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ORIGINAL ARTICLE

Morphology, Morphogenesis and Molecular Phylogeny of a New Obligate Halophile Ciliate, *Schmidtiella ultrahalophila* gen. nov., spec. nov. (Ciliophora, Hypotrichia) Isolated from a Volcanic Crater on Sal (Cape Verde Islands)

Fengchao Li[a,b,†], Zhishuai Qu[b,c,†], Dan Luo[a], Sabine Filker[d], Xiaozhong Hu[c] & Thorsten Stoeck[b]

a College of Life Sciences, Hebei University, Baoding 071002, China
b Ecology Group, University of Technology Kaiserslautern, Kaiserslautern 67663, Germany
c Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China
d Molecular Ecology Group, University of Technology Kaiserslautern, Kaiserslautern 67663, Germany

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Correspondence
T. Stoeck, Department of Ecology, University of Technology Kaiserslautern, Kaiserslautern 67663, Germany
Telephone number: +49-631-205-2502;
FAX number: +49-631-205-2496;
e-mail: stoeck@rhrk.uni-kl.de

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†These authors contributed equally to this work.

ABSTRACT

A new hypotrichous ciliate, *Schmidtiella ultrahalophila* gen. nov., spec. nov., was isolated from a solar saltern on the island of Sal, Cape Verde. The possession of only one short dorsal kinety clearly distinguishes *S. ultrahalophila* from other known hypotrichous genera and species. Further diagnostic characters include: a flexible and slender body, an average size of 85 × 15 μm in vivo; a bipartite adoral zone with two hypertrophied frontal adoral membranelles and nine to twelve ventral adoral membranelles; three frontal, one parabuccal, two frontoventral, two or three postoral ventral, and two or three frontoterminal cirri; and marginal cirral rows variable in number, usually one on each side. Ontogenetic data indicate the following: the frontal-ventral cirri originate from six or five anlagen; the proter inherits the parental adoral zone; the frontal and ventral cirri originate from five or six anlagen; and the marginal cirral rows and the dorsal kinety tend to originate intrakinetally. Additional marginal rows are rarely derived from de novo anlagen. Based on its morphology, morphogenesis and its SSU rRNA phylogenetic placement, the new species should be assigned to the order Sporadotrichida Fauré-Fremiet, 1961. Due to low taxon sampling, however, its exact position in this order remains enigmatic.

PREVIOUS surveys have shown that hypotrichous ciliates are highly diverse and distributed in various habitats such as soil, freshwater, estuarine, marine, and even hypersaline environments (Berger 1999, 2006, 2008, 2011; Chen et al. 2017a,b, 2018; Harding and Simpson 2018; Kumar and Foissner 2016, 2017; Lei et al. 2009; Li et al. 2018; Liu et al. 2017; Luo et al. 2017; Lyu et al. 2018; Wang et al. 2017). Thus far, three hypotrichous genera have been reported from hypersaline habitats: *Apourosomoida* Foissner et al. 2002, *Cladotricha* Gaievskaïa, 1925, and *Schimidingerothrix* Foissner 2012. Hypotrichs in hypersaline habitats typically have a simple ciliature, that is, some of the typical hypotrich features such as buccal, pretransverse, transverse, caudal cirri, and dorsal bristle rows are lacking or vestigial (Foissner 2012; Foissner et al. 2014; Lu et al. 2017, 2018). For instance, *Schimidingerothrix* lacks dorsal bristle rows and a paroral membrane (Foissner 2012; Foissner et al. 2014). It is possible that the simplified ciliature is an adaptation to highly saline habitats, where the competition is low and the bacterial food is abundant (Foissner et al. 2014). The simplified ciliature may also be helpful in reducing buoyancy and resistance when swimming, possibly another adaptation to hypersaline environments. Owing to their morphological similarities to species caused by simple ciliature, systematic classification of hypersaline hypotrichs is challenging.

In order to investigate ciliates in hypersaline habitats, extensive sampling was conducted on the island of Sal, Cape Verde and a new hypotrichous ciliate was isolated from hypersaline water. The morphology and ontogeny of this species are described below. After comparison with related taxa, a new genus is suggested, primarily based

on its highly simplified cirral pattern. In addition, phylogenetic analyses derived from SSU rRNA gene sequences support the erection of a novel genus and illustrate its uncertain family assignment.

MATERIALS AND METHODS

Sample collection, morphological methods, and identification

The hypersaline water sample (salinity 280‰) was collected in September 2015, from an ancient solar saltern (Salina de Pedra de Lume) on the island of Sal, Cape Verde (16°46′22.88″N, 22°54′3.53″W; Fig. 1). Salinity was measured on site with a portable refractometer. For detailed culturing methods, see Pan and Stoeck (2017). Generally, the clonal culture of *Schmidtella ultrahalophila* gen. nov., spec. nov. was established and maintained in sterilized salt water (salinity about 250‰) at room temperature (about 20 °C) in the laboratory. The addition of two or three autoclaved wheat grains per 25 ml of culture medium served the enrichment of indigenous bacteria as food. Live observations were carried out using a bright field microscope equipped with differential interference contrast (Axioplan, Carl Zeiss, Jena, Germany). Scanning electron microscopy (SEM) was performed using a Supra 55 VP (Carl Zeiss, Jena, Germany) as described by Vd'ačnỳ and Foissner (2012). The protargol silver staining method of Wilbert (1975) was used to reveal the ciliature and the nuclear apparatus; protargol was synthesized for the most part according to Pan et al. (2013). Drawings of stained specimens were conducted at a magnification of 2,000X with the aid of a camera lucida. Measurements were made with an ocular micrometer. In order to illustrate the changes that occur during divisional morphogenesis, ciliary structures of parental cells were depicted by contours, whereas those of the daughter cells were shaded black. The terminology used herein is informed by that of Berger (2011), and the systematics follow those of Gao et al. (2016). Identification is primarily based on Foissner (2012), Foissner et al. (2014), and Lu et al. (2018).

DNA amplification and sequencing

DNA extraction and PCR amplification of the small subunit rRNA gene and phylogenetic analyses were conducted as previously described (Foissner et al. 2014). In short, genomic DNA was extracted from approximately twenty cells from pure culture, using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The SSU rRNA gene was amplified, using Phusion Taq (NEB, Ipswich MA). The primers used were the universal eukaryote specific primer pair EUKA-EUKB (Medlin et al. 1988). Bi-directional sequencing of cloned PCR-products (pMiniT 2.0 vector and the NEB PCR-cloning kit, New England Biolabs Inc) was carried out with the Big Dye Terminator Kit (Applied Biosystems, FosterCity, CA) on an ABI 3730 automated sequencer.

Phylogenetic analyses

All of the sequences used in the phylogenetic analyses were obtained from the GenBank database, with the exception of the SSU rRNA gene sequence of *Schmidtiella ultrahalophila* gen. nov., spec. nov. (See Fig. 2 for accession numbers of all sequences used in this study). Sequences were aligned on the European Bioinformatics Institute web server (http://www.ebi.ac.uk) using the MUSCLE package. The alignment was then edited manually using BioEdit. Manual editing included end-trimming and elimination of gap positions in individual sequences when sequences were highly conserved and identical over all remaining sequences in the alignment. This resulted in a final matrix of 1,723 characters. A Maximum-likelihood (ML) tree was constructed using RAxML-HPC2 v. 8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010) with the optimal model GTR + I + G (selected by Modeltest v.3.4) (Posada and Crandall 1998). A Bayesian inference (BI) analysis was carried out using the MrBayes 3.2.6 on XSEDE (Ronquist and Huelsenbeck

Figure 1 Sampling site in a solar saltern (Salina de Pedra de Lume) on the island of Sal, Cape Verde. (**A**) overview of the saltern area; (**B**) the sampling site.

