

First record of *Linderiella jebalae* Boix, Sala, Escoriza & Alonso 2016 (Crustacea, Branchiopoda, Anostraca) in the Iberian Peninsula. Dispersal across the Strait of Gibraltar?

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ABSTRACT

First record of *Linderiella jebalae* Boix, Sala, Escoriza & Alonso (Crustacea, Branchiopoda, Anostraca) on the Iberian Peninsula. Dispersal across the Strait of Gibraltar?

We report the first record of *Linderiella jebalae* in the Iberian Peninsula from two temporary ponds in La Línea de la Concepción (Cádiz, southern Spain). This species was described in 2016 in the Moroccan Atlas Mountain range on the other side of the Strait of Gibraltar, 102 km away from this new Iberian location. Sequencing a fragment of cytochrome c oxidase subunit I (COI) showed a higher genetic diversity in Morocco, but only one haplotype in the Iberian populations, suggesting recent dispersal from Morocco to Spain, possibly mediated by birds through their annual migrations. This finding increases the diversity of anostraceans in the Iberian Peninsula to 16 species.

Key words: Anostraca, distribution, Spain, temporary pond, dispersal, bird migration, *Linderiella*

RESUMEN

Primera cita de *Linderiella jebalae* Boix, Sala, Escoriza & Alonso (Crustacea, Branchiopoda, Anostraca) en la Península Ibérica. ¿Dispersión a través del Estrecho de Gibraltar?

Se documenta la primera cita de *Linderiella jebalae* Boix et al. 2016 en la Península Ibérica, concretamente en dos charcas temporales de la Línea de la Concepción (Cádiz). Esta especie fue descrita en 2016 en la cordillera marroquí del Atlas, al otro lado del Estrecho de Gibraltar, a 102 km de la nueva localidad ibérica. La secuenciación de un fragmento del gen mitocondrial citocromo c oxidasa subunidad I (COI) mostró una mayor diversidad genética en los individuos de Marruecos, con un sólo haplotipo en la población ibérica, sugiriendo una dispersión reciente desde Marruecos a España, posiblemente mediada por aves a través de sus migraciones anuales. El nuevo hallazgo aumenta la diversidad de anostráceos en la Península Ibérica hasta las 16 especies.

Palabras clave: Anostraca, distribución, España, charca temporal, dispersión, migración de aves, *Linderiella*

INTRODUCTION

The Iberian Peninsula is home to a rich native fauna of large branchiopods, with 25 taxa described and another three pending to complete the description (*Linderiella* sp. in Girona, *Tanymastix* sp. in the Portuguese Algarve; and *Triops* sp. in the Valencian region). Fifteen out of 25 belong to the order Anostraca (Sala *et al.*, 2017), commonly known as fairy shrimps. This high relative richness with respect to other European countries may be the result of the geological and geographical diversity of the Iberian Peninsula and the relatively low impact of glaciations compared to other more northern European territories. In fact, glaciation in the Iberian Peninsula during the Quaternary concentrated around the main mountain systems and with a very uneven ice cover, with a maximum extension in the Pyrenees and only isolated glaciers throughout the rest of the region (Deltell, 2005). Another notable factor is the particular configuration of the relief of the Iberian Peninsula. It is the result of intense Alpine deformation in a process of crustal shortening connected to the N–S that gave rise to a large number of internal Cenozoic basins of very different sizes formed by lithospheric folding, and many of them have undergone a common sedimentary evolution in which long periods of endorheic regime stand out (De Vicente *et al.*, 2011; Vergés *et al.*, 2019). On the other hand, the occurrence of the Mediterranean climate is reflected in an aridification process from 5 500 cal. yr BP to the present (Pérez-Obiol *et al.*, 2011), *i.e.*, after the Late Quaternary glacial phase, in the Iberian Peninsula. However, that moment was followed by significant changes in climate regimes during the Late Holocene, alternating warm (*e.g.*, Medieval Climate Anomaly, recent warming) and cold stages (*e.g.*, Dark Ages, LIA) (Oliva *et al.*, 2019).

