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Observations on a fragilarioid diatom found in inter-dune lakes of the Badain Jaran Desert (Inner Mongolia, China), with a discussion on the newly erected genus *Williamsella* Graeff, Kociolek & Rushforth

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Abstract

Populations of a fragilarioid diatom from inter-dune lakes of the Badain Jaran Desert (northern China), originally identified and reported under the name *Fragilaria* sp. *cf. famelica*, were investigated in detail using light and scanning electron microscopy. The analyses indicate that most of the morphological features of this taxon are identical to *Williamsella angusta* Graeff, Kociolek & S.R. Rushforth, the type species of the genus *Williamsella* recently described from Blue Lake warm spring (Utah, USA). The criteria used to separate *Williamsella* from *Fragilaria* (i.e. the presence/absence of spines, the colonial formation, the preference for saline vs freshwater habitat, and the coverings of the areolae) are not supported and the genus is refuted. Instead the new combination *Fragilaria crenophila* comb. nov. is proposed for the Blue Lake species. The taxon from the Badain Jaran desert lakes represents a varietal population that is characterized by shorter valve length and lower stria density than the nominal variety, and is given the name *Fragilaria crenophila* var. *sinensis*. A discussion of the taxonomy, ecology and geographical distribution of this taxon is presented and a detailed comparison with *Fragilaria asiatica* Hustedt, a very similar species described from northern Tibet (China) is made.

Keywords: *Williamsella*, *Ulnaria*, *Fragilaria*, Hustedt, taxonomy, Badain Jaran Desert, Tibet, China

Introduction

The taxonomy and circumscription of genera belonging to Fragilariaceae remains a subject of debate among diatomists (Williams 2011, 2013; Morales *et al.* 2014). While new species, even new genera (Morales *et al.* 2002, 2003; Li *et al.* 2010; Graeff *et al.* 2013), are being described, there are still many species included within whatever *Fragilaria sensu lato* might be. During a study of the diatom flora of surface sediments of the inter-dune lakes of the Badain Jaran desert, Inner Mongolia, China, specimens of an unusual fusiform fragilarioid species was found in several of the samples investigated. In a previous study that focused on the relationship between the diatom assemblages of these lakes and the electrical conductivity gradient (Rioual *et al.* 2013; Rioual *et al.* 2014), these specimens were tentatively identified as *Fragilaria* sp. cf. *famelica*. In the same year, however, Graeff *et al.* (2013) described some very similar specimens from Blue Lake warm spring (Utah, USA). These authors argued that the specimens they found could not be satisfactorily incorporated into any known genera and consequently described them as the type species of a new genus: *Williamsella angusta* Graeff, Kociolek & S.R. Rushforth (Graeff *et al.* 2013: 6). *Williamsella* was distinguished from *Fragilaria* Lyngbye (1819: 182) on the basis of its colonial formation (growing singly vs in colonies), the occurrence of spines (absent vs present in most species), its habitat (saline vs freshwater) and by the presence/absence of external coverings over the areolae. Since then, Al-Handal *et al.* described from a saline lake in southern Iraq another fragilaroid

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species that they attributed to this genus, *Williamsella iraqiensis* Al-Handal & Kociolek (Al-Handal *et al*. 2016: 291). *W. iraqiensis* was distinguished from *W. angusta* mainly by having a wider sternum and higher stria density.

The purpose of this paper is fourfold. Firstly, we address the issue of the con-specificity between the specimens found in the spring-fed, inter-dune lakes of the Badain Jaran Desert and *Williamsella angusta*. Secondly, we compare the morphologies of these two populations with that of similar taxa illustrated in the literature and in particular with *Fragilaria asiatica* Hustedt (1922: 119), a species described from northern Tibet. Thirdly, we address the issue of the validity of the genus *Williamsella* by discussing the characteristics that were used to distinguish it from related genera such as *Fragilaria*, *Ulnaria* (Kützing) Compère (2001: 100) and *Tibetiella* Li, Williams & Metzeltin (2010: 329). Fourthly, we provide ecological information on the specimens found in the Badain Jaran desert lakes.

Material and methods

Study area

In the Badain Jaran Desert, the surface morphology is characterized by large sand dunes extending to a maximum height of 460 m. Between these dunes coexist >100 permanent lakes (Yang 2000). They have neither surface inflows nor outflows but are not strictly closed-basin lakes and can be considered as seepage lakes (Hofmann 1996). Climatic, hydrological and geomorphological mechanisms all contribute to the differences in salinity and solute composition observed among the lakes of this desert (Rioual *et al*. 2013). The regional climate is strongly continental and hyper arid. At the Ekenhuduge weather station, located at the southern edge of the desert, the mean annual temperature is 7.7°C, mean monthly temperatures range from −10°C in January to +25°C in July. Average annual precipitation is 118 mm but inter-annual variation is high. Diurnal temperatures in summer months range from 0 to >40°C (Yang & Williams 2003; Yang *et al*. 2003b; Ma *et al*. 2009).

Collection, preparation and analysis of samples

The diatom species discussed here was originally found in the surface sediments of twelve lakes of the Badain Jaran Desert (out of the 42 lakes investigated). Whole cells, still containing plastids, were found on the filters used for water chemistry analyses and in epiphyton samples (on *Potamogeton* sp. and *Chara* sp). These samples were kept frozen since their collection in 2007 (Table 1). The field and laboratory methods for monitoring the diatom community were described previously (Rioual *et al*. 2013). In summary, for analysis under the light microscope, sub-samples of the cleaned, homogenized suspension were diluted with distilled water and left to settle onto 18 mm diameter round glass coverslips and dried overnight in ambient conditions. Coverslips were mounted on glass slides with Naphrax® (a commonly used mountant with a refraction index of 1.73). Counting was made with a Leica DM LB2 light microscope (Leica Microsystems, Wetzlar, Germany) using bright-field and phase contrast oil immersion optics at 1000 × magnification. Light micrographs were captured either by using a MVC3000 camera and ACD see software or a Leica DFC450 camera. At least 500 diatom valves were enumerated to establish abundance values expressed as percentages. Several general and regional diatom floras (Cumming *et al*. 1995; Witkowski *et al*. 2000; Metzeltin *et al*. 2009; Hofmann *et al*. 2011) as well as genera-specific references (Krammer 1997; Lange-Bertalot 2001; Levkov 2009) were consulted for identification purposes. Terminology followed Anonymous (1975) and Round *et al*. (1990).

TABLE 1. List of samples from the Badain Jaran Desert lakes used for the analysis of *Fragilaria crenophila* var. *sinensis*.

Site	Sampling date	Sample type	Water depth (m)	*F. crenophila* v. *sinensis* (%)	presence of live cells
Aer Jilin	18-Jun-2007	surface sediment	1.7	7.9	n/a
		epiphyton (*Potamogeton* sp.)	1.5	2.3	yes
		water (GF filter)	0.3	n/a	yes
Shaobai Jilin	19-Jun-2007	surface sediment	1.2	7.9	n./a
		epiphyton (*Potamogeton* sp.)	0.5	1.5	yes
		water (GF filter)	0.3	n/a	yes
	13-Oct-2009	epiphyton (*Potamogeton* sp.)	1.2	9.3	yes
		water (Nitrate cellulose filter)	0.3	n/a	yes
Wosigetu Nuo’er	18-Oct-2008	surface sediment	1.3	1.5	n/a
		epiphyton (*Potamogeton* sp.)	0.3	1.6	yes
		epiphyton (*Chara* sp.)	1.3	5.5	yes

For scanning electron microscope (SEM) analyses, aliquots of cleaned samples from the surface sediments of two lakes of the Badain Jaran Desert were air dried onto 12 mm diameter glass coverslips. These were mounted onto aluminum stubs using Ted Pella double-coated carbon conductive adhesive tape (Ted Pella, Inc., Redding, CA, USA) and gold coated in a Polaron SC7640 sputter coater (Quorum Technologies, East Grinstead, UK) for viewing on a LEO 1530 VP (LEO Electronenmikroskopie GmbH, Oberkochen, Germany) at the Key State Laboratory of Paleobiology and Stratigraphy (Chinese Academy of Sciences, Nanjing, China). Additional SEM images of untreated material were obtained by cutting off small pieces of the glass fiber filters used for water chemistry analyses and mounting them on aluminum stubs. These samples were analysed on a Nova Nano SEM 450 (FEI, Hillsboro, Oregon, USA) at the Institute of Geology & Geophysics (Chinese Academy of Sciences, Beijing, China). Photographs of the type material of *F. asiatica* were taken on a Phantom ProX at the Institute of Earth Environment (Chinese Academy of Sciences, Xi'an).

For the description of the new taxon, the herbarium acronyms follow Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp) with IGGDC = Herbarium of Bacilliarophyceae, Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing, People's Republic of China.

Environmental data

In order to provide a dataset of environmental variables that can be linked with the diatom abundance data, surface water samples (-0.3 m depth) were collected by hand (directly from a boat by lowering a sampling bottle into the lake-water) from the deepest part of each lake during each fieldwork campaign. The maximum water depth was determined either with a hand-held acoustic depth meter or, for shallow lakes, with a ruler. Lakes were sampled in May 2007, October 2008 and October 2009. In the original dataset, 26 environmental variables were measured either on unfiltered lake water (pH, conductivity, alkalinity, total nitrogen and total phosphorus) or water filtered through Whatman GF/F® glass fiber filters (for the concentrations of the main cations, anions and dissolved organic carbon). The complete list and the methods used to measure these various environmental variables are given in Rioual *et al*. (2013).

Morphometric analyses and comparison with other species

Morphometric analysis was done using light microscopy (LM). The specimens from three lakes of the Badain Jaran Desert were photographed and measured along the same transects used for the determination of relative percentages. In this study the following populations were investigated:

1. *Fragilaria* sp. from the surface sediment of Aer Jilin collected in June 2007 (30 valves).
2. *Fragilaria* sp. from the surface sediment of Shaobai Jilin collected in June 2007 (40 valves).
3. *Fragilaria* sp. from the surface sediment of Wosigetu Nuoer collected in October 2008 (20 valves).
4. *Williamsella angusta* Graeff, Kociolek & Rushforth from the type population from Blue Lake Warm Springs, Utah, USA (90 valves). Isotype slide COLO 8508.
5. *Fragilaria asiatica* Hustedt from the sample referred as "Zwischen Lager XXVII und XXVIII, 17.8.1900", northern Tibet (90 valves). Type slide KA-61 and type material AS1395 (i.e. a cleaned suspension kept in formalin and glycerine) borrowed from the Hustedt Diatom Collection at the Institute for Marine Research in Bremerhaven (Germany).

In addition, published LM photographs were also measured for comparison. These include nine images of *W. angusta* published in Graeff *et al*. (2013; figs 19–27), six images of *F. asiatica* published in Simonsen (1987), five images of *F. asiatica* published online by the Hustedt Diatom Collection EarthCape Database (2017), two images of *Fragilaria cf. tenera* from subsaline lakes in British Columbia (Canada) published in Cumming *et al*. (1995; Plate 8, figs 8–9), three images of *Fragilaria aff. famelica* from Nine Mile pond, Florida Everglades (USA) published in Lange-Bertalot (1993; Plate 12, figs 12–14) and five images of *W. iraqiensis* published in Al-Handal *et al*. (2016).

