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Short note

Planothidium lacustre (Achnanthidiaceae), first world record outside the type locality

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Abstract. During a study of Iberian benthic diatom flora in disconnected Mediterranean pools, some small populations of *Planothidium lacustre* were found in Andalusia and Extremadura (Spain). Specifically, in seven pools of temporary rivers in the Guadiana and Tajo basins. Since its discovery in a small pond in Doñana (Andalusia, Spain) in 2013, there have been no new clear records of this species. Our detailed morphological analysis based on optical microscopy and scanning electron microscopy allowed us to show the morphological variability of the taxon. The specimens of *P. lacustre* are illustrated with microscopic images, and information on their distribution and ecology is provided. Our findings also suggest that *P. engelbrechtii* could be a species closely related to *P. lacustre*. They allow a better understanding of this distinctive Iberian taxon and highlight the need to protect Mediterranean temporary pools and ponds, often forgotten habitats.

Keywords. Bacillariophyta, diatom, disconnected pool, *Planothidium lacustre*, temporary river.

Resumen. Durante un estudio florístico de diatomeas bentónicas ibéricas realizado en pozas mediterráneas desconectadas, se encontraron algunas pequeñas poblaciones de la especie *Planothidium lacustre* en Andalucía y Extremadura (España). En concreto, en siete pozas de ríos temporales de las cuencas del Guadiana y del Tajo. Desde su descubrimiento en un pequeño estanque de Doñana (Andalucía, España) en 2013, no se han encontrado nuevos registros claros de esta especie. Nuestro análisis morfológico detallado basado en observaciones de microscopía óptica y microscopía electrónica de barrido nos permitió mostrar la variabilidad morfológica del taxón. Los ejemplares de *P. lacustre* se ilustran con imágenes microscópicas y se añade información sobre su distribución y ecología. Nuestras aportaciones también sugieren que *P. engelbrechtii* podría ser una especie muy próxima a *P. lacustre*. Este estudio contribuye a mejorar el conocimiento de este distintivo taxón ibérico y destaca la necesidad de proteger las lagunas y pozas temporales mediterráneas, hábitats a menudo olvidados.

Palabras clave. Bacillariophyta, diatomea, poza desconectada, *Planothidium lacustre*, río temporal.

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Title in Spanish: *Planothidium lacustre* (Achnanthidiaceae), primer registro fuera de la localidad tipo.

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The members of the monoraphid diatom genus *Planothidium* Round & Bukhtiyarova are common and often abundant in streams with variable water quality (Morales 2006). Species are frequently found in both running and stagnant waters and shows varying degrees of tolerance to eutrophication (Morales 2006; Cantonati & al. 2021). As a result, some representatives of the genus are widely used for their ecological potential as bioindicators of water quality in biomonitoring studies (Stancheva & al. 2020; Noga & al. 2013). *Planothidium* cells are usually solitary, attached to the substrate (e.g., algae, macroalgae or sand). However, representatives have also been reported in brackish, marine and even aerial environments (Van de Vijver & al. 2018; Cantonati & al. 2021). *Planothidium* is usually classified into three groups: those with a cavum (e.g., *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot), those with a sinus (e.g., *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot), and those with neither (e.g., *Planothidium daui* (Foged) Lange-Bertalot). Although this character is usually easily observable under light microscopy (LM), various challenges may hinder accurate taxonomic identification, such as population variations, the presence of unknown taxa, inaccurate descriptions, or incomplete environmental characterization (Van de Vijver & al. 2018; Stancheva & al. 2020). One example is the continuous description of new species each year, which contributes to a better understanding of *Planothidium* diversity (Van de Vijver & al. 2013; Van de Vijver & al. 2018; Van de Vijver & Bosak 2019). *Planothidium lacustre* Álvarez-Blanco, Cejudo-Figueiras & S. Blanco is a rare Iberian species described from a small pond in Doñana Natural Park (Andalusia, Spain), located in the Guadalquivir basin. In the original paper, it was described having an elliptic-lanceolate valve outline with convex margins and protracted, broadly rounded, rostrate apices (Blanco & al. 2013). Its description was based on a small number of specimens from a single temporary pond in southwest Spain, making it highly likely that the entire cell variability was not included in the description. Additionally, very little environmental information was provided.

