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LATE QUATERNARY *NUPELA* TAXA OF RETEZAT MTS (S. CARPATHIANS), WITH DESCRIPTION OF *NUPELA POCSII* SP. NOV. (BACILLARIOPHYCEAE)

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Abstract. Nupela pocsii Buczkó & Wojtal, sp. nov., a new species from the Retezat Mountains (S. Carpathians), is described. High asymmetry (along apical and transapical axes and in raphe pattern) and small dimensions are the most characteristic features of this taxon. Nupela pocsii was found during paleolimnological research in early Holocene sediment of Lake Brazi. Seven other representatives of the genus Nupela were detected in high-resolution diatom analyses of three lake sediment sequences of the Retezat Mts: Nupela fennica (Hustedt) Lange-Bertalot, N. imperfecta (Schimanski) Lange-Bertalot, N. impexiformis (Lange-Bertalot) Lange-Bertalot, N. lapidosa (Krasske) Lange-Bertalot, N. paludigena (R. P. Scherer) Lange-Bertalot, N. vitiosa (Schimanski) Siver & Hamilton and an unidentified Nupela Vyverman & Compere species. Our results suggest high diversity of oligotraphenic species in these mountain lakes during their ontogeny, which began 17,000–15,000 years ago. In addition to the newly described species this is also the first record of N. paludigena in European lakes, although its occurrence was previously documented by SEM and LM from Lake Saint Anna without correct identification.

Key words: diatoms, Nupela, oligotrophy, paleolimnology, Retezat Mountains, South Carpathians

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INTRODUCTION

The genus *Nupela* was erected by Vyverman and Compére in 1991 and since then the number of its representatives has gradually increased, due in part to transfers of already known species from the genera *Achnanthes*, *Brachysira*, *Navicula* and *Neidium* to *Nupela*, and in part to the description of several new species (e.g., Monnier *et al.* 2003; Potapova *et al.* 2003; Kulikovskiy *et al.* 2009; Wojtal 2009; Siver *et al.* 2010; Bahls 2011; Potapova 2011b). In AlgaeBase there are 43 records of *Nupela* taxa listed (Guiry & Guiry 2013), and 48 species names are found in the California Academy of Sciences Catalogue of Diatom Names (http://research.calacademy.org). Bahls (2011) recently summarized the history of the genus, including its modified definition. The most distinctive feature of *Nupela* seems to be the larger external openings of the areolae relative to the internal openings (Spaulding & Edlund 2008). Species of *Nupela* are small, generally smaller than 20 μ m. The valves can be broad or narrow, iso- or heterovalvar with respect to raphe development and/or shape of the central area, and sometimes tend to be dorsiventral, with the raphe displaced toward the primary side such that the secondary side is wider (Siver *et al.* 2007). The valve face is typically flat.

Nupela was originally described from highelevation ponds in Papua New Guinea (Vyverman & Compére 1991). Since its initial discovery, *Nupela* has been reported across Europe, South

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America, North America, Asia and Africa, typically in oligotrophic waters with acidic to circumneutral pH (Metzeltin & Lange-Bertalot 1998; Monnier *et al.* 2003; Potapova *et al.* 2003; Siver *et al.* 2007; Wojtal 2009; Kulikovskiy *et al.* 2010).

In this article we summarize our findings on the occurrence and distribution of *Nupela* species in three mountain lakes during the late Quaternary period after the glaciers receded and these lakes – the most spectacular attractions of Retezat National Park – formed (Urdea 2004).

