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Research article

Fragilaria rinoi sp. nov. (Fragilariales, Fragilariophyceae) from periphytic river samples in Central Portugal

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Abstract. A new benthic freshwater diatom, *Fragilaria rinoi* Almeida & C.Delgado sp. nov., is described from river periphyton samples in Portugal. *Fragilaria rinoi* sp. nov. is illustrated and discussed based on populations collected from the Vouga, Mondego and Lis river basins in central Portugal and compared with the type material of *Fragilaria vaucheriae* (Kütz.) J.B.Petersen. The morphological features of the new diatom species are documented through light and scanning electron micrographs, including a comparative analysis with related species of the genus (*F. candidagilae* Almeida, C.Delgado, Novais & S.Blanco, *F. intermedia* Grunow in Van Heurck, *F. neointermedia* Tuji & D.M.Williams, *F. recapitellata* Lange-Bert. & Metzeltin, *F. perminuta* (Grunow) Lange-Bert., *F. vaucheriae* and *F. microvaucheriae* C.E.Wetzel & Ector). *Fragilaria rinoi* sp. nov. is characterized by solitary cells without spines, lanceolate valves with slightly rostrate apices, a narrow, linear axial area, and a large, unilateral central area. *Fragilaria rinoi* sp. nov. may be confused with *F. microvaucheriae* in terms of length, striae density and outline, although a morphometric analysis revealed that *F. rinoi* sp. nov. is significantly wider. *Fragilaria rinoi* sp. nov. is present in rivers with high dissolved oxygen concentrations, medium to high conductivity, neutral to slightly alkaline pH and high mean values of nitrates and ammonium.

Keywords. Diatoms, periphyton, *Fragilaria*, new species, Portugal, rivers.

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Introduction

Diatoms are very important and well represented in all freshwater ecosystems, being among the four biological quality elements required in the Water Framework Directive (WFD, The European Parliament and European Council 2000) for freshwater biomonitoring, and are known to be sensitive to a wide variety of pressures (Kelly & Whitton 1995; Almeida & Gil 2001; Nunes *et al.* 2003). In the years 2011 and 2012, as part of a monitoring program in the rivers of Central Portugal, the epilithic diatom flora was sampled in the Vouga, Mondego and Lis river basins. During this project, identification problems were encountered within *Fragilaria* species.

During the last decades, a number of taxonomic studies have focused on *Fragilaria* taxa, this genus being the object of several reviews (Poulin *et al*. 1986; Williams & Round 1986, 1987). As a consequence, the taxonomy and systematics of Fragilariaceae has changed greatly over the years. Different authors have been using contrasting characters in order to delimitate species within this genus (Ognjanova-Rumenova *et al*. 1994; Morales 2003), although the systematic position of certain related genera is still not well resolved (e.g., Round 1984; Williams 2006).

Type material of *F. capucina* Desm. and *F. vaucheriae* (Kütz.) J.B.Petersen were photographed by Krammer & Lange-Bertalot (1991). Along with the photographs of these materials, 11 varieties of *F. capucina* were also presented: *F. capucina* var. *vaucheriae* (Kütz.) Lange-Bert., *F. capucina* var. *rumpens* (Kütz.) Lange-Bert. ex Bukht., *F. capucina* var. *perminuta* (Grunow) Lange-Bert., *F. capucina* var. *septentrionalis* (Østrup) Lange-Bert., *F. capucina* var. *distans* (Grunow) Lange-Bert., *F. capucina* var. *radians* (Kütz.) Lange-Bert., *F. capucina* var. *amphicephala* (Kütz.) Lange-Bert., *F. capucina* var. *austriaca* (Grunow) Lange-Bert., *F. capucina* var. *capitellata* (Grunow) Lange-Bert., *F. capucina* var. *mesolepta* (Rabenh.) Rabenh. and *F. capucina* var. *gracilis* (Østrup) Hust., commonly used in the last two decades in publications on diatoms. The group of species around *F. capucina* and *F. vaucheriae* is commonly recorded in European freshwaters. The taxonomic status of certain populations found in different regions is now the subject of scientific contributions (Delgado *et al.* 2015; Wetzel & Ector 2015).

The detailed study of the type material of *Fragilaria* sheds light on this complex genus, with few distinct structures. In this regard, light and scanning electron microscopy studies are very useful, together with the careful examination of the environmental characteristics of the sites. Therefore, the aim of this work is to describe *Fragilaria rinoi* Almeida & C.Delgado sp. nov., based on its ecology and detailed morphological features using light and scanning electron microscopy.

Material and methods

Study area and sampling

The study area located in Central Portugal is a region with a high economic value, but where anthropogenic activities have caused strong environmental pressures (Feio *et al.* 2007). The 51 samples analysed in this study were collected in 26 watercourses from three adjacent river catchments (total area of 11 215 km^2) in Central Portugal: Mondego, Vouga, and Lis river basins (Fig. 1). Water samples were collected in spring and autumn during the years 2011 and 2012. Environmental parameters such as water temperature (°C), pH, dissolved oxygen ($mg.l^{-1}$), oxygen saturation (% O_2) and electric conductivity ($\mu S.cm^{-1}$) were measured *in situ* with a Multiparameter Probe 3430 WTW portable meter. Water samples were collected into polypropylene bottles, stored at 4°C in darkness, and transported to the laboratory for analysis of the nutrients and cations according to conventional methods (APHA 1995).

Epilithic biofilms were collected simultaneously with the water samples. The sampling design, treatment procedure and study of diatom communities were based on European standards (CEN TC230 N68

2003), and consisted in removing epilithic biofilm from stones, by scraping their upper surface with a toothbrush. An aliquot of each sample (ca 3 ml), preserved in 4% formalin, was washed with distilled water and treated with HNO_3 (65%) and potassium dichromate ($K_2Cr_2O_7$) at room temperature for 24 hours. At least three centrifugations (1500 rpm) took place followed by rinsing with distilled water to remove oxidation by-products, and permanent slides were mounted with Naphrax®.