Within the Order Anostraca, the case of the genus *Linderiella* is particularly relevant since it is associated with ponds with a Mediterranean climate and a split distribution between the Nearctic and the western Palearctic (Boix *et al.*, 2016; Fig. 1). All species of this genus show a high degree of endemism: *L. massaliensis* Thiéry & Champeau appears in south-eastern France (Thiéry & Cham-

peau, 1988), *L. africana* Thiéry is distributed in the central area of the Atlas Mountains in Morocco (Thiéry, 1986), while *L. jebalae* appears in the northern Atlas Mountains (Boix *et al.*, 2016). Regardless of *Linderiella* sp. from Catalonia, currently under description (D. Boix Pers. Comm.), to date only one species was known in the Iberian Peninsula, *L. baetica* Alonso & García-de-Lomas, which is also endemic and present in a single locality in the province of Cádiz (Alonso & García-de-Lomas, 2009; García-de-Lomas *et al.*, 2016). Habitat degradation and risk of disappearance of its habitat has caused this species to be considered “Critically Endangered” by the IUCN (García-de-Lomas *et al.*, 2017; IUCN, 2021).

In January 2021, during samplings aimed at looking for new locations for *L. baetica*, specimens of *Linderiella* were found in two small temporary ponds near the urban centre of La Línea de la Concepción (Cádiz, S. of Spain). Further observation in the laboratory confirmed that the species matched the morphological features of *L. jebalae*, which was previously described in northern Morocco from specimens found in 2011 and 2012 (Boix *et al.*, 2016).

In this work, we aim at identifying the *Linderiella* species found in temporary ponds from La Línea de la Concepción (Cádiz) as well as to provide an environmental characterisation (including water quality and accompanying biota) of the ponds where the Iberian population of *L. jebalae* was found. Moreover, a genetic study was carried out to infer whether the North African and South European populations dispersed before or after the disconnection between the European and African continents at the end of the Miocene, coinciding with the end of the Messinian salinity crisis 5–6 million years ago (Blanc, 2002; Krijgsman *et al.*, 1999). We hypothesize that the dispersal of *L. jebalae* throughout the Strait of Gibraltar is recent (< 5 million years ago), being likely mediated by migratory birds.

MATERIALS AND METHODS

Study area and biometric analysis

The studied ponds (El Tejar and La Peña) are located in the outskirts of La Línea de la Con-

cepción (southern Spain, N 36° 10', W 5° 21', 27–40 m a.s.l.), about 3.5 km from Gibraltar (Fig. 2). Following the Köppen climate classification, the climate could be classified as Csa (Peel et al., 2007), characterised by mild winters (average temperature in January = 13.0 °C) and warm summers (average temperature in August = 22.3 °C) with an average rainfall of 592 mm (1981–2010) (AEMET 2022), with particular microclimatic, humid conditions due to the formation of cap and banner clouds over the peak of Gibraltar during easterlies (Dorman et al., 1995). Daily rainfall data were recorded at the San Roque weather station (<https://es.meteosolana.net/estacion/6056X>), located 12 km from the studied ponds in order to roughly infer the time required for cyst germination and maturation of *L. jebalae*. These rain ponds take up in small endorheic depressions located on a rocky outcrop that belongs to the so-called Aljibe Unit or Numidic Mantle, a lithological unit from the Aquitaine (ca. 23 My) that is

characterised by rounded grains of pure siliceous sandstone mainly composed of quartz (90 %) (Alonso-Chaves et al., 2004; Pendón, 2004). The ponds are small (110–190 m²), with a maximum depth of 0.7 m. Conductivity (measured with a portable meter Crison® model CM35+) ranged 448–600 µS/cm. pH (measured at 12 p.m. with a portable meter Crison® model PH25+) ranged 7.1–7.8. Samples for nutrients were preserved cool and measured by a specialised company (Innoagral® Laboratories) within 5 days: NH₄⁺ = 0.42–0.70 mg/L; NO₃⁻ up to 7 mg/L; total phosphorous below detection level (0.05 mg/L). The ponds are located at the southern foot of Sierra Carbonera. Habitat types (EUNIS) surrounding the ponds are western *Calicotome* and *Genista* garrigues. The two ponds studied are 175 m apart. Once *L. jebalae* was detected, periodic visits (02 February, 16 March, 30 March, 23 April and 18 May 2021) were made to describe the accompanying community and to roughly

