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The first freshwater *Berkeleya* (Bacillariophyceae) species - *Berkeleya fluviatilis sp. nov.* from the Krka River, Croatia

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ABSTRACT
The Krka River flows into the Adriatic Sea and its estuary is known to have been formed in the early Holocene. Generally, karst freshwater in this region encounters potential salinization, creating a link for the transition of various species from marine to freshwater habitats. Due to the high conductivity in karst freshwater ecosystems, it is assumed that species in these environments could more easily overcome osmotic stress during adaptation to freshwater conditions. In this study, we provide a description of new freshwater species of the predominantly marine genus *Berkeleya* observed in the Krka River. *Berkeleya fluviatilis sp. nov.* resembles the brackish/marine species *Berkeleya rutilans*, making it a potential model for studying time-calibrated evolution into freshwater environments in such areas.

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INTRODUCTION

Diatoms (Bacillariophyceae) are one of the most diverse group of eukaryotic unicellular algae that inhabit a variety of terrestrial, freshwater and marine habitats (Mann & Droop, 1996). As major constituents of planktonic and benthic algal communities, they evolved a varied of morphological adaptation for different habitat requirements. Buoyancy in the water column and the ability to adhere to submerged surfaces may be a result of secretion activity (Aumeier & Menzel, 2012). Secretion of diversified adhesive structures provides important functions such as cell motility, formation of an adhesive stalks for attachment to surfaces or to form complex colonies. As a result of intense matrix secretion, dense colonies of macroscopic mucilage tubes can form (Aumeier & Menzel, 2012). Various genera of pennate diatoms can form tubes, and cells can often be observed sliding within them. These pennate genera include *Berkeleya* (Cox, 1975a), *Encyonema* (Round *et al*., 1990) *Gyrosigma* (Round *et al*., 1990), *Navicula* (Cox, 1988), *Parlibellus* (Camacho & Vidal, 2011), *Haslea* (Hamsher & Saunders, 2014), *Mastogloia* (John, 1993) and *Nitzschia* (Fernandes *et al*., 2002; Fricke *et al*., 2017).

The genus *Berkeleya* was first described by Greville (1827), using tube structure as the most important taxonomic feature. The tube structure exhibited significant variability, evident in Grunow's treatment of the genus, which encompassed a wealth of varieties (Grunow, 1880). For instance, *B. rutilans* (Trentepohl *ex* Roth) Grunow alone was described by 30 varieties based on colony characteristics, such as branching, granulation of the matrix, size of the colony and colour of the matrix. Based on these characters Grunow (1880) divided *B. rutilans* in two groups: a) colony matrix smooth and hyaline, and b) colony matrix rough and granulated. Afterwards, with the development of better microscopes, valve structure was studied in greater detail (Cleve, 1894; Cox, 1975b; Péragallo & Péragallo, 1897; Van Heurck, 1896). Cleve (1894) questioned tube morphology as a differential generic character and rejected this feature, resulting with the transfer of the marine tube-dwelling *Berkeleya* to the genus *Amphipleura* Kützing. This approach was accepted by most later authors. Later, Cox (1975a) separated *Berkeleya* from *Amphipleura*, based on several morphological characteristics such as raphe structures, presence or absence of definite ribs, symmetrical or asymmetrical axial areas, as well as ecological preferences (freshwater *vs.* marine/brackish). Additionally, Cox (1975a) provided emended description the genus and four *Berkeleya* species.

Presently, *Berkeleya* comprises species with linear, linear-lanceolate, lanceolate to elliptic valves, and with narrowly or bluntly rounded to slightly capitate ends. Striae are uniseriate, consisting of simple areolae closed by hymenes. External raphe endings are straight or slightly deflected towards the secondary side. The mature cingulum contains five open bands, each bearing two rows of round or oval poroids (Round *et al*., 1990). The plastid contains two girdle-appressed parts connected by a narrow isthmus (Cox, 1975b). According to Guiry & Guiry (2025) there are 17 accepted taxa (15 species and two varieties) within *Berkeleya* and an additional 65 taxa with unresolved taxonomic or nomenclatural status.

Though these colony forming genera have ecological importance, species within *Berkeleya* are still scarcely studied (Fricke *et al*., 2017; Olenici *et al*., 2021). Based on emended description of the genus given by Cox (1975a), species are

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characterized as brackish or marine. Further, taxonomical and ecological studies of Cox (1979) in England, Mizuno (1981) in Japan, Lobban (1985) in North America, and Houpt (1994) in Europe revealed the habitat preferences for species of *Berkeleya*. Currently, among all known *Berkeleya* taxa, only *Berkeleya fennica* Juhlin-Dannfelt is erroneously mentioned as a freshwater species (Olenici *et al.*, 2021), although other records of this species, including type material, originate from brackish or saline waters (Gong *et al.*, 2020; Juhlin-Dannfelt, 1882). Here, we present the first freshwater species from the Krka River, which reveals new insights into the ecological preferences and diversity of *Berkeleya*.