During a survey of the Iberian benthic diatom flora conducted in 65 disconnected Mediterranean pools [June and July 2023], several small populations of a species identified as *Planothidium lacustre* were collected. Specifically, *Planothidium lacustre* was found in 7 of these pools (located in Andalusia and Extremadura, Spain) (Fig. 1). Five sites were located in the Guadiana basin: Arroyo de Tamujoso, Arroyo de las Canchalejas, Río Burdalillo, Arroyo de Albarrajena, and Río Zapatón. Two other sites were located in the Tajo basin: Río Gibranzos and Arroyo de Barbaón (Table 1). The last one is particularly important as it is located in the Monfragüe National Park and Biosphere Reserve. Both basins are characterized by siliceous geology and a Meso-Mediterranean climate (Peinado & al. 2017). In all cases, the pools had formed after the disruption of water flow from several temporary rivers in the Guadiana and Tajo basins.

Table 1. Relative abundance of *Planothidium lacustre* in each disconnected pool and their physicochemical values, depth, and ion and nutrient concentrations.

	Arroyo de Tamujoso	**Arroyo de Albarrajena**	**Arroyo de Barbaón**	**Arroyo de las Canchalejas**	**Río Burdalillo**	**Río Gibranzos**	**Río Zapatón**
Autonomous community	Andalusia	Extremadura	Extremadura	Extremadura	Extremadura	Extremadura	Extremadura
Coordinates	37°54’59.0”N 7°11’58.7”W	39°16’47.1”N 6°52’17.2”W	39°51’43.2”N 6°04’38.6”W	39°13’11.7”N 5°55’58.7”W	39°14’10.6”N 5°53’32.2”W	39°27’02.1”N 6°02’37.5”W	39°17’49.7”N 6°49’01.7”W
Abundance (%)	0.66	0.83	0.22	1.35	2.68	0.41	0.88
T (ºC)	27.11	28.17	21.47	35.03	30.23	22.5	29.5
pH	9.73	8.86	7.12	9.66	7.9	8.29	8.43
O_2 (mg/L)	11.9	14.1	3.38	30.84	7.5	8	11.15
O_2 (%)	156.1	182.1	40.5	460.6	103.9	96	150.8
Conductivity (microS/cm)	353	550	95	940	289	314	212
Mean deep (m)	0.05	0.23	0.3	0.24	0.21	0.2	0.18
Phosphate (mg PO_4/L)	0.15	0.57	0.12	1.54	0.03	0.021	0.06
Nitrite (mg NO2/L)	0.004	0.01	0	0	0.005	0	0.005
Nitrate (mg NO3/L)	0.13	0.91	0.17	4,97	1.07	1.22	0.12
Ammonium (mg NH_4/L)	0.12	1.06	0.06	2.60	0.14	0.04	0.06
Chloride (mg Cl/L)	19.85	79.40	8.50	141.80	15.59	26.94	14.18
Sulphate (mg SO_4/L)	104.38	23.72	4.744	40.32	16.60	28.46	11.86

