THE BIOLOGY AND ECOLOGY OF TINTINNID CILIATES

MODELS FOR MARINE PLANKTON

EDITORS:

JOHN R. DOLAN, DAVID J. S. MONTAGNES,
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Chapter 1

INTRODUCTION TO TINTINNIDS

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The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton, First Edition. Edited by John R. Dolan, David J.S. Montagnes, Sabine Agatha, D. Wayne Coats, and Diane K. Stoecker.

1.1 WHY A BOOK ON TINTINNID CILIATES?

Tintinnids are "curious" because their morphology is unusual for planktonic organisms: it is a shelled cell. There are other groups of shelled organisms in the plankton, such as foraminifera and pteropods, but very few are as diverse as the tintinnids. They show an astounding variety of forms in their shells (loricae), and dozens of different types can be found in just a few liters of seawater (Dolan & Stoeck 2011). Nonetheless, all have a similar fundamental trophic role in the pelagic ecosystem: that of grazers at the base of the food web feeding on nanoplankton, for the most part. They are, therefore, ecologically united as planktonic ciliates, mainly consumers of nanoplankton, and morphologically and phylogenetically united as shelled, choreotrich ciliates. The biology and ecology of this group merit investigation if we are to understand how species-rich planktonic food webs work.

Tintinnids attracted the attention of some of the first microscopists, who were fascinated by such "shelled infusoria". From the early oceanographic expeditions, the variety of forms of their loricae catalogued from the plankton net-tow material served as an example of biodiversity in the plankton. As they are among the few protist organisms sampled using plankton nets, they were for a time considered a major component of the microzooplankton (zooplankton ranging in size from 20 to 200 μm), grazers of the smallest planktonic algae in the ocean (Beers 1982). Beginning in the 19th century and still today, tintinnids have been the focus of detailed studies that together form a considerable body of literature consisting of hundreds of reports. In recent years, monographic treatment of tintinnids has seen something of a renaissance with the appearance of comprehensive works on tintinnids of the South Atlantic (Alder 1999), Brazilian territorial waters (Gomes et al. 2006), Lebanese coastal waters (Abboud-Abi Saab 2008), and the Adriatic Sea (Krsinic 2010). The literature on tintinnids constitutes a wealth of information covering a surprisingly wide range of topics and approaches. This information has, however, remained scattered across hundreds of journal articles and monographs published over nearly 150 years.

This volume is an attempt to bring together material covering distinct aspects and topics to allow a comprehensive view of the biology and ecology of tintinnids. The ultimate goal in assembling this material is to provide a digest (a complete compendium would required a much longer book) showing the complexity of the different facets of a well-circumscribed group of single-celled eukaryotes, organisms of the marine plankton.

Although tintinnid ciliates are characteristic of the marine plankton, there are a few species found in freshwaters. Freshwater tintinnids often appear to be ephemeral in lake plankton, blooming and then virtually disappearing. In this book, we focus on marine plankton; freshwater forms will not be treated, but mentioned only in passing.

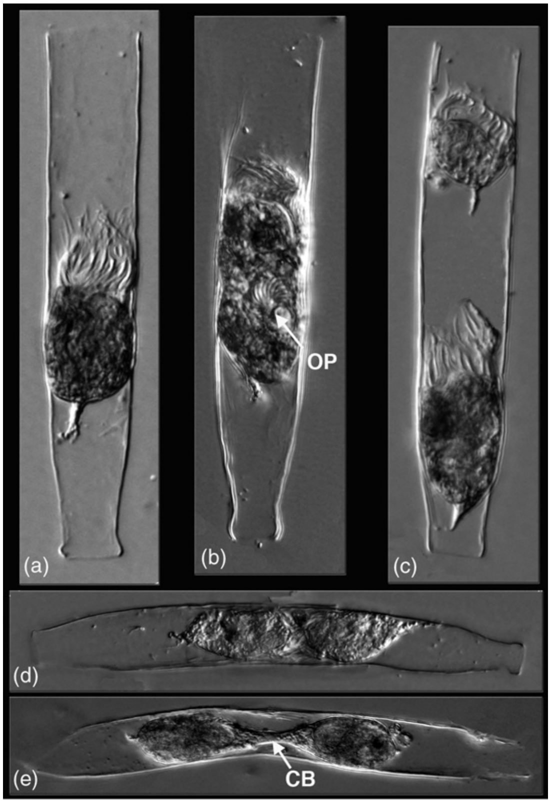

Fig. 1.1 Life-cycle stages in *Eutintinnus inquilinus*. (a) A trophont (feeding stage) cell. (b) An early stage of binary fission showing the oral primordium (OP), which will develop into a new mouth. (c) The final stage of cell division. (d) An early stage of conjugation. (e) A conjugating pair showing a well-developed cytoplasmic bridge (CB) through which nuclear material will be exchanged. Loricae are about 100 μm long. Images are of Lugol's-fixed cells; sample from a mixed culture provided by F. Lombard. For color version, see Plate 1.1.

1.2 WHAT IS A TINTINNID CILIATE?

Formally, tintinnids are ciliate protists of the class Spirotrichea, subclass Choreotrichida, order Tintinnida. Among the unicellular eukaryotes, the common characteristics of ciliates are possession of cilia during all or some part of the life cycle, nuclear dualism (macro- and micronuclei), and sexual recombination through conjugation, separate from reproduction which in tintinnids and most other ciliates is through binary fission (Fig. and Plate 1.1). Ciliates in the marine plankton are largely dominated by members of the class Spirotrichea, all of which have mouth cilia in the form of polykinetids (trichia), which are compound cilia that resemble bristles more than fine hairs arranged in a rough circle, more or less a spiral, around the oral cavity. Most marine planktonic ciliates are members of either the subclasses Choreotrichia or Oligotrichia. In both groups the oral polykinetids are well developed, serving both for locomotion and feeding. The subclass Oligotrichia (meaning "few trichia"), comprises forms with the oral polykinetids arranged in an open circle around the oral cavity and few cilia on the cell, such as in the familiar (often cone-shaped) oligotrichs of the genera *Strombidium* and *Laboea*. In contrast, species in the Choreotrichia have oral polykinetids arranged in closed circle around the oral cavity and usually relatively conspicuous rows of cilia on the cell surface. Choreotrich ciliates include species of the genera *Strobilidium* (often sphere-shaped), *Strombidinopsis* (often carrot-shaped), as well as species in which the ciliate cell is always surrounded by a shell (lorica), the tintinnid ciliates.

Besides the lorica, there are cytological characteristics that distinguish tintinnids from other choreotrichs (Fig. 1.2). The oral ciliature includes structures called tentaculoids, which appear to be related to prey capture (see Chapter 4). The posterior end of the cell is a contractile, stalk-like, peduncle which connects the cell to its lorica. The tintinnid cell surface has a distinct field of rows of short cilia, the somatic ciliature (see Chapter 3). These very brief characterizations are based on the detailed descriptions and analyses found in Lynn (2008), Lynn & Small (2000), and Corliss (1979).

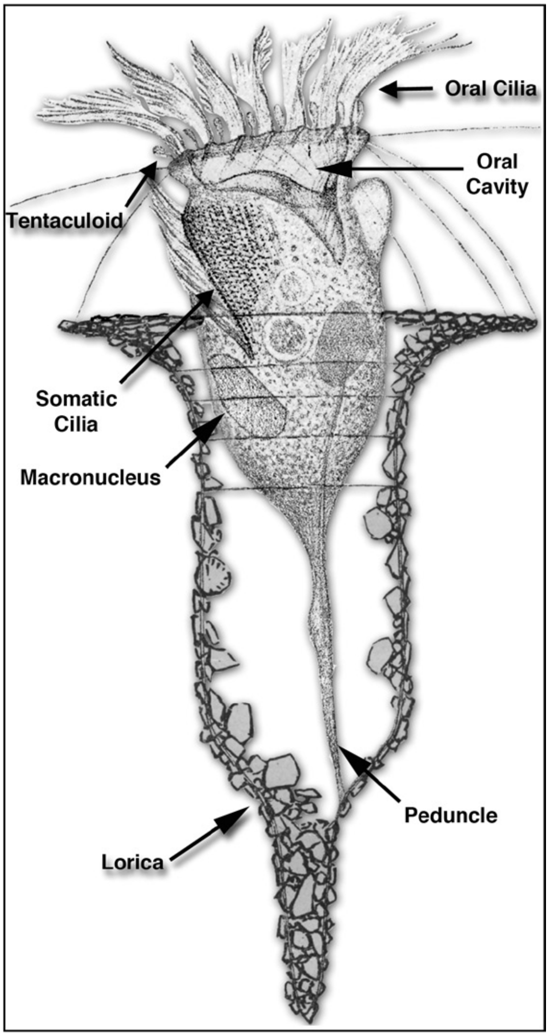

Fig. 1.2 Basic morphology of a tintinnid in a cut-away drawing of *Tintinnopsis campanula*, adapted from Fauré-Fremiet (1924). The oral cilia are comprised of oral membranelles arranged in closed circle around the funnel-shaped oral cavity. Interspersed between the oral membranelles are tentaculoids, structures presumed to be related to prey capture. The cell surface is covered in part by rows of short cilia, the somatic cilia. The macronucleus is often evident. The posterior end of the ciliate cell is attached to the lorica by a contractile peduncle.