MATERIALS AND METHODS

Study site

The Krka River, a meandering watercourse with a length of 73 km and a catchment area of 2,088 km^2, winds its way through the Dinaric region of Dalmatia in Croatia (Fig. 1). Embedded in this unique karstic landscape, the river comprises a complex web of surface and groundwater dynamics influenced by a variety of factors such as lithological formations, tectonics, extent of karstification, groundwater linkages, and hydrological conditions (Cukrov *et al.*, 2007). The Krka River is mainly characterized by the presence of many tufa barriers along its course. In this context, 'tufa' refers to porous $CaCO_3$ deposits that develop under certain physical and chemical conditions. These deposits not only shape the geological character of the river, but also provide a habitat for a variety of organisms that make an important contribution to the complex process of lime precipitation (Kulaš *et al.*, 2022; Primc-Habdija & Matoničkin, 2005; Vilenica *et al.*, 2018).

Sample collection and microscopy analysis

Surface sediment was collected with a turkey baster, while epilithon was collected with a stainless-steel scoop. Both samples were preserved in ethanol at a final concentration of 70%. In the laboratory, diatom samples were cleaned with $KMnO_4$ and 37% HCl and boiled for 30 min at 80°C. The samples were centrifuged for 10 minutes and subsequently rinsed five times with distilled water. Permanent diatom slides were prepared using Naphrax and observed in light microscopy (LM) under oil immersion at 1000× magnification with a Nikon Eclipse 80i microscope equipped with objective Plan-Apochromat with aperture 1.4 and working distance 0.13 mm. The slides were deposited in the Croatian National Diatom Collection (HRNDC) at the Faculty of Science, University of Zagreb and the Macedonian National Diatom Collection (MKNDC) at the Institute of Biology, Faculty of Natural Sciences, SS Cyril and Methodius University in Skopje. For scanning electron microscopy (SEM), the material was prepared by drying a clean diatom suspension onto cover slips attached to SEM stubs, coated with gold-palladium, and studied with a Zeiss Gemini Ultra plus SEM microscope (working distance 3–12 mm, 3–5 kV, the Natural History Museum, London, UK, Imaging and Analysis Centre).

RESULTS

During the long-term observations of the freshwater part of the Krka River (Croatia) and Lake Ohrid (North Macedonia),

