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Three new species of *Ulnaria* (Kützing) Compère (Bacillariophyta) from China, with reference to the fine structure of their valvocopula

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 ${\tt Couverture} \ / \ {\tt Cover:} \ {\tt one} \ {\tt frustule} \ {\tt apex} \ {\tt of} \ {\tt Ulnaria} \ {\tt dongtingensis} \ {\tt Bing} \ {\tt Liu}, \ {\tt sp.} \ {\tt nov.} \ {\tt Credit:} \ {\tt Bing} \ {\tt Liu}.$

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Three new species of *Ulnaria* (Kützing) Compère (Bacillariophyta) from China, with reference to the fine structure of their valvocopula

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ABSTRACT

Three new species of *Ulnaria* (Kützing) Compère are described based on light and scanning electric microscopy observations. *Ulnaria oxybiseriata* D.M. Williams & Bing Liu, sp. nov., has linear-lanceolate to lanceolate valves with apiculate and produced ends, biseriate striae, and a central area mostly forming an incomplete fascia surrounded by shortened striae on one side. *Ulnaria jinbianensis* S. Blanco & Bing Liu, sp. nov., bears linear-lanceolate valves with rostrate to subrostrate ends in larger valves and cuneate ends in smaller valves, uniseriate stria, and a central area forming a rectangular to trapezoid fascia. *Ulnaria dongtingensis* Bing Liu, sp. nov., possesses a narrow-lanceolate valve outline with produced rostrate to capitate ends, uniseriate stria, and a central area forming almost square fascia. The valvocopula of all three new species bears the same ultrastructure: 1) a complete valvocopula is a closed ring with the same shape as the valve outline, closely attached to the mantle interior surrounding the valve margin; 2) each valvocopula present a row of poroids which bisects the pars interior and exterior; 3) valvocopula with sawtooth-shaped projections attached to the valve over virgae internally; and 4) the valvocopula closed and hyaline at both apices. All three species were found in alkaline freshwater (around pH 8) with moderate conductivity (*c.* 102-221 µS/cm).

KEY WORDS
Closing plate,
dongting Lake,
fascia,
silica sawtooth,
Wuling Mountains,
new species.

RÉSUMÉ

Trois espèces nouvelles d'Ulnaria décrites sur la base d'observations de leur valvocopula. Trois nouvelles espèces d'Ulnaria (Kützing) Compère sont décrites à partir d'observations aux microscopes optique et électronique à balayage. Ulnaria oxybiseriata D.M. Williams & Bing Liu, sp. nov., a des valves linéaires-lanceolées à lanceolées aux extrémités apiculées, des stries biseriées, et une aire centrale formant généralement un fascia incomplet entouré de stries plus courtes sur un côté. Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., possède des valves linéaires-lanceolées avec des extrémités rostrées à subrostrées chez les plus grandes valves et des extrémités cunéiformes pour les plus petites valves, des stries uniseriées, et une aire centrale formant un fascia rectangulaire à trapézoïdal. Ulnaria dongtingensis Bing Liu, sp. nov., possède un contour de valve étroitement lanceolée avec des extrémités rostrées à capitées, des stries uniseriées, et une aire centrale formant un fascia presque carré. La valvocopula des trois nouvelles espèces est représentée par la même ultrastructure: 1) une valvocopula complète en anneau fermé, de la même forme que le contour de la valve, étroitement attachée à l'intérieur du manteau entourant le bord de la valve; 2) chaque valvocopula présente une rangée de poroïdes (ou pores) qui divise les parties intérieure et extérieure; 3) valvocopula avec des saillies en dent de scie fixées à la valve par-dessus les côtes secondaires (virgae) internes; et 4) la valvocopula fermée et hyaline aux deux extrémités (ou apex). Les trois espèces ont été trouvées dans des eaux

douces alcalines (pH 8 environ) avec une conductivité modérée (c. 102-221 µS/cm).

MOTS CLÉS
Plaque de fermeture,
lac Dongting,
fascia,
dent de scie en silice,
monts Wuling,
espèces nouvelles.

INTRODUCTION

The diatom genus *Ulnaria* (Kützing) Compère is the correct name for a number of taxa previously placed in the large, but heterogeneous and poorly defined, genus Synedra Ehrenberg (Williams 1986, 2011) and is typified by *Ulnaria ulna* (Nitzsch) Compère (2001). Synedra is currently circumscribed to account for a few less frequently encountered marine species typified by *Synedra balthica* Ehrenberg (synonym of *S. gaillonii* (Bory) Ehrenberg). Three characters were initially proposed as synapomorphies for *Ulnaria*: 1) the basal siliceous layer (that is the topological relationship between the ribs, interconnecting struts and closing plates); 2) all bands in the cingulum are closed; and 3) modification of the ocellulimbus, with a larger, more dense inset plate, of 12-14 porelli per row and 20-25 rows per plate (Williams 1986). The first two characters remain as defining features of *Ulnaria* (Williams 2011), the third character is under further examination (DMW pers. obs.). The first character is an amalgamation of several, probably, distinct features.

Over 30 species have been placed in the genus *Ulnaria* (Guiry in Guiry & Guiry 2018) and, although it is distributed globally, there are endemic species confined to certain parts of the world, especially in South America (e.g. Van de Vijver & Cocquyt 2009; Morales *et al.* 2014) and Asia (e.g. You *et al.* 2008; Tuji 2009; Kulikovskiy *et al.* 2016; Liu *et al.* 2017). It is generally known that there are extremely diverse inland freshwater ecosystems in China likely to harbor endemic diatom species. From Wuling Mountains and Dongting Lake, China, we described three new species of *Ulnaria: U. sinensis* Bing Liu & D.M. Williams & Bing Liu and *U. gaowangjiensis* Bing Liu & D.M. Williams (Liu *et al.* 2017), and a new species of *Entomoneis: E. triundulata* Bing Liu & D.M. Williams (Liu *et al.* 2018), respectively. Moreover, the expectation is that many other species await

description from these two study areas (e.g. Liu *et al.* 2019). The present paper reports on three species of *Ulnaria* found in the Wuling Mountains area and Dongting Lake, China. Their descriptions are based on valve outline, central area shape, the structure of the virgae-vimines arrangement, and the fine structure of their valvocopula.

MATERIAL AND METHODS

The Wuling Mountains area, where the Wuling Mountains stretch across Chongging, Hunan, Hubei and Guizhou provinces, is one of the ten biodiversity hotspot ecoregions considered as conservation priorities in China (Tang et al. 2006). The region covers 171 800 km² including higher elevation mountainous areas (elevation 200-2570 m a.s.l.) and a subtropical humid monsoon climate (see also Liu et al. 2017). Li River and Yuan River, two major rivers both originated from Wuling Mountains, finally run into Dongting Lake. Donghe river and Bake river are two tributaries of Yuan River. Jinbian brook is a small tributary of Li River. In this mountainous area, there are numerous streams/rivers where the diatom flora is underexplored. Dongting Lake is the second largest freshwater lake in China and is located between 28°30'-30°20'N and 111°40'-113°40'E in the northeastern part of Hunan Province. It has four inputs from Xiang, Zi, Yuan, and Li Rivers respectively and three inputs from the Yangtze River's three outlets (Songzi, Hudu, and Ouchi) respectively.

Diatom samples used in this study were collected from four different sites: one each from the Donghe river, Bake river, Jinbian brook, and Dongting Lake (Table 1). In the Donghe river, Bake river and Jinbian brook, materials were scraped off from stones using a toothbrush, washed into 100 ml sampling bottles, and preserved with 70% ethanol. In Dongting Lake, epipelic diatoms were separated from the mud using

Table 1. — The three sampling sites for three new species from the Wuling Mountains and Dongting Lake, with associated pH, conductivity, and temperature (three replicates).

