Ciliophora

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Abstract

The ciliated protists (Phylum Ciliophora) are typically longer than 50 μm in body length and so are conspicuous microbial eukaryotes. There are over 8,000 species of these usually quickly moving protists, which locomote using files of cilia organized on the cell surface. In addition to the files of cilia or kineties on the cell surface, ciliates are also characterized by nuclear dimorphism or the possession of two kinds of nuclei: (1) the micronucleus, which is not transcriptionally active and which is considered the equivalent of the germ line in multicellular organisms and (2) a macronucleus, which is transcriptionally active and which is typically a developmental product of the amplification of the micronuclear or germ-line DNA. The micronucleus participates in conjugation, which is the sexual process of ciliates, and the third major feature to characterize this phylum. Ciliates as large cells are the top predators or heterotrophs in microbial food webs when metazoans are absent. As heterotrophs, they feed upon bacteria, smaller protists, and even other ciliates in ecosystems from the poles to the tropics and from terrestrial soils to the sediments around deep-sea hydrothermal vents. The genus *Mesodinium* includes the only "autotrophic" ciliate species, but many species are mixotrophic, capturing the chloroplasts of prey or hosting autotrophic protists as endosymbionts. Ciliates can also be symbionts of other organisms, ranging from commensals found in the stomachs of ruminants to parasites of fish. Ciliates, such as *Tetrahymena* and *Paramecium*, whose genomes have been sequenced, serve as model organisms for cell and molecular biology.

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Introduction

General Characteristics

The ciliates are undoubtedly one of the easiest groups of protists for the nonspecialist to identify since their typical feature is the presence of files or rows of cilia, known as kineties, on the cell surface. Most also have a cytostome or "cell mouth" around which oral cilia are arranged. Ciliates also exhibit nuclear dualism in which the relatively larger macronucleus is physiologically active, containing thousands of copies of genes, while the relatively smaller, diploid micronucleus is the germ nucleus whose meiotic products are exchanged during conjugation. These general features are found together in no other group of protists. *Stephanopogon* was a disturbingly exceptional ciliate as it appeared to have files of cilia on the cell surface but lacked nuclear dualism, but we now know that *Stephanopogon* is an example of presumed phylogenetic affinity that turned out to be convergence when inspected more closely: its kinetid does not have any typical ciliate fibrillar associates (i.e., no transverse microtubular ribbon, no postciliary microtubular ribbon, no kinetodesmal fiber) (Patterson and Brugerolle 1988; see ▶ Heterolobosea). There are over 1,100 ciliate genera and over 8,000 species included in these genera (Lynn 2008), although some argue that this diversity may represent only 10% of the actual diversity of species (Foissner et al. 2008).

Occurrence

Most species are free-living and found in ponds, lakes, estuaries, saltmarshes, and oceans. They have been collected in almost every conceivable aqueous habitat from Antarctica (Kepner et al. 1999 in Lynn 2008; Song and Wilbert 2000 in Lynn 2008) to hot springs; from small, temporary puddles to lakes and oceans (Kofoid and Campbell 1939); from slightly moistened soils (Foissner 1988a in Lynn 2008) to fresh waters (Beaver and Crisman 1989) and saline waters (Pierce and Turner 1992); and from streams (Cleven 2004 in Lynn 2008) to sewage treatment plants (Curds 1975b in Lynn 2008).

A variety of species is available from culture collections and biological supply houses (Table 1). Those strains kept in culture collections are most likely to have a certified pedigree and should be chosen for experimental work.

Literature

The nonspecialist is advised to consult introductory protozoology books, such as Hausmann et al. (2003), although there is still much of value in older texts, for example, Grell (1973) or Sleigh (1989). Having established an appreciation for the

Table 1 List of ciliate species available from culture collections

Species[a]	Source[b]	Species	Source
Blepharisma americanum CCAP		*Paramecium tredecaurelia* ATCC	
Blepharisma hyalinum CCAP		*Paramecium triaurelia* ATCC	
Blepharisma stoltei ATCC		*Paramecium undecaurelia* ATCC	
		Parauronema acutum ATCC	
Bursaria truncatella BS		*Plagiopyla nasuta* CCAP	
		Potomacus pottsi ATCC	
Cinetochilum margaritaceum ATCC		*Prodiscophrya collini* CCAP	
Chilodonella uncinata ATCC			
Cohnilembus reniformis CCAP		*Sorogena stoianovitchae* ATCC	
Coleps hirtus CCAP		*Spirostomum ambiguum* CCAP	
Colpidium striatum CCAP		*Stentor coeruleus* BS	
Colpoda cavicola ATCC			
Colpoda cucullus ATCC		*Tetrahymena americanis* ATCC, CCAP	
Colpoda inflata ATCC, CCAP		*Tetrahymena asiatica* ATCC	
Colpoda magna ATCC			
Colpoda maupasi ATCC		*Tetrahymena australis* ATCC	
		Tetrahymena bergeri ATCC	
Colpoda steinii ATCC, CCAP		*Tetrahymena borealis* ATCC, CCAP	
Cyclidium glaucoma ATCC, CCAP		*Tetrahymena canadensis* ATCC, CCAP	
Dexiostoma campyla ATCC, CCAP			
Didinium nasutum ATCC, BS		*Tetrahymena capricornis* ATCC	
		Tetrahymena cosmopolitanis ATCC *Tetrahymena corlissi* ATCC	
Euplotes gracilis ATCC		*Tetrahymena elliotti* ATCC	
		Tetrahymena farleyi ATCC	
Euplotes vannus CCAP		*Tetrahymena furgasoni* ATCC	
Euplotes raikovi ATCC		*Tetrahymena hegewischi* ATCC	
Heliophrya sp. ATCC		*Tetrahymena hyperangularis* ATCC	
Ilsiella palustris CCAP		*Tetrahymena limacis* ATCC, CCAP	
Mesanophrys chesapeakensis ATCC		*Tetrahymena lwoffi* CCAP	
Meseres corlissi CCAP		*Tetrahymena malaccensis* ATCC	
Metopus es CCAP		*Tetrahymena nanneyi* ATCC	
Nassula sorex CCAP		*Tetrahymena nipissingi* ATCC	
Opisthonecta henneguyi ATCC		*Tetrahymena paravorax* ATCC	
		Tetrahymena patula ATCC, CCAP	
Paramecium biaurelia ATCC, CCAP		*Tetrahymena pigmentosa* ATCC, CCAP	
Paramecium bursaria CCAP, BS		*Tetrahymena pyriformis* ATCC, CCAP	
Paramecium caudatum BS		*Tetrahymena rostrata* ATCC	
Paramecium decaurelia ATCC		*Tetrahymena setosa* ATCC	
Paramecium dodecaurelia ATCC		*Tetrahymena shanghaiensis* ATCC	
Paramecium jenningsi ATCC			
Paramecium multimicronucleatum ATCC, BS		*Tetrahymena sonneborni* ATCC	

(*continued*)

Table 1 (continued)

Species[a]	Source[b]	Species	Source
Paramecium novaurelia ATCC		*Tetrahymena thermophila* ATCC, CCAP	
Paramecium octaurelia ATCC		*Tetrahymena tropicalis* ATCC	
Paramecium pentaurelia ATCC		*Tetrahymena vorax* ATCC, CCAP	
Paramecium polycaryum ATCC		*Tokophrya infusionum* ATCC	
		Tokophrya lemnarum ATCC	
Paramecium quadecaurelia ATCC		*Trimyema koreanum* CCAP	
Paramecium septaurelia ATCC		*Trimyema shoalsi* ATCC	
Paramecium sexaurelia ATCC		*Uronema marinum* CCAP	
Paramecium sonneborni ATCC		*Vorticella microstoma* ATCC, CCAP	
Paramecium tetraurelia ATCC, CCAP		*Vorticella similis* CCAP	

[a]Some of the species listed are available in several strains or stocks. This listing was prepared from WWW listings of these three culture collections in July, 2013

[b]*ATCC* American Type Culture Collection (www.atcc.org), 10801 University Blvd., Manassas, VA, U.S.A. 20110-2209; *CCAP* Culture Collection of Algae and Protozoa (www.ccap.ac.uk), Scottish Marine Institute, Dunbeg, Argyll, Scotland, UNITED KINGDOM PA37 1QA; *BS* Boreal Science (www.boreal.com), 399 Vansickle Road, St. Catharines, ON, CANADA L2S 3T4

general biology of the phylum, the reader may choose to read Grassé (1984), Hausmann and Bradbury (1996), Jones (1974), and Matthes and Wenzel (1966) or to specialize in any of a number of areas. Lynn (2008) provides a comprehensive account of the literature and the history of ciliatology, and the present chapter is largely a précis of Lynn's monographic work.

More detailed descriptions of particular genera are provided in books on the biology of: *Blepharisma* (Giese 1973); *Paramecium* (Beale and Preer 2008; Görtz 1988); *Stentor* (Tartar 1961); and *Tetrahymena* (Asai and Forney 2000; Collins 2012; Elliott 1973). Details of the physiology and biochemistry of *Tetrahymena* can be found in Hill (1972) and of the developmental biology and genetics of *Paramecium, Tetrahymena, Euplotes*, and other ciliates in Nanney (1980), Frankel (1989), and Beale and Preer (2008).

Several monographs contain review papers that include chapters specifically devoted to the ciliates. These include the general biology of ciliates (Grassé 1984), their systematics (Lynn 2008; de Puytorac 1994), chemical aspects of protozoan biology (Kidder 1967), aspects of the biochemistry and physiology of protozoa (Hutner 1964; Hutner and Lwoff 1955; Levandowsky and Hutner 1980; Lwoff 1951), and selected topics on a wide range of protozoan research topics (Chen 1967–1972).

Specific topics in ciliate biology have been reviewed: extrusive organelles (extrusomes) (Hausmann 1978; Rosati and Modeo 2003 in Lynn 2008); membrane trafficking (Allen and Fok 2000); contractile vacuoles (Allen 2000; Patterson 1980); evolution of cortical microtubular structures (Lynn 1981); somatic function of the micronucleus (Ng 1986); genetics and aging (Smith-Sonneborn 1981); and endosymbionts of *Euplotes* (Heckmann 1983).

Some specific mention should be made of publications on the systematics and ecology of ciliates. Foissner et al. (1994), for example, have published useful keys for freshwater ciliates found in activated sludge plants and other anoxic environments. For a key to species used as biological indicators, see Bick (1972). Curds (1982) and Curds et al. (1983) have provided comprehensive keys to the genera of freshwater ciliates from Britain and other regions. For families of marine ciliates of the northeastern United States see Borror (1973). Foissner et al. (1999) have published a key to limnetic ciliates. Lynn and Small (2002) have provided a broader key to representative genera and species of free-living and symbiotic as well as freshwater and marine ciliates while Jankowski (2007) has reviewed all genera. Berger (2011) is an example of his taxonomic treatments of hypotrich groups while Vd'ačný and Foissner (2012) continue the taxonomic monographs published by the Foissner lab.

History of Knowledge

Antony von Leeuwenhoek was probably the first to observe ciliates. Until the mid-nineteenth century, ciliates were called Infusoria because of their prominence in infusions of vegetation. The early years were spent mainly in descriptive taxonomy. In the nineteenth century, taxonomic research on the protists was expanded by such men as Bütschli, Claparède, Dujardin, Kent, Lachmann, Maupas, and Stein. Stein (1859, 1867 in Corliss 1979) carefully and precisely used the variations in the ciliature of oral and somatic regions of the cortex to establish affinities among taxa. Bütschli (1887–1889 in Lynn 2008) published a comprehensive monograph on the ciliates in which he modified Stein's scheme of classification. Bütschli's classification scheme of the Class INFUSORIA dominated until well into the twentieth century (see Corliss 1974a in Lynn 2008). Kahl (1930–1935) monographed the ciliates, primarily of northern Europe. His encyclopedic work is still authoritative. The name CILIOPHORA was originally proposed by Doflein in 1901. In the mid-1930s, Chatton and Lwoff perfected the "wet" silver impregnation technique, which revealed the pattern of surface and subsurface kinetosomes (basal bodies). The "Chatton-Lwoff" technique revealed details of the cortical patterns and provided information for Fauré-Fremiet's (1950a in Lynn 2008) next revision of ciliate classification, formalized by Corliss (1956, 1961 in Lynn 2008). Basing his analysis primarily on details of the cortex revealed by light microscopy, Jankowski (1967 in Lynn 2008) recognized even more diversity and elevated the number of ciliate orders. The development and use of electron microscopy during the next decade revealed an even more complex picture to systematists (Lynn 2008).

Building on Jankowski's ideas and new ultrastructural information, de Puytorac et al. (1974 in Lynn 2008) and Corliss (1974a, b in Lynn 2008) presented a further revision of what is now recognized as the Phylum CILIOPHORA. Small and Lynn (1981 in Lynn 2008) argued: (1) that these revisions of ciliate classification had been presented with inadequate consideration of their conceptual bases and (2) that more

weight must be placed on the ultrastructural features of the cortex, especially the somatic kinetid (Lynn 1981; Lynn and Small 1981 in Lynn 2008), if phylogenetic affinity was to be recognized. Small and Lynn (1981 in Lynn 2008) proposed a radically new classification system that formed the basis of the revised classifications presented by Lynn and Small (1997, 2002 in Lynn 2008; see section below on "Characterization and Recognition"). Jankowski (2007) has presented a revised system. See Corliss (1979, 1986) and Lynn (2008) for more detailed historical accounts of ciliate systematics.

Practical Importance

The agricultural and medical importance of ciliates relates to their associations with mammals. Large populations of particular species of symbiotic ciliates are found in the digestive tracts of sheep, goats, cattle, pigs, and horses. Although their presence is not essential for the growth of the herbivores, the ciliates most likely stabilize the cellulolytic bacterial populations (Bonhomme 1990 in Lynn 2008; Coleman 1989 in Lynn 2008; Dehority 1993 in Kreier and Baker 1993).

A wide variety of ciliates exploit both freshwater and marine fishes (Basson and Van As 2006 in Woo 2006; Burgess and Matthews 1995b in Lynn 2008; Dickerson 2006 in Woo 2006; Bradbury 1994 in Kreier 1994; Iglesias et al. 2001 in Lynn 2008), presenting economic problems in aquaculture operations only when present in large numbers (Harikrishnan et al. 2010). *Balantidium,* the only endoparasitic ciliate of man, has been reported to cause gastrointestinal infections. These often occur in places where people and pigs cohabit (Schuster and Ramirez-Avila 2008; Zaman 1993 in Kreier and Baker 1993). Numerous ciliates are parasites of invertebrate marine animals (Bradbury 1994 in Kreier 1994). Their effect, from the human perspective, can be defined as harmful, when, for example, populations of the commercially important Dungeness crab are infected (Morado and Small 1995) or rearing of snails for human consumption (Segade et al. 2009) or beneficial, when the infected hosts are the larvae and adults of insects that are vectors of human parasites (Barros et al. 2006 in Lynn 2008; Batson 1983 and references cited therein; Washburn et al. 1988 in Lynn 2008).

Ciliates have been used in a number of practical applications, ranging from the assessment of water quality to their use as model organisms for assessment of the effects of chemicals on metazoans.

Certain associations of ciliates can be used as complex indicators of the quality of the environment (Bick 1972 in Lynn 2008; Foissner 1988; Foissner et al. 1982) and to reveal the complex effects of pollution on the microbiota (Cairns et al. 1972; Tan et al. 2010). Ciliates play an important, perhaps essential, role in the clarification of water during and after sewage treatment (Curds 1969 in Lynn 2008; Fried et al. 2000 in Lynn 2008; Small 1973 in Lynn 2008).

Protists are becoming increasingly popular as bioassay organisms due in part to rising costs of maintaining laboratory animals and increasing pressure from animal welfare groups (Schultz et al. 1978). Ciliates, in particular the *Tetrahymena*

"pyriformis" species complex and *Colpidium campylum*, have been used in bioassays for protein quality (Rølle 1980; Wang et al. 1980), in bioassays to detect toxic substances in aquatic environments (Gilron and Lynn 1996; Gilron and Lynn 1998; Slabbert et al. 1983) and soils (Forge et al. 1993 in Lynn 2008), and as possible models for mammalian cells in assessing the effects of chemicals (Dayeh et al. 2004).

Habitats and Ecology

The comprehensive bibliography of Finlay and Ochsenbein-Gattlen (1982), while dated, should provide the interested reader with a starting point for the literature. Fenchel (1987) provides another focus with some emphasis on the ciliates while Dolan et al. (2013) have provided a thorough and readable overview of the systematics and ecology of tintinnid ciliates, a conspicuous group in the marine plankton.

Habitats

The four main environments where ciliates are obvious include the benthos, especially the marine littoral, terrestrial soils, the plankton, and certain symbiotic associations. Ciliates are also found in some unusual habitats, which will be described as well.

Benthic Habitats. Benthic ciliates have been studied in freshwater, brackish, and marine habitats where they may be found freely swimming over the substrate or attached to it. The community of microbes in aquatic environments attached to rocks, fallen logs, and the like is called the aufwuchs or biofilm.

One of the earliest studies of the benthic ciliates within sediments was Fauré-Fremiet's study (1950c in Lynn 2008) of the interstitial fauna of sandy beaches; he noted that ciliate species may be free-swimming in the interstices or thigmotactic, crawling on grain surfaces. Others are attached to the grains. The distribution of ciliates is affected both by the compaction and the redox potential of the substrate in marine (Fenchel 1969) and salt marsh sediments (Elliott and Bamforth 1975 in Corliss 1979). Ciliates are particularly conspicuous when fine interstices are present and when oxygen tension is low. Azovsky and Mazei (2013) concluded that ~60% marine benthic ciliates species are endemic. Ciliates are common eukaryotic organisms in sediment trap samples off southern California at depths to 2,000 m and in the deep benthos of the Mediterranean Sea (Hausmann et al. 2002 in Lynn 2008), often conspicuous in deep anoxic regions (Orsi et al. 2012; Takishita et al. 2010). They have been found on rock surfaces as well as on the tubes of vent worms in the 21° N hydrothermal vents at depths up to 2,600 m (Small and Gross 1985).

Terrestrial Soils. Ciliates are often conspicuous in damp soils; they are ubiquitous in soil cultures from all parts of the world (Foissner 1998a in Lynn 2008). However, ciliates are usually outnumbered by the testate amebae (Bamforth 1980 in Lynn 2008). Protists in general constitute a relatively small portion of the total biomass in soil (Adl 2003). The species diversity and abundance of ciliates are functions of

geography, season, moisture content, temperature, pH, organic content, and the degree of compaction and abrasion of the soil environment (Foissner 1987, 1997d in Lynn 2008).

The Plankton. Ciliates, conspicuous components of planktonic communities at most times of the year, are found in freshwater (Pace and Orcutt 1981 and Porter et al. 1979, both in Finlay and Ochsenbein-Gattlen 1982), neritic (Beaver and Crisman 1989; Leakey et al. 1994 in Lynn 2008), and oceanic environments (Strom et al. 1993 in Lynn 2008). They are found in small, temporary puddles, tide pools, lakes, rivers, and the major oceans of the world. Because standard zooplankton sampling procedures are unsuitable for soft-bodied ciliates their presence, abundance, and diversity were undoubtedly underestimated until the late 1980s when more appropriate sampling techniques, using water bottles, were adopted. Although loricate tintinnids predominate in the plankton literature (Dolan et al. 2013; Kofoid and Campbell 1939; Heinbokel and Beers 1979 in Coats and Heinbokel 1982), it is now clear that nonloricate ciliates are consistently more abundant when other sampling techniques are used (Lynn and Montagnes 1991 in Lynn 2008; Pierce and Turner 1992). Indeed, ciliates are exceedingly abundant in association with the spring phytoplankton bloom in temperate waters and may flourish at other times of the year in short-term blooms. These blooms can lead to spatial patchiness in the distribution of ciliates that may range from 10s to 100s of meters in size (Bulit et al. 2009).

Symbiotic Associations. Ciliates are found as symbionts in association with a wide variety of species (Bradbury 1996 in Hausmann and Bradbury 1996; Lynn 2008). The most thoroughly studied associations include those with ruminant mammals and related herbivores (Hungate 1978 in Kreier 1978), sea urchins (Levine 1972 in Chen 1972), fish (Hoffman 1978 in Kreier 1978), crustaceans (Ferńandez-Leborans 2001 in Lynn 2008), and a variety of molluscan species (for example, Raabe 1972 in Lynn 2008). The symbiotic relationships are generally thought to be commensalistic, either as endo- or ectocommensals, but ruminant ciliates may be mutualistic (Hungate 1978 in Kreier 1978). Some ciliates found in fish and insects can be classified as parasitic (i.e., harmful to their host): they may be histophagous (tissue-eating; necrotrophic) (Alvarez-Pellitero et al. 2004 in Lynn 2008; Batson 1983; Hoffman 1978 in Kreier 1978).

Ciliates host a variety of microorganisms, including bacteria, mastigotes, chlorellae, and other ciliates. Again, the nature of the symbiotic relationships varies from mutualistic, commensalistic, parasitic, or pathogenic (Ball 1969 in Chen 1969; Berninger et al. 1986 in Lynn 2008; Görtz and Dieckmann 1987 in Lynn 2008; Heckmann 1983; Soldo et al. 1974; Weis and Ayala 1979).

Some Unusual Habitats. The ciliates are not as successful as prokaryotes and mastigotes in exploiting extreme habitats. Nevertheless, species have been described from habitats of temperature extremes: from hot thermal springs and waters near the deep-sea hydrothermal vents at a depth of 2,600 m off the California coast (Small and Gross 1985) to the ice and lakes of Antarctica (Christner et al. 2003 in Lynn 2008; Kepner et al. 1999 in Lynn 2008; Laybourn-Parry et al. 2002 in Lynn 2008; Lee and Fenchel 1972 in Lynn 2008).

