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Morphology and ecology of *Achnanthidium caravelense* (Bacillariophyceae), a new species from Portuguese rivers

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With 81 figures and 4 tables

Abstract: A new benthic freshwater diatom species belonging to the genus *Achnanthidium* Kütz. has been recorded from several watercourses in the North of Portugal. *Achnanthidium caravelense* Novais et Ector is described as a new species based on light and scanning electron microscopic observations, as well as on its ecological preferences as reconstructed from field observations. The most characteristic morphological features of this species are the different outline of the raphe valve (narrowly elliptic with linear margins) and the rapheless valve (narrowly elliptic to narrowly rhombic with moderately convex margins), the non-protracted broadly rounded apices and the length/width ratio. Furthermore, the areolae of the single row along the mantle are elongated and are more or less widely open, which is a characteristic discernible in girdle view under light microscopy. The species that *A. caravelense* resembles most is *A. eutrophilum* (Lange-Bert.) Lange-Bert.; nevertheless it can be distinguished from the latter by the different raphe valve outline, its higher valve length/width ratio and autecology. *A. caravelense* is common and abundant in soft waters with low to moderate nutrient content in the North of Portugal.

Key words: *Achnanthidium caravelense*, *Achnanthidium eutrophilum*, Bacillariophyceae, diatoms, ecology, morphology, new species, Portugal, ultrastructure.

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Introduction

Species of the genus *Achnanthidium* Kütz. are common and abundant in benthic communities, colonizing distinct substrates in diverse freshwater habitats (e.g. Potapova & Ponader 2004, Wojtal et al. 2010).

Achnanthidium has been restituted to genus rank by Mann (Round et al. 1990), since previously it was considered as a subgenus of *Achnanthes* Bory (e.g. Patrick & Reimer 1966). Since then, the genus *Achnanthes* has been split into several genera, the genus *Achnanthidium* being confined by Round & Bukhtiyarova (1996) to the taxa morphologically similar to the generitype *A. microcephalum* Kütz. According to Round & Bukhtiyarova (1996), the genus *Achnanthidium* can therefore be characterized by its linear-lanceolate to lanceolate-elliptic valves, radial or almost transverse striae, which are uniseriate in SEM and coarser in the centre (especially in the raphe valve); cells in girdle view are shallow-V-shaped, the raphe valve presents a fine central raphe hardly expanded at the centre, straight or turned to one side at the apex and a row of slightly elongated areolae in the mantle.

Achnanthidium minutissimum sensu lato is one of the most frequent species within this genus and has been reported from acidic to alkaline and from oligotrophic to hypereutrophic waters, which has been questioned by several researchers and is considered a good example of a common species that needs further studies (Round 2004). Although the *A. minutissimum* complex has been recently a subject of more detailed studies (e.g. Potapova & Hamilton 2007), there are still gaps in the knowledge, especially of the ecological preferences of the species. Round (2004) pointed out that the current taxonomic system on diatoms often uses "aggregate" species, which comprise numerous forms with different ecological requirements.

During a survey of benthic diatoms sampled in watercourses from Mainland Portugal *Achnanthidium* frustules morphologically ascribable to the *A. minutissimum* complex and most similar to *A. eutrophilum* (Lange-Bert.) Lange-Bert. have been found. Since these individuals were present in numerous reference sites, a more detailed examination of the taxon was performed by means of light (LM) and scanning electron microscopy (SEM). Furthermore, the examination of the environmental characteristics of the sites where it has been sampled allowed us to gather sufficient information to propose this as a new species for science, being described and characterized hereby.

Material and methods

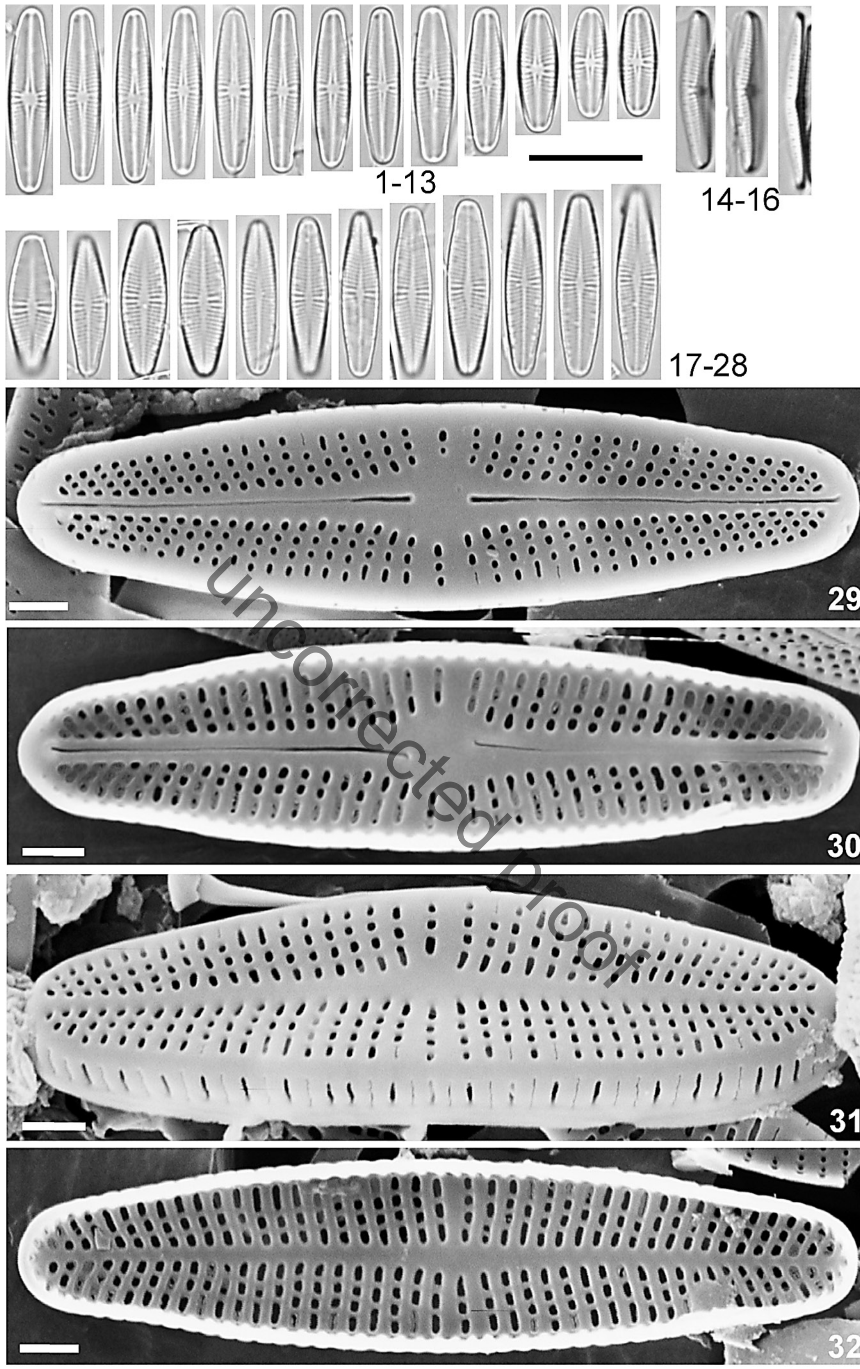
Benthic diatoms were sampled in spring and summer 2006 and 2007 in streams and rivers from Mainland Portugal. Epilithic diatom samples were treated by oxidation using hot hydrogen peroxide (35 %) and diluted HCl (37 %) in order to obtain a suspension of clean frustules. Permanent slides were mounted with Naphrax®. LM observations and morphometric measurements were performed using a Leica® DMRX brightfield microscope with 100x oil immersion objective and light microscopy photographs were taken with a Leica® DC500 camera. Samples selected for scanning electron microscopy analysis were filtrated through polycarbonate membrane filters with a pore diameter of 3 µm, mounted on stubs using double sided carbon tape and sputtered with platinum (30 nm) with Modular High Vacuum Coating System (BAL-TEC MED 020) and studied with a Hitachi SU-70, operated at 5.0 kV.