1.3 THE LORICA AS THE DEFINING CHARACTERISTIC OF TINTINNID CILIATES

In addition to being the only choreotrich ciliates with loricae, tintinnids are also the only ciliates of the plankton with loricae. Although there are cytological characteristics that distinguish tintinnids, the defining one is its lorica or shell (although other cytological characters distinguish tintinnids from other choreotrichs or "naked oligotrichs": see Chapter 3). The lorica, although of various shapes ranging from tubular to vase- or bowl-shaped, is always open at one end, the oral end, and closed or tapered at the opposite, or bottom end. Within the lorica the tintinnid cell is attached near the bottom end by a contractile portion of the ciliate cell, a pedicle or peduncle (see Fig. 1.1). While feeding, the cell extends out of the lorica and the tintinnid is propelled mouth-end forward. If disturbed, the cell retracts into the lorica.

The contractile behavior and the lorica as the defining character were apparent in the text of the first description of a tintinnid by O.F. Müller published in 1778 (Box 1.1). The "creature" was described as *Trichoda inquilinus*; all ciliates were placed in the genus *Trichoda* by Müller; *inquilinus* – "lodger" or "occupant" – refers to the ciliate as the inhabitant of a structure. Remarkably, not only was the contractile behavior noted but also that reproduction was by cell division. Müller noted that the "mother cell" (the proter that retains the original mouth) abandons the lorica to the newly formed daughter cell. The form he described might be the species now called *Eutintinnus inquilinus* (shown in Fig. 1.1), known to attach to particles (Fauré-Fremiet 1908; Jonsson et al. 2004). The appellation *Tintinnus inquilinus* was given to the species by Schrank (1803) who removed it from *Trichoda*, giving it its own genus, without unfortunately noting why he chose the term "*Tintinnus*", which most commonly refers to the ringing of a bell.

Very early on it was noted that distinct types of lorica exist among tintinnids and that these may represent natural groups (Claparède & Lachmann 1858). A completely lorica-based taxonomy and phylogeny was introduced by Kofoid & Campbell (1929, 1939) and identifications are today still based on lorica morphology. However, culture studies and recent molecular phylogenies have shown that lorica characteristics do not always reflect phylogenetic relationships (see Chapters 2 and 3). Nonetheless, for practical reasons, tintinnids traditionally have been divided into two groups corresponding to easily distinguished lorica types: one with agglutinated (or agglomerated) loricae composed of particles and another, those with hyaline, generally transparent, loricae. The two lorica types very approximately correspond to different habitats in which tintinnid species with the different loricae are the dominant forms: coastal and open waters (see Chapter 10).

Mineral particles are used in the "agglutinated" loricae of species in the genera *Tintinnopsis*, *Stenosemella*, *Tintinnidium*, and *Leprotintinnus*. These forms are generally restricted to coastal waters. In some genera there are species that incorporate the hard parts of some microalgae. The coccoliths from coccolithophorid algae are used by some species (*Codonella*, *Dictyocysta*, *Codonellopsis*, and *Acanthostomella*) or the remains of diatoms (*Laackmanniella*). The arrangement of "agglutined particles" can be quite precise. For example, in *Codonella elongata*, the coccolithophores are all oriented in the same "face-out" direction (Fig. 1.3). However, not all species show precision in particle use. Examples of the variety of forms with agglutinated lorica are shown in Fig. and Plate 1.4. These species generally dominate tintinnid assemblages in coastal waters but some can be found in open waters.

"Hyaline loricae" groups tintinnids of a very large variety of shapes, sizes, and architectures whose sole common characteristic is a lack of extraneous particles in the lorica. The morphologies range from the transparent small (50 µm diameter) bowl-shaped *Ascampbellia tortulata* to the large, conical *Cyttarocylis cassis* which shows a sculpted surface. Although some hyaline lorica species are found in coastal waters (e.g. species of *Helicostomella*, *Favella*) typically they are the dominant forms in open-water tintinnid assemblages. A sampling of the diversity of hyaline-lorica forms is shown in Fig. and Plate 1.5.

The term "lorica" refers to the armor or protective clothing worn by roman soldiers. Dujardin (1841) credits Ehrenberg (1832) with using the word in his Latin text to describe the protective structures, *Panzer* in the German text, of tintinnids as well as other protists and rotifers. Indeed, the hard structures of many different protists appear early in the fossil record and likely evolved as protection against predation by other protists (Porter 2011). Among planktonic organisms, the seemingly odd morphologies of present-day forms are thought to be the result of a "watery arms race" between prey and predator (Smetacek 2001; Hamm & Smetacek 2007). In contrast, Kofoid & Campbell (1939) stated that the lorica probably did not evolve as protective device because it affords little protection against large metazoan predators such as copepods. However, tintinnids are subject to predation from a very wide range of predators (Chapter 5) as well as

BOX 1.1 Translation of the 1779 Latin text of O.F. Müller

Trichoda inquilinus (T. lodger): a sheathed trichoda, with a hyaline, cylindrical, shell and a little footstalk

This is among the smallest creatures, and with the slow work of a simple lens, the eye observes a very small point with great mobility. The shell, whether egg-shaped, oblong, smooth, is so glassy transparent that otherwise invisible things might be seen. For this creature is a swimmer, which you might say has a pivoted footstalk . . .

I have been able to observe these things for a long time with my eyes unaided, being apparent in great numbers. The most apt name should account for the quick motion and the rotation of the hairs. Often the organ of the hairs is spread out resembling a comb, then the more distinct, outermost, blackish teeth, in the hyaline jelly of the true center, which are show various movements, are seen.

The clear footstalk runs through the middle of the sheath, fixed though at the wide base. Often it is contracted, the hairs are together, the little head is submerged, and the side strings of the sheath stand forth like a pregnant creature stretching. Slowly the head retreats back, with the hairs, whether thin tentacles or spread about, extend, barely occupying the entrance of the sheath, intently moving for nutrition. I saw it withdraw within the sheath and be spread out at the top of the head.

Having been disturbed by dust (and I don't know where the center is), it becomes very bare, it adheres to very many things and moves about slowly; it swims around vehemently with the head clinging on to the outside of the sheath with the work of the hairs; if it is clinging on to the base of the sheath, fixed only a little, whether extended in a perpendicular or oblique fashion, an alternate movement of the top of the head follows, moving in water continuously or less so.

I have found two swimming together lengthwise. Whether it was at the base of the sheath, or lying higher up, or lower than the top, and the head was pulled about in every sense: at length it tore itself apart, front from back.

I saw a certain one, the heads of which were more than threefold, and they fully occupied the whole sheath even with the footstalk inconspicuously or for a short time; also two by two the little creatures lived in the sheath with their own footstalks.

An extension developed, while I watched the little creature growing. The head is divided in the middle and the bottom at the base of the footstalk, while what happens must be due to the workings of the hairs. By what means the middle of the sheath is truly divided I know not even though keeping them in clean and pure sea water, even in entirely non-renewed water, through the whole of winter 1776, 1777 and 1778.

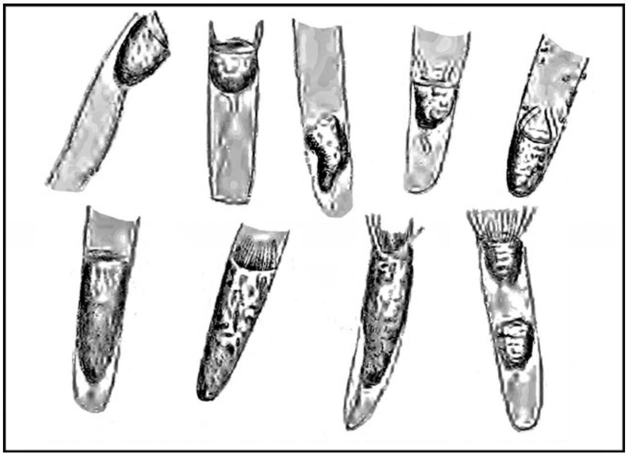

Fig. B1.1 The lodger occupies various places; small, scaly sheaths called Monads are found together and sticking together in the fifth image. A series of fertile mothers having taken positions to divide, laden and mature, and at the last image a mother is now swimming away. *O.F. Müller (1779) pp. 8–9 (Latin translation by B. Scott, University of Liverpool).*

Image not available in this digital edition.

Fig. 1.3 Scanning electron micrograph of a specimen of *Codonella elongata* from the Bay of Villefranche. The upper panel shows the lorica with a bottom portion cut away with a high-energy electron beam. The lower panel shows the interior surface of the lorica. Note that the lorica is agglomerated with the coccolithes of *Helicosphaera carteri* oriented as they are on the living algae. Images are courtesy of François Guyot and Charles Bachy. An image of a live specimen of *C. elongata* is shown in Fig. 1.4.

