RESEARCH ARTICLE



Two new freshwater species of the genus Achnanthidium (Bacillariophyta, Achnanthidiaceae) from Qingxi River, China

Pan Yu^{1,2}, Qingmin You¹, Wanting Pang¹, Quanxi Wang¹

College of Life and Environmental Sciences, Shanghai Normal University, Shanghai 200234, China
Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China

Corresponding author: Quanxi Wang (wangqx@shnu.edu.cn)

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Abstract

We describe two new Achnanthidium species, A. anhuense **sp. nov.** and A. qingxiense **sp. nov.**, from Qingxi River, Anhui Province, China, based on light and scanning electron microscopy. Both species belong to the "A. pyrenaicum complex" of the Achnanthidium genus, based on their possession of transapicallyelongated areolae and deflected external distal raphe fissures. A. anhuense **sp. nov.** has a slightly irregular linear-lanceolate valve with rounded or weakly protracted apices and a transapically rectangular or bow tie central area on the raphe valve. A. qingxiense **sp. nov.** has a linear-lanceolate valve with rounded apices and the axial area distinct expanded apices on the rapheless valve. Both species differ sufficiently from other similar species, based on valve outline, shape of the axial and central areas and striae density. These new species were all collected from stone substratum.

Keywords

Diatoms, monoraphid, morphology, new species, taxonomy

Introduction

The diatom genus *Achnanthidium* Kützing (1844) was originally described by Kützing, as a subgenus of the *Achnanthes* Bory de Saint-Vincent (1822). The species *Achnanthes microcephalum* Kützing was the type species of the subgenus (Pérès et al. 2014; Yu et al. 2019a). In the 1990s, Round et al. (1990) resurrected *Achnanthidium* and elevated it to the genus level. Later, Round and Bukhtiyarova

(1996) redefined the circumscription of this genus, with the main identification characteristics of *Achnanthidium* including small cells with a length and width of usually less than 30 μ m and 5 μ m, respectively, linear-lanceolate to lanceolate-elliptic valves and straight or curved external distal raphe ends (Round and Bukhtiyarova 1996; Yu et al. 2019 a, b). Species of *Achnanthidium* are widely distributed in various types of freshwater habitats in which they are common and abundant (Novais et al. 2011; Pinseel et al. 2015; Karthick et al. 2017; You et al. 2019; Yu et al. 2019a, b).

Owing to the small size (usually less than 30 μ m in length and less than 5 μ m in breadth) and identification characteristics of *Achnanthidium*, this genus has been subdivided into three major subgroups. The *A. minutissimum* complex includes species with straight external distal raphe ends and linear to linear-lanceolate valve shapes, increasing striae density toward the apices and round external areolar openings. The species of the *A. pyrenaicum* complex have external distal raphe ends that are deflected or hooked to one side of the valve and slit-like areolar openings. The members of the *A. exiguum* complex have external distal raphe ends curved in opposite directions (Compère and Van de Vijver 2011; Karthick et al. 2017; Yu et al. 2018, 2019a; Miao et al. 2020; Tseplik et al. 2021; You et al. 2021). *A. exiguum* and its relatives have been segregated into a new genus of *Gogorevia* Kulikovskiy, Glushchenko, Maltsev and Kociolek (Kulikovskiy et al. 2020).

Presently, the number of species in the genus *Achnanthidium* is greater than 200 (Marquardt et al. 2017; Kociolek et al. 2018; You et al. 2021). Before the year of 2000, 11 new *Achnanthes* species had been described from China (Hustedt 1922; Jao 1964; Jao et al. 1974; Qi and Xie 1984; Zhu and Chen 1994, 1996; Liu et al. 2021). Some of these species should be transferred to *Achnanthidium*, but owing to the loss of the type material, it is difficult to confirm their taxonomic position. It is, therefore, necessary to collect samples from the type locality, and re-evaluate their taxonomic position (Liu et al. 2021). From 2001 to 2021, 14 new *Achnanthidium* species have been described from China (Liu et al. 2016; Yu et al. 2018, 2019a, b, You et al. 2019, 2021; Liu et al. 2021). During an investigation of the freshwater diatoms from the Qingxi River, two unknown *Achnanthidium* species were discovered. The purpose of this present study was to document and formally describe those species with light microscopy (LM) and scanning electron microscopy (SEM) and compare the new species with morphologically similar taxa.

