Morphological note on the marine diatom
Achnanthes longipes C.Agardh from Japanese material

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Abstract

The fine structure of the marine diatom species Achnanthes longipes is presented. The valve shape is panduriform to linear-lanceolate, with biseriate striae on both valves; the cells have many disc-shaped plastids. This species forms colonies of long chains, usually with more than 10 individuals connected to each other. However, these colonies are not formed by terminal spines or marginal ridges on the ARV. Japanese specimens possess terminal orbiculi. As terminal orbiculi may appear as one of the structural patterns of large and modified cribra, we suggest that both individuals—those forming terminal orbiculi on the ARV and those lacking them—are classified as A. longipes.

Key index words: Achnanthes longipes, marine diatom, morphology, orbiculi, plastid

Introduction

Diatoms are one of the most species-rich groups of algae; more than 10,000 taxa were described by 1964 (e.g. VanLandingham 1967), with many new additions being added since then by subsequent authors. Furthermore, many more groups have also been circumscribed in recent taxonomic investigations, primarily based upon features observed for the first time with the electronic microscope (EM) (e.g. Round et al. 1990, Witkowski et al. 2000, Metzeltin & Lange-Bertalot 2002, Werum & Lange-Bertalot 2004, Metzeltin et al. 2005). The results have yielded many new taxa at different ranks. As a consequence, both specific as well as generic identifications have become difficult using only the light microscopic (LM).

The genus Achnanthes, established by Bory (1822), included monoraphid, epiphytic heterovalvate diatoms (Bory 1822, Boyer 1926-27, Toyoda et al. 2005a, Toyoda et al. 2006). More than 900 taxa, including infra-specific categories, have since been described by a number of researchers (VanLandingham 1967; also see Index Nominum Algarum Bibliographia Phycologica Universalis 'http://ucjeps.berkeley.edu/INA.html'). More recently, the genus has been subdivided into 9 new and separate genera, and with the re-establishment of Achnanthidium Kütz., species now placed in new genera are often referred to as Achnanthes sensu lato (s.l.) (e.g. Round et al. 1990, Bukhtiyarova & Round 1996, Round & Bukhtiyarova 1996, Round & Bukhtiyarova 1996, Round & Basson 1997). Species of Achnanthes occur mainly in marine and brackish waters, with only a few in freshwater; that is, with the exception of species in Achnanthes, those in Achnanthes s.l. are freshwater. Currently, more than 100 taxa have been classified in the new or re-established genera separated from Achnanthes (cf. Potapova & Ponader 2004). However, some of the species in Achnanthes remain unknown for many of their features because of the lack of authentic specimens or figures. In the present study we describe the fine-
Materials and Methods

A large quantity of specimens from a corrugated sheet used for cultivate the giant abalone (*Nordotis discusa*) and a horned turban (*Batillus cornutus*) were obtained. Details are as follows (The numbers of the samples refer to the private collection of K. Toyoda as KT* and H. Suzuki as S*):


Living cells were observed using LM and Confocal Laser Scanning Microscopy (CLSM "OLYMPUS-FLUOVIEW BX50") to determine plastid morphology using auto-fluorescence of plastids under ultraviolet light. As species of *Achnanthes* have different structures to their valves in each frustule (i.e. they are heterovalvate), to avoid the possibility of mis-identification based on single valves only, both the raphid and araphid valves of the same frustule were examined using a "double-faced slide", made by mounting samples in between two cover-slips "MGK-S" (Matsunami Glass Industry, Ltd., Osaka, Japan). For scanning electronic microscope (SEM) observations, the samples were cleaned of organic matter with the breaching method (Nagumo 1995). Observations were first made with a "PHILIPS XL-30 FEG". Valve terminology follows Anonymous (1975), Ross *et al.* (1979) and Cox & Ross (1981), with additional terms from Toyoda *et al.* (2003). The raphid valve will be referred to as the RV, the araphid valve as the ARV.

Morphology

Valves panduriform, gently constricted at the centre of the valve, 77-128 μm in length, 18-40 μm in width. Striae biseriate on the both valves, with 6.5-7.5 transapical virgae (often referred to as costae) in 10 μm on RV, and 5-6.5 on ARV. In LM observations, on the RV, transapical virgae occur either side of raphe, opposite each other. Raphe fissure extends the entire length of the valve to the stauros, narrowly expanded (Fig. 1a). Raphelss sternum on ARV almost central and appears gently biconvex (Fig. 1b). Terminal orbiculi on the poles of ARV (Fig. 1b). In girdle view, the convex valve bears the ARV, while concave valve bears the RV (Fig. 2). In living material, cells with many small plastids, discoid-shaped, usually more than 30 (Figs 4, 5). Plastids meet the cell wall, but the centre of the frustule is usually filled (Figs 4b, 5c). This taxon forms long chain colonies with a long stalk, usually more than 10 individuals connected to each other (Fig. 3).