MATERIALS AND METHODS

Lacustrine sediment cores were obtained from four glacial lakes for a high-resolution multiproxy study conducted in the Retezat Mts. (Magyari et al. 2009a). Lake Brazi (45°23'47"N, 22°54'06"E; 0.5 ha; 1740 m a.s.l.; max. 1.1 m water depth) and Lake Gales (45°23'6"N, 22°54'33"E; 3.68 ha; 2040 m a.s.l.; max. 20 m water depth) are situated on the northern flank and were cored in 2007. Lake Lia (45°35'30"N, 22°87'87"E; 1.3 ha; 1910 m a.s.l.; max. 4.3 m water depth) and Lake Bucura (45°36'40"N, 22°87'79"E; 10 ha; 2040 m a.s.l.; max. 15.7 m water depth) are situated on the southern flank and were drilled in 2008 (Magyari et al. 2009a). For subsampling, core sections were first split lengthwise followed by subsampling at usually 4 cm resolution (Magyari et al. 2009a). Subsamples were used for multiproxy analyses, including geochemical, pollen, macrofossils, cladocera, chironomids and siliceous algae. The chronology of the sediment sequences is published in part (for Lake Brazi and Gales see Magyari et al. 2009a) and some radiocarbon dates are not vet published (Lake Lia).

So far high-resolution diatom analyses have been carried out in three of the four lakes (Lake Brazi, Gales, Lia); more than 450 samples were counted. For analyses of the siliceous algae, samples were prepared by standard digestion procedures (Battarbee 1986). Aliquot-evaporated suspensions were embedded in Zrax and Pleurax. From each sample *ca* 350 valves were counted using a light microscope (Leica DM LB2 with 100 HCX PLAN APO objective and Fujifilm FinePix S2 Pro digital camera; later VSI–3.OM(H) digital camera). During the counting procedure pictures of ambiguous valves were taken; later the pictures were arranged according to the diatom genera to which they belong, and the small forms were grouped together. For



Fig. 1. Location of the studied lakes in the Retezat Mountains, Southern Carpathians.

SEM, cleaned samples were air-dried on an aluminum stub. Specimens were coated with gold-palladium using a XC7620 Mini Sputter Coater for 120 s at 16 mA, and studied with a Hitachi S–2600N scanning electron microscope operated at 20 kV and 5–8 mm distance. Morphological terminology follows Hendey (1964), Barber and Haworth (1981) and Round *et al.* (1990). Valves were measured from digital images using the camera software.

RESULTS

We distinguished eight taxa within the Nupela genus in the three studied lakes. The following taxa were found and are presented below: Nupela fennica, N. imperfecta, N. impexiformis, N. lapidosa, N. paludigena, N. vitiosa and an unidentified member of Nupela (Figs 19 & 20). The material also yielded a new species, described formally here as N. pocsii. Nupela fennica (Hustedt) Lange-Bertalot 2004 Figs 2–4

Navicula fennica Hustedt 1962.

REFERENCE. Potapova (2010a).

Valves linear-elliptical with broadly rounded ends. Length 16–19 μ m, width 3.0–3.8 μ m, striae hardly discernible in LM; *ca* 35–40 in 10 μ m. Axial area narrowly linear. Central area forms rectangular fascia. Striae radiate in middle, parallel or slightly convergent at apices. According to Potapova (2010a) the distinction between *N. fennica* and *N. vitiosa* is not entirely clear. On the basis of protologues, *N. fennica* differs from *N. vitiosa* by the shape of the central area, which for the first species is symmetrical, rectangular, and expanded to both valve margins.

DISTRIBUTION IN THE RETEZAT MTS. Only two valves were found at 416 cm (8480 cal yr BP) in Lake Brazi. In Lake Gales *N. fennica* was sporadically found from 140 cm (5900 cal yr BP) to the top of the core (up to present). In Lake Lia it was recorded from 710 cm (*ca* from 14,000 cal yr BP) also up to the top of the core.

GENERAL DISTRIBUTION. Known from Finland (Lange-Bertalot & Metzeltin 1996), and North America (Potapova 2010a).

Nupela imperfecta (Schimanski) Lange-Bertalot 1999 Fig. 5

Achnanthes imperfecta Schimanski 1978.

REFERENCE. Krammer & Lange-Bertalot (1991: 53; fig. 31: 1–10).

Valves linear-lanceolate, apices protracted with broadly rounded ends. Length 19.8–26.0 μ m, width 6.3–6.5 μ m. The outline and dimensions as well as the shape of the central area and the striae pattern well fit the description of *N. imperfecta*.

DISTRIBUTION IN THE RETEZAT MTS. Only two valves were found, one in Lake Gales at 172 cm (8500 cal yr BP) and the other in Lake Lia at 214 cm (5200 cal yr BP).

