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# The genus *Dorofeyukea* (Bacillariophyta, Stauroneidaceae) in karst environments in the Brazilian Pantanal: with the description of four new species

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**Abstract:** In a Brazilian karst environment, composed predominantly of carbonate rocks, diatoms were collected in periphytic habitat (epiphyton, epilithon, epipsammon) in lentic and lotic water bodies from the Serra da Bodoquena. This Pantanal region is known for its tourist and scenic value, covering springs and rivers with extensive formation of active limestone tufa. Six species of *Dorofeyukea* were investigated under light and electron microscopy. The genus is characterized by a narrow stauroid fascia surrounded by some irregularly shortened striae, uniseriate slightly radiated striae, areolae circular or rectangular covered internally by a hymenes and apical pseudosepta. Four new species are described and compared with similar taxa *Dorofeyukea bahlsii* sp. nov., *D. bodoquenensis sp. nov.*, *D. calcarea sp. nov.*, and *D. pantanalensis* sp. nov. The new species are distinguished from each other and from similar taxa of *Dorofeyukea* mainly by the valve contour and shape of the apices. *Dorofeyukea kotschyi* (Grunow) Kulikovskiy, Kociolek, Tusset et T. Ludwig and *D. texana* (Patrick) Kulikovskiy et Kociolek were also recorded, described and illustrated in this study.

**Key words:** calcareous habitat, diatom, new species, South America, stauroid fascia, taxonomy

## Introduction

*Dorofeyukea* Kulikovskiy, Maltsev, Andreeva, T. Ludwig et Kociolek is a naviculoid genus, characterized by solitary cells, described based on molecular analyses and morphological approach of the valve and the chloroplast. *Dorofeyukea* species share narrow stauroid fascia surrounded by 1–3 irregularly shortened striae, the uniseriate and weakly radiate striae, almost parallel at the apices, the circular or rectangular areolae covered internally by hymenes, and the presence of an apical pseudoseptum at both apices. Partial DNA sequences from the SSU and rbcL loci show that *Dorofeyukea* belongs to the stauroneioid diatom clade (Kulikovskiy et al. 2019).

Currently, twelve species have been accepted taxonomically (Guiry & Guiry 2021) and *Dorofeyukea kotschyi* (Grunow) Kulikovskiy, Kociolek, Tusset et T. Ludwig is a cosmopolitan species of the genus, found in Europe (Kulikovskiy et al. 2019), Africa (Stoyneva–Gärtner & Descy 2020), Oceania (John 2020), Asia (Liu et al. 2020), and South America (Metzeltin & Lange–Bertalot 1998; Rumrich et al. 2020). *Dorofeyukea kotschyi* was registered as *Navicula kotschyi* (Grunow) in floristic freshwater studies from Brazil (Tremarin et al. 2009; Silva et al. 2011). Detailed studies revealing the ultrastructure of *Dorofeyukea* species from Brazil are non–existent.

*Navicula grimmei* var. *rostellata* Hustedt, *Navicula grimmei* Krasske, and *N. grimmeioides* H.P. Gandhi were recently transferred to *Dorofeyukea* (Kulikovskiy et al. 2019; Trábert et al. 2019). The detailed analyses of the type materials of *Dorofeyukea rostellata* (Hustedt) Kulikovskiy et Kociolek and *Dorofeyukea grimmeioides* (H.P. Gandhi) Ács, Buczkó et Ector under light (LM) and scanning electron microscopy (SEM) clarified the identity of the Croatian species. Also, the study corroborate that this group did not belong to *Navicula,* and fits in the main features of the recently proposed genus *Dorofeyukea* (Trábert et al. 2019).

The analysis of environmental variables in the karstic water bodies from Serra da Bodoquena (Tusset et al. 2017, 2018) revealed similarities (pH 7.2–8.3 and conductivity 320–470 $\mu S.cm^{-1}$) with those recorded during the studies of *D. rostellata* in Croatian lakes,

located in calcareous bedrock and to those described for *D. indokotschyi* Kulikovskiy, Maltsev, Andreeva et Kociolek and *D. kotschyi* in Indonesia, as neutral to slightly alkaline water (pH 7.3–7.7) and low conductivity (230–240 μS.cm$^{-1}$) (Trábert et al. 2019, Kulikovskiy et al. 2019).

We analyzed populations of six species of *Dorofeyukea* from Brazilian freshwater carbonatic karstic environments, located at Serra de Bodoquena Park, describing and illustrating their morphology by light and scanning electron microscopy. We also compare them with similar taxa, and related its distribution with the physical and chemical water variables. Four species were proposed as new taxa.

## Material and Methods

**Studied area**. The Serra da Bodoquena karst (21°05'42" S, 56°42'17" W) is located at the southwest edge of the Pantanal Complex, in Mato Grosso do Sul, a central west region state of Brazil, covering the cities of Bonito, Jardim and Bodoquena, where sampling occurred. The aquatic environments in the region are characterized by crystal clear and carbonated water and the presence of sinkholes is frequent, despite of the predominance of surface drainage (Lino et al. 1984; Sallun Filho et al. 2004). Active limestone tufas are formed by the continuous accumulation of calcium carbonate in association with biological activity (moss and algae) along the drainage system (Sallun Filho et al. 2009; Boggiani et al. 2011).

**Sampling**. For this study, 16 sampling sites (waterfall, stream, river and lake) were selected in karst water bodies. A total of 28 periphytic samples were collected in the rainy season: one sample in September 2013; 11 in November 2015 (sampling campaign I); and 16 in November 2019 (sampling campaign II), resulting in six epilithic, three episammic and 19 epiphytic samples (Table 1). We registered the following environmental variables during the sampling: water temperature (°C), pH, electrical conductivity (μS.cm$^{-1}$). Also geographical coordinates are given (Table 1). In the 2019 sampling, other variables were analysed according to APHA (2012): Total P (mg.l$^{-1}$), Ca (mg.l$^{-1}$), Mg (mg.l$^{-1}$), and $SiO_2$ (mg.l$^{-1}$) (Table 2).

**Diatom analysis**. Periphytic diatoms were obtained by scraping different substrates, such as stones, sand grains, specimens of Charophytes, submerged Bryophytes, with a scalpel wrapped in foil. Samples were fixed in 70% alcohol solution and cleaned according to Simonsen (1974) and Moreira–Filho & Valente–Moreira (1981). Slides were prepared with clean diatom valves, included in Naphrax® (R.I. = 1.74), and were analysed using an Olympus BX40 microscope at 1000× magnification (N.A. 1.30, planachromat), equipped with Phase Contrast optics and the Olympus DP71 Imaging System. Cleaned samples were placed on aluminum stubs, dried and covered with gold in Balzers Sputtering SCD 030 for ultrastructural analyses of the frustules in the scanning electron microscopy (SEM). The SEM images were performed in a JEOL JSM 6360LV and TESCAN VEGA3 LMU microscopies, operated at 10–15 kV and 5–8 mm work distance. Besides that, part of the cleaned material was air dried on the formvar–coated copper grids (300 mesh) and analyzed under transmission electron microscopy (TEM) using a JEM 1200EXII, operated at 80 kV. Samples and slides were deposited in the Herbarium of the Universidade Federal do Paraná (UPCB), and Universidade Federal do Rio Grande do Sul (ICN), Brazil. The terminology used in the description of the taxa was based on Barber & Haworth (1981) and Round et al. (1990).

## Results and Discussion

***Dorofeyukea bahlsii* Tusset sp. nov. (Figs 1–14)**

**Description**

**LM (Figs 1–8):** Valves elliptic–lanceolate with capitate to subcapitate apices. Valve dimensions (n=20): length 24.2–29.2 μm, width 6–7 μm. Prominent pseudosepta. Axial area relatively broad. Raphe filiform. Proximal raphe ends straight, not expanded. Central area slightly asymmetric due to the varying number of short central striae ranging from one to three, most commonly two, forming narrow stauroid fascia bowtie–shaped, slightly expanded to the valve margins. Striae radiate, 22–24 in 10 μm. Areolae not discernible in LM.

**SEM (Figs 9–14):** Externally, striae continuing onto the mantle, radiate at the center becoming parallel towards the ends (Fig. 9). Areolae transapically elongated in the valve face and longitudinally elongated near the apices, 32–36 in 10 μm. Internally, areolae covered by hymenes (see Fig. 13). Internally and externally, the proximal raphe fissures are slightly deflected in the same direction (Figs 11–12). Internally, distinct, large pseudosepta present at each valve apex (Figs 10, 13). External distal raphe endings hook–shaped to the secondary side, deflected in the same direction, continuing onto the mantle (Fig. 14).

**Holotype**: UPCB collection No. 78228 (here depicted in Fig. 2), deposited in (Paraná Federal University, Botany Department, Brazil).

**Paratypes:** Brazil. Mato Grosso do Sul: Bonito, Formoso River, waterfall in Sitio Ybirá Pe farm, epiphyton, UPCB 78254; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epilithon, UPCB 78432; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epilithon, ICN 203543; Mato Grosso do Sul: Bonito, Cachoeiras Park, epilithon, UPCB 78434. Mato Grosso do Sul: Bonito, Cachoeiras Park, epilithon, ICN 203545.

**Type locality**: Brazil. Mato Grosso do Sul: Bonito, Blue Spring, 20°54'09.7" S, 56°31'48.4" W September 2013, E. Tusset s.n.

