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Morphology and ecology of *Fragilaria misarelensis sp. nov.* (Bacillariophyta), a new diatom species from southwest of Europe

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ABSTRACT
A new freshwater diatom species was described from southwest Europe (Portugal), based on morphological and ultrastructural characteristics. *Fragilaria misarelensis* Almeida, C.Delgado, Novais & S.Blanco is characterised by lanceolate valves, with slightly capitate ends, alternate striae and lacking spines joining adjacent cells, which are solitary. The valve shape of *Fragilaria misarelensis*, when compared to similar forms, is unique, as revealed by a thorough literature survey of *Fragilaria* species, geometric morphometry and discriminant analysis. *Fragilaria misarelensis sp. nov*. was frequent and abundant in central Portugal, in poorly mineralized watercourses under low anthropogenic influence, characterised by neutral pH, low conductivity and high dissolved oxygen concentration. The analysis of type material belonging to similar species, *Fragilaria capucina* fo. *sublanceolata-baikali, F. capucina* fo. *lanceolata-baikali* and *F. nevadensis*, contributed to improving their characterisation and illustration, also supporting the delimitation of the new Portuguese species. *Fragilaria capucina* fo. *sublanceolata-baikali* and *F. capucina* fo. *lanceolata-baikali* were considered to differ substantially from *F. capucina* and raised to species level as *Fragilaria sublanceolata-baikali* (Flower & D.M.Williams) Novais, C. Delgado & S.Blanco, *comb. et stat. nov*. and *Fragilaria lanceolata-baikali* (Flower & D.M.Williams) Novais, C. Delgado & S.Blanco, *comb. et stat. nov.*

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KEYWORDS
Diatoms; Freshwater; Geometric morphometry; Taxonomy; Type material

INTRODUCTION

The genus *Fragilaria* Lyngbye has been a primary target of diatom studies during the last decades (e.g. Tuji 2004; Tuji & Williams 2006a, 2006b, 2008, 2013; Williams & Round 1987). The *Fragilaria capucina/vaucheriae* species complex *sensu* Lange-Bertalot in Krammer & Lange-Bertalot (1991) is one of the most frequent and abundant, and widely illustrated in the literature. It includes several recently described new species (e.g. Delgado *et al*. 2015, 2016; Linares-Cuesta & Sánchez-Castillo 2007; Van de Vijver *et al*. 2012; Wetzel & Ector 2015). However, the diagnostic features allowing the discrimination of *Fragilaria* Lyngbye are difficult to solve. Ribbon-like colony formation due to interlocking spines, present on the surface, was usually attributed to *Fragilaria* Lyngbye; whereas, *Synedra* Ehrenberg was attached to the surface of rocks or plants by means of mucilage pads or aggregated into radiate colonies not forming long, linear filamentous structures (Patrick & Reimer 1966; Poulin *et al*. 1986; Round *et al*. 1990; Williams 1986). With the development and progress of microscopy techniques, researchers gradually updated previous morphological observations and definitions, expanding our knowledge by detailed microstructural observations. Though colony formation in itself was not considered by Williams & Round (1987) sufficient to justify the separation between *Fragilaria* and *Synedra* (this feature is difficult to observe on treated material and on isolated valves), their investigations reveal differences in other characters to support their subdivision. In their revision of the genus, species of *Fragilaria* Lyngbye form linear colonies, with frustules attached by adjacent interdigitating spines, but the genus definition was restricted to those species with simple striae, a few open girdle bands and a simple type of ocellulimbus at each pole. Later, the scanning electron microscopy (SEM) features that characterise the genus were compiled by Morales (2001), which consist of mostly uniseriate striae, composed of round or slightly elongated areolae, discoid closing plates, a well-developed central sternum, apical pore fields of a weakly developed ocellulimbus type, and the presence of rimoportulae and spines (of variable shape and located on or between the striae). *Synedra* Ehrenberg was characterised by Williams (1986) by having closed bands in the cingulum, a basal siliceous layer, modification of the ocellulimbus, with a denser inset plate of 12–14 porelli per row and 20–25 rows per plate, biseriate striae, and ornamentation of the pars exterior on all band components. The recent view of Williams (2011) simplifies the *Fragilaria/Synedra* issue, based on open girdle

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bands in *Fragilaria* and closed girdle bands in *Ulnaria*, one of the defining features of the *Synedra ulna* (Nitzsch) Ehrenberg group and hypothesised as a synapomorphy for this group (Williams 2011). This author, based on nomenclatural reasons, suggested the use of the generic name *Ulnaria* for the group previously typified by the freshwater species *Synedra ulna*, and the use of *Synedra* for the group of diatoms typified by the marine species *S. gaillonii* (Bory) Ehrenberg.

A literature survey of diatom studies in Portugal (Novais *et al.* 2015) revealed that more than 40 taxa belonging to the genus *Fragilaria* have been recorded (Table S1). *Fragilaria rhabdosoma* Ehrenberg 1832 was the first *Fragilaria* species cited in the earliest study of diatoms from Portugal (Ehrenberg 1845). *Fragilaria capucina* Desmazières 1825 (Colmeiro 1889), *F. danica* (Kützing) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996 (Colmeiro 1889), *F. mutabilis* (W.Smith) Grunow 1862 (Trelease 1897) and *F. radians* (Kützing) D.M.Williams & Round 1987 (Trelease 1897) were also recorded in the first diatom studies carried out in the country during the 19th century. Amongst these, we find *Fragilaria capucina*, which continues to be recorded and is one of the most commonly cited and widespread *Fragilaria* species in the country (Table S1). *Fragilaria vaucheriae* (Kützing) J.B.Petersen 1938 is also widely distributed in Portugal and cited in several studies (Table S1). Given the importance of the species complex around *Fragilaria capucina/vaucheriae* in the country, the present work was carried out in order to thoroughly study a *Fragilaria* species belonging to this complex, present in several samples and reaching considerable abundances. An extensive literature survey on *Fragilaria* species was carried out, allowing a thorough comparison of the Portuguese species morphological with comparable taxa. In this study, specimens from the type materials of the similar *Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M.Williams 2004, *Fragilaria capucina* fo. *lanceolata-baikali* Flower & D.M.Williams 2004 and *Fragilaria nevadensis* Linares-Cuesta & Sanchez-Castillo 2007, which are poorly illustrated in the literature, were also examined.

MATERIAL AND METHODS

Portuguese samples

A total of 53 samples, collected in 28 watercourses from three adjacent river catchments (total area of 11,215 km^2) in central Portugal (Mondego, Vouga, and Lis river basins; Fig. 1) were analysed in this study. Epilithic diatoms were sampled in spring, autumn and winter in 2007, 2011 and 2012, in streams and rivers, following standard methodology (Association Française de Normalisation 2003; European Committee for Standardization 2003; Inag 2008) and preserved with formaldehyde solution (4% v/v) immediately after sampling. Details of the samples thoroughly analysed are listed in Table S2.

At each sampling site, current velocity was measured (m s^{-1}), water samples for chemical analysis were collected from lotic zones, and environmental variables such as water temperature (°C), pH, dissolved oxygen (mg l^{-1}), oxygen saturation (% O_2) and conductivity (µS cm^{-1}) were determined *in situ* with portable metres calibrated in the field. Temperature and oxygen were measured with a WTW Oxi 197 oxymeter, conductivity with an Orion Model 115 at 25°C, and pH with a Thermo Orion 290+. Water samples were collected in polypropylene bottles, stored at 4 °C in the darkness and transported to the laboratory for analysis. The parameters alkalinity (mg $CaCO_3$ l^{-1}), total hardness (mg $CaCO_3$ l^{-1}), chlorides (mg Cl^- l^{-1}), ammonium (µg N-NH_4^+ l^{-1}), nitrates (µg N-NO_3^- l^{-1}), nitrites (µg N-NO_2^- l^{-1}), phosphates (µg P-PO_4^{3-} l^{-1}), total phosphorus (µg P l^{-1}), sulphates (mg SO_4^{2-} l^{-1}), chemical oxygen demand (mg l^{-1}), silica (mg SiO_2 l^{-1}) and total dissolved solids (mg l^{-1}) were analysed according to standard methods (American Public Health Association 1995).

An aliquot of each Portuguese and type material samples (*ca.* 3 ml) was oxidised with 4–6 ml of nitric acid (65% v/v) and potassium dichromate ($K_2Cr_2O_7$) at room temperature for 24 hours. Afterwards, samples were repeatedly centrifuged (1500 rpm) and rinsed with distilled water at least three times to remove oxidation by-products. Permanent slides were mounted with Naphrax® (Brunel Microscopes Ltd, Chippenham, UK) for light microscopy (LM) analysis. For SEM analysis, metal stubs were prepared with organic free samples air-dried on a thin pellicle of graphite (EMITECH K 950X) and coated with gold–palladium (Polaron equipment limited SEM coating unit E5000).

The distribution map showing the Portuguese species (Fig. 1) was created using Quantum GIS 1.8.0 (QGIS Development Team 2018).

Type material analysis

Type material of *Fragilaria capucina* fo. *sublanceolata-baikali, F. capucina* fo. *lanceolata-baikali* and *F. nevadensis* was observed and analysed (Table S3).

The type materials of *Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M.Williams (slide BM 100321, illustrated in Fig. 2) and *F. capucina* fo. *lanceolata-baikali* Flower & D.M. Williams (slide BM 100321, Fig. 2) from Lake Baikal, station number 3.10 (BK 8), coll number 3.1 (26 June 1997), are housed in the Natural History Museum of London (BM). *Fragilaria nevadensis* Linares-Cuesta & Sánchez-Castillo (slide 3013) is housed in the Diatom Phycoteca of Granada (Spain), University Herbarium (GDA; Table S3).

The permanent slide loaned by the Natural History Museum of London (BM 100321) was used for the LM analysis of the type material of *Fragilaria capucina* fo. *sublanceolata-baikali* and *F. capucina* fo. *lanceolata-baikali*. An aliquot with the type material of *Fragilaria nevadensis* was oxidised, and a permanent slide (for LM observations) and a metal stub (for SEM analysis) were prepared following the same procedure as for the Portuguese samples.