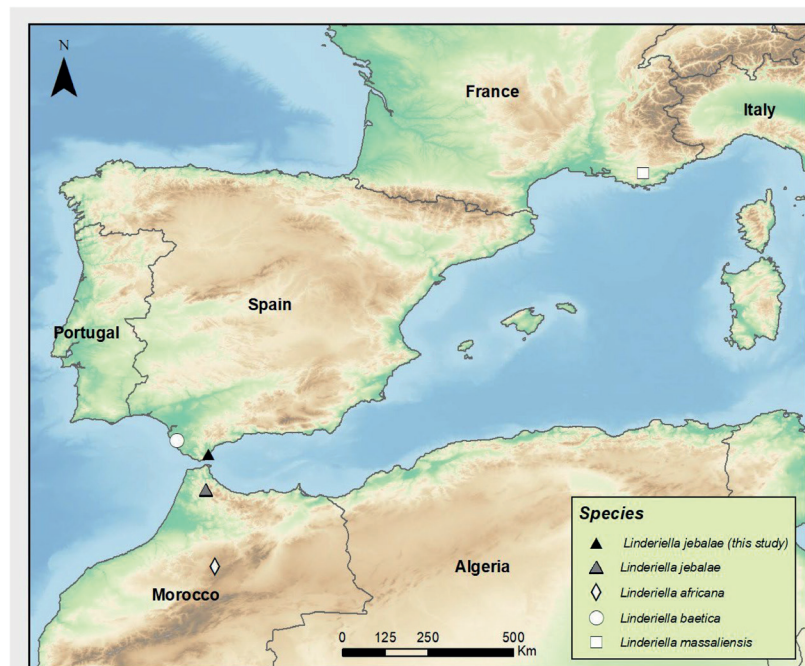


Figure 1. Distribution of the Palearctic species of the genus *Linderiella*. Only type localities of each species are included (Thiéry, 1986a; Thiéry & Champeau, 1988; Alonso & García-de-Lomas, 2009), as well as the new locality of *Linderiella jebalae* reported in the present study. *Distribución de las especies paleárticas del género Linderiella. Se incluyen las localidades tipo incluidas en la descripción de cada especie (Thiéry 1986a; Thiéry & Champeau 1988; Alonso & García-de-Lomas 2009), así como la nueva localidad de Linderiella jebalae del presente estudio.*

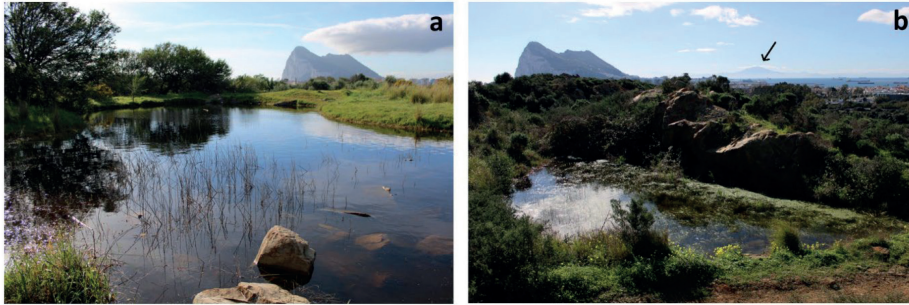


Figure 2. Overview of the rain ponds where *Lindieriella jebalae* was found: El Tejar (a) and La Peña (b) ponds. In (b) note Mount Jebel Musa, included in the North African Atlas Mountains (arrow), where *L. jebalae* was originally described. *Panorámica de las charcas pluviales donde se encontró Lindieriella jebalae: a) charca El Tejar; b) charca La Peña. En el fondo de la imagen (b) se observa el monte Jebel Musa, incluido en la cordillera norteafricana del Atlas (flecha), próximo a la zona donde *L. jebalae* fue descrita.*

estimate the life span of *L. jebalae*. The samplings were carried out using a nytex net (mesh size = 100 μ m; section = 26 \times 21 cm). The collected fauna were fixed with formaldehyde at a final concentration of 4 %. Some specimens were also fixed in 100 % ethanol for molecular analysis (see below). *Lindieriella jebalae* specimens preserved in 4 % formaldehyde and 100 % ethanol have been deposited in the collection of the University of Granada (CCZ-UGR, n.º cat. 20068-CG y 20069-CG).