Ecology

Three aspects of the ecology of ciliates will be discussed below: the role that ciliates have played in models of ecological theory, the ecology of ciliate communities, and the contribution ciliates make to primary and secondary production.

Ecological Models. Protists/protozoa are excellent experimental organisms for the modelling of ecological theory for several important reasons (Montagnes et al. 2012; Salt 1974).

"These are that if a phenomenon is found to occur in protozoa it has a high probability of being a general one, and that the absence of sexes, age classes, and other characteristics of more complex animals permit certain reactions to be seen in protozoa more clearly than in higher animals." (Salt 1974).

Ciliates share with other protists the properties outlined above by Salt (1974). Being small organisms, they have many generations in a short period of time, and the diversity of "functionally" different species can enable the construction of complex communities. Microcosm experiments with ciliates can be replicated with ease and because these are small and manageable "systems" there can be rigorous to complete control of most abiotic factors.

Gause (1934) was the first to take advantage of the protists in testing and modelling ecological phenomena, both in his studies of predator–prey modelling using *Paramecium caudatum* and *Didinium nasutum* and in his studies of competitive exclusion using *Paramecium aurelia*, *Paramecium caudatum*, and *Stylonychia mytilus*.

Over 30 years later, Salt (1967) modelled the predator–prey interaction between *Woodruffia metabolica* and *Paramecium aurelia*, discovering, among other things, that the predator exhibited a threshold response to prey density rather than a proportional response and that the escape from predation of a portion of the prey population was a result of innate behavioral characteristics of the predator. Salt (1974, 1975 in Salt 1979) investigated predator–prey interactions between two species used by Gause (1934), *Didinium* and *Paramecium*. In these studies, Salt (1974) found that both predator and prey density can act as controls on the capture rate of the predator, that as predator density increases, the size of individual *Didinium* increases while prey capture and/or food intake rates decline (Salt 1975 in Salt 1979), and that "*Didinium* at higher densities are more efficient in the utilization of energy than are those at low densities" (Salt 1979). Li and Montagnes (2015) have used these two species to more deeply explore predator–prey models, concluding predator conversion efficiency and predator mortality, two key model components, can depend upon prey abundance. Luckinbill (1973 in Lynn 2008) concentrated on the prolonged coexistence of predator and prey also using *Didinium* and *Paramecium*. Whereas Gause (1934) and Cooper et al. (2012) found that the prey needed physical refuges or habitat fragmentation to prolong the interaction, Luckinbill (1973 in Lynn 2008) prolonged coexistence by providing a physically homogeneous environment using methyl cellulose to slow both predator and prey locomotion; in this system, if prey growth is restricted and if prey can maintain adequate numbers for survival while simultaneously remaining at low enough

densities to avoid capture, a cycling of the predator and prey populations is achieved. Luckinbill and Fenton (1978) have further explored the relationship between intrinsic rates of increase, frequency of environmental perturbation, and population cycling in bacterivorous ciliates. They demonstrated that populations of fast-growing species track environmental variations more closely and become extinct more quickly than populations of slower growing species.

Gause's initial experiments on competitive ability have been explored in more detail. Natural, rather than experimental, populations of *Paramecium aurelia* were studied in a woodland seepage area (Gill and Hairston, in Gill 1972). Although one stock of *P. aurelia* was apparently competitively excluded, evidence suggests that it was not well adapted to the marginal, highly unpredictable habitat used for the experiments. Further investigating the relationships between intrinsic rates of increase r, saturation densities K, and competitive ability of experimental populations of *Paramecium aurelia*, Gill (1972) concluded that there was "no consistent relationship between r and K and competitive ability, and that simple environmental changes affect competitive ability much less than they affect either r or K." On the other hand, Luckinbill (1979) showed that selection for increased r also increased K of several stocks of *Paramecium primaurelia*. In over six species of bacterivorous ciliates, estimates of r_m (the maximum rate of increase) were also positively correlated with K and negatively correlated with competitive ability (Luckinbill 1979).

Several examples in the recent literature have used ciliates and other protists as model organisms to explore and illuminate aspects of biodiversity and ecosystem function (Giller et al. 2004). Morin and McGrady-Steed (2004) concluded that there was an inverse relationship between species richness and the carbon dioxide flux in microcosms featuring protists, primarily ciliates. Food web diversity and productivity can also strongly influence the composition of bacterial communities in model ecosystems of microbial eukaryotes and thus ultimately influence decomposition rates (Krumins et al. 2006). As a third example, Fukami and Morin (2003) demonstrated that the order in which the ciliate community was assembled had significant impacts on the productivity-diversity relationship. Finally, Limberger and Wickham (2012) showed that diversities and differences among habitats of low connectivity persisted longer compared to habitats with medium and high connectivities.

Assemblages and Communities. Assemblages of ciliates are characteristic, not only of certain habitats but also within the same habitat where predictable assemblages seem to occur at specific seasons or under specific conditions related to biotic and abiotic factors (Bick 1972 in Lynn 2008; Grolière 1978). The apparent predictability of these assemblages has led some investigators to suppose that the many different species have dependent interactions (Cairns and Yongue 1977) although Dolan et al. (2007) concluded that the neutral theory of random colonization could explain the structure of tintinnid communities. There is certainly variability in the appearance of these assemblages both in time (Goulder 1980 in Lynn 2008) and in space (Bulit et al. 2009; Taylor and Berger 1980), driven primarily by resource availability (Galbraith and Burns 2010).

Ciliates are heterotrophs, being either phagotrophs or osmotrophs. Various species can be categorized as bacterivorous, algivorous, carnivorous (Elliott and Bamforth 1975 in Corliss 1979; Fenchel 1968 in Fenchel 1969; Noland and Gojdics 1967 in Chen 1967), or histophagous. Ciliates perform a similar role in soils and aquatic sediments. By grazing on bacterial populations and ingesting plant residues ciliates increase rates of decay and mineral cycling (Fenchel and Harrison 1976; Krumins et al. 2006). Some ciliates in both marine and freshwater habitats can be classified as "autotrophic" or mixotrophic: in these cases they contain algal symbionts or they somehow "steal" the chloroplasts of their algal prey – a phenomenon called kleptoplasty (Johnson 2011; Perriss et al. 1994; Stoecker et al. 1989). Mixotrophy may enable survival in habitats that would be marginal for an obligate heterotroph (Esteban et al. 2010).

In planktonic communities, ciliates are links in the food chains (Sanders and Wickham 1993; Sherr and Sherr 1988). In marine "snow," ciliates are a part of the decomposition food web (Caron et al. 1982). In oceanic regions, perhaps more than 90% of the carbon may be cycled through the protists, including ciliates such as tintinnids and oligotrichs (Lynn and Montagnes 1991 in Lynn 2008). In planktonic food webs, ciliates may be important in the regeneration of some nutrients (Garst and Horstmann 1983; Johannes 1965 in Corliss 1979) but not others (Taylor and Lean 1981 in Garst and Horstmann 1983). In coastal regions, a few ciliates, such as the autotrophic *Mesodinium rubrum*, may even contribute substantially to primary production (Smith and Barber 1979 in Lynn 2008). As links, ciliates are consumed by a variety of other organisms: in the pelagic realm, copepods, jellyfish, and larval fish have been recorded as predators (de Figueiredo et al. 2007; Stoecker and Sanders 1985 in Lynn 2008; Stoecker et al. 1987 in Lynn 2008).

Production. Production is defined as the amount of biomass generated per unit time. There are now many estimates of the contribution ciliates make to both primary and secondary production of biomass. For example, ciliates in littoral sand sediments representing 0.05% of the biomass are estimated to have contributed 15% to the secondary production of the zoobenthos (Burkovsky 1978). In sediments from a freshwater lake, Finlay (1978 in Finlay and Ochsenbein-Gattlen 1982) concluded that production and consumption by benthic ciliates are significant components of the energy flow through the benthos. Tintinnids may constitute more than 25% of the secondary production at certain times of the year (Middlebrook et al. 1987 in Lynn 2008). However, the nonloricate oligotrich ciliates typically "out-produce" tintinnids in a variety of ecosystems (Gilron et al. 1991 in Lynn 2008; Lynn et al. 1991a in Lynn 2008; Montagnes et al. 1988 in Lynn 2008). The record for contribution to **primary** production (as well as high speed swimming) is held by the autotrophic ciliate *Mesodinium rubrum*, which harbors a symbiotic photosynthetic cryptomonad and its chloroplasts (Lindholm 1985). Smith and Barber (1979 in Lynn 2008) recorded photosynthetic rates of 1,000–2,000 mg C m^{-3} h^{-1} for a bloom of this ciliate, matching the most productive phytoplankton. More often, primary production by mixotrophic ciliates is a very minor component (Perriss et al. 1994).

Characterization and Recognition

The Phylum

General Characterization. Ciliates, with rare exceptions (i.e., *Phalacrocleptes*, a suctorian in the PHYLLOPHARYNGEA), have cilia at some stage in their life cycle. Ciliates are dikaryotic; their cells contain macronuclei and micronuclei. In this nuclear dualism, the macronucleus is physiologically dominant, actively synthesizing mRNA and rRNA while the micronucleus, the repository of the genomic DNA, is involved in genetic recombination and sexual phenomena. In most ciliates, the macronuclei contain far greater than diploid quantities of DNA and are considered to be ampliploid (i.e., containing many amplified copies of the ciliate's genome) (Lynn 2008). The micronuclei of ciliates are considered to be diploid, although polyploidy undoubtedly occurs. Nuclear division is closed: the nuclear membranes of both macro- and micronuclei remain intact while the mitotic apparatus separates the DNA. In ciliates of the HETEROTRICHEA, however, most mitotic microtubules are **external** to the macronuclear membrane, a diagnostic feature for this class. Micronuclear chromosomes are attached to microtubules by kinetochores. The macronucleus develops from a micronucleus after conjugation. Initially, the micronuclear chromosomes may be endoreplicated many times to become polytenic. Subsequently, DNA sequences are deleted using a RNA-mediated epigenetic machinery that results in subchromosomal macronuclear DNA molecules that range in length from 2 to 300 kb (Chalker 2008; Juranek and Lipps 2007; Nowacki et al. 2010; Prescott 1994). Telomeric sequences, such as the hexanucleotide CCCCAA, are added by telomerase to the chromosome ends (Blackburn 1992; Blackburn et al. 1983). The macronucleus divides amitotically by an unknown method of segregation, possibly just randomly, of these subchromosomal DNA molecules. The macronuclei of one group of ciliates, the KARYORELICTEA (see below), cannot divide: new macronuclei arise by division and differentiation of micronuclei at each cell division (Raikov 1982; Raikov 1996 in Hausmann and Bradbury 1996).

The ciliature of the body in most ciliates is specialized around the cell mouth or cytostome (Fig. 1). Because of its variability, the pattern and arrangement of this oral ciliature has been the basis for the classification of ciliates for many years (Corliss 1979; Lynn 2008; Lynn and Small 2002). Most ciliates are phagotrophic, ingesting particulate material and/or prey in food vacuoles that are formed at the cytostome. Some ciliates are astomatous and osmotrophic. On completion of the digestive cycle, the food is egested through the cell anus, typically via a well-defined cytoproct.

Other organelles that distinguish one group of ciliates from another are the position and arrangement of contractile vacuoles (Patterson 1980); the type and distribution of such extrusomes as mucocysts and toxicysts (Hausmann 1978; Rosati and Modeo 2003 in Lynn 2008); the presence of stalks, loricae, or other attachment structures; and types of encystment structures (Lynn 2008).

Reproduction, that is, the production of new individuals, occurs by transverse binary fission, also called homothetogenic fission (Lynn 2008): the fission plane

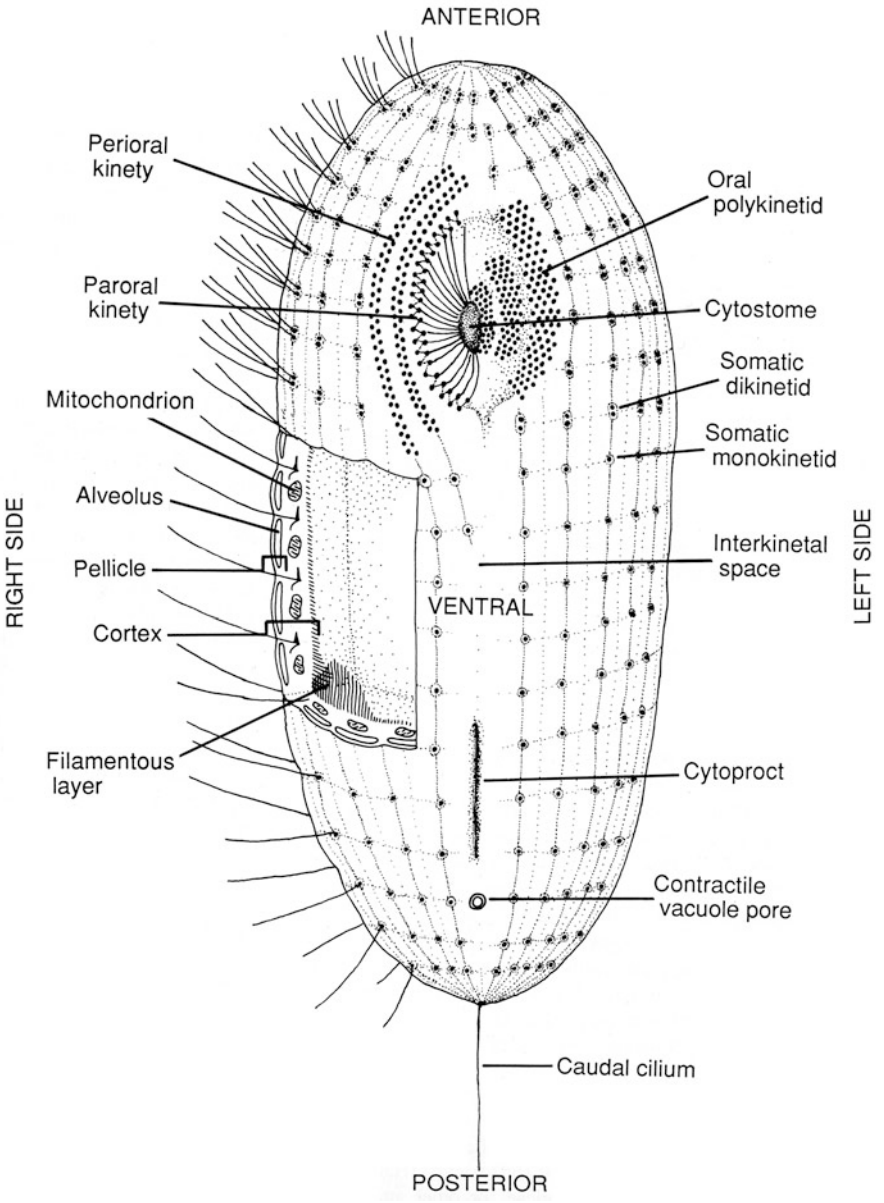

Fig. 1 Schematic figure of the ventral surface of a generalized ciliate. The cortex of a ciliate may be divided into somatic and oral regions. The locomotor units or kinetids of the somatic region are aligned in files called kineties. These kinetids can be dikinetids composed of two kinetosomes and cilia or monokinetids composed of one kinetosome and its cilium. Often a longer caudal cilium is carried posteriorly. Perioral ciliature as specializations of some somatic kineties may border the oral region. In this example, there is a paroral to the *right* of the cytostome and three oral polykinetids to the *left* of the cytostome. The cilia of these organellar complexes have not been illustrated; the

occurs across the longitudinal axis of the kineties and the body. In many taxa, binary fission may be modified so that unequal division occurs.

The sexual process, sometimes seen in field collections or lab cultures as pairing of individual ciliates of complementary mating types, is called conjugation (Miyake 1996 in Hausmann and Bradbury 1996; Nanney 1980; Orias in Collins 2012). Conjugation occurs for minutes or hours to as much as a day or so during which time the partners exchange haploid gametic micronuclei. The conjugating partners of many spirotrich, peritrich, and suctorian species can be quite different in size; in these circumstances, total conjugation or complete fusion of partners may occur. Usually, syngamy is restricted to the fusion of gametic micronuclei, which have undergone meiosis. After syngamy, the partners typically separate. During this process, new macronuclei develop from mitotic products of the zygotic nucleus through a RNA-mediated process using scan RNAs (scnRNA) (see Singh et al. 2014).

Detail of Cell Structure. In a "typical" ciliate, the cortex or the outer 1–2 μm of the cell can be divided into two main regions, the somatic and the oral region (Fig. 1). The somatic region, composed of a "skeletal" support system opposed by the hydrostatic pressure within the cell, functions in locomotion, sensing the environment, attachment to surfaces, and secretion of protective coverings. The oral region functions in sensing, acquiring, and ingesting nutrients. A complicated framework of kinetosomes, microfilaments, microtubules, and other fibers that are collectively called the infraciliature underlies these regions (Figs. 2 and 3a–d).

The infraciliature is comprised of kinetosomes arranged into longitudinal files (rows) called kineties (Fig. 1). Somatic and oral kinetal patterns are characteristic of various groups of ciliates (Lynn 2008; Lynn and Small 2002). The kinetosome is apparently the organizing center for the cortical fibrillar structures: usually two groups of microtubules and a striated kinetodesmal fibril (Figs. 2 and 3a–d) are associated with a parasomal sac. The fibrillar associates of the kinetosome anchor the cilium and provide structural support for the cortex.

Ciliates are bounded by a cell membrane, the plasmalemma (Lynn 2008). The plasmalemma in most ciliates is underlain by unit membrane-bound sacs called alveoli with which are associated a family of cortical proteins, the alveolins (Gould et al. 2008). The alveoli in their turn are subtended by a fibrous layer of varying thickness called the epiplasm, whose component proteins, the epiplasmins, form a complex skeletal network (Damaj et al. 2009) (Fig. 2). The plasma membrane, alveoli, and epiplasm comprise the pellicle, which is part of the cortex (Fig. 2).

Fig. 1 (continued) kinetosomes only have been represented as dots. The cytoproct for egestion and the contractile vacuole pore for osmoregulation are posterior to the oral region, in the interkinetal space. The cut-away portion of the somatic cortex illustrates the sac-like alveoli beneath the plasma membrane, the cortical mitochondria, and the kinetosome with its fibrillar associates. These cortical structures are sometimes separated from the endoplasm by a filamentous layer

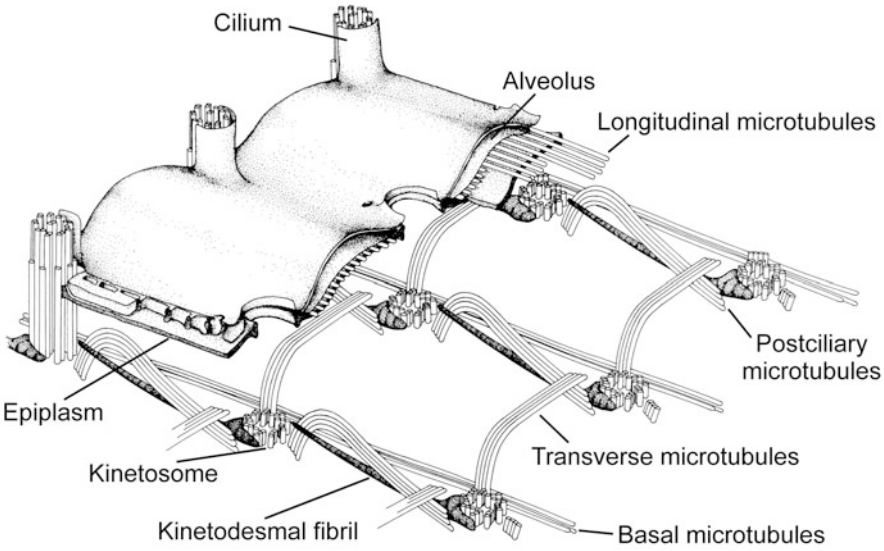

Fig. 2 This is a detailed schematic figure of the generalized somatic cortex of a ciliate. Nine locomotor units or kinetids are illustrated: six are illustrated without cilia and three are illustrated with cilia. The cell surface is covered by a unit plasma membrane (plasmalemma), which is pierced by indentations called parasomal sacs. The cortical alveoli underlie the plasma membrane between kineties and are connected to adjacent alveoli along the kinety. The alveoli, in their turn, may be underlain by microtubules that lie on top of a dense, perhaps fibrous layer called the epiplasm. The epiplasm is pierced by the parasomal sacs and by the kinetosomes. The kinetosomes are associated with three fibrillar structures: a periodically striated kinetodesmal fibril; a laterally directed transverse ribbon; and a posteriorly directed postciliary ribbon. A set of basal microtubules courses beside the kinetosomes but is not directly connected to them

A variety of other organelles is found in the cortex. The majority of the mitochondria with tubular cristae (Fig. 3b, e, g, h) are found in the cortical ridges between kineties, where they are anchored in position by connections to cortical microtubules and to the epiplasm. Mitochondria in some ciliates have transformed into hydrogenosomes (de Graaf et al. 2011), and these are typically intimately associated with symbiotic methanogenic bacteria (Boxma et al. 2005 in Lynn 2008; Fenchel and Finlay 1991a). Extrusomes (Fig. 3e, f, j) are also distributed in the cortex between and within the kineties. There are several types of these exocytotic organelles, which function to aid in the capture of prey, in building the wall in resting cysts, or for some other unknown function (Hausmann 1978; Rosati and Modeo 2003 in Lynn 2008).

