1 • Historical perspectives

Myths, legends and reality.

1.1 INTRODUCTION

Despite a well-established tradition for protistological studies in nineteenth-century Europe, it was an American, Henry James-Clark, who published the first correct and unequivocal description of a 'collar-bearing' flagellate. At the same time he also observed the morphological similarity between free-living collared flagellates and the choanocytes of a sponge. The definitive date was 1866, when James-Clark (1866a, b) published a summary of his findings, to be followed a year later by a more expansive and illustrated account under the title *On the Spongiae Ciliatae as Infusoria Flagellata; or, observations on the structure, animality and relationship of* Leucosolenia botryoides *Bowerbank* (James-Clark, 1867b). While this title appears somewhat archaic by today's standards, nevertheless it encapsulates the significance of his findings. Carter (1857, 1859) had previously concluded that the ampullaceous (aquiferous) sacs of sponges were 'ciliated chambers', hence the term *Spongiae Ciliatae*. James-Clark (1866a, 1867b, 1871b) was now able to show that the flagella-bearing collared monads lining the body cavity of the calcareous sponge *Leucosolenia botryoides* bore a striking resemblance to the free-living, collar-bearing flagellates he had just described, and for this reason he considered sponges to be colonial members of the *Infusoria Flagellata*. The historical importance of James-Clark's (1867b) observations with respect to the study of choanoflagellates in particular, and to the debate concerning the possible evolution of sponges and animals in general, cannot be overestimated.

1.2 FIRST PUBLISHED RECORD OF A COLLAR-BEARING FLAGELLATE

Confusion surrounds the first published record of a collar-bearing flagellate. There are several reasons for this, including: the superficial similarity of stalked colonial choanoflagellates to other unrelated protists; the limitations of early light microscopy; incomplete original taxonomic descriptions and a general lack of coordination in the early literature. Central to this confusion was the allocation of 'genuine' choanoflagellates to existing genera whose holotypes, in hindsight, could not be choanoflagellates. The two most important non-choanoflagellate genera involved were *Anthophysa* Bory de Saint-Vincent 1822 and *Epistylis* Ehrenberg 1830; the former is now a well-established genus of colourless colonial chrysophytes and the latter a genus of stalked peritrichous ciliates.

Anthophysa Bory (1822) was erected for an 'apparently' stalked species of *Volvox* first described as *V. vegetans* by Otto Frederik Müller (1786) (Fig. 1.1). At the same time as introducing this new genus, Bory de Saint-Vincent (1822) changed the specific name from *vegetans* to *mulleri* in honour of Müller. This name change was subsequently reversed by Stein (1878). Bory de Saint-Vincent (1822) included a second *Anthophysa* species, *A. dichotoma*, in his 1822 work and subsequently added a third, *A. solitaria*, with the briefest of detail and no illustrations (Bory de Saint-Vincent 1824). Ehrenberg (1830) created the genus *Epistylis* for a stalked colonial ciliate to which he subsequently added a new species, *E. botrytis* (holotype shown in Figs 1.2–1.3) (Ehrenberg, 1831, 1838). While the cellular details of *E. botrytis* differ from those of a choanoflagellate, in particular they include an anterior ring of 'cilia' and lack a single flagellum, nevertheless the overall form of the colony is not dissimilar to that of a stalked choanoflagellate such as a species of *Codosiga* (Fig. 3.1).

Trying to unravel the multiple confusions between specimens attributable to *Anthophysa vegetans*, *Epistylis botrytis* and 'genuine' stalked colonial choanoflagellates is fraught with difficulty. The limitations of nineteenth-century microscopy led authors such as Stein (1849) to represent anterior rings of cilia as peripheral spines which could equally well have been the shrunken collars of

