Morphology and morphogenesis of a novel mangrove ciliate, Sterkiella subtropica sp. nov. (Protozoa, Ciliophora, Hypotrichia), with phylogenetic analyses based on small-subunit rDNA sequence data

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A novel marine hypotrichous ciliate, Sterkiella subtropica sp. nov., was recently isolated from a mangrove wetland in Hong Kong. Its morphology, morphogenesis and systematic position have been investigated. The novel species is diagnosed by combined features of morphology, ciliature and nuclear apparatus, while its ontogenetic events present a stable pattern: (i) the six streaks of the undulating membrane (UM) and cirral anlagen are segmented in a 1 : 3 : 3 : 4 : 4 pattern from left to right, and form three frontal, four frontoventral, one buccal, five ventral and five transverse cirri; (ii) the dorsal structure is similar to most other oxytrichids; that is, in a ‘4 + 2’ pattern with three caudal cirri being formed. Based on the small-subunit rDNA sequence, the novel species is different from its congeners by between 21 and 35 bp, with sequence identities from 0.978 to 0.987. All molecular trees exhibited a similar topology: the monophyly of species of the genus Sterkiella is not completely supported in our analyses, and approximately unbiased tests (both including and excluding the novel species) also reject the possibility that Sterkiella is a monophyletic lineage, as indicated by the morphology-based classification.

INTRODUCTION

Based on their morphological and morphogenetic characteristics, the hypotrichous ciliates are considered to be one of the most confusing and divergent groups of protists (Berger, 1999, 2006, 2008, 2011; Chen et al., 2013a; Fan et al., 2014; Jiang et al., 2013; Küppers & Claps, 2013; Li et al., 2013; Park et al. 2013). The ontogenetic processes of hypotrichids reveal highly diversified patterns, which provide information that is indispensable for a better understanding of their complex evolutionary and systematic relationships (Foissner, 1996; Foissner et al., 2014; Jung et al., 2014; Küppers et al., 2011; Lu et al., 2014; Lv et al., 2013; Shao et al., 2013, 2014a, b, c; Singh & Kamra, 2013; Singh et al. 2013).

Abbreviations: AU, approximately unbiased; AZM, adoral zone of membranelles; BI, Bayesian inference; ML, maximum-likelihood; SSU, small subunit; UM, undulating membrane.

The GenBank/EMBL/DDBJ accession number for the SSU rDNA sequence of Sterkiella subtropica sp. nov. is KM924307.

In the present work, we describe the morphology and ontogeny of Sterkiella subtropica sp. nov. isolated from a mangrove wetland in Hong Kong, southern China. Its morphological characteristics correspond well with those of its congeners, and its ontogenetic process conforms to the
Sterkiella mode (Berger, 1999; Berger et al., 1985; Foissner & Berger, 1999; Foissner et al., 2002; Nieto et al., 1984; Petz & Foissner, 1997). In addition, the small-subunit (SSU) rDNA sequence of the novel species was characterized, and phylogenetic analyses, as well as approximately unbiased (AU) tests, were performed in order to assess the systematic relationships among species of the genus Sterkiella.

**METHODS**

**Sample collection and identification.** Sterkiella subtropica sp. nov. was collected on 3 December 2009 from mangrove mud (covered by marine water with a salinity of 29 %o) in Hong Kong Wetland Park, Hong Kong. After adding filtered seawater, the ciliates were maintained in Petri dishes at room temperature (approx. 23 °C) with rice grains as a food source to promote the growth of bacteria as food. Cells were examined in vivo using bright-field and differential interference contrast microscopy and were impregnated with protargol following the method of Wilbert (1975) in order to reveal the infraciliature. Measurements of the morphological characteristics of stained specimens were performed at a magnification of \( \times 1000 \). Drawings were made with the help of a camera lucida. Terminology follows Berger (1999).

**DNA extraction, PCR amplification and sequencing.** After washing with filtered seawater, genomic DNA was extracted from ciliate cells using the REDExtract-N-Amp Tissue PCR kit (Sigma). The manufacturer’s protocol was followed in this procedure, except that only 1/10 of the suggested volume for each solution was used. The SSU rDNA was amplified using the universal primers EuK and EuKb (Medlin et al., 1988). Cloning and sequencing were performed according to Huang et al. (2014).