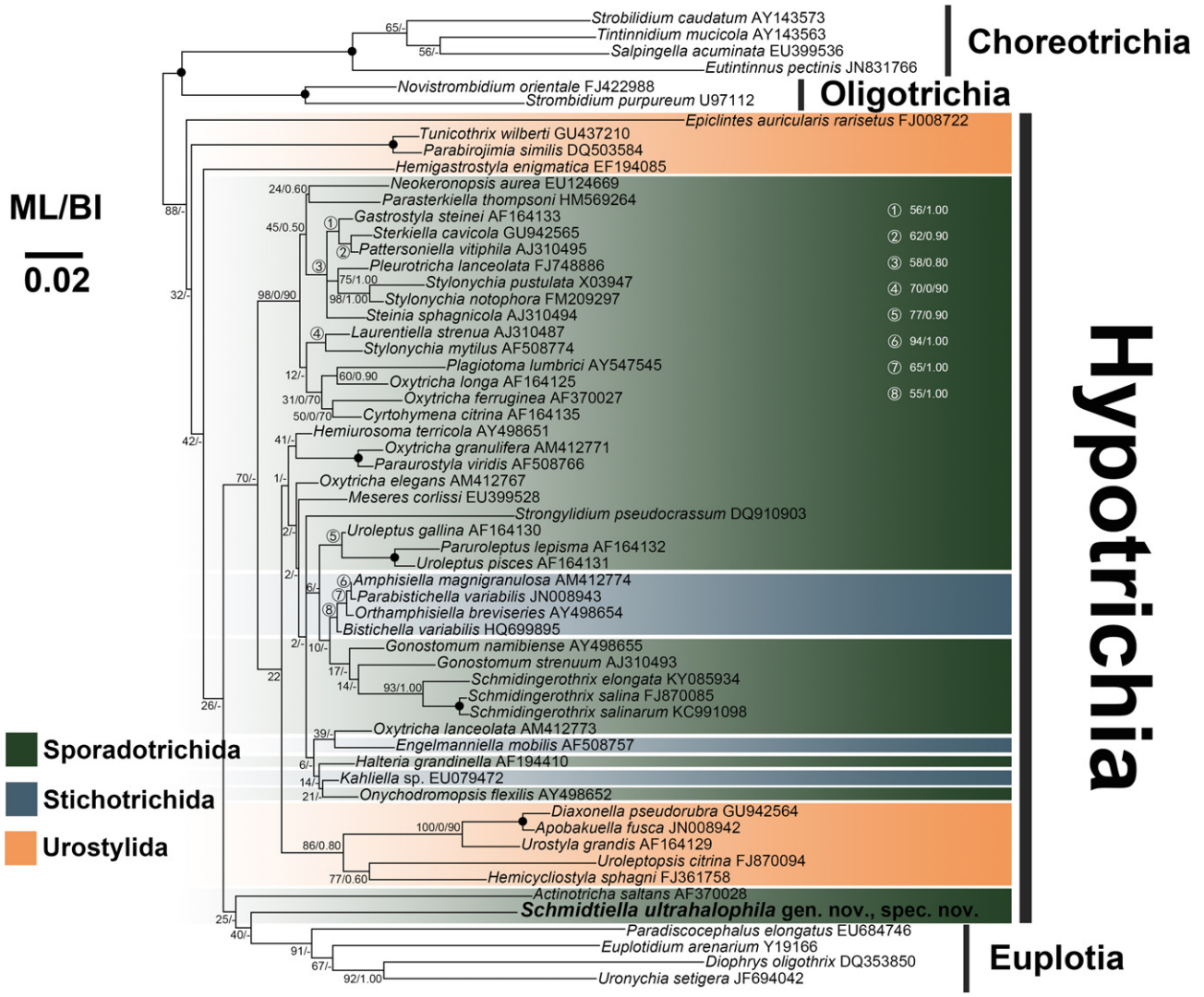

Figure 2 Maximum-likelihood tree derived from SSU rRNA gene sequences. The new taxon is in bold. Species from Choreotrichia and Oligotrichia were chosen as outgroup. Numbers at the nodes show the bootstrap values for ML and BI (ML/BI). "–" refers to disagreement in topologies of the ML and BI trees, and thus only the bootstrap values of ML are presented. The scale bar corresponds to two substitutions per 100 nucleotide positions (0.02).

2003) on the CIPRES Science Gateway with the model GTR + I + G (selected by MrModeltest 2.2) (Nylander 2004). Markov Chain Monte Carlo (MCMC) simulations were run for a million generations with a sample frequency of every 100th generation (the first 2,500 of each were discarded as burn-in). The number of chains run was four. All data are available from the authors upon request.

RESULTS

Morphological description

Cell size 75–110 × 12–22 μm in vivo (on average 85 × 15 μm; $n = 8$) and 70–105 × 15–30 μm after protargol staining ($n = 21$). Body slender and highly flexible, length:width ratio approximately 5.7:1 in vivo, as opposed to 3.7:1 in the protargol preparations; a pointed tail occupying 3–8% of cell length, usually turning rightwards (Fig. 3A, B, 4A, C). One or two (normally two) macronuclear nodules positioned along cell midline or slightly left of it, each 6–25 × 4–6 μm in size after protargol staining; usually one, sometimes two (four out of 21 specimens) globular micronuclei, 2–5 μm across after protargol impregnation, usually located between the macronuclear nodules (Fig. 3H, 4G). Contractile vacuole and cortical granules are absent. Cytoplasm colorless to greyish in bright field, filled with numerous colorless cytoplasmic granules and food vacuoles (Fig. 4A, B). Swims slowly. When swimming, the two hypertrophied frontal adoral membranelles (FM) swing powerfully, driving the body forward.

