Symposium on Ciliates in Memory of Denis Lynn

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Abstract

Denis Lynn (1947–2018) was an outstanding protistologist, applying multiple techniques and data sources and thus pioneering an integrative approach in order to investigate ciliate biology. For example, he recognized the importance of the ultrastructure for inferring ciliate phylogeny, based on which he developed his widely accepted classification scheme for the phylum Ciliophora. In this paper, recent findings regarding the evolution and systematics of both peritrichs and the mainly marine planktonic oligotrichean spirotrichs are discussed and compared with the concepts and hypotheses formulated by Denis Lynn. Additionally, the state of knowledge concerning the diversity of ciliates in bromeliad phytotelmata and amitosis in ciliates is reviewed.
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Introduction

Denis Lynn (1947–2018) was an enthusiastic protistologist and his research has made a significant impact in the field of ciliate biology in general and systematics in particular. Throughout his career, Denis pioneered the application of multiple techniques and data sources in order to address scientific questions of interest, wherever possible adopting an integrative approach (Clamp and Lynn 2017). For example, he was among the first to recognize the importance of ultrastructure and histological staining for inferring phylogenetic relationships among ciliates. Almost simultaneously with Bronowski (1970), he hypothesized

an inverse relationship between a structure's conservation through time and the level of its biological organisation (Lynn 1976, 1981; Lynn and Small 1981). This was referred to as the "hypothesis on structural conservatism". Since oral ciliary structures more directly influence the fitness of ciliate species, Denis concluded that they might show more homoplasies than the more conservative ultrastructure of the somatic kinetids (basal bodies plus associated fibrillar structures), which is accordingly more suitable for elucidating phylogenetic relationships. Based on these findings, the hypotheses on ciliate evolution and systematics were revolutionized, leading to the establishment of the two subphyla (Postciliodesmatophora and Intramacronucleata) and 11 classes that we recognize today (Adl et al. 2019; Lynn 2008). Significantly, Denis' morphology-based scheme was supported by molecular phylogenetics; so, he continued to promote ultrastructural research and cell morphology in general, even in the age of molecular biology.

Denis continuously integrated new findings obtained by the application of cutting-edge techniques into bigger

phylogenetic concepts and classifications (Adl et al. 2012, 2019; Lynn 1991, 1996a, 1996b, 2008, 2014, 2017; Lynn and Corliss 1991; Lynn and Kolisko 2017; Lynn and Small, 1988, 1990, 1997, 2002; Small and Lynn 1981, 1985). Integrative approaches combining different methods (live observation, protargol staining, scanning and transmission electron microscopy, barcoding, phylogenetics, and phylogenomics) and data from various life cycle stages (trophonts, resting cysts, and dividers) might yield apomorphies (derived characters) that can be mapped on the branches of the gene trees, characterizing particular taxa. Likewise, emerging conflicts between molecular phylogenies and the current classification might be eliminated by providing morphological and ultrastructural characters that allow the splitting of non-monophyletic taxa and/or the detection of homoplasious features.

Given his influence in the field of protistology, and in particular ciliatology, it was fitting that the opening symposium of the VIII ECOP-ISOP Congress in Rome, 2019 should be on the topic of ciliates and be organized jointly by FEPS (Federation of European Protistological Societies) and ISOP (International Society of Protistologists) in memory of Denis. This paper summarizes the four presentations that comprised the symposium.

The Biology and Systematics of Oligotrichean Ciliates (Alveolata, Ciliophora): New Findings and Old Concepts – Sabine Agatha

The biodiversity of Oligotrichea is currently in the focus of many studies (Santoferrara et al. 2016). Molecular genealogies indicate several non-monophyletic taxa, the misinterpretation of morphological characters, and an insufficient understanding of the cytological features in this group of planktonic spirotrich ciliates. The current state of knowledge, especially recent findings, are summarized here with reference to discoveries and concepts of Denis Lynn.

The Oligotrichea are sister to the mostly benthic hypotrichs. Both constitute the Perilemmaphora Berger, 2008 based on the shared possession of a perilemma, a membranous layer covering the entire cell. The monophyletic Oligotrichea comprise the Oligotrichida and Choreotrichida; the latter consist of the paraphyletic aloricate choreotrichids and the monophyletic loricate tintinnids. After many years of ambiguity, the phylogenomic analyses by Lynn and Kolisko (2017) ultimately rejected a close relationship between the halteriids and the oligotrichids, placing the halteriids into the sporadotrichid hypotrichs. Hence, the apical position of the adoral zone of membranelles and the enantiotropic division mode apparently represent homoplasious adaptations to the planktonic lifestyle in halteriids and oligotrichids.

The last common ancestor of the Oligotrichea probably lived during the Ordovician. Recent analyses by Fernandes and Schrago (2019) suggest that the diversification of oligotrichids and choreotrichids commenced about 310 Ma and 346 Ma ago, respectively, showing a comparatively accelerated speciation rate like the related hypotrichs. Santoferrara and McManus (2017) estimated the diversity of extant Oligotrichea, using curated gene sequences of identified morphospecies and unidentified cells. The comparison of the proportions of described oligotrichid, aloricate choreotrichid, and tintinnid species with the proportions of sequences assigned to these groups demonstrates a mismatch. The oligotrichids and aloricate choreotrichids are seemingly much more diverse than hitherto known and may include novel clades. In tintinnids, however, the proportion of sequences is much smaller than that of described species and most sequences fall in known taxa. However, the real diversity of tintinnids is difficult to estimate as both synonyms and cryptic species seem to be common. Additionally, the discovery of several new species is likely in tintinnids as suggested by analyses of material from the North Pacific (about 1/3 of the 98 morphospecies could not be assigned to any known species; M. Kagerer, Univ. Salzburg, pers. commun.), although to a lesser extent than in oligotrichids and aloricate choreotrichids.

Beyond the known features of oligotrichids (C-shaped adoral zone of membranelles; hypoapokinetal stomatogenesis in a subsurface tube; enantiotropic cell division; girdle kinety in diverse patterns, ventral kinety when present usually longitudinal), the ultrastructural study by Bardele et al. (2018) on *Limnostrombidium viride* provided additional insights. In *Limnostrombidium* species, the girdle cilia are conspicuously clavate. Kahl (1932) already speculated about a sensory function of these cilia, which is supported by the new data showing paraflagellar bodies and the typical arrays of intramembranous particles; yet, the stimulus perceived is unknown. Oligotrichids possess invariably paired somatic basal bodies with only one cilium, which is at the anterior basal body of each dikinetid in the ventral kinety and at the left basal body in each dikinetid in the curved girdle kinety. Thus, their kineties have an orientation, which was used for inferring the evolution of the somatic ciliary patterns (Agatha 2004, 2011; Agatha and Strüder-Kypke 2014).

The extrusive character of oligotrichid trichites has been known for about one hundred years (Kahl 1932; Modeo et al. 2001; Penard 1920). Bardele et al. (2018), however, demonstrated for the first time the presence of "8 + 1 rosettes" of intramembranous particles at the trichite attachment sites, which is typical for the majority of ciliate extrusomes. The trichite arrangement and the associated ultrastructures are characters probably usable for splitting non-monophyletic groupings among the oligotrichids, e.g., the genus *Strombidium*.

The oligotrichids comprise four families: the Cyrtostrombidiidae Agatha, 2004 with extraordinarily strong pharyngeal fibres, the Tontoniidae Agatha, 2004 with a highly contractile tail, the paraphyletic Strombidiidae

Fauré-Fremiet, 1970 with only plesiomorphic features, and the Pelagostrombidiidae Agatha, 2004. The latter is defined by its neoformation organelles, permanent subsurface tubes in which stomatogenesis takes place (vs. transient tubes in the remaining oligotrichids). This structure was ultrastructurally investigated for the first time by Bardele et al. (2018), confirming its function.