Microscopy observations (LM and SEM)

LM observations of both the Portuguese samples and type material were carried out using a Leitz® Biomed 20 EB bright-field microscope equipped with a 100× oil immersion objective (NA 1.32). Light micrographs were obtained using an Olympus DP70 camera attached to a Zeiss® Axioplan 2 imaging microscope with differential interference contrast and the same magnification (NA 1.40). Morphometric measurements (length, width and number of striae, counted along the axial area) were registered for each species, using the micrographs. A Hitachi SU-70 scanning

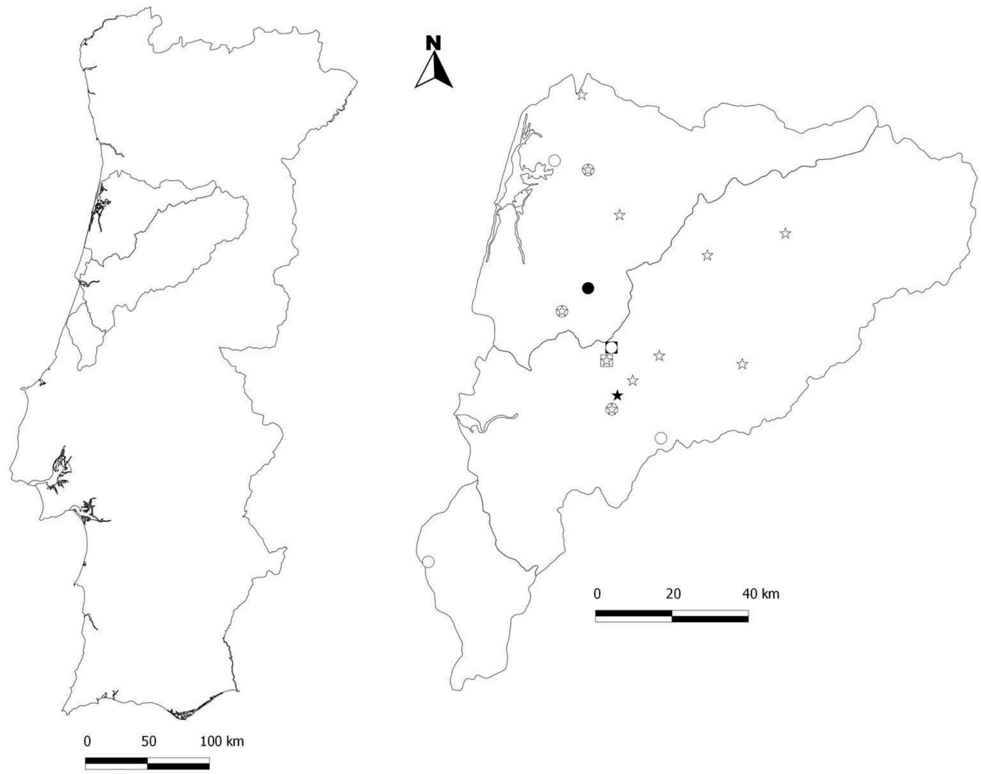


Fig. 1. Distribution map with sampling sites where the Portuguese *Fragilaria* species were identified in central Portugal. Star: *Fragilaria misarelensis sp. nov.*; square: *F. candidagilae*; circle: *F. rinoi*. Black represents type localities.

electron microscope operated at 7 kV and 10-mm working distance was used for image acquisition of the Portuguese samples and the type material of *Fragilaria nevadensis*. Ultrastructural analysis of areolae types, number and placement of rimoportulae, girdle bands, volae and apical pore fields were observed in SEM micrographs. Micrographs were digitally manipulated and plates containing LM and SEM pictures were mounted using Corel Draw X5® (Corel, Mountain View, CA, USA).

Comparison with morphologically similar species

In addition to the type material analysis, a thorough literature survey was carried out, allowing a detailed comparison of the Portuguese species with similar *Fragilaria* species commonly found in freshwater or recently described from European watercourses.

Information about *Fragilaria recapitellata* Lange-Bertalot & Metzeltin and *F. perminuta* (Grunow) Lange-Bertalot was retrieved from Tuji & Williams (2008) and Delgado *et al.* (2015); for *F. pectinalis* (O.F.Müller) Lyngbye we used the manuscript by Tuji & Williams (2008); for *F. microvaucheriae* Wetzel & Ector, information was obtained from Wetzel & Ector (2015); for *F. rhabdosoma* Ehrenberg from Tuji (2004); for *F. capucina* Desmazières and *F. rumpens* (Kützing) G.W.F.Carlson from Tuji & Williams (2006b); *F. vaucheriae* (Kützing) J.B.Petersen was checked in Krammer & Lange-Bertalot (1991), Tuji & Williams (2006a), Delgado *et al.* (2015) and Wetzel & Ector (2015); *F. sandellii* Van de Vijver & Jarlman from Van de Vijver *et al.* (2012); *F. candidagilae* Almeida, C.Delgado, Novais & S.Blanco and *F. rinoi* Almeida & C.Delgado were recently described by Delgado *et al.* (2015, 2016).

Valve shape analysis

Valve shape analysis and morphometric comparisons were carried out to compare *F. misarelensis sp. nov.* with morphologically and morphometrically similar *Fragilaria* species whose type material is well illustrated in the literature, including the type materials photographed in this study.

Valve shape analysis and morphometric comparison were based on measurements of 10–30 valves. More than 100 individuals of each taxon were observed under LM and SEM for morphological characterisation.

Morphological terminology follows Round *et al.* (1990), Ross *et al.* (1979), Barber & Haworth (1981) and Williams & Round (1987; used for terminology on areolae, girdle band features and apical pore field characteristics).

Valve shape differences between the studied taxa were analysed using geometric morphometry. To perform this analysis, valve outline was captured as a geometric configuration of ~100 non-homologous pseudolandmarks per valve using CLIC software (Dujardin *et al.* 2010), directly digitised from the LM micrographs taken in this study and from illustrations of the type material of comparable taxa available in the literature. In total, 200 images were processed. The Cartesian coordinates of the pseudolandmarks were aligned (translated, rotated and scaled) by the Procrustes generalised orthogonal least-squares superimposition procedure (Rohlf & Slice 1990). A principal components analysis

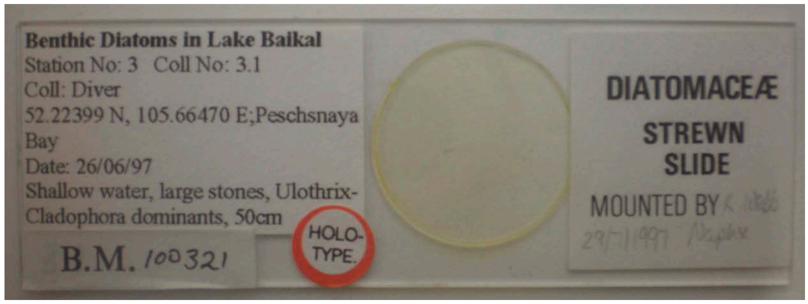


Fig. 2. Original glass slide (holotype) with type material of *Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M.Williams and *Fragilaria capucina* fo. *lanceolata-baikali* Flower & D.M.Williams, BM 100321.

(PCA) was carried out on the resulting normalised co-ordinates by means of the software PAST v3.14 (Hammer *et al.* 2001). To visualise the size and shape of the scatterplot for each predefined group, the resulting groups were fitted to 95% confidence ellipses. Finally, a one-way nonparametric multivariate analysis of variance test (NPMANOVA; Anderson 2001) using Euclidean distances was performed between the transformed co-ordinates in order to test for significant differences between the *a priori* defined groups. Main morphometric parameters (valve length and width, density of striae and areolae) were measured directly on LM/SEM micrographs. The type material of taxa illustrated in the literature was also measured and compared to the parameters obtained in the populations here described by means of a NPMANOVA (Euclidean distance metric), in order to test the null hypothesis of no differences between the morphometry of the populations under study and that of the types of similar species.

Measurements of the type material of *Fragilaria capucina* fo. *sublanceolata-baikali, F. capucina* fo. *lanceolata-baikali* and *F. nevadensis* were taken in this study. For the type material illustrations of the other species, the following manuscripts were consulted: Delgado *et al.* (2015) for *Fragilaria recapitellata, F. perminuta* and *F. vaucheriae*; Tuji & Williams (2008) for *Fragilaria pectinalis* and Wetzel & Ector (2015) for *F. microvaucheriae.*

RESULTS

Portuguese species

Fragilaria misarelensis Almeida, C.Delgado, Novais & S. Blanco, *sp. nov.*

Figs 3–34, S1–S55 (LM); Figs 35–43 (SEM)

HOLOTYPE: BM 101 857 (Natural History Museum, London, UK), microscopic slide and preserved sample.

ISOTYPES: ZU10/77 (Hustedt Collection, Bremerhaven, Germany), microscopic slides.

TYPE LOCALITY: Mondego River, Mondego basin, Casal da Misarela, Coimbra Municipality, district: Coimbra, region: Centre, Portugal; coordinates 40°11′54.233″N, 08°21′59.582″W; coll. Carmen Elias and Cristina Delgado; coll date 20 March 2012.