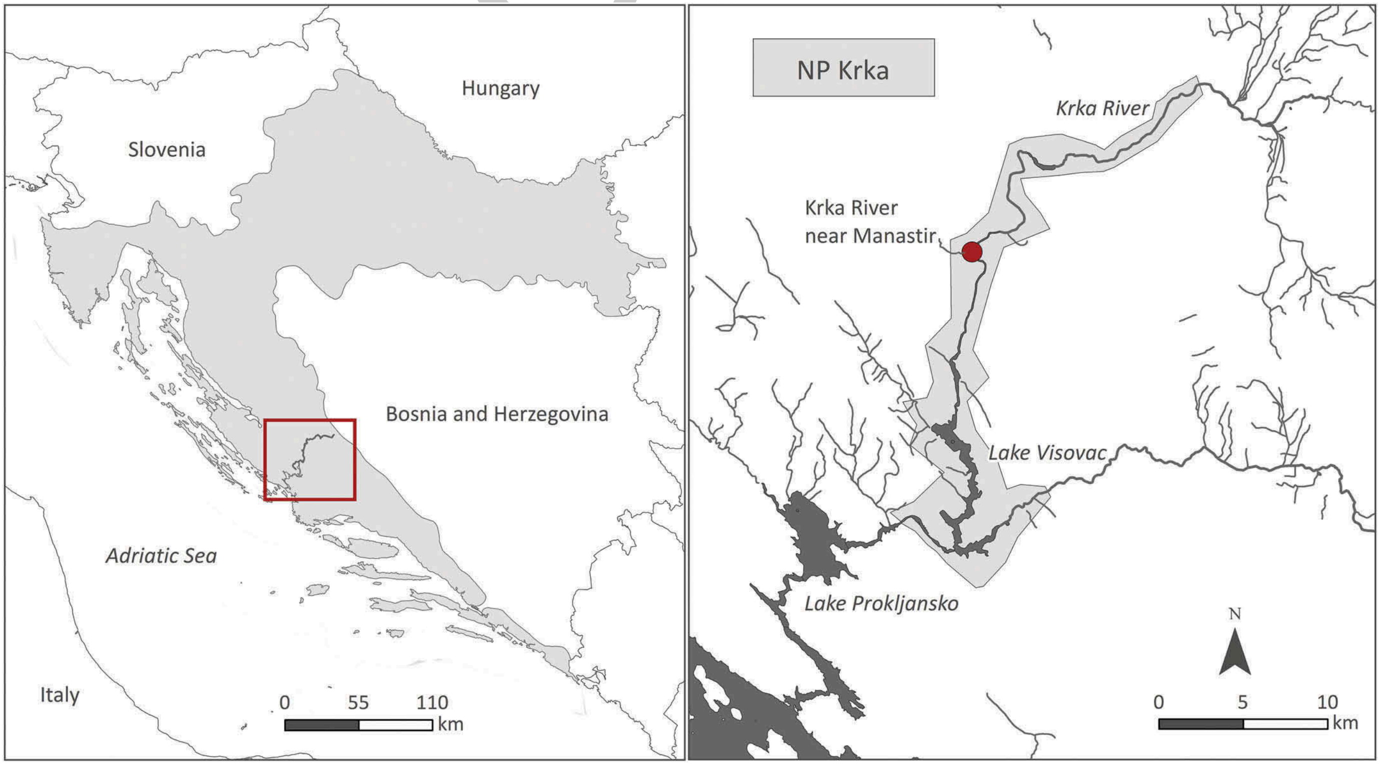


Fig. 1. Study area and location of the type locality, Krka River near Manastir.

one species of *Berkeleya* was observed, but almost always in low abundance. In one sample from 2022 from the Krka River, this species was present in higher abundance that allowed us to observe its morphological features in more detail. The species possess different morphological characters such as valve shape, valve ends, stria density, as well as a different ecology compared to other known *Berkeleya* species and therefore we propose a new species.

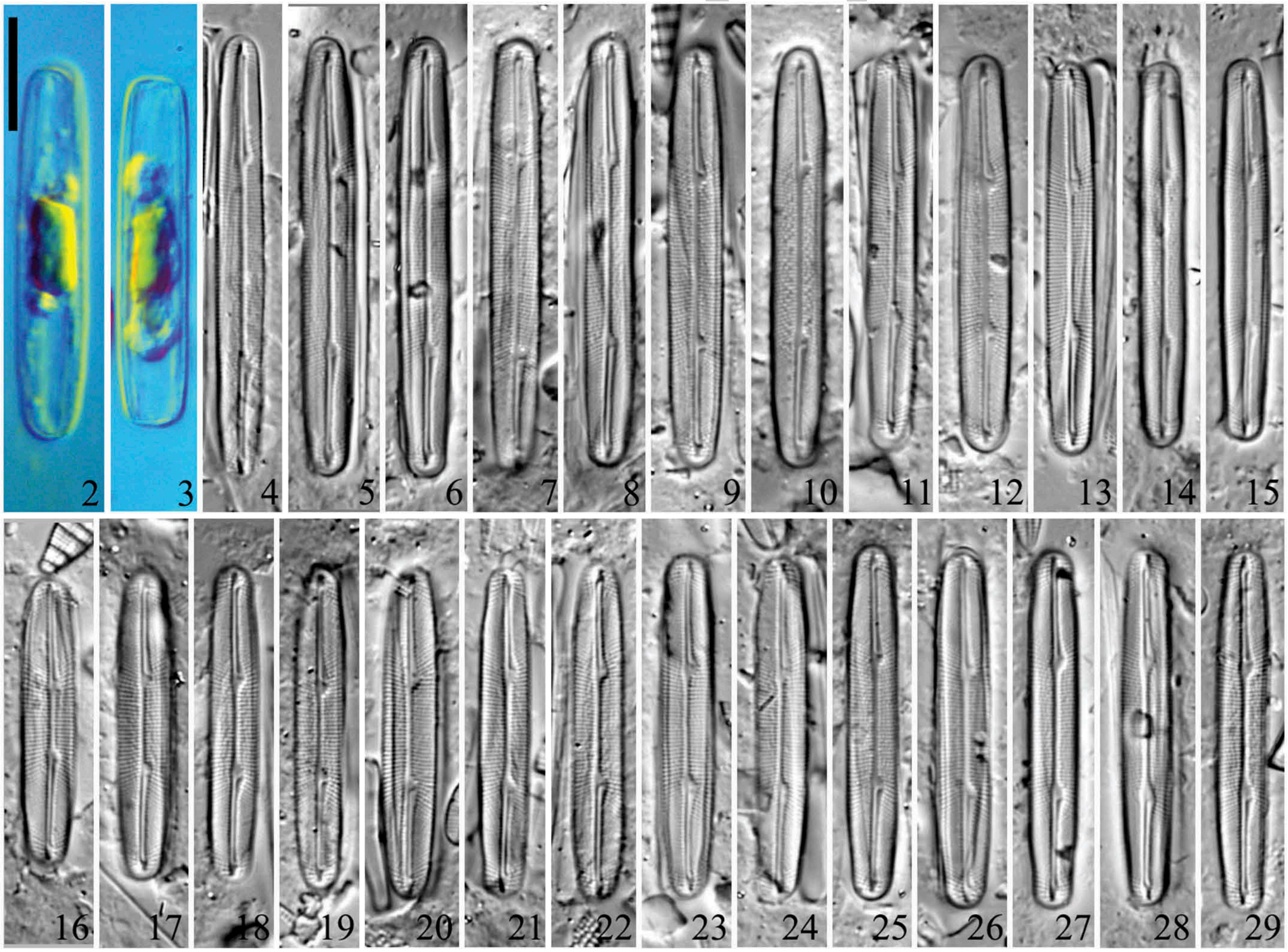
Berkeleya fluviatilis Gligora Udovič & Levkov *sp. nov.*
Fig 2–36