Morphological terminology follows Ross et al. (1979), Round et al. (1990) and Krammer & Lange-Bertalot (1991). For comparison, the following publications were consulted: Cholnoky (1957), Carter & Denny (1982), Kobayasi & Mayama (1982), Lange-Bertalot & Krammer (1989), Krammer & Lange-Bertalot (1991), Kobayasi (1997), Monnier et al. (2004, 2007), Potapova & Ponader (2004), Ivanov & Ector (2006), Potapova (2006), Bukhtiyarova (2007), Ponader & Potapova (2007), Potapova & Hamilton (2007), Taylor et al. (2007), Morales et al. (2009), Zidarova et al. (2009), Hlúbiková et al. (2011), Van de Vijver et al. (2011a) and Van de Vijver et al. (2011b).

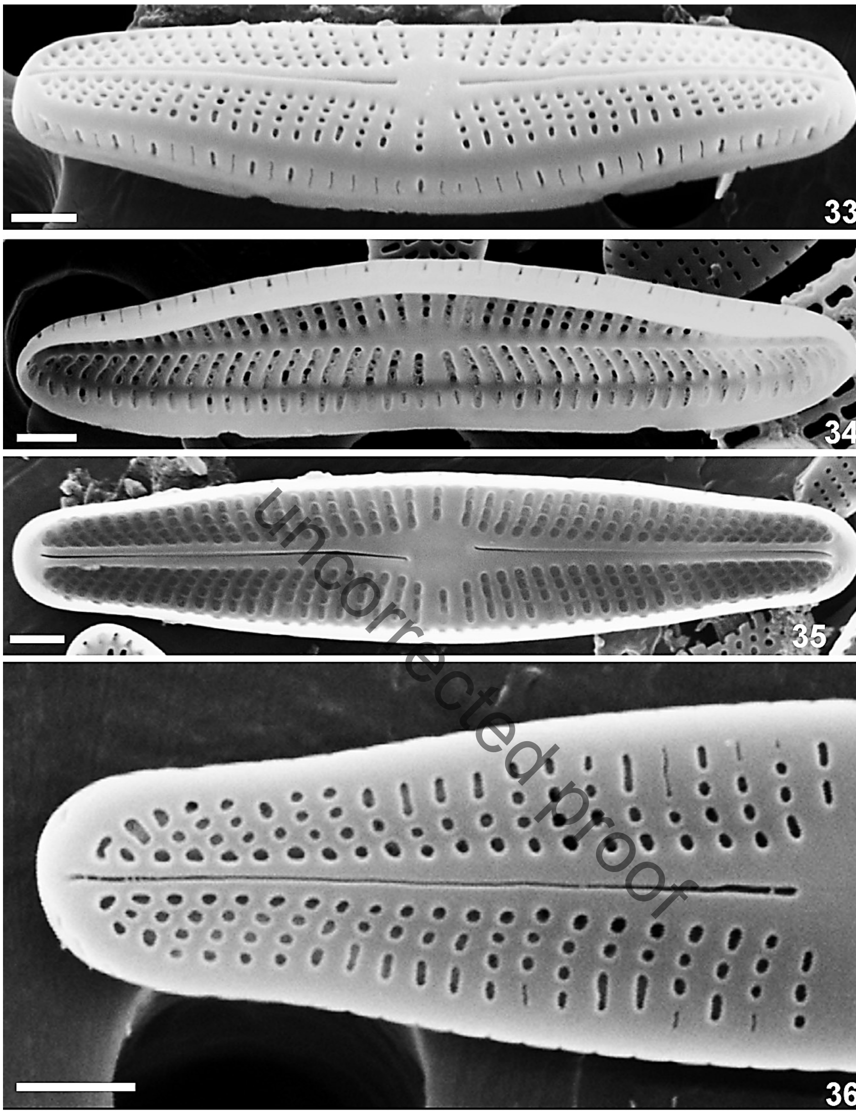
To perform the morphometric analysis a total of 120 valves (60 raphe valves and 60 rapheless valves) of each species have been measured under the LM microscope with the 100x oil immersion objective.

Ecological preferences of *Achnanthidium caravelense* and *A. eutrophilum* were inferred based on environmental variables from 73 sites from Mainland Portugal where these taxa have been found with a relative abundance above 1 % (31 sites for *A. caravelense* and 43 sites for *A. eutrophilum*). In total 20 environmental parameters were assessed by calculating the abundance weighted-average (WA): $N\text{-}NH_4^+$ (µg $N\text{-}NH_4^+$ L^{-1}), Cl^- (mg Cl^- L^{-1}), total organic carbon TOC (mg O_2 L^{-1}), total hardness (mg $CaCO_3$ L^{-1}), $P\text{-}PO_4^{3-}$ (µg $P\text{-}PO_4^{3-}$ L^{-1}), total phosphorus (µg P L^{-1}), soluble reactive *phosphorus (SRP)* (µg P L^{-1}), $N\text{-}NO_3^-$ (µg $N\text{-}NO_3^-$ L^{-1}), $N\text{-}NO_2^-$ (µg $N\text{-}NO_2^-$ L^{-1}), Na^+ (mg Na^+ L^{-1}), SO_4^{2-} (mg SO_4^{2-} L^{-1}), Ca^{2+} (mg Ca^{2+} L^{-1}), DO (% sat.), DO (mg L^{-1}), pH, conductivity (µS cm^{-1}), current velocity (m s^{-1}), altitude (m), catchment area (km^2) and distance from source (m). Standard methods for water chemical analysis were used according to APHA (1995).