**Habitat:** periphytic (epiphyton, epilithon) in lotic environments.

**Etymology:** The species name was a tribute to Dr. Loren Bahls for his contribution to the study of diatoms.

**Ecology:** *Dorofeyukea bahlsii* was very rare in these samples. The populations were found in periphytic habitat (epiphyton, epilithon) of lotic environments. It was found in neutral to alkaline waters (pH 7.6–8), conductivity 387–536 μS.cm$^{-1}$, water temperature 25–30 °C, $N–NO_{3-}$ 0.10–0.27 mg.l$^{-1}$, Total P 0.01–0.03 mg.l$^{-1}$,

Table 1. Sampling data and access number in the Herbarium of the Universidade Federal do Rio Grande do Sul (ICN) and of the Universidade Federal do Paraná (UPCB).

| Date | Access UPCB | Access ICN | Locality | City | Geographical coordinates | Sample or substrate | pH | Conductivity ($\mu S.cm^{-1}$) | Altitude (m) | Water Temperature (°C) |
|---|---|---|---|---|---|---|---|---|---|---|
| 09/09/2013 | 78228 | | Blue Spring | Bonito | 20°54'09.7" S, 56°31'48.4" W | Epiphyton /Charophytes | … | … | 322 | … |
| 11/10/2015 | 76099 | | Formoso River | Bonito | 21°10'17.7" S, 56°27'00.4" W | Epiphyton /Charophytes | 7.8 | 376 | 285 | 27 |
| 11/10/2015 | 76100 | | Sucuri River | Bonito | 21°15'12.9" S, 56°33'37.3" W | Epiphyton /Charophytes | 7.6 | 323 | 325 | 27 |
| 11/10/2015 | 78233 | | Olho d'Água River | Jardim | 21°26'12.6" S, 56°26'09.3" W | Epiphyton /Charophytes | 7.2 | 320 | 241 | 27 |
| 11/10/2015 | 78261 | | Prata River | Jardim | 21°26'08.6" S, 56°25'46.2" W | Epilithon | 7.2 | 320 | 240 | 27 |
| 11/10/2015 | 78266 | | Olho d'Água River | Jardim | 21°26'12.6" S, 56°26'09.3" W | Epipsammon | 7.2 | 320 | 241 | 27 |
| 11/10/2015 | 78245 | | Prata River | Jardim | 21°26'08.6" S, 56°25'46.2" W | Epiphyton /Charophytes | 7.2 | 320 | 240 | 27 |
| 11/11/2015 | 78246 | | Chapeninha Spring | Bonito | 20°50'28.7" S, 56°35'39.8" W | Epiphyton/Bryophytes | 7.5 | 476 | 308 | 24 |
| 11/11/2015 | 78248 | | Chapena waterfall | Bonito | 20°49'58.8" S, 56°33'17.3" W | Epiphyton/Bryophytes | 7.6 | 382 | 308 | 28 |
| 11/12/2015 | 78254 | | Formoso River/ Sitio Ybirá Pe waterfall | Bonito | 21°09'02.9" S, 56°25'40.7" W | Epiphyton /Charophytes | 7.6 | 387 | 271 | 30 |
| 11/13/2015 | 78256 | | Formoso River/ Ecologic Park | Bonito | 21°10'38.1" S, 56°27'08.1" W | Epiphyton /Charophytes | 8 | 376 | 281 | 26 |
| 11/15/2015 | 78240 | | Boca da Onça waterfall | Bodoquena | 20°44'23.5" S, 56°44'04.6" W | Epiphyton/Bryophytes | 7.3 | 407 | 270 | 27 |
| 11/22/2019 | 78438 | 203549 | Formoso River / Sitio Ybirá Pe waterfall | Bonito | 21°09'02.9" S, 56°25'40.7" W | Epiphyton /Charophytes | 7.1 | 411 | 271 | 27 |

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*Table 1 Cont.*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 11/22/2019 | 78453 | 203564 | Bonito River | Bonito | 21°08'58.4" S, 56°25'40.3" W | Epiphyton /Charophytes | 7.8 | 422 | 267 | 28 |
| 11/25/2019 | 78420 | 203531 | Mimoso River | Bonito | 20°59'55.5" S, 56°30'50.1" W | Epiphyton /Charophytes | 8 | 536 | 246 | 28 |
| 11/25/2019 | 78431 | 203542 | Prata River | Jardim | 21°26'08.6" S, 56°25'46.2" W | Epilithon | 7.4 | 371 | 240 | 26 |
| 11/25/2019 | 78432 | 203543 | Mimoso River | Bonito | 20°59'55.5" S, 56°30'50.1" W | Epilithon | 8 | 536 | 246 | 28 |
| 11/25/2019 | 78440 | 203551 | Misteriosa Lake | Jardim | 21°27'30.9" S, 56°27'12.4" W | Epiphyton /Charophytes | 8.2 | 337 | 254 | 25 |
| 11/25/2019 | 78443 | 203554 | Olho d'Água River | Jardim | 21°26'12.6" S, 56°26'09.3" W | Epiphyton /Charophytes | 7.4 | 338 | 241 | 26 |
| 11/25/2019 | 78457 | 203568 | Prata River | Jardim | 21°26'08.6" S, 56°25'46.2" W | Epiphyton /Charophytes | 7.4 | 371 | 240 | 26 |
| 11/25/2019 | 78456 | 203567 | Olho d'Água River | Jardim | 21°26'12.6" S, 56°26'09.3" W | Epipsammon | 7.4 | 338 | 241 | 26 |
| 11/26/2019 | 78422 | 203533 | Boca da Onça waterfall | Bodoquena | 20°44'23.5" S, 56°44'04.6" W | Epiphyton/Bryophytes | 8.6 | 395 | 270 | 26 |
| 11/26/2019 | 78446 | 203557 | Salobra River | Bodoquena | 20°44'14.2" S, 56°44'03.9" W | Epilithon | 8.1 | 339 | 146 | 29 |
| 11/26/2019 | 78455 | 203566 | Formoso River/ Ecologic Park | Bonito | 21°10'38.1" S, 56°27'08.1" W | Epiphyton /Charophytes | 8 | 423 | 281 | 27 |
| 11/27/2019 | 78434 | 203545 | Mimoso River/ Cachoeira Park | Bonito | 21°00'20.4" S, 56°30'04.7" W | Epilithon | 7.7 | 476 | 274 | 25 |
| 11/29/2019 | 78428 | 203539 | Blue Spring | Bonito | 20°54'09.7" S, 56°31'48.4" W | Epiphyton /Charophytes | 6.7 | 669 | 322 | 25 |
| 11/29/2019 | 78429 | 203540 | Lake Blue Spring | Bonito | 20°53'47.8" S, 56°31'36.9" W | Epilithon | 7 | … | 322 | 26 |
| 11/29/2019 | 78454 | 203565 | Blue Spring | Bonito | 20°54'09.7" S, 56°31'48.4" W | Epipsammon | 6.7 | 669 | 322 | 25 |

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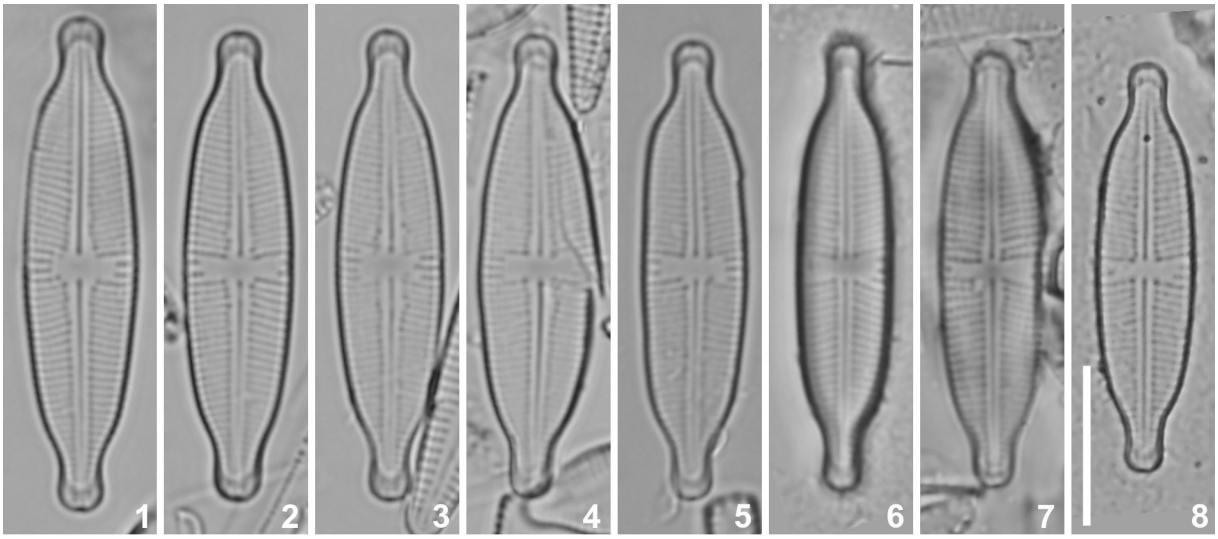
Table 2. Chemical variables at sampling sites in 2019.

| **Locality** | **Ca (mg.l$^{-1}$)** | **Total P (mg.l$^{-1}$)** | **Mg (mg.l$^{-1}$)** | **N–NO$_{3-}$ (mg.l$^{-1}$)** | **SiO$_2$ (mg.l$^{-1}$)** |
|---|---|---|---|---|---|
| Blue Spring | 60.60 | 0.01 | 39.10 | 0.10 | 10.90 |
| Prata River | 63.40 | 0.02 | 18.90 | 0.10 | 21.40 |
| Olho d'Água River | 53.60 | 0.03 | 19.10 | 0.10 | 23.20 |
| Misteriosa Lake | 50.40 | 0.03 | 18.50 | 0.10 | 21.40 |
| Salobra River | 47.20 | 0.01 | 4.07 | 0.66 | 7.87 |
| Boca da Onça waterfall | 26.80 | 0.01 | 27.60 | 0.21 | 11.20 |
| Mimoso River | 64.60 | 0.02 | 44.60 | 0.10 | 17.20 |
| Mimoso River/Cachoeira Park | 64.60 | 0.02 | 44.60 | 0.10 | 17.20 |
| Formoso River /Sitio Ybirá Pe waterfall | 29.10 | 0.03 | 16.40 | 0.27 | 9.72 |
| Formoso River/Ecologic Park | 46.90 | 0.01 | 12.60 | 0.27 | 8.26 |



