

A new European record of *Diadlesmis fukushimae* and its transference to *Humidophila* genus (Bacillariophyta)

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Abstract – *Diadlesmis fukushimae*, a rare oligotraphenic diatom, was found in some high mountain lakes of Romania. Its occurrence in the Parâng and Retezat Mountains is the second European record of the species. To date *D. fukushimae* has been known only from the type locality (Shenandoah National Park, Virginia, USA) and from a spring (Grotta Guernica, Dolomiti Bellunesi National Park (south-eastern Alps, Italy). Investigation by scanning electron microscopy showed that this species should be transferred to the recently established genus *Humidophila*. A new combination is proposed, *Humidophila fukushimae*. The morphological details of the European population are also presented.

Keywords: *Diadlesmis*, diatoms, *Humidophila*, mountain lakes, oligotrophy, taxonomy

Introduction

During studies on diatom assemblages in high mountain lakes a characteristic species was recently recorded. The heavily silicified diatom at first glance resembles representatives of the genus *Diadlesmis* (GRUNOW 1860: 552), but was not identical to the known and illustrated European taxa (e.g., KRAMMER and LANGE-BERTALOT 1991, LANGE-BERTALOT and METZELTIN 1996). After detailed investigation we identified it as *Diadlesmis fukushimae* Lange-Bertalot, M. Werum & Broszinski (WERUM and LANGE-BERTALOT 2004). The only previous European record of this diatom has been from the Grotta Guernica spring (Dolomiti Bellunesi National Park, south-eastern Alps, Italy) as reported by CANTONATI and SPITALE (2009).

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Recently, LOWE et al. (2014) published a study on the taxa and populations of the genus *Diademesmis*, subgenus *Paradiademesmis*, from the Hawaiian Islands. On the basis of the structure of valves, they concluded that most of the known *Diademesmis* taxa considerably differ from *Diademesmis confervacea* Kutzing (1844: 109), which is the type species of the genus *Diademesmis*. The establishment of a new genus, *Humidophila* Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová 2014, was necessary and was accomplished by splitting off the genus *Humidophila* from the genus *Diademesmis* (KÜTZING 1844) by LOWE et al. (2014). A total of 47 former *Diademesmis* species were transferred to *Humidophila*, but *Diademesmis fukushimae* was not mentioned in this compilation. A species of genus *Diademesmis* is fairly easily distinguished from other naviculoid diatoms by the combination of striae and raphe structure, and valve shape (ROUND et al. 1990).

According to the definition of the genus *Humidophila* (LOWE et al. 2014), the species belonging to the new genus are linear, linear-elliptical to elliptical. The valve face is flat; a narrow ridge can be seen between the valve face and mantle. Striae on the valve face are composed of one elliptical to ovoid areola. On the valve mantle, one single, elongated areola opening is in line with each stria. The raphe is filiform, straight with variable external proximal raphe endings but never deflected, bent or hooked. Distal external raphe endings never extend onto the mantle. Internally, areolae openings are covered by a porous hymen. The central nodule is small and rounded. Proximal raphe endings are straight or slightly anchor-shaped; distal raphe endings terminate in small helictoglossae.

In AlgaeBase there are 65 records of *Diademesmis* taxa listed (GUIRY and GUIRY 2014), and 75 species names are found in the California Academy of Sciences Catalogue of Diatom Names (FOURTANIER and KOCIOLEK 2014), which speaks to the richness of this group.

Material and methods

A total of 40 high mountain lakes, situated in three characteristic mountain regions of Romania: the Făgăraș, Parâng and Retezat Mountains were sampled in 2012, 2013 and 2014. The results of the sampling are under preparation and investigation. The diatom assemblage analysis is complete but the results have not been published.

For analyses of the siliceous algae, samples were prepared using standard digestion procedures (BATTARBEE 1986). Aliquot-evaporated suspensions were embedded in Zrax and Pleurax. Approximately 400 valves were counted from each sample using a light microscope (LEICA DM LB2 with 100 HCX PLAN APO objective and VSI-3.OM(H) digital camera). For scanning electron microscope analysis (SEM), cleaned samples were air-dried on an aluminium stub. Coating with gold-palladium was accomplished using an XC7620 Mini Sputter Coater for 120 s at 16 mA. A HITACHI S-2600N scanning electron microscope operated at 20 kV and 5–8 mm distance was used. Morphological terminology follows BARBER and HAWORTH (1981) and ROUND et al. (1990). Valve measurements were made from digital images using the camera software.