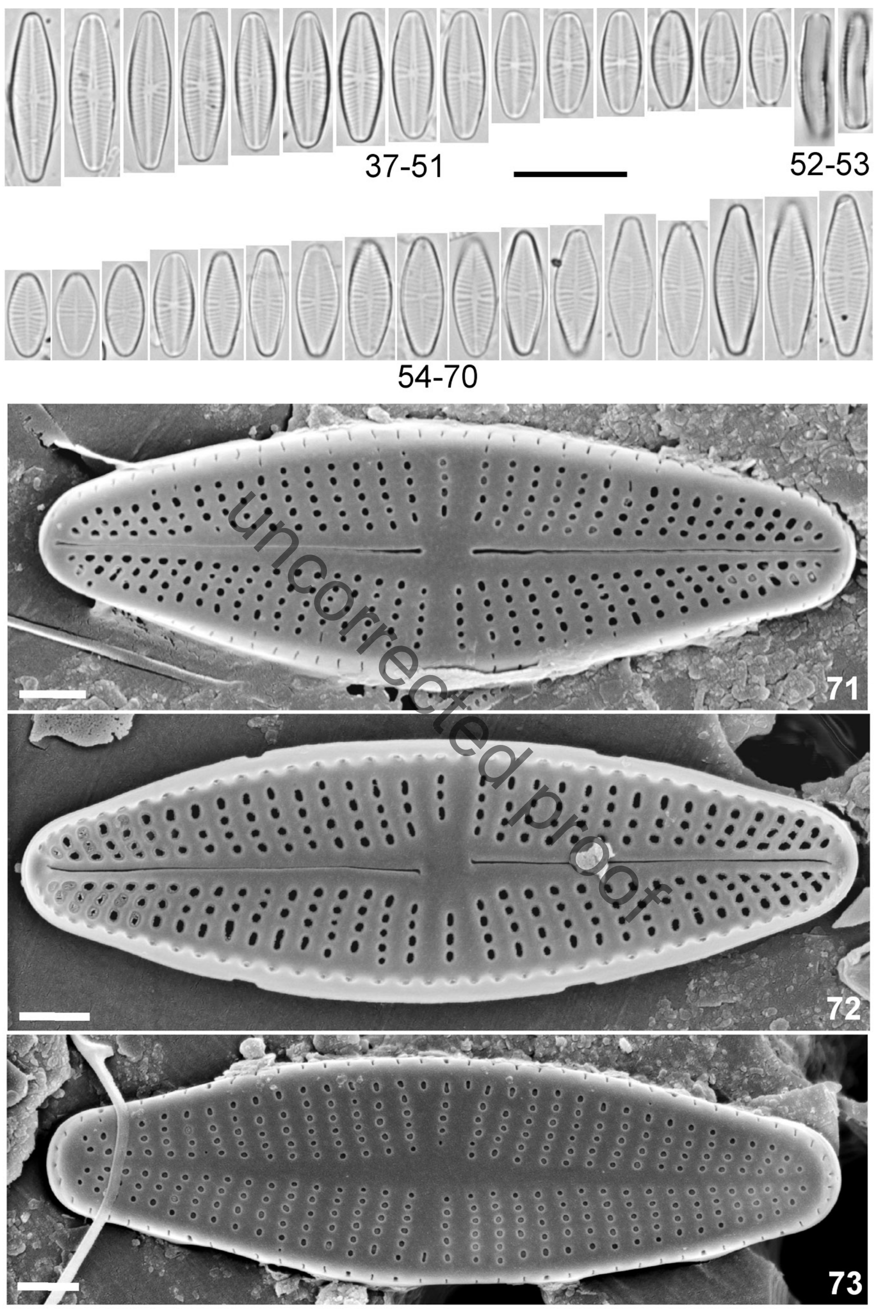
The distribution map of *Achnanthidium caravelense* was generated using GIS software ArcGIS 9.3 (ESRI 2008). Statistical analysis for *A. caravelense* and *A. eutrophilum* morphometric data was performed by analysis of variance over the length/width ratio. Differences in ecological preferences were analysed using only samples from streams (31 sites for *A. caravelense* and 30 sites for *A. eutrophilum*) since *A. caravelense* was not present in reservoirs. The analysis was performed using a weighted general multivariate model after variables normalization (natural logarithm). Statistical analyses were performed with SPSS software (SPSS 16.0 for windows, SPSS Inc. 1989–2007) and the graphical analysis was performed with the SigmaPlot® software v. 7.101 (SPSS Inc. 2001).



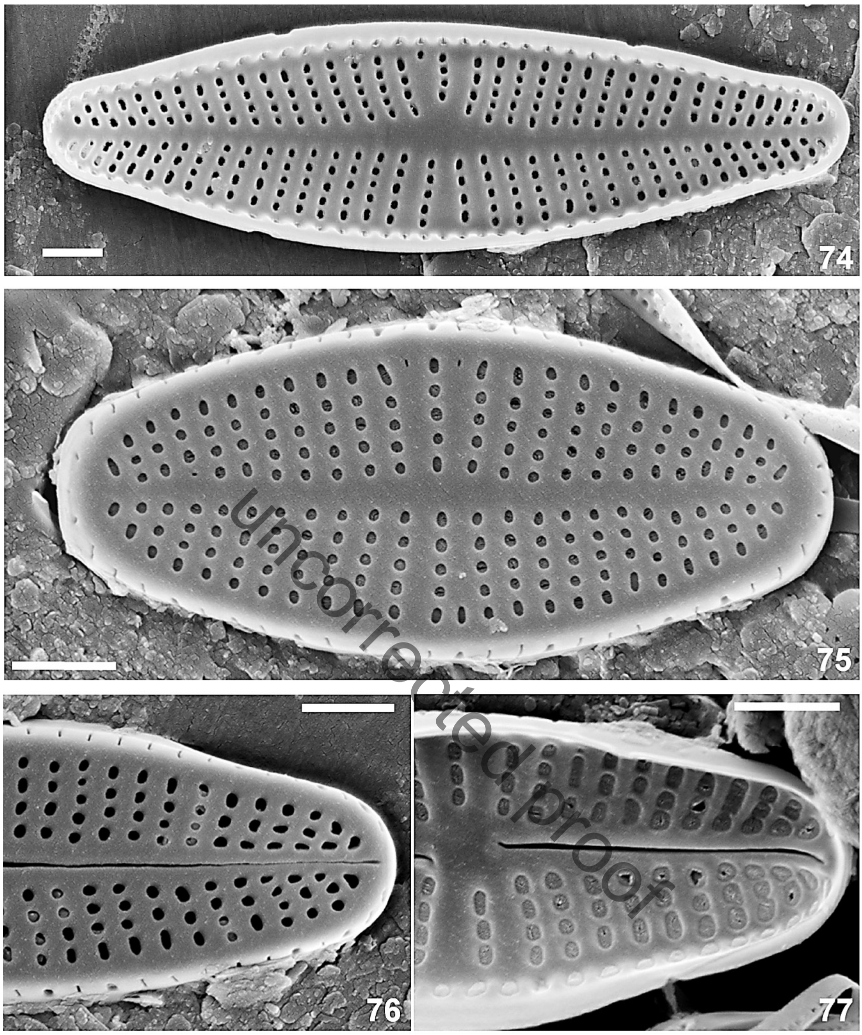
Figs 1–32. *Achnanthidium caravelense* Novais et Ector. Light and scanning electron micrographs of the type population from Caravelas stream (Portugal). **1–13.** LM views of raphe valves. **14–16.** LM views of frustules in girdle view. **17–28.** LM views of rapheless valves. **29.** SEM external view of a raphe valve. **30.** SEM internal view of a raphe valve. **31.** SEM external view of a rapheless valve. **32.** SEM internal view of a rapheless valve. Scale bar (Figs 1–28) = 10 μm; scale bars (Figs 29–32) = 1 μm.