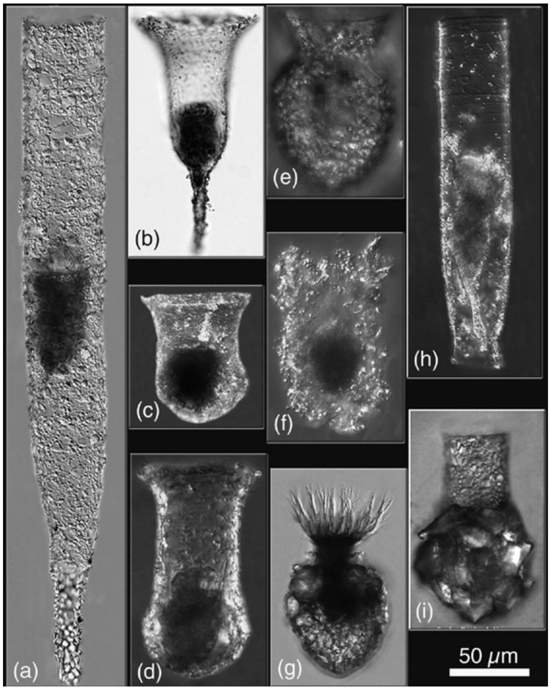

Fig. 1.4 Some tintinnid species with agglutinated loricae: *Tintinnopsis radix* (a), *T. campanula* (b), *T. dadayi* (c), *T. spiralis* (d), *Codonella elongata* covered with coccoliths (e), *Tintinnidium* sp. (f), *Stenosemella ventricosa* (g), *Leprotintinnus pellucidus* (h), and *Codonellopsis schabi* (i). Species names are attributed based on lorica morphology. All the specimens are Lugol's-fixed cells except for *Codonella elongata* which was a live specimen. For color version, see Plate 1.4.

parasitic infections (Chapter 6). In reality, the identity of the major source of mortality for tintinnids is unknown so it is difficult to assess the possible use of the lorica as a protective device.

Many explanations other than "armor" for the lorica have been suggested. These include that of a flotation aid in the case of the hyaline lorica (Kofoid 1930; Campbell 1926), alternatively as aid in quickly sinking away from predators in the case of the agglutinated lorica of *Stenosemella* (Capriulo et al. 1982), and maintaining directionality in swimming in the case of the elongate nail or spike-shaped lorica of oceanic forms (Kofoid & Campbell 1939). A lorica, like projecting spines or trailing structures in other small zooplankton, may reduce swimming speed while enhancing the capture of food particles by increasing fluid motion

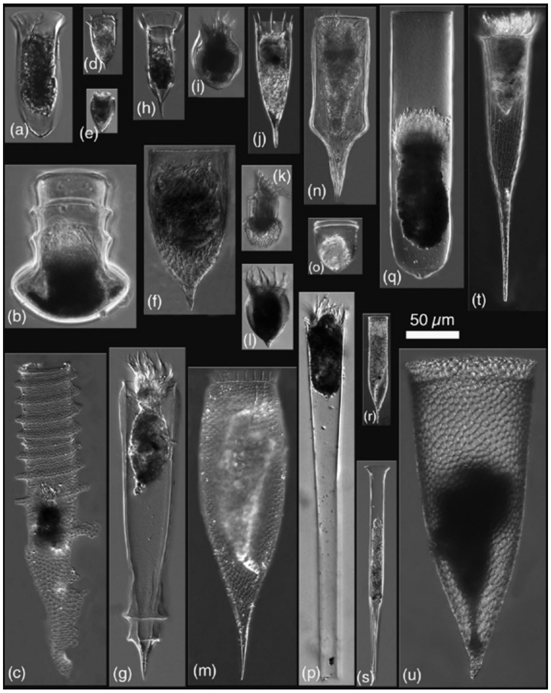

Fig. 1.5 Examples of tintinnid species with hyaline loricae: *Amphorides quadrilineata* (a), *Amplectella collaria* (b), *Climacocylis scalaria* (c), *Acanthostomella conicoides* (d), *Protorhabdonella simplex* (e), *Epiplocylis blanda* (f), *Xystonellopsis paradoxa* (g), *Ormosella trachelium* (h), *Proplectella elipisoida* (i), *Dadayiella ganymedes* (j), *Dictyocysta lepida* (k), *Metacylis mediterranea* (l), *Parafavella parumdentata* (m), *Parundella messinensis* (n), *Ascampbellia tortulata* (o), *Eutintinnus stramentus* (p), *Undella hyalina* (q), *Helicostomella subulata* (r), *Salpingella acuminata* (s), *Rhabdonella spiralis* (t), and *Cyttarocylis cassis* (u). All the specimens are Lugol's-fixed cells. For color version, see Plate 1.5.

around the oral cilia (Emlet & Strathmann 1985). The lorica can also serve as an attachment device. Jonsson et al. (2004) found that in some species of *Eutintinnus*, the tintinnid can reversibly attach its lorica to detrital particles thus increasing its feeding rate owing to the "tethering effect" (Strathmann & Grünbaum 2006) and reducing its susceptibility to copepod predation. Lastly, the lorica may furnish protection against ultraviolet radiation (Armstrong & Brasier 2005), allowing tintinnids to exploit the near-surface waters. There appears no reason not accept the possibility that the variety of loricae found among tintinnids may serve a variety of functions.

1.4 HISTORY OF TINTINNID STUDIES

Early mentions of tintinnids are mostly in taxonomic treatises and consist of little more than simple listings in species catalogues of "infusoria" (i.e., Müller 1776, Schrank 1803; Ehrenberg 1832; Dujardin 1841). The first detailed consideration of tintinnids was by Claparède & Lachmann (1858–1860, part 1, pp. 192–221) who provided notes on the morphology and ecology of most of the 17 known species, and placed them all in a single genus, *Tintinnus.* They remarked on the abundance of tintinnids in marine waters compared with their rarity in freshwaters, and the diversity of lorica types. The appearance of more marine biological studies of tintinnids marked the last quarter of the 19th century.

In 1873 Ernst Haeckel published the first paper devoted exclusively to tintinnids. He not only described new species from waters off Messina (Italy) and Lanzarote (Canary Islands), but also the development and release of what he thought were reproductive spores. We now know that he was the first to describe parasites in tintinnids (see Chapter 6). A few years later appeared the observations of Herman Fol (1881, 1883, 1884) on tintinnids from the Bay of Villefranche-sur-Mer, describing investigations into the chemical nature of the lorica (see Chapter 2) and some new species. These reports were followed by a series papers from the Zoological Station in Naples first by Géza Enzt, senior (1884, 1885) and then Eugene von Daday (1886, 1887) who published the first monographic review of tintinnids (Daday 1887). The number of known species rose to 70 with the monograph; he also included a key to the species listed (many of which were new) and reviewed reports of anatomy and what little was known of physiology as well as biogeography.

Detailed observations on feeding and fine structure did not begin until the early 20th century, perhaps because maintaining tintinnids in the laboratory proved difficult (as Müller had remarked back in 1778). Emmanuel Fauré-Fremiet published in 1908 the first extensive report on a tintinnid, now known as *Eutintinnus*, possibly the same species Müller observed, based on both living and fixed specimens. This was followed by Géza Entz junior (1909b) who notably included the study of fine-structure based on thin sections of what is now known as *Favella*. These studies of tintinnids, based on specimens from near-shore waters, were soon eclipsed by the development of biological oceanography. In terms of tintinnid studies, oceanography turned the focus toward the open sea, first with regard to taxonomy and new species, and later to the role of tintinnids in marine ecosystems.

Distinct forces prompted the development of biological oceanography in the late 19th and early 20th centuries. These ranged from the primary one of the need to understand the dynamics underlying exploited marine populations, especially herring and cod, to nationalistic desires to show mastery of the seas and science (Mills 1989). Regardless of the precise reasons behind the great oceanographic expeditions of the early 20th century, the study of tintinnids profited greatly. Although singularly neglected by the English Challenger Expedition, in the subsequent oceanographic expeditions of many countries tintinnids were granted the same attention as that given to other planktonic groups such as diatoms, dinoflagellates, and copepods. The major publications include Karl Brandt's monographs from the German 1899 Plankton Expedition (Brandt 1906, 1907), the Hans Laackmann reports from the German South Polar Expedition of 1901–1903 (Laackmann 1907, 1910), Alphonse Meunier's reports from the Duke of Orleans Arctic expedition of 1907 (Meunier 1910), and Eugen Jörgensen's monograph from the Danish Thor Expedition to the Mediterranean in 1908–1910 (Jörgensen 1924). These largely taxonomic treatises, cataloguing species occurrences and describing new forms, reached their apex with the reports of Charles Kofoid and Arthur Campbell on material from the Agassiz Expedition (Fig. 1.6) of the USA to the Eastern Tropical Pacific in 1904–1905 (Kofoid & Campbell 1929, 1939).

In describing the species found in material from the Agassiz expedition, Kofoid and Campbell produced two monographs. The first, "A conspectus" (Kofoid & Campbell 1929), was a compendium of previously described forms as well as new species from the expedition material and other samples they collected. At that time the literature contained descriptions over 1750 different forms, variously described as species, subspecies, and varieties of tintinnids. The "conspectus" ascribed species status to 705 forms in 51 genera, all based uniquely on features of the lorica (Kofoid & Campbell 1929). Some years later, Kofoid and Campbell published a second report, which concerned exclusively the tintinnids from the Agassiz Expedition. In this second monograph they re-named and re-arranged many tintinnid genera and described some new species yielding a new total of 726 species (Kofoid & Campbell 1939). Other notable studies include those of Hada working in Japanese waters and the Western Tropical Pacific (1937, 1938), and Campbell's report on material from the last voyage of the Carnegie throughout the Pacific (1942). Although such efforts in descriptive zoology continued in the latter half of the 20th century, their pace slowed considerably with a shift in focus toward the role of tintinnids in marine food webs.

A turn toward ecological studies was belated because tintinnids and other small protist grazers had long been suspected to be of importance. Naive views on marine food webs can be understandably summarized by the saying "Big fish eat little fish" (Fig. 1.7). However, by the late 1800s it was recognized that, rather than plant and organic matter supplied by rivers feeding small fish, plankton formed the base of marine food webs. Furthermore, plankton production was likely in some manner linked to exploited fish populations. This view provided the scientific justification of Victor Hensen's Plankton Expedition in 1889, which is generally considered as the first campaign of biological oceanography (Mills 1989). The studies of Kofoid (1897) and Lohmann (1901) had shown that most of the biomass in the plankton was missed using plankton nets, even the "fine silk" used to sample for tintinnids and other small plankton.

