

Further insights into the highly derived haptorids (Ciliophora, Litostomatea): Phylogeny based on multigene data

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Evolutionary relationships of taxa within the ciliate subclass Haptoria are poorly understood. In this study, we broaden the taxon sampling by adding 14 small subunit ribosomal RNA gene sequences, 13 large subunit ribosomal RNA gene sequences and 13 ITS1-5.8S-ITS2 gene sequences of haptorians. This includes the first molecular data from two genera, *Pseudotrachelocerca* Song, 1990, and *Foissnerides* Song & Wilbert, 1989. Phylogenies inferred from the three individual genes and concatenated data sets show that: (i) the subclass Haptoria could be a multiphyletic complex with about up to four main clades while “interrupted” by some intermingled with the related subclasses Rhynchostomatia, Trichostomatia and some *incertae sedis*; (ii) the genus *Pseudotrachelocerca* Song, 1990, is clearly separated from Litostomatea and clusters within an assemblage comprising the classes Prostomatea, Colpodea and Plagiopylea; (iii) both morphological evidence and molecular evidence indicate that the genus *Foissnerides* should be transferred from family Trachelophyllidae to Pseudoholophryidae; (iv) the validity of the order Helicoprordontida Grain, 1994, and its monophyly is strongly supported; (5) the family Chaeneidae does not belong to the order Lacrymarida but represents a distinct clade in the subclass Haptoria.

1 | INTRODUCTION

Ciliated protists (ciliates) are important model organisms for studying molecular biology, cell biology, symbiotic relationships, ecology and evolution (Adl et al., 2012; Berger, 2011; Chen et al., 2016; Corliss, 1979; Gao, Song, & Katz, 2014; Lynn, 2008; Song, Warren, & Hu, 2009; Wang et al., 2017). One of the most diverse and systematically confused ciliate groups is the class Litostomatea Small & Lynn, 1981, which comprises two subclasses, Haptoria Corliss, 1974 and Trichostomatia Bütschli, 1889 (Lynn, 2008). Trichostomatia is a well-defined monophylum comprising species that are endocommensals or parasites

in vertebrates (Cameron & O’Donoghue, 2004; Corliss, 1974; Foissner & Foissner, 1988; Grain, 1994; Jankowski, 2007; Lynn & Small, 2002; Wright & Colorni, 2002; Wright, Dehority, & Lynn, 1997; Wright & Lynn, 1995). By contrast, the free-living subclass Haptoria is paraphyletic, and the evolutionary relationships within the subclass have not yet been clarified (Cameron, 2003; Cameron & O’Donoghue, 2004; Gao et al., 2008; Pan et al., 2010; Strüder-Kypke, Kornilova, & Lynn, 2007; Strüder-Kypke, Wright, Foissner, Chatzinotas, & Lynn, 2006; Vďačný, 2015; Vďačný & Rajter 2015; Vďačný, Slovak, & Foissner, 2014; Wright et al., 1997; Wu et al., 2017).

Haptoria is a highly diverse group that comprises over 1000 species, the body sizes of which range from 15 × 30 to

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200 × 2500 μm. Morphologically, Haptoria is usually characterized by telokinetal stomatogenesis, orally located toxicysts and uniform holotrichous somatic ciliation (Foissner & Foissner, 1988; Gao et al., 2008; Lynn & Small, 2002). Evolutionary relationships within the Haptoria based on conventional morphological characters, such as nematodesmata and the dorsal brush, are not confirmed by the molecular data (Foissner & Foissner, 1988; Lipscomb & Riordan, 1990). In addition, recent molecular phylogenetic analyses have not corroborated the monophyly of Haptoria, leaving numerous historical problems unresolved (Gao, Warren, et al., 2016; Pan et al., 2010; Vďačný, 2015; Vďačný, Breiner, et al., 2017; Vďačný & Rajter 2015; Zhang, Simpson, & Song, 2012). For example, three haptorian genera, *Cyclotrichium*, *Paraspathidium* and *Askenasia*, fall into either class Protomatea or Plagiopylea in single and multigene phylogenies (Gao, Warren, et al., 2016; Zhang et al., 2012). The haptorian genera *Trachelotractus* and the subclass Rhynchostomatia have both been suggested as the candidate basal litostomatean group (Gao, Warren, et al.,

2016; Vďačný, Breiner, et al., 2014; Zhang et al., 2012). Moreover, the phylogeny of haptorian taxa below the rank of order remains confused, mainly due to the limited molecular information available (Vďačný & Rajter, 2015).

Phylogenetic analyses based on concatenation of multiple genes are increasingly used to give better interpretations evolutionary relationships within different ciliate groups (Antipa, Dolan, Lynn, Obolkina, & Strüder-Kypke, 2016; Gao, Gao, Wang, Katz, & Song, 2014; Gao, Warren, et al., 2016; Gao et al., 2017; Gentekaki et al., 2014; Huang, Luo, Bourland, Gao, & Gao, 2016; Li, Liu, Gao, Warren, & Song, 2013; Li, Zhan, & Xu, 2017; Pan, Jiang, Fan, Al-Farraj, & Gao, 2017; Sung, Sung, Hywel-Jones, & Spatafora, 2007; Yan, Xu, Al-Farraj, Al-Rasheid, & Song, 2016; Yan, Fan, et al., 2016; Zhao, Gao, Fan, Strüder-Kypke, & Huang, 2015). In this study, we broaden the taxon sampling of haptorians by adding 14 small subunit ribosomal RNA (SSU-rRNA), 12 large subunit ribosomal RNA (LSU-rRNA) and 12 ITS1-5.8S-ITS2 gene sequences. Two genera, *Pseudotrachelocerca* and *Foissnerides*, are sequenced for the first time. Phylogenetic

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	Latitude/longitude	Accession No.		
			SSU-rDNA	ITS1-5.8S-ITS2	LSU-rDNA
<i>Chaenea</i> sp. 2	Daya Bay, Guangzhou, southern China	22°46'N 114°41'E	MF474336	MF474363	MF474350
<i>Chaenea</i> sp. 3	Techeng Island, Zhanjiang, southern China	21°09'N 110°26'E	MF474337	MF474364	MF474351
<i>Chaenea vorax</i>	Huguang Lake, Zhanjiang, southern China	21°08'N 110°17'E	MF474338	MF474365	MF474352
<i>Epispathidium</i> sp.	Daya Bay, Guangzhou, southern China	22°46'N 114°41'E	MF474339	MF474366	MF474353
<i>Foissnerides</i> sp.	Donghai Island, Zhanjiang, southern China	21°00'N 110°31'E	MF474340	MF474367	MF474354
<i>Helicoprordodon</i> sp. 1	Jiaozhou Bay, Qingdao, northern China	35°55'N 120°12'E	MF474341	–	–
<i>Homalozoon vermiculare</i>	Huguang Lake, Zhanjiang, southern China	21°08'N 110°17'E	MF474342	MF474368	MF474355
<i>Lacrymaria maurea</i> pop 2	Jiaozhou Bay, Qingdao, northern China	36°03'N 120°23'E	MF474344	MF474370	MF474357
<i>Lacrymaria marina</i> pop 3	Guandu town, Zhanjiang, southern China	21°22'N 110°26'E	MF474343	MF474369	MF474356
<i>Lacrymaria</i> sp. 1	Jiaozhou Bay, Qingdao, northern China	36°03'N 120°19'E	MF474345	MF474371	MF474358
<i>Phialina</i> sp. 1	Jiaozhou Bay, Qingdao, northern China	36°03'N 120°20'E	MF474346	MF474372	MF474359
<i>Phialina vermicularis</i> pop 1	Jiaozhou Bay, Qingdao, northern China	36°03'N 120°20'E	MF474347	MF474373	MF474360
<i>Phialina vertens</i>	Jiaozhou Bay, Qingdao, northern China	36°03'N 120°20'E	MF474348	MF474374	MF474361
<i>Pseudotrachelocerca trepida</i>	Gaoqiao town, Zhanjiang, southern China	21°35'N 109°45'E	MF474349	MF474375	MF474362

analyses based both on single genes and on concatenated gene data sets are performed to reveal the evolutionary relationships among some major taxa within the subclass Haptoria.

2 | MATERIALS AND METHODS

2.1 | Ciliate collection, DNA extraction and gene sequencing

Forty molecular sequences of SSU-rDNA, ITS1-5.8S-ITS2 and LSU-rDNA were newly generated for 14 species of haptorians. Sample information and GenBank accession numbers are shown in Table 1. Morphological identification was based on observations of both live and protargol-stained specimens. The generic morphology of the new sequenced species refers to Figure 1a–o respectively. Genomic DNA was extracted from cleaned cells using the REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, USA) or the DNeasy Blood & Tissue Kit (Qiagen, CA). PCR amplification and sequencing were performed according to Gao, Li, et al. (2016).

