

Stylonychia harbinensis sp. n., a new oxytrichid ciliate (Ciliophora, Hypotrichia) from the Heilongjiang Province, China

Shi Xin-Bai¹ and Dieter Ammermann²

¹ Department of Biology, Harbin Normal University, Harbin, China

² Zoologisches Institut der Universität, Tübingen, Germany

Summary

Stylonychia harbinensis sp. n. is widely distributed in fresh waters around Harbin, China. It belongs to the *S. mytilus*-complex (*S. mytilus*, *S. lemnae* and *S. ammermanni*). It is 142 x 58 µm in size (Protargol-impregnated cells) and usually has 2 large micronuclei (5 µm in diameter). *S. harbinensis* sp. n. has much smaller cells than *S. mytilus* and *S. lemnae*, and larger micronuclei than *S. ammermanni*. The new species also has a unique pattern of dorsal bristles. *S. harbinensis* sp. n. does not conjugate either with other species of the *S. mytilus* complex or with other *Stylonychia* species.

Key words: *Stylonychia harbinensis* sp. n., Stichotricha, Ciliophora

Introduction

Stylonychia is a widely distributed and well known freshwater oxytrichid ciliate genus. According to Foissner and others (1991), the first description of a *Stylonychia* species (*S. mytilus*) was published by Müller (1773). In his famous work Kahl (1932) recognized 11 species of the genus *Stylonychia*. Berger (1999), in his detailed review of the family Oxytrichidae, also validated 11 species. Most recently a new species, *S. ammermanni*, has been described from India (Gupta et al., 2001).

In this paper we describe a new species, *S. harbinensis* sp. n., which is widely distributed in fresh waters around Harbin, China. Preliminary reports of this species were presented earlier at “The second Asian

Conference on Ciliate Genetics, Cell Biology and Molecular Biology” (Shanghai, 1986) and in the “Proceedings of the 4th Symposium of the Chinese Protozoological Society” (Guang Zhou, 1987). However, the species had not been described in sufficient detail and Berger (1999, p. 599) therefore listed this name as “nomen nudum”.

Material and methods

Stylonychia harbinensis sp. n. was found in several ponds and small rivers around Harbin (126°80' E, 45°70' N). Most of the clones researched in preparation of the present paper were isolated from a pond in the Sun Island, located on the Song-Hua River in the northern part of Harbin. This pond is connected to and fed by

the Song-Hua River. *S. harbinensis* sp. n. was also found in the river itself. The holotype slide contains Protargol-impregnated cells from this location. This is therefore the type locality ("Terra typica") of the new species. This slide is deposited at the "Biology Centre of the upper Austrian Museum", J.-W.-Klein. Str. 73, A-4040 Linz, Austria (Collection "Evertibrata varia", no. 2003/45). Paratype slides are deposited at the Laboratory of Protozoology of the Harbin Normal University (Harbin 150080, China) and at the "Zoolo-gische Schausammlung" of the University of Tuebingen, Sigwartstr. 3, D-72076 Tuebingen.

The cells were grown in sterilized pond water (or Pringsheim's solution) and fed with the phytoflagellate *Chlorogonium elongatum* or the saprozoic flagellate *Chilomonas paramecium*. They were studied either alive or fixed and impregnated with Protargol. The modified Protargol method described in Shi and Frankel (1990) was used, except that the step "3" was changed to soaking the cells in albumin-glycerine-water (50 parts of distilled water with 2 parts of fresh egg albumin and 2 parts of glycerine). To stain the cirral basal plate, the bleaching time in KMnO_4 and oxalic acid in step "13" and "15" was prolonged to 8-10 min and 9-11 min, respectively. To study the nuclei, cells were fixed with saturated aqueous mercuric chloride and then stained by routine Feulgen reaction method.