By the 1920s careful studies of coastal fish populations and food webs, for example the herring food web, revealed the complexity of predator–prey relationships in the plankton (Fig. 1.8 and see Chapter 5, Fig. 5.4). Thus, by the 1930s, the planktonic community was known to be dominated, at least in terms of biomass, by very small organisms and the community as a whole was characterized by complex predator–prey relationships. Nonetheless, studies on the ecology of tintinnids and other small grazers of the plankton did not begin in earnest until the 1960s.

The period of relative inactivity from the 1930s to the 1950s was attributed by John Beers (1982) to the development of pigment methods in phytoplankton studies which replaced the use of microscopic analysis of water samples. Meanwhile, zooplankton studies continued to rely on net sampling. The effect was then that microzooplankters, such as tintinnids, were overlooked or ignored because few people were examining

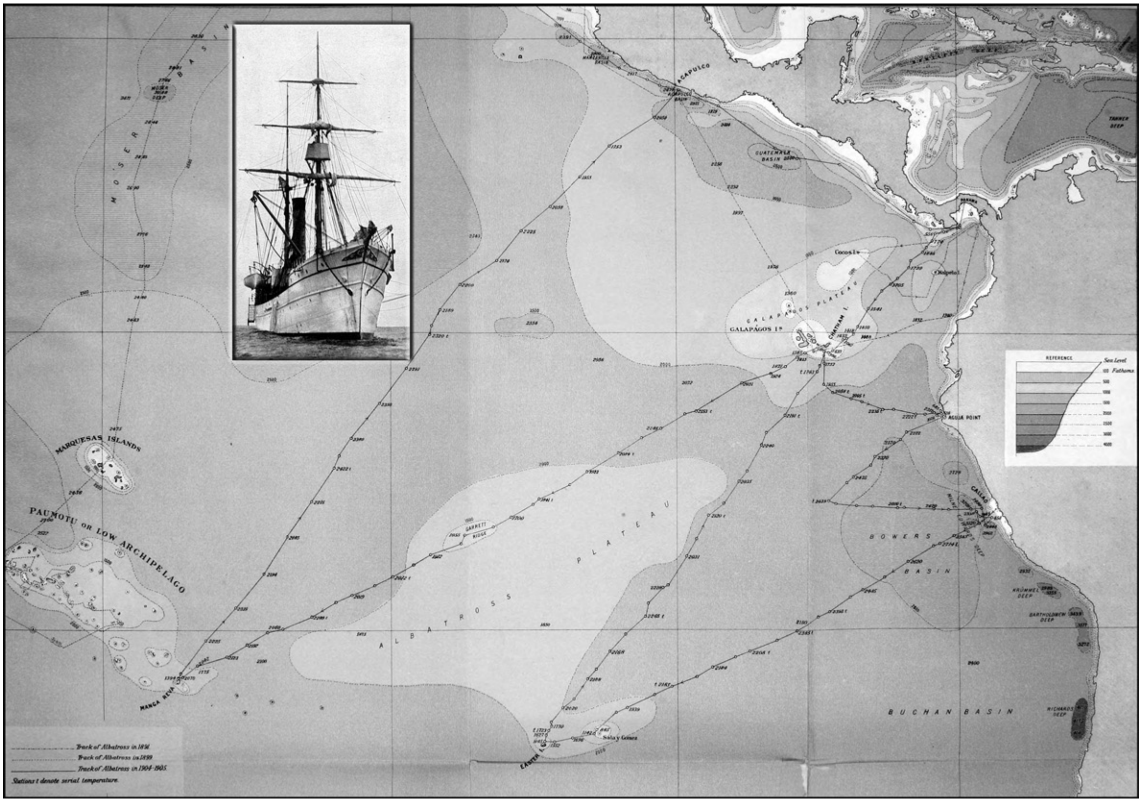

Fig. 1.6 Station map of the Agassiz expedition (from Agassiz 1906). Inset image, courtesy of the National Oceanic and Atmospheric Administration (NOAA) image library, shows the *Albatross* on station. Louis Agassiz organized the oceanographic expedition which explored the Eastern Tropical Pacific in 1904–1905 with the assistance of the US navy using the US Fisheries Commission steamer *Albatross*. The scientific staff included two notable "assistants": C.A. Kofoid and H.B. Bigelow. The material gathered during the expedition was the basis for the two widely used monographs of Kofoid & Campbell (1929, 1939).

water samples with a compound microscope. What occurred in the early 1960s was firstly that zooplankton researchers were pointedly reminded that plankton nets were not sampling all the grazers, that is all of the zooplankton (Banse 1962; Hansen & Andersen 1962). Furthermore, the use of size-fractionation reminded phytoplankton researchers that small phytoplankton often accounted for most of the standing stock (see, for example, McAllister et al. 1960) and these small cells were not usually thought of as major food items of the zooplankton sampled with nets.

Field studies focusing on microzooplankton, in particular those of Beers & Stewart (1967, 1969), showed unequivocally that microzooplankton represented a considerable biomass. Microzooplankton were then apparently "re-discovered" in the 1960s so that by the end of the decade, John Ryther (1969) in "Photosynthesis and fish production in the sea" could simply state:

> "Intermediate between the nannoplankton and the carnivorous zooplankton are a group of herbivores, the microzooplankton, whose ecological significance is a subject of considerable current interest. Representatives of this group

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Fig. 1.7 The naive view of marine food webs as "Big fish eat little fish" was illustrated by Pieter Bruegel the elder in 1557, as an allegory of human greed. Until the late 19th century, the base of the marine food chain (nourishing the littlest fish) was thought to be matter supplied by river input and shallow water plants. Image of a lithograph version, © Trustees of the British Museum.

> include protozoans such as Radiolaria, Foraminifera, and Tintinnidae, and larval nauplii of microcrustaceans. These organisms, which may occur in concentrations of tens of thousands per cubic meter, are the primary herbivores of the open sea."

Microzooplankton were placed, for a time, at the center of a new view of marine food webs (Fig. 1.9). However, it was recognized that protozoan ecology, as a whole and including planktonic protists, was sorely under-developed (Corliss 1973). Perhaps not surprisingly, in the ensuing years, the ecology and physiology of tintinnids received considerable attention.

An extensive series of studies was published by Ken Gold covering culture (Gold 1968, 1970, 1971, 1973), coastal communities (1975, 1977), as well as anatomy and physiology (Gold 1969a, b, 1979; Gold & Morales 1975a, b, 1976a, b, c; Gold & Pollinger 1971). Gold's

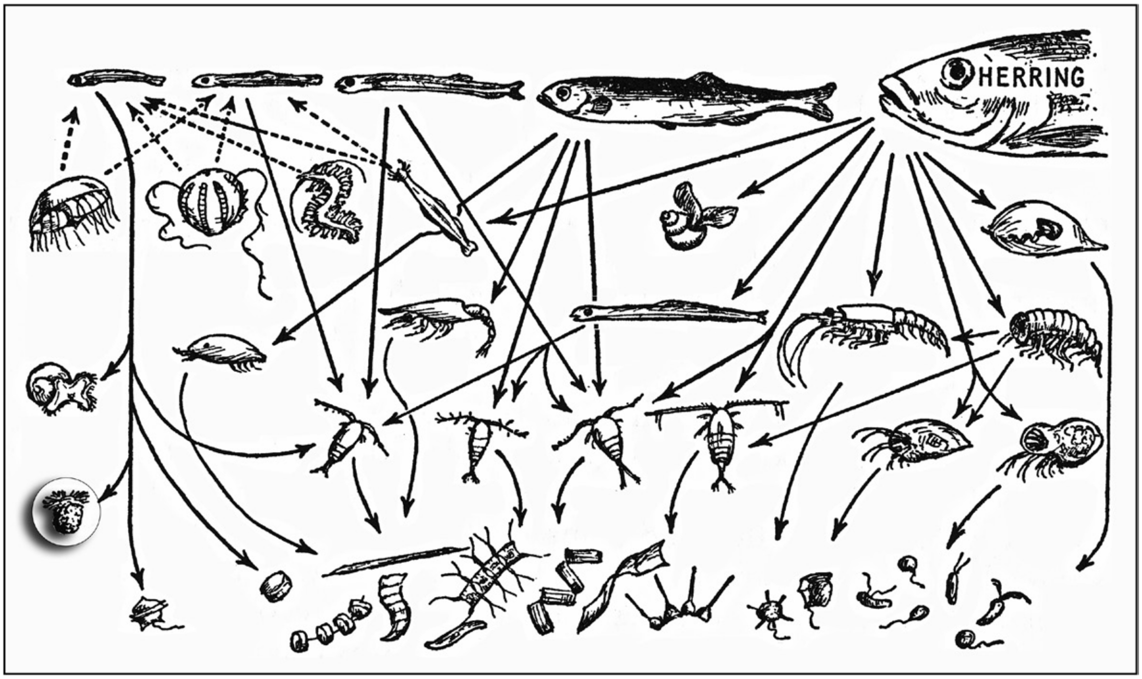

Fig. 1.8 By the end of the 19th century, planktonic organisms were recognized as the base of a complex food web supporting exploited fish populations. The drawing by Hardy (1965) depicts the herring food web based on the relationships described in his 1924 herring monograph. Note in the lower left a tintinnid. Figure adapted from Hardy (1965).

