Highlights

- New sequencing of 28 species and curation of all GenBank sequences for three rDNA loci

- Concatenated phylogeny with 18 families and updated classification with 23 families

- *Favella* forms a distinct family and *Tintinnopsis* is spread among 11 clades

- A previously-unknown environmental clade matches Leegaardiellidae

- Prevalence of uncharacterized and cryptic diversity in aloricates

Phylogeny, classification and diversity of Choreotrichia and Oligotrichia (Ciliophora, Spirotrichea)

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ABSTRACT

Ciliated protists in the subclasses Choreotrichia and Oligotrichia are major components of marine plankton. Despite their ecological relevance, there are uncertainties in their systematics and diversity. We retrieved and curated all the ribosomal DNA (rDNA) sequences available in GenBank for these groups, and analyzed them in two ways. The first approach was based on morphologically-identified sequences (including those of two families and six genera newly studied here by single-cell sequencing), and aimed at improving phylogenetic inferences using concatenated sequences of three rDNA loci. Based on phylogenetic and morphological support, we update the taxonomic classification of these subclasses into 23 families, including the re-established Favellidae. We also propose an informal naming system for unassigned taxa, namely *Tintinnopsis* and five closely-related genera that are spread among eleven clades. The second approach included unidentified environmental sequences, and was used to explore potentially novel diversity in these subclasses. Our results support high proportions of both synonyms in tintinnids and uncharacterized diversity in aloricate choreotrichs and oligotrichs. One previously unidentified, environmental clade is here linked to the newly sequenced Leegaardiellidae. Our curation of almost 4,000 rDNA sequences affirms known issues of public repositories, and suggests caution in both the use and contribution to these unique resources for evolutionary and diversity studies.

Keywords: protist; ciliate; tintinnid; single-cell sequencing; sequence curation; environmental sequencing

1. Introduction

Despite their importance in evolution of life and ecosystem functioning, major protist taxa remain poorly understood in terms of diversity and systematics (Corliss, 2002; Cotterill et al., 2008). Here we focus on two ecologically important groups of ciliated protists, the sister subclasses Choreotrichia Small and Lynn, 1985 and Oligotrichia Bütschli, 1887/1889. Although they are present in varied environments (including freshwater plankton, benthos and even as endocommensals in sea urchins), these groups thrive in marine plankton, where they are usually species-rich and abundant (Lynn, 2008). They include heterotrophs and mixotrophs in a size spectrum of about 10 to 200 μm, and thus play diverse trophic roles as algae and bacteria consumers, primary producers, and prey for small metazoans (Calbet and Saiz, 2005; McManus and Santoferrara, 2013; Pierce and Turner, 1992; Sanders and Wickham, 1993).

Morphologically, these subclasses are characterized by an adoral zone of membranelles that surrounds the apical part of the cell, and a somatic ciliature that is generally reduced. The adoral zone of membranelles forms a closed or slightly opened circle in Choreotrichia, whereas it is C-shaped in Oligotrichia (Lynn, 2008). In Choreotrichia (or choreotrichs), some taxa have an external lorica attached to the cell (order Tintinnida = tintinnids), while the rest (order Choreotrichida = choreotrichids), as well as all of the Oligotrichia (oligotrichs), are aloricate. For most ciliates, taxonomy is based on the cell morphology and ciliary patters, which are studied *in vivo* and by complex staining techniques, especially difficult for the smallest and/ or uncultivable species (Agatha, 2011). In contrast, tintinnid taxonomy is based on the lorica, which is relatively easy to sample and characterize, but it is less reliable for species diagnosis and classification of higher taxa (Agatha and Strüder-Kypke, 2013; Alder, 1999; Laval-Peuto, 1994). As with other organisms, the taxonomic and evolutionary studies of these groups have been gradually complemented with DNA sequences over the last 15 years (e.g., Bachy et al., 2012; Liu et al., 2015; Santoferrara et al., 2012; Snoeyenbos-West et al., 2002).

Current limitations in the understanding of choreotrich and oligotrich systematics include: (1) some families still lack data on the ciliary patterns or have never been sequenced reliably and thus are not represented in cladistic or phylogenetic inferences (Agatha and Strüder-Kypke, 2012, 2014); (2) for some families, data on morphology (cell- and/ or lorica-based) and DNA sequences do not agree and thus increased taxon and character sampling are needed (for example, by using multi-gene approaches that are known to improve phylogenetic accuracy in other ciliate clades; Yi et al., 2014); (3) several families and genera are not monophyletic and require revision, including extremely diverse taxa that are

currently difficult to link in taxonomic and ecological studies (e.g., *Tintinnopsis*); and (4) classification systems have not been stable and require constant update, as expected due to increasing knowledge, but in some cases also due to premature conclusions based on incomplete data.

In addition to the known gaps in current systematics, many choreotrich and oligotrich taxa may remain undiscovered. For more than a decade, environmental surveys worldwide allowed the accumulation of ciliate sequences in public repositories (e.g., Stoeck et al., 2003; Doherty et al., 2007; Lie et al., 2014), which provide unique opportunities to reveal uncharacterized diversity. In fact, divergent lineages detected iteratively by environmental sequencing, some of them probably representing families or genera, remain unbounded to morphology (e.g., Forster et al., 2015; Santoferrara et al., 2014). Molecular data also suggest that the number of species currently known for choreotrichs and oligotrichs is inaccurate, for example due to cases of interspecific similarity (crypticity) and intraspecific polymorphism (Katz et al., 2005; Kim et al., 2013; McManus et al., 2010; Santoferrara et al., 2013, 2015). Particularly, tintinnids are suspected of synonymy problems, as many species that were established based on minute lorica differences may actually reflect phenotypic variation due developmental or environmental factors (Alder, 1999; Dolan, 2016; Laval-Peuto, 1981). As a result, about five times more species have been described for tintinnids than for aloricate choreotrichs and oligotrichs combined (>1,000 and <200, respectively), also because the aloricate morphospecies remain unexplored in extensive geographical areas (Agatha, 2011).

To help clarify choreotrich and oligotrich taxonomy, evolutionary relationships and global diversity, we focused on the following objectives: (1) to increase the number of families represented in phylogenetic inferences based on three rDNA loci newly studied by single-cell sequencing; (2) to update the classification of these groups based on our novel results and other recent findings; (3) to propose a system for informal classification of ecologically important taxa with uncertain taxonomic position; and (4) to explore the potential for novel diversity within these groups by integrating environmental sequences from multiple studies in a single phylogenetic context. To complete these aims, we retrieved and manually curated all the choreotrich and oligotrich rDNA data in NCBI GenBank, including both morphologically-identified and environmental sequences. There is an increasing need for careful evaluation of DNA sequences available in public repositories, given the well-known issue of inadequate data accumulating along with the useful information (Kozlov et al., 2016). This is true for sequences linked to a named species (e.g., due to misidentifications) and also for environmental sequences (e.g., due to methodological artifacts). Thus, by carefully documenting our curation efforts, we also provide a useful resource for future studies on ciliate phylogenetics and diversity.

2. Material and methods

2.1. Single cell sequencing

We analyzed isolates of twenty-one species newly collected in summer 2015 and seven species sampled in previous studies, all from Northwest Atlantic waters (3 choreotrichids, 23 tintinnids and 2 oligotrichs; Fig. 1, Supplementary Fig. S1). Of them, twenty-seven species were sequenced for the first time for at least one marker and only one had been sequence before for the three of them. At least one genus and/ or marker was newly sequenced within eight families. Two families (Leegaardiellidae and Ascampbelliellidae), six genera (*Leegaardiella, Ascampbelliella, Salpingacantha, Ptychocylis, Parafavella* and *Parundella*) and twelve species (in bold in Supplementary Table S1) had not been sequenced before for any marker. Tintinnid and aloricate taxa were identified based on the lorica or cell morphology, respectively (see detailed information in Supplementary Text 1). Single cells were studied in the microscope, individually subjected to DNA extraction and sequenced as described before (Santoferrara et al., 2013, 2015). Three primer sets were used for DNA amplification and Sanger sequencing of the small subunit (SSU) rDNA, the 5.8S rDNA combined with the internally transcribed spacer regions 1 and 2 (ITS regions) and the D1-D2 region of the large subunit (LSU) rDNA (Supplementary Table S2). Chromatogram quality was checked individually and sequences in the forward and reverse sense were assembled manually in MEGA v. 5 (Tamura et al., 2011). A total of 60 newly obtained sequences were uploaded in GenBank (accession numbers KY290291 to KY290350). Also, we updated 50 of our previous GenBank records (Supplementary Text 2, Fig. S2A).

2.2. Phylogenetic inferences

For phylogenies, we focused on SSU rDNA, ITS regions and LSU rDNA sequences identified to the genus or species level based on morphology. We retrieved and manually curated all the sequences labeled as Choreotrichia or Oligotrichia in NCBI GenBank (1,297 and 261, respectively; last updated on November 1, 2016). Records from environmental sequencing as well as low quality and redundant sequences were eliminated; sequences potentially misidentified or lacking published morphological data were retained but flagged (Supplementary Text 3). Our newly obtained sequences were then added, along with four outgroup sequences of the subclass Hypotrichia Stein, 1859. Four final datasets including from 47 to 198 sequences were obtained: SSU rDNA, ITS regions, LSU rDNA, and the three

markers concatenated (Supplementary Table S3). For the concatenated dataset, sequences from the same specimen were combined when possible (Supplementary Table S4); although sequences from the three markers exist for additional species, almost forty of them were excluded due to serious quality concerns (Supplementary Text 3).

Each dataset was aligned with MAFFT v. 7 (Katoh and Standley, 2013). Ambiguous positions were removed with the guidance of Gblocks v. 0.91b under default parameters (Castresana, 2000). Maximum likelihood inferences were done with RAxML v. 8.3.17 (Stamatakis, 2014), with the best-known tree and the node support values inferred out of 200 trees and 10,000 bootstraps, respectively. Bayesian inferences were done with MrBayes v. 3.2.1 (Ronquist et al., 2012). Five million generations were run and trees were sampled each 1,000 cycles. The initial 1,000 trees were discarded as burn-in, and the remaining 4,000 trees were used to estimate the Bayesian posterior probabilities. For each analysis, the GTR model with a Γ model of rate heterogeneity and a proportion of invariable sites was used, as previously identified with MrModeltest v. 2 (Nylander, 2004) under the Akaike Information Criterion. Based on RAxML bootstrap support and Bayesian posterior probabilities, inference support was considered good (>70%, 0.95), moderate (45-70%, 0.90-0.95) or low (<45%, 0.9).

2.3. Exploring the unknown taxa

To explore the proportion of potentially novel taxa in Choreotrichia and Oligotrichia, we considered all the SSU rDNA sequences available in NCBI GenBank. Both morphologically-identified and environmental sequences from these groups were retrieved and curated in the context of the EukRef initiative (http://eukref.org). A reference dataset including reliable SSU rDNA sequences of all the major taxa that have been sequenced was created. This dataset was the seed to iteratively retrieve all the GenBank sequences that are ≥80% similar to the groups of interest using the BLASTN algorithm (Camacho et al., 2009) against the NCBI non-redundant/nucleotide collection (last updated on July 2015). Sequences shorter than 500 bp (less reliable for phylogenetic analysis; e.g., Dunthorn et al., 2014), chimeras detected with UCHIME (Edgar et al., 2011), and a dataset known to include misidentifications (accession numbers AB640624 to AB640682) were removed. Sequences from the present study were incorporated.

To simplify the bioinformatic steps, the sequences were clustered at 97% similarity with USEARCH (Edgar, 2010). These clusters were subjected to iterative rounds of alignment (MAFFT v. 7; Katoh and Standley, 2013), refinement (trimAl v. 1.2; Capella-Gutiérrez et al., 2009), and maximum likelihood

158 inference (FastTree v. 2; Price et al., 2010) in order to detect and remove any remaining sequence out of
159 the groups of interest or with suspicious quality (i.e., some long branches manually identified as
160 chimeras). The final dataset of 346 clusters (3,145 total sequences) was separated into Tintinnida,
161 Choreotrichida and Oligotrichia, re-aligned and analyzed with RAxML as described above (see 2.2; the
162 only difference was that 1,000 bootstraps were used here). The 3,145 final sequences were also clustered
163 at 99% similarity (the cutoff generally accepted as approximation to species in these taxa; Bachy et al.,
164 2013; Santoferrara et al., 2013, 2014), which resulted in 943 clusters. The final datasets will be publicly
165 available as part of EukRef (http://eukref.org).

166

167 **3. Results and Discussion**

168 *3.1. Phylogeny*

169 We expanded the phylogenetic tree of Choreotrichia and Oligotrichia by adding 27 newly sequenced
170 species (Fig. 1, S1) and by including 18 out of 23 families in concatenated SSU rDNA, ITS regions and
171 LSU rDNA analyses (Fig. 2). In general, inferences based on concatenated sequences or on each
172 separate marker agreed, although the former had higher support (Fig. 2, 3, S3, S4, S5). All analyses
173 confirmed the monophyly of Choreotrichia and Oligotrichia, but disagreed in which of these subclasses
174 embraces Lynnellidae. This family is basal within Choreotrichia in concatenated and SSU rDNA
175 analyses (Fig. 2, 3), but affiliated to Oligotrichia or sister to both subclasses in our ITS regions and LSU
176 rDNA trees (Fig. S4, S5) and previous studies (e.g. Liu et al., 2015, 2016), although usually with
177 moderate or low support. An affiliation with Choreotrichia is supported by shared morphological traits
178 (a slightly-open adoral zone of membranelles in *Parastrombidinopsis* and *Parastrombidium*, and the
179 structure of the somatic kinetids in Lohmanniellidae; Agatha and Strüder-Kypke, 2014), even if
180 differences in the position of the oral ciliature weaken this association (Liu et al., 2015).

181 Regardless of Lynnellidae, Choreotrichida is not monophyletic based on our trees (Fig. 2, 3, S4, S5) and
182 previous studies of both DNA sequences and morphology (Agatha and Strüder-Kypke, 2014). Within
183 this order, we newly sequenced the family Leegaardiellidae, which forms a long branch between
184 Strombidinopsidae and Strobilidiidae in the concatenated analysis (Fig. 2) and between two known
185 subclades of the paraphyletic Strombidinopsidae (Liu et al., 2016) in the SSU rDNA tree (Fig. 3, S3A).
186 This contrasts with morphological cladistics, which places Leegaardiellidae as the most basal
187 Choreotrichida due to the singularity of their bipartite collar membranelles (Agatha and Strüder-Kypke,
188 2012, 2014; Fig. 1). The conflicts in Lynnellidae, Leegaardiellidae and Strombidinopsidae may be due

to the lack of sequences for some taxa (Lohmanniellidae and *Parastrombidium*). In contrast, Strobilidiidae is the least problematic taxon in the order, as it is usually inferred as monophyletic and as the most derived Choreotrichida (e.g., Fig. 2, 3).

Tintinnida is the best represented group in our trees, and it is confirmed as monophyletic (although with moderate or low support in RAxML analyses; Fig. 2, 3, S4, S5). The monophyletic Tintinnidiidae, Tintinnidae (including the newly sequenced *Salpingacantha*), Eutintinnidae, and Favellidae (re-established here; see 3.2) were sequentially arranged in the trees, in agreement with previous molecular inferences and morphology (mainly the somatic ciliary patterns, lorica ultrastructure and extrusome types; Agatha and Strüder-Kypke 2012, 2013, 2014). The next taxa in the trees are less clearly resolved. Dictyocystidae and Stenosemellidae appear as sister, monophyletic clades in the concatenated analysis (Fig. 2), but they cluster together in the SSU rDNA tree, where more taxa are included (Fig. S3B). Despite similarities in lorica morphology and extrusome type (Supplementary Text 1), only Dictyocystidae presents a lorica sac, which is considered as an important synapomorphy of this family (Agatha and Strüder-Kypke, 2013, 2014). Xystonellidae, Undellidae (only in the SSU rDNA tree), and a clade with Rhabdonellidae (including *Metacylis*; see 3.2), Cyttarocylididae, Ascampbelliellidae (newly sequenced here), Epiplocylididae and Ptychocylididae (excluding *Favella*; see 3.2), are all monophyletic, but in some cases are arranged as polytomies (Fig. 2, 3). Also arranged as polytomies are the most chaotic tintinnids, the paraphyletic *Tintinnopsis* and other *incertae sedis* genera that form up to eleven clades in our trees (see 3.2 and 3.3) and for which at least four kinds of both somatic ciliary patterns and lorica matrix texture are known (Agatha et al., 2013; Agatha and Strüder-Kypke, 2014).

