

Phylogenetic position of three *Condylostoma* species (Protozoa, Ciliophora, Heterotrichea) inferred from the small subunit rRNA gene sequence

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Abstract

The systematically poorly known ciliate genus *Condylostoma* was erected by Vincent in 1826. About 10 morphotypes have been reported, but any molecular investigations concerning this group so far are lacking. In this work, the small subunit ribosomal RNA (SS rRNA) gene of three marine *Condylostoma* species was sequenced, by which the phylogenetic trees were constructed by distance-matrix, maximum parsimony and Bayesian inference methods. The results show that (1) all the trees have similar topologies with high supports; (2) *Condylostoma* is mostly related to the genus *Condylostentor*; and (3) three *Condylostoma* species as well as *Condylostentor auriculatus* cluster together and form a sister group with other heterotrichs. This is moderately consistent with the assessment of phylogenetic relationships of *Condylostoma*-related heterotrichs from the morphological information. The phylogenetic relationship of some other related heterotrichs, *Peritromus*, *Folliculina*, *Stentor* and *Blepharisma*, has been also discussed.

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1. Introduction

The comparison of small subunit ribosomal RNA sequences has gained widespread acceptance for the inference of the phylogenetic relationships among ciliated protozoa [1,2]. The number of ssrRNA sequences for ciliates has accumulated rapidly during the last few years, which reveals new insights on the taxonomy and phylogeny of ciliates and reflects more objective traits at the generic level [2–6].

In the past decades, many genera within the class Heterotrichea, e.g. *Stentor*, *Blepharisma*, *Climacostomum*, *Maristentor* and *Spirostomum* [7–10], have been investigated using molecular methods. However, the gene information of *Condylostoma* species, which are traditional heterotrichs, has not been available for a long time.

A new survey on evolutionary studies of ciliated protozoa, the SS rRNA gene of three congeners of *Condylostoma*, viz. *Condylostoma minutum*, *Condylostoma spatiosum* and *Condylostoma curva* was conducted. Inferred from sequences information combining with morphological characters (Fig. 1), we constructed and analyzed the topological trees to achieve a better interpretation of the phylogenetic relationships within the class Heterotrichea.

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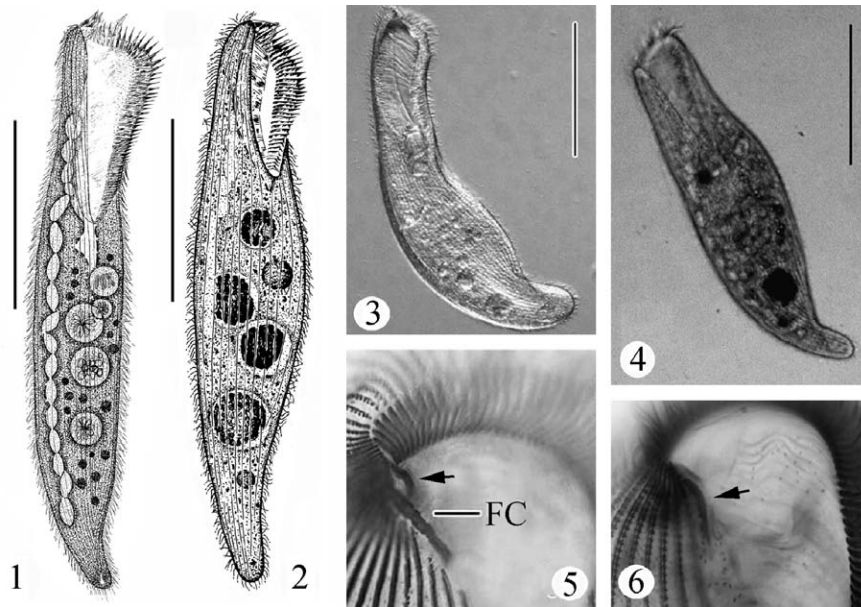


Fig. 1. Morphological characters of *Condylostoma spathiosum* (1, 3, 5) and *C. minimum* (2, 4, 6). 1–3, Typical specimens from life; 4–6, anterior portion of buccal apparatus, to show the paroral membrane (arrows in 4) and the long frontal cirrus (arrow in 6); 5, anterior portion, to reveal the frontal cirrus (FC) and the distal end of adoral zone. Scale bars = 200 μ m.