Total length, TL (i.e., from the front of the head to the distal tip of cercopods, excluding setae) of some specimens was measured. Cysts removed from the brood pouches were counted. Cyst counts only included ‘countable’, separate cysts (either mature or immature), but aggregate masses of immature, non-separable eggs were not included.

Genetic analyses

For genetic analyses we sequenced 11 individuals from the available samples from four different localities of *L. jebalae*, one sequence from the type locality in Morocco (Al bayara; Boix *et al.* 2016), two sequences from a nearby pond (Tabrait; Chauen), both collected in April 2012, and four sequences from the Iberian new localities (El Tejar and La Peña ponds), collected in 2021. DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit (QiagenTM, Hilden, Germany) according to the manufacturer’s protocol in a to-

tal volume of 50 μ l and was subsequently stored at -20 °C. Fragments of the 658-bp section of the cytochrome c oxidase gene subunit 1 (COI) were amplified using the primers jgLCOI490 (5’-TITCIACIAAYCAYAARGAYATTGG-3’) and jgHCO2198 (5’-TAIACYTCIGGRTGIC-CRAARAYCA-3’) (Geller *et al.*, 2013). All fragments were PCR amplified bidirectionally. Total reaction volume (25 μ L) consisted of 1 \times PCR buffer (Silverstar, Eurogentec), 1.5 mM MgCl₂, 200 μ M of each dNTP, 0.2 μ M of each primer, 2 μ L of template DNA, 1–2 U Taq polymerase and UV light-sterilized mQ-H₂O. PCR conditions involved a denaturing step of 1 min at 94 °C, five cycles of 40 s at 94 °C, 40 s at 45 °C and 60 s at 72 °C, followed by 35 cycles of 40 s at 94 °C, 40 s at 51 °C and 60 s at 72 °C and a final elongation of 5 min at 72 °C.

The resulting sequences (deposited in GenBank under accession numbers OQ064236 for Albayara-Morocco-MV17 -also Spanish samples-; OQ064237 for Tabrait-Morocco-MV17-F11; and OQ064238 for Tabrait-Morocco-MV17-E12) were aligned with the ClustalW algorithm (Thompson, Higgins, & Gibson, 1994) in MEGA version 7 (Kumar, Stecher, & Tamura, 2016). The alignments were checked by eye and corrected according to the translated amino-acid alignment, and sequence divergences (Kimura 2-parameter model) were calculated with the same software. We performed haplotype network analyses to estimate gene genealogies using HAPLOVIEW-

