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A new species of the genus *Adlafia* (Anomoeoneidaceae, Bacillariophyta) from Moscow Region (Russia) and North Macedonia

DMITRY CHUDAEV[1,3]* & ZLATKO LEVKOV[2,4]
[1]*Faculty of Biology, M.V. Lomonosov Moscow State University, Leninskie Gory 1, building 12, Moscow, 119234, Russia.*
[2]*Institute of Biology, Faculty of Natural Sciences, Ss Cyril and Methodius University in Skopje, Arhimedova 3, 1000 Skopje, Republic of North Macedonia.*
[3] *chudaev@list.ru; https://orcid.org/0000-0002-8407-6832*
[4] *zlevkov@pmf.ukim.mk; https://orcid.org/0000-0002-1184-2356*
**Corresponding author*

Abstract

Adlafia is a relatively small diatom genus in terms of number of species and consisting of small-celled species with delicate morphology that is barely visible under the light microscope. Only a few morphological characteristics recognizable under a light microscope can be used to identify species. This could lead to a broader species concept and misidentifications of species. Recent observations of the diatom flora of Russia and North Macedonia reveal the existence of a new *Adlafia* species. This study describes it based on light and scanning electron microscopy observations. The new species resembles *Adlafia minuscula*, but its valves are narrowly lanceolate, more slender and have a smaller width. The species is probably widely distributed in Europe, but was overlooked in earlier studies.

Key words: biodiversity, diatoms, freshwaters, morphology, taxonomy

Introduction

The diatom genus *Adlafia* Moser, Lange-Bertalot & Metzeltin (1998: 87) includes relatively small-celled symmetric naviculoid diatoms with areolae occluded externally with hymenes. Only five species including generitype *Adlafia muscora* (Kociolek & Reviers 1996: 201) Moser *et al*. (1998: 89) were transferred into the genus from *Navicula* Bory de Saint-Vincent (1822: 128) when *Adlafia* was established (Moser *et al*. 1998). Currently, the genus contains 34 taxa (Guiry & Guiry 2023). Several species have been transferred from *Navicula* (e.g. Metzeltin & Lange-Bertalot 1998, Lange-Bertalot & Genkal 1999, Van de Vijver *et al*. 2002, Bruder & Medlin 2008, Reid & Williams 2008, Monnier *et al*. 2012, Tusset *et al*. 2017, Heudre *et al*. 2018, Van de Vijver *et al*. 2019), with the majority described as new in recent decades from different world regions: South America (Metzeltin & Lange-Bertalot 1998, Tusset *et al*. 2017), Sub-Antarctic (Van de Vijver *et al*. 2002, Van de Vijver *et al*. 2013), North America (Morales & Le 2005), Asia (Kulikovskiy *et al*. 2012, Liu *et al*. 2017, Glushchenko *et al*. 2020, Radhakrishnan *et al*. 2023), Europe (Monnier *et al*. 2012), New Caledonia (Marquié *et al*. 2018, Le Cohu *et al*. 2020), Puerto Rico (Ciugulea *et al*. 2019). A few taxa were described from fossil materials (Benson & Kociolek 2012, Siver & Velez 2022). Many of the species have a geographically restricted distribution, and only a few are considered widespread such as *A. minuscula* (Grunow in Van Heurck 1880: pl. 14: fig. 3) Lange-Bertalot (in Lange-Bertalot & Genkal 1999: 32), *A. minuscula* var. *muralis* (Grunow in Van Heurck 1880: pl. 14: fig. 27) Lange-Bertalot (in Lange-Bertalot & Genkal 1999: 32) and *A. brockmanii* (Hustedt 1934: 382) Bruder & Hinz (in Bruder & Medlin 2008: 326). In general, it is very hard to assess whether species are widespread or have restricted distributions, since many *Adlafia* species are difficult to recognize under LM and there is a possibility that species have been misidentified or grouped under same name. Most of the *Adlafia* species have been observed in oligotrophic and subaerial habitats (Levkov *et al.* 2005, Morales & Le 2005, Monnier *et al*. 2012), including deep ancient lakes (Kulikovskiy *et al.* 2012). Very few species have been recorded in eutrophic waters (Cantonati *et al*. 2017).