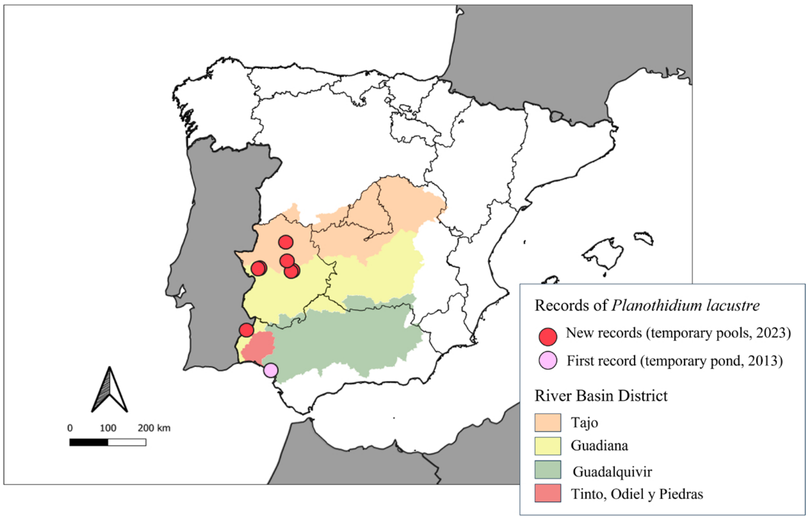

Fig. 1. Distribution of *Planothidium lacustre* in Spain. New records are indicated by red points, while the only previous record is shown in pink. [river basin districts are also highlighted on the map; Tajo = Orange; Guadiana = Yellow; Guadalquivir = Green; Tinto, Odiel, and Piedras = Dark red].

Sampling of each pool was carried out with the help of a toothbrush, scraping seven submerged stones (approximate size of 15 cm^2 each). All collected material was fixed in 96% alcohol in 50 mL Falcon tubes and kept cold in refrigerators. Water samples were also collected for nutrient analysis and different physical and chemical variables were obtained in situ using a multiparameter probe (YSI Professional plus Multiparameter Probe) [water temperature (ºC), pH, dissolved oxygen (mg/L), oxygen saturation (%), conductivity (EC µS/cm)] (Table 1). The diatom samples were treated in the laboratory with hydrogen peroxide (37%) to eliminate organic matter with hot treatment (90ºC). After this, they were cleaned with distilled water and two drops of hydrochloric acid were added to eliminate traces of calcium carbonate. Part of the sample was mounted on slides with Naphrax©. The samples were identified to species level using diatom monographs (Lange-Bertalot & Krammer 2000–2011; Hofmann & al. 2011) under a Zeiss microscope at × 1000 magnification with DIC and camera. For each sample, the relative abundance (%) of each species was calculated from 400 valves (Table 1). Notably, these samples contained communities with a high abundance and frequency of *Staurosira venter* (Ehrenberg) Cleve & J.D. Möller, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Cocconeis euglypta* Ehrenberg, and *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot. All samples were stored in the collection of the University of Barcelona and one more (Río Burdalillo) was kept at the Centre for Plant Biodiversity Documentation of CRAI (CeDocBiV). Reference: BCN Naphrax slide BCN-Phyc 13687. Another fraction of this treated sample was deposited on a glass coverslip adhered to the sample holder of the scanning electron microscope by means of a conductive diadhesive disk (carbon tape, Ted Pella). The sample was left to dry for 24 hours. Subsequently, it was coated with a thin layer of gold to improve the electronic conductivity and was observed in the scanning electron microscope (SEM) JSM-7001F (Jeol) (acceleration voltage of 15 kV) in the Electron Microscopy Unit TEM-SEM of the Scientific and Technological Centers of the University of Barcelona.

Morphological description

LM (Figs. 2–33): Valves rhombic-lanceolate (smaller valves) to elliptic-lanceolate with protracted, broadly rounded, rostrate apices. Some specimens of intermediate sizes were also curved along the transverse axis, possibly due to teratological forms (e.g., Figs. 6, 19, 21, 23, or 24). Valve dimensions (n = 32): length 8–16 µm, width 3.8-6.0 µm. Rapheless valve (RLV) (Figs. 2–12): Axial area narrow, linear to linear-lanceolate. Central area absent or variably expanded due to shortening of at least one stria. When more than one, striae occasionally opposed (Fig. 9). Transapical striae subparallel to radiate, more radiate towards the apices, 16-19 in 10 µm. Areolae not discernible in LM. Raphe valve (RV) (Figs. 13–33): Axial area narrow, linear. Central area slightly transapically expanded by the shortening of the middle striae. Transapical striae radial, more marked at apices, 16–18 in 10 µm. At least one shortened stria of variable size present in the central area. When more than one shortened stria present, striae occasionally opposed (Fig. 30). Areolae not resolvable in LM.