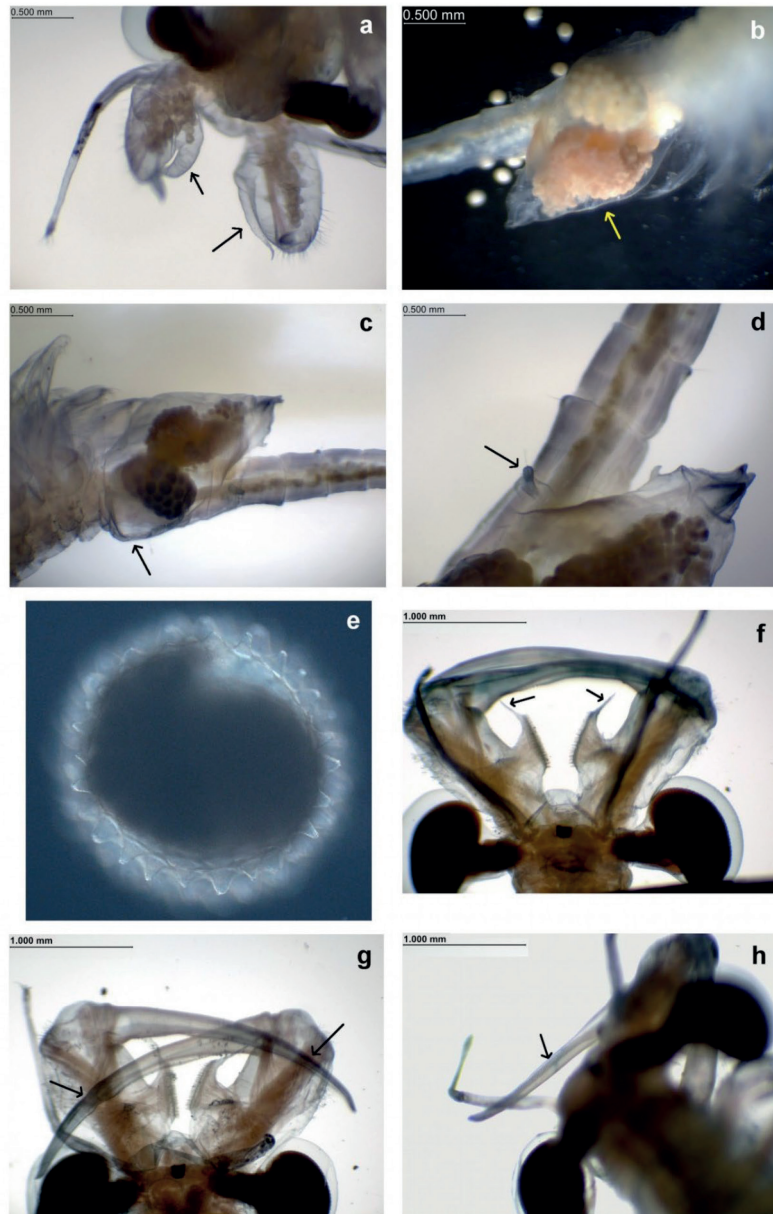


Figure 3. Diagnostic characters of *Linderiella jebalae* specimens collected in La Línea de la Concepción (Cádiz, southern Spain): Female: a) Long, filiform and acute basal outgrowth of the antennae, ending in a warty apex; b) globose, heart-shaped brood pouch with cysts in different maturation stages; c) brood pouch insertion in the first genital segment; d) second genital somite with highly developed cuticular papillae; e) cyst covered with thick, blunt spines. Male: f) basomedial outgrowth on proximal segment of antenna; g) antennal distal segment with maximum curvature located at the distal third; h) row of transverse ridges of the antennal segment with a sinuous shape, with the proximal section before the bend one quarter longer than the distal section after the bend. *Caracteres diagnósticos de Linderiella jebalae de los ejemplares encontrados en La Línea de la Concepción (Cádiz, sur de España).* Hembra: a) antenas con un apéndice largo (unas tres veces más largo que el segmento distal de la antena), filiforme y agudo, que termina en un ápice con aspecto verrugoso; b) ovisaco con la característica forma globosa del género, ligeramente ensanchado que le da un aspecto acorazonado o de pica (especialmente in vivo); c) punto de inserción del ovisaco al principio del primer somito genital; d) segundo somito genital con papila muy desarrollada que terminan en una cerda o seda. e) Quiste con la superficie cubierta de espinas simples con extremo redondeado. Macho: f) artejo basal de la antena con apéndice con una terminación muy afilada; g) artejo apical de la antena, con forma de hoz, con su máxima curvatura en el tercio distal; h) artejo apical con fila de crestas transversales con forma sinuosa, siendo un cuarto más largas en la porción proximal (antes de la curvatura) que en la porción distal, después de la curvatura.

ER, which turns trees built from traditional phylogenetic methods into haplotype genealogies (Salzburger, Ewing, & von Haeseler, 2011). We estimated the phylogeny using a maximum-likelihood method with RaxML Blackbox (Stamatakis, 2006) with invariant sites (GTRCAT + I) and no gamma model of rate heterogeneity as suggested by the model, applying 1000 bootstrap replicates. The input data were COI sequences from each individual, subsequently collapsed into haplo-

types. Sequences with ambiguous bases were not included in the analysis. The best tree (using the log-likelihood criterion) was selected for network construction using HAPLOVIEWER.