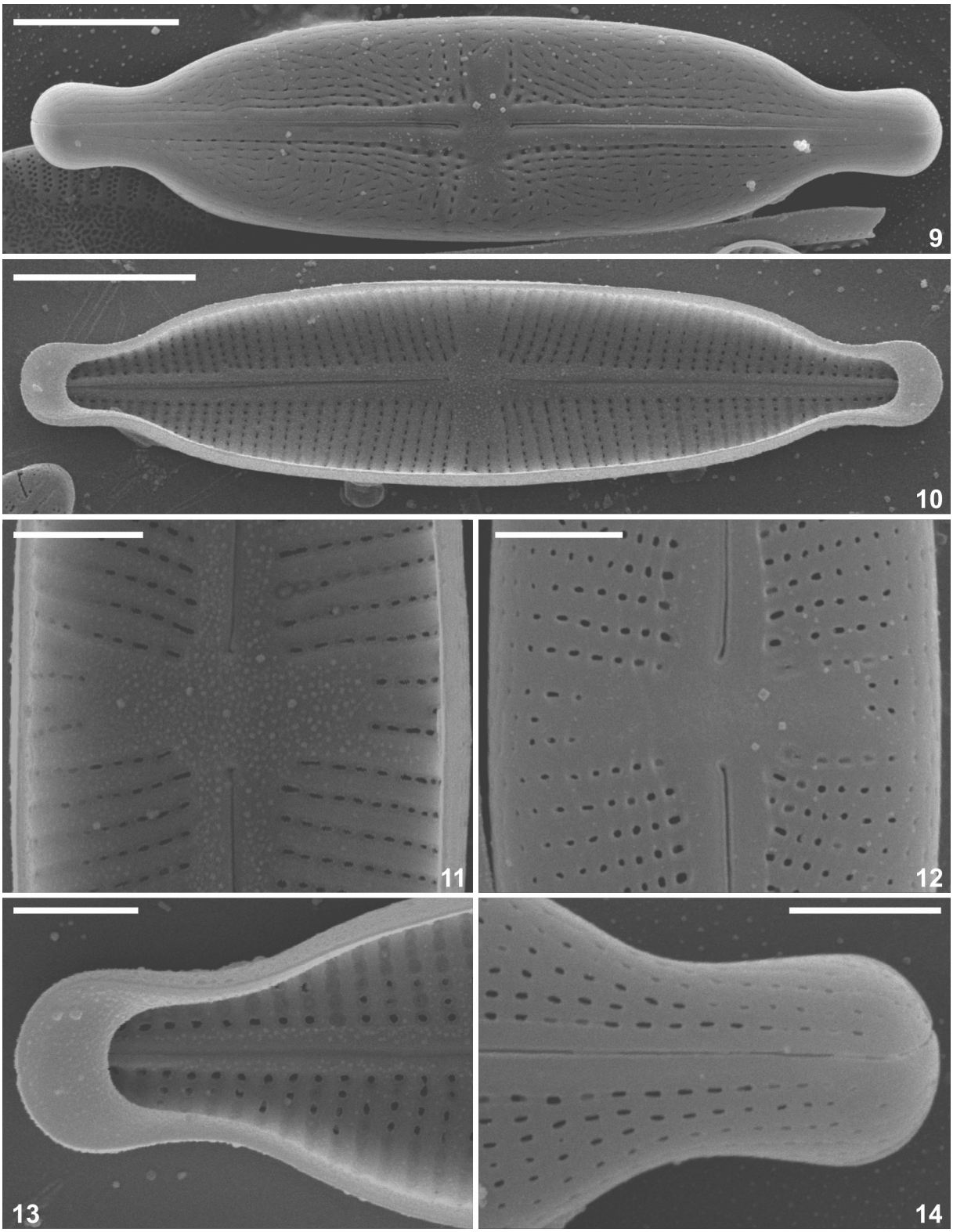

Figs 1–8. *Dorofeyukea bahlsii* sp. nov., LM, valve views: (2) holotype specimen. Scale bar 10 µm.

Ca 29.1–64.6 mg.l$^{-1}$, Mg 16.4–44.6 mg.l$^{-1}$, $SiO_2$ 9.72–17.2 mg.l$^{-1}$ (Tables 1–2).

*Dorofeyukea bahlsii* is similar to *Dorofeyukea septata* (Hustedt) J. John in relation to the prominent pseudoseptum, broad axial area, dimensions and radiate striae becoming parallel at the apices. *Dorofeyukea septata* can be distinguished from *D. bahlsii* by the linear–lanceolate to lanceolate valve shape, sub–rostrate to rounded apices, and central area expanded (Table S1). The species was abundant in King Fish Hotspring, in neutral waters (pH 7.2) and high conductivity (1273 µS.cm$^{-1}$), and in Stevenson Creek, South Australia with alkaline waters (pH 9.1) and low conductivity (207 µS.cm$^{-1}$) (John 2020).

*Dorofeyukea bahlsii* have pseudosepta very prominent and quite visible in LM, differing from *D. kotschyi*. In addition, *D. bahlsii* has a greater number of areolae in the striae (Table S1) and these are transapically elongated in central area. In *D. bahlsii* the central and axial areas are broader, compared to *D. kotschyi* (Potapova 2013; Kulikovskiy et al. 2019).

*D. indokotschyi* differs from *D. bahlsii* by the elongated apices, narrow pseudoseptum not discernible in LM, and the less dense striae (20–21 in 10 µm). The population of *D. bahlsii* has elliptic–lanceolate valves, with capitate to subcapitate apices and denser striae, 22–24 at 10 µm (Kulikovskiy et al. 2019) (Table S1).




Figs 9–14. *Dorofeyukea bahlsii* sp. nov., SEM: (9–10) external and internal valve view, respectively; (11) internal view of median region of valve showing the narrow fascia slightly expanded to the valve margins with often 1–2 strongly shortened striae and proximal raphe endings; (12) external view of median region showing the proximal raphe endings; (13) internal view of valve apex, note the pseudosepta and areolae occlusion near the apex; (14) external view valve showing the distal raphe ending extending to the mantle. Scale bars 5 μm (9–12), 2 μm (13–14).

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***Dorofeyukea bodoquenensis* Tusset, Tremarin, L.S. Cardoso et T. Ludwig sp. nov. (Figs 15–33)**

**Description**

**LM (Figs 15–26):** Valves lanceolate with subrostrate apices. Valve dimensions (n=100): length 11.2–31.8 µm, width 3.6–5.5 µm. Prominent pseudosepta. Axial area relatively broad. Raphe filiform. Proximal raphe endings straight, not expanded. Central area slightly asymmetric due to the varying number of short central striae ranging from one to three, most commonly one, forming narrow stauroid fascia, slightly expanded to the valve margins. Striae radiate from the valve center to the apices, 22–26 in 10 µm. Areolae not discernible in LM.

**SEM (Figs 27–33):** Externally, striae radiate, continuing onto the mantle. Areolae rounded to transapically elongate in the valve face, and longitudinally elongated near the apices, 35–40 in 10 µm. internally, areolae covered by hymenes (see Fig. 32). Internally proximal raphe ends slightly deflected (Fig. 30). Externally, proximal raphe ends shaped like a tear drop (Fig. 31). Internally, distinct, large pseudoseptum present at each valve apex (Figs 27, 32). In external view the distal raphe end hook–shaped to the secondary side, continuing onto the mantle (Fig. 33).

**Holotype:** UPCB collection No. 78228 (here depicted in Fig. 19), deposited in (Paraná Federal University, Botany Department, Brazil).

**Paratypes:** Brazil. Mato Grosso do Sul: Jardim, Chapeninha Spring, epiphyton, UPCB 78246; Mato Grosso do Sul: Bonito, Prata River, epilithon, UPCB 78261; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epiphyton, UPCB 78420; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epiphyton, ICN 203531; Mato Grosso do Sul: Bonito, Blue Spring, epiphyton, UPCB 78428; Mato Grosso do Sul: Bonito, Blue Spring, epiphyton, ICN 203539; Mato Grosso do Sul: Jardim, Prata River, epilithon, UPCB 78431; Mato Grosso do Sul: Jardim, Prata River, epilithon, ICN 203542; Mato Grosso do Sul: Jardim, Misteriosa Lake, epiphyton, UPCB 78440; Mato Grosso do Sul: Jardim, Misteriosa Lake, epiphyton, ICN 203551; Mato Grosso do Sul: Bodoquena, Salobra River, epilithon, UPCB 78446; Mato Grosso do Sul: Bodoquena, Salobra River, epilithon, ICN 203557; Mato Grosso do Sul: Bonito, Blue Spring, epipsammon, UPCB 78454; Mato Grosso do Sul: Bonito, Blue Spring, epipsammon, ICN 203565; Mato Grosso do Sul: Bonito, Formoso River Ecologic Park, epilithon, UPCB 78455. Mato Grosso do Sul: Bonito, Formoso River Ecologic Park, epilithon, ICN 203566.

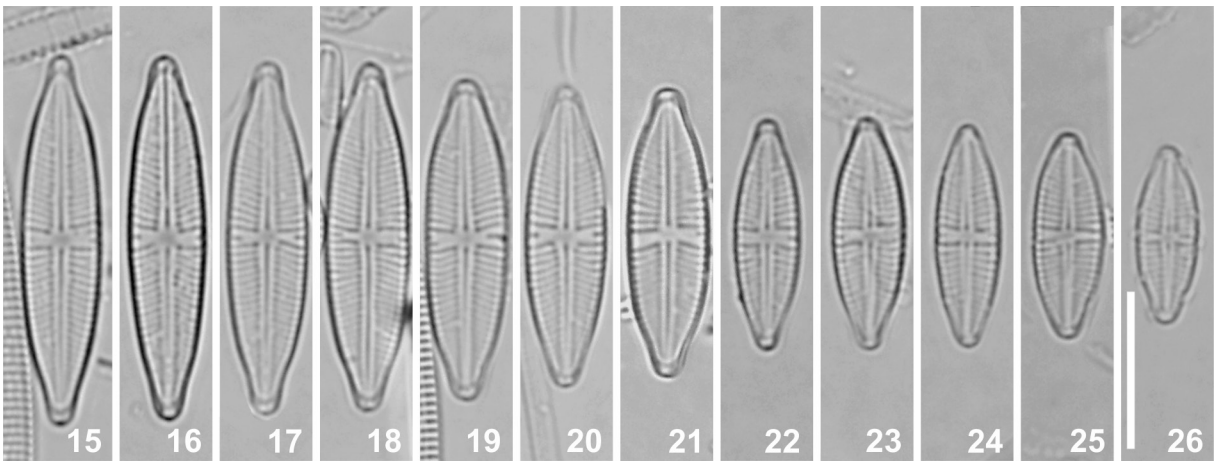
**Type locality:** Brazil. Mato Grosso do Sul: Bonito, Blue Spring, 20°54'09.7" S, 56°31'48.4" W September 2013, E. Tusset s.n.