Taxon	Sampling site	Coordinates and elevation above sea level	рН	Conductivity (µS/cm)	Temperature (°C)	Sampling Date
Ulnaria jinbianensis S. Blanco & Bing	Shuirao Simen, Jinbian Stream (type locality)	29°20'36"N, 10°28'13"E; 467 m	8.5 ± 0.3	102.7 ± 0.8	8.7 ± 0.2	December 31, 2015
Liu, sp. nov.	Bake Village, Bake River	28°41'49"N, 109°24'12"E, 450 m	7.9 ± 0.1	221 ± 0.4	13.2 ± 0.1	December 10, 2016
Ulnaria oxybiseriata D.M. Williams & Bing Liu, sp. nov.	Lianai Qiao, Donghe River	28°19'5"N, 109°43'27"E, 200 m	8.5 ± 0.1	202.3 ± 1.2	13.2 ± 0.3	December 9, 2016
Ulnaria dongtingensis Bing Liu, sp. nov.	Yang's Village, Dongting Lake	28°52'29.5"N, 112°16'52"E, 57 m	8.3 ± 0.2	109.3 ± 0.1	23.5 ± 0.3	April 23, 2017

lens tissue, which was removed from the mud surface after one day (Mann et al. 2004). The collected lens tissue was then processed with concentrated nitric and sulphuric acids and rinsed with distilled water five times. Temperature, pH and conductivity were measured in situ with a portable multi meter (HQ40D, HACH Company).

Permanent slides were prepared using Naphrax[®] mountant and examined using a Leica DM3000 light microscopy (LM) equipped with a Leica DFC425C digital camera. Samples were also examined using scanning electron microscopy (SEM). Several drops of the cleaned diatom material were air-dried onto glass coverslips. Coverslips were attached to aluminum stubs using double-sided conductive carbon tape and sputtercoated with platinum (Cressington Sputter Coater 108auto, Ted Pella, Inc.). Samples were examined and imaged using a field emission scanning electron microscopy (FE-SEM) Sigma HD (Carl Zeiss Microscopy) available at Huaihua University, China.

Diatom valve terminology follows Ross et al. (1979), Barber & Haworth (1981), Cox & Ross (1981), and Williams (1986).

RESULTS

Division BACILLARIOPHYTA Class BACILLARIOPHYCEAE Subclass FRAGILARIOPHYCIDAE Family ULNARIACEAE Genus Ulnaria (Kützing) Compère

Ulnaria oxybiseriata D.M. Williams & Bing Liu, sp. nov. (Figs 1-5)

Type. — Lianaigiao (28°18'51.3"N, 109°43'41.6"E, 200 m a.s.l.), Donghe River, Jishou City, China, leg. Bing Liu, 9.XII.2016 (holo-, JIU[JIU G201804], the Herbarium of Jishou University, Hunan, People's Republic of China, here illustrated as Fig. 1A; iso-, BM[BM 101952], the Natural History Museum, London, United Kingdom, here illustrated as Fig. 1H).

ETYMOLOGY. — From the oxy- (acute) and biseriate, referring to the apiculate and produced ends and the biseriate striae of this new species.

DISTRIBUTION. — Known only from the type locality.

ECOLOGY. — The following environmental parameters were measured in the field. Conductivity was $202.3 \pm \hat{1}.2 \mu \text{S/cm}$, pH was 8.5 \pm 0.1 and water temperature was 13.2 \pm 0.3°C.

DESCRIPTION

Valve linear-lanceolate (Fig. 1A, H, J) to lanceolate (Fig. 1B-G, I) with apiculate and produced ends (Fig. 1A-J). Valve dimensions (n = 31): 56-78 μm long, 6-9 μm wide at centre. Sternum discernible, narrow, often regular, sometimes irregular (e.g. Fig. 1E, I). Central area variable, mostly forming an incomplete fascia bordered by shortened striae on one side, trapezoid (Fig. 1B-J); rarely as a rectangular fascia in larger valve (Fig. 1A). Striae often parallel except at each pole where they slightly radiate, sometimes striae oblique (e.g. Fig. 1E, I), 10-12 (often 11) in 10 μ m.

SEM observation

Frustule rectangular, each reproducing frustule with six closed girdle bands, i.e., two valvocopulae and four copulae (Fig 2, labelled B1 to B6). Striae continuing throughout the mantle, even in the central part (Fig. 2A, D, arrows), mantle blisters present (Fig. 2C, arrows). Valve linear-lanceolate to lanceolate with apiculate and produced ends (Figs 3A-C; 4A, B; 5A), central area rectangular, forming a fascia in larger valves (e.g. Fig. 3A), or trapezoid due to the presence of shortened striae on one side (Figs 3B, C, E; 4A, B, E, F). A rimoportula at each apex, oblique with respect to the apical axis (Figs 3A-C; 4A, B), occasionally three per valve, one located near the sternum, the other two close to the mantle at each apex (Fig. 5E, N, arrows), externally opening as a simple, rectangular hole (Fig. 3D, F), internally bilabiate (Fig. 4C, D, G, H). Striae biseriate, sometimes triseriate near the valve margins (Fig. 4C, D, arrows); areolae almost rounded, c. 40-45 areolae in 10 µm. Virgae wide, vimines unconspicuous (Figs 4; 5), and closing plates simple, with c. 2-5 small struts connecting the solid plates with the areola wall (Fig. 3G). A silica layer connecting two adjacent virgae present on some valves (Fig. 4E, F, H, arrowheads). An ocellulimbus at each apex, situated on the polar margin, composed of c. 7-9 transapical rows and 11-13 pervalvar rows, 6-9 serrated projections overhanging the ocellulimbus (Fig. 3D, E, arrows).

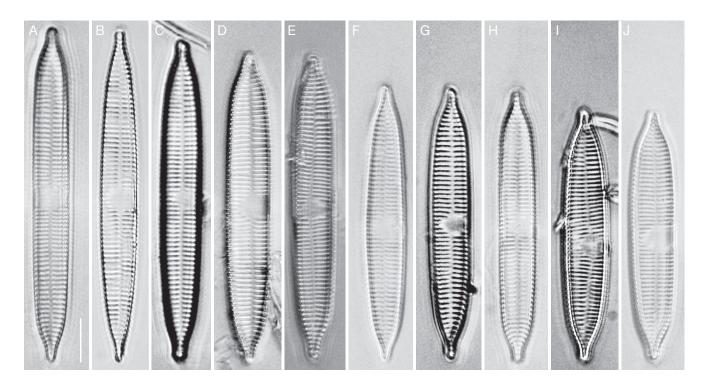


Fig. 2. — *Ulnaria oxybiseriata* D.M. Williams & Bing Liu, sp. nov., ten specimens showing a valve size diminution series: **A**, illustration of holotype specimen (a valve indicated by a circle on the slide); **B**, **F**, two valves bearing lanceolate outline; **C**, **D**, **G**, **H**, **J**, five valves bearing linear-lanceolate outlines; **E**, **I**, two valves with oblique and asymmetrical striae. Scale bar: 10 µm.

A complete valvocopula forming a closed ring with the same shape as the valve outline, closely attached to the mantle interior, surrounding the valve margin (Fig. 5A). Each valvocopula bearing a (mostly) continuous row of poroids bisecting pars interior from exterior, located at mid-line, occasionally with a second row of poroids (Fig. 5A-L). The latter not always complete, sometimes with interruptions (Fig. 5B, arrow; 35, arrows). Valvocopula with sawtooth-shaped projections attached to valve, internally visible over each virga (Fig. 5D, arrowheads). Valvocopula closed, hyaline (with no ornamentation) at both apices (Fig. 5B, K, M, N).

Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov. (Figs 6-12)

Type. — Shuirao Simen (29°20'36"N, 110°28'13"E, 467 m a.s.l.), Zhangjiajie National Forestry Park, China, leg. Bing Liu, 29.XII.2015 (holo-, JIU[JIU G201805], the Herbarium of Jishou University, Hunan, People's Republic of China, here illustrated as Fig. 6C; iso-, BM[BM 101953], the Natural History Museum, London, United Kingdom, here illustrated as Fig. 6B).

ETYMOLOGY. — Named after Jinbian Stream, where the species was found.

DISTRIBUTION. — Known from the type locality and Bake River.

ECOLOGY. — The following environmental parameters were measured at the type locality and at Bake Village, Bake River. Type locality (Shuirao Simen, Jinbian brook): Conductivity was $102.7 \pm 0.8 \,\mu\text{S/cm}$, pH was 8.5 ± 0.3 and water temperature was $8.7 \pm 0.2^{\circ}\text{C}$. Bake Village (Bake River): Conductivity was $221 \pm 0.4 \,\mu\text{S/cm}$, pH was $7.9 \pm 0.1 \,\mu\text{m}$ and water temperature was $13.2 \pm 0.1^{\circ}\text{C}$.

DESCRIPTION

LM

Valves linear-lanceolate, with rostrate to subrostrate ends in larger valves and cuneate ends in smaller valves (Fig. 6A-I). Valve dimensions (n = 49): 42-91 μ m long, 5.5-7.5 μ m wide at centre. Sternum discernible, narrow, sometimes irregular (e.g. Fig. 6D). Central area forming a rectangular to trapezoid fascia (Fig. 6A-I). Striae often parallel except at each apex, where radiate, sometimes oblique (e.g. Fig. 6D), 10-11 in 10 μ m. The population from Bake River has the same morphology and dimensions (Fig. 10A-I).

SEM observation

Valve linear-lanceolate with rostrate to subrostrate ends in larger valve and cuneate ends in smaller valves (Fig. 7A, B). Central area trapezoid as a fascia due to often two shortened striae present on one side (Figs 7A, B, D, G; 8C). Rimoportula at each pole, situated at an angle, externally as a simple, rectangular hole (Fig. 7C, E, F, H), internally bilabiate (Fig. 8B, D, E). An ocellulimbus at each pole, situated on polar margin, composed of *c*. 5-9 transapical rows and 11-14 pervalvar rows, a few serrated projections overhang pole (Fig. 7F, H). Striae uniseriate; areolae rounded to oblong (Fig. 3G), *c*. 35-50 areolae in 10 μm. Internally, valve linear-lanceolate with subrostrate to subrostrate or cuneate ends (Figs 8A; 9A, B). Striae occasionally oblique (Fig. 9B), a few striae radiate at each pole (Figs 8B; 8D).

Valvocopula closed, same shape as valve outline, closely attached to mantle interior, surrounding valve margin (Fig. 9A, B, C, E). Each valvocopula with single row of

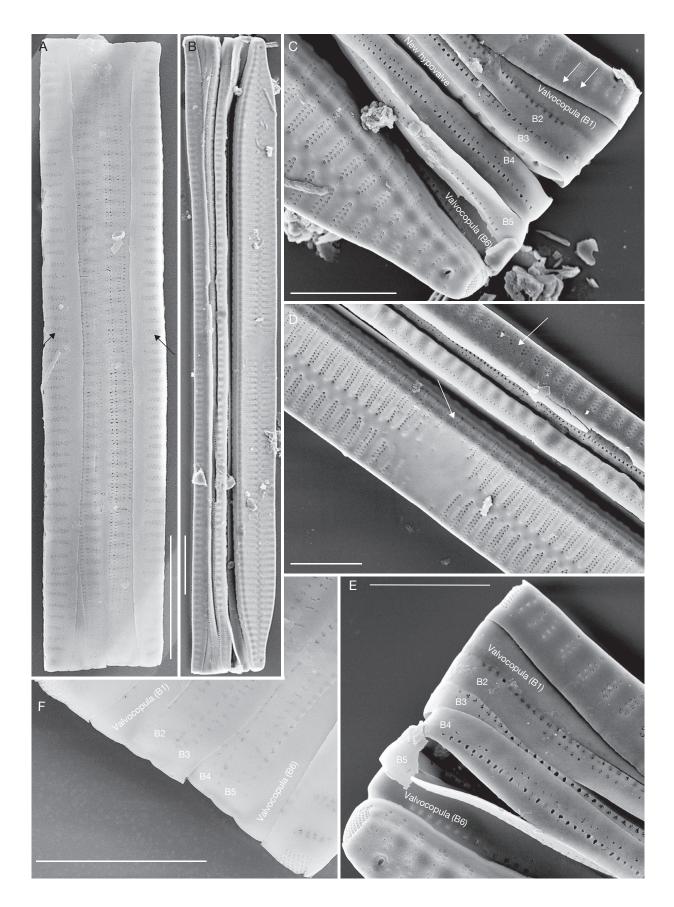


Fig. 3. — Ulnaria oxybiseriata D.M. Williams & Bing Liu, sp. nov., SEM, girdle view: A, a complete frustule, note the striae continuing until mid-mantle (arrows); B, a separated frustule; **C**, apex detail of **B** showing six girdle bands, a new hypovalve, and mantle blisters (arrows); **D**, middle detail of **B**, note the striae continuing until mid-mantle (arrows); **E**, apex detail of **B** showing six closed girdle bands; **F**, apex detail of **A** showing six closed girdle bands. Scale bars: A, B, 10 µm; C-F, 5 µm.

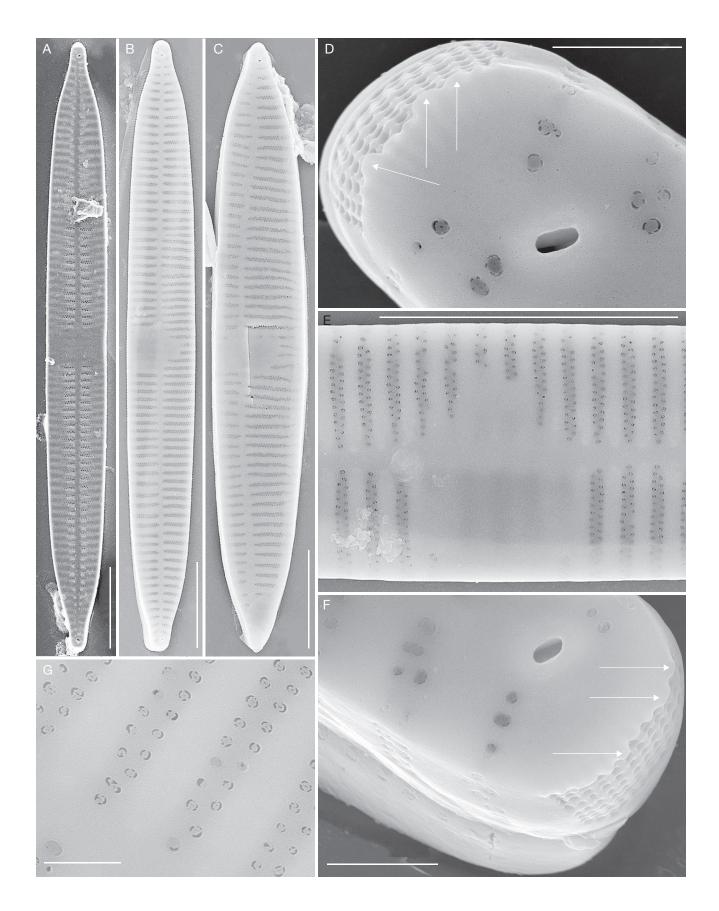


Fig. 4. — *Ulnaria oxybiseriata* D.M. Williams & Bing Liu, sp. nov., SEM, external view: **A-C**, three complete valves, note a fascia present in **A**, and the oblique and asymmetrical striae in **C**; **D**, apex detail of **B**, note the ocellulimbus and a few projections overhang it (arrows); **E**, middle detail of **B**; **F**, apex detail of **B**, note the ocellulimbus and a few projections overhang it (arrows); **G**, detail of closing plates. Scale bars: A-C, E, 10 μm; D, F, G, 1 μm.

