Pinnularia aljustrelica sp. nov. (Bacillariophyceae), a new diatom species found in acidic waters in the Aljustrel mining area (Portugal) and further observations on the taxonomy and ecology of *P. acidophila* Hofmann et Krammer and *P. acoricola* Hustedt

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Abstract: A new benthic freshwater diatom species belonging to the genus *Pinnularia* Ehrenb. has been recorded in the Água Forte stream surrounding the Aljustrel mining area in southern Portugal. *Pinnularia aljustrelica* Luís, Almeida et Ector sp. nov. is described as a new species based on light and scanning electron microscopy observations and on its particular habitat in an acidic environment due to acid mine drainage, high metal concentrations (As, Cd, Cu, Fe, Mn, Pb and Zn) and high sulphate and conductivity.
The taxa most similar to *P. aljustrelica* are *P. acidophila* Hofmann et Krammer, *P. acoricola* Hustedt and *P. acoricola* var. *lanceolata* Hustedt, so type materials of these taxa were studied for comparative purposes. Although, the ecology of the three similar taxa is also quite similar, the new species has a combination of particular morphological characteristics studied under LM and SEM that separates it from the rest. *Pinnularia aljustrelica* has a general shape relatively similar to *P. acidophila* and *P. acoricola*, but the valve outline is not as linear as in *P. acidophila* and not as oval as in *P. acoricola.*

Key words: *Pinnularia aljustrelica*, new species, morphology, extreme acidic environment, Portugal

Introduction

The pyrite tailings of Aljustrel (Southern Portugal) are characterized by a total lack of vegetation and the nearby streams are strongly affected by acid mine drainage (AMD) produced when pyritic minerals are exposed to weathering, resulting in very low pH (formation of sulphuric acid), high conductivity, elevated sulphate content and high concentrations of dissolved metals as arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn) (Skousen et al. 1994). A new diatom species was found in this area during a survey in the 2008–2009 period. The new taxon resembled other species currently ascribed to *Pinnularia*, a genus commonly reported from similar highly acidic conditions. The acidic conditions increase bio–availability of metals (Luís et al. 2009), thus making the monitoring of the ecosystem through biological studies highly necessary.

Diatoms are one of the principal groups of organisms in these affected streams; making them excellent bio–indicators based on their high sensitivity to changes in environmental conditions (Bahls 1993; Stevenson & Pan 1999; Blinn & Herbst 2003). Based on literature data (Carter 1972; Whitton & Diaz 1981; Negoro 1985; Cassie & Cooper 1989; Watanabe & Asai 1995; Sabater et al. 2003), one of the most widespread taxa in acidic waters is apparently *Pinnularia acoricola* Hustedt, described in 1935 in Java from sulphate–rich waters with a pH of 2.4. *Pinnularia acoricola* var. *lanceolata* Hustedt was also discovered in Indonesia (Lake Toba, Sumatra), as a fossil freshwater diatom (Hustedt 1935). Lessmann et al. (1999) recorded *P. acoricola* in an extreme acidic lake with high concentrations of calcium, iron, aluminium, manganese and sulphate. This taxon has a variable morphology resembling *P. obscura* Krasske (Carter 1972). Both species, *P. acoricola* and *P. obscura,* were observed in waters with low pH in England (Hargreaves et al. 1975), North America (Whitton & Diaz 1981) and South Africa (DeNicola 2000). Apparently, these diatoms seem to be well adapted to low pH conditions allowing them to develop large communities in otherwise inhospitable environmental conditions. The morphology of *P. acoricola* is clearly influenced by the ecological conditions resulting in a high variety of sizes and shapes, e.g. *P. acoricola* var. *osoresanensis* Negoro described in Japan by Negoro (1944), then transferred at the species level by Fukushima et al. (2002) as *P. osoresanensis* (Negoro) Fukushima, Yoshitake et Ko–Bayashi. Recently, *P. acidophila* Hofmann et Krammer in Krammer (Krammer 2000) was described from Eastern Germany in a lake resulting from an opencast mining of low pH.

The main purpose of the present work is to study the *Pinnularia* populations of the Aljustrel mining area in southern Portugal. For this purpose, the type materials of *P. acidophila*, *P. acoricola* and *P. acoricola* var. *lanceolata* were investigated in order to clarify the differences and similarities of these species with the Southern Portuguese *Pinnularia.* This detailed examination by means of light (LM) and scanning electron microscopy (SEM), as well as the particular environmental characteristics of the streams surrounding this mining area lead us to propose a new *Pinnularia* species, here described in detail.

Material and Methods

One litre of water was collected from the surface, as close to the centre of the stream as possible, in Ponte Monte Ruas and Porto de Beja sites (Aljustrel mining area – Southern Portugal, Fig. 1), simultaneously with the diatom sampling. Then, the surface water samples were returned in a cool box to the laboratory and stored at 4 °C before the analyses according to the protocols proposed by German Chemists Association (1981), EPA–Environmental Protection Agency (1982) and ASTM (1984). Temperature (T, °C), pH and electrical conductivity (EC, µS.cm^{-1}, at 25 °C) were measured directly in the field with a WTW® Multiline P4 set.

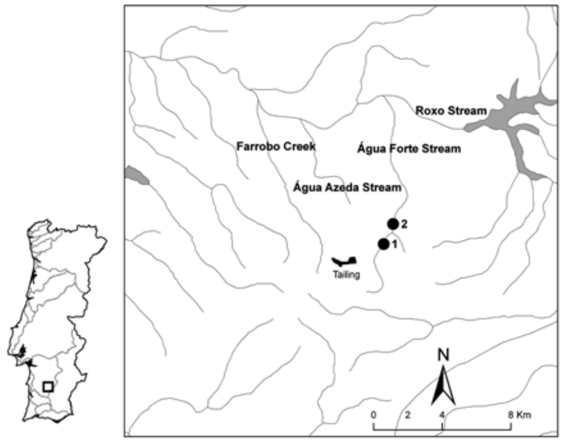


Fig. 1. Sampling sites where *Pinnularia aljustrelica* was found (Água Forte stream near the Aljustrel tailing): (1) Ponte Monte Ruas; (2) Porto de Beja.

The determination of As, Ca, Cd, Cl, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn was carried out using ICP–MS. The determination of COD, NH_4^+ and PO_4^{3-} was done by spectrophotometry. For NO_3^- and SO_4^{2-} determination, ion chromatography was used (all the concentrations of the chemical elements were expressed in mg.l^{-1}).

Epipsammic diatoms were collected from the sediment with a syringe, four times at Ponte Monte Ruas (site 1 – 37º52'22.87''N; 8º8'59.42''W), twice in 2008 (spring and summer) and twice in 2009 (spring and winter). Porto de Beja (site 2 – 37º53'0.69''N; 8º8'36.83''W) was sampled only once, in spring 2008. Both sites are located in the Água Forte stream that surrounds the Aljustrel mining area.