GENERAL DISTRIBUTION. Mongolia (Kulikovskiy *et al.* 2010), Europe: Taunus, Iceland, Finland (Lange-Bertalot & Krammer 1989).

Nupela impexiformis (Lange-Bertalot) Lange-Bertalot 1999 Figs 6–10

Achnanthes impexiformis Lange-Bertalot in Lange-Bertalot & Krammer 1989.

REFERENCES. Lange-Bertalot & Krammer (1989: 53; fig. 31: 1–10), Potapova (2011a).

Valves elliptical-lanceolate with capitate ends. Length 14.5–17.0 μ m, width 4.5–5.5 μ m, striae not discernible in LM. Heterovalvar, the raphe is absent on one of the valves. Raphe straight. This population has slightly larger valves than given in the description, where 12–16 μ m is given for length and 3.5–4.5 for width, with 45–55 striae in 10 μ m. Potapova (2011a) found valves up to 17 μ m in length but with valve width in agreement with the original description (3.8–4.6 μ m).

DISTRIBUTION IN THE RETEZAT MTS. In Lake Gales *A. impexiformis* was found in seven samples: between 138 cm (5770 cal yr BP) and 108 cm (4820 cal yr BP), at 56 cm (3060 cal yr BP) and at 2 cm (120 cal yr BP), but it was not abundant in the samples. Two valves were found in Lake Lia at 503 cm (10,080 cal yr BP) and at 347 cm (7660 cal yr BP).

GENERAL DISTRIBUTION. Finland, Sweden, Island (Lange-Bertalot & Krammer 1989), Mongolia (Kulikovskiy *et al.* 2010); prefers oligotrophic, circumneutral or acidic (*Sphagnum* bogs) habitats.

Nupela lapidosa (Krasske) Lange-Bertalot 1999 Figs 11–15

Achnanthes lapidosa Krasske 1929

REFERENCE. Krammer & Lange-Bertalot (1991: 47; fig. 27: 1–14), Wojtal (2009: 234; figs 1–5, 14–25).

Frustule heterovalvar, one valve has complete raphe but absent in another one. Valves lanceolate to linear-lanceolate with slightly elongated ends. Length $15.5-23.0 \mu m$, width $4.8-5.6 \mu m$. Striae clearly visible on both valves, slightly radiate in middle of valve and becoming radiate towards apices, 20-24 in $10 \mu m$. Axial area linear-lanceolate on raphe valve, broadly lanceolate on rapheless valve. Central area asymmetrical, unilaterally expanded up to valve margin (rapheless valve) or almost up to valve margin (raphe valve).

DISTRIBUTION IN THE RETEZAT MTS: In the Late Glacial part of Lake Gales it was found sporadically at 280 cm (ca 13,540 cal yr BP), 264 cm (12,960 cal yr BP) and 228 cm (12,040 cal yr BP); between 212 cm (11,500 cal yr BP) and 140 cm (5910 cal yr BP) the species was continuously present. In Lake Lia it was a common but never abundant member of the diatom assemblages; it was recorded between 762 cm (ca 14,200 cal yr BP) and 443 cm (ca 9220 cal yr BP). In the Late Glacial population (14,700–11,700 cal yr BP) valves markedly smaller than in the Holocene (from 11,700 cal yr BP) were found.

GENERAL DISTRIBUTION: *Nupela lapidosa* is a common representative of *Nupela*, often reported from springs having very low conductivity, low alkalinity and high oxygen concentration in the Tatra Mts (Wojtal 2013), and in aerophytic habitats (Krammer & Lange-Bertalot 1991). It was previously reported from the Alps and Central Europe as preferring oligotrophic, circumneutral waters of low to moderate conductivity (Werum & Lange-Bertalot 2004).

Nupela paludigena (R. P. Scherer) Lange-Bertalot 1993 Figs 16–18

Anomoeoneis paludigena R. P. Scherer 1988

REFERENCES. Scherer (1988: 149; figs 42–45, 72), Siver *et al.* (2007: 128; figs 4, 5).

