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ORDER SLOPALINIDA

Patterson, 1989

BY

B. L. J. DELVINQUIER and DAVID J. PATTERSON

Slopalines embrace seven genera of endocommensal flagellates occurring mostly in the posterior regions of the intestines of cold-blooded vertebrates. Most opalines have been described from Anura. The order comprises two families, the Opalinidae and the Proteromonadidae. The Opalinidae consist of five genera and about 400 species which have been described in amphibia, reptiles, freshwater and marine fish, and in a few invertebrates. The group has been most recently reviewed by Delvinquier and Patterson (1993).

The Proteromonadidae consist of two genera. There is only a few species of *Karotomorpha* (from amphibia). There are several species of *Proteromonas* occurring mainly in reptiles and more rarely in amphibia and mammals. The opalines have been more extensively studied than proteromonads (Kulda and Nohyková, 1978; Metcalf, 1923, 1940; Wessenberg, 1978; Delvinquier and Patterson, 1993). The relationships among genera of slopalines is illustrated in Fig. 1.

The surface of opalines is covered with rows (kineties) of flagella. As a result, they were first considered to be ciliates, and Metcalf (1923, 1940) referred to them as Protociliates; however, they lack a number of the pecularities of the ciliates such as dimorphic nuclei and were transferred from the ciliates to the Sarcomastigophora by Corliss and Balamuth (1963). With the recognition of the polyphyly of the Sarcomastigophora, they have been segregated as an independant group. Opalines and proteromonads are placed together because they have a similar and distinctive structure of the flagellum and because in both families the cell surface is underlain by a cortex of evenly spaced microtubules. In the opalines and *Karotomorpha*, these take the form of ribbons. In *Proteromonas*,

the microtubules are single. On the basis of these ultrastructural similarities proteromonads and opalines were combined to form the slopalines (see Delvinquier and Patterson, 1993).

Proteromonas has tripartite hairs attached to the posterior end of the body which together with double-gyred transitional helices in the flagellar transitional region indicate an affinity with the chrysophyte algae and other stramenopiles (Delvinquier and Patterson, 1993).

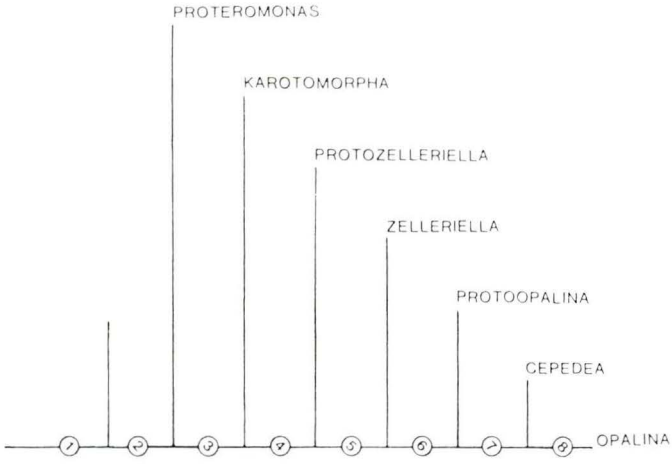

Fig. 1. Proposed relationships within the slopalines. Numbered items indicate proposed evolutionary steps. (1) Origins of stramenopiles from biflagellated tubulocristate protists by appearance of tripartite hairs and derivation of a subset with a double-gyred transitional helix. (2) Tripartite hairs reduced to body surface; evenly spaced microtubules underlie cell surface. (3) Complete loss of tripartite hairs, cortical microtubules transform into ribbons, and number of flagella increased to four. (4) Kineties begin to develop; cells become binucleated, enlarge, and become flattened. (5) Kineties become continuous; falx distinctive and marginal. (6) Falx becomes axial. (7) Nuclei proliferate and become smaller. (8) Return to marginal falx.

Opalines are thought to have evolved in Anura from Gondwanaland prior to the break-up of the continent in late Jurassic (Delvinquier and Patterson, 1993). They now have a world-wide distribution.

The opalines have a life cycle that is linked to that of the host in those species that have been studied. Our understanding is based on opalines that live in Anura. Opalines grow and divide along and across the kineties while in their host. Cysts are produced during the sexual activity of the host and are released into water. Tadpoles are infected by feeding on the cysts. Excysting opalines are destined to produce gametes. Conjugation occurs between dissimilar gametes (Wessenberg, 1961) (Fig. 2).

