On the freshwater dinoflagellates presently included in the genus *Amphidinium*, with a description of *Prosoaulax* gen. nov.

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*Amphidinium* is a genus of naked dinoflagellates, characterized by a small epicone and a larger hypocone. Presently about 120 species from both freshwater and marine biotopes have been described. The genus has long been known to be polyphyletic, however, and the recent rediscovery of the type species has now allowed for more precise definition of the genus. The new circumscription of the genus leaves the freshwater species without a generic name. A new generic name, *Prosoaulax* gen. nov., is therefore proposed for the first described freshwater species, *Amphidinium lacustre* Stein (1883), and related species. Based on ultrastructural features, notably the very unusual type of eyespot, *Prosoaulax* is considered to be related to a recently recognized, but poorly understood group of dinoflagellates, comprising the marine species *Gymnodinium simplex*, *G. natalense*, *Polarella antarctica*, and some symbionts of marine invertebrates, *G. bei* and *G. linucheae*. It also includes the symbionts of corals (‘zooxanthellae’) belonging to the genus *Symbiodinium*, a genus believed to contain the modern representatives of the Suessiales, an order of dinoflagellates extending back into the Mesozoic.

INTRODUCTION

The generic name *Amphidinium* was created by Claparède & Lachmann (1859), and given to a single species of dinoflagellates, *Amphidinium opeculatum*, discovered in the sea near Bergen, Norway. The description gives the size of the epicone as ‘réduite à l’état d’une plaqué mince, comparable à un opercule’. The illustrations show chloroplast-like structures radiating from a central body, which has been interpreted as a pyrenoid.

The first freshwater species included in *Amphidinium* was found in Lake Wolschauer near Prague by Stein (1883), who gave it the name *A. lacustre*. A formal description of the new species was not given, but Stein provided several drawings accompanied by interpretive legends. *Amphidinium lacustre* resembled its marine relative in having a small epicone, but chloroplasts were not mentioned and the round bodies shown in the illustrations are likely to represent food vacuoles (Caldado et al. 1998).

Since these first findings, about 120 species have been described and referred to *Amphidinium* (Murray & Patterson 2002). All species are naked with a small epicone and a large hypocone, usually with little or no displacement of the two ends of the transverse girdle. Otherwise, however, there is considerable diversity within the genus: whereas some species have a minute epicone, in others the epi- and hypocone differ much less in size. Some species possess chloroplasts, others have so-called cyanelles in the cytoplasm and others again lack photosynthetic organelles altogether and are entirely heterotrophic. Recently, a chloroplast-containing species with little size difference between the epi- and hypocone, *A. cryophilum* G.D. Wedemayer, L.W. Wilcox & L.E. Graham, was shown to be related to and transferred to *Gymnodinium* F. Stein (Daugbjerg et al. 2000), adding to the uncertainty of the circumscription of *Amphidinium*. A more satisfactory circumscription of the genus had been awaiting rediscovery of the type species, however, but this has now been accomplished by Flø Jørgensen et al. (2004). The genus as circumscribed by these authors comprises only species with a minute, irregular, triangular or crescent-shaped epicone. The epicone is deflected to the left and the ventral part of the cingulum is V-shaped. Cells are with or without chloroplasts and all known species are marine.

This definition excludes most of the species currently assigned to *Amphidinium*, among them all the freshwater species.

The genus *Trochodinium* W. Conrad (Conrad 1926, p. 79), regarded as a synonym of *Amphidinium* since Schiller (1932, p. 311) treated its only described species as *A. prismaticum* (W. Conrad) J. Schiller, was proposed for a flagellate found in brackish water, with a minute epicone and a hypocone shaped as an inverted quadrangular pyramid with slightly depressed sides. The absence of a well-defined sulcus, the presence of a plainly visible eyespot, drawn as a red half-moon on one of the concave faces of the hypocone, and the particular shape of the cell, suggesting an unusual cytoskeleton organization (Conrad 1926, pl. 1, figs 11, 12), separate *Trochodinium* from *Amphidinium sensu stricto*. Whereas none of the species known from freshwater is an obvious member of *Trochodinium*, several show features that clearly separate them from this genus (see below).