Figs 33–36. *Achnanthidium caravelense* Novais et Ector. Scanning electron micrographs of the type population from Caravelas stream (Portugal). **33.** SEM external girdle view of a raphe valve showing the row of areolae more or less enlarged in the mantle. **34.** SEM internal oblique view of a raphe valve showing the row of areolae more or less enlarged in the mantle. **35.** SEM internal valve view of a raphe valve showing the areolae covered by hymens. **36.** SEM detail of the apical area of a raphe valve showing the slit-like areolae in the margins, in external view. Scale bars (Figs 33–36) = 1 μm.



Figs 37–73. *Achnanthidium eutrophilum* (LANGE-BERT.) LANGE-BERT. Light and scanning electron micrographs of the population from Monte Novo Reservoir (Portugal). **37–51.** LM views of raphe valves. **52, 53.** LM views of frustules in girdle view. **54–70.** LM views of rapheless valves. **71.** SEM external view of a raphe valve. **72.** SEM internal view of a raphe valve. **73.** SEM external view of a rapheless valve. Scale bar (Figs 37–70) = 10 µm; scale bars (Figs 71–73) = 1 µm.



Figs 74–77. *Achnanthidium eutrophilum* (Lange-Bert.) Lange-Bert. Scanning electron micrographs of the population from Monte Novo Reservoir (Portugal). **74.** SEM internal view of a rapheless valve. **75.** SEM external view of a raphe valve showing the row of areolae more or less enlarged in the mantle. **76.** SEM detail of the apical area of a raphe valve in external view. **77.** SEM detail of the apical area of a raphe valve in internal view. Scale bars (Figs 74–77) = 1 µm.

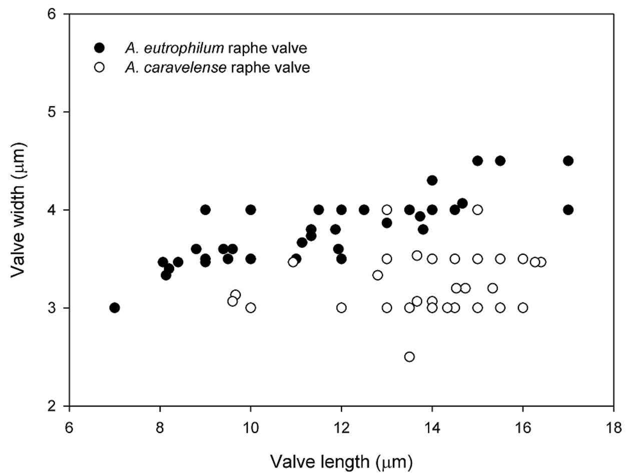


Fig. 78. Dimensions of raphe valves of *Achnanthidium caravelense* (Caravelas stream) and *A. eutrophilum* (Monte Novo Reservoir) (n = 120).

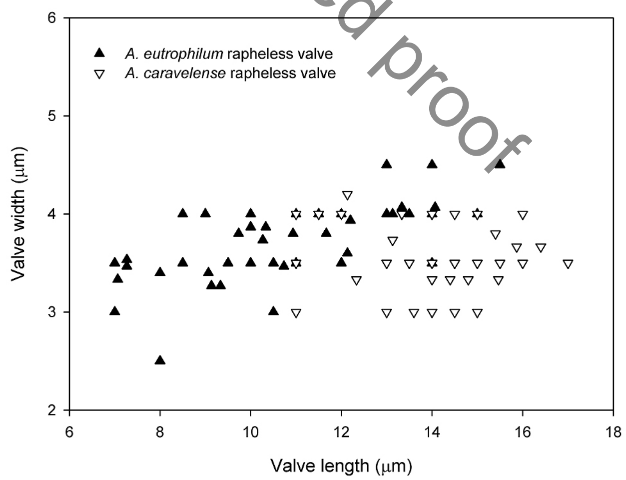


Fig. 79. Dimensions of rapheless valves of *Achnanthidium caravelense* (Caravelas stream) and *A. eutrophilum* (Monte Novo Reservoir) (n = 120).

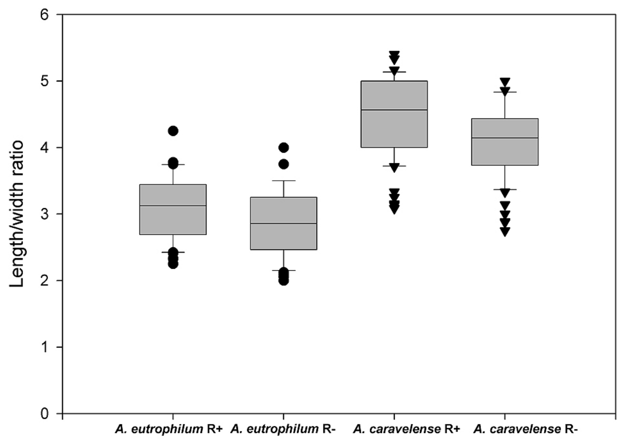


Fig. 80. Length/width ratio of raphe (R+) and rapheless valves (R–) of *Achnanthidium caravelense* (Caravelas stream) and *A. eutrophilum* (Monte Novo Reservoir) (n = 240).

