

Biogeography and morphology of a poorly known diatom *Dorofeyukea rostellata* (Hustedt) Kulikovskiy & Kociolek

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ABSTRACT

Biogeography and morphology of a poorly known diatom *Dorofeyukea rostellata* (Hustedt) Kulikovskiy & Kociolek

Dorofeyukea rostellata is a rare and poorly known representative of *Dorofeyukea* genus. To date, this taxon has only been recorded only outside of Europe. In two Croatian lakes, we found a diatom in appearance similar to *Dorofeyukea rostellata* and within corresponding physical and chemical conditions. Due to the uncertain taxonomical position of this taxon, type material of *Dorofeyukea rostellata* and a closely related species, *Navicula grimmeioides*, were analysed by means of light and scanning electron microscopy. Upon a detailed investigation, we could clearly distinguish the Croatian population from *N. grimmeioides*. However, the Croatian population and the type material of *D. rostellata* differed only in minor characteristics. The most obvious difference is the shape of areolae. Nevertheless, differences between the shapes of the areolae can be caused by the degree of silicification, which often depends on the environmental conditions. Since almost every other characteristic of the Croatian population overlapped with that of the type material of *D. rostellata* we identified it as *D. rostellata*. Additionally, scanning electron microscopy analyses also revealed that *N. grimmeioides* belongs to the genus *Dorofeyukea*, so its new combination was proposed.

Key words: type material, *Dorofeyukea rostellata*, *Navicula grimmeioides*, calcareous habitat, new combination

RESUMEN

Biogeografía y morfología de una diatomea poco conocida *Dorofeyukea rostellata* (Hustedt) Kulikovskiy & Kociolek

Dorofeyukea rostellata es un representante raro y poco conocido del género *Dorofeyukea*. Hasta la fecha, esta especie solo ha sido registrada fuera de Europa. En dos lagos croatas, encontramos una diatomea en apariencia similar a *Dorofeyukea rostellata* y en las condiciones físicas y químicas correspondientes. Debido a la incierta posición taxonómica de este taxón, el material tipo de *Dorofeyukea rostellata* y una especie estrechamente relacionada, *Navicula grimmeioides*, se analizaron mediante microscopía de luz y electrónica de barrido. Tras una investigación detallada, pudimos distinguir claramente la población croata de *N. grimmeioides*. Sin embargo, la población croata y el tipo de material de *D. rostellata* diferían solo en características menores. La diferencia más obvia es la forma de las areolas. Sin embargo, las diferencias pueden deberse al grado de silicificación, que a menudo depende de las condiciones ambientales. Como casi todas las demás características de

la población croata se superponían con del material tipo de *D. rostellata*, lo identificamos como *D. rostellata*. Además, los análisis de microscopía electrónica de barrido también revelaron que *N. grimmeioides* también pertenece al género *Dorofeyukea*, por lo que se propuso una nueva combinación.

Palabras clave: ejemplares tipo, *Dorofeyukea rostellata*, *Navicula grimmeioides*, hábitat calcáreo, nueva combinación

INTRODUCTION

Navicula grimmei var. *rostellata* Hustedt (1937, p. 236, fig. 13) was described from Indonesia in 1937 as a variety of *Navicula grimmei* Krasske. Kulikovskiy *et al.* (2019) transferred them into a newly erected genus *Dorofeyukea*, which was described on the basis of DNA sequence and morphological data as *Dorofeyukea rostellata* (Hustedt) Kulikovskiy & Kociolek and *Dorofeyukea grimmei* (Krasske in Hustedt) Kulikovskiy & Kociolek.

Distribution of *Dorofeyukea rostellata* is sporadic but wide and disparate (Fig. 1a); it was first recorded in the tropical equatorial lakes of Central Java and in warm springs in Central Sumatra (Hustedt, 1937). It was also recorded from Antarctica, in the sediment from King George Island (Kim & Park, 1988) as *Navicula grimmei* var. *rostellata*. The identity of the diatom documented by Kim & Park (1988) is ambiguous. However, the species found by Kim & Park is not *Dorofeyukea rostellata* based on the attached images according to Kim & Park's publication (1988). Consequently, we put it on the world map as formally noticed occurrence. Another occurrence of this taxon comes from Ghana (Smith *et al.*, 2015).

To study and assess the ecological potential of Croatian reservoirs, a country-wide survey was organised in 2016 and 2017. In some of the benthic samples, we found a diatom population similar to the members of genus *Dorofeyukea* with characteristic isodiametric areola (Potapova, 2013). We referred to it as 'Croatian population' in the followings. The most characteristic features of the taxon resembled *Dorofeyukea grimmei*. Consequently, we overviewed the taxa closely related to *Dorofeyukea grimmei*. Based on main characters we concluded that the reinvestigation of the original material is necessary to confirm the identification. The analyses of the

type materials of *Dorofeyukea rostellata* and *Navicula grimmeioides* H.P. Gandhi in light (LM) and scanning electron microscopy (SEM) were carried out to clarify the identity of the Croatian diatom taxon. Our goal was to give an emended description of the ultrastructure of the studied taxa.

MATERIALS AND METHODS

Sampling

The Croatian standard diatom sampling methodology in water quality assessments of rivers and lakes was used in Croatia (Narodne Novine, 73/2013, 151/2014 and 78/2015). Sampling was performed in two artificial lakes Ričice and Tribalj following the "single habitat sampling" principle (Barbour *et al.*, 1999). As there were no stones as representative microhabitats at either of the sampling points, mud in the Lake Ričice (43.51065 °N, 17.11951 °E) and technolital (concrete) in Lake Tribalj (at 45.22876 °N, 14.66736 °E and 45.22651 °N, 14.67109 °E sampling points) were sampled on 22th July and 8th September in 2016, respectively (Fig. 1b). Five sub-samples were sampled at each sampling site. All samples were preserved with 4 % formaldehyde.

The following environmental variables were recorded at each sampling point during the sampling: water temperature (°C), pH, electric conductivity (µS/cm), dissolved oxygen (mg/L), oxygen saturation (%). Other variables were detected only in one point in each lake: alkalinity (mg/L), total hardness (mg/L), turbidity (NTU), BOD₅ (mg/L), COD-Mn (mg/L), NH₄-N (mg/L), NO₂-N (mg/L), NO₃-N (mg/L), Total N (mg/L), PO₄-P (mg/L), Total P (mg/L), Ca (mg/L), Mg (mg/L) and SiO₂ (mg/L) as per standard methods for water analysis (CEN, 2014). The measured values are presented in Table 1.

