

Choanoflagellate evolution: the morphological perspective ¹

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Summary

Choanoflagellates are a ubiquitous group of heterotrophic nanoflagellates found in aquatic environments everywhere. Recent molecular phylogeny has confirmed them as being the sister group to the Metazoa. They are easily recognised by their spherical to ovoid cell with a single anterior flagellum surrounded by a collar of microvilli. Choanoflagellates are filter feeders, the flagellum creating a flow of water from which food particles, mostly bacteria, are trapped on the outside of the collar. To enhance the flow of water over the collar, the cell needs to be stationary and this is achieved by the production of an organic theca with a stalk. Thecate choanoflagellates are common in both freshwater and marine environments but require a substratum for settlement. A group of exclusively marine choanoflagellates, Acanthoecidae, has developed a siliceous basket-like covering, the lorica, that encloses the cell and serves not only to resist the locomotory forces created by the flagellum but also to direct and enhance water flow over the collar. Variations in the morphology of the lorica, the amount of silicification and the positioning of the inner organic investment have allowed loricate species to diversify and thereby inhabit many different microniches within the water column.

Key words: Choanoflagellate, glycolyx, theca, lorica, siliceous costae, water flows, ecology, planktonic species, phylogeny

Introduction

The very word ‘morphology’ conjures up thoughts of the past and even in its modern guise as ultrastructure, it still seems strangely outdated and only of ‘natural history’ interest. And yet, as recorded here, a relatively little known but important group of heterotrophic nanoflagellates seems to contradict this current view of biology. For the choanoflagellates, the study of morphology demonstrates *par excellence* the integration of ultrastructure with ecology and evolution. The justification for this claim rests on the contrast between the living cell, which in terms of morphology and ultrastructure is so conservative, and its external structures, which by many

subtle structural variations have facilitated ecological diversity on a global scale. This communication will explore the relationship between choanoflagellate morphology and ecology and will summarise aspects that are now considered to be of evolutionary importance.

Cell Morphology

Choanoflagellates are one of the most important and ubiquitous groups of heterotrophic nanoflagellates in aquatic ecosystems. Their morphology is unmistakable on account of the remarkable constancy of the protoplast that is spherical to ellipsoidal in shape and bears a single flagellum surrounded by a

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funnel-shaped collar made up of actin-supported microvilli (Fig. 1) (Karpov and Leadbeater, 1997, 1998). The function of the flagellum is to create a current of water from which potential food particles, mostly bacteria, become entrapped on the outer surface of the collar (Pettitt et al., 2002). Pseudopodia arising from the base of the collar ingest trapped particles and create food vacuoles that are subsequently transported to the rear of the cell where digestion takes place (Leadbeater, 1983). This mechanism of feeding has obviously suited choanoflagellates well and variations on the basic pattern are relatively minor. A similar conservativeness also applies to the structure and distribution of organelles within the cell (Fig. 2) (Karpov and Leadbeater, 1997).

The requirement for an external covering stems from the functional behaviour of the cell. Movement of the flagellum creates a locomotory force which, if not resisted in some way, propels the cell through the water. In the swimming mode, local flows of water extend less than a wavelength from the flagellum and the velocity of water movement reduces exponentially with distance (Lighthill, 1976). Thus the capacity to bring fresh particle-laden water to the surface of the cell is limited. However, if the cell is attached to a surface then local flows of water extend for between 10-20 body diameters around the organism thereby ensuring extensive renewal of water over the cell surface (Lapage, 1925; Sleight, 1964; Higdon, 1979). For the cell to be an efficient feeder the locomotory force must be resisted and in many species this is achieved by attachment of the cell to a surface either directly or by means of a peduncle (stalk) (Lighthill, 1976; Sleight, 1991). At its simplest, the posterior end of the cell attaches to a surface but in many species attachment is the first step in the production of an organic coat or theca which partly surrounds the posterior portion of the cell. To enhance the efficiency of feeding, the coat or theca must not interfere with the local water flows around the collar. To enclose a cell above the base of the collar a rigid framework with apertures is necessary and one group of choanoflagellates, the Acanthoecidae, has achieved this by development of a siliceous basket-like lorica.

Systematics of the Choanoflagellida

The first unequivocal description of choanoflagellates was by Henry James Clark (1866) when he sampled marine and freshwater localities and described four species. He also astutely observed the similarity between choanoflagellates and the choanocytes of sponges and so initiated the idea that the choanoflagellates were the protozoan ancestors of the sponges

and by inference the animal kingdom. William Saville-Kent (1880-82), who for ten years dedicated his life to the development of seawater aquaria, named many new species which were ultimately published in the *Manual of the Infusoria*. He erected a new order, Choanoflagellata, within the Protozoa for uniflagellate monads with a hyaline collar and delineated two families based on the nature of the cell covering. Members of the Codonosigidae were 'naked' and divided laterally (Fig. 3) whereas members of the Salpingoecidae possessed a thicker organic covering, the theca, and because of this physical limitation, division could only be accomplished by the cell becoming amoeboid and emerging from the anterior end of the theca (Fig. 5). Kent (1880-82) was an enthusiastic advocate of the choanoflagellate/sponge relationship and when he observed a colonial choanoflagellate in samples from a freshwater pond he named it *Proterospongia* (first sponge). However, as will be demonstrated later, motile colonial stages are common in the life-cycles of many sedentary species. Much later a third family, the Acanthoecidae Norris (1965), was added to accommodate species with siliceous basket-like loricae (Figs. 10-22). Although choanoflagellates with siliceous loricae were illustrated unknowingly by Kent (1880) and later in detail by Ellis (1929), the full significance of the lorica was not appreciated until Norris's 1965 study.

The last thirty years have seen a resurgence of interest in the choanoflagellates, firstly because they are the sister group to the Metazoa (Carr et al., 2008), and secondly because they are a major group of heterotrophic nanoflagellates particularly in marine environments (Thomsen et al., 1997 for refs). With the application of electron microscopy to nanoplanktonic studies a considerable literature has accumulated, particularly with respect to loricate species which are exclusively marine. Species lists are now available for many regions around the world.