2. Materials and methods

2.1. Sample collection and identification

All the samples were collected from a sand beach near Qingdao (Tsingtao, 36°08'N; 120°43'E), China. Observations on living cells were carried out using bright field and differential interference microscopy [11].

2.2. Extraction of genomic DNA and phylogenetic analysis

Total DNA extraction, PCR and phylogenetic analyses were performed according to Refs. [5,6,12]. The SS rRNA gene sequences of other ciliates were obtained from the GenBank/EMBL databases. The dinoflagellate *Dinophysis acuta* was set as an outgroup.

3. Results

3.1. Sequences and comparisons

The SS rRNA gene sequences were firstly determined for *Condylostoma minutum*, *C. spatiosum* and *C. curva*. Their sequence lengths, GC-contents and GenBank Accession Nos. are as follows: for *C. minutum*, 1664 nucleotides, GC = 46.45% (DQ822482); for *C. spatiosum*, 1659 nucleotides, GC = 46.59% (DQ822483); and for *C. curva*, 1692 nucleotides, GC = 47.52% (EU379939).

3.2. Molecular phylogenetic analyses

As shown in Figs. 2 and 3, all the phylogenetic trees are nearly identical in topology. The monophyly of the phylum

Ciliophora is confirmed with highest supports (100% BI, 100% NJ, 100% LS). In all the trees, 47 ciliate species are generally divided into two large branches: subphylum Postciliodesmatophora and subphylum Intramacronucleata.

The class Heterotrichea appears to be a well-supported (95% BI, 100% NJ, 94% LS, 100% MP) monophyletic group. *Peritromus* branches firstly from the heterotrichs clade at a very deep level, followed by the separation of the *Climacostomum* branch. However, the position of *Spirostomum* and *Gruberia* are not well resolved in our analysis. The remaining heterotrichs separate into two strongly supported groups in all our trees (Fig. 2). In one group, three *Stentor* spp. and *Blepharisma* form a sister clade to *Eufolliculina* and *Maristentor*. However, *Condylostoma curva* clusters with *Condylostentor auriculatus* rather than with its congeners of *Condylostoma* in the other group (91% BI, 92% NJ, 93% LS, 62% MP) (Figs. 2 and 3).

3.3. Cladistic analyses based on morphological characters

A tree was constructed to demonstrate the potential phylogenetic position of *Condylostoma* in the well-known class Heterotrichea based on morphological information (Table 1). As Fig. 4 exhibits, clades of *Licinophora* and *Peritromus* branch at a very deep level. The characters of AZM (non-closed vs. closed) and buccal apparatus separate all other heterotrichs into two large groups, one group containing *Climacostomum*, *Fabrea*, *Spirostomum*, *Blepharisma*, *Bursaria* and the other group containing *Stentorids*, *Eufolliculina*. The genus *Condylostoma* branches at a basal position to the former group due to the apomorph of the highly developed paroral membrane and similar ciliature as well as the morphology (Fig. 4 and Table 1).