The contractile vacuole pore (Figs. 1 and 3g) is a cortical structure that serves as the opening through which the products of osmoregulation or the contents of the contractile vacuole are expulsed (Allen 2000). Egestion takes place through the cytoproct (Fig. 1), usually a slit-like opening in the cortex (Allen and Wolf 1974 in Lynn 2008).

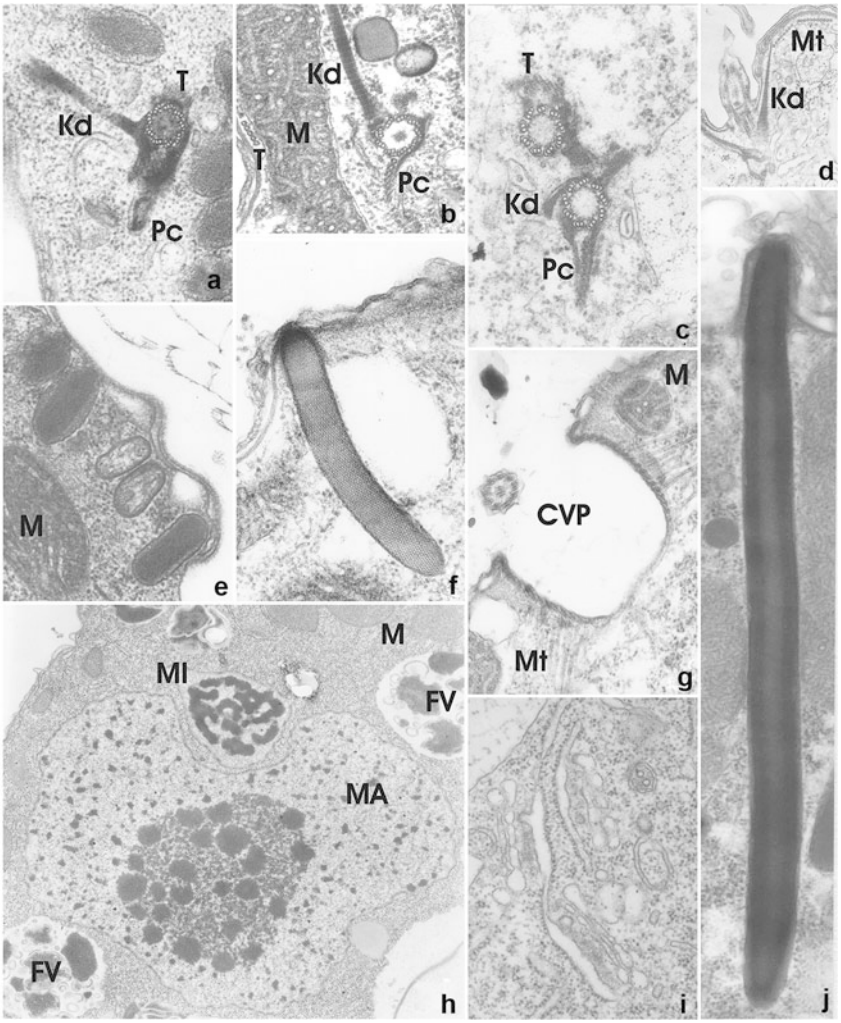

Fig. 3 (**a**–**j**) Ultrastructural features of the Phylum CILIOPHORA. (**a**–**d**) Somatic kinetids of some ciliates. (**a**) *Lepidotrachelophyllum,* a haptorian litostome. ×49,000. (**b**) *Colpidium,* a hymenostome oligohymenophorean. ×50,000. (**c**) *Colpoda,* a colpodean. ×55,000. (**d**) Oblique section through the base of the cilium and a longitudinal section of the tapering kinetodesmal fibril of *Colpoda*. Note the microtubules (*MT*) underlying the cortex with its flattened alveoli beneath the plasma membrane. *Kd* kinetodesmal fibril, *M* mitochondrion, *Pc* postciliary microtubular ribbon, *T* transverse microtubular ribbon. (**e**, **f**, **j**) Extrusomes of several ciliates. (**e**) Mucocysts of *Lepidotrachelophyllum*. ×38,000. (**f**) A mucocyst of *Colpidium*. ×35,500. (**j**) A "mucocyst" of *Ophryoglena.* ×38,000. (**g**) Contractile vacuole pore (*CVP*) of *Colpidium,* an oligohymenophorean. A set of microtubules is embedded in the epiplasm along the wall of the pore while other microtubules (*Mt*) originate in the epiplasm of the wall and extend away from the pore over the surface of the contractile vacuole. *M* mitochondrion. ×20,000. (**h**) Macronucleus (*MA*) and micronucleus (*MI*) of *Colpoda,* a colpodean. Note also the mitochondria (*M*), which have tubular cristae (see also (**e**) and (**g**) above). *FV* food vacuole ×18,000. (**i**) Golgi apparatus of *Colpoda*. ×36,000

The endoplasm of ciliates, in general, has a less obvious organization than the cortex or ectoplasm. The macronucleus and micronucleus (Fig. 3h) are among the largest endoplasmic structures. Food vacuoles are scattered throughout the endoplasm. The Golgi bodies, often called dictyosomes in ciliates and plants, are inconspicuous and scattered throughout the cytoplasm (Fig. 3i). Microtubular ribbons usually originate from cortical kinetosomes and extend into the endoplasm to direct the movement of organelles and vesicles in both directions between the endoplasm and the cortex (Allen and Fok 2000).

Some Life Cycles. The life cycle of a typical ciliate is fairly simple (Fig. 4). In the presence of nutrients, cells grow and reproduce by binary fission to increase the size of the population. As food becomes limiting, some ciliates disperse from the food

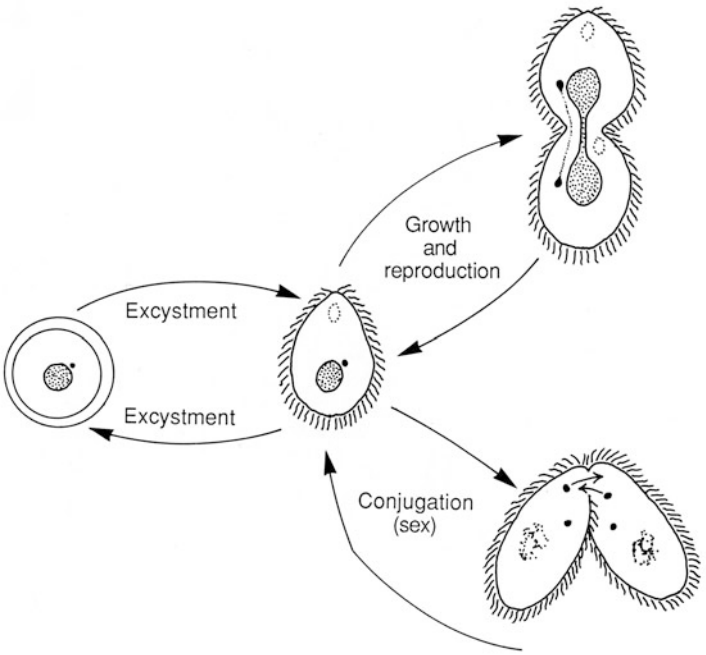

Fig. 4 This figure illustrates a generalized ciliate life cycle, divisible into three phases. The vegetative or asexual reproductive cycle involves feeding, growth, and division by binary fission. Conjugation, the sexual cycle, often stimulated by the depletion of food and the onset of starvation, involves temporary fusion of complementary mating types, meiotic reduction of chromosome number from diploid to haploid, and exchange of haploid gametic nuclei before separation of the partners as exconjugants. If food is present, growth and division ensue; if it is still absent or some other environmental stress such as pH, temperature, toxins, or desiccation stimulates it, the ciliate may enter the encystment-excystment cycle. Cysts may persist for months to years. Ciliates excyst when stimulated by the appropriate environments. According to Goodey (1915), the oldest viable ciliate cysts, two species of *Colpoda,* were more than 38 years old while other protozoan cysts from soils have remained viable for almost 50 years while Shatilovich et al. (2015) have isolated viable cells from late Pleistocene permafrost, 32–35,000 years old

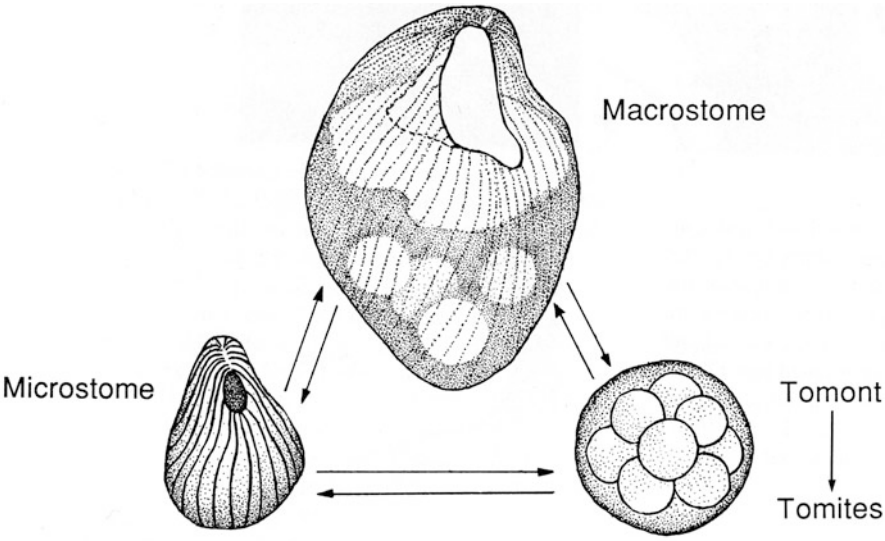

Fig. 5 Life cycle of the macrostome-microstome oligohymenophorean *Tetrahymena vorax*. The microstome form feeds on bacteria. When this food disappears or another suitable ciliate prey is present, some of the population of *T. vorax* are stimulated to undergo a morphogenetic transformation during which their bacteria-feeding oral apparatus dedifferentiates and a new, macrostome oral apparatus designed to capture ciliate prey differentiates. Cell division can occur in a cyst: the divider or tomont undergoes cell division yielding several offspring cells called tomites (After Corliss 1973 in Elliott 1973; Redrawn by S. Alexander)

source and begin to starve. Starvation initiates sexual receptivity in many species. Ciliates conjugate when they encounter complementary mating types. If no mating partner is available, autogamy (self-fertilization) may occur. If nutrients are not discovered, either prior to or subsequent to conjugation or autogamy, many species encyst by secreting a protective wall about themselves. Resting cysts may or may not withstand desiccation.

Some ciliate life cycles are more complicated. The feeding stages or trophonts can be dimorphic. In *Tetrahymena vorax*, one morph is bacterivorous while the other morph feeds on other ciliates (Fig. 5). Depletion of the bacterial population by the microstome bacterivores stimulates some individuals to differentiate as macrostome carnivores that, as cannibals, begin ingesting their siblings or, as predators, other prey ciliates; the presence of the appropriate bacteria stimulates differentiation back to the microstome morph. The life cycles of parasitic species are even more complex. The life cycle of apostome ciliates, for example, is closely linked to the molt cycle of their crustacean hosts. Some apostomes reproduce in the nutrient-rich fluid associated with the shed exoskeleton or exuvium of the host (Bradbury 1996 in Hausmann and Bradbury 1996) (Fig. 6), while other apostomes are parasitoids that kill their krill hosts (Gomez-Gutierrez et al. 2012).

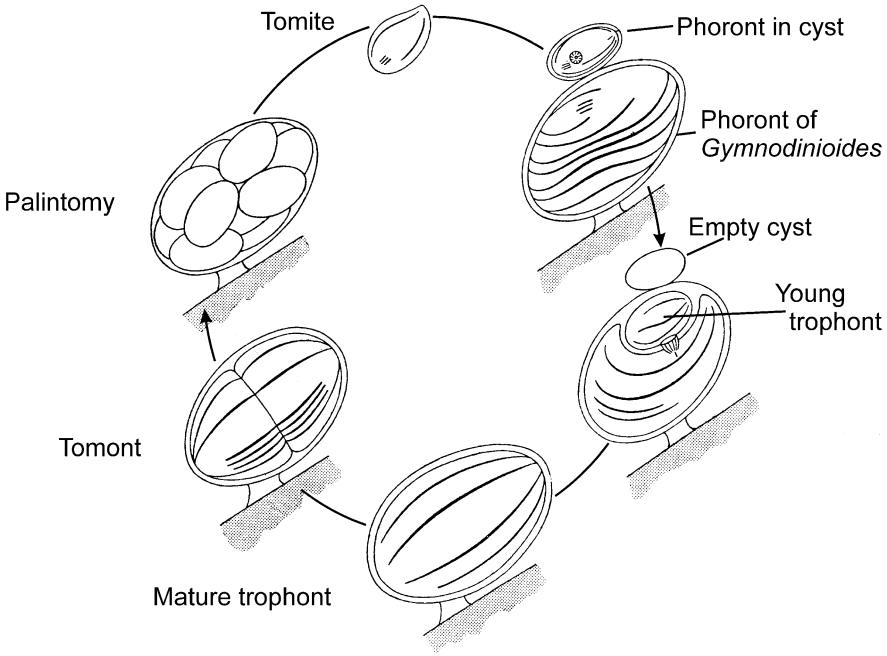

Fig. 6 Life cycle of the predatory apostome Phtorophrya, a "hyperparasite" of the exuviotrophic apostome Gymnodinioides, which itself feeds on the exuvial fluids of its crustacean host. The tomite of Phtorophrya finds a phoront of Gymnodinioides, encysted on the crustacean host's cuticle and attaches to also become a phoront. Phtorophrya then penetrates the phoront of Gymnodinioides, consumes it as a trophont, develops into a tomont that divides by palintomy to produce multiple tomites of Phtoroprhya (Modified from Chatton and Lwoff, 1935a in Lynn, 2008)

Methods and Criteria

Taxa are characterized by morphology: optical and electron microscopy are used. Increasingly, these microscopic approaches are used in conjunction with appropriate biochemical and molecular genetic techniques.

Optical Microscopy. Ciliates can be studied live or fixed and stained by several standard cytological procedures. Studying live ciliates, which requires patience, is facilitated by the combined use of differential interference contrast microscopy and a microcompressor (Skovorodkin 1990; Zinskie et al. 2015) or "slowing agents" such as methyl cellulose or nickel sulfate to retard ciliate movement.

The most informative methods for optical microscopy employ a variety of stains. Silver stains or silver impregnation techniques provide the most information about cortical structures: (1) the protargol or silver proteinate technique provides permanent preparations that reveal the most detail of cortical and subcortical structures including microtubules (Aufderheide 1982; Foissner 1991); (2) the pyridinated silver carbonate method provides either temporary or permanent preparations that

reveal the kinetodesmal fibrils and other finely filamentous cortical and subcortical structures (Fernandez-Galiano 1976 in Augustin et al. 1984; Foissner 1991); and (3) the Chatton-Lwoff silver impregnation procedure provides permanent preparations revealing the pattern of surface structures and kinetosomes (Foissner 1991; Frankel and Heckmann 1968). The only other stain that may be necessary, especially if details of nuclear morphology are needed, is the Feulgen nuclear stain (Foissner 1991) or acridine orange fluorescence stain (Coats and Heinbokel 1982).

Morphological criteria are used to determine ciliate affinities. Features used in taxonomy include, for example, the presence of complex associations of somatic cilia, of few or many oral structures, of loricae, and of stalks (see Curds 1982; Curds et al. 1983; Foissner et al. 1994, 1999; Lynn 2008; Lynn and Small 2002). Species can be determined by mating experiments: mating incompatibility is used as the criterion for a biological species (Lynn and Doerder in Collins 2012; Nanney and McCoy 1976 in Nanney 1980; Sonneborn 1975 in Nanney 1980), but because controlled conjugation is available for so few species, mating tests are not extensively used to identify species. If no mating test organisms exist, quantitative differences can be sought among clones, strains, or species, using multivariate morphometric procedures (for example, Gates 1977; Lynn and Malcolm 1983).

Electron Microscopy. Both scanning and transmission electron microscopy have been used to study ciliates. Preparation of most specimens for scanning electron microscopy is now standardized (Foissner 1991). Preparation of specimens for transmission electron microscopy, especially fixation, is quite varied: for specific methods see references in Lynn (2008).

Features revealed by transmission electron microscopy distinguish between higher taxa. The kinetid – the kinetosome and its microtubular and fibrillar associates – is the fundamental unit of the ciliate cortex. Kinetids of the somatic and oral cortex can be distinguished in the same ciliate (Lynn 2008). The structure and arrangements of the kinetids distinguish one taxon from another (Fig. 7). Clustering techniques have been used on large data sets of ultrastructural characters to determine relationships between taxa (Lynn 1979 in de Puytorac et al. 1984).

Biochemical and Molecular Genetic Techniques. Biochemical criteria restricted to easily cultured, oligohymenophorean ciliates also have been employed to determine relationships among ciliate taxa. DNA hybridization has been used with *Tetrahymena* species (Allen and Li 1974 in Nanney 1980); starch gel electrophoresis of isozymes has been used with species of *Tetrahymena* (Borden et al. 1977 in Nanney 1980) and *Paramecium* (Allen et al. 1983); polyacrylamide gel electrophoresis of cytoskeletal proteins has been used with species of *Tetrahymena* (Vaudaux et al. 1977 in Nanney 1980). However, with the invention of the polymerase chain reaction (PCR), it is now possible, even from single cells, to amplify genes that have been used in various ways to identify species: randomly amplified polymorphic DNAs (RAPDs) have been used to identify species of *Paramecium* (Stoeck et al. 1998 in Lynn 2008) and *Euplotes* (Chen et al. 2001 in Lynn 2008).

A more recent approach is to use mitochondrial genes as "barcodes." The mitochondrial cytochrome *c* oxidase subunit 1 (cox1) "barcode" can identify putative biological species of *Paramecium* (Barth et al. 2006 in Lynn 2008) and

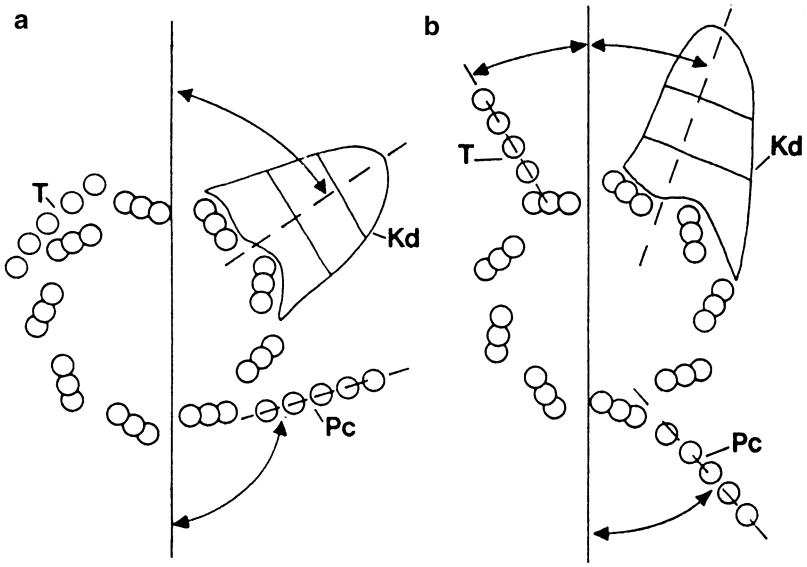

Fig. 7 Schematic figures of two ciliate kinetids illustrate the kinds of characteristics that can be recorded and quantified in comparative studies of cortical ultrastructure. The *central solid line* indicates the longitudinal axis of the kinety. The numbers of microtubules in the transverse (*T*) and postciliary ribbons (*Pc*) can be counted. The relationship of the transverse ribbon to the kinetosome perimeter can be either tangential (**a**) or radial (**b**). With all the radially oriented components, the angle with respect to the kinety axis can be measured and used either quantitatively or semiquantitatively. *Kd* kinetodesmal fibril (After Lynn 1981)

Tetrahymena (Kher et al. 2011). The cox1 barcode is broadly applicable to ciliates (Strüder-Kypke and Lynn 2010) and has been used to infer the existence of cryptic species in genera where mating crosses have not yet been used (Gentekaki and Lynn 2012; Guggiari and Peck 2008). The mitochondrial small subunit rDNA and the apocytochrome b gene also show promise as barcodes to identify cryptic species (Barth et al. 2008; Katz et al. 2011).

Phylogenomic studies are now possible with the annotation of genomes of *Tetrahymena* (Coyne et al. 2008) and *Paramecium* (Arnaiz and Sperling 2011). Gentekaki et al. (2014) published the first phylogenomic analysis of ciliates, using these published genomes along with RNA sequence libraries for representative marine species (see Marine Microbial Eukaryote Transcriptome Sequencing Project – marinemcroeukaryotes.org).

Classification

A Classification Scheme. Several revisions of the classification of the Phylum CILIOPHORA have been presented in the last 15 years: de Puytorac (1994) edited a collaborative revision to genus level with French colleagues; Jankowski (2007) has

presented a revision down to genus; and Lynn (2008) has presented a revision to family level but listing the included genera. These classifications have differences that are discussed by Lynn (2008), and a revised classification is described below, characterizing the major groups only (Table 2).

