NUPELA MATRIOSCHKA SP. NOV., NUPELA THURSTONENSIS
COMB. NOV. AND NUPELA NEOGRACILLIMA COMB. & NOM. NOV.
(BACILLARIOPHYCEAE): CRITICAL ANALYSIS
OF THEIR MORPHOLOGY

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Abstract. Light and electron microscopic observations of the diatom flora from the Polistovo-Lovatsky Sphagnum bogs (Russia, Novgorod region) revealed the occurrence of an unknown species comparable to Navicula thurstonensis Kaczmarska, a taxon described from Hawaii. Nupela matrioschka is described here as a species new to science, based on its valve morphology typical for the genus Nupela Vyverman & Compère. The major features conforming to Nupela are the ultrastructure of the raphe system and areolae. We propose formal transfer of Navicula thurstonensis to Nupela as N. thurstonensis (Kaczmarska) Kulikovskiy, Lange-Bertalot & Witkowski comb. nov. Both species are compared to similar taxa belonging to Nupela described from temperate climate zones. Also proposed is transfer of Achnanthes gracillima Hustedt to Nupela. Since the epithet gracillima is not available due to the priority of Nupela gracillima (Hustedt) Lange-Bertalot 1993, we propose as a necessary new name Nupela neogracillima (Hustedt) Kulikovskiy & Lange-Bertalot comb. nov., nom. nov.

Key words: taxonomy, diatom morphology, Nupela, Navicula, Achnanthes, new species, new combinations, Polistovo-Lovatsky Sphagnum bogs (Russia), Hawaii

INTRODUCTION

The genus Nupela Vyverman & Compère was described based on the ultrastructure of areolae and of the raphe system. As shown by Rumrich et al. (2000), Monnier et al. (2003) and Potapova et al. (2003), areolae in Nupela are occluded by external hymens. Likewise the particular raphe structure can only be detected in SEM. The external terminal raphe endings in most cases form a double hook (e.g., Rumrich et al. 2000; Monnier et al. 2003; Potapova et al. 2003) or occasionally a single hook (Lange-Bertalot 1993). The internal proximal raphe endings are variable, either simple or hooked or sometimes T-shaped (Vyverman & Compère 1991; Lange-Bertalot 1993; Potapova et al. 2003). Morphological analysis reveals that at present Nupela is to a certain extent heterogenous, as it includes frustules with a naviculoid or with a achnanthoid raphe system. In the first case both valves possess fully developed raphe branches; in the second case either the frustule is heterovalvar with one raphid valve and the other one araphid or else the raphe is strongly reduced on the second valve. This problem has already been raised by Monnier et al. (2003), Siver et al. (2005), Kulikovskiy (2007), Antoniades et al. (2008) and others. Since the early 1990s, many naviculoid and achnanthoid species have been either transferred to this genus or described as new to science (e.g., Lange-Bertalot 1993; Lange-Bertalot & Moser 1994; Lange-Bertalot & Metzeltin 1996; Moser et al. 1998; Lange-Bertalot & Genkal 1999; Rumrich et al. 2000; Kusber & Metzeltin 2001; Monnier et al. 2003; Potapova et al. 2003; Metzeltin & Lange-Bertalot 1998, 2007; Antoniades et al. 2008).
Floristic studies of Sphagnum bog diatoms in the European part of Russia resulted in the identification of an unknown species similar to Navicula thurstonensis described by Kaczmarska in Rushfort et al. (1984) from Hawaii. Both species have principally naviculoid frustule morphology. Based on light and electron microscopic observations we describe a new species Nupela matrioschka and propose the transfer of Navicula thurstonensis to Nupela. Likewise we propose the transfer of Achnanthes gracillima Hustedt, which possesses features of achnanthoid taxa in Nupela. The general taxonomy of Nupela is also briefly discussed.

MATERIAL AND METHODS

The material studied was collected from different parts of the Polistovo-Lovatsky Sphagnum Bogs (Novgorod region, Russia) in July 2005. Altogether 49 samples were collected including plankton, benthos and periphyton from Sphagnum spp. Preparation of the diatoms for microscopic observations followed the methods of Balonov (1975). A part of each sample was placed in glass test-tubes and potassium chromide was added. After 3–5 min of reaction the samples were washed with distilled water by centrifugation, and the supernatant was removed. Centrifugation was repeated several times. Permanent diatom preparations were mounted with Naphrax®. Light microscopic (LM) observations employed a Nikon Eclipse E600 with a Plan-apochromatic 100× oil immersion objective and a Nikon DS-5M digital camera. Diatom valve ultrastructure was analyzed with a JSM-25S (JEOL, Japan) scanning electron microscope and an H-300 (Hitachi, Japan) transmission electron microscope.

