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Achnanthidium bratanense sp. nov. (Bacillariophyceae, Achnanthidiaceae), a new diatom from the Lake Bratan (Bali, Indonesia)

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Abstract
A new species, *Achnanthidium bratanense*, is described from Lake Bratan, located on the island of Bali (Indonesia). The morphology of this species was analyzed with light (LM) and scanning electron microscopy (SEM). *A. bratanense* is characterized by linear-elliptic to nearly elliptic valves with convex margins and rounded, broadly subcapitate apices. The striae of this species are hardly discernable under LM; they are weakly radiate throughout the valve and composed of one to four large transapically elongated areolae of different length and shape. The most similar taxon to *A. bratanense* is *A. macrocephalum*, a species described from Sumatra, another Indonesian island. The differences of *A. bratanense* from similar taxa are discussed.

Keywords
Indonesia, monoraphid diatoms, morphology, new species

Introduction

The genus *Achnanthidium* Kützing, 1844 is one of the largest genus among monoraphid diatoms. Although it had been described as a separate genus it was considered as a subgenus of *Achnanthes* Bory, from 1822 until the 90s (Round et al. 1990; Round and Bukhtiyarova 1996). Currently, *Achnanthidium* includes, according to different estimates, between 139 (Guiry and Guiry 2017) and nearly 200 species

(Kociolek et al. 2021a). Revision of the genus continues up to now. Recently, two genera, namely *Gogorevia* (Kulikovskiy et al. 2020b) and *Gomphothidium* (Kociolek et al. 2021b) have been segregated from *Achnanthidium*.

The *Achnanthidium* taxa are common in different climatic zones all over the world (*e.g.*, Ponader and Potapova 2007; Wojtal et al. 2011; Novais et al. 2015; Karthick et al. 2017; Marquardt et al. 2017; Krahn et al. 2018; Yu et al. 2019). However, their identification is challenging because of the small size of these diatoms, often requiring examination using electron microscopy, and significant variability of diagnostic features (Ponader and Potapova 2007; Hlúbiková et al. 2011).

The number of publications dealing with freshwater diatoms from Indonesia is still rather low. The most comprehensive treatment was made by Hustedt (1937, 1942). Some of his new taxa have been re-examined (e.g., Hamsher et al. 2014; Kapustin et al. 2017, 2020; Kapustin and Kulikovskiy 2018; Wetzel et al. 2019; Kulikovskiy et al. 2020a). Also, a lot of new diatom species were described from Indonesian freshwaters over the last two decades (Bramburger et al. 2006; Kociolek et al. 2018; Kapustin et al. 2019, 2021; Kulikovskiy et al. 2019; Rybak et al. 2019), including two new *Achnanthidium* species (Tseplik et al. 2021a,b). The aim of this paper is to describe a new monoraphid species, *Achnanthidium bratanense* sp. nov., from Lake Bratan located on the island of Bali, Indonesia.

Materials and methods

A benthic sample containing *Achnanthidium* was collected from a volcanic Lake Bratan on 14 November 2010 (08°16.579'S, 115°09.985'E). For general characteristics of this lake see Green et al. (1978). Environmental variables were measured with a Hanna multiparameter probe meter (HANNA HI98128).

The sample was boiled in concentrated hydrogen peroxide (~37%) to dissolve the organic matter. It was then washed with deionized water four times at 12 h intervals. After decanting and filling with deionized water up to 100 ml, the suspension was spread on to coverslips and left to dry at room temperature. Permanent diatom slides were mounted in Naphrax. Light microscopic (LM) observations were performed with a Zeiss Scope A1 microscope equipped with an oil immersion objective (100×/n.a.1.4, differential interference contrast [DIC]) and Zeiss Axio-Cam ERc 5s camera. Valve ultrastructure was examined with a JSM-6510LV scanning electron microscope (Papanin Institute for Biology of Inland Waters RAS, Borok, Russia), operated at 10 kV and 11 mm distance. For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminum stubs after air-drying. The stubs were sputter coated with 50 nm of gold.

The original sample preserved with Lugol's solution, as well as cleaned material preserved with 96% ethanol, are housed at the Laboratory of Molecular Systematics of Aquatic Plants, K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences (Moscow, Russia).