Cell Coverings

THE GLYCOLYX

This term is used in a general and unspecific manner to refer to the layer of fine fibrils on the outer surface of the plasma membrane. This feature is probably universal for all choanoflagellate cells, although in those enclosed within a theca or lorica it may be obscured. It is seen to greatest advantage in cells described as being 'naked', which includes motile cells resulting from division, some species of *Monosiga*, *Codosiga* and some colonial species (Leadbeater and Morton, 1974a; Karpov and Leadbeater, 1997). In

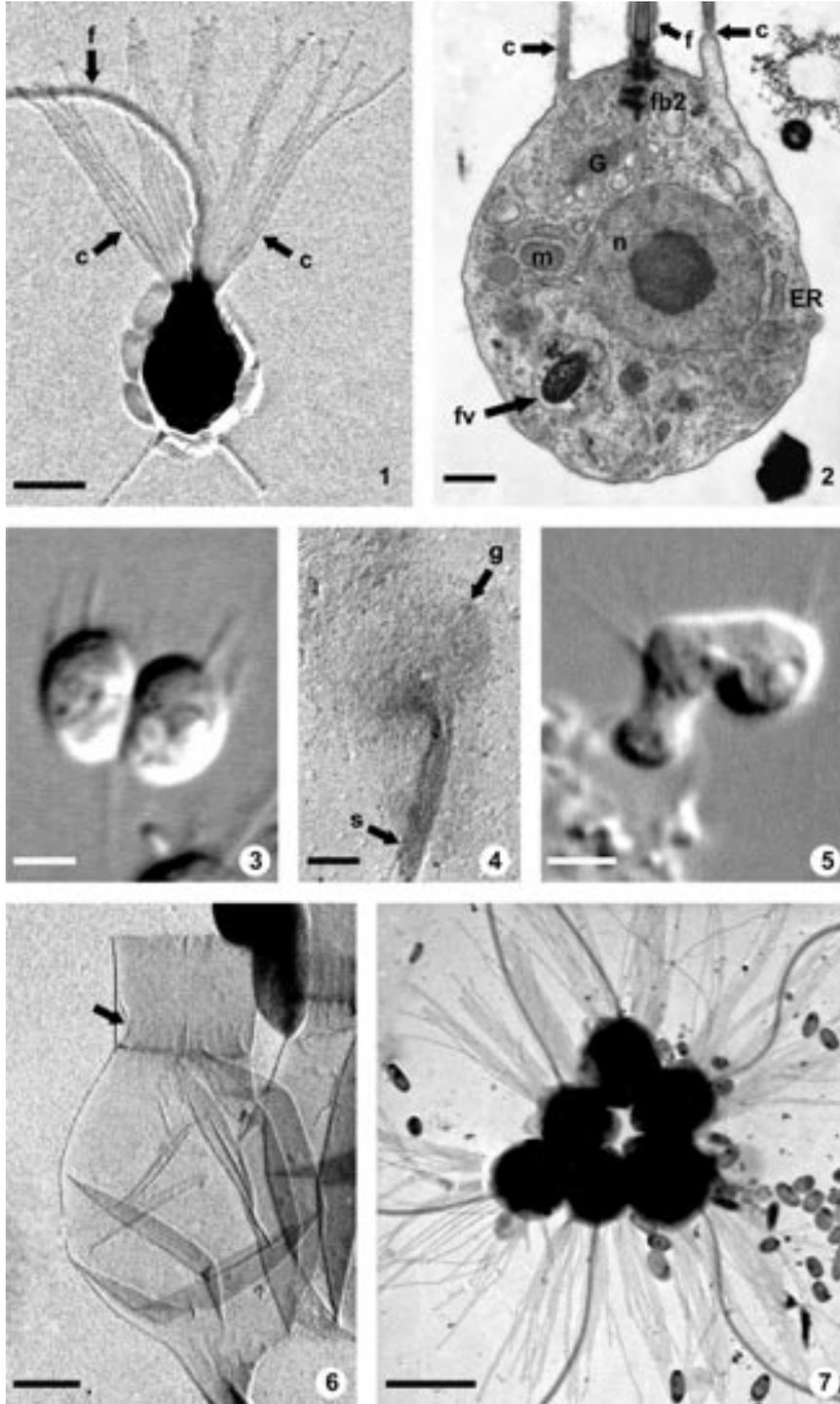


Fig. 1. *Monosiga ovata*. Cell with single flagellum (f) surrounded by a collar of tentacles (c). Two posterior tentacles for attachment to substratum. Fig. 2. *M. ovata*. Section of cell showing general disposition of organelles. Abbreviations: c - collar, f - flagellum, fb2 - second flagellar base; G - Golgi apparatus, n - nucleus, m - mitochondrion, fv - food vacuole. Fig. 3. *Codosiga gracilis*. Two daughter cells immediately following division. Fig. 4. *C. gracilis*. Whole mount of a stalk (s) and remaining glycocalyx (g) following extraction of cell with detergent. Fig. 5. *Choanoeca perplexa*. Cell division, showing amoeboid form of cell emerging from neck of flask-shaped theca. Fig. 6. *Salpingoeca urceolata*. Shadowcast empty flask-shaped theca showing inner flange at bottom of collar (arrow). Fig. 7. *Proterospongia* sp. (ATCC 50818). "Proterospongia" colony of six cells with long flagella and collars of tentacles. Scale bars: 1 - 2 μ m; 2 - 0,5 μ m; 3 - 2 μ m; 4 - 0,5 μ m; 5 - 2 μ m; 6 - 1 μ m; 7 - 4 μ m.

Codosiga gracilis the thin outer covering is sufficiently robust to hold the top of the stalk to the base of the cell and it survives extraction after the cell has been removed with dilute detergent (Fig. 4).

ORGANIC COVERINGS

In addition to the glycocalyx, the majority of choanoflagellates possess a continuous organic covering of some description. This includes the theca, which comprises a cup- or flask-shaped structure (Fig. 6) and stalk, and the inner organic investment of the lorica (Leadbeater, 1972; 1977; Thomsen, 1977, Buck et al., 1990). Despite variations in the micro-anatomy and surface appearance of these structures there does appear to be some consistency in their composition. The basis of the organic layer is a sheet of microfibrils embedded in an amorphous matrix (Fig. 9). This is most clearly seen in the stalks of thecate species where there is a prominent longitudinal band of microfibrils located within an amorphous coating (Fig. 8). The composition of the microfibrils is not known for certain but is likely to be carbohydrate since it gives a positive staining response to wheat germ agglutinin (WGA) tagged with a fluorescent dye (Leadbeater, unpub. data). Stalks are extremely robust and withstand prolonged boiling in acid or alkali. In species with cup-shaped thecae, microfibrils of the stalk are continuous with those comprising the cup.

