The Journal of
Eukaryotic Microbiology

Published by the International Society of Protistologists

J. Eukaryot. Microbiol., 57(6), 2010 pp. 460–467

DOI: 10.1111/j.1550-7408.2010.00500.x

Redescription of *Favella ehrenbergii* (Claparède and Lachmann, 1858) Jörgensen, 1924 (Ciliophora: Choreotrichia), with Phylogenetic Analyses Based on Small Subunit rRNA Gene Sequences

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ABSTRACT. The identification of *Favella ehrenbergii*, a marine planktonic ciliate, has largely been based on its lorica features. This approach is potentially problematic given the polymorphic lorica during this organism's life cycle. We isolated a population of *F. ehrenbergii* from the coastal waters of Incheon, Korea, and revealed its infraciliature using the protargol staining method. Phylogenetic analysis based on small subunit rRNA gene sequences was also performed. Results showed that this population possessed 16 collar membranelles (CM) and about 100 somatic kineties. These features are highly conserved, even in later dividers. As such, the number of CM and somatic kineties can be used as key characteristics for identification of *Favella* species.

Key Words. Choreotrich, marine ciliate, SSU rRNA, taxonomy, tintinnids.

FAVELLA species have been often studied in temperate coastal waters because of their high abundance, growth rates, and predation of toxic dinoflagellates (Hansen 1989, 1995; Kamiyama and Arima 1997, 2001; Pierce and Turner 1992, 1993; Stoecker, Guillard, and Kavee 1981). Identifications of this group, and other lorica-bearing tintinnid ciliates, have traditionally been based on their lorica features, which is problematic given the variation of the lorica (Laval-Peuto 1981). More recently, it has been reported that the highly conserved somatic ciliary pattern rather than the variable lorica shape should serve as a key character for identification of tintinnids (Agatha and Riedel-Lorjé 2006; Agatha and Tsai 2008; Choi et al. 1992). The small subunit (SSU) rRNA gene is increasingly being sequenced, which will enable the identification of such taxa down to genus or perhaps even species level (Agatha and Strüder-Kypke 2007; Gao et al. 2009; Strüder-Kypke and Lynn 2008).

Although *Favella ehrenbergii* (Claparède and Lachmann, 1858) Jörgensen, 1924, has been morphologically redescribed in Laval-Peuto (1981), its infraciliature has yet to be revealed. In this study, we redescribe this species based on combined data of infraciliature and SSU rRNA gene sequences. Synonyms of this taxon are also discussed.

MATERIALS AND METHODS

Sample collection and culture. A population sample of *F. ehrenbergii* was collected with a 20-μm plankton net from Incheon coastal waters, Korea (126°35′40″E, 37°27′00″N), during the summer of 2006. Water temperature and salinity at the time of collection were 20 °C and 31 psu, respectively. Primary morphological observation of this species was conducted at 60X under a dissecting microscope. One cell was picked with a micropipette and transferred to the culture plate. The culture was maintained for 3 months with the dinoflagellate *Heterocapsa triquetra* as prey at 20 °C, salinity 31 psu, and 12:12 h light:dark cycle.

Species identification. Cultured cells were picked up randomly from wells and fixed in Bouin's solution at a final concentration of 6% (v/v) for morphological examination of their lorica using a Sedgwick–Rafter chamber. Over 50 individuals were used for protargol impregnation (Wilbert 1975). Observations and drawings of stained specimens were performed at 1,600X with a camera lucida. We followed the terminology proposed by Agatha and Riedel-Lorjé (2006). Cell movement was studied in a Petri dish under a dissecting microscope.

Deposition of slides. Two protargol-impregnated voucher slides are deposited in the Natural History Museum, London (numbered 2010:7:5:1) and the Laboratory of Marine Plankton, Inha University, Korea (numbered 20061001), respectively.

DNA extraction, gene amplification, and sequencing. Fewer than 10 cells were picked out from the clonal culture established above to provide a sample for DNA preparation. DNA extraction, amplification of the SSU rRNA gene, cloning, and sequencing were performed following Gong et al. (2007).

Phylogenetic analysis. Two protargol-impregnated voucher were retrieved from the NCBI database for phylogenetic tree construction. One karyorelictean species and two heterotrichean species were used as out-group taxa. Sequences were aligned using CLUSTAL X 1.81 (Jeanmougin et al. 1998). The program MrModeltest v.2 (Nylander 2004) selected the GTR+I+G as the best model using AIC criterion, and this was then used for both Bayesian and maximum likelihood (ML) inference. The Bayesian tree was constructed from an output of 10,000 trees generated by MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with 1,000,000 cycles for the Markov Chain Monte Carlo algorithm and sampling every 100th generation. Stationary likelihood scores were determined by plotting the $-\ln L$ against the generation. The first 1,500 trees below the observed stationary level were discarded as burn-in. A ML tree was constructed with the PhyML V2.4.4 program (Guindon and Gascuel 2003). The reliability of internal branches was assessed using the non-parametric bootstrap method with 1,000 pseudoreplicates. TreeView v1.6.6 (Page 1996) and MEGA4.0 (Tamura et al. 2007) were used to visualize tree topology.