In the course of the studies of diatom flora of Moscow Region (Russia) and Republic of North Macedonia populations of unknown *Adlafia* species were revealed. The aim of the present paper is to describe the morphology of

the taxon, formally describe it as a new species and compare it with other morphologically similar taxa for taxonomic purposes.

Material and methods

Material from the Moscow Region (Russia) was collected from vicinity of Lytkarino town on 27 April 2022 by D.A. Chudaev. The sample MW-D 979 is represented by washing from plant debris from the bottom of small (about 1.5 m in diameter) temporary spring puddle (N 55.57276°, E 37.93421°) in deciduous forest. Environmental parameters are the following: water temperature 14.5°C, pH 7.8, electric conductivity 370 µS/cm. The sample was fixed with ethyl alcohol (2:1) at the sampling site.

Sample from the North Macedonia was collected from a small temporary wetland near the cave Krasna, Mountain Jakupica (N 41.867835°, E 21.497891°) on 22nd May 2002 by Z. Levkov as wet mosses in the forested area (MKNDC 004431). The sample was fixed with 4% formaline at the sampling site.

The material from the Moscow Region was cleaned from organics by heating with concentrated (37%) hydrogen peroxide during 1 hour, with subsequent adding of a few drops of hydrochloric acid for 15 min. The material was rinsed with distilled water by centrifugation (4 times). Few drops of the suspension were dried onto coverslips at room temperature, and then embedded into aniline-formaldehyde medium with refraction index 1.67–1.68 (Elyashev 1957). Light microscopy (LM) investigation was performed at Levenhuk Med25t microscope with Opton 63× oil immersion objective (n.a.=1.4) and Levenhuk M1000 Plus digital camera. For scanning electron microscopy (SEM) coverslips with dried material were mounted on the surface of aluminium stubs with nail-polish, coated with Au-Pd in Eiko IB3 ion coater and studied under Quattro S (Thermoscientific). SEM working distance was 10 mm, accelerating voltage was 15 kV.

The material from the North Macedonia was cleaned with $KMnO_4$ and 37% HCl and the samples were boiled at 80 °C for 45 minutes. The samples were rinsed at least five times with distilled water and consequently centrifuged. The diatom slides were mounted with Naphrax and LM observations were made with Nikon Eclipse 80i microscope (Nikon Corporation, Japan), under oil immersion at 1000× magnification. Diatom images were captured using a Nikon Coolpix P6000 camera. For SEM, aliquots of diatom sample extracts were dried on coverslips attached to stubs with carbon tape and coated with a thin gold-palladium layer approximately 20 nm for 120 seconds with a sputtering current of 20 to 25 mA (Polaron SC7640 Sputter, Quorum Technologies, Ashford, UK). Scanning electron microscopy was performed at 5 kV and 5 mm working distance using a Zeiss Gemini Ultra plus SEM (Cambridge Instruments Ltd, Cambridge, UK).

Digital images were processed using ImageJ 1.45s (rotation, cropping, resizing, measurements) and GIMP 2.10.10 (plates arrangement). Morphological terminology follows Ross *et al*. (1979) and Gogorev *et al*. (2018). The material and slides are stored in the diatom collection of the Department of Mycology and Algology, Lomonosov MSU, Moscow (MW-D) and in Macedonian National Diatom Collection (MKNDC), Skopje, North Macedonia. The isotype slide was deposited in Komarov Botanical Institute (LE), Saint-Petersburg, Russia.

