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ADVANCES IN AQUATIC AND SUBTERRANEAN BEETLES RESEARCH: A TRIBUTE TO IGNACIO RIBERA

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Suplementos del Boletín de la
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Advances in aquatic and subterranean beetles research: a tribute to Ignacio Ribera

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PREFACE

It has been a bit longer than two years since our friend and colleague Ignacio (Nacho) Ribera passed away. The memory of him remains among those of us who were lucky enough to meet Nacho. This monograph is dedicated to him, bringing a set of scientific contributions from his colleagues whose topics are part of the main research lines (and passions) of his scientific work: taxonomy, systematics, biogeography and evolution of aquatic and subterranean beetles.

In the last two years, several contributions have highlighted Nacho' scientific and personal profile, including the complete list of his publications, and both the taxa described by and dedicated to him (BELLÉS, 2020; CEHRE, 2020; DELOCADO *et al*., 2020; FAILLE *et al*., 2020, 2021; JÄCH, 2020; MELIC, 2020; MILLÁN *et al*., 2020a, b; VALLADARES & MILLÁN, 2020).

Nacho was a passionate biologist, interested in everything concerning beetles, especially their diversity, distributions, adaptations and evolution of aquatic and cave beetles. He published a total of 285 papers, in which he described 107 species new to science, highlighting the discovery of the Aspyditidae family. Besides, 7 species and subspecies, and one new genus have been dedicated to him in this monograph, extending to a total of 15 species and two genera.

Concerning the aquatic beetles, we would like to emphasise his crucial contributions, such as the first complete checklist of aquatic and semi-aquatic beetles of the Iberian Peninsula. He was a pioneer in studying the adaptive morphology of the family Dytiscidae, and more importantly, he deepens in the phylogeny of the aquatic beetles, shedding light on the systematics and evolution of the families Dytiscidae and Hydraenidae. He postulated the "*Habitat Constraint*" hypothesis which, from an evolutionary point of view, highlights the importance of habitat stability as a determinant of species range sizes (via differences in their dispersal capabilities). He also dealt in-depth with evolutionary studies related to the habitat transition between lotic and lentic water bodies, but also the transition from freshwater to saline water, or vice versa. Certainly, Nacho, due to his work and publications on aquatic beetles, became one of the most prestigious specialists worldwide.

Regarding the subterranean environment, Nacho revealed that *Dalyat* Mateu is a vicariant genus of carabid whose origin must be in the separation of the Iberian plate from the rest of Pangea in the Jurassic-Cretaceous boundary. He also addressed the position of *Ildobates neboti* Español, confirming it within the Zuphiini tribe. At the same time, he began to study the phylogeny of the hypogeal Trechini of the Pyrenees, and afterwards he addressed the diversification of *Troglocharinus* Reitter, opening the way to a new perspective on the evolution and dispersion of the subterranean fauna. He published the first morphological phylogeny through cladistic analysis of the Leptodirini tribe (Leiodidae), and the first molecular phylogenies for the two main groups that have colonised the subterranean environment, the tribes Leptodirini and Trechini. His contribution to the knowledge of underground and stygobic environments between 2005 and 2021, postulated him as one of the most important specialists worldwide also in this field.

The complete list of scientific works signed by Nacho, the taxa described by him and those dedicated to his person can be found on the web:

https://www.um.es/ecoaqua/index.php/external-collaborators.

We do not want to extend further. We hope these pages serve as a tribute to his person, but also a tribute to a life and scientific style that would serve as an example for future generations of insect lovers.

Our gratitude to the authors and reviewers of the articles that make up this monograph, as well as to the Asociación española de Entomología, which has greatly facilitated its preparation, edition and publication.

November 2022
The editors

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Rediscovery of *Helophorus cincticollis* Guillebeau (Coleoptera, Helophoridae) in Spain

Redescubrimiento de *Helophorus cincticollis* Guillebeau (Coleoptera, Helophoridae) en España

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ABSTRACT

Helophorus cincticollis Guillebeau, 1893 is confirmed as a Spanish species as a result of recent discoveries in the province of Cadiz.

Key words: *Helophorus cincticollis*, new record, Cádiz, Spain.

RESUMEN

Como resultado de los descubrimientos recientes en la provincia de Cádiz, se confirma la presencia en España de *Helophorus cincticollis* Guillebeau, 1893.

Palabras clave: *Helophorus cincticollis*, nueva cita, Cádiz, España.

INTRODUCTION

Helophorus cincticollis Guillebeau, 1893 is a mainly North African species, apparently widespread in Morocco and Algeria, but was added to the list of Spanish (and European) beetles by ANGUS (1992) on the basis of material collected near Malaga by F. Balfour-Browne in January and February 1931, among his mainly unidentified duplicate material, which he had given to Robert B. Angus (RBA). Balfour-Browne's notebooks, now housed in the Royal Museum of Scotland, Edinburgh, give details of the Spanish captures, beside a small road near Málaga Airport. They also show that from Málaga he travelled to Morocco, where he also captured *H. cincticollis.*

The area round Málaga Airport has been developed since 1931 and RBA was unable to find either *H. cincticollis* or pools where it might have occurred, but there seemed to be no good reason to doubt Balfour-Browne's data.

RESULTS

In 2018, before the onset of general lockdowns resulting from the Covid-19 pandemic, RBA received from Jonas Köhler (JK) a box of *Helophorus* for identification. Among the material was a series of five *H. cincticollis* with the data "Esp. San-Roque-Guadiaro, Laguna Guadalquitón, 36.267514°N 5.291280°W, J. Köhler 16.02.2014" and one with the data "Esp. Torreguadiaro, Laguna Guadalquitón, J. Köhler 16.02.2014". The map on Google Earth shows a small lake north of the Playa de Guadalquitón, with the coordinates 36.260117°N 5.291556°W. This would appear to be the Laguna Guadalquitón. The coordinates on the beetles' data labels refer to an area of rough ground about 1.5 km S of Sotogrande, and this contains two or three pools with the coordinates 36.263610°N 5.291581°W and a slightly larger pool with the coordinates 36.262131°N 5.293036°W. The lake at Torreguadiaro has the coordinates 36.294089°N 5.273584°W and according to the website Andalucía Rústica (info@andaluciarustica.com) is the Laguna Torreguadiaro.

In November 2018 RBA and Elizabeth M. Angus (EMA) visited the pools at 36.263610°N 5.291581°W and collected a further male from which a rather poor chromosome preparation was obtained, sufficient to show the karyotype comprised 22 chromosomes, 20 + Xy. This specimen and its aedeagus are shown in Fig. 1. *H. cincticollis* is a very distinctive species with its highly arched orange pronotum with evenly rounded sides. The only species at all resembling it is *H. croaticus* Kuwert, distributed from Central Europe eastwards to Yakutia. *H. croaticus* has the elytral flanks opposite the metasternum as wide as the epipleurs while in *H. cincticollis* they are half that width. The aedeagi of the two species are clearly different, that of *H. croaticus* being only about 0.4 mm long and with the outer margins of the parameres straighter.

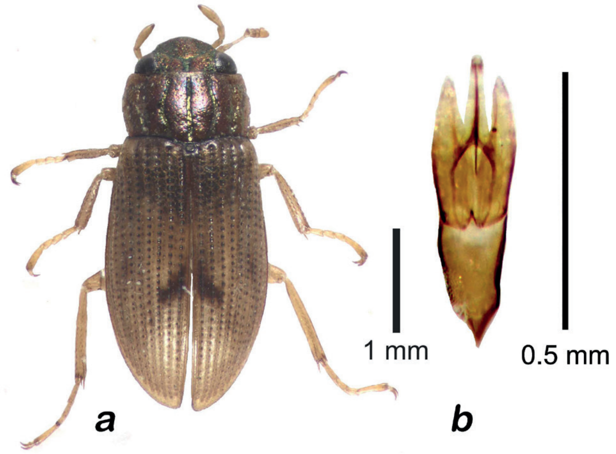

Fig. 1. *Helophorus cincticollis*: a) whole beetle; b) aedeagus.

Fig. 1. *Helophorus cincticollis*: a) vista completa; b) edeago.

DISCUSSION

The rediscovery of *H. cincticollis* represents a very welcome return of a species not seen in Spain for over 90 years. The map on Google Earth shows an area with marshes and pools in the Parque del Guadalhorce just east of the seaward end of Málaga Airport, an area not visited by RBA. Maybe this was the area where Balfour-Browne found it, and in any event the area would be well worth checking. Relatively undeveloped land along the Mediterranean coast of Andalucía is a rare commodity. The once delightful shingle area near Adra where RBA found *Canthydrus siculus* (Ragusa, 1882) and *Cybister vulneratus* KLUG is long gone, swallowed up by polythene huertas (FOSTER, 1986). JK and RG visited the Sotogrande and Torreguadiaro area late in the afternoon of February 16th 2014, so did not have very long to investigate the pools, and when RBA and EMA visited it in 2018 RBA was restricted by an arthritic hip, so again the visit was rather fleeting. This area, including the Laguna Guadalquitón would surely repay a proper investigation and should be considered as of conservation interest. Maybe *Canthydrus siculus* could be found there, and possibly even *Cybister vulneratus*! For the taxonomy of Mediterranean *Canthydrus* see TOLEDO (2022).

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A new species of *Coelometopon* Janssens from the Wild Coast of South Africa (Coleoptera: Hydraenidae)

Una nueva especie de *Coelometopon* Janssens de la Wild Coast de Sudáfrica (Coleoptera: Hydraenidae)

http://zoobank.org/F0B1C788-0DBE-408C-A89B-8C4B2071EAB7

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ABSTRACT

Coelometopon riberae **sp. nov.** is described from Mkambati Nature Reserve, on the Wild Coast Region of the Eastern Cape Province, South Africa. Notes are provided to distinguish the new species from other members of this strictly madicolous genus.

Key words: Coleoptera, Hydraenidae, *Coelometopon*, new species, South Africa, ecology.

RESUMEN

Se describe *Coelometopon riberae* **sp. nov.** de la reserva natural de Mkambati, en la región de Wild Coast de la provincia de Eastern Cape, Sudáfrica. Se aportan datos para distinguir la nueva especie de otros miembros del género que es estrictamente madícola.

Palabras clave: Coleoptera, Hydraenidae, *Coelometopon*, nueva especie, Sudáfrica, ecología.

INTRODUCTION

Madicolous habitats (VAILLANT, 1956) support a specialised, and often poorly known water beetle fauna in many parts of the world, with many new species and even families having been discovered in recent years (e.g. RIBERA *et al*., 2002; RIBERA & BILTON, 2007; SPANGLER & STEINER, 2005; BALKE *et al*., 2008; HÁJEK & FIKÁČEK, 2008; CLARKSON & SHORT, 2012; FIKÁČEK *et al*,. 2012). South Africa supports a diverse madicolous beetle fauna, particularly of Hydraenidae (e.g. PERKINS & BALFOUR-BROWNE, 1994; BILTON, 2014; 2015; 2016). *Coelometopon* Janssens, 1972 is a strictly madicolous hydraenid genus, restricted to southern and eastern Africa, most species being endemic to South Africa (PERKINS, 2005). Here *Coelometopon riberae* sp. nov. is described, following our recent fieldwork in the Eastern Cape province. Comparative notes are provided which will allow the new species to be distinguished from its congeners, together with a brief description of the ecology of the new species.

MATERIALS AND METHODS

Beetles were collected by hand from wet rock faces. Specimens were studied using a Leica MZ8 stereomicroscope, with a Fluopac FP1 fluorescent illuminator. Habitus photographs were taken with a Canon EOS 600D camera fitted to a Leica Z6 Apo macroscope, fitted with a 2x objective lens. Specimens were illuminated using a Leica LED5000 HDI dome illuminator to avoid shadow.

Genitalia were mounted on glass slides in Kisser's glycerol gelatine (see RIEDEL, 2005) and imaged using an Olympus CX31 microscope with the same camera. All image stacks were produced by hand, and combined using Zerene Stacker software (www.zerenesystems.com).

Exact label data are cited for specimens.

ABBREVIATIONS

AMG	Albany Museum, Grahamstown, South Africa
CDTB	Collection D.T. Bilton, Plymouth, UK
NMW	Naturhistorisches Museum Wien, Vienna, Austria
ISAM	South African Museum, Cape Town, South Africa
SANC	South African National Collection of Insects, Pretoria, South Africa
TMSA	Ditsong Museum (former Transvaal Museum), Pretoria, South Africa
TL	Total length (labrum to elytral apex)
EL	Elytral length
MW	Maximum width

TAXONOMY

***Coelometopon riberae* sp. nov.**

http://zoobank.org/CEB11C08-E782-4A0D-BB3B-DF80DC3A02FB

Type material: Holotype: ♂ "7/ix/2019 South Africa EC, Mkambati Nature Reserve, beside Horseshoe Falls on Mkambati River, wet rock face, D T Bilton leg." (genitalia extracted and mounted on same card) and red holotype label (AMG).

Paratypes: 11 ♂, 14 ♀, Same data as holotype (AMG, CDTB, NMW, ISAM, SANC, TMSA). All with red paratype labels.

Description: Measurements: Holotype TL = 2.15 mm; EL = 1.40 mm; MW = 0.95 mm. Paratype males TL = 1.95–2.35 mm; EL = 1.30–1.75 mm; MW = 0.90–1.05 mm. Paratype females TL = 2.00–2.50 mm; EL = 1.35–1.60 mm; MW = 0.95–1.10 mm.

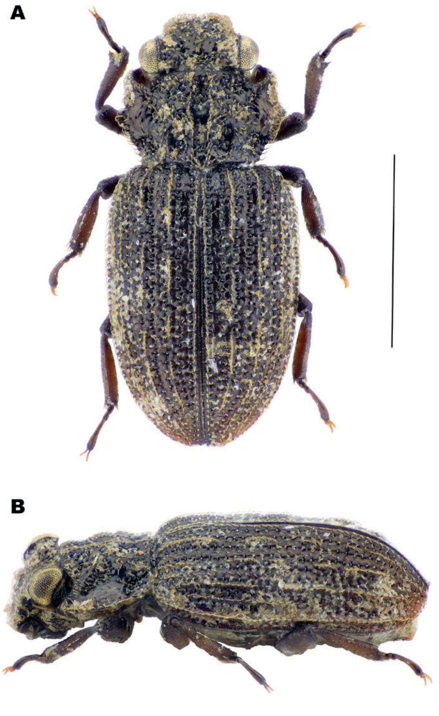

Fig. 1. Habitus of *Coelometopon riberae* **sp. nov.** Holotype: A) dorsal; B) lateral. Scale bar = 1 mm.

Fig. 1. Hábitus de *Coelometopon riberae* **sp. nov**. Holotipo: A) dorsal; B) lateral. Escala = 1 mm.

Dorsum (Fig. 1) dark brown to black, legs dark to reddish brown, tarsi and femoro-tibial junctions darker; claw paler. Maxillary palpi dark brown to black. Antennal club black, stem segments paler brown. Venter brown to dark brown. Head broadly triangular, broadest at hind margin of eyes and narrowing to labral apex. Eyes slightly raised, protruding, and occupying approximately 1/3 of side margin of head. Labrum transverse, with very shallow, semicircular apicomedian emargination. Anterior and lateral margins evenly rounded. Upper surface of labrum shining, devoid of microreticulation and with scattered tubercles and curved, decumbent setae, especially around anterior projections. Clypeus with front angles produced and front margin arcuate. Dorsal surface shining with sparse granules, each bearing curved setae. Frontoclypeal suture arcuate, distinctly impressed. Frons and vertex granulate, granules denser than on clypeus and larger in posterior half of vertex. Each granule bearing a short curved seta. Setae stouter and longer in front of and around interior margins of eyes. Small reddish-brown ocellus visible interior to each eye, in posterior part of vertex. Pronotum transverse, cordiform, broadest at middle. Sides acutely produced at widest part, narrowing strongly to protruding front angles, and weakly emarginated in front of and behind middle. Hind angles obtuse. Front margin broadly sinuate in middle half, then strongly curved forwards to front angles. Hind margin sinuate around acuminate middle. Central part strongly granulate, granules larger and denser in front half, where they are spaced approximately one granule's width apart. Granules sparser and almost absent on marginal sections. Each granule bearing a long curved yellowish seta. Median longitudinal furrow of disc shallow, somewhat interrupted in middle. Anterior and posterior admedian fovea shallow, anterior fovea opening anterolaterally. Anterior adlateral fovea shallow and broadly open laterally; posterior adlateral fovea deeper, opening laterally to pronotal margin. Dorsal surface of pronotum shining, without microreticulation. Elytra elongate, widest approximately in the middle, where pseudepipleuron formed from 8th interval is broadest. Sides almost parallel-sided behind this widest point, weakly narrowing to posterior 1/5, and then strongly rounded to conjointly weakly emarginated apex. Sides of elytra granulate, granules spaced ½–1 granule's width apart, and bearing long, curved, yellow setae. Elytra with some intervals bearing low costae, all costae with rows of long, golden setae. Costae most strongly raised on interval 2; less so on intervals 4 & 6. Second elytral interval costate in 3–4 sections. Interval 4 costate in 4–6 sections. Costae very low posteriorly, but row of setae reaching elytral apex. Interval 5 with setae at shoulder. Interval 6 with 3 weak costae. Interval 7 raised slightly throughout, with row of short, decumbent yellow setae. Intervals 1 and 3 flat with similar granules and setae. Mentum transverse, produced apicomedially and with broad, shallow depression posteriorly either side of midline. Shining, with weak, transverse microreticulation and sparse medium punctures bearing decumbent white setae. Submentum triangular, shining, without microreticulation. Prosternum with low median ridge anterior to procoxae; dull, with scale-like vestiture. Pronotal hypomera broad, shining, with small, sparse granules. Elytral epipleura and pseudepipleura shining; pseudepipleura broad, especially around anterior 1/3, but continued to approximately 1/8 from apex. Mesoventrite shining, with microscopic scale-like vestiture; raised into small projection between mesocoxae. Metaventrite shining, with weak isodiametric microreticulation towards sides. With shallow elongately oval central depression occupying posterior half of ventrite. Area around and in front of depression with close, medium punctures, each bearing a long, white recumbent seta. Sides of shining part of metaventrite with sparse but very coarse punctures, bearing shorter, decumbent setae. Abdominal ventrite 1 with strong oblique carina behind each coxa. Abdominal ventrites 1–6 shining, with weak isodiametric microreticulation. Ventrites 1–5 with loose transverse rows of punctures bearing long whitish decumbent setae. Ventrite 6 with punctures and setae throughout. Aedeagus (Fig 2) elongate, main piece nearly straight in ventral aspect; similar in lateral aspect; ridged process elongate, curved to left in ventral view; gonopore process curved strongly towards the apex in lateral view; membranous process very small, at base of gonopore process. Parameres reaching angular process, and with thickened, rounded apices bearing long bristles. Females slightly larger than males – see above. Venter more strongly reticulate; prosternum and mesosternum dull, the latter with a distinct

Fig. 2. Aedeagus of *Coleometopon riberae* **sp. nov**. Holotype: A) ventral; B) lateral. Scale bar = 100 µm.

Fig. 2. Edeago de *Coleometopon riberae* **sp. nov**. Holotipo: A) ventral; B) lateral. Escala = 100 µm.

central depression and lateral pits. Pseudepipleuron of elytral interval 8 broader than in males. Last ventrite with small but distinct apicomedian emargination and a flattened, triangular impression in centre. Last tergite strongly bilobed, each lobe bearing a group of 4 – 6 short, stout spines.

Variation: Some variation in colour across the type series, with some specimens appearing paler than the holotype due to tenerality. Dorsal setae, and granulation weaker on darker (older?) individuals, the pronotum in particular appearing to have large, shining, non-granulate areas posterolaterally on the disc.

Etymology: Named after our sadly departed friend and colleague, Ignacio Ribera, who contributed greatly to our knowledge of Hydraenidae and madicolous beetles.

Diagnosis: A member of the *endroedyi* group of *Coelometopon* (see PERKINS, 2005), with pronotal granulation sparse, especially laterally. Elytral interval 1 with longitudinal granule/setal row throughout its length; intervals 3 and 5 with granule/setal rows interrupted three times on disc; interval 7 with row of granules/setae interrupted once just behind middle; edge of interval 8 with unilinear row of granules. Aedeagus characteristic. The new species would key to *Coelometopon zulu* Perkins, 2005 in PERKINS (2005). It can be distinguished from *C. zulu* by the sparser granulation of the pronotum, especially laterally. The aedeagus of *C. riberae* sp. nov. differs from *C. zulu* in the more elongate and pointed shape of the ridged process in lateral view as well as the different curvature of the main piece.

DISTRIBUTION

Known only from the type locality (Fig. 3), on wet rock faces beside Horseshoe Falls on the Mkambati River in Mkambati Nature Reserve, Eastern Cape, South Africa. Mkambati is on the Pondoland Wild Coast, whose sandstones and forested gorges support a high number of endemic taxa (VAN WYK & SMITH, 2001; MUCINA *et al.*, 2006), and is part of the Maputaland-Pondoland-Albany Global Biodiversity Hotspot (MITTERMEIER, *et al.* 2004). However, the fauna of the entire Wild Coast region remains poorly studied compared with other areas in southern Africa (PERERA, *et al.* 2011; HAMER & SLOTOW, 2017). Water bodies of the coastal conservation areas, like Mkambati Nature Reserve, have recently been shown to be important habitats for aquatic beetles (PERISSINOTTO *et al.,* 2016; BILTON, 2021).

Fig. 3. Type locality of *C. riberae* sp. nov., beside Horseshoe Falls, Mkambati River, Eastern Cape, South Africa.

Fig. 3. Localidad tipo de *C. riberae* sp. nov., próxima a Horseshoe Falls, Mkambati River, Eastern Cape, Sudáfrica.

ECOLOGY

Found on damp rock faces in the spray zone of Horseshoe Falls waterfall. Specimens were taken from damp areas of seepage beside the main falls, and were not present in wet areas beside the falls themselves. *C. riberae* **sp. nov**. was the most abundant water beetle present at the site, occurring together with *Anacaena glabriventris* Komarek, 2004 and *Enochrus* (*Methydrus*) sp.

ACKOWLEDGEMENTS

We are grateful to Ayanda Mlambo, Zizile Mlungu and Alvaro Vetina, for their company on our visit to Mkambati and assistance in the field, as well as to Nick Helme for recommending this location. Nomatile Nombewu (Eastern Cape Parks and Tourism Agency) is thanked for assistance with sampling permits.

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Two new species of the genus *Aphaenops* Bonvouloir from the central French Pyrenees (Coleoptera, Carabidae, Trechinae)

Dos nuevas especies del género *Aphaenops* Bonvouloir de los Pirineos centrales franceses (Coleoptera, Carabidae, Trechinae)

http://zoobank.org/References/aefb24af-d4d0-45a3-967f-03651ee90010

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ABSTRACT

Among the rich cave fauna of the pyrenean chain, the genus *Aphaenops* is of particular interest due to its endemism, its diversity and the remarkable morphological adaptations of its representatives. A group belonging to the Eastern clade (*sensu* FAILLE *et al.*, 2010c) is especially diversified and of difficult determination. It is a complex of species, sometimes defined as subgenus *Cerbaphaenops* Coiffait, 1962, grouping some of the most common and wisdespread species of subterranean Trechini of the Pyrenean range. An integrative study of the group coupling morphological and molecular approaches, with a special focus on the widespread species *A. crypticola* (Linder), revealed the presence of two unknown species, which are described here under the name *Aphaenops riberai* **n. sp.** and *A. corbazi* **n. sp.** Unexpectedly, these two new species do not belong to the *A. crypticola* clade, but to the *A. cerberus* clade. Details of the distribution and diagnostic characters are provided. *Aphaenops crypticola* is restricted to the localities west to the Garonne valley, between the Garonne and the Neste d'Aure. All the populations formerly attributed to *A. crypticola* and located east to Garonne valley belong to *Aphaenops parallelus sensu novo*.

Key Words: Carabidae, Trechini, endemism, allopatric speciation, taxonomy, cave beetles, biospeleology, France.

RESUMEN

Entre la rica fauna cavernícola de la cadena pirenaica, el género *Aphaenops* resulta de especial interés por su endemismo, su diversidad y las notables adaptaciones morfológicas de sus representantes. Un grupo perteneciente al clado oriental (*sensu* FAILLE *et al.*, 2010c) está especialmente diversificado y sus especies son de difícil determinación. Es un complejo de especies, a veces definido como subgénero *Cerbaphaenops* Coiffait, 1962, que agrupa algunas de las especies más comunes y extendidas de Trechini subterráneos de la cordillera pirenaica. Un estudio integrador del grupo que combina métodos morfológicos y moleculares, con un enfoque especial en la especie extendida *A. crypticola* (Linder), reveló la presencia de dos especies desconocidas, que se describen aquí con el nombre *Aphaenops riberai* **n. sp.** y *A. corbazi* **n. sp.** Inesperadamente, estas dos nuevas especies no pertenecen al clado de *A. crypticola*, sino al clado de *A. cerberus*. Se proporcionan detalles de la distribución y caracteres de diagnóstico. *Aphaenops crypticola* está restringida a las localidades situadas al oeste del valle del Garona, entre el río Garona y el de Neste d'Aure. Todas las poblaciones anteriormente atribuidas a *A. crypticola* y situadas al este del valle del Garona pertenecen a *Aphaenops parallelus sensu novo*.

Palabras clave: Carabidae, Trechini, endemismos, especiación alopátrica, taxonomía, coleópteros subterráneos, biospeleología, Francia.

INTRODUCTION

The genus *Aphaenops*, described in 1862 (BONVOULOIR, 1862), is endemic to the Pyrenees. It counts 50 species in France according to the last Catalogue of Coleoptera, whereas 7 species are spanish endemics (TRONQUET, 2014; SERRANO, 2013). The group is still uncompletely known, as evidenced by the number of pyrenean caves for which no biological data is available, the potential presence of the species in screes and «Milieu Souterrain Superficiel» (MSS, JUBERTHIE *et al.*, 1980; JUBERTHIE & BOUILLON, 1983), and the recent descriptions of taxa (FAILLE *et al.*, 2007, 2010b, 2013; FAILLE & BOURDEAU, 2008, 2011a). Confined within fragmented karstic units, the populations of *Aphaenops* are partially or totally isolated, which lead to a strong reduction or even a total lack of gene flow between them. Such a fragmentation of populations makes this speciose genus an ideal model for tackling speciation processes in underground environment.

Most of the species of the genus are narrow endemics often known from a single cave, although the exact distribution of the taxa is often incompletely known. Only a few of the species are relatively widespread. This is the case of two species sometimes assigned to the subgenus *Cerbaphaenops* Coiffait, 1962, located between the Neste d´Aure valley (Hautes Pyrénées) and the Ariège river (Ariège): *A. cerberus* (Dieck, 1869) and *A. crypticola* (Linder, 1859). The phylogeography of the first species was recently studied (FAILLE *et al.*, 2015), whereas the history and biogeography of the second remains poorly known, in spite of –or due to– the numerous localities from where it is quoted (JEANNEL, 1941; COIFFAIT, 1958 among many others). *Aphaenops crypticola* as currently understood is the species with the broadest distribution in the French Pyrenees, distributed from the Portet d´Aspet area in the East to the Aure Valley in the West.

By revising an abundant material of this species, we discovered two new taxa, restricted to a few caves of the area of Sauveterre-de-Comminges (31) (Fig. 1), the description of which is the subject of this article.

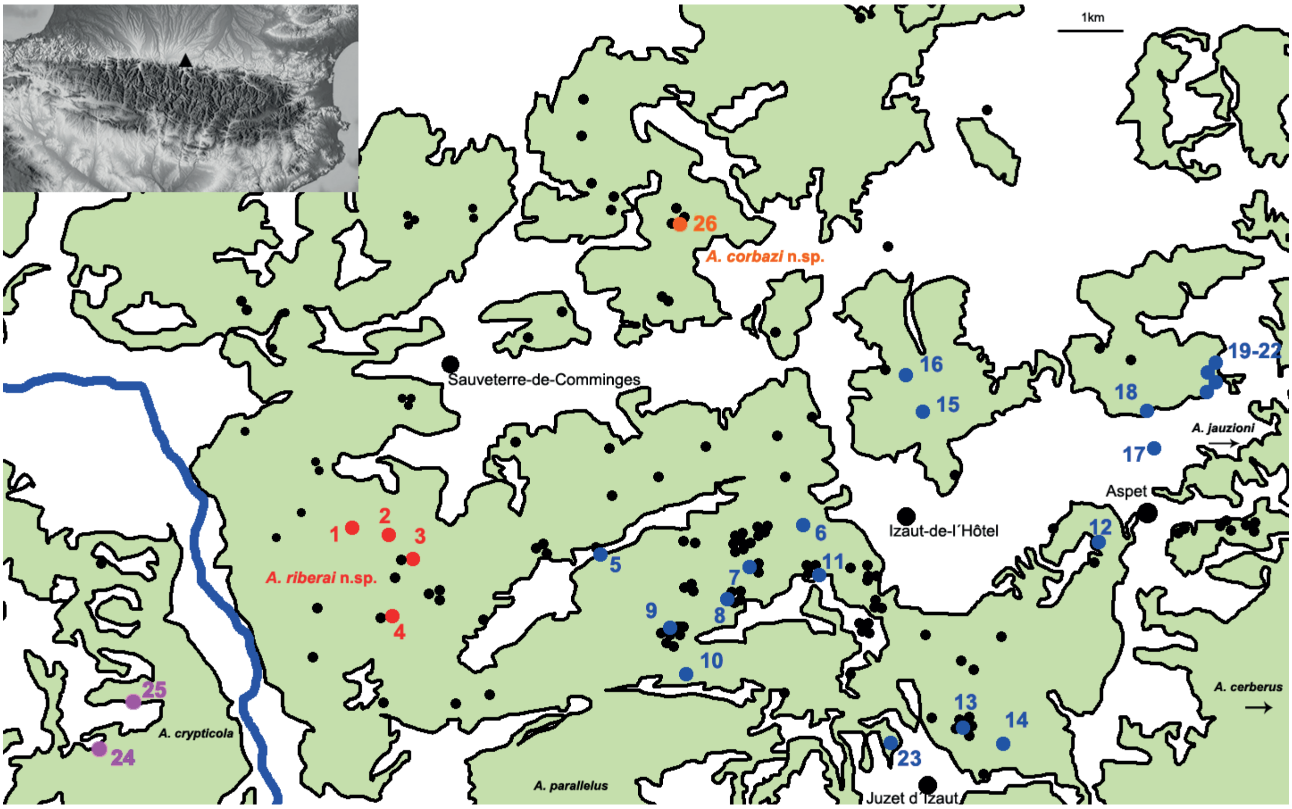

Fig. 1. Map of the localities of the new species. Red (**1-4**): *Aphaenops riberai* **n. sp.**: **1,** Puits du Brocard; **2,** Gouffre Touzan; **3,** Grotte de Boucou; **4,** Gouffre de Boucou (=Clots des Arrestous). Blue (**5-23**): *A. parallelus* (localities known in the area, partial distribution): **5,** Grotte de Malvézie; **6,** Grotte de la Maouro; **7,** Gouffre de Campels 4; **8,** Puits du Plan de Bau; **9,** Puits du Sarrat des Arragnous; **10,** Grotte de Pomarède; **11,** Grotte Casteret; **12,** Grotte de Gèles; **13,** Grotte du Cap de Payssas; **14,** Grotte du Mount; **15,** Grotte de Gouillou; **16,** Grotte du Castel de Lestelle; **17,** Grotte de Terreblanque; 18, Gouffre de la Chouette; **19-22,** Puits d´Ascalero, Grotte d´Ascalero N°2, Grotte du Moulin de Pujos, Grotte de Saint Paul; **23,** Grotte/Gouffre de Houaliech. Rose (**24-25**): *A. crypticola* (localities known in the area, partial distribution): **24,** Puits de la Coume de Samuran; **25,** Ruère de Bentoulan. Orange (**26**): *A. corbazi* **n. sp. 26,** Gouffre de Pouy 2, type locality (and only locality known so far). Black points, caves listed in regional speological databases with a development of 10 meters or more, without biological data available.

Fig. 1. Mapa de las localidades de las nuevas especies. Rojo (**1-4**): *Aphaenops riberai* **n. sp.**: **1,** Puits du Brocard; **2,** Gouffre Touzan; **3,** Grotte de Boucou; **4,** Gouffre de Boucou (=Clots des Arrestous). Azul (**5-23**): *A. parallelus* (localidades conocidas en el área, distribución parcial): **5,** Grotte de Malvézie; **6,** Grotte de la Maouro; **7,** Gouffre de Campels 4; **8,** Puits du Plan de Bau; **9,** Puits du Sarrat des Arragnous; **10,** Grotte de Pomarède; **11,** Grotte Casteret; **12,** Grotte de Gèles; **13,** Grotte du Cap de Payssas; **14,** Grotte du Mount; **15,** Grotte de Gouillou; **16,** Grotte du Castel de Lestelle; **17,** Grotte de Terreblanque; **18,** Gouffre de la Chouette; **19-22,** Puits d´Ascalero, Grotte d´Ascalero N°2, Grotte du Moulin de Pujos, Grotte de Saint Paul; **23,** Grotte/Gouffre de Houaliech. Rosa (**24-25**): *A. crypticola* (localidades conocidas en el área, distribución parcial) **24,** Puits de la Coume de Samuran; **25,** Ruère de Bentoulan. Naranja (**26**): *A. corbazi* **n. sp. 26,** Gouffre de Pouy 2, localidad típica (única localidad conocida). Puntos negros, cuevas registradas en bases de datos espeológicos regionales con un desarrollo de 10 metros o más, sin datos biológicos.

MATERIAL AND METHODS

Specimens used for the molecular part of the work were collected alive by hand in the cave and preserved in absolute ethanol in the field. Extractions of single specimens were non-destructive, using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). After extraction, the specimens were dry mounted on a card, the genitalia extracted and included in water-soluble dimethyl hydantoin formaldehyde resin (DMHF) on a card pinned beneath the specimen. Vouchers and DNA samples are deposited in the collections of the State Museum of Natural History, Stuttgart (SMNS).

In the phylogenetic analyses we included 26 specimens belonging to 17 species of hypogean Pyrenean Trechini, and the species *Geotrechus orpheus* Dieck and *Aphaenops leschenaulti* Bonvouloir were selected to root the tree, as those species are known to be unambiguously outside the studied group (FAILLE *et al.,* 2010c).

We amplified three DNA fragments, two mitochondrial (the 5' end of cytochrome c oxidase subunit 1, cox1 and 5' end of large ribosomal unit plus the Leucine transfer plus the 3' end of NADH dehydrogenase subunit 1, rrnL+trnL+nad1 (ca 740bp)) and one nuclear (large ribosomal unit, LSU) (see Appendix I for primers used, and FAILLE *et al.*, 2010c for general PCR conditions). Sequences were assembled and edited with Bioedit v. 7.0 (HALL, 1999) and Geneious prime 2019.2.3 (KEARSE *et al.*, 2012). New sequences have been deposited in Genbank database with accession number indicated in Appendix II. Other sequences were taken from FAILLE *et al.* (2010c, 2011b) (Appendix II). We aligned the sequences using the MAFFT online v.6 with the L-INS-i algorithm and default parameters (KATOH & TOH, 2008). Maximum likelihood analyses were conducted on a combined data matrix with RAxML GUI, with thorough bootstraps, 20 runs and 500 reps (STAMATAKIS, 2006; STAMATAKIS *et al.*, 2008; SILVESTRO & MICHALAK, 2012), with a GTR+I+G evolutionary model. We used the default values for other parameters of the search (STAMATAKIS *et al.*, 2008).

We also studied a large number of dry mounted specimens to cover the complete morphological range of the species complex. These specimens are deposited in the following institutions or particular collections:

MNHN: Muséum national d´Histoire naturelle, Paris (France); **ZSM**: Zoologische Staatssammlung, Munich (Germany); **SMNS**: Staatliches Museum für Naturkunde Stuttgart (Germany); **CCB**: coll. C. Bourdeau (Rebigue, France); **CHB**: coll. H. Brustel (Purpan, France); **COC**: coll. O. Courtin (Castres, France); **CFL**: coll. Fresneda-Lagar (Llesp, Spain).

Morphological study

Abbreviations of measurements used are as follows: HL, length of head from labral anterior margin to cervical ridge; HW, maximum head width; PW, maximum pronotal width; BPW, basal pronotal width, measured between hind angles; PL, pronotal length measured along midline; EL, length of elytra from basal ridge of scutellum to its apex, measured parallel to suture; EW, maximum elytral width; HuW, humeral width, measured between humeral angles.

The habitus and diagnostic characters were illustrated using a Visionary Digital photography system (LK Imaging System, Dun. Inc. - Canon EOS 5DSR); A VHX-5000 Keyence microscope was used for photographs of the genitalia.

RESULTS

Aphaenops riberai **n. sp.** (Fig. 2, 3A, 4A, 5C, 6A, 7M-N)

http://zoobank.org/ NomenclaturalActs/ 50B8ECF3-BEAF-453E-A1A2-3A148F7BC576

Type locality. France, Haute-Garonne, Sauveterre-de-Comminges, Boucou, Puits du Brocard, 43º 00’ 52”N, 0º 38’ 52”E, 852 m.

Type series. Holotype: 1 ♂ dry mounted, genitalia mounted below the specimen, on a transparent slide. F-31, Sauveterre-de-Comminges, Boucou, Puits du Brocard. Labelled: “Boucou F-31/10.IV.2010//Puits du Brocard/Bourdeau & Fresneda//DNA extraction code/ZSM-L299b//*Aphaenops riberai* n. sp./HOLOTYPE/Faille & Bourdeau des. 2021” (SMNS). Paratypes: 2 ♂♂, 4 ♀♀, same collecting data as holotype (CFL). 33 exx., same data as holotype (CCB). 1 ♂, same collecting data as holotype but with DNA extraction code: ZSM-L299 (SMNS); F-31, Sauveterre-de-Comminges, Boucou, Clots des Arrestous (= Gouffre de Boucou), 09.IX.1989, G. Corbaz leg., 15 ♀♀, 18 ♂♂ (SMNS); F-31, Sauveterre-de-Comminges, Gouffre Touzan, 09.VIII.1990, G. Corbaz leg., 6 ♀♀, 1 ♂ (SMNS); F-31, Sauveterre-de-Comminges, Grotte de Boucou, 19.II.1988, G. Corbaz leg., 1 ♂ (SMNS); 16.X.2021, A. Faille leg., 1 ♂, extraction code L2126, plus 1 ♀ spare in ethanol (SMNS).

Other material studied. Rests of two exemplars, F-31, Sauveterre-de-Comminges, Grotte de Boucou, 19.II.1988, G. Corbaz leg. (SMNS).

Diagnostic characters of the subgenus as currently understood, namely depigmentation, anophthalmy, apterism, “aphaenopsian” habit: long legs and antenna, body slender, hairy head, ocular furrows shortened, striae obsolete, elytra glabrous.

Diagnosis. The new species is distinguishable from geographically related species by the ventral pubescence well developed, the elytra with only two discal setae (a character shared with the second species described below), the pronotum smaller and the shape of the median lobe of aedeagus. *Aphaenops riberai* **n. sp.** is genetically closely related to *A. cerberus* and *A. jauzioni*, although it shares with *A. crypticola* the lack of protarsal dilatation in male. It can be distingued from *A. corbazi* **n. sp.** by the temples which are sharp, the length of the pronotum and the humera (Fig. 2-3), as well as the shape of the median lobe of aedeagus (Fig. 6A-B, 7M-N).

Description of the holotype (male). Body length measured from apex of mandible to apex of elytron 5.0 mm, maximum width 1.36 mm. Habitus as in Figure 2. Anophthalmic, pronotum and elytra glabrous, head pubescent, body and appendages very elongate, forebody (head+pronotum) nearly as long as elytra. Body coloration homogeneous, pale yellow. *Microsculpture:* Head and pronotum surface covered with distinct transverse meshes. Elytra without evident reticulation. *Head* (Fig. 3A): hairy, much longer than wide and much longer and wider than pronotum, temples slightly convex, neck constriction sharp. Two pairs of frontal setae, frontal furrows broad anteriad, incomplete, not reaching the posterior frontal pore. Antennae very elongate, filiform, densely pubescent from antennomere ii; antennal ratio: i 1.00, ii 1.40, iii 1.68, iv 1.80, v 1.80, vi 1.84, vii 1.68, viii 1.52, ix 1.48, x 1.32, xi 1.64. Clypeus concave, hexasetose. HL: 1.1 mm; HW: 0.7 mm. Ligula and paraglossae as in Fig. 4A. Pronotum dolioliform, much longer than large. PL: 0.8 mm; BPW: 0.39 mm; PW: 0.6 mm. Elytra with sharp shoulders, discal serie with two setae only. The number of discal setae is quite

Fig. 2. *Aphaenops riberai* **n. sp.**, Holotype.

Fig. 2. *Aphaenops riberai* **n. sp.**, Holotipo.

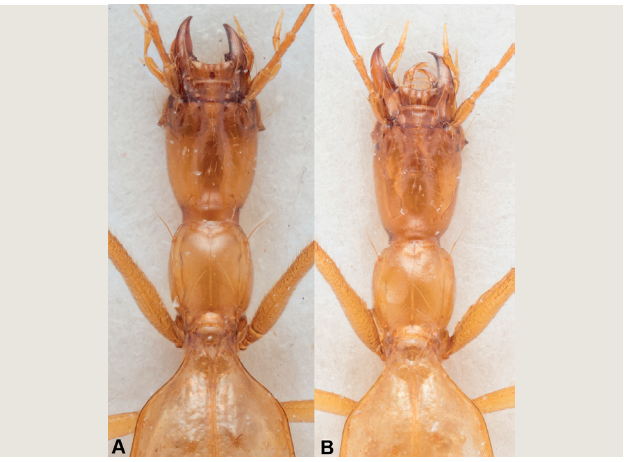

Fig. 3. Forebody of **A,** *Aphaenops riberai* **n. sp.** and **B,** *A. corbazi* **n. sp.**

Fig. 3. Parte anterior de **A,** *Aphaenops riberai* **n. sp.** y **B,** *A. corbazi* **n. sp.**

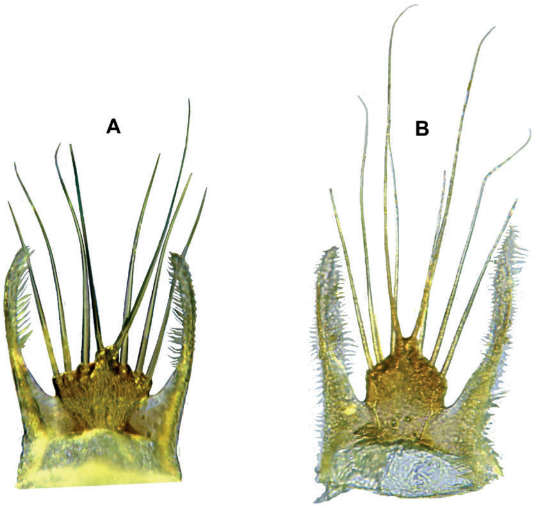

Fig. 4. Ligula and paraglossae of **A,** *Aphaenops riberai* **n. sp.** and **B,** *A. corbazi* **n. sp.**

Fig. 4. Lígula y paraglosas de **A,** *Aphaenops riberai* **n. sp.** y **B,** *A. corbazi* **n. sp.**

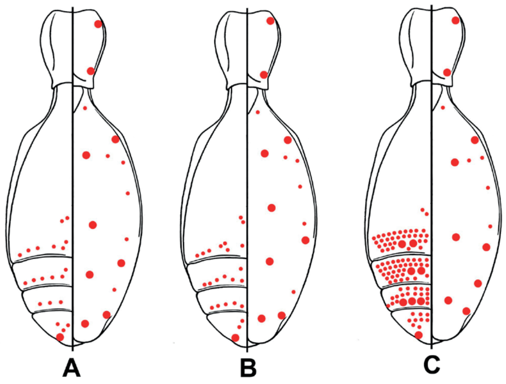

Fig. 5. *Aphaenops* spp., pubescence, dorsal (left) vs ventral (right) view. **A,** *A. crypticola*, Gouffre de Peyregnes; **B,** *A. parallelus*, Gouffre de Béguet; **C,** *A. riberai* **n. sp.**, Puits du Brocard.

Fig. 5. *Aphaenops* spp., vista de la pubescencia dorsal (izquierda) y ventral (derecha). **A,** *A. crypticola*, Gouffre de Peyregnes; **B,** *A. parallelus*, Gouffre de Béguet; **C,** *A. riberai* **n. sp.**, Puits du Brocard.

stable among the specimens; one specimen from Gouffre Touzan with an extra seta on the right elytra. EL: 2.6 mm; EW:1.36 mm; HuW: 1.0 mm. Abdominal ventrites densely pubescent (Fig. 5C). *Genitalia*. Aedeagal median lobe slightly blunt apically, rounded both dorsally and ventrally; apical part gradually curved downward, straight and elongate, with a blunt apex (Fig. 6A, 7M-N). The aedeagal internal sac bears a small triangular, sclerotized individualized pieces. Parameres with two, straight apical setae each.

Etymology. The species is dedicated to our friend Ignacio Ribera, as a tribute to his outstanding contribution to the knowledge of the phylogeny and evolution of cave beetles, especially in the Pyrenean area.

Habitat. The species is restricted to a small karstic massif eastern of the Garonne valley (Fig. 1). The type locality of the new species, the "Puits du Brocard" (namely "pit of the roebuck"), is a small pit of 17 m which develops in Aptian marlstone, the topography of which was drawn by the SMSP in May 1987 (JAUZION, 1987). The presence of *Aphaenops* was first reported by CORBAZ & JAUZION (1988), then by GARNERY (1988) and JAUZION (1990) under the name *Aphaenops crypticola*. *Aphaenops riberai* **n. sp.** is also known from three other close localities, the Clots des Arrestous (= Gouffre de Boucou), the Gouffre Touzan, and the Grotte de Boucou (Fig. 8), all located in the municipality of Sauveterre-de-Comminges. The presence of the species in the grotte de Boucou was also reported by GARNERY (1988) and JAUZION (1989), as *Aphaenops* sp. and FAILLE *et al*. (2010a) as *Aphaenops crypticola*.

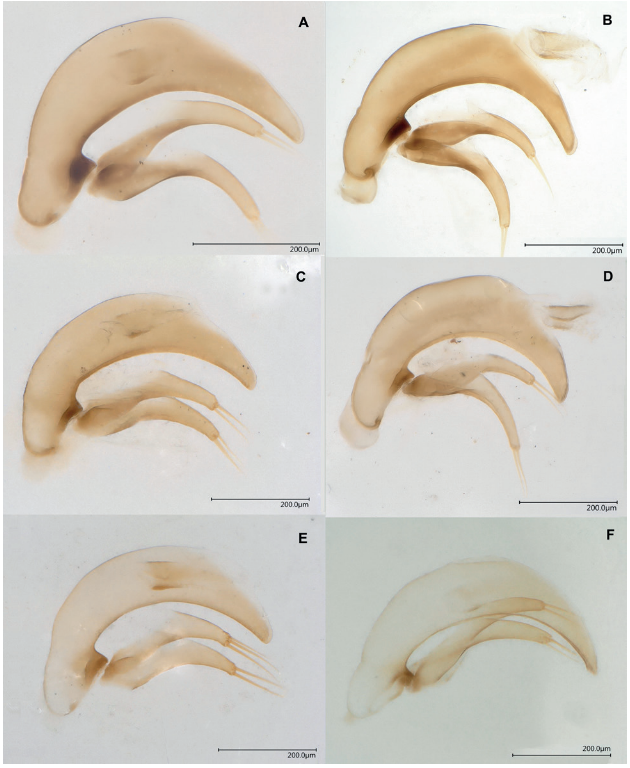

Fig. 6. Male genitalia of *Aphaenops* ssp. **A,** *Aphaenops riberai* **n. sp.**, ZSM_L299 from the type locality, Puits du Brocard (F-31, Sauveterre-de-Comminges); **B,** *A. riberai* **n. sp.**, from the Clots des Arrestous (F-31, Sauveterre-de-Comminges) **C,** *A. crypticola*, gouffre de Peyregnes (F-65, Tibiran-Jaunac); **D,** *A. parallelus* Coiffait, from the Grotte du Mount (F-31, Juzet d´Izaut); **E,** *A. cerberus bruneti* Jeannel, grotte de l´Estelas (Cazavet); **F,** *A. corbazi* **n. sp.**, holotype, ZSM_L364, Gouffre de Pouy 2 (F-31, Régades).

Fig. 6. Genitalia del macho de *Aphaenops* ssp. **A,** *Aphaenops riberai* **n. sp.**, ZSM_L299 de la localidad típica, Puits du Brocard (F-31, Sauveterre-de-Comminges); **B,** *A. riberai* **n. sp.**, del Clots des Arrestous (F-31, Sauveterre-de-Comminges) **C,** *A. crypticola*, gouffre de Peyregnes (F-65, Tibiran-Jaunac); **D,** *A. parallelus* Coiffait, Grotte du Mount (F-31, Juzet d´Izaut); **E,** *A. cerberus bruneti* Jeannel, grotte de l´Estelas (Cazavet); **F,** *A. corbazi* **n. sp.**, holotipo, ZSM_L364, Gouffre de Pouy 2 (F-31, Régades).

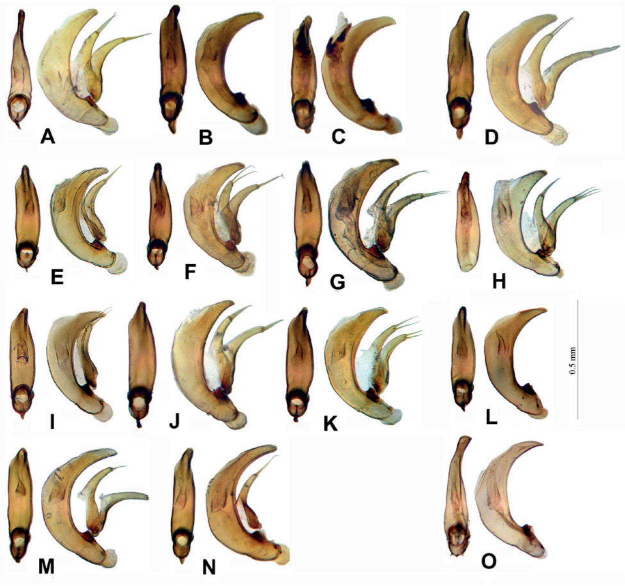

Fig. 7. Male genitalia, variability, dorsal and lateral view. (**A-D**) *Aphaenops crypticola*: **A,** gouffre de Peyregnes (F-65, Tibiran-Jaunac); **B,** Ayguat de Pelou (F-65, Nistos); **C,** Grotte de Coume Nere (F-65, Saint-Bertrand-de-Comminges); **D,** Ruère de Saint-Bertrand (F-65, Saint-Bertrand-de-Comminges). (**E-L**) *A. parallelus*: **E,** Grotte d´Ambielle (F-64, Arette, holotype of *A. bourdeaui*); **F,** Grotte de Gouillou (F-31, Aspet); **G,** Grotte de la Bourre (F-31, Coulédoux); **H,** grotte de Pourcibouco (F-31, Bézins-Garraux); **I,** Gouffre de Béguet (F-31, Juzet d´Izaut); **J,** Grotte d´Aron (F-31, Portet-d´Aspet); **K,** Grotte d´Escarchein (F-09, Galey); **L,** Grotte du Mount (F-31, Juzet d´Izaut). (**M-N**) *A. riberai* **n. sp.**: **M,** Gouffre Touzan (F-31, Sauveterre-de-Comminges); **N,** Grotte de Boucou (F-31, Sauveterre-de-Comminges). **O,** *A. corbazi* **n. sp.**: Gouffre Pouy 2 (F-31, Régades).

Fig. 7. Variabilidad de la genitalia del macho en visión dorsal y lateral (**A-D**) *Aphaenops crypticola*: **A,** gouffre de Peyregnes (F-65, Tibiran-Jaunac); **B,** Ayguat de Pelou (F-65, Nistos); **C,** Grotte de Coume Nere (F-65, Saint-Bertrand-de-Comminges); **D,** Ruère de Saint-Bertrand (F-65, Saint-Bertrand-de-Comminges). (**E-L**) *A. parallelus*: **E,** Grotte d´Ambielle (F-64, Arette, holotype of *A. bourdeaui*); **F,** Grotte de Gouillou (F-31, Aspet); **G,** Grotte de la Bourre (F-31, Coulédoux); **H,** grotte de Pourcibouco (F-31, Bézins-Garraux); **I,** Gouffre de Béguet (F-31, Juzet d´Izaut); **J,** Grotte d´Aron (F-31, Portet-d´Aspet); **K,** Grotte d´Escarchein (F-09, Galey); **L,** Grotte du Mount (F-31, Juzet d´Izaut). (**M-N**) *A. riberai* **n. sp.**: **M,** Gouffre Touzan (F-31, Sauveterre-de-Comminges); **N,** Grotte de Boucou (F-31, Sauveterre-de-Comminges). **O,** *A. corbazi* **n. sp.**: Gouffre Pouy 2 (F-31, Régades).

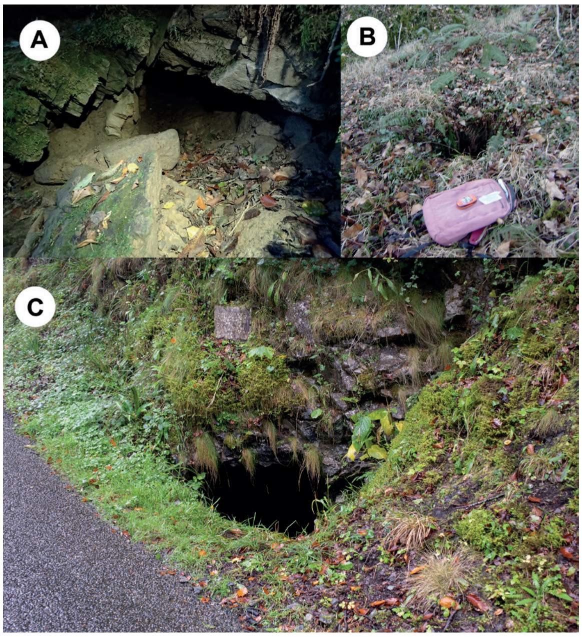

Fig. 8. Entrances of some of the localities of the two new species and *Aphaenops parallelus*. *Aphaenops riberai* **n. sp.**: **A,** Grotte de Boucou; *Aphaenops corbazi* **n. sp.**: **B,** Gouffre de Pouy 2 (type locality). **C,** Grotte de la Buhadère, type locality of *A. parallelus* (picture: S. Huang).

Fig. 8. Bocas de acceso a cavidades subterráneas de las dos nuevas especies y de *Aphaenops parallelus*. *Aphaenops riberai* **n. sp.**: **A,** Grotte de Boucou; *Aphaenops corbazi* **n. sp.**: **B,** Gouffre de Pouy 2 (localidad típica). **C,** Grotte de la Buhadère, localidad típica de *A. parallelus* (fotografía: S. Huang).

***Aphaenops corbazi* n. sp.** (Fig. 3B, 4B, 6D, 7O, 9)

http://zoobank.org/NomenclaturalActs/8100B47D-0742-4B37-99D7-BC10ACF63F63

Type locality. France, Haute-Garonne, Régades, Gouffre du Pouy n° 2, UTM (ETRS89) 43º 03' 33"N, 0º 42' 32"E, 583 m.

Type series. Holotype: 1 ♂ dry mounted, genitalia mounted below the specimen, on a transparent slide. F-31, Régades, Gouffre du Pouy n° 2. Labelled: "Régades F-31/11.II.2011//Gouffre Pouy 2/Bourdeau & Fresneda//DNA extraction code/ZSM-L364//*Aphaenops corbazi* n. sp./HOLOTYPE/Faille & Bourdeau des. 2021". Paratypes: 6 ♂♂, 3 ♀♀, same collecting data as holotype (CFL). 1 ♀, same collecting data as holotype, with DNA extraction code: ZSM-L364bis (SMNS); 1 ♂, 4 ♀♀, same locality, 13.X.1991, G. Jauzion leg. (SMNS). 43 exx., same locality, 6.V.2015, Courtin & Bourdeau leg. (SMNS, CCB, COC, CHB).

Diagnostic characters of the subgenus as currently understood, namely depigmentation, anophthalmy, apterism, "aphaenopsian" habit: long legs and antenna, body slender, hairy head, ocular furrows shortened, striae obsolete, elytra glabrous.

Diagnosis. *Aphaenops corbazi* **n. sp.** is distinguishable from *A. riberai* **n. sp.** by its overall less slender appearance, the forebody much shorter than elytra, the neck strongly accused (Figs. 3; 9), the head large with a more reduced pubescence, the pronotum less elongated, not dolioliform and much larger in its anterior part than at the base (Fig. 3B), and the shape of the median lobe of aedeagus, regularly curved and with a shorter apical part (Figs. 6A-B, D; 7M-O). This new species is genetically close to *A. riberai* **n. sp.**, *A. jauzioni* and *A. cerberus*; it shares with *A. crypticola* and *A. riberai* **n. sp.** the lack of protarsal dilatation in male.

Fig. 9. *Aphaenops corbazi* **n. sp.**, Holotype.
Fig. 9. *Aphaenops corbazi* **n. sp.**, Holotipo.

Description of the holotype (male). Body length measured from apex of mandible to apex of elytron 4.75 mm, maximum width 1.28 mm. Habitus as in Figure 9. Anophthalmic, pronotum and elytra glabrous, head pubescent, body and appendages very elongate, forebody (head+pronotum) much shorter than elytra. Body coloration homogeneous, pale yellow. *Microsculpture:* Head surface covered with distinct transverse meshes. Pronotum and elytra with isodiametric reticulation barely distinct. *Head* (Fig. 3B) finely hairy, longer than wide and longer and nearly as wide than pronotum, temples convex, neck constriction very sharp. With two pairs of frontal setae, frontal furrows broad anteriad, incomplete, not reaching the posterior frontal pore. Antennae very elongate, filiform, densely pubescent from antennomere ii; antennal ratio: i 1.00, ii 1.20, iii 1.57, iv 1.67, v 1.70, vi 1.60, vii 1.53, viii 1.37, ix 1.27, x 1.20, xi 1.67. Clypeus concave, hexasetose. HL: 1.1 mm; HW: 0.66 mm. Ligula and paraglossae more protruding than in *A. riberai* **n. sp.**, as in Fig. 4B. *Pronotum* slightly longer than large, wider in its anterior part. PL: 0.7 mm; BPW: 0.40 mm; PW: 0.6 mm. Elytra with sharp shoulders, discal serie with two setae. The holotype shows asymetry for this character, with two setae on the left elytra, and three setae on the right one. EL: 2.45 mm; EW: 1.28 mm; HuW: 1.03 mm. Abdominal ventrites densely pubescent (as *A. riberai* **n. sp.**, Fig. 5C). *Genitalia:* Aedeagal median lobe with acute apical tip, with a broadly rounded, convex dorsal face and ventral faces; apical part gradually curved downward, straight and elongate, with an blunt apex (Fig. 6F, 7O). The aedeagal internal sac bears a small subtriangular, faintly sclerotized individualized pieces. Parameres with two, straight apical setae each.

Etymology. This second new species is amicably named in honor of Gabriel Corbaz (Boucou, Sauveterre-de-Comminges), for his contribution to the knowledge of the cave beetle fauna of Haute Garonne.

Habitat. The type locality of *Aphaenops corbazi* **n. sp.** is a 75 m deep pit located in a small limestone south of Saint-Gaudens (Fig. 10B). It was discovered and described in 1991 by the Société Méridionale de Spéléologie et de Préhistoire (SMSP). Other cavities are reported from this small karstic area, which might also host the new species. The species was already quoted from the cave, under the species name *A. crypticola* (JAUZION, 1991; observed on 02-06-1991).

The two new taxa *Aphaenops riberai* **n. sp.** and *Aphaenops corbazi* **n. sp.** differ from the other taxa (*A. crypticola*, *A. cerberus*, *A. jauzioni*, *A. hustachei,* most of the populations of *A. parallelus*…) by the number of discal setae, 2 instead of 3. Nevertheless, this character is variable in some populations, as already observed by JEANNEL (1922, 1926, 1928, 1941) or even by PIOCHARD DE LA BRÛLERIE (1872) when studying *A. cerberus*.

Molecular phylogeny of the species group

The result of the phylogenetic reconstruction is in accordance with the previous phylogenies published (FAILLE *et al*., 2010c, 2011).

The two new species are unambiguously found out of the *A. crypticola* species group, with strong support (Fig. 10). Both of them belong to the «*cerberus* clade», together with the two species *A. cerberus* and *A. jauzioni*. The different populations of *Aphaenops crypticola* and *A. parallelus* included in the analyse were clearly outside this clade, as expected based on the morphology (Fig. 10).

Surprisingly, some populations regarded as «*crypticola*» by authors have a very different history, and we can recognize here three clades: *A. aeacus*, *A. crypticola*, and *A. cerberus* (Fig. 10). The first clade is the westernmost group, distributed between the Nistos area in the East to the Bagnères-de-Bigorre area in the West; the second clade has the largest distribution and the most complex history and is still in need of revision, but the species attributed to *A. crypticola* in this clade are clearly distibuted into two subclades, one gathering the populations west to the Garonne valley (*A. crypticola*), the second one the populations east to the Garonne valley (*A. parallelus sensu novo*). Detailed phylogeographic studies of the different populations included in these clades would be required to understand the evolutionary history and the role of the abiotic environmental parameters in the current distribution of the clade.