Frustules isovalvar. Valves elliptical-lanceolate to linear-lanceolate, with rostrate to capitate apices. Length 10–20 μ m, width 3–5 μ m. Striae slightly radiate to parallel in center, becoming parallel to-

wards apices, 38–45 in 10 µm. Axial area narrow. Central area variable, small, elliptical to rectangular in shape and most often unevenly spaced on each side of valve. Raphe slightly wavy with distal raphe fissures running down onto apical mantle and deflected towards secondary side of valve. Proximal raphe fissures straight and end as small teardrop-shaped pores. Areolae elliptical to rectangular, expanded along transapical valve axis, with wider opening on external surface than on internal surface. Mantle a single row of elongated areolae.

DISTRIBUTION IN THE RETEZAT MTS. This species was found only in Lake Lia. LM pictures are based on material retrieved from 134 cm (3250 cal yr BP) and the SEM image is based on a sample at 50 cm (1060 cal yr BP).

OTHER OCCURRENCES. Buczkó and Magyari (2007: 11; Pl. 4: 96 & 97) published LM and SEM images of an unidentified diatom as Nupela sp. from Lake Saint Anna. The dimensions of these specimens are length 12–15 µm, width 3 µm, striae not visible in LM, 54 in 10 µm in SEM. This form was infrequent in the Holocene sequence of Lake Saint Anna but occurred in several layers; its abundance never exceeded 2%. The description, other published data and the illustration N. paludigena indicate conspecificity of the Nupela specimens from Lake Saint Anna and Lake Lia. As a result. our data extend the occurrence of N. paludigena to the Southern and Eastern Carpathians, and as far as we know this is the first European record of this taxon.

GENERAL DISTRIBUTION. This species was originally described by Scherer (1988) from acidic localities in the southeastern part of the Atlantic Coastal Plain. Siver *et al.* (2007) reported it from the Atlantic Coastal Plain of North Carolina from

Figs 2–27. 2–4 – *Nupela fennica* (Hustedt) Lange-Bertalot from lake Brazi, 5 – *N. imperfecta* (Schimanski) Lange-Bertalot from Lake Lia at 214 cm, 6–10 – *N. imperiformis* (Lange-Bertalot) Lange-Bertalot from Lake Gales and Lake Lia, 11–14 – LM of *N. lapidosa* (Krasske) Lange-Bertalot, 15 – internal view of rapheless valve of *N. lapidosa* from Lake Lia (SEM), 16 – external view of *N. paludigena* (Scherer) Lange-Bertalot from Lake Lia at 50 cm (SEM), 17 & 18 – LM of *N. paludigena* from Lake Brazi, 20 – *Nupela* sp. from Lake Gales, 21–25 – LM of *N. vitiosa* (Schimanski) Siver & Hamilton in Lake Brazi, 26 – external view of *N. vitiosa* (SEM), 27 – internal view of *N. vitiosa*. Note the unilaterally deflected proximal raphe terminals (arrow). Scale bars: 2–14 & 17–25 (LM) – 10 µm; 16 & 26 (SEM) – 5 µm, 15 & 27 (SEM) – 5 µm.



six bays, also from strongly acidic environments. Lake Saint Anna is an acidic volcanic lake with pH 4.0–6.4 (Magyari *et al.* 2009b), which corresponds with previously published data on the autoecology of the taxon. In Lake Lia the pH ranges from 6.4 to 6.7 (János Korponai pers. com.) According to Algaebase (Guiry & Guiry 2013) this species is mentioned from New Zealand (Harper *et al.* 2012).

Nupela pocsii Buczkó & Wojtal, sp. nov. Figs 28–38

DESCRIPTION. Light microscopy (Figs 28–35). Valves small, elliptical with broadly rounded ends, 4.5–7.0 μ m long, 3.2–4.4 μ m wide (n = 18). Striae not discernible.