**Phylogenetic analyses.** The SSU rDNA sequence of the novel species was aligned and modified manually with the sequences of 51 other ciliates (54 populations) downloaded from the GenBank database (see Fig 6 for accession numbers) using CLUSTAL W implemented in BioEdit 7.0 (Hall, 1999) and resulting in a final alignment of 1776 sites. A maximum-likelihood (ML) analysis with bootstrapping of 1000 replicates was performed using RAxML-HPC2 on XSEDE 8.0.0 (Stamatakis, 2006; Stamatakis et al., 2008) on the CIPRES Science Gateway (http://www.phylo.org/sub_sections/portal; Miller et al., 2010) with the model GTR + I + G as the optimal choice. Bayesian inference (BI) analysis was conducted with MrBayes 3.2.2 (Ronquist & Huelsenbeck, 2003) via the CIPRES Science Gateway using the GTR + I + G as the best model (selected by MrModeltest version 2.0; Nylander 2004). Markov chain Monte Carlo simulations were run for 1 000 000 generations with a sampling frequency of 100 and a burn-in of 2500 trees. A majority-rule consensus tree with posterior probabilities was created from all remaining trees. TreeView version 1.6.6 (Page, 1996) and MEGA 4.0 (Tamura et al., 2007) were used to visualize tree topologies. Systematic classification follows Berger (1999).

The statistical probability of the hypothesis that Sterkiella is a monophyletic lineage was evaluated using AU tests (Shimodaira, 2002) as described by Zhang et al. (2014).

**RESULTS**

**Description of Sterkiella subtropica sp. nov.**

**Diagnosis.** Medium-sized member of Sterkiella measuring 100–200 \( \times 35–70 \) µm in vivo, body semi-rigid, fusiform to teardrop-shaped. About 25–39 membranelles; 18–26 left and 19–27 right marginal cirri. Five ventral cirri arranged in typical 2 : 1 : 2 pattern. Four dorsal and two dorsomarginal kinetics, with one caudal cirrus at the posterior end of each of dorsal kinetics 1, 2 and 4. Two macronuclear nodules and one to four micronuclei. Marine habitat.

**Type locality and ecology.** Hong Kong Wetland Park, Hong Kong, China (22° 28’ 10” N 114° 00’ 31” E). This organism was isolated from mangrove mud covered by marine water with a salinity of 29 %o.

**Type specimens.** The protargol slide containing the holotype specimen (Fig. 1k; registration no. NHMUK 2013.3.25.1) has been deposited in the Natural History Museum, London, UK, and two paratype slides (registration nos CXM09120302/1 and /2) have been deposited in the Laboratory of Protozoology, Ocean University of China, China.

**Etymology.** The Latin adjective subtropicus, -a, -um [masc., fem., neut.] recalls the fact that the type material was found in a subtropical area of China.

**Morphological description (Table 1 and Figs. 1 and 2).** Cell in vivo 100–200 \( \times 35–70 \) µm, semi-rigid; somewhat fusiform to teardrop-shaped, with posterior end obviously wider than anterior (ratio of widths of posterior end to anterior about 3 : 2) and both ends rounded; a ratio of length to width of about 3 : 1 and dorsoventrally flattened 3 : 1–3 : 2 (Figs. 1a–c, 2a). In some well-fed specimens, a ratio of length to width of about 2 : 1, anterior as wide as posterior and left margin distinctly convex (Fig. 1d–f). Adoral zone prominent, comprising 33–40 % of body length; with transparent membrane-like structure at anterior part (Figs. 1b, d and 2a). Neither cortical granules nor pigments observed. Cytoplasm colourless to grey, usually with many shining granules and food vacuoles containing diatoms and bacteria (10–20 µm across; Figs. 1e–i and 2a). Contractile vacuole about 20 µm across, located near left margin in anterior 2/5 region, pulsating at intervals of 1–3 min (Figs. 1e–g and 2a).

Two ellipsoidal and centrally located macronuclear nodules, each 25–45 \( \times 12–30 \) µm across after protargol impregnation. One to four spherical micronuclei, located close to macronuclear nodules, 4–8 µm across after protargol impregnation (Figs. 1k–o and 2c).