The somatic kinetid, the most highly conserved structural component of the cortex, is used as a major criterion for this taxonomic scheme, but this is now supplemented by gene sequence data (Fig. 8) (Lynn 1981, 2008). Oral kinetids and their arrangements, which are less conserved, are used to assess more recent common ancestry of the taxa already related by similarities in the somatic kinetid structure. The detailed structure of the somatic kinetid is conserved in many groups and is a more important criterion of relatedness than the number. The total number of kinetids is extremely variable. Mono- and dikinetids can occur in the same subclass or even in the same ciliate cortex, indicating that relatively small heritable changes can change the total number of kinetids, which is a less conservative feature than the number of kinetosomes per kinetid, which in turn is less conserved than the pattern and structure of the kinetid itself (Lynn 2008). Features of the somatic kinetid and the somatic cortex are used to characterize the 11 major groups, called classes by Lynn (2008) (Fig. 9). Orsi et al. (2012) proposed a 12th class, but its independence needs confirmation (cf. Fig. 8). For a more detailed description of the taxa to the family level, see Lynn (2008).

The Major Groups. Several "representative" genera from each class will be use here to illustrate diversity within each class. Because there is so much diversity among genera and because even within a species form and size may change quite dramatically, the term "representative" is an over-generalization.

The first two groups – karyorelicteans and heterotricheans, united primarily by similarities in the somatic kinetids (Fig. 9) and cortex – are placed together in the POSTCILIODESMATOPHORA (Table 2). Many postciliodesmatophorans are highly contractile, possessing similar, presumably homologous, contractile fibrous cytoplasmic structures – the filamentous myonemes, which shorten the cells. The overlapping postciliary microtubular ribbons – the postciliodesmata – extend the cells using microtubule arms that enable sliding of the ribbons on each other and so elongate the cell (Huang and Pitelka 1973 in Lynn 2008).

POSTCILIODESMATOPHORA Gerassimova and Seravin 1976. These ciliates have somatic kinetids whose postciliary microtubular ribbons overlap to form a complex of microtubules that are interconnected by arms – the postciliodesmata.

KARYORELICTEA Corliss 1974. Karyorelicteans (Fig. 10) are thought to represent the ancestral stock of the phylum (see "Evolutionary History", "Fossil Record" and "Phylogeny"). Karyorelicteans possess kinetids with conspicuous kinetodesmal fibrils and postciliary ribbons that overlap to form postciliodesmata (Fig. 9). Their cells contain two to many macronuclei with approximately the micronuclear (diploid) amount of DNA. Their macronuclei arise only by division of micronuclei at the time of cell division. These ciliates are common in estuarine or marine benthic environments.

HETEROTRICHEA Stein 1859. Heterotricheans, because of the similarities of their somatic kinetids (Fig. 9), are thought to have descended from karyorelictean-

Table 2 Classification of the phylum CILIOPHORA[a]

Phylum CILIOPHORA Doflein, 1901
•*POSTCILIODESMATOPHORA* Gerassimova & Seravin, 1976
••**KARYORELICTEA** Corliss, 1974
•••Protostomatida Small & Lynn, 1985
•••Loxodida Jankowski, 1978
•••Protoheterotrichida Nouzarède, 1977
••**HETEROTRICHEA** Stein, 1859
•••Heterotrichida Stein, 1859
•*INTRAMACRONUCLEATA* Lynn, 1996
••**CARIACOTRICHEA** Orsi et al. 2012
••**SPIROTRICHEA** Bütschli, 1889
•••Protocruziidia de Puytorac, Grain & Mignot, 1987
•••Phacodiniidia Small & Lynn, 1985
•••Protohypotrichia Shi et al., 1999
•••Licnophoria Corliss, 1957
•••Euplotia Jankowski, 1979
•••Choreotrichia Small & Lynn, 1985
•••Hypotrichia Stein, 1859
•••Oligotrichia Bütschli, 1887
••**ARMOPHOREA** Jankowski, 1964[b]
•••Armophorida Jankowksi, 1964
•••Clevelandellida de Puytorac & Grain, 1976
••**LITOSTOMATEA** Small & Lynn 1981
•••Haptoria Corliss, 1974
••••Haptorida Corliss, 1974
••••Pleurostomatida Schewiakoff, 1896
••••Cyclotrichiida Jankowski, 1980 *incertae sedis*
•••Rhynchostomatia Jankowski, 1980
•••Trichostomatia Bütschli, 1889
••••Vestibuliferida de Puytorac et al., 1974
••••Entodiniomorphida Reichenow in Doflein & Reichenow, 1929
••••Macropodiniida Lynn, 2008[b]
••***CONTHREEP*** Lynn in Adl et al., 2012[c]
•••**PHYLLOPHARYNGEA** de Puytorac et al., 1974
•••Synhymenia de Puytorac et al. in Deroux, 1978
•••Cyrtophoria Fauré-Fremiet in Corliss, 1956
••••Chlamydodontida Deroux, 1976
••••Dysteriida Deroux, 1976
•••Chonotrichia Wallengren, 1895
••••Exogemmida Jankowski, 1972
••••Cryptogemmida Jankowski, 1975
•••Rhynchodia Chatton & Lwoff, 1939
••••Hypocomatida Deroux, 1976
••••Rhynchodida Chatton & Lwoff, 1939
•••Suctoria Claparède & Lachmann, 1858
••••Exogenida Collin, 1912
••••Endogenida Collin, 1912
••••Evaginogenida Jankowski in Corliss 1979
•••**NASSOPHOREA** Small & Lynn 1981
••••Nassulida Jankowski, 1967
••••Microthoracida Jankowski, 1967
••••Colpodidiida Foissner, Agatha & Berger, 2002 *incertae sedis*

(*continued*)

Table 2 (continued)

•••**COLPODEA** Small & Lynn 1981
••••Platyophryida de Puytorac et al., 1979
••••Bursariomorphida Fernández-Galiano, 1978
••••Colpodida de Puytorac et al., 1974
••••Cyrtolophosidida Foissner, 1978
•••**PROSTOMATEA** Schewiakoff, 1896
••••Prostomatida Schewiakoff, 1896
••••Prorodontida Corliss, 1974
•••**PLAGIOPYLEA** Small & Lynn, 1985[b]
••••Plagiopylida Small & Lynn, 1985
••••Odontostomatida Sawaya, 1940 *incertae sedis*
•••**OLIGOHYMENOPHOREA** de Puytorac et al., 1974
••••Peniculia Fauré-Fremiet in Corliss, 1956
••••Scuticociliatia Small, 1967
••••Hymenostomatia Delage & Hérouard, 1896
••••Apostomatia Chatton & Lwoff, 1928
••••Peritrichia Stein, 1859[d]
••••Astomatia Schewiakoff, 1896

[a]Refer to Lynn (2008) for diagnoses of these taxa and a more complete listing of included families and genera

[b]This taxon, a so-called "ribo-class/group," is based on molecular phylogenetics, primarily the SSUrRNA gene but still lacks a morphological synapomorphy

[c]This taxon is another "ribo-class/group," based on molecular phylogenetics of multiple genes (Lynn 2008) but still lacks a morphological synapomorphy. Its name derives from the major included groups (i.e., COLPODEA, OLIGOHYMENOPHOREA, NASSOPHOREA, PROSTOMATEA, PLAGIOPYLEA, PHYLLOPHARYNGEA) and should be pronounced CON-3-P

[d]The Peritrichia are traditionally divided into the Sessilida and Mobilida. Recent molecular phylogenomic analyses have confirmed the monophyly of this group (Gentekaki et al. 2017)

like ancestors (Fig. 10). Heterotrichs have kinetids with postciliary ribbons that overlap to form postciliodesmata and weakly developed kinetodesmal fibrils that often extend slightly posteriorly. The left oral ciliature usually consists of a series of oral polykinetids numerous enough to form a spiral extending out of the oral cavity onto the cell surface (Fig. 10). Their polygenomic macronuclei are capable of division using microtubular bundles that form *outside* the macronuclear envelope – *extramacronuclear* microtubules. This kind of macronuclear division is thought to have arisen independently of that exhibited by the majority of ciliates (see below INTRAMACRONUCLEATA; Lynn 2008). Heterotricheans are found in all habitats described above ("Habitats and Ecology, Habitats").

INTRAMACRONUCLEATA Lynn 1996. The other major division of ciliates is strongly supported by gene sequence data (Fig. 8) (Lynn 2008). There is at present only one significant morphological feature that appears to unite these ciliates: the division of the macronucleus by *intramacronuclear* microtubules – hence the name. Lynn (2008) speculated that a molecular genetic character may ultimately be found that supports this subdivision of the phylum. The remaining major groups are considered intramacronucleates.

SPIROTRICHEA Bütschli 1889. Spirotricheans are a morphologically and genetically diverse class (Figs. 8, 11 and 12). With the exception of *Protocruzia* and *Phacodinium*, replication bands are the specialized morphological feature that accompanies DNA replication in these ciliates (Lynn 2008). *Phacodinium* may have lost this structure as it is placed *within* the spirotrich clade (Fig. 8). On the other hand, *Protocruzia* has a quite unusual macronuclear structure, is only weakly associated with other spirotrichs based on gene sequences (Fig. 8) (Gentekaki

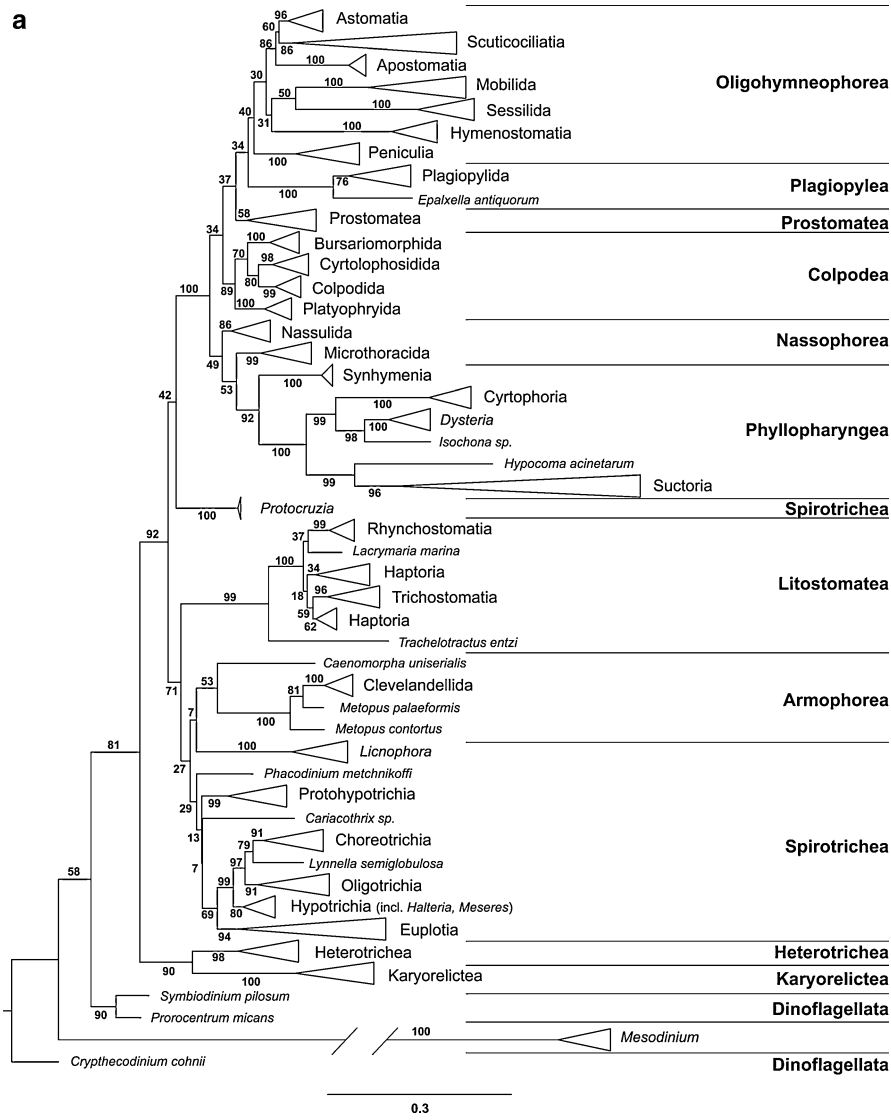

Fig. 8 (continued)

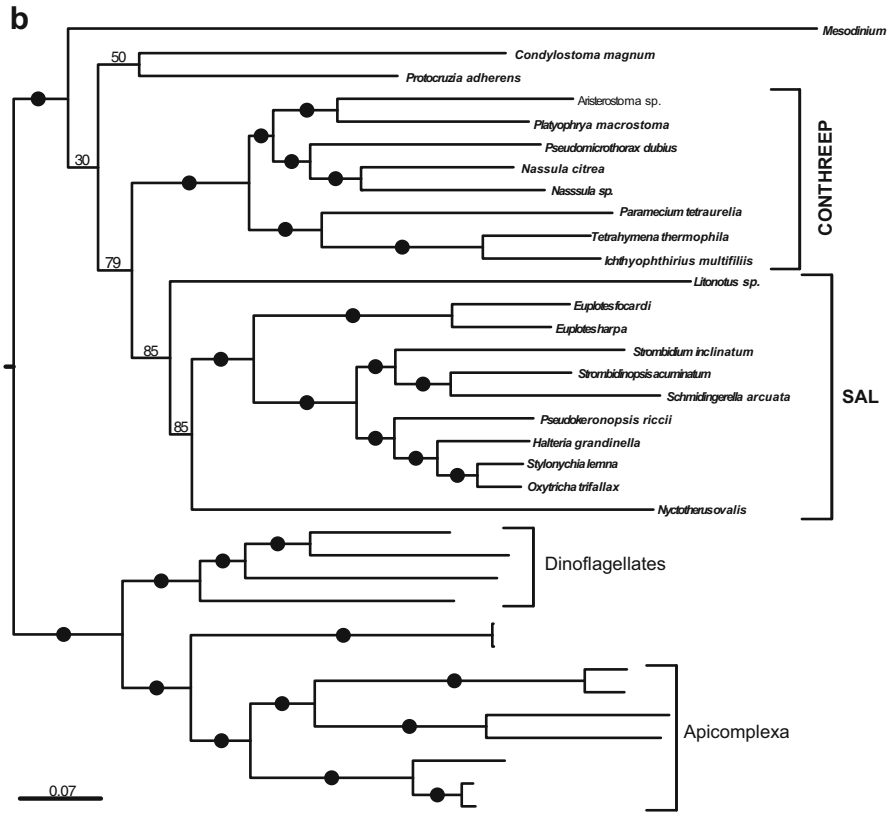

Fig. 8 Phylogenies of the ciliated protozoa based on small subunit rRNA gene sequences (**a**) and on a phylogenomic analysis (**b**). (**a**) A maximum likelihood tree inferred from small subunit rRNA gene data of ciliate species representative of the different classes. The POSTCILIODESMATOPHORA, which includes the Karyorelictea and Heterotrichea, is sister to the INTRAMACRONUCLEATA, which includes the remaining nine major groups. A new class, the Cariacotrichea including *Cariacothrix*, has been suggested by Orsi et al. (2012), but here it is embedded within the Spirotrichea. Dr. Michaela Strüder-Kypke derived this phylogeny using PhyML 3.0 with the GTR (General-Time-Reversible) model with gamma distribution and an estimate of invariable sites. The numbers at the nodes represent the support values for the maximum likelihood analysis. The scale bar represents 30 substitutions per 100 nucleotides. (**b**) A maximum likelihood tree constructed by RAxML using the LG model with empirical frequencies and gamma distribution based on a concatenated alignment of ~120 genes. The *black circles* denote 100% bootstrap support for 1,000 bootstraps (Lynn and Kolisko, unpublished)

et al. 2014), and may in fact represent the type of a new monotypic class of ciliates (Gao et al. 2016; Li et al. 2010). Spirotrichs like *Stylonychia* (Fig. 11) and *Euplotes* (Fig. 12) are typically benthic while *Halteria* (Fig. 11), *Strombidinopsis*, *Tintinnopsis*, *Cymatocylis*, and *Limnostrombidium* (Fig. 12) are typically planktonic, in both marine and freshwater habitats.

The first three groups appear to form the SAL clade, for the first letter in the name of each included group (Fig. 8) (Gentekaki et al. 2014), while the ARMOPHOREA

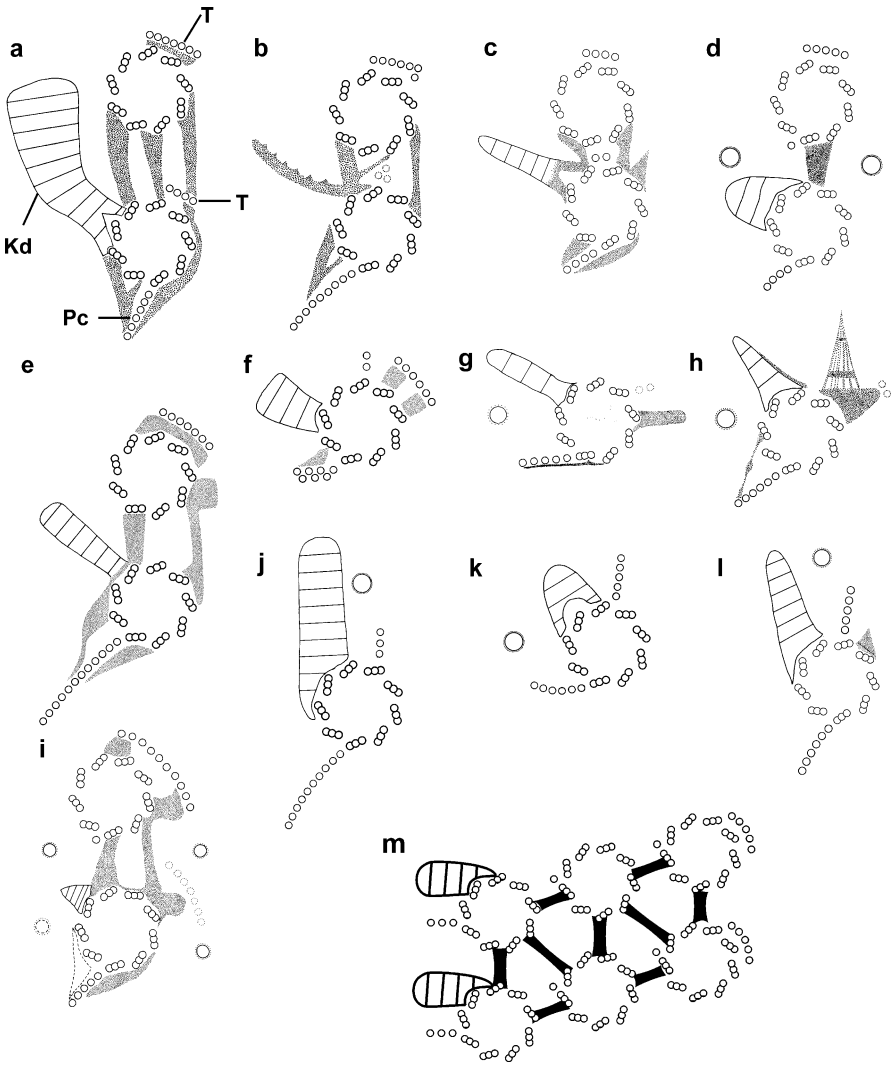

Fig. 9 Schematics of somatic kinetids of genera representative of each major group in the Phylum CILIOPHORA. (**a**) *Loxodes* – KARYORELICTEA; (**b**) *Blepharisma* – HETEROTRICHEA; (**c**, **d**) *Protocruzia* (**c**), *Euplotes* (**d**) – SPIROTRICHEA; (**e**) *Metopus* – ARMOPHOREA; (**f**) *Balantidium* – LITOSTOMATEA; (**g**) *Chilodonella* – PHYLLOPHARYNGEA; (**h**) *Obertrumia* – NASSOPHOREA; (**i**) *Colpoda* – COLPODEA; (**j**) *Plagiopyla* – PLAGIOPYLEA; (**k**) *Holophrya* – PROSTOMATEA; (**l**) *Tetrahymena* – OLIGOHYMENOPHOREA; (**m**) *Plagiotoma* – SPIROTRICHEA. *Kd* kinetodesmal fibril, *Pc* postciliary microtubular ribbon, *T* transverse microtubular ribbon

and LITOSTOMATEA have been proposed to be related based on the lamella-like arrangement of postciliary ribbons that underly the somatic cortex, the so-called Lamellicorticata (Vd'ačný et al. 2010; Vd'ačný et al. 2012).