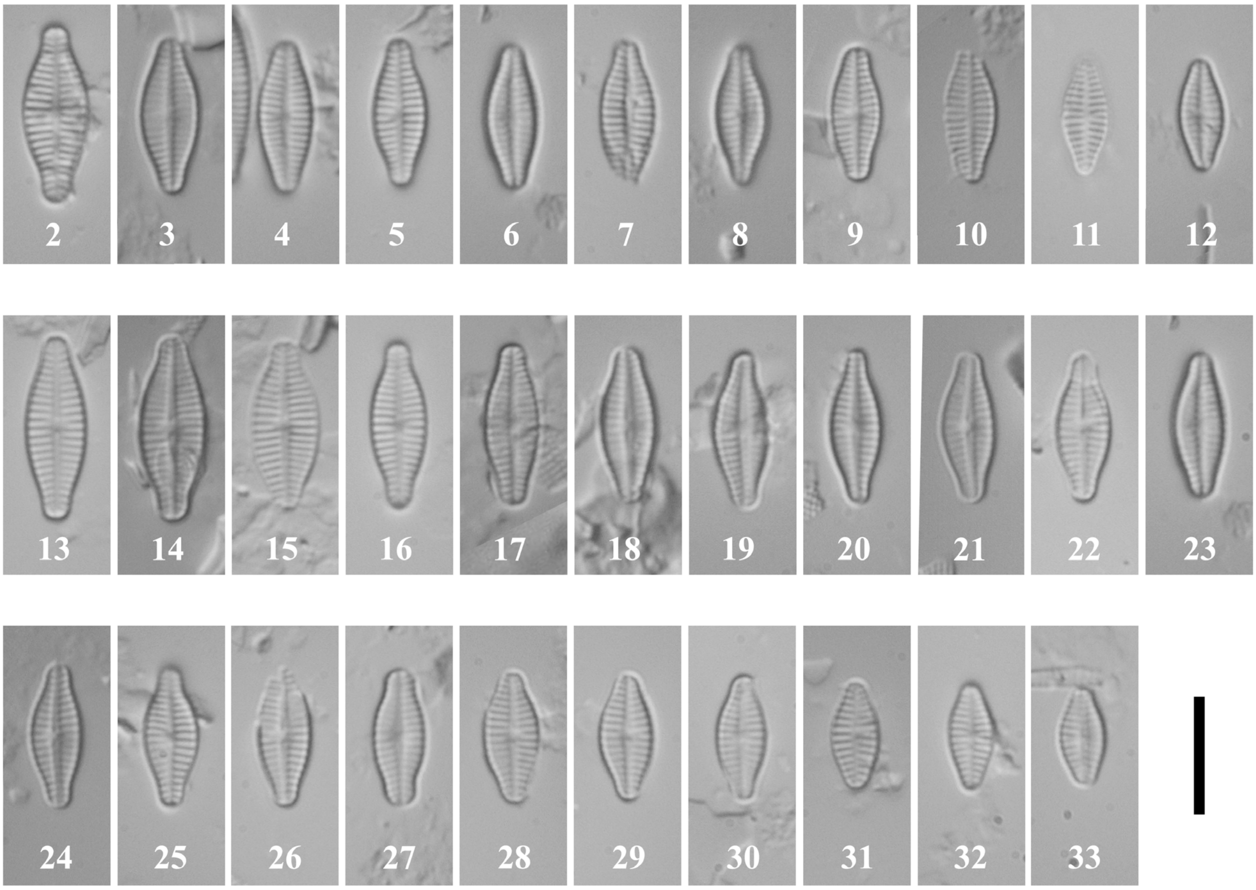

Fig. 2-33. Images of *Planothidium lacustre* obtained with the optical microscope: **2–12**, rapheless valves in a series of size reductions; **13–33**, raphe valves in a series of size reductions [all specimens are from Andalucia and Extremadura pools; scale bar = 10 µm].