Flask-shaped thecae are distinctive in both morphology and microanatomy (Leadbeater, 1977). The shape is that of a round bottomed-flask with parallel-sided neck whose extreme anterior end is curved outwards (Fig. 6). A distinctive inner flange with periodic longitudinal striations attaches the theca to the cell. In section the theca comprises two darker layers sandwiching a lighter staining layer. Whilst there is no obvious appearance of microfibrils in untreated thecae, nevertheless with careful negative staining they can be seen within the neck (Leadbeater, unpub. data).

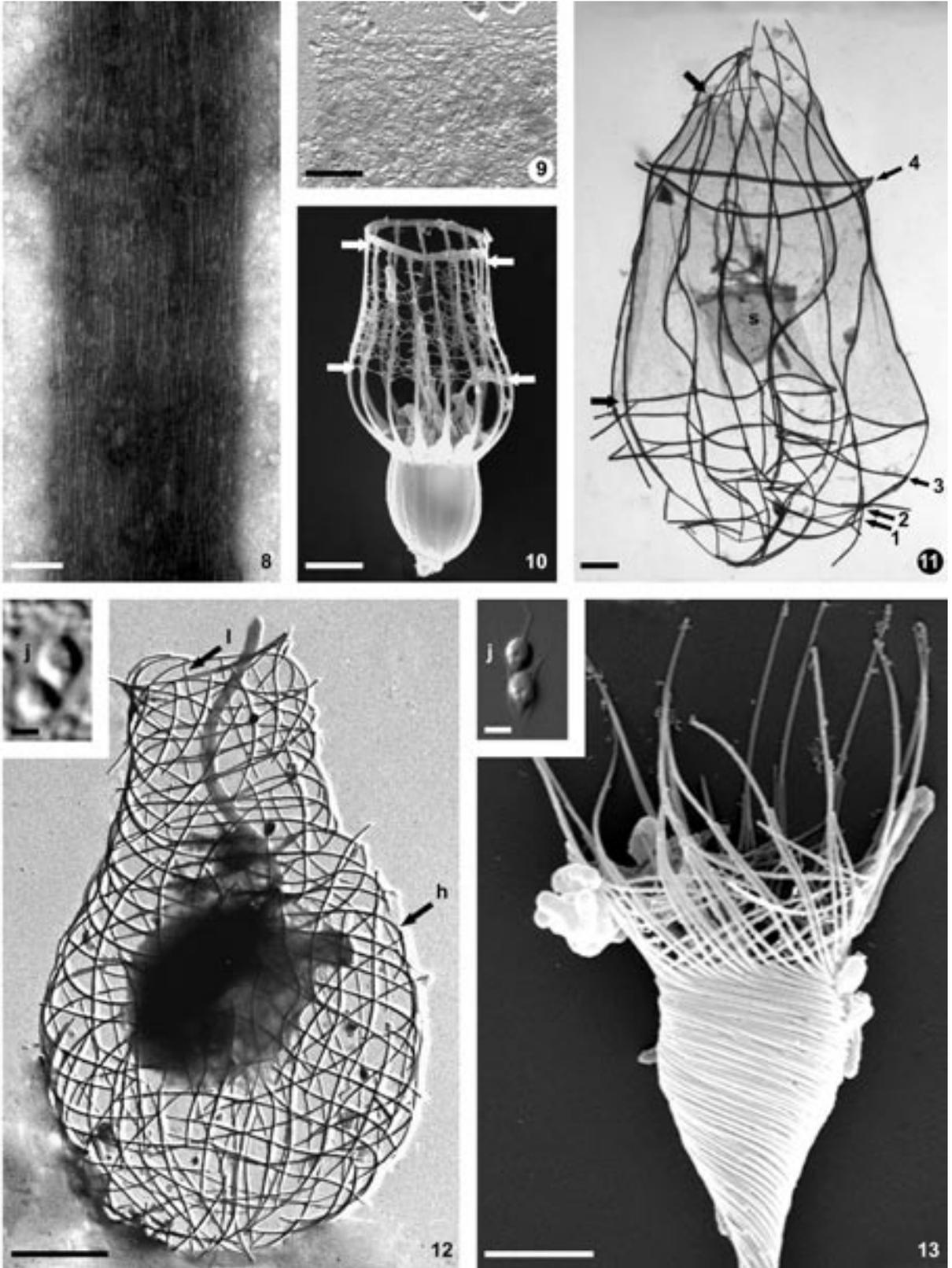
An organic layer is present within the basket-like lorica of members of the Acanthoecidae (Leadbeater and Morton, 1974b; Leadbeater, 1979a). Whilst superficially there is considerable variation in the appearance and location of this layer, two functions can be ascribed to it. One is to secure the cell within the lorica; the second is to restrict or guide water flow through the lorica (Andersen, 1988). In most species the cell is held in position by a continuous organic layer that is similar in structure to the cup-shaped theca mentioned above (Leadbeater, 1972). In many species the cell is held closely within the lorica at its posterior

end and in this case the organic covering provides both an inner lining to the lorica and a direct attachment to the cell (Leadbeater, 1972). However, in some of the larger tectiform species, such as *Diaphanoeca grandis*, the cell is located centrally (Fig. 11) and is attached to a ring at the anterior end of the lorica. In this case it is the collar tentacles that secure the cell to the lorica. In many species a second organic investment is present which comprises a thinner and more open meshwork of microfibrils (Manton et al., 1981; Leadbeater, 2008). This is closely associated with the inner surface of the lorica and is usually some considerable distance from the cell. In *Acanthoeca spectabilis* the meshwork has a regular appearance comprising an array of longitudinal and transverse array of microfibrils (Leadbeater et al., 2008), in other examples the pattern is irregular (Manton et al., 1981). In *Savillea parva* the closeness of the microfibrils and the completeness of the covering would appear to restrict movement of particles into the lorica (Leadbeater, 2008). In other species, for instance *Diplothea costata* (Fig. 10) and *Diaphanoeca grandis* (Fig. 11), the microfibrillar layer is positioned so as to direct and enhance water flow over the outer surface of the collar. In these examples the weft of microfibrils is known as the veil.

THE PROTEROSPONGIA ENIGMA

The value of the theca is to attach a cell to a substratum and thereby enhance the flow of water around the cell. The length of the stalk is important for it raises the cell above the substratum thereby overcoming the boundary layer effect associated with water movements over surfaces. However, from the ecological perspective there is a major limitation with the theca and that is the necessity for attachment to a substratum. This is not a problem in benthic locations but presents a problem in pelagic environments. To some extent thecate cells can overcome this limitation by attaching to suspended particles. However, the larger the water body the less frequent is the availability of such particles and the necessity to become self-sufficiently planktonic increases. This has only been achieved permanently in the sea by loricate species. However, temporary excursions into the planktonic environment have been achieved by many thecate species.

