*Zoological Journal of the Linnean Society*, 2017, **179**, 475–491. With 5 figures

# Taxonomy and molecular phylogeny of two new brackish hypotrichous ciliates, with the establishment of a new genus (Ciliophora, Spirotrichea)

XIAOTIAN LUO[1,2†], FENG GAO[2†], ZHENZHEN YI[1]*, YING PAN[3], SALEH A. AL-FARRAJ[4] and ALAN WARREN[5]

[1]*Guangzhou Key Laboratory of Subtropical Biodiversity and Biomonitoring, School of Life Science, South China Normal University, Guangzhou 510631, China*
[2]*Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China*
[3]*Department of Biology & Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China*
[4]*Zoology Department, King Saud University, Riyadh 11451, Saudi Arabia*
[5]*Department of Life Sciences, Natural History Museum, London SW7 5BD, UK*

*Received 14 July 2015; revised 8 March 2016; accepted for publication 18 April 2016*

One new genus and two new species of hypotrich ciliates isolated from brackish-water habitats in China were investigated. The new genus ***Polystichothrix* gen nov.** is defined by having a continuous adoral zone and more than 18 frontal-ventral-transverse cirri; undulating membranes in *Oxytricha*-pattern; three clearly differentiated frontal cirri; frontal and ventral cirri aligned in rows; one marginal cirral row on each side of the cell; more than three dorsal kineties; buccal cirrus and transverse cirri present. ***Polystichothrix monilata* sp. nov.** can be recognized by having a citrine-brownish cell colour caused by its citrine-yellow cortical granules; two frontal rows with a frontoventral cirrus to the left of them; four or five postoral ventral cirri; four or five short ventral rows; five dorsal kineties; about eight moniliform macronuclear segments. ***Lamtostyla ovalis* sp. nov.** is characterized by an ovoidal, flexible body; an amphisiellid median cirral row comprising about 11 cirri; about four frontoventral cirri; two pretransverse ventral and five transverse cirri; three dorsal kineties. We also provide the first record of the *small subunit ribosomal RNA* gene sequences for the genera ***Lamtostyla*** and ***Polystichothrix* gen. nov.** Phylogenetic analyses show that ***Pol. monilata* sp. nov.** is a member of the family Oxytrichidae, and that ***L. ovalis* sp. nov.** should be assigned to the family Amphisiellidae, although neither family is monophyletic.


doi: 10.1111/zoj.12451

ADDITIONAL KEYWORDS: ***Lamtostyla ovalis*** – new species – ***Polystichothrix monilata*** – *small subunit rRNA* gene – systematics.

## INTRODUCTION

Hypotrichs, with their highly diverse cortical structures and nuclear apparatus, play a major role in enhancing our understanding of the systematics and evolutionary relationships amongst ciliates (Berger, 1999, 2006, 2008, 2011; Song, Warren & Hu, 2009). Recent faunistic studies have revealed numerous new taxa of hypotrichous ciliates, suggesting that this group is even more diverse than previously supposed (Foissner, Filker & Stoeck, 2014; Heber, Stoeck & Foissner, 2014; Jung, Park & Min, 2014; Kim *et al.*, 2014; Kumar *et al.*, 2014, 2015; Küppers, 2014; Lu *et al.*, 2014; Shao *et al.*, 2014a,b; Hu & Kusuoka, 2015; Singh & Kamra, 2015). Moreover, much work has been carried out on the morphogenesis and molecular phylogeny of hypotrichs, which has led to a better understanding of their systematics and the evolutionary relationships amongst them (Yi *et al.*, 2009; Chen, Huang & Song, 2011; Miao *et al.*, 2011; Song *et al.*, 2011; Yi & Song, 2011;

*Corresponding author. E-mail: zyi@scnu.edu.cn
†These authors contributed equally.

Huang, Dunthorn & Song, 2012; Chen *et al.*, 2014; Fan *et al.*, 2014a,b,c; Huang *et al.*, 2014; Luo *et al.*, 2015; Lv *et al.*, 2015; Wang *et al.*, 2015; Zhao *et al.*, 2015).

One of the major groups of hypotrichous ciliates is the family Oxytrichidae Ehrenberg, 1838, which is defined as follows: usually with 18 frontal-ventral-transverse cirri clustered into six distinct groups (i.e. three frontal, four frontoventral, one buccal, three postoral ventral, two pretransverse ventral, and five transverse cirri), which originate from six longitudinal streaks and/or at least one fragmenting dorsal kinety, usually kinety 3, although fragmentation has been secondarily lost in some taxa (Berger, 1999). However, there are oxytrichids with more than 18 frontal-ventral-transverse cirri, i.e. *Ancystropodium* Fauré-Fremiet, 1907, *Apoamphisiella* Foissner, 1997, *Apoterritricha* Kim *et al.*, 2014, *Gastrostyla* Engelmann, 1862, *Hemigastrostyla* Song & Wilbert, 1997, *Kerona* Muller, 1786, *Laurentiella* Dragesco & Njine, 1971, *Onychodromopsis* Stokes, 1887, *Onychodromus* Stein, 1859, *Paraparentocirrus* Kumar *et al.*, 2014, *Paraurostyla* Borror, 1972, *Parentocirrus* Voss, 1997, *Pattersoniella* Foissner, 1987, *Styxophrya* Foissner *et al.*, 2004, and *Territricha* Berger & Foissner, 1988, and in some of these (e.g. *Paraurostyla*, *Pattersoniella*, *Territricha*, and *Styxophrya*) the frontal-ventral-transverse cirri originate from more than six streaks (Berger, 1999; Kim *et al.*, 2014; Kumar *et al.*, 2014).

The family-level classification of the hypotrich genus *Lamtostyla* has been ambiguous since it was established by Buitkamp (1977), mainly because of the lack of morphogenetic information for the type species and of molecular phylogenetic information for any species in the genus. Buitkamp (1977) assigned *Lamtostyla* to the family Holostichidae, a placement that was accepted by Corliss (1979) and Tuffrau (1979). Small & Lynn (1985) classified *Lamtostyla* in the family Cladotrichidae, whereas Lynn & Small (2002) assigned it to Trachelostylidae. Jankowski (1979) placed it in Oxytrichidae. Petz & Foissner (1996) were the first to include *Lamtostyla* in the family Amphisiellidae, which was accepted by Shi (1999), Berger (2008), and Lynn (2008). In his comprehensive review of the family Amphisiellidae, Berger (2008) recognized 12 species of *Lamtostyla*.

In the current study, we provide descriptions of two new hypotrichous species, *Polystichothrix monilata* sp. nov. (which has more than 18 frontal-ventral-transverse cirri) and *Lamtostyla ovalis* sp. nov. In addition, the *small subunit ribosomal RNA* (*SSU rRNA*) gene of the two novel species was sequenced, and molecular phylogenetic analyses based on *SSU rRNA* gene sequence data were performed to evaluate their systematic relationships.

## MATERIAL AND METHODS

### Collection, cultures, and microscopy methods

*Polystichothrix monilata* sp. nov. was collected on 7 December 2013 from Zhanjiang Mangrove National Nature Reserve (21°55′N, 109°75′E), Guangdong Province, southern China. The water temperature was 23 °C and the salinity 15 psu. *Lamtostyla ovalis* sp. nov. was collected on 28 September 2010 from Zhan Qiao Pier in Qingdao (36°06′N, 120°31′E) on the Yellow Sea coast of Shandong Province, northern China. The water temperature was 21 °C and the salinity 20 psu. Samples comprised a mixture of water and sediments with organic debris, collected directly from small puddles.

Cultures from fresh samples of both isolates were maintained in Petri dishes at a temperature of 24 °C using habitat water with rice grains to enrich the growth of bacterial food for the ciliates. Living cells were isolated from raw cultures using micropipettes and observed with bright field and differential interference contrast microscopy. The protargol staining method of Wilbert (1975) was used to reveal the infraciliature and nuclear apparatus. *In vivo* measurements were taken at 40–1000 × magnifications. Counts and measurements of the stained specimens were carried out at a magnification of 1000 ×. Drawings of stained specimens were performed at a magnification of 1250 × with the help of a camera lucida and photomicrographs. Terminology is according to Berger (1999, 2006, 2008).

### Amplification of DNA and molecular phylogeny methods

In order to remove potential contamination prior to molecular analyses, cells were washed four times in filtered (0.22 μm) habitat water using a micropipette. One to several cells were then transferred to a 1.5 mL microfuge tube with a minimum volume of water. Genomic DNA was extracted using the Dneasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The *SSU rRNA* gene was amplified using the primers 18s-F (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and 18s-R (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) (Medlin *et al.*, 1988). Cloning and sequencing were performed according to Huang *et al.* (2014).

