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Taxonomy and Phylogeny of Heliozoa. III. Actinophryids

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Summary. The diversity, relationships and classification of the actinophryid heliozoa (protists) are reviewed. Descriptions of two new species (*Actinophrys salsuginosa* and *Ciliophrys azurina*) are presented. The actinophryid heliozoa are revised to include six species: *Actinophrys sol* (Müller, 1773) Ehrenberg, 1830, *A. pontica* Valkanov, 1940, *A. tauryanini* Mikrjukov et Patterson, 2000, *A. salsuginosa* sp. n., *Actinosphaerium eichhornii* (Ehrenberg, 1840) Stein, 1857, and *A. nucleofilum* Barrett, 1958. *Echinosphoerium* /*Echinosphaerium* Hovasse, 1965 and *Camptonema* Schaudinn, 1894 are regarded as junior subjective synonyms of *Actinosphaerium* Stein, 1857. The relatedness between actinophryid heliozoa and pedinellid helioflagellates is discussed. The new species, *Ciliophrys azurina*, exhibiting characters (tapering axonemes and peripheral location of heterochromatin) previously only reported in the actinophryids. This allows a proposition for the sequence of character acquisition and a new group of stramenopiles - the actinodines - uniting pedinellids, ciliophryids and actinophryids.

Key words: actinodines, Actinophryida, *Actinophrys salsuginosa* sp. n., *Actinophrys tauryanini* nom. nov., *Actinosphaerium, Camptonema, Ciliophrys azurina* sp. n., *Echinosphaerium, Echinosphoerium*, heliozoa, Pedinellales, protista, protozoa, stramenopiles.

INTRODUCTION

The heliozoa are a polyphyletic assemblage of protists having arisen from different evolutionary origins but have developed a similar life style and body form (Smith and Patterson 1986; Patterson 1988, 1994; Mikrjukov 1998, 2000a; Mikrjukov *et al.* 2000). Despite its historical use as a taxon, the term “heliozoa” is now used only colloquially to describe organisms with a round body, no internal skeleton but with radiating stiff pseudopodia. Most organisms previously classified as heliozoa are now assigned to the centroheliozoa (Centrohelida Kühn 1926), desmothoracids (Desmothoracida Hertwig and Lesser 1874), gymnosphaerids (Gymnosphaerida Poche 1913) or to the actinophryids (Actinophryida Hartmann 1913). We believe these groups to be monophyletic (Smith and Patterson 1986, Patterson 1999). Other genera of heliozoon-like protists have been placed within the nucleariid filose amoebae (Mikrjukov 1999a). Various helioflagellates are to be found in the dimorphids (*Dimorpha* and *Tetradimorpha*) and pedinellids (*Ciliophrys*, *Parapedinella*, *Actinomonas*, *Pteridomonas*, *Pedinella* and *Pseudopedinella*). A few genera (*Wagnerella*, *Actinolophus*, *Servetia*, *Sticholonche*, and *Pseudodimorpha*) remain of uncertain affinities (Mikrjukov 2000d, Mikrjukov *et al.* 2000).

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Actinophryid heliozoa are unflagellated organisms with tubular mitochondrial cristae. They can be distinguished by having axopodial axonemes formed of double polygonal spirals of microtubules, two types of extrusomes, with cysts having a layer of siliceous scales and within which autogamy occurs. The nuclei divide as a semi-open orthomitosis (Mignot 1979, 1980a, b, 1984; Patterson 1979, 1986). The most characteristic species are *Actinophrys sol* and *Actinosphaerium eichhornii* (Fig. 1).

There are only a few species of actinophryids, but they are the most frequently occurring heliozoa in freshwater habitats (see: Rainer 1968, Siemensma 1991). Actinophryids are recorded occasionally in soils and mosses (Sandon 1927, Geltzer 1993), or in marine and brackish environments (Jones 1974, Golemanski 1976, Bovee and Sawyer 1979, Mikrjukov 1996a, Mikrjukov and Patterson 2000). Like other heliozoa, they are passive predators, consuming motile prey which adhere to the axopodia (Patterson and Hausmann 1981, Grębecki and Hausmann 1993).

Major lineages of protozoa are identified by electron microscopy (Krylov *et al.* 1980; Corliss 1994; Patterson 1994, 1999; Mikrjukov 1999c). The actinophryids are one of the most intensively studied groups of free-living protists. Classical light microscopical observations (Hertwig 1899; Penard 1904; Bělař 1923, 1924) have been extended with ultrastructural studies on general morphology (Andersen and Beams 1960; Hovasse 1965; Tilney and Porter 1965; Patterson 1979, 1986; Shigenaka *et al.* 1980; Jones and Tucker 1981; Mikrjukov 1996a), growth and feeding (Ockleford and Tucker 1973, Suzaki *et al.* 1980a, Patterson and Hausmann 1981, Hausmann and Patterson 1982, Linnenbach *et al.* 1983, Pierce and Coats 1999, etc.), locomotive mechanisms (Ockleford 1974, Grębecki and Hausmann 1992, 1993), cyst formation (Patterson 1979, Patterson and Thompson 1981, Shigenaka and Iwate 1984, Shigenaka *et al.* 1985, Newman and Patterson 1993, etc.), asexual cell fusion (Toyohara *et al.* 1977, Shigenaka and Kaneda 1979, etc.), mitosis (Suzaki *et al.* 1978, Mignot 1984), and of autogamy in the cyst (Mignot 1979, 1980a, b). As freshwater actinophryids may often be maintained in culture with relative ease (Sakaguchi and Suzaki 1999), they have been exploited for a number of studies on microtubules (e.g. Tilney and Byers 1969; Roth *et al.* 1970, 1975; Roth and Pihlaja 1977; Suzaki *et al.* 1980b; Patterson and Hausmann 1982; Shigenaka *et al.* 1982; Matsuoka and Shigenaka 1985; Suzaki *et al.* 1992, etc.). The actinophryid heliozoa can be regarded as a well studied group.

We here extend the revision of the taxonomy of heliozoa (Roijackers and Siemensma 1988; Siemensma and Roijackers 1988a, b; Siemensma 1991; Mikrjukov 1996b, c, 1997, 1999a, 2000c, d) to the actinophryids. Despite the extent of investigations of actinophryid biology and ultrastructure, species identities are very unclear. Individuals exhibit considerable variation in form-especially as a result of recent feeding history. In the absence of type material for any of the species, many of the identities remain ambiguous. We believe that it is now appropriate to review the composition of this family, removing those species which cannot be unambiguously identified. We use this opportunity also to comment on two ideas about the origins of the actinophryids - that they are related either to filose amoebae or to the pedinellid flagellates (Patterson 1986, 1989).

MATERIALS AND METHODS

Actinophrys salsuginosa was isolated from the brackish-water pond Swanpool (Falmouth, England) with salinity varying from 1 to 19‰. The heliozoon was isolated from an organically enriched sample from the shore line, and was maintained in 20% sea-water in Evian water and fed twice weekly with washed *Tetrahymena* or *Colpidium*. Light-microscopy and electron-microscopy was carried out as described elsewhere (Patterson 1979, 1980), except that fixatives etc. for electron-microscopical investigations were made up in distilled water or in 20% calcium-free sea water.

The growth of the new species and of *A. sol* was investigated in a range of salinities corresponding to 0-40% sea water. Nine replicate cultures, each initiated with five heliozoa, were made up with 0.5 ml medium of each salinity, to which were added equal numbers of washed *Tetrahymena vorax* cells. The food organisms did not survive at salinities greater then 40% sea water. The cultures were kept in humid chambers at 16°C, and the number of heliozoa counted daily. The size of uncompressed cells was measured microscopically at the end of study. A fixed sample of the cultures from which the type-series of *A. salsuginosa* was taken has been deposited as type material at the Natural History Museum, London (Department of Palaeontology) as a resin-embedded block of material (N PR 138). Living material was lodged at the Culture Collection for Algae and Protozoa, England.

Samples containing the *Ciliophrys azurina* were collected from East Point and Lee Point, Darwin (Northern Territory, Australia) in September 1994 using the procedures outlined by Larsen and Patterson (1990) and Patterson and Simpson (1996).

We refer to photographs as reference type material for new species. This practice is accepted under the guidelines of the International Code for Botanical Nomenclature, but not under the guidelines of the International Code for Zoological Nomenclature. We have

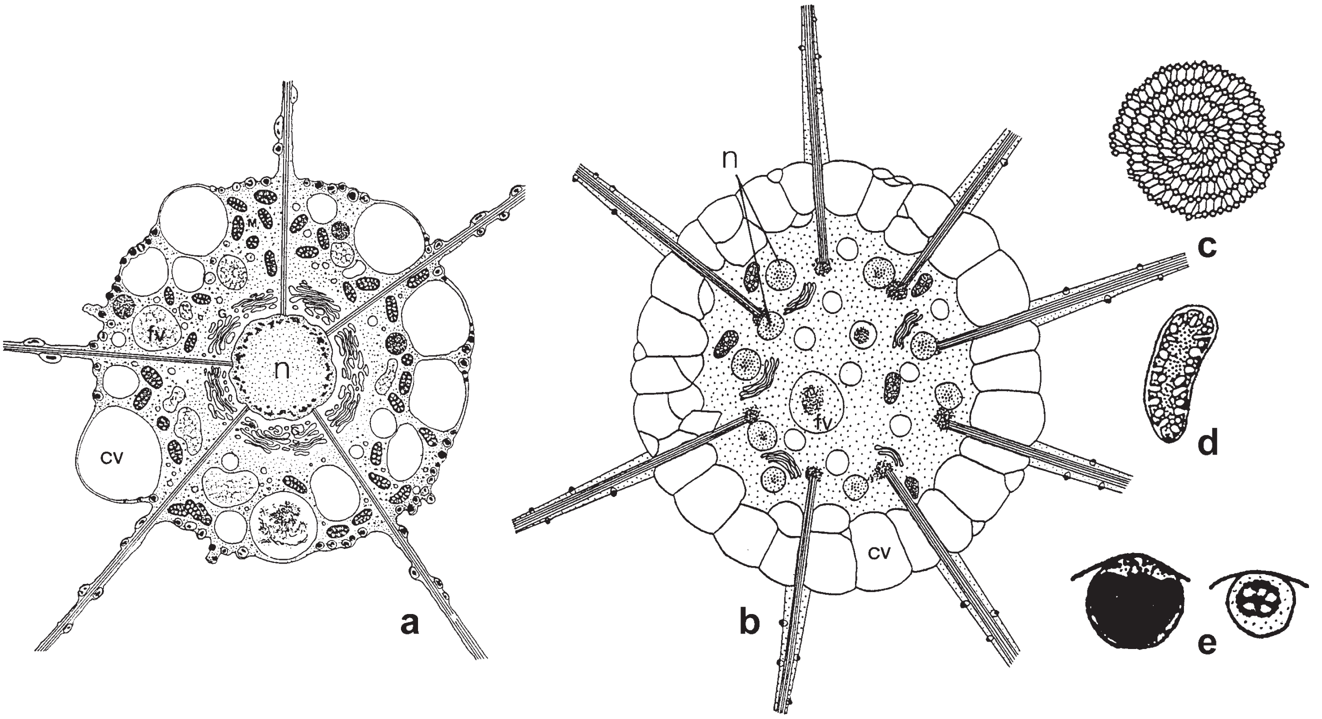


Fig. 1. General structure of actinophryid heliozoa: **a** - *Actinophrys sol* (after Siemensma 1991); **b** - *Actinosphaerium eichhornii*; **c** - axonemal lattice; **d** - mitochondrion; **e** - two types of extrusomes. cv - contractile vacuole, fv - food vacuole, n - nucleus

adopted the practise of using uninterpreted illustrations as type material as we know of no effective alternative of providing unambiguous identities for these small protists, and because we believe that the use of uninterpreted records is compliant with the spirit of the ICZN.

