Short communication

Reconsideration of the phylogenetic positions of three stichotrichous genera Holosticha, Anteholosticha and Pseudokeronopsis (Spirotrichea: Ciliophora) inferred from complete SSU rRNA gene sequences

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Abstract

The small subunit rRNA (SSU rRNA) gene for six marine stichotrichs, Pseudokeronopsis carnea, P. flava, Holosticha heterofoissneri, H. diademata, H. braudhuyae and Anteholosticha manca, was sequenced and characterized. Using this molecular information, the phylogenetic positions of three related genera, Pseudokeronopsis, Holosticha and Anteholosticha were determined. The results indicate that the family Urostylidae is clearly separated from the family Pseudokeronopsidae. The present phylogenetic analyses unambiguously placed Pseudokeronopsis close to Holosticha, and support the conclusion that these genera should be regarded as the members of the order Urostylidae within the stichotrich clade. Furthermore, the results of this study also support the monophyly of the genus Pseudokeronopsis, the redefinition of Holosticha s. str. and the placement of Anteholosticha in a clade separate from Holosticha.

Keywords: Urostylidae; Clade; Evolution; Taxonomy

1. Introduction

Stichotrichous ciliates are commonly found in marine, limnetic and terrestrial biotopes. Studies on these have concentrated mainly on their morphology, ecology, taxonomy and systematic classification. A large part of the remaining spirotrichs comprises the hypotrichs (s. l.), which were split, mainly according to DNA sequence data [1,2], into hypotrichs s. str. (the former euplotids) and stichotrichs (all other former hypotrichs). The taxonomy and phylogeny of the subclass Stichotrichia are, however, among the most confused within the phylum Ciliophora, mainly due to the paucity of morphological and morphogenetic data [3]. The genera Pseudokeronopsis and Holosticha (s. l.) are highly and particularly differentiated at the intrageneric level, which indicates that they might be pivotal organisms in terms of their phylogenetic positions [4–7].

Sequence information from homologous macromolecules shared by all members of a group can be used to measure the extent of genetic relationships among them [8]. The evolutionary relationships of and within the class Spirotrichea based on the molecular data have been discussed by several authors [9–11]. These reports mainly focused on order level and higher. Interesting information related to
lower level taxa within the group (e.g. the composition and the branching orders of families and genera) is, however, now being generated as the molecular data increase.

We have sequenced the SSU rRNA gene for six marine stichotrichous ciliates, Pseudokeronopsis carnea, P. flava, Holosticha heterofoissneri, H. bradburyae, H. diademata and Anteholosticha manca. The main aims of this study are to determine the systematic positions of these taxa and to enhance our understanding of the phylogenetic relationships within the subclass Stichotrichia.

2. Materials and methods

2.1. Ciliate collection and identification

All specimens used are from the culture store of the Laboratory of Protozoology (OUC), which were isolated from the coastal waters near Qingdao, China. Clonal cultures were established and maintained in autoclaved marine water with the appropriate prey at room temperature. Identification was performed according to Song and Wilbert [12].

2.2. DNA extraction, PCR and cloning

Cells were rinsed three times with sterile artificial marine water after being starved overnight and then pelleted by centrifugation. Genomic DNA was extracted as described previously [13]. A 1:1 mixture of the regular DNA polymerase (Promega, USA) and the Pfu Taq DNA polymerase (High Fidelity, Sangon, Canada) was utilized for the PCR mix. Genomic DNA was extracted as described [13]. A 1:1 mixture of the regular DNA polymerase (Promega, USA) and the Pfu Taq DNA polymerase (High Fidelity, Sangon, Canada) was utilized for the PCR mix. Genomic DNA was extracted as described [13].

2.3. Sequence availability and phylogenetic analysis

The nucleotide sequences referred to in this work are available from the GenBank/EMBL databases. A kary-elicited ciliate, Loxodes magnus L31519, was selected as the outgroup species. All methods used for phylogenetic analyses were performed as previously described [13].

3. Results

3.1. Sequences and comparisons

The SSU rRNA gene sequences of six stichotrichs were submitted to the GenBank database. Their length, GC content (%) and the accession numbers are as follows: *Pseudokeronopsis carnea*, 1770 bp, 44.35%, AY881633; *P. flava*, 1770 bp, 44.69%, AY881634; *Holosticha heterofoissneri*, 1776 bp, 45.49%, DQ059582; *H. bradburyae*, 1783 bp, 44.97%, EF123706; *H. diademata*, 1784 bp, 45.74%, DQ059583, and *Anteholosticha manca*, 1772 bp, 43.99%, DQ503578. Alignment of these sequences clearly showed similarities in the primary structure and GC content of the SSU rRNA gene in stichotrichs to other Spirotrichous ciliates. The GC contents are in the same range as in other ciliates [3,13,14].

The sequence of *P. carnea* differed in 35 nucleotides from the sequence of *P. flava* (structural similarity 98%). Seventy-one sites are different between *H. heterofoissneri* and *H. diademata* (structural similarity 96%); 104 sites are different between *H. heterofoissneri* and *H. bradburyae* (structural similarity 94%); 52 sites are different between *H. bradburyae* and *H. diademata* (structural similarity 97%); 110 sites are different between *A. manca* and *A. multistyliata* (structural similarity 93%). The structural similarities were 89–91% between *Pseudokeronopsis* and *Holosticha* specimens; 88–92% between *Anteholosticha* and *Holosticha* specimens; and 90–92% between *Pseudokeronopsis* and *Anteholosticha* specimens.