Materials and methods

Diatom samples were collected from the Qingxi River located in Chizhou City, Anhui Province, China, in January 2018. In the field, several water chemistry parameters were recorded, including pH, temperature, dissolved oxygen, salinity, total dissolved solids (TDS) and conductivity, using a YSIPro Plus multiparameter meter (YSI, Ohio, USA). Diatom samples were collected from stones using clean toothbrushes and the samples were placed in a bottle and preserved with formalin (4% final concentration). Total phosphorus (TP) was measured by alkaline potassium persulphate digestion ultraviolet spectrophotometry, total nitrogen (TN) was measured by potassium persulphate digestion ammonium molybdate spectrophotometry and chemical oxygen demand (COD) was measured with the potassium permanganate index method (CSEPB 2002).

In the laboratory, diatom samples were cleaned with concentrated nitric acid using the Microwave Accelerated Reaction System (Model MARS, CEM Corporation, Charlotte, USA) (Parr et al. 2004) with a pre-programmed digestion scheme (temperature, 180 °C) (Yu et al. 2017, 2019a, b). Next, samples were alternately centrifuged for 5 min at 3500 rpm (TDZ5-WS, Luyi Corporation, Shanghai, China) and washed six times using distilled water until the pH of the sample was close to neutral. Finally, the cleaned samples were kept in 95% ethanol. Cleaned diatom frustules were mounted in Naphrax for LM or air-dried on to cover slips and mounted on to alloy stubs for observation by SEM. The LM observations were made with an Olympus BX-53 microscope (Tokyo, Japan) fitted with DIC optics and a 100× oil immersion objective (1.4 numerical aperture) and an Olympus DP-71 digital camera. The SEM examination was conducted using a Hitachi SU8010 (1–2 kV, WD 8 mm) (Tokyo, Japan). Images were compiled with Adobe Photoshop CS6. Morphological terminology followed Round et al. (1990). All of the diatom samples and permanent slides are housed in the Lab of Algae and Environment, College of Life Sciences, Shanghai Normal University (SHTU), Shanghai, China.

Results

Achnanthidium anhuense P. Yu, Q. M. You & Q. X. Wang, sp. nov. Figs 1A-AD, 2-5

Description. LM (Fig. 1A–AD), valves are slightly irregularly linear-lanceolate in shape, with rounded or weakly protracted apices. Valve length 13–35.7 μ m, breadth 3.5–4.5 μ m (n = 200). Raphe valve is concave, with narrow, linear-lanceolate axial area, with a central area that is transapically rectangular or bow tie-shaped, usually slightly asymmetric. Striae radiate at the middle portion and nearly parallel towards apices, the number of striae is 18–20 in 10 μ m at the middle portion, 26–32 in 10 μ m near the apices. Rapheless valve is convex, axial area narrow linear-lanceolate and weakly expanded at the middle portion of the valve. Striae are nearly parallel, 16–26 in 10 μ m in the centre and 22–30 in 10 μ m near the apices.

In the SEM, both valves have a narrow hyaline area at the valve face and mantle junction (Figs 2A, B, 4A, B). Raphe valve: Externally, the raphe is filiform and straight (Fig. 2A, B), distal raphe ends are deflected to the same side (Fig. 2A–C, E), and proximal raphe ends straight and teardrop-shaped (Fig. 2A, B, D, F). Striae uniseriate, containing 3–6 round, oval or transapically-orientated areolae in the



Figures I. A–AD LM valve views of *Achnanthidium anhuense* sp. nov. **AE–AS** LM valve views of *Achnanthidium qingxiense* sp. nov. Scale bar: 10 µm.

middle portion of the valve and 1–3 round, oval or transapically-orientated areolae at the apices (Fig. 2A–C, E). Valve mantle with a single row of linear areolae extend along the valve, but with a slight interruption in the apices (Fig. 2B, C, E). Internally, raphe terminates in raised helictoglossae close to the apices (Fig. 3A–D), proximal raphe ends form small hooks and are distinctly deflected in opposite directions (Fig. 3A, B, E). Areolae transapically elongated in the central portion of the valve, becoming larger and oblong at the apices (Fig. 3C–E). Areolae are occluded by hymenes perforated by delicate slits and each hymene joins with the adjacent hymene (Fig. 3F).