The total dataset comprised 300 specimens and was investigated using conventional morphometric analyses. Measurements of valve length, width and stria density were made on digitized images calibrated against a slide micrometer using public domain ImageJ software (Rasband 2013). Following Graeff *et al*. (2013) stria density was measured at the valve margin, at the valve center. In practice, the distance spanned by twenty striae was measured and algebraically converted to a number of striae per 10 µm (Kaczmarska *et al*. 2009). To establish if the morphological characteristics of these populations differed from each other, single character analyses and bivariate analyses were performed on the dataset derived from LM images. For single characters analyses, box plots were used to show the quantile distributions of valves characters. The populations from the Badain Jaran Desert lakes, Blue Lake and Tibet were also compared using histograms showing the probability density distribution of valve length, width and stria density. Bivariate analyses were based on scatter plots. Boxplots, scatterplots, histograms and probability density distribution curves were drawn using R (R development core team, 2013). Difference in morphological characteristics (medians for valve length, width and stria density) between the three populations was tested using the Kruskal-Wallis (with post hoc pairwise comparisons, Table 2) and Kolmogorov-Smirnov tests in the program PAST (Hammer *et al.* 2001).

Beside the analyses based on LM observations, areola density was estimated from SEM images. In practice, the lengths of three to four areolae were measured on striae located near the valve central area using ImageJ and algebraically converted to a number of areolae per 10 µm. For the Badain Jaran populations, areolae density was estimated from 52 measurements taken from 5 valves. For Blue Lake, the areola density was estimated from 20 measurements on the two valves illustrated in Graeff *et al*. (2013). From the type material of *F. asiatica*, 40 measurements from 4 valves were taken. In addition, the diameters of the areolae were also measured for these three populations (131 measurements in total).

TABLE 2. Kruskal-Wallis test statistics for the comparisons of fragilarioid populations from the Badain Jaran Lakes (BJ), Blue Lake (BL) and Tibet (all three populations with n=90).

		Valve length	Valve width	Stria density
H (Chi^2)		54.84	79.71	70.71
p		<0.0001	<0.0001	<0.0001
p of post hoc pairwise tests, with Bonferroni correction	BJ vs BL	<0.0001	0.662	<0.0003
	BJ vs Tibet	<0.0001	<0.0001	<0.0001
	BL vs Tibet	<0.0001	<0.0001	<0.0001

Species response analysis

With the aim of defining the responses (in terms of proportions of the complete assemblages) of the species investigated to the main environmental gradients (= the predictor variables) Generalized Additive Models (GAMs) were used. GAMs were fitted using the program CanoDraw 4.1 (ter Braak & Šmilauer 2002). To avoid the influence of uneven sampling intensity along the gradient, the environmental data were log-transformed or square-root transformed. The model complexity of GAMs was chosen by the stepwise selection procedure using the Akaike information criterion (AIC). AIC considers not only the goodness of fit, but also parsimony, penalizing more complex models (Lepš & Šmilauer 2003). Poisson distribution and log link function were used in the models.

Results

Description of the taxon from the Badain Jaran lakes

In LM (Figs 1–15)

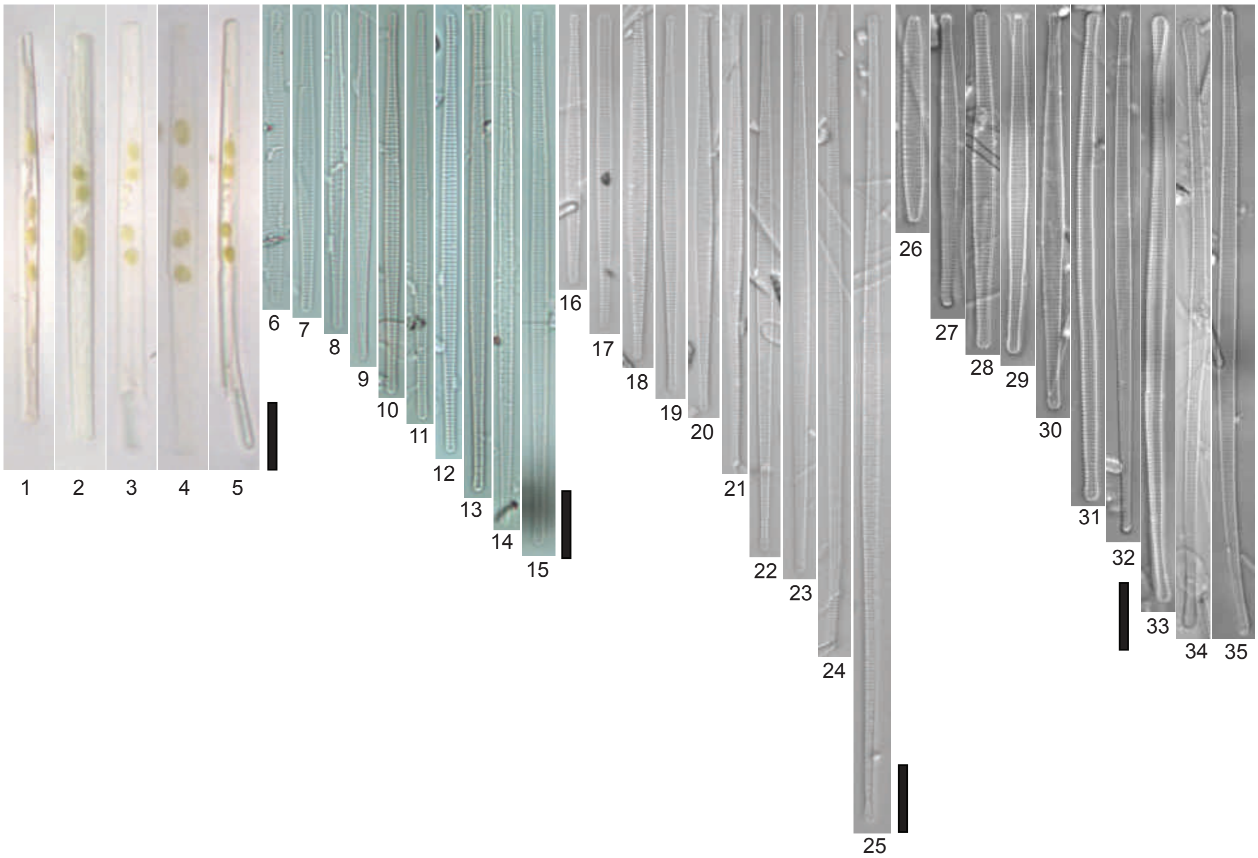
Cells are solitary with four plastids distributed in the middle third of the cell (Figs 1–5). Valves are fusiform becoming linear in larger valves, with apices slightly inflated and rounded (Figs 6–15). Valve lengths are 43–81 µm, and breadths 2–3 µm. The central sternum is very narrow with no central area. Striae are opposite, density 15–19 in 10 µm.

In SEM (Figs 36–47)

Areolae appear to be round and of irregular size (0.12–0.19 µm in diameter, n=66). The number of areolae per stria ranges from five at the centre to three in the narrow sections of the valve (Figs 36–39). Externally, each areola is covered by a thin, flat siliceous closing plate that appears to be more or less sunken into the areolae (Figs 38, 39, 45, 47). When these plates are dissolved a smaller recessed opening is apparent (Figs 42–44). Spines were not observed. One rimoportula per valve, internally sessile with a narrow, slit-like opening, is oriented at a slight angle to the apical axis (Fig 40), and externally opening as a rectangular occlusion (Figs 42, 44). A small pore field is found at each apex, lying in a shallow ocellulimbus (Figs 44–46). The cingulum is composed of several open copulae (at least 3) each with a single row of areolae, that are also occluded by the same type of covering as on the areolae of the valve face (Figs 44–47). The pars interior is scalloped (Fig 44).

Analysis of the type material of Williamsella angusta

For this study we only observed *W. angusta* under the LM (n=90, Figs 16–25). Compared with the original description given in Graeff *et al.* (2013) we found slightly different ranges in valve length (40–122 µm instead of 27.5–106 µm) and stria density (15–20 instead of 17–20 striae in 10 µm).



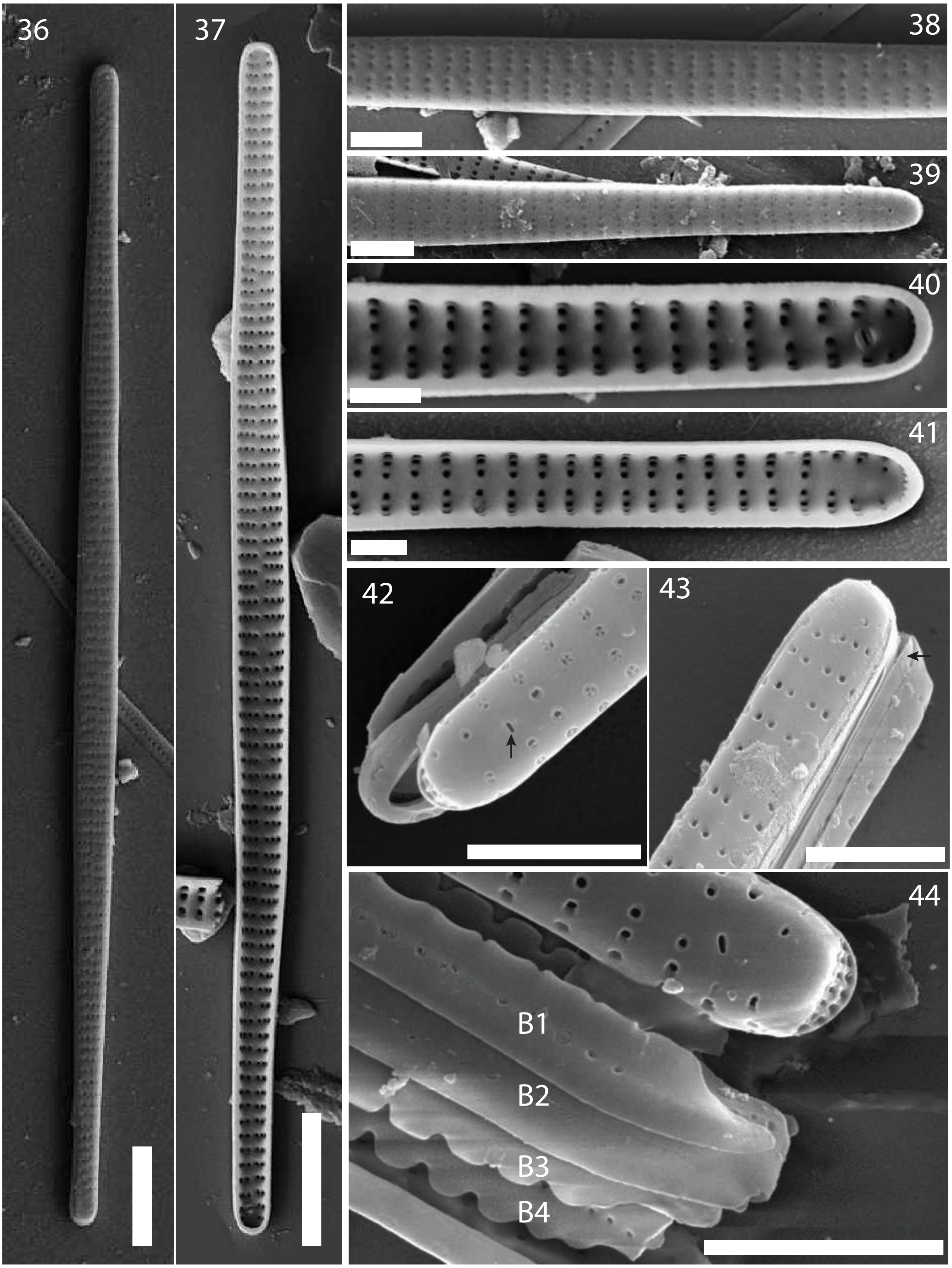
FIGURES 1–35. Light micrographs for the comparison of *Fragilaria crenophila* var. *sinensis var. nov., Williamsella angusta* Graeff, Kociolek & S.R. Rushforth and *Fragilaria asiatica* Hustedt. Figs 1–15. *F. crenophila* var. *sinensis* from lakes in the Badain Jaran Desert. Figure 12 is the holotype. Figs 16–25 *W. angusta* from Blue Lake, Utah, USA from isotype slide COLO 8508. Figs 26–35. *F. asiatica* from Tibet, from type slide KA–61. Figs 1–5, untreated material from epiphyton samples (Lake Shaobai Jilin, June 2007), bright field. Figs 6–35 cleaned preparations, taken in bright field and differential interference contrast. Scale bars = 10 µm.