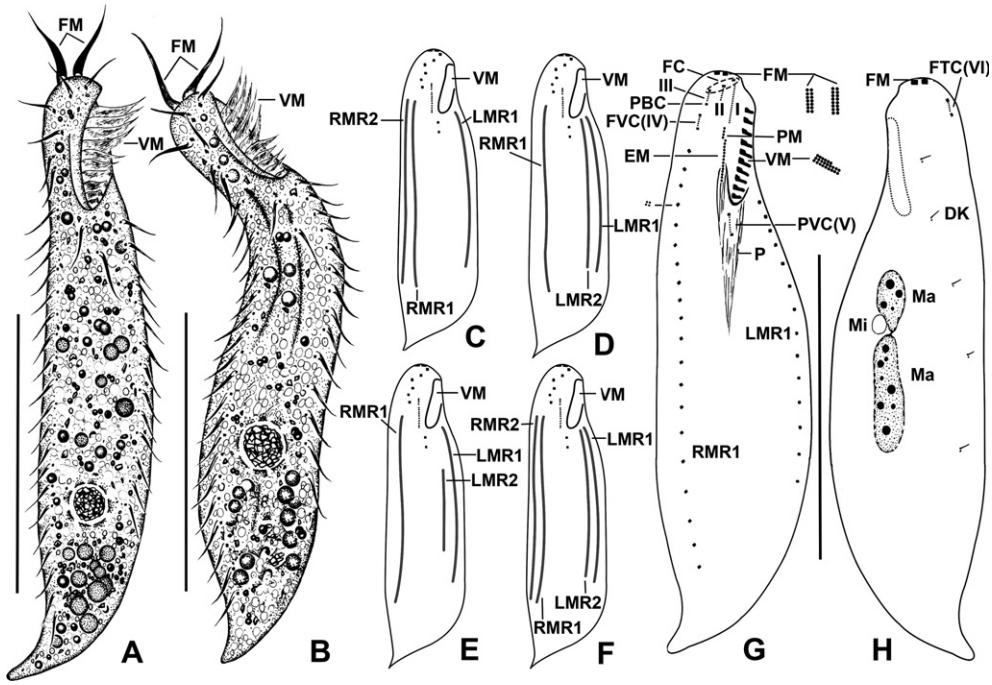

Figure 3 Morphology and ciliature of *Schmidtiella ultrahalophila* gen. nov., spec. nov. from life (**A, B**) and after protargol preparation (**C–H**). (A, B) Ventral views of representative individuals. (C–F) Different patterns of marginal cirral rows. (G, H) Ventral (G) and dorsal (H) views of the holotype specimen, showing ventral ciliature, nuclear apparatus, and dorsal kinety, cirri originating from the same anlage connected by broken line (G). DK = dorsal kinety; EM = endoral membrane; FC = frontal cirri; FM = frontal adoral membranelles; FVC = frontoventral cirri; FTC = frontoterminal cirri; LMR = left marginal row; Ma = macronuclear nodule; Mi = micronucleus; P = pharynx; PBC = parabuccal cirrus; PM = paroral membrane; PVC = postoral ventral cirri; RMR = right marginal row; VM = ventral membranelles; I–VI = number of the frantoventral cirri anlagen, to indicate the origination of the cirri. Scale bars = 40 μm.

Bipartite adoral zone composed of two frontal adoral and nine to 12 (on average 10) ventral membranelles (VM), occupying 15–20% of body length in vivo and about 25% in protargol preparations; these two parts are separated from each other by a distinct gap (Fig. 3A, G, 4D, F), about 4 μm in width. Base of frontal membranelle consists of two rows of kinetids, each with 6–8 basal bodies (Fig. 3G); cilia approximately 20 μm long in vivo. Base of ventral membranelle composed of two long rows (usually 9–10 basal bodies each) and one short row (usually six basal bodies) (Fig. 3G). Undulating membranes similar to *Gonostomum* pattern (Fig. 3G); paroral and endoral membranes nearly straight, composed of monokinetids; paroral membrane about 3.8 μm long in protargol preparations and generally composed of four cilia, usually terminates at right of anterior end of endoral membrane; endoral membrane on average 7.2 μm long, composed of 10–14 cilia; pharyngeal fibers clearly recognizable in protargol preparations, extending obliquely backwards (Fig. 3G, 4F).

Cirral pattern is simple due to lack of buccal, transverse, and caudal cirri (Fig. 3A, G, 4F, G, I). Continuously three frontal, one parabuccal, and two frontoventral cirri (FVC), whereas postoral ventral (PVC) and frontoterminal cirri (FTC) vary from two to three in number. FVC located posterior right of parabuccal cirrus, PVC lie behind buccal vertex, and FTC are on right anterior portion of dorsal side (Fig. 3G, H). One, sometimes two (six out of 21 specimens) right marginal cirral rows (RMR); numbers of cirri in RMR1 (inside row in two RMR specimens) and RMR2 (outside row) were 17.7 and 14.7 on average, respectively; one, occasionally two (two out of 21 specimens) left marginal cirral rows (LMR); LMR1 (outside row) and LMR2 (inside row) comprise 9–17 and 10–13 cirri, respectively (Fig. 3C–G); marginal cirri approximately 8–15 μm long in vivo; base of cirrus usually composed of four and rarely, two basal bodies. First (left) and second (middle) frontal cirri usually composed of six basal bodies and the third (right) frontal cirrus usually four; first frontal cirrus located near the left frontal adoral membranelle (Fig. 3G, 4F).

One dorsal kinety composed of four to nine bristles, begins at level of subapical, terminates slightly behind mid-body, extending as long as about 2/3 of the cell length (Fig. 3H, 4I, J). Dorsal cilia 2–4 μm long in vivo.

A live microscopy video of *Schmidtiella ultrahalophila* gen. nov., spec. nov. is provided as Supporting Information, revealing specific features of its morphology and movement.

Morphogenesis

Stomatogenesis and development of frontoventral cirri

Cell division commences with de novo-formation of several basal bodies immediately behind the posterior

