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Phylogeny of the families Zoothamniidae and Epistylididae (Protozoa: Ciliophora: Peritrichia) based on analyses of three rRNA-coding regions

Yuan Zhuang[a], John C. Clamp[b], Zhenzhen Yi[c], Daode Ji[a,*]

[a] Ocean School, Yantai University, Yantai 264005, China
[b] Department of Biological and Biomedical Sciences, North Carolina Central University, Durham, NC 27707, USA
[c] Guangzhou Key Laboratory of Subtropical Biodiversity and Biomonitoring, South China Normal University, Guangzhou 510631, China

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ABSTRACT

Peritrichs are a major group of ciliates with worldwide distribution, and they play important roles in many habitats. Intrafamilial phylogeny of some peritrichs was investigated using information from three genes, which provided more robust interpretations than single-gene analyses. Sixty-seven new sequences including SSU rDNA, ITS1-5.8S-ITS2 and LSU rDNA were aligned with available sequences in GenBank to infer phylogenetic relationships within the families Zoothamniidae and Epistylididae. Results reveal the following relationships: (1) Epistylididae is polyphyletic, consisting of two clades that nest within the Zoothamniidae as part of the crown clade of peritrichs (order Vorticellida) and a third one that is part of the basal clade of peritrichs (order Opercularida); (2) *Epistylis elongata* falls within one of the clades of *Zoothamnium* rather than with congeners; (3) *Zoothamnium* is probably paraphyletic, consisting of three divergent clades, with the genera *Myoschiston* and *Zoothamnopsis* intermingled with species of *Zoothamnium*. The following evolutionary hypotheses can be inferred from these results: (1) the contractile stalk of *Zoothamnium* is plesiomorphic. (2) *Myoschiston, Zoothamnopsis* and clade II of Epistylididae are derived from the *Zoothamnium* morphotype by partial or incomplete development of the spasmoneme that forms the contractile center of the stalk around which the rigid cortex is secreted. (3) Clade I of the Epistylididae, which are primarily colonial forms that appear never to have evolved a spasmoneme of any sort, may represent the ancestral morphotype of peritrichs.

1. Introduction

Peritrichs (class Oligohymenophorea, subclass Peritrichia) are one of the most abundant groups of ciliates in freshwater, estuarine, marine and even hypersaline environments (Jankowski, 1985; Kahl, 1935; Shen, 1980; Song 1986; Sun et al., 2016; Zhuang et al., 2016). Many peritrichs also attach to crustaceans, fishes, amphibians, aquatic plants, algae, and other hosts (Abdallah et al., 2011; Crites, 1977; Rajabunizal and Ramanibai, 2011; Song, 2003; Utz and Eizirik, 2007; Visse, 2007). For most sessile ones, they are morphologically well-defined by presence of an expanded oral area (peristome) with a spiral oral infraciliature consisting of three polykineties and a haplokinety, an aboral trochal band of cilia that appears only during the dispersal stage (telotroch), and a scopula at the aboral pole that secretes a stalk for attachment. In morphological classifications, constituent taxa of peritrichs are distinguished clearly by characteristics of stalk, spasmoneme, lorica (if present), peristome, oral infraciliature and silverline pattern (Lynn, 2008); however, major elements of this classification have been challenged and essentially invalidated by recent molecular phylogenetic investigations (Li et al., 2008, 2015; Sun et al., 2012a, 2016; Utz et al., 2010). In general, the classification of the entire subclass Peritrichia is in a state of flux.

In particular, two once taxonomically distinct families, Zoothamniidae and Epistylididae, have been found to overlap with one other in recent molecular investigations with better taxon representation (Sun et al., 2016) that were based upon sequences of the gene coding for the small ribosomal subunit (SSU rRNA). These phylogenies have not been tested by analyses of other coding regions, however.

Phylogenies based upon multiple genes, concatenated or in separate analyses, have been used in different groups of ciliates to provide more robust interpretations of their relationships (Gao et al., 2014, 2016; Gentekaki et al., 2014; Huang et al., 2014, 2016; Li et al., 2015; Yi et al., 2009a, 2009b; Yi and Song, 2011; Sun et al., 2016). The present study used 57 new sequences of morphospecies of *Zoothamnium* and *Epistylis*, obtained from three separate parts of the entire rRNA coding region to test the relationships between the families Zoothamniidae and Epistylididae hypothesized from SSU rRNA data alone. The new sequences included 25 SSU rRNA sequences, 18 ITS1-5.8S-ITS2

* Corresponding author.
E-mail address: daodeji@126.com (D. Ji).

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Table 1
List of species for which SSU rDNA, ITS1-5.8S-ITS2 region and LSU rDNA were newly sequenced in the present work.