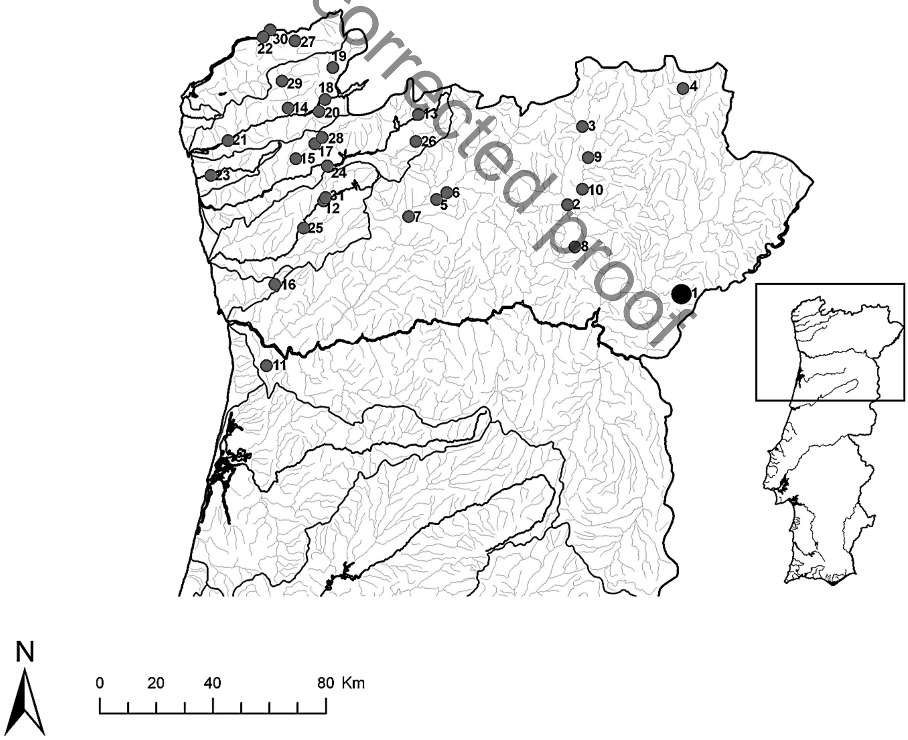


Fig. 81. Distribution map of *Achnanthidium caravelense* in the North and Centre of Portugal. The black circle represents the type locality. The numbers correspond to the identification number (ID) presented in Table 4.

Table 1. Comparison between Achnanthidium caravelense and morphologically similar species.

Achnanthidium	***caravelense*** (Caravelas stream, this study)	***affine*** (KRAMMER & LANGE-BERTALOT 1991)	***eutrophilum*** (HLÚBIKOVÁ et al. 20101, Germany)
Valve length (µm)	9.6-17	8-30	7.5-16
Valve width (µm)	2.5-4.2	3.5-5	3.2-4.8
Raphe valve			
Valve outline	narrowly elliptic	rhombic to rhombic-lanceolate	narrowly rhombic
Valve apices	broadly rounded; not protracted	acutely protracted	bluntly rounded to broadly cuneate
Central area	rounded to elliptic	large wedge-shaped fascia	small rhombic, almost absent
Striae (in 10 µm)	30	22-24	25-30 (27)
Number of areolae	2-4	3-5	4-6
Striation pattern	slightly radiate in the middle part, strongly radiate and slightly more densely spaced near the apices	slightly more densely spaced near apices	rounded to elongated areolae
Areola morphology	rounded to elongated; often slit-like near the valve margin	areolae rounded near apices	slit-like or both slit-like and broadly elliptic in the mantle
Rapheless valve			
Valve outline	narrowly rhombic	rhombic to rhombic-lanceolate	narrowly rhomboidal to lanceolate
Valve apices	obtusely rounded not protracted	acutely protracted	bluntly rounded to broadly cuneate
Central area	elliptic	asymmetrical	narrowly rhomboidal to lanceolate, almost absent
Striae (in 10 µm)	30-32	22-24	25-30
Number of areolae	3-4	3-4	4-6
Striation pattern	slightly radiate in the middle part, strongly radiate and slightly more densely spaced near the apices	equidistant throughout	rounded to elongated areolae
Areola morphology	rounded to elongated; often slit-like near the valve margin	usually rounded areolae	slit-like or both slit-like and broadly elliptic in the mantle

eutrophilum (Monte Novo Reservoir, Portugal, this study)	exile (KRAMMER & LANGE-BERTALOT 1991)	***minutissimum*** (KRAMMER & LANGE-BERTALOT 1991)	***standeri*** (CHOLNOKY 1957)	***taiaensis*** (CARTER & DENNY 1982)
7-17	12-33	5-20 (25)	24-38	10-35
2.5-4.5	4-6	(2.5) 3-3.5 (4)	4.5-5.5	3-5
rhombic to rhombic-elliptic	rhombic-lanceolate to linear-lanceolate	linear-elliptic to linear-lanceolate	linear to linear-lanceolate	lanceolate
broadly rounded; not protracted to slightly protracted	bluntly rounded; not protracted to slightly protracted	protracted; capitate	protracted; rounded	rounded
very small or absent	elliptic	irregular; 1-2 shortened striae	small; sometimes only in one side	small oval
22-26	25-30	c. 30	28-30	36-40
3-5	–	3-4	–	–
radiate; slightly more densely spaced near the apices	radiate; more densely spaced near the apices	slightly more densely spaced near the apices	slightly radiate in the middle; strongly radiate near the apices	radiate
rounded to slightly elongated; sometimes slit-like near the valve margin	–	areolae rounded	–	–
rhombic	rhombic-lanceolate to linear-lanceolate	linear-elliptic to linear-lanceolate	linear to linear-lanceolate	lanceolate
broadly rounded; not protracted to slightly protracted	bluntly rounded; not protracted to slightly protracted	protracted; capitate	protracted; rounded	rounded
very small or absent	elliptic	elliptic to lanceolate	undefined	small
22-26	25-30	c. 30	24-26	c. 40
4-6	–	3-5	–	–
radiate; slightly more densely spaced near the apices	radiate; more densely spaced near the apices; more densely spaced in the middle than in the raphe valve	equidistant throughout	rougher than the striae of the raphe valve; short and thick shadow lines in the valve edges	perpendicular to the midline
rounded to slightly elongated	–	usually rounded areolae	–	–

Observations and discussion

Achnanthidium caravelense **Novais et Ector sp. nov.** (Figs 1–36)

Diagnosis: Valvae anguste ellipticae vel anguste rhombicae, linearibus el modice convexis marginibus, leniter vel non protractis late rotundatis apicibus. Longitudo: 9.6–17.0 µm, latitudo: 2.5–4.2 µm. Rhaphovalva concava, area axialis linearis, area centralis fere absens vel rotundata. Rhaphe recta filiformis poris centralibus et extremis terminalibus rectis. Striae transapicales radiantes omnino densiores ad apices, 30 in 10 µm vel ad 35–40 in 10 µm ad apices. Striae 2–4 rotundatis vel transapicaliter elongatis areolis compositae. Areolae nonnunquam rimiformes ad marginem. Striae tantum rotundatis areolis compositae ad apices. Areovalva modice convexa, area axialis angusta linearis modice dilatata ad aream centralem. Area centralis modice elliptica. Striae transapicales radiantes omnino densiores ad apices 30 in 10 µm vel 35 in 10 µm ad apices. Striae 3–4 rotundatis vel transapicaliter elongatis areolis compositae. Areolae nonnunquam rimiformes ad marginem. Aperturae internae areolarum occlusae hymenibus. Areolae seriei singularis ad limbum elongatae et nonnunquam late apertae.