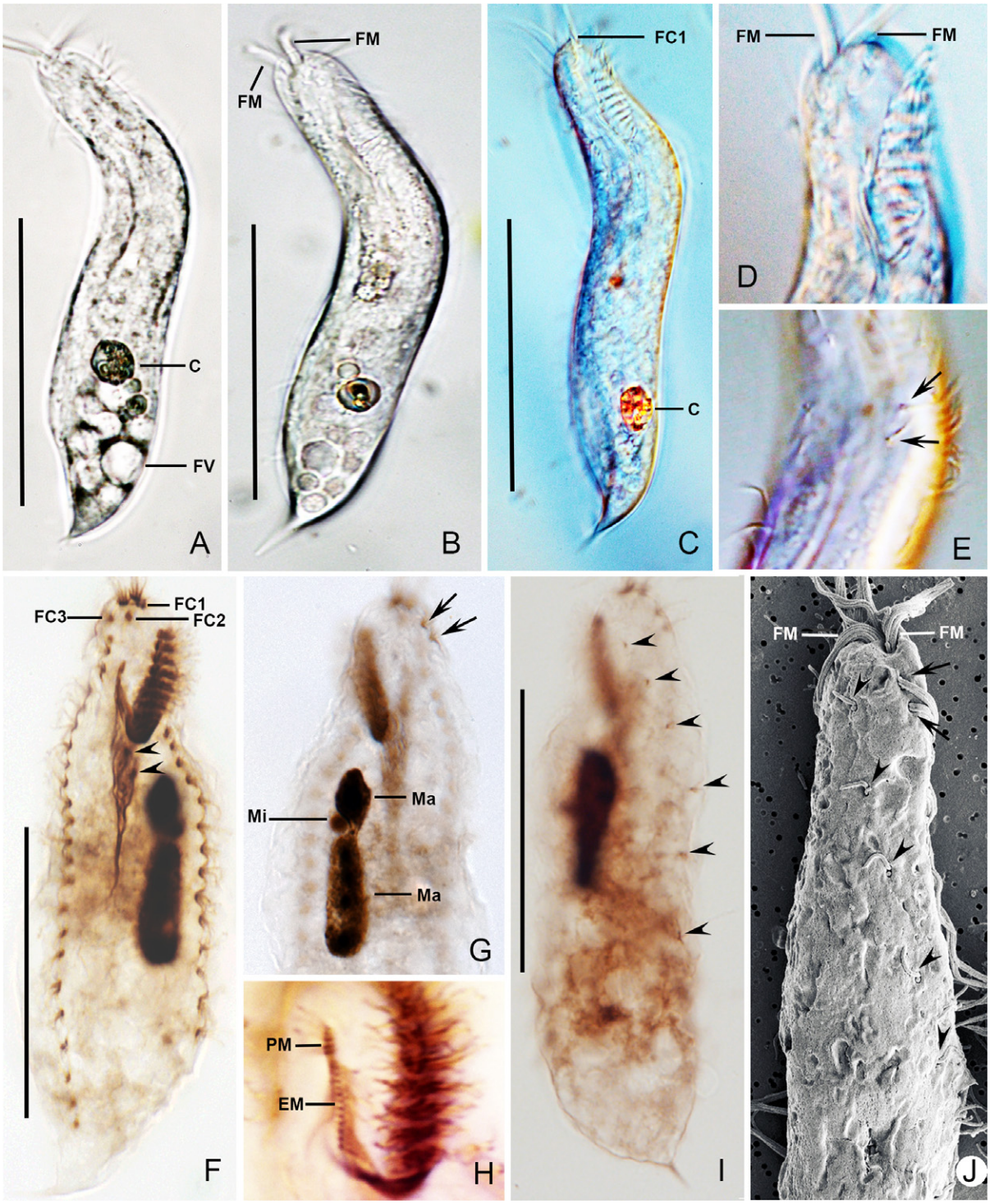

Figure 4 Photomicrographs of *Schmidtiella ultrahalophila* gen. nov., spec. nov. from life sample (**A**–**E**), after protargol preparation (**F**–**I**), and in scanning electron microscopy (**J**). Ventral (A, C) and dorsal (B) views of freely motile individuals, illustrating the flexibility of the body. (D) Ventral view of anterior body, showing the frontal adoral membranelles. (E) Shows the postoral ventral cirri (arrows). (F, G) Ventral and dorsal views of the holotype specimen, showing the organization of the ciliature and the nuclear apparatus; arrowheads in (F) indicate the postoral ventral cirri, arrows in (G) indicate the frontoterminal cirri. (H) Shows the paroral and endoral membranes. (I) Dorsal view, showing the dorsal kinety (arrowheads). (J) Dorsal view, showing the two frontal adoral membranelles, dorsal kinety (arrowheads), and the frontoterminal cirri (arrows). C = cytoplasmic crystal; EM = endoral membrane; FC1–3 = frontal cirri 1–3; FM = frontal adoral membranelles; FV = food vacuoles; Ma = macronuclear nodule; Mi = micronucleius; PM = paroral membrane. Scale bars = 40 μm.

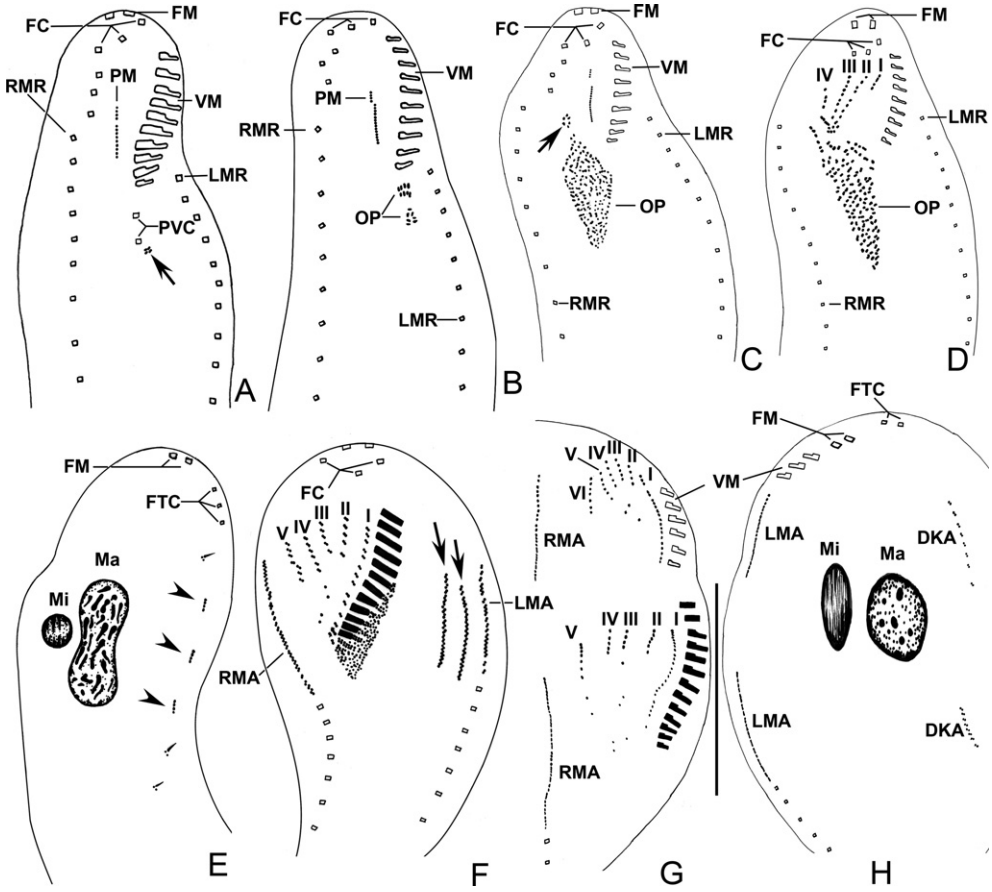

Figure 5 Morphogenesis of *Schmidtiella ultrahalophila* gen. nov., spec. nov. after protargol preparation (**A**–**H**). (A) Ventral view of a very early divider, showing newly formed group of basal bodies (arrow). (B) Shows the postoral ventral cirri modified to anlagen and incorporated in the formation of the OP. (C) Shows some basal bodies (arrow) occured in front of the OP. (D) Shows the formation of anlagen I–IV. (E) Dorsal side of an early middle divider, showing the reproduction of the dorsal kinety (arrowheads). (F) Ventral side of a middle reorganiser, showing the two de novo-formed extra anlagen (arrows). (G, H) Ventral and dorsal side of a divider, showing the proter has six anlagen (I–VI), but the opisthe has only five. DKA = dorsal kinety anlagen; EM = endoral membrane; FC = frontal cirri; FM = frontal adoral membranelles; FTC = frontoterminalcirri; LMA = left marginal row anlage; LMR = left marginal row; Ma = macronuclear nodule; Mi = micronucleus; OP = oral primordium; PVC = postoral ventral cirri; RMA = right marginal cirri anlage; RMR = right marginal row; VM = ventral membranelles; I–VI = frontoventral cirri anlagen I–VI. Scale bars = 40 μm.

postoral ventral cirrus (Fig. 5A, 6A). Then, two postoral ventral cirri dedifferentiate to form loosely arranged basal bodies. All of these basal bodies merge to form the oral primordium of the opisthe (OP; Fig. 5B, 6B).

The OP then becomes larger and extends forward due to further proliferation of basal bodies (Fig. 5C, D, 6C) which differentiate into new adoral membranelles (Fig. 5G, 7A, C, E, G). Simultaneously, a small group of basal bodies independently forms in front of the anterior end of the OP (Fig. 5C, 6C, D). Then, four streaks of frontoventral cirral anlagen (I–IV) are formed (Fig. 5D, 6E). Based on the position of anlagen and parental cirri, the origin of certain anlagen can be deduced as follows: anlage I develops from the parental paroral membrane; anlage II seems to be formed de novo; anlagen III and IV at least partly originate from parental PBC (III/2) and parental FVC, respectively; later, anlagen V and VI are formed (Fig. 6F), likely derived from the basal body group formed in front of the OP.