TAXONOMY

Diagnostic criteria

As noted above, species identities among actinophryid heliozoa are not well established, and are mostly based on variations in size and vacuolation of the cell. Different isolates of actinophryids exhibit subtle yet persistent differences in appearance (Sondheim 1916, Shigenaka *et al.* 1980, this study). There is considerable intraspecific variation for this group, especially associated with feeding and excystment (e.g. Patterson and Hausmann 1981).

Identities of taxa cannot be corroborated by reference to the biological species concept. The only form of sexual activity recorded for actinophryid heliozoa is a process of the autogamy involving a fusion of gametes within the cyst (Bělař 1923, 1924; Peters 1964, 1966; Mignot 1979, 1980a, b). Each cell represents an independent genetic lineage, comparable to asexual organisms. Biological species concepts are not applicable. We therefore apply the concept that species are groups of more than one individual which can be distinguished unambiguously and consistently from other groups of individuals by discontinuities in one or more intrinsic attributes, but which contain no groups which satisfy the same definition. In this case the discontinuities are established by the microscopical appearance of the trophic and encysted organisms.

We attach importance to the word “unambiguously” in the definition above, and place in the same entity, taxa which cannot be easily distinguished. Entities with an appearance which falls within known intraspecific variation do not meet the condition of allowing those nominal taxa to be readily identified. Previous authors have also come to the same conclusion and the taxonomic history of this taxon is characterized by extensive synonymies (e.g. Leidy 1879, Rainer 1968). We recognize only four previously described species which can be distinguished by one or more characters that are exclusive to them.

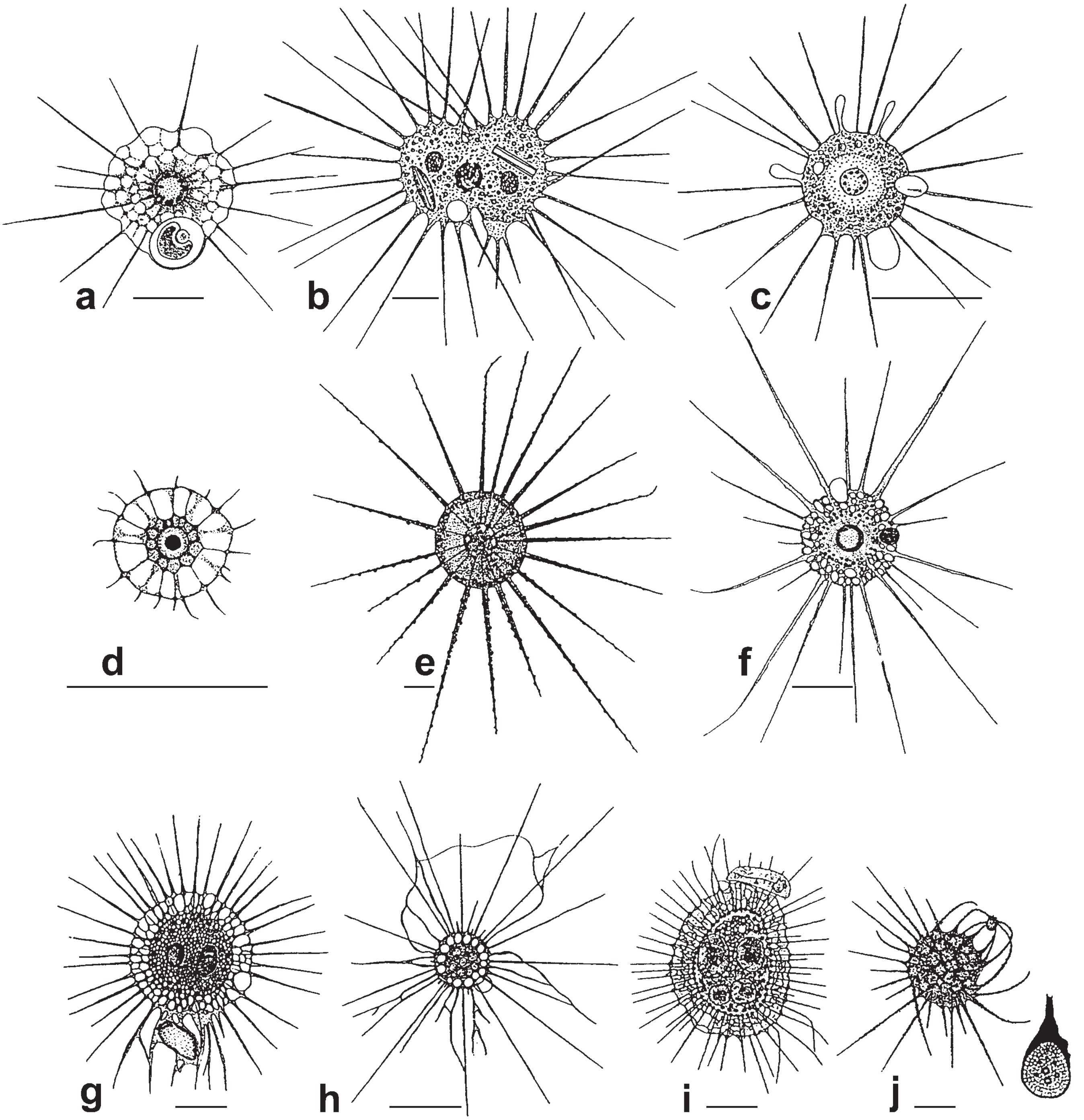
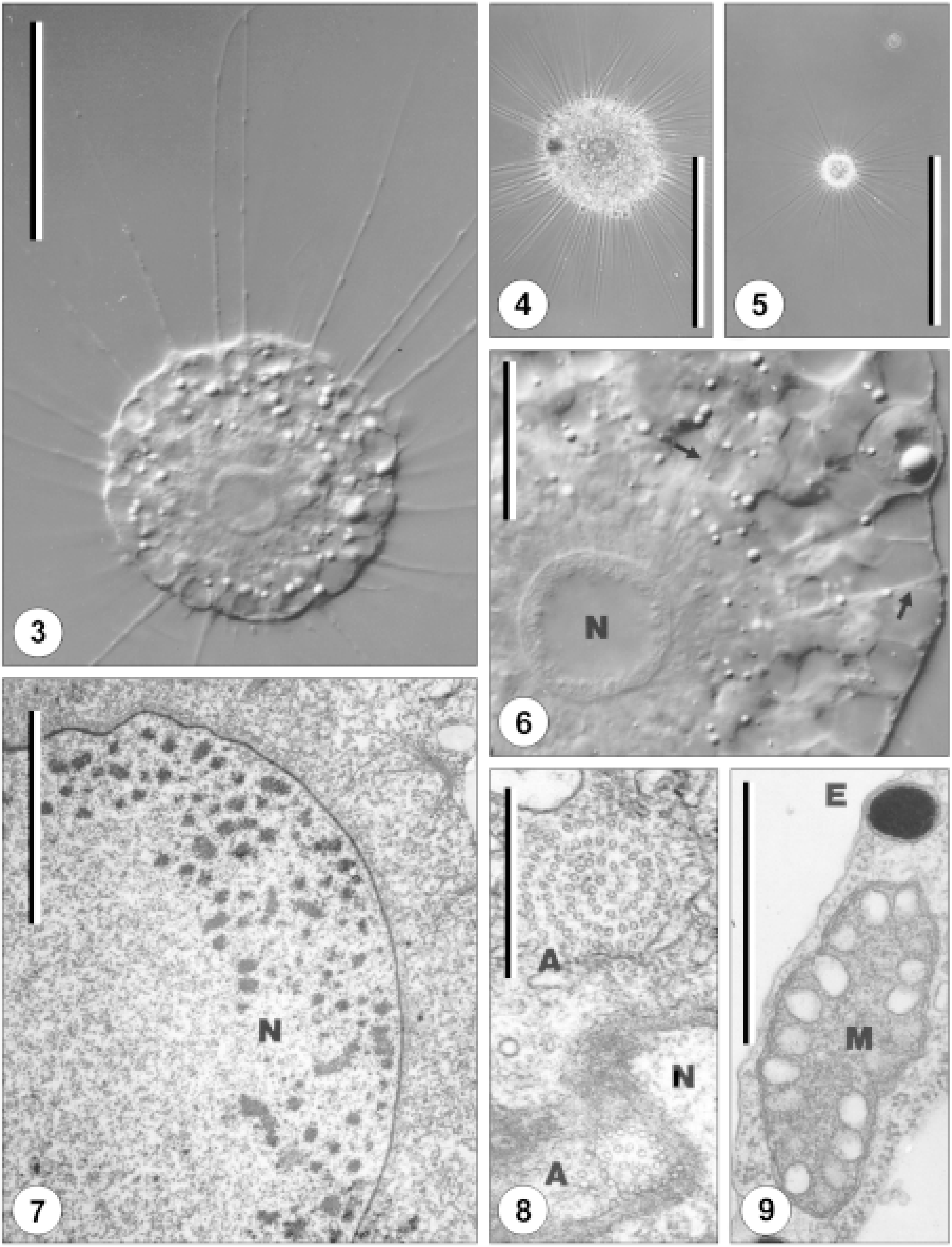


Fig. 2. Actinophryid heliozoa: **a** - *Actinophrys sol* (after Grenacher 1869); **b** - *A. subalpina* (after West 1901); **c** - *A. vesiculata* (after Penard 1901); **d** - *A. pontica* (after Valkanov 1940); **e** - *A. tauryanini* (after Mikrjukov 1996 a); **f** - *A. salsuginosa* sp. n.; **g** - *Actinosphaerium eichhornii* (after Rainer 1968); **h** - *A. arachnoideum* (after Penard 1904); **i** - *A. nucleofilum* (after Barrett 1958); **j** - *Camptonema nutans* and base of axoneme (after Schaudinn 1894). Scale bars - **a-f** - 50; **g-j** - 100 µm