2.2 | Data sets and alignments

Newly characterized sequences combined relevant sequences obtained from the GenBank database (Table S1) were assembled into four data sets: (i) 1614 characters of SSU-rDNA (113 taxa in total); (ii) 334 characters of ITS1-5.8S-rDNA –ITS2 (54 taxa in total); (iii) 1760 characters of LSU-rDNA (44 taxa in total); and (iv) 3708 characters of concatenated sequence data of the above three genes (113 taxa in total). Representatives of the class Spirotrichea were used as outgroups for all analyses.

Sequences were aligned using GUIDANCE in the GUIDANCE Web server (<http://guidance.tau.ac.il/>) with default parameters (Penn et al., 2010). Ambiguous columns were removed based on confidence scores calculated optimally by GUIDANCE (0.75, 0.605 and 0.6 for SSU-rDNA, ITS1-5.8S-rDNA –ITS2 and LSU-rDNA, respectively). Both ends of the alignments were trimmed to be blunt. For a concatenated gene analysis, the three genes derived from the same isolate were concatenated to be contigs in SeaView V4 (Galtier, Gouy, & Gautier, 1996; Gouy, Guindon, & Gascuel, 2010).

2.3 | Phylogenetic analyses

The GTR + I + G model was selected by the Akaike Information Criterion (AIC) in MrModeltest v2 (Nylander, 2004) and Modeltest v3.4 (Posada & Crandall, 1998), which was subsequently used in the phylogenetic analyses. Maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out on the online server CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010), using RAxML-HPC2 on XSEDE v8.2.9 (Stamatakis, 2014) and

MrBayes on XSEDE v3.2.6 (Ronquist et al., 2012), respectively. In the ML analyses, 1000 bootstraps were performed to assess the reliability of internal branches. For Bayesian analyses, Markov chain Monte Carlo (MCMC) simulations were run with two sets of four chains for 4,000,000 generations, with sampling every 100 generations and a burn-in of 10,000. All the remaining trees were used to calculate posterior probabilities (PP) using a majority rule consensus. MEGA v5 (Tamura et al., 2011) was used to visualize the tree topologies. Terminology and classification mainly follow Gao, Warren, et al. (2016) and Vd'áčný, Bourland, Orsi, Epstein, and Foissner (2011).

2.4 | Topology testing

To assess monophyly, 14 ML trees were generated in RAxML (Stamatakis, 2014) with enforced constraints (Table 2). Internal relationships within the constrained group and among the remaining taxa were unspecified. Resulting constrained topologies were then compared to the best (unconstrained) ML topologies using the approximately unbiased (AU) test (Shimodaira, 2002) implemented in CONSEL v 0.1 (Shimodaira & Hasegawa, 2001), as described in Huang, Dunthorn, and Song (2012).

TABLE 2 Approximately unbiased (AU) test results

Topology constraints	AU value (ρ)	
	Concatenated tree	SSU-rDNA tree
Unconstrained	.863	.645
Monophyly of Lacrymariidea + Chaeneidae	.313	.572
Monophyly of Lacrymariidea + Chaeneidae + Didiniida	.110	.387
Monophyly of <i>Pseudotrachelocerca</i> + Haptoria	<.001	<.001
Monophyly of <i>Pseudotrachelocerca</i> + Litostomatea	<.001	.004
Monophyly of <i>Foissnerides</i> + <i>Acaryophrya</i> + <i>Trachelophyllum</i>	.022	.161
Monophyly of <i>Foissnerides</i> + <i>Trachelophyllum</i>	.008	.063
Monophyly of <i>Foissnerides</i> + <i>Enchelyodon</i>	<.001	<.001

$\rho < .05$ refute the possibility of monophyly; $\rho > .05$ do not refute the possibility of monophyly. Values that are less than .05 are in bold.

3 | RESULTS

3.1 | Concatenated phylogeny

The ML and BI trees share basically congruent topologies; therefore, we only present the BI tree with nodal support from both algorithms (Figure 2).

According to the concatenated gene phylogeny, the class Litostomatea is monophyletic and is sister to Armophorea (BI/ML: 0.96/65). Both the subclasses Rhynchostomatia (represented by the orders Dileptida and Tracheliida) and Trichostomatia are monophyletic, whereas Haptoria is paraphyletic because its members intermingle with Rhynchostomatia and Trichostomatia.

Most of the newly sequenced haptorians group within the Litostomatea. The only exception is *Pseudotrachelocerca trepida* which clusters with *Cyclotrichium cyclokaryon* (BI/ML: 1.00/96). These two species form a clade that is sister to the class Colpodea (BI/ML: 1.00/51) within a large assemblage that also includes the classes Prostomatea, Plagiopylea and Oligohymenophorea (BI/ML: 0.99/60).

The haptorian genus *Foissnerides* groups with *Acaryophrya* sp. PV-2014 with high support (BI/ML: 1.00/87) to form a terminal clade within the Spathidiida. The newly sequenced species *Helicoprordon* sp. groups with *H. maximus* with full support, which then clusters with *Trachelotractus entzi* forming a clade that occupies the basal position within the Litostomatea. The next branch in the litostomatean assemblage is the strongly supported clade of *Chaenea*. Notably, one of our unidentified *Chaenea* species (sp.3) is highly divergent from other well-known chaeneids.

The positions of the haptorian genera *Homalozoon* and *Epispathidium* are still unresolved, even using the updated data set. The genus *Epispathidium* is revealed to be paraphyletic, the newly sequenced *Epispathidium* sp. grouping with the subclass Trichostomatia with low-to-moderate support (BI/ML: 0.61/58) to form a clade that is sister to *E. papilliferum*.

When Chaeneidae is excluded, the family Lacrymariidae is monophyletic, the two representative genera (*Phialina* and *Lacrymaria*) forming a maximally supported clade (BI/ML: 1.00/100). The newly added *Phialina* sp. occupies the basal position of the Lacrymariidae with high-to-full support. *Phialina vermicularis* and *P. vertens* cluster together with moderate support (BI/ML: 0.60/84) and with three populations of *Phialina salinarum* form a fully supported clade that is sister to the strongly supported clade of *Lacrymaria*.

3.2 | Phylogenetic analyses based on SSU-rDNA sequence data

The topology of the SSU-rDNA tree is similar to that of the concatenated tree. For instance, clades of *Chaenea* are strongly supported, and *Helicoprordon* + *Trachelotractus* occupy the two

basal positions in the class Litostomatea (Figure 3). It is noteworthy that the close relationship of the family Lacrymariidae and subclass Rhynchostomatia, predicted by the concatenated tree, is not recovered in the SSU-rDNA tree.

Similar to the concatenated tree, the species *Pseudotrachelocerca trepida* does not fall into the Litostomatea clade, rather it groups with *Cyclotrichium* and seven unidentified environmental sequences (BI/ML: 1.00/86) in the SSU-rDNA tree. The phylogenetic position of this clade within the “CONthreeP” supergroup (namely classes Colpodea, Oligohymenophorea, Nassophorea, Plagiopylea, Prostomatea and Phyllopharyngea), however, is not resolved.

3.3 | Phylogenetic analyses based on LSU-rDNA and ITS1-5.8S-ITS2 sequence data

Twelve new sequences of ITS1-5.8S-ITS2 and 12 of LSU-rDNA, obtained in the present work, enabled us to include an abundant sampling of haptorian species in 5.8S-rDNA and LSU-rDNA trees for the first time (Figures 4 and 5). Although the two phylogenetic trees based on the LSU-rDNA or ITS1-5.8S-ITS2 have fewer species and lower support than the concatenated phylogeny, analyses of both loci show similar results, including the phylogenetic positions of the genera *Pseudotrachelocerca* and *Foissnerides*, and the identity of the basal groups of Litostomatea, that is *Trachelotractus* + *Helicoprordon* and *Chaenea*.