**Habitat:** periphytic (epiphyton, epilithon, epipsammon) in lentic and lotic environments.

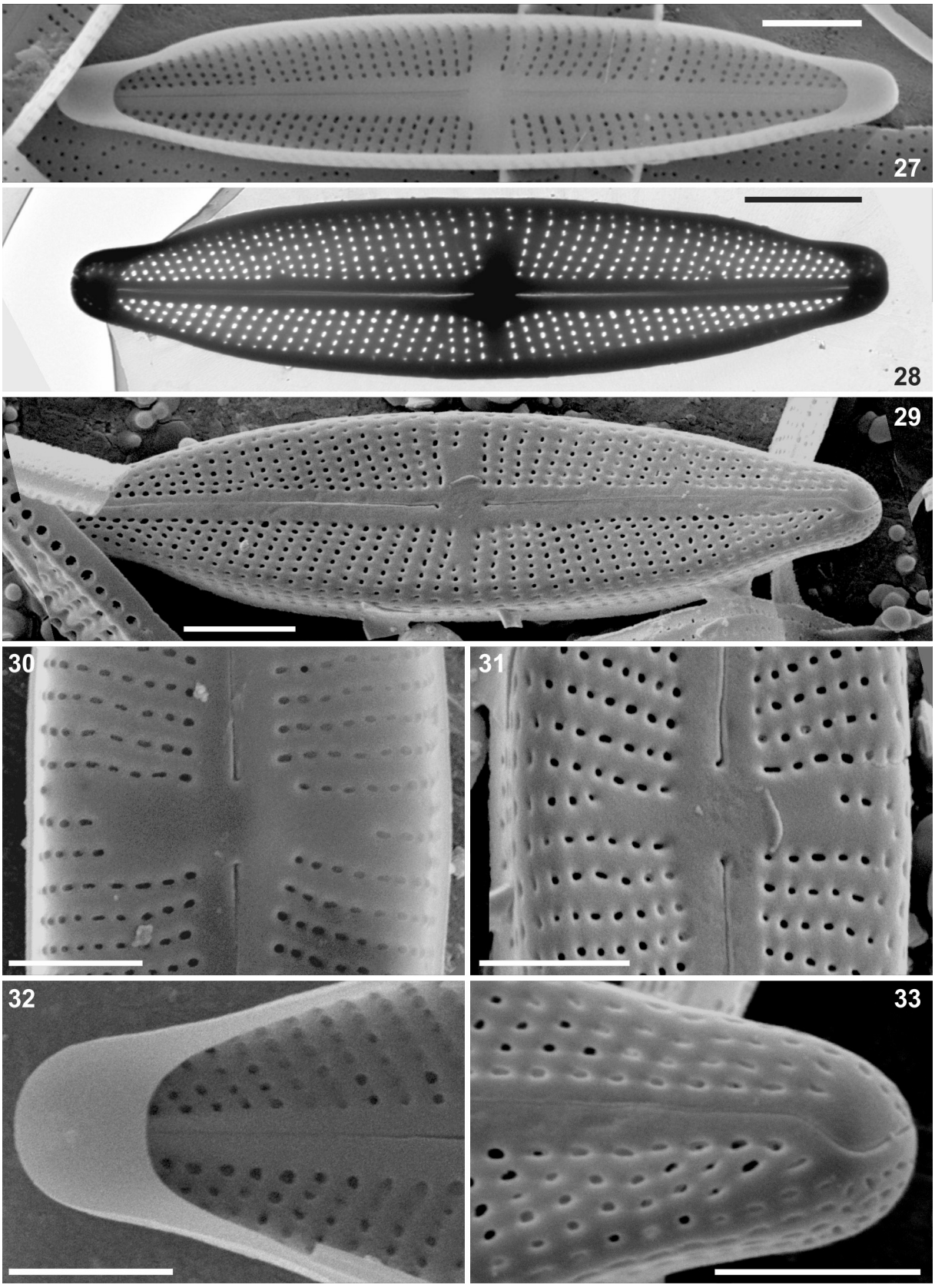
**Etymology:** The specific epithet refers to the karstic study region of the Serra da Bodoquena National Park.

**Ecology:** *Dorofeyukea bodoquenensis* was more abundant in the type material. The population were collected in periphytic habitat (epiphyton, epilithon, epipsammon) in lentic and lotic environments. It was found in neutral to alkaline waters (pH 6.7–8.2), conductivity 320–669 $\mu S.cm^{-1}$, water temperature 25–29 ºC, $N–NO_{3-}$ 0.10–0.66 $mg.l^{-1}$, Total P 0.01–0.03 $mg.l^{-1}$, Ca 46.9–64.6 $mg.l^{-1}$, Mg 4.07–44.6 $mg.l^{-1}$, $SiO_2$ 7.86–21.4 $mg.l^{-1}$ (Tables 1–2).

The features used to separate *D. bodoquenensis* from similar species of *Dorofeyukea* are presented in Table S1. *Dorofeyukea septata* is similar to *D. bodoquenensis* in terms of its lanceolate valve contour, striation pattern and prominent pseudoseptum at the valve apex. However, these species differ from *D. bodoquenensis* by the rounded areolae and the striae radiate in the center becoming parallel at the ends. Other differences include the central area that is bowtie–shaped (John 2020). Another similar species, *D. calcarea* is distinguished by the slightly expanded central area towards the valve margins (see Figs 46), and also, by the striae slightly radiate along the valve face and almost parallel at the apices. Moreover, pseudosepta are present in the apices, but not easily visible LM (Figs 34–45).




Figs 15–26. *Dorofeyukea bodoquenensis* sp. nov., LM. valve views: (19) holotype specimen. Scale bar 10 µm.




Figs 27–33. *Dorofeyukea bodoquenensis* sp. nov., (27, 29–33) SEM, (28) TEM: (27) internal valve view; (28) valve overview showing the striation pattern; (29) external valve view; (30) internal view of median region of valve showing the narrow fascia slightly expanded to the valve margins; (31) external view of median region showing the proximal raphe endings; (32) internal view of valve apex, note the pseudosepta and areolae occlusion; (33) tilted valve showing the distal raphe ending extending to the mantle. Scale bars 3 µm (27–29), 2 µm (30–33).

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***Dorofeyukea calcarea* Tusset, Tremarin, L.S. Cardoso et T. Ludwig sp. nov. (Figs 34–51)**

**Description**

**LM (Figs 34–45):** Valves lanceolate, minor shapes elliptical, protracted ends, subrostrate to rostrate. Valve dimensions (n=70): length 10.5–22 µm, width 3.9–5.5 µm. Narrow pseudosepta. Axial area narrow, linear. Raphe filiform. Proximal raphe endings straight, not expanded. Central area slightly asymmetric due to the varying number of short central striae ranging from one to three, most commonly two, forming narrow stauroid fascia bowtie–shaped, slightly expanded to the valve margins. Striae radiate, 20–24 in 10 µm. Areolae not discernible in LM.

**SEM (Figs 46–51):** Externally, striae continuing onto the mantle, radiate at the center becoming parallel towards the ends. Areolae rounded to transapically elongated in the valve face and longitudinally elongated near the apices, 30–35 in 10 µm. Internally, areolae covered by hymenes (Fig. 51). Externally and internally, the proximal raphe fissures are slightly deflected in the same direction (Figs 48–49). In external view the distal raphe end hook–shaped to the secondary side, continuing onto the mantle (Fig. 50). Internally, distinct narrow pseudoseptum present at each valve apex (Figs 47, 51).

**Holotype**: UPCB collection No. 78233 (here depicted in Fig. 36), deposited in (Paraná Federal University, Botany Department, Brazil).

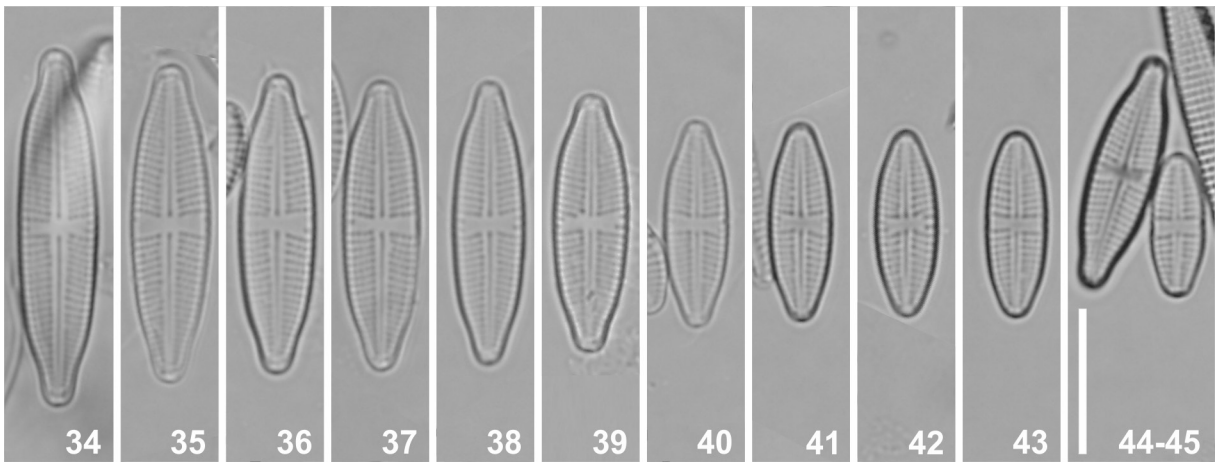
**Paratypes:** Brazil. Mato Grosso do Sul: Bodoquena, Boca da Onça waterfall, epiphyton, UPCB 78240; Mato Grosso do Sul: Jardim, Prata River, epiphyton, UPCB 78245; Mato Grosso do Sul: Bonito, Chapena waterfall, epiphyton, UPCB 78248; Mato Grosso do Sul: Bonito, Formoso River, waterfall in Sitio Ybirá Pe, epiphyton, UPCB 78254; Mato Grosso do Sul: Bonito, Formoso River in Ecologic Park, epiphyton, UPCB 78256; Mato Grosso do Sul: Jardim, Olho d'Água River, epipsammon, UPCB 78266; Mato Grosso do Sul: Bodoquena, Boca da Onça waterfall, epiphyton, UPCB 78422; Mato Grosso do Sul: Bonito, Blue Spring, epiphyton, UPCB 78428; Mato Grosso do Sul: Bonito, Blue Spring, epilithon, UPCB 78429; Mato Grosso do Sul: Bonito, Blue Spring, epilithon, ICN 203540; Mato Grosso do Sul: Jardim, Prata River, epilithon, UPCB 78431; Mato Grosso do Sul: Jardim, Prata River, epilithon, ICN 203542; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epilithon, UPCB 78432; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epilithon, ICN 203543; Mato Grosso do Sul: Bonito, Cachoeiras Park, epilithon, UPCB 78434; Mato Grosso do Sul: Bonito, Cachoeiras Park, epilithon, ICN 203545; Mato Grosso do Sul: Bonito, Formoso River, waterfall in Sitio Ybirá Pe, epiphyton, UPCB 78438; Mato Grosso do Sul: Bonito, Formoso River, waterfall in Sitio Ybirá Pe, epiphyton, ICN 203549; Mato Grosso do Sul: Jardim, Olho d'Água River, epiphyton, UPCB 78443; Mato Grosso do Sul: Jardim, Olho d'Água River, epiphyton, ICN 203554; Mato Grosso do Sul: Bonito, Bonito River, epiphyton, UPCB 78453; Mato Grosso do Sul: Bonito, Bonito River, epiphyton, ICN 203564; Mato Grosso do Sul: Jardim, Prata River, epiphyton, UPCB 78457; Mato Grosso do Sul: Jardim, Prata River, epiphyton, ICN 203568.