We have therefore searched the well over 300 proposed generic names of extant dinoflagellates for a likely candidate to accommodate the excluded freshwater species. The search was not successful, however, and we are therefore erecting a

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new genus, *Prosoaulax* gen. nov., based on the first freshwater species described, *A. lacustris*.

In the present paper we have concentrated on the freshwater species presently referred to *Amphidinium*. Whereas some are regarded as synonyms, a few differ so little from the type species that they are transferred to the new genus. Others are not clearly related to the new genus, nor to any other known genus, and determination of their phylogenetic affinities must await study of additional material. Some species described from brackish or marine environments display morphological characteristics similar to those found in species transferred to the new genus [e.g. *A. carunculus* W. Conrad & Kufferath (Conrad & Kufferath 1954, p. 73); *A. coeruleum* W. Conrad (Conrad 1939, p. 10)]; they need to be re-examined to ascertain their affinities.

In the present paper, authors of taxa are spelled according to Brummitt & Powell (1992).

**Prosoaulax** *Calado & Moestrup, gen. nov.*

Dinoflagellata atheca epiconis multo minoribus quam hypoconis (plerumque minus quam parte una tertia); cellulae dorsoventraliter vix aut haud complanatae, longitundine aequantem aut parum longiores quam latiores; cingulum circulare extremis ambobus ad planum idem; stigma si adest stratorum plurium compositum quae particulas lateriformes continent infra radicem microtubularem posita; filum microtubulare pedunculare praesens in pedunculum in latus ventrale epiconi extendans, pedunculo aliquando ad passendum fungenti; amphisma vesiculis aliquot centum pro parte maximima polygonis sed vesicula singulari linearique elongataque in area apicali composita; pusula longa convoluta tubularis.

Atheca dinoflagellates with the epicone much smaller than the hypcone (usually less than one third); cells little flattened dorsoventrally or not at all, usually as long as or little longer than broad; cingulum circular, with both ends at the same level; eyespot, if present, composed of several layers of flat vesicles containing bricklike particles, underlying the longitudinal microtubular root; peduncle microtubular strand present, capable of extending into a peduncle on the ventral side of the epicone, which is at times involved in food uptake; amphisma composed of a few hundreds of mostly polygonal amphisma vesicles, but a linear, elongate vesicle present in the apical area; pusula long convoluted, tube-like structure.

**Type species:** *Prosoaulax lacustris* (F. Stein) Calado & Moestrup, *comb. nov.*, designated here.

**Etymology:** Greek prefix proso-, to the front, and Greek aulax, furrow; referring to the anterior location of the transverse furrow.

The name *Prosoaulax* was coined in an analogous way to *Proualax* Diesing (Diesing 1866, pp. 305, 383), which also referred to the anterior location of the cingulum in the single species, originally described by Perty (1852, p. 162, pl. 7, fig. 14) as *Peridinium corpusculum*. The orientation of dinoflagellate cells was interpreted at the time as the reverse of what it is now, so *Proualax* was actually proposed with the opposite orientation to *Amphidinium* and *Dinophysis* Ehrenberg with respect to the position of the cingulum and could not be applied to the new genus proposed here. Diesing’s genus was hardly ever used and the type species, after being transferred to *Gymnodinium* by Kent (1881, p. 443), was considered unidentifiable with the available data by Kofoid & Swezy (1921) and eventually crept out of freshwater floras.

**Prosoaulax lacustris** (F. Stein) Calado & Moestrup, *comb. nov.*

**Basionym:** *Amphidinium lacustrum* F. Stein (1883, p. 15, pl. 17, figs 21–30).

**Lectotype:** figure 21 in Stein’s (1883) pl. 17 (reproduced here as Fig. 1a) is designated here as the lectotype of the species. However, the morphological variability of populations, some of which is visible in the remainder of Stein’s drawings, must be borne in mind for identification.