4 | DISCUSSION

4.1 | Systematic position of family *Pseudotrachelocercidae* Song, 1990

The historical assignment of *Pseudotrachelocerca trepida* to the class Litostomatea is rejected by our analyses; rather, it clusters with *Cyclotrichium cyclokaryon* with high or full support and shows close relationships to classes Colpodea, Prostomatea and Plagiopylea, in all our single-gene and concentrated trees (Figures 1–4). Hitherto, *Pseudotrachelocerca trepida* has been assigned to the monogeneric family Pseudotrachelocercidae. *Pseudotrachelocercica* (Figure 1h,i) is similar to other litostomateans in having a dorsal brush (DB), apical extrusomes and a mesh-like silver-line system (Song, 1990). Among these characters, the DB is an important feature for the classification of litostomateans, and a three-rowed DB is considered as an apomorphy of the last common ancestor (Vd'ačný et al., 2011). However, *Pseudotrachelocerca* is divergent in that its DB is composed of irregularly arranged kinetids and is connected to only one somatic kinety (vs. to several somatic kineties in most other litostomateans; Song, 1990). Both the divergent phylogenetic position and the heterogeneities in the DB appearance imply that the DB structure of *Pseudotrachelocerca*

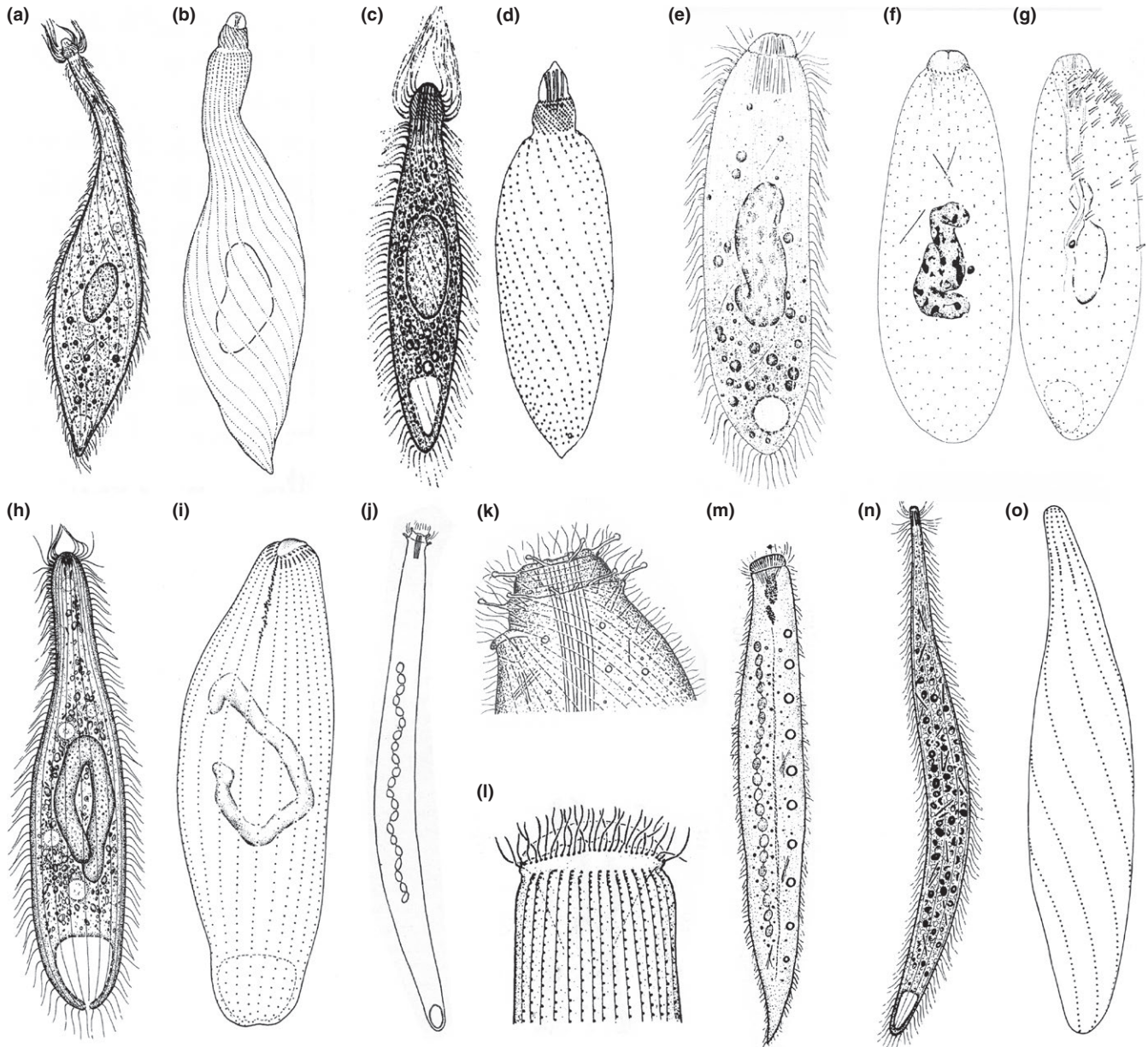


FIGURE 1 Morphology and infraciliature of seven haptorian genera. (a, b). *Lacrymaria*, from Song et al. (2003). (c, d). *Phialina*, from Song et al. (2003). (e–g). *Foissnerides*, from Song & Wilbert (1989). (h, i). *Pseudotrachelocera* (from Song 1990). (j, k). *Helicoprorodon* (from Dragesco 1960). (l, m). *Homalozoon*, from Dragesco (1970). (n, o). *Chaenea*, from Fan et al. (2015)

might be a homoplasy of the typical litostomeatean dorsal brush. In addition, the topology testing results strongly reject the grouping of *Pseudotrachelocerca* to either Haptoria or Litostomeata (AU test, $p < .05$; Table 2). Accordingly, we suggest an exclusion of Pseudotrachelocercidae from the class Litostomeata.

Although *Pseudotrachelocerca* grouped with *Cyclotrichium* with moderate-to-high support in all the phylogenetic analyses, there is no distinct morphological evidence to support this phylogenetic affiliation. Morphologically, *Pseudotrachelocerca* is similar to *Cyclotrichium* in possessing both oralized somatic monokinetids and circumoral dikinetids, characters that are also commonly present in some

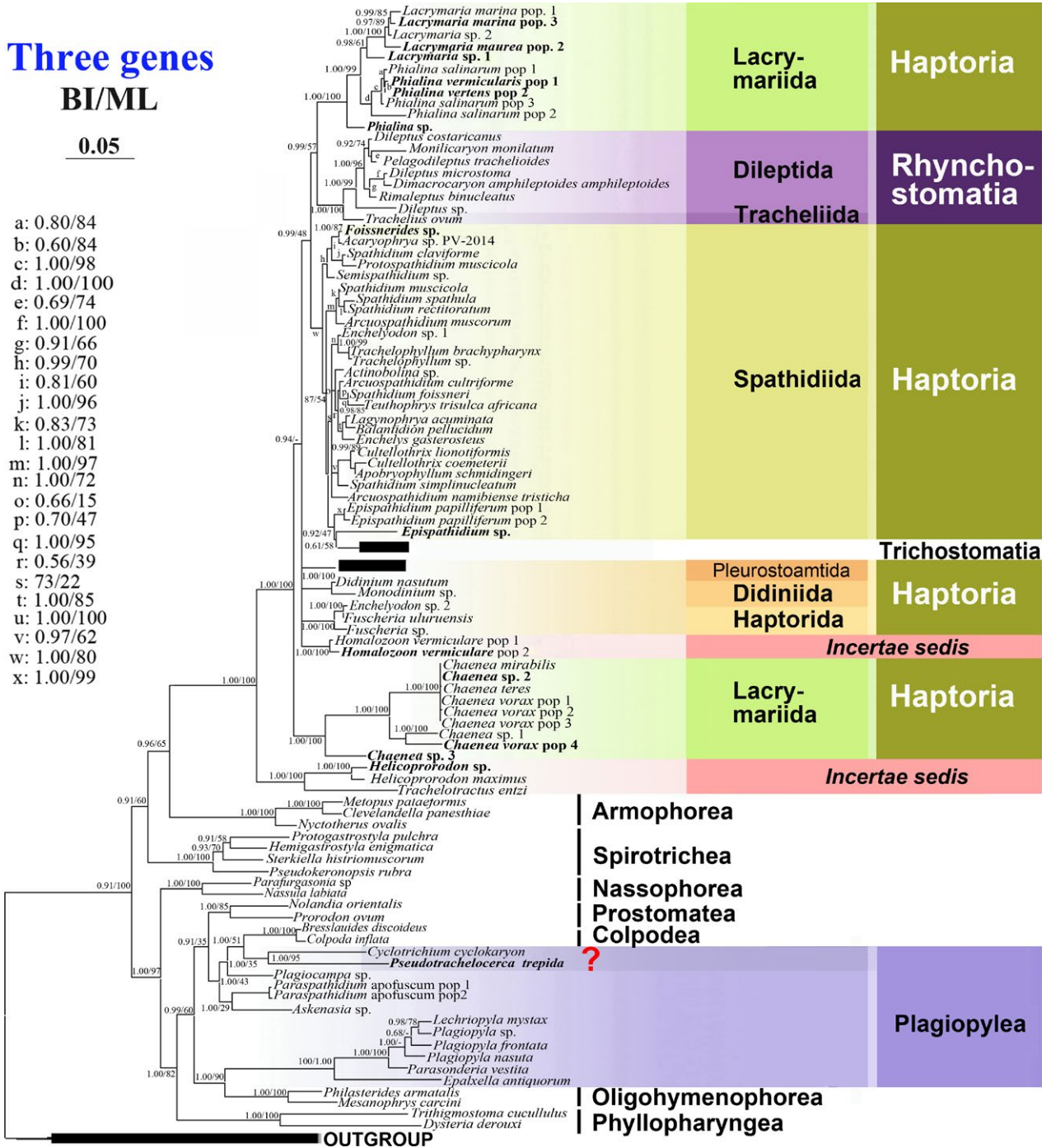
typical haptorians, for example *Chaenea* (Kwon, Vďačný, Shazib, & Shin, 2014; Xu, Song, & Hu, 2005). Other genera that cluster with *Pseudotrachelocerca* and *Cyclotrichium* and which are systematically ambiguous include *Askenasia*, *Paraspathidium* and the prostomeatean genus *Plagiocampa*. Previous studies have placed these taxa in the class Plagiopylea, which was not well supported by our results (Gao, Warren, et al., 2016; Zhang, Yi, Song, Al-Rasheid, & Warren, 2010; Zhang et al., 2012). Relationships between these genera remain unresolved pending enrichment of taxa and genes. Based on the current knowledge, we propose that Pseudotrachelocercidae should be regarded as *incertae sedis* within the Prostomeatea + Plagiopylea + Colpodea complex.