When a thecate species divides, a motile cell is produced and this allows for dispersal (Fig. 5). If food supplies are abundant, the swimming cell can ingest particles and undergo division in the motile state. If the daughter cells do not separate then they have the ability to form colonies (Fig. 7). The locomotory forces



created by flagellar movement of individual cells in a colony are resisted by other colony cells attempting to swim in the opposite direction. The result is a moving mass of cells that can just about maintain itself in the planktonic condition. The literature contains numerous descriptions of colonies that have been attributed to colonial genera such as *Proterospongia*, *Sphaeroeca* and *Astrosiga*. Traditionally little attention has been paid to the origin or fate of these colonies although in some instances, especially in samples of stagnant water, colonies only appeared after some considerable period of time.

Recently, isolates of three thecate choanoflagellates have given rise to colonies in culture, each with a characteristic but different morphology. Thus *Proterospongia* sp. (American Type Culture Collection ATCC 50818), whose sedentary stage comprises a cup-shaped theca, regularly gives rise to *Sphaeroeca*-like colonies (Fig. 7). *Salpingoeca amphoridium*, a flask-shaped thecate species, gives rise to flat plates with geometrically arranged cells held together by posterior threads (Carr et al., 2008). *Proterospongia choanojuncta*, which has a sedentary stage indistinguishable from *Choanoeca perplexa*, produces large colonies whose cells are held together by adjacent collar tentacles (Leadbeater, 1983). No doubt there are other thecate species capable of producing colonies of other morphologies (see Leadbeater, 1983). The taxonomic problem posed by species with multiphasic life-cycles is that the various stages are known by different names. Since the sedentary form is the basic, long-lasting stage of a life-cycle and possesses a clearly identifiable theca, the name of this stage should take priority when describing a species. Thus the validity of genera such as *Proterospongia*, *Sphaeroeca* and *Astrosiga* must be in doubt. However, this matter cannot be resolved until the type species of the respective colonial genera have been established in clonal culture and the respective sedentary stages identified.

THE LORICA

Probably the most remarkable structure to have evolved within the Choanoflagellida is the siliceous basket-like lorica (Leadbeater, 1979b; Thomsen and Buck, 1991). This unique structure is confined to a group of marine species and its capacity for variation has permitted choanoflagellates to exploit a wide range of ecological niches, particularly involving the planktonic environment (Leakey et al., 2002).

The unit of lorica construction is the costal strip, a siliceous rod-shaped structure. Costal strips are attached to each other end-to-end to form costae and the lorica typically contains an outer arrangement of longitudinal costae with an inner arrangement of helical or transverse costae; the combined positioning of these two sets of costae gives the lorica its basket-like appearance and maintains its overall mechanical stability (Figs. 10-12) (Leadbeater, 2008). Each costal strip is deposited within the cell in a membrane-bounded vesicle and, when complete, is exocytosed to the exterior (Leadbeater 1975, 1979b, c, 1994a). Costal strips are accumulated outside the cell until a sufficient number has been produced to form a lorica (Leadbeater, 1979b, 1994b). Assembly of the lorica occurs as a discrete, continuous movement which lasts from two to five minutes after which no further adjustments can be made. Whilst all loricate species adhere to these basic principles there are, nevertheless, two distinctive variations on the theme (Manton et al., 1981). In one variant, the *nudiform* condition, a lorica-bearing cell divides to produce a 'juvenile' flagellated cell (Figs. 12 inset, 13 inset) which swims away from the parent lorica, settles down on a substratum, accumulates costal strips in vertical bundles on its surface and when a complete set has been produced, assembles a lorica (Leadbeater and Morton, 1974b; Leadbeater et al., 2008). In the second variant, the *tectiform* condition, a lorica-bearing cell produces a full complement of costal strips prior to cell divi-

Fig. 8. *Salpingoeca infusionum*. Negatively stained stalk (uranyl acetate) showing parallel arrangement of microfibrils. Fig. 9. *S. infusionum*. Edge of cup-shaped theca showing inner weft of microfibrils after treatment at 80° C in water. Fig. 10. *Diplothea costata*. SEM of lorica showing the location of the microfibrillar veil on the top two-thirds of the lorica (arrow). The apertures below the veil allow the ingress of water and food particles. Fig. 11. *Diaphanoeca grandis*. Empty lorica showing the arrangement of longitudinal and four transverse rings (right arrows). The veil extends from near the top of the spines (upper left arrow) to the third transverse costa from the bottom (lower left arrow). Fig. 12. *Savillea parva*. Cell with lorica showing the two layered arrangement of costae (arrow). The left-handed rotation of the inner helical costae (arrow h) can be seen. Fig. 12 inset. Cell division in *S. parva*. The flagellar bearing juvenile cell (j) is the uppermost. Fig. 13. *Acanthoeca spectabilis*. SEM of empty lorica showing left-handed rotation of costae. The helix consists of longitudinal costae that undergo two turns and are continuous with the 15 spines. Fig. 13 inset. Cell division in *A. spectabilis*. The flagellar bearing juvenile cell (j) is the uppermost. Scale bars: 8 – 50 nm; 9 – 0.5 µm; 10 – 2 µm; 11 – 2 µm; 12 – 2 µm; 12 inset – 1 µm; 13 – 2 µm; 13 inset – 2 µm.

sion and stores them at the top of the collar (Fig. 15). When a complete set of strips has been produced, the cell divides and the resulting juvenile is inverted and pushed out backwards from the parent lorica taking with it the accumulated strips (Fig. 16). Within minutes of the juvenile being liberated from the parent a new lorica is assembled (Leadbeater, 1979c, 1994b). The separate phylogenetic grouping of nudiform and tectiform taxa is supported by a recent molecular phylogenetic study (Carr et al., 2008).

NUDIFORM CHOANOFLAGELLATES

Currently there are only six confirmed nudiform species attributable to four genera, *Savillea*, *Acanthoeca*, *Polyoeca* and *Helgoeca*. All share the basic characters mentioned above. A recent molecular phylogeny of the choanoflagellates based on four genes recovers them as a well supported monophyletic clade within the loricate grouping (Carr et al., 2008). This confirms them as members of a closely related evolutionary group rather than an eclectic assemblage of taxa.