SEM (Figs. 34–39): RV (Figs. 34–35, 39): Valves concave with protracted rostrate, forward-curving apices (especially in intermediate sizes). Valve face flat. Virgae of the striae not raised. Striae multiseriate, composed of 2–3 rows of small (most frequently 3), rounded areolae. Triseriate striae usually with larger areolae bordering smaller central areolae. Striae in central part reducing number of areola rows towards the center of the valve, presenting a triangular shape (Fig. 34). At least one shortened stria of variable size in the central area. At the apices, terminal striae occasionally fused by insertion of scattered areolae (Fig. 39, arrow). Terminal raphe fissures unilaterally bent, shortly continuing onto the valve mantle (Fig. 39). Central raphe pores enlarged, relatively close to each other. Internally, central raphe endings deflected into opposite directions on a weakly raised central nodule. Raphe sternum almost not raised (Fig. 35). Terminal raphe endings terminating onto small helictoglossae. Striae located in shallow grooves between their virgae. RLV (Figs. 36–38): Valves convex with protracted, rostrate, backward-curving apices (especially in intermediate sizes). Virgae not raised above their striae. Valve face flat. Striae multiseriate, composed of 2–3 rows of areolae. Triseriate striae with larger areolae bordering a third row of smaller areolae. One or more triangular striae in the central area reducing the number of areola series towards the center (Figs. 36, 38). Shortened stria usually absent. At the apices, striae more closely together giving the impression of large fused striae with 6 rows of areolae. Internally, areolae in shallow grooves between weakly raised virgae (Fig. 37).