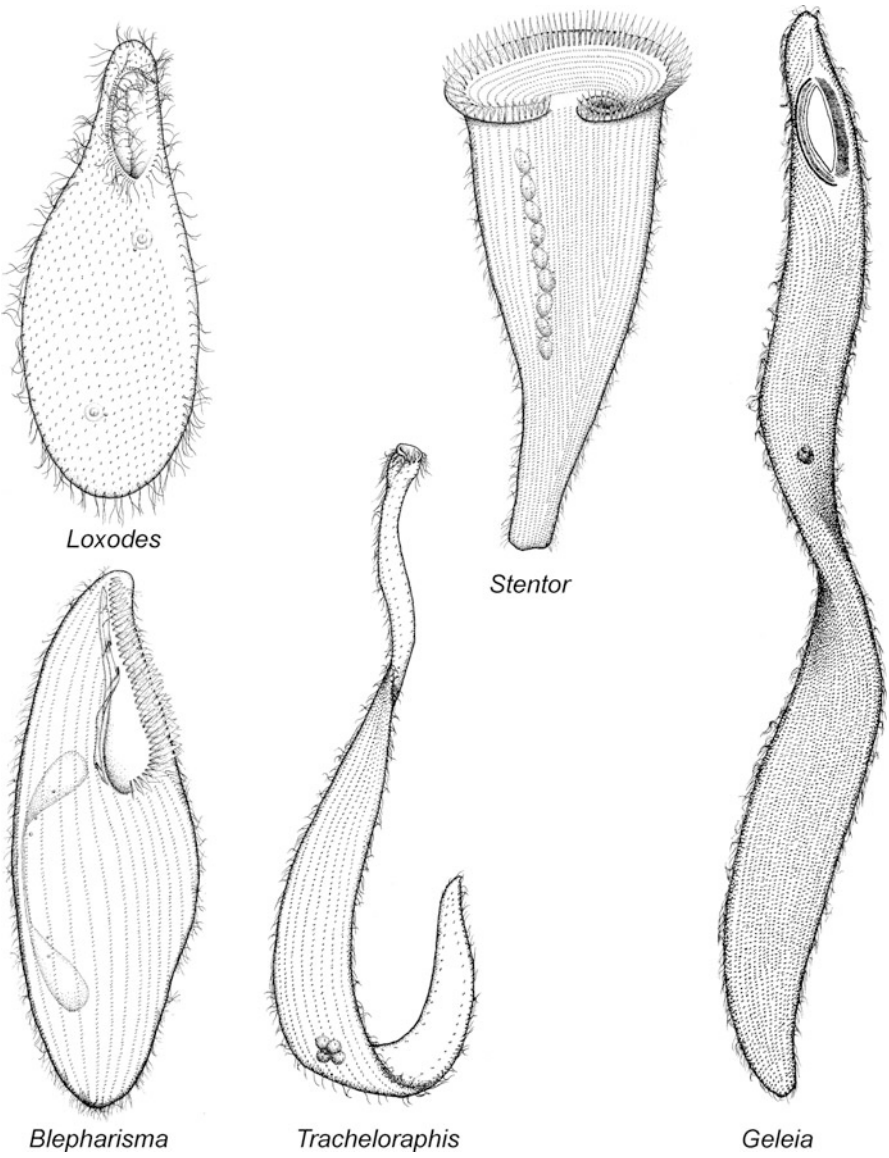

Fig. 10 Representative genera of the POSTCILIODESMATOPHORA. KARYORELICTEA. *Loxodes*, *Tracheloraphis*, and *Geleia*. HETEROTRICHEA. *Blepharisma* and *Stentor*

ARMOPHOREA Lynn 2002. Armophoreans represent one of what have been called "ribo-classes" of ciliates since they were identified as a monophyletic group only by gene sequence data (Fig. 8). In fact, like the spirotricheans, armophoreans are morphologically diverse, both at the cell level *and* at the somatic kinetid level (Figs. 9 and 13). Thus, they can be viewed as "an exception that proves the rule" that

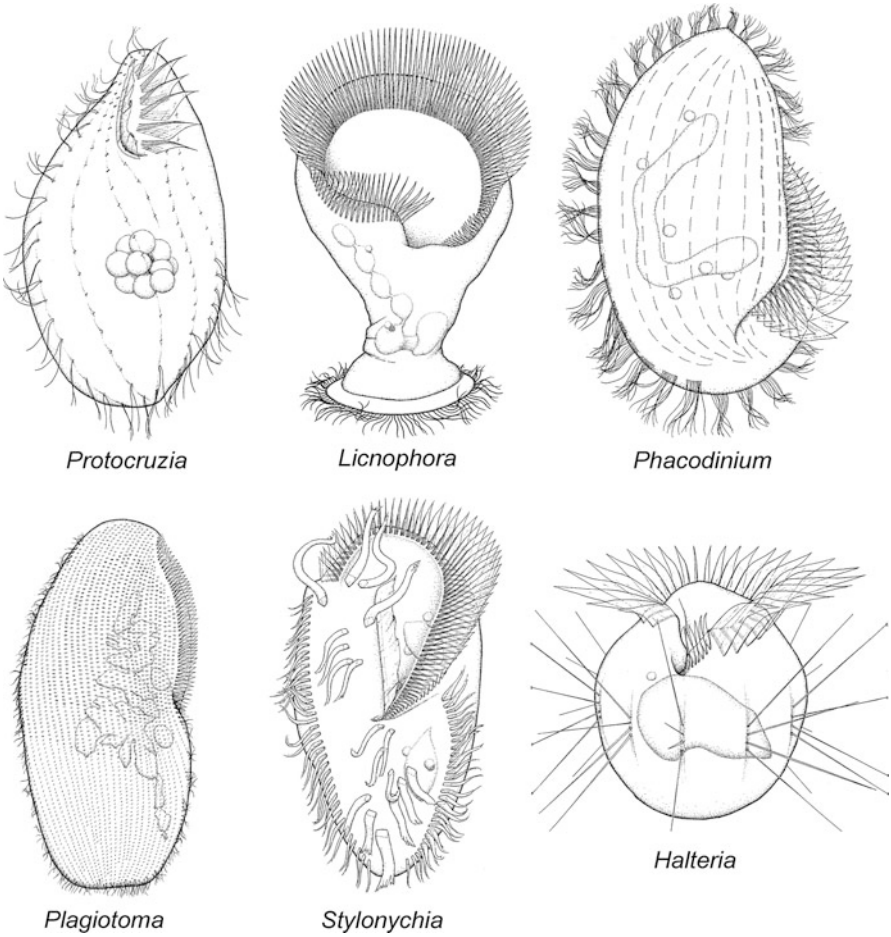

Fig. 11 Representative genera of the SPIROTRICHEA. Protocruzidiidia. *Protocruzia*. Licnophoria. *Licnophora*. Phacodiniidia. *Phacodinium*. Hypotrichia. *Plagiotoma*, *Stylonychia*, and *Halteria*

somatic kinetids generally reflect larger assemblages within the phylum. Armophoreans are typically found in anoxic freshwater and marine habitats, and the gene sequence data may ultimately be corroborated by physiological and biochemical characters since these ciliates all have hydrogenosomes rather than mitochondria. The clevelandellid armophoreans are all intestinal endosymbionts in invertebrates, such as millipedes and cockroachs, and in vertebrates, such as frogs.

LITOSTOMATEA Small and Lynn 1981. The litostomates (Fig. 14) include three rather diverse subgroups of ciliates: the Rhynchostomatia, carnivorous ciliates that use a proboscis for hunting; the Haptoria, principally free-living, carnivorous ciliates; and the Trichostomatia, principally endosymbionts of birds and mammals. These groups have somatic monokinetids with two transverse ribbons – a tangential

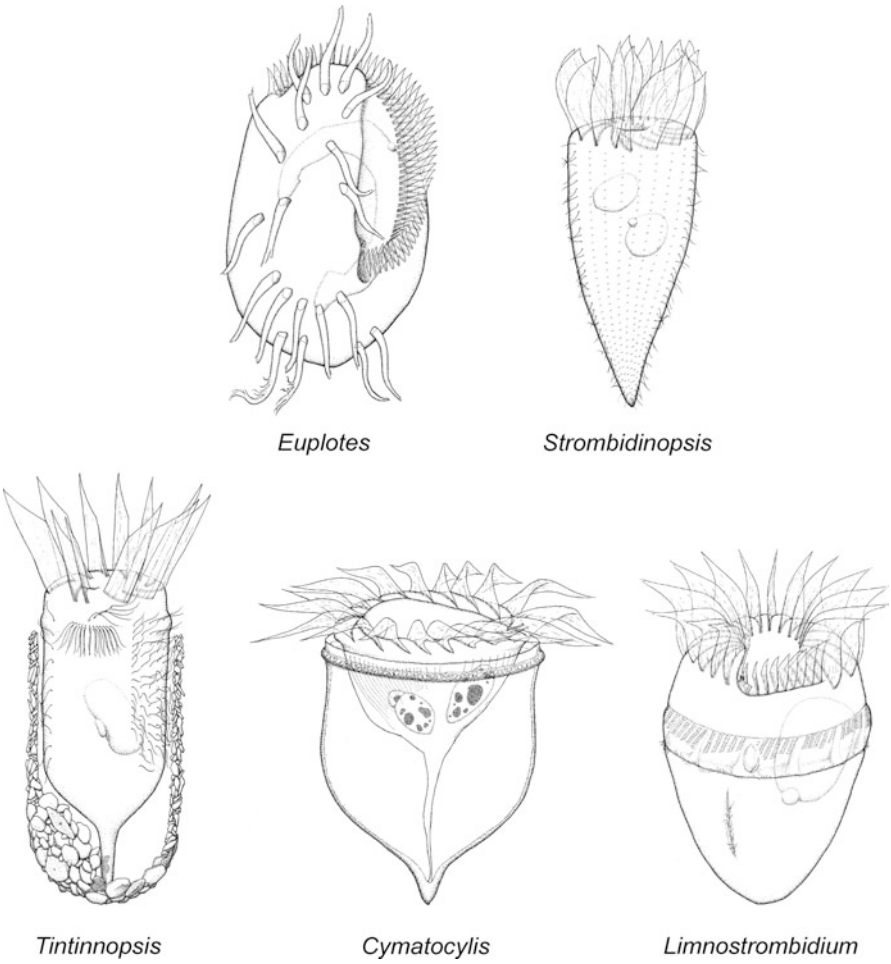

Fig. 12 Representative genera of the SPIROTRICHEA. Euplotia. *Euplotes*. Choreotrichia. *Strombidinopsis*, the tintinnids *Tintinnopsis* and *Cymatocylis*. Oligotrichia. *Limnostrombidium*

one and a radial one, a short, almost laterally directed kinetodesmal fibril, and convergent postciliary microtubules (Fig. 9).

The last six major groups, phyllopharygeans, nassophoreans, colpodeans, prostomateans, plagiopyleans, and oligohymenophoreans are often linked together in molecular phylogenies (Fig. 8) (Lynn 2008). Even though there is no obvious strong unifying morphological feature for this group, it has been named CONTHREEP (pronounced CON-3-P), based on the first letter of the names of the groups that are robustly clustered by genetic features.

PHYLLOPHARYNGEA de Puytorac et al. 1974. Phyllopharyngeans, like the spirotrichs, are a morphologically diverse clade (Fig. 15). However, all have somatic monokinetids with a short, laterally directed kinetodesmal fibril and a

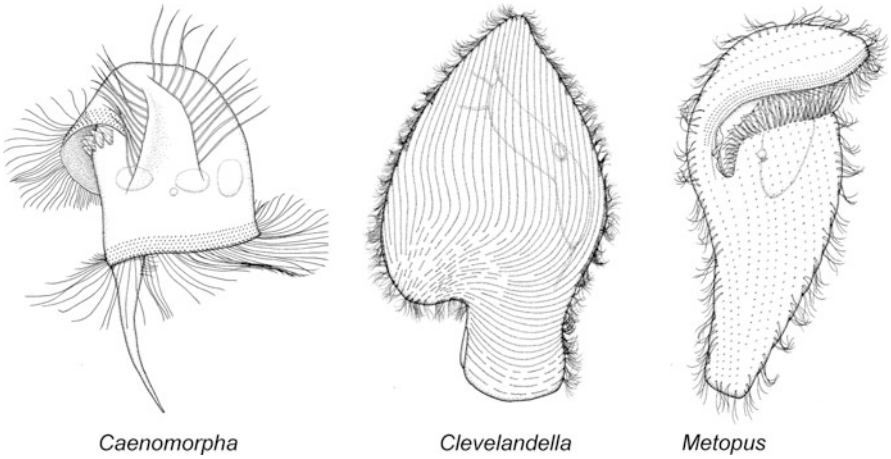

Fig. 13 Representative genera of the ARMOPHOREA. Armophorida. *Caenomorpha* and *Metopus*. Clevelandellida. *Clevelandella*

poorly developed or absent transverse microtubular ribbon accompanied by a fibrous support (Fig. 9). Their postciliary microtubules originate from the kinetosome in a convergent position; subkinetal microtubules course beneath the kinetosomes of a somatic kinety; and the cytopharynx is surrounded by radially arranged microtubular ribbons called phyllae. These ciliates are primarily benthic: chonotrichs and suctoria are sessile on nonbiological surfaces or other organisms. Some are ciliated only during dispersal: for example, the suctorians *Discophrya* and *Acineta* (Fig. 15).

NASSOPHOREA Small and Lynn 1981. The nassophoreans have singly or doubly ciliated somatic kinetids (Fig. 9). Somatic dikinetids have an anterior kinetosome with a tangential transverse ribbon and a posterior kinetosome with an anteriorly directed kinetodesmal fibril, a divergent postciliary ribbon, and often a tangential transverse ribbon. Somatic alveoli are well developed. The cytopharyngeal apparatus, which is similar to that of cyrtophorine phyllopharyngeans, is the cyrtos, a complex microtubular "basket" that functions in ingestion (Tucker 1978). Simple "oral" polykinetids are found adjacent to the cytostome or extending in bands of varying length across the body (Fig. 16). The nassophoreans are commonly free-living, benthic ciliates typically found in fresh, brackish, and salt water, typically feeding on filamentous cyanobacteria.

COLPODEA de Puytorac et al. 1974. The somatic kinetid of colpodeans is strikingly unique (Fig. 9). Colpodeans have dikinetids with a short, laterally directed kinetodesmal fibril and a set of prominent overlapping transverse microtubular ribbons that arise from the posterior kinetosome. Colpodeans clearly demonstrate the range in variability of oral structures while the somatic structures remain invariable (Lynn 2008) (Fig. 17). These ciliates are commonly found in temporary freshwater or soil habitats where they encyst when the environment dries.

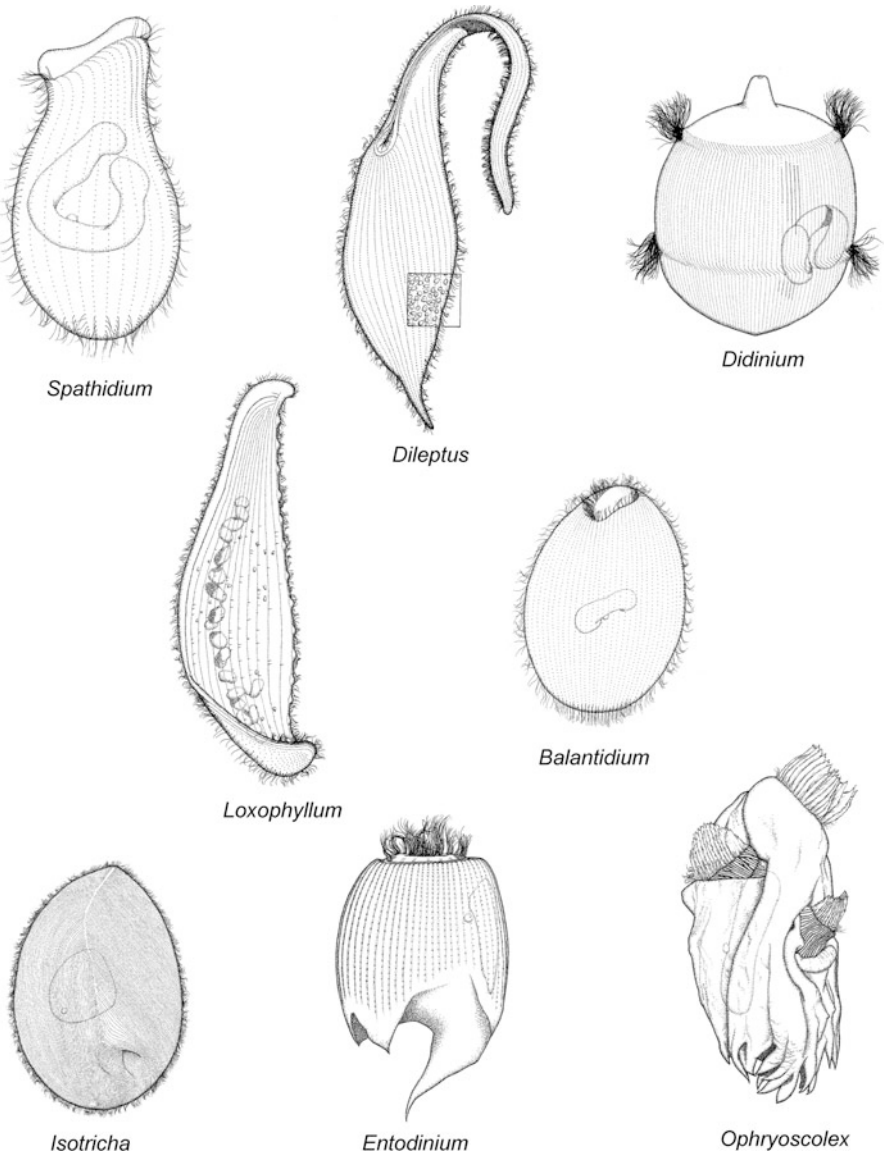

Fig. 14 Representative genera of the LITOSTOMATEA. Rhynchostomatia. *Dileptus*. Haptoria. *Spathidium*, *Didinium*, *Loxophyllum*. Trichostomatia. *Balantidium*, *Isotricha*, *Entodinium*, and *Ophryoscolex*

PROSTOMATEA Schewiakoff 1896. The distinguishing features of the prostomateans include somatic monokinetids with probably a radial transverse ribbon and well-developed, right-anteriorly directed kinetodesmal fibrils (Fig. 9), and the perimeter of the oral area supported by oral dikinetid postciliary ribbons that,

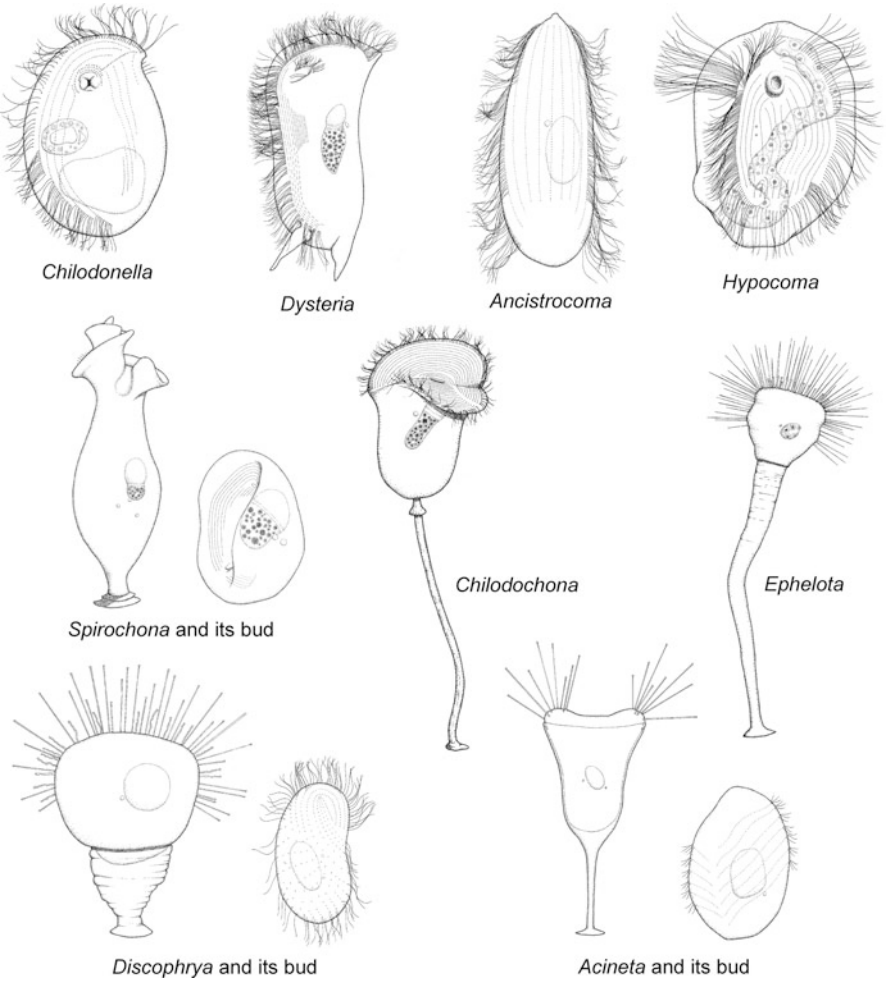

Fig. 15 Representative genera of the PHYLLOPHARYNGEA. Cyrtophoria. *Chilodonella* and *Dysteria*. Chonotrichia. *Chilodochona* and *Spirochona* and its bud. Rhynchodia. *Ancistrocoma* and *Hypocoma*. Suctoria. *Discophrya* and its bud, *Acineta* and its bud, and *Ephelota*. Note how the pattern of the somatic ciliature in the buds of chonotrichs and suctorians resembles that of the cyrtophorians

at least in *Urotricha*, extend from each dikinetid to overlap each other in a circular microtubular band. The prostomateans (Fig. 18) apparently evolved toxicysts independently from the litostomateans, since members of both classes have these organelles. Prostomateans are found in a wide variety of habitats; commonly, they are benthic and most species are marine.