Superficially the loricae of these species appear disparate in structure. In *Savillea parva* the lorica comprises two systems of costae (Fig. 12); the outer costae are longitudinal and the inner helical with a left-handed conformation (Leadbeater, 2008). The ratio of helical to longitudinal costae is 1:1. There are between 8-10 helical costae at the anterior end but they undergo two turns and so it is possible to count between 16-20 costae along the length of the lorica (Fig. 12). Assembly of the lorica from groups of vertically aligned strips is achieved by the juvenile cell undergoing a two-turn rotation as the developing costae are moved forwards. The longitudinal costae on the outside rotate freely whereas the developing inner helical costae are attached in a 1:1 ratio to the longitudinal costae at the anterior end and to the cell surface at the posterior end. Thus as the lorica forming tentacles advance they are also rotated by the cell to achieve two turns (Leadbeater, 2008).

In *Helgoeca nana* the arrangement of costae is not dissimilar to that in *Savillea* (Leadbeater et al., 2008). The ratio between the outer longitudinal and inner helical costae with a left-handed conformation is approximately 1:1. The longitudinal costae project as spines beyond the anterior edge of the helical costae. The number of turns is difficult to discern but the juvenile must undergo at least one rotation.

At first sight the situation in *Acanthoeca spectabilis* appears different (Fig. 13). However, a helical arrangement of costae is again obvious and careful analysis shows that the helix is formed by 14-16 lon-

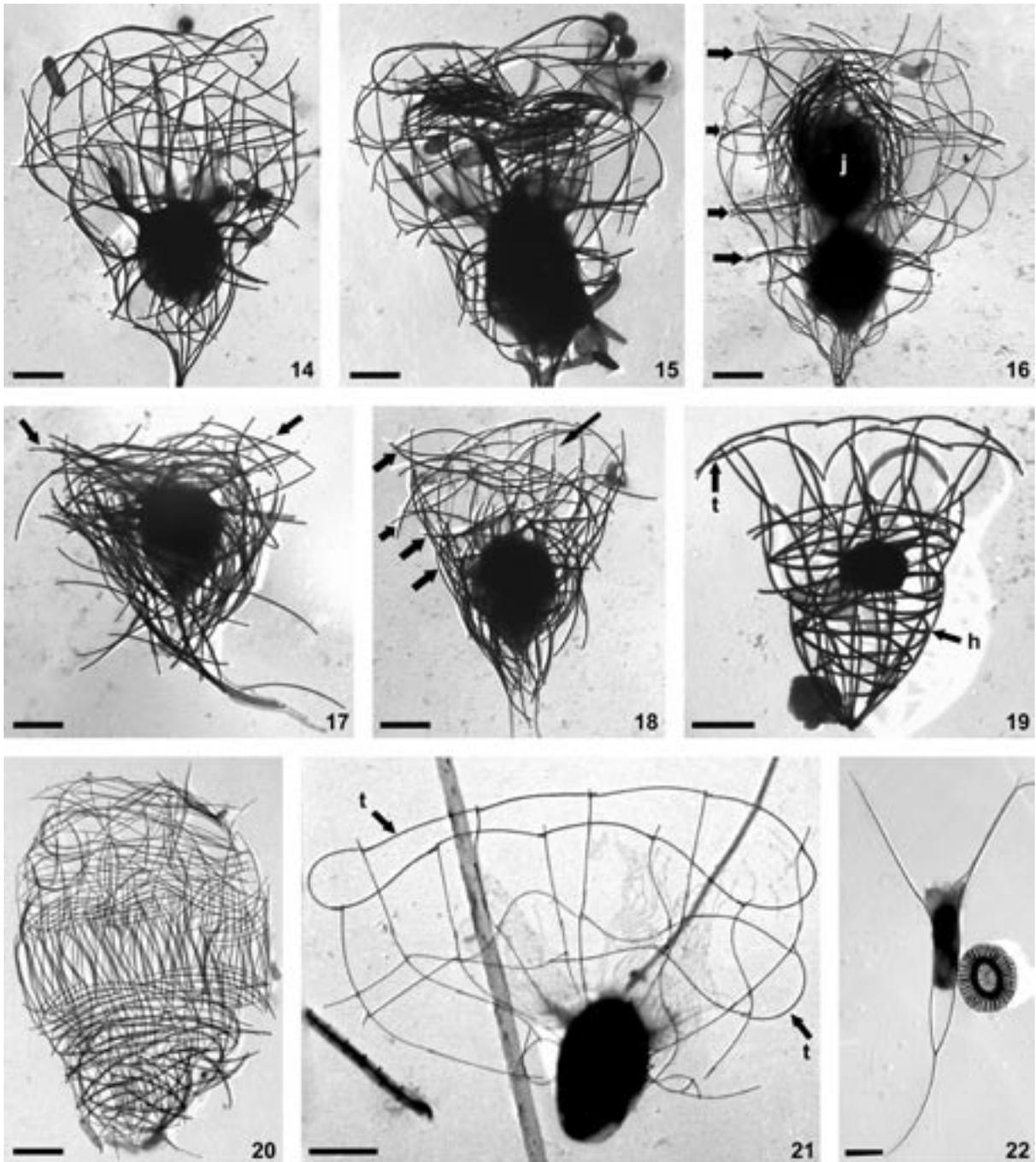
gitudinal costae that extend from the base of the stalk to the tip of the spines. As in *Savillea*, two left-handed rotations are required to form the lorica chamber (Leadbeater et al., 2008).

Thus, despite superficial dissimilarities, a number of consistent features are common to nudiform species. All have helically arranged costae with a left-handed conformation. The minimum amount of rotation is one turn and two species undergo two turns. Loricae are assembled from strips that are vertically aligned on the juvenile cell and the strips destined for the inner costae are located innermost. Exactly how the rotational force is generated is not known. There is no evidence that the protoplast actually rotates longitudinally nor do the tentacles effect the movement. Thus it is probable that the necessary rotation is generated by the cytoskeleton within the cell. Similar rotational movements have been observed in other cells, for instance in the flagellate *Poterioochromonas stipitata* during lorica formation (Schnepf et al., 1975).