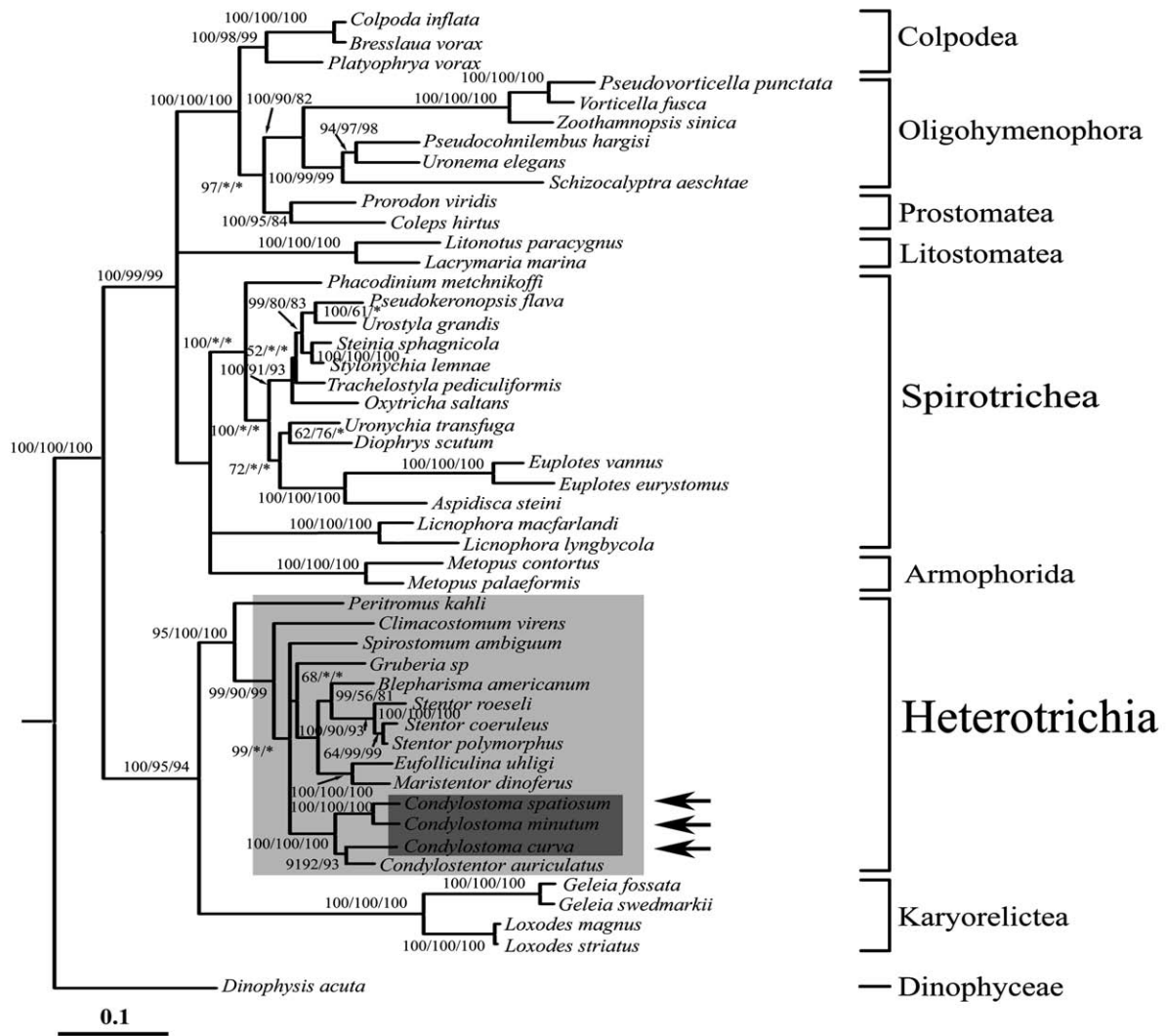


Fig. 2. The consensus phylogenetic tree derived from Bayesian analysis, and two distance analyses show the phylogenetic positions of the genus *Condylostoma*. Numbers at the nodes represent the posterior probabilities from 1,000,000 generations for Bayesian analysis followed by the bootstrap values from 1000 replicates for the neighbor-joining method and least-squares method in order. Asterisks indicate bootstrap values less than 50%. Evolutionary distance is represented by the branch length to separate the species in the figure. The scale bar corresponds to ten substitutions per 100 nucleotide positions. Arrows mark the three new *Condylostoma* species we sequenced.

4. Discussion

In agreement with other molecular analyses [13–15], our study confirms that the heterotrichous and karyorelictean ciliates belong to the Postciliodesmatophora, which has been indicated by morphological characters: (1) somatic dikinetids with postciliodesmata, and (2) division of the macronuclei by extramacronuclear microtubules [16–18].

Regarding the phylogeny of the genus *Condylostoma*, there have never been any investigations using molecular methods before. Hence, our present work is the first try to reevaluate its systematic position, which basically corresponds to the traditional arrangement, i.e. congeners of this genus belong to a clearly outlined, monophyletic clade in spite of only few data being available for the analysis.

