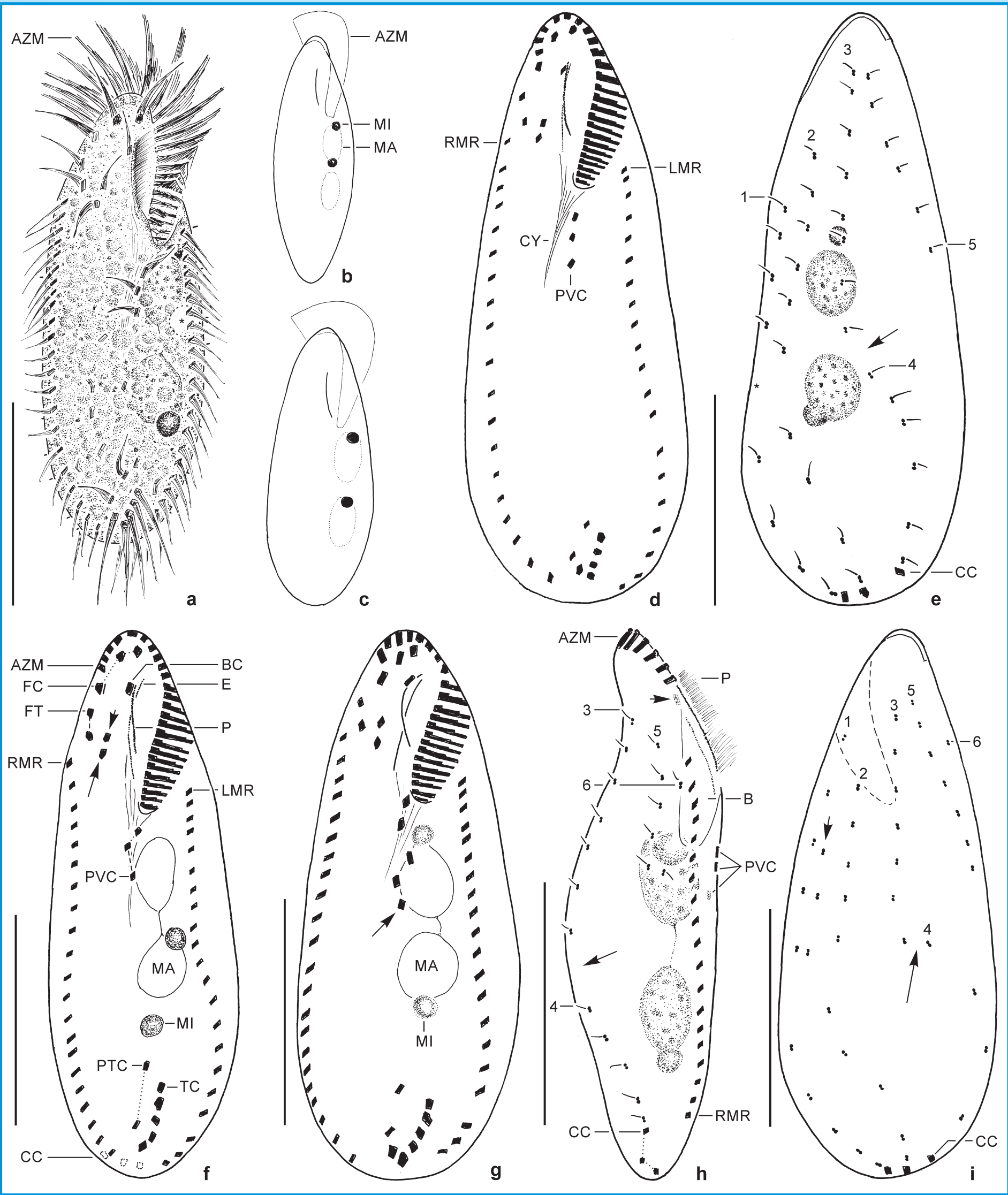


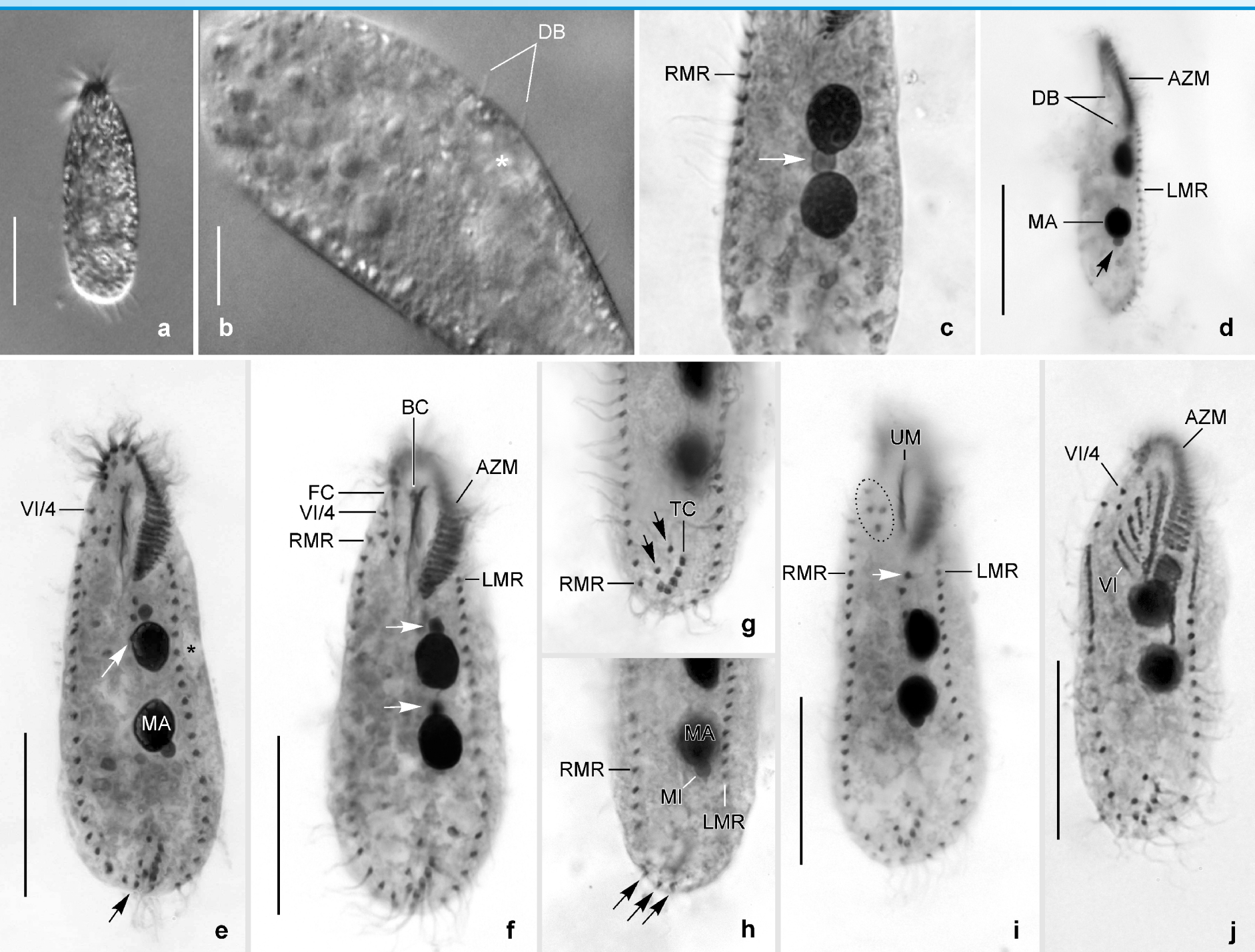
Morphology, phylogenetic relationships, and pH response of two *Oxytricha*-like species (Ciliophora, Hypotricha)

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Figs 1a-i. *Oxytricha acidotolerans* spec. nov. in vivo (a-c) and after protargol impregnation (d-i). **a:** Ventral view of representative specimen. Asterisk marks contractile vacuole. **b, c:** Spindle-shaped and slightly oviform shape variants with two rare arrangements of micronuclei. **d, e:** Infraciliature of ventral and dorsal side and nuclear apparatus of holotype specimen. Asterisk in (e) marks break in dorsal kinety 1, arrow denotes indistinct "fragmentation" of kinety 3. **f:** Specimen with almost linearly arranged transverse cirri. Short arrow marks frontoventral cirrus III/3, long arrow denotes cirrus IV/3. **g:** Specimen with five postoral ventral cirri (rearmost marked with arrow) and six transverse cirri. **h:** Right lateral view. Short arrow marks slightly subadjacent buccal cirrus, long arrow marks "fragmentation" of kinety 3. **i:** Infraciliature of dorsal side of a specimen with an additional (remnant of previous generation?) short row of dorsal bristles (short arrow); kinety 6 is composed of one (rarely two) basal body pair only, if present at all. Long arrow marks "fragmentation" of kinety 3. AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; CY, pharyngeal fibres; E, endoral; FC, frontal cirri; FT, frontoterminal cirri (= cirri VI/3, VI/4); LMR, left marginal row; MA, macronuclear nodule; MI, micronucleus; P, paroral; PVC, postoral ventral cirri; PTC, pretransverse ventral cirri; RMR, right marginal row; TC, transverse cirri; 1-6, dorsal kineties. Scale bars: 30 µm.