Type material

For comparative purposes, the type material of similar *Fragilaria* taxa was analysed. The type material of *F. vaucheriae*, deposited in the Natural History Museum (London) (Fig. 2) was observed and photographed under a Zeiss Ultra Plus VP FEG SEM operated at 7 kV and 20 mm distance. The dried sample was coated with a gold-palladium mixture before SEM observations.

Portuguese samples

Diatoms were observed under the light microscope Leitz Biomed 20 EB equipped with an immersion objective of 100× and NA 1.32. Light micrographs were taken using an Olympus DP70 camera attached to a Zeiss Axioplan 2 imaging microscope with differential interference contrast (DIC) and a 100× immersion objective (NA 1.40). Measurements of valve length, width and number of striae/10 µm were taken of at least 30 specimens per species, under the light microscope. A Hitachi SU-70 electron microscope operated at 7 kV and 10 mm distance was used for image acquisition of organic free samples,

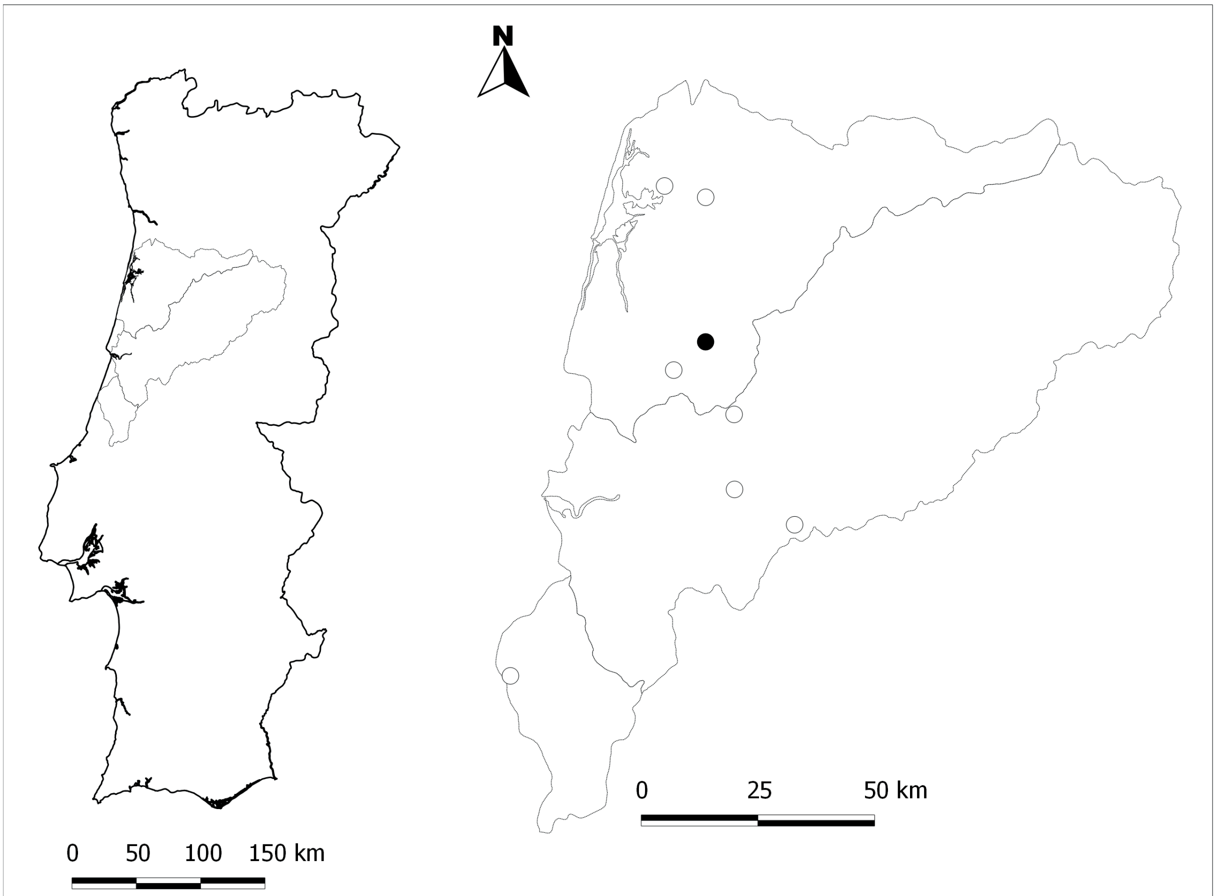


Fig. 1. Geographic locations of the sampling sites where *Fragilaria rinoi* Almeida & C.Delgado sp. nov. was found. The type locality is represented by a black circle in the Vouga river basin followed south by river Mondego and Lis basins.

which were air-dried on a metal stub coated with a thin pellicle of graphite (EMITECH K 950X). The dried sample was finally coated with a gold-palladium mixture (Polaron equipment limited SEM coating unit E5000) before SEM observations.

Ultrastructural analysis of areolae types, number and placement of rimoportulae, girdle bands, and apical pore fields were checked in SEM micrographs. Plates containing LM and SEM pictures were mounted using CorelDraw X5®.

Valve shape analysis

Geometric morphometry was used to assess differences between taxa and/or populations based on valve shape. To perform this analysis, the valve outline was captured as a geometric configuration of 400 non-homologous pseudolandmarks per individual using CLIC software (Dujardin *et al.* 2010), directly digitized from the LM micrographs. Illustrations of the type material of comparable taxa available in the literature were also digitized and used in this study:
1) Photographs and measurements of *Fragilaria intermedia* Grunow in Van Heurck (1881: pl. 45, fig. 11) from Tuji & Williams (2013);
2) Photographs of *F. vaucheriae* from the Botanic Garden Meise, Belgium (BR) (published in Wetzel & Ector 2015: 276, figs 2–23) and of *F. microvaucheriae* C.E.Wetzel & Ector (from Wetzel & Ector 2015: 284, figs 107–136);
3) From Delgado *et al*. (2015) we used the photographs of *F. candidagilae* Almeida, C.Delgado, Novais & S.Blanco (Delgado *et al*. 2015: 4, figs 2–33) with holotype in the Museum of Natural History, London (BM! 101 793) and isotype in the Hustedt Collection, Bremerhaven, Germany (ZU10/13), *F. recapitellata* Lange-Bert. & Metzeltin (Delgado *et al*. 2015: 10, figs 40–75), *F. perminuta* (Grunow) Lange-Bert. (Delgado *et al*. 2015: 10, figs 76–107), *F. capucina* (Delgado *et al*. 2015: 10, figs 108–122) and *F. neointermedia* Tuji & D.M.Williams (Delgado *et al*. 2015: 12, figs 132–149).

