Bacillariophyta

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Contents

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Abstract

The diatoms (Bacillariophyta) are the most species-rich group of autotrophic algae, found in fresh, brackish, and marine waters worldwide, and also in damp terrestrial habitats. They are well represented in marine phytoplankton and may account for 20% of global photosynthetic carbon fixation. However, the vast majority of the estimated 100,000 species are benthic, living attached to surfaces or gliding over sediments using a unique organelle, the raphe system. Flagellate cells are absent, except in the sperm of some lineages. Diatoms possess a similar photosynthetic apparatus to that present in several other stramenopile lineages (with fucoxanthin and chlorophyll c as the principal accessory pigments) but are easily recognized by the unique construction and composition of their cell wall, which is usually strongly silicified and consists of two overlapping halves (thecae); these in turn consist of a larger end piece (valve) and a series of narrow strips (girdle bands). Expansion of the cell occurs by sliding apart of the thecae and addition of new bands to the inner, overlapped theca. At cell division, each daughter cell inherits one of the thecae of the parent and forms a new theca internally. Hence, because the silicified wall is inelastic, average cell size usually declines during vegetative growth and has to be restored through expansion of a special cell, the auxospore, usually after sexual reproduction. A few diatoms have lost their plastids and are osmotrophic. Classification has traditionally relied on details of valve structure. There is a rich fossil record.

Keywords

Bacillariophyta • Diatoms • Frustule • Girdle • Silicification • Valves

Summary Classification

•Bacillariophyta
••leptocylindrids
••corethrids
••melosirids
••ellerbeckiids
••arachnoidiscids
••coscinodiscids
••rhizosolenids
••proboscids

••Bacillariophytina
•••Mediophyceae (polar centrics)
•••Bacillariophyceae (pennate diatoms)
••••Urneidophycidae
••••Fragilariophycidae
••••Bacillariophycidae (raphids)[1]

Introduction

General Characteristics

The Bacillariophyta, commonly known as ***diatoms***, are a group of unicellular (though sometimes colonial), diploid, golden or brown-pigmented algae, most of which occur in freshwater and marine habitats; just a few live on land. The aquatic species can be planktonic or benthic. The vast majority of diatoms are free-living phototrophs but some live as endosymbionts of other protists and a small number have lost photosynthetic capacity and have become obligate heterotrophs. Like related phototrophic stramenopiles (heterokonts), photosynthetic diatoms possess chloroplasts that are bounded by four membranes and contain thylakoids grouped into threes. The principal light-harvesting pigments are fucoxanthin, chlorophyll a, and various forms of chlorophyll c. The most characteristic feature of diatoms is their silicified cell wall, referred to as the ***frustule*** (see section "Cell Wall and Cell Division"), which is unlike anything found in other organisms. It is strong and sometimes massive but, crucially for these photosynthetic cells, transparent. It is composed of several interlocking and overlapping elements, comprising two ***valves***, one at each end of the cell, which are usually large and robust, and a variable number of more delicate ***girdle bands*** covering the space in between (Fig. 1a, b). The vegetative cells are always walled except in the few species that occur as endosymbionts; no free-living flagellated or amoeboid cells exist, except as gametes. In a very few cases (e.g., some stages of the highly unusual, polymorphic diatom *Phaeodactylum*), the cell wall is purely organic.

Diatoms have a simple diplontic life cycle, multiplying profusely by mitotic divisions during the diploid vegetative phase and producing haploid cells only as a result of gametogenesis. A characteristic and remarkable feature of most diatoms is that average cell size decreases during the vegetative phase and has to be restored through formation of a special cell – the ***auxospore*** (see section "Life Cycle"). Auxosporulation is usually preceded by sexual reproduction, the auxospore being a zygote formed through the fusion of motile or nonmotile gametes, but in some cases the auxospore is formed asexually. During auxosporulation, the cell walls of the old,

[1]In the case that the eight informally named groups (leptocylindrids to proboscids) together comprise a monophyletic taxon (see "Taxonomy"), this is called the Coscinodiscophytina, containing a single class, Coscinodiscophyceae.

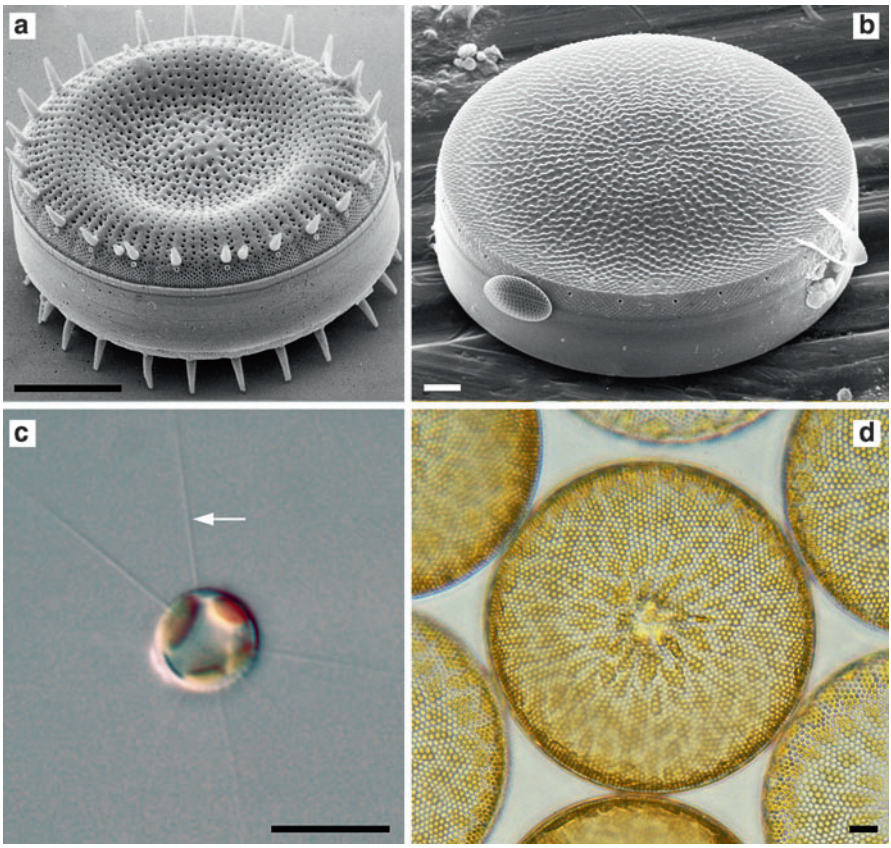

Fig. 1 Cells of planktonic centric diatoms. Scale bars = 10 μm. (**a**) Whole cell of *Stephanodiscus* with concentric undulations of the valve and a crown of spines. SEM. (**b**) Whole cell of *Actinocyclus,* SEM. (**c**) Living cell of *Cyclotella* with radiating fibrils of chitin (e.g., *arrow*) secreted through fultoportulae (Fig. 12c). (**d**) Living cells of *Coscinodiscus*; many small plastids are visible beneath the honeycomb-like pattern of markings on the valve

small vegetative cells are discarded. In the "centric" lineages of diatoms, sexual reproduction is ***oogamous***: here the auxospore is formed by fertilization of a large ***nonmotile egg cell*** by a much smaller, ***anteriorly uniflagellate sperm***. However, in one late-evolving lineage (the pennate diatoms, comprising the majority of extant species), the gametes are relatively large and alike in size and appearance (though not necessarily in activity), and lack flagella. Auxospores (which are not dormant stages, contrary to what might be thought from the use of the word "spore") often possess special wall elements found at no other stage during the life cycle, which allow and control cell expansion and protect the newly enlarged vegetative cell while it forms its new frustule.

Some diatoms are nonmotile, drifting freely in the water column or lying loose on a substratum or growing attached to it. Others are motile, gliding actively over

surfaces via a unique type of locomotion associated with a unique organelle, the ***raphe system***, which comprises slits through the cell wall (the ***raphe slits***) and associated elements of the cytoskeleton. Movement is generated by secretion of polysaccharide through the raphe slits, adhesion of the secreted material to the substratum, and active displacement of the secretions relative to the cell by interactions with the cytoskeleton, thus driving the cell forward (Edgar and Pickett-Heaps et al. 1984). Through their raphe secretions, stalks and pads, benthic diatoms often greatly modify their immediate environment, e.g., by gluing sediment particles together or by forming a thick biofilm that is colonized by other algae and microorganisms.

Several hundreds of genera of extant diatoms are recognized, and the number of named species and infraspecific taxa (including fossils) exceeds 60,000 (Kociolek and Williams 2015). Some of these taxa are synonyms, but many species have not yet been discovered or named, and it has been estimated that the final total of extant species will be between 100,000 and 200,000 (Mann and Vanormelingen 2013). Many small-celled diatoms have been poorly researched and some important, highly species-rich habitats have been largely neglected, e.g., the phytobenthos of sublittoral marine habitats. Furthermore, gene sequence data reveal that cryptic and pseudocryptic species are common. Hence the diatoms have a strong claim to be considered one of the most diverse and successful groups of protists. They also have a rich subfossil and fossil record, because their silica shells are resistant to decay. Many extinct fossil genera are known, and many modern genera are represented in the fossil record by extinct species.

Recently, diatoms have become the focus of intense research using genomic and transcriptomic approaches, because of their importance to the functioning of the biosphere and because of their unrivalled ability to metabolize silicon and produce patterned, silicified walls.

Occurrence and Sampling

Diatoms occur in almost all aquatic habitats, both freshwater and marine (Round 1981a), and probably account for about 20% of global net primary production (Mann 1999b). Virtually the whole ocean (70% of the earth's surface), down to depths to which photosynthetically available radiation (of wavelength 400–700 nm) penetrates, is colonized by diatoms, though they are numerically most abundant in regions of upwelling and other productive zones. However, the greatest diversity is probably in marine intertidal communities. For example, in two nearby samples from a North Carolina beach, Friedrich Hustedt (1955) recognized 369 species (of which 89 were new) belonging to 63 different genera. Diatoms occur on land too. Most soils capable of supporting plant growth bear diatoms, and they occur anywhere water drips, collects, or flows – even the moist regions between bryophyte leaves and on the surfaces of angiosperm leaves and lichens in wet tropical forests (Round 1981a).

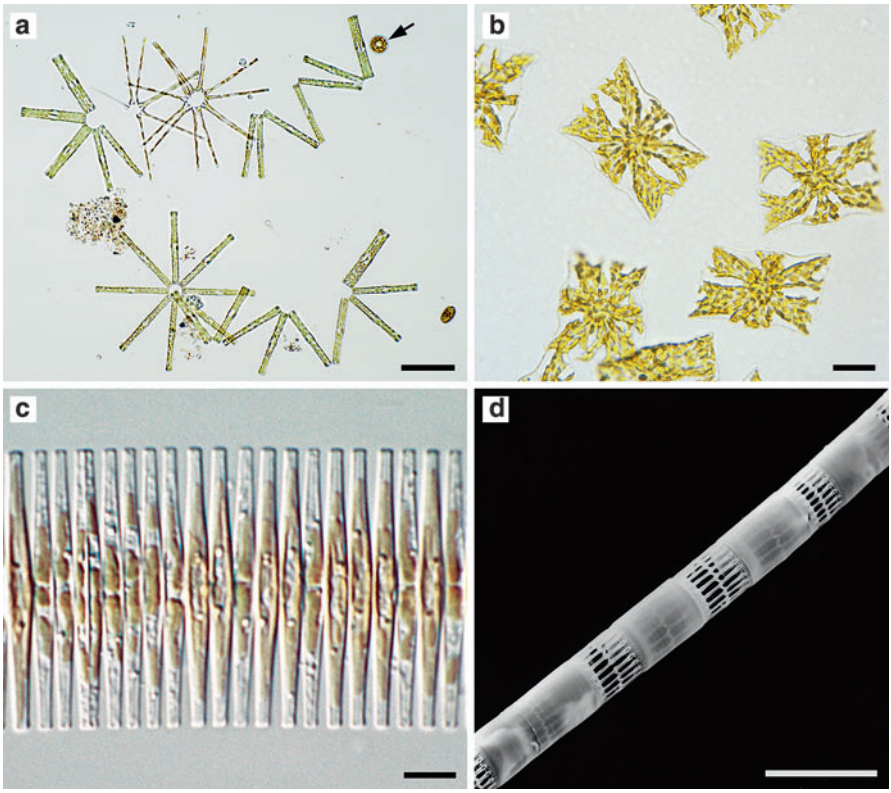

Fig. 2 Planktonic diatoms. (**a**) Freshwater phytoplankton containing a stellate *Asterionella* colony (slender-celled colony at *top*), stellate and zigzag colonies of *Tabellaria*, and a single *Cyclotella* cell (*arrow*). Scale bar = 50 μm. (**b**) The marine *Mediopyxis*: solitary cells. Scale bar = 10 μm. (**c**) A ribbon of *Fragilaria* cells from freshwater Scale bar = 10 μm. (**d**) Filament of *Skeletonema* cells, SEM. Scale bar = 10 μm

Diatoms live as motile, attached, or suspended cells. Though the suspended (***planktonic***) species are those most often illustrated, and thus the most familiar to biologists (Figs. 1a–d and 2a–d), the range of form is greater in benthic habitats, and there are far more benthic species than planktonic ones (by a couple of orders of magnitude). Motile species occur in the surface film of soils and on dripping rock faces, and on the sediments of ponds, lakes, streams, rivers, coastal lagoons, and coastal seas. They often coat the surface of estuary muds with a dense brown layer of cells, which play an important ecological role in stabilizing sediments (Underwood and Paterson 2003). These ***epipelic*** diatoms (Fig. 3a) are motile and often migrate vertically upwards through the sediment in the morning and move back into the sediment later in the day, in a rhythm of movement under the control of a biological clock which, in tidal situations, is in synchrony with the tidal cycle (Palmer and Round 1967). Soil diatoms are of similar morphology to those occurring in aquatic epipelon, but they are generally smaller and less motile. Sand in both freshwater and

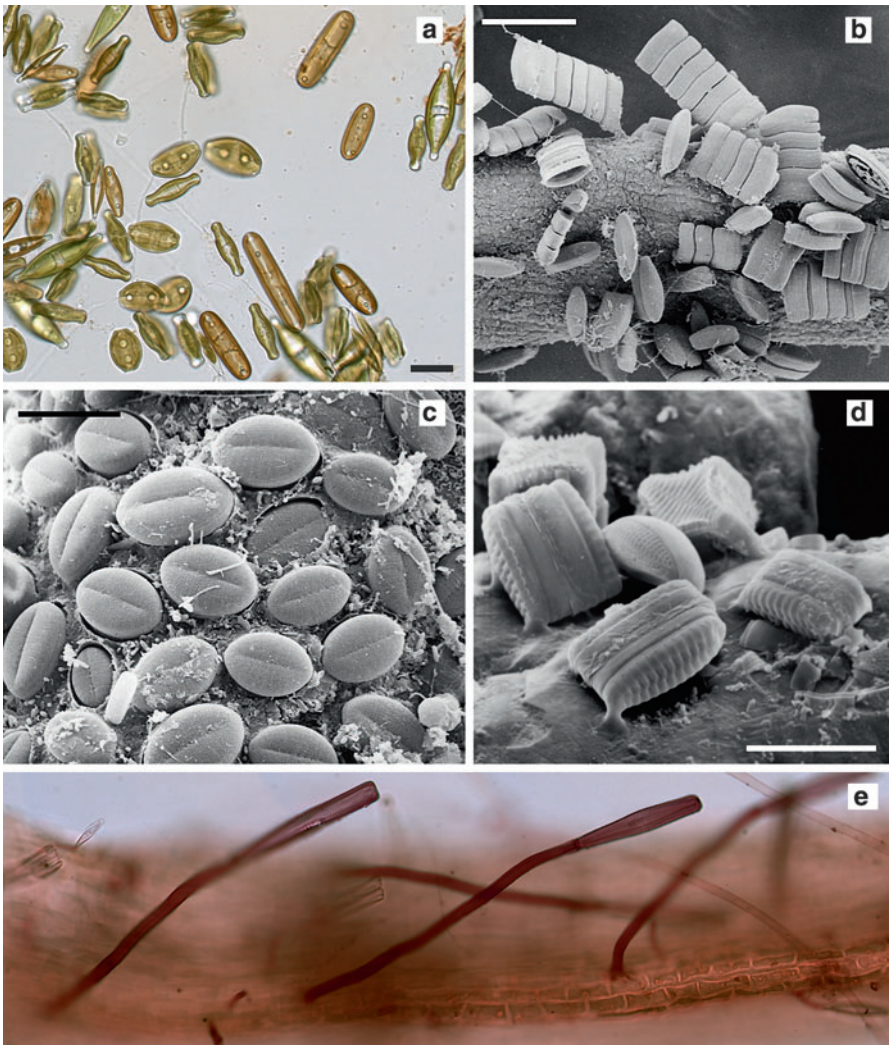

Fig. 3 Diatom communities. (**a**) Freshwater epipelon, containing *Amphora*, *Sellaphora*, *Navicula* and *Hippodonta* cells. Scale bar = 20 μm. (**b**) *Achnanthes* growing epiphytically on a plant surface. SEM. Scale bar = 50 μm. (**c**) *Cocconeis* growing on the green alga *Cladophora*. SEM. Scale bar = 10 μm. (**d**) Epipsammon: *Martyana*, *Amphora*, and *Staurosira* on a sand grain. SEM. Scale bar = 10 μm. (**e**) Carmine-stained cells of *Gomphonema*, attached to a plant surface by long polysaccharide stalks

marine environments may be colonized not only by epipelon but also by extremely small diatoms attached to the surfaces of the sand grains themselves, comprising the ***epipsammon*** (Fig. 3d). Attached (***epilithic***) species coat rock surfaces, the hard surfaces of calcified algae, and the dead fragments of corals and calcareous algae. Filamentous algae in both freshwater and marine habitats are often so densely