In choreotrichids, the adoral zone forms a closed circle (vs. a C-shaped pattern in oligotrichids and hypotrichs) and stomatogenesis takes place in a subsurface pouch (vs. in a tube in oligotrichids and at the cell surface in hypotrichs). Depending on the phylogenetic analysis employed, the monotypic genus *Lynnella* is sister to the oligotrichids or the choreotrichids. The morphological features of *L. semiglobulosa*, however, propose an affiliation with the choreotrichids: broadened proximalmost collar membranelles, longitudinal kineties with a derived kinetid structure (see below), stomatogenesis in a subsurface pouch, two macronucleus nodules and the absence of trichites. The only character indicating a close relationship with the oligotrichids is the ventrally open adoral zone of membranelles, although this might be interpreted as retrogression as in the choreotrichids *Parastrombidium* and *Parastrombidinopsis*.

Currently, the topology of gene trees cannot fully be supported by hypotheses of character evolution due to the scarcity of morphological data. As suggested by Denis Lynn, the ultrastructure of the somatic kinetids might contain phylogenetically relevant information. While such data on the hypotrich outgroup have long been available, oligotrichids were not studied until 2018 (Bardele et al. 2018), data for the diverse group of aloricate choreotrichs was available only for a single strobilidiid representative (Grim, 1987), and only recently were the monokinetids and dikinetids thoroughly investigated in a tintinnid (Gruber et al. 2018a). So, when Agatha and Strüder-Kypke (2014) established their hypothesis on the evolution of somatic kinetids in choreotrichids (kinetid transformation hypothesis), almost no ultrastructural data were available, knowledge of somatic kinetids being based on observations on protargol-stained material. The dikinetids in the oligotrichid *Limnostrombidium* deviate from the plesiomorphic state of the dorsal dikinetids in typical hypotrichs by the presence of a permanent kinetodesmal fibril and electron-dense structures and the absence of transverse microtubules and the single postciliary microtubules at the anterior basal bodies (Bardele et al. 2018). For choreotrichids, Agatha and Strüder-Kypke (2014) assumed first the outgrowth of cilia also at the posterior dikinetidal basal bodies, the subsequent loss of the anterior cilia, and finally the loss of the anterior basal bodies. The latter steps probably occurred several times independently. To test this hypothesis, ultrastructural studies are required. Investigations on the somatic kinetids in the tintinnid *Schmidingerella meunieri* confirmed this hypothesis at least for tintinnids possessing a ventral kinety (Gruber et al.

2018a). These authors found three types of somatic kinetids: (i) dikinetids with two cilia, (ii) dikinetids with cilia only at the posterior basal bodies, and (iii) ciliated monokinetids. As predicted by the hypothesis, the fibres associated with the posterior dikinetidal basal bodies are identical to those of the monokinetids. Besides the typical fibrillar associates of ciliate kinetids, however, three extraordinary microtubular ribbons occur in this tintinnid and form a network in the ciliated anterior cell portion together with long, overlapping postciliary microtubules. The monokinetids of the strobilidiid ciliate *Rimostrombidium lacustre* [reported as *Strobilidium velox* by Grim (1987)] deviate distinctly from both the oligotrichid and tintinnid kinetids. These recent studies corroborate Denis Lynn's observation that spirotrich ciliates display a considerable variability in the somatic kinetid ultrastructure, contradicting the structural conservatism hypothesis (Lynn 2008). Furthermore, the findings in *Schmidingerella* are in contradiction to the "rule of excluded sectors" by Lynn (1981) stating that when one fibrillar associate is well-developed, the others are reduced: here, thick bundles of overlapping postciliary ribbons, well-developed kinetodesmal fibrils, and transverse microtubular ribbons co-occur with three thick novel microtubular bundles.

Tintinnids are the only house-forming ciliates in the plankton. The about 1,000 mainly marine species were established merely on characteristics of the loricae. Gene sequence and cladistic analyses reveal that groups with either hyaline or agglutinated loricae are non-monophyletic, implying that such structures represent homoplasies. An example of the latter is the genus *Tintinnopsis*. On species level, phenotypic plasticity and interspecific similarities hamper identification. Gruber et al. (2018b) demonstrated pseudocrypticity in *Tintinnopsis everta*. In some gene trees, this species groups with congeners also characterized by a flaring lorica collar; however, synonymy is rejected by their genetic dissimilarity. Likewise, cryptic species and phenotypic plasticity were indicated in tintinnids with hyaline loricae (*Helicostomella* and *Parafavella*) by various genetic markers (Jung et al. 2018; Santoferrara et al. 2015). The CO1 primer set designed for spirotrich ciliates by Park et al. (2019) yields a mitochondrial marker with a potentially better resolution for inferring phylogenetic relationships among tintinnids (Jung et al. 2018).

The somatic ciliary patterns of tintinnid ciliates can be revealed by protargol-staining. Hypotheses on their evolution were continuously updated (Agatha and Strüder-Kypke 2007, 2012, Agatha and Strüder-Kypke 2013, Agatha and Strüder-Kypke 2014); only the most recent data await their inclusion (Ganser and Agatha 2019; Gruber et al. 2018b; Smith et al. 2018). Unfortunately, the specimens described by Choi (2015) based on protargol-stained material are not conspecific with the type species *Tintinnopsis beroidea* Stein, 1867, which was authoritatively redescribed by Entz (1884); hence, *Tintinnopsis* is non-monophyletic and needs to be taxonomically revised. Ganser and Agatha (2019) established a new genus for *Antetintinnidium mucicola* (Claparède and

Lachmann, 1858), using the absence of ventral organelles (vs. present in *Tintinnidium* and *Membranicola*) as a key distinguishing feature and improved the diagnosis of the family Tintinnidiidae. In contrast to other tintinnid genera, the somatic ciliary pattern of *Nolaclusilis* is known, but genetic data are lacking (Sniezek et al. 1991; Snyder and Brownlee 1991); the cytological features suggest a sister group relationship to the cluster of Eutintinnidae and tintinnids with more derived ciliary patterns. The family Eutintinnidae now also includes the monotypic genus *Dartintinnus* (Smith et al. 2018).

The most complex tintinnid ciliary pattern currently known comprises right, left, and lateral ciliary fields, ventral and dorsal kineties, and a posterior kinety. Its kinetidal structure, i.e., dikinetids with cilia only at the posterior basal bodies, is identical to that of the dorsal kineties. Based on data from *Tintinnopsis everta,* Gruber et al. (2018b) suggested that the posterior kinety might have evolved from the anteriorly shortened and leftwards curved left dorsal kinety. Contradictory illustrations of protargol-stained cells showing two dorsal kineties plus a posterior kinety (Lynn and Small 2002; Small and Lynn 1985) require confirmation. Further investigations are also needed to verify the preliminary ontogenetic observations on *Schmidingerella arcuata* suggesting that the posterior portion of the ventral kinety, which is similar to the posterior kinety in structure and position, is not a distinct ciliary row dividing separately from the monokinetidal anterior portion (Agatha and Strüder-Kypke 2012). Currently, species with the most complex ciliary pattern and those with the *Schmidingerella*-like one are scattered across the molecular trees. However, since many branches in the genetic genealogies have only low statistical support, it cannot be excluded that these patterns might finally group together, characterizing distinct tintinnid clades.

The ultrastructure of Oligotrichea provides features that are potentially relevant for phylogenetic inferences, e.g., the fibrillar associates of the oral ciliature, the walls of resting cysts, the texture of the tintinnid lorica, and the possibly extrusive capsules of tintinnids; the preliminary findings of the working group in Salzburg are promising. Only by integrating data from various disciplines and sources as proposed by Denis can cladistic and molecular analyses be reconciled.

