Available online at www.sciencedirect.com

ScienceDirect

Progress in Natural Science 19 (2009) 1097–1101

Progress in Natural Science

www.elsevier.com/locate/pnsc

Phylogenetic investigation on five genera of tintinnid ciliates (Ciliophora, Choreotrichia), based on the small subunit ribosomal RNA gene sequences

Zicong Li[a], Zhenzhen Yi[a], Jinpeng Yang[a], Jun Gong[b], John C. Clamp[c], Khaled A.S. Al-Rasheid[d], Saud Al-Arifi[d], Abdulaziz A. Al-Khedhairy[d], Weibo Song[a,*]

[a] *Laboratory of Protozoology, KLM, Ocean University of China, Qingdao 266003, China*
[b] *Department of Zoology, Natural History Museum, London SW7 5BD, UK*
[c] *Department of Biology, North Carolina Central University, NC 27707, USA*
[d] *Zoology Department, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*

Received 7 October 2008; received in revised form 6 November 2008; accepted 7 November 2008

Abstract

The phylogenetic relationship among tintinnid ciliates are relatively poorly studied based on molecular data. In the present work, seven species belonging to five genera of the order Tintinnida (*Amphorellopsis acuta*, *Codonellopsis nipponica*, *Favella taraikaensis*, *Stenosemella nivalis*, *Tintinnopsis beroidea*, *Tintinnopsis cylindrica* and *Tintinnopsis lohmanni*) were analyzed using the information on their small subunit ribosomal RNA gene sequences. Phylogenetic trees were constructed using Bayesian inference (BI), maximum parsimony (MP), neighbor-joining (NJ), and least-squares (LS) methods. Generally, similar topologies were revealed with high or moderate supports, in which the main results show that (1) all tintinnids analyzed belong to a single assemblage; (2) congeners in *Tintinnopsis* do not cluster together, which indicates that the lorica-based definition for this genus is not consistent with the SSU rRNA phylogeny; (3) *A. acuta* groups with *Tintinnidium mucicola* but not with *Eutintinnus*, indicating that the traditional family Tintinnidae might be a paraphyletic group; (4) *Stenosemella* and *Codonellopsis* are clearly most related and possibly even merged into one genus regarding their similar morphology and molecular analyses, and possession of a hyaline collar is the only characteristic of the genus.

Keywords: Phylogeny; Tintinnida; Molecular systematics; SSU rRNA

---

1. Introduction

Lorica-bearing oligotrich ciliates, also known as tintinnids, are important elements in the planktonic food web [1,2]. The taxa-rich order Tintinnida comprises about 15 families, 74 genera and over 1200 morphospecies [3,4]. Historically, identification and systematic schemes of tintinnid ciliates have emphasized lorica features (e.g. the presence/absence of bowl, aboral horn, collar and oral rim, their relative size, shape and ornamentation, the capability of agglutination, the structure and texture of the wall). The presence of an agglutinated (hyaline) lorica was considered to be an ancestral (derived) feature by Kofoid and Campbell [3,5], which disagreed with the cladograms based on infraciliature characters [6–9]. This lorica-based classification of tintinnids, which virtually ignored zooid morphology, has recently been demonstrated to be different from their molecular phylogeny, though the monophyly of tintinnids is generally well recognized [8,9].

Resolving the phylogenetic relationships, understanding their evolutionary pathway and thereafter systematic

---

[*] Corresponding author. Tel.: +86 532 82032283.
E-mail address: wsong@ouc.edu.cn (W. Song).

1002-0071/$ - see front matter © 2009 National Natural Science Foundation of China and Chinese Academy of Sciences. Published by Elsevier Limited and Science in China Press. All rights reserved.
doi:10.1016/j.pnsc.2008.11.011

revision of tintinnids are just beginning, as the infraciliature of numerous species is yet to be characterized, and many families and genera remain non-sampled, under-sampled or unresolved in molecular trees [8–10]. As a contribution, we herein focus on the phylogenetic matter of five tintinnids genera, *Amphorellopsis*, *Codonellopsis*, *Favella*, *Stenosemella* and *Tintinnopsis*, of which the identities were determined based on traditional lorica morphology, and SSU rRNA were sequenced as well.