RESULTS

Favella ehrenbergii (Claparède and Lachmann, 1858) Jörgensen, 1924 (Table 1, Fig. 1–25).

Description of the Incheon population. The lorica is hyaline without any particles, and ranged from 60.0 to 330.0 μm in length and 80.0–93.8 μm in width (Table 1). Lorica shape varied among individuals and may be found in two forms. The favela form is mostly cylindrical bowl-like with an aboral constriction (10.0–43.8 × 8.0–25.0 μm) (Table 1, Fig. 1–6). In some specimens the aboral constriction became less distinct with the horn indistinct or even absent (Fig. 7–12). Lorica length is extended by

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Table **1.** Morphometric data of *Favella ehrenbergii* based on protargol-impregnated preparations.

Characteristics	Mean	SE	M	SD	Min	Max	*n*
Lorica, length[a]	176.6	7.8	178.0	53.0	60.0	330.0	46
Favella form[a]	209.2	8.7	210.0	41.8	93.8	330.0	23
Coxliella form[a]	144.0	8.8	150.0	42.3	60.0	231.3	23
Lorica oral diameter[a]	88.6	0.3	89.0	2.2	80.0	93.8	46
Favella form[a]	89.4	0.3	90.0	1.6	87.5	93.8	23
Coxliella form[a]	87.8	0.5	88.0	2.5	80.0	90.6	23
Aboral constriction, length	25.0	1.9	25.0	8.9	10.0	43.8	23
Aboral constriction, width	16.9	0.9	18.8	4.1	8.0	25.0	23
Cell length	83.4	6.6	80.6	21.0	50.0	115.0	10
Cell width	66.8	5.7	62.5	18.1	40.0	95.0	10
Ma, number	2.0	0	2.0	0	2.0	2.0	25
Ma, length	38.2	3.7	36.5	11.9	22.0	60.0	10
Ma, width	16.1	1.4	15.0	4.5	12.0	25.0	10
DK, number	2.0	—	2.0	—	2.0	2.0	12
DK1, number of kinetids	74.3	6.6	71.0	11.4	65.0	87.0	3
DK2, number of kinetids	86.3	11.8	75.0	20.5	74.0	110.0	3
Kineties in RF, number	56.4	1.8	55.0	5.7	48	65	10
Longest kinety in RF, number of kinetids	12.0	0.6	12	1.0	11	13	3
Shortest kinety in RF, number of kinetids	5.7	0.3	6	0.6	5	6	3
Kineties in LF, number	36.6	1.2	37.0	3.7	31	43	10
Longest kinety in LF, number of kinetids	13.7	0.3	140	0.6	13	14	3
Shortest kinety in LF, number of kinetids	4.7	0.3	5	0.6	4	5	3
Kineties in LA, number	11.7	0.9	11	2.8	8	17	10
Longest kinety in LA, number of kinetids	31.7	2.0	32	3.5	28	35	3
Shortest kinety in LA, number of kinetids	16.7	0.3	17	0.6	16	17	3
VK, number	1.0	—	1.0	—	1.0	1.0	13
CM, number	16.0	—	16.0	—	16.0	16.0	23
BM, number	1.0	—	1.0	—	1.0	1.0	23

[a]After Bouin's solution fixation.

Measurements in μm.

BM, buccal membranelles; CM, collar membranelles; LA, lateral ciliary field; LF, left ciliary field; Ma, macronuclear nodules; RF, right ciliary field; VK, ventral kinety; M, median; Max, maximum; Min, minimum; *n*, number of individuals investigated; SD, standard deviation; SE, standard error.

1 to about 11 spiral turns in the oral region (Fig. 1–2, 6–7). The second lorica form, the coxliella form, is distinguished by continuous spiral turns in the lorica (Fig. 8–12) and occasionally the posterior region was expanded (Fig. 12).

Cell movement occurs slowly by rotation about the main axis, twitching back on contact with obstacles. After fixation, the contractile stalk of the cell in vivo disappears, and the cell contracts, measuring about 50–115 × 40–95 μm in size (Table 1). There are two ellipsoidal macronuclear nodules, each about 22–60 × 12–25 μm (Fig. 16, 20, 21). The micronucleus was not observed. The adoral zone of membranelles is composed of 16 collar membranelles (CM) and one buccal membranelle (BM) (Fig. 13, 14, 19). A long endoral membrane extends under the CM in the buccal cavity (Fig. 14, 18).