Results

Achnanthidium bratanense **Kapustin, Glushchenko & Kulikovskiy, sp. nov.**
Figures 1, 2

Description. ***LM*** (Fig. 1A-T). Valves linear-elliptic to nearly elliptic with convex margins and rounded, broadly subcapitate apices. Frustules rectangular in girdle view and not bent (Fig. 1T). Length 5.0–8.7 µm, breadth 2.7–3.2 µm (n=32). In raphe valves axial area narrow, linear, slightly widening at center. Central area very small in raphe valves, outlined by shortened striae; central area in rapheless valves rhomboid (Fig. 1J). Raphe straight, filiform. In rapheless valves axial area expanded widening towards rhombic central area (Fig. 1E). Striae hardly discernable in LM, weakly radiate (Fig. 1A, H, K, M). Areolae indistinct in LM.

SEM (Fig. 2). Externally, raphe straight, filiform with drop-shaped proximal and distal raphe endings (Fig. 2A, B). Internally, proximal raphe endings deflected in opposite directions, distal raphe endings terminating in helictoglossae (Fig. 2E, F). Striae weakly radiate throughout the valve, 41–44 in 10 µm, and composed of one to four large transapically elongated areolae of different length and shape (from slit-like to irregularly rectangular). Areolae absent along valve margins; mantle with a single row of slit-like to almost rectangular areolae. Internally, areolae occluded by a hymen (Fig. 2E, F).

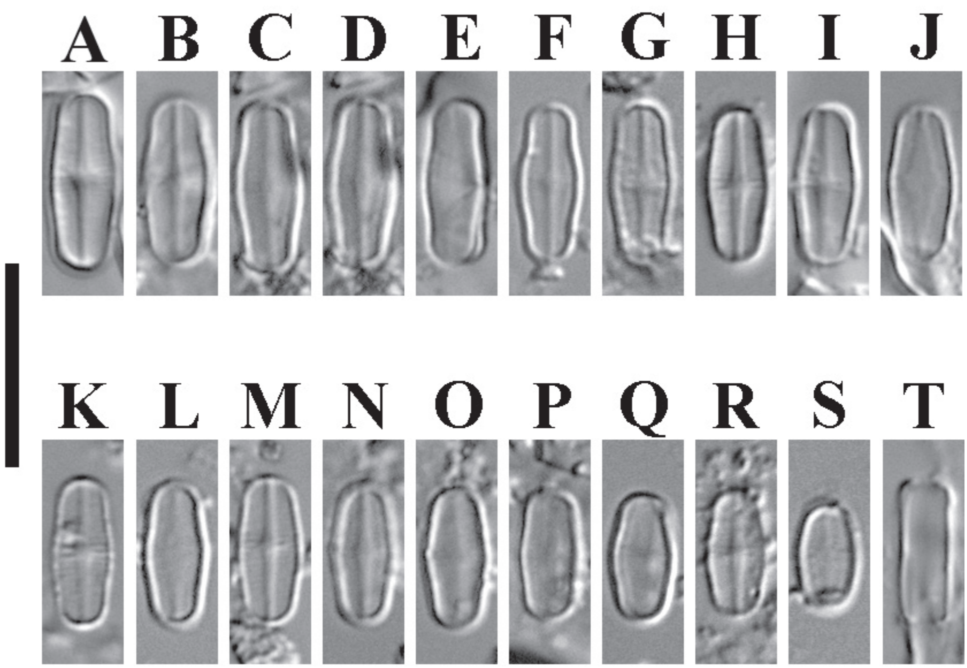

Figure 1. A–T *Achnanthidium bratanense* sp. nov. (LM). **A–S** size diminution series showing variation in valve outline **A** holotype specimen **A-D, F-I, K, M, N, R** raphe valves **E, J, L, O-Q, S** rapheless valves **T** frustule in girdle view. Scale bar: 10 µm.