In middle dividers, six frontoventral cirri anlagen (I–VI) of the proter and opisthe are formed (Fig. 6J, K, 7A). Interestingly, we found that a middle divider has only five anlagen in the opisthe, but six in the proter (Fig. 5G, 6I), and a middle reorganizer also has five anlagen (Fig. 5F, 6P). Because late dividers with five anlagen were not detected, the manner of cirri generation is unknown. In six frontoventral cirri anlagen dividers, new cirri are generated in the following mode: anlage I develops the left frontal cirrus (I/1) and the undulating membranes; anlage II generates the middle frontal (II/1) cirrus; anlage III contributes the right frontal cirrus (III/1) and the parabuccal cirrus (III/2); anlage IV forms the frontoventral cirri (FVC); anlage V develops postoral ventral cirri (PVC); and anlage VI forms frontoterminal cirri (FTC) (Fig. 6N, O, 7A, C, E, G). Then, new PVC and FTC migrate to their final positions: the postoral area and dorsal side, respectively. The anlagen I–VI produce

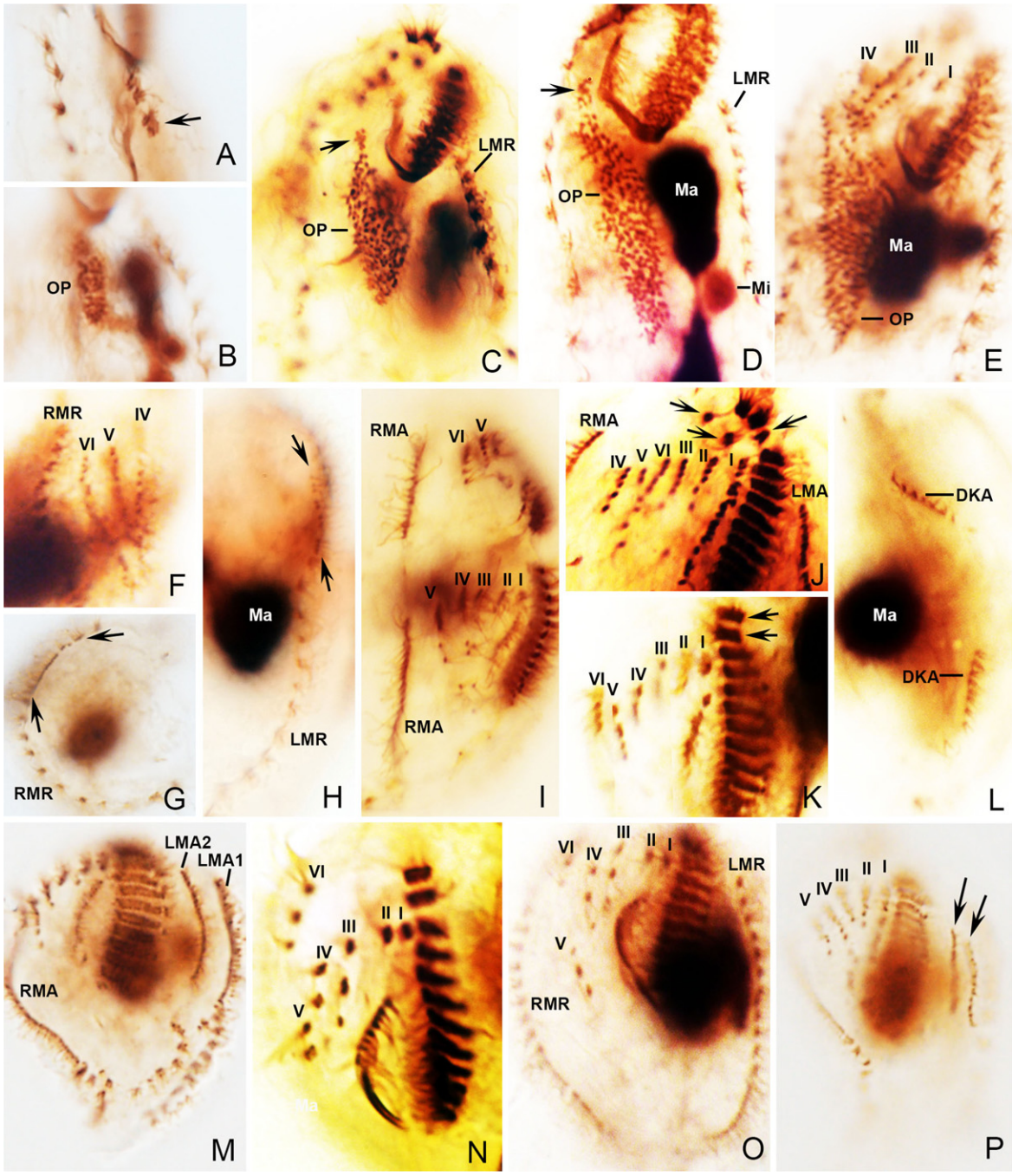

Figure 6 Morphogenesis of *Schmidtiella ultrahalophila* gen. nov., spec. nov. after protargol preparation (**A**–**P**). (A, B) Postoral region of very early dividers, showing the newly formed groups of basal bodies. (C, D) Ventral view of early dividers, showing some basal bodies occur in front of the oral primordium. (E) Ventral view of an early divider, showing the formation of frontoventral cirri anlagen of the proter (I–IV). (F) Shows the newly formed anlagen V and VI of an early middle divider. (G, H) Show one anlage (primary primordium) formed within the right (G) and left (H) marginal row of early middle dividers (between arrows). (I) Ventral view of an early middle divider, which has only five anlagen (I–V) in the opisthe. (J, K) Ventral views of a proter and an opisthe in middle stage, showing the six anlagen (I–VI); arrows in (J) mark the parental frontal cirri and in (K) mark the newly formed frontal adoral membranelles of the opisthe. (L) Dorsal view of a mid-divider, showing the newly formed dorsal kinety anlagen. (M) Ventral side of an opisthe in middle stage, showing the marginal cirri anlagen. (N, O) Ventral views of two opisthes in late stage, showing the newly formed ventral cirri and the migration of the postoral ventral cirri originating from the anlage V. (P) Ventral side of a reogranizer in middle stage, showing the two de novo-formed extra anlagen. DKA = dorsal kinety anlagen; LMA = left marginal row anlage; LMR = left marginal row; Ma = macronuclear nodule; Mi = micronucleus; OP = oral primordium; RMA = right marginal cirri anlage; RMR = right marginal row; I–VI = frontoventral cirri anlagen I–VI. Scale bars = 50 μm.

