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

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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 & Colomi, 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
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 Askensasia, 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 litostomean 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

| Species               | Sampling location                          | Latitude/longitude | Accession No.                  |
|-----------------------|--------------------------------------------|--------------------|--------------------------------|
|                       |                                            |                    | SSU-rDNA | ITS1-5.8S-ITS2 | LSU-rDNA |
| Chaenea sp. 2         | Daya Bay, Guangzhou, southern China        | 22°46′N 114°41′E   | MF474336 | MF474363       | MF474350 |
| Chaenea sp. 3         | Techeng Island, Zhanjiang, southern China  | 21°09′N 110°26′E   | MF474337 | MF474364       | MF474351 |
| Chaenea vorax         | Huguang Lake, Zhanjiang, southern China    | 21°08′N 110°17′E   | MF474338 | MF474365       | MF474352 |
| Epispathidium sp.     | Daya Bay, Guangzhou, southern China        | 22°46′N 114°41′E   | MF474339 | MF474366       | MF474353 |
| Foissnerides sp.      | Donghai Island, Zhanjiang, southern China  | 21°00′N 110°31′E   | MF474340 | MF474367       | MF474354 |
| Helicoprorodon sp. 1  | Jiaozhou Bay, Qingdao, northern China      | 35°55′N 120°12′E   | MF474341 | –               | –        |
| Homalozoon vermiculare| Huguang Lake, Zhanjiang, southern China    | 21°08′N 110°17′E   | MF474342 | MF474368       | MF474355 |
| Lacinumia maurea pop 2| Jiaozhou Bay, Qingdao, northern China      | 36°03′N 120°23′E   | MF474344 | MF474370       | MF474357 |
| Lacinumia marina pop 3| Guandu town, Zhanjiang, southern China     | 21°22′N 110°26′E   | MF474343 | MF474369       | MF474356 |
| Lacinumia sp. 1       | Jiaozhou Bay, Qingdao, northern China      | 36°03′N 120°19′E   | MF474345 | MF474371       | MF474358 |
| Phialina sp. 1        | Jiaozhou Bay, Qingdao, northern China      | 36°03′N 120°20′E   | MF474346 | MF474372       | MF474359 |
| Phialina vermicularis pop 1| Jiaozhou Bay, Qingdao, northern China    | 36°03′N 120°20′E   | MF474347 | MF474373       | MF474360 |
| Phialina vertens      | Jiaozhou Bay, Qingdao, northern China      | 36°03′N 120°20′E   | MF474348 | MF474374       | MF474361 |
| Pseudotrachelocerca trepida | 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 relation-
ships 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 se-
quencing were performed according to Gao, Li, et al. (2016).

2.2 | Data sets and alignments

Newly characterized sequences combined relevant se-
quencies 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 de-
fault 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 analy-
ses. 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), respec-
tively. 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 genera-
tions, with sampling every 100 generations and a burn-in of
10,000. All the remaining trees were used to calculate pos-
terior probabilities (PP) using a majority rule consensus.
MEGA v5 (Tamura et al., 2011) was used to visualize the
tree topologies. Terminology and classification mainly fol-
low Gao, Warren, et al. (2016) and Vd’ačný, 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 con-
strained topologies were then compared to the best (uncon-
strained) 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 | SSU-rDNA tree |
| Unconstrained       | .863        | .645          |
| Monophyly of Lacrymariidea + Chaeneidae | .313 | .572 |
| Monophyly of Lacrymariidea + Didiniida | .110 | .387 |
| Monophyly of Pseudotrachelocerca + Haptoria | <.001 | <.001 |
| Monophyly of Pseudotrachelocerca + Litostomatea | <.001 | .004 |
| Monophyly of Foissnerides + Acaryophrya + Trachelophyllum | .022 | .161 |
| Monophyly of Foissnerides + Trachelophyllum | .008 | .063 |
| Monophyly of Foissnerides + Enchelyodon | <.001 | <.001 |

ρ< .05 refute the possibility of monophyly; ρ> .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 Trachelida) 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 trepita* 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 *Acyarophyra* sp. PV-2014 with high support (BI/ML: 1.00/87) to form a terminal clade within the Spathidiida. The newly sequenced species *Helicoprorodon* 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 litostomean 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 *Homalozooon* 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 *Lacrzymaria*) 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 *Lacrzymaria*.