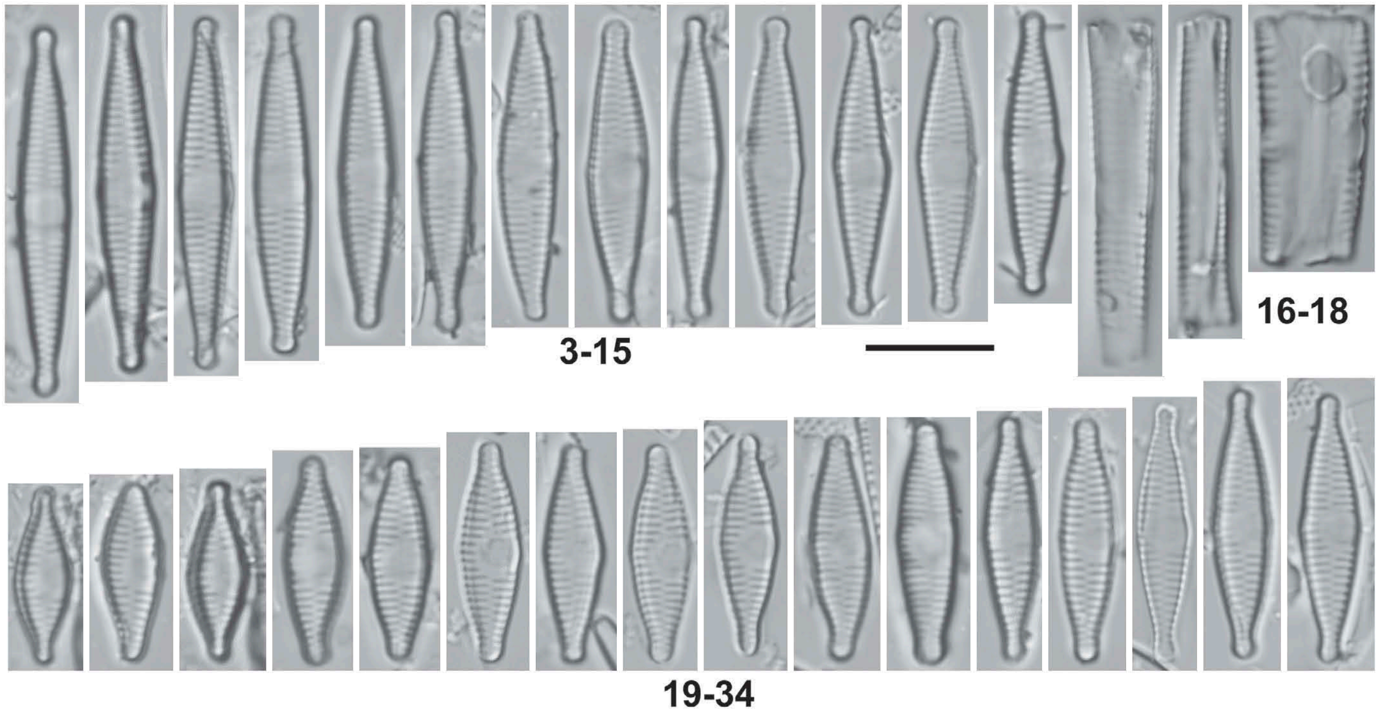
ETYMOLOGY: The specific epithet *misarelensis* refers to Casal da Misarela, the type locality.

DESCRIPTION: Valves lanceolate with capitate apices, sometimes with a slight central swelling (Figs 13, 15). Frustules rectangular in girdle view with interruption of striation in the middle portion (Figs 16–18). Valve dimensions (Casal da Misarela, holotype population, n = 29; Foz do Caneiro, n = 36; Côja, n = 19): Length 13.6–29.2 µm (Casal da Misarela), 10.5–32.0 µm (Foz do Caneiro), 15.4–26.7 µm (Côja); width 3.6–4.7 µm (Casal da Misarela), 3.1–5.2 µm (Foz do Caneiro), 3.3–5.2 µm (Côja); stria density 15–18 in 10 µm (Casal da Misarela), 15–18 in 10 µm (Foz do Caneiro), 16–18 in 10 µm (Côja). Striae alternate, subparallel throughout the valve or slightly radiant towards the valve apices (Figs 3–15, 19–34, 35–37). Striae uniseriate, composed of slightly elongated areolae that become more rounded along the apical axis (six to seven areolae in 1 µm) in both valves (Figs 35–37, 40, 41). Central area bilaterally expanded in the majority of the valves (e.g. Figs 3, 5, 6, S1–S19), sometimes central area unilaterally expanded, especially in smaller valves (Figs 24–27). Siliceous plaques present along the valve mantle edge (Figs 39, 43). Axial area narrow, slightly expanded towards the valve centre (Figs 3, 5, 36, 40). Each valve has two apical pore fields of the ocellulimbus type made up of four to five rows, each composed of 10 to 13 poroids (Figs 35, 36, 38–40, 43). Isolated cells without spines (Figs 35, 36, 39, 40, 43). Girdle bands open, with small, unoccluded perforations (Fig. 43). One rimoportula per valve present near the apex and might have different orientations (Figs 35–38).

Comparison with similar species

Morphological features of *Fragilaria misarelensis sp. nov*. are described in Table 1 and a comparison of the valve shape of the three populations of *Fragilaria misarelensis sp. nov*. illustrated in LM is presented in Fig. 92. Here the overlapping is visible, revealing that the three populations represented belong to the same species.

Type materials of Baikal endemics *Fragilaria capucina* fo. *sublanceolata-baikali, F. capucina* fo. *lanceolata-baikali* and *F. nevadensis* were analysed and summarised in Table 1, in Figs 44–91 (LM) and S56–S60 (SEM). In addition, Figs 93-96 provide comparisons with species similar to *F. misarelensis sp. nov*. that are poorly illustrated in the original descriptions. From these, only *Fragilaria nevadensis* and *F. capucina* fo. *sublanceolata-baikali* overlap in valve dimensions (Tables 1, 2, Figs 94, 95). Comparing the central area of *Fragilaria nevadensis*, it is also bilaterally expanded as in *F. misarelensis*. Nevertheless, it is well delimited in the latter; whereas, it is more undefined in *F. nevadensis*, where ghost striae are also visible. *Fragilaria misarelensis* shares several ultrastructural characters with *F. nevadensis*, namely, the shape of the areolae (linear along the valve edge and round along the apical axis), type of apical pore fields (ocellulimbus type, composed of three to five rows including 8 to 14 poroids) and presence

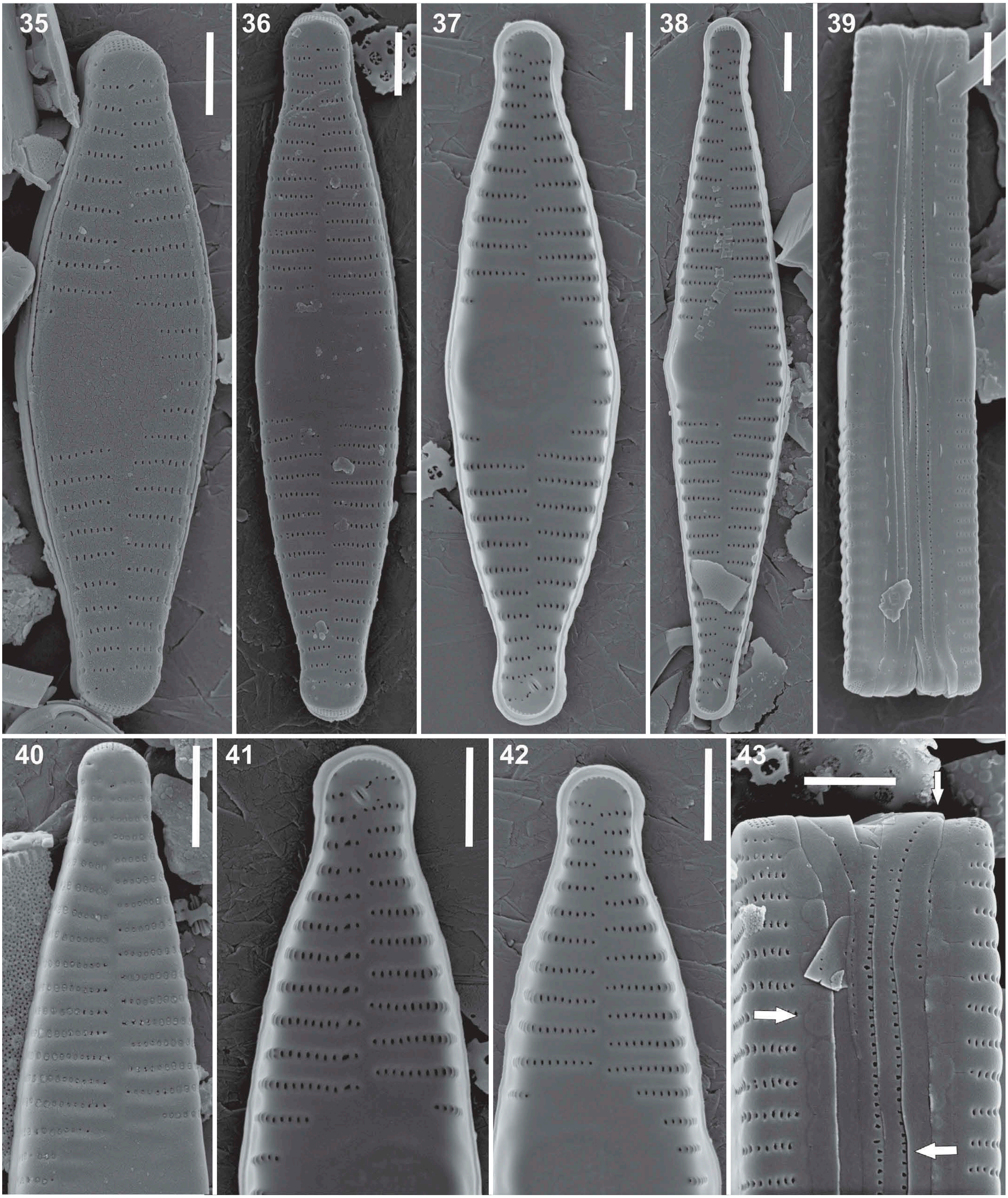


Figs 3–34. Light micrographs of *Fragilaria misarelensis sp. nov.* from Casal da Misarela, Mondego River, Mondego basin, 20 March 2012 (holotype population).
Figs 3–15. Valve view.
Figs 16–18. Complete frustules in girdle view.
Figs 19–34. Valve view.
Scale bar = 10 µm.

of one single rimoportula. However, *Fragilaria misarelensis* lacks spines, which are present in *F. nevadensis*, being conical close to the apex to spatulate in the middle of the valve (Figs S57, S58 and SEM illustrations in Linares-Cuesta & Sánchez-Castillo 2007). *Fragilaria capucina* f. *sublanceolata-baikali* has lanceolate-elliptical valves with convex margins between the central area and the rostrate apices; whereas, the valve is lanceolate with capitate apices in *F. misarelensis sp. nov*. The ultrastructure of *Fragilaria capucina* f. *sublanceolata-baikali* is similar to that of *F. misarelensis* in the features that characterise the genus, such as the presence of two apical pore fields, one rimoportula and uniseriate striae. Furthermore, both species present copulae with a single row of puncta; however, they differ in the shape of the areolae (elongated in *Fragilaria capucina* f. *sublanceolata-baikali* and with variable shape in *F. misarelensis*) and in the small spines sometimes present in *F. capucina* fo. *sublanceolata-baikali* (Flower *et al*. 2004).