Scanning electron microscopy (Figs 36-38). Frustules isovalvar. Valves elliptical, with high asymmetry along apical and transapical axes (Figs 36 & 37). Axial area rhombic-lanceolate. Central area variable, usually asymmetrical, reaching valve margin on secondary side of valve; transapically expanded large fascia on secondary side of valve and small circular area on primary side. Transapical striae slightly radial in valve middle, becoming parallel towards apices; 40-44 striae in 10 µm. Areolae elliptical, sometimes round, expanded transapically, with wider opening on external surface. Areolae sometimes slightly irregular and arranged in shorter rows. Ca 35-40 areolae in 10 µm. Externally, proximal raphe fissures slightly wavy and simply terminated, the two branches differing markedly in length. Distal raphe fissures double-curved, hook-shaped and strongly unilaterally deflected towards secondary side of valve, extending onto mantle (Figs 36-37). Internally, distal fissures simple, proximal raphe fissures T-shaped (Fig. 38). Voight faults distinct (Fig. 37).

HOLOTYPE: BP slide 2216, illustrated in Figure 28; at coordinates 62 and 105 with Leica DM LB2 light microscope; ISOTYPE: W. Szafer Institute of Botany, Kraków, Poland, No. DW 10.

TYPE LOCALITY. The new species was described from a glacial lake (Lake Brazi, TDB–1; 45°23'47"N, 22°54'06"E, 1740 m a.s.l.), sediment core 516 cm depth, drilled by Enikő Magyari and Mihály Braun in 2007.

ETYMOLOGY. This diatom is named in honor of the prominent Hungarian bryologist Tamás Pócs, in appreciation of his contribution to cryptogam research in Hungary, Europe and all over the world.

Morphologically the species most similar to *Nupela pocsii* is *N. vitiosa*, but *N. pocsii* is clearly smaller and always elliptical, while *N. vitiosa* is elliptic-lanceolate to linear-lanceolate. These two species differ in the shape of the internal raphe endings (T-shaped in *N. pocsii* and straight, simple in *N. vitiosa*), as well as in the proximal raphe endings on the external valve face. *Nupela pocsii* has simple while *N. vitiosa* has slightly expanding endings. These two species differ in symmetry as well. *Nupela vitiosa* has apical while *N. pocsii* has apical and transapical asymmetry.

Nupela sp.

Figs 19 & 20

Valves linear-lanceolate, with rostrate apices, length 19.0–20.5 μ m, width 3.5–4.5 μ m. Striae radiate, more than 35 in 10 mm. Axial area narrow, central area large, asymmetric with rectangular fascia. Raphe straight.

We could not identify these diatom specimens; however, we hope that the publication of these yet unknown forms will help in later identification and better recognition of the diatom flora of the Retezat Mountains, as in the case of Lake Saint Anna and *Nupela paludigena*.

This *Nupela* species in Figure 19 resembles a picture published from European springs (Werum & Lange-Bertalot 2004, Plate 26: 9), identified as *Nupela tristis* (Krasske) Lange-Bertalot from the Spessart Mts, Germany. On the basis of the original description of *N. tristis* (basionym: *Navicula tristis* Krasske 1939, described from Chile) they are not conspecific (Lange-Bertalot *et al.* 1996).

DISTRIBUTION IN THE RETEZAT MTS: The diatom presented in Figure 19 was found in the Holocene part of Lake Brazi at 300 cm (*ca* 3000 cal yr BP), and the other valve (Fig. 20) was found in Lake Gales at 168 cm (*ca* 8120 cal yr. BP).



Figs 28–38. *Nupela pocsii* Buczkó & Wojtal, *sp. nov.*, 28–35 – LM of *N. pocsii* from Lake Brazi, Retezat Mts., Romania, 28 – holotype of *N. pocsii* (slide number BP 2216), 36 & 37 – SEM of external view of *N. pocsii* (note the high asymmetry of central area), 38 – SEM of internal valve view (note the T-shaped proximal raphe terminal – arrow). Scale bars: 28–35 (LM) – 10 μ m; 36–38 (SEM) – 2 μ m.

Nupela vitiosa (Schimanski) Siver & Hamilton 2005 Figs 21–27

Navicula vitiosa Schimanski 1978

REFERENCE. Potapova (2010b).