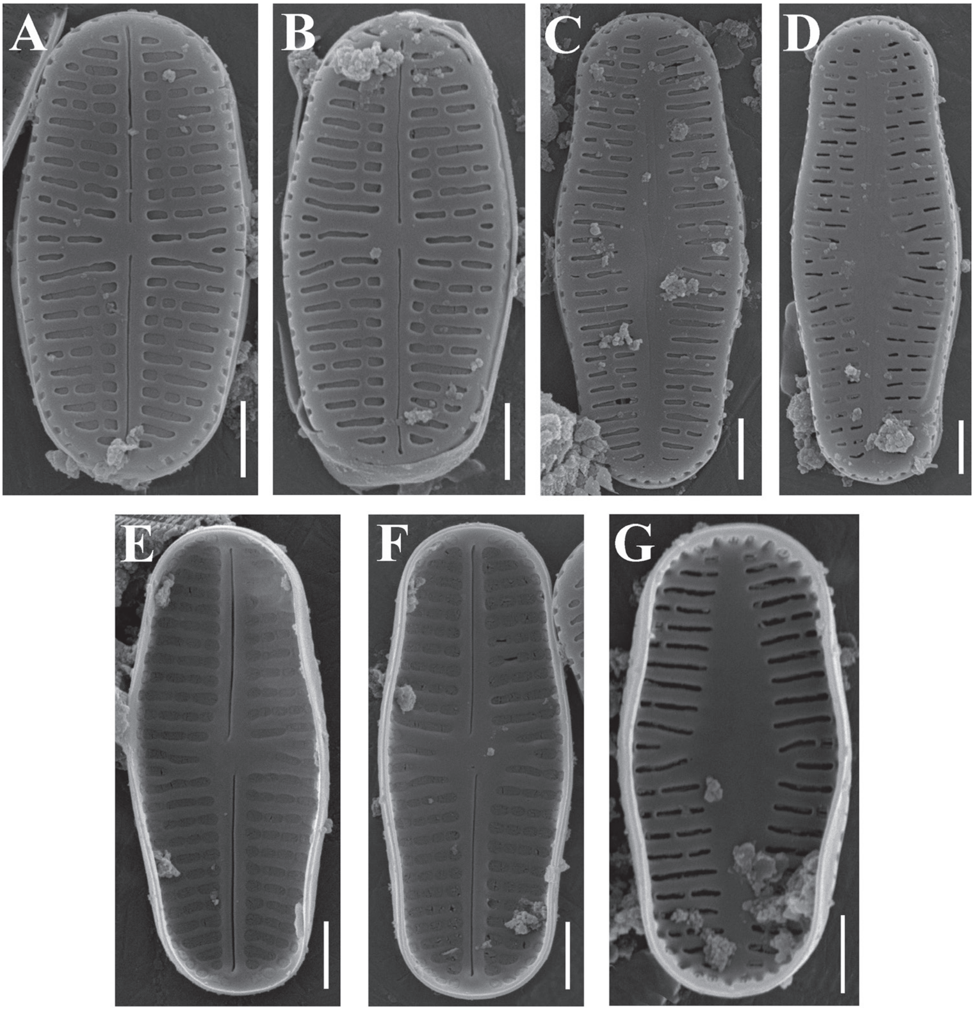

Figure 2. A–G *Achnanthidium bratanense* sp. nov. (SEM). **A, B** raphe valve, external view **C, D** rapheless valve, external view **E, F** raphe valve, internal view **G** rapheless valve, internal view. Scale bar: 1 μm.

Holotype (here designated): permanent slide No. MHA 01125, deposited at the Main Botanical Garden, Russian Academy of Sciences (MHA). Fig. 1A illustrates the holotype.

Isotype (here designated): permanent slide No. 01125a, deposited in collection of Maxim Kulikovskiy, Timiryazev Institute of Plant Physiology, Russian Academy of Sciences.

Type locality. Indonesia, Island of Bali, Lake Bratan, 08°16.579'S, 115°09.985'E, *leg*. I.I. Ivanov on 14 November 2010.

Etymology. The specific epithet refers to the type locality, Lake Bratan.

Ecology. *Achnanthidium bratanense* together with *Gogorevia rinatii* were the most abundant species in the sample. Rarely single frustules of *Planothidium* sp., *Stauroneis* sp., *Cymbella* sp. and other diatoms were encountered. During sampling the temperature was recorded as 25.7 °C, pH as 7.82, and conductivity as 22 $\mu S \cdot cm^{-1}$.

Distribution. So far, this species is known from its type locality only.

Discussion

Our new species is closely related to *Achnanthidium macrocephalum* (Hustedt) Round & Bukhtiyarova, 1996. This taxon was described by Hustedt (1937) as *Achnanthes minutissima* var. *macrocephala* from Sumatra. Recently, Wetzel et al. (2019) have re-examined Hustedt's type material using both LM and SEM. Although the length and breadth of both species overlapped, *A. macrocephalum* is generally larger than *A. bratanense* (Table 1). However, the larger valves of *A. macrocephalum* have distinctly capitate apices. Despite the high abundance of *A. bratanense* we could not found such valves with capitate apices. Additionally, both taxa differ in striae density: *A. macrocephalum* has ca. 38 striae in 10 µm (Wetzel et al. 2019), whereas *A. bratanense* has 41–44 striae in 10 µm (see Table 1). In contrast to *A. macrocephalum*, the striae of *A. bratanense* is composed of 1–4 transapically elongated areolae. In *A. macrocephalum* the striae composed of 1 (smaller valves) or two (rarely 3) areolae (Wetzel et al. 2019). Also *A. bratanense* has weakly radiate striae throughout the valve whereas in *A. macrocephalum* the striae become parallel towards the valve ends.

