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Balanion masanensis n. sp. (Ciliophora: Prostomatea) from the Coastal Waters of Korea: Morphology and Small Subunit Ribosomal RNA Gene Sequence

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ABSTRACT. The planktonic ciliate *Balanion masanensis* n. sp. is described from living cells, from cells prepared by quantitative protargol staining (QPS), scanning electron microscopy (SEM), and transmitted electron microscopy (TEM) preparations, and the sequence of its nuclear small subunit rDNA (SSU rDNA) is reported. This species is almost ovoid with a flattened anterior oral region when the cells are alive and stained. The flattened anterior region of a living cell often forms a dome with the perimeter receded in a groove, and this region is easily inflated or depressed. In SEM photos, a brosse of six to nine monokinetids (or possibly three to five dikinetids) was observed inside the circumoral dikinetids. In TEM photos, circumoral microtubular ribbons were observed below the oral cilia, which along with the oral flaps were 8–16 μm in length. The cytostome is a slight funnel-like central depression on the flattened anterior end. The morphological characteristics of this ciliate are identical to those of the genus *Balanion* (Order Prorodontida). The ranges (and mean ± standard deviation) of cell length, cell width, and oral diameter of living cells (n = 23–26) were 27–43 μm (35.2 ± 4.6), 25–32 μm (28.6 ± 2.3), and 25–30 μm (27.6 ± 1.3), respectively, while those of the QPS-stained specimens (n = 70) were 23–37 μm (30.6 ± 3.5), 26–35 μm (30.7 ± 2.2), and 26–33 μm (29.5 ± 1.5), respectively. Forty-six to 55 somatic kineties (SKs) were equally spaced around the cell body and extended from the oral to near the posterior regions with 24–50 monokinetids per kinety. Each kinetid bore a cilium 2.8–7.2 μm long. A caudal cilium (ca 14 μm long) arose on the posterior end. The single ellipsoid macronucleus is 6.8–13.4 × 6.8–10.5 μm, accompanied by a single micronucleus (2.0–2.8 × 1.5–2.5 μm) visible only in QPS specimens. Because, the cell size, the number of SKs, and the number of kinetosomes per SK of this ciliate were much greater than those of *Balanion comatum* and *Balanion planctonicum*, the only two *Balanion* species so far reported, we have established *B. masanensis* n. sp. When properly aligned, the sequence of the SSU rDNA of *B. masanensis* n. sp. (GenBank Accession No. AM412525) was approximately 9% different from that of *Coleps hirtus* (Colepidae, Prorodontida) and 12% different from that of *Prorodon teres* (Prorodontidae, Prorodontida).

Key Words. Brosse, ciliate, DNA, plankton, prostome ciliate, protozoa.

PROSTOME ciliates are often dominant heterotrophic protists in freshwater (Müller 1989; Sommaruga and Psenner 1993; Sonntag et al. 2002; Weisse and Müller 1998) and marine environments (Smetacek 1989; Stoecker, Davis, and Provan 1983). However, the taxonomy and ecology of these ciliates is still poorly known, even though they are sometimes abundant. There are two orders established in the Class Prostomatea—Order Prostomatida and Order Prorodontida—distinguished by whether a ciliate has ''brosse'' kinetids around the oral region or not (Lynn and Small 2002). This oral structure, which is often difficult to find, can be identified by careful examination with specialized methods, such as silver impregnation technique or scanning electron microscopy (SEM) after deciliation (Bardele 1999).

DNA sequences of only a few prostome species have been reported, and there are no sequences for the Order Prostomatida, and only three sequences for the Order Prorodontida for the genera *Coleps*, *Prorodon*, and *Cryptocaryon* (Hirt et al. unpubl. observ.; Leipe et al. 1994; Stechmann, Schlegel, and Lynn 1998; Wright and Colorni 2002).

The species belonging to the genus *Balanion* (Order Prorodontida) are sometimes abundant in both freshwater and marine environments (Jakobsen and Hansen 1997; Müller and Geller 1993; Stoecker and Evans 1985; Weisse et al. 2001). They are known to be effective grazers on nanoflagellates and red-tide dinoflagellates (Jakobsen and Hansen 1997; Kenter, Zimmermann, and Müller 1996; Müller and Schlegel 1999; Stoecker et al. 1983; Stoecker and Evans 1985). So far, only two species have been reported in this genus—*Balanion comatum* and *Balanion planctonicum* (Bardele 1999; Foissner, Berger, and Kohmann 1994; Foissner, Oleksiv, and Müller 1990; Jakobsen and Montagnes 1999; Small and Lynn 1985). Wulff (1919) provides an early morphological study of *B. comatum*. In their redescription of *B. comatum*, Jakobsen and Montagnes (1999) provided the key characters for the genus *Balanion* as (1) the presence of the circumoral microtubular ribbons (COMT), (2) the prominent oral dikinetid–flap complex, and (3) the brosse lying internal to the circumoral dikinetids.

During red tides dominated by the mixotrophic dinoflagellate *Heterocapsa triquetra* in Masan Bay, Korea, we found a new prostome ciliate that appeared to be a *Balanion* sp. In this study, we describe this is a new species of *Balanion*, *Balanion masanensis* n. sp. and establish its phylogenetic position within the Class Protstomatea based on its small subunit rDNA (SSU rDNA) sequence.

MATERIALS AND METHODS

Preparation of samples. Plankton samples collected with water samples were taken from a pier in Masan Bay, Korea, from December 2005 to February 2006 when the water temperature and salinity were 3.1 °C–14.1 °C and 23.3–32.7 psu, respectively. The samples were transported to the laboratory within 30 min, gently screened through a 202-μm nitex mesh, and divided into 270-ml polycarbonate (PC) bottles and 500-ml polyethylene (PE) bottles. Some of a dense culture of *H. triquetra* (concentration = ca 15,000 cells/ml) was added to the 270-ml PC bottles as food. The bottles were placed on plankton wheels rotating at 0.9 rpm and incubated at 5°–10 °C under an illumination of 20 μE/m^2/s of cool white fluorescent light on a 12:12 h light–dark cycle. Two days later, aliquots of the enriched water were transferred to six-well tissue culture plates and a clonal culture of *B. masanensis* n. sp. was established by two serial single-cell isolations. Once dense cultures of this ciliate were obtained, they were transferred every 2 days to 270-ml PC bottles of fresh *H. triquetra* as prey. For the observation of living cells and the analysis of DNA sequence, the cells maintained by this method were used.

For the quantitative protargol stain (QPS) method (Montagnes and Lynn 1987), samples in the PE bottles were fixed with a

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Table **1.** The list of the nucleotide sequences used in this study.