Valves linear-elliptical-lanceolate with rounded, slightly drawn-out ends. Length 9.3–16.0 μ m, width 3.2–3.6 μ m. Axial area narrowly linear. Central area elliptical or round, usually asymmetrically expanded to one side of valve. Striae radiate in middle, slightly convergent at apices, 35–40 in 10 μ m. Areolae transversely elliptical, covered by external hymens, *ca* 45 in 10 mm. Raphe filiform, straight or very slightly curved, with slightly expanded external proximal endings.

NOTE. Potapova (2010b) reported smaller valves ($6.5-15.0 \mu m$ long, $2.7-3.6 \mu m$ wide) of *N. vitiosa* than were given in the original description ($10-16 \mu m$ long, $3-4 \mu m$ wide) by Schimanski (1978).

DISTRIBUTION IN THE RETEZAT MTS. *Nupela vitiosa* was found in Lake Brazi in the early Holocene at 539 cm (10,790 cal yr BP) and 457 cm (*ca* 9260), in Lake Gales during the Holocene but sporadically, and in Lake Lia at 134 cm (3250 cal yr BP).

GENERAL DISTRIBUTION: Germany, Netherlands, Finland, and Canada (Krammer & Lange-Bertalot 1991); North America (Siver *et al.* 2005).

DISCUSSION

During the ongoing palaeolimnological investigation of glacial lakes of the Southern Carpathians we found several small-celled diatoms belonging to the genus *Nupela*. Their distribution proved to be very uneven in terms of time of presence and location (Table 1). *Nupela fennica*, *N. impexiformis*, *N. lapidosa* and *N. vitiosa* were recorded in low abundance but their occurrence was not sporadic. *Nupela paludigena*, *N. imperfecta* and *N. pocsii* were very rare; only one or two valves were noted in a sample. In the modern core-top samples we recorded *N. fennica*, *N. impexiformis*, *N. paludigena* and *N. vitiosa*.

 Table 1. Distribution of Nupela Vyverman & Compère taxa in the Retezat Mountains. + sporadic; ++ rare.

Taxon/Lake	Brazi	Gales	Lia
Nupela fennica	+	+	+
N. imperfecta	-	+	+
N. impexiformis	-	+	+
N. lapidosa	-	++	+
N. paludigena	-	-	+
N. pocsii	+	-	_
N. vitiosa	++	+	+

On the timescale, *Nupela vitiosa* was the first member of the genus to appear in Lake Lia, at *ca* 14,200 cal yr BP, but for the Late Glacial period (until 11,600 cal yr BP) *N. lapidosa* was more characteristic *Nupela* species in Lake Gales and Lake Lia. The valves of the Late Glacial population of *N. lapidosa* are markedly smaller than the valves found in the Holocene.

In the early Holocene (11,600–8200 cal yr BP; Walker *et al.* 2012) *N. vitiosa* was a characteristic diatom in Lake Brazi, while *N. lapidosa* was more abundant in Lake Lia. However, the relative abundance of the latter never reached 2%. The new species, *N. pocsii*, was also found in the early Holocene.

For the middle and late Holocene (8200–4200 and 4200 cal yr BP up to the present) *N. impexiformis* was the dominant *Nupela* species in Lake Gales and Lake Lia, but this well-defined form was absent from Lake Brazi.

Nupela paludigena was found only in the late Holocene, after 4200 cal yr BP (Walker *et al.* 2012), and only in Lake Lia. No characteristic distribution of the presence/absence of *N. fennica* was determined.

Many features used to distinguish *Nupela* species from other finely ornamented diatoms such as *Adlafia* and *Kobayasiella* are observable only by electron microscopy (SEM, TEM). *Nupela* is a growing genus of diatoms whose identification to species level and even to genus level has to be done by SEM study. Further detailed investigations of the genus *Nupela* are required. The data presented here are a first step toward understanding diversity within *Nupela* in the Southern Carpathians, a pristine mountain habitat. During the ontogeny of the studied lakes the members of this genus replaced each other but their occurrences were always sporadic. The very low relative abundance of *Nupela* species prevents us from making detailed paleolimnological inferences, but their sporadic occurrence suggests that the waters of the lakes were oligotrophic and oxygen-rich.

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