Results

Stylonychia harbinensis sp. n. (Figs 1-4) possesses a rigid body, 18 frontal-ventral-transverse (FVT)-cirri, two rows of marginal cirri distinctly separated posteriorly and a large peristome (about a half of the cell length). It clearly belongs to the *S. mytilus* complex, a group of similar species (*S. mytilus*, *S. lemnae* and *S. ammermanni*). In general appearance it looks like a small *S. mytilus*. The characteristics of Protargol-stained cells are listed in Table 1. The new species is certainly not easy to distinguish from the other three species of the *mytilus*-group, but the combination of three characteristics allows a clear identification:

1. Size: The size of living well-fed cells is 176 ± 15 μm , being 154 ± 8 μm nine hours after food removal. (The size of *S. mytilus* nine hours after food removal is about 300 μm and that of *S. lemnae*, about 250 μm . The size of *S. ammermanni* (Protargol, 134 μm) overlaps with the size of *S. harbinensis* sp. n.).

2. Micronuclei (Mi): *S. harbinensis* sp. n. cells have a high frequency of 2 Mi (95% of 100 counted cells). Only 5% of the cells counted have 1, 3 or 4 Mi. The three other species have more Mi: *S. mytilus* and *S. lemnae* have 2-4 Mi per cell. In a population of *S. ammermanni* 33% of the cells had 2, 3 or 4 Mi in approximately equal proportion (Sapra, pers. comm.).

S. harbinensis sp. n. has large Mi: 7 μm in diameter in living cells, 5 μm in Protargol-impregnated cells. This is the same size range as that of the *S. mytilus* Mi, while *S. lemnae* has slightly smaller Mi (6 μm in living cells, 4 μm after Protargol) (Ammermann and Schlegel, 1983). The Mi of *S. ammermanni* are considerably smaller: 2.62 μm after Protargol (Gupta et al., 2001); 4 μm in living cells (own measurements).

3. Dorsal bristles: Cells of *S. harbinensis* sp. n. have an incomplete row 4 with 18 dorsal kineties. This is similar to *S. ammermanni* (incomplete, 21 bristles). But the new species has a complete row 3, while in *S. ammermanni* it ends before the posterior end of the cell (Gupta et al., 2001). In *S. mytilus* and *S. lemnae* all rows of dorsal bristles are complete (Ammermann and Schlegel, 1983).

Other distinguishing markers: The buccal cirrus in *S. harbinensis* sp. n. is located more posteriorly compared to *S. ammermanni*: the distance from the anterior end of the *S. harbinensis* sp. n. cell to the buccal cirrus is 27% of the cell length (Table 1), compared to 18% in *S. ammermanni* (according to Fig. 1A in Gupta et al., 2001). This is another small difference between these two species.

S. harbinensis sp. n. can also be distinguished by its 3 caudal cirri, which are 30-50 μm long in living cells, fringed and equally spaced (at a distance of 9-11 μm , see Fig. 1).

The number of micronuclear chromosomes, examined in young macronucleus enlarges of early exconjugants, is about 950 in *S. harbinensis* sp. n. (in *S. lemnae* $2n = 360$, Ammermann, 1987). Similar data from other species are not available.

The macronucleus is bipartite, as in all members of the *S. mytilus* group.