The species *Condylontentor auriculatus* was originally described by Kahl [19] as *Stentor auriculatus*. After a rein-

vestigation, Fauré-Fremiet [20] transferred it to the genus *Condylostoma*. Later on, Jankowski [21] proposed to assign this species to the newly established genus *Condylontentor* (family Condylontentoridae), which was challenged by Foissner and Wöfl [22]. The present sequence analyses (Figs. 2 and 3) revealed a kinship of *Condylontentor* and three *Condylostoma* spp. Although Foissner and Wöfl synonymized *Condylontentor* with the well-known genus *Condylostoma*, we believe that the two genera can be separated by their different morphological characters, especially that of the buccal apparatus. *Condylontentor* has (1) a nearly closed AZM (vs. AZM only along the left side of the buccal cavity in *Condylostoma*); (2) a paroral membrane that is parallel to AZM and relatively inconspicuous (vs. paroral membrane extremely well developed and lies along the right side of the buccal cavity in *Condylostoma*); (3) many vestibulum kineties on the upper wall of the

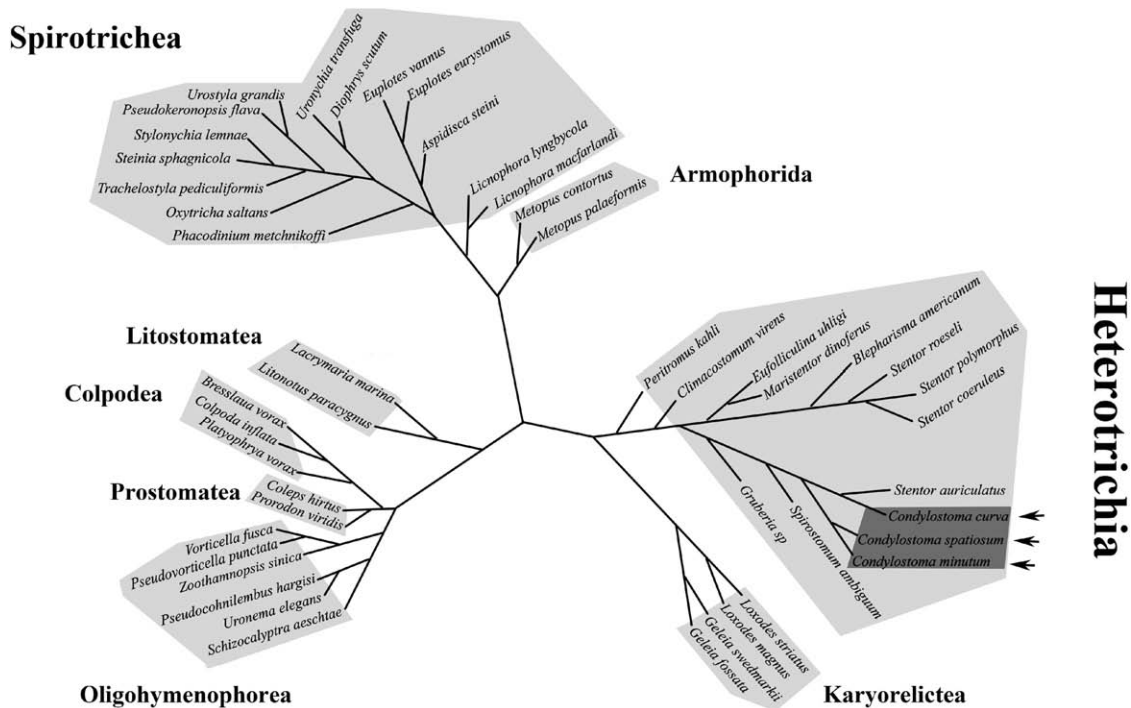


Fig. 3. Maximum parsimony (MP) tree of the genus *Condylostoma* constructed from SS rRNA gene sequences using PAUP* 4.0. Numbers are bootstrap values (percentage of 1000 replicates) for the internal branches.