Globally, nudiform species are ubiquitous and abundant in marine and brackish water habitats. However, they are relatively limited in habitat range. All are sedentary and associated with microbial biofilms. *Savillea* spp. are usually buried within a biofilm whereas *Helgoeca nana*, *Acanthoeca* spp. and *Polyoeca dichotoma* are emergent. The paucity of species and limitation of habitat suggest that nudiform choanoflagellates are either the remnant of a previously larger radiation or that they have always had a limited radiation. The variations in lorica pattern indicate that there were probably intermediate species that no longer exist.

TECTIFORM CHOANOFLAGELLATES

Tectiform species are most obviously recognised by the presence of horizontal bundles of costal strips on the inner surface of the top of the collar of exponentially growing cells (Fig. 15) (Leadbeater, 1994a). Thus, in contrast to nudiform species, the parent cell that already possesses a lorica produces and stores strips in anticipation of division. A recent study of *Diplothea costata*, which has distinctive categories of strips within its lorica (Fig. 10), has permitted an investigation of the order in which strips are produced and their positioning within the stored bundles (B.S.C. Leadbeater, unpubl. data). Developing strips within the parent cell are inverted with respect to the orientation of the strips in the parent lorica. Strips that will form the costal rings are produced first starting with those destined for the posterior costa. These are followed by strips destined for the longitudinal costae, again starting with those that



Figs 14-18. *Stephanoeca diplocostata*. Shadowcast whole mounts of cells at different stages in the cell cycle. Fig. 14. Early interphase; cell without accumulation of strips. Fig. 15. Late interphase; large accumulations of strips at top of collar apparent. Fig. 16. Late cytokinesis; juvenile cell (j) emerging from parent lorica. Four arrows on left indicate location of transverse costae (rings). Fig. 17. Recently released juvenile showing location of costal strips that will form future transverse costae (arrows). Fig. 18. Lorica assembly; lorica-assembling tentacles (long arrow top right) are moving costal strips into position. Large arrows on left indicate the relative positions of the four transverse costae. Fig. 19. *Saepicula pulchra*. Lorica showing outer layer of longitudinal costae and inner layer of helical costae (h) in posterior chamber and a single anterior transverse ring (arrow t). Fig. 20. *Stephanoeca norrisi*. Benthic tectiform species with over 400 strips in lorica. Fig. 21. *Parvicorbicula socialis*. Planktonic tectiform species showing the open framework of the lorica with two transverse (ring) costae (arrows t) and thinly silicified strips. Fig. 22. *Bicosta minor*. Planktonic tectiform species; lorica is reduced to seven costal strips comprising a chamber with three spines. Scale bars: 14-22 – 2 μ m.

will form the posterior end of the lorica. Thus, immediately prior to cell division, the accumulation consists of a complete set of strips inverted with respect to the parent lorica and with the transverse strips on the outside. During division the juvenile cell is inverted with respect to the daughter cell that will remain within the parent lorica (Fig. 15). At the same time the outer transverse strips are pulled down, whilst the horizontal bundles of strips destined for the longitudinal costae rotate to the longitudinal position. As the juvenile cell is pushed out of the parent lorica it takes with it the accumulated strips. Those destined for the transverse costae are now horizontally orientated and are located on the inner surface of the bundles of vertical strips (Figs. 16, 17). Assembly of the lorica follows immediately the juvenile has been released from the parent lorica and involves a forward movement of the lorica-assembling tentacles and a rotational movement with left-handed conformation (Fig. 18).

There are obviously many features in common between the nudiform and tectiform modes of lorica production, including the left-handed rotational movement. However, the major difference in tectiform species is the horizontal orientation of the strips on the juvenile cell that are destined for the future transverse (ring) costae. The latter are characteristic of the loricae of tectiform choanoflagellates (Figs. 10, 11, 14-21). Two groups of tectiform species can be distinguished. The first group, as exemplified by *Stephanoeca diplocostata*, *S. norrisi* (Figs. 14, 20) and *Saepicula pulchra* (Fig. 19) have, in addition to the outer layer of longitudinal costae, one or more anterior transverse rings and helical costae in the posterior chamber. In the case of *S. pulchra* the ratio of helical to longitudinal costae is 1:1 (Fig. 19). The second group, exemplified by *Diaphanoeca grandis* and *Parvicorbicula socialis* (Figs. 11, 21), contains loricae where the longitudinal costae are maintained in place by transverse costae alone. It is this group that contains species with some of the largest loricae on record (Thomsen et al., 1990). The combination of size, reduced numbers of costae and thinly silicified costal strips ideally suits this group to the planktonic mode of existence. The ultimate development is seen in species of *Bicosta* where the number of costal strips is reduced to seven and only longitudinal costae are evident (Fig. 22).

Ecological and Evolutionary Significance of Choanoflagellate Morphology

The choanoflagellate cell plan, comprising a uniflagellate cell with a collar of actin-based microvilli,

is relatively simple, in terms of morphology and ultrastructure, and yet is highly effective with respect to its function as a filter feeder. This is reflected by the extent to which the plan has been conserved without modification in extant species. The only minor variation that has been noted to date is the absence of a flagellum in one or two species such as *Choanoeca perplexa*, and here the large wide-angled collar compensates for the absence of locally generated water movements (Leadbeater, 1977).

The functional niche occupied by choanoflagellates is that of suspension feeder. Of the three categories of suspension feeder, namely filter, direct-interception and diffusion feeder (see Fenchel, 1991), choanoflagellates are classed as filter feeders. Within this category of nanoflagellates they are almost without competitors. For sedentary choanoflagellates, which include thecate (freshwater and marine) and nudiform loricate (marine only) species, the direct interception feeders, such as *Paraphysomonas* spp., bicosoecids and suspension-feeding bodonids, occupy a similar niche and must be the nearest competitors for equivalent size food particles. However, they also experience the same limitation as choanoflagellates in the trade-off between locomotory and feeding activities. Nevertheless, some of these species can also form colonies and occupy the freshwater column in a similar way to the 'proterospongia' stage of some choanoflagellates. Diffusion feeders, such as the small heliozoan *Ciliophrys*, only become effective as competitors when the prey is highly motile (Fenchel, 1991).

