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Review paper

What Morphology and Molecules Tell Us about the Evolution of Oligotrichea (Alveolata, Ciliophora)

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Abstract. The evolution of the dominant marine plankton ciliates, the oligotrichids and choreotrichids, is analysed for morphologic and genetic convergences and apomorphies based on literature and our own data. These findings have taxonomic implications. Within the oligotrichid genus *Parallelostrombidium* two subgenera, *Parallelostrombidium* Agatha, 2004 nov. stat. and *Asymptokinetum* nov. subgen., are established, using the courses of the ventral and girdle kineties as a distinguishing feature. Likewise, a different arrangement of extrusome attachment sites is used for a split of the oligotrichid genus *Novistrombidium* into the subgenera *Novistrombidium* Song and Bradbury, 1998 nov. stat. and *Propecingulum* nov. subgen.; *Novistrombidium* (*Propecingulum*) *ioanum* (Lynn and Gilron, 1993) nov. comb. and *Novistrombidium* (*Propecingulum*) *platum* (Song and Packroff, 1997) nov. comb. are affiliated. Based on discrepancies in the somatic ciliary pattern and the presence of conspicuous argyrophilic inclusions, the aloricate choreotrichid species *Pelagostrobilidium kimae* nov. spec. is distinguished from *P. conicum*. The diagnosis for the tintinnid family Eutintinnidae Bachy *et al*., 2012 is improved by including cell features. The co-operation of taxonomists and molecular biologists is strongly recommended to prevent misinterpretations of gene trees due to incorrectly identified species and for better species circumscriptions.

Key words: Choreotrichids, cladistic analyses, gene sequence analyses, oligotrichids, tintinnids, somatic ciliature.

INTRODUCTION

The marine plankton is a highly diverse community of organisms, among which we find the Oligotrichea, a ciliate taxon that episodically dominates the microzooplankton (Pierce and Turner 1992). The classification of the Oligotrichea in this paper follows Agatha (2004b) and is based on the relationships revealed by cladistic analyses and genetic phylogenies, except for the uncertain position of the halteriids (see below). As the Oligotrichea have species-specific trophic requirements (e.g., algivorous, bacterivorous, mixotrophic), proper identification is essential (i) for appreciating their role in the multi-step microbial food web and energy flux to the conventional planktonic food web and (ii) for estimating their biodiversity and biogeography (Agatha 2011a).

Since the last combined cladistic and phylogenetic analyses of the Oligotrichea, specifically of the tintin-

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nids (Agatha and Strüder-Kypke 2012a, b), there was a considerable progress, yielding a wealth of new genetic and morphologic data published in separate papers: (i) ~ 65 new SSU rRNA gene sequences (Bachy *et al.* 2012; Liu *et al.* 2012; Saccà *et al.* 2012; Santoferrara *et al.* 2013; Xu *et al.* 2012, 2013; Kim *et al.* 2013); (ii) cell features in two further genera (Saccà *et al.* 2012, Kim *et al.* 2013); and (iii) new somatic ciliary patterns in the tintinnid genus *Tintinnopsis* (Jiang *et al.* 2012) and the aloricate choreotrichid genus *Pelagostrobilidium* (Lee *et al.* 2011, Liu *et al.* 2012). The inclusion of these morphologic findings, new features (e.g., the somatic ciliary pattern evolution in *Pelagostrobilidium*), and ciliary patterns inferred from illustrations in Small and Lynn (1985) raised the number of considered taxa from 49 in Agatha and Strüder-Kypke (2012a, b) to 67 in the present review and the number of cladistically analysed characters from 76 to 94. Here, we thus present the current state of knowledge about the evolution of Oligotrichea based on concerted analyses of morphology and molecules.

Based on morphology and pattern of cell division, the Oligotrichea comprise the halteriids, oligotrichids, and choreotrichids (Agatha 2004b, Agatha and Foissner 2009). The most prominent feature of the Oligotrichea is the apical adoral zone of membranelles (C-shaped or circular arrangement of fan-like ciliary units), which is used for locomotion and filter feeding. The zone is divided into portions with large collar membranelles and small buccal membranelles. While the halteriids and oligotrichids contain exclusively aloricate species, the choreotrichids embrace besides naked species the tintinnids with loricae usually 50–300 µm long. Typically, the aloricate Oligotrichea have globular to obconical cell shapes, measure 15–260 µm in length, and have a reduced somatic ciliature with possibly sensory function. The tintinnid cells are attached by a peduncle to the bottom of the lorica, which they carry through the water. The cells are obconical in extended state and the anterior cell portion with the adoral zone of membranelles projects out of the lorica. Disturbance causes a retraction of the tintinnid into the lorica by contraction of the peduncle, and the cell assumes a globular to ellipsoidal shape. The somatic ciliature of tintinnids consists of numerous ciliary rows, which, in contrast to the aloricate taxa, are not exposed to the surrounding water, but are mostly covered by the lorica; it is assumed that the somatic ciliature is involved in lorica formation (Laval-Peuto 1981).

The first species assigned to the Oligotrichea were (i) the halteriid *Halteria grandinella* (Müller, 1773) Dujardin, 1841, (ii) the oligotrichid *Strombidium sulcatum* Claparède and Lachmann, 1859, (iii) the aloricate choreotrichid *Strobilidium caudatum* (Fromentel, 1876) Foissner, 1987, and (iv) the tintinnid *Tintinnus inquilinus* (Müller, 1776) Schrank, 1803. While the investigation of live and preserved specimens revealed only some characters in the aloricate taxa, the vase- or tube-shaped tintinnid loricae provided several features for identification and classification. In the 1950s, histological staining procedures commenced to reveal the ciliary patterns and allowed their usage in taxonomy and systematics. Notably, protargol impregnation, which stains basal bodies and the nuclear apparatus (macronuclei and micronuclei), is still routinely used today. The introduction of electron microscopy provided further insights into cell morphology, especially, into kinetid structure (basal body and associated root structures). A new era of phylogenetic analyses commenced with the introduction of gene sequencing. In particular, the small subunit ribosomal RNA (SSU rRNA) gene is frequently used to infer relationships among ciliate taxa.

The most recent monographs on halteriids, oligotrichids, and aloricate choreotrichids were based on live and preserved material only and regarded 127 species, 14 genera, and three families as valid (Maeda and Carey 1985, Maeda 1986). The application of silver-impregnation techniques has yielded many new species, whose number continuously increases. However, the rate of discovery was and still is distinctly influenced by the trend to neotypify species rather than to establish new ones, assuming that the majority of species have a cosmopolitan distribution. Accordingly, the intensity of taxonomic studies during the past thirty years was much higher than implied by the rate of discovery (Agatha 2011a). Currently, the number of reliable species amounts to 15 halteriid species in three genera and one family, 115 oligotrichid species in 18 genera and four families, and 50 species of aloricate choreotrichids in nine genera and five families; the majority of them have been redescribed, applying modern methods (live observation, silver impregnation, electron microscopy; own data). The more than one thousand tintinnid species are classified in 75 genera and 14 families, still mainly using lorica characteristics, as cell features have properly been described in only 29 species (Agatha and Strüder-Kypke 2012a, b; Jiang *et al.* 2012; Saccà *et al.* 2012; Kim *et al.* 2013).

The introduction of new investigation methods not only revealed new characters and contributed to the discovery of new species, but cladistic and phylogenetic analyses of these data also provided arguments for the establishment of new higher taxa and an improvement of the systematics (Agatha 2004a, b, 2011b; Agatha and Strüder-Kypke 2007, 2012a, b). Specifically, the evolution of the somatic kinetids and the somatic ciliary patterns turned out to be of high taxonomic and systematic significance within the Oligotrichea. The main clue for the evolutionary reconstruction was the orientation of the somatic kineties (ciliary rows), in particular the fact that only the anterior basal bodies of the dikinetids (basal body pairs) are associated with a cilium in the (dorsal) somatic kineties of the closely related euplotid (e.g., *Euplotes*) and hypotrich (e.g., *Oxytricha*) ciliates. In the following, the evolution of the oligotrichids and choreotrichids is reviewed, emphasizing homoplasies and apomorphies and their implications on taxonomy and systematics. The halteriids with their distribution mainly in freshwater and their controversial phylogenetic relationships (sistergroup to oligotrichids and choreotrichids according to morphology and mode of cell division, but members of the hypotrichs according to SSU rRNA analyses; Snoeyenbos-West *et al.* 2002, Agatha and Foissner 2009) are excluded here.

RESULTS AND DISCUSSION

1. Oligotrichids. The oligotrichids are the sistergroup to the choreotrichids. Both differ in the arrangement of the adoral zone of membranelles (C-shaped vs. circular pattern) and the origin of the oral primordium (oral apparatus of posterior division product develops in a subsurface tube vs. a pouch).

Although the somatic ciliature comprises only two kineties, namely, the ventral and girdle kineties with an ancestral kinetid structure, 13 patterns are currently known (Fig. 1). Four girdle kinety patterns occur in the tailed family Tontoniidae and also in tailless taxa (Figs 1II, IV–VII; Agatha 2011b). Due to the unique ultrastructure of the contractile tail (probably sea anchor function), Agatha (2004a) supposed an independent development of these four patterns in the tailed and tailless genera. This is actually supported by ontogenetic (positions of oral primordia) and genetic data (Figs 1IV, VI, 2). However, Agatha's hypothesis apparently failed concerning the monotypic tailless genus *Laboea* and the tailed genus *Spirotontonia* (Fig. 1VII), both with an identical somatic ciliary pattern. The genetic analyses by Gao *et al.* (2009) indicated that the pattern developed only once and that *Laboea strobila* lost the tail, regaining the plesiomorphic state; shared unique SSU rRNA regions, topology testing (Li *et al.* 2013), and some cladograms based on morphologic features support this assumption (Supplement Figs S3, S6, Tables S1, S2). The Tontoniidae apparently branched off rather early in the oligotrichid evolution (Figs 1, 2). The position of the oral primordium relative to the girdle kinety and extrusome attachment sites was a valuable taxonomic feature to split the speciose genus *Strombidium* and to establish two further genera, *Foissneridium* and *Opisthostrombidium* (Figs 1V, VIII, IX; Agatha 2011b).

The tailless genera *Apostrombidium* and *Varistrombidium* Xu, Warren, and Song, 2009 were established for strongly deviating, partially very complex somatic ciliary patterns (Xu *et al*. 2009). Agatha (2011b) supposed their origin in the tailless genus *Omegastrombidium* (Figs 1IV, XII, XIV); actually, the close relationship of the three genera is supported by ontogenetic and genetic data (Gao *et al.* 2009, Xu *et al.* 2011, Song *et al.* 2013; Fig. 2).

The tailless genus *Parallelostrombidium* is assumed to represent the most ancestral oligotrichid pattern (Fig. 1I). Differences in the extent to which the ventral kinety and the dextrally spiralled girdle kinety run parallel indicate the presence of two subgenera (see 'Taxonomic implications'; Figs S7, S8). The *Parallelostrombidium* pattern probably gave rise to the *Novistrombidium* pattern (Fig. 1II). Discrepancies mainly in the position of the extrusome attachment sites in relation to the oral primordium support two genetically distinct groups of *Novistrombidium* species (Figs 2, S10, S11; Li *et al.* 2013, Song *et al.* 2013), for which two subgenera are established (see 'Taxonomic implications').

In contrast to the great diversity of somatic ciliary patterns, whose evolutionary advantages are unknown, the oral ciliature is rather conserved in oligotrichids. Only in the genus *Cyrtostrombidium*, do the extraordinarily thick pharyngeal fibres and the absence of buccal membranelles and an endoral membrane justify the establishment of a distinct family, the Cyrtostrombidiidae (Agatha 2004a). A further family, the Pelagostrombidiidae, was established for freshwater genera characterized by a neoformation organelle (permanent tube, in which the posterior divider forms its oral apparatus; Agatha 2004a). The remaining genera are assigned to the family Strombidiidae, which is paraphyletic in cla-

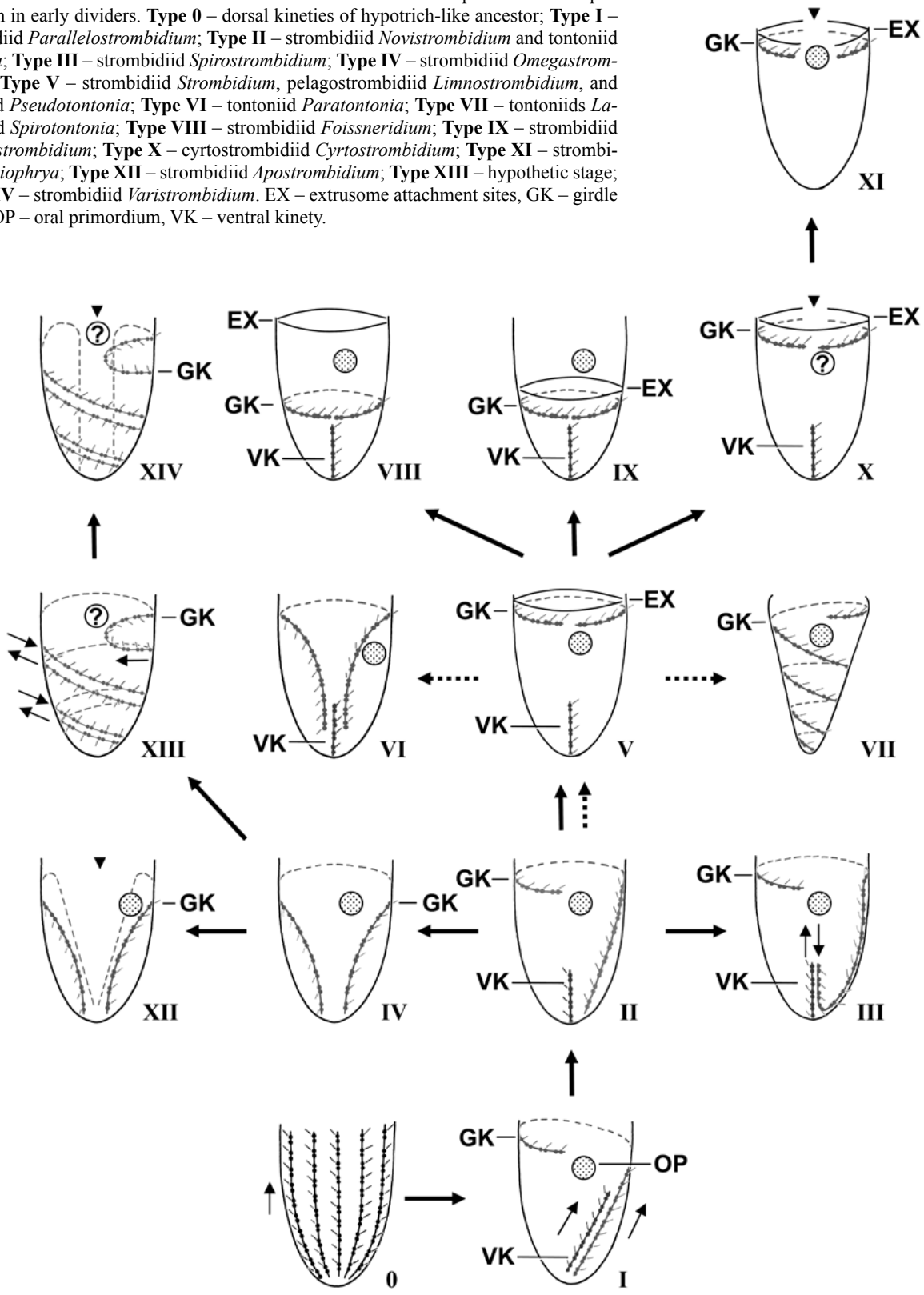

Fig. 1. Hypothetical evolution of oligotrichid somatic ciliary patterns (0–IV, VI, VII, after Agatha 2011b; V, VIII–XIV, originals; protargol impregnation). Small arrows mark orientation of kineties (posterior to anterior). Arrowheads denote dorsal breaks in girdle kinety. Dotted arrows mark the tontoniid evolution. Dotted circles denote position of oral primordium in early dividers. **Type 0** – dorsal kineties of hypotrich-like ancestor; **Type I** – strombidiid *Parallelostrombidium*; **Type II** – strombidiid *Novistrombidium* and tontoniid *Tontonia*; **Type III** – strombidiid *Spirostrombidium*; **Type IV** – strombidiid *Omegastrombidium*; **Type V** – strombidiid *Strombidium*, pelagostrombidiid *Limnostrombidium*, and tontoniid *Pseudotontonia*; **Type VI** – tontoniid *Paratontonia*; **Type VII** – tontoniids *Laboea* and *Spirotontonia*; **Type VIII** – strombidiid *Foissneridium*; **Type IX** – strombidiid *Opisthostrombidium*; **Type X** – cyrtostrombidiid *Cyrtostrombidium*; **Type XI** – strombidiid *Williophrya*; **Type XII** – strombidiid *Apostrombidium*; **Type XIII** – hypothetic stage; **Type XIV** – strombidiid *Varistrombidium*. EX – extrusome attachment sites, GK – girdle kinety, OP – oral primordium, VK – ventral kinety.

distic analyses (Figs S3–S6), as morphologic features for a split are currently not available; however, the arrangement of the extrusome attachment sites (clustered or in one or several rows) might be a promising feature, but more data are required for verification.

Gene trees consider only 11 out of the 18 oligotrichid genera and two out of the four families, and tree topologies vary, depending on the phylogenetic analysis used (compare unsupported nodes in Fig. 2 and supplemental Fig. S1). Nevertheless, several branches are supported by morphologic features (see above), especially by the evolution of the somatic ciliary patterns. While the family Tontoniidae and genera therein are fully supported in the phylogenetic analysis, support values for basal nodes in the family Strombidiidae are generally very low (Fig. 2).

2. Aloricate choreotrichids. Both cladistic and genetic analyses usually show (i) a monophyly of the tintinnids mainly based on the apomorphic lorica and (ii) a paraphyly of the aloricate choreotrichids (Figs 3, S2–S5, S12). Since different numbers of genera are considered in the cladograms and gene trees (aloricate choreotrichids: 9 vs. 6; tintinnids: 15 vs. 30), more detailed comparisons are difficult, especially since the tree topology is also influenced by the methods of analysis (compare Figs 3 and S2 for molecular analyses alone).

Immediately after separation from the oligotrichids, the structure of the somatic kinetids commenced to change in the choreotrichids, viz., a second cilium formed at the posterior dikinetidal basal body, producing a pattern found for instance in the aloricate genus *Strombidinopsis* (Fig. 4; Lynn *et al.* 1991). The subsequent steps occurred several times independently in (i) the tintinnids, (ii) the Lohmanniellidae and Strobilidiidae, and (iii) the genus *Lynnella*: the cilium at the anterior basal body is lost, and finally, the unciliated anterior basal body disappears. These comparatively rapid changes and the resulting diversity of kinetid structures in the choreotrichids contradict the structural conservatism of the somatic cortex hypothesized by Lynn (1981); but the reasons for and the advantages of these structural changes are unknown. In tintinnids, this transformation process is accompanied by the introduction of specialised ciliary fields and rows (see below).

