Morphological versus molecular data – Phylogeny of tintinnid ciliates (Ciliophora, Choreotrichia) inferred from small subunit rRNA gene sequences*

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Abstract: Tintinnid ciliates are an abundant and important component of marine planktonic food webs. Traditionally classified together based on lorica morphology and the arrangement of the oral ciliature, they include 76 recent genera in 15 families. Infraciliary data are still rare for tintinnids. Therefore, lorica morphology is the primary, but very disputed, character for species determination. Previous sequence analyses of 16 representative species of the major oligotrich orders showed that the subclasses Oligotrichia (excluding *Halteria*) and Choreotrichia form a monophyletic group and that tintinnids are indeed monophyletic choreotrichs, a sistergroup to the order Choreotrichida. We collected 12 additional tintinnid species, representing five families to refine the phylogenetic analyses: *Amphorellopsis acuta*, *Codonella apicata*, *Dictyocysta reticulata*, *Eutintinnus fraknoi*, *Eutintinnus* sp., *Favella* sp., *Salpingella acuminata*, *Steenstrupiella steenstrupii*, *Stenosemella ventricosa*, *Tintinnopsis radix*, *T. subacuta*, and *T. uruguayensis*. Our data clearly refute the idea of inference of phylogenetic relationships based on lorica characteristics.

Key words: Gulf Stream, lorica variation, paraphyly, Tintinnida, *Tintinnopsis*.

Introduction

The generalized tintinnid cell is conical or funnel-shaped, attached with a peduncle to a lorica that is endogenously produced. The oral ciliature is prominent, a closed circle of large polykinetids, typical for ciliates of the subclass Choreotrichia (Lynn 2008). The somatic ciliature is more or less reduced – we distinguish a right, a left, and a lateral ciliary field, a dorsal, a ventral, and a posterior kinety. These ciliary components, however, are not necessarily present in all taxa (Agatha & Strüder-Kypke 2007). Most tintinnid ciliates have two macro- and two micronuclei – however, one or four macronuclei are also common in some genera (Agatha & Strüder-Kypke 2007). Replication bands can be observed during DNA replication – a character typical for most species in the class Spirotrichea (Lynn 2008).

The lorica often obscures the infraciliature in protargol stains – normally used to determine kinetal patterns – important in ciliate taxonomy and systematics. Therefore, the infraciliature of only a few tintinnid species has been described to date (Foissner & Wilbert 1979; Laval-Peuto & Brownlee 1986; Song & Wilbert 1989; Blatterer & Foissner 1990, Foissner & O'Donoghue 1990; Sniezek et al. 1991; Snyder & Brownlee 1991; Choi et al. 1992; Petz & Foissner 1993; Song 1993; Wasik & Mikolajczyk 1994; Petz et al. 1995; Cai et al. 2006; Agatha & Riedel-Lorjé 2006, Agatha & Tsai 2008).

Based on the monographs of Kofoid & Campbell (1929, 1939) that describe over 1700 tintinnid species according to their lorica morphology, taxonomy and systematics – even phylogeny – of tintinnids was inferred from this feature. The first attempt to reconstruct tintinnid phylogeny based on lorica characteristics (Kofoid & Campbell 1939) hypothesized that agglomerated loricae were basal and hyaline loricae derived. This concept was neither supported by infraciliary (Laval-Peuto & Brownlee 1986) nor by molecular (Strüder-Kypke & Lynn 2003, Agatha & Strüder-Kypke 2007) data.

With the first studies of Laval-Peuto (1977, 1981, 1983) on cultured *Favella ehrenbergi*, details about lorica formation became known. Since then it is generally accepted that the tintinnid lorica is not a fixed structure, but can be polymorphic within species. Depending on the habitat and how and at what stage in the life cycle the lorica is formed we find highly variable loricae in the same species (e.g., replacement lorica, epilorica). Other studies also demonstrated that many

* The authors dedicate this article to Prof. Dr. Wilhelm Foissner, on the occasion of his 60th birthday. Dr. Foissner's influence on ciliate taxonomy has been tremendous; his numerous contributions and his approach to integrate various fields of research through collaborations are exemplary and serve as inspiration for many of us.

