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Pinnularia baetica sp. nov. (Bacillarophyceae): Comparison with other panduriform species in the Mediterranean area

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Abstract

A new freshwater diatom species *Pinnularia baetica sp. nov.* is described from two different ponds from the Mediterranean area: Andalucia (South of Spain) and Lake Livadičko, Serbia. The species is described by observations under light (LM) and scanning electron microscopy (SEM). The most similar taxa to *P. baetica* are *P. atlasii* and *P. infirma* that were studied through material obtained in lagoons of northern Morocco (locus classicus of *P. atlasi*). Although there are similarities in the morphological characters of the frustule, it was possible to verify both through LM and SEM observations, clear differences between *P. baetica* and the latter two taxa: *Pinnularia baetica* has a panduriform shape more pronounced than *P. infirma* and larger valve size. On the other hand, the absence of spines in *P. baetica*, the more convergent striation at the poles and a slightly wider valve are the main differences with *P. atlasi*. This paper documents the distribution areas of *P. baetica* in calcareous systems of oligotrophic mid-mountain ponds of Spain and Serbia.

Keywords: *Pinnularia baetica*, alkaline mountain lagoon, diatoms, *Pinnularia atlasi*, Morocco, Spain, Sardinia

Introduction

Despite of the high species diversity in the genus *Pinnularia* Ehrenberg (1843: 45) with 737 accepted taxa (Guiry & Guiry, 2019), data for *Pinnularia* species diversity in the south of Spain are very scarce and only few species have been recorded in Sierra Nevada Mountain (Linares-Cuesta 2003), in acidic rivers as Río Tinto (Luís *et al*. 2012, Rivera *et al.* 2019) and Río Odiel (Rivera *et al.* 2019). Species from the genus *Pinnularia* have been reported as living in extreme environmental conditions, like the freezing temperatures of the Antarctic region where *P. catenaborealis* Pinseel, Hejduková, Vanormelingen, Kopalová, Vyverman & Van de Vijver (Pinseel *et al.* 2016: 99), *P. sofia* Van de Vijver & Le Cohu (in Van de Vijver *et al.* 2004: 104), *P. obaesa* Van de Vijver (2008: 222, 223) and *P. australorabenhorstii* Van de Vijver (2008: 224) and several other species of *Pinnularia* (Van de Vijver & Zidarova 2011; Zidarova *et al.* 2012) are well adapted. They can also be present in the surrounding streams of mining areas, characterized by high metal concentrations and very low pH, as it is the case of *P. aljustrelica* Luís, Almeida & Ector (in Luís *et al.* 2012: 29) and *P. paralange-bertalotii* Fukushima, S.Yoshitake & T.Ko-Bayashi (in Fukushima *et al.* 2001: 38). Other relevant aspect is that some of the described species from Antarctic can form colonies through the presence of marginal spines (Van de Vijver *et al.* 2004; Pinseel *et al.* 2016), although most of the *Pinnularia* species have solitary life forms (Round *et al.* 1990). Even though chain formation by spines affects many biological processes, such as sexual interactions, predation and nutrient uptake (e.g. Fryxell & Miller 1978; Pahlow *et al.* 1997), it is not clear the main function in the different species of *Pinnularia* (Pinseel *et al.* 2016). Among those species that have spines it is important to highlight *P. atlasi* Darley (Darley 1990: 73). This species is characterized by panduriform valves, bilateral fascia in the central area, irregular markings in the axial and central areas and almost entirely parallel striae. The description of *P. atlasi* based only on LM observations by Darley (1990) did not show the presence of spines and did not provided further illustrations or argumentations (See figures 1 and 2 in Darley, 1990)

To date only Lange Bertalot *et al.* (2003) from Sardinia (Fig. 1), had presented scanning electronic microscopy (SEM) illustrations as the basis for a more completed diagnosis of this species. Lange-Bertalot *et al*. (2003) reported the presence of spines in the valve margin from a new population of *P. atlasi*, mostly concentrated in the central area and disappearing towards the ends of the valve. Another species with panduriform shape is *P. infirma* Krammer (in Krammer & Lange-Bertalot 1985: 109), that was described from the central Europe, in a locality situated at 250 m a.s.l. in Franken, Germany.

In the present study, the current knowledge on *P. atlasi*, *P. infirma* Krammer is reviewed and the existing Europe distributional data for the species. A new species is proposed within the genus *Pinnularia* based on detailed LM and SEM observations. The main objective of the present work is to study the "panduriform" *Pinnularia* populations of several locations of the Mediterranean area. For this purpose, samples from the syntype locations of *P. atlasi*, were obtained from the two sites of northern Morocco (Ouiouane and Guedrouz), where Darley (1990) originally discovered and described the species. Also a population of *P. infirma*, were investigated in order to compare differences and similarities of these species with others from the genus *Pinnularia* in very specific places in southern Spain, Serbia and Macedonia. This detailed examination through light (LM) and scanning electron microscopy (SEM), allowed proposing a new species of *Pinnularia*, here described in detail.

Material and methods

The diatom communities from sediments (epipelic samples) were collected from the littoral zone of six sites, between lagoons, ponds and a reservoir; one from South Europe (Spain), two from North Africa (Morocco), one alpine pond on Mountain Shar Planina (North Macedonia), one reservoir near Skopje and one glacial lake on Mountain Shar Planina (Serbia) (Table 1, Fig. 1). The sample from Laguna Seca (Granada, Spain), located in the north of the Natural Park of Sierra de Castril (Complex of Baetic Mountains) was collected in 2007. Laguna Seca is located at an altitude of 2000 m, and represents a small pond located in a shallow basin, filled with rainwater and snowmelt; it is usually drought at the beginning of summer. It is dominated by outcrops of limestone and dolomites originated in the Upper Jurassic.

The precise locations of Ouiouane and Guedrouz lagoons where *P. atlasi* was described by Darley (1990) were difficult to find due to lack of coordinates in the original description. According to Darley (1990), the species was found in Guedrouz lagoon in "Timenkar plates in the central High Atlas from the region of Marrakech" meanwhile Ouiouane lagoon in mountains close to the city of Azrou. Both were temporary lagoons, located at 2000 and 1880 m a.s.l. respectively, which are entirely dry over a period of six to eight months (Darley, 1990). Following Darley's notes, new samples were located and obtained in both ponds from Morocco, Guedrouz and Ouiouane.

This material from Ouiouane Lake is proposed as a Locotype and it will be submitted to a collection hosted in the Herbarium, University of Granada (Spain), because the population of *P. atlasi* designed by the original finder was not available.

Sample from Radika, southern part of Shara Mountain, North Macedonia originates from a small temporary alpine pond with an area of 100 m^2 and maximal depth of 20 cm. The bottom is covered with clay and macrophytes are absent. Lake Livadičko (=Štrbačko), Serbia is located 2,173 m a.s.l. with maximum length of 228 m and maximum width of 120 m, area of 1.3–2 ha and depth of 6–8 m. The bottom is mostly covered with sediment or clay.

TABLE 1. Sampling sites location in this study.