Figs. 3-9. *Actinophrys salsuginosa* sp. n.; **3-5** - general views, differential interference microscopy (**3**) and phase contrast microscopy (**4**, **5**) of living cells; **6** - the nucleus (N), the cortical cytoplasm, and axonemes (arrows) in a living cell, differential interference microscopy; **7** - transmission electron micrograph showing nucleolar material as a layer of small aggregations at the periphery of the nucleus; **8** - axonemes (A); **9** - mitochondrion. E - an extrusome with dark homogeneous contents. Scale bars - **3** - 50; **4** - 100; **5** - 300; **6** - 10; **7** - 35; **8, 9** - 2 µm

The history of Actinophryidae Dujardin, 1841

The first observations of heliozoa are probably those of Joblot (1718), although the kind of heliozoon he observed is not clear. The earliest unambiguous descriptions of actinophryid heliozoa were made by Ehrenberg (1830) of *Actinophrys sol*. This is the type-genus for the family and type-species for the genus (Rainer, 1968). Ehrenberg used a specific name for organisms of uncertain identity described by Müller (1773, 1786) under the name *Trichoda sol*. The recognition of the actinophryid type of organization as distinctive is ascribable to Dujardin (1841) who was the first to use the root "actinophry-" in the name of a suprageneric taxon. He did not employ a latinised name (referring to the "family Actinophryiens"), but it is to him that we assign nomenclatural authority for (all) the suprafamilial ranks based on this root. The first use of a latinised family name (Family Actinophryina) is that of Claparède and Lachmann (1858). The actinophryids are now typically assigned ordinal rank in traditionalist classification schemes (e.g. Levine *et al.* 1980, Cachon and Cachon 1982, Febvre-Chevalier 1985, Siemensma 1991). The first use of this rank is attributable to Hartmann (1913). The taxon has been placed of class by Krylov *et al.* (1980), Karpov (1990), Corliss (1994), and Kussakin and Drozdov (1998), and has been incorporated in unranked schemes by others (Patterson 1994, 1999).

Generic composition of the actinophryids

The most recent review of heliozoan taxonomy (Siemensma 1991) included two genera in the actinophryids. They are the uninucleate *Actinophrys* Ehrenberg, 1830 and multinucleate *Actinosphaerium* Stein, 1857. We agree with this view. Other reviews refer to four or five actinophryid genera.

Hovasse (1965) divided the genus *Actinosphaerium*, creating *Echinosphoerium* or *Echinosphaerium* (the paper is ambiguous in respect to the preferred spelling) on the basis of whether all axopodia terminate on nuclei (*Echinosphoerium*/ *Echinosphaerium*) or not (*Actinosphaerium*). This distinction is maintained by Shigenaka with co-authors (1980).

Trégouboff (1953) recognised four actinophryid genera: *Actinophrys*, *Actinosphaerium*, *Camptonema* Schaudinn, 1894 and *Vampyrellidium* Zopf, 1887. *Vampyrellidium* has been shown to be a nucleariid (Patterson *et al.* 1987). *Camptonema* was recognised by Rainer (1968), but as the axonemes of this monotypic genus terminate on the nucleus, we regard it as a junior synonym of *Actinosphaerium* Febvre-Chevalier (1985) recognised *Actinophrys*, *Actinosphaerium*, *Echinosphaerium* and *Camptonema*.

Levine *et al.* (1980) and Sleigh *et al.* (1984) include the helioflagellate genus *Ciliophrys* Cienkowski, 1876.

What makes actinophryid heliozoa distinctive

Actinophryid heliozoa are round bodied unicellular organisms. There are no cilia or flagella. There is a single central nucleus or many small nuclei located in the central part of the cell (the endoplasm). Numerous stiff arms or axopodia, noticeably tapering from the base to the tip, radiate from the whole body surface. The axopodia are supported internally by microtubules arranged in a double hexagonal spiral and terminate in electron-dense material located on the nuclear envelope or near a nucleus. Mitochondrial cristae are tubular (bleb-like) and have an electron-dense matrix. There are two types of simple extrusomes - a larger osmiophilic type and a smaller granular type. The surface of trophic cells is naked. Actinophryids feed mainly by predation, often accompanied by fusion of several cells. Cysts may form which have multiple walls, one of which is comprised of siliceous elements. Reproduction is mainly by binary fision. Sexuality is limited to autogamy and occurs in the cyst and is accompanied by the formation and subsequent fusion of amoeboid gametes.

Diagnoses and discussion of the genera and species

Actinophrys Ehrenberg, 1830

Diagnosis. Uninucleate actinophryid heliozoa in which the axonemes terminate on a central nucleus.

Remarks. The taxonomic history of the genus and of its type species is confused. Müller's (1773) original reference to it as *Trichoda sol* reappears in a later work (Müller 1786) but there is no specific indication that the drawings were made from the same material as used for the original description. These drawings might well (but not certainly) relate to a uninucleate actinophryid. The species name was reassigned to the genus *Peritricha* by Bory de St.Vincent (1824), but without any further new observations. Ehrenberg (1830) provided the first unambiguous description of this organism, identifying it with the organism described by Müller. The status of the genera *Trichoda* and *Peritricha*, and of the numerous species originally included in them, is obscure. Corliss (1979) regards *Trichoda* as a *nomen oblitum*. Neither generic name appears to have been in contemporary use and we are unaware of the designation of any type-species for either genus. Both names are held to be *nomina dubia*, in that the taxa are not well circumscribed and it is no longer clear to what organisms these taxa refer. For this reason, and in order not to introduce nomenclatural confusion to a well circumscribed genus, the generic name *Actinophrys* is retained.

Recent accounts include different number of species in this genus. Rainer (1968) includes four species: *A. sol* (Müller, 1773) Ehrenberg, 1840, *A. subalpina* West, 1901, *A. vesiculata* Penard, 1901, and *A. pontica* Valkanov, 1940. Siemensma (1991) considers only *A. sol*, and regards *A. vesiculata* and *A. subalpina* as synonyms of it. He makes no comments in respect of *A. pontica*. Mikrjukov (1996a) described a new marine species *A. marina* using a species name preoccupied by Dujardin (1841) for species previously synonymized with *A. sol* by Rainer (1968) and for which we introduce *A. tauryanini*.

Actinophrys sol **(Müller, 1773) Ehrenberg, 1840 (Fig. 2a)**

Diagnosis. *Actinophrys* with a body measuring about 50 (19-90) µm in diameter, with heterochromatin forming a continuous layer under the nuclear envelope; cyst wall with flat siliceous scales.

Remarks. The species to which the name *Actinophrys sol* refers to is ambiguous because of the absence of type material. This problem is compounded by phenotypic variability of actinophryids - there being considerable variation of form as a function of feeding history. Many nominal species assigned to this genus are now regarded as synonyms of this species (for lists see: Rainer 1968). Despite being extensively studied (e.g. Bělař 1923, 1924; Ockleford and Tucker 1973; Mignot 1979, 1980a, b, 1984; Patterson 1979; Patterson and Hausmann 1981; Newman and Patterson 1993), there have to date been no features which allow the unambiguous separation of species in this genus. As indicated below, we now rely on the appearance of plates in the cyst to distinguish *A. sol* from *A. salsuginosa*. In the absence of previously designated type material, we apply *Actinophrys sol* to organisms which satisfy the description based on culture LB 1502/2 from the Culture Collection for Algae and Protozoa (Patterson 1979).

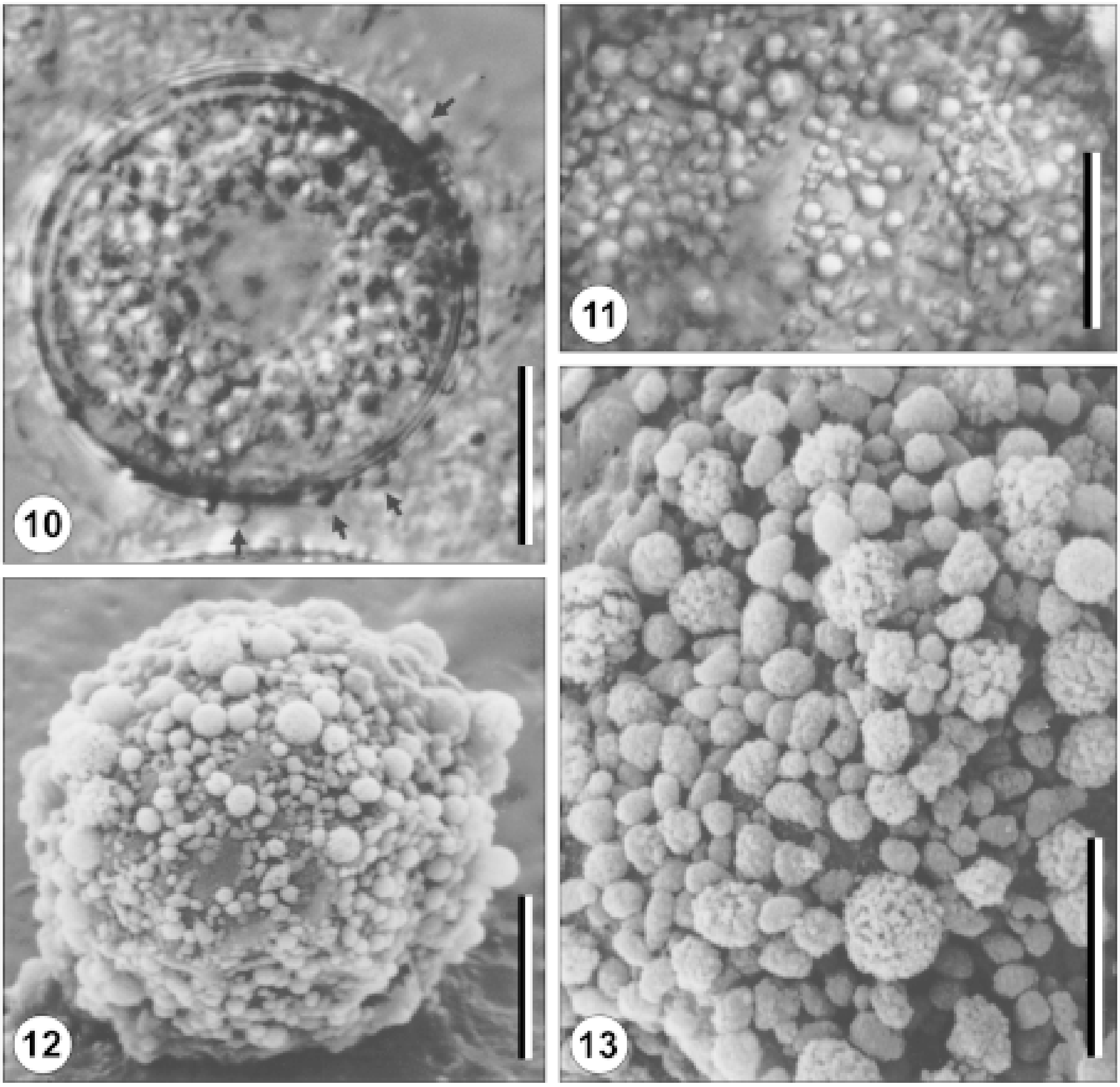
West (1901) described *A. subalpina* (Fig. 2b) as a species of *Actinophrys* having a spherical body 42-61 µm in diameter, and finely granular cytoplasm and no peripheral vacuoles. As peripheral vacuolisation is a function of the recent feeding history of the organism (Patterson and Hausmann 1981), this description could equally well apply to individuals of *A. sol*. No type material designated in the original description. It was published with a single figure (plate 30, Fig. 36) herein designated as lectotype. *A. subalpina* cannot be unambiguously distinguished from *A. sol*, and in agreement with Penard (1904), we regard *A. subalpina* as a subjective junior synonym of *A.sol*.