The ventrally slightly opened adoral zone of membranelles in *Parastrombidinopsis*, *Parastrombidium*, and *Lynnella* is interpreted as synapomorphic retrogression to the plesiomorphic (open) state, as this feature is combined with an advanced kinetid structure, elongated bases (polykinetids) in the proximal collar membranelles, and a stomatogenesis within a pouch, features typical of choreotrichids (Fig. S12, Tables S1, S2; Agatha and Strüder-Kypke 2012a). In cladograms and gene trees, however, the position of *Lynnella* is highly variable, occasionally even suggesting an affiliation with the oligotrichids or a sistergroup relationship to the remaining choreotrichids (Figs 3, S2–S5, S12; Xu *et al.* 2012). The inclusion of gene sequences from *Leegaardiella*, *Lohmanniella*, and *Parastrombidium* will show whether (i) *Lynnella* is closely related to *Parastrombidium* and *Parastrombidinopsis* as indicated by the shape of the adoral zone and the latter two genera have to be affiliated with the family Lynnellidae or (ii) *Lynnella* is related to *Lohmanniella* as indicated by a similar structure of the somatic kinetids.

Usually, the genera *Pelagostrobilidium*, *Rimostrombidium*, and *Strobilidium* form a monophylum, the family Strobilidiidae, based on kineties composed of condensed monokinetids (single basal bodies) and cytoplasmic lips covering the bases of their cilia (Figs S3–S5, S12); this cluster is also revealed by molecular phylogenies (Figs 3, S2; Agatha and Strüder-Kypke 2007). The diversity of somatic ciliary patterns is comparatively large in *Pelagostrobilidium* (Figs S14–27). Probably, the *Rimostrombidium*-like ancestor had six more or less straight somatic kineties. The curvature of kinety 2 seems to be the most important feature for taxonomy and inferring intrageneric relationships: first, the kinety became anteriorly shortened, then sigmoidal, subsequently semicircular, and finally it performed a ~ 270° curvature. Posterior shortenings occurred several times independently in kineties 3–6. In the absence of kinety 5, kinety 6 performed distinct curvatures. Korean specimens identified by Lee *et al*. (2011) with *Pelagostrobilidium conicum*, as authoritatively redescribed by Agatha and Riedel-Lorjé (1998), deviate in the length of somatic kineties 1 (posteriorly shortened vs. unshortened) and 2 (anteriorly vs. posteriorly shortened) and an argyrophilic C-shaped structure near the collar membranelles (present vs. absent). These differences justify the establishment of a distinct species for the Korean specimens (see 'Taxonomic implications').

3. Tintinnids. The most conspicuous apomorphy of the tintinnids is the lorica, which probably acts as sea anchor (Jonsson *et al.* 2004) and may show a phenotypic plasticity caused by environmental conditions during its formation and the cell cycle (Laval-Peuto 1981, Agatha *et al*. 2012); the most reliable lorica fea-

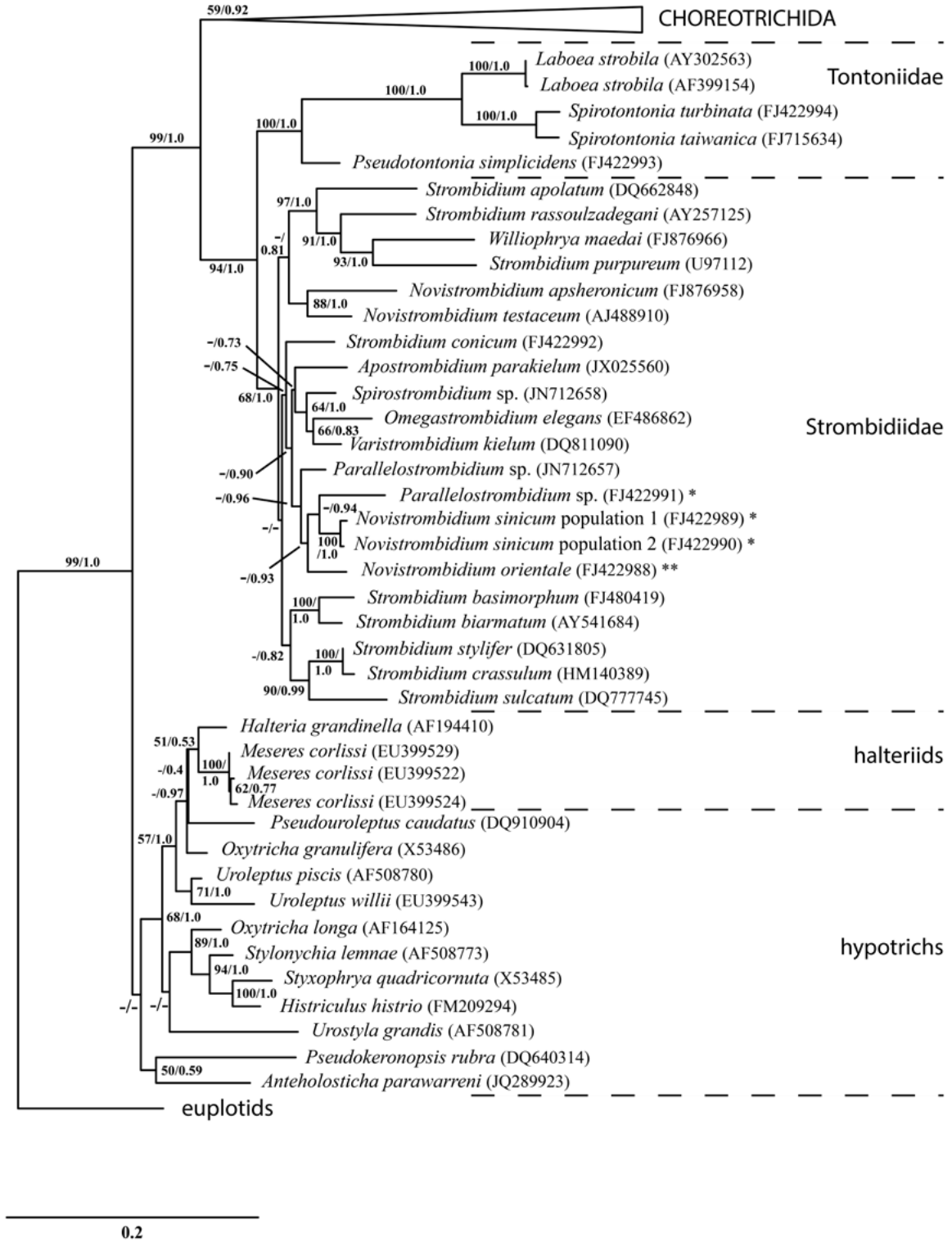

Fig. 2. Maximum Likelihood tree of the Oligotrichida inferred from small subunit ribosomal RNA (SSU rRNA) gene sequences (66 taxa and 1823 nucleotide positions) aligned with the Muscle algorithm (Edgar 2004) implemented in MEGA ver. 5.1 (Tamura *et al.* 2011). The alignment is available upon request. The tree was computed with RAxML (Stamatakis *et al.* 2008) and the datasets were bootstrap re-sampled 100 times. Support values are listed at the nodes. The second values at the nodes represent the posterior probability values of a Bayesian Inference analysis performed with MrBayes (Ronquist and Huelsenbeck 2003). Values below 50% and 0.5, respectively, are represented by a dash. * – initially published as *Spirostrombidium* sp.; ** – initially published as *Parallelostrombidium* sp.

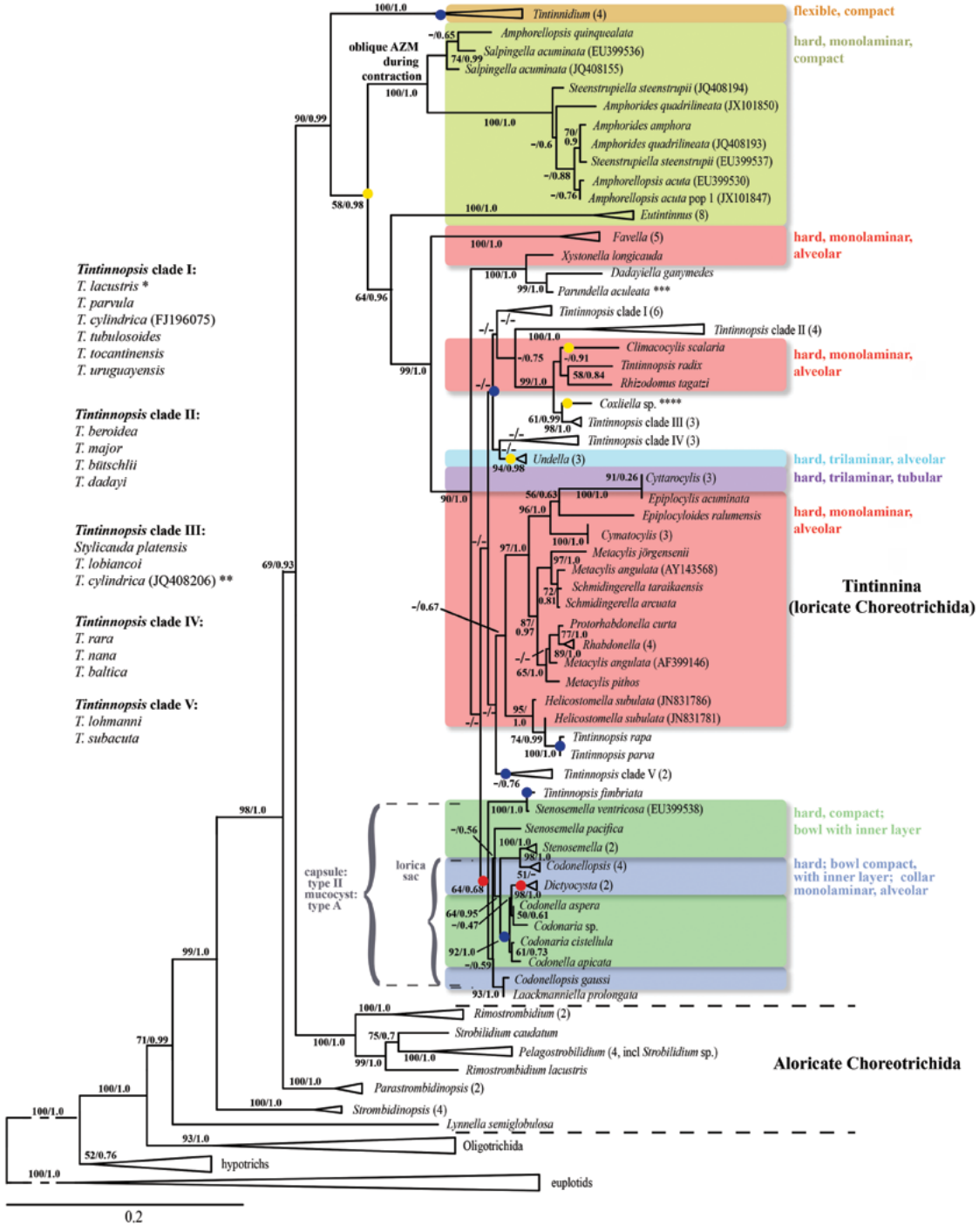

Fig. 3. Maximum Likelihood tree of the Choreotrichida inferred from small subunit ribosomal RNA (SSU rRNA) gene sequences (138 taxa and 1859 nucleotide positions) aligned with the Muscle algorithm (Edgar 2004) implemented in MEGA ver. 5.1 (Tamura *et al.* 2011). The alignment is available upon request. The tree was computed with RAxML (Stamatakis *et al.* 2008) and the datasets were bootstrap re-sampled 100 times. Support values are listed at the nodes. The second values at the nodes represent the posterior probability values of a Bayesian Inference analysis performed with MrBayes (Ronquist and Huelsenbeck 2003). Values below 50% and 0.5, respectively, are represented by dashes. Branches with unambiguously clustered taxa are collapsed, species of the genus *Tintinnopsis* grouped in 5 different clades numbered I–V. Most common lorica structures: ● – hyaline; ● – entirely agglomerated; ● – composed of hyaline collar and agglomerated bowl; * – after Kofoid and Campbell (1929) a synonym of *Codonella cratera*; ** – does not correspond with the redescription of Agatha and Riedel-Lorjé (2006); *** – possibly incorrectly identified, might be *Dadayiella acutiformis*; **** – invalid taxon, very likely a replacement lorica (see text).

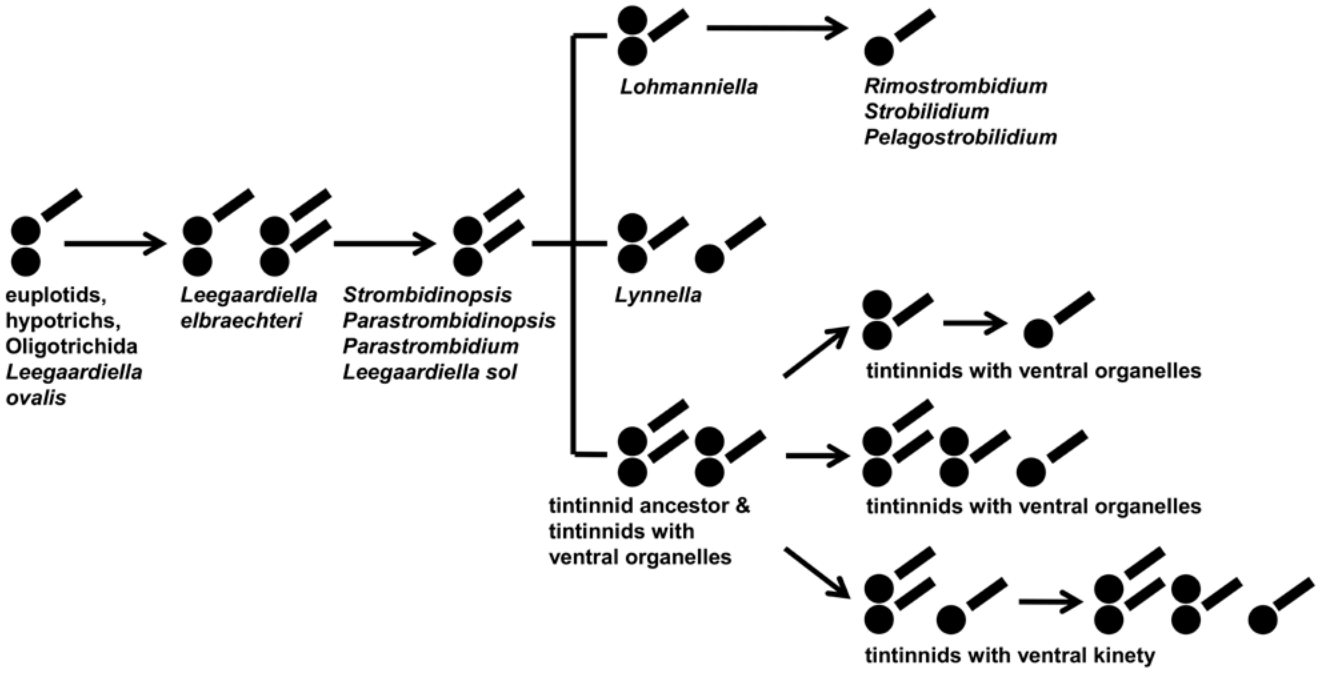

Fig. 4. Evolution of kinetid structures in the somatic ciliature of choreotrichid ciliates. The aloricate taxa have only one kinetid type, except for *Leegaardiella elbraechteri* and *Lynnella*. Tintinnids with ventral organelles have two (*Tintinnidium*, subgenus *Tintinnidium*), rarely one (*Tintinnopsis cylindrata*, *Membranicola*) or three (*Tintinnidium*, subgenus *Semitintinnidium*) kinetid types. Extant tintinnids with a ventral kinety have some dikinetids with two cilia and many monokinetids or some dikinetids with two cilia, some dikinetids with one cilium, and many monokinetids.

tures for species identification are apparently the general outline, details of the opening rim, the wall texture, and the opening diameter (Laval-Peuto and Brownlee 1986). Santoferrara *et al*. (2013) phylogenetically analysed variable regions of the SSU and LSU rRNA of tintinnids. They distinguished three taxonomic tendencies: (i) similarities and deviations in lorica shapes and sizes and gene sequences are consistent in the majority of morphospecies; (ii) some/several similar morphospecies show a high genetic divergence, supporting Kofoid's and Campbell's (1929, 1939) splits based on minute deviations in lorica morphology; and (iii) some/several morphologically distinct morphospecies are genetically identical or very similar. This has been shown for the genera *Cymatocylis*, *Favella*, *Rhabdonella*, and *Helicostomella*, in which distinct morphospecies deviate genetically by less than 1% in both the SSU and LSU rRNA sequences (Bachy *et al.* 2012, Xu *et al.* 2012, Kim *et al.* 2013). However, conspecificity cannot automatically be inferred from a high genetic similarity as demonstrated in two *Helicostomella* morphotypes. Despite a dissimilarity of only 0.5% in the SSU rRNA, compensatory base changes in the ITS2 helices II and III indicate the presence of two distinct species (Xu *et al*. 2012). Accordingly, the percentage of synonyms among the more than one thousand tintinnid species is still hardly guessable.

The genetic diversity within single morphotypes found by Kazama *et al*. (2012) markedly exceeds that of any previous molecular study on tintinnids, aloricate choreotrichids, and oligotrichids (see also Gong *et al.* 2013). For instance, the same *Tintinnopsis* morphospecies (with entirely agglomerated lorica) atypically clusters at the beginning of the tintinnid evolution with *Amphorellopsis*, *Steenstrupiella*, and *Salpingella* (all with hyaline loricae, a comparatively simple somatic ciliary pattern, and an oblique adoral zone in contracted cells; Figs S52–61) and also occurs in the tintinnid branch with the most complex ciliary pattern. Since previous and the present studies found the first occurrence of *Tintinnopsis* species only after the branching of *Favella* (Figs 3, S2, S3, S5, S12), methodological errors (misidentification of the species or contamination of the extracted DNA) might have caused these distinctly de-

viating sequences in single morphotypes. Accordingly, the sequences deposited by Kazama *et al.* (2012) in GenBank are not suitable as references in phylogenetic analyses.

Helical structures may constitute collars (epiloricae) or entire loricae of hyaline and agglomerated types. The *Coxliella* species with their completely helical loricae probably represent replacement loricae (paraloricae) built independently from cell division when lorica forming material is less abundant in the cell and construction is thus slower, or they are formed after cell division when the material is possibly less viscous (Laval-Peuto 1981, 1994). The presence of "*coxliella*" forms could be shown for *Favella* (Laval-Peuto 1981, Kim *et al.* 2010) and *Schmidingerella* (Agatha and Strüder-Kypke 2012a), whereas clear evidence from cultures is lacking for *Helicostomella*, *Parafavella* (all hyaline), and some *Tintinnopsis* species (entirely agglomerated; Laval-Peuto and Brownlee 1986). As already assumed by Brandt (1907), the genus *Coxliella* thus seems to be artificial, whereas Xu *et al.* (2013) regarded high abundances of *Coxliella* sp. and the apparent absence of a typical form as support for the validity of the genus *Coxliella*. The cell cycle of *Favella*, however, demonstrated that under certain (unknown) conditions the "*coxliella*" form can produce the spiralled "*decipiens*" form after cell division (Laval-Peuto 1981); so, the typical form must not necessarily occur. Further, the typical form might have been too rare to be detected in the study of Xu *et al.* (2013). In several other genera, helical structures seem to be absent, e.g., *Petalotricha*, *Salpingella* (both hyaline), *Codonella* (agglomerated), and *Dictyocysta* (partially or entirely hyaline; Agatha *et al.* 2012).