Site	Locality	Taxa	Coordinates	
Laguna Seca	Granada	*P. baetica*	37°56'20"N	2°41'19"W
Ouiouane	Azrou	*P. atlasi, P. infirma*	33°07'53"N	5°20'37"W
Guedrouz	Marrakech	*P. atlasi*	31°26'13"N	7°28'07"W
Lake Livadičko	Serbia	*P.baetica*	42°11'27"N	21°04'25"E
Radika	Macedonia	*P.infirma*	41°50'30"N	20°44'21"E
Matka	Serbia	*P. cf. atlasi*	41°52'07"N	21°11'16"E

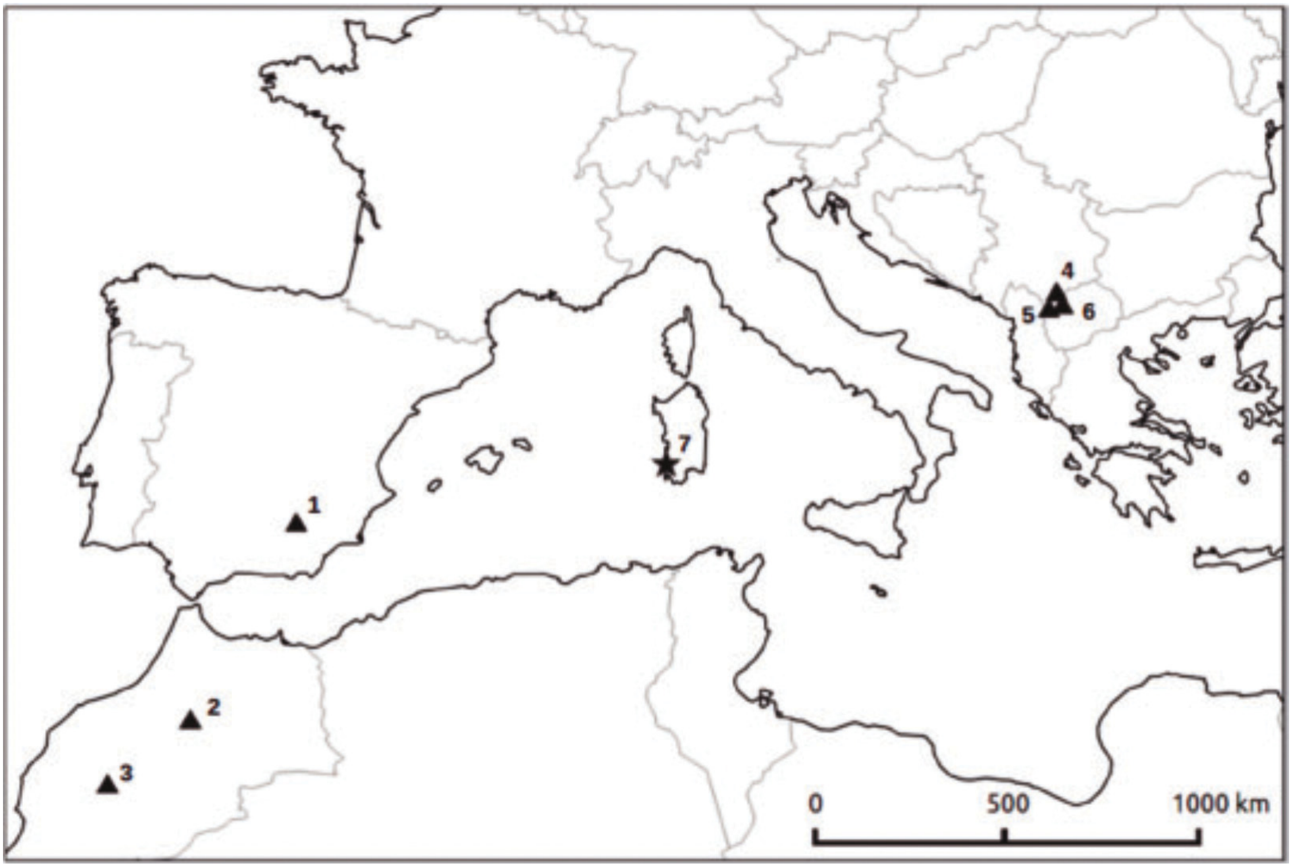


FIGURE 1. Geographical locations of the 6 sampling sites of this study and the location sampled by Lange-Bertalot as a review of the *P. atlasi* distributions species. 1. Laguna Seca; 2. Guedrouz; 3. Ouiouane; 4. Livadicko; 5. Radika; 6. Matka; 7. Sardinia (not sampled in this study).

Sediment samples from Laguna Seca, Guedrouz and Ouiouane were collected by suction through a one-metre-long glass tube (a Lund tube). Several transects were made in the littoral zone in order to get an integrated sample of the epipelic community. Field parameters such as: pH, conductivity and temperature were measured directly from the lagoon water with a portable multimeter model WTW pH/Cond 340i/SET, actually was measured just in Granada population.

The samples were transported to the laboratory in cold (4ºC–8ºC) and dark conditions. Once in the laboratory, the sediment was left to stand in a petri dish, then the supernatant was removed and after the sediment was covered by coverslips. The coverslips were removed at least 12 hours later so diatoms, by means of the phototactic movement, were attached to the coverslip. Then it was fixed with 4% formaldehyde.

The samples were oxidized with hydrogen peroxide 30% w/w. Possible calcium carbonate inclusions were removed by adding a few drops of diluted hydrochloric acid 1N into the sample. They were then washed four times with distilled water, leaving the pellet and the supernatant was removed several times to prevent rupture of the valves when the samples were centrifuged. Permanent slides were mounted with a synthetic resin, Naphrax® (Brunel Microscopes Ltd, UK) with a high refractive index (1.74). Light microscopy observations were performed using a Leica DMI3000 B light microscope (Phase contrast) with a 100x oil immersion objective with NA (numerical aperture) of 1.30 and LM photographs were taken with a Leica DFC295. Images of samples from Shara Mountain were performed using a Nikon E–80i light microscope, equipped with a Nikon Coolpix 600 digital camera. Measurements of valve length, width and number of striae (in 10 μm) were taken from at least 20 specimens per species, under the light microscope. Several subsamples for scanning electron microscopy were filtered through polycarbonate membrane filters with 5 μm pore diameter, mounted on stubs with double sided carbon tape. A Zeiss SUPRA40VP Electron Microscope with variable pressure high resolution (FESEM) was used for observation of the valve structures. Morphological terminology follows Round *et al.* (1990), Krammer (2000) and Cox (2004). Apart from the original descriptions of *P. atlasi* provided by Darley (1990), the following publications were consulted for taxonomical and ecological comparison: Hustedt (1930), Krammer & Lange-Bertalot (1986, 1988, 1991 a, b), Lange-Bertalot *et al.* (2003), Levkov *et al.* (2005).

The range of morphometric and micrographic data from *P. atlasi* in Darley´s description included both populations of Guedrouz and Ouiouane. Due to the low number of valves of *P. atlasi* in both lakes, it was necessary several subsamples for the morphometric and micrographic data. Therefore, in Guedrouz the number of studied valves were n=19 and Ouiouane were n=18.

All images were digitally edited, and plates were made using CorelDRAW 2018 and the map (Fig. 1) was done using QGIS Dufour 2.0.

Results

Taxonomic treatment

Phylum Ochrophyta

Class Bacillariophyceae

Subclass Bacillariophycidae

Order Naviculales

Family Pinnulariaceae

Genus *Pinnularia* Ehrenberg (1843: 43–49)

Pinnularia baetica **Fernández Moreno & Sánchez Castillo *sp. nov.* Figs 2–16, 47–52, 66–69**

Type:—SPAIN**,** province of Granada, Sierra Seca, Natural Park of Sierra de Castril, Laguna Seca, 37º56´41.86´´ N, 2º40´54.80´´ W, lagoon epipelon, Coll. David Fernández Moreno and Pedro Miguel Sánchez Castillo, 2007 (Holotype: Phycotheque GDA-algae slide number 3725, prepared with material from the sample collected in Laguna Seca, hosted in the Herbarium, University of Granada (Spain), Isotype: Slide BM 101919, prepared with material from the sample collected in Laguna Seca, hosted in the Natural History Museum, London (UK).