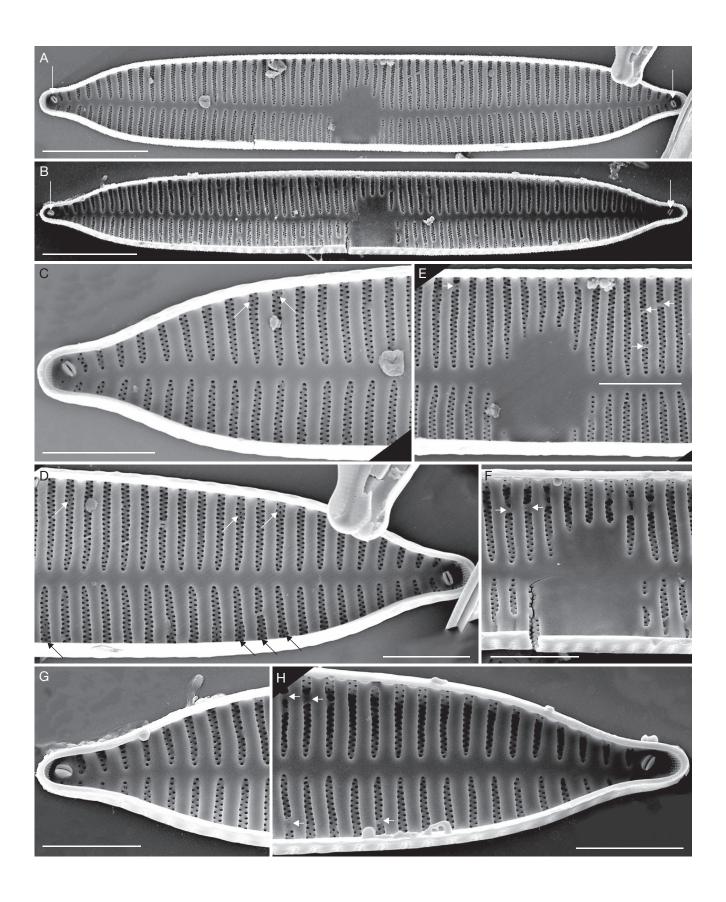


Fig. 5. - Ulnaria oxybiseriata D.M. Williams & Bing Liu, sp. nov., SEM internal view: A, B, two complete valves note the rimportula locations (arrows); C, D, details of A, note some stria triseriate (arrows); E, middle part detail of A, note the silica plates connecting two virgae (arrowheads); F- H, details of B, note silica plates connecting two virgae (arrowheads). Scale bars: A, B, 10 μm; C-H, 5 μm.

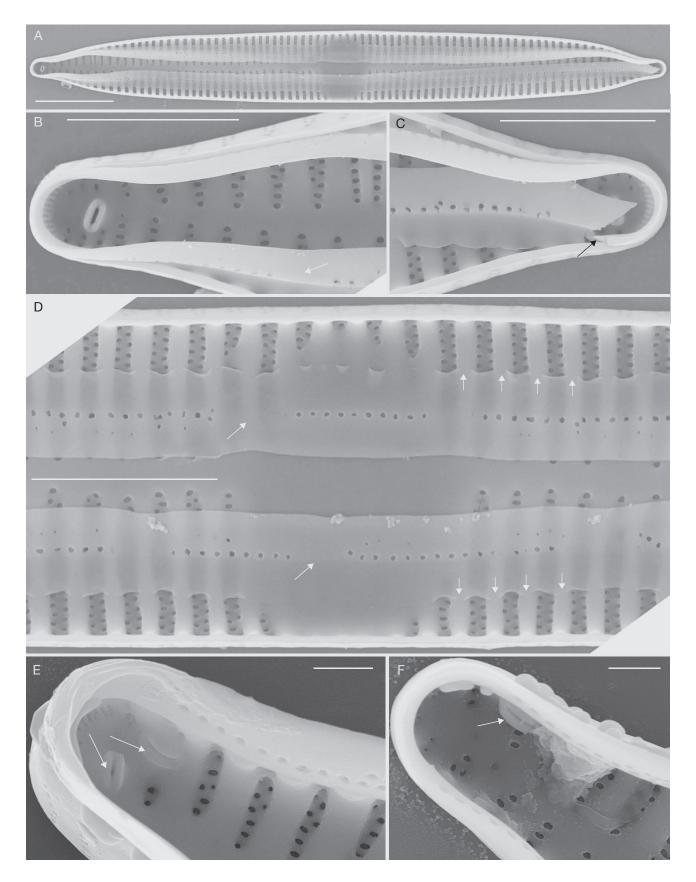


Fig. 6. — *Ulnaria oxybiseriata* D.M. Williams & Bing Liu, sp. nov., SEM, internal view: **A**, a valve with a valvocopula; **B**, **C**, apex details of **A**, note the closed valvocopula (an artificial break in **C**, **arrow**); **D**, middle detail of **A**, note a row of poroids located at the mid-line of the valvocopula with some interruptions (**arrows**) and each sawtooth-shaped projection over a corresponding virga (**arrowheads**); **E**, **F**, apex details show two rimportulae located at an apex (**E**, **two arrows**) and one rimportula at the other apex (**F**, **arrow**). Scale bars: A, 10 μm; B-D, 5 μm; E, F, 1 μm.

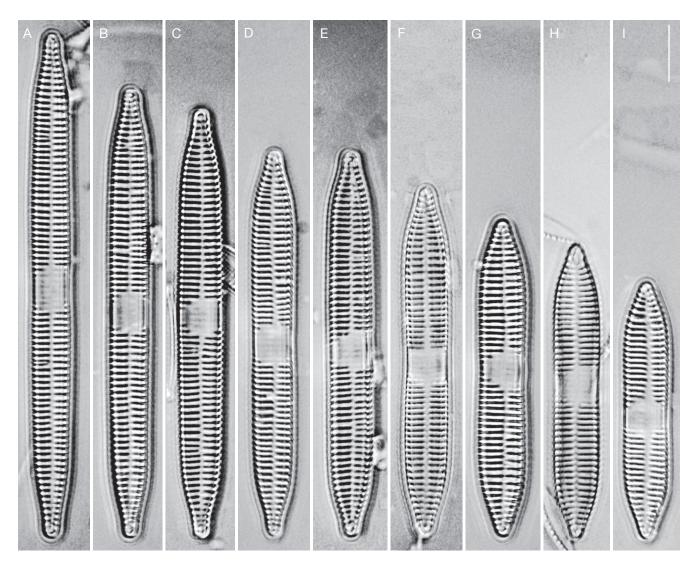


Fig. 7. — Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., LM, type population from Jinbian brook. Nine specimens showing a valve size diminution series, note the protracted rostrate ends in larger valves becoming cuneate in smaller valves: C, illustration of holotype specimen (an individual indicated by a circle on the slide). Scale bar: 10 µm.

poroids which bisects the pars interior and exterior, located at mid-line (Fig. 9C-E). The single row of poroids not always complete, sometimes with interruptions (Fig. 9D, arrows). Valvocopula with sawtooth-shaped projections attached to the valve, internally visible over each virga (Fig. 9D, arrowheads).