Penard (1901) described *A. vesiculata* (Fig. 2c) as a species of *Actinophrys,* 25-30 µm in diameter, with pendulous vacuoles and nucleoli in the form of condensed spheres. Penard (1904) subsequently questioned his own observations on the nucleoli. No other original observations have been made on this species. No type material was designated in the original description, which was published with three figures [Figs. 2-4 (by Penard)] of which Fig. 2 (by Penard) is herein designated as lectotype. The «pendulous» vacuoles would be mechanically unstable structures and we concur with Rainer (1968) that they were probably caused by pressure from the cover-slip and that Penard (1901) observed *A. sol*. *A. vesiculata* is held to be a junior subjective synonym of *A. sol*.

Actinophrys pontica **Valkanov, 1940 (Fig. 2d)**

Diagnosis. *Actinophrys* species measuring about 12 µm, the nucleus with a central spherical nucleolus.

Remarks. Valkanov described this organism from the Black Sea brackish-water habitats, and it was subsequently rediscribed by Jones (1974) and recorded by Febvre-Chevalier (1990). Neither account is explicit as to whether living material was observed. There was no designation of type material in the original publication, but there were two figures (1 and 2), of which figure 1 is herein designated lectotype. By virtue of its small size, and distinctive nucleolar location, this species may be distinguished from *A. sol*. This species is said to have very marked peripheral vacuolization, but this may reflect recent feeding history and we do not regard this as



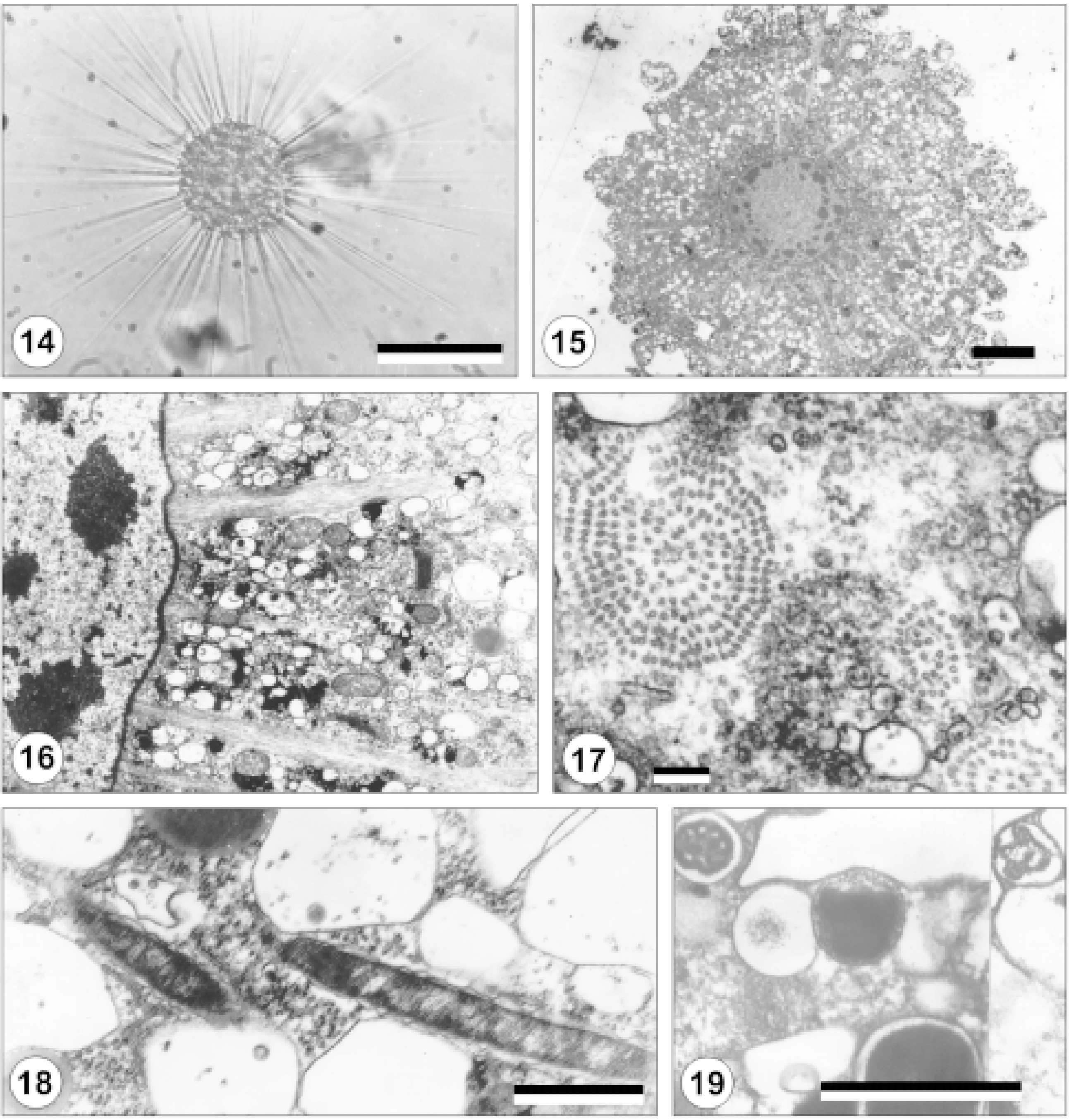
Figs. 10-13. Cysts of *Actinophrys salsuginosa* sp. n.; **10** - general view; **11-13** - spherical nature of siliceous elements of the wall; **10,11**- light microscopy; **12, 13** - scanning electron microscopy. Scale bars - **10, 12** - 10; **11** - 5; **13** - 2.5 µm

a reliable diagnostic feature. Further work is required, specifically to ensure that individuals of *Ciliophrys* were not observed. Mikrjukov (1999b) observed *Actinophrys* sp. in coastal Black Sea water (with a salinity of 1.8%), about 30 µm in diameter, but corresponding more to the characteristics of *A. sol*.

Actinophrys salsuginosa Patterson, sp. n. (Fig. 2f).

Diagnosis. *Actinophrys* species measuring about 29-114 µm, with nucleolar material forming a peripheral layer of small aggregates, and with a cyst incorporating spherical siliceous elements.

Description. The size of the trophic organism is quite variable. The average diameter of non-feeding cells, under the culture regime described above, is 44.2 µm. The arms extend about 150-200 µm from the body. The dimensions of the body vary depending upon the recent feeding history and the salinity of the medium. During feeding, very large masses of cells may form, but they do not adhere strongly to the substrate. After feeding, uninucleate cells separate from the fused masses. Initially these have a diameter of about 95-100 µm, but after a day or so, the majority of the cells have a body diameter about half this value. Consequently, a fre-



Figs. 14-19. *Actinophrys tauryanini*; **14** - general view by light microscopy; **15** - cross-section through the median part of the cell; **16** - central area of the cell showing a peripheral part of the nucleus with nucleoli as large aggregations and the inner parts of the axonemes; **17** - axonemes in cross-section; **18** - rod-like ectoplasmic bacteria; **19** - two types of extrusomes: large ones with a homogeneous content, and smaller ones with a heterogeneous, microgranular content. **15-19** - transmission electron microscopy. Scale bars - **14** - 100, **15-16** - 10; **17-19** - 1 µm

quency distribution histogram of body diameters in a population tends to be bimodal. The extreme dimensions encountered for the body were 29 µm and 114 µm. Cells grown in a medium of zero salinity had an average diameter of 61 µm, while the average diameter of cells grown in 40% sea water was 40.5 µm. Higher concentrations were not investigated because the prey became moribund in these salinities. The nucleolar substance/

heterochromatin forms a layer 3-4 µm thick under the membrane of the centrally located nucleus (Figs. 3, 6, 7). Ultrastructural observations confirm that: the axonemes radiate from the nuclear envelope and are formed of double interlocking spirals of microtubules (Fig. 8), there are electron-dense extrusomes, and mitochondria have bleb-like cristae and a dark matrix (Fig. 9). As with other actinophryids, *A. salsuginosa* is able to form cysts with multiple layers in the wall (Fig. 10). Sintered siliceous beads mostly 0.3-1.0 µm in diameter form a layer of the cyst wall (Figs. 11-13).

Remarks. The organism isolated from Swanpool was identified as a member of the genus *Actinophrys* because of the stiff radiating arms, with axonemes comprised of double interlocking spirals of microtubules and terminate on a large central nucleus, and because of the siliceous material in the cyst (Fig. 6). Calkins gives a figure of a marine *Actinophrys* from Woods Hole which is similar to *A. salsuginosa* (Calkins 1902).

Actinophrys salsuginosa resembles *A. sol* closely in general appearance and size of trophic individuals. It can be distinguished from *A. sol* (*sensu* Patterson 1979) because of the arrangement of the nucleolar material and by the spherical (as opposed to flattened) shape of the siliceous elements of the wall. The body size of *A. salsuginosa* is similar to that of *A. sol*, although the new species has a more vacuolated outer region, and more delicate (longer and thiner) arms. The two species differ in their tolerance of saline conditions. *A. sol* did not survive in salinities greater then 20% sea water. *A. salsuginosa* continued to grow actively in 40% salinity. Under similar salinity conditions, the new species was slightly larger, more vacuolate, with fine arms, formed larger masses during feeding; these masses did not adhere to the substrate to the same extent as those of *A. sol*. *A. salsuginosa* differs also from the marine *A. tauryanini* (see below) because it is usually half the size, has a different nucleolar morphology, is tolerant of low salinity regimes, and may have a layer of large peripheral vacuoles. *A. salsuginosa* can be distinguished from *A. pontica* by the nucleolar configuration.

Two ultrastructural characteristics of *A. salsuginosa* suggest a close relationship to *Actinosphaerium nucleofilum*. They are: location of the nucleolar substance as a peripheral layer of small grains (Anderson and Beams 1960, Shigenaka *et al.* 1980), and siliceous components of the cyst wall of both are sintered spheres (Patterson and Thompson 1981).

Actinophrys tauryanini (Mikrjukov, 1996) nom. nov. (Figs. 2e, 14-19)

Diagnosis. Marine *Actinophrys* species measuring 70-90 µm; the nucleus with large peripheral clumps of the nucleolar material; without contractile vacuoles.