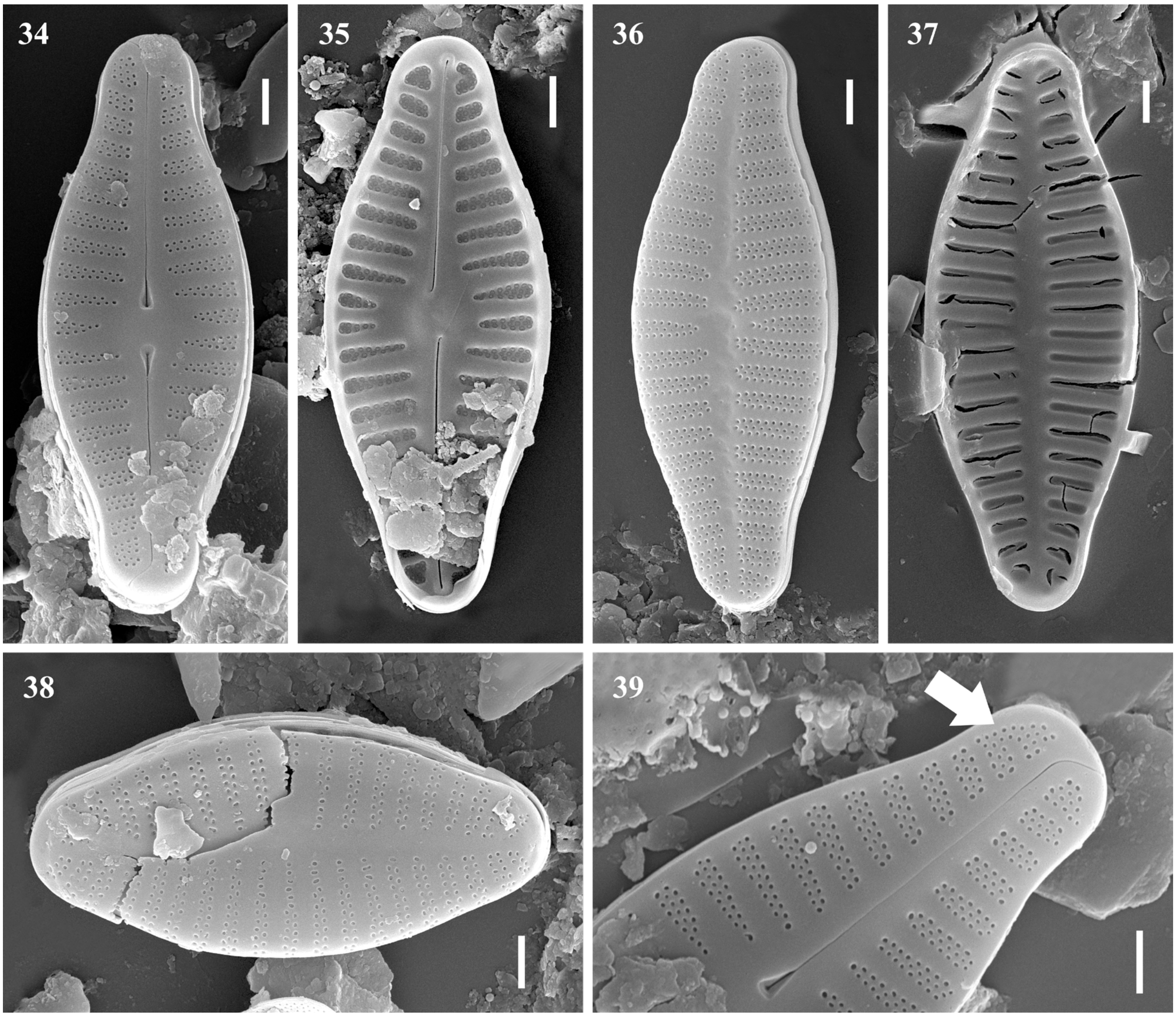

Fig. 34-39. Images of *Planothidium lacustre* obtained with SEM: **34**, external view of an entire raphe-bearing valve; **35**, internal view of an entire raphe; **36**, external view of an entire rapheless valve; **37**, internal view of an entire rapheless valve; **38**, external view of an entire rapheless valve with two rows of pores; **39**, external view of a raphe-bearing valve where the terminal striae are fused by the insertion of scattered areolae (white arrow) [all specimens are from Río Burdalillo (Extremadura); scale bar = 1 µm].

Taxonomic comments

The specimens examined match with the original description of *Planothidium lacustre* (Blanco & al. 2013). However, different aspects of its morphology need to be updated. We observed a larger variability in valve outline, ranging from rhombic-lanceolate (smaller valves) to elliptic-lanceolate, always with protracted, rostrate apices. We had found several frustules curved along the transapical axis. Others were strongly curved along the transverse axis, which could indicate teratological forms. According to Blanco & al. (2013), valve dimensions were 10.6–15.2 µm for length and 5.1–5.4 µm for width. Under the light microscope, we significantly expanded this range for the length 8-16 µm, width 3.8–6 µm. Other features, such as the presence of at least one shortened stria in the central area, have allowed for a better characterization of the species.

We agree with the authors of the original description that *Planothidium lacustre* is very similar to *P. delicatulum* (Kützing) Round & Bukhtiyarova (Blanco & al. 2013; Van de Vijver & al. 2018). However, our observations suggest that *P. engelbrechtii* is also closely related. Although Blanco & al. (2013) already reported this similarity, the new information reaffirms it with even greater clarity. In fact, *P. delicatulum* and *P. engelbrechtii* in Europe are often confused (Lange-Bertalot & al. 2003). *Planothidium engelbrechtii* was originally described as *Achnanthes engelbrechtii* Cholnoky from South Africa (Cholnoky 1955). Later authors updated the original description, for instance revising the stria structure (Cholnoky