The population from Bake village at Bake River shares the same ultrastructural characters (Figs 11; 12).

Ulnaria dongtingensis Bing Liu, sp. nov. (Figs 13-16)

Type. — Yang's village (28°52'29.5"N, 112°16'52"E, 57 m a.s.l.), West Dongting Lake, China, leg. Bing Liu, 23.IV.2017 (holo-, JIU[JIU G201806], the Herbarium of Jishou University, Hunan, People's Republic of China, here illustrated as Fig. 13B; iso-, BM[BM 101954], the Natural History Museum, London, United Kingdom, here illustrated as Fig. 13A).

ETYMOLOGY. — Named after Dongting Lake, where the species was found.

DISTRIBUTION. — Known only from the type locality and from a sampling site located in East Dongting Lake.

ECOLOGY. — The following environmental parameters were measured in the field with three replicates: conductivity was $109.3 \pm 0.1 \,\mu\text{S/cm}$, pH was 8.3 ± 0.2 and water temperature was 23.5 ± 0.3 °C.

DESCRIPTION

LM

Valves narrow-lanceolate, i.e., valves with parallel central margins, length equal to, or slightly larger than, length at central area, strongly tapering closer to middle of valve rather than to poles, then gently tapering near the ends where finally form produced, rostrate to capitate apices (Fig. 13). Valve dimensions (n = 34): $106-260 \mu m \log_{10}$, 5-7 μm wide at centre.

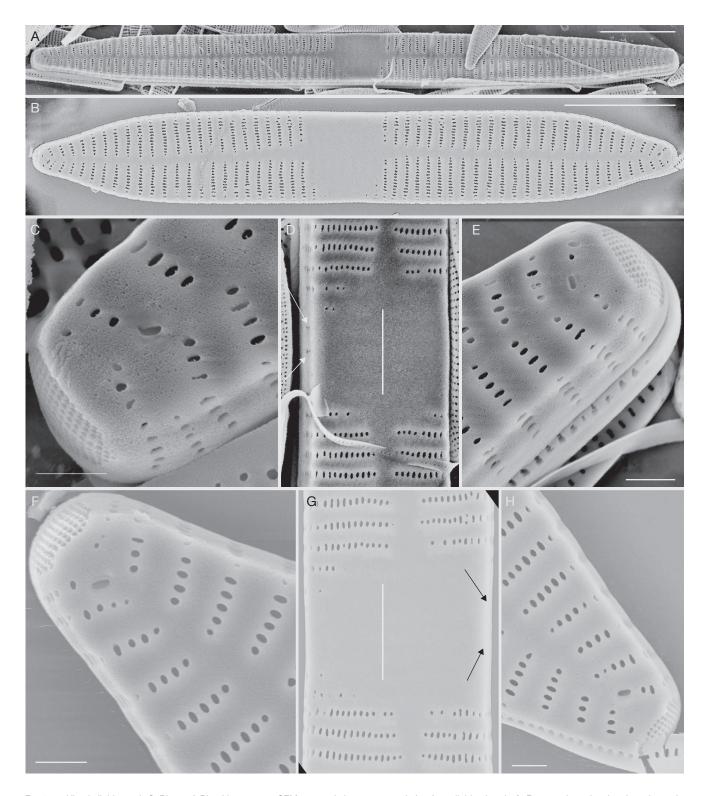


Fig. 8. — *Ulnaria jinbianensis* S. Blanco & Bing Liu, sp. nov., SEM, external view, type population from Jinbian brook: **A**, **B**, two valves showing the valve variations; **C-E**, details of **A**, note the striation of mantle (**two arrows**). **F-H**, details of **B**, note no striation on one mantle middle part (**two arrows**). Scale bars: A, B, 10 μm; C, E, F, H, 1 μm; D, G, 5 μm.

Sternum discernible, narrow, regular. Central area forming a fascia, almost square. Striae often opposite, but sometimes alternate, 10-12 in 10 μ m. Ghost striae sometimes present (e.g. Fig. 13B, F).

SEM observation

Frustule rectangular with at least three closed girdle bands (Fig. 14A-C). Striation continues from valve face onto mantle except at mantle centre which is hyaline (Fig. 14A, D,

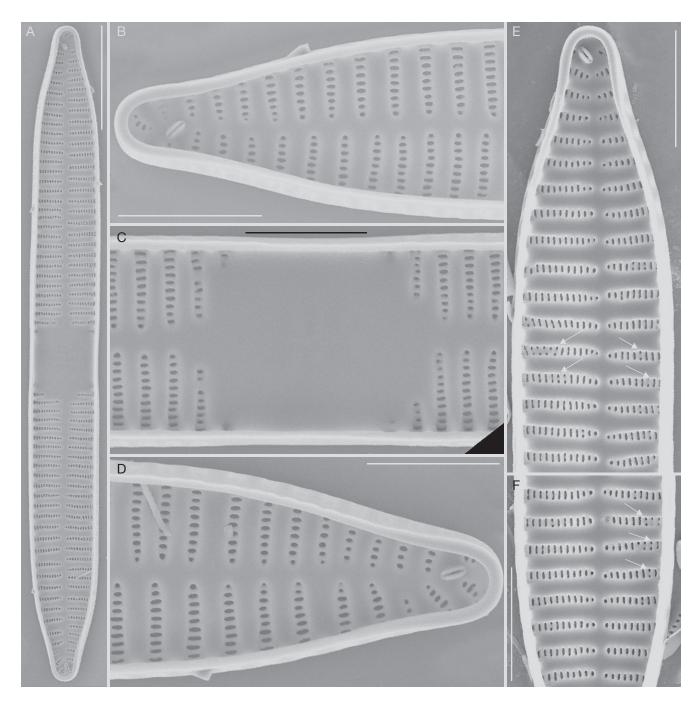


Fig. 9. — Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., SEM, internal view, type population from Jinbian brook: A, a complete valve; B-D, details of A; E, F, apex details showing part biseriate striae (arrows). Scale bars: A, 10 μ m; B-F, 5 μ m.

curved arrows). Valve narrow-lanceolate with produced rostrate to capitate ends (Figs 15A; 16A). Central area forming an almost square fascia (Fig. 15D). A rimoportula present at each apex, situated at an angle with respect to sternum, externally opening as a simple, almost rectangular foramen (Figs 14B, C; 15B, C). Ocellulimbus at each pole, situated on polar margin, composed of c. 6-10 transapical rows and 12-15 pervalvar rows. Two horn-like projections overhang pole (Figs 14B, C; 15B, C, arrows). Striae uniseriate; areolae often oblong, sometimes rounded, occluded by simple closing plates (Figs 14E; 15B-D), c. 35-40 areolae in 10 μm. Internally, wide virgae with much narrower vimines, both distinct (Fig. 16C-H). Rimoportula bilabiate (Fig. 16A, C, D). Central area forming fascia bordered by few shortened striae, almost square (Fig. 16D). Valvocopula with single row of areolae except at poles where areolae absent (Fig. 16F-H). Pars interior of valvocopula with distinct crenulated edge (Fig. 16F-H, arrowheads).