Four samples from Hofmann's and Hustedt's type material collections were also examined for this study:
– Material received from G. Hofmann (holotype material of *Pinnularia acidophila* Hofmann et Krammer, corresponding to the slide number 61B IOK in Krammer collection); Locality: Braunkohlerestsee, Lausitz, Restloch 107, 0–2 cm (lake resulting from an opencast mining, Lausitz, Eastern Germany); Collection date: 21/05/1996; Collector name: G. Hofmann.
– Material number AS403 (lectotype material of *Pinnularia acoricola* Hustedt); Locality: Java, Dieng–Plateau, D3b, Telaga Pengilan, Schwingrasen; Substratum: *Sphagnum*; Collection date: 06/03/1929; Collector name: F. Ruttner; Friedrich–Hustedt–Zentrum für Diatomeenforschung, Alfred–Wegener Institut für Polar und Meeresforschung, Bremerhaven (BRM).
– Material number AS895 (material with abundant *Pinnularia acoricola* Hustedt); Locality: Sumatra, Lake Toba, TW1d, W–Ufer Samosir, N–Sigaol; Substratum: mud; Collection date: 04/11/1929; Collector name: F. Ruttner; Friedrich–Hustedt–Zentrum für Diatomeenforschung, Alfred–Wegener

Institut für Polar und Meeresforschung, Bremerhaven (BRM).
– Material number AS956 (holotype material of *Pinnularia acoricola* var. *lanceolata* Hustedt); Locality: Sumatra, Lake Toba, TD4, fossil, West Coast of Samosir Island; Substratum: diatomite; Collection date: 01/01/1929; Collector name: F. Ruttner; Friedrich–Hustedt–Zentrum für Diatomeenforschung, Alfred–Wegener Institut für Polar und Meeresforschung, Bremerhaven (BRM).

The samples from Aljustrel mining area were treated in the laboratory with nitric acid (HNO_3 65%) and potassium dichromate ($K_2Cr_2O_7$) at room temperature for 24 h, followed by three centrifugations (1500 rpm) to wash the excess of acid, then treated with hydrochloric acid (HCl 37%) during more than 24 h to disaggregate the valves, and then cleaned again, through three centrifugation cycles (1500 rpm) to wash the excess of HCl. The samples from Hofmann's and Hustedt's collections were rinsed out of fixatives, treated by oxidation with hot hydrogen peroxide (H_2O_2 120 vols.) and hydrochloric acid, and then rinsed three times with deionised water in order to obtain a suspension of clean frustules. Permanent slides were mounted using the high refractive index (1.74) medium Naphrax® (Brunel Microscopes Ltd, UK). Light microscopy observations were taken using a Leica® DMRX light microscope (DIC contrast) with a 100 x oil immersion objective and LM photographs were taken with a Leica® DC500 camera. Samples chosen for scanning electron microscopy were filtered through polycarbonate membrane filters with a 3 µm pore diameter, mounted on stubs with double sided carbon tape, sputtered with platinum (30 nm) with a Modular High Vacuum Coating System (Bal–Tec MED 020) and studied with a Hitachi SU–70, operated at 5.0 kV. Morphometric parameters were obtained from LM and SEM photographs: a total of 162 valves (110 of *Pinnularia aljustrelica* sp. nov., 23 of *P. acidophila*, 28 of *P. acoricola* and 1 of *P. acoricola* var. *lanceolata* type materials) were measured.

Morphological terminology follows Round et al. (1990) and Krammer (2000). Apart from the original descriptions of *P. acidophila*, *P. acoricola* and *P. acoricola* var. *lanceolata*, the following publications were consulted for taxonomical and ecological comparison (Table 1): Hustedt in Schmidt et al. (1934), Hustedt (1935), Negoro (1944), Carter (1972), Simonsen (1987), Krammer (2000), Jordan (2001), Wydrzycka & Lange–Bertalot (2001), Fukushima et al. (2002), Van de Vijver et al. (2002) and Metzeltin et al. (2005).

Bibliographical references of similar taxa with illustrations, which made the comparison possible with our populations, were also used e.g., Negoro (1985), Watanabe & Asai (1995), Idei & Mayama (2001), Jordan (2001) and Hobbs et al. (2009).

All images were digitally manipulated and plates made using Microsoft Photo Editor 3.01, Microsoft PowerPoint 2000 SP–3 and Adobe Photoshop Elements v. 2.0.

The map of the sites where the new *Pinnularia* species (Fig. 1) was found was generated using GIS software ArcGis 9.3 (ESRI 2008).

Results and Discussion

Pinnularia aljustrelica Luís, Almeida et Ector sp. nov.

Figs 2–21: LM, Figs 157–161: SEM

Diagnosis: Valvae anguste lanceolatae ad rhombicae–lanceolatae apicibus non protractis, late rotundatis. Longitudo 15.9–24.0 µm, latitudo 3.0–4.1 µm. Area axialis angusta, linearis. Area centralis rhombica formans fasciam leviter ad moderate latam, rotundam. Raphe recta poris proximalibus unilateraliter deflexis. Fissurae terminales raphis curvatae ad idem latus valvae. Striae transapicales fortiter radiatae in media parte valvae, abrupte valde convergentes ad apices, 15–18 in 10 µm.

Holotype (here designated): slide BR–4230 (National Botanic Garden, Meise, Belgium).
Isotype (here designated): slide BRM–ZU8/11 (Friedrich Hustedt Diatom Collection, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany).
Type locality: Ponte Monte Ruas, Água Forte stream (Ribeira da Água Forte), municipality of Aljustrel, subregion of Baixo Alentejo (District: Beja), Portugal; collector: Ana T. Luís; collection date: 07/04/2008; geographical coordinates: 4192136.37 N, (29)574774.42 W; Etymology: The specific epithet *aljustrelica* refers to the name of the type locality.

Light microscopy (Figs 2–21)