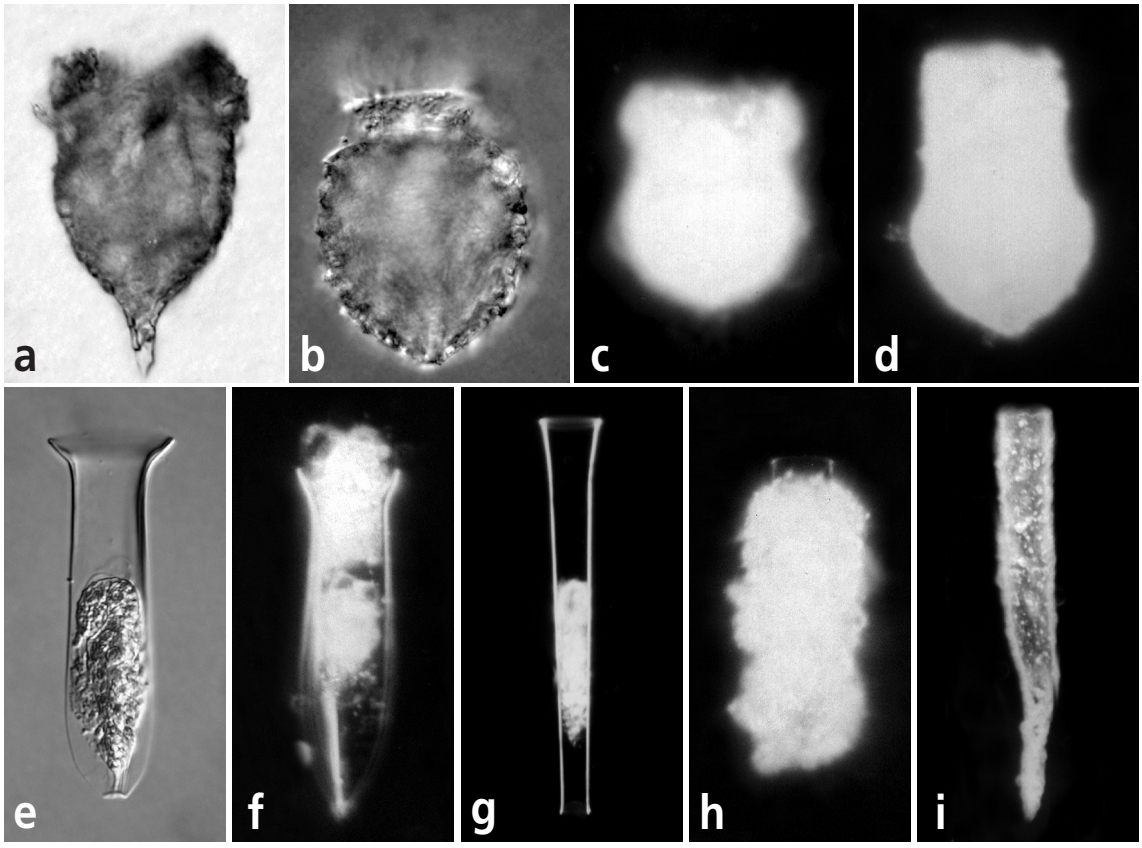

Fig. 1a-i: Light microscopical images of eight tintinnid species acquired with differential interference contrast (**a, b, e**) or dark field (**c, d, f–h**) optics. **a**: *Tintinnopsis uruguayensis*. **b**: *Stenosemella ventricosa*. **c**: *Codonella apicata*. **d**: *Tintinnopsis subacuta*. **e**: *Steenstrupiella steenstrupii*. **f**: *Amphorellopsis acuta*. **g**: *Eutintinnus fraknoi*. **h**: *Eutintinnus* sp. **i**: *Tintinnopsis radix*.

tintinnid species show an enormous flexibility in their lorica formation (Hofker 1931; Burkovski 1973; Gold & Morales 1975; Bakker & Phaff 1976; Davis 1978, 1981; Bernatzky et al. 1981; Williams et al. 1994) – suggesting that many of the species described by Kofoid & Campbell (1929, 1939) will be synonymized when other features are used as species criteria. On the other hand, further morphological, ecological, and genetic studies will probably reveal cryptic species within the tintinnids.

Laval-Peuto & Brownlee (1986) used the few data available on the infraciliature to construct a phylogenetic/evolutionary model. However, the first molecular studies (Snoeyenbos-West et al. 2002; Strüder-Kypke & Lynn 2003) could not confirm their data in all details. Agatha & Strüder-Kypke (2007) used both infraciliary and molecular data to develop an evolutionary model of ciliary patterns within the order Tintinnida. In our present study we want to refine our first analysis and resolve phylogenetic relationships among the different genera by including more species from different families. By comparing the molecular and morphological data (both lorica and infraciliature) we discuss the relevance of the different criteria for models of evolution within the order Tintinnida.