Three genes

BI/ML

0.05

- a: 0.80/84
- b: 0.60/84
- c: 1.00/98
- d: 1.00/100
- e: 0.69/74
- f: 1.00/100
- g: 0.91/66
- h: 0.99/70
- i: 0.81/60
- j: 1.00/96
- k: 0.83/73
- l: 1.00/81
- m: 1.00/97
- n: 1.00/72
- o: 0.66/15
- p: 0.70/47
- q: 1.00/95
- r: 0.56/39
- s: 73/22
- t: 1.00/85
- u: 1.00/100
- v: 0.97/62
- w: 1.00/80
- x: 1.00/99



CLASS LITOSTOMATEA

FIGURE 2 Bayesian inference (BI) tree based on the concatenated three genes (SSU-rDNA, 5.8S-rDNA and LSU-rDNA) of Litostomatea focusing on subclass Haptoria. Newly sequenced species are in bold. Numbers at nodes represent the posterior probability of BI and the bootstrap values of ML out of 1000 replicates, respectively. Clades with a different topology in the ML tree are indicated by “-.” The scale bar corresponds to five substitutions per 100 nucleotide positions

4.2 | Systematic position of the genus *Foissnerides*

The genus *Foissnerides* Song & Wilbert, 1989 (Figure 1e-g) is defined by the following combination of characters: the presence of nematodesmata originating from the circumoral kinety; highly specialized dorsal brush comprising

more than four rows, each of which is composed of irregularly arranged paired and unpaired kinetosomes (Song & Wilbert, 1989). This genus was originally placed in the family Trachelophyllidae Kent, 1882, which was accepted by Lynn (2008). Foissner, Agatha, and Berger (2002), however, transferred *Foissnerides* to the family Enchelyodontidae, based on its lack of lepidosomes.

SSU rDNA
BI/ML
0.1

- a: 1.00/-
- b: 0.95/73
- c: 0.80/59
- d: 1.00/94
- e: 0.68/-
- f: 0.91/45
- g: 0.99/67
- h: 0.91/42
- i: 0.81/62
- j: 1.00/86
- k: 1.00/94
- l: 1.00/99
- m: 0.91/63
- n: 1.00/86
- o: 0.83/70
- p: 0.66/46
- q: 1.00/100
- r: 0.92/59
- s: 0.84/84
- t: 0.74/68

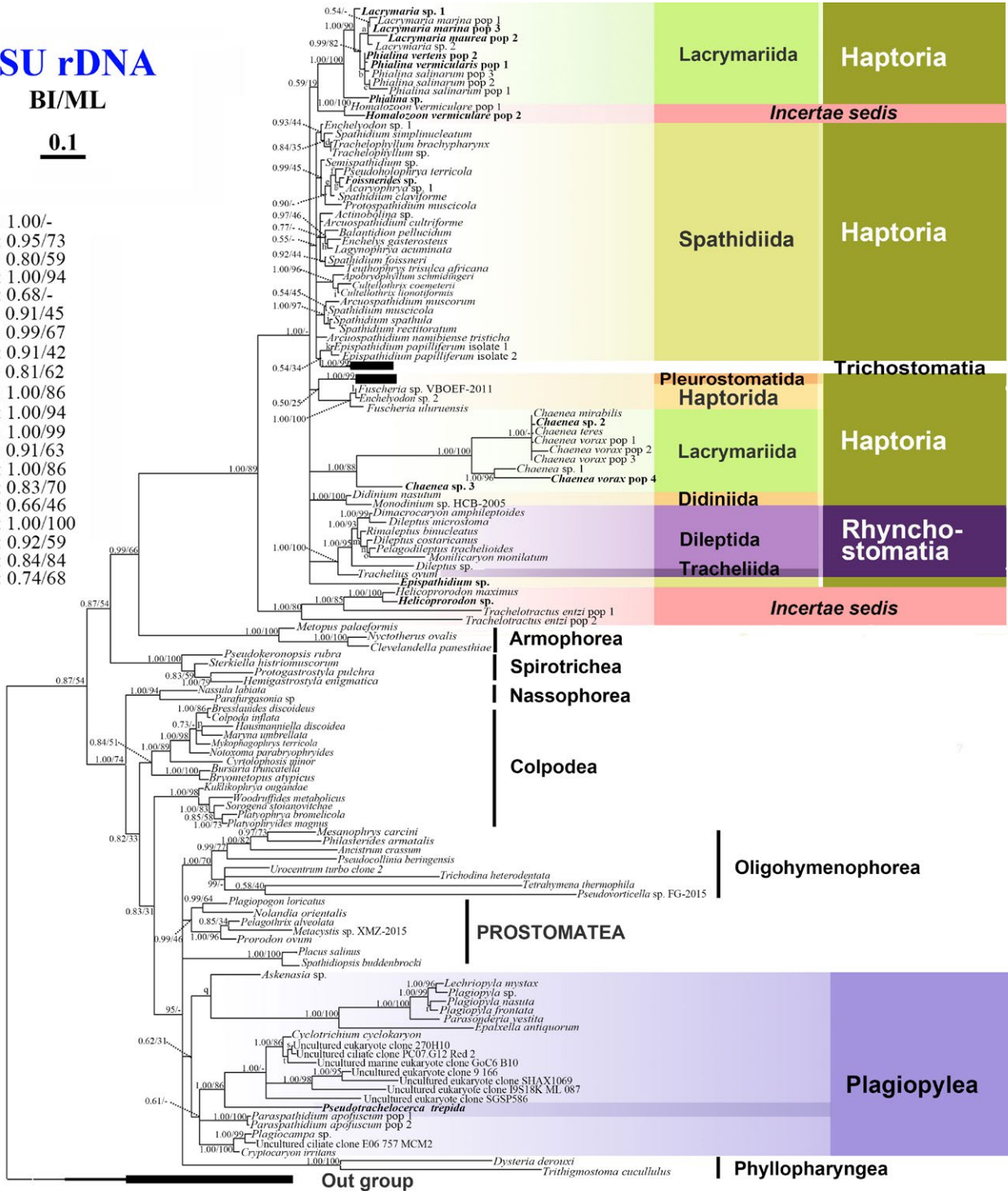


FIGURE 3 Bayesian inference (BI) tree based on the SSU-rDNA of Litostomatea focusing on Haptoria. Newly sequenced species are in bold. Numbers at nodes represent the posterior probability of BI and the bootstrap values of ML out of 1000 replicates, respectively. Clades with a different topology in the BI tree are indicated by “-.” The scale bar corresponds to five substitutions per 100 nucleotide positions

Vd’áčný et al. (2011) agreed with this assignment despite the fact that *Foissnerides* differs from other enchelyodontids by having more DB rows (more than 4 vs 3; Foissner et al., 2002). However, the phylogenetic assignment of *Foissnerides* could not be definitively resolved due to the absent of molecular data.