Cells are approximately round, slightly flattened dorsoventrally, the epicone accounting for about 25% of the total length. Cells are usually 8–14 µm long and 8–12 µm wide but smaller (down to 6 µm) and larger (up to 16 µm) cells may occur. The epicone is slightly narrower than the hypcone and its top is almost flat, though occasionally convex or conical. The cingulum is deeply marked, without displacement. The sulcus is deep and narrow, widening somewhat towards the posterior end. The nucleus is in the left side of the hypcone. An elongated, greenish-yellow eyespot is present along the right edge of the sulcus. Starved cells are colourless except for the eyespot; recently fed cells have food vacuoles of different sizes and colours, in both epicone and hypcone. Cysts are thick-walled and four-angled. [For further description and a discussion of the taxonomic concept of this species, see Calado et al. (1998).]

The presence or absence of chloroplasts has been discussed repeatedly in this species, and Javornicky (1967) provides compelling evidence that cells may sometimes have parietal, bright yellow chloroplasts. ‘They were very distinct and sharply limited, being unmistakably distinguishable from ingested algae which were also observed; the other specimens of this population were colourless, but morphologically identical’ (Javornicky 1967, p. 55). Woloszynska (1936) found dark yellow chloroplasts in cells from the littoral zone of lakes; from deeper waters the chloroplasts were light yellow, and from depths of 40 m cells lacked chloroplasts.

Chloroplasts can be found in dinoflagellate cells either as stable organelles that grow and divide in coordination with cell growth and division (true chloroplasts), or as temporarily functional portions of ingested photosynthetic organisms (kleptochloroplasts) which are eventually digested, as demonstrated for e.g. *Gymnodinium aeruginosum* F. Stein (Schnepf et al. 1989; Farmer & Roberts 1990). Whereas some of the coloured bodies described inside *P. lacustris*—like cells were undoubtedly food vacuoles in various stages of digestion, others, present in some cells and absent in others, probably represented kleptochloroplasts. However, these temporary photosynthetic organelles may be undistinguishable from true chloroplasts, especially when all cells examined in a population have recently been feeding.

Although several similar species may exist, a number of species descriptions were based on a combination of morphological characters that fall within the variation of a single population and on the presence, and sometimes colour, of chloroplasts, leaving us only with unreliable features to distinguish them. In view of the variability observed in cultured and field populations and the uncertainty about the nature of the chloroplasts reported for some, we were unable to distinguish the following taxa from *P. lacustris*, and we accordingly regard
them as synonyms (figures accompanying the original descriptions have been included in the present article as Figs 2–14):

*Amphidinium elenkinii* Skvortzov (1925, pp. 146, 148, un-numbered text-figures; as ‘Elenkini’); Fig. 2a–d; originally described as having very small, spherical, brown chloroplasts, ‘seldom absent’.

*Amphidinium eucephalum* J. Schiller (1955, p. 23, pl. 1, fig. 6a–c); Fig. 12a–c; described as a probable mixotroph with 8–10 small, very pale greenish-yellow, hardly visible chloroplasts.

*Amphidinium gyrinum* T.M. Harris (1940, p. 18, fig. 5N–S); Fig. 4a–f; cells were originally described as often showing ingested algae, and as differing from *A. elenkinii* in constantly lacking chloroplasts and in having a shorter longitudinal flagellum.

*Amphidinium hyalinum* Entz (1930, p. 207, figs 1, 2); Fig. 5a, b; cells were originally described as often containing up-taken foreign bodies, but no chloroplasts, and as being perhaps identical to *A. larvale* Er. Lindemann. According to Schiller’s (1932, p. 296) interpretation, the species contains chloroplasts, at least temporarily.
Amphidinium inconstans J. Schiller (1955, p. 25, pl. 1, fig. 9a–e); Fig. 14a–e; originally described as a holozoic species without chloroplasts.

Amphidinium larvale Er. Lindemann (1928a, p. 291, figs 1–3); Fig. 8a–c; originally described as a holozoic species without chloroplasts.

Amphidinium sauerzopfi J. Schiller (1955, p. 22, pl. 1, fig. 4a–c, as ‘Sauerzopf’); Fig. 11a–c; described with two yellowish-brown chloroplasts. However, in the German text (although not in the Latin description), Schiller (1955) questions the nature of these cytoplasmic bodies: ‘oder sind es aufgenommene Chrysomonaden?’.