Locomotion by crawling on the bottom of Petri dishes and among debris, floating on the surface of the water, and by rotating around the longitudinal axis when swimming.

Adoral zone composed of 25–39 membranelles with cilia 15–20 µm long in vivo (Figs. 1j, k and 2b). Undulating membranes in typical Oxytricha pattern: paroral almost as long as endoral, both membranes slightly curved inwards to buccal field and intersecting at anterior 1/5–1/4 part with a single buccal cirrus located nearby (Figs. 1l and 2b). Three
Fig. 1. Photomicrographs of Sterkiella subtropica sp. nov. in vivo (a–j) and after protargol impregnation (k–o). (a–c) Ventral views of representative specimens. Arrows mark the transparent membrane at the anterior part of the adoral zone of membranelles. (d, e) Ventral (d) and dorsal (e) views of the same well-fed and slightly squashed cell. Arrows indicate the transparent membrane at the anterior part of the adoral zone of membranelles (d) and contractile vacuole (e). (f) Dorsal view of a well-fed and slightly squashed specimen. Arrows mark the food vacuoles. (g–i) Inclusions within the cytoplasm: arrows indicate the shining granules. (j) Ventral view, indicating the frontal (double arrowheads), two postoral ventral (arrows) and three caudal (arrowheads) cirri. (k) Infraciliature in ventral view of holotype specimen. (l) Ventral view of the buccal field. Arrow marks the buccal cirrus and the dashed circle shows four postoral ventral cirri. (m, n) Dorsal views show the dorsal kineties (m) and three caudal cirri (arrowheads). (o) Ventral view to indicate the micrornuclei (arrow). CV, Contractile vacuole; DK, dorsal kineties; e, endoral membrane; FC, frontal cirri; FVC, frontoventral cirri; LMR, row of left marginal cirri; Ma, macronuclear nodules; p, paroral membrane; RMR, row of right marginal cirri; TC, transverse cirri. Bars, 60 μm.

Table 1. Morphometric characterization of Sterkiella subtropica sp. nov.

Data are based on protargol-impregnated specimens.

<table>
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<tr>
<th>Character</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>CV</th>
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<td>68</td>
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<tr>
<td>Width of macronuclear nodules (μm)</td>
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<td>25</td>
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<tr>
<td>Micronuclei (n)</td>
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<td>0.7</td>
<td>27.8</td>
<td>25</td>
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<tr>
<td>Length of micronuclei (μm)</td>
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<td>5.8</td>
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strong frontal cirri with cilia 15–20 µm long in vivo (Fig. 1j) and four frontoventral cirri forming a V-shape and located to right of buccal field (Figs. 1k, l and 2b). Ventral cirri arranged in a 2 : 1 : 2 pattern; three postoral cirri with rear one positioned far distant from the anterior two, while the two pretransverse ones are located near the transverse cirri (Fig. 2b). Five relatively strong transverse cirri with cilia 20 µm long forming a J-shaped row (Figs. 1k and 2b) and extending beyond the cell posterior margin in vivo (Fig. 1j). Left and right marginal rows comprising 18–26 and 19–27 cirri, respectively, and almost reaching each other at the posterior end of the cell, with cilia about 15 µm long in vivo (Figs. 1j, k and 2b).

Dorsal ciliature composed of four dorsal and two dorso-marginal kineties (Figs. 1m and 2c), with cilia 2–3 µm long in vivo; dorsal kineties 1, 2 and 3 extending almost as long as body length, while kinety 4 is slightly shortened anteriorly. Three conspicuous caudal cirri with cilia 15–20 µm long in vivo, each located at the posterior end of dorsal kineties 1, 2 and 4 (Figs. 1n and 2c).