Description

Valves panduriform (Figs 2–16), constricted in the center, then distally widening finishing with narrowly rounded to almost cuneate apices. Valve length 45.8–77.7 µm, width 9.6–12.2 µm (n=20). Central area variable, from narrow rhombic fascia (e.g. Fig. 6) to broad bow-tie-shaped fascia (e.g. Fig. 12), formed by shortening of one to three central striae. Fascia with similar size on both sides (e.g. Figs 4, 12) or smaller on secondary side (Figs 5, 6). Axial area linear-lanceolate, widening towards central area. Raphe slightly lateral, with unilaterally deflected proximal raphe ends, terminating in slightly expanded pores (Figs 2–16). The distal raphe fissures hooked on opposite side of proximal ends. Transapical striae parallel to slightly radiate in mid-valve to convergent at the poles, 9–10 in 10 µm.

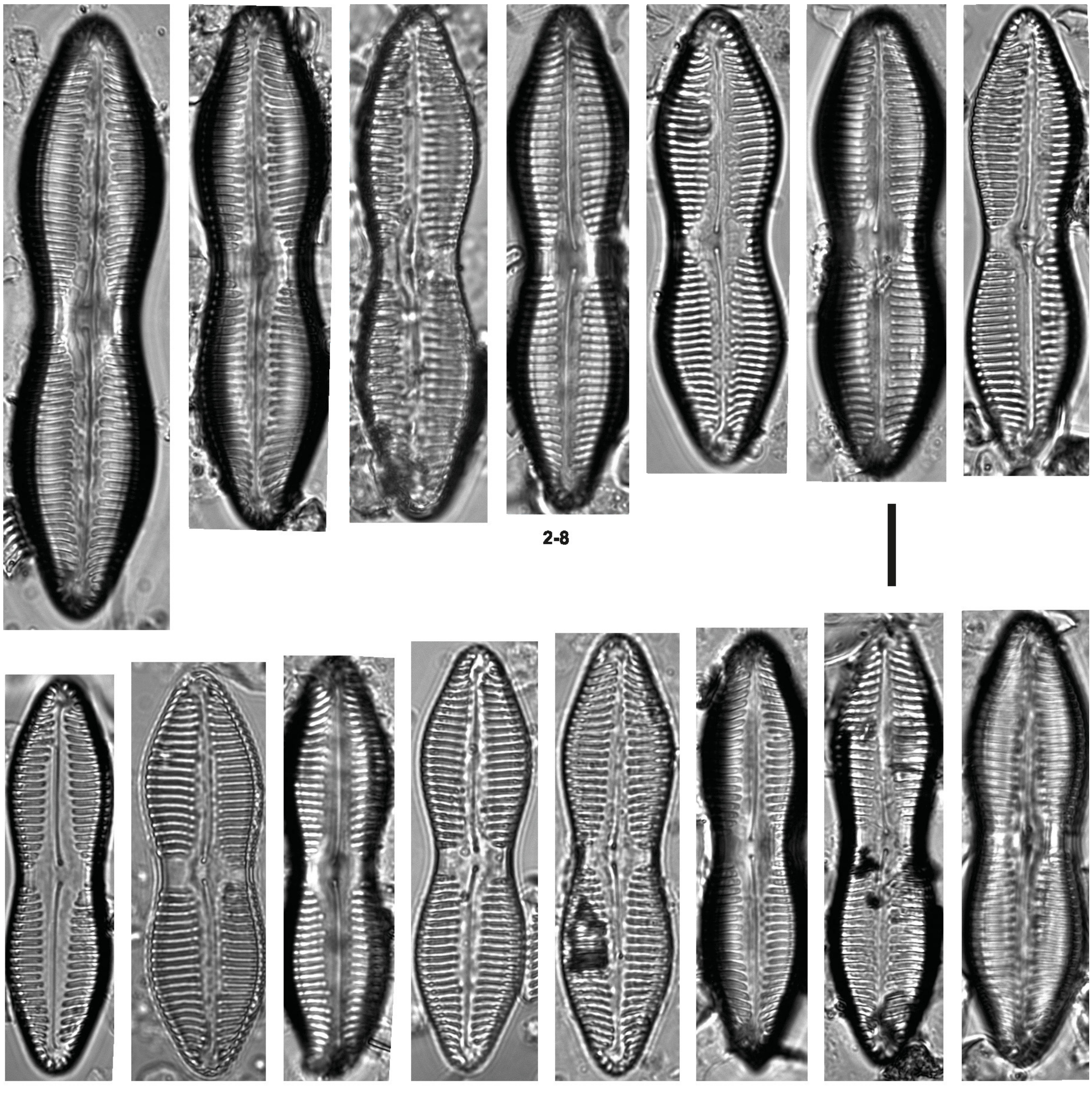
External proximal raphe ends slightly expanded and unilaterally deflected (Figs 47, 51), while distal raphe fissures clearly hooked shaped (Figs 47, 49), continuing onto mantle. Internal raphe straight, proximally terminating with short, slightly bent, on small inflated central nodule (Fig. 48). Distal raphe endings terminating onto small, slightly eccentric, weakly raised helictoglossa (Figs 48, 50). Striae composed of large alveolus (Fig. 50). Externally, each alveolus composed of 7–8 rows of small, round areolae (Fig. 52) with diameter of 75–100 nm. Each areola occluded by porous hymen perforated by 6/5 pores with 20–30 nm in diameter.

Etymology: The specific epithet *baetica* refers to the name of the mountain chain complex where the species was found.

Ecology The limnological characteristics of Laguna Seca at the sampling time, corresponded to the end of the flood period (in summer), when pond bottom became full of a community of macrophytes. The water temperature was 15.7°C, the conductivity was low (83 µS/cm), with a very alkaline pH of 9.2. The associated diatom flora from the littoral zone, together with *P. baetica* (12%) was dominated by *Nitzschia tenuirostris* Manguin (in Bourrelly & Manguin 1952: 105) (31%), *Pinnularia* cf. *obscura* Krasske (1932: 117) (32%) and species from *Eunotia* Ehrenberg (1837: 44) (2.5%) closely related to the taxa identified by Lange-Bertalot *et al.* (2003) in Sardinia, such as *Eunotia sardiniensis* Lange-Bertalot Cavacini, Tagliaventi & Alfinito (2003: 42) and *Stauroneis* Ehrenberg (1843: 45) (13%).

In this study, species reported as *P. atlasi* (Figs 17–31, 53–58) were from the type locations designed by the original finder collected in Morocco (Ouiouane, Guedrouz) since Darley´s type material is unavailable. Valves are panduriform, constricted in the center, then distally widening and finishing with narrowly rounded to almost cuneate apices. Valve length of 35.4–77.9 µm and width 8.7–15.8 µm (n=37) in both locations. The central area is variable, from narrow rhombic fascia (Fig. 27) to broad bow-tie-shaped fascia (Fig 18, 19), formed sometimes by shortening central striae. Fascia with similar size on both sides (Figs 17, 18) or smaller on secondary side (Fig. 27). Axial area linear-lanceolate, widening towards the central area. Raphe slightly lateral, with unilaterally deflected proximal raphe ends, terminating in slightly expanded pores (Figs 17–31). The distal raphe fissures hooked on opposite side of proximal ends. Transapical striae are parallel to slightly radiate in mid-valve to convergent in poles, (9)10–11/10 µm. In North Macedonia just one valve of *Pinnularia* aff. *atlasi* (Fig. 76) was found, with sizes falling inside the Moroccans' range but with length/width relation higher (Table 2). Also, the spines are presents even in optical microscope (See in the center of the valve in Fig.76).

There are no previous SEM studies in the Moroccan populations of *P. atlasi*. The main character of *P. atlasi*, is the presence of spines growing up from circular pits (Lange Bertalot *et al.* 2003) as well as in the specimens of Sardinia. The populations of *P. infirma* were found in Ouiouane (Morocco) and in Radika Pond (North Macedonia). *Pinnularia infirma* (Figs 32–46, 70–75) is characterized by its narrow central area and its less panduriform and smaller size when compared with *P. atlasi*, *P.* cf. *atlasi* and *P. baetica*. The valve is characterized by: length 25.2–43.2 µm, width 5.2–7.2 µm and 11–13 striae in 10 µm (this study).