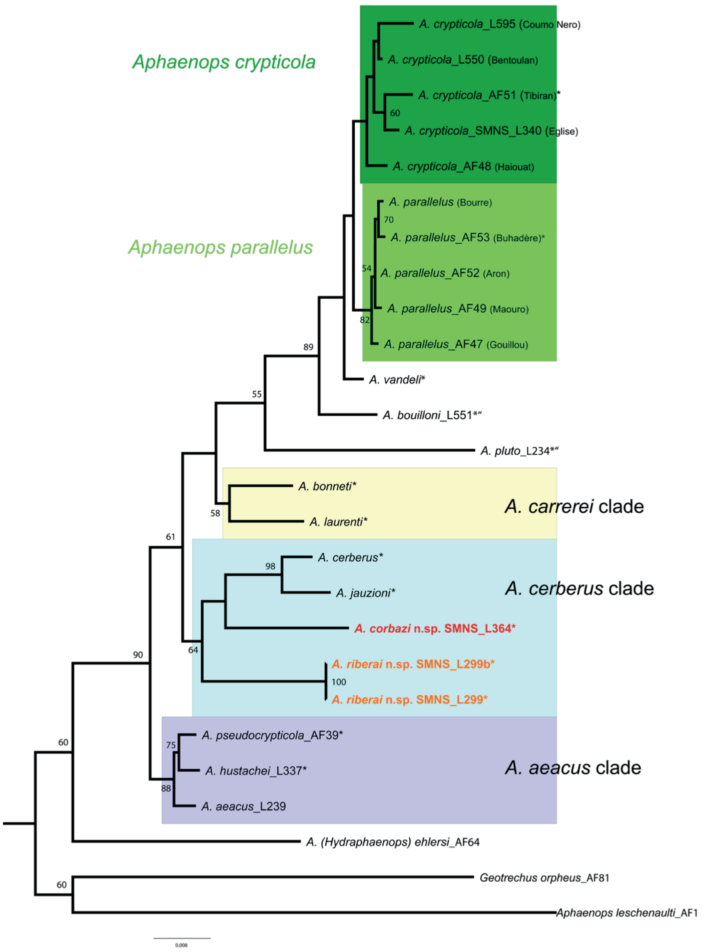

Fig. 10. Phylogram obtained with RAxML and the combined mitochondrial and nuclear data. Asterisks: specimens from type localities. Above nodes, bootstrap support values when superior to 50. See Appendix II for the localities of the specimens.

Fig. 10. Filograma obtenido con RAxML con una combinación de secuencias mitocondriales y nucleares de ADN. Asteriscos: ejemplares procedentes de localidades típicas. Sobre los nodos figura el valor «bootstrap» de soporte cuando es superior a 50. Véase la Apéndice II para la procedencia de los ejemplares.

Finally, the two new taxa are unambiguously included in a third clade, the «*A. cerberus*» clade, which was so far limited to the two species *A. cerberus* and *A. jauzioni*. The new species *Aphaenops riberai* **n. sp.** and *A. corbazi* **n. sp.** significatively extend the distribution of this clade to the West, as the eastern limit of *A. cerberus* is the Arbas massif, although this population would require a careful study (FAILLE & DEHARVENG, 2021).

The discovery of two new species close to *Aphaenops cerberus* in this area is surprising, as *A. cerberus* is widespread between Ariège valley and Aspet area, but is replaced to the west by the widespread *A. crypticola*.

On the status of *Aphaenops crypticola* and *A. parallelus*

Aphaenops crypticola is the oldest species described (as *Anophtalmus*) of all the hypogean Trechini of the Pyrenees (LINDER, 1859). It was first considered as a species with a large distribution extending from the Ger d´Aspet to the Gave de Pau, with two subspecies, the widespread *A. crypticola s. s.* and its subspecies *aeacus* (Saulcy) limited to the region of Bagnères-de-Bigorre (JEANNEL, 1941). The molecular results, in agreement with the morphology and a study of the enzymatic polymorphism (GARNERY, 1988) attesting to the specific difference of the two entities (FAILLE, 2006; FAILLE *et al.*, 2010a-c), *A. aeacus* was reinstated as a distinct species (QUEINNEC & OLLIVIER, 2011).

In agreement with the previous publications, the morphology (especially the genitalia, Fig. 8) and the distribution (Fig. 1), a clear genetic segregation between the populations of *A. crypticola* on both sides of the Garonne is evidenced (Fig. 9). *Aphaenops crypticola* was described from the Gargas cave (F-65, Aventignan); the specific epithet *crypticola* is therefore attributed to all the populations located between the Aure valley to the West and the Garonne to the East. All the populations located between the Garonne valley and the Portet d´Aspet area are attributed to *Aphaenops parallelus* **stat. nov.**, a species described by COIFFAIT (1954) from the Buhadère cave (also known as «grotte de l´Homme mort», F-31, Coulédoux; Fig. 10C) and recently synonymized with *A. crypticola* (QUÉINNEC & OLLIVIER, 2011).

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Here below are listed the verified localities of each taxa. We also report in brackets the alternative names of some cavities, as it can be found in old speleological catalogs (e.g. LUCANTE, 1880). Localities for which we could not study any specimens are indicated by [!].

Aphaenops crypticola (Linder, 1859)

Type locality. F-65, Aventignan, grotte de Gargas (LINDER, 1859; BONVOULOIR, 1866; BEDEL & SIMON, 1875; JEANNEL & RACOVITZA, 1907, 1910, 1929; JEANNEL, 1922, 1926, 1928, 1941; COIFFAIT, 1947).

Other localities. **Hautes-Pyrénées (F-65)**: **Aventignan**: Grotte des Tignahustes (FAILLE, 2006). **Hèches**: Grotte de Penotte (= grotte de Rebouc) [Nota: Probable mispelling of „Pène Haute“; probably the „grotte supérieure de Rebouc“?] (FOURÈS, 1947; COIFFAIT, 1947, 1958). **Ilheu**: Puits de la Ruère de Bentoulan (new record). **Nistos**: Grotte de lÁyguat de Pelou (= Haiouat de Pelou) (JOFFRE, 1931; SANTAMARIA & FAILLE, 2007; FAILLE, 2006; FAILLE *et al.*, 2010a,c); Grotte de l´Eglise (new record) [Nota: the species is here very rare, whereas it is much more common in Ayguat de Pelou]; Tute du Chef (= Grotte du Mont Mouch, de Moumouch, grotte de l´Ourtiga) (CHAPPPUIS & JEANNEL, 1951). **Sacoué**: Puits du Mouton (JAUZION, 1990). **Samuran**: gouffre de la Coume (= Puits Lamolle) (JAUZION, 1990 as *Aphaenops* sp.). **Seich**: Grotte Bernard (new record). **Tibiran-Jaunac**: grotte de Tibiran (= Thibiran, Grotte de Labarthe, las Tachouères (in LUCANTE, 1880)) (ABEILLE DE PERRIN, 1873; LUCANTE, 1880; JEANNEL, 1926, 1928); gouffre de Peyregnes (= grotte de Peyreigne (JAUZION, 1990 [as *Aphaenops* sp.]; FAILLE, 2006; FAILLE *et al.*, 2010a,c). **Troubat**: Grotte de Sainte Arailles (= grotte de Troubat) (new record). **Haute-Garonne (F-31)**: **Saint-Bertrand-de-Comminges**: Grotte inférieure de Coume Nère (= Coumo Nero (COIFFAIT, 1962b; JAUZION, 1990 as *Aphaenops* sp.); Puits de la Ruère de Saint-Bertrand (JAUZION, 1990); Gouffre de la Spuguette (as *Aphaenops* sp. JAUZION, 1989) [!]; grotte des Jonquilles (new record) [!].

The presence of the species in the following localities remain to be checked, due to the documented co-occurrence of the species of the clade *aeacus* with *A. crypticola* in the caves of the area: **Hautes-Pyrénées (F-65)**: **Camous**: Grotte du Tunnel (= Grotte d´Escalère) (FOURÈS, 1947; COIFFAIT, 1947, 1958). **Hèches**: Gouffre des Charentais (ANDRÉ, 2014). **Lortet**: Grotte de l´Ours (JEANNEL, 1926, 1928, 1941; COIFFAIT, 1947); MSS

S141 (JUBERTHIE & BOUILLON, 1983). **Nistos**: Aven de la femme de Peychet (FOURÈS, 1947). Puits de la Picarre (JAUZION, 1990).

Contrary to what has been previously said regarding the distribution of the species, suggesting as western limit the Nistos valley (FAILLE, 2006; FAILLE *et al.*, 2010a; QUÉINNEC & OLLIVIER, 2011), *Aphaenops crypticola* reaches the Aure valley in the West (Grotte de Rebouc). The Aure valley seems therefore to be the western limit of the distribution of *A. crypticola*.

Apart from *A. parallelus* and the two new taxa described here, a few populations belonging to the group of *A. aeacus* were erroneously attributed to *A. crypticola*:

Aphaenops group *aeacus*

Localities. **Hautes-Pyrénées (F-65)**: **Banios**: Grotte de Banios (GARNERY, 1988). **Esparros**: Gouffre d´Esparros (CABIDOCHE, 1967; BONADONA, 1971). **Fréchet Aure**: Grotte de Fréchet Aure (CHAPPUIS & JEANNEL, 1951; quoted in FAILLE *et al.*, 2006, 2010c as *Aphaenops* sp.). **Labastide-de-Neste**: Grotte de Labastide (= grande grotte de Labastide, grotte des Chevaux) (JEANNEL, 1922, 1926, 1928, 1941; COIFFAIT, 1947, GARNERY, 1988, JAUZION, 1990); Petite grotte de Labastide (JEANNEL & RACOVITZA, 1907 (as *Aphaenops* sp.)); COIFFAIT, 1947). **Sarrancolin**: Grotte d´Ilhet (JEANNEL, 1926, 1928, 1941; COIFFAIT, 1947); Gouffre de Coume Bère (= Gouffre de Bassia) (CABIDOCHE, 1962); Grotte de Sarrancolin (= Grotte de la cascade) (FOURÈS, 1947; COIFFAIT, 1947, 1958)

Aphaenops parallelus Coiffait 1954

Type locality. F-31, Couledoux, Grotte de la Buhadère (= grotte de l´Homme mort) (COIFFAIT, 1954b, 1958, 1959; 1962a,b; BOURDEAU, 1978; CORBAZ & JAUZION, 1988; FAILLE *et al.*, 2010c)

Other localities. **Haute-Garonne (F-31)**: **Arbas**, Grotte du Goueil di Her (COIFFAIT, 1969; BOURDEAU, 1978; FAILLE & DEHARVENG, 2021) [Nota: the species was found only once in this well known cave by H. Coiffait. The record is in need of confirmation (see FAILLE & DEHARVENG, 2021, p.12)]. **Arbon**: Grotte d´Arbon (as *A. crypticola*: COIFFAIT, 1958, 1959, 1962b); Gouffre SCC (new record); gouffre Casteret (new record); gouffre de Ruau n°1 (new record). **Aspet**: Grotte de Gouillou (as *A. crypticola*: JEANNEL & RACOVITZA, 1914; JEANNEL, 1922, 1926, 1928, 1941; COIFFAIT, 1947; JUBERTHIE & MASSOUD, 1977; GARNERY, 1988; GERS, 1992, 1995; FAILLE, 2006; FAILLE *et al.*, 2010a,c); Grotte de Terreblanque (FOURÈS, 1947 [as *A. cerberus*]; as *A. crypticola*: COIFFAIT, 1958, 1959; DRESCO, 1949; JAUZION, 1989; FAILLE, 2006; FAILLE *et al.*, 2010c) [Nota: the population of Pourcet Mount, North of Aspet (Terreblanque, Saint Paul,..) is geographically isolated and genetically diverging from the other populations of *A. parallelus* (FAILLE, 2006; FAILLE *et al.*, 2010c); material from more caves of this massif would be required to fix its status]; Grotte de Gèles (= Grotte de Girosp) (COIFFAIT, 1958, 1954a, 1959, 1962b; GARNERY, 1988); Gouffre de la Chouette (as *A. crypticola*: JAUZION, 1990); Puits de Boucharrague 2 (as *A. crypticola*: JAUZION, 1991). **Bézins-Garraux**: Grotte de Pourcibouco (as *A. crypticola*: JAUZION, 1989, 1991). **Boutx**, Grotte du Ger de Boutre (Ger de Boutx, COIFFAIT, 1962b). **Cabanac-Cazaux**: Gouffre de Cabanac (as *A. crypticola*: COIFFAIT, 1962b); Grotte du Castel de l'Estelle (as *A. crypticola*: COIFFAIT, 1962b). **Cazaunous**: Gouffre des Chevaux (as *A. crypticola*: COIFFAIT, 1962b); Grotte de Pomarède (GARNERY, 1988). **Coulédoux**: Grotte de la Bourre (as *A. crypticola*: JAUZION, 1989, 1990; Faille, 2006, FAILLE *et al.*, 2010b); grotte de Chapitre (FAILLE, 2006); grotte du Pont de l´Oule (new record). **Estadens**: Ruisseau souterrain de Saint Paul (as *Aphaenops* sp.: CHAPPUIS & JEANNEL, 1951; as *A. crypticola*: DRESCO, 1949; JAUZION, 1989); grotte du Moulin de Pujos (new record); Puits d'Ascalero (as *A. crypticola*: JAUZION, 1991); grotte d´Ascalero 2 (as *Aphaenops* sp.: JAUZION, 1989). **Izaut-de-l'Hôtel**: Grotte de la Maouro (as *A. crypticola*: COIFFAIT, 1958, 1959, 1962b; FAILLE, 2006; SANTAMARIA & FAILLE, 2007; FAILLE *et al.*, 2010a,c); Grotte du Barget (= Grotte d'Izaut, Aspugue d'Izaut) (as *A. crypticola*: COIFFAIT, 1958, 1959, 1962b; GARNERY, 1988); Grotte de Goutères n° 1 (as *A. crypticola*: JAUZION, 1990). **Juzet d'Izaut**: Grotte de Béguet (= Houaliech, grande grotte du Cagire) (as *A. crypticola*: COIFFAIT, 1958, 1959, 1962b; GARNERY, 1988; JAUZION, 1990; FAILLE *et al.*, 2010b); Gouffre de Béguet (as *A. crypticola*: COIFFAIT, 1958, 1959, 1962b); Fissure de la Coume du Cagire (as *A. crypticola*: COIFFAIT, 1958, 1959, 1962b); Gouffre des Italiens (= Puits de la Coume de Cagire 03, Aven des Italiens) (as *A. crypticola*: COIFFAIT, 1958, 1959, 1962b); Grotte du Mount (as *A. crypticola*: GARNERY, 1988; CORBAZ & JAUZION, 1988; JAUZION, 1989; SANTAMARIA & FAILLE, 2007); Grotte du Cap de Payssas (as *A. crypticola*: GARNERY, 1988; CORBAZ & JAUZION, 1988; JUBERTHIE *et al.*, 1988; JAUZION, 1991). **Malvézie**: Puits du Plan de Bau n° 1 (as *A. crypticola*: JAUZION, 1989); Grotte de Bau 2 (new record); Puits du Sarrat des Aragnous (new record); grotte du Sarrat des Aragnous (new record); Grotte de Malvézie (new record). **Portet d'Aspet**: Grotte d´Aron (as *A. crypticola*: JAUZION, 1989, 1990; FAILLE, 2006; FAILLE *et al.*, 2010a,c); Puits Philippe (as *A. crypticola*: GARNERY, 1988; CORBAZ & JAUZION, 1988); Gouffre de la Coume de Bitan (as *Aphaenops* sp.: JAUZION, 1991). **Ariège (F-09)**: **Saint-Lary**, Grotte de Guitère (= Metch di Mals, grotte du Boulanger) (as *Aphaenops* sp.: JAUZION, 1989). **Galey**: Grotte d´Escarchein (as *A. crypticola*: FAILLE, 2006, SANTAMARIA & FAILLE, 2007). **Pyrénées-Atlantiques (F-64)**: **Arette**, grotte d´Ambielle (as *A. bourdeaui* Coiffait: COIFFAIT, 1976; as *A. crypticola*: FAILLE *et al.*, 2010a) [Nota: Probable introduction, never found again; see discussion in FAILLE *et al.*, 2010a].

Differential diagnosis between *A. crypticola* and *A. parallelus*

The external morphology -including the overall body size- is quite variable among the populations of the clade *crypticola*, especially for *A. crypticola* and *A. parallelus*. Such variability is the consequence of the fragmentation of the distribution of these two taxa. We can nevertheless distinguish a character that constantly differs among the two taxa: the shape of the aedeagus, which is constantly short and broad in its middle part, with a short and slightly hooked apex in *A. parallelus* whereas much more elongate, without median enlargement nor hooked apex in *A. crypticola* (Fig. 6, 7).

Concluding remarks

Once again, the discovery of new taxa of cave fauna in Pyrenees underline the potential of increasing the number of species to be discovered in France, in a group –the Coleoptera– for which the discovery of taxa new to Science remains exceptional in the surface ecosystems of the country.

The priority in the near future should be the systematic sampling of the caves candidates for hosting cave fauna, as well as MSS of the area, in order to precise the distribution of each taxa. This might lead to the discovery of new taxa especially in the small isolated karsts forming the last fragmented shreds of limestone spread along the northern slope of the central Pyrenees. This is also a prerequisite to efficient conservation actions of this unique and discreet endemic fauna. The actual protection of the genus *Aphaenops* lead to opposite effects, and is an obstacle to this aim as the visitors of subterranean environments like speleologists are then reluctant to collect fauna in caves, consequently restraining the sharing of informations and the public participation in scientific research regarding cave animals. A reassessment of the Red Lists of cave taxa is needed, as effective conservation measures regarding cave fauna have to be related to the potential threats, like quarrying activities, dams, water pollution or urbanization that might directly impact the limestone units and lead to the irreversible destruction of hypogean taxa.

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Appendix I. Primers used for the amplification.

Apéndice I. Cebadores usados para la amplificación.

Gene	Name	Sense	Sequence	Reference
cox1	Jerry (M202)	F	CAACATTTATTTTGATTTTTTGG	Simon *et al.* 1994
	Pat (M70)	R	TCCA(A)TGCACTAATCTGCCATATTA	Simon *et al.* 1994
	Chy	F	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	Ribera *et al.* 2010
	Tom	R	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	Ribera *et al.* 2010
	Ron	F	GGATCACCTGATATAGCATTCCC	Simon *et al.* 1994
rrnL-nad1	16saR (M14)	F	CGCCTGTTTA(A/T)CAAAAACAT	Simon *et al.* 1994
	ND1A (M223)	R	GGTCCCTTACGAATTTGAATATATCCT	Simon *et al.* 1994
LSU	D1	F	GGGAGGAAAAGAAACTAAC	Ober, 2002
	D3	R	GCATAGTTCACCATCTTTC	Ober, 2002

Appendix II. Sequenced specimens, with codes, localities and sequence accession numbers. New sequences in bold.

Apéndice II. Ejemplares secunciados, con su código, localidad y número de acceso a las seciencias moleculares. Nuevas secuencias en negrita.

Species	code	localities	LSU	Cox1	rrnL	trnaLeu	NAD1
***Aphaenops* Bonvouloir, 1862**							
A. aeacus (Saulcy, 1864)	ZSM-L239	Grotte de Castelmouly - Bagnères-de-Bigorre (France-65)	**ON427717**	**ON421543**	**ON660965**	**ON660965**	**ON660965**
A. hustachei Jeannel, 1916	ZSM-L337	Grotte de l'Ayguat de Pelou - Nistos (France-65)	**ON427718**	**ON421544**	**ON660966**	**ON660966**	**ON660966**
A. leschenaulti Bonvouloir, 1861	MNHN-AF1	Grotte de Castelmouly - Bagnères-de-Bigorre (France-65)	GQ293593	GQ293629	GQ293739	GQ293757	GQ293822
A. bonneti Fourès, 1948	MNHN-AF38	Trou du Rantou - Suc-et-Sentenac (France-09)	GQ293571	FR733897	GQ293721	GQ293774	GQ293805
A. bouilloni Coiffait, 1955	ZSM-L551	Grotte de Pétillac - Bordes-sur-Lez (France-09)	**ON427719**	**ON421545**	**ON660967**	**ON660967**	**ON660967**
A. cerberus (Dieck, 1869)	MNHN-AF30	Grotte du Sendé - Moulis (France-09)	GQ293589	GQ293646	GQ293718	GQ293779	GQ293835
A. corbazi **n. sp.**	SMNS-L364	Gouffre Pouy 2 – Régades (France-31)	**ON427720**	**ON421546**	**ON660968**	**ON660968**	**ON660968**
A. crypticola (Linder, 1859)	ZSM-L550	Puits de la Ruère de Bentoulan - Ilheu (France-65)	**ON427721**	**ON421547**	**ON660969**	**ON660969**	**ON660969**
A. crypticola (Linder, 1859)	SMNS-L340	Grotte de l'Eglise - Nistos (France-65)	**ON427722**	**ON421548**	**ON660970**	**ON660970**	**ON660970**
A. crypticola (Linder, 1859)	ZSM-L595	Grotte inférieure de Coume Nère - Saint-Bertrand-de-Comminges (France-31)	**ON427723**	**ON421549**	**ON660971**	**ON660971**	**ON660971**
A. crypticola (Linder, 1859)	MNHN-AF48	Grotte de l'Ayguat de Pelou - Nistos (France-65)	**ON427724**	**ON421550**	**ON660972**	**ON660972**	**ON660972**
A. crypticola (Linder, 1859)	MNHN-AF51	Gouffre de Peyregnes - Tibiran-Jaunac (France-65)	GQ293580	GQ293642	**ON660973**	**ON660973**	**ON660973**
A. jauzioni Faille, Déliot & Quéinnec, 2007	MNHN-AF33	Grotte d'Artigouli - Estadens (France-31)	GQ293581	GQ293640	FR729564	FR729564	FR729564
A. laurenti Genest, 1983	MNHN-AF63	Grotte de Bordes de Crues - Seix (France-09)	GQ293569	GQ293634	GQ293719	GQ293767	GQ293813
A. parallelus Coiffait, 1954	MNHN-AF53	Grotte de la Buhadère - Coulédoux (France-31)	GQ293582	GQ293652	GQ293707	GQ293763	GQ293810
A. parallelus Coiffait, 1954	AcrB	Grotte de la Bourre - Coulédoux (France-31)	**ON427725**	**ON421551**	**-**	**-**	**-**

Species	**code**	**localities**	**LSU**	**Cox1**	**rrnL**	**trnaLeu**	**NAD1**
A. parallelus Coiffait, 1954	MNHN-AF52	Grotte d'Aron - Portet d'Aspet (France-31)	GQ293591	GQ293651	FR729567	FR729567	FR729567
A. parallelus Coiffait, 1954	MNHN-AF47	Grotte de Gouillou - Aspet (France-31)	GQ293577	GQ293671	GQ293709	GQ293765	GQ293812
A. parallelus Coiffait, 1954	MNHN-AF49	Grotte de la Maouro - Izaut-de-l'Hôtel (France-31)	FR733993	GQ293653	GQ293708	GQ293764	GQ293811
A. pseudocrypticola Coiffait, 1947	MNHN-AF39	Grotte de l'Eglise - Nistos (France-65)	GQ293573	GQ293636	GQ293711	GQ293751	GQ293844
A. pluto (Dieck, 1869)	MNHN-AF58	Grotte du Sendé - Moulis (France-09)	GQ293567	GQ293647	**ON660974**	**ON660974**	**ON660974**
A. riberai **n. sp.**	SMNS-L299	Puits du Brocard - Sauveterre-de-Comminges (France-31)	**ON427726**	**ON421552**	**ON660975**	**ON660975**	**ON660975**
A. riberai **n. sp.**	SMNS-L299b	Puits du Brocard - Sauveterre-de-Comminges (France-31)	**ON427727**	**ON421553**	-	-	-
A. vandeli Fourès, 1954	MNHN-AF44	Grotte de Payssa - Salsein (France-09)	GQ293584	GQ293657	GQ293706	GQ293762	GQ293808
A. (Hydraphaenops) ehlersi (Abeille de Perrin, 1872)	MNHN-AF64	Goueil-di-Her - Arbas (France-31)	GQ293565	GQ293683	FR729571	FR729571	FR729571
***Geotrechus* Jeannel, 1919**							
G. orpheus (Fairmaire, 1863)	MNHN-AF81	Grotte de la Quère - Mérigon (France-09)	GQ293560	FR733901	FR729572	FR729572	FR729572

***Scarodytes ignacio* sp. n., elevation to species rank of *S. halensis* var. *ibericus* (Régimbart, 1901), and notes on two other species of Dytiscidae (Insecta, Coleoptera, Dytiscidae)**

Scarodytes ignacio sp. n., elevación a rango de especie de *S. halensis* var. *ibericus* (Régimbart, 1901), y notas sobre otras dos especies de Dytiscidae (Insecta, Coleoptera, Dytiscidae)

http://zoobank.org/References/589F51C0-E9AB-46B3-8C8E-F763745D317C

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ABSTRACT

Scarodytes ignacio **sp. n.** is described from Morocco. Syntypes of the variety *ibericus* Régimbart, 1901 of *Scarodytes halensis* (Fabricius, 1787) have been studied. This variety was described from the Iberian Peninsula and so far had subspecific rank. One of the syntypes is selected and designated as the lectotype of this taxon, the rank of which is elevated to species level. The Moroccan and the Iberian species are closely related and both are distinctly separate from *S. halensis*. The habitus and the male genitalia of all three species are illustrated. New records of *Ilybius dettneri* (Fery, 1986), and *Hydroporus constantini* Hernando & Fresneda, 1996 are given.

Key words: Coleoptera, Dytiscidae, Hydroporini, *Scarodytes*, new species, lectotype, elevation of rank, new records, Morocco, Spain.

RESUMEN

Se describe *Scarodytes ignacio* **sp. n**. de Marruecos. Se han estudiado los sintipos de la variedad *ibericus* Régimbart, 1901 de *Scarodytes halensis* (Fabricius, 1787). Esta variedad se describió de la península ibérica con rango subespecífico. Se seleccionó un sintipo para designarlo como lectotipo de este taxón, cuyo rango se eleva a nivel de especie. Las especies de Marruecos y de Iberia están muy relacionadas y ambas se separan claramente de *S. halensis*. Se ilustra la forma del cuerpo y la genitalia del macho de las tres especies. Se aportan nuevos registros de *Ilybius dettneri* (Fery, 1986), e *Hydroporus constantini* Hernando & Fresneda, 1996.

Key words: Coleópteros, Dytiscidae, Hydroporini, *Scarodytes*, especie nueva, lectotipo, elevación de rango, nuevas citas, Marruecos, España.

INTRODUCTION

In the early 2010s Ignacio Ribera and I decided to start a revision of the genus *Scarodytes* Gozis, 1914. After molecular studies of specimens from several African, Asian and European countries, Ignacio was able to construct a first phylogenetic tree, but due to various reasons we had to postpone the project and to deal with some other publications (VILLASTRIGO *et al.*, 2017, 2018; FERY & RIBERA, 2018). At present I see no possibility of finishing the complete project alone, but I am very pleased here to have the possibility to bring Ignacio's work a few steps forward – although not to its conclusion.

Considering Dytiscidae, one of Ignacio's great concerns was that the variety *ibericus* Régimbart, 1901 of *Scarodytes halensis* (Fabricius, 1787) undoubtedly must be treated as a valid species. This result of his molecular investigations is supported by distinct differences in the male genitalia and also in some external morphological characters of both species. Furthermore, Ignacio found that *S. ibericus* **n. stat.** is the sister of another species occurring in northwestern Morocco and which is described below as *Scarodytes ignacio* **sp. n.**

The syntypes of Régimbart's taxon were located in the Régimbart collection in Paris museum (MNHN) and one of them is here designated the lectotype. It must be noted that according to Article 45.6.4 of the ICZN (1999) Régimbart's name has subspecific rank and, thus, is an available name, and its authorship is not changed by the elevation of its rank to species level.

MATERIAL AND METHODS

The material was studied with an Olympus SZX16 stereoscopic microscope. Photographs were taken with a Canon EOS 550D digital camera attached to the microscope. Helicon Focus 6.4.1 software was used to combine stacks of photos and Adobe Photoshop CS5 software was used to retouch photos and ink drawings. The terminology to denote the orientation of the median lobe follows MILLER & NILSSON (2003). Genitalia were studied in wet condition. The "synonymy list" given under *S. ignacio* **sp. n.** includes only a very small number selected from numerous publications which deal with Moroccan *Scarodytes* – in all of them the records are given under the name *S. halensis*.

The following acronyms for collections from which we have studied material are used in the text:

CHF	coll. H. Fery (Germany, Berlin; property of the NMW)
MNHN	Muséum National d'Histoire Naturelle, Paris, France (H. Perrin, A. Mantilleri)
NMW	Naturhistorisches Museum Wien, Austria (M. Jäch)

Abbreviations used in the text are: TL (total length), MW (maximum width). Label texts are cited in quotation marks. Slashes in label texts indicate line breaks. Additional comments are given in square brackets. Coordinates are given in decimal notation and were checked with Google Earth Pro.

RESULTS

***Scarodytes ignacio* sp. n.**

http://zoobank.org/NomenclaturalActs/E04CD909-3D86-463B-BBB7-8083EBFF6E64

Scarodytes halensis (Fabricius); BEDEL 1895–1925: 359 (partim?); GUIGNOT 1959: 462 (partim?); KOCHER 1958: 25 (partim?).

Scarodytes halensis halensis (Fabricius); BENNAS & SAINZ-CANTERO 2006: 61 (partim?); BENAMAR *et al.* 2021: 57 (partim?).

Deronectes (*Scarodytes*) *halensis* (Fabricius); ZIMMERMANN 1933: 190 (partim?).

Type locality: Morocco, ca. 50 km NW Khenifra, ca. 10 km WSW Mouley Bou Azza, ca. 33.19N 6.33W.

Type material: ***Holotype***: §, "12.4.2014 Morocco, ca. 50 km NW / Khenifra, ca. 10 km WSW Mouley / Bou Azza, 33.19114N 6.32927W / 610 m, brook, Fery leg." [printed], "Holotype / Scarodytes ignacio sp. n. / Fery det. 2021" [red, printed] (NMW) (see Fig. 17). ***Paratypes***: 2 §, 6 $, same first label as holotype (CHF). 1 §, "12.4.2014 Morocco, ca. 50 km NW / Khenifra, ca. 10 km W Aguelmous / 33.1336N 5.9796W, 910 m / brook, Fery leg." [printed; immature specimen] (CHF). 1§, 1$, "81 MOROCCO Pays Zaër Zaiane / 25.3.2008 strm rd. to El-Khatouat / 581m, N33°15‘50.4“ W6°46‘08.2“/ Ribera, Hernando & Aguilera leg.", "AH 114“ (CHF). 2 $, "72 MOROCCO 9.4.2007 / Zaër Zaïane, Tachenrhout / rv. crsrd. Aguelmoussa-Goaïda / 905m, N33°7‘59.4“ W5°58‘46.7“ / Aguilera, Hernando & Ribera leg.", "AC 15" (CHF) (photos of latter two localities in Figs 21 and 22).

Description of holotype

Habitus: oval, lateral outline with weak discontinuity at posterior angles of pronotum and base of elytra; maximum width situated distinctly before middle of elytral length. Dorsal surface yellowish with strongly contrasting black pattern (Fig. 1); yellowish parts on head and pronotum slightly more reddish than those on elytra. Surface with silky sheen due to fine punctation. Ventral surface black in large part (Fig. 2); without reticulation, distinctly shiny between punctures; appendages generally brownish, upper surface of tarsi blackish.

Figs. 1-4: Habitus of: (**1-2**): *Scarodytes ignacio* **sp. n.**: (**1**) dorsal view of holotype and (**2**) ventral view of paratype; (**3-4**): (**3**) *S. ibericus* **n. stat.** (lectotype) and (**4**) *S. halensis* (specimen from Poveda de la Sierra, Spain).

Figs. 1-4: Hábitus de: (**1-2**): *Scarodytes ignacio* **sp. n.**: (**1**) vista dorsal del holotipo y (**2**) vista ventral del paratipo; (**3-4**): (**3**) *S. ibericus* **n. stat.** (lectotipo) y (**4**) *S. halensis* (ejemplar de Poveda de la Sierra, España).

Head: reddish yellow, next to inner side of each eye with large black spot, covering also clypeal groves, but not reaching anterior margin of head; anterior margin of head narrowly darkened; vertex black, near eyes fused with black spots in front of it. Clypeal grooves broadened anteriad and more impressed there; inner margin of eyes with distinctly impressed punctured furrow alongside. Reticulation of head rather weak; mainly restricted to black parts; in clypeal grooves, between grooves and on vertex more distinct. Punctation not double, but diameter of punctures somewhat varying; in clypeal grooves and alongside eyes denser, in middle between eyes sparser. Setation on head generally short and almost imperceptible, restricted to clypeal grooves and punctured stripe alongside eyes.

Pronotum: largely black, black pattern reaching until anterior margin; only laterally of same reddish yellow colour as on head; a small dot on centre of disc also reddish yellow. Pronotal base also black, reaching until lateral rim; near anterior angles (behind eyes) reddish yellow colour shortly extending mediad. Base of pronotum slightly wider than base of elytra; maximum width at posterior angles; lateral margins evenly rounded; posterior angles more or less rectangular, shortly rounded. Lateral rim distinct, becoming broader in anterior third, somewhat darker than adjacent yellowish sides of pronotum. Pronotum in posterior half on each side at border between black and yellowish areas with longitudinal impression, these, however, not sharply limited as e.g. in species of *Graptodytes* Seidlitz, 1887, or *Oreodytes* Seidlitz, 1887; impressions near pronotal base strongly broadened and forming a deep sublateral groove on each side. Another impression at each anterior pronotal angle, but smaller and less deeply impressed. Punctation not double; punctures smaller on disc, sparser in centre (at reddish yellow dot), laterally becoming denser and coarser; on lateral reddish yellow areas very dense, thus surface appearing roughly structured; punctures especially dense in basal grooves . Behind anterior margin with line of irregularly arranged somewhat larger punctures parallel to anterior margin. Between this line and anterior margin with fairly impressed reticulation, otherwise reticulation absent making pronotal disc quite shiny. Setation short and sparse, restricted to sides of pronotum.

Elytra: with ground colour yellowish, less reddish than on head and pronotum; pattern of black vittae strongly contrasting with yellow ground (Fig. 1). Sutural vitta reaching from elytral base until apex, here somewhat broadened. Beside four long vittae on each elytron, partly confused; second and fourth reaching base of elytron, base narrowly black. More laterally vestiges of three vittae recognisable; these, however, reduced to one small black dot at shoulders, one larger spot behind and a third spot near midlength of elytra. Seventh vitta present in posterior half, posteriorly confused with third vitta. Alongside margin elytra yellowish except apically. Marginal rim dark brown, thin, slightly thicker near shoulders, thinner near apex.

Punctation on disc finer and denser than on disc of pronotum; punctures somewhat larger and denser more laterally and apically. At high magnification very sparse and very small punctures visible on disc, interspersed between larger punctures. First sutural puncture line only recognisable as short oblique stripe near elytral base; second sutural puncture line present in anterior two-thirds of elytra, but well recognisable only when adequately illuminated (for these two sutural lines see FERY & RIBERA (2018), p. 8 and fig. 3.1 on p. 53). Two further discal puncture lines present, but also only very poorly recognisable. More laterally a very few further punctures might be interpreted as rests of additional puncture lines. Setae on disc absent (or extremely short; eventually rubbed of mechanically), short behind base some longer setae; more laterally and in posterior third setae quite distinct. Surface of elytra on disc shiny between punctures, but less shiny else because of setation.

Ventral surface: predominantly black (Fig. 2); surface distinctly shiny; reticulation absent. Hypomeron and elytral epipleura in anterior two-thirds of same colour as adjacent parts on dorsal surface. Mouth parts (except blackish mandibles), gula, sides of prosternum, propleuron, pro- and mesocoxae, epipleura in posterior third and all trochanters reddish brown; pro- and mesofemora, pro- and mesotibiae slightly lighter, profemora medially distinctly darkened, mesofemora only weakly darkened. Metafemora and -tibiae darker brownish. Lobes of metacoxal processes and hind margins of third to fifth abdominal ventrites transparently brownish; tip of last abdominal ventrite also somewhat brownish. Pro- and mesotarsomeres blackish brown on upper surface, brownish on lower surface. Metatarsomeres darker brownish, in apical third more blackish. Antennomeres yellowish, progressively darkened distally beginning with fifth antennomere; palpomeres also yellowish, last one distally largely blackish.

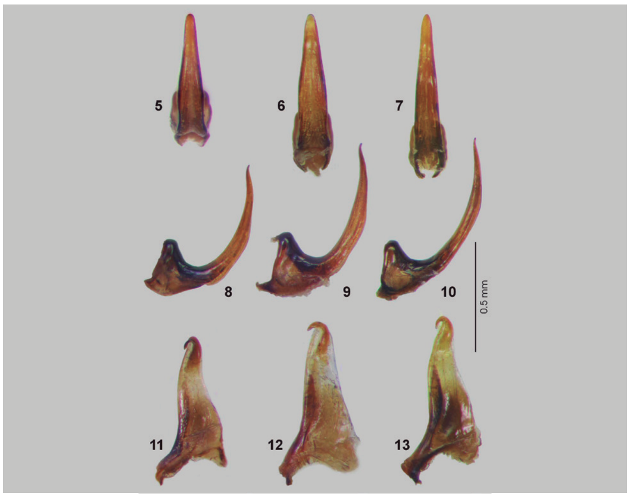
Third antennomere almost of same length as fourth, each slightly shorter than second and fifth. Gula with several punctures next to borders of genae. Genae with deeply and sharply incised curved furrow somewhat behind posterior margin of eyes; surface between eyes and furrow smooth; posterior to furrow with numerous punctures, in part elongate and transversely oriented; between inner margin of eyes and mouthparts with some very distinct wrinkles.

Prosternum before procoxae vaulted and very roughly sculptured; prosternal column weakly ascending (if specimen studied with underside turned upwards), near anterior margin of procoxae with transverse ridge. Prosternal process weakly inclined, lanceolate, with smooth longitudinal carina; right and left of carina coarsely punctured and with some setae. Tip of process shortly rounded, reaching onto anteromedial process of metaventrite between contiguous mesocoxae.

Metepisternum with only very few in part elongate punctures; smooth between punctures and thus especially shiny. Epipleura broad in anterior third (broader than mesotibiae), evenly narrowing backwards from level of posterior tip of metepisternum until level of second abdominal ventrite and then rather narrow until apex; densely punctured and setose (more or less as adjacent lateral parts of elytra). Metaventrite (except medially and submedially) and metacoxae (except anteromedially and before posterior margin) covered with large punctures, these much larger than those on pronotum; distance between punctures mostly smaller to their diameter. A small area on each side of midline of metaventrite and another one short before its posterior margin without punctures. Anteromedial metaventral process along midline slightly impressed, anteriorly forming a furrow for reception of tip of prosternal process; punctures on process finer than on more lateral parts of metaventrite ; punctures between metacoxal lines still finer. Metacoxal lines diverging anteriad, ending shortly before posterior margin of metaventrite. Punctation on first to third abdominal ventrites laterally coarser and denser than on metacoxae, on second and third ventrite medially finer; punctation on fourth and fifth ventrite finer and still denser, on last ventrite very dense; thus, last three ventrites appearing less shiny. Setae on last four abdominal ventrites medially distinct, longer than diameter of punctures; setae in punctures on metaventrite, metacoxae and first two abdominal ventrites very short, almost imperceptible; third to fifth ventrite medially with tuft of longer setae. Tibiae of all legs straight; protibiae alongside dorsal (= outer) margin with long soft setae ("hairs"); only visible when observed in liquid, otherwise closely attached to tibiae.

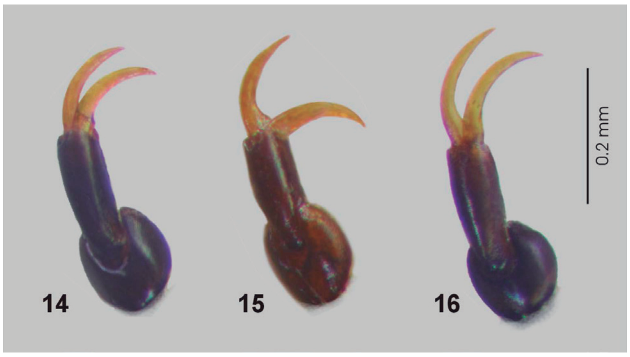
Basal three protarsomeres somewhat expanded; second protarsomere distinctly broader than long; mesotarsomeres also broadened, but less than those of protarsi. First three tarsomeres ventrally covered with many whitish setae; setae distally without sucker cups; however, ends of setae very shortly cut off and appearing as having a sucking function. Protarsal claws as in Fig. 14 (left fore-leg), more or less equal in length, shorter than fifth protarsomere; anterior (= inner) claw slightly thicker than posterior claw and almost evenly curved; posterior claw more straight in distal half and with small denticle at base (denticle shining through anterior claw in Fig. 14). For comparison protarsal claws of *S. ibericus* **n. stat.** and *S. halensis* in Figs 15 and 16, respectively. Mesotarsal claws slightly shorter than those of protarsi; metatarsal claws still shorter, thinner and less curved.

Aedeagus: Median lobe in ventral view (Fig. 5) almost evenly tapering to apex, but very slightly narrowed somewhat before apex; tip rounded; in lateral view (Fig. 8) dorsally (concave outline) widely rounded basally, more distally evenly curved, but very shortly sinuate at apex. Left paramere as in Fig. 11; ventrally (right outline in figure) distinctly concavely curved.



Figs. 5-13: Median lobe of aedeagus in ventral and lateral view and left paramere of: **(5, 8, 11)** *Scarodytes igna*cio **sp. n.** (holotype), **(6, 9, 12)** *S. ibericus* **n. stat.** (lectotype), **(7, 10, 13)** *S. halensis* (specimen from Poveda de la Sierra, Spain).

Figs. 5-13: Lóbulo medio del edeago en vista ventral y lateral, y parámero izquierdo de: **(5, 8, 11)** *Scarodytes igna*cio **sp. n.** (holotype), **(6, 9, 12)** *S. ibericus* **n. stat.** (lectotipo), **(7, 10, 13)** *S. halensis* (ejemplar de Poveda de la Sierra, España).



Figs. 14-16: Claws of left fore leg of: **(14)** *Scarodytes ignacio* **sp. n.**, (15) *S. ibericus* **n. stat.**, (16) *S. halensis* (specimen from Poveda de la Sierra, Spain). **Notes**: The anterior (= inner) claws on the right in the figures. The angle between the two claws of *S. ibericus* **n. stat.** (Fig. 15) is artificially large, being caused in preparation. I refrained from moving the anterior claw to a more natural position in case it might become damaged.

Figs. 14-16: Uñas de la pata delantera izquierda de: **(14)** *Scarodytes ignacio* **sp. n.**, (15) *S. ibericus* **n. stat.**, (16) *S. halensis* (ejemplar de Poveda de la Sierra, España). **Notas**: Las uñas anteriores (= internas), a la derecha en las figuras. El ángulo entre las dos uñas de *S. ibericus* **n. stat.** (Fig. 15) se ha alargado artificialmente durante la preparación. Evité moverlo a una posición más natural para no dañarlo.

Tip with small tuft of quite long transparent, very thin and partially curved setae (not recognisable in Fig. 11). *Notes*: Remark on setae at tip also applying to *S. ibericus* **n. stat.** and *S. halensis* (Figs. 12 and 13).

Females: External morphology very similar to males, except for not broadened pro- and mesotarsomeres and slightly thinner and shorter claws.

Measurements: TL: 3.8–4.4 mm, MW: 2.05–2.4 mm, TL/MW: 1.76–1.85; (holotype: 4.1 mm/2.25 mm/1.85). For comparison I give here also the measurements of the two other species treated in this work: (1) *S. ibericus* **n. stat.**: TL: 3.9–4.3 mm, MW: 2.15–2.35 mm, TL/MW: 1.74–1.83; (lectotype: 4.1 mm/2.35 mm/1.75); (2) Spanish *S. halensis*: TL: 3.8–4.2 mm, MW 2.1–2.2 mm, TL/MW: 1.81–1.91) (specimen from Poveda de la Sierra, Fig. 4: 3.9 mm/2.15 mm/1.82)

Variability: The variability of the species' external morphology is hardly worth mentioning; slight deviations were found in the pronotal and elytral pattern and in the size and shape of the male pro- and mesotarsal claws.

Collecting circumstances: At the type locality the new species was found between stones and gravels on rocky ground in a slowly running brook of about 1.5 m width. The specimens were accompanied by specimens of *Agabus ramblae* Millán & Ribera, 2001, *Deronectes fairmairei* (Leprieur, 1876), *Graptodytes ignotus* (Mulsant & Rey, 1861), *Nebrioporus clarkii* (Wollaston, 1862), *Stictonectes optatus* (Seidlitz, 1887), and *Aulonogyrus striatus* (Fabricius, 1792).

At Aguelmous it was found in a slowly running brook of 2–3 m width with sandy/gravely ground and some stones. The specimen was accompanied by specimens of *Agabus bipustulatus* (Linnaeus, 1767), *Deronectes fairmairei* (Leprieur, 1876), *Dytiscus pisanus* Laporte, 1835, *Graptodytes ignotus* (Mulsant & Rey, 1861), *Hydroglyphus geminus* (Fabricius, 1792), *Hydroporus discretus* Fairmaire & Brisout de Barneville, 1859, *H. lucasi* Reiche, 1866, *H. pubescens* (Gyllenhal, 1808), *H. tessellatus* (Drapiez, 1819), *Laccophilus hyalinus* (De Geer, 1774), *Stictonectes* spec. cf. *optatus* (Seidlitz, 1887), *Stictotarsus maghrebinus* Mazzoldi & Toledo, 1998, and *Aulonogyrus striatus* (Fabricius, 1792). From the other two localities I have no information about the accompanying fauna.

Distribution: So far the new species is known only from the type locality and three nearby localities.

Etymology: The new species is named in honour of my friend and famous entomologist Ignacio Ribera who passed much too early from us. Together with Pedro Aguilera (†) and Carles Hernando he found several specimens of the new species near the type locality and was the first who noted that the Moroccan specimens do not belong to *Scarodytes halensis* nor to *S. ibericus* **n. stat.** The specific name is a noun in the nominative singular standing in apposition.

Designation of the lectotype of *Hydroporus halensis* var. *ibericus* Régimbart, 1901

As mentioned in the Introduction, Ignacio's molecular studies showed that Régimbart's var. *ibericus* from the Iberian Peninsula is sister of the Moroccan *Scarodytes ignacio* **sp. n.** and that both are by no means closely related to *Scarodytes halensis* (Fabricius, 1787). I have studied numerous specimens of the two Iberian species from Spain and Portugal which – based e.g. on the pattern of the dorsal surface – can easily be assigned either to *S. ibericus* **n. stat.** or *S. halensis*. Accordingly, *S. ibericus* **n. stat.** occurs in the southwestern part of Spain and in Portugal, and *S. halensis* in the more northeastern part of the Iberian Peninsula. Iberian collecting data for both species are given in FERY & FRESNEDA (2007) under the name *S. halensis*. I refrain, however, from attributing here each of these records to one of the two species because I have observed considerable variation of a few characters in both taxa which require more detailed investigations. The results must be postponed to a future publication.

On the other hand, the observed variability of some characters in *S. ibericus* **n. stat.** requires a fixation of the identity of this taxon to serve the stability of the nomenclature. Hence in 2015 Ignacio and I made an attempt to designate a lectotype, this nomenclatural act was, however, not published and thus is unavailable. This is why I designate hereby one of the syntypes which were found in the MNHN as the lectotype of *Hydroporus halensis* var. *ibericus* Régimbart, 1901.

Lectotype (by present designation): §, "Ciud. Rodrigo / Espagne" [hw Régimbart], "v. ibericus Rég." [hw Régimbart], "Muséum Paris / 1908 / coll. M.A. Régimbart" [printed], "Lectotype / Hydroporus ibericus / Régimbart, 1901 / Fery & Ribera des. 2015" [red, printed], "Lectotype / Hydroporus halensis var. / ibericus Régimbart, 1901 / Fery des. 2021" [red, printed], "Scarodytes ibericus / (Régimbart, 1901) / Fery det. 2021" [printed] (MNHN) (see Fig. 18). **Paralectotypes** (10 exs.): 1 $, same labels as the lectotype except the second and the last but one. 4 §§, 2 $$, "Escorial / (Espagne) / L. Bleuse / 29.30/7 1879" [printed], "Muséum Paris / 1908 / coll. M.A. Régimbart" [printed] (MNHN) (see Fig. 17). 3 $$, "Lusitania" [printed], "Muséum Paris /1908 / coll. M.A. Régimbart" [printed] (MNHN) (see Fig. 20). All paralectotypes with a respective red printed paralectotype label and the white determination label. **The type locality** of the species becomes: Spain, Salamanca Province, Ciudad Rodrigo. **Notes**: The lectotype lacks the last eight segments of the right antenna, the last two tarsomeres of the right mid-leg and the claws of the right hind-leg.

Diagnostic remarks: *Scarodytes ignacio* **sp. n.** can be distinguished from *S. ibericus* **n. stat.** by the more extended black pattern which reaches e.g. the anterior margin of the pronotum in the former (Fig. 1), but not in the latter (Fig. 3); the lateral black stripe at the pronotal base is broader than in *S. ibericus* **n. stat.** and also the black elytral pattern is somewhat more prominent and the vittae more confluent in part. *Scarodytes halensis* can be distinguished from both other species by the less intensive dark pattern, mainly on the pronotum where the two black spots are considerably reduced (Fig. 4) (male from Spain, Guadalajara prov., Poveda de la Sierra, near Molina de Aragon, Fery leg. 20.5.1990). On average, the body shape appears in *S. ibericus* **n. stat.** slightly broader than in the two other species (see the TL/MW under Measurements) and the MW is situated more anteriorly. The male pro- and mesotarsal claws are rather short in *S. ignacio* **sp. n.** and in *S. ibericus* **n. stat.**, not much longer than in females and shorter than in *S. halensis*. The separation on the basis of

12.4.2014 Morocco, ca.50km NW
Khenifra, ca. 10 km WSW Mouley
Bou Azza, 33.19114N 6.32927W
610 m, brook, Fery leg.

HOLOTYPE
Scarodytes ignacio sp. n.
Fery det. 2021

Ciud. Rodrigo
Espagne

v. ibericus Rég.

MUSÉUM PARIS
1908
coll. M. A. RÉGIMBART

LECTOTYPE
Hydroporus ibericus
Régimbart, 1901
Fery & Ribera des. 2015

LECTOTYPE
Hydroporus halensis var.
ibericus Régimbart, 1901
Fery des. 2021

Scarodytes ibericus
(Régimbart, 1901)
Fery det. 2021

Escorial
(Espagne)
L.Bleuse
29.30/7 1879

Lusitania

Figs. 17-20: Labels of: **(17)** holotype of *Scarodytes ignacio* **sp. n.**; **(18)** lectotype of *Hydroporus halensis* var. *ibericus* Régimbart, 1901; **(19, 20)** two paralectotypes of *H. halensis* var. *ibericus*; (only first label).

Figs. 17-20: Etiquetas de: **(17)** holotipo de *Scarodytes ignacio* **sp. n.**; **(18)** lectotipo de *Hydroporus halensis* var. *ibericus* Régimbart, 1901; **(19, 20)** dos paralectotipos de *H. halensis* var. *ibericus*; (solo la primera etiqueta).

Figs. 21-23: **(21-22)**: collecting site of *Scarodytes ignac*io **sp. n.** at **(21)** Tachenrhout, **(22)** El-Khatouat; **(23)** Once in happy days - remembering a wonderful evening at El-Khatouat; Carles Hernando, Carmelo Andújar and Pedro Aguilera (†) (from left to right); Ignacio took the photo (collecting site in the background).

Figs. 21-23: **(21-22)**: Localidades donde se ha capturado *Scarodytes ignac*io **sp. n. (21)** Tachenrhout, **(22)** El-Khatouat; **(23)** Día feliz - recordando una noche maravillosa en El-Khatouat; Carles Hernando, Carmelo Andújar y Pedro Aguilera (†) (de izquierda a derecha); Ignacio tomó la foto (lugar de recolección al fondo).

external morphological characters is especially simple in females – those of *S. halensis* have the entire abdomen reddish while those of the other two species have it almost completely black.

The important differences are in the shape of the median lobe and the parameres of males (compare Figs 5-13): (a) median lobe as well as parameres are smallest in *S. ignacio* **sp. n.**, larger in *S. ibericus* **n. stat.** and largest in *S. halensis*, although the respective specimens of the first two have the same TL and that of the latter is even smaller; (b) the tip of the median lobe in ventral view is rather broadly rounded in *S. ignacio* **sp. n.**, less so in *S. ibericus* **n. stat.** and still more pointed in *S. halensis*; (c) the ventral outline of the median lobe in lateral view is near base more strongly curved in *S. ignacio* **sp. n.**, a little less in *S. ibericus* **n. stat.** and distinctly less in *S. halensis*. Differences in the parameres are obvious and need not be described in detail. However, I want to note that the hook at the tip of the parameres is generally somewhat variable in all three species and cannot be used for distinguishing the three species.

New Iberian records of two species of Dytiscidae (Agabini and Hydroporini)

Ilybius dettneri (Fery, 1986)

So far this species was known from central and northern Portugal (MILLÁN *et al.,* 2014: 498) and in the western part of the Cantabrian Mountain: La Coruña (FRESNEDA *et al.,* 1990) and Lugo, Sierra de Ancares (unpublished record; specimens in coll. D.T. Bilton (Plymouth, UK), coll. L. Hendrich (Munich, Germany) and in CHF). I found the species in June 2015 in very small temporary ponds in the northwest of Espinosa de los Monteros (Burgos Province) together with *Hydroporus cantabricus* Sharp, 1882. Surprisingly, this collecting site is situated about 300 km east and 450 km northeast of the others and in different geographical regions. While the new site lays in the west of the Pyrenean region, the others lie in the western part of the Iberian massif and in the western Cantabrian Mountains, respectively. In a first attempt Ignacio and I suspected that both populations might represent two different species. However, after careful study of several specimens we had to refuse this thesis. Future collecting activities may show that the species is also occurring between these localities, in the eastern parts of the Cantabrian Mountains.

Hydroporus constantini Hernando & Fresneda, 1996

This species so far was only known from a relatively small area in the Sierra de Demanda, S of the village Fresneda de la Sierra at about 1,800-1,900 m, and from Peña Labra, Cantabria (MILLÁN *et al.,* 2014: 478). Myself, I have found the species several times at the border of small springs at an altitude of about 1.900 m at Pico Tres Mares (W Reinosa, Palencia prov.; only about 3 km E of Peña Labra).

This species is somewhat variable in shape of body and of male aedeagus. Thus, if only a few specimens are studied one might suspect that different species occur in different localities. I had this idea when I studied a few of my first specimens from Pico Tres Mares and compared them with some of my specimens from the Sierra de Demanda. Both localities are separated by regions of lower altitude (along a line Logroño, Burgos, León) and this could explain the development of two genetically sufficiently different populations.

Ignacio, after having studied the molecules of specimens from both regions, expressed doubts (although he was not sure - the results were not clear enough) and, finally, I agreed with him after having studied the morphology of more specimens - all differences found in the specimens lay in the scope of variability of this species. It might be interesting to search for the species in somewhat elevated regions between the Sierra de Demanda and Pico de Tres Mares (e.g. the Sierra de Atapuerca).

ACKNOWLEDGEMENTS

I thank Javier Fresneda (Llesp, El Pont de Suert, Spain) for his help identifying Iberian specimens of the genus *Scarodytes*. Carles Hernando (Barcelona, Spain) sent comments on the photo in Fig. 23 - many thanks to him. Garth Foster (Ayr, Scotland, UK) is thanked for the linguistic check of an earlier version of this work. Thanks also to "Ecki" Rößner (Germany, Schwerin) for accompanying me in 2014 on a nice and successful collecting trip to Morocco (although somewhat difficult at the end).

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Dos nuevos Leptodirini hipogeos de Asturias (España): *Quaestus riberai* sp. n. y *Quaestus valenzuelai* sp. n. (Coleoptera, Leiodidae, Cholevinae)

Two new hypogean Leptodirini from Asturias (Spain), *Quaestus riberai* sp. n. and *Quaestus valenzuelai* sp. n. (Coleoptera, Leiodidae, Cholevinae)

http://zoobank.org/References/B94E63D8-DC8F-4985-8B14-22D0E3B8C676

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RESUMEN

Se describen dos Leptodirini nuevos, *Quaestus riberai* **sp. n.** y *Quaestus valenzuelai* **sp. n.** (Coleoptera: Leiodidae: Cholevinae) de un pequeño carst aislado de la formación Barcaliente (Manto del Ponga) en los Concejos de Parres y Piloña, Asturias, España. La posición filogenética de estas especies se infiere mediante una filogenia molecular de la serie filética de *Quaestus.* Se aportan figuras de las estructuras diferenciales, un estudio comparativo con las especies de sus respectivos clados y un mapa de distribución.

Palabras clave: Leiodidae, Leptodirini, *Quaestus*, especies nuevas, filogenia molecular, Asturias, España.

ABSTRACT

Two new hypogean Leptodirini, *Quaestus riberai* **sp. n.** and *Quaestus valenzuelai* **sp. n.** (Coleoptera: Leiodidae: Cholevinae) are described from a small and isolated karst of the geological formation Barcaliente (Ponga mantle) in Concejos de Parres and Piloña, Asturias, Spain. The phylogenetic position of these species has been studied by means of a molecular phylogeny of the phyletic series of *Quaestus*. Figures of the differential structures, a comparative study with the species of their respective clades and a distribution map are provided.

Key words: Leiodidae, Leptodirini, *Quaestus*, new species, molecular phylogeny, Asturias, Spain.

INTRODUCCIÓN

Una de las áreas de la península Ibérica con más alta biodiversidad de fauna hipógea es la región asturiana. Se encuentra en los relieves cantábricos dónde es exclusiva la presencia de Leptodirini (Coleoptera, Leiodidae, Cholevinae) de las series filéticas (SALGADO *et al*., 2008) *Quaestus* (hipogeos) y *Notidocharis* (endogeos). Los elementos de la serie *Quaestus* se distribuyen desde los relieves occidentales de Guipúzcoa (río Deva) hasta los confines orientales de Lugo y Ourense en Galicia; hay un enclave meridional en Sierra de Guadarrama con una sola especie de esta serie. En Asturias son especialmente numerosos los táxones de la tribu Leptodirini de los que en la actualidad se han catalogado 39 especies endémicas de la región, aunque alguna de ellas también se encuentra en relieves de las provincias colindantes. El conocimiento de los Leptodirini asturianos se puede considerar alto pues son numerosos los trabajos taxonómicos, faunísticos y biogeográficos que han aportado una amplia información de este territorio. Entre todas las aportaciones destacan por su relevancia los trabajos de SALGADO (1998, 1999), en los que se describen especies próximas o se dan datos de distribución de las especies que en el presente trabajo se describen como nuevas. Además, SALGADO & FRESNEDA (2009) aportan un inventario de los Leptodirini de Asturias.

En este artículo se estudian los Leptodirini de varias cavidades subterráneas de la zona cárstica de Tospe-Lago-Llerandi y de una cueva más alejada y enclavada en otra unidad geológica próxima a la localidad de La Piñera. El área de Tospe-Lago-Llerandi abarca una superficie de unos 12 km^2 y está situada entre las cuencas de los ríos Sella, al este, y Piloña, al nordeste; esta área está completamente aislada por desarrolladas barreras geológicas, como fallas, cabalgamientos y amplios estratos de materiales no carstificables como esquistos y areniscas (Fig. 1), lo que configura una verdadera «isla» cárstica en la que se han producido diversos fenómenos de especiación (SALGADO, 1999). En este enclave se han muestreado diez cuevas: en cuatro de ellas coexisten *Quaestus acuminatus* Salgado, 1999, un endemismo de esta unidad geológica, y *Q. riberai* **sp. n.**, y en otras tres *Q. acuminatus* y *Q. valenzuelai* **sp. n.** Los ejemplares de estas dos nuevas especies fueron inicialmente considerados como próximos a *Q. jeannei* (Coiffait, 1965) por SALGADO (1998, 1999), pero el estudio molecular muestra su independencia específica (Fig. 2). En este artículo se describen estos dos nuevos táxones y se determina su posición taxonómica a partir de una filogenia molecular de su serie filética.