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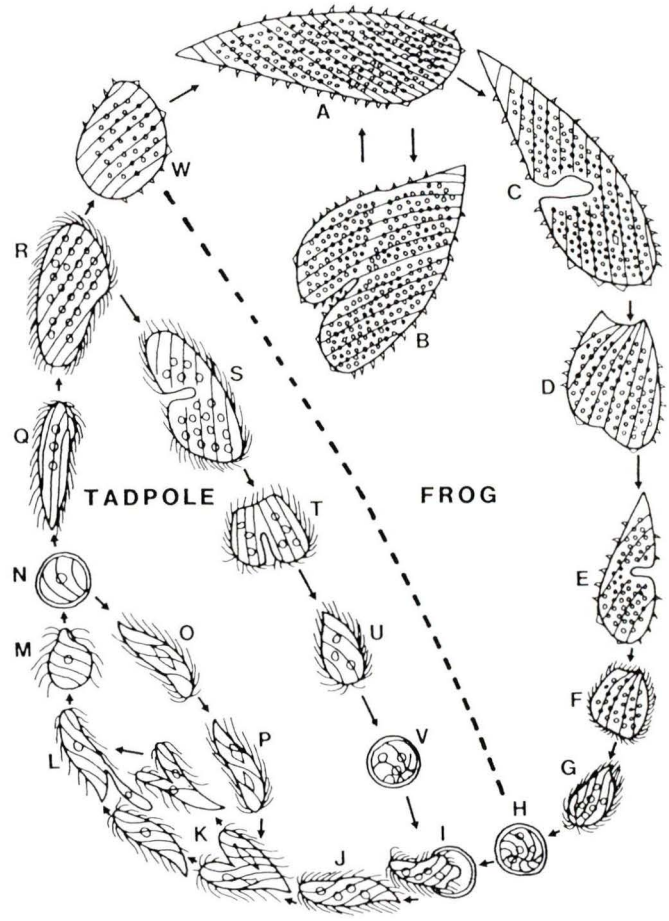


Fig. 2. Life cycle of *Opalina* proposed by Wessenberg. A,B. Growth and division of trophonts. C-G. Palintomy (divisions) leading to the production of small cells which form cysts (H). Cysts are shed from the frog and excyst (I) upon ingestion by tadpoles. The cells which excyst (K) will produce anisogametes (K), which fuse (L) to produce zygocysts (N). The sexual cycle may be repeated (O,P), or, alternatively, cells may grow and divide with or without cyst formation.

Proteromonads are small protists, 10-25 μm in length, mononucleated with apically inserting flagella. *Proteromonas* is biflagellated with a

smooth cortex and material adhering to its posterior end. *Karotomorpha* is quadriflagellated with a pleated or striated cortex. The two genera can be distinguished on the basis of the number of flagella.

Opalines are usually greater than 100 µm in length and are binucleated or multinucleated. Nuclei are monomorphic. All opalines have flagella arranged as kineties. The kineties arise anteriorly at the **falx**, which is a morphogenetic center.

Opaline genera are distinguished by the number of nuclei and by the orientation of the falx relative to the antero-posterior axis of the cell (Fig. 3). *Protozelleriella*, *Zelleriella,* and *Protoopalina* typically have two nuclei with a diameter greater than 10 µm, but a few exceptions are reported. *Opalina* and *Cepedea* are multinucleated with nuclei typically less than 10 µm in diameter. A long and thin falx extends along the margin and mostly lies transverse to the antero-posterior axis of the cell in *Protozelleriella*, *Zelleriella,* and *Opalina*. This is referred to as a marginal falx. Alternatively, a short and broad falx lies mostly along the antero-posterior axis of the cell in *Protoopalina* and *Cepedea* and is referred to as axial.

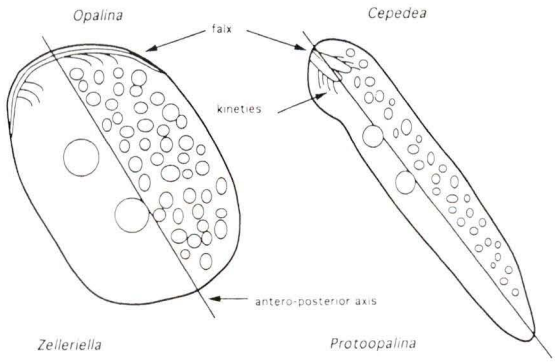
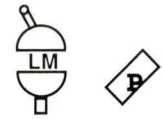


Fig. 3. Orientation of falx: marginal (left) and axial (right) for the four major genera.