The PCA plot with the results from the geometric morphometric analysis shows that the valve outline of the individuals from the holotype population of *Fragilaria misarelensis sp. nov.* partially overlaps with species such as *F. perminuta, F. capucina* fo. *sublanceolata-baikali, F. capucina* f. *lanceolata-baikali, F. microvaucheriae* and *F. vaucheriae* (Fig. 93); however, the NPMANOVA analysis revealed statistically significant ($P < 0.05$) differences from all of these species in more than one character analysed except *F. vaucheriae* which only differed significantly from *F. misarelensis* in stria density (Table 2). With respect to length, it partially overlaps with *Fragilaria perminuta, F. capucina* fo. *sublanceolata-baikali* and *F. vaucheriae*, and the width overlaps with *F. vaucheriae, F. capucina* fo. *sublanceolata-baikali* and *F. capucina* f. *lanceolata-baikali*; whereas, there is no overlapping in stria density (Fig. 96, Table 2). Nevertheless, *Fragilaria perminuta* forms colonies, *F. capucina* fo. *sublanceolata-baikali* has lanceolate-elliptical valves with margins between central area and convex and rostrate apices, and *F. vaucheriae* has coarser striation. *F. microvaucheriae* has generally narrower valves, sometimes presents small conic spines and has a wide unilateral central area, with a rectangular aspect; whereas, it is bilaterally expanded in most specimens of *F. misarelensis sp. nov.* (Table 3).

Fragilaria species recently described from Portugal (*F. candidagilae* and *F. rinoi*) differ morphologically and morphometrically from *F. misarelensis sp. nov.*, because the valve shape of *F. candidagilae* is very distinctive, with the strongly capitate apices, coarser striation, usually wider valves and central area unilaterally expanded; whereas, *F. misarelensis sp. nov.* does not present strongly capitate apices, has denser striation, and a central area mostly bilaterally expanded. *Fragilaria rinoi* is characterised by slightly rostrate apices, has generally wider valves and a central area unilaterally expanded; whereas, the apices are capitate in *F. misarelensis*, the central area is bilaterally expanded (sometimes with a slight central swelling) and valves are generally narrower (Tables 1, 3). The ultrastructure of *Fragilaria misarelensis* is similar to that of *F. rinoi* and *F. candidagilae*, differing only in the shape of the areolae, variable in *F. misarelensis* and round in *F. candidagilae* and *F. rinoi* (also presenting a higher dentity of areolae, with 13–14 areolae in 1 µm vs 6–7 areolae in 1 µm in *F. misarelensis*). From an ecological point of view,



Figs 35–43. Scanning electron micrographs of *Fragilaria misarelensis sp. nov.* from Casal da Misarela, Mondego River, Mondego basin, 20 March 2012 (holotype population).
Figs 35, **36**. Valves in external view.
Figs 37, **38**. Valves in internal view.
Fig. 39. Frustule in girdle view.
Fig. 40. Detail of an apex in external view, with the apical pore field and rimoportula.
Figs 41, **42**. Details of the apices of Fig. 37 in internal view, with the single rimoportula and the apical pore fields.
Fig. 43. Detail of the apex in girdle view, with the open girdle bands with small, unoccluded perforations and siliceous plaques visible (indicated by the arrows). Scale bar = 2 μm.

Table 1. Morphological features of the new Portuguese species and the type material analysed in detail.

	Fragilaria misarelensis Almeida, C.Delgado, Novais & S.Blanco	*Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M.Williams	*Fragilaria capucina* fo. *lanceolata-baikali* Flower & D.M.Williams	*Fragilaria nevadensis* Linares-Cuesta & Sánchez-Castillo
References	This study	Flower *et al.* (2004); this study	Flower *et al.* (2004); this study	Linares-Cuesta & Sánchez-Castillo (2007); this study
Valve morphometry: length (μm); width (μm)	13.6–29.2; 3.6–4.7 (Casal da Misarela, type population)	12–35; 3.5–4.5 (Flower *et al.* 2004); 11.6–23.8; 3.8–4.7 (this study)	35–57; 3.5–4.5 (Flower *et al.* 2004); 33.9–39.3; 3.5–4.3 (this study)	30–50; 3.5–5.0 (Linares-Cuesta & Sánchez-Castillo 2007); 23.9–47.7; 3.2–4.1 (this study)
Striation	Alternate, subparallel throughout the valve to slightly radiant towards the ends; 15–18 in 10 μm	Alternate, subparallel in the middle of the valve to slightly radiant towards the ends, 19–20 in 10 μm (Flower *et al.* 2004); 17–21 in 10 μm (this study)	Alternate, subparallel in the middle of the valve to slightly radiant towards the ends, 19–20 in 10 μm (Flower *et al.* 2004); 17–18 in 10 μm (this study)	Alternate, parallel, not punctate, 14–18 in 10 μm
Valve outline	Lanceolate	Lanceolate–elliptical, with valve margins convex between the central area and the apices	Lanceolate	Lanceolate
Apices	Capitate	Rostrate	Slightly developed, subrostrate	Subcapitate
Central area	Bilaterally expanded in the majority of the valves	Unilaterally expanded from the axial area to the valve margin, small inflation to one side of the central area margin	Asymmetric and sometimes extending to the margins of the valve, small inflation to one side of the central area margin	Elliptic, usually extending to the valve margins; swollen central part
Colonies	No	–	–	Yes
Number of rimoportula	1	1	1	1
Apical pore fields	Ocellulimbus type, made up of four to five rows, each composed of 10 to 13 poroids	Ocellulimbus type	Ocellulimbus type	Ocellulimbus type, made up of three to five rows, each composed of 8 to 14 poroids
Girdle bands	Open, with small, unoccluded perforations	Open, each with a single row of puncta	Open, each with a single row of puncta	–
Spines	Absent	Small spines sometimes present	Small spines sometimes present	Spines are conical near the apex and spatulate in the middle of the valve

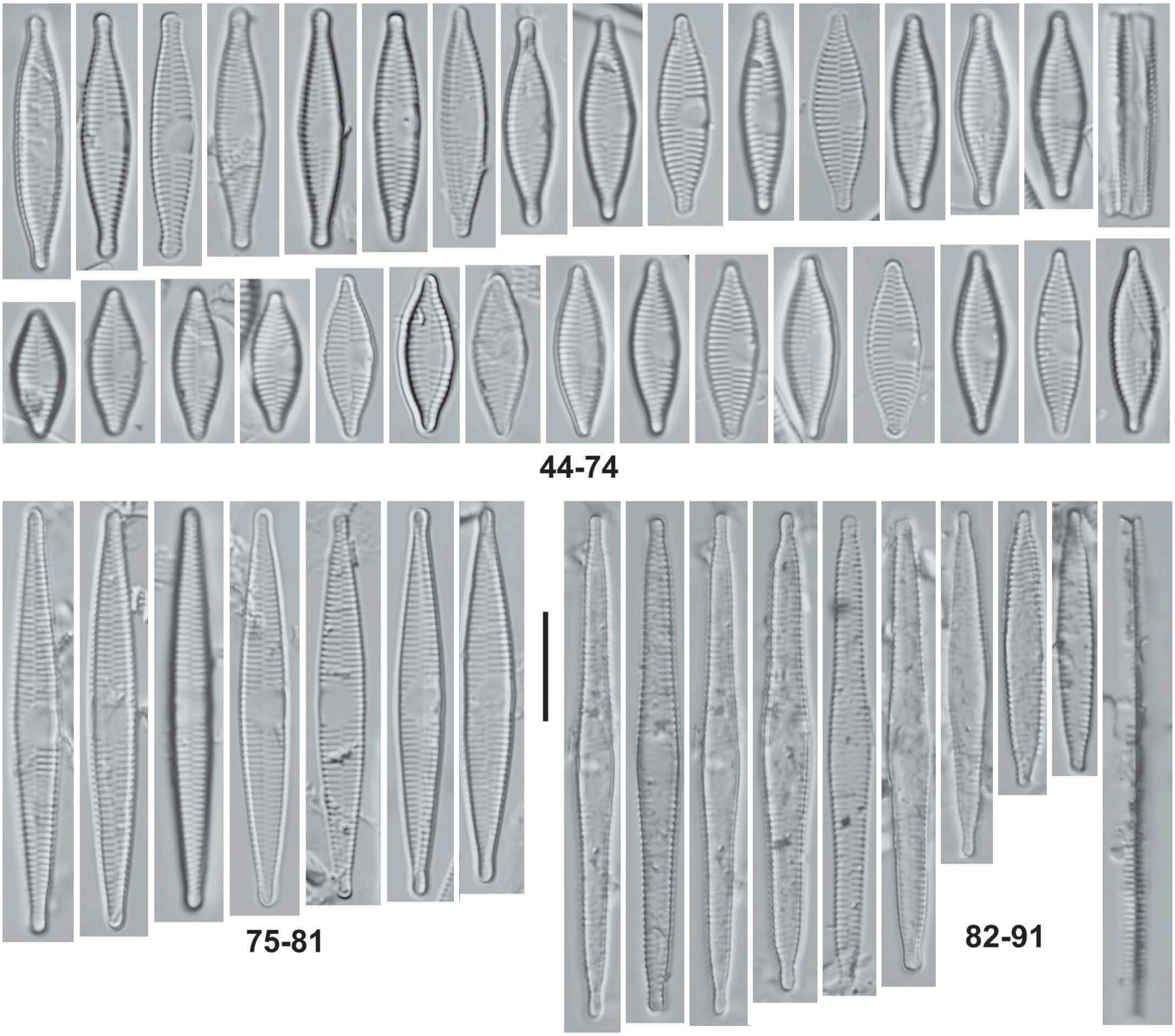
Fragilaria misaralensis sp. nov. and *F. candidagilae* are present in poorly mineralized waters with low conductivity and low nitrate, nitrite and phosphate concentrations; whereas, *F. rinoi* is present in watercourses with medium to high conductivity and high nutrient concentrations, with mean values of nitrate–nitrogen of 3.7 mg l^{-1} (Delgado *et al.* 2015, 2016).