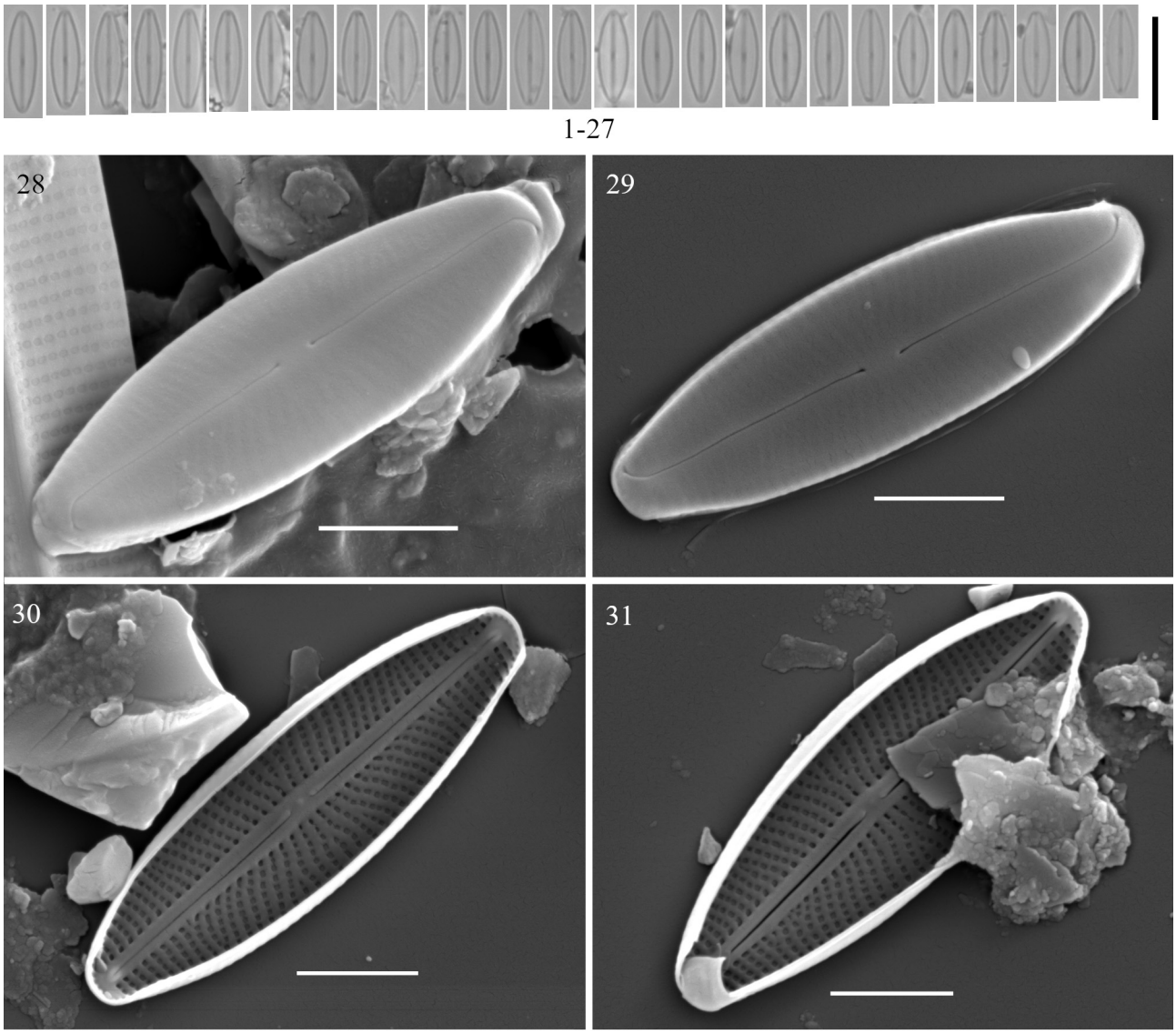
Results

Adlafia belyakovae Chudaev & Levkov *sp. nov.*

Description:—LM (Figs 1–27, 32–46): Valves lanceolate to linear-lanceolate with narrowly rounded to barely protracted ends, length 8.0–10.0 µm, width 2.5–3.2 µm, length to width ratio 2.7–3.7 (n=30, type material)[1]. Valve structure invisible in LM, except the median raphe sternum with barely distinguishable straight central raphe endings.