Standard staining techniques to be used for slopalines include Bodian's protargol, silver nitrate impregnation, or Fernandez-Galiano's ammoniacal silver impregnation.

Opaline distribution in hosts is reviewed in Delvinquier and Patterson (1993).

KEY TO THE FAMILIES AND GENERA



1. Mononucleated, flagella not arranged as kineties ..... Family **Proteromonadidae**..... 2
1'. Multinucleated, flagella arranged as kineties.. .....Family **Opalinidae** 3

2. Two anterior flagella..... ***Proteromonas***
2'. Four anterior flagella..... ***Karotomorpha***

3. Binucleated..... 4
3' Multinucleated..... 6

4. With long, thin, marginal falx extending along the margin and mostly transverse to the antero-posterior axis of the cell..... 5
4' With short, broad axial falx running mostly in the direction of the antero-posterior axis of the cell..... ***Protoopalina****

5. Peripheral hyaline margin devoid of flagella ..... ***Protozelleriella***
5'. Body evenly covered with kineties ..... ***Zelleriella***

6. With long, thin, marginal falx transverse to the anteroposterior axis of the cell; body evenly covered with kineties..... ***Opalina***
6'. With short, broad axial falx parallel to the anteroposterior axis of the cell .... ***.Cepedea***

* A few species of *Protoopalina* have been described with four, six, or eight nuclei. All of these species have large and aligned nuclei by which they can be distinguished (Delvinquier and Patterson, 1993).

FAMILY PROTEROMONADIDAE Grassé, 1952

Genus ***Proteromonas*** Kunstler, 1883

Endocommensal flagellates up to about 25 µm in length, with 2 apical flagella. Surface not markedly ridged, but posterior end may carry fine hairs. A large dictyosomal complex is located near the anterior of the cell. Complex life cycle involves a cyst in which multiple divisions occur. Records mainly from the guts of lizards but also

reported from caudate amphibia and mammals. Four species, e.g. *Proteromonas lacertaeviridis.*

Fig. 4. *Proteromonas lacertaeviridis.*

Genus ***Karotomorpha*** Travis, 1934

Small (12-16 µm) flagellates with 4 long apical flagella. Single nucleus, body surface ridged. Massive dictyosomal complex (parabasal apparatus) is located in the anterior part of the cell. Reported from the guts of Australian, North American, and European amphibia. Several species.
TYPE SPECIES: *Karotomorpha bufonis* (Alexeieff, 1916)
TYPE HOST: *Triton, Rana, Amblystoma*

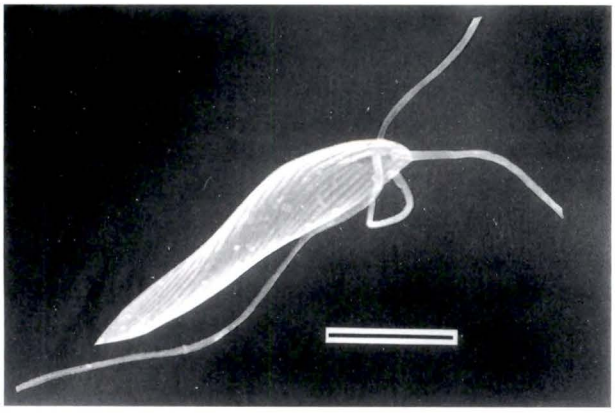


Fig. 5. *Karatomorpha bufonis.* Scale bar=5 µm.

FAMILY OPALINIDAE Claus, 1874

Genus ***Protozelleriella***
Delvinquier, Markus & Passmore, 1991

Binucleated, with a long, thin, marginal falx almost perpendicular to the antero-posterior axis of the cell, with a peripheral, hyaline margin that is devoid of flagella and with a crenulate posterior margin. One report only from bufonid toad in South Africa.
TYPE SPECIES: *Protozelleriella devilliersi* Delvinquier, Markus & Passmore, 1991
TYPE HOST: *Capensibufo rosei*

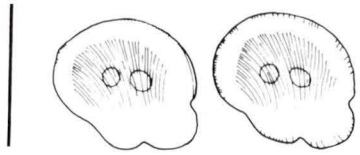


Fig. 6. *Protozelleriella devilliersi.* Scale bar=100 µm.