The literature analysis (compiled in Table 3) also revealed that smaller specimens of *Fragilaria capucina* Desmazières, *F. pectinalis* (O.F.Müller) Lyngbye and *F. rumpens* (Kützing) G.W.F.Carlson may overlap in valve length; nevertheless, larger specimens of these species exceed the length of *F. misarelensis sp. nov*. (Table 3). Other noticeable differences amongst the aforementioned taxa are the following: *F. capucina* possesses two rimoportula, one at each end, and forms ribbon-like colonies (Tuji & Williams 2006b); *F. rumpens* has a lanceolate to fusiform valve outline with rostrate ends and denser striation and presents irregular spines located on the costae (at mantle–face junction). These are rectangular at the central area and very small and triangular at the valve poles (Tuji & Williams 2006a). *F. pectinalis* has coarser striation, linear–lanceolate valves with a central area unilaterally expanded and linking spines (Tuji & Williams 2008). *Fragilaria sandellii* Van de Vijver & Jarlman, a species recently described from a small oligotrophic Swedish stream, has similar length but is characterised by wider valves with a central area forming a large asymmetrical fascia, small rounded areolae and the occasional presence of very small marginal spines (Van de Vijver *et al.* 2012).

Additionally, *Fragilaria neointermedia* Tuji & D.M. Williams presents a central area slightly bilaterally expanded in some specimens; however, this species has coarser striation (8–10 in 10 μm), rostrate ends and spatulate linking spines (Delgado *et al.* 2015).While this paper was in press, it came to our attention the publication of the similar *F. drouotiana* Heudre, Wetzel, Moreau & Ector. However, it can be easily differentiated from *F. misarelensis* by the narrower valves (*F. drouotiana*: width 2.4 - 3.2 mm and *F. misarelensis*: width 3.6 - 4.7 mm) with central area unilateral, whilst it is bilaterally expanded in the majority of *F. misarelensis* valves. *Fragilaria* drouotiana also has denser striation than *F. misarelensis* (17 - 22 vs 15 - 18 striae in 10 μm). With respect to the ecology, *F. drouotiana* was found in epiphyton in lakes while F. misarelensis was identified from sediments and hard substrate in rivers and can tolerate higher concentrations of nitrates, nitrites and ammonium than *F. drouotiana*.

Distribution, ecology and associated diatom flora

From 53 samples belonging to the river basins of Mondego, Vouga and Lis, *Fragilaria misarelensis sp. nov*. was found in the



Figs 44–91. Light micrographs of type material.
Figs 44–74. *Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M.Williams (BM 100321).
Figs 75–81. *Fragilaria capucina* fo. *lanceolata-baikali* Flower & D.M.Williams (BM 100321).
Figs 82–91. *Fragilaria nevadensis* Linares-Cuesta & Sanchez-Castillo (GDA 3013). Scale bar = 10 µm.

epilithon and sediment of 15 samples collected in 12 sites (of which 9 were located in the Mondego and 3 in the Vouga river basin; Fig. 1, Table S2). The relative abundance of this species ranged from 0.48% in the sample collected in sediment from Cunha Baixa (19 March 2012) to 13.66% sampled on hard substrate from Foz do Caneiro (12 June 2007). It was present with relative abundance above 1% at 10 sites. Three samples were from the type locality, Casal da Misarela, May and October 2011 and March 2012. *Fragilaria misarelensis* was present in poor to mineralized rivers with low to high conductivity (16–1060 µS cm^{-1}), neutral–alkaline pH (6.83–7.75) and low nitrates (0.34 - 3.29 mg NO_3- l -^{1}), nitrites (0.002 -0.09 mg NO_2- l -^{1}) and ammonium (0.002 - 2.31 mg NH_4+ l -^{1}) concentrations (Table S4). This diatom species occurred at a high range of dissolved oxygen (between 45.6% and 114.8%) and silica (between 1.34 and 8.7 mg SiO_2 l^{-1}) (Table S4). However, abundance-weighted averages for several environmental parameters revealed that it occurs preferentially in watercourses with low mineralization (conductivity = 111 µS cm^{-1}; alkalinity = 21.5 mg $CaCO_3$ l^{-1}; total hardness = 35.3 mg $CaCO_3$ l^{-1}), neutral pH (pH = 7.1), low nutrient concentrations (nitrates = 1632 µg NO_3^- l^{-1}; nitrites = 20 µg NO_2^- l^{-1}; phosphates = 132 µg PO_4^{3-} l^{-1}; total P = 290 µg P l^{-1}) and high dissolved oxygen (DO = 93.2% sat.) (Table S4).

In the type locality (Casal da Misarela, 20 March 2012), the relative abundance of *Fragilaria misarelensis sp. nov.* was 1.23% and the associated diatoms co-occurring with this new taxon were *Achnanthidium minutissimum* (Kützing) Czarnecki

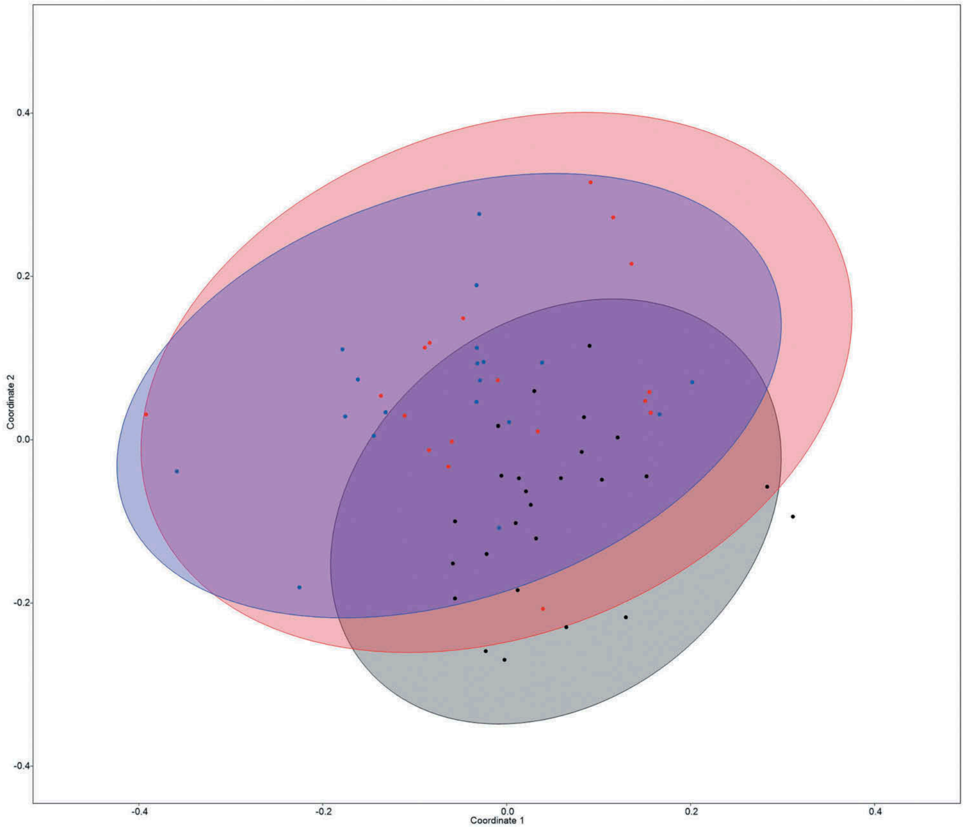


Fig. 92. Comparison of the three populations of *Fragilaria misarelensis sp. nov*. illustrated in LM. Black: Casal da Misarela, Mondego River, Mondego basin, 20 March 2012 (holotype population). Red: Foz do Caneiro, Mondego River, Mondego basin, 12 June 2007. Blue: Côja, Alva River, Mondego basin, 11 June 2007.