DESCRIPTION: Cells solitary, not forming colonies in mucilage tubes (Figs. 2, 3). Frustules in girdle view rectangular (Figs. 2, 3). Valves linear with almost parallel margins and obtusely rounded and subprotracted apices (Figs. 4–29). Transition from valve face to valve mantle gradual (Figs 30, 33). Valve mantle shallow. Five open girdle bands per valve with two rows of round pores (Figs 32). Each girdle band bears two rows of poroids for most of its length, and reduced to a single row near the valve ends (Figs 30, 33). Valve length (*n* = 42) 25–39 µm, valve width 4.0–5.0 µm. Axial area narrow, linear. Central area very narrow, linear, elongated along apical axis (Figs 31, 32, 34). Raphe branches straight, relatively short, central raphe ends separated by approximately one-third of valve length (Figs 33, 35). Length of raphe branches almost constant during life cycle (Figs. 4–29). Central area shortens as cell length decreases (Figs. 4–29). Externally, proximal raphe ends bent on one side and, in some specimens, is slightly dilated (Figs 33, 35). Distally raphe terminating on valve face not passing onto valve mantle (Figs 33, 35). Distal raphe ends unilaterally deflected (Figs 31, 32, 34). Internally, proximal raphe ends slightly expanded, distally terminate in large porte-crayon helictoglossa (Figs 36). Striae parallel to slightly radiate in mid-valve 30–32 in 10 µm, punctate, becoming strongly radiate distally and convergent near apices (Figs 33). Externally, striae are uniseriate composed of round to elliptical areolae (Figs 33, 35). Areolae near raphe and central area larger and more elongated (Figs 32, 34). Areolae on valve apex smaller and slit-like or tear-drop-shaped (Figs 33, 35). Internally, areolae with large, round to elongate openings (Figs 36).

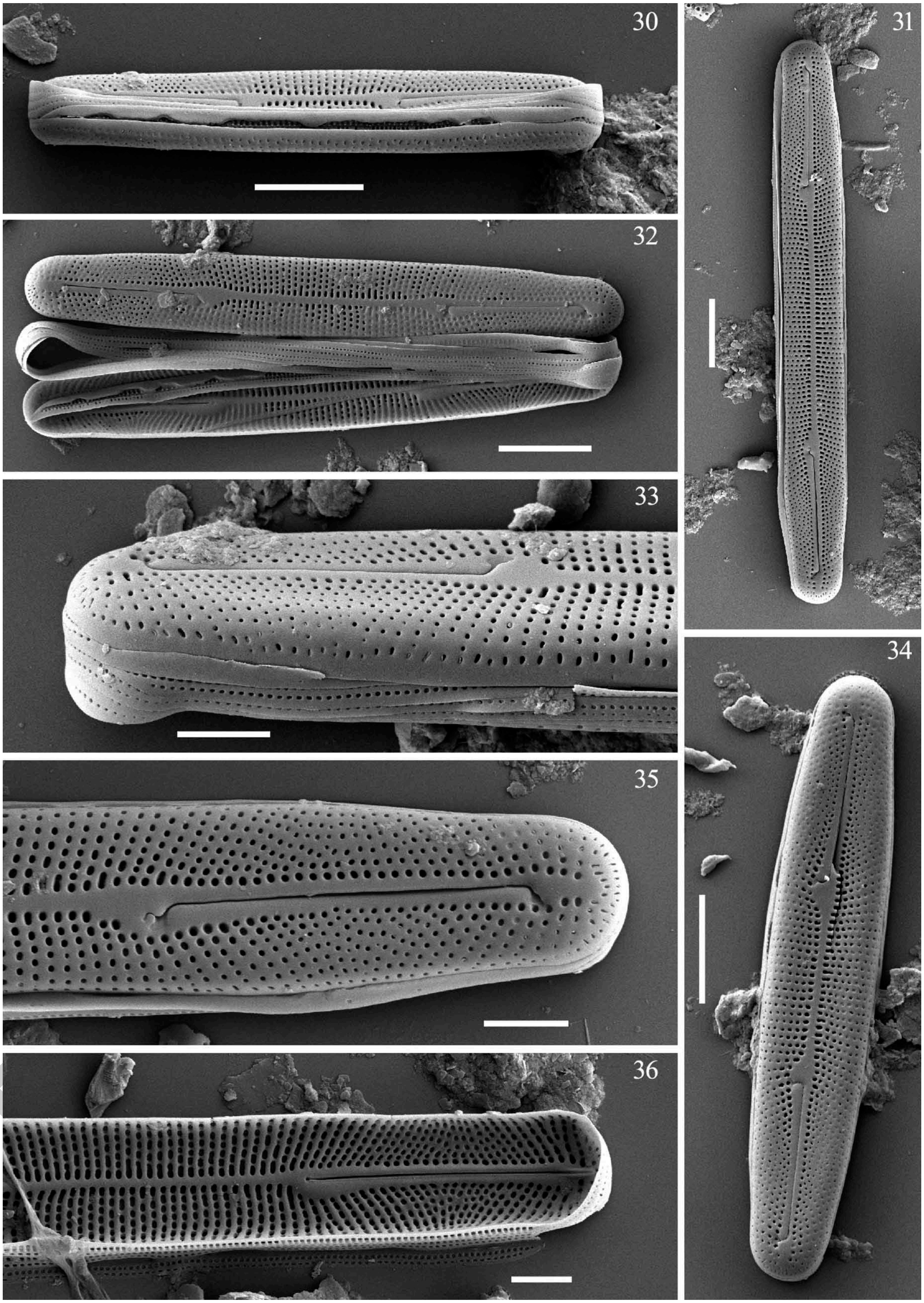
HOLOTYPE: Slide HRNDC 002093, collected 30 August 2022, by M. Gligora Udovič and Z. Levkov, deposited in Croatian National Diatom Collection, Faculty of Science, University of Zagreb, Croatia.