Table 1
Assessment on the phylogenetic relationships of some representative heterotrichous genera based on morphological features

| | Apomorph | Plesiomorph |
|-----|--|-----------------------------------|
| 1. | Somatic kineties highly defenerated | Not highly degenerated |
| 2. | Adhesive disc present | Absent |
| 3. | Somatic ciliature clearly divided into dorsal and ventral patterns | Not divided |
| 4. | Somatic cilia differentiated, non-uniform | Somatic cilia uniform |
| 5. | AZM non-closed | AZM closed |
| 6. | Peristomial lobes absent | Present |
| 7. | Paroral membrane degenerated | Not degenerated |
| 8. | Body dorsoventrally compressed | Not dorsoventrally compressed |
| 9. | Buccal cavity predominant | Inconspicuous |
| 10. | Peristomial kineties highly developed | Not highly developed |
| 11. | Vestibular cavity deeply sunk and prominent | Not sunk and inconspicuous |
| 12. | Vestibulum kineties are located on the upper wall | In the central area or the bottom |
| 13. | Ventral groove present | Absent |
| 14. | Anterior end pointed | Blunt |
| 15. | Paroral membrane consisting of dikinetids | Monokinetids |
| 16. | No peristomial cilia in the vestibular cavity | Such cilia present |

vestibular cavity (vs. vestibulum kineties absent from buccal cavity in *Condylostoma*) [19]. Our cladistic analyses (Fig. 4) also show that these two genera are remarkably different from one another with regard to their morphological characters.

The clade *Condylostoma curva*–*Condylostentor auriculatus* is supported well by bootstrap or consensus values in all trees (Figs. 2 and 3) though, in the morphological aspect, three congeners of *Condylostoma* have more similarities. We suggest that the affiliation of two genera which is based only on the SS rRNA gene sequences of the four species could be premature, because the SS rRNA gene may not

provide sufficient genetic variation to rigorously test genera assignments. Instead, an expanded investigation on *Condylostoma* and *Condylostentor* is needed to gather more information, such as sequencing of some gene coding for proteins and inclusion of a wider selection of species, for the construction of phylogenetic trees with a better degree of resolution than the present ones.

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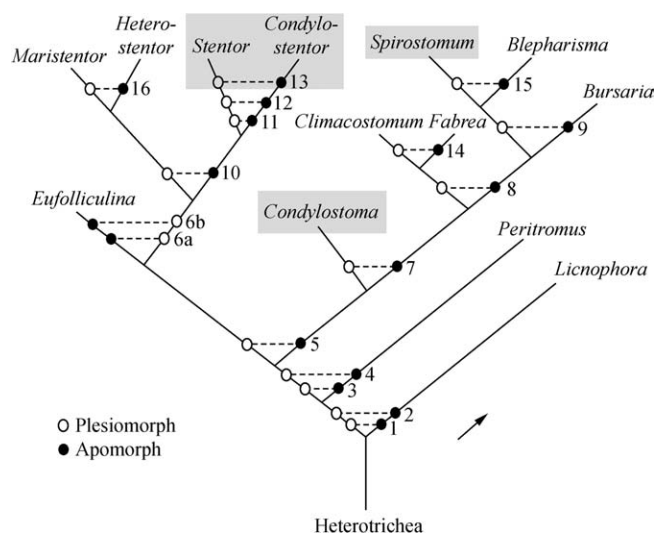


Fig. 4. Assessment of phylogenetic relationships of *Condylostoma*-related heterotrichs due to morphological information. Arrow indicates the evolutionary direction. Detailed data see Table 1.