The somatic ciliary pattern includes a lateral ciliary field pattern (Agatha and Strüder-Kypke 2007) without a reduction of the second dorsal kinety (Fig. 13). The two dorsal kineties (Fig. 13, 25) are composed of dikinetids. The ventral kinety is composed of monokinetids, and curves along the margin of the oral primordium in dividers (Fig. 13, 15, 17, 22–23). There are 48–65 kineties in the right ciliary field, 31–43 kineties in the left ciliary field, and 8–17 kineties in the lateral ciliary field (Table 1, Fig. 13). The kineties of right and left fields are mostly composed of monokinetids and one anterior dikinetid (Fig. 13–15, 24). The lateral kineties are composed of monokinetids and the anterior to eighth kinetids are dense (Fig. 13, 15, 17, 22–23).

Sequences and phylogenetic analyses. The SSU rRNA gene sequence of *F. ehrenbergii* is 1,757 bp long (including primer regions) and has a GC content of 46.96%. The sequences were deposited in GenBank under accession numbers GU574767 to GU574770. The sequence similarities of our genes from four different cloning products were 99.60–99.94% (data not shown).

The Bayesian and ML trees showed that our four sequences of *F. ehrenbergii* are clustered with those of *Favella panamensis* and *Favella campanula* (Fig. 26). This placement is strongly supported by all phylogenetic methods (>99%, Fig. 26). Three species are clustered together in a separate clade within the Tintinnida. However, our sequences are not clustered with previous data of *F. ehrenbergii* (accession no. AF399164) and *Favella taraikensis* (accession no. FJ196073), which are placed within *Metacylis* clade. Also, our sequences of *F. ehrenbergii* showed similarities of 99.72–100.0% when compared with that of *F. panamensis* (accession no. AY143572).

DISCUSSION

Favella ehrenbergii was originally described under the name *Tintinnus ehrenbergii* by Claparède and Lachmann (1858) based on information regarding its size (about 190 μm) and the cylindrical shape of its lorica (Fig. 27, 28). Later, Jörgensen (1924) transferred it to the genus *Favella*, but recorded a less cylindrical lorica compared with Claparède and Lachmann (1858) (Fig. 29, 30). Kofoid and Campbell (1929) used *F. ehrenbergii* as type species for this genus and also illustrated a less cylindrical lorica compared with the original description of Claparède and Lachmann (1858) (Fig. 31).

Laval-Peuto (1981) has carefully studied the lorica morphology of *F. ehrenbergii* and its construction, and revealed polymorphism of the lorica during the life cycle of cultured ciliates. She transferred the *Coxliella* species to *Favella*, after determining that this

