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Fine-structural characterization and phylogeny of *Peridinium polonicum*, type species of the recently described genus *Naiadinium* (Dinophyceae)

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Abstract

Peridinium polonicum is a freshwater peridinioid with an unusual tabulation that includes one or two anterior intercalary plates in the mid-dorsal axis, and in such a low position that it seems inset in precingular Plate 4. Although the species has been classified in both *Peridinium* and *Peridiniopsis*, evidence from nucleotide sequences consistently shows that its closest relatives are within the *Scrippsiella* group. The genus *Naiadinium* Carty has been recently described with *P. polonicum* as its type species. However, *Naiadinium* was separated from other peridinioids only on the basis of shape and plate arrangements and these characters do not allow reliable determination of its closest phylogenetic relatives. Serial section fine-structural analysis revealed the presence of a small peduncle supported by a conspicuous microtubular basket that extended far into the cell; a complex pusular system that included a collecting chamber from which about 70 pusular tubes radiated; a flagellar apparatus with general peridinioid characters but with an unusually large distance of nearly 700 nm between basal bodies. An ITS1-5.8S-ITS2 rDNA-based phylogenetic analysis grouped, with high statistical support, *Naiadinium polonicum* with three species currently placed in *Scrippsiella*, viz. *S. irregularis*, *S. precaria* and *S. ramonii*.

Keywords: ITS rDNA; *Naiadinium polonicum*; Phylogeny; Pusule; Scrippsiella; Ultrastructure

Introduction

Peridinium polonicum was described from freshwater lakes in Poland and in present day western Ukraine by Wołoszyńska (1916), who illustrated the characteristic shape and tabulation of the species in a series of detailed drawings. Although a single intercalary plate was reported by Wołoszyńska (1916), later authors found this character to be variable within populations of *P. polonicum*, with many (often most) cells showing two intercalary plates (Adachi, 1965; Bourrelly, 1968; Hansen and Flaim, 2007; Hu et al., 2008). When two intercalary plates are present Plate 1a is frequently quite smaller than 2a (Hansen and Flaim, 2007; Hu et al., 2008).

A large similarity has long been noted between the general morphology of *P. polonicum* and a species described from Lake Geneva under the name *Glenodinium gymnodinium* (Penard, 1891). Lindemann (1925) provided compelling evidence that the two names refer to the same species

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by reporting *P. polonicum* as common in the sites studied by Penard. However, the absence of any trace of amphiesmal plates in Penard's (1891) illustrations of *G. gymnodinium* (as implied in the specific epithet) renders the synonymy doubtful and later authors were divided in applying one name or the other to specimens with *polonicum*-like plate arrangements (e.g., Schiller, 1937; Kisselev, 1954; Starmach, 1974; Popovský and Pfiester, 1990). The synonymy was extensively discussed by (Huber-Pestalozzi, 1950, p. 169–171), who provided separate entries for the two names.

Thompson (1951) described under the name *Glenodinium gymnodinium* var. *biscutelliforme* a form with two intercalary plates that he illustrated as equal in size. Thompson's variety brings to mind Lefèvre's (1932, p. 179) tabulation complexum–conjunctum of *P. polonicum* and was renamed *Glenodinium gymnodinium* tab. *complexum* by Kisselev (1954).

Peridinium polonicum was transferred to the genus *Peridiniopsis* by Bourrelly (1968), who regarded the presence of two intercalary plates as the result of duplication of the single plate that would, in his view, represent the basic type of tabulation of the species. Discussions on whether *Peridinium* or *Peridiniopsis* would better accommodate the species are made moot by phylogenetic studies, which reveal closer relationships to the *Scrippsiella* group (Gottschling and Soehner, 2013; Logares et al., 2007). The species has recently been transferred to the new genus *Naiadinium* by Carty (2014); *Glenodinium gymnodinium* var. *biscutelliforme* was also transferred as a second species of *Naiadinium* (Carty, 2014).

Whether under one name or the other, peridinioids with *polonicum*-type of shape and plate arrangements attracted practical interest as one of the few freshwater dinoflagellate species reported to produce toxins. Following earlier records of fish mortality (Adachi, 1965), Hashimoto et al. (1968) described an ichthyotoxic agent, which they named glenodinine. Other ichthyotoxins associated with *N. polonicum* were isolated by Oshima et al. (1989), who characterized a suite of toxic compounds called polonicum toxins A, B and C. Mass mortality of rainbow trout in a fish farm in Duraton river, Spain, apparently mediated by an unidentified toxin, was also associated with a bloom of *Naiadinium polonicum* (Roset et al., 2002). Claims that *Naiadinium biscutelliforme* (R.H. Thompson) Carty represents a separate species from *N. polonicum* (Wołoszyńska) Carty and that only *N. biscutelliforme* produces toxins recommend re-examination of purportedly toxic populations (Carty, 2014). However, the description of the full variation from a single intercalary plate, to two very unequal, to two similar plates in the population from which an ichthyotoxin was first identified speaks against such hypotheses (Adachi, 1965).

Modern authors tend to use the epithet *polonicus/a/um* even when formally considering *G. gymnodinium* and *P. polonicum* as synonyms (e.g., Carty, 2014; Popovský and Pfiester, 1990) despite the priority of the epithet *gymnodinium*. The use of *Naiadinium polonicum* when the names of the two species involved are considered synonyms may be desirable as the organism is potentially toxic and therefore of practical importance, and the identity of *P. polonicum* is much clearer than that of *G. gymnodinium*. In addition, the chemical designation of a set of toxins that has been coined after *P. polonicum* loses the connection to their producing organism if the epithet *gymnodinium* is used. Conservation of the name *Peridinium polonicum* against *Glenodinium gymnodinium* would solve the problem while still admitting the use of the latter name by those who do not recognize the synonymy. Such a proposal is being prepared for submission elsewhere.

Naiadinium polonicum is a relatively common species in Europe (e.g., Grigorszky et al., 1998; Hansen and Flaim, 2007; Lefèvre, 1932; Lindemann, 1924; Pandeirada et al., 2013), in North America (e.g., Thompson, 1951; Carty, 1993) and in Asia (Adachi, 1965; Hu et al., 2008). No records are known for Australia or Africa.

In the present work we describe the fine-structural organization of *Naiadinium polonicum* on the basis of observations by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). A phylogenetic analysis based on sequences of the nuclear ribosomal internal transcribed spacers (ITS1 and ITS2) and 5.8 S rDNA has also provided information on the close relatives of the new genus.

Material and Methods

Biological material

The cells used for scanning electron microscopy were collected during a bloom of the species in Bryrup Langsø, Central Jutland, Denmark on 28 July 2011 and in a pond from a farm in Gafanha da Boavista, Ílhavo, Portugal on 11 October 2006. Both net samples were fixed with Lugol's solution. Cells used for transmission electron microscopy were isolated from a net sample collected on the west side of Sankt Jørgens Lake, Copenhagen, Denmark on 5 September 1995. The cells photographed by light microscopy are from Bryrup Langsø, Denmark, 28 July 2011, and from the pond in Gafanha da Boavista, sampled on 8 May 2014. The cysts were found in a culture started from one cell collected on 27 October 2014 in the pond in Gafanha da Boavista.

Light microscopy

Light micrographs of fixed and live cells and cysts were taken with a DP70 Olympus camera (Olympus Corp., Tokyo, Japan) on a Zeiss Axioplan 2 imaging light microscope (Carl Zeiss, Oberkochen, Germany).