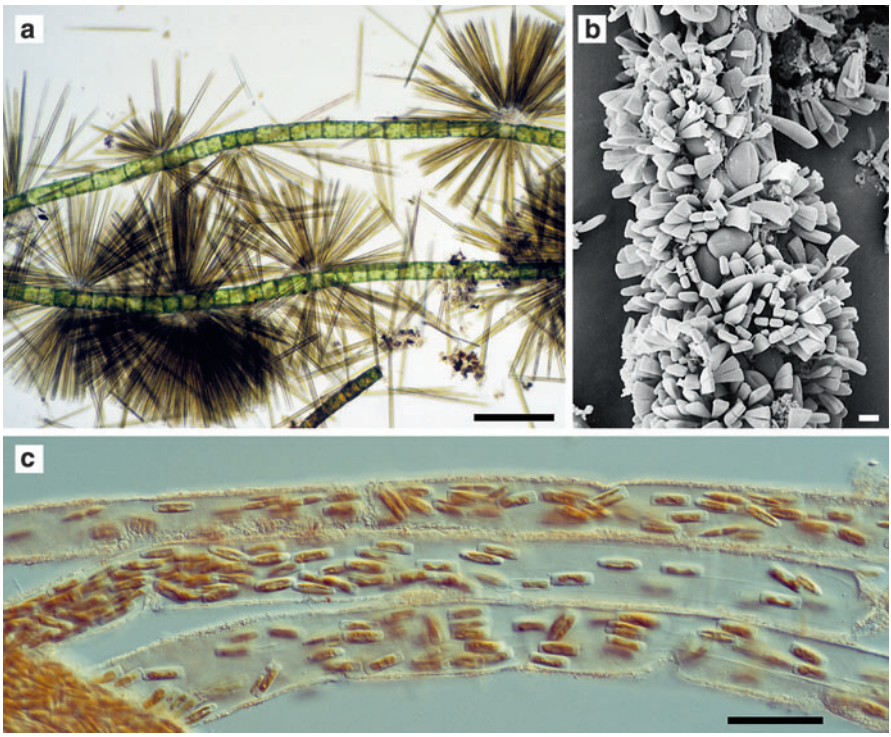

Fig. 4 Attached diatom communities. (**a**) *Ulnaria* epiphytic on filamentous green algae. Scale bar = 50 μm. (**b**) Dense growth of *Rhoicosphenia*, *Gomphonema* and *Cocconeis* on the green alga *Cladophora*. SEM. Scale bar = 10 μm. (**c**) Tube-dwelling *Berkeleya*, scraped from a rock surface. The tubes are made of polysaccharide. Scale bar = 50 μm

covered by ***epiphytic*** diatoms (Figs. 3b, c, e, and 4a) that the host surface may be obscured (Fig. 4b), and animal hosts, from copepod hard parts and limpet shells to sea birds and cetaceans, sometimes have an external diatom microbiota (***epizoon***) involving species-specific forms. Vast areas of ***sea ice*** around Antarctica and the Arctic ice cap are coated on the undersurface with a dense layer of diatoms. Smaller forms are even found in the brine channels of the ice. Man-made objects placed in water soon acquire a covering of diatoms, and glass slides or ceramic tiles have sometimes been deployed for this reason in rivers, so that they can later be removed for assessment of water quality and ecological status (e.g., during biomonitoring under the European Union Water Framework Directive).

Collection of diatoms involves sampling of sediments, plants, or animals, or filtering (or sedimenting) quantities of lake or seawater. Sediments should be sampled by techniques that remove only the top few millimeters or so. If the sediment is then placed in a Petri dish or translucent plastic box, excess water removed, and cover glasses or lens cleaning tissue placed on the surface, motile diatoms will move upwards and attach to the new substratum and can be removed to

a microscope slide for examination or placed in culture media. Sand samples can be washed free of silt and organic matter by repeated agitation and settling and the sand grains then observed directly on a microscope slide. Communities on plant and animal surfaces can be observed directly if the plant or animal is microscopic, or the surface coating of diatoms may be removed from them or stones by scraping with a scalpel. Careful sampling will often show that an upper "canopy" is present. This is more easily detached than the initial colonizers, which grow appressed to the substratum. Planktonic diatoms can be sampled by drawing a net through the water either horizontally behind a drifting boat (or thrown in from the shore) or vertically by lowering a weighted net to a set distance below water level and then drawing it up. More complicated devices can be used if quantitative samples are required, including standard water bottle samplers that can capture known volumes of water from known depths.

Because of the nature of their cell walls, diatoms have left evidence of their evolution in the fossil record since the Cretaceous, often in the form of fairly pure deposits called ***diatomites***, produced by sedimentation of the plankton of fresh and marine waters. Diatomites may be powdery or more rocklike, the latter requiring treatment (grinding, disaggregation using chemicals, freeze–thaw cycles, or sonication) to reduce them to a finer state before examination. The material can often be mounted directly in water or high refractive index mountants but is often better if "cleaned" first (see below).

The fine detail of wall structure is usually critical for identification and has to be revealed by cleaning the cells with strong oxidizing agents (e.g., a mixture of concentrated sulfuric and nitric acids, or hydrogen peroxide; however, though widely used, the latter seems often to lead to erosion of fine detail) to remove organic material, leaving only the silica parts of the cell wall. If the sample contains much carbonate, this may have to be removed first (it can be dissolved with dilute hydrochloric or nitric acids and washing to remove the resulting salts), especially if sulfuric acid is to be used subsequently. After oxidation, samples must be thoroughly washed with deionized water by settling or centrifugation. Then the cleaned frustules (which often separate into their component pieces – valves and girdle bands) can be dried onto cover glasses and mounted in high refractive index media (e.g., Naphrax: Fleming 1954). Final identification of species can then be attempted. Care should always be taken to study the full range of forms present in a population because most diatoms undergo size reduction during the life cycle, and the shape and patterning of the valves can also change. It is not uncommon for the small and large cells in the life cycle to be mistaken for different species.

Because species are generally characterized and identified by the morphology of their silica valves, and because important details of valve structure cannot be seen easily in living cells, it is common for diatom communities to be studied only after cleaning, as described above. This has had the unfortunate side effect that many aspects of the structure and growth of living cells remain unknown, even in common species. Details of plastid form and position are often characteristic of the species or genus in benthic diatoms but must be examined in very fresh material (because gross changes often occur rapidly after sampling) or after fixation (with rapidly penetrating

fixatives such as glutaraldehyde or OsO_4-containing mixtures) and staining. It should also be remembered that the chloroplasts and other organelles often move around the cell in preparation for, or after, cell division (e.g., Mann 1996). Such changes need to be taken into account when interpreting and identifying live diatoms. Living diatoms can be studied for several hours or days in microscope slide preparations in which the coverslip is sealed to the slide using petroleum jelly. Alternatively, they can be examined using water immersion lenses dipped directly into Petri dish cultures, or through the base of the culture vessel using an inverted microscope. With the advent of molecular systematics, it is worth considering whether aliquots of samples should be preserved for subsequent genetic analysis, e.g., by freezing at −80 °C.

The gradual decrease of cell size that occurs in most diatom species during the life cycle has consequences for the maintenance of strains in culture. If conditions for sexual reproduction and auxosporulation are unfavorable in culture, or if the diatom is heterothallic, clonal strains will continue to get smaller and finally die (Chepurnov et al. 2004). Furthermore, even if clones are self-compatible and can complete the life cycle, their progeny may suffer from inbreeding depression and die out after a few sexual generations (Chepurnov et al. 2011). Consequently, most culture collections contain rather few diatom strains, many of which are atypical of the group (e.g., some avoid size reduction, whereas others auxosporulate automictically). Small numbers of diatom species are maintained in the major culture collections, e.g., at the National Center for Marine Algae and Microbiota (NCMA), Bigelow, Maine, USA (https://ncma.bigelow.org/); the Culture Collection of Algae (UTEX), Austin, Texas, USA (https://utex.org/); the Culture Collection of Algae and Protozoa (CCAP), Oban, Scotland, United Kingdom (http://www.ccap.ac.uk/); the Sammlung von Algenkulturen (SAG), Universität Göttingen, Germany (https://www.uni-goettingen.de/en/); the Roscoff Culture Collection, Roscoff, France (http://www.roscoff-culture-collection.org/); and the Microbial Culture Collection, National Institute for Environmental Studies, Tsukuba, Japan (http://mcc.nies.go.jp/). Pedigreed lineages of heterothallic diatom species, as well as homothallic and asexual lineages, are maintained by the specialized diatom culture collection at the Protistology and Aquatic Ecology Research Group, Ghent University, Belgium (http://bccm.belspo.be/about-us/bccm-dcg). Many individual workers also maintain small collections for research. Some progress has been made in cryopreservation of diatoms, but because of the complications caused by the life cycle, cryopreservation is not a permanent solution to culture maintenance, though it can considerably extend the availability of a strain. Not surprisingly, therefore, there is no system for designating “type strains” in diatoms; instead, proposals have been made for using DNA barcodes to help typify taxa (Evans and Mann 2009).

Once cleaned, diatom frustules can be preserved indefinitely either dry or suspended in alcohol; the use of aqueous preservatives (e.g., formalin, Lugol’s iodine) should be avoided because the frustules will slowly dissolve. Large collections of permanent slides of cleaned diatoms, including type specimens, are held by several institutions, notably the Academy of Natural Sciences,

Philadelphia; the Natural History Museum, London; and the Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven; but many other museums and institutes also hold important collections (Fryxell 1975, lists some and De Wolf and Sterrenburg provide further information at http://home.planet.nl/~wolf0334/). Collections of slides with text catalogues were distributed by several diatomists in the late nineteenth and early twentieth centuries (e.g., Tempère and Peragallo 1915).

Literature

Most of the early literature on the structure, life cycle, and taxonomy of diatoms is in German and includes the following major works: Kützing (1844); Pfitzer (1871); Schmidt (1874–1959); Schütt (1896); Hustedt (1927–1966); Karsten (1928); and Geitler (1932). A widely used, more recent flora for identifying freshwater diatoms is the *Süsswasserflora von Mitteleuropa* by Krammer and Lange-Bertalot (1986–1991; see also the condensed and updated version by Hofmann et al. 2013). An excellent handbook to marine planktonic diatoms was produced in English by Hasle and Syvertsen (1996), though this is not comprehensive, focusing on the more commonly encountered species of temperate and polar regions. Online floras for freshwater diatoms are being assembled in the USA (http://westerndiatoms.colorado.edu) and the UK. There are no up-to-date, comprehensive accounts of marine and brackish benthic diatoms. For these, the French flora of Peragallo and Peragallo (1897–1908) is still indispensable, together with myriad papers scattered through many journals, which are often hard to access (however, digitization of the older literature means that many works can now be accessed at e.g., http://www.biodiversitylibrary.org/, http://gallica.bnf.fr/, https://archive.org/).

Several series of specialist diatom publications are active, including *Bibliotheca Diatomologica*, *Iconographia Diatomologica*, *Diatom Monographs*, and *Diatoms of Europe*. Most of the volumes in these series focus on taxonomy and biodiversity (e.g., Metzeltin and Lange-Bertalot 2007; Levkov 2009). The journal *Diatom Research* (1986–) is published on behalf of the International Society for Diatom Research, which also organizes the biennial International Diatom Symposium, and *Diatom* is published by the Japanese Society of Diatomology. There is an extensive Russian and Japanese literature on diatoms. The earlier Russian papers are catalogued in the Soviet bibliography of algal literature (reprinted in Koeltz 1976 and indexed by Gollerbakh and Krasavina 1971); see also the ongoing *Diatomovye vodorosli* flora of marine and freshwater diatoms (e.g., Glezer et al. 1974).

Of special interest is the collection of electron micrographs edited by Helmcke and Krieger (1953–1977), whereas listings of more recent micrographs have been compiled by Gaul et al. (1993) and Henderson and Reimer (2003). A remarkable catalogue of diatom names was compiled by VanLandingham (1967–1979), which laid the foundation for an online catalogue of diatom names (http://researcharchive.calacademy.org/research/diatoms/names/index.asp currently not updated past September 2011) compiled at the California Academy of Science by E. Fourtanier

and J.P. Kociolek. However, VanLandingham's catalogue contains extra information not present in the online catalogue, viz. key references illustrating the use of taxon names. Another useful resource for nomenclature and taxonomy, collating information from the literature, is the "Diatom New Taxon File" of the Academy of Natural Sciences, Philadelphia, at http://symbiont.ansp.org/dntf.

Ecological, biochemical, physiological, and genetic information on diatoms is widely scattered in a vast and rapidly growing literature. A review of genus-level biodiversity was produced by Round et al. (1990), who also provided an extended, referenced introduction to diatom structure and biology. The multiauthor volume edited by Smol and Stoermer (2010) gives many examples of applications of diatoms in ecological monitoring, paleoecology, and forensics. Eclectic collections of topics are reviewed in *The Diatom World* (edited by Seckbach and Kociolek 2011) and in the much earlier but still useful *Biology of Diatoms* (edited by Werner 1977). Much interesting information about diatoms and the early history of diatom research is summarized in a handbook by Taylor (1929), which also gives information about the derivations of diatom names. The terminology of cell wall structures and morphology is summarized by Ross et al. (1979) and Barber and Haworth (1981). The special terminology applied to sexual stages and auxospores has recently been codified by Kaczmarska et al. (2013).

History of Knowledge

The first diatom taxa were described at the end of the eighteenth century, but the earliest illustrations of a diatom (a *Tabellaria*) appeared much earlier (Anonymous 1703). The name "Diatomeae" was first used by C. A. Agardh in 1824, although the basic two-part nature of the diatom wall had been implicitly recognized by De Candolle in 1805, when he named the genus *Diatoma* (Lamarck and De Candolle 1805). During the first 50 years of the nineteenth century, a large number of species were described. In 1830–1832, Agardh published a *Conspectus Criticus Diatomacearum* containing c. 100 species; by 1844, Kützing could list c. 800 species. The great German scientist Christian Gottfried Ehrenberg studied both living and fossil material from all over the world and produced innumerable illustrations, excellent for their time, many of which appear in the volumes of the *Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin* (see references in VanLandingham 1967–1979). He noted diatoms attached to the under surface of ice, in soil, on animals, and on sediments, and speculated on many aspects of their biology. Ehrenberg (1854) wrote on the formation of geological strata by the growth and deposition of microorganisms, including diatoms.

The motility and organelles of some diatoms suggested to early workers, including Ehrenberg (1838), that diatoms were animals (the chloroplasts and reserve material being interpreted as organs of digestion), and it was not until the middle of the nineteenth century that they were shown to be autotrophs (Kützing 1844). Around this time there were also numerous arguments about the mechanism of motility and about whether diatoms could live in the dark ocean depths (they can

survive for some time but do not actually photosynthesize and propagate). There was at first little understanding of the diatom life cycle: auxospores were observed but thought to be sporangia (Smith 1856), involved primarily in multiplication and dispersal rather than in regeneration of large cells *per se*.

Throughout the first half of the nineteenth century, the principal focus of diatom research was the description of diatom genera and species. The second half of the century saw classic studies on cell structure by Pfitzer (1871), Lauterborn (1896), Müller (1886, 1889, 1901), and Schütt (1896). Some of their observations were truly remarkable for their detail and accuracy and could be confirmed only when electron microscopy became available (e.g., Pickett-Heaps et al. 1984). Meanwhile, compilations of descriptive data continued, such as in the *Atlas der Diatomaceen-Kunde*, begun by Adolf Schmidt in 1874 and continued by various other authors until 1959. Descriptions of genera and species were augmented from material during nineteenth-century expeditions, including the great oceanographic voyages of H.M.S. Challenger in 1873–1876. Fundamental studies on Arctic (Cleve and Grunow 1880) and Antarctic (Karsten 1905–1907; Heiden and Kolbe 1928) diatoms were also completed at an early date. Of course many more expeditions took place on land and none was more remarkable than that undertaken by Georgi as early as 1772, exploring the waters around Lake Baikal in Siberia. His material was included in the collection of Klaproth in Berlin.

Explanation of one of the unique features of the diatom life cycle – how average cell size decreases with each cell division – was presented formally and independently by MacDonald and Pfitzer (MacDonald 1869; Pfitzer 1869) and analyzed further by Geitler (1932), whose work detailing the shape and pattern changes that accompany size reduction should still be prescribed reading for all undertaking taxonomic studies of diatoms. Discovery of the size restoration stage – auxosporulation – had occurred earlier (Thwaites 1847), but its significance was not then fully understood. Meiosis was shown to be associated with gametogenesis in the pennate diatom *Surirella* by Karsten (1912), thus showing that pennate diatoms are diplonts, but it was not until 1950 that it was finally established that centric diatoms are also diplonts (von Stosch 1950), exhibiting oogamy. Knowledge of chloroplast morphology and division in diatoms, which is still far from complete, was given an excellent foundation by the eccentric Russian biologist C. Mereschkowsky (Sapp et al. 2002), better known for his championship of the endosymbiosis, in a series of papers in the early 1900s (e.g., Mereschkowsky 1902–1903, 1904).

The first half of the twentieth century was notable for the massive contribution of Friedrich Hustedt who described nearly 2000 new taxa (most of them small-celled and freshwater) and also published numerous works on the structure, taxonomy, biogeography, and ecology of diatoms, including the seminal *Die Kieselalgen Deutschlands, Österreichs und der Schweiz* (1927–1966). The foundation for our current knowledge of diatom life cycles and sexual reproduction was laid principally by just three workers: L. Geitler (see Schmid 1991), H.A. von Stosch (see Anonymous 1987), and A.M. Roshchin (e.g., 1994, and see Chepurnov et al. 2004).

From the 1960s onwards, the *Deep-Sea Drilling Project* and its successors (currently the *International Ocean Discovery Program*) have provided long cores from all the oceans and stimulated work on the geological record of diatoms. Many new species have been described and evolutionary events documented. Cores have also been made for paleoecological analyses in countless lakes worldwide (though rarely from earlier than the Quaternary) and have documented both natural and anthropogenic environmental changes (e.g., Smol and Stoermer 2010).

The development of transmission electron microscopy (which allowed the study of organelle structure, mitosis, cell division mechanisms, and wall formation) and, since c. 1967, scanning electron microscopy has transformed our knowledge and interpretation of diatom structure and also stimulated a resurgence in systematics. Little physiological or biochemical work on diatoms was undertaken until the 1950s, and there is no comprehensive review of the many recent developments.