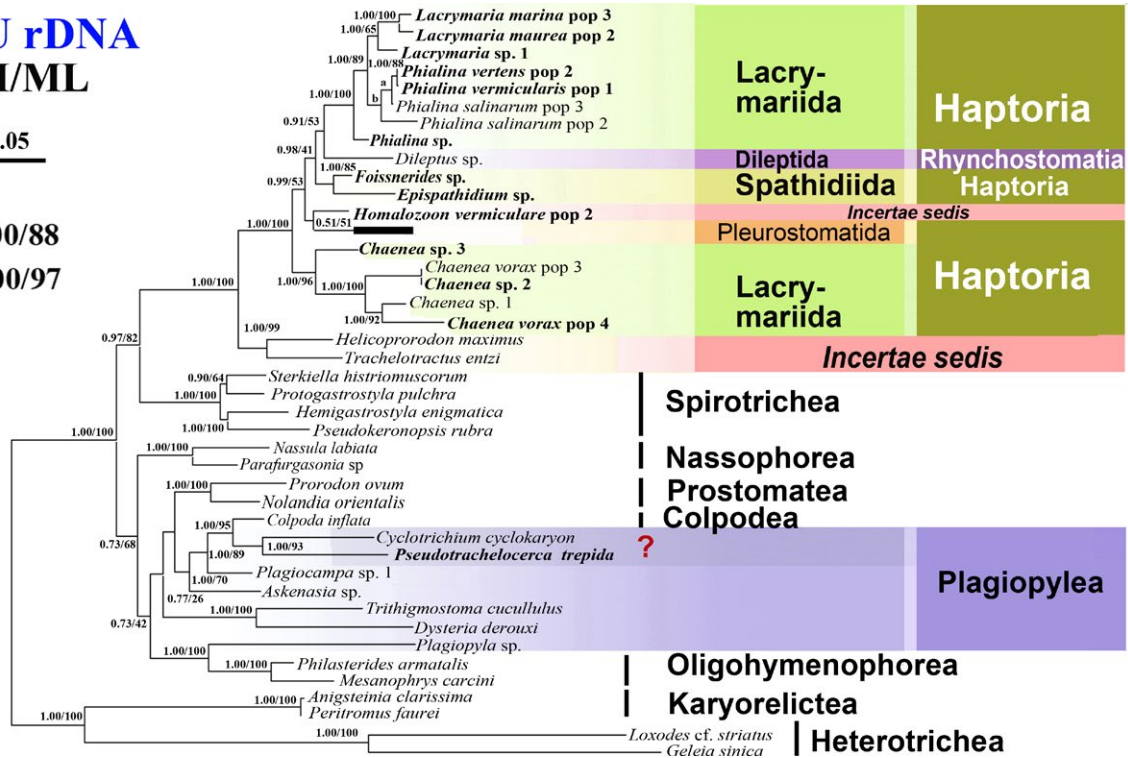
In our phylogenetic analyses (Figures 2 and 3), *Foissnerides* sp. clusters with *Acaryophrya* sp. and *Pseudoholophrya terricola*, rather than with trachelophyllids (*Trachelophyllum*) or enchelyodontids (*Enchelyodon*). The possibility that either *Foissnerides* or *Foissnerides* + *Acaryophrya* (a former trachelophyllid, see below) clusters with other trachelophyllids

LSU rDNA BI/ML

0.05

a: 1.00/88

b: 1.00/97



LITOSTOMATEA

FIGURE 4 Bayesian inference (BI) tree based on 44 LSU-rDNA gene sequences of Litostomatea, focusing on Haptoria. Newly sequenced species are in bold. Numbers at nodes represent bootstrap values of the posterior probability of BI and ML out of 1000 replicates, respectively. Clades with a different topology in the BI tree are indicated by “-.” The scale bar corresponds to five substitutions per 100 nucleotide positions

is rejected by the concatenated gene analyses (AU test, $p < .05$; Table 2). Likewise, neither is the grouping of *Foissnerides* with enchelyodontids supported (AU test, $p < .001$; Table 2). This result is consistent with the morphological homologies shared by *Foissnerides*, *Acaryophrya* and *Pseudoholophrya*: they have similar oral structure (circular circumoral dikinetids and the presence of nematodesmata) and a multirowed (i.e., > 3 rows) DB (Serrano, Martín-González, & Fernández-Galiano, 1990). The DB structure in both *Foissnerides* and *Pseudoholophrya* is conspicuously diffuse (Foissner et al., 2002; Serrano et al., 1990). *Acaryophrya* was transferred from Trachelophyllidae to Pseudoholophryidae by Rajter and Vďačný (2016) based on a combination of molecular and morphological data (circular circumoral dikinetids bearing nematodesma, and multirowed dorsal brush). In the light of the morphological similarity between *Foissnerides*, *Acaryophrya* and *Pseudoholophrya*, and their highly supported phylogenetic affiliation, we suggest that *Foissnerides* should be transferred from Trachelophyllidae to Pseudoholophryidae.

4.3 | Systematic position of *Helicoprordon*

Helicoprordon clusters with *Trachelotractus* in all our gene trees with high support. Their basal position within the Litostomatea is strongly supported, which is consistent with previous studies (Gao, Warren, et al., 2016;

Vďačný & Rataj, 2017). *Helioprordon* (Figure 1j,k) and *Trachelotractus entzi* Foissner, 1997 were placed in the family Helicoprordotidae Small and Lynn, 1985, by Foissner (1997). However, the placement of Helicoprordotidae has been subject to debate. It has been placed in Haptorida (Lynn, 2008; Lynn & Small, 2002), Pseudoholophryida Foissner & Foissner, 1988 (Foissner & Foissner, 1988) and Helicoprordontida Grain, 1994, which was established for the family Helicoprordontidae (Grain, 1994). Our results and other recent phylogenetic studies support this latter assignment, by revealing that Helioprordontidae represents an ordinal level clade in the class Litostomatea (Gao, Warren, et al., 2016; Vďačný & Rataj, 2017). Vďačný and Rataj (2017) elevated the suborder Helicoprordontina Foissner & Foissner, 1988 to the rank of order. However, as Helicoprordontida had already been established by Grain (1994), the order Helicoprordontida sensu Foissner & Foissner, 1988 should be treated as a junior synonym, and Helicoprordontina Foissner & Foissner, 1988 should be retained as a suborder.

4.4 | Systematic position of *Chaenea* and other lacrymarid genera

Due to its distinct combination of morphological traits (contractile body, presence of oralized somatic monokinetids,