Oligotrichia remains largely under-sampled in our concatenated analyses (Fig. 2). In the SSU rDNA tree (Fig. 3, S3A), Tontoniidae and Cyrtostrombidiidae are monophyletic, and the only available sequence labeled as Pelagostrombidiidae forms an isolated branch, in agreement with clear morphological differences among these three families (a contractile tail except in *Laboea*, a cyrtos, and a neoformation organelle, respectively; Agatha, 2004). In contrast, Strombidiidae and several of its genera, particularly the species-rich *Strombidium*, are paraphyletic (Fig. S3A, S4, S5). Probably because several taxa have not been sequenced reliably (not even the type *S. sulcatum*; Supplementary Text 3) or at all, phylogenetic relationships are poorly supported, unstable and partly inconsistent with evolutionary hypotheses based mainly on the somatic ciliary patterns (Agatha and Strüder-Kypke, 2014; Liu et al., 2015). For now, clades that show molecular and morphological cohesion include (1) *Williophrya* and *Strombidium* species characterized by an eyespot, which may be a major synapomorphy of this group (Liu et al., 2016); and (2) the subgenus *Novistrombidium* (*Novistrombidium*), differentiated by

221 extrusome position, a feature of potential taxonomic value that deserves more study in Strombidiidae
222 (Agatha and Strüder-Kypke, 2014).

223

224 *3.2. Updated classification*

225 We propose an updated classification for Choreotrichia and Oligotrichia (Table 1). This is based on the
226 latest comprehensive classifications for these groups (Agatha, 2011; Agatha and Strüder-Kypke, 2013;
227 Lynn, 2008), the revision of subsequent literature, and our novel findings (Supplementary Table S5).
228 Our intent is to reconcile the existing data in the most conservative way, considering both morphological
229 and molecular support (see 3.1). The motivations for this updated classification are three. First, the latest
230 and most widely-used systems disagree in some taxa that are now represented in phylogenetic trees. For
231 example, *Cyrtostrombidium* has been considered a Strombidiidae (Lynn, 2008), but a separate family is
232 now supported by both its morphology (Agatha, 2004) and DNA sequences (Tsai et al., 2015; Fig. 3).
233 Second, recently-created taxa need to be added in the classification, if justified. For example, the
234 distinctiveness of *Lynnella* has warranted a new family (Liu et al., 2011), but its inclusion in a new order
235 (Liu et al., 2015) seems premature given the morphological similarities to Choreotrichida and
236 unresolved phylogenetic relationships (see 3.1). Finally, our new data confirm or reject some
237 rearrangements in tintinnids, as explained below.

238 We reestablish the family Favellidae and improve its diagnosis (see 3.2.1). The previous placement of
239 *Favella* in Ptychocylididae (Campbell, 1954) is refuted by the distant position of our novel *Ptychocylis*
240 sequences, which cluster with those of *Cymatocylis* (Fig. 2, S3B). This separation is supported by
241 differences in the ciliary pattern and lorica ultrastructure. *Favella* presents two dorsal kineties in the
242 somatic ciliature, and a lorica wall monolaminar with alveoli and a smooth surface (Agatha and Strüder-
243 Kypke, 2012; Kim et al., 2010). In contrast, *Cymatocylis,* and presumably other Ptychocylididae, have a
244 more developed ciliary pattern with only one dorsal kinety (Kim et al., 2013) and a lorica wall that is
245 also monolaminar with alveoli, but with ridges (also present in *Ptychocylis*; Supplementary Text 1).

246 *Parundella* and *Dadayiella* are separate genera and both need family reassignment. They have been
247 incorrectly synonymized (Xu et al., 2013), as noticed by Agatha and Strüder-Kypke (2014). Having
248 sequenced them here (Fig. 1) or in previous studies (Santoferrara et al. 2016a), we confirm differences
249 in genes and lorica morphology (Supplementary Text 1). *Parundella* was first established as an *Undella*
250 subgenus given that both taxa show distinct wall laminae (Jörgensen, 1924), but the former was then
251 moved to Xystonellidae without clear reasons (Kofoid and Campbell, 1929). Here, we transfer

Parundella to Undellidae due to their phylogenetic affinity (Fig. S3B) and similar lorica wall ultrastructure (trilaminar; Agatha and Strüder-Kypke, 2014; Marshall, 1969). *Dadayiella* was affiliated to Tintinnidae, but this placement is not supported by DNA sequences (Fig. S3) or morphology (Kofoid and Campbell, 1929). Thus, we transfer *Dadayiella* as *incertae sedis* in Xystonellidae based on their fully supported phylogenetic relationship (Fig. S3B), although detailed morphological studies are needed to confirm this affiliation.

Cyttarocylis and *Petalotricha* may be separate genera. These genera, their families and several of their species have been unified based on identical SSU rDNA and ITS regions in specimens from the Mediterranean (Bachy et al., 2012). We found identical sequences for both markers in *C. acutiformis* and *P. ampulla* from the NW Atlantic, but our novel LSU rDNA sequences differ by 1.8% between species, in agreement with the marked dissimilarities in lorica morphology (Fig. 1D-E, Supplementary Text 1). This molecular divergence and, especially, the fact that lorica differences are not confirmed as intra-taxon polymorphism (Dolan, 2016) delay potential species and genera synonymizations until more features are studied and unified diagnoses can be provided. Instead, family synonymization is supported phylogenetically (Fig. 2) and by the shared lorica ultrastructure (trilaminar, tubular; Agatha and Strüder-Kypke, 2014). Bachy et al. (2012) included also *Metacylis* and *Rhabdonella* in Cyttarocylididae, but the lack of morphological justification and the increased taxon and character sampling in our inferences (Fig. 2, S3B) suggest that these transfers are premature. Conservatively, we avoid lumping Cyttarocylididae, Ascampbelliellidae, Rhabdonellidae, Epiplocylididae, and Ptychocylididae, even if they form a highly supported clade in our trees (Fig. 2, 3) and some of their representatives are known to share either the lorica texture (the three later; Agatha and Strüder-Kypke, 2014) or the extrusome type (the first and third; Laval-Peuto and Barría de Cao, 1987).

The family Metacylididae is no longer supported, as noted before (Bachy et al., 2012). *Metacylis* and *Pseudometacylis* are here transferred to Rhabdonellidae, given the phylogenetic position of the former (the second remains unsequenced; Fig. 2, S3B) and shared lorica texture of all of them (hyaline, monolaminar with alveoli, low surface ridges, and pores; Agatha and Strüder-Kypke, 2012; Balech, 1968; Lackey and Balech, 1966). Other former Metacylididae, *Climacocylis* and *Helicostomella*, share a similar lorica texture (Agatha and Strüder-Kypke, 2014), but they are phylogenetically distant, and instead related to *Tintinnopsis*-like species (Fig. 2, 3). Also related to *Tintinnopsis* are *Stylicauda*, *Rhizodomus* and *Leprotintinnus*, the later no longer supported in Tintinnidiidae due to both phylogenetic distance and unclear morphological affinity (Zhang et al., 2016). The later six genera are *incertae sedis* in Tintinnida.

3.2.1. Family Favellidae Kofoid and Campbell, 1929 amended

Diagnosis: Two loricae types, protolorica and paralorica. Protolorica frequently with an annulated or spiralled epilorica and a posterior process; paralorica spiralled, usually lacking a posterior process. Lorica wall monolaminar with alveoli and smooth surface. Ciliary pattern characterized by two dorsal kineties, a monokinetidal ventral kinety, and lateral, right, and left ciliary fields. One genus: *Favella*.

3.3. Informal classification of incertae sedis*:* Tintinnopsis *and related genera*

The taxonomy of *Tintinnopsis* has always been problematic. Because its lorica is densely agglomerated with particles, most diagnostic characters are difficult to study. There is a long history of species splits and unifications (e.g., Bakker and Phaff, 1976), and it has even been considered a "complex" instead of a genus (Alder, 1999). DNA sequencing has revealed that *Tintinnopsis*-like species may actually belong to several genera and families, but a taxonomic revision is currently impossible because most of the about 160 described species still need reexamination with modern methods, including the type *T. beroidea* (Agatha, 2010). The more species are sequenced, the more widespread they are in phylogenetic trees. This has led to attempts to name clades informally (Agatha and Strüder-Kypke, 2014; Bachy et al., 2012; Zhang et al., 2016). However, these names are inconsistent in the literature and have other limitations in their utility (Supplementary Table S6). For example, such names have not considered that some stable, well-supported clades include not only *Tintinnopsis*-like species, but also other *incertae sedis* taxa with sparsely-agglomerated (*Leprotintinnus, Rhizodomus, Stylicauda*) or particle-free (*Climacocylis*, *Helicostomella*) loricae. For some of these taxa, lorica similarities in particle-free cultures (Fig. S2B) and strong phylogenetic bonds (Santoferrara et al., 2015) suggest that a common affiliation may be reached once data on the lorica matrix and cytology allow for a formal classification.

Taxa such as *Tintinnopsis* and *Helicostomella* are widely distributed and sometimes very abundant in coastal plankton (e.g., Dolan and Pierce, 2013; Santoferrara and Alder, 2009). Thus, finding a stable way to catalog and link them is important not only for phylogenetic studies, but also for ecological surveys, that are increasingly being based on environmental sequencing. Relevant patterns may now remain unrealized just because sequences are difficult to link to distinct clades. Here we suggest an informal system to name unclassified tintinnid taxa (Fig. 3), which has similarities, for example, to recent (but differently aimed) proposals for sequences of foraminifera (Morard et al., 2016) and

eukaryotes in general (eukref.org). Eleven clades and isolated branches including *Tintinnopsis* and related genera are enumerated consecutively with a single Arabic number. As more sequences are added in the tree, potentially split clades that include a representative sequence (GenBank accessions in bold in Fig. 3) should retain their number, while new clades should take the next available number. On the other hand, as clades merge or are formally classified, their numbers should become unavailable.

3.4. Unknown lineages in Choreotrichia and Oligotrichia

Choreotrichia and Oligotrichia have a long tradition of morphological description, but it is possible that emerging molecular data reveal new taxa. Analysis of all the SSU rDNA sequences available in NCBI GenBank (known morphospecies and environmental sequences mostly from clone libraries) suggests a high potential for uncharacterized or novel taxa in these subclasses, although the trends are opposite for loricates and aloricates (Fig. 4). Most tintinnid sequences represent morphologically-identified taxa, while most choreotrichid and oligotrich sequences derive from environmental surveys (Fig. 4A). Furthermore, for choreotrichids and oligotrichs, there are environmental clades that are as divergent as the known families, although most of former have low support in our analyses. At least some of these environmental clades could represent known lineages not sequenced yet, while the others may represent novel families and genera completely unknown from the morphological point of view.

Two conspicuous branching patterns are evident in our trees (Fig. 4A). Cyrtostrombidiidae and Lynnellidae form isolated branches. One possible explanation for this pattern is that primers used in environmental surveys do not capture the real diversity within these taxa; if so, many other novel clades in the same situation may remain undiscovered. Alternatively, these taxa may exemplify heterogeneous levels of SSU rDNA divergence, or dissimilar rates of diversification among families, possibly derived from differences in geographical distributions, ecological niches or other factors (Vamosi et al., 2009; Pyron and Burbrink, 2013). In contrast to these "lonely" taxa, most other clades include a variable number of sequences, with a maximum for Strobilidiidae and the non-monophyletic Strombidiidae, followed by Tontoniidae and Leegaardiellidae (Fig. 4A). Of them, only Strombidiidae is known to be much diversified (12 genera, >90 species; Agatha, 2011; Table 1) and to include cryptic species (Katz et al., 2005; McManus et al., 2010). Our results suggest a strong underestimation of taxonomic diversity and a high degree of crypticity also for Tontoniidae, Strobilidiidae and Leegaardiellidae.

The proportion of described species versus DNA sequences confirms the underrepresentation of choreotrichids and oligotrichs as well as the overrepresentation of tintinnids in global species inventories

(Fig. 4B). About 86% of described species correspond to tintinnids, while 14% belong to choreotrichids and oligotrichs combined (Agatha and Strüder-Kypke, 2014). On the other hand, SSU rDNA sequences (this study) suggest that oligotrichs are the most diversified (61%), followed by choreotrichids (25%), and lastly by tintinnids (14%). Although these results support that a high number of synonyms exist among tintinnid morphospecies (Alder, 1999; Dolan, 2016), this situation should not be oversimplified. Examples of either undistinguishable or distinct morphospecies with identical SSU rDNA that consistently differ in more variable, species-level markers (ITS regions and/ or LSU rDNA), and in some cases even ecologically, have been reported (Xu et al., 2012; Santoferrara et al., 2013, 2015; this study). In other words, the conserved nature of SSU rDNA and our incomplete knowledge on intra- and interspecific sequence similarity (or the lack of a universal clustering cutoff equivalent to species) prevent an ultimate estimation of global species richness using only molecular data. Integration of multi-gene, morphological and eco-physiological data is needed to fully characterize ciliate diversity (Agatha, 2011; Santoferrara et al., 2016b).

Because of our curation strategy, we analyzed only sequences longer than 500 bp (see 2.3). However, the current use of environmental high-throughput sequencing (HTS) has produced a massive amount of shorter sequences, which further suggest hidden diversity in ciliates (e.g., Forster et al., 2015; Gimmler et al., 2016). For now, most of this diversity remain morphologically and functionally uncharacterized. Here, single-cell sequencing coupled with morphological identification allows us to link a previously unidentified environmental clade to a known family. We first detected a clade ("cluster X") by HTS and hypothesized that it could correspond to a choreotrichid family not sequenced before (Santoferrara et al., 2014). Although related environmental sequences were found by diverse molecular methods (e.g., Grattepanche et al., 2016; Lie et al., 2014), their taxonomic identity remained a mystery. We now confirm an affiliation to Leegaardiellidae, given the close relationship of these environmental sequences with our novel sequence for this family (Fig. 1A-C; Fig. S6).

4. Conclusions

We have expanded the phylogenetic inferences based on sequences of three rDNA loci for Choreotrichia and Oligotrichia, including two families and six genera never sequenced before. In total, we analyzed 18 families in a multi-gene phylogenetic context, not including those that lack reliable sequences for at least one locus (Cyrtostrombidiidae, Pelagostrombidiidae and Undellidae) or for the three of them (Lohmanniellidae and Nolaclusiliidae). Based on careful comparison of our molecular results with

available information on cytological and ultrastructural characters, we re-established the family Favellidae and updated the classification of these subclasses into 23 total families. Eleven clades that remain *incertae sedis* in Tintinnida as well as most families in Choreotrichida and Oligotrichia need additional studies to clarify their taxonomy and evolutionary relationships. Furthermore, entire genera and families remain undescribed among Choreotrichida and Oligotrichia, as suggested by the analysis of all the unidentified, environmental sequences available in GenBank. This analysis provides insights into the environmental diversity of these groups that were not obvious in the individual sequencing efforts. These data also support the fact that aloricates include a high proportion of cryptic species, while loricates include many synonyms.

As more and more environmental sequences are generated, solid references are needed to link these data to the known taxa and to identify hotspots of novel diversity. We used single-cell sequencing to link morphological and molecular data, including in a previously unidentified environmental clade here revealed as Leegaardiellidae. Additionally, we curated almost 4,000 sequences from GenBank, which showed problems in both identified sequences (e.g., misidentifications, insufficient or nonexistent published data to confirm identifications, documentation of specimens that cannot be confirmed as the sequenced ones, inconsistent labeling) and environmental sequences (e.g., chimeras and other methodological artifacts). Another alarming issue is the lack of metadata associated with environmental sequences. For example, most choreotrich oligotrich records lack geographical coordinates, thus limiting studies of spatial distribution. This is particularly important in the current context of climate change that affects, for example, population dynamics and species distribution ranges (Pfenninger et al., 2012; Hofer, 2016). In this context, caution is needed in both the use and contribution to public repositories, given that they are unique resources for evolutionary and diversity studies.

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Appendix A. Supplementary Material

Supplementary data associated with this article can be found in the online version at XXXX.