Ciliates in bromeliad phytotelmata: a hidden diversity – Laura Utz

The Neotropic is one of the eight biogeographical regions of the world. It includes South America, Central America, the Caribbean, and the southern part of North America. Despite its name, the Neotropical region does not include only tropical, but also temperate and high altitude zones. It is a region with diverse ecosystems and high biodiversity since, for nearly 100 million years, Neotropical microorganisms, fauna, and flora were almost completely isolated (Joly 2008).

The Atlantic Forest is one of the major ecological regions present in the Neotropic, extending along the Brazilian coast (from Rio Grande do Norte to Rio Grande do Sul) and spreading to the west, reaching Paraguay and Argentina (Dafonseca 1985). Although only about 28% of its original cover remains (Rezende et al. 2018), the region harbors an extraordinary diversity with many endemic species that are threatened with extinction. More than 52% of the tree species and about 92% of the amphibians found in the forest are endemic with new species being described regularly.

The family Bromeliacea is endemic of the Neotropical region, with the exception of one species found in Africa. This monocot plant family is currently divided into 58 genera with more than 3.000 described species. Bromeliads occur in a wide variety of habitats ranging from humid forests to dry environments. They live as epiphytes, ground- or rock-dwellers (Givnish et al. 2011). Brazil is considered the hotspot for biodiversity and endemism of the Bromeliacea as approximately 803 species representing 31 genera are restricted to the Atlantic Forest (Martinelli et al. 2008).

Some species of bromeliads present imbricated leaves that form a cistern with their bases. Such cisterns or tanks accumulate rainwater and organic matter (Maguire 1971), generating a unique ecosystem called the phytotelma. This ecosystem has characteristics that may influence the host plant as well as the diversity of habitats where the bromeliad can survive (Richardson 1999). Several kinds of organisms inhabit bromeliad phytotelmata. The first study on such organisms was performed by Picado (1913), who analyzed the arthropod diversity in bromeliads from Costa Rica and discovered a wide range of insects and spiders. In recent years, several studies on organisms from bromeliad water tanks have been published, focusing on crustaceans (Jocque et al. 2013), insects (Bacigalupo et al. 2006; Frank and Lounibos 2009), spiders (Laessle 1961), ciliates (e.g., Buosi et al. 2015; Dunthorn et al. 2012; Durán-Ramírez et al. 2015; Foissner et al. 2003; Simão et al. 2017), and prokaryotes (e.g., Brandt et al. 2015; Giongo et al. 2019; Goffredi et al. 2015).

Foissner et al. (2003) published the first comprehensive study on ciliates from bromeliad water tanks, describing several new species and genera and discussing the endemism of ciliates that inhabit these small ecosystems. Subsequently, several studies focusing on diversity, richness, and systematics of bromeliad ciliates based on morphological data have been performed (e.g., Buosi et al. 2015; Durán-Ramírez et al. 2015). The first study to use a phylogenetic approach for investigating the diversity and phylogeny of ciliates from bromeliad phytotelmata was carried out by Dunthorn et al. (2012). They sequenced the 18S rDNA of several isolates collected from bromeliad water tanks, bamboo, and tree holes and found that almost all classes of ciliates (*sensu* Lynn 2008) were present in the sampled phytotelmata. As expected for these very particular environments, both endemic and ubiquitous ciliate species were found. Subsequent studies demonstrated that microorganism diversity in bromeliad phytotelmata

is influenced by environmental conditions. For example, Buosi et al. (2015) showed a significant correlation between water volume and the composition of the ciliate community in the cisterns of *Aechmaea distichantha*. Kratina et al. (2016) found that the abundance and richness of microfaunal communities in bromeliads from Costa Rica were negatively correlated with the canopy cover and the distance of the plant from the ground. These microorganisms also showed a very narrow optimal temperature range; deviations from this optimum caused a decline in taxon richness.

Although a high diversity of microorganisms inhabiting phytotelmata was demonstrated by morphological techniques, a much more diverse assemblage was observed, using high-throughput sequencing. Goffredi et al. (2015) were the first to apply a metatranscriptome technique to estimate the community dynamics and functional diversity of prokaryotes, eukaryotes, and viruses in phytotelmata. In their study, the RNA transcripts were assigned to 25 phyla of bacteria, seven orders of archaea, 21 classes of metazoans, and eight classes of fungi. These findings represented a significant advance in knowledge concerning phytotelmata communities, although only two bromeliad specimens of a single species were studied.

The first study that used high-throughput sequencing to analyze not only the diversity of microorganisms but also of small metazoans in bromeliad phytotelmata was performed by Simão et al. (2017). In the cisterns of *Aechmaea gamosepala* and *Vriesea platynema* from the Atlantic Forest, they found a high diversity of organisms. The most abundant ciliate genera were *Tetrahymena* and *Glaucoma*. The most frequently encountered metazoan groups were arthropods followed by nematodes and platyhelminthes. Surprisingly high abundances of *Trypanosoma* spp. were detected, demonstrating that these organisms and their vectors could use phytotelmata as a common habitat.

To date, the majority of studies on the microfaunal diversity and abundance in bromeliad phytotelmata have been carried out on plants from within the same ecological context (altitude, temperature, rainfall, etc.). Future studies should take these ecological parameters into account to assess which of them are crucial in controlling the microeukaryote diversity in bromeliad cisterns.

Amitosis in ciliates – Rebecca Zufall

As Denis' work clearly highlights, ciliates are unusual and diverse in cell morphology and ultrastructure. He also recognized, however, that they are unusual and diverse at the genomic level (Lynn 2008). Ciliates are famous for their separation of germline and somatic functions into micro- and macronuclei, respectively. The micronucleus is usually diploid and divides by mitosis. In contrast, the macronuclear genome is reduced by DNA elimination before considerable amplification of chromosomes and is thus considered to be ampliploid (reviewed in Lynn 2008).
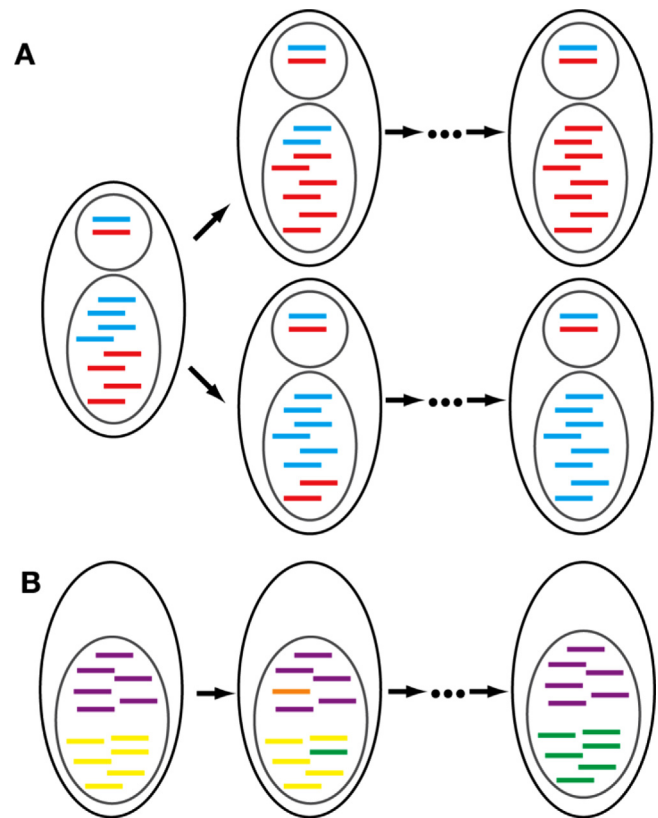

Fig. 1. Amitosis of the *Tetrahymena* macronucleus. (A) An individual that is heterozygous in the diploid micronucleus and the ampliploid macronucleus may produce progeny with differing copy numbers of each homologous chromosome in the macronucleus (shown in red and blue) while remaining heterozygous in the micronucleus. Eventually this process will lead to phenotypic assortment and the loss of heterozygosity in the macronucleus. Shown are two cell lineages that have become fixed for different alleles in the macronucleus generating population-level genetic variation. (B) Amitosis happens independently for each chromosome, shown in an amicronucleate cell lineage. Here two different chromosomes are shown in purple and yellow. Consider that a deleterious mutation happens on one chromosome (orange mutation on purple chromosome) and a beneficial mutation on a different chromosome (green mutation on yellow chromosome). Because these chromosomes assort independently during amitosis, the deleterious mutation can be eliminated from the population and the beneficial mutation fixed. If these nuclei were instead to divide by mitosis, the deleterious and beneficial mutations would be inextricably linked so long as reproduction is asexual.