Genus ***Zelleriella*** Metcalf, 1920

Binucleated, with a long, thin, marginal falx, transverse to the antero-posterior axis of the cell. Kineties cover the body evenly. Mostly Southern Hemisphere distribution. Mostly in anuran amphibia but occasional records for caudate and caecilian amphibia, reptiles, and fish. *Zelleriella antilliensis, Z. falcata, Z. devincki.*
TYPE SPECIES: *Zelleriella antillliensis* (Metcalf, 1914) Metcalf, 1923
TYPE HOST: *Bufo marinus* (as *Bufo aqua*)

Genus ***Protoopalina*** Metcalf, 1918

Binucleated, with a short, broad, axial falx running parallel to the antero-posterior axis of the cell. Kineties cover the body evenly. A few species with 4, 6, or 8 nuclei have been reported, mostly in Asia. Predominantly Old World. Reported mostly from anuran amphibia, but also from caudate amphibia, reptiles, and fish. Examples: *Protoopalina intestinalis, P. mitotica, P. tenuis, P. saturnalis.*
TYPE SPECIES: *Protoopalina intestinalis* (Stein, 1856)
TYPE HOST: *Bombina bombina*

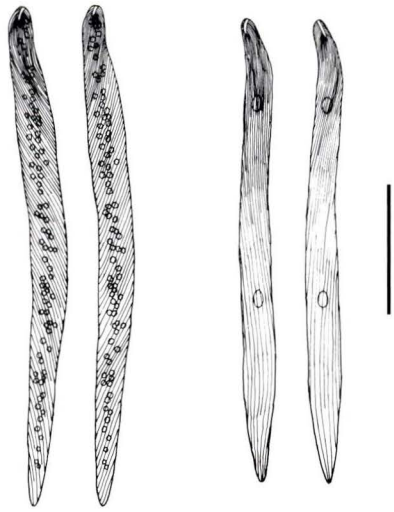


Fig. 7 (left). *Zelleriella falcata*. Fig. 8 (right). *Protoopalina tenuis*. Scale bar=100 µm.

Genus ***Cepedea*** Metcalf, 1920

Multinucleated, with a short, broad, axial falx parallel to the antero-posterior axis of the cell. Kineties cover the body evenly. More common in Old World than in New World and unreported in Australia. Mostly in anuran amphibia, but also in fish and one report in a freshwater snail. *Cepedea dimidiata, C. affinis, C. ciliata.*
TYPE SPECIES: *Cepedea dimidiata* (Stein, 1860) Metcalf, 1923

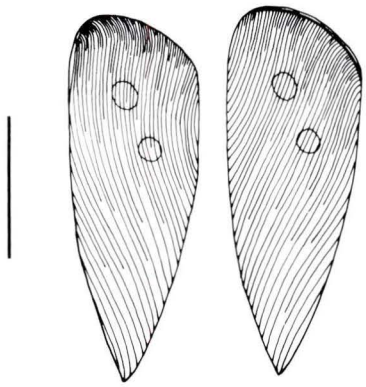


Fig. 9. *Cepedea affinis*. Scale bar=100 µm.

Genus ***Opalina*** Purkinje & Valentin, 1835

Multinucleated, with a long, thin, marginal falx transverse to the antero-posterior axis of the cell. Kineties cover the body evenly. Body shape either elongate or broad. May have a suture line running dorso-ventrally at the back of the cell. Mostly in Asia and in North America and unreported in Australia. Mostly in Anura but occasional records in caudate amphibia, reptiles, and one freshwater mussel. Many species, e.g. *Opalina ranarum, O. obtrigonoidea, O. duquesnei, O. elongata*.
TYPE SPECIES: *Opalina ranarum* (Ehrenberg, 1831) Purkinje & Valenin, 1835
TYPE HOST: Frogs

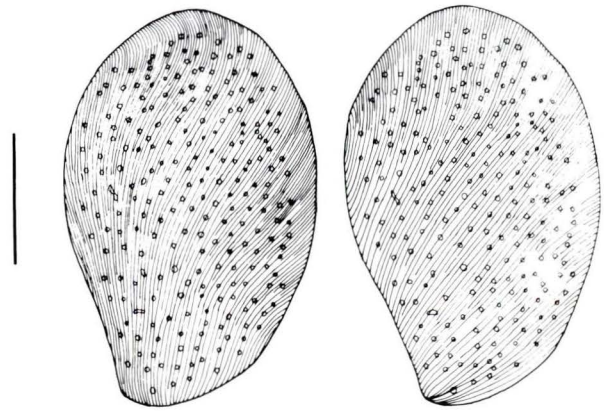


Fig. 10. *Opalina duquesnei*. Scale bar=100 µm.