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587

Table 1. Updated

Choreotrichia Small and Lynn, 1985 (2 orders)
Choreotrichida Small and Lynn, 1985 (5 families)
Leegaardiellidae Lynn and Montagnes, 1988 (1 genus)
Leegaardiella Lynn and Montagnes, 1988
Lohmanniellidae Montagnes and Lynn, 1991 (1 genus)
Lohmanniella Leegaard, 1915
Lynnellidae Liu et al., 2011 (1 genus)
Lynnella Liu et al., 2011
Strobilidiidae Kahl in Doflein and Reichenow, 1929 (3 genera)
Pelagostrobilidium Petz, Song and Wilbert, 1995
Rimostrombidium Jankowski, 1978
Strobilidium Schewiakoff, 1892
Strombidinopsidae Small and Lynn, 1985 (3 genera)
Parastrombidinopsis Kim et al., 2005
Parastrombidium Fauré-Fremiet, 1924
Strombidinopsis Kent, 1881

Tintinnida Kofoid and Campbell, 1929 (14 families)
Ascampbelliellidae Corliss, 1960 (4 genera)
Acanthostomella Jörgensen, 1927
Ascampbelliella Corliss, 1960
Incertae sedis: Luxiella Lecal, 1953
Niemarshallia Corliss, 1960
Cyttarocylididae Kofoid and Campbell, 1929 (2 genera)
Cyttarocylis Fol, 1881
Petalotricha Kent, 1881
Dictyocystidae Haeckel, 1873 (6 genera)
Codonaria Kofoid and Campbell, 1929
Codonella Haeckel, 1873
Codonellopsis Jörgensen, 1924
Dictyocysta Ehrenberg, 1854
Incertae sedis: Laackmanniella Kofoid and Campbell, 1929
Wangiella Nie, 1934
Epiplocylididae Kofoid and Campbell, 1929 (3 genera)
Epicancella Kofoid and Campbell, 1929
Epiplocylis Jörgensen, 1924
Epiplocyloides Hada, 1938
Eutintinnidae Bachy et al., 2012 (1 genus)
Eutintinnus Kofoid and Campbell, 1939
Favellidae Kofoid and Campbell, 1929 (1 genus)
Favella Jörgensen, 1924
Nolaclusiliidae Sniezek et al., 1991 (1 genus)
Nolaclusilis Snyder and Brownlee, 1991
Ptychocylididae Kofoid and Campbell, 1929 (4 genera)
Cymatocylis Laackmann, 1910
Protocymatocylis Kofoid and Campbell, 1929
Ptychocylis Brandt, 1896
Wailesia Kofoid and Campbell, 1939
Rhabdonellidae Kofoid and Campbell, 1929 (7 genera)
Epirhabdonella Kofoid and Campbell, 1939
Metacylis Jörgensen, 1924
Pseudometacylis Balech, 1968
Protorhabdonella Jörgensen, 1924
Rhabdonella Brandt, 1906
Rhabdonellopsis Kofoid and Campbell, 1929
Schmidingerella Agatha and Strüder-Kypke, 2012
Stenosemellidae Campbell, 1954 (1 genus)
Stenosemella Jörgensen, 1924
Tintinnidae Claparède and Lachmann, 1858 (21 genera)
Albatrossiella Kofoid and Campbell, 1929
Amphorellopsis Kofoid and Campbell, 1929
Amphorides Strand, 1928
Brandtiella Kofoid and Campbell, 1929
Bursaopsis Kofoid and Campbell, 1929
Buschiella Corliss, 1960
Cantharіella Kofoid and Campbell, 1929
Clevea Balech, 1948
Daturella Kofoid and Campbell, 1929
(continued)
Epicranella Kofoid and Campbell, 1929
Odontophorella Kofoid and Campbell, 1929
Ormosella Kofoid and Campbell, 1929
Proamphorella Kofoid and Campbell, 1939
Prostelidiella Kofoid and Campbell, 1939
Rhabdosella Kofoid and Campbell, 1929
Salpingacantha Kofoid and Campbell, 1929
Salpingella Jörgensen, 1924
Salpingelloides Campbell, 1942
Steenstrupiella Kofoid and Campbell, 1929
Stelidiella Kofoid and Campbell, 1929
Tintinnus Schrank, 1803
Tintinnidiidae Kofoid and Campbell, 1929 (2 genera)
Membranicola Foissner, Berger and Schaumburg, 1999
Tintinnidium Kent, 1881
Undellidae Kofoid and Campbell, 1929 (7 genera)
Amplectella Kofoid and Campbell, 1929
Amplectellopsis Kofoid and Campbell, 1929
Cricundella Kofoid and Campbell, 1929
Parundella Jörgensen, 1924
Proplectella Kofoid and Campbell, 1929
Undella Daday, 1887
Undellopsis Kofoid and Campbell, 1929
Xystonellidae Kofoid and Campbell, 1929
Parafavella Kofoid and Campbell, 1929
Spiroxystonella Kofoid and Campbell, 1939
Xystonella Brandt, 1906
Xystonellopsis Jörgensen, 1924
Incertae sedis: Dadayiella Kofoid and Campbell, 1929
Incertae sedis in Tintinnida:
Codonopsis Kofoid and Campbell, 1939
Poroecus Cleve, 1902
Climacocylis Jörgensen, 1924
Helicostomella Jörgensen, 1924
Leprotintinnus Jörgensen, 1900
Rhizodomus Strelkow and Wirketis, 1950
Rotundocylis Kufferath, 1950
Stylicauda Balech, 1951
Tintinnopsis Stein, 1867
Nomen inquirendum: Coxliella Brandt, 1906

Oligotrichia Bütschli, 1887/1889 (1 order)
Strombidiida Petz and Foissner, 1992 (4 families)
Cyrtostrombidiidae Agatha, 2004 (1 genus)
Cyrtostrombidium Lynn and Gilron, 1993
Pelagostrombidiidae Agatha, 2004 (2 genera)
Limnostrombidium Krainer, 1995
Pelagostrombidium Krainer, 1991
Strombidiidae Fauré-Fremiet, 1970 (12 genera)
Antestrombidium Liu et al., 2015
Apostrombidium Xu, Warren and Song, 2009
Foissneridium Agatha, 2010
Novistrombidium Song and Bradbury, 1998
Omegastrombidium Agatha, 2004
Opisthostrombidium Agatha, 2010
Parallelostrombidium Agatha, 2004
Sinistrostrombidium Liu et al., 2015
Spirostrombidium Jankowski, 1978
Strombidium Claparède and Lachmann, 1859
Varistrombidium Xu, Warren and Song, 2009
Williophrya Liu et al., 2011
Tontoniidae Agatha, 2004 (5 genera)
Laboea Lohmann, 1908
Paratontonia Jankowski, 1978
Pseudotontonia Agatha, 2004
Spirotontonia Agatha, 2004
Tontonia Fauré-Fremiet, 1914

634 classification of the subclasses Choreotrichia and Oligotrichia. Details in Table S5.

Figure legends

Fig. 1. Examples of specimens sequenced in this study. A to C, the choreotrichid *Leegaardiella* sp. As in most Choreotrichia, the adoral zone of membranelles consists of (A) a closed circle of collar membranelles (bipartite in this genus: they consist of an outer and an inner portion with long and short membranelles, OCM and ICM, respectively) and (B) buccal membranelles (BM). The somatic ciliature is reduced, as revealed by protargol impregnation (sequential planes in C); there are four short somatic kineties (SK) consisting of dikinetids in the posterior part of the cell. D to J, the tintinnids *Cyttarocylis acutiformis*, *Petalotricha ampulla*, *Epiplocylis undella*, *Ptychocylis minor*, *Salpingacantha undata*, *Parundella aculeata* and *Parafavella parumdentata*, respectively. Species identification is based on the lorica. K, the oligotrich *Laboea strobila*. Although not easy to see in fixed material, the adoral zone of membranelles is C-shaped; the somatic ciliature includes a spiraled girdle kinety that confers this species a screw-like shape. See additional sequenced specimens and detailed descriptions in the Supplementary Material. Isolate number is shown. Scale = 20 μm. We sequenced all species for the first time for at least one marker, except *L. strobila*.

Fig. 2. Phylogenetic tree inferred from concatenated SSU rDNA, ITS regions and LSU rDNA sequences. RAxML bootstrap support and MrBayes posterior probability values are shown (only if >45% and >0.90, respectively). A black circle indicates full support in both analyses. Species in bold were sequenced in this study. GenBank accession numbers are shown in Supplementary Table S4. Families (colors) and Tintinnida *incertae sedis* (grey) as in Table 1.

Fig. 3. Phylogenetic tree inferred from SSU rDNA sequences. RAxML bootstrap support and MrBayes posterior probability values are shown (only if >45% and >0.90, respectively). A black circle indicates full support in both analyses. A star indicates non-monophyly. Families are collapsed (expanded version in Fig. S3). Tintinnida *incertae sedis* are expanded and enumerated by clade or isolated branch; for each of them, one sequence (in bold) is selected as representative (the most basal, reliable or distinctive one).

Fig. 4. The knowns and unknowns in Choreotrichia and Oligotrichia. A, SSU rDNA clusters (97% similarity) including morphologically-identified (the knowns, in grey) or only environmental (the unknowns, in pink) sequences from NCBI GenBank. Several clades or isolated branches may represent novel taxa (?); a star indicates non-monophyly. B, proportion of SSU rDNA clusters (99% similarity) and described species by order.

Supplementary Material

Phylogeny, classification and diversity of Choreotrichia and Oligotrichia (Ciliophora, Spirotrichea)

Luciana F. Santoferrara, Viviana V. Alder, George B. McManus

Index

Supplementary Text 1. Identification of sequenced specimens

We sequenced the SSU rDNA, ITS regions and/ or LSU rDNA for twenty-one species newly collected in summer 2015 and seven species sampled in previous studies (Fig. 1, Fig. S1, Suplementary Table S1). The newly collected specimens were sampled in shelf and oceanic waters of the Northeast Atlantic on board the R.V. Connecticut, except *Metacylis angulata* that was collected from the UConn dock in the shore of Connecticut, USA. All species were sequenced for the first time for at least one marker, except *Laboea strobila* (sequenced before for the three markers). Two families (Leegaardiellidae and Ascampbelliellidae), six genera (*Leegaardiella*, *Ascampbelliella*, *Salpingacantha*, *Ptychocylis, Parafavella* and *Parundella*) and twelve species (in bold in Suplementary Table S1) had not been sequenced before for any marker.

Our sampling was based on the fact that offshore species have been less frequently sequenced, and thus underrepresented in phylogenetic inferences, compared to species collected in shoreline locations. As most of these species were collected during an oceanographic cruise, which complicates even more the inherent difficulty in culturing choreotrichs and oligotrichs and getting enough material to examine, the study was based on single cells picked from samples preserved with non-acid Lugol's solution (4% final concentration). In each case, a single specimen was studied in the microscope (400-600X total magnification), photographed and sequenced. All morphological identifications were supported by molecular results (position in phylogenetic trees and BLAST comparison against identified sequences in GenBank). For *Leegaardiella*, that belongs to a family never sever sequenced before, additional single cells were picked for protargol impregnation (Wilbert, 1975); because it is impossible to impregnate and sequence a same specimen, we were very careful in staining cells from the same sample and as similar as possible to the sequenced one. Given the mentioned limitations, most of our new sequences correspond to tintinnids; the few choreotrichids and oligotrichs that we studied were generally identified above the species level, but we consider this information valuable as it gives us the opportunity to represent most families of both subclasses in phylogenies based on concatenated sequences.

Below, we include the morphological description of the specimens sequenced. Classification is based on Table 1. Each sequenced specimen is shown in Fig. 1 and Fig. S1, and their measures are provided in Table S1. For tintinnids, additional measurements are provided in the text (average ± standard deviation; number of specimens = n). We also include the bibliography used for identification and discussion of taxonomic aspects. The obtained sequences are compared with previous ones, if available. When applicable, we explain the decisions about sequences included or excluded from the final alignments used to build our phylogenetic trees (see also Supplementary Text 3).

Subclass Choreotrichia Small & Lynn, 1985

Order Choreotrichida Small & Lynn, 1985

Family Leegaardiellidae Lynn & Montagnes, 1988

***Leegaardiella* sp. (Fig. 1A-C)**

Conical cell. Apical region with a complete circle of 16-20 collar membranelles separated in inner and outer portions; 6 short buccal membranelles clearly separated from the collar membranelles. Protargol impregnation revealed two ovoid macronuclei and four somatic kineties in the posterior end of the cell, each composed of 7 to 12 dikinetids, apparently not covered by a cytoplasmic flap. The specimens present the diagnostic characters that distinguish Leegaardiellidae from other Choreotrichida (Lynn and Montagnes, 1988).

An identified specimen of this family is sequenced here for the first time. However, its sequence matches a previously unidentified environmental clade that was informally labeled as Cluster X (Santoferrara et al., 2014; Grattepanche et al., 2016).

Family Strobilidiidae Kahl in Doflein & Reichenow, 1929

***Pelagostrobilidium* sp. (Fig. S1A)**

Globular cell with a complete circle of collar membranelles in the apical region, consistent with the family. Since the genera of this family are differentiated by their somatic kineties (Petz et al., 1995), which are not visible in Lugol's fixed material, genus identification was based on DNA sequencing.

Our sequence forms a highly-supported, monophyletic clade with all the other *Pelagostrobilidium* species sequenced so far (Fig. 2, S3, S4, S5). The closest match according to BLAST is *P. neptuni* (Montagnes & Taylor, 1994) Petz, Song & Wilbert, 1995, with a 94% similarity in SSU rDNA (AY541683; Agatha et al., 2005). For ITS regions and LSU rDNA, the closest match is *P. minutum* Liu et al., 2012, with 86-87% similarity (KM222055, KM222149; Gao et al. 2016a).

Order Tintinnida Kofoid & Campbell, 1929

Family Ascampbelliellidae Corliss, 1960

***Ascampbelliella acuta* (Kofoid & Campbell, 1929) Corliss, 1960 (Fig. S1B)**

Lorica cup-shaped, with an erect inner collar (about 5 µm tall) and a flaring outer rim. Aboral end slightly pointed. Length = 38.7±1.9 µm, oral diameter = 30.6±0.6 µm, outer rim diameter = 38.3±1.2 µm, n = 8. Slightly smaller than original description (length 43 µm, oral diameter 33 µm; Kofoid and

Campbell, 1929). This family is sequenced for the first time.

Family Cyttarocylididae Kofoid & Campbell, 1929

***Cyttarocylis acutiformis* Kofoid & Campbell, 1929 (Fig. 1D)**

Lorica conical with a flaring collar separated by a nuchal constriction. Oral rim with very small denticulation. Aboral end with a minute horn. Wall with clearly-visible alveoli. Only one specimen observed and measured (Table S1). Agrees with original description (Kofoid and Campbell, 1929).

Our specimen from the NW Atlantic is identical in SSU rDNA to *C. acutiformis* isolate FG873 [JQ408203, labeled as *C. cassis* (Haeckel, 1873) Fol, 1881 in GenBank] and *C. cassis* isolate FG302 [JQ408186, labeled as *C. eucecryphalus* (Haeckel, 1887) Kofoid, 1812 in GenBank], and in both SSU rDNA and ITS regions to *C. eucecryphalus* isolate CB836 (JQ408169), all of them from the Mediterranean (Bachy et al., 2012). Isolates CB873 and FG302 were later re-identified as *C. cassis* and *C. brandti* Kofoid & Campbell, 1929, respectively, by the same authors (Dolan et al., 2014). Because they are identical to our sequences (also in ITS regions labeled as *Petalotricha ampulla*, JQ408165, JQ408168, Bachy et al., 2012), these sequences are excluded from our final alignments.

***Petalotricha ampulla* (Fol, 1881) Kent, 1882 (Fig. 1E)**

Bowl-shaped lorica with a flaring collar separated by a nuchal constriction. The collar is divided in two parts, a conical base and a flaring lip. Aboral end slightly pointed. The characteristic fenestrae in the aboral part of the lorica were barely visible in our specimen, probably masked by the big cell inside. Only one specimen observed and measured (Table S1), with size moderately larger than in the original description (87 µm long, 81 µm in oral diameter; Fol, 1881).

Our specimen from the NW Atlantic is identical in SSU rDNA and ITS regions to specimens from the Mediterranean (isolates CB837, FG301 corresponding to JQ408168, JQ408185; isolate FG1399 corresponding to JQ408165 has one substitution; all of them labeled as *Cyttarocylis ampulla* in GenBank), although the later are even bigger than in the original description (135 µm long, 125 µm in oral diameter; Bachy et al., 2012). As re-identified by the same authors (Dolan et al., 2014), isolates CB837 and FG1399 match better the original description of *P. major* Jörgensen, 1924 (114 µm long, 128 µm in oral diameter; Jörgensen, 1924). Because they are identical to our sequences, these three sequences are excluded from our final alignments.