SEM, external valve surface (Figs 28–29, 47–51): Valve surface flat, without ridges or furrows. Striae and areolae barely visible due to external position of hymenes, lying at the same level as valve surface. Axial area narrow, linear; central area almost absent to slightly expressed and wider than axial area. Raphe branches almost straight or slightly undulated with slightly expanded central ends and unilaterally gently curved terminal fissures, sloping down to the mantle.

1 Population from the North Macedonia has following values of metric characters: length 8.0–9.5 µm, width 2.3–3.4 µm, length to width ratio 2.5–3.8 (n=72).



FIGURES 1–31. *Adlafia belyakovae sp. nov*., type material from the Moscow Region. 1–27. LM, slide MW-D 979s1, Fig. 1 represents the holotype. 28–29. SEM, external valve surface. 30–31. SEM, internal valve surface. Scale bars = 10 μm (1–27), 2 μm (28–31).

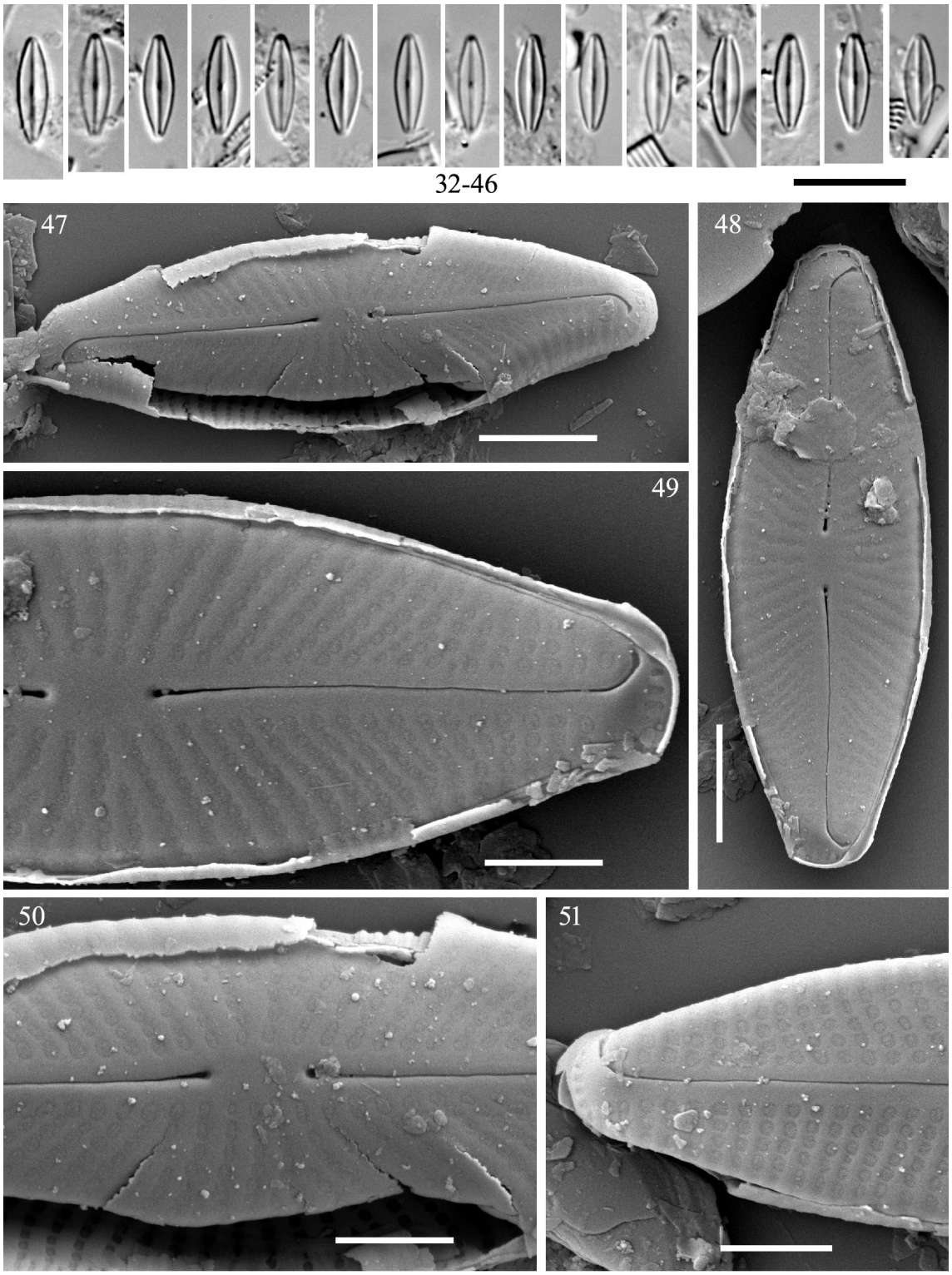
SEM, internal valve surface (Figs 30–31): Striae radiate in valve centre, becoming subparallel or slightly convergent towards apices; in central region shortened marginal striae present between the longer ones. Striae density 41–50 in 10 μm. Areolae openings square to circular, 59–72 in 10 μm. Single rows of areolae surround valve apices. Raphe-sternum linear, thickened. Raphe straight, with unilaterally deflected proximal endings, at the apices terminates in well-developed straight helictoglossae. Small knob-like round thickening presents between proximal raphe endings.