Species	Accession number	References
Amphisiella magnigranulosa	AM412774	Schmidt et al. (2007)
Amylovorax dehorityi	AF298817	Cameron, Adlard and O'Donoghue (2001)
Anophryoides haemophila	U51554	Ragan et al. (1996)
Anoplophrya marylandensis	AY547546	Affa'a et al. (2004)
Astylozoon enriquesi	AY049000	
Blepharisma americanum	M97909	Greenwood et al. (1991)
Bresslaua vorax	AF060453	Lynn et al. (1999)
Bryometopus sphagi	AF060455	Lynn et al. (1999)
Bursaria truncatel	U82204	Stechmann, Schlegel, and Lynn (1998)
Caenomorpha uniserialis	U97108	
Cardiostomatella vermiforme	AY881632	Li et al. (2006)
Chattonidium setense	AM295495	Modeo et al. (2006)
Chilodonella uncinata	AF300281	
Chlamydodon excocellatus	AY331790	Snoeyenbos-West et al. (2004)
Climacostomum virens	X65152	Hammerschmidt et al. (1996)
Coleps hirtus	U97109	
Colpes sp.	DQ487194	
Colpes sp.	X76646	Stechmann et al. (1998)
Colpoda inflata	M97908	Greenwood et al. (1991)
Colpoda steinii	DQ388599	
Cryptocaryon irritans	AF351579	Wright and Colorni (2002)
Dasytricha ruminantium	U57769	Wright and Lynn (1997)
Dexiotrichides pangi	AY212805	Song, Ma, and AL-Rasheid (2003)
Didinium nasutum	U57771	Wright and Lynn (1997)
Discophrya collini	L26446	Leipe et al. (1994)
Dysteria derouxi	AY378112	
Ephelota gemmipara	DQ834370	
Eufolliculina uhligi	U47620	Hammerschmidt et al. (1996)
Furgasonia blochmanni	X65150	Bernhard et al. (1995)
Gonostomum strenuum	AJ310493	Bernhard et al. (2001)
Gruberia sp.	L31517	Hirt et al. (1995)
Halteria grandinella	AF194410	Shin et al. (2000)
Heliophrya erhardi	AY007446	
Hemiophrys macrostoma	AY102173	
Homalozoon vermiculare	L26447	Leipe et al. (1994)
Isochona sp.	AY242116	Snoeyenbos-West et al. (2004)
Loxodes striatus	U24248	Hammerschmidt et al. (1996)
Macropodinium ennuensis	AF298820	
Maristentor dinoferus	AY630405	Miao et al. (2005)
Metopus contortus	Z29516	Hirt et al. (1995)
Miamiensis avidus	AY550080	Jung et al. (2005)
Nyctotheroides deslierresae	AF145353	
Obertrumia georgiana	X65149	Bernhard et al. (1995)
Ophryoglena catenula	U17355	Wright and Lynn (1995)
Paramecium tetraurelia	X03772	Sogin and Elwood (1986)
Pelagostrobilidium neptuni	AY541683	Agatha et al. (2005)
Peritroums kahli	AJ537427	
Phacodinium metchnikoffi	AJ277877	Shin et al. (2000)
Philasterides dicentrarchi	AY642280	
Plagiotoma lumbrici	AY547545	Affa'a et al. (2004)
Platyophrya vorax	AF060454	Lynn et al. (1999)
Polycosta roundi	AF298819	
Prorocentrum micans	AY585526	Zhang, Bhattacharya, and Lin (2005)
Prorodon teres	X71140	Leipe et al. (1994)
Prorodon viridis	U97111	
Pseudoplatyophrya nana	AF060452	Lynn et al. (1999)
Rimostrombidium lacustris	DQ986131	Agatha and Strueder-Kypke (2007)
Schizocaryum dogieli	AF527756	Lynn and Struder-Kypke (2002)
Sirloxophyllum utriculariae (submitted as *Loxophyllum*)	L26448	Leipe et al. (1994)
Sorogena stoianovitchae	AF300287	Lasek-Nesselquist and Katz (2001)
Strombidinopsis jeokjo	AJ628250	Jeong et al. (2004)
Strombidium inclinatum	AJ488911	Modeo et al. (2003)
Symbiodinium pilosum	X62650	Medlin et al. (1988)
Tetrahymena canadensis	M98022	Sogin et al. (1986)
Tintinnopsis dadayi	AY143562	Strueder-Kypke and Lynn (2003)
Tracheloraphis sp.	L31520	Hirt et al. (1995)
Trithigmostoma steini	X71134	Leipe et al. (1994)
Urocentrum turbo	AF255357	Strueder-Kypke et al. (2000)

All are available from the GenBank/EMBL databases and have the accession numbers.

modified concentrated Bouin's solution (final concentration 10%, v/v) in which most of the cells were well preserved.

For SEM, a 20 ml aliquot of a dense culture of *B. masanensis* n. sp. was fixed with osmic acid (final concentration 2%, w/v) in seawater for 1.5 h and then the fixed cells were collected on a PC membrane filter (pore size = 5 μm) without additional pressure. Fixed cells were rinsed three times with distilled waters to remove the salt, dehydrated through an ethanol series, and finally dried using critical point dryer (Bal-Tec, CPD 300, Balzers, Germany). The dried filters were mounted on a stub and coated with gold–palladium. Cells were viewed with a JSM-840A SEM (JEOL Ltd., Tokyo, Japan) and photographed using a digital camera connected by a computer. To observe the brosse, an important key to the genus *Balanion*, deciliated cells were observed when fixed for SEM with 2% (w/v) osmium tetroxide.

For transmission electron microscopy (TEM), 500 ml monocultured cells were fixed for 1.5 h in 4% (v/v) glutaraldehyde in culture medium. Cells were centrifuged, and the pellet was embedded in agar. After several rinses with medium, the cells were postfixed in 1% (w/v) osmium tetroxide in deionized water. Dehydration was accomplished using a graded ethanol series (50%, 60%, 70%, 80%, 90%, and 100% ethanol, followed by two 100% ethanol steps). The material was embedded in Spurr's low-viscosity resin (Spurr 1969). Sections were obtained with a RMC MT-XL ultramicrotome (Boeckeler Instruments Inc., Tucson, AZ) and post-stained with 3% (w/v) aqueous uranyl acetate followed by lead citrate. Stained sections were viewed with a JEOL-1010 electron microscope (JEOL Ltd.).

Measurement of specimens. We measured cell length (the maximum longitudinal linear distance excluding cilia), cell width (the maximum diameter), and oral diameter of living cells, using an image analysis system mounted on a compound microscope (Image-Pro Plus 4.5, Media Cybernetics, Silver Spring, MD). We measured morphometric characteristics of cells stained using QPS as follows: more than 1 wk after being fixed with Bouin's solution, the samples were stained using QPS, and examined with a compound microscope at a magnification of 400–1,000X. Measurement of the cells followed the recommendations of Lynn and Small (2002), Foissner (1984), Foissner et al. (1994), and Jakobsen and Montagnes (1999). We measured number and length of