Based on identical or almost identical sequences of SSU rDNA and ITS regions for the sequenced *Petalotricha* and *Cyttarocylis* species, including the respective types *P. ampulla* and *C. cassis*, Bachy et al. (2012) proposed the new combination *Cyttarocylis ampulla* (Kent, 1882) Bachy, Dolan & López-García, 2012, despite their markedly different loricae (oral diameter, shape and wall structure; see above, Fig. 1). We confirmed the identity in such markers, but we found a 1.8% difference in LSU rDNA (11 out of 607 total nucleotides; as part of our routinely quality control, all polymorphic sites were confirmed by manual inspection of chromatograms obtained for each species in the forward and reverse direction), which is clearly consistent with different species and even different genera (Santoferrara et al., 2013, 2015). Thus, we reject the synonymization of *Cyttarocylis* and *Petalotricha* until more detailed morphological studies are done. Although we agree in that Petalotrichidae Kofoid & Campbell, 1929 is not valid, we reject the transference of the genera *Metacylis* and *Rhabdonella* to Cyttarocylididae, which was proposed on the basis of SSU rDNA sequences, but with no morphological support (Bachy et al., 2012).

Family Dictyocystidae Haeckel, 1873

***Dictyocysta elegans* Ehrenberg, 1854 (Fig. S1C)**

Lorica with a cylindrical collar almost 30 μm long, formed by two rows of quadrangular fenestrae. Bowl conical to rounded, with more irregular fenestrae. One specimen observed and measured (Table S1). In agreement with original description, as illustrated by Brandt (1906, 1907).

This specimen from the NW Atlantic has SSU rDNA sequence identical or almost identical (one or two substitutions) compared to *D. elegans* and *D. lepida* Ehrenberg, 1854 from the same area (KT792928-9, Santoferrara et al., 2016a), *D. lepida* from the Mediterranean (JQ408188; Bachy et al. 2012) and *D. reticulata* Kofoid & Campbell, 1929 from off Florida, NW Atlantic (EU399532, Strüder-Kypke and Lynn, 2008). They all have similar size and shape, except that *E. elegans* has two rows of fenestrae. Although some of them may be synonyms, one sequence per species was kept in our final alignment.

Within the family Dictyocystidae, *Codonaria* Kofoid & Campbell, 1929, *Codonella* Haeckel, 1873, *Codonellopsis* Jörgensen, 1924 and *Dyctiocysta* Ehrenberg, 1854 share as synaporphies both a lorica sac (Agatha, 2010b) and the type of capsules (or extrusomes; Laval-Peuto and Barria de Cao, 1987). Actually, this motivated uniting these genera (including also *Laackmanniella* Kofoid & Campbell, 1929 and *Wangiella* Nie, 1934 as *incertae sedis*) into Dictyocystidae, while Codonellidae Kent, 1881 and Codonellopsidae Kofoid & Campbell, 1929 were eliminated (Agatha and Strüder-Kypke, 2012). The

capsule type, but not the lorica sac, are shared by *Stenosemella*, which used to be placed in Codonellopsidae (Lynn, 2008), but then left as the only genus of Stenosemellidae Cambell, 1954 (a subfamily raised to family by Agatha and Strüder-Kypke, 2012). Both Dictyocystidae and Stenosemellidae present species with an agglomerated bowl and a hyaline collar, but it seems that the collar is compact in *Stenosemella* while alveolar in *Codonellopsis* and some *Dictyocysta* (Agatha and Strüder-Kypke, 2014). This feature may have been overlooked in sequenced specimens identified as *Stenosemella*, which may partially explain the non-monophyly of *Stenosemella* and its clustering with the Dictyocystidae in molecular phylogenies (Fig. S3, S4). More studies are required to confirm this affiliation, and thus Stenosemellidae is conservatively kept. The previous discussion proves invalid the proposal of Bachy et al. (2012) of keeping Codonellopsidae (including *Codonellopsis, Stenosemella*) and Codonellidae (including *Codonaria, Codonella,* and *Dictyocysta*; incorrectly given priority over the older family Dictyocystidae).

Family Epiplocylididae Kofoid & Campbell, 1939

***Epiplocylis undella* (Ostenfeld & Schmidt 1901) Jörgensen, 1924 (Fig. 1F)**

Cup-shaped lorica, with a pointed pedicel. Oral rim simple. Thick wall with deep reticulations in the aboral region. Size (Table S1) and shape match perfectly the original description (Ostenfeld and Schmidt, 1901).

In the SSU rDNA tree (Fig. S3), our sequence clusters with *Epiplocyloides ralumensis* (Brandt, 1906) Hada, 1938 (JX101854; Xu et al., 2013; BLAST match 98%), which shows the suboral bulge that characterizes this genus (Hada, 1938). A sequence labeled as *Epiplocylis acuminata* (Daday, 1887) Jörgensen, 1924 is not associated to published morphology, is only 1248 bp long, it branches apart from *E. undella* and *E. ralumensis* in our preliminary trees and it has only 95% BLAST match to the former (JQ715615; Bachy et al., 2013), so it is not included in our final alignment.

Family Eutintinnidae Bachy et al., 2012

***Eutintinnus medius* (Kofoid & Campbell, 1929) Kofoid & Campbell, 1939 (Fig. S1D)**

Lorica with two openings, with an everted oral end and a straight aboral end. Length = 239.4±17.4 μm, oral diameter = 48.1±1.4 μm, aboral diameter = 32.5±3.6 μm, n = 5. Shape and size match the original description (length 192-254 μm, oral diameter 44-58 μm; Kofoid and Campbell, 1929). Lorica size

matches specimens found in the same area, with only one base substitution in the SSU rDNA sequence (KT792925; Santoferrara et al., 2016a).

***Eutintinnus perminutus* (Kofoid & Campbell, 1929) Kofoid & Campbell, 1939 (Fig. S1E)**

Lorica with two openings, with a "brim" at the oral end but not at the aboral end. Length = 144.3±5.7 μm, oral diameter = 32.5±0.4 μm, outer rim diameter = 23.3±0.7 μm, n = 8. Although the shape and oral diameter agree with the original description, the later mentions longer loricae (140-183 μm; Kofoid and Campbell, 1929). Lorica size matches specimens found in the same area, with identical SSU rDNA sequence (KT792926; Santoferrara et al., 2016a).

Family Ptychocylididae Kofoid & Campbell, 1929

***Ptychocylis minor* Jörgensen, 1899 (Fig. 1G)**

Lorica with the shape of an inverted bell, with two marked suboral bulges (the first right below the oral rim, and the second above the middle of the lorica) and a less marked one (below the middle of the lorica). Wall alveolar, with ridges on the surface. Oral rim denticulate. Aboral end with a pointed pedicel about 50 μm long. Length = 145.6±3.9 μm, oral diameter = 71.4±1.3 μm, maximum diameter = 82.2±3.1 μm, n = 4. The original description differs in shorter loricae (92-114 μm long; Jörgensen, 1899).

The genus *Ptychocylis* is sequenced for the first time. In our phylogenetic trees, it clusters with *Cymatocylis* Laackmann, 1910, but not with the distant *Favella* Jörgensen, 1924 (Fig. 2, 3, S3, S4). We propose to transfer *Favella* to the family Favellidae Kofoid & Campbell, 1929. The subfamily Favellineae was created by Kofoid and Campbell (1929), then raised to the family Favellidae by Campbell (1942), including *Favella*, *Cymatocylis*, *Protocymatocylis* Kofoid and Campbell, 1929 and *Poroecus* Cleve, 1902, while leaving *Ptychocylis* as the only genus in Ptychocylididae. Although Campbell (1954) reduced Favellidae again by transferring its members to Ptychocylididae, some authors have posteriorly acknowledged both families (e.g., Marshall, 1969). Other previous schemes (Lynn, 2008; Agatha and Strüder-Kypke, 2013) only recognize Ptychocylididae, including *Cymatocylis*, *Favella*, *Protocymatocylis, Ptychocylis* and *Wailesia* Kofoid & Campbell, 1939.

Our proposal has not only molecular, but also morphological support. In phylogenetic trees, Favellidae (including only the genus *Favella*) is basal to most Tintinnida families, except Tintinnidiidae, Tintinnidae and Eutintinnidae (Fig. 2, 3, S3, S4). The lorica wall is monolaminar with alveoli and outer

surface smooth, while the ciliary pattern is characterized by two dorsal kineties, based on the recent redescriptions of *F. panamensis* and *F. ehrenbergii* (Agatha and Strüder-Kypke, 2012; Kim et al., 2010).

The Ptychocylididae genera sequenced so far, *Ptychocylis* and *Cymatocylis*, are more derived and branch together with high support in the SSU rDNA tree and moderate support in the concatenate analysis (Fig. 2, S3). *Cymatocylis* has the most complex ciliary pattern, with one dorsal kinety (Kim et al., 2013). Also, in *Cymatocylis* the lorica wall is monolaminar with alveoli, but it presents ridges, which are absent in *Favella* (Laackmann, 1910; Agatha and Strüder-Kypke, 2012, 2014). The ridges on the lorica wall are also observed in *Ptychocylis* (see above). The two remaining Ptychocylididae genera (*Protocymatocylis* and *Wailesia*), have not been sequenced but have ridges on the wall according to the illustrations in their original descriptions (Wailes, 1925; Kofoid and Campbell, 1929, 1939), thus also kept in this family.

Rhabdonellidae Kofoid & Campbell, 1929

***Metacylis angulata* Lackey & Balech, 1966 (Fig. S1F)**

Bowl-shaped lorica with a short collar. Rounded aboral end. Maximum diameter larger than length (Table S1). The only specimen observed was bigger than indicated in original description (length = 58-64 μm, oral diameter = 44.5-48.5 μm, maximum diameter = 64-70.5 μm; Lackey and Balech, 1966).

Our sequence from Long Island Sound, Connecticut is identical or almost identical to previous *M. angulata* sequences from the same area (SSU rDNA, AF399143-46 and ITS regions, AF399068-78; AF399145-6 are very divergent, and thus excluded; Snoeyenbons-West et al., 2002) and Buzzards Bay, Massachusetts (SSU rDNA, AY143568; Strüder-Kypke and Lynn, 2003), both locations relatively close to the type (Great South Bay, Long Island, New York). It is curious that this very distinct and relatively big species was only discovered in 1966 (Lackey and Balech, 1966), and, to the best of our knowledge, it has been reported only in coastal waters of north-east U.S. (Capriulo et al., 2002; Costas et al., 2007; Gold and Morales, 1975; Pierce, 1996; Pierce and Turner, 1994; Rosetta and McManus, 2003; Snoeyenbons-West et al., 2002; Strueder-Kypke and Lynn, 2003), thus being suspected as endemic (Pierce, 1996).

The *Metacylis* Jörgensen, 1924 sequences included in our alignments cluster within Rhabdonellidae (Fig. 2, S3, S4, S5), including *Rhabdonella* Brandt, 1906, *Protorhabdonella* Jörgensen, 1924 and *Schmidingerella* Agatha & Strüder-Kypke, 2012. *Metacylis* sp. (AY143567; Strüder-Kypke and Lynn, 2003) is excluded in our final alignment because its sequence lacks some of the *Metacylis* signatures and it is impossible to confirm identification in published pictures. *Metacylis joergenseni* (Cleve, 1902)

Kofoid & Campbell, 1929 (JQ408183; Bachy et al 2012) seems well identified, but its sequence has regions that are very divergent from other *Metacylis* and even other tintinnids; thus, this sequence is excluded as well. We include *Metacylis tropica* Duran, 1957 (KP883283; unpublished) and *Metacylis pithos* Skryabin & Al-Yamani, 2006, probably a synonym of *Metacylis oviformis* Nie & Cheng, 1947, (JX101862; Xu et al., 2013).

The family Metacylididae Kofoid & Campbell, 1929, which included *Metacylis* Jörgensen, 1924, *Climacocylis* Jörgensen, 1924, *Helicostomella* Jörgensen, 1924, and *Pseudometacylis* Balech, 1968, is invalid, as noted before (Bachy et al., 2012). Here, *Metacylis* is transferred to Rhabdonellidae Kofoid & Campbell, 1929. Metacylididae loricae were characterized by a spiraled collar and a non-spiraled bowl (except in *Climacocylis*), while Rhabdonellidae loricae do not present spirals, but vertical ribs (Kofoid and Campbell, 1929). Despite the differences in gross lorica morphology, *Metacylis* and Rhabdonellidae share a similar lorica ultrastructure (with alveoli, low surface ridges, and pores) at least based on *Metacylis angulata, Rhabdonella spiralis* (Fol, 1881) Brandt, 1906, and *Schmidingerella arcuata* (Brandt, 1906) Agatha & Strüder-Kypke, 2012 (Agatha and Strüder-Kypke, 2012; Lackey and Balech, 1966). *Pseudometacylis* has not been sequenced, but it would follow the placement of *Metacylis* given their similarity in wall structure (Balech, 1968). *Climacocylis* and *Helicostomella* remain as *insertae sedis* in Tintinnida (see below).

***Protorhabdonella simplex* (Cleve, 1900) Jörgensen, 1924 (Fig. S1G)**

Bullet-shaped lorica, with a slightly flaring oral end and pointed aboral end. About 6 vertical ribs. Length = 55.5±4.1 µm, oral diameter = 32.7±1.6 µm, n = 9. Slightly smaller than in original description (70 µm long, 35 µm in oral diameter; Cleve, 1900).

The SSU rDNA sequence of our NW Atlantic specimen has a 99% match against the smaller species *Protorhabdonella curta* (Cleve, 1900) Jörgensen, 1924 isolated from the East China Sea (JX101863; Xu et al., 2013).

Family Tintinnidae Claparède & Lachmann, 1958

***Amphorides minor* (Jörgensen, 1924) Strand, 1928 (Fig. S1H)**

Vase-shaped lorica, with flaring oral end and truncated aboral end. Aboral aperture of about 8 µm. Length = 93.7±5.3 µm, oral diameter = 35.9±1.0 µm, n = 5. Different from *Amphorides quadrilineata* (Claparède & Lachmann, 1958) Strand, 1928, which is bigger (Jörgensen, 1924; Kofoid and Campbell, 1929) and was also observed in our samples (length = 101.8 µm, oral diameter = 44.9 µm).

Our NW Atlantic specimen of *A. minor* matches in size and has only one mismatch in SSU rDNA compared to an isolate identified as *A. quadrilineata* from East China Sea (JX101850; Xu et al., 2013); our sequence has no mismatch in SSU rDNA and ITS regions to *A. quadrilineata* isolate FG618 from the Mediterranean (JQ408156; Bachy et al., 2012). We consider that all these sequences may actually belong to *A. minor*. Bachy et al. (2012) also identified as *A. quadrilineata* the isolates FG293, FG295, FG249 and FG1141, but they differ in published micrographs and SSU rDNA sequences between them and respect to the other isolates. We consider that isolates FG293 and FG295 may represent the actual *A. quadrilineata* based on published photomicrographs (JQ408184, JQ408189; Bachy et al., 2012). Finally, *Amphorides amphora* (Claparède & Lachmann, 1958) Strand, 1928, which is bigger than *A. quadrilineata*, was apparently well identified and sequenced by Xu et al. (2013; JX101849). The later sequence is identical to the ones from potentially misidentified *A. quadrilineata* isolates FG1141 and FG249 (JQ408193, JQ408176; Bachy et al., 2012), and *Steenstrupiella steenstrupii* (Claparède & Lachmann, 1858) Kofoid & Campbell, 1929 (EU399537; Strüder-Kypke and Lynn, 2008), again based on the published photomicrographs. *S. steenstrupii* was apparently well identified in isolates with identical sequences from the NW Atlantic and Mediterranean (KT792924, Santoferrara et al., 2016a; JQ408194, JQ408201, Bachy et al., 2012). The duplicated, potentially misidentified sequences mentioned in this paragraph were removed from our final alignments.

***Salpingacantha undata* (Jörgensen, 1899) Kofoid & Campbell, 1929 (Fig. 1H)**

Lorica tubular, elongated, with flaring oral end. Aboral fins. Aboral end with a terminal cylinder to which fins do not extend and ending in a small aperture (about 3 μm wide). Oral end forming three peaks, with a V-shaped canal between two of them. Alotugh the later feature distinguishes this genus from *Salpingella* Jörgensen, 1924, the peaks may not be visible when specimens are rotated (see detail in Fig. 1H). Length = 252.5±11.8 μm, oral diameter = 25.3±2.4 μm, n = 10. Our specimens match *S. undata*, except for the described length of 320-400 μm (Jörgensen, 1899; Kofoid and Campbell, 1929, 1939). For sequence comparison, see "*Salpingacantha unguiculata*".