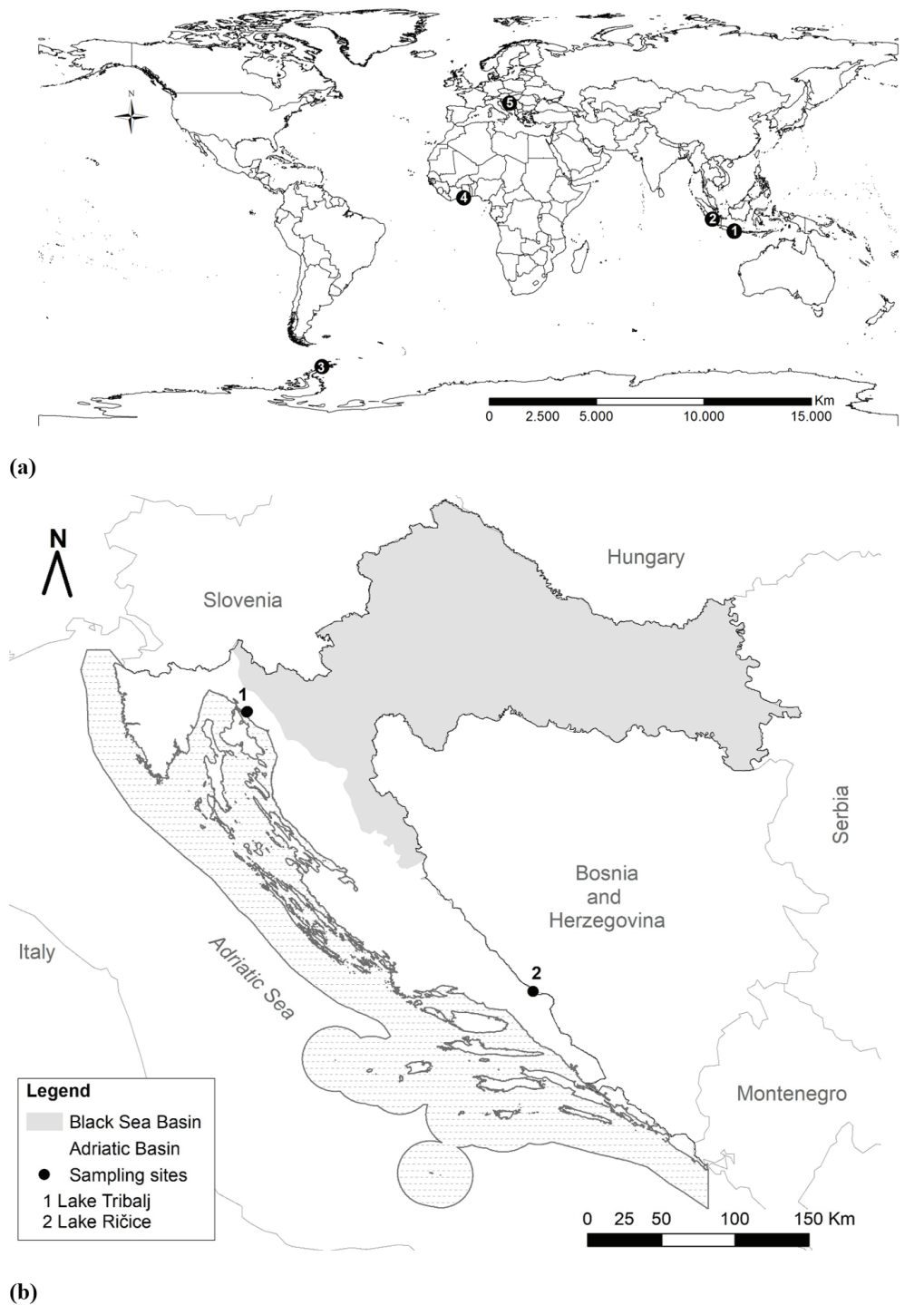


Figure 1. (a) World map distribution of *Dorofeyukea rostellata* (1 – Central Java, 2 – Central Sumatra, 3 – Antarctica (King George Island) (The species was recorded here but that must have been misidentification. However, we put it on the map because this occurrence of the species had formally been published.), 4 – Ghana, 5 – Lake Tribalj, Lake Ričice). *Distribución mundial de Dorofeyukea rostellata*. (b) *Sampling localities (1 - Tribalj and 2 - Ričice lakes, Croatia). Sitios de muestreo.*

Table 1. Physical and chemical variables in sampling sites on the sampling days. *Variables físicas y químicas en sitios de muestreo en los días de muestreo.*

	Ričice	Tribalj
Water Temperature (°C)	27.5	22.3
pH	7.66	7.33
Conductivity (μS/cm)	255	243
Dissolved oxygen (mg/L)	7.67	8.78
Oxygen saturation (%)	102.3	102.3
Alkalinity (mg/L)	140.1	118
Total hardness (mg/L)	150.2	149
Turbidity (NTU)	8.35	11.7
BOD ₅ (mg/L)	0.93	1.5
COD-Mn (mg/L)	1.25	2
NH ₄ -N (mg/L)	0.075	0.041
NO ₂ -N (mg/L)	0.024	0.003
NO ₃ -N (mg/L)	0.155	0.19
Total N (mg/L)	0.892	0.41
PO ₄ -P (mg/L)	0.0014	0.0031
Total P (mg/L)	0.003	0.028
Ca (mg/L)	50.26	48.3
Mg (mg/L)	5.88	6.77
SiO ₂ (mg/L)	3.35	3.6

Microscopic investigations

In the laboratory, samples were rinsed with distilled water to remove formaldehyde and subsequently treated with hot H₂O₂ and mounted in Naphrax®. Slides were analysed with the Olympus IX-70 light microscope (1500×). At least 500 frustules were counted for the Croatian samples.

Using LM 46 valves of *Dorofeyukea rostellata* were documented in the type material and further 9 specimens in SEM. The type material of *Navicula grimmeioides* was observed with an Olympus BX53 DIC microscope equipped with Olympus DP73 camera and cellSens 1.13 imaging software.

A part of the cleaned and washed samples was filtered through a 3 μm Isopore™ polycarbonate membrane filter (Merck Millipore, Germany) for SEM studies. The filter was fixed onto a stub using double-sided carbon tape and coated with gold using a rotary-pumped sputter coater

Quorum Q150R S (ZEISS). Fine structures of diatom frustules were observed with Zeiss EVO MA 10 SEM (ZEISS) operated at 10 kV and 10 mm distance using SEM detectors in case of Croatian samples and type material of *Dorofeyukea rostellata*. For scanning electron microscopy of type material of *Navicula grimmeioides*, processed samples were air dried on the cover glass and pasted over the aluminium stubs using double-sided carbon tape. Samples were gold coated for approximately 3 minutes using an Emitech K575X sputter coater and observed with a Zeiss EVO 50 Lab 6 Scanning Electron Microscope. Observations were made under 15–20 kV acceleration voltages at a 5–8 mm Working Distance. Micrographs were recorded using Zeiss SmartSEM software. Gandhi's samples used in this study are housed at Agharkar Research Institute's Diatom Herbarium, Pune. All statistical analyses were performed in R version 3.4.2 implemented in Windows. Maps were generated using ArcMap 10.1.

Type material of *Dorofeyukea rostellata* investigated: AS 745 (lectotype), 7.3.1929 (under the name *Navicula grimmei* var. *rostellata*).

LM images were taken from the powder of the lectotype material washed before mounting into Naphrax®.

The studied slides of the Croatian population of *Dorofeyukea rostellata* are deposited in the Hungarian Natural History Museum BP-NHMUS-ALG 2300-2305.

RESULTS

Investigation of the Croatian population (Figs. 2-13, Figs. 38-57)

In LM the outline of symmetrical valves is lanceolate with slightly capitate or broadly rounded apices (Figs. 2-13). The valve length is 12.8–30.8 μm (mean = 18±4.0 μm, n = 26) and the width is 5.3–7.3 μm (mean = 6.1±0.5 μm, n = 26). Striae are radial, (slightly) convergent on the apices. The number of striae is 19–25 in 10 μm (mean = 22±1.3, n = 26). Central area is slightly asymmetric bowtie-shaped. Axial area is narrow. Raphe is filiform. There is no stigma on the surface of the valve.