Valvocopula closed, same shape as valve, closely attached to mantle interior, surrounding valve margin (Fig. 16B). Each valvocopula with a single row of poroids bisecting pars interior and exterior, located at mid-line (Fig. 16F-H). Single row

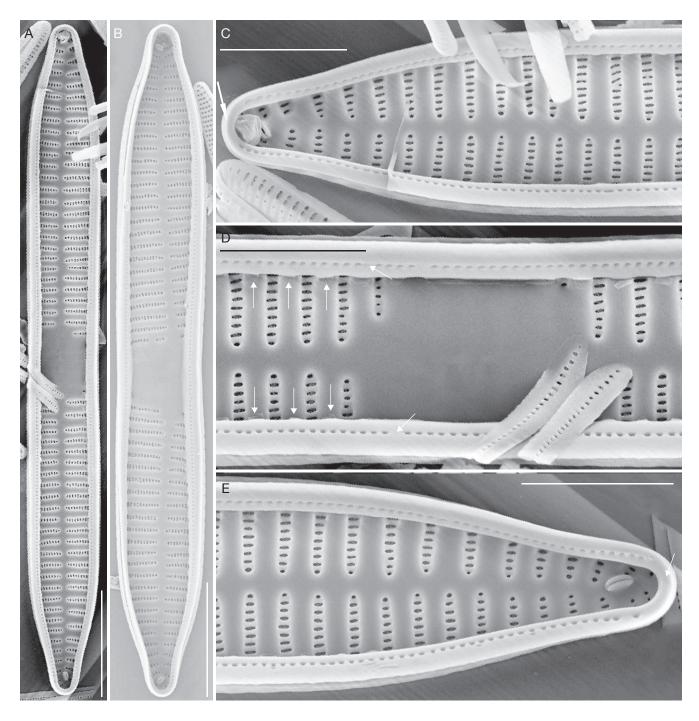


Fig. 10. — *Ulnaria jinbianensis* S. Blanco & Bing Liu, sp. nov., SEM, internal view, type population from Jinbian brook: **A**, **B**, two complete valves, each with a closed valvocopula; **C-E**, details of **A**, note the single row of poroids with some interruptions (**D**, two arrows), each sawtooth-shaped projection attaching over a corresponding virga (**D**, arrowheads), and valvocopula closed and hyaline at both apices (**C**, **E**, arrows). Scale bars: A, B, 10 μm; C-E, 5 μm.

of poroids sometimes with interruptions (Fig. 16G, arrows). Valvocopula with sawtooth-shaped projections attached to the valve, internally visible over each virga (Fig. 16F-H, arrowheads).

DISCUSSION

Although *Ulnaria oxybiseriata* D.M. Williams & Bing Liu, sp. nov., and *U. jinbianensis* S. Blanco & Bing Liu, sp. nov.,

have similar linear-lanceolate valve outline, they differ under LM by the apiculate and protracted ends of the former and a fascia (transapically hyaline band) always present in the latter. Under SEM, these two species belong to different stria type: the former is biseriate and the latter uniseriate. *Ulnaria oxybiseriata* D.M. Williams & Bing Liu, sp. nov., possesses two characters that are rarely observed: 1) a silica layer connecting two adjacent virgae; 2) three rimoportulae per valve (Fig. 5E, N; the latter condition has been observed in *Ulnaria*

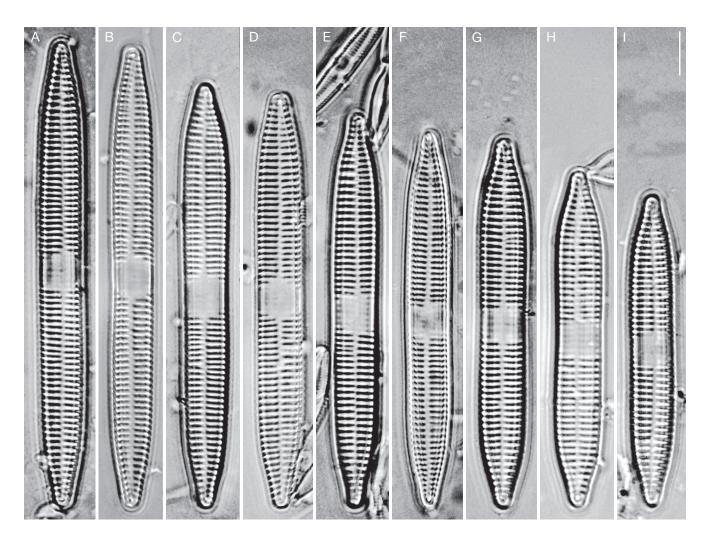


Fig. 11. — Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., LM, a population from Bake river. Nine specimens showing a valve size diminution series, note the protracted rostrate ends in larger valves becoming cuneate in smaller valves. Scale bar: 10 µm.

contracta (Østrup) E. Morales & M. L. Vis (Morales et al. 2007). Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., also possesses a character rarely observed: uniseriate striae that are sometimes biseriate at different points along the same striae (Fig. 8E, F), which was also observed in *U. contracta* (Morales et al. 2007). Ulnaria dongtingensis Bing Liu, sp. nov., is much longer than both *U. oxybiseriata* D.M. Williams & Bing Liu, sp. nov., and *U. jinbianensis* S. Blanco & Bing Liu, sp. nov. Moreover, its narrow valve outline is conspicuously different from the lanceolate valve outline of the latter two. The ultrastructure of *Ulnaria* taxa is diverse and more characters can be found with continued investigation.

Ulnaria oxybiseriata D.M. Williams & Bing Liu, sp. nov., and *U. oxyrhynchus* (Kützing) Aboal both have similar linearlanceolate valve outlines and apiculate ends, but three differences can be documented: the former has a lower stria density (10-12 in 10 µm vs. 13-14 in 10 µm), and biseriate striae (uniseriate in *U. oxyrhynchus*); and has (mostly) a central area bordered by shortened striae only on one side, while *U. oxy*rhynchus has a central area flanked by shortened stria on both sides (Morales et al. 2007). Both Ulnaria oxybiseriata D.M.

Williams & Bing Liu, sp. nov., and *U. verhaegeniana* Van de Vijver, M.de Haan, Mertens & Cocquyt have acute apices, but the latter bears uniseriate striae. *Ulnaria oxybiseriata* D.M. Williams & Bing Liu, sp. nov., differs *U. lanceolata* (Kützing) Compère by the end shape: the former has apiculate ends and the latter has rostrate ends (Table 2). Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., and *U. ramesii* (Héribaud-Joseph) T. Ohtsuka both have uniseriate striae, similar linearlanceolate valve outlines and rostrate ends. However, there are three differences between them: the former has a lower stria density (10-11 in 10 μm vs. 11-14 in 10 μm); with narrower valves (5.5-7.5 μm vs. 8-9 μm); and a fascia-shaped central area whereas the central area in *U. ramesi* shows always shortened striae on both sides (Morales et al. 2007). Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., also different from U. lanceolata: the former has uniseriate striae and a central area always constituting a fascia, but the latter has biseriate striae and a central area rarely forming a fascia (see Kobayasi et al. 1987: 15, figs 14-17).