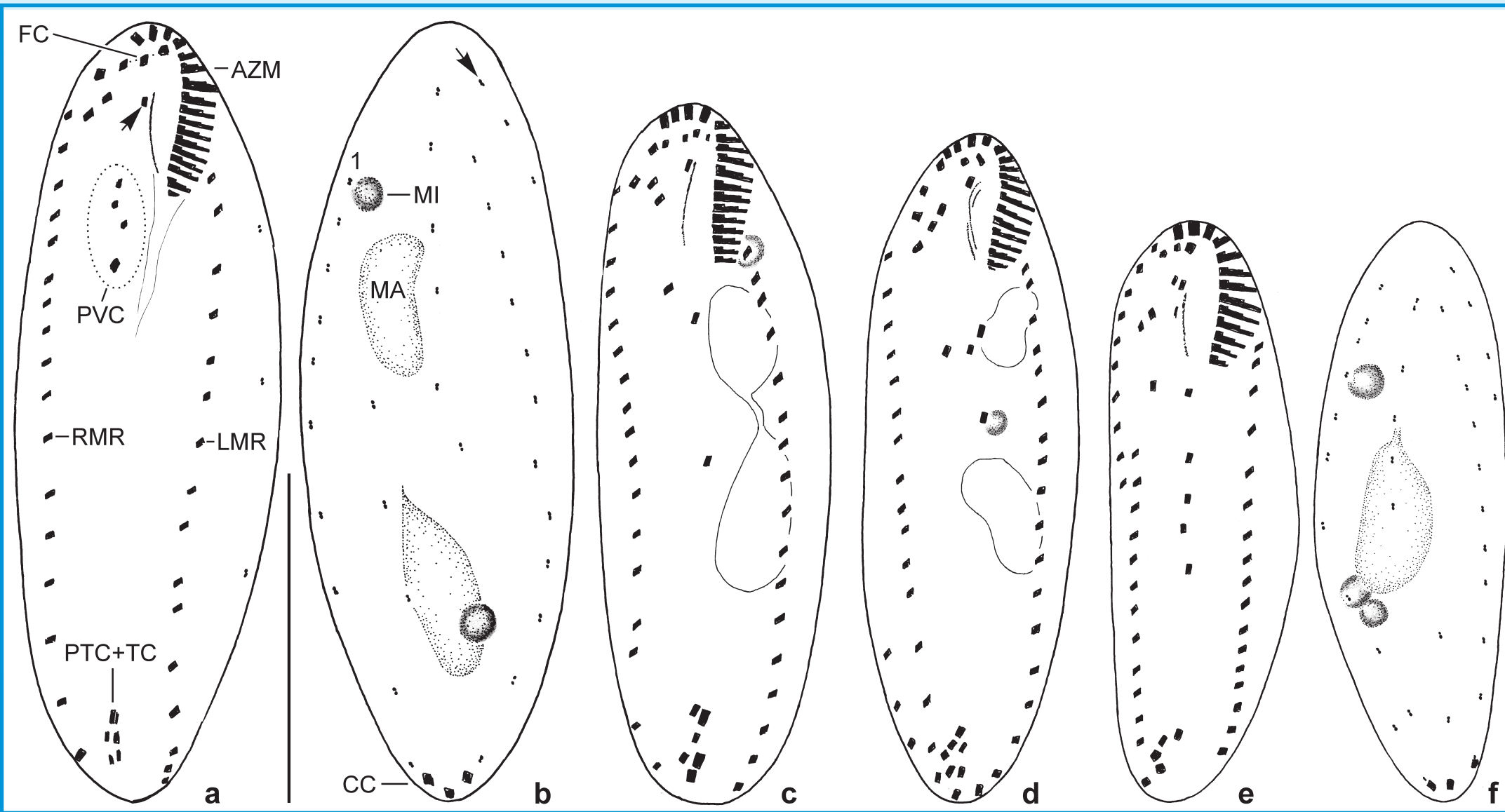


Figs 2a-j. *Oxytricha acidotolerans* spec. nov. in vivo (a, b; differential interference contrast; video frames) and after protargol impregnation (c-j). **a:** Dorsal view of a freely motile specimen. **b:** Optical section showing, inter alia, dorsal bristles (about 3 µm) and contractile vacuole (asterisk). **c:** Rare specimen with a single micronucleus (arrow) in between the two macronuclear nodules. **d:** Right lateral view showing that the nuclear apparatus is rather close to the ventral side (arrow marks micronucleus). **e:** Holotype specimen (composite of eight micrographs). White arrow marks rearmost postoral ventral cirrus (V/3), black arrow denotes a caudal cirrus. Asterisk indicates area of contractile vacuole. **f:** Specimen with micronuclei (arrows) at anterior end of macronuclear nodules. **g, h:** Posterior end showing, inter alia, pretransverse ventral cirri (arrows in g) and caudal cirri (arrows in h). **i:** Specimen with anterior postoral ventral cirrus (arrow) slightly shifted leftwards. Frontoventral cirri circled. **j:** Ventral view of reorganizer. AZM, adoral zone of membranelles; BC, buccal cirrus; DB, dorsal bristles; FC, frontal