ISOTYPES: Slide MKNDC 014170, collected 30 August 2022, by M. Gligora Udovič and Z. Levkov, deposited in Macedonian National Diatom Collection, Institute of Biology, Faculty of Natural Sciences, Ss Cyril and Methodius University in Skopje, Republic of North Macedonia.

TYPE LOCALITY: 43° 57’ 43.8084” N, 15° 59’ 22.3692” E, surface sediment, Krka River near Manastir, Croatia.



Figs 2–29. Light microscope images of native preserved and cleaned valves. Scale bar = 10 µm.
Figs 2,3. Light microscope images of native preserved valves with chloroplast (note that the chloroplast is modified due to fixation).
Figs 4-29. Light mic roscope images of cleaned valves showing the morphological variation of the type population and the size diminution series.



Figs 30–36. Scanning electron microscope images of cleaned frustules and valves. Scale bars = 5 µm (Figs 30–32, 34) and 2 µm (Figs 33, 35, 36).
Fig. 30. Frustule in girdle view. showing the valve mantle and open girdle bands.**Fig. 31**. External valve view of the whole valve.**Fig. 32**. Opened frustule showing external and internal valve structure and girdle bands bearing two rows of round areolae.**Fig. 33**. Detailed view of half valve in oblique view showing the valve mantle and girdle bands.**Fig. 34**. External valve view of the whole valve.**Fig. 35**. External detailed valve view of the half valve showing the raphe structure and areolae morphology.**Fig. 36**. Internal valve view showing the raphe structure and areolae morphology.

ETYMOLOGY: the epithet *'fluviatilis'* was proposed to aptly signify its association with river habitats.

Berkeleya fluviatilis is a freshwater species, recorded at several locations in the freshwater part of the Krka River, such as Lake Visovac, Manojlovac and Roški slap. A few valves were also recorded in Lake Ohrid, N. Macedonia (Acc. No. MKNDC 009728), Lake Ohrid shore near Daljan (41°7' 5.9988" N 20°46' 39" E). Both localities are typical karstic habitats, oligotrophic with slightly alkaline pH and medium conductivity (200–600 μScm^{-1}) due to the high carbonate concentrations. At the type locality, the Krka River is smooth flowing with the presence of different macrophytes near the shore and a bottom covered with sand and pebbles. The type locality is characterized by relatively high conductivity (636 μScm^{-1}), due to the presence of dissolved carbonates, higher oxygen concentration (9.44 mgL^{-1}) and oxygen saturation (105%), high pH values (8.43) and moderate to high temperature (20.4° C) at the time of sampling. Chemical parameters concentrations measured at the time of sampling were: nitrates ($NO_3^{-}N$ = 0.002mgL^{-1}), nitrites ($NO_2^{-}N$ = 0.003 mgL^{-1}), ammonium ion (NH_4^{+}-N ≤0.02 mgL^{-1}), bicarbonate (HCO_3^{-} =165 mgL^{-1}), and total phosphorus (TP =0.02 mgL^{-1}). Other species in the type material are typical freshwater taxa such as: *Achnanthidium microcephalum* Kützing, *Achnanthidium straubianum* (Lange-Bertalot) Lange-Bertalot, *Encyonopsis microcephala* (Grunow) Krammer, *Achnanthidium affine* (Kützing) Czarnecki and *Denticula tenuis* Kützing.

species of *Berkeleya* have been described from marine or brackish waters (Medlin, 1990; Round *et al.*, 1990) and most of them form mucilage tubes (Table 1). *Berkeleya fluviatilis* is a freshwater taxon and observation of preserved samples indicates that it is not a tube dwelling species (Figs. 2, 3).