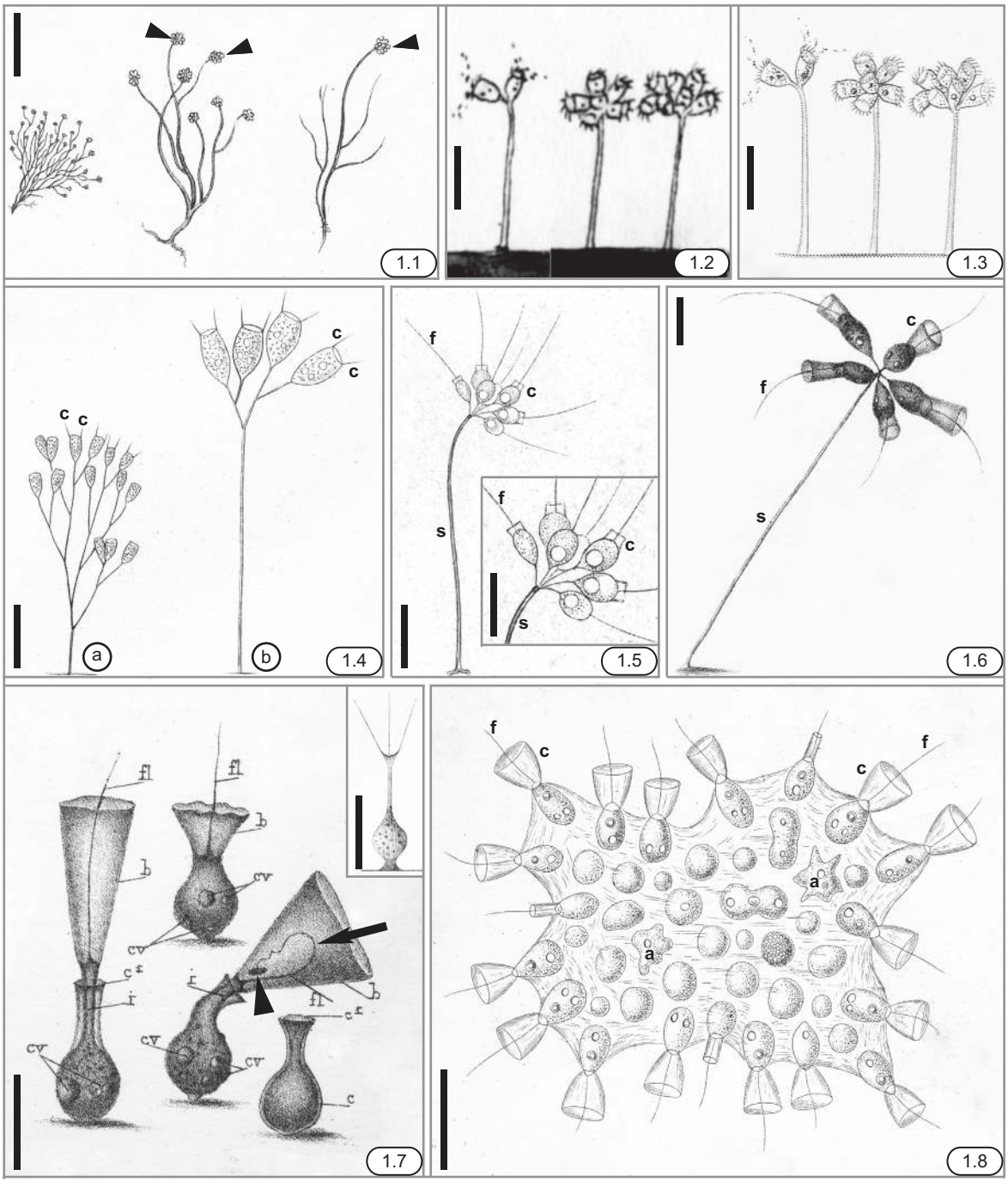

Plate 1 (Figures 1.1–1.8)

Figs 1.1–1.8 Illustrations of choanoflagellates, some with original labelling. Flagellum (f), collar (c), stalk (s). **Fig. 1.1** *Anthophysa vegetans.* Reproduced from Müller (1786). Thick-stalked protist with terminal colonies of cells (arrowheads).

choanoflagellates (Fig. 1.4a, b). What was needed at this time was recognition that 'genuine' choanoflagellate cells possessed a single anterior flagellum surrounded by a 'hyaline' collar. Fresenius (1858) must take the credit for this achievement. He was the first to illustrate unequivocally a choanoflagellate cell with a collar and single anterior flagellum (Fig. 1.5 and inset). However, he failed to recognise the collar as a novel and distinctive structure. Instead, he referred to every cell as having "an identical fine trimmed appendix out of which a long locomotor thread protrudes" (Fresenius, 1858, p. 25). He named his specimen *Anthophysa solitaria* on the basis of Bory de Saint-Vincent's (1824) sketchy specific description and he also acknowledged a resemblance to Ehrenberg's (1838) illustration of *Epistylis botrytis*. It was against this muddled background that James-Clark's (1866a *et seq*.) observations proved to be so enlightening.

1.2.1 Dates of James-Clark's publications

Confusion also surrounds the quoted dates of James-Clark's publications with respect to collar-bearing flagellates and sponges. This is for two reasons: first, his major publications were pre-empted in 1866 by a two-page printed summary of a lecture he gave to the Boston Natural History Society, in which he included descriptions of two choanoflagellate genera (James-Clark, 1866a). Second, during the following six years he wrote three substantive papers, each of which was first published in an American journal followed one year later by a verbatim copy, except for corrections, in a British journal. Thus the titles of each of these three papers have two publication dates according to whether the American or British version is being quoted (James-Clark, 1866b, 1867a, b, 1868, 1871b, 1872). Table 1.1 lists James-Clark's seven relevant papers, giving the dates of imprint. For the two choanoflagellate genera described by James-Clark, namely *Codosiga* and *Salpingoeca*, 1866 is the valid date of publication since the descriptions meet the criteria of the International Code of Zoological Nomenclature (ICZN) and this is the date recorded in Nomenclator Zoologicus (online version 0.86; 2005). The single species attributed to *Codosiga*, namely *C. pulcherrimus*, and the three *Salpingoeca* species, namely *S. gracilis*, *S. marinus* and *S. amphoridium*, were first described in James-Clark, 1867b. Two of these names, *pulcherrimus* and *marinus*, were subsequently corrected to *pulcherrima* and *marina* in the British version of this paper (James-Clark, 1868). However, according to the ICZN this does not alter the original valid date of publication as being 1867.