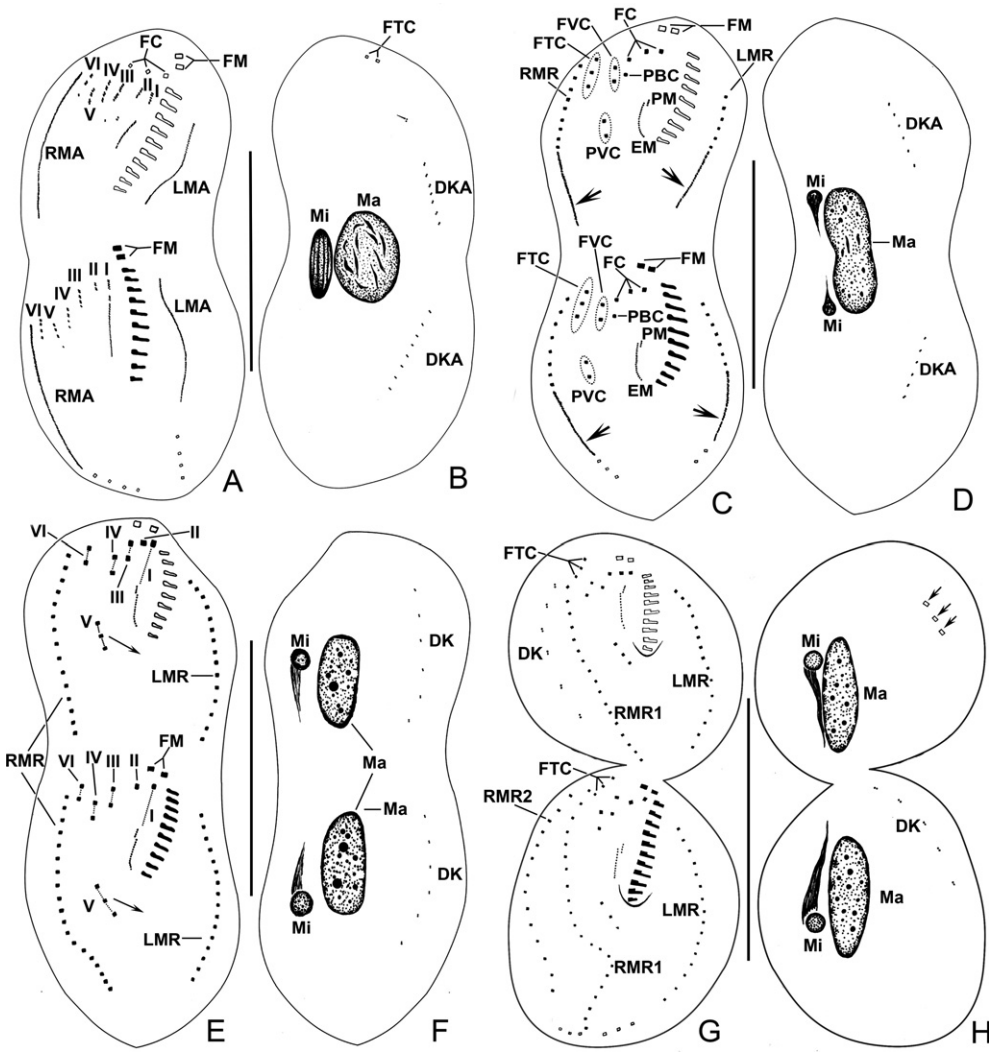

Figure 7 Morphogenesis of *Schmidtiella ultrahalophila* gen. nov., spec. nov. in middle and late stages (broken lines connect cirri originating from same anlage). (**A, B**) Show the six frontoventral cirri anlagen (I–VI) and dorsal kinety anlagen in a middle divider. (**C, D**) Ventral and dorsal views of a late divider, showing the newly formed cirri; arrows in (C) indicate the anlagen for the marginal rows. (**E, F**) Ventral and dorsal views of a very late divider, noting the origin of the new undulating membranes and the new ventral cirri; arrows in E indicate the final position of the cirri. (**G, H**) Ventral and dorsal views of a late divider, showing the proter has one right marginal row, but the opisthe has two marginal rows (RMR2 of the opisthe originates de novo); arrows in (H) indicate the parental FTC. DK = dorsal kinety; DKA = dorsal kinety anlagen; EM = endoral membrane; FC = frontal cirri; FM = frontal adoral membranelles; FVC = frontoventral cirri; FTC = frontoterminal cirri; LMR = left marginal row; Ma = macronuclear nodule; Mi = micronucleus; PBC = parabuccal cirrus; PM = paroral membrane; PVC = postoral ventral cirri; RMR = right marginal row; I–VI = frontoventral cirri anlagen I–VI. Scale bars = 40 μm.

the following number of cirri: 1:1:2:2:2(or 3):2(or 3) (Table 1).

Development of marginal cirri and dorsal kinety

Only several dividers observed, which show the following characteristics: only one marginal cirri anlage is formed in each parental marginal row, which develop into new cirri to replace the old ones (Fig. 5F, G, H, 6G, H, I, M, O, 7A, C, E, G); a primary primordium is formed within the middle portion of the parental dorsal kinety, then proliferates, splits and creates dorsal kinety anlagen for both proter and opisthe, which finally replace the old structures (Fig. 5E, H, 6L, 7B, D, F, H). A reorganizer was found with two de novo-formed left marginal cirri anlagen (Fig. 5F, 6P) and an opisthe of a late divider with an additional right marginal cirral row (Fig. 7G).

Division of nuclear apparatus

The nuclear apparatus divides in the standard way: the macronuclear nodules have a replication band in the earlier stages and then fuse to a single, globular mass in middle dividers. Sometime later, the fused

Table 1. Morphometric data of *Schmidtiella ultrahalophila* gen. nov., spec. nov.

Character	Min	Max	Med	Mean	SD	CV	*n*
Body, length (μm)	70	105	85	83.3	8.8	10.6	21
Body, width (μm)	15	30	25	23.4	3.9	16.9	21
Body, length: width ratio	2.8	6	3.5	3.7	0.8	21.6	21
Adoral zone of membranelles, length (μm)	15	25	20	20.4	2.7	13.2	21
Frontal adoral membranelles, number	2	2	2	2	0	0	21
Ventral membranelles, number	9	12	10	9.9	0.7	7.1	21
Total membranelles, number	11	14	12	11.9	0.7	5.9	21
Paroral, length (μm)	3	5	4	3.8	0.6	14.8	15
Endoral, length (μm)	6	8	7	7.2	0.6	7.8	15
Frontal cirri, number	3	3	3	3	0	0	21
Parabuccal cirrus, number	1	1	1	1	0	0	21
Cirri formed by anlage I (FC1), number	1	1	1	1	0	0	21
Cirri formed by anlage II (FC2), number	1	1	1	1	0	0	21
Cirri formed by anlage III (FC3 + PBC), number	2	2	2	2	0	0	21
Cirri formed by anlage IV (FVC), number	2	2	2	2	0	0	21
Cirri formed by anlage V (PVC), number	2	3	2	2.3	0.5	20.7	21
Cirri formed by anlage VI (FTC), number	2	3	2	2.0	0.2	10.6	21
Left marginal cirral rows, number	1	2	1	1.1	0.4	31.4	21
Cirri in left marginal cirral row 1, number	9	17	14	13.6	1.9	14.2	21
Cirri in left marginal cirral row 2 (inside row), number	10	13	12	11.7	1.5	13.1	2
Posterior end of left marginal row 1 to posterior body end, distance (μm)	15	25	17	18.1	3.6	19.9	15
Right marginal rows, number	1	2	1	1.2	0.4	33.8	21
Cirri in right marginal row 1, number	14	22	17	17.7	2.1	12.1	21
Cirri in right marginal row 2 (outside row), number	13	16	15	14.7	1.0	7.0	6
Posterior end of right marginal row 1 to posterior body end, distance (μm)	2	10	6	7	2.7	38.3	15
Dorsal kineties, number	1	1	1	1	0	0	21
Dikinetids in dorsal kinety, number	4	9	5	5.5	1.1	19.5	21
Posterior end of dorsal kinety to posterior body end, distance (μm)	18	40	30	29.6	6.5	21.9	21
Macronuclear nodules, number	1	2	2	1.8	0.4	24.8	21
First macronuclear nodule, length	6	20	12	12.7	4.1	32.1	15
Second macronuclear nodule, length	6	25	15	15.8	5.0	31.8	15
Macronuclear nodules, width (μm)	4	6	5	5.1	0.6	12.5	15
Micronuclei, number	1	2	1	1.2	0.4	33.8	21
Micronuclei, diameter (μm)	2	5	3	3.3	0.7	21.7	15

All data were measured from protargol-preparations.
CV = coefficient of variation in %; FC = frontal cirri; FVC = frontoventral cirri; FTC = frontoterminal cirri; HT = holotype specimen; Max. = maximum; Mean = arithmetic mean; Med. = median value; Min = minimum; *n* = number of specimens examined; PBC = parabuccal cirrus; PVC = postoral ventral cirri; SD = standard deviation; – = data not available.

macronucleus elongates and fragments during cytokinesis. The micronucleus divides by mitosis (Fig. 5E, H, 7B, D, F, H).