The advent of cheap sequencing technologies has provided new insights into diatom systematics and has also allowed the first microsatellite-based investigations of the genetic structure of marine (e.g., Rynearson and Armbrust 2004; Godhe et al. 2013) and freshwater diatom populations (e.g., Evans et al. 2009; Vanormelingen et al. 2015); the only previous studies of population structure were based on isozymes (e.g., Gallagher 1982).

A diatom, *Thalassiosira pseudonana*, was the first eukaryotic microalga to have its genome wholly sequenced (Armbrust et al. 2004), inaugurating a new phase of research into the developmental genetics and metabolism of the group. The genome of another diatom, the highly unusual polymorphic pennate *Phaeodactylum tricornutum*, has also been sequenced (Bowler et al. 2008) and other species have followed (e.g., *Pseudo-nitzschia multiseries*, *Fragilariopsis cylindrus*). Several unexpected features of diatoms have been discovered as a result of genomic studies, such as that they possess a urea cycle, which is thought to help diatoms make particularly effective use of C and N following periods of N limitation (Allen et al. 2011). Diatoms have also been discovered to have unusual actin and microfilament-related components (Aumeier et al. 2015), and many examples of horizontal gene transfer from bacteria have been found (e.g., Bowler et al. 2008; Raymond and Kim 2012). Transcriptome studies are being used to dissect the process of sexual reproduction in raphid diatoms (e.g., Patil et al. 2015; Moeys et al. 2016). The advent of high-throughput sequencing has also provided new insights into the diversity and distribution of marine planktonic diatoms (Nanjappa et al. 2014; Malviya et al. 2016) and the mechanisms that maintain this diversity (Alexander et al. 2015), and has the potential to revolutionize the use of diatoms in biomonitoring (e.g., Kermarrec et al. 2014).

Practical Importance

The importance of diatoms in planktonic communities has long been recognized, and the control of their populations by silica limitation was shown in detail for several freshwater species by Lund (1949 and subsequent publications). The total contribution by diatoms to the algal biomass within many communities is still not clear

because they do not usually grow alone but in assemblages containing other algal groups. Nevertheless, their overall biomass and contribution to carbon fixation are certainly enormous (Mann 1999b estimated that they may account for c. 20% of total global C-fixation), and they are clearly very important in the food chains of aquatic habitats and have been significant players during the evolution of the biosphere (e.g., Falkowski and Knoll 2007; Berger 2007; Renaudie 2016).

Diatoms can be used as indicators of water quality and ecological status, and systems have been devised to utilize diatom populations growing on natural substrata in running waters and in lakes for biomonitoring (e.g., Kelly et al. 2008). Because their frustules are preserved well in many lake and ocean sediments, diatoms are very important for detecting long-term changes (over tens to millions of years) in aquatic environments (Smol and Stoermer 2010). Diatoms are valuable in water supply reservoirs because they oxygenate the water and remove excess nutrients; however, with excessive growth, they can become a nuisance, blocking the filtration devices in water treatment plants. Other undesirable effects include the production of the neurotoxin domoic acid (a noncanonical amino acid) by marine species of the genera *Nitzschia* and *Pseudo-nitzschia* (and apparently by *Amphora coffeaeformis*), causing potentially lethal "amnesic shellfish poisoning" (Trainer et al. 2012).

The sediments left in freshwater and marine basins that have been drained or raised above sea level often yield diatomite because of the fact that, under favorable conditions, planktonic diatoms settle to the bottom and their silica, being relatively insoluble, builds up to form deposits several hundreds of feet thick in places, e.g., Lompoc in California. This material can be processed by relatively simple means to remove organic or calcareous matter and then used in many industries, e.g., as fine abrasives and filtration material (Smol and Stoermer 2010). Fossil diatoms are also important as stratigraphical markers, e.g., for oil exploration (Krebs et al. 2010).

The unique ability of diatoms to fashion intricate cell walls of amorphous silica has stimulated particular interest among cytologists (Pickett-Heaps et al. 1990) and also biochemists and engineers (e.g., Kröger 2007; Wee et al. 2005), because of the potential to develop new methods for synthesizing silica in ambient conditions and new biomimetic materials, and to provide inspiration for architecture (Kooistra and Pohl 2015).

Habitats and Ecology

A division of diatom habitats can be made along freshwater/marine lines and indeed the vast majority of diatom genera (even whole families and orders) occupy either one or the other habitat. However, some genera occur in both and some others, especially among lineages of motile diatoms, are predominantly found in one but "spill" a few species into the other (Mann 1999a; Alverson et al. 2007). It is quite common to find similar life forms in similar habitats, whether marine or freshwater, as a result of convergent evolution (e.g., between *Tabellaria* or *Diatoma* and *Grammatophora*, which all produce zig-zag colonies: Figs. 2a, and 5b, d). Almost

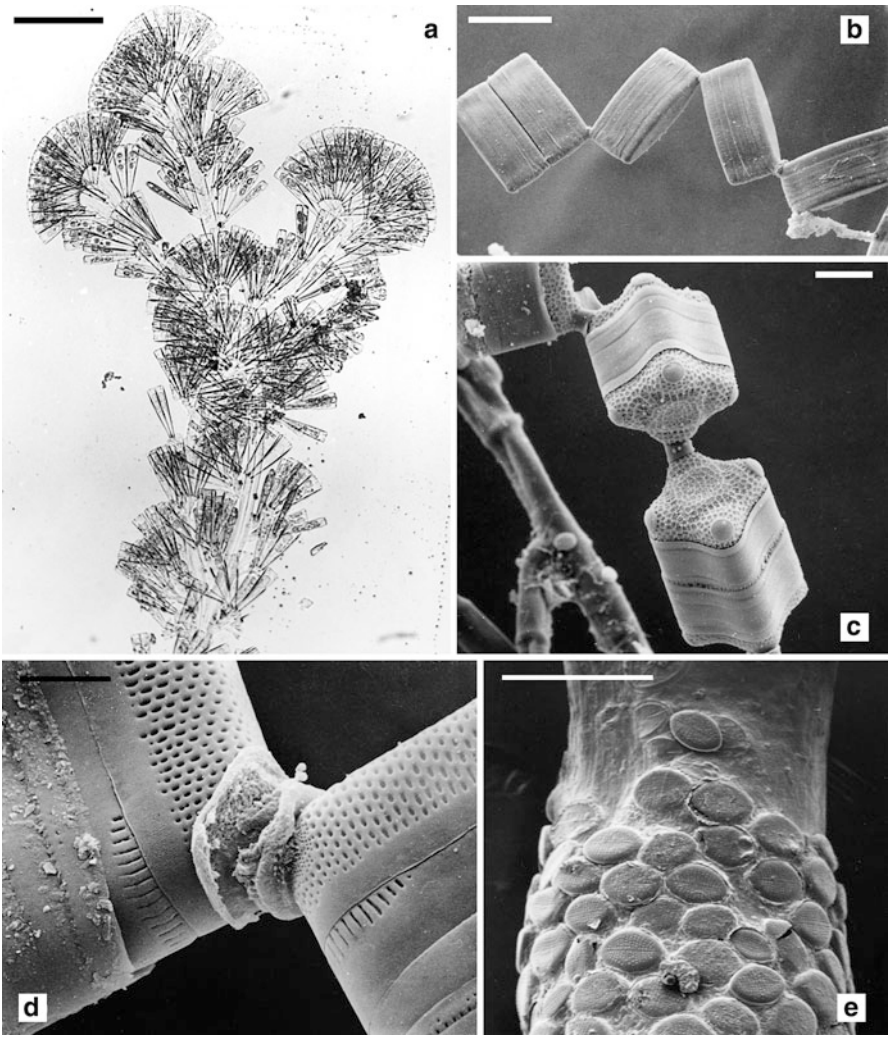

Fig. 5 Colony formation through the formation of mucilage (polysaccharide) pads and stalks. (**a**) *Licmophora* colony. Scale bar = 100 μm. (**b**) Chain of four cells of *Diatoma* linked by pads secreted from the ends of the valves. SEM. Scale bar = 20 μm. (**c**) Chain of *Amphitetras* cells linked by pads secreted through areas of small, unoccluded pores at the corners of the valves. SEM. Scale bar = 20 μm. (**d**) Detail of two *Grammatophora* cells united by a pad of mucilage at their apices. SEM. Scale bar = 3 μm. (**e**) *Cocconeis* on a marine hydroid. SEM. Scale = 100 μm

all diatoms are free-living autotrophs and out of the more than 10,000 described living species, fewer than 10 are colorless heterotrophs (Kamikawa et al. 2015), though this may in part reflect limited sampling of suitable habitat. A similar number of diatoms have been discovered living symbiotically, mainly in Foraminifera (Lee 2011), and a few dinoflagellates (so-called dinotoms) have incorporated diatoms as

permanent endosymbionts, with which they coevolve (Tamura et al. 2005; Pienaar et al. 2007; Saburova et al. 2009). The colorless forms, mostly species of *Nitzschia* (Lewin and Lewin 1967; Li and Volcani 1987; Kamikawa et al. 2015), have secondarily lost the ability to photosynthesize but retain a nonpigmented plastid (leucoplast: Schnepf 1969; Kamikawa et al. 2015).

Free-living diatoms occur in two major types of communities: (i) plankton, occurring in open water masses, and (ii) benthos, which are the communities associated with underwater surfaces and by extension also the subaerial habitats on soil, plants, etc. These gross habitat boundaries disguise a number of sub-habitats and countless niches (Round 1981a).

Plankton

The open waters of oceans and lakes are potentially available for diatom growth, down to the depth to which photosynthetically available light penetrates: populations in tropical oceans have been recorded down to 120–140 meters. However, it is unusual to find living diatoms circulating from the surface to such depths. Instead, the surface waters are extremely barren in parts of the tropical oceans and a deep-lying population occurs in the region of the thermocline in a zone of relatively high nutrient availability derived from the ample store of nutrients in the deep water, although the diatoms may be close to the point of light limitation. In temperate to cold oceans, populations tend to congregate in the surface 30–40 meters.

No diatom cells have a motility mechanism that can move them actively through water, except for the sperm of centric diatoms. Indeed, many planktonic diatoms tend to sink because the specific gravity of silica is significantly greater than that of water; maintenance of such cells in the water column is mainly because of wind- or current-induced turbulence, as can be readily seen when a lake freezes and the water column becomes isolated from wind and wave action – in this case, the diatom population sinks (Lund 1954). A characteristic of many marine planktonic diatoms is the possession of a very large vacuole, accommodated by a wide girdle containing many girdle bands. Some marine diatoms are consequently able to offset the excess weight of the silica wall by incorporating low-density solutes or adjusting ion concentrations in the cell vacuole (Boyd and Gradmann 2002). However, this is feasible only in larger-celled species (Raven and Waite 2004). A further consequence of the large vacuole is that it enables the plastids to spread out over a large surface area in conditions of low light or to clump the plastids round the nucleus (karyostrophy: see Mann 1996), supposedly for protection of the latter against high irradiation in bright sunlight.

There is an enormous range in cell size and form among planktonic genera. Small-celled, pill-box-shaped species of *Stephanodiscus* (Fig. 1a), *Cyclotella*, *Minidiscus*, and *Thalassiosira* may be only 3–5 μm in diameter, whereas the common marine *Coscinodiscus* and *Actinocyclus* (Fig. 1b) species vary between 30 and 600 μm. The largest cells of the centric genus *Ethmodiscus* can reach 2 mm in diameter. Needlelike species are also common among planktonic diatoms, ranging

from small Cymatosirales a few μm long (Hasle et al. 1983), through *Thalassionema* (10–100 μm in length) to *Thalassiothrix*, which can attain lengths of more than 5 mm. Some planktonic diatoms are solitary (e.g., *Stephanodiscus*, *Coscinodiscus*: Fig. 1a, d), but in many others the cells remain attached to each other after division to form colonies, which may be long filaments or stepped chains, e.g., in *Rhizosolenia*, *Chaetoceros*, *Skeletonema* (Fig. 2d), and *Pseudo-nitzschia*; ribbons, e.g., in *Fragilaria* (Fig. 2c) and *Fragilariopsis*; starlike (stellate), e.g., *Tabellaria*, *Asterionella* (Fig. 2a), and *Asterionellopsis*; or zigzags, e.g., *Thalassionema* and *Diatoma* (Fig. 5b). In some diatoms, the ability to form chains is facultative (e.g., *Mediopyxis*: Fig. 2b). In still others, the cells have long extensions or produce long chitin fibrils (Fig. 1c) that slow the rate of sinking, e.g., Walsby and Xypolyta (1977). Colonial morphology, such as in *Asterionella* and *Fragilaria*, can also be argued on physical grounds to be adaptations that slow sedimentation (Reynolds 2006). However, other diatom species growing in the same water may have no apparent mechanism to reduce sinking rate and indeed, sinking is arguably advantageous in some circumstances, e.g., to remove diseased cells from populations or to alleviate diminishing nutrient availability (Raven and Waite 2004). Sinking is enhanced by aggregation in the form of "marine snow" and live cells and empty frustules may be rapidly exported in this way (Smetacek 1985), facilitating deposit of diatom frustules on the ocean floor (rather than dissolution during sedimentation).

Growth in the plankton is dependent upon a supply of silica (generally in good supply in cold temperate oceans and after the winter input in lakes), and the rate of recycling of this element may be critical for the maintenance of populations. Other nutrients (especially N, P, and Fe), light intensity, and temperature are also controlling factors, in combination with the genetically determined physiological capacities and nutrient uptake systems of the cells. Equally important for population dynamics, however, are the "loss processes," which include sinking, outwash (in lakes at certain times of the year), physical or biochemical damage, parasitism, and grazing (Reynolds 2006). Only when the rate of cell growth overcomes these loss factors will the population increase and a diatom "bloom" occur, which can sometimes color the water brown, especially in spring. If a bloom continues for a long time, the available silica may be used up and the majority of cells may die. Small residual populations remain and grow again when conditions are favorable. Some marine planktonic species form thick-walled cells, which seem to help ensure short- or long-term survival of adverse conditions (McQuoid and Hobson 1996). These may be modified vegetative cells or specialized "resting spores" with a morphology quite unlike that of the vegetative cells (Round et al. 1990). A few freshwater diatoms, such as *Aulacoseira italica*, have been shown to sediment to the lake bottom and remain there in a viable vegetative state until the next growing period; in this case, in winter when the turbulence stirs the cells from the lake bottom into the water column (Lund 1954). This is impossible over most of the ocean surface, where the bottom is beyond the action of turbulence sufficient to resuspend the cells, but it may happen in inshore waters. A further ecological attribute of some marine planktonic species is the fixation of atmospheric nitrogen via endobiotic cyanobacteria (e.g., *Richelia intracellularis* in species of several diatom genera: Carpenter et al. 1999).

Benthos

The situation here is much more complex than that of the plankton because of the range of habitats in which either motile or nonmotile attached species occur. Nutrient concentrations are usually higher in benthic habitats than in the water column above. Nevertheless, the growth of benthic diatoms can also be limited by nutrient availability, and it has recently been shown that benthic diatoms perceive gradients of nutrient concentrations, e.g., of silicate (Bondoc et al. 2016), and exhibit directional movements in relation to them.

Epipelon and Soils. The surfaces of sediments of all kinds support a motile microbiota of diatoms. Whereas they can be found at some depth in the deposits and may exist there for some time, they only grow actively in the top few millimeters of the sediment. There are many records of soil diatoms at greater depths, but these are probably species that have been washed down or carried there by animals. In many lakes the epipelic microbiota only colonize sediments down to 5–10 meters below the water surface, depending upon the transmission of light through the water; in the sea, epipelon may extend to much greater depths. The vast majority of diatoms in this habitat are motile biraphid species (having raphe slits on both valves: Fig. 3a) because, after disturbance or burial by inwashed sediment, phototactic movement up to the surface is essential. These species often undergo circadian movements in and out of the surface sediment (Palmer and Round 1967; Round 1981a). A few filamentous species also "float" on the surface sediments in flocs where they seem to maintain themselves and avoid burial. Many epipelic diatoms are grazed by other protists, such as ciliates, and small animals, such as mollusks, and in some marine habitats by fish. Whereas the latter are probably nonselective, grazing by protists (Hamels et al. 2004) and parasitism by chytrids and oomycetes (Canter and Jaworski 1983, Mann 1999b) probably play a major role in controlling the diversity of epipelic and other diatom communities.

Epipsammon. Sand grains are often the site of attachment of small diatoms, and in some habitats every grain is covered by up to a hundred or more diatoms. Some grow adnate (closely appressed) to the surface of the grain, often forming short chains, whereas others perch on small mucilage pads and stand out from the grains, e.g., *Martyana* (Fig. 3d). The subtidal marine sand community (comprising both epipsammic and epipelic species) is probably the least explored in diatom ecology, due to its inaccessibility.

Epiphyton. All photoautotrophic groups, including algae and a few diatoms, are hosts to diatom species. A brown coating of diatoms on angiosperms and on green and red algae along coasts is often obvious to the naked eye. As with the sand-associated microbiota, some species are "glued" onto the plant surfaces (*Epithemia*, *Cocconeis*; Fig. 3c), whereas others are on short pads or stalks, projecting into the water (*Ulnaria*, *Achnanthes*; Figs. 3b and 4a). Yet others occur on long branching stalks (*Gomphonema*, *Licmophora*; Figs. 3e and 5b). Many attach by a corner pad of mucilage and then form zig-zag colonies when the cells remain attached to each other after cell division (*Diatoma*, Fig. 5b; *Amphitetras*, Fig. 5c; *Grammatophora*, Fig. 5d). All these features probably function (here and in other attached

communities) to project cells into positions where they will intercept more nutrients, capture more light, and compete less with adjacent organisms, with the counterbalancing risk of becoming more susceptible to grazers and parasites.

Epilithon. Rock surfaces support a microbiota of attached species. In protected regions, e.g., rock pools, filamentous species may develop upward into the water and some species grow inside mucilage tubes up to several centimeters long, e.g., *Berkeleya* (Fig. 4c) and *Parlibellus*. Recent evidence suggests that the diatoms inside a single tube may not be genetically identical (Hamsher and Saunders 2014): the tube may therefore be a cooperatively assembled structure, produced by several or many pioneer cells.