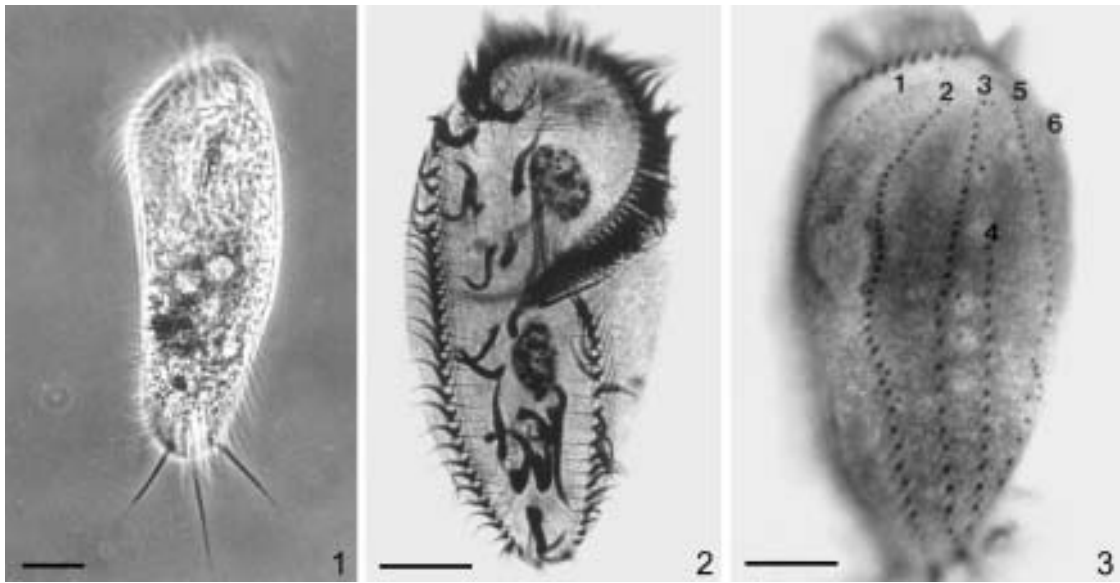
S. harbinensis sp. n. has a high division rate: the cells divide every 9 hours at room temperature (21° C) in the laboratory. The same rate is found in *S. ammermanni* (Sapra, pers. comm.). The length of cell cycle in *S. mytilus* and *S. lemnae* is 12 hours.

The pattern of division morphogenesis of *S. harbinensis* sp. n. is similar to that described for other members of the *S. mytilus*-group (Wirnsberger et al., 1986; Gupta et al., 2001). Therefore, we do not give any details.

Attempts to cross mature strains of *S. harbinensis* with other species from our collections, viz. *S. mytilus*, *S. lemnae*, *S. ammermanni*, *S. pustulata*, *S. putrina*, *S. notophora*, *S. pusilla* and *S. vorax* did not yield any conjugants (Sapra, personal communication and our own experiments). However, *S. harbinensis* sp. n. has mating types. Strains from different ponds mate with each other.

Comparison with other *Stylonychia* species.

The distinguishing characteristics of *S. harbinensis* sp. n. have already been described and a comparison



Figs. 1-3. *Stylonychia harbinensis* sp. n. 1 - Living cell, dorsal view; 2-3 - Protargol-impregnated cells, ventral (2) and dorsal (3) view, with 1-6 = kineties. Scale bars: 25 µm.

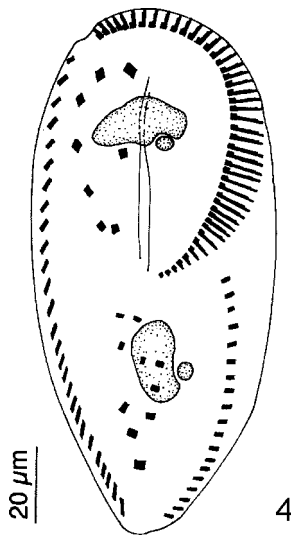


Fig. 4. *Stylonychia harbinensis* sp. n. Line diagram, ventral view.

Table 1 Morphometric characterization of *Stylonychia harbinensis* n. sp.

Character	Mean ± S.D.	n
Cell length (µm)	142 ± 6 µm	15
Cell width (µm)	58 ± 3 µm	15
Cell length/width	2.54 ± 0.85	15
Peristome length in % from cell length	51.5 % ± 2.17	15
Distance anterior end/buccal cirrus in % from whole length	27.2 % ± 1.66	15
No. of adoral membranelles	45 ± 4	10
No. of left marginal cirri	19 ± 2	10
No. of right marginal cirri	27 ± 4	10
No. of dorsal bristles in row 1 (see Fig. 3)	40 ± 3.6	10
	2	10
	3	10
	4	10
	5	5
	6	? ^{x)}
Mi number	2.1 ± 0.5	20
Mi diameter, µm,	5 ± 0.25 µm	10
Ma size (µm), length	22 ± 3.5 µm	20
Ma size (µm), with	11 ± 2.0 µm	20

Notes: The data were taken from Protargol impregnated cells. S.D. - standard deviation, n = number of specimens measured, ^{x)} could not be counted

with the other three species of the *S. mytilus*-complex has been made. However, it may be helpful to compare the new species with other species of the genus *Stylonychia* because the differences can be quite subtle.