Material and methods

Sampling: Eleven species (*Amphorellopsis acuta*, *Codonella apicata*, *Dictyocysta reticulata*, *Eutintinnus* sp., *E. fraknoi*, *Salpingella acuminata*, *Steenstrupiella steenstrupii*, *Stenosemella ventricosa* [2 populations], *Tintinnopsis radix*, *T. subacuta*, *T. uruguayensis*) were sampled in Florida during July 2000 and March 2002. Some of the species are shown in Figure 1. *Favella* sp. was sampled in Narragansett Bay in July 1999. The exact locations and dates are listed in Table 1. Genus and species identifications were made by microscopical observation at magnifications of ×160 and ×400. They were based on the general lorica morphology, following mostly Marshall (1969), Kofoid & Campbell (1929, 1939), and Alder (1999). All cells were pipetted out of the water samples and fixed in 80% EtOH (final concentration) for DNA extraction.

DNA extraction, PCR amplification, cloning, sequencing: The DNA extraction from the EtOH-fixed cells followed the protocol described by Strüder-Kypke & Lynn (2003) using 70–150 µl of 5% Chelex® 100 (Sigma, Oakville, ON, Canada) and 5–10 µl of Proteinase K (20mg/ml, Sigma) for about 30–100 cells. Typically 10–20 µl of template were used for the subsequent PCR reactions. The PCR amplification of the small subunit rRNA gene (SSrRNA) was performed in a Perkin-Elmer GeneAmp 2400 thermocycler (PE Ap-

plied Biosystems, Mississauga, ON, Canada) using the forward primer 82F (5'-GAAACTGCGAATGGCTC-3'; ELWOOD et al. 1985) and the universal reverse primer B (5'-TGATCCTTCTGCAGGTTCACCTAC-3'; MEDLIN et al. 1988). *Favella* sp. was amplified using the internal forward primer 300F (5'-AGGGTTCGATTC-CGGAG-3', ELWOOD et al. 1985) and primer B. The PCR products of *Favella* sp., *Stenosomella ventricosa*, and *T. uruguayensis* were purified using the GeneClean Kit (Qbiogen, Carlsbad, CA, USA). Initial PCR products of all other species were cloned using the TOPO TA Cloning Kit (Invitrogen, Burlington, ON, Canada). Subsequently, 2–4 µl of the purified cloned product were used to re-amplify the SSrDNA with the primers 82F and reverse B and the re-amplification products were purified as above. Sequencing was performed in both directions using an ABI Prism 377 Automated DNA Sequencer (Applied Biosystems Inc., Foster City, CA, USA) using dye terminator and Taq FS with three forward and three reverse internal SSrRNA primers (ELWOOD et al. 1985) and the amplification primers.

Phylogenetic analyses: All nucleotide sequences used in this article are available from the GenBank/EMBL databases and their accession numbers are listed in Figure 2. The SSrRNA gene sequences of the tintinnid ciliates were added to our existing DCSE (Dedicated Comparative Sequence Editor; DE RIJK & DE WACHTER 1993) database and automatically aligned to other tintinnid sequences. Based on the secondary structure of the SSrRNA molecule, we further refined the alignment. Before starting the phylogenetic analyses, we tested the model of substitution with MrModeltest (NYLANDER 2004). The recommended parameter settings (general time reversible model with gamma distribution and consideration of invariable sites [GTR+I+G]) were implemented into the MrBayes ver. 3.2 (RONQUIST & HUELSENBECK 2003), a phylogenetic program employing Bayesian Inference and determining the maximum posterior probability of a phylogeny out of 1,000,000 trees, approximating it with the Markov Chain Monte Carlo (MCMC). A maximum parsimony (MP) analysis was performed with PAUP* ver. 4.0b10 (SWOFFORD 2002) and the data were bootstrap resampled 1000 times. As a third approach, the genetic distances of the included sequences were calculated by DNADIST of the PHYLIP package ver 3.65 (FELSENSTEIN 2005), following the Kimura-2-parameter model of substitution (KIMURA 1980), and phylogenetic trees were constructed using the distance matrix method NEIGHBOR (neighbor joining = NJ; SAITOU & NEI 1987).