The *SSU rRNA* gene sequences of *Pol. monilata* sp. nov. and *L. ovalis* sp. nov. were aligned with sequences of 82 other hypotrich taxa downloaded from the National Center for Biotechnology Information (NCBI) Database. The strombidiids *Parastrombidinopsis minima*, *Novistrombidium orientale*, *Strombidinopsis acuminata*, and *Strombidium*

*apolatum* were selected as outgroup taxa. NCBI accession numbers are provided after the species names in the phylogenetic tree (Fig. 5). All sequences were aligned using MUSCLE implemented on the website http://www.ebi.ac.uk/Tools/msa/muscle/. The resulting alignment was manually edited using the program BIOEDIT 7.2.5 (Hall, 1999) and both ends of the alignment were trimmed. The final alignment used for phylogenetic analyses included 1767 positions and 88 taxa. Maximum likelihood (ML) analysis, with 1000 bootstrap replicates, was carried out using RAxML-HPC2 on XSEDE v. 8.1.11 (Stamatakis, Hoover & Rougemont, 2008) on the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010) with the GTR + I + G (general time reversible + invariable sites + gamma) model selected by MODELTEST v. 3.4. Bayesian inference (BI) was performed using MrBayes 3.2.3 on XSEDE (Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway with the model GTR + I + G selected by MrModeltest v. 2 according to the Akaike information criterion (Nylander, 2004). Markov chain Monte Carlo simulations were run for 6 000 000 generations with a sampling frequency of 100 and a burn-in of 6000 trees. The remaining trees were used to calculate the posterior probabilities with a majority-rule consensus. Tree topologies were visualized using MEGA 6.0 (Tamura *et al.*, 2007). The systematic classification follows Lynn (2008) and Adl *et al.* (2012).

## RESULTS

### Subclass Hypotrichia Stein, 1859
### Order Sporadotrichida Fauré-Fremiet, 1961
### Family Oxytrichidae Ehrenberg, 1830
### Genus *Polystichothrix* gen. nov

*Diagnosis*
Oxytrichid *sensu* Berger (2006) with continuous adoral zone and more than 18 frontal-ventral-transverse cirri; undulating membranes in *Oxytricha*-pattern; three clearly differentiated frontal cirri; buccal cirrus and transverse cirri present; frontal and ventral cirri aligned in rows; one marginal cirral row on each side of cell; more than three dorsal kineties.

*Etymology*
*Polystichothrix* is a composite of the Greek adjective 'poly' (many), the Greek noun 'sticho' (row), and the Greek noun 'thrix' (hair, ciliate) and alludes to the elevated number of cirral rows. Feminine gender.

*Type species*
***Polystichothrix monilata* sp. nov.**

### *Polystichothrix monilata* sp. nov.
(Figs 1A–G, 2A–T; Table 1)

*Diagnosis*
Body flexible; 125–155 × 25–35 μm *in vivo*; citrine-yellow cortical granules about 1 μm in diameter; about 35 adoral membranelles; one buccal cirrus, one anterior and one posterior frontal row with a frontoventral cirrus to the left of them; four or five postoral ventral cirri; four or five ventral rows; two pretransverse ventral cirri; *c.* seven transverse cirri; on average 37 left and 38 right marginal cirri; five dorsal kineties, dorsal kinety 4 starting at level of buccal vertex; about eight macronuclear segments; two micronuclei; brackish water habitat.

*Type locality and ecology*
Zhanjiang Mangrove National Nature Reserve (21°55′N, 109°75′E), Guangdong Province, China. Water temperature 23 °C, salinity 15 psu.

*Type specimens*
A protargol slide containing the holotype specimen (see Figs 1B, C, 2J, K; registration no. LXT2013120701/1) and one paratype slide (registration no. LXT2013120701/2) were deposited in the Laboratory of Protozoology, Ocean University of China (OUC). A second paratype slide was deposited in the Natural History Museum, London (registration number NHMUK 2015.7.10.1).

*Etymology*
The species-group name *monilata* is a composite of the Latin noun 'monile' (necklace) and the suffix 'ata' (having a feature) and refers to the moniliform macronucleus.

*Description*
Body 125–155 × 25–35 μm *in vivo*, flexible but not contractile, sometimes slightly twisted, elongate, generally elliptical in outline with both ends rounded and head region slightly bent to left. Ratio of length to width about 5:1, dorsoventrally flattened about 2:1 with ventral side flat and dorsal side bulging evenly when viewed laterally (Figs 1A, 2A–E). Anterior end with a conspicuous transparent, thin collar. Adoral zone occupying about 28% of body length (Figs 1A, 2A–D). Buccal cavity wide, right margin of cavity in mid-cell region. Pellicle thin and flexible. Cortical granules citrine-yellow, spherical, approximately 1 μm in diameter, rendering cell citrine-brownish at low magnification (Fig. 2A–E, H). Several cortical granules grouped together around dikinetids, arranged in short rows between dorsal kineties and cirral rows (Figs 1E, F, 2G). Cytoplasm colourless,

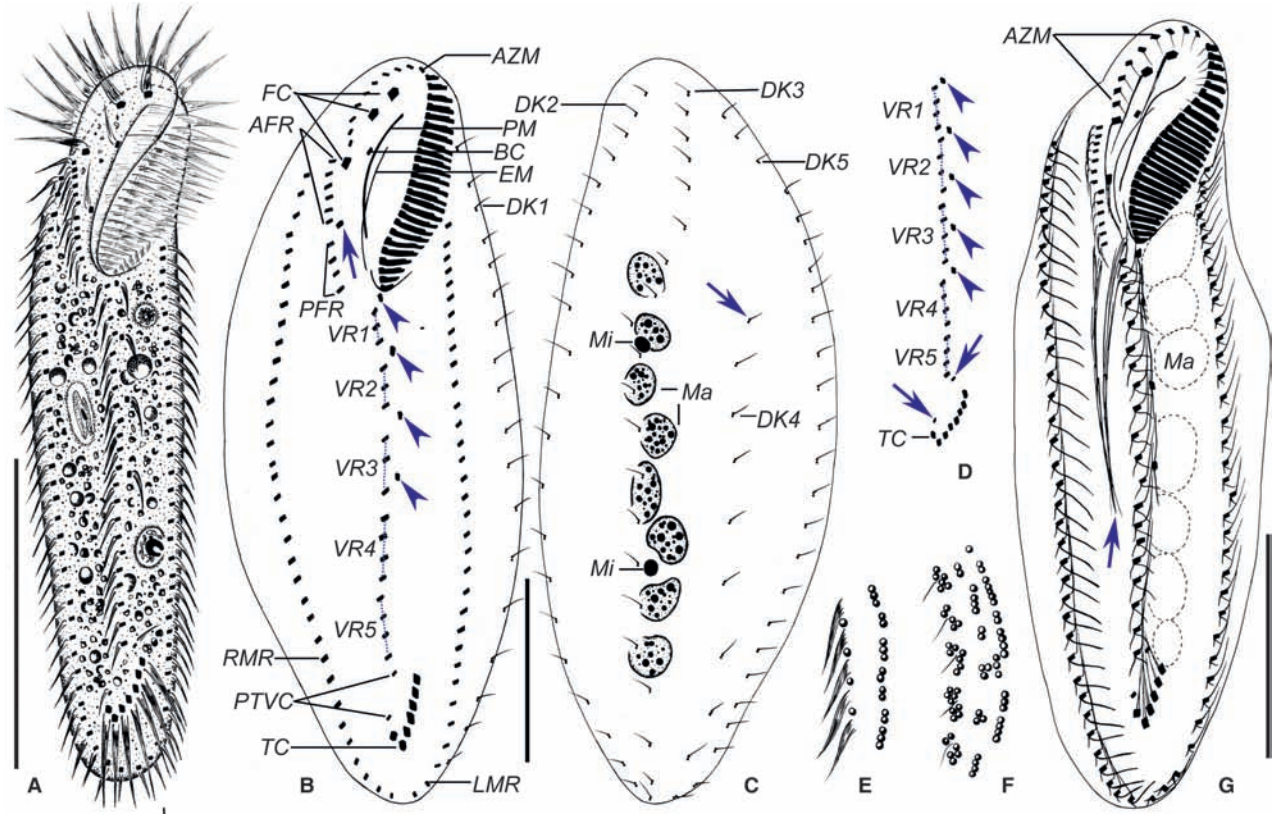



**Figure 1.** ***Polystichothrix monilata*** **sp. nov.** from live specimens (A, E, F) and after protargol staining (B–D, G). A, ventral view of a representative specimen. B, C, ventral (B) and dorsal (C) view of holotype, showing infraciliature and nuclear apparatus; arrowheads indicate postoral ventral cirri, arrow in (B) indicates frontoventral cirrus, whereas in (C) the arrow indicates anterior-most dikinetids of dorsal kinety 4, dotted lines in (B) indicate presumptive ontogenetic relationships amongst cirri, deduced from interphase specimens. D, detail from a specimen with five postoral ventral cirri (arrowheads) and eight transverse cirri; arrows indicate two fine pretransverse ventral cirri and dotted lines indicate presumptive ontogenetic relationships amongst cirri, deduced from interphase specimens. E, F, distribution of cortical granules on ventral (E) and dorsal (F) sides. G, ventral view of a representative specimen, showing infraciliature, nuclear apparatus, and the fibre system; arrow indicates the strong pharyngeal fibres. Abbreviations: AFR, anterior frontal row; AZM, adoral zone of membranelles; BC, buccal cirrus; DK1–5, dorsal kineties 1–5; EM, endoral membrane; FC, frontal cirri; LMR, left marginal cirri row; Ma, macronuclear segments; Mi, micronuclei; PFR, posterior frontal row; PM, paroral membrane; PTVC, pretransverse ventral cirri; RMR, right marginal cirri row; TC, transverse cirri; VR1–5, ventral rows 1–5. Scale bars = 60 μm (A); 30 μm (B, C, G).