Both genetic and cladistic analyses reveal that different lorica types (hyaline, entirely or partially agglomerated) do not form distinct evolutionary lineages (Figs 3, S2–5, S12; Strüder-Kypke and Lynn 2008); only the branch of freshwater tintinnids with two ventral (ciliary) organelles is characterized by a special type, namely, a flexible and agglomerated lorica with a compact matrix. In the mainly marine tintinnids with a ventral kinety, the lorica walls were probably first hard, hyaline, and compact (*Amphorellopsis*, *Amphorides*, *Eutintinnus*, *Salpingella*, *Steenstrupiella*; the collapsible lorica of *Nolaclusilis* represents an autapomorphy), became hard, hyaline, and monolaminar with alveoli (Figs S28–38), and then the first hard, agglomerated loricae occurred, as indicated by the increasing complexity of the somatic ciliary pattern. Further hard, hyaline loricae are found in *Undella* (trilaminar with alveoli), *Cyttarocylis*, and *Petalotricha* (trilaminar with tubules; genetic data even indicate a synonymy; Bachy *et al.* 2012). Finally, the wall of the hard, agglomerated lorica portions became apparently bilayered, viz., composed of a thick outer layer with particles embedded into compact material and a continuous compact inner layer (Figs S39–51; *Codonella*, *Codonellopsis*, *Stenosemella*, and probably *Dictyocysta* and *Codonaria*). Hyaline collars that might be associated with such agglomerated bowls are compact (*Stenosemella*; own data) or alveolar (*Codonellopsis*, *Dictyocysta*; own data, Laval-Peuto 1994); however, the texture of the entirely hyaline and fenestrated lorica in *Dictyocysta mitra* seems to be tubular (Agatha 2010).

Together with the lorica, a right and left ciliary field evolved in the common tintinnid ancestor, giving rise to the mainly freshwater species with two ventral organelles and the mainly marine species with a ventral kinety. In the former branch, the diversity of kinetid structures is comparatively large, ranging from one to three types in a single species, while the complexity of the ciliary pattern did not change anymore (Figs 4, S12). In the marine branch, two kinetid structures first occurred together with the ventral kinety and were later completed by a third type, while the complexity of the pattern distinctly increased by the successive addition of a further field and specialised kineties (Agatha and Strüder-Kypke 2012b, this study).

Although the somatic ciliature is assumed to be involved in lorica formation, correlations between ciliary patterns, lorica types (hyaline, agglomerated), wall textures (compact, alveolar etc.), and deformability are not recognisable, except for the occurrence of a ventral kinety and hard loricae and ventral organelles and flexible loricae, respectively (Figs 3, S3–5, S12; Agatha *et al.* 2012). It seems more likely that differences in lorica type, texture, and deformability result essentially from differences in the lorica forming material, whose main chemical component is probably of proteinaceous nature with variable additions of, e.g., lipids and carbohydrates. However, a correlation between lorica features and the characteristics of the lorica material was again not evident (Agatha and Simon 2012, Agatha *et al.* 2012).

A detailed comparison of gene trees with cladograms is currently impeded by differences in the species and genera analysed (Figs 3, S3–5, S12). Nevertheless, the evolution of somatic ciliary patterns is the main feature complex in cladistic analyses of tintinnids and is rather well reflected by genetic phylogenies.

Further morphologic characters support several groupings in the molecular genealogies. The lorica sac with its foldable closing apparatus represents a synapomorphy of the genera *Codonaria*, *Codonella*, *Codonellopsis*, and *Dictyocysta* (Agatha 2010). *Stenosemella* and *Laackmanniella* are closely related to these four genera phylogenetically, but apparently lack a lorica sac (Fig. 3; Kim *et al.* 2013). While the relationship of the former genus might be explained by the same type of capsule (tintinnid extrusome; Laval-Peuto and Barria de Cao 1987), further morphological studies are required in the latter genus to understand its phylogenetic placement (Kim *et al.* 2013). An oblique orientation of the oral ciliature and peristomial field in contracted specimens probably represents the synapomorphy of *Salpingella*, *Amphorellopsis*, *Amphorides*, *Steenstrupiella*, *Salpingacantha*, and *Bursaopsis obliqua* (Figs S3, S12, S52–61). Actually, the former four genera constitute a monophylum in the gene trees separate from the genus *Eutintinnus*, for which Bachy *et al*. (2012) established the family Eutintinnidae; here, the family diagnosis is emended by including cell features (see 'Taxonomic implications').

The non-monophylies of tintinnid genera in gene trees necessitate further morphologic studies, especially, of the cells. Recently, the problem of two genetically distinct groups of *Favella* species was resolved and the genus *Schmidingerella* has been established for the second cluster deviating in ciliary pattern and lorica wall texture (Figs S28, S31; Agatha and Strüder-Kypke 2012a). A monolaminar, alveolar wall with surface ridges and pores unites the new genus, *Rhabdonella* (own data), *Rhabdonellopsis* (Gold and Morales 1977), *Protorhabdonella* (Kofoid and Campbell 1929), *Epiplocylis* (Abboud-Abi Saab 2008), and *Epiplocyloides* (Laval-Peuto 1994, Abboud-Abi Saab 2008) within the family Rhabdonellidae, which is mostly supported by genetic data (Figs 3, S2, S31–36). *Cymatocylis* also falls into this genetic cluster and indeed shows also ridges on the lorica surface (Laackmann 1910, Kim *et al.* 2013). A further conspicuous non-monophyly concerns the genus *Tintinnopsis*. Although as yet four different ciliary patterns have been discovered (Fig. S12), a reasonable split of the genus can, however, currently not be performed, as the cell features of its type species, *T. beroidea*, are unknown and the determination of the specimens sequenced is uncertain (Agatha and Strüder-Kypke 2012b).

Generally, doubtful identifications or misidentifications of genetically analysed species cause serious problems in the interpretation of the trees, particularly, when it concerns type species (see also above for Kazama *et al*. 2012). For instance, a very high genetic similarity (one base pair difference in the SSU rRNA) between *Dadayiella ganymedes* sequenced by Xu *et al.* (2013) and *Parundella aculeata* analysed by Bachy *et al.* (2012) was used as argument by Xu *et al.* (2013) to transfer the type species *D. ganymedes* to the genus *Parundella* and to make the genus *Dadayiella* thus invalid. The astonishing close genetic relationship of species affiliated with different families in the lorica-based classification (Tintinnidae and Xystonellidae) caused scepticism about the determinations. Actually, the micrograph and the morphometric data of the loricae indicate that the former specimens have been confused with *Dadayiella bulbosa* (with knob at posterior lorica process vs. without in *D. ganymedes*; Entz 1884; Brandt 1906, 1907). In the second case, the specimen genetically analysed perfectly matches *P. aculeata* in lorica shape and size (lorica length: 105 µm; opening diameter: 27 µm), but also *Dadayiella acutiformis* Kofoid and Campbell, 1939 (lorica length: 82–103 µm; opening diameter: 25–30 µm; Jörgensen 1924, Kofoid and Campbell 1939). The main differences between the two species are delicate longitudinal ribs in the anterior quarter of the *Dadayiella* lorica, which might be hardly recognisable in the light microscope when the cell is inside the lorica. The small micrograph provided by Bachy *et al.* (2012) does not allow any decision about the presence of ribs, and we assume that the authors have not seen the ribs and thus identified the specimen with *P. aculeata*. But nevertheless, the astonishing genetic relationship together with the existence of a similar-sized and similar-shaped *Dadayiella* species should keep us sceptical. If the latter specimen has actually been misidentified, both morphotypes would belong to the same genus (*Dadayiella*) and possibly even to the same species, as already suggested by Jörgensen (1924). However, any nomenclatural act should await a detailed re-investigation of the lorica and cell and possibly the analysis of additional molecular markers. These examples demonstrate the urgent need for co-operation of molecular biologists with experienced taxonomists for reliable identifications.

The consultation of monographs (e.g., Kofoid and Campbell 1929, 1939) is a very helpful first step, but species determinations should finally be based on original descriptions or authoritative redescriptions, as revising authors may have changed the circumscrip-

tions by lumping species. Synonymisations should be performed only after detailed investigations of the cell (live observation, protargol impregnation) and lorica ultrastructure (electron microscopy) and genetic analyses. The variable regions (D1-D2) of the LSU rRNA gene (Santoferrara *et al*. 2013) and ITS2 sequence and secondary structure comparisons (Snoeyenbos-West *et al.* 2003, Weisse *et al.* 2006) are promising molecular markers for elucidating phylogenetic relationships and species limits in tintinnids. Therefore, we regard any taxonomic acts merely based on gene sequence data and lorica features as premature, especially, as they are occasionally in conflict with the International Code of Zoological Nomenclature (ICZN 1999). On the other hand, descriptions of new species or redescriptions should comprise gene sequence analysis besides the complete morphologic data.

4. Taxonomic implications

Genus *Parallelostrombidium* Agatha, 2004

Subgenus *Parallelostrombidium* Agatha, 2004 nov. stat. (Fig. S7)

Diagnosis: Ventral kinety entirely parallel to girdle kinety.

Type species: *Strombidium rhyticollare* Corliss and Snyder, 1986.

Species assignable: *Parallelostrombidium* (*Parallelostrombidium*) *rhyticollare* (Corliss and Snyder, 1986) Agatha, 2004 and *Parallelostrombidium* (*Parallelostrombidium*) *siculum* (Montagnes and Taylor, 1994) Agatha, 2004.

Subgenus *Asymptokinetum* nov. subgen. (Fig. S8)

Diagnosis: Only posterior portion of erected ventral kinety parallel to girdle kinety.

Type species: *Parallelostrombidium paralatum* Xu *et al*., 2006.

Etymology: Composite of the Greek adjective *asymptotos* (not falling together) and verb *kinein* (to move), referring to the course of the girdle kinety, which continuously approaches the longitudinal ventral kinety; neuter gender.

Species assignable: The subgenus *Asymptokinetum* is monotypic, comprising only *Parallelostrombidium* (*Asymptokinetum*) *paralatum* Xu *et al*., 2006.

Genus *Novistrombidium* Song and Bradbury, 1998

Remarks: According to the topology tests by Li *et al.* (2013), the monophyly of the genus *Novistrombidium* cannot be rejected, which matches the Hennigian argumentation scheme (Fig. S6). Thus, the genus is split here only into two subgenera differing in morphology and probably in their ITS2 secondary structure (Li *et al.* 2013).

Subgenus *Novistrombidium* Song and Bradbury, 1998 nov. stat. (Fig. S11)

Diagnosis: Extrusome attachment sites in question mark-shaped pattern directly posterior to adoral membranelles and in an arc on posterior dorsal side. Oral primordium between question mark-shaped pattern of extrusome attachment sites and girdle kinety.

Type species: *Strombidium testaceum* Anigstein, 1913.

Species assignable: *Novistrombidium* (*Novistrombidium*) *testaceum* (Anigstein, 1913) Song and Bradbury, 1998 and *Novistrombidium* (*Novistrombidium*) *apsheronicum* (Alekperov and Asadullayeva, 1997) Agatha, 2003.

Subgenus *Propecingulum* nov. subgen. (Fig. S10)

Diagnosis: Extrusome attachment sites directly anterior to girdle kinety. Anterior portion of girdle kinety elongated, performing further dextral spirals. Oral primordium anterior to stripe of extrusome attachment sites extending along girdle kinety.

Type species: *Novistrombidium sinicum* Liu *et al*., 2009.

Etymology: Composite of the Latin prefix *prope* (near) and the noun *cingulum* (girdle), referring to the extrusome stripe directly anterior to the girdle kinety; neuter gender.

Discussion: *Novistrombidium* (*Propecingulum*) *sinicum* Liu *et al*., 2009 and *Novistrombidium* (*Propecingulum*) *orientale* Liu *et al*., 2009 are assigned to the new subgenus. While the affiliations of *Strombidium ioanum* and *S. platum* with the genus *Novistrombidium* are not disputable because of their dextrally spiralled girdle kinety abutting on a longitudinal ventral kinety (the orientation of the dikinetids indicates the presence of both a girdle and a ventral kinety in *S. platum*), their assignment to the subgenus *Propecingulum* has to be verified by live observations. However, the reticular silverline system directly anterior to the dextrally spiralled girdle kinety probably indicates the arrangement of the extrusomes in *S. ioanum*, which is therefore tentatively affiliated, becoming *Novistrombidium* (*Propecingulum*) *ioanum* (Lynn and Gilron, 1993) nov.

comb. Likewise, extrusomes were merely found in the posterior cell portion close to the girdle kinety in *S. platum*, which is thus also tentatively affiliated, becoming *Novistrombidium* (*Propecingulum*) *platum* (Song and Packroff, 1997) nov. comb.

Pelagostrobilidium kimae nov. spec. (Fig. S19)

Diagnosis: Size after protargol impregnation ~ 30 × 18 μm; obovoidal to pyriform. Invariably one micronucleus. Invariably six somatic kineties commencing at same level, except for anteriorly shortened kinety 2: longitudinal kineties 1 and 3–6 posteriorly shortened; kinety 2 slightly sigmoidal, on the left of kinety 3. About 24 collar membranelles and invariably one buccal membranelle.

Etymology: Dedicated to Y.-O. Kim (Korea Institute of Ocean Science & Technology, Geoje, Republic of Korea) due to her contributions to the ecology and taxonomy of marine planktonic ciliates.

Comparison with congeners: There is only one congener sharing the posteriorly shortened somatic kineties 3–6, namely, *P. conicum* as authoritatively described by Agatha and Riedel-Lorjé (1998). However, the Korean specimens deviate in the length of somatic kineties 1 (posteriorly shortened vs. unshortened) and 2 (anteriorly vs. posteriorly shortened) and an argyrophilic C-shaped structure near the collar membranelles (present vs. absent), justifying the establishment of a new species.

Family Eutintinnidae Bachy *et al.*, 2012

Improved diagnosis: Lorica cylindroidal with anterior and posterior openings at truncate ends, wall hyaline, rarely agglomerated, compact, with regular transverse striation in transmission electron micrographs. Usually four macronucleus nodules and two micronuclei. Somatic ciliature comprises (i) a right and left ciliary field with monokinetidal kineties having one dikinetid anteriorly, (ii) a short, monokinetidal ventral kinety, and (iii) two, rarely three dorsal kineties.

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Supplementary material: Figures S1–S61 and Tables S1–S3.

REFERENCES

Abboud-Abi Saab M. (2008) Tintinnids of the Lebanese Coastal Waters (Eastern Mediterranean). CNRS-Lebanon/UNEP/MAP/RAC/SPA: i–viii + 1–192

Agatha S. (2004a) Evolution of ciliary patterns in the Oligotrichida (Ciliophora, Spirotricha) and its taxonomic implications. *Zoology* **107:** 153–168

Agatha S. (2004b) A cladistic approach for the classification of oligotrichid ciliates (Ciliophora: Spirotricha). *Acta Protozool.* **43:** 201–217

Agatha S. (2010) A light and scanning electron microscopic study of the closing apparatus in tintinnid ciliates (Ciliophora, Spirotricha, Tintinnina): A forgotten synapomorphy. *J. Eukaryot. Microbiol.* **57:** 297–307

Agatha S. (2011a) Global diversity of aloricate Oligotrichea (Protista, Ciliophora, Spirotricha) in marine and brackish sea water. *PLoS ONE* **6:** e22466 + Supporting Information

Agatha S. (2011b) Updated hypothesis on the evolution of oligotrichid ciliates (Ciliophora, Spirotricha, Oligotrichida) based on somatic ciliary patterns and ontogenetic data. *Eur. J. Protistol.* **47:** 51–56

Agatha S., Riedel-Lorjé J. C. (1998) Morphology, infraciliature, and ecology of some strobilidiine ciliates (Ciliophora, Oligotrichea) from coastal brackish water basins of Germany. *Eur. J. Protistol.* **34:** 10–17

Agatha S., Riedel-Lorjé J. C. (2006) Redescription of *Tintinnopsis cylindrica* Daday, 1887 (Ciliophora, Spirotricha) and unification of tintinnid terminology. *Acta Protozool.* **45:** 137–151

Agatha S., Strüder-Kypke M. C. (2007) Phylogeny of the order Choreotrichida (Ciliophora, Spirotricha, Oligotrichea) as inferred from morphology, ultrastructure, ontogenesis, and SSrRNA gene sequences. *Eur. J. Protistol.* **43:** 37–63

Agatha S., Foissner W. (2009) Conjugation in the spirotrich ciliate *Halteria grandinella* (Müller, 1773) Dujardin, 1841 (Protozoa, Ciliophora) and its phylogenetic implications. *Eur. J. Protistol.* **45:** 51–63

Agatha S., Simon P. (2012) On the nature of tintinnid loricae (Ciliophora: Spirotricha: Tintinnina): A histochemical, enzymatic, EDX, and high-resolution TEM study. *Acta Protozool.* **51:** 1–19 + Supplementary Material

Agatha S., Laval-Peuto M., Simon P. (2012) The tintinnid lorica. In: The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton, (Eds. J. R. Dolan, D. J. S. Montagnes, S. Agatha, W. D. Coats, D. K. Stoecker). Wiley–Blackwell, Chichester, 17–41

Agatha S., Strüder-Kypke M. C. (2012a) Reconciling cladistic and genetic analyses in choreotrichid ciliates (Ciliophora, Spirotricha, Oligotrichea). *J. Eukaryot. Microbiol.* **59:** 325–350

Agatha S., Strüder-Kypke M. C. (2012b) Systematics and evolution of tintinnid ciliates. In: The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton (Eds. J. R. Dolan, D. J. S. Montagnes, S. Agatha, W. D. Coats, D. K. Stoecker). Wiley-Blackwell, Chichester, 42–84

Bachy C., Gómez F., López-García P., Dolan J. R., Moreira D. (2012) Molecular phylogeny of tintinnid ciliates (Tintinnida, Ciliophora). *Protist* **163:** 873–887 + Supplementary Data

Brandt K. (1906) Die Tintinnodeen der Plankton-Expedition. Tafelerklärungen nebst kurzer Diagnose der neuen Arten. *Ergebn. Plankton-Exped. Humboldt-Stiftung* **3 La:** 1–33 + Plates 1–70

Brandt K. (1907) Die Tintinnodeen der Plankton-Expedition. Systematischer Teil. *Ergebn. Plankton-Exped. Humboldt-Stiftung* **3 La:** 1–488

Edgar R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. **32:** 1792–1797

Entz G. Sr. (1884) Über Infusorien des Golfes von Neapel. *Mitt. zool. Stn Neapel* **5:** 289–444 + Plates 20–25

Gao S., Gong J., Lynn D., Lin X., Song W. (2009) An updated phylogeny of oligotrich and choreotrich ciliates (Protozoa, Ciliophora, Spirotrichea) with representative taxa collected from Chinese coastal waters. *Syst. Biodivers.* **7:** 235–242

Gold K., Morales E. A. (1977) Studies on the Tintinnida of Enewetak Atoll. *J. Protozool.* **24:** 580–587

Gong J., Dong J., Liu X., Massana R. (2013) Extremely high copy numbers and polymorphisms of the rDNA operon estimated from single cell analysis of oligotrich and peritrich ciliates. *Protist* **164:** 369–379

ICZN = International Commission on Zoological Nomenclature (1999) International Code of Zoological Nomenclature. Fourth edition adopted by the International Union of Biological Sciences. International Trust for Zoological Nomenclature, London. Tipografia La Garangola, Padova.