Holotype (here designated): BR-4214 (National Botanic Garden, Meise, Belgium), microscopic slide and preserved sample.
Isotypes (here designated): BM-101456 (Natural History Museum, London, UK), ZU7/44 (Hustedt Collection, Bremerhaven, Germany), microscopic slides.
Type locality: Quinta das Quebradas, Caravelas stream (Ribeira das Caravelas), subregion Alto Trás-os-Montes (District: Bragança), in the Parque Natural do Douro Internacional, Portugal (coll. Novais, coll. date 22/07/2007), coordinates 41º 13' 55,581'' N, 06º 45' 15,031'' W.
Etymology: The specific epithet *caravelense* refers to the name of the stream "Caravelas" where the type material was sampled (Ribeira das Caravelas).

Description: Valves narrowly elliptic or lanceolate to narrowly rhombic with linear to moderately convex margins and only very weakly to non-protracted, broadly rounded apices. Valve dimensions (n = 120): 9.6–17.0 µm long and 2.5–4.2 µm wide. Raphe valve concave with a linear axial area and a central area varying from almost absent to rounded. Raphe straight, filiform with gradually expanding, straight central raphe pores and straight terminal endings. Transapical striae radiate throughout the entire valve, becoming denser and more strongly radiate towards the apices, 30 in 10 µm in the middle of the valve up to 35–40 in 10 µm near the apices. Striae composed of 2–4 rounded to transapically elongated areolae, sometimes being slit-like near the valve margin. Striae near the apices formed only by rounded areolae. Rapheless valve slightly convex with narrow, linear axial area slightly widening towards the central area. Central area weakly elliptical and almost absent, with more widely spaced striae bordering it. Transapical striae radiate throughout the whole valve, slightly denser near the apices, 30 in 10 µm up to 35 in 10 µm near the apices. Striae mainly composed of 3–4 round to transapically elongated areolae. Areolae terminating the striae near the valve margin sometimes slit-like. Internal areolae openings occluded by hymenes (Fig. 35). The areolae of the single row along the mantle are elongated and are more or less widely open, which is a characteristic discernible under LM in girdle view (Figs 14–16).

Comparison with similar species

Achnanthidium caravelense is one of the few species presenting an irregular appearance of the striae in girdle view, due to some enlarged areolae in the mantle. Among the few species presenting this characteristic we can find the African species *Achnanthes standeri* Cholnoky and *A. taiaensis* J. R. Carter, described from South Africa by Cholnoky (1957) and from Sierra Leone by Carter & Denny (1982), respectively. Nevertheless, *Achnanthidium caravelense* differs from *Achnanthes standeri* by the absence of the clearly visible irregularities in the rapheless valve in valve view (under LM), the more linear to linear-lanceolate rapheless valve in *A. standeri*, its larger valve dimensions and the central area sometimes unilaterally expanded to the valve margin. *Achnanthes taiaensis* also presents clearly visible irregularities in the striae of the rapheless valve in valve view (under LM) and its striae are perpendicular to the midline, about 40 in 10 µm, whilst in *Achnanthidium caravelense* the striae are always radiant and circa 30 in 10 µm up to 35 in 10 µm close to the apices. *Achnanthes taiaensis* and *A. standeri* are two poorly known species, therefore they should be subject of detailed studies, since the original descriptions and drawings provided by Cholnoky (1957) and Carter & Denny (1982) correspond better to the current concept of the genus *Achnanthidium* and a future transfer to this genus should be considered.

The valves of *Achnanthidium caravelense* are usually wider than those of *A. minutissimum*, which presents both raphe and rapheless, linear-elliptic to linear-lanceolate valves with protracted capitate ends, while *A. caravelense* shows a narrowly rhombic rapheless valve and not protracted rounded ends. *Achnanthidium caravelense* can be easily distinguished from *A. affine* (Grun.) Czarn. by the absence of the typical central area presenting a large wedge-shaped fascia. *Achnanthidium exile* (Kütz.) Round et Bukht. differs from *A. caravelense* by its wider valves and the typical elliptic central area in the raphe valve.

To facilitate the comparison between *Achnanthidium caravelense* and morphologically similar species, their morphological characteristics are summarized in Table 1.

The species that *Achnanthidium caravelense* resembles most in terms of valve dimensions and outline is *A. eutrophilum*, which led us to compare the morphology and ultrastructure of *A. caravelense* (Figs 1–36) with a typical population of *A. eutrophilum* from Portugal (Figs 37–77). The Portuguese population of *A. eutrophilum* corresponds well to the characteristics of the epitype material (Hlúbiková et al. 2010), just differing by its slightly narrower valves: 2.5–4.5 μm for the Portuguese population and 3.2–4.8 μm for the epitype material from the Main River in Germany, as can be seen in Table 1 where both populations are compared.

The observation of the LM micrographs showed that *Achnanthidium eutrophilum* presents a more rhombic raphe valve than *A. caravelense*; nevertheless, its rapheless valves are quite similar. Therefore, a morphometric analysis was performed in order to verify if there were measurable differences between these two species. A total of 120 valves (60 raphe valves and 60 rapheless valves) of each species have been measured and the results are given in Figures 78 and 79. The morphometric analysis shows that there are differences between the two *Achnanthidium* species, mainly regarding the raphe valve, which is longer and narrower in *A. caravelense* than in *A. eutrophilum*, as seen in Figure 78. The rapheless valves are more similar, presenting more overlap among specimens, even though the majority of the *Achnanthidium caravelense* valves are larger, while the width is quite similar.

The valve outline (especially of the raphe valve, seen in LM micrographs) and the morphometric analysis in terms of width and length demonstrated differences in both species. This led us to consider that the length/width ratio can be a good character to distinguish between them. The box-plots with the length/width ratio of *Achnanthidium caravelense* and *A. eutrophilum* (Fig. 80) showed that it is indeed a good character to distinguish between them, especially in the case of the raphe valve. In addition, analysis of variance evidenced significant differences between the length/width ratio of *Achnanthidium caravelense* and *A. eutrophilum* with $p<0.001$. *Achnanthidium caravelense* presents a length/width ratio usually above 4 while in *A. eutrophilum* it is around 3, as seen in Table 2 where the main morphometric characteristics of both species are summarized.