Remarks. This species was found in the White Sea (18-40 m depth), at salinities of 2.7-2.9‰, and was first reported as *A. marina.* This name was preoccupied by one introduced by Dujardin (1841) and a replacement name was required.

In size and with peripheral vacuoles, it resembles the taxon described as *A. subalpina* (Fig. 2 b). *A. tauryanini* grows well at oceanic salinities (35‰), but dies at salinities below 22-23‰. Organisms were maintained from 1992-1996 in the laboratory using marine diatoms and *Bodo* sp. as food. No peripheral layer of vacuoles was observed during feeding. Cells in culture are always solitary and did not fuse. Attempts to obtain cysts of this species were not successful. *A. sol* was recorded at the same time from in estuarine bays of the White Sea with salinities not exceeding 14 ‰ (Mikrjukov 2000b). Estuarine *A. sol* measured about 30-40 µm in diameter, had a transparent cytoplasm with large peripheral vacuoles, some of which behaved as contractile vacuoles. *A. tauryanini* can be distinguished from *A. sol* not only by being double the size, by having a fine vacuolisation of the cytoplasm, no spongiome nor contractile vacuoles, and by its tolerance of saline conditions. The marine species *A. tauryanini* differs from the brackish-water *A. salsuginosa* and *A. pontica* by the appearance of the nucleolar material. The species has also been recorded in the Tasman Sea (Mikrjukov and Patterson 2000).

The ultrastructure of *A. tauryanini* (Figs. 15-19) is similar to that of *A. sol* but it differs in having large clumps of the peripheral nucleolar material (Fig. 16) and by having rod-like cytoplasmic bacteria (Fig. 18).

Actinosphaerium Stein, 1857

Diagnosis. Multinucleate actinophryid heliozoa in which the axonemes may or may not end on the nuclei.

Remarks. The genus *Actinosphaerium* was erected by Stein (1857) to accommodate *Actinophrys eichhornii* Ehrenberg, 1840 and was distinguished by the presence of large number of nuclei. Of the species which have been assigned to this genus, current reviewers (e.g. Trégouboff 1953; Rainer 1968; Febvre-Cheva-

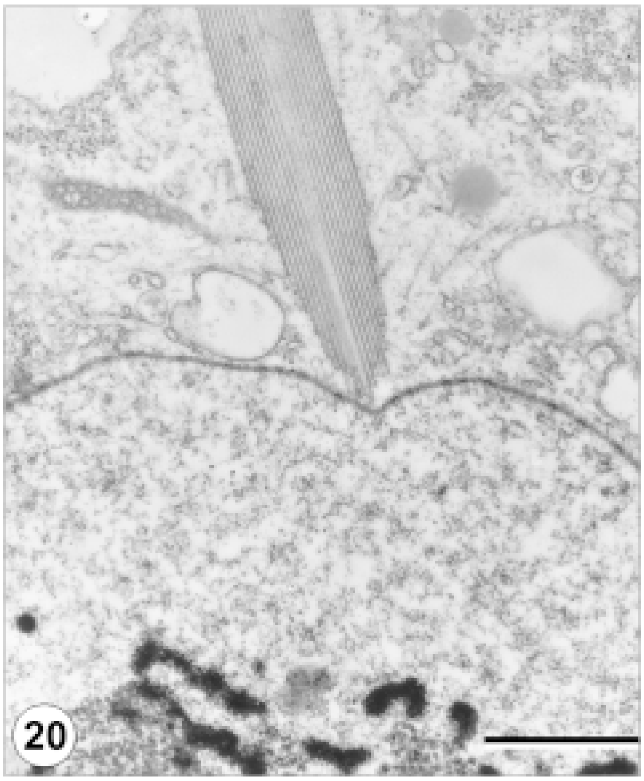


Fig. 20. Termination of an axoneme adjacent to the envelope of a nucleus of *Actinosphaerium eichhorni*. The central position of the nucleolus is clearly seen. Scale bar - 1 µm. Transmission electron micrograph by J. Robertson

lier 1985, 1990; Siemensma 1991) also accept *A. arachnoideum* Penard, 1904. Barrett (1958) added *A. nucleofilum*. Some aspects of the ultrastructure of *A. nucleofilum* were provided by Anderson and Beams (1960). Using this information, Hovasse (1965) erected a new genus for *A. nucleofilum*. The name was spelled *Echinosphoerium* and *Echinosphaerium* in the original paper, and is probably more commonly referred to under the latter spelling (Tilney and Byers 1969, Matsuoka *et al.* 1985). The appropriateness of Hovasse's action is discussed under *A. nucleofilum* below. Subsequently, and incorrectly, Shigenaka *et al.* (1980) assigned all of the above-named species to the genus *Echinosphaerium* and added two new species, *E. akamae* and *E. ikachiensis*, which were synonymized with *A. eichhornii* by Siemensma (1991) without any comments. The status of *A. portuum* Kufferath, 1952 included by Shigenaka *et al.* (1980) has received little attention. Kufferath's description makes no mention or inference of the number of nuclei, and cannot be admitted to a discussion of the genus *Actinosphaerium*, nor indeed, on the basis of the information provided, is there a good case for regarding the organism as an actinophryid. In the discussion which follows we include the genus *Camptonema* Schaudinn, 1894 as it is a multinucleated heliozoan.

The genus has been subject to extensive ultrastructural study (Anderson and Beams 1960; Hovasse 1965; Kitching and Craggs 1965; Tilney and Porter 1965; Tilney and Byers 1969; Shigenaka *et al.* 1975; Schliwa 1976, 1977; Shigenaka 1976; Shigenaka and Kaneda 1979; Shigenaka *et al.* 1979, 1980; Toyohara *et al.* 1977, 1978, 1979; Suzaki *et al.* 1980a; Patterson and Thompson 1981, *etc*.). Similarities in the packing pattern of microtubules in axopodia, in extrusome morphology, mitochondrial appearance and cyst morphology, confirm that this genus is very closely related to *Actinophrys* and is probably derived from it (Smith and Patterson 1986).

Actinosphaerium eichhornii **(Ehrenberg, 1840) Stein, 1857 (Fig. 2g)**

Diagnosis. *Actinosphaerium* species measuring typically 200-300 µm, with numerous nuclei, each usually 13-17 µm in diameter, with nucleoli clustered centrally in each nucleus.

Remarks. Originally described as *Actinophrys eichhornii* Ehrenberg, 1840, Borowsky (1910) has shown the number and dimension of nuclei to be sensitive to the recent feeding history, and a range of sizes from 11 to 21 µm has been reported in the literature (Cash and Wailes 1921, Penard 1904). The nucleolar material (heterochromatin) lies as a cluster of granules in the centre of each nucleus. At the moment, this is the only useful diagnostic criterion. Shigenaka and co-workers (1980) proposed several morphometric criteria by which species might be distinguished. They included the ratio of the diameter of endoplasm to the thickness of ectoplasm having to be always more than 2:1. There is no electron microscopical information on the structure of the cyst wall in *A. eichhornii*.

Penard (1904) described *A. arachnoideum* Penard, 1904 (Fig. 2h) based on observations of six cells, and stated that the body measures 70-80 µm, and the cell had 4-12 nuclei measuring 7-8 µm in width. Penard believed that *A. arachnoideum* was distinct from *A. eichhornii* because the cell had some pseudopodia without axonemes. The dimensions of the body fell within the range encountered in *A. eichhornii*. The second type of pseudopodia noted by Penard may be seen in many actinophryids, particularly if compressed by a cover slip. The small size and number of nuclei is the only means of identifying the organism. However, excysting specimens of *Actinosphaerium* are uninucleate (Hertwig 1899). There is uncertainty as to the dimensions of the nuclei (Cash and Wailes 1921 *vs* Penard 1904). The number of nuclei varies according to the recent feeding history of the organism (Borowski 1910). In the absence of the other diagnostic features, there is no sound basis for retaining this as a valid species. The name is held to be a junior synonym of *A. eichhornii*.

Actinosphaerium nucleofilum Barrett, 1958 (Fig. 2i)

Diagnosis. *Actinosphaerium* species measuring 230-400 µm, with small and numerous nuclei, 4-8 µm in diameter, with peripherally located nucleolar substance, and with a cyst wall incorporating spherical siliceous elements.

Remarks. Barrett (1958) distinguished this species from *A. eichhornii* because in *A. nucleolfilum* some axodopodia terminate on the surface of nuclei (see remarks to *Echinosphaerium* below), and the nuclei have the nucleolar material located peripherally. The diameter of the nuclei is very small, and this was confirmed by Shigenaka *et al.* (1980). The cysts have spherical siliceous elements in the wall (Patterson and Thompson 1981). We consider *A. nucleofilum* Barrett, 1858 as the second species of the genus *Actinosphaerium.* Currently, this is the most extensively studied of the multinucleated actinophryids.

Hovasse (1965) introduced the generic name *Echinosphoerium* / *Echinosphaerium* (both spellings were used in his paper) for actinosphaerids with axopodia terminating on the surface of nuclei - on the assumption that in *Actinosphaerium* axopodia do not end on nuclei. This is supported by observations of some authors (Bütschli 1882, Penard 1904, Valkanov 1940). Stein's original description of *Actinosphaerium* included no details on this feature. Hovasse (1965) identifued *E. nucleofilum* (Barrett, 1958) Hovasse, 1965 as the type species of a new genus. Tilney and Porter (1965) provided ultrastructural evidence that some axopodia do end on the nuclei, but this is not always the case (Jones and Tucker 1981). The situation in *A. eichhornii* appears to be the same. Several workers have shown that axopodia end close to the nuclei (Roskin 1925, Rumjantzew and Wermel 1925). That some axopodia terminate against nuclei with central heterochromatin (i.e. are micrographs of *A. eichhornii*) has been confirmed ultrastructurally (Fig. 20). Electron micrographs similar to Fig. 20 have been obtained from *Actinophrys* and *A. nucleofilum* (Allison *et al.* 1970, Shigenaka and Kaneda 1979, Tilney and Porter 1965, Toyohara *et al.* 1977, etc.). As *A. eichhornii* and *A. nucleofilum* may have axopodia terminating against nuclei or ending freely in the cytoplasm, and we do not believe that Hovasse's reasoning for erecting a new genus is justified. The generic names *Echinosphoerium* and *Echinosphaerium* are held to be synonymour with *Actinosphaerium* Stein, 1857.

Shigenaka *et al.* (1980) described *Echinosphaerium ikachiensis* Shigenaka, Watanabe et Suzaki, 1980 as a species of *Echinosphaerium*, with body diameter 186-436 µm, and with nuclei (diameter 9-15 µm) with peripheral clots of the nucleolar material. Some details of the description of this taxon, especially the ratio of endoplasm to ectoplasm (3-16:1) generate the same uncertainties as raised with *A. akamae* (see below). No information was given on axonemal termination nor on the structure of the cysts. Fig. 3c from the work by Shigenaka *et al.* (1980) shows sparse peripheral clots of the nucleolar material in a nucleus, and in this character the taxon is similar to *A. nucleofilum*. Despite consistent differences between isolates observed by Shigenaka *et al.* (1980) we do not believe that this taxon could be unambiguously distinguished from *A. nucleofilum*, and we regard *E. ikachiensis* as a junior synonym of *A. nucleofilum*.