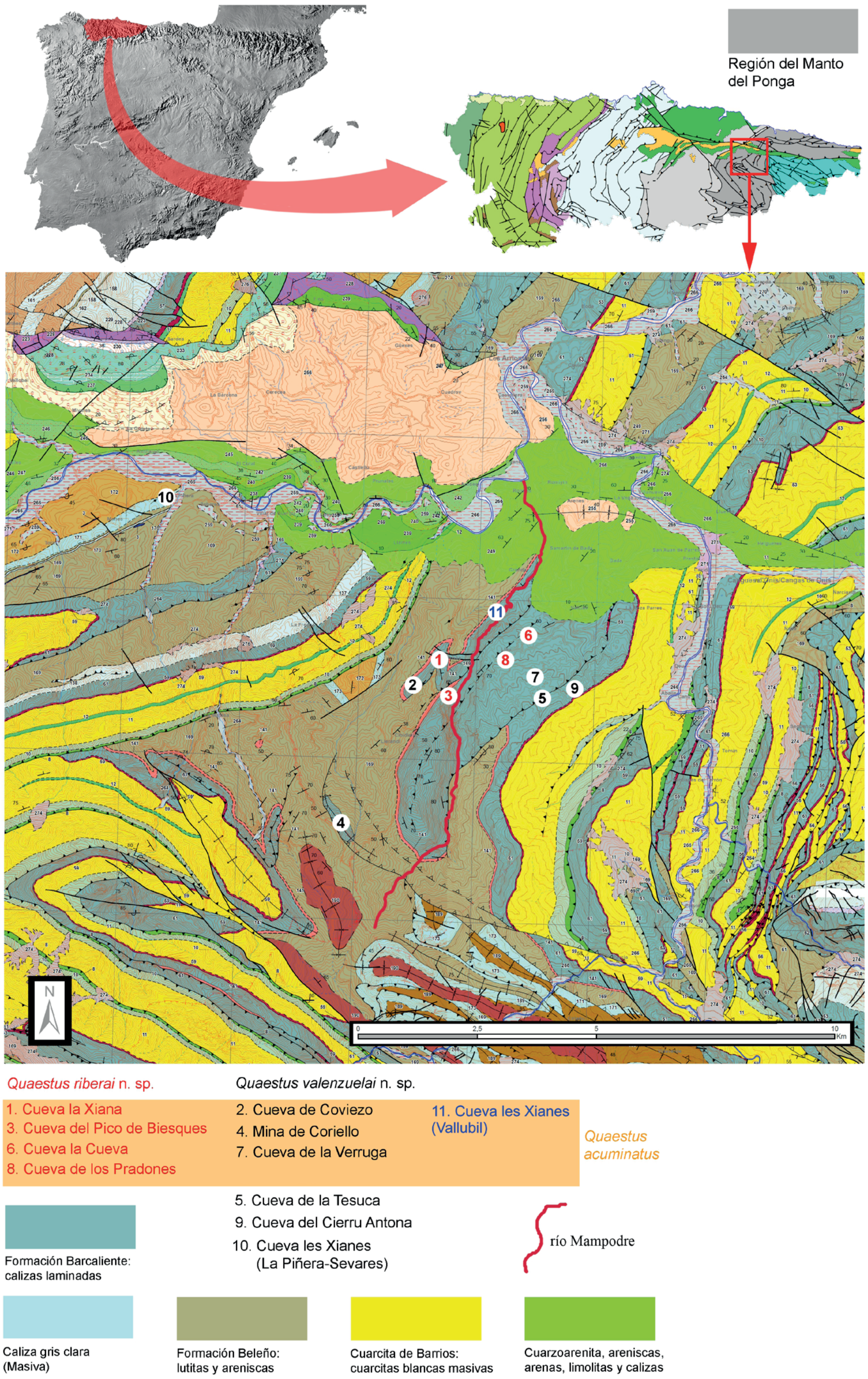

Fig. 1. Mapa geológico con la distribución de las especies. Modificado de Mapa Geológico Digital Continuo del Principado de Asturias (GEODE), 1:50000, hojas 30, 31, 54 y 55.

Fig. 1. Geologic map with the distribution of the species. Modified from Mapa Geológico Digital Continuo del Principado de Asturias (GEODE), 1:50000, maps 30, 31, 54 y 55.

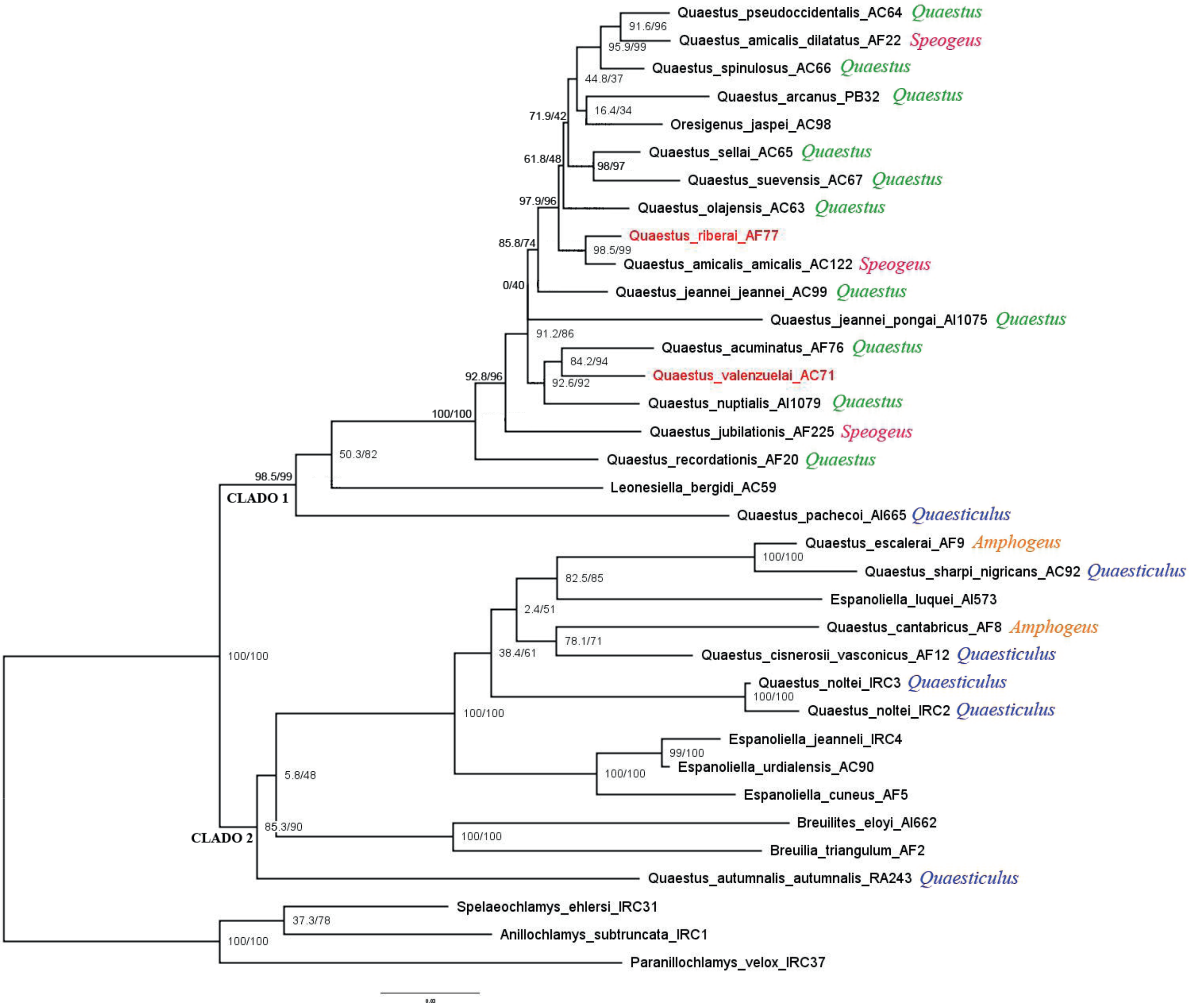

Fig. 2. Filograma obtenido con IQ TREE v1.6. En los nodos se incluyen los valores de soporte obtenidos con un test SH-aLRT y con Ultrafast Bootstrap (SH-aLRT/UFBoot). Ver el Apéndice I para la procedencia de los ejemplares.

Fig. 2. Phylogram obtained with IQ TREE v1.6. Numbers in nodes support with test SH-aLRT and Ultrafast Bootstrap (SH-aLRT/UFBoot). See Appendix I for details of the locality of the specimens.

MATERIAL Y MÉTODOS

Muestreo y estudio morfológico. La recogida de muestras se hizo en cavidades subterráneas, en medio subterráneo profundo, al rastreo o con la ayuda de cebos o trampas de caída. Una parte de los ejemplares se conservaron en etanol al 70% para el estudio morfológico, y otra en etanol al 96% para el estudio molecular. Una vez extraído el ADN y las estructuras para el estudio morfológico todos los ejemplares se montaron en seco sobre cartulinas. Se han extraído genitalias de ambos sexos y sumergido en una solución acuosa de KOH al 10% durante dos horas, para a continuación pasarlas por una serie alcohólica (60%–96%) para su deshidratación durante unos 15 minutos y por último por un baño de xilol durante unas 12 horas; finalizado este proceso las estructuras se han incluido en Bálsamo del Canadá sobre una plaquita rectangular de acetato transparente que se conserva inserta en la misma aguja que el ejemplar al que pertenece. Las fotografías de los habitus se han hecho con un estereomicroscopio Olympus SZX16 (Olympus, Tokyo, Japan) y las de las estructuras genitales con un microscopio de transmisión Olympus CH. En ambos casos se ha usado una cámara digital Olympus C5060WZ para obtener series de fotografías que se han montado con el programa CombineZP (Informer Technologies Inc., Dominica) y posteriormente se han procesado con Adobe Photoshop CS (Adobe, San José, California).

Extracción y secuenciación del ADN. Para el estudio molecular de los ejemplares capturados y conservados en frío en etanol al 96% se han usado los mismos métodos detallados en RIBERA *et al*. (2010), FAILLE *et al*. (2010a, b) y FRESNEDA *et al*. (2011, 2019, 2021). Se obtuvo el ADN mediante una extracción no destructiva con fenol–cloroformo o mediante kits comerciales (DNeasy Tissue Kit o Qiagen GmbH). Vouchers y alícuotas de ADN se han depositado en instituciones públicas (ver Apéndice I) y en los archivos entomológicos de los autores. Se han amplificado fragmentos de cuatro genes mitocondriales y dos nucleares en seis procesos de PCR. Los genes mitocondriales son los extremos 5’ (cox1 5‘, barcode) y 3‘ (cox1 3‘) de la subunidad 1 de la citocromo c oxidasa; un fragmento continuo que incluye la terminación 3‘ de la unidad ribosomal mayor (rrnL), el gen completo de RNA de transferencia de la Leucina (trnL) y el extremo 5‘ de la NADH deshidrogenasa 1 (nad1); y por último, un fragmento interno del gen citocromo b (cob). Los genes nucleares son fragmentos de las unidades ribosómicas mayor (un fragmento interno del gen ribosomal 28S, LSU) y menor (extremo 5’ del gen ribosomal 18S, SSU). Para detalles sobre los cebadores utilizados véase FRESNEDA *et al.* (2021: Tabla II). Las secuencias se han ensamblado y editado con el software Sequencher TM 4.8 (Gene Codes, Inc., Ann Arbor, Michigan) o Geneious R10 (https://www.geneious.com). Las nuevas secuencias (un total de 82), se han depositado en la base de datos *Nucleotide* (*nt*) de Genbank (https://www.ncbi.nlm.nih.gov/genbank/) con los códigos de acceso que figuran en la Apéndice I.

Análisis filogenético. En el análisis se ha incluido una selección de secuencias previamente publicadas (RIBERA *et al.*, 2010; SALGADO *et al.*, 2012; CIESLAK *et al.*, 2014), más las nuevas obtenidas posteriormente (Apéndice I). Esta selección incluye una representación de otras especies de los subgéneros de *Quaestus*: *Amphogeus* Salgado, 2000, *Quaestus* Schaufuss, 1861, *Quaesticulus* Schaufuss, 1861 y *Speogeus* Salgado, 1985, y también especies de los géneros *Breuilia* Jeannel, 1909, *Breuilites* Salgado, 1980, *Espanoliella* Guéorguiev, 1976, *Leonesiella* Salgado, 1996, y *Oresigenus* Jeannel, 1948 pertenecientes a la misma serie filética (SALGADO *et al.*, 2008). El árbol filogenético se ha enraizado con el grupo externo formado por especies de los tres géneros de la serie filética de *Spelaeochlamys* Dieck, 1870. Las secuencias de los genes que presentan variación en la longitud se han alineado utilizando el programa MAFFT online v.7 aplicando el algoritmo Q–INS–i que tiene en cuenta la estructura secundaria de los genes ribosomales (KATOH & TOH, 2008). La matriz con las secuencias concatenadas se ha analizado mediante algoritmos de máxima verosimilitud con IQ TREE v1.6 (NGUYEN *et al.*, 2015) seleccionando el modelo evolutivo más apropiado a este conjunto de datos -GTR+F+R4 (TAVARÉ, 1986)- con ModelFinder (KALYAANAMOORTHY *et al.*, 2017) más el criterio de información de Akaike (AIC). Se evaluó la estabilidad topológica y el soporte de los nodos con 1000 bootstraps (UFBoot) (MINH *et al.*, 2013; HOANG *et al.*, 2018) y 1000 réplicas de SH-aLRT (NGUYEN *et al.*, 2015).

Abreviaciones. CAF, col. A. Faille (Stuttgart, Alemania); CCB, col. C. Bourdeau (Rebigue, Francia); CFL, col. Fresneda-Lagar (Llesp, España); CHB, col. H. Brustel (Toulouse, Francia); CPMG, col. P.M. Giachino (San Martino Canavese, Italia); CZULE, col. Zoológica de la Universidad de León (León, España); MNCN, Museo Nacional de Ciencias Naturales (Madrid, España); MZB, Museu de Ciències Naturals (Zoologia) de Barcelona (Barcelona, España); NHM, Natural History Museum (Londres, Inglaterra).

RESULTADOS Y DISCUSIÓN

Taxonomía

***Quaestus riberai* sp. n.** (Fig. 3)

http://zoobank.org/NomenclaturalActs/6B6EBD7A-0E3A-45C2-A10F-E26307F79007

Localidad típica: España, Asturias, Concejo de Parres, Tospe, Cueva La Xiana, 43º 20’ N 5º 12’ O, 355 m.

Serie tipo. Holotypus ♂: España, Asturias, Concejo de Parres, Tospe, Cueva La Xiana, 12-3-1994, J.M. Salgado leg., genitalia montada sobre una etiqueta transparente insertada en la misma aguja; lleva la etiqueta roja «Holotypus *Quaestus* / *riberai* **sp. n.** / Fresneda-Salgado, 2021» (CFL). Paratypus: con los mismos datos de recolección que el holotypus 6 ♂♂ y 11 ♀♀; 12-5-2007, Salgado leg., 4 ♂♂ y 1 ♀ (un ejemplar ♂ con código voucher IBE-AF77). Concejo de Parres, Tospe, Cueva del Pico de Biesques, 420 m, 9-9-1995, Salgado leg., 2 ♂♂ y 1 ♀. Concejo de Parres, Lago, Cueva la Cueva, 340 m,16-7-1994, Salgado leg., 44 ♂♂ y 50 ♀♀. Concejo de Parres, Lago, Cueva de los Pradones, 360 m, 4-7-1995, Salgado leg., 5 ♂♂ y 7 ♀♀; 9-9-1995, Salgado leg., 99 ♂♂ y 112 ♀♀. Todos los paratipos incorporan la etiqueta roja «Paratypus *Quaestus* / *riberai* **sp. n.** / Fresneda & Salgado, 2021» (CAF, CCB, CFL, CHB, CPMG, CZULE, MNCN, MZB).

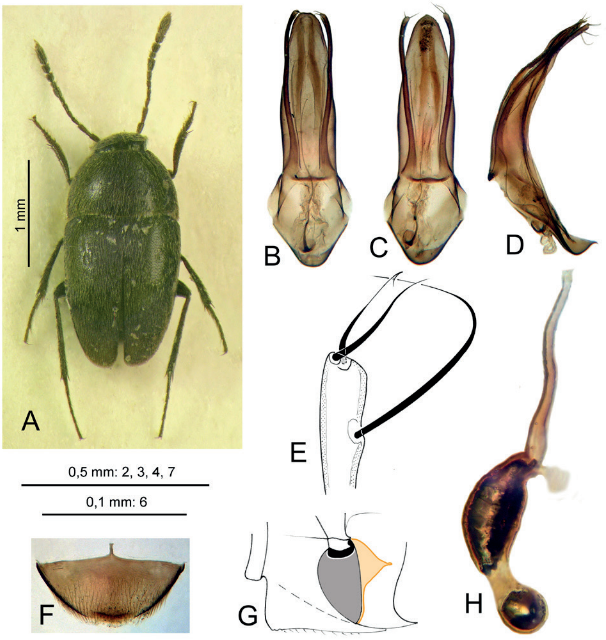

Fig. 3. *Quaestus riberai* **sp. n.** A: Habitus; B: Edeago en vista dorsal, holotipo; C: Idem, paratipo de la misma localidad; D: Edeago en vista lateral, holotipo; E: Ápice del estilo lateral del tegmen; F: VIII urito femenino; G: Quilla mesoventral y suturas del metaventrito (en color naranja); H: Espermateca.

Fig. 3. *Quaestus riberai* **sp. n.** A: Habitus; B: Aedeagus in dorsal view, holotype; C: Idem, paratype from the same locality; D: Aedeagus in lateral view, holotype; E: Apex of the lateral stylus of the tegmen; F: VIII urito femenino; G: Quilla mesoventral y suturas del metaventrito (en color naranja); H: Espermateca.

Descripción del holotipo ♂. Longitud 1,92 mm, medida desde la quilla occipital de la cabeza hasta el ápice elitral; anchura máxima 0,95 mm. Cuerpo de aspecto oval, convexo y poco estrechado hacia atrás (Fig. 3A). Coloración rojiza brillante casi uniforme. Tegumento del pronoto y élitros con una granulación muy difusa y distante. Pubescencia amarillenta, muy corta, fina y tumbada. Cabeza moderadamente ensanchada, con la quilla occipital bien marcada; punteado muy fino y espaciado. Antenas cortas (0,95 mm), bastante robustas con los artejos algo engrosados a partir del séptimo, que casi alcanzan el tercio basal de los élitros; artejos tercero a sexto similares en longitud y anchura; el undécimo es el más robusto y el más largo de todos, dos veces y media la longitud del décimo; todos son más largos que anchos, excepto el octavo que es transverso. Pronoto transverso (relación anchura/longitud: 1,78); estrechado hacia delante y poco estrechado hacia atrás, con los lados regularmente arqueados; la máxima anchura está en la base que es tan ancha como los élitros; vértices posteriores afilados, algo prolongados sobre los élitros y los ángulos agudos; escultura del tegumento con un punteado casi imperceptible. Élitros cortos y anchos —1,3 veces más largos que anchos—, con los márgenes regularmente arqueados y fuertemente unidos en la sutura elitral; máxima anchura en la zona anterior de los hombros; ápice de cada élitro separadamente redondeado; no hay estría parasutural, si bien en la zona basal pueden observarse entre tres y nueve puntos muy borrosos. Quilla mesoventral alta y larga, con el vértice en arco amplio y el ángulo ligeramente obtuso; borde anterior ligeramente convexo y rebordeado; borde inferior estrecho, recto y con sedas. Suturas del metaventrito que delimitan un área triangular bien definida y en el punto de unión forman un vértice estrecho y afilado; la sutura anterior es convexa y la posterior subrectilínea (Fig. 3H). Patas gráciles y moderadamente largas con los cuatro primeros protarsómeros dilatados, siendo el primero muy ligeramente más estrecho que el ápice de la protibia (relación = 0,95); las mesotibias un poco arqueadas y las metatibias rectas. Lóbulo medio del edeago, en vista lateral (Fig. 3D), en arco, con una marcada depresión en el tercio apical, posteriormente sinuoso y con el ápice afilado, curvado hacia la cara ventral; en vista dorsal (Fig. 3B-C), tiene los márgenes laterales ligeramente arqueados a lo largo de la zona media, después se estrecha regularmente hasta el ápice que puede presentarse ya ligeramente truncado o ya afilado. Estilos laterales del tegmen delgados, con la zona apical arqueada hacia adentro, tan largos como el lóbulo medio y sin maza diferenciada; se insertan tres sedas de longitud similar, dos apicales con los poros de inserción muy próximos y otra basal un poco más larga con el poro de inserción distante de los poros apicales (Fig. 3E). Saco interno sin zonas diferenciadas, engloba un estilete filiforme tan largo como el lóbulo medio, con una cazoleta o placa basal hemisférica y dos fajas de escamas o espínulas mal definidas a lo largo de la zona media de la lámina basal; las bandas de refuerzo están bien definidas, son largas y sobrepasan la mitad del lóbulo medio (Fig. 3 B-C).

Diagnosis de la hembra. Presenta las mismas características externas de color, forma, talla y punteado que los machos, las diferencias más significativas se observan en los protarsos, que no están dilatados y son tetrámeros, y en la maza de las antenas con los artejos algo más cortos y más gruesos. Complejo espermatecal con la espermateca pequeña y los lóbulos bien esclerotizados (Fig. 3F); el lóbulo apical es esférico, más ancho que el conducto de unión que es hialino, pero más estrecho que el lóbulo basal, éste oblongo y unas cuatro veces más largo que el diámetro del lóbulo apical; el conducto espermático es unas 6-8 veces más largo que la espermateca, está algo ensanchado en la zona anterior, luego es fino, sin una estructura diferenciada en su unión a la bolsa copuladora; la glándula espermática está bien definida y desemboca en la unión del conducto con la espermateca (Fig. 3F). VIII uroventrito (Fig. 3G) con la zona apical en arco amplio y un área oscurecida en la zona media, la espina ventral corta y estrecha, con el ápice truncado.

Etimología. Especie dedicada a nuestro amigo Ignacio Ribera, como recuerdo agradecido y en reconocimiento a su amplia labor investigadora en los campos más diversos de la Entomología, muy especialmente en el evolutivo.

Variabilidad. La longitud de los paratipos se encuentra entre 1,90 y 2,05 mm; su anchura máxima entre 0,93 y 1,03 mm. Aunque en el holotipo los artejos tercero a sexto son similares en longitud y anchura, en algunos ejemplares el artejo quinto es algo más largo que los otros; también en algunos paratipos los artejos noveno y décimo son tan largos como anchos. En algunos ejemplares el ápice del lóbulo medio del edeago, en vista dorsal, puede ser más o menos afilado (Fig. 3B-C).

Distribución. *Quaestus riberai* **sp. n.** se ha encontrado en cuatro cuevas próximas que están separadas dos a dos por el río Mampodre (Fig. 1); están enclavadas en la zona central del área geológica de Tospe-Lago-Llerandi en la formación del Barcaliente que es una sucesión de calizas oscuras depositadas durante el Carbonífero inferior. En las cuatro cuevas convive con *Q. acuminatus* Salgado, 1999.

***Quaestus valenzuelai* sp. n.** (Fig. 4)

http://zoobank.org/NomenclaturalActs/8363C63A-9BE6-44C0-8347-A97DA5961419

Localidad típica: España, Asturias, Concejo de Piloña, La Piñera-Sevares, Cueva les Xianes, 43º 21' N 5º 16' O, 150 m.

Serie tipo. Holotypus: ♂, España, Asturias, La Piñera-Sevares, Concejo de Piloña, Cueva les Xianes, 23-6-2007, Salgado leg., genitalia montada sobre una etiqueta transparente insertada en la misma aguja que el holotipo; lleva la etiqueta roja «Holotypus *Quaestus* / *valenzuelai* **sp. n.** / Fresneda *et al*., 2021» (CFL). Paratypus: con los mismos datos de localidad y recolector, 11 ♂♂ y 14 ♀♀ del 12-6-1996 (un ejemplar ♂ con código voucher IBE-AC71), 56 ♂♂ y 46 ♀♀ del 12-6-1999, J.M. Salgado leg. Concejo de Parres, Lago, Cueva de la Tesuca, 340 m, 2-7-1994, Salgado leg., 2 ♂♂. Concejo de Parres, Lago, Cueva de la Verruga, 350 m, 17-6-1995, Salgado leg., 25 ♂♂ y 19 ♀♀. Concejo de Parres, Lago, Cueva de Xierru Antona, 330 m, 17-9-1994, Salgado leg., 17 ♂♂ y 18 ♀♀. Concejo de Parres, Llerandi, Cueva de la Mina de Coriello, 320 m, 11-6-1994, Salgado leg., 12 ♂♂ y 8 ♀♀. Concejo de Parres, Tospe, Cueva de Coviezo, 410 m, 30-4-1994, Salgado leg., 23 ♂♂ y 16 ♀♀. Todos los paratipos incorporan la etiqueta roja «Paratypus *Quaestus* / *valenzuelai* **sp. n.** / Fresneda-Salgado, 2021» (CAF, CCB, CFL, CHB, CPMG, CZULE, MNCN, MZB).

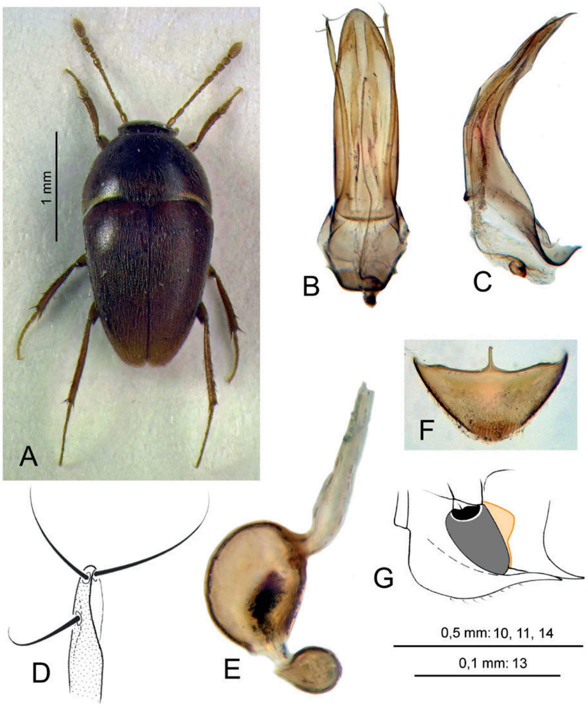

Fig. 4. *Quaestus valenzuelai* **sp. n.** A: Habitus; B: Edeago en vista dorsal, holotipo; C: Edeago en vista lateral, holotipo; D: Ápice del estilo lateral del tegmen; E: Espermateca; F: VIII urito femenino; G: Quilla mesoventral y suturas del metaventrito (en color naranja).

Fig. 4. *Quaestus valenzuelai* **sp. n.** A: Habitus; B: Aedeagus in dorsal view, holotype; C: Aedeagus in lateral view, holotype; D: Apex of the lateral stylus of the tegmen; E: Spermatheca; F: VIII female urite; G: Mesoventral keel and metaventrite sutures (in orange).

Descripción del holotipo ♂. Longitud 2,48 mm, medida desde la quilla occipital hasta el ápice de los élitros; anchura máxima 1,22 mm. Cuerpo ovoide y muy convexo, alargado y estrechado hacia atrás (Fig. 4A). Coloración marronácea, algo más claras las antenas, las tibias y los tarsos de las patas. Pubescencia amarillenta, abundante, corta, fina y tumbada. Cabeza moderadamente ensanchada, con la quilla occipital bien marcada. Antenas cortas (1,05 mm) y robustas que casi alcanzan la mitad de la longitud del cuerpo; artejos algo engrosados a partir del séptimo, siendo los antenómeros tercero y quinto iguales en longitud y ligeramente más largos que el cuarto y sexto que son iguales entre sí; el undécimo es casi tres veces la longitud del décimo; todos más largos que anchos, excepto el octavo, noveno y décimo que son tan largos como anchos. Pronoto transverso, casi dos veces más ancho que largo (relación anchura/longitud: 1,85); estrechado hacia delante y poco estrechado hacia atrás, con los lados regularmente arqueados; máxima anchura un poco antes de la base que es tan ancha como los élitros; ángulos posteriores agudos y vértices puntiagudos, algo prolongados sobre los élitros; escultura del tegumento con un punteado casi imperceptible. Élitros cortos y anchos —1,35 veces más largos que anchos—, con los márgenes regularmente arqueados; máxima anchura muy cerca de la base; ápice de cada élitro separadamente redondeado; no hay estría parasutural, si bien en la base se pueden observar entre cuatro y seis puntos separados. Quilla mesoventral alta y larga, con el margen anterior recto y el vértice redondeado con el ángulo obtuso; margen ventral convexo y algo ensanchado. Suturas del metaventrito que delimitan un área triangular bien definida, la superior arqueada y la inferior sinuosa; en la unión se forma un vértice poco afilado (Fig. 4G). Patas gráciles, moderadamente largas con los cuatro primeros protarsómeros dilatados, siendo el primero un poco más estrecho que el ápice de la protibia (relación: 0,95); mesotibias algo curvadas y metatibias ligeramente combadas. Lóbulo medio del edeago robusto, en vista lateral (Fig. 4C), en arco, más fuertemente curvado en la zona media con una ligera depresión hacia el tercio apical de la cara dorsal, y el ápice afilado, algo curvado hacia la cara ventral; en vista dorsal (Fig. 4B), los márgenes laterales ligeramente arqueados a lo largo de la zona media, luego mengua regularmente para mostrar una zona apical lanceolada con el ápice poco apuntado. Estilos laterales del tegmen delgados, el grosor uniforme en casi toda su longitud, y algo más cortos que el lóbulo medio; la zona apical no está dilatada, con el ápice más estrechado, en ella se insertan tres sedas de longitud similar, dos apicales próximas y otra basal más distante (Fig. 4D). Endofalo sin zonas diferenciadas, engloba un estilete bien desarrollado y muy largo, más largo que el lóbulo medio, con una cazoleta oblonga y dos cortas fajas de diminutas escamas o espínulas a ambos lados de la zona basal; bandas de refuerzo largas que sobrepasan la mitad del lóbulo medio; hacia su base se encuentran dos faneras muy estrechas y alargadas dispuestas a ambos lados del estilete (Fig. 4B).

Diagnosis de la hembra. Con las mismas características de color, forma y talla que los machos, las diferencias más significativas se observan en los protarsos, que no están dilatados y son tetrámeros, y en la maza de las antenas con los artejos más cortos y gruesos, siendo los antenómeros octavo a décimo más anchos que largos. Complejo espematecal (Fig. 4E) con la espermateca pequeña; los lóbulos apical y basal bien esclerotizados, el apical regularmente esférico, más ancho y tan largo como el conducto de unión que es hialino, el basal globoso, mucho más desarrollado que el lóbulo apical; conducto espermático entre seis y ocho veces más largo que la espermateca, algo dilatado en la zona anterior y sin ninguna estructura diferenciada en su unión a la bolsa copuladora; hay glándula espermática. VIII uroventrito (Fig. 4F) con la zona apical en arco ligeramente apuntado y una amplia mancha oscurecida; la espina ventral corta, muy estrecha y el ápice truncado.

Etimología. Especie dedicada a nuestro amigo, el bioespeleólogo asturiano Enrique Valenzuela (Asturias, España).

Variabilidad. Sólo se han observado pequeñas diferencias de tamaño. La longitud de los paratipos va desde los 2,40 hasta los 2,50 mm; su anchura desde los 1,20 hasta los 1,25 mm.

Distribución. *Quaestus valenzuelai* **sp. n.** se localiza en seis cuevas (Fig. 1) de las que cinco se encuentran en el área de Tospe; en tres de ellas convive con *Quaestus acuminatus*; en la sexta, cueva les Xianes, más alejada, cohabita con *Q. spinulosus* Salgado, 1998.

Filogenia molecular y examen comparativo

En el árbol filogenético obtenido (Fig. 2) se observa que tanto el género *Quaestus* como cada uno de sus subgéneros no son monofiléticos y requieren una nueva ordenación taxonómica (FRESNEDA *et al*., *in prep.*) Los valores de SH-aLRT y de bootstrap se indican como soporte de los nodos en el árbol. La serie al completo está dividida sin ambigüedad (soporte 100/100) en dos grandes clados:

Clado 1. *Quaestus* (*Quaesticulus*) *pachecoi* es la especie hermana de todo el clado con alto soporte (98.5/99). Además. figuran los géneros *Leonesiella* y *Oresigenus*, y especies de los subgéneros *Quaestus s. str.* y *Speogeus*.

Clado 2. *Quaestus* (*Quaesticulus*) *autumnalis autumnalis*, es la especie hermana de todo el clado que incluye los géneros *Breuilites*, *Breuilia*, un clado con parte de las especies de *Espanoliella* y otro con parte de las especies de *Quaesticulus* junto con *Amphogeus* y *Espanoliella luquei*. *Quaestus* (*Amphogeus*) *escalerai* es la especie hermana de *Quaestus* (*Quaesticulus*) *sharpi nigricans* con alto soporte (100/100).

La inferencia filogenética indica que las dos nuevas especies descritas pertenecen al género *Quaestus* (clado 1) con un alto soporte estadístico (Fig. 2). *Quaestus riberai* **sp. n.** es la especie hermana de *Quaestus* (*Speogeus*) *amicalis amicalis* (Salgado, 1984) con soporte alto (98.5/99). Estas dos especies forman el clado hermano de los *Quaestus s. str*. más *Speogeus* y *Oresigenus* también con soporte alto (97.9/96). El edeago de *Quaestus riberai* **sp. n.** y *Q.* (*Speogeus*) *amicalis amicalis* responde al mismo modelo, que es bastante exclusivo dentro del género, aunque sin embargo presentan claras diferencias; ambos tienen el cuarto apical, en vista lateral, sinuoso, pero el de *Q. amicalis amicalis* es considerablemente más grueso, robusto, con el bulbo basal fuertemente engrosado ventralmente y más fuertemente arqueado; también los estilos laterales del tegmen son más largos pues sobrepasan el ápice del lóbulo medio; además éstos tienen el ápice engrosado, en forma de maza. También la morfología externa es distinta; *Q. riberai* **sp. n.** es más pequeño y redondeado, siendo mayor y marcadamente oblongo *Q. amicalis amicalis*; las antenas son más cortas y robustas en *Q. riberai* **sp. n.** siendo los antenómeros más cortos y proporcionalmente más anchos. *Quaestus riberai* **sp. n.** cohabita en todas las cuevas con *Q. acuminatus* (Fig. 1); ambas se separan muy fácilmente ya que *Q. acuminatus* tiene el cuerpo más alargado, las antenas con los artejos más largos y los protarsos del macho más anchos que la zona apical de las protibias; también por el edeago, al presentar el lóbulo medio muy afilado, y los parámeros y estilete más cortos; al igual que el complejo espermatecal con el lóbulo basal más corto y esférico (ver SALGADO 1999).

Quaestus valenzuelai **sp. n.** es la especie hermana de *Q.* (*Quaestus*) *acuminatus* con soporte 84.2/94, formando ambas el grupo hermano de *Q. nuptialis* con soporte 92.6/92. Está muy separada tanto morfológica como filogenéticamente de *Q. spinulosus*, especie con la que convive; esta es la especie hermana de *Q. pseudoccidentalis* (Salgado, 1980) y *Q. amicalis dilatatus* (Salgado, 1984) con alto soporte, 95.9/99. Aunque *Quaestus valenzuelai* **sp. n.** es hermana de *Q. acuminatus*, morfológicamente es más similar a *Q. nuptialis* (Español, 1973): su edeago responde al mismo modelo aunque es más largo y con el ápice más apuntado. También, como en *Q. nuptialis*, la espermateca es corta, con el lóbulo apical diminuto y el basal fuertemente engrosado. Pero su estrecha relación con *Q. acuminatus*, según muestra el estudio molecular, está corroborada por el criterio geográfico: *Q. nuptialis* tiene una gran área de distribución situada considerablemente más al sur, al límite con la cuenca del río Duero; *Quaestus valenzuelai* **sp. n.** y *Q. acuminatus*, especies hermanas, se encuentran al norte y conviven en tres cavidades en los relieves meridionales de la Sierra del Sueve (Fig. 1): este parece ser pues un caso paradigmático de especiación simpátrica. Dada la similitud morfológica con *Q. nuptialis* resulta sorprendente que sus relaciones filogenéticas sean muy próximas a *Q. acuminatus* con una morfología tan absolutamente diversa. Probablemente el ancestro de este grupo de especies fuese morfológicamente similar a *Quaestus valenzuelai* **sp. n.** y *Q. nuptialis* y se distribuyese por una área aproximadamente igual a la que hoy día ocupa todo el clado; después de una primera cladogénesis que separó los elementos septentrionales, una segunda debió aislar un diminuto contingente de individuos que rápidamente cambió hasta lo que conocemos como *Q. acuminatus*: como hipótesis se puede explicar como un estado derivado consecuencia de un efecto fundacional. El modelo de edeago de *Q. valenzuelai* **sp. n.** y *Q. nuptialis* se transmite, con pequeñas diferencias, por toda la región basal del clado *Quaestus*, desde la primera cladogénesis, *Q. recordationis* (Salgado, 1982), hasta *Q. jeannei jeannei* (Coiffait, 1965). Así pues, este parece ser el «modelo plesiomórfico» de edeago dentro del clado.

En el árbol filogenético (Fig. 2) se observan relaciones entre determinados táxones que sugieren la necesidad de cambios taxonómicos. Serían los casos de (i) *Oresigenus jaspei*, la especie hermana de *Quaestus arcanus* Schaufuss, 1861 (con bajo soporte: 16.4/34), que quedaría incluida en el género *Quaestus*, de (ii) *Q. amicalis dilatatus* (especie hermana de *Q. pseudoccidentalis* con buen soporte: 91.6/96) y bien diferenciada de *Q. amicalis amicalis,* que podría ser elevada al rango especie, y (iii) *Q. jeannei pongai* (Salgado, 1982) anclada a un nodo con soporte muy bajo (0/40), pero claramente diferenciada de *Q. jeannei jeannei*. También cabe destacar la posición filogenética de *Quaestus pachecoi* (Bolívar, 1915) —con buen soporte: 98.5/99— o la de *Q. autumnalis autumnalis* (Escalera, 1898) —con soporte aceptable: 85.3/90— situados respectivamente como hermanos de los dos grandes clados observados en la filogenia. Esta que se aporta es una filogenia parcial; probablemente al incorporar los restantes táxones de la serie filética se van a producir desplazamientos de las ramas ancladas en nodos con bajo soporte o se van a incorporar especies a estos clados modificando su topología interna, pudiendo variar el soporte de distintos nodos. Por estos motivos, de momento, se considera más prudente no hacer cambios taxonómicos.

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Apéndice I. Material examinado en el estudio filogenético, con número de voucher y números de acceso en GenBank de las secuencias depositadas: JMS (leg. J.M. Salgado), AF (leg. A. Faille), JF (leg. J. Fresneda), IR (leg. I. Ribera), AC (leg. A. Cieslak).

Appendix I. Studied material for the phylogenetic analyses, with voucher and accession numbers in Genbank of the deposited sequences: JMS (leg. J.M. Salgado), AF (leg. A. Faille), JF (leg. J. Fresneda), IR (leg. I. Ribera), AC (leg. A. Cieslak).

Especie	Voucher	Localidad y recolector	BAR	COI	16S	18S	cytb	28S
Anillochlamys subtruncata	NHM-IRC1	España, Castellón, Montan, Cova Cirat, 19.12.1998, JF	**ON109503**	GU356850	GU356745	GU356904	GU356802	GU356941
Breuilia triangulum	IBE-AF2	España, Asturias, Porrúa, Cueva Verde, 6.7.2008, AF, JF, IR & AC		HG915358	HG915661	HG915433		HG915504

Especie	Voucher	Localidad y recolector	BAR	COI	16S	18S	cytb	28S
Breuilites eloyi	MNCN-AI662	España, Asturias, Caravia Alta-Arriondas, Cueva de Entrecuevas, 21.1.2006, JMS	**ON109504**	GU356859	GU356755	GU356911	GU356810	GU356949
Espanoliella cuneus	IBE-AF5	España, Cantabria, Límpias, Cueva de Seña, 3.7.2008, JMS, AF, JF, IR & AC		HG915365	HG915668	HG915440		HG915511
Espanoliella jeanneli	NHM-IRC4	España, Cantabria, Sámano, Cueva de la Lastrilla, 12.7.1998, JF	**ON109505**	GU356863	GU356759	**ON117155**	GU356813	GU356955
Espanoliella luquei	MNCN-AI573	España, Cantabria, Santoña, Barrio del Dueso, Cueva del Polvorín, 31.5.2003, JMS	**ON109506**	GU356864	GU356760	GU356914	GU356814	GU356956
Espanoliella urdialensis	IBE-AC90	España, Cantabria, Santullán, Cueva de los Peines II, 17.10.2008, JMS		HG915366	HG915669	HG915441		HG915512
Leonesiella bergidi	MNCN-AC59	España, León, Sanvitul, Cueva Moroucedi, 22.9.2007, JMS		HG915369	HG915672	HG915443		HG915516
Oresigenus jaspei	IBE-AC98	España, Asturias, Covadonga, Cueva del Reguerín, 7.11.2008, JMS		HG915374	HG915675	HG915448		HG915521
Paranillochlamys velox	NHM-IRC37	España, Tarragona, Hospitalet de l'Infant, Avenc de la Júlia, 16.12.2001, F. Fadrique		GU356874	GU356770	GU356922	GU356823	GU356965
Quaestus (Amphogeus) cantabricus	IBE-AF8	España, Vizcaya, San Pedro de Galdames, Cueva de la Magdalena, 4.7.2008, JMS, AF, JF, IR & AC		HG915385	HG915688	HG915458	HG915607	HG915532
Quaestus (Amphogeus) escalerai	IBE-AF9	España, Cantabria, Rasines, Cueva del Valle, 5.7.2008, JMS, AF, JF, IR & AC		HG915386	HG915690	HG915459		HG915533
Quaestus (Quaesticulus) autumnalis autumnalis	IBE-RA243	España, Cantabria, Rioseco-Santiurde de Torazo, Cueva de las Arrigueras, 14.11.2007, C.G. Luque		HG915387	HG915687	HG915460		HG915534
Quaestus (Quaesticulus) cisnerosii vasconicus	IBE-AF12	España, Burgos, Llorengoz, Cueva de Albia, 4.7.2008, JMS, AF, JF, IR & AC		HG915388	HG915689	HG915461	HG915609	HG915535
Quaestus (Quaesticulus) noltei	NHM-IRC2	España, Guipúzcoa, Mendaro, Cueva del Viento, 18.7.2000, JF		GU356883	GU356781		GU356831	GU356975
Quaestus (Quaesticulus) noltei	NHM-IRC3	España, Guipúzcoa, Olatz, Sima Kobeta, 15.7.1998, JF		HE572816	HE576716	GU356928	HE572887	HE572869
Quaestus (Quaesticulus) pachecoi	MNCN-AI665	España, Asturias, San Tirso-San Román de Candamo, Cueva del Ferradal, 15.10.2005, JMS		HG915389	HG915691			HG915536
Quaestus (Quaesticulus) sharpi nigricans	IBE-AC92	España, Cantabria, Puente Viesgo, Cueva de los Murciélagos, 7.9.2008, JMS		HG915390	HG915692	HG915462		HG915537

Especie	Voucher	Localidad y recolector	BAR	COI	16S	18S	cytb	28S
Quaestus (Quaestus) acuminatus	IBE-AF76	España, Asturias, Tospe, Cueva la Xiana, 12.5.2007, JMS	**ON109507**	**ON421530**	**ON386256**	**ON117156**	**ON420827**	**ON146576**
Quaestus (Quaestus) arcanus	IBE-PB32	España, Cantabria, Alfoz de Lloredo, Cueva de la Rogeria de Oreña, C.G. Luque & L. Labrada	**ON109509**		**ON386257**	**ON117157**	**ON420828**	**ON146577**
Quaestus (Quaestus) jeannei jeannei	IBE-AC99	España, Asturias, Covadonga, Cueva de la Vega de Teón, 7.11.2008, JMS	**ON109510**	**ON421532**	**ON386258**	**ON117158**	**ON420829**	**ON146578**
Quaestus (Quaestus) jeannei pongai	MNCN-AI1075	España, Asturias, Viboli, Cueva de los Moros, 24.6.2006, JMS	**ON109511**	GU356884	GU356782	GU356929	HE663511	GU356976
Quaestus (Quaestus) nuptialis	MNCN-AI1079	España, León, Oseja de Sajambre, Cueva de Buseco, 24.6.2006, JMS	**ON109513**	**ON421534**	**ON386260**	**ON117160**	**ON420830**	**ON146580**
Quaestus (Quaestus) olajensis	MNCN-AC63	España, León, Santa Olaja de la Varga, Cueva del Carrascal, 15.5.2007, JMS	**ON109514**	**ON421535**	**ON386262**	**ON117161**		**ON146582**
Quaestus (Quaestus) pseudoccidentalis	MNCN-AC64	España, Asturias, Villobal-Borines, Cueva del Sidrón, 23.6.2007, JMS	**ON109515**	**ON421536**	**ON386263**	**ON117162**	**ON420831**	**ON146583**
Quaestus (Quaestus) recordationis	IBE-AF20	España, Asturias, Las Cuevas, Cueva del Agua, 28.6.2008, JMS	**ON109516**	**ON421537**	**ON386264**	**ON117167**	**ON420832**	**ON146585**
Quaestus (Quaestus) sellai	MNCN-AC65	España, Asturias, Nueva, Cueva del Penicial, 21.7.2007, JMS	**ON109518**	**ON421539**	**ON386266**	**ON117164**	**ON420825**	**ON146586**
Quaestus (Quaestus) spinulosus	MNCN-AC66	España, Asturias, Piñera-Sevares, Cueva les Xianes, 23.6.2007, JMS	**ON109519**	**ON421540**	**ON386267**	**ON117163**	**ON420834**	**ON146584**
Quaestus (Quaestus) suevensis	MNCN-AC67	España, Asturias, Bodes, Cueva de la Carretera o de Pitufos, 12.5.2007, JMS	**ON109520**	**ON421541**	**ON386268**	**ON117165**	**ON420824**	**ON146587**
Quaestus (Speogeus) amicalis amicalis	IBE-AC122	España, Asturias, Sellano-Ponga, Cueva Subterráneo, 3.9.2009, JMS	**ON109508**	**ON421531**				
Quaestus (Speogeus) amicalis dilatatus	IBE-AF22	España, Asturias, Las Cuevas, Cueva del Agua, 8.6.2008, JMS		HG915391	HG915685	HG915464		HG915539
Quaestus (Speogeus) jubilationis	IBE-AF225	España, Asturias, Pando-Villaviciosa, Cueva de Covallos, 17.10.09, JMS	**ON109512**	**ON421533**	**ON386259**	**ON117159**	**ON420826**	**ON146579**
Quaestus riberai sp. n.	IBE-AF77	España, Asturias, Tospe, Cueva la Xiana, 12.5.2007, JMS	**ON109517**	**ON421538**	**ON386265**		**ON420833**	**ON146575**
Quaestus valenzuelai sp. n.	MNCN-AC71	España, Asturias, Piñera-Sevares, Cueva les Xianes, 23.6.2007, JMS	**ON109521**	**ON421542**	**ON386261**	**ON117166**		**ON146581**
Spelaeochlamys ehlersi ehlersi	NHM-IRC31	España, Alicante, Cocentaina, Cova de les Meravelles, 30.3.2002, JF		GU356886	GU356784	GU356931	GU356833	GU356978

New Hygropetricolous Leptodirini from Albania (Coleoptera, Leiodidae, Cholevinae)

Nuevos Leptodirini higropétricos de Albania (Coleoptera, Leiodidae, Cholevinae)

http://zoobank.org/References/F926EA8B-A389-415C-BE10-137EC3FA1546

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ABSTRACT

Three new ultraspecialized Leptodirini (Coleoptera, Leiodidae, Cholevinae) are described from the Shpella Shtares cave in Albania. *Riberius* (**n. gen.**) *stillicidii* **n. sp.** is an enigmatic taxon of Leptodirini, markedly distinct for several morphological features in antennae, mouth parts, and male and female genitalia. However, for some characters, it should not belong to the phyletic lineage of *Hadesia*, which so far includes only hygropetricolous species. On the contrary, it seems closer to the *Anthroherpon* lineage. *Kircheria dritae* **n. sp.** and *Anthroherpon shtarensis* **n. sp.**, from the same cave, owing to their morphological features, are the south-western geographical adelphotaxa of *K. beroni* Giachino & Vailati and *A. garbellii* Giachino & Vailati, respectively.

Key Words: *Riberius* **n. gen.**, *stillicidii* **n. sp.**, *Kircheria dritae* **n. sp.**, *Anthroherpon shtarensis* **n. sp.**, Albania.

RESUMEN

Se describen tres nuevos Leptodirini ultraespecializados de las cuevas Shpella Shtares en Albania. *Riberius* (**n. gen.**) *stillicidii* **n. sp.** Es un enigmático taxon de Leptodirini que se diferencia por peculiaridades de las antenas, de las piezas bucales y de los genitalia de ambos sexos. No parece pertenecer al linaje filético de *Hadesia*, que hasta ahora es el único que incluye especies higropétricas. Parece cercano al linaje *Anthroherpon*. *Kircheria dritae* **n. sp.** y *Anthroherpon shtarensis* **n. sp.**, de la misma cueva, por sus características morfológicas, son los adelfotaxa geográficos del suroeste de *K. beroni* Giachino & Vailati y *A. garbellii* Giachino & Vailati, respectivamente.

Palabras clave: *Riberius* **n. gen.**, *stillicidii* **n. sp.**, *Kircheria dritae* **n. sp.**, *Anthroherpon shtarensis* **n. sp.**, Albania.

INTRODUCTION

The Dinaric range in the Balkan Peninsula is a well-known hotspot of subterranean biodiversity (GIACHINO *et al.,* 1998; JEANNEL 1924; PERREAU, 2000, 2015, HLAVÁČ *et al.*, 2017) also with regard to hygropetricolous Leptodirini (sensu SKET, 2004). This knowledge includes many more or less recent contributions (CASALE & JALŽIĆ, 1988; CASALE *et al.,* 2000a; 2004; ĆURČIĆ *et al.,* 2019; GIACHINO & GUÉORGUIEV, 1993; GIACHINO *et al.,* 1998, 2011, 2021; GUÉORGUIEV V.B., 1990; MORAVEC & MLEJNEK, 2002; MLEJNEK & MORAVEC, 2003; NJUNJIĆ *et al.,* 2017; NONVEILLER & PAVIĆEVIĆ, 1999; PERREAU & PAVIĆEVIĆ, 2008, POLAK *et al.,* 2016).

The same cannot be said regarding the knowledge of the hyperspecialized Leptodirini of Albania. If we exclude the contributions of GIACHINO & VAILATI (1998, 2005, 2006) and GIACHINO *et al.* (2016), relating to the description of *Kircheria beroni*, *Albanodirus trezzii*, *A. gobettii* and *A. ivanpetrovi*, and some species or subspecies of *Anthroherpon*, little is known about the specialised species of this area.

Thanks to recent speleological explorations in large Albanian caves carried out by Italian speleologists of the “Gruppo Speleologico Martinese“ (GSM), “Gruppo Speleologico Bolognese” (GSB – USB), “La Venta” Association and “Gruppo Speleologico Faentino”, we were able to study a series of hygropetricolous Leptodirini collected by them and kindly made available to us. The results of the study are reported in this contribution.

While this contribution was still in proof, additional specimens of the treated species became available thanks to a GSM expedition to Albania in August 2022. The type series now includes these specimens.

MATERIAL AND METHODS

Morphological analysis was conducted on whole specimens and dissected male genitalia mounted in Canada balsam. All drawings were made using a drawing tube on a Leitz Dialux biological microscope and Wild M3 stereomicroscope. The photographs of the holotypes of *Riberius* gen. nov. *stillicidii* **n. sp.** and *Kircheria dritae* **n. sp.** was obtained by Gianni Allegro using a Leica DFC295 camera mounted on a Leica M205 C Stereomicroscope, using the software Leica Application System V4.0. Arnaud Faille conducted amplification, preliminary sequencing and molecular analysis from DNA extracted in the laboratory of Ignacio Ribera at the Institut de Biología Evolutiva (IBE) of Barcelona (Spain) and Department of Entomology, Coleoptera, Stuttgart State Museum of Natural History of Stuttgart (Germany).

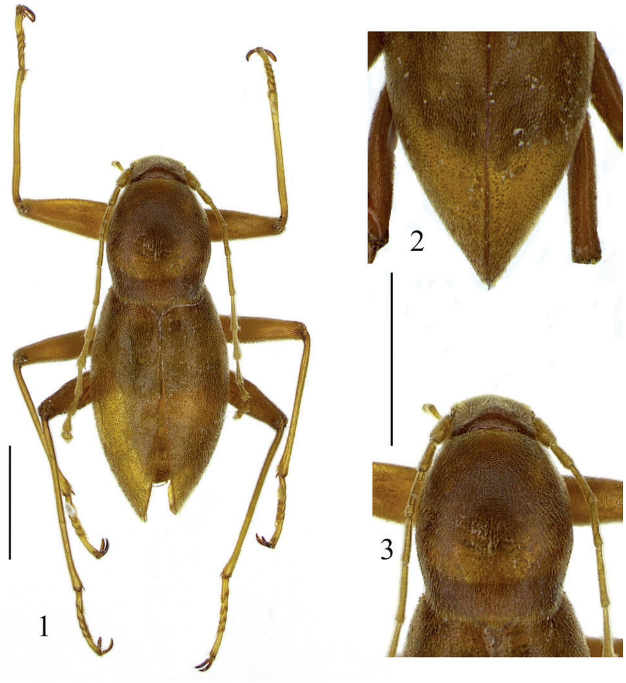
Acronyms

The following acronyms are used for collections, type series and measurements: MCSNG, Museo civico di Storia naturale "G. Doria", Genova, Italia; Cca, A. Casale Collection, Torino, Italia; Cgi, P. M. Giachino Collection, San Martino Canavese (TO), Italia. HT, Holotype; PT, PTT, Paratype (s). BAR, body/Antennae ratio (lengths of pronotum+elytra/antennae); PW/PL, pronotal width/pronotal length; EL/EW, elytral length/elytral width.

RESULTS

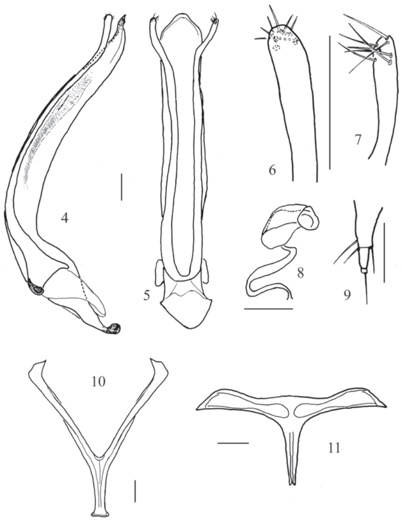
***Riberius* n. gen.** (Figs. 1-17)

http://zoobank.org/NomenclaturalActs/6C599CB5-CD8C-4800-AC57-6ADA3A1F5531



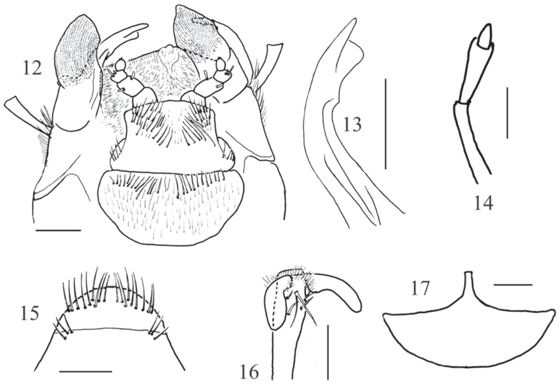
Figs. 1-3. *Riberius stillicidii* **n. gen. n. sp.**: HT ♂ (1, 3), PT ♀ (2). Habitus (1); elytral apex in female, dorsal view (2); head and pronotum dorsal view (3). Scale bar: 1 mm.

Figs. 1-3. *Riberius stillicidii* **n. gen. n. sp.**: HT ♂ (1, 3), PT ♀ (2). Habitus (1); ápice elitral de la hembra en vista dorsal (2); cabeza y pronoto en vista dorsal (3). Escala: 1 mm.



Figs. 4-11. *Riberius stillicidii* **n. gen. n. sp.**: HT ♂ (4, 7), PT ♀ (8-11). Aedeagus in lateral view (4) and dorsal view (5); apex of left paramere in lateral (6) and dorsal (7) view; spermatheca (8); stylomere (9); metendosternite (10); metatergal apparatus (11). Scale bar: 0.1 mm.

Figs. 4-11. *Riberius stillicidii* **n. gen. n. sp.**: HT ♂ (4, 7), PT ♀ (8-11). Edeago en vista lateral (4) y vista dorsal (5); ápice del parámero izquierdo en vista lateral (6) y dorsal (7); espermateca (8); estilómero (9); metendosternito (10); aparato metatergal (11). Escala: 0,1 mm.



Figs. 12-17. *Riberius stillicidii* **n. gen. n. sp.**: PT ♀. Head, ventral view (4); left mandible, ventral view (13); maxillary palpomere, ventral view (14); labrum, dorsal view (15); protarsal claw, ventral view (16); VIII ventrite in female (14). Scale bar: 0.1 mm.

Figs. 12-17. *Riberius stillicidii* **n. gen. n. sp.**: PT ♀. Cabeza en vista ventral (4); mandíbula izquierda en vista ventral (13); palpómero maxilar en vista ventral (14); labrum en vista dorsal (15); uñas del protarso en vista ventral (16); VIII ventrito de la hembra (14). Escala: 0,1 mm.

Type species: *Riberius stillicidii* n. sp.

Diagnosis and description

A genus of small sized, pentamerous, eyeless, pubescent Leptodirini with pholeuonoid body, large, sub-ovoidal pronotum, ovate and dimorphic elytra, and antennae shorter than the body length.

Head elongate, not retractile, without occipital carina; clypeus and labrum with dense, long pubescence and a series of anterior setae (labrum) or two lateral groups of three setae (clypeus) (Fig. 15). Penultimate labial palpomere long and apically dilated; apical palpomere very short and stout

(Fig. 14). Antennae relatively short, inserted on the base of the medium 3rd of head, similar in both sexes and not reaching the elytron apex; first antennomere widened, longer than 2nd antennomere; 11th antennomere as long as the 10th one.

Pronotum large and not dimorphic: only slightly more elongate in male than in female. Sub-ovoidal in shape, with its maximum width just after the middle. Lateral sides regularly rounded anteriorly, subrectilinear basally. Hind angles obtuse, largely evident; basal margin narrower than the base of elytra. Anterior margin convex.

Pterothorax not pedunculate. Mesoventral carina absent; mesocoxal cavities contiguous.

Metatergal apparatus (Fig. 11) reduced, with posterior apophysis just shorter than lateral branches.

Metendosternite (Fig. 10) thin, V-shaped, with narrow furcal arm and short and narrow furcal plate. Arm apophysis well developed, flexed and pointed inwards.

Elytra elongate-ovate, moderately attenuate at apex. Apex pointed in both sexes, with a small, acute, pointed, posterior expansion in female. Microsculpture sparse, not forming transversal rows; disc with thin and short pubescence; sutural stria absent.

Legs very long and slender, with femora thickened basally. Protibiae slightly widened just at apex, without comb but with an incomplete, inner, apical basket of spines and short setae and without outer spurs; inner spurs unifid, very small and thin. Male protarsi 5-segmented, not dilated. Meso- and metatibiae with inner, unifid apical spur and apical basket of very short apical spines and setae; tarsal claws (Fig. 16) long, widened; empodial seta bifid.

Aedeagus (Figs. 4-7) large sized, slender, ventrally abruptly arcuate before the base; basal lamina of median lobe bent, without ventral carina; parameres as long as the median lobe, apically turned outward, each furnished with seven setae at apex. Endophallus almost unarmed, with only a short, centre-apical, striated bundle.

VIIIth ventrite of female (Fig. 17) with an evident, apically truncated, apophysis.

Gonapophysis (Fig. 9) normally developed. Spermatheca (Fig. 8) membranous, bean-shaped, only slightly sclerotized, not annulated.

Etymology

Riberius: Latinization of the name "*Ribera*", dedicated to the memory of our colleague and friend Ignacio Ribera. The name of the genus is masculine.

Riberius stillicidii Giachino & Casale **n. sp.** (Figs. 1 - 17)

http://zoobank.org/NomenclaturalActs/2E030C4D-7B82-45D1-9031-F55A89258E02

Loc. Typ.: Albania, Vrana e Madhe, Shpella Shtares cave, m 1427, 42.31766° N 19.85403° E.

Type material: HT ♂, Albania, Vrana e Madhe, Shpella Shtares cave, m 1427, 42.31766° N 19.85403° E, 23.VIII.2019, A. Lacirignola, M. G. Mastronardi, M. Pastore leg. (CCa). PTT: 1 ♀, same data (CGi); 1 ♀, same data, AN1279 (MCSNG); 3 ♂♂ 1♀, same data, 18.VIII.2022, GSM leg. (CCa, CGi); 1♀, same data, 20.VIII.2022, GSM leg. (CCa).

Description

A medium sized (mm 3.16-3.21; 3.16 HT ♂), pholeuonoid, highly specialized leptodirine beetle. Colour dark testaceous, integument opaque, pubescent.