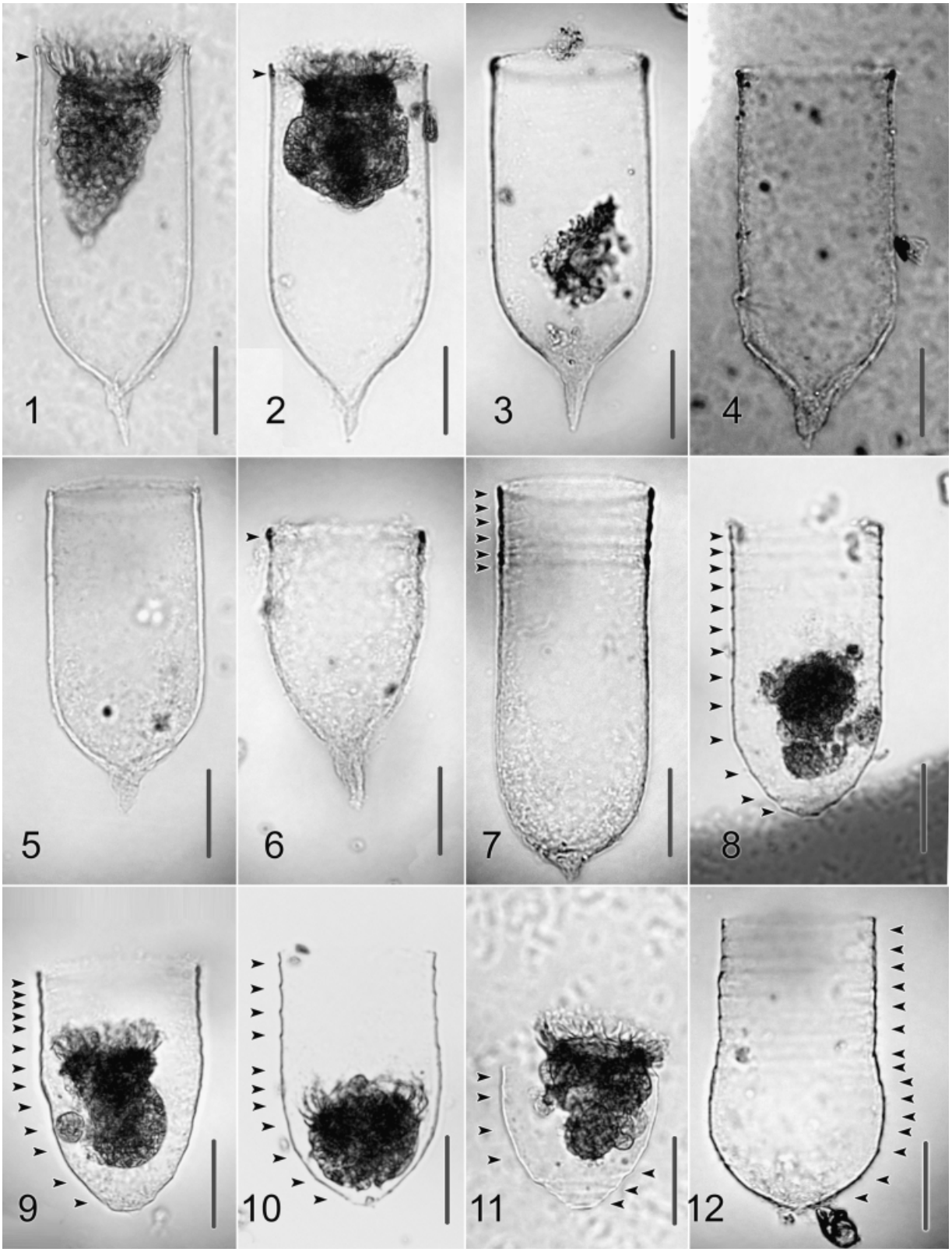

Fig. **1–12.** Lorica morphology of laboratory-cultured *Favella ehrenbergii* after Bouin's fixation, showing variation in shape and size. **1–6.** Showing a typical loricae with a distinct aboral constriction and a pedicel. **7–12.** Loricae in which the aboral constriction and pedicel are less distinct or even absent. The *Coxliella*-form is illustrated in 8–12. Arrowheads mark spiral turns. Scale bars 50 μm.