Actinosphaerium akamae (Shigenaka, Watanabe et Suzaki, 1980) nov. comb.

Diagnosis. A species of *Actinosphaerium* measuring 82-244 µm, with nuclei (diameter 8-13 µm) with nucleolar material as a central cluster of granules, and with a cyst wall not incorporating siliceous components.

Remarks. Much of the information provided about this species relates to the dimensions of the cell, and proportions of endoplasm and ectoplasm (morphometric characters). The proportions of endoplasm to ectoplasm (2-14:1) appear to have been calculated (rather than measured) using the minimum of one value against the maximum of the other. If the values are summed, they gives sizes for cells outside the range described in the paper. The cyst of *A. akamae* does not incorporate siliceous components, but is composed of several organic (i. e. mucous, granular, fibrillar and electron-dense ones) layers (Shigenaka *et al.* 1985). Originally named *Echinosphaerium akamae* but, for reasons given above, now assigned to *Actinosphaerium*. The appearance of nuclei is similar to that of *A. eichhornii* although they are reported as slightly smaller. In view of the discrepancies over reports of nuclear size in *A. eichhornii* and in view of the variability of this character (see above), this aspect requires reinvestigation. While there is little doubt that different stocks investigated by the Japanese workers exhibited consistent and identifiable differences from which species, there are no absolute characters by which *A. akamae* can be identified except the absence of siliceous elements from the cyst. Given the overall similarity of this taxon to *A. eichhornii*, the uncertainty over some of the distinguishing characters, we treat the taxon as *nomen dubium* until the unusual nature of the cyst is confirmed and/or other discriminatory characters emerge.

Camptonema Schaudinn 1894

The genus *Camptonema* was created by Schaudinn (Schaudinn 1894) to accommodate a single marine species, *C. nutans* Schaudinn, 1894 (Fig. 2J). This organism has not been recorded since its original description. It has body with a diameter of 120-180 µm, vacuolated ectoplasm, and granular endoplasm. At the periphery of the endoplasm there are about 10 oval nuclei, about 15 µm long. This species is normally not admitted to the genus *Actinosphaerium* because a cone of dense material surrounds the axoneme where it terminates from the nucleus. However, cone-like aggregations of material around *Actinosphaerium*, and the species name *C. nutans* a junior subjective synonym of *A. eichhornii*. *A eichhornii* has been recorded in estuarine bays of the White sea with a salinity not exceeding 1.0% (Mikrjukov 2000b).

The genus *Ciliophrys* Cienkowski, 1876

Several authors (Levine *et al.* 1980, Cachon and Cachon 1982) include the flagellated genus *Ciliophrys* Cienkowski, 1876 among the actinophryids. This follows arguments of the close affinities of these two groups (Davidson 1972, 1982). Although the case for such affinity is attractive, it is quite clear that *Ciliophrys* has more characters in common with the pedinellids - sharing with them flagellar and cytoskeletal organization (Zimmermann *et al.* 1984, Patterson and Fenchel 1985, Preisig *et al.* 1991 - see below). As discussed below we do not believe that the ciliophryids are a subset of the actinophryids, rather the converse. Our discussion and diagnosis of actinophryids (above) does not include this genus. We describe here a new species which contributes to our understanding of relationships between these groups.

Ciliophrys is a naked and heterotrophic pedinellid with either no stalk or a short stubby stalk (unpublished ultrastructural information). It is distinguished from other pedinellid taxa without plastids because the arms radiate from the whole cell surface, and, while in the heliozoan state, has weakly active flagellum held in a figure of 8 configuration. The cell may convert into an arm-less form at which time the flagellum becomes more active and the pseudopodia are withdrawn. These arm-less cells usually swim with the flagellum directed to the front. The fine, non-tapering axopods are supported by single triads of microtubules. As with actinophryids and other pedinellids, the interior ends of these axonemes are associated with nuclei. The composition of the genus was discussed by Larsen and Patterson (1990). We currently admit two species, and here add a third.

Ciliophrys infusionum Cienkowski, 1876 (Fig. 21, a) (Syns: *C. marina* Caullery, 1909; *Dimorpha monomastix* Penard, 1921)

This species is distinguished because it has non-tapering arms; the nucleus has a large central nucleolus, and because the arm-less form swims actively. *Ciliophrys infusionum* has been found in marine sites in SE North America, subtropical and tropical Australia, Denmark, England, English Channel, Fiji, Gulf of Finland, Hawaii, Mediterranean, Norway and equatorial Pacific (Lee and Patterson 2000).

Ciliophrys australis Schewiakoff, 1893

This species is distinguished because it is not motile in the arm-less state. This species has not been observed since its original description. We suspect that this may prove to be the same as *C. infusionum*. The spelling *C. australiensis* by Larsen and Patterson (1990) is incorrect.

Ciliophrys azurina Patterson, sp. n. (Figs. 21b; 22, 23)

Diagnosis. *Ciliophrys* with tapering arms; nucleus with a central nucleolus and additional peripheral heterochromatin.

Description. Cell 15 µm in diameter, with radiating arms with extrusomes. The single flagellum is held in

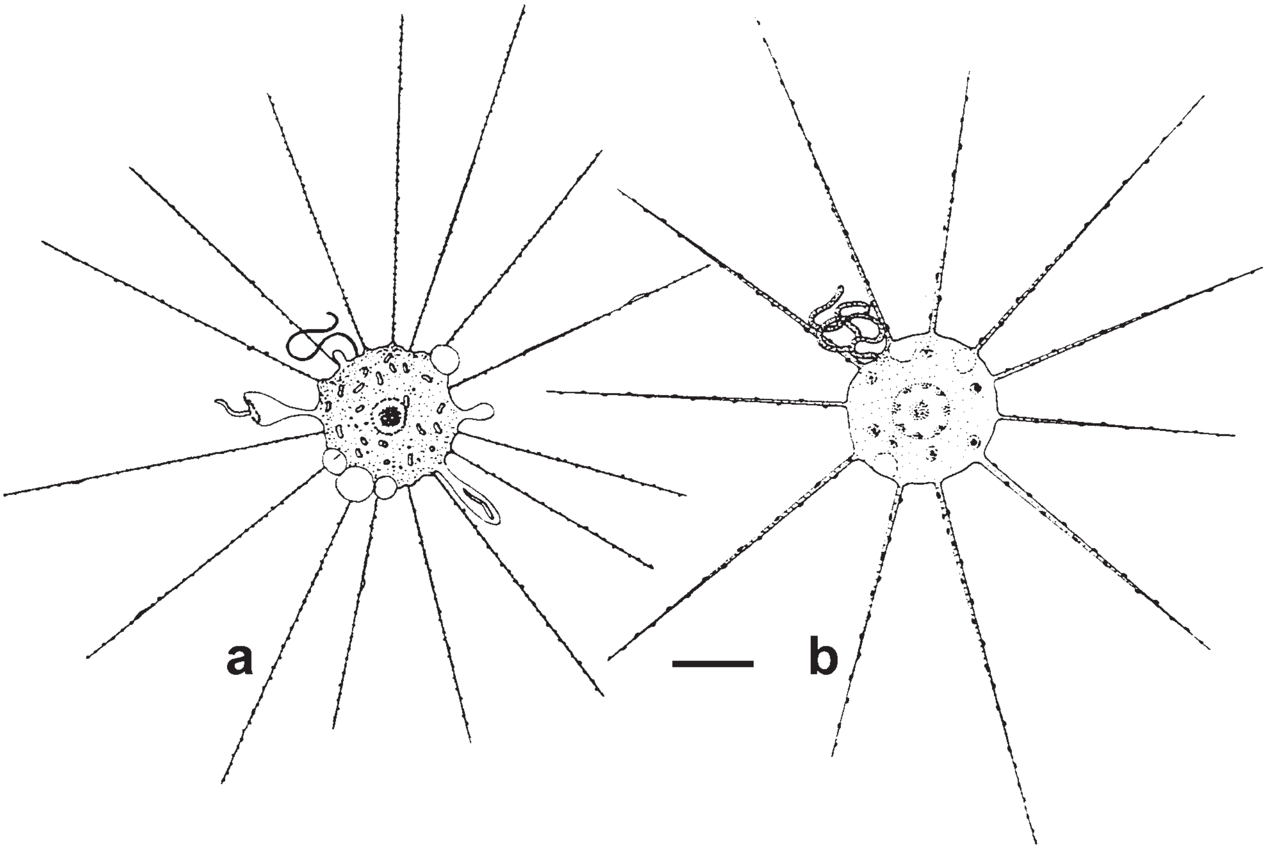
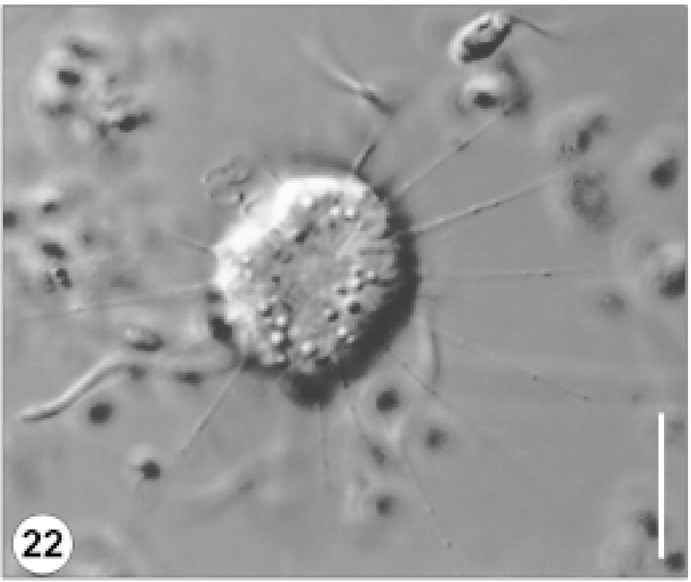
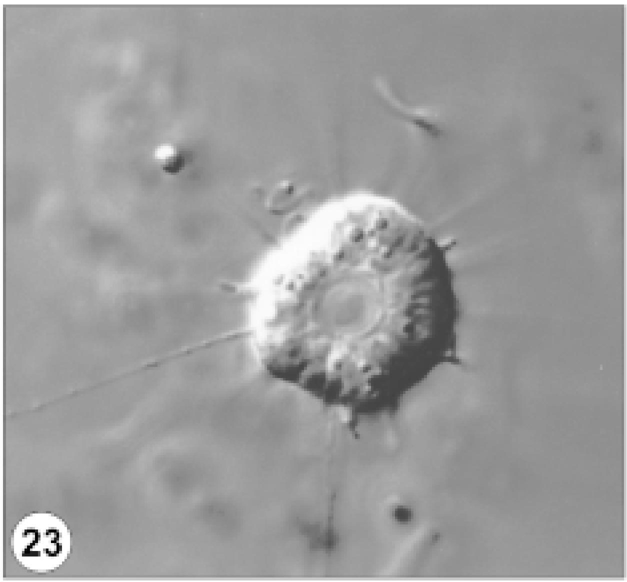


Fig. 21. Line drawings of (**a**) *Ciliophrys infusionum* Cienkowski, 1876 (after Siemensma 1991) and (**b**) *C. azurina* Patterson, sp. n.). Scale bar - 10 µm





Figs. 22,23. *Ciliophrys azurina* sp. n., live cells viewed with differential interference microscopy; showing the double “figure of 8” flagellum, nucleus with a central nucleolus and additional peripheral aggregates of heterochromatin. Scale bar - 10 µm front of swimming cells, and in non-swimming (feeding) cells the flagellum is held tightly curled, typically in a double "figure of 8". The nucleus is large, prominent and has a nucleolus and clumps of material located around the inner face of the nuclear envelope. Observed consuming diatoms.