Figures 2. A–F *Achmanthidium anhuense* sp. nov., SEM external views of raphe valve **A**, **B** external view of an entire raphe valve **C**, **E** apices of the valve, showing the distal raphe ends **D**, **F** central area of the valve, showing the proximal raphe ends, **D** seems detail of **A** and **F** of **B**. Scale bars: 5 μm (**A**, **B**); 1 μm (**C–F**).

Rapheless valve: Externally, the axial area is linear, being weakly expanded in the central area (Fig. 4A, B). Striae are uniseriate, comprise of 4–6 round or transapically elongated areolae in the central area (Fig. 4A, B, D) and 1–4 round or irregular oblong areolae at the apices (Fig. 4A–C). A row of slit-like areolae is present on the mantle (Fig. 4C, D). Internally, axial area slightly raised, with a shallow cutting line



Figures 3. A–F *Achnanthidium anhuense* sp. nov., SEM internal views of raphe valve **A**, **B** internal view of an entire raphe valve **C**, **D** apices of the valve, showing the distal raphe ends **E** central area of the valve, showing the proximal raphe ends **F** internal areala openings with fine hymenate structures. Scale bars: 5 μm (**A**, **B**); 1 μm (**E**); 0.5 μm (**C**, **D**); 0.3 μm (**F**).

at the middle portion (Fig. 5A, B, E) and have a shallow depression at the ends of the axial area (Fig. 5A–D). Areolae are transapically oval in the centre of the valve (Fig. 5A, B, E) and large, irregular and oblong at the ends (Fig. 5A–D). Areolae are occluded by hymenes perforated by delicate slits and each hymene joins with the adjacent hymene (Fig. 5F).



Figures 4. A–D *Achnanthidium anhuense* sp. nov., SEM external views of rapheless valve **A**, **B** external view of an entire rapheless valve **C** apices of the valve **D** central area of the valve. Scale bars: 5 μm (**A**, **B**); 1 μm (**C**, **D**).

Holotype (designated here). SHTU! Slide QXH201801-Z7 in Lab of Algae and Environment, College of Life Sciences, Shanghai Normal University, Shanghai, China. Holotype illustrated in Fig. 1H, W.

Registration. http://phycobank.org/103057

Type locality. CHINA. Qingxi River, Anhui Province, 30°14'39"N, 117°49'58"E, *leg. Q.X. Wang and P. Yu, 23*th January 2018.

Etymology. The species is named for the place where it was found, namely Anhui Province.

Ecology. Collected in one sample (QXH201801-Z7) on stone. Water temperature - 8.1 °C, pH - 7.8, Salinity - 0.05%, TDS $- 95.55 \text{ mg} \cdot l^{-1}$, EC $- 99.3 \mu \text{S} \cdot \text{cm}^{-1}$, TN $- 0.5 \text{ mg} \cdot l^{-1}$, TP $- 0.03 \text{ mg} \cdot l^{-1}$, COD $- 0.1 \text{ mg} \cdot l^{-1}$.

Distribution. So far only known from the type locality.



Figures 5. A–F *Achnanthidium anhuense* sp. nov., SEM internal views of rapheless valve **A**, **B** internal view of an entire rapheless valve **C**, **D** apices of the valve **E** central area of the valve **F** internal areola openings with fine hymenate structures. Scale bars: $5 \mu m$ (**A**, **B**); $1 \mu m$ (**E**); $0.5 \mu m$ (**C**, **D**, **F**).

Achnanthidium qingxiense Q. M. You, P. Yu & Q. X. Wang, sp. nov. Figs 1AE–AS, 6–9

Description. LM (Fig. 1AE–AS). Valves linear-lanceolate in shape, with rounded or weakly protracted apices. The valve length is 22.5–28 μ m and breadth of 3.8–4.6 μ m (n = 30). Raphe valve with a narrow, linear-lanceolate axial area is weakly expanded at the middle portion of the valve. Striae slightly radiate at the centre

area, becoming denser towards the apices, 21-25 in 10 µm at the centre, 42-44 in 10 µm near the apices. Rapheless valve with narrow, linear axial area is weakly expanded at the middle portion of the valve. Striae are nearly parallel, becoming denser towards the apices, 20-24 in 10 µm at the centre, up to 32-34 in 10 µm at the apices.