Ecology, distribution and associated diatom flora

Although the morphology of *Achnanthidium caravelense* under LM could resemble *A. eutrophilum* at first sight, the fact that it was found in samples from several reference sites, according to the classification in the context of the implementation of the Water Framework Directive (2000/60/EC) in Portugal, made us consider it could be a species with different ecological requirements. Subsequently, the abundance-weighted averages of 16 physical and chemical variables and current velocity, altitude, distance from source and catchment area were calculated for both species in Portugal (Table 3).

Analysis of the variables weighted averages and their ranges, presented in Table 3, supports the hypothesis that both species differ by their ecological preferences. The results from a multivariate analysis (Hotelling's Trace statistics) confirm the difference ($p < 0.001$). In Table 3 are marked the variables for which the two species showed statistically significant different preferences. Therefore, the ecological preferences of these species are significantly different (with $p < 0.001$)

Table 2. Morphometric comparison between *Achnanthidium caravelense* (Caravelas stream) and *A. eutrophilum* (Monte Novo Reservoir, Portugal). Minimum, maximum and median values are presented (n = 240).

	A. caravelense		*A. eutrophilum*	
	Rapheless valve	Raphe valve	Rapheless valve	Raphe valve
Length (µm)	11.0-17.0 (14.5)	9.6-16.4 (14.7)	7.0-15.5 (10.7)	7.0-17.0 (12.0)
Width (µm)	3.0-4.2 (3.5)	2.5-4.0 (3.1)	2.5-4.5 (4.0)	3.0-4.5 (4.0)
Ratio (length/width)	2.8-5.0 (4.1)	3.1-5.4 (4.6)	2.0-4.0 (2.9)	2.3-4.3 (3.1)

Table 3. Abundance-weighted averages (WA), minimum and maximum of 16 physical and chemical variables and current velocity, altitude, distance from source and catchment's area corresponding to the ecological spectrum of the presence of *Achnanthidium caravelense* and *A. eutrophilum* in Portugal. Asterisks represent the parameters that were significantly different for both species with $p < 0.001$** and $p < 0.05$*.

	A. caravelense				***A. eutrophilum***			
	WA	**min**	**max**	**n**	**WA**	**min**	**max**	**n**
N-NH4+ (µg N-NH_4^+ L^{-1})	**36**	1	320	28	**71**	1	670	43
Cl- (mg Cl- L^{-1}) **	**11.2**	3.0	85.0	31	**20.9**	4.0	92.8	30
TOC (mg O_2 L^{-1}) **	**2.0**	1.2	4.2	31	**3.8**	0.7	18.8	29
Total hardness (mg $CaCO_3$ L^{-1}) **	**19.1**	5.0	44.0	31	**90.1**	0.1	268.0	43
P-PO_4^{3-} (µg P_2O_5 L^{-1}) **	**6**	1	21	24	**78**	1	900	40
Total phosphorus (µg P L^{-1})	**9**	1	51	27	**144**	1	1120	43
Soluble reactive phosphorus (SRP) (µg P L-1) **	**2**	1	7	22	**13**	1	44	26
N-NO_3^- (µg N-NO_3^- L^{-1}) **	**1707**	2	4210	30	**2470**	150	7600	43
N-NO_2^- (µg N-NO_2^- L^{-1}) **	**21**	1	290	29	**71**	1	220	38
Na^+ (mg Na^+ L^{-1}) **	**8.3**	0.4	39.3	31	**5.5**	0.7	15.8	30
SO_4^{2-} (mg SO_4^{2-} L^{-1})	**4.4**	0.1	20.1	31	**29.5**	2.2	170.6	29
Ca^{2+} (mg Ca^{2+} L^{-1}) **	**4.9**	2.0	15.0	31	**19.8**	2.0	110.6	30
DO (% sat.)	**90**	76	100	31	**85**	66	119	30
DO (mg L^{-1}) *	**8.2**	6.2	9.1	31	**9.4**	5.2	12.8	43
pH	**7.1**	6.2	8.1	31	**8.1**	6.4	9.0	43
Conductivity (µS cm^{-1}) *	**76**	28	307	31	**263**	35	723	43
Current velocity (m s^{-1})	**0.5**	0.0	1.3	31	**0.7**	0.0	1.9	29
Altitude (m) **	**196**	5	898	31	**78**	13	545	30
Catchment area (km^2)	**1103**	13	15491	31	**7171**	19	122704	30
Distance from source (m)	**38911**	29	129546	31	**83974**	103	968460	30

Table 4. Characteristics of sampling sites. Coordinates referred to the datum Lisboa. The identification number (ID) refers to the identification of the sites presented in the distribution map.

Site	Watercourse (Basin)	Sampling date	Latitude	Longitude	ID
Além da Veiga	Ave River (Ave)	25/09/2007	41º 32' 11,089" N	08º 15' 48,312" W	12
Alto Cávado	Cávado River (Cávado)	24/09/2007	41º 48' 43,800" N	07º 51' 41,200" W	13
Arcos de Valdevez	Vez River (Lima)	21/09/2007	41º 49' 54,300" N	08º 24' 57,800" W	14
Barral	Homem River (Cávado)	26/09/2007	41º 40' 15,787" N	08º 22' 58,694" W	15
Cantim	Leça River (Leça)	16/09/2007	41º 16' 13,790" N	08º 28' 08,375" W	16
Cavacadouro	Homem River (Cávado)	26/09/2007	41º 43' 07,787" N	08º 18' 08,708" W	17
Cidadelhe	Lima River (Lima)	27/09/2007	41º 51' 34,687" N	08º 15' 32,467" W	18
Férrea	Peneda River (Lima)	20/09/2007	41º 57' 41,166" N	08º 13' 33,703" W	19
Froufe	Froufe River (Lima)	27/09/2007	41º 49' 15,387" N	08º 17' 04,012" W	20
Lanheses	Lima River (Lima)	21/09/2007	41º 43' 39,800" N	08º 40' 14,500" W	21
Monção	Minho River (Minho)	20/09/2007	42º 04' 49,800" N	08º 29' 35,600" W	22
Monte Branco	Neiva River (Lima)	22/09/2007	41º 36' 58,886" N	08º 44' 37,633" W	23
Parada de Bouro	Cávado River (Cávado)	26/09/2007	41º 38' 53,788" N	08º 14' 54,716" W	24
Ponte na E.N. 103	Rabagão River (Cávado)	24/09/2007	41º 43' 33,590" N	07º 52' 21,180" W	26
Pontilhões	Ave River (Ave)	22/09/2007	41º 27' 02,800" N	08º 20' 54,000" W	25
Quinta das Quebradas	Caravelas Stream (Douro)	22-07-2007	41º 13' 55,581" N	06º 45' 15,031" W	1
Rabaçal	Rabaçal River (Douro)	14/08/2007	41º 31' 18,013" N	07º 13' 49,497" W	2
Sabor	Sabor River (Douro)	10/08/2007	41º 53' 10,695" N	06º 43' 57,239" W	4
Segude	Mouro River (Minho)	20/09/2007	42º 02' 44,394" N	08º 23' 15,807" W	27
Sequeirós	Homem River (Cávado)	26/09/2007	41º 44' 19,500" N	08º 16' 13,400" W	28
Sub-Igreja	Vez River (Lima)	21/09/2007	41º 55' 04,285" N	08º 26' 34,386" W	29
Tâmega 1	Tâmega River (Douro)	24/09/2007	41º 32' 25,909" N	07º 47' 09,085" W	5
Tâmega 2	Tâmega River (Douro)	24/09/2007	41º 29' 12,991" N	07º 54' 12,934" W	7
Troporiz	Gadanha River (Minho)	20/09/2007	42º 03' 27,704" N	08º 31' 28,094" W	30
Tua	Tua River (Douro)	16/08/2007	41º 23' 12,765" N	07º 12' 03,892" W	8
Tuela 1	Tuela River (Douro)	14/08/2007	41º 40' 12,568" N	07º 08' 27,694" W	10
Tuela 2	Tuela River (Douro)	11/08/2007	41º 34' 12,526" N	07º 09' 58,814" W	9
Uima	Uima Stream (Douro)	14/08/2007	41º 00' 44,018" N	08º 30' 11,363" W	11
Vale Armeiro	Rabaçal River (Douro)	10/08/2007	41º 46' 09,905" N	07° 09' 46,586" W	3
Veral	Tâmega River (Douro)	21/07/2007	41º 33' 45,071" N	07º 44' 30,135" W	6
Vilela	Pequeno River (Ave)	25/09/2007	41º 32' 51,100" N	08º 15' 17,400" W	31