The relationship between the epilithic and epiphytic floras is not clear. Some genera and even species certainly live in both habitats, but whether any species are actually confined to one or the other requires further study. Both epiphytic and epilithic habitats may be stable for long periods of time (relative to the generation time of individual cells) and allow the establishment of "climax communities."

Metaphyton. Nonattached diatoms occur in the colorless mass of mucilage produced by some algae growing epibiotically (probably also epilithically) and remaining as a gel around the substratum. This community was first studied by Behre (1956) but few have investigated it in detail since then. Medlin (1983) showed that the metaphytic and epiphytic communities were distinct entities and that the epiphyton showed host specificity but the metaphyton did not. The diatoms within the mucilage are weakly motile. This community is very similar to the one developing in some acid streams and bog pools, consisting of masses of mucilage-forming sheets in which diatoms coexist with many other algae. These mucilage-based associations tend to be confined to waters of low pH.

Epizoon. This community is very little studied. Habitats include the feathers of diving sea birds (Holmes and Croll 1984) and the perisarc of hydroids, which often forms a rich substratum for *Cocconeis* (Fig. 5e) and *Grammatophora*. Small crustaceans can have species of *Synedra* (in fresh water) and *Pseudohimantidium* (in the sea) on their appendages; these diatoms seem to be specific to the animals. Shells of mollusks also support attached diatoms and all hard parts of dead animals become coated with diatoms. The skin of cetaceans is the substratum for species of *Bennettella* and *Epipellis* (Holmes 1985; Denys and De Smet 2010), whereas marine turtles bear diverse epizoic communities (Majewska et al. 2015) and may be important natural dispersal vectors for benthic species.

Symbiosis. The first endosymbiotic diatom recorded was *Licmophora* in *Convoluta* (Ax and Apelt 1965) and since then diatoms have been discovered to be endosymbionts of foraminifera (Lee et al. 1979; Lee 2011) and dinoflagellates (e.g., Pienaar et al. 2007; Chesnick et al. 1997; Imanian and Keeling 2014). The *Convoluta* and foraminiferan endosymbionts do not form siliceous wall elements within their hosts but can produce them again when extracted and cultured. Foraminifera also ingest free-living diatoms, and free-living species of diatoms may attach to the outside of the carbonate skeleton. As far as is known, the endosymbionts of dinoflagellates have totally lost the capacity to grow independently. A symbiotic relationship between the Antarctic ice diatom *Amphiprora kufferathii*

and its epiphytic bacteria has been demonstrated by Hünken et al. (2008). The diatom benefits with enhanced antioxidative defenses, and the bacteria utilize hydrogen peroxide produced by the diatom's photosynthesis.

Ice Diatoms. The microbiota of sea ice is a rather mixed one with diatoms being the dominant group (Thomas and Dieckmann 2003). When sea ice forms, the surface plankton is incorporated into the ice where it occupies brine pockets and channels which arise during freezing. The water in the brine pockets can attain salinities up to 4 times that of seawater as temperatures in the sea ice drop to below −10 °C. Some species do not survive, but many can withstand the hypersaline conditions and low temperatures, proliferating to form dense brown layers on the periphery and underside of the ice. Some of the species have narrow temperature requirements with optima around 2 °C and ceasing growth at 5 °C. *Melosira arctica* attaches to the lower surface of multiyear ice in the Arctic and produces long pendant columns.

The Siliceous Wall as Protection

The diatom protoplast of vegetative cells is never exposed, even during cell division, and its robust nature has led to suggestions, reviewed by Hamm et al. (2003), that the silica cell wall functions as a defense against predators. Whatever the truth of this, broken fragments of diatoms are common in fecal pellets and provide ample evidence of grazing in the marine water column, and there are also records of parasitism both in freshwater and the sea (Raven and Waite 2004). Canter showed evidence of infection of diatoms leading to accelerated decline of populations and demonstrated specificity in choice of closely related hosts (Canter and Jaworski 1983; Crawford et al. 1985; Mann 1999b). Penetration by parasites is sometimes achieved between the girdle bands or via apparent "weak points," such as the rimoportulae or raphe, but may also be through the valves (Kühn et al. 1996). The use of silica as a wall material has been suggested by Raven (1983) to reflect its lower energetic cost, relative to carbon.

Tolerance of Ecological Factors

Each individual species has a genetically determined range for existence and for optimal growth, which is then restricted further by competition and grazing. The ranges for very few species have been worked out in detail, but together, the diatoms occupy a remarkably wide span of environments. One important determinant of distribution is salinity. Some diatoms are stenohaline, being restricted to a narrow range of salinity (usually either freshwater or fully marine), but others are less fussy. Some marine diatoms extend down the salinity scale almost to fresh water and many grow optimally at salinities below the average 33–35‰ of seawater. Equally, some tolerate hypersaline conditions but as salinity increases, e.g., in tropical lagoons or salt works, the number of species decreases until at 120‰ only one or two survive

(Ehrlich 1975). However, no species have yet been confirmed as confined to salinities above that of normal seawater.

Extremes of temperature are also tolerated by a few species. For example, some diatoms are able to withstand extremely high temperatures in thermal springs: *Denticula elegans* was found living at 60–62 °C by Cassie and Cooper (1989) at Rotorua, New Zealand, and Cassie (1989) reported *Fragilaria construens* surviving 77 °C. However, most diatoms have much lower tolerance limits, and Hustedt (1959) considered 45 °C to be the upper limit for most species.

Fresh waters are chemically much more diverse than seawater and here there are clear species preferences, e.g., for acid, alkaline, or sulfate-rich waters. Some *Pinnularia* species can tolerate a pH of less than 2 (Sabater et al. 2003). In most cases, the physiological basis of these preferences has not been established. For example, in the case of pH, it is usually unclear whether it is pH itself that is selective or whether it is some other factor, such as the availability of carbon dioxide or bicarbonate, or of silicate or other nutrients, that is causal. The abundance of a few species is clearly correlated with water flow, e.g., *Meridion*. Whatever the physiological mechanisms, however, the combination of adequate taxonomy, identifiable preferences, and the long-term preservation of diatoms in lake and ocean sediments makes diatoms unrivalled for reconstruction of environmental change in aquatic habitats over periods of tens to millions of years (reviewed in Smol and Stoermer 2010).

Just as conditions may become suitable to sustain massive growths of planktonic diatoms, so too may benthic species be favored. This occurs spectacularly and disastrously in rivers in many parts of the world as a consequence of blanket growths of *Didymosphenia geminata* (e.g., Bothwell et al. 2014). This species severely compromises the ecosystem of affected rivers and causes expensive problems for water management.

Characterization and Recognition

Cell

The Bacillariophyta are all unicellular or colonial. Their vegetative cells are diploid and characterized above all by their complex siliceous walls. In many species the ornate pores, thickenings, and spines of the siliceous wall components are clearly visible under high magnifications in the light microscope, but further significant detail is always detectable by electron microscopy. It is possible to identify some species in live material, but traditionally the cells have been treated to separate the wall components and it is above all the morphology of the valves that forms the basis for classification and identification.

Inside diatom cells are the organelles typical of heterokont (stramenopile) algae. The plastids are conspicuous and vary in color from yellowish or greenish hues to a deep brown, and they are therefore sometimes called chromoplasts or chromatophores, rather than chloroplasts. They may be small discoid or lobed structures

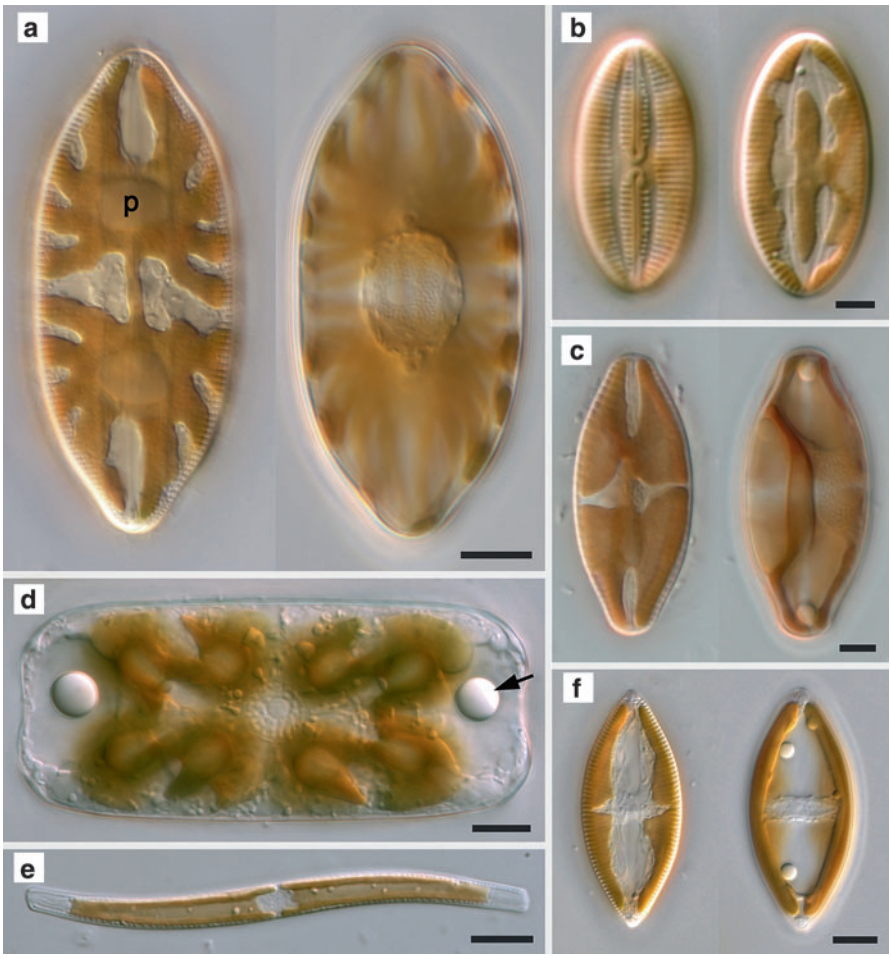

Fig. 6 Living cells of raphid pennate diatoms, all seen in valve view except (**d**, **e**). All scale bars = 10 μm, except (**c**). (**a**) Peripheral and central focuses of *Lyrella* cell. Note the strongly lobed chloroplast, which contains two roundish pyrenoids (e.g., p), and the central nucleus containing a prominent nucleolus and surrounded by a shell of cytoplasm containing Golgi bodies (appearing as short *curved bars*). (**b**) Valve and peripheral focuses of *Fallacia*. The lobes of the chloroplast are clearly related to the pattern of markings on the valves, avoiding the lyre-shaped clear area. (**c**) Peripheral and central focuses of *Placoneis*. Scale bar = 5 μm. (**d**) Amphoroid diatom in girdle view, with a highly convoluted chloroplast and two 'volutin' granules (e.g., *arrow*). (**e**) Sigmoid *Nitzschia* species containing two chloroplasts arranged end to end. (**f**) Peripheral and central focuses of *Navicula* cf. *palpebralis*; there are two chloroplasts, one on each side of the cell. Note also the central, transversely elongate nucleus and two volutin granules

(Figs. 1d and 2b), or platelike (Fig. 6e), or ribbonlike (Fig. 7d), or highly dissected and complex in shape (Figs. 6a–d, f, and 7a). In raphid diatoms, chloroplast morphology and position are usually highly constant within genera and can be used to help identify living diatoms. There is often a clear relationship between the

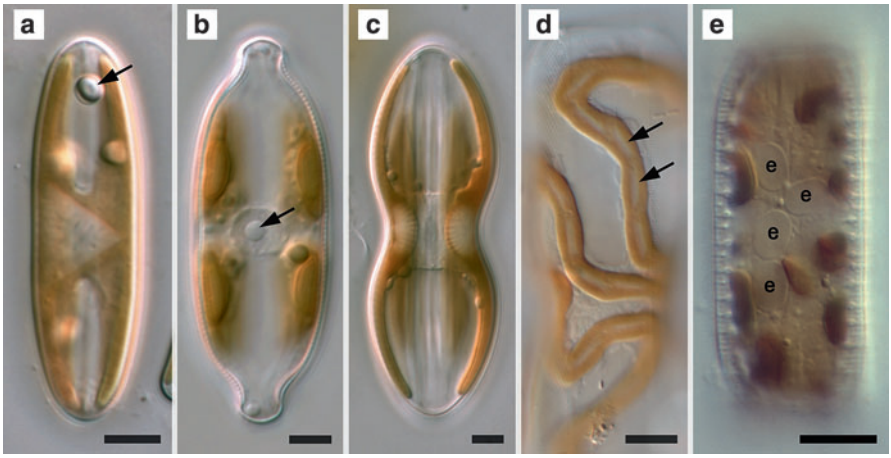

Fig. 7 Detail of chloroplasts and cells of raphid pennate diatoms. (**a**) *Sellaphora bacillum*. Note the H-shaped chloroplasts, the eccentrically placed triangular profile of the pyrenoid (with invaginations) and volutin granule (*arrow*). Scale bar = 5 μm. (**b**) *Neidium* cell with four chloroplasts and a central nucleus surrounded by Golgi bodies (appearing as *curved bars*) and containing a prominent nucleolus (*arrow*). Scale bar = 5 μm. (**c**) *Diploneis* cell with two chloroplasts, one on either side of the cell, each with a prominent invaginated pyrenoid at the center. Scale bar = 5 μm. (**d**) Part of a *Donkinia* cell with ribbon-like chloroplasts. Note the bar-like pyrenoids along the axis of the chloroplast (arrows). Scale bar = 10 μm. (**e**) *Epithemia* cell, containing four endosymbionts (e). Scale bar = 10 μm

position and shape of the chloroplasts and cell wall structures and other organelles (Fig. 6b).

The chloroplasts contain chlorophylls a and c, fucoxanthin, and various other carotenoid pigments, e.g., diatoxanthin and diadinoxanthin (Jeffrey et al. 2011; Egeland 2016). One or more pyrenoids are usually present in each chloroplast and are often conspicuous (Figs. 6a and 7a–d). The number of pyrenoids per chloroplast and their structure and positions vary among genera; some have angular shapes (Fig. 7a), probably reflecting a semicrystalline substructure. In a few genera the pyrenoids are penetrated by fingerlike extensions of the cytoplasm (Fig. 7a, c). The chloroplasts are bounded by four membranes, reflecting their ultimate origin through secondary endosymbiosis of a red alga (van den Hoek et al. 1995). Chloroplast (cp-) DNA is usually contained in a peripheral "ring nucleoid," running around the margin of the organelle (Kuroiwa et al. 1981; Coleman 1985), but in large-celled diatoms the arrangement can differ: in *Nitzschia sigmoidea* cp-DNA lines the sides of the linear pyrenoids (Mayama et al. 2004) and in *Pinnularia nobilis* it occurs as scattered granules (Mayama and Shihira-Ishikawa 1994).

The mitochondria have tubular invaginations of their inner membranes (Fig. 12f). Prominent shells of Golgi bodies occur around the nucleus in many pennate and most bipolar centric diatoms (Figs. 6a and 7b), whereas elsewhere among the centrics there are sometimes special associations of a Golgi body, endoplasmic

reticulum, and a mitochondrion (e.g., Pickett-Heaps et al. 1990), or of a Golgi body and either a mitochondrion or a chloroplast (Idei et al. 2012). The principal carbon storage products are oil globules and glistening whitish deposits of chrysolaminarin (a β–1,3 glucose polymer). Polyphosphates are also produced (Kuhl 1962), forming conspicuous "volutin" granules in some species (Figs. 6d, f, and 7a), and it seems likely that diatoms play an important role in transferring phosphorus from the water column to the sediments in the world's oceans (Diaz et al. 2008).

Some diatoms contain endosymbionts. Heterotrophic bacteria have been found in the raphid diatom *Pinnularia* (Schmid 2003a, b), and cyanobacteria are present in the vacuoles of some planktonic diatoms, such as *Hemiaulus* and *Rhizosolenia* (e.g., Janson et al. 1995), and in the cytoplasm of *Epithemia* (Fig. 7e) and *Rhopalodia* (Geitler 1977; Nakayama et al. 2011). These cyanobacteria contribute to the symbiosis principally through nitrogen fixation (e.g., Foster et al. 2011; Kemp and Villareal 2013). The endosymbionts of *Epithemia* and *Rhopalodia* are incapable of independent existence and indeed of photosynthesis (Nakayama et al. 2014). How these cyanobacteria entered diatom cells, despite the presence of the frustule, is a mystery; the only naked cells known in *Epithemia* and *Rhopalodia* are the amoeboid gametes.

Cell Wall and Cell Division

The diatom cell wall (***frustule***) is often likened to a Petri dish (cf. Fig. 1a, b) because it consists of two overlapping halves (***thecae***). However, this is a little misleading, because each theca is itself composite, consisting of a series of hoops (the ***girdle bands***) attached to the edge of a large endpiece (the ***valve***). One theca (the ***hypotheca***) is generally slightly smaller than the other (the ***epitheca***: Fig. 8a–d) and is always younger, being formed after the latest mitosis. During the cell cycle, the hypotheca slides out from beneath the older, overlapping epitheca and new bands are added to its edge; in this way, the cell increases in volume. The volume cannot be increased in any other direction because the siliceous valves and girdle bands, like glass, are essentially inelastic, although they can flex (e.g., in the living cells of the raphid diatom *Craticula*, the valves bow outwards as a result of the turgor of the cell, despite being well-silicified and robust: Mann 1994).

Once the cell has grown sufficiently and the hypotheca has attained more or less the same length and structure (with the same number of girdle bands) as the epitheca, mitosis is initiated. As the division of the nucleus is completed, cytokinesis takes place and two new valves (usually with at least some of their accompanying girdle bands) are formed within the frustule of the parent cell, before the old thecae separate. Then the two daughter cells separate, each inheriting one of the valves of the parent cell and one of the newly formed valves. This highly characteristic, semiconservative mode of cell division, is known only from this phylum and has fundamental consequences for much of diatom biology, e.g., causing average cell size to decrease during the vegetative phase (see below).