1.2.2 William Saville Kent; Otto Bütschli; Friedrich Ritter von Stein

James-Clark's pioneering work proved to be a catalyst for multiple independent but overlapping investigations on choanoflagellates, particularly by William Saville Kent (1871b, 1878c, 1880–2), Otto Bütschli (1878) and Friedrich Ritter von Stein (1878). Independently, apparently without collusion, these three authors came to the same conclusion that *Codosiga pulcherrima* James-Clark

Figs 1.2–1.3 *Epistylis* (*Codosiga*) *botrytis*. Bars = *c*.5 μm. Reproduced from the Ehrenberg Collection with permission from the Museum für Naturkunde, Berlin.

Fig. 1.2 Copy of original drawing (513) by Ehrenberg (1831).

Fig. 1.3 Illustration from *Die Infusionsthierchen* (Ehrenberg, 1838).

Fig. 1.4a–b. Stein's (1849) illustrations of *Epistylis* (*Codosiga*) *botrytis*. Individual collars appear as rigid spines (c). Bars = 5 and 10 μm, respectively. Reproduced from Stein (1849).

Fig. 1.5 *Anthophysa solitaria* (= *Codosiga botrytis*). Earliest convincing illustration of a stalked colony of choanoflagellate cells. Bars = 10 μm. Reproduced from Fresenius (1858).

Fig. 1.6 *Codosiga pulcherrima*. Bar = 10 μm. Reproduced from James-Clark (1867b).

Fig. 1.7 *Salpingoeca amphoridium*. The central cell possesses a recurved flagellum (arrow) and a bacterium near the base of the collar (arrowhead). Bar = 10 μm. Reproduced from James-Clark (1867b). **Fig. 1.7** inset: *Salpingoeca amphoridium* – drawing from notebook (1857). Reproduced from Carter (1871). Bar = 10 μm.

Fig. 1.8 *Proterospongia haeckeli*. Collar-bearing cells at the surface and amoeboid cells (a) embedded in 'zoocytium'. Bar = 10 μm. Reproduced from Kent (1880–82).

Table 1.1 *Details of James-Clark's seven important publications relating to choanoflagellates and sponges (date of imprint in brackets).*

1866a Paper presented at the Boston Society of Natural History on the animality of Sponges and their relationship with the Infusoria Flagellata. *Proceedings of the Boston Society of Natural History* **11**: 16–17 (December 1866)

1866b Conclusive proofs on the animality of the ciliate sponges, and their affinities with the *Infusoria Flagellata*. *American Journal of Science and Arts,* Series 2, **42**: 320–5 (November 1866).

1867a Conclusive proofs on the animality of the ciliate sponges, and their affinities with the *Infusoria Flagellata*. *The Annals and Magazine of Natural History*, Series 3, **19**, 13–19 (January 1867).

1867b On the *Spongiae Ciliatae* as *Infusoria Flagellata*: or, observations on the structure, animality and relationship of *Leucosolenia botryoides* Bowerbank. *Memoirs of the Boston Society of Natural History* **1**: 305–40 (September 1867).

1868 On the *Spongiae Ciliatae* as *Infusoria Flagellata*: or, observations on the structure, animality and relationship of *Leucosolenia botryoides* Bowerbank. *The Annals and Magazine of Natural History*, Series 4, **1**: 133–42, 188–215, 250–64 (February, March and April 1868).

1871b The American *Spongilla*, a craspedote, flagellate, infusorian. *American Journal of Science and Arts* **12**: 426–36 (December 1871).

1872 The American *Spongilla*, a craspedote, flagellate, infusorian. *Monthly Microscopical Journal* **7**: 104–114 (March 1872).

(Fig. 1.6) was synonymous with Ehrenberg's (1831) *Epistylis botrytis* (Fig. 1.3) (Bütschli, 1878; Kent, 1878c; Stein, 1878). As a result the combined name *Codosiga botrytis* (Ehrenberg) Bütschli came into being. The three authors introduced different collective names for collared flagellates, namely: Family Cylicomastiges Bütschli (1878); Order Craspedomonadina Stein (1878); and Family Choanoflagellata (Kent, 1880–2). Subsequently, the term Cylicomastiges disappeared without trace, but Craspedomonadina (craspedomonads) and Choanoflagellata (choanoflagellates, Kragenmonaden) have continued to be used interchangeably, although now choanoflagellate is the most commonly used colloquial term and is used throughout this text.

1.3 MORPHOLOGY AND REPRODUCTION OF THE 'COLLARED FLAGELLATE'

James-Clark's (1867b) illustrations of choanoflagellates are so clear and accurate that this publication alone served to establish the basic morphological features of the group (Figs 1.6–1.7, 3.11). Subsequently it has become apparent that the choanoflagellate cell plan is not only unmistakable, but also remarkably consistent, with only minor variations, such as absence of a flagellum in *Choanoeca perplexa* (Fig. 2.51). The essential features of a choanoflagellate include a radially symmetrical, spherical to ovoid cell body with a single central anterior flagellum surrounded by a funnel-shaped collar that appears hyaline when viewed with light microscopy, but which comprises a ring of 20–50 microvilli that are held out rigidly in life (see Section 2.7.2). The flagellum undulates with a base-to-tip planar wave which creates a current of water from which prey particles are trapped on the outer surface of the collar. They are subsequently ingested at the base of the outer collar surface by pseudopodia (see Section 2.4).