Type:—RUSSIA: Moscow Region, Lytkarino, small temporary spring puddle in deciduous forest, washing from plant debris, 55.57276° N 37.93421° E, *D.A. Chudaev, 27 April 2022* (Holotype: MW-D! slide # 979s1 = Fig. 1, isotype: LE A0004241).

Etymology:—The species is thankfully dedicated to Dr. Galina Alekseevna Belyakova, the author of many Russian phycological textbooks and the first phycology teacher of D.A. Chudaev.

Distribution and ecology:—The species has been observed in its type locality and also a population was found in North Macedonia. The type population originates from a shallow temporary waterbody with slightly alkaline water (pH 7.8) and medium electrolyte content (370 μS/cm). Accompanying diatom flora is represented by *Nitzschia acidoclinata* Lange-Bertalot (1976: 277), *Nitzschia palea* var. *debilis* (Kützing 1844: 65) Grunow (in Cleve & Grunow 1880: 96), *Pinnularia marchica* I.Schönfelder (in Krammer 2000: 218), *Pinnularia sinistra* Krammer (1992: 175), *Sellaphora nigri* (De Notaris in De Notaris & Baglietto 1872: num. 632) Wetzel & Ector (in Wetzel *et al*. 2015: 221), *Sellaphora subseminulum* (Hustedt in Brendemühl 1949: 441) Wetzel (in Wetzel *et al*. 2015: 218). Besides diatoms the slide contains a lot of chrysophycean cysts. The population from North Macedonia was found in a small temporary

wetland near the cave Krasna, Mountain Jakupica. Diatom flora is represented by *Navicula cryptocephala* Kützing (1844: 95), *Gomphonema varioreduncum* Jüttner, Ector, Reichardt, Van de Vijver & Cox (in Jüttner *et al*. 2013: 306), *Nitzschia palea* var. *debilis*, *Nitzschia alpina* Hustedt (1943: 232), *Planothidium lanceolatum* (Brébisson ex Kützing 1846: 247) Lange-Bertalot (1999: 287), *Meridion circulare* (Greville 1822: 213) Agardh (1831: 40) and *Fragilaria* Lyngbye (1819: 182) sp.