3.2. Bayesian inference and distance-matrix analyses

Trees inferred from the SSU rRNA gene sequences were constructed using multiple algorithms. Both Bayesian inference and distance-matrix trees indicate that the specimens belonging to the same genus cluster together with strong bootstrap support (Fig. 1). Interestingly, *Holosticha* (s. str.) clusters with Amphisella, which is consistent with the morphological data [15], and is separated from *Anteholosticha*, which clusters in a clade with *Diaxonella* and Urostyla. *Pseudokeronopsis* forms an additional clade within the stichotrich clade just after *Holosticha-Amphisella*.

Phylogenetic analyses provide a strong bootstrap support for the monophyly of the classes Spirotrichaea, Oligo-hymenophorea and Heterotrichia *sensu* Lynn and Small 2002 (Fig. 1). In the Baltic sea, the subclass Hypotrichia branches first from the spirotrichcean clade at a very deep level (93% Bayesian credibility). The subclass Stichotrichia is divided into two orders, Stichotrichida and Urostylida, and forms a sister group to the clade Hypotrichia (99% Bayesian credibility) (Fig. 1). However, both Stichotrichida and Urostylida seem to be paraphyletic. In the stichotrich clade, *Pseudoamphisella* diverges first from the main group followed in turn by *Pseudokeronopsis-Pseudourostyla*, *Anteholosticha-Diaxonella-Urostyla*, *Holosticha-Amphisella*, *Hemigastrostyla*, with the remaining stichotrich ciliates clustering together and to form a monophyletic group. Interestingly, the clade comprising *Uroleptus*, *Paruroleptus* and *Paraurostyla* is more closely related to oxytrichids (s. l.) than to other typical urostylic strains.

3.3. Maximum parsimony analyses

As shown in Fig. 2, the major aspects of the topology of the maximum parsimony tree (MP) are generally similar to
those of the Bayesian tree (Fig. 1). For example, *Pseudokeronopsis* (represented by *P. carnea* and *P. flava*) and *Holosticha* (s. str., represented by *H. heterofoissneri*, *H. bradburyae* and *H. diademata*) cluster together in the subclass Stichotrichia, while *Holosticha* clusters with *Amphisiella* and is separated from *Anteholosticha*.

4. Discussion

As demonstrated in this work and elsewhere [1,2], each of the classes Spirotrichea, Oligohymenophorea, and Heterotrichia is confirmed as monophyletic groups based on SSU rRNA gene sequences. Furthermore, our analyses also support the monophyly of the subclass Stichotrichia *sensu* Lynn and Small. Stichotrichia comprises four orders: Plagiotomida, Sporadotrichida, Urosylida and Stichotrichida. Only representatives of the latter two were included in this study, both of which were found to be polyphyletic, as demonstrated by some recent investigations and the systematic relationship among these highly diverse taxa needs further re-estimation and reevaluation (Shao et al. unpublished, personal communication).

The genus *Pseudokeronopsis*, which was erected by Borror and Wicklow [16], is characterized by its ciliation that is of the *Holosticha*-pattern apart from the frontal cirri, which form the so-called “bicorona”, i.e. comprising two curved and parallel rows of frontal cirri that are continuous posteriorly with the midventral complex (=midventral rows) [7,16]. Our results indicate that the family Urostyliidae (including the subfamily Holostichinae) is clearly separated from the family Pseudokeronopsidae, as suggested by Borror and Wicklow [16]. Furthermore, our analysis unambiguously places *Pseudokeronopsis* close to *Holosticha*, which supports the conclusion that these genera should be regarded as the members of the order Urostylida within the stichotrich clade; and confirms the monophyly of the genus *Pseudokeronopsis*.

*Holosticha* Wrzesiowski, 1877 is a large genus within the subclass Stichotrichia. It has long been assumed that *Holosticha* (s. l.) is monophyletic because its members share
very similar patterns of infraciliature. Furthermore, it is generally considered that most hypotrichs (s. l.) share a similar pattern of morphogenesis at the generic level [4].

Species within the genus Holosticha, however, exhibit a range of morphogenetic processes, which led to the suggestion that these taxa may have evolved from different lineages and that Holosticha is therefore not monophyletic [5]. In his review of the genus, Berger [17] recognized only seven species of Holosticha, these being unified by a highly characteristic combination of synapomorphies (i.e. anterior end of the left marginal row curved rightwards, adoral zone bipartite, buccal cirrus distinctly anterior of the paroral) and plesiomorphies. The seven species are: H. bradburyae Gong et al., 2000; H. diademata (Rees, 1884) Kahl, 1932; H. foissneri Petz et al., 1995; H. gibba (Müller, 1786) Wrzesniowski, 1877; H. heterofoissneri Hu and Song, 2001; H. pullaster (Müller, 1773) Foissner et al., 1991; H. spindleri Petz et al., 1995. All other species formerly included in Holosticha were assigned to the genera Anteholosticha (29 species), Caudiholosticha (10 species), and Biholosticha (2 species) [8]. This work supports this arrangement in the sense that (1) H. bradburyae, H. diademata and H. heterofoissneri clustered together; (2) Holosticha was confirmed as being monophyletic; and (3) Anteholosticha clustered separately from the Holosticha clade. More molecular data, including complete sequences of the large subunit ribosomal RNA and sequences of protein genes such as tubulins and DNA-dependent RNA polymerase.

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References