RESULTS AND DISCUSSION

Nupela matrioschka Kulikovskiy, Lange-Bertalot & Witkowski, sp. nov.

Differens versus Nupela thurstonensis (Kaczmarska) Kulikovskiy, Lange-Bertalot & Witkowski comb. nov. Valvae distincte late ellipticae apicibus plus minusve late rotundatis (nec angustius ellipticeae ad elliptico-lanceolatas apicibus saepi leviter protractis). Longitudo 9.7–12.0 μm, latitudo 4.3–6.3 (non 3.2–4.2) μm. Raphe, area axialis centralisque circiter conformantes. Areolae 30–40 (non 40–50) in 10 μm, quasi irregulariter sitae (non fere regulariter) inter se cum areis hyalynis apicalibus. Disordinatio Voigtii conspicue latius inter duas striae extendens.

TYPE: RUSSIA, Novgorod, Polisto-Lovatsky Sphagnum bog, 21 July 2005, leg. M. Kulikovskiy. Slide no. 13629 in collection A. Witkowski, Institute of Marine Sciences, University of Szczecin (SZCZ); ISOTYPES: Slide no. 6 in collection M. Kulikovskiy, Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Russia; slide no. BMR ZU6/78 deposited in Alfred Wegener Institute, Bremerhaven (BMR), Germany.

ETYMOLOGY: The epithet may be seen as a neo-Latin substantive referring to traditional Russian dolls.

Differential diagnosis versus Nupela thurstonensis (Kaczmarska) Kulikovskiy, Lange-Bertalot & Witkowski comb. nov. Valves distinctly broad-elliptical with broad, never protracted apices (not narrower elliptical to elliptical-lanceolate with often slightly protracted ends). Length 9.7–12 μm, breadth 4.3–6.3 (not 3.2–4.2) μm. Raphe, axial and central area approximately conforming. Transapical striae radiate, becoming moderately convergent near the ends on either valve side (this feature is less distinct on the valve side opposite to the Voigt fault in Nupela thurstonensis). The Voigt fault is (comparatively) more broadly expanded between two distal striae. Areolae 30–40 (not 40–50) in 10 μm, forming irregular wavy lines apically with narrow hyaline areas in between, as in many other Nupela taxa.

TEM AND SEM

Valve surface flat, strongly bent towards the very shallow valve mantle. Axial area very narrow, linear; central area weakly developed, as an indistinct expansion of the raphe sternum. A completely developed raphe occurs on both valves of the frustule (Fig. 2: 1). Raphe filiform, straight (Fig. 1: 1–10), external, central raphe endings simple (Fig. 2: 1–4), internally slightly expanded (Fig. 1: 11, 12), ending in a small helictoglossa...
External apical raphe endings on both valves of the frustule strongly doubly hooked towards the same side of the valve (Fig. 2: 1–4). Transapical striae uniseriate, radiate becoming parallel or slightly convergent near the apices, 42–48 in 10 μm. Areolae form 4–5 longitudinal rows on each side of the raphe (Fig. 1: 1–10; 2: 1–4). A row of elongated poroids pervalvar on the valve mantle (Fig. 2: 1–4).

DISTRIBUTION. So far found only at the type locality, Polistovo-Lovatsky Sphagnum bogs.
NOTES. No other established Nupela species except *N. thurstonensis* may be confused with the new taxon. The features of three more or less similar taxa from North America are given in Table 1 for comparison.

*Nupela thurstonensis* (Kaczmarska) Kulikovskiy, Lange-Bertalot & Witkowski, **comb. nov.**


Our proposal to transfer *Navicula thurstonensis* to *Nupela* is based on ultrastructural features of the raphe system and the type of areolation. The ultrastructure of areolae was discussed by Potapova *et al.* (2003), who noted that *Nupela* described from North America have areolae occluded by external hymens and that in specimens after strong cleaning processes the hymenate occlusions are dissolved. Vyverman and Compère (1991) based the generic description of *Nupela* on the presence of perforated hymenate occlusions located on the...
valve interior, but they used the term ‘hymen’ incorrectly. As the number of Nupela species is still increasing, raphe ultrastructure can be pointed out as one of the crucial criteria for delimitation of the genus. In Navicula thurstonensis, hymenate areolae occlusions were not observed, but the raphe system is typical of species belonging in Nupela (cf. Vyverman & Compère 1991; Lange-Bertalot & Moser 1994; Rumrich et al. 2000; Potapova et al. 2003; Table 1). Hence we propose formal transfer of Navicula thurstonensis to the genus Nupela.