usually packed with granules (1–5 μm in diameter), and food vacuoles usually containing diatoms and sometimes small ciliates (Fig. 2I, L, T). Contractile vacuole not observed. Locomotion by slowly crawling on substrate and amongst debris, or occasionally by swimming in water.

Cilia of anterior membranelles approximately 15 μm long *in vivo*. All cirri on ventral side about 10–12 μm long except the stronger frontal cirri, which have cilia about 15 μm long, and the transverse cirri, which are about 15–20 μm long and project beyond posterior cell margin.

Infraciliature as shown in Figures 1B–D, G, 2J–T. Adoral zone composed of 27–41 membranelles, distal end of adoral zone extending far onto right-ventral side of cell (Distal End of adoral zone-value *c.* 0.4; Berger, 1999, 2006). Bases of membranelles unequal in length, those in distal part comprise three short, equal-length rows of kinetosomes, those in proximal part with four rows, one short and three long (Figs 1B, G, 2J, L). Undulating membranes more or less in *Oxytricha*-pattern; paroral and endoral membranes almost equal in length, slightly curved and optically intersect in posterior third; endoral membrane with single-rowed kinetosomes, starting at midpoint of buccal field; paroral with multiple-rowed kinetosomes, commencing ahead of endoral (Fig. 1B, G). Constantly three enlarged frontal cirri, rightmost one just behind distal end of adoral zone; single buccal cirrus right of paroral membrane (Figs 1B, G, 2F,

J, L). Two frontal rows; anterior row composed of five to eight cirri, posterior row four to six cirri. One frontoventral cirrus located left of posterior end of anterior frontal row (Figs 1B, G, 2J). Four or five short ventral cirri rows, each composed of three to eight cirri. Four or five sparsely distributed postoral ventral cirri located left of ventral cirral rows, anterior-most one just behind buccal vertex (Figs 1B, D, G, 2R–T). Six to eight transverse cirri arranged in J-shaped row; two fine pretransverse ventral cirri located close to transverse cirri (Figs 1B, D, G, 2P, Q). Two marginal rows, almost confluent posteriorly. Right marginal row composed of 32–44 cirri, commencing near anterior end of anterior frontal row; left marginal row composed of 32–41 cirri, starting about level with posterior end of paroral membrane (Figs 1B, G, 2J). Five dorsal kineties; dorsal kinety 4 commences at about level of buccal vertex, the other four extend almost entire body length; dorsal bristles about 4 μm long *in vivo* (Figs 1B, C, 2K, M–O). Strong fibres associated with cirri are visible in protargol-stained specimens; pharyngeal fibres extremely strong, extending posteriorly and occupying about one-third of body length (Figs 1G, 2T). Macronucleus left of cell median, moniliform, usually composed of eight globular to ellipsoidal segments; consistently two ellipsoidal micronuclei (Figs 1C, G, 2J, L, M, P).

## Order Stichotrichida Fauré-Fremiet, 1961
## Family Amphisiellidae Jankowski, 1979
## Genus *Lamtostyla* Buitkamp, 1977
## ***Lamtostyla ovalis* sp. nov.**
## (Figs 3A–I, 4A–H; Table 2)

*Diagnosis*

Body 70–110 × 40–60 μm *in vivo*; generally oval in outline, colourless to slightly grey-greenish; about 22 adoral membranelles; three frontal cirri; one buccal cirrus; amphisiellid median cirral row comprises about 11 cirri and terminates ahead of mid-body; three to seven frontoventral cirri; two pretransverse ventral cirri; five transverse cirri; 19 left and 24 right marginal cirri on average; three dorsal kineties; two macronuclear segments; two micronuclei; brackish water habitat.

*Type locality and ecology*

A small puddle near Zhan Qiao Pier in Qingdao (36°06′N, 120°31′E), Shandong Province, China. Water temperature 21 °C, salinity 20 psu.

*Type specimens*

The protargol slide containing the holotype specimen (see Fig. 3B, C, G; registration no. PY2010092801/1) was deposited in the Laboratory of Protozoology, Ocean University of China (OUC). A paratype slide was deposited in the Natural History Museum, London (registration number NHMUK 2015.7.10.2).

*Etymology*

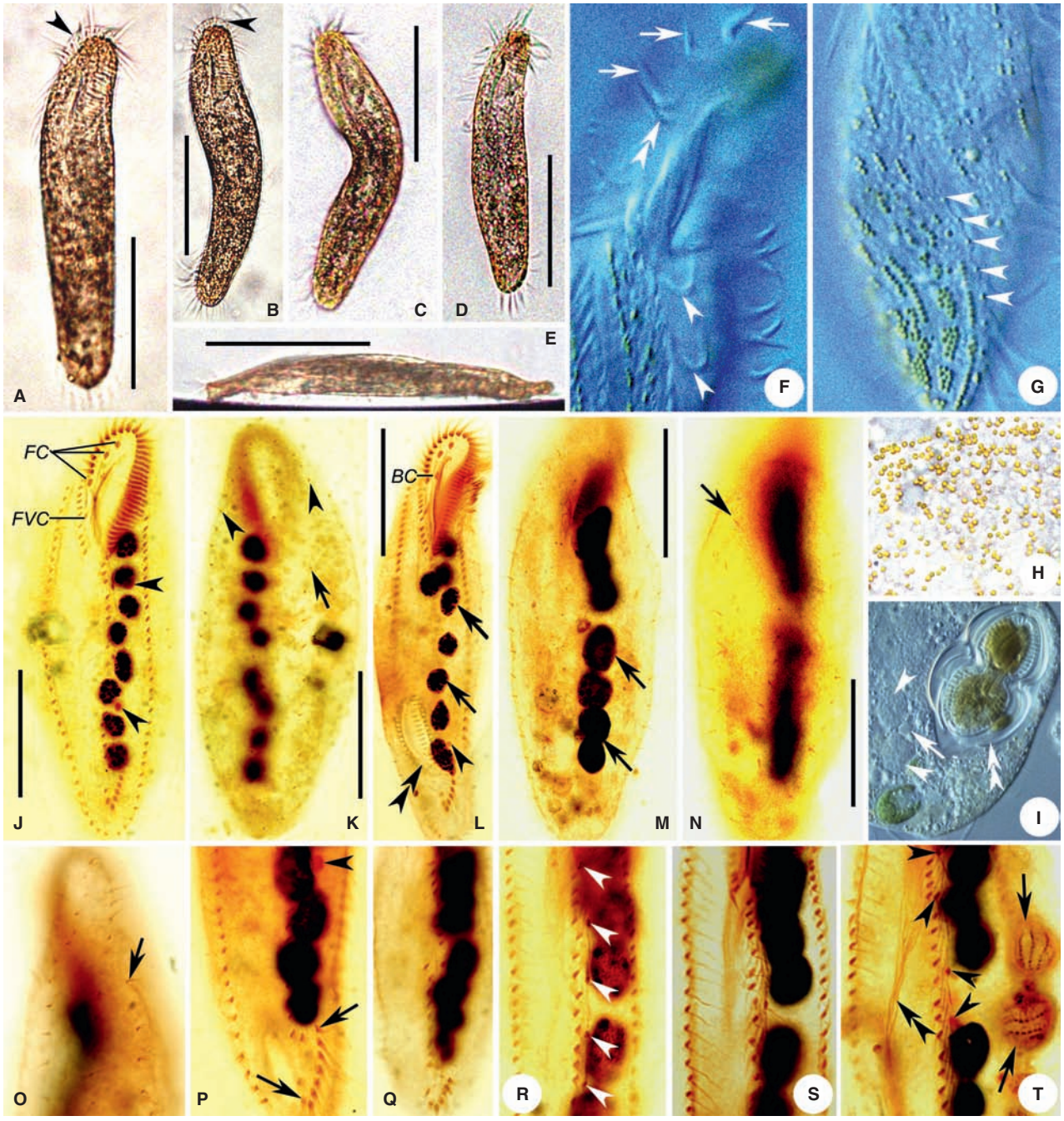
The species-group name *ovalis* (Latin adjective; egg-shaped, oval) refers to the oval body outline.