Analysis of the type material of Fragilaria asiatica

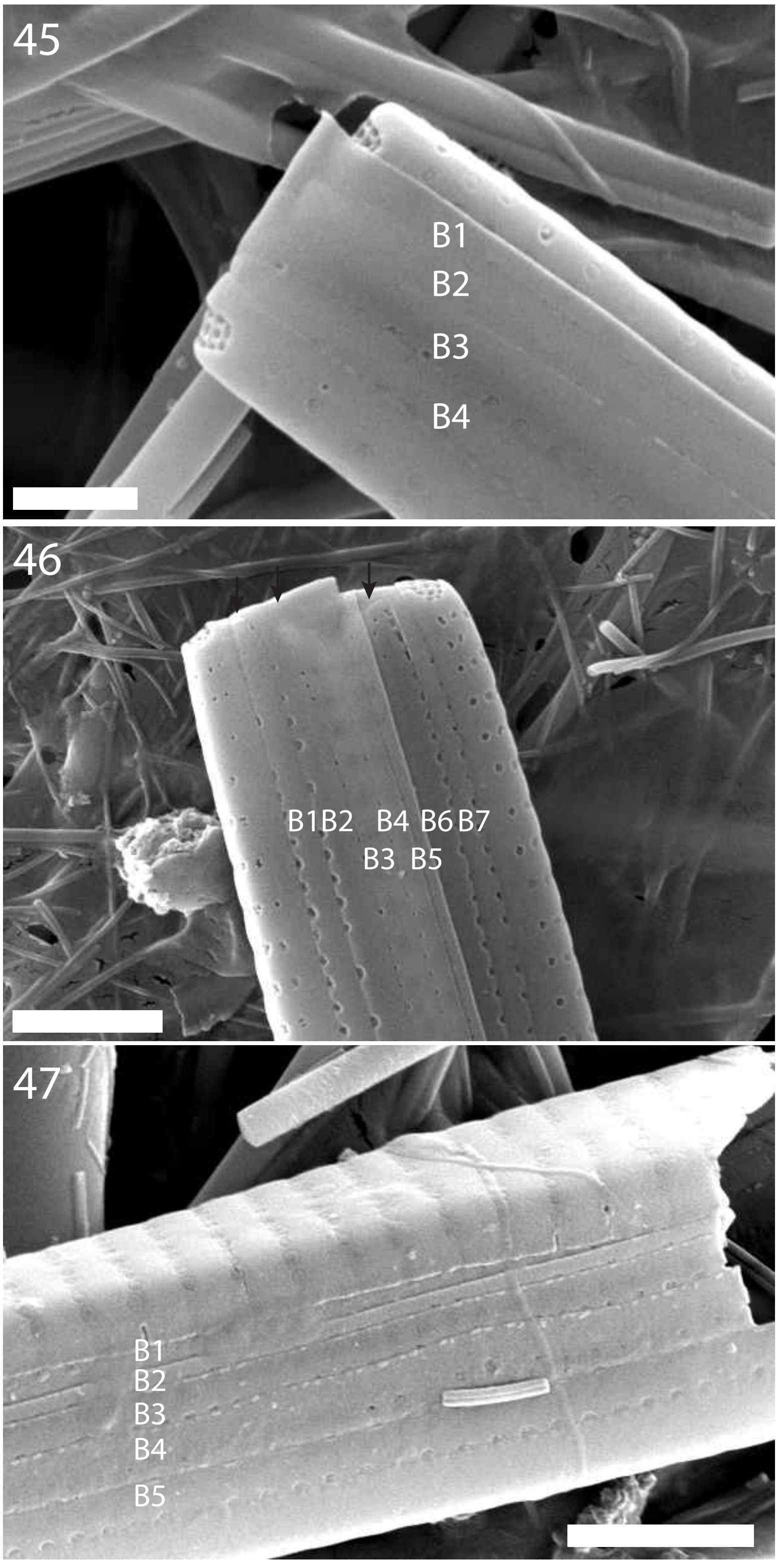
F. asiatica was described by Hustedt (1922) from samples collected by the Swedish explorer Sven Hedin during his 2nd expedition in Tibet (1899–1902) when crossing the Hoh Xil (also called Kekexili) Basin, south of the Arka Tagh in the eastern part of the Kunlun Mountains. Some details on the expedition route were reported by Hedin (1903) in his book "Central Asia and Tibet". In this book, Hedin also provided maps that he drew of the route used by the expedition (at the scale 1:200,000). In Hustedt's publication (1922), the sample in which *F. asiatica* was found is only reported as: "between camps 27 and 28 (Chaîne v. d. Putte), 17.8.1900". Hustedt (1922) however, mentioned in the introduction of his paper that most of the Hedin's samples he could analyse were derived from algal mats ("Algenrasen" in the original text in German), Chlorophyceae and aquatic plants that had been kept dried in a herbarium with the adherent diatoms. He also noted that this sample was collected from freshwater. On the maps corresponding to the period from the 9th to 19th August 1900 (Hedin 1903, Pl.34), elevations are given at regular intervals on the route but the geographical coordinates are provided only for camps 25 and 27. On the 17th of August 1900, the day when the *F. asiatica* sample was collected, Hedin only mentioned "two pools on the top of the main pass" and we may assume that these were the water bodies that were sampled. Using the map and information Hedin provided in his book as well as Google Earth®, we estimated that these pools, i.e. the type location of *F. asiatica,* have approximately the geographical coordinates 35°40'N and 89°53'E. Such position is about 1180 km southwest from the Badain Jaran lakes where we found the fragilarioid diatom investigated here.

The original Latin description of *F. asiatica* given by Hustedt (1922) is very succinct and we propose here an emended description based on the analysis of 90 valves under the LM and 11 whole valves under the SEM:

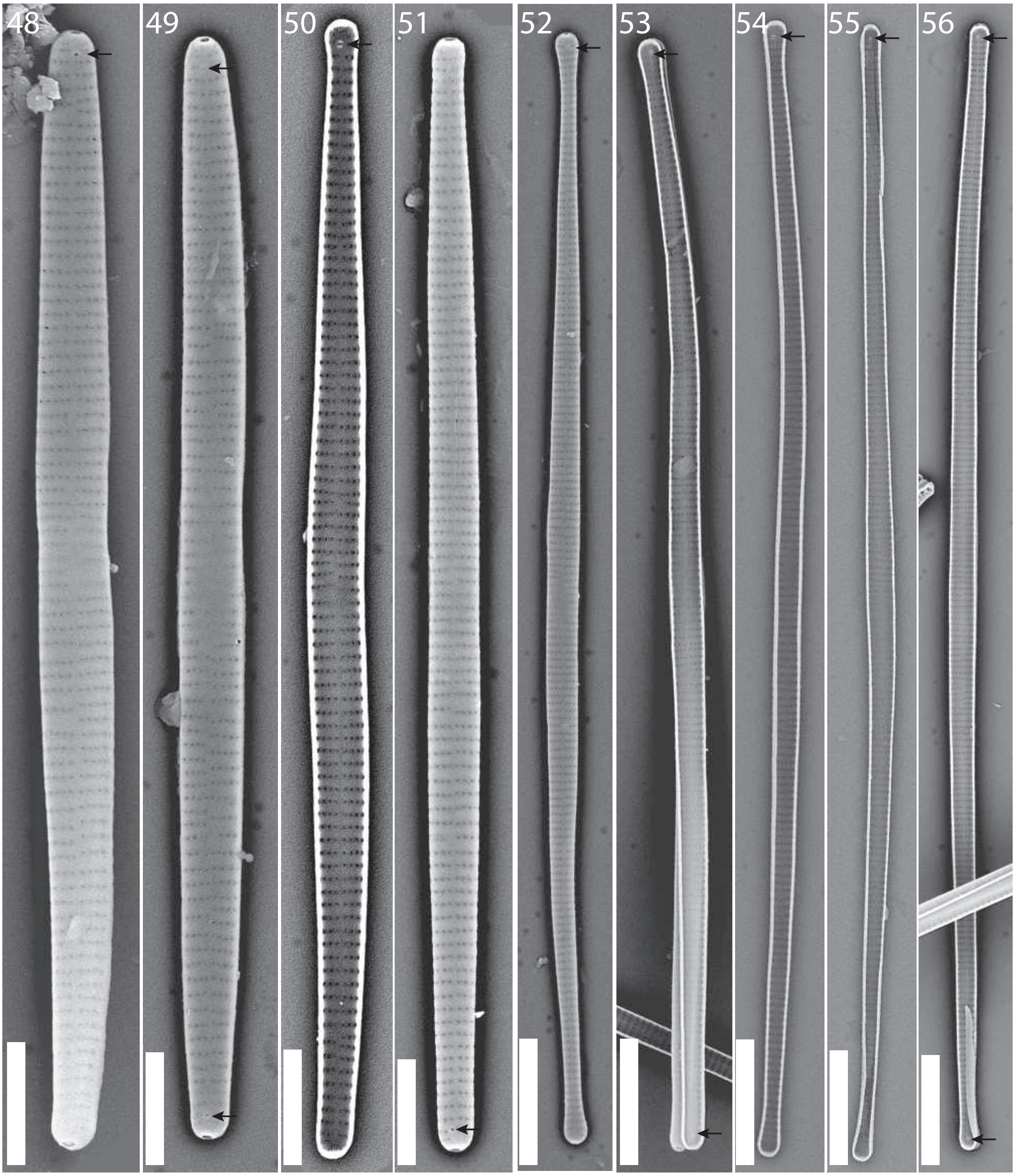
Valves fusiform, becoming linear in larger valves, with apices subcapitate in larger specimens and slightly protracted in smaller specimens (Figs 26–35). Length 31–102 µm, width 2–4 µm. Central sternum very narrow. Generally no central area is formed, but in some specimens the sternum widens to form an irregular central area (Figs 26, 27, 35). Striae opposite, density 15–19 in 10 µm (instead of 25 striae in 10 µm as reported by Hustedt).