Most of the more than 40 established Nupela species are known from the tropics. Only a few taxa have so far been either described or recorded from the temperate climate zone (e.g., Lange-Bertalot & Metzeltin 1996; Potapova et al. 2003). Some of the species originally described from either low or high geographic latitudes have subsequently been found to occur also under temperate or tropical climate conditions (Rumrich et al. 2000). It is difficult to characterize Nupela thurstonensis autecologically and biogeographically, as the data originate from only one locality.

In terms of the autoecology of Nupela matrioschka, it is known only from subaerial habitats with low pH, for example Sphagnum bog, which is always characterized by exposed substrate and low pH (e.g., Johanssen 1999; Buczko 2006; Witkowski et al. 2006; Kulikovskiy 2007). As pointed out by Rushforth et al. (1984), numerous taxa identified in subaerial habitats in Hawaii are also capable of growth in similar conditions worldwide. Included in this group are Melosira dickiei (Thwaites) Kützing, Adlafia bryophila (Petersen) Lange-Bertalot and Fallacia insociabilis (Krasske) D. G. Mann (see also Lange-Bertalot & Metzeltin 1996; Metzeltin & Lange-Bertalot 1998, 2007). In the Polistov-Lovatsky Sphagnum bog ecosystem, Nupela matrioschka belongs to a diatom assemblage typical for raised oligotrophic bogs consisting of, for example, Tabellaria flocculosa (Roth) Kützing, Aulacoseira subarctica (O. Müller) Haworth, A. tenella (Nygaard) Simonsen, Frustulia krammeri Lange-Bertalot & Metzeltin, F. saxonica Rabenhorst, Eunotia serra Ehrenberg, E. meis-teri Hustedt, Pinnularia subcapitata var. elongata Krammer and Kobayasiella parasubtilissima (Kobayasi & Nagumo) Lange-Bertalot (Kulikovskiy 2007).

Both taxa under discussion belong to a group of small-celled forms with elliptic to elliptic-lanceolate valves, which are not difficult to distinguish within Nupela. They show close relationships to species like Nupela exotica Monnier, Lange-Bertalot & Bertrand, Nupela neglecta Ponader, Lowe & Potapova, Nupela carolina Potapova & Clason and Nupela rumrichorum Lange-Bertalot. These species are very well circumscribed by a complex of features including raphe ultrastructure, which shows different degrees of heterovalvity (e.g., N. matrioschka, N. thurstonensis, N. exotica, N. carolina, N. rumrichorum, N. neglecta; Table 1). The raphe system of N. matrioschka, N. thurstonensis, N. exotica, and N. carolina appears to be a criterion typical of naviculoid Nupela taxa. Other Nupela species have a raphe on only one valve (e.g., N. rumrichorum). In N. matrioschka and N. thurstonensis, autecology is an additional discriminating criterion. They inhabit either intermittently wet habitats (N. thurstonensis) or Sphagnum bogs (N. matrioschka) with low pH, whereas other Nupela species occur in habitats with moderately low to higher pH, ranging from 5.4 to 8.5, usually close to or exceeding 7.0 (cf. Table 1).

Achnanthes gracillima Hustedt is a rare species inhabiting oligotrophic water bodies of the Northern Hemisphere (Krammer & Lange-Bertalot 1991). The first SEM images were published by Marciniak (1986), and later by Genkal and Kharitonov (2006). Raphe ultrastructure and the areolae are typical for the genus Nupela (Fig. IV: 1–3 in Marciniak 1986; Figs 1: 1–5, 2: 1–8 in Genkal & Kharitonov 2006). Based on these criteria we propose the transfer of Achnanthes gracillima to Nupela. However, the specific epithet ‘gracillima’ is already occupied in the genus, since Stauroneis gracillima Hustedt (Hustedt 1943; see also Simonsen 1987) was transferred to Nupela as N. gracillima (Hustedt) Lange-Bertalot (Lange-Bertalot 1993). Therefore, we propose a new specific epithet ‘neogracillima’ to replace ‘gracillima’.
Table 1. Comparison of *Nupela thurstonensis* (Kaczmarska) Kulikovskiy, Lange-Bertalot & Witkowski, *comb. nov.* with other taxa.