**Type locality**: Brazil. Mato Grosso do Sul: Jardim, Olho d'Água River, 21°26'12.6" S, 56°26'09.3" W, November 2015, E. Tusset s.n.

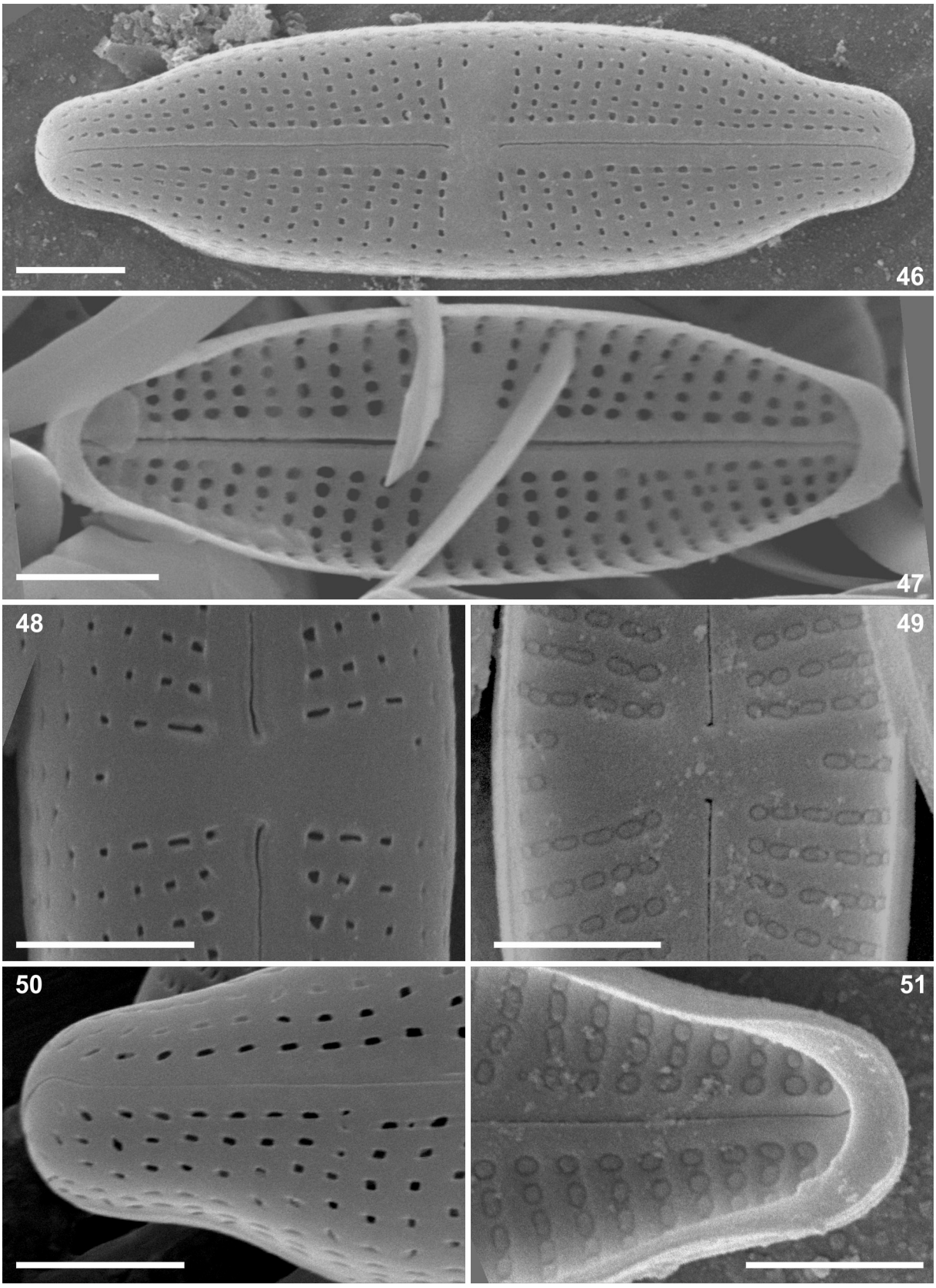
**Habitat:** periphytic (epiphyton, epilithon, epipsammon) in lentic and lotic environments.

**Etymology:** The specific epithet refers to the occurrence of the taxon in carbonated waters from the karst landform of the Bodoquena plateau.

**Ecology:** *Dorofeyukea calcarea* was more abundant in the type material. The population were collected in periphytic habitat (epiphyton, epilithon, epipsammon) in lentic and lotic environments. It was found in neutral to alkaline waters (pH 6.7–8.6), conductivity 320–669 µS.cm$^{-1}$, water temperature 25–29 °C, N–$NO_{3-}$ 0.10–0.27 mg.l$^{-1}$, Total P 0.01–0.03 mg.l$^{-1}$, Ca 26.8–64.6 mg.l$^{-1}$, Mg 12.6–44.6 mg.l$^{-1}$, $SiO_2$ 8.26–23.2 mg.l$^{-1}$ (Tables 1–2).




Figs 34–45. *Dorofeyukea calcarea* sp. nov., LM, valve views: (36) holotype specimen. Scale bar 10 µm.




Figs 46–51. *Dorofeyukea calcarea* sp. nov., SEM: (46–47) external and internal valve view, respectively; (48) external view of central area showing the striation pattern and the proximal raphe endings; (49) internal view of central area showing the narrow fascia; (50) external view of valve apex showing the distal raphe ending; (51) internal view of valve apex, note the pseudosepta. Scale bars 2 µm.

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*Dorofeyukea calcarea* is similar to *D. grimmeioides* and *D. rostellata* in relation to lanceolate valves outline, central area and raphe ends. However, these species differ from *D. calcarea* by having larger valves, radiate striation pattern throughout the valve, lower densities of striae and areolae (Table S1). In addition, *D. grimmeioides* has capitate or rostrate apices, smaller and more elliptical central area with elongate striae. *Dorofeyukea calcarea* has uniform areolae, which is difficult to be observed in LM (Figs 34–45). The central area of *D. rostellata* is transapically oval outside and fairly irregular inside, and areolae can easily be counted in LM. This species has rostrate apices and internal distal raphe ends straight, terminating in a small helictoglossa near the apices (Trábert et al. 2019). *D. Dorofeyukea calcarea* has a maximum valve width of 5.5 µm, while the minimum valve width of *D. rostellata* is 6.6 µm (Hustedt 1937).

***Dorofeyukea kotschyi* (Grunow) Kulikovskiy, Kociolek, Tusset T. Ludwig in Kulikovskiy et al. (2019) (Figs 52–68)**

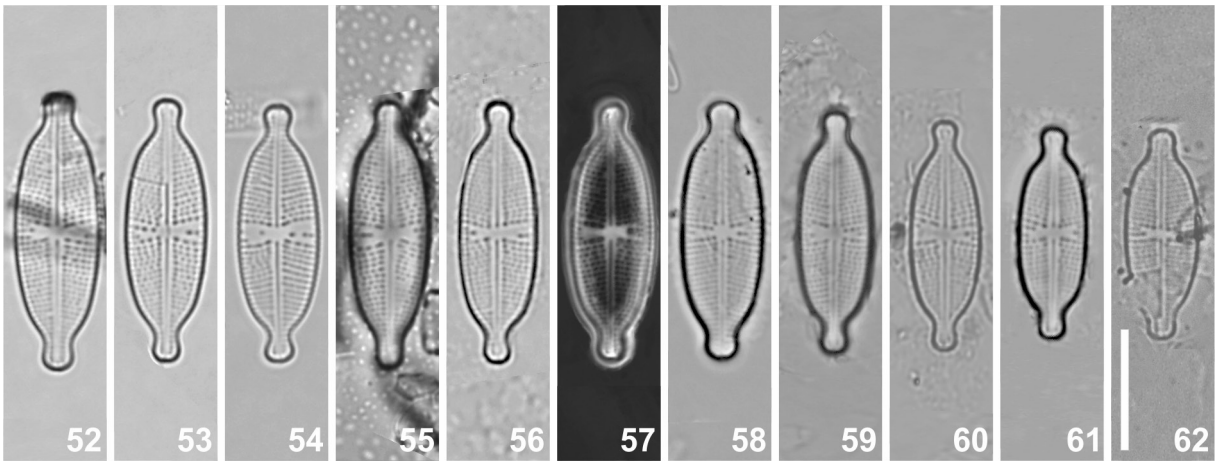
**Basionym:** *Navicula kotschyi* Grunow, Grunow (1860): Verh. K. Zool.–Bot. Ges. Wien 10: 538, pl.2: fig.12.

**LM (Figs 52–62):** Valves elliptic–lanceolate, with rostrate to capitate ends. Valve dimensions (n=35): length 16–23.6 µm, width 5.5–7 µm. Axial area narrow, linear. Raphe filiform. Proximal raphe endings straight, not expanded. Central area slightly asymmetric due to the varying number of short central striae ranging from one to three, most commonly two, forming narrow stauroid fascia bowtie–shaped, expanded to the valve margins. Striae radiate, 22–24 in 10 µm. Areolae rounded to transapically elongate, 26–30 in 10 µm.