FIGURES 2–16. *Pinnularia baetica sp. nov.* Light micrographs of the type population sampled in Laguna Seca in 2007. Scale bar 10 µm.

TABLE 2. Comparison between *Pinnularia baetica* and the morphologically and ecologically most similar taxa (data from Sardinia comes from the literature)

***Pinnularia* (References)**	***baetica* (this study,Spain)**	***baetica* (this study,Serbia)**	***atlasi* (this study, Guedrouz)**	***atlasi* (this study, Ouiouane)**	**cf.*atlasi* (this study, Macedonia**	***atlasi* (Darley 1990)**	***atlasi* (Lange-Bertalot et al. 2003)**	***infirma* (this study,Spain)**	***infirma* (this study,Macedonia)**	***infirma* (Krammer 2000)**
Number of measurements (n)	20	4	19	18	1	-	-	20	5	-
Valve Length (µm)	45.8-77.7	55.5-79	44.1-72	35.4-77.9	57.2	65-77	n.d.	25.2-43.15	29.1-41	18-51
Valve Width (µm)	9.1-12.2	10.5-14.5	8.3-11.2	8.7-12.5	8.	15-16	n.d.	5.2-6.9	6.3-7.2	4.5-8
Valve Wider Width (µm)	11.6-16.6	15.4-17.2	11.2-14.6	11.2-15.8	11.8	-	-	6.9-7.9	7.2-8.1	-
Valve Outlines	Panduriform	Panduriform	Panduriform	Panduriform	Panduriform	Panduriform	Panduriform	Weakly panduriform	Weakly panduriform	Linear-lanceolate with moderately convex sides
Length/Width	5.8	5.3	5.9	5.3	7.0	4.6	n.d.	5.6	5.2	5.5
Length/Wider Width	4.3	4.1	4.5	4.2	4.8	-	-	4.6	4.6	-
Valve Apices	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Obtusely to acutely rounded	Obtusely to acutely rounded	Obtusely to acutely rounded
Central Area	Rhombic-Broad fascia	Rhombic-Broad fascia	Rhombic-Broad fascia	Rhombic-Broad fascia	Broad fascia	Rhombic-Broad fascia	Rhombic-Broad fascia	Rhombic, marginal, widened to a narrow to valve-wide fascia	Rhombic, marginal, widened to a narrow to valve-wide fascia	Rhombic, marginal, widened to a narrow to valve-wide fascia

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TABLE 2. Comparison between *Pinnularia baetica* and the morphologically and ecologically most similar taxa (data from Sardinia comes from the literature)

***Pinnularia* (References)**	***baetica* (this study,Spain)**	***baetica* (this study,Serbia)**	***atlasi* (this study, Guedrouz)**	***atlasi* (this study, Ouiouane)**	**cf.*atlasi* (this study, Macedonia**	***atlasi* (Darley 1990)**	***atlasi* (Lange-Bertalot et al. 2003)**	***infirma* (this study,Spain)**	***infirma* (this study,Macedonia)**	***infirma* (Krammer 2000)**
Number of measurements (n)	20	4	19	18	1	-	-	20	5	-
Valve Length (µm)	45.8-77.7	55.5-79	44.1-72	35.4-77.9	57.2	65-77	n.d.	25.2-43.15	29.1-41	18-51
Valve Width (µm)	9.1-12.2	10.5-14.5	8.3-11.2	8.7-12.5	8	15-16	n.d.	5.2-6.9	6.3-7.2	4.5-8
Valve Wider Width (µm)	11.6-16.6	15.4-17.2	11.2-14.6	11.2-15.8	11.8	-	-	6.9-7.9	7.2-8.1	-
Valve Outlines	Panduriform	Panduriform	Panduriform	Panduriform	Panduriform	Panduriform	Panduriform	Weakly panduriform	Weakly panduriform	Linear-lanceolate with moderately convex sides
Length/Width	5.8	5.3	5.9	5.3	7.0	4.6	n.d.	5.6	5.2	5.5
Length/Wider Width	4.3	4.1	4.5	4.2	4.8	-	-	4.6	4.6	-
Valve Apices	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Cuneiform	Obtusely to acutely rounded	Obtusely to acutely rounded	Obtusely to acutely rounded
Central Area	Rhombic-Broad fascia	Rhombic-Broad fascia	Rhombic-Broad fascia	Rhombic-Broad fascia	Broad fascia	Rhombic-Broad fascia	Rhombic-Broad fascia	Rhombic, marginal, widened to a narrow to valve-wide fascia	Rhombic, marginal, widened to a narrow to valve-wide fascia	Rhombic, marginal, widened to a narrow to valve-wide fascia

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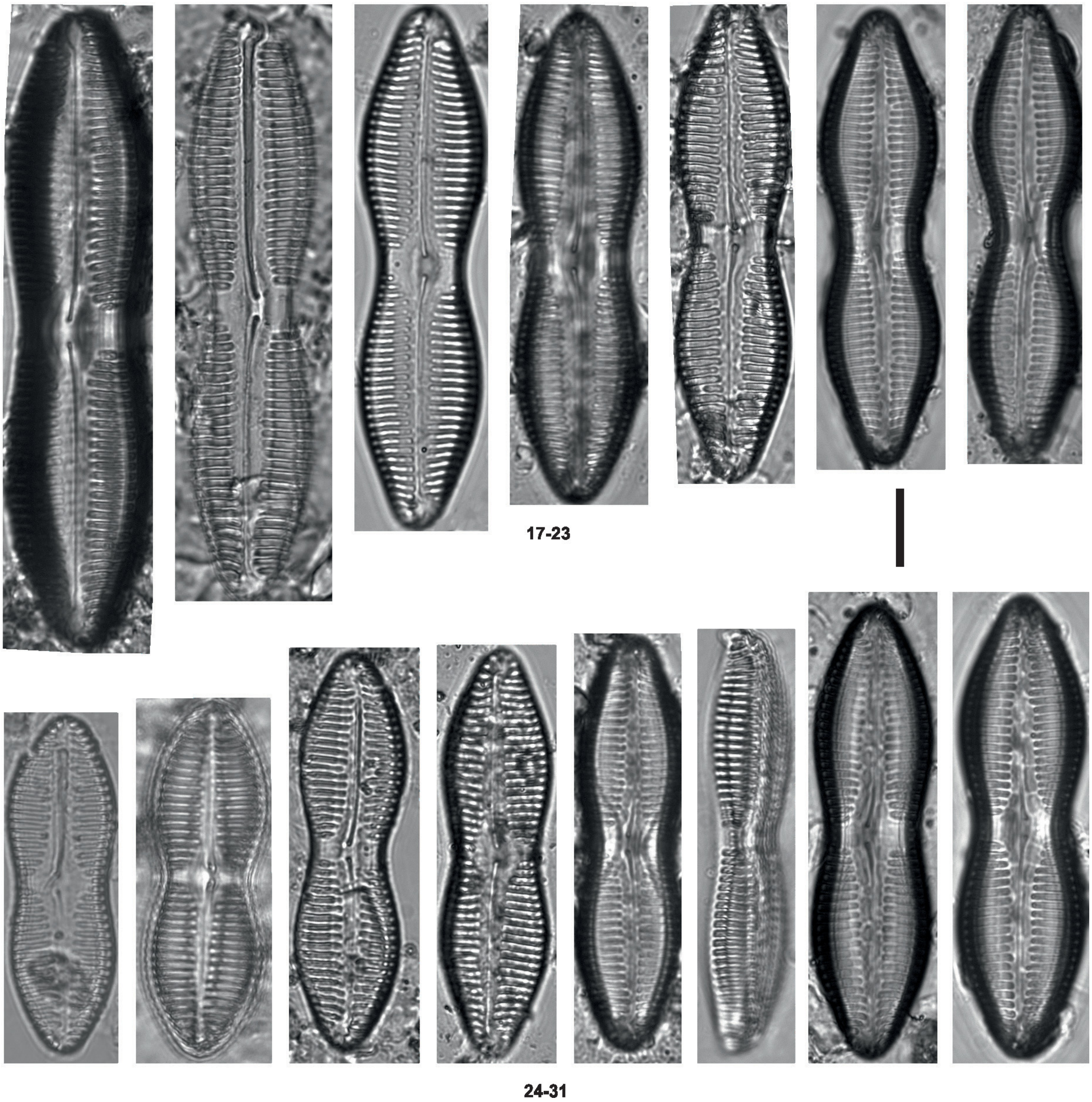
TABLE 2 (Continued**)**