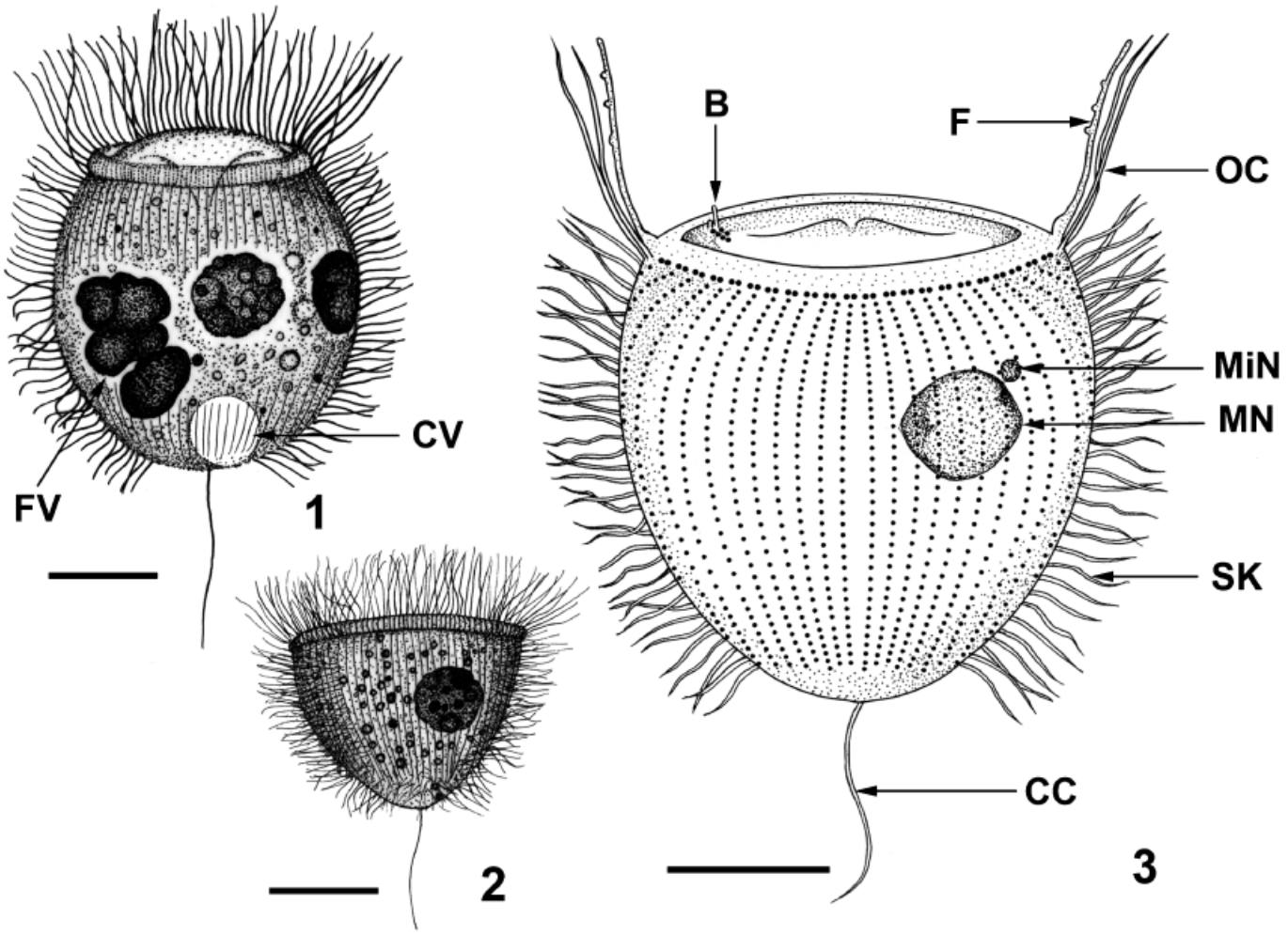

Fig. **1–3.** Schematic diagrams of *Balanion masanensis* n. sp. **1, 2.** Lateral view based on living cells in a natural population. **1.** General cell shape having eaten prey. **2.** Starved cell. **3.** Lateral view based on live, protargol-stained, and scanning electron microscopic preparations indicating the position and shape of the oral cilia (OC), oral flaps (F), brosse (B), somatic kineties (SKs), caudal cilium (CC), macronucleus (MN), and micronucleus (MiN). CV, contractile vacuole; FV, food vacuoles. Scale bars = 10 μm.

oral cilia and length of somatic cilia using the samples fixed by formalin solution (final concentration 5%, v/v) and SEM specimens.

DNA extraction, PCR amplification, sequencing and data analysis. Genomic DNA was extracted from ca 100 cells from a clonal culture with a DNeasy Tissue kit (Qiagen, Stanford, CA) following the manufacturer's instructions. The extracted DNA was divided into two tubes and used to conduct independent PCR reactions for the SSU rDNA, which were performed under the following conditions: one cycle of 3 min at 94 °C; 15 cycles of 30 s at 94 °C, 40 s at 56 °C, and 3 min at 72 °C; 25 cycles of 30 s at 94 °C, 40 s at 52 °C, and 3 min at 72 °C; and a final cycle of 5 min at 72 °C. For the full-length fragment, the universal eukaryotic forward primer EukA (5′-CTG GTT GAT CCT GCC AG-3′) and the reverse primer EukB (5′-TGA TCC TTC YGC AGG TTC-3′) were used (Petroni et al. 2002). For the internal 600 bp fragment (approximately 600–1,200 bp downstream from the 5′-end of the gene), forward primer Gas+600 (5′-CGG TAA TKC CAG CTC CAA TAG CG-3′) and reverse primer Gas-1220 (5′-CCT GGT GGT GCC CTT CCG TC-3′) were used. The PCR fragments were inserted into a pGEM-T vector (Promega, Madison, WI) and at least three colonies for each PCR fragment were sequenced in both directions with EukA, EukB, Gas+600, Gas-1220 primers, and two additional primers, Gas+1390 (5′-CTG GTT AAT TCC GAT AAC G-3′) and Gas-1540 (5′-GGG CAT CAC AGA CCT GT-3′) using an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems, Foster City, CA).

Sequence availability and phylogenetic analysis. The nucleotide sequences are available from the GenBank/EMBL databases (Table 1). The sequences were aligned by eye, with the alignment based on a secondary structure using the SSU rDNA of *Tetrahymena canadensis* (Wuyts, Perriere, and Van de Peer 2004) in the Genetic Data Environment (GDE 2.2) program (Smith et al. 1994). A total of 1,291 nucleotides were included in the phylogenetic analyses under maximum parsimony (MP), neighbour-joining (NJ), maximum likelihood (ML) criteria, and Bayesian analyses.

For ML, NJ, and Bayesian analyses, we performed a likelihood ratio test using MODELTEST 3.7 (Posada and Crandall 1998) to determine the best available model for the data. The selected model was a Tamura and Nei model (TrN, Tamura and Nei 1993) with a gamma correction for among-site rate variation