Despite this relative simplicity, the early literature relating to choanoflagellate morphology, prey capture, cell coverings, cell division, sex and recombination is full of conflicting information and opinions due to the limitations of light microscopy and/or various interpretative misconceptions. Some of the more contentious issues which over time have made their way into standard textbooks are discussed below.

1.3.1 Collar morphology and the mechanism of prey capture and feeding

In spite of his excellent observations on choanoflagellate cell morphology, James-Clark (1867b, p. 315) erroneously considered that the location of prey ingestion, the mouth as he called it, was at the base of the flagellum within the confines of the collar, rather than outside the collar. He envisaged that particles of food were thrown by the flagellum "toward the mouth by vigorous spasmodic

incurvations or jerks" (Fig. 1.7, arrow). This error was subsequently compounded by Kent (1878a, c, 1880–2), who envisaged the collar as being a funnel-shaped extension of the cell on the surface of which particles were trapped and "slowly, almost imperceptibly, carried along with the circulating current of the collar's substance up the outside and down the inside until, on reaching the base of its inner surface, they were engulfed within the cell" (Kent, 1880–2, p. 327) (Fig. 1.9).

While James-Clark (1867b) and Kent (1878c, 1880–2) incorrectly interpreted the details of prey capture and feeding they were, nevertheless, correct in viewing the collar as an entire funnel-shaped structure. In contrast, Entz (1883), subsequently supported by Francé (1893, 1897), Ehrlich (1908) and Schouteden (1908), regarded the collar as being the upper vertically expanded portion of a spirally coiled membrane that arises on the surface of the cell at the level of one of the two 'Schlingvacuoles' (gullet vacuoles), which had been described by earlier workers as contractile vacuoles (Fig. 1.10) (Lapage, 1925). Francé (1897) likened the collar membrane to a spirally wound piece of paper (Fig. 1.11). These authors considered that food particles followed a spiral path down the collar and were ultimately ingested by the 'gullet vacuole', which was capable of undergoing swallowing movements.

Bütschli (1878), Fisch (1885), Burck (1909), Griessmann (1913), Lapage (1925), de Saedeleer (1929) and Ellis (1929, 1935) correctly concluded that the collar was a funnel-shaped structure which served to entrap particles on its outer surface and that ingestion occurred at the base of the outer collar surface. However, opinion was divided about whether ingestion involved linguiform pseudopodia that originated from the base of the collar (Fig. 1.12) (Griessmann, 1913) or whether it occurred on the side of the cell between the plasma membrane and the surrounding organic covering (Bütschli, 1878). In fact, both observations are probably correct since in some strains of *Codosiga botrytis* prey particles are ingested at the side of the cell, although the surrounding sheath is not involved in the process as Bütschli (1878) suggested. However, in the majority of species particles are ingested by linguiform pseudopodia that rise up along the lower part of the collar (see Section 2.7.3).

Most early workers refer to the collar as being hyaline and membranous. Griessmann (1913) was the first to show unequivocally that, after fixation with osmium tetroxide and staining with dahlia and methyl violet, the collar comprised a series of threads (Fig. 1.14). This was a relatively late record since Bidder (1895) had previously demonstrated that the choanocyte collars of the sponge *Sycon compressum*, when fixed with osmium tetroxide, embedded in paraffin wax and stained with haematoxylin, comprised a palisade of 20–30 fine threads (Fig. 1.13).

Frenzel (1891) described a new freshwater species from Argentina, *Diplosiga socialis*, which he claimed possessed two concentric collars, one outside the other. Francé (1897) subsequently added another genus, *Diplosigopsis*, for cells also with 'double collars'. However, de Saedeleer (1929) argued that the apparent existence of two collars was an observational misinterpretation. He suggested that the appearance of a second collar was either due to the remnants of pseudopodia at the base of the collar or, alternatively, the funnel-shaped anterior of the surrounding theca. Subsequent electron microscopy (EM) has confirmed de Saedeleer's (1929) opinion.

1.3.2 Terminology relating to extracellular coverings

Table 1.2 lists some of the terms that have been used to describe choanoflagellate coverings. The variety of terminology has come about for a number of reasons. First, the terms themselves, irrespective of language, are mostly non-specific and have not been used in a consistent manner. Second, standard light microscopy was often unable to resolve covering structures clearly. Electron microscopy has permitted a more thorough understanding of cell coverings, although the terminology remains subjective.