In the SEM, on both valves, the valve mantle has a single row of slit-like areolae (Figs 6B, C, 8A, B). Externally, the raphe is filiform, slightly undulate and has distal raphe ends deflected to the same side (Fig. 6A). On the side of the deflection, there is a depression near the distal raphe ends (Fig. 6A, B). The proximal raphe ends are straight and teardrop-shaped (Fig. 6A, C). Areolae are small, round to transapically orientated, the uniseriate striae are composed of 5–8 areolae in the middle portion of the valve (Fig. 6A, C) and 1–5 areolae at the apex (Fig. 6A, B). Internally, distal raphe ends terminate in raised helictoglossae (Fig. 7A–C), while the proximal raphe ends are weakly deflected in opposite directions (Fig. 7A, B, E). Areolae transapically elongated in the central portion of the valve and becoming larger and oblong at the apices (Fig. 7A–C, E).

Rapheless valve: Externally, the axial area is linear, being weakly expanded at the central area and distinctly expanded at the apices (Fig. 8A–E). Striae are uniseriate, comprise of 5–10 round or transapically orientated areolae in the central area (Fig. 8A, B, E) and 1–4 round, oblong or slit-like areolae at the apices (Fig. 8A–D).



Figures 6. A–C *Achnanthidium qingxiense* sp. nov., SEM external views of raphe valve **A** external view of an entire raphe valve **B** apices of the valve, showing the distal raphe ends **C** central area of the valve, showing the proximal raphe ends. Scale bars: $5 \,\mu m$ (**A**); $1 \,\mu m$ (**B**, **C**).

Internally, the axial area is slightly raised, with a subtriangular area at the ends of the axial area (Fig. 9A–D). Areolae are transapically oval in the centre of the valve (Fig. 9A, B, E) and large, irregular and oblong at the ends (Fig. 9A–D). On both interiors of both valves, areolae are occluded by hymenes perforated by delicate slits and each hymene joins with the adjacent hymene (Figs 7D, 9F).

Holotype (designated here). SHTU! Slide QXH201801-Z7 in Lab of Algae and Environment, College of Life Sciences, Shanghai Normal University, Shanghai, China. Holotype illustrated in Fig. 1AJ, AO.



Figures 7. A–E *Achnanthidium qingxiense* sp. nov., SEM internal views of raphe valve **A**, **B** internal view of an entire raphe valve **C** apices of the valve, showing the distal raphe ends **E** central area of the valve, showing the proximal raphe ends **D** internal areola openings with fine hymenate structures. Scale bars 5 μ m (**A**, **B**); 1 μ m (**C**, **E**); 0.3 μ m (**D**).

Registration. http://phycobank.org/103058

Type locality. CHINA. Qingxi River, Anhui Province, 30°14'39"N, 117°49'58"E, *leg. Q.X. Wang and P. Yu, 23th January 2018.*

Etymology. The species is named after Qingxi River, where it was discovered. **Ecology.** Collected in one sample (QXH201801-Z7) on stone. The environmental conditions are exactly the same as for the *A. dubium* sp. nov.

Distribution. The new species is known only from the type locality.



Figures 8. A–E *Achmanthidium qingxiense* sp. nov., SEM external views of rapheless valve **A**, **B** external view of an entire rapheless valve **C**, **D** apices of the valve **E** central area of the valve. Scale bars: 5 μ m (**A**, **B**); 1 μ m (**E**); 0.5 μ m (**C**, **D**).



Figures 9. A–F *Achnanthidium qingxiense* sp. nov., SEM internal views of rapheless valve **A**, **B** internal view of an entire rapheless valve **C**, **D** apices of the valve **E** central area of the valve **F** internal areala openings with fine hymenate structures. Scale bars: $5 \,\mu m$ (**A**, **B**); $1 \,\mu m$ (**E**); $0.5 \,\mu m$ (**C**, **D**); $0.3 \,\mu m$ (**F**).