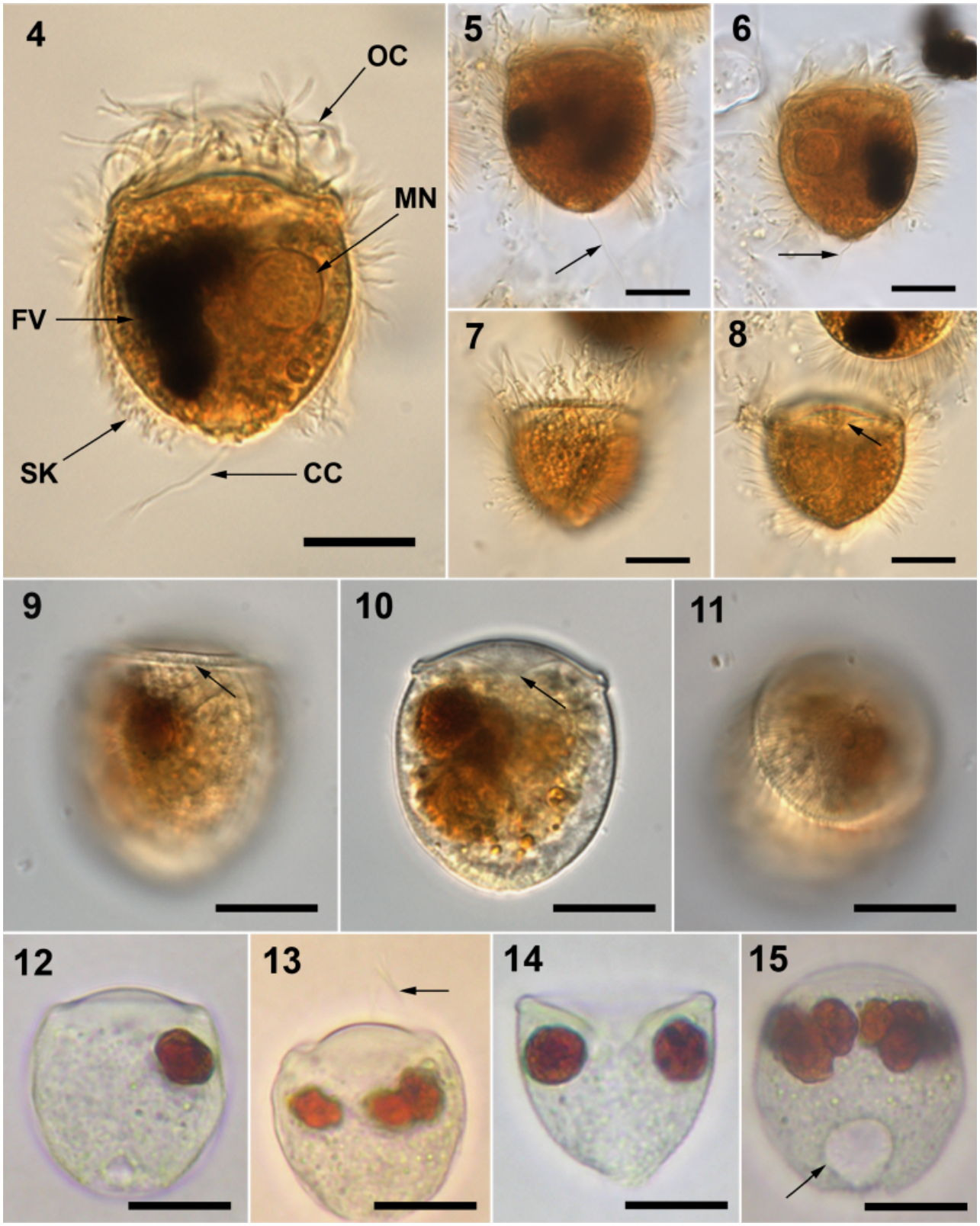

Fig. **4–15.** Micrographs of cells fixed by Lugol's solution (Fig. 4–11) and living cells (Fig. 12–15) of *Balanion masanensis* n. sp. All particles in the cells colored black or dark brown were prey (*Heterocapsa triquetra*). **4–8.** Lateral view showing general cell shapes (Fig 4–6: cells satiated with prey, Fig. 7–8: starved cells), oral cilia (OC), macronucleus (MN), somatic kineties (SKs), caudal cilium (CC), and food vacuoles (FV). Arrows in Fig. 5, 6 indicate the CC and the arrow in Fig. 8 indicates the oral depression. **9, 10.** Lateral view of deciliated cell (Fig. 9: ''dorsal'' focal plane, Fig. 10: middle focal plane) showing the distinct ''lip'' (arrow in Fig. 9) surrounded by dense oral dikinetids and surrounding a central oral depression (arrow in Fig. 10). **11.** Semi-anterior view showing the flattened oral region. **12.** Lateral view showing general shape. **13.** Dorsal–lateral view indicating the position and shape of the OC (arrow). **14.** Lateral view showing the oral depression. **15.** Dorsal–posterior view showing the position and shape of contractile vacuole (arrow). Scale bars = 10 μm.

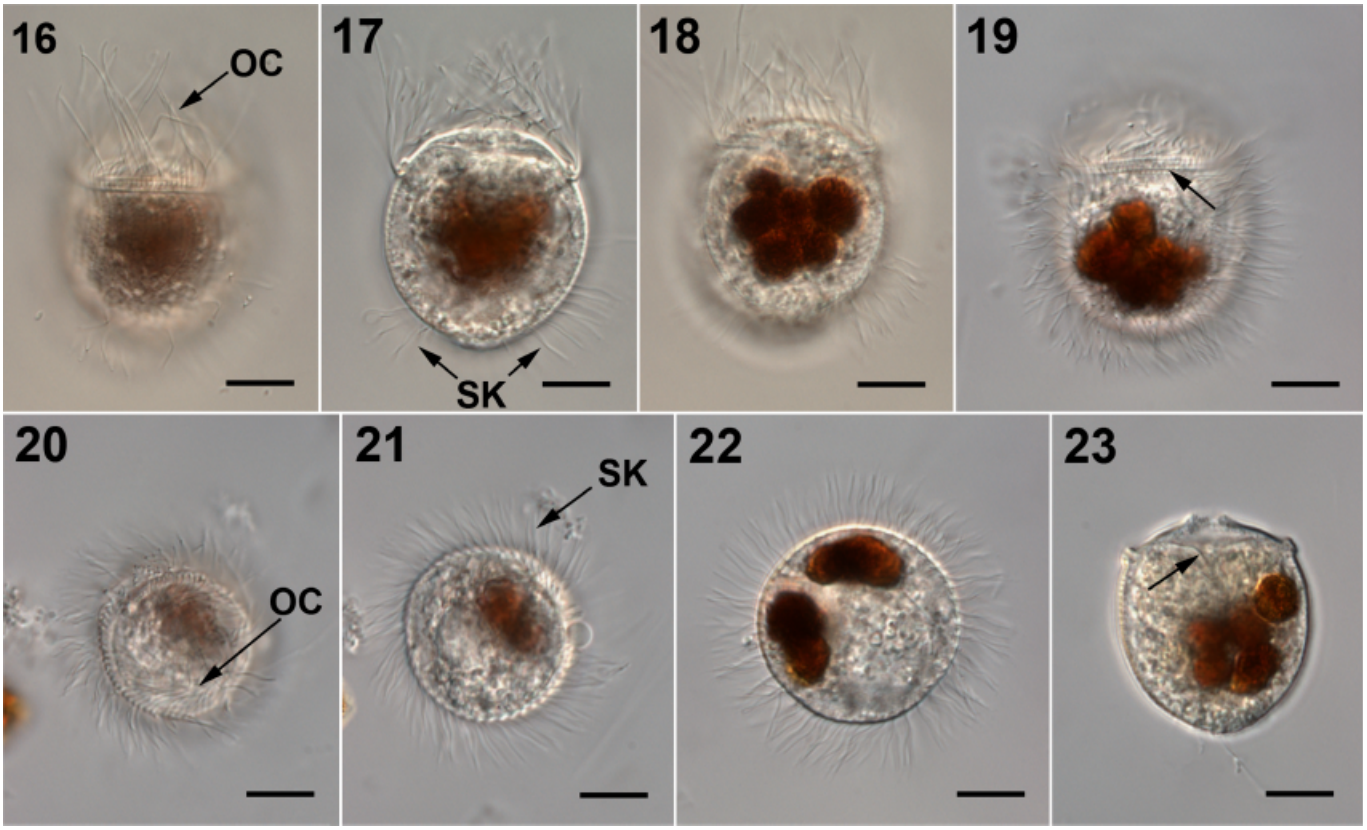

Fig. **16–23.** Micrographs of *Balanion masanensis* n. sp. fixed by formalin solution. **16–19**. Lateral views showing the oral cilia (OC), cilia of the somatic kineties (SKs), and distinct ''lip'' (arrow in Fig. 19). **20–22.** Anterior views demonstrating the OC and cilia of the SKs. **23.** Lateral view showing the oral depression (= cytostome) of a deciliated cell. Scale bars = 10 μm.

($\alpha = 0.5528$) and invariant sites ($I = 0.3165$). The phylogenetic analyses were performed by ML, MP, and NJ implemented with PAUP 4.0b10 (Swofford 2002) for MacPro OS X. The ML, NJ, and Bayesian analysis used a single model (TrN+I+Γ) for the data set: (BaseFreq = (0.3139, 0.1853, 0.2372), RateMatrix = (1.0000, 2.5048, 1.0000, 1.0000 3.8650), $\Gamma = 0.5528$, Invariant = 0.3165). Maximum likelihood and MP analyses used heuristic searches with a branch-swapping algorithm (tree bisection–reconnection). Distance matrices for NJ analyses were calculated via ML. The robustness of branches was tested by bootstrap analyses (Felsenstein 1985) using 1,000 (NJ and MP) and 250 (ML) replications. Ten heuristic searches in MP and NJ and two in ML (starting trees via random stepwise addition) were used for each bootstrap replicate. Maximum likelihood bootstrapping was performed on 10 processors. Bayesian analyses were performed with a TrN model with rate set to invgamma and nucleotide frequencies set to dirichlet (A = 0.3139, C = 0.1853, G = 0.2372, T = 0.2637). Four Markov chains (2,000,000 generations per chain) were run and trees were saved every 1,000 generations with the first 800 trees discarded. A majority-rule consensus tree was created from the remaining 1,201 trees to examine the posterior probabilities of each clade.

RESULTS

Balanion masanensis Kim, Jeong, & Lynn, n. sp.

Description. *Balanion masanensis* n. sp. is oval with a flattened anterior oral region, which is encircled by oral dikinetids bearing two cilia, and a rounded or bluntly pointed posterior end when the cells are both alive and stained (Fig. 1–15, 24, 25). Living cells are transparent except for food vacuoles (Fig. 12–15). Their flattened anterior region often forms a dome with the perimeter receded in a groove, and this part easily changes shape, becoming either inflated or depressed (Fig. 13, 14). The cytostome, a slight funnel-like central depression on the flattened anterior end, is observed well in SEM specimens (Fig. 32). In lateral views, the cytostome was often observed in live cells and those fixed by formalin and Lugol's solution (Fig. 8, 10, 14, 23). The flattened oral region was covered by radial ridges in Lugol's, SEM, and TEM specimens (Fig. 11, 32, 35–40, 41). At the edge of the flattened oral region of deciliated SEM specimens, the ''brush,'' called a ''brosse'' by Jakobsen and Montagnes (1999), was observed (Fig. 32, 35–40). The brosse consisted of a relatively long kinety (1.5–2 μm; three to five kinetids) and relatively short kinety (ca 0.5 μm; three to six kinetids). With our preparations, we were unable to determine whether these were organized as dikinetids.