Remarks. *Ciliophrys azurina* can be distinguished from the other well described species in the genus, *C. infusionum*, by being considerably larger (15 µm *vs* 5 µm, although we note that *C. infusionum* has been reported as up to 20 µm long). More importantly, *C. azurina* can also be distinguished because the flagellum is longer and held in a double "figure of 8", because the arms taper from base to tip, and because of the existence of peripheral clumps of heterochromatin in the nuclei. These two characters are held in common with *Actinophrys* - and there is especial similarity with *Actinophrys pontica*. We interpret the tapering arms and peripheral heterochromatin as being apomorphic characters for a previously unrecognised clade which includes *C. azurina* and the two genera of actinophryids and which we here refer to as the heliomonads

Summary of the composition of actinophryids

Actinophrys Ehrenberg, 1830

A. sol (Müller, 1773) Ehrenberg, 1830

Synonyms: *A. difformis* Ehrenberg, 1830; *A. marina* Dujardin, 1841; *A. stella* Perty, 1852; *A. oculata* Stein, 1854; *A. tenuipes* Claparède and Lachmann, 1858; *A. fissipes* Lachmann, 1859; *A. longipes* Lachmann, 1859; *A. tunicata* Lachmann, 1859; *A. limbata* Lachmann, 1859; *A. paradoxa* Carter, 1864; *A. picta* Leidy, 1879; *A. alveolata* Schewiakoff, 1893; *A. subalpina* West, 1901; *A. vesiculata* Penard, 1901.

A. pontica Valkanov, 1940

A. salsuginosa Patterson, n. sp.

A. tauryanini Mikrjukov et Patterson, 2000

Actinosphaerium Stein, 1857

Synonyms: *Camptonema* Schaudinn, 1894, *Echinosphoerium* Hovasse, 1965

A. eichhornii (Ehrenberg, 1840) Stein, 1857

Synonyms: *A. arachnoideum* Penard, 1904; *C. nutans* Schaudinn, 1894;

A. nucleofilum Barrett, 1958

Synonym: *E. ikachiensis* Shigenaka, Watanabe et Suzaki, 1980.

The evolution of the actinophryids

The evolutionary relationships of the actinophryid heliozoa among the protists has not previously been resolved (Patterson 1994, 1999). Polyphyly of the taxon Heliozoea has been clearly established (Febvre-Chevalier 1982; Smith and Patterson 1986; Patterson 1988; Mikrjukov 1998, 2000a *inter alia*). There have been some arguments that heliozoa with axonemes terminating on the nucleus (i.e. actinophryids, desmothoracids, taxopodids) should be grouped together and separated from those (centrohelids, gymnosphaerids and dimorphid helioflagellates) with an axoplast or centroplast as a microtubule organizing centre. On the basis of this argument, the former have been grouped (sometimes with the ciliophryids) as the Cryptaxohelida (Febvre-Chevalier and Febvre 1984), or as the Actinophryidea (Karpov 1990) or the Nucleohelea (Cavalier-Smith 1993). As other characters, such as cell topology, organization of mitochondria, extrusomes, microtubule packing pattern, cyst morphology, life cycle, do not suggest that these taxa are closely related (Smith and Patterson 1986), we are of the view that the nuclear termination of axonemes is a homoplasious character (convergence) (Patterson 1999, Mikrjukov 2000a). We do not support an explicit or implicit argument that the affinities of the actinophryids lie with other heliozoa.

Two other proposals as to the affinities of the actinophryids have been discussed: (1) with filose amoebae; or (2) with the helioflagellate *Ciliophrys* and the other pedinellid flagellates (Patterson 1986, 1988).

The evolutionary affinity with filose amoebae was suggested by Trégouboff (1953). The amoebae generally and rhizopods are polyphyletic and are being replaced by a larger number of more restrictively circumscribed groups (Patterson 1999). Two types of amoebae have a gross similarity to the heliozoa - the vampyrellid and nucleariid filose amoebae.

The vampyrellid filose amoebae include *Vampyrella* (Hausmann 1977, Hülsmann 1982) and *Lateromyxa* (Hülsmann 1993, Röpstorf *et al.* 1993). Like actinophryids, they have mitochondria with tubular cristae. They have a number of additional features not found in actinophryids. They contain large electron-dense bodies which probably account for their orange colour. They have elaborate ribosomal arrays, often associated with digestion vacuoles. Vampyrellids have a peculiar mode of feeding which involves perforating the walls of algae and fungi, they produce digestion cysts which lack the actinophryid wall structure, and they do not undergo autogamy. These lack any clear affinity with the heliozoa.

The nucleariids include *Nuclearia*, *Vampyrellidium* and some taxa previously linked to heliozoa such as *Pompholyxophrys* and *Pinaciophora*. Nucleariids have discoidal cristae in the mitochondria, no extrusive organelles, no siliceous elements in the cyst, no axonemes, and nuclear division profiles unlike actinophryids (Mignot and Savoie 1979; Patterson 1983, 1985; Cann 1986; Mikrjukov 1999a, c; Mikrjukov and Mylnikov 2000). Recent molecular data suggests affinities of nucleariids with other lamellicristate taxa (Mikrjukov and Mylnikov 2000, Amaral pers. comm.). No particular character or characters support a relatedness between nucleariids and actinophryids (Patterson 1986).

The suggestion of a phylogenetic link between actinophryids and *Ciliophrys* and other heterotrophic pedinellid helioflagellates has been discussed on several occasions (Davidson 1972, 1982; Patterson and Fenchel 1985; Patterson 1986, 1989). *Ciliophrys* has been regarded by some as a distinct type of heliozoon (Febvre-Chevalier 1985, Siemensma 1991) or as a type of actinophryid (Levine *et al.* 1980, Sleigh *et al.* 1984). *Ciliophrys* is undoubtedly related to the pedinellid helioflagellates. Shared characters include: (1) the common presence of microtubular triad axonemes, (2) the axonemes ending on the nucleus, (3) the axonemes being linked by strands of fibrous material, (4) mitochondria with tubular cristae, (5) homogeneous extrusomes, (6) a single apical flagellum with adjacent barren basal body, (7) basal bodies attaching almost directly to the nucleus; (8) paraxonemal inclusions; (9) tripartite flagellar hairs; and (10) transitional helix or rings below the transitional plate (data on heterotrophic genera of pedinellids from Larsen 1985, Patterson and Fenchel 1985, Pedersen *et al.* 1986, Mylnikov 1989; data on plastidic genera: Swale 1969a, Throndsen 1971, Ostroff and van Valkenburg 1978, Zimmermann *et al.* 1984, Koutoulis *et al.* 1988, Thomsen 1988, Daugbjerg 1996a). Based on these characters, *Ciliophrys,* the other genera of heterotrophic pedinellid helioflagellates (e.g. *Pteridomonas* and *Actinomonas*), and those taxa with plastids (e.g. *Pedinella, Pseudopedinella, Apedinella,* and *Mesopedinella)* form a well circumscribed group of stramenopiles called the pedinellids. We now regard the pedinellids as a paraphyletic group and in line with arguments presented elsewhere (Patterson 1994, 1999) prefer to use this name only in its colloquial sense.

Pedinellids and actinophryids resemble each other in the nuclear termination of the axonemes, in having mitochondria with tubular cristae, and having extrusomes with electron-dense unstructured contents. The pedinellids and actinophryids differ in several major respects. Firstly, the actinophryids have no flagellum nor any flagellated stage in the life cycle. Secondly, the axopodial axonemes in pedinellids and *Ciliophrys* are supported by triads of microtubules and not by a double polygonal spiral as is observed in all actinophryids. Thirdly, the cristae in the mitochondria of *Ciliophrys* and other pedinellids are tubular and not-bleb like; there are fine wisps of material within the cristae and cystalline deposits may be seen in the matrix of the mitochondria. Neither feature has been observed in the actinophryids. Indeed, the mitochondria of actinophryids more closely resemble those of many chrysophytes (*sensu* Hibberd 1976, 1986) than those of *Ciliophrys*. Fourthly, the extrusomes of *Ciliophrys* are smaller than the larger extrusomes of *Actinophrys* (Davidson, 1980; Patterson, unpubl.) but are similar to the smaller ones of *Actinophrys* (Linnenbach *et al.* 1983, Patterson 1986, Mikrjukov 1996a, Fig. 19). This type of extrusomes does not appear to have been recorded in other taxa except for those under consideration here, but large dark homogeneous structures have been observed in *Pseudospora* (Swale 1969b), xanthophytes (Hibberd 1981), *Olisthodiscus* (Leadbeater 1969), gymnosphaerid heliozoa (Mikrjukov unpubl.), etc. The value as phylogenetic markers is therefore debatable. Fifthly, both groups form cysts, but those of actinophryids have a complex envelope including a layer of siliceous artefacts, whilst the cyst envelope of pedinellids is purely organic (Hibberd 1986, Thomsen 1988). No sexuality has been reported in the cysts of pedinellids.

Despite the differences between actinophryids and ciliophryids, the new data on *C. azurina* gives credibility to the argument that ciliophryids and actinophryids are related. *C. azurina* combines features previously thought to be exclusive to the pedinellids (one hairy flagellum held in a “figure of 8”, radiating actinopods which may be withdrawn in swimming cells) and features previously thought to be exclusive of the actinophryids (tapering arms with substantial axonemes, and clumps of condensed material around the inner periphery of the nucleus). We presume that the tapering axopodia have more than 3 microtubules. We conclude that there is a clade that includes some pedinellids and which has tapering axopodia and peripheral heterochromatin as apomorphic. This clade includes *Ciliophrys azurina* and the actinophryid heliozoa (Fig. 24).