Results

Collected species: *Amphorellopsis acuta* (Fig. 1f) shows the typical vase-shaped hyaline lorica (144-148 µm long, oral diameter 43–48 µm) with a slightly flaring collar. Its end is pointed and the vertical fins are as long as the lorica. *Codonella apicata* (Fig. 1c) has a distinct separation of collar and bowl, and the collar is bulging and narrows toward the anterior rim. The lorica is 60–70 µm in length, its oral diameter measures 44 µm and the largest diameter of the bowl is 48–52 µm. The agglutination of the lorica is very dense, therefore no further details of the lorica structure could be observed. *Dictyocysta reticulata* shows the typical fenestrated collar (17–22 µm high) with six large fenestrae. The agglomerated bowl is conical and bluntly pointed. The lorica is 65 µm long, the oral diameter is 39 µm, and the largest diameter of the bowl is 48 µm. *Eutintinnus fraknoi* (Fig. 1g) has a hyaline lorica that is slightly flaring at both ends but distinctly tapered towards the aboral end. The lorica is very long (315 µm) and the oral diameter measures 52 µm. *Eutintinnus* sp. (Fig. 1h) was originally not recognized as *Eutintinnus* species. Only the gene sequence data and the hyaline portion of the lorica that extends past the agglutinated material suggest an assign-

Table 1: Origin of the ciliate species and GenBank accession numbers of their small subunit DNA sequences.

Species	GenBank	Origin	Latitude/Longitude
Amphorellopsis acuta	EU399530	Jupiter Island, FL, U.S.A.	27°03'N/80°06'W
Codonella apicata	EU399531	Gulf Stream, 16 miles offshore	27°33'N/79°59'W
Dictyocysta reticulata	EU399532	Gulf Stream, 16 miles offshore	27°33'N/79°59'W
Eutintinnus fraknoi	EU399534	Fort Pierce, FL, U.S.A.	27°28'N/80°18'W
Eutintinnus sp.	EU399533	Fort Pierce, FL, U.S.A	27°28'N/80°18'W
Favella sp.	EU399535	Galilee, Narragansett Bay, RI, U.S.A.	41°22'N/71°30'W
Salpingella acuminata	EU399536	Gulf Stream, 16 miles offshore	27°33'N/79°59'W
Steenstrupiella steenstrupii	EU399537	Gulf Stream, 20 miles offshore	27°28'N/79°56'W
Stenosemella ventricosa			
strain SFL02-1	EU399538	Fort Pierce, FL, U.S.A.	27°27'N/80°19'W
strain SFL02-2	EU399539	Stuart, FL, U.S.A.	27°12'N/80°15'W
Tintinnopsis radix	EU399540	Gulf Stream, 19 miles offshore	27°32'N/79°56'W
Tintinnopsis subacuta	EU399541	Wabasso Beach, FL, U.S.A.	27°50'N/80°26'W
Tintinnopsis uruguayensis	EU399542	Fort Pierce, FL, U.S.A.	27°27'N/80°19'W