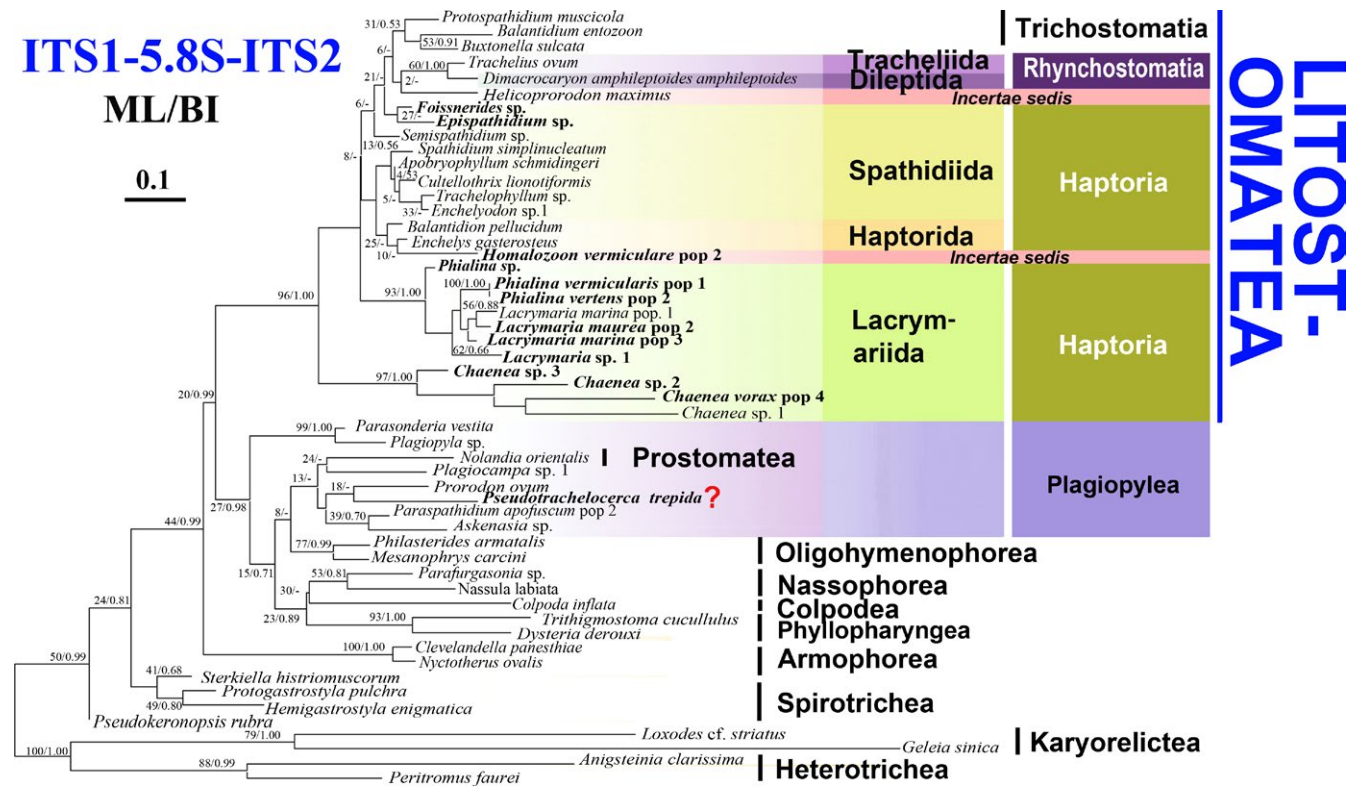


FIGURE 5 Bayesian inference (BI) tree based on ITS1-5.8S-ITS2 gene sequences of Litostomatea, focusing on Haptoria. Newly sequenced species are in bold. Numbers at nodes represent bootstrap values of the posterior probability of BI and ML out of 1000 replicates, respectively. 8 Clades with a different topology in the BI tree are indicated by “-.” The scale bar corresponds to 10 substitutions per 100 nucleotide positions

meridionally arranged somatic kineties and invariably four-rowed dikinetidal dorsal brush), *Chaenea* (Figure 1n,o) has a rather complicated systematic history having been variously assigned to the families Trachelophyllidae, Acropisthiidae and Fuscheriidae (for review, see Vd’achný et al., 2011). Based on its long DB bristles and the fact that the DB is distinctly separated from the circumoral kinety by narrowly spaced somatic monokinetids, Kwon et al. (2014) established the family Chaeneidae and placed it in the order Lacrymarida.

SSU-rDNA single-gene trees recover *Chaenea* as a distinct and long branch in the class Litostomatea (Gao, Warren, 2016; Gao et al., 2008; Vd’achný et al., 2011; Vd’achný, Breiner, et al., 2014; Zhang et al., 2012; Pan, Gao, Lin, Warren, & Song, 2013; Kwon et al., 2014; Vd’achný & Rataj, 2017; and the present work). Vd’achný et al. (2011), Vd’achný, Bourland, Orsi, Epstein, and Foissner (2012), Vd’achný, Breiner, (2014) suggested that this placement might be a consequence of class III long branch attraction, caused by large proportion of mutually incompatible sites in the SSU-rDNA sequences. In the present study, the addition of other rDNA loci (LSU, ITS1-5.8S-ITS2 region) and SSU-rDNA sequences from more taxa (six lacrymarids and three chaeneids) recovered a long and separate branch of chaeneids. Its affiliations to order Lacrymarida, or Didiniida as suggested by Kwon et al. (2014), are not

rejected by the topology test (AU test, $\rho > .05$; Table 2). Collectively, regarding the divergent positions in the multigene trees and the unique morphology of chaeneids, we agree with the establishment of family Chaeneidae. The affiliation of Chaeneidae to the order Lacrymarida or other haptorid groups, however, could not be resolved by rDNA loci alone.

We provide with the SSU-rDNA sequences of six species/populations within *Phialina* and five species/populations within *Lacrymaria*; however, relationship between the two genera could not be resolved by neither the phylogenetic tree nor the homology sites in the SSU-rDNA primary sequences (data not shown). Morphologically, *Lacrymaria* (Figure 1a,b) and *Phialina* (Figure 1c,d) differ only by the contractility of the anterior portion of the body, which is a questionable genus-level character (Lynn & Small, 2002). The results of the present study suggest that these two genera might be synonymous.

4.5 | The systematics of the class Litostomatea

The present study supports the monophyly of both subclasses Rhynchostomatia and Trichostomatia, which is consistent with the findings of Vd’achný et al. (2011), and the monophyly of each of the haptorian orders Lacrymariida, Haptorida, Didiniida, Pleurostomatida and Spathidiida. In addition, we provide new

insights into the systematics of the order Helicoprordontida and the families Pseudotrachelocercidae, Helicoprordontidae, Pseudoholophryidae and Chaeneidae. The subclass Haptoria, however, is not monophyletic, and the evolutionary relationships among most of its orders remain obscure (Figure 2). The application of multiple phylogenetic markers, in parallel with improved taxon sampling, is needed to understand the evolutionary history of this major group of ciliates.

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REFERENCES

- Adl, S. M., Simpson, A. G., Lane, C. E., Lukes, J., Bass, D., Bowser, S. S., ... Spiegel, F. W. (2012). The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology*, 59, 429–493. <https://doi.org/10.1111/j.1550-7408.2012.00644.x>
- Antipa, G. A., Dolan, J. R., Lynn, D. H., Obolkina, L. A., & Strüder-Kypke, M. C. (2016). Molecular phylogeny and evolutionary relationships between the ciliate genera *Peniculistoma* and *Mytilophilus* (Pencilistomatidae, Pleuronematida). *Journal of Eukaryotic Microbiology*, 63, 642–650. <https://doi.org/10.1111/jeu.12316>
- Berger, H. (2011). Monograph of the Gonostomatidae and Kahliellidae (Ciliophora, Hypotricha). *Monographiae Biologicae*, 90, 1–741.
- Cameron, S. L. (2003). Taxonomy and phylogeny of endosymbiotic ciliates (Ciliophora: Litostomatea) associated with Australian herbivorous marsupials. *International Journal for Parasitology*, 33, 347–355. [https://doi.org/10.1016/S0020-7519\(03\)00007-9](https://doi.org/10.1016/S0020-7519(03)00007-9)
- Cameron, S. L., & O'Donoghue, P. J. (2004). Phylogeny and biogeography of the “Australian” trichostomes (Ciliophora: Litostomata). *Protist*, 155, 215–235. <https://doi.org/10.1078/143446104774199600>
- Chen, X., Gao, S., Liu, Y., Wang, Y., Wang, Y., & Song, W. (2016). Enzymatic and chemical mapping of nucleosome distribution in purified micro-and macronuclei of the ciliated model organism, *Tetrahymena thermophila*. *Science China Life Sciences*, 59, 909–919. <https://doi.org/10.1007/s11427-016-5102-x>
- Corliss, J. O. (1974). Remarks on the composition of the large ciliate class Kinetofragmophora de Puytorac et al., 1974, and recognition of several new taxa therein, with emphasis on the primitive order Primociliatida n. ord. *Journal of Protozoology*, 21, 207–220. <https://doi.org/10.1111/j.1550-7408.1974.tb03643.x>
- Corliss, J. O. (1979). *The ciliated protozoa. Characterization, classification, and guide to the literature*, 2nd edn. Oxford, UK: Pergamon Press.
- Dragesco, J. (1960). Ciliés mesopsammiques littoraux. Systématique, morphologie, _écologie. *Travaux de la Station Biologique de Roscoff*, 12, 1–356.
- Dragesco, J. (1970). Ciliés libres du Cameroun. *Annales de la Faculté des Sciences du Cameroun. Numéro hors-série*, 1–141.
- Fan, X. P., Xu, Y., Gu, F. K., Li, J. Q., Al-Farraj, S. A., Al-Rasheid, K. A. S., & Hu, X. Z. (2015). Morphology of Two Novel Species of *Chaenea* (Ciliophora, Litostomatea): *Chaenea paucistriata* spec. nov and *C. sinica* spec. nov. *Acta Protozoologica*, 54, 97–106.
- Foissner, W. (1997). Updating the trachelocercids (Ciliophora, Karyorelictea). IV. Transfer of *Trachelocerca entzi* Kahl, 1927 to the Gymnostomatea as a new genus, *Trachelotractus* gen. n. (Helicoprordontidae). *Acta Protozoologica*, 36, 63–74.
- Foissner, W., Agatha, S., & Berger, H. (2002). Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib desert. Part I: Text and line drawings. Part II: Photographs. *Denisia*, 5, 1–1459.
- Foissner, W., & Foissner, I. (1988). The fine structure of *Fuscheria terricola* Berger et al., 1983 and a proposed new classification of the subclass Haptoria Corliss, 1974 (Ciliophora, Litostomatea). *Archiv für Protistenkunde*, 135, 213–235. [https://doi.org/10.1016/S0003-9365\(88\)80070-8](https://doi.org/10.1016/S0003-9365(88)80070-8)
- Galtier, N., Gouy, M., & Gautier, C. (1996). SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. *Computer Applications in the Biosciences*, 12, 543–548.
- Gao, F., Gao, S., Wang, P., Katz, L. A., & Song, W. (2014). Phylogenetic analyses of cyclidiids (Protista, Ciliophora, Scuticociliatia) based on multiple genes suggest their close relationship with thigmotrichids. *Molecular Phylogenetics and Evolution*, 75, 219–226. <https://doi.org/10.1016/j.ympev.2014.01.032>
- Gao, F., Huang, J. A., Zhao, Y., Li, L., Liu, W., Miao, M., ... Song, W. (2017). Systematic studies on ciliates (Alveolata, Ciliophora) in China: Progress and achievements based on molecular information. *European Journal of Protistology*, 61, 409–423.
- Gao, F., Li, J., Song, W., Xu, D., Warren, A., Yi, Y., & Gao, S. (2016). Multi-gene-based phylogenetic analysis of oligotrich ciliates with emphasis on two dominant groups: Cyrtostrombidiids and strombidiids (Protozoa, Ciliophora). *Molecular Phylogenetics and Evolution*, 105, 241–250. <https://doi.org/10.1016/j.ympev.2016.08.019>
- Gao, F., Song, W., & Katz, L. A. (2014). Genome structure drives patterns of gene family evolution in ciliates, a case study using *Chilodonella uncinata* (Protista, Ciliophora, Phyllopharyngea). *Evolution*, 68, 2287–2295.
- Gao, S., Song, W., Ma, H., Clamp, J., Yi, Z., Al-Rasheid, K. A. S., ... Lin, X. (2008). Phylogeny of six genera of the subclass Haptoria (Ciliophora, Litostomatea) inferred from sequences of the gene coding for small subunit ribosomal RNA. *Journal of Eukaryotic Microbiology*, 55, 562–566. <https://doi.org/10.1111/j.1550-7408.2008.00360.x>
- Gao, F., Warren, A., Zhang, Q., Gong, J., Miao, M., Sun, P., ... Song, W. (2016). The all-data-based evolutionary hypothesis of ciliated protists with a revised classification of the phylum Ciliophora (Eukaryota, Alveolata). *Scientific Reports*, 6, 24874. <https://doi.org/10.1038/srep24874>