Morphogenesis during binary fission (Figs. 3 and 4).
In the earliest divider observed, a long and narrow field of closely spaced basal bodies originates apokinetally and forms the oral primordium of the opisthe anterior of the leftmost transverse cirrus (Figs. 3a and 4a). Soon, it

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Fig. 2. Sterkiella subtropica sp. nov. in vivo (a) and after protargol impregnation (b, c). (a) Ventral view of a representative specimen. (b, c) Ventral (b) and dorsal (c) views of the same specimen, showing the infraciliature and nuclear apparatus. Arrow indicates the buccal cirrus; double arrowheads mark the micronuclei; arrowheads refer to the caudal cirri; and the dashed circle indicates the postoral ventral cirri. PTVC, pretransverse ventral cirri; see legend to Fig. 1 for other definitions. Bars, 60 µm.
lengthens, widens and differentiates new adoral membranelles posteriorly, with a proliferation of basal bodies (Figs. 3b, c and 4b, c). Meanwhile, the old frontoventral cirri and undulating membranes remain intact (Fig. 3a).

In the middle stage, the fronto-ventral-transverse cirral anlagen (FVT anlagen) form five streaks in both the proter and opisthe, in which the two posterior-most frontoventral cirri are involved. At the same time, the old paroral membrane dedifferentiates in the proter to form one frontal cirrus anteriad; in the opisthe, the undulating membranes anlage appears de novo to the left of the FVT-anlagen with one frontal cirrus then originating from its anterior part. The opisthe’s oral primordium differentiates into more and more new membranelles (Figs. 3d, f and 4d, e).

During the late stages, in the opisthe, the new AZM extends and bends towards the right, while the parental adoral zone remains entirely in the proter. The anlage of undulating membranelles (e, Endoral membrane; LMA, anlagen of left marginal row; LMR, row of left marginal cirri; Ma, macronuclear nodules; OP, oral primordium; p, paroral membrane; RMA, anlagen of right marginal row; RMR, row of right marginal cirri; TC, transverse cirri; UMA, anlagen of undulating membranes. Scale bars=60 μm.)
membranes splits longitudinally to differentiate the paroral and endoral membranes. In both dividers, the six FVT-anlagen form new cirri (cirri derived from the same streak are connected by dashed lines in Fig. 3i), which will migrate to their final positions as the cell undergoes cytokinesis (Figs. 3g, i and 4f, g).

**Fig. 4.** Photomicrographs of *Sterkiella subtropica* sp. nov. in morphogenesis after protargol impregnation. (a) Ventral view of an early divider, to show the oral primordium (OP). (b, c) Ventral views of two early dividers, to indicate how the OP develops and differentiates new membranelles posteriad from their anterior end. (d, e) Ventral views of two middle dividers, both in the proter and opisthe: arrows mark the five streaks of the developing FVT anlagen and double arrowheads show how the UMA will give rise to one frontal cirri. (f) Ventral view of a late divider; double arrowheads indicate the UMA split into new endoral and paroral membranes. (g, k, l). Ventral (g, k) and dorsal (l) views of the same late divider. Arrow and arrowheads mark the newly formed dorsomarginal kineties and caudal cirri, respectively; (k) and (l) show the details. (h, i) Dorsal views of the same divider shown in (d), in both the proter (h) and opisthe (i). Arrows mark the anlagen of dorsal kineties and double arrowheads show the micronuclei. (j, m) Ventral (j) and dorsal (m) views of the same individual shown in (f). Arrows indicate the anlagen of the dorsomarginal kineties formed around the anterior part of the anlagen of the right marginal row, double arrowheads mark the dividing micronuclei and arrowheads show the forming caudal cirri at the ends of the anlagen of dorsal kineties.

LMA, Anlagen of left marginal row; Ma, macronuclear nodules; OP, oral primordium; RMA, anlagen of right marginal row; UMA, undulating membrane anlagen.
In both proter and opisthe, the anlagen of the marginal cirral rows develop intrakinetically, strengthen longitudinally and replace the parental ones. The right marginal anlagen originate slightly earlier than the left ones (Figs. 3d, f, i and 4d–g).

New dorsal kineties originate from two sets of dorsal primordia in both the proter and opisthe and then proliferate, strengthen at both ends and replace the parental structures at the late stage (Fig. 3j). One set of primordia dedifferentiate intrakinetically from DK1 to DK3 in the middle stage (Figs. 3e and 4h, i); the rightmost one splits to form the new DK3 and DK4 (Fig. 3h), and one caudal cirrus is generated from each posterior end of the new DK3, DK2 and DK4 (Figs. 3h, j and 4g, l, m). The other set of primordia originate de novo to the anterior right of the right marginal cirral row in the late stage (Figs. 3g, h and 4j) and develop into the dorsomarginal kineties in each divider (Figs. 3i, j and 4g, k, l).