***Salpingacantha unguiculata* (Brandt, 1906) Kofoid & Campbell, 1929 (Fig. S1I)**

Lorica tubular, elongated, with flaring oral end. Aboral fins. Aperture of about 2 μm in aboral end. Oral end forming three peaks. Length = 121.6±4.9 μm, oral diameter = 12.4±0.8 μm, n = 6. Size considerably smaller compared to the original descriptions of this species (length 230-290 μm, oral diameter 16-20 μm; Brandt 1906, 1907) or others in the genus (Jörgensen, 1899; Kofoid and Campbell, 1929; Laackmann, 1910). Later reports of this species have mentioned sizes more similar to our specimens

(e.g. as small as 130 μm in length and 11 μm in oral diameter; Marshall, 1969). We conservatively identify this species as the most similar one in the shape of the oral end.

The SSU rDNA sequences of *Salpingacantha undata* and *S. unguiculata* cluster with different sequences labeled as *Salpingella acuminata* (Claparède & Lachmann, 1858) Jörgensen, 1924 (Fig. S3). *S. acuminata* sampled off Florida, NW Atlantic has unreported morphology (EU399536; Strüder-Kypke and Lynn, 2008) and 22 substitutions compared to the apparently well identified *S. acuminata* from the Mediterranean (JQ408155; Bachy et al., 2012). The close genetic relationship, and the high similarity in the loricae of both genera, suggest that they are synonyms, as proposed before (Alder, 1999). Intriguingly, *Amphorellopsis quinquealata* (Laackmann, 1907) Balech, 1971 (JQ924059; Kim et al., 2013) is close to the former sequences (Fig. 2, S3), instead of clustering with *Amphorellopsis acuta* (Schmidt, 1902) Kofoid & Campbell, 1929 (JX101847, Xu et al., 2013) and *Amphorellopsis* sp. (KU715756-8, Zhang et al., 2016), which clusters with *Amphorides* Strand, 1928 and *Steenstrupiella* Kofoid & Campbell, 1929 (see above). Two sub-clades of Tintinnidae, potentially different families or subfamilies given their genetic distance (Fig. S3), are impossible to clarify for now due to the non-monophyly of *Amphorellopsis* Kofoid & Campbell, 1929.

Undellidae Kofoid & Campbell, 1929

***Parundella aculeata* Jörgensen, 1924 (Fig. 1I)**

Lorica conical, elongated, ending in a pedicel about 40 μm long. Oral rim simple, entire. Wall with distinct laminae, especially from the oral rim to the middle of the lorica. Length = 150.4±12.1 μm, oral diameter = 30.0±0.5 μm, n = 12. Matches original description (Jörgensen, 1924).

A SSU rDNA sequence labeled as *P. aculeata* was very likely misidentified (JQ408204; Bachy et al., 2012; excluded from our final alignment). Instead, the sequenced specimen very likely belongs to the genus *Dadayiella* Kofoid & Campbell, 1929, as noticed before (Agatha and Strüder-Kypke, 2014). Unfortunately, the high similarity between this misidentified sequence and sequences labeled as the type species of *Dadayiella, D. ganymedes* (Entz Sr., 1884) Kofoid & Campbell, 1929 (JX101852-3; Xu et al., 2013) was used as a basis to transfer the later to *Parundella* (Xu et al., 2013). Even more, *D. ganymedes* was also apparently misidentified by Xu et al. (2013), and the corresponding sequence would actually belong to *D. bulbosa* (Brandt, 1906) Kofoid & Campbell, 1929 (without vs. with knob at the pedicel; Entz 1884; Brandt 1906, 1907). However, the latter is almost identical (only one nucleotide substitution) to a partial sequence apparently well-identified as *D. ganymedes* (KT792930; Santoferrara et al., 2016a; although the specimen sequenced differs with the original description in having only an incipient

pedicel, other loricae in the same samples had a pedicel up to 15 μm long, and they did not match any other congener better; excluded from final alignment because it is only 800 nt long).

Having observed and sequenced *Parundella* and *Dadayiella* in this or previous studies (Santoferrara et al., 2016a), we confirm that both genera exist and that the name *Parundella ganymedes* (Entz, 1884) Xu et al., 2013 is invalid.

Also, both genera need family reassignments. Based on SSU rDNA (Fig. S3), *Parundella* belongs to Undellidae Kofoid & Campbell, 1929 and *Dadayiella* belongs to Xystonellidae Kofoid & Campbell, 1929. *Parundella* was first established as a subgenus of *Undella* Daday, 1887, with both taxa characterized, for example, by distinct wall laminae and an inconspicuous or simple wall structure (Jörgensen, 1924). Latter, Kofoid and Campbell (1929) assigned them to separate families, *Parundella* to Xystonellidae, and *Undella* to Undellidae. It is unclear why *Parundella* was assigned to Xystonellidae, and the authors even exclude this genus from the presence of secondary wall structure in the family diagnosis (Kofoid and Campbell, 1929). Instead, they state that *Parundella* differs from *Xystonella* and *Xystonellopsis* in having a primary wall structure and a simple oral rim (Kofoid and Campbell, 1929). A high affinity between *Parundella* and *Undella* was suggested by Alder (1999), who highlighted that both genera have hyaline loricae with conspicuous wall laminae. In fact, the lorica wall is trilaminar in both *Parundella* and *Undella*, while monolaminar in *Xystonella* (Marshall, 1969; Agatha and Strüder-Kypke, 2014). Thus, both SSU rDNA and lorica morphology support the transference of *Parundella* from Xystonellidae to Undellidae. According to SSU rDNA sequences, the Undellidae cluster seems to include also two freshwater, unidentified specimens that look like *Tintinnopsis* (JQ408177-8; Bachy et al., 2012). However, we excluded these sequences from our final alignment due to their short length (1,333 bp), that results in the lack of some signature regions.

In contrast, while the affiliation of *Dadayiella* within Xystonellidae is fully supported by SSU rDNA (Fig. S3 and shared signatures, e.g. nucleotides 573-580 shared only by *Dadayiella* and *Parafavella*), there are not obviuos similarities in the known morphological features. However, neither there is morphological support for *Dadayiella* being a Tintinnidae Claparède & Lachmann, 1958, as it shares almost no feature with the other genera of the family (Kofoid and Campbell, 1929). Alder (1999) even considered *Dadayiella* as an isolate genus within Tintinnida given its oral rim often crenulated and conspicuous facets in the collar region. Excluding this genus, the Tintinnidae form an almost fully supported monophyletic clade (Fig. 2, S3). We thus place *Dadayiella* as *incertae sedis* in Xystonellidae until detailed cytological and ultrastructural studies are performed.

Family Xystonellidae Kofoid & Campbell, 1929

***Parafavella parumdentata* (Brandt, 1906) Kofoid & Campbell, 1929 (Fig. 1J, S1J)**

Lorica conical with a slight suboral bulge. Oral rim denticulate, slightly flaring. Aboral end with pointed pedicel. Wall with polygonal structure. Length = 144.0 ±14.3 µm, oral diameter = 48.4±0.8 µm, maximum diameter = 50.3±0.8 µm, n = 6. In agreement with original description (Brandt, 1906, 1907). This genus is sequenced for the first time.

***Xystonella acus* (Brandt, 1906) Brandt, 1907 (Fig. S1K)**

Lorica conical. Oral rim channeled. Aboral end with a pedicel simple of about 20 µm. Wall with hexagonal reticulation. Length = 367.9±0.7 µm, internal oral diameter = 60.6±2.2 µm, external oral diameter = 74.9±0.3 µm, n = 2. Matches original description (Brandt, 1906, 1907).

Our NW Atlantic specimen has SSU rDNA sequence identical to *X. longicauda* (Brandt, 1906) Laackmann, 1910 from same area (KT792933; Santoferrara et al., 2016a) and from the Mediterranean (JQ408211; sequences JQ408160/96 have two to five differences; Bachy et al., 2012), which is considerably smaller according to the same authors (296 and 280 µm long, 53 and 55 µm in internal oral diameter, respectively) and the original description (Brandt, 1906, 1907).

***Incertae sedis* in Tintinnida**

***Climacocylis scalaroides* Kofoid & Campbell, 1929 (Fig. S1L)**

Lorica cylindrical, very delicate, with a spiral band in the upper third. Aboral end irregular, open. Wall hyaline, with large alveoli. Length = 136.4±23.0 µm, oral diameter = 32.2±0.9 µm, n = 3. It matches perfectly the original description (Kofoid and Campbell, 1929).

SSU rDNA sequence has a 99% BLAST hit to *Climacocylis scalaria* (Brandt, 1906) Jörgensen, 1924 isolates FG1116 and FG1118 (JQ408210-3) from the Mediterranean, which presents a bigger loricae (410-420 µm long, 55 µm oral diameter; Bachy et al., 2012), in agreement with the original description (Brandt, 1906, 1907).

Climacocylis used to be included in Metacylididae (Kofoid and Campbell, 1929). However, the existing sequences for this genus cluster apart from *Metacylis*, and instead form a well-supported clade with *Rhizodomus tagatzi* Strelkow & Wirketis, 1950 (JQ392572, Saccà et al., 2012; Fig. S3). The lorica morphology and the presence of large alveoli in the wall also contrasts to the characteristics of

Metacylididae (see above), while the lorica spirals resemble *R. tagatzi* (Saccà et al., 2012). Thus, we propose to exclude *Climacocylis* from Metacylididae and keep it as *incertae sedis* in Tintinnida.

Another genus in a similar situation is *Helicostomella,* which we propose to transfer as *incertae sedis* in Tintinnida. This genus was included in Metacylididae (Kofoid and Campbell, 1929), but its sequences cluster apart from *Metacylis* or *Climacocylis* based on our trees (Fig. 2, 3, S3, S4, S5). Although *Helicostomella* and *Metacylis* loricae share a spiraled collar and a non-spiraled bowl (Jörgensen, 1924), they differ in their ciliary patterns (Pierce, 1996). *Helicostomella* actually shows a close relationship with some *Tintinnopsis*-like species, which share a 23-nucleotide deletion in the 5'end of ITS1 that is not present in any other tintinnid sequenced so far, but differ mainly in the absence vs. presence of particles on the lorica (Santoferrara et al., 2015). Interestingly, we were able to culture one of these agglomerated forms, which losts its particles and became very similar to *Helicostomella*, although lacking spiral in the collar region (Fig. S2B). This confirms that agglutination is highly dependent on particle availability more than a diagnostic feature (e.g., Alder, 1999; Agatha and Strüder-Kypke, 2013). Sequences of *Tintinnopsis parva* Merkle, 1909, *T. rapa* Meunier, 1910, *T. tenuis* Hada, 1932 and *T. turbinata* Balech, 1948 cluster with *Helicostomella* (Santoferrara et al., 2015), and not with a sequence labeled as the type of the genus, *T. beroidea* Stein, 1867, altough of uncertain identification (EF123709; unpublished). This, and the lack of data on lorica ultrastructure and cytology for most *Tintinnopsis*-like species, prevent a revision of this taxon.

Subclass Oligotrichia Bütschli, 1887/1889

Order Strombidiida Petz & Foissner, 1992

Family Tontoniidae Agatha, 2004

***Laboea strobila* Lohmann, 1908 (Fig. 1K)**

The specimen was identified based on its characteristic screw-like shape, which is caused by the girdle kinety performing four to five whorls (Montagnes et al., 1988, Agatha et al., 2004). Although our specimen was fixed with Lugol's solution, its size (Table S1) falls within the ranges reported by Agatha et al. (2004) for specimens *in vivo* and after protargol impregnation.

The sequences of our NW Atlantic specimen are identical or almost identical (>99.8% similarity) to those from adjacent Long Island Sound waters (SSU rDNA and ITS regions, AF399151-4 and AF399079-81; Snoeyenbos-West et al., 2002), from the Mediterranean (SSU rDNA, AY302563; Agatha et al., 2004), and from the China Sea (ITS regions, KU715799; the LSU rDNA, KU715780 has 5 substitutions = 0.7% difference, which could be due to inter-population differences; Zhang et al., 2016).

Only the former sequences were kept in our final alignments. In contrast, another set of sequences from China (Gao et al., 2016b; KU525740, KU525756) have multiple substitutions and/or indels (more than 1% difference in SSU rDNA and more than 4% difference in both ITS regions and LSU rDNA), consistent with either a misidentification or sequences of poor quality; thus the sequences from Gao et al. (2016b) were not kept in our final alignments.

***Pseudotontonia* sp. (Fig. S1M)**

Conical cell with the contracted tail that is characteristic of most members of the family. The diagnostic character of the genus *Pseudotontonia* Agatha, 2004, a horizontal girdle kinety, seems evident in our Lugol-fixed specimen (not to be confused by the distended cell surface, which is probably a fixation artifact; Montagnes and Lynn, 1991). According to Agatha (2004), other Tontoniidae genera differ in a girdle kinety dextrally spiraled (*Tontonia* Fauré-Fremiet, 1914), sinistrally spiralled (*Spirotontonia* Agatha, 2004*, Laboea* Lohmann, 1908), or horizontally orientated on the dorsal side, but with the kinety ends extending to the posterior end of the ventral side (*Paratontonia* Jankowski, 1978). Identification of our specimen at the species level is impossible given the lack of detailed cytological data.

Our sequence forms a highly-supported, monophyletic clade with all the other Tontoniidae species sequenced so far, and it is basal to a clade of *Laboea* plus *Spirotontonia* (Fig. S3, S4, S5). The closest match according to BLAST is *Pseudotontonia simplicidens* (Lynn & Gilron, 1993) Agatha, 2004 (GenBank accession FJ422993; Gao et al 2009), with 92% similarity in SSU rDNA. Gao et al. (2009) did not include any morphological data, except for one photomicrograph. Also labeled as *P. simplicidens,* sequences KM222146 (LSU rDNA) and KM222052 (ITS regions) obtained by Gao et al. (2016a) seem problematic. The former was included in our alignment but it clusters apart from all other tontoniids in the respective tree (Fig. S5). The later does not align or BLAST to any spirotrich; it is clearly incorrect and thus eliminated from our alignment.

Supplementary Text 2. Complementary identification and update of GenBank records published by Santoferrara et al. (2013)

Species identification should be confirmed with original descriptions (Santoferrara et al., 2016b). All our previous GenBank records followed this procedure, except in our first study (Santoferrara et al., 2013). We reinvestigated all the identifications in the later study and, based on original literature and recent redescriptions, we confirm the identification of *Eutintinnus pectinis* (Kofoid and Campbell, 1929), *Favella ehrenbergii* (Claparède and Lachmann, 1858; Kim et al., 2010), *Stenosemella pacifica* (Kofoid and Campbell, 1929; Agatha and Tsai, 2008), *Tintinnidium balechi* (Barría de Cao, 1981), *Tintinnidium mucicola* (Claparède and Lachmann, 1858), *Tintinnopsis baltica* (Brandt, 1896), *Tintinnopsis butschlii* (Daday, 1887), *Tintinnopsis cylindrica* (Daday, 1887; Agatha and Riedel-Lorjé, 2006), *Tintinnopsis lobiancoi* (Daday, 1887), *Tintinnopsis major* (Meunier, 1910), *Tintinnopsis nana* (Lohmann, 1908), *Tintinnopsis parva* (Merkle, 1909), *Tintinnopsis parvula* (Brandt 1906, 1907; Agatha, 2010a), *Tintinnopsis rapa* (Meunier, 1910), *Tintinnopsis tocantinensis* (Brandt 1906, 1907; Kofoid and Campbell, 1929), and *Tintinnopsis uruguayensis* (Balech, 1948).

Instead, the following records were corrected. Records JN831777-78 and JN831867-68, which were labeled as *Schmidingerella taraikaensis* (Hada, 1932) Agatha & Strüder-Kypke, 2012, are re-identified as *Schmidingerella arcuata* (Brandt, 1906) Agatha & Strüder-Kypke, 2012 based on the recent redescription of the later species (Agatha and Strüder-Kypke, 2012). In addition, records JN831831-32 and JN831918, which were labeled as *Tintinnopsis platensis* da Cunha and Fonseca, 1917, are re-labeled as *Stylicauda platensis* (da Cunha and Fonseca, 1917) Balech, 1951 based on the typical characteristics of the later genus (the presence of a hyaline appendix; Balech, 1951). Also, four samples were re-sequenced to obtain a longer sequence: *Stenosemella steini* isolate Hat506 KT792927, *Schmidingerella arcuata* isolate 125 JN831867, *Favella ehrenbergii* isolate 15 JN831860, *Strombidinopsis* sp. isolate LFS-2012 JQ028732 (Santoferrara et al., 2012, 2013, 2016a).

Reinvestigation of specimens previously classified up to the genus level (Santoferrara et al., 2013) allowed to improve the following determinations (Fig. S2A):

***Tintinnopsis acuminata* Daday, 1887**

Bullet-shaped lorica, sparsely agglutinated. Length = 75.4±9.7 µm, oral diameter = 37.7±5.2 µm (up to 47.5 µm), n = 7. In agreement with original description, with oral diameter overlapping the described range (45-50 µm; Daday, 1887).