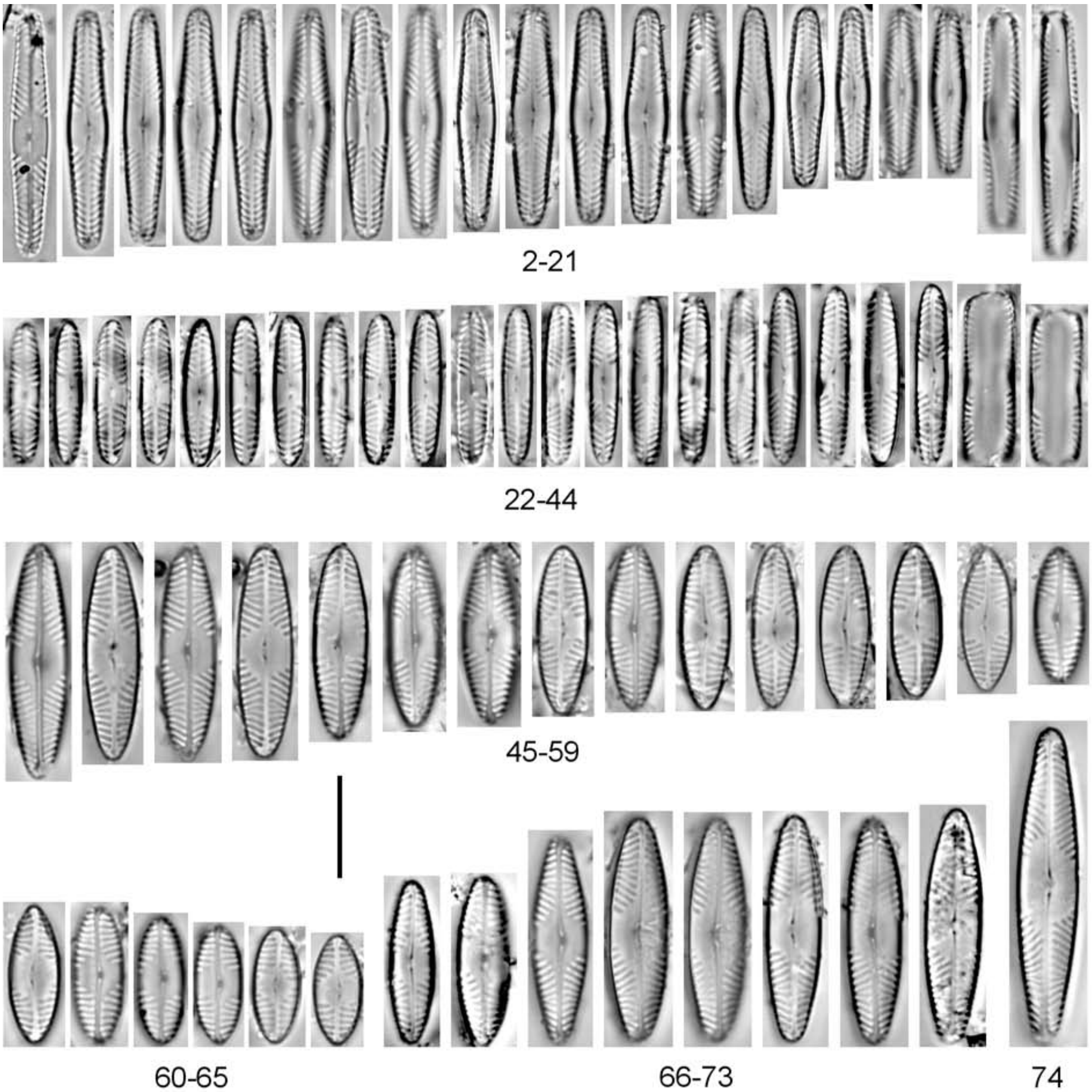
Valves narrowly lanceolate to rhombic–lanceolate, gradually tapering towards the broadly rounded, non protracted apices. Valve dimensions of the type population (n=19): length 15.9–24.0 µm, width 3.0–4.1 µm (Table 2). Axial area narrow, linear. Central area rhomboid forming a small to moderately large, rounded fascia. Raphe straight with unilaterally deflected proximal raphe endings. Transapical striae strongly radiate near the valve centre, changing abruptly to convergent towards the apices, 15–18 in 10 µm.

Table 1. Comparison between *Pinnularia aljustrelica* and the morphologically and ecologically most similar taxa (all data from the literature with the exception of *P. aljustrelica* Luís, Almeida et Ector).

Pinnularia [References]	*aljustrelica* Luís, Almeida et Ector (This study)	*acidicola* Van de Vijver et Le Cohu (Van de Vijver et al. 2002)	*acidophila* Hofmann et Krammer (Krammer 2000)	*acoricola* Hustedt (Hustedt 1935)	*acoricola* var. *elongatirhombica* Wydrzycka, Lange–Bertalot et Metzeltin (Wydrzycka & Lange-Bertalot 2001)	*acoricola* var. *lanceolata* Hustedt (Hustedt 1935; Krammer 2000)	*osoresanensis* (Negoro) Fukushima, Yoshitake et Ko–Bayashi (Negoro 1944; Fukushima et al. 2002)	*subacoricola* Metzeltin, Lange–Bertalot et García–Rodríguez (Metzeltin et al. 2005)
Valve length (µm)	15.9–24.0	24–40	12–22	15–30	16–47	30–32	7–29	10–30
Valve width (µm)	3–4.1	4.5–7	3–3.3	4–6	4.5–7	7–7.8	3–5.5	3.5–4.5
Valve outline	Narrowly rhombic	Linear with convex sides to linear–elliptical	Linear–lanceolate; straight to weakly convex sides	Elliptico–lanceolate	Rhomboid–lanceolate and larger, gradually attenuated since the central area until the ends	More lanceolate	Linear–elliptic transapical shells	Linear–elliptic to rhombic–lanceolate
Valve apices	Rounded	Protracted	Cuneiform	Rounded	Rounded	Pointed	More or less distended middle blunt rounded ends	Less cuneately or obtusely rounded
Central area	Rhomboid	Rhomboid	Broad fascia	Medium to large circular space or a complete fascia	Medium to large circular space or a complete fascia	Large circular space or more often, a break in the striae reaching to the margin	A slight swelling in the central area	Very large because of striae limitation in the distal parts of the valve
Number of striae in 10 µm	15–18	11–12	13–16	13–15	13–16	12–13	15–19	15

Table 1 Cont.