Head elongate, not retractile, anophthalmous, without occipital carina. Mouth pieces (Figs. 12-15) with long setae (see JEANNEL, 1924; CASALE & JALŽIĆ, 1988; NONVEILLER & PAVIČEVIĆ, 1999; CASALE *et al.,* 2000a, 2000b; MORAVEC & MLEJNEK, 2002; MLEJNEK & MORAVEC, 2003; MOLDOVAN *et al.,* 2004; CASALE *et al.,* 2004; GIACHINO & VAILATI, 2006; GIACHINO *et al.,* 2021). Labrum (Fig. 15) transverse, with anterior margin totally curved and without evident angles from anterior margin and lateral ones. Bearing, in addition to the dense pubescence, a set of 18-10 long setae. Mandibles (Fig. 13) widened and flattened in the apical half, twisted and toothed on the inner edge. Maxillae short, stipe with the articles bearing tufts of long setae; lacinia reduced, the outer lobe capped by a sort of triangular membranous and fully striated lobe. Maxillary palpi (Fig. 14) elongate, with second palpomere shorter than the first, third one short and conical. Labium (Fig. 12) fully covered by a dense pubescence, with anterior margin slightly curved; front and lateral areas equipped, on the outer side of the mouth, with two sets of long setae. Ligula sub-pentagonal, with anterior margin with a small tooth and without range of setae.

Antennae slender but relatively short, more elongate in the male (BAR: 1.07 ♂; 1.15♀).

Length of antennomeres in mm:

HT ♂: 0.26; 0.16; 0.30; 0.25; 0.33; 0.30; 0.26; 0.23; 0.35; 0.19; 0.16

PT ♀: 0.25; 0.15; 0.25; 0.23; 0.31; 0.28; 0.25; 0.23; 0.34; 0.18; 0.15

Pronotum large, elongate, longer than wide (PW/PL: 0.80 ♂, 0.89 ♀), with its maximum width just after the middle; lateral sides rounded anteriorly, sub-rectilinear basally; anterior margin curved outward. Hind angles obtuse, rounded; basal margin narrower than the base of elytra. Disc convex, covered by dense, short, decumbent pubescence.

Elytra elongate-ovate (EL/EW: 1.51 ♂ 1.70 ♀), with apex dimorphic: acute, not expanded in male (Fig. 1), with two, short, acute, expansions (Fig. 2) in female. Maximum width at the middle length. Disc with microsculpture sparse, not forming transversal rows; completely covered by short, thin and decumbent pubescence; sutural stria absent.

Legs as in the description of the genus; male protarsi pentamerous, not dilated; is wider than the apex of protibia. Femora widened at base, tibiae straight.

Aedeagus (Figs. 4-7) large sized, slender. Median lobe, in lateral view, abruptly bent at the base, with basal lamina bent, without ventral carina. Apex, in lateral view, pointed and bisinuated. In dorsal view very elongate, with subparallel sides almost to the apex, that is subtriangular and slightly truncate. Parameres as long as the median lobe, each bearing seven inner setae: one apical and two sets of three subapical. Endophallus almost unarmed, with only a short, centre-apical, striated bundle.

Female genitalia as in the description of the genus.

Etymology

From the Latin word *stillicidium – ii*, "falling water drops". The name wants to recall the hygropetric environment in which the species lives.

Kircheria dritae **n. sp.** (Figs. 18 - 26)

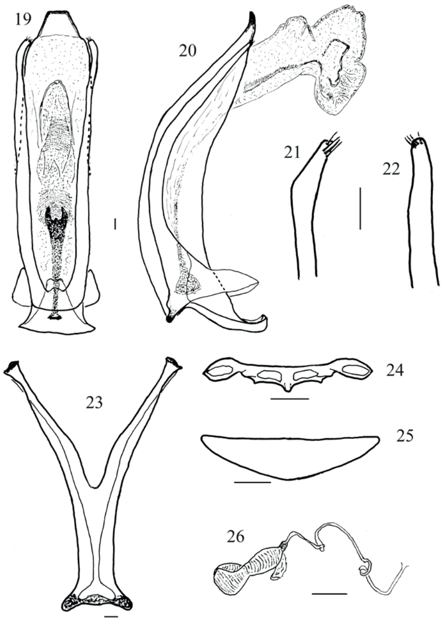
http://zoobank.org/NomenclaturalActs/A6E446A2-26F2-4551-8C57-CE43F77659D0

Loc. Typ.: Albania, Vrana e Madhe, Shpella Shtares cave, m 1427, 42.31766° N 19.85403° E.

Type material: HT ♂, Albania, Vrana e Madhe, Shpella Shtares cave, m 1427, 42.31766° N 19.85403° E, 23.VIII.2019, A. Lacirignola, M. G. Mastronardi, M. Pastore leg. AN1277 (CCa). PTT: 1 ♀, same data, AN1280 (MCSNG); 2 ♂♂, same data (CCa, CGi); 1 ♀, same data, 20.VIII.2019 (CCa); 1 ♂, same data, VIII.2018, L. Luprano & C. Pastore leg. (CGi); 1♂ 1♀, same data, 19.VIII.2022, GSM leg. (CCa, CGi); 1♀, same data, 24.VIII.2022, GSM leg. (CCa); 1♂, same data, 21.VIII.2022, GSM leg. (CGi).

Fig. 18. *Kircheria dritae* **n. sp.**: HT ♂, habitus. Scale bar: 1 mm.

Fig. 18. *Kircheria dritae* **n. sp.**: HT ♂, habitus. Escala: 1 mm.



Figs. 19-26. *Kircheria dritae* **n. sp.**: HT ♂ (19-22); PT ♀ (23-26). Aedeagus in dorsal view (19) and lateral view (20); apex of right paramere in dorsal view (21) and left in lateral view (22); metendosternite (23); metatergal apparatus (24); VIII ventrite in female (25); spermatheca (26). Scale bar: 0.1 mm

Figs. 19-26. *Kircheria dritae* **n. sp.**: HT ♂ (19-22); PT ♀ (23-26). Edeago en vista dorsal (19) y lateral (20); ápice del parámero derecho en vista dorsal (21) y del izquierdo en vista lateral (22); metendosternito (23); aparato metatergal (24); VIII ventrito de la hembra (25); espermateca (26). Escala: 0.1 mm

Diagnosis

A *Kircheria* species of large size (mm 8.00-9.04) (as usual in the genus), anophthalmous, with non-retractile head and specialized mouthparts. Antennae, if compared with the specialization degree, relatively short, hardly exceeding the apex of elytra in male when stretched backwards; shorter, just reaching the apex of elytra in female.

Description

Total length, with head bent in natural posture: 9.04-9.07 mm ♂♂, 8.00-8.90 ♀♀. Colour: normally completely brown ferrugineous (yellow testaceous in immature specimens). In some specimens the pronotum, head, femora, tibiae, mandibles and antennomeres 1-7 are darker and elytra, tarsi and antennomeres 8-11 paler, rufo-testaceous. Body (Fig. 18) hadesian shaped, elongate, very tapered, acute apically, remarkably convex, uniformly covered with a short, very thick and hydrophobous pubescence.

Head very elongate, enlarged anteriorly with concave genae; labrum big and robust, arcuate; mandibles (Fig. 18) markedly flattened at base, thickened dorsally in the apical part, with a bidentate apex. Maxillary palpi long, with last palpomere, basally stumpy, shorter than the penultimate one. Labium, glossae, paraglossae, labrum, and lateral part of stipes bearing long setae. Antennae (Fig. 18) inserted on the basal fourth of the head. Second antennomere shorter than the first one; third antennomere longest of all; eighth longer than the three subsequent ones, and eleventh shortest of all, except for the second one.

Length of antennomeres in mm:

Holotype ♂: 0.57; 0.38; 1.04; 0.80; 1.28; 0.95; 0.85; 0.78; 0.66; 0.71; 0.52

Paratype ♀: 0.57; 0.19; 0.93; 0.72; 0.93; 0.73; 0.61; 0.65; 0.61; 0.66; 0.38

Pronotum (Fig. 18) elongate, cordate (max. width/max. length ratio: 0.75 ♂♂, 0.76 ♀♀), uniformly convex, with lateral margins regularly rounded anteriorly and remarkably sinuate posteriorly in the basal third. Basal angles broadly obtuse yet sharp; base remarkably arcuate and projecting posteriorly, wider than the anterior margin. Sides of pronotum finely beaded all their length long (but this bead is not obvious in the anterior part in dorsal aspect).

Mesothoracic peduncle lacking; scutellum big and transverse; mesoventrite flat, without carina; mesocoxal cavities confluent. Metatergal apparatus (Fig. 24) very reduced in size, with a very short posterior apophysis. Metendosternite (Fig. 23) not V-shaped, with a robust furcal trunk and long, relativaly narrow and moderately divergent furcal arms.

Elytra (Fig. 18) very elongate, oval, narrow (max length/max. width ratio: 2.28 ♂♂, 2.86 ♀♀), with maximum width at the middle, apex altogether acute, but separately sub-rounded, more rounded in male and slightly more acute in female, finely carinated laterally. Elytral disc convex, not depressed or flattened along the elytral suture, without transversal striation nor sutural stria.

Legs frail and very elongate. As in *Anthroherpon* Reitter, 1889, femora are typically enlarged at the base and narrowed towards the apex, flattened, and entirely covered on both sides with a very short and thick hydrophobous pubescence. Tibiae lacking of apical spurs; protibiae slightly curved outwards; mesotibiae slightly curved inwards and metatibiae straight. Pro- and mesotibiae strongly enlarged at the distal end; all tibiae covered with a very short and thick hydrophobous pubescence, but all showing a glabrous stripe on the external side. Protarsi pentamerous and not dilated in male; first meso- and metatarsomere very elongate, markedly longer than the fifth too. Tarsal claws big, specialized, wide and strongly hollow spoonwise.

Male genital segment reduced, wide and oval, without a pronounced ventral apophysis.

Aedeagus big (Figs. 19-22). Median lobe, in lateral view, slightly and regularly curved from base to apex. Apex slightly upwards. Median lobe, in dorsal view, robust and elongate, with subparallel sides from the base to pre-apical area; apex truncated, broadly trapezoidal, with right anterior margin. Basal blade of median lobe short and stumpy. Tegmen complete, wide and well visible dorsally; ventral blade of the tegmen narrow; parameres short and relatively strong, neatly shorter than median lobe. Apex of parameres bearing, on the inner side, four apical short, subequal, setae (Figs. 21-22). Endophallus with a characteristic basal, long sclerified piece, enlarged at base and bifid at apex; two, median-apical, spinuliform bundles are also present (Fig. 19).

VIIIth ventrite in female totally lacking of anterior apophysis (Fig. 25). Stylomeres lacking. Spermatheca (Fig. 26) sacciform, shaped like a curved cucumber, uniformly and very poorly sclerified, and with a striated surface; apical and basal parts not differing one from the other, but only with the distal part slightly dilated; annexed gland small and confluent with the insertion of the ductus, that is in a proximal position.

Etymology

From the name of Mrs. Drita, who hosted the Italian speleologists during the explorations of the Shpella Shtares cave. This name in Albanian language means "light", the light that led them towards the discovery of the new species described in this contribution.

Comparative notes

Kircheria dritae **n. sp.** differs from *K. beroni* Giachino & Vailati, 2006 by the shape of the apex of median lobe of aedeagus, narrower and clearly trapezoidal, truncated at apex; by the presence of 4 setae at apex of parameres (3 in *K. beroni*), and by the structure of endophallus, which takes a characteristic sclerotized piece with bifid apex.

Anthroherpon shtarensis **n. sp.** (Figs 27- 29)

http://zoobank.org/NomenclaturalActs/4E86C67A-E1EC-478F-8519-550C6A5FBED7

Loc. Typ.: Albania, Vrana e Madhe, Shpella Shtares cave, m 1427, 42.31766° N 19.85403° E.

Type material: HT ♂, Albania, Vrana e Madhe, Shpella Shtares cave, m 1427, 42.31766° N 19.85403° E, 23.VIII.2019, A. Lacirignola, M. G. Mastronardi, Pastore M. leg. AN1278 (CCa); 2♂♂, same data, 18.VIII.2022, GSM leg. (CCa, CGi); 1♀, same data, 24.VIII.2022, GSM leg. (CCa); 1♂, same data, 18.VIII.2022, GSM leg. (CGi).

Diagnosis

A large-sized *Anthroherpon* species (mm 8.24 HT ♂) of the *A. latipenne* species group (sensu GUÉORGUIEV, 1990, GIACHINO & GUÉORGUIEV, 1993 and GIACHINO & VAILATI, 2005). Characterized by shape of aedeagus and elytra fully covered by pubescence; shape of elytra, which are almost scaphoid; shorter parameres and presence of only two setae at apex of parameres.

Description

Total length with extended head: 8.02 mm. Colour uniformly reddish dark, with tarsi, palpi, and apical antennomeres slightly lighter. Body (Fig. 27) leptodiroid, very elongate, with physogastric, convex and almost scaphoid elytra. Pronotum narrow and very elongate. Integument shiny, with evident microsculpture on head, pronotum and elytra, not aligned in transversal strioles on the elytral disc. Integument pubescence very sparse, upright and moderately long on head and pronotum; dense, golden, short and upright on elytra.

Head hypognathous, very elongate, anophthalmous, without occipital carina, remarkably widened in front. Mouthparts, with dorsal part of the mandibles clearly concave; maxillary palpi elongate, with the penultimate palpomere subconical, widened at apex, and the last palpomere conical and very elongate. Antennae very long, (BAR 0.76), frail, with the 2nd antennomere shorter than the first one, 3rd and 5th the longest ones, and 11th slightly shorter than the 10th one.

Length of antennomeres in mm:

0.60; 0.40; 1.08; 0.76; 1.08; 0.76; 0.88; 0.72; 0.92; 0.86; 0.74

Pronotum very elongate-cordiform (PW/PL: 0.53) and bottlenecked before the base at about 1/4 of its length, with the maximum width at the middle and base narrower than the anterior margin. Lateral margins strongly sinuate in the basal part, subrectilinear and convergent in the distal half and not rebordered in the basal half; basal angles obtuse and blunted; base distinctly curved and beaded. Pronotal disc with evident microsculpture and almost completely glabrous (with only some scattered and short erected setae).

Mesothoracic peduncle short, more than four times wider than long, flat and slightly depressed dorsally, bearing a very strong microsculpture with isodiametric polygonal meshes.

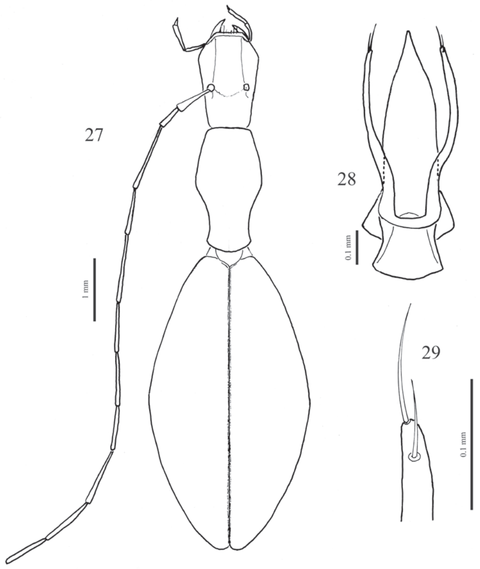
Mesoventrite convex and without mesoventral carina. Prosternum not carinate; mesocoxal cavities not confluent, separated by a long posterior apophysis.

Elytra very elongate (EL/EW: 1.78), slightly pyriform, almost scaphoid, with the largest width just at the mid-section, separately and briefly rounded apically; disc very convex, with the pubescence uniformly dense, short and upright. Sutural stria absent.

Legs very long and frail, with femora enlarged and swollen in the basal part, progressively tapering distally. Tibiae straight, not enlarged distally; claws long and slightly enlarged. Anterior tarsi pentamerous and not dilated in ♂.

Aedeagus (Figs. 28, 29) small-sized, short and poorly arcuate; median lobe, in dorsal view, relatively stocky, with the apex subtriangular and pointed. Basal blade of the median lobe stocky, short and enlarged. Parameres frail, shorter than the median lobe, bisinuate in dorsal view, and bearing two setae: one apical, and another one subapical. Endophallus totally unarmed.

Female unknown.



Figs. 27-29. *Anthroherpon shtarensis* **n. sp.**: HT ♂. Habitus (27); aedeagus in dorsal view (27); apex of left paramere in lateral view (29).

Figs. 27-29. *Anthroherpon shtarensis* **n. sp.**: HT ♂. Habitus (27); edeago en vista dorsal (27); ápice del parámero izquierdo en vista lateral (29).

Etymology

From the name of the cave, Shtares used without the final "s", which in Albanian is also the name of a poisonous flower (the Henbane).

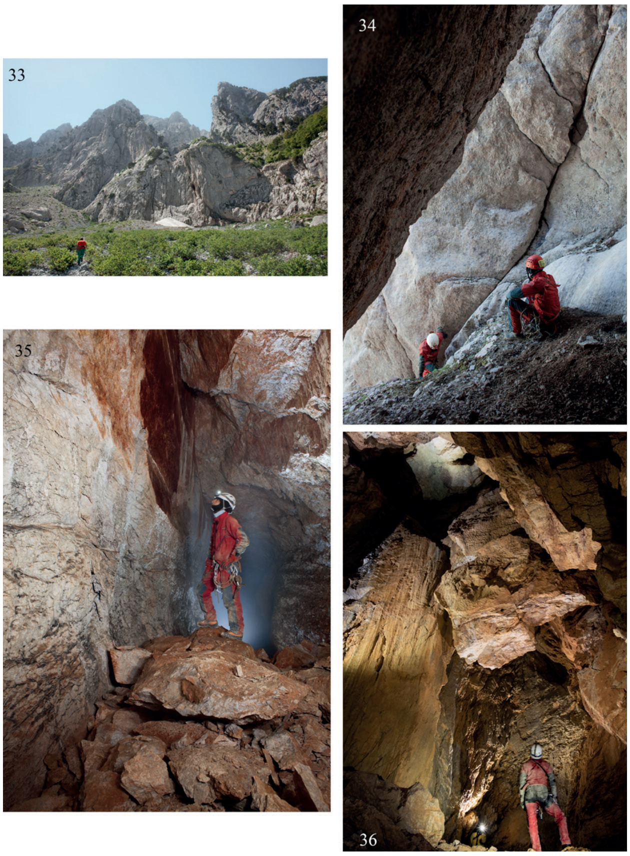
Comparative notes

Closely related to *A. taxi* Müller, 1913 (s. l.) and *A. garbellii* Giachino e Vailati, 2005, by the shape of aedeagus and elytra fully covered by pubescence. *A. shtarensis* **n. sp.** is particularly close to *A. garbellii* by the shape of elytra, which are almost scaphoid. The new species differs from both *A. garbelli* and *A. taxi trezzii* Giachino e Vailati, 2005 (both from Shpella e Pucit cave) by the shorter antennae, shorter parameres and the presence of only two setae at apex of parameres. Furthermore, from *A. taxi trezzii* differs by the maximum width of elytra in the middle (vs. the apical third in the latter).

DISCUSSION

Distribution and ecology

The three new taxa were discovered in the same cave: the Shpella Shtares, near the village of Vrana e Madhe (Regional Natural Park of Nikaj Mërtur), at an altitude of 1,427 m a.s.l. (42.31766° N 19.85403° E), in the Albanian Alps (PASTORE et *al.*, 1919) (Figs. 33).



Figs. 33-36. Shpella e Shtares cave. 33: Mali e Kakisë Mt., near Shpella e Shtares; 34: near the entrance; 35: hygropetric environment; 36: internal hall. Courtesy of the Archive of Gruppo Speleologico Martinese.

Figs. 33-36. Cuevas Shpella y Shtares. 33: Montes Mali y Kakisë, cerca de las cuevas Shpella y Shtares; 34: proximidades de la boca de acceso; 35: ambiente higropétrico; 36: sala interna. Cortesía del archivo del Gruppo Speleologico Martinese.

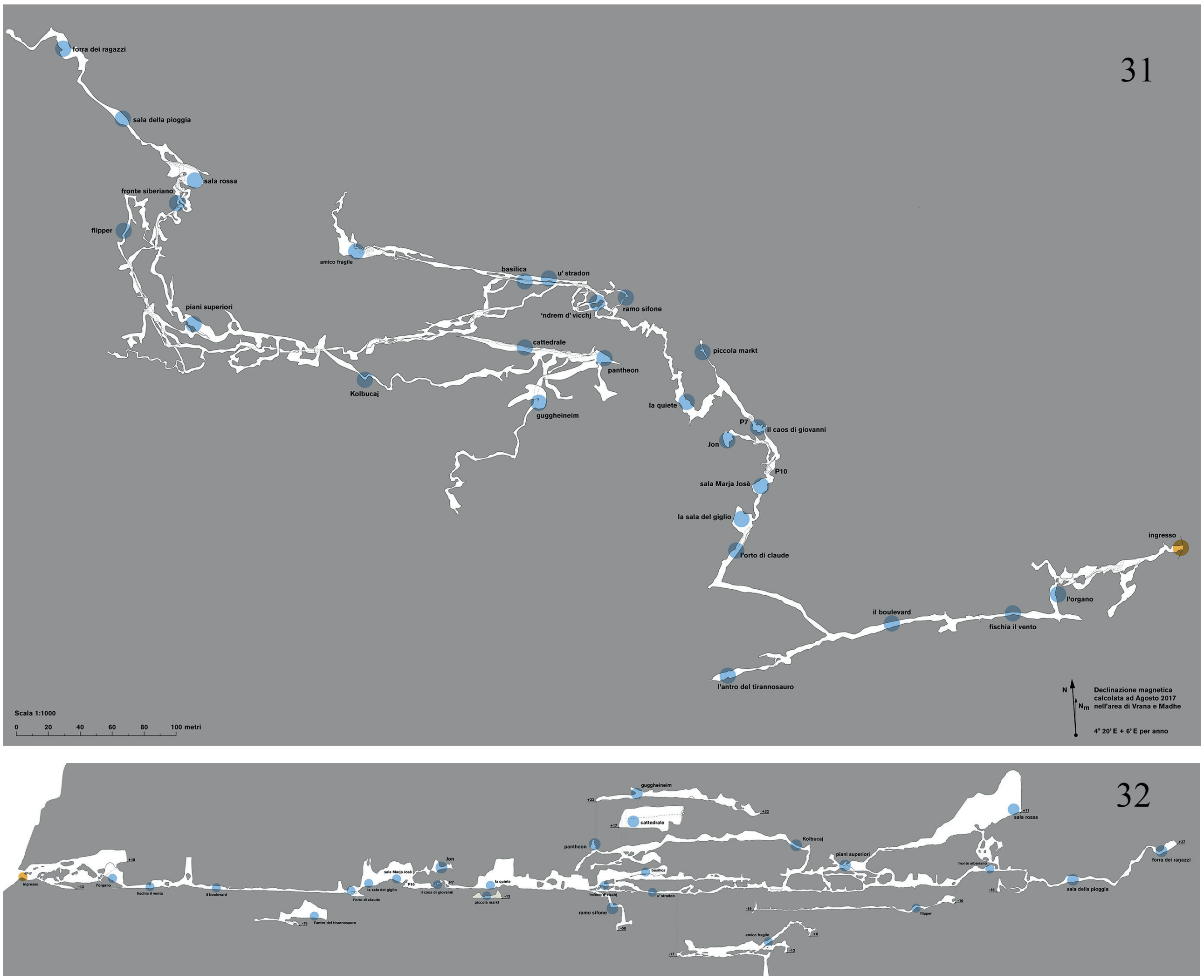
The Shpella Shtares cave opens with a large sub-horizontal entrance at the base of a rock wall, at the foot of Mount Mali e Kakisë (2,359 m a.s.l.) (Fig. 34), characterized, in August, by a strong flow of cold air coming out. The cave, now, has a planimetric development of about 4.6 km on different levels (Figs. 31, 32). In the lower levels, the average internal temperature of the cavity is 3.2-4°C. In the first kilometer, there are no points with intense dripping and / or particularly humid stretches probably because of the strong air draught. In 2018, about 2 km from the main entrance of the cavity, on the walls of a gorge with running water at a temperature of 3.2 ° C, was collected the first specimen of *K. dritae* **n. sp.** More systematic sampling carried out in August 2019 revealed, in the innermost areas of the cave (in the zones named "Forra dei Ragazzi", "Flipper" and "Fronte Siberiano") (Fig. 34) the presence, in a hygropetric environment (fig 35), of *Kircheria dritae* **n. sp.**, *Riberius stillicidii* n. sp, and *Anthroherpon shtarensis* **n. sp.**

Taxonomic, zoogeographic and habitat remarks

In the context of Leptodirini of the Balkan Peninsula, any evaluation of the systematic position of *Kircheria* and *Riberius* gen. nov., in absence of molecular data (which are still being analyzed) appears difficult. Morphological characters of hyper-specialization, derived by the particular environment where they live, the so-called hygropetric environment (SKET, 2004), tend to mask the synapomorphies significant from the systematic point of view. With the progress of research and the discoveries of new taxa, emerges the idea that many characters considered significant in a "Jeannelian systematic" (JEANNEL, 1924) or "parajeannelian systematic" (LANEYRIE, 1967; GUÉORGUIEV, 1976; PERREAU, 2000, 2015) need further evaluation. At the same time, characters considered of little meaning, or never analyzed in detail, are revealing their phylogenetic importance. For this reason, we will briefly analyze a series of characters and their relative significance.

Antennae insertion point. JEANNEL (1924) used this character to define the tribe Antroherpona (= subtribe Anthroherponina auct.), emphasizing the insertion in the hind quarter of the head instead of the middle third. Subsequent authors have usually understood this statement as a "shift to the back" of the insertion point. Observing the relative position of antennae with respect to the progressive specialization of the body, proceeding from less specialized ones (bathyscioids) to the more specialized ones (leptodiroids or scaphoids), what is noticeable is not a backward displacement of the antennae, but an elongation, probably due to different ways of specialization, of the mouth apparatus, of the front part of the head (GIACHINO & VAILATI, 1993; PERREAU & PAVICEVIC, 2008). Different types of specialization of mouth pieces would probably deserve careful evaluation, aimed so far only to apparatus of hygropetricolous taxa (as i.e. *Riberius* gen. nov. and *Kircheria*), where mouth pieces taking long setae, i.e. adapted to filtering water and organic matter (see JEANNEL, 1924; CASALE & JALŽIĆ, 1988; NONVEILLER & PAVIČEVIĆ, 1999; CASALE *et al.*, 2000a, 2000b; MORAVEC & MLEJNEK, 2002; MLEJNEK & MORAVEC, 2003; MOLDOVAN *et al.*, 2004; CASALE *et al.*, 2004; GIACHINO & VAILATI, 2006; GIACHINO *et al.*, 2021). We recall that a similar phenomenon, although of opposite polarity, can be found in the Paracatopini (Leiodidae, Cholevinae) of New Zealand, where the pre-antennal part of the head is shortened (GIACHINO & VAILATI, 1993). Consequently, in our opinion, the insertion position of antennae, as shows also by preliminary molecular data (FRESNEDA *et al.*, in prep.), is a character, at least in part linked to the specialization degree of the mouth apparatus which may appears as autoapomorphy in different lineages. For this reason, it would be better not to use it for phylogenetic reconstructions. A practical example is provided by the genus *Hygrodromus* Giachino, Casale & Jalžić, 2021, which presents all typical characters of the phyletic lineage of *Anthroherpon* / *Hadesia*, but has the antennae inserted in the middle third of the head, as in *Riberius* gen. nov.

Number of male protarsi. A synapomorphy linked to the number of protarsi, constantly four in females of all species, characterizes the whole tribe of Leptodirini. Perhaps for this reason JEANNEL (1924) gave particular emphasis to the tetramery of male protarsi in some species, establishing the group of "Teleomorphs". JEANNEL (1924) did not maintain a univocal attitude, however, assigning the tetramere genus



Figs. 31-32. Shpella e Shtares cave. 31: plant map; 32: section map. Courtesy of the Archive of Gruppo Speleologico Martinese.

Figs. 31-32. Cuevas Shpella y Shtares. 31: planta; 32: sección. Cortesía del archivo del Gruppo Speleologico Martinese.

Spelaeobates Müller, 1901 to his tribe Antroherpona. Recent phylogenetic reconstructions based on molecular data (FRESNEDA *et al.,* in prep.) highlight the phyletic inconsistency of this character that appears independently in different phyletic lineages and in different geographical areas. Also from this point of view, the aforementioned genus *Hygrodromus,* with tetramere male protarsi, appears as exceptional within a pentamere lineage.

Relative length of first and second antennomeres. Discussed by JEANNEL (1924), LANEYRIE (1967) and GUÉORGUIEV (1976), the molecular data (FRESNEDA *et al.*, in prep.) seems to confirm instead a significant phylogenetic importance to this character. Inter alia, all genera of the *Anthroherpon* phyletic lineage (*Anthroherpon*, *Graciliella*, *Leptomeson*, *Hadesia*, *Kircheria*, *Velebitodromus*, *Hygrodromus*, *Nauticiella* and *Riberius* gen. nov.) have the first antennomere much longer than the second one and, often, also the third much longer.

Atrophy of the ovipositor. This is a condition well documented in the phyletic lineage of *Anthroherpon*, where the morphology of female genitalia shows complete atrophy of stylomeres and gonocoxites. This condition is present in *Anthroherpon*, *Graciliella*, *Leptomeson*, *Hadesia*, *Kircheria*, *Velebitodromus*, *Hygrodromus*, *Riberius* gen. nov. and, probably, *Nauticiella*. The importance of this character is confirmed by molecular data (FRESNEDA *et al.,* in prep.), but it should be noted that the genus *Croatodirus*, belonging to a different phyletic lineage (FRESNEDA *et al.*, in prep.) and *Sinuicollia* Piva, 2008 (PERREAU, 2019), also presents an atrophic ovipositor. This should be interpreted as a probable homoplasy.

Apophysis of VIII^th^ female ventrite. Morphological analysis of the VIII^th^ female ventrite reveals that several genera of the phyletic lineage of *Hadesia* (*Hadesia*, *Kircheria*, *Hygrodromus* and, probably, *Nauticiella* and *Velebitodromus*) shows this ventrite totally devoid of anterior apophysis. The molecular phylogenetic analysis is so far, unfortunately, affected by the lack of data of almost all genera, except *Hadesia* (FRESNEDA *et al.*, in prep.). This certainly limits the evaluation of this character, but at the same time partially confirms its importance, isolating *Hadesia*, devoid of apophysis, from the complex *Anthroherpon* + *Graciliella* + *Leptomeson*, equipped with apophysis.

Riberius **n. gen.** shows the first antennomere longer than the second, male protarsi pentameres, completely atrophied ovipositor and well-developed ventral apophysis of the VIII female ventrite. Analysis and evaluation of the taxonomic significance of these characters, together with other morphological characters, in particular those of the male genitalia, lead us to approach *Riberius* gen. nov. to the phyletic series of *Anthroherpon*. This series, consisting on a molecular basis of at least two distinct clade

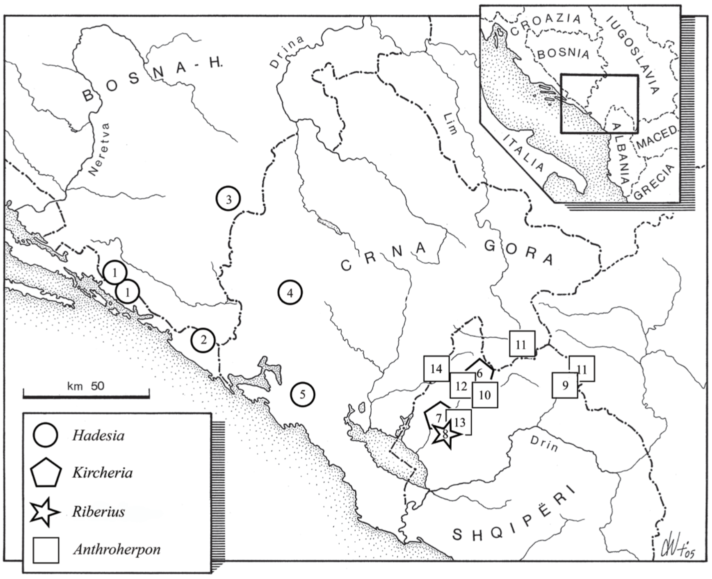


Fig. 30. Distribution map of specialized Leptodirini in Northern Albania. Genera: *Hadesia*, *Kircheria*, *Riberius, Anthroherpon* and *Graciliella*. 1: *Hadesia vasiceki*; 2: *H. azamo*; 3: *H. lakotai*; 4: *H. weiratheri*; 5: *H. zetae*; 6: *Kircheria beroni*; 7: *K. dritae* **n. sp.**; 8: *Riberius* nov. gen. *stillicidii* **n. sp.**; 9: *Anthroherpon latipenne latellai*; 10: *A. taxi trezzii*; 11: *A. taxi albanicum*; 12: *A. garbellii*; 13: *A. shtarensis* **n. sp.**; 14: *Graciliella apfelbecki schwienbacheri.*

Fig. 30. Mapa de distribución de los Leptodirini especializados del norte de Albania. Géneros: *Hadesia*, *Kircheria*, *Riberius, Anthroherpon* y *Graciliella*. 1: *Hadesia vasiceki*; 2: *H. azamo*; 3: *H. lakotai*; 4: *H. weiratheri*; 5: *H. zetae*; 6: *Kircheria beroni*; 7: *K. dritae* **n. sp.**; 8: *Riberius* **n. gen.** *stillicidii* **n. sp.**; 9: *Anthroherpon latipenne latellai*; 10: *A. taxi trezzii*; 11: *A. taxi albanicum*; 12: *A. garbellii*; 13: *A. shtarensis* **n. sp.**; 14: *Graciliella apfelbecki schwienbacheri.*

branches (*Anthroherpon* + *Graciliella* + *Leptomeson*) and *Hadesia*, on a morphological basis would seem to base this clade on the presence / absence of ventral apophysis of the VIII female ventrite. However, due to the presence of a developed apophysis, *Riberius* gen. nov. seems closer to the phyletic lineage of *Anthroherpon* than to *Hadesia*, with which it shares the hygropetricolous lifestyle.

Some preliminary molecular data based on cox1 only (Faille, personal communication) show that: 1. *Kircheria* is close to *Hadesia* and *Anthroherpon*, and 2. *Riberius* is not directly related with the *Kircheria-Hadesia* clade, nor with *Anthroherpon* lineage. More conserved markers (in progress) are required to precise the phylogenetic affinities of *Riberius*.

Analysis of taxonomic data and the distribution map of Fig. 30 seem to confirm these hypotheses, showing a sort of continuity of morphological features and geographical vicariance of the members of these phyletic series (*Anthroherpon*, *Graciliella*, *Leptomeson*, *Hadesia*, *Kircheria*, *Velebitodromus*, *Hygrodromus*, *Nauticiella* and *Riberius* gen. nov.). This continuity, which starting from the Velebit Massif in the North, reaches the Albanian Alps in the South, represents in the absence of further molecular data another support to the phylogenetic relationships discussed so far.

Anthroherpon shtarensis **n. sp.** and *Kircheria dritae* **n. sp.** are, respectively, the south-western adelphotaxa of *A. garbellii* Giachino & Vailati, 2005 and *K. beroni* Giachino & Vailati, 2006, both from Shpella e Pucit cave.

From the faunal and zoogeographic points of view, the two caves (Shpella Shtares and Shpella e Pucit) have a very similar fauna of Coleoptera Leptodirini, in some ways vicariant. We have likely to expect further discoveries in these two caves: another *Anthroherpon* species in the Shpella Shtares (from the Shpella e Pucit we also know *A. taxi trezzii* Giachino & Vailati, 2005) and another *Riberius* species in the Shpella e Pucit, where, for now, this taxon is missing.

ACKNOWLEDGEMENTS

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Copelatus ignacioi, an unusually shaped new species of diving beetle from French Guiana (Coleoptera, Dytiscidae)

Copelatus ignacioi, una nueva especie de ditíscido con forma inusual de la Guayana Francesa (Coleoptera, Dytiscidae)

http://zoobank.org/References/2E4817CC-7C6E-4B57-91D8-0FD3C0A73E4A

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ABSTRACT

Copelatus ignacioi sp. nov. from French Guiana is described and illustrated. It has an unusual compact cylindrical body, small eyes, and short and broad appendages; it is the first *Copelatus* with 15 longitudinal striae on each elytron. The new species is formally assigned to the *Copelatus abonnenci* species group, although its relationships with other *Copelatus* remain doubtful.

Key words: Dytiscidae, Copelatinae, *Copelatus*, taxonomy, new species, French Guiana, Neotropical Region.

RESUMEN

Se describe e ilustra a *Copelatus ignacioi* sp. nov. de Guinea Francesa. Esta especie tiene un cuerpo inusualmente compacto y cilíndrico, ojos pequeños, y apéndices cortos y anchos; es el primer *Copelatus* con 15 estrías longitudinales sobre cada élitro. La nueva especie se asigna formalmente al grupo de especies *Copelatus abonnenci*, aunque su relación con otros *Copelatus* se mantiene en duda.

Palabras clave: Dytiscidae, Copelatinae, *Copelatus*, taxonomía, nueva especie, French Guiana, Región Neotropical.

INTRODUCTION

Copelatus Erichson, 1832 represents the most speciose genus of the family Dytiscidae. To date, 460 species, predominantly from forested areas of the tropics, have been described (NILSSON & HÁJEK, 2022). *Copelatus* species inhabit a large variety of both running and stagnant waters.

Despite the fact that the Neotropical Region hosts a rich *Copelatus* fauna – 118 species were so far recorded from the region, representing a fourth of the world diversity of the genus (cf. NILSSON & HÁJEK, 2022) – our knowledge about the diversity of *Copelatus* in Latin America remains very poor. The only recent taxonomic studies deal with the Caribbean Islands (MEGNA & EPLER, 2012; MANUEL *et al.*, 2018). With the exception of recent descriptions of bromeliadicolous (RESENDE & VANIN, 1991) and troglomorphic (CAETANO *et al.*, 2013) species from Brazil, only the old, often insufficient, descriptions without figures are available for continental South America, and the genus is thus in need of revision. During our work on Copelatinae phylogenetics, we have discovered a very peculiar *Copelatus* from French Guiana, in habitus and elytral striation unlike any known species. Here we present its formal description in honour of Ignacio Ribera who was our dearest friend and mentor, and so much more.

MATERIAL AND METHODS

The specimen was examined using an Olympus SZX12 stereomicroscope. Habitus photographs were taken using a Canon EOS 550D digital camera with an attached Canon MP-E65mm f/2.8 1–5× macro lens as an image stack at different focal planes, afterwards combined using Helicon Focus 6.3.0 software. Images of genital structures were taken with a Canon EOS R camera. We used a Mitutoyo 10× ELWD Plan Apo objective attached to a Carl Zeiss Jena Sonnar 3.5/135 MC as focus lens. Illumination was with three LED segments SN-1 from Stonemaster (https://www.stonemaster-onlineshop.de). Image stacks were generated using the Stackmaster macro rail (Stonemaster), and images were then assembled with the computer software Helicon Focus v. 4.77TM.

The following abbreviations were used in the descriptions: TL, total length, measurement of length from clypeal margin to apex of elytra; TL-h, total length minus head length, measurement of length from anterior margin of pronotum to apex of elytra; TW, maximum width of body measured at right angle to TL. The terminology to denote the orientation of the genitalia follows MILLER & NILSSON (2003).

Exact label data are cited for the type material and given in quotation marks. Authors' additional remarks are provided in square brackets; [p]– preceding data are printed. Separate label lines are indicated by a slash (/), separate labels by a double slash (//). The holotype is deposited in the collection of the Zoologische Staatssammlung München, Munich, Germany (ZSMG) for further morphological investigation and will ultimately be transferred to the Muséum national d'Histoire naturelle, Paris, France (MNHN).

RESULTS

***Copelatus ignacioi* sp. nov.** (Figs. 1–3)

http://zoobank.org/NomenclaturalActs/26535EFF-5DE1-4FE5-94C2-6AC1E98BF63F

Type locality. French Guiana, Arrondissement of Cayenne, Réserve Naturelle Régionale Tresor, trek to a cave close to Pattawa.

Type material. Holotype ♂, labelled: "**French Guyana**: Montagnes / Tresor, trek to Cave close to / Pattawa, xii. 2012, Balke & Kotrba, DNA / M Balke: MB397 [p] // 397 DNA / M Balke [green label, p] // HOLOTYPE ♂ / *COPELATUS* / *ignacioi* sp. nov. / J. Hájek et al. det. 2021 [red label, p]".

Description of holotype. *Habitus* (Fig. 1) cylindrical, sides almost parallel-sided, only slightly attenuated proximally and distally, base of pronotum slightly broader than base of elytra; broadest at 1/3 of elytral length, slightly convex. Dorsal surface submatt.

Coloration. Body brown blackish; sides of pronotum somewhat reddish translucent; clypeus, ventral side and appendages reddish brown; elytra with transverse orange band at base between striae 1–12.

Head. Broad, ca. 0.73× width of pronotum, trapezoidal. Anterior margin of clypeus slightly concave. Antennae with antennomeres broad (antennomeres II–X ca. 1.2× longer than wide), club-shaped. Eyes small (ratio of interocular distance / head width = 0.76). Reticulation consisting of fine, well impressed isodiametric polygonal meshes. Numerous long and shallow strioles present posterolaterally from the eyes. Punctation double; several large setigerous punctures present in fronto-clypeal depressions, in frontal depressions at level of anterior margin of eyes, and in depressions along inner margin of eyes; fine and sparsely distributed punctures placed among meshes of reticulation.

Pronotum. Transverse, broadest before posterior angles. Anterior angles acute, posterior angles obtuse. Sides moderately and evenly curved, in posterior quarter subparallel; lateral beading distinct except for anterior corners. Anterior margin straight, posterior margin nearly straight with only indistinct sinuation medially. Reticulation almost imperceptible, consisting of superficially impressed, longitudinally elongate polygonal meshes. Surface of pronotum densely covered with long shallow strioles; centre of disc with medial longitudinal smooth line. Punctation double; row of coarse setigerous punctures present along anterior margin, laterally close to sides, and in basolateral depressions along basal margin; fine punctures distributed irregularly on surface of pronotum.

Elytra. Elytral striation consisting of 15 superficially impressed dorsal striae, submarginal stria absent; all striae complete, although odd striae abbreviated apically. Whole surface of elytra covered with fine, shallow,

Figs. 1-3. *Copelatus ignacioi* **sp. nov.** Fig. 1. Dorsal habitus; Fig. 2. Median lobe of aedeagus in lateral (a) and ventro-apical (b) view; 3. Paramere. Scale bar = 0.5 mm (Figs. 2–3).

Figs. 1-3. *Copelatus ignacioi* **sp. nov.** Fig. 1. Vista dorsal; Fig. 2. Lóbulo medio del edeago en vista lateral (a) y ventro-apical (b); 3. Parámero. Escala = 0.5 mm (Figs. 2–3).

long striolae. Surface reticulation consisting of very fine, superficially impressed polygonal meshes; meshes longitudinally elongate, hardly perceptible in basal half, but isodiametric or transverse and more apparent in apical half. Punctation double; large setigerous punctures present along lateral margin of elytra; badly perceptible, very fine punctures sparsely distributed among meshes of reticulation.

Legs. Protibia modified, slightly angled near base, distinctly broadened distally, club-shaped. Pro- and mesotarsomeres 1–3 distinctly broadened, ventrally with adhesive setae. Natatorial setae well developed.

Ventral side. Prosternum sinuate anteriorly, obtusely keeled medially. Prosternal process shortly lanceolate, in cross-section convex, apex obtuse; process distinctly bordered; reticulation hardly perceptible, consisting of shallowly impressed polygonal meshes; punctation consisting of setigerous punctures along margin. Metaventrite with microsculpture consisting of polygonal meshes, meshes distinctly transverse laterally; lateral parts of metaventrite (“metasternal wings”) tongue-shaped, slender. Metacoxal lines well impressed, abbreviated (absent in basal fourth). Metacoxal plates covered with long longitudinal strioles; reticulation consisting of extremely elongated, longitudinal polygonal meshes. Metacoxal processes rounded and incised at posterior margin. Abdominal ventrites I–II with longitudinal strioles; ventrites III–IV with oblique strioles laterally; ventrite V with transverse strioles. Abdominal reticulation consisting of elongate polygonal meshes, longitudinal on ventrites I–II, oblique on ventrite III and transverse on ventrites IV–VI. Punctation double; coarse setigerous punctures present medially and medio-laterally on ventrites III–VI; fine punctures sparsely distributed on surface of ventrites, clusters of somewhat coarser punctures present along posterior margin on ventrites IV and V and basolaterally on ventrite VI.

Male genitalia. Median lobe in lateral aspect “C”-shaped, broad, apex pointed (Fig. 2a); in ventral aspect median lobe attenuated in apical fifth, apex twisted left (Fig. 2b). Parameres broad, “D”-shaped; apical lobe long, club-shaped (Fig. 3).

Female. Unknown.

Measurements. TL: 4.5 mm; Tl-h: 4.0 mm; MW: 2.1 mm.

Differential diagnosis. With the presence of 15 elytral striae, the new species is unique within the genus *Copelatus*. It can be formally assigned to the *C. abonnenci* species group established by GUIGNOT (1939) for species with 14–23 elytral striae, and so far comprising a single species – *C. abonnenci* Guignot, 1939 – also from French Guyana, which possesses 22 dorsal + a submarginal elytral stria.

From all hitherto known species of *Copelatus*, the new species can also be easily recognised based on its cylindrical habitus, broad antennomeres, small eyes, elytra with transverse basal orange band, pronotum and elytra sparsely covered with fine longitudinal striolae, and characteristic shape of the median lobe of aedeagus.

Etymology. The new species is named in honour of the late Ignacio Ribera (1963–2020), our mentor, colleague and friend; the specific epithet is a noun in the genitive case.

Collecting circumstances. The species was collected from a very small puddle on the forest floor; however, as only one specimen was found, this habitat may be accidental for the species.

Distribution. The species is so far known only from the type locality in northeast French Guyana.

DISCUSSION

The aberrant morphology of *Copelatus ignacioi* sp. nov. might well indicate special habitat requirements of the new species. Based on our molecular phylogenetic analysis (BALKE *et al.*, 2008; and unpublished data), *C. ignacioi* sp. nov. may represent the sister species of a clade of obligatory bromeliadicolous species, and some characters like the compact body and short appendages are indeed shared between the new species and bromeliadicolous *Copelatus*. Therefore, although the single specimen of *C. ignacioi* sp. nov. was found on the forest floor, we suggest other water bodies, e.g. various phytotelmata might be the typical microhabitat of the new species. Molecular phylogenetic work was made possible through DFG project number: 496550039 to M. Balke.

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Two new species of South American water beetles in the genus *Hydraena* Kugelann 1794, subgenus *Dnahydnaedna* Perkins 2017 (Coleoptera, Hydraenidae)

Dos nuevas especies de escarabajos acuáticos de Sudamérica del género *Hydraena* Kugelann 1794, subgénero *Dnahydnaedna* Perkins 2017 (Coleoptera, Hydraenidae)

http://zoobank.org/References/CFB94747-5308-446B-A509-2423DBE76817

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ABSTRACT

Two new species of South American water beetles in the genus *Hydraena*, subgenus *Dnahydnaedna* are described: *H.* (*D.*) *guyanica* **n. sp.** from Guyana, and *H.* (*D.*) *ignacioriberai* **n. sp.** from Suriname. Habitus images and male genitalia illustrations of the holotypes of both species are presented. The subgenus *Dnahydnaedna* is briefly reviewed and dorsal habitus images of the eight currently known species are given. New collection records for *H* (*D.*) *paeminosa* Perkins are also provided.

Key words: South America, water beetles, Hydraenidae, *Dnahydnaedna*, new species

RESUMEN

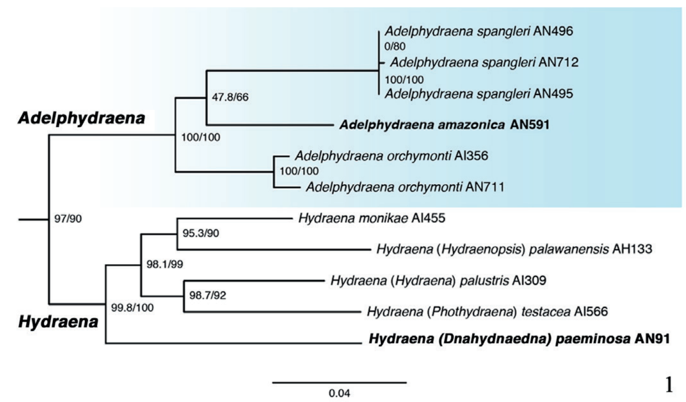
Se describen dos nuevas especies de Sudamérica del género *Hydraena*, subgénero *Dnahydnaedna*: *H.* (*D.*) *guyanica* **n. sp.** de Guyana, y *H.* (*D.*) *ignacioriberai* **n. sp.** de Surinam. Se presentan ilustraciones del habitus y genitalia masculina de los holotipos de ambas especies. El subgénero *Dnahydnaedna* se revisa brevemente, proporcionando imágenes del habitus de los 8 especies conocidas del mismo. Se proporcionan nuevas citas de *H* (*D.*) *paeminosa* Perkins.

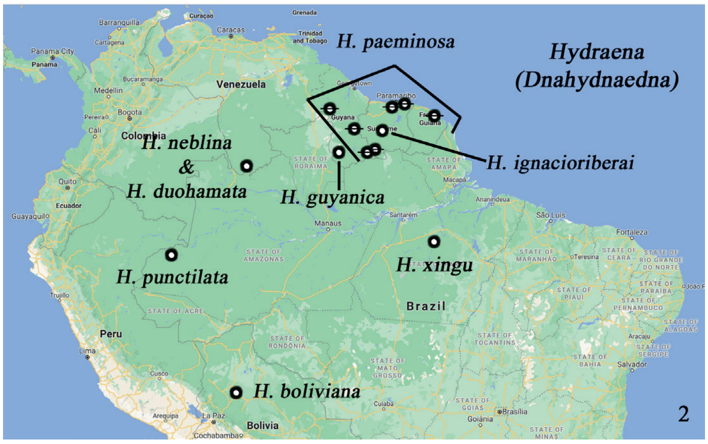
Palabras clave: Sudamérica, escarabajos acuáticos, Hydraenidae, *Dnahydnaedna*, nueva especie

INTRODUCTION

The subgenus *H.* (*Dnahydnaedna*) Perkins 2017 was established on the basis of morphological characters shared by six species from South America, and on the basis of DNA sequence data published by TRIZZINO *et al.* (2013). These data and additional DNA sequence data were presented in the context of *Adelphydraena* Perkins 1989, the sister genus of *Hydraena* (PERKINS & RIBERA, 2020) (Fig. 1). The clade was originally described as the "paeminosa group", based on the single species *H. paeminosa* Perkins (PERKINS, 1980).

Members of *H.* (*Dnahydnaedna*) have the following combination of characteristics: moderately large to large adults (ca. 1.60–1.96 mm), coarsely punctate, elytral punctures random or mostly random, and habitus characteristic, with pronotum rather elongate; ventrally, gena with posterior ridge, and mesoventral intercoxal process tapering to sharp point that is contiguous with a median carina of the metaventrite; male genitalia are usually quite tubular, and have a basic plan similarity; the parameres are characteristic, the left paramere is very small, and located toward the distal end of the main piece, whereas the right paramere has been lost, the paramere setae having their sockets on the main piece (right paramere is very short, lobate, in *H. (D.) ignacioriberai* n. sp.); female gonocoxite «divided,» having a weakly sclerotized midlongitudinal area; spermatheca circular, somewhat disc-shaped. These characters are more completely discussed, and illustrated in PERKINS (2011).





Figs. 1–2. Known geographical distributions of *Hydraena* (*Dnahydnaedna*) species (6). Phylogenetic tree obtained with IQ-TREE, showing *Dnahydnaedna* as sister group of other *Hydraena* subgenera (7). Numbers in nodes, ultrafast bootstrap / SH-like aLRT support. From Perkins & Ribera (2020).

Figs. 1–2. Distribución geográfica conocida de las especies de *Hydraena* (*Dnahydnaedna*) (6). Árbol filogenético realizado en IQ-TREE, mostrando *Dnahydnaedna* como grupo hermano del resto de subgéneros de *Hydraena* (7). Los números en los nodos hacen referencia a los valores de soporte Ultrafast bootstrap / SH-like aLRT. A partir de Perkins & Ribera (2020).

Eight species are presently included in *H.* (*Dnahydnaedna*): *H. boliviana* (Bolivia), *H. duohamata* (Venezuela), *H. guyanica* **n. sp.** (Guyana), *H. neblina* (Venezuela), *H. paeminosa* (Guyana, Suriname), *H. punctilata* (Brazil), *H. ignacioriberai* **n. sp.** (Suriname), and *H. xingu* (Brazil) (Fig. 1; also see figures and text in PERKINS, 2011). The known species distributions are, in general, circum-Amazonian (Fig. 2).

DEPOSITORIES

Specimens are deposited in the following institutions: Center for Biological Diversity, University of Guyana, Georgetown (CBDG); National Zoological Collection of Suriname, Paramaribo (NZCS); Snow Entomological Museum, University of Kansas, Lawrence, Kansas, USA (SEMC); Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA (MCZ); and Museo Nacional de Ciencias Naturales, Madrid, Spain (IBE).

Methods of specimen preparation and imaging follow those of PERKINS (1977).

Hydraena (Dnahydnaedna) guyanica, new species

(Figs. 3–5)

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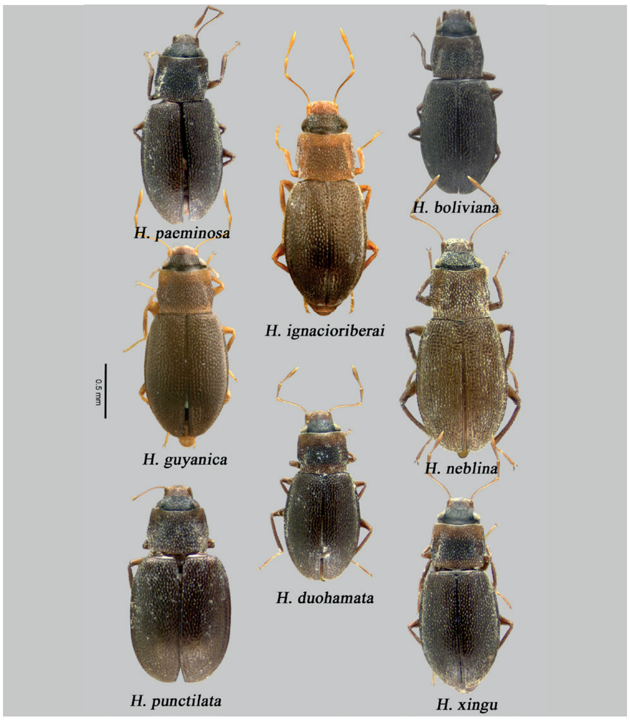

Fig. 3. Dorsal habitus of *Hydraena* (*Dnahydnaedna*) species.

Fig. 3. Vista dorsal del hábitus de las especies de *Hydraena* (*Dnahydnaedna*).

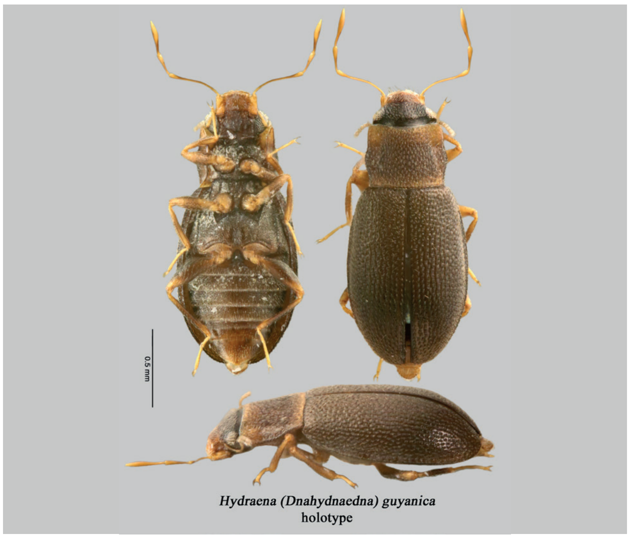

Fig. 4. Dorsal, ventral and lateral habitus of *Hydraena* (*Dnahydnaedna*) *guyanica*, holotype.

Fig. 4. Vistas dorsal, ventral y lateral del hábitus de *Hydraena* (*Dnahydnaedna*) *guyanica*, holotipo.

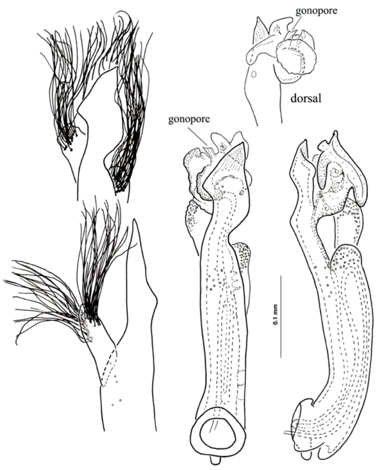

Fig. 5. Male genitalia of *Hydraena* (*Dnahydnaedna*) *guyanica*, holotype (left side: parameres and setae detail in ventral view (above); right paramere and setae detail in lateral view (below).

Fig. 5. Genitalia masculina de *Hydraena* (*Dnahydnaedna*) *guyanica*, holotipo (izquierda: parámeros y detalle de sus sedas en vista ventral (arriba); parámero derecho y detalle de sus sedas en vista lateral (abajo).

Type material. Holotype (male): "Guyana: Region 9, N. Parabara, creek by basecamp (Bototo wau creek), 289 m, 31 Oct 2013, 2.181800 (2° 10› 54.4794») N; -59.338432 (-59° 20› 18.3546»)W, stream margins, leg. Short, Isaacs, & Salisbury, (GY13-1031-01A)". [IBE voucher #AN51]. Holotype deposited in CBDG, four paratypes deposited in SEMC, one in MCZ, one in IBE.

Differential Diagnosis. Similar in dorsal habitus to *H. duohamata* (Figs. 3, 4), including the presence of serial discal elytral punctures; differentiated therefrom by the larger size (ca. 1.74 mm vs. 1.58 mm). Reliable determinations will require careful examination of the male genitalia (Fig. 5).

Description. Habitus as illustrated (Fig. 4). Size: holotype (length/width, mm): body (length to elytral apices) 1.74/0.76; head width 0.39; pronotum 0.39/0.53, pronotum anterior margin 0.43, pronotum basal margin 0.47; elytra 1.12/0.76. Dorsum of head dark brown to piceous; pronotum light brown to testaceous in front of and behind diffusely margined dark brown fascia, ratios of color bands, as measured in midline, ca. 3/15/2; elytra dark brown; legs testaceous; palpi testaceous, tip not darker. Dorsum of head microreticulate, dull. Frons punctures shallow, faint, ca. 1xef. Clypeus microreticulate, punctures obsolete. Mentum effacedly microreticulate, very sparsely very finely punctulate, dull; postmentum very finely densely micropunctulate and dull in median area, anterior margin smooth, shining. Genae raised in ridge posteriorly, except median 1/4 lacking ridge. Pronotum and elytra coarsely densely punctate, subscabrous, interstices microreticulate, more markedly on pronotum than elytra. Pronotum with median 1/3 of anterior margin emarginate; scintilla absent; discal foveae absent; lateral foveae deep, confluent, separated from lateral margin by low irregular ridge. Elytra with summit of posterior declivity slightly past midlength; lateral explanate margins narrow; punctures serial to subserial on disc, otherwise random. Apices in dorsal aspect weakly separately rounded, in posterior aspect margins forming shallow angle with one another. Prosternal intercoxal process laminate; median carina sinuate in profile. Mesosternal intercoxal process tapering to sharp point, tip contiguous with low median carina of metaventrite. Plaques moderately wide, separation ca. equal length, parallel, weakly raised, located at sides of shallow median depression. Metaventrite with median carina in anterior 1/2, also with very short point on each side, extended posteriorly from margin of each mesocoxal cavity. AIS width at straight posterior margin ca. equal plaque separation. Tibiae slender, straight. Male abdominal apex symmetrical; last tergite with apicomedian notch.

Etymology. Named in reference to the geographical distribution.

***Hydraena (Dnahydnaedna) ignacioriberai*, new species**

(Figs. 3, 6, 7)

http://zoobank.org/NomenclaturalActs/61F9EDA4-78B2-4DA8-8795-08A9E2CB801A

Type material. Holotype (male): "Suriname: Sipaliwini District, Central Suriname Nature Reserve: Tafelberg Summit, near Caiman Creek Camp, 733 m, 18.viii.2013, 3.899033 (3° 53› 56.5188»)N; -56.180817 (-56° 10› 50.9406») W, small stream with lots of plants and leaf litter, leg. Short & Bloom (SR13-0818-03A)". [IBE voucher #AN90]. Holotype deposited in NZCS, one paratype deposited in SEMC, one in IBE.

Differential Diagnosis. Similar in dorsal habitus to *H. paeminosa* (Figs. 1, 4); differentiated therefrom by the larger size (ca. 1.88 mm vs. 1.70 mm), and the dull, markedly microreticulate pronotum, contrasting with the shiny elytral interstices. Reliable determinations will include careful examination of the male genitalia (Fig. 7).

Fig. 6. Dorsal, ventral and lateral habitus of *Hydraena* (*Dnahydnaedna*) *ignacioriberai,* holotype.

Fig. 6. Vistas dorsal, ventral y lateral del hábitus de *Hydraena* (*Dnahydnaedna*) *ignacioriberai*, holotipo.