Amphidinium skujae Christen (1958, p. 69, fig. 2); Fig. 3; originally described with 2–5 greenish-yellow chloroplasts, which, however, ‘can sometimes be absent or very reduced’.

Amphidinium tatrae Woloszynska [1936 (probably 1937; see list of references below), p. 190, pl. 9, fig. 5]; Fig. 6; in the original text, the chloroplast colour was noted to vary between dark yellow in cells from the littoral zone and light yellow in cells from deeper waters (see also A. tatrae forma achromaticum Woloszynska).

Amphidinium tatrae Woloszynska forma achromaticum Woloszynska [1936 (probably 1937; see list of references below), p. 191, pl. 9, fig. 6]; Fig. 7; the name was proposed for chloroplast-lacking cells of A. tatrae collected from about 40 m depth.

Amphidinium turicense Huber-Pestalozzi (1950, p. 104, fig. 80); Fig. 9a–c; originally described with large brownish-yellow bodies, which were ‘perhaps chloroplasts’.

Gymnodinium stagnale J. Schiller (1955, p. 29, pl. 2, fig. 15a–d); Fig. 13a–d; described as having two brown bodies in the hypocone, possibly chloroplasts, but more likely to be food particles.

The following two taxa were described with features that would warrant them recognition as different species. However, further work is needed to establish the stability of critical characters.

Amphidinium lateum Skuja (1939, p. 148, pl. 10, figs 18–20); Fig. 10a–c; described with 3–4 parietal, discoid, golden-yellow chloroplasts. Although Skuja did not state how many cells were examined, he reported them from a single location (a ditch in a wood) in May 1938. More extended observations or, preferably, unialgal culture studies are needed to ascertain whether the organism possesses true chloroplasts and therefore represents a different species.

Amphidinium tenagodes T.M. Harris (1940, p. 20, fig. 5J–M); Fig. 15a–d; originally described with two large chloroplasts, very pale yellow, and granules resembling ingested food. It differs in size (17 μm long), in being a slow swimmer, and in sometimes having the cingulum in the middle of the cell. Its identity is very uncertain.

**Prosoaulax multiplex** (J. Schiller) Calado & Moestrup, comb. nov.

Fig. 18a–e

BASIONYM: Amphidinium multiplex J. Schiller (1955, p. 23, fig. 7a–e).

Cells were described by Schiller (1955) as 8–10 μm long and 6.5 μm wide. The epicone was flat or cap-shaped. The cells had about eight chloroplasts, but these were difficult to see and sometimes no chloroplasts were visible. This species appears to differ from P. lacustris in that the sulcus extends onto the epicone.

The following species could not be separated from P. multiplex and are therefore considered synonyms:

Amphidinium lohammarii Skuja (1956, p. 354, pl. 61, figs 18–22, as ‘Lohammari’); Fig. 19a–f; cells were described by...
Skuja (1956) as 10–13 µm long, in other words overlapping in size with *P. multiplex* described the year before but not cited by Skuja. Skuja described the cells of *A. lohammarii* to be colourless, devoid of chloroplasts, but with a few large, strongly refringent granules. Ettl’s material from Denmark (Ettl 1980), identified by him as *A. elenkinii*, agrees with *P. multiplex* in the sulcus extending onto the epicone. It is, however, stated to be 15–18 µm long, i.e. longer than both *P. multiplex* and *A. lohammarii*, and the anterior end is notably flat. Its identity is presently obscure.

*Amphidinium vorax* J. Schiller [1955, p. 26, fig. 11a–c (not 11a–e, as stated in the description)]; Fig. 16a–c; Schiller (1955) described this taxon as 14–18 µm long and 14 µm wide. The anterior part of the cingulum is somewhat extended anteriorly (Fig. 16a, b). It was described by Schiller to be without chloroplasts, and feeding on cyanophytes and chrysophytes was observed. It is tentatively placed as a synonym of *P. multiplex*, although it may represent a separate species.

*Prosoaulax viridis* (J. Schiller) Calado & Moestrup, comb. nov.

Fig. 17a, b

Basionym: *Amphidinium viride* J. Schiller (1955, p. 21, fig. 2).