The following samples were used for documentation in this paper:

- BP 2013/152; Lake Peleaga, sample from the deepest part of the lake, at 4.1 m water depth, collected on 21st July 2013.
- BP 2013/160; Lake Negru, the deepest part of the lake, at 27.2 m water depth, collected on 25th July 2013.

Results

Diademsis fukushimae was found in three lakes in the Romanian Carpathians, one in the Parâng Mountains and two in the Retezat Mountains. The localities and some chemical parameters of the lakes where *D. fukushimae* was detected are shown in Tab. 1. The relative abundance of it was low in all the samples. It was most numerous in Lake Negru, although its relative abundance remained below 3% in this lake.

Tab. 1. Locations and some chemical parameters of the lakes where *Humidophila fukushimae* was found.

Lake	Mountain region	Altitude (m) a.s.l.	N	E	pH	Water depth (m)	Conductivity ($\mu\text{S cm}^{-1}$)
Rosiile	Parâng	1978	45°20'41,3"	23°33'15,8"	8.54	17.2	12.5
Negru	Retezat	2036	45°21'33,2"	22°49'36,6"	7.71	27.7	15.0
Peleaga	Retezat	2122	45°21'51,9"	22°54'07,6"	7.51	4.1	13.0

On the basis of the description of *Humidophila* we should transfer *Diademsis fukushimae* into this newly defined genus.

Observation

Humidophila fukushimae (Lange-Bertalot, M. Werum et Broszinski) Buczkó and Kövér comb. nov. Figs. 1A–N, Figs. 2A–D, Figs. 3A–D.

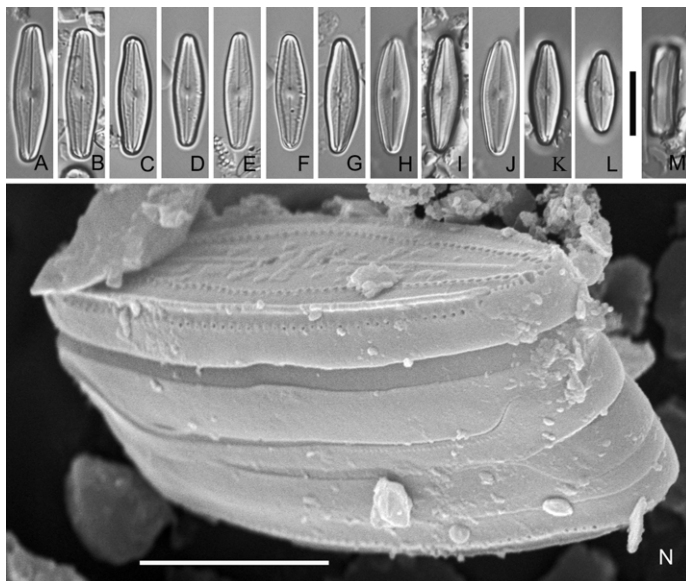


Fig. 1. *Humidophila fukushimae*: LM micrographs of population on Lake Negru (A–M), valve view (A–L); scanning electron micrograph (SEM), entire frustule in girdle view in oblique position (N). Scale bar represents 10 μm except for N where scale bar = 5 μm .

Basionym. Diademsis fukushimae Lange-Bert, M. Werum et Broszinski 2001 Diatom 17, pp 6–9, Pl. 1, Figs. 33–38, Pl. 2, Figs. 47–53, Pl. 3. 54–57.

Morphological observations

Light Microscopy (Figs. 1A–M)

Valves are linear-elliptical to elliptical-lanceolate, ends not protracted (Figs. 1A–L). Frustules in girdle view are broadly rectangular (Fig. 1M). Valve dimensions ($n = 20$): length 14–21.7 μm , ($x = 17.4 \pm 2.2$), width 4.5–6 μm ($x = 5 \pm 0.4$), frustule's width more than 4 μm (Fig. 1M). Raphe is filiform, straight, with distinct central pores. Axial area is broad, but strongly narrowed and lanceolate to the ends. Transapical striae are very short, and difficult to resolve in LM.