Developmental processing of the macronuclear genome leads to chromosomes that are lacking centromeres and thus have no way to attach spindles during division. These acentromeric chromosomes result in a type of division called amitosis, whereby chromosomes are unequally distributed among daughter cells (Allen and Nanney 1958; Orias and Flacks 1975; Fig. 1).

Tetrahymena (Oligohymenophorea) was of particular interest to Denis and his lab. Due to morphological similarity, identification of *Tetrahymena* species has long

been problematic (Lynn and Doerder 2012). Development of the mitochondrial locus cytochrome *c* oxidase subunit 1 (*cox*1) as a barcode for the genus both provided a tool for species identification and allowed clarification of the phylogenetic relationships among taxa (Chantangsi et al. 2007; Kher et al. 2011). Using this barcode and other sequence data, Denis and others discovered and described several new species of *Tetrahymena* (e.g., Doerder 2019; Lynn et al. 2000, 2018) and provided phylogenetic resolution of the genus (Chantangsi and Lynn 2008; Doerder 2019).

In most ciliates, amitotic division of the macronucleus results in senescence due to the eventual loss of essential chromosomes (Bell 1988). Species in the genus *Tetrahymena* are unique among studied ciliates in their ability to survive amitotic division for many generations with no evidence of senescence (Doerder 2014). This feature has helped make *T. thermophila* a powerful model system in experimental biology (e.g., Greider and Blackburn 1985; Kruger et al. 1982). The ability to survive long periods of amitotic division is likely due to *Tetrahymena* having the unusual capacity to control the copy number of each chromosome during amitotic division and thus prevent the loss of any essential chromosomes (Orias et al. 2011; Preer and Preer 1979). The mechanism of *Tetrahymena*'s chromosomal copy number control is unknown. The ability of *Tetrahymena* to survive long periods of asexual division makes this genus a useful system in which to study the long-term evolutionary consequences of amitosis.

Arguably, the most important consequence of amitosis is the generation of genetic variation among asexual progeny. Normally, i.e. under mitotic division, asexually generated progeny are identical to their parents and to each other, with the exception of *de novo* mutations. However, because homologous chromosomes are apparently randomly distributed among progeny during amitosis in ciliates, asexual progeny may or may not contain the same genetic complement as their parents (Fig. 1). Eventually, amitosis results in phenotypic, or allelic, assortment, whereby the macronuclear genome becomes completely homozygous for one of the parental alleles (Doerder et al. 1992). Thus, amitosis generates variation at two levels. First, amitosis can produce progeny containing macronuclei with differing copy numbers of alleles at a locus. Second, following phenotypic assortment, populations may contain individuals that are fixed in the macronucleus for alternate alleles, even if their micronuclei are genetically identical (Doerder 2014; Fig. 1A).

Because the rate of evolution is dependent on the additive genetic variation in a population (Fisher 1930), the fact that amitosis generates genetic variation among progeny has important implications for how ciliates evolve. For example, amitosis is likely to allow ciliates to adapt more quickly to novel environments relative to species that have a single nucleus dividing by mitosis. This is simply due to the fact that amitosis produces more genetic variation in a population upon which natural selection can

act. Experimental evolution of laboratory populations of amitotically dividing *T. thermophila* are currently being used to study the rates of adaptation under amitosis (J. Tarkington and R. Zufall, unpubl. data).

Amitosis may also have allowed *Tetrahymena* to remain asexual for an unexpectedly long time by generating extensive genetic variation that is otherwise normally produced by sexual reproduction. Most eukaryotes undergo some form of sexual reproduction, e.g., most ciliates undergo facultative conjugative sex (reciprocal fertilization). However, obligate asexuality is surprisingly common in the genus *Tetrahymena*, as evidenced by the abundance of lineages and species that have completely lost their germline micronucleus (amicronucleate; Doerder 2014). Some of these amicronucleate lineages may be millions of years old, indicating that they are not experiencing the usual deleterious consequences of asexuality, e.g., genetic deterioration or inability to adapt to new environments (e.g., Henry et al. 2012; Neiman et al. 2010; Paland and Lynch 2006). The most likely explanation for this observation is that amitosis, in the context of the *Tetrahymena* genome, provides benefits of sex in the absence of sex (Zufall 2016). For example, assortment during amitosis allows for the disassociation of beneficial and deleterious alleles on different macronuclear chromosomes, allowing beneficial alleles to fix without deleterious alleles hitchhiking as they would in a mitotic asexual population (Doerder 2014; Fig. 1B). Mathematical models and simulations are being used to test these predictions (H. Zhang, J. West, R. Zufall, and R. Azevedo, unpubl. data).

The Biology and Systematics of Peritrich Ciliates: Old Concepts and New Findings – Alan Warren

Peritrichs have been known for over 340 years and were among the first protists to be observed and documented by the so-called "Father of Protistology", Antonie van Leeuwenhoek (for details, see Dobell 1932). They are ubiquitous in aquatic habitats where most can be found attached, either permanently or temporarily, to a wide variety of submerged substrates, either living or inanimate, although some species are permanently free-swimming. Peritrichs play a key role in controlling populations of suspended bacteria by predation, and some epibiontic forms can cause harm to their host. Peritrichs comprise one of six subclasses within the class Oligohymenophorea, namely Apostomatia, Astomatia, Hymenostomatia, Peniculia, Peritrichia, and Scuticociliatia (Adl et al. 2019; Lynn 2008). With approximately 1,000 described species, peritrichs are one of the largest ciliate groups, accounting for 10–15% of all known ciliate species. They are classified into 123 genera, 19 families, and two orders, i.e., Sessilida and Mobilida (Lynn 2008). Despite the large body of scientific literature on peritrichs, knowledge

of their origin, evolution, systematics, and biogeography is scant and/or uncertain.

Traditionally, the subclass Peritrichia was thought to be monophyletic and most closely related to the thigmotrichid scuticociliates based on the supposed homology of organelles associated with attachment in these two groups, i.e., the scopula in peritrichs and the thigmotactic region in thigmotrichid scuticociliates. The first molecular phylogeny that included a peritrich was based on SSU rDNA sequence data and showed that sessilid peritrichs are more closely related to hymenostomes than they are to peniculines (Lynn and Sogin 1988). Over the following 20 years, the SSU rDNA and other gene markers were sequenced for numerous peritrichs and other oligohymenophoreans. Phylogenetic analyses based on this expanded dataset suggested that the subclass Peritrichia may not be monophyletic, with the Hymenostomatia rather than the Mobilida being the sister group of the Sessilida, although these findings were questioned as being attributable to the methods employed for alignment and for masking ambiguous positions (see Jiang et al. 2019 and references therein). In the first phylogenomic analysis of peritrichs, Gentekaki et al. (2017) reported that the subclass Peritrichia is indeed monophyletic, although the identity of its sister group was ambiguous according to the analysis method used, i.e., Peniculia by Bayesian inference and Scuticociliatia + Hymenostomatia by maximum likelihood. Due to the lack of genomic and transcriptomic data, however, only five peritrich species were included in this analysis (Gentekaki et al. 2017).