***Pinnularia* (References)**	***baetica* (this study,Spain)**	***baetica* (this study,Serbia)**	***atlasi* (this study, Guedrouz)**	***atlasi* (this study, Ouiouane)**	**cf.*atlasi* (this study, Macedonia**	***atlasi* (Darley 1990)**	***atlasi* (Lange-Bertalot et al. 2003)**	***infirma* (this study,Spain)**	***infirma* (this study,Macedonia)**	***infirma* (Krammer 2000)**
Number of Striae in 10 µm	(9)10	10	(9)10-11	(9)10-11	10	8-9	n.d.	11-13	11-13	9-12
Presence of Spines	No	No	Yes	Yes	Yes	n.d.	Yes	No	No	No
Striae Patterns	Transapical striae parallel to slightly or strongly convergent at the poles	Transapical striae parallel to slightly or strongly convergent at the poles	Transapical striae parallel to slightly convergent at the poles	Transapical striae parallel to slightly convergent at the poles	Transapical striae parallel to slightly convergent at the poles	Transapical striae parallel to slightly convergent at the poles	Transapical striae parallel to slightly convergent at the poles	Transapical striae slightly radiate to convergent at the poles	Transapical striae slightly radiate to convergent at the poles	Transapical striae slightly radiate to convergent at the poles
Number of Rows per Striae	7	?	8-9	8(9)	n.d.	n.d.	n.d.	5-6	?	n.d.
Raphe	Central nodule	Central nodule	Central nodule	Central nodule	Central nodule	Central nodule	Central nodule	Central nodule	Central nodule	Central nodule strongly deflected to one side and comma shaped at the poles
	bent to one side and hoooked at the poles	bent to one side and hoooked at the poles	bent to one side and hoooked at the pole	bent to one side and hoooked at the poles	bent to one side and hoooked at the poles	bent to one side and hoooked at the poles	bent to one side and hoooked at the poles	bent to one side and hoooked at the poles	bent to one side and hoooked at the poles	bent to one side and hoooked at the poles
Ecology	Calcareous mountain pond	Calcareous mountain pond	Calcareous mountain pond	Calcareous mountain pond	Calcareous mountain pond	Calcareous mountain pond	Calcareous mountain pond	Calcareous mountain pond	Calcareous mountain pond	

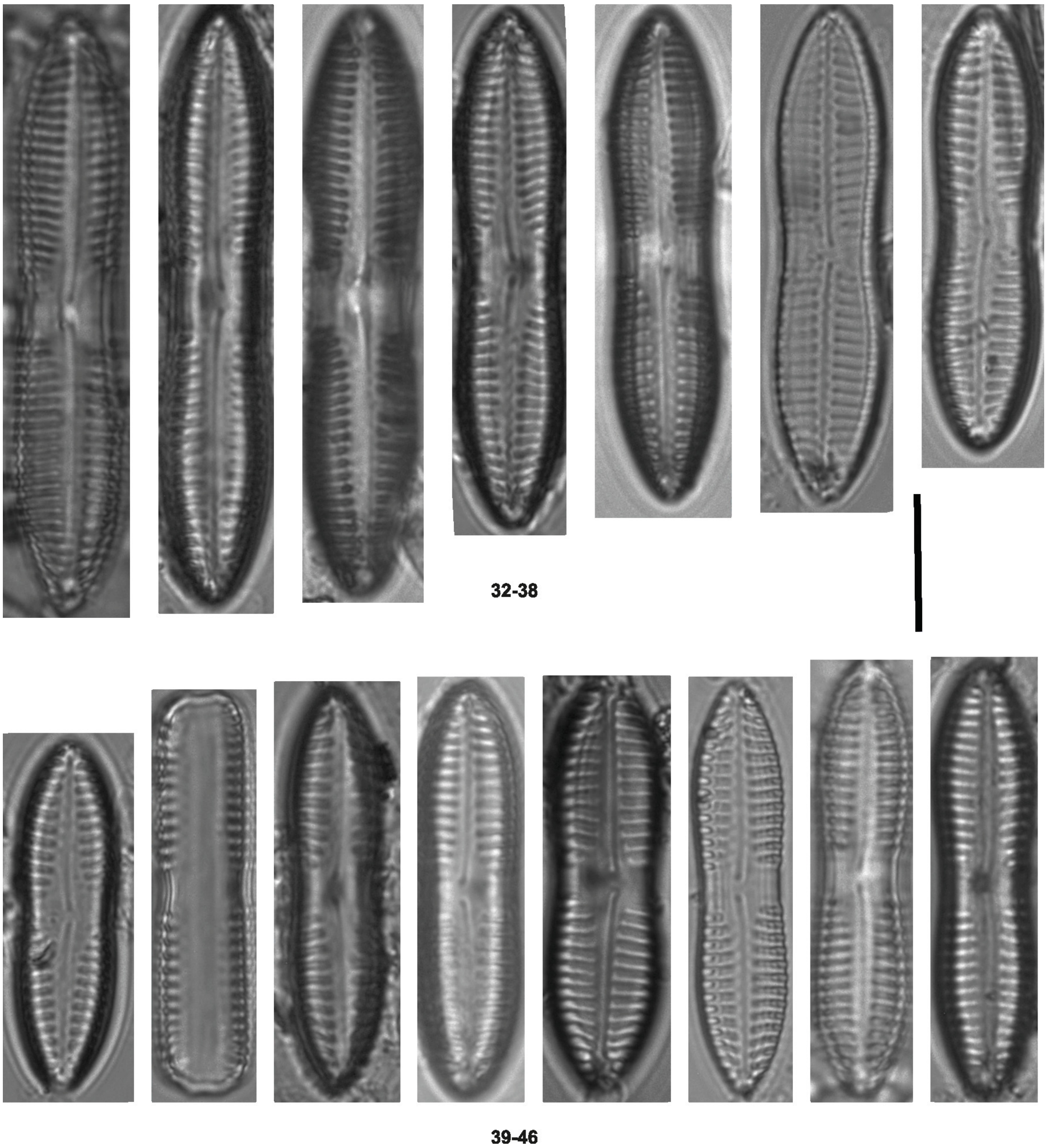


FIGURES 17–31. *Pinnularia atlasi* Darley. Light micrographs of the type location collected in Morocco. Scale bar 10 µm.

Discussion

The most similar taxa to *P. baetica* are *P. atlasi* and *P. infirma* (Table 2). One of the most distinctive features of *P. atlasi* is the presence of spines, as it was previously recorded in the population from Sardinia (Lange-Bertalot *et al.* 2003). This character is not common in the genus *Pinnularia* (Van de Vijver *et al.* 2004). Most similar species to *P. atlasi*, in terms of shape and position of the spines, is *P. spinea* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito (in Lange-Bertalot *et al.* 2003: 108) having the same type of spines and similar set up and structure (although it has several rows of spines, being the external smaller) different from other spine-carrying species of the genus *Pinnularia. Pinnularia sofiae* Van de Vijver & Le Cohu (in Van de Vijver *et al.* 2008: 104), *P. catenaborealis* Pinseel, Hejdukova, Vanormelingen, Kopalova, Vyverman & Van de Vijver (in Pinseel *et al.* 2016) and *P. gemella* Van de Vijver (in Van de Vijver *et al.* 2009: 432) with different shapes.