Schiller described this taxon as 12–14 µm long, and 10–11 µm wide. The epicone was 4 µm long, but very narrow and semicircular, sharply set from the hyposoma. Cells were described as having six green chloroplasts. This species has been recorded only from the original description.

**DISCUSSION**

**Concept of the genus Amphidinium Claparède & J. Lachmann**

Claparède & Lachmann (1859) described the type species of *Amphidinium*, *A. operculatum*, as having a minute epicone (almost like an operculum) and a much larger hypocone. In the type species of *Gymnodinium*, *G. fuscum* (Ehrenberg) F. Stein, the epi- and hypocones are of almost the same size. Many new species of naked dinoflagellates were discovered in the latter part of the 19th and the early part of the 20th centuries, and in some species the size difference between epi- and hypocone was somewhere in the extremes found in *A. operculatum* and *G. fuscum*. This led to uncertainty about the difference between *Gymnodinium* and *Amphidinium*. Thus Geitler (1924) described the new species *G. amphidinioides*, in which the epicone is distinctly smaller than the hypocone (about one third of the hypocone), then Schiller (1932) transferred it to *Amphidinium*. Schiller’s concept of *Amphidinium* included only cells in which the cingulum was located ‘near the front end and showing no or little displacement’; however, this did not prevent him from including *A. britannicum* (Herdman) M. Lebour in *Amphidinium*, although this species has very strong displacement of the cingulum!

Huber-Pestalozzi (1950) attempted to be more precise and listed the epicone of *Amphidinium* as one third–one fifth the length of the hypocone. Popovský & Pfiester (1990) described it as less than one third the length of the cell.

The new circumscription of *Amphidinium* by Flø Jørgensen *et al.* (2004), based on modern techniques, brings us back to the beginning once more: the epicone of *Amphidinium* is again described as ‘minute’, and ‘irregular, triangular or crescent-shaped’. This very narrow generic concept is supported by the techniques used by the authors, but it excludes a very large number of species from the genus, including all known freshwater species. Below we list the freshwater species that cannot to our knowledge be included in any existing genus of dinoflagellates.

**Blue-grey or blue-green species presently included in Amphidinium**

The freshwater species of *Amphidinium* described as having so-called cyanelles, or blue-grey or blue-green chloroplasts, do not belong in *Amphidinium sensu stricto*. Their phylogenetic affinities are uncertain and require additional studies. They include:

*Amphidinium amphidinioides* (Geitler) J. Schiller (1932, p. 278); basionym: *Gymnodinium amphidinioides* Geitler (1924, p. 110, figs a–f); homotypic synonym: *Amphidinium geitleri* Huber-Pestalozzi (1950, p. 105, nom. illeg. (superfluous substitute name)).

*Amphidinium bidentatum* J. Schiller (1955, p. 21, pl. 1, fig. 3).

*Amphidinium bourrelyi* Wawrik (1983, p. 790, fig. 7e, f).

*Amphidinium caerulescens* J. Schiller (1955, p. 27, pl. 1, fig. 12a, b).

*Amphidinium glaucum* W. Conrad (1926, p. 75, pl. 1, figs 3–5); brackish water.

*Amphidinium lacunarium* Skuja (1964, p. 347, pl. 66, figs 28, 29).

*Amphidinium oculatum* J. Schiller (1955, p. 22, pl. 3, fig. 5a, b).

*Amphidinium phthartum* Skuja (1939, p. 149, pl. 10, figs 21–24).

*Amphidinium vigrense* Woloszynska (1925, pp. 3, 8, fig. 1).

**Invalidly described taxa**

The two taxa *A. glaucovirescens* J. Schiller (1955, p. 25, pl. 1, fig. 10a–c) and *A. obliquum* J. Schiller (1955, p. 24, pl. 1, fig. 8) were described by Schiller as preliminary (*ad interd.; ad interd.*, respectively) and the names are therefore invalid according to the International Code of Botanical Nomenclature (Greuter *et al.* 2000, Art. 34.1).