RESULTS

The specimens found in the studied ponds matched the morphological characteristics of *L. jebalae*. Females showed: (i) antennae with long, filiform and

Table 1. Plants, arthropods and chordates detected in the temporary ponds from La Línea de La Concepción (Cádiz, Spain) where *Linderiella jebalae* was found. *Plantas, artrópodos y cordados detectados en las charcas temporales de la Línea de la Concepción donde se encontró Linderiella jebalae.*

Phylum/Class/Species	El Tejar	La Peña
Ph. Plantae		
<i>Baldellia ranunculoides</i>	+	+
<i>Eleocharis palustris</i>	+	+
<i>Ranunculus peltatus</i> subsp. <i>peltatus</i>	-	-
<i>Alisma lanceolatum</i>	-	-
<i>Callitriche</i> sp.	-	-
Ph. Arthropoda		
Cl. Branchiopoda		
<i>Linderiella jebalae</i>	+	+
<i>Moina micrura</i>	-	+
<i>Alona rectangula</i>	-	+
<i>Leydigia acanthocercoides</i>	+	-
<i>Macrothrix hirsuticornis</i>	+	-
Cl. Copepoda		
<i>Mixodiaptomus incrassatus</i>	+	+
<i>Hemidiaptomus roubaui</i>	+	+
Cl. Ostracoda		
<i>Heterocypris incongruens</i>	+	+
Cl. Insecta		
<i>Gerris argentatus</i>	-	+
<i>Notonecta maculata</i>	-	+
<i>Corixa panzeri</i>	-	+
<i>Nepa cinerea</i>	+	-
<i>Stratiomys longicornis</i>	+	-
<i>Ilybius</i> sp.	-	+
Ph. Cordata/ Cl. Amphibia		
<i>Triturus pygmaeus</i>	-	+
<i>Pleurodeles waltl</i>	+	+
<i>Pelobates cultripes</i>	+	-

acute basal outgrowths (about three times longer than the distal segment of the antennae), ending in a warty apex (Fig. 3a); (ii) a globose brood pouch, showing a heart or spade shape (particularly in *in vivo* specimens) (Fig. 3b); (iii) the brood pouch is inserted at the beginning of the first genital segment (Fig. 3c); (iv) the lateral and dorsolateral part of the genital somites presented several isolated bristles; (v) the second genital somite with highly developed cuticular papillae ending in a bristle (Fig. 3d). Cysts are covered with thick, blunt spines (Fig. 3e). Males showed the proximal segment of the antennae with a basomedial lanceolate outgrowth ending in a long, narrow and acute distal process (Fig. 3f). The sclerotized distal segment of the antennae is slightly curved (sickle shape) with maximum curvature located at the distal third (Fig. 3g). Also, the row of transverse ridges on the inner surface of antennae shows a sinuous shape (Fig. 3h), being the proximal section before the bend, one quarter longer than the distal section after the bend (Boix et al., 2016).

Sexual dimorphism in size was found. Mature females (mean TL = 14.9 mm, sd = 1.5, n = 5) were significantly longer ($p = 0.0002$, Mann-Whitney U test) than males (mean TL = 11.4, sd = 0.8 mm, n = 14). Curiously, the ratio male:female in *L. jebalae* was highly different between neighbour ponds, one pond (La Peña) showed a male:female ratio of 3.5, the other pond (El Tejar) had a male:female ratio of ~100. Brood pouches showed three cyst aggregates with different stages of maturation. Each female contained an average (\pm sd) of 145 ± 32 cysts per brood (n = 3).

Intense rainfall responsible for pond flooding occurred between 6–11 January 2021 (total rainfall in this period = 185.6 mm) and we found mature specimens on 25 January 2021. No *L. jebalae* specimens were found on 16 March 2021. No other large branchiopod was present throughout the flooding cycle. Aquatic and helophytic vegetation was almost absent in El Tejar pond where few specimens of *Eleocharis palustris* and *Baldellia ranunculoides* appeared at the end of the flooding cycle. In contrast, vegetation was abundant in La Peña pond throughout the flooding period (cover > 50 %), dominated by *Callitriche* sp., *Ranunculus peltatus* subsp. *peltatus*, *Eleocharis palustris*, *Alisma lanceolatum* and *Baldellia ranunculoides*.