FIGURES 32–51. *Adlafia belyakovae sp. nov.*, population from the North Macedonia. 32–46. LM, slide MKNDC 004431. 47–51. SEM, external valve surface. 47–48. Whole valve. 49. Half-valve with centre and apex. 50. Central part of the valve from Fig. 47 at higher magnification. 51. Valve apex. Scale bars = 10 µm (32–46), 2 µm (47, 48), 1 µm (49–51).

Discussion

The new species can be placed into the genus *Adlafia*. There is no contradiction between its character combination and characters mentioned in the genus description (Moser *et al*. 1998, Lange-Bertalot 2001). *Adlafia belyakovae* has small-sized naviculoid valves (8.0–10.0 μm ×2.5–3.2 μm), with very dense uniseriate striae, composed of externally occluded areolae, filiform raphe with scarcely expanded central pores and strongly deflected terminal fissures. Several small-celled *Adlafia* species share these ultrastructural features.

TABLE 1. Comparison of *Adlafia belyakovae* with morphologically similar taxa.

Taxon name	Valve outline	Valve dimensions, μm	Number of striae in 10 μm	Reference
A. belyakovae	lanceolate to linear-lanceolate with narrowly rounded to barely protracted ends	8.0–10.0×2.5–3.2	41–50	This study
A. baicalensis	linear with broadly rounded, at most very weakly protracted ends	12.7–13.0×3.3–3.5	34–35	Kulikovskiy *et al.* 2012
A. diahotensis	linear-lanceolate to almost elliptical with wedge-shaped obtusely rounded ends	9.5–16×2.8–3.8	36–40	Le Cohu *et al.* 2020
A. langebertalotii	linear-lanceolate to elliptic-lanceolate with rounded ends	7.3–11.9×2.0–2.6	30–32(34)	Monnier *et al.* 2012
A. minuscula var. *minuscula*	narrow- or rhombic-elliptical to elliptic-lanceolate with slightly protracted and obtusely rounded ends	10–16×3.2–4.8	35–45	Lange-Bertalot 2001
A. minuscula var. *muralis*	broadly elliptical with broadly rounded ends	8–12×4–5.5	no data	Lange-Bertalot 2001
A. multnomahii	lanceolate with capitate to rostrate ends	9–16×4–5	37–45	Morales & Le 2005
A. neoniana	elliptic-lanceolate with rostrate to subcapitate ends	9.4–18.5×3.7–5.1	30–32	Ciugulea *et al*. 2019
A. pseudobaicalensis	linear-elliptical-lanceolate to linear with broadly, shortly protracted at most somewhat subrostrate ends	11.5–14.7×2.7–3.3	32–34	Kulikovskiy *et al.* 2012
A. suchlandtii	linear with indistinctly protracted, obtusely rounded ends	12–15×2.5–3	26–28	Lange-Bertalot 2001
A. submuscora	lanceolate to narrowly lanceolate with bluntly rounded, subrostrate ends	10–12×2.5–3.1	40–45	Van de Vijver *et al*. 2013
A. tellerensis	linear elliptical to lanceolate with broadly rounded ends	15–22×4.5–6.0	16–18	Benson & Kociolek 2012
Navicula pseudoexilissima	elliptical with narrowly rounded ends	8.5×3.5	-	Hustedt 1948