cirri; IV/4, anterior frontoterminal cirrus; LMR, left marginal row; MA, macronuclear nodule; MI, micronucleus; RMR, right marginal row; TC, transverse cirri; UM, undulating membranes; VI, frontal-ventral-transverse cirri anlage VI. Scale bars: 30 µm (a, d-f, i, j), 10 µm (b).

Abstract

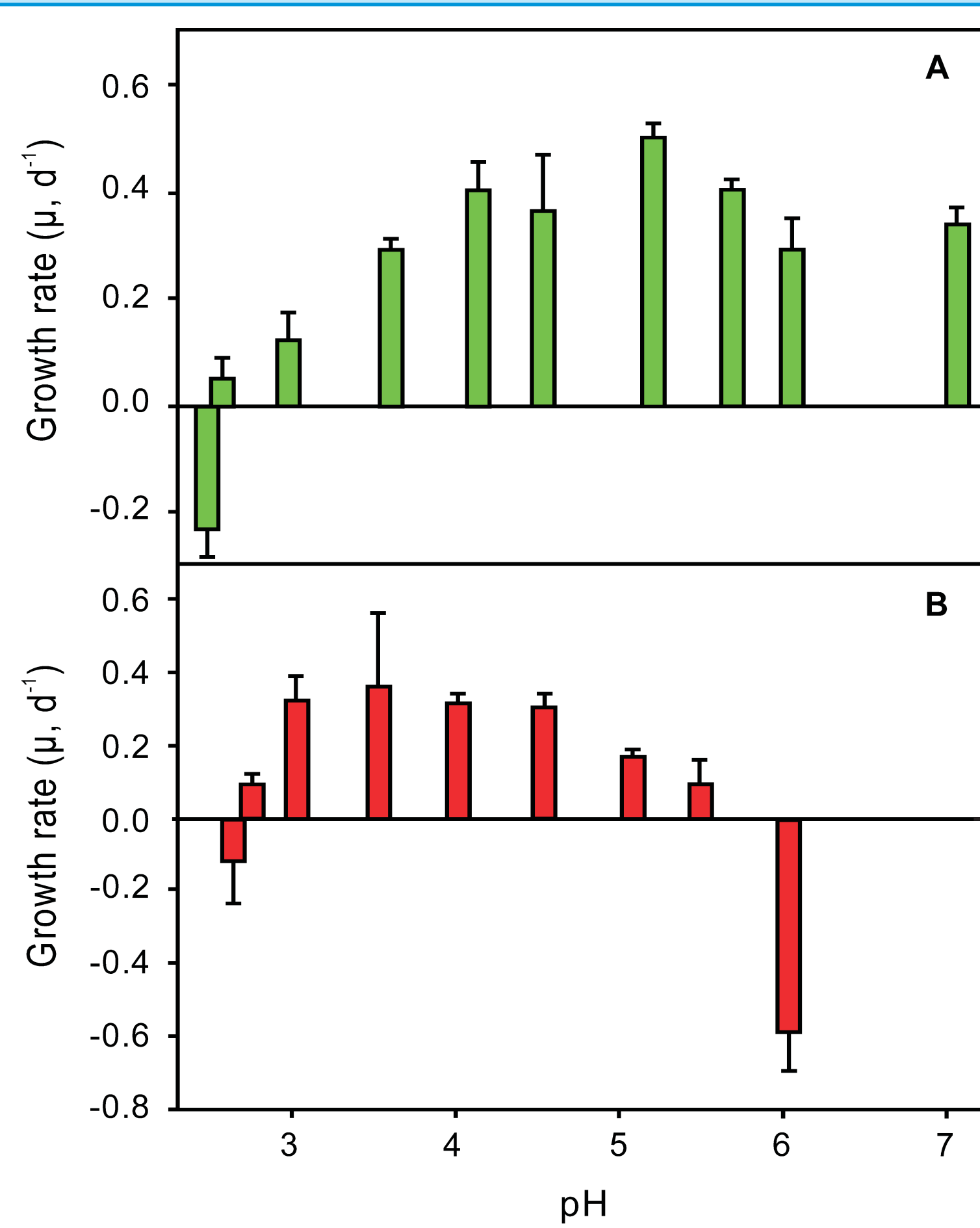
The ciliates were isolated from two chemically similar acid mining lakes (pH ~2.6) located in Lusatia, East Germany, and at Langau, Lower Austria. *Oxytricha acidotolerans* spec. nov. from Langau (Figs 1a-i, 2a-j) has 18 frontal-ventral-transverse cirri but a very indistinct kinety 3 fragmentation so that the assignment to *Oxytricha* is uncertain. The somewhat smaller species from Lusatia has the dorsal kineties arranged in the *Urosomoida* pattern and, perhaps due to the low pH and/or long cultivation time, a highly variable cirral pattern (Figs 3a-f); it is preliminary designated as *Urosomoida* spec. Both species differ by 3% in their SSU rDNA. The NJ tree (Fig. 7) resulting from 18S rDNA gene sequence analyses revealed that *Urosomoida* spec. from Lusatia is very closely related to an unidentified *Oxytricha*-like species from the acidic Spanish River Rio Tinto and to *Urosomoida longa*. The next relatives are *Oxytricha acidotolerans* and a cluster composed of three flexible oxytrichids. Our experimental results revealed a broad pH niche for *Oxytricha acidotolerans*, with positive growth rates over the entire pH range investigated, peaking at moderately acidic conditions (Fig. 4A). Cyst formation (Fig. 5) was positively and linearly related to pH (Fig. 6). *Urosomoida* spec. was more sensitive to pH and did not survive at circum-neutral pH (Fig. 4B). Our experimental results characterize *Oxytricha acidotolerans* as an acidotolerant species, while *Urosomoida* spec. is acidophilic.

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Figs 3a-f. *Urosomoida* spec. from Lusatia (protargol impregnation). Ventral and dorsal view of four specimens documenting very high variability of infraciliature and nuclear apparatus (drawn to scale). Arrow in (a) marks buccal cirrus, arrow in (b) denotes the dorsomarginal kinety. AZM, adoral zone of membranelles; CC, caudal cirri; FC, frontal cirri; LMR, left marginal row; MA, macronuclear nodule; MI, micronucleus; PVC, postoral ventral cirri; PTC+TC, pretransverse ventral cirri and transverse cirri; RMR, right marginal row; 1, dorsal kinety 1. Scale bar: 30 µm.