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Fig. 1.9 Trophic relations of microzooplankton after Conover (1982). Heavy arrows denote direct relations. (a) A diatom. (b) An autotrophic nano-flagellate. (c) A *Ceratium* (dinoflagellate). (d) A gymnodinid dinoflagellate. (e) Single cells of autotrophic prokaryotes. (f) A dinophysid dinoflagellate. (g) Clumped cells of autotrophic prokaryotes. (h) Single cells of heterotrophic prokaryotes. (i) Detrital particle with attached bacteria. (j) Heterotrophic nanoflagellate. (k) Detrital particle. (l) Small chaetognath. (m) Tintinnid ciliate. (n) Copepod nauplius. (o) Appendicularian. (p) Copepod. (r) Ctenophore. (s) Large chaetognath. (t) Planktivorous fish. © Collection of Musée Océanographique de Monaco, Fondation Albert Ier, Prince de Monaco.

laboratory investigations along with the further field studies of Beers & Stewart (1970, 1971) reiterated the probable importance of microzooplankton, of which tintinnids were an obvious component. Perhaps consequently, between 1974 and 1984, worldwide at least ten PhD theses specifically addressed the ecology of tintinnids in planktonic ecosystems: Blackbourn (1974) in northwest Canada, Johansen (1976) in eastern Canadian, Hedin (1976) in Sweden, Heinbokel (1977) in California, Rassoulzadegan (1975) in France, Souto (1979) in Argentina, Damodara (1981) in Southern India, Brownlee (1982), Capriulo (1982), and Verity (1984) in the northeast USA. During this time extensive cytological investigations of tintinnids were carried out (Hedin 1975a; Laval 1971, 1972; Laval-Peuto 1975, 1976a,b, 1977; 1981; 1983).

The period of the mid-1970s to the mid-1980s also saw the development of our current view of the structure of planktonic ecosystems, a view that recognizes the importance of picoplankton, both autrophic and heterotrophic. This period then included the proposition of the “microbial loop” and the beginnings of the modern field of aquatic microbial ecology. The reassessment of the structure of planktonic communities, as distinct both qualitatively and quantitatively from a linear view of primary producer to herbivore to fish, was prompted by several discoveries (or re-discoveries), as follows.

1. Both primary production and phytoplankton biomass are dominated by small (<20 μm) cells in most systems (as reviewed in Malone (1980)).

2. Phytoplankton are a considerable direct source of dissolved organic carbon, excreting a significant portion of the carbon fixed through photosynthesis (e.g., Sharp 1977; Sellner 1981).

3. The abundance of bacteria is high, much higher than previously thought (revealed through the development of direct-counting techniques using fluorescent stains) and relatively invariant (i.e., Hobbie et al. 1977).

4. Bacterioplankton are active with bacterial secondary production of the same order of magnitude as primary production in many systems (Fuhrman & Azam 1980).

The conclusion that a substantial and active microbial community exists in the plankton was inescapable. However, its structure was unclear. Field and laboratory experiments with tintinnids, taken as typical microzooplankton, had shown high grazing rates on nanoplankton size prey (Heinbokel 1978a, b; Heinbokel & Beers 1979; Rassoulzadegan 1978, Rassoulzadegan & Etienne 1981; Stoecker et al. 1981). However, there was little evidence that microzooplankton were major consumers of bacteria-sized organisms. In fact, work with freshwater ciliates suggested that ciliates typical of the marine microzooplankton were poor candidates for the role of bacteriovores (Fenchel

1980a, b, c, d). In contrast, studies of the feeding and abundances of marine heterotrophic nanoflagellates strongly supported their proposition as bacteriovores (Fenchel 1982a, b, c, d). There remained the need to explain high bacterial abundances and growth rates.

Most of the pieces of the puzzle, perhaps first noted by Pomeroy (1974), were put together with the proposition of the microbial loop by Azam et al. in 1983. The "loop" was one of dissolved organic matter. It is excreted by both autotrophic and heterotrophic microbes as well as metazoan zooplankton and it supports bacterial production. Heterotrophic nanoflagellates consume the bacterial production and grazing by microzooplankton accounts for the production of heterotrophic nanoflagellates. A simplified contemporary view of the microbial loop in the plankton is shown in Fig. 1.10. Not shown in the figure are complexities such as mixotrophy in the phytoplankton, parasitic organisms (see Chapter 6), and the large variety of predators known to feed on microzooplankton such as tintinnids (see Chapter 5).

Not long after the formal proposition of the microbial loop, the true complexities of the microbial component of marine plankton became apparent. It was found that across all microbial size-classes, taxonomic affinity was a very poor predictor of the basic trophic attribute of autotroph, heterotroph, or mixotroph. The existence of a complex microbial food web characterized by many connections among microbes of blurred trophic roles (prokaryotic and eukaryotic alike), as well with higher trophic levels, was recognized (e.g., Sherr & Sherr 1988). Attention was diverted from tintinnids in part because there is, in reality, no "typical" microzooplankter, and furthermore tintinnids are usually but a minority component of the assemblage "protist microzooplankton" (see Chapter 9). In addition, research activity in plankton food-web dynamics slowed somewhat throughout the 1990s. In the oceanographic community there was a clear shift in focus to prokaryote communities. The development of molecular tools made prokaryotes much more amenable to study (e.g., Sherr & Sherr 2008a) and dissolved organic matter, the substrate supporting heterotrophic prokaryotes, was found to be a pool of carbon much more dynamic than previously thought (Hedges 2002). In recent years most tintinnid studies have focused on questions of diversity and distributions (e.g. Fonda Umani et al. 2011; Sitran et al. 2009; Thompson & Alder 2005) or taxonomy and phylogeny, notably using a combiniation of both molecular and classic

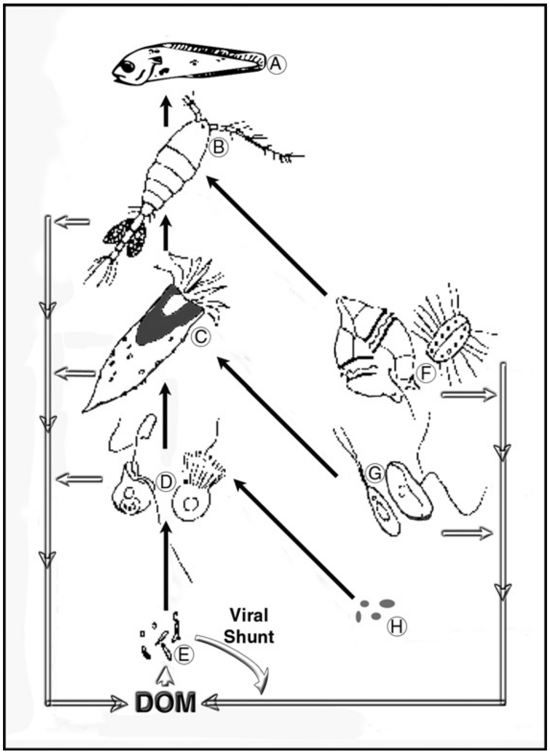

Fig. 1.10 Simplified contemporary view of the microbial loop in the marine plankton and the microbial food web based on the relationships described in Fenchel (1988). Dissolved organic matter (DOM) originates from a large pool of organisms of diverse trophic levels, with flows shown as the open lines, and is consumed by heterotrophic bacteria (e). The "viral shunt" is the flow of DOM from bacteria themselves, lysed from viral infection, back into the DOM pool. The solid arrows show predator–prey relations. Larval fish (a) eat copepods (b). Copepods eat large phytoplankton (f) such as dinoflagellates and diatoms as well as small microzooplankton such as tintinnids (c). The autotrophic nanoflagellates (g), and the heterotrophic nanoflagellates (d), are eaten by the microzooplankton. The heterotrophic nanoflagellates feed on autotrophic bacteria (h), such as *Synechococcus*, as well heterotrophic bacteria. The "microbial loop" proper is the production of DOM, its flow to heterotrophic bacteria transforming it into bacterial biomass, and the subsequent transfer of this bacterial biomass up the food web through whose activities, in part, supply the DOM pool thus closing the loop.

morphological data (e.g. Kim et al. 2010; Agatha & Strüder-Kypke 2007, 2012; Santoferrara et al. 2012a, 2012b; Xu et al. 2012; Bachy et al. 2012), an approach that appears especially valuable for tintinids (see Chapters 2 and 3).

1.5 TINTINNIDS AS MODEL ORGANISMS FOR MARINE PLANKTON

Hutchinson posed the question, “How can so many species co-exist in a relatively homogenous environment?”; his “Paradox of the Plankton” is now over 50 years old (Hutchinson 1961). So far, no explanation, including his own, has been generally accepted and new mechanisms continue to be proposed (e.g., Shoresh et al. 2008; Fox et al. 2010). Furthermore, the diversity Hutchinson described was based on observations of morphologically defined species and this diversity is now known to be dwarfed by the genetic diversity revealed by sequencing the DNA of natural plankton communities. Genetic data suggests that thousands of species of protists can be found in a few liters of seawater (see, for example, Edgcomb et al. 2011). Such an astounding diversity is difficult to explain. Complicating any attempt is the fact that for most species-rich groups of planktonic organisms, phylogeny, morphology, and ecology are not easily related to one another. For example, harpacticoid copepods include both planktonic and benthic species, forms that are herbivorous, others that are carnivorous, or still others that are parasitic. Similarly, gymnodinid dinoflagellates can be benthic, planktonic, phototrophic, mixotrophic, heterotrophic, or parasitic. In contrast to such taxa, tintinnid ciliates represent a singular exception of a coherent group of organisms.