FIGURES 36–44. Scanning electron micrographs of *Fragilaria crenophila* var. *sinensis* var. nov. from Badain Jaran lakes. Fig. 36. External view of a whole valve. Fig. 37. Internal view of the whole valve. Fig. 38. External view of the centre of the valve. In the background, a detached valvocopula with a single row of areolae. Fig. 39. External view of apex depicting opposite striae, disc-like volae and the external opening of the rimoportula. Fig. 40. Internal view of apex showing the rimoportula. Fig. 41. Internal view of apex without a rimoportula. Fig. 42. Apex with external opening of the rimoportula (arrow). The areolae covering are partially dissolved. Fig. 43. Apex without rimoportula. Open copula (arrow). Fig. 43. Oblique view of an apex showing the ocellulimbus and in girdle view, components of the cingulum (labeled B1–B4) showing a single row of areolae and a scalloped pars interior. Scale bars = 10 µm (Figs 36, 37); = 2µm (Figs 38, 39, 42, 43); = 1µm (Figs 40, 41).

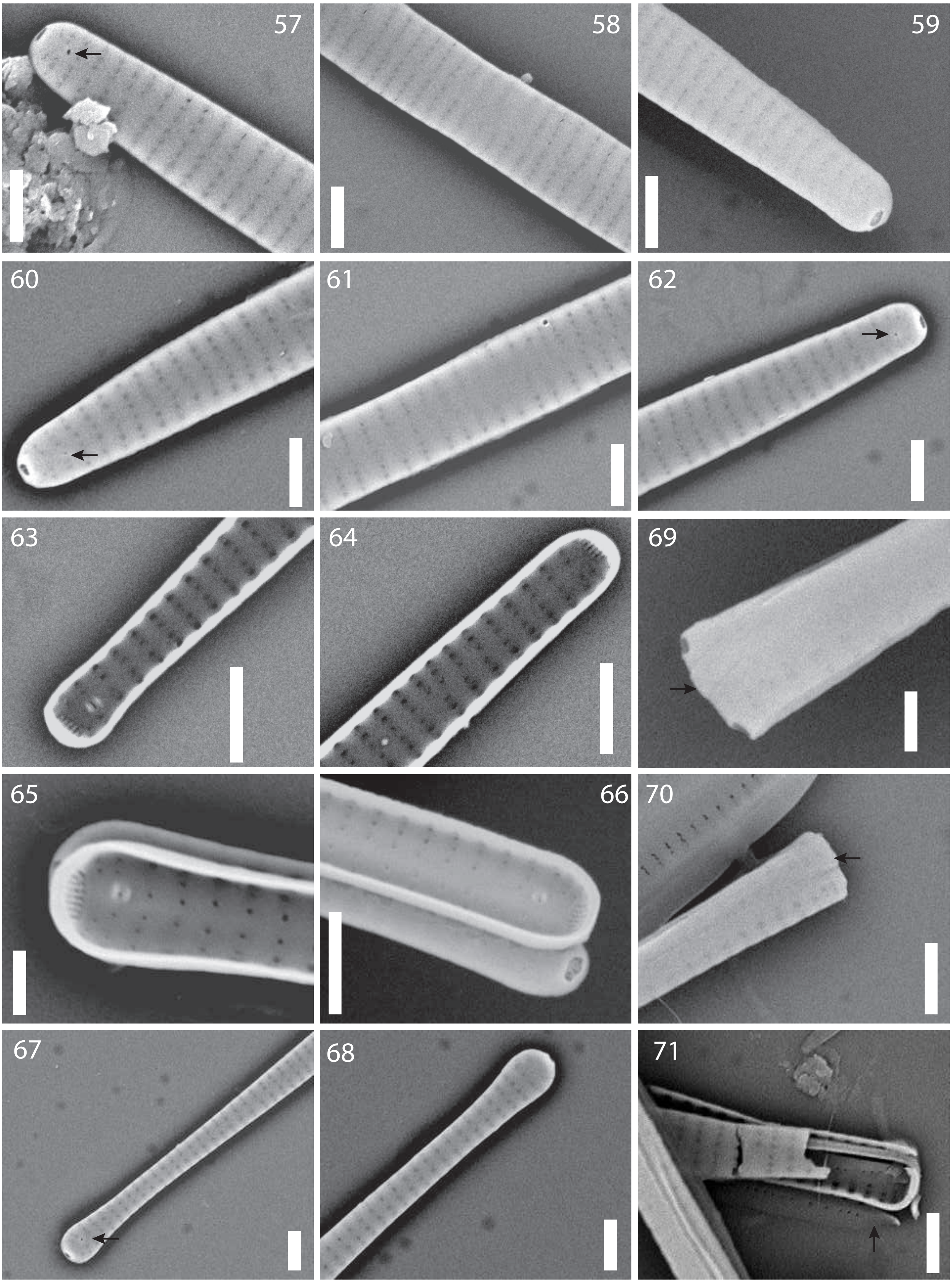


FIGURES 45–47. Scanning electron micrographs of *Fragilaria crenophila* var. *sinensis* from untreated material from Lake Shaobai Jilin, Badain Jaran Desert deposited on a GF filter (water sample collected in June 2007). Fig. 45. External view of the apex of a whole cell in girdle view. The cingulum is composed of four copulae (labeled B1–B4), each with a single row of areolae occluded with disc–like coverings . Fig. 46. Same as in Fig. 45 but with a cingulum composed of at least seven copulae (labeled B1–B7). Copulae with open ends (arrows). The areolae covering are partially dissolved. Fig. 47. Girdle view of central part of a broken cell showing disc–like coverings of the arolae and a cingulum composed of five girdle bands (B1–B5). Scale bars = 2 μm (Figs 46, 47); = 1 μm (Fig 45).



FIGURES 48–56. Scanning electron micrographs of whole valves of *Fragilaria asiatica* Hustedt from type material AS1395, Tibet. Figs 48, 49, 51,52. External views. Figs 50, 53–56. Internal views. Rimoportulae (arrows) can be present on each apex (Figs 49, 53, 56), or only present on one apex (Figs 48, 50–52, 54,55). Scale bars = 10 µm (Figs 48–51); = 20 µm (Figs 52–56).

In SEM (Figs 48–71) spines absent. Striae uniseriate composed of 4–5 areolae in the middle (Figs 58, 61) to 2–3 areolae near the apices (Figs 60, 68). Areolae appear round and irregular in size (0.15–0.23 µm in diameter, n=40). Externally, each areola is covered by a thin, flat siliceous closing plate that appears more or less sunken into the areolae (Figs 60, 68). In the majority of valves (8 out of the 11 valves observed) one rimoportula per valve (Figs 48, 50–52, 54, 55) but in some cases (3 out of 11) two rimoportulae are present (Figs 49, 53, 56). Rimoportulae, internally sessile with a narrow, slit-like opening, located transapically at the central axis of the valve (Figs 63, 65, 66), externally as a



FIGURES 57–71. Scanning electron micrographs of *Fragilaria asiatica* Hustedt. Figs 57–59. External view of a short valve with one rimoportula (arrow). Figs 60–62. External view of a short valve with two rimoportulae (arrows). Figs 63–64. Internal view of a short valve with one rimoportula. Figs 65–66. Internal view of a long valve with two rimoportulae. Figs 67–68. External view of apices of a long valve with one rimoportula (arrow). Fig. 69. Girdle view of apex of a whole cell showing the closed end of the valvocopula (arrow). Fig. 70. Opposite end of the same cell as in Fig. 69 with open end of the valvocopula (arrow). Fig. 71. Apex of broken cell showing detached valvocopula with open end (arrow). Scale bars = 2 µm (Figs 57–64, 66–68, 70, 71); = 1 µm (Figs 65, 69).

simple oval poroid (Figs 57, 60, 62, 67). A small pore field is found at each apex, externally it appears depressed below the surface of the valve (Figs 57, 59, 60, 62, 66, 69), internally the ocellulimbi are distinct with a fine grid pattern positioned at the valve terminus on the mantle (Figs 63–66). The cingulum is composed of a single open copula with a single row of poroids (Figs 69–71).

Results of statistical analyses

The boxplots for valve length, valve width and stria density indicate that the populations analyzed from three lakes of the Badain Jaran Desert (Aer Jilin, Shaobai Jilin and Wosigetu Nuoer), have very similar morphological characteristics (Fig. 72). We therefore combined the three populations from the Badain Jaran for comparison with the material from Blue Lake (USA) with *F. asiatica* and with the other similar taxa mentioned previously. The median values for valve length, valve width and stria density of the Badain Jaran combined population (n=90) are 61 µm, 2.5 µm and 17 stria per 10 µm, respectively. Our analysis of *Williamsella angusta* type material from Blue Lake (n=90 valves) allows us to extend the ranges of morphometric characters given by Graeff *et al*. (2013) for the original description of this taxon (Fig. 72), although we did not find a valve as short as the ones illustrated in that publication (their Figs 26 and 27). For this population, the median values for valve length, valve width and stria density were 71 µm, 2.5 µm and 18 stria per 10 µm, respectively. For the type material of *F. asiatica* from Tibet, the median values for valve length, valve width and stria density were 47 µm, 2.8 µm and 17 stria per 10 µm, respectively (n=90). Identical values were obtained from analysing the 11 images provided in Simonsen (1987) and in the online version of the Hustedt Diatom Collection (2017).

Histograms for the Blue Lake population and for the combined Badain Jaran populations (n=90 for both) show symmetric, unimodal distribution for valve length, width and stria density (Fig. 73). This suggests that our samples were representative of the populations investigated (Krammer 1997). For *F. asiatica* (n=90), we observed a bimodal distribution for valve length. The shorter valves (below 70 µm), with a modal length of 40–45 µm dominated and made up 81% of the total population, while the larger valves (above 70 µm) made up 19% of the total population with a modal length of 85–90 µm (Fig. 73).

The Kruskal-Wallis tests indicated that the three populations from the Badain Jaran Desert, from Blue Lake and Tibet had different median values for valve length, and stria density (see Table 2 for test statistics). The difference between the distributions of the three populations was confirmed by the Kolmogorov-Smirnov tests. The only case in which the null hypothesis (=lack of difference) could not be rejected was for valve width between the Badain Jaran and Blue Lake populations.

Boxplots for areola density (Fig. 74A), suggested a rather large variability for this character as indicated by the wide ranges observed within a single specimen (“Dugx”) or between two specimens from the same lake (“Shao-1” and “Shao-2”). The two specimens shown in Graeff *et al*. (2013) had the highest areola density among all the valves investigated, but their range overlapped with those observed in the Badain Jaran specimens (43.5–54 and 40–52 in 10 µm, respectively). By contrast, the valves of *F. asiatica* had a consistently lower areola density than that of the other two populations (Fig. 74A). Areolae had the same size, about 0.15 µm in diameter, in the Blue Lake and Badain Jaran populations but were consistently larger in *F. asiatica* with a mean diameter of 0.19 µm (Fig. 74B).

The bivariate analyses (Fig. 75) confirmed that the specimens from the Badain Jaran tended to have shorter valve and lower stria density than those from Blue Lake. *F. asiatica* specimens tended to have wider valves than the other taxa. The two specimens from British Columbia appeared more similar to those from the Badain Jaran than from Blue Lake while the three specimens from Florida were characterized by their narrow valves. The five specimens of *W. iraqiensis* were clearly separated from all others taxa by having a much higher stria density.

Ecology and associated diatom flora

In the Badain Jaran material, including preserved epiphyton samples and untreated material collected on glass fiber filters, no colonial formation was observed suggesting that cells were solitary. Living cells of this species (i.e. with plastids, Figs 1–5) were found in all epiphyton samples (n=5) collected from lakes Aer Jilin, Shaobai Jilin and Wosigetu Nuoer (Table 1). It was most abundant (9.3% of the assemblage) in an epiphyton sample collected on *Potamogeton* sp. in October 2009 in Lake Shaobai Jilin. Specimens were also found on *Chara* sp. (1.6% in an epiphyton sample from Lake Wosigetu Nuoer collected in October 2008). All Badain Jaran lakes in which this taxon was abundant are shallow (maximum water depth = 1.7 m).