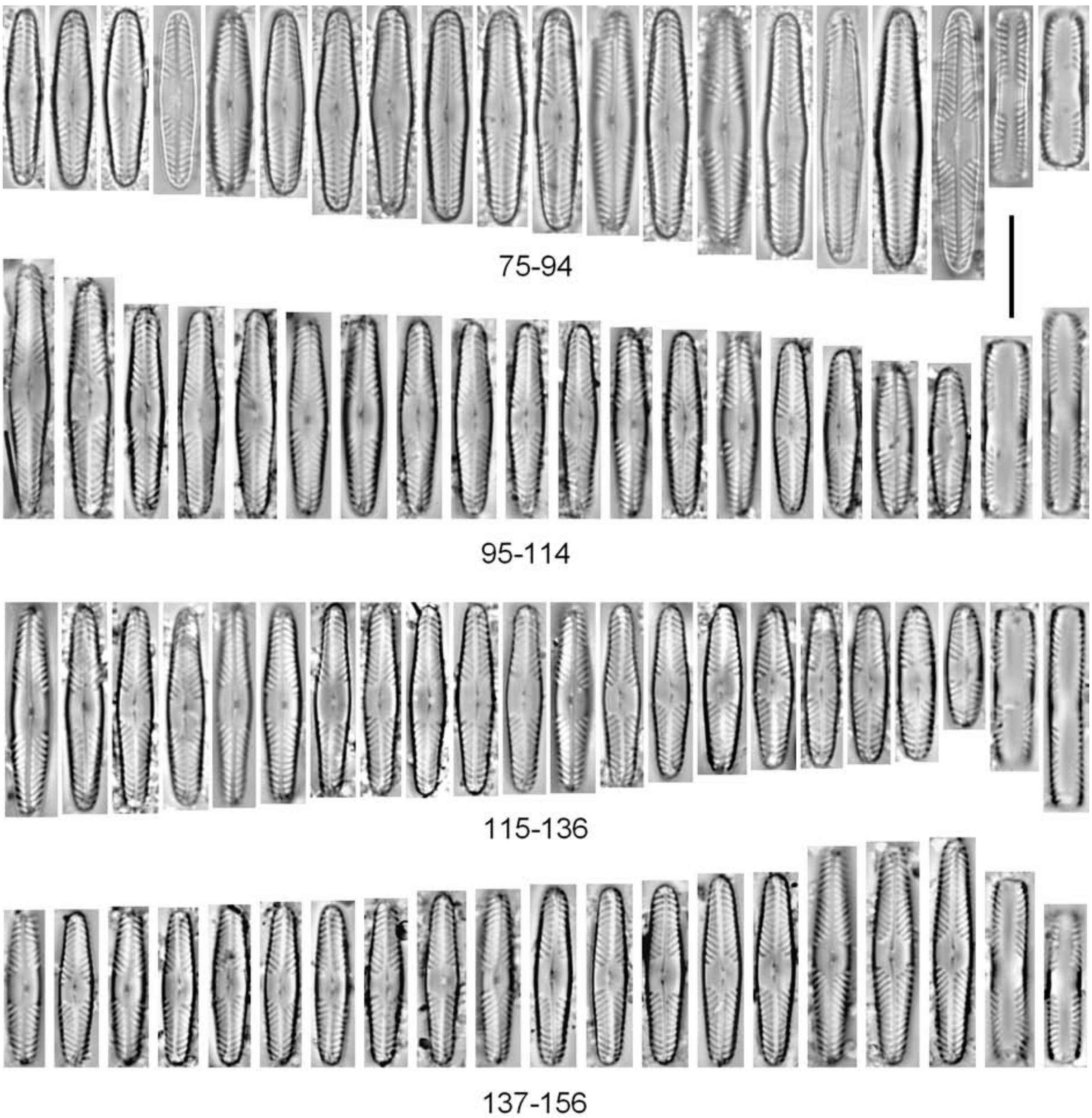
Striation pattern	Striae abruptly changed from strongly convergent at the poles to strongly radial at the center	Transapical striae parallel to slightly radiate in the middle, convergent near the poles	Radiate in the middle and strongly convergent at the ends	Strongly convergent at the poles and strongly radial at the center	Strongly convergent at the poles and strongly radial at the center	Strongly convergent at the poles and strongly radial at the center	Striae transapical radiated in the medium and convergent in the poles	3–7 subpolars pairs of convergent striae and 1–3 pairs of radiate striae
Raphe	Straight; at central nodule bent to one side	Filiform, with distinct, weakly deflected central pores	Filiform, weakly deflected in the middle	Straight, slightly diverting in the central pores	Straight in the larger specimens and curved in the smaller, with central terminations curved to one side	Nearly straight; at central nodule bent to one side	Filiform	Filiform
Ecology	Present in acidic streams with pH variation of 1.9–2.2, high sulphate and metal concentrations (As, Cd, Cu, Fe, Mn, Pb, Zn) and high conductivity due to dissolved ions and not to organic contamination	Quite common in all habitats: relatively wet (>60%) acid soils to wet mosses and acid pools and lakes with low specific conductance (<90 $\mu S\ cm^{-1}$)	Abundant in type locality, an opencast mining lake; low pH water and 99.5% acidobiontic taxa in association	First time found in Java (pH~3) in sulphated waters; acidophilic taxa that can change form due to high temperatures and acidic waters and in some cases, present in waters with high metal concentrations (e.g. Al, Fe)	Preference of really acidic waters and rich in minerals (sulphuric and chlorhydric acidity); huge variability of valve forms	Optimum pH is 5 but lives in waters of pH 2.6	High–saline Isobe–mineral springs (temperature 15.3 °C, pH 6.5) in the Gumma Prefecture	Different pH values in cave (reduced light regime), far from the sea



Figs 2–21. *Pinnularia aljustrelica* sp. nov. Luís, Almeida et Ector. Light micrographs of the type population sampled in Ponte Monte Ruas in spring 2008, Água Forte stream (Aljustrel, Portugal): (2–19) valvar view; (20–21) girdle view.
Figs 22–44. *Pinnularia acidophila* Hofmann et Krammer. Light micrographs of the type population: (22–42) valvar view; (43–44) girdle view. Figs 45–65. *Pinnularia acoricola* Hustedt. Light micrographs of the type population in valvar view.
Figs 66–73. *Pinnularia acoricola* Hustedt. Light micrographs, in valvar view (found in the type material of *Pinnularia acoricola* var. *lanceolata* Hustedt).
Fig. 74. *Pinnularia acoricola* var. *lanceolata* Hustedt. Light micrograph, in valvar view (holotype material). Scale bar 10 µm.