**Other freshwater taxa of uncertain affinities**

*Amphidinium achronomaticum* Skvortzov (1958, p. 194, pl. 5, figs 39, 40) was described as having only one flagellum and no cingular constriction. In a later key to species of *Amphidinium* (Skvortzov 1968) it was distinguished from another colourless species by having ‘the girdle in the posterior part of the cell’. It is not clear why it was described as a dinoflagellate.
Amphidinium aeschrum T.M. Harris (1940, p. 18, fig. 6A–F).

Amphidinium alinii Skvortzov (1958, p. 195, pl. 6, fig. 59).

Amphidinium coprosum Baumeister (1943, p. 337, fig. 3b, c).

Amphidinium kesslitzii J. Schiller (1928, p. 135, pl. 5, fig. 12 plus text-fig. 11, as ‘Kesslitzii’); recorded also from freshwater by Bicudo & Skvortzov (1970).

Amphidinium kesslitzii var. sanctipaulense C.E.M. Bicudo & Skvortzov (1970, p. 12, fig. 7).

Amphidinium lefevrei Skvortzov (1968, p. 83, pl. 1, fig. 6, as ‘lefevreii’).

Amphidinium mucicola W. Conrad (1941, p. 1, figs A–E, as ‘mucicolum’) has a strongly sigmoid sulcus somewhat resembling that of Cochlodinium F. Schütt. Its phylogenetic affinities are obscure.

Amphidinium nasutum Skvortzov (1958, p. 194, pl. 6, ‘fig. 65’); although there is no fig. marked 65 in pl. 6 of Skvortzov (1958), it is probably the unnumbered drawing between figs 46 and 52 that is fairly similar to the figure illustrating the same taxon in Skvortzov (1968, pl. 1, fig. 7).

Amphidinium obtusum Er. Lindemann (1919, p. 218, text-figs 4, 5); although this was included in some freshwater floras (e.g. Huber-Pestalozzi 1950; Starmach 1974), it was discovered in brackish water together with marine species. The two cell portions delimited by the cingulum differ greatly in size, but the sulcus was not observed, making cell orientation doubtful. Lindemann (1928b, p. 71) later transferred the species to a new genus, Lissiella Er. Lindemann. The organism has not been rediscovered.

Amphidinium ovoideum (Lemmermann) Lemmermann (1900, p. 115); basionym: Prorocentrum ovoideum Lemmermann (1896, p. 147, figs 1–3); recorded also from freshwater by Bicudo & Skvortzov (1970).

Amphidinium pusillum J. Schiller (1955, p. 20, pl. 1, fig. 1a, b).

Amphidinium radiatum Javornický (1957, p. 260, fig. 7b, d).

Amphidinium roseolum (Schmarda) J. Schiller (1932, p. 312); basionym: Glenodinium roseolum Schmarda (1854, pp. 12, 24, pl. 1, fig. 9); although reported from an area of saline lakes (‘Natron Seen’), the organism was originally found with other freshwater organisms in a temporary pond left after a flood of the Nile. The species is illustrated by two drawings, showing cells with two portions of different size separated by a cingulum. Neither a sulcus nor a sulcal flagellum were seen. Contrary to Schiller’s (1932) conclusion that the organism belongs in Amphidinium, the presence of an eyespot in the smaller part of the cell suggests a Kiatodinium Fott, possibly related to K. vorticella (F. Stein) Fott ex A.R. Loeblich.

Amphidinium ruttneri J. Schiller (1955, p. 28, pl. 2, fig. 13a–c).

Amphidinium schilleri Skvortzov (1968, p. 82, pl. 1, fig. 4).

Amphidinium sphagnicola W. Conrad (1943, p. 2, text fig. 1, pl. 1, fig. 3, as ‘sphagnicolum’).

Amphidinium vernale Skvortzov (1946, p. 13, pl. 1, figs 1, 2); this was described as a subglobose organism 19–20 μm long. In its general shape it suggests a large, slightly deformed cell of a Prosoaulax species. However, the sulcus was described as indistinct, making reliable identification impossible.

Gymnodinium raram Litvinenko (1963); non vidimos – fide Matvienko & Litvinenko (1977, pp. 241, 242); this was described as a dorsoventrally constricted organism with a lid-like, broadly rounded epicone. Although its general shape is reminiscent of Prosoaulax, the presence of a bright-red, horse-shoe-shaped eyespot suggests otherwise. The examined specimens had two longitudinal flagella and probably represented planozygotes.