The number of oral dikinetids was 51–63 (Table 2). Oral flaps were observed inside the oral dikinetids, encircling the oral region. The range of the length of oral dikinetid cilia was 8.4–15.5 μm (n = 24), measured in the SEM specimens. This oral cilia complex was easily lost in QPS specimens (Fig. 24–30), but was well preserved in specimens fixed with formalin (final concentration 5%, v/v) or in Lugol's (1%–2%) solutions. The flaps in some SEM specimens were swollen (Fig. 34).

The ranges of cell length, cell width, and oral diameter of living cells were 27.1–43.3, 24.8–31.7, and 24.8–29.6 μm, respectively, while those of the QPS-stained specimens were 22.8–37.3,

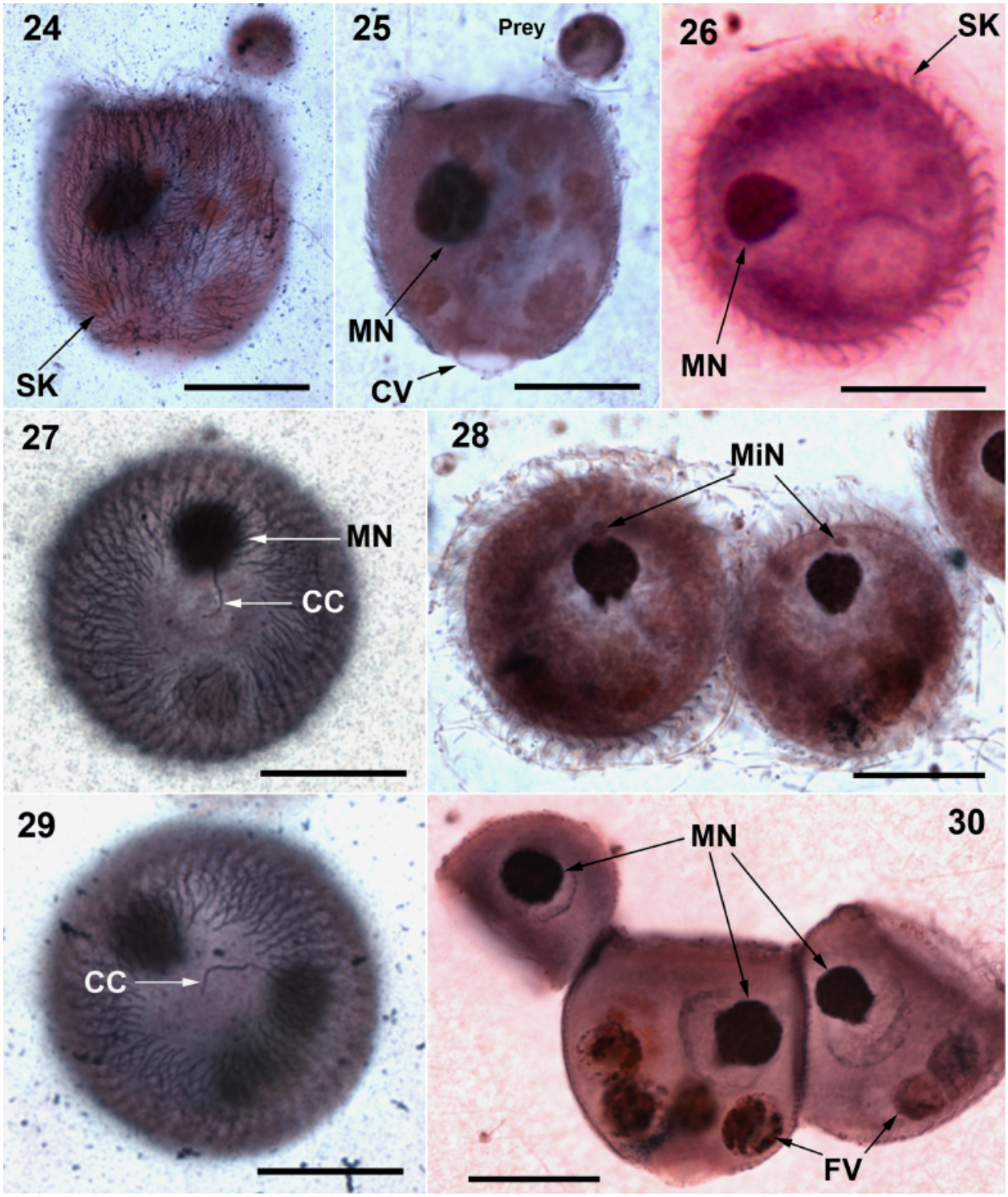

Fig. **24–30.** Micrographs of protargol-stained specimens of *Balanion masanensis* n. sp. **24, 25.** Lateral views (Fig. 24: ''dorsal'' focal plane, Fig. 25: middle focal plane) of a cell demonstrating the somatic kineties (SKs), macronucleus (MN), and contractile vacuole (CV). **26.** Anterior view taken in the middle of the cell showing the position and shape of MN and SKs. **27, 29.** Posterior views showing caudal cilium (CC) and the bare area of the end of the cells. **28.** Anterior view taken in the middle of the cell showing the position and shape of MN and micronucleus (MiN). **30.** Lateral views showing variations in cell size. Note MN and food vacuoles (FV). Scale bars = 10 μm.