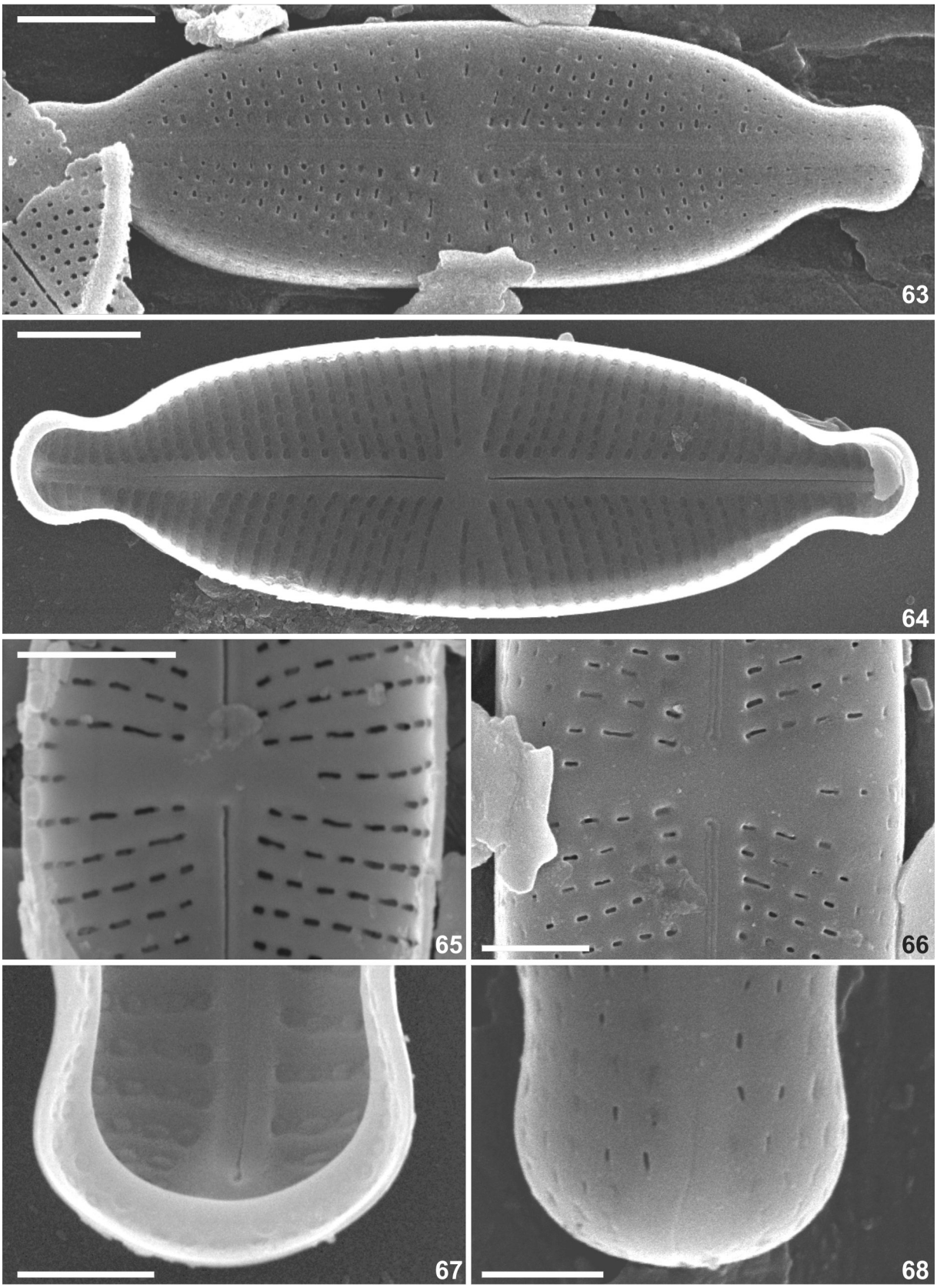
**SEM (Figs 63–68):** Externally, striae uniseriate, continuing onto the mantle, radiate at the center becoming parallel towards the ends (Fig. 63). Areolae transapically elongated in the valve face and longitudinally elongated near the apices (Figs 66, 68). Internally, areolae covered by hymenes (see Fig. 67). Proximal raphe fissures internally straight, externally slightly curved (Figs 65–66). Distal raphe endings externally hook–shaped, continuing onto the mantle (Fig. 68) and internally ending in a small helictoglossa (Fig. 67). Distinct, but narrow pseudoseptum at each valve apice, ornamented with rounded pores (Figs 64, 67).

**Occurrence in samples:** BRAZIL. Mato Grosso do Sul: Bonito, Formoso River Ecologic Park, epiphyton, UPCB 76099; Mato Grosso do Sul: Bonito, Sucuri River Ecologic Park, epiphyton, UPCB 76100; Mato Grosso do Sul: Jardim, Olho d'Água River, epiphyton, UPCB 78233; Mato Grosso do Sul: Bodoquena, Boca da Onça waterfall, epiphyton, UPCB 78240; Mato Grosso do Sul: Jardim, Chapeninha Spring, epiphyton, UPCB 78246; Mato Grosso do Sul: Bonito, Chapena waterfall, epiphyton, UPCB 78248; Mato Grosso do Sul: Bonito, Formoso River in Ecologic Park, epiphyton, UPCB 78256; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epiphyton, UPCB 78420; Mato Grosso do Sul: Bonito, waterfall in Mimoso River, epiphyton, ICN 203531; Mato Grosso do Sul: Bodoquena, Boca da Onça waterfall, epiphyton, UPCB 78422; Mato Grosso do Sul: Bodoquena, Boca da Onça waterfall, epiphyton, ICN 203533; Mato Grosso do Sul: Jardim, Prata River, epilithon, UPCB 78431; Mato Grosso do Sul: Jardim, Prata River, epilithon, ICN 203542; Mato Grosso do Sul: Bonito, Formoso River Ecologic Park, epilithon, UPCB 78455; Mato Grosso do Sul: Bonito, Formoso River Ecologic Park, epilithon, ICN 203566; Mato Grosso do Sul: Jardim, Olho d'Água River, epipsammon, UPCB 78456. Mato Grosso do Sul: Jardim, Olho d'Água River, epipsammon, ICN 203567.

**Ecology:** *Dorofeyukea kotschyi* was rare in the samples. The population was observed in periphytic habitat (epiphyton, epilithon, epipsammon) in lentic and lotic environments. It was found in neutral to alkaline waters (pH 7.2–8.6), conductivity 320–536 µS.cm$^{-1}$, water temperature 24–28 °C, N–$NO_{3-}$ 0.10–0.66 mg.l$^{-1}$, Total P 0.01–0.03 mg.l$^{-1}$, Ca 26.8–64.6 mg.l$^{-1}$, Mg 12.6–44.6 mg.l$^{-1}$, $SiO_2$ 8.2–23.2 mg.l$^{-1}$ (Tables 1–2).




Figs 52–62. *Dorofeyukea kotschyi*, LM, (phase contrast in 57), valve views. Scale bar 10 µm.




Figs 63–68. *Dorofeyukea kotschyi*, SEM: (63) external valve view; (64) internal valve view; (65) internal view of median region of valve showing the narrow fascia slightly expanded to the valve margins; (66) external view of median region showing the proximal raphe endings; (67) internal view of valve apex, note the small helictoglossa, pseudosepta and areolae occlusion near the apex; (68) external view of valve apex showing the distal raphe ending extending to the mantle. Scale bars 5 μm (63–64), 2 μm (65–66), 1 μm (67–68).

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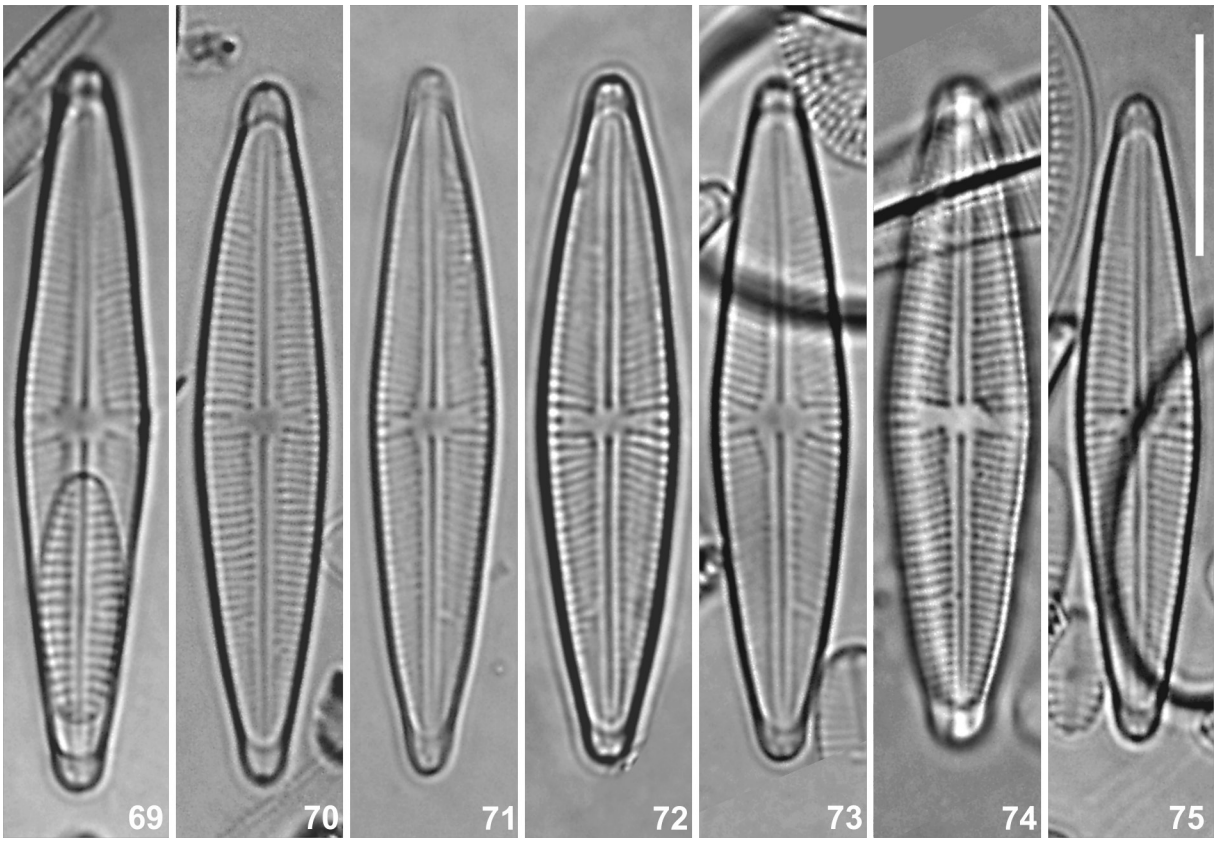
*Dorofeyukea indokotschyi* and *D. orangiana* (Patrick) Kulikovskiy et Kociolek have the external areolae openings transapically elongated and longitudinally elongated near the apices, but differ from *D. kotschyi* by valve shape, apices and number of areolae (Table S1).

*D. kotschyi* was described from the thermal waters of Budapest, Hungary in 1860, and it is now commonly found across the USA (Kulikovskiy et al. 2016 a, b). The species has been reported to Lake Upper Killarney and the river near Glencar (pH 6.3) in County Kerry in Ireland (Foged 1977). *D. kotschyi* was also reported to Dongting Lake, first record of the genus *Dorofeyukea* to China (Liu et al. 2020).