Fig. 11. *Opalina elongata*. Scale bar=100 µm.

Bezzenbergeria and *Hegneriella* (Opalinidae) are not regarded as valid genera (Delvinquier and Patterson, 1993).

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CLASS SYNUROPHYCEAE Andersen, 1987

ROBERT A. ANDERSEN and HANS R. PREISIG

The Synurophyceae are separated from the Class Chrysophyceae (See Chapter Chrysomonada; Andersen, 1987; Andersen, 1989; Moestrup, 1995; Preisig, 1995). A common and obvious feature of all common genera of Synurophyceae is the presence of bilaterally symmetrical silica scales. The scales remain as fossils in lake sediments, and numerous paleolimnological studies have been based upon the distribution of these fossil scales (e.g. Battarbee et al., 1980; Smol, 1980; Smol et al., 1984a,b; Smol, 1995). The Synurophyceae have a number of significant cellular features that are not found in the Chrysophyceae. These include a unique flagellar apparatus, chlorophyll c_1 only—rather than chlorophylls c_1 and c_2 combined—chloroplasts that usually lack membrane connections to the nucleus, as well as the production of bilaterally symmetrical scales (Andersen, 1987; Andersen, 1989). In the older literature these organisms were classified in the Chrysophyceae, and many papers still refer to these two groups informally as "chrysophytes". The history of these two classes is described in Kristiansen (1995). The Synurophyceae are predominantly freshwater flagellates, occurring in a wide range of habitats from polar regions to the tropics (Kristiansen, 1981, 1986a,b; Cronberg, 1989; Kristiansen et al., 1995). There are only a few reports of Synurophyceae from marine environments, e.g. *Mallomonas epithalattia* Droop, 1955 and *M. subsalina* Conrad & Kufferath, 1954 (Parke and Dixon, 1976) and *Synura uvella* Ehrenberg, 1834 (McAlice, 1975). Although representatives can be found in all types of freshwater, they are most commonly encountered in acidic or circumneutral waters in cold temperate bogs, ponds, and lakes (Kristiansen, 1986b; Asmund and Kristiansen, 1986; Siver 1991; see also papers in Kristiansen and Andersen, 1986; Kristiansen et al., 1989; Sandgren et al., 1995; Kristiansen and Cronberg 1996). Almost two hundred species have been described (e.g. Starmach, 1985; Asmund and Kristiansen, 1986), but there are numerous synonymies caused by independent descriptions using light and electron microscopy.

Very few have studied the biochemistry, cell biology, molecular biology and physiology of the Synurophyceae. The major carbohydrate storage product has not been determined for any member of the Synurophyceae but it is often assumed to be leucosin (Klebs, 1893; Archibald et al., 1963), chrysolaminaran (Quillet, 1955; Beattie et al., 1961; Smestad-Paulsen and Myklestad, 1978) or similar relatively small (dp < 40) ß-1,3 linked glucan. The chloroplast genome of *Synura petersenii* Korshikov, 1929 is only 91.5 kb (Wee et al., 1993), one of the smallest chloroplast genomes known (see Coleman and Goff, 1991). Another member of the class, *Chrysodidymus synuroideus* Prowse, 1962, has a chloroplast genome size of 102 kb (Graham et al., 1993), and these data suggest the chloroplast genome size of the Synurophyceae is quite small compared to other algae and plants. The organization of the plastid genome in *Synura* is scattered rather than as a ring (Coleman, 1985). The mitochondrial genome of *Chrysodidymus synuroideus* is 33 kb (Graham et al., 1993) and the putative mitochondrial genome of *Synura petersenii* is 34.5 kb (Wee et al., 1993); these mitochondrial genomes are comparable in size to those of other