Previously labeled as *Tintinnopsis* sp. 4 for GenBank records JN831839-45 and JN831924-30 (Santoferrara et al., 2013).

***Tintinnopsis turbinata* Balech, 1948**

Lorica conical, densely agglutinated. With a small, narrowed collar and a pointed aboral end. Length = 37.7±1.7 µm, oral diameter = 21.7±2.1 µm, maximum diameter = 26.0±2.3 µm, n = 4. In agreement with original description (Balech, 1948).

Previously labeled as *Tintinnopsis* sp. 5 for GenBank records JN831846, JN831931, KM982893-95, KM982850 (Santoferrara et al., 2013, 2015).

***Tintinnopsis tenuis* Hada, 1932**

Lorica cylindrical, with a rounded aboral end. Length = 40.1±1.0 µm, oral diameter = 21.5±0.6 µm, n = 3. Size in better agreement with a subsequent redescription by the same author (54-64 and 43-55 µm long, 25-29 and 21-23 µm wide in specimens from Mutsu Bay and Akkeshi Bay, Japan, based on Hada, 1932 and Hada, 1937, respectivelly).

Previously labeled as *Tintinnopsis* sp. 6 for GenBank records JN831847-48, KM982896 and JN831932-33 (Santoferrara et al., 2013, 2015).

***Tintinnopsis kiangsuensis* Chiang, 1956**

Lorica globular, narrowed in the oral end. Oral rim irregular and aboral end pointed. Length = 57.8±1.9 µm, oral diameter = 31.3±2.3 µm, maximum diameter = 44.7±1.6 µm, n = 6. Our specimens from riverine waters of the Rio de la Plata, Argentina match perfectly in shape and size compared to the type population of a lake in Kiangsu, China (Chiang, 1956).

Previously labeled as *Tintinnopsis* sp. 7 for GenBank records JN831849-50 and JN831934-35 (Santoferrara et al., 2013).

***Tintinnopsis urnula* Meunier, 1910**

Lorica conical to globular, with a constriction below a slightly flaring oral end. Aboral end slightly pointed. Length = 63.3±5.5 µm, oral diameter = 40.9±0.7 µm, maximum diameter = 49.3±5.9 µm, n = 2. Shape agrees with original the description of Meunier (1910). This author did not include measures, but our specimens perfectly match the dimensions reported afterwards (Marshall, 1969).

Previously labeled as *Tintinnopsis* sp. 8 for GenBank records JN831851-52 and JN831936-37 (Santoferrara et al., 2013).

***Tintinnopsis pseudocylindrica* Hada, 1964**

Lorica mostly cylindrical, whit a conical aboral region. Aboral end pointed or broken. Sparsely agglutinated. Length = 164.3±12.8 µm, oral diameter = 35.5±1.2 µm, n = 5. In agreement with original description (Hada, 1964).

Previously labeled as *Tintinnopsis* sp. 9 for GenBank records JN831853-55 and JN831938-40 (Santoferrara et al., 2013).

Supplementary text 3. Sequence curation

In this study, we obtained and curated two sets of sequences, one based on sequences from morphologically-characterized specimens, and another one including also unidentified environmental sequences. This section refers to the first set of sequences, which was manually retrieved and carefully curated as described bellow. The final curated sequences are displayed in Fig. S3, S4 and S5.

Step 1: sequence retrieval and exclusion of sequences identified only above genus. We downloaded from GenBank all the SSU rDNA, ITS regions and LSU rDNA sequences labeled as Oligotrichia or Choreotrichia (261 and 1297 respectively, as of November 1, 2016). We excluded environmental sequences with no further genus or species identification (unidentified sequences KX158679-738 from Zhang et al. 2016; clone libraries sequences AY821916/8, Šlapeta et al., 2005; EU646907/79, Euringer and Lueders, 2008; GU993549-87 and HM001218-9, Doherty et al., 2010; FJ431595, Marie et al., 2010; JX567350-503 and KF662488-2721, Bachy et al., 2013, 2014; KJ638876-80, Yu et al., 2015; LN869977/70165, Rossi et al., 2016), or those identified based only on phylogeny (clone library sequences JF791015-6, JF791039; Rocke et al., 2013). Some sequences identified only to genus were excluded as well (JX178769-JX178900, Gong et al. 2013; AY143564-5, EU399535, Strüder-Kypke and Lynn, 2003, 2008; EU024986/90, Auinger et al., 2008; DQ487198, Duff et al., 2008; GU067802/8018, FJ543106-7, KF800042, KJ101609, AM412524, JQ781699, KT389860/90000, KM067399, all unpublished). Sequences GQ281554-5 (Medinger et al., 2010) are the only available ones for *Pelagostrombidium*, but we excluded them as they are <180 bp long. ITS sequence DQ811089 (unpublished), labeled as *Strombidium sulcatum*, is excluded because it does not align or BLAST to any other Spirotrichea sequences. Finally, we excluded all the sequences from a work that is questionable in terms of species identification and sequence quality (AB640624-83, Kazama et al., 2012).

This yielded preliminary datasets of 408, 293 and 218 total sequences (SSU rDNA, ITS regions, LSU rDNA, respectively), including also our new sequences. For phylogenies, each alignment was further refined. Additional sequences only identified to genus were excluded (AF399013-16, AF399021-67, AF399115-17, AF399122-27, AF399132-5, Snoeyenbos-West et al., 2002; DQ241741-50, Katz et al., 2005; FJ422985-7, Gao et al., 2009; JN853788, Li et al., 2013; JN033234-36, Zhao et al., 2012; KJ609043, KU525753, KU525773, KU525736, KJ609043 Gao et al., 2016b; GU206560-2, unpublished).

Step 2: excluding redundant sequences and flagging potential misidentifications. In the case of more than one sequence labeled as the same species that were identical or highly similar (>99.8% similarity),

we retained only one sequence (the best documented one or the longest one). For conflictive cases, the criteria used to retain or exclude some of the sequences from our final alignments are explained in Suplementary Text 1. Additional cases follow (mostly SSU rDNA, except if otherwise stated):

- Some slightly different sequences (<0.3%) labeled as the same species may result from intraspecific variability or sequencing errors. *Eutintinnus fraknoi* sequence EU399534 (Strüder-Kypke and Lynn, 2008) clusters together but has up to three substitutions compared to sequence JN871722 (Bachvaroff et al., 2012; not illustrated) and JQ408157/9 (Bachy et al., 2012; short sequence, specimen similar to the first one based on published pictures). Same for similar-looking *E. pectinis* JN831766 (Santoferrara et al., 2013) vs. JN871720 (Bachvaroff et al., 2012) and AY143570 (Strüder-Kypke and Lynn 2003); *Amphorellopsis acuta* JX101847 vs. JX101848 (Xu et al., 2013), EU399530 (Strüder-Kypke and Lynn, 2008), FJ196071 (Li et al., 2009), JN033241(ITS region, Zhao et al., 2012) as well as *Amphorellopsis* sp. (SSU rDNA KU715756-8, ITS regions KU715794, Zhang et al., 2016); *Tintinnidium mucicola* JN831798-800 (Santoferrara et al., 2013) and KU715767 (Zhang et al., 2016) vs. AY143563 (Strüder-Kypke and Lynn, 2003); *Tintinnopsis radix* EU399540 and KU715772-3 vs. KU715774 (Strueder-Kypke and Lynn, 2008; Zhang et al., 2016). In these cases, only the former sequence of each case was kept in our final alignment.

- Some sequences labeled as the same species but quite different (i.e., they cluster apart in trees and/ or are >1% different) may be related to misidentifications. *Novistrombidium testaceum* sequence FJ377547 (Zhang et al., 2010) is >1.3% divergent to AJ488910 (Modeo et al., 2003), but only the latter is associated to complete morphological data. *E. tubulosus* sequences JX101855-6 (Xu et al., 2013) differ from sequence JQ408187 (Bachy et al., 2012); of them, the latter belong to specimens more similar in size to the original description (Ostenfeld, 1899). *E. pectinis* was apparently misidentified in one study (AF399169-71, AF399105-07, Snoeyenbos-Weis et al., 2002), thus explaining the genetic divergence compared to sequences AY143570, JN871720 and JN831766 (Strüder-Kypke and Lynn, 2003; Bachvaroff et al., 2012; Santoferrara et al., 2013). *Tintinnopsis cylindrica* JQ408181/191/206 (Bachy et al., 2013) differ from sequences JN831811-2 (Santoferrara et al., 2013) and FJ196075 (Li et al., 2009); although the three studies reported specimens with similar lorica size, the two later indicate a shape more similar to the original description (Daday, 1887) and recent redescription (Agatha and Riedel-Lorjé, 2006). In these cases, both variants were kept in our final alignment. *Tintinnopsis* sp. (JN871723; Bachvaroff et al., 2012) has identical SSU sequence, similar morphology, and was isolated from the same place as *Tintinnopsis cylindrica* according to Bachy et al. (2012), and thus it was eliminated from our final alignment.

- *Favella campanula* (FJ422984; Gao et al., 2009), *Favella azorica* (JQ408208/12; Bachy et al.,

2012), and *Favella campanula*, forms *campanula* and *azorica* (JX101860 and JX101861, respectively; Xu et al., 2013) have identical or very similar sequence. The former and the later were kept in final alignment because they are longer and and/or better documented.

- *Rhabdonella hebe* (AY143566, Strüder-Kypke and Lynn, 2003), *R. poculum* (JX101864, Xu et al., 2013), *R. elegans* (SSU rDNA JQ408175, ITS regions JQ408175, Bachy et al., 2012), *R. valdestriata* (ITS regions KU715802, LSU rDNA KU715782 Zhang et al., 2016), and *R. spiralis* (e.g. SSU rDNA KT792932, Santoferrara et al., 2016a; ITS regions KY290307, LSU rDNA KY290349, this study) have identical or almost identical sequence, but they differ in shape and/ or size. Similar species with identical or almost identical sequence are *Favella ehrenbergii* (e.g. GU574769, Kim et al., 2010; ITS regions KY290309, this study) and *F. panamensis* (AY143572, Strüder-Kypke and Lynn, 2003; ITS regions KU715798, Zhang et al., 2016); *Schmidingerella arcuata* (e.g. SSU rDNA JQ837815, Agatha and Strüder-Kypke, 2012; ITS regions KY290310, this study; LSU rDNA JN831867, Santoferrara et al., 2013), *S. taraikaensis* (SSU rDNA FJ196073, Li et al., 2009; ITS regions JN033237, Zhao et al., 2012) and *Schmidingerella quequenensis* (SSU rDNA KU715765, ITS regions KU715805, LSU rDNA KU715786, Zhang et al., 2016); *Cymatocylis calyciformis*, *C. convallaria* and *C drygalskii* (SSU rDNA, ITS regions and LSU rDNA JQ924046-52; Kim et al., 2013); *Undella claparedei*, *U. hyaline, U. marsupialis*, (JQ408164, JQ408207/ JQ408171, JQ408214; the two former differ in ITS regions; Bachy et al., 2012) and *U. subcaudata* KT792931. Although some of them may correspond to synonyms within their respective genera, this cannot be confirmed at the moment and thus one representative per species is kept in our final alignment.

- *Codonellopsis morchella* and *C. orthoceras* have identical SSU rDNA sequence but different morphology (e.g. JQ408173/80, Bachy et al., 2012). One sequence per species was kept.

- Species identification within the genus *Helicostomella* is difficult, and thus available sequences are divided in *H. subulata* clusters I, II and III (Santoferrara et al., 2015). One representative sequence per cluster is kept.

- The clade including *Codonella*, *Codonaria*, *Codonellopsis*, *Dyctiocysta*, *Laakmanniella* and *Stenosemella* has some inconsistencies (lack of monophyly for some genera), but most sequences and associated identifications seem appropriate. An evident inconsistency corresponds to three different kinds of sequences labeled as *Stenosemella ventricosa* (Claparède & Lachmann, 1858) Jörgensen, 1924. Sequences KU715764 and KU715804 (Zhang et al., 2016) correspond to a specimen more similar to the original description (Claparède and Lachmann, 1858) and subsequent

redescription by Fol (1884), while sequences EU399538-9 (Strüder-Kypke & Lynn, 2008) and JQ408170/4 (Bachy et al., 2012) correspond to specimens with different morphology according to the published picture. Consequently, the two later were labeled as potentially misidentified in our final alignment. Compared to the isolates from Bachy et al. (2012), *Stenosemella* sp. (Zhang et al., 2016) has similar dimensions and only 2 substitutions in SSU rDNA, but we cannot confirm conspecificity, so both sequences are kept in the final alignment. The sequence labeled as *Tintinnopsis fimbriata* (AY143560, Strüder-Kypke and Lynn, 2003) also clusters here, but it could corespond to a misidentification. Strüder-Kypke and Lynn (2003) did not provide a description, measurements or a specific reference for identification. Although the drawing provided resembles *T. fimbriata* (e.g. as redescribed by Agatha, 2008), this is not the case for the accompanying micrograph. Based on morphological data, *T. fimbriata* (as redescribed by Agatha, 2008) does not relate to *Codonella* or *Codonellopsis*, but to other *Tintinnopsis* species (Agatha and Strüder-Kypke, 2012). *Codonella cratera* (DQ487193; Duff et al., 2008) does not cluster here, but with another freshwater species, *Tintinnopsis lacustris* (JQ408161-2; Bachy et al., 2012). However, the generic affiliation of *C. cratera* is probably incorrect, as inferred from the ciliary patterns (Agatha 2010a, b; Agatha and Strüder-Kypke, 2007; Laval-Peuto and Brownlee, 1986) and SSU rDNA sequences (Bachy et al., 2012; Fig. S3).

Potentially misidentified sequences kept in our final alignments are flagged in Fig. S3, S4, S5 (green).

Step 3: solving labeling issues. A few cases of labeling inconsistencies were preliminarily solved in our alignments as follows:

- Some sequence labels in GenBank do not match the species names given in the corresponding publications. In our alignments, we updated the labels of the following sequences: KJ534583, *Cyrtostrombidium* sp. is *C. paralongisomum* (Tsai et al., 2015); KM084728, *Strombidium* sp. is *S. pseudostylifer* (Song et al., 2015a); KJ609050, *Strombidium* sp. is *S. tropicum* (Liu et al., 2015b); DQ487200, *Tintinnopsis* sp. is *Tintinnidium pusillum* and DQ487193, *Codonella* sp. is *Codonella cratera* (Duff et al., 2008); JN853790, *Omegastrombidium* sp. is *O.* cf. *elegans* (Li et al., 2013). Sequences KJ609049 and KJ609044, labeled as *Strombidium hausmanni* in Gao et al. (2016b) actually belong to *S. guangdongense* (Liu et al., 2016).

- Sequences labeled as *Favella taraikaensis* in GenBank (SSU rDNA: FJ196073, Li et al., 2009; ITS regions: JN033237, Zhao et al., 2012) are now known to correspond to *Schmidingerella taraikaensis* (Agatha and Strüder-Kypke, 2012). Accordingly, we relabeled these sequences in our alignments.

- For *Novistrombidium* and *Parallelostrombidium* (sequences FJ422988, FJ422989, FJ422991,

HM140404, FJ876958, FJ377547), subgenera were added to the sequence names based on the work of Agatha and Strüder-Kypke (2014).

Step 4: flagging or removing low quality records. Of higher concern were inconsistencies coming mostly from three recent papers devoted to ciliates (Gao et al., 2016a), oligotrichs (Gao et al., 2016b), and tintinnids (Zhang et al., 2016):

- Most of the sequences reported by Gao et al. (2016b) were not accompanied with sufficient morphological information to confirm their identification (23 out of 36 sequenced populations that were only partially characterized in this paper or before by Gao et al., 2009; Zhang et al., 2010). In addition, some Oligotrichia and Choreotrichia sequences reported by Gao et al. (2016a) are not accompanied by morphological information at all (*Spirostrombidium schizostomum* KM222098, KM222053, KM222147; *Strombidinopsis batos* FJ881862 KM222054 KM222148, *Pelagostrobilidium minutum* FJ876959, KM222055, KM222149). Except if mentioned elsewhere in this paper, we kept them in our final alignments of SSU rDNA, ITS regions and LSU rDNA, but they should be considered with caution.

- ITS sequence KM222052 (Gao et al., 2016a), labeled as *Pseudotontonia simplicidens*, does not align or BLAST to any spirotrich. It is clearly incorrect and thus eliminated from our alignment.