Discussion

Based on the generic description of *Achnanthidium* by Round et al. (1990) and Round and Bukhtiyarova (1996), *A. anhuense* sp. nov. and *A. qingxiense* sp. nov. clearly belong to this genus. The two new species possess characters that support their assignment to the *A. pyrenaicum* complex, deflected external distal raphe fissures (Yu et al. 2018, 2019 b, You et al. 2019, 2021).

Achnanthidium anhuense sp. nov. is similar to a few species, including A. pyrenaicum (Hustedt) Kobayasi (Kobayasi 1997), A. pseudoconspicuum (Foged) Jüttner & Cox (Jüttner, Cox 2011), A. rostropyrenaicum Jüttner & Cox (Jüttner et al. 2011) and A. initium Karthick, Taylor and Hamilton (Karthick et al. 2017). To facilitate a comparison between A. anhuense sp. nov. and these similar species, their morphological characteristics are summarised in Table 1. There is a difference in the valve outline between A. anhuense sp. nov. and these other species, with the valves of A. anhuense being slightly irregularly linear-lanceolate, whereas the values of A. pseudoconspicuum are linear-elliptical and those of A. initium are linear-lanceolate to lanceolate. On the raphe valve, A. anhuense sp. nov. has a transapically rectangular or bow-tie central area, whereas the central area of A. pseudoconspicuum and A. pyrenaicum is small and hardly differentiated. A. pseudoconspicuum has narrow transverse fascia and A. initium has an asymmetrical transverse fascia central area. In addition, the axial area of A. anhuense sp. nov. is linear-lanceolate, but narrow linear in other similar species. The valves of A. anhuense sp. nov. are longer (13-35.7 µm) than in other similar species and wider (3.5–4.5 µm) than in A. initium (3.1–3.6 µm). Moreover, on the raphe valve, the striae

Species/Feature	A. anhuensis	A. initium Karthick,	A. rostropyrenaicum	A. pseudoconspicuum	A. pyrenaicum
	sp. nov.	Taylor & Hamilton	Jüttner & Cox	(Foged)	(Hustedt) Kobayasi
				Jüttner & Cox	
Valve length (µm)	13-35.7	11-25.2	18-24.5	12.9-21	10-16
Valve width (µm)	3.5-4.5	3.1-3.6	4.3-4.5	2.9-4.8	2.5-4
Valve outline	Linear-lanceolate	Linear-lanceolate to	Linear-lanceolate	Linear-elliptical	Linear-lanceolate
		lanceolate			
Valve apices	Rounded or weakly	Rounded to weakly	Rostrate	Rounded or slightly	Slightly drawn–out
	protracted	rostrate rounded		cuneate	ends
Raphe valve					
Axial area	Narrow, linear-	Narrow linear	Narrow linear	Narrow linear	Narrow linear
	lanceolate				
Central area	Rectangular or	Asymmet-rical	Small and hardly	Narrow transverse	Small and hardly
	bow tie	transverse fascia	differentiated	fascia	differentiated
Raphe	Distal fissures	Distal fissures	Raphe distal raphe	Distal fissures	Raphe distal raphe
	deflected to the	deflected to the	ends curved to the	deflected to the	ends curved to the
	same side, internally	opposite side at an	same side, internally	opposite side at an	same side, internally
	proximal raphe	angle of 80–90°,	proximal raphe	angle of 60–80°,	proximal raphe
	fissures distinct	internally proximal	endings curved	internally central	endings curved
	deflected in opposite	ends curved in	towards opposite side	raphe ends curve to	towards opposite side
	direction	opposite directions	**	opposite sides	**
Density of striae	18-20 (middle),	29-34	20-22 (middle), 32	22-24 (middle), 32	20-25 (middle),
(10 µm)	26-31 (apices)		(apices)	(apices)	34-40 (apices)
Number of areolae	3-6 (middle), 1-3	2-5 (middle), 1-3	2-6 (middle), 1-3	3-5 (middle), 1-3	No data
per stria	(apices)	(apices)	(apices)	(apices)	
Rapheless valve					
Axial area	Narrow linear-	Narrow linear	Narrow linear	Narrow linear	Narrow linear
	lanceolate				
Central area	Absent	Weakly elliptical to	Absent	Absent	Absent
		almost absent			
Density of striae	16-26 (middle),	32-35	22 (middle), 28	20-24	20-28 (middle),
(10 µm)	22-30 (apices)		(apices)		32-38 (apices)
Number of areolae	4-6 (middle), 1-2	3-5 (middle), 1-3	4-6 (middle), 1-2	4-6 (middle), 2-3	No data
per stria	(apices)	(apices)	(apices)	(apices)	
References	Current study	Karthick et al. (2017)	Jüttner et al. (2011)	Jüttner and Cox	Kobayashi (1997)
				(2011)	