- Gentekaki, E., Kolisko, M., Boscaro, V., Bright, K. J., Dini, F., Di Giuseppe, G., ... Lynn, D. H. (2014). Large-scale phylogenomic analysis reveals the phylogenetic position of the problematic taxon *Protocruzia* and unravels the deep phylogenetic affinities of the ciliate lineages. *Molecular Phylogenetics and Evolution*, *78*, 36–42. <https://doi.org/10.1016/j.ympev.2014.04.020>
- Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, *27*, 221–224. <https://doi.org/10.1093/molbev/msp259>
- Grain, J. (1994). Classe de Litostomatea. In P. de Puytorac (Ed), *Infusoires ciliés - systématique. Traité de Zoologie. II Fasc. 2* (p. 267–310). Paris, France: Masson.
- Huang, J. A., Dunthorn, M., & Song, W. (2012). Expanding character sampling for the molecular phylogeny of euplotid ciliates (Protozoa, Ciliophora) using three markers, with a focus on the family Uronychiidae. *Molecular Phylogeny and Evolution*, *63*, 598–605. <https://doi.org/10.1016/j.ympev.2012.02.007>
- Huang, J. B., Luo, X. T., Bourland, W. A., Gao, F., & Gao, S. (2016). Multigene-based phylogeny of the ciliate families Amphisiellidae and Trachelostylidae (Protozoa: Ciliophora: Hypotrichia). *Molecular Phylogenetics and Evolution*, *101*, 101–110. <https://doi.org/10.1016/j.ympev.2016.05.007>
- Jankowski, A. W. (2007). Phylum Ciliophora Doflein, 1901. In M. V. Krylow, & A. O. Frolov (Eds.), *Protista: Handbook on zoology, Part 2* (pp. 415–976). St. Petersburg, Russia: Nauka.
- Kwon, C. B., Vďačný, P., Shazib, S. U. A., & Shin, M. K. (2014). Morphology and molecular phylogeny of a new haptorian ciliate, *Chaenea mirabilis* sp. n., with implications for the evolution of the dorsal brush in haptorians (Ciliophora, Litostomatea). *Journal of Eukaryotic Microbiology*, *61*, 278–292. <https://doi.org/10.1111/jeu.12105>
- Li, J., Liu, W., Gao, S., Warren, A., & Song, W. (2013). Multigene-based analyses of the phylogenetic evolution of oligotrich ciliates, with consideration of the internal transcribed spacer 2 secondary structure of three systematically ambiguous genera. *Eukaryotic Cell*, *12*, 430–437. <https://doi.org/10.1128/EC.00270-12>
- Li, J., Zhan, Z., & Xu, K. (2017). Systematics and molecular phylogeny of the ciliate genus *Pseudokeronopsis* (Ciliophora, Hypotrichia). *Journal of Eukaryotic Microbiology*, *64*, 850–872. <https://doi.org/10.1111/jeu.12420>
- Lipscomb, D. L., & Riordan, G. P. (1990). The ultrastructure of *Chaenea teres* and an analysis of the phylogeny of the haptorid ciliates. *Journal of Protozoology*, *37*, 287–300. <https://doi.org/10.1111/j.1550-7408.1990.tb01149.x>
- Lynn, D. H. (2008). *The ciliated protozoa. Characterization, classification and guide to the literature*, 3rd edn. Dordrecht, The Netherlands: Springer.
- Lynn, D. H., & Small, E. B. (2002). Phylum Ciliophora. In J. J. Lee, G. F. Leedale & P. Bradbury (Eds.), *The Illustrated guide to the protozoa*, 2nd edn. (pp. 371–656). Lawrence, KS: Society of Protozoologists.
- Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. New Orleans, LA: Proceedings of the Gateway Computing Environments Workshop (GCE).
- Nylander, J. A. (2004). *MrModeltest v2. Evolutionary Biology Centre*. Uppsala, Sweden: Uppsala University.
- Pan, H. B., Gao, F., Li, J., Lin, X., Al-Farraj, S. A., & Al-Rasheid, K. A. S. (2010). Morphology and phylogeny of two new pleurostomatid ciliates, *Epiphyllum shenzhenense* n. sp. and *Loxophyllum spirellum* n. sp. (Protozoa, Ciliophora) from a mangrove wetland, South China. *Journal of Eukaryotic Microbiology*, *57*, 421–428. <https://doi.org/10.1111/j.1550-7408.2010.00492.x>
- Pan, H. B., Gao, F., Lin, X., Warren, A., & Song, W. (2013). Three new *Loxophyllum* species (Ciliophora: Pleurostomatida) from China with a brief review of the marine and brackish *Loxophyllum* species. *Journal of Eukaryotic Microbiology*, *60*, 44–56. <https://doi.org/10.1111/jeu.12005>
- Pan, H. B., Jiang, J. M., Fan, X. P., Al-Farraj, S. A., & Gao, S. (2017). Phylogeny and taxonomy of five poorly known species of cyrtophorian ciliates (Protozoa, Ciliophora, Phyllopharyngea) from China seas. *Zoological Journal of the Linnean Society*, *180*, 475–492. <https://doi.org/10.1093/zoolinnean/zlw006>
- Penn, O., Privman, E., Ashkenazy, H., Landan, G., Graur, D., & Pupko, T. (2010). GUIDANCE: A web server for assessing alignment confidence scores. *Nucleic Acids Research*, *38*, W23–W28. <https://doi.org/10.1093/nar/gkq443>
- Posada, D., & Crandall, K. A. (1998). Modeltest: Testing the model of DNA substitution. *Bioinformatics*, *14*, 817–818. <https://doi.org/10.1093/bioinformatics/14.9.817>
- Rajter, L., & Vďačný, P. (2016). Rapid radiation, gradual extinction and parallel evolution challenge generic classification of spathidiid ciliates (Protista, Ciliophora). *Zoologica Scripta*, *45*, 200–223. <https://doi.org/10.1111/zsc.12143>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Serrano, S., Martín-González, A., & Fernández-Galiano, D. (1990). General morphology and cytological events during the conjugation process in *Acaryophrya collaris* (Kahl, 1926) (Ciliophora, Haptorina). *Archiv für Protistenkunde*, *138*, 65–74. [https://doi.org/10.1016/S0003-9365\(11\)80211-3](https://doi.org/10.1016/S0003-9365(11)80211-3)
- Shimodaira, H. (2002). An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, *51*, 492–508. <https://doi.org/10.1080/10635150290069913>
- Shimodaira, H., & Hasegawa, M. (2001). ConSel: For assessing the confidence of phylogenetic tree selection. *Bioinformatics*, *17*, 1246–1247. <https://doi.org/10.1093/bioinformatics/17.12.1246>
- Song, W. (1990). Infraciliature and silverline system of the ciliate *Pseudotrachelocerca trepida* (Kahl, 1928), nov. gen., nov. comb., and establishment of a new family, the Pseudotrachelocercidae nov. fam. (Ciliophora, Haptoria). *European Journal of Protistology*, *26*, 160–166. [https://doi.org/10.1016/S0932-4739\(11\)80110-0](https://doi.org/10.1016/S0932-4739(11)80110-0)
- Song, W., Warren, A., & Hu, X. (2009). *Free-living Ciliates in the Bohai and Yellow Seas, China*. Beijing, China: Science Press.
- Song, W., & Wilbert, N. (1989). Taxonomische Untersuchungen an Aufwuchsciliaten (Protozoa, Ciliophora) im Poppelsdorfer Weiher, Bonn. *Lauterbornia*, *3*, 2–221.
- Song, W., Zhao, Y., Xu, K., Hu, X., & Gong, J. (2003). *Pathogenic protozoa in mariculture*. Beijing, China: Science Press.
- Stamatakis, A. (2014). RAXML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*, 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>