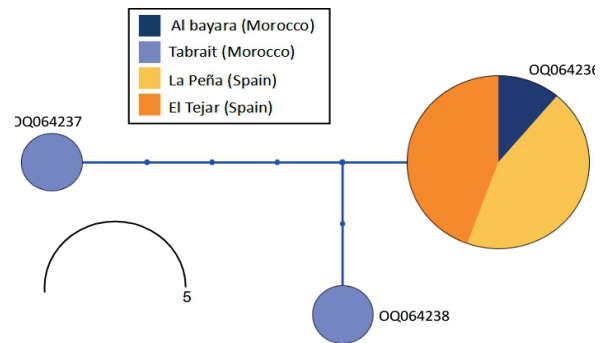


Figure 4. Network of haplotypes of a fragment of the mitochondrial gene for cytochrome c oxidase subunit I (COI) of *Linderiella jebalae*. Each circle represents a unique haplotype, and its size is proportional to the number of individuals sharing that specific haplotype. Each branch with more than one mutational step is labelled. The accession numbers of GenBank sequences are included. *Red de haplotipos de un fragmento del gen mitocondrial citocromo c oxidasa subunidad I (COI) de Linderiella jebalae.* Cada círculo representa un haplotipo único, y su tamaño es proporcional al número de individuos compartiendo cada haplotipo específico. Cada rama con más de un eslabón mutacional está marcada. Se incluyen los números de acceso de cada secuencia en el GenBank.

In El Tejar pond, turbidity was low but increased as the flooding progressed. In contrast, La Peña Pond showed high transparency throughout the entire flooding period. Amphibian larvae also appeared; spur toad larvae (*Pelobates cultripipes*) were abundant in El Tejar pond but were absent in La Peña Pond. In contrast, pygmy newt larvae (*Triturus pygmaeus*) were only present in La Peña Pond. Iberian ribbed newts (*Pleurodeles waltli*) were present in both ponds. Calanoid copepods and other anomopoda species were represented in both ponds (Table 1).

We found three different haplotypes among the 11 individuals sequenced, all of them present in the two Moroccan populations, but only one in Spain, which was shared with one of the two populations from Morocco (Al bayara; Fig. 4). The three haplotypes had small genetic distances, with the two individuals sequenced within Tabrait being the most distant haplotypes (0.92 %; Morocco). The haplotypes of Al bayara, La Peña and El Tejar diverged from the two Tabrait haplotypes by 0.44 % and 0.77 %, respectively.

DISCUSSION

The new record documented here represents the second species of the genus *Lindieriella* present in the Iberian Peninsula (regardless of the undescribed *Lindieriella* sp. from Catalonia; D. Boix pers. comm.). The relatively short period (15–20 days) occurring between pond filling and the observation of mature individuals suggests that *L. jebalae* typically occurs of early stages of the flooding cycle, as reported for *Lindieriella baetica*, *Tanymastigites lusitanica* and *Tanymastix stagnalis* (Alonso and García-de-Lomas, 2009; Machado and Sala, 2013; Olmo *et al.*, 2015). During periodic samplings, we observed that *L. jebalae* was absent two months after pond filling, suggesting a short life span, which is also true for *Tanymastix stagnalis* (1–2.75 months) (Olmo *et al.*, 2015; Lopes da Cunha *et al.*, 2021) and *L. baetica* (~2 months; Alonso and García-de-Lomas, 2009).

The Iberian ponds where *L. jebalae* have been found showed some common features with the type of habitat of this species in Morocco (Boix *et al.*, 2016): low conductivity ($\leq 610 \mu\text{S}/\text{cm}$), transparent temporary waters and abundance of macrophytes in the case of La Peña Pond. Despite the differences in species composition between Spanish and Moroccan ponds, their aquatic communities have features in common such as the absence of other anostracans and the presence of calanoid copepods and amphibians.

Some similarities were also found between the Iberian habitats of *L. jebalae* and *L. baetica*, highlighting the presence of large calanoid copepods such as *Mixodiaptomus incrassatus*, amphibians (Alonso & García-de-Lomas, 2009) and the absence of large anomopods such as *Daphnia magna*, which is common in other temporary pluvial ponds throughout the Cádiz province (García-de-Lomas & García, 2005). Interestingly, *L. jebalae* is the only large branchiopod in both the Iberian and Moroccan ponds, thus contrasting with *L. baetica*, which shares the pond with five large branchiopod species (Alonso & García-de-Lomas, 2009).