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References

- [1] Elwood HJ, Olsen GJ, Sogin ML. The small subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytrichia nova* and *Stylonychia pustulata*. *Mol Biol Evol* 1985;2:399–410.
- [2] Bernhard D, Stechmann A, Foissner W. Phylogenetic relationships within the class Spirotrichea (Ciliophora) inferred from small subunit rRNA gene sequences. *Mol Phylogen Evol* 2001;21:86–92.
- [3] Greenwood SJ, Schlegel M, Sogin ML, et al. Phylogenetic relationships of *Blepharisma americanum* and *Colpoda inflata* within the phylum Ciliophora inferred from complete small subunit rRNA gene sequences. *J Protozool* 1991;38:1–6.
- [4] Chen Z, Song W. Phylogenetic positions of *Aspidisca steini* and *Euplotes vannus* within the order Euplotida (Hypotrichia: Ciliophora) inferred from complete small subunit ribosomal RNA gene sequences. *Acta Protozool* 2002;41:1–9.
- [5] Li L, Song W. Phylogenetic position of the marine ciliate, *Certesia quadrinucleata* (Ciliophora; Hypotrichida) inferred from the complete small subunit ribosomal RNA gene sequence. *Europ J Protistol* 2006;42:55–61.
- [6] Shang H, Song W, Warren A, et al. Phylogenetic positions of two marine ciliates, *Metanophrys similis* and *Pseudocohnilembus hargisi* (Protozoa, Ciliophora, Scuticociliatia), inferred from complete small subunit rRNA gene sequences. *Prog Nat Sci* 2006;16:373–8.
- [7] Hirt R, Dyal PL, Wilkinson M, et al. Phylogenetic relationships among karyorelictids and heterotrichs inferred from small subunit rRNA sequences: resolution at the base of the ciliate tree. *Mol Phylogen Evol* 1995;4:77–87.
- [8] Miao W, Simpson AGB, Fu C, et al. The giant zooxanthellae-bearing ciliate *Maristentor dinoferus* (Heterotricha) is closely related to Folliculinidae. *J Eukaryot Microbiol* 2005;52:11–6.
- [9] Rosati G, Modeo L, Melai M, et al. A multidisciplinary approach to describe protists: a morphological ultrastructural, and molecular study on *Peritromus kahli* Villeneuve-Brachon, 1940 (Ciliophora, Heterotricha). *Protist* 2006;158:139–45.
- [10] Hammerschmidt B, Schlegel M, Lynn DH, et al. Insights into the evolution of nuclear dualism in the ciliates revealed by phylogenetic analysis of rRNA sequences. *J Eukaryot Microbiol* 1996;43:225–30.
- [11] Hu X, Song W. Redescription of two known species, *Gastrocirrhus monilifer* (Ozaki et Yangiu, 1942) and *Gastrocirrhus stentoreus* Bullington, 1940, with reconsideration of the genera *Gastrocirrhus* and *Euplotidium*. *Acta Protozool* 2003;42:345–55.
- [12] Chen Z, Song W, Warren A. Studies on six *Euplotes* spp. (Ciliophora: Hypotrichida) using RAPD fingerprinting, including a comparison with morphometric analyses. *Acta Protozool* 2000;39:209–16.
- [13] Lynn DH, Strüder-Kypke M. Phylogenetic position of *Lincnophora*, *Lechriopyla*, and *Schizocaryum*, three unusual ciliates (phylum Ciliophora) endosymbiotic in echinoderms (Phylum Echinodermata). *J Eukaryot Microbiol* 2002;49:460–8.
- [14] Schlegel M, Eisler K. Evolution of ciliates. In: Hausmann K, Bradbury PC, editors. *Ciliates: cells as organisms*. Gustav Fischer Stuttgart; 1996. p. 73–94.
- [15] Stechmann A, Schlegel M, Lynn DH. Phylogenetic relationships between prostome and colpodean ciliates tested by small subunit rRNA sequences. *Mol Phylogen Evol* 1998;9:48–54.
- [16] de Puytorac deP, Grain J, Mignot JP. *Précis de Protistologie*. Paris: Société Nouvelle des Editions Boubée; 1987, [in French].
- [17] Raikov IB. *The protozoan nucleus. Morphology and evolution*. New York: Springer; 1982.
- [18] Small EB, Lynn DH. Phylum Ciliophora Doflein, 1901. In: *An illustrated guide to the Protozoa*. Lawrence: Society of Protozoologists; 1985, pp. 393–575.
- [19] Kahl A. *Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha*. *Tierwelt Dtl* 1932;25:399–650.
- [20] Fauré-Fremiet E. *Condylostoma (Stentor) auriculatus* (Gruber). *Bull Soc Zool Fr* 1936;61:511–9.
- [21] Jankowski AW. *Conspectus of a new system of the phylum Ciliophora*. *Proc Zool Inst Acad Sci USSR Leningrad* 1980;94:102–21.
- [22] Foissner W, Wölfl S. Revision of the genus *Stentor* Oken (Protozoa, Ciliophora) and description of *S. araucanus* nov. spec. from South American lakes. *J Plankt Res* 1994;16:255–89.