In the surface sediments of Shaobai Jilin, Aer Jilin and Wosigetu Nuoer, *Fragilaria* sp. accounted for 7.9, 7.9 and 1.5 % of the assemblages, respectively. *Fragilaria* sp. was only a minor component (<1.0%) in nine other surface-sediment samples. The assemblages of the three lakes in which *Fragilaria* sp. was most abundant were broadly similar

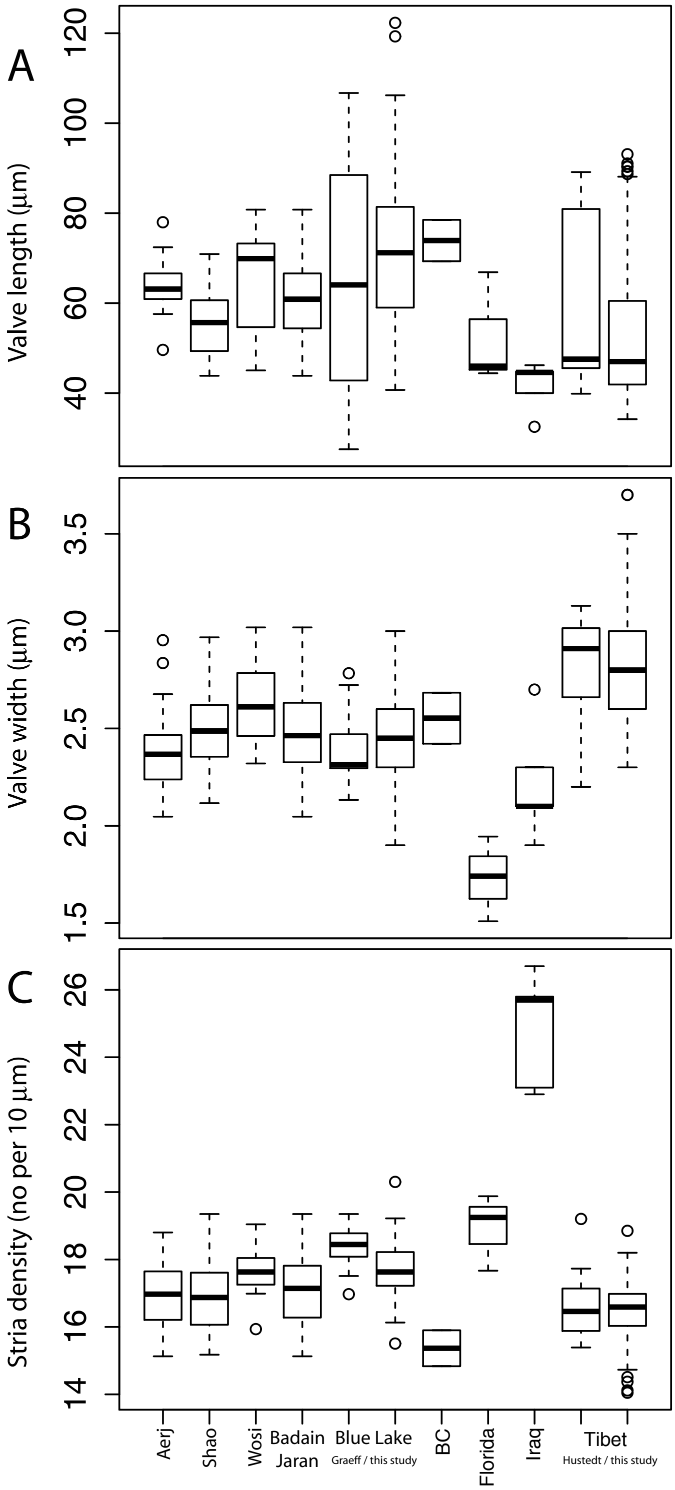


FIGURE 72. Morphometric analysis on LM micrographs of *Fragilaria crenophila* var. *sinensis* and comparison with type populations of *Williamsella angusta* and *Fragilaria asiatica.* A. Boxplots showing the quantile distributions of valve length data for three lakes of the Badain Jaran, for the combined data from these 3 populations. Plotted for comparison are the same data from the type population of *Williamsella angusta* from Blue Lake (USA), including digitized images from the original paper (Graeff *et al.* 2013) and new LM images taken from the type slide, data from similar diatom from British Columbia (BC, Canada) (Cumming *et al.* 1995) and Florida, USA (Lange-Bertalot 1993), data for *Williamsella iraqiensis* from southern Iraq (Al-Handal *et al.* 2016) and data for the type population of *Fragilaria asiatica* from Tibet, including digitized images from Simonsen (1987) and new LM images taken from type material. The 25–75 percent quartiles (excluding outliers) are drawn using a box. The median is shown with a horizontal line inside the box. The whiskers represent the upper and lower 'inner fence' that are drawn from the edge of the box up to the largest/lowest data point less than 1.5 times the box height. Outliers, i.e. values outside the inner fences, are shown as circles if they lie further from the edge of the box than three times the box height. B. Same as in A, but for valve width. C. Same as in A, but for stria density.

and dominated by the following taxa: *Encyonopsis krammeri* Reichardt (1997: 61), *Seminavis lata* (Krammer) Rioual (2014: 287), *Achnanthidium caledonicum* (Lange-Bertalot) Lange-Bertalot (1999: 277), *Brachysira* sp., *Denticula kuetzingii* Grunow (1862: 546), *Navicula wygashii* Lange-Bertalot (2001: 81), *Crenotia thermalis* (Rabenhorst) Wojtal (2013: 81), and *Amphora affinis* Kützing (1844: 107). The first six taxa listed were also the dominant taxa found in the epiphyton samples analyzed. This strongly suggests that the diatoms deposited on the surface sediments were mostly derived from the epiphytic communities with much lower contributions from the epipsammic, epipelic and planktonic communities.

On the type slide from Blue Lake, *W. angusta* was largely dominant in relative abundance (34%). The other abundant taxa (>5%) were *Tetramphora chilensis* (Hustedt) Stepanek & Kociolek (2016: 131), *Brachysira aponina* Kützing (1836: 3), *Denticula kuetzingii* var. *rumrichae* Krammer (1987: 66), *Encyonema evergladianum* Krammer (1997: 73) and *Navicula salinicola* Hustedt (1939: 638). Interestingly, the following taxa are present in the diatom assemblages from both Blue Lake and the Badain Jaran lakes, *Seminavis lata, Gomphonema lateripunctatum* Reichardt & Lange-Bertalot (1991: 530), *Cocconeis lineata* Ehrenberg (1849: 301), *Navicula veneta* Kützing (1844: 95), *Sellaphora stroemii* (Hustedt) H. Kobayasi in Mayama *et al*. (2002: 90), *Cyclotella meneghiniana* Kützing (1844: 50), *Diploneis interrupta* (Kützing) Cleve (1894: 84).

For *F. asiatica*, as indicated earlier, we do not know exactly what kind of sample was investigated by Hustedt (1922), although it was most likely of epiphytic origin. The assemblage in the type material was totally dominated by two species: *F. asiatica* (57%) and *Achnanthes hedinii* Hustedt (1922: 122)(40%), which is another taxon that Hustedt (1922) described as new in the same publication. Our SEM observations indicate however, that valves of *A. hedinii* fully matched with the type material of *Achnanthes thermalis* (Rabenhorst) Schönfeldt (1907) as illustrated in Krammer & Lange-Bertalot (1991). This taxon was also the type species of the genus *Crenotia* Wojtal (2013: 81). Interestingly, *Crenotia thermalis* (Rabenforst) Wojtal is considered as an indicator for mineral spring waters with high concentration of chlorides and sulphates (Wojtal 2013; Hofmann *et al*. 2011). The other taxa present in the sample represented about 3% of the assemblage, were *Encyonopsis behrei* (Foged) Krammer & Metzeltin (1997: 139), *Navicula salinarum* Grunow (1880: 33) and two species of *Denticula*.

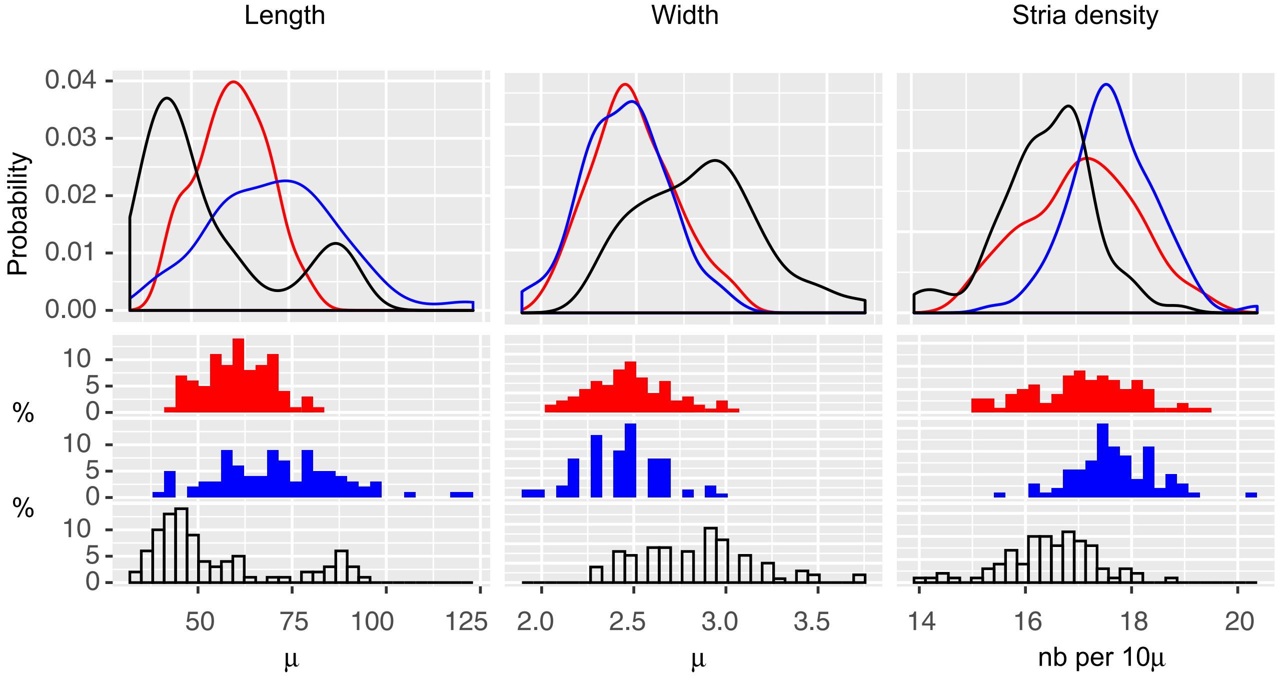


FIGURE 73. Morphometric analysis on LM images of *Fragilaria crenophila* var. *sinensis* from Badain Jaran lakes (combined population, n=90, in red) and comparison with the type populations of *Williamsella angusta* from Blue Lake, Utah, USA (n=90, in blue) and *Fragilaria asiatica* from Tibet (n=90, in black). Plot of probability density distribution with histograms of the valve length, valve width and stria density data.