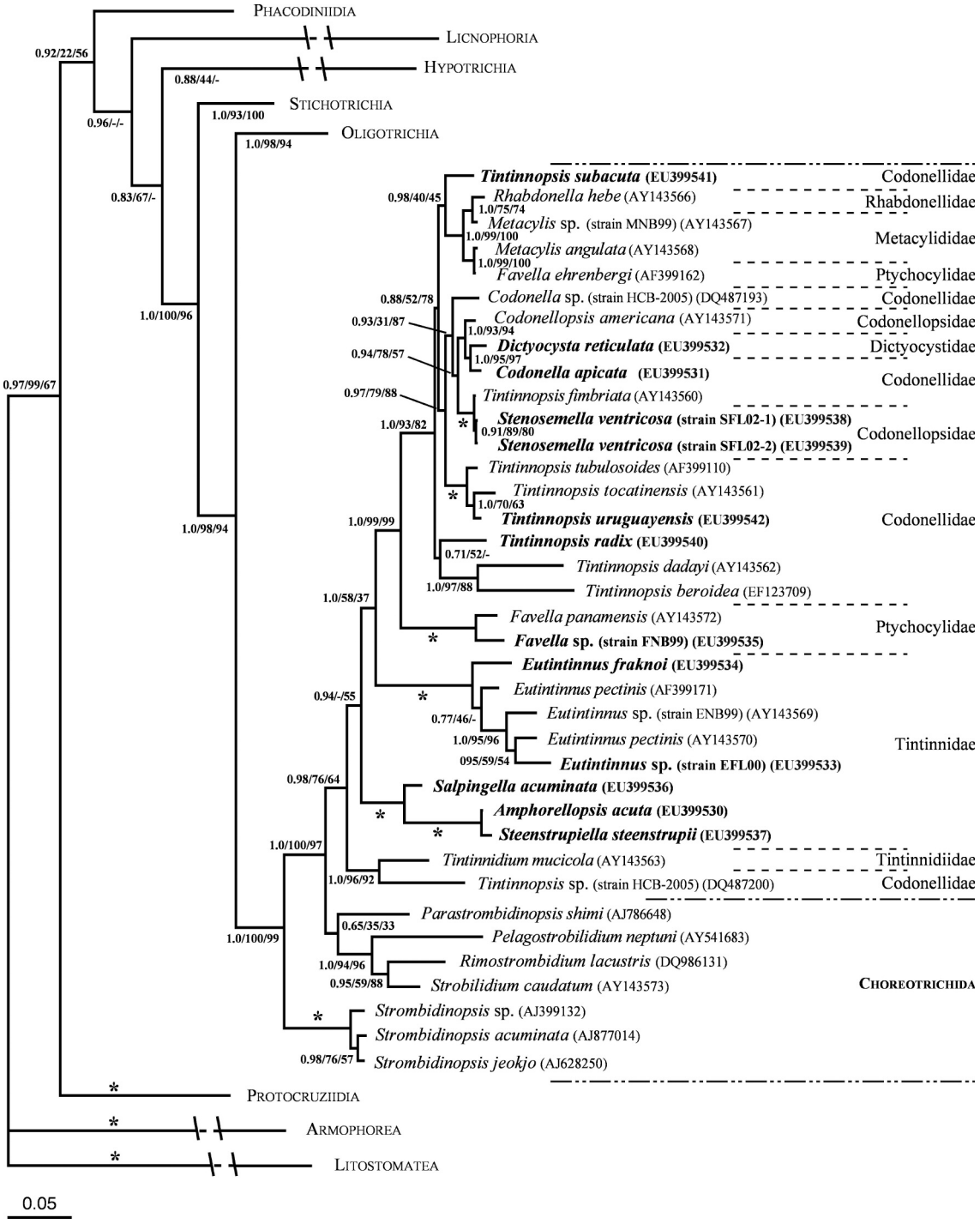

Fig. 2: Bayesian inference tree computed with MrBayes (RONQUIST & HUELSENBECK 2003), based on the General Time-reversible (GTR) model with gamma-distribution and an estimate of invariable sites, determined by MrModeltest (NYLANDER 2004). The first numbers at the nodes represent the posterior probability values of the Bayesian analysis (BI) and the second and third numbers represent bootstrap values (percent out of 1000 replicates) for maximum parsimony (MP; SWOFFORD 2002) and neighbor joining (NJ; SAITOU & NEI 1987), respectively. An asterisk represents full support in all three analyses and dashes indicate bootstrap values of less than 20% in MP and NJ. The scale bar represents 5 substitutions per 100 nucleotides. The new sequences appear in bold face.

ment to this genus. It is considerably smaller than *E. fraknoi* (127–131 µm long, oral diameter 35–37 µm). *Salpingella acuminata* is longer and narrower (223 × 35 µm) than *Steenstrupiella* or *Amphorellopsis*. Its lorica is also narrow and elongated with a wide flaring oral end. The several aboral fins are slightly curved and do not extend more than half of the lorica length. *Steenstrupiella steenstrupii* (Fig. 1e) possesses a trumpet-shaped hyaline lorica with flaring collar and an inflated aboral end with short vertical fins. Our specimens measure 160–170 µm in length and 50–60 µm in their oral diameter. *Steenstrupiella steenstrupii* is the only species in the genus that matches the observed size and shape. *Stenosemella ventricosa* (two populations; Fig. 1b) shows the typical densely agglutinated lorica with a low hyaline collar. The bowl is wide and tapers towards the aboral end. Identification follows mainly the shape of the lorica (rather broad) and its size (65–75 µm long and largest diameter of the bowl 60 µm). *Tintinnopsis radix* (Fig. 1i) has the typical shape of the lorica, which is almost cylindrical and tapers gradually to the aboral horn. The size range (263–320 µm long, oral diameter 39–41 µm)