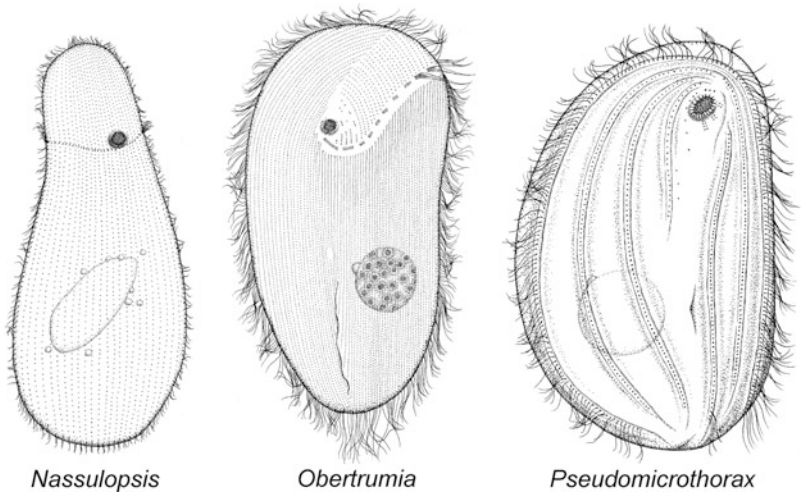

Fig. 16 Representative genera of the NASSOPHOREA. Nassulida. *Nassulopsis* and *Obertrumia*. Microthoracida. *Pseudomicrothorax*

PLAGIOPYLEA Small and Lynn 1985. Like the ARMOPHOREA, the plagiopyleans represent a "ribo-class" as they were recognized as a monophyletic unit based *only* on the sequences of the small subunit rRNA gene (Stoeck et al. 2007 in Lynn 2008). Two groups are now placed in this class – the plagiopylids and odontostomatids, but they are morphologically extremely dissimilar (Fig. 19). Like armophoreans, plagiopyleans are typically inhabitants of anoxic freshwater and marine habitats and have hydrogenosomes, which can be associated with intracellular methanogenic bacteria or with extracellular ectosymbiotic methanogens that increase the ciliate's metabolic efficiency in these habitats. Thus, a unifying biochemical or physiological feature may eventually be discovered to explain this assemblage.

OLIGOHYMENOPHOREA de Puytorac et al. 1974. Oligohymenophoreans are a diverse group morphologically characterized as having a paroral on the right side of the cytostome and typically three oral polykinetids on the left, although their cellular form is quite varied (Figs. 20 and 21). Their somatic kinetids have a *radially* oriented transverse ribbon associated with the posterior kinetosome of a dikinetid or with the monokinetid kinetosome, but the peniculines, such as *Paramecium*, are an exception to this rule (Fig. 9). Postciliary microtubules are divergent and are directed posteriorly, whereas the kinetodesmal fibril associated with the posterior kinetosome of a dikinetid is anteriorly directed and strongly overlapping. The Peritrichia, such as *Vorticella* (Fig. 20), are adapted to sessility and lack somatic kinetids but are related to the other oligohymenophoreans by the pattern of division morphogenesis (i.e., the structure and formation of the oral region). Oligohymenophorean ciliates are common in all habitats described (see above "Habitats and Ecology, Habitats").

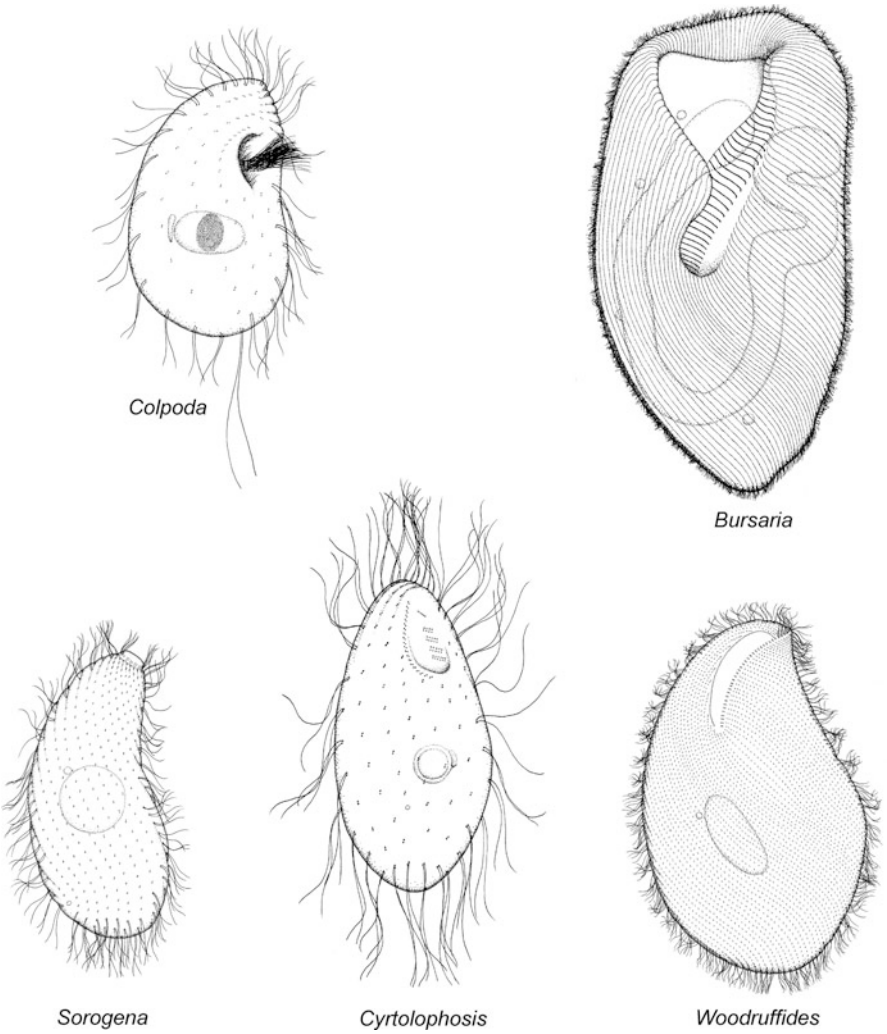

Fig. 17 Representative genera of the COLPODEA. Colpodida. *Colpoda*. Bursariomorphida. *Bursaria*. Sorogenida. *Sorogena*. Cyrtolophosidida. *Cyrtolophosis* and *Woodruffides*

Maintenance and Cultivation

Collection and Isolation from Nature

Collection. Procedures for collecting ciliates vary depending upon the habitat in which the organisms are found (see "Habitats and Ecology, Habitats"). Collection methods for benthic habitats and soils have been outlined by Uhlig (1972), Alabouvette et al. (1981), and Acosta-Mercado and Lynn (2003). Planktonic ciliates

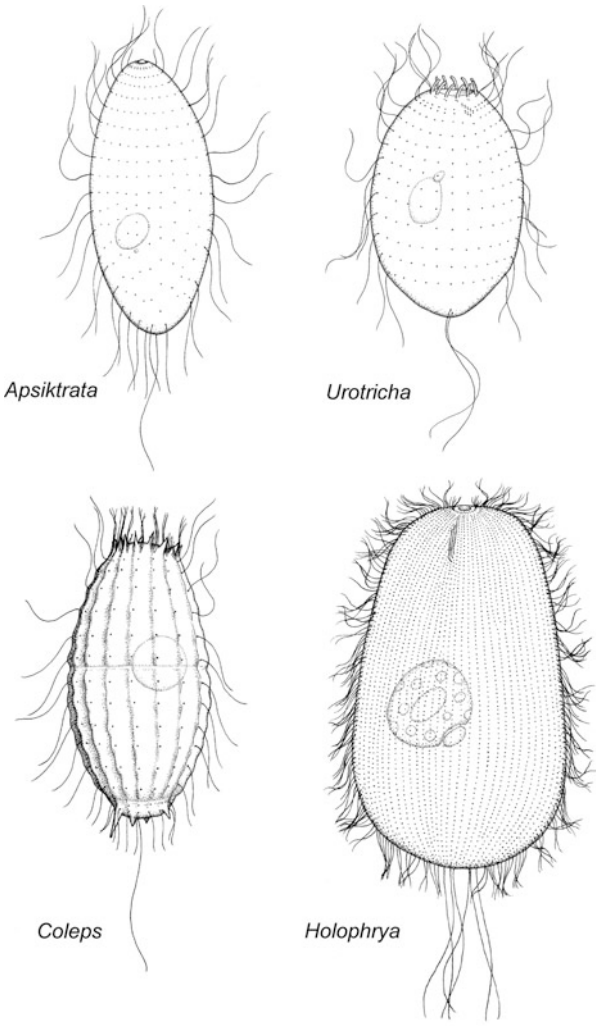

Fig. 18 Representative genera of the PROSTOMATEA. Prostomatida. *Apsiktrata*. Prorodontida. *Urotricha*, *Coleps*, and *Holophrya*

are best collected by using bottles or whole water sampling apparatuses (Montagnes and Lynn 1993).

Enumeration. For enumeration of ciliates, several methods have been published for benthic collections (Dye 1979; Finlay et al. 1979 in Finlay and Ochsenbein-Gattlen 1982; Wickham et al. 2000) and soils (Acosta-Mercado and Lynn 2003). For planktonic collections, counting chambers may be used: the ciliates can be counted alive (Dale and Burkhill 1982) or can be fixed using a concentrated Bouin's fixative and stained using the quantitative protargol stain (Montagnes and Lynn 1993).

Isolation. Field collections can be examined immediately and the species of interest isolated using flame-drawn Pasteur pipettes. Alternatively, the collection can be enriched by the addition of *small* quantities of boiled leaves, seeds, grains,

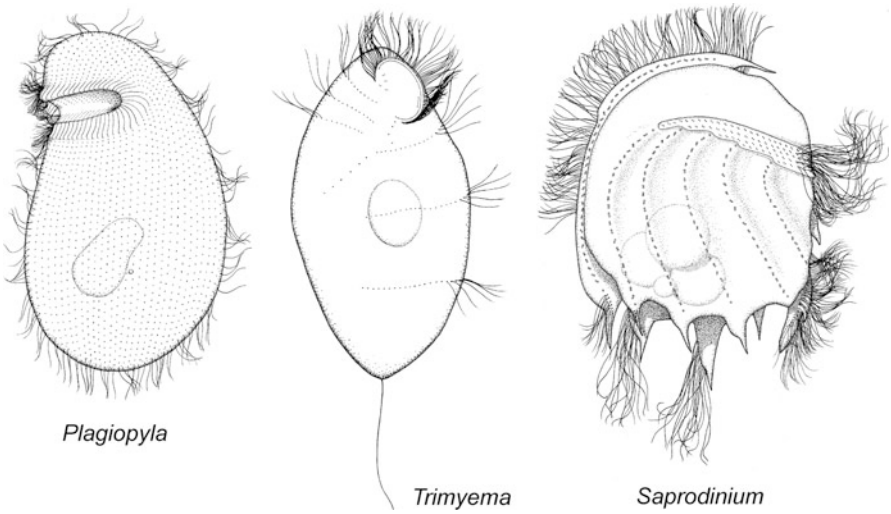

Fig. 19 Representative genera of the PLAGIOPYLEA. Plagiopylida. *Plagiopyla* and *Trimyema*. Odontostomatida. *Saprodinium*

animal tissues, or nutrient media, such as proteose peptone. After a few days, the cultures can be examined and species of interest isolated, usually by hand pipetting single ciliates under a low power microscope (Foissner 1991).

Nyberg (1981) isolated *Tetrahymena* species by enriching field collections with proteose peptone and adding antibiotics to inhibit bacterial growth. Kosinski (1979) described a method of producing axenic cultures by several passages of a bacterized batch culture through sterilized tubes, each transfer being made using sterile hypodermic needles.

Cultivation

Monoxenic Cultures. Many species of ciliates have been grown on a variety of food sources. Observations of what the ciliate of interest feeds upon in nature must be made prior to establishing the organism in culture. The nutritional value of a variety of bacterial species for selected ciliates has been discussed by Dive (1973 in Lynn 2008). There are many variables to consider in order to successfully establish a species in culture (see, for example, Hamilton and Preslan 1969 in Lynn 2008); these become especially complex when culturing planktonic species, such as tintinnids (Gold 1973) or "carnivorous" (ciliativorous) species, that is, ciliates that eat other ciliates.

Axenic Culture. Very few ciliates have been grown in axenic cultures. *Tetrahymena* species were the first, and almost all other axenically grown ciliate species are members of the Class OLIGOHYMENOPHOREA. *Tetrahymena* species are cultured on proteose peptone (Cassidy-Hanley in Collins 2012; Nyberg 1981; Keenan

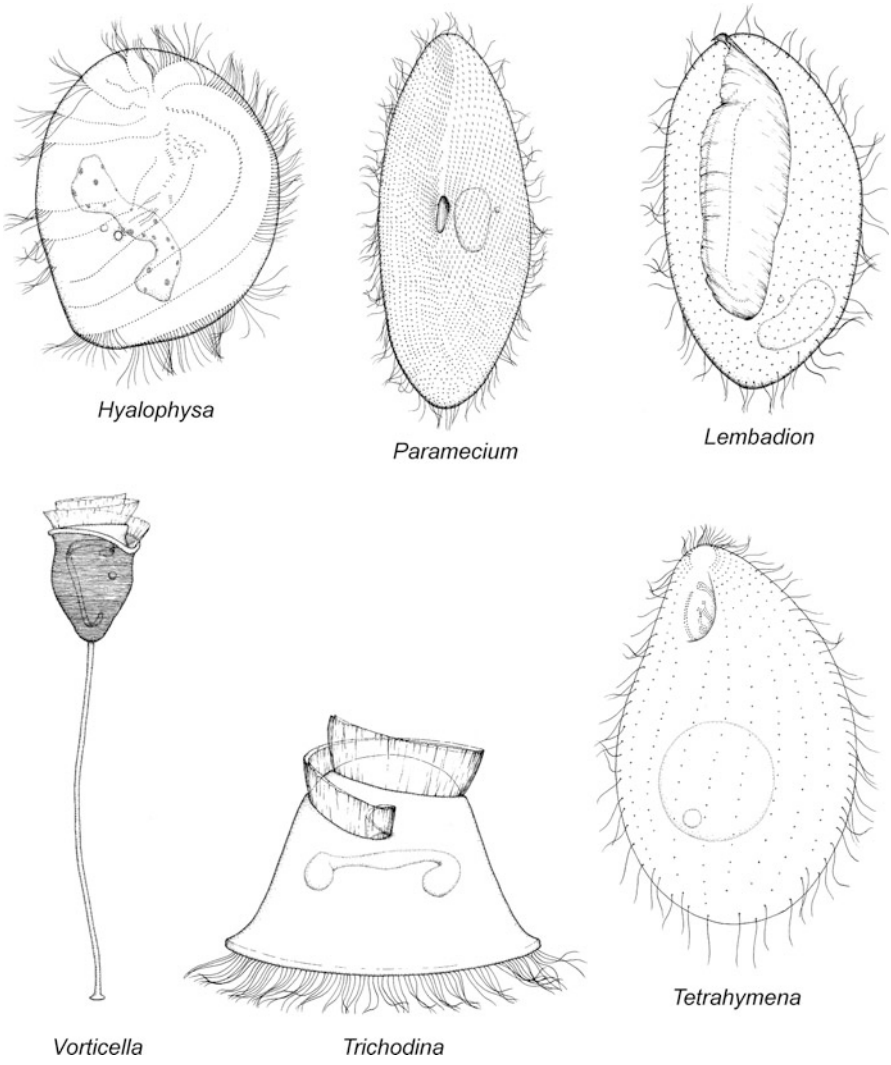

Fig. 20 Representative genera of the OLIGOHYMENOPHOREA. Apostomatia. *Hyalophysa*. Peniculia. *Paramecium* and *Lembadion*. Peritrichia. *Vorticella* and *Trichodina*. Hymenostomatia. *Tetrahymena*

et al. 1978). *Paramecium* species can also be grown on proteose peptone-trypticase media (Fok and Allen 1979; Soldo et al. 1966 in Fok and Allen 1979). *Uronema* and some related scuticociliates are marine forms that have been successfully cultivated axenically on proteose peptone-trypticase media (Iglesias et al. 2003 in Lynn 2008; Soldo and Merlin 1972 in Soldo et al. 1974).

Large-Scale Cultivation. Large-scale cultivation of *Tetrahymena* and *Paramecium* is accomplished by increasing the volume of axenic medium (Thiele et al.

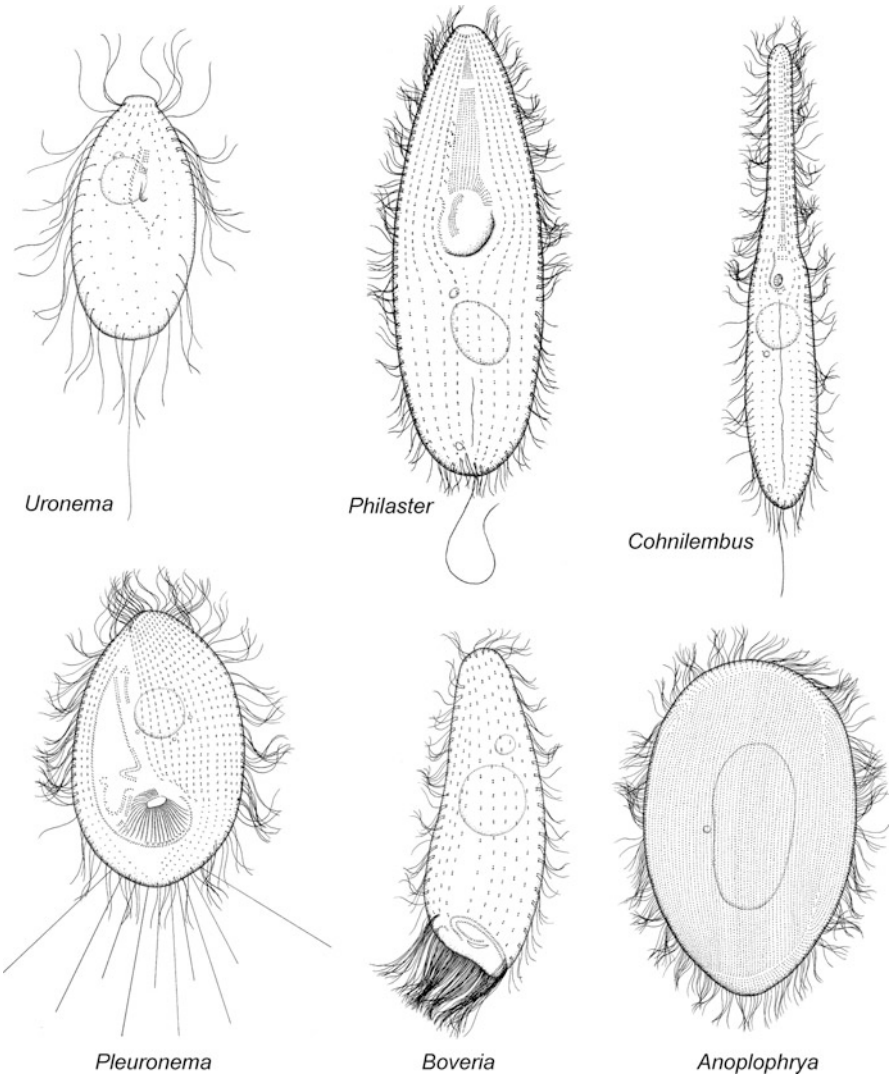

Fig. 21 Representative genera of the OLIGOHYMENOPHOREA cont'd. Scuticociliatia. *Uronema*, *Philaster*, *Cohnilembus*, *Pleuronema*, and *Boveria*. Astomatia. *Anoplophrya*

1980 in Schmidt 1982). Care must be taken that adequate oxygen is provided either by aeration or through having a high surface area to volume ratio in the culture flask.

Large-scale monoxenic cultures must be carefully regulated to ensure maximum growth of the predatory ciliates relative to the food. Several methods have been described for cultivating euplotian and hypotrich ciliates (Laughlin et al. 1983; Schmidt 1982).

Long Term Preservation. Little information exists on methods of long-term preservation of ciliates. A combination of low temperature and cryoprotectant seems to be most successful (Anderson et al. 2009; Cassidy-Hanley in Collins 2012; Krenek and Berendonk 2009). Encystment by some ciliates, such as colpodids, allows for simple, long-term dry storage on vegetation or filter paper.

Culture Media. Media have been devised for the cultivation of a number of species of ciliates. Recipes are listed for two very common media: a medium for bacterized cultures based on an infusion derived from dried cereal grass leaves and a proteose peptone-based medium for axenic culture of some tetrahymenid ciliates (Tables 3 and 4). Two good sources for recipes of a large number of media are an old publication prepared by the Committee on Cultures, Society of Protozoologists (1958), which gives the appropriate media for a variety of species, and the American Type Culture Collection Catalogue of Strains I (1982), in which the species are cross-referenced to the appropriate medium.

Cultivation of anaerobic ciliates began first with those resident in the rumen (Coleman 1969 in Michalowski et al. 1985). Free-living anaerobes, such as the armophorean *Metopus* (Narayan et al. 2007), the plagiopyleans *Trimyema* (Wagener and Pfennig 1987) and *Plagiopyla* (Fenchel and Finlay 1991b), and scuticociliates (Dyer 1989 in Lynn 2008), have now been isolated from various habitats and cultivated using similar techniques.