Description. Habitus as illustrated (Fig. 6). Size: holotype (length/width, mm): body (length to elytral apices) 1.88/0.77; head width 0.44; pronotum 0.42/0.55, pronotum anterior margin 0.47, pronotum basal margin 0.53; elytra 1.16/0.77. Dorsum of head with frons dark brown to piceous, clypeus light brown to testaceous; pronotum light brown to testaceous in front of and behind diffusely margined dark brown fascia, ratios of color bands, as measured in midline, ca. 5/12/5; elytra dark brown; legs brown; palpi testaceous, tip not darker. Dorsum of head microreticulate, dull. Frons punctures shallow, faint, ca. 1xef. Clypeus microreticulate, punctures obsolete. Mentum effacedly microreticulate, very sparsely very finely punctulate, dull; postmentum very finely densely micropunctulate and dull in median area, anterior margin smooth, shining. Genae raised in ridge posteriorly, except median 1/4 lacking ridge. Pronotum and elytra coarsely densely punctate, subscabrous; interstices on pronotum strongly microreticulate, dull; interstices on

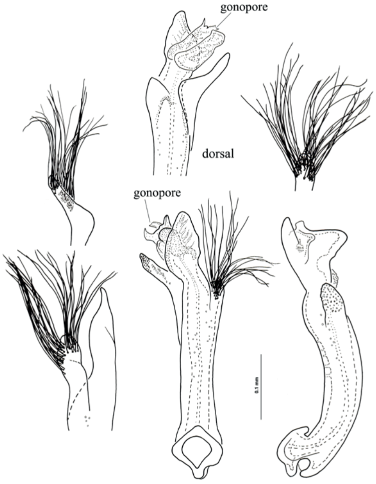

Fig. 7. Male genitalia of *Hydraena* (*Dnahydnaedna*) *ignacioriberai*, holotype (left side: left paramere in ventral view (above); right paramere and setae detail in lateral view (below).

Fig. 7. Genitalia masculina de *Hydraena* (*Dnahydnaedna*) *ignacioriberai*, holotipo (izquierda: parámero izquierdo en vista ventral (arriba); parámero derecho y detalle de sus sedas en vista lateral (abajo).

elytra non-microreticulate, shining. Pronotum with median 1/3 of anterior margin emarginate; scintilla absent; discal foveae absent; lateral foveae deep, confluent, separated from lateral margin by low irregular ridge. Elytra with summit of posterior declivity slightly past midlength; lateral explanate margins narrow; punctures random. Apices in dorsal aspect weakly separately rounded, in posterior aspect margins forming shallow angle with one another. Prosternal intercoxal process laminate; median carina sinuate in profile. Mesosternal intercoxal process tapering to sharp point, tip contiguous with low median carina of metaventrite. Plaques moderately wide, separation ca. equal length, slightly convergent anteriorly, weakly raised, located at sides of shallow median depression. Metaventrite with median carina in anterior 1/2, also with very short point on each side, extended posteriorly from margin of each mesocoxal cavity. AIS width at straight posterior margin ca. equal plaque separation. Tibiae slender, straight. Male abdominal apex symmetrical; last tergite with apicomedian notch.

Etymology. Named in honor of Ignacio Ribera, in recognition of his many contributions to the knowledge of water beetle taxonomy, biogeography, ecology, and phylogeny.

New collection records for *H. (D.) paeminosa* Perkins

French Guiana: 30 km S Cayenne, crossroad N1/D5, xii.2002 - i.2003, M. Balke (2 MCZ). **Guyana**: Region 13, Ayanganna Airstrip, trail from Blackwater Creek Camp to Potaro River, 5.297050 N, 59.833332 W, (5°17›49.3794››N, 59°49›59››), elev. 684 m, 19.iii.2014, leg. A. Short (GY14-0319-01A) (29 SEMC); Region 6, Upper Berbice, Basecamp 1, ca. 1 km S. of camp along mammal transect, 4° 9› 14.4606»N, 58° 10› 37.617»W, elev. 109 m, 25.ix.2014, leg. Short, Salisbury, La Cruz (GY14-0925-01D) (5 SEMC); Upper Berbice, Basecamp 1, creek next to basecamp (upstream), 4.154817 N, 58.178616 W, (4°9›17››N, 58°10›43››W), elev. 96 m, 24.ix.2014, leg. Short, Salisbury, La Cruz (GY14-0924-01A) (16 SEMC); Upper Berbice, Basecamp 1, seasonal channel of creek next to basecamp, 4.154817 N, 58.178616 W, elev. 96 m (4°9›17.3412››N, 58°10›43.017››W), 21.ix.2014, leg. Short, Salisbury, La Cruz (GY14-0921-02A) (14 SEMC).

ACKNOWLEDGEMENTS

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TRIZZINO, M., M.A. JÄCH , P. AUDISIO, R. ALONSO & I. RIBERA, 2013. A molecular phylogeny of the cosmopolitan hyperdiverse genus *Hydraena* Kugelann (Coleoptera, Hydraenidae). *Systematic Entomology*, 38: 192–208. https://doi.org/10.1111/j.1365-3113.2012.00654.x

The Mediterranean *Canthydrus* Sharp and taxonomic notes on *C. arabicus* Sharp, 1882 (Coleoptera, Noteridae)

Los *Canthydrus* Sharp del Mediterráneo y notas taxonómicas sobre *C. arabicus* Sharp, 1882 (Coleoptera, Noteridae)

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ABSTRACT

Two species of *Canthydrus* Sharp (Coleoptera, Noteridae) are recognized to occur in the Mediterranean basin: the eastern *C. diophthalmus* (Reiche & Saulcy, 1855) from Egypt to Middle Orient, reaching Uzbekistan, and the western Mediterranean *C. siculus* (Ragusa, 1882) **stat. nov**. previously treated as a junior synonym of *C. diophthalmus*, occurring in Egypt, Algeria, Morocco, Italy (Sicily and Sardinia), Spain (Andalusia and Baleares) and most likely Libya and Tunisia. *Canthydrus diophthalmus* is formally recorded also for some localities in sub-Saharan Africa, and specimens observed from Sudan confirm the occurrence of this species in the Afrotropical region, but it cannot be affirmed whether also *C. siculus* occurs in tropical Africa. The two species are very similar, but can be easily separated by aedeagal features and, often, by the extension of the yellow lateral markings of the pronotum. A third species, the strictly Afrotropical *C. koppi* Wehncke, 1883, is very closely related and together these species form the *C. diophthalmus* species complex. The three species are described and illustrations of habitus and male genitalia are given. *Canthydrus ornatus* Sharp, 1882 is no longer treated as a junior subjective synonym of *C. luctuosus* (Aubé), but as a junior subjective synonym of *C. diophthalmus* (Reiche & Saulcy) (**new synonymy**). First records are presented for *C. diophthalmus* (Sudan, Uzbekistan) and *C. koppi* (Benin, Burkina Faso). *Canthydrus arabicus* Sharp, 1882 is redescribed on the basis of type material and its habitus and male genitalia are illustrated for the first time. The species is considered closely related to *C. notula* (Erichson, 1843) the male genitalia of which are also illustrated for comparison.

Key words: burrowing water beetles, *Canthydrus*, Mediterranean basin, tropical Africa, Arabia, taxonomy, first records.

RESUMEN

En la cuenca Mediterránea se reconocen dos especies de *Canthydrus* Sharp (Coleoptera, Noteridae): la especie oriental *C. diophthalmus* (Reiche & Saulcy, 1855) que se localiza desde Egipto hasta Oriente Medio, alcanzando Uzbekistán, y la especie occidental *C. siculus* (Ragusa, 1882) **stat. nov**. previamente considerada como sinónimo junior de *C. diophthalmus*, que se distribuye en Egipto, Argelia, Marruecos, Italia (Sicilia y Cerdeña), España (Andalucía y Baleares), y probablemente en Libia y Túnez. *Canthydrus diophthalmus* ha sido localizada en algunas localidades subsaharianas, así como especímenes de Sudán confirma la presencia de esta especie en la región Afrotropical; sin embargo, no se puede afirmar la presencia de *C. siculus* en esta región. Las dos especies presentan morfologías muy similares, pero son fácilmente reconocibles por sus edeagos y, frecuentemente, por la extensión de las marcas amarillas en los laterales del pronoto. Una tercera especie, *C. koppi* Wehncke, 1883, estrictamente Afrotropical, es muy similar y las 3 especies juntas formar el complejo de especies *C. diophthalmus*. Se describen las tres especies, y se proporcionan ilustraciones de sus habitus y genitalias masculinas. *Canthydrus ornatus* Sharp, 1882 ya no es considerado como sinónimo subjetivo junior de *C. luctuosus* (Aubé), sino como sinónimo subjetivo junior de *C. diophthalmus* (Reiche & Saulcy) (**nuevo sinónimo**). Se presentan los primeros registros de *C. diophthalmus* en Sudán y Uzbekistán, y de *C. koppi* en Benín y Burkina Faso. Se redescribe *Canthydrus arabicus* Sharp, 1882 en base al material tipo y a la morfología de su habitus y genitalia masculina, que se ilustran por primera vez. La especie se considera estrechamente relacionada con *C. notula* (Erichson, 1843), cuya genitalia masculina también se ilustra para que sirva de comparación.

Palabras clave: escarabajos acuáticos excavadores, *Canthydrus*, Cuenca Mediterránea, África tropical, Arabia, taxonomía, primeras citas.

INTRODUCTION

The large noterid genus *Canthydrus* Sharp, 1882 contains about 65 species (NILSSON, 2011) occurring in the Afrotropical (GUIGNOT, 1959), Oriental, East Palearctic (TOLEDO, 2008; TOLEDO, *in prep.*) and in the Australian Region (TOLEDO, 2010). Only a very few species are known from the western Palearctic region: (1) *C. diophthalmus* (Reiche & Saulcy, 1855) is so far known to be the only Mediterranean representative of the genus, occurring in the Middle East, North Africa and southern Europe; this species is recorded also from central Africa (Angola, Cameroon, Ethiopia, Senegal, Zaire) (NILSSON, 2011); (2) *C. arabicus* Sharp, 1882 was described from Arabia; (3) *C. frontalis* Sharp, 1882 was also described from Arabia; at present the taxon is treated as a junior synonym of the Oriental *C. luctuosus* (Aubé, 1838) and, accordingly, the distribution of the latter should be assumed to extend into Arabia (Palearctic!); (4) the Oriental and East Palearctic *C. laetabilis* (Walker,1858) which reaches the southeastern provinces of Iran (FERY *et al.*, 2012; VONDEL *et al.*, 2017).

The nomenclatural history of *C. diophthalmus* is of special interest and rather complex. Since SCHAUM (1857), the name was treated for a long time as a synonym of the widespread African *Canthydrus notula* (Erichson, 1843) (e.g. SHARP, 1882; RÉGIMBART, 1895a; GUIGNOT, 1959). OMER-COOPER (1965) first suggested *C. diophthalmus* as valid species occurring in the Mediterranean region, distinct from *C. notula* which occurs only in Africa south of the Sahara. Her classification was adopted by later authors up to the present day. Other species-names, associated with these two taxa are *Hydrocanthus badenii* Wehncke, 1876, and *Canthydrus biguttatus* Régimbart, 1895a. The former was described from Angola and later synonymized by BRANDEN (1885) with *H. notula* (*sensu* SCHAUM, 1857 = *C. diophthalmus* + *notula*). RÉGIMBART (1895a), accepting Schaum's synonymy of *C. diophthalmus* with *C. notula*, described *Canthydrus biguttatus* from Western Africa (Senegal) to separate the smaller species from tropical Africa (*C. biguttatus*) from the larger *C. notula* from northern Africa. RÉGIMBART (1895b) described *C. biguttatus* a second time from Eastern Africa (Somalia). This has remained unchanged until OMER-COOPER (1965) new classification, when *C. biguttatus* became a junior subjective synonym of *C. notula*. NILSSON (2005) found that Régimbart described the same species twice and, accordingly, the second name become a junior primary homonym and a junior subjective synonym of the first. In the same work, NILSSON (2005) treated both names as synonyms of *C. notula*. Nevertheless, the name *C. biguttatus* survived in some later works (e.g. BILARDO, 1976; BILARDO & PEDERZANI, 1978).

Hydrocanthus notula var. *siculus* Ragusa, 1882 was discovered in Sicily and described as a variety with strongly extended yellow lateral margins of pronotum. The name *Canthydrus notula siculus* var. *numidicus* Bedel, 1925 was given to similar specimens from Algeria which have an additional humeral spot on each elytron. MONTES & SOLER (1985) were the first who treated the two taxa as junior subjective synonyms of *C. diophthalmus* (see also NILSSON, 2005, 2011).

Finally, SHARP (1882) described *Canthydrus ornatus* from Persia (Iran) which RÉGIMBART (1899) considered a variety of *C. luctuosus* (Aubé, 1838). Thus *C. ornatus* was subsumed under a species (*C. luctuosus*) with a primarily oriental distribution (ZIMMERMANN, 1927; VAZIRANI, 1968); this arrangement is still current (NILSSON, 2011).

GUIGNOT (1959: 499, fig. 422) published the first illustrations of the median lobe of aedeagus of *Canthydrus diophthalmus* (under the name *notula*) and FRANCISCOLO (1979: 497, fig. 1463) took Guignot's fig 422 to illustrate the aedeagus of *C. diophthalmus* in Fauna d'Italia vol. XIV. A second, more precise, illustration of the median lobe of *C. diophthalmus* were given in MONTES & SOLER (1985: 188, fig 1b) in which the authors compared the former with that of *C. notula* (fig. 1g). Considerable differences in the figures in GUIGNOT and in MONTES & SOLER caused me to study material from almost the whole Palearctic range of what was currently understood to be *C. diophthalmus*.

At the light of these observations it is clear that not one but two different, though very close, species of *Canthydrus* populate the coastal regions of the Mediterranean. These two species are here redescribed and illustrated, together with a third one, very closely related but strictly Afrotropical, and the *C. diophthalmus* species-complex is here defined to include these three taxa.

Furthermore, a diagnosis based on the type material of the very poorly known *Canthydrus arabicus* Sharp is given here. Presumably, it is the third truly western Palearctic species of the genus, never collected again after its description.

MATERIAL AND METHODS

The material on which this study is based includes more than 210 specimens standing in different collections. These were studied with an Alstar XTL-3400F stereomicroscope with a millimetre slide mounted in one of the oculars for measurements. Dried specimens were rehydrated in clear water before dissection and then remounted on their original cards together with genitalia. The latter was studied in wet condition (glycerol) and photographed with a Canon EOS 1200D camera attached to the stereomicroscope. Ink drawings of genitalia were made tracing the photos with transparent paper from the computer screen, then scanned and retouched with Adobe Photoshop CS6 software.

Photos of the habitus were made with a Canon EOS 40D with macro-objective Canon MP-E 65 and ring LED illumination. For each specimen, several photographs were taken at different levels, then combined with Zerene Stacker software program.

Maps were made by using Google Earth© and retouched with Adobe Photoshop CS6 software. The terminology to denote the orientation of the male genitalia follows MILLER & NILSSON (2003). The abbreviations TL and MW in the descriptions indicate, respectively, total length and maximum width. Historical material with incomplete and/or imprecise data is quoted between quotation marks ("").

Acronyms of collections used in the text:

DBSU	Department of Biology, Shiraz University, Shiraz, Iran.
FLS	Collection Francesco Livretti, S. Antioco (Cagliari), Italy.
GFA	Collection Garth Foster, Ayr, Scotland (UK).
HFB	Collection Hans Fery, Berlin, Germany.
IRB	Collection Ignacio Ribera, Spain (now in MNCN).
MBAC	Museo del Dipartimento di Biologia Animale dell'Università, Catania, Italy.
MNB	Museum für Naturkunde, Berlin, Germany.
MNCN	Museo Nacional de Ciencias Naturales, Madrid, Spain
MNHNP	Museum National d'Histoire Naturelle, Paris, France
MSNG	Museo di Storia Naturale "Giacomo Doria", Genova, Italy.
MTB	Collection Mario Toledo, Brescia, Italy.
NHML	The Natural History Museum, London, England (UK).
NMW	Naturhistorisches Museum Wien, Vienna, Austria.

RESULTS

The *Canthydrus diophthalmus* species complex

This complex is composed of three species: (1) *Canthydrus diophthalmus* (Reiche & Saulcy, 1855), (Figs. 1-2); (2) *C. siculus* (Ragusa, 1882) **stat. nov.** (Fig. 3) and *C. koppi* Wehncke, 1883 (Fig. 4). These are rather large, not very convex *Canthydrus*, with a total length of 3.0–3.7 mm. In lateral view the margin of the pronotum is weakly convex and margin of the elytra weakly concave. The elytra are black with a submedian yellow spot and, often, a second lateral subbasal marking. The abdominal segment IX of males is rather sclerotised, with thick ring-like lateral walls and dense apical comb of short and thick setae (Fig. 5a). The aedeagus of these species is large and robust, very characteristic in all its parts: the median lobe is flat, strongly sinuate on both sides with apex bent upward (Figs. 6-8); the left paramere is elongate, bean-shaped, with apical fringe of short setae (Figs. 6c, 8c), the right paramere is broadly triangular (Figs. 6d, 8d).

Figs. 1-4. Habitus **a**) dorsal and **b**) lateral aspect: **1**) *Canthydrus diophthalmus* (ex. from Israel); **2**) *C. diophthalmus* (lectotype of *C. ornatus*); **3**) *C. siculus* (ex. from Sardinia); **4**) *C. koppi* (ex. from Burkina Faso).

Figs. 1-4. Vista **a**) dorsal y **b**) lateral del habitus de: **1**) *Canthydrus diophthalmus* (ejemplar de Israel); **2**) *C. diophthalmus* (lectotipo de *C. ornatus*); **3**) *C. siculus* (ejemplar de Cerdeña); **4**) *C. koppi* (ejemplar de Burkina Faso).

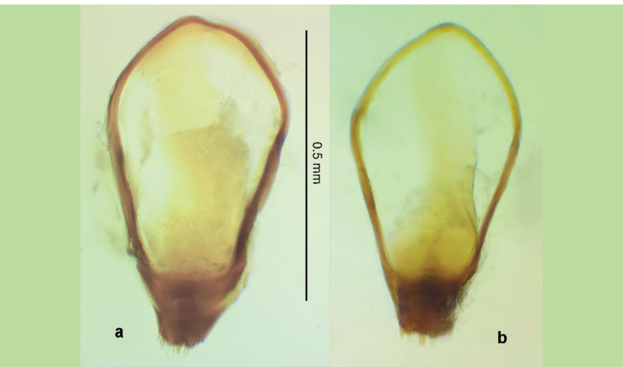


Fig. 5. Ninth abdominal segment of: **a**) *Canthydrus siculus* **b**) *C. notula*.

Fig. 5. Noveno segmento abdominal de: **a**) *Canthydrus siculus* **b**) *C. notula*.

Canthydrus arabicus Sharp, 1882 (Fig. 9) is close to *Canthydrus notula* (Erichson, 1843) for male genitals features and habitus. They are both similar in body shape and colouration to the members of the *diophthalmus* complex (exception made for the paler colour of *C. arabicus*, but patterns on dorsal surface are similar). *C. notula* (this character was not studied for *C. arabicus*) has less sclerotised male abdominal segment IX, with thinner lateral walls (Fig. 5b) and, together with *C. arabicus*, shares a smaller and remarkably different aedeagus: the median lobe is less robust and with a different structure (Figs. 10, 11) and the left paramere is not bean-shaped but subtriangular (Fig. 10b, 11b) as in most of the species of *Canthydrus*. These characters distinguish *C. notula* and *C. arabicus* from this complex.

Canthydrus diophthalmus and *C. siculus* have both small morphological variability in the median lobe of the aedeagus (Figs. 12-13) likely due to their broad distributions in the southern Western Palearctic Region, and, at least for *C. diopthalmus*, in tropical Africa (Figs. 14-15), *C. koppi* has a western Afrotropical distribution (Fig. 15). *Canthydrus notula* is widespread likely in the whole Afrotropical region, reaching the southern Arabian Peninsula, whereas *C. arabicus* is known only for its type locality in central Saudi Arabia (Fig. 14).

Canthydrus diophthalmus (Reiche & Saulcy, 1855)

(Figs. 1, 2, 6, 12)

Hydrocanthus diophthalmus Reiche & Saulcy, 1855: 641 (orig. descr.).

Canthydrus diophthalmus (Reiche & Saulcy, 1855): J. OMER-COOPER, 1965: 71 (as valid species, distinct to *notula* ERICHSON); MONTES & SOLER, 1985:191 (partim; Angola, Cameroon, Egypt, Ethiopia, Libya, Morocco, Senegal, Sicily, Spain, Tunisia, Zaire); BILTON, 1992:117 (Israel); SALEH AHMED & ANGUS, 2000: 167 (Egypt); ZALAT *et al.*, 2000:13 (?partim; Egypt); BARCLAY *et al.*, 2001: 27 (Turkey); NILSSON, 2003:33 (cat., partim); NILSSON, 2011: 11 (cat., partim); HÁJEK, 2005: 19 (Turkey); SALAH & RÉGIL, 2015: 190 (?partim; Egypt); VONDEL *et al.*, 2017: 237 (Iran); HÁJEK, 2017: 844 (cat., partim).

Canthydrus (s. str.) *diophthalmus* (Reiche & Saulcy, 1855): NILSSON, 2005: 98 (cat., partim).

Hydrocanthus notula Erichson, 1843 (misident.): SCHAUM, 1857: LXXX (?partim; Egypt); RÉGIMBART, 1878: 349 (?partim; Egypt);

Canthydrus notula (Erichson, 1843) (misident.): SHARP, 1882: 275 (?partim; Egypt, Morocco); MARSEUL, 1882:40 (?partim; Egypt); BRANDEN, 1885:17 (?partim; Egypt, Morocco); BEDEL, 1895: 374 (partim; Angola, Egypt, Morocco, Syria); RÉGIMBART, 1895a:123 (?partim; Egypt, Lybia, Morocco); GUIGNOT, 1959:498 (partim; Ethiopia, Zaire); ALFIERI, 1976:31 (?partim; Egypt).

Canthydrus sotula (Erichson, 1843) (misident., misspell.): ALI, 1978:11 (Iraq).

Hydrocanthus badenii Wehncke, 1876:223 (orig. descr.): BRANDEN, 1885: 17 (syn.).

Canthydrus notula var. *badenii* (Wehncke, 1876): BEDEL, 1895: 374.

Canthydrus ornatus Sharp 1882: 276 (orig. descr.); MARSEUL, 1882: 40 (Iran); BRANDEN. 1885: 17 (Iran); RÉGIMBART. 1893: 363 (Syria); RÉGIMBART, 1899: 249 (doubtfully var. of *luctuosus* Aubé, 1838; Iran, Iraq); ZIMMERMANN, 1927: 9 (Iran, Iraq, Sumatra); TOLEDO, 2008: 65 (lectotype des.). **(new synonymy)**

Canthydrus luctuosus ornatus Sharp, 1882: ALI, 1978: 11 (Iraq).

Canthydrus. sp. probably *notula* (Erichson, 1843): HOSSEINIE, 1974: 241–242 (Iran).

Type localities. *H. diophthalmus*: Lebanon, Beirut; *H. badenii*: Angola; *C. ornatus*: Persia [Iran].

Type material. *H. diophthalmus*: syntypes (MNHNP) not studied; *H. badenii*: syntypes (MNHNP) not studied; *C. ornatus*: Lectotype (NHML) designated by TOLEDO, 2008: 65.

Material examined. EGYPT: Wadi Natron [Wadi El Natrun], Kafr Daoud, Sattm. (?) (2 exs NMW); Baharyia Oasis, nr. Bahiti, 4.IV.1989 (1 ex. NMW); Aswan, Luxor, 25.VI.1993, leg. R Salah Ahmed (4 exs MTB); Alexandria, Cairo, 22.VII.1992, leg. R Salah Ahmed (1 ex. MTB).

IRAN: Khuzestan, rd. Shushtar to Defzul, 7 km NW Shusthar, marshland 100 m a.s.l., 29.III.1995 (#1849) Elmi leg. (1 ex. DBSU); Khuzestan, rd. Andimeshk to Dehloran 16 km W Andimeshk, pond 100 m a.s.l., 30.III.1995 (#1495) Elmi leg. (26 exs DBSU); Khuzestan, rd. Ramshir to Bandar–e Mahshahr, 3 km S Ramshir, pond 40 m a.s.l., 30.III.1995 (#1523) Elmi leg. (3 exs DBSU); Khuzestan, rd. Andimeshk to Dehloran, Kharkheh riv., 25 km N Andimeshk, marshland 40 m a.s.l., I.1995 (#1436) Elmi leg. (3 exs DBSU); Khuzestan, rd. Andimeshk to Shush, 13 km S Andimeshk, marshland 120 m a.s.l. I.1975 (# 1435) Elmi leg. (1 ex. DBSU); Khuzestan, rd. Ramhormoz to Ramshir, 76 km SE Awhaz, 3.IV.1995 (#1522) Elmi leg. (1 ex. DBSU); Khuzestan, Kharkheh riv., 8 km W Andimeshk, 31.I.2001 (# 2400) Elmi leg. (2 exs DBSU); Khuzestan, rd. Shush to Ahwaz, Shanmarj, 14 km S Shush, 31.I.2001 (# 2401) Elmi leg. (1 ex. DBSU); Khuzestan, rd. Shush to Ahwaz, 49 km S Shush, 31.I.2001 (# 2403) Elmi leg. (1 ex. DBSU); Fars, Kazerun, Parishan lake, 128 km SW Kazerun, lake marsh, 21.X.1975 (# 294) Elmi leg. (6 exs DBSU);

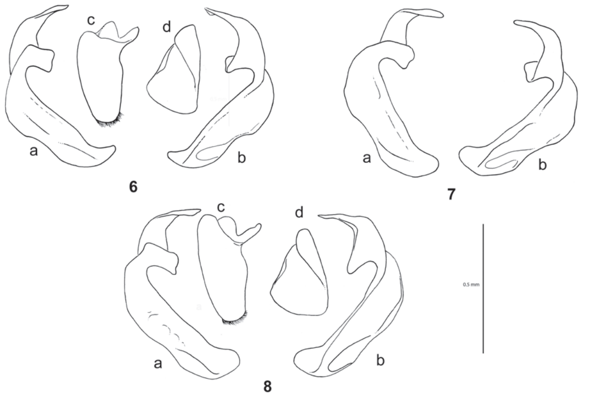
IRAQ: “Mesopotamia Exp. Nat. O. V. 1010“ “Iurna 9 sept, 1910” (1 ex. NMW).

ISRAEL: Hula Cloll. 33, 1.XII.1970 [hw Brancucci] Canthydrus notula Er., det. M. Brancucci 1985 [hw Brancucci] (2 exs HFB); Hula, 21.III.1985, leg. M. Jäch (3 exs NMW); Bteha, NW Huqoq, 7.IV.1986, leg. M. Jäch (3 exs NMW); Taninim Mdg, pond, 22.IV.1986, leg. M. Jäch (2 exs NMW).

SUDAN: Wad Medani a. Blanc Nile, at light, 18, 20, 22.X.1979, leg. Hieke (3 exs MNB).

TURKEY: Sourrondings of Namrun, Kilik. Taurus, 28.VIII.1981, leg. M. Jäch (T15) (1 ex. NMW); Adana Prov., Tuzla, SW of Adana, 1.VIII.1990, leg. Schödl (18) (2 exs. NMW).

UZBEKISTAN: “Samarkand, Reitter” (1 ex. NMW); “Turkestan, Reitter” (1 ex. NMW).



Figs. 6-8. Aedeagus: **a**) median lobe, left side, **b**) median lobe, right side, **c**) left paramere, **d**) right paramere: **6**) *Canthydrus diophthalmus* (ex. from Iraq); **7**) *C. siculus* (ex. from Sicily); **8**) *C. koppi* (ex. from Burkina Faso).

Figs. 6-8. Edeago: **a**) lóbulo medio, vista lateral izquierda, **b**) lóbulo medio, vista lateral derecha, **c**) parámero izquierdo, **d**) parámero derecho: **6**) *Canthydrus diophthalmus* (ejemplar de Iraq); **7**) *C. siculus* (ejemplar de Sicilia); **8**) *C. koppi* (ejemplar de Burkina Faso).

Description. This species was redescribed by several authors, only the most relevant characters are discussed here.

Habitus (Figs. 1–2). Body elongate oval, rather convex, maximum width at shoulders or just behind; in lateral view sides of pronotum weakly rounded and sides of elytra weakly concave to almost straight at shoulders; pronoto-elytral sinuation normally weak. Head completely yellow or with frons partially or totally dark; pronotum black with lateral sides yellow, often rather extended but rarely reaching disc; each elytron black with faint blue sheen, always with single yellow submedian spot and, often, more or less defined lateral marking, close to shoulder. Some specimens also with small pale subbasal spot, close to disc. Microreticulation on pronotum same as on elytra, fine but visible, arranged in small vermiculations. Stiff setae on prosternum same as on prosternal process, at most weakly longer; setal punctation on prosternal process and noterid platform rather close and uniform. Microreticulation impressed on lateral expansions of metaventrite, metacoxae and first abdominal ventrite; hardly visible on noterid platform and on rest of abdomen. Ventral surface black or dark brown, prosternum (except prosternal process) paler, proepisterna and hypomera reddish. Legs reddish-brown, meso- and metatibiae and tarsi darker.

Measurements: TL: 3.0–3.7 mm; MW 1.3–1.8 mm.

Male: Aedeagus large and robust. Median lobe (Fig. 6 a,b) elongate and flat, apically strongly bent dorsally, hook-shaped; ventral side somewhat sinuate, saddle-like. Left paramere (Fig. 6c) elongate, inflated, bean-shaped, rounded apically with fringe of short hairs. Right paramere robust, broadly triangular (Fig. 6d).

Female: Not differing externally from male.

Variability. Rather uniform in size and body shape, more variable in colouration. Head sometimes bicoloured (black on frons and part of clypeus, with yellow labrum) to almost completely testaceous; yellow sides of pronotum from thin to widely extended, but rarely reaching disc. Submedian spot on elytra always present; several specimens also with a second marking at shoulders. Small differences can be observed also in median lobes, but apparently of no relevance (Fig. 12). The three specimens seen from Sudan are somewhat smaller, the two males share more extended yellow sides on pronotum with respect to the female and they all have a visible lateral submedian marking on elytra; male genitalia do not show significant differences. Specimens studied from Iran and Iraq, steadily show more extended pale patterns on elytra (Fig. 2), with larger submedian spot, lateral markings at the base of elytra always well developed, plus a discal subbasal spot. This eastern population, represented by the types of *C. ornatus*, might have taxonomic interest, and further studies would be desirable to investigate this question. The two old specimens studied from Uzbekistan do not look different from typical *diophthalmus*; the population they belong to is likely isolated as no records from surrounding regions are known.

Distribution. (Fig. 14). Southeastern Mediterranean and the Middle East: Cyprus, Egypt, Iran (Khuzestan, western Fars), northern Iraq, Israel, Lebanon, Palestine (S. Giov. Acri = Akkā; this is reported in MONTES & SOLER, 1985, based on a specimen kept in the MSNG; this record seems to have been overlooked by subsequent authors), Syria, Turkey, Uzbekistan. The species seems not to go west of Egypt, where its distribution overlaps with *C. siculus* at least in the north of the country. *C. diophthalmus* is recorded also from tropical Africa (Angola, Cameroon, Democratic Republic of Congo, Ethiopia, Senegal) (Fig. 15), but so far I have been unable to see Afrotropical material, except the three specimens from Sudan. First record for Sudan and Uzbekistan.

The chromosomes of this species were described by BILTON (1992), based on Israeli (Golan) material and by SALEH AHMED & ANGUS (2000), based on Egyptian (Cairo, 10th Ramadan New Town) material. The karyotypes figured in these two papers differ only in the selection of a different chromosome pair as the XY sex chromosomes by SALEH AHMED & ANGUS (2000).

Notes on synonymies. RÉGIMBART (1899) suggested the synonymy of *Canthydrus ornatus* Sharp with *C. luctuosus* (Aubé, 1838) and this classification is currently adopted (NILSSON, 2011; HÁJEK, 2017). The examination of the aedeagus of the lectotype of *C. ornatus* shows that this taxon is conspecific with *C. diophthalmus*, thus becoming now its junior subjective synonym (**new synonymy**). The identity of *Hydrocanthus badenii* is still obscure; not having had the opportunity to see the type material yet, I am not able to confirm or refute the synonymy with *diophthalmus* and the presence of this species in Angola.

***Canthydrus siculus* (Ragusa, 1882) stat. nov.**

(Figs 3, 5a, 7, 13)

Hydrocanthus notula var. *siculus* Ragusa, 1882: 249 (orig. descr.).

Canthydrus notula ab. *siculus* (Ragusa, 1882): BEDEL, 1895: 375; RÉGIMBART, 1895a: 123 (Sicily); ZIMMERMANN, 1919: 117 (Sicily).

Canthydrus notula var. *siculus* (Ragusa, 1882): BRANDEN, 1885: 17 (Sicily).

Canthydrus notula sbsp. *siculus* (Ragusa, 1882): BEDEL, 1895: 375 (?partim; Algeria, Baleares, Egypt, Lybia, Morocco, Sardinia, Sicily, Tunisia).

Canthydrus diophthalmus var. *siculus* (Ragusa, 1882): MONTES & SOLER, 1985: 191 (syn.).

Hydrocanthus diophthalmus Reiche & Saulcy, 1855; HAMPE, 1863: 290 (Sicily).

Canthydrus diophthalmus (Reiche & Saulcy, 1855): FRANCISCOLO, 1979: 496 (Sicily, Sardinia); MONTES & SOLER, 1985: 191 (partim: Spain); RICO *et al*., 1990: 33 (Spain); MELONI, 1991: 169 (Sardinia); NILSSON, 2003: 33 (partim); NILSSON, 2004: 17; NILSSON, 2011: 11 (cat., partim); CILLO *et al*., 2016: 299 (Sardinia); HÁJEK, 2017: 844 (cat., partim); BENAMAR *et al*., 2021: 18 (Morocco).

Canthydrus (s. str.) *diophthalmus* (Reiche & Saulcy, 1855): NILSSON, 2005: 98 (cat., partim).

Hydrocanthus notula Erichson, 1843 (misident.): SCHAUM, 1857: LXXX (?partim; Egypt); RÉGIMBART, 1878: 349 (?partim; Egypt); SHARP, 1878: 113 (Morocco).

Hydrocanthus (*Canthydrus*) *notula* Erichson, 1843 (misident.): SEIDLITZ, 1887: 79 (?partim; Egypt, Algeria, Sicily).

Canthydrus notula (Erichson, 1843) (misident.): SHARP, 1882: 275 (?partim; Egypt, Morocco); MARSEUL, 1882: 40 (?partim; Egypt); BRANDEN, 1885: 17 (?partim; Egypt, Morocco); RÉGIMBART, 1895a: 123 (partim?; Egypt, Libya, Morocco); ZIMMERMANN, 1920: 12 (Italy, Sicily, Syria, North Africa); KOCHER, 1958: 11 (Morocco); ALFIERI, 1976: 31 (?partim; Egypt).

Canthydrus notula sbsp. *siculus* var. *numidicus* Bedel, 1925: 375; MONTES & SOLER, 1985: 191 (syn.).

Type locality. *H. notula* var. *siculus*: Italy, Sicily, Lentini lake; *C. notula siculus* var. *numidicus*: Algeria, La Calle [El Kala].

Type material. *H. notula* var. *siculus*: syntypes (MBAC) not studied; C. *notula siculus var. numidicus*: syntypes (MNHNP) not studied.

Material examined. ALGERIA: Algeria, Gauthier V, 31 m a.s.l., 36°50.130‘N 8°26.360‘E, 15.VI.2006, Bouzid leg. (V57) (3 exs HFB); Algeria, Gauthier IV, 34 m a.s.l., 36°50.230‘N 8°26.560‘E, 21.III.2006, Bouzid leg. (V39) (1 ex. HFB); Algeria, Bouzaroura (carrière), 15 m a.s.l., 36°50.240‘N 7°43.795‘E, 13.IV.2006, Bouzid leg. (V8) (1 ex. HFB); Algeria, Garaet Dissia, 14 m a.s.l., 36°51.980‘N 7°21.210‘E, nr. Ben-Azzouz (Skikda), 4.VII.2009, S. Bouzid leg. (1 ex. HFB); Algeria, Garaet Sidi Makhouf, 9 m a.s.l., 36°53.170‘N 7°18.190‘E, nr. Ben-Azzouz (Skikda), 29.6.2009, S. Bouzid leg. (3 exs HFB); Algeria, Mare Messida, 28 m a.s.l., 36°48.760‘N 8°24.180‘E, nr. Ain El-Assel (El-Tarf), 11.V.2009, S. Bouzid leg. (13 exs HFB).

EGYPT: "Cairo, Umgebung [= environs]" [printed], „Hydrocanth. notula Er." [hw] „coll. Dr. J. Fodor" [printed] (1 ex. male HFB); "Suez" [historical material, with no additional data] (5 exs NMW).

ITALY: Sicily: "Ragusa, Sicilia, 1882" [likely referred to Enrico Ragusa the name of the collector, not the town in Sicily] (5 exs NMW, likely part of the type series of *H. notula* var. *siculus* Ragusa, 1882, although not indicated as syntypes); "Sizilien, Mernl? [hw, not clear], Collection Kaufmann" (6 exs NMW); "Sicilia, Reitter, Collect. Hauser" (2 exs NMW); "*Canthydrus siculus* Rag. Lentini" (5 exs NMW, MTB); Sardinia, Cagliari, Natural Park Molentargius-Saline, freshwater pond Bellarosa Minore, 1.XII.2010, leg. F. Livretti (30 exs FLS, MTB).

MOROCCO: Foret Mamora, stream in rd. to Tiflet, 156 m a.s.l., 34º04‘00"N 6º05‘49"W, 26.III.2008, leg. Ribera, Hernando & Aguilera (2 exs IRB).

SPAIN: Andalusía, Almería Prov., pool E of Adra, 10.IV.1962, leg. R. Angus (2 exs HFB); Almería Prov., pool E of Adra, 10.IV.1962, leg. R. Angus (15 exs NHML, GFA).

Description. *Habitus* (Fig. 3). Oval elongate, convex; pronoto-elytral sinuation in lateral view rather marked. Maximum width close to shoulders. Head yellow, sometimes with dark band behind eyes, more or less developed. Typical specimens with yellow colouration on pronotum very extended, leaving only median "hourglass-shaped" dark marking on disc (Fig. 3; but see "Variability" below). Elytral colouration as typical *C. diophthalmus*: deep black with bluish sheen, with yellow submedian spot and, often, with more or less defined pale lateral marking close to shoulder of each elytron. Microreticulation on pronotum and elytra as in *C. diophthalmus*. Setal punctation on prosternum, prosternal process and noterid platform as in *C. diophthalmus*. Underside black, prosternum (but not prosternal process), proepisterna and hypomera reddish, epipleura brownish. Pro- and mesofemora reddish, metafemora dark brown; pro-, meso- and metatibiae and tarsi dark.

Measurements: TL: 3.3–3.7 mm; MW 1.65–1.8 mm.

Male: Median lobe of aedeagus (Fig. 7 a, b) large, very robust and stocky, broad and flat; saddle on ventral side a bit less strongly pronounced to that of *C. diophthalmus*, apex bent dorsally with tip broad and rounded, not hook-shaped. Left and right parameres same as those of *C. diophthalmus*.

Female: Not differing externally from male.

Differential diagnosis. Very close to *C. diophthalmus* in size, body shape and colouration, but often weakly broader and more convex; in lateral view with slightly more convex lateral margins of elytra and weakly more marked pronoto-elytral sinuation; in typical specimens the reddish colouration of pronotum extends towards disc leaving only a small medial marking. The two species can be easily separated by the shape of the median lobe, which in *C. siculus* is even more robust and broad than that of *C. diophthalmus*, with tip broad and rounded, not hook-shaped and the proximal side of the distal portion (just below the articulation with left paramere) is visibly broader with respect to *C. diophthalmus*.

Variability. Specimens seen from northern Egypt (Suez and Cairo) look closer to the typical specimens from Sicily, as well as material seen from Sardinia, always with broadly extended lateral yellow patches on pronotum. Specimens studied from Algeria, Morocco and Spain share less extended yellow markings on sides of pronotum, although there seems to be variability, according to the figures given in MONTES & SOLER (1985). Also, small differences in male genitalia can be seen as specimens from Algeria, Morocco and Spain seem to have a slightly less robust median lobe (Figs. 13a, 13b). Recording the finding of this beetle in continental Spain, MONTES & SOLER (1985) clearly figured a median lobe of *C. diophthalmus* (as considered here). In the paper it is not specified whether the aedeagus illustrated belongs to a Spanish specimen or to a specimen from elsewhere dissected for comparison, but it is rather improbable that *C. diophthalmus* occurs in Spain, together with *C. siculus*. I did not see the material collected from Albufera de Adra in 1981 by Montes, to which such paper refers (likely deposed in the Universidad Autónoma de Madrid), but specimens collected from the same locality in 1962 by R. Angus, all have the median lobe of *C. siculus*.

Distribution and ecological notes. (Fig. 14). Mediterranean coast of Algeria, northern Egypt, Italy (Sicily, Sardinia), Atlantic and Mediterranean Morocco, Spain (Andalusía, Baleares). I have not seen specimens from Libya and Tunisia, but most likely the record of *diophthalmus* from these countries must be referred to *siculus*. In northern Egypt it overlaps with *C. diophthalmus*, and further studies on the distribution of both species in the country would be of great interest to understand their geonomy and ecology.

An endangered species, at least in Europe. In Sicily it was known from the Lake Lentini (Siracusa), which was completely drained at the turn of the Second World War. The species seems to have been collected for the last time by Nino Sanfilippo in 1948 (FRANCISCOLO, 1979) and then declared extinct from the island. Another record for Sicily is a historical record in Castelbuono (Palermo), reported in ROCCHI, 2006. From the Baleares there have been no new data since the last century (TENENBAUM, 1915). In Andalusia it is known from very few localities (province of Almería and Parque Nacional de Doñana), where it seems to be threatened by the loss of environments (the locality of Albufera de Adra was wrecked and polluted since at least 1985; R. ANGUS (pers. com., 2021). In Sardinia the species is rare but, recently, a few new localities with good populations have been found (CILLO *et al*., 2016). The habitat in which this species has been recently collected in southern Sardinia (Natural Park Molentargius-Saline, which is inside the town of Cagliari) is a permanent, hypereutrophic freshwater pond whose waters are fed directly by the local purifier (already purified). The pond is surrounded by a thicket of *Phragmites* and a few rare *Typha* (Fig. 16), the population of macroinvertebrates is rather scarce and several alien species have been observed, such as nutria (*Myocastor coypus*), Louisiana crayfish (*Procambarus clarkii*), American terrapin (*Trachemys scripta* s.l.) and gambusia (*Gambusia affinis*) (F. LIVRETTI, pers. com., 2019).

Canthydrus koppi **Wehncke, 1883**

(Figs. 4, 8)

Canthydrus koppi Wehncke, 1883: 148 (orig. descr.); GUIGNOT, 1959: 503; NILSSON, 2011 (cat., see for bibliography).

Canthydrus koppi var. *sexmaculatus* Guignot, 1955: 864.

Type locality. Ivory Coast (Ghana), Addah.

Type material. Syntypes (MNHNP) not studied.

Material examined. BENIN: Dep. Atlantique, Allada, Glotomé village, slowly running stream, 17 m a.s.l., 06°41‘07“N 02°02‘37“E, 1.II.2006, leg. Georgen, Komarek & Houngué (1 ex. NMW); Dep. Zou, Zogbodomé, Lokoli (forest), muddy pond at Hlanzoun river, 17 m a.s.l., 07°03‘29”N 02°15‘31“E, 3.II.2006, leg. Georgen, Komarek & Houngué (2 exs NMW); Dep. Zou, Za Kpota & Kové, Zou river, bridge at road Abomey-Cové, ca. 30 m a.s.l., 07°12‘04.5“N 02°17‘20“E, 7.II.2006, leg. Georgen, Komarek & Houngué (1 ex. NMW); Dep. Ouémé, Porto Novo, Lopodji village, pond with rich riparian vegetation, 06°26‘50.5“N 02°39‘30“E, 8.II.2006, leg. Georgen, Komarek & Houngué (7 exs NMW).

BURKINA FASO: Campienga, at light, 110503N04242E, 161 m a.s.l., 21.VIII.2005, leg. Moretto (5 exs MTB).

CAMEROON: Mokolo (nr. Maroua), pre-Sahel, residual pools of streams, 8–10.VII.1979, leg. G. Onore (3 exs MTB).

MALI: Goundaka, Bandiagara river, 14°29‘N 3°56‘W, 21.II.2000, leg. Komarek & Mayer (21 – 3) (1 ex. NWM); idem, 12.II.2000, (12 – 1) (1 ex. NMW); San, Bani river, 13°18‘N 4°54‘W, 22.II.2000, leg. Komarek & Mayer (22 – 1) (1 ex. NMW); Bore, 15°6‘N 3°33‘W, 20.II.2000, leg. Komarek & Mayer (20 – 1) (1 ex. NMW); S Tombouctou, 16°40‘N 3°00‘W, 18.II.2000, leg. Komarek & Mayer (18 – 1) (1 ex. NMW); Korioume, Niger river, 16°40‘N 3°00‘W, 18.II.2000, leg. Komarek & Mayer (18 – 2) (1 ex. NMW); Kona, Niger river, 14°58‘N 3°55‘W, 20.II.2000, leg. Komarek & Mayer (20 – 3) (20 exs NMW, MTB); 50 km E Djenne, 13°50‘N 4°25‘W, 10.II.2000, leg. Komarek & Mayer (10 – 3) (5 exs. NMW, MTB); Sanga, stream, 14°29‘N 3° 17‘W, 13.II.2000, leg. Komarek & Mayer (13 – 3) (2 exs NMW); Mopti, Niger river, 14°30‘N 4°12‘W, 21.II.2000, leg. Komarek & Mayer (21 – 1) (4 exs NMW).

NIGER: N'Dourga, at light, 8.VIII.1980, leg. Leblanc (2 exs MTB).

NIGERIA: Ibadan, Oyo State, IITA Campus, light trap, XII.2004, leg. G. Georgen (1 ex. NMW).

Description. The species was redescribed by GUIGNOT (1959); only the most relevant diagnostic characters are given here.

Habitus (Fig. 4). Body oval, rather convex, maximum width at shoulders; in lateral view sides of pronotum weakly rounded and sides of elytra weakly concave; pronoto-elytral sinuation not very pronounced. Head completely yellow; pronotum completely yellow, with short dark band along hind side in scutellar area; each elytron black with faint blue sheen, with yellow submedian and subbasal, lateral, regular markings. Microreticulation on pronotum and elytra fine but visible, arranged in small vermiculations. Prosternum with stiff setae as well as on prosternal process; setal punctation on prosternal process and noterid platform rather close and uniform. Microreticulation impressed on lateral expansions of metaventrite, metacoxae and first abdominal ventrite; hardly visible on noterid platform and on rest of abdomen. Underside colouration pitch-brown, whole prothorax yellow, except for prosternal process dark brown. Legs reddish-brown, meta- tibiae and tarsi darker.

Measurements: TL: 3.4–3.6 mm; MW 1.4–1.5 mm.

Male: Aedeagal features very similar to those of *C. siculus*. Median lobe (Fig. 8 a, b) slightly more slender and less robust with respect to typical *siculus* from Sicily and Sardinia, but not different from specimens studied from Spain, Morocco and Algeria. Left and right parameres (Figs 8 c, d) same as in *siculus* and *diophthalmus*.

Female: Not differing externally from male.

Differential diagnosis. Very close to the preceding species in size, shape and colouration, but the pronotum is completely yellow, except for a dark line along the posterior margin, broader medially; rare exceptions share also a thin dark median line on pronotum. Elytra normally with the subbasal lateral yellow spot well developed. Male genitalia very similar to those of *C. siculus*.

Distribution. (Fig. 15). Western Afrotropical: Benin, Burkina Faso, Cameroon, Chad, Gambia, Ivory Coast, Mali, Mauritania, Niger, Nigeria, Senegal. First records for Benin and Burkina Faso.

Remarks. Despite the difference of colouration of pronotum and the apparently disjunct distribution, the aedeagi of *C. koppi* and *C. siculus* are indistinguishable from each other, or almost so, and this obviously suggests a very close relationship between these two taxa, which could be even conspecific. F. PEDERZANI (pers. com., 2021) states that observing extensive material of *C. koppi* from West Africa, he found it difficult several times to clearly distinguish this species from *C. diophthalmus*, since certain specimens seemed to intermediate between the two species, due to the colouration of pronotum and for aedeagal features. He was unable to clarify whether by *diophthalmus* he strictly meant the taxon as considered here or whether he referred to specimens with male genitalia of *siculus* or to both, but in any case, it is possible that in tropical Africa the patterns of diversity among these taxa are more complex or discrete than in the Mediterranean basin. The territory of the Sahara until a few thousand years ago was not the desert we know now, there were savannas and wetlands that in all probability reached the coasts of North Africa, and it is at least probable that there was a genetic continuity between the north Africa and tropical west Africa. It is conceivable that, if there is currently a genetic isolation between the populations of *C. koppi* in tropical west Africa and of *C. siculus* in the Mediterranean, these are, actually, in the process of differentiation, even if they are not yet distinct species. Deeper studies on much more African material and supported by molecular diagnoses are desirable in order to get a better overview of the status and the natural history of these two taxa. Having seen only specimens of both *diophthalmus* and *siculus* from the Palearctic, except for the above mentioned from Sudan, and having seen only typical specimens of *koppi*, at the moment it is impossible to give further comments beyond considering that *C. diophthalmus*, *C. siculus* and *C. koppi* are three distinct species, though very close.

Canthydrus arabicus Sharp, 1882

(Figs. 9, 11)

Canthydrus arabicus Sharp, 1882: 272 (orig. descr.); MARSEUL, 1882: 39; BRANDEN, 1885: 16; ZIMMERMANN, 1920: 9 (Arabia); NILSSON, 2003: 33 (cat.); TOLEDO, 2008: 64 (lectotype des.); NILSSON, 2011: 10 (cat.); HÁJEK, 2017: 844 (cat.); RIBERA *et al*., 2019 (Saudi Arabia).

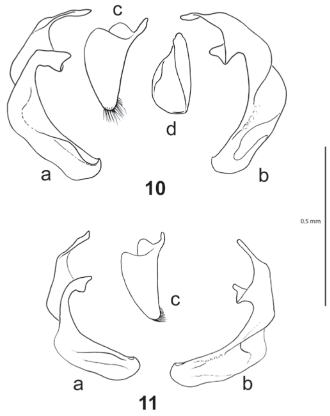
Canthydrus (s. str.) *arabicus* Sharp, 1882: NILSSON, 2005: 97 (cat.).

Type locality. Al-Hedjaz, Arabia [likely Al Hijaz, close to the Mecca, Saudi Arabia].

Type material. Lectotype (NHML) designated by TOLEDO, 2008: 65.

Fig. 9. Habitus, **a**) dorsal view and **b**) lateral view of *Canthydrus arabicus* (lectotype).

Fig. 9. Habitus, vistas **a**) dorsal y **b**) lateral de *Canthydrus arabicus* (lectotipo).



Figs. 10-11. Aedeagus: **a**) median lobe, left side, **b**) median lobe, right side, **c**) left paramere, **d**) right paramere: *Canthydrus notula* (ex. from South Africa); **11**) *C. arabicus* (lectotype; right paramere unavailable).

Figs. 10-11. Edeago: **a**) lóbulo medio, vista lateral izquierda, **b**) lóbulo medio, vista lateral derecha, **c**) parámero izquierdo, **d**) parámero derecho: *Canthydrus notula* (ejemplar de Sudáfrica); **11**) *C. arabicus* (lectotipo; parámero derecho no disponible).

Description. *Habitus* (Fig. 9). Rather large, somewhat convex, glossy. Maximum width behind shoulders (ca. 1/6 of elytral length); dorsal outline oval, distinctly tapering towards apex. In lateral view lateral margin of pronotum poorly convex, margin of elytra not strongly concave; pronoto-elytral sinuation rather weak.

Colour: Head reddish-yellow, vaguely darkened around eyes. Pronotum dark brown to reddish-brown, vaguely darkened on disc, lateral sides yellow. Elytra slightly lighter than pronotum, at least on disc; each elytron with submedian, small, almost regularly rounded, testaceous spot. Underside piceous-brown, darker on prosternal process and noterid platform. Antennae and mouthparts testaceous. Legs uniformly reddish-brown.

Structures and sculpture: Reticulation on head fine, composed by circular meshes; no dots visible. Sides of pronotum, in lateral view, with lateral bead rather fine but visible; surface glossy, microreticulation fine, arranged in small vermiculations. Elytral surface shining, microreticulation similar but evenly finer than that on pronotum; longitudinal series of very fine dots visible on each elytron. Prosternum with stiff setae arranged in single line; prosternal process and noterid platform uniformly covered with rather close and uniform setae. Microreticulation strong on metacoxal plates, finer on abdominal ventrites.

Measurements: Lectotype TL 3.0 mm; MW 1.6 mm; paralectotypes TL 3.0–3.3 mm; MW 1.6–1.8 mm.

Male: Median lobe of aedeagus (Fig. 11a, b) with distal portion rather short and stout, weakly sinuated on ventral side, ending with weak hook-like tip. Left paramere (Fig. 11c) triangular, elongate, gently tapering to narrowly rounded apex, with tuft of short setae. Right paramere lost during dissection.

Female: Not differing externally from male.

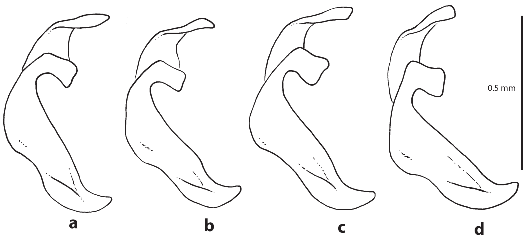


Fig. 12. Variation in shape of median lobe of aedeagus (left side) in *Canthydrus diophthalmus*: **a**) ex. from Uzbekistan; **b**) ex. from Egypt (Aswan); **c**) ex. from Israel; **d**) lectotype of *C. ornatus*.

Fig. 12. Variación en la forma del lóbulo medio del edeago (vista lateral izquierda) de *Canthydrus diophthalmus*: **a**) ejemplar de Uzbekistán; **b**) ejemplar de Egipto (Aswan); **c**) ejemplar de Israel; **d**) lectotipo de *C. ornatus*.

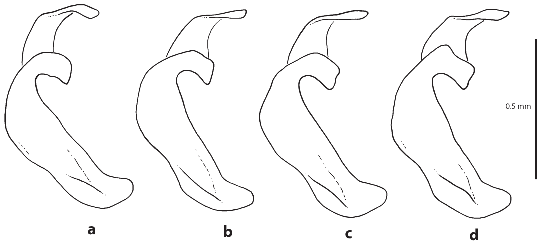


Fig. 13. Variation in shape of median lobe of aedeagus (left side) in *Canthydrus siculus*: **a**) ex. from Algeria; **b**) ex. from Spain; **c**) ex. from Egypt (Suez); **d**) ex. from Sardinia.

Fig. 13. Variación en la forma del lóbulo medio del edeago (vista lateral izquierda) de *Canthydrus siculus*: **a**) ejemplar de Argelia; **b**) ejemplar de España; **c**) ejemplar de Egipto (Suez); **d**) ejemplar de Cerdeña.

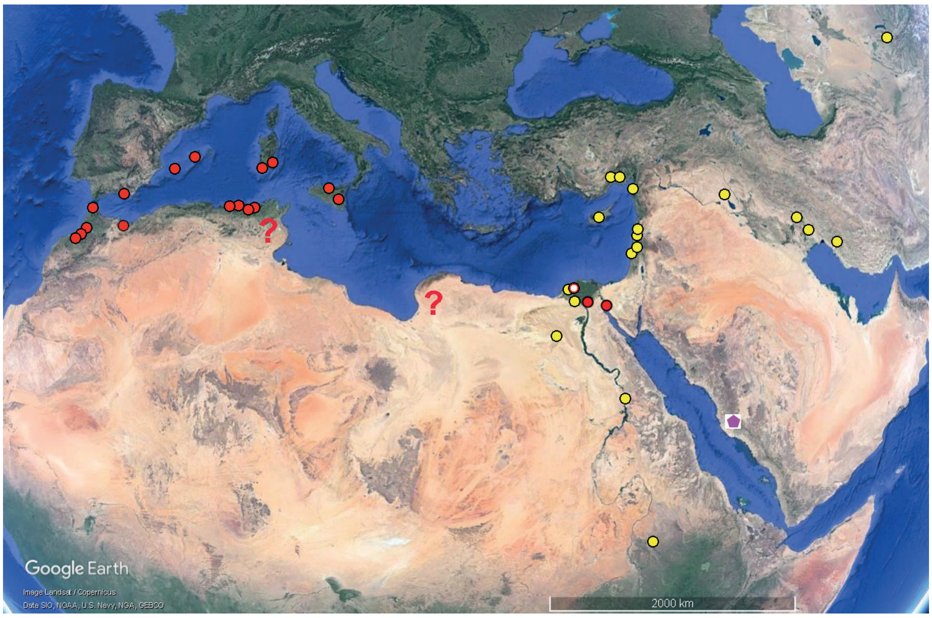


Fig. 14. Distribution in the Mediterranean, northern Africa and Middle Orient of *Canthydrus diophthalmus* (full yellow dots), *C. siculus* (full red dots) and *C. arabicus* (mauve pentagon in Saudi Arabia). Empty red dot in Egypt: unchecked records from Alexandria and Damanhur (ALFIERI 1976) which could be referred to both *C. siculus* or *C. diophthalmus*. Question marks in Libya and Tunisia: records of *diophthalmus* from literature, most likely referring to *C. siculus*.

Fig. 14. Distribución en el Mediterráneo, norte de África y Oriente Medio de *Canthydrus diophthalmus* (círculos amarillos), *C. siculus* (círculos rojos) y *C. arabicus* (pentágono malva en Arabia Saudí). Círculos rojos vacíos en Egipto: registros no confirmados de Alejandría y Damanhur (ALFIERI 1976) que pueden referirse a *C. siculus* o a *C. diophthalmus*. Interrogantes en Libia y Túnez: registros bibliográficos de *C. diophthalmus*, posiblemente se refieran a *C. siculus*.

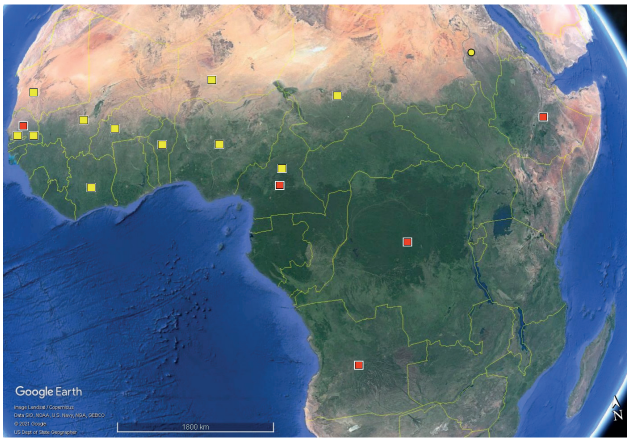


Fig. 15. Known Afrotropical distribution (indication of country only) of *Canthydrus diophthalmus* s.l. (red squares) and *C. koppi* (yellow squares). The yellow dot in Sudan is the same locality as that of *C. diophthalmus* in Fig. 14.

Fig. 15. Distribución conocida en la región Afrotropical (se destacan países y no localidades) de *Canthydrus diophthalmus* s.l. (cuadrados rojos) y *C. koppi* (cuadrados amarillos). El punto amarillo en Sudán es la misma localidad que para *C. diophthalmus* en la Fig. 14.

Fig. 16. Pond Bellarosa Minore in the Natural Park Molentargius-Saline (southern Sardinia), where *Canthydrus siculus* was recently collected (photo: F. LIVRETTI).

Fig. 16. Charca Bellarosa Minore en el Parque Natural Molentargius-Saline (sur de Cerdeña), donde recientemente se recolectó *Canthydrus siculus* (fotografía: F. LIVRETTI).

Distribution. (Fig. 14) Saudi Arabia, so far known only from the type locality, never collected again after the original description.

Taxonomic remarks. This species is known only from the type series, composed of seven specimens. The pale colouration might suggest that it belongs to the African *C. concolor* group (*sensu* GUIGNOT, 1959) or the Asian *C. flavus* group (WEWALKA, 1992), though these groups, based on dorsal colouration only, likely are not natural. A closer relationship of *C. arabicus* with *C. notula* is suggested by the rather similar male genitalia (Figs. 10-11) and by the colour patterns, very similar in both species, although the darker parts in *arabicus* are reddish-brown instead of black. *Canthydrus notula* is a widespread Afrotropical species, known to occur also in Oman and Yemen (NILSSON, 2011; RIBERA *et al.*, 2019) and *C. arabicus* might be a northern vicariant of the former, but in the light of such scarce available data not much can be said about this enigmatic beetle.