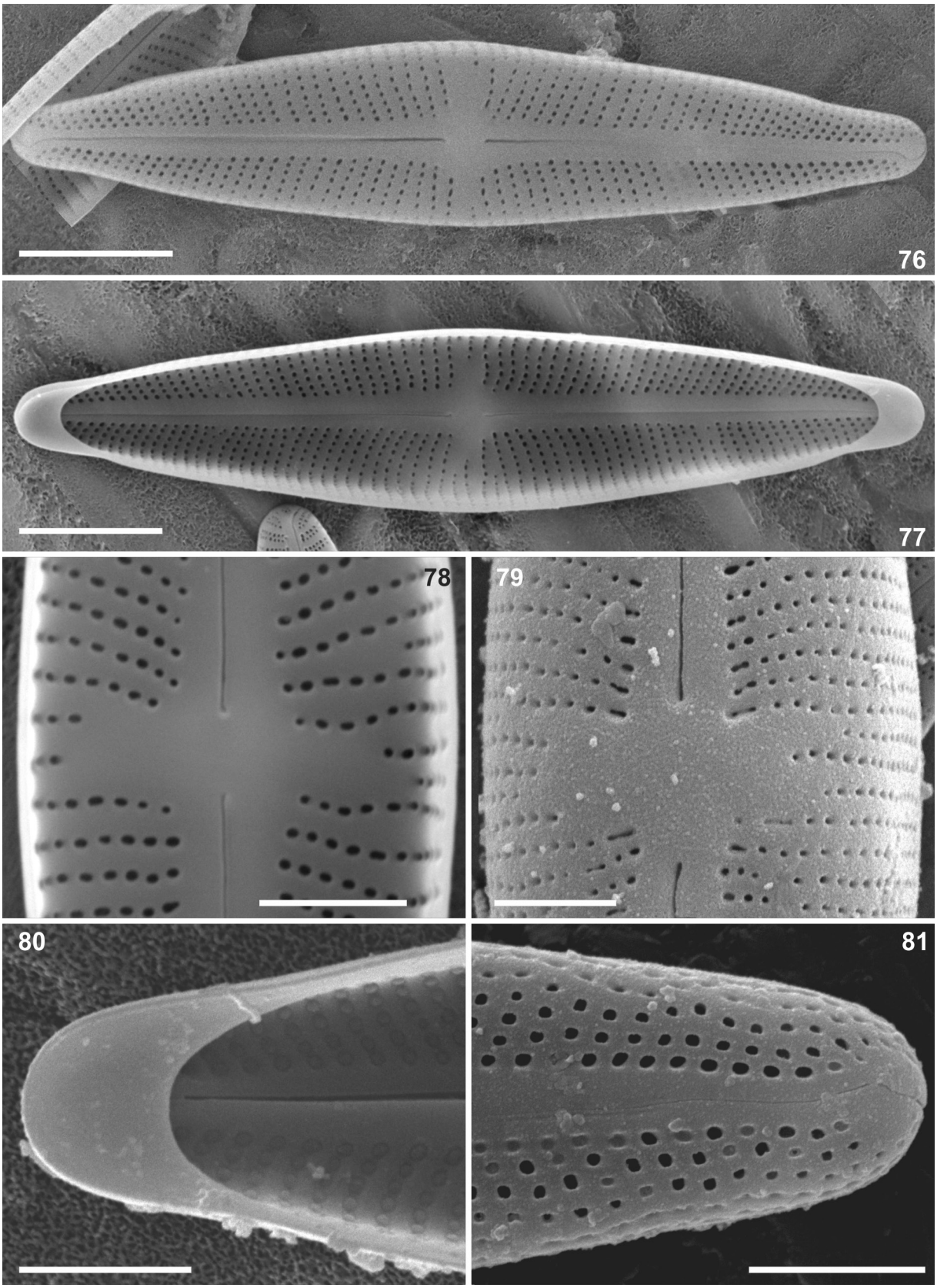
Kulikovskiy et al. (2019) recorded the occurrence of *D. kotschyi* in planktonic habitat (240 µS.cm$^{-1}$, pH 7.6, water temperature 30 °C) somewhat similar to that where we found *D. kotschyi* in Brazil. *Dorofeyukea kotschyi* [as *Luticola kotschyi* (Grunow) Taylor, Harding et Archibald, nom. inval.] has recently been reported occurring in phytoplankton samples for northwest Argentina in high–altitude from mountain lagoon between 400 and 3.000m (Taboada et al. 2021).

***Dorofeyukea pantanalensis* Tusset sp. nov. (Figs 69–81)**
**Description**
**LM (Figs 69–75):** Valves rhombical–lanceolate, acutely rounded apices, not protracted. Valve dimensions (n=20): length 29.1–33.6 µm, width 5.4–7 µm. Prominent pseudosepta. Broad axial area. Raphe filiform. Proximal raphe endings straight, not expanded. Central area round, slightly asymmetric due to the varying number of short central striae ranging from one to three, most commonly two, forming narrow stauroid fascia, slightly expanded to the valve margins. Striae radiate, 22–24 in 10 µm. Areolae not discernible in LM.
**SEM (Figs 76–81):** Externally, striae radiate, continuing onto the mantle. Areolae rounded to transapically elongate in the valve face and longitudinally elongated near the apices, 31–35 in 10 µm. Internally, areolae covered by hymenes (see Fig. 80). Internally and externally, the proximal raphe fissures are straight (Figs 78–79). External distal raphe endings hook–shaped to the secondary side, deflected in the same direction, continuing onto the mantle (Fig. 81). Internally, distinct, large pseudosepta present at each valve apex (Figs 77, 80).

**Holotype**: UPCB collection No. 78233 (here depicted in Fig. 72), deposited in (Paraná Federal University, Botany Department, Brazil).
**Type locality**: Brazil. Mato Grosso do Sul: Jardim, Olho d'Água River, 21°26'12.6" S, 56°26'09.3" W, November 2015, E. Tusset s.n.
**Habitat:** periphytic (epiphyton) in lotic environments.
**Etymology:** The species was named in honor to the environment in which the species was found, the Brazilian Pantanal biome.




Figs 69–75. *Dorofeyukea pantanalensis* sp. nov., LM, valve views: (72) holotype specimen. Scale bar 10 µm.




Figs 76–81. *Dorofeyukea pantanalensis* sp. nov., SEM: (76–77) external and internal valve view, respectively; (78) internal view of median region of valve showing the narrow fascia slightly expanded to the valve margins; (79) external view of median region showing the proximal raphe endings; (80) internal view of valve apex, note the pseudosepta and areolae occlusion near the apex; (81) external view of valve apex, showing the distal raphe ending extending to the mantle. Scale bars 5 μm (76–77), 2 μm (78–81).

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**Ecology:** *Dorofeyukea pantanalensis* was very rare in the type material. The population was observed in periphytic habitat (epiphyton) in lotic environment. It was found in neutral waters (pH 7.2), conductivity 320 µS.cm$^{-1}$, and water temperature of 27 °C (Table 1).

*Dorofeyukea pantanalensis* is very distinct from any other *Dorofeyukea* species. Rhombic–lanceolate valves, prominent pseudosepta, records of valves with dimensions greater than 30µm were only reported to *D. ivatoensis* (Metzeltin et Lange–Bertalot) Kulikovskiy et Kociolek*, D. rostellata* and *D. tenuipunctata* (Hustedt) Kulikovskiy et Kociolek, and they can be easily distinguished by the valve shape (Table S1).

*Navicula septataeoides* Hustedt, rarely reported in the literature, is described and illustrated (pl. 776, fig. 1749) by Hustedt (1961–1966) from Lago Alto Branco, Brazil, and it seems to be the taxon most related to *D. pantanalensis*. *Navicula septataeoides* presents prominent pseudosepta and radiate striae along the valve, differing by lacking the stauroid fascia, slightly expanded to the valve margins, and dimensions are much larger, length (73µm long and 15µm wide) in relation to *D. pantanalensis*.

*D. pantanalensis* presents wider valves, rhombic–lanceolate, acutely rounded apices, not protracted, while *D. bodoquenensis* has lanceolate valves with subrostrate ends (Table S1).

***Dorofeyukea texana* (Patrick) Kulikovskiy et Kociolek in Kulikovskiy et al. (2019) (Figs 82–99)**

**Basionym:** Navicula texana Patrick, Patrick (1959): Proc. Acad. Nat. Sci. Philadelphia, 111: 98, pl. 8, fig. 5.

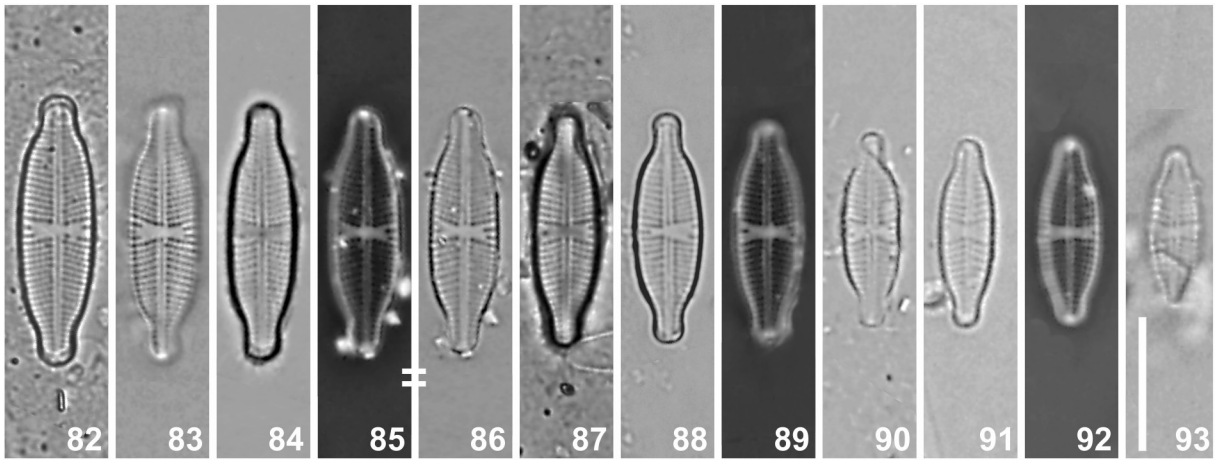
**LM (Figs 82–93):** Valves lanceolate, with subcapitate to rostrate ends. Valve dimensions (n=30): length 17.5–21.5 µm, width 4–5.6 µm. Narrow pseudosepta. Axial area narrow, linear. Raphe filiform. Proximal raphe endings straight, not expanded. Central area slightly asymmetric due to the varying number of short central striae ranging from one to three, most commonly two, forming narrow stauroid fascia bowtie–shaped, slightly expanded to the valve margins. Striae radiate, 20–24 in 10 µm. Areolae not discernible in LM.