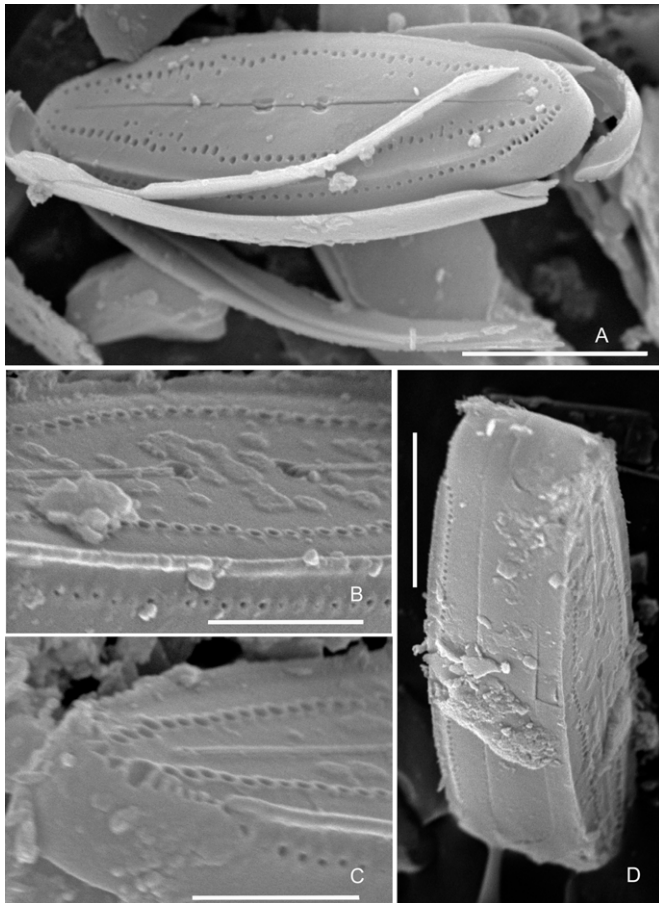


Fig. 2. *Humidophila fukushimae*: scanning electron micrograph (SEM), external valve view; valve face and open bands (A); details of the central area with central pores that are entirely flanked by depressions (B), note relief-like patches in the area between raphe and areolae; details of apices with the simple raphe ends (C), note ridge of silica at the junction of valve face and mantle; entire frustule (D). Scale bars represent 5 μm for A and D, and for B and C scale bars = 2 μm .

Scanning Electron Microscopy (Fig. 1N, Figs. 2A–D, Figs. 3A–D)

Valves are flat (Fig. 1N, Figs. 2A–D). Junction of the valve face and mantle bears a ridge of silica (Fig. 1N, Figs. 2B–C). Striae are uniseriate, containing round poroids (Fig. 1N, Fig. 2A–D), the number of areolae is 38–40 in 10 μm . The central pores are flanked by short but comparatively deep depressions (Figs. 2A,B,D). The hymens lie on the inner surface of the transapical ribs making the valve interior appear somewhat featureless (Figs. 3A,B; ROUND et al. 1990). Sometimes there are polar excavations on the sternum in the inside view (Fig. 3B). Axial area is broad (Fig. 1N, Figs. 2A–D). Distal raphe endings are