It is in the marine environment that choanoflagellates have achieved their greatest ecological diversity and have acquired a unique and unrivalled importance; this is almost entirely due to the evolution and diversity of the lorica. Whilst the six nudiform species are common and not unimportant in their limited ecological range, it is the tectiform taxa (> 120 species) that are of supreme importance. The most important structural feature of the tectiform lorica is the presence of transverse (ring) costae. Whether or not such rings ever existed in nudiform taxa is not known. Certainly those species still extant do not possess this feature. The development of transverse costae has accompanied a great expansion in volume of the lorica, with the largest species achieving 80µm length x 30µm in width (*Diaphanoeca multiannulata*) (Thomsen et al., 1990, 1997), this is in comparison with the relatively small size of nudiform species. Variations in the pattern of costae, the location of the inner organic investment and the reduction of silica within the costae have allowed species to become 'fine-tuned' to a variety of microniches within the water column. Thus

benthic species, such as *S. diplocostata* and *S. norrisi* (Figs. 14, 20), are usually of moderate size and are characterised by the possession of many heavily silicified costal strips. On the other hand, species such as *Parvicorbicula socialis* (Fig. 21), with their voluminous loricae and minimal number of thinly-silicified costae are eminently suited to an entirely planktonic existence. The development and positioning of the diaphanous organic investment of the lorica (veil) (Figs. 10, 11), has permitted cells to enhance the efficiency of water flow through the lorica and thereby to clear a relatively larger volume of water of suspended food particles, another important pre-requisite for planktonic mode of life in the open ocean.

It is tempting to think that evolution within the Choanoflagellida has followed the same logical progression as that related here for morphology. The universality of protoplast morphology and an organic covering strongly support the monophyletic ancestry of this group of flagellates. The similarity in the range of theca morphology between freshwater and marine species of non-loricate choanoflagellates suggests their relatively close relationship and the probable multiple origins of freshwater species. Ultrastructural studies cast doubt on the separation between the Codonosigidae and Salpingoecidae. This is supported by the fact that the majority of thecate species, whether surrounded by a glycocalyx alone or held within a cup or flask, have the capability of producing extracellular microfibrillar structures, even if only a stalk for attachment.

The unique morphology of the lorica clearly supports a single evolutionary origin for loricate species. Whilst the use of silica in extracellular structures of protists is common, for example diatoms, chrysophytes and testate amoebae, the construction of a lorica comprising a system of costae made up of costal strips is without comparison. The difference between nudiform and tectiform species is of considerable significance. Whilst the basic features of costal strip production and lorica assembly, including a left-handed rotation, are common to both groupings, differences in the timing, with respect to the cell cycle, and the order of strip production, the inversion of the juvenile cell during cytokinesis and the rearrangement of strips on the juvenile cell all point to an increase in complexity in tectiform species. We can only speculate whether the only outcome of this increase in complexity was the production of transverse costae but certainly their evolution appears to have allowed for the great diversification that took place within the tectiform lineage.

Within the tectiform lineage there appear to be at least two groups. One group of genera, including

Saepicula and *Stephanoeca*, is distinguished by having helical costae within the posterior part of the lorica and transverse costae in the anterior part. Helical costae represent a variant on longitudinal costae in that each costa is derived from one bundle of costal strips. Thus the possibility exists that helical costae could have arisen twice, once in nudiform and once in tectiform taxa, or they might be an ancestral feature shared with nudiform taxa. In general, tectiform species with helical costae are relatively small and inhabit inshore benthic localities. The second group with transverse costae alone demonstrate the greater diversity and it is within this group that some of the largest loricae are found. Whilst these two groups account for the majority of tectiform taxa, the phylogenetic position of *Bicosta* species remains somewhat enigmatic. They accumulate the shorter costae at the top of the collar but the longer spines do not appear to be extruded until division is underway (Thomsen and Larsen, 1992). They completely lack transverse costae but the longitudinal costae forming the lorica chamber in *Bicosta spinifera* and *B. antennigera* do undergo a left-handed rotation. It is only *B. minor* that apparently shows no evidence of turning.

In retrospect, morphological studies on the choanoflagellates have provided one of the better insights amongst protists into the relationship between ultrastructure, ecology and evolution. This can be attributed to the fact that the cell has remained almost unchanged throughout evolution whilst the external structures have varied to suit individual species to microniches within the aquatic environment. The costal construction of the lorica has also been favourable to interpretation and analysis. The result has been that virtually every structural nuance observed can be ascribed a functional and ecological role. There is, of course, still much to learn. For instance, the left-handed rotational movement, so important to lorica assembly, is certainly an ancestral character within the loricate clade, but it might also be an ancestral character to all choanoflagellates. Rotational movements have been observed in *Poterioochromonas* which has an entirely organic lorica comprising chitin microfibrils (Schnepf et al., 1975). It could be that species with thecae also undergo a rotational movement making this a basic feature of all choanoflagellate cells. The mechanism by which costal strips are able to bond to each other once they have reached their final position is also a feature that requires further investigation. Nevertheless, the detailed information that is now available for choanoflagellates should serve as model for the many other protistan groups that have external structures of uncertain function and ecological benefit.