**SEM (Figs 94–99):** Externally, striae uniseriate, continuing onto the mantle, radiate at the center becoming parallel towards the ends. Areolae transapically elongated in the valve face and longitudinally elongated near the apices, 33–35 in 10 µm. Internally areolae individually covered by hymenes (see Fig. 96). Externally, the proximal raphe fissures slightly deflected in the same direction (Fig. 97). Internally, distinct, narrow pseudosepta present at each valve apex (Fig. 98). External distal raphe endings hook–shaped to the secondary side, deflected, continuing onto the mantle (Fig. 99). Open girdle bands with one row of small pores (Figs 95, 99).

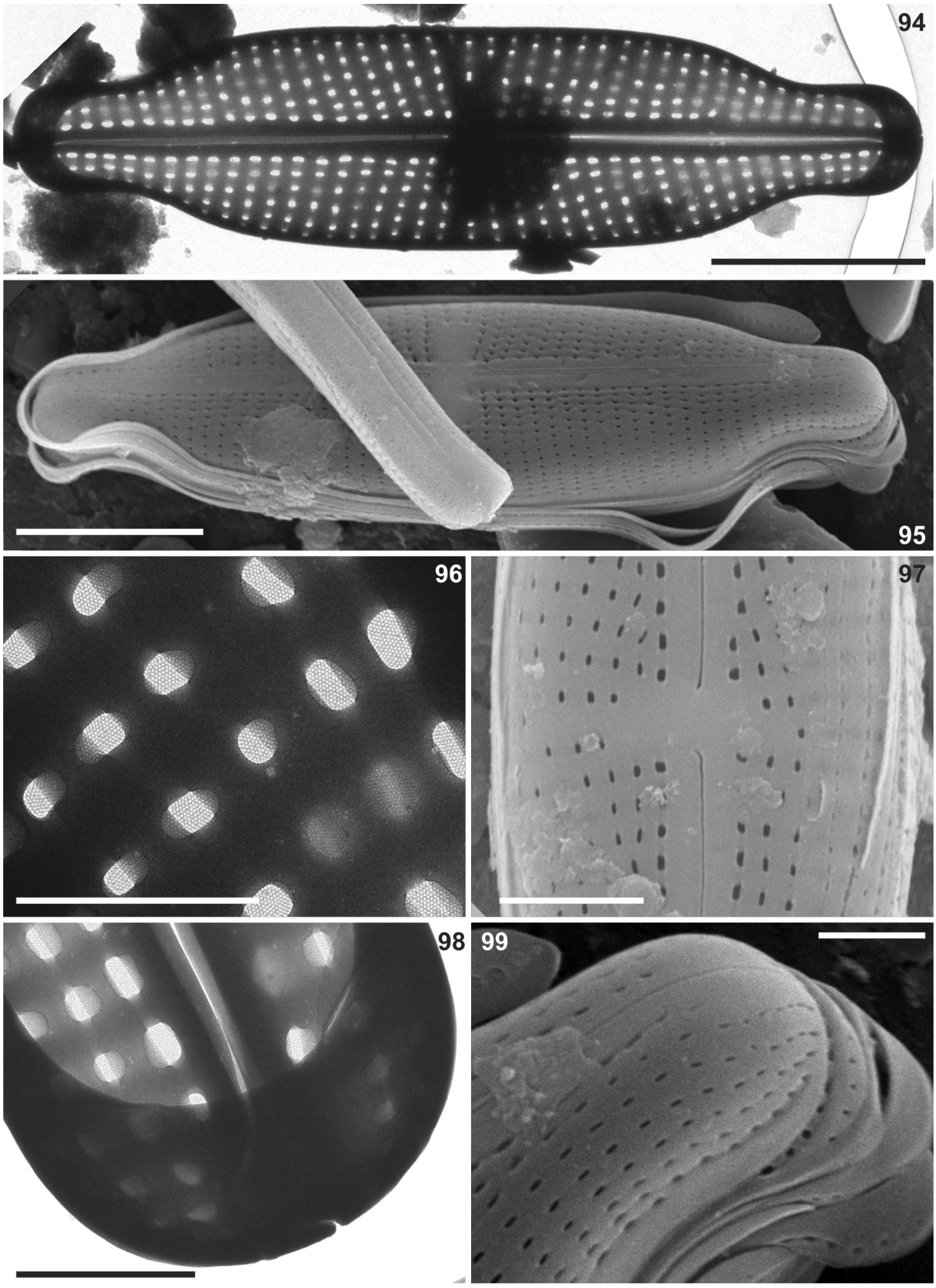
**Occurrence in samples**: Brazil. Mato Grosso do Sul: Bonito, Chapena waterfall, epiphyton, UPCB 78248; Mato Grosso do Sul: Bonito, Blue Spring, epiphyton, UPCB 78428; Mato Grosso do Sul: Bonito, Blue Spring, epiphyton, ICN 203539.

**Ecology:** *Dorofeyukea texana* was very rare in the samples. The population was observed in periphytic habitat (epiphyton) in lotic environments. It was found in neutral to alkaline waters (pH 6.7–7.6), conductivity 382–669 µS.cm$^{-1}$, water temperature 25–28 °C, N–$NO_{3-}$ 0.10 mg.l$^{-1}$, Total P 0.01 mg.l$^{-1}$, Ca 60.6 mg.l$^{-1}$, Mg 39.1 mg.l$^{-1}$, SiO2 10.9 mg.l$^{-1}$ (Tables 1–2).

Potapova (2013) investigated the type population of *Navicula texana* Patrick and *N. savannahiana* Patrick, currently included in *Dorofeyukea*. Both species present narrow capitate apices, punctate striae and transverse central area characteristic of *D. kotschyi*, but they differ in width and valve outline. Potapova (2013) also observed a pseudoseptum at each distal valve end of *D. texana*, but she cannot confirm the existence of pseudosepta in *D. savannahiana* due to the rare occurrence of this species in the sample. Based on the illustrations presented by Potapova (2013: Figs 81–87), we concluded that *D. savannahiana* has curve–radiate striae and transapically elongated areolae different from




Figs 82–93. *Dorofeyukea texana*, LM, (phase contrast in 85, 89, 92), valve views. Scale bar 10 µm.




Figs 94–99. *Dorofeyukea texana*, (94, 96, 98) TEM, (95, 97, 99) SEM: (94) valve overview showing the striation pattern; (95) external valve view; (96, 98) detail of areolae occlusion, note the hymens; (97) external view of median region of valve showing the narrow fascia slightly expanded to the valve margins; (98) view of valve apex, pseudosepta; (99) external view of valve and apex showing the girdle bands. Scale bars 5µm (94–95), 2µm (97), 1µm (96, 98–99).

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*D. texana* that have parallel striae and punctiform or apically elongated areolae.

Distribution of *D. texana* (as *Navicula texana*) in North America: Laurentian Great Lakes (Stoermer, Kreis & Andresen 1999), type locality Texas, freshwater with high mineral content, hard water (Patrick & Reimer 1966), and United States of America (Kociolek 2005, Eberle 2008). It was also recorded from Asia: Korea (Lee, Choi & Lee 1995, Lee 2012), Mongolia (Dorofeyuk & Kulikovskiy 2012), and Russia (Far East) (Medvedeva & Nikulina 2014).

*Dorofeyukea* belongs to the stauroneioid diatom forming a single branch clade, together with *Stauroneis* Ehrenberg, *Prestauroneis* Bruder et Medlin, and *Craticula* Grunow, and it is frequently characterized as having a narrow stauroid fascia surrounded by few shortened striae. Also, species are represented by relatively small length cells, not exceeding 40 µm (Kulikovskiy et al. 2019; Trábert et al. 2019). *Stauroneis* is a polyphyletic genus and further molecular and morphological investigation are needed (Kulikovskiy et al. 2019).

Cox & Williams (2000) stated that in 1894, Cleve had already commented that species with different forms of thickening in the central area were placed in *Stauroneis*, and the stauros are used as diagnostic characteristic of this genus, and is easily recognized by light microscopy. Based on this supposed feature, the central area can be thickened in various ways and delimited by the shortening of striae (Round et al.1990; Kociolek et al. 2019; Cox & Williams 2000; Cox 2001; Wadmare et al. 2022).

*Dorofeyukea kotschyi* was included in *Navicula* before. But, the isodiametric shape of the areolae is not characteristic of the apically elongated areolae of *Navicula* sensu stricto, as well as the pseudoseptum; therefore, these species should be placed in another genus (Potapova 2013). The study of scanning electron microscopy (SEM) is often necessary to determine the taxonomic position of species, recent studies on several original materials have transferred some naviculoid species from *Navicula*, *Naviculadicta* Lange–Bertalot in Lange–Bertalot et Moser to another genus, as well as some taxa belonging to *Dorofeyukea*.

Twelve taxa use to be the total diversity of *Dorofeyukea.* We found two of them occurring in Brazilian karst, and four new species unknown so far, increasing the number of species in the genus *Dorofeyukea* to sixteen. The taxa were observed in periphytic habitat (epiphyton, epilithon, epipsammon) in lentic and lotic freshwater environments. The species *D. pantanalensis, D. texana,* and *D. bahlsii* were rare in this study, *D. pantanalensis* and *D. texana* occurred in only one sample while *D. bahlsii* in three. *Dorofeyukea bodoquenensis*, *D. calcarea,* and *D. kotschyi* occurred in more than ten samples (Table 1).

Investigating diatom diversity occurring in these karst environments around the world, elevated richness is notable in Florida Everglades (Lee et al. 2013), Croatia (Jasprica & Hafner 2005), Slovakia (Czerwik–Marcinkowska et al. 2018), Bosnia, Herzegovina, Croatia and Montenegro (Hafner & Jasprica 2013), Poland (Wojtal 2003), Vietnam (Weide 2015), Mexico (Novelo et al. 2007; Ibarra et al. 2009), Mediterranean (Lai et al. 2019), Spain (Delgado et al. 2013), Italy (Lai et al. 2016). Some recent studies resulted in the description of new species of diatoms as in Croatia (Gligora Udovič et al. 2022), China (You et al. 2016; You et al. 2019; Lowe et al. 2017), and Brazil (Tusset et al. 2017, 2018). Therefore, new species proposals in this study were not surprising.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Morphometric data of the Brazilian population and related Dorofeyukea species.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)


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