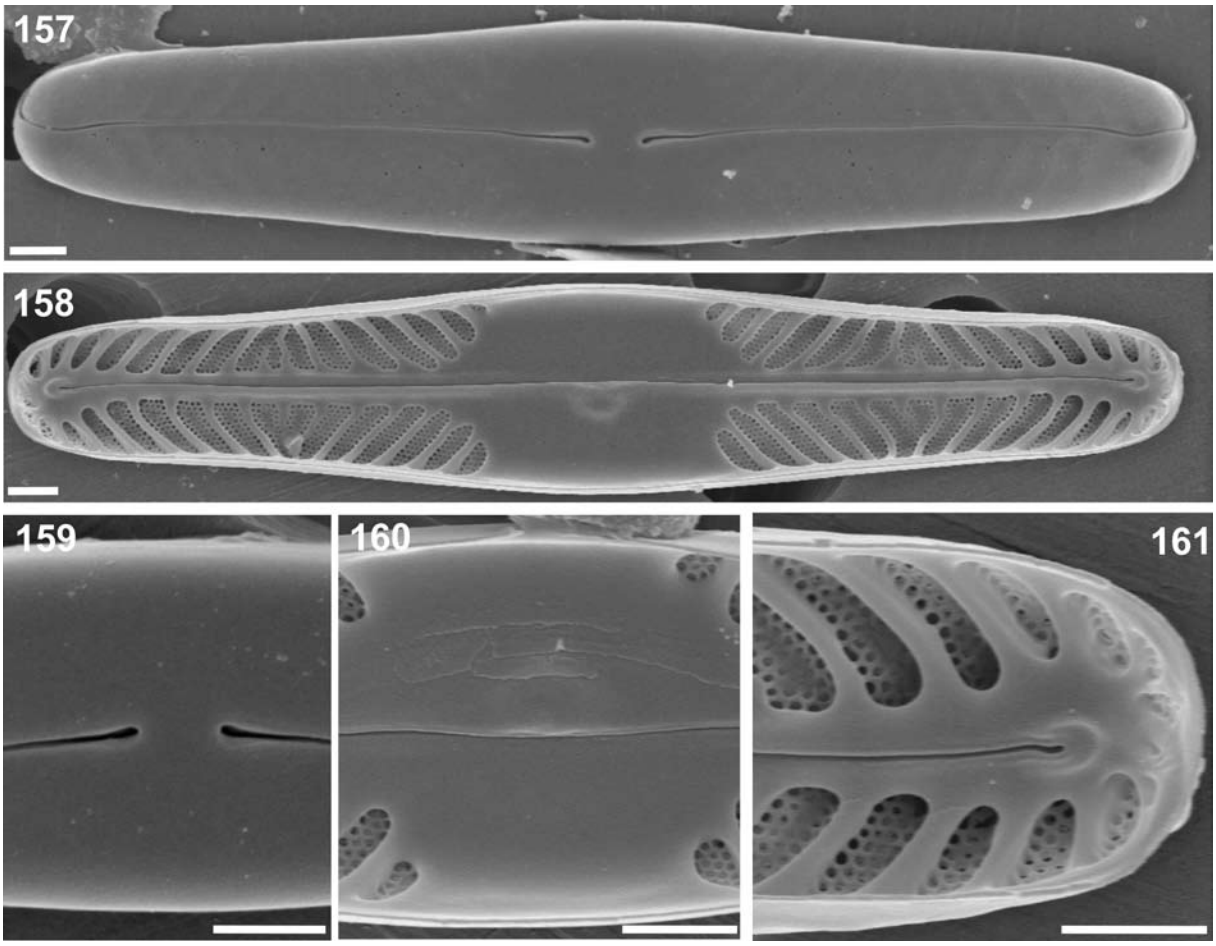
Scanning electron microscopy (Figs 157–161)
Proximal raphe endings weakly expanded, never drop–like (Fig. 159). The terminal raphe endings are curved towards the secondary side of the valves while the central pores are deflected to the primary side of the valve (Fig. 157). Each alveolus composed of 3–5 rows of very small, rounded pores, usually externally covered by a membrane (Figs 160, 161). Internally, the raphe fissure shows no intermission near the central nodule (Fig. 160) and the distal raphe endings terminate on small helictoglossae (Fig. 161).

Morphometric variability
The type population of *Pinnularia aljustrelica* shows a length range between 15.9 and 24.0 µm, a width of 3.0 to 4.1 µm and a stria density of 15–18 in 10 µm. The other three populations observed at



Figs 75–156. *Pinnularia aljustrelica* sp. nov. Luís, Almeida et Ector: Figs 75–94. Light micrographs of the population sampled in Ponte Monte Ruas in summer 2008, Água Forte stream (Aljustrel, Portugal): (75–92) valvar view; (93–94) girdle view. Figs 95–114. Light micrographs of the population sampled in Ponte Monte Ruas in spring 2009, Água Forte stream (Aljustrel, Portugal): (95–112) valvar view; (113–114) girdle view. Figs 115–136. Light micrographs of the population sampled in Ponte Monte Ruas in winter 2009, Água Forte stream (Aljustrel, Portugal): (115–133) valvar view; (135–136) girdle view. Figs 137–156. Light micrographs of the population sampled in Porto de Beja in spring 2008, Água Forte stream (Aljustrel, Portugal): (137–154) valvar view; (155–156) girdle view. Scale bar 10 µm.

Ponte Monte Ruas presented a slightly different range in valve dimensions (Table 2). During the summer of 2008 (Figs 75–94), valves showed a length ranging from 11.3 to 25.7 µm, a width of 3.0 to 3.9 µm and a stria density of 14–17 in 10 µm. In spring 2009 (Figs 95–114) the valve length varied between 14.3–24.3 µm with a width of 3.0–3.8 µm and 16–18 striae in 10 µm. Finally, in the sample taken in winter 2009 (Figs 115–136, 173–177), the observed length varied between 11.5–20.0 µm, while the width ranged between 3.1–3.7 µm and the striae were 15–18 in 10 µm. The Porto de Beja population (Figs 137–156) had a range in length of 14.5 to 21.7 µm, width of 3.0 to 3.8 µm and 16–18 striae in 10 µm. A total of 110 valves were measured for all populations (length of 11.3 to 25.7 µm, width of 3.0 to 4.1 µm, stria density of 14–18 in 10 µm).



Figs 157–161. *Pinnularia aljustrelica* sp. nov. Luís, Almeida et Ector. Scanning electron micrographs of the type population sampled in Ponte Monte Ruas in spring 2008, Água Forte stream (Aljustrel, Portugal): (157) external valve view: terminal fissure curved in the same direction and the terminal raphe endings deflected to the primary side; (158) internal valve view; (159) external view of the raphe with the proximal endings weakly expanded; (160) internally, the raphe fissure shows no intermission near the central nodule; (161) internal view of the apex, showing the distal raphe endings terminating on small helictoglossae, the areolae features of the striae and the structure of the alveoli. Scale bars 1 µm.

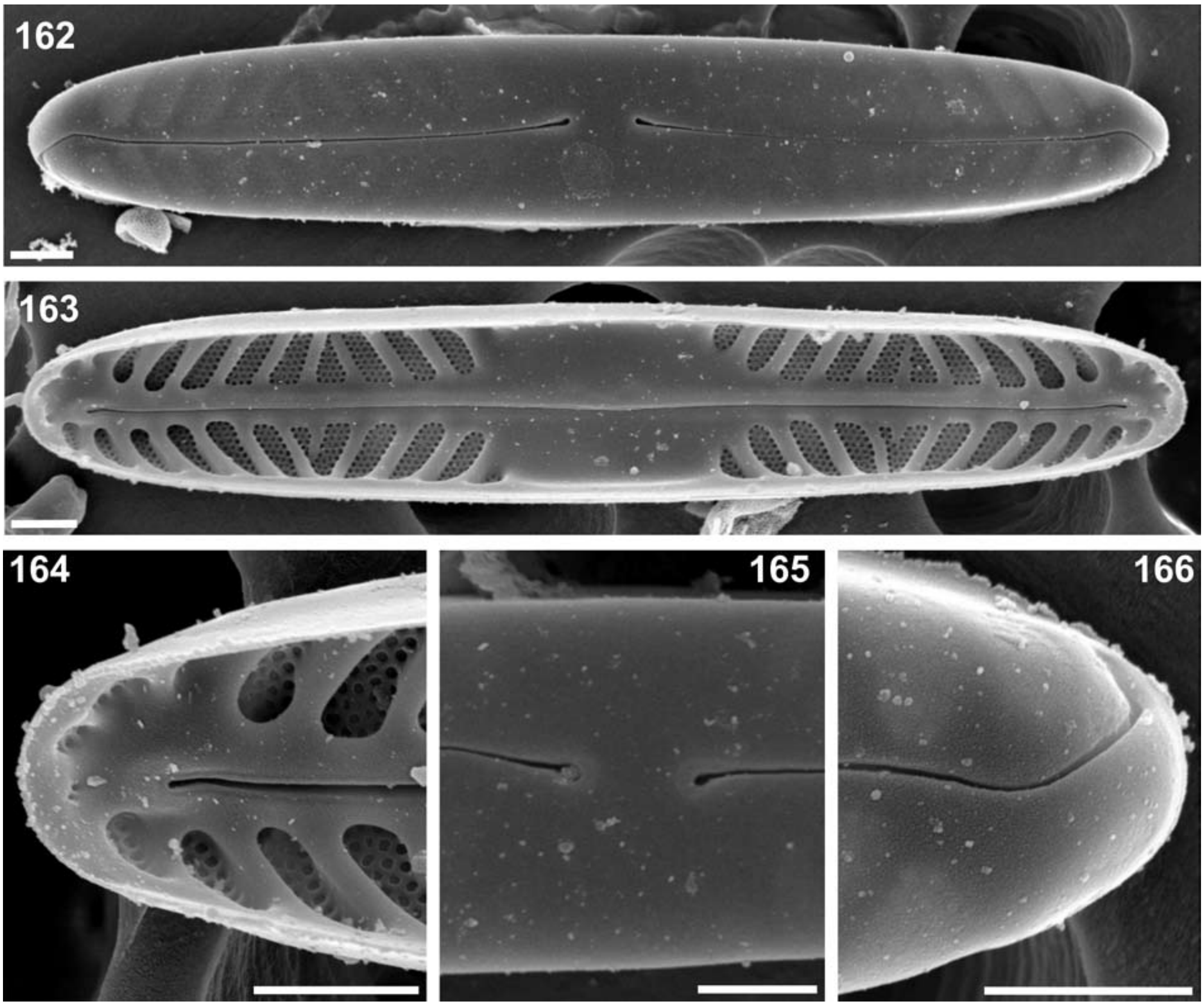
Ecology, distribution and associated diatom flora