Two macronuclear nodules expand conspicuously in the early stage (Figs. 3e and 4e, h, i) and fuse into one single mass in the late stage (Figs. 3h and 4f, m). This single mass then divides into two parts (Figs. 3j and 4j), each of which will divide into the two new macronuclear nodules for both proter and opisthe. The micronuclei divide separately in the late stages of morphogenesis (Figs. 3h, j and 4m).

Molecular data and phylogenetic analyses (Table 2 and Figs. 5 and 6). The SSU rDNA sequence was deposited in GenBank with accession number KM924307. The length and G+C content (excluding primers) were 1647 bp and 45.17 mol%, respectively.

Including our newly characterized sequence, there are six SSU rDNA sequences from four species of the genus Sterkiella. They differ from each other by between 3 and 35 bp, with sequence identities from 0.978 to 0.998 (Table 2, Fig. 5).

A broad selection of SSU rDNA sequences from 52 species (55 populations) was included in the phylogenetic analyses. The topologies of the ML and BI trees were basically congruent; therefore, a single topology from both algorithms indicated on branches (Fig. 6). In the phylogenetic trees, all four species of the genus Sterkiella fall into the Stylonychinae clade, but form three groups: (i) two populations of Sterkiella histriomuscorum and S. cavicola (66 % ML, 1.00 BI), (ii) two populations of Sterkiella nova (93 % ML, 1.00 BI), and (iii) our novel species, S. subtropica. The AU test also rejects the possibility that Sterkiella is a monophyletic lineage, regardless of whether or not the novel species is included.

DISCUSSION

Generic assignment

According to the definition of Berger (1999), the genus Sterkiella Foissner et al., 1991 is characterized by its rigid or only slightly flexible body, curved and intersecting undulating membranes, marginal rows separated posteriorly and the presence of caudal cirri. It differs from the genus Oxytricha by the larger AZM (≥40 % of body length vs ≤35 %), the position of cirrus V/3, which is distinctly separated from the other two postoral cirri (vs three postoral cirri forming a narrow group), a bigger ratio of body width to length ≥40 % (vs <40 %) and the rigid or slightly flexible body (vs body supple) (Berger, 1999). Our isolate has an AZM covering 33–40 % of the body length and a ratio of body width to length ranging from 33 to 50 %, giving it a state intermediate between Sterkiella and Oxytricha. Nonetheless, it possesses a rigid body and cirrus V/3 is distinctly separated from the other two postoral cirri. Both characters support the generic assignment of our isolate into the genus Sterkiella.

Concerning the morphogenetic process, our isolate indicates similar main events to S. cavicola, S. histriomuscorum and S. nova: (i) in the proter, the old AZM remains and the undulating membrane (UM) dedifferentiates to form the anlage for the new structure; (ii) the six streaks, including the UM primordium, of the cirral anlagen are segmented in a 1 : 3 : 3 : 3 : 4 : 4 pattern from left to right and then form three frontal, four frontoventral, one buccal, five ventral and five transverse cirri, respectively; (iii) the dorsal morphogenesis is in a typical Oxytricha pattern and forms three caudal cirri; and (iv) the two macronuclear nodules fuse and divide later (Berger et al., 1985; Foissner &

Table 2. Numbers of unmatched nucleotides (upper right) and sequence identities (lower left) between the SSU rDNA of four species of the genus Sterkiella (six populations)

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**Fig. 5.** Sections of the SSU rDNA sequence alignment of four species of the genus *Sterkiella* (six populations) showing non-matching sites. Numbers above the lines indicate the positions of nucleotides in the alignment. Differences in the length of the sequences are compensated for by introducing alignment gaps (−) in the sequences. Matching sites are marked by dots.