The reported *Ulnaria* taxa possess very variable valve outlines. For example, Van de Vijver & Cocquyt (2009) reported

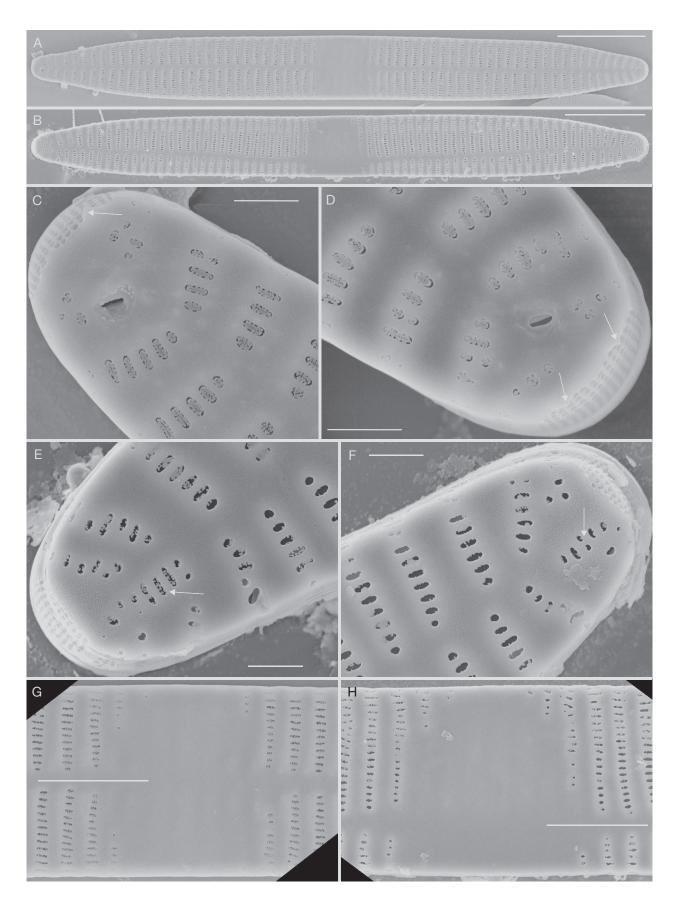


Fig. 12. — *Ulnaria jinbianensis* S. Blanco & Bing Liu, sp. nov., SEM, external view, a population from Bake river: **A**, **B**, two complete valves; **C**, **D**, details of **A**, note horn-like projections overhang the ocellulimbus; **E**, **F**, details of **B**, note a stria almost parallel to the apical axis (**two arrows**); **G**, middle part detail of **A**; **H**, middle part detail of **B**. Scale bars: A, B, 10 μm; C-F, 1 μm; G, H, 5 μm.

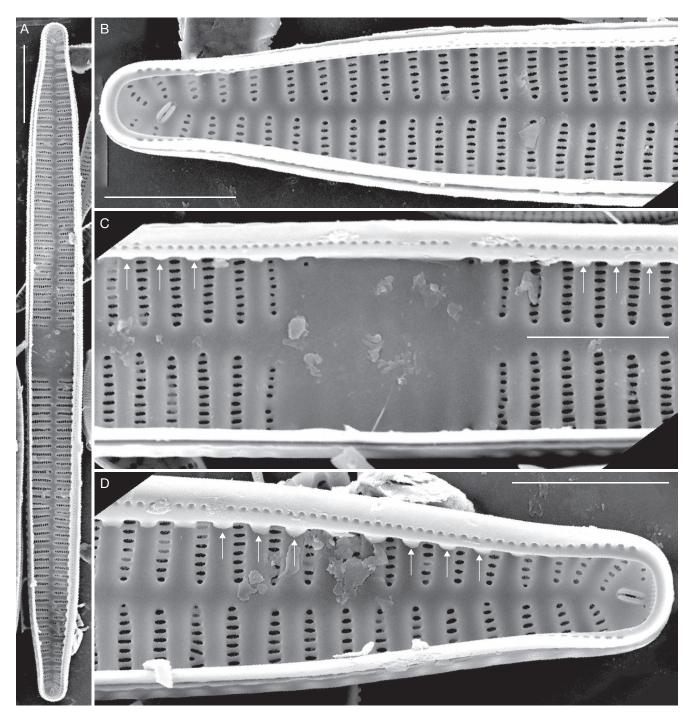


Fig. 13. — Ulnaria jinbianensis S. Blanco & Bing Liu, sp. nov., SEM, internal view, a population from Bake river: A, a complete valve with closed valvocopula; B-D, details of A, note each sawtooth-shaped projection attaching over a corresponding virga (C, D, arrows). Scale bars: A, 10 µm; B-D, 5 µm.

a linear to linear-lanceolate *Ulnaria* species, *U. colcae* Van de Vijver & Cocquyt. Lange-Bertalot & Ulrich (2014) reported a few needle-shaped *Ulnaria* species, e.g. *U. delicatissima* (W. Smith) Aboal & P. C. Silva, U. acus (Kützing) Aboal. Morales et al. (2014) described two new Ulnaria species, one with a lanceolate outline and the other linear-lanceolate. Kulikovskiy et al. (2016) described one approximately fusiform (narrowlanceolate) Ulnaria species and one needle-shaped Ulnaria species. Liu et al. (2017) described three Ulnaria taxa (one lanceolate, one linear-lanceolate, and one very long linear). Van de Vijver *et al.* (2017) reported a linear *Ulnaria* species. Finally, Cantonati et al. (2018) reported two Ulnaria taxa (one is strictly linear, the other is linear-lanceolate). From above mentioned reports, we can see that the *Ulnaria* taxa with fusiform (narrow-lanceolate) valve outline are rare. Thus, only a couple of similar taxa can be found to fit Ulnaria dongtingensis' outline. The most similar taxon to *Ulnaria dongtingensis* Bing Liu, sp. nov., is *U. ferefusiformis* Kulikovskiy & Lange-Bertalot,



Fig. 14. — *Ulnaria dongtingensis* Bing Liu, sp. nov., LM. Nine specimens showing a valve size diminution series, note the narrow-lanceolate valve outline: **B**, illustration of holotype specimen (an individual indicated by a circle on the slide). Scale bars: A, B, C-I, 10 µm.



Fig. 15. — Ulnaria dongtingensis Bing Liu, sp. nov., SEM, girdle view: A, a separated frustule; B, C, two apices of A showing the closed girdle bands, ocellulimbi, and two horn-like projections (arrows) overhang the ocellulimbus; D, detail of A, note two shortened striae bordering the central area (two arrows) and hyaline mantle middle part (dashed arrow); E, a detail of the closing plates on the mantle. Scale bars: A, D, 10 µm; B, C, E, 1 µm.

although *U. dongtingensis* Bing Liu, sp. nov., has usually larger valve dimensions (106-260 μm vs. 76-152 μm in length, 5-7 μm vs. 4.0-4.8 μm in width), a fascia-shaped central area (lacking in *U. ferefusiformis*), and usually lower stria density (10-12 in 10 μm vs. 12-14 in 10 μm) (Kulikovskiy *et al.* 2016) (Table 2). Ulnaria acus possesses a lanceolate valve outline and lacks central area or has an indistinctly circular one with shortened marginal striae (Lange-Bertalot & Ulrich 2014).

Complete *Ulnaria* frustules in girdle view (e.g. as in Liu et al. 2017: figs 11-13, 37-40, 59-62) are not easy to find in the literature but separated frustules and valves with valvocopulae can be spotted by experience such as in this paper. All new species in this paper have the same valvocopula ultrastructure: 1) a complete valvocopula is a closed ring with the same shape as the valve outline, closely attached to the mantle interior surrounding the valve margin; 2) each valvocopula present a row of poroids which bisects the pars interior and exterior, located at mid-line; 3) valvocopula with sawtooth-shaped projections attached to the valve over virgae internally; and 4) the valvocopula closed and hyaline at both apices. The number of transapical rows and pervalvar rows composing the ocellulimbus, the number of silica projections overhanging the ocellulibus, and the closing plates are all observed in all three new species of this study. Ulnaria monodii (Guermeur) Cantonati & Lange-Bertalot possess two