*Description*

Cell about 70–110 × 40–60 μm *in vivo*, oval in outline (Figs 3A, 4A–F), anterior end slightly narrowed and bends leftwards when cell changes direction during locomotion, posterior end more or less broadly rounded. Ratio of length to width about 5:3–2:1; dorsoventrally flattened about 2:1. Pellicle very flexible. Buccal field narrow, transparent, and conspicuous *in vivo*, occupying about 1/3 of body length. Cytoplasm colourless; whole body except buccal field usually packed with numerous dark-greenish algae (3–10 μm across; we are unsure whether they are endosymbionts or not) and several food vacuoles containing diatoms and small ciliates, rendering cell slightly grey-greenish (Figs 3I, 4A–G). Several bacterial plaques of varying size on dorsal surface, each composed of *c.* 1–2 × 0.5 μm bacilli (Fig. 4H). Cortical granules absent. Contractile vacuole about 10 μm across, positioned left of midline in mid-body region (Fig. 3A). Locomotion by slowly crawling on substrate; swimming cells not observed.

Cilia of anterior membranelles approximately 12 μm long *in vivo*. All ventral cirri about 8–10 μm long except frontal and transverse cirri, which have cilia about 12 μm long; transverse cirri do not project beyond posterior body margin (Fig. 3A). Dorsal bristles about 3 μm long *in vivo*.

Infraciliature as shown in Figure 3B–I. Adoral zone composed of 19–25 membranelles with about four membranelles in distal portion located apically. Bases of distal membranelles comprise three short equal-length rows of kinetosomes; bases of proximal membranelles comprise one short and three long rows of kinetosomes (Fig. 3B, D, E). Paroral and endoral membranes equal in length, arranged in parallel, occupying only about half length of buccal field (Fig. 3B, D, I). Consistently three enlarged frontal cirri located near anterior end of cell, arranged in a slightly oblique pseudorow (Fig. 3B, D, G). Single enlarged buccal cirrus located right of anterior end of paroral membrane (Fig. 3B, D, G–I). Amphisiellid median cirral row (ACR) usually composed of eight to 11 cirri, commencing behind rightmost frontal cirrus and terminating at about level of buccal vertex (Fig. 3B, D, I). Three to seven (usually four) frontoventral cirri left of anterior portion of ACR (Fig. 3B, D, G, H). Usually five transverse cirri




arranged in J-shaped or U-shaped row. One to three, usually two, pretransverse ventral cirri positioned ahead of transverse cirri. Two marginal rows not reaching posterior end of body and thus separated posteriorly. Right marginal row commencing near anterior part of ACR, composed of 19–31 cirri; left marginal row starting about level with buccal vertex, composed of 16–24 cirri (Fig. 3B, G–I). Invariably three dorsal kineties more or less shortened anteriorly; number of bristles per kinety increases from left to right (Fig. 3C, F). Two ellipsoidal macronuclear segments slightly left of cell midline, transparent *in vivo*, conspicuously separated but connected to each other via a thread (Figs 3A, C, E, 4A, E); rear end of anterior macronuclear segment a little behind level of buccal vertex, rear end of posterior segment slightly anterior of transverse cirri when observed *in vivo*. Two spherical micronuclei, *c.* 2 μm in diameter, each closely associated with a macronuclear segment (Fig. 3C, H).

**Figure 2.** ***Polystichothrix monilata*** **sp. nov.** from live specimens (A–I) and after protargol staining (J–T). A–D, ventral views of typical individuals, showing the slightly twisted, elongate body shape and different body size; arrowheads mark the conspicuous transparent and thin collar. E, lateral view of a representative specimen. F, ventral view of anterior region, showing frontal cirri (arrows), buccal cirrus (double arrowhead), and postoral ventral cirri (arrowheads). G, distribution of cortical granules on ventral and dorsal sides; arrowheads mark cortical granules distributed between cirri. H, showing citrine-yellowish cortical granules. I, nuclear apparatus and the food vacuoles filled with diatoms (double arrowhead); arrowheads mark macronuclear segments; arrow indicates micronucleus. J, K, ventral (J) and dorsal (K) views of holotype specimen, showing infraciliature and nuclear apparatus; arrowheads in (J) indicate the micronuclei, whereas in (K) arrowheads indicate dikinetids of dorsal kineties and arrow indicates anterior-most dikinetids of dorsal kinety 4. L, ventral view, showing infraciliature and nuclear apparatus; arrows indicate macronuclear segments; arrowhead indicates a micronucleus, double arrowhead indicates an ingested diatom. M, N, dorsal view of the same specimen with seven macronuclear segments (arrows in M), showing dorsal infraciliature; arrow in (N) indicates anterior-most dikinetids of dorsal kinety 4. O, dorsal view of anterior region of cell; arrow indicates anterior-most dikinetids of dorsal kinety 4. P, ventral view of a specimen with eight transverse cirri; arrows indicate rightmost and leftmost transverse cirri; arrowhead marks a micronucleus. Q, ventral view of a specimen with six transverse cirri. R, ventral view of a specimen with five postoral ventral cirri (arrowheads). S, ventral view of the fibre system. T, strong pharyngeal fibres (double arrowhead), four postoral ventral cirri (arrowheads), and small ingested ciliates (arrows). Abbreviations: BC, buccal cirrus; FC, frontal cirri; FVC, frontoventral cirrus. Scale bars = 60 μm (A–E); 30 μm (J–N).

**Table 1.** Morphometric characterization of ***Polystichothrix monilata*** **sp. nov.** based on protargol-stained specimens (measurements in μm)

| Character | Min. | Max. | Med. | Mean | SD | CV | *N* |
|---|---|---|---|---|---|---|---|
| Body, length | 88 | 135 | 110 | 116.7 | 13.6 | 11.7 | 25 |
| Body, width | 30 | 50 | 35 | 37.1 | 5.3 | 14.3 | 25 |
| Adoral zone, length | 27 | 39 | 32 | 32.4 | 4.0 | 12.5 | 25 |
| Adoral membranelles, number | 27 | 41 | 35 | 35.1 | 3.5 | 10.1 | 25 |
| Frontal cirri, number | 3 | 3 | 3 | 3.0 | 0.0 | 0.0 | 25 |
| Buccal cirrus, number | 1 | 1 | 1 | 1.0 | 0.0 | 0.0 | 25 |
| Frontoventral cirrus, number | 1 | 1 | 1 | 1.0 | 0.0 | 0.0 | 25 |
| Cirri in anterior frontal row, number | 5 | 8 | 6 | 6.4 | 0.7 | 11.4 | 23 |
| Cirri in posterior frontal row, number | 4 | 6 | 5 | 6.0 | 0.8 | 12.9 | 23 |
| Cirri in ventral row 1, number | 3 | 5 | 4 | 4.0 | 0.5 | 13.4 | 25 |
| Cirri in ventral row 2, number | 3 | 5 | 4 | 3.7 | 0.6 | 16.4 | 25 |
| Cirri in ventral row 3, number | 3 | 5 | 4 | 3.6 | 0.6 | 17.6 | 25 |
| Cirri in ventral row 4, number | 3 | 8 | 4 | 4.6 | 1.2 | 26.1 | 25 |
| Cirri in ventral row 5, number | 0 | 7 | 5 | 4.5 | 1.7 | 36.7 | 25 |
| Cirri in postoral ventral cirri, number | 4 | 5 | 4 | 4.1 | 0.3 | 6.9 | 25 |
| Cirri in left marginal row, number | 32 | 41 | 37 | 37.0 | 2.8 | 7.4 | 25 |
| Cirri in right marginal row, number | 32 | 44 | 37 | 37.8 | 3.5 | 9.3 | 25 |
| Pretransverse cirri, number | 2 | 2 | 2 | 2.0 | 0.0 | 0.0 | 25 |
| Transverse cirri, number | 6 | 8 | 7 | 7.2 | 0.6 | 7.7 | 25 |
| Dorsal kineties, number | 5 | 5 | 5 | 5.0 | 0.0 | 0.0 | 19 |
| Dikinetids in dorsal kinety 1, number | 18 | 24 | 21 | 21.2 | 1.9 | 9.0 | 11 |
| Dikinetids in dorsal kinety 2, number | 20 | 26 | 24 | 23.6 | 1.9 | 8.1 | 11 |
| Dikinetids in dorsal kinety 3, number | 21 | 28 | 26 | 25.0 | 2.2 | 8.8 | 11 |
| Dikinetids in dorsal kinety 4, number | 8 | 10 | 9 | 9.2 | 0.8 | 8.7 | 11 |
| Dikinetids in dorsal kinety 5, number | 23 | 35 | 28 | 28.2 | 3.8 | 13.5 | 11 |
| Macronuclear segments, number | 7 | 8 | 8 | 7.9 | 0.3 | 3.5 | 25 |
| Micronuclei, number | 2 | 2 | 2 | 2.0 | 0.0 | 0.0 | 11 |

CV, coefficient of variation in %; Max., maximum; Mean, arithmetic mean; Med., median value; Min., minimum; *N*, number of specimens examined; SD, standard deviation.