Sexual dimorphism also occurred in *L. jebalae*, with females being larger than males; however, the total length of *L. jebalae* is greater than that of *L. baetica* (Alonso & García-de-Lomas, 2009). The sex ratio found in La Peña pond

was similar to *L. baetica* (male:female = 2–7.7; Alonso and García-de-Lomas, 2009); however, the high male-biased sex ratio of *L. jebalae* in El Tejar pond is extremely rare. Slight male-biased sex ratios have been reported in other anostracan species (e.g., a male:female ratio of up to 1.6 in Italian populations of *Chirocephalus diaphanus* (Mura *et al.*, 2003)). Interestingly, such male dominance was only reported in west Mediterranean populations of *Branchipus schaefferi* from North Africa and Spain, which may be due to the presence of one or more supranumerary chromosomes that interfere with sex determination (Beladjal *et al.*, 2002). Our preliminary observations suggested a remarkable difference in the egg clutches between *L. jebalae* and *L. baetica*. Mature females of *L. baetica* produced about 14–18 eggs per brood (Alonso & García-de-Lomas, 2009), whereas *L. jebalae* showed an average of 145 eggs per brood. Moreover, three cyst aggregates in different stages of maturation were observed in *L. jebalae* (Fig. 2b). Thus, the total number of cysts produced in *L. jebalae* may be higher than those reported here, and multiple clutches or cyst release events throughout the flood cycle are expected, as occurs in other anostracan species (Atashbar *et al.*, 2012; Gharibi *et al.*, 2016). Cyst production reaches 533–545 cysts/female in *Branchipus schaefferi* (Beladjal *et al.*, 2007); up to 187 cysts/female in *Tanymastix stagnalis* (Olmo *et al.*, 2015); 579–1094 cysts/female (for 12–18 °C) in *Branchinecta orientalis* (Atashbar *et al.*, 2012); about 150–200 eggs per brood in *Streptocephalus torvicornis* (Nagorskaya *et al.*, 2004); and 174–114 cysts/female (for 20–25 °C) in *Phallocryptus spinosus* (Gharibi *et al.*, 2016). Despite other biotic and abiotic factors involved in species prevalence, fecundity may have conservation implications, suggesting that *L. jebalae* has a greater dispersal potential than *L. baetica*.

The results of the genetic analyses showed that the two Moroccan ponds had higher genetic diversity than those from the Iberian Peninsula, with three haplotypes in Morocco and one in Spain. These results, despite being based solely on a few individuals, suggest that the most likely scenario is that the Iberian pond has been colonised by Moroccan populations. The fact that there is no genetic variability within the Iberian ponds

suggests that this colonisation could be recent, and the scenario of dispersal prior to continental separation is unlikely. This separation took place in the Miocene-Pliocene boundary. The Atlantic waters found a way through the present Strait of Gibraltar and rapidly refilled the Mediterranean 5.33 million years ago in an event known as the Zanclean flood (Loget et al., 2005; García-Castellanos et al., 2009; Schee et al., 2016). Besides *L. jebalae*, other anostracan species such as *Chirocephalus diaphanus*, *Streptocephalus torvicornis*, *Branchipus schaefferi*, *Branchinecta ferox* and *Artemia salina* are represented both in the Iberian Peninsula and northern Morocco (van den Broek et al., 2015). Among these species, *Artemia* spp. is a paradigmatic case of demonstrated bird-mediated dispersal (Green et al., 2005; Rogers, 2014). A similar pattern has been observed in the copepod *Metadiaptomus chevreuxi*, which also colonizes temporary pools and has separated clades in the south of the Iberian Peninsula, Sicily and Tunisia. This distribution suggests the possible role of migratory birds in north-south dispersal (Marrone et al. 2020). In this sense, the arrival of *L. jebalae* to the Iberian Peninsula is possible by passerine birds during their annual migrations (Viana et al., 2016), taking into account the distance at which it has been found (102 km) with respect to the place where the species has been described (Boix et al., 2016). These birds are abundant in the study area during prenuptial migration (Pilastro et al., 1998; Hilgerloh, 2009) and make their first stopover as soon as they cross the Strait of Gibraltar. In this period (February–March) the temporary ponds still have water and cysts are available. Unintentional dispersal of *L. jebalae* cysts by researchers that sampled and described this species in Morocco (e.g., via footwear or motor vehicles; Waterkeynet al., 2010) was excluded because they ignored or didn't visit the Iberian ponds where this species has been found (M. Alonso pers. com.), and vice versa, i.e., the people who sampled the Iberian ponds had not visited Morocco.

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