Tintinnid ciliates constitute a single suborder of the ciliate order Choreotrichidae and are thus phylogenetically united. In terms of morphology, all are characterized by the possession of a shell (lorica) whose architecture forms the basis of classic taxonomic schemes. In this species-rich group (over 1000 morphotypes have been described, see Chapter 3), virtually all are restricted to the marine plankton. A few species are known from freshwater plankton, but no tintinnids are benthic. In marine plankton assemblages they are ubiquitous and the great majority are consumers of nanoplankton, including the smaller size-fractions of phytoplankton. Distinct species have long been distinguished using light microscopy. Consequently, compared with most other groups of planktonic organisms, especially other planktonic protists, there exists a very substantial and relatively detailed literature, both modern and historical. Tintinnids are a phylogenetically coherent group (e.g., Lynn 2008) of morphologically and ecologically similar species (e.g., Dolan 2010). Thus, they are the ideal group of planktonic organisms to examine questions of variability in time, space, and composition.

Tintinnid ciliates most likely originated in the marine plankton from a shell-less, oligotrich-like ciliate (see Chapter 3). Tintinnids are often attributed one of the most extensive and continuous fossil records for zooplanktonic organisms, dating back to the Ordovician period (Fig. 1.11). Fossils resembling tintinnid loricae have been described as fossil tintinnid species and continue today to be described as tintinnids (see, for example, Bignot & Poignant 2010). Thus, in principle, tintinnids are good model organisms for reconstructing paleo-environments. Unfortunately, the fossil record of tintinnids is, in reality, ambiguous. This is because many fossil remains reported to be tintinnids, for example calpionellids, most likely are not (see Chapter 8). The fossil loricae of calpionellids are formed of calcite and as no known ciliates form external structures of calcite, calpionellids were probably

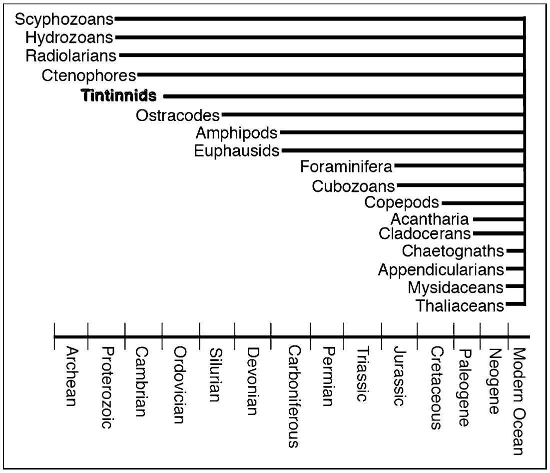

Fig. 1.11 The fossil record of major zooplankton taxa according to Rigby and Milsom (2000). The record attributed to tintinnids is typically one of the oldest among contemporary taxa of the plankton. However, many fossils described as tintinnids probably are not (see Chapter 8). Based on data from Rigby & Milsom (2000).

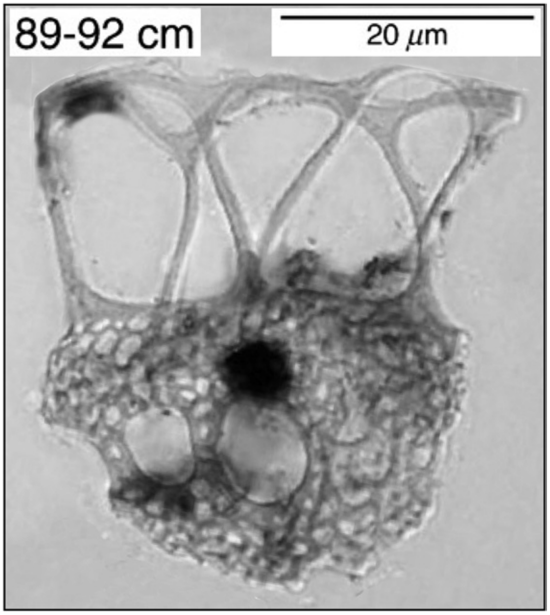

Fig. 1.12 A lorica of *Dicytocysta lepida* from a section of a sediment core dated to 3000–6000 years before present. Sediment core samples from the Orca Basin (2249 m depth) of the Gulf of Mexico contained tintinnid loricae and crustacean remains in the section corresponding to 89–92 cm depth in the core. Reprinted from Earth and Plantary Science Letters, 272, Meckler, A.N., Schubert, C.J., Hochuli, P.A., Plessen, B., Birgel, D., Flower, B.P., Hinrichs, K.-U. & Haug, G.H. Glacial to Holocene terrigenous organic matter input to sediments from Orca Basin, Gulf of Mexico. A combined optical and biomarker approach, 251–263. Copyright (2008) with permission from Elsevier.

not even ciliates, much less tintinnids (Remane 1963, 1998). Some authorities then attribute a fossil record to tintinnids which begins only in early Jurassic period (e.g., Armstrong & Brasier 2005).

Although the fossil record is unsettled, it is clear that the remains of tintinnids can provide evidence of relatively recent climatic change. Very well preserved lorica have been found in late Holocene deposits (Fig. 1.12). Loricae are among the microfossils that can be used as indicators of ecological and hydrographic shifts. For example, changes in an Antarctic lake were inferred using the sediment record of tintinnids and other planktonic organisms by Cromer et al. (2005). Likewise paleo-hydrographic changes in waters surrounding the Faroe Islands were investigated by analyzing the occurrences of dinoflagellate cysts, acritarchs, and tintinnid loricae in sediment cores (Roncaglia 2004).

Tintinnids are probably the group of planktonic protists with best potential for analysis using automated or semi-automated imaging systems. They are relatively large and the geometry of the lorica is simple. Furthermore, some characteristics of the simple geometry, specifically the diameter of the lorica oral opening, is both of taxonomic significance in distinguishing species (see Laval-Peuto & Brownlee 1986) and correlates with some ecological characteristics, such as prey size (Fig. 1.13) or maximum growth rate (see Chapter 4). Among tintinnids, morphological diversity is correlated with species diversity and morphological differences reflect ecological differences. Therefore, data in the simple form of morphological descriptors can provide information on species diversity and ecological characteristics in tintinnids. Unfortunately, existing imaging systems are not suitable for analyzing most natural assemblages in their entirety. The size range of tintinnid loricae (50–400 μm length, 15–200 μm diameter) is incompatible with either the size range or resolving capacity of existing instruments such as the Flowcam (e.g., Zarauz et al. 2009) or the ZooScan (e.g., Gorsky et al. 2010). However, the near future, like the recent past, will likely see great progress in instruments for analyzing plankton populations (see Erickson et al. 2011).

Although studying tintinnids may not provide a general explanation for "the paradox of the plankton", any attempt to explain the paradox will have to account for co-existence of dozens of tintinnid species, all of roughly similar morphology and ecology, in a few liters of seawater. Does the answer lie in the partitioning of food resources among tintinnid species, variability in competition from other microzooplankters, differential mortality, or life-cycle differences among species? The question of what regulates or rules the composition of assemblages of tintinnids is part of the question of what regulates planktonic assemblages. This question is far from trivial or only of academic interest. Predicting the consequences of climate change on marine systems requires an understanding the functioning of planktonic food webs, which in turn requires an understanding of what regulates their composition.

As a concluding note, it is worth noting that outside the realm of science, tintinnids can be considered as models. In the art world, tintinnids have appeared as both models of artistic form in nature as well as subjects. The best known are perhaps those in Haeckel's "Art Forms of Nature" seen in Plate 3, Ciliata (Haeckel 1904). Several of the species shown are from the plates of his 1873 paper describing forms he found in the Canary Islands and Naples (Fig. and Plate 1.14).

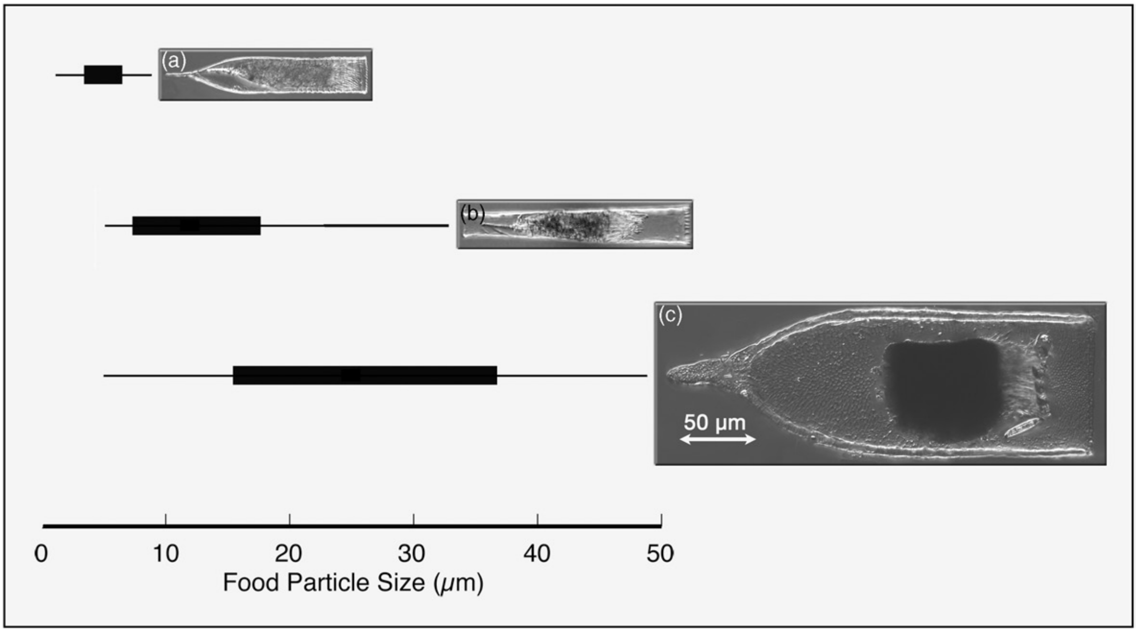

Fig. 1.13 The size range of the food items ingested by three tintinnid species ((a) *Helicostomella subulata*, (b) *Eutintinnus pectinus*, (c) *Favella ehrenbergii*) in Danish coastal waters varies with the size of the oral diameters of the loricae. Based on data from Fenchel (1987), fig. 7.1.