- Strüder-Kypke, M. C., Kornilova, O. A., & Lynn, D. H. (2007). Phylogeny of trichostome ciliates (Ciliophora, Litostomatea) endosymbiotic in the Yakut horse (*Equus caballus*). *European Journal of Protistology*, *43*, 319–328. <https://doi.org/10.1016/j.ejop.2007.06.005>
- Strüder-Kypke, M. C., Wright, A. D., Foissner, W., Chatzinotas, A., & Lynn, D. H. (2006). Molecular phylogeny of litostome ciliates (Ciliophora, Litostomatea) with emphasis on free-living haptorian genera. *Protist*, *157*, 261–278. <https://doi.org/10.1016/j.protis.2006.03.003>
- Sung, G., Sung, J., Hywel-Jones, N., & Spatafora, J. (2007). A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution*, *44*, 1204–1223. <https://doi.org/10.1016/j.ympev.2007.03.011>
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Vďačný, P. (2015). Estimation of divergence times in litostomean ciliates (Ciliophora: Intramacronucleata), using Bayesian relaxed clock and 18S rRNA gene. *European Journal of Protistology*, *51*, 321–334. <https://doi.org/10.1016/j.ejop.2015.06.008>
- Vďačný, P., Bourland, W. A., Orsi, W., Epstein, S. S., & Foissner, W. (2011). Phylogeny and classification of the Litostomatea (Protista, Ciliophora), with emphasis on free-living taxa and the 18S rRNA gene. *Molecular Phylogenetics and Evolution*, *59*, 510–522.
- Vďačný, P., Bourland, W. A., Orsi, W., Epstein, S. S., & Foissner, W. (2012). Genealogical analyses of multiple loci of litostomean ciliates (Protista, Ciliophora, Litostomatea). *Molecular Phylogenetics and Evolution*, *65*, 397–411.
- Vďačný, P., Breiner, H.-W., Yashchenko, V., Dunthorn, M., Stoeck, T., & Foissner, W. (2014). The chaos prevails: Molecular phylogeny of the Haptoria (Ciliophora, Litostomatea). *Protist*, *165*, 93–111.
- Vďačný, P., & Rajter, L. (2015). Reconciling morphological and molecular classification of predatory ciliates: Evolutionary taxonomy of dileptids (Ciliophora, Litostomatea, Rhynchostomatia). *Molecular Phylogenetics and Evolution*, *90*, 112–128.
- Vďačný, P., & Rataj, M. (2017). Evaluation of systematic position of helicoprodorontids and chaeneids (Ciliophora, Litostomatea): An attempt to break long branches in 18S rRNA gene phylogenies. *Journal of Eukaryotic Microbiology*, *64*, 608–621. <https://doi.org/10.1111/jeu.12396>
- Vďačný, P., Slovak, M., & Foissner, W. (2014). Multivariate morphometric analyses of the predatory ciliate genus *Semispalthidium* (Ciliophora: Litostomatea), with description of *S. longiarmatum* nov. spec. *European Journal of Protistology*, *50*, 329–344.
- Wang, P., Wang, Y., Wang, C., Zhang, T., Al-Farraj, S. A., & Gao, F. (2017). Further consideration on the phylogeny of the Ciliophora: Analyses using both mitochondrial and nuclear data with focus on the extremely confused class Phyllopharyngea. *Molecular Phylogenetics and Evolution*, *112*, 96–106. <https://doi.org/10.1016/j.ympev.2017.04.018>
- Wright, A. G., & Colorni, A. (2002). Taxonomic re-assignment of *Cryptocaryon irritans*, a marine fish parasite. *European Journal of Protistology*, *37*, 375–378. <https://doi.org/10.1078/0932-4739-00858>
- Wright, A. D., Dehority, B. A., & Lynn, D. H. (1997). Phylogeny of the rumen ciliates *Entodinium*, *Epidinium* and *Polyplastron* (Litostomatea:Entodiniomorpha) inferred from small subunit ribosomal RNA sequences. *Journal of Eukaryotic Microbiology*, *44*, 61–67. <https://doi.org/10.1111/j.1550-7408.1997.tb05693.x>
- Wright, A. D., & Lynn, D. H. (1995). Phylogeny of the fish parasite *Ichthyophthirius* and its relatives *Ophryoglena* and *Tetrahymena* (Ciliophora, Hymenostomatia) inferred from 18S ribosomal RNA sequences. *Molecular Biology and Evolution*, *12*, 285–290.
- Wu, L., Jiao, X. X., Shen, Z., Yi, Z. Z., Li, J. Q., Warren, A., & Lin, X. F. (2017). New taxa refresh the phylogeny and classification of pleurostomatid ciliates (Ciliophora, Litostomatea). *Zoologica Scripta*, *46*, 245–253. <https://doi.org/10.1111/zsc.12193>
- Xu, D., Song, W., & Hu, X. (2005). Morphology of *Cyclotrichium taniguchii* sp. nov. and *C. cyclokaryon* with establishment of a new genus, *Dicyclotrichium* gen. nov. (Ciliophora: Haptorida). *Journal of the Marine Biological Association of the United Kingdom*, *85*, 787–794. <https://doi.org/10.1017/S0025315405011719>
- Yan, Y., Fan, Y., Chen, X. R., Li, L. F., Warren, A., Al-Farraj, S. A., & Song, W. (2016). Taxonomy and phylogeny of three heterotrich ciliates (Protozoa, Ciliophora), with description of a new *Blepharisma* species. *Zoological Journal of the Linnean Society*, *177*, 320–334. <https://doi.org/10.1111/zoj.12369>
- Yan, Y., Xu, Y., Al-Farraj, S. A., Al-Rasheid, K. A. S., & Song, W. (2016). Morphology and phylogeny of three trachelocercids (Protozoa, Ciliophora, Karyorelictea), with description of two new species and insight to the evolution of the family Trachelocercidae. *Zoological Journal of the Linnean Society*, *177*, 306–319. <https://doi.org/10.1111/zoj.12364>
- Zhang, Q., Simpson, A., & Song, W. (2012). Insights into the phylogeny of systematically controversial haptorian ciliates (Ciliophora, Litostomatea) based on multigene analyses. *Proceedings of the Royal Society of London B: Biological Sciences*, *279*, 2625–2635. <https://doi.org/10.1098/rspb.2011.2688>
- Zhang, Q., Yi, Z., Song, W., Al-Rasheid, K. A. S., & Warren, A. (2010). The systematic position of *Paraspathidium* Noland, 1937 (Ciliophora, Litostomatea?) inferred from primary SSU rRNA gene sequences and predicted secondary rRNA structure. *European Journal of Protistology*, *46*, 280–288. <https://doi.org/10.1016/j.ejop.2010.05.001>
- Zhao, X., Gao, S., Fan, Y., Strüder-Kypke, M., & Huang, J. (2015). Phylogenetic framework of the systematically confused *Anteholosticha-Holosticha* complex (Ciliophora, Hypotrichia) based on multigene analysis. *Molecular Phylogeny and Evolution*, *91*, 238–247. <https://doi.org/10.1016/j.ympev.2015.05.021>

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