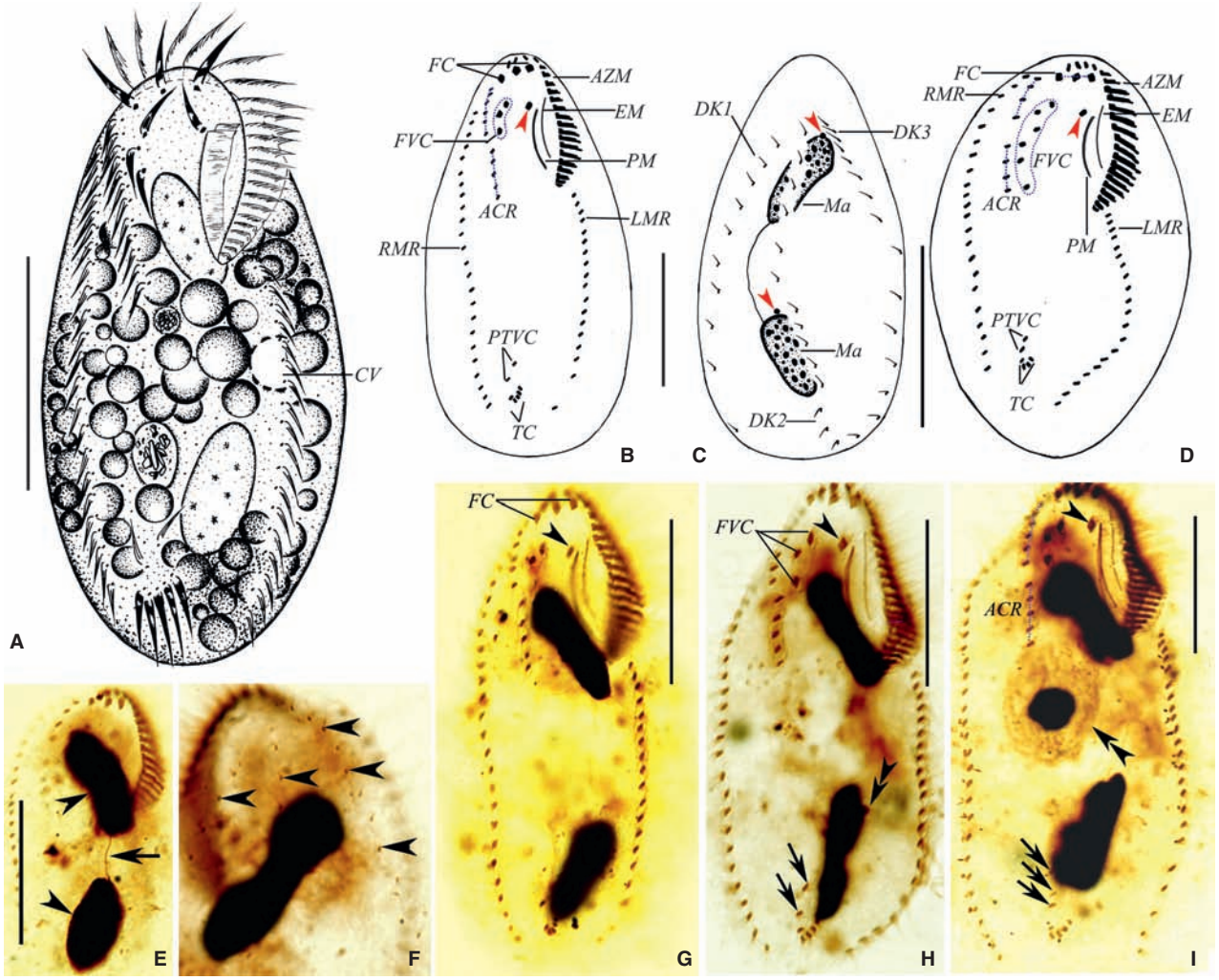



**Figure 3.** ***Lamtostyla ovalis* sp. nov.** from live specimens (A) and after protargol staining (B–I). A, ventral view of a representative specimen. B, C, ventral (B) and dorsal (C) views of holotype specimen, showing infraciliature and nuclear apparatus; arrowhead in (B) indicates buccal cirrus, arrowheads in (C) indicate micronuclei. D, ventral view of a specimen with six frontoventral and four transverse cirri; arrowhead indicates the buccal cirrus. E, ventral view, showing macronuclear segments (arrowheads) and the thread (arrow) between them. F, dorsal view of anterior region of cell, showing distribution of dikinetids (arrowheads). G, ventral view of holotype specimen, showing infraciliature and nuclear apparatus, arrowhead indicates buccal cirrus. H, I, ventral view of representative specimen; arrowhead indicates buccal cirrus, arrows show pretransverse ventral cirri; double arrowhead in (H) indicates micronucleus and in (I) indicates a small ingested ciliate. Abbreviations: ACR, amphisiellid median cirral row; AZM, adoral zone of membranelles; CV, contractile vacuole; DK1–3, dorsal kineties 1–3; EM, endoral membrane; FC, frontal cirri; FVC, frontoventral cirri; LMR, left marginal cirri row; Ma, macronuclear segments; PM, paroral membrane; PTVC, pretransverse ventral cirri; RMR, right marginal cirri row; TC, transverse cirri. Scale bars = 30 μm.

### *SSU rRNA* GENE SEQUENCE AND PHYLOGENETIC ANALYSES

The *SSU rRNA* gene sequence of *Pol. monilata* sp. nov. (GenBank accession number KT192639) is 1698 bp long and has a G + C content of 45.11%; that of *L. ovalis* sp. nov. (GenBank accession number KP266625) is 1729 bp long and has a G + C content of 45.58%.

The topologies of the BI and ML trees inferred from *SSU rRNA* gene sequences were basically congruent with variable support values; therefore, only the ML topology (with nodal support from both methods) is shown (Fig. 5). The phylogenetic analyses showed that neither order Stichotrichida nor Sporadotrichida is monophyletic. Within these two orders, three families out of seven (i.e. Trachelostylidae, Gonostomatidae, and Spirofilidae) and one

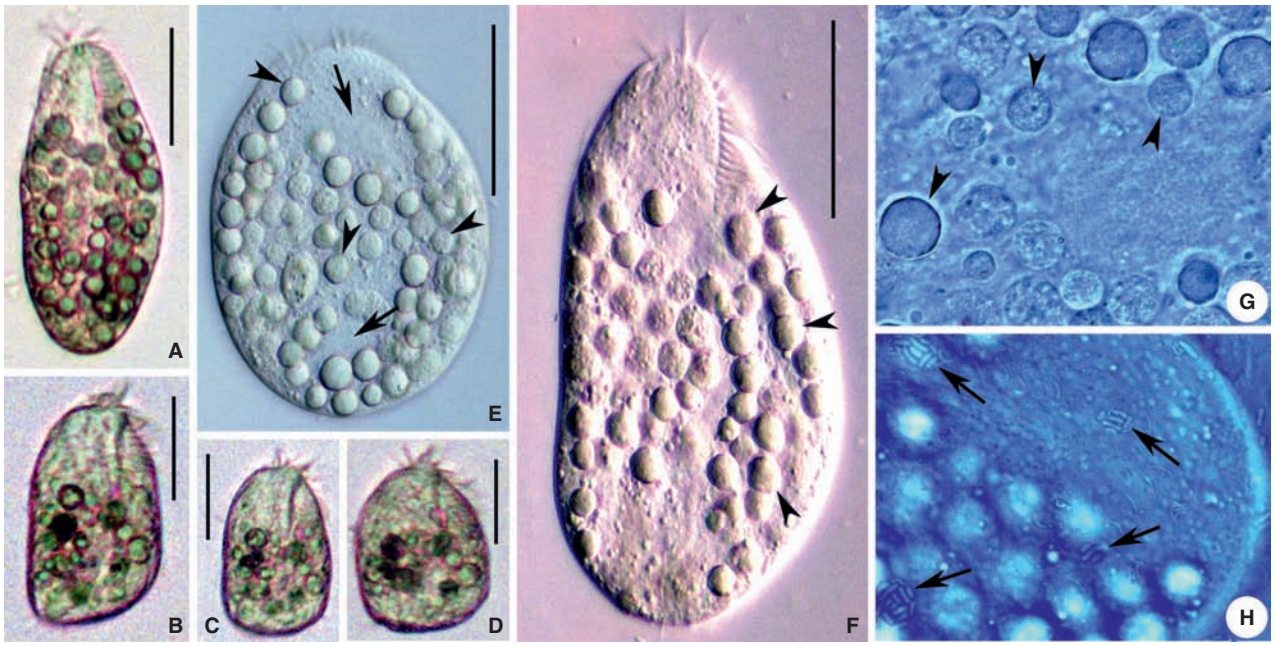



**Figure 4.** ***Lamtostyla ovalis*** **sp. nov.** from live specimens. A, ventral view of a representative specimen. B–D, ventral views of the same specimen, showing flexibility and different body shapes. E, F, specimen filled with algae (arrowheads); arrows in (E) mark macronuclear segments. G, detail of cell showing possibly endosymbiotic algae (arrowheads). H, dorsal surface of cell showing bacterial plaques of different size composed of bacilli (arrows). Scale bars = 30 μm.