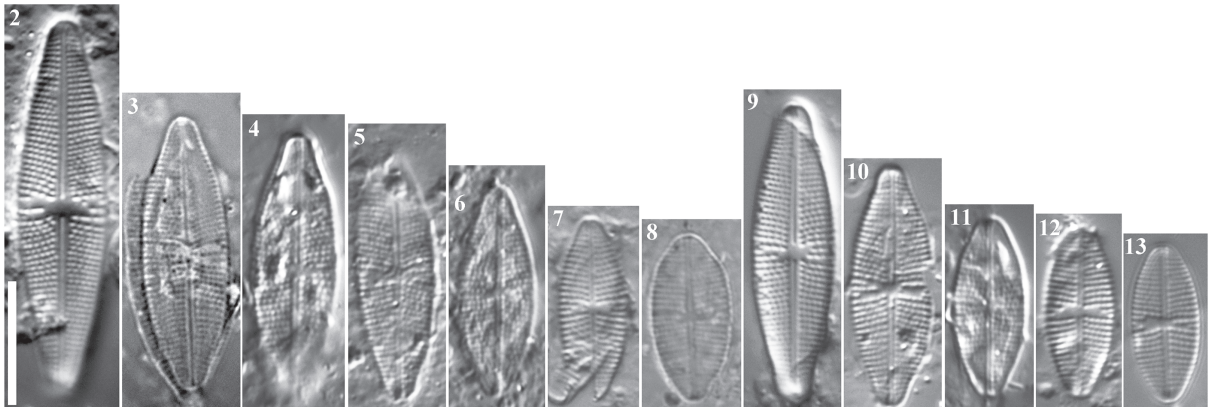


Figure 2-13. *Dorofeyukea rostellata* found in the Croatian lakes. LM: (Figs 2-8) from Ričice, (Figs 9-13) from Tribalj. Scale bar = 10 μ m. *Especies encontradas en los embalses de Croacia. MO: (Figs 2-8) de Ričice, (Figs 9-13) de Tribalj. Barra de escala = 10 μ m.*

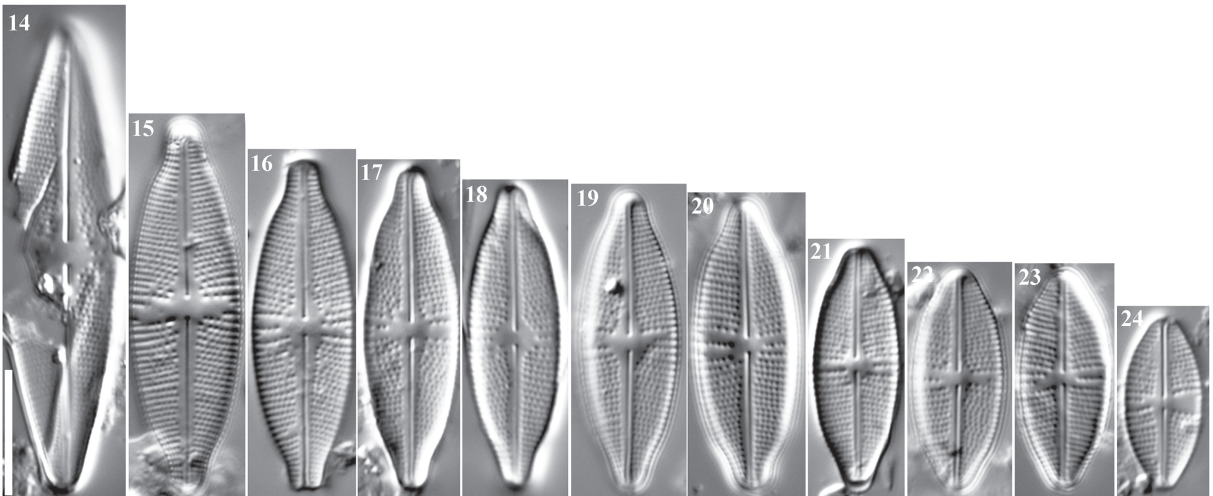


Figure 14-24. *Dorofeyukea rostellata*. LM micrographs of the type material. Scale bar = 10 μ m. *Dorofeyukea rostellata. Micrografías MO del material tipo. Barra de escala = 10 μ m.*

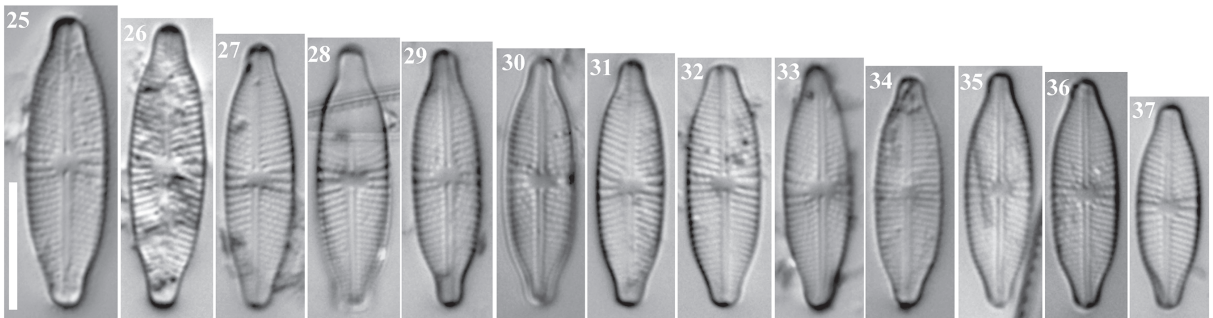


Figure 25-37. *Dorofeyukea grimmeioides*. LM micrographs of type material. Scale bar = 10 μ m. *Dorofeyukea grimmeioides. Micrografías MO del material tipo. Barra de escala = 10 μ m.*

In SEM (Figs. 38-57), striae are composed of single rows of elongated areolae, which continue on the mantle in several rows (Figs. 38, 41, 43). On the central area there are (1)2-3(4) striae creating an irregular hyaline field (Figs. 40, 42, 44, 56). In the middle part of the valve the interstriae can be wider or narrower (Figs. 41, 42, 44, 45). Externally, the areolae are rectangular, occa-

sionally transversally elongated at the central part (Fig. 55) and slit-like on the apices (Figs. 52-54). The proximal raphe fissures are straight and slightly drop-shaped.