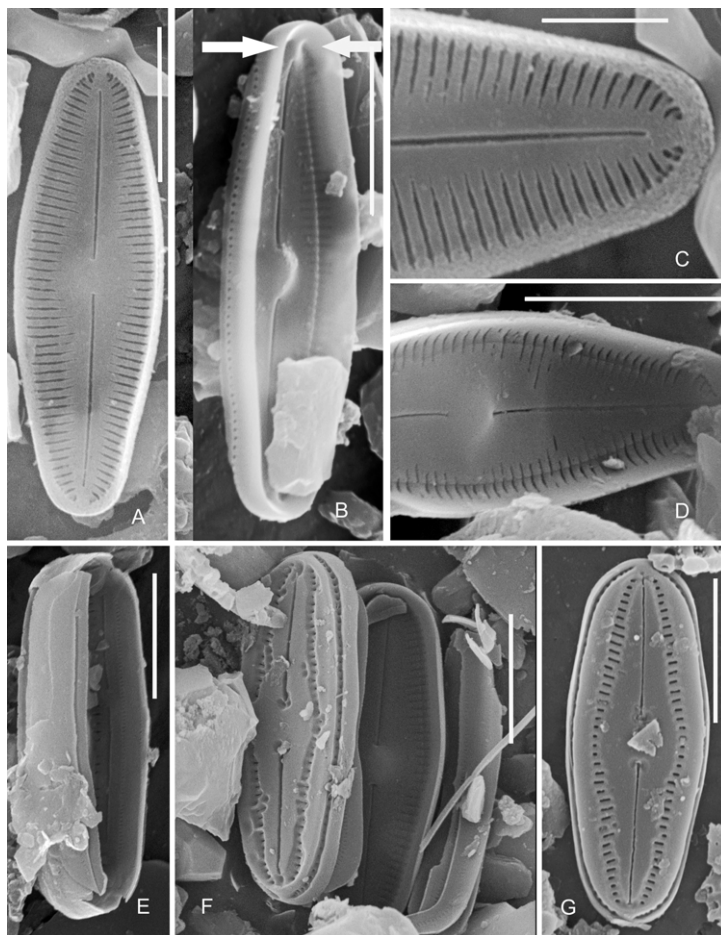


Fig. 3. *Humidophila fukushimae* (A–D) and *Humidophila perpusilla* (E–G): scanning electron micrograph (SEM), internal valve view (A–D); internal overview of an entire valve (A–B), note the not quite T-shaped central raphe endings; the hymens lie on the inner surface of the transapical ribs, partly corroded (B), note the polar excavations (marked by arrow); details of apices with simply raphe endings and intercalated shorter striae (C); details of central part (D); external girdle view (E); valves of an entire frustulum with inside and outside view (F), note the mantle areolae located on the valve face (LOWE et al. 2014); external view of a valve (G). Scale bars represent 5 μm except for D where scale bar = 2 μm .

simple (Fig. 3C), proximal raphe endings not quite T-shaped (Fig. 3D). The cingulum consists of a few to many open bands (two bands on Fig. 2D, four bands on Fig. 1N), with one transverse row of round poroids (Fig. 1N, Figs. 2A–D). Irregular relief-like patches in the area between raphe and areolae can be observed (Fig. 1N, Figs. 2B–D) but no such structures are seen on Fig. 2A. Intercalated shorter striae are in the apices (Fig. 3C).

Taxonomical remarks: The valves of the Carpathian population are markedly smaller (14–21 μm) than is indicated in the description (18–25 μm). The width of the valves – a more stable feature of a species – is 4.5–6 μm (in the type population the width is 5–6 μm). The number of striae is higher (38–40/10 μm) than the 34–36/10 μm in the holotype population (WERUM and LANGE-BERTALOT 2000). Another difference, of deep polar excavations on either side of the sternum in the inside view, as documented by LANGE-BERTALOT and WERUM (2001) and WERUM and LANGE-BERTALOT (2004), can be observed only on a few valves; the majority of our population is without such a structure.

Humidophila fukushimae is similar in outlines to the most common *H. perpupilla* (Grunow 1860: 552) (LOWE et al. 2014). However *H. perpupilla* (Figs. 3E–G) is significantly smaller. *Humidophila perpupilla* co-occurred with *H. fukushimae* in Lake Negru.

Distribution: *Humidophila fukushimae* was originally described in Shenandoah National Park near »Hogback Overlook«, Virginia, USA. It was collected from a freshwater spring trickling over rocks (leg. Anja Broszinski, November 1999), and was dedicated to Hiroshi Fukushima on the occasion of his 77th birthday (LANGE-BERTALOT and WERUM 2001) in the journal *Diatom*. Three years later the description with the same photographic documentation was published (WERUM and LANGE-BERTALOT 2004) on page 136, Plate 62 Figs. 33–38, Plate 63 Figs. 1–7, and Plate 64 Figs. 1–4. Recently CANTONATI and SPITALE (2009) published a record away from its type locality, in Europe. They reported *H. fukushimae* from Spring Grotta Guernica, where this diatom also had very low relative abundance (0.2%), as in our samples.

Discussion

It is noteworthy that the representatives of genus *Humidophila* are known from aerial/subaerial habitats and most of the species were collected from springs and/or wet surfaces. We have found *H. fukushimae* in mountain lakes, furthermore from the deepest part of the lakes (water depth varied between 4.1 and 27.7 meter). It was the most frequent in a deep lake, Lake Negru. On the other hand, the sample from the lake bottom was rich in silt, perhaps as a result of erosion from the lakeshore, which could be an explanation for the unusual habitat. The obtained results confirm the presence of a typical and highly specific limnoterrestrial diatom flora in the high mountain lakes of the Carpathians.

The taxonomic revision of the diatom assemblages of poorly studied regions, such as Antarctica (e.g. VAN DE VIJVER et al. 2011) or Lake Baikal (KULIKOVSKIY et al. 2012), revealed a biogeographic pattern of several diatoms; they are not »cosmopolitan« with worldwide distribution, as has been generally accepted for a long time. Our finding somewhat contradicts the previous idea, as we found a species whose distribution to date was believed to be restricted to the American continent.

Humidophila fukushimae appears to be a peculiar species with regard to its valve outline and dimensions, and its valve face structure, which enabled us to identify it easily. It

can be considered a rare diatom species, and its discovery in the Carpathians draws attention to how restricted our knowledge of diatom distribution is, even in a well-investigated area like Europe. This new record also highlights the diatom species richness of high mountain regions and their importance for diatom biodiversity conservation.

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