Table 3 Recipe for proteose peptone-yeast extract culture medium

5–10 g	Proteose peptone (Difco)
5–10 g	Yeast extract (water soluble portion of autolyzed yeast)
2 g	Glucose
1 l	Distilled water
Add the dry ingredients to the distilled water and heat until they are dissolved. Dispense into culture vessels and autoclave to sterilize	
Note: This medium is useful only for axenic cultivation of some tetrahymenid ciliates	

Table 4 Recipe for culture medium based on extract of cereal grass leaves

Preparation of stock infusion of cereal grass leaves	*Preparation of the culture medium*
50 g powdered, dehydrated cereal grass leaves (Sigma) Calcium carbonate (pinch) 1 l distilled water (or, for marine ciliates, ½ strength sea water) Add the cereal grass leaves to the distilled water and boil for 10 min taking care that the suspension does not boil over. Filter the suspension using a Buchner funnel and bring the filtrate back to the 1l volume. Dispense as 10-ml aliquots in screw cap tubes. Autoclave to sterilize and store in the refrigerator	Dilute the stock infusion into 250–1000 ml of distilled water, sterile pond water, or a dilute salt solution; the more dilute the medium, the less populous the bacteria will be. Ciliates may be transferred from nature to this medium. Alternatively, introduce prey bacteria by inoculating the medium with *Aerobacter aerogenes* or other suitable Gram-negative aerobic bacterial prey. Incubate at room temperature for 6–24 h, and then transfer the ciliates to this medium

Evolutionary History

Fossil Record

Ciliates, being soft-bodied organisms, are poorly represented in the fossil record. A number of species have "hard" parts that have the potential to be preserved: *Coleps* species of the PROSTOMATEA secrete internal calcium carbonate structures; *Loxodes* species of the KARYORELICTEA accumulate barium sulfate as conspicuous intracellular granules (Finlay et al. 1983); a variety of tintinnid species of the choreotrich SPIROTRICHEA secrete loricae to which are agglomerated or attached mineral particles from the water column; or the calcium carbonate tests of coccolithophorids (Tappan and Loeblich 1973). The mineral components of *Coleps* and *Loxodes* may be too small and inconspicuous to be recorded as body fossils. Remarkably, whole cells of *Coleps*, *Paramecium*, and *Colpoda* species have been discovered embedded in fossilized amber over 200 million years old (Martín-González et al. 2008; Schmidt et al. 2006).

The loricas of tintinnids, possibly by partial recrystallization of attached coccolith tests, deposited by the tintinnid when alive, and by secondary growth of calcite crystals, have provided a record of the history of this group of ciliates. The tintinnids apparently reached peak diversity during the Jurassic and Cretaceous (Fig. 22; Tappan and Loeblich 1973). Some have argued that fossil tintinnids might be of Proterozoic origins, but this is highly unlikely (Lipps, Stoeck, and Dunthorn in Dolan et al. 2013). Since fossil genera have been placed in present-day families of tintinnids, very little can be said about the rate and degree of divergence of taxa (Lipps et al. in Dolan et al. 2013; Lynn 2008). Lipps et al. (in Dolan et al. 2013) make a strong case that the calpionellids are likely not tintinnids.

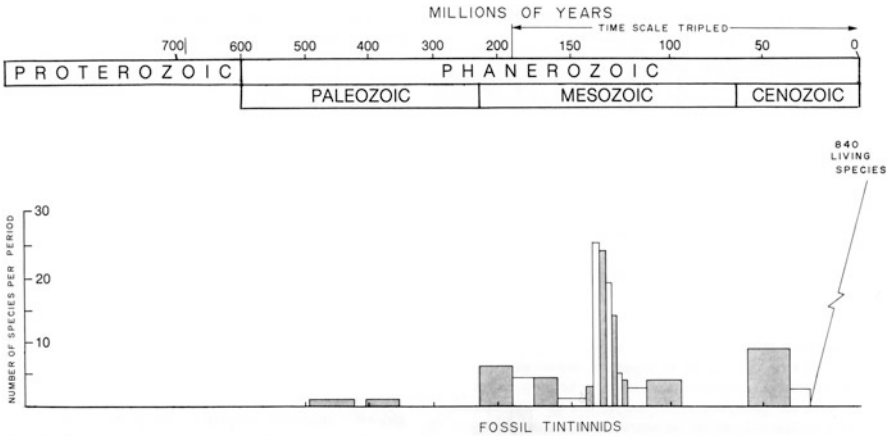

Fig. 22 Total number of species of tintinnids per geological period from their earliest appearance in the Ordovician up to the present. Taxa in the *shaded columns* are likely not tintinnids (After Tappan and Loeblich 1973; Redrawn by K. Wellencamp)

Phylogeny

Phylogeny Within the Phylum CILIOPHORA. The presumed phylogenetic relationships of the major clades of ciliates have been briefly mentioned above (Characterization and Recognition, *Classification*). The most informative data on the adaptive diversification of ciliates is provided by gene sequences, particularly that of the small subunit rRNA gene (Fig. 8a) and now phylogenomic analyses (Fig. 8b) (Gentekaki et al. 2014; Lynn 2008). This is because it is almost impossible to understand how one kind of cortical ultrastructure is related to or transformed into another kind of cortical ultrastructure.

From the molecular genetic perspective, the phylum is unambiguously divided into two major clades (Fig. 8, Table 2). Within the POSTCILIODESMATOPHORA, it has been argued that macronuclear division evolved from a division-less ancestral karyorelictean-like state by the use of *extramacronuclear* microtubules while macronuclear division evolved independently in the other clade – the INTRAMACRONUCLEATA – by use of *intramacronuclear* microtubules (Orias 1991a in Lynn 2008).

How the various groups of intramacronucleates diverged varies somewhat depending upon what gene one uses as the data source (Lynn 2008). The spirotrichs, armophoreans, and litostomes (SAL) appear to be related robustly in phylogenomic analyses, while the remaining six classes form a clade that is often well supported and has been labeled CONTHREEP, an acronym standing for the first letters of the included groups (Fig. 8).

Origin of the Phylum CILIOPHORA. The origin of the Phylum CILIOPHORA is as conjectural as the origin of the groups within it. The putative ancestor could have been a "corticoflagellate": a flagellate with a locomotory dikinetid, cytostome, and with a cortex and infraciliature similar to that of the ciliates (Lynn and Small 1981 in Lynn 2008). There are at least two contemporary taxa that exhibit these features: the dinoflagellates and *Colponema* spp. have dikinetids with ribbons of microtubules associated with each kinetosome, a striated rootlet fibril associated with at least one kinetosome and extending toward the cell surface, and cortical alveoli similar to those of ciliates. Gene sequences confirm the associations of these three contemporary taxa with other alveolates (see ► Dinoflagellata, and ► Apicomplexa).

From this flagellate ancestor, polymerization or an increase in numbers of kinetid units must have occurred to form the ciliary files or kineties. The most plausible model to date has been presented by Eisler (1992). It is imagined that this ancestor had a file of dikinetids, the homologue to the paroral, associated with its cytostome. These paroral dikinetids replicated laterally to produce a file or row of dikinetids to their right, and this would produce the first somatic kinety. Repeated replication of this process would generate multiple kineties that would eventually "cover" the cortical surface (Eisler 1992). It is further proposed that the adoral ciliary structures (i.e., those on the left side of the cytostome) differentiated from somatic kineties adjacent to the left side of the cytostome, a process that often happens in contemporary ciliates but in very diverse ways.

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References

Acosta-Mercado, D., & Lynn, D. H. (2003). The edaphic quantitative protargol stain: A sampling protocol for assessing soil ciliate abundance and diversity. *Journal of Microbiological Methods, 53*, 365–375.

Adl, S. M. (2003). *The ecology of soil decomposition*. Oxford: Oxford University Press.

Alabouvette, C., Couteaux, M. M., Old, K. M., Pussard, M., Reisinger, O., & Toutain, F. (1981). Les protozoaires du sol: Aspects écologiques et methodologiques. *Année Biologique, 20*, 255–303.

Allen, R. D. (2000). The contractile vacuole and its membrane dynamics. *BioEssays, 22*, 1035–1042.

Allen, R. D., & Fok, A. K. (2000). Membrane trafficking and processing in *Paramecium. International Review of Cytology, 198*, 277–318.

Allen, S. L., Rushford, C. L., Nerad, T. A., & Lau, E. T. (1983). Intraspecies variability in the esterases and acid phosphatases of *Paramecium jenningsi* and *Paramecium multimicronucleatum*: Assignment of unidentified paramecia; comparison with the *P. aurelia* complex. *Journal of Protozoology, 30*, 155–163.

American Type Culture Collection. (1982). *Catalogue of strains I* (15th ed., p. 556). Bethesda: American Type Culture Collection.

Anderson, S. A., Hulston, D. A., McVeagh, S. M., Webb, V. L., & Smith, P. J. (2009). In vitro culture and cryopreservation of *Uronema marinum* isolated from farmed New Zealand groper (*Polyprion oxygeneios*). *Journal of Microbiological Methods, 79*, 62–66.

Arnaiz, O., & Sperling, L. (2011). ParameciumDB in 2011: New tools and new data for functional and comparative genomics of the model ciliate *Paramecium tetraurelia. Nucleic Acids Research, 39*, D632–D636.

Asai, D. J., & Forney, J. D. (Eds.). (2000). *Tetrahymena thermophila* (Methods in cell biology, Vol. 62). New York: Academic.

Aufderheide, K. J. (1982). An improvement in the protargol technique of Ng and Nelsen. *Transactions of the American Microscopical Society, 101*, 100–104.

Augustin, H., Foissner, W., & Adam, H. (1984). An improved pyridinated silver carbonate method which needs few specimens and yields permanent slides of impregnated ciliates (Protozoa, Ciliophora). *Mikroskopie (Wien), 41*, 134–137.

Azovsky, A., & Mazei, Y. (2013). Do microbes have macroecology? Large-scale patterns in diversity and distribution of marine benthic ciliates. *Global Ecology and Biogeography, 22*, 163–172.

Barth, D., Tischer, K., Berger, H., Schlegel, M., & Berendonk, T. U. (2008). High mitochondrial haplotype diversity of *Coleps* sp. (Ciliophora: Prostomatida). *Environmental Microbiology, 10*, 626–634.

Batson, B. S. (1983). *Tetrahymena dimorpha* sp. nov. (Hymenostomatida: Tetrahymenidae), a new ciliate parasite of Simuliidae (Diptera) with potential as a model for the study of ciliate morphogenesis. *Philosophical Transactions of the Royal Society, London B, 301*, 345–363.

Beale, G. H., & Preer, J. R. (2008). *Paramecium: Genetics and epigenetics*. Boca Raton: CRC Press.

Beaver, J. R., & Crisman, T. L. (1989). The role of ciliated protozoa in pelagic freshwater ecosystems. *Microbial Ecology, 17*, 111–136.

Berger, H. (2011). *Monograph of the Gonostomatidae and Kahliellidae (Ciliophora, Hypotricha)*. Dordrecht: Springer.

Bick, H. (1972). *Ciliated protozoa: An illustrated guide to the species used as biological indicators in freshwater biology.* Geneva: World Health Organization.

Blackburn, E. H. (1992). Telomerase. *Annual Reviews of Biochemistry, 61*, 113–129.

Blackburn, E. H., Budarf, M. L., Challoner, P. B., Cherry, J. M., Howard, E. A., Katzen, A. L., Pan, W.-C., & Ryan, T. (1983). DNA termini in ciliate macronuclei. *Cold Spring Harbor Symposia on Quantitative Biology, 47*, 1195–1207.

Borror, A. C. (1973). Marine flora and fauna of the northeastern United States. Protozoa: Ciliophora (*NOAA Technical Report NMFS CIRC–378*).

Bulit, C., Díaz-Avalos, C., Signoret, M., & Montagnes, D. J. S. (2009). Scaling patterns of plankton diversity: A study of ciliates in a tropical coastal lagoon. *Hydrobiologia, 624*, 29–44.

Burkovsky, I. V. (1978). Structure, dynamics and production of a community of marine psammophilous ciliates. *Zoologicheskiĭ Zhurnal, 57*, 325–337 (in Russian with English summary).

Cairns, J., Jr., & Yongue, W. H., Jr. (1977). Factors affecting the number of species in freshwater protozoan communities. In J. Cairns (Ed.), *Aquatic microbial communities* (pp. 257–303). New York: Garland.

Cairns, J., Jr., Lanza, G. R., & Parker, B. C. (1972). Pollution related structural and functional changes in aquatic communities with emphasis on freshwater algae and protozoa. *Proceedings of the Academy of Natural Sciences of Philadelphia, 124*, 79–127.

Caron, D. A., Davis, P. G., Madin, L. P., & Sieburth, J. M. N. (1982). Heterotrophic bacteria and bactivorous protozoa in oceanic macroaggregates. *Science, 218*, 795–797.

Chalker, D. L. (2008). Dynamic nuclear reorganization during genome remodeling of *Tetrahymena. Biochimica et Biophysica Acta, Molecular Cell Research, 1783*, 2130–2136.

Chen, T.-T. (Ed.). (1967). *Research in protozoology* (Vol. 1). New York: Pergamon Press, Vol. 2 (1967), Vol. 3 (1969), Vol. 4 (1972).

Coats, W., & Heinbokel, J. (1982). A study of reproduction and other life cycle phenomena in planktonic protists using an acridine orange fluorescence technique. *Marine Biology, 67*, 71–79.

Collins, K. (Ed.). (2012). *Tetrahymena thermophila* (Methods in cell biology, Vol. 109). Amsterdam/London: Elsevier/Academic.

Committee on Cultures, Society of Protozoologists. (1958). A catalogue of laboratory strains of free-living and parasitic protozoa. *Journal of Protozoology, 5*, 1–38.

Cooper, J. K., Li, J. Q., & Montagnes, D. J. S. (2012). Intermediate fragmentation per se provides stable predator–prey metapopulation dynamics. *Ecology Letters, 15*, 856–863.

Corliss, J. O. (1979). *The ciliated protozoa. Characterization, classification and guide to the literature* (2nd ed.). New York: Pergamon Press.

Corliss, J. O. (1986). The development of ciliate systematics from the era of József Gelei until the present time. *Symposia Biologica Hungarica, 33*, 67–86.

Coyne, R. S., Thiagarajan, M., Jones, K. M., Wortman, J. R., Tallon, L. J., Haas, B. J., Cassidy-Hanley, D. M., Wiley, E. A., Smith, J. J., Collins, K., Lee, S. R., Couvillion, M. T., Liu, Y. F., Garg, J., Pearlman, R. E., Hamilton, E. P., Orias, E., Eisen, J. A., & Methe, B. A. (2008). Refined annotation and assembly of the *Tetrahymena thermophila* genome sequence through EST analysis, comparative genomic hybridization, and targeted gap closure. *BMC Genomes, 9*, 1471–2164.

Curds, C. R. (1982). British and other freshwater ciliated protozoa. Part I. Ciliophora: Kinetofragminophora. Keys and notes for identification of the free-living genera. In D. M. Kermack & R. S. K. Barnes (Eds.), *Synopses of the British Fauna* (Vol. 22). New York: Cambridge University Press.

Curds, C. R., Gates, M. A., & Roberts, D. M. L. (1983). British and other freshwater ciliated protozoa. Part II. Ciliophora: Oligohymenophora and Polyhymenophora. Keys and notes for identification of the free-living genera. In D. M. Kermack & R. S. K. Barnes (Eds.), *Synopses of the British Fauna* (Vol. 23). New York: Cambridge University Press.

Dale, T., & Burkhill, P. H. (1982). Live counting – A quick and simple technique for enumerating pelagic ciliates. *Annales de l'Institut Oceanographique, 58*, 267–276.

Damaj, R., Pomel, S., Bricheux, G., Coffe, G., Vigues, B., Ravet, V., & Bouchard, P. (2009). Cross-study analysis of genomic data defines the ciliate multigenic epiplasmin family: Strategies for functional analysis in *Paramecium tetraurelia. BMC Evolutionary Biology, 9*, 125.

Dayeh, V. R., Chow, S. L., Schirmer, K., Lynn, D. H., & Bols, N. C. (2004). Evaluating the toxicity of Triton X-100 to protozoan, fish, and mammalian cells using fluorescent dyes as indicators of cell viability. *Ecotoxicology and Environmental Safety, 57*, 375–382.

de Figueiredo, G. M., Nash, R. D. M., & Montagnes, D. J. S. (2007). Do protozoa contribute significantly to the diet of larval fish in the Irish Sea? *Journal of the Marine Biological Association of the United Kingdom, 87*, 843–850.

De Graaf, R. M., Ricard, G., van Alen, T. A., Duarte, I., Dutilh, B. E., Burgtorf, C., Kuiper, J. W. P., van der Staay, G. W. M., Tielens, A. G. M., Huynen, M. A., & Hackstein, J. P. (2011). The organellar genome and metabolic potential of the hydrogen-producing mitochondrion of *Nyctotherus ovalis. Molecular Biology and Evolution, 28*, 2379–2391.

de Puytorac, P. (Ed.). (1994). *Traité de Zoologie, Infusoires Ciliés* (Vol. 2). Paris: Masson.

de Puytorac, P., Grain, J., Legendre, P., & Devaux, J. (1984). Essai d'application de l'analyse phénétique à la classification du phylum des Ciliophora. *Journal of Protozoology, 31*, 496–507.

Dolan, J. R., Ritchie, M. E., & Ras, J. (2007). The "neutral" community structure of planktonic herbivores, tintinnid ciliates of the microzooplankton, across the S.E. Tropical Pacific Ocean. *Biogeosciences, 4*, 297–310.

Dolan, J. R., Montagnes, D. J. S., Agatha, S., Coats, D. W., & Stoecker, D. K. (2013). *The biology and ecology of tintinnid ciliates: Models for marine plankton*. Oxford: Wiley-Blackwell.

Dye, A. H. (1979). Quantitative estimation of protozoa from sandy substrates. *Estuarine and Coastal Marine Science, 8*, 199–204.

Eisler, K. (1992). Somatic kineties or paroral membrane: Which came first in ciliate evolution? *BioSystems, 26*, 239–254.

Elliott, A. M. (Ed.). (1973). *Biology of Tetrahymena*. Stroudsburg: Dowden, Hutchinson and Ross.

Esteban, G. F., Fenchel, T., & Finlay, B. J. (2010). Mixotrophy in ciliates. *Protist, 161*, 621–641.

Fenchel, T. (1969). The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. *Ophelia, 6*, 1–182.

Fenchel, T. (1987). *Ecology of protozoa*. Berlin: Springer.

Fenchel, T., & Finlay, B. J. (1991a). The biology of free-living anaerobic ciliates. *European Journal of Protistology, 26*, 210–215.

Fenchel, T., & Finlay, B. J. (1991b). Synchronous division of an endosymbiotic methanogenic bacterium in the anaerobic ciliate *Plagiopyla frontata* Kahl. *Journal of Eukaryotic Microbiology, 38*, 22–28.

Fenchel, T., & Harrison, P. (1976). The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus. In J. M. Anderson & A. MacFadyen (Eds.), *The role of terrestrial and aquatic organisms in decomposition processes* (pp. 285–299). London: Blackwell.

Finlay, B. J., & Ochsenbein-Gattlen, C. (1982). Ecology of free-living protozoa. A bibliography of published research concerning freshwater and terrestrial forms 1910–1981 (*Freshwater Biological Association, Occasional Publication* No. 17).

Finlay, B. J., Hetherington, N. B., & Davison, W. (1983). Active biological participation in lacustrine barium chemistry. *Geochimica et Cosmochimica Acta, 47*, 1325–1329.

Foissner, W. (1988). Taxonomic and nomenclatural revision of Sladeek's lists of ciliates (Protozoa: Ciliophora) as indicators of water quality. *Hydrobiologia, 166*, 1–64.

Foissner, W. (1991). Basic light and electron microscopic methods for taxonomic studies of ciliated protozoa. *European Journal of Protistology, 27*, 313–330.

Foissner, W., Franz, H., & Adam, H. (1982). Terrestrische Protozoen als Bioindikatoren im Boden einer planierten Ski-Piste. *Pedobiologia, 24*, 45–56.

Foissner, W., Berger, H., & Kohmann, F. (1994). *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems – Band III: Hymenostomata, Prostomatida Nassulida*. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. Heft 1/94.

Foissner, W., Berger, H., & Schaumburg, J. (1999). *Identification and ecology of limnetic plankton ciliates*. Munich: Bavarian State Office of Water Management.

Foissner, W., Chao, A., & Katz, L. A. (2008). Diversity and geographic distribution of ciliates (Protista: Ciliophora). *Biodiversity and Conservation, 17*, 345–363.

Fok, A. K., & Allen, R. D. (1979). Axenic *Paramecium caudatum*. I. Mass culture and structure. *Journal of Protozoology, 26*, 463–470.

Frankel, J. (1989). *Pattern formation. Ciliate studies and models*. Oxford: Oxford University Press.

Frankel, J., & Heckmann, K. (1968). A simplified Chatton-Lwoff silver impregnation procedure for use in experimental studies with ciliates. *Transactions of the American Microscopical Society, 87*, 317–321.

Fukami, T., & Morin, P. J. (2003). Productivity-diversity relationships depend on the history of community assembly. *Nature, 424*, 423–426.

Galbraith, L. M., & Burns, C. W. (2010). Drivers of ciliate and phytoplankton community structure across a range of water bodies in southern New Zealand. *Journal of Plankton Research, 32*, 327–339.

Gao, F., Warren, A., Zhang, Q., Gong, J., Miao, M., Sun, P., Xu, D., Huang, J., Yi, Z., & Song, W. (2016). The all-data-based evolutionary hypothesis of ciliated protists with a revised classification of the Phylum Ciliophora (Eukaryota, Alveolata). *Scientific Reports, 6*, 24874. doi:10.1038/sreps24874.

Garst, V., & Horstmann, U. (1983). N-remineralization of phyto- and bacterioplankton by the marine ciliate *Euplotes vannus. Marine Ecology-Progress Series, 13*, 55–60.

Gates, M. A. (1977). Analysis of positional information applied to cirral pattern of the ciliate *Euplotes. Nature, 268*, 362–364.

Gause, G. F. (1934). *The struggle for existence.* Reprint 1964. New York: Hafner.

Gentekaki, E., & Lynn, D. H. (2012). Spatial genetic variation, phylogeography and barcoding of the peritrichous ciliate *Carchesium polypinum. European Journal of Protistology, 48*, 305–313.