From the two locations of Morocco, slight differences in the valve length (44.1–72 µm in Guedrouz and 35.4–77.9 µm in Ouiouane) and width (8.3–11.2 µm in Guedrouz and 8.7–12,5 µm in Ouiouane) of *P. atlasi* from the collected material can be noticed. According to the protologue *P. atlasi* is 65–77 µm long, 15–16 µm wide (Darley 1990) with 8–9 striae in 10 µm. The main differences between *P. atlasi* and *P. baetica* can be noticed in presence of spines along the entire valve margin on both sides in *P. atlasi* species. In *P. atlasi*, spines near the mid-valve are relatively large (approx. length/width: 1 µm x 1 µm) and bifurcated at the ends (Fig. 58), while those located towards the ends of the valve are smaller and have blunt ends (Figs 55). They are clearly aligned in a single marginal row between the valve and mantle, progressively decreasing in diameter from the center to the apex of the valve, even disappearing (Figs 53, 55). The presence of spines at a particular position in the center of the frustule in *P. atlasi* can be observed in an unfocused (because of the panduriform shape) and incomplete girdle view at LM (Fig.65), as it was previously mentioned by Lange-Bertalot *et al.* (2003, fig. 88: 3). On other hand, the most evident characteristic of *P. baetica* is the absence of spines on the valve margin (Figs 47, 51). Additionally, *P. baetica* has more convergent striae in the apices (Figs 47, 48), and slightly lower striae density (9–10 in 10 µm vs (9)10–11 in 10 µm in *P. atlasi*) in all valve.

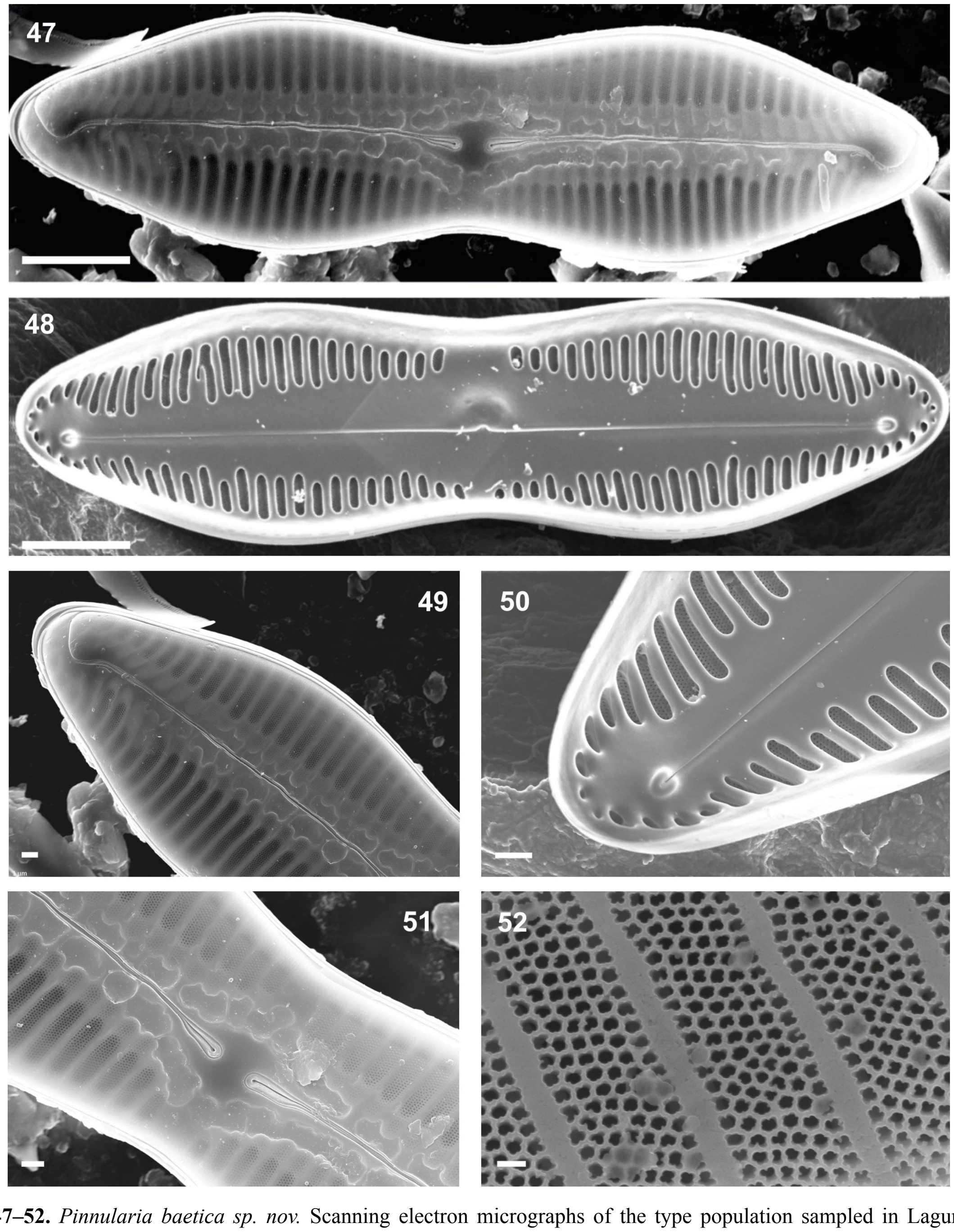


FIGURES 32–46. *Pinnularia infirma* Krammer. Light micrographs of the population collected in Ouiouane, Morocco in this study. Scale bar 10 µm.