Internally, the areolae are closed by hymens (Fig. 57). Most of them are also elongated, some of them are dot shaped (Figs. 56, 57). Number of areolae is (24) 25-30 in 10 μm (mean = 27.7 ± 1.9 ,

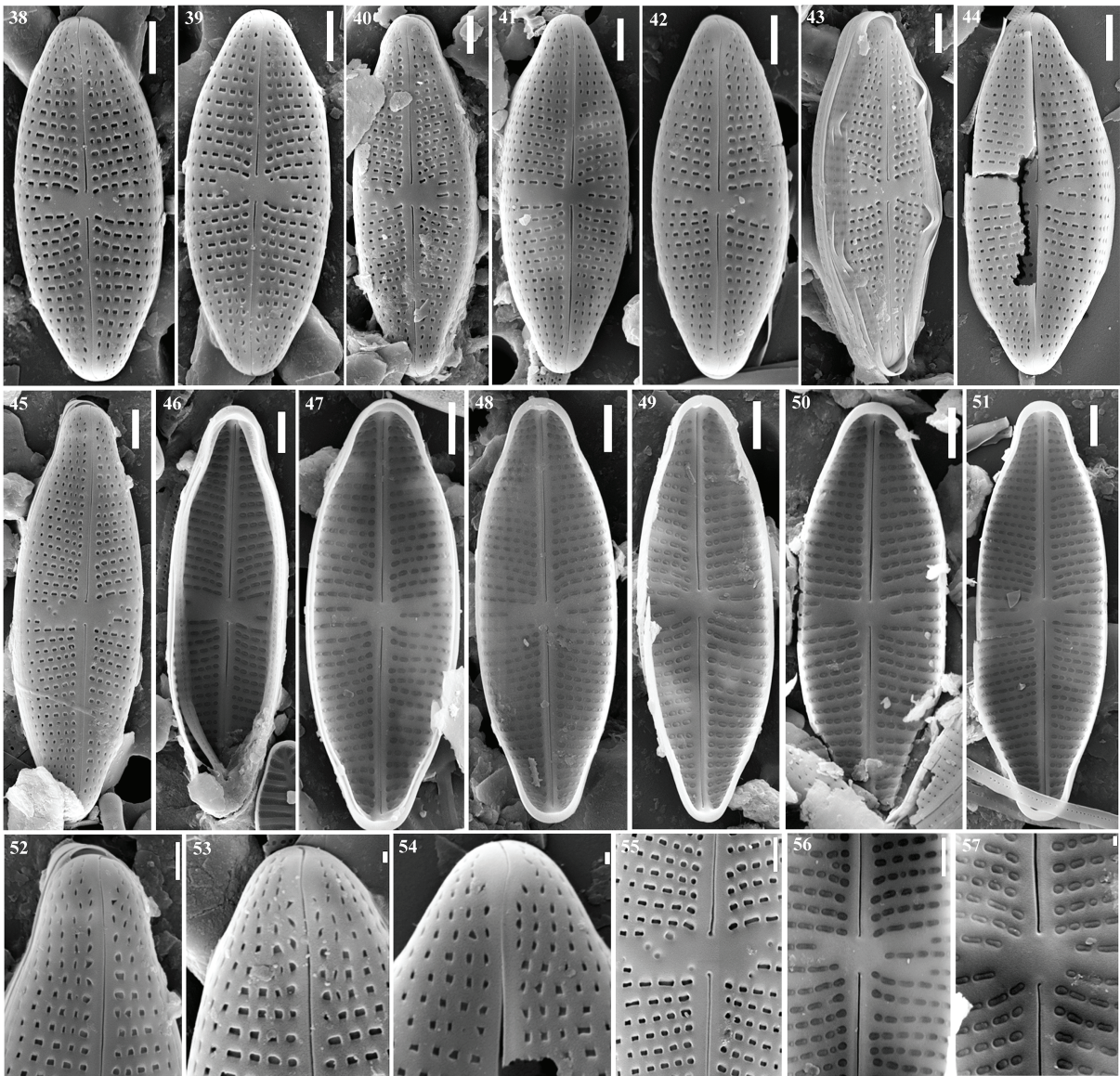


Figure 38-57. *Dorofeyukea rostellata* found in the Croatian lakes. SEM micrographs from Tribalj Lake. Scale bar = 2 μm for Figs 38-51; 1 μm for Figs 52, 55, 56 and 200 nm for Figs 53, 54, 57. *Especies encontradas en los lagos croatas. Fotografías MEB del lago Tribalj. Escala = 2 μm para las figuras 38-51; 1 μm para las figuras 52, 55, 56 y 200 nm para las figuras 53, 54 y 57.*

$n = 14$). The axial area is relatively broad (Figs. 38-51). Internally, the raphe fissures are straight, terminating in small helictoglossae near the poles (Figs. 46-51). Narrow pseudoseptum is present on both apices (Figs. 46-51).

Investigation of type material of *Dorofeyukea rostellata* (Hustedt) Kulikovskiy & Kociolek (Figs. 14-24, 58-67)

In LM the valve is lanceolate with capitate or rostrate apices. The length of the valve is $14.6\text{--}39.1\text{ }\mu\text{m}$ (mean = $24.1 \pm 4.5\text{ }\mu\text{m}$, $n = 55$), the width is $6.6\text{--}10.4\text{ }\mu\text{m}$ (mean = $8.1 \pm 0.8\text{ }\mu\text{m}$, $n = 55$). Striae are radial, becoming slightly convergent on the apices. Number of striae is 17-25 in $10\text{ }\mu\text{m}$ (mean = 20.2 ± 1.4 , $n = 55$) and it can be denser toward the ends. Areolae are thin and drop-like and can easily be counted in LM. The number of areolae is 20-32 in $10\text{ }\mu\text{m}$ (mean = 24.5 ± 2.4 , $n = 55$). The raphe is straight. There is no stigma on the valve surface.

In SEM externally, the areolae are transapically slit-like continuing on the mantle (Fig. 66). Internally, the areolae are mostly round, some of them are oval; they are closed by hymen (Figs. 63-65, 67). The striae can be shorter or longer near the central area and become denser toward the ends. Striae are radial, slightly convergent on the apices. The axial area is relatively broad and become wider near the field of central area (Fig. 63). Raphe is straight; externally, distal raphe endings are bent to the same side (Figs. 58-62). The proximal raphe fissures are straight and slightly drop-shaped on the outer valve (Fig. 66), dot-like on the inner valve (Fig. 67). Internally, the distal raphe endings are straight, terminating in small helictoglossae near the poles (Figs. 63-65). Central area is transapically oval outside and fairly irregular inside.

Holotype: Ground slime of Telaga Pasir, middle Java. Isotype: warm springs of Kadjaj, central Sumatra. Hustedt F. 1937. *Arch. Hydrobiol. Suppl.* 15: 236, pl. 17, fig. 13. QH90A67s, Figured also in Schmidt's Atlas, pl. 405, figs 30-34.

Lectotype: N3/45. Sumatra. Warme Quelle v. Kadjaj. SKW2ba, Simonsen, R. 1987. Atlas and catalogue of the diatom types of Friedrich

Hustedt: 201, pl. 310, figs 6-10 (this material was investigated and illustrated in our study).

Isolectotype: N3/46. Sumatra. Warme Quelle v. Kadjaj. SKW2ba, Simonsen, R. 1987. Atlas and catalogue of the diatom types of Friedrich Hustedt: 201.

Investigation of type material of *Navicula grimmeioides* H.P. Gandhi (Figs. 25-37, 68-81)

In LM, the valve outline is lanceolate with capitate or rostrate apices. The length of the valve is $16.8\text{--}22.6\text{ }\mu\text{m}$ (mean = 18.7 ± 2.0 , $n = 20$), the width is $5.2\text{--}6.1\text{ }\mu\text{m}$ (mean = 5.4 ± 0.4 , $n = 20$). Striae are radial throughout the valve, their number is 18-26 in $10\text{ }\mu\text{m}$ (mean = 21.4 ± 2.7 , $n = 20$). They are composed of observable but hardly to analyse coarse areolae. Central area is round. The raphe is straight. There is no stigma on the valve.