The ecological preferences of the new *Pinnularia* species were inferred based on environmental variables from two sites of the Água Forte stream in the Aljustrel mining area (Portugal). *Pinnularia aljustrelica* dominates the investigated stream Água Forte. Table 3 shows all environmental parameters. Both sites have highly acidic waters with a rather constant low pH (1.9–2.3), high sulphate levels (16995–36900 mg.l^{-1} SO_4^{2-}), high metal concentrations (As: 1.90–52.17 mg.l^{-1}; Cd: 1.62–2.48 mg.l^{-1}; Cu: 130–348 mg.l^{-1}; Fe: 3688–6173 mg.l^{-1}; Mn: 66–204 mg.l^{-1}; Pb: <0.01–1.20 mg.l^{-1}; Zn: 766–1202 mg.l^{-1}) and high conductivity (12200–17140 µS.cm^{-1}) caused by dissolved ions in the water. The water temperature varied between 9.1–29.2 ºC. In total, *P. aljustrelica* was recorded in two sites of the Água Forte stream at five sampling moments with abundances above 91%.

Comparison with related taxa

The most similar taxa to *Pinnularia aljustrelica* are *P. acidophila* and *P. acoricola* (Table 4).

The first taxon in this comparison is *P. acidophila* (Figs 22–44, 162–166). Our analysis of the type material of *P. acidophila* shows some differences in valve length (13.6–17.3 µm), width (2.5–3.1 µm) and number of striae in 10 µm (15–18) compared with the original description of Krammer (2000): 12–22 µm in length, 3.0–3.3 in width, 13–16 striae in 10 µm. The main differences between *P. acidophila* and *P. aljustrelica* include a narrower valve width and a more linear to lanceolate valve outline lacking the larger width in the central area (Figs 22–42) instead of lanceolate to rhombic shape in *P. aljustrelica* (Figs 2–19).



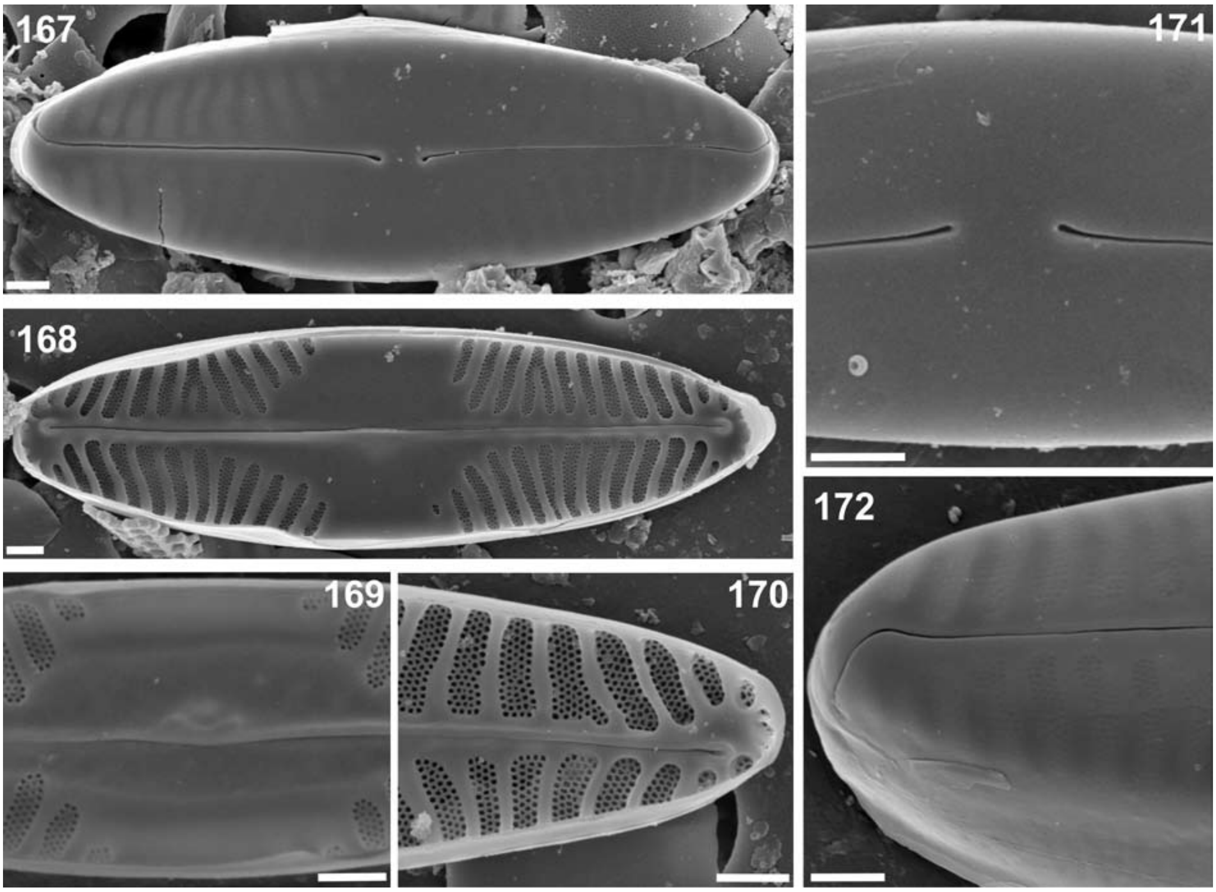
Figs 162–166. *Pinnularia acidophila* Hofmann et Krammer. Scanning electron micrographs of the type population: (162) external view of the entire valve; (163) internal view of the entire valve; (164) internal view of the apex, showing the raphe termination, the areolae features of the striae and the structure of the alveoli; (165) external view of the raphe in the central area; (166) external view of the apex, showing the distal raphe ending. Scale bars 1 µm.