Jiang Y., Yang J., Al-Farraj S. A., Warren A., Lin X. (2012) Redescription of three tintinnid ciliates, *Tintinnopsis tocantinensis*, *T. radix*, and *T. cylindrica* (Ciliophora, Spirotrichea), from coastal waters off China. *Eur. J. Protistol.* **48:** 314–325

Jonsson P. R., Johansson M., Pierce R. W. (2004) Attachment to suspended particles may improve foraging and reduce predation risk for tintinnid ciliates. *Limnol. Oceanogr.* **49:** 1907–1914

Jörgensen E. (1924) Mediterranean Tintinnidae. *Rep. Danish Ocean. Exped., 1908–1910, Mediterr. Seas, Vol. II (Biol.)* **J3:** 1–110

Kazama T., Ishida S., Shimano S., Urabe J. (2012) Discrepancy between conventional morphological systematics and nuclear phylogeny of tintinnids (Ciliophora: Choreotrichida). *Plankton Benthos Res.* **7:** 111–125

Kim S. Y., Yang E. J., Gong J., Choi J. K. (2010) Redescription of *Favella ehrenbergii* (Claparède and Lachmann, 1858) Jörgensen, 1924 (Ciliophora: Choreotrichia), with phylogenetic analyses based on small subunit rRNA gene sequences. *J. Eukaryot. Microbiol.* **57:** 460–467

Kim S. Y., Choi J. K., Dolan J. R., Shin H. C., Lee S., Yang E. J. (2013) Morphological and ribosomal DNA-based characterization of six Antarctic ciliate morphospecies from the Amundsen Sea with phylogenetic analyses. *J. Eukaryot. Microbiol*. **60:** 497–513

Kofoid C. A., Campbell A. S. (1929) A conspectus of the marine and fresh-water Ciliata belonging to the suborder Tintinnoinea, with descriptions of new species principally from the Agassiz Expedition to the eastern tropical Pacific 1904–1905. *Univ. Calif. Publs Zool.* **34:** 1–403

Kofoid C. A., Campbell A. S. (1939) Reports on the scientific results of the expedition to the eastern tropical Pacific, in charge of Alexander Agassiz, by the U. S. Fish Commission Steamer "Albatross," from October, 1904, to March, 1905, Lieut.-Commander L. M. Garrett, U. S. N. Commanding. XXXVII. The Ciliata: The Tintinnoinea. *Bull. Mus. comp. Zool. Harv.* **84:** 1–473 + Plates 1–36

Laackmann H. (1910) Die Tintinnodeen der deutschen Südpolar-Expedition 1901–1903. *Deutsche Südpolar-Expedition* **11**, Zoologie III: 342–496 + Plates XXXIII–LI

Laval-Peuto M. (1981) Construction of the lorica in Ciliata Tintinnina. In vivo study of *Favella ehrenbergii*: variability of the phenotypes during the cycle, biology, statistics, biometry. *Protistologica* **17:** 249–272 (with French summary)

Laval-Peuto M. (1994) Classe des Oligotrichea Bütschli, 1887. Ordre des Tintinnida Kofoid et Campbell, 1929. In: Traité de Zoologie. Anatomie, Systématique, Biologie. II. Infusoires Ciliés. 2. Systématique, (Ed. P. de Puytorac). Masson, Paris, Milano, Barcelona, 181–219

Laval-Peuto M., Barria de Cao M. S. (1987) Les capsules, extrusomes caracteristiques des Tintinnina (Ciliophora), permettent une classification evolutive des genres et des familles du sous-ordre. *Ile Réun. Scientif. GRECO 88, Trav. C.R.M.* **8:** 53–60

Laval-Peuto M., Brownlee D. C. (1986) Identification and systematics of the Tintinnina (Ciliophora): evaluation and suggestions for improvement. *Annls Inst. océanogr., Paris* **62:** 69–84 (with French summary)

Lee E. S., Shin M. K., Kim Y.-O. (2011) Morphological descriptions of four oligotrich ciliates (Ciliophora: Oligotrichia) from southern coast of Korea. *Korean J. Syst. Zool.* **27:** 131–141

Li J., Liu W., Gao S., Warren A., Song W. (2013) Multigene-based analyses of the phylogenetic evolution of oligotrich ciliates, with consideration of the internal transcribed spacer 2 secondary structure of three systematically ambiguous genera. *Eukaryotic Cell* **12:** 430–437 + Supplementary Material

Liu W., Yi Z., Lin X., Warren A., Song W. (2012) Phylogeny of three choreotrich genera (Protozoa, Ciliophora, Spirotrichea), with morphological, morphogenetic and molecular investigations on three strobilidiid species. *Zool. Scr.* **41:** 417–434

Lynn D. H. (1981) The organization and evolution of microtubular organelles in ciliated protozoa. *Biol. Rev.* **56:** 243–292

Lynn D. H., Montagnes D. J. S., Dale T., Gilron G. L., Strom S. L. (1991) A reassessment of the genus *Strombidinopsis* (Ciliophora, Choreotrichida) with descriptions of four new planktonic species and remarks on its taxonomy and phylogeny. *J. Mar. Biol. Ass. U.K.* **71:** 597–612

Maeda M. (1986) An illustrated guide to the species of the Families Halteriidae and Strobilidiidae (Oligotrichida, Ciliophora), free swimming protozoa common in the aquatic environment. *Bull. Ocean Res. Inst., Univ. Tokyo* **21:** 1–67

Maeda M., Carey P. G. (1985) An illustrated guide to the species of the Family Strombidiidae (Oligotrichida, Ciliophora), free swimming protozoa common in the aquatic environment. *Bull. Ocean Res. Inst., Univ. Tokyo* **19:** 1–68

Pierce R. W., Turner J. T. (1992) Ecology of planktonic ciliates in marine food webs. *Rev. Aquat. Sci.* **6:** 139–181

Ronquist F., Huelsenbeck J. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574

Saccà A., Strüder-Kypke M. C., Lynn D. H. (2012) Redescription of *Rhizodomus tagatzi* (Ciliophora: Spirotrichea: Tintinnida), based on morphology and small subunit ribosomal RNA gene sequence. *J. Eukaryot. Microbiol.* **59:** 218–231

Santoferrara L. F., McManus G. B., Alder V. A. (2013) Utility of genetic markers and morphology for species discrimination within the order Tintinnida (Ciliophora, Spirotrichea). *Protist* **164:** 24–36 + Supplementary Data

Small E. B., Lynn D. H. (1985) Phylum Ciliophora Doflein, 1901. In: An Illustrated Guide to the Protozoa, (Eds. J. J. Lee, S. H. Hutner, E. C. Bovee). Society of Protozoologists, Allen Press, Lawrence, Kansas, 393–575

Snoeyenbos-West O. L. O., Salcedo T., McManus G. B., Katz L. A. (2002) Insights into the diversity of choreotrich and oligotrich ciliates (Class: Spirotrichea) based on genealogical analyses of multiple loci. *Int. J. Syst. Evol. Microbiol.* **52:** 1901–1913

Song W., Li J., Liu W., Jiang J., Al-Rasheid K. A. S., Hu X. (2013) Taxonomy, morphology and molecular systematics of three oligotrich ciliates, including a description of *Apostrombidium parakielum* spec. nov. (Ciliophora, Oligotrichia). *Int. J. Syst. Evol. Microbiol.* **63:** 1179–1191

Stamatakis A., Hoover P., Rougemont J. (2008) A rapid bootstrap algorithm for the RAxML web-Servers. *Syst. Biol*. **75:** 758–771

Strüder-Kypke M. C., Lynn D. H. (2008) Morphological versus molecular data – phylogeny of tintinnid ciliates (Ciliophora, Choreotrichia) inferred from small subunit rRNA gene sequences. *Denisia* **23:** 417–424

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*. **28:** 2731–2739

Weisse T., Strüder-Kypke M. C., Berger H., Foissner W. (2008) Genetic, morphological, and ecological diversity of spatially separated clones of *Meseres corlissi* Petz & Foissner, 1992 (Ciliophora, Spirotrichea). *J. Eukaryot. Microbiol*. **55:** 257–270

Xu D., Warren A., Song W. (2009) Oligotrichs. In: Free-living Ciliates in the Bohai and Yellow Seas, China, (Eds. W. Song, A. Warren, X. Hu). Science Press, Beijing, 307–351

Xu D.-P., Sun P., Clamp J. C., Ma H.-G., Song W.-B. (2011) The establishment of a new oligotrich genus *Varistrombidium* gen. nov. and the morphology and phylogeny of a marine ciliate, *Varistrombidium kielum* (Maeda and Carey, 1985) nov. comb. (Protista, Ciliophora). *Acta Zootax. Sin.* **36:** 502–510

Xu D., Sun P., Shin M. K., Kim Y. O. (2012) Species boundaries in tintinnid ciliates: A case study – morphometric variability, molecular characterization, and temporal distribution of *Helicostomella* species (Ciliophora, Tintinnina). *J. Eukaryot. Microbiol.* **59:** 351–358

Xu D., Sun P., Warren A., Noh J. H., Choi D. L., Shin M. K., Kim Y. O. (2013) Phylogenetic investigations on ten genera of tintinnid ciliates (Ciliophora: Spirotrichea: Tintinnida), based on small subunit ribosomal RNA gene sequences. *J. Eukaryot. Microbiol.* **60:** 192–202 + Supporting Information

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Supplement

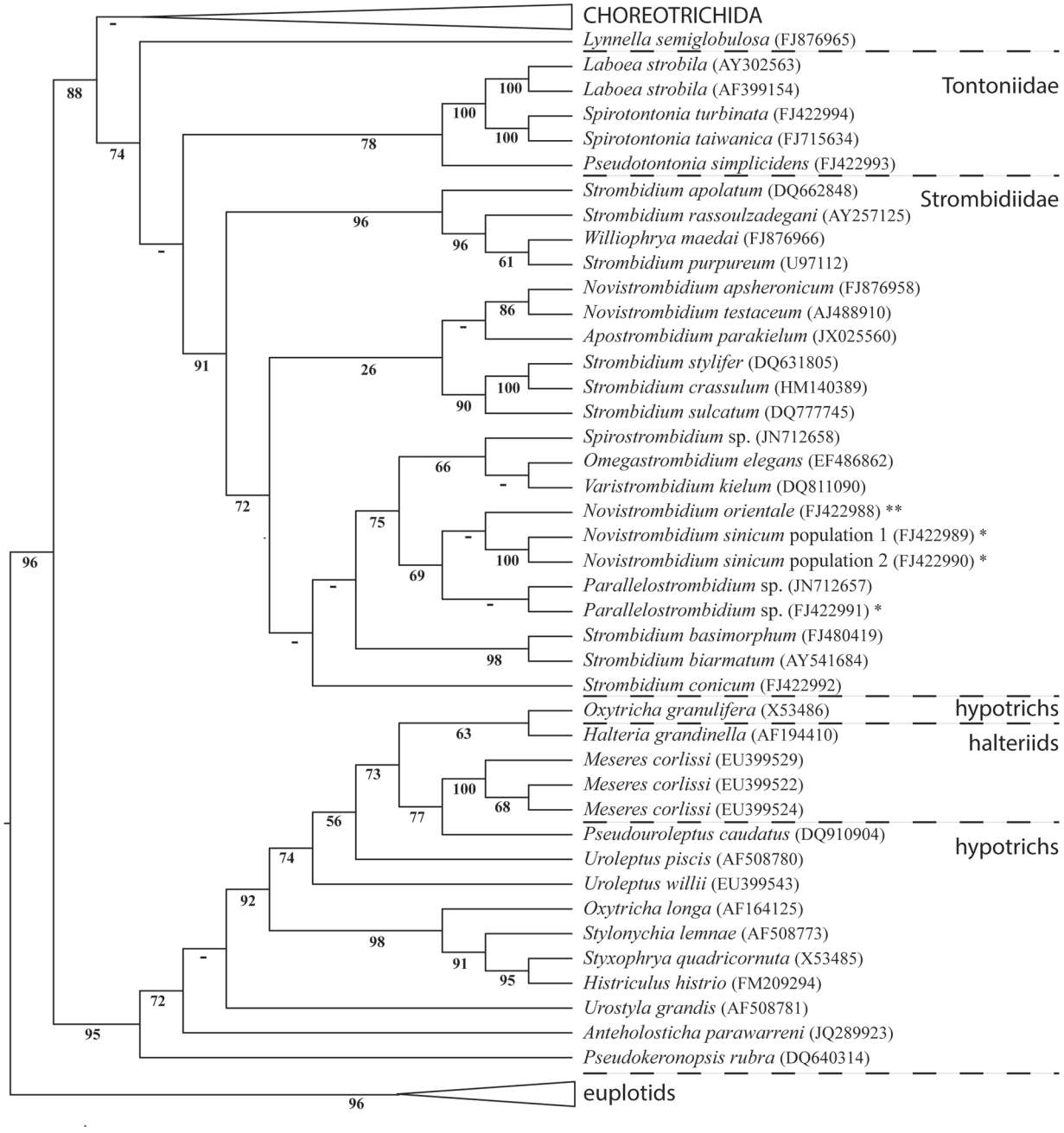


Fig. S1. Profile Neighbor Joining tree of the Oligotrichida inferred from small subunit ribosomal RNA (SSU rRNA) gene sequences (66 taxa and 1823 nucleotide positions) aligned with the Muscle algorithm (Edgar 2004) implemented in MEGA ver. 5.1 (Tamura *et al.* 2011). The tree was computed with ProfDistS (Wolf *et al.* 2008). Support values are listed at the nodes. Values below 50% are represented by dashes. * – initially published as *Spirostrombidium* sp.; ** – initially published as *Parallelostrombidium* sp.

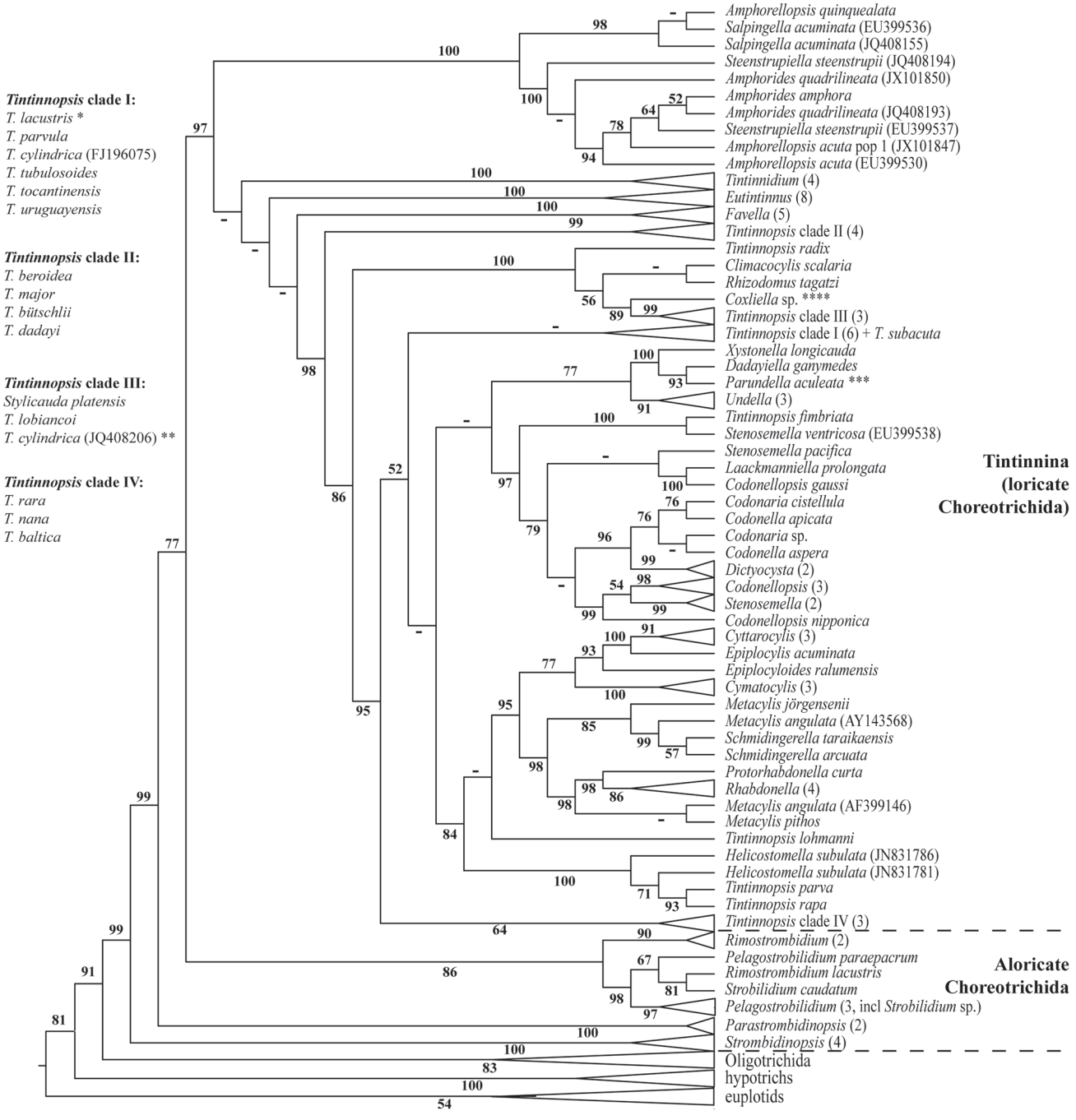


Fig. S2. Profile Neighbor Joining tree of the Choreotrichida inferred from small subunit ribosomal RNA (SSU rRNA) gene sequences (138 taxa and 1859 nucleotide positions) aligned with the Muscle algorithm (Edgar 2004) implemented in MEGA ver. 5.1 (Tamura *et al.* 2011). The tree was computed with ProfDistS (Wolf *et al.* 2008). Support values are listed at the nodes. Values below 50% are represented by dashes. Branches with unambiguously clustered taxa are collapsed, species of the genus *Tintinnopsis* grouped in 5 different clades numbered I–V. * – after Kofoid and Campbell (1929) a synonym of *Codonella cratera*; ** – does not correspond with the redescription of Agatha and Riedel-Lorjé (2006); *** – possibly incorrectly identified, might be *Dadayiella acutiformis*; **** – invalid taxon, very likely a replacement lorica (see text).