In SEM, externally, the areolae are transapically elongated, thin or slit-like (Figs. 77, 78). The number of areolae is 25-35 in $10\text{ }\mu\text{m}$ (mean = 32.1 ± 3.2 , $n = 10$). They are arranged in single rows of striae that continue on the mantle (Figs. 78, 81). Central area is round. Axial area is relatively broad and irregular. Raphe runs straight with bending distal endings. The proximal raphe fissures are strongly drop-shaped (Fig. 72).

Internally, the areolae are mostly round, some are oval which are closed by a hymen (Figs. 69, 70, 73, 79-81). The distal raphe endings are straight (Figs. 77, 78), terminating in small helictoglossae near the poles (Fig. 79). Proximal raphe fissures are slightly deflected to the same side (Figs. 74, 75).

Lectotype (designated here):—[illustration] Figs. 73-75 in p. 41, pl. 2 in Gandhi (1998: 324).

Type material: Lake Chandola border side with *Ceratophyllum*, Ahmedabad, India. Sample collected by H.P. Gandhi on 17 April 1960. Gandhi labelled this sample as "Sr-185 Ahmedabad: Lake Chandola border side with *Ceratophyllum*, a further spot cleaned 17-4-1960". Gandhi, H.P. (1998). Fresh-water Diatoms of Central Gujarat. Dehra Dun, India: Bishen Singh Mahendra Pal Singh, pl. 2, figs. 73-75.

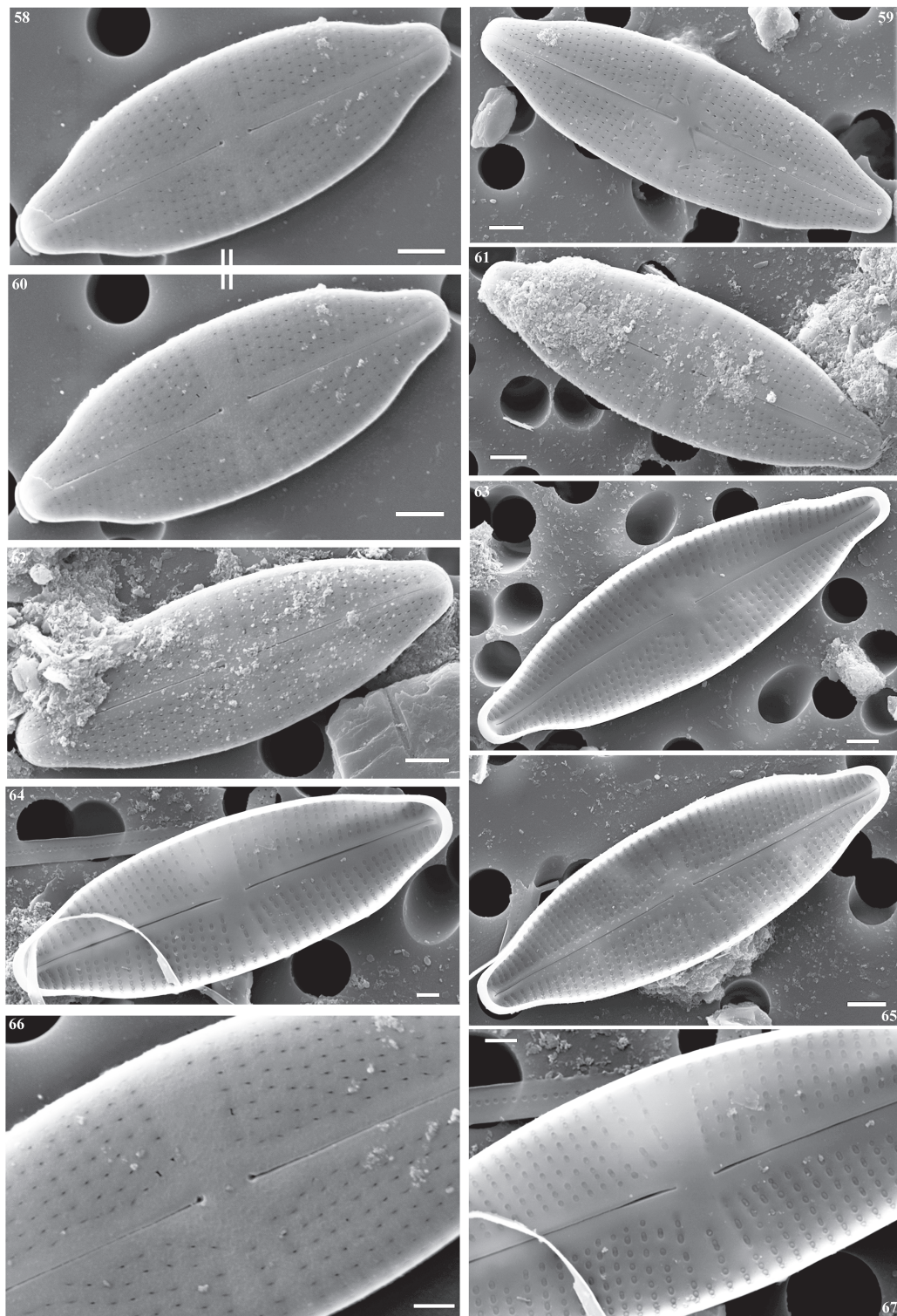


Figure 58-67. *Dorofeyukea rostellata*. SEM micrographs of the type material. Scale bar = 2 μm for Figs 58-63, 65; 1 μm for Figs 64, 66, 67. *Dorofeyukea rostellata*. Micrografías MEB del material tipo. Barra de escala = 2 μm para las figuras 58-63, 65; 1 μm para las figuras 64, 66, 67.