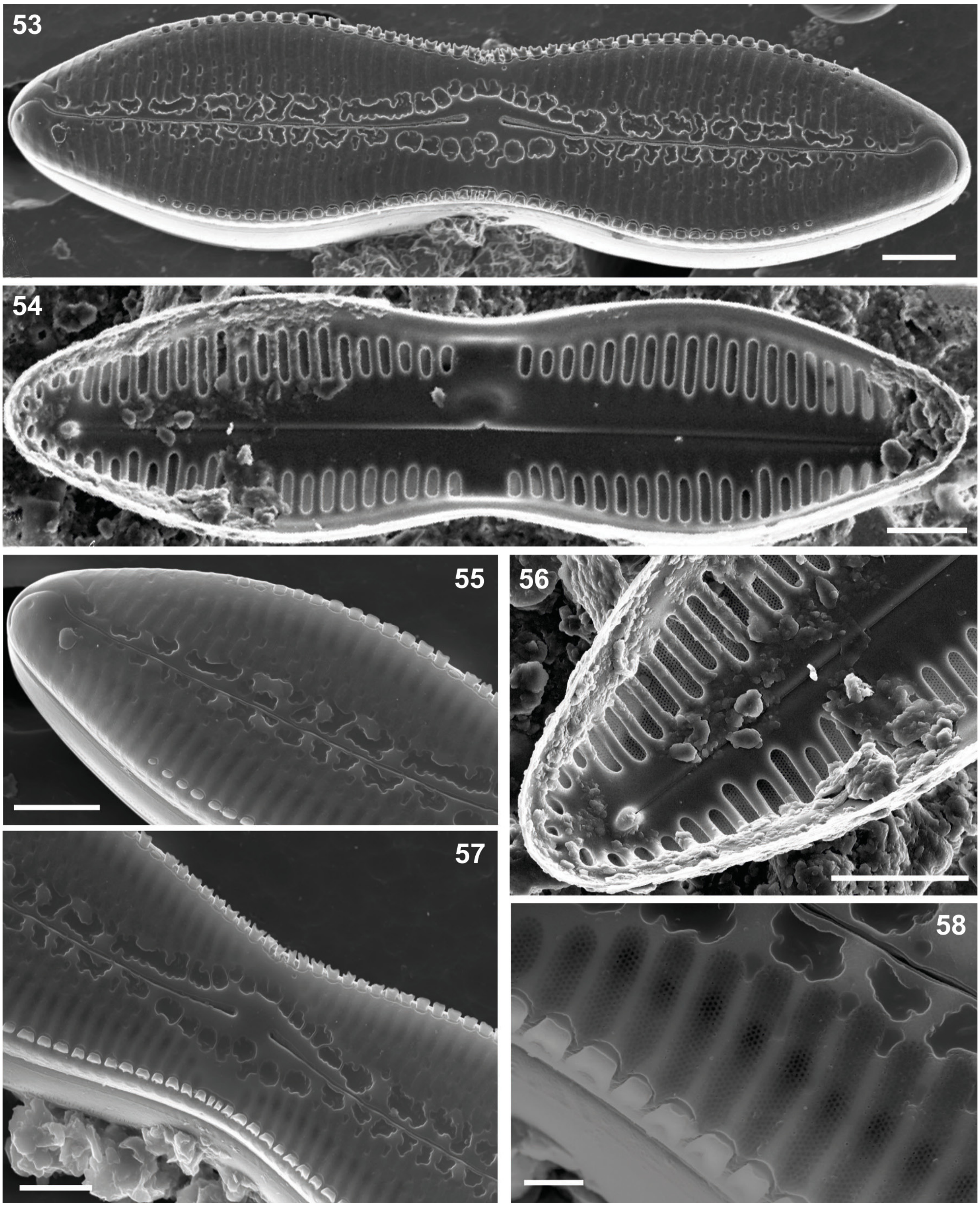
Pinnularia infirma Krammer & Lange-Bertalot (1985) was studied because it appeared with *P. atlasi* in Ouiouane and it has a panduriform shape but more slightly than *P. atlasi* (also *P. cf. atlasi*) and *P. baetica* and the size is smaller than the rest of the species of this study. It was originally described in central Europe, in a locality at 250 m a.s.l. (Franken, Germany). It was also observed in Morocco, and in Macedonia and is reported from different places, such as Germany (Hustedt 1930; Ludwig & Schnittler 1996; Krammer & Lange-Bertalot 1985), Great Britain (Whitton *et al.* 2003), North Macedonia (Levkov *et al.* 2005), Russia (Stenina & Patova 2007) and Sardinia (Krammer 2000; Lange-Bertalot *et al.* 2003). No ultrastructural data are provided and no reference to the presence of spines is given.

In the sample from Ouiouane ephemeral pond, both *P. infirma* and *P. atlasi* are present, but these two species can be easily differentiated by the valve size and shape, presence/absence of spines and striae density. *Pinnularia infirma* had slight panduriform valves instead of the more panduriform valves of *P. baetica*. Furthermore, *P. infirma* has obtusely to acutely poles, smaller valve size and the number of rows in each alveolus is lower (5–6) than in *P. baetica* (7–8).

Because this species (except *P.infirma*) showed biogeographical similarities with other endemic organisms from the circum-mediterranean region such as some plants (Nieto-Feliner 2014; Roselló 2013), and other aquatic groups with a high rate of endemicity in the Mediterranean (Tierno de Figueroa *et al.* 2013), there is a possibility that it might be the result of a succession of continuous geological events from the Tertiary era that caused the connection and fragmentation of territories between Sardinia and northern Africa through the Balearic Islands and southern Europe (Azzaroli 1981), further studies would be necessary to confirm this fact. The distribution of *P. baetica* is more difficult to understand. It is possible that the species shows a more continuous northern Mediterranean distribution not completely known yet. Or it might be possible that the particular characteristics of its habitat could explain its disjoint distribution.



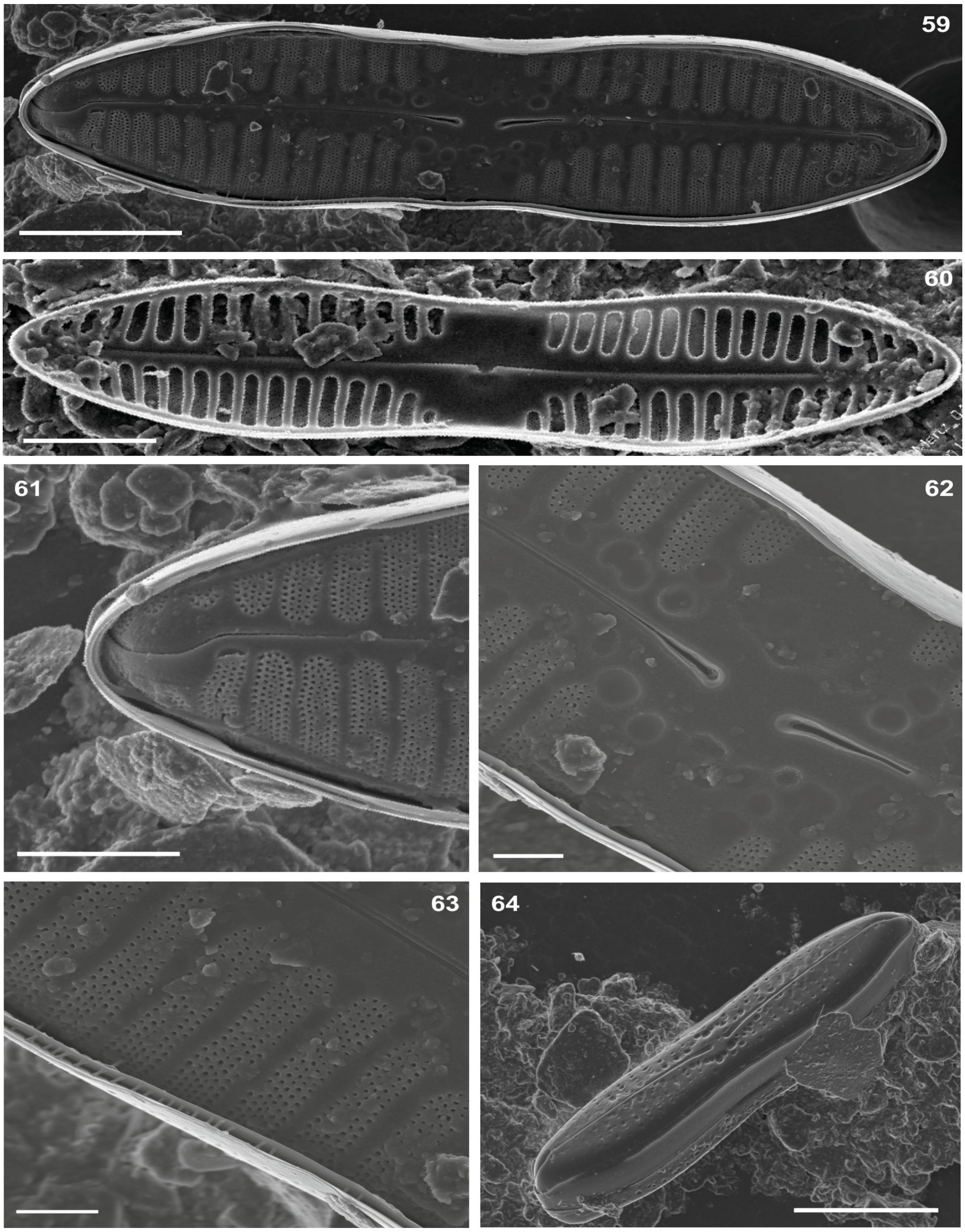
FIGURES 47–52. *Pinnularia baetica sp. nov.* Scanning electron micrographs of the type population sampled in Laguna Seca. (47) external valvar view; (48) internal valvar view; (49) external view of the apex, showing the distal raphe hooked endings; (50) internal view of the apex, showing the distal raphe endings terminating on small helictoglossae; (51) external central nodule bent on one side; (52) rows of each alveoli. Scale bars 5 µm (47–49,51); 1 µm (50,52).