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Water beetle assemblages in the alpine lakes of Sierra Nevada (Spain)

Coleópteros acuáticos de las lagunas alpinas de Sierra Nevada (España)

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ABSTRACT

Sierra Nevada, the southernmost alpine system in Europe, hosts a complex of ponds and small lakes scattered throughout the highest parts of this massif, many of which are of glacial origin. These lakes are of particular interest, as they are subject to extreme environmental conditions and harbor simplified aquatic macroinvertebrate communities with highly cold-adapted species dominated by water beetles. Here we update the inventory of the aquatic Coleoptera species and their distribution within the system of alpine lakes within Sierra Nevada massif, as well as explore the environmental factors that drive the patterns in water beetle assemblages. We report a total of 22 taxa, which represents a very complete and reliable inventory according to a completeness assessment based on an asymptotic model fitted to a species accumulation curve. Water beetle assemblages were structured along an elevation gradient according to the non-parametric multivariate analysis, while there were not significant differences between beetle composition of open and closed lakes.

Key words: Coleoptera, high mountain, ponds, diversity, distribution, endemism.

RESUMEN

Sierra Nevada, el sistema alpino más meridional de Europa, alberga un conjunto de lagunas y pequeños lagos repartidos por las partes más altas de este macizo, muchos de los cuales son de origen glaciar. Estas lagunas son de particular interés, ya que están sujetas a condiciones ambientales extremas, albergando comunidades de macroinvertebrados acuáticos con baja riqueza de especies (en su mayoría coleópteros acuáticos) pero compuestas por especies con una elevada adaptación a climas fríos. En este trabajo actualizamos el inventario de especies de coleópteros acuáticos y su distribución dentro del sistema de lagunas alpinas del macizo de Sierra Nevada, así como exploramos los factores ambientales que determinan los patrones en la composición de sus comunidades. Se han registrado un total de 22 taxones, que representa un inventario muy completo y fiable según indican los resultados de una evaluación de completitud basada en un modelo asintótico ajustado a una curva de acumulación de especies. Las comunidades de escarabajos acuáticos se estructuraron a lo largo de un gradiente de altitud de acuerdo con el análisis multivariado no paramétrico, mientras que no hubo diferencias significativas entre la composición de coleópteros de lagunas abiertas y cerradas.

Palabras clave: Coleoptera, alta montaña, lagunas, diversidad, distribución, endemismo.

INTRODUCTION

Reliable species inventories are fundamental to describe biodiversity patterns (GASTON, 2000) and to develop efficient biodiversity management policies, especially in the current climate change context (MEYER *et al.,* 2015, POWNEY & ISAAC, 2015). In this sense, the determination of species diversity and distributions, specially the identification of endemisms, are very important since they are central issues in conservation studies (MYERS *et al.,* 2000). More specifically, having complete species inventories and distribution information is a critical priority to successfully identify and manage biodiversity hotspots and protected areas.

Sierra Nevada (declared as National Park in 1999) is a clearly delimited alpine mountain range in southern Spain covering a surface area of approximately 2000 km^2 and stretching from east of west for almost 90 km, belonging to an extensive mountain system in the southern Iberian Peninsula denominated as Sierras Béticas. Sierra Nevada presents unique characteristics which make it to represent one of the areas with the highest values of biodiversity and endemicity of the Iberian Peninsula (RUANO *et al.,* 2013; MILLÁN *et al.,* 2013): it is the southernmost alpine system in Europe and it presents a considerable altitude (with peak Mulhacén at 3482 m.a.s.l., being not only the highest mountain in the Iberian Peninsula, but also the highest peak in Europe outside the Alps).

Sierra Nevada hosts a system of ponds and small lakes scattered throughout the highest parts of this massif, many of which are of glacial origin (DIAZ-HERNANDEZ & HERRERA-MARTINEZ, 2021). These lakes are of particular interest, as they are subject to environmental extreme conditions (e.g. temperatures of -20 ºC or lower are commonly reached specially in the cryo-Mediterranean bioclimatic belt) (MORALES-BAQUEROL *et al.,* 1992). They harbor simplified aquatic macroinvertebrate communities with highly cold-adapted species dominated by water beetles, some of which are Iberian endemisms or micro-endemisms only present in this massif (MILLÁN *et al.,* 2013, 2014).

While the macroinvertebrate communities inhabiting lotic aquatic habitats within Sierra Nevada have been the target of numerous studies (RUANO *et al.,* 2013), the information relative to those from this system of lakes is still scarce and disperse. The first records on aquatic Coleoptera in Sierra Nevada were reported in 1856 by Rosenhauer, describing the presence of eleven species. From them, during the XIXth and XXth century, some faunistic studies have described and added more aquatic Coleoptera species to the list (SHARP, 1880-82; BERTRAND, 1954; MATEU, 1954; SÁINZ-CANTERO & ALBA-TERCEDOR, 1991a,b). As most studies were considered partial inventories and the records along years were disperse and taxonomic revisions changed some taxa status, SÁINZ-CANTERO (2013) reported an updated inventory of the aquatic Coleoptera in Sierra Nevada, listing the presence of 103 species belonging to 9 families of aquatic Adephaga and Polyphaga. However, there is a lack of studies focused specifically on the water beetle community inhabiting the system of high mountain lakes of this massif, which can be explained by their remote location and difficult access. Similarly, we know very little about the factors shaping the diversity patterns in these alpine lakes, and how lake characteristics determine the structure and composition of their invertebrate assemblages.

The aim of the present study is to update the inventory of the aquatic Coleoptera (Adephaga and Polyphaga) and their distribution within the system of alpine ponds and lakes of Sierra Nevada massif, as well as to explore the environmental factors that drive the patterns in water beetle assemblages.

MATERIALS AND METHODS

Study area

We selected a total of 29 ponds and lakes distributed across the five main valleys or basins (Dílar, Genil-Maitena, Trevélez, Poqueira and Lanjarón) and representing the variety of different high-mountain lentic water bodies in Sierra Nevada (see Table I and Fig. 1). The studied lakes are located at altitudes between 2520 and 3086 m.a.s.l., belonging to the bioclimatic belts oro-Mediterranean (1900 to 2900 m.a.s.l.) and cryo-ro-Mediterranean (above 2900 m.a.s.l.) (RIVAS-MARTÍNEZ, 1990), and remain ice-covered for long periods every year. The substrate of the lakes vary from stony silt to mud and organic depositions. Within the water, the aquatic vegetation is generally absent, although some of the lakes are surrounded by "green fringes" of varied extension, locally known as "borreguiles", which also cover the shore of the lake and represent a microhabitat for aquatic invertebrates within the water. Furthermore, their water is generally oligotrophic, and with low mineralization (MORALES-BAQUEROL *et al.,* 1992).

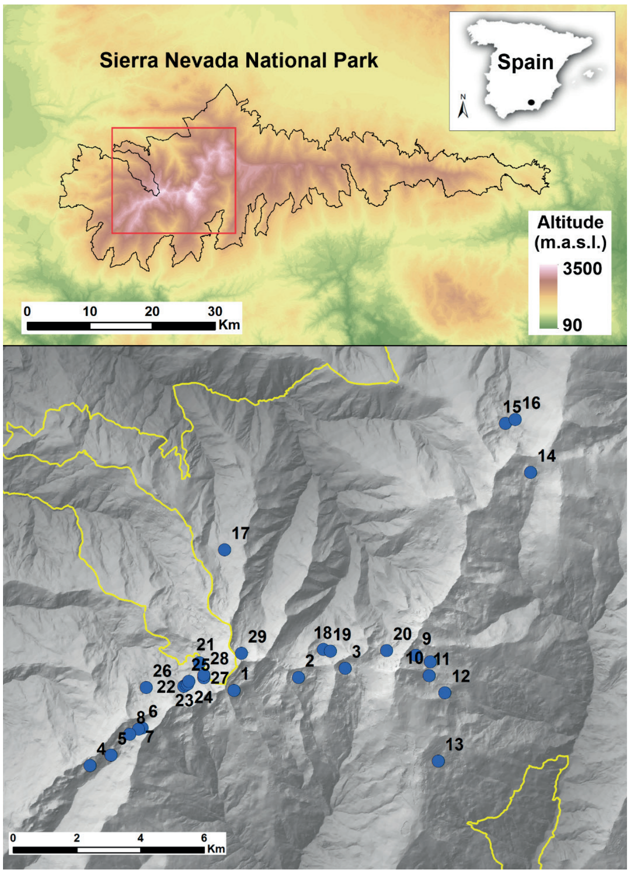

Fig. 1. Study area showing the location of the studied lakes. Codes numbers correspond to the codes shown in Table I.

Fig. 1. Área de estudio mostrando la localización de las lagunas estudiadas. Los códigos corresponden a los mostrados en la Tabla I.

Data collection

Lake sampling was carried out during the ice-free season (June-October) in 2018, 2020 and 2021 in the context of the projects ALPINEDIVING (funded by Fundación Biodiversidad, Spanish Ministry for the Ecological Transition and the Demographic challenge; PI: P. Abellán) and ALPINECHANGE (funded by the Spanish Ministry of Science and Innovation; PI: P. Abellán), which have focused on the study of macroinvertebrate communities of alpine lakes in Sierra Nevada in the context of climate change. Most of lakes were visited just once. Samples were collected using pond nets (pentagonal, 30 cm deep and 0.5 mm mesh) and sampling was stratified by all microhabitat types until additional species could no longer be found. Samples were preserved in 96 % ethanol and transported to the laboratory for processing and species identification.

Table I. Alpine lakes studied in Sierra Nevada and environmental data.

Tabla I. Lagunas alpinas estudiadas en Sierra Nevada y datos ambientales.

Lake	Code	X	Y	Elevation	Bioclimatic belt	Basin	Area (ha)	Type	Borreguil
Laguna de Aguas Verdes	1	37.048674	-3.368381	3055	Cryoro-Mediterranean	Poqueira	0.19	Open	Yes
Laguna de Río Seco	2	37.052113	-3.345583	3025	Cryoro-Mediterranean	Poqueira	0.42	Open	Yes
Laguna de la Caldera	3	37.054566	-3.329206	3030	Cryoro-Mediterranean	Poqueira	2.1	Closed	No
Laguna Cuadrada	4	37.027073	-3.418602	2910	Cryoro-Mediterranean	Lanjarón	0.24	Closed	No
Laguna de Tajos Altos	5	37.030018	-3.411314	2920	Cryoro-Mediterranean	Lanjarón	0.05	Closed	No
Laguna de Lanjarón	6	37.037718	-3.400453	2980	Cryoro-Mediterranean	Lanjarón	0.29	Closed	No
Lagunillo de Lanjarón	7	37.037359	-3.401639	2975	Cryoro-Mediterranean	Lanjarón	0.01	Open	Yes
Lagunillo de Charca Pala	8	37.035964	-3.40477	2945	Cryoro-Mediterranean	Lanjarón	0.04	Open	Yes
Laguna Altera	9	37.0585	-3.304462	3067	Cryoro-Mediterranean	Trevélez	0.3	Closed	No
Laguna de Tajos Coloraos	10	37.056636	-3.299319	3025	Cryoro-Mediterranean	Trevélez	0.25	Closed	Yes
Laguna del Borreguil	11	37.052767	-3.299767	2980	Cryoro-Mediterranean	Trevélez	0.18	Open	Yes
Laguna Hondera	12	37.047937	-3.294226	2897	Oro-Mediterranean	Trevélez	0.57	Open	Yes
Laguna del Peñón Negro	13	37.028721	-3.296453	2830	Oro-Mediterranean	Trevélez	0.67	Closed	Yes
Laguna de Juntillas	14	37.110091	-3.264324	2930	Cryoro-Mediterranean	Trevélez	0.11	Open	Yes
Laguna de los Lavaderos de la Reina	15	37.123851	-3.273205	2635	Oro-Mediterranean	Genil y Maitena	0.17	Open	Yes
Lagunillo de los Lavaderos de la Reina	16	37.124932	-3.269786	2615	Oro-Mediterranean	Genil y Maitena	0.1	Open	Yes
Lagunillo de San Juan	17	37.088003	-3.371759	2520	Oro-Mediterranean	Genil y Maitena	0.1	Open	Yes
Laguna de la Gabata	18	37.060025	-3.336952	2785	Oro-Mediterranean	Genil y Maitena	0.11	Open	Yes
Laguna Larga	19	37.059562	-3.334408	2790	Oro-Mediterranean	Genil y Maitena	1.77	Closed	No
Laguna de la Mosca	20	37.059761	-3.314717	2895	Oro-Mediterranean	Genil y Maitena	0.48	Open	Yes
Laguna de las Yeguas	21	37.056166	-3.380402	2880	Oro-Mediterranean	Dílar	0.33	Open	No
Lagunillo Bajo de la Ermita	22	37.049537	-3.385807	2865	Oro-Mediterranean	Dílar	0.01	Open	Yes
Lagunillo Medio de la Ermita	23	37.049896	-3.384989	2870	Oro-Mediterranean	Dílar	0.02	Open	Yes
Lagunillo Alto de la Ermita	24	37.050199	-3.384505	2875	Oro-Mediterranean	Dílar	0.01	Closed	Yes
Charca de la Ermita	25	37.050925	-3.384108	2870	Oro-Mediterranean	Dílar	0.004	Open	Yes
Lagunillo Misterioso	26	37.049107	-3.399001	2695	Oro-Mediterranean	Dílar	0.06	Open	Yes
Lagunillo de la Virgen Alto	27	37.051912	-3.378810	2947	Cryoro-Mediterranean	Dílar	0.02	Open	Yes
Lagunillo de la Virgen Bajo	28	37.052645	-3.378861	2945	Cryoro-Mediterranean	Dílar	0.09	Open	Yes
Laguna del Corral del Veleta	29	37.058762	-3.365136	3086	Cryoro-Mediterranean	Genil y Maitena	0.14	Closed	No

Lakes were characterized *in situ* according to their hydrological and geomorphological features and grouped into two categories: (i) *open lakes*, those associated to springs or small watercourses (with inflows and/or outflows); and (ii) *closed lakes*, those isolated from other water sources than direct snowmelt. These two categories can potentially host contrasting assemblages, as they present different environmental conditions: closed lakes are subjected to higher rates of evaporation and water level fluctuations, although may survive the summer drought, and display higher water temperatures and their margins are usually devoid of any vegetation. In contrast, open lakes are more stable environments, and are usually surrounded by extensive green fringes or *borreguiles*. Furthermore, we used a Geographic Information System to collect information about the altitude and area of each lake. Most of the sampled lakes survived the summer drought.

Statistical analysis

We evaluated the quality of the obtained inventory using an asymptotic model fitted to a species accumulation curve (SOBERÓN & LLORENTE, 1993; JIMÉNEZ-VALVERDE & HORTAL, 2003). First, we generated a collector's curve, which represents the expected accumulated number of species found within a certain geographical area relative to the sampling effort invested (number of lakes in our case). The collector's curve was obtained with the software EstimateS 9.1.0 (COLWELL, 2013) randomizing the sample order 100 times. Then, we used the Clench function to fit the model to the smoothed curve of the observed data using SPSS 26.0 software (SPSS, Inc.), and computed the asymptotic value, which represents the estimated species richness for an ideally unlimited sample size (SOBERÓN & LLORENTE, 1993). We used the ratio of recorded to predicted species richness as a measure of completeness of our inventory.

A non-parametric multivariate analysis was applied on a biological matrix based on presence–absence data and using the Bray-Curtis index among lake samples. We used non-metric multidimensional scaling (nMDS) to assess the main patterns in assemblage structure of water beetles using the R package 'vegan' (OKSANEN *et al.*, 2019). To determine which environmental gradients were significantly ($P < 0.05$) associated with nMDS space, we used the function "envfit" with 1000 permutations to fit environmental vectors (elevation and lake area) to the nMDS ordination. Furthermore, a nonmetric single-factor analysis of similarity (ANOSIM) was used to test whether assemblage composition differed among lake types (open and closed) using also the R package 'vegan'.

RESULTS

A total of 22 aquatic Coleoptera species and subspecies were found in the studied alpine lakes (Table II). The listed species belonged to 6 families: Dytiscidae (11 species), Haliplidae (1 species), Elmidae (2 species), Helophoridae (2 species), Hydraenidae (3 species) and Hydrophilidae (3 species). The number of species/taxa in a single lake varied from 2 to 10, with 7.2 taxa per lake as mean species richness. The most frequent taxa were *Agabus nevadensis* Lindberg, 1939, *Hydroporus sabaudus sierranevadensis* Shaverdo, 2004, *Boreonectes ibericus* (Dutton & Angus, 2007) and *Helophorus glacialis* Villa & Villa, 1833, all them occurring in at least 25 of the 29 studied lakes. Other common taxa were *Agabus nebulosus* (Forster, 1771), *Hydroporus nevadensis* Sharp, 1882, *Hydroporus marginatus* (Duftschmid, 1805), *Helophorus nevadensis* Sharp, 1882, *Limnebius truncatellus* (Thunberg, 1794) and *Enochrus fuscipennis* (Thomson, 1884), which occurred in at least 10 of the lakes. The remaining species (12) occurred in 4 or less lakes, with 7 recorded in a single lake only (see Table 2).

The species accumulation curve and fitted model ($R^2 = 0.94$) reached an asymptote, with a slope of 0.046 for the total number of samples. The model predicted the presence of 24 species, indicating a 94% of completeness. The distribution of lake assemblages according to nMDS ordination is shown in Fig. 2. The best two-dimensional solution ordination had a stress value of 0.165. The *envfit* analysis indicated that lake assemblages distributed in the nMDS space mostly according to altitude ($R^2 = 0.446$, $p = 0.001$) with no influence of lake area ($R^2 = 0.0480$, $p = 0.516$). Closed and open lakes overlapped in nMDS space although closed ones tended to be located on the left of the plot. Examining water beetle composition data by lake type, there were not significant differences between open and closed lakes (ANOSIM R = -0.0299, $p = 0.323$; Fig. 2).

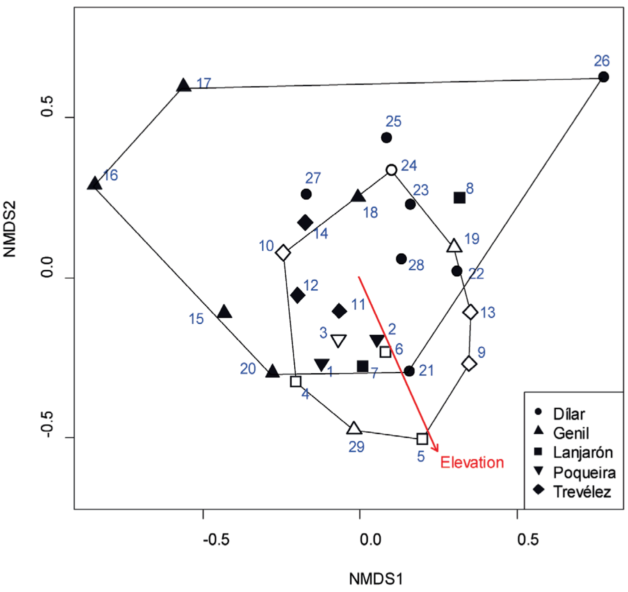

Fig. 2. Results of non-metric multidimensional scaling (nMDS) (stress = 0.165). Sites are labelled in relation to the the five main valleys or basins in which they are located (Dílar, Genil, Trevélez, Poqueira and Lanjarón) and the lake type ("closed" and "open" as white and black symbols, respectively; polygons delimitate both groups). The magnitude of the correlation between the nMDS axes and elevation is shown as a vector. See Table I for codes.

Fig. 2. Resultados del escalamiento multidimensional no métrico (nMDS) (estrés = 0.165). Los símbolos corresponden a los cinco cuencas o valles principales en los que se localizan las lagunas (Dílar, Genil, Trevélez, Poqueira y Lanjarón) y sus colores corresponden al tipo de laguna (blanco para lagunas cerradas, y negro para abiertas), delimitados por los polígonos. La magnitud de la correlación entre los ejes del nMDS y la altitud se muestra como un vector. Ver Tabla I para los códigos.

List of species

Suborder ADEPHAGA Schellenberg, 1806

Dytiscidae Leach, 1815

1. *Agabus nevadensis* Lindberg, 1939

Endemic Iberian species only found in Sierra Nevada, were it occurs in almost all the studied lakes in relatively high densities.

First record in Sierra Nevada: LINDBERG (1939).

Observations: the status of this taxon has been questioned (e.g. RIBERA *et al.*, 1998) in relation to *A. bipustulatus* (L.), a very common and widespread species, although allozyme studies of the complex support the hypothesis of reproductive isolation between both species (DROTZ *et al.*, 2010).

2. *Agabus biguttatus* (Olivier, 1795)

Trans-Iberian species widely distributed in the Iberian Peninsula mainly in mountain ranges. It was found in two of the study lakes: Laguna del Peñón Negro and Laguna de la Gabata.

First record in Sierra Nevada: MATEU (1954).

Table II. List of families and species of aquatic Coleoptera and their presence in studied lakes in Sierra Nevada. Lake codes correspond those shown in Table 1 and Figure 1.

Tabla II. Lista de familias y especies de coleópteros acuáticos encontrados y su presencia en cada una de las lagunas estudiadas en Sierra Nevada. Los códigos corresponden a los mostrados en la Tabla 1.

			Lake Code																												
Suborder	**Family**	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**
Adephaga	Dytiscidae	*Agabus nevadensis*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
		Agabus biguttatus													X					X											
		Agabus nebulosus			X							X	X	X	X	X	X	X	X	X						X	X		X		
		Boreonectes ibericus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X	
		Hydroporus sabaudus sierranevadensis	X	X	X	X		X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
		Hydroporus nevadensis	X	X	X	X	X	X	X			X	X	X		X	X			X		X	X								X
		Hydroporus marginatus	X	X	X	X	X	X	X		X	X	X		X						X	X	X							X	
		Hydroporus normandi alhambrae																	X								X				
		Hydroglyphus geminus														X															
		Laccophilus hyalinus																X													
		Laccophilus minutus																				X									
	Haliplidae	*Haliplus (Neohaliplus) lineatocollis*	X										X																		
Polyphaga	Elmidae	*Elmis aenea*																				X									
		Limnius opacus			X			X			X												X								
	Helophoridae	*Helophorus (Rhopalohelophorus) glacialis*	X	X	X		X	X	X	X	X		X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X
		Helophorus (Rhopalohelophorus) nevadensis	X		X	X			X					X				X	X			X							X		X
	Hydraenidae	*Limnebius truncatellus*	X	X	X	X	X	X	X		X		X	X	X		X						X	X							X
		Ochthebius (Ochthebius) quadrifoveolatus		X							X							X													
		Ochthebius (Ochthebius) semotus					X																								
	Hydrophilidae	*Enochrus (Lumetus) fuscipennis*	X	X		X		X					X	X		X	X	X	X			X			X				X	X	
		Limnohydrobius convexus															X														
		Laccobius (Dimorpholaccobius) obscuratus													X																

3. *Agabus nebulosus* (Forster, 1771)

Trans-Iberian species widely distributed in the Iberian Peninsula. Species well represented in both bioclimatic belts.

First record in Sierra Nevada: SÁINZ-CANTERO & ALBA-TERCEDOR (1991a).

4. *Boreonectes ibericus* (Dutton & Angus, 2007)

Trans-Iberian species widely distributed in the main mountain ranges in the Iberian Peninsula. This species occurs in almost all of the studied lakes in relatively high densities, being found mainly under stones.

First record in Sierra Nevada: DUTTON & ANGUS (2007).

5. *Hydroporus sabaudus sierranevadensis* Shaverdo, 2004

Endemic Iberian taxon exclusively found in Sierra Nevada, where it occurs in almost all of the studied lakes. This species seems to be found in shores with vegetation (mainly moss).

First record in Sierra Nevada: SHARP (1880-82).

Observations: the records of *Hydroporus nivalis* Heer, 1839 in Sierra Nevada by LINDBERG (1939), BERTRAND (1954) and SÁINZ-CANTERO & ALBA-TERCEDOR (1991a) must refer to *H. s. sierranevadensis*.

6. *Hydroporus nevadensis* Sharp, 1882

Endemic Iberian species distributed in the main mountain ranges of the Iberian Peninsula. Species well represented in the Sierra Nevada lakes, especially in the cryoro-Mediterranean bioclimatic belt.

First record in Sierra Nevada: SHARP (1880-82).

7. *Hydroporus marginatus* (Duftschmid, 1805)

Trans-Iberian species distributed in the main mountain ranges of the Iberian Peninsula. Species well represented in the Sierra Nevada lakes, especially in the cryoro-Mediterranean bioclimatic belt, being found mainly under stones.

First record in Sierra Nevada: BERTRAND (1954).

8. *Hydroporus normandi alhambrae* Fery, 1999

Endemic Iberian species exclusively found in the Sierra Nevada lakes and springs. This species was found in two of the studied lakes: Lagunillo de San Juan and Charca de la Ermita.

First record in Sierra Nevada: FERY (1999).

9. *Hydroglyphus geminus* (Fabricius, 1792)

Trans-Iberian species widely distributed in lentic habitats in the Iberian Peninsula. This species was found only in one of the studied lakes: Laguna de Juntillas.

First record in Sierra Nevada: SÁINZ-CANTERO & ALBA-TERCEDOR (1991a).

10. *Laccophilus hyalinus* (De Geer, 1774)

Trans-Iberian species widely distributed in lotic habitats in the Iberian Peninsula. This species was found only in one of the studied lakes (Lagunillo de los Lavaderos de la Reina), associated with its input spring.

First record in Sierra Nevada: SÁINZ-CANTERO & ALBA-TERCEDOR (1991a).

11. *Laccophilus minutus* (Linnaeus, 1758)

Trans-Iberian species widely distributed in lentic habitats in the Iberian Peninsula. This species was found only in one of the studied lakes: Laguna de la Mosca.

First record in Sierra Nevada: SÁINZ-CANTERO & ALBA-TERCEDOR (1991a).

Haliplidae Kirby, 1837

12. *Haliplus (Neohaliplus) lineatocollis* (Marsham, 1802)

Trans-Iberian species widely distributed in the Iberian Peninsula. This species was found in two of the studied lakes: Laguna de Aguas Verdes and Laguna del Borreguil.

First record in Sierra Nevada: BERTRAND (1954).

Suborder POLYPHAGA Emery, 1886

Elmidae Curtis, 1830

13. *Elmis aenea* (P.W.J. Müller, 1806)

Species with a European distribution, present in the main mountain ranges in eastern and northern Iberian Peninsula. This species was found only in one of the studied lakes: Laguna de la Mosca.

First record in Sierra Nevada: ROSENHAUER (1856).

14. *Limnius opacus* (P.W.J. Müller, 1806)

Trans-Iberian species distributed in lotic habitats of the main mountain ranges in the Iberian Peninsula. This species was found in four of the studied lakes, mainly in the cryoro-Mediterranean bioclimatic belt.

First record in Sierra Nevada: BERTHÉLEMY (1964).

Helophoridae Leach, 1815

15. *Helophorus (Rhopalohelophorus) glacialis* Villa & Villa, 1833

Trans-Iberian species limited to a few mountain ranges in the Iberian Peninsula. In Sierra Nevada this species occurs in almost all the studied lakes.

First record in Sierra Nevada: ROSENHAUER (1856).

16. *Helophorus (Rhopalohelophorus) nevadensis* Sharp, 1916

Endemic Iberian species exclusively distributed in Cordillera Cantábrica, Sistema Central and Sierra Nevada. Species well represented in the Sierra Nevada lakes, especially in the cryoro-Mediterranean bioclimatic belt.

First record in Sierra Nevada: SHARP (1916).

Hydraenidae Mulsant, 1844

17. *Limnebius truncatellus* (Thunberg, 1794)

Species with a European distribution, occurring in lotic habitats of the main mountain ranges in the Iberian Peninsula. Species well represented in the Sierra Nevada lakes, especially in the cryoro-Mediterranean bioclimatic belt.

First record in Sierra Nevada: BALFOUR-BROWNE (1979).

18. *Ochthebius (Ochthebius) quadrifoveolatus* Wollaston, 1854

Afrotropic Mediterranean species mainly distributed in eastern Iberian Peninsula. This species was found in three of the studied lakes: Laguna de Río Seco, Laguna Altera and Lagunillo de los Lavaderos de la Reina.

First record in Sierra Nevada: JÄCH (1989).

19. *Ochthebius (Ochthebius) semotus* Orchymont, 1942

Endemic Iberian species exclusively distributed in Cordilleras Béticas. This species was found only in one of the studied lakes: Laguna de Tajos Altos (Lanjaron Basin).

First record in Sierra Nevada: ORCHYMONT (1942).

Hydrophilidae Latreille, 1802

20. *Enochrus (Lumetus) fuscipennis* (Thomson, 1884)

Trans-Iberian species widely distributed in the Iberian Peninsula. Species well represented in the Sierra Nevada lakes.

First record in Sierra Nevada: SÁINZ-CANTERO & ALBA-TERCEDOR (1991b).

21. *Limnohydrobius convexus* (Brullé, 1835)

Trans-Iberian species distributed in the Iberian Peninsula in disperse populations. This species was found only in one of the studied lakes: Laguna de los Lavaderos de la Reina.

First record in Sierra Nevada: The present study reports the first record of the species in Sierra Nevada. 1 male, Laguna de los Lavaderos de la Reina, 05/08/2020, *leg*. P. Abellán, *det*. A. Millán.

22. *Laccobius (Dimorpholaccobius) obscuratus* Rottenberg, 1874

Palearctic species distributed in the main mountain ranges of the Iberian Peninsula. This species was found only in one of the studied lakes: Laguna del Peñón Negro.

First record in Sierra Nevada: ORCHYMONT (1935).

DISCUSSION

Our study contributes to the characterization of the water beetle fauna of high mountain lakes in Sierra Nevada. We report a total of 22 species and subspecies, which represents a very complete and reliable inventory according to our completeness assessment based on the asymptotic model fitted to the species accumulation curve (SOBERÓN & LLORENTE, 1993). This system of alpine lakes hosts a relatively poor community of water beetles in terms of species richness, especially when compared with other Iberian lowland ponds and lakes (PICAZO *et al*., 2012). This is in agreement with findings from previous studies focused on other European alpine lake systems (e.g. FÜREDER *et al*., 2006; BARTELS *et al*., 2021). Indeed, the severe physical conditions to which these lakes are subjected, such as low thermal stability, high irradiance, a fluctuating climate, short growing seasons and, usually, low nutrient levels, must be behind their relatively simplified biological communities (ČIAMPOROVÁ-ZAŤOVIČOVÁ et al., 2010; MENDOZA & CATALAN, 2010).

Water beetle assemblages were structured along an elevation gradient according to the multivariate analysis, while there were not significant differences between beetle composition of open and closed lakes, contrary to our expectations. Most of the studied lakes are located at altitudes over 2,800 m.a.s.l., with only a few close to 2,500 m.a.s.l. The latter lack some of the most frequent species in the highest lakes such as *Helophorus glacialis*, and displayed lower species richness. This pattern is in agreement with findings of ILG & OERTLI (2014) for Dytiscidae beetles in subalpine and alpine ponds of Switzerland, but contrasts with those of ČIAMPOROVÁ-ZATOVIČOVÁ & ČIAMPOR (2011) who found a hump-shaped response of water beetle richness to altitude in the Tatra Mountains, with the number of species being highest between 1,500 and 1,800 m a.s.l. The species *Agabus nevadensis, Boreonectes ibericus, Hydroporus sabaudus sierranevadensis, Hydroporus nevadensis, Hydroporus marginatus,* and *Helophorus glacialis* are widely represented in the studied lakes in Sierra Nevada, presenting high densities and co-occurring especially in the cryoro-Mediterranean bioclimatic belt, so this combination of species may be considered as the indicator water beetle community in Sierra Nevada alpine lakes.

The present study reports the first record of *Limnohydrobius convexus* in Sierra Nevada, a Trans-Iberian species with disperse populations throughout the Iberian Peninsula. This species is typical of both lentic and lotic water bodies from lowlands to mountain ranges. Only one male was found in Laguna de los Lavaderos de la Reina (2,635 m.a.s.l.), which could be considered as a vagrant individual.

Most of the species (12) presented a Trans-Iberian distribution, 6 were Iberian endemisms, 2 presented a European distribution, one was an Afrotropic Mediterranean species and one was a Palearctic species. The geographic location of Sierra Nevada, in the south of the Iberian Peninsula, thus of Europe, may explain the low presence of European and Palearctic species and the dominance of Trans-Iberian and Afro-Mediterranean species (MILLÁN *et al*., 2013). More importantly, the system of alpine lakes harbors three exclusive endemic taxa (*Agabus nevadensis*, *Hydroporus sabaudus sierranevadensis* and *Hydroporus normandi alhambrae*) likely as a result of long-term isolation between this massif and other Iberian mountain ranges since the Pleistocene (VILLASTRIGO *et al.*, 2021). These three species restricted to Sierra Nevada massif have been categorized as highly vulnerable according to their conservation status (SÁNCHEZ-FERNÁNDEZ *et al.*, 2008; MILLÁN *et al.*, 2014). Moreover, a recent study (PALLARÉS *et al.*, 2020) has shown that these species will probably experience a virtual loss of climatically suitable habitat under climate change scenarios, although physiological experiments showed that they would have certain capacity to deal in situ with future warming conditions. These species should be included in the red list of threatened species of Andalucía and Spain, and habitat management and protection strategies should be implemented to protect them. Conservation measures should be focused on the maintenance of current populations and the minimisation of those impacts derived from tourism and cattle grazing (PALLARÉS *et al.*, 2020).

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Water beetles from Castilla-La Mancha (Central Iberia): species and areas of interest

Coleópteros acuáticos de Castilla-La Mancha (centro de la península ibérica): especies y espacios de interés

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ABSTRACT

One of the main current environmental problems is the high rate of species extinctions associated with human activities. In a context of limited resources for biodiversity conservation, setting conservation priorities is therefore necessary. Hence, this study aims at prioritising the most interesting species and areas for the conservation of water beetles in the Autonomous Region of Castilla-La Mancha (C-LM), and evaluating to which extent these areas are represented within the Natura 2000 network. *Hydraena madronensis* Castro, García & Ferreras, 2000, *Hydraena mecai* Millán & Aguilera, 2000, and *Hydraena quetiae* Castro, 2000 were identified as priority species within C-LM as they are threatened species that exclusively appear in the study area. Moreover, 32 priority areas for conservation were identified. They are mainly located in the mountainous region from southwestern C-LM, Montes de Toledo and Alto Tajo, as well as in certain saline environments in lowlands. Some of these areas are currently unprotected, and therefore some alternatives for their conservation are discussed

Key words: aquatic Coleoptera, conservation, Iberian Peninsula, priority areas, endemism, Natura 2000 network.

RESUMEN

Uno de los principales problemas ambientales es la alta tasa de extinción de especies asociada con las actividades humanas. En un contexto de recursos limitados para la conservación de la biodiversidad, es necesario establecer prioridades de conservación. Este estudio tiene como objetivo identificar las especies y espacios más interesantes para la conservación de los coleópteros acuáticos en la Comunidad Autónoma de Castilla-La Mancha (C-LM), y evaluar hasta qué punto están representados en la red Natura 2000. *Hydraena madronensis* Castro, García y Ferreras, 2000, *Hydraena mecai* Millán y Aguilera, 2000, e *Hydraena quetiae* Castro, 2000 fueron identificadas como especies prioritarias por ser especies amenazadas que aparecen exclusivamente en C-LM. Además, se identificaron 32 áreas como prioritarias, localizadas principalmente en las zonas montañosas del cuadrante sureste de C-LM, Montes de Toledo y Alto Tajo, así como en algunos ambientes salinos en zonas bajas. Algunas de estas áreas están actualmente desprotegidas y, por lo tanto, se discuten alternativas para su conservación.

Palabras clave: coleópteros acuáticos, conservación, península ibérica, áreas prioritarias, endemismos, Red Natura 2000.

INTRODUCTION

Global biodiversity loss is one of the biggest problems that humanity is currently facing (RANDS *et al*., 2010; CARDINALE *et al*., 2012). This problem is especially important in aquatic ecosystems, as their biodiversity has been shown to decline at higher rates than that from other ecosystems (DUDGEON, 2019; ALBERT *et al*., 2021). Despite freshwater biodiversity is disproportionately threatened, it is underprioritized relative to the marine and terrestrial biota (VAN REES *et al*., 2021). This constitutes a worrying matter because although freshwater ecosystems occupy less than 1% of the Earth's surface, they contain as much as 12% of all known species (GARCÍA-MORENO *et al*., 2014). Therefore, integrating the protection of aquatic biota within overall biodiversity conservation strategies is pivotal to preserve the valuable ecosystem services provided by freshwater habitats (GRIZZETTI *et al*., 2019).

Establishing protected areas stands out as one of the main conservation strategies worldwide to halt biodiversity loss under the auspices of the Convention on Biological Diversity (Aichi targets for 2020; CBD, 2010). In a context of limited resources for biodiversity conservation, setting priorities is essential, so this poses the challenge of identifying those species and areas most interesting on which conservation efforts should be focused (BROOKS *et al*., 2006). Towards this aim, the first step is to locate those areas that constitute biodiversity hotspots in terms of species richness, of rare, endemic or vulnerable species, as well as of some combination of these attributes. Once these areas have been identified, the next task in conservation biology is to evaluate the effectiveness of the current protected areas in representing these biodiversity hotspots. Many studies have employed this approach using different taxonomic groups and different spatial scales. For example, RODRIGUES *et al*. (2004) considered vertebrates on a global scale, whereas ABELLÁN & SANCHEZ-FERNÁNDEZ (2015) and KUKKALA *et al*. (2016) considered herpetofauna and vertebrates, respectively, on a European scale. Similarly, the effectiveness of protected areas has been evaluated within the Iberian Peninsula using dung beetles (MARTÍN-PIERA, 2001), vertebrates (REY BENAYAS & DE LA MONTAÑA, 2003; LÓPEZ-LÓPEZ *et al*., 2011), lichens (MARTÍNEZ *et al*., 2006), butterflies (ROMO *et al*., 2007), endangered water beetles (SÁNCHEZ-FERNÁNDEZ *et al*., 2008a), endangered invertebrates (HERNÁNDEZ- MANRIQUE *et al*., 2012), bats (LISÓN *et al*., 2015), Iberian endemic fauna (ROSSO *et al*., 2018) and both freshwater European species of community interest and threatened species (mainly fishes and birds, SÁNCHEZ-FERNÁNDEZ *et al*., 2021). Despite this large amount of research dedicated to evaluating the effectiveness of protected area networks, there is a general paucity of studies that include invertebrates, especially aquatic insects, in their evaluations (CARDOSO *et al*., 2011).

With that purpose, we here focused on water beetles as this is one of the few aquatic insect groups with relatively good taxonomic and faunistic knowledge in the Iberian Peninsula (SÁNCHEZ-FERNÁNDEZ *et al*., 2008b). Besides, this taxonomic group has been demonstrated to be an excellent surrogate of other macroinvertebrate groups (SÁNCHEZ-FERNÁNDEZ *et al*., 2006; SLIMANI *et al*., 2019). In this study, we compiled all the available faunistic information regarding water beetle species from the Autonomous Region of Castilla-La Mancha (central Iberia) in order to identify both the species and areas with the highest conservation interest. Finally, a gap analysis was conducted to assess whether the European Natura 2000 network (N2000) of protected areas across Castilla-La Mancha is effective in the conservation of these species and areas. Such a regional level approach is of high interest given that responsibility in environmental management and biodiversity conservation matters in Spain are held by the regional Autonomous Governments. Therefore, outcomes from this study could be valuable for setting future conservation priorities in Castilla-La Mancha, one of the largest and most bio-diverse regions of Spain for which there have not been previous attempts in this sense, except for one of their five provinces, i.e., Albacete (ABELLÁN *et al*., 2004; PICAZO *et al*., 2010).

METHODS

Study area and data collection

Castilla–La Mancha (C-LM) is a Spanish Autonomous Region with Mediterranean climate. It is located in central Iberia and covers a surface of 79,409 km^2 (Figure 1). Distributional data on water beetles were mainly obtained from the ESACIB database that encompasses more than 70,000 georeferenced records for the Iberian Peninsula (MILLÁN *et al*., 2014; SÁNCHEZ-FERNÁNDEZ *et al*., 2015). The ESACIB database includes all the geographic and biological data available from the literature up to 2015, as well as from private and museum collections and other unpublished sources. This database was updated with a bibliographic compilation from 2016 to 2018, and also with data from our own samplings carried out in 2018 in C-LM. All species' records were aligned with a UTM grid of 10 × 10 km resolution (906 grid cells in total across the study area).

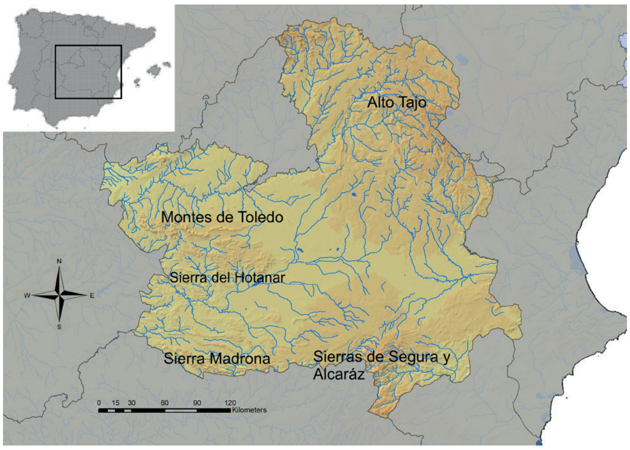

Fig. 1. Map of the study area, indicating the location of some relevant zones.

Fig. 1. Mapa del área de estudio, indicando la ubicación de algunas zonas relevantes.

Priority species for conservation

We assigned each species to one of the following categories of general distributional range following MILLÁN *et al.* (2014): Trans-Iberian (T; widely distributed species both across Iberia and beyond its northern and southern boundaries), Northern (N; distributed across Iberia and Europe), Southern (S: distributed across Iberia and North Africa) and endemic species (X: exclusively found in Iberia). Iberian endemic species were ranked according to their conservation priority or degree of vulnerability (low, moderate or high) based on the list compiled by SÁNCHEZ-FERNÁNDEZ *et al.* (2008a) and updated by MILLÁN *et al.* (2014). We identified as priority species for conservation in C-LM those that are threatened species (i.e., with high vulnerability) and exclusively endemic to the study area.

Biodiversity patterns and priority areas for conservation

We used four criteria to identify priority areas for conservation: total species richness (TSR), Iberian endemics richness (IER) and another two additional criteria based on species rarity. Towards this aim, we generated maps displaying TSR and IER at a resolution of 10 x 10 km UTM grid cells. Regarding rarity, we calculated a rarity index for each species, defined as the inverse of the number of grid cells in which each species appears (1/n; being n the number of grid cells in which a species appears). Then, we calculated for each grid cell i) an accumulated rarity value (AC-R) as the sum of the rarity values of all species that appear in that grid cell and ii) an average rarity value (AV-R) as the sum of the rarity values of all species that appear in that grid cell divided by the total number of species occurring there (i.e., (Σ1/n)/N; being N the total number of species recorded for that grid cell). Grid cells were then sorted according to each one of these four criteria (TSR, IER, AC- R and AV-R). After that, the top 5% cells (that is, 42 out of the 906 that span C-LM) were selected as priority areas following each criterion. Finally, we identified the Priority Areas for Conservation (PAC) in C-LM as those cells emerging as priority cells for at least three out of the four considered criteria.

Effectiveness of protected areas

We evaluated the effectiveness of the terrestrial European Natura 2000 (N2000) network in C-LM for protecting water beetles. The N2000 network of protected areas is the main basis to preserve biodiversity in the European Union (EU) (GASTON *et al.*, 2008). The N2000 network is based on two European directives, i) the Birds Directive (Council of the European Communities, 2010), which provides a list of species (Annex I) for which EU member states are required to designate Special Protection Areas (SPAs), and ii) the Habitats Directive (Council of the European Communities, 1992), which aims at protecting specific habitats (listed in Annex I) and animals (other than birds) and plants (listed in Annex II) for which each member state is required to designate special areas of conservation (SACs). Together, SPAs and SACs form the N2000 network. There are 72 SACs and 39 SPAs in the study area, occupying a total surface of 18,392 km^2 (23% of the study area), which represents the 14% of the whole Spanish N2000 area. Two GIS data layers (SPAs and SACs) at 10 x 10 km resolution (obtained from the website of Spanish Ministry for the Ecological Transition and the Demographic Challenge) were processed and combined to produce a single layer of the current N2000 network in C-LM. Then, the overlapping proportion between each PAC and N2000 was calculated using QGIS (v 3.14.0.) Each PAC was assigned to a category of protection as follows: very low protection (cells with overlapping values <12.5%), low protection (12.5% - 25%), moderate protection (25% - 50%), high protection (50% -75%) and very high protection (>75%). We finally identified conservation gaps, i.e., areas that would need further protection, as those PACs with very low protection by N2000 (i.e., overlapping values below 12.5%).

RESULTS

Priority species for conservation

A database with ca. 10,000 geo-referenced records for a total of 303 water beetle species and subspecies (62% of all Iberian species) was obtained (Appendix I). Of these 303 taxa, 150 (50%) have a Trans-Iberian distribution; 70 (23%) a Northern distribution; 31 (10%) a Southern distribution; and 52 (18%) were Iberian endemics, representing 40% of all Iberian endemics. Only one out of these 52 Iberian endemic species (2%) had a low vulnerability status, whereas 27 (52%) and 24 (46%) taxa had a moderate and high vulnerability status, respectively (SÁNCHEZ-FERNÁNDEZ *et al.*, 2008a; MILLÁN *et al.*, 2014); see Appendix I in Supplementary Material. Three species (*Hydraena madronensis* Castro, García & Ferreras, 2000, *Hydraena mecai* Millán & Aguilera, 2000, and *Hydraena quetiae* Castro, 2000) were identified as priority species for conservation in C-LM (see Table I), as they are species with a high vulnerability status whose distribution is exclusively restricted to the study area (Figure 2).

Priority areas for conservation and effectiveness of protected areas

Biodiversity patterns (species richness, endemicity and both average and accumulated rarity) are shown in Figure 3. Total Species Richness (TSR) showed a significant correlation with AC-R (r^2=0.82) and IER (r^2=0.52) but not with AV-R (r^2=0.02), meaning that those areas showing higher species richness also harbour more endemic species, and rare species only occur in sites with low species richness. A total of 32 PACs were identified (Figure 4). They are mainly located in the southwestern mountainous areas (Sierras de Segura y Alcaraz), disperse points in other mountainous areas such as Montes de Toledo in western C-LM and Alto Tajo in the North-East of the study area, as well as certain saline environments in lowlands. Among these 32 PACs, we identified five of them as conservation gaps, with very low protection coverage by the N2000. They were located in southern areas, namely the surroundings of Ojos de Villaverde lake, Saladares de Cordovilla, Alboraj lake, Río Gallo stream close to Setiles village in Alto Tajo area and, finally, several small siliceous streams in Sierra Madrona (southern C-LM) and Sierra del Hontanar (West of C-LM) (Figure 4). On the other hand, all the cells in which priority species were detected showed high o very high overlapping values (>70%) with the N2000, except for the case of one of the three known localities where *Hydraena quetiae* was recorded (Sierra del Hontanar). This cell matches with one of the five conservation gaps previously detected for PAC.

DISCUSSION

This study reveals the role of C-LM aquatic ecosystems as a water beetle biodiversity hotspot: they host 303 species and subspecies which represent up to 62% of all Iberian species and 52 Iberian endemics which represent up to 40% of all Iberian endemics. These numbers are even more impressive taking into account that Iberia harbours a vast part of the European water beetle biodiversity, with up to 120 species (25% of the Iberian water beetle fauna) being endemic from this southern Europe peninsula (MILLÁN *et al.*, 2014). Among the Iberian endemisms, 24% were ranked as highly vulnerable, with 3 of these species (*Hydraena madronensis*, *H. mecai* and *H. quetiae*) being restricted to C-LM. Therefore, special attention should be put in monitoring actions focused on these three priority species for C-LM. It is worth to note that none of the species considered here have any legal figure of protection, neither is included in any red list, nor are acknowledged as species of Community interest (i.e., they are not listed in the Habitats Directive). Thus, if these species do not receive protection at a national level, the only protection available for most of them has to do with their occurrence in protected areas designated on the basis of other taxa or habitat features (i.e., those species and habitats of Community interest). Although the aim of N2000 is to protect species listed in the Birds and Habitats Directives, the conservation status of the remaining biodiversity should be also a concern for the European Union, and hence a secondary objective of N2000 sites.

Most of the N2000 sites in the study area have been designated using aquatic elements (species or habitats), which means that this network could theoretically provide appropriate mechanisms to preserve aquatic biodiversity (SÁNCHEZ-FERNÁNDEZ *et al.*, 2021). Our analyses reported the effectiveness of N2000 network for water beetle conservation, so given the utility of this group as surrogate of aquatic biodiversity (BILTON *et al.*, 2006; SÁNCHEZ-FERNÁNDEZ *et al.*, 2006; SLIMANI *et al.*, 2019) such effectiveness could apply to other groups of aquatic macroinvertebrates. The priority areas for water beetle conservation identified here represent a wide typology of the aquatic ecosystems found in C-LM, from headwater streams to saline water bodies in lowlands. The degree of coverage of such PACs by the N2000 is generally high. Therefore, aquatic biodiversity in C-LM could be, in principle, relatively well protected. However, 5 of the 32 PACs presented a very low protection coverage. These grids, identified as conservation gaps, included isolated streams in mountain areas (Alto Tajo and Sierra del Hontanar) and saline systems mainly located in the South of C-LM. Considering the high conservation value of these aquatic ecosystems according to the criteria used here (total species richness, endemic species richness and rarity), we recommend their inclusion in the Natura 2000 network or their designation as entomological microreserves for aquatic insects (SAMWAYS *et al.*, 2020). However, it is important to emphasize that because aquatic ecosystems are highly influenced by the processes occurring at the upper parts of their basins, these entomological microreserves could be an efficient solution only when those upper parts are not affected by anthropogenic pressures.

The location of most PAC in mountainous areas could be explained by the predominance of narrow-range species in lotic environments, mainly small streams, due to historical and evolutionary causes (RIBERA, 2008),

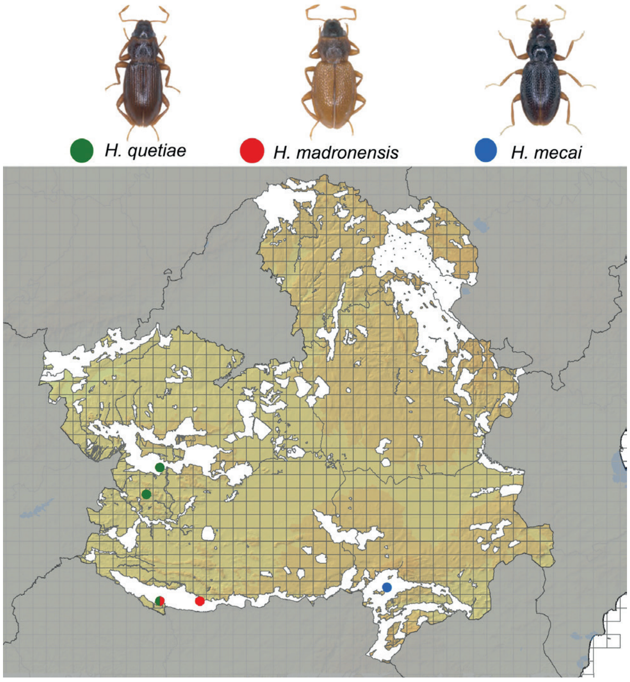

Fig. 2. Distribution of the three priority species for conservation in Castilla-La Mancha (white surface represents the Natura 2000 network in the study area). Pictures of the three species obtained from Millán *et al.* (2014).

Fig. 2. Distribución de las tres especies prioritarias en Castilla-La Mancha (la superficie blanca representa la Red Natura 2000 en el área de estudio). Fotografías de las tres especies obtenidas de Millán *et al.* (2014).

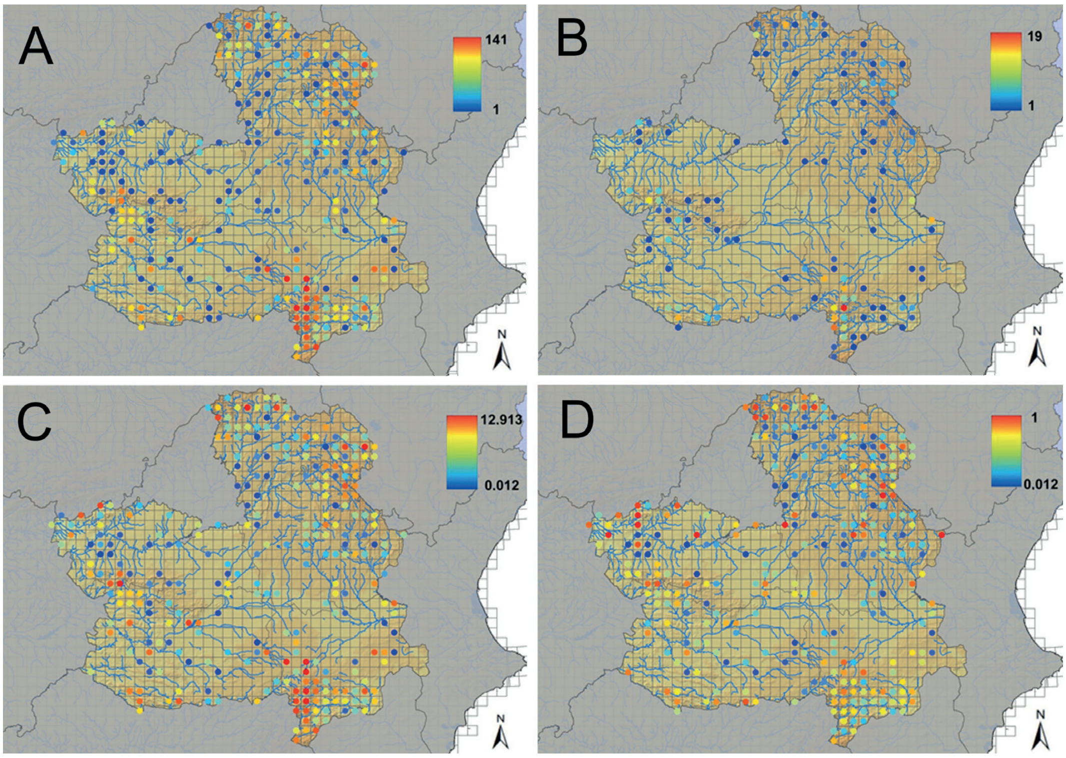

Fig. 3. Diversity patterns for water beetles in the study area. A) Total Species Richness; B) Iberian Endemic Species Richness; C) Accumulated Rarity Value; D) Average Rarity Value.

Fig. 3. Patrones de diversidad de coleópteros acuáticos en el área de estudio. A) Riqueza total de especies; B) Riqueza de especies endémicas ibéricas; C) Valor de rareza acumulado; D) Valor de rareza promedio.

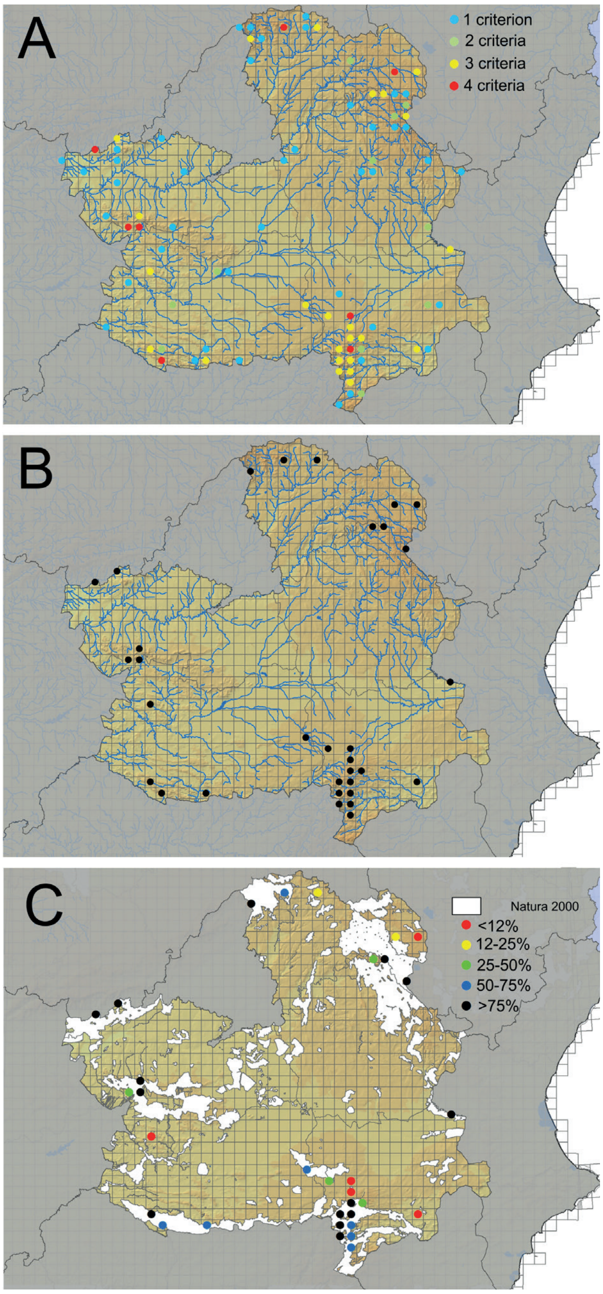

Fig. 4. Priority areas for conservation and gap analysis. A) Number of criteria for which each cell was considered as a priority area; B) Priority Areas for Conservation (PAC) in C-LM; C) Overlapping between PCA and Natura 2000 network (white surface): very low (overlapping values <12.5%) is denoted by red dots, low (overlapping values ranging between 12.5% - 25%) is denoted by yellow dots, moderate (overlapping values ranging between 25% - 50%) is denoted by green dots, high (overlapping values ranging between 50% -75%) is denoted by blue dots, and very high (overlapping values >75%) is denoted by black dots.

Fig. 4. Áreas prioritarias y análisis de vacíos de conservación. A) Número de criterios para los que cada cuadrícula fue considerada como área prioritaria; B) Áreas Prioritarias para la Conservación (PAC) en C-LM; C) Cobertura de las PCA por la Red Natura 2000 (superficie blanca): muy baja (solapamiento <12,5 %), puntos rojos); baja (solapamiento entre el 12,5 % y el 25 %, puntos amarillos); moderada (solapamiento entre 25 % - 50 %, puntos verdes), alta (solapamiento entre 50 % y 75 %, puntos azules) y muy alta (solapamiento >75 %, puntos negros).

as well as by the lower human impact occurring in highlands, which has preserved headwater ecosystems under natural conditions (SÁNCHEZ-FERNÁNDEZ *et al.*, 2012; MILLÁN *et al.*, 2014). Importantly, these areas are predicted to be critically affected by climate change (PEPIN *et al.*, 2015), so special attention should be paid regarding the monitoring and conservation of the great biodiversity they harbour. On the other hand, increasing conservation efforts is particularly interesting in inland saline systems from C-LM owing to their biodiversity contribution not only at a regional scale (MILLÁN *et al.*, 2011; PICAZO *et al.*, 2012), but also in a European context where these systems are very rare. In this sense, some previous studies have shown inland saline waters to host halotolerant biota and a high number of rare and endemic species, (e.g., *Ochthebius caesaraugustae* Jäch, Ribera & Aguilera, 1998, or *O. glaber* Montes & Soler, 1988) absent in freshwater systems (MILLÁN *et al.*, 2011; PICAZO *et al.*, 2012). Thus, the protection of these singular habitats, frequently subjected to significant anthropogenic pressures, would target a key-principle that guides the design of protected area networks, such as increasing the ecological complementarity among the set of areas already protected (MARGULES & PRESSEY, 2000).

Among the PAC identified as conservation gaps, it needs to be highlighted the one that includes one of the only three known localities where the priority endemic species *Hydraena quetiae* occurs (a small stream in Sierra del Hontanar, Ciudad Real province). The remaining localities with priority species occur in areas showing a high overlap with the N2000. Nevertheless, it is important to stress that the mere fact that a species occurs within a protected area does not guarantee its effective conservation (RADA *et al.*, 2019). Therefore, it is crucial that environmental and natural resources management plans of each protected area include specific measures pursuing the conservation of water bodies regardless of the specific items (species or habitats) used for their declaration. On this subject, avoiding direct human pressures, the maintenance of natural flows, and especially the management at the basin level, are indispensable tools for the conservation of aquatic biodiversity in C-LM.

It must be acknowledged that our dataset shows some clear limitations, such as the lack of information on the sampling effort carried out for each species, as well as the coarse scale of the spatial units considered (10 × 10 km cells). Despite working with the group of organisms that likely counts on the best information available among any other group of aquatic insects in the study area, it is reasonable to consider that some important areas for conservation could have been overlooked owing to a lack of sampling effort. Anyway, the areas here identified as priority and gaps are certainly important, and their conservation should be prioritized. Besides, it is worth to emphasize other two important points: i) top sites identified here using scoring procedures based on richness and/or rarity may suffer from high species redundancy, while some species may remain outside of the priority sites; and ii) some of the priority sites identified in this study have suffered an important degradation in the last years; for example, Ojos de Villaverde lagoon was colonised by *Procambarus clarkii* with a devastating effect in the aquatic community. In this case, we recommend to conduct a new sampling campaign in the PACs in order to know their current conservation status.

The results obtained in this study can be relevant both at a local scale as a particular case study in C-LM but also at an international level owing to the great contribution of the study area to the European N2000 network. According to the Natura 2000 Barometer 2018–2019 of the European Commission, the N2000 sites in the study area represent 2.3% of the whole European network, covering an area similar to those in entire countries such as Portugal (2.4%), Croatia (2.6%) or the UK (2.7%), and higher than that in another 14 countries. The results obtained in this study could guide future revisions of the design of reserve networks that would provide a framework to address deficiencies in the N2000 network for adequately protecting freshwater biodiversity in Europe. Indeed, these results could help Spain to meet their obligations as an EU Member State under the CBD including the post-2020 global biodiversity framework (CBD, 2021).