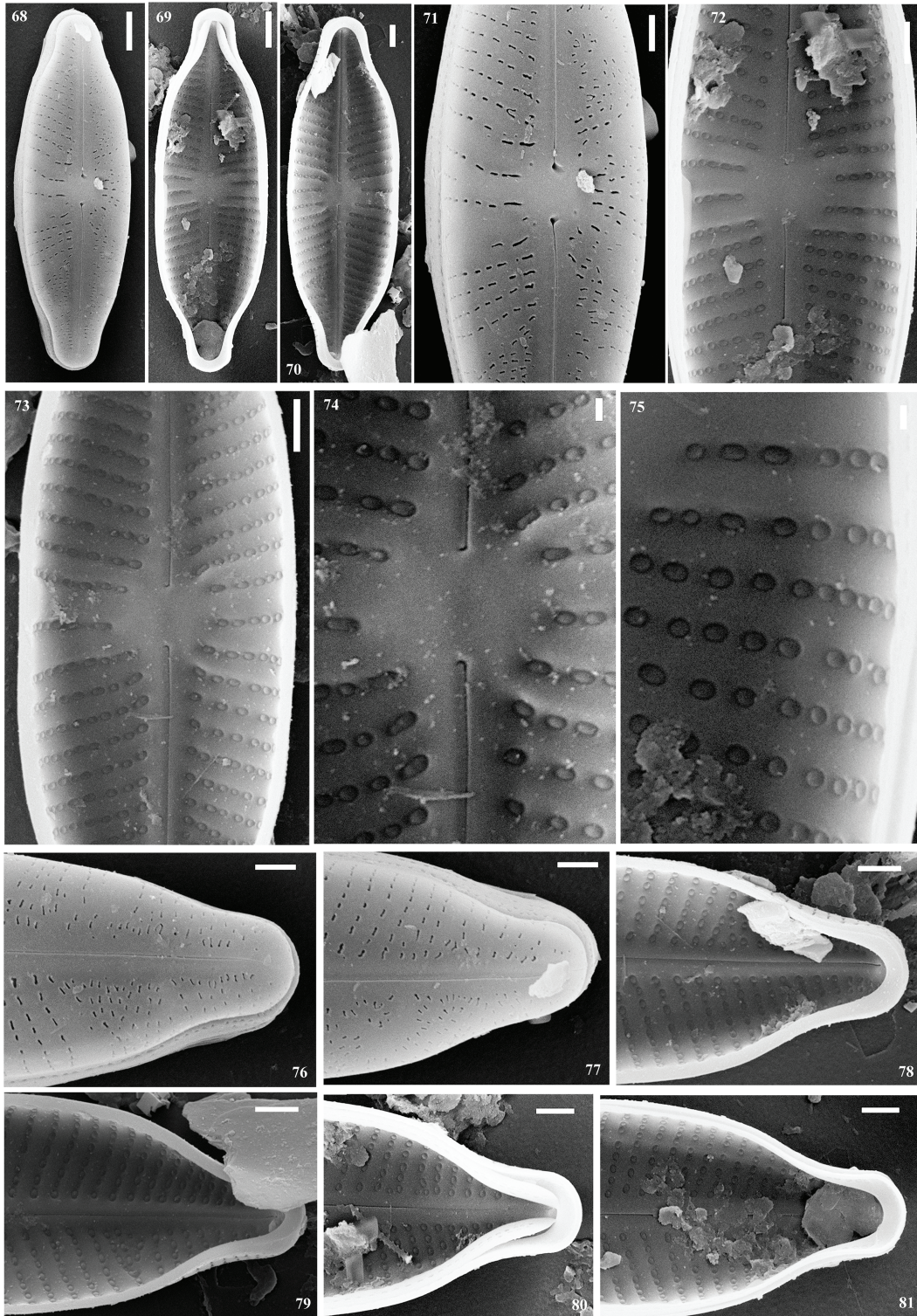


Figure 68-81. *Dorofeyukea grimmeioides*. SEM micrographs of the type material. Scale bar = 2 μm for Figs 68, 69; 1 μm for Figs 71-73, 76-81; 200 nm for Figs 74, 75. *Dorofeyukea grimmeioides*. Micrografías MEB del material tipo. Barra de escala = 2 μm para las Figuras 68, 69; 1 μm para las figuras 71-73, 76-81; 200 nm para las figuras 74, 75.

Habitat and ecology

Dorofeyukea rostellata populations were found in two Croatian lakes with similar physical and chemical characteristics (Table 1). Both lakes were oligotrophic, oligosaprobic and situated in calcareous bedrock; COD values were below 2 mg/L and oxygen saturation was always more than 85 %. The water of the lakes is neutral to slightly alkaline with pH 7.66 (Ričice), 7.12 and 7.53 (Tribalj1, Tribalj3, respectively) and with low conductivity (255 μ S/cm for Lake Ričice; 241 μ S/cm for Lake Tribalj1 and 243 μ S/cm for Lake Tribalj3). The studied taxon occurred in the northern and in the eastern part of Tribalj, and in the northern part of Ričice. They occurred with low relative abundances (0.96 % in Lake Ričice, 1.68 % in the eastern part and 0.49 % in the northern part of Lake Tribalj). *Achnanthidium straubianum* (Lange-Bertalot) Lange-Bertalot was the only species that reached relatively high abundance in all three samples, it was subdominant in Lake Ričice (4.34 %) and dominant in Lake Tribalj with 6.49 % and 10.73 % of relative abundance in northern and in the eastern part of the lake. In Lake Ričice the dominant species were *Amphora inariensis* Krammer (36.14 %), *Cavinula cocconeiformis* (W. Gregory ex Greville) D.G. Mann & Stickle in Round *et al.* (10.12 %) and *Pantocsekiella ocellata* (Pantocsek) K.T. Kiss & Ács (26.02 %). In Lake Tribalj the dominant species were *Achnanthidium minutissimum* (Kützinger) Czarnecki (north part 18.03 % and east part 21.46 %), *Encyonopsis subminuta* Krammer & E. Reichardt (north part 12.74 % and east part 15.85 %) and *Ulnaria delicatissima* (W. Smith) Aboal & P.C. Silva (north part 11.3 % and east part 16.59 %).

DISCUSSION

Comparing the Croatian population with the type material of *Dorofeyukea rostellata* revealed differences in valve, central area and the areola shape, and the width and shape of the axial area. In SEM it can be seen that areolae of the Croatian population are mostly rectangular or square shaped in the middle (Fig. 55) and perpendicularly elongated toward the ends (Figs. 52-54) in

external view, while that of *D. rostellata* are transapically slit-like (Figs. 58-62, 66). Furthermore, the axial area of the Croatian population is narrow and regular (Figs. 38-51), whereas it is wide and irregular in case of the type material of *D. rostellata* (Figs. 60, 63-65). However, as they overlapped with each other in the most important characteristics: valve apices and outline, striation, stria and areola density (Fig. 82), we cannot distinguish them as distinct taxa.

The presented Croatian population of *Dorofeyukea rostellata* resembles *Navicula grimmei* Krasske, a species described from Hessen, Germany (Krasske, 1925: 45, fig. 1: 14). *Navicula grimmei* had been synonymized to *Navicula kotschyi* (Krasske) Grunow (1860: 538, fig. 2: 12) by Krammer & Lange-Bertalot (1986). *Navicula kotschyi* was also transferred to *Dorofeyukea* genus by Kulikovskiy *et al.* (2019). *Dorofeyukea kotschyi* (Grunow) Kulikovskiy, Kociolek, Tusset & T.A.V. Ludwig differs from the Croatian population because *D. kotschyi* has more capitate ends and the areola density is 20-24 in 10 μ m (Tyree & Bishop, 2015), while the Croatian population has denser areolae, 24-30 in 10 μ m (Table 2). Furthermore, the areolae of *D. kotschyi* are uniform throughout the valve (Tyree & Bishop, 2015), whereas they are narrow and transapically elongated toward the apices in the Croatian population. Kulikovskiy *et al.* (2019) ignored that *Navicula grimmei* was synonymized to *Navicula kotschyi* and they transferred the species into *Dorofeyukea* genus. But they used an incorrect reference as its basionym, so *Dorofeyukea grimmei* (Krasske in Hustedt) Kulikovskiy & Kociolek is invalid at this moment according to the "International Code of Nomenclature for Algae, Fungi and Plants" (McNeill *et al.*, 2012). Kulikovskiy *et al.* (2019) described a new species *Dorofeyukea indokotschyi* Kulikovskiy, Maltsev, Andreeva & Kociolek, which is a closely relative of *D. kotschyi*; but *Dorofeyukea indokotschyi* has definitely more capitate ends than the Croatian population.