With the advancements in high-throughput single-cell sequencing, Jiang et al. (2019) were able to significantly increase taxon sampling among peritrichs by providing genomic or transcriptomic data for 12 species, i.e., ten sessilids (seven freshwater and three brackish water species) and two mobilids. With the inclusion of genomic or transcriptomic data for 24 previously sequenced protist species, i.e., six peritrichs, 16 other ciliates from six different classes, and two apicomplexans as outgroup taxa, a phylogenomic tree based on a 151-gene dataset was constructed (Supplementary Fig. 1). The main findings of this analysis were that the subclass Peritrichia is monophyletic and its sister group is the subclass Peniculia (Jiang et al. 2019).

Although genomic chronograms have been used to yield reliable timelines for eukaryotic evolution, divergence times of early diverging lineages such as ciliates are difficult to date because the fossil record is both sparse and tenuous, and genome-scale data are available for only a few taxa (Jiang et al. 2019 and references therein). Consequently, little research has focused on time estimation of divergence of the main ciliate lineages and none included peritrichs. Based on divergence rates of SSU rDNA gene sequences, Wright and Lynn (1997) estimated that the class Oligohymenophorea originated 1,600–250 million years ago (Ma). This was the first study to calibrate the SSU rDNA molecular clock for ciliates, for which they used the divergence of the ciliate

ectoparasite *Ichthyophthirius multifiliis* from its free-living relatives, which was estimated using an independently-timed event, i.e., the origin of freshwater teleost fish in the fossil record, teleosts being the only known host of *I. multifiliis*. Based on the 151-gene supermatrix for 36 taxa, Jiang et al. (2019) constructed a chronogram and calculated divergence times for major ciliate lineages using the program RelTime (Supplementary Fig. 2). The timetree was computed using two calibration constraints: (1) the fossil record for *Triacola ostracodarum* (252.2–247.2 Ma) within the family Vaginicolidae (represented by *Thuricola* sp. in Jiang et al. 2019) and (2) the divergence of *I. multifiliis* from its free-living relative *Tetrahymena* 512–461 Ma (Wright and Lynn 1997). Among the main findings of this study were that: (1) the class Oligohymenophorea originated during the Proterozoic, 1,170–696 Ma, which is within the range estimated by Wright and Lynn (1997); (2) the subclass Peritrichia originated during the late Proterozoic to Cambrian, 820–488 Ma; (3) sessilids and mobilids appeared at about the same time, 614–366 Ma and 637–379 Ma, respectively; and (4) vorticellids diverged 350–200 Ma, a date which is consistent with a fossil *Vorticella*-like species discovered in Antarctica inside the wall of a >200 Ma leech cocoon (Bomfleur et al. 2012; Jiang et al. 2019).

Contractility is one of the most conspicuous features of peritrichs and was described by Antonie van Leeuwenhoek in the earliest published reports of ciliates (for details, see Dobell 1932). Contraction can occur both in the zooid and in the stalk of certain sessilids, i.e., many zoothamniids and vorticellids, and is achieved by means of myonemes, which are bundles of fibrils made up of centrin-like proteins. Somatic myonemes form a network within the zooid, while stalk myonemes are found exclusively in an organelle called the spasmoneme. About 60% of myoneme protein consists of the calcium-binding protein spasmin (Amos et al. 1975). Contraction and extension processes are driven by calcium ion binding and release, resulting in small conformational changes in spasmin subunits, which are amplified by their linear arrangement in filaments (Mahadevan and Matsudaira 2000). Jiang et al. (2019) identified the gene encoding spasmin in genomic-scale data. Among a wide range of ciliates and two apicomplexans, spasmin sequences were recovered only from peritrichs and *Paramecium*, thus supporting the finding from the phylogenomic analysis based on a 151-gene dataset that the sister group to the Peritrichia is Peniculia. Currently, a total of 81 spasmin sequences are known from 17 peritrichs and two *Paramecium* species, namely, *P. caudatum* and *P. tetraurelia*. *Paramecium caudatum* has only a single spasmin gene, while *P. tetraurelia* has four near-identical spasmins as a result of successive rounds of whole-genome duplication. By contrast, most peritrichs have more than one spasmin, Furthermore, different FPKM (fragments per kilobase of transcript model per million reads mapped) values point to functional differentiation among these genes, which probably reflects the complex and well-developed myoneme systems

in peritrichs. This is particularly evident in vorticellids and zoothamniids which possess both spasmoneme (SPM) and somatic (SOM) spasmins (Jiang et al. 2019). By constructing a spasmin phylogenetic tree, Jiang et al. (2019) traced the evolution of spasmins in order to reveal systematic relationships among the peritrichs and to investigate the rapid evolution of this subclass. A tree showing the distribution of spasmin in peritrichs and *Paramecium* was also inferred by phylogenomic analysis (Supplementary Fig. 3). Among the findings of these analyses were: (1) spasmins of *Paramecium* are located at the base of the spasmin gene tree and can be considered SOM spasmins; (2) mobilid spasmins are closely related to those of *Paramecium* and may be the first spasmins to have evolved in peritrichs; (3) spasmins of sessilid peritrichs that ancestrally lack a spasmoneme, e.g., members of the suborder Opercularina, are SOM components and represent the earliest-evolving myoneme constituents of peritrichs; (4) spasmins that are common to both SOM and SPM (collectively called SOM/SPM spasmins) originated within the loricate sessilid family Vaginicolidae, which could be an intermediate group between the operculariines and vorticellines; (5) there are two SPM spasmin types in sessilids, i.e., those in stalks that contract in a zig-zag fashion (zoothamniids) and those in stalks that contract in a spiral fashion (vorticellids); (6) certain species of *Epistylis*, the stalks of which lack a spasmoneme, have SOM/SPM (zig-zag type) spasmins and therefore probably evolved from a zoothamniid-like ancestor having secondarily lost their spasmoneme; (7) certain members of the family Astylozoidae, e.g., the stalkless forms *Opisthonecta* and *Scyphidia*, have spiral-type SPM spasmins, supporting the hypothesis that these genera evolved from vorticellid-like ancestors; (8) parallel adaptive radiations occurred in each SPM group, supporting the hypothesis that the stalk and spasmoneme evolved rapidly; and (9) the acquisition and loss of the stalk and/or spasmoneme could be key events in the evolutionary history of sessilid peritrichs leading to the high diversity that they exhibit today (Jiang et al. 2019).

Biogeographic patterns in ciliates in general, and peritrichs in particular, are poorly understood. Most marine species are believed to have a relatively high dispersal potential, such that both globally distributed and geographically isolated taxa exist. Some ciliate species are more conspicuous and easier to identify with confidence than others, leading some authors to propose these "flagship" species as the ultimate proof of endemism (Foissner 2006). Ecological Niche Models (ENMs) infer suitable abiotic habitat conditions for non-model organisms by generating a correlational model that unites occurrence information and environmental data for the taxon of interest to determine the geographic distribution of habitat conditions correlated with species occurrences (Soberón and Peterson 2005; Williams et al. 2018). Using primary occurrence data, drawn from the Global Biodiversity Information Facility (http://www.gbif.org/) and OBIS (http://www.iobis.org/), for three marine "flagship" ciliate species (including the planktonic colonial sessilid

peritrich *Zoothamnium pelagicum*), and environmental data drawn from the National Oceanic and Atmospheric Administration's World Ocean Atlas (NOAA 1999), Williams et al. (2018) estimated each species' spatial and environmental distributions in the North Atlantic Ocean using ENMs. The main findings were that: (1) each of the studied species has a unique environmental signature and geographic distribution; (2) the main factor influencing geographic distribution is sea surface temperature, with *Z. pelagicum* occupying the most southerly range extending to the Caribbean and the equator; and (3) the other main factors constraining the distribution of *Z. pelagicum* were oxygen concentration and silicate content (Williams et al. 2018).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejop.2020.125694.