Berkeleya fluviatilis possess a unique set of characters that distinguish it from similar species (Table 1). Several *Berkeleya* species such as *B. adeliensis* Medlin, *B. hyalina* (F.E.Round & M.E.Brooks) E.J.Cox, *B. fragilis* Greville, *B. transfretana* Olenici, Jiménez-Gómez & Blanco, have high stria density (>40 in 10 μm) and thus valves have a hyaline appearance. Striae in *B. fluviatilis* are clearly visible with LM and appear as finely to distinctly punctate. The most morphologically similar species to *B. fluviatilis* is *B. rutilans*. Differences between these two species, beside tube formation, can be noticed in the valve shape (elliptic-lanceolate to linear-elliptic in *B. rutilans vs.* linear with almost parallel margins) with narrowly rounded apices (*vs.* obtusely rounded and subprotracted apices in *B. fluviatilis*) and stria density (24–28 in 10 μm in *B. rutilans vs.* 30–32 in 10 μm in *B. fluviatilis*; Minami *et al.*, 2011; Witkowski *et al.*, 2000). A population of *B. rutilans* from the Roanne River at Lenoncourt, France (Heudre *et al.*, 2021) is characterized by more elliptical valve and lower stria density (26–28 in 10 μm). Several populations of *B. rutilans* from England have been studied by Cox (1975b) and the stria density of these populations is fairly constant around 27 in 10 μm. Cox (1977) recorded *B. rutilans* in the Severn Estuary (England) in the upper eulittoral without a large freshwater inflow, but also observed variation in the stria length (not stria density) and shape of the central area that might indicate existence of discrete varieties. The highest morphological and genetic diversity in *B. rutilans* have been observed by Hamsher & Saunders (2014) from Canadian coasts with 14 so called 'genetic species groups'. Only one species group (*Berkeleya* sp. 9 *sensu* Hamsher & Saunders, Figs. 10–12) has a linear shape, but it can easily be differentiated by the lower valve width (2–3 μm), shape of the valve ends (narrowly rounded) and stria density and orientation (22–28 (26) in 10 μm, radiate in the centre of the valve and parallel towards the apices) from *B fluviatilis*. Other genetic species groups depicted in Hamsher & Saunders (2014, Figs 1–18) have elliptic to elliptic-lanceolate valve outline with narrowly rounded apices, and can therefore be easily distinguished from *B. fluviatilis*. *Berkeleya obtusa* (Greville) Grunow has comparable stria density (24–32 in 10 μm) with *B. fluviatilis*, but the former has lanceolate valves with narrowly rounded apices (Witkowski *et al.*, 2000; figs 62:25–28). *Berkeleya antarctica* Grunow is characterized by slightly higher striae density (36–40 in 10 μm) and linear-lanceolate valves with narrowly rounded apices. Due to the high stria density the valves of *B. antarctica* appear almost hyaline (Witkowski *et al.*, 2000; figs 62, 10–13), opposite to *B. fluviatilis* which has coarse and punctate striae clearly visible with LM. Several species of *Berkeleya* have valve outline similar to *B. fluviatilis*. *Berkeleya adeliensis* has linear valves with broadly rounded ends, but due to the high stria density (50–60 in 10 μm) appear hyaline in the LM. *Berkeleya scopulorum* (Brébisson) Cox is characterized by linear valves with broadly rounded ends, but the valves are much larger (100–400 μm long and 8–18 μm wide) than *B. fluviatilis* (Witkowski *et al.*, 2000, p. 157). Differences between *B. fennica* Juhlin-Dannfelt and *B. fluviatilis* can be seen in valve width (2.6–3.8 μm in *B. fennica vs.* 4.0–5.0 μm in *B. fluviatilis*) and valve shape (lanceolate with narrowly rounded ends in *B. fennica vs.* linear with almost parallel margins and obtusely rounded and subprotracted apices in *B. fluviatilis*). More recently, a new *Berkeleya* species has been described, *B. witkowskii* Edlund, Ruiz, Ballant & Stone (Edlund *et al.*, 2025) from Puerto Rico. The species is characterized by large gelatinous macroscopic colonies, linear-lanceolate valves with acutely rounded ends with hyaline appearance due to high stria density (32–37 in 10 μm). Differences between *B. fluviatilis* and *B. witkowskii* can be seen in valve shape (linear with

Table 1. Morphological and numerical features of selected Berkeleya species.