In an attempt to clarify and simplify the terminology relating to choanoflagellate coverings, three categories are currently recognised throughout this work (Table 1.2): (1) a thin, flexible extracellular organic matrix (glycocalyx) or sheath (craspedid species with non-restrictive cell division); (2) a continuous inflexible constraining organic envelope or theca (craspedid species with restricted (emergent) cell division); (3) a siliceous basket-like cage comprising a two-layered arrangement of costae made up of rod-shaped costal strips known as a lorica. Until recently, these three categories of coverings formed the basis of the three choanoflagellate families, Codonosigidae Kent, Salpingoecidae Kent and Acanthoecidae Norris, respectively (see Section 1.8.1). However, based on recent molecular

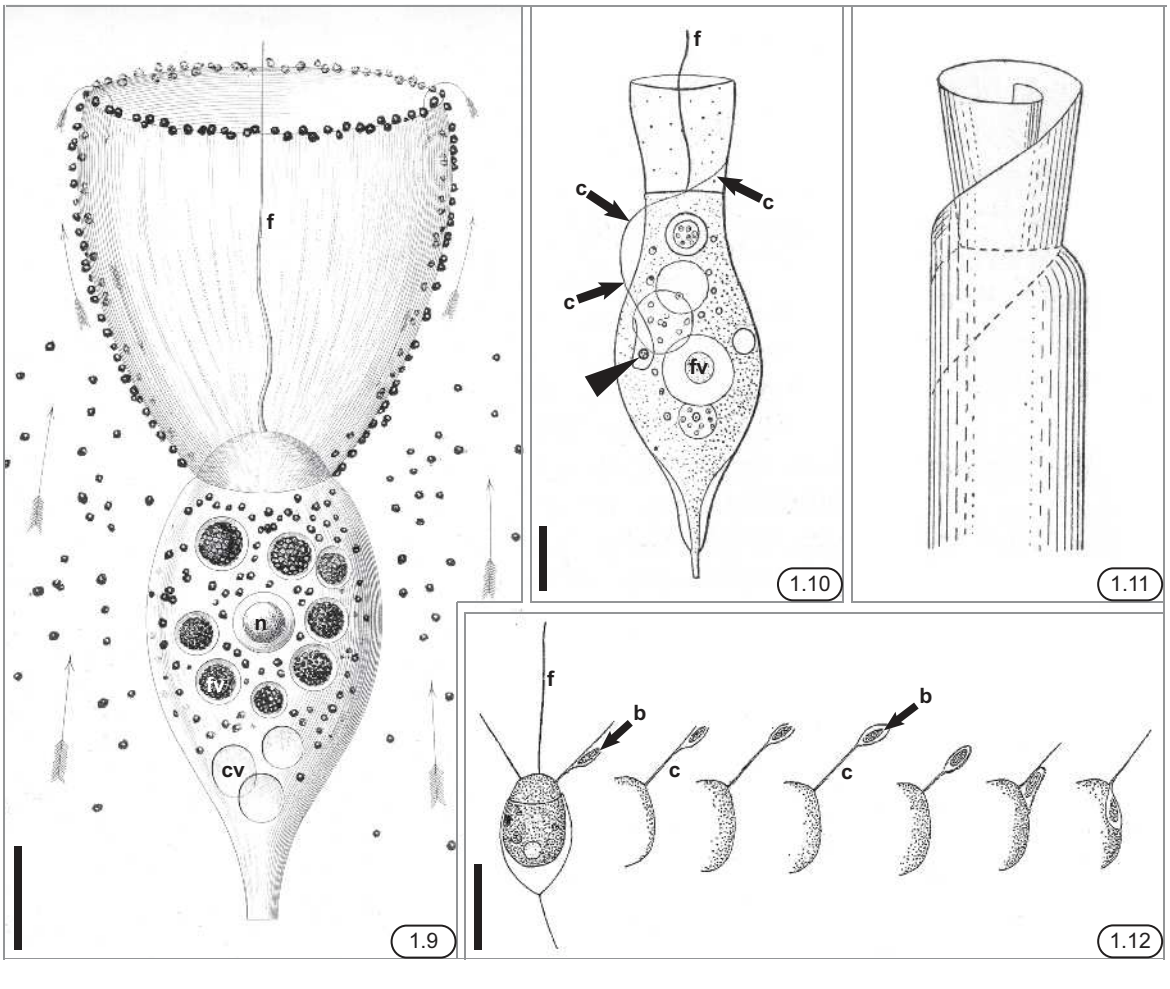

Plate 2 (Figures 1.9–1.12)

Figs 1.9–1.12 Early illustrations of the choanoflagellate collar and mechanisms of prey capture and ingestion.
Fig. 1.9 *Monosiga gracilis*. Copy of frontispiece from Kent (1880–2) showing his interpretation of the movement of carmine particles in the medium and on the collar. Arrows denote particle movement from rear of cell to their entrapment on the outer surface of the collar. Particles are then transported to the top of the collar and subsequently down the inner surface to the base, where they are ingested. Flagellum (f), nucleus (n), food vacuole (fv), contractile vacuole (cv). Bar = 2.5 μm.
Fig. 1.10 *Codosiga botrytis*. Drawing of a cell showing the flagellum (f) and spiral form of the collar (arrows c) leading to the 'Schlingvacuole' (gullet vacuole) (arrowhead). Food vacuole (fv). Bar = 2 μm. Reproduced from Burck (1909).
Fig. 1.11 Spiral form of the collar illustrated as spiral roll of paper. Reproduced from Burck (1909).
Fig. 1.12 *Salpingoeca infusionum*. Sequence of drawings showing ingestion of bacterium (b) in linguiform pseudopodium on outer surface of collar (c). Bar = 10 μm. Reproduced from Griessmann (1913).