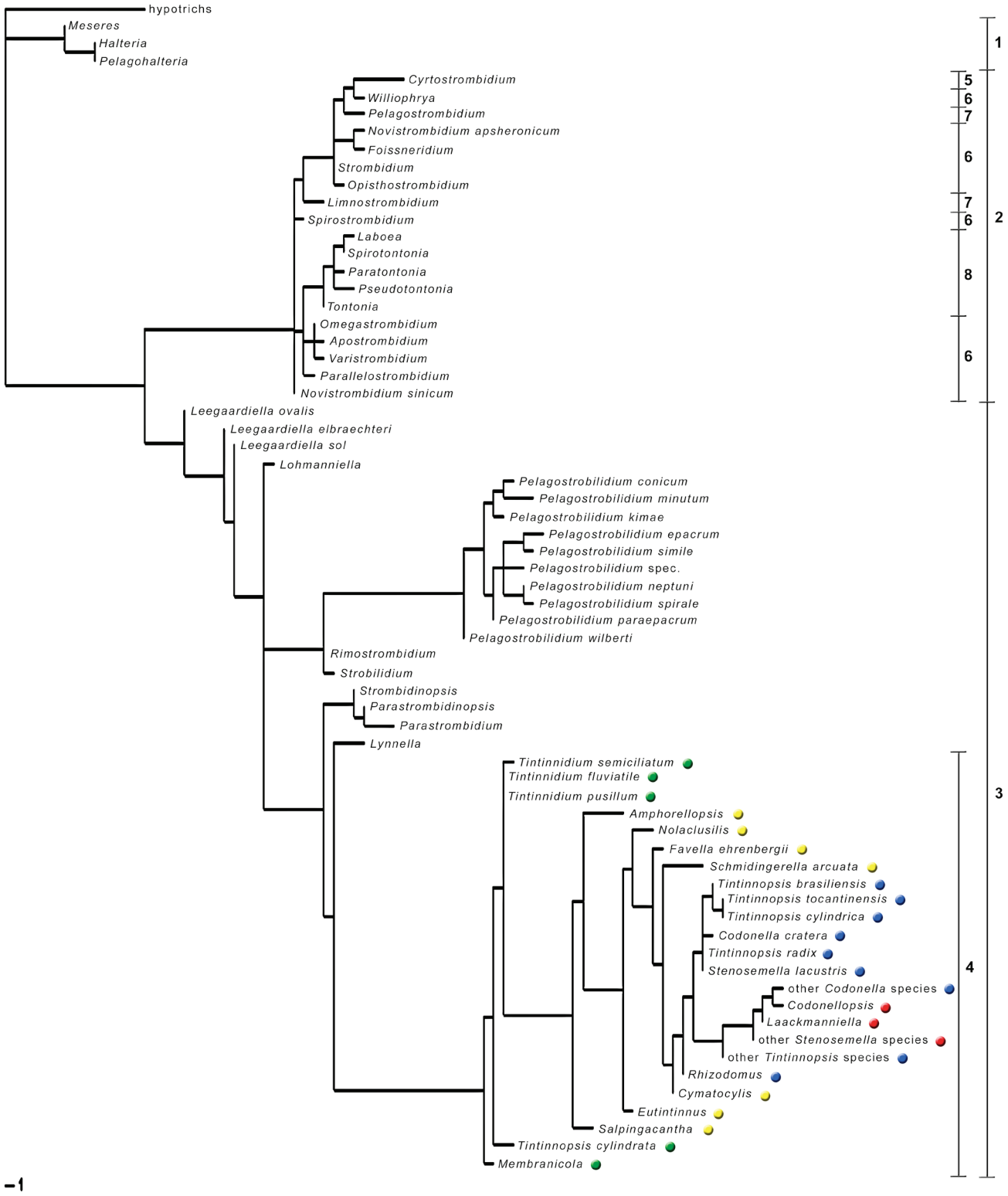


Fig. S3. Maximum parsimony tree calculated with PAUP* vers. 4.0b10 (Swofford 2002), equally weighted morphologic characters (Tables S1, S2), and hypotrichs as outgroup. 83 out of 94 characters parsimony-informative, tree length 227, consistency index 0.71, homoplasy index 0.29, retention index 0.95. 1 – halteriids, 2 – oligotrichids, 3 – choreotrichids, 4 – tintinnids, 5 – Cyrtostrombidiidae, 6 – Strombidiidae, 7 – Pelagostrombidiidae, 8 – Tontoniidae. Most common lorica structures: ● – flexible, agglomerated, ● – hard, agglomerated, ● – hard, entirely hyaline, ● – hard, composed of hyaline collar and agglomerated bowl. *Pelagostrobilidium* spec. sensu Ota and Taniguchi (2003).

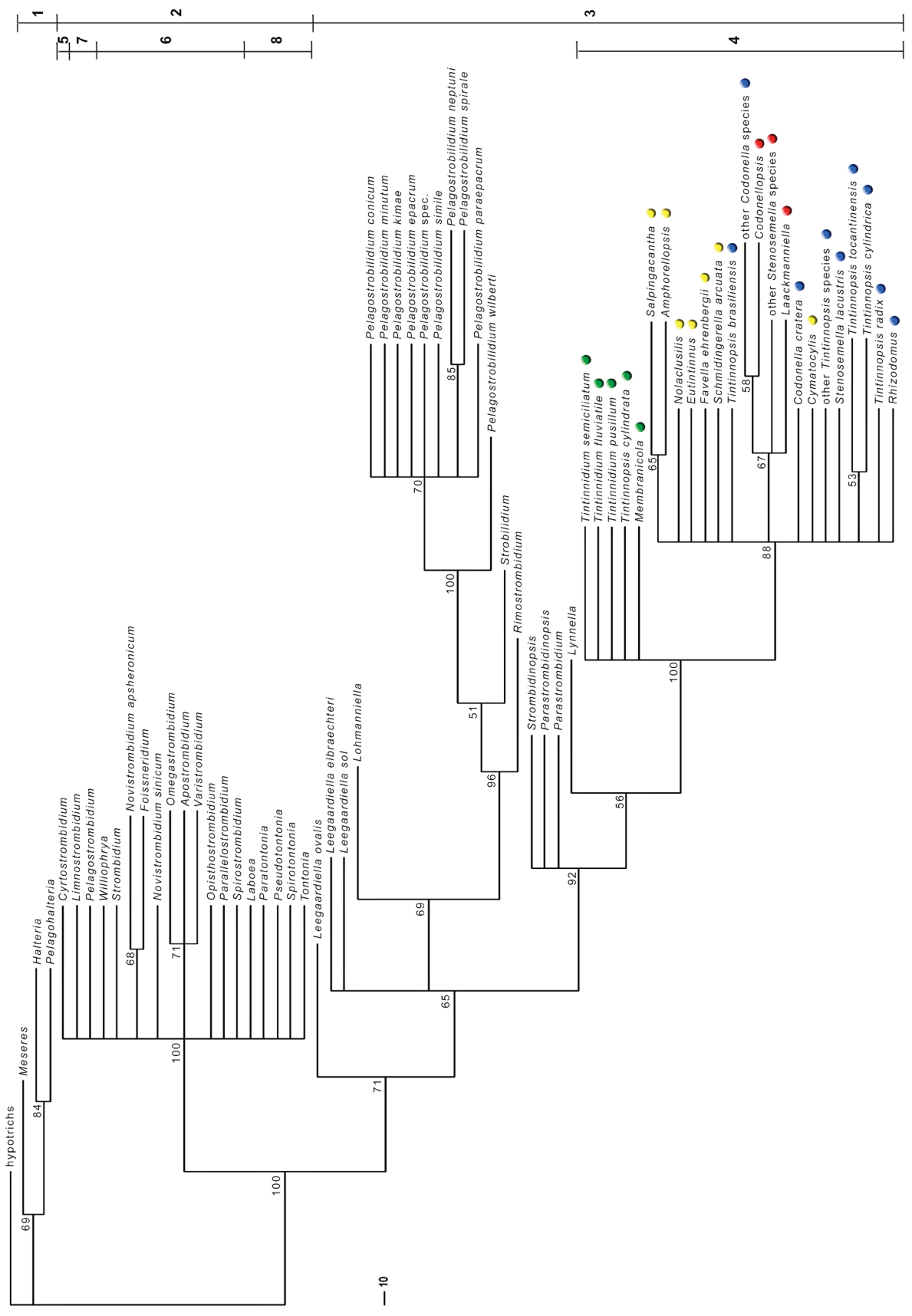


Fig. S4. Bootstrap 50% majority-rule consensus parsimony tree inferred from morphologic data of the Oligotrichea (Tables S1, S2), using PAUP* ver. 4.0b10 (Swofford 2002) and the hypotrichs as outgroup. 83 out of 94 characters were parsimony-informative. The bootstrap method with heuristic search included 100 replicates. 1 – halteriids, 2 – oligotrichids, 3 – choreotrichids, 4 – tintinnids, 5 – Cytostrombidiidae, 6 – Strombidiidae, 7 – Pelagostrombidiidae, 8 – Tontoniidae. Most common lorica structures: ● – flexible, agglomerated, ● – hard, agglomerated, ● – hard, entirely hyaline, ● – hard, composed of hyaline collar and agglomerated bowl. *Pelagostrobilidium* spec. sensu Ota and Taniguchi (2003).

Fig. S5. Strict consensus tree of the Oligotrichea calculated with the computer program Hennig86 by a heuristic analysis of equally weighted morphologic characters (Tables S1, S2) and the branch-swapping algorithm, using the hypotrichs as outgroup. It has a length of 180, a consistency index of 66, and a retention index of 90. 1 – halteriids, 2 – oligotrichids, 3 – choreotrichids, 4 – tintinnids, 5 – Cyrtostrombidiidae, 6 – Strombidiidae, 7 – Pelagostrombidiidae, 8 – Tontoniidae. Most common lorica structures: ● – flexible, agglomerated, ● – hard, agglomerated, ● – hard, entirely hyaline, ● – hard, composed of hyaline collar and agglomerated bowl. *Pelagostrobilidium* spec. sensu Ota and Taniguchi (2003).

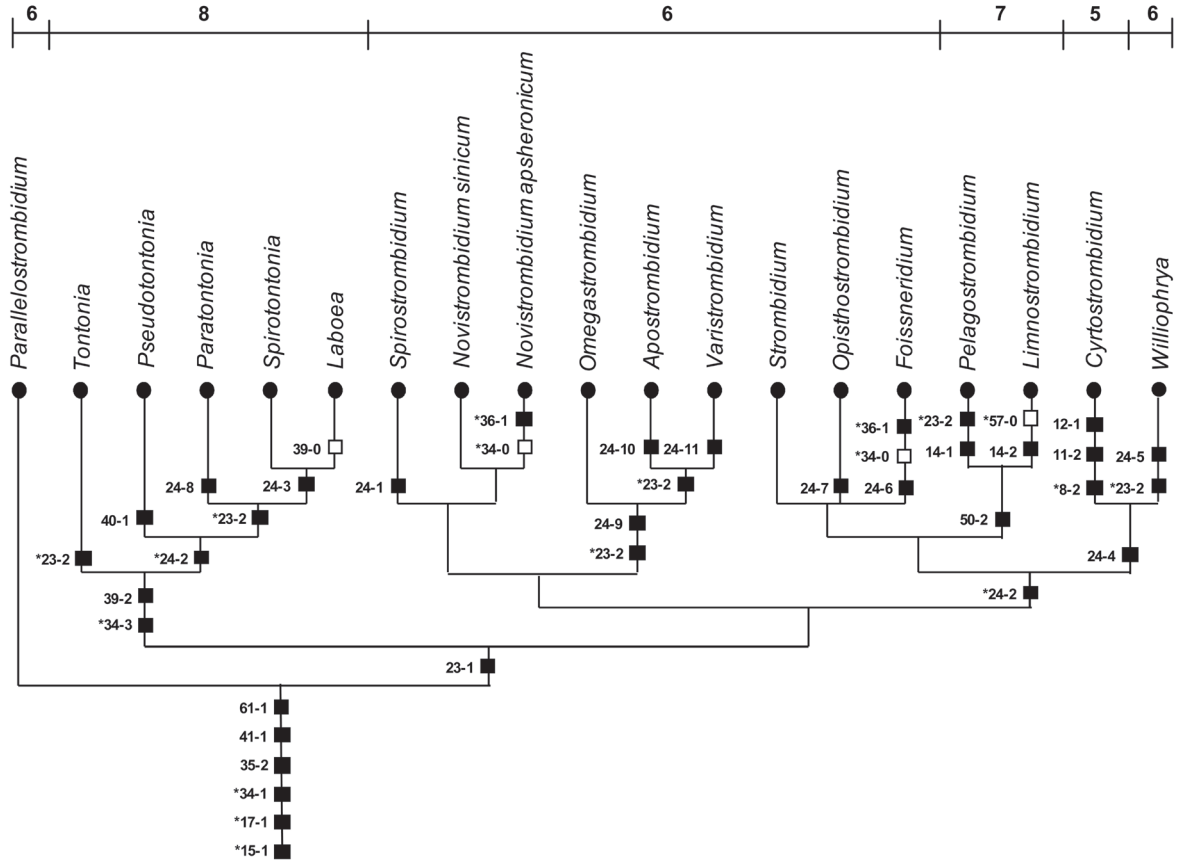
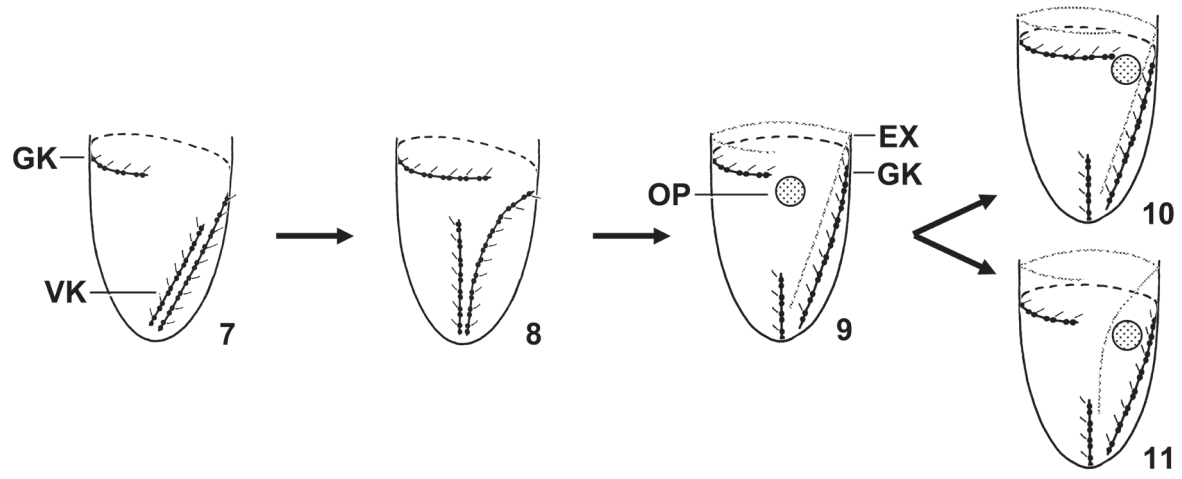
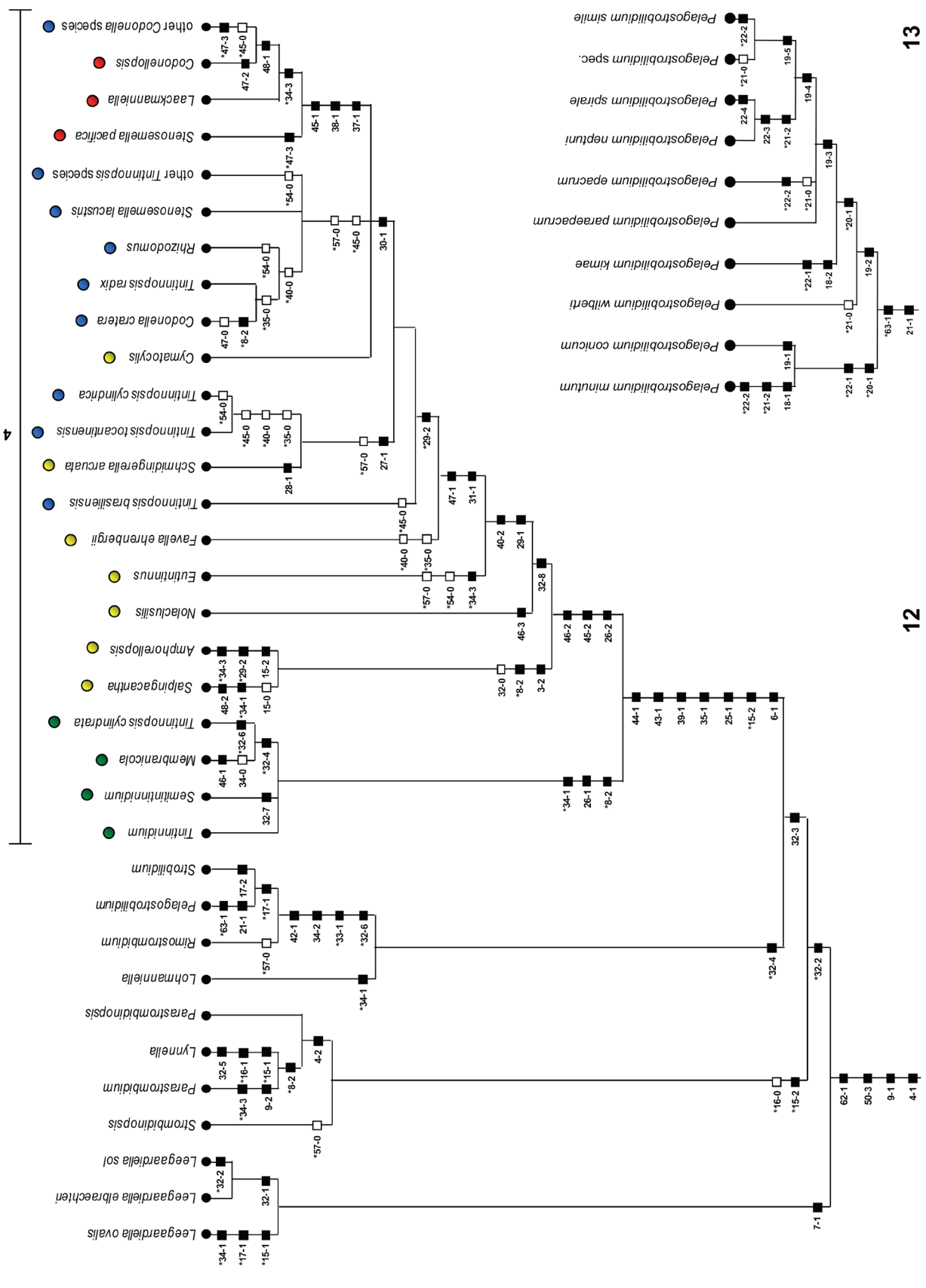