<table>
<thead>
<tr>
<th>Species/ Feature</th>
<th><em>Nupela thurstonensis</em>&lt;br&gt;comb. nov.</th>
<th><em>Nupela matrjoschka</em>&lt;br&gt;sp. nov.</th>
<th><em>Nupela exotica</em> Monnier, Lange-Bertalot &amp; Bertrand</th>
<th><em>Nupela neglecta</em> Ponader, Lowe &amp; Potapova</th>
<th><em>Nupela carolina</em> Potapova &amp; Clason</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valve shape</td>
<td>elliptic to elliptic-lanceolate, often with slightly protracted ends</td>
<td>broad-elliptical with broad, never protracted apices</td>
<td>elliptical-lanceolate, ends fairly obtusely to more acutely rounded and often slightly short-protracted</td>
<td>lanceolate to elliptical-lanceolate with slightly protracted apices; slightly asymmetrical about apical and transapical planes</td>
<td>elliptical-lanceolate</td>
</tr>
<tr>
<td>Length, μm</td>
<td>7–13</td>
<td>9.7–10.3</td>
<td>8.6–13.3</td>
<td>3–15</td>
<td>5–15</td>
</tr>
<tr>
<td>Width, μm</td>
<td>3.2–4.2</td>
<td>4.0–6.3</td>
<td>3.0–4.1</td>
<td>2.6–4.5</td>
<td>2.4–4.4</td>
</tr>
<tr>
<td>Stria orientation</td>
<td>radiate, becoming parallel or slightly convergent near the poles</td>
<td>radiate, becoming parallel or slightly convergent near the poles</td>
<td>radiate; striae often contain only a few areolae</td>
<td>slightly radiate, becoming parallel or slightly convergent near the poles</td>
<td>radiate</td>
</tr>
<tr>
<td>Stria in 10 μm</td>
<td>(30)35–45</td>
<td>42–48</td>
<td>40–45</td>
<td>40–48</td>
<td>42–54</td>
</tr>
<tr>
<td>Axial area</td>
<td>narrow, linear</td>
<td>narrow, linear</td>
<td>narrow, linear</td>
<td>linear-lanceolate</td>
<td>narrow, linear</td>
</tr>
<tr>
<td>Central area</td>
<td>rather small ± round, sometimes lacking</td>
<td>rather small ± round, sometimes lacking</td>
<td>barely developed or lacking</td>
<td>small, round or elliptical</td>
<td>square or lyre-shaped, formed by interrupted central striae, often with several isolated areolae</td>
</tr>
<tr>
<td>Raphe</td>
<td>long in both valves</td>
<td>long in both valves</td>
<td>long in both valves</td>
<td>differing between valves; one valve with a long raphe, the other with distinctly shortened branches</td>
<td>long in both valves</td>
</tr>
<tr>
<td>Proximal raphe</td>
<td>terminate with very small punctiform central pores</td>
<td>terminate with very small punctiform central pores</td>
<td>moderately arcuate with roundish central pores</td>
<td>roundish central pores on long branches and without any visible central pore on short branches</td>
<td>terminal raphe fissure forming a curved opening to the primary side of the valve</td>
</tr>
<tr>
<td>Ecology and distribution</td>
<td>wet habitats on rocks, Hawaii</td>
<td><em>Sphagnum</em> oligotrophic bogs (pH 3.7–5.5)</td>
<td>tropical fish aquarium, France</td>
<td>rivers and brooks of USA, pH 5.4–8.5</td>
<td>rivers and brooks of USA, pH 5.4–8.5</td>
</tr>
<tr>
<td>Reference</td>
<td>Rushfort <em>et al.</em> 1984</td>
<td>This study</td>
<td>Monnier <em>et al.</em> 2003</td>
<td>Potapova <em>et al.</em> 2003</td>
<td>Potapova <em>et al.</em> 2003</td>
</tr>
</tbody>
</table>
**Nupela neogracillima** (Hustedt) Kulikovskiy & Lange-Bertalot, *comb. nov. & nom. nov.*

**Basionym:** *Achnanthes gracillima* Hustedt 1927, Arch. Hydrobiol. 18: 161, Figs 5: 10–11.

With the description of a new species, and the transfer of two additional taxa, the number of species in the genus *Nupela* now exceeds 40 (e.g., Lange-Bertalot 1993; Lange-Bertalot & Moser 1994; Lange-Bertalot & Metzeltin 1996; Moser et al. 1998; Lange-Bertalot & Genkal 1999; Rumrich et al. 2000; Metzeltin & Kusber 2001; Monnier et al. 2003; Potapova et al. 2003; Metzeltin & Lange-Bertalot 1998, 2007; Antoniades et al. 2008).

To conclude we stress that the results of our study reported here show that further discoveries and transfers in established genera of small-celled taxa, such as *Nupela*, can be expected. This will occur through the routine use of electron microscopes in studies of the ultrastructure of small raphid diatoms.

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