The present study seeks to further explore the lorica morphology of SSU rRNA phylogenetic relationships by incorporating newly obtained data from seven tintinnids of these five genera. Specific questions are asked: (1) Are *Tintinnidium* and *Amphorellopsis* closely related? (2) Could *Stenosemella* and *Codonellopsis* be congeneric? (3) What is the relationship between the families Codonelleopsidae and Codonellidae? (4) Is there more evidence for the paraphyletic genus *Tintinnopsis*?

2. Materials and methods

2.1. Ciliate collection

Amphorellopsis acuta (Schmidt, 1901) [3], *Codonellopsis nipponica* (Hada, 1964), *Favella taraikaensis* (Hada, 1932), *Stenosemella nivalis* (Meunier, 1910) [3], *Tintinnopsis beroidea* (Stein, 1867), *T. cylindrica* (Daday, 1887), and *T. lohmanni* (Laackmann, 1906) were collected from the coast of Qingdao (Tsingtao, 36° P P08′ N; 120° P43′ E), China. Subsequent isolation and identification were carried out according to Xu et al. [11].

2.2. DNA extraction, PCR amplification and phylogenetic analyses

Total DNA extraction, PCR and phylogenetic analyses were performed according to references [12–14]. The SSU rRNA gene sequences of other ciliates were obtained from the GenBank/EMBL databases.

3. Results

3.1. Deposition of sequences

The obtained SSU rRNA sequences of seven tintinnids have been deposited in GenBank, and their accession numbers are listed in Table 1.

3.2. Comparison of SSU rRNA gene sequences

The genetic distances (d) among tintinnid ciliates are rather high compared with other spirotrichs. The maximum genetic distance between tintinnid species is 0.131 (*T. beroidea–A. acuta*), whereas when compared with all choreotrich species, it is 0.137 (*T. beroidea–Pelagostrobilidium neptuni*). The minimum genetic distance between tintinnids is 0 (*T. cylindrica–Tintinnopsis tubulosoides*), which shows that the sequences are identical.

3.3. Phylogenetic analyses

Four different methods (BI, MP, LS, and NJ) resulted in basically congruent topologies (Figs. 1 and 2). All the six subclasses of Spirotrichea, viz. Choreotrichia, Hypotrichia, Oligotrichia, Phacodiniidia, Protocruziidia, and Stichotrichia, appear monophyletic. Within the Choreotrichia, the monophyletic Tintinnida is highly or moderately supported (0.99 BI, 81% MP, 71% LS, and 64% NJ). However, species with agglomerated (e.g. in *Tintinnopsis*) and hyaline (e.g. in *Favella*, *Metacylis*, and *Rhabdonella*) loricae do not form separate groups.

The *Tintinnopsis* species seem highly dispersed. *Tintinnopsis tocatinensis*, *T. cylindrica*, and *T. tubulosoides* form a solid group, whereas *T. beroidea* and *Tintinnopsis dadayi* form another, though their relationship is unclear. *T. lohmanni* groups with some hyaline species and is always basal in the group. *Tintinnopsis fimbriata* clusters with the family Codonellopsidae. For some moderate or low posterior probabilities and bootstrap supports, the relationship of the seven *Tintinnopsis* species is uncertain. The position of *Stenosemella* is uncertain within the family. In Bayesian, MP and NJ analyses, *S. nivalis* groups with *Codonellopsis americana*, while in LS, it groups outside the two *Codonellopsis* species.

Amphorellopsis acuta and *Tintinnidium mucicola* branch basal to all other tintinnids (1.00 BI, 82% MP, 48% LS, and 76% NJ), followed by *Eutintinnus* species (1.00 BI, 76% MP, 64% LS, 82% NJ). In this way, the family Tintinnidae is paraphyletic.

4. Discussion

As demonstrated in the present work, there are strong supports for the monophyly of the order Tintinnida (0.99

Table 1
GenBank accession numbers of the seven ciliate species' small subunit rRNA gene sequences and information about the gene sequence.

Species	GenBank Accession Nos.	Length (nucleotides)	GC content (%)
Amphorellopsis acuta	FJ196071	1687	47.54
Codonellopsis nipponica	FJ196072	1760	46.93
Favella taraikaensis	FJ196073	1761	47.19
Stenosemella nivalis	FJ196074	1761	46.91
Tintinnopsis beroidea	EF233709	1676	46.90
Tintinnopsis cylindrica	FJ196075	1761	46.73
Tintinnopsis lohmanni	FJ196076	1682	46.55