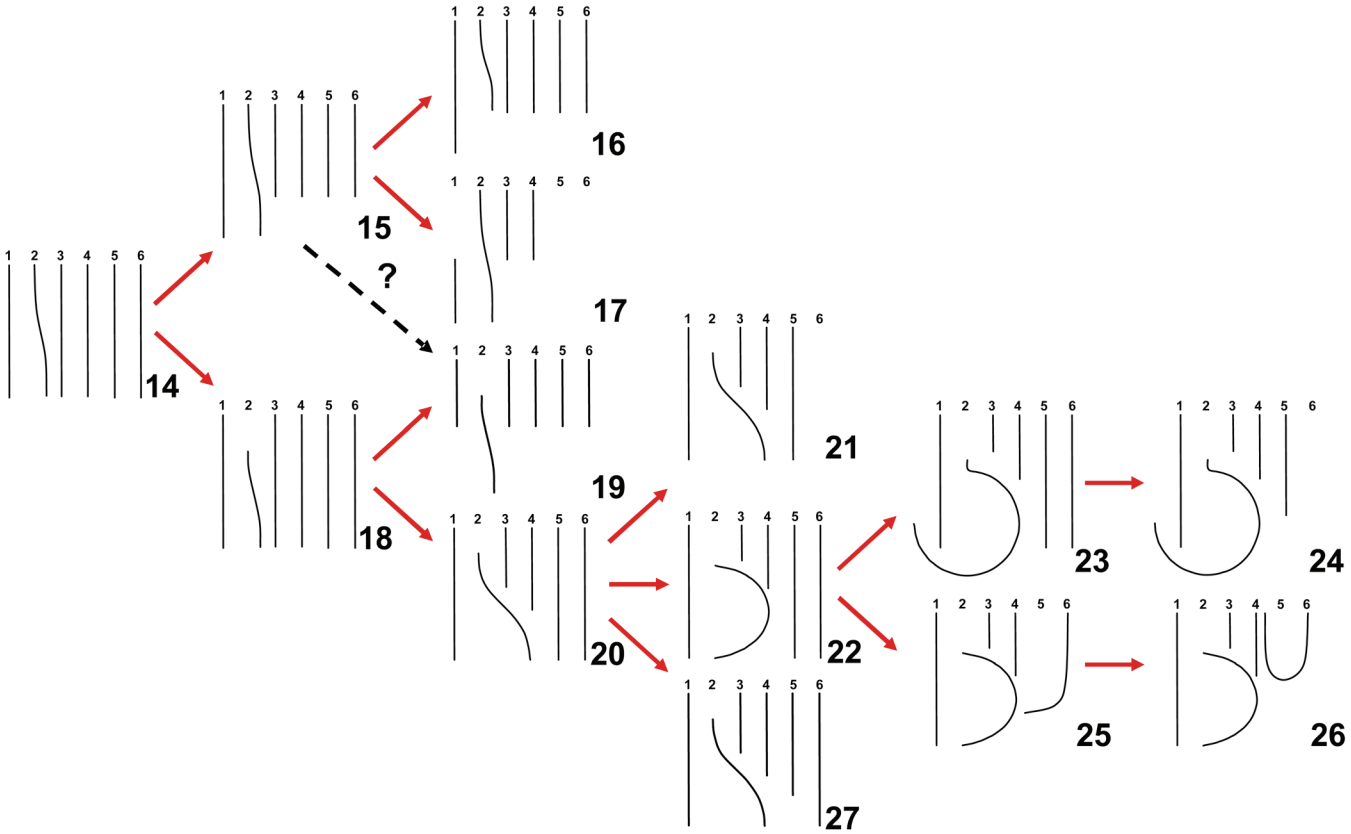
Fig. S6. Monophyletic oligotrichid ciliates in the maximum parsimony tree of the Oligotrichea generated by the Hennigian argumentation method. For character coding, see Table S1. Black squares mark apomorphies, open squares denote reversals to plesiomorphic states, and asterisks mark homoplasies. 5 – Cyrtostrombidiidae, 6 – Strombidiidae, 7 – Pelagostrombidiidae, 8 – Tontoniidae.



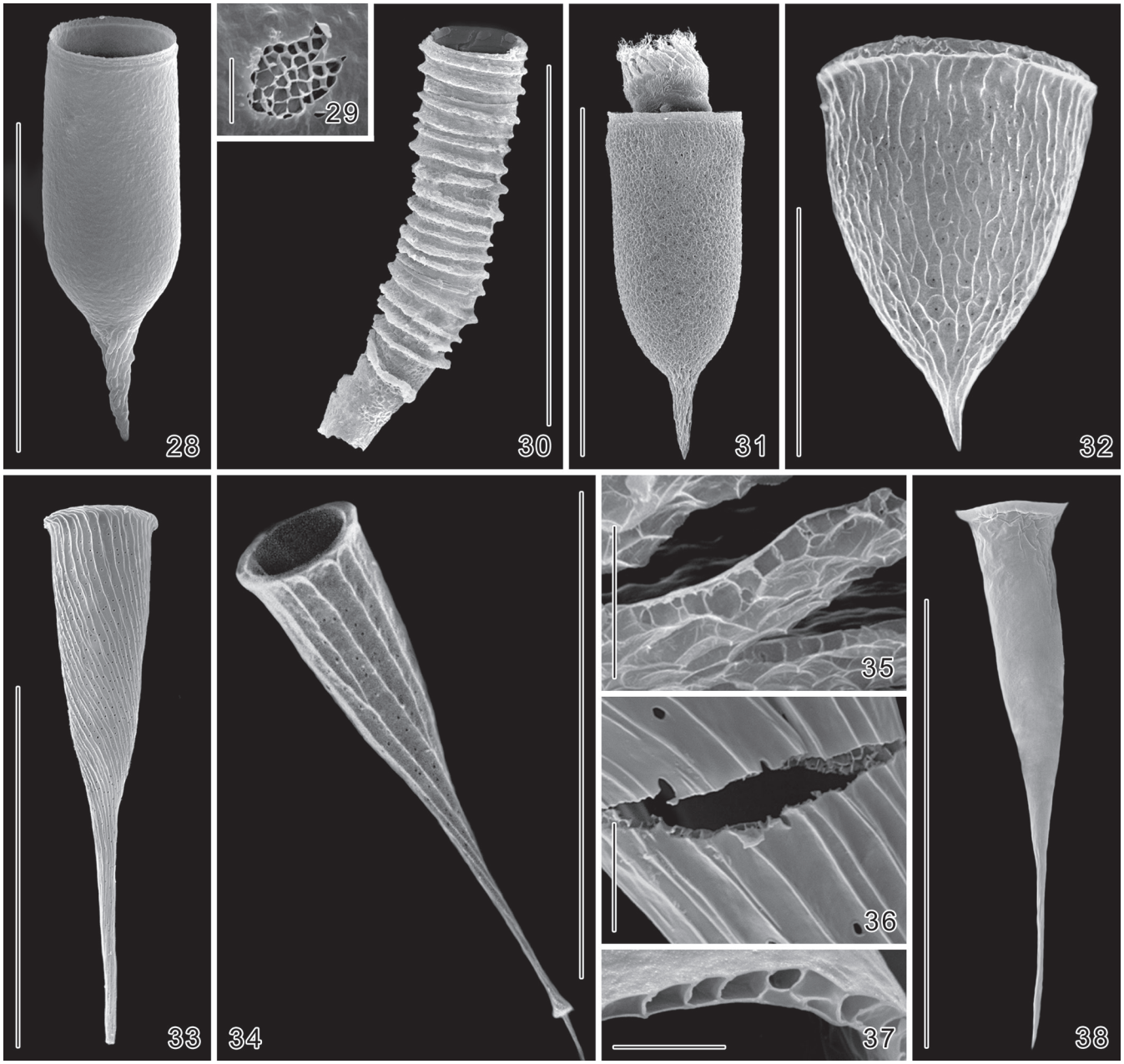
Figs S7–S11. Evolution of somatic ciliary patterns in the genera *Parallelostrombidium* and *Novistrombidium* [originals based on illustrations of Montagnes and Taylor 1994 (7), Xu *et al.* 2006 (8), Liu *et al.* 2009 (10), and Agatha 2003 (11); protargol impregnation]. The pattern of *Parallelostrombidium* (*Parallelostrombidium*) *siculum* (7) probably gave rise to the pattern of *Parallelostrombidium* (*Asymptokinetum*) *paralatum* (8), which possibly represents the transition stage to the genus *Novistrombidium* (9). The hypothetic ancestor of that genus had the stripe of extrusome attachment sites associated with the girdle kinety as usual, and both structures are posterior to the oral primordium. It represents the origin of the subgenus *Propecingulum* (10; with *N. ioanum* nov. comb., *N. orientale*, *N. platum* nov. comb., and *N. sinicum*) and the subgenus *Novistrombidium* (11; with *N. apsheronicum* and *N. testaceum*). EX – extrusome attachment sites, GK – girdle kinety, OP – oral primordium, VK – ventral kinety.



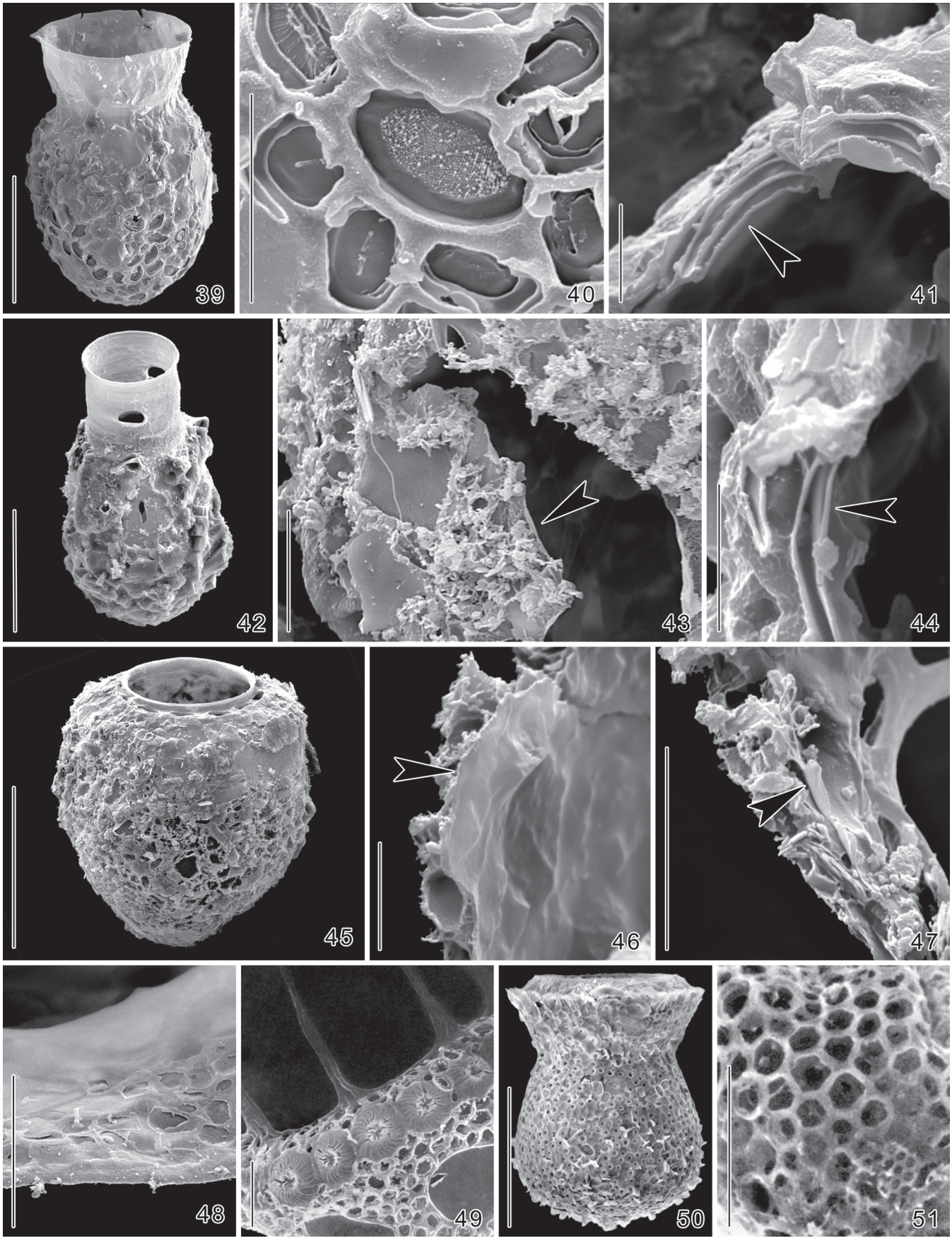
Figs S12, S13. Monophyletic choreotrichid ciliates (12) and monophyletic genus *Pelagostrobilidium* (13) in the maximum parsimony tree of the Oligotrichea generated by the Hennigian argumentation method. For character coding, see Table S1. Black squares mark apomorphies, open squares denote reversals to plesiomorphic states, and asterisks mark homoplasies. 4 – tintinnids, *Semitintinnidium* – subgenus of the genus *Tintinnidium*, *Tintinnidium* – subgenus of the identically named genus. Most common lorica structures: – flexible, agglomerated, – hard, agglomerated, – hard, entirely hyaline, – hard, composed of hyaline collar and agglomerated bowl.



Figs S14–S27. Evolution of somatic ciliary patterns in choreotrichid genus *Pelagostrobilidium* [originals based on Lynn and Montagnes 1988 (21, 26), Montagnes and Taylor 1994 (25), Agatha and Riedel-Lorjé 1998 (16), Song and Bradbury 1998 (24), Ota and Taniguchi 2003 (23), Küppers *et al.* 2006 (18), Lee *et al.* 2011 (19), Liu *et al.* 2012 (17, 27); protargol impregnation]. **14** – Hypothetic *Rimostrombidium*-like ancestor; **15, 20, 22** – hypothetic intermediate states; **16** – *P. conicum*; **17** – *P. minutum*; **18** – *P. wilberti*; **19** – *P. kimae* nov. spec.; **21** – *P. epacrum*; **23** – *Pelagostrobilidium* spec.; **24** – *P. simile*; **25** – *P. neptuni*; **26** – *P. spirale*; **27** – *P. paraepacrum*. 1–5 – somatic kineties 1–5.



Figs S28–S38. Structure and texture of hyaline loricae [28–31, 33, 35–38, originals from specimens collected at the U.S. east coast (28, 29, 31, 35) and in the Mediterranean Sea (30, 33, 36–38); 32, after Abboud-Abi Saab 2008; 34, after Gold and Morales 1977; scanning electron micrographs]. **28, 29** – *Favella panamensis*, lateral view of lorica (28) and lorica wall with removed outer surface (29) showing the monolaminar texture with alveoli; **30** – *Climacocylis* sp., lateral view of lorica. The monolaminar texture with alveoli is recognizable in the posterior portion of the lorica, where the surface layer has been removed; **31, 35** – *Schmidingerella arcuata*, lateral view of lorica (31) and fracture of lorica wall (35). The lorica wall has minute pores and reticulate ridges on the outer surface; **32** – *Epiplocyloides*, lateral view of lorica. The wall has minute pores and reticulate ridges; **33, 34, 36 –** *Rhabdonella spiralis* (33, 36) and *Rhabdonellopsis apophysata* (34), lateral views of loricae (33, 34) and fracture of lorica wall (36). The walls have minute pores and spiralled, anastomosing ridges; **37, 38** – *Xystonella longicauda*, lateral view of lorica (38) and fracture of lorica wall (37). Scale bars: 200 µm (28, 30, 31, 33, 34, 38), 50 µm (32), and 5 µm (29, 35–37).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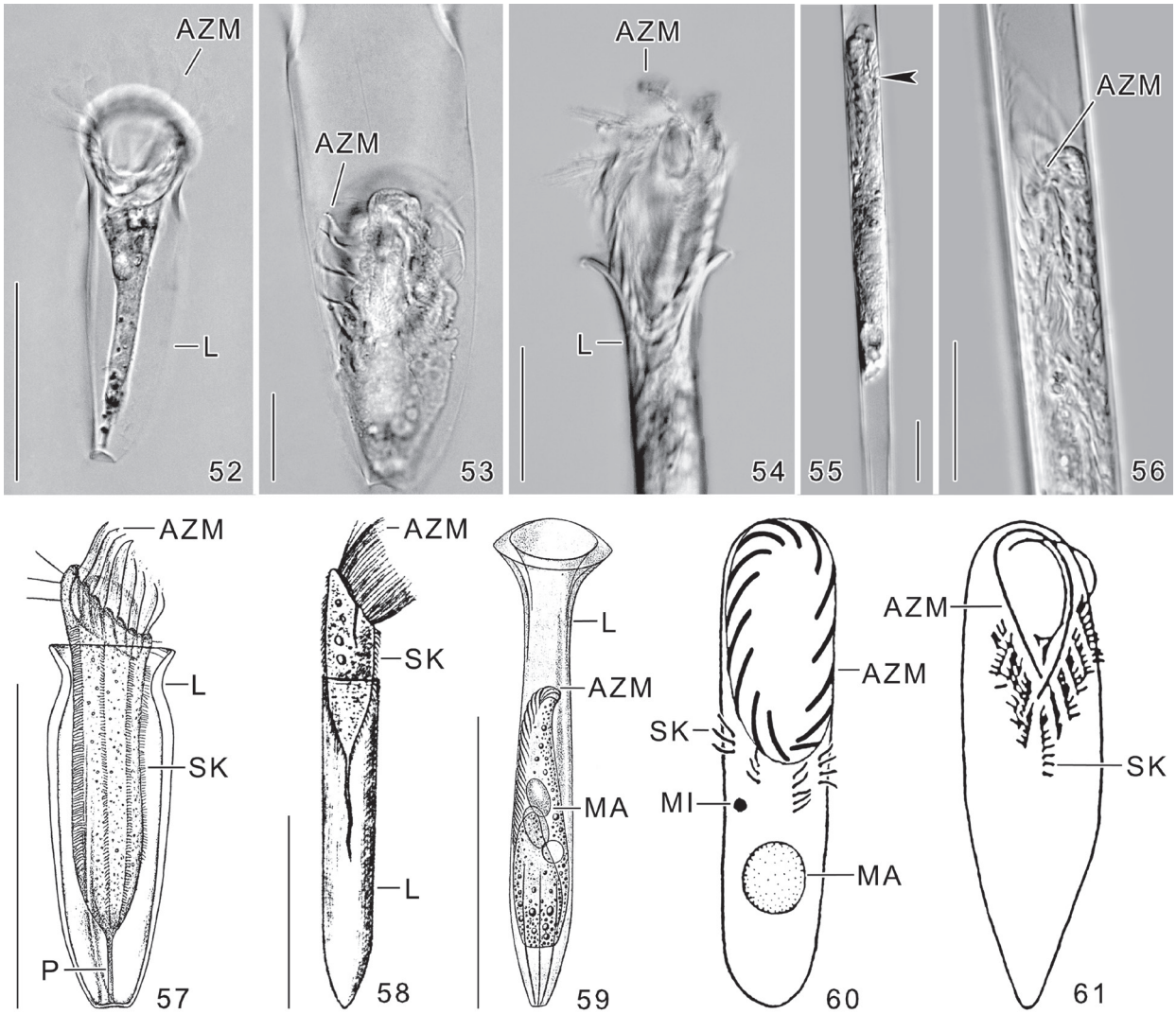

Figs S52–S61. Obliquely orientated adoral zones of membranelles in tintinnids (52–56, originals from specimens collected in the Mediterranean Sea; 57, from Fauré-Fremiet 1924; 58, redrawn by Kent 1880–1882; 59, from Entz 1884; 60, 61, from Small and Lynn 1985; 52–59, from life; 60, 61, protargol impregnation). **52, 53, 57** – *Amphorides quadrilineata*, extended (52, 57) and contracted (53) specimens; **54–56** – *Salpingella attenuata*, just extending (54) and contracted specimens (55, 56). Arrowhead marks oblique adoral zone (55); **58** – *Bursaopsis obliqua*, extending specimen. Size taken from Kent (1881–1882); **59** – *Steenstrupiella entzi*. Entz (1884) and Kofoid and Campbell (1929) identified the cell in the lorica with a hypotrich ciliate, possibly based on the oblique and thus untypical orientation of the adoral zone; **60** – *Salpingacantha* sp., contracted specimen without lorica (size not mentioned); **61** – *Amphorellopsis acuta*, contracted specimen without lorica (size not mentioned). Scale bars: 100 µm (52, 57–59) and 20 µm (53–56). AZM – adoral zone of membranelles, L – lorica, MA – macronucleus, MI – micronucleus, P – peduncle, SK – somatic kineties.