1959; Schoeman & Ashton 1983) but it was not until Compère & Van de Vijver (2009) that Cholnoky's South African type material was better characterised. *Planothidium engelbrechtii* has a rhombic-lanceolate to elliptic-lanceolate valve outline with protracted, broadly rounded, rostrate apices. Several valves show some morphological similarity to *P. lacustre*. Moreover, valve dimensions seem to be rather similar for both taxa: 8–16 x 3.8–6 µm vs 8–19 x 3.5–5.0 µm. *Planothidium engelbrechtii* occasionally presents a shorter stria in the central area, similar to *P. lacustre* but the striae are composed of 3–4 series of areolae. In a recent diatom survey of Californian rivers, Stancheva & al. (2020) also observed differences (valve outline, striae, axial and central areas) between their *P. engelbrechtii* specimens and the original lectotype from South Africa. The authors speculated on the existence of an unresolved species complex that requires further investigation. We believe that *P. lacustre* could be part of this complex. *Planothidium pericavum* (J.R.Carter) Lange-Bertalot is also likely part of this complex, as it exhibits a morphology very similar to *P. engelbrechtii*, *P. lacustre* and *P. delicatulum*. In fact, according to Carter (1966), *P. pericavum* is difficult to distinguish from *P. engelbrechtii*, differing mainly in having less protracted valve apices and striae composed of only two rows of areolae. Therefore, we agree that new taxonomic studies are needed to help clarify these issues.

Distribution and Ecology

The ecology of *Planothidium lacustre* was established in a temporary pond from Doñana National Park (Andalusia, Spain) for a coastal Mediterranean-Atlantic climate (Blanco & al. 2013). Although no new confirmed records have been reported since its original description, a morphologically similar species was observed in a recent study from Lake Sasyk (Ukraine) (Bilous & al. 2023). However, due to the lack of SEM data, the authors classified it as a taxonomic uncertainty (*Planothidium* cf. *lacustre*). Our study has allowed us to expand the known distribution of this species, extending it to the central-southern Iberian Peninsula (Extremadura–Andalusia). Therefore, successful populations of *P. lacustre* are likely to exist in Portugal and other nearby Spanish river basins (e.g., Tinto, Odiel, and Piedras). Our data have also improved the ecological knowledge of *P. lacustre* based on the characterization of disconnected pools (Meso-Mediterranean climate). Among them, we can highlight high temperature values, mainly alkaline pH, or relatively high phosphate. In this sense, disconnected pools and ponds could function as temporary habitats with strong ecological similarity, as they are commonly aquatic systems with high values of temperature, dissolved oxygen, and pH (Blanco & al. 2013; Quevedo-Ortiz & al. 2024). These highly dynamic habitats also show strong similarity with those of *P. engelbrechtii*. Californian and Sardinian *P. engelbrechtii* specimens were also found in temporary habitats, specifically non-perennial and ephemeral rivers (Stancheva & al. 2020; Lange-Bertalot & al. 2003). Given the presence of *P. engelbrechtii* in these Mediterranean regions (including South Africa) and its morphological similarities, we affirm the necessity for further detailed study probably related to a species complex.

Based on all the above, *Planothidium lacustre* is a rare Iberian species adapted to highly temporary habitats that differs from its closest taxonomic relatives. Considering that the genus *Planothidium* is widely used to assess the ecological quality of continental waters (Noga & al. 2013), it is likely that *P. lacustre* can provide important information in various biomonitoring studies in the Iberian Peninsula. This study contributes to a better understanding of this species and to the protection of Mediterranean temporary pools and ponds, often forgotten habitats.

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AUTHORSHIP CONTRIBUTION STATEMENT

Guillermo QUEVEDO-ORTIZ: Conceptualization, Investigation, Methodology, Writing-original draft, Writing – review & editing. Joan GOMÀ: Conceptualization, Writing – review & editing.

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