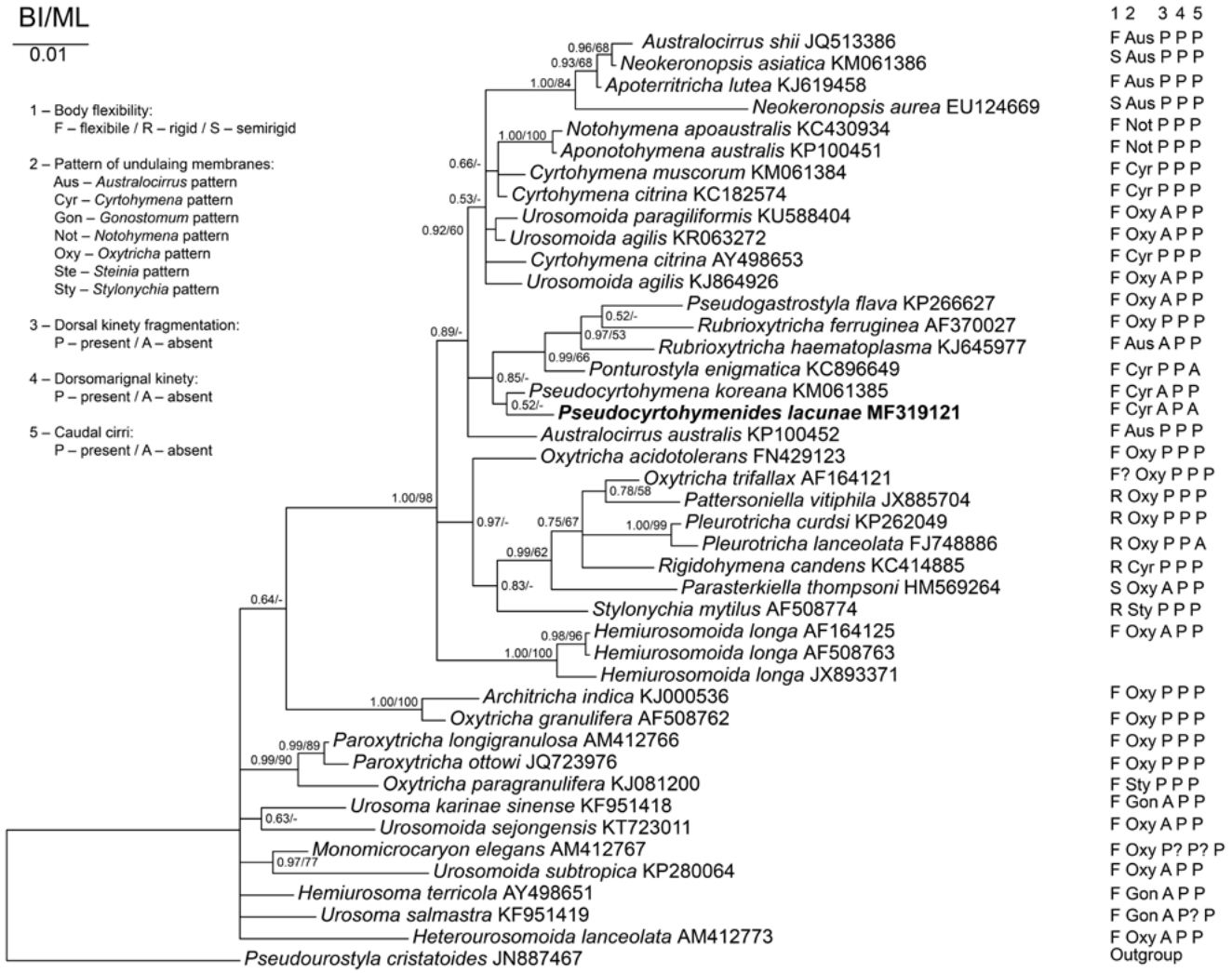

Fig. 3. Phylogenetic tree of SSU rRNA gene sequences, showing the position of *Pseudocyrtohymenides lacunae* nov. spec. on the basis of Maximum Likelihood (ML) and Bayesian Inference (BI). Bootstrap values of ML and posterior probabilities of BI were denoted on each interior branch. If the values of the bootstrap and the posterior probability were less than 50% and 0.50, respectively, they were excluded. The scale bar represents one nucleotide substitution per 100 nt.

Ponturostyla enigmatica (Fig. 3). These groups all have the reduced number of caudal cirri in common; *Pseudocyrtohymena* has 1–3 caudal cirri and *Pseudocyrtohymenides* lacks the caudal cirri (1 in *P. flava*; 0 or 1 in *R. haematoplasma*; 1 or 2 in *R. ferruginea*; 0 in *P. enigmatica*) (Berger 1999, Song 2001, Fan *et al*. 2015). Based on the phylogenetic tree (Fig. 3; see the terms on the upper left), morphological attributes such as '2–undulation membranes' or '3–dorsal kinety fragmentation' did not exclusively separate these genera. This was expected, as with other oxytrichids, the genus *Pseudocyrtohymenides* is defined as a combination of characteristics not individual ones.