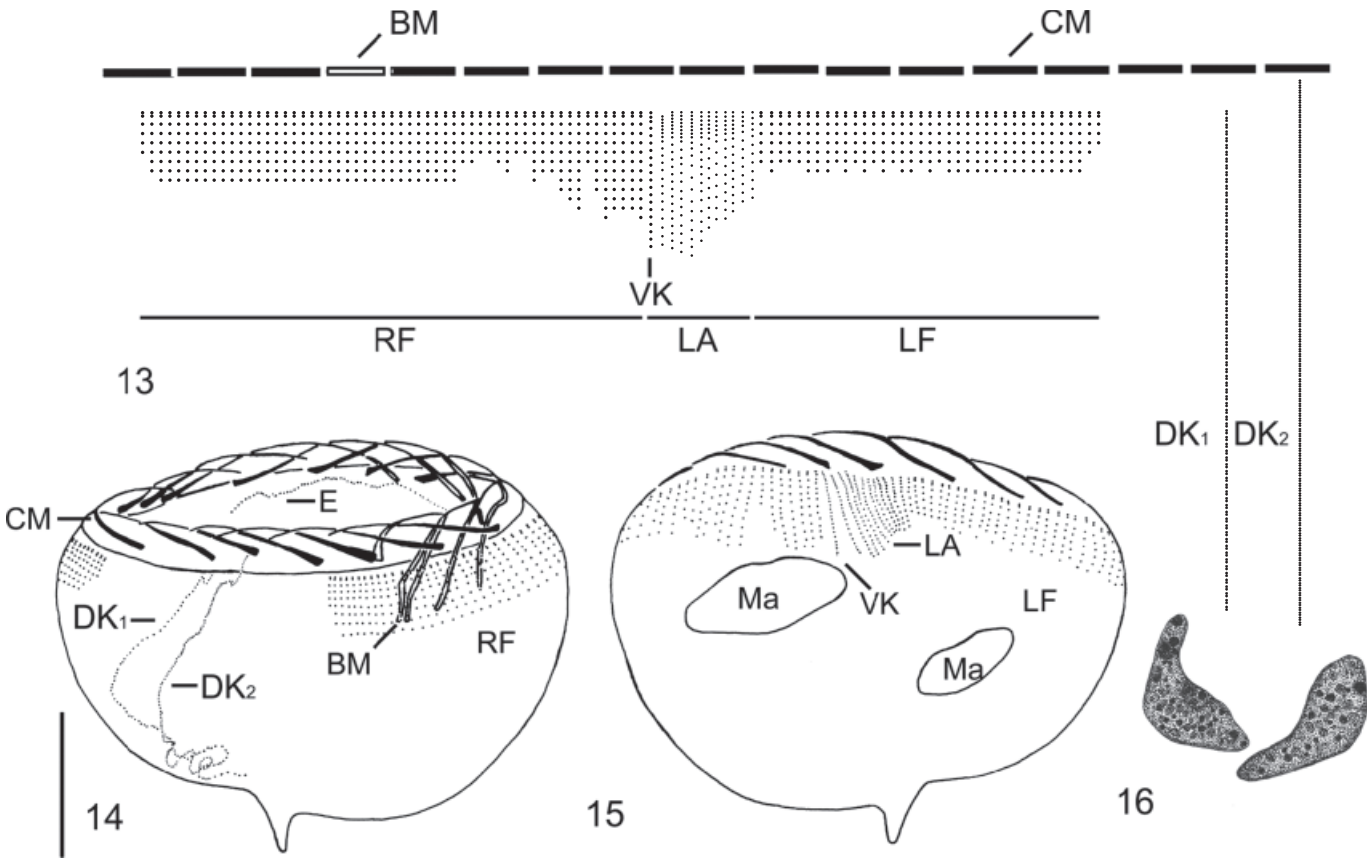

Fig. **13–16.** Schematic figures of *Favella ehrenbergii* after protargol impregnation. **13**. Kinetal map; **14–15**. Dorsal (14) and ventral (15) views of the infraciliature; **16.** Macronuclei. BM, buccal membranelles; CM, collar membranelles; DK1, dorsal kinety 1; DK2, dorsal kinety 2; E, endoral membrane; LA, lateral ciliary field; LF, left ciliary field; Ma, macronuclear nodules; RF, right ciliary field; VK, ventral kinety. Scale bars: 50 μm.

lorica form is a polymorph of *F. ehrenbergii* (Laval-Peuto, 1981). However, detailed observations of the polymorph from other *Favella* species have never been published. Therefore, it is difficult to identify all the species that should be included in this genus.

The situation is further complicated by published SSU rRNA gene sequences attributed to three *Favella* species—*F. ehrenbergii*, *F. panamensis*, and *F. taraikaensis*. Strüder-Kypke and Lynn (2003) reported the SSU rRNA sequence of *F. panamensis* Kofoid and Campbell, 1929 from Florida, USA. Interestingly, our sequences of *F. ehrenbergii* are almost identical (>99.72%) to this sequence of *F. panamensis*. This ''inter-species'' similarity fits well with the intra-species similarity (99.60–99.94%) of *F. ehrenbergii* determined in this study. Furthermore, both species cluster robustly together in our phylogeny. *Favella panamensis* was first reported by Kofoid and Campbell (1929) with a more cylindrical bowl and fuller aboral region to differentiate it from *F. ehrenbergii*. However, the description of *F. panamensis* by Kofoid and Campbell (1929) was much more similar to the original illustrations of *F. ehrenbergii* (Fig. 32) even the lorica length of *F. panamensis* was 136–232 μm, which overlaps with described isolates of *F. ehrenbergii* (Table 2). Considering the many characteristics (i.e. lorica morphology, lorica size, and genetic similarity) shared between *F. ehrenbergii* and *F. panamensis*, it is likely that the latter is a junior synonym of the former.

In the phylogenetic tree, our SSU rRNA sequences are not clustered with *F. ehrenbergii* isolated from Long Island Sound, USA and submitted by Snoeyenbos-West et al. (2002) and this SSU rRNA sequence is identical to that from *F. taraikaensis* Hada, 1932 isolated near Qingdao, China and submitted by Li et al. (2009). *Favella taraikaensis* can be easily confused with *F. ehrenbergii*: they have similar lorica shapes and sizes (Table 2, Fig. 33), but the former is distinguished by its expanding lorica just below the oral rim.

Hedin (1975), using electronic microscopy, recorded 26 adoral zone membranelles for *F. ehrenbergii* (vs. 17 in our population), although the lorica exhibited a significant suboral bulge (vs. none in Claparède and Lachmann 1858; Laval-Peuto 1981; present data) (Fig. 34). Such an expanding anterior part of the lorica was also observed in *F. ehrenbergii* by Snoeyenbos-West et al. (2002) and in *F. taraikaensis* by Li et al. (2009). If this suboral bulge is indeed a taxonomically stable feature of the lorica, then the *F. ehrenbergii* from Long Island Sound ought to be identified as *F. taraikaensis*. Because these sequences of ''*F. taraikaensis*'' are placed within the *Metacylis* clade, further study of their infraciliature is needed to confirm this taxonomic position.