Species	Sampling location	SSU rDNA			ITS1-5.8S-ITS2			LSU rDNA		
		Accession No.	Length (bp)	GC content	Accession No.	Length (bp)	GC content	Accession No.	Length (bp)	GC content
Zoothamnopsis sinica	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675177	1515	43.63%	KY675148	385	36.36%	KY675187	1734	42.33%
Zoothamnium plumula	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675162	1515	44.36%	KY675147	382	41.36%	–	–	–
Zoothamnium maximum	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675155	1651	42.76%	–	–	–	–	–	–
Zoothamnium mucedo	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675156	1626	43.48%	KY675141	505	32.48%	KY675185	1727	41.52%
Zoothamnium palmphlatum	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675157	1626	43.48%	KY675144	484	38.64%	–	–	–
Zoothamnium parahiketes	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675159	1627	43.39%	–	–	–	–	–	–
Zoothamnium paraentzii	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675160	1651	42.88%	KY675146	490	35.31%	–	–	–
Zoothamnium duplicatum	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675161	1651	43.06%	KY675145	489	35.58%	KY675188	1734	42.33%
Zoothamnium wangi	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675175	1514	44.06%	KY675140	383	39.43%	KY675195	1733	43.91%
Zoothamnium sp.*1 JI130604B*	Qinshui River, Yantai, China (37°44′N, 121°64′E)	KY675164	1628	43.24%	KY675134	491	33.60%	–	–	–
Zoothamnium sp.*2 JI13062201*	Qinshui River, Yantai, China (37°44′N, 121°64′E)	KY675165	1627	43.76%	KY675135	484	39.26%	KY675189	1842	43.76%
Zoothamnium sp.*3 JI13062901*	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675166	1626	43.97%	KY675136	484	39.26%	KY675190	1846	43.72%
Zoothamnium sp.*4 JI13062001*	Qinshui River, Yantai, China (37°44′N, 121°64′E)	KY675167	1626	43.30%	KY675137	505	32.28%	KY675191	1838	41.62%
Zoothamnium sp.*5 JI2016*	Yuniao River, Yantai, China (37°43′N, 121°58′E)	KY675168	1625	43.38%	–	–	–	–	–	–
Zoothamnium sp.*6 JI2016*	Yuniao River, Yantai, China (37°43′N, 121°58′E)	KY675169	1626	43.42%	–	–	–	–	–	–
Zoothamnium sp.*7 JI2016*	Xinan River, Yantai, China (37°44′N, 121°55′E)	KY675170	1627	43.70%	–	–	–	–	–	–
Zoothamnium sp.*8 JI2016*	Xinan River, Yantai, China (37°44′N, 121°55′E)	KY675171	1626	43.30%	–	–	–	–	–	–
Zoothamnium sp.*9 LZ55*	Xinan River, Yantai, China (37°44′N, 121°55′E)	KY675173	1649	43.42%	KY675139	485	37.73%	KY675193	1734	42.96%
Zoothamnium sp.*10 LZ50*	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675174	1650	43.33%	–	–	–	KY675194	1734	42.96%
Zoothamnium sp.*11 JI2016*	Xinan River, Yantai, China (37°44′N, 121°55′E)	KY675172	1650	43.45%	KY675138	452	37.17%	KY675192	1734	42.96%
Zoothamnium sp.*12 JI20160709*	Yuniao River, Yantai, China (37°43′N, 121°58′E)	KY675176	1515	44.49%	–	–	–	–	–	–
Zoothamnium sp.*13 JI14041401*	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675158	1624	43.10%	KY675143	484	38.64%	KY675186	1843	43.41%
Zoothamnium sp.*14 JI13050401*	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675163	1624	42.92%	KY675142	484	38.43%	–	–	–
Epistylis elongata	Sanyuan Lake, Yantai, China (37°47′N, 121°46′E)	KY675179	1651	43.00%	KY675149	495	35.76%	KY675196	1734	43.08%
Epistylis chrysemydis	Sanyuan Lake, Yantai, China (37°47′N, 121°46′E)	KY675178	1516	43.01%	KY675150	388	34.28%	KY675197	1733	42.82%
Epistylis sp.*1 JI2016080701*	Sanyuan Lake, Yantai, China (37°47′N, 121°46′E)	–	–	–	KY675151	388	37.36%	KY675198	1732	42.21%
Epicarchesium corlissi	Sanyuan Lake, Yantai, China (37°47′N, 121°46′E)	KY675182	1651	43.91%	KY675152	481	42.41%	KY675199	1730	44.86%
Epicarichesium sp.*1 JI13062902*	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675181	1626	43.48%	–	–	–	–	–	–
Epicarchesium variable	Guangdang River, Yantai, China (37°47′N, 121°48′E)	KY675180	1627	43.15%	–	–	–	–	–	–
Pseudovorticella sp.*1 LZ54*	Yuniao River, Yantai, China (37°43′N, 121°58′E)	KY675183	1514	44.58%	KY675153	476	45.59%	KY675200	1730	45.55%
Pseudovorticella sp.*2 LZ53*	Xinan River, Yantai, China (37°44′N, 121°55′E)	KY675184	1652	43.16%	KY675154	454	42.51%	–	–	–

– Data not available.