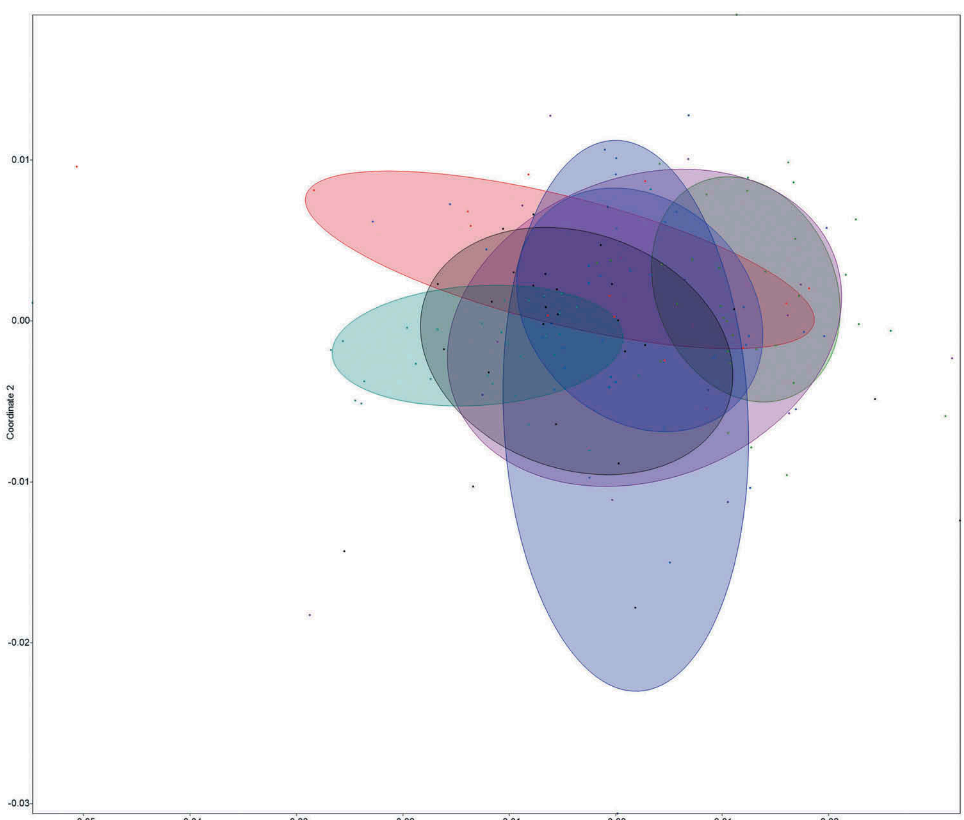


Fig. 93. Comparison of the holotype of *Fragilaria misarelensis sp. nov*. with the type material of other related taxa. Data fitted to 95% confidence ellipses. NMDS plot of Bookstein-transformed valve outline pseudolandmark ordinates. Red: *Fragilaria microvaucheriae*. Dark blue: *F. lanceolata-baikali*. Light blue: *F. perminuta*. Olive green: *F. recapitellata*. Dark green: *F. sublanceolata-baikali*. Violet: *F. vaucheriae*. Grey: *F. misarelensis*.

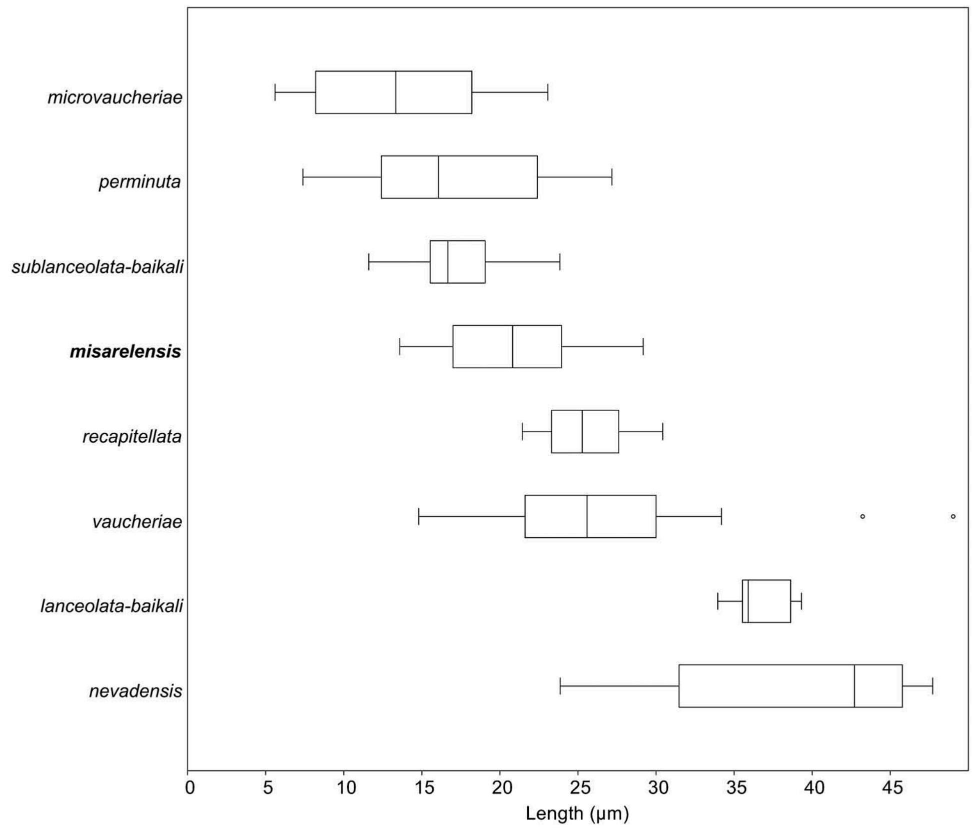


Fig. 94. Box plot of length measurements of different *Fragilaria* species (type populations). The boxes represent the 25%–75% quartiles, the median is shown with vertical line inside the box, and minimum and maximum values are shown with short vertical lines (whiskers). Outliers are plotted as individual points.

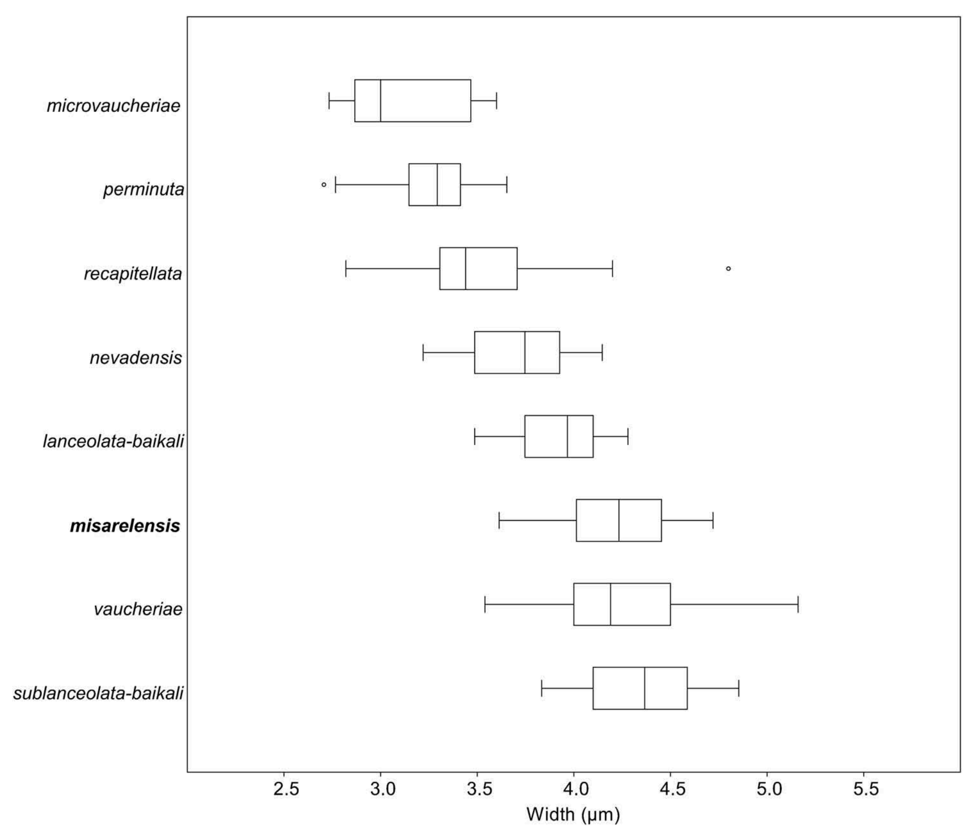


Fig. 95. Box plot of width measurements of different *Fragilaria* species (type populations). The boxes represent the 25%–75% quartiles, the median is shown with vertical line inside the box, and minimum and maximum values are shown with short vertical lines (whiskers). Outliers are plotted as individual points.

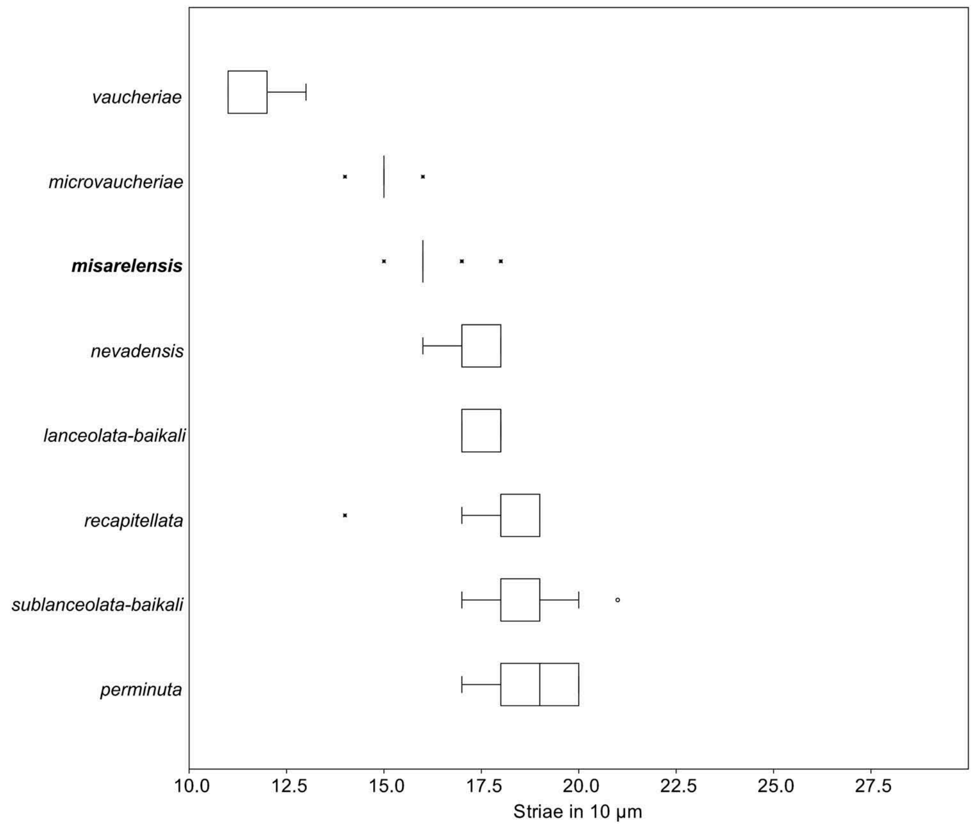


Fig. 96. Box plot of measurements of different *Fragilaria* species. Variation in the number of striae in 10 µm measured in the type populations. The boxes represent the 25%–75% quartiles, the median is shown with vertical line inside the box, and minimum and maximum values are shown with short vertical lines (whiskers). Outliers are plotted as individual points.