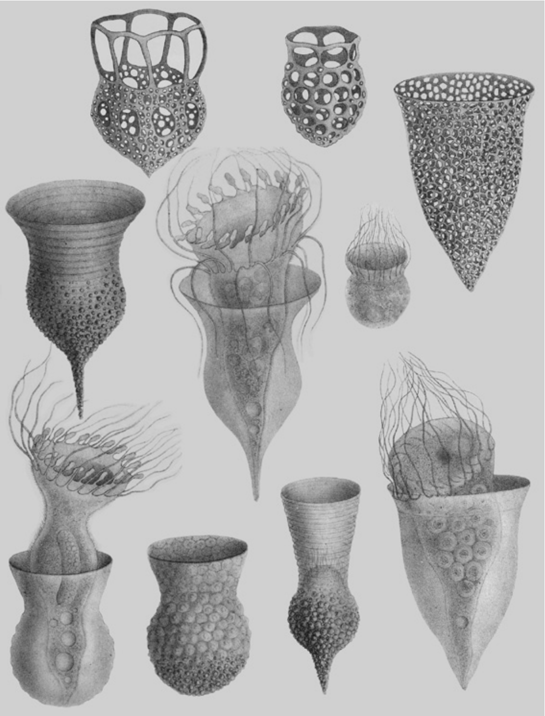

Fig. 1.14 Tintinnids of Ernst Haeckel. Restored drawings from Ernst Haeckel's 1873 paper, the first scientific article devoted to tintinnid ciliates. Image by J.R. Dolan. For color version, see Plate 1.14.

Fig. 1.15 "La Boheme: A Portrait of Today's Oceans in Peril", the 2012 sculpture by Mara G. Haseltine depicts two tintinnids ensnarled in plastic. Dimensions: 91 cm × 122 cm × 185 cm (length × width × height). Materials: uranium-infused blown glass and plastic. By permission of Mara Heseltine. For color version, see Plate 1.15.

A recent example of tintinnids in the art world is in Mara Haseltine's sculpture "La Boheme", which depicts tintinnids entangled in microscopic pieces of ultraviolet-degraded plastic (Fig. and Plate 1.15).

1.6 KEY POINTS

1. Tintinnids are ciliate protists, constituting a suborder of the order Choreotrichidae, with the common characteristic of having a lorica. They are all planktonic and the overwhelming majority are marine. Based on differences in lorica architecture, literally hundreds of species have been described since the first tintinnid was noted over 230 years ago.

2. The role of tintinnids in the pelagic ecosystems is that of a component of the microzooplankton, grazers at the base of the food web. They are a species-rich group of organisms which are ecologically united as planktonic ciliates, mainly consumers of nanoplankton, and are morphologically as well as phylogenetically united as "shelled", choreotrich ciliates.

3. Tintinnid ciliates represent an excellent group of organisms to examine questions of what regulates or rules the composition of planktonic assemblages.

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Chapter 2

THE TINTINNID LORICA

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2.1 INTRODUCTION

Tintinnids are unique among planktonic ciliates in building solid shells. These shells, called loricae (singular lorica; Latin, "corselet"), are minute works of art. Sometimes they are simply tube- or vase-shaped, sometimes incredibly elaborate, but the builders are only unicellular organisms (Figs 1.4, 1.5, 2.1, 2.2, 3.16, and 3.17).

Because our knowledge about the inhabitants is rather scarce, the taxonomic classification of the tintinnids is still commonly based on features of the loricae (Chapter 3). Therefore, we should look closely at the factors that influence the appearance of the

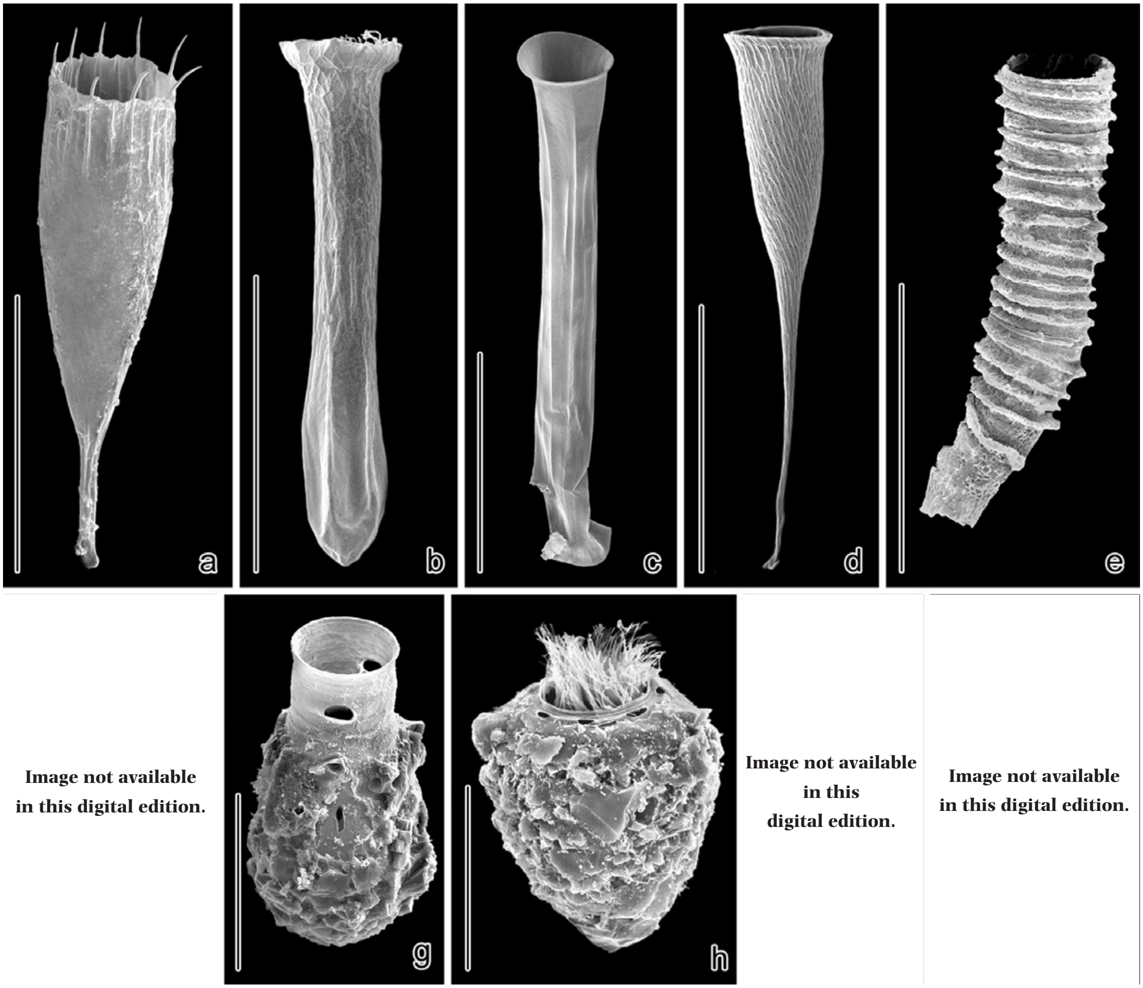

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Fig. 2.1 Hyaline loricae (a–e), a hard, agglomerated lorica (f), loricae composed of a hyaline collar and an agglomerated bowl (g, h), and soft, agglomerated loricae (i, j) in the scanning electron microscope (a–e, g, originals of S. Agatha; f, adapted from Agatha 2010b; h, adapted from Agatha, S. & Tsai, S. (2008) Redescription of the tintinnid *Stenosemella pacifica* Kofoid and Campbell, 1929 (Ciliophora, Spirotricha) based on live observation, protargol impregnation, and scanning electron microscopy. Journal of Eukaryotic Microbiology, 55, 75–85; i, j, adapted from Foissner et al. 1999). (a) *Dadayiella ganymedes*. (b) *Steenstrupiella* species. (c) *Eutintinnus brandti*. (d) *Rhabdonella spiralis*. (e) *Climacocylis elongata*. (f) *Tintinnopsis parvula*. (g) *Codonellopsis schabi*. (h) *Stenosemella pacifica*. (i, j) *Tintinnopsis cylindrata*. The empty lorica in Fig. 2.1i collapsed in the scanning electron microscope, as it is soft. Scale bars 50 μm (a, b), 100 μm (c), 150 μm (d, e), 20 μm (f), 50 μm (g, i, j), and 30 μm (h).