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 *Helicoprorodon + 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 trepita* 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 + Helicoprorodon* 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 monogenic family *Pseudotrachelocercidae*. *Pseudotrachelocercica* (Figure 1h,i) is similar to other litostomeans in having a dorsal brush (DB), apical extrusomes and a mesh-like silverline system (Song, 1990). Among these characters, the DB is an important feature for the classification of litostomeans, 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 kinetics in most other litostomeans; Song, 1990). Both the divergent phylogenetic position and the heterogeneities in the DB appearance imply that the DB structure of *Pseudotrachelocerca*
might be a homoplasy of the typical litostomean dorsal brush. In addition, the topology testing results strongly reject the grouping of *Pseudotrachelocerca* to either Haptoria or Litostomatea (AU test, ρ< .05; Table 2). Accordingly, we suggest an exclusion of Pseudotracheloceridae from the class Litostomatea.

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 prostomatean 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 Pseudotracheloceridae should be regarded as *incertae sedis* within the Prostomeata + Plagiopylea + Colpodea complex.

**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). *Pseudotrachelocerca* (from Song 1990). (j, k). *Helicoprorodon* (from Dragesco 1960). (l, m). *Homalozoon*, from Dragesco (1970). (n, o). *Chaenea*, from Fan et al. (2015)
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.
Vďačný 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 absence 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
is rejected by the concatenated gene analyses (AU test, \( p < .05 \); Table 2). Likewise, neither is the grouping of \textit{Foissnerides} with enchelyodontids supported (AU test, \( p < .001 \); Table 2). This result is consistent with the morphological homologies shared by \textit{Foissnerides}, \textit{Acaryophrya} and \textit{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 \textit{Foissnerides} and \textit{Pseudoholophrya} is conspicuously diffuse (Foissner et al., 2002; Serrano et al., 1990). \textit{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 nematodesmata, and multirowed dorsal brush). In the light of the morphological similarity between \textit{Foissnerides}, \textit{Acaryophrya} and \textit{Pseudoholophrya}, and their highly supported phylogenetic affiliation, we suggest that \textit{Foissnerides} should be transferred from Trachelophyllidae to Pseudoholophryidae.

### 4.3 | Systematic position of \textit{Helicoprorodon}

\textit{Helicoprorodon} clusters with \textit{Trachelotracus} 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). \textit{Helicoprorodon} (Figure 1j,k) and \textit{Trachelotracus entzi} Foissner, 1997 were placed in the family Helicoprorodontidae Small and Lynn, 1985, by Foissner (1997). However, the placement of Helicoprorodontidae has been subject to debate. It has been placed in Haptoria (Lynn, 2008; Lynn & Small, 2002), Pseudoholophryidae Foissner & Foissner, 1988 (Foissner & Foissner, 1988) and Helicoprorodontidae Grain, 1994, which was established for the family Helicoprorodontidae (Grain, 1994). Our results and other recent phylogenetic studies support this latter assignment, by revealing that Helicoprorodontidae represents an ordinal level clade in the class Litostomatea (Gao, Warren, et al., 2016; Vďačný & Rataj, 2017). Vďačný and Rataj (2017) elevated the suborder Helicoprorodontina Foissner & Foissner, 1988 to the rank of order. However, as Helicoprorodontidae had already been established by Grain (1994), the order Helicoprorodontida sensu Foissner & Foissner, 1988 should be treated as a junior synonym, and Helicoprorodontina Foissner & Foissner, 1988 should be retained as a suborder.

### 4.4 | Systematic position of \textit{Chaenea} and other lacrymarid genera

Due to its distinct combination of morphological traits (contractile body, presence of oralized somatic monokinetics,
meridionally arranged somatic kineties and invariably four-rowed dikeneticidal dorsal brush), Chaenea (Figure 1n,o) has a rather complicated systematic history having been variously assigned to the families Trachelophyllidae, Acropisthiidae and Fuscherriidae (for review, see Vd’ačný 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’ačný et al., 2011; Vd’ačný, Breiner, et al., 2014; Zhang et al., 2012; Pan, Gao, Lin, Warren, & Song, 2013; Kwon et al., 2014; Vd’ačný & Rataj, 2017; and the present work). Vd’ačný et al. (2011), Vd’ačný, Bourland, Orsi, Epstein, and Foissner (2012), Vd’ačný, 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, \( \rho > 0.05 \); 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 Lacyrmyaria; 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, Lacyrmyaria (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 Rhynchochotomia and Trichostomata, which is consistent with the findings of Vd’ačný et al. (2011), and the monophyly of each of the haptorian orders Lacrymarida, Haptorida, Didiniida, Pleurostomatida and Spathidiida. In addition, we provide new
insights into the systematics of the order Helicoprorodontida and the families Pseudotracheloceridae, Helicoprorodontidae, 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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