Classification of the actinophryids and pedinellids

The only authors to previously use a taxon to house the actinophryids and all pedinellids are Karpov (1990) and Kussakin and Drozdov (1998). Both exploit a ‘heterokont phylum’ the Pedinellomorpha. However,

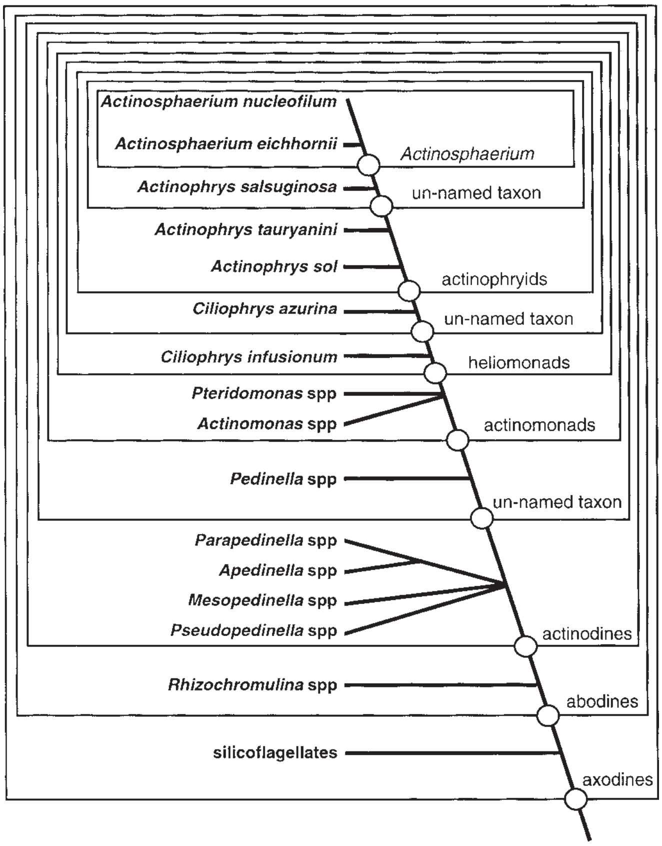


Fig. 24. Suggested relationships among the axodines with names for monophyletic and holophyletic groups, the taxa contain all of the descendents from the circle adjancet to the clade name. For details see the text

they include other heliozoa (desmothoracids and taxopodids) with the group. We do not regard these types of heliozoa as being related (Smith and Patterson 1986) and so regard this taxon as mis-conceived, and not identical to the clade which includes pedinellids and actinophryids.

We hold the view that nomenclature will be made less ambiguous is we introduce a new clade name as a new clade is identified. We propose the new term “actinodines” to refer to the pedinellids (including the ciliophryids) and actinophryids. Within this is a further clade with the synapomorphy of microtubule supported axopodia arranged with radial symmetry and which we refer to as the heliomonads. Our current hypothesis as to the interrelationships among the actinodines is shown in Figure 24.

Affinities of the actinodines

The phototrophic pedinellid genera have traditionally considered as a taxon of various rank within the (strict) chrysophytes (Pascher 1910, Christensen 1980, Lee 1980, Zimmermann *et al.* 1984, Cavalier-Smith 1986), or as a group with a more distant affinity to the strict chrysophytes (Hibberd 1976, 1986; Cavalier-Smith *et al.* 1995; Moestrup 1995). This latter view is based on a more sophisticated and defensible argument that the pedinellids differ from ochromonadine chrysophytes by virtue of the number of flagella, absence of rootlet structures such as the rhizoplast or the quadripartite microtubular root system, non-flagellar axonemes, number of plastids, absence of photosensory apparatus (stigma), absence of stomatocysts, etc.

On the basis of molecular and structural comparisons, the pedinellids have been linked with the silicoflagellates and the Rhizochromulinales (Cavalier-Smith *et al.* 1995, Moestrup 1995, O’Kelly and Wujek 1995, Cavalier-Smith and Chao 1996, Medlin *et al.* 1997). All have microtubular axonemes ending on the nucleus. These groups have been united as the axodines by Patterson (1994, 1999), the class Dictyochophyceae (Moestrup 1995, Preisig 1999), and as the class Actinochrysophyceae (Cavalier-Smith *et al.* 1995). Of these, the axodines are conceived as defined by synapomorphy and this concept is unaffected by the inclusion of the actinophryids.

Of the two other axodine groups, *Rhizochromulina marina* is amoeboid and has plastids (Hibberd and Chretiennot-Dinet 1979, O’Kelly and Wujek 1995) and seems to be most closely related to actinodines. Zoospores of *Rhizochromulina* resemble pedinellid cells in (1) the presence of a non-flagellated kinetosome, (2) lacking microtubular kinetosomal rootlets, (3) the position of the helix (or of two rings) underneath the transitional plate of the kinetosomes, (4) the posterior position of the Golgi apparatus. The close relatedness of pedinellids with *Rhizochromulina* is supported by molecular data (Cavalier-Smith *et al.* 1995, Cavalier-Smith and Chao 1996). The microtubules in pseudopods of *Rhizochromulina* are not fixed in number and not gathered in axonemes, and hence we do not consider rhizochromulinids inside the actinodines. We refer to the group (*Rhizochromulina* + actinodines) as the ‘abodines’. The synapomorphy of abodines is the posterior location of the dictyosomes.

Silicoflagellates differ from the abodines because their dictyosomes are located to the sides of the nucleus and not posterior to it; and they have a well developed intracellular siliceous skeleton (Deflandre 1953, van Valkenburg 1971, Moestrup and Thomsen 1990). Like the abodines, they have microtubule-supported pseudopods with axonemes terminating on the nuclear envelope; a double ring below the transverse septum (van

Valkenburg 1980; Moestrup and Thomsen 1990; Moestrup 1992, 1995; O'Kelly 1993). Some pedinellids and silicoflagellates have a flagellar wing supported by a dense paraxial rod. Silicoflagellates have an unusual ring-like structure (perhaps a ring of opaque bodies) outside the axoneme at the level of the distal end of kinetosome.

The Pelagophyceae is the most probable sister taxon to the axodines (Andersen *at al.* 1993, Honda *et al.* 1995, Cavalier-Smith and Chao 1996, Potter *et al*. 1997). The pelagophytes include uniflagellated and coccoid algae. The axodines and the pelagophytes are uniflagellated stramenopiles with two rings inside the basal body, but there is no evidence of microtubular axonemes in the body of the pelagophytes. They probably form the next most proximate group of stramenopiles. Saunders *et al.* (1997) argue that the diatoms are the sister group to this cluster. Honda *et al.* (1995) include *Sulcochrysis* within this territory. Medlin *et al.* (1997) consider diatoms, pelagophytes, silicoflagellates and pedinellids as a separate group of stramenopiles which they call as "reduced flagellar apparatus group"; this group is characterised by (1) a flagellar transitional region with two transitional plates and a small transitional helix (or two rings ?) below the major plate, (2) a flagellar apparatus that lacks microtubular roots, (3) basal bodies positioned on or very near the nucleus, (4) a paraxial rod which is common in some members.

Cavalier-Smith *et al.* (1995) are of the view that the plastidic stramenopiles gave rise to the aplastidic taxa. The most primitive stramenopiles identified in molecular studies are the bicosoecids and oomycetes both of which are heterotrophic (Leipe *et al*. 1994, 1996). The bicosoecids appear to be related to the heterotrophic *Caecitellus* and pseudodendromonads, but whether the two latter taxa form a sister structure to the stramenopiles or fall within the stramenopiles is unclear. Overall, this suggests that the first stramenopiles were heterotrophic. The molecular data do indicate that the pedinellids were derived early in stramenopile evolution. Given that the most likely sister groups to the actinodines, and the sister groups to the axodines contain plastids, it seems probable that actinodines were ancestrally with plastids and subsequently lost them. This point of view is supported by cladistic analysis on ultrastructural data of pedinellids (Daugbjerg 1996b) which suggests that the most primitive pedinellid is a species of *Pseudopedinella*.

There are two recent schemes of conventional classification considering the position of pedinellids and related taxa. That of Moestrup (1995) is:

Class Dictyochophyceae Silva, 1982
  Order Pedinellales Zimmermann *et al.*, 1984
  Order Rhizochromulinales O'Kelly et Wujek, 1995
  Order Dictyochales Haeckel, 1894.

The classification of Cavalier-Smith and co-workers (Cavalier-Smith *et al.* 1995, Cavalier-Smith and Chao 1996) infers that the rhizochromulinids should be regarded as a pedinellid, creates a paraphyletic taxon by segregating the Ciliophryida from the other pedinellids, and intrudes a monotypic taxon:

Superclass Dictyochia Haeckel, 1894, Cavalier-Smith, 1993
  Class Pelagophyceae Andersen et Saunders, 1993
  Class Actinochrysophyceae Cavalier-Smith, 1995
    Subclass Pedinellidae Cavalier-Smith, 1986
      Order Pedinellales
      Order Ciliophryida
      Order Rhizochromulinales
    Subclass Silicophycidae Rothmaler, 1951

We have been unable to emerge with a single scheme of classification which protects familiar groupings and rank for convenience, as well as reflects our understanding of relationships. We present two schemes. The first supported by one of us (KM) and reflects traditional conventions. The second is supported by the other author (DP) and reflects a desire to create a phylogenetic classification using conventions discussed elsewhere (Patterson 1994, 1999). The defining attributes of new taxonomic concepts are:

Abodines: Axodines with posterior dictyosomes

Actinodines: Abodines with non-flagellar axonemes terminating on the nucleus and arranged with radial symmetry

Actinomonads: Actinodines without plastids

Heliomonads: Actinomonads with radial axopodia

Classification 1

Superclass Dictyochia Haeckel, 1894
  Class Pelagophyceae Andersen and Sanders, 1993
  Class Actinochrysophyceae Cavalier-Smith, 1995
    Subclass Silicophycidae Rothmaler, 1951
    Subclass Abaxodinae subcl. n.
 Superorder Rhizochromulinea O'Kelly and Wujek, 1995
 Superorder Actinodinea superord. n.
  Order Pedinellales Zimmermann *et al.,* 1984
  Order Ciliophryida Febvre-Chevalier, 1985
  Order Actinophryida Hartmann, 1913

Classification 2

- Axodines
    - Silicoflagellates
    - Abodines
        - Rhizochromulinids
        - Actinodines
            - *Pseudopedinella* sm
            - *Mesopedinella* sm
            - *Apedinella* sm
            - *Parapedinella* sm
            - Un-named taxon
                - *Pedinella* sm
                - Actinomonads
                    - *Pteridomonas*
                    - *Actinomonas*
                    - Heliomonads
                        - Ciliophrys
                        - Actinophryids
                        - *Actinophrys*
                            - *Actinophrys sol*
                            - *Actinophrys tauryanini*
                            - *Actinophrys salsuginosa*
                            - *Actinosphaerium*
                                - *Actinosphaerium eichhornii*
                                - *Actinosphaerium nucleofilum*

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