(43.97%), *Fragilaria* aff. *rumpens* (Kützing) G.W.F.Carlson (3.27%), *Reimeria sinuata* (W.Gregory) Kociolek & Stoermer (2.86%), *Achnanthidium atomoides* Monnier, Lange-Bertalot & Ector (4.70%), *A. lineare* W.Smith (1.02%), *A. rivulare* Potapova & Ponader (16.97%), *Aulacoseira granulata* (Ehrenberg) Simonsen (3.48%), *Cocconeis euglypta* Ehrenberg (1.02%), *Encyonema minutum* (Hilse) D.G.Mann (9.00%), *Eunotia pectinalis* (Kützing) Rabenhorst (1.63%), *Fragilaria* sp. (2.04%), *Gomphonema minutum* (C.Agardh) C.Agardh (1.23%) and *Navicula cryptotenella* Lange-Bertalot (1.84%). From these, the most abundant taxa are circumneutral, oligo to β-mesosaprobous, freshwater (<100 mg Cl^{-} l^{-1}) or freshwater to brackish (<500 mg Cl^{-} l^{-1}), nitrogen autotrophic tolerating very small to elevated concentrations of organically bound nitrogen and requiring continuously high dissolved oxygen concentrations (Van Dam *et al.* 1994; retrieved from the 2013 OMNIDIA v5.5 database [Lecointe *et al.* 1993]).

Type material analysis

The type material of *Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M.Williams, *F. capucina* fo. *lanceolata-baikali* Flower & D.M.Williams and *F. nevadensis* Linares-Cuesta & Sánchez-Castillo were analysed in detail for comparison with the Portuguese species. Detailed information about the type material is presented in Table S3, LM and SEM illustrations of the type populations are provided in Figs 44–91 (LM) and S56–S60 (SEM) and morphological features are summarised in Table 1.

Fragilaria capucina fo. *sublanceolata-baikali* Flower & D.M.Williams in Flower *et al.* (2004) Figs 13, 15 (SEM) Figs 44–74 (LM)

MORPHOLOGY: Valve dimensions (*n* = 30): Length 11.6–23.8 µm, width 3.8–4.7 µm, striae 18–21 in 10 µm in the type material analysed in this study; and length 12–35 µm, width 3.5–4.5 µm, striae 19–20 in 10 µm described in Flower *et al.* (2004). The valves are lanceolate–elliptical with valve margins between the central area and apices convex, apices rostrate, single rimoportula at the valve apex (Fig. 13; SEM illustration in Flower *et al.* 2004). Small conical spines are present in the costae (Fig. 15; SEM illustration in Flower *et al.* 2004).

Fragilaria capucina fo. *lanceolata-baikali* Flower & D.M. Williams in Flower *et al.* (2004) Figs 14, 16 (SEM) Figs 75–81 (LM)

MORPHOLOGY: Valve dimensions (*n* = 7): Length 33.9–39.3 µm, width 3.5–4.3 µm, striae 17–18 in 10 µm in the type material analysed in this study; and length 35–57 µm, width 3.5–4.5 µm, striae 19–20 in 10 µm described

Table 2. Morphometric and morphological comparison between the type material of *Fragilaria misarelensis sp. nov.* and related taxa. NPMANOVA results, Fischer's statistic and Bonferroni-corrected *P* values are presented (significant values highlighted in bold). *n* = sample size; GM = geometric morphometry; M = morphometry. *F. misarelensis* n (GM = 26; M = 29); *F. lanceolata-baikali* n (GM = 7; M = 7); *F. microvaucheriae* n (GM = 13; M = 12); *F. nevadensis* n (GM = –; M = 11); *F. perminuta* n (GM = 35; M = 31); *F. recapitellata* n (GM = 35; M = 38); *F. sublanceolata-baikali* n (GM = 30; M = 30); *F. vaucheriae* n (GM = 15; M = 26). L/W = length/width.

		Fragilaria lanceolata-baikali	*Fragilaria microvaucheriae*	*Fragilaria nevadensis*	*Fragilaria perminuta*	*Fragilaria recapitellata*	*Fragilaria sublanceolata-baikali*	*Fragilaria vaucheriae*
Length	*R*	0.9303	0.3086	0.7533	0.04877	0.2965	0.1027	0.1185
	P	**0.0021**	**0.0144**	**0.0021**	1	**0.0021**	0.1176	0.1188
Width	*R*	0.09099	0.8686	0.3977	0.8686	0.5538	−0.0041	−0.007907
	P	1	**0.0021**	**0.0042**	**0.0021**	**0.0021**	1	1
Stria density	*R*	0.5679	0.4847	0.3981	694	0.7246	721	0.9979
	P	**0.0063**	**0.0021**	**0.0084**	**0.0021**	**0.0021**	**0.0021**	**0.0021**
L/W	*R*	0.9022	0.1364	0.8784	0.05551	0.4289	0.08798	0.08798
	P	**0.0021**	0.9576	**0.0021**	0.9639	**0.0021**	0.2268	0.3416
Outline	*R*	0.2126	0.1668	–	0.2019	0.4128	0.1619	0.1243
	P	**0.0126**	**0.0462**	–	**0.0021**	**0.0021**	**0.0042**	0.1617

in the type material (Flower *et al.* 2004). The valves were lanceolate with valve margins essentially straight between the central area and apices. Apices slightly developed, subrostrate. Small conical spines present in the costae (Fig. 16; SEM illustration in Flower *et al.* 2004).

According to Flower *et al.* (2004), both forms were identified from shallow-water sampling stations in Lake Baikal, but the number of valves of *Fragilaria capucina* fo. *sublanceolata-baikali* (referred to as Morphotype 1 throughout the text in Flower *et al.* 2004) exceeded those of *F. capucina* fo. *lanceolata-baikali* (referred to as Morphotype 2). The shallow-water epilithic communities were dominated by forms of *Fragilaria capucina* Desmazières (including the two morphotypes above mentioned), *Hannaea arcus* (Ehrenberg) Patrick & Reimer, *Encyonema minutum* (Hilse) D.G.Mann, *Cocconeis placentula* Ehrenberg, *Nitzschia dissipata* (Kützing) Rabenhorst and gomphonemoid taxa (Flower *et al.* 2004).

Fragilaria capucina fo. *sublanceolata-baikali* and *F. capucina* fo. *lanceolata-baikali* were described as two forms of *F. capucina* from Lake Baikal by Flower *et al.* (2004). These two morphotypes were described as 'forms' pending revision of the entire species group. Recently, several studies on the *Fragilaria capucina/vaucheri*ae complex were published, including the revision of the type material of *Fragilaria capucina* Desmazières (Tuji & Williams 2006b). According to Tuji & Williams (2006b), valves with alternate striae, linking spines, allowing the formation of ribbon-like colonies, open girdle bands and one rimoportula at each apex, characterise *Fragilaria capucina*. Alternate striae, open girdle bands, sometimes the presence of small spines and only one rimoportula per valve characterise both Baikal 'forms'. Given the above-mentioned differences, we propose to raise these forms to species level.

Fragilaria sublanceolata-baikali **(Flower & D.M.Williams) Novais, C.Delgado & S.Blanco, *comb. et stat. nov.***

BASIONYM: *Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M. Williams in Flower *et al.* 2004, Proceedings of the Seventeenth International Diatom Symposium, p. 92, figs 13, 15.

Fragilaria lanceolata-baikali (Flower & D.M.Williams) Novais, C.Delgado & S.Blanco, *comb. et stat. nov.*

BASIONYM: *Fragilaria capucina* fo. *sublanceolata-baikali* Flower & D.M. Williams in Flower *et al.* 2004, Proceedings of the Seventeenth International Diatom Symposium, p. 92, figs 14, 16.

Fragilaria nevadensis **Linares-Cuesta & Sánchez-Castillo in Linares-Cuesta & Sanchez-Castillo (2007) Figs 1–3 (LM) Figs 4–9 (SEM) Figs 82–91 (LM) Figs S56–S60 (SEM)**

MORPHOLOGY: Valve dimensions (*n* = 11): Length 23.9–47.7 µm, width 3.2–4.1 µm, striae 16–18 in 10 µm in the type material analysed in this study; and length 30–50 µm, width 3.5–5.0 µm, striae 14–18 in 10 µm in the original description by Linares-Cuesta & Sánchez-Castillo (2007). The valves were lanceolate with subcapitate ends and axial sternum narrow (Figs 83, 86). The central parts were swollen with an elliptical central area usually extending to the margins of the valve (Figs 83, 86). The striation was alternate, parallel and not punctate (Fig. 83). Spines present, being conical near the apex to spatulate in the middle of the valve (Figs S57, S58).