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REFERENCES

Alfaro M. E., Zoller S., Lutzoni F. (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Mol. Biol. Evol.* **20:** 255–266

Berger H. (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). *Monogr. Biol.* **78:** i–xii, 1–1080

Chen W., Chen X., Li L., Warren A., Lin X. (2015) Morphology, morphogenesis and molecular phylogeny of an oxytrichid ciliate, *Rubrioxytricha haematoplasma* (Blatterer & Foissner, 1990) Berger, 1999 (Ciliophora, Hypotricha). *Int. J. Syst. Evol. Microbiol.* **65:** 309–320

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

Fan Y., Zhao X., Hu X., Miao M., Warren A., Song W. (2015) Taxonomy and molecular phylogeny of two novel ciliates, with establishment of a new genus, *Pseudogastrostyla* n. g. (Ciliophora, Hypotrichia, Oxytrichidae). *Eur. J. Protistol.* **51:** 374–385

Foissner W. (1991) Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *Eur. J. Protistol.* **27:** 313–330

Foissner W. (2016) Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos. *Denisia* **35:** 1–912

Gao F., Gao S., Wang P., Katz L. A., Song W. (2014) Phylogenetic analyses of cyclidiids (Protista, Ciliophora, Scuticociliatia) based on multiple genes suggest their close relationship with thigmotrichids. *Mol. Phylogenet. Evol.* **75:** 219–226

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

Jeanmougin F., Thompson J. D., Gouy M., Higgins D. G., Gibson T. J. (1998) Multiple sequence alignment with Clustal X. *Trends Biochem. Sci.* **23:** 403–405

Jo E., Jung J.-H., Min G.-S. (2015) Morphology and molecular phylogeny of two new brackish water ciliates of *Bakuella* (Ciliophora: Urostylida: Bakuellidae) from South Korea. *J. Eukaryot. Microbiol.* **62:** 799–809

Jung J.-H., Park K.-M., Min G.-S. (2012) Morphology, morphogenesis, and molecular phylogeny of a new brackish water ciliate, *Pseudourostyla cristatoides* n. sp., from Songjiho lagoon on the coast of East Sea, South Korea. *Zootaxa* **3334:** 42–54

Jung J.-H., Park K.-M., Min G.-S. (2015) Morphology and molecular phylogeny of *Pseudocyrtohymena koreana* n. g., n. sp. and Antarctic *Neokeronopsis asiatica* Foissner et al., 2010 (Ciliophora, Sporadotrichida), with a brief discussion of the *Cyrtohymena* undulating membranes pattern. *J. Eukaryot. Microbiol.* **62:** 280–297

Jung J.-H., Park K.-M., Kim S. (2016b) Morphology and molecular phylogeny of the soil ciliate *Anteholosticha rectangula* nov. spec. from King George Island, maritime Antarctica. *Acta Protozool.* **55:** 89–99

Jung J.-H., Baek Y.-S., Kim S., Choi H.-G. (2016a) Morphology and molecular phylogeny of a new freshwater ciliate *Urosomoida sejongensis* n. sp. (Ciliophora, Sporadotrichida, Oxytrichidae) from King George Island, Antarctica. *Zootaxa* **4072:** 254–262

Jung J.-H., Baek Y.-S., Kim S., Choi H.-G., Min G.-S. (2011) A new marine ciliate, *Metaurostylopsis antarctica* nov. spec. (Ciliophora, Urostylida) from the Antarctic Ocean. *Acta Protozool.* **50:** 289–300

Kumar S., Foissner W. (2015) Biogeographic specializations of two large hypotrich ciliates: *Australocirrus shii* and *A. australis* and proposed synonymy of *Australocirrus* and *Cyrtohymenides*. *Eur. J. Protistol.* **51:** 210–228

Kumar S., Kamra K., Bharti D., La Terza A., Sehgal N., Warren A., Sapra G. R. (2015) Morphology, morphogenesis, and molecular phylogeny of *Sterkiella tetracirrata* n. sp. (Ciliophora, Oxytrichidae), from the Silent Valley National Park, India. *Eur. J. Protistol.* **51:** 86–97

Lynn D. H. (2008) The ciliated protozoa: Characterization, classification, and guide to the literature. Springer, New York

Naqvi I., Gupta R., Borgohain P., Sapra G. R. (2006) Morphology and morphogenesis of *Rubrioxytricha indica* n. sp. (Ciliophora : Hypotrichida). *Acta Protozool.* **45:** 53–64

Pan X., Fan Y., Gao F., Qiu Z., Al-Farraj S. A., Warren A., Shao C. (2016) Morphology and systematics of two freshwater urostylid ciliates, with description of a new species (Protista, Ciliophora, Hypotrichia). *Eur. J. Protistol.* **52:** 73–84

Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A., Huelsenbeck J. P. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61:** 539–542

Song W. (2001) Morphology and morphogenesis of the marine ciliate *Ponturostyla enigmatica* (Dragesco & Dragesco-Kernéis, 1986) Jankowski, 1989 (Ciliophora, Hypotrichida, Oxytrichidae). *Eur. J. Protistol.* **37:** 181–197

Sonnenberg R., Nolte A. W., Tautz D. (2007) An evaluation of LSU rDNA D1–D2 sequences for their use in species identification. *Front. Zool.* **4:** 6

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

Zhao Y., Gao F., Li J., Yi Z., Warren A. (2012) Phylogenetic analyses on the tintinnid ciliates (Protozoa, Ciliophora) based on multigene sequence data. *Acta Protozool.* **51:** 319–328

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