Fate of parental structures

The parental structures were not retained, except for the parental frontal adoral and ventral membranelles which remained unchanged during the morphogenetic process and are wholly inherited by the proter. During this divisional process, the parental frontal and frontoterminal cirri did not contribute to the formation of any anlagen and were resorbed after cell division.

SSU rRNA gene sequence

The SSU rRNA gene sequence of *Schmidtiella ultrahalophila* gen. nov., spec. nov. has been deposited in the GenBank database and is available under accession number MH792120. The length and GC content are 1,715 bp and 46.36%, respectively. The highest sequence similarity is to *Schmidingerothrix salina* isolate-LLQ0605232C (GenBank accession: FJ870085, sequence similarity: 93.18%). While *Schimidtiella* gen. nov. is vitally different from *Schmidingerothrix* (see discussion below) in morphology.

Both ML and BI phylogenetic inference present *Schmidtiella* gen. nov. as an individual branch at the base of the hypotrichs. Very low support values from both analyses (40 from ML, no statistical support from BI) indicate the uncertain position of this new species and genus within the hypotrichs. In an additional ML phylogenetic analysis with a restricted number of taxa, the new genus branches basal to *Schmidingerothrix* with low bootstrap support (26). In all phylogenetic analyses, *Schmidtiella*

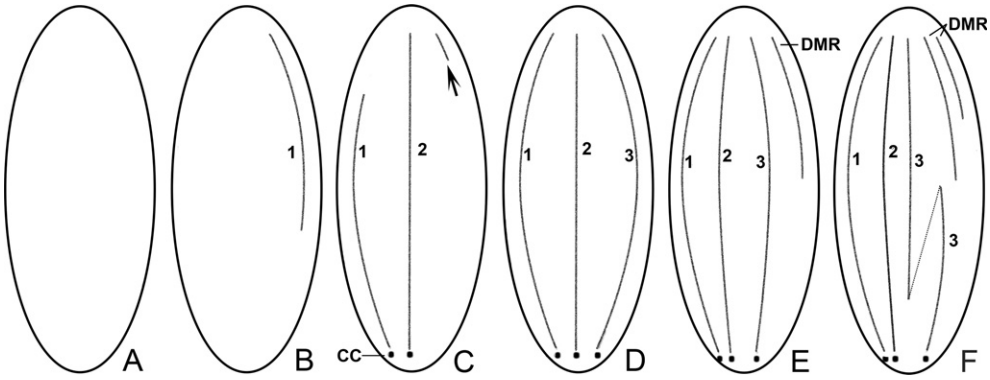

Figure 8 Comparison of the dorsal kinety pattern of *Schmidtiella* gen. nov. with related taxa (**A**, modified from Foissner 2012; **B**, present work; **C**, midified from Foissner et al. 2002; **D**–**F**, midified from Shao et al. 2012). (A) *Schmidingerothrix* pattern, dorsal kinety absent. (B) *Schmidtiella* pattern, one short row of dorsal kinety formed by intrakinetal proliferation. (C) *Apourosomoida* pattern, arrow marks a "third" dorsal kinety formed by some dislocated bristles of the dorsal kinety 2. (D) *Gonostomum* pattern (*Cladotricha* species also in this pattern), all three of the bipolar kineties developed by intrakinetal proliferation. (E) *Urosomoida* pattern, with three bipolar kineties (divided by intrakinetal proliferation) and a dorsomarginal row. (F) *Oxytricha* pattern, with bipolar kineties (1, 2), fragmentation of kinety 3 (anterior and posterior portions linked by dotted lines), and with dorsomarginal rows. CC = caudal cirri; DMR = dorsomarginal rows, originate from/near the right marginal row; 1–3 = dorsal kinety rows.

gen. nov. is attracted by the hypotrich *Oxytricha saltans*, likewise represented by a single individual branch.

Occurrence and ecology

As mentioned above, *Schmidtiella ultrahalophila* gen. nov., spec. nov. was found in hypersaline waters (salinity approximately 280‰). This species grew well with salinity between 200‰ and 280‰ in the lab and a temperature of around 20 °C. The attempt of culture at lower salinity (100‰) failed and the population gradually disappeared over the course of one week. The species feeds on bacteria.

DISCUSSION

Establishment of the new genus *Schmidtiella* gen. nov.

The subclass Hypotrichia includes three orders: (1) Stichotrichida, whose cirri are arranged, often in many linear files; (2) Sporadotrichida, in which cirri are distributed "sporadically" in conspicuous frontal, ventral, and transverse groupings; and (3) Urostylida, in which the frontoventral cirri are arranged in two or more zig-zag files on the ventral surface (Lynn 2008). Based on the ventral cirral pattern, particularly the absence of long ventral cirral rows, we assign the new genus to class Spirotrichea Buetschli 1889, Subclass Hypotrichia Stein, 1859, order Sporadotrichida.

Schmidtiella ultrahalophila gen. nov., spec. nov. is a heterotrophic obligate halophile, which possesses the distinctive feature of having only one short kinety on the dorsal side (bipolar and dorsomarginal kineties absent), which is unique among known hypotrichs (Fig. 8). Thus a new genus, *Schmidtiella*, is suggested.

Schmidtiella gen. nov. has another characteristic feature, viz., its *Gonostomum*-like oral apparatus. As a result, a comparison with hypotrichs that possess such an oral apparatus is required. Lu et al. (2018) recently provided a detailed review of hypotrichs with a *Gonostomum*-like apparatus pattern. Of those, *Schmidtiella* gen. nov. closely resembles *Apourosomoida* Foissner et al. 2002, *Cladotricha* Gaievskaïa, 1925, and *Schmidingerothrix* Foissner 2012, in that all of them have a slender body, simple cirral pattern, and a bipartite adoral zone of membranelles. However, there are still sufficient features to distinguish the new genus from the three genera above (Table 2).

Apourosomoida also comes from a highly saline habitat (soil), has frontal cirri, and its postoral ventral cirri originated from the same anlage (Foissner et al. 2002). However, *Apourosomoida* possesses buccal and transverse cirri and two dorsal kinety rows associated with one caudal cirrus each at the posterior end, thus it can be easily separated from the new genus. *Schmidtiella* gen. nov. differs from *Cladotricha* due to the number of dorsal kineties (one short row vs. three kineties), the absence of caudal cirri (vs. presence), and the absence of the long frontoventral rows (vs. presence) (Berger 2011). *Schmidtiella* gen. nov. can be separated from *Schmidingerothrix* because it has a dorsal kinety, paroral membrane, and postoral ventral cirri (vs. all absent in the latter), and lacks long ventral cirral row and cortical granules (vs. both present in the latter) (Foissner 2012; Foissner et al. 2014; Lu et al. 2018).