References

Adl, S.M., Simpson, A.G., Lane, C.E., Lukes, J., Bass, D., Bowser, S.S., Brown, M.W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., Le Gall, L., Lynn, D.H., McManus, H., Mitchell, E.A., Mozley-Stanridge, S.E., Parfrey, L.W., Pawlowski, J., Rueckert, S., Shadwick, R.S., Schoch, C.L., Smirnov, A., Spiegel, F.W., 2012. The revised classification of eukaryotes. J. Eukaryot. Microbiol. 59, 429–493.

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., Agatha, S., Berney, C., Brown, M.W., Burki, F., Cárdenas, P., Čepička, I., Chistyakova, L., del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A.A., Hoppenrath, M., James, T.Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D.J.G., Lara, E., Le Gall, L., Lynn, D.H., Mann, D.G., Massana, R., Mitchell, E.A.D., Morrow, C., Park, J.S., Pawlowski, J.W., Powell, M.J., Richter, D.J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F.W., Torruella, G., Youssef, N., Zlatogursky, V., Zhang, Q., 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. J. Eukaryot. Microbiol. 66, 4–119.

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

Agatha, S., 2011. 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., 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., Strüder-Kypke, M.C., 2012. Reconciling cladistic and genetic analyses in choreotrichid ciliates (Ciliophora, Spirotricha, Oligotrichea). J. Eukaryot. Microbiol. 59, 325–350.

Agatha, S., Strüder-Kypke, M.C., 2013. Systematics and evolution of tintinnid ciliates. In: Dolan, J.R., Montagnes, D.J.S., Agatha, S., Coats, D.W., Stoecker, D.K. (Eds.), The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton. John Wiley & Sons, Ltd, Oxford, Chichester, pp. 42–84.

Agatha, S., Strüder-Kypke, M.C., 2014. What morphology and molecules tell us about the evolution of Oligotrichea (Alveolata, Ciliophora). Acta Protozool. 53, 77–90.

Allen, S.L., Nanney, D.L., 1958. An analysis of nuclear differentiation in the selfers of Tetrahymena. Am. Nat. 92, 139–160.

Amos, W.B., Routledge, L.M., Yew, F.F., 1975. Calcium-binding proteins in a vorticellid contractile organelle. J. Cell Sci. 19, 203–213.

Bacigalupo, A., Segura, J.A., Garcia, A., Hidalgo, J., Galuppo, S., Cattan, P.E., 2006. First finding of Chagas disease vectors associated with wild bushes in the Metropolitan Region of Chile. Revta Med. Chilena 134, 1230–1236.

Bardele, C.F., Stockmann, N., Agatha, S., 2018. Some ultrastructural features of the planktonic freshwater ciliate Limnostrombidium viride (Alveolata, Ciliophora, Oligotrichida) and improved diagnoses of oligotrich taxa. Acta Protozool. 57, 169–193.

Bell, G., 1988. Sex and Death in Protozoa. Cambridge University Press, Cambridge.

Bomfleur, B., Kerp, H., Taylor, T.N., Moestrup, O., Taylor, E.L., 2012. Triassic leech cocoon from Antarctica contains fossil bell animal. Proc. Natl. Acad. Sci. U.S.A. 109, 20971–20974.

Brandt, F.B., Martinson, G.O., Pommerenke, B., Pump, J., Conrad, R., 2015. Drying effects on archaeal community composition and methanogenesis in bromeliad tanks. FEMS Microbiol. Ecol. 91, 1–10.

Bronowski, J., 1970. New concepts in the evolution of complexity: stratified stability and unbounded plans. Synthese 21, 228–246.

Buosi, P.R.B., Cabral, A.F., Utz, L.R.P., Vieira, L.C.G., Velho, L.F.M., 2015. Effects of seasonality and dispersal on the ciliate community inhabiting bromeliad phytotelmata in riparian vegetation of a large tropical river. J. Eukaryot. Microbiol. 62, 737–749.

Chantangsi, C., Lynn, D.H., 2008. Phylogenetic relationships within the genus Tetrahymena inferred from the cytochrome c oxidase subunit 1 and the small subunit ribosomal RNA genes. Mol. Phylogenet. Evol. 49, 979–987.

Chantangsi, C., Lynn, D.H., Brandl, M.T., Cole, J.C., Hetrick, N., Ikonomi, P., 2007. Barcoding ciliates: a comprehensive study of 75 isolates of the genus Tetrahymena. Int. J. Syst. Evol. Microbiol. 57, 2412–2423.

Choi, J.K., 2015. Invertebrate Fauna of Korea 1. 2. Ciliophora: Postciliodesmatophora, Intramacronucleata - Marine ciliates. National Institute of Biological Resources, Ministry of Environment, Incheon.

Clamp, J.C., Lynn, D.H., 2017. Investigating the biodiversity of ciliates in the 'Age of Integration'. Eur. J. Protistol. 61, 314–322.

Dafonseca, G., 1985. The vanishing Brazilian Atlantic Forest. Biol. Conserv. 34, 17–34.

Dobell, C.C., London 1932. Antony van Leeuwenhoek and His "Little Animals": Being Some Account of the Father of Protozoology and Bacteriology and His Multifarious Discoveries in These Disciplines: Collected, Translated, and Edited, From His Printed Works, Unpublished Manuscripts, and Contemporary Records: Published on the 300th Anniversary of His Birth.

Doerder, F.P., 2014. Abandoning sex: multiple origins of asexuality in the ciliate Tetrahymena. BMC Evol. Biol. 14, 112.

Doerder, F.P., 2019. Barcodes reveal 48 new species of Tetrahymena, Dexiostoma, and Glaucoma: phylogeny, ecology, and biogeography of new and established species. J. Eukaryot. Microbiol. 66, 182–208.

Doerder, F.P., Deak, J.C., Lief, J.H., 1992. Rate of phenotypic assortment in Tetrahymena thermophila. Dev. Genet. 13, 126–132.

Dunthorn, M., Stoeck, T., Wolf, K., Breiner, H.W., Foissner, W., 2012. Diversity and endemism of ciliates inhabiting neotropical phytotelmata. Syst. Biodivers. 10, 195–205.

Durán-Ramírez, C.A., García-Franco, J.G., Foissner, W., Mayén-Estrada, R., 2015. Free-living ciliates from epiphytic tank bromeliads in Mexico. Eur. J. Protistol. 51, 15–33.

Entz, G.S., 1884. Über Infusorien des Golfes von Neapel. Mitt. zool. Stn Neapel 5, 289–444, + Plates XX–XXV.

Fernandes, N.M., Schrago, C.G., 2019. A multigene timescale and diversification dynamics of Ciliophora evolution. Mol. Phylogenet. Evol. 139, 106521.

Fisher, R.A., 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

Foissner, W., 2006. Biogeography and dispersal of micro-organisms: A review emphasizing protists. Acta Protozool. 45, 111–136.

Foissner, W., Strüder-Kypke, M., van der Staay, G., Moon-van der Staay, S., Hackstein, J., 2003. Endemic ciliates (Protozoa, Ciliophora) from tank bromeliads (Bromeliaceae): a combined morphological, molecular, and ecological study. Eur. J. Protistol. 39, 365–372.