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

density at the middle and apices of *A. anhuense* sp. nov. is less than in other similar species and, on the rapheless valve, the striae density at the middle of *A. anhuense* sp. nov. is less $(16-26/10 \ \mu\text{m})$ than in *A. initium* $(32-35/10 \ \mu\text{m})$. The striae density at the apices of *A. anhuense* sp. nov. is less $(22-30/10 \ \mu\text{m})$ than in *A. initium* $(32-35/10 \ \mu\text{m})$ and *A. pyrenaicum* $(32-38/10 \ \mu\text{m})$, but higher than in *A. pseudoconspicuum* $(20-24/10 \ \mu\text{m})$.

Species similar to A. qingxiense sp. nov. include A. gracillimum (Meister) Mayama (Kobayasi et al. 2006), A. chitrakootense Wojtal (Wojtal et al. 2010), A. sinense Liu & Blanco (Liu et al. 2016), A. linannulumm Karthick, Taylor & Hamilton (Karthick et al. 2017) and A. sublanceolatum Yu, You & Wang (Yu et al. 2019a). This group of species is compared in Table 2. Externally, on the raphe valve, A. gingxiense sp. nov. has a linear-lanceolate valve with rounded apices, while A. linannulumm possesses linear elliptical to lanceolate valves and rounded or slightly protracted apices. The species of A. sinense are not protracted, but are acute and round, A. gracillimum has an elliptical to lanceolate valve and narrowly rostrate to subcapitate apices and A. chitrakootense possesses linear to linear-elliptical valve and subcapitate to rounded apices. In addition, A. gingxiense sp. nov. has a narrow linear-lanceolate axial area, whereas the axial area of A. gracillimum is linear and that of A. linannulumm is lanceolate. A. qingxiense sp. nov. has absent central area, while A. sinense possesses a rhombic-lanceolate central area and A. gracillimum has an asymmetrical central area. Moreover, on the rapheless valve, A. qingxiense sp. nov. can easily be separated from other similar species, based on its being distinctly expanded at the apices on the axial area (Figs 8, 9). Conversely, on the raphe valve, the striae density at the middle of A. gingxiense sp. nov. is less $(21-25/10 \,\mu\text{m})$ than in *A. chitrakootense* (26–30/10 µm), but higher than in *A. gracillimum* (22/10 µm) and the striae density at the apices of A. qingxiense sp. nov. is higher than other similar species. On the rapheless valve, the striae density at the middle of A. qingxiense sp. nov. is less (20-24/10 µm) than in A. linannulumm (24-26/10 µm) and A. chitrakootense $(26-30/10 \ \mu m)$, but higher than in *A. gracillimum* $(22/10 \ \mu m)$ and the striae density at the apices of A. qingxiense sp. nov. is higher than other similar species.