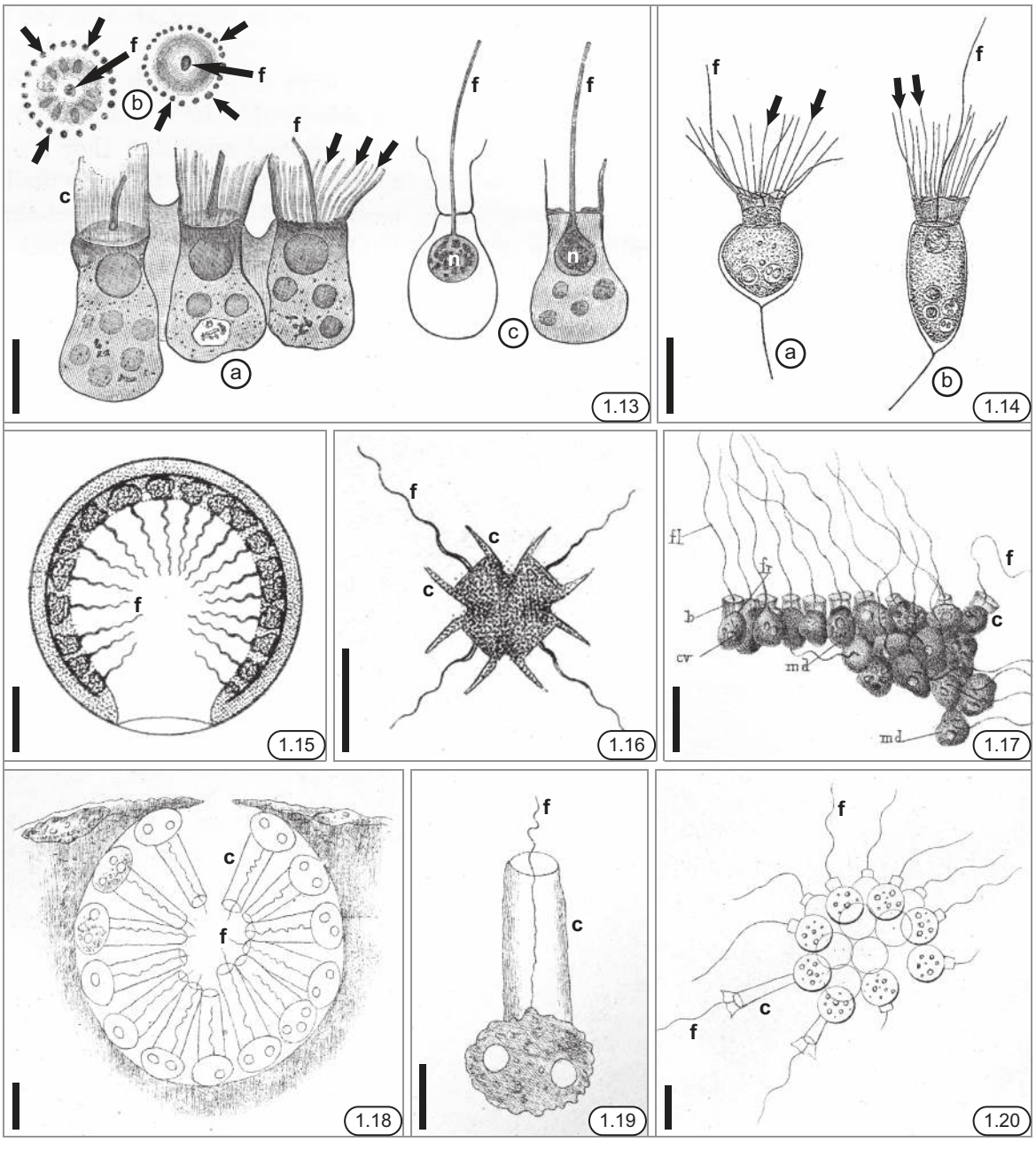

Plate 3 (Figures 1.13–1.20)

Figs 1.13–1.20 Collar-bearing sponge cells (choanocytes) and *Salpingoeca*. Flagellum (f), collar (c). **Fig. 1.13** *Sycon compressum*. Sectioned choanocytes stained with haematoxylin. a. Three cells showing collars composed of microvilli (arrows). b. Transverse section of two choanocytes showing central flagellum (arrow f) and collar comprising 20–36 microvilli (arrows). c. Two cells showing connection between flagellar base and nucleus. Bar = 5 μm. Reproduced from Bidder (1895).

Table 1.2 *Terminology that has been used to describe the coverings of choanoflagellates. Calyx (Latinised Greek) = cup-like structure; Coque (French) = shell; Gehäuse (German) = fixed envelope, shell; Hülse (German) = case, sheath, envelope; Schleimhülse (German) = mucous shell.*

	Craspedida		Acanthoecida
Author	Glycocalyx (envelope)	Theca	Lorica
James-Clark (1866a)	–	Calyx	–
Bütschli (1878)	Schleimhülse	–	–
Stein (1878)	–	Hülse	–
Kent (1880–2)	–	Sheath, lorica	–
Lemmermann (1910)	–	Gehäuse	–
Saedeleer (1929)	Gel périphérique, loge	Coque	–
Ellis (1929)	Jelly-plasm	Pseudo-lorica	Loge-coque, true lorica
Boucaud-Camou (1966)	–	Coque	Coque
Norris (1965)	–	Lorica	Lorica
Bourrelly (1968)	–	Logette	–

phylogeny two major clades are now identified within the Class Choanoflagellatea; one, Craspedida, contains species with organic coverings (equivalent to the previous Codonosigidae and Salpingoecidae) and the other, Acanthoecida, contains attached and pelagic species with siliceous basket-like coverings (equivalent to Acanthoecidae) (see Section 10.4.1 and Fig. 10.3) (Nitsche *et al*., 2011).