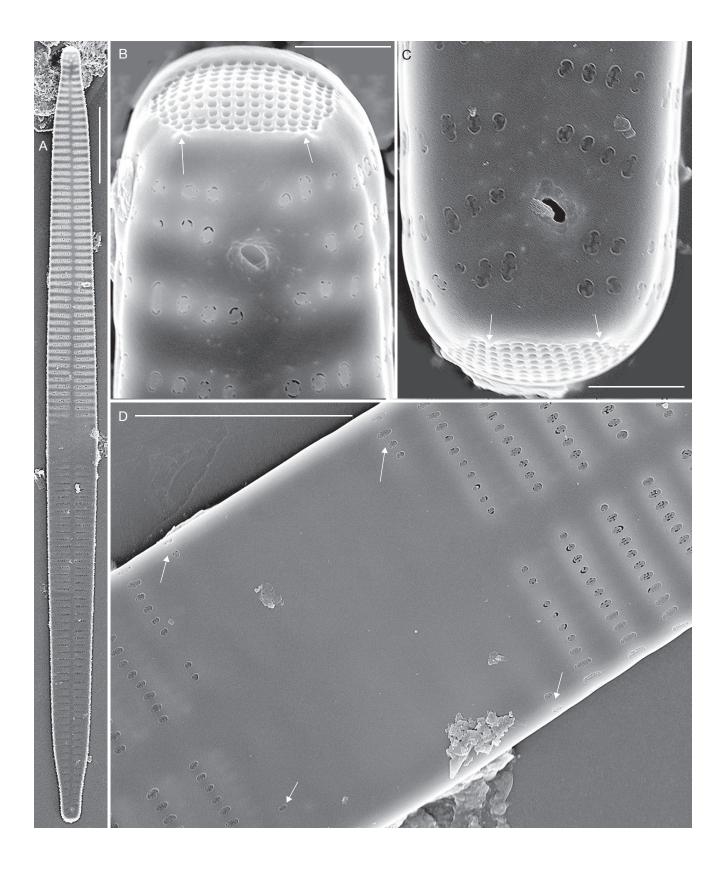


Fig. 16. — *Ulnaria dongtingensis* Bing Liu, sp. nov., SEM, external view. **A**, a complete valve, note the narrow-lanceolate outline; **B**, **C**, two apices of **A** note the ocellulimbus and two horn-like projections overhang the ocellulimbus (**arrows**); **D**, middle part detail of **A**, note a square fascia present and shortened striae (**arrows**) bordering the central area. Scale bars: A, 10 µm; D, 5 µm; B, C, 1 µm.

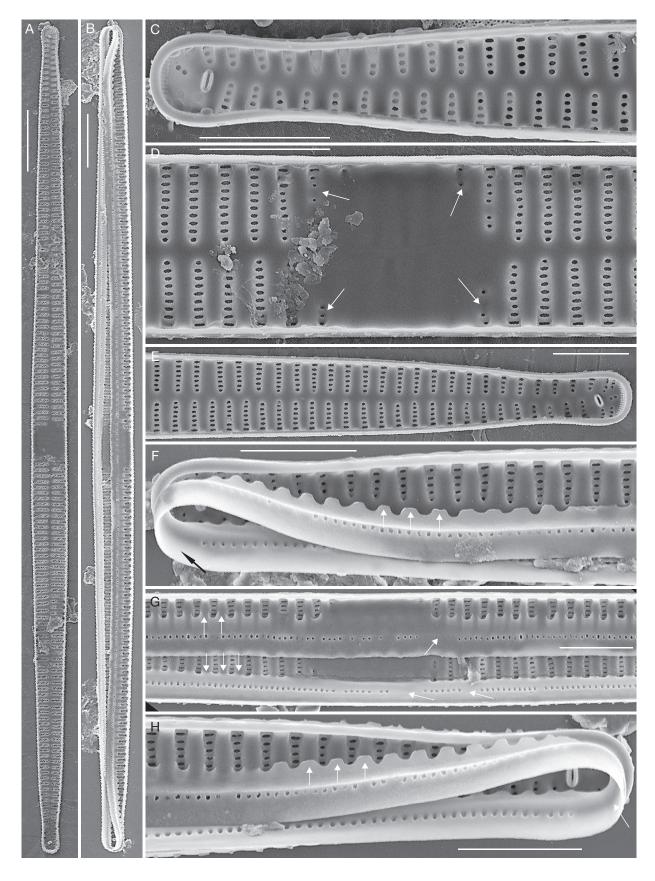


Fig. 17. — Ulnaria dongtingensis Bing Liu, sp. nov., SEM, internal view: A, a complete valve; B, a valve with closed a valvocopula; C, E, apex detail of A, note the capitate end; D, middle part detail of A, note a square fascia present and a few shortened striae (arrows) bordering the central area; F, H, apex detail of B, note the sawtooth-shaped projections (arrowheads) and the valvocopula closed and hyaline at apex (arrow); G, middle detail of B, note the single row of poroids with some interruptions (arrows) and each sawtooth-shaped projection attaching over a corresponding virga (arrowheads). Scale bars: A, B, 10 µm; C-H, 5 µm.

Table 2. — Comparision of the morphological characters for identification amongst some species of Ulnaria

Features	U. oxybiseriata sp. nov.	U. jinbianensis sp. nov.	U. dong- tingensis sp. nov.	U. oxyrhynchus	U. verhae- geniana	U. ramesii	U. lanceolata	U. Ianceolata	U. ferefu- siformis
Length (µm)	56-78	42-91	106-260	43-64	55-90	50-92	55-75	57-110	76-152
Width (µm) Stria type and number in 10 µm	6-9 Biseriate, 10-12	5.5-7.5 Uniseriate, 10-11	5-7 Uniseriate, 10-12	6-7 Uniseriate, 13-14	5.0-6.0 Uniseriate, 10-12	8-9 Uniseriate, 11-14	8-10 Biseriate, 8-10	7-9 Biseriate, 9-10	4.0-4.8 Uniseriate, 12-14
Valve outline	Linear- lanceolate to lanceolate	Linear- lanceolate	Narrow- lanceolate	Linear- lanceolate	Linear	Linear- lanceolate or lanceolate	Linear- lanceolate	Linear- lanceolate	Approximately fusiform
Apex	Apiculate	Rostrate to subrostrate or cuneate	Rostrate to capitate	Acute, conical to subrostrate	Acute	Rostrate	Rostrate	Rostrate	Subcapitate
Central area	LM and SEM: flanked on one side by shortened striae in most specimens rarely rectangular fascia	,	LM and SEM: square fascia	LM and SEM: Flanked by short striace	LM and SEM: rectangula fascia	LM and SEM: r ellipsoid to rectangula		LM and SEM: circular, slightly inclined toward one side	LM: indistinctly defined due to weak e ghost striae
Number of closing plate struts	2-5	4-8	2-6	No data	No data	No data	No data	No data	Present
Number of horn-like projections	6-9	at least 2	2	No data	2	No data	No data	No data	No data
Numbers of rows of ocel- lulimbus	Transapical rows 7-9, pervalvar rows 11-13	Transapical rows 5-9, pervalvar 3 rows 11-14	Transapical rows 6-10, pervalvar rows 12-15	No data	No data	No data	No data	No data	No data
References	This study	This study	This study	Morales et al. 2007	Van de Vijver et al. 2017	Morales et al. 2007	Kobayasi et al. 1987	Williams 1986	Kulikovskiy et al. 2016

horn-like projections overhanging the ocellulibus (Cantonati et al. 2018: 85, fig. 35). The ultrastructural details of all three new species are summarized (in Table 2) to provide the data for comparison in future studies in the genus *Ulnaria*. As stated above, closed bands are a synapomorphy of the genus *Ulnaria*. Few taxa in other possibly related genera have been studied in detail for the band structure. Our unpublished observations on the genera *Fragilaria* and *Hannaea* reveal simple but open bands (DMW, pers. comm., etc.).

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