Fig. 7. Phylogenetic NJ based consensus tree of the SSU rDNA of hypotrich ciliates. The species used in this study are shown in bold face. Support values at the nodes represent bootstrap percentages of the Neighbor-joining (first number) and Maximum-likelihood (second number) analyses and posterior probabilities of the Bayesian analysis (third number). Arrows denote support values to corresponding nodes.



Figs 4A, B. Population growth rate of *Oxytricha acidotolerans* (A) and *Urosomoida* spec. (B) in relation to pH. Bars represent means of triplicates, the error bars denote 1 SD.

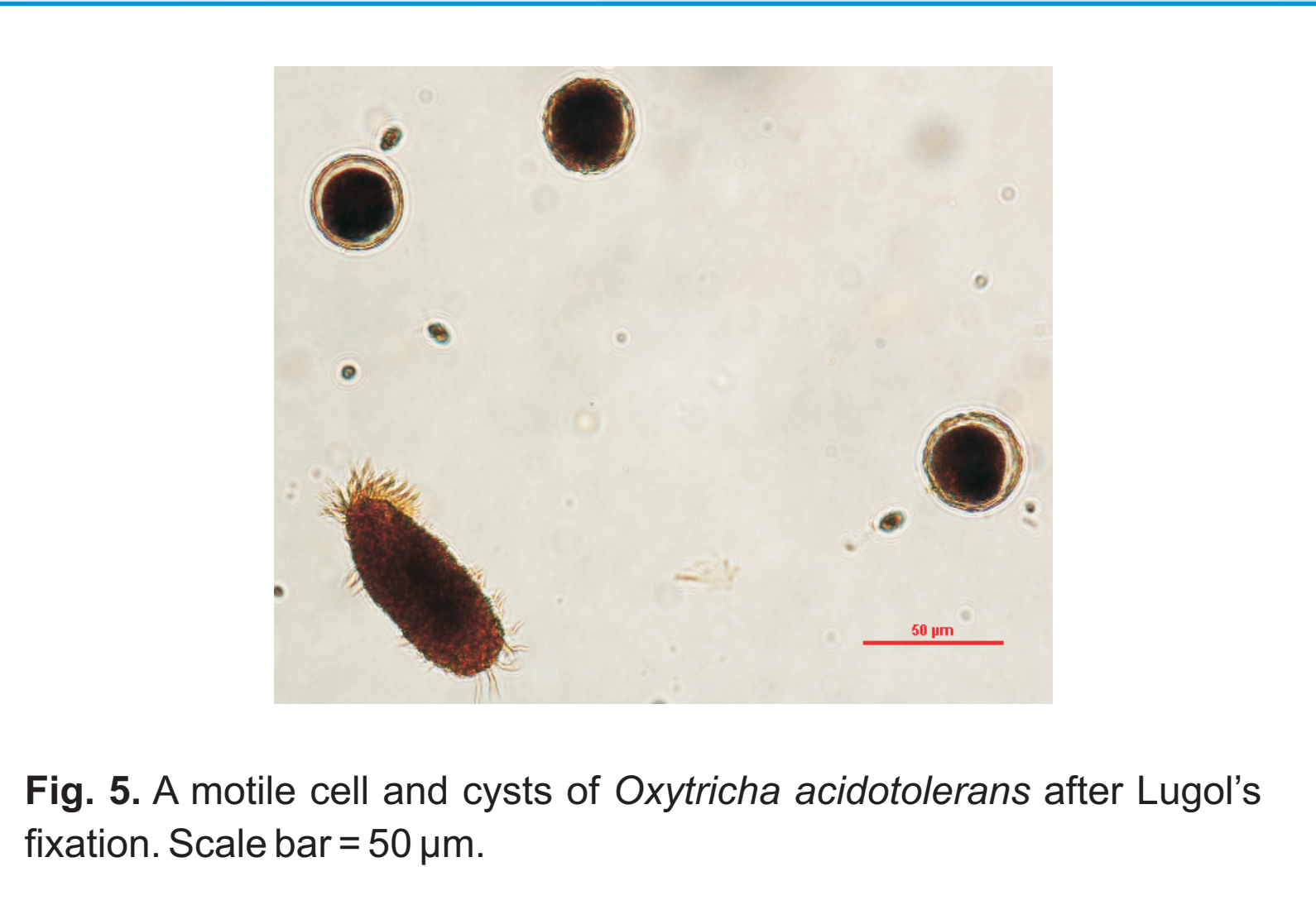


Fig. 5. A motile cell and cysts of *Oxytricha acidotolerans* after Lugol's fixation. Scale bar = 50 µm.