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Appendix I. List of water beetle species found in Castilla-La Mancha following a taxonomic order, with notes on their biogeographical range (BR) and their vulnerability category (V) according to Sánchez-Fernández *et al.* (2008). T: Trans-Iberian; N: Northern; S: Southern; X: Iberian endemics. * denotes those Iberian endemic species restricted to Castilla-La Mancha.

Apéndice I. Lista de especies de coleópteros acuáticos de Castilla-La Mancha siguiendo un orden taxonómico, con indicación de su tipo biogeográfico (BR) y su categoría de vulnerabilidad (V) según Sánchez-Fernández *et al.* (2008). T: Transibérica; N: Norte; S: Sur; X: Endemismo ibérico. * indica que ese endemismo ibérico está restringido a Castilla-La Mancha.

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
1	SPHAERIUSIDAE	*SPHAERIUS*	*HISPANICUS*	MATTHEWS 1899	T	
2	GYRINIDAE	*GYRINUS*	*CASPIUS*	MÉNÉTRIES, 1832	T	
3	GYRINIDAE	*GYRINUS*	*DEJEANI*	BRULLÉ, 1832	T	
4	GYRINIDAE	*GYRINUS*	*DISTINCTUS*	AUBÉ, 1836	T	
5	GYRINIDAE	*GYRINUS*	*SUBSTRIATUS*	STEPHENS, 1829	T	
6	GYRINIDAE	*GYRINUS*	*SUFFRIANI*	SCRIBA, 1855	N	
7	GYRINIDAE	*GYRINUS*	*URINATOR*	ILLIGER, 1807	T	
8	GYRINIDAE	*AULONOGYRUS*	*STRIATUS*	(FABRICIUS, 1792)	T	
9	GYRINIDAE	*ORECTOCHILUS*	*VILLOSUS*	(MÜLLER, 1776)	T	
10	HALIPLIDAE	*PELTODYTES*	*CAESUS*	(DUFTSCHMID, 1805)	T	
11	HALIPLIDAE	*PELTODYTES*	*ROTUNDATUS*	(AUBÉ, 1836)	T	
12	HALIPLIDAE	*HALIPLUS*	*OBLIQUUS*	(FABRICIUS, 1787)	T	
13	HALIPLIDAE	*HALIPLUS*	*LINEATOCOLLIS*	(MARSHAM, 1802)	T	
14	HALIPLIDAE	*HALIPLUS*	*ANDALUSICUS*	WEHNCKE, 1874	T	
15	HALIPLIDAE	*HALIPLUS*	*FULVUS*	(FABRICIUS, 1801)	T	
16	HALIPLIDAE	*HALIPLUS*	*GUTTATUS*	AUBÉ, 1836	T	
17	HALIPLIDAE	*HALIPLUS*	*MUCRONATUS*	STEPHENS, 1832	T	
18	HALIPLIDAE	*HALIPLUS*	*RUBIDUS*	PERRIS, 1857	T	
19	NOTERIDAE	*NOTERUS*	*CLAVICORNIS*	(DE GEER, 1774)	N	
20	NOTERIDAE	*NOTERUS*	*LAEVIS*	STURM, 1834	T	
21	HYGROBIIDAE	*HYGROBIA*	*HERMANNI*	(FABRICIUS, 1775)	T	
22	DYTISCIDAE	*LIOPTERUS*	*ATRICEPS*	SHARP, 1882	T	
23	DYTISCIDAE	*LACCOPHILUS*	*HYALINUS*	(DE GEER, 1774)	T	
24	DYTISCIDAE	*LACCOPHILUS*	*MINUTUS*	(LINNAEUS, 1758)	T	
25	DYTISCIDAE	*LACCOPHILUS*	*POECILUS*	KLUG, 1882	T	
26	DYTISCIDAE	*HYPHYDRUS*	*AUBEI*	GANGLBAUER, 1892	T	
27	DYTISCIDAE	*HYDROVATUS*	*CLYPEALIS*	SHARP, 1876	T	
28	DYTISCIDAE	*HYDROVATUS*	*CUSPIDATUS*	(KUNZE, 1818)	T	
29	DYTISCIDAE	*YOLA*	*BICARINATA*	(LATREILLE, 1804)	T	
30	DYTISCIDAE	*BIDESSUS*	*COXALIS*	SHARP, 1880-82	T	
31	DYTISCIDAE	*BIDESSUS*	*GOUDOTII*	(CASTELNAU, 1834)	T	
32	DYTISCIDAE	*BIDESSUS*	*MINUTISSIMUS*	(GERMAR, 1824)	T	
33	DYTISCIDAE	*BIDESSUS*	*PUMILUS*	(AUBÉ, 1836)	T	
34	DYTISCIDAE	*HYDROGLYPHUS*	*GEMINUS*	(FABRICIUS, 1792)	T	
35	DYTISCIDAE	*HYDROGLYPHUS*	*SIGNATELLUS*	(KLUG, 1834)	S	
36	DYTISCIDAE	*HYGROTUS*	*CONFLUENS*	(FABRICIUS, 1787)	T	
37	DYTISCIDAE	*HYGROTUS*	*FRESNEDAI*	(FERY, 1992)	X	HIGH
38	DYTISCIDAE	*HYGROTUS*	*IMPRESSOPUNCTATUS*	(SCHALLER, 1783)	N	
39	DYTISCIDAE	*HYGROTUS*	*INAEQUALIS*	(FABRICIUS, 1777)	T	
40	DYTISCIDAE	*HYGROTUS*	*LAGARI*	(FERY, 1992)	S	

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
41	DYTISCIDAE	*HYGROTUS*	*PALLIDULUS*	(AUBÉ, 1850)	T	
42	DYTISCIDAE	*HYGROTUS*	*MUSICUS*	(KLUG, 1833)	S	
43	DYTISCIDAE	*HYDROPORUS*	*DECIPIENS*	SHARP, 1878	X	LOW
44	DYTISCIDAE	*HYDROPORUS*	*DISCRETUS*	FAIRMAIRE, 1859	T	
45	DYTISCIDAE	*HYDROPORUS*	*GYLLENHALII*	SCHIÖDTE, 1841	N	
46	DYTISCIDAE	*HYDROPORUS*	*INCOGNITUS*	SHARP, 1869-70	N	
47	DYTISCIDAE	*HYDROPORUS*	*LIMBATUS*	AUBÉ, 1836	T	
48	DYTISCIDAE	*HYDROPORUS*	*LUCASI*	REICHE, 1866	S	
49	DYTISCIDAE	*HYDROPORUS*	*MARGINATUS*	(DUFTSCHMID, 1805)	T	
50	DYTISCIDAE	*HYDROPORUS*	*NEVADENSIS*	SHARP, 1882	X	MODERATE
51	DYTISCIDAE	*HYDROPORUS*	*NIGRITA*	(FABRICIUS, 1792)	N	
52	DYTISCIDAE	*HYDROPORUS*	*NORMANDI NORMANDI*	RÉGIMBART, 1903	N	
53	DYTISCIDAE	*HYDROPORUS*	*OBSOLETUS*	AUBÉ, 1836	T	
54	DYTISCIDAE	*HYDROPORUS*	*PLANUS*	(FABRICIUS, 1781)	T	
55	DYTISCIDAE	*HYDROPORUS*	*PUBESCENS*	(GYLLENHAL, 1808)	T	
56	DYTISCIDAE	*HYDROPORUS*	*TESSELLATUS*	DRAPIEZ, 1819	T	
57	DYTISCIDAE	*HYDROPORUS*	*VAGEPICTUS*	FAIRMAIRE & LABOULBÈNE, 1854	N	
58	DYTISCIDAE	*GRAPTODYTES*	*AEQUALIS*	ZIMMERMANN, 1918	S	
59	DYTISCIDAE	*GRAPTODYTES*	*BILINEATUS*	(STURM, 1835)	N	
60	DYTISCIDAE	*GRAPTODYTES*	*CASTILIANUS*	FERY, 1995	X	MODERATE
61	DYTISCIDAE	*GRAPTODYTES*	*FLAVIPES*	(OLIVIER, 1795)	T	
62	DYTISCIDAE	*GRAPTODYTES*	*FRACTUS*	(SHARP, 1880-82)	T	
63	DYTISCIDAE	*GRAPTODYTES*	*IGNOTUS*	(MULSANT, 1861)	T	
64	DYTISCIDAE	*GRAPTODYTES*	*VARIUS*	(AUBÉ, 1836)	T	
65	DYTISCIDAE	*METAPORUS*	*MERIDIONALIS*	(AUBÉ, 1836)	T	
66	DYTISCIDAE	*SCARODYTES*	*HALENSIS*	(FABRICIUS, 1787)	T	
67	DYTISCIDAE	*STICTONECTES*	*EPIPLEURICUS*	(SEIDLITZ, 1887)	N	
68	DYTISCIDAE	*STICTONECTES*	*LEPIDUS*	(OLIVIER, 1795)	T	
69	DYTISCIDAE	*STICTONECTES*	*OCCIDENTALIS*	FRESNEDA & FERY, 1990	X	HIGH
70	DYTISCIDAE	*STICTONECTES*	*ABELLANI*	MILLÁN, PICAZO & FERY	X	MODERATE
71	DYTISCIDAE	*STICTONECTES*	*OPTATUS*	(SEIDLITZ, 1887)	T	
72	DYTISCIDAE	*DERONECTES*	*BICOSTATUS*	(SCHAUM, 1864)	X	MODERATE
73	DYTISCIDAE	*DERONECTES*	*DEPRESSICOLLIS*	(ROSENHAUER, 1856)	X	MODERATE
74	DYTISCIDAE	*DERONECTES*	*FAIRMAIREI*	(LEPRIEUR, 1876)	T	
75	DYTISCIDAE	*DERONECTES*	*HISPANICUS*	(ROSENHAUER, 1856)	T	
76	DYTISCIDAE	*DERONECTES*	*MOESTUS*	LEPRIEUR, 1876	T	
77	DYTISCIDAE	*DERONECTES*	*OPATRINUS*	(GERMAR, 1824)	N	
78	DYTISCIDAE	*DERONECTES*	*WEWALKAI*	FERY & FRESNEDA, 1988	X	HIGH
79	DYTISCIDAE	*IBERONECTES*	*BERTRANDI*	(LEGROS, 1956)	X	MODERATE
80	DYTISCIDAE	*STICTOTARSUS*	*DUODECIMPUSTULATUS*	(FABRICIUS, 1792)	N	
81	DYTISCIDAE	*BOREONECTES*	*IBERICUS*	(DUTTON & AGNUS, 2007)	T	
82	DYTISCIDAE	*NEBRIOPORUS*	*BUCHETI CAZORLENSIS*	(LAGAR, FRESNEDA & HERNANDO, 1987)	X	MODERATE
83	DYTISCIDAE	*NEBRIOPORUS*	*CANALICULATUS*	(LACORDAIRE, 1835)	N	
84	DYTISCIDAE	*NEBRIOPORUS*	*CLARKII*	(WOLLASTON, 1862)	S	

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
85	DYTISCIDAE	*NEBRIOPORUS*	*FABRESSEI*	(RÉGIMBART, 1901)	N	
86	DYTISCIDAE	*NEBRIOPORUS*	*SANSII*	(AUBÉ, 1836)	N	
87	DYTISCIDAE	*NEBRIOPORUS*	*BAETICUS*	(SCHAUM, 1864)	X	HIGH
88	DYTISCIDAE	*NEBRIOPORUS*	*CERESYI*	(AUBÉ, 1836)	T	
89	DYTISCIDAE	*OREODYTES*	*DAVISII*	CARR, 2001	N	
90	DYTISCIDAE	*NECTOPORUS*	*SANMARKII SANMARKII*	(SAHLBERG, 1826)	N	
91	DYTISCIDAE	*PLATAMBUS*	*MACULATUS*	(LINNAEUS, 1758)	N	
92	DYTISCIDAE	*AGABUS*	*BIGUTTATUS*	(OLIVIER, 1795)	T	
93	DYTISCIDAE	*AGABUS*	*BIPUSTULATUS*	(LINNAEUS, 1767)	T	
94	DYTISCIDAE	*AGABUS*	*BRUNNEUS*	(FABRICIUS, 1798)	T	
95	DYTISCIDAE	*AGABUS*	*CONSPERSUS*	(MARSHAM, 1802)	T	
96	DYTISCIDAE	*AGABUS*	*DIDYMUS*	(OLIVIER, 1795)	T	
97	DYTISCIDAE	*AGABUS*	*GUTTATUS*	(PAYKULL, 1798)	N	
98	DYTISCIDAE	*AGABUS*	*HEYDENI*	WEHNCKE, 1872	S	
99	DYTISCIDAE	*AGABUS*	*LABIATUS*	(BRAHM, 1790)	N	
100	DYTISCIDAE	*AGABUS*	*NEBULOSUS*	(FORSTER, 1771)	T	
101	DYTISCIDAE	*AGABUS*	*PALUDOSUS*	(FABRICIUS, 1801)	T	
102	DYTISCIDAE	*AGABUS*	*RAMBLAE*	MILLÁN & RIBERA, 2001	S	
103	DYTISCIDAE	*ILYBIUS*	*CHALCONATUS*	(PANZER, 1796)	T	
104	DYTISCIDAE	*ILYBIUS*	*MERIDIONALIS*	AUBÉ, 1836	T	
105	DYTISCIDAE	*ILYBIUS*	*MONTANUS*	(STEPHENS, 1828)	T	
106	DYTISCIDAE	*RHANTUS*	*HISPANICUS*	SHARP, 1880-81	T	
107	DYTISCIDAE	*RHANTUS*	*SUTURALIS*	(MCLEAY, 1825)	T	
108	DYTISCIDAE	*COLYMBETES*	*FUSCUS*	(LINNAEUS, 1758)	T	
109	DYTISCIDAE	*COLYMBETES*	*SCHILDKNECHTI*	DETTNER, 1983	S	
110	DYTISCIDAE	*MELADEMA*	*CORIACEA*	CASTELNAU, 1834	T	
111	DYTISCIDAE	*ERETES*	*GRISEUS*	MOTSCHULSKY 1849	T	
112	DYTISCIDAE	*HYDATICUS*	*LEANDER*	(ROSSI, 1790)	T	
113	DYTISCIDAE	*HYDATICUS*	*SEMINIGER*	(DE GEER, 1774)	N	
114	DYTISCIDAE	*GRAPHODERUS*	*CINEREUS*	(LINNAEUS, 1758)	N	
115	DYTISCIDAE	*ACILIUS*	*SULCATUS*	(LINNAEUS, 1758)	T	
116	DYTISCIDAE	*DYTISCUS*	*CIRCUMFLEXUS*	FABRICIUS, 1801	N	
117	DYTISCIDAE	*DYTISCUS*	*MARGINALIS*	LINNAEUS, 1758	N	
118	DYTISCIDAE	*DYTISCUS*	*PISANUS*	CASTELNAU, 1834	T	
119	DYTISCIDAE	*DYTISCUS*	*SEMISULCATUS*	MÜLLER, 1776	T	
120	DYTISCIDAE	*CYBISTER*	*LATERALIMARGINALIS*	(DE GEER, 1774)	T	
121	HELOPHORIDAE	*HELOPHORUS*	*NUBILUS*	FABRICIUS, 1776	N	
122	HELOPHORIDAE	*HELOPHORUS*	*ALTERNANS*	GENÉ, 1836	N	
123	HELOPHORIDAE	*HELOPHORUS*	*AQUATICUS*	(LINNAEUS, 1758)	T	
124	HELOPHORIDAE	*HELOPHORUS*	*OCCIDENTALIS*	ANGUS, 1983	S	
125	HELOPHORIDAE	*HELOPHORUS*	*BAMEULI*	ANGUS, 1987	X	MODERATE
126	HELOPHORIDAE	*HELOPHORUS*	*BREVIPALPIS*	BEDEL, 1881	N	
127	HELOPHORIDAE	*HELOPHORUS*	*ASTURIENSIS*	KUWERT, 1885	T	
128	HELOPHORIDAE	*HELOPHORUS*	*FLAVIPES*	FABRICIUS, 1792	N	
129	HELOPHORIDAE	*HELOPHORUS*	*FULGIDICOLLIS*	MOTSCHUSLKY, 1860	T	

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
130	HELOPHORIDAE	*HELOPHORUS*	*LONGITARSIS*	WOLLASTON, 1864	T	
131	HELOPHORIDAE	*HELOPHORUS*	*MINUTUS*	FABRICIUS, 1775	N	
132	HELOPHORIDAE	*HELOPHORUS*	*OBSCURUS*	MULSANT, 1844	N	
133	HELOPHORIDAE	*HELOPHORUS*	*SEIDLITZII*	KUWERT, 1885	X	MODERATE
134	HYDROCHIDAE	*HYDROCHUS*	*ANGUSTATUS*	GERMAR, 1824	T	
135	HYDROCHIDAE	*HYDROCHUS*	*FLAVIPENNIS*	KÜSTER, 1852	T	
136	HYDROCHIDAE	*HYDROCHUS*	*GRANDICOLLIS*	KIESENWETTER, 1870	T	
137	HYDROCHIDAE	*HYDROCHUS*	*IBERICUS*	VALLADARES, DÍAZ-PAZOS & DELGADO, 1999	X	MODERATE
138	HYDROCHIDAE	*HYDROCHUS*	*INTERRUPTUS*	HEYDEN, 1870	X	HIGH
139	HYDROCHIDAE	*HYDROCHUS*	*NITIDICOLLIS*	MULSANT, 1844	T	
140	HYDROCHIDAE	*HYDROCHUS*	*NOOREINUS*	HENEGOUVEN & SÁINZ-CANTERO, 1992	X	MODERATE
141	HYDROCHIDAE	*HYDROCHUS*	*SMARAGDINEUS*	FAIRMAIRE, 1879	T	
142	HYDROPHILIDAE	*BEROSUS*	*AFFINIS*	BRULLÉ, 1835	T	
143	HYDROPHILIDAE	*BEROSUS*	*HISPANICUS*	KÜSTER, 1847	T	
144	HYDROPHILIDAE	*BEROSUS*	*SIGNATICOLLIS*	(CHARPENTIER, 1825)	T	
145	HYDROPHILIDAE	*BEROSUS*	*GUTTALIS*	REY, 1883	T	
146	HYDROPHILIDAE	*HEMISPHAERA*	*GUIGNOTI*	SHAEFER, 1975	T	
147	HYDROPHILIDAE	*CHAETARTHRIA*	*SEMINULUM-SIMILLIMA*	(HERBST, 1797)/VORST & CUPPEN, 2003	T	
148	HYDROPHILIDAE	*CHAETARTHRIA*	*SIMILIS*	WOLLASTON, 1864	T	
149	HYDROPHILIDAE	*PARACYMUS*	*AENEUS*	(GERMAR, 1824)	T	
150	HYDROPHILIDAE	*PARACYMUS*	*PHALACROIDES*	(WOLLASTON, 1867)	T	
151	HYDROPHILIDAE	*PARACYMUS*	*SCUTELLARIS*	(ROSENHAUER, 1856)	T	
152	HYDROPHILIDAE	*ANACAENA*	*BIPUSTULATA*	(MARSHAM, 1802)	T	
153	HYDROPHILIDAE	*ANACAENA*	*GLOBULUS*	(PAYKULL, 1798)	T	
154	HYDROPHILIDAE	*ANACAENA*	*LUTESCENS*	(STEPHENS, 1829)	T	
155	HYDROPHILIDAE	*ANACAENA*	*LIMBATA*	(FABRICIUS, 1792)	N	
156	HYDROPHILIDAE	*LACCOBIUS*	*GRACILIS GRACILLIS*	MOTSCHULSKY, 1849	T	
157	HYDROPHILIDAE	*LACCOBIUS*	*FEMORALIS*	REY, 1885	T	
158	HYDROPHILIDAE	*LACCOBIUS*	*REVELIERI*	PERRIS, 1864	S	
159	HYDROPHILIDAE	*LACCOBIUS*	*ATRATUS*	ROTTENBERG, 1874	T	
160	HYDROPHILIDAE	*LACCOBIUS*	*ATROCEPHALUS*	REITTER, 1872	S	
161	HYDROPHILIDAE	*LACCOBIUS*	*FRESNEDAI*	GENTILI & FIKÁCEK, 2015	T	
162	HYDROPHILIDAE	*LACCOBIUS*	*HISPANICUS*	GENTILI, 1974	T	
163	HYDROPHILIDAE	*LACCOBIUS*	*MORAGUESI*	RÉGIMBART, 1898	T	
164	HYDROPHILIDAE	*LACCOBIUS*	*NEAPOLITANUS*	ROTTENBERG, 1874	T	
165	HYDROPHILIDAE	*LACCOBIUS*	*OBSCURATUS*	ROTTENBERG, 1874	N	
166	HYDROPHILIDAE	*LACCOBIUS*	*SINUATUS*	MOTSCHULSKY, 1849	T	
167	HYDROPHILIDAE	*LACCOBIUS*	*STRIATULUS*	(FABRICIUS, 1801)	N	
168	HYDROPHILIDAE	*LACCOBIUS*	*YTENENSIS*	SHARP, 1910	T	
169	HYDROPHILIDAE	*HELOCHARES*	*LIVIDUS*	(FORSTER, 1771)	T	
170	HYDROPHILIDAE	*HELOCHARES*	*PUNCTATUS*	SHARP, 1869	N	
171	HYDROPHILIDAE	*ENOCHRUS*	*ATER*	(KUWERT, 1888)	T	
172	HYDROPHILIDAE	*ENOCHRUS*	*BICOLOR*	(FABRICIUS, 1792)	T	

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
173	HYDROPHILIDAE	*ENOCHRUS*	*JESUSARRIBASI*	ARRIBAS & MILLÁN, 2013	X	HIGH
174	HYDROPHILIDAE	*ENOCHRUS*	*FUSCIPENNIS*	(THOMSON, 1884)	T	
175	HYDROPHILIDAE	*ENOCHRUS*	*HALOPHILUS*	(BEDEL, 1878)	T	
176	HYDROPHILIDAE	*ENOCHRUS*	*POLITUS*	KÜSTER, 1849	T	
177	HYDROPHILIDAE	*ENOCHRUS*	*SALOMONIS*	(SAHLBERG, 1900)	N	
178	HYDROPHILIDAE	*ENOCHRUS*	*MORENAE*	(HEYDEN, 1870)	N	
179	HYDROPHILIDAE	*ENOCHRUS*	*NIGRITUS*	(SHARP, 1872)	T	
180	HYDROPHILIDAE	*LIMNOHYDROBIUS*	*CONVEXUS*	(BRULLÉ, 1835)	T	
181	HYDROPHILIDAE	*HYDROBIUS*	*FUSCIPES*	(LINNAEUS, 1758)	T	
182	HYDROPHILIDAE	*LIMNOXENUS*	*NIGER*	(ZSCHACH, 1788)	N	
183	HYDROPHILIDAE	*LIMNOXENUS*	*OLMOI*	HERNANDO & FRESNEDA, 1993	S	
184	HYDROPHILIDAE	*HYDROCHARA*	*FLAVIPES*	(STEVEN, 1808)	T	
185	HYDROPHILIDAE	*HYDROPHILUS*	*PISTACEUS*	(CASTELNAU, 1840)	T	
186	HYDROPHILIDAE	*COELOSTOMA*	*HISPANICUM*	(KÜSTER, 1848)	T	
187	HYDROPHILIDAE	*COELOSTOMA*	*ORBICULARE*	(FABRICIUS, 1775)	N	
188	HYDRAENIDAE	*HYDRAENA*	*ALTAMIRENSIS*	DÍAZ PAZOS & GARRIDO, 1993	X	HIGH
189	HYDRAENIDAE	*HYDRAENA*	*EXASPERATA*	ORCHYMONT, 1935	X	MODERATE
190	HYDRAENIDAE	*HYDRAENA*	*GRACILIDELPHIS*	TRIZZINO, VALLADARES, GARRIDO & AUDISIO	N	
191	HYDRAENIDAE	*HYDRAENA*	*HISPANICA*	GANGLBAUER, 1901	X	MODERATE
192	HYDRAENIDAE	*HYDRAENA*	*IBERICA*	ORCHYMONT, 1936	X	MODERATE
193	HYDRAENIDAE	*HYDRAENA*	*MADRONENSIS**	CASTRO, GARCÍA & FERRERAS, 2000	X	HIGH
194	HYDRAENIDAE	*HYDRAENA*	*MANFREDJAECHI*	DELGADO & SOLER, 1991	X	MODERATE
195	HYDRAENIDAE	*HYDRAENA*	*TRUNCATA*	REY, 1885	N	
196	HYDRAENIDAE	*HYDRAENA*	*AFUSSA*	ORCHYMONT, 1936	X	MODERATE
197	HYDRAENIDAE	*HYDRAENA*	*ANGULOSA*	MULSANT, 1844	N	
198	HYDRAENIDAE	*HYDRAENA*	*BARROSI*	ORCHYMONT, 1934	N	
199	HYDRAENIDAE	*HYDRAENA*	*BISULCATA*	REY, 1884	S	
200	HYDRAENIDAE	*HYDRAENA*	*BOLIVARI*	ORCHYMONT, 1936	X	MODERATE
201	HYDRAENIDAE	*HYDRAENA*	*BRACHYMERA*	ORCHYMONT, 1936	N	
202	HYDRAENIDAE	*HYDRAENA*	*CAPTA*	ORCHYMONT, 1936	S	
203	HYDRAENIDAE	*HYDRAENA*	*CARBONARIA*	KIESENWETTER, 1849	N	
204	HYDRAENIDAE	*HYDRAENA*	*CORDATA*	SCHAUFUSS, 1833	T	
205	HYDRAENIDAE	*HYDRAENA*	*CORINNA*	ORCHYMONT, 1936	X	MODERATE
206	HYDRAENIDAE	*HYDRAENA*	*CORRUGIS*	ORCHYMONT, 1934	N	
207	HYDRAENIDAE	*HYDRAENA*	*INAPICIPALPIS*	PIC, 1918	N	
208	HYDRAENIDAE	*HYDRAENA*	*MARINAE*	CASTRO, 2004	X	HIGH
209	HYDRAENIDAE	*HYDRAENA*	*MECAI**	MILLÁN & AGUILERA, 2000	X	HIGH
210	HYDRAENIDAE	*HYDRAENA*	*MINUTISSIMA*	STEPHENS 1829	N	
211	HYDRAENIDAE	*HYDRAENA*	*PYGMAEA*	WATERHOUSE, 1833	N	
212	HYDRAENIDAE	*HYDRAENA*	*QUETIAE**	CASTRO, 2000	X	HIGH
213	HYDRAENIDAE	*HYDRAENA*	*QUILISI*	LAGAR, FRESNEDA & HERNANDO, 1987	N	

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
214	HYDRAENIDAE	*HYDRAENA*	*RIPARIA*	KUGELANN, 1794	N	
215	HYDRAENIDAE	*HYDRAENA*	*RUFIPENNIS*	BOSCÁ BERGA, 1932	N	
216	HYDRAENIDAE	*HYDRAENA*	*SERVILLA*	ORCHYMONT, 1936	X	HIGH
217	HYDRAENIDAE	*HYDRAENA*	*SHARPI*	REY, 1886	X	MODERATE
218	HYDRAENIDAE	*HYDRAENA*	*STUSSINERI*	KUWERT, 1888	N	
219	HYDRAENIDAE	*HYDRAENA*	*SUBIMPRESA*	REY, 1885	N	
220	HYDRAENIDAE	*HYDRAENA*	*UNCA*	VALLADARES, 1989	X	MODERATE
221	HYDRAENIDAE	*HYDRAENA*	*ATRATA*	DESBROCHES DES LOGES, 1891	T	
222	HYDRAENIDAE	*HYDRAENA*	*HERNANDOI*	FRESNEDA & LAGAR, 1990	S	
223	HYDRAENIDAE	*HYDRAENA*	*TESTACEA*	CURTIS, 1830	T	
224	HYDRAENIDAE	*LIMNEBIUS*	*CORDOBANUS*	ORCHYMONT, 1938	X	MODERATE
225	HYDRAENIDAE	*LIMNEBIUS*	*EVANESCENS*	KIESENWETTER, 1865	S	
226	HYDRAENIDAE	*LIMNEBIUS*	*EXTRANEUS*	ORCHYMONT, 1938	S	
227	HYDRAENIDAE	*LIMNEBIUS*	*FRETALIS*	PEYERIMHOFF, 1912	S	
228	HYDRAENIDAE	*LIMNEBIUS*	*FURCATUS*	BAUDI, 1872	T	
229	HYDRAENIDAE	*LIMNEBIUS*	*GERHARDTI*	HEYDEN, 1870	X	MODERATE
230	HYDRAENIDAE	*LIMNEBIUS*	*HISPANICUS*	ORCHYMONT, 1941	X	MODERATE
231	HYDRAENIDAE	*LIMNEBIUS*	*LUSITANUS*	BALFOUR-BROWNE, 1978	X	MODERATE
232	HYDRAENIDAE	*LIMNEBIUS*	*MAURUS*	BALFOUR-BROWNE, 1978	S	
233	HYDRAENIDAE	*LIMNEBIUS*	*MILLANI*	RIBERA & HERNANDO, 1998	X	HIGH
234	HYDRAENIDAE	*LIMNEBIUS*	*NANUS*	JÄCH, 1993	X	MODERATE
235	HYDRAENIDAE	*LIMNEBIUS*	*OBLONGUS*	REY, 1883	T	
236	HYDRAENIDAE	*LIMNEBIUS*	*PAPPOSUS*	MULSANT, 1844	N	
237	HYDRAENIDAE	*LIMNEBIUS*	*TRUNCATELLUS*	(THUNBERG, 1794)	N	
238	HYDRAENIDAE	*OCHTHEBIUS*	*EXARATUS*	MULSANT, 1844	T	
239	HYDRAENIDAE	*OCHTHEBIUS*	*AENEUS*	STEPHENS, 1835	T	
240	HYDRAENIDAE	*OCHTHEBIUS*	*BELLIERI*	KUWERT, 1887	X	MODERATE
241	HYDRAENIDAE	*OCHTHEBIUS*	*BICOLON*	GERMAR, 1824	T	
242	HYDRAENIDAE	*OCHTHEBIUS*	*BONNAIREI*	GUILLEBAU, 1896	S	
243	HYDRAENIDAE	*OCHTHEBIUS*	*DILATATUS*	STEPHENS, 1829	T	
244	HYDRAENIDAE	*OCHTHEBIUS*	*FIGUEROORUM*	GARRIDO, VALLADARES & RÉGIL, 1991	S	
245	HYDRAENIDAE	*OCHTHEBIUS*	*HEYDENI*	KUWERT, 1887	N	
246	HYDRAENIDAE	*OCHTHEBIUS*	*IRENAE*	RIBERA & MILLÁN, 1998	X	HIGH
247	HYDRAENIDAE	*OCHTHEBIUS*	*JAIMEI*	DELGADO & JÄCH, 2007	X	HIGH
248	HYDRAENIDAE	*OCHTHEBIUS*	*EXSCULPTUS*	GERMAR, 1824	N	
249	HYDRAENIDAE	*OCHTHEBIUS*	*ALBACETINUS*	FERRO, 1984	X	HIGH
250	HYDRAENIDAE	*OCHTHEBIUS*	*AUROPALLENS*	FAIRMAIRE, 1879	S	
251	HYDRAENIDAE	*OCHTHEBIUS*	*CAESARAUGUSTAE*	JÄCH, RIBERA & AGUILERA, 1998	X	HIGH
252	HYDRAENIDAE	*OCHTHEBIUS*	*CORRUGATUS*	ROSENHAUER, 1856	S	
253	HYDRAENIDAE	*OCHTHEBIUS*	*CUPRESCENS*	GUILLENBAU, 1893	S	
254	HYDRAENIDAE	*OCHTHEBIUS*	*DELGADOI*	JÄCH, 1994	X	HIGH
255	HYDRAENIDAE	*OCHTHEBIUS*	*DENTIFER*	REY, 1885	N	
256	HYDRAENIDAE	*OCHTHEBIUS*	*DIFFICILIS*	MULSANT, 1844	T	

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
257	HYDRAENIDAE	*OCHTHEBIUS*	*GLABER*	MONTES & SOLER, 1988	X	HIGH
258	HYDRAENIDAE	*OCHTHEBIUS*	*GRANDIPENNIS*	FAIRMAIRE, 1879	S	
259	HYDRAENIDAE	*OCHTHEBIUS*	*JUDEMAESI*	DELGADO & JÄCH, 2007	S	
260	HYDRAENIDAE	*OCHTHEBIUS*	*MARINUS*	(PAYKULL, 1798)	N	
261	HYDRAENIDAE	*OCHTHEBIUS*	*MEDITERRANEUS*	IENISTEA, 1988	T	
262	HYDRAENIDAE	*OCHTHEBIUS*	*MERIDIONALIS*	REY, 1885	T	
263	HYDRAENIDAE	*OCHTHEBIUS*	*MERINIDICUS*	FERRO, 1985	S	
264	HYDRAENIDAE	*OCHTHEBIUS*	*NANUS*	STEPHENS, 1829	T	
265	HYDRAENIDAE	*OCHTHEBIUS*	*NOTABILIS*	ROSENHAUER, 1856	S	
266	HYDRAENIDAE	*OCHTHEBIUS*	*PUNCTATUS*	STEPHENS, 1829	T	
267	HYDRAENIDAE	*OCHTHEBIUS*	*QUADRIFOSSULATUS*	WALTL, 1835	S	
268	HYDRAENIDAE	*OCHTHEBIUS*	*QUADRIFOVEOLATUS*	WOLLASTON, 1854	S	
269	HYDRAENIDAE	*OCHTHEBIUS*	*SEMISERICEUS*	SAINT-CLAIRE DEVILLE, 1908	T	
270	HYDRAENIDAE	*OCHTHEBIUS*	*SEMOTUS*	JÄCH, 2001	X	HIGH
271	HYDRAENIDAE	*OCHTHEBIUS*	*TACAPASENSIS BAETICUS*	FERRO, 1984	X	HIGH
272	HYDRAENIDAE	*OCHTHEBIUS*	*TUDMIRENSIS*	JÄCH, 1997	X	HIGH
273	HYDRAENIDAE	*OCHTHEBIUS*	*VIRIDESCENS*	IENISTEA, 1988	T	
274	HYDRAENIDAE	*OCHTHEBIUS*	*VIRIDIS FALLACIOSUS*	GANGLBAUER, 1901	T	
275	ELMIDAE	*POTAMOPHILUS*	*ACUMINATUS*	(FABRICIUS, 1792)	T	
276	ELMIDAE	*DUPOPHILUS*	*BREVIS*	MULSANT & REY, 1872	N	
277	ELMIDAE	*STENELMIS*	*CANALICULATA*	(GYLLENHAL, 1808)	N	
278	ELMIDAE	*ELMIS*	*AENEA*	(MÜLLER, 1806)	T	
279	ELMIDAE	*ELMIS*	*MAUGETII MAUGETII*	LATREILLE, 1798	N	
280	ELMIDAE	*ELMIS*	*RIOLOIDES*	(KUWERT, 1890)	N	
281	ELMIDAE	*ESOLUS*	*PARALLELEPIPEDUS*	(MÜLLER, 1806)	T	
282	ELMIDAE	*ESOLUS*	*PYGMAEUS*	(MÜLLER, 1806)	T	
283	ELMIDAE	*OULIMNIUS*	*BERTRANDI*	BERTHÉLEMY, 1964	X	HIGH
284	ELMIDAE	*OULIMNIUS*	*MAJOR*	(REY, 1889)	T	
285	ELMIDAE	*OULIMNIUS*	*RIVULARIS*	(ROSENHAUER, 1856)	T	
286	ELMIDAE	*OULIMNIUS*	*TROGLODYTES*	(GYLLENHAL, 1827)	T	
287	ELMIDAE	*OULIMNIUS*	*TUBERCULATUS PEREZI*	SHARP, 1872	X	HIGH
288	ELMIDAE	*LIMNIUS*	*INTERMEDIUS*	FAIRMAIRE, 1881	T	
289	ELMIDAE	*LIMNIUS*	*OPACUS*	MÜLLER, 1806	T	
290	ELMIDAE	*LIMNIUS*	*PERRISI CARINATUS*	PEREZ-ARCAS, 1865	X	MODERATE
291	ELMIDAE	*LIMNIUS*	*VOLCKMARI*	(PANZER, 1793)	N	
292	ELMIDAE	*RIOLUS*	*NITENS*	(MÜLLER, 1817)	N	
293	ELMIDAE	*RIOLUS*	*SODALIS*	(ERICHSON, 1847)	N	
294	ELMIDAE	*RIOLUS*	*CUPREUS*	(MÜLLER, 1806)	N	
295	ELMIDAE	*RIOLUS*	*ILLIESI*	STEFFAN, 1958	N	
296	ELMIDAE	*RIOLUS*	*SUBVIOLACEUS*	(MÜLLER, 1817)	N	
297	DRYOPIDAE	*POMATINUS*	*SUBSTRIATUS*	(MÜLLER, 1806)	T	
298	DRYOPIDAE	*DRYOPS*	*ALGIRICUS*	(LUCAS, 1849)	T	
299	DRYOPIDAE	*DRYOPS*	*GRACILIS*	(KARSCH, 1881)	T	
300	DRYOPIDAE	*DRYOPS*	*LURIDUS*	(ERICHSON, 1847)	T	

	FAMILY	GENUS	SPECIES	AUTHOR	BR	V
301	DRYOPIDAE	*DRYOPS*	*LUTULENTUS*	(ERICHSON, 1847)	T	
302	DRYOPIDAE	*DRYOPS*	*NITIDULUS*	(HEER, 1841)	N	
303	DRYOPIDAE	*DRYOPS*	*SULCIPENNIS*	(COSTA, 1883)	T	

Los coleópteros acuáticos y semiacuáticos de la comarca de El Bierzo (León, NO de España) (Coleoptera: Gyrinidae, Haliplidae, Noteridae, Hygrobiidae, Dytiscidae, Helophoridae, Georissidae, Hydrochidae, Hydrophilidae, Hydraenidae, Scirtidae, Dryopidae, Elmidae, Heteroceridae) - Reunión del Balfour~Browne Club, 25-30 junio 2015

Aquatic and semi-aquatic Coleoptera from El Bierzo region (León, NW Spain) (Coleoptera: Gyrinidae, Haliplidae, Noteridae, Hygrobiidae, Dytiscidae, Helophoridae, Georissidae, Hydrochidae, Hydrophilidae, Hydraenidae, Scirtidae, Elmidae, Dryopidae, Heteroceridae) - Balfour~Browne Club Meeting, 25-30th June 2015

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RESUMEN

Se presenta la relación de especies de coleópteros acuáticos y semiacuáticos capturadas en la reunión del Balfour~Browne Club de 2015 realizada en Villafranca del Bierzo (León, España). Se incluye un listado de 139 especies y subespecies de las familias Gyrinidae, Haliplidae, Noteridae, Hygrobiidae, Dytiscidae, Helophoridae, Georissidae, Hydrochidae, Hydrophilidae, Hydraenidae, Scirtidae, Dryopidae, Elmidae y Heteroceridae, de las que 23 son novedades para la comarca del Bierzo. Con las citas previas, este número se elevaría a 184 especies. Se comentan las especies con mayor interés de conservación. En el Bierzo destaca la elevada riqueza, con mayoría de especies de amplia distribución, pero con un elevado nivel de endemismos ibéricos (20 %) asociados a arroyos de montaña. Se discute el alto interés en conservación de esta zona para los coleópteros acuáticos y la amplia cobertura de protección territorial de sus hábitats.

Palabras clave: escarabajos acuáticos, El Bierzo, península ibérica, faunística, Balfour~Browne Club, conservación.

ABSTRACT

The list of aquatic and semi-aquatic beetle species collected at the meeting of the Balfour~Browne Club in Villafranca del Bierzo (León, Spain) in 2015 is reported here. It includes a list of 139 species and subspecies of the families Gyrinidae, Haliplidae, Noteridae, Hygrobiidae, Dytiscidae, Helophoridae, Georissidae, Hydrochidae, Hydrophilidae, Hydraenidae, Scirtidae, Elmidae, Dryopidae and Heteroceridae, of which 23 are new records for El Bierzo region. Including the previous records, the number of known species to El Bierzo raise to 184. Some of the most interesting species from a conservation perspective are discussed. We can highlight the great species richness of El Bierzo. The high value of species richness in El Bierzo is highlighted. Although most of the species have a wide distribution, we also found a great number of Iberian endemic species (20%), most of them related to mountain streams. We also discuss the high conservation interest of this area for aquatic beetles and the wide coverage of protection of their habitats.

Key words: water beetles, El Bierzo, Iberian Peninsula, faunistics, Balfour~Browne Club, conservation.

INTRODUCCIÓN

El Balfour~Browne Club es una asociación internacional dedicada el estudio de los coleópteros acuáticos, fundada en Reino Unido en 1976. Desde 1988 realiza reuniones anuales en distintos países de Europa y norte de África, en las que los participantes intercambian información sobre este grupo y realizan muestreos conjuntos en la zona de la reunión. En España se han realizado reuniones del Balfour~Browne Club en Villamanín (León, 1990), Barcelona (1994), Riópar (Albacete, 2002), Rascafría (Madrid, 2007), Hernani (Gipuzkoa, 2012) y Villafranca del Bierzo (León, 2015).

El presente trabajo reúne los resultados de los muestreos realizados durante la reunión de Villafranca del Bierzo, que se realizó a finales de junio de 2015. En el *meeting* se reunieron 35 coleopterólogos de 10 países (Alemania, Australia, Austria/Bielorusia, Brasil, España, Francia, Holanda, Luxemburgo, Reino Unido y Suecia) que prospectaron los diferentes medios acuáticos de la comarca del Bierzo, en el oeste de la provincia de León, y algunas zonas contiguas de la Cordillera Cantábrica. La mayoría de los muestreos se realizaron en las cuatro áreas protegidas de esta comarca: i) la Zona de Especial Conservación (ZEC) Sierra de los Ancares que se superpone en gran parte con ii) la reserva de la Biosfera de los Ancares Leoneses que incluye iii) la Reserva Natural Fluvial del río Burbia y iv) la ZEC Montes Aquilanos y Sierra del Teleno en cuyo extremo occidental se sitúa el Monumento Natural de Las Médulas y el Lago de Carucedo.

Los estudios previos y la recopilación en el atlas de MILLÁN *et al.* (2014) contabilizaban en la zona 161 especies y subespecies de coleópteros acuáticos (excluidos los taxones semiacuáticos).

MATERIAL Y MÉTODOS

Área de estudio

El Bierzo es una comarca natural situada en el noroeste de la península ibérica, al oeste de la provincia de León, formada por una periferia montañosa que contacta con las provincias de Asturias, Lugo y Orense y una depresión central cuyo eje es la cuenca del río Sil con dirección NE-SO (Fig. 1). Esta zona central, formada por dos cubetas sucesivas, está dominada por plataformas de conglomerados (HÉRAIL, 1984) donde llegan los principales afluentes del Sil (Tremor, Boeza, Noceda y Cabrera por su margen izquierdo y Cúa, Burbia, Selmo y Valcarce por la margen derecha). La altitud media de la cubeta es de 500 m, pero está rodeada de importantes macizos montañosos (Cordillera Cantábrica, Montes de León, Montes Aquilanos, Caurel y Ancares) de naturaleza mayoritariamente silícea que llegan a sobrepasar los 2000 m. De las zonas húmedas destacan el lago de Carucedo y las lagunas de las Médulas, cuyo origen está relacionado con una explotación aurífera de época romana. En el Bierzo se encuentran las regiones biogeográficas Eurosiberiana y Mediterránea. El clima es de tipo mediterráneo pero de clara influencia atlántica, con inviernos fríos y veranos suaves (730 mm de precipitación media anual; 12,3 °C temperatura media anual). Lógicamente este clima no es uniforme, ya que en las zonas montañosas las temperaturas son más bajas y la precipitación supera los 900 mm de media.

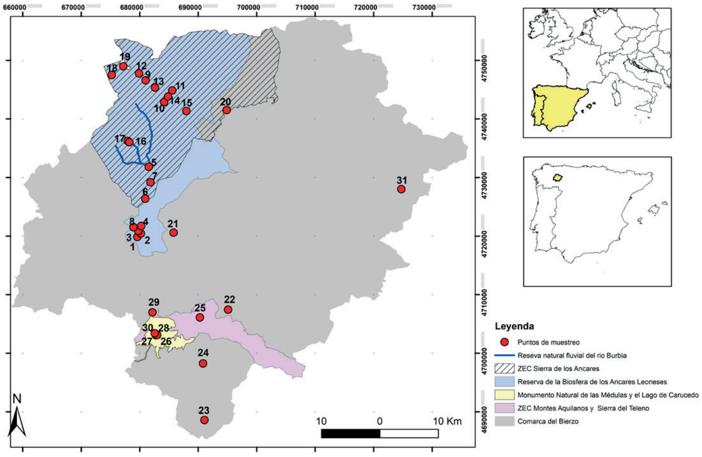

Fig. 1. Localización y mapa de la comarca del Bierzo, con indicación de las áreas protegidas y las localidades muestreadas (códigos de las localidades en la Apéndice I).

Fig. 1. Location and map of El Bierzo region, with the protected areas and sampling localities (locality codes in Appendix I).

Metodología

Los ejemplares se recogieron mediante los procedimientos habituales en entomología acuática, principalmente captura directa con manga acuática tipo D-net de 250 µm de luz de malla. En charcas y lagunas se muestreó en los márgenes y hacia el interior del medio acuático removiendo el sustrato y la vegetación sumergida. En arroyos y ríos, para muestrear en la corriente, se coloca la manga sobre el sustrato con la boca hacia la corriente para capturar los ejemplares que arrastra el agua al remover los cantos, arena, limo y vegetación precedente. Complementariamente, un colador sirvió para capturar los ejemplares que flotan en el agua al remover el sustrato y la vegetación de las orillas de los medios acuáticos. En cada punto de muestreo fueron prospectados los diferentes hábitats hasta que (aparentemente) no aparecieron nuevas especies.

Las capturas se realizaron entre los días 26 y 30 de junio de 2015 en 31 localidades repartidas por gran parte del Bierzo. La mayoría de los muestreos se realizaron en aguas corrientes (26), pero también hay una representación de lagos y lagunas (5), principalmente en el área de las Médulas. La lista de localidades de muestreo se presenta en la Apéndice I y se representa en el mapa de la Fig. 1. Algunos ambientes prospectados se muestran en las fotografías de la Fig. 2. Muchas localidades son el resultado de la agrupación de los puntos de muestreo visitados por los participantes, siendo algunas coordenadas geográficas una aproximación a varias muestras situadas a pocos metros de distancia. Se han establecido 11 agrupaciones (A-K), correspondiendo una zona a los medios estancados y otras 10 a ríos y sus afluentes, aunque en alguna de estas zonas puede haber fuentes con pilones (Apéndice I). Las localidades de captura prospectadas por los participantes fuera de la comarca del Bierzo (otras zonas de León o en las provincias de Lugo, Ourense o Palencia) no han sido incluidas en el presente trabajo.

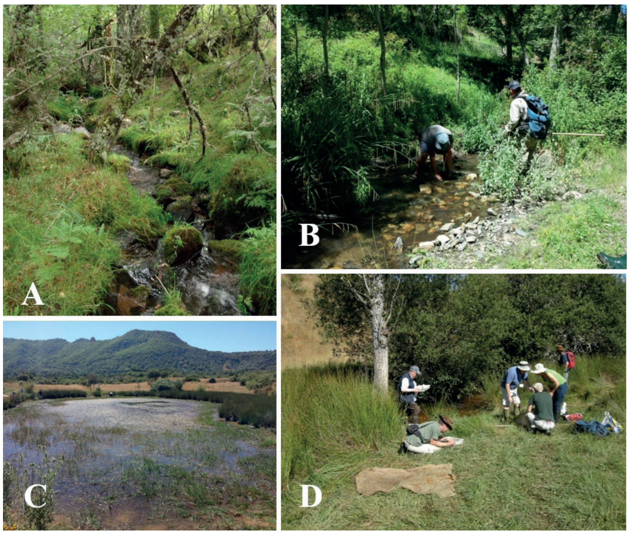

Fig. 2. Algunos hábitats prospectados: A) Loc. 12, Puerto de Ancares, afluente del río Cuiña, hábitat de *Hydraena monstruosipes*; B) Loc. 22, río Ozuela, en las estribaciones de los Montes Aquilanos; C) Loc. 26, Las Médulas, Laguna Larga 1; D: Loc. 27, Las Médulas, extremo limoso de la Laguna Larga 2, con un grupo de recolectores, Ignacio Ribera en primer plano, agachado. Fotos: A-C, Josefina Garrido; D, Matt Smith.

Fig. 2. Some sampling habitats: A) Loc. 12, Puerto de Ancares, Cuiña river tributary, habitat of *Hydraena monstruosipes*; B) Loc. 22, Río Ozuela, Montes Aquilanos foothills; C) Loc. 26, Las Médulas, Laguna Larga 1, loc. 26; D) Loc. 27, Las Médulas, muddy end of Laguna Larga 2, with a collectors group, Ignacio Ribera in the foreground. Photos: A-C, Josefina Garrido; D, Matt Smith.

Para los aspectos biogeográficos se utilizaron las categorías propuestas por RIBERA *et al.* (1999a) y seguidas por MILLÁN *et al.* (2014): especies de amplia distribución o Transibéricas (T), iberoeuropeas o de distribución Norte (N), iberoafricanas o de distribución Sur (S) y endemismos ibéricos (E). Para asignar el grado de vulnerabilidad de las especies endémicas se han utilizado las clases asignadas por SÁNCHEZ-FERNÁNDEZ *et al.* (2008), actualizadas por MILLÁN *et al.* (2014): baja (B), moderada (M), alta (A) y muy alta, de la que no se han recogido especies en la zona.

RESULTADOS

Se han identificado un total de 139 especies y subespecies, pertenecientes a 14 familias. La lista de las especies identificadas y localidades de captura agrupadas se detalla en la Apéndice II, que también incluye el rango biogeográfico y la vulnerabilidad de las especies endémicas. La lista completa de especies, localidades y recolectores se recoge en el Apéndice III. Las especies citadas previamente en la zona, pero no capturadas en este muestreo, resultado de la revisión de los datos aportados por RÉGIL CUE-

TO (1982), VALLADARES DÍEZ (1988), GARRIDO GONZÁLEZ (1990), AGUILERA *et al.* (1998), GARCÍA CRIADO (1999) y la recopilación de MILLÁN *et al.* (2014) y SÁNCHEZ-FERNÁNDEZ *et al.* (2015) se relacionan en el Apéndice IV. Para la clasificación y la nomenclatura se han seguido los catálogos paleárticos de LÖBL & SMETANA (2003), NILSSON & HÁJEK (2022) y LÖBL & LÖBL (2015), con las aportaciones posteriores de RUDOY *et al.* (2016) y SHORT *et al.* (2017), siguiendo en cada familia un orden alfabético de géneros y especies. Se comentan a continuación las especies con mayor interés faunístico o biogeográfico.

Dytiscidae

Deronectes costipennis gignouxi Fery & Brancucci, 1989

Subespecie ibérica descrita de la provincia de León (FERY & BRANCUCCI, 1989) y aparentemente exclusiva de la Cordillera Cantábrica. Las citas disponibles indican que los montes orientales de Galicia marcan la barrera que, actualmente, separa las dos subespecies de *D. costipennis.* Mientras *Deronectes costipennis costipennis* Brancucci, 1983, se distribuye en las montañas de Portugal (Serra da Estrela) y de Galicia, *D. costipennis gignouxi* ocupa las vertientes leonesa y asturiana de la Cordillera Cantábrica (DE PAZ & OTERO, 1995; GONZÁLEZ & NOVOA, 1995; FERY & FRESNEDA, 2007; MILLÁN *et al*., 2014). Habita arroyos de cabecera, con preferencia por sustratos orgánicos con hojarasca en descomposición (MILLÁN *et al*., 2014). En el presente estudio, se ha capturado en 10 localidades y con numerosos ejemplares, lo que indica que es común en arroyos de montaña del Bierzo, la mayoría no calcáreos.

Hydrovatus cuspidatus (Kunze, 1818)

Repartida por la región Paleártica, en la península ibérica está citada sobre todo en el área mediterránea (MILLÁN *et al*., 2014). Su captura ahora en las Médulas (loc. 27) representa su primera cita en León y en las provincias del noroeste de España. Se ha encontrado en su hábitat característico de charcas bien vegetadas, hábitat que comparte con su otro congénere ibérico *Hydrovatus clypealis* Sharp, 1876.

Ilybius dettneri (Fery, 1986)

Especie endémica del noroeste peninsular, conocida de la mitad norte de Portugal y de algunas localidades del occidente de Galicia (FRESNEDA *et al*., 1990; FERY & NILSSON, 1993; PÉREZ-BILBAO *et al*., 2010). Las capturas del presente estudio suponen su primer registro para el Bierzo y para la provincia de León. Esta nueva localización extiende el área de distribución de la especie hacia el este peninsular, incluso de su distribución potencial (MILLÁN *et al*., 2014). Habita en charcas, generalmente de zonas de montaña, pero también se encuentra en charcas laterales y llanuras inundadas de arroyos y zonas remansadas de pequeños ríos (MILLÁN *et al*., 2014). Aquí se ha localizado en áreas encharcadas de praderas de alta montaña de los Ancares (locs. 18 y 19).

Oreodytes davisii rhianae Carr, 2001

Endemismo de las montañas de la mitad septentrional de la península ibérica (FERY & FRESNEDA, 2007). Aunque sus límites con la subespecie nominal no están bien establecidos (MILLÁN *et al*., 2014), nuestras capturas en tres localidades de los ríos Burbia (locs. 4 y 7) y Silván (loc. 23) confirman su presencia hacia el Oeste, en la cuenca del Sil, ya que las citas previas de León y Asturias se sitúan en cursos de los Picos de Europa o la Montaña Cantábrica Central que dirigen sus aguas al mar Cantábrico o a la cuenca del Duero. Taxón típico de remansos de arroyos de montaña (MILLÁN *et al.*, 2014), aunque con características variadas.

Stictonectes rebeccae Bilton, 2012 (Fig. 3)

Endemismo ibérico, conocido del Sistema Central y el noroeste de la península ibérica (BILTON, 2012; MILLÁN *et al*., 2013, 2014). Las presentes capturas suponen la primera cita para el Bierzo y la provincia de León, aunque es probable que algunos registros antiguos de *Stictonectes epipleuricus* (Seidlitz, 1887) correspondan a esta especie. Aunque, como ocurre con varias especies de *Stictonectes*, ambas especies podrían convivir (MILLÁN *et al*., 2013), en el presente estudio no han compartido estaciones de muestreo. *S. rebeccae* se recogió en dos localidades del río Burbia (locs. 3 y 4) y *S. epipleuricus* en tres localidades, dos también en el río Burbia (locs. 1 y 6) y en el río Cúa (loc. 20) todas ellas en altitudes entre 500-720 m. Coincidiendo con lo indicado por BILTON (2012), *S. rebeccae* se localiza en las orillas con cantos de arroyos de segundo orden de cursos de montaña de sustrato silíceo, conviviendo en este hábitat con ditíscidos reófilos endémicos como *Oreodytes davisii rhianae* Carr, 2001, *Deronectes bicostatus* (Schaum, 1864) y *Deronectes costipennis gignouxi.*

Fig. 3. *Stictonectes rebeccae* habitus (Dytiscidae). Foto: D.T. Bilton.

Fig. 3. *Stictonectes rebeccae* habitus (Dytiscidae). Photo: D.T. Bilton.

Helophoridae

Helophorus (Empleurus) schmidti A.Villa & G.B. Villa, 1838

Especie presente en Europa central y occidental (LÖBL & LÖBL, 2015), en la península ibérica se conoce sobre todo de zonas de montaña de la mitad septentrional, particularmente Pirineos, Cordillera Cantábrica y Sierra de Guadarrama (ANGUS, 1992; VALLADARES & RIBERA, 1999). En el presente estudio fue recogida en un único punto, en el río Cuiña, un arroyo de montaña afluente del río Ancares, a 980 m de altitud (loc. 13). Según ANGUS (1992) los adultos de esa especie son terrestres, pero como en nuestra captura, asociados a hábitats de aguas corrientes o estancadas de zonas altas (QUENEY & PRÉVOST, 2021).

Georissidae

Georissus (Neogeorissus) costatus Laporte de Castelneau, 1840

Especie paleártica (LÖBL & LÖBL, 2015), su distribución ibérica es muy poco conocida, estando citada de Andalucía, Madrid y el centro de Portugal (VALLADARES & RIBERA, 1999). Se constata, por tanto, su presencia en el cuadrante noroccidental de la Península. Vive en las orillas de las aguas corrientes, en los intersticios entre la arena y la grava (QUENEY & PRÉVOST, 2021), hábitat que coincide con el de la localidad de captura en el río Burbia (loc. 7).

Hydrochidae

Hydrochus interruptus Heyden, 1870

Endemismo ibérico distribuido por los Sistemas Central, Ibérico y la Cordillera Cantábrica (MIGUÉLEZ, & VALLADARES, 2014), con numerosas citas para la provincia de León que corresponden en parte a las citas previas de *Hydrochus nitidicollis* Mulsant, 1844 señaladas por VALLADARES (1995), especie con la que era confundida hasta el trabajo de RIBERA *et al.* (1999b). Ambas especies cohabitan y son relativamente comunes en las montañas del Bierzo, dato de interés si se tiene en cuenta que según HIDALGO-GALIANA & RIBERA (2011) tienen un origen común (se separaron en el Mesiniense, hace 6,2 MA). Habita principalmente las orillas de arroyos de cabecera y otros cursos de agua de mayor entidad, menos común en charcas de montaña, en altitudes comprendidas entre 504-2.121 m de altitud (MIGUÉLEZ & VALLADARES, 2014).

Hydrophilidae

Cercyon sternalis Sharp, 1918

Especie distribuida en gran parte de Europa y algunas zonas de Asia, pero que no estaba citada de la península ibérica (LÖBL & LÖBL, 2015). Además de la presente captura en el noroeste peninsular, existen registros no publicados de la especie en la costa oeste de Galicia (MARTÍNEZ SABARÍS & GARRIDO GONZÁLEZ, 2022) y los comunicados por G.N. Foster (*com. pers.)* en Cantabria (La Costana, 15.iv.1985, G.N. Foster leg.) y Almería (Albufera Nueva, cerca de Adra, 22.iv.1985, G.N. Foster leg.) que hacen suponer una distribución ibérica bastante amplia. Esta ausencia de datos se debe sin duda al escaso nivel de conocimiento de los Sphaeridiinae ibéricos. En el Bierzo se ha capturado en las orillas de un río (loc. 21) y de una laguna con abundante materia orgánica (loc. 28), hábitat que coincide con el típico de la especie en sustratos con vegetación en descomposición (FOSTER *et al*., 2014).

Chaetarthria similis Wollaston, 1864

Presente en Europa occidental, Israel, norte de África e islas Canarias (LÖBL & LÖBL, 2015). Está citada de forma dispersa por gran parte de España, pero no se conocía de todo el cuadrante noroccidental (MILLÁN *et al.*, 2014). Ha sido recolectada en dos localidades que corresponden a orillas con grava y limo de un curso de montaña (río Burbia, loc. 7) o de un tramo medio (río Cúa, loc. 21), en hábitats de aguas corrientes propios de la especie (MILLÁN *et al.*, 2014; QUENEY & PRÉVOST, 2021).

Hemisphaera seriatopunctata (Perris, 1874)

Especie del mediterráneo occidental. Muchas de las citas de esta especie anteriores a que CASTRO & DELGADO (1998) señalaran la presencia ibérica de *Hemisphaera guignoti* Schaefer, 1975, requerían confirmación. Tanto los ejemplares ahora estudiados, como el material revisado de la cita previa de León (VALLADARES, 1995), curiosamente ambos recogidos en la misma localidad de Cacabelos (río Cúa, loc. 21), confirman la presencia de *H. seriatopunctata* en la mitad norte (y hacia el oeste) de la península ibérica. Vive en charcas del estiaje en tramos medios de los ríos (MILLÁN *et al.*, 2014), hábitat que coincide con el de las presentes capturas, entre el limo y la grava de los márgenes de un río y canales de riego.

Hydraenidae

Hydraena (Hydraena) bisulcata Rey, 1884

Especie iberomagrebí, ampliamente citada en la mitad suroccidental peninsular, pero que cuenta con muy pocas citas en el noroeste ibérico (MILLÁN *et al.*, 2014; VALLADARES *et al.*, 2018). Aunque ya se conocía de la zona, sus escasos registros previos (siempre con muy pocos individuos) se localizan en el Bierzo Alto, ríos Noceda (VALLADARES, 1989) y Tremor (GARCÍA-CRIADO, 2002), capturándose ahora en la cabecera del valle de Ancares, en el río Cuiña (arroyo Miravalles, 1.040 m de altitud, loc. 9). Se trata de una especie que ocupa gran variedad de cursos de agua, muchas veces en tramos medios (VALLADARES *et al.*, 2018), pero que aquí se ha recogido en un pequeño curso de montaña con sustrato de pizarras y areniscas, donde se alternan corrientes rápidas y pequeñas pozas.