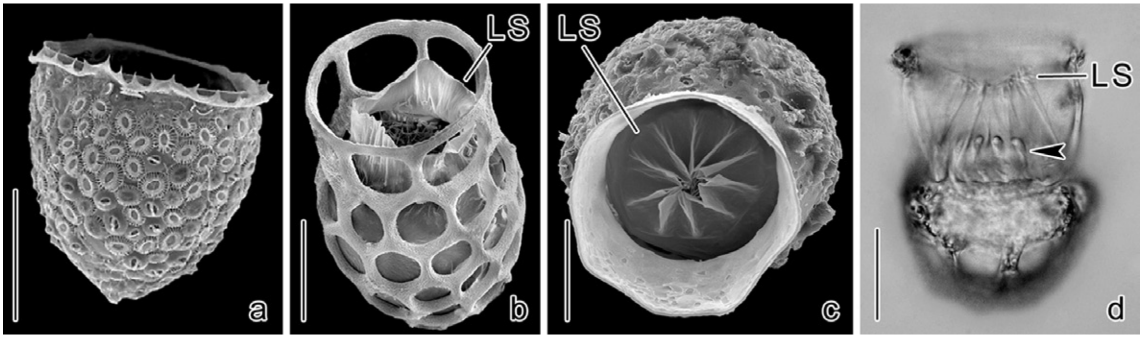

Fig. 2.2 Lorica of *Acanthostomella* species covered with coccoliths (a; calcium carbonate plates formed by coccolithophorids, unicellular algae) and specimens of *Dictyocysta mitra* (b), *D. lepida* (d), and *Codonella aspera* (c) showing a lorica sac. Arrowhead in Figure 2.2d denotes tentaculoids (pin-shaped cytoplasmic extensions containing tintinnid extrusomes). Micrographs from the scanning electron microscope (a–c; a, adapted from Young & Geisen 2002; b, c, adapted from Agatha, S. (2010a) A Light and scanning electron microscopic study of the closing apparatus in tintinnid ciliates (Ciliophora, Spirotricha, Tintinnina): a forgotten synapomorphy. Journal of Eukaryotic Microbiology, 57, 297–307) and a live cell (d; adapted from Agatha 2010a). LS, lorica sac. Scale bars 10 µm (a) and 20 µm (b–d).

loricae, which are (1) the amount and quality of the lorica material, (2) the environmental conditions during formation, and (3) the cell cycle. Astonishingly, many of these unicellular organisms are able to construct not only a single type of lorica, but several, which are occasionally so dissimilar that they were affiliated with different families. After the death of the ciliate or when the lorica is abandoned, it sediments, transporting chemical compounds to deeper water layers and possibly to the bottom of the sea or lake. As tintinnids occasionally dominate the microzooplankton (heterotrophic organisms of the pelagic zone 20–200 µm in size), the material flux can be considerable.

2.2 DIVERSITY, FORMATION, AND VARIABILITY OF LORICAE

Diversity of loricae

The loricae are often much longer than the cell proper (Figs 1.4, 1.5, and 2.10) and they are thus able to harbor the entire disturbed ciliate retracted by its contractile peduncle. In extended state, the anterior cell portion with the adoral membranelles (fan-like ciliary structures used for swimming and feeding; Chapter 4) protrudes from the lorica. The lorica might be a protection against the numerous predators (mainly copepods and invertebrate larvae), but other functions are also possible (Chapter 1).

Just after division and before it starts the construction of its own lorica, the proter (anterior division product) is naked, whereas the opisthe (posterior division product) keeps the old lorica. Tintinnids that lost or abandoned their loricae are also naked, until they form new loricae. Reports of naked tintinnids from the plankton are not available, probably because the lorica formation is generally a rapid process. An occasional misidentification with aloricate choreotrichids of the genus *Strombidinopsis* can also not be ruled out, considering that usually fixed material was investigated. Furthermore, naked tintinnids are slow swimming, relatively small cells that could easily be caught by numerous predators. In cultures, however, naked tintinnids can be observed.

According to the famous monographs on extant (non-fossil) tintinnids by Kofoid & Campbell (1929, 1939), the vast majority of genera have hyaline loricae (42 genera in 10 families), which are remarkably diverse in morphology (Figs 1.5 and 2.1a–e). The loricae range from small, smooth, and rounded forms resembling bowls (e.g., in *Undella* and *Proplectella*) and tube-shaped loricae (e.g., in *Eutintinnus*) to large bowls with everted collars (e.g., in *Petalotricha*; Fig. 2.4) and chalice-shaped loricae (e.g., in *Epiplocylis*). The anterior lorica portions can be quite elaborate with denticulations, flaring collars, and portions of different structure or sculptured appearance (Figs 2.1 and 2.2). The second major type of lorica is hard and has entirely, or only in the posterior portion, particles of mineral or biogenic origin agglomerated. These loricae are found

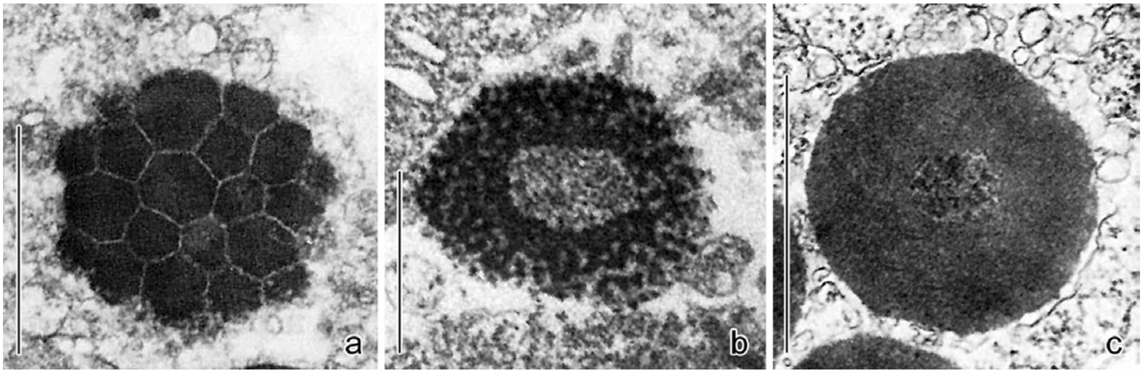

Fig. 2.3 Transmission electron micrographs of lorica forming granules. (a) Morula-like aggregate of granules in *Petalotricha ampulla* (adapted from Laval-Peuto 1994); similar structures occur in *Cymatocylis convallaria*, *Cyttarocylis brandti*, and *Parafavella denticulata*. (b, c) Granular and compact types of granules in *Parafavella gigantea* (adapted from Hedin 1975b). Scale bars 2 µm (a) and 1 µm (b, c).

in 11 genera belonging to four families (Figs 1.4, 2.1f–j, 2.2a, c, d, and 2.12). A few agglomerated species have soft loricae; they belong to the family Tintinnidiidae, which comprises three genera.

A unique character, the closing apparatus, was recently re-investigated, using light and scanning electron microscopy (Agatha 2010a). The first observations on this structure were performed about 130 years ago by Fol (1883; for details, see Agatha 2010a). Two kinds of closing apparatus exist. A membranous closing apparatus, which shuts the lorica opening by 8–24 folds, usually 12, in disturbed and thus retracted ciliates, is found in four genera, which were until recently affiliated with three families (Chapter 3; Fig. 2.2b–d): *Codonella*, *Codonaria* (hard, agglomerated loricae), *Codonellopsis* (loricae composed of a hyaline collar and an agglomerated bowl), and *Dictyocysta* (loricae entirely hyaline or composed of a hyaline collar and an agglomerated bowl). Owing to a similar morphology and functionality, the foldable closing apparatus was regarded as synapomorphy (shared derived character) of these genera (Chapter 3). This kind of closing apparatus merges posteriorly into a lorica sac, which is difficult to recognize as it completely lines the lorica bowl (broadened lorica portion). Even if not hermetically sealed, the lorica sac and its closing apparatus might be a valuable protection against contact predators, who use feeding tubes (dinoflagellates) and pseudopodia (radiolaria), especially, in tintinnids with fenestrated loricae, for example *Dictyocysta*. The second type of closing apparatus is diaphragm-like and occurs in the genera *Salpingacantha* and *Salpingella* (Tintinnidae; with hyaline loricae). Because the diaphragm-like closing apparatus shows neither folds nor closing movements and is not associated with a lorica sac, a homology with the foldable apparatus is less likely. Therefore, the genera *Salpingacantha* and *Salpingella* are probably not closely related to the four genera mentioned above. This is supported by phylogenetic analysis of the small subunit ribosomal RNA (SSU rRNA) gene (Chapter 3).

Because hyaline loricae lack agglomerated particles obscuring their content and texture (see below), those tintinnids were preferentially studied. Even with a very simple microscope at low magnification, Müller (1779) was able to observe the ciliate, the contractility of its peduncle, and some stages of cell division (see Box 1.1 in Chapter 1). To avoid impediments by the agglomerated particles, the first sections of tintinnid cells to study the cell structures were mainly conducted in species with hyaline loricae (Entz Jr 1909b; Merkle 1909). Differences in the structure of the lorica wall and sometimes its texture were described, using light microscopy (Brandt 1906, 1907). Later, ultrathin sections for transmission electron microscopy provided new data on the cytology of tintinnids and the texture of the lorica wall (Laval 1972; Laval-Peuto 1975, 1980). Several types of texture are distinguishable (Figs 2.13 and 2.14): monolaminar and compact (e.g., in *Eutintinnus*), monolaminar with alveoli (small chambers; e.g., in *Climacocylis*), trilaminar with different tubules in the three layers (e.g., in