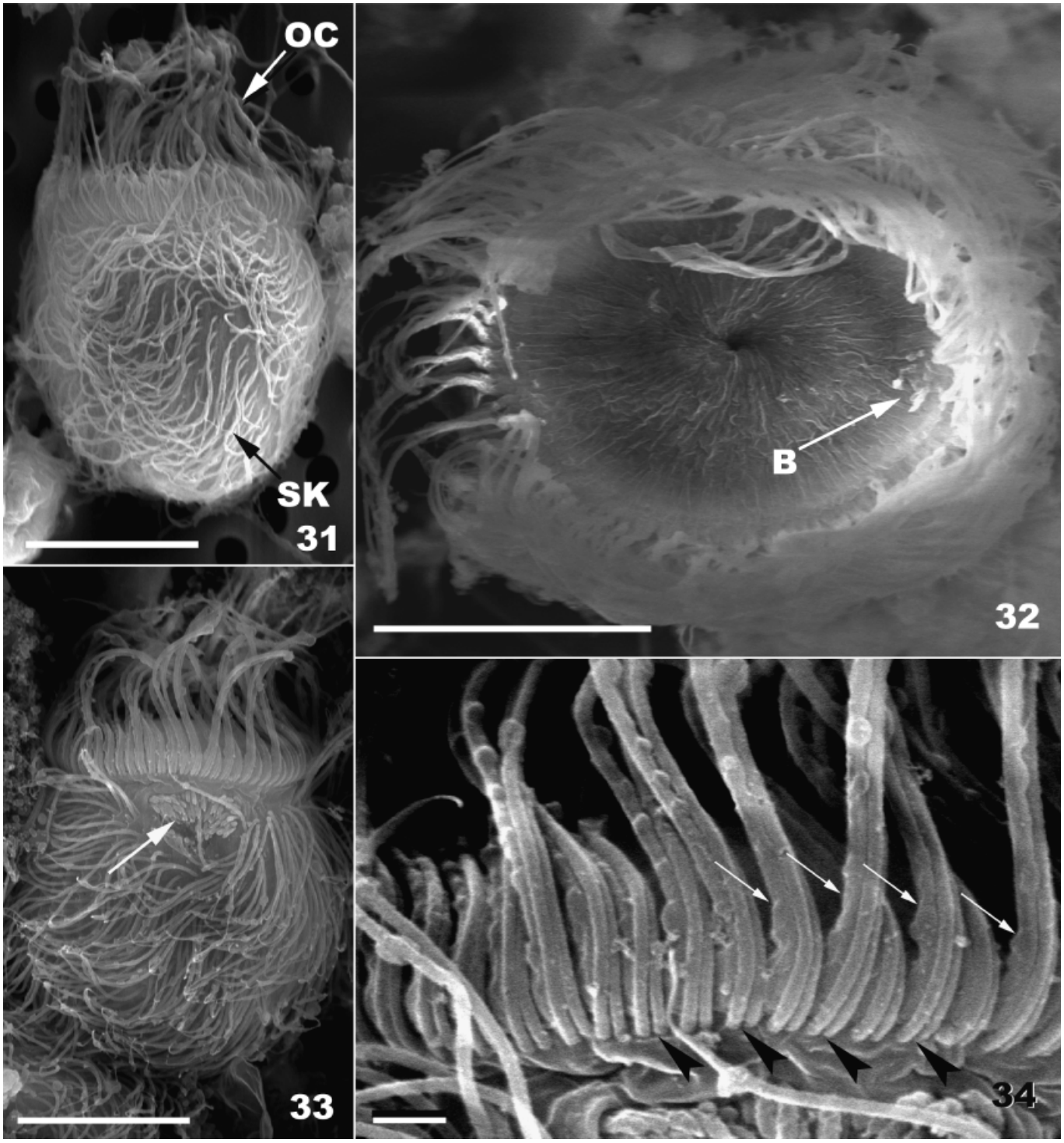

Fig. **31–34.** Scanning electron micrographs of *Balanion masanensis* n. sp. **31, 33.** Lateral views showing oral cilia (OC) and somatic kineties (SKs). Arrow in Fig. 33 indicates the oral anlage. **32.** Anterior views showing the shape and position of the cytostome and ''brosse'' (B). **34.** Oral dikinetids (black arrow heads) are forming the ''complex'' with oral flaps (white arrows). Scale bars = 10 μm for Fig. 31–33, and 1 μm for Fig. 34.

26–5.4, and 26–33 μm, respectively (Table 2). Forty-six to 55 longitudinally oriented somatic kineties (SKs) were equally spaced around the cell body, and extended from the oral end to near the posterior end. Typically, the posterior one-fifth of the cell was bare except for a caudal cilium (ca 14 μm long) (Fig. 4–6, 27, 28, Table 2). Each SK consisted of 24–50 monokinetids (Fig. 24, 25, 31, 33), and each kinetid bore cilia of 10–15 μm long. The numbers of oral dikinetids, SKs, and monokinetids in a SK of cells starved for 1 day were not different from those of cells satiated with *H. triquestra*.

This ciliate had one ellipsoid macronucleus of 6.8–13.4 × 6.8–10.5 μm and one micronucleus of 2.0–2.8 × 1.5–2.5 μm, visible

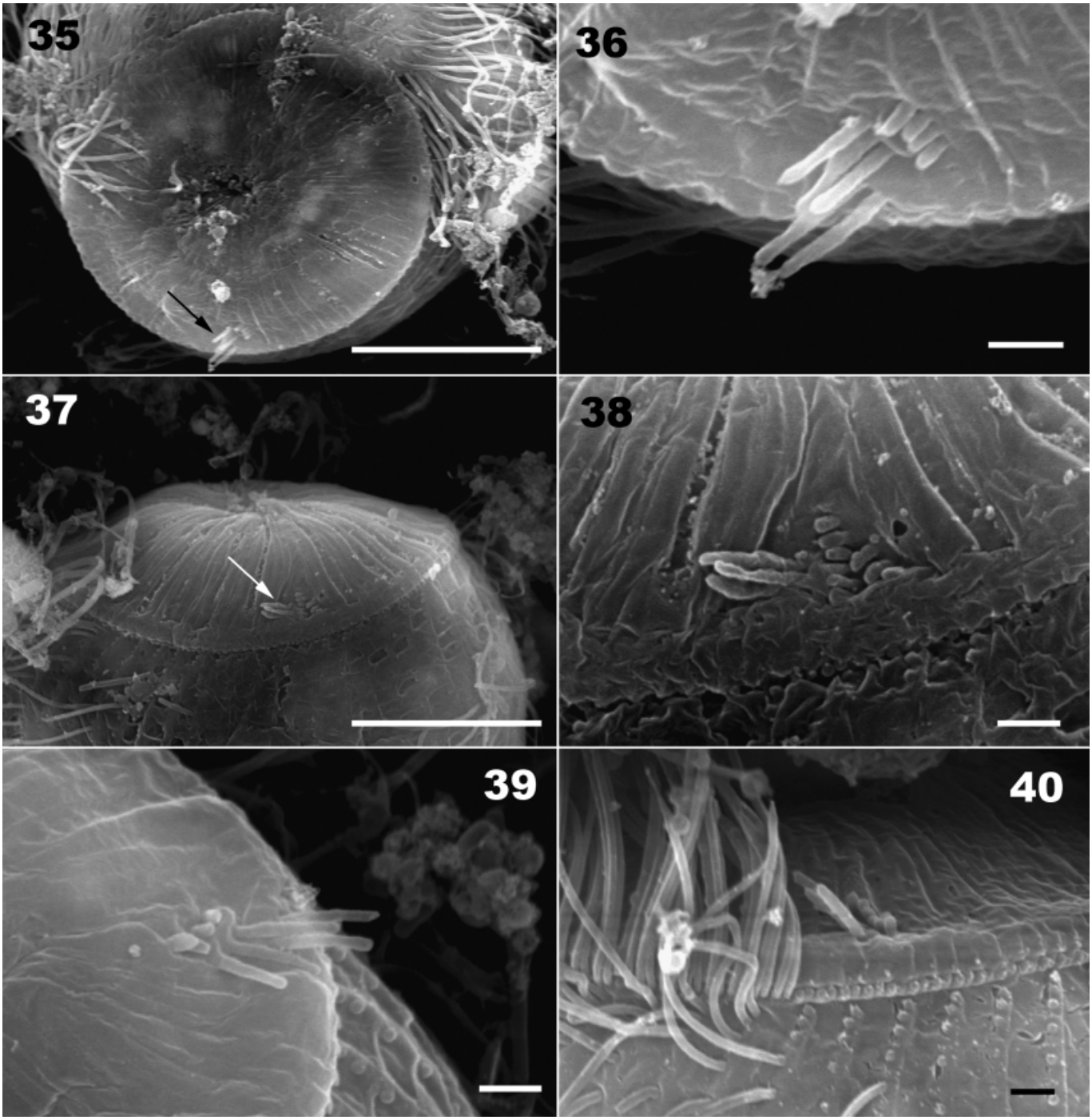

Fig. **35–40.** Electron micrographs of *Balanion masanensis* n. sp. showing the brosse located inside the circumoral dikinetids. Scale bars = 10 μm for Fig. 40, 42, and 44, and 1 μm for Fig. 41, 43, and 45. We are unable to determine whether the brose is organized as monokinetids or dikinetids.

only in QPS-stained specimens (Fig. 28). The macronucleus was located near the center of the cell and the micronucleus located on its outer side (Fig. 28).

In TEM photos, a circumoral microtubular ribbon (COMT) was observed adjacent to the oral kinetids (Fig. 42, 45, 46). The number of microtubules inside oral flaps was approximately 7–9 (Fig. 43, 44).