◀◀

Figs S39–S51. Structure and texture of agglomerated loricae (39–48, originals from specimens collected in the Mediterranean Sea; 49, after Burns 1983; 50, 51, after Gold and Morales 1977; scanning electron micrographs). **39–41, 48** – *Codonella aspera*, lateral view of lorica (39), detail of outer bowl surface with impressions of formerly incrustrated coccoliths (40), fracture surface of bowl (41), and inner collar surface (48). The wall seems to be composed of compact matrix material, in which particles are/were embedded, and an inner layer (arrowhead); **42–44** – *Codonellopsis schabi*, lateral view of lorica (42) and surfaces and fracture surfaces of bowl (43, 44). Apparently, the agglomerated particles are embedded into compact matrix material and an inner layer (arrowheads) lines the bowl; **45–47** – *Stenosemella ventricosa*, lateral view of lorica (45) and fracture surfaces of bowls (46, 47) showing compact matrix material, in which particle are embedded, and a continuous inner layer (arrowheads); **49** – *Dictyocysta reticulata*, transition zone between collar and bowl. The impressions of the formerly embedded coccoliths strongly resemble those in *Codonella* (Figs S39, S40); accordingly, we assume a similar wall texture; **50, 51 –** *Codonaria oceanica*, lateral view of lorica (50) and lorica matrix (51) after hydrochloric acid treatment that removed the coccoliths. The impressions of the formerly embedded coccoliths strongly resemble those in *Codonella* (Figs S39, 40); thus, a similar wall texture is supposed. Scale bars: 50 µm (50), 40 µm (39, 42, 45), 10 µm (40, 43, 47–49, 51), and 4 µm (41, 44, 46).

Table S1. Character states and coding used for construction of a cladogram with the Hennigian argumentation method (first or only code; Figs. S6, S12, S13) and for computer analyses (only or second code; Figs. S3–S5). The coding is mainly based on outgroup comparison with the hypotrichs. If not stated otherwise, the characters are ordered/additive (the states have a certain sequence; Wagner/Farris optimisation).

	Characters	
	Apomorphic states	Plesiomorphic states
1	Cell usually globular to obconical (coded 1)	Cell usually distinctly dorsoventrally flattened (coded 0)
2	Usually planktonic (coded 1)	Usually benthic (coded 0)
3	Adoral zone of membranelles mainly apical (coded 1) or secondarily ventral in contracted specimens (coded 2)	Adoral zone of membranelles mainly ventral (coded 0)
4	Adoral zone of membranelles circular (coded 1) or secondarily with minute ventral gap (coded 2)	Adoral zone of membranelles C-shaped (coded 0)
5	30–50% (coded 1) or 0% (coded 2) of adoral polykinetids composed of four rows of basal bodies	> 90% of adoral polykinetids composed of four rows of basal bodies (coded 0)
6	Postciliary and transverse microtubules absent in adoral membranelles (coded 1)	Postciliary and transverse microtubules present in adoral membranelles (coded 0)
7	Collar polykinetids bipartite (coded 1)	Collar polykinetids continuous (coded 0)
8	Adoral zone bipartite, i.e., composed of buccal membranelles with small polykinetids and short cilia and collar membranelles with broad polykinetids and long cilia (coded 1), buccal membranelles absent (coded 2)	Adoral zone not bipartite, i.e., polykinetids and cilia of membranelles gradually decrease in size towards the cytostome (coded 0)
9	Last collar membranelles with proximally elongated polykinetids (coded 1) or proximally and distally elongated polykinetids (coded 2)	All collar membranelles with similar-sized polykinetids (coded 0)
10	Undulating membrane(s) often diplostichomonad (two parallel rows of basal bodies) or polystichomonad (more than two parallel rows of basal bodies; coded 1)	Undulating membrane(s) monostichomonad (single row of basal bodies; coded 0)
11	Paroral membrane absent (coded 1), paroral and endoral membranes absent (coded 2)	Usually endoral and paroral membranes present (coded 0)
12	Cyrtos-like (conspicuously strong) pharyngeal fibres (coded 1)	Common pharyngeal fibres (coded 0)
13	Cirri absent (coded 1)	Cirri present (coded 0)
14 [a]	Somatic kinetids unciliated (coded 1) or with clavate cilia (coded 2)	Somatic kinetids with rod-shaped or fusiform cilia (coded 0)
15 [a]	Usually one or two somatic kineties (coded 1), usually ten or more somatic kineties (coded 2)	Usually 3–9 somatic kineties (coded 0)
16	≥ 40% of unspecialised somatic kineties shortened or entirely reduced (coded 1)	Unspecialised somatic kineties extend from adoral zone of membranelles to posterior cell end (coded 0)
17 [a]	Some unspecialised somatic kineties curved (coded 1) or forming a posterior spiral (coded 2)	Unspecialised somatic kineties longitudinal (coded 0)
18 [a]	*Pelagostrobilidium* – somatic kinety 1: anteriorly (coded 1) or posteriorly shortened (coded 2)	*Pelagostrobilidium* – somatic kinety 1: unshortened (coded 0)
19 [b]	*Pelagostrobilidium* – somatic kinety 2: posteriorly shortened (coded 1; coded 10000); anteriorly shortened (coded 2; coded 01000); distinctly sigmoidal (coded 3; coded 01100); semicircular (coded 4; coded 01110); or performs ~ 270° curvature (coded 5; coded 01111)	*Pelagostrobilidium*: somatic kinety 2 unshortened and slightly sigmoidal (coded 0; coded 00000)
20	*Pelagostrobilidium* – somatic kineties 3 and 4: posteriorly shortened (coded 1)	*Pelagostrobilidium* – somatic kineties 3 and 4: unshortened (coded 0)
21	*Pelagostrobilidium* – somatic kinety 5: posteriorly shortened (coded 1) or absent (coded 2)	*Pelagostrobilidium* – somatic kinety 5: unshortened (coded 0)
22 [b]	*Pelagostrobilidium* – somatic kinety 6: posteriorly shortened (coded 1; coded 1000); absent (coded 2; coded 1100); L-shaped (coded 3; coded 0010); or U-shaped (coded 4; coded 0011)	*Pelagostrobilidium* – somatic kinety 6: longitudinal and unshortened (coded 0)
23 [a]	Oligotrichid ventral kinety erected (coded 1), usually indistinct or absent (coded 2)	Oligotrichid ventral kinety dextrally spiralled (coded 0)

	Characters	
	Apomorphic states	Plesiomorphic states
24 [b]	Oligotrichid girdle kinety: dextrally spiralled with posterior end inversely orientated (coded 1; coded 10000000000); horizontally orientated anterior to oral primordium (coded 2; coded 01000000000); sinistrally spiralled (coded 3; coded 01100000000); horizontally orientated anterior to oral primordium with dorsal gap (coded 4; coded 01010000000); horizontally orientated at level of oral primordium, with dorsal gap (coded 5; coded 01011000000); horizontally orientated posterior to oral primordium and separate from extrusome attachment sites (coded 6; coded 01000100000); horizontally orientated posterior to oral primordium together with extrusome attachment sites (coded 7; coded 01000010000); Ω-shaped anterior to oral primordium (coded 8; coded 01000001000); Ω-shaped posterior to oral primordium (coded 9; coded 00000000100); extends to posterior cell end on ventral and dorsal sides, in two or three fragments (coded 10; coded 00000000110); in several mostly clockwise inclined fragments (coded 11; coded 00000000101)	Oligotrichid girdle kinety dextrally spiralled (coded 0; coded 00000000000)
25	Somatic kineties arranged in a right and left ciliary field (coded 1)	Somatic kineties more or less equidistantly arranged (coded 0)
26 [a]	Two ventral organelles (coded 1) or one specialised tintinnid ventral kinety (coded 2)	Specialised ventral organelles or tintinnid ventral kinety absent (coded 0)
27	Tintinnid ventral kinety composed of a monokinetidal anterior and a dikinetidal posterior portion (coded 1)	Tintinnid ventral kinety monokinetidal (coded 0)
28	Right ciliary field and tintinnid ventral kinety separated by a broad unciliated stripe (coded 1)	Right ciliary field abuts on ventral kinety (coded 0)
29	Two dorsal kineties (coded 1) or one dorsal kinety (coded 2)	Specialised dorsal kinety/kineties absent (coded 0)
30	Posterior kinety present (coded 1)	Specialised posterior kinety absent (coded 0)
31	Lateral ciliary field present (coded 1)	Lateral ciliary field absent (coded 0)
32 [b]	Unspecialised somatic kineties: some dikinetids with cilia only at the anterior basal bodies, other dikinetids with two cilia (coded 1; coded 10000000); all dikinetids with two cilia (coded 2; coded 11000000); most dikinetids with cilia only at the posterior basal bodies, few dikinetids with two cilia (coded 3; coded 11100000); all dikinetids with cilia only at the posterior basal bodies (coded 4; coded 11110000); some dikinetids with cilia only at the posterior basal bodies, some ciliated monokinetids (coded 5; coded 11111000); ciliated monokinetids (coded 6; coded 11111100); mostly ciliated monokinetids, some dikinetids with two cilia, some dikinetids with cilia only at the posterior basal bodies (coded 7; coded 11100010); mostly ciliated monokinetids, some dikinetids with two cilia (coded 8; coded 11100011)	Unspecialised somatic kineties composed of dikinetids, each has a distinct cilium associated only with the anterior basal body (coded 0; coded 00000000)
33	Somatic kinetids condensed (coded 1)	Somatic kinetids distinctly separate (coded 0)
34 [a]	Majority of members with one ellipsoidal macronucleus nodule (coded 1), one C-shaped macronucleus (coded 2), or more than two macronucleus nodules (coded 3)	Majority of members with two macronucleus nodules (coded 0)
35 [a]	Tintinnid extrusomes (capsules) and/or structures usually associated with tintinnid extrusomes (coded 1) or oligotrichid extrusomes (trichites; coded 2) present	Extrusomes absent (coded 0)
36	Stripe of extrusome (trichite) attachment sites distinctly apart from oligotrichid girdle kinety (coded 1)	Stripe of extrusome (trichite) attachment sites directly anterior to oligotrichid girdle kinety (coded 0)
37 [a]	Capsule Type II (coded 1)	Capsule Type I (coded 0)
38	Mucocyst Type A (coded 1)	Mucocysts absent (coded 0)
39 [a]	Contractility of peduncle (coded 1) or tail (coded 2)	Posterior cell portion acontractile (coded 0)
40 [a]	Anterior cell portion with contractile tentacles (coded 1) or tentaculoids (coded 2)	Anterior cell portion without cytoplasmic appendages (coded 0)
41	Polysaccharidic cortical platelets (coded 1)	Cortical platelets absent (coded 0)
42	Kinetal lips covering bases of somatic cilia present (coded 1)	Kinetal lips covering bases of somatic cilia absent (coded 0)

	Characters	
	Apomorphic states	Plesiomorphic states
43	Vesicular reticulum present (coded 1)	Vesicular reticulum absent (coded 0)
44	Lorica present (coded 1)	Lorica absent (coded 0)
45 [a]	Lorica types: bowl agglomerated, collar hyaline (coded 1); entirely hyaline (coded 2)	Lorica entirely agglomerated (coded 0)
46 [b]	Lorica flexible with subterminal membrane (coded 1; coded 010), hard (coded 2; coded 100), or hard and collapsible (coded 3; coded 101)	Lorica flexible (coded 0; coded 000)
47 [a]	Texture of lorica wall: monolaminar alveolar (coded 1); collar monolaminar alveolar, bowl bilaminar (coded 2); or collar monolaminar compact, bowl bilaminar (coded 3)	Texture of lorica wall monolaminar compact (coded 0)
48 [a]	Closing apparatus: foldable with lorica sac (coded 1) or diaphragm-like without lorica sac (coded 2)	Lorica sac and closing apparatus absent (coded 0)
49	Enantiotropy (coded 1)	Homeotropy (coded 0)
50 [b]	Stomatogenesis hypoapokinetal in transient tube (coded 1; coded 100), in permanent tube (coded 2; coded 110), or in transient pouch (coded 3; coded 101)	Stomatogenesis epiapokinetal (coded 0; coded 000)
51	Undulating membranes originate de novo (coded 1)	Undulating membranes originate from oral primordium or cirral anlagen (coded 0)
52	Unspecialised somatic kineties originate de novo (coded 1)	Unspecialised somatic kineties originate usually by intrakinetal proliferation of basal bodies (coded 0)
53	Reorganisation of somatic kineties present (coded 1)	Reorganisation of somatic kineties absent (coded 0)
54	Preformed emergence pore of resting cyst closed with a plug (coded 1)	Preformed emergence pore and plug absent in resting cyst (coded 0)
55	Ectocyst (outer cyst layer) bipartite and granular (coded 1)	Ectocyst comprises a single microfibrillar or membranous layer (coded 0)
56	Wall of resting cyst with inorganic layers (coded 1)	Wall of resting cyst without inorganic layers (coded 0)
57 [a]	Lepidosome structure tubular (coded 1) or fibrous (coded 2)	Lepidosomes absent (coded 0)
58	Lepidosome shape conical/spine-like (coded 1)	Lepidosomes globular (coded 0)
59	"Curious structures" in cytoplasm of resting cyst present (coded 1)	"Curious structures" in cytoplasm of resting cyst absent (coded 0)
60	Cyst wall precursors of halteriid type (coded 1)	Cyst wall precursors of hypotrich type (coded 0)
61	Inner cyst membrane that encloses the ciliate emerging from the cyst absent (coded 1)	Inner cyst membrane that encloses the ciliate emerging from the cyst present (coded 0)
62	Pycnosis of vegetative macronucleus without fragmentation (coded 1)	Fragmentation of vegetative macronucleus prior to pycnosis (coded 0)
63 [a]	Interlocking arrangement (coded 1) or oblique arrangement (coded 2) of conjugants	Parallel arrangement of conjugants (coded 0)
64	Transient dimorphism of conjugants (coded 1)	Isomorphic conjugants (coded 0)
65	Conjugants share membranelles (coded 1)	Conjugants do not share membranelles (coded 0)
66	Single derivative of first maturation division performs second division (coded 1)	All derivatives of first maturation division participate in second division (coded 0)

[a] Non-additive (unordered) character states, i.e., each state can change into any other state by one step.
[b] Binary coding of character state trees (first code for Hennigian argumentation scheme; second code for computer analyses).

Table S2. Distribution of character states over the taxa cladistically analysed with the computer programs PAUP* and Hennig86 (Figs S3–S5). Note that the character state trees of Characters 19, 22, 24, 32, 46, and 50 (Table S1) were converted into additive binary coding. Shades of grey mark the most common (in ≥ 50% of species) character state in a polymorphic taxon and the presumed character state of a taxon adopted from the state in its closest relatives.

	Characters									
Taxon[a]	10	20	30	40	50	60	70	80	90	
Hypo	00000000-1	0000000---	----------	----------	--------00	000000000-	--000000--	----000000	1000100000	2110
Mese	1110100100	0010000---	----------	----------	--------00	000000010-	--000000--	----100011	1010201100	?111
Halt	1110100100	0010010---	----------	----------	--------00	000000110-	--000000--	----100011	1010211?0	1111
Phal	1110100100	0010010---	----------	----------	--------00	000000110-	--000000--	----100011	10?0??11?0	?111
Cyrt	111020020-	2110111---	---------1	0101000000	0-------00	0000000120	--001000--	----110010	01?1?1????	000?
Will	1110200100	1010111---	---------2	0101100000	0-------00	0000000120	--00?000--	----110010	01?1??????	000?
Plst	1110200100	1011111---	---------2	0100000000	0-------00	0000000120	--001000--	----111010	011121??1?	000?
Limn	1110200100	1012111---	---------1	0100000000	0-------00	0000000120	--001000--	----111010	01?10-????	000?
Labo	1110200100	1010111---	---------2	0110000000	0-------00	0000000320	--001000--	----110010	01?1??????	000?
Noap	1110200100	1010111---	---------1	0000000000	0-------00	0000000021	--001000--	----110010	01?1??????	000?
Nosi	1110200100	1010111---	---------1	0000000000	0-------00	0000000120	--001000--	----110010	01?1??????	000?
Omeg	1110200100	1010111---	---------2	0000000010	0-------00	0000000120	--001000--	----110010	01?1??????	000?
Opis	1110200100	1010111---	---------1	0100001000	0-------00	0000000120	--001000--	----110010	01?1??????	000?
Fois	1110200100	1010111---	---------1	0100010000	0-------00	0000000021	--001000--	----110010	01?1??????	000?
Plle	1110200100	1010111---	---------0	0000000000	0-------00	0000000120	--001000--	----110010	01?1??????	000?
Spir	1110200100	1010111---	---------1	1000000000	0-------00	0000000120	--001000--	----110010	01?1??????	000?
Stro	1110200100	1010111---	---------1	0100000000	0-------00	0000000120	--001000--	----110010	01?121??1?	000?
Pato	1110200100	1010111---	---------2	0100000100	0-------00	0000000320	--201000--	----110010	01?1??????	000?
Psto	1110200100	1010111---	---------1	0100000000	0-------00	0000000320	--211000--	----110010	01?1??????	000?
Spto	1110200100	1010111---	---------2	0110000000	0-------00	0000000320	--201000--	----110010	01?1??????	000?
Tont	1110200100	1010111---	---------2	0000000000	0-------00	0000000320	--201000--	----110010	01?1??????	000?
Apos	1110200100	1010111---	---------2	0000000011	0-------00	0000000120	--00?000--	----110010	01?1??????	000?
Vari	1110200100	1010111---	---------2	0000000010	1-------00	0000000120	--00?000--	----110010	01?1??????	000?
Lova	1111201110	1010111---	----------	----------	--------00	000000010-	--000000--	----110110	01?1?????1	?000
Lelb	1111201110	1010010---	----------	----------	--------10	000000000-	--000000--	----110110	01?1?????1	?000
Lsol	1111201110	1010010---	----------	----------	--------11	000000000-	--000000--	----110110	01?1?????1	?000
Lohm	1111200110	1010010---	----------	----------	--------11	110000010-	--000000--	----110110	01?1?????1	?000
Pcon	1111200110	1010011010	000111000-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Pmin	1111200110	1010011100	000121100-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Pwil	1111200110	1010011001	000000000-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Pkim	1111200110	1010011201	000111000-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Pepa	1111200110	1010011001	100101100-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Pota	1111200110	1010011001	111100000-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Psim	1111200110	1010011001	111111100-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Pnep	1111200110	1010011001	110120010-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Pspi	1111200110	1010011001	110120011-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Ppep	1111200110	1010011001	100110000-	----------	--------11	111100120-	--000100--	----110110	01?1?????1	1000
Rimo	1111200110	1010010---	----------	----------	--------11	111100120-	--000100--	----110110	01?10-???1	?000