**Table 2.** Morphometric characterization of ***Lamtostyla ovalis*** **sp. nov.** based on protargol-stained specimens (measurements in μm)

| Character | Min. | Max. | Med. | Mean | SD | CV | *N* |
|---|---|---|---|---|---|---|---|
| Body, length | 65 | 105 | 83 | 82.7 | 10.4 | 12.6 | 21 |
| Body, width | 35 | 62 | 48 | 48.2 | 7.5 | 15.6 | 21 |
| Adoral zone, length | 23 | 35 | 29 | 28.8 | 3.2 | 11.3 | 21 |
| Adoral membranelles, number | 19 | 25 | 22 | 21.9 | 1.6 | 7.1 | 21 |
| Frontal cirri, number | 3 | 3 | 3 | 3.0 | 0.0 | 0.0 | 21 |
| Buccal cirrus, number | 1 | 1 | 1 | 1.0 | 0.0 | 0.0 | 21 |
| Frontoventral cirri, number | 3 | 7 | 3 | 3.8 | 1.3 | 33.8 | 21 |
| Cirri in anterior part of ACR, number | 4 | 10 | 6 | 6.4 | 1.2 | 19.4 | 21 |
| Cirri in posterior part of ACR, number | 4 | 6 | 5 | 4.8 | 0.7 | 14.7 | 21 |
| Cirri in ACR, number | 8 | 16 | 11 | 11.2 | 1.7 | 15.2 | 21 |
| Cirri in left marginal row, number | 16 | 24 | 19 | 19.2 | 2.6 | 13.7 | 21 |
| Cirri in right marginal row, number | 19 | 31 | 23 | 23.8 | 3.3 | 14.0 | 21 |
| Pretransverse cirri, number | 1 | 3 | 2 | 1.7 | 0.6 | 37.5 | 21 |
| Transverse cirri, number | 4 | 6 | 5 | 5.0 | 0.4 | 8.0 | 21 |
| Dorsal kineties, number | 3 | 3 | 3 | 3.0 | 0.0 | 0.0 | 21 |
| Dikinetids in dorsal kinety 1, number | 9 | 15 | 11 | 11.4 | 1.9 | 16.7 | 16 |
| Dikinetids in dorsal kinety 2, number | 13 | 20 | 14 | 15.2 | 2.6 | 17.1 | 6 |
| Dikinetids in dorsal kinety 3, number | 13 | 20 | 15 | 16.0 | 2.4 | 15.0 | 8 |
| Macronuclear segments, number | 2 | 2 | 2 | 2.0 | 0.0 | 0.0 | 25 |
| Micronuclei, number | 1 | 2 | 2 | 1.6 | 0.5 | 31.3 | 8 |

ACR, amphisiellid median cirral row; CV, coefficient of variation in %; Max., maximum; Mean, arithmetic mean; Med., median value; Min., minimum; *N*, number of specimens examined; SD, standard deviation.

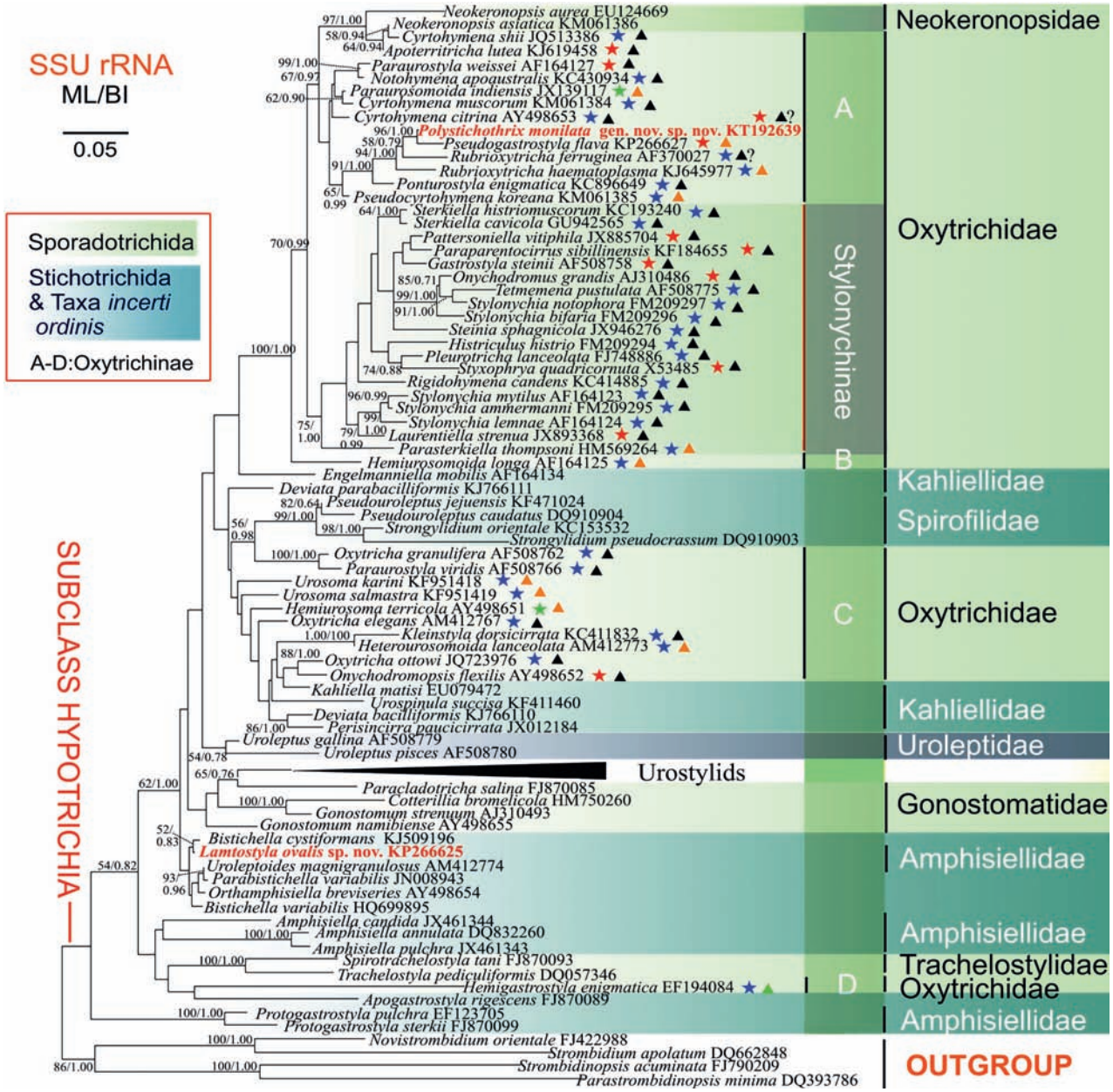



**Figure 5.** The maximum likelihood (ML) tree inferred from *small subunit ribosomal RNA* gene sequences, showing the position of the new species ***Polystichothrix monilata* sp. nov.** and ***Lamtostyla ovalis* sp. nov.** (indicated in bold font). Bootstrap values above 50 for ML and posterior probability values above 0.5 for Bayesian inference (BI) are given at nodes. Stars in different colours mark oxytrichids with frontal-ventral-transverse cirri of different numbers (green < 18; blue = 18; red > 18). Orange triangles mark oxytrichids with dorsomarginal kineties but lacking dorsal kinety fragmentation; black triangles mark oxytrichids with dorsomarginal kineties and dorsal kinety fragmentation; black triangles with question marks indicate oxytrichids lacking morphogenetic information but that we speculate have dorsomarginal kineties and dorsal kinety fragmentation; green triangle marks oxytrichids with dorsal kinety fragmentation but lacking dorsomarginal kineties. All branches are drawn to scale. Scale bar corresponds to five substitutions per 100 nucleotide positions.