Adlafia belyakovae is morphologically most similar to *A. minuscula* var. *minuscula* and *A. minuscula* var. *muralis*. The main difference of *A. belyakovae* from these taxa is smaller valve width (2.6–3.2 μm vs. 3.2–4.8 μm in *A. minuscula* var. *minuscula* and 4.0–5.5 μm in *A. minuscula* var. *muralis*, Lange-Bertalot 2001). Valves of *A. belyakovae* are also more slender (length to breath ratio is higher). Another similar taxon is *Navicula pseudoexilissima* Hustedt (1948: 46), described from garden pool in Plön, Germany. The species is considered a synonym of *A. minuscula* var. *muralis* by Lange-Bertalot & Rumrich (1981), despite some differences between these two taxa regarding valve size. *Navicula pseudoexilissima* is characterized by elliptic-lanceolate valves (not narrowly lanceolate as in *A. belyakovae*) with slightly separated and rounded apices (opposite to narrowly rounded in *A. belyakovae*) and valve width 3.5–4.0 μm (2.5–3.2 μm in *A. belyakovae*). *Adlafia submuscora* Van de Vijver, Kopalová, Zidarova & E.J.Cox (2013: 191) has been described from James Ross Island, Maritime Antartic region and has similar valve size (10–12 μm long and 2.5–3.1 μm wide) and shape (lanceolate to narrowly lanceolate). Differences can be noticed in the shape of the apices (subrostrate and broadly rounded in *A. submuscora*). *Adlafia suchlandtii* (Hustedt 1943: 168) Monnier & Ector (in Monnier *et al*. 2012: 139) differs from *A. belyakovae* by linear valves with parallel margins and coarser striae (26–28 in 10 μm) resolvable in LM (Lange-Bertalot 2001).

Adlafia diahotensis Le Cohu, Marquié & Tudesque (2020: 2) described from New Caledonia differs from *A. belyakovae* by coarser striae, resolvable in LM (36–40 in 10 μm vs. 41–50 in 10 μm); the striae in *A. diahotensis* are more strongly convergent towards valve ends; another differentiating feature is the absence of small knob between internal proximal raphe endings in *A. diahotensis*. European species *Adalfia langebertalotii* Monnier & Ector in Monnier *et al*. (2012: 133) differs from *A. belyakovae* by general valve outline (parallel to weakly constricted vs. convex valve margins), valve length to breadth ratio up to 5.3, coarser striae (still resolvable in LM, 30–34 in 10 μm) and areolae (50–55 in 10 μm). Fossil species *Adlafia tellerensis* Benson & Kociolek (2012: 53) in comparison to *A. belyakovae* has larger valves (length 15–22 μm, width 4.5–6.0 μm) with much coarser striation (16–18 in 10 μm) easily distinguishable in LM.

Four new *Adlafia* species were recently described from Vietnam (Glushchenko *et al*. 2020), but all of them have linear valves with distinctly protracted to capitate apices, thus can be easily differentiated from *A. belyakovae*. Similarly, *A. neoniana*, described by Cantonati (in Ciugulea *et al*. 2019), has elliptic-lanceolate valves with rostrate to subcapitate apices and is 3.7–5.1 μm wide. *Adlafia multnomahii* Morales & Le (2005: 151) also has capitate to rostrate apices and thus can be easily differentiated from *A. belyakovae*. *Adlafia pseudobaicalensis*, described by Kulikovskiy & Lange-Bertalot in Kulikovskiy *et al*. (2012: 37) from the Lake Baikal, has widely protracted valve ends, lower striae density (32–34 in 10 μm) and slightly longer valves (11.5–14.7 μm) compared to *A. belyakovae* (Kulikovskiy *et al*. 2012). *Adlafia baicalensis* Kulikovskiy & Lange-Bertalot in Kulikovskiy *et al*. (2012: 36) possesses different valve outline (valves only barely narrowing towards widely rounded apices) and coarser striation (34–35 in 10 μm) resolvable in LM. Comparison of *Adlafia belyakovae* with morphologically similar taxa is provided in Table 1.

Our data indicate that *A. belyakovae* was found in geographically distant regions of Europe (Moscow Region and North Macedonia). The taxon was also illustrated from Germany by Reichard (2018, Taf. 128: 28–35) as "Sippe mit schlankeren Schalen" of *A. minuscula*. So, it may be assumed that the species is widely distributed in Europe, but previously was overlooked due to small valve dimensions and similarity to *A. minuscula*.

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