Gene sequence. The SSU rRNA gene sequence (Genbank Accession No. AM412525) of this new isolate has a length of 1,752 nucleotides and a GC content of 43.6%. Its sequence shows $<$90.9% similarity with closely related species of the Class Prostomatea, but shows the highest SSU rRNA gene sequence similarity to *Cryptocaryon irritans* (Cryptocaryonidae, Prorodontida; 90.9%), followed by *Prorodon teres* (Prorodontidae, Prorodontida; 90.5%) and *Prorodon viridis* (Prorodontidae, Prorodontida; 89.7%). The phylogenetic analyses using Bayesian, ML, MP, and NJ grouped the new isolate with members of the Class Prostomatea, but its phylogenetic placement was ambiguous. In Bayesian

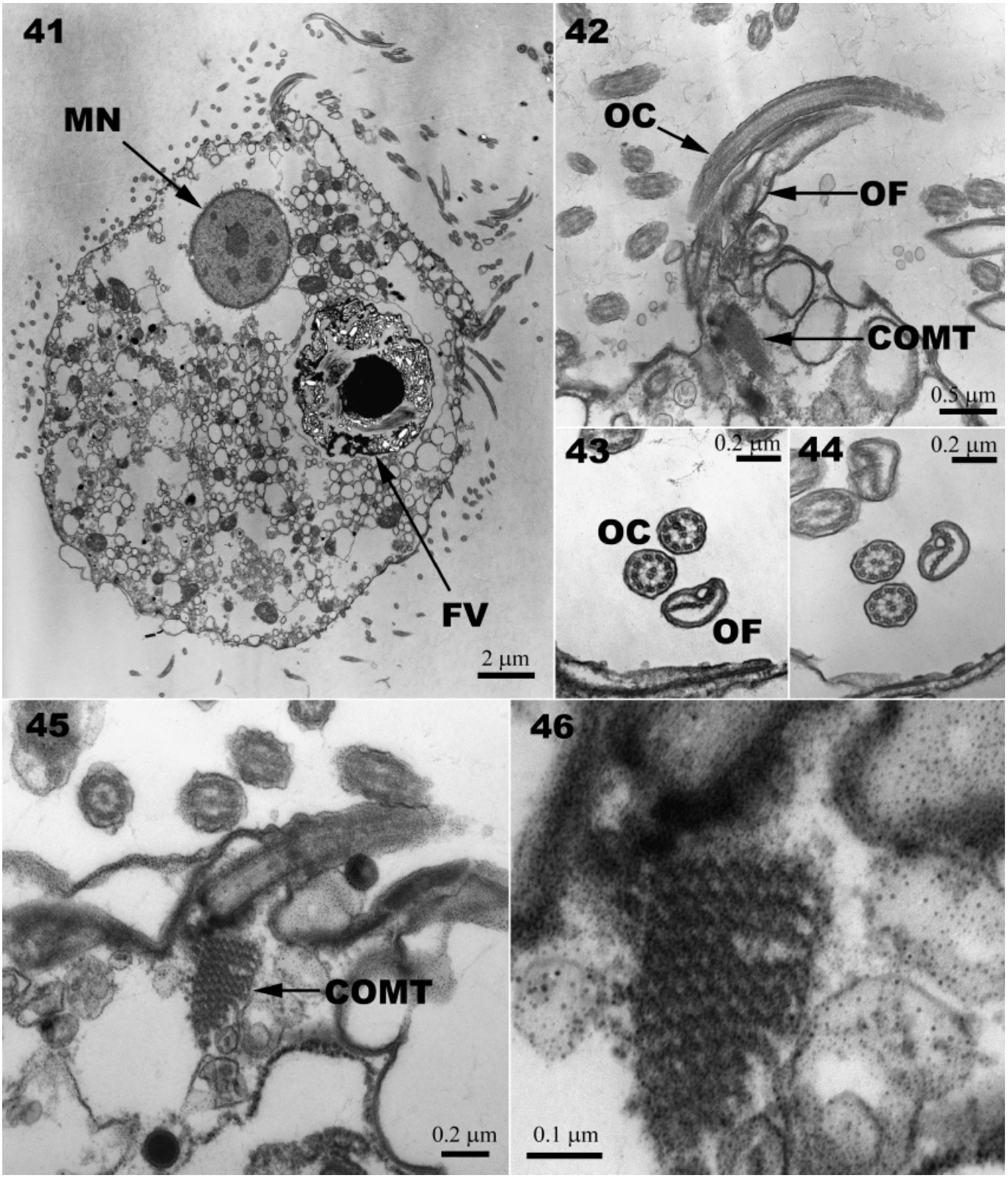

Fig. **41–46.** Transmission electron micrographs of *Balanion masanensis* n. sp. **41, 42, 45, 46.** Anterior longitudinal section showing macronucleus (MN), oral cilia (OC), oral flap (OF), and circumoral microtubular ribbon (COMT). **43, 44.** Cross-sections of the OC and OF.

Table **2.** Morphometric characterization of *Balanion masanensis* n. sp. based on protargol-stained specimens (and living cells in parenthesis) and specimens observed by scanning electron microscopy (*).

Character	Range	Average	SD	n
Cell length (μm)	22.8–37.3	30.6	3.5	70
	(27.1–43.3)	(35.2)	(4.6)	(23)
Cell width (μm)	26.0–35.4	30.7	2.2	70
	(24.8–31.7)	(28.6)	(2.3)	(26)
Oral diameter (μm)	26–33	29.5	1.5	70
	(24.8–29.6)	(27.7)	(1.3)	(25)
No. of oral dikinetids	63–51	57.8	3.9	10
Length of oral cilia* (μm)	8.4–15.5	12.5	2.0	24
No. of kinetids in long brosse kinety	3–5	4.2	0.8	6
No. of kinetids in short brosse kinety	3–6	4.5	1.0	6
Length of long brosse kinety (μm)	1.5–2	1.7	0.2	15
Length of short brosse kinety (μm)	0.4–0.6	0.5	0.1	14
No. of somatic kinety	46–55	51	2.5	22
Length of somatic kinety (μm)	19.8–27.4	24.1	7.4	13
No. of cilia per somatic kinety	24–50	38.6	7.4	15
Length of somatic cilia* (μm)	2.8–7.2	5.1	1.0	20
Somatic kinety as % of total cell length	73.5–81.8	78.1	2.8	13
No. of macronucleus	1	1	0	68
Length of macronucleus (μm)	6.8–13.4	9.3	1.0	68
Width of macronucleus (μm)	6.8–10.5	8.1	0.7	68
Length of micronucleus (μm)	2.0–2.8	2.4	0.3	10
Width of micronucleus (μm)	0.5–2.5	1.9	0.3	10

and ML analyses, the new species clustered together with *C. irritans* to form a monophyletic clade with weak support (0.9 posterior probability, 50% BP) (Fig. 47).

Time and locality of isolation. Our new isolate was found in Masan Bay, which is located in southern coastal Korea (35°12′N, 128°34′E). It was often observed from November to early March, when the ranges of the water temperatures and salinities were 3.1 °C–14.1 °C and 23.3–32.7 psu, respectively.

Remarks on culturing and behavior. The new isolate has been cultured for >1 yr by providing the red-tide dinoflagellate *H. triquetra* as prey. The maximum growth rate of this ciliate on *H. triquetra* was $\sim 2.5\,day^{-1}$ (our unpubl. data). When starved, this ciliate swims in straight paths, but when satiated, it swims in a large circle.

DISCUSSION

Morphological and genetical characteristics. Our isolate from Masan Bay is definitely a prostome ciliate, based on the sequence of its SSU rDNA and on its general morphology. It can be assigned unambiguously to the genus *Balanion* because it has (1) a brosse inside the circumoral ring of dikinetids, (2) shows the circumoral microtubular ribbon (COMT), and (3) has elongated oral flaps that are supported by a ribbon of microtubules. However, its body size and the numbers of oral cilia, SKs, kinetosome per SK, and microtubules inside oral flaps of this ciliate were considerably different from those of *B. comatum* and *B. planctonicum*, the only other *Balanion* species, so far reported (Table 3). The mean cell length, cell width, and oral diameter of the QPS-stained specimens (30.6, 30.7, and 29.5 μm, respectively) were considerably larger than those of *B. comatum* and *B. planctonicum* (13.1–20.0, 10.0–13.5, and 9–13.3 μm, respectively). The number of oral dikinetids of Masan Bay *Balanion* (range = 51–63) was greater than that of *B. comatum* (35–46) and *B. planctonicum* (29–39). The six to nine brosse monokinetids of our new isolate were greater than the two kinetids of *B. comatum*, but similar to the eight kinetids of *B. planctonicum*. We were unable to determine whether the brosse kinetids of *B. masanensis* n. sp. are organized as monokinetids or dikinetids. In addition, the brosse appears to consist of two different-sized kineties, which has not been previously described. The 51–63 SKs of the Masan Bay isolate was greater than for both *B. comatum* (20–29) and *B. planctonicum* (21–29). The 24–50 monokinetids per SK of the Masan Bay isolate were also greater than for both *B. comatum* (12–26) and *B. planctonicum* (7–12). For these reasons, we establish the new species *B. masanensis* n. sp.