Frank, J.H., Lounibos, L.P., 2009. Insects and allies associated with bromeliads: a review. Terr. Arthropod Rev. 1, 125–153.

Ganser, M.H., Agatha, S., 2019. Redescription of Antetintinnidium mucicola (Claparède and Lachmann, 1858) nov. gen., nov. comb. (Alveolata, Ciliophora, Tintinnina). J. Eukaryot. Microbiol. 66, 802–820.

Gentekaki, E., Kolisko, M., Gong, Y., Lynn, D., 2017. Phylogenomics solves a long-standing evolutionary puzzle in the ciliate world: The subclass Peritrichia is monophyletic. Mol Phylogenet. Evol. 106, 1–5.

Giongo, A., Medina-Silva, R., Astarita, L.V., Borges, L.G.A., Oliveira, R.R., Simão, T.L.L., Gano, K.A., Davis-Richard, A.G., Brown, C.T., Fagen, J.R., Arzivenco, P.M., Neto, C.P., Abichequer, A.D., Lindholz, C.G., Baptista-Silva, A., Mondin, C.A., Utz, L.R.P., Triplett, E.W., Eizirik, E., 2019. Seasonal physiological parameters and phytotelmata bacterial diversity of two bromeliad species (Aechmea gamosepala and Vriesea platynema) from the Atlantic Forest of southern Brazil. Diversity 11, 111.

Givnish, T.J., Barfuss, M.H., Van Ee, B., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, J.A., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K.,

Luther, H., Till, W., Zizka, G., Berry, P.E., Sytsma, K.J., 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. Am. J. Bot. 98, 872–895.

Goffredi, S.K., Jang, G.E., Haroon, M.F., 2015. Transcriptomics in the tropics: Total RNA-based profiling of Costa Rican bromeliad-associated communities. Computat. Struct. Biotechnol. J. 13, 18–23.

Greider, C.W., Blackburn, E.H., 1985. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell 43, 405–413.

Grim, J.N., 1987. The kinetid structures of the choreotrichous ciliate *Strobilidium velox* and an assessment of its evolutionary lineage. J. Protozool. 34, 117–123.

Gruber, M.S., Mühlthaler, A., Agatha, S., 2018a. Ultrastructural studies on a model tintinnid - *Schmidingerella meunieri* (Kofoid and Campbell, 1929) Agatha and Strüder-Kypke, 2012 (Ciliophora). I. Somatic kinetids with unique ultrastructure. Acta Protozool. 57, 195–213.

Gruber, M.S., Strüder-Kypke, M., Agatha, S., 2018b. Redescription of *Tintinnopsis everta* Kofoid and Campbell 1929 (Alveolata, Ciliophora, Tintinnina) based on taxonomic and genetic analyses — discovery of a new complex ciliary pattern. J. Eukaryot. Microbiol. 65, 484–504.

Henry, L., Schwander, T., Crespi, B.J., 2012. Deleterious mutation accumulation in asexual *Timema* stick insects. Mol. Biol. Evol. 29, 401–408.

Jiang, C., Wang, G., Xiong, J., Yang, W., Sun, Z., Feng, J., Warren, A., Miao, W., 2019. Insights into the origin and evolution of Peritrichia (Oligohymenophorea, Ciliophora) based on analyses of morphology and phylogenomics. Mol. Phylogenet. Evol. 132, 25–35.

Jocque, M., Fiers, F., Romero, M., Martens, K., 2013. Crustacea in phytotelmata: A global overview. J. Crustacean Biol. 33, 451–460.

Joly, C.A., 2008. Biodiversity and climate change in the Neotropical region. Biota Neotrop. 8, 1–2.

Jung, J.-H., Moon, J.H., Park, K.-M., Kim, S., Dolan, J.R., Yang, E.J., 2018. Novel insights into the genetic diversity of *Parafavella* based on mitochondrial CO1 sequences. Zool. Scr. 47, 743–755.

Kahl, A., 1932. Urtiere oder Protozoa. I: Wimpertiere oder Ciliata (Infusoria). Eine Bearbeitung der freilebenden und ectocommensalen Infusorien der Erde, unter Ausschluß der marinen Tintinnidae. 3. Spirotricha. In: Dahl, F. (Ed.), Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise. G. Fischer, Jena, pp. 399–650.

Kher, C.P., Doerder, F.P., Cooper, J., Ikonomi, P., Achilles-Day, U., Küpper, F.C., Lynn, D.H., 2011. Barcoding *Tetrahymena*: discriminating species and identifying unknowns using the cytochrome *c* oxidase subunit I (cox-1) barcode. Protist 162, 2–13.

Kratina, P., Petermann, J.S., Marino, N.A.C., McDonald, A.A.M., Srivastava, D.S., 2016. Environmental control of the microfaunal community structure in tropical bromeliads. Ecol. Evol. 7, 1627–1634.

Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E., Cech, T.R., 1982. Self-splicing RNA: Autoexcision and autocyclization of the ribosomal RNA intervening sequence of *Tetrahymena*. Cell. 31, 147–157.

Laessle, A.M., 1961. A micro-limnological study of Jamaican bromeliads. Ecology 42, 499–517.

Lynn, D.H., 1976. Comparative ultrastructure and systematics of Colpodida - structural conservatism hypothesis and a description of *Colpoda steinii* Maupas. J. Protozool. 23, 302–314.

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

Lynn, D.H., 1991. The implications of recent descriptions of kinetid structure to the systematics of the ciliated protists. Protoplasma 164, 123–142.

Lynn, D.H., 1996a. My journey in ciliate systematics. J. Eukaryot. Microbiol. 43, 253–260.

Lynn, D.H., 1996b. Systematics of ciliates. In: Hausmann, K., Bradbury, P.C. (Eds.), Ciliates: Cells as Organisms. G. Fischer Verlag, Stuttgart, Jena, Lübeck, Ulm, pp. 51–72.

Lynn, D.H., 2008. The Ciliated Protozoa. Characterization, Classification, and Guide to the Literature, 3rd Edition ed. Springer, Dordrecht.

Lynn, D.H., 2014. Kinetids, concepts, and coincidences. In: Hausmann, K., Radek, R. (Eds.), Cilia and Flagella - Ciliates and Flagellates: Ultrastructure and cell biology, function and systematics, symbiosis and biodiversity. Schweizerbart Sci. Publ., Stuttgart, pp. 175–188.

Lynn, D.H., 2017. Ciliophora. In: Archibald, J.M., Simpson, A.G.B., Slamovits, C.H. (Eds.), Handbook of the Protists. 2nd ed. Springer International Publishing, pp. 679–730.

Lynn, D.H., Corliss, J.O., 1991. Ciliophora. In: Harrison, F.W. (Ed.), Microscopic Anatomy of Invertebrates. Wiley-Liss, New York, Chichester, pp. 333–467.

Lynn, D.H., Doerder, F.P., 2012. The life and times of *Tetrahymena*. In: Collins, K. (Ed.), Methods in Cell Biology. Academic Press, pp. 9–27.

Lynn, D.H., Kolisko, M., 2017. Molecules illuminate morphology: phylogenomics confirms convergent evolution among 'oligotrichous' ciliates. Int. J. Syst. Evol. Microbiol. 67, 3676–3682.

Lynn, D.H., Small, E.B., 1981. Protist kinetids: structural conservatism, kinetid structure, and ancestral states. BioSystems 14, 377–385.

Lynn, D.H., Small, E.B., 1988. An update on the systematics of the phylum Ciliophora doflein, 1901: the implications of kinetid diversity. Biosystems 21, 317–322.