Preisig *et al*. (1994) surveyed the terminology used for protistan cell coverings and were critical of the use of non-specific terms, such as theca and lorica. They recommended that new terms should be created for specific structures and suggested that the term 'basket' might be used for the siliceous costal coverings of choanoflagellates. However, there is now a substantial quantity of choanoflagellate literature in which the term 'lorica' has been used to describe the silica basket. To change such an extensively used term now would not only lead to considerable confusion but would also run the risk of not becoming established in the literature.

1.3.3 Cell division

The morphology of cell division is closely allied to the categorisation of extracellular coverings (Table 1.3). In the absence of a restrictive covering, nuclear and cytoplasmic

Fig. 1.14a–b. *Salpingoeca pyxidium* and *S. infusionum*, respectively. Cells stained with dahlia and methyl violet showing single flagellum and collar comprising thread-like microvilli (arrows). Bar = 10 μm. Reproduced from Griessmann (1913).
Fig. 1.15 *Spongilla* sp. Section of ampullaceous sac showing inner lining of flagellated cells. Bar = 5 μm. Reproduced from Carter (1857).
Fig. 1.16 *Spongilla alba*. Four monociliated spiniferous (collar-bearing) cells from a spherical sac. Bar = 5 μm. Reproduced from Carter (1859).
Fig. 1.17 *Leucosolenia botryoides*. Fragment of 'monadigerous' layer showing collar-bearing cells. Bar = 10 μm. Reproduced from James-Clark (1876b).
Figs 1.18 and 1.19 *Spongilla arachnoidea*. Reproduced from James-Clark (1872).
Fig. 1.18 Section of flagellated chamber showing individual choanocytes each with a single flagellum and well-developed collar. Bar = 10 μm.
Fig. 1.19 Single choanocyte. Bar = 5 μm.
Fig. 1.20 *Grantia compressa*. Group of collar-bearing sponge cells. Bar = 5 μm. Reproduced from Carter (1871).

Table 1.3 *Terminology used in text to describe the morphology of cell division in choanoflagellates.*

Craspedida		Acanthoecida	
Non-restrictive coverings	Thecate (restrictive covering)	Nudiform	Tectiform
Longitudinal division	Emergent division	Diagonal division	Inverted division

division occur in the longitudinal (vertical) plane of the parent cell; this arrangement is termed longitudinal division. The two daughter cells that lie side-by-side until separation occurs share the cell covering equally. This category of division is typical of cells surrounded by a thin, flexible glycocalyx or sheath and is exemplified by species of *Monosiga*, *Codosiga* and *Desmarella* (Section 3.3). It is not uncommon for daughter cells resulting from longitudinal division to remain attached to each other after cytokinesis, thereby forming colonies, examples being species of *Codosiga* and the *Desmarella* and *Proterospongia* stages of craspedids (see Section 3.5). The unrestricted form of cell division was the reason why early workers used terms such as 'mucous sheath', 'gel périphérique' and 'Schleimhülse' to describe the accompanying expandable cell covering (see Table 1.2).

However, craspedid cells with restrictive coverings cannot undergo the standard process of longitudinal division because of space constraints; instead the cell becomes amoeboid and partially emerges from the parent theca. Nuclear division, which may still be in the longitudinal plane, occurs within the emergent portion of cytoplasm. One daughter nucleus remains within the cytoplasm in the parent theca, while the other passes to a developing naked 'juvenile' cell which eventually swims away, settles onto a surface and secretes a new theca. This form of division is called 'emergent' in this text (see Section 3.4). Earlier workers have referred to this type of division as 'budding' (bourgeonnement) and the motile cell has been called a 'hernia' (de Saedeleer, 1929; Ellis, 1929). The term 'juvenile' is used throughout this book to refer to a daughter cell resulting from division of a thecate or loricate cell that does not remain with the parent covering.

With respect to acanthoecids (loricate species), two forms of cell division are observed. In nudiform species, cell division is diagonal, which is a modified form of longitudinal division. The flagellar poles of both daughter cells face in an anterior direction (see Chapter 6). In tectiform species, nuclear division is more-or-less longitudinal but as cytokinesis proceeds the daughter cell that will leave the parent lorica is inverted and pushed backwards out of the lorica. The flagellar poles of the two daughter cells face each other as separation occurs. This form of division is called 'inverted' in this text (see Chapter 7).

1.3.4 Sex and recombination

Sexual reproduction can be defined as the fusion of two haploid gametic nuclei or gametes to form a single zygotic nucleus or diploid cell (zygote). Meiosis is an essential precursor to sexual reproduction. During meiosis homologous chromosomes undergo replication followed by recombination. Subsequently, two rounds of nuclear division, usually accompanied by cell division, produce four haploid nuclei or gametes. Since sexual reproduction is widespread in nature, including many unicellular organisms, it is reasonable to ask whether there is any evidence of sex in choanoflagellates.