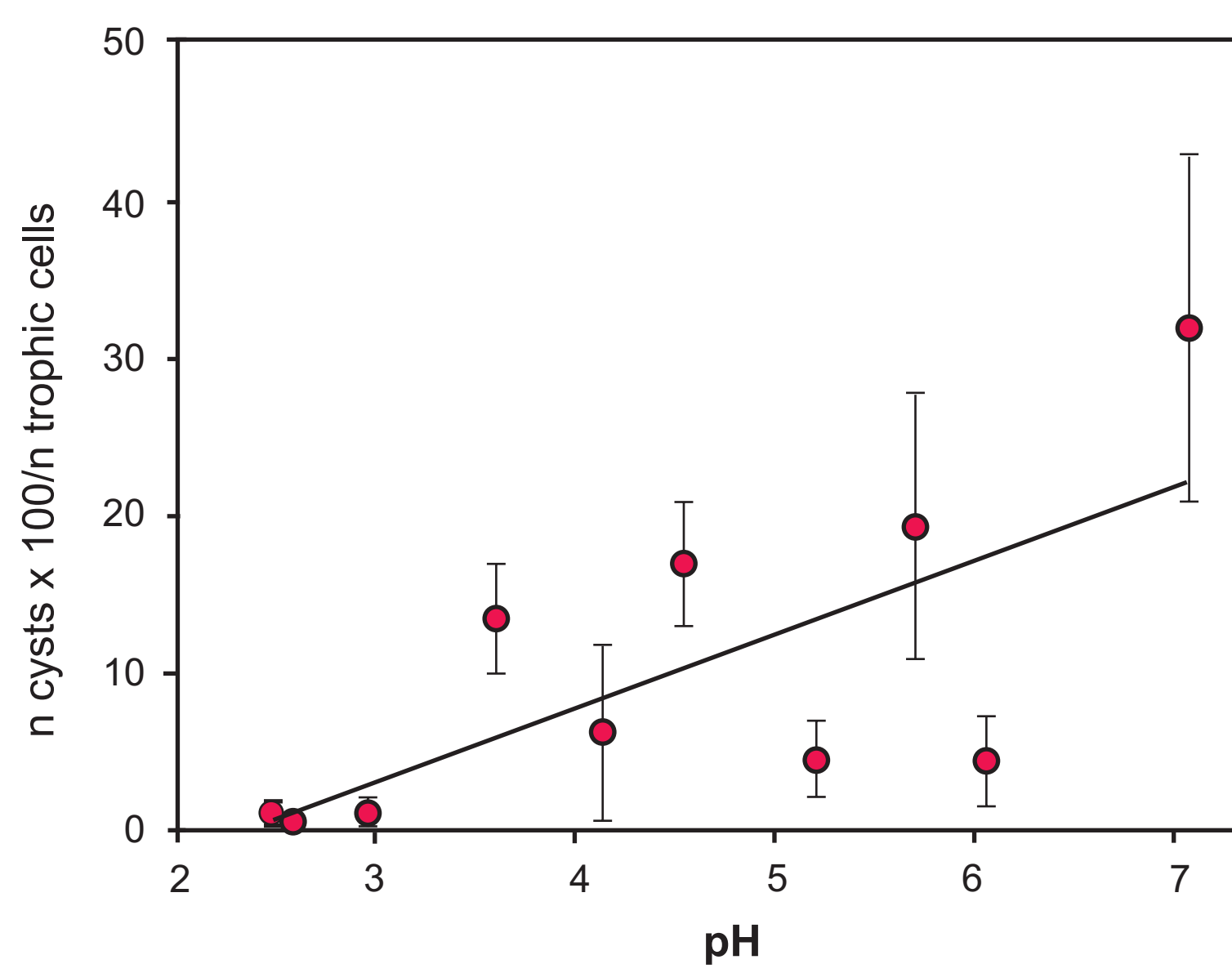
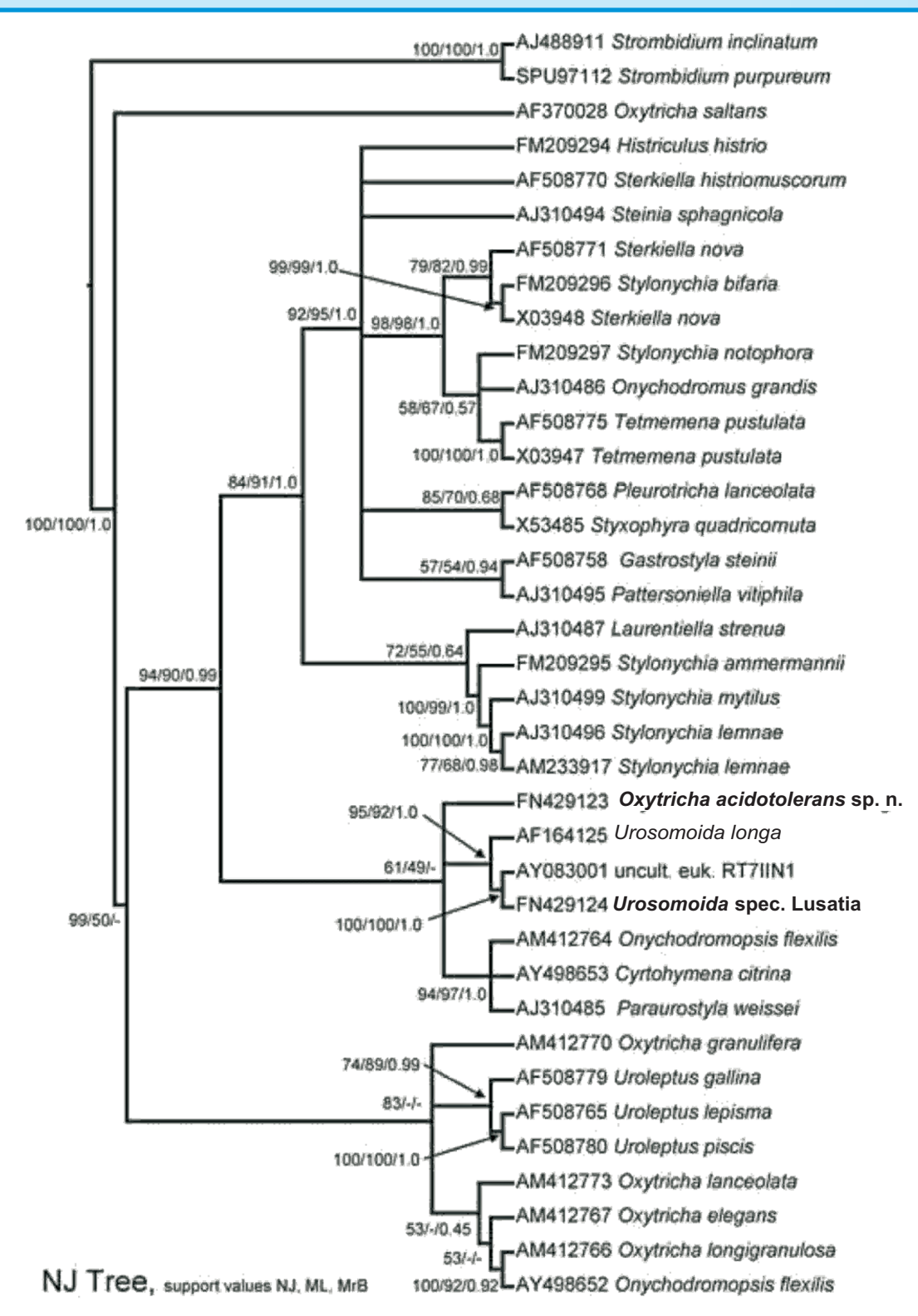


Fig. 6. The percentage of cysts in the population of *Oxytricha acidotolerans* in relation to pH. The line represents the linear regression of cysts (%) vs. pH.



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