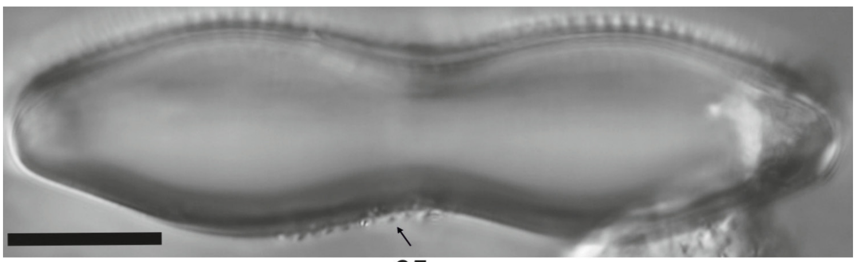
FIGURES 53–58. *Pinnularia atlasi* Darley. Scanning electron micrographs of the type location collected in Morocco. (53) external valvar view; (54) internal valvar view; (55) external view of the apex, showing the distal raphe hooked endings; (56) internal view of the apex, showing the distal raphe endings terminating on small helictoglossae; (57) external central nodule bent on one side and spines in marginal area; (58) external rows of each alveoli. Scale bars 5 µm (53–57); 1 µm (58).

Conclusion

P. baetica sp. nov. is described from Southern Spain and Serbia, and it is compared with *P. atlasi* from the type locality. *Pinnularia atlasi* has been recorded in North of Morocco (in this study as well as in Darley´s description) and Sardinia. In North Macedonia, one similar species *P.* aff. *atlasi,* has been observed. The presence of *P*. aff. *atlasi* would be dependent of molecular studies clarifying its taxonomic status. A better knowledge of the diatom communities in the south of Europe could add more information about the biological history in this Mediterranean region.



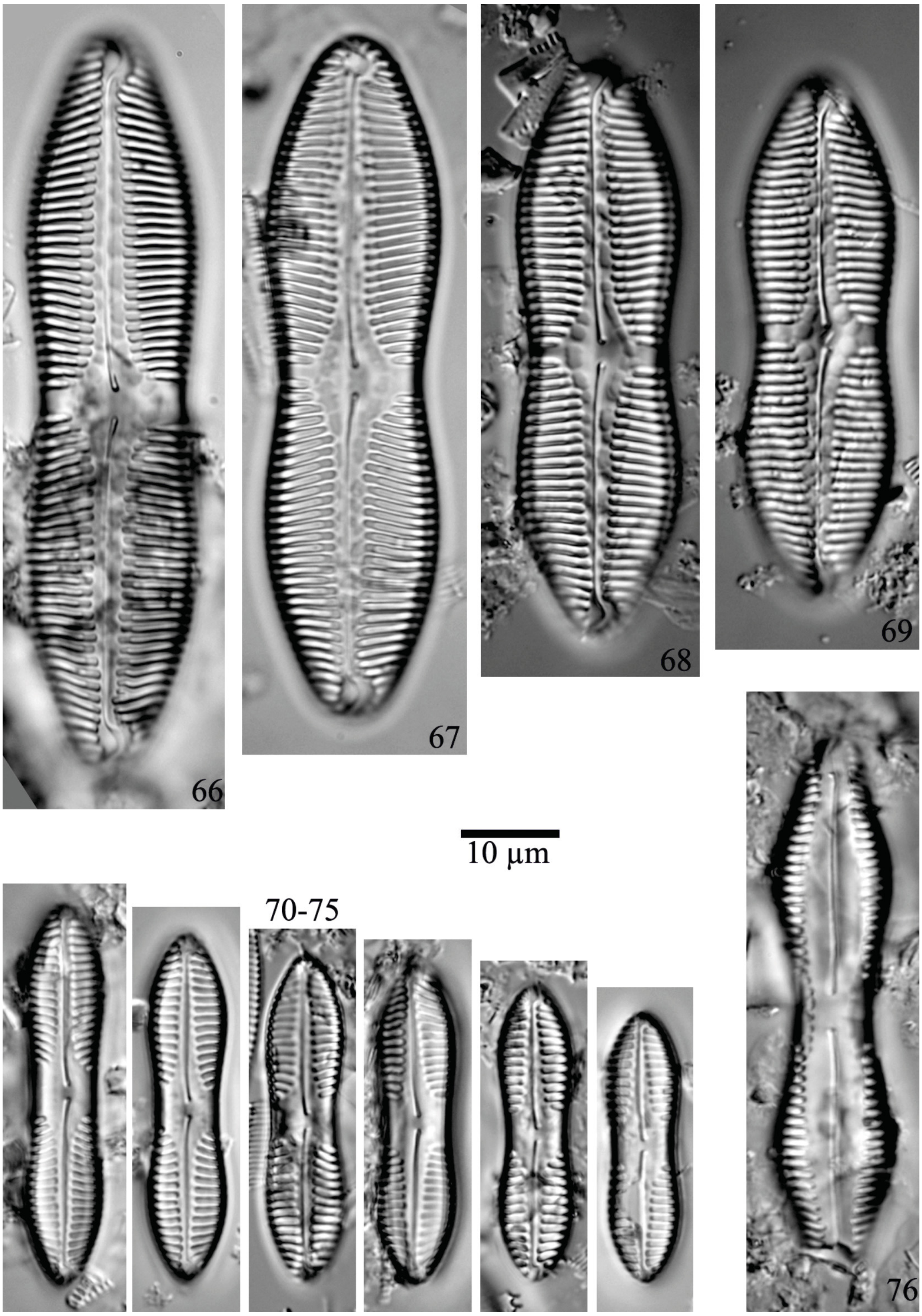
FIGURES 59–64. *Pinnularia infirma* Krammer. Scanning electron micrographs of the population collected in Ouiouane, Morocco. (59) external valvar view; (60) internal valvar view; (61) external view of the apex, showing the distal raphe hooked endings; (62) external central nodule bent on one side; (63) external rows of each alveoli; (64) Girdle view. Scale bars 5 µm (59–61); 1 µm (62–63); 10 µm (64).



65

FIGURES 65. *Pinnularia atlasi Darley* from the type location in Morocco. Light micrograph in girdle view. Showing the spines (see arrow) defined in the middle of the concave part of the lower valve. Scale bar 10 µm.

Pinnularia baetica and *P. infirma* were recorded also in North Macedonia and Serbia (Figs 66–75). *Pinnularia baetica* (Figs 66–69) and *P. infirma* (Figs 70–75) were observed in glacial lakes and ponds of Shara Mountain, Probably, both *P. baetica* and *P. infirma* have broader distribution in Europe, but they are always rare in the samples, represented by one to very few valves on the slide. The habitats where they occur are intermittent ponds, calcareous and oligotrophic with medium electrolyte content. The new species P. baetica has a combination of particular morphological characteristics studied under LM and SEM that separates it from the rest of the species analysed in this work.



FIGURES 66–76. *Pinnularia* species collected in Serbia and Macedonia. (66–69) *Pinnularia baetica sp. nov.* Light micrographs of the type population sampled in Lake Livadičko (Serbia). (70–75) *Pinnularia infirma* Krammer. Light micrographs of the population collected in Radika. (76) *Pinnularia atlasi* Darley from Macedonia. Light micrograph. Showing the spines defined in the middle of the valve. Scale bar 10 µm.

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