lies also well within the range for this species. *Tintinnopsis subacuta* (Fig. 1d) does not have the typical lorica shape but we assign our specimen to this species based on the size of the lorica (83–87 µm long, oral diameter 39 µm, largest diameter 48–56 µm), the cylindrical anterior end and the expanded, bowl-shaped aboral end, which is slightly pointed. *Tintinnopsis uruguayensis* (Fig. 1a) has a very characteristic lorica shape with a ragged oral rim and a distinct aboral horn. The length of the lorica is 70–80 µm, the oral diameter 40–50 µm, and the largest diameter of the bowl 40 µm. *Favella* sp. shows the typical lorica shape of congeneric species. However, no microscopy data are available and, therefore, no further identification is possible.

Primary sequence: The new SSrDNA sequences of this study have been submitted to GenBank and are available under the accession numbers listed in Table 1. Lengths of the sequences and their GC contents (in %) are as follows: *Amphorellopsis acuta* – 1688 nucleotides, GC 47%; *Codonella apicata* – 1682 nucleotides, GC 46%; *Dictyocysta reticulata* – 1683 nucleotides, GC 46%; *Eutintinnus fraknoi* – 1685 nucleotides, GC 48%; *Eutintinnus* sp. (strain EFL00) – 1309 nucleotides, GC 47%; *Favella* sp. (strain FNB99) – 1355 nucleotides, GC 47%; *Salpingella acuminata* – 1690 nucleotides, GC 48%; *Steenstrupiella steenstrupii* – 1690 nucleotides, GC 47%; *Stenosemella ventricosa* (strainSFL02-1) – 1681 nucleotides, GC 46%; *S. ventricosa* (strainSFL02-2) – 1606 nucleotides, GC 47%; *Tintinnopsis radix* – 1681 nucleotides, GC 47%; *Tintinnopsis subacuta* – 1762 nucleotides, GC 46%; *T. uruguayensis* – 1624 nucleotides, GC 47%. Most sequences are partial at the 5'-end due to PCR amplification with primers 82F or 300F.

Phylogenetic analyses: All three phylogenetic methods provide similar tree topologies. Therefore, only the Bayesian inference (BI) tree is shown with support values for all three analyses listed at the nodes (Fig. 2). Two litostome species and two armophorean species are chosen as out-group taxa to the spirotrichs for the analyses. Several species of all spirotrich subclasses are included as the ingroup in the analyses. However, in order to keep the tree topology manageable, only the branches representing the different subclasses are drawn. The monophyly of the order Tintinnida is confirmed and together with the order Choreotrichida they form the subclass Choreotrichia, a sistergroup to the subclass Oligotrichia. As in previous studies with complete tintinnid data sets (Strüder-Kypke & Lynn 2003, Agatha & Strüder-Kypke 2007) the monophyly of the Tintinnida and the Choreotrichida is not unambiguously supported. On the contrary, species of the families Strobilidiidae and Parastrombidinopsidae rather group with the tintinnids than with the family Strombidinopsidae.

The complete data set for the tintinnids consists of 31 sequences of 13 genera in 8 families. The genus *Tintinnidium* (family Tintinnidiidae) groups basal, together with an unidentified *Tintinnopsis* species. A fully supported (1.0 BI, 100% MP, 100% NJ) cluster comprising the genera *Salpingella*, *Amphorellopsis*, and *Steenstrupiella* branches next, followed by the also fully supported group of *Eutintinnus* species. These four genera are placed in the family Tintinnidae. Interestingly, two undetermined *Eutintinnus* species have an agglutinated lorica. Two species of the genus *Favella* (*F. panamensis* and *Favella* sp.), belonging to the family Ptychocylididae, group together with full support, while the third species (*F. ehrenbergi*) clusters with the genera *Metacylis* and *Rhabdonella* (1.0 BI, 99% MP, 100% NJ). The phylogenetic relationships of the remaining genera (*Metacylis*, *Rhabdonella*, *Codonella*, *Codonellopsis*, *Dictyocysta*, *Stenosemella*, and especially *Tintinnopsis*) are not resolved. The *Tintinnopsis* species form only two branches with high support (*T. dadayi* and *T. beroidea* [1.0 BI, 97% MP, 88% NJ]; *T. tubulosoides*, *T. tocatinensis* and *T. uruguayensis* [full support in all analyses]). However, these branches are distinctly separated from each other. *Tintinnopsis radix* is very weakly associated with *T. dadayi* and *T. beroidea* (0.71 BI, 52% MP), while *Tintinnopsis* sp., *T. subacuta*, and *T. fimbriata* do not group with any other *Tintinnopsis* species but with the *Metacylis*/*Rhabdonella* clade, the *Stenosemella* clade and basally with *Tintinnidium*, respectively. *Dictyocysta* (family Dictyocystidae) is placed with *Codonella* (family Codonellidae [1.0 BI, 95% MP, 97% NJ]) and *Codonellopsis* (family Codonellopsidae) while *Stenosemella* (also family Codonellopsidae) branches outside this cluster together with *Tintinnopsis fimbriata* (family Codonellidae) with full support. Based on these results and assuming that species determination is correct, the families Codonellidae and Codonellopsidae represented by several genera in this study have to be regarded as paraphyletic.