A. anhuense sp. nov. and *A. qingxiense* sp. nov. were collected from stones in Qingxi River, which is fast-flowing. *Achnanthidium* species can occur across a broad range of trophic conditions, from oligotrophic to eutrophic waters (Karthick et al. 2017; Miao et al. 2020). The *A. pyrenaicum* complex is abundant in clear and fast- flowing streams and they can be good indicators of specific environmental conditions in freshwater ecosystems (Cantonati and Spitale 2009; Jüttner et al. 2011), which is supported by the results of this study. The two new species occur in low TN (0.5 mg·l⁻¹), TP (0.03 mg·l⁻¹) and COD (0.1 mg·l⁻¹) environments. Moreover, in the type locality, some other monoraphid species co-occur with these new species. The co-occurring monoraphid taxa include *A. latecephalum* Kobayasi (Kobayasi 1997), *A. pyrenaicum* (Hustedt) Kobayasi (Kobayasi 1997), *Planothidium lanceolatum* (Brébisson ex Kütz-ing) Lange-Bertalot (Lange-Bertalot 1999), *A. rivulare* Potapova & Ponader (Potapova and Ponader 2004) and *A. subhudsonis* var. *kraeuselii* (Cholnoky) Cantonati & Lange-Bertalot (Kusber et al. 2017). In further studies, we will continue to study the relation-ship between diatom diversity and ecology from this region.

Species/Feature	A. qingxiensis sp. nov.	A. sublanceolatum Yu, You & Wang	<i>A. limamulumm</i> Karthick, Taylor & Hamilton	A. sinense Liu & Blanco	A. gracillimum (Meister) Mayama	A. chitrakootense Wojtal
Valve length (µm)	22.5-28	18–35	15.5-32.5	17.5-31.7	19–31.5	13-42
Valve width (µm)	3.8-4.6	4-4.5	2.5-4.5	4.1 - 6.0	3-4	3.4-4.2
Valve outline	Linear-lanceolate	Linear-lanceolate	Linear elliptical to lanceolate	Narrow lanceolate	Elliptical to lanceolate	Linear to linear-elliptical
Valve apices	Rounded	Rounded or weakly protracted	Rounded or slightly protacted	Not protracted, acute round	Narrowly rostrate to subcapitate	Subcapitate to rounded
Raphe valve						
Axial area	Narrow, linear-lanceolate	Narrow, linear-lanceolate	Lanceolate	Narrow lanceolate	Linear	linear-lanceolate
Central area	Absent	Absent	Indistinct to weakly expanded	Rhombic-lanceolate	Asymmetrical	Indistinct to weakly expanded
Raphc	Slightly undulate, distal raphe ends deflected towards the same side, internally proximal raphe endings weakly deflected in	Distal raphe fissures deflected to the same side, internally proximal raphe fissures weakly deflected in opposite direction	Distal fissures deflected to the same side at an ange of 80–90°, internally proximal ends curved in opposite directions	Raphe distal raphe ends curved to the same side, internally proximal raphe endings curved towards opposite side	Distal raphe fissures are sharply bent, internally central raphe ends curve to opposite sides	Distal raphe ends strongly curved to the same side, proximal raphe ends with slightly defl ected to opposite sides
Density of striae (10 µm)	opposite directions 21–25 (middle), 42–44 (apices)	20–23 (middle), 34–42 (apices)	24–27 (middle), 32–34 (apices)	21–28 (middle), 40 (apices)	22 (middle), 36 (apices)	26–30
Number of areolae per stria Rapheless valve	5-8 (middle), 1-3 (apices)	3-4 (middle), $1-2$ (apices)	2-3	5–6 (middle), 1–3 (apices)	4-5 (middle), 1-2 (apices)	2-4 (middle), 1-3 (apices)
- Axial area	Linear	Narrow linear-lanceolate	Narrow lanceolate	Linear	Linear	Linear
Central area	Absent	Absent	Weakly expanded to absent	Absent	Weakly expanded to absent	Absent
Density of striae (10 μ m)	20–24 (middle), 32–34 (apices)	21–24 (middle), 30–36 (apices)	24–26 (middle), 28–30 (apices)	21–27 (middle), 34 (apices)	22 (middle), 36 (apices)	26–30
Number of areolae per stria	5-10 (middle), 1-4 (apices)	2-5 (middle), 1-2 (apices)	2-4	6-7 (middle), 1-3 (apices)	4-5 (middle), 1-3 (apices)	4-5 (middle), 2-4 (apices)
References	Current study	Yu et al. (2019a)	Karthick et al. (2017)	Liu et al. (2016)	Kobayasi et al. (2006)	Wojtal et al. (2010)

Table 2. Comparison of morphological characteristics of Achnanthidium qingxiensis sp. nov. and closely related taxa.

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