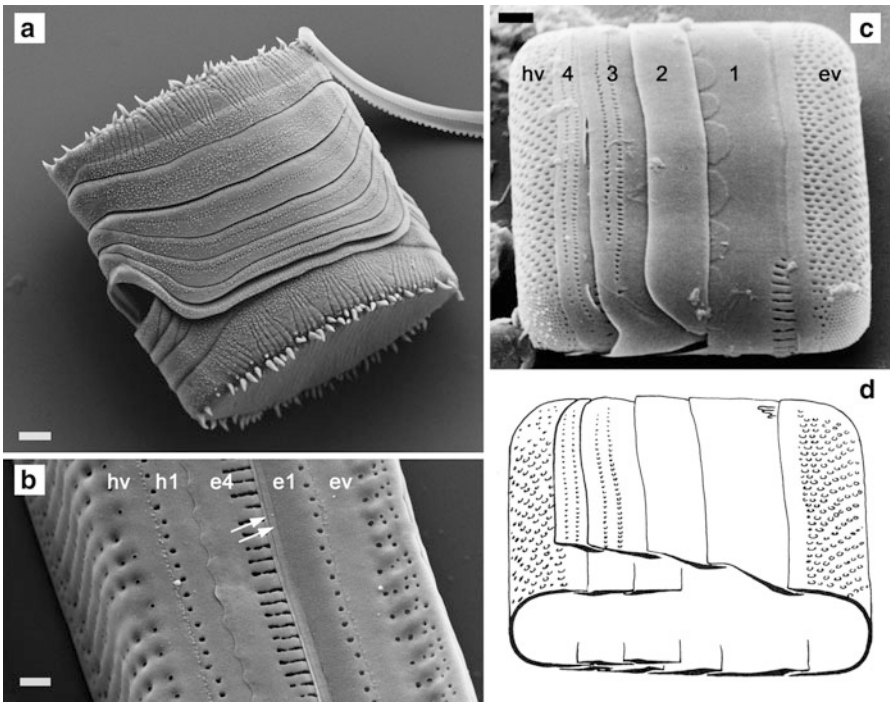

Fig. 8 Frustule and girdle structure. SEM. (**a**) *Diatoma* frustule: the epitheca is at the top, overlapping the hypotheca. Scale bar = 2 μm. (**b**) Detail of a *Nitzschia* frustule. The epitheca comprises the epivalve (*ev*) and four girdle bands, two wide (e1, e4) and two very narrow ones (*arrows*) in between. The epitheca partially overlaps the hypotheca, of which the hypovalve (*hv*) and one band (h1) are visible. Scale bar = 500 nm. (**c**, **d**) Photograph and drawing of a *Grammatophora* frustule. The epivalve to the *right* (ev) is linked to four girdle bands (1–4), which partly obscure the hypovalve to the *left* (hv). Note the variation of markings in the girdle-bands. The schematic cut-away drawing of a *Grammatophora* frustule (**d**) illustrates the spatial relationships of the frustule components in **c**. The two girdle-bands of the incompletely formed hypocingulum (*left*) are assumed. Scale bar = 10 μm

In many diatoms, the daughter cells separate fully once the new valves are complete, but in some the valves remain connected by organic material or interlocking or fused silica projections (Figs. 2d and 12d, e). In this way, chains can be formed, which, with some important exceptions (these include colonies of cells on branched stalks, thalloid mucilaginous colonies in *Dickieia* and mucilaginous tubes in various raphid diatoms, e.g., *Berkeleya* [Fig. 4c]), are the only means of colony formation. In a few diatoms, the new valves are not smaller than the valves of the parent cell because of an unusual flexibility of the girdle. Consequently, these species can grow in culture indefinitely, without any reduction in the average size of the cells in the population (Chepurnov et al. 2004).

The valves are perforated by numerous small pores, arranged in species-specific patterns. Traditionally, two main types of valve pattern have been recognized (Schütt

1896). In the "***centric***" type of organization, the pores are arranged in radiating rows (striae: Figs. 1a, b, d), subtended at the pattern center (which is not always at the center of the valve) by a small ring (***annulus***), within which pores are less regularly arranged or absent. Centric diatoms can be circular (Fig. 1a–d), oval, triradiate or triangular, quadrate (Fig. 5c), or many angled; less often they are elongate. Molecular phylogenetic studies have shown that the centric diatoms are not a monophyletic grouping but, depending on the criteria selected for the analysis (see Medlin 2014), fall either into a grade of separate lineages or into two monophyletic classes, comprising the radial and bipolar centrics, respectively (see section "Summary Classification"). In the "***pennate***" type of organization (Fig. 9a–g), the pattern is feather-like (Latin *pinna* or *penna* = feather), the striae lying in two rows either side of a longitudinal bar or rib (the ***sternum***). Pennate diatoms are almost invariably elongate but may be isopolar (Figs. 9b, g) or heteropolar (Figs. 9a, c), bilaterally symmetrical (Figs. 9b) or dorsiventral (Fig. 9d, i). The down-turned side of the valve is known as the ***valve mantle*** and the markings on this may differ from those on the top of the valve (the valve face). Unlike the centrics, the pennate diatoms are always recovered as monophyletic in molecular phylogenies (e.g., Sims et al. 2006; Theriot et al. 2010), but sternum-like structures have evolved independently in some centric lineages, perhaps through elongation of the annulus (e.g., Kooistra et al. 2003a).

The majority of pennate species have two complex slits along or near the midline of the valves – these are known as ***raphe slits***, and it is through them that the organism achieves locomotion (Fig. 9b–i). A model to explain raphe function was proposed by Edgar and Pickett-Heaps (1984) and no major revision of this seems yet to be needed. Mucilage fibrils are secreted into and through the raphe slits, apparently from Golgi-derived vesicles, but remain connected to the protoplast via transmembrane components. In turn, the transmembrane components interact with actin microfilaments lying immediately beneath the raphe and are constrained to stream along the raphe slits (Round et al. 1990). Hence, if the mucilage fibrils become attached distally to a firm substratum, the effect of the streaming will be to generate motion of the whole cell, which occurs at speeds of up to 20 μm or more per second. Mucilage is left behind as a trail when it reaches the ends of the slits, forming part of the "extracellular polymeric substances" released by diatom cells and performing various functions including adhesion and providing structure (Daniel et al. 1987; Underwood and Paterson 2003).

Some genera have raphe slits on both valves (***biraphid***), while others (the ***monoraphid*** diatoms, which are polyphyletic) have slits on one valve only. In the latter, motility is limited and slow and the cells are attached to the substratum for most of the time by mucilage, e.g., *Cocconeis* (Fig. 3c) and *Achnanthes* (Fig. 3b). For accounts of the various diatom polysaccharides, see Hoagland et al. 1993; Underwood and Paterson 2003; Gügi et al. 2015. The raphe slits can run along the midline of the valve (Figs. 9b, c, f) or may be displaced to one side (Fig. 9g, i) or even circumferential (Fig. 9e). The raphe normally consists of a pair of slits running from either side of a clear central area to the apex, where the external fissure often bends and continues as a blind surface groove (Fig. 5c). In several genera, e.g., *Nitzschia* and *Hantzschia* (Fig. 6d), the slits are bridged internally by short bars

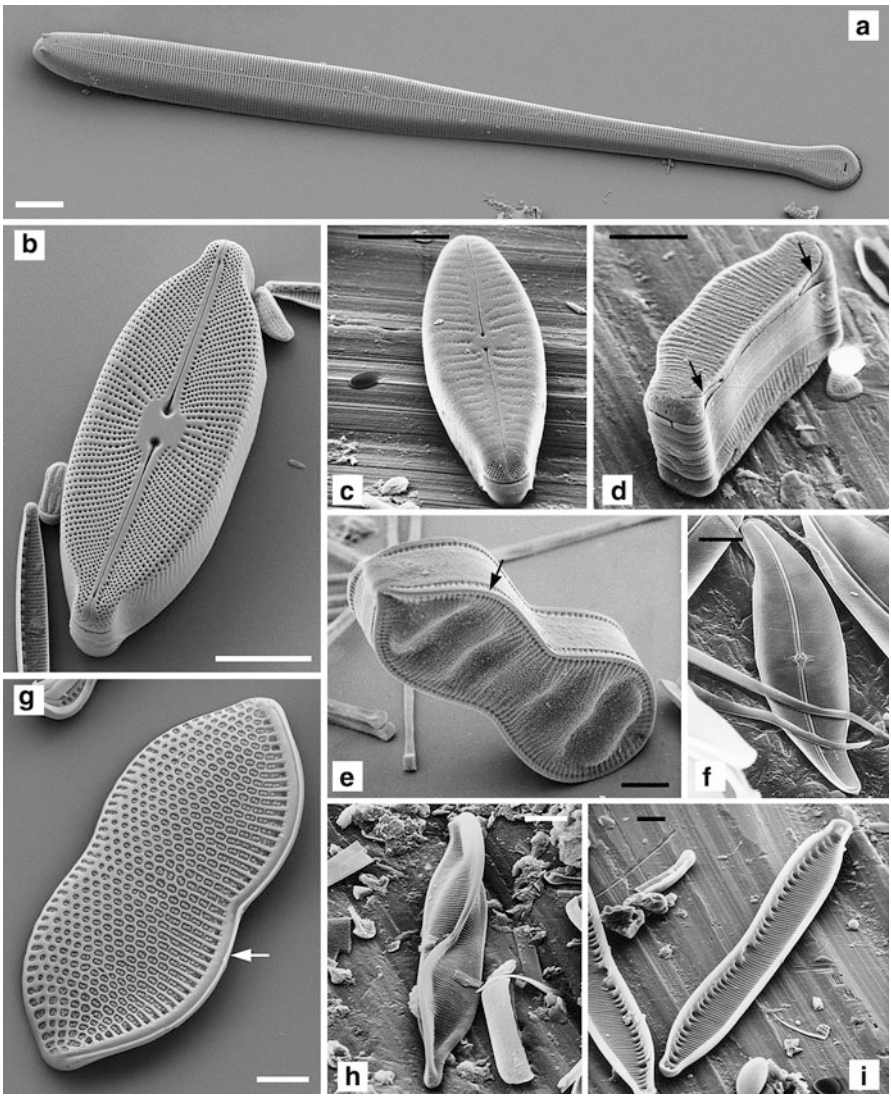

Fig. 9 Pennate diatoms. SEM. All except *Licmophora* (**a**) are raphid diatoms. (**a**) *Licmophora* valve; a stalk (like those shown in Figs. 3e and 5a) is secreted through special pores at the narrower end. Note the sternum running along the center of the valve and bearing transverse ribs on either side. Scale bar = 5 μm. (**b**) *Cosmioneis* frustule. Note the two axial raphe slits and slightly radiating striae. Scale bar = 10 μm. (**c**) *Gomphonema*, with heteropolar symmetry. Scale bar = 10 μm. (**d**) Frustule of *Eunotia*, which has short raphe slits (*arrows*) that run from the valve face over onto the mantle. They are found on the same side in the two valves of each frustule. Scale bar = 10 μm. (**e**) *Cymatopleura* frustule. The valve face is undulate and the raphe (*arrow*) runs round the rim of the valve with a discontinuity at either end. SEM. Scale bar = 10 μm. (**f**) Sigmoid symmetry of *Gyrosigma*. Scale = 10 μm. (**g**) *Psammodictyon* valve. The raphe (*arrow*) is borne on a raised keel at the margin of the valve. Scale bar = 2 μm. (**h**) *Entomoneis* valve: the raphe is elevated on a ridge, which takes a sigmoid curve along the valve. Scale bar = 10 μm. (**i**) The raphe of *Hantzschia* lies to one side of the valve (shown here from the inside) and is subtended on the inside by a number of small bridges (fibulae). Scale bar = 5 μm

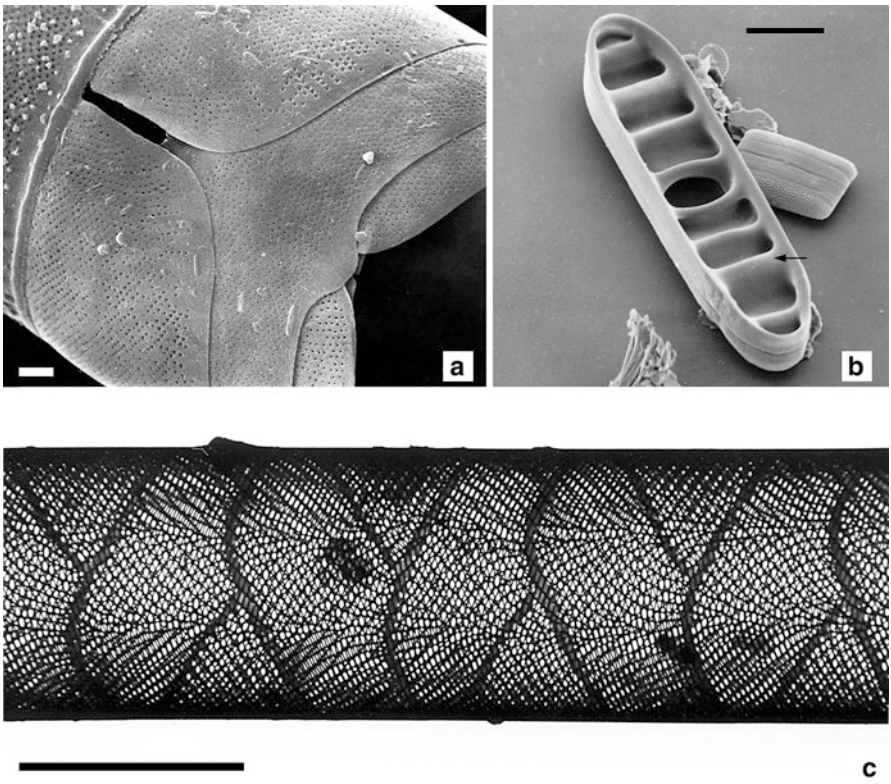

Fig. 10 Girdle bands. (**a**) *Pleurosira:* the gaps left by the split ends of the first and third bands are partially or completely closed by an enlargement of the second band. SEM. Scale bar = 10 μm. (**b**) Girdle band of *Grammatophora* with its characteristic undulate septum (*arrow*). SEM. Scale bar = 10 μm. (**c**) The scale-like girdle bands of *Rhizosolenia*. Transmission electron micrograph (*TEM*). Scale bar = 1 μm

(fibulae), which appear to function as ties, preventing the valve from splitting along the raphe. In the genus *Eunotia* and its allies, which seem to be an early offshoot of the raphid diatom lineage (Theriot et al. 2010), the raphe slits are very short and lateral to the sternum instead of integrated into it (Fig. 9d), but the cells are nevertheless motile. As in the centric series (Figs. 1b and 5c), there is all manner of variation in valve outline and topography in pennate diatoms, including sigmoid (Fig. 9f, h) and keeled (Fig. 9h) forms.

The siliceous girdle bands are frequently split rings, with the splits in adjacent bands lying at 180° to each other. Opposite the split in one band there is a tonguelike extension (ligula) of the adjacent girdle band to fill the gap (Figs. 8a, c, and 10a). In a few genera some of the bands are complete hoops, e.g., in *Grammatophora*, where the bands also bear well-developed septa extending part way into the cell lumen (Fig. 10b). Still other diatoms have a girdle composed of individual segments (Fig. 10c), appearing like diamond-shaped scales.

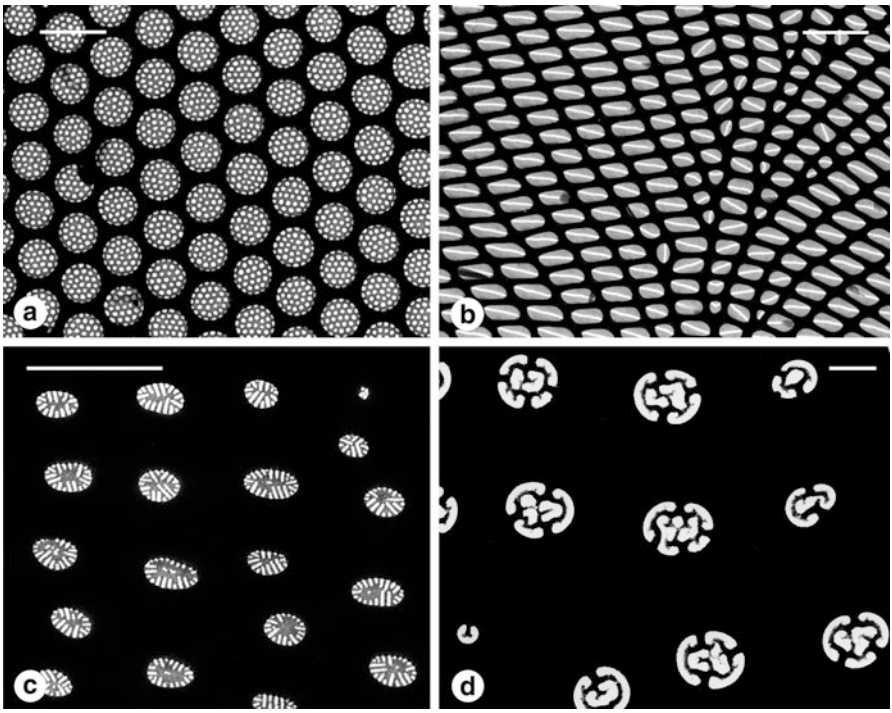

Fig. 11 Details of areola structure. TEM. Scale bars = 1 μm. (**a**) *Roperia*: each areola has many small pores in a thin siliceous velum. (**b**) Each velum of *Rhizosolenia* has just one narrow slit. (**c**) The areolae of *Cocconeis* are variable in size and shape and so is the pattern of slits in the velum. (**d**) The vela of *Rhaphoneis* are branching, interconnected projections from the side of the areolae

The pores of the valves and girdle bands, termed ***areolae***, allow transfer of water, nutrients, gases, cellular products, etc. between environment and cell. Only rarely, however, are they simple channels through the silica. In most cases, a fine plate of silica, itself perforated by tinier holes, stretches across the pores. These plates are known as ***vela*** or ***pore plates*** and take many different forms, each to some extent characteristic of genera or groups of genera; the position of the velum, towards the inner or outer ends of the areolae, is also of systematic importance. Some of the variation to be found among vela can be seen in Fig. 11a–d. The areolae of the girdle bands are usually similar to those on the valves but much smaller. The last-formed bands (furthest from the valves) are often plain. In some cases, all the girdle bands lack pores.