1) In the same size range as the new species is *S. pseudograndis*, which was described in 1935 from China (Wang and Nie, 1935), but has not been found since then. This species has a strange number of cirri (an indication of incorrect observation according to Berger,

1999, who stated that a detailed redescription was necessary). The cell shape in this species is also different from that in *S. harbinensis* sp. n., and the caudal cirri are conspicuously shifted to the right in comparison to *S. harbinensis* sp. n. The adoral zone of membranelles

in *S. pseudograndis* is also much shorter than in *S. harbinensis* sp. n.

2) All other *Stylonychia* species described in Berger, 1999 in the size range of *S. harbinensis* sp. n. have either a different nuclear apparatus (*S. nodulinucleata*, *S. stylomuscorum*) and/or a different cell shape (*S. curvata*, *S. vorax*, *S. notophora*, *S. pustulata*, *S. putrina*, *S. pusilla*), or inconspicuous caudal cirri (*S. bifaria*).

ACKNOWLEDGEMENTS

We thank Prof. Dr. G. R. Sapra, Dept. of Zoology, University of Delhi, Delhi, India, for valuable advice and help. We also thank Dr. H. Berger, Salzburg (Austria) for some comments on the manuscript.

References

Ammermann D. 1987. Germ line specific DNA and chromosomes of the ciliate *Stylonychia lemnae*. *Chromosoma*. 95, 37-43.

Ammermann D. and Schlegel M. 1983. Characterization of two sibling species of the genus *Stylonychia* (Ciliata, Hypotricha): *S. mytilus* Ehrenberg, 1838 and *S. lemnae* n. sp. I. Morphology and reproductive behavior. *J. Protozool.* 30, 290-294.

Berger H. 1999. Monograph of the *Oxytrichidae* (Ciliophora, Hypotrichia). Kluwer Acad. Publishers, Dordrecht, Boston, London.

Foissner W., Blatterer H., Berger H. and Kohmann F. 1991. Taxonomische und ökologische Revision der Ciliaten des Saprobien-systems – Band I: Cyrtophorida, Oligotricha, Hypotrichia, Colpodea. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. 1/91, 1-478.

Gupta R., Kamra K., Arora S. and Sapra G.R. 2001. *Stylonychia ammermanni* sp. n., a new oxytrichid (Ciliophora: Hypotrichida) ciliate from the river Yamuna, Delhi, India. *Acta Protozool.* 40, 75-82.

Kahl A. 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Tierwelt Deutschlands*. 25, 399-650.

Müller O.F. 1773. *Vermium terrestrium et fluviatiliatum*, *Historia*.

Shi X-B. and Frankel J. 1990. Morphology and development of mirror-image doublets of *Stylonychia mytilus*. *J. Protozool.* 37, 1-13.

Wang C.C. and Nie D. 1935. Reports on the rare and new species of fresh-water infusoria, part II. *Sinensia*, Shanghai. 6, 399-524.

Wirnsberger E., Foissner W. and Adam H. 1986. Biometric and morphogenetic comparison of the sibling species *Stylonychia mytilus* and *S. lemnae*, including a phylogenetic system for the oxytrichids (Ciliophora, Hypotrichida). *Arch. Protistenkd.* 132, 167-185.

Address for correspondence: Dieter Ammermann, Lindenstr. 17, D 72119 Ammerbuch, Germany.
E-mail: dieter.ammermann@uni-tuebingen.de

Editorial responsibility: Sergei Fokin