Lynn, D.H., Small, E.B., 1990. Phylum Ciliophora. In: Margulis, L., Corliss, J.O., Melkonian, M., Chapman, D.J. (Eds.), Handbook of Protoctista. Jones/Bratlett, Boston, pp. 498–523.

Lynn, D.H., Small, E.B., 1997. A revised classification of the Phylum Ciliophora Doflein, 1901. Rev. Soc. Mex. Hist. Nat. 47, 65–78.

Lynn, D.H., Small, E.B., 2002. Phylum Ciliophora Doflein, 1901. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), An Illustrated Guide to the Protozoa. Organisms Traditionally Referred to as Protozoa, or Newly Discovered Groups 2nd ed. (year 2000), pp. 371–656.

Lynn, D.H., Sogin, M.L., 1988. Assessment of phylogenetic relationships among ciliated protists using partial ribosomal RNA sequences derived from reversed transcripts. BioSystems 21, 249–254.

Lynn, D.H., Gransden, S.G., Wright, A.-D.G., Josephson, G., 2000. Characterization of a new species of the ciliate *Tetrahymena* (Ciliophora: Oligohymenophorea) isolated from the urine of a

dog: first report of *Tetrahymena* from a mammal. Acta Protozool. 39, 289–294.

Lynn, D.H., Doerder, F.P., Gillis, P.L., Prosser, R.S., 2018. *Tetrahymena glochidiophila* n. sp., a new species of *Tetrahymena* (Ciliophora) that causes mortality to glochidia larvae of freshwater mussels (Bivalvia). Dis. Aquat. Organ. 127, 125–136.

Maguire, B. Jr., 1971. Phytotelmata: Biota and community structure determination in plant-held waters. Ann. Rev. Ecol. Syst. 2, 439–464.

Mahadevan, L., Matsudaira, P., 2000. Motility powered by supramolecular springs and ratchets. Science 288, 95–99.

Martinelli, G., Vieira, C.M., Gonzalez, M., Leitman, P., Piratininga, A., Costa, A., Forzza, R.C., 2008. Bromeliaceae da Mata Atlântica: lista de espécies, distribuição e conservação. Rodriguésia 59, 209–258.

Modeo, L., Petroni, G., Bonaldi, M., Rosati, G., 2001. Trichites of *Strombidium* (Ciliophora, Oligotrichida) are extrusomes. J. Eukaryot. Microbiol. 48, 95–101.

Neiman, M., Hehman, G., Miller, J.T., Logsdon, J.M., Taylor, D.R., 2010. Accelerated mutation accumulation in asexual lineages of a freshwater snail. Mol. Biol. Evol. 27, 954–963.

NOAA (National Oceanic and Atmospheric Administration), 1999. World Ocean Atlas 1998. National Oceanographic Data Center.

Orias, E., Flacks, M., 1975. Macronuclear genetics of *Tetrahymena* I. Random distribution of macronuclear gene copies in *T. pyriformis*, Syngen 1. Genetics 79, 187–206.

Orias, E., Cervantes, M.D., Hamilton, E.P., 2011. *Tetrahymena thermophila*, a unicellular eukaryote with separate germline and somatic genomes. Res. Microbiol. 162, 578–586.

Paland, S., Lynch, M., 2006. Transitions to asexuality result in excess amino acid substitutions. Science 311, 990–992.

Park, M.-H., Jung, J.-H., Jo, E., Park, K.-M., Baek, Y.-S., Kim, S., Min, G.-S., 2019. Utility of mitochondrial CO1 sequences for species discrimination of Spirotrichea ciliates (Protozoa, Ciliophora). Mitochondrial DNA Part A 30, 1–8.

Penard, E., 1920. Observations sur le *Strombidium viride* Stein. Rev. Suisse Zool. 28, 1–9.

Picado, C., 1913. Les broméliacées épiphytes considérées comme milieu biologique. Bull. Sci. Fr. Belg. 5, 215–360.

Preer, J.R., Preer, L.B., 1979. The size of macronuclear DNA and its relationship to models for maintaining genic balance. J. Protozool. 26, 14–18.

Rezende, C.L., Scarano, F.R., Assad, E.D., Joly, C.A., Metzger, J.P., Strassburg, B.B.N., Tabarelli, M., Fonseca, G.A., Mittermeier, R.A., 2018. From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest. Perspect. Ecol. Conserv. 16, 208–214.

Richardson, A.A., 1999. The bromeliad microcosm and the assessment of faunal diversity in a Neotropical forest. Biotropica 31, 321–336.

Santoferrara, L.F., Tian, M., Alder, V.A., McManus, G.B., 2015. Discrimination of closely related species in tintinnid ciliates: new insights on crypticity and polymorphism in the genus *Helicostomella*. Protist 166, 78–92.

Santoferrara, L.F., Bachy, C., Alder, V.A., Gong, J., Kim, Y.-O., Saccà, A., da Silva Neto, I.D., Strüder-Kypke, M.C., Warren, A., Xu, D., Yi, Z., Agatha, S., 2016. Updating biodiversity studies in loricate protists: the case of the tintinnids (Alveolata, Ciliophora, Spirotrichea). J. Eukaryot. Microbiol. 63, 651–656.

Santoferrara, L.F., McManus, G.B., 2017. Integrating dimensions of biodiversity in choreotrichs and oligotrichs of marine plankton. Eur. J. Protistol. 61, 323–330.

Simão, T.L.L., Giongo-Borges, A., Gano, K.A., Davis-Richardson, A.G., Brown, C.T., Fagen, J.R., Triplett, E.W., Dias, R., Mondin, C.A., da Silva, R.M., Eizirik, E., Utz, L.R.P., 2017. Characterization of ciliate diversity in bromeliad tank waters from the Brazilian Atlantic Forest. Eur. J. Protistol. 61, 359–365.

Small, E.B., Lynn, D.H., 1981. A new macrosystem for the phylum Ciliophora Doflein, 1901. BioSystems 14, 387–401.

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

Smith, S.A., Song, W., Gavrilova, N.A., Kurilov, A.V., Liu, W., McManus, G.B., Santoferrara, L.F., 2018. *Dartintinnus alderae* n. g., n. sp., a brackish water tintinnid (Ciliophora, Spirotrichea) with dual-ended lorica collapsibility. J. Eukaryot. Microbiol. 65, 400–411.

Sniezek, J.H., Capriulo, G.M., Small, E.B., Russo, A., 1991. *Nolaclusilis hudsonicus* n. sp. (Nolaclusiliidae n. fam.) a bilaterally symmetrical tintinnine ciliate from the lower Hudson River estuary. J. Protozool. 38, 589–594.

Snyder, R.A., Brownlee, D.C., 1991. *Nolaclusilis bicornis* n. g., n.sp. (Tintinnina: Tintinnidiidae): a tintinnine ciliate with novel lorica and cell morphology from the Chesapeake Bay estuary. J. Protozool. 38, 583–589.

Soberón, J., Peterson, A.T., 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. Biodivers. Inform. 2, 1–10.

Williams, R.A.J., Owens, H.L., Clamp, J., Peterson, A.T., Warren, A., Martín-Cereceda, M., 2018. Endemicity and climatic niche differentiation in three marine ciliated protists. Limnol. Oceanogr. 63, 2727–2736.

Wright, A.-D.G., Lynn, D.H., 1997. Maximum ages of ciliate lineages estimated using a small subunit rRNA molecular clock: Crown eukaryotes date back to the Paleoproterozoic. Arch. Protistenk. 148, 329–341.

Zufall, R.A., 2016. Mating systems and reproductive strategies in *Tetrahymena*. In: Witzany, G., Nowacki, M. (Eds.), Biocommunication of Ciliates. Springer International Publishing, Switzerland, pp. 221–233.