The valves tend to be more complex than the girdle bands and may have special types of apertures in addition to the areolae. The most common type of special aperture, found in most centric and a few pennate diatoms, is developed internally as a slit between a pair of lips and externally either as a simple opening or a tube and is termed a ***rimoportula*** (Fig. 12a, b) or ***labiate process***. The functions of rimoportulae remain unclear in most cases, although in a few cases they have been shown to be

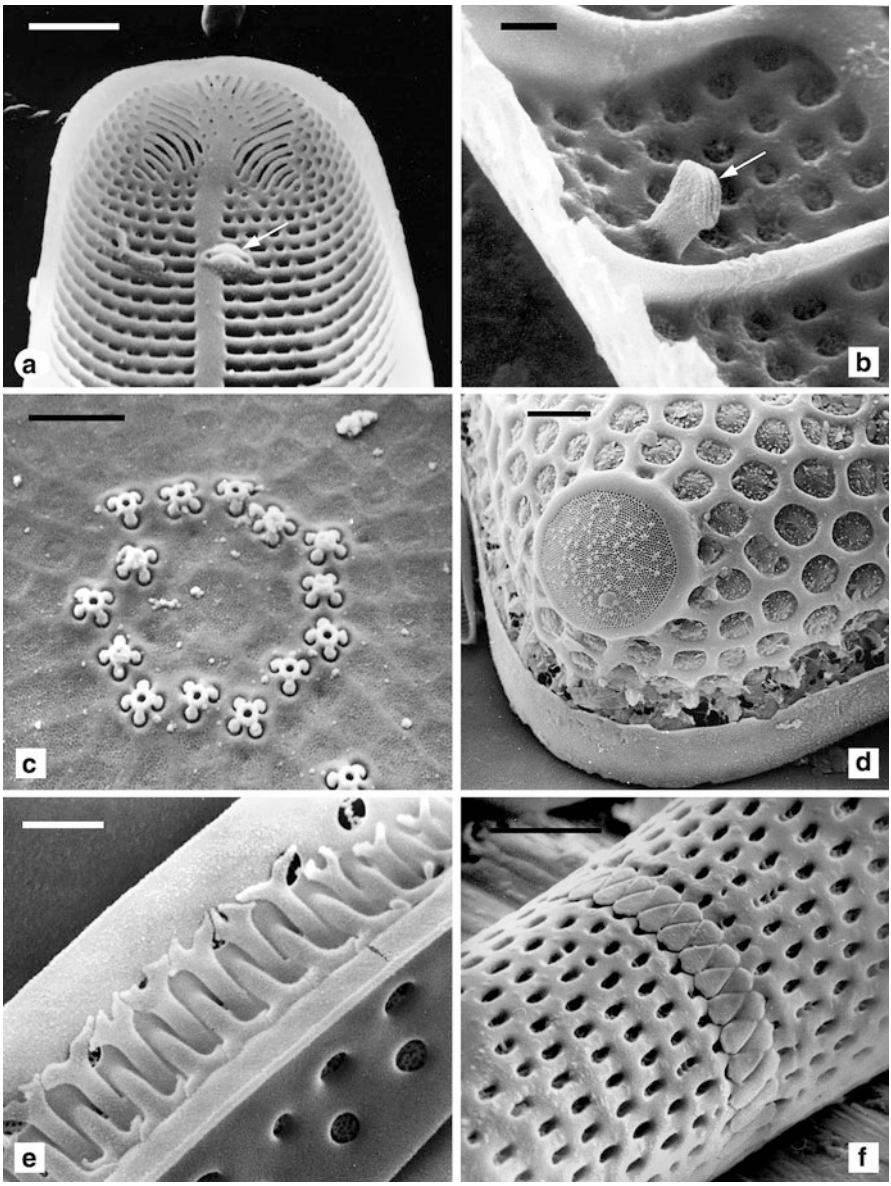

Fig. 12 Special wall structures. SEM. Scale bars = 1 μm. (**a**) Rimoportula of *Cyclophora* (*arrow*). (**b**) Stalked rimoportula of *Triceratium* (*arrow*). (**c**) Internal apertures of the fultoportulae of *Thalassiosira*. (**d**) Ocellus of *Odontella*. (**e**) Linking spines connecting two valves of *Cymatosira*. (**f**) Linking spines connecting two valves of *Aulacoseira*

involved in secretion for motility (Medlin et al. 1986; Pickett-Heaps et al. 1990) or endocytosis (Kühn and Brownlee 2005). The other well-known type of process is

confined to the centric order Thalassiosirales and is called the ***fultoportula*** or ***strutted process***. This consists externally of a simple tube or opening and internally of a tube surrounded by a number of basal satellite pores separated by buttresses (Fig. 12c), or with the satellite pores developed as tubes. Its function is the secretion of chitin fibers (Fig. 1c) that connect cells together or control sedimentation (Walsby and Xypolyta 1977). Additionally, in many epiphytic, epilithic, and epipsammic diatoms there are areas of simple pores near the periphery or ends of the valves (Figs. 5d, 8c, 9c, and 12a, d), from which stalks or pads of mucilage are secreted to link the cells to the substratum or to one another.

Spines, tubercles, etc. are common on the outer surfaces of the valves but never on the girdle bands, nor on the inner surface of the valves. Some spines (Fig. 12e, f) act to connect cells together in chains and in a few genera the exit tubes of the rimoportulae or fultoportulae act as interlocking devices.

Diatom mitosis and particularly the structure and functioning of spindle and associated structures have been the focus of considerable detailed research, which has contributed significantly to a general understanding of the mechanism of mitosis (Pickett-Heaps 1991). In some species a small dense body of granular material is associated with microtubules and lies near the nucleus during interphase. This ***microtubule organizing center*** (***MTOC*** or ***centrosome***) breaks down at prophase and at the same time a complex and highly ordered spindle develops nearby. Cytokinesis occurs through ***cleavage*** (Round et al. 1990). Mitosis and cytokinesis are followed by the formation of new valves (indeed, this sequence is generally obligatory). The MTOC reforms and migrates to a position between the nucleus and the ***silica deposition vesicle*** (***SDV***), which is a flattened sac beneath the cell membrane in which the new valves are formed. The nucleus and the MTOC usually remain intimately associated with the developing valve, and systems of microtubules (subtended by the MTOC) and microfilaments are present, which may play a role in the expansion of the SDV and the morphogenesis of the valve (Pickett-Heaps et al. 1990). In some diatoms, treatment with microtubule inhibitors results in the formation of distorted valves, although the basic rib–stria system appears to be little affected. A special structure, the ***raphe fiber***, has been found immediately below the forming raphe slits in recently divided cells of raphid diatoms and may be involved in generating the complex shape of the raphe (Pickett-Heaps et al. 1990). A somewhat similar fibrous structure – ***the labiate process apparatus*** – is present while the rimoportulae are formed.

Since 2000, there have been major advances in our understanding of how silicate is acquired by cells and converted into the amorphous hydrated silica of the valves and girdle bands (Hildebrand 2008; Hildebrand & Lerch 2015; Finkel 2016), stimulated by the realization that diatoms achieve feats of chemical engineering in ambient conditions that materials chemists achieve only by using high temperatures and pressures. Building on earlier studies by Volcani and coworkers (e.g., chapters in Simpson and Volcani 1981) and using modern molecular and genomic approaches, it has been possible to characterize components of the silicon transport system (Hildebrand 2008) and to show that silica deposition in the SDV is catalyzed and mediated by at least two classes of proteins: (1) ***silaffins***, which are peptides rich in

serine and lysine that have been extensively modified after translation by methylation, phosphorylation, and covalent linkage with polyamines and silacidins; and (2) ***silacidins***, which contain mostly phosphorylated serine and aspartic and glutamic acids (Sumper and Brunner 2008). It appears that interactions between silaffins, silacidins, the polyamines, and polysaccharides, e.g., chitin, control the detail of silica deposition (e.g., Richthammer et al. 2011). Recently, transcriptomics approaches have added considerably to knowledge of which genes are involved in silicification (reviewed by Finkel 2016).

However, although the biochemical and electrostatic properties of silaffins and silacidins probably take us a long way towards understanding the finer detail of cell wall development, it is not yet clear that they are relevant to larger-scale morphogenesis in diatoms: the creation of the beautifully ordered patterns of ribs and pores of diatom valves still mostly eludes explanation. Pickett-Heaps et al. (1979) proposed that an organic template is formed, onto which silica is deposited from both sides. This may be true for pennate diatoms whose wall is a simple laminate structure but the structure of more complex walls, such as are found in many centric diatoms, suggests the formation of one layer first, onto which a chambered or ***loculate*** system is later superimposed (Crawford 1974a; Schmid and Volcani 1983; Round and Crawford 1984). Lenoci and Camp (2008) have been able to generate patterns very similar to those of many diatoms possessing chambered or folded valves, using a model based on phase separation on a planar surface, and Pickett-Heaps et al. (1990) argue that the cytoskeleton and cell organelles are probably also involved in mesoscale patterning in diatoms; this is supported also by more recent studies using fluorescence labeling (Tesson and Hildebrand 2010).

The initial development of the valve almost always involves sequential formation of a tightly controlled rib–stria pattern, and the way that the pattern varies in relation to disturbances (e.g., Mann 2006) and natural variation in valve size indicates that the rib–stria system and any template controlling its appearance must form as the SDV expands outwards from the initial pattern center (e.g., Schmid and Volcani 1983; Pickett-Heaps et al. 1990), which is usually either the annulus (in centric diatoms) or the sternum (in pennate diatoms). Explanation of the control of rib spacing during the production of the initial layer (which must be very precise, since otherwise the species taxonomy of diatoms would not work as well as it does) is probably the main remaining challenge in understanding diatom morphogenesis.

At the gross level, cell shape in diatoms is created largely during the expansion of the auxospore (see below) and then gradually modified by differential flexing of the girdle during the subsequent phase of slow decline in size during the vegetative phase (Mann 1994), except in species with circular valves where no modification occurs except in teratologies.

Life Cycle

As noted above, in some species the girdle bands are sufficiently flexible to allow the new valves to be as large as the old valves, even though they are formed within the

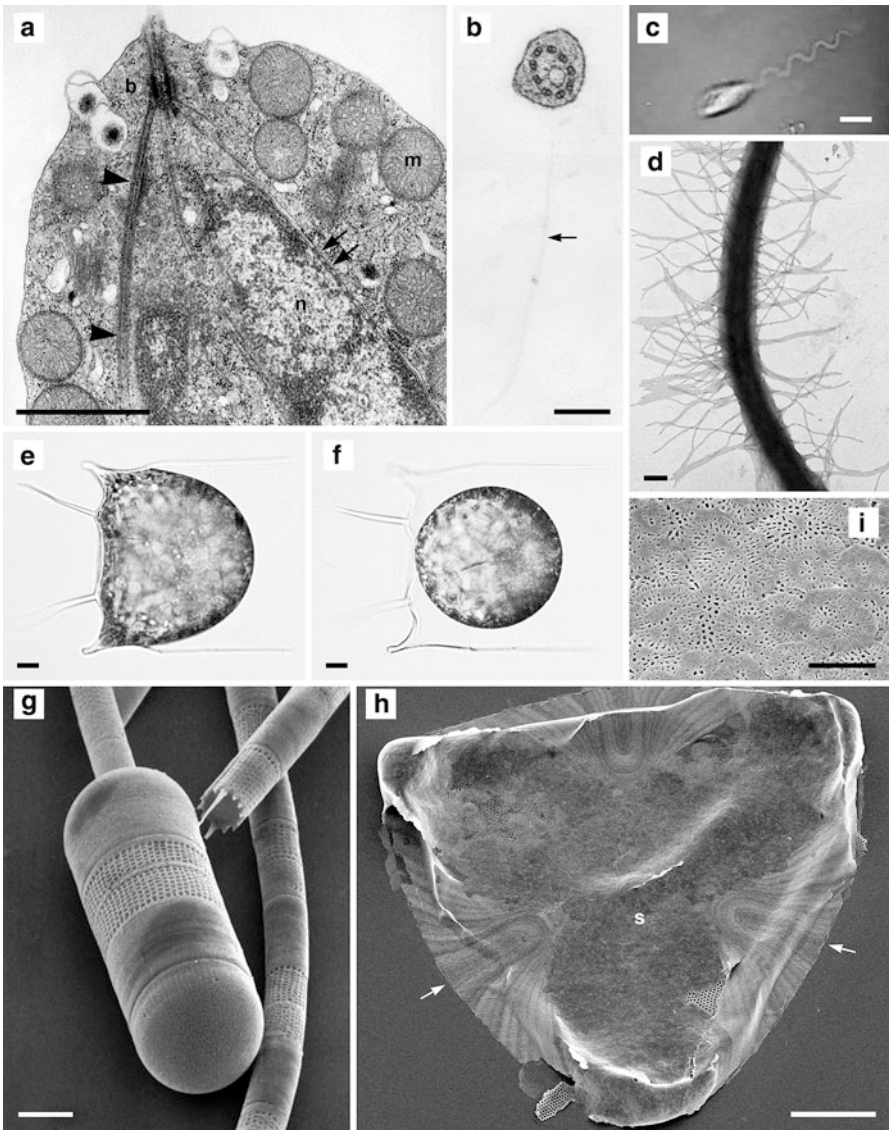

Fig. 13 Sexual reproduction in centric diatoms. Micrographs (**a**–**d**, **h**, **i**) were very kindly provided by Professor Masahiko Idei (Bunkyo University, Japan) and are reproduced here by permission (**a**) Apex of a *Thalassiosira* sperm in longitudinal thin section, showing the flagellar basal body (*b*) subtending a cone of microtubular bundles (e.g., *arrowheads*) that extend over the surface of the nucleus (*n*). Many nuclear pores are evident (*arrows*). Note also abundant rounded mitochondria (*m*) containing tubular invaginations of the inner membrane. TEM. Scale bar = 1 μm. (**b**) Transverse thin section through the flagellum of *Melosira moniliformis* var. *octogona*. The axoneme lacks central microtubules (9+0 configuration) but frequently contains vesicles, as here. Long mastigonemes are attached to either side of the flagellum (e.g., *arrow*). (**c**) Swimming sperm of *Thalassiosira*. High-speed video still, showing quasi-sinusoidal beat. Scale bar = 5 μm.

parent cell's frustule. However, in most diatoms one of the new valves is smaller than the smaller of the two parent valves by double the thickness of the girdle bands (Crawford 1981). Consequently, a succession of mitotic cell divisions generally results in a diminution of the average valve dimensions. Ultimately, death of the population will result unless the maximum dimensions of the cells are restored. This usually occurs via an auxospore formed following sexual reproduction. Sexual reproduction is morphologically isogamous in most pennate genera, but oogamous, with motile sperm and larger nonmotile egg cells, in the various lineages of centric diatoms (although information is absent for many genera).

Though regarded until recently as almost universally homothallic, diatoms do in fact exhibit a variety of mating systems (Chepurnov et al. 2004; Sato et al. 2011; Davidovich et al. 2012). Some pennate diatoms (probably the majority) are heterothallic, whereas others (and also most centric diatoms) are facultatively or habitually homothallic. Reduced sexuality (via auto- or apomixis) has evolved independently in several lineages (e.g., Mann et al. 2013; Poulíčková et al. 2015).

In oogamous diatoms, ***sperm*** (Fig. 13c) are produced following a series of divisions within a modified cell (***spermatogonium***); they are then released and swim to find the ***egg cell*** (produced within an ***oogonium***: Fig. 13e), presumably guided by chemotaxis. Pennate diatoms lack flagellate stages and here the gametes (Fig. 14b) are usually all alike (morphologically isogamous) and show very limited autonomous movement; in raphid pennate diatoms, it is the sexualized vegetative cells that move, using their raphe systems to find each other and pair actively before meiosis is initiated (Fig. 14a). The cells then often surround themselves with a capsule of mucilage (Fig. 14d), in which gametogenesis and fertilization take place. Araphid pennate diatoms are not generally able to move very effectively and in some genera (e.g., *Tabularia*, *Pseudostaurosira*), the gametes are differentiated into small nonmotile female gametes and ± equally small male gametes that possess curious threadlike appendages that generate spinning and unidirectional movements, which help the gametes find each other (Sato et al. 2011; Davidovich et al. 2012), though only over very short distances. ***Pheromones*** have recently been demonstrated to be involved in the sexualization and chemotaxis of pennate diatoms (Sato et al. 2011; Gillard et al. 2013; Moeys et al. 2016), and the genetic basis of sex determination is now being explored for the first time (Vanstechelman et al. 2013).