In total, 246 images were processed. The Cartesian coordinates of the pseudolandmarks were aligned (translated, rotated and scaled) by the Procrustes generalized orthogonal least-squared superimposition procedure (Rohlf & Slice 1990). Size, rotation and scale were additionally standardized by Bookstein's

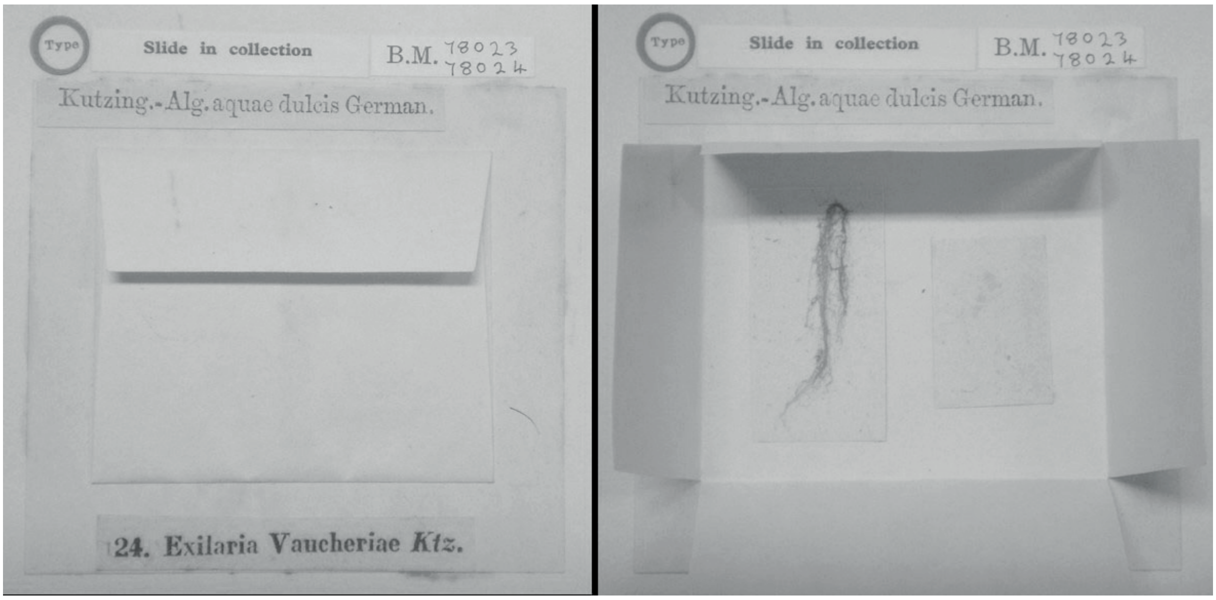


Fig. 2. Type material of *Fragilaria vaucheriae* deposited in the Natural History Museum of London (BM 78023, BM 78024).

transformation, forcing the two first pseudolandmarks onto the coordinates (0.0) and (1.0). A non-metric multidimensional scaling (NMDS) was carried out on the resulting normalized coordinates by means of the software PAST version 2.17 (Hammer *et al.* 2001), using Spearman's rank correlation coefficient (ρ) as the similarity measure. To visualize the size and shape of the scatter plot for each predefined group, the resulting groups were fitted to 95% confidence ellipses. Finally, a one-way non-parametric multivariate analysis of variance test (NPMANOVA, Anderson 2001) was performed between the transformed coordinates in order to test for significant differences between the *a priori* defined groups.

Main morphometric parameters (valve length and width, striae and areolae density) were measured directly on LM/SEM micrographs. The type material of comparable taxa illustrated in the literature were also measured and compared to the parameters obtained in the populations here described by means of a NPMANOVA (ρ 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.

Results

Phylum Ochrophyta Caval.-Sm. (Cavalier-Smith 1995)
Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska (Medlin & Kaczmarska 2004)
Subclass Bacillariophycidae Round (Round *et al.* 1990)
Order Fragilariales P.C.Silva (Silva 1962)
Family Fragilariaceae Grev. (Greville 1833)
Genus ***Fragilaria*** Lyngb. (Lyngbye 1819)

Fragilaria rinoi Almeida & C.Delgado sp. nov.
Figs 1, 3–82

Diagnosis

Valvae linearis lanceolatae ad rhombicae. Longitudo valvae 8.8–24.1 µm, latitudo valvae 4.2–5.6 µm. Striae uniseriate, alternantes, punctatae, 14–16 in 10 µm, parallelae in media parte, ad apices radiatae. Striae ex areolis rotundis, 13–14 in 1 µm, externe occlusae. Rimoportula una pro valva.

Etymology

The new species is dedicated to Prof. Jorge Rino (Aveiro, Portugal) who carried out valuable research on the ecology and biology of freshwater algae during the second half of the twentieth century. His passionate and unique way of teaching encouraged students to pursue this study area.

Type material

Holotype

Slide BM 101 794, prepared with material from the sample collected in Mogofores, housed in the Natural History Museum, London (UK), illustrated in Figs 3–62. The valve representing the holotype is illustrated in Fig. 9.