The loricae of the Incheon specimens are shorter and narrower than those of the population of the Bay of Villefranche sur Mer (Laval-Peuto 1981) (Table 2). Laval-Peuto (1981) measured the lorica from environmental samples, and observed over 90% of the *Favella* form from environmental samples. It is possible to observe small-sized loricae in a culture because of the lorica building may not be as effective. The size of the lorica of our cultured *F. ehrenbergii* may have been influenced by culture conditions, such as temperature and the simpler diet using only one type of dinoflagellate. However, the *Coxliella* polymorph from

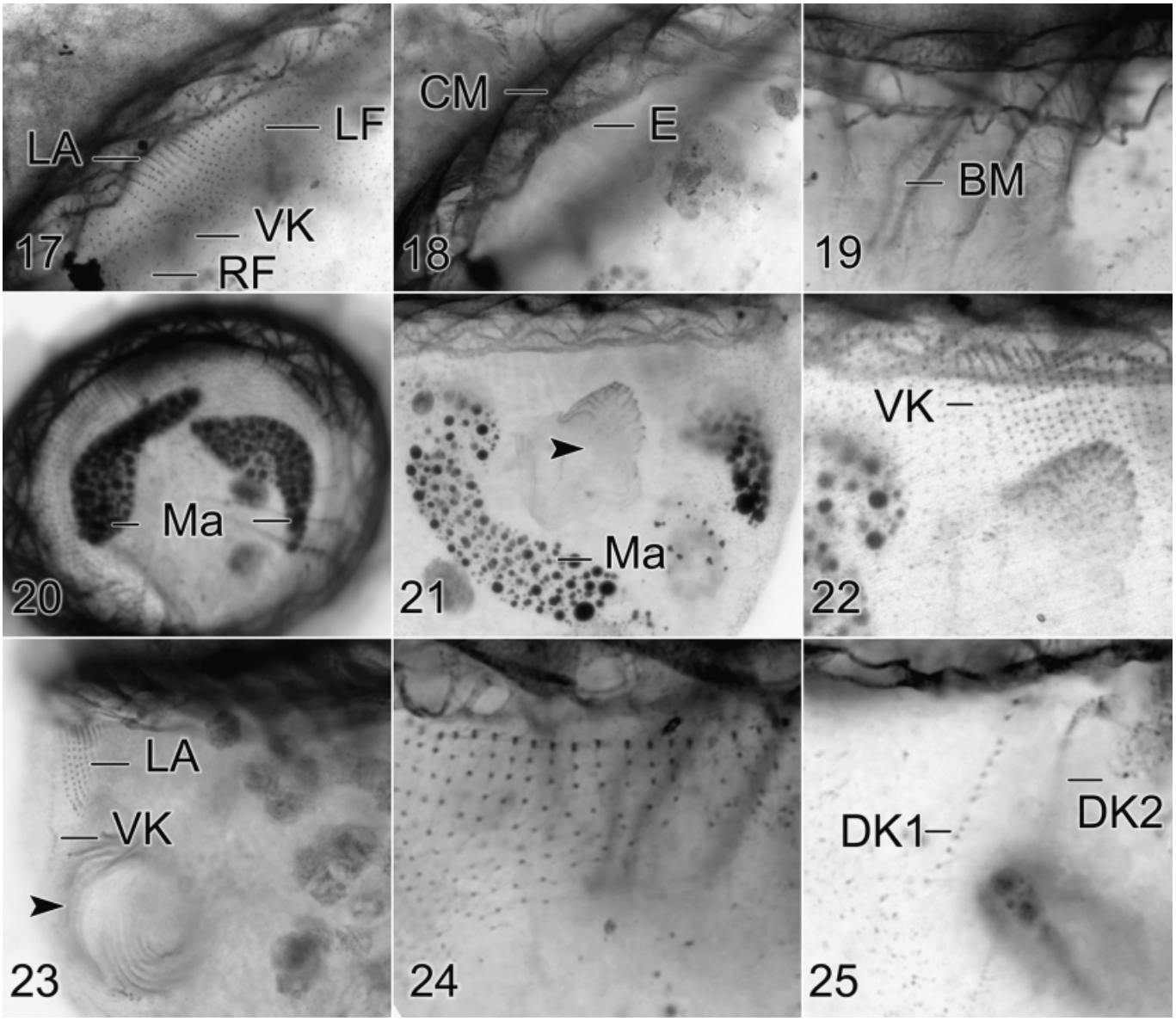

Fig. **17–25.** Photomicrographs of *Favella ehrenbergii* after protargol impregnation. **17–19.** Ventral view of infraciliature. **20.** Macronuclei. **21–23.** Oral primordium (arrowheads) and ventral kinety of divider. **24.** Right ciliary field. **25.** Dorsal kineties of divider. BM, buccal membranelles; CM, collar membranelles; DK, dorsal kinety; E, endoral membrane; LA, lateral ciliary field; LF, left ciliary field; Ma, macronuclear nodules; RF, right ciliary field; VK, ventral kinety.

our study fits well with the loricae from the cultural study by Laval-Peuto (1981). Furthermore, the *Favella* form of our specimens corresponds well with the original description of Claparède and Lachmann (1858); therefore, we believe that the identification of our isolate from Incheon as *F. ehrenbergii* is correct.