The valve apices in *P. acidophila* are cuneiform and never rounded, and the raphe is filiform and weakly deflected in the middle.

Pinnularia acoricola was described in 1935 by Hustedt. Carter emended the original description in 1972 based on the extremely high variability in shape and size of *P. acoricola*: the valves range from lanceolate to oval with a length varying between 8 to 34 µm and a width of 3 to 6 µm. The apices are rounded and the stria density varied between 13 and 17 in 10 µm. Our analyses of the *P. acoricola* type material (Figs 45–65, 167–172) revealed a range of 10.6–22.9 µm in length, 4.3–5.6 µm in width and 14–18 striae in 10 µm (Table 4). Nevertheless, *P. acoricola* differs sufficiently from *P. aljustrelica* by the valve outline.

Pinnularia acoricola has an elliptic–lanceolate valve outline instead of the more lanceolate to narrowly rhombic–lanceolate valves of *P. aljustrelica* furthermore, the valves are wider than those of *P. aljustrelica*.

Pinnularia acoricola var. *lanceolata* (Fig. 74), a third taxon used for comparison, recently recombined under the name of *P. acoricolatoba* Kulikovskiy, Lange–Bertalot et Metzeltin (Kulikovskiy et al. 2010), has acutely rounded apices combined with a typical lanceolate valve outline contrary to *P. aljustrelica* that has broadly rounded apices and a more rhombic–lanceolate outline. Moreover, the central area in *P. acoricola* var. *lanceolata* forms a rather elliptically–shaped fascia, a feature never observed in *P. aljustrelica*. *Pinnularia acoricola* var. *lanceolata* valves are larger, wider and have a lower number of striae in 10 µm than *P. aljustrelica* sp. nov. In the type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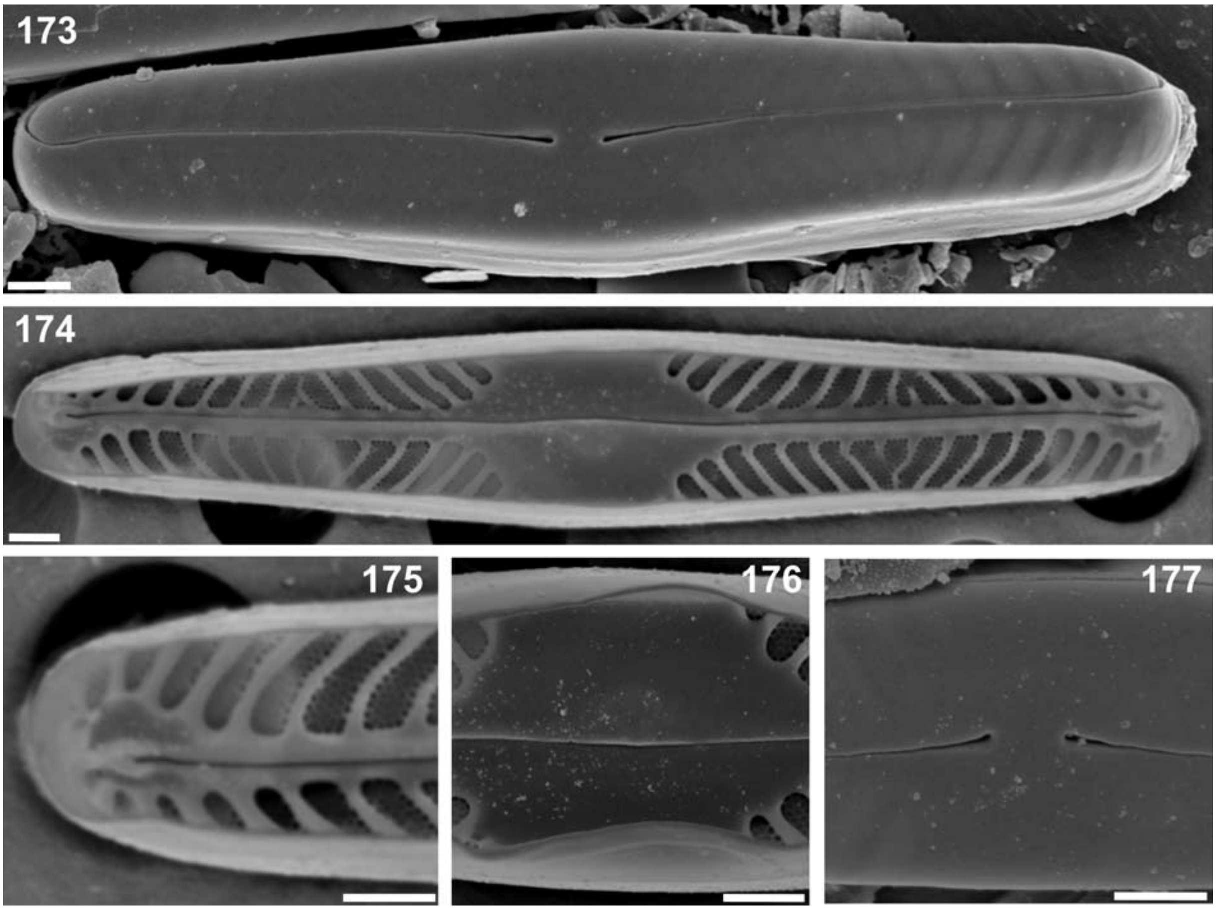
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Table 2. Morphometric data of the populations of *Pinnularia aljustrelica* (Água Forte stream). Minimum, maximum (and medium) values for the most important parameters: length, width and number of striae (total n=110).