Table 3. Morphological features of selected *Fragilaria* taxa similar to *F. misarelensis sp. nov.*

	Fragilaria candidagilae Almeida, C. Delgado, Novais & S. Blanco	*Fragilaria capucina* Desmazières	*Fragilaria recapitellata* Lange-Bertalot & Metzeltin	*Fragilaria microvaucheriae* C.E.Wetzel & Ector	*Fragilaria pectinalis* (O. F.Müller) Lyngbye	*Fragilaria perminuta* (Grunow) Lange-Bertalot	*Fragilaria rhabdosoma* Ehrenberg	*Fragilaria rinoi* Almeida & C.Delgado	*Fragilaria rumpens* (Kützing) G.W.F.Carlson	*Fragilaria sandellii* Van de Vijver & Jarlman	*Fragilaria vaucheriae* (Kützing) J.B. Petersen
Basionym	–	–	*Synedra capitellata* Grunow in Van Heurck	–	*Conferva pectinalis* O. F.Müller	*Synedra* (*vaucheriae* var.?) *perminuta* Grunow in Van Heurck	–	–	*Synedra rumpens* Kützing	–	*Exilaria vaucheriae* Kützing 1833
References	Delgado *et al.* (2015)	Tuji & Williams (2006a)	Van Heurck (1881); Tuji & Williams (2008); Delgado *et al.* (2015)	Wetzel & Ector (2015)	Tuji & Williams (2008)	Van Heurck 1881; Tuji & Williams (2008); Delgado *et al.* (2015)	Tuji (2004)	Delgado *et al.* (2016)	Tuji & Williams (2006b)	Van de Vijver *et al.* (2012)	Krammer & Lange-Bertalot (1991); Tuji & Williams (2006b, 2013)
Valve morphometry: length (μm); width (μm)	13.0–25.8; 4.5–5.0	25.6–67.5; 3.1–3.8	20–39; 3–4 (Tuji & Williams 2008); 21.4–30.4; 3–4 (Delgado *et al.* 2015)	5.7–23.4; 2.5–3.8	28–37; 3.5–4.0	9–24; 3.0–3.5 (Tuji & Williams 2008); 7.5–32.6; 3.0–3.5 (Delgado *et al.* 2015)	15.6–28.3 (20.8 in Ehrenberg's original description 1832); 3.3–3.9	8.8–24.1; 4.2–5.6	25–63; 3.0–4.0	11.5–22.0; 4.5–6.0	22–42.7; *ca.* 4.0 (Krammer & Lange-Bertalot 1991)
Striation	Alternate, punctuate and parallel to slightly radiate towards the ends; 12–14 in 10 μm	Alternate, parallel to slightly radiate towards ends; *ca.* 15–16 striae/10 μm	Alternate, parallel to slightly radiate towards ends; 17–19 in 10 μm (both Tuji & Williams [2008] and Delgado *et al.* [2015])	Coarse, uniseriate, radiate throughout, 15–16 in 10 μm	Alternate, parallel to slightly radiate towards ends; 14–15 in 10 μm	Alternate, parallel to slightly radiate towards ends; 18–19 in 10 μm	Alternate, parallel to slightly radiate towards ends; *ca.* 18–20 in 10 μm	Parallel, becoming slightly radiate at the ends; 14–16 in 10 μm	Parallel throughout; 18–20 in 10 μm	Very slightly radiate to almost parallel throughout, alternating although not always very clearly, 16–18 in 10 μm	Alternate, parallel to slightly radiate towards ends; 12–14/10 μm (Krammer & Lange-Bertalot 1991); 9–14/10 μm (Tuji & Williams 2006)
Valve outline	Linear–lanceolate to elliptical in smaller specimens	Linear	Lanceolate	Lanceolate in larger specimens to rhombic–lanceolate in smaller specimens	Linear–lanceolate	Lanceolate	Linear–lanceolate	Lanceolate to rhombic lanceolate in smaller specimens	Lanceolate, fusiform, sometimes irregularly curved	Lanceolate to elliptic–lanceolate in smaller specimens	Linear–lanceolate
Apices	Strongly capitate	Weakly rostrate	Strongly apiculate	Slightly rostrate to cuneate (in smaller specimens)	Weakly rostrate to capitate	Slightly rostrate	Weakly rostrate	Slightly rostrate	Rostrate	Protracted, rostrate	Capitate

(*Continued*)

Table 3. (Continued).

	Fragilaria candidagilae Almeida, C. Delgado, Novais & S. Blanco	*Fragilaria capucina* Desmazières	*Fragilaria recapitellata* Lange-Bertalot & Metzeltin	*Fragilaria microvaucheriae* C.E.Wetzel & Ector	*Fragilaria pectinalis* (O. F.Müller) Lyngbye	*Fragilaria perminuta* (Grunow) Lange-Bertalot	*Fragilaria rhabdosoma* Ehrenberg	*Fragilaria rinoi* Almeida & C.Delgado	*Fragilaria rumpens* (Kützing) G.W.F.Carlson	*Fragilaria sandellii* Van de Vijver & Jarlman	*Fragilaria vaucheriae* (Kützing) J.B. Petersen
Central area	Unilaterally expanded from the axial area to the valve face margin	Rectangular to rhombic	Unilateral, often expanded just until the sternum	Wide and unilateral (bilateral in large specimens)	Unilateral, often expanded just until the sternum	Strongly unilateral	Characteristic swellings, slightly unilateral in small individuals, bilateral in larger	Unilateral	A wide transverse fascia, sometimes unilaterally or bilaterally gibbous	Asymmetrical fascia, on one side bordered by shortened striae; ghost striae sometimes present in the fascia	Unilateral in small and bilateral in larger individuals
Colonies	No	Ribbon-like	No	No	–	Yes	Yes	No	Ribbon-like, adhering by their valve faces	No	–
Number of rimoportula	1	2	1	1	1	–	1	1	One, eccentric, adjacent to sternum	One, very difficult to resolve in LM	1
Apical pore fields	Ocellulimbus type, made up of six to seven rows, each composed of 8 to 14 poroids	Rectangular, bearing neatly arranged rows of poroids	Rectangular	Composed of fine porelli arranged in regular rows parallel to the apical axis	Rectangular, bearing neatly arranged rows of poroids	–	Rectangular, bearing neatly arranged rows of poroids	Ocellulimbus type, made up of four rows, each composed of 10 to 11 poroids	Rectangular	Present	Rectangular
Girdle bands	Open with perforations	Open bands, each with a single row of small punctae	–	Open, with small unoccluded perforations	Open	–	–	Open	Open bands with puncta	–	–
Spines	Absent	Present	Absent	Small conical spines (spinules) sometimes present	–	–	Present	Absent	Spines irregular, located on the costae, at mantle–face junction, often deformed and rectangular at central area, very small and triangular at valve poles	Very small marginal spines occasionally present	Either do not exist or are very small, and they do not link with sibling cells

DISCUSSION

Fragilaria misarelensis sp. nov. was compared with several similar species, from which it differs morphologically, morphometrically or ecologically. Optimal conditions for *Fragilaria misaralensis sp. nov.* are watercourses under low anthropogenic impacts, characterised by low mineralization, neutral pH, low nutrient concentrations and high dissolved oxygen. It is probable that *F. misarelensis sp. nov.* is common in European rivers with the same characteristics, because a population was found in Italy (E. Falasco, pers. comm. by e-mail).

From the type material analysed in this study, only *Fragilaria nevadensis* was identified in Portugal. It was present, in low abundance, at 10-m depth in the pelagic zone of Alqueva reservoir near Mourão, southern Portugal (0.25%), in July 2014 and in Coura River (Minho basin) in June 2017 (0.49%; Novais M.H., pers. comm.). According to Linares-Cuesta & Sánchez-Castillo (2007), the ecological preferences of this species are similar to *Fragilaria capucina* var. *austriaca*, which lives in oligotrophic to mesotrophic and circumneutral to alkaline waters, and is frequent in the Alps and Pyrenees (Krammer & Lange-Bertalot 1991). The sample collected in the Alqueva reservoir was slightly alkaline (pH = 7.8), with medium conductivity (420 μS cm^{-1}), low dissolved oxygen (41.5%), high temperature (21.25°C) and low nitrates, nitrites, total nitrogen, total phosphorus and phosphates concentrations. Regardless, the low occurrence of the species does not allow further comments on its optimal growth conditions.

Fragilaria misarelensis sp. nov. is placed in *Fragilaria*, even though its cells are not joined to form ribbon-like colonies, and occur as single cells, lacking spines located on or between the striae. This was one of the main features that originally characterised the genus (Williams & Round 1987). Nevertheless, Williams & Round (1987) noted that some *Fragilaria* species have been reported as growing singly, and the presence of spines characterises a much larger group, being present in other genera, i.e. *Staurosirella* D.M. Williams & Round, *Staurosira* (Ehrenberg) D.M.Williams & Round, *Stauroforma* Flower, Jones & Round or *Punctastriata* D.M.Williams & Round (Morales 2001). This species presents other SEM features that corroborate placement in genus *Fragilaria*, such as uniseriate striae, composed of round or slightly elongated areolae, a well-developed central area, apical pore fields of ocellulimbus type, the presence of one rimoportula (Morales 2001), and open girdle bands (Williams & Round 1987). The presence of spines is a dubious character in the characterisation of the genus, because it is absent in *Fragilaria rinoi* (Delgado *et al.* 2016), *F. candidagilae* (Delgado *et al.* 2015) and *F. pectinalis* (Wetzel & Ector 2015; Table 3). Furthermore, according to Wetzel & Ector (2015), spines are usually absent in *F. microvaucheriae* but can also be present (being small, conic and irregularly located on the costae). The variability of this character (presence/absence of spines) within the same species leads us to propose the exclusion of this character in the description of the genus *Fragilaria*.

Fragilaria species are abundant in European river basins, but it remains difficult to determine their ecological preferences, because they are often present in low abundances and only recently are several species belonging to the *Fragilaria capucina/vaucherie* complex being described and characterised in detail. Once these new species are described and well illustrated, it is expected to find them in other European regions or elsewhere, as is the case of *Fragilaria candidagilae*, described from central Portugal, that was illustrated by Lange-Bertalot *et al.* (2017) from central Europe and by Bishop *et al.* (2017) from southeast rivers in the United States (identified in Figs 45–48 as *Fragilaria* sp.35).

The comparison between *Fragilaria misarelensis sp. nov.* and similar taxa revealed that it presents a unique combination of characters: the valve shape (lanceolate valves and slightly capitate ends); density of striae; absence of linking spines; and the type of areolae, not found in the literature.

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