Stein (1878) published a drawing of a stalked *Codosiga botrytis* cell with a smaller collar-bearing cell, flagellum outermost, projecting horizontally from its side (Plate VIII, Fig. 10 in Stein, 1878). He described this as "probably conjugation" between two cells. Fisch (1885) observed similar pairs of *C. botrytis* cells but considered that they were undergoing cell division as part of colony formation (Figs 74 and 75 in Fisch, 1885). There are several reports in the literature of multiphasic life cycles with unicells of varying size which might suggest variations in ploidy (Leadbeater, 1983a; Dayel *et al.*, 2011). Thomsen attributed sudden changes in lorica size (Thomsen and Larsen, 1992) and unexplained changes in costal strip morphology (Thomsen *et al.*, 1997) in *Bicosta spinifera* to the existence of complex polymorphic life cycles which might involve sexual reproduction (see Section 9.5).

Molecular studies have produced firmer evidence of sex within choanoflagellates. *Monosiga brevicollis* possesses long terminal repeat retrotransposons within its genome, which suggests that it has a sexual stage in its life cycle since asexual organisms cannot tolerate retrotransposons due to the rapid accumulation of deleterious mutations caused by their transposition (Carr *et al*., 2008b). Furthermore, *M. brevicollis* possesses 18 of the 19 genes that comprise the 'meiotic detection toolkit' (Carr *et al*., 2010). Eight of these genes function only in meiosis, whereas the others function in both mitosis and meiosis. This suggests that *M. brevicollis* is capable of switching between asexual and sexual reproduction.

Levin and King (2013), working with *Salpingoeca rosetta*, have reported the most convincing evidence of sex in a choanoflagellate to date. *S. rosetta* has a sexual cycle with transitions between haploid and diploid states. A haploid clonal culture was obtained that exhibited genetic stability over several months. When sub-samples of this culture were grown in unenriched (low-nutrient) seawater, after six days 89% of cells were diploid (as determined by propidium iodide staining). This change in ploidy was accompanied by instances of pairing and fusion between small rounded uniflagellated cells (tentatively called male gametes) and larger ovoid uniflagellated cells (tentatively called female gametes). Successful fusions were always initiated by contact between the basal end of the male gamete (opposite pole to the flagellum) and the base of the collar of the female gamete. This phenomenon is not too dissimilar to that illustrated by Stein (1878) for *Codosiga botrytis*.

A history of sex and recombination in *S. rosetta* is also suggested by the fact that single nucleotide polymorphisms (SNPs) were broken up into discrete haplotype blocks instead of spanning the length of each chromosome as would be expected in an asexual organism. The sharp boundaries at the edges of the segmented haplotype blocks probably mark the regions where there has been genetic exchange between homologous chromosomes (Levin and King, 2013).

Demonstrating sexual reproduction in unicellular protists is notoriously difficult. For instance, haptophycean flagellates, including coccolithophorids, are known to possess life cycle stages that vary in ploidy, but there is still a lack of hard evidence that they produce gametes and undergo meiosis.

1.4 CHOANOFLAGELLATES AS ANCESTORS OF THE SPONGES AND LOWER METAZOA

James-Clark's (1867b) publication on the possible relationship between choanoflagellates and sponges coincided with major advances in scientific knowledge, particularly in relation to animal evolution and systematics. These topics dominated biological thought in the mid nineteenth century and several powerful protagonists, including Ernst Haeckel, dominated the field. It is not surprising, therefore, that James-Clark's (1867b) observations quickly became embroiled in partisan controversy. While there is general agreement that the Metazoa are monophyletic and must have originated from a single-celled protozoan ancestor (Srivastava *et al*., 2010), nevertheless there has been widespread debate about how multicellularity was first achieved and what sort of protozoan ancestor might have been involved (see Section 10.8).

Over the years there have been many theories relating to the possible origin of the Metazoa; the more important are reviewed by Salvini-Plawen (1978), Wilmer (1996), Nielsen (2001) and Mikhailov *et al*. (2009). Choanoflagellates have featured in many of these theories, in a morphological context starting with James-Clark's (1867b) publication and more recently in a molecular phylogenetic context (see Chapter 10). The brief discussion below is limited to theories that involve choanoflagellates or conflict directly with a choanoflagellate/sponge ancestry. This account starts with a comparison of choanoflagellate and choanocyte cell structure; reviews the case for sponges being colonial protozoa; highlights the conflicts arising from Haeckel's Gastraea theory; and summarises hypotheses that attempt to resolve outstanding conflicting views.

1.4.1 Similarity between choanoflagellates and sponge choanocytes

James-Clark's (1867b) discovery that a group of flagellated infusoria closely resembled the flagellated cells lining the chambers of sponges has stood the test of time. The morphological features shared by these cells include a single central flagellum encircled by a 'hyaline' collar. There are also similarities in their functional properties in as much as they create water currents and are able to ingest particles by means of pseudopodia, although the mechanism of the latter may not be identical in both cell types (Leys and Eerkes-Medrano, 2006). The similarity