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References

- Andersen P. 1988. Functional biology of the choanoflagellate *Diaphanoeca grandis* Ellis. Mar. Microb. Food Webs. 3, 35-50.
- Buck K.R., Marchant H.J., Thomsen H.A. and Garrison D.L. 1990. *Kakoeca antarctica* gen. et sp. n., a loricate choanoflagellate (Acanthoecidae, Choanoflagellida) from Antarctic sea ice with a unique protoplast suspensory membrane. Zool. Scripta. 19, 389-394.
- Carr M., Leadbeater B.S.C., Hassan R., Nelson M., and Baldauf S.L. 2008. Molecular phylogeny and evolution of morphology in Choanoflagellates, the sister group to Metazoa. Submitted
- Ellis W.N. 1929. Recent researches on the Choanoflagellata (Craspedomonadines). Ann. Soc. Roy. Zool. Belg. 60, 49-88.
- Fenchel T. 1991. Flagellate design and function. In: The biology of free-living heterotrophic flagellates (Eds. Patterson D.J. and Larsen J.). Clarendon Press, Oxford. pp. 7-19.
- Higdon J.J.L. 1979. The generation of feeding currents by flagellar motions. J. Fluid Mech. 94, 305-330.
- James-Clark, H. 1866 Conclusive proofs on the animality of the ciliate sponges, and their affinities with the Infusoria Flagellata. Mem. Boston Soc. Nat. Hist. 1, 305-340.
- Karpov, S.A. and Leadbeater, B.S.C. 1997. Cell and nuclear division in a freshwater choanoflagellate, *Monosiga ovata* Kent. Europ. J. Protistol. 33, 323-334.
- Karpov S.A. and Leadbeater B.S.C. 1998. Cytoskeleton structure and composition in choanoflagellates. J. Eukaryot. Microbiol. 45, 361-367.
- Kent W. S. 1880-1882. A manual of the infusoria. Vol. 1-3. D. Bogue London.
- Lapage G. 1925. Notes on the Choanoflagellate, *Codosiga botrytis*, Ehrbg. Quart. J. Microsc. Sci. 69, 471-508.
- Leadbeater B.S.C. 1972. Ultrastructural observations on some marine choanoflagellates from the coast of Denmark. Br. Phycol. J. 7, 195-211.
- Leadbeater B.S.C. 1975. A microscopical study of the marine choanoflagellate *Savillea micropora* (Norris) comb. nov. and preliminary observations on lorica development in *S. micropora* and *Stephanoeca diplocostata* Ellis. Protoplasma. 83, 111-129.
- Leadbeater B.S.C. 1977. Observations on the life-history and ultrastructure of the marine choanoflagellate *Choanoeca perplexa* Ellis. J. Mar Biol Ass UK. 57, 285-301.
- Leadbeater B.S.C. 1979a. Developmental and ultrastructural observations on two stalked marine choanoflagellates, *Acanthoecopsis spiculifera* Norris and *Acanthoeca spectabilis* Ellis. Proc. R. Soc. Lond. Biol. 204, 57-66.
- Leadbeater B.S.C. 1979b. Developmental studies on the loricate choanoflagellate *Stephanoeca diplocostata* Ellis. I. Ultrastructure of the non-dividing cell and costal strip production. Protoplasma. 98, 241-262.
- Leadbeater B.S.C. 1979c. Developmental studies on the loricate choanoflagellate *Stephanoeca diplocostata* Ellis. II. Cell division and lorica assembly. Protoplasma. 98, 311-328.
- Leadbeater B.S.C. 1983. Distribution and chemistry of microfilaments in choanoflagellates, with special reference to the collar and other tentacle systems. Protistologica. 19, 157-166.
- Leadbeater B.S.C. 1994a. Developmental studies on the loricate choanoflagellate *Stephanoeca diplocostata* Ellis. VII. Dynamics of costal strip accumulation and lorica assembly. Eur. J. Protistol. 30, 111-124.
- Leadbeater B.S.C. 1994b. Developmental studies on the loricate choanoflagellate *Stephanoeca diplocostata* Ellis. VIII. Nuclear division and cytokinesis. Eur. J. Protistol. 30, 171-183.
- Leadbeater, B.S.C. 2008 Choanoflagellate lorica construction and assembly: The nudiform condition. I. *Savillea* species. Protist. 159, 259-268.
- Leadbeater B. S. C. and Morton C. 1974a. A microscopical study of a marine species of *Codosiga* James-Clark (Choanoflagellata) with special reference to the ingestion of bacteria. Biol. J. Linn. Soc. 6, 337-347.
- Leadbeater B.S.C. and Morton C. 1974b. A light and electron microscope study of the choanoflagellates *Acanthoeca spectabilis* Ellis and *A. brevipoda* Ellis. Arch. Mikrobiol. 95, 279-292.
- Leadbeater B.S.C., Henouil M., and Berovic N. 2008. Choanoflagellate lorica construction and assembly: The nudiform condition. II. The *Acanthoeca spectabilis*. Protist. 159, 495-505.
- Leadbeater B.S.C., Hassan, R., Nelson M., Carr M. and Baldauf S.L. 2008. A new genus, *Helgoeca* gen.

nov., for a nudiform choanoflagellat. Eur. J. Protistol. 44, 227–237.

Leakey R.J.G., Leadbeater B.S.C., Mitchell E., McCready S.M.M. and Murray A.W.A. 2002. The abundance and biomass of choanoflagellates and other nanoflagellates in waters of contrasting temperature to the north-west of South Georgia in the Southern Ocean. Europ. J. Protistol. 38, 333–350.

Lighthill J. 1976. Flagellar hydrodynamics. SIAM Review. 18, 161–230.

Manton I., Bremer G. and Oates K. 1981. Problems of structure and biology in a large collared flagellate (*Diaphanoeca grandis* Ellis) from arctic seas. Proc. R. Soc. Lond. B. 213, 15–26.

Norris R.E. 1965. Neustonic marine Craspedomonadales (Choanoflagellata) from Washington and California. J. Protozool. 12, 589–612.

Pettitt M.A., Orme B.A.A., Blake J.R. and Leadbeater B.S.C. 2002. The hydrodynamics of filter feeding in choanoflagellates. Europ. J. Protistol. 38, 313–332.

Schnepf E., Röderer G. and Herth W. 1975. The formation of the fibrils in the lorica of *Poteriochromonas stipitata*: tip growth, kinetics, site, orientation. Planta. 125, 45–62.

Sleigh M.A. 1964. Flagellar movement of the sessile flagellates *Actinomonas*, *Codonosiga*, *Monas* and *Poteriodendron*. Quart. J. Micros. Sci. 105, 405–414.

Sleigh M.A. 1991. Mechanisms of flagellar propul-

sion; a biologist's view of the relation between structure, motion, and fluid mechanics. Protoplasma. 164, 45–53.

Thomsen H.A. 1977. External morphology of the choanoflagellate *Salpingoeca gracilis* James-Clark. J. Mar. Biol. Ass. UK. 57, 629–634.

Thomsen H. A. and Buck K. R. 1991. Choanoflagellate diversity with particular emphasis on the Acanthoecidae. In: The biology of free living heterotrophic flagellates (Eds. Patterson D.J. and Larsen J.) Clarendon Press, Oxford. pp. 259–284.

Thomsen H. A. and Larsen J. 1992. Loricated choanoflagellates of the Southern Ocean with new observations on cell division in *Bicosta spinifera* (Thronsdren, 1970) from Antarctica and *Saroecca attenuata* Thomsen, 1979, from the Baltic Sea. Polar Biol. 12, 53–63.

Thomsen H.A., Garrison D.L. and Kosman, C. 1997. Choanoflagellates (Acanthoecidae, Choanoflagellida) from the Weddell Sea, Antarctica. Taxonomy and community structure with particular emphasis on the ice biota; with preliminary remarks on choanoflagellates from Arctic sea ice (North east water Polynya, Greenland). Archiv Protistenkd. 148, 77–114.

Thomsen H.A., Buck K.B., Coale S.L., Garrison D.L. and Gowing M.M. 1990. Loricated choanoflagellates (Acanthoecidae Choanoflagellida) from the Weddell Sea, Antarctica. Zool. Scripta. 19, 367–387.

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