Samplings	Spring 2008	Summer 2008	Spring 2009	Winter 2009	Spring 2008	Total 2008 & 2009
Sites	Ponte Monte Ruas (type) (n=19)	Ponte Monte Ruas (n=24)	Ponte Monte Ruas (n=18)	Ponte Monte Ruas (n=30)	Porto de Beja (n=19)	Ponte Monte Ruas – Porto de Beja (n=110)
Length (µm)	MIN: 15.9 MAX: 24.0 AVG: 20.7	MIN: 11.3 MAX: 25.7 AVG: 19.7	MIN: 14.3 MAX: 24.3 AVG: 18.6	MIN: 11.5 MAX: 20.0 AVG: 17.4	MIN: 14.5 MAX: 21.7 AVG 16.9	MIN: 11.3 MAX: 25.7 AVG: 18.7
Width (µm)	MIN: 3.0 MAX: 4.1 AVG: 3.6	MIN: 3.0 MAX: 3.9 AVG: 3.6	MIN: 3.0 MAX: 3.8 AVG: 3.3	MIN: 3.1 MAX: 3.7 AVG: 3.4	MIN: 3.0 MAX: 3.8 AVG: 3.3	MIN: 3.0 MAX: 4.1 AVG: 3.4
Number of striae in 10 µm	MIN: 15 MAX: 18 AVG: 16	MIN: 14 MAX: 17 AVG: 15	MIN: 16 MAX: 18 AVG: 16	MIN: 15 MAX: 18 AVG: 16	MIN: 16 MAX: 18 AVG: 17	MIN: 14 MAX: 18 AVG: 16

Figs 167–172. *Pinnularia acoricola* Hustedt. Scanning electron micrographs of the type population: (167) external view of the entire valve; (168) internal view of the entire valve; (169) internal view of the raphe in the central area; (170) internal view of the apex, showing the raphe termination, the areolae features of the striae and the structure of the alveoli; (171) external view of the raphe in the central area; (172) external view of the apex, showing the distal raphe ending. Scale bars 1 µm.

Figs 173–177. *Pinnularia aljustrelica* Luís, Almeida et Ector. Scanning electron micrographs of the population sampled in Ponte Monte Ruas site in winter 2009, Água Forte stream (Aljustrel, Portugal): (173) external view of the entire valve; (174) internal view of the entire valve; (175) internal view of the apex, showing the raphe termination and the areolae features of the striae; (176) internal view of the raphe in the central area; (177) external view of the raphe in the central area. Scale bars 1 µm.

slide of *P. acoricola* var. *lanceolata* we only found one valve (Fig. 74) that maybe can be identified as *P. acoricola* var. *lanceolata*, which means that the variety is very rare. Figs 66–73 represent *P. acoricola* var. *acoricola* valves found on the type slide of *P. acoricola* var. *lanceolata*. Fortunately Simonsen (1987, pl. 254, figs 18–21) could illustrate four valves of *P. acoricola* var. *lanceolata* (Holotype: P1/4, Tobasee, Sumatra, TD4, marked specimens named on the label); this variety differs from the nominate variety of *P. acoricola* by much larger size dimensions, rhombic–lanceolate area and more widely spaced central raphe endings (Kulikovskiy et al. 2010).

Several authors (Whitton & Satake 1996; Sabater et al. 2003; Gerhardt et al. 2008; Hobbs et al. 2009; Urrea–Clos & Sabater 2009) reported *P. acoricola* as an abundant taxon in AMD affected sites, the species being often reported as a common taxon in acidic environments all over the world: in Java sulphuric streams with pH of 2.8 to 3.0 (Carter 1972), in Europe with pH of 1.5 to 3 (Whitton & Diaz 1981) and in New Zealand waters with pH below 1 (Cassie & Cooper 1989). Negoro (1985) regularly found this species in acidic habitats in Japan, with pH 2–4 and temperatures of 20.5–46.8 °C. According to Whitton et al. (2000), *P. acoricola* has been recorded at by far the lowest pH values for any diatom, with records as low as pH 0.2, though critical studies to confirm viability have not yet been made for the lowest pH values. Watanabe & Asai (1995) reported this taxon also from Japan, in sites having a pH below 1.1 whereas Sabater

Table 3. Values of 13 physical and chemical variables and 7 trace metals, for the two sampling sites, in spring and summer of 2008 and spring and winter of 2009 (COD: Chemical Oxygen Demand).

Samplings	Spring 2008	Summer 2008	Spring 2009	Winter 2009	Spring 2008
	Ponte Monte Ruas (type)	Ponte Monte Ruas	Ponte Monte Ruas	Ponte Monte Ruas	Porto de Beja
pH	1.9	2.3	2.1	2.0	2.2
Conductivity (µS.cm^{-1})	17140	14200	12200	14100	12670
Temperature (ºC)	18.5	29.2	18.6	9.1	21.0
NH_4^+ (mg. l^{-1})	0	0	0	0	0
NO_3^- (mg. l^{-1})	0	295	0	546	0
PO_4^{3-} (mg.l^{-1})	<0.02	0.24	<0.02	2.00	<0.02
COD (mg.l^{-1})	722	657	14	30	166
Cl^- (mg.l^{-1})	157	184	122	74	144
SO_4^{2-} (mg. l^{-1})	28239	16995	22601	36900	20169
Na^+ (mg.l^{-1})	63	88	47	40	65
K^+ (mg.l^{-1})	<5000	<5	<5000	8	<5000
Mg^{2+} (mg.l^{-1})	953	438	668	366	652
Ca^{2+} (mg.l^{-1})	539	250	424	434	375
Si^{3+} (mg.l^{-1})	89	45	59	34	83
As (mg.l^{-1})	48.50	30.00	1.90	52.17	21.70
Cd (mg.l^{-1})	2.48	1.70	1.80	2.10	1.62
Cu (mg.l^{-1})	348	130	265	216	248
Fe (mg.l^{-1})	6173	3688	6157	4842	4543
Mn (mg.l^{-1})	204	89	117	66	142
Pb (mg.l^{-1})	<0.01	0.01	0.30	1.20	<0.01
Zn (mg.l^{-1})	1202	970	766	1112	776

et al. (2003) found this taxon in an acid stream in south–western Spain, the Rio Tinto, located in the same geological unit Iberian Pyrite Belt (IPB) as the Aljustrel mining area. Jordan (2001) also reported that *P. acoricola* was found in acidic waters, with a pH optimum around 5, in thermal waters, frequently associated with high iron concentrations. The waters where Negoro (1944) found the species had high concentrations of aluminium and iron. All Great Britain localities, where this species was found, are typically acidic pounds, that, due to mining activity, have high concentrations of copper, iron and zinc (J.R. Carter, pers. comm.). It is highly likely that this acidobiontic species may not always correspond to *P. acoricola* and might have been confused in the past with other *Pinnularia* species.

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Table 4. Morphometric data of the type populations of *Pinnularia aljustrelica*, *P. acidophila* and *P. acoricola.* Minimum, maximum (and medium) values for the most important parameters: length, width and number of striae.

Type material	*Pinnularia aljustrelica* Luís, Almeida et Ector	*Pinnularia acidophila* Hofmann et Krammer	*Pinnularia acoricola* Hustedt
n	19	23	28
Length (µm)	MIN: 15.9 MAX: 24.0 AVG: 20.7	MIN: 13.6 MAX: 17.3 AVG 15.5	MIN: 10.6 MAX: 22.9 AVG: 16.2
Width (µm)	MIN: 3.0 MAX: 4.1 AVG: 3.6	MIN: 2.5 MAX: 3.1 AVG: 2.8	MIN: 4.3 MAX: 5.6 AVG 4.8
Number of striae in 10 µm	MIN: 15 MAX: 18 AVG: 16	MIN: 15 MAX: 18 AVG: 17	MIN: 14 MAX: 18 AVG: 16

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