**Fig. 6.** ML and BI analyses based on SSU rDNA sequences. The new sequence provided in the present work is indicated in bold. Numbers at nodes indicate ML bootstrap values from 1000 replicates and the BI posterior probability. Disagreements between the topology of this tree and that of the BI tree are marked by asterisks (*). Nodes that were well supported (>99 % ML, 1.00 BI) are represented by solid circles. Two long branches have been shortened, as shown by ‘//’, and the other branches are drawn to scale. Bar, 2 substitutions per 100 nucleotide positions.
Our isolate looks similar to *Oxytricha oxymarina* Berger, 1999 (formerly *Oxytricha marina* Kahl, 1932) in general morphology as well as the marine habitat. However, *O. oxymarina* has the three narrowly spaced postoral ventral cirri, a generic feature of *Oxytricha*, while in our isolate, the rear one of the three postoral cirri is far distant from the anterior two cirri. Besides, *O. oxymarina* probably has a soft body (vs rigid in our form) and a smaller buccal field less than 1/3 of body length (vs about 40 % in *Sterkiella subtropica*), and its contractile vacuole is situated in the first quarter of body (vs at about the anterior 2/5) (Berger, 1999; Kahl, 1932).

Nonetheless, the precise infraciliature of *O. oxymarina* is still unclear, because corresponding redescriptions do not agree well with the original report (Agamaliev, 1978; Berger, 1999; Dragesco, 1972; Jones, 1974; Kahl, 1932; Kwon & Shin, 2008). The Chadian population of *O. oxymarina* described by Dragesco (1972) has the five ventral cirri arranged in a 3 : 2 pattern (vs in a 2 : 1 : 2 pattern in our new isolate) and more marginal cirri (31–33 vs 18–26 on the left and 33–39 vs 19–27 on the right). Jones (1974) described a population of *O. oxymarina* and illustrated the ventral ciliary pattern, in which the four frontoventral cirri are scattered (vs forming a V-shape pattern) and the two posterior ventral cirri are distinctly apart from the transverse cirri (vs adjacent to the transverse cirri). Both the Caspian Sea and Korean specimens of *O. oxymarina* have a larger buccal field, occupying half of the body length (vs about 40 %), and five scattered ventral cirri (vs arranged in 2 : 1 : 2 pattern) (Agamaliev, 1978; Kwon & Shin, 2008). All these differences justify the separation of our isolate from the nominal populations of *O. oxymarina*.

**Comparison with congeners (Table 3)**


*Sterkiella subtropica* sp. nov. can be distinguished from the type species *S. cavicola* by its invariably two (vs four) macronuclear nodules and marine habitat (vs freshwater or terrestrial). The novel species also differs from the type species by the smaller size (100–200 × 35–70 μm vs 140–250 × 70–120 μm) and fusiform to teardrop-shaped body (vs ovoid or margins almost in parallel) *in vivo* (Berger, 1999; Berger & Foissner, 1987; Gelei & Szabados, 1950; Groliere, 1969; Kahl, 1935; Shin & Kim, 1994).

*Sterkiella histriomuscorum* has been considered as a sibling species complex with *S. nova*, possessing very similar morphological characteristics both *in vivo* and after protargol impregnation, and possibly also with *S. tricirrata* (with five dorsal kineties) and *S. terricola* (with four macronuclear nodules) (Foissner & Berger, 1999). Our novel species is very similar to these forms in infraciliature, but distinctly different in habitat (marine water vs commonly in terrestrial and freshwater habitats and rarely in wastewater treatment plants). *In vivo*, *S. subtropica* sp. nov. is fusiform to teardrop-shaped, while the body of members of the *S. histriomuscorum* complex usually has margins almost in parallel and both ends broadly rounded (Berger, 1999; Foissner & Berger, 1999). Moreover, the sequence information strongly indicates that the two forms belong to clearly different species (Table 2, Figs. 5, 6).

*Sterkiella admirabilis* has invariably four macronuclear nodules (vs two) and lives in freshwater and soil habitats (vs marine). It is much larger (length 390–450 μm *in vivo* vs 100–200 μm), and has a dominant adoral zone occupying about 50 % (vs 33–40 %) of the body length with about 50 (vs 25–39) adoral membranelles and many more marginal cirri (about 30 vs 21 on the left and 42 vs 23 on the right) (Alekperov & Musayev, 1988; Berger, 1999; Foissner, 1980). Although detailed information on the morphology and infraciliature of *S. quadrinucleatus* is lacking, this saltwater species does have a differentiating feature, the presence of four macronuclear nodules (vs two).