Schmidtiella gen. nov. is very similar to *Erimophrya* Foissner et al. 2002, as it possesses a slender body shape; postoral ventral cirri arranged in line; has frontal frontoventral cirri; a relatively simple cirral pattern, viz., without a long frontoventral cirral row and pretransverse ventral cirri. But the new genus can be distinguished from the latter as it lacks a buccal cirrus (vs. one), transverse

Table 2. Morphometric comparison of the *Schmidtiella* gen. nov. with other related genera

Character	*Schmidtiella* gen. nov.	*Schimidingerothrix*	*Cladotricha*	*Apourosomoida*
Buccal cirri	×	×	√	√
Paroral membrane	√	×	√	√
Long ventral cirral row	×	√	√	×
Transverse cirri	×	×	×	√
Dorsal kineties	√(one short row)	×	√(three bipolar rows)	√(two rows)
Caudal cirri	×	×	√	√
References	Present work	Foissner (2012)	Berger (2011)	Foissner et al. (2002)

× structures absent; √structures present.

cirri (vs. presence), caudal cirri (vs. two), and a dorsomarginal kinety (vs. presence), and also has a different habitat (hypersaline water vs. nonsaline soil) (Foissner et al. 2002).

Circinella Foissner, 1994 is also similar to *Schmidtiella* gen. nov. because both have a bipartite adoral zone of membranelles, possessing frontal cirri, and lack transverse and caudal cirri (Foissner 1994). However, *Circinella* differs from the latter by having a ventral cirral row longer than the adoral zone of membranelles (vs. absence), lacking postoral cirri (vs. presence), and having more than one bipolar dorsal kinety (vs. one short row).

Morphogenesis

The morphogenesis of the dorsal kinety in *Schmidtiella ultrahalophila* gen. nov., spec. nov. shows it lacks both dorsomarginal kineties and oxytrichid type kinety fragmentation, indicating that it does not belong to the dorsomarginalian part of the Hypotrichia.

Usually, most hypotrichs generate new marginal rows in the standard way, that is, two primordia originate within each parental row (Berger 2011). The new taxon forms one marginal row on each side in this way. However, some interphase specimens show an additional marginal row, whose origin is uncertain at present owing to a lack of critical divisional stages. It could be derived from an extra anlage formed de novo as shown in some reorganizers (Fig. 5F, 6P). Alternative explanations cannot be excluded, for example, that perhaps it is resulting from the remaining parental marginal cirri.

Regarding its length and position, the short dorsal kinety in *Schmidtiella ultrahalophila* gen. nov., spec. nov. looks like a dorsomarginal kinety, but the dorsal morphogenesis shows that this is not the case, owing to the fact that the new dorsal kinety originates within the parental kinety.

The frontoventral cirri of *Schmidtiella ultrahalophila* gen. nov., spec. nov. usually originates from six frontoventral anlagen (Fig. 6J, K, 7A, C, E). However, in some specimens five anlagen can occur (Fig. 5F, G, 6I, P). Six anlagen are an old feature already present in the ground pattern of the hypotrichs (Berger 2011). Considering *Gonostomum* species also have six or five frontoventral anlagen, *S. ultrahalophila* gen. nov., spec. nov. may be cosely related to *Gonostomum* due to these characteristics.

Unfortunately, the exact origin of the opisthe's frontoventral anlagen is unclear. Based on the early stage dividers (Fig. 5D, E, 6E, F, I), it is, however, reasonable to assume that anlagen V and VI are common anlagen (primary primordia) for proter and opisthe, because the anlagen are relatively long and no anlagen of the opisthe are formed in early middle dividers.

Schmidtiella ultrahalophila gen. nov., spec. nov. usually has six frontoventral cirri anlagen, three frontal cirri, and anlage I forms the left frontal cirrus and undulating membranes, which are common for 18-cirri hypotrichs (e.g. *Oxytricha*). In contrast, the frontoventral cirri anlagen develop far less cirri in the new taxon than those in the 18-cirri hypotrichs, which may be an apomorphy for taxa from supersaline habitat (Berger 1999; Shao et al. 2015).

Berger (2011) stated that the last common ancestor of the hypotrichs is gonostomatids, with three bipolar kineties each bearing a caudal cirrus. Based on the *Gonostomum*-like oral apparatus pattern and morphogenetic characters as well as reduced cirri on the ventral side and fewer dorsal kineties, we speculate that *Schmidtiella ultrahalophila* gen. nov., spec. nov. could be evolved from a gonostomatidae ancestor.

Phylogenetic analyses

Phylogenetic analyses show that any classification of the new genus into a known (previously sequenced) family is not possible, based on available gene database information. Long-branch attraction is a common observation for taxa with unavailable relatives in phylogenetic analyses: uncertain taxa tend to be positioned in the periphery because of biases in available taxa (Wiens 2005). This explains the unsupported branching positions of *Schmidtiella ultrahalophila* gen. nov., spec. nov. within the Sporadotrichida (Fig. 2). Another possible explanation is that the resolution of the available phylogenetic marker is low. Thus, the new genus *Schmidtiella* gen. nov. assigned to Sporadotrichida is mainly based on the morphological characters, though not supported by phylogenetic analyses of the traditional marker gene used in ciliate phylogeny. Different genes (or a set thereof) in combination with an extended taxon sampling for these genes may possibly confirm this assignment.

TAXONOMIC SUMMARY

Ciliophora Doflein, 1901
Spirotrichea Bütschli, 1889
Hypotrichia Stein, 1859
Sporadotrichida Fauré-Fremiet, 1961

Schmidtiella gen. nov.

Diagnosis. Hypotrichs with *Gonostomum*-like oral apparatus. With frontal, parabuccal cirri, postoral ventral, and frontoterminal cirri. Buccal, pretransverse, transverse, and caudal cirri are absent. One short dorsal kinety, originated intrakinetally during morphogenesis.

Type species. *Schmidtiella ultrahalophila* spec. nov.

Etymology and dedication. *Schmidtiella* is a composite of the surname Schmidt, the thematic vowel *"i"*, and the diminutive suffix *-ella*. Feminine gender. We dedicate this new genus to Prof. Dr. Helmut J. Schmidt, president of University of Technology Kaiserslautern, for his outstanding contributions to the field of ciliate ecology.

Schmidtiella ultrahalophila spec. nov.

Diagnosis. Size 75–110 × 12–22 μm in vivo and 70–105 × 15–30 μm after protargol preparation. Body slender and flexible, with pointed posterior end. Usually two macronuclear nodules, one or two micronuclei. Cortical granules are absent. Three frontal, one parabuccal, two frontoventral, two or three postoral ventral, two or three frontoterminal cirri. Frontoterminal cirri located on the dorsal side. One or two left and right marginal rows, respectively. Adoral zone composed of two strong frontal and usually 10 ventral membranelles.

Type locality. An ancient solar saltern (Salina de Pedra de Lume) on the island of Sal, Cape Verde (16°46′22.88″N, 22°54′3.53″W). The salinity was approximately 280‰.

Etymology. The species name *ultrahalophila* is a composite of "*ultra-*" (very, super), the Greek words "*halós*" (salt) and "*philos*" (loving, preferring, friendly), and the suffix *–a*. It refers to the ultra-haline water body (280‰) where the species was discovered.

Type material. The protargol slide containing the holotype specimen (LFC2017032804A) and three paratype slides (LFC2017032804B–F) have been deposited in the Laboratory of Hydrobiology, Hebei University, China. One protargol slide with the paratype specimen has been deposited in the Natural History Museum, London, with the registration number of NHMUK 2018.10.26.1. The relevant specimens have been marked and labeled by black ink circles on the coverslips.

ZooBank LSID of this paper. urn:lsid:zoobank.org:act:B7D6BEF3-3FB7-4686-B8B3-F59393383C65

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Movie S1. Live microcsopy video imaging of *Schmidtiella ultrahalophila* gen. nov., spec. nov.