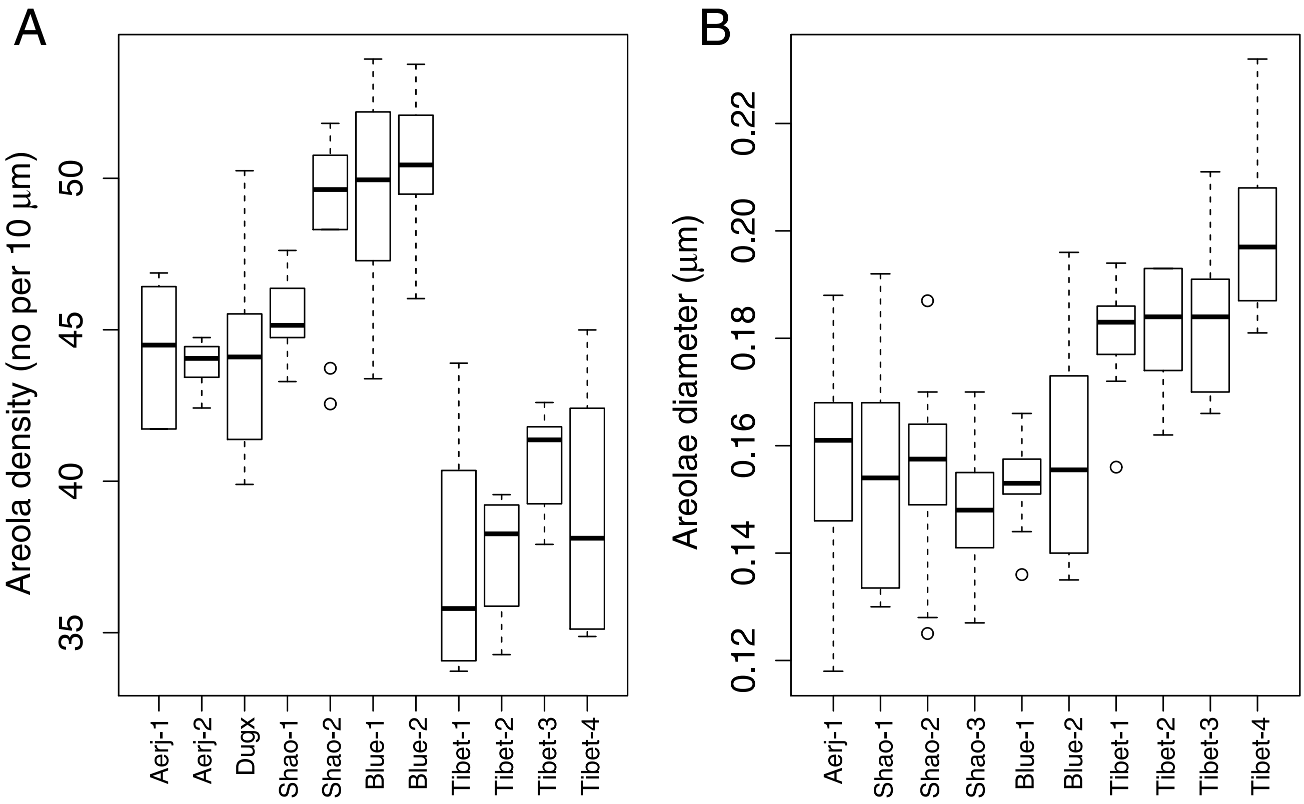


FIGURE 74. Morphometric analysis on SEM images of *Fragilaria crenophila* var. *sinensis*. A: areola density measured on valves from various Badain Jaran lakes and from the type populations of *Williamsella angusta* (measured from images in Graeff *et al.* 2013) and *Fragilaria asiatica* Hustedt (measured on SEM images taken from type material). B: Comparison of areolae diameter. See legend of Fig. 72 for explanation of the boxplots.

Discussion

Under LM the *Fragilaria* species found in the lakes of the Badain Jaran Desert appear very similar to *F. asiatica* as both have largely overlapping ranges in valves length, width and stria density. SEM observations however clearly show that the Badain Jaran taxon is not *F. asiatica* as it differs by their number of rimoportulae (*F. asiatica* can have two rimoportulae, one at each apex, which was never observed in the Badain Jaran taxon), the structure of the ocellulimbi (externally it appears small and depressed below the surface of the valve in *F. asiatica,* that is not the case for the Badain Jaran diatom), the number of valvocopula (only one for *F. asiatica*, several for the Badain Jaran taxon) and by the density and size of their areolae (Fig. 74).

The ongoing debate about the circumscription of genera within Fragilariaceae may cause problems in assigning some specimens to a particular genus. *F. asiatica* is a good example of this problem. *F. asiatica* can possess two rimoportulae, one on each apex, unlike most species included in *Fragilaria* that are characterized by having only one rimoportula per valve. In addition, the external appearance of its ocellulimbi is unlike what is found in most species of *Fragilaria* and resemble that of a species of *Ulnaria*, such as *Ulnaria toolensis* Graeff, Kociolek & Rushford (2013: 9) and the type of ocellulimbi observed in several species of the genus *Tabularia* (Williams & Round (1986: 320) such as *Tabularia waernii* Snoeijs (Snoeijs & Kuylenstierna 1991: 352, figs 26–27), *Tabularia cf laevis* Kützing (Snoeijs 1992, fig. 80), *Tabularia kobayasii* Hidek. Suzuki & Mitsuishi (2015: 89) and *Tabularia investiens* (W. Smith) D.M. Williams & Round (1986: 324)(Suzuki *et al.* 2015, figs 23, 36). *F. asiatica*, however, does not belong to the genus *Ulnaria* because it has an open valvocopula, while species of *Ulnaria* are characterized by closed valvocopulae as discussed by Williams (1986, 2011). Similarly, the presence of an open valvocopula excludes *F. asiatica* from belonging to the monospecific genus *Tibetiella* that closely resembles *Ulnaria* except for the presence of 2–5 rimoportulae on each apex (Li *et al.* 2010). *F. asiatica* does not belong to the genus *Tabularia* either as it does not have fine structure of the striae with various types of closing plates and/or cribra (Williams and Round 1986). With its open valvocopula and striae composed of simple rows of areolae *F. asiatica* is best maintained within the current concept of the genus *Fragilaria*. Moreover, the presence of two rimoportulae per valve, albeit unusual within the genus *Fragilaria*, has already been observed in *Fragilaria capucina* Desmazières (1830: 453)(Tuji & Williams 2006) and more recently in *Fragilaria spectra* Almeida, Morales & Wetzel (Almeida *et al*. 2016: 174) while the type of ocellulimbus observed in *F. asiatica* also occurs in *Fragilaria pennsylvanica* Morales (Morales 2003a: 163, fig 26).

By contrast with *F. asiatica*, the major qualitative morphological characters of the Badain Jaran populations such as overall shape, details of the apices, areolae structure and position and number of rimoportulae correspond to *Williamsella angusta,* as described in Graeff *et al*. (2013) from Blue Lake Warm Springs. However, two quantitative characters (valve length and stria density) show a consistent difference. This degree of difference and the geographical separation is sufficient to diagnose this taxon as separate from *W. angusta* and can best be considered as a variety, i.e. “a population forming a more or less distinct, local facies of a species” as defined by Geißler & Jahn (1986).

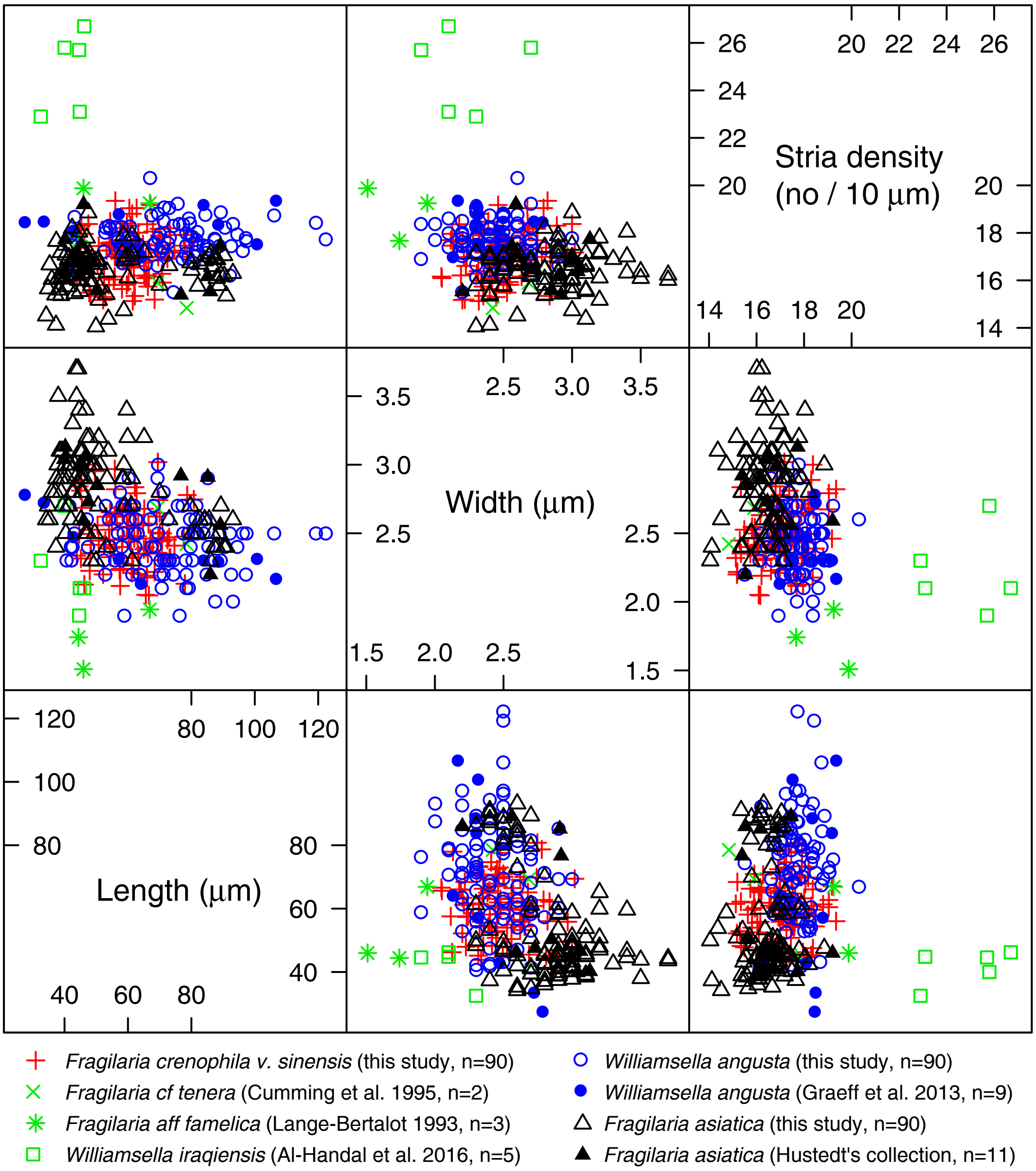


FIGURE 75. Matrix of biplots of morphometric characters comparing populations of *Fragilaria crenophila* var. *sinensis* from the Badain Jaran Desert (red plus signs), with valves of *Williamsella angusta* (blue filled circles for LM images published in Graeff *et al*. (2013), blue open circles for valves examined in this study) from Blue Lake (USA), of *Fragilaria asiatica* (black filled triangles for LM images of the Hustedt Diatom Collection, including Simonsen (1987), black open triangles for valves examined in this study), *Fragilaria* sp. *cf tenera* (green crosses) from subsaline lakes of British Columbia (Canada, Cummings *et al*. 1995), *Fragilaria* sp. *aff. famelica* (green stars) from Florida (Lange-Bertalot 1993) and *Williamsella iraqiensis* (green open squares) from Iraq (Al-Handal *et al*. 2016). Measurements are in µm for valve length and width. Stria density is in number of striae per 10 µm.