Hydraena (Hydraena) monstruosipes Ferro, 1986 (Fig. 4)

Endemismo cantábrico muy restringido, se distribuye por las montañas de la sierra de Ancares (León y Lugo), la sierra del Caurel (Lugo) y el suroccidente de Asturias. Se ha vuelto a capturar en dos arroyos del Puerto de Ancares (Fig. 2A), pero además una captura en el río Ozuela al sur del Bierzo (loc. 22) representa una novedad poblacional de interés en una zona suroriental desconocida para la especie. Este punto, situado en las estribaciones de los Montes Aquilanos (Fig. 2B), se corresponde con un pequeño arroyo de escasa profundidad y sustrato de pizarra, características típicas del hábitat de la especie (VALLADARES *et al.*, 2018). La especie es frecuente, pero no abundante en su área de distribución.

Fig. 4. *Hydraena monstruosipes* habitus del macho (Hydraenidae). Foto: D.T. Bilton.

Fig. 4. *Hydraena monstruosipes* male habitus (Hydraenidae). Photo: D.T. Bilton.

Limnebius (Bilimneus) myrmidon Rey, 1883

Especie de amplia distribución, que ocupa gran parte de Europa occidental y central, alcanzando Turquía (LÖBL & LÖBL, 2015). En la península ibérica solo se conocía del centro y este de la Cordillera Cantábrica, más algunas citas del río Palancia entre Castellón y Valencia (MILLÁN *et al.* (2014). Su captura ahora en el Bierzo, implica su presencia en la cuenca del Miño-Sil, siendo la cita más occidental de la especie. Se capturó en las orillas del río Burbia, aguas abajo de la localidad de Veguellina (loc. 7). El río Burbia es un cauce representativo de los ríos de montaña silícea húmeda, con el hábitat característico de esta especie, orillas o charcas marginales de este tipo de medios (MILLÁN *et al.*, 2014; VALLADARES *et al.*, 2018).

Dryopidae

Dryops nitidulus (Heer, 1841)

Especie paleártica, distribuida por Europa meridional y occidental y Turquía, recientemente registrada en Japón (YOSHITOMI & HAGA, 2018). En la península ibérica es una especie rara, con muy pocas citas en los Pirineos, Cordillera Cantábrica y Serranía de Cuenca (MILLÁN *et al.*, 2014). En el presente estudio fue capturada en el río Burbia (loc. 3) y en los márgenes del Lago de Carucedo (loc. 29). Estas capturas representan la primera cita de la especie para la mitad occidental peninsular, siendo nueva para la provincia de León. Según MILLÁN *et al.* (2014) es una especie torrentícola, característica de arroyos de cabecera, que se localiza entre las gravas y arenas de las orillas, dato que coincide con el del hábitat de la localidad 3 de las capturas.

Heteroceridae

***Heterocerus aragonicus* Kiesenwetter, 1850**

Especie del Mediterráneo occidental, en la península ibérica su distribución es bastante amplia, pero se desconocía de todo el cuadrante noroccidental con la excepción de dos citas costeras en Asturias y A Coruña (AGUILERA *et al.*, 1998). Aunque su hábitat característico son las orillas de charcas residuales de aguas corrientes, en el Bierzo se ha capturado en los márgenes de lagunas y charcas de las Médulas (locs. 26, 27 y 30) (Figs. 2C-D).

DISCUSIÓN

La fauna de coleópteros acuáticos del Bierzo, y por extensión de la provincia de León, está bastante bien estudiada, ya que esta zona se prospectó para la realización de varias tesis doctorales: RÉGIL CUETO (1982) para Hydradephaga, VALLADARES DÍEZ (1988) para Polyphaga, GARRIDO GONZÁLEZ (1990) para Hydradephaga y algunos grupos de Polyphaga y GARCÍA CRIADO (1999) para Hydraenidae y Elmidae. MILLÁN *et al.* (2014) recopilan la presencia de unas 156 especies en la comarca, sin incluir citas de las familias Georissidae, Heteroceridae y Scirtidae de hábitos semiacuáticos o incluso terrestres como ocurre con algunos géneros de hidrofílidos Sphaeridiinae. Incluidos los grupos semiacuáticos, en el presente trabajo se han identificado un total de 139 especies, 23 de ellas nuevas para el Bierzo. Estas novedades constituyen una cifra destacable, pues pese a los estudios previos, ponen de manifiesto el interés de llevar a cabo estudios colaborativos por expertos en el grupo, como el que se realizó en 2015. Añadiendo las citas previas, que incluyen 45 especies citadas pero no capturadas en el presente trabajo, puede considerarse que el número acumulado de especies de coleópteros acuáticos y semiacuáticos en el Bierzo se sitúa en 184 y que su conocimiento es ahora muy completo, ya que con anterioridad a este estudio la completitud estimada era ya superior al 80 % para esta zona (SÁNCHEZ-FERNÁNDEZ *et al.*, 2011).

La cifra de riqueza obtenida es muy alta para un área con una extensión de 3.179 Km^2 y no mucha variedad de hábitats, en su mayoría aguas corrientes de montaña o valle y unas pocas lagunas situadas en la cubeta central. Si se compara con áreas peninsulares bien estudiadas y con gran interés en conservación, el número de especies es aquí notablemente más alto, por ejemplo, Sierra Nevada alberga 103 especies (SÁINZ-CANTERO, 2013) o Doñana registra 110 especies (MILLÁN *et al.*, 2005). Otra comparación de interés es con las zonas estudiadas en dos reuniones precedentes del Balfour~Browne Club, cuyos resultados se han publicado. En la provincia de Gipuzkoa (BALFOUR~BROWNE CLUB, 2013) se inventariaron 99 especies (95 capturadas en la reunión) y en la Reserva natural del Val de Loire, en el centro de Francia (BALFOUR~BROWNE CLUB, 2015) se recogieron 147 especies.

En la composición biogeográfica de los 139 taxones identificados tienen una gran importancia los elementos transibéricos (49,64 %) y norteños, europeos básicamente (27,34 %), la presencia de endemismos ibéricos es destacable (20,86 %) y los elementos con distribución Sur es muy reducida (2,16 %). Si añadimos a estos taxones las citas previas no capturadas en el Bierzo (184 especies en total) la composición biogeográfica prácticamente no varía: T (49,45 %), N (28,80 %), S (2,17 %) y E (19,56 %). La situación del Bierzo en el norte de la península ibérica, pese a la influencia climática mediterránea, implica una fuerte dominancia de los elementos de amplia distribución, incluidos los europeos, que suman el 80 % de esta fauna. La proporción de endemismos peninsulares también es importante, 29 especies capturadas (34, incluidas las citas previas) pertenecientes en su mayoría a las familias Dytiscidae e Hydraenidae. Se trata mayoritariamente de especies de aguas corrientes, muchas de ellas de arroyos de montaña, de géneros como *Deronectes, Oreodytes, Hydroporus, Stictonectes, Hydraena* o *Hydrochus* que han sufrido importantes procesos de especiación. Algunos autores (RIBERA & VOGLER, 2000; RIBERA, 2008) han explicado este predominio de especies con distribución reducida por el papel de los medios lóticos, más estables y aislados, en la aparición de nuevas especies, principalmente cursos de montaña que son muy abundantes en la comarca del Bierzo. La capacidad dispersiva de las especies de ambientes lóticos (sobre todo en arroyos de montaña) es menor que en ambientes lénticos, influenciada por la diferente estabilidad geológica de estos medios. Esa menor capacidad de dispersión limita el flujo génico entre las diferentes poblaciones, ocasionando una mayor especiación. Un ejemplo lo representa *Hydraena monstruosipes,* la especie con distribución más restringida de la zona, que forma parte de un pequeño linaje de endemismos ibéricos (clado de *Hydraena tatii*) constituido por cuatro especies con áreas de distribución muy reducidas (RIBERA *et al.*, 2011).

Además de la riqueza, el interés en conservación de esta zona es elevado considerando el número y grado de vulnerabilidad de las 26 especies endémicas capturadas de las que se disponen datos (MILLÁN *et al.*, 2014). Predominan las especies con una vulnerabilidad “moderada” (22 especies) y son muy pocas las incluidas en las categorías “alta” (3 especies) o “baja” (1 especie) (ver Apéndice II). Añadiendo las especies citadas y no capturadas (Apéndice IV) el número de especies con vulnerabilidad “moderada” se eleva a 28. Estos datos confirman al área geográfica del Bierzo como una de las zonas prioritarias de conservación para los coleópteros acuáticos ibéricos (MILLÁN *et al.* 2014). La mayoría de las localidades prospectadas se encuentran dentro de espacios protegidos (Fig. 1), así las zonas A, B, C, D y la loc. 20 (Apéndice I) se encuentran dentro de la Reserva de la Biosfera de los Ancares Leoneses y, dentro de ésta, las localidades 5, 7, 20 y las zonas B, C y D están también dentro de la ZEC Sierra de los Ancares, destacando la riqueza de las localidades de las zonas A (63 especies), B (52) y D (56). También las localidades 5, 16 y 17 se sitúan dentro de la Reserva Natural Fluvial del río Burbia. Otras zonas como la J (Las Médulas) y la localidad 25 se enmarcan en la ZEC Montes Aquilanos y Sierra del Teleno, con especial interés para el pequeño complejo lagunar de las Médulas, situado en el espacio natural protegido Monumento Natural de Las Médulas y el Lago de Carucedo, con 58 especies. Los datos de riqueza por zonas pueden consultarse en la Apéndice II. Desde un punto de vista legal, la mayoría de la fauna de coleópteros acuáticos estudiada quedaría dentro de los espacios protegidos del Bierzo. Se trata de figuras de protección genéricas que inciden especialmente en la conservación de los ambientes terrestres y los vertebrados, con la excepción de la Reserva Natural Fluvial del Burbia que, aunque limitada espacialmente, parece ser un área bien seleccionada. En todo caso, la especificidad de la conservación de la biodiversidad de los medios acuáticos requiere de medidas concretas para este tipo de fauna y sus hábitats.

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Apéndice I. Localidades muestreadas y agrupación por zonas (A-K), con coordenadas geográficas, fecha de captura y altitud.

Appendix I. Localities sampled grouping by areas (A-K), with geographic coordinates, sampling date and altitude.

Zona	Punto	Localidad	Coordenadas	Fecha	Altitud msnm
A	**Río Burbia**				
	1	Río Burbia, Villafranca del Bierzo	42°36‘36“N, 06°48‘38“W	26.vi.2015	500
	2	Río Burbia, 500m NE Villafranca del Bierzo	42°36‘55“N, 06°48‘10“W	26.vi.2015	510
	3	Río Burbia, Landoiro	42°37‘09“N, 06°48‘30“W	26.vi.2015	587
	4	Río Burbia, Puente de Rey	42°37‘37“N, 06°48‘06“W	26-29.vi.2015	537
	5	Río Burbia, aguas arriba de Veguellina	42°43‘03“N, 06°46‘58“W	27.vi.2015	680
	6	Río Burbia, Paradaseca (puente)	42°40‘07“N, 06°47‘30“W	28.vi.2015	721
	7	Río Burbia, aguas debajo de Veguellina	42°41‘37“N, 06°46‘49“W	29.vi.2015	650
	8	Arroyo Pradela, Landoiro	42°37‘31“N, 06°49‘05“W	29.vi.2015	574
B	**Río Ancares**				
	9	Arroyo Miravalles, Tejedo de Ancares	42°51‘02“N, 06°47‘05“W	27.vi.2015	1040-1107
	10	Río Seco, Candín	42°48‘59“N, 06°44‘49“W	27.vi.2015	900
	11	Río Cuiña, Pereda de Ancares	42°49‘29“N, 06°44‘19“W	27.vi.2015	915-964
	12	Río Cuiña y afluente, Puerto de Ancares	42°51‘41“N, 06°47‘53“W	27.vi.2015	1340-1400
	13	Río Cuiña, Tejedo de Ancares	42°50‘21“N, 06°45‘55“W	27.vi.2015	980
	14	Río de la Vega, Suertes	42°50‘02“N, 06°43‘46“W	27.vi.2015	927
	15	Río Ancares, Candín	42°48‘06“N, 06°42‘04“W	27.vi.2015	832
C	**Río Porcarizas**				
	16	Río Porcarizas, Porcarizas & Campo del Agua	42°45‘29“N, 06°49‘32“W	27.vi.2015	996-1250
	17	Tributario del Río Porcarizas	42°45‘22“N, 06°49‘20“W	27.vi.2015	1110

Zona	Punto	Localidad	Coordenadas	Fecha	Altitud msnm
D	**Río Ser**				
	18	Río de la Vega, SO Suárbol	42°51'36"N, 06°51'19"W	27.vi.2015	1096-1102
	19	Rego da Cespedosa, Puerto de Ancares	42°52'23"N, 06°49'50"W	27-29.vi.2015	1430
E	**Río Cúa**				
	20	Río Cúa, Bárcena de la Abadía	42°48'05"N, 06°37'01"W	27.vi.2015	727
	21	Río Cúa, Cacabelos	42°36'55"N, 06°44'05"W	30.vi.2015	485
F	**Río Ozuela**				
	22	Río Ozuela, Ozuela-Barrio del Río	42°29'41"N, 06°37'32"W	28.vi.2015	632
G	**Río Silván**				
	23	Río Silván, Silván	42°19'34"N, 06°40'52"W	28.vi.2015	980
H	**Río Cabrera**				
	24	Río Cabrera, Pombriego & Yebra	42°24'47"N, 06°40'51"W	28.vi.2015	480-496
I	**Arroyo Ferradillo**				
	25	Arroyo Ferradillo, Villavieja	42°29'02"N, 06°41'05"W	28.vi.2015	759
J	**Las Médulas**				
	26	Laguna Larga 1	42°27'30"N, 06°46'37"W	28.vi.2015	742
	27	Laguna Larga 2	42°27'34"N, 06°46'38"W	28.vi.2015	736
	28	Laguna Pinzais	42°27'31"N, 06°46'28"W	28.vi.2015	74
	29	Lago Carucedo	42°29'37"N, 06°46'59"W	28.vi.2015	520
	30	Lago Sumido	42°27'41"N, 06°46'46"W	28.vi.2015	743
K	**Río Tremor**				
	31	Río Tremor, Tremor de Abajo	42°40'18"N, 06°15'27"W	26.vi.2015	810

Apéndice II. Relación de especies capturadas agrupadas por zonas, con área de distribución (DT) y vulnerabilidad de las especies endémicas (VU). Ver los códigos en el apartado Metodología. Las especies con * son nuevas citas para la comarca del Bierzo.

Appendix II. List of collected species, grouping by areas, with distribution category (DT) and vulnerability (VU). See codes in methodology. Species with * are new records for El Bierzo region.

Especie	A	B	C	D	E	F	G	H	I	J	K	DT	VU
Gyrinidae													
Gyrinus distinctus						x						T	
Gyrinus substriatus							x					T	
Orectochilus villosus	x				x			x				T	
Haliplidae													
Haliplus lineatocollis	x	x	x	x			x			x		T	
Peltodytes caesus *										x		T	
Peltodytes rotundatus										x		T	
Noteridae													
Noterus laevis										x		T	
Hygrobiidae													
Hygrobia hermanii										x		T	
Dytiscidae													
Acilius sulcatus										x		T	
Agabus biguttatus	x	x		x		x	x		x			T	
Agabus bipustulatus	x	x		x	x		x			x		T	

Especie	A	B	C	D	E	F	G	H	I	J	K	DT	VU
Agabus brunneus	x					x	x					T	
Agabus didymus	x				x					x		T	
Agabus guttatus		x		x								N	
Agabus heydeni	x	x	x	x								S	
Agabus paludosus		x		x								N	
Bidessus minutissimus					x							T	
Boreonectes ibericus	x						x					T	
Colymbetes fuscus										x		T	
Cybister lateralimarginalis										x		T	
Deronoctes angusi	x											E	M
Deronectes bicostatus	x	x					x				x	E	M
Deronectes costipennis guignouxi	x	x		x	x				x			E	M
Deronectes ferrugineus	x		x		x		x				x	E	M
Dytiscus pisanus				x								T	
Dytiscus semisulcatus		x		x	x							T	
Graptodytes flavipes							x			x		T	
Graptodytes ignotus					x							T	
Graptodytes varius	x									x		T	
Hydroglyphus geminus		x		x						x		T	
Hydroporus brancoi brancoi		x		x								E	M
Hydroporus decipiens	x						x					E	B
Hydroporus discretus	x	x		x		x	x					T	
Hydroporus gyllenhalii				x								N	
Hydroporus necopinatus necopinatus				x								E	M
Hydroporus nevadensis		x		x								E	A
Hydroporus nigrita	x	x	x	x	x		x					N	
Hydroporus obsoletus	x			x								T	
Hydroporus planus						x						T	
Hydroporus pubescens	x	x		x			x			x	x	T	
Hydroporus tesellatus	x	x		x	x	x	x	x	x	x		T	
Hydroporus vagepictus	x	x								x		N	
Hydrovatus clypealis *										x		T	
Hydrovatus cuspidatus *										x		T	
Hygrotus impressopunctatus *										x		N	
Hygrotus inaequalis						x				x		T	
Hygrotus lagari *										x		S	
Hyphydrus aubei										x		T	
Iberonectes bertrandi	x				x		x	x				E	M
Ilybius chalconatus				x			x			x		T	
Ilybius dettneri *				x								E	A
Ilybius meridionalis	x										x	T	
Ilybius montanus *				x						x		T	
Laccophilus minutus										x		T	
Liopterus atriceps *										x		T	
Nebrioporus carinatus	x	x			x		x				x	E	M
Nectoporus sanmarkii alienus	x	x			x		x				x	E	
Oreodytes davisii rhianae	x						x					E	M
Rhantus hispanicus										x		T	
Rhantus suturalis										x		T	
Stictonectes epipleuricus	x				x							N	
Stictonectes lepidus							x					T	
Stictonectes rebeccae *	x											E	M
Stictotarsus duodecimpustulatus	x											N	

Especie	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**DT**	**VU**
Helophoridae													
Helophorus alternans *		x										T	
Helophorus nubilus					x							N	
Helophorus schmidti *		x										N	
Helophorus flavipes	x	x		x	x		x			x		N	
Helophorus glacialis							x					N	
Helophorus obscurus *		x							x			N	
Helophorus seidlitzii	x	x	x	x			x		x	x	x	E	M
Georissidae													
Georissus costatus *	x											T	
Hydrochidae													
Hydrochus angustatus				x						x		T	
Hydrochus interruptus	x			x	x					x		E	A
Hydrochus nitidicollis	x			x	x					x		T	
Hydrophilidae													
Anacaena bipustulata	x				x					x		T	
Anacaena globulus	x	x	x	x	x		x	x	x	x	x	T	
Anacaena lutescens					x					x		T	
Cercyon sternalis *					x					x		N	
Chaetarthria simillima / C. seminulum		x		x								N	
Chaetarthria similis *	x				x							T	
Coelostoma hispanicum					x							T	
Coelostoma orbiculare		x		x						x		N	
Enochrus fuscipennis		x		x						x		T	
Enochrus nigritus				x						x		T	
Helochares lividus										x		T	
Helochares punctatus		x			x					x		N	
Hemisphaera seriatopunctata					x							T	
Limnohydrobius convexus *										x		T	
Hydrobius fuscipes complex				x						x		T	
Hydrophilus pistaceus										x		T	
Laccobius atratus	x			x								T	
Laccobius bipunctatus				x								T	
Laccobius obscuratus	x				x							N	
Laccobius striatulus					x							N	
Laccobius ytenensis	x		x	x	x		x			x	x	T	
Limnoxenus niger										x		N	
Megasternum concinnum *	x											T	
Paracymus scutellaris										x		T	
Sphaeridium lunatum *				x								N	
Sphaeridium scarabaeoides *				x								T	
Hydraenidae													
Hydraena barrosi	x	x		x								N	
Hydraena bisulcata		x										S	
Hydraena brachymera		x	x	x	x	x	x	x	x			N	
Hydraena corinna	x	x	x					x				E	M
Hydraena emarginata		x					x					N	
Hydraena exasperata	x					x			x			E	M
Hydraena iberica	x	x		x	x			x			x	E	M
Hydraena inapicipalpis	x	x			x							N	
Hydraena minutissima		x										N	
Hydraena monstruosipes		x		x		x						E	A
Hydraena sharpi	x	x			x							E	M

Especie	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**DT**	**VU**
Hydraena stussineri	x	x			x						x	N	
Hydraena testacea	x			x	x							T	
Hydraena unca	x			x				x				E	M
Limnebius gerhardti	x			x	x					x		E	M
Limnebius lusitanus				x								E	M
Limnebius myrmidon *	x											N	
Limnebius truncatellus	x	x		x	x		x				x	N	
Ochthebius exaratus		x								x		T	
Ochthebius heydeni	x	x						x	x			N	
Ochthebius legionensis	x											E	M
Scirtidae													
Contacyphon iberus	x											E	
Contacyphon sp.		x				x				x			
Elodes sp.				x									
Hydrocyphon pernigrans								x				E	
Hydrocyphon sp.				x				x		x			
Elmidae													
Dupophilus brevis	x	x				x		x				N	
Elmis aenea	x					x	x		x			N	
Elmis maugetti maugetti						x						N	
Elmis perezi		x		x								N	
Elmis rioloides		x					x	x				N	
Esolus angustatus		x		x								N	
Esolus parallelepipedus	x			x		x					x	T	
Limnius opacus	x	x		x			x	x				T	
Limnius perrisi carinatus	x	x	x	x	x							E	M
Limnius wolckmari	x					x						N	
Oulimnius perezi		x		x								E	M
Dryopidae													
Dryops algiricus *										x		T	
Dryops luridus	x	x	x	x	x		x		x	x		T	
Dryops nitidulus *	x									x		N	
Heteroceridae													
Heterocerus aragonicus *										x		T	
Total de especies	63	52	11	56	39	16	32	14	11	58	13		

Apéndice III: Lista de especies y recolectores, con el número de las localidades de captura.

Appendix III: List of species and collectors, with sampling sites number.

Recolectores:

AV: Adrián Villastrigo
AME: Andrés Millán, Equipo
GF: Garth N. Foster
HF: Hans Fery
HS: Helena Shaverdo
IE: Íñigo Esteban
IR: Ignacio Ribera
JDP: Juan A. Díaz Pazos
JFE: Jean François Elder
JG: Josefina Garrido
LFV: Luis Felipe Valladares
LH: Lars Hendrich
MEV: Barend van Maanen, Gert van Ee & Oscar Vorst
MM: Michael Manuel
PQ: Pierre Queney

GYRINIDAE

Gyrinus (*Gyrinus*) *distinctus* Aubé, 1838

22(AV, JG)

Gyrinus (*Gyrinus*) *substriatus* Stephens, 1829

23(JFE, MM, PQ)

Orectochilus villosus (Müller, 1776)

3(MEV), 4(JFE, MM), 5(GF), 6(LH), 8(JFE, MM, PQ), 21(MEV), 24(JFE, MM, PQ)

HALIPLIDAE

Haliplus (*Neohaliplus*) *lineatocollis* (Marsham, 1802)

1(LH), 3(MEV), 4(JFE), 6(LH), 11(HS), 13(MEV, HS), 16(GF), 19(IR), 23(PQ), 26(MEV), 27(IR)

Peltodytes (*Peltodytes*) *caesus* (Duftschmid, 1805)

26(MEV), 28(AME)

Peltodytes (*Peltodytes*) *rotundatus* (Aubé, 1836)

27(HS)

NOTERIDAE

Noterus laevis Sturm, 1834

26(IE, MEV, JFE, AME, MM, PQ), 27(GF, IR, HS, JG, AME), 28(GF, PQ)

HYGROBIIDAE

Hygrobia hermanni (Fabricius, 1775)

26(IE, MEV, AME, PQ), 27(AV, GF, HS, JG, LH, AME), 28(AME, PQ)

DYTISCIDAE

Acilius (*Acilius*) *sulcatus* (Linnaeus, 1758)

27(GF, JG, LH), 30(IE)

Agabus (*Gaurodytes*) *biguttatus* (Olivier, 1795)

2(MM), 4(MM), 8(JFE), 10(AME), 19(IR), 22(HS), 23(MM), 25(IE)

Agabus (*Gaurodytes*) *bipustulatus* (Linnaeus, 1767)

2(MM), 3(MEV, MM), 4(JFE, MM), 8 (MM), 11(HS, LFV), 13(MEV, HS), 15(PQ), 19(AV, IE, GF, IR, LH, AME), 20(AME), 23(JFE, MM, PQ), 26(AME), 27(AME, GF, IR, HS), 28(PQ)

Agabus (*Gaurodytes*) *brunneus* (Fabricius, 1798)

4(MM), 22(HS), 23(MM)

Agabus (*Gaurodytes*) *didymus* (Olivier, 1795)

2(MM), 3(MEV), 4(HS, MM), 8(JFE), 20(AME), 27(GF)

Agabus (*Gaurodytes*) *guttatus* (Paykull, 1798)

9(IR), 13(HS), 19(IR)

Agabus (*Gaurodytes*) *heydeni* Wehncke, 1872

4(JFE), 9(IE, LH), 10(AME), 13(MEV, MM), 15(PQ), 16(GF), 19(IE, GF, LH, AME, MM)

Agabus (*Gaurodytes*) *paludosus* (Fabricius, 1801)

9(LH), 10(AME), 11(HS), 13(HS, MM, MEV), 15(PQ), 19(AV, GF, LH, AME, MM, HF)

Bidessus minutissimus (Germar, 1823)

21(MEV)

Boreonectes ibericus (Dutton y Angus, 2007)

6(LH), 23(JFE, MM)

Colymbetes fuscus (Linnaeus, 1758)

27(GF), 28(PQ), 30(IE)

Cybister (*Cybister*) *lateralimarginalis* (De Geer, 1774)

26(MEV), 27(GF)

Deronectes angusi Fery y Brancucci, 1990

4(HS, MM), 6(LH)

Deronectes bicostatus (Schaum, 1864)

3(MEV), 4(HS, JFE), 6(LH), 8(JFE, MM), 15(PQ), 23(JFE, MM, PQ)

Deronectes costipennis gignouxi Fery y Brancucci, 1989

5(GF), 9(IE, IR, JFE, LH, HF), 10(AME), 11(HF, HS), 12(HS, LFV), 13(HS, LFV, MM), 18(MM), 19(AV, GF, IE, IR, LH, AME, MM, HF), 20(IE), 25(IE)

Deronectes ferrugineus Fery y Branccuci, 1987

4(HS, MM), 5(GF), 6(LH), 7(MEV), 8(JFE, MM), 16(GF), 20(AV, IE), 23(PQ), 31(GF)

Dytiscus pisanus Castelnau, 1835

19(LH)

Dytiscus semisulcatus O.F. Müller, 1776

9(IR, LH), 11(HS), 13(MEV), 19(LH, AME), 20(AME)

Graptodytes flavipes (Olivier, 1795)

23(MM), 26(AME, MEV, MM, PQ), 27(GF, LFV, HS, LH), 28(PQ), 30(MEV)

Graptodytes ignotus (Mulsant y Rey, 1861)

21(MEV)

Graptodytes varius (Aubé, 1838)

1 (LH), 4(HS, MM), 6(LH), 8(JFE), 26(MM)

Hydroglyphus geminus (Fabricius, 1792)

13(MM), 18(MM), 19(MM), 27(HS)

Hydroporus brancoi brancoi Rocchi, 1981

13(MM), 15(PQ), 18(MM), 19(MM)

Hydroporus decipiens Sharp, 1878

2(MM), 23(MM)

Hydroporus discretus Fairmaire y Brisout de Barneville 1859

4(MM), 7(MEV), 13(HS), 19(GF, MM), 22(JG), 23(MM)

Hydroporus gyllenhalii Schiödte, 1841

18(MM), 19(IR)

Hydroporus necopinatus necopinatus Fery, 1999

18(MM)

Hydroporus nevadensis Sharp, 1882

11(HS), 13(HS, MM), 15(PQ), 18(MM), 19(MM)

Hydroporus nigrita (Fabricius, 1792)

2(MM), 3(MEV), 4(HS, MM), 6(LH), 8(MM), 9(IR, JFE, LH), 10(AME), 11(LFV, HS), 13(MEV, HS, MM), 15(PQ), 16(GF), 19(IE, GF, IR, LH, AME, MM), 20(IE), 23(JFE, PQ)

Hydroporus obsoletus Aubé, 1838

3(MEV), 19(AME)

Hydroporus planus (Fabricius, 1782)

22(HS)

Hydroporus pubescens (Gyllenhal, 1808)

2(MM), 8(MM), 10(AME), 11(HS), 13(MEV, HS, MM), 19(IE, GF, IR, AME, MM), 23(JFE), PQ), 30(JFE), 31(GF)

Hydroporus tessellatus (Drapiez, 1819)

1(LH), 4(HS), 8(JFE, MM), 11(HS), 13(HS, MM), 19(IR, AME), 20(IE), 22(HS), 23(PQ), 24(PQ), 25(IE), 27(IR, AME)

Hydroporus vagepictus Fairmaire y Laboulbène, 1855

4(HS), 11(HS), 26(MEV, AME, MM), 27(GF, HS, IR, LH), 30(JFE)

Hydrovatus clypealis Sharp, 1876

26(JFE, AME, MM, PQ), 27(IR), 28(PQ)

Hydrovatus cuspidatus (Kunze, 1818)

27(HS)

Hygrotus (*Leptolambus*) *impressopunctatus* (Schaller, 1783)

26(MEV, MM, PQ), 27(IR, HS)

Hygrotus (*Leptolambus*) *lagari* (Fery, 1992)

27(GF)

Hygrotus (*Hygrotus*) *inaequalis* (Fabricius, 1777)

22(AV), 26(IE, JFE, AME, MEV, MM, PQ), 27(GF, IR, LFV, HS, JG, AME), 28(PQ), 30(MEV)

Hyphydrus aubei Ganglbauer, 1891

26(IE, MEV, JFE, AME, MM), 27(GF, IR, HS, LH), 30(JFE)

Iberonectes bertrandi (Legros, 1956)

3(MEV), 4(JFE, HS, MM), 5(GF), 6(LH), 7(MEV), 8(JFE), 20(IE, AME), 23(JFE, PQ), 24(JFE, MM)

Ilybius chalconatus (Panzer, 1796)

19(IR, AME, MM), 23(MM), 26(AME),27(IR), 30(IE)

Ilybius dettneri (Fery, 1986)

18(MM), 19(GF, LH)

Ilybius meridionalis Aubé, 1837

2(MM), 4(JFE, HS, MM), 6(LH), 8(MM), 31(GF)

Ilybius montanus (Stephens, 1828)

19(GF), 26(MM), 27(GF), 28(GF, PQ), 30(MEV)

Laccophilus minutus Linnaeus, 1758

26(IE), 27(GF, IR, LFV, HS), 28(AME)

Liopterus atriceps Sharp, 1882

26(JFE, MM, PQ), 27(GF, MEV, 27, LH)

Nebrioporus carinatus (Aubé, 1838)

3(MEV), 4(JFE, HS, MM), 6(LH), 7(MEV), 11(LFV, HS), 20(IE), 23(JFE, MM), 31(GF)

Nectoporus sanmarkii alienus (Sharp, 1873)

5(GF), 7(MEV), 11(LFV, HS), 13(MEV), 20(AV, IE, AME), 23(JFE, MM, PQ), 31(GF)

Oreodytes davisii rhianae Carr, 2001

4(MM), 7(MEV), 23(JFE, MM)

Rhantus hispanicus Sharp, 1882

27(GF)

Rhantus suturalis (Macleay, 1825)

27(AME)

Stictonectes epipleuricus (Seidlitz, 1887)

1(LH), 6(LH), 20(IE)

Stictonectes lepidus (Olivier, 1795)

23(MM)

Stictonectes rebeccae Bilton, 2012

3(MEV), 4(HS)

Stictotarsus duodecimpustulatus (Fabricius, 1792)

5(GF)

HELOPHORIDAE

Helophorus (*Empleurus*) *nubilus* Fabricius, 1777

20(AME)

Helophorus (*Empleurus*) *schmidti* A. Villa & G.B. Villa, 1838

13(MEV)

Helophorus (*Rhopalohelophorus*) *flavipes* Fabricius, 1792

3(MEV), 7(MEV), 9(IE, PQ), 10(AME), 11(LFV), 12(LFV), 13(MEV), 15(PQ), 19(AV, IE, IR, AME), 20(AV, AME), 21(MEV), 23(PQ), 26(IE, MEV), 27(IR), 30(MEV)

Helophorus (*Rhopalohelophorus*) *glacialis* A. Villa & G.B. Villa, 1833

23(PQ)

Helophorus (Rhopalohelophorus) obscurus Mulsant, 1844

13(MEV), 25(IE)

Helophorus (*Rhopalohelophorus*) *seidlitzi* Kuwert, 1885

5(GF), 7(MEV), 11(LFV), 13(JDP, MEV), 16(GF), 17(GF), 19(IE, GF), 23(PQ), 25(IE), 27(GF), 31(GF)

Helophorus (*Trichohelophorus*)*alternans* Gené, 1836

13(MEV)

GEORISSIDAE

Georissus (*Neogeorissus*) *costatus* Laporte, 1840

7(MEV)

HYDROCHIDAE

Hydrochus angustatus Germar, 1824

19(AME), 26(AME), 27(GF, IR), 28(AME), 30(IE, JFE)

Hydrochus interruptus Heyden, 1870

3(MEV), 7(MEV), 19(IR, AME), 21(MEV), 26(AME), 28(AME)

Hydrochus nitidicollis Mulsant, 1844

7(MEV), 8(JFE), 19(AV, GF), 21(MEV), 26(IE, PQ), 27(LFV)

HYDROPHILIDAE

Anacaena bipustulata (Marsham, 1802)

3(MEV), 7(MEV), 21(MEV), 26(IE, AME), 27(IR), 28(AME)

Anacaena globulus (Paykull, 1798)

2(MEV), 3(MEV), 5(GF), 6(LH), 7(MEV), 9(AV, IR, PQ), 10(AME), 11(LFV), 12(LFV), 13(MEV, LFV), 15(PQ), 16(GF), 17(AME, GF), 19(IE, GF, IR, AME), 20(AV, IE), 21(MEV), 23(PQ), 24(PQ), 25(IE), 26(MEV), 27(AME), 31(GF)

Anacaena lutescens (Stephens, 1829)

20(AME), 26(MEV, 29(MEV)

Cercyon (*Cercyon*) *sternalis* Sharp, 1918

21(MEV), 28(PQ)

Chaetarthria similis Wollaston, 1864 T

7(MEV), 21(MEV)

Chaetarthria simillima Vorst & Cuppen, 2003/ *C. seminulum* Herbst, 1797

13(MEV), 19(AME)

Coelostoma (*Coelostoma*) *hispanicum* Küster, 1848

21(MEV)

Coelostoma (*Coelostoma*) *orbiculare* (Fabricius, 1775)

13(MEV), 19(AME), 26(AME), 28(GF, PQ), 30(MEV)

Enochrus (*Lumetus*) *fuscipennis* Thomson, 1884

11(LFV), 13(MEV), 19(GF, IR), 26(IE, MEV, JFE, AME, PQ), 27(GF), 28(GF, PQ), 30(MEV)

Enochrus (*Methydrus*) *nigritus* Sharp, 1873

19(GF, IR, AME), 26(IE, MEV, AME, PQ), 27(GF, IR), 28(GF, PQ), 30(MEV)

Helochares (*Helochares*) *lividus* (Forster, 1771)

26(MEV, JFE, AME, PQ), 27(AV, GF, LFV, AME), 28(AME), 29(MEV), 30(MEV)

Helochares (*Helochares*) *punctatus* Sharp, 1869

13(MEV), 21(MEV), 26(IE, MEV, JFE, PQ), 27(JDP, MEV, IR, LFV), 28(PQ), 29(MEV)

Hemisphaera seriatopunctata (Perris, 1874)

21(MEV)

Hydrobius fuscipes Linnaeus, 1758 complex

19(GF, AME), 26(MEV, AME), 27(GF, IR, LFV), 28(GF, PQ), 30(MEV)

Hydrophilus (*Hydrophilus*) *pistaceus* Laporte, 1840

26(MEV), 27(MEV)

Laccobius (*Dimorpholaccobius*) *atratus* Rottenberg, 1874

7(MEV), 19(GF, AME)

Laccobius (*Dimorpholaccobius*) *bipunctatus* (Fabricius, 1775)

19(IR)

Laccobius (*Dimorpholaccobius*) *obscuratus* Rottenberg, 1874

3(MEV), 20(AME)

Laccobius (*Dimorpholaccobius*) *striatulus* Fabricius, 1801

21(MEV)

Laccobius (*Dimorpholaccobius*) *ytenensis* Sharp, 1910

3(MEV), 7(MEV), 16(GF), 19(IE), 21(MEV), 23(PQ), 26(MEV), 27(GF), 30(MEV), 31(GF)

Limnohydrobius convexus (Brullé, 1835)

27(JDP, IR)

Limnoxenus niger Gmelin, 1790

26(IE, MEV, AME, PQ), 27(GF, IR), 30(MEV)

Megasternum concinnum Marsham, 1802

3(MEV), 7(MEV)

Paracymus scutellaris Rosenhauer, 1856

26(MEV, AME), 27(GF, IR), 30(MEV)

Sphaeridium lunatum Fabricius, 1792

19(GF)

Sphaeridium scarabaeoides (Linnaeus, 1758)

19(GF)

HYDRAENIDAE

Hydraena (*Hydraena*) *barrosi* d'Orchymont, 1934

7(MEV), 9(IR), 11(JDP), 12(JDP), 13(JDP, LFV), 19(IE, IR)

Hydraena (*Hydraena*) *bisulcata* Rey, 1884

9(PQ)

Hydraena (*Hydraena*) *brachymera* d'Orchymont, 1936

9(IE, IR, PQ), 10(AME), 11(JDP, LFV), 13(JDP, MEV, LFV), 14(PQ), 15(PQ), 16(GF), 19(GF, IE, IR, AME), 20(IE, AME), 22(LFV), 23(PQ), 24(PQ), 25(IE)

Hydraena (*Hydraena*) *corinna* d'Orchymont, 1936

3(MEV), 13(JDP, MEV), 14(PQ), 15(PQ), 16(GF), 24(PQ)

Hydraena (*Hydraena*) *emarginata* Rey, 1885

11(JDP), 13(JDP, LFV), 23 (JFE, PQ)

Hydraena (*Hydraena*) *exasperata* d'Orchymont, 1935

3(MEV), 7(MEV), 22(LFV), 25(IE)

Hydraena (*Hydraena*) *iberica* d'Orchymont, 1936

5(GF), 7(MEV), 9(IE, IR, PQ), 11(JDP, LFV), 13(JDP, LFV, MEV), 14(PQ), 15(PQ), 19(GF, IE, IR, AME), 20(AME), 24(PQ), 31(GF)

Hydraena (*Hydraena*) *inapicipalpis* Pic, 1918

3(MEV), 15(PQ), 20(IE), 21(MEV)

Hydraena (*Hydraena*) *minutissima* Stephens, 1829

10(AME)

Hydraena (*Hydraena*) *monstruosipes* Ferro, 1986

12(JDP, LFV), 19(AV, IE, GF, IR, AME), 22(LFV)

Hydraena (*Hydraena*) *sharpi* Rey, 1886

3(MEV), 11(JDP), 13(JDP, LFV), 14(PQ), 15(PQ), 20(IE)

Hydraena (*Hydraena*) *stussineri* Kuwert, 1888

3(MEV), 5(GF), 7(MEV), 13(MEV), 20(AME), 21(MEV), 31(GF)

Hydraena (*Hydraena*) *unca* Valladares, 1989

9(IR), 19(IR), 24(PQ)

Hydraena (*Phothydraena*) *testacea* Curtis, 1830

3(MEV), 7(MEV), 19(AME), 21(MEV)

Limnebius (*Bilimneus*) *myrmidon* Rey, 1883

7(MEV)

Limnebius (*Limnebius*) *gerhardti* Heyden, 1870

7(MEV), 19(AME), 20(AME), 21(MEV), 26(MEV), 27(IR), 29(MEV), 30(MEV), 31(GF)

Limnebius (*Limnebius*) *lusitanus* J. Balfour-Browne, 1979

19(IR, AME)

Limnebius (*Limnebius*) *truncatellus* (Thunberg, 1794)

3(MEV), 7(MEV), 10(AME), 13(MEV, LFV), 19(IR, AME), 20(AME), 21(MEV), 23(PQ), 31(GF)

Ochthebius (*Asiobates*) *heydeni* Kuwert, 1887

3(MEV), 5(GF), 7(MEV), 10(AME), 13(MEV), 24(PQ), 25(IE)

Ochthebius (*Aulacochthebius*) *exaratus* Mulsant, 1844

9(PQ), 27(MEV), 30(MEV),

Ochthebius (*Enicocerus*) *legionensis* Hebauer & Valladares Diez, 1985
7(MEV)

SCIRTIDAE

Contacyphon iberus (Nyholm, 1976)
5(GF)
Contacyphon sp.
10(AME), 22(JG), 26(IE)
Elodes sp.
19(AV, AME)
Hydrocyphon pernigrans Nyholm, 1967
24(PQ)
Hydrocyphon sp.
19(AME), 24(PQ), 26(AME), 27(AME)

ELMIDAE

Dupophilus brevis Mulsant y Rey, 1872
7(MEV), 9(IR), 12(LFV), 22(LFV), 24(JFE, PQ)
Elmis aenea (P.W.J. Müller, 1806)
3(MEV), 22(JG), 23(JFE, PQ), 25(IE)
Elmis maugetii maugetii Latreille, 1802
22(AV, LFV)
Elmis perezi Heyden, 1870
9(PQ), 12(JDP, LFV), 13(JDP, LFV), 19(AV, IE, AME)
Elmis rioloides (Kuwert, 1890)
11(JDP), 13(JDP, LFV), 15(PQ), 23(JFE), 24(JFE)
Esolus angustatus (P.W.J. Müller, 1821)
9(IR, PQ), 11(JDP, LFV), 12(LFV), 13(JDP, MEV, LFV), 19(AV, IE)
Esolus parallelepipedus (P.W.J. Müller, 1806)
7(MEV), 19(IE), 22(LFV), 31(GF)
Limnius opacus P.W.J. Müller, 1806
8(PQ), 10(AME), 11(LFV), 19(AME), 23(PQ), 24(JFE)
Limnius perrisi carinatus (Pérez-Arcas, 1865)
7(MEV), 9(IE, IR, PQ), 10(AME), 11(JDP, LFV), 13(JDP), 15(PQ), 16(GF), 19(AME, AV, GF, IE, IR), 20(AME)
Limnius volckmari (Panzer, 1793)
7(MEV), 22(AV)
Oulimnius perezi (Sharp, 1872)
9(IR, PQ), 19(IE, IR)

DRYOPIDAE

Dryops algiricus (Lucas, 1846)
26(MEV), 30(MEV)
Dryops luridus (Erichson, 1847)
3(MEV), 5(GF), 7(MEV)), 9(IE), 10(AME), 17(GF), 19(IE, GF, IR, AME), 20(AME), 21(MEV), 23(JFE), 25(IE), 26(IE, MEV, AME, PQ), 27(JDP, IR, LFV, JG), 30(MEV)
Dryops nitidulus (Heer, 1841)
3(MEV), 29(MEV)

HETEROCERIDAE

Heterocerus aragonicus Kiessenweter, 1850
26(PQ), 27(GF), 30(JFE)

Apéndice IV: Lista de especies citadas previamente en El Bierzo y no capturadas en el presente muestreo. Para cada especie se indican las áreas de distribución general y la vulnerabilidad de las especies endémicas (entre paréntesis), con los códigos señalados en la Metodología.

Appendix IV: List of species previously recorded from El Bierzo and not collected in this sampling. For each species the general distribution and the endemic species vulnerability (in parentheses) are indicated, with the codes as described in the Methodology.

GYRINIDAE

Gyrinus (Gyrinus) substriatus Stephens, 1829 T
Gyrinus (Gyrinus) urinator Illiger, 1807 T

HALIPLIDAE

Brychius elevatus (Panzer, 1794) N
Haliplus (Haliplus) heydeni Wehncke, 1875 N
Haliplus (Liaphlus) fulvus (Fabricius, 1801) T

DYTISCIDAE

Agabus (Gaurodytes) nebulosus (Forster, 1771) T
Bidesssus coxalis Sharp, 1882 T
Dytiscus marginalis Linnaeus, 1758 N
Graptodytes castilianus Fery, 1995 E (M)
Graptodytes fractus (Sharp, 1882) T
Hydroporus foveolatus Heer, 1839 N
Hydroporus paganettianus Scholz, 1923 E (M)
Hydroporus sabaudus sabaudus Fauvel, 1865 N
Hydroporus vespertinus Fery & Hendrich, 1988 E (M)
Laccophilus hyalinus (De Geer, 1774) T
Nebrioporus canaliculatus (Lacordaire, 1835) N
Nebrioporus ceresyi (Aubé, 1838) T
Nebrioporus elegans (Panzer, 1794) N
Nebrioporus luctuosus (Aubé, 1838) N
Scarodytes halensis (Fabricius, 1787) T
Yola bicarinata (Latreille, 1804) T

HELOPHORIDAE

Helophorus (Helophorus) grandis Illiger, 1798 T
Helophorus (Rhopalohelophorus) brevipalpis Bedel, 1881 N
Helophorus (Rhopalohelophorus) minutus Fabricius, 1775 N

HYDROCHIDAE

Hydrochus angusi Valladares, 1988 E (M)

HYDROPHILIDAE

Berosus (Berosus) affinis Brullé, 1835 T
Berosus (Berosus) signaticollis (Charpentier, 1825) T
Laccobius (Dimorpholaccobius) sinuatus Motschulsky, 1849 T
Laccobius (Microlaccobius) gracilis gracilis Motschulsky, 1849 T

HYDRAENIDAE

Hydraena (Hydraena) cordata Schaufuss, 1883 T
Hydraena (Hydraena) corrugis d'Orchymont, 1934 N
Hydraena (Hydraena) gracilidelphis Trizzino, Valladares, Garrido & Audisio, 2012 N

Hydraena (Hydraena) hispanica Gangblauer, 1901 E (M)
Limnebius (Bilimneus) extraneus d'Orchymont, 1938 S
Limnebius (Limnebius) furcatus Baudi, 1872 T
Limnebius (Limnebius) ibericus J. Balfour-Browne, 1979 E (M)
Ochthebius (Enicocerus) exsculptus Germar, 1823 N
Ochthebius (Ochthebius) sidanus d'Orchymont, 1942 N

ELMIDAE
Esolus pygmaeus (P.W.J. Müller, 1806) T
Normandia nitens (P.W.J. Müller, 1817) T
Oulimnius bertrandi Berthélemy, 1964 E (A)
Oulimnius rivularis (Rosenhauer, 1856) T
Oulimnius troglodytes (Gyllenhal, 1827) T
Stenelmis canaliculata (Gyllenhal, 1808) N

HETEROCERIDAE
Augyles (Littorimus) senescens (Kiesenwetter, 1865) T

The *Ochthebius* (Coleoptera, Hydraenidae) from western Palaearctic supratidal rockpools

Los *Ochthebius* (Coleoptera, Hydraenidae) de pozas costeras supralitorales del Paleártico occidental

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ABSTRACT

We have compiled all available information regarding the presence of *Ochthebius* species in supratidal rockpools in the western Palaearctic, distinguishing species typical of these habitats from those whose presence is occasional. Overall, there are 32 taxa (30 species and 3 subspecies) linked with coastal habitats, 21 of them restricted to supratidal rockpools. Information concerning their distribution, morphological identification, habitat preference and coexistence are highlighted, as well as further open questions that need to be addressed to clarify the ecology and evolution of water beetles in these extreme environments.

Key words: *Ochthebius*, coastal habitats, checklist

RESUMEN

Hemos recopilado toda la información disponible sobre la presencia de especies del género *Ochthebius* en pozas costeras supralitorales del Paleártico occidental, distinguiendo aquellas típicas de estos hábitats de las especies cuya presencia es ocasional. En total, se conocen 32 taxones (30 especies y 3 subespecies) típicos de ambientes costeros, 21 de ellos restringidos a pozas costeras supralitorales. Se destaca información relativa a su distribución, identificación morfológica, preferencia de hábitat y su coexistencia, así como otras cuestiones que se deben abordar para clarificar la ecología y evolución de escarabajos acuáticos en este tipo de ambientes tan extremos.

Palabras clave: *Ochthebius*, habitat de costa, lista de especies

INTRODUCTION

Beetles comprise the most specious group of insects known (STORK, 2018), with representatives widely distributed in all geographical regions and capable of inhabiting almost all kinds of habitats. They are also a successful order in the aquatic environment, which they have colonised and diversified multiple independent times, aquatic representatives being known in a number of families (BILTON *et al.*, 2019). Their adaptability in aquatic ecosystems led beetles to be one of the most specious groups of insects (SHORT, 2018) together with Diptera and Trichoptera, albeit these last two orders do not include aquatic adults.

Water beetles are capable of colonising a great variety of habitats worldwide, including some extreme environments such as high mountain lakes (e.g., PALLARÉS *et al.*, 2020), hypersaline inland water bodies (e.g., MILLÁN *et al.*, 2011), or groundwaters (RIBERA & REBOLEIRA, 2019), but no species are known to with a strictly marine lifestyle (i.e., open sea, RIBERA & BEUTEL, 2014). However, a very reduced number of genera belonging to the families Limnichidae and Hydraenidae are able to spend at least, part of their life associated with marine water, particularly in the ecotone between marine and terrestrial environments (JÄCH, 1989a, 1993; HERNANDO & RIBERA, 2000). Traditionally, the scientific community has neglected marine-linked environments, but during recent years they have begun to receive more attention, especially those habitats known as supratidal rockpools inhabited by *Ochthebius* species (e.g., URBANELLI, 2002; URBANELLI & PORRETTA, 2008; PORRETTA & URBANELLI, 2012; SABATELLI *et al.*, 2016, 2021a; VILLASTRIGO *et al.*, 2020a).

Supratidal rockpools are located just above the high tide line, not submerged by marine water but exposed to storms or high tides (STEPHENSON & STEPHENSON, 1949). They are strongly dynamic environments due to their unstable characteristics, in which water temperature, salinity or desiccation pressures are extreme and change even over short time intervals, and where wind intensity plus wave action reinforces the pressures on their inhabitants (MIRÓN-GATÓN *et al.*, 2022). As a result, these species need adaptations to cope with stressful conditions, such as temperatures as high as 38 °C or salinities above 100 g/L (MIRÓN-GATÓN *et al.*, 2022). For these reasons, the rockpool community is very species-poor in comparison with other environments (MARGALEF, 1949) but strongly adapted to these extreme conditions.

The genus *Ochthebius* Leach, 1815 (Family Hydraenidae) is one of the few water beetles lineages able to live, and even diversify (VILLASTRIGO *et al.*, 2020b) in supratidal rockpools. Previously, this genus included a few species exclusively restricted to supratidal rockpools, all of them belonging to the old subgenera *Cobalius* and *Calobius* (JÄCH, 1989a; 1993). However, recent studies have increased the number of species surviving in these conditions (AUDISIO *et al.*, 2010; JÄCH & DELGADO, 2014; JÄCH & DELGADO, 2017, RIBERA & FOSTER, 2018; VILLASTRIGO *et al.*, 2020a; BILTON, 2021). Also, additional evidence has been presented on the potential cryptic speciation in this genus in the western Palaearctic (SABATELLI *et al.*, 2016; 2021a; VILLASTRIGO *et al.*, 2020a; unpublished data), so it is an opportune time to take stock of the number of *Ochthebius* species currently known in coastal areas and which of these are exclusive to supratidal rockpools.

Here, we presented a complete list of *Ochthebius* species found in the western Palaearctic supratidal rockpools, distinguishing those species exclusive from these particular habitats from those whose presence should be considered sporadic.

METHODOLOGY

Study area

The study covers the western Palaearctic region, including the Mediterranean coasts, the Atlantic coast of Morocco, Spain, Portugal and France, the British Isles and Macaronesia. We compiled the available information of the *Ochthebius* species found in supratidal rockpools and provided additional insights based on literature and our field observations.

True water beetles from supratidal rockpools

We have considered as true species from supratidal rockpools, those that 1) occupy supratidal shoreline pools and never or very rarely have been found outside these habitats; 2) are influenced at some extent by the sea water through tides and storms (mean salinity usually similar or higher to that of the adjacent sea); 3) inhabit pools without presence of emergent or rooted vegetation (except *Posidonia* remains); 4) capable of completing their entire life cycle in such habitats.

The species here included as sporadic from supratidal rockpools, are those previously cited, at least one time, in the scientific literature, or have been observed by us, in the western Palaearctic supratidal rockpools.

Sampling considerations

Sampling in supratidal rockpools requires particular techniques. Entomological aquatic kick nets with a D-shaped frame are of little use since most of the pools are small and shallow. Direct collection of individuals is usually preferable by using soft tweezers and brushes. In some localities with a high density of specimens, the use of kitchen strainers of different sizes plus mouth aspirators can be also helpful. In this case, pool water should be stirred by hand and most of the individuals will float, enabling their collection with a strainer, to later put them in a white tray. It is also important to 'wash' the surrounding areas of the pool by intensely throwing water with a tray or bucket, mainly in rock pores and crevices but also in areas with vegetation surrounding rockpools (HERNANDO *et al.*, 2017).

Distribution maps

Distribution maps of the *Ochthebius* restricted to supratidal rockpools were built using QGIS v3.16 (QGIS.org, 2021). Localities were collated based on references (URBANELLI *et al.*, 1996; JÄCH, 1989a; 1993; SABATELLI *et al.*, 2016; 2021a; VILLASTRIGO *et al.*, 2020a and b) plus our unpublished observations.

RESULTS

32 taxa (30 species and 3 subspecies), are strongly associated with coastal habitats: one within the subgenus *Asiobates* Thomson, 1859, 14 within the subgenus *Ochthebius* Leach, 1815, 16 within the subgenus *Cobalius* Rey, 1886 (including the three subspecies), and 1 within the subgenus *Micragasma* Sahlberg, 1900. We report for the first time *Ochthebius* (*Ochthebius*) *mediterraneus* Ieniştea, 1988 as a sporadic rockpool species, recorded in Galicia (one male, 19/08/15, As Furnas, Pontevedra; Velasco & Millán leg.). A checklist including all species and subspecies can be found in Table I.

Out of the 32 species and subspecies listed, 21 are considered exclusive to supratidal rockpools. All the "true" rockpool species are aggregated in two large lineages: the *Cobalius* subgenus and the *quadricollis* species group within *Ochthebius* subgenus (VILLASTRIGO *et al.*, 2019); the former divided in turn into 3 species groups (*algicola*, *biltoni* and *lejolisii*, VILLASTRIGO *et al.*, 2020a). All known species within these lineages are found in supratidal rockpools with the only exception of *Ochthebius serratus* Rosenhauer, 1856, which inhabits saline wetlands near the coast (MILLÁN *et al.*, 2014) but always well behind the shoreline (distribution ranges for the studied species are shown in Figures 1-3). The remaining species listed in Table I may appear in supratidal rockpools due to occasional dispersals (flight or crawling) from their nearby habitats. Overall, these wandering species can be divided in six additional lineages (VILLASTRIGO *et al.*, 2019): *Asiobates* and *Micragasma* subgenera, plus *foveolatus*, *lobicollis, marinus* and *metallescens* species groups. Main body shapes of the lineages found are shown in Figures 4 and 5 (true *Ochthebius* of rockpools and sporadic species respectively).

Most taxa (24) are present on the Mediterranean coasts, whereas 8 are exclusive to Macaronesia. The remaining species, *Ochthebius* (*Cobalius*) *anzar* Villastrigo, Hernando, Millán & Ribera, 2020 and *Ochthebius* (*Micragasma*) *subtrigosus* Reitter, 1897, are from the Atlantic coast of Morocco and the eastern Canary Islands, and the Caspian coast, respectively (Figures 1-3, Table I). Despite some species exhibiting a large distributional range (e.g., *Ochthebius quadricollis* Mulsant, 1844, see Figure 1), almost half of the species (15) are rather more narrowly endemic, 12 to islands in the Mediterranean or the Macaronesia, and 8 are true supratidal rockpool species (Table I). As noted in Figures 1-3, sympatry is a common phenomenon within rockpools, it being frequent to find species from the two largest lineages (*Cobalius* subgenus and *quadricollis* species group) in the same localities. Sympatry of species from the same lineage can also be observed at range edges or for *Cobalius* species within the *lejolisii* and *biltoni* species groups plus *Ochthebius anzar* (Figure 2-3).

Primary morphological characters to differentiate principal groups from supratidal rockpools (i.e., *Cobalius* subgenus and *quadricollis* species groups) are easily recognised in the field with magnification: 1) elytral margin serrated in the *Cobalius* subgenus, 2) elytral apex very low cut in the *quadricollis* species group) and 3) labrum deeply divided on the anterior margin in the *quadricollis* species group. Main groups of true rockpools species are also easily identified based on the distal (and mobile) lobe of male aedeagus: reduced stem, with a sclerotised structure with a rather long appendix in *quadricollis* species (Figure 6); or simple and elongated within *Cobalius* (Figure 7). However, species identification is

Table I. Checklist of *Ochthebius* species inhabiting supratidal rockpools and surrounding wetlands in the Western Palaearctic Region. Endemic species are highlighted in bold.

Tabla I. Lista de las especies de *Ochthebius* que viven en los charcas supralitorales en la región del paleártico oeste. Las especies endémicas van en negrita.

Subgenera	Species group	Species	Author	True rockpool	Distribution
Asiobates	*minimus*	*O. rugulosus*	Wollaston, 1857	No	Canary Islands / Madeira
Cobalius		*O. anzar*	Villastrigo, Hernando, Millán & Ribera, 2020	Yes?[1]	Morroco / Canary Islands?
Cobalius	*algicola*	***O. algicola***	Wollaston, 1871	Yes	Madeira
Cobalius	*algicola*	***O. balfourbrownei***	Jäch, 1989	Yes	Cabo Verde (Ihla de Sal)
Cobalius	*algicola*	***O. freyi***	Orchymont, 1940	Yes	Azores
Cobalius	*algicola*	***O. gorgadensis***	Villastrigo, Hernando, Millán & Ribera, 2020	Yes	Cabo Verde (Ihla de Santiago)
Cobalius	*algicola*	***O. lanthanus***	Ribera & Foster, 2018	Yes	Canary Islands (Gran Canaria)
Cobalius	*biltoni*	***O. biltoni***	Jäch & Delgado, 2017	Yes	Sicily Isl.
Cobalius	*biltoni*	***O. cortomaltese***	Villastrigo, Hernando, Millán & Ribera, 2020	Yes	Malta Isl.
Cobalius	*biltoni*	*O. evae*	Villastrigo, Hernando, Millán & Ribera, 2020	Yes	Atlantic Morocco / Southeast Spain
Cobalius	*lejolisii*	*O. adriaticus adriaticus*	Reitter, 1886	Yes	Central Mediterranean
Cobalius	*lejolisii*	*O. adriaticus moreanus*	Pretner, 1929	Yes	Eastern Mediterranean
Cobalius	*lejolisii*	*O. adriaticus pleuralis*	Reitter, 1886	Yes	Eastern Mediterranean
Cobalius	*lejolisii*	***O. asper***	Sahlberg, 1900	Yes	Greece
Cobalius	*lejolisii*	*O. celatus*	Jäch, 1989	Yes	Central and Eastern Mediterranean
Cobalius	*lejolisii*	*O. lejolisii*	Mulsant & Rey, 1861	Yes	Atlantic coast / Southeast Spain
Cobalius	*lejolisii*	*O. subinteger*	Mulsant & Rey	Yes	Western and Central Mediterranean
Micragasma		***O. minoicus***	Hernando, Villastrigo & Ribera, 2017	No	Crete
Ochthebius	*foveolatus*	*O. mediterraneus*	Ienişteа, 1988	No	Western Mediterranean
Ochthebius	*lobicollis*	*O. lobicollis*	Rey, 1885	No	Western Mediterranean
Ochthebius	*lobicollis*	***O. lapidicola***	Wollaston, 1864	No	Canary Islands
Ochthebius	*marinus*	*O. deletus*	Rey, 1885	No	Mediterranean
Ochthebius	*marinus*	*O. subpictus*	Wollaston, 1857	No	Mediterranean / Atlantic
Ochthebius	*metallescens*	***O. javieri***	Jäch, 2000	No	Balearic Islands (Menorca)
Ochthebius	*metallescens*	*O. poweri*	Rye, 1869	No	Western Mediterranean / Atlantic France / British Isles
Ochthebius	*metallescens*	***O. scopuli***	Jöhler, Jäch & Delgado, 2016	No	Sardinia Isl.
Ochthebius	*metallescens*	*O. semisericeus*	Sainte-Claire Deville, 1914	No	Western Mediterranean
Ochthebius	*quadricollis*	***O. brevicollis***	Baudi a Selve, 1864	Yes	Cyprus
Ochthebius	*quadricollis*	*O. heeri*	Wollaston, 1854	Yes	Madeira / Canary Islands
Ochthebius	*quadricollis*	*O. quadricollis*	Mulsant, 1844	Yes	Mediterranean / Atlantic coast
Ochthebius	*quadricollis*	*O. steinbuehleri*	Reitter, 1886