Species	Tube forming	Outline	Length (μm)	Width (μm)	Stria density (in 10 μm)	Ecology	Source
B. fluviatilis sp. nov.	no	linear	25–39	4.0–5.0	30–32	freshwater	this study
B. rutilans	yes	linear-lanceolate	18–35	4.0–6.0	~27	brackish to marine	Cox (1975b)
B. rutilans var. ? *sensu* Medlin (1990)	yes	linear–lanceolate	18–35	4.0–6.2	26–28	marine	Medlin (1990)
B. obtusa	–	lanceolate	17–36	5.0–7.0	24–32	marine to brackish	Witkowski *et al.* (2000)
B. antarctica	yes	linear-lanceolate	20–26	6.0–8.0	36–40	marine	Medlin (2019)
B. adeliensis	yes	linear	57–82	7.0–13.0	50–60	marine	Medlin (2019)
B. scopulorum	yes/free living	linear-lanceolate	62–93	8.0–11.0	19–20	marine	Cox (1979)
B. fennica	yes	lanceolate	13–31	2.6–3.8	36–40	brackish	Gong *et al.* (2020)
B. fragilis	yes	linear–lanceolate	40–90	4.0–6.0	>40	marine	Cox (1975b)
B. hyalina	yes	lanceolate	30–50	~5.0	>40	marine	Cox (1975b)
B. insularis	–	narrow-lanceolate	6–17	2.4–2.8	18–25	marine	Takano (1983)
B. micans	yes	lanceolate	70–130	8.0–10.0	~27	marine	Cox (1975b)
B. sparsa	–	narrow elliptical	19–37	5.0–8.0	18–11	marine	Witkowski *et al.* (2000)
B. transfretana	–	lanceolate	12–14	3.0–4.0	>40	brackish	Olenici *et al.* (2021)

parallel margins *vs.* linear-lanceolate with convex margins), valve size (5.6–6.4 µm), proximal raphe ends (long and distinctly unilaterally deflected vs. straight to slight unilaterally deflected), distal raphe ends (long and distinctly unilaterally deflected vs. straight and simple).

DISCUSSION

Berkeleya fluviatilis possess morphological and ultrastructural features characteristic of the genus *Berkeleya*. Previous studies have extensively documented the distinctive features of *Berkeleya* in marine environments (Cox, 1975a; Greville, 1827; Grunow, 1880; Van Heurck, 1896). Our observations revealed notable similarities with the marine species *B. rutilans* challenging the conventional understanding of *Berkeleya* as a marine genus. Here, we present a strictly freshwater species of *Berkeleya*, not forming tubes, recorded from a karstic Krka River of the Adriatic Sea watershed. The intriguing observation is that only a few species of *Berkeleya* have been observed in brackish habitats (Gong *et al.*, 2020; Juhlin-Dannfelt, 1882), while most species have been exclusively identified in marine environments. This observation indicates that the colonization of freshwater habitats by marine *Berkeleya* species might have occurred, particularly in light of the geographical connectivity between the karstic Croatian region and the Adriatic Sea.

The migration of diatom lineages between freshwater and marine environments is a noteworthy phenomenon in macroevolution, characterized by its infrequency and profound implications for terrestrial, freshwater, and marine ecosystems (Nakov *et al.*, 2019). This adaptive movement represents a rare occurrence on a large evolutionary scale. Phylogenetic studies have shed light on the evolutionary history of diatom groups within the orders *Surirellales* and *Rhopalodiales*, providing the first evidence that colonization of freshwater habitats was preceded by adaptation to intermediate brackish environments, suggesting that these transitions from marine to freshwater environments occurred relatively recently (Ruck *et al.*, 2016). However, it remains unclear whether transitions from marine to freshwater environments were followed by substantial diversification. Nakov *et al.* (2019) suggested that the marine–freshwater diversity imbalance in diatoms is maintained by faster turnover and higher net diversification rates in freshwater environments. The present richness of freshwater species can be traced to the diversification of a limited number of ancestral lineages that colonized freshwater habitats from marine or terrestrial biomes during earlier geological periods, particularly during Earth's glaciation cycle (Nakov *et al.*, 2019; Vermeij & Dudley, 2000; Žuljević *et al.*, 2016). This evolutionary dynamic is not limited to diatoms but is also observed among various other algae and animals, particularly in the karstic regions of Croatia (Primc-Habdija & Matoničkin, 2005; Zanella *et al.*, 2009).