Phylogeny of Prosoaulax

Prosoaulax lacustris was studied in detail by Calado et al. (1998, as A. lacustre) by using transmission electron microscopy of series of thin sections. Two of the most unusual features of the cell were the presence of an elongate apical amphiesmal vesicle, bearing a row of knob-like structures, and the structure of the eyespot. The elongate, apical amphiesmal vesicle of P. lacustris is an unusual feature that may be phylogenetically significant, but it is difficult to observe.

The eyespot was found to comprise a stack of extraplastidial cisternae with brick-like material, a very unusual type of eyespot so far known only in a few dinoflagellates. It was recognized as an eyespot by its appearance in the light microscope and also by its location in the cell, associated with the ventral, longitudinal flagellar root [LMR; r1 according to Moestrup (2000)]. Several types of eyespots are presently known in dinoflagellates, always associated with this particular flagellar root.

An eyespot containing brick-like material is so unusual that it is likely to indicate phylogenetic relationship between the organisms with such a feature. These species presently include the marine planktonic Polarella glacialis Montresor, G. Proccinni & D.K. Stoecker (Montresor et al. 1999), known from both the Arctic and the Antarctic, the marine symbiont Gymnodinium linucheae Trench & L.-V. Thinh (a symbiont in the jellyfish Linuche Eschscholtzi) (Trench & Thinh 1995), and the marine benthic species G. natalense T. Horigucli & Pienaar (Horigucli & Pienaar 1994a, b). DNA-sequencing has confirmed that P. glacialis and G. linucheae are indeed phylogenetically related to each other, but only more distantly related to Gymnodinium sensu stricto (as defined by Daugbjerg et al. 2000) and Amphidinium sensu stricto. We have no DNA data on Prosoaulax lacustris and so, on several occasions during 2003, we visited the pond in Denmark where the species occurred in 1995 (Calado et al. 1998). However, Prosoaulax was not refound in the pond, which showed signs of having become polluted, being very heavily dominated by chlorococcalean algae; by contrast, in 1995 it contained a diversity of flagellates with very few green algae. Attempts to obtain material of Prosoaulax for DNA sequencing therefore had to be discontinued for lack of the organism.

The ultrastructural study by Calado et al. (1998) showed a number of differences between P. lacustris and what is now
considered to be the true *Amphidinium*, represented by *A. carterae* Hulburt (Dodge & Crawford 1968) and *A. rynchocoeum* Ansimová (Farmer & Roberts 1989), a probable syn-onym of *A. carterae* or *A. massartii* Biecheler (Murray et al. 2004). The most notable difference lies in the construction of the pusule system, which in *A. rynchocoeum* is a group of large cisternae that empty directly into each of the flagellar canals, whereas in *P. lacustris* a single long tube-like structure opens into one of the flagellar canals; the tube undergoes con-siderable coiling distally and takes up a substantial part of the cell’s cytoplasm.

The data presently available suggest that *Prosoaulax* forms a natural group together with the other species possessing a brick-containing eyespot, plus *G. simplex* (Lohmann) Kofoid & Swezy, recently examined with DNA-sequencing, and a few more species presently included in *Gymnodinium* (Saldarriaga et al. 2001; Montresor et al. 2003). The group also comprises *Symbiodinium* Freudenthal, the main genus of ‘zooxanthellae’ in corals. In phylogenetic trees based on DNA sequences, the group is not particularly closely related to *Amphidinium sensu stricto* (Daugbjerg et al. 2000; Saldarriaga et al. 2001). Fensome et al. (1993) included the family Symbiodiniaceae in the order Suessiales, an order which previously comprised only fossil species. The characteristic feature of the order is the presence of 7–10 latitudinal series of am-phisomal vesicles. Very interestingly, *P. lacustris* and the mo-tile cells of *Symbiodinium* share the presence of an anterior, very narrow amphiesmal vesicle (or row of vesicles) on the cell (Loeblich & Sherley 1979; Fensome et al. 1993, mis-named ‘acrobase’). If the idea of a phylogenetic relationship holds up, all these genera may belong in the Suessiales.

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