for chlorides, total organic carbon, total hardness, phosphates, soluble reactive phosphorus, nitrates, nitrites, sodium, calcium and altitude. In addition, their ecological preferences are different (with $p < 0.05$) for conductivity and dissolved oxygen. No significant differences were detected for ammonia, total phosphorus, sulphates, catchment area, distance from source, current velocity, pH and dissolved oxygen (% saturation).

Both *Achnanthidium* species never occurred in the same samples at times. *Achnanthidium caravelense* occurs in circumneutral waters (WA pH: 7.1), with low conductivity (WA cond.: 76 µS cm^{-1}) while in Portugal *A. eutrophilum* prefers slightly alkaline waters (WA pH: 8.1) with moderate conductivity (WA cond.: 263 µS cm^{-1}). Furthermore, the range of nutrient concentrations is more ample for *Achnanthidium eutrophilum*: WA N-NO_3^-: 1,707 µg L^{-1} (*A. caravelense*) – 2,470 µg L^{-1} (*A. eutrophilum*); WA N-NO_2^-: 21 µg L^{-1} (*A. caravelense*) – 71 µg L^{-1} (*A. eutrophilum*), as well as the soluble reactive phosphorus: WA SRP: 2 µg L^{-1} (*A. caravelense*) – 13 µg L^{-1} (*A. eutrophilum*). Furthermore, the analysis proves that both species may occur in similar river types but with different degrees of organic contamination.

The ecological preferences of *Achnanthidium eutrophilum* in Portuguese rivers are in accordance with the information provided by Lange-Bertalot & Metzeltin (1996) who stated that this species occurs in oligotrophic to polytrophic waters and by Ponader & Potapova (2007), who referred that in the Appalachian rivers (United States of America) this species was found in four sites characterized by pH between 8.1–8.3, conductivity between 291–583 µS cm^{-1} and a wide range of nutrient concentrations (NO_3^- + NO_2^-: 700–2,200 µg L^{-1}). Regarding phosphate, *Achnanthidium eutrophilum* was found in the United States of America in sites with a lower range of values (PO_4^{3-}: 10–26 µg L^{-1}) than the Portuguese populations (WA PO_4^{3-}: 78 µg L^{-1}).

In total, *Achnanthidium caravelense* has been recorded with abundances above 1 % in 31 sampling sites (30 in the North, only one in the Centre, and none in the South of Portugal) belonging to the Ave, Cávado, Douro, Lima, Leça and Minho watersheds. The characterization of the sampling sites and the respective distribution map are presented in Table 4 and Figure 81. *Achnanthidium eutrophilum* has been recorded in abundances above 1 % in 43 sampling sites in streams and reservoirs distributed all over the country and belonging to the watersheds of Ribeiras do Algarve (5 sites), Mira (1 site), Guadiana (6 sites), Tejo (16 sites), Mondego (5 sites), Vouga (4 sites) and Douro (6 sites).

Besides *Achnanthidium caravelense*, the diatom community was dominated in its type locality by *A. lineare* W. Sm. and *A. rivulare* Potapova et Ponader, which are species characteristic of nutrient-poor to moderately nitrogen rich waters (Potapova & Ponader 2004, Van de Vijver et al. 2011a). *A. rivulare* is a species characteristic of soft waters and a pH of approximately 6 (Potapova & Ponader 2004), while *A. lineare* is characteristic of circumneutral to weakly alkaline waters (pH 7.0–8.5) and very low to moderate specific conductivity, between 23–200 µS cm^{-1} (Van de Vijver et al. 2011a).

Conclusion

The new species *Achnanthidium caravelense* clearly belongs to the genus *Achnanthidium*, according to the description of Round & Bukhtiyarova (1996), on the basis of the valve outline, radiate striae, uniseriate (in SEM) and wider spaced striae in the centre of the valve. In girdle view cells are shallow-V-shaped. The raphe valve has a straight central raphe hardly expanding at the centre and a row of elongated areolae in the mantle.

Within the genus *Achnanthidium* this species belongs to the complex around *A. minutissimum*, due to its straight terminal raphe fissures, in opposition to the species with terminal raphe fissures clearly deflected, such as *A. convergens* (H. Kobayasi) H. Kobayasi, *A. japonicum* (H. Kobayasi) H. Kobayasi, *A. latecephalum* H. Kobayasi, *A. pyrenaicum* (Hust.) H. Kobayasi (Kobayasi 1997), *A. deflexum* (Reimer) J. C. Kingston, *A. rivulare* Potapova et Ponader 2004 (Potapova & Ponader 2004) and *A. zhakovschikovii* Potapova (Potapova 2006).

Achnanthidium caravelense is characterized by a set of distinct morphological and ecological features that separate it well from all other similar *Achnanthidium* species and it is widely distributed in Portuguese rivers. There is therefore a rather high probability that this species also occurs commonly in other European regions, in rivers with suitable conditions and has not yet been recorded due to probable misidentifications with *Achnanthidium eutrophilum* or *A. minutissimum*.

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