subfamily (i.e. Stylonychinae) are monophyletic. *Protogastrostyla pulchra* and *Protogastrostyla sterkii* cluster in a clade that branches off at the base of the ingroup. *Lamtostyla ovalis* sp. nov. clusters with five stichotrichid species, i.e. *Uroleptoides magnigranulosus*, *Bistichella cystiformans*, *Bistichella variabilis*,

*Parabistichella variabilis*, and *Orthoamphisiella breviseries*, near the base of the *SSU rRNA* gene trees forming a group that is sister to a large assemblage comprising most of the stichotrichids, sporadotrichids, and (core) urostyloids for which *SSU rRNA* gene sequence data are available. *Polystichothrix monilata* sp. nov. is sister to *Pseudogastrostyla flava* with high support (96% ML, 1.00 BI), and is next most closely related to *Rubrioxytricha ferruginea*, *Rubrioxytricha haematoplasma*, *Ponturostyla enigmatica*, and *Pseudocyrtohymena koreana*. Except for *Pseudogastrostyla flava*, all of these species have 18 frontal-ventral-transverse cirri and are members of the subfamily Oxytrichinae. In order to further investigate evolutionary relationships within the family Oxytrichidae, we mapped morphological characters onto the phylogenetic tree (Fig. 5). Based on cirral patterns, Oxytrichidae is divided into three groups, viz. oxytrichids with 18 frontal-ventral-transverse cirri (blue stars), oxytrichids with more than 18 cirri (red stars), and oxytrichids with fewer than 18 cirri (green stars). The Oxytrichidae can also be separated into two groups based on the presence or absence of dorsal kinety fragmentation, viz. those with dorsomarginal kineties but lacking dorsal kinety fragmentation (orange triangles), and those both with dorsomarginal kineties and dorsal kinety fragmentation (black triangles). However, grouping patterns based on either of these two morphological character states are not possible in the topology of the *SSU rRNA* gene trees (Fig. 5).

## DISCUSSION

### Establishment of the new genus *Polystichothrix* gen. nov

*Polystichothrix monilata* sp. nov. possesses more than 18 frontal-ventral-traverse cirri with the frontal and ventral cirri arranged in short rows, and five dorsal kineties with dorsal kinety 4 shortened. Unfortunately we failed to obtain any morphogenetic stages in our preparations; therefore, the ontogenetic pattern of development of the frontal-ventral-traverse cirri or dorsal kineties is unknown. From the characteristics of interphase specimens we speculate that the frontal-ventral-traverse cirri originate from more than six frontal-ventral-traverse anlagen, the shortened dorsal kinety 4 may derive from dorsal kinety 3 fragmentation, and dorsal kinety 5 may come from the dorsomarginal anlage.

Regarding its general infraciliature, the new genus *Polystichothrix* resembles neither stichotrichids, which have one or more ventral cirral rows, nor the urostylids, which have midventral cirri arranged in a typical zigzag pattern (Berger, 2006, 2008). As no morphogenetic stages of *Polystichothrix monilata* sp. nov. are available, however, we cannot completely exclude the possibility that *Polystichothrix* gen. nov. is a stichotrichid. Furthermore, *Polystichothrix* also differs from the typical oxytrichids, which have 18 frontal-ventral-traverse cirri arranged in six groups (Berger, 1999). There are, however, some oxytrichids with more than 18 frontal-ventral-transverse cirri that should be compared with *Polystichothrix* gen nov.

In addition to differences in their cirral pattern, *Onychodromus* and *Styxophrya* can be easily separated from *Polystichothrix* gen nov. by having a rigid (vs. flexible) body, the presence (vs. absence) of unique cytoplasmic processes (horns) on the dorsal surface, and more than six (vs. five) dorsal kineties. *Laurentiella* and *Pattersoniella* are also rigid oxytrichids that can be distinguished from *Polystichothrix* gen nov. by having more than six dorsal kineties with multiple fragmented kineties (vs. five dorsal kineties) and a conspicuously large, wide adoral zone of membranelles that extends for about 50% of the body length (vs. not conspicuously wide and extending for only about 30% of the body length). In addition, *Laurentiella* has five frontal-ventral-traverse cirral rows (vs. two frontal rows and four or five ventral rows in *Polystichothrix* gen. nov.), and *Pattersoniella* has six frontal cirri and four cirri forming a so-called 'bicorona' (vs. three frontal cirri in *Polystichothrix* gen. nov.) (Berger, 1999).

*Ancystropodium* has a club-shaped body, two slightly obliquely arranged frontoventral rows, and three conspicuous caudal cirri, whereas *Polystichothrix* gen. nov. has an elliptical body outline, two frontal and four or five ventral rows, and lacks caudal cirri (Berger, 1999).

*Territricha* can be distinguished from *Polystichothrix* gen. nov. by having frontoventral cirri arranged in a V-shaped pattern and more than six dorsal kineties (vs. two short frontal rows, four or five short ventral rows, and five dorsal kineties; Berger, 1999).

Compared with *Polystichothrix* gen. nov., *Kerona* has numerous frontal and frontoventral cirri arranged in six slightly to distinctly curved rows (vs. two short frontal rows and four to five short ventral rows), a large adoral zone of membranelles that occupies about 50% (vs. 30%) of the body length, and dorsal kineties arranged in about 16–20 indistinct rows (vs. five well-defined dorsal kineties) (Berger, 1999).

*Apoamphisiella*, *Parentocirrus*, and *Paraurostyla* differ from *Polystichothrix* gen. nov. by having at least two long frontoventral rows (vs. two short frontal rows and four or five short ventral rows) and several caudal cirri (vs. caudal cirri absent) (Berger, 1999).

Two oxytrichid genera, i.e. *Gastrostyla* and *Hemigastrostyla*, and two non-oxytrichid genera, i.e. *Protogastrostyla* and *Apogastrostyla*, can be separated from *Polystichothrix* gen. nov. by having most of their frontoventral and postoral ventral cirri in a more or less continuous, slightly oblique row (vs. two short frontal rows and four to five short ventral rows) and four or five (vs. six to eight) transverse cirri. In addition, *Hemigastrostyla* and *Apogastrostyla* have two 'extra' cirri behind the right marginal row, these being absent in *Polystichothrix* gen. nov. (Song & Wilbert, 1997; Berger, 1999; Gong *et al.*, 2007; Li *et al.*, 2010; Shao *et al.*, 2011).

## Phylogenetic analysis, familial assignment and Systematic position of *Polystichothrix* gen. nov.

The present phylogenetic analyses based on *SSU rRNA* gene sequence data suggest that *Polystichothrix monilata* sp. nov. is an oxytrichid, and is most closely related to *Pseudogastrostyla*, followed by *Rubrioxytricha*, *Ponturostyla*, and *Pseudocyrtohymena* (Fig. 5). These five genera share some morphological characters that are phylogenetic markers for oxytrichids, for example the possession of dorsomarginal kineties (Song & Wilbert, 1989; Blatterer & Foissner, 1990; Berger, 1999, 2006, 2008; Song, 2001; Chen *et al.*, 2015; Jung, Park & Min, 2015; Shao, Lu & Ma, 2015). By contrast, amongst the taxa within this clade, only *Pon. enigmatica*, possibly *Pol. monilata* sp. nov., and *R. ferruginea* possess dorsal kinety fragmentation (Song, 2001; Chen *et al.*, 2015; Fan *et al.*, 2015; Jung *et al.*, 2015). This conflicts with the hypotheses that dorsal kinety fragmentation is the main apomorphy of the Oxytrichidae, and that species that lack dorsal kinety fragmentation are non-oxytrichid Dorsomarginalia (Berger, 1999, 2008). In other clades, some species with dorsomarginal kineties but lacking dorsal kinety fragmentation (species marked with orange triangles in Fig. 5) also cluster with oxytrichids possessing dorsomarginal kineties (species marked with black triangles in Fig. 5). Consequently, we here classify *Polystichothrix* gen. nov. as a member of the family Oxytrichidae.

It has been suggested that oxytrichids with 18 frontal-ventral-traverse cirri are ancestral and that those with more than 18 frontal-ventral-traverse cirri, e.g. *Polystichothrix* gen. nov., *Apoterritricha*, *Paraurostyla*, *Pattersoniella*, and *Territricha*, evolved by a secondary increase in the numbers of frontal-ventral-transverse cirri anlagen and of cirri originating from the right anlagen (Berger, 1999; Kim *et al.*, 2014). In our phylogenetic analyses based on *SSU rRNA* gene sequence data, *Pol. monilata* sp. nov., *Apoterritricha*, and *Paraurostyla* branched in separate groups, although all nested within the Oxytrichinae clade A (Fig. 5). This indicates that the character state of more than 18 frontal-ventral-traverse cirri might have evolved several times. Gene sequence data from a wider range of taxa, however, are needed in order to investigate this hypothesis and to establish a robust classification of the family Oxytrichidae.