Taxon[a]	Characters 10	20	30	40	50	60	70	80	90	
Strb	1111200110	1010012---	----------	----------	--------11	111100120-	--000100--	----110110	01?1?????1	?000
Spsi	1111200110	1010200---	----------	----------	-00--00011	000000000-	--000000--	----110110	01?10-???1	?000
Parp	1112200110	1010200---	----------	----------	-00--00011	000000000-	--000000--	----110110	01?1?????1	?000
Parb	1112200220	1010200---	----------	----------	-00--00011	000000030-	--000000--	----110110	01?1?????1	?000
Lynn	1112200210	1010110---	----------	----------	-00--00011	111000000-	--000000--	----110110	01?1?????1	?000
semi	1111210210	1010210---	----------	----------	-11--00011	100010011-	??10001100	0000110110	0????????1	0000
fluv	1111210210	1010210---	----------	----------	-11--00011	100000011-	??10001100	0000110110	0????????1	0000
pusi	1111210210	1010210---	----------	----------	-11--00011	100000011-	??10001100	0000110110	0????????1	0000
cyda	1111210210	1010210---	----------	----------	-11--00011	111100011-	??10001100	0000110110	0????????1	0000
Memb	1111210210	1010210---	----------	----------	-11--00011	11000000?-	??10001100	1000110110	0????????1	0000
Amph	11212102?1	?010210---	----------	----------	-2?020000	00000003?-	??1?001121	0000?101?0	0????????1	0000
Scan	11212102?1	?010010---	----------	----------	-2?0?00??	??????01?-	??1?001121	0002?101?0	0????????1	0000
Nola	1111210110	1010210---	----------	----------	-20000011	100011001-	??1?001121	0100110110	0????????1	0000
Euti	1111210110	1010210---	----------	----------	-20010011	100011031-	??12001121	0000110110	00??0-???1	0000
Fehr	1111210110	1010210---	----------	----------	-20010111	100011000-	-?10001121	0010110110	0????????1	0000
Schm	1111210110	1010210---	----------	----------	-21120111	100011001-	??12001121	0010110110	01??0-???1	0000
bras	1111210?10	1010210---	----------	----------	-20020111	10001100?-	??1?001101	00?0110110	0????????1	0000
Ocod	1111210110	1010210---	----------	----------	-20021111	100011031-	1112001101	0031110110	0????????1	0000
Ccra	1111210210	1010210---	----------	----------	-20021111	100011000-	-?10001101	0000110110	0????????1	0000
Cpsi	1111210110	1010210---	----------	----------	-20021111	100011031-	1112001111	0021110110	0????????1	0000
Cyma	1111210110	1010210---	----------	----------	-20021111	100011001-	??1?001121	0010110110	0????????1	0000
Otsp	1111210110	1010210---	----------	----------	-20021111	100011001-	0012001101	00?0110110	00??0-???1	0000
lacu	1111210110	1010210---	----------	----------	-20021111	10001100?-	??1?001101	00?0110110	0????????1	0000
Oste	1111210110	1010210---	----------	----------	-20021111	100011001-	1112001111	0030110110	0????????1	0000
toca	1111210110	1010210---	----------	----------	-21020111	100011000-	-?10001101	00?0110110	0???0-???1	0000
radi	1111210110	1010210---	----------	----------	-20021111	100011000-	-?10001101	00?0110110	0???0-???1	0000
cyli	1111210110	1010210---	----------	----------	-21020111	100011000-	-?10001101	00?0110110	00??0-???1	0000
Rhiz	1111210110	1010210---	----------	----------	-20021111	100011001-	??10001101	0010110110	00??0-???1	0000
Laac	1111210110	1010210---	----------	----------	-20021111	10001103?-	??1?001111	00?0110110	0????????1	0000

[a] *Amph* – *Amphorellopsis*, *Apos* – *Apostrombidium*, *bras* – *Tintinnopsis brasiliensis*, *Ccra* – *Codonella cratera*, *Cpsi* – *Codonellopsis*, *cyda* – *Tintinnopsis cylindrata*, *cyli* – *Tintinnopsis cylindrica*, *Cyma* – *Cymatocylis*, *Cyrt* – *Cyrtostrombidium*, *Euti* – *Eutintinnus*, *Fehr* – *Favella ehrenbergii*, *fluv* – *Tintinnidium fluviatile* of subgenus *Tintinnidium*, *Fois* – *Foissneridium*, *Halt* – *Halteria*, Hypo – hypotrichs, *Laac* – *Laackmanniella*, *Labo* – *Laboea*, *lacu* – *Stenosemella lacustris*, *Lelb* – *Leegaardiella elbraechteri*, *Limn* – *Limnostrombidium*, *Lohm* – *Lohmanniella*, *Lova* – *Leegaardiella ovalis*, *Lsol* – *Leegaardiella sol*, *Lynn* – *Lynnella*, *Memb* – *Membranicola*, *Mese* – *Meseres*, *Noap* – *Novistrombidium apsheronicum*, *Nola* – *Nolaclusilis*, *Nosi* – *Novistrombidium sinicum*, Ocod – other *Codonella* species, *Omeg* – *Omegastrombidium*, *Opis* – *Opisthostrombidium*, Oste – other *Stenosemella* species, Otsp – other *Tintinnopsis* species, *Parb* – *Parastrombidium*, *Parp* – *Parastrombidinopsis*, *Pato* – *Paratontonia*, *Pcon* – *Pelagostrobilidium conicum*, *Pepa* – *Pelagostrobilidium epacrum*, *Phal* – *Pelagohalteria*, *Pkim* – *Pelagostrobilidium kimae*, *Plle* – *Parallelostrombidium*, *Plst* – *Pelagostrombidium*, *Pmin* – *Pelagostrobilidium minutum*, *Pnep* – *Pelagostrobilidium neptuni*, *Pota* – *Pelagostrobilidium* studied by Ota and Taniguchi (2003), *Ppep* – *Pelagostrobilidium paraepacrum*, *Psim* – *Pelagostrobilidium simile*, *Pspi* – *Pelagostrobilidium spirale*, *Psto* – *Pseudotontonia*, *pusi* – *Tintinnidium pusillum* of subgenus *Tintinnidium*, *Pwil* – *Pelagostrobilidium wilberti*, *radi* – *Tintinnopsis radix*, *Rhiz* – *Rhizodomus*, *Rimo* – *Rimostrombidium*, *Scan* – *Salpingacantha*, *Schm* – *Schmidingerella arcuata*, *semi* – *Tintinnidium semiciliatum* of subgenus *Semitintinnidium*, *Spir* – *Spirostrombidium*, *Spsi* – *Strombidinopsis*, *Spto* – *Spirotontonia*, *Strb* – *Strobilidium*, *Stro* – *Strombidium*, *toca* – *Tintinnopsis tocantinensis*, *Tont* – *Tontonia*, *Vari* – *Varistrombidium*, *Will* – *Williophrya*.

Table S3. Alphabetical list of all sequences used in the phylogenetic analyses of this study: genus and species name, isolate or strain designation if necessary, and GenBank Accession Number.

Taxa	Accession numbers
Amphorellopsis acuta	EU399530
Amphorellopsis acuta	JX101847
Amphorellopsis quinquealata	JQ924058
Amphorides amphora	JX101849
Amphorides quadrilineata	JQ408193
Amphorides quadrilineata	JX101850
Anteholosticha parawarreni	JQ289923
Apostrombidium parakielum	JX025560
Aspidisca steinii	AF305625
Climacocylis scalaria	JQ408213
Codonaria cistellula	JQ408202
Codonaria sp.	JQ408172
Codonella apicata	EU399531
Codonella aspera	JQ408179
Codonellopsis americana	AY143571
Codonellopsis gaussi	JQ924053
Codonellopsis morchella	JQ408173
Codonellopsis nipponica	FJ196072
Codonellopsis orthoceras	JQ408180
Coxliella sp.	JX101851
Cymatocylis calyciformis	JQ924046
Cymatocylis convallaria	JQ924050
Cymatocylis drygalskii	JQ924052
Cyttarocylis ampulla (formerly *Petalotricha ampulla*)	JQ408168
Cyttarocylis cassis	JQ408203
Cyttarocylis eucecryphalus	JQ408169
Dadayiella ganymedes	JX101852
Dictyocysta lepida	JQ408188
Dictyocysta reticulata	EU399532
Diophrys appendiculata	AY004773
Epiplocylis acuminata	JQ715615
Epiplocyloides ralumensis	JX101854
Euplotes aediculatus	X03949
Eutintinnus apertus	JQ408195
Eutintinnus fraknoi	EU399534
Eutintinnus lusus-undae (agglomerated-form)	JX101858
Eutintinnus pectinis	AY143570
Eutintinnus stramentus	JX101859
Eutintinnus tenuis	JN871721
Eutintinnus tubulosus (agglomerated-form)	JX101856
Eutintinnus tubulosus	JQ408187
Favella adriatica	JQ408215
Favella campanula	FJ422984
Favella ehrenbergii	GU574767
Favella markusovszkyi	JN871725
Favella panamensis	AY143572
Halteria grandinella	AF194410
Helicostomella subulata	JN831781
Helicostomella subulata	JN831786
Histriculus histrio	FM209294
Laackmanniella prolongata	JQ924056
Laboea strobila	AF399154
Laboea strobila	AY302563
Lynnella semiglobulosa	FJ876965
Meseres corlissi (strain AU5)	EU399524
Meseres corlissi (strain CHI)	EU399529
Meseres corlissi (strain DR)	EU399522
Metacylis angulata	AF399146
Metacylis angulata	AY143568
Metacylis jörgensenii	JQ408183
Metacylis pithos	JX101862
Moneuplotes crassus	AJ310492
Novistrombidium orientale	FJ422988
Novistrombidium sinicum population 1	FJ422989
Novistrombidium sinicum population 2	FJ422990
Novistrombidium apsheronicum	FJ876958
Novistrombidium testaceum	AJ488910
Omegastrombidium elegans	EF486862
Oxytricha granulifera	X53486
Oxytricha longa	AF164125
Parallelostrombidium sp. (3-GD-08040807)	FJ422991
Parallelostrombidium sp. (WS-2012)	JN712657
Parastrombidinopsis minima	DQ393786
Parastrombidinopsis shimi	AJ786648
Parundella aculeata	JQ408204
Pelagostrobilidium minutum	FJ876959
Pelagostrobilidium neptuni	AY541683
Pelagostrobilidium paraepacrum	FJ876963
Protorhabdonella curta	JX101863
Pseudokeronopsis rubra	DQ640314
Pseudotontonia simplicidens	FJ422993
Pseudouroleptus caudatus	DQ910904
Rhabdonella elegans	JQ408175
Rhabdonella hebe	AY143566

Taxa	Accession numbers
Rhabdonella poculum	JX101864
Rhabdonella spiralis	JQ408158
Rhizodomus tagatzi	JQ392572
Rimostrombidium lacustris	DQ986131
Rimostrombidium sp.	EU024986
Rimostrombidium veniliae	FJ876964
Salpingella acuminata	JQ408155
Salpingella acuminata	EU399536
Schmidingerella arcuata	JQ837816
Schmidingerella taraikaensis	FJ196073
Spirostrombidium sp.	JN712658
Spirotontonia taiwanica	FJ715634
Spirotontonia turbinata	FJ422994
Steenstrupiella steenstrupii	JQ408194
Steenstrupiella steenstrupii	EU399537
Stenosemella nivalis	FJ196074
Stenosemella pacifica	JN831792
Stenosemella ventricosa	JQ408170
Stenosemella ventricosa	EU399538
Strobilidium caudatum	AY143573
Strobilidium sp.	AF399124
Strombidinopsis acuminatum	AJ877014
Strombidinopsis jeokjo	AJ628250
Strombidinopsis sp. (LFS-2012b)	JQ028734
Strombidinopsis sp.	AM412524
Strombidium apolatum	DQ662848
Strombidium biarmatum	AY541684
Strombidium basimorphum	FJ480419
Strombidium conicum	FJ422992
Strombidium crassulum	HM140389
Strombidium purpureum	U97112
Strombidium rassoulzadegani	AY257125
Strombidium stylifer	DQ631805
Strombidium sulcatum	DQ777745
Stylonychia lemnae	AF508773
Styxophrya quadricornuta	X53485

Taxa	Accession numbers
Tintinnidium balechi	JN831797
Tintinnidium fluviatile	JQ408163
Tintinnidium mucicola	AY143563
Tintinnidium pusillum	DQ487200
Tintinnopsis baltica	JN831805
Tintinnopsis beroidea	EF123709
Tintinnopsis bütschlii	JN831810
Tintinnopsis cylindrica	FJ196075
Tintinnopsis cylindrica	JQ408206
Tintinnopsis dadayi	AY143562
Tintinnopsis fimbriata	AY143560
Tintinnopsis lacustris	JQ408161
Tintinnopsis lobiancoi	JN831814
Tintinnopsis lohmanni	FJ196076
Tintinnopsis major	JN831816
Tintinnopsis nana	JN831821
Tintinnopsis parva	JN831824
Tintinnopsis parvula	JN831830
Stylicauda platensis (as *Tintinnopsis*)	JN831832
Tintinnopsis radix	EU399540
Tintinnopsis rapa	JN831834
Tintinnopsis rara	JQ408200
Tintinnopsis subacuta	EU399541
Tintinnopsis tocantinensis	AY143561
Tintinnopsis tubulosoides	AF399109
Tintinnopsis uruguayensis	EU399542
Undella claparedei	JQ408164
Undella hyalina	JQ408171
Undella marsupialis	JQ408190
Uroleptus piscis	AF508780
Uroleptus willii	EU399543
Uronychia transfuga	AF260120
Urostyla grandis	AF508781
Varistrombidium kielum	DQ811090
Williophrya maedai	FJ876966
Xystonella longicauda	JQ408160

REFERENCES

Abboud-Abi Saab M. (2008) Tintinnids of the Lebanese Coastal Waters (Eastern Mediterranean). CNRS-Lebanon/UNEP/MAP/RAC/SPA: i–viii + 1–192

Agatha S. (2003) Morphology and ontogenesis of *Novistrombidium apsheronicum* nov. comb. and *Strombidium arenicola* (Protozoa, Ciliophora): a comparative light microscopical and SEM study. *Eur. J. Protistol.* **39:** 245–266

Agatha S., Riedel-Lorjé J. C. (1998) Morphology, infraciliature, and ecology of some strobilidiine ciliates (Ciliophora, Oligotrichea) from coastal brackish water basins of Germany. *Eur. J. Protistol.* **34:** 10–17

Agatha S., Riedel-Lorjé J. C. (2006) Redescription of *Tintinnopsis cylindrica* Daday, 1887 (Ciliophora, Spirotricha) and unification of tintinnid terminology. *Acta Protozool.* **45:** 137–151

Burns D. A. (1983) The distribution and morphology of tintinnids (ciliate protozoans) from the coastal waters around New Zealand. *N. Z. J. Mar. Freshwat. Res.* **17:** 387–406

Edgar R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. **32:** 1792–1797

Entz G. Sr. (1884) Über Infusorien des Golfes von Neapel. *Mitt. zool. Stn Neapel* **5:** 289–444 + Plates 20–25

Fauré-Fremiet E. (1924) Contribution a la connaissance des infusoires planktoniques. *Bull. biol. Fr. Belg. Suppl.* **6:** 1–171

Gold K., Morales E. A. (1977) Studies on the Tintinnida of Enewetak Atoll. *J. Protozool.* **24:** 580–587

Kent W. Saville (1880–1882) A Manual of the Infusoria: Including a Description of all known Flagellate, Ciliate, and Tentaculiferous Protozoa, British and Foreign, and an Account of the Organization and Affinities of the Sponges. Volumes I–III. D. Bogue, London + Plate 25–51 (Vol. I – 1880: i–x + 1–472; Vol. II – 1881: 473–720; 1882: 721–913; Vol. III – 1882: Plates)

Kofoid C. A., Campbell A. S. (1929) A conspectus of the marine and fresh-water Ciliata belonging to the suborder Tintinnoinea, with descriptions of new species principally from the Agassiz Expedition to the eastern tropical Pacific 1904–1905. *Univ. Calif. Publs Zool.* **34:** 1–403

Küppers G. C., Lopretto E. C., Claps M. C. (2006) *Pelagostrobilidium wilberti* n. sp. (Oligotrichea, Choreotrichida): morphology and morphogenesis. *J. Eukaryot. Microbiol.* **53:** 477–484

Lee E. S., Shin M. K., Kim Y.-O. (2011) Morphological descriptions of four oligotrich ciliates (Ciliophora: Oligotrichia) from southern coast of Korea. *Korean J. Syst. Zool.* **27:** 131–141

Liu W., Xu D., Lin X., Li J., Gong J., Al-Rasheid K. A. S., Song W. (2009) *Novistrombidium sinicum* n. sp. and *Novistrombidium orientale* n. sp. (Protozoa: Ciliophora): two new oligotrich ciliates from a mangrove wetland, South China. *J. Eukaryot. Microbiol.* **56:** 459–465

Liu W., Yi Z., Lin X., Warren A., Song W. (2012) Phylogeny of three choreotrich genera (Protozoa, Ciliophora, Spirotrichea), with morphological, morphogenetic and molecular investigations on three strobilidiid species. *Zool. Scr.* **41:** 417–434

Lynn D. H., Montagnes D. J. S. (1988) Taxonomic descriptions of some conspicuous species of strobilidiine ciliates (Ciliophora: Choreotrichida) from the Isles of Shoals, Gulf of Maine. *J. Mar. Biol. Ass. U. K.* **68:** 639–658

Montagnes D. J. S., Taylor F. J. R. (1994) The salient features of five marine ciliates in the class Spirotrichea (Oligotrichia), with notes on their culturing and behaviour. *J. Eukaryot. Microbiol.* **41:** 569–586

Ota T., Taniguchi A. (2003) Conjugation in the marine aloricate oligotrich *Pelagostrobilidium* (Ciliophora: Oligotrichia). *Eur. J. Protistol.* **39:** 149–160

Small E. B., Lynn D. H. (1985) Phylum Ciliophora Doflein, 1901. In: An Illustrated Guide to the Protozoa, (Eds. J. J. Lee, S. H. Hutner, E. C. Bovee). Society of Protozoologists, Allen Press, Lawrence, Kansas, 393–575

Song W., Bradbury P. C. (1998) Studies on some new and rare reported marine planktonic ciliates (Ciliophora: Oligotrichia) from coastal waters in North China. *J. Mar. Biol. Ass. U. K.* **78:** 767–794

Swofford D. L. (2002) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4.0b10. Sinauer Ass., Sunderland, Massachusetts

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*. **28:** 2731–2739

Wolf M., Ruderisch B., Dandekar T., Müller T. (2008) ProfDistS: (Profile-) Distance based phylogeny on sequence-structure alignments. *Bioinformatics* **24:** 2401–2402

Xu D., Song W., Warren A. (2006) Morphology and infraciliature of two new species of marine oligotrich ciliates (Ciliophora: Oligotrichida) from China. *J. Nat. Hist. (London)* **40:** 1287–1299