*Sterkiella terricola* inhabits soil biotopes (vs marine) and has four macronuclear nodules (vs two) and three transverse cirri (vs five) (Berger, 1999; Buitkamp, 1977; Dragesco & Dragesco-Kernéis, 1986). Likewise, the soil dweller *S. tricirrata* has three transverse cirri (vs five) and a smaller body size (80 μm vs 100–200 μm long) (Berger, 1999; Buitkamp, 1977).

**Phylogenetic position of the genus Sterkiella**

Berger & Foissner (1997) and Berger (1999) assigned the genus *Sterkiella* to the subfamily Stylonychinae on the basis of morphological and morphogenetic data. So far, molecular information is available for only four species, including the novel species (Fig. 6). Both the present study and previous studies based on analysis of SSU rDNA sequences indicate a well-supported Stylonychinae clade and the assignment of *Sterkiella* to the subfamily Stylonychinae (Li et al. 2011; Schmidt et al., 2007).

The monophyly of *Sterkiella* is not supported in the phylogenetic analyses, however, since the four species of *Sterkiella* fall into three branches (Fig. 6). The AU test also rejects its monophyly, even though all these species of the genus *Sterkiella* share similar morphological characteristics and a highly stable morphogenetic process (Berger, 1999; Berger et al., 1985; Foissner & Berger, 1999; Nieto et al., 1984;
Table 3. Biotopes and morphological comparison of seven species of the genus *Sterkiella*

| Taxa: 1, *Sterkiella cavicola* (data from Foissner et al., 1991); 2, *S. admirabilis* (Berger, 1999); 3, *S. histrioicusporum* complex (*S. nova*) (Foissner & Berger, 1999; Foissner et al., 1991); 4, *S. terricola* (Berger, 1999); 5, *S. tricirrata* (Berger, 1999); 6, *S. quadrinucleatus* (Berger, 1999); 7, *S. subtropica* sp. nov.  

<table>
<thead>
<tr>
<th>Character</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotope(s)</td>
<td>Terrestrial and freshwater</td>
<td>Terrestrial and freshwater</td>
<td>Terrestrial and freshwater</td>
<td>Terrestrial</td>
<td>Terrestrial</td>
<td>Freshwater, brackish and marine water</td>
<td>Mangrove mud, covered with marine water</td>
</tr>
<tr>
<td>Body size <em>in vivo</em></td>
<td>140–250 × 70–120 µm</td>
<td>350–450 µm long</td>
<td>80–180 × 40–70 µm</td>
<td>~120 µm long</td>
<td>~80 µm long</td>
<td>80–250 × 40–60 µm</td>
<td>100–200 × 35–70 µm</td>
</tr>
<tr>
<td>Body shape</td>
<td>Ovoid or margins</td>
<td>Body margins more or</td>
<td>Body margins parallel, both ends rounded</td>
<td>Elliptical</td>
<td>Elliptical</td>
<td>Body margins parallel or slightly converging posteriorly</td>
<td>Fusiform to teardrop-shaped with posterior obviously wider than anterior</td>
</tr>
<tr>
<td>Contractile vacuoles <em>(n)</em></td>
<td>One, with two collecting canals</td>
<td>Two</td>
<td>One</td>
<td>One</td>
<td>One</td>
<td>One</td>
<td>One</td>
</tr>
<tr>
<td>Transverse cirri <em>(n)</em></td>
<td>5–6</td>
<td>5–6</td>
<td>4–5</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Dorsal kinetics <em>(n)</em></td>
<td>6</td>
<td>Unknown</td>
<td>6–7</td>
<td>6</td>
<td>5–6</td>
<td>6–8</td>
<td>6</td>
</tr>
<tr>
<td>Macronuclear nodules <em>(n)</em></td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Petz & Foissner, 1997). In addition, the phylogenetic relationships between the genera within the subfamily Stylonychinae are still unclear, because of their similar morphological and morphogenetic features and the lack of sufficient molecular data. It is clear, therefore, that more data on morphological characteristics, detailed ontogenetic processes as well as more gene information based on exact identifications are still needed to gain a better understanding of the systematic relationships among the stylonychids.

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**REFERENCES**


notes on the morphogenesis of *Sterkiella histriomuscorum*. Polar Rec (Gr Brit) 33, 307–326.