Fig. 13 (continued) (**d**) Mastigonemes in two rows on the flagellum of *Hydrosera*. Whole mount, TEM. Scale bar = 200 nm. (**e**) Theca of living *Odontella* oogonium containing partly naked egg cell. Scale bar = 10 μm. (**f**) Egg of *Odontella* with polarized cell contents. Scale bar = 10 μm. (**g**) Pre- and postauxospore cells of *Aulacoseira*. The large hemispherical valves either end of the wider filament are initial valves, i.e. the first valves formed within the spherical auxospore. SEM. Scale bar = 10 μm. (**h**) Ventral side of the auxospore wall (incunabula and perizonium) of *Triceratium*. The center is covered by a mass of small scales (detail in **i**). The triangular shape is created as a result of differential wall hardening through deposition of a complex set of perizonial strips, beginning with a triradiate element with its center on the dorsal side, whose three arms curve back (*arrows*) onto the ventral side. Other bands are then added adjacent to the primary band (cf. Round et al. 1990, fig. 65). (**i**) Incunabular scales of *Triceratium*. SEM. Scale bar = 5 μm

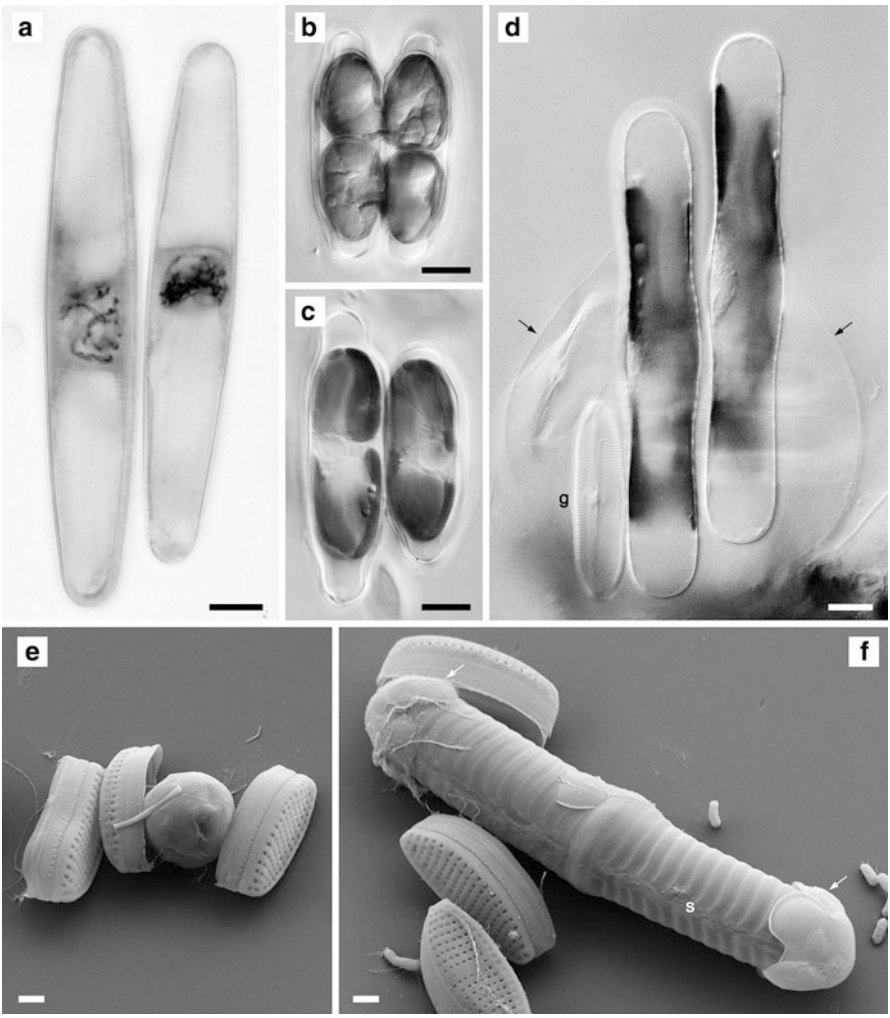

Fig. 14 Sexual reproduction in pennate diatoms**.** The images in (**e**) and (**f**) were very kindly provided by Drs Shinya Sato and Laia Rovira. (**a**) Paired cells of the raphe-bearing pennate diatom *Navicula oblonga* in meiotic prophase (diplotene at *left*, zygotene at *right*). Haematoxylin-stained preparation. Scale bar = 10 μm. (**b**) Paired cells of *Neidium*, each containing two large non-flagellate gametes. The gametes are beginning to move (clockwise) into the adjoining cell. Scale bar = 10 μm. (**c**) Paired cells of *Neidium* following fusion of the gametes and movement of one gamete from each gametangium into the other cell. Each parental frustule therefore now contains a single zygote. Scale bar = 10 μm. (**d**) Expanded auxospores of *Caloneis*, flanked by a valve of one gametangium (*g*). Gametogenesis and fertilization occurs here within a large ellipsoidal mass of mucilage (*arrow*). Scale bar = 10 μm. (**e**) Spherical zygote of *Nitzschia inconspicua* flanked by the thecae of the gametangial frustule. The zygote is covered by incunabula of silica scales. Scale bar = 1 μm. (**f**) Expanded auxospore of *Nitzschia inconspicua*. The scale-case of the zygote has been split into two scaly caps (*arrows*) by the growth of the auxospore, which develops a cylindrical shape through sequential hardening of its wall, outwards from the center, by perizonial strips; these are open on one side, forming a 'suture' (*s*). Scale bar = 1 μm

Meiosis occurs during gametogenesis (Fig. 14a). The isogametes of pennate diatoms (Fig. 14b) and the egg cells of oogamous diatoms (Fig. 13e, f) all possess plastids, as do some sperm, although it is unclear whether sperm plastids ever survive in the zygote after fertilization of the egg cell. The gametes mostly lack their own siliceous walls, although they are often protected by mucilage capsules or (in centric eggs and many pennate diatoms) by remaining partly enclosed within the frustule of the mother cell (e.g., Idei et al. 2012). Diatom sperm possess two opposite rows of tripartite mastigonemes (Fig. 13b, d) and perform quasi-sinusoidal movements (Fig. 13c) in the plane of the mastigonemes, like the flagellate cells of other heterokont protists, but they differ from them in the structure of the flagellar axoneme, which lacks central microtubules (i.e., the axoneme has a ***9+0 configuration***: Fig. 13b and Idei et al. 2013b). The flagellar apparatus is also unusual in lacking the usual systems of microtubular and fibrous roots, though it sometimes possesses instead a cone of microtubules extending down over the surface of the nucleus (Fig. 13a). No transitional helix is present, and there is no trace of a second flagellum or basal body in the few sperm that have been studied in detail.

Fertilization is followed by development of the zygote into an auxospore, so-called because it is this cell that is able to grow and restore the maximum size characteristic of the species. The zygote produces an organic wall as it differentiates into an auxospore and, as the auxospore expands, silica elements are often inserted into the wall, creating regions that afford some rigidity and probably control expansion. Consequently, an initially spherical (Fig. 14e) or ellipsoidal zygote (Fig. 14c) can be transformed into more complex shapes – elongate cigars with or without a swollen central portion (Fig. 14d, f), bananas, spindles, triradiate forms, or stars (Mann 1994). The special silica elements added to the auxospore wall to stiffen it are sometimes many and intricate (e.g., Poulíčková et al. 2007) and can be divided into (1) the ***incunabula*** – i.e., those elements formed by the zygote before expansion (Trobajo et al. 2006), which can include circular or elliptical scales (Figs. 13i and 14e) or narrow strips of plain silica – and (2) the ***perizonium*** (Figs. 13h and 14f), which comprises several or many bands (often differentiated into transverse and longitudinal series) that are formed sequentially by the auxospore as it expands (Idei et al. 2013a). The development of the auxospore often involves a considerable increase in dimensions (to twice or several times the length of the gametangia in some pennate diatoms: Figs. 14d, e, f). Once expansion is complete, a new cell – the ***initial cell*** – is formed within the auxospore (Fig. 13g). This involves two successive mitoses, each preceding the deposition of a new large valve. The initial valves are usually unlike the valves produced during the vegetative phase because they are formed within and molded by the auxospore, not by another frustule. In addition, the initial valves of chain-forming diatoms differ from those produced subsequently by virtue of the fact that they lack interlocking spines, etc., and come to lie at the ends of the filaments to which they give rise after subsequent cell divisions. Sometimes modifications of cell shape occur during formation of the initial valves, after auxospore expansion is complete, as a result of contractions of the auxospore away from parts of its wall. The divisions of the initial cell and its immediate

descendants are followed by the formation of valves that correspond ever more closely to those typical of the species.

The chloroplasts can be inherited uniparentally or biparentally in diatoms (Mann 1996), and it has recently be shown that recombination can occur between chloroplast genomes located in different plastids within the same cell (D'Alelio and Ruggiero (2015).

The sexual process – and hence restoration of the maximum size – is initiated only when the cells are within a certain size range (Geitler 1932; Chepurnov et al. 2004). Until a ***critical size threshold*** is passed, cells can only reproduce vegetatively. Particular environmental conditions are probably also required for sexualization in many cases, although in culture there seems to be little difference between the conditions required for active vegetative growth and those that permit auxosporulation. Auxospore formation occurs infrequently in nature, because the length of the sexual phase is much shorter than the period of vegetative multiplication during which cell size diminishes (a few days or weeks as opposed to months or years: Mann 1988). Hence it is not surprising that there are rather few records of auxosporulation in natural populations (but see references in Mann 1988 and, e.g., D'Alelio et al. 2010, Jewson & Granin 2015). In temperate planktonic communities, records of auxospores tend to be restricted to the beginning or end of the growing period, i.e., in spring and late summer (e.g., Jewson 1992). Thinning of the population by entering the sexual phase with a large commitment of cells to gamete formation, as in *Corethron pennatum* (Crawford 1995, as *C. criophilum*), could have advantages for survival of the assemblage through periods of low nutrients brought about by a bloom (Crawford et al. 1998) and sidestep the interruption of synthesis that is one costly consequence of the sexual phase (Lewis 1983). In natural populations of single species, small cells generally outnumber large cells; this seems also to reflect the costs of sexual reproduction, in lost synthesis and aborted or unfit gametes and zygotes (Mann 2011).

Taxonomy

Karsten's (1928) system is a convenient starting point for tracing the development of modern diatom classifications. Karsten placed the diatoms in a division (or phylum), the Bacillariophyta, as have many modern workers (see Round 1981b). Within this, he recognized two orders, the Centrales (***centric diatoms***) and the Pennales (***pennate diatoms***), based on the organization of the pattern on the valves – which is radially or concentrically ordered (rarely irregular) in the Centrales, and feather-like in Pennales. This subdivision is also echoed in the features of the sexual reproduction of the two groups – oogamous in centrics but usually isogamous and always lacking flagellate sperm in pennates. Silva (1962) elevated the centrics and pennates to classes (Centrobacillariophyceae and Pennatibacillariophyceae) and created or amended a number of orders within them, which brought the classification of diatoms into line with that of other major algal groups.

Scanning electron microscopy revealed further groupings beyond those recognized by Karsten and Silva. Round et al. (1990) therefore suggested many changes and new taxa, from classes to genera, in an attempt to summarize likely relationships, based not only on cell wall detail but also on cytological and other information. Analyses of molecular sequence data (especially from 18S rDNA) have subsequently shown that neither the traditional classification nor the revised system by Round et al. can be upheld, although some aspects of each gain support. Unfortunately, there is as yet no agreement about what should replace the older classifications, nor about whether it is sensible to make any changes at all until a clearer picture of diatom evolution emerges.

In the Round et al. (1990) classification, the diatoms (Bacillariophyta) were split into three classes: Coscinodiscophyceae, Fragilariophyceae, and Bacillariophyceae. These three are readily identifiable. The Coscinodiscophyceae equated more or less to the Centrales (Centrobacillariophyceae) and comprised all those diatoms with radial organization of the primary valve pattern, centered upon a small ring (annulus). The Fragilariophyceae and Bacillariophyceae together comprised the Pennales (Pennatibacillariophyceae) of earlier classifications, all having feather-like organization (transverse ribs and rows of pores, subtended by a longitudinal sternum). The two classes were separated by the absence (Fragilariophyceae) or presence (Bacillariophyceae) of a raphe system. The three classes of Round et al. (1990) seem mostly to avoid the charge of polyphyly. However, it is now clear that they do not capture the essential features of diatom evolution, since two of the three classes (Coscinodiscophyceae and Fragilariophyceae), as defined by Round et al. (1990), are almost certainly paraphyletic. Medlin and Kaczmarska (2004) therefore suggested a new system, in which the diatoms are split into two subdivisions, Coscinodiscophytina and Bacillariophytina. The Coscinodiscophytina comprised only centric diatoms (i.e., having a centric organization of the valve pattern); the Bacillariophytina, on the other hand, contained both centric diatoms, classified by Medlin and Kaczmarska into the Mediophyceae, and pennate forms, classified in the Bacillariophyceae. However, in some subsequent analyses the Coscinodiscophytina and the Mediophyceae have both been paraphyletic (e.g., Sorhannus 2007; Theriot et al. 2011), or the Mediophyceae have been monophyletic but not the Coscinodiscophytina (e.g., Ashworth et al. 2012; Nakov et al. 2015). If either of these later reconstructions accurately reflects evolution, the Medlin–Kaczmarska scheme will not satisfy most systematists, who require monophyly of taxa. Medlin (2014) notes, on the other hand, that if certain criteria are met in the molecular analysis, the Coscinodiscophytina and Mediophyceae **are** recovered as monophyletic clades and it has also been suggested (e.g., Medlin 2015, 2016a) that some reproductive and morphological features are consistent with the Medlin–Kaczmarska classification. In summary, there is as yet no consensus on the phylogeny and classification of centric diatoms. However, even if the Coscinodiscophytina and Mediophyceae are not monophyletic, Medlin and Kaczmarska's revision made two significant advances on the previous system proposed by Round et al. (1990): (1) it recognized that the primary evolutionary radiation took place among diatoms with a centric organization and oogamous

reproduction, and (2) it restored unitary status for the pennates, which are monophyletic in most molecular phylogenies and are characterized morphologically by the possession of a single sternum as the pattern center.

At the ordinal to family level, some of the groupings recognized by Round et al. (1990) and earlier authors appear monophyletic in molecular phylogenies and formal analyses of morphological characteristics. Examples are the Cymatosirales, Thalassiosirales, Bacillariales, Sellaphorineae, and Naviculaceae (e.g., Theriot et al. 2010, Ruck and Theriot 2011). However, many do not. Thus, *Proboscia* and *Urosolenia* are not closely related to *Rhizosolenia* (Round et al. placed them together in the same family, Rhizosoleniaceae), and *Achnanthes* and *Achnanthidium* are not related, despite their similarly monoraphid frustules (Round et al. placed them together in the Achnanthales) (e.g., Medlin and Kaczmarska 2004; Sorhannus 2007; Theriot et al. 2010). In contrast, at the genus level, many of the revisions suggested or incorporated by Round et al. (1990) have been supported by later analyses, such as the removal of *Ardissonea* and *Toxarium* from *Synedra* (Medlin et al. 2008), or the separation of *Lyrella*, *Petroneis*, *Fallacia*, *Sellaphora*, and *Placoneis* from *Navicula* (Jones et al. 2005; Bruder and Medlin 2007; Evans et al. 2008). At present, however, there are few or no molecular data for many genera and even where molecular data are available, the phylogenetic trees they yield often contain few nodes that (from bootstrap support values or posterior probabilities, or congruence with morphological or other data sets) can be regarded as reliable. Furthermore, different approaches to alignment and phylogeny reconstruction are adopted by different researchers, with significant effects on the phylogenies obtained (e.g., contrast Medlin & Kaczmarska 2004 with Theriot et al. 2015). Hence it is not surprising that there is no consensus yet about what should replace the Round et al. classification. The completion of current initiatives to develop multigene phylogenies of diatoms (e.g., Ashworth et al. 2013) will hopefully lead to a more satisfactory system. This will probably involve many major changes in how particular groups of species or genera are classified: a good example, showing the difficulties of reconciling existing taxonomy with new understanding, based on molecular and refined morphological analysis, is given by Ruck et al. (2016) in a study of the Rhopalodiales and Surirellales.

Given current uncertainty (except that previous classifications are wrong in many respects), we depart significantly from the previous edition of this book and present a greatly simplified classification (Table 1) modified from Adl et al. (2005). It is based on a comparison of recent published phylogenies and classifications (e.g., Theriot et al. 2010, 2011; Nanjappa et al. 2013; Nakov et al. 2015; Li et al. 2015; Medlin 2016a, b), taking into account the persistent lack of support for many basal nodes in molecular analyses (e.g., Theriot et al. 2015, fig. 1) and the frequent lack of a clear pattern in the distribution of morphological and cytological characters. Decisions about which clades should be recognized among the "radial centrics," ("Coscinodiscophytina") is especially problematic. In order to get an idea of the diversity that Table 1 represents, but ignoring the classification imposed upon it in 1990, readers should refer to the atlas of genera by Round et al. (1990), although many further genera have been described since that book was written.