Improved diagnosis of *Favella ehrenbergii*. Lorica is hyaline and thick on average 60.0–431.6 μm long and 80.0–116.2 μm wide, mostly cylindrical with aboral constriction of 10.0–174.3 μm length or none. None to several spiral turns, as well as a coxliella form present. Occasionally, the posterior part of the lorica may be slightly expanded in the *Coxliella* form. Suboral bulge is absent. Cells measure between 50–115 × 40–95 μm in size after protargol impregnation. Two ellipsoidal macronuclear nodules are observed. Sixteen CM and one BM. Two dorsal kineties and one ventral kinety. There are 48–65 kineties in the right ciliary field, 31–43 kineties in the left ciliary field, and 8–17 kineties in the lateral ciliary field.

ACKNOWLEDGMENTS

We would like to sincerely thank Prof. Denis Lynn, Dr. Sabine Agatha, and Dr. Dapeng Xu for valuable comments on the manuscript. This work was supported by the Korea Research Foundation Grant (KRF-2008-013-c00064) and a grant from the Natural Science Foundation of China (no. 40976099). Dr. E.J. Yang was also supported by KOPRI (PN10020).

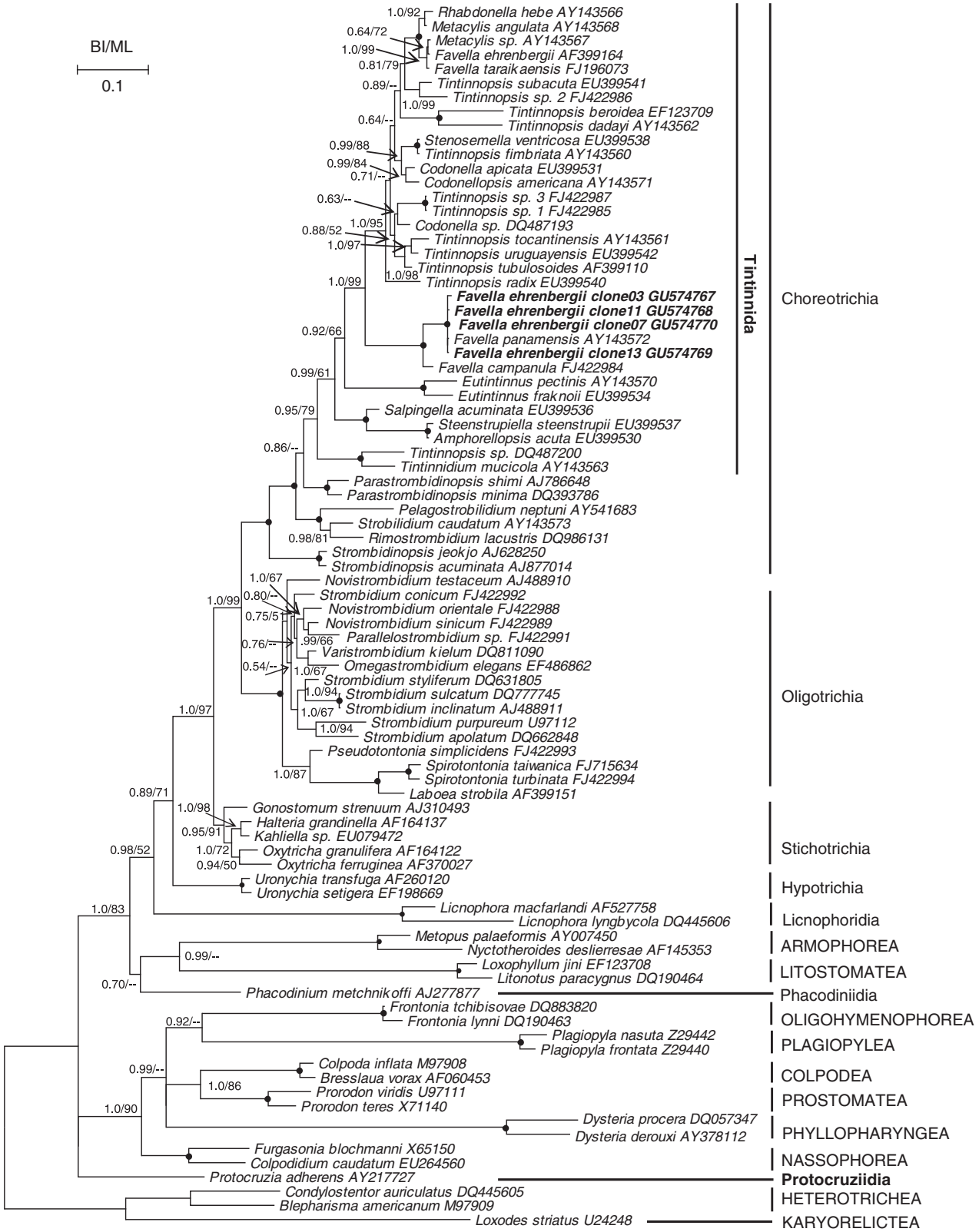

Fig. **26.** A Bayesian tree based on small subunit rRNA gene sequences showing the relationships between the Korean population of *Favella ehrenbergii* (bold) and other choreotrich ciliates. Numbers at the nodes represent support values in the following order: Bayesian posterior probabilities using