The specimens from Blue Lake Warm Springs were originally identified as *Synedra radians* Kützing by Kaczmarska & Rushforth (1983: 37, pl. 35, fig. 9, pl. 36, figs 3–5, after Patrick & Reimer 1966: 137). Graeff *et al.* (2013: 9) convincingly argued that this was a misidentification as *Synedra radians,*which has a distinct central area according to Patrick & Reimer (1966: pl. 5, fig. 4) and proceeded to describe the Blue Lake specimens as a new species. They went on the note that "…specimens of *W. angusta* [their new species] possess a rimoportula at only one apex, excluding it from genus *Synedra*[1] Ehrenberg (1830: 60) which has a rimoportula at each apex (Round *et al.* 1990: 346, 370)" (Graeff *et al*. 2013: 9). They continued by pointing out that their specimens could not be assigned to *Fragilaria* Lyngbye either because that genus "should contain only taxa that occur in colonies… [and] that *Fragilaria* should remain a genus of freshwater species" (Graeff *et al*. 2013: 9). Their key characteristic, however, is that "The external appearance of the areolae, with the externally-placed coverings, sets this new genus apart from other known araphid genera", and the fact that their specimens occur in saline waters, suggested that these specimens should be place in a new taxon, the new genus *Williamsella* was erected for them. In the following, we discuss the points made by Graeff *et al.* (2013) to support *Williamsella* and distinguish it from the related genera *Ulnaria* (=*Synedra*), *Tibetiella* and *Fragilaria*. We then argue that the establishment of this new genus is not justified.

Williamsella versus Ulnaria (and Tibetiella)

According to Graeff *et al*. (2013), the fact that *W. angusta* has a rimoportula at only one apex precludes its inclusion in the genus *Ulnaria*. In our opinion, the number of rimoportula is not the best criterion to decide to which genus, *Ulnaria* or *Fragilaria*, a species should be attributed. This is because there are several species of *Ulnaria* that possess only one rimoportula as shown for *Ulnaria colcae* Van de Vijver & Cocquyt (2009: 212), *Ulnaria titicacaensis* E. Morales, Ector & P.B. Hamilton (2014: 43) and *Ulnaria macilenta* E. Morales, C.E. Wetzel & S.F. Rivera (2014: 45)(Morales *et al*. 2014) while on the contrary, some species of *Fragilaria* possess two rimoportulae, one at each apex. More importantly in our opinion, *W. angusta* does not belong to the genus *Ulnaria* because it has open girdle bands while species of *Ulnaria* are characterized by having only closed girdle bands. This character represents a synapomorphy for the genus *Ulnaria* as put forward by Williams (1986, 2011). In recent years, the possession of a closed valvocopula has been used by many authors as a justification for placing new species in the genus *Ulnaria* (Van de Vijver & Coquyt 2009; Kulikovskiy *et al*. 2016; Liu *et al*. 2017). Morales *et al*. (2014) had argued that it is "difficult to accept closed girdle bands as appropriate evidence" because the type species of the genus *Ulnaria*, *Synedra ulna* (Nitzsch) Ehrenberg had not been studied in detail under the SEM. However, soon after Morales *et al*. (2014) published their study, Lange-Bertalot & Ulrich (2014) proposed an epitype for *Ulnaria ulna* (Nitzsch) Compère (2010: 100) to solve the problem since the original material from Nitzsch may never be found. This epitype of *U. ulna* has closed girdle bands (Lange-Bertalot & Ulrich 2014, Pl. 29, Fig. 3).

Similarly, the presence of open girdle bands in *W. angusta* excludes it from belonging to the monospecific genus *Tibetiella*, that closely resembles *Ulnaria* except for the presence of 2–5 rimoportulae on each apex (Li *et al*. 2010).

Williamsella versus Fragilaria

Since both the genera *Williamsella* and *Fragilaria* have open girdle bands and a rimoportula at only one apex (which is not always the case for species included in *Fragilaria*, see below), Graeff *et al*. (2013) used other characteristics and characters to distinguish them.

i) The absence of spines and colonial formation:

Not all *Fragilaria* species form colonies and bear spines; there are numerous examples such as *Fragilaria pennsylvanica* Morales (Morales 2003a), *F. pectinalis* (O.F. Müller) Lyngbye (1819: 185) as illustrated in Wetzel & Ector (2015), *F. candidagilae* Almeida, C. Delgado, Novais & S. Blanco (in Delgado *et al*. 2015: 4), *F. boreomongolica* Kulikovskiy, Lange-Bertalot, Witkowski & Dorofeyuk (Kulikovskiy *et al*. 2010: 36). In their comprehensive revision of the needle-shaped group of fragilarioid diatoms, Lange-Bertalot & Ulrich (2014) also reported that several of the long, needle-like *Fragilaria* are spineless and have a solitary life-form including *F. perdelicatissima* Lange-Bertalot & Van de Vijver in Lange-Bertalot & Ulrich (2014: 19), *F. saxoplanctonica* Lange-Bertalot & Ulrich (2014: 30), *Fragilaria aquaplus* Lange-Bertalot & Ulrich (2014: 32) and *Fragilaria gracilis* Østrup (1910: 190).

More generally, within the Fragilariaceae the presence or absence of spines is not a stable character at the genus level. For example, in *Stauroforma* Flower, Jones & Round (1996: 53), the spinose species *Stauroforma exiguiformis*

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[1] Here the nomenclature is complicated as species in the genus *Synedra*, as understood by Round *et al.* (1990) are now placed in *Ulnaria*. This is a nomenclatural act not a taxonomic one (Williams 2011).

Flower, Jones & Round (1996: 53) can co-occur with the non-spinose species *Stauroforma inermis* Flower, Jones & Round (1996: 54)(Flower *et al.* 1996). Species within *Pseudostaurosira* D.M. Williams & Round (1988: 276) generally possess spines but exceptions exist such as *Pseudostaurosira parasitica* (W. Smith) Morales (2003b: 287). Another example was given by Flower (2005) who distinguished *Pseudostaurosira microstriata* var. *spinosa* Flower (2005: 65) from the non-spinose nominal variety, *Pseudostaurosira microstriata* (Marciniak) Flower (2005: 65). Conversely, while species in *Ulnaria* are typically spineless, *Ulnaria ungeriana* (A. Grunow) Compère (2001: 100) have well-developed linking spines (Williams 1986, Siver *et al.* 2006). Even at the species level, the presence or absence of spines is not always a good criterion for taxonomic separation. For example, *Staurosira dimorpha* Morales (2010: 103) can form two sympatric variants, one with spines the other spineless. Interestingly, for this latter species the lack of spines does not prevent formation of colonies (Morales *et al.* 2010).

ii) A preference for saline versus freshwater

To argue against the inclusion of their new species in the genus *Fragilaria,* Graeff *et al.* (2013) referred to Round *et al.* (1990) who stated that *Fragilaria* species are restricted to freshwater. This statement however, is not entirely correct as *Fragilaria famelica* (Kützing) Lange-Bertalot (1980: 749) is commonly reported from marine coasts (Witkowski *et al.* 2000) and saline springs (Wojtal 2013). Graeff *et al.* (2013) described *Williamsella angusta* from the main basin and the surrounding marsh of Blue Lake, Utah. There, the salinity varies from 5.5 ppt in the lake to 13.4 ppt in the marshes (see Table 1 in Graeff *et al.* 2013). From these values, Graeff *et al.* (2013) deduced that their new species was restricted to saline waters. For the taxon we found in the Badain Jaran lakes however, our data (Fig. 76) suggest that it optimally grows in subsaline conditions, i.e. in a range of salinity of 0.5–3 ppt following the classification proposed by Hammer *et al.* (1983). The three lakes in which this fragilarioid diatom is most abundant have a salinity ranging between 1.3 and 2.0 ppt, although it was also found (0.2%) in one lake with much higher salinity (30.3 ppt).

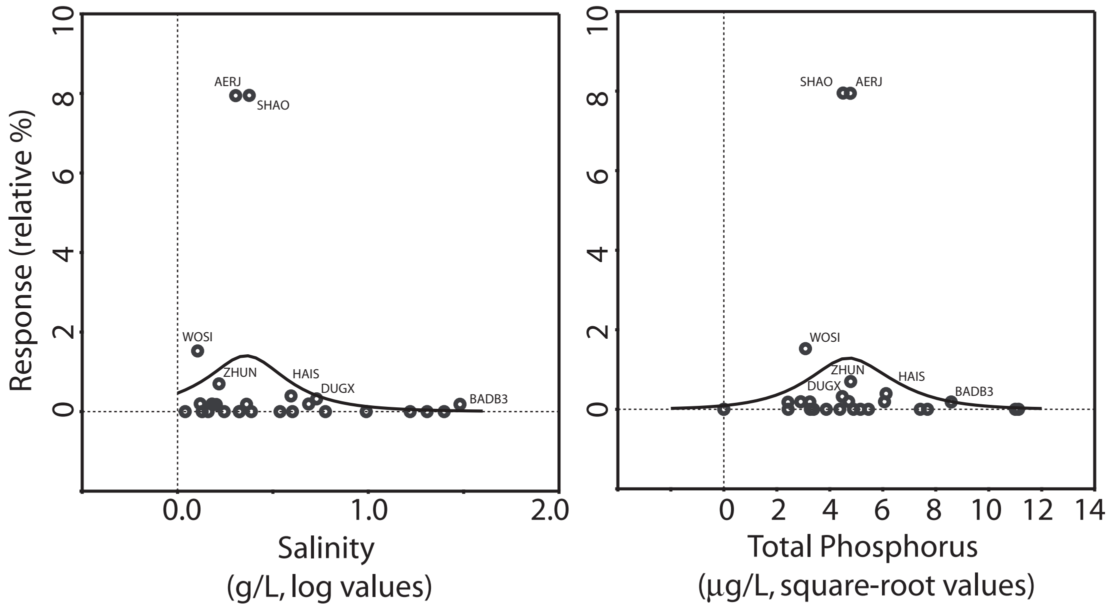

FIGURE 76. Distribution of *Fragilaria crenophila* var. *sinensis* in the 26 surface sediment samples included in the training-set and fitted generalized additive models of its responses along the salinity and total phosphorus gradients.

iii) The external coverings of the areolae

In their description of *Fragilaria*, Round *et al.* (1990) wrote that the areolae of this genus have delicate, often disc-like, cribra (as illustrated in their fig. g, p. 347). A structure very similar to the one observed in *W. angusta* exists in *Fragilaria longifusiformis* P. Siver, E. Morales, B. Van de Vijver, M. Smits, P.B. Hamilton & H. Lange-Bertalot (2006: 185), a species described from the south-eastern USA (Hains & Sebring, 1981) but also reported in Europe (Siver *et al.* 2006) and in Brazil (Ludwig *et al.* 2015). Siver *et al.* (2006) went on to argue that the presence of these areolae coverings they called disc-like vola, was one of the important characters for attributing *F. longifusiformis* to the genus *Fragilaria* (instead of *Synedra,* i.e. *Ulnaria*). Recently, Lange-Bertalot and Ulrich (2014) distinguished the subspecies *F. longifusiformis* ssp. *eurofusiformis* Lange-Bertalot and Ulrich (2014: 37). SEM images of this subspecies indicate that external coverings of the areolae can also be present in this taxon (plate 18, fig. 3) but are often dissolved (plate

18, figs 6–8). External coverings of the areolae can be observed in other needle-like *Fragilaria* species such as *F. tenera* (W.Smith) Lange-Bertalot (1980: 746) as illustrated in Lange-Bertalot and Ulrich (2014, plate 3, figs 3–6) and in Almeida *et al*. (2016, figs 15–17), *F. neotropica* P.D. Almeida, E. Morales & C.E. Wetzel (2016: 171)(figs 43, 47, 49 in Almeida *et al*. 2016). Note that Almeida *et al*. (2016) used the term rotae when referring to these structures.