With SEM observations, the outer surface of the RV appears round (Fig. 6). The central raphe fissures (central pores) are slightly expanded and unilaterally deflect; there is a thickened stauros, which reaches the valve margins (Fig. 9), while the terminal fissures are deflected on the opposite sides at the valve apices (Fig. 10). Internal views of the RV reveal a slightly thickened stauros and additional thickening on the virgae, distal to the raphe slit (Fig. 7). The raphe fissures are almost straight, the central endings swell a little (Fig. 8) and, internally, the terminal fissures curve slightly in the opposite direction into small helictoglossae (Fig. 11). Transapical virgae emerge radially from the raphel sternum, which does not exhibit bilateral symmetry (Figs 7, 8, 11). The external surface of the ARV lacks any ridge or spine (Fig. 12). The raphelss sternum emerges from the centre of the ARV (Fig. 16). There are terminal orbiculi at the poles (Figs 13-15). There are cribrate areolae, which are more or less round and usually supported at three or four points around the periphery (Fig. 20). Intervening areolae appear sunken and transapical virgae are well developed in the internal parts of the ARV (Fig. 17). All copulae open ended at one pole, and areolae are composed of two rows of striae on the copulae and valvocopulae (Figs 18, 19, 21). Especially on valvocopula, the areolae are usually round or rectangular in shape (Fig. 22), and occluded with cribrae, creating curved peripheral slits.
Discussion

The fine structure of *Achnanthes longipes* has been illustrated by several researchers. Hendey (1959) illustrated some ultra-structural features of the cribræ with transmission electron microscopy and Novarino (1992) studied the structure of the girdle bands and provided descriptions of several different types of bands. However, as for the existence of terminal orbiculi, their presence...
Figs 6-11. SEMs with RV view. Fig. 6. External view of RV uppermost, showing rounded valve face. Fig. 7. Internal view of RV, showing transapical virgae emerging radially from raphe sternum. Fig. 8. Internal view of stauros, with slightly swollen central pores. Fig. 9. External view of stauros, with clear central pores. Fig. 10. Terminal view of RV, with terminal fissure. Fig. 11. Internal view at the pole of the RV, terminal fissure curved to opposite side of the external view.

varies in each description. For instance, the figured individuals in Hustedt (1937) have no terminal orbiculi at the ARV poles but Round et al. (1990) figure some individuals with them. We have not yet addressed the question, what kind of structure the terminal orbiculi are. However, from a developmental point of view, terminal orbiculi may appear as one of the structural pat-
terns derived from large and modified cribra (see Figs 14, 20), with the loss of its transverse and longitudinal virgae. To be sure it, the cultivation of cells under different conditions may provide suitable answers for the appearance or otherwise of such structures. At present, we suggest that both individuals, forming terminal orbiculi on the ARV or their lack, are to be classified as *A. longipes*.

As for the morphology of chloroplasts, *Achnanthes longipes* has many small plastids (Cleve 1895. p. 195, Round et al. 1990. p. 502. fig. B, Novarino 1992. fig. 2, also figures 14, 15 in this paper). Conversely, many of the other species in
Achnanthes have two large plastids per cell, one on either side of the median transapical plane (e.g. Toyoda et al. 2003, Toyoda et al. 2005b); *A. inflata* (Kütz.) Grunow has two U-shaped plastids in valve view, one at each end of the cell (Toyoda 2004, unpubl.). Hence, the structure of the plastid is useful as it is shared by some of the taxa in *Achnanthes*. However, this feature has not been used to discriminate between *Achnanthes* and other monoraphid genera because there is insufficient information on the number, shape and arrangement of the plastids in many species. Nevertheless, Cox (1981) and Mann (1988) have noted that the number and structure of the plastids can be important and are relevant to the taxonomy of diatoms. More observations on the features of plastids will provide additional data for determining the relationships among diatoms as well as a means for distinguishing the various groups of monoraphid diatoms from other groups.

Taxonomic (re-)placement or (re-)definition of many species in *Achnanthes* s.l. from species in *Achnanthes* includes more than a hundred taxa; the numbers of species in the genus *Achnanthes* will probably be less than a hundred.

This investigation has described the detailed morphology of *Achnanthes*. We believe that description of the fine structure of valve features is necessary for determining the taxonomy of diatoms. As for species in closely related genera to *Achnanthes*, data on the fine structure is still
rather poor in spite of the large number of taxa already described. It is necessary to observe more species of *Achnanthes* s.l. from several viewpoints, such as valve features, the shape and number of plastids, its colony type and any ecological information, to avoid further taxonomical confusion among the genera.

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