Class Prostomatea Schewiakoff, 1896
Order Prorotontida Corliss, 1974
Family Balanionidae Small and Lynn, 1985
Balanion masanensis Kim, Jeong, & Lynn n. sp.

Table **3.** Comparison of morphological characteristics of *Balanion masanensis* n. sp. with other *Balanion* spp. based on specimens observed by the protargol-staining, scanning electron microscopy (*), and transmission electron microscopy (**) specimens.

Character	*B. planctonicum*		*B. comatum*		*B. masanensis*
Cell length (μm)	13.1 (10–18)	20	14 (12–26)	15 (10–25)	30.6 (23–37)
Width (μm)	13.5 (10–17)	15	12.1	10 (7–13)	30.7 (25–32)
Oral end diameter (μm)	10.1 (7–13)	13.3	9	(8–12)	29.5 (26–33)
Oral dikinetid number*	33.6 (29–38)	34 (29–39)	NA	40 (35–46)	57.8 (51–63)
Oral cilia length* (μm)	10.3 (8–13)	8	NA	(5–10)	12.5 (8.4–15.5)
SK number	24.7 (21–28)	26 (23–29)	NA	25 (20–29)	51 (46–55)
SK length (μm)	6.8 (4–10)	8	9	(10–12)	24.1 (16.8–27.4)
SK as % of cell length	54	60	63	75 (50–90)	78.1 (73.5–81.8)
Kinety per SK	11.3 (7–12)	(8–11)	NA	18 (12–26)	38.6 (24–50)
Somatic cilia length (μm)	6.4	NA	6–7	5 (5–8)	7.7 (6–10)
Brosse kinetids*	0	4	NA	At least 2 (n = 1)	6–9
Caudal cilia	1	1	1	1	1
MN number	1	1	1	1	1
MN length (μm)	4.5 (3–6)	(8–11)	5	4 (3–7)	9.3 (7–13)
MN width (μm)	3.6 (3–5)	(7–8)	4.6	4 (3–7)	8.1 (7–11)
Microtubules in oral flap**	NA	4	NA	(9–10)	NA
Presence of oral depression	NA	Food induced	Present	Rare	Present
Habitat	Freshwater	Freshwater	Marine/brackish	Marine/brackish	Marine/estuary
Reference	Foissner et al. (1990)	Bardele (1999)	Wulff (1919)	Jakobsen and Montagnes (1999)	This study

NA, not available; MN, macronucleus; SK, somatic kinety.

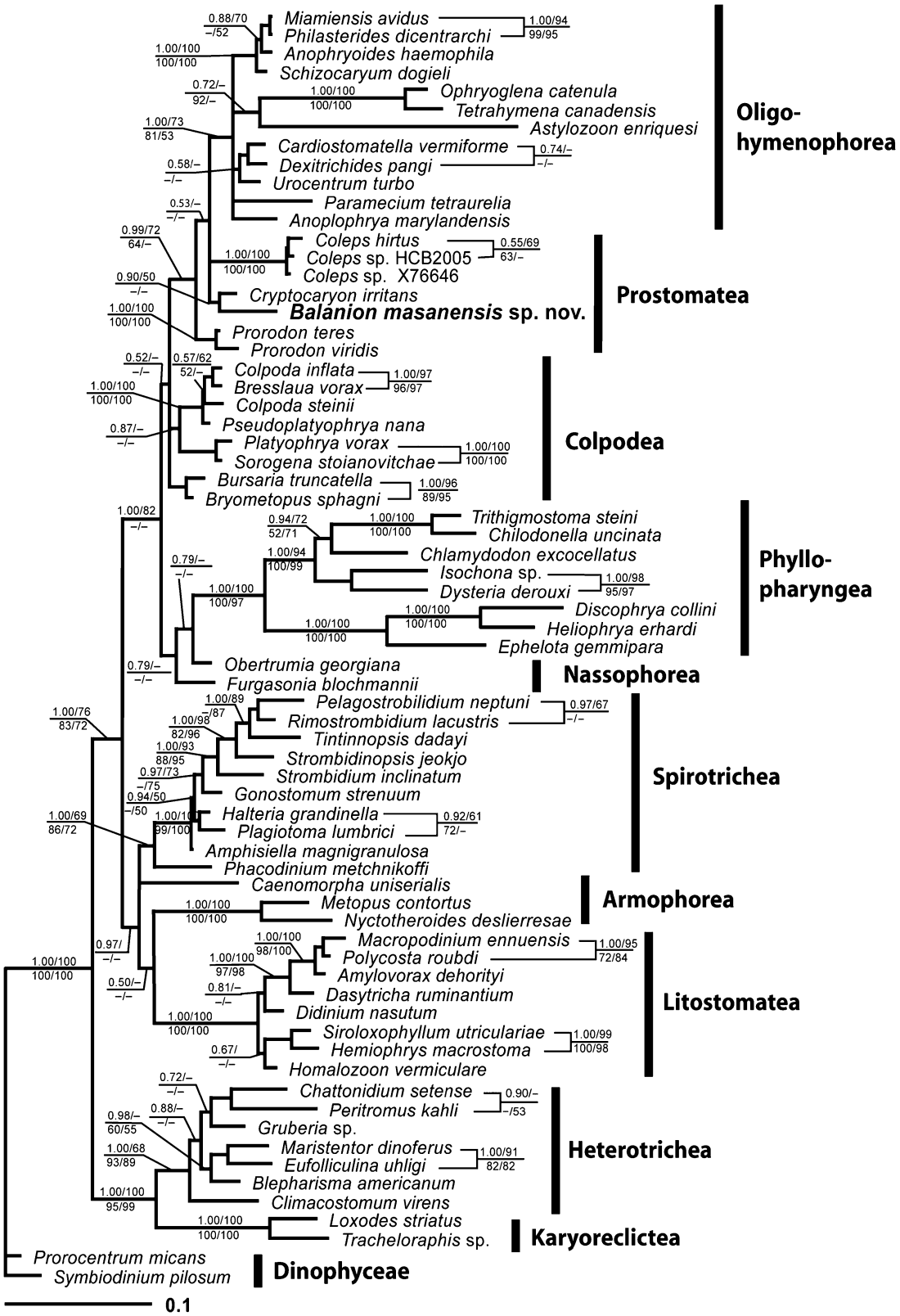

Fig. **47.** Consensus Bayesian tree ($-\ln L = 13{,}579.625$) based on 1,291 aligned positions with *Prorocentrum micans* and *Symbiodinium pilosum* as outgroup taxa. Numbers above branches indicate the posterior probability (left) from Bayesian analysis and bootstrap values (right) from maximum likelihood (ML) analysis. Numbers below branches indicate bootstrap values for neighbor-joining (NJ) (left) and maximum parsimony (MP) (right) analyses. Bootstrap values $\geq$ 50% and posterior probabilities $\geq$ 0.5 are shown.

Diagnosis. Ovoid with a flattened anterior oral region and a rounded or bluntly pointed posterior end when the cells are both alive and stained. Brosse inside the circumoral dikinetids, which are supported by circumoral microtubules and accompanied by elongate flaps. Cell length, cell width, and oral diameter of living cells are 27.1–43.3, 24.8–31.7, and 24.8–29.6 μm, respectively, while those of the QPS-stained specimens are 22.8–37.3, 26–5.4, and 26–33 μm, respectively. The numbers of oral dikinetids are 51–63. The six to nine brosse monokinetids are arranged into two different-sized kineties. The 51–63 SKs have 24–50 monokinetid per kinety.

Etymology. The specific epithet *masanensis* refers to the type locality—Masan Bay, Korea.

Deposition of type material. A hapantotype slide as USNM slide 1107776 of protargol-stained cells has been deposited in the Ciliate Type Specimen Slide Collection, US Natural History Museum, Smithsonian Institution, Washington, DC.

Gene sequence. The SSU rRNA gene sequence—GenBank Accession No. AM412525.

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