For *Fragilaria* species of smaller size, disc-like volae are clearly visible in SEM images of *F. neointermedia* Tuji & D.M. Williams (2013: 7, fig. 37), *F. capitellata* (Grunow) J.B. Petersen (1946: 54) as illustrated in Tuji & Williams (2008, figs 47–49). Although they did not discuss the significance of these structures in their study, Wetzel and Ector (2015) also published SEM images that show external coverings of the areolae for *F. vaucheriae* (Kütz.) J.B. Petersen (1938: 167)*, F. uliginosa* Kulikovskiy, Lange-Bertalot, Witkowski & Dorofeyuk (2010: 37)*, F. microvaucheriae* Wetzel & Ector (2015: 282) and *F. pectinalis*, the later being the type species for the genus *Fragilaria*. Delgado *et al*. (2015) refers to rounded floating discs covering the areolae openings for *F. candidagilae*.

Interestingly, SEM illustrations of *W. iraqiensis*, the other species of *Williamsella* recently described from a spring-fed saline lake in Southern Iraq (Al-Handal *et al*. 2016), also suggest that these external coverings are easily dissolved. They are missing from all the valves they illustrated except for one, where they occur on the apex (their fig.13).

In summary, it would appear that the disc-like external coverings of the areolae, usually referred as volae or rotae, seem to be a common feature of *Fragilaria* species. It is likely that difficulty concerning assessing the presence or absence of volae or rotae in some species is due to dissolution either in the field or as an artifact of the cleaning method as suggested by our observations (contrast Figs 42–44 taken from cleaned material derived from surface sediment samples with Figs 45–47 taken from untreated material observed on the glass fiber filters used for the chemical analyses of water samples). Molecular studies on Fragilariaceae also suggest that the various types of covering over the areolae do not correspond with monophyletic clades (Medlin *et al*., 2008, 2012).

In addition to the three characters discussed above, Al-Handal *et al*. (2016, see their Table 1) listed the presence of four plate-like plastids in *Williamsella* as another feature that differentiate this genus from the two plate-like plastids generally reported for the other Fragilarioid genera such as *Ulnaria* and *Fragilaria.* We found the same number and distribution of plastids in the Badain Jaran population (see Figs 1–5) as the one shown by Al-Handal *et al*. (2016, Fig 7) for *W. iraqiensis* and this suggests that the two taxa are closely related. It is, however, not strictly correct that all *Fragilaria* species have two plate-like plastids as other configurations have been reported like in *Fragilaria longifusiformis* ssp. *eurofusiformis* Lange-Berlatot & Ulrich, which has undivided chloroplasts (Lange-Bertalot & Ulrich 2014, plate 17, figs 5–7) and in *F. spectra,* which has numerous and elongate plastids (Almeida *et al*. 2016, figs 54, 55). In addition, the number and position of the plastids has not been reported for a majority of taxa currently considered to belong to *Fragilaria*. Therefore, while this character may be useful for the purpose of distinguishing species, it may not be appropriate at the generic level, at least until a more systematic study of the issue is carried out.

From the above, we conclude that erecting the genus *Williamsella* on the basis of the three characters presented in Graeff *et al*. (2013), i.e. colonial formation, habitat and areolae coverings and the additional character of the number of plastids (Al-Handal *et al*. 2016), cannot be justified using these criteria. In addition, the structure of the cingulum (composed of several open copulae with a single row of areolae present along the advalvar edge) conforms to the description given for *Fragilaria* in Round *et al*. (1990).

One option is to emend the definition of both *Fragilaria* and *Williamsella* another is that *W. angusta* and *W. iraqiensis* should be re-assigned to the genus *Fragilaria* as it is currently defined. Based on the above, we propose the second option:

Fragilaria iraqiensis (Al-Handal & Kociolek) Rioual comb. nov.

Basionym: *Williamsella iraqiensis* Al-Handal & Kociolek (2016: 291, figs 2–21).

Fragilaria crenophila (Graeff, Kociolek & S.R. Rushforth) Rioual comb. et nom. nov.

Basionym: *Williamsella angusta* Graeff, Kociolek & S.R. Rushforth (2013: 7, figs 19–37) non *Fragilaria angusta* Ehrenberg (1838: 205, Taf. 15, fig. XVI, 1, 2)

It is noteworthy to add that in 1838, Ehrenberg described and illustrated *Fragilaria angusta* (Ehrenberg 1838: 205, Taf. 15, fig. XVI), a species named a few years earlier, but not described, for specimens from Siberia (Ehrenberg 1832: 62, 68, "Saratof an der Volga", 70, "Tobolsk im Nordwestlichen Sibirien am Irtysch und Tobol"). While the catalogue compiled by Clara Ehrenberg, his daughter, included reference to the original drawings of *Fragilaria angusta* and it is possible to examine these, no reference was made to the source of the specimens. Even though it is virtually impossible to accurately determine what *Fragilaria angusta* Ehrenberg might be from either the drawings or the published images, Ehrenberg's name is still validly published. Thus, for *Williamsella* to be transferred to the genus *Fragilaria* it needs a new name, in this case, *Fragilaria crenophila.*

Fragilaria crenophila **var. *sinensis*** Rioual, var. nov.

Valve solitary, fusiform becoming linear in elongated valves, with apices slightly inflated and rounded. Length 43–81 µm, breadth 2–3 µm. Central sternum very narrow, no central area is formed. Striae opposite, stria density 15–19.5 in 10 µm.

Type:–CHINA. Inner Mongolia, Shaobai Jilin, 39° 34'04 N 102° 14'78 E, 1204 m a.s.l, surface sediment sample collected by P. Rioual in June 2007 (holotype IGGDC!, designated here, individual from slide SHAO-ss07, illustrated in Fig. 12, located using England Finder K35/4; Isotype BM!, slide 101/801, designated here, Natural History Museum, London, United Kingdom).

Etymology:—The species epithet refers to springs: *crenophile* is an organism preferring spring environment; the variety epithet refers to China.

Habitat and distributions in Asia and North America

Although the analysis of epiphyton samples suggests that *F. crenophila* var. *sinensis* is epiphytic, we cannot exclude that it can survive in the plankton as it was also present in the water samples collected from these lakes as shown by the presence of numerous whole cells (Figs 45, 46) found on the surface of the filters observed under the SEM. It is also unclear what is the habitat (planktonic or epiphytic) of the nominate variety of *F. crenophila* (= *W. angusta*) as Graeff *et al.* (2013) only mentioned that this species was found in the main basin of Blue Lake (max. depth = 18 m) and in the surrounding marshes. The response curves for *F. crenophila* var. *sinensis* along the salinity and TP gradients in lakes of the Badain Jaran Desert are shown in Fig. 76. The highest abundances of *F. crenophila* var. *sinensis* were observed in subsaline and mesotrophic lakes.

In North America, together with the occurrence of *F. crenophila* in Blue Lake, Utah, a very similar taxon was identified as *Fragilaria* cf. *tenera* by Cumming *et al.* (1995: 89, pl. 8, figs 8–9) in subsaline lakes of British Columbia (Canada). In Lange-Bertalot (1993) valves identified as *Fragilaria* aff. *famelica* (Kützing) Lange-Bertalot (pl.12, figs 12–14), from the Nine Mile pond in the Everglades (Florida, USA), may also belong to this taxon, especially as it was found in association with *Fragilaria synegrotesca* Lange-Bertalot (1993: 49), a taxon also found in Blue Lake. Graeff *et al.* (2013) seem to have overlooked the existence of *F. synegrotesca* however, as they described the Blue Lake populations as a new species *U. toolensis* instead of a simple re-combination within the genus *Ulnaria*. The Everglades specimens, however, have narrower valves (1.5–1.9 µm) than *F. crenophila* or *F. crenophila* var. *sinensis* and may represent yet another species or variety. Slate & Stevenson (2007) found the same taxon (see their figs 25, 78) in their study on the diatom flora of the Everglades (although they called it *Fragilaria* cf. *tenera* instead of *Fragilaria* aff. *famelica*) where it was especially common in un-enriched conditions with an estimated TP optimum of 14 µm ±13 $\mu g.L^{-1}$. Their results were in agreement with those of Lange-Bertalot (1993) who reported this taxon in oligotrophic to mesotrophic waters not only from Florida but also from Jamaica, Mexico and Central America. Unfortunately, salinity was not mentioned in these two studies.

The geographic distribution of *Fragilaria crenophila* and its variety *sinensis* could therefore indicate a widespread or disjunct species dispersal pattern that includes North America and Eastern Asia. Similar trans-Pacific distribution patterns have been reported for gomphonemoid taxa (Kociolek *et al.* 2013, You *et al.* 2013), for the araphid genus *Tetracyclus* (Williams 1996; Williams and Reid 2006; Williams 2009) and for a few other taxa belonging to the genera *Cymbella*, *Frustulia*, *Navicula*, *Neidium*, and *Synedra* (Potapova 2014).

Conclusion

We found that the morphological characteristics investigated in this study do not form a sound basis for erecting a new genus to accommodate several taxa previously associated with the genus *Fragilaria* as currently defined (Round *et al.* 1990). As long argued by Williams (1986), questions remain as to the validity of the genus *Fragilaria* as it is not yet characterized by any synapomorphy, and it is thought not unlikely that further systematic revision of *Fragilaria* will be undertaken. In any such revision however the role of ecological preferences as opposed to morphological characters needs to be carefully considered. Graeff *et al*. (2013) justified the creation of the genus *Williamsella* at least partly according to the ecology of *W. angusta* (its occurrence in a saline lake and its non-colonial mode of growth). Round & Maidana (2001) similarly erected the araphid genera *Belonastrum* and *Synedrella* primarily using ecological habitats and colonial formation of the planktonic species *Synedra berolinensis* Lemmermann (1900: 31) and the epiphytic species *Synedra parasitica* (W. Smith) Hustedt (1930: 161), respectively. However, Morales (2003b) refuted the creation of these genera and demonstrated using detailed SEM analysis that *S. berolinensis* and *S. parasitica* could be allocated to existing genera, as *Staurosirella berolinensis* (Lemmermann) Bukhtiyarova (1995: 418) and *Pseudostaurosira parasitica* (W. Smith) Morales (2003b: 287), respectively. Morales (2003b) concluded that the practice of deciding the taxonomic status of a species based on an ecological perspective needs to be done with caution. While our investigation on *Williamsella* leads us to support this recommendation, ecology is nevertheless a useful criterion for supporting taxonomic decisions since it is underpinned by physiological and biochemical processes

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