Gentekaki, E., Kolisko, M., Boscaro, V., Bright, K. J., Dini, F., Di Giuseppe, G., Gong, Y., Miceli, C., Modeo, L., Molestina, R. E., Petroni, G., Pucciarelli, S., Roger, A. J., Strom, S. L., & Lynn, D. H. (2014). Large-scale phylogenomic analysis reveals the phylogenetic position of the problematic taxon *Protocruzia* and unravels the deep phylogenetic affinities of the ciliate lineages. *Molecular Phylogenetics and Evolution, 78*, 36–42.

Gentekaki, E., Kolisko, M., Gong, Y., & Lynn, D. H. (2017). Phylogenomics solves a long-standing evolutionary puzzle in the ciliate world: The subclass Peritrichia is monophyletic. *Molecular Phylogenetics and Evolution, 106*, 1–5.

Giese, A. C. (Ed.). (1973). *Blepharisma: The biology of a light-sensitive protozoan*. Stanford: Stanford University Press.

Gill, D. E. (1972). Intrinsic rates of increase, saturation densities, and competitive ability. I. An experiment with *Paramecium. The American Naturalist, 106*, 461–471.

Giller, P. S., Hillebrand, H., Berninger, U.-G., Gessner, M. O., Hawkins, S., Inchausti, P., Inglis, C., Leslie, H., Malmquist, B., Monaghan, M. T., Morin, P. J., & O'Mullan, G. (2004). Biodiversity effects on ecosystem functioning: Emerging issues and their experimental test in aquatic environments. *Oikos, 104*, 423–436.

Gilron, G. L., & Lynn, D. H. (1996). Ciliated protozoa as test organisms in toxicity assessments, Ch. 21. In P. Wells, C. Blaise & K. Lee (Eds.). *Microscale testing in aquatic toxicology – Advances, techniques and practise* (pp. 323–336). Boca Raton: Lewis Publishers.

Gold, K. (1973). Methods for growing Tintinnida in continuous culture. *American Zoologist, 13*, 203–208.

Gomez-Gutierrez, J., Strüder-Kypke, M. C., Lynn, D. H., Shaw, T. C., Aguilar-Mendez, M. J., Lopez-Cortes, A., Martinez-Gomez, S., & Robinson, C. J. (2012). *Pseudocollinia brintoni* gen. nov., sp. nov. (Apostomatida: Colliniidae), a parasitoid ciliate infecting the euphausiid *Nyctiphanes simplex. Diseases of Aquatic Organisms, 99*, 57–78.

Goodey, T. (1915). Note on the remarkable retention of vitality by protozoa from old stored soils. *Annals of Applied Biology, 1*, 395–399.

Görtz, H.-D. (Ed.). (1988). *Paramecium*. Berlin: Springer.

Gould, S. B., Tham, W. H., Cowman, A. F., McFadden, G. I., & Waller, R. F. (2008). Alveolins, a new family of cortical proteins that define the protist infrakingdom Alveolata. *Molecular Biology and Evolution, 25*, 1219–1230.

Grassé, P.-P. (Ed.). (1984). *Traité de Zoologie* (Fasc. 1, *Infusoires ciliés*, Vol. 2). Paris: Masson.

Grell, K. G. (1973). *Protozoology*. New York: Springer.

Grolière, C.-A. (1978). Contribution à l'étude des ciliés des sphaignes. III. Étude mathématique des resultat. *Protistologica, 14*, 295–311.

Guggiari, M., & Peck, R. (2008). The bacterivorous ciliate *Cyclidium glaucoma*, isolated from a sewage treatment plant: Molecular and cytological descriptions for barcoding. *European Journal of Protistology, 44*, 168–180.

Harikrishnan, R., Balasundaram, C., & Heo, M.-S. (2010). Scuticociliatosis and its recent prophylactic measures in aquaculture with special reference to South Korea. Taxonomy, diversity and diagnosis of scuticociliatosis: Part I; Control strategies of scuticociliatosis: Part II. *Fish & Shellfish Immunology, 29*, 15–31.

Hausmann, K. (1978). Extrusive organelles in protists. *International Review of Cytology, 52*, 197–276.

Hausmann, K., & Bradbury, P. C. (Eds.). (1996). *Ciliates. Cells as organisms*. Stuttgart: Gustav Fischer.

Hausmann, K., Hülsmann, N., & Radek, R. (2003). *Protistology* (3rd ed.). Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller).

Heckmann, K. (1983). Endosymbionts of *Euplotes. International Review of Cytology. Supplement, 14*, 111–144.

Hill, D. L. (1972). *The biochemistry and physiology of Tetrahymena*. New York: Academic.

Hutner, S. H. (Ed.). (1964). *Biochemistry and physiology of protozoa* (Vol. 3). New York: Academic.

Hutner, S. H., & Lwoff, A. (Eds.). (1955). *Biochemistry and physiology of protozoa* (Vol. 2). New York: Academic.

Jankowski, A. W. (2007). Phylum Ciliophora Doflein, 1901. In A. F. Alimov (Ed.), *Protista. Part 2, Handbook on zoology* (pp. 415–993). St. Petersburg: Russian Academy of Sciences, Zoological Institute (in Russian with English summary).

Johnson, M. D. (2011). Acquired phototrophy in ciliates: A review of cellular interactions and structural adaptations. *Journal of Eukaryotic Microbiology, 58*, 185–195.

Jones, A. R. (1974). *The ciliates*. London: Hutchinson.

Juranek, S. A., & Lipps, H. J. (2007). New insights into the macronuclear development of ciliates. *International Review of Cytology, 262*, 219–251.

Kahl, A. (1930–1935). Urtiere oder protozoa. I: Wimpertiere oder Ciliata (Infusoria), eine Bearbeitung der freilebenden und ectocommen-salen Infusorien der Erde, unter Ausschluss der marinen Tintinnidae. In F. Dahl (Ed.), *Die TierweltDeutschlands* (Parts 18 (year 1930), 21 (1931), 25 (1932), 30 (1935), pp. 1–886). Jena: G. Fischer.

Katz, L. A., DeBerardinis, J., Hall, M. S., Kovner, A. M., Dunthorn, M., & Muse, S. V. (2011). Heterogeneous rates of molecular evolution among cryptic species of the ciliate morphospecies *Chilodonella uncinata. Journal of Molecular Evolution, 73*, 266–272.

Keenan, K., Erlich, E., Donnelly, K. H., Basel, M. B., Hutner, S. H., Kassoff, R., & Crawford, S. A. (1978). Particle-based axenic media for tetrahymenids. *Journal of Protozoology, 25*, 385–387.
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 1 (cox-1) barcode. *Protist, 162*, 2–13.
Kidder, G. W. (Ed.). (1967). Protozoa. In: M. Florkin, B. T. Scheer (Eds.), *Chemical zoology* (Vol. 1). New York: Academic.
Kofoid, C. A., & Campbell, A. S. (1939). The Ciliata: The Tintinnoinea. Reports on the scientific results of the expedition to the eastern tropical Pacific. 1904–1905. *Bulletin of the Museum of Comparative Zoology Harvard, 84*, 1–473.
Kosinski, R. J. (1979). A method of sterilizing ciliates without special equipment. *BioScience, 29*, 306–307.
Kreier, J. P. (Ed.). (1978). *Parasitic protozoa* (Vol. II). New York: Academic.
Kreier, J. P. (Ed.). (1994). *Parasitic protozoa* (2nd ed., Vol. VIII). New York: Academic.
Kreier, J. P., & Baker, J. R. (1993). *Parasitic protozoa* (Vol. III). New York: Academic.
Krenek, S., & Berendonk, T. U. (2009). A long-term cryopreservation tool for cell characteristics: Cryopreservation of *Paramecium caudatum. Protist, 160*, 355–363.
Krumins, J. A., Long, Z. T., Steiner, C. F., & Morin, P. J. (2006). Indirect effects of food web diversity and productivity on bacterial community function and composition. *Functional Ecology, 20*, 514–521.
Laughlin, T. J., Henry, J. M., Phares, E. F., Long, M. V., & Olins, D. E. (1983). Methods for the large-scale cultivation of an *Oxytricha* (Ciliophora: Hypotrichida). *Journal of Protozoology, 30*, 63–64.
Levandowsky, M., & Hutner, S. H. (Eds.). (1980). *Biochemistry and physiology of protozoa* (2nd ed.). New York: Academic.
Li, J. Q., & Montagnes, D. J. S. (2015). Restructuring fundamental predator–prey models by recognising prey-dependent conversion efficiency and mortality rates. *Protist, 166*, 211–223.
Li, L. F., Stoeck, T., Shin, M. K., AL-Rasheid, K. A. S., AL-Khedhairy, B. A., & Song, W. B. (2010). *Protocruzia*, a highly ambiguous ciliate (Protozoa: Ciliophora): Very likely an ancestral form for Heterotrichea, Colpodea or Spirotrichea? With reevaluation of its evolutionary position based on multigene analyses. *Science China Life Sciences, 53*, 131–138.
Limberger, R., & Wickham, S. A. (2012). Transitory versus persistent effects of connectivity in environmentally homogeneous metacommunities. *Plos One, 7*(8), e44555.
Lindholm, T. (1985). *Mesodinium rubrum* – A unique photosynthetic ciliate. *Advances in Aquatic Microbiology, 3*, 1–48.
Luckinbill, L. S. (1979). Selection and the *r*/*K* continuum in experimental populations of protozoa. *The American Naturalist, 113*, 427–437.
Luckinbill, L. S., & Fenton, M. M. (1978). Regulation and environmental variability in experimental populations of protozoa. *Ecology, 59*, 1271–1276.
Lwoff, A. (Ed.). (1951). *Biochemistry and physiology of protozoa* (Vol. 1). New York: Academic.
Lynn, D. H. (1981). The organization and evolution of microtubular organelles in ciliated protozoa. *Biological Reviews, 56*, 243–292.
Lynn, D. H. (2008). *The ciliated protozoa: Characterization, classification, and guide to the literature* (3rd ed.). New York: Springer.
Lynn, D. H., & Malcolm, J. R. (1983). A multivariate study of morphometric variation in species of the ciliate genus *Colpoda* (Ciliophora: Colpodida). *Canadian Journal of Zoology, 61*, 307–316.
Lynn, D. H., & Small, E. B. (2002). Phylum Ciliophora, Doflein, 1901. In: J. J. Lee, G. F. Leedale, & P. C. Bradbury (Eds.), *An illustrated guide to the protozoa* (Vol. 1 (2000), pp. 371–656). Lawrence: Society of protozoologists.
Martín-González, A., Wierzchos, J., Gutiérrez, J. C., Alonso, J., & Ascaso, C. (2008). Morphological stasis of protists in lower cretaceous amber. *Protist, 159*, 251–257.
Matthes, D., & Wenzel, F. (1966). *Die Wimpertiere (Ciliata)*. Stuttgart: Kosmos-Verlag.

Michalowski, T., Szczepkowski, P., & Muszynski, P. (1985). The factors affecting the cultivation of the rumen ciliate protozoan *Entodinium exiguum* in vitro. *Acta Protozoologica, 24*, 297–305.

Montagnes, D. J. S., & Lynn, D. H. (1993). A quantitative protargol stain (QPS) for ciliates and other protists. In P. F. Kemp, B. F. Sherr, E. B. Sherr, & J. J. Cole (Eds.), *Aquatic microbial ecology* (pp. 229–240). Boca Raton: Lewis Publishers.

Montagnes, D., Roberts, E., Lukes, J., & Lowe, C. (2012). The rise of model protozoa. *Trends in Microbiology, 20*, 184–191.

Morado, J. F., & Small, E. B. (1995). Ciliate parasites and related diseases of Crustacea: A review. *Reviews in Fisheries Science, 3*, 275–354.

Morin, P. J., & McGrady-Steed, J. (2004). Biodiversity and ecosystem functioning in aquatic microbial systems: A new analysis of temporal variation and species richness-predictability relations. *Oikos, 104*, 458–466.

Nanney, D. L. (1980). *Experimental ciliatology. An introduction to genetic and developmental analysis in ciliates*. New York: Wiley.

Narayan, N., Priya, M., Haridas, A., & Manilai, V. B. (2007). Isolation and culturing of a most common anaerobic ciliate *Metopus* sp. *Anaerobe, 13*, 14–20.

Ng, S. F. (1986). The somatic function of the micronucleus of ciliated protozoa. *Progress in Protozoology, 1*, 215–286.

Nowacki, M., Haye, J. E., Fang, W. W., Vijayan, V., & Landweber, L. F. (2010). RNA-mediated epigenetic regulation of DNA copy number. *Proceedings of the National Academy of Sciences USA, 107*, 22140–22144.

Nyberg, D. (1981). Three new 'biological' species of *Tetrahymena* (*T. hegewischi* n. sp., *T. sonneborni* n. sp., *T. nipissingi* n. sp.) and temperature tolerance of members of the *pyriformis* complex. *Journal of Protozoology, 28*, 65–69.

Orsi, W., Edgcomb, V., Faria, J., Foissner, W., Fowle, W. H., Hohmann, T., Suarez, P., Taylor, C., Taylor, G. T., Vd'ačný, P., & Epstein, S. S. (2012). Class Cariacotrichea, a novel ciliate taxon from the anoxic Cariaco Basin, Venezuela. *International Journal of Systematic and Evolutionary Microbiology, 62*, 1425–1433.

Patterson, D. J. (1980). Contractile vacuoles and associated structures: Their organization and function. *Biological Reviews, 55*, 1–46.

Patterson, D. J., & Brugerolle, G. (1988). The ultrastructural identity of *Stephanopogon apogon* and the relatedness of the genus to other kinds of protists. *European Journal of Protistology, 23*, 279–290.

Perriss, S. J., Laybourn-Parry, J., & Jones, R. I. (1994). Chlorophyll contents and photosynthetic rates of the freshwater mixotrophic ciliate *Strombidium viride* (Ciliophora: Oligotrichida). *Archiv für Hydrobiologie, 130*, 473–483.

Pierce, R. W., & Turner, J. T. (1992). Ecology of planktonic ciliates in marine food webs. *Reviews in Aquatic Science, 6*, 139–181.

Prescott, D. M. (1994). The DNA of ciliated protozoa. *Microbiological Reviews, 58*, 233–267.

Raikov, I. B. (1982). *The protozoan nucleus. Morphology and evolution*. Vienna: Springer.

Rølle, G. (1980). *Tetrahymena pyriformis W* and rats as test organisms for the evaluation of protein quality. *Acta Agriculturae Scandinavica, 30*, 193–200.

Salt, G. W. (1967). Predation in a experimental protozoa population (*Woodruffia-Paramecium*). *Ecological Monographs, 37*, 113–144.

Salt, G. W. (1974). Predator and prey densities as controls of the rate of capture by the predator *Didinium nasutum. Ecology, 55*, 434–439.

Salt, G. W. (1979). Density, starvation, and swimming rate in *Didinium* populations. *The American Naturalist, 113*, 135–143.

Sanders, R. W., & Wickham, S. A. (1993). Planktonic protozoa and metazoa: Predation, food quality and population control. *Marine Microbial Food Webs, 7*, 197–223.

Schmidt, H. J. (1982). New methods for cultivating, harvesting, and purifying mass cultures of the hypotrich ciliate *Euplotes aediculat us. Journal of Protozoology, 29*, 132–135.

Schmidt, A. R., Ragazzi, E., Coppellotti, O., & Roghi, G. (2006). A microworld in Triassic amber. *Nature, 444*, 835.

Schultz, T. W., Dumont, J. N., & Kyte, L. M. (1978). Cytotoxicity of synthetic fuel products on *Tetrahymena pyriformis*. II. Shale oil retort water. *Journal of Protozoology, 25*, 502–509.

Schuster, F. L., & Ramirez-Avila, L. (2008). Current world status of *Balantidium coli. Clinical Microbiology Reviews, 21*, 626–638.

Segade, P., Kher, C. P., Lynn, D. H., & Iglesias, R. (2009). Morphological and molecular characterization of renal ciliates infecting farmed snails in Spain. *Parasitology, 136*, 771–782.

Shatilovich, A., Stoupin, D., & Rivkina, E. (2015). Ciliates from ancient permafrost: Assessment of cold resistance of the resting cysts. *European Journal of Protistology, 51*, 230–240.

Sherr, E., & Sherr, B. (1988). Role of microbes in pelagic food webs: A revised concept. *Limnology and Oceanography, 35*, 1225–1227.

Singh, D. P., Saudemont, B., Guglielmi, G., Arnaiz, O., Gout, J. F., Prajer, M., Potekhin, A., Przybos, E., Aubusson-Fleury, A., Bhullar, S., Bouhouche, K., Lhuillier-Akakpo, M., Tanty, V., Blugeon, C., Alberti, A., Labadie, K., Aury, J. M., Sperling, L., Duharcourt, S., & Meyer, E. (2014). Genome-defence small RNAs exapted for epigenetic mating-type inheritance. *Nature, 509*, 447. doi:10.1038/nature13318.

Skovorodkin, I. N. (1990). A device for immobilization of small biological objects during light microscopical observation. *Tsitologiya, 32*, 87–91 (in Russian with English summary).

Slabbert, J. L., Smith, R., & Morgan, W. S. G. (1983). Application of a *Tetrahymena pyriformis* bioassay system for the rapid detection of toxic substances in waste waters. *Water SA, 9*, 81–87.

Sleigh, M. A. (1989). *Protozoa and other protists* (2nd ed.). London: Edward Arnold.

Small, E. B., & Gross, M. (1985). Preliminary observations of ciliated protozoa from the 21°N hydrothermal vent site. *Biological Society of Washington Bulletin, 6*, 401–410.

Smith-Sonneborn, J. (1981). Genetics and aging in protozoa. *International Review of Cytology, 73*, 319–354.

Soldo, A. T., Godoy, G. A., & Brickson, S. (1974). Infectious particles in a marine ciliate. *Nature, 249*, 284–286.

Stoecker, D. K., Taniguchi, A., & Michaels, A. E. (1989). Abundance of autotrophic, mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters. *Marine Ecology Progress Series, 50*, 241–254.

Strueder-Kypke, M. C., & Lynn, D. H. (2010). Comparative analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene in ciliates (Alveolata, Ciliophora) and evaluation of its suitability as a biodiversity marker. *Systematics and Biodiversity, 8*, 131–148.

Takishita, K., Kakizoe, N., Yoshida, T., & Maruyama, T. (2010). Molecular evidence that phylogenetically diverged ciliates are active in microbial mats of deep-sea cold-seep sediment. *Journal of Eukaryotic Microbiology, 57*, 76–86.

Tan, X. L., Shi, X. L., Liu, G. J., Xu, H. L., & Nie, P. (2010). An approach to analyzing taxonomic patterns of protozoan communities for monitoring water quality in Songhua River, northeast China. *Hydrobiologia, 638*, 193–201.

Tappan, H., & Loeblich, A. R., Jr. (1973). Evolution of the oceanic plankton. *Earth-Science Reviews, 9*, 207–240.

Tartar, V. (1961). *The biology of Stentor*. New York: Pergamon Press.

Taylor, W. D., & Berger, J. (1980). Microspatial heterogeneity in the distribution of ciliates in a small pond. *Microbial Ecology, 6*, 27–34.

Tucker, J. B. (1978). Endocytosis and streaming of highly gelated cytoplasm alongside rows of arm-bearing microtubules in the ciliate *Nassula. Journal of Cell Science, 29*, 213–232.

Uhlig, G. (1972). Protozoa. In C. Schlieper (Ed.), *Research methods in marine biology* (pp. 129–141). Seattle: University of Washington Press.

Vd'ačný, P., & Foissner, W. (2012). *Monograph of the dileptids: (Protista, Ciliophora, Rhynchostomatia)* (Denisia). Linz: Land Oberösterreich, Biologiezentrum/Oberösterreichische Landesmuseen.

Vd'ačný, P., Orsi, W., & Foissner, W. (2010). Molecular and morphological evidence for a sister group relationship of the classes Armophorea and Litostomatea (Ciliophora, Intramacronucleata, Lamellicorticata infraphyl. nov.), with an account on basal litostomateans. *European Journal of Protistology, 46*, 298–309.

Vd'ačný, P., Bourland, W. A., Orsi, W., Epstein, S. S., & Foissner, W. (2012). Genealogical analyses of multiple loci of litostomatean ciliates (Protista, Ciliophora, Litostomatea). *Molecular Phylogenetics and Evolution, 65*, 397–411.

Wagener, S., & Pfennig, N. (1987). Monoxenic culture of the anaerobic ciliate *Trimyema compressum* Lackey. *Archives of Microbiology, 149*, 4–11.

Wang, Y.-Y. D., Miller, J., & Beuchat, L. R. (1980). *Tetrahymena thermophila* as an organism for bioassay of protein quality. *Nutrition Reports International, 21*, 645–651.

Weis, D. S., & Ayala, A. (1979). Effect of exposure period and algal concentration on the frequency of infection of aposymbiotic ciliates by symbiotic algae from *Paramecium bursaria*. *Journal of Protozoology, 26*, 245–248.

Wickham, S., Gieseke, A., & Berninger, U. G. (2000). Benthic ciliate identification and enumeration: An improved methodology and its application. *Aquatic Microbial Ecology, 22*, 79–91.

Woo, P. T. K. (Ed.). (2006). *Fish diseases and disorders, Volume 1: Protozoan and metazoan infections* (2nd ed.). Cambridge, MA: CAB International.

Zinskie, J. A., Shribak, M., Bruist, M. E., Aufderheide, K. J., & Janetopoulos, C. (2015). A mechanical microcompressor for high resolution imaging of motile specimens. *Experimental Cell Research, 337*, 249–256.