- Sequences labeled as *Favella* cf. *campanula* (KM222099, KM222057, KM222151; Gao et al., 2016a) are not accompanied by published morphology, but are actually identical to *Schmidingerella* sequences, and thus disregarded in our final alignments.

- Sequence KJ609053 is labeled as *Cyrtostrombidium longisomum* in GenBank, but as *C. paralongisomum* in the corresponding paper (Gao et al., 2016b). The sequence is more similar to previous report of the later (6 and 4 substitutions to KJ534582 and KJ534583, respectively; Tsai et al., 2015); however, we kept the GenBank label in our alignment. Sequence KU525757 labeled as *Lynnella semiglobulosa* (Gao et al., 2016b) has 6 substitutions compared to FJ876965 (Liu et al., 2011b). In ITS regions and LSU rDNA, *L. semiglobulosa* KU525757 (Gao et al., 2016b) has a ca. 30 nt insertion and >20 substitutions, respectively, compared to sequences KM222051/KM222145 labeled as the same species (Gao et al., 2016a). Records KJ609048, KJ609048 and KJ609059 are labeled as *Strombidium triquetrum* in GenBank, but as *Strombidium* cf. *capitatum* in the corresponding paper (Gao et al., 2016b); the SSU rDNA has ca. 30 substitutions compared to *S. capitatum* KP260510 (Song et al., 2015a), so the GenBank label was kept in our alignment. ITS sequence KJ609042 labeled as *Omegastrombidium* cf. *elegans* has ca. 50 substitutions and/or indels (11% difference) compared to JN853790 (Li et al., 2013), both from China but lacking adequate

morphological data. In these cases, both sequences were kept in our alignments.

- Sequence FJ876962, labeled as *Strombidium paracalkinsi* in Gao et al. (2016b), is no longer available in GenBank, with the legend "This record was removed at the submitter's request because the source organism cannot be confirmed". We excluded this sequence from our alignments.

- Populations *Strombidium basimorphum* and *S. basimorphum* pop. 2 isolated from different places were given identical GenBank accession number in the paper by Gao et al. (2016b). Also, the sampled location of *Strombidium stylifer* population 2 (JX012185) does not agree in the original publication of this record (Song et al., 2015b) and in the paper by Gao et al. (2016b).

- Sequence JX310365 corresponds to *Antestrombidium agathae* (Liu et al., 2015a), but this sequence and KU525725, supposedly corresponding to the same species, are labeled as *A. wilberti* in Gao et al. (2016b). We kept the former label in our alignment.

- Sequences KU525752 and KU525735 labeled as *S. rassoulzadegani* in Gao et al. (2016b) differ >1% in SSU rDNA, >1.5% in ITS regions and >1.5% in LSU rDNA compared to the isolates from Long Island Sound (McManus et al., 2010; Santoferrara et al., 2013). Similarly to sequences labeled as *Laboea strobila* (see Supplementary Text 1), this suggests misidentification or low quality of the sequences published by Gao et al. (2016b), and thus they were not kept in our final alignment.

- Sequence KU525748 (SSU rDNA) and KU525732 (LSU rDNA), labeled *Parallelostrombidium paralatum* (Gao et al., 2016b), have > 20 and >35 substitutions and/ or indels compared to HM140404 and HM122021, respectively. However, the later are unpublished, and thus only the former were kept in our final alignments. Other sequences from Gao et al. (2016b) that we favored in our final alignment correspond to *Omegastrombidium elegans* KU525750 and *Varistrombidium kielum* KJ609051 (over the very similar but unpublished sequences EF486862 and DQ811090).

- Sequence KU715766, labeled as *Tintinnidium* cf. *primitivum* (Zhang et al., 2016) the picture used for species identification is not from the specimen sequenced. The latter procedure is unacceptable for barcoding tintinnids with agglomerate loricae, given that examples of species with very similar appearance but very different sequence have been found, even in the same sample (Santoferrara et al., 2013). Zhang et al. (2016) also used this questionable procedure for sequences labeled as *Leprotintinnus simplex, Tintinnopsis baltica, T. brasilensis, T. cylindrica, T. fistularis, T. hemispiralis, T. parvula* and *Tintinnopsis* sp. (KU715768-71, KU715775, KU715801, KU715806, KU715808-14, KU715817, KU715781, KU715788-92). Because of the risk that the identified and the sequenced specimens do not belong to the same species, these sequences were eliminated from

Eliminado: is problematic for two reasons. One, there is a >10 nt insertion not present in any Choreotrichia or Oligotrichia, thus making the quality of this sequence suspicious. Two,

our final alignment. Another potentially problematic sequence from the same study is KU715759 (Zhang et al., 2016). Using the acompaining picture, the sequence was designated to *Eutintinnus* cf. *apertus*. However, this sequence is 6% divergent from *E. apertus* (JQ408195, Bachy et al., 2012), and it has at least five regions of 5-25 nucleotides and some indels that are very different compared to any other available *Eutintinnus* sequences (>5% divergence). Sequence KU715759 was thus eliminated from our final alignment. Compare and eliminate also LSU and ITS!!!

Eliminado: *Eutintinnus* cf. *apertus* (

Eliminado: ,

Eliminado:

Eliminado: suggesting that one of them has been misidentified. However, the SSU rDNA of the former isolate

Eliminado: , thus making the quality of this sequence highly suspicious

Eliminado: Consequently, the suspicious s

Eliminado: (Zhang et al., 2016)

Eliminado: ¶

The problem of insufficient or no morphological data associated to a sequence is also true for other sequences present in our final alignments. This has impacted mostly aloricates, for which accurate identification of species require staining. In some cases, the morphological information published is insufficient (*Strobilidium caudatum* AY143573, Strüder-Kypke and Lynn, 2003; *Spirotontonia turbinata* FJ422994, *Pseudotontonia simplicidens* FJ422993, *Strombidium conicum* FJ422992, Gao et al., 2009; *Strombidium sulcatum* FJ377546, *Strombidium basimorphum* FJ480419, *Novistrombidium testaceum* FJ377547, Zhang et al., 2010; *Novistrombidium orientale* JN853791, *N. testaceum* JN853795, *Omegastrombidium* cf. *elegans* JN853790, *Strombidium basimorphum* JN853787, *S. conicum* JN853793, *S. stylifer* JN853794, Li et al., 2013), while in other cases no publication exists at all (*Pelagostrobilidium paraepacrum* FJ876963; *Pelagostrobilidium minutum* FJ876959; *Strombidium crassulum* HM140389, HM122034; *Strombidium apolatum* DQ662848; *Strombidium purpureum* U97112). In contrast, most tintinnid sequences have at least a published picture of the lorica, which allows preliminary identification (although not always an unequivocal one); exceptions are two unpublished sequences (*Metacylis tropica* KP883283, *Tintinnopsis beroidea* EF123709). All these sequences were kept in our final alignments of each marker, but should be considered with caution. Especially for Oligotrichia, the low quality of some sequences may have caused, at least partially, the poor resolution of phylogenetic inferences. For example, a potential uncertainty involves *Strombidium* cf. *parastylifer*, which is included in the "eyespot" clade according to Gao et al. (2016b) and our analyses (Fig. S3), but an eyespot was not reported in the original description of this species (Xu et al., 2009). Poor quality sequences kept in our final alignments are flagged in Fig. S3, S4, S5 (red).

For the concatenated alignment, we were more stringent. Given the problems exposed above, all the sequences obtained by Gao et al. (2016b) were excluded. Sequences that completely lack morphology data in Gao et al. (2016a) were excluded (three sets of sequences; we only kept two sets of sequences for which the morphology of the same population seems to have been characterized in previous publications). Sequences for which the specimens documented and sequenced are not the same were excluded (three sets of sequences from Zhang et al., 2016).

Supplementary Table S1. Specimens sequenced for SSU rDNA, ITS regions and LSU rDNA. All species, except *Laboea strobila*, were newly sequenced for at least one marker. Taxa in bold were not represented in GenBank for any marker.

Species[1]	Isolate	Measurements (µm)			Sampling data				
		Length[4]	Width[4]	Other[5]	Date	Lat. N	Long. W	Site depth (m)	Sampled depth (m)
***Leegaardiella* sp.**	LS803	43.2	46.2	n.a.	8/12/2015	39.90	71.47	329	50
Pelagostrobilidium sp.	LS781	n.a.	46.5	n.a.	8/12/2015	39.90	71.47	329	0
Ascampbelliella acuta	LS800	38.0	30.7	38.4 (c)	8/12/2015	39.90	71.47	329	50
Cyttarocylis acutiformis	LS807	198.8	120.5	95.0 (b)	8/12/2015	39.79	71.46	1100	50
Petalotricha ampulla	LS787	126.3	90.1	72.5 (b)	8/12/2015	39.90	71.47	329	50
Dictyocysta elegans[2]	LS801	66.9	38.9	43.9 (c)	8/12/2015	39.90	71.47	329	50
Epiplocylis undella	LS784	144.3	70.3	36.9 (d)	8/12/2015	39.90	71.47	329	0
Eutintinnus medius[2]	LS786	235.9	47.6	30.5 (a)	8/12/2015	39.90	71.47	329	0
Eutintinnus perminutus[3]	LS759	138.1	32.7	24.1 (a)	8/12/2015	39.96	71.57	128	40
Ptychocylis minor	LS754	141.6	70.7	82.9 (c)	8/12/2015	39.96	71.57	128	40
Metacylis angulata	SS699	85.4	64.6	109.3 (c)	6/29/2015	41.32	72.07	2	0
Protorhabdonella simplex[2]	LS794	54.8	33.5	n.a.	8/12/2015	39.90	71.47	329	50
Amphorides minor	LS763	86.0	36.7	28.2 (b)	8/12/2015	40.99	71.68	40	16
Salpingacantha undata	LS772	237.4	23.5	18.1 (b)	8/12/2015	40.99	71.68	40	35
Salpingacantha unguiculata	LS804	116.5	12.5	9.3 (b)	8/12/2015	39.79	71.46	1100	50
Parundella aculeata[2, 6]	LS789	159.9	29.7	37.3 (d)	8/12/2015	39.90	71.47	329	50
Parafavella parumdentata	LS751/ LS862	140.2/ 144.7	48.0/ 48.1	n.a.	8/12/2015	39.96	71.57	128	40
Xystonella acus[2]	LS758	367.4	59.1	75.1 (d)	8/12/2015	39.96	71.57	128	40
Climacocylis scalaroides[2]	LS813	132.1	31.3	38.4 (c)	8/12/2015	39.79	71.46	1100	0
Laboea strobila	LS766	93.2	46.9	n.a.	8/12/2015	40.99	71.68	40	16
Pseudotontonia sp.	LS753	95.7	46.8	n.a.	8/12/2015	39.96	71.57	128	40

[1]Excludes seven specimens that we sequenced before for at least one marker, and thus their morphology and sampling data are already published: *Strombidinopsis* sp. isolate LFS-2012, *Favella ehrenbergii* isolate 15, *Schmidingerella arcuata* isolate 125, *Tintinnopsis cylindrica* isolate 71, *Rhabdonella spiralis* isolate Hat525, *Steenstrupiella steenstrupii* isolate Hat552, *Eutintinnus medius* isolate Hat566 (Santoferrara et al., 2012, 2013, 2016a). [2]Sequenced for SSU rDNA only. [3] Not sequenced for SSU rDNA. [4]For tintinnids, lorica length and oral diameter. [5]Other lorica dimensions: a aboral diameter, b suboral diameter, c maximum diameter, d appendix length. [6]Sequence JQ408204 labeled *P. aculeata* (Bachy et al., 2012) apparently corresponds to *Dadayiella* (Agatha and Strüder-Kypke, 2014).

Supplementary Table S2. Primers used.

Name	Sequence 5' → 3'	Target	Reference	Use
Primer A-F	AACCTGGTTGATCCTGCCAGT	Universal, SSU rDNA	Medlin et al., 1988	Amplification and sequencing
Primer B-R	TGATCCTTCTGCAGGTTCACCTAC	Universal, SSU rDNA	Medlin et al., 1988	Amplification and sequencing
Tin18S-F	ATTAGTACTTAACTGTCAGAGGTG	Tintinnids, SSU rDNA, internal	Santoferrara et al., 2013	Nested amplification and sequencing, when needed
Tin18S-R2	CGGCATAGTTTATGGTTAAGACT	Tintinnids, SSU rDNA, internal	Santoferrara et al., 2013	Nested amplification and sequencing, when needed
18ScomF-3end	GTCGTAACAAGGTTTCCGTAGGTG	Universal, ITS regions and D1-D2 region of LSU rDNA	Bai et al., 2002	Amplification and sequencing
com28SR1	TCACGCATAGTTCACCATCTTTCG	Universal, ITS regions and D1-D2 region of LSU rDNA	Wang et al., 2014	Amplification and sequencing
com28SR2	TTAGACTCCTTGGTCCGTGTTT	Universal, ITS regions and D1-D2 region of LSU rDNA	Wang et al., 2014	Nested amplification and sequencing, when needed

Supplementary Table S3. Alignments obtained for Choreotrichia and Oligotrichia, including four outgroup sequences.

Marker	Sequences	Length (bp)
SSU rDNA	198	1755
ITS regions	113	571
LSU rDNA	105	856
Concatenated	47	3031

Supplementary Table S4. Sequences used for concatenated alignment. In bold, sequences from this study. Only species with complete or almost complete sequences of SSU rDNA, ITS regions and LSU rDNA kept in the respective alignments were included; some sequences were excluded due to quality concerns (see Supplementary Text 3). All the sequences are from one single specimen, or *at least from the same population. Classification as in Table 1.

Family	Species	SSU rDNA	ITS regions	LSU rDNA	References
Leegaardiellidae	*Leegaardiella* sp.	**KY290313**	**KY290291**	**KY290333**	**This study**
Lynnellidae	*Lynnella semiglobulosa*	FJ876965*	KM222051*	KM222145*	Liu et al., 2011b; Gao et al., 2016a
Strobilidiidae	*Pelagostrobilidium* sp.	**KY290314**	**KY290292**	**KY290334**	**This study**
	Rimostrombidium veniliae	FJ876964*	KM222056*	KM222150*	Liu et al., 2012; Gao et al., 2016a
Strombidinopsidae	*Strombidinopsis* sp.	JQ028734	**KY290311**	JQ028732	Santoferrara et al., 2012; **this study**
Ascampbelliellidae	*Ascampbelliella acuta*	**KY290315**	**KY290293**	**KY290335**	**This study**
Cyttarocylididae	*Cyttarocylis acutiformis*	**KY290316**	**KY290294**	**KY290336**	**This study**
	Petalotricha ampulla	**KY290317**	**KY290295**	**KY290337**	**This study**
Dictyocystidae	*Codonellopsis gaussi*	JQ924053	JQ924053	JQ924053	Kim et al., 2013
	Laackmanniella prolongata	JQ924056	JQ924056	JQ924056	Kim et al., 2013
Epiplocylididae	*Epiplocylis undella*	**KY290319**	**KY290296**	**KY290338**	**This study**
Eutintinnidae	*Eutintinnus perminutus*	KT792926*	**KY290298**	**KY290340**	Santoferrara et al., 2016a; **this study**
	Eutintinnus medius	**KY290320***	**KY290297**	**KY290339**	**This study**
Favellidae	*Favella ehrenbergii*	JN831768	**KY290309**	JN831860	Santoferrara et al., 2013; **this study**
Ptychocylididae	*Cymatocylis calyciformis*	JQ924046	JQ924046	JQ924046	Kim et al., 2013
	Ptychocylis minor	**KY290321**	**KY290299**	**KY290341**	**This study**
Rhabdonellidae	*Metacylis angulata*	**KY290322**	**KY290300**	**KY290342**	**This study**
	Rhabdonella spiralis	KT792932	**KY290307**	**KY290349**	Santoferrara et al., 2016a; **this study**
	Schmidingerella arcuata	JN831778	**KY290310**	JN831867	Santoferrara et al., 2013; **this study**
Stenosemellidae	*Stenosemella steini*	KT792927*	KM982880	KM982843	Santoferrara et al., 2015, 2016a
	Stenosemella ventricosa	KU715764	KU715804	KU715785	Zhang et al., 2016
	Stenosemella sp.	KU715763	KU715803	KU715784	Zhang et al., 2016
Tintinnidae	*Amphorellopsis quinquealata*	JQ924059	JQ924059	JQ924059	Kim et al., 2013
	Amphorides minor	**KY290324**	**KY290301**	**KY290343**	**This study**
	Salpingacantha undata	**KY290325**	**KY290302**	**KY290344**	**This study**
	Salpingacantha unguiculata	**KY290326**	**KY290303**	**KY290345**	**This study**
	Steenstrupiella steenstrupii	KT792924	**KY290308**	**KY290350**	Santoferrara et al., 2016a; **this study**
Tintinnidiidae	*Tintinnidium mucicola*	JN831800	KM982881*	JN831889	Santoferrara et al., 2013, 2015
	Tintinnidium sp. 1	JN831801	KM982882*	JN831891	Santoferrara et al., 2013, 2015
Xystonellidae	*Parafavella parumdentata*	**KY290328** *	**KY290304**	**KY290346**	**This study**
Tintinnida 5	*Tintinnopsis cylindrica*	JN831811	**KY290312** *	JN831901	Santoferrara et al., 2013; **this study**
	Tintinnopsis levigata	KM982811	KM982886	KM982847*	Santoferrara et al., 2015
Tintinnida 7	*Tintinnopsis nana*	JN831821	KM982887*	JN831909	Santoferrara et al., 2013, 2015
Tintinnida 9	*Tintinnopsis ventricosoides*	KU715776	KU715818	KU715793	Zhang et al., 2016
Tintinnida 10	*Tintinnopsis lata*	KM982810*	KM982883	KM982844	Santoferrara et al., 2015
Tintinnida 11	*Tintinnopsis parva*	JN831823	KM982889	JN831911	Santoferrara et al., 2013, 2015
	Tintinnopsis rapa	JN831834	KM982892	JN831920	Santoferrara et al., 2013, 2015
	Helicostomella subulata c. I	JN831780	KM982870	JN831872	Santoferrara et al., 2013, 2015
	Helicostomella subulata c. II	JN831781	KM982854	JN831874	Santoferrara et al., 2013, 2015
	Helicostomella subulata c. III	JN831784	KM982853*	JN831870	Santoferrara et al., 2013, 2015
Strombidiidae	*Strombidium rassoulzadegani*	AY257125*	KM982897*	JQ028733*	McManus et al., 2010; Santoferrara et al., 2012, 2015
Tontoniidae	*Laboea strobila*	**KY290331**	**KY290305**	**KY290347**	**This study**
	Pseudotontonia sp.	**KY290332**	**KY290306**	**KY290348**	**This study**
Outgroup	*Oxytricha longa*	AF508763*	AF508763*	AF508763*	Hewitt et al., 2003
	Urostyla grandis	AF508781*	AF508781*	AF508781*	Hewitt et al., 2003
	Stylonychia lemnae	AF508773*	AF508773*	AF508773*	Hewitt et al., 2003
	Halteria grandinella	AF508759*	AF508759*	AF508759*	Hewitt et al., 2003