It should be noted that Hustedt (1942) reported *Achnanthes minutissima* var. *macrocephala* from Lake Bratan on Bali and suggested that it might be widely distributed in the Indo-Malayan region. Unfortunately, he gave neither description nor images to support the written statement. It is very likely he actually observed *A. bratanense* instead of *A. macrocephalum.* Wetzel et al. (2019) supposed that Hustedt (1937) included in his description of *A. macrocephalum* two morphotypes.

Also *A. bratanense* is similar to several other taxa including *A. rosenstockii* (Lange-Bertalot) Lange-Bertalot var. *rosenstockii*, 2004, *A. rosenstockii* var. *inareolatum* Lange-

Table 1. Comparison of morphological characteristics of *Achnanthidium bratanense* sp. nov. and closely related taxa.

	A. bratanense	***A. macrocephalum***	***A. rosenstockii* var. *rosenstockii***	***A. rosenstockii* var. *inareolatum***	***Kolbesia sichuanensis***
Valve length, µm	5.0–8.7	7–12	6–14	9.6–15.1	10.8–14.1
Valve width, µm	2.7–3.2	2.5–3.2	3–4	4.2–5.1	3.2–3.7
Valve outline	linear-elliptic	linear-elliptic with convex margins	linear-lanceolate	linear-lanceolate	linear-lanceolate
Valve apices	subcapitate	rounded, broadly capitate	subcapitate	subcapitate	broadly capitate
Striae density	41–44	38	27–32	20	22–26
Number of areolae per stria	1–4	1–2(3)	2–4	1	1
Reference	This study	Wetzel et al. 2019	Krammer and Lange-Bertalot 2004	Krammer and Lange-Bertalot 2004; Yu et al. 2019	Yu et al. 2019

Bertalot, 2004, and *Kolbesia sichuanenis* P. Yu, Q-M. You & Q-X Wang, 2019 (Table 1). *A. rosenstockii* var. *rosenstockii* is slightly wider than *A. bratanense* and the stria density is lesser (27–32 in 10 µm *vs.* 41–44 in 10 µm). *A. rosenstockii* var. *inareolatum* differs from the type variety in having striae composed of a single macroareola. Probably, this taxon will be better to place in the genus *Karayevia* Round & Bukhtiyarova emend. Bukhtiyarova, 2006. From both *A. rosenstockii* var. *inareolatum* and *Kolbesia sichuanenis*, *A. bratanense* differs in stria structure (number of areolae per stria) and stria density. Also, these taxa are significantly larger than *A. bratanense* (see Table 1).

Traditionally, three morphological groups are recognized within *Achnanthidium* (e.g. Novais et al. 2015; Karthick et al. 2017; Krahn et al. 2018; Yu et al. 2019): 1) *A. minutissimum* complex which is characterized by having straight external distal raphe ends, and striae density that increase towards the apex; 2) *A. pyrenaicum* complex which is characterized by having external distal raphe ends that deflect or hook to one side of the valve, and 3) *A. exiguum* complex have external distal raphe ends curved in opposite directions. Recently, the latter complex has been segregated into a new genus, *Gogorevia* (Kulikovskiy et al. 2020a). Although, *A. bratanense*, *A. macrocephalum* and *A. rosenstockii* can be placed in *A. minutissimum* complex based on the raphe structure, they have completely different striae structure and represent a separate morphological group. Interestingly, Pinseel et al. (2017) revealed 12 distinct lineages within *A. minutissimum* complex and one of them was described as the new species, *A. digitatum* Pinseel et al., 2017. Recently, Tseplik et al. (2021b) described from the ancient Lake Matano (island of Sulawesi, Indonesia) the new species, *A. gladius* Tseplik et al., 2021b, which was closely related to the latter taxon. Thus, further detailed study of the pore apparatus ultrastructure as well as molecular studies of *A. bratanense* and allied taxa will help to better understand the taxonomic status and phylogenetic placement of this morphological group.

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