the MrBayes algorithm (BI) and bootstrap values from maximum likelihood (ML) analyses (as % out of 1,000 replicates). Solid circles (•) denote nodes with full bootstrap support in all algorithms. A hyphen (-) represents support values $<50\%$ and disagreement between BI and ML at a given node. Note that *Favella* spp. are not monophyletic: some species cluster with *Metacylis* species quite distant from this Korean population.

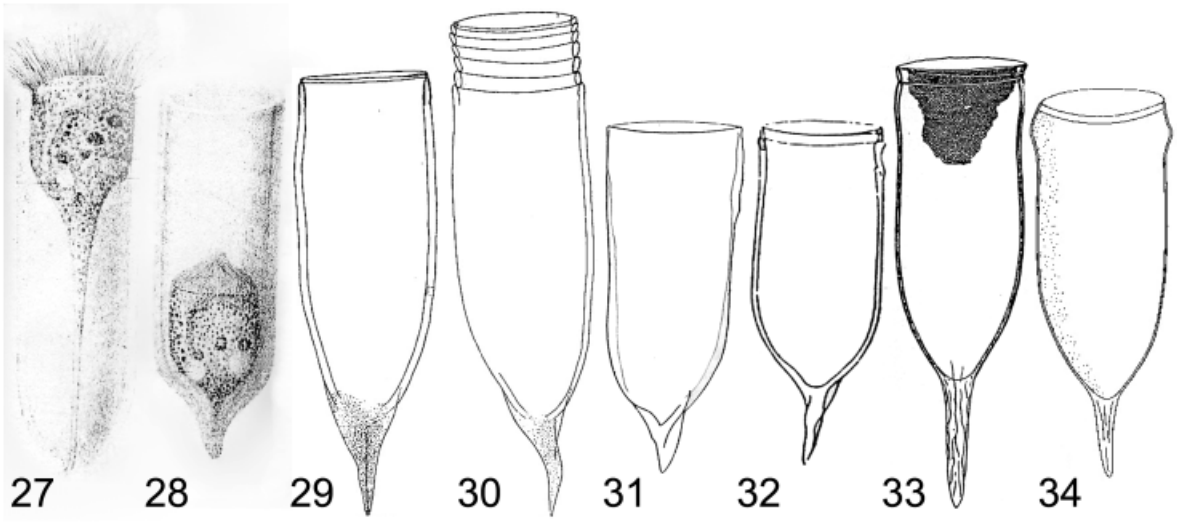

Fig. **27–34.** Lorica morphology of *Favella* species. **27–31.** *Favella ehrenbergii* from Claparède and Lachmann (1858) (27–28), from Jörgensen (1924) (29–30) and from Kofoid and Campbell (1929) (31). **32.** *Favella panamensis* from Kofoid and Campbell (1929). **33, 34.** *Favella taraikaensis* from Hada (1932) (33) and from Hedin (1974) (34).

Table **2.** Morphological comparison of related *Favella* species.

Species	Lorica, length		Lorica oral diameter		Reference
	Favella form	*Coxliella form*	*Favella form*	*Coxliella form*	
F. ehrenbergii	209.2 (93.8–330.0)	144.0 (60.0–231.3)	89.4 (87.5–93.8)	87.8 (80.0–90.6)	This study
F. ehrenbergii	186.8–431.6	224.1 (83–303.0)	95.5–116.2	87.2–105.8	Laval-Peuto (1981)
F. panamensis	136–232	—	—	—	Kofoid &Campbell (1929)
F. taraikaensis	283 (260–314)	—	83 (80–86)	—	Hada (1932)

Measurements in μm.

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Received: 05/09/10, 07/05/10; accepted: 07/06/10