Discussion

Of all tintinnid species analyzed in this study, 12 possess a hyaline lorica and 18 have an agglutinated lorica. As shown previously (Strüder-Kypke & Lynn 2003, Agatha & Strüder-Kypke 2007), species with hyaline and agglutinated loricae often cluster together. *Tintinnidium* with a flexible and agglutinated lorica branches basally, followed by representatives of the families Tintinnidae and Ptychocylidae, both usually with a hyaline lorica. However, it is important to note that 2 unidentified species with an agglutinated lorica cluster with the *Eutintinnus* species – normally known to have only hyaline loricae. While the first species has a lightly agglutinated lorica (Strüder-Kypke & Lynn 2003),

the lorica of the other species is densely agglutinated and only the anterior part of the lorica is undecorated and recognizable (Fig. 1h). The remaining species with a hyaline lorica (*Metacylis*, *Rhabdonella* and *Favella ehrenbergi*) are placed within the large group of codonellid and codonellopsid species, which all possess an agglutinated lorica. This topology supports the view that the ability to agglutinate particles to the lorica may be relevant for genus determination, but that it shows no evolutionary trend. BERNATZKY et al. (1981) furthermore demonstrated that the quality and quantity of agglutination in freshwater tintinnids depends on the season and the environment.

AGATHA & STRÜDER-KYPKE (2007) inferred phylogenetic trees based on both cytological and molecular data and the topologies were congruent. Their cytological data suggest an evolutionary model that places *Tintinnidium* basal in the tintinnids, together with *Membranicola* and *Tintinnopsis cylindrata*, followed by *Nolaclusilis* and *Eutintinnus* and finally resulting in a polychotomy of various genera (i.e., *Tintinnopsis*, *Codonellopsis*, *Stenosemella*, *Cymatocylis*, and *Codonella*). Our analysis further confirms these results, especially the close relationships among the species of the families Codonellidae and Codonellopsidae.

The genus *Tintinnopsis*, which is the most diverse among the tintinnids, has long been thought to be paraphyletic (LAVAL-PEUTO & BROWNLEE 1986; PETZ & FOISSNER 1993; ALDER 1999; AGATHA & RIEDEL-LORJÉ 2006) and this has been previously confirmed in phylogenies based on morphological and molecular features (STRÜDER-KYPKE & LYNN 2003, AGATHA & STRÜDER-KYPKE 2007). One reason for the high number of species in the genus is probably the fact that detailed lorica features are not recognizable due to the dense agglutination (ALDER 1999). Since earlier species descriptions were mainly based on lorica features, this genus functioned as a 'sink' for all tintinnid species with dense agglutination and no other obvious morphological characters (e.g. hyaline collar, fenestration of the collar). Six of the nine *Tintinnopsis* species in our study form two distinct clusters that are separated by high genetic divergences. Three *Tintinnopsis* species (*Tintinnopsis* sp., *T. fimbriata*, and *T. subacuta*) are placed with other genera. Since *T. beroidea* is the type species of the genus, the branch consisting of *T. beroidea* and *T. dadayi* should be considered to represent the 'true' *Tintinnopsis* species. However, it is premature to change any generic affiliations, since more data, especially infraciliary characteristics, should be gathered to confirm this topology.