In exploring the migration of diatom species from marine to freshwater environments, intriguing hypotheses emerge. The 'stepping stone' hypothesis, provides a perspective, proposing that ancestrally marine diatoms may have utilized brackish waters as an intermediate habitat before entering freshwater environments (Ruck *et al.*, 2016). Another hypothesis derived from studies of the genera *Amphora* and *Halamphora* underscores the importance of inland saline habitats (Stepanek & Patrick Kociolek, 2019). According to this perspective, these groups include representatives that have colonized smaller inland water bodies with elevated conductivity, which are distinct from both coastal brackish and surrounding freshwater floras (Stepanek & Kociolek, 2015; Stepanek & Patrick Kociolek, 2019). A notable example can be found in Croatia, where a specific shallow lake near the sea harbours species from the genus *Tetraamphora* (Caput Mihalić *et al.*, 2019). This case supports the idea that inland waters, due to their unique environmental conditions, can support distinct diatom communities and offer valuable insights into morphological and physiological adaptations to varying salinity levels. Nevertheless, the broader ecological and evolutionary significance of inland waters within this pattern has yet to be fully evaluated (Stepanek & Patrick Kociolek, 2019). Another proposed scenario to explain multiple freshwater invasions involves the landlocking of marine species due to sea-level changes at various spatial and temporal scales, with the subsequent dilution of seawater (Žuljević *et al.*, 2016). Under such altered conditions, most marine species are expected to disappear, and successful adaptation is considered a rare event needing osmotic adaptation. While this scenario provides a plausible mechanism for the emergence of freshwater populations, direct phylogenetic evidence linking high-conductivity inland species to nearby marine relatives require further investigation. In diatoms specifically, the cellular and evolutionary responses to such osmotic shifts are still not well understood (Ivošević Denardis *et al.*, 2024). The speciation and extinction rates for marine diatoms may be lower in comparison to their freshwater counterparts, suggesting that although transitions between habitats are rare, those experienced by freshwater diatoms inhabiting discontinuous and irregularly distributed environments could lead to bursts of diversification, making such transitions important drivers of diatom biodiversity (Nakov *et al.*, 2019; Wilke *et al.*, 2020). Notably, the karstic area in Croatia boasts distinctive characteristics, marked by robust conductivity due to the release of ions such as calcium and bicarbonate during the dissolution of carbonate rocks. Karstic environments have high conductivity so may aid the transition from marine to freshwaters (Sims *et al.*, 2006). The development of this adaptive trait could be linked to geological epochs, notably during the last glacial maximum (~20,000 years ago; Žuljević *et al.*, 2016) when the sea level was up to 130 m lower, and karstic rivers along the eastern Adriatic coast were formed and incised valleys into the land (Juračić & Prohić, 1991). The Krka River estuary was formed during the Holocene (Hasan *et al.*, 2023), when sea-level rose, but there are not enough data available about the evolutionary history of Quaternary deposits along the eastern Adriatic coast to be certain (Hasan *et al.*, 2023). However, it is known that karstic freshwaters in Croatia face a particular challenge, increased salinization occurring through various natural processes. These processes include geological factors, precipitation, atmospheric deposition, limestone porosity allowing direct entry of seawater, evaporation, diffusion through porous limestone, geological processes, and the salinization of karstic springs (Herbert *et al.*, 2015; Thorslund *et al.*, 2021). This kind of phenomenon adds complexity to the challenges faced by diatoms in karstic freshwater systems, highlighting the intricate relationships between physicochemical conditions, biological responses and adaptation from marine to fresh waters (Nakov *et al.*, 2019).

Berkeleya fluviatilis sp. nov. not only represents a new described species and the first freshwater representative of the genus but also may hold significant potential as a model organism for studying transitions between marine and freshwater environments in diatoms. Although freshwater habitats account for only about 2% of Earth's surface (Grosberg *et al.*, 2012), they support disproportionately high biodiversity and, in the case of diatoms, have been more extensively studied than many marine systems (Appeltans *et al.*, 2012). This exceptional diversity highlights the importance of freshwater systems for investigating evolutionary dynamics and ecological adaptation, making the study of *Berkeleya fluviatilis* particularly valuable (Mann & Vanormelingen, 2013; Miller & Wiens, 2017; Wiens, 2015). Examples of diatom species such as *Envekadea hedini* (Hustedt) Van de Vijver, Gligora, Hinz, Kralj & Cocquyt and *Tetraamphora croatica* Gligora Udovič, Caput Mihalić, Stanković & Levkov, known from Lake Vransko, a specific karstic lake known for its occasional salinization (Caput Mihalić *et al.*, 2019; Gligora *et al.*, 2009), emphasize the unique ecological features of the Croatian karstic ecoregion. This region emerges as a hotspot for rare, specific, and potentially endemic species within the Balkan Peninsula. In this context, the study of *Berkeleya fluviatilis* provides a valuable opportunity for a deeper understanding of evolutionary dynamics and ecological adaptations in further investigations.

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DISCLOSURE STATEMENT

Q5 No potential conflict of interest was reported by the authors.

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