The Croatian population differs from *Navicula grimmeioides* (Gandhi, 1998: 324, fig. 41: 73-75) by the following characteristics: valve apices, shape of the central area, striation and areola shape. The Croatian population has less

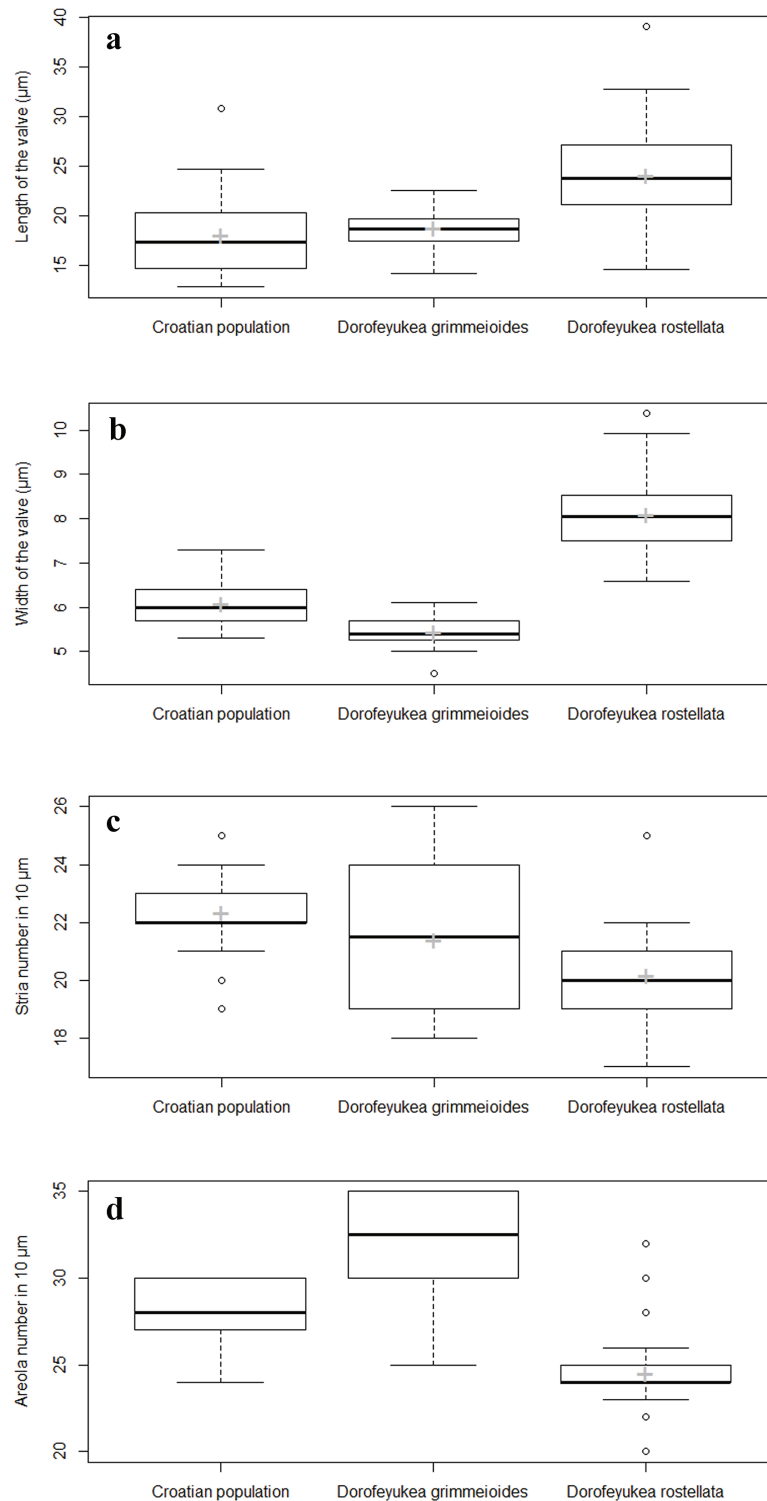


Figure 82. Box plots of length (a), width (b), striae (c) and areola number (d) in 10 μm of the two investigated type material and the Croatian population. The grey cross on the box marks the mean of the metrics. *Diagramas de cajas de longitud (a), ancho (b), estrias (c) y areola (d) en 10 μm de los dos materiales investigados y la población croata. La cruz gris en el cuadro marca la media de las métricas.*

Table 2. Differential diagnosis of the Croatian population and related taxa. *Diagnóstico diferencial de la población croata y taxones relacionados.*

	Length (µm)	Width (µm)	Striae in 10 µm	Areolae (shape and number in 10 µm)	Valve outline	Axial area	Raphe	Central area	Biogeography	References
Croatian population	12.8-30.8	5.3-7.3	19-25 radial slightly convergent on the apices	(24)25-30 elongated in single rows which continue on the mantle; externally rectangular, transapically elongated at the central part and slit-like on the apices	lanceolate with slightly capitate or broadly apices	narrow	filiform; raphe fissures are straight, internally terminating in small helictoglossae near the poles	slightly asymmetric bowtie shape	Tribalj and Ričice, Croatia	present study
<i>Dorofeyukea ancisa</i> (Hustedt) Kulikovskiy & Kociolek (Hustedt 1953: 48, pl. 150, fig. 9)	14	5-6	20 radiate, at apices nearly parallel	NA	rectangular with little convex edges	narrow	filiform	small, transapically slightly widened	Oasis Gafsa, southern Tunisia	Hustedt (1953)
<i>Dorofeyukea grimmei</i> (Krasske in Hustedt) Kulikovskiy & Kociolek (Krasske 1925, p. 45, pl. 1, fig. 14)	18-21	6	~25 the two middle shortened and slightly coarser; towards the ends become narrower	quite coarse (20-24)	elliptic- lanceolate with capitate apices	narrow	NA	square	Laurentian Great Lakes, North America	Hustedt (1930), Potapova (2013)
<i>Dorofeyukea grimmeioides</i> (H.P. Gandhi) Ács, Buczkó & Ector comb. nov. (Gandhi 1998: 324, p. 41, figs 73-75)	14.2-22.6	4.5-6.1	18-26 radial	25-35 thin, transapically elongated, slit- like; closed by hymen in the inner valve	lanceolate with capitate or rostrate apices	relatively broad	straight, proximal raphe fissures strongly drop-shaped	round, forming narrow stauroid fascia with 3 shortened striae	Gujarat, India	Gandhi (1998), present study
<i>Dorofeyukea indokotschyi</i> Kulikovskiy, Maltsev, Andreeva & Kociolek (Kulikovskiy <i>et al.</i> 2019: figs 2-4)	25-26	7-7.5	20-21 weakly radiate, almost parallel at the poles	rectangular	elliptic to linear elliptic, ends protracted, abruptly short- rostrate	narrow	filiform, central pore small, appearing as puncta	narrow stauroid fascia surrounded by 1-3 irregularly shortened striae	Indonesia, Papua, Lake Sentani	Kulikovskiy <i>et al.</i> (2019)
<i>Dorofeyukea ivatoensis</i> (Metzeltin & Lange-Bertalot) Kulikovskiy & Kociolek (Metzeltin & Lange-Bertalot 2002: 286, pl. 27, figs 6-10, pl. 28, figs 1-3)	18-34	6.6-9.5	21-24 radiate, proximally becoming subparallel, finally slightly convergent near the poles; stigma in the central area absent	15(20) rather coarse, some moderately elongated transapically in the middle, becoming smaller and denser towards the ends; isodiametric (punctiform) to apically elongated (only in SEM observed), the areolae form continuous rows from the valve face over the mantle	elliptic, ends shortly protracted broadly, more or less subcapitate, finally broadly rounded	linear, narrow to very narrow, not or very little broadened to the middle	filiform, straight with inconspicuous central pores, broadly deflected	slightly variable to barely developed; mostly small, circular or elliptic	"Island Continent", Madagascar	Metzeltin & Lange-Bertalot (2002)

Cont.