The close relationships of species of the families Dictyocystidae (*Dictyocysta*), Codonellidae (*Tintinnopsis*, *Codonella*), and Codonellopsidae (*Codonellopsis*, *Stenosemella*) show that classification based on lorica features is ambiguous, at least in species with agglutinated loricae. Phylogenies inferred from cytological and infraciliary data (AGATHA & STRÜDER-KYPKE 2007) have also been unable to completely resolve the relationships among those genera and the phylogenetic trees based on morphological data resulted either in a polychotomy (MP) of those species or *Stenosemella* was depicted as paraphyletic (Hennigian tree) – *Stenosemella nivalis* grouping with *Codonellopsis*, and *Stenosemella lacustris* grouping with *Codonella* and *Tintinnopsis*. Already PETZ & FOISSNER (1993) suggested transferring several *Tintinnopsis* species (e.g., *T. subacuta* and *T. baltica*) to the genus *Codonella*, while LAVAL-PEUTO & BROWNLEE (1986) assign *C. cratera* to the genus *Tintinnopsis*. Our molecular results add more evidence that the families Codonellidae and Codonellopsidae are probably interrelated and in need of a comprehensive revision.

While we attempted to be as thorough as possible in our determination of the species, we cannot exclude the possibility of misidentification especially since the species determination was based on lorica characteristics. However, we are confident that at least the generic affiliations of the ciliates we studied are correct. Unexpected placements are the branching of *T. mucicola* and *Tintinnopsis* sp., the clustering of the two populations of *Eutintinnus pectinis*, the grouping of *Favella ehrenbergi* with *Metacylis* and *Rhabdonella* and generally the small distances between those genera, and finally the placement of *Tintinnopsis subacuta* with this group. Although only the sequence data are available for *Tintinnopsis* sp., its clustering with *T. mucicola* is not unreasonable. LAVAL-PEUTO & BROWNLEE (1986) noted that the infraciliature of *T. cylindrata* resembles that of *T. mucicola* and therefore reassign it to the genus *Tintinnidium*. This view is supported by PETZ & FOISSNER (1993). However, protargol impregnations show that *Tintinnopsis mucicola* lacks two ventral organelles (LAVAL-PEUTO & BROWNLEE 1986), which are present in the other *Tintinnidium* species and *Tintinnopsis cyclindrata* (AGATHA & RIEDEL-LORJÉ 2006). Once infraciliature and molecular data are available for both type species, generic affiliations of the latter will likely need to be changed. The placement of *T. subacuta* with the hyaline genera has only partial support (0.78 BI, 40% MP, 45% NJ). It is very likely that this affiliation will change when more sequences are added, especially from other '*Tintinnopsis*' species. The two *Eutintinnus pectinis* populations are very different in their lorica morphology (SNOEYENBOS-WEST et al. 2002; STRÜDER-KYPKE & LYNN 2003), and probably represent two different species. The highly supported placement of *Favella ehrenbergi* with *Metacylis angulata* may also be due to a misidentification. The genetic distance between both

species is only d = 0.002. *Metacylis* and *Favella* can be very similar if observed at low magnifications. The images provided by SNOEYENBOS-WEST et al. (2002) show a rather 'untypical' lorica shape for *F. ehrenbergi*: the lorica size is too small, a suboral bulge is present, and the aboral horn is fairly short. While the size of the lorica and the length of the aboral horn are known to be highly variable especially under culture conditions (GOLD 1969, 1970; LAVAL-PEUTO 1981), the presence of a suboral bulge is characteristic for other *Favella* species, but not for *F. ehrenbergi*. Therefore, it is possible that this sequence does not represent a *Favella* species or, at least, that it is a different *Favella* species. If the latter is the case, the genus *Favella* is separated into two very divergent lineages. The genetic distances between *Metacylis* and *Rhabdonella* are also very small (d = 0.007–0.014). However, these two genera possess distinct lorica features, which were used for the identification (STRÜDER-KYPKE & LYNN 2003). Infraciliary data need to be collected in order to make a final decision on the relationships of these genera.

In conclusion, the additional data further support the evolutionary model suggested by AGATHA & STRÜDER-KYPKE (2007) and show that at least the genus *Tintinnopsis* and the families Codonellidae and Codonellopsidae have to be regarded as paraphyletic. The sampling density of tintinnid genera, however, with regards to both infraciliary and molecular data is still too small to allow a definite statement about phylogenetic relationships. At this point, infraciliary and molecular data are in accordance and should be the primary features considered for species identification as well as taxonomic placements. Lorica morphology in agglutinated tintinnids is not a reliable character for assignments above the level of species.

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