Table 1 Major clades and paraphyletic taxa of diatoms. The examples of genera listed include the genera illustrated in this chapter

Division	*Bacillariophyta*	Descriptions and subgroups	Examples of taxa
Subdivision Subdivision Coscinodiscophytina: monophyletic in Medlin and Kaczmarska (200$) (and then comprising the single class Coscinodiscophyceae), paraphyletic in Theriot et al. (2015). Contains several clades of radial centric diatoms whose interrelationships are unclear. Valves generally circular; pattern-center an annulus; sexual reproduction via oogamy; auxospores with scales only	leptocylindrids	Chain-forming, delicate; valves circular, striae radiating from a central circular annulus; unique simple process present near the annulus; girdle bands segmental; auxospore forming a dormant resting stage (not present in other centric clades)	*Leptocylindrus*, *Tenuicylindrus*
	corethrids	Solitary; valves circular; radially symmetrical; articulating spines secreted from around the valve margin; rimoportulae absent; girdle bands segmental	*Corethron*
	melosirids	Usually chain-forming, sometimes forming special "separation valves"; valves circular, radially symmetrical; rimoportulae small, scattered on the valve face or marginal; girdle bands hooplike or segmental	*Aulacoseira*, *Melosira*, *Podosira*, *Stephanopyxis*
	ellerbeckiids	= "paralids" of Mann in Adl et al. (2005): Chain-forming, heavily silicified; valves circular, radially symmetrical; small tube processes present, restricted to the mantle; girdle bands hooplike	*Ellerbeckia*
	arachnoidiscids	Solitary, heterovalvar; valves circular, radially symmetrical; one valve with its center surrounded by radial slits (apparently modified rimoportulae); girdle bands hooplike	*Arachnoidiscus*
	coscinodiscids	Solitary, isovalvar; valves usually circular, striae radiating from a central, subcentral, or submarginal circular annulus; rimoportulae central, scattered on the valve face or marginal; girdle bands hooplike	*Actinocyclus*, *Actinoptychus*, *Coscinodiscus*, *Stellarima*, and many others
	rhizosolenids	Chain-forming, with a long perivalvar axis, rarely solitary; valves circular, almost radially symmetrical or with the pattern-center displaced towards one side; rimoportula single, associated closely with the annulus, sometimes developed into a spine; girdle bands segmental	*Guinardia*, *Rhizosolenia*
	proboscids	Usually solitary, with a long pervalvar axis; valves circular, extended into an eccentric beak (proboscis); rimoportulae and other processes absent; girdle bands segmental	*Proboscia*

(*continued*)

Table 1 (continued)

Division	*Bacillariophyta*	Descriptions and subgroups			Examples of taxa
	Subdivision Bacillariophytina	Valves usually elongate or structurally bipolar or multipolar, as a result of anisometric expansion of the auxospore, constrained by a perizonium (not present and likely secondarily lost in Thalassiosirales)			
		Class Mediophyceae (possibly paraphyletic with respect to the Bacillariophyceae)	Pattern-center an annulus (which is sometimes elongate rather than circular); valve outline and structure highly varied, mostly bi- or multipolar; sexual reproduction via oogamy		Thalassiosirales, Cymatosirales, Lithodesmiales, Chaetocerotales, Biddulphiales, *Attheya* and others; includes *Amphitetras*, *Cyclotella*, *Cymatosira*, *Hydrosera*, *Mediopyxis*, *Odontella*, *Pleurosira*, *Skeletonema*, *Stephanodiscus*, *Thalassiosira*, *Triceratium*
		Class Bacillariophyceae (pennate diatoms)	Pattern-center a sternum; sexual reproduction via morphological isogamy, rarely anisogamy		
			Subclass Urneidophycidae	Nonmolecular characters as for Bacillariophyceae	*Asterionellopsis*, *Delphineis*, *Plagiogramma*, *Rhaphoneis*, *Talaroneis*
			Subclass Fragilariophycidae (core araphids)	Nonmolecular characters as for Bacillariophyceae	*Asterionella*, *Diatoma*, *Fragilaria*, *Grammatophora*, *Martyana*, *Rhabdonema*, *Tabellaria*
			Subclass Bacillariophycidae (raphids)	Possession of a raphe system	*Achnanthes*, *Berkeleya*, *Caloneis*, *Cocconeis*, *Cymatopleura*, *Diploneis*, *Donkinia*, *Entomoneis*, *Epithemia*, *Eunotia*, *Fallacia*, *Gomphonema*, *Gyrosigma* *Hantzschia*, *Hippodonta*, *Lyrella*, *Navicula*, *Neidium*, *Nitzschia*, *Placoneis*, *Psammodictyon*, *Sellaphora*, and many others

A complementary approach, in which molecular phylogenies are used to test explicit hypotheses concerning the evolution of specified traits, has recently been applied and has provided insights into variation and changes in salinity preference (Alverson et al. 2007), cell size (Nakov et al. 2014), growth form (Nakov et al. 2015), and reproductive behavior (Mann et al. 2013; Poulíčková et al. 2015).

At the species level, studies of reproductive isolation and fast-evolving genes indicate that the diatoms are even more speciose than was already known. Common freshwater and marine diatoms have proved to be complexes of several or many species that are difficult or impossible to identify reliably using the light microscope (e.g., Sarno et al. 2005; Amato et al. 2007; Evans et al. 2008; Souffreau et al. 2013; Vanormelingen et al. 2013). It is likely that many other "species" are likewise composite, with potentially adverse consequences for ecological studies, biomonitoring, biogeography, and other sciences dependent on consistent and accurate identification. To help obviate difficulties, DNA barcoding is being developed (e.g., Mann et al. 2010; Zimmermann et al. 2011).

Maintenance and Cultivation

Enrichment and Isolation from Nature

Diatoms are relatively easy to culture in mixed populations simply by enriching natural water with nutrient solutions or transferring subsamples to artificial media (see below). It is convenient to do this in Petri dishes, which can then be observed directly at low magnification with a stereo-microscope or inverted microscope to check for growth. It should always be remembered that diatoms require dissolved silicate for growth and this is usually added to media, although it is sometimes assumed that supply will be adequate if soft-glass dishes are used. Light may be natural or artificial and alternating light/dark cycles or silicon-starvation can be used to achieve a degree of synchrony (e.g., Darley and Volcani 1971). Temperature should be adjusted by experimentation; most diatoms grow over a wide range but some, e.g., ice diatoms, can have a very narrow range.

To isolate clones, individual cells or colonies can be picked out from mixed cultures or natural samples with a micropipette, washed in sterile medium, and transferred to new sterile media. Alternatively, natural samples can be spread on agar plates, using normal microbiological technique. If the plates have been previously dried for a short time in an oven at 30 °C or in a flow hood, the liquid of the sample will quickly be absorbed and the individual cells will be trapped on the agar surface, where they can either be picked off immediately using a micropipette or allowed to grow into colonies. If the latter approach is taken, discrete colonies can be removed after a few days or weeks by cutting out agar blocks, each with a colony originating from a single cell, or subsampled using a micropipette and transferred to clean agar or liquid media. Clones may survive for months or years (especially if the growth rate is reduced by use of low light and temperature), but as previously noted, many cannot be kept indefinitely because of size reduction and a mating system that

enforces outbreeding. Thus clonal cultures may not always be ideal for maintaining diatoms in culture and unialgal cultures may be more suitable for long-term survival. It should be remembered in any case that meiosis and recombination are likely to occur in clones of homothallic and automictic diatoms maintained for months or years in culture and that consequently cells should be reisolated before critical experimental work is undertaken.

Axenic Cultures

The usual mixtures of antibiotics (e.g., of streptomycin, ampicillin, or penicillin) can be added to cultures to suppress bacteria and, through repeated transfer, produce axenic cultures (Andersen 2005).

Culture Media

Growth media suitable for freshwater and marine diatoms and other algae are given in the handbook edited by Andersen (2005). Relatively high quantities of silicate are of importance for culturing diatoms, but otherwise no special requirements are necessary for routine culture. Apart from vitamins, no organic additives to media are generally needed, except of course for the few obligate heterotrophs. However, some diatoms have so far remained recalcitrant ("unculturable"), particularly large-celled species from marine intertidal sandflats (e.g., Droop et al., 2000).

For freshwater diatoms, we frequently use WC medium, which was developed originally by Guillard and Lorenzen (1972). This contains:

36.76 mg	$CaCl_2.2H_2O$
8.71 mg	K_2HPO_4
36.9 mg	$MgSO_4.\ 7H_2O$
28.42 mg	$Na_2SiO_3.\ 9H_2O$
12.6 mg	$NaHCO_3$
85.01 mg	$NaNO_3$

Micronutrients:

3.15 mg	$FeCl_3.\ 6H_2O$
0.18 mg	$MnCl_2.4H_2O$
0.01 mg	$CuSO_4.5H_2O$
0.022 mg	$ZnSO_4.7H_2O$
0.01 mg	$CoCl_2.6H_2O$
0.006 mg	$Na_2MoO4.2H_2O$
1.0 mg	HBO_3
4.36 mg	Na EDTA

Vitamins:

0.1 mg	Thiamine.HCl
0.5 μg	Biotin
0.5 μg	Vitamin B12

Make up to 1 l with deionized water. Alternatively, stock solutions can be made at 1000× concentration and added at 1 mL L^{-1}. The vitamins should be added after autoclaving. WC is a fairly nutrient-rich medium, usually adjusted to around pH 7 (with drops of HCl). Diatoms from acid oligotrophic waters may be better grown in a modified GG medium (von Stosch and Fecher 1979).

For marine diatoms, we have found Roshchin medium (Roshchin 1994) to be effective: dissolve 202 mg KNO_3, 17.9 mg $Na_2HPO_4.12H_2O$, 1.2 mg $Na_2S_2O_3.5H_2O$, and 10 mg Na_2SiO_3. $9H_2O$ in 1 L filtered seawater; trace elements and vitamins can be added as for WC medium. Again, stock solutions can be made at 1000× strength. The medium is sterilized by pasteurization or filtration, since autoclaving leads to precipitation of some components. Alternatively the well-known series of "f" media can be used, in particular f/2 medium (Andersen 2005). If fully defined marine media are required, an artificial seawater mix can be used instead of natural seawater.

Evolutionary History and Biogeography

Fossil Record

The fossil record of diatoms has been briefly summarized by Sims et al. (2006) and Harwood et al. (2007). The earliest generally accepted records of diatoms are of "*Pyxidicula*" species, from the late Early Jurassic of Germany (Toarcian; c. 190 Mya) (Rothpletz 1900). However, the original source of the material is unknown. Rothpletz boiled a fossil sponge in HCl (hence the original specimen was destroyed), and the resultant siliceous residue (diatoms) was mounted and sectioned (Medlin 2015, 2016a). The earliest diverse, well-preserved diatom assemblages studied in modern times are from the Early Cretaceous, especially a deposit from the Weddell Sea (Gersonde and Harwood 1990; Harwood and Gersonde 1990). Paleozoic records have been reported but are now discounted as contamination. The absence of diatoms from Paleozoic or PreCambrian deposits has sometimes been ascribed (e.g., Round 1981b) to conversion of the diatomaceous silica to porcelanite and later to chert (a process described by Calvert 1977). However, although many diatom deposits have undoubtedly been lost through diagenesis, the order of appearance of major diatom groups in the fossil record agrees reasonably well with molecular phylogenies (Sims et al. 2006; Kooistra et al. 2007) and tentative dating of molecular trees suggests that the fossil record, though imperfect, does not hugely underestimate the origin of the diatoms: a Mesozoic or latest Paleozoic (late Permian) origin is the most likely (Kooistra and Medlin 1996; Sorhannus 2007; Medlin 2011, 2015, 2016a). The date of origin of the pennates, however, is particularly controversial

(Medlin and Desdevises 2016). In the Tertiary, an extensive fossil record has been preserved and is used for stratigraphic correlation and for calibrating the molecular clock in phylogenetic studies.

Nevertheless, although the fossil record is more reliable than some have thought, dissolution and fragmentation of the more delicate species certainly does occur and results in a modified picture of the natural assemblages that originally existed. For example, biochemical markers indicate that the microfossil record of *Rhizosolenia* and related genera (whose frustules are composed largely of girdle bands) underestimates their age (Sinninghe Damsté et al. 2004), and the blanket bogs of boreal regions often contain a rich diatom flora of strongly silicified acidophilic species whereas a few centimeters down in the peat there are often no diatom remains, presumably because of dissolution. Further sources of serious bias for evolutionary studies is the greater likelihood that planktonic species will become fossilized, because of their much greater initial abundance and distribution, relative to benthic species, and the lack of suitable depositional environments for marine littoral species. The Eocene diatomites at Oamaru in New Zealand are an important exception, preserving a highly diverse assemblage of well-preserved near-shore marine diatoms (Edwards 1991). On land, the short life of most lakes and destruction of deposits by glacial and other erosion lead to a surprisingly poor fossil record for freshwater diatoms, though there are some remarkable exceptions (e.g., the Eocene Giraffe Pipe deposits in NW Canada: Siver et al. 2010). Preservation of internal structure is extremely rare, but diatoms with cell content have been discovered in late Cretaceous cherts in Mexico (Beraldi et al. 2015).

Freshwater and terrestrial diatoms are usually considered to occur somewhat later in the geological sequence than marine ones, but some recently discovered Early Cretaceous deposits in Korea may be of terrestrial origin (Harwood et al. 2007). Multiple invasions into freshwaters have been documented using molecular phylogenies (Sims et al. 2006), and some have been demonstrated to occur in the reverse direction (Alverson et al. 2007). Molecular clock methods have been used to date invasion times in the Thalassiosirales (Alverson 2014). Recently, diatoms have been found preserved in amber (Girard et al. 2009).

The fossil record is still underused as a source of information for phylogenetic reconstruction and systematics at the generic and species level. Increasingly, however, the fossil record is being used in conjunction with neontological analysis and molecular phylogenies to estimate the tempo of evolution in particular diatom genera or families (e.g., Souffreau et al. 2011). In a few cases, it has been possible to use fossil material to detect anagenetic changes within what appears to be a single lineage, such as the evolution of *Stephanodiscus yellowstonensis* from *S. niagarae*-like ancestors in Yellowstone Lake, Wyoming (Theriot et al. 2006). For some marine planktonic groups impressive fossil records are available, documenting morphological evolution over many millions of years (e.g., Yanagisawa and Akiba 1990).

Biogeography

During most of the twentieth-century species, species concepts and delimitation in diatoms – and consequently data on species distributions – were based almost entirely on the morphology of the valve as seen with the light microscope. Latterly, details observable with EM have gained importance and this, coupled with insights from mating experiments and (still more recently) molecular sequence data, has been accompanied by an explosion in the descriptions of new species. There has certainly also been a trend towards narrower species definitions – a coarse-grained taxonomy has been replaced by a much finer one (Mann 1999b). Furthermore, whereas it was always accepted (e.g., Hustedt 1942) that some diatoms appeared to be restricted to particular regions because of dispersal constraints, as opposed to ecological restrictions, a much greater proportion of new species are now being claimed to be endemic to particular small regions or lakes.

It is doubtful whether many of the claims of endemism are justified, given the difficulties and inconsistencies in identifying diatoms (partly because there are so few critical revisions of any diatom genera [Kociolek and Williams 2015] and partly because of problems in accessing all the relevant literature), the rather limited sampling of many parts of the world (especially in Africa, S America, and SE Asia, and more generally in the tropical zone both in the sea and on land), and the very real problem of how to detect microeukaryote species when they are rare (i.e., occurring at frequencies of less than, say, 1 in 10^6). Likewise, claims that particular species have been introduced (e.g., Coste and Ector 2000) also need to be treated with caution (e.g., Gómez and Souissi 2010). Some diatoms do seem to be restricted to particular regions by geographical barriers, rather than the availability of suitable habitats: examples are discussed by Vanormelingen et al. (2008) and include the unmistakable genus *Eunophora*, apparently restricted now to temperate Australasia. There is also clear evidence for isolation by distance between populations of some heterothallic species, even on scales of a few tens or hundreds of kilometers (Vanormelingen et al. 2015). On the other hand, there are also examples, confirmed by barcode and/or mating data, of species and haplotypes with extremely wide distributions (e.g., Evans et al. 2009; Rimet et al. 2014), and geographical pattern disappears very quickly as one ascends the taxonomic hierarchy from species to genera, implying rather rapid spread of diatom lineages, relative to higher plants and vertebrates. On the other hand, there is also evidence of range contractions. For example, the genus *Arachnoidiscus* was formerly present in Europe (e.g., in the Miocene: Hajós 1986), but is now extinct there, the nearest populations being in the Indian Ocean.

Origin of the Diatoms

There is still a huge gap in our understanding of how and when diatoms acquired their unique morphology and life-cycle characteristics. Originally, the diatoms were kept as a quite separate group, allied to various algal/animal groups. Pascher (1914, 1921) seems to have been the first to suggest that the diatoms have features in common with the

Chrysophyceae and Xanthophyceae. To reflect this, he placed all three groups together in the phylum Chrysophyta. Ultrastructural and molecular sequence data have confirmed the general thrust of Pascher's idea, placing the diatoms unambiguously among the heterokont protists ("stramenopiles") within the chromalveolates (e.g., Andersen 2004; this position is recognized in the overall classification of protists by Adl et al. 2005). However, a close relationship to silica scale-producing algae, such as the Chrysophyceae, is not likely according to molecular and ultrastructural evidence (e.g., Derelle et al. 2016). At present, molecular phylogenies indicate that the closest known relatives of the diatoms are the Bolidophyceae and Parmales, which are small groups of marine autotrophic picoplankton with the same kind of four-membrane-bound plastids as diatoms and other autotrophic heterokonts (Guillou et al. 1999; Ichinomiya et al. 2011). The relationship with the Parmales was earlier predicted by Mann and Marchant (1989), because Parmales produce silica scales that, in their pattern and apparently space-filling ontogeny, resemble diatom valves and girdle bands. In particular, the round plates produced by Parmales often possess ring structures (annuli) at their centers (Booth and Marchant 1987), like centric diatom valves (Round and Crawford 1981). However, although Parmales scales seem to develop centrifugally from an annulus, as in diatoms, the two groups differ significantly in their morphogenesis, because Parmales plates develop within the cell (Yamada et al. 2016), whereas diatom valves and girdle bands are always formed peripherally in association with the cell membrane; and also in the control of silicification, because cell growth and division are not prevented by silicon depletion in Parmales (Yamada et al. 2014), whereas in diatoms they are.

There is therefore some support for the suggestions of Round and Crawford (1981, 1984) and Mann and Marchant (1989) that the diatom frustule originated as a ***scale-case***. Both sets of authors postulate that diatoms evolved from cells bearing uniform scales, via an early stage where scales were differentiated into larger valve-like scales and narrower ones resembling the segmental girdle bands of modern rhizosolenids (cf. the differentiation of round shield plates and triradiate girdle and dorsal plates in Parmales), and a later stage when the proto-girdle bands became thinner and stretched to form hoops encircling the cell. This assumes that valves and girdle bands have a common origin and indeed their fine structure is often so similar that this is a reasonable assumption, and it seems that girdle bands are also formed centrifugally, like valves (e.g., Sato 2010). Furthermore, cells covered evenly with scales are known in diatoms, in the auxospores of some centric species, e.g., of *Melosira* and *Ellerbeckia* (Crawford 1974b; Schmid and Crawford 2001) and in several pennates (e.g., Mann et al. 2013). The Round–Crawford and Mann–Marchant schemes differ principally in the assumptions made about the nature of the scales and scaly cell in the early ("Ur") diatoms. In the Mann–Marchant scheme, the scales of the ancestral diatom are abutting space-filling components of a cyst wall, whereas Round and Crawford envisaged the scales as discrete imbricating elements covering growing vegetative cells, as in modern synurophytes. In a series of opinion papers, Medlin (e.g., 2007) has suggested that that silica may originally have had the property in diatoms of inducing a temporary resting state, which is consistent with the "Ur" diatom being a cyst.

No precursors of diatoms are known from the fossil record. Though it now seems clear that the Bolidophyceae–Parmales are their nearest relatives, the diatoms are an extremely well-characterized, distinctive, and monophyletic group, and it is nomenclaturally convenient to regard them as a separate phylum, which allows maintenance and gradual refinement of the lower-level classification of diatoms developed during the twentieth century.

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