## Systematic position of the genus *Lamtostyla*

Historically, the genus *Lamtostyla* has been classified in the families Holostichidae (Buitkamp, 1977; Corliss, 1979; Tuffrau, 1979), Oxytrichidae (Jankowski, 1979), Cladotrichidae (Small & Lynn, 1985), Amphisiellidae (Petz & Foissner, 1996; Shi, 1999; Berger, 2008; Lynn, 2008), and Trachelostylidae (Lynn & Small, 2002). The present study provides the first *SSU rRNA* gene sequence data for *Lamtostyla*. Our phylogenetic analyses place *Lamtostyla* in a clade that includes representatives of genera that are either already assigned to the family Amphisiellidae, i.e. *Uroleptoides* (Lynn, 2008) or that have been suggested might belong to Amphisiellidae, i.e. *Orthoamphisiella*, *Bistichella*, and *Parabistichella* (Eigner, 1997; Berger, 2008; He & Xu, 2011; Jiang *et al.*, 2013; Fan *et al.*, 2014b). This is consistent with the morphology of genera within this clade, all of which have three more or less enlarged frontal cirri and one marginal cirral row on each side of cell, the main difference amongst them being the number and origin of the frontoventral rows (Berger, 2008; He & Xu, 2011; Jiang *et al.*, 2013; Fan *et al.*, 2014b). By contrast, *Lamtostyla* and *Amphisiella*, the type genus of the family Amphisiellidae, are in different clades (Fig. 5). This placement partly agrees with the morphological evidence, which shows that *Lamtostyla* can be distinguished from *Amphisiella* by having fewer dorsal kineties, fewer transverse cirri, and a short (vs. long) amphisiellid median cirral row (Berger, 2008). These genera, however, share a number of characters including a continuous adoral zone of membranelles, straight and parallel undulating membranes, three enlarged frontal cirri, a buccal cirrus, two or more cirri located left of the anterior portion of the amphisiellid median cirral row and which originate from two separate anlagen, and one left and one right marginal row (Berger, 2008). Consequently, based both on the morphological and molecular data, we suggest that *Lamtostyla* should be treated as a member of the family Amphisiellidae. Phylogenetic analyses inferred from other gene markers and morphogenetic studies from a wider range of taxa are urgently needed in order to elucidate whether the family Amphisiellidae is monophyletic.

**Table 3.** Morphological and morphometric comparisons of *Lamtostyla* species, measurements in μm

| Character | *Lamtostyla ovalis* | *Lamtostyla islandica* | *Lamtostyla perisincirra* | *Lamtostyla australis* | *Lamtostyla lamottei* | *Lamtostyla procera* | *Lamtostyla elegans* |
|---|---|---|---|---|---|---|---|
| Size *in vivo* | 70–110 × 40–60 | 60–80 × 20–25 | 50–80 × 20–30 | 90–130 × 30–40 | 100? | 130–210 × 15–25 | 140–200 × 25–45 |
| Size in protargol preparations | 55–75 × 40–45 | 56–70 × 17–21 | 34–57 × 12–18 | 77–127 × 23–32 | – | 112–172 × 16–26 | 126–190 × 25–45 |
| Cortical granules | Lacking | Lacking | Lacking | Lacking | Not known | Lacking | Lacking |
| BL:BW | 1.7:1 | 3.4:1 | 2.8:1 | 3.5:1 | 3.5:1 | 6.1:1 | 5:1 |
| AZM:BL | 35% | 27% | 30% | 24%–30% | 23% | 15% | 16–23% |
| ACR:BL | 30% | 27% | – | 38–46% | 26% | 30% | 31% |
| AM, number | 19–25 | 15–16 | 15–18 | 21–26 | 19 | 17–23 | 18–29 |
| PTVC, number | 1–3 | 0 | 0 | 0 | 0 | 0 | 0 |
| TC, number | 4–6 | 3 | 2–5 | 2–6 | 2 | 3–6 | 0–5 |
| DK, number | 3 | 3 | 3–4 | 3 | 4 | 2 | 2–3 |
| Ma, number | 2 | 2 | 2 | 2 | 2 | 2 | 4–8 |
| Data source | Present work | Berger & Foissner (1988) | Berger, Foissner & Adam (1984) | Blatterer & Foissner (1988) | Buitkamp (1977) | Foissner, Agatha & Berger (2002) | Foissner *et al.* (2002) |

| Character | *Lamtostyla quadrinucleata* | *Lamtostyla vitiphila* | *Lamtostyla decorata* | *Lamtostyla granulifera* | *Lamtostyla longa* | *Lamtostyla raptans* |
|---|---|---|---|---|---|---|
| Size *in vivo* | 100–125 × 30 | 90–120 × 30–50 | 100–170 × 20–35 | 120–170 × 20–55 | 85–130 × 35–50 | 200 × 40 |
| Size in protargol preparations | 70–105 × 21–32 | 76–100 × 32–42 | 90–154 × 20–34 | 125–160 × 49–80 | – | – |
| Cortical granules | Lacking | Lacking | Colourless | Colourless | Not known | Not known |
| BL:BW | 3.4:1 | 2.4:1 | 4–9:1 | 2–6:1 | 3:1 | 5:1 |
| AZM:BL | 20% | 25% | 14%–23% | 28% | 20–25% | 25% |
| ACR:BL | 38% | 42% | 21% | 27% | – | – |
| AM, number | 14–19 | 20–23 | 18–21 | 23–27 | 18–19 | 33–36 |
| PTVC, number | 0 | 0 | 1 | 1–2 | 2 | 1 |
| TC, number | 2–4 | 1–2 | 6–9 | 5 | 5 | 5 |
| DK, number | 2 | 3 | 3 | 3 | 5 | 5 |
| Ma, number | 4 | 4–5 | 2–4 | 2 | 2 | 2 |
| Data source | Berger & Foissner (1989) | Foissner (1987) | Foissner *et al.* (2002) | Foissner (1997) | Hemberger (1985) | Hemberger (1985) |

ACR:BL, amphisiellid median cirral row length: body length in protargol preparations; AM, adoral membranelles; AZM:BL, length of adoral zone of membranelles : body length in protargol preparations; BL:BW, body length: body width ratio in protargol preparations; DK, dorsal kineties; Ma, macronuclear segments; PTVC, pretransverse cirri; TC, transverse cirri.

Characters marked with '–' were not available from the source cited.

?, It is uncertain whether this datum (100 μm) is for living specimens or not.

### COMPARISON OF *LAMTOSTYLA OVALIS* SP. NOV. WITH ITS CONGENERS AND RELATED SPECIES

In his comprehensive review of the family Amphisiellidae, Berger (2008) recognized 12 species of *Lamtostyla*. Of these, *Lamtostyla islandica* Berger & Foissner, 1988, most closely resembles *L. ovalis* sp. nov. in that both have a similar cirral pattern, three dorsal kineties, two macronuclear segments each with a closely associated micronucleus, and lack cortical granules. However, the former can be distinguished from the latter by having a relatively smaller body size (60–80 × 20–25 μm vs. 70–110 × 40–60 μm *in vivo*), a more slender body (length: width ratio about 3.4:1 vs. 1.7:1 in protargol preparations), fewer adoral membranelles (15–16 vs. 19–25), three transverse cirri that project distinctly beyond the posterior body margin (vs. usually five transverse cirri that do not project beyond the posterior body margin), and pretransverse ventral cirri absent (vs. one to three pretransverse ventral cirri in an oblique pseudorow) (Berger & Foissner, 1988). Even though there are some overlapping characters between *L. ovalis* sp. nov. and other *Lamtostyla* species, we still found sufficient features to distinguish them and establish our new species (Table 3).

## ACKNOWLEDGEMENTS

This work was supported by: the Natural Science Foundation of China (project numbers: 31430077, 41276139, 31471973); China Postdoctoral Science Foundation (2015T80746); the Postdoctoral Foundation in Qingdao and Shandong Province; the Deanship of Scientific Research, King Saud University, Prolific Research Group funding award (PRG-1436-24); the Royal Society/The National Natural Science Foundation of China (31411130122) International Exchanges Scheme; an award from the Biotechnology and Biological Sciences Research Council of China Partnering Award Scheme; and the Fundamental Research Funds for the Central Universities (201564022, 201562029). We would like to thank Dr Helmut Berger for his suggestions during drafting, Prof. Weibo Song and Prof. Xiaozhong Hu (OUC), who critically read the drafts and gave many helpful suggestions, Ms Chundi Wang (OUC) for gene sequencing, and Prof. Xiaofeng Lin, Southern China Normal University, for ensuring institutional support for this research.

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