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

Wenbo Guo, Weibo Song, Khaled A.S. Al-Rasheid, Chen Shao, Miao Miao, Saleh A. Al-Farraj, Saleh A. Al-Qurishy, Zigui Chen, Zhenzhen Yi, Shan Gao

Laboratory of Protozoology, Ocean University of China, Minxing Building B, 5 Yushan Road, Qingdao 266003, China
Zoology Department, King Saud University, Riyadh 11451, Saudi Arabia

Received 24 January 2008; received in revised form 9 April 2008; accepted 9 April 2008

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.

© 2008 National Natural Science Foundation of China and Chinese Academy of Sciences. Published by Elsevier Limited and Science in China Press. All rights reserved.

Keywords: Marine ciliates; *Condylostoma*; SS rRNA; Phylogeny; Heterotrichs

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 Heterotrichae, 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 RNA 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 Heterotrichae.
2. Materials and methods

2.1. Sample collection and identification

All the samples were collected from a sand beach near Qingdao (Tsingtao, 36°17'08"N; 120°43'00"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 Heterotrichia 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 Heterotrichia based on morphological information (Table 1). As Fig. 4 exhibits, clades of Licnophora 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).
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 *Condylostentor auriculatus* was originally described by Kahl [19] as *Stentor auriculatus*. After a reinvestigation, Faure`-Fremiet [20] transferred it to the genus *Condylostoma*. Later on, Jankowski [21] proposed to assign this species to the newly established genus *Condylostentor* (family *Condylostentoridae*), which was challenged by Foissner and Wölf [22]. The present sequence analyses (Figs. 2 and 3) revealed a kinship of *Condylostentor* and three *Condylostoma* spp. Although Foissner and Wölf synonymized *Condylostentor* 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. *Condylostentor* 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. 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.
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.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (Grant No. 30670280 and
40676076) and a grant from the Center of Excellence in Biodiversity, King Saud University.

References