Supplementary Table S5. Classification of Choreotrichia and Oligotrichia in the latest revisions, recent changes, and updated version proposed in this study. Our proposal is based on the system by Lynn (2008) for Choreotrichida and Oligotrichia (except *, after Agatha, 2011), Agatha and Strüder-Kypke (2013) for Tintinnida, and recent changes: [1]present study, [2]Bachy et al. (2012), [3]Liu et al. (2015a), [4]Liu et al. (2011a). Only genera that have been sequenced are included here (see all genera in Table 1).

Classification systems			
Lynn (2008)	**Agatha (2011), Agatha and Strüder-Kypke (2013)**	**Recent changes**	**This study**
Choreotrichia	-		Choreotrichia
Choreotrichida	Choreotrichida		Choreotrichida
-	Strobilidiina		-
Leegaardiellidae: *Leegaardiella*	Leegaardiellidae: *Leegaardiella*		Leegaardiellidae: *Leegaardiella*
Lohmanniellidae	Lohmanniellidae		Lohmanniellidae
-	Lynnellidae: *Lynnella*		Lynnellidae: *Lynnella*
Strobilidiidae: *Pelagostrobilidium, Rimostrombidium, Strobilidium*	Strobilidiidae: *Pelagostrobilidium, Rimostrombidium, Strobilidium*		Strobilidiidae: *Pelagostrobilidium, Rimostrombidium, Strobilidium*
Strombidinopsidae: *Parastrombidinopsis, Strombidinopsis*	Strombidinopsidae: *Parastrombidinopsis, Strombidinopsis*		Strombidinopsidae: *Parastrombidinopsis, Strombidinopsis*
Tintinnida	Tintinnina		Tintinnida
Ascampbelliellidae: *Ascampbelliella*	Ascampbelliellidae: *Ascampbelliella*		Ascampbelliellidae: *Ascampbelliella*
Codonellidae: *Codonaria, Codonella, Tintinnopsis*	-		-
Codonellopsidae: *Codonellopsis, Laackmanniella, Stenosemella*	-		-
Cyttarocylididae: *Cyttarocylis*	Cyttarocylididae: *Cyttarocylis*	*Petalotricha* added[2]	Cyttarocylididae: *Cyttarocylis, Petalotricha*
Dictyocystidae: *Dictyocysta*	Dictyocystidae: *Codonaria, Codonella, Codonellopsis, Dictyocysta; incertae sedis: Laackmanniella*		Dictyocystidae: *Codonaria, Codonella, Codonellopsis, Dictyocysta; incertae sedis: Laackmanniella*
Epiplocylididae: *Epiplocylis, Epiplocyloides*	Epiplocylididae: *Epiplocylis, Epiplocyloides*		Epiplocylididae: *Epiplocylis, Epiplocyloides*
-	-	Eutintinnidae created[2]	Eutintinnidae: *Eutintinnus*
-	-	Favellidae reestablished[1]	Favellidae: *Favella*
Metacylididae: *Climacocylis, Helicostomella, Metacylis*	Metacylididae: *Climacocylis, Helicostomella, Metacylis*	Metacylididae eliminated[1]	-
Nolaclusiliidae	Nolaclusiliidae		Nolaclusiliidae
Petalotrichidae: *Petalotricha*	Petalotrichidae: *Petalotricha*	Petalotrichidae eliminated[2]	-
Ptychocylididae: *Cymatocylis, Favella, Ptychocylis*	Ptychocylididae: *Cymatocylis, Favella, Ptychocylis*	*Favella* transferred[1]	Ptychocylididae: *Cymatocylis, Ptychocylis*
Rhabdonellidae: *Protorhabdonella, Rhabdonella*	Rhabdonellidae: *Protorhabdonella, Rhabdonella, Schmidingerella*	*Metacylis* added[1]	Rhabdonellidae: *Metacylis, Protorhabdonella, Rhabdonella, Schmidingerella*
-	Stenosemellidae: *Stenosemella*		Stenosemellidae: *Stenosemella*
Tintinnidae: *Amphorellopsis, Amphorides, Dadayiella, Eutintinnus, Salpingacantha, Salpingella, Steenstrupiella*	Tintinnidae: *Amphorellopsis, Amphorides, Dadayiella, Eutintinnus, Salpingacantha, Salpingella, Steenstrupiella*	*Eutintinnus* transferred[2] *Dadayiella* transferred[1]	Tintinnidae: *Amphorellopsis, Amphorides, Salpingacantha, Salpingella, Steenstrupiella*
Tintinnidiidae: *Tintinnidium, Leprotintinnus*	Tintinnidiidae: *Tintinnidium, Leprotintinnus*	*Leprotintinnus* is *incertae sedis*[1]	Tintinnidiidae: *Tintinnidium*
Undellidae: *Undella*	Undellidae: *Undella*	*Parundella* added[1]	Undellidae: *Parundella, Undella*
Xystonellidae: *Parafavella, Parundella, Xystonella*	Xystonellidae: *Parafavella, Parundella, Xystonella*	*Dadayiella* added[1] *Parundella* transferred[1]	Xystonellidae: *Parafavella, Xystonella; incertae sedis: Dadayiella*
Incertae sedis: Rhizodomus, Stylicauda	*Incertae sedis: Rhizodomus, Stylicauda, Tintinnopsis*	*Climacocylis*[2], *Helicostomella*[1] and *Leprotintinnus*[1] are *incertae sedis*	*Incertae sedis: Climacocylis, Helicostomella, Leprotintinnus, Rhizodomus, Stylicauda, Tintinnopsis*
Nomen inquirendum: Coxliella	*Nomen inquirendum: Coxliella*		*Nomen inquirendum: Coxliella*
Oligotrichia	Oligotrichia		Oligotrichia
Strombidiida	Oligotrichida		Strombidiida
-	Cyrtostrombidiidae: *Cyrtostrombidium*		Cyrtostrombidiidae*: *Cyrtostrombidium*
-	Pelagostrombidiidae: *Limnostrombidium*		Pelagostrombidiidae*: *Limnostrombidium*
Strombidiidae: *Cyrtostrombidium, Laboea, Novistrombidium, Omegastrombidium, Parallelostrombidium, Spirostrombidium, Strombidium*	Strombidiidae: *Apostrombidium, Novistrombidium, Omegastrombidium, Parallelostrombidium, Spirostrombidium, Strombidium, Varistrombidium*	*Antestrombidium*[3], *Sinistrostrombidium*[3], and *Williophrya* created[4]	Strombidiidae: *Antestrombidium, Apostrombidium*, Novistrombidium, Omegastrombidium, Parallelostrombidium, Sinistrostrombidium, Spirostrombidium, Strombidium, Varistrombidium*, Williophrya*
Tontoniidae: *Pseudotontonia, Spirotontonia*	Tontoniidae: *Laboea, Pseudotontonia, Spirotontonia*		Tontoniidae: *Laboea*, Pseudotontonia, Spirotontonia*

Supplementary Table S6. Informal classification of *incertae sedis* in Tintinnida. In this study, clades including *Tintinnopsis* and closely-related related genera (*Climacocylis, Helicostomella, Leprotintinnus, Rhizodomus* and *Stylicauda*) are labeled as Tintinnida 1 to 11 (based on RAxML support >70% in the SSU rDNA tree shown in Fig. 3 and S3). Some isolated branches (i.e., including only one species) are also given a label because they may host more species and become clades as more taxa are sequenced. Matching clade numeration among studies was generally impossible, but we attempted to arrange the table rows as coherently as possible. Superscripts indicate probably [1]a different *Tintinnopsis* species; [2]a *Tintinnopsis* species; [3]not a *Tintinnopsis* species; [4]an invalid genus (see Fig. S3).

Bachy et al. (2012)	Agatha and Strüder-Kypke (2014)	Zhang et al. (2016)	This study
Clade I: *T. cylindrica* JQ408206[1], *T. radix* **Problems:** not monophyletic; excludes related *incertae sedis* (*C. scalaria*)	***Tintinnopsis* clade I:** *T. lacustris, T. parvula, T. tocatinensis*, *T. tubulosoides*, *T. uruguayensis, T. cylindrica* FJ196075 **Problem:** low phylogenetic support	**TIPS I-1:** *T. cylindrica* JQ408206[1], *T. lobiancoi*, *T. pseudocylindrica* (as *T.* sp 9), *T.* sp JN871723 **TIPS I-2:** *T. radix* **Problems:** TIPS I is not monophyletic; excludes related *incertae sedis* (*C. scalaria, L. nordqvisti, R. tagatzi, S. platensis, Coxliella* sp[4])	**Tintinnida 2:** *T. cylindrica* JQ408206[1], *T. lobiancoi*, *T. pseudocylindrica*, *T. radix, C. scalaria, C. scalaroides, L. nordqvisti, R. tagatzi, S. platensis, Coxliella* sp.[4]
Clade II: *T. rara*	***Tintinnopsis* clade II:** *T. beroidea*, *T. bütschlii*, *T. dadayi*, *T. major*	**TIPS II:** *T. rara*	**Tintinnida 1:** *T. beroidea*, *T. bütschlii*, *T. dadayi*, *T. major*
Clade III: *Codonella cratera*[2], *T. lacustris, T. cylindrica*, *T. tocatinensis*, *T. tubulosoides, T. uruguayensis*, *T.* sp 1, *T.* sp 3	***Tintinnopsis* clade III:** *T. cylindrica* JQ408206[1], *T. lobiancoi*, *S. platensis* **Problems:** not just a *Tintinnopsis* clade; *T. radix* and *R. tagatzi* not included in any clade	**TIPS III-1:** *T. acuminata* (as *T.* sp 4), *T. baltica*, *T. fistularis*, *T. nana*, *T.* sp 1, *T.* sp 3 **TIPS III-2:** *T. parvula* JN831825 **TIPS III-3:** *T. cylindrica*, *T. tocatinensis*, *T. tubulosoides*, *T. uruguayensis*, *T.* sp. KU715775 **Problem:** TIPS III is not monophyletic	**Tintinnida 3:** *Codonella cratera*[2], *T. lacustris*
Clade IV: *T. lohmanni, T. subacuta*, *T.* sp 2 **Problem:** not monophyletic	***Tintinnopsis* clade IV:** *T. baltica*, *T. nana*, *T. rara* **Problem:** low phylogenetic support	**TIPS IV:** *T. subacuta*, *T.* sp 2	**Tintinnida 4:** *T. parvula* JN831825
Clade V: *T. fimbriata*[3], *Stenosemella ventricosa* **Problem:** probable Dictyocystidae	***Tintinnopsis* clade V:** T. *lohmanni*, *T. subacuta* **Problem:** low phylogenetic support	**TIPS V:** *T. fimbriata*[3] **Problems:** not a clade or isolated branch; probable Dictyocystidae	**Tintinnida 5:** *T. cylindrica*, *T. levigata*, *T. tocatinensis*, *T. tubulosoides*, *T. uruguayensis*
***Tintinnopsis sensu stricto*:** *T. beroidea*, *T. dadayi* **Problem:** identification of the type cannot be confirmed	-	**TIPS VI:** *T. beroidea*, *T. bütschlii*, *T. dadayi*, *T. major*	**Tintinnida 6:** *T. rara*
-	**Problem:** *T. parva* and *T. rapa* not included in any clade	**TIPS VII:** *T. parva*, *T. parvula* KU715771, *T. rapa*, *T. turbinata* (as *T.* sp 5), *T. tenuis* (as *T.* sp 6)	**Tintinnida 11:** *T. parva*, *T. rapa*, *T. turbinata*, *T. tenuis*, *H. subulata*
-	-	**TIPS VIII:** *T. brasiliensis*, *T. urnula* (as *T.* sp 8), *T. ventricosoides*	**Tintinnida 9:** *T. urnula*, *T. ventricosoides*
-	-	**TIPS IX:** *T. lohmanni, T. kiangsuensis* (as *T.* sp 7)	**Tintinnida 10:** *T. lata*, *T. lohmanni, T. kiangsuensis*
-	-	**Other problems:** *T. lacustris*, *T. lata* and *T. levigata* not included in analysis	**Tintinnida 7:** *T. acuminata*, *T. baltica*, *T. nana*
-	-	-	**Tintinnida 8:** *T. subacuta*

Supplementary Figure S1. Aditional specimens sequenced (see also Fig. 1). A *Pelagostrobilidium* sp.; B *Ascampbelliella acuta*; C *Dictyocysta elegans*; D *Eutintinnus medius*; E *Eutintinnus perminutus*; F *Metacylis angulata*; G *Protorhabdonella simplex*; H *Amphorides minor*; I *Salpingacantha unguiculata*; J *Parafavella parumdentata*; K *Xystonella acus*; L *Climacocylis scalaroides*; M *Pseudotontonia* sp. Isolate number is shown. Scale = 20 µm. All species were sequenced for the first time for at least one marker.

Supplementary Figure S2. A Specimens reinvestigated (modified from Santoferrara et al., 2013). **B** *Tintinnopsis rapa* in particle-free cultures develops loricae that are similar to those of *Helicostomella*. Cells were cultured in filtered seawater with *Isochrysis* sp. and *Dunaliella tertiolecta* as food, at 19°C, for two weeks. Both taxa share a 23-nucleotide deletion in the 5' end of ITS1 that is not present in any other tintinnid sequenced so far, and are closely related in phylogenetic trees (Santoferrara et al., 2015). Scale = 20 μm.

Supplementary Figure S3. Phylogenetic tree inferred from SSU rDNA sequences. RAxML bootstrap support and MrBayes posterior probability values are shown (only if >45% and >0.90, respectively). Sequences in bold are from this study. Sequences are flagged based on their quality (Supplementary Text 3): in red, insufficient/ non-existing morphological data publication or potentially inaccurate sequencing; in green, potentially misidentified based on published morphological data. Colored backgrounds and black bars correspond to families and informal Tintinnida clades, respectively. Long branches were shortened ten times (red square).

A

Supplementary Figure S3 (continued).

Supplementary Figure S4. Phylogenetic tree inferred from ITS regions. Explanations as in Fig. S3.

Supplementary Figure S5. Phylogenetic tree inferred from LSU rDNA. Explanations as in Fig. S3.

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