Table 2. (cont.)

<i>Dorofeyukea kotschy</i> (Grunow) Kulikovskiy, Kociolek, Tusset & T.A.V. Ludwig (Grunow 1860: 538, pl. 2: fig. 12)	18-19 18.0-25.3	5-5.5 6.0-8.1	20-24 radiate, at apices nearly parallel radiate	coarse and visible in LM externally round, internally transapically linear	linear with parallel central valve margins and distinctly capitate apices	narrow and linear	filiform with unilaterally deflected distal ends	bowtie-shaped with marginal areolae	Laurentian Great Lakes, North America	Grunow (1860), Potapova (2013), Kulikovskiy <i>et al.</i> (2019)
<i>Dorofeyukea orangiana</i> (R.M. Patrick) Kulikovskiy & Kociolek (Patrick 1959: 97, pl. 8, fig. 4)	17-22	5-7	18-20 (towards the ends 26) radiate, distinctly punctate	20-25 (Potapova 2013)	linear-lanceolate with narrow, rostrate, rounded ends	narrow, widening toward the central area	proximal raphe fissures strongly drop-shaped	rounded (more or less)	Sabine River, Texas, Lake Maurepas, Louisiana, Ridley Creek, Pennsylvania, Patuxent River, Maryland	Patrick (1959), Potapova (2013)
<i>Dorofeyukea rostellata</i> (Hustedt) Kulikovskiy & Kociolek (Hustedt 1937: 236, pl. 17, fig. 13)	14.6-39.1	6.6-10.4	17-25 radial, slightly convergent on the apices	20-32 externally slit-like	lanceolate with capitate or rostrate apices	broad	straight, proximal raphe fissures slightly drop-shaped	transapically oval on the outer and irregular on the inner valve, forming narrow stauroid fascia with 2-3 irregularly shortened striae	Java, Bali, Sumatra	Hustedt (1937), and own measurements
<i>Dorofeyukea savannahiana</i> (R.M. Patrick) Kulikovskiy & Kociolek (Patrick 1959: 97, pl. 8, fig. 7)	17-22	4-5	20-22	24-28 (Potapova 2013) radiate, distinctly punctate	linear with distinctly capitate ends	narrow	straight, proximal raphe fissures slightly drop-shaped	transverse, not reaching the margin of the valve	Savannah River, United States	Patrick (1959), Potapova (2013)
<i>Dorofeyukea texana</i> (R.M. Patrick) Kulikovskiy & Kociolek (Patrick 1959: 98, pl. 8, fig. 5)	18-22	5-7	18-21	20-27 (Potapova 2103) irregularly shortened on each side of the central nodule; parallel at apices of the valve, otherwise radiate; distinctly punctate	margins are parallel with narrowed, distinctly rostrate ends	narrow	straight with slightly unilaterally deflected proximal ends	transverse	Guadalupe River, United States	Patrick (1959), Potapova (2013)

capitate apices, especially the smaller specimens (Figs. 55, 56), than *N. grimmeioides*. Striae of the Croatian population are radial but become convergent on the apices (Figs. 2-13). Furthermore, its striae can be longer or shorter in the central part (Figs. 40-42) forming a bowtie shape central area, while in the case of *N. grimmeioides* striae are radial throughout the valve and the central area is round (Figs. 25-37, 73-75). In SEM the Croatian population has rectangular areolae (Figs. 55-57), which become lineolate-like toward the apices (Figs. 52-54) whilst that of in case of *N. grimmeioides* has transapically slit-like areolation (Figs. 68-70). Externally, the proximal raphe ends of the Croatian popu-

lation are dot-like fissures (Figs. 38-45, 55) while drop-shaped in case of *N. grimmeioides* (Fig. 72).

Based on the description of *Dorofeyukea* genus (Kulikovskiy *et al.*, 2019), *N. grimmeioides* also belongs to *Dorofeyukea*, because its central area forming narrow stauroid fascia (Figs. 68-70), surrounded with 2-3 shortened striae, its areolae covered internally by dome-shaped hymens (Figs. 74, 75), distal raphe ends hook-shaped going to mantle (Fig. 76), and it has pseudoseptum at distal ends (Figs. 69, 70, 78-81). Judging by morphological characteristics, we propose the transfer of *N. grimmeioides* to the genus *Dorofeyukea* as follows:

***Dorofeyukea grimmeioides* (H.P. Gandhi)
Ács, Buczkó & Ector comb. nov.**

Basionym: *Navicula grimmeioides* H.P. Gandhi 1998, Bishen Singh Mahendra Pal Singh. Dehra Dun. India 324, p. 41, pl. 2, figs. 73-75.

Some other species, as *Dorofeyukea texana* (R.M. Patrick) Kulikovskiy & Kociolek (Patrick 1959: 98, fig. 8: 5) also resembles the Croatian population, but its areolae on the central part are not elongated transversally. *Dorofeyukea savannahiana* (R.M. Patrick) Kulikovskiy & Kociolek (Patrick 1959: 97, fig. 8: 7) have more capitate ends. According to Potapova (2013), it is possible that *Dorofeyukea kotschy* is rather variable in valve outline, shape and density of areolae, and width of the pseudoseptum and both *D. texana* and *D. savannahiana* are conspecific with it. Kulikovskiy *et al.* (2019) refer them as valid species. Potapova (2013) was not able to distinguish pseudoseptae in *D. savannahiana*. Narrow pseudoseptum is present on both apices in case of the Croatian population. *Dorofeyukea orangiana* (R.M. Patrick) Kulikovskiy & Kociolek (Patrick 1959: 97, fig. 8: 4) and *Dorofeyukea ivatoensis* (Metzeltin & Lange-Bertalot) Kulikovskiy & Kociolek (Metzeltin & Lange-Bertalot 2002: 286, figs. 27: 6-10, 28: 1-3) have round central area. In addition, the central area of *D. ivatoensis* is small and its distal raphe end is curved. The central area of *Dorofeyukea ancisa* (Hustedt) Kulikovskiy & Kociolek (Hustedt 1953: 150, fig. 9) is smaller than that of Croatian population (Table 2).

Our study pointed out that investigation of the type materials can be very important, particularly in case of rare taxa. We also want to stress here that the intensive studies on diatom taxonomy using high-resolution microscopic techniques, for better understanding of the ultrastructure of diatom frustule, can lead to new species description or combination of species. Although SEM analyses also revealed that the studied taxa do not belong to the genus *Navicula* sensu stricto, molecular data are important for erecting a new genus.

SUMMARY

Dorofeyukea rostellata is a rare diatom, known from various habitats. Here we reported the first

European occurrence of this diatom. Detailed ultrastructural investigations of the type material of *Navicula grimmeioides*, which resemble to *Dorofeyukea rostellata*, revealed that its characteristics fit the main features of the genus *Dorofeyukea*, so we proposed to transfer it into the genus *Dorofeyukea* as a new combination.

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