Isotype

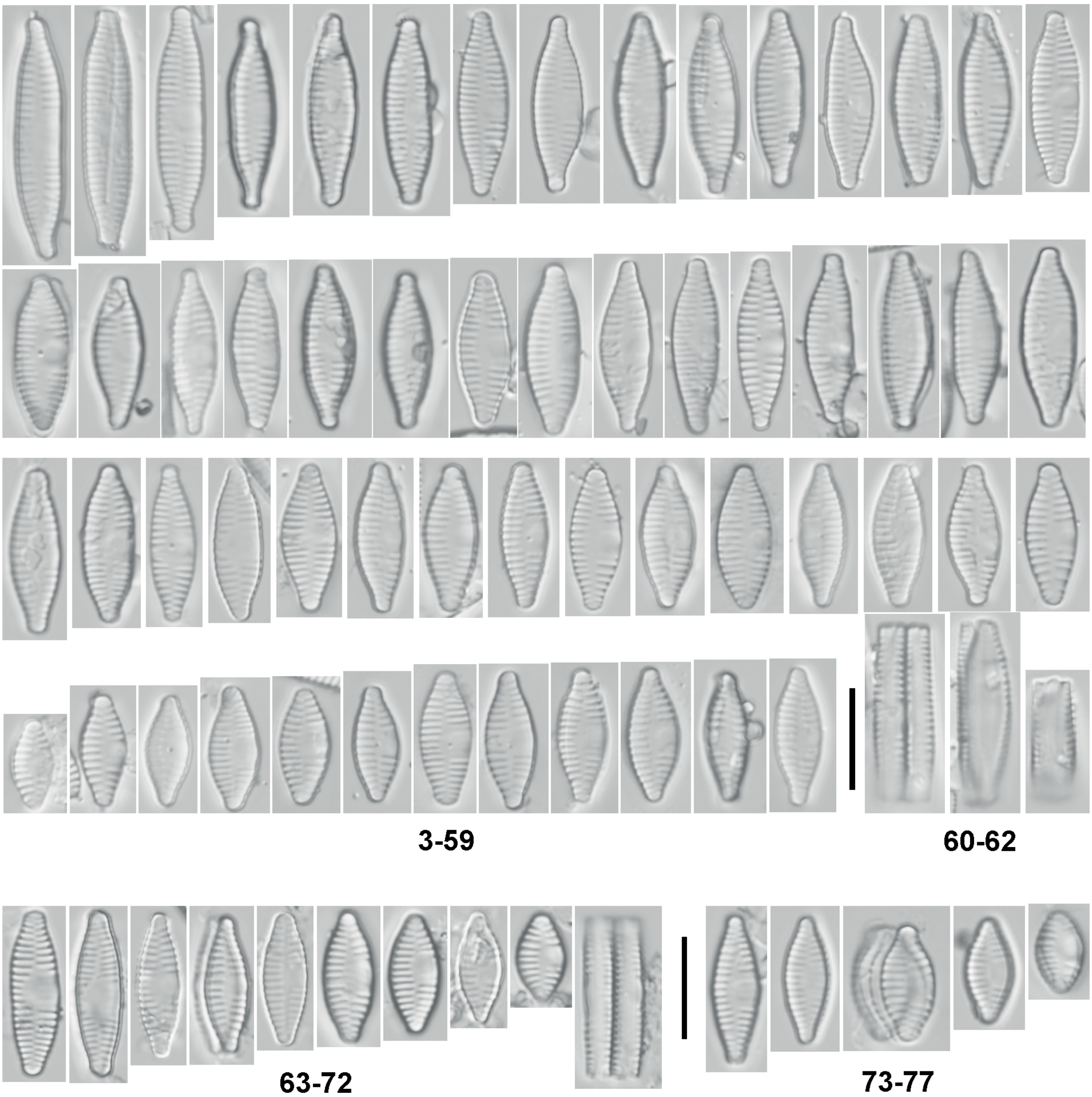
Slide ZU10/14, prepared from the sample Mogofores, housed at the Friedrich Hustedt Diatom Collection, Alfred-Wegener- Institut für Polar- und Meeresforschung, Bremerhaven, Germany (BRM).

Type locality

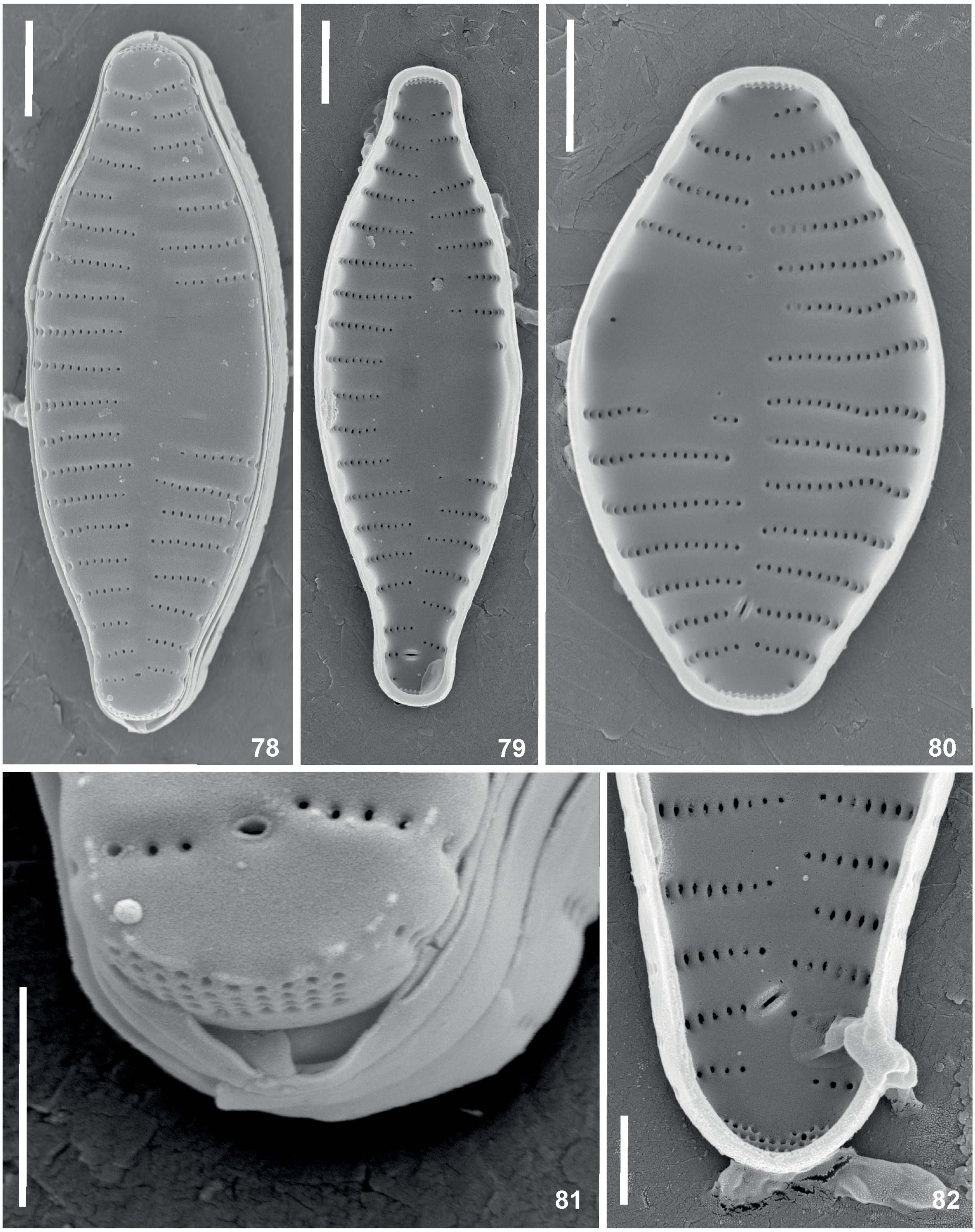
PORTUGAL: Cértima River, Vouga Basin, Mogofores, municipality of Coimbra, district of Coimbra, Coimbra subregion, Centro Region, Portugal, coordinates 40°27.17′ N, 8°27.575′ W, river epilithon, 21 Mar. 2012, *Carmen L. Elias and Cristina Delgado*.

Description

LM observations (Figs 3–77). *Fragilaria rinoi* sp. nov. is characterized by the presence of solitary cells with lanceolate valves and slightly rostrate apices in larger specimens to rhombic lanceolate in smaller specimens. Frustules rectangular in girdle view with interruption of striation in the middle portion. Axial



Figs 3–77. Light microscope images of *Fragilaria rinoi* Almeida & C.Delgado sp. nov. **3–62**. Mogofores (Cértima river, Vouga river basin, 21 Mar. 2012) type material (BM 101 794; Fig. 9 is from the holotype). **63–72**. Estarreja (Antuã river, Vouga river basin, 22 Mar. 2012). **73–77**. Foz do Ceira (river Ceira, Mondego river basin, 20 Mar. 2012). Scale bar = 10 µm.



Figs 78-82. Scanning electron microscope images of *Fragilaria rinoi* Almeida & C.Delgado sp. nov. from Mogofores (river Cértima, Vouga river basin), 21 Mar. 2012 (Holotype BM 101 794). **78**. Complete valve in external view. **79–80**. Complete valves in internal view. **81**. Detail of an apex in external view, with the apical pore field and rimoportula. **82**. Detail of an apex in internal view, with the rimoportula clearly visible. Scale bars: 78–80, 82 = 2 µm; 81 = 1 µm.

Table 1. Minimum (Min), maximum (Max) and averages (Avg) of 14 physical and chemical variables and water velocity (n = 8 samples) corresponding to the ecological spectrum of the presence of *Fragilaria rinoi* Almeida & C.Delgado sp. nov. in Portugal.

Variable	Min	Max	Avg
Water temperature (ºC)	7.4	21.5	14.5
pH	7.0	7.8	7.4
Conductivity (µS.cm^{-1})	43	765	418
TDS (mg.l^{-1})	20.0	355.0	202.6
Dissolved oxygen (%)	82	103	88
Dissolved oxygen (mg.l^{-1})	2.1	11.7	7.9
Water velocity (m.s^{-1})	0.1	0.4	0.3
N-NH_4^+ (mg N.l^{-1})	0.2	3.8	1.7
N-NO_3^- (mg.l^{-1})	0.2	7.8	3.7
P-PO_4^{3-} (mg PO_4^{3-}.l^{-1})	0.3	2.9	1.8
Total-P (mg P.l^{-1})	0.2	1.4	0.9
Alkalinity (mg $CaCO_3$.l^{-1})	15.2	350.0	165.2
Chlorides (mg Cl.l^{-1})	7.6	47.8	31.4
Hardness (mg $CaCO_3$.l^{-1})	16.7	301.7	148.1
Silica (mg SiO_2.l^{-1})	2.4	8.5	6.3

area narrow, linear, central area larger, unilateral in all specimens. Valve dimensions (n = 30): length 8.8–24.1 µm and width 4.2–5.6 µm. Sternum narrow, slightly widening towards the central area (Figs 3–77). Striae parallel to the transapical axis becoming slightly radiate at the poles, 14–16 in 10 µm.

SEM observations (Figs 78–82). External valve face without spines. A single rimoportula is present at one pole and might vary from apically oriented (Fig. 79) to almost transapical orientation (Figs 80, 82). Striae uniseriate, composed of round areolae (13–14 areolae in 1 µm) on both valves (Figs 78–80). Each valve has two apical pore fields (APF) composed of simple fine porelli arranged in regular rows parallel to the apical axis (Fig. 78) and made up of 4 rows, each composed of 10 to 11 poroids (Fig. 81). Outer and inner areolar openings without siliceous depositions (Figs 78–82).

Distribution and ecology

Fragilaria rinoi sp. nov. was found in the epilithon, but always in low relative abundances. From a total of 51 samples collected in the river basins of Vouga, Mondego and Lis, *F. rinoi* sp. nov. was identified in 8 samples (Fig. 1), but only in two of them with abundance above 1%. These two samples were located in Estarreja (Antuã river; Mar. 2012, with relative abundance – r.a. – of about 1.6%) and Mogofores (Cértima river; Mar. 2012, r.a. of about 2.8%) (Fig. 1), in the Vouga watershed. The rest of the localities where *F. rinoi* sp. nov. occurred were: Chãs (Lis basin, 0.9%), Carvalhal (Vouga basin, 0.7%), Foz do Ceira (Mondego basin, 0.5%), Lousã Piscinas (Mondego basin, 0.5%), Casal do Ermio (Mondego basin, 0.2%) and Botão (Mondego basin, 0.2%). The averages, the maximum and minimum values of the 14 physical and chemical variables are presented in Table 1, indicating that the optimal temperature for this new taxon is 14.5°C. *Fragilaria rinoi* sp. nov. was present in rivers with medium to high conductivity (43–765 µS.cm^{-1}), neutral to slightly alkaline pH (7.0–7.8) and relatively high nitrate-nitrogen (3.7 mg.l^{-1}), ammonium (0.2–3.8 mg.l^{-1}) and phosphorus (0.2–1.4 mg P.l^{-1}). This diatom occurs under high dissolved oxygen concentrations (between 82% and 103%) and silica concentrations between 2.4 and 8.5 $mg.l^{-1}$ (Table 1).

Associated diatoms

The assemblage of the type locality (Mogofores) was dominated by *Planothidium frequentissimum* (Lange-Bert.) Lange-Bert. (26.2%), *Sellaphora seminulum* (Grunow) D.G.Mann (15.9%) and *Gomphonema saprophilum* (Lange-Bert. & E.Reichardt) Abarca *et al*. (11.1%). Species with abundances higher than 3% were *Amphora pediculus* (Kütz.) Grunow (5.3%), *Encyonema ventricosum* (C.Agardh) Grunow (5.3%), *Eolimna subminuscula* (Manguin) Gerd Moser, Lange-Bert. & Metzeltin (5.1%), *Geissleria ignota* (Krasske) Lange-Bert. & Metzeltin (4.6%), *Cyclotella meneghiniana* Kütz. (4.4%), *Nitzschia amphibia* Grunow (4.4%) and *Navicula veneta* Kütz. (3.9%). Other diatom taxa with abundance lower than 3% were *Fragilaria rinoi* sp. nov. (2.8%), *Navicula reichardtiana* Lange-Bert. (2.1%), *Nitzschia palea* (Kütz.) W.Sm. (1.9%), and *Cocconeis euglypta* Ehrenb. (1.6%).

The diversity value of this diatom community was high (Shannon-Wiener Index = 3.6).

Fragilaria vaucheriae (Kütz.) J.B.Petersen
Figs 2, 83–90

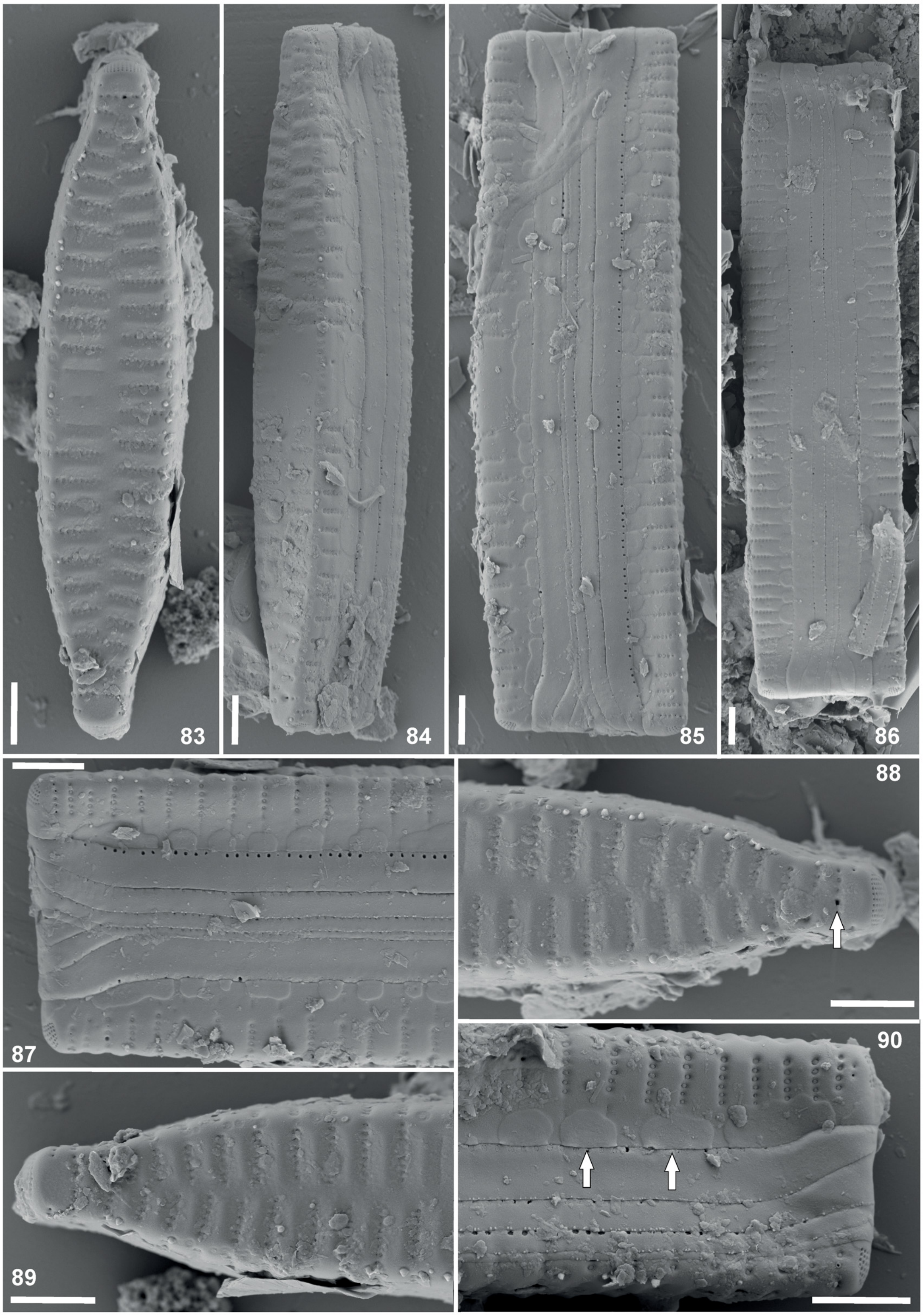
Exilaria vaucheriae Kütz., *Linnaea* 8: 560 (1833a). – Type: Alg. Dec. III. No. 24 (syntypes: BM 78023, BM 78024).

The type material of *Fragilaria vaucheriae* (Kützing 1833b) was published in Kützing's exiccata set *Algarum Aquae Dulcis Germanicarum* (Decas III, No. 24) without either description or figure. This material was observed by Lange-Bertalot (1980, pl. 4, figs 82–94, 97–102) with a broader concept that was later narrowed in Krammer & Lange-Bertalot (2004: pl. 108, figs 10–15). In the present study, the type material from the Natural History Museum of London was photographed with a scanning electron microscope (Figs 83–90). Micrographs from the recently published paper by Wetzel & Ector (2015), which were acquired from Van Heurck's collection housed at the Botanic Garden Meise, Belgium (BR) were also used for the morphometrical measurements of *F. vaucheriae*.

Description

Fragilaria vaucheriae is characterized by the presence solitary cells with valves linear and narrow and rostrate to subcapitate ends (Fig. 83). Frustules rectangular in girdle view with interruption of striation in the middle portion (Figs 84–85). Axial area narrow, linear, central area larger than *F. rinoi* sp. nov., unilateral in all specimens. Striae coarse, uniseriate, parallel to the transapical axis and slightly radiate at the poles (Figs 83, 88). External valve face presents small spines in some specimens (Figs 83, 88). A single rimoportula is present at one pole, aligned with the first stria at the valve face apex (Figs 83, 88). Girdle bands are open (Figs 84, 85, 87), with small, unoccluded perforations. Striae composed of round areolae (12–13 areolae in 1 µm) on both valves (Figs 83, 89). Each valve has two apical pore fields (APF) composed of simple fine porelli arranged in regular rows parallel to the apical axis (Fig. 83) and made up of 6 rows, each composed of 10 to 11 poroids (Figs 85–88). Outer areolar are closed with siliceous depositions (Figs 83–90). Siliceous plaques are present along the valve mantle edge (Figs 85, 87, 90).

The photographs of the other *Fragilaria* species (*Fragilaria candidagilae*, *F. recapitellata*, *F. perminuta*, *F. neointermedia*, *F. intermedia*, *F. capucina* and *F. microvaucheriae*) were further digitally measured and included in comparative boxplots and scatterplots here discussed (Figs 91–92). Whenever possible



Figs 83-90. SEM micrographs of the type material of *Fragilaria vaucheriae* (Kütz.) J.B.Petersen. **83**. Complete valve in external view. **84**. Complete frustule in external view. **85–86**. Frustule in girdle view. **87, 90**. Detail of the apex in girdle view, with the girdle bands and fimbriae clearly visible (indicated by the arrows). **88–89**. Detail of the apex in external view (arrow indicates the rimoportula). Scale bars = 2 µm.

Table 2. Morphometric and morphological comparison between the type material of *Fragilaria rinoi* Almeida & C.Delgado sp. nov. (type population) and related taxa. NPMANOVA results, Fisher's F statistic (F) and Bonferroni-corrected p values (p) are shown (significant values highlighted in bold).

		Fragilaria candidagilae	*Fragilaria capucina*	*Fragilaria intermedia*	*Fragilaria microvaucheriae*	*Fragilaria neointermedia*	*Fragilaria perminuta*	*Fragilaria recapitellata*	*Fragilaria vaucheriae*
Length (in µm)	F	9.481	191.2	49.2	7.238	188.4	0.02824	238.3	79.91
	p	0.0924	**0.0028**	**0.0028**	0.2632	**0.0028**	1	**0.0028**	**0.0028**
Width (in µm)	F	22.87	64.27	8.615	67.55	50.95	29.64	134	33.15
	p	**0.0028**	**0.0028**	0.1092	**0.0028**	**0.0028**	**0.0028**	**0.0028**	**0.0028**
Striae density (in 10 µm)	F	43.31	11.38	49.67	4.797	76.05	11.94	389	119.3
	p	**0.0028**	0.0672	**0.0028**	0.7196	**0.0028**	**0.0252**	**0.0028**	**0.0028**
Outline	F	143.9	80.8	128.1	1.417	93.57	239.1	139.7	0.8493
	p	**0.028**	**0.028**	**0.028**	1	**0.028**	**0.028**	**0.028**	1

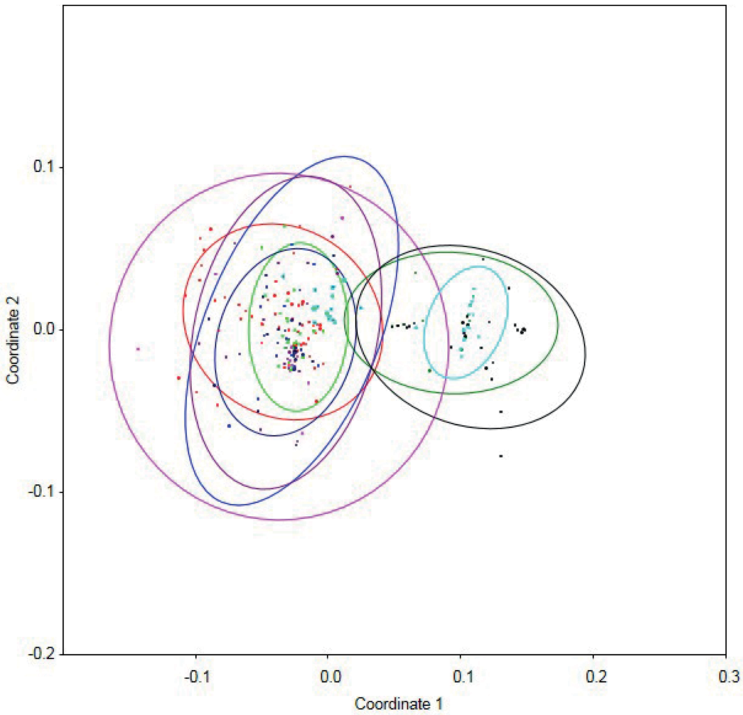


Fig. 91. Comparison of the type population of *Fragilaria rinoi* Almeida & C.Delgado sp. nov. with the type material of related taxa. Data fitted to 95% confidence ellipses. NMDS plot of Bookstein-transformed valve outline pseudolandmark coordinates. Black: *F. rinoi* sp. nov. Red: *F. candidagilae* Almeida, C.Delgado, Novais & S.Blanco. Blue: *F. capucina* Desm. Pink: *F. intermedia* Grunow in Van Heurck. Olive green: *F. microvaucheriae* C.E.Wetzel & Ector. Purple: *F. neointermedia* Tuji & D.M.Williams. Green: *F. perminuta* (Grunow) Lange-Bert. Dark blue: *F. recapitellata* Lange-Bert. & Metzeltin. Sky blue: *F. vaucheriae* (Kütz.) J.B.Petersen.

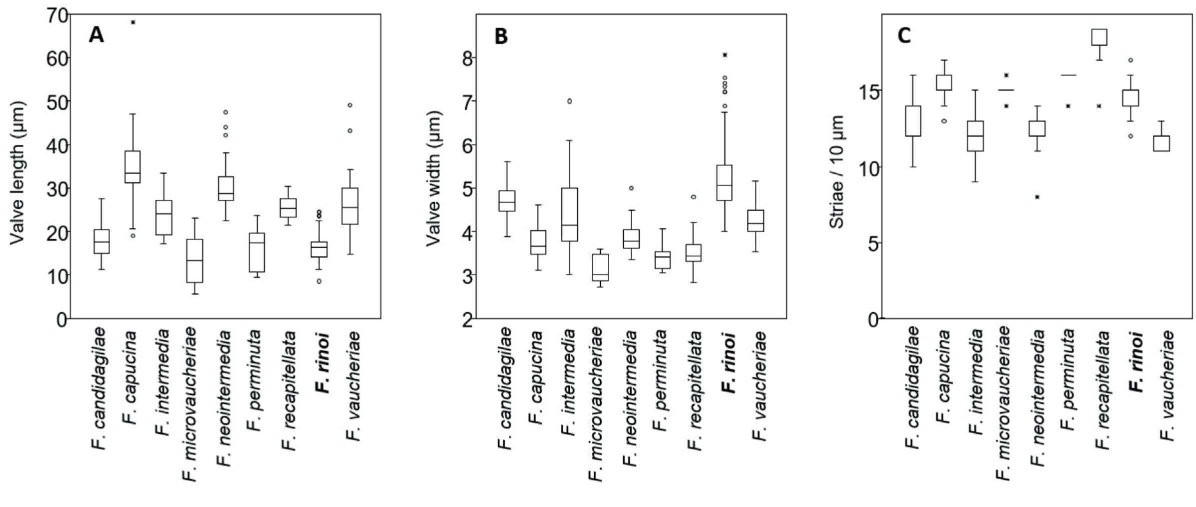


Fig. 92. Boxplot of measurements of different *Fragilaria* species. Variation in valve length (a), width (b) and striae in 10 µm (c) measured in the type populations. The boxes represent the 25–75% quartiles, the median is shown with a horizontal line inside the box, and minimum and maximum values are shown with short horizontal lines (whiskers). Outliers are plotted as individual points.

(i.e., when valves were more than 20 µm long) four measures of striae density were taken from each valve.

Discussion

Fragilaria rinoi sp. nov. from Mogofores, the type locality (Figs 3–62: LM; Figs 78–82: SEM) and from other localities (Figs 63–77, LM: Mogofores 21 Mar. 2012; Estarreja 22 Mar. 2012; Foz do Ceira 20 Mar. 2012) were compared with the type material of *Fragilaria candidagilae*, *F. recapitellata*, *F. perminuta*, *F. neointermedia*, *F. intermedia*, *F. capucina* (light micrographs presented in Delgado *et al*. 2015) and *F. vaucheriae* (data from micrographs of the type material presented in Wetzel & Ector 2015).

Fragilaria rinoi sp. nov. may be confused, in the first instance, with *F. microvaucheriae* in terms of length, striae density and outline (Table 2). However, the geometric morphometric analysis and the comparison with the type material from Wetzel & Ector (2015) revealed that *F. rinoi* sp. nov. is wider (4.2–5.6 µm vs 2.5–3.8 µm) than *F. microvaucheriae*. The comparison of *F. rinoi* sp. nov. with the type material of *F. recapitellata* and *F. neointermedia* revealed that *F. rinoi* sp. nov. is smaller and wider.

According to the geometric morphometric analysis (Figs 91–92), the valve outline of the type population of *F. rinoi* sp. nov. partially overlaps with different *Fragilaria* species, although it is statistically different ($p = 0.0015$, Table 2). The shape and morphometry of *Fragilaria rinoi* sp. nov. is statistically different from the one of *F. recapitellata* and *F. neointermedia*. Compared to *F. vaucheriae*, the species here described is statistically different in all the parameters except in outline (Table 2). *Fragilaria rinoi* sp. nov. has similarities in length with *F. candidagilae* and *F. perminuta*, but is statistically different in width, striae density and outline ($p = 0.0015$, Table 2). *Fragilaria rinoi* sp. nov. is only similar to *F. capucina* in striae density and to *F. intermedia* in width.

The morphology of *F. rinoi* sp. nov. overlaps with the one of *F. candidagilae* in terms of length, but the new species is slightly wider (4.2–5.6 µm vs 4.5–5.0 µm), has denser striation than *F. candidagilae* (14–16 vs 12–14 striae in 10 µm) and slightly rostrate apices, instead of the typical strongly capitate apices present in *F. candidagilae*.

The ecology of *F. rinoi* sp. nov. is different from that of *F. microvaucheriae* because *F. microvaucheriae* is present in rivers with low conductivity, never over 230 µS cm^{-1} and low nutrient concentration (Wetzel & Ector 2015); while *F. rinoi* sp. nov. is present in waters with medium to high conductivity and high nutrient concentration.

Conclusions

Fragilaria rinoi sp. nov. is a new diatom species that was differentiated from similar species using valve morphology and geometric morphometric comparisons using light and scanning electron microscopy. This study shows that *F. rinoi* sp. nov. is not abundant in central Portuguese rivers, but can appear in several rivers from adjacent basins. An additional difficulty was the low number of specimens and images used in the literature for the description of new taxa or even for the redefinition of others. *Fragilaria rinoi* sp. nov. is present in rivers with high dissolved oxygen concentrations, medium to high conductivity, neutral to slightly alkaline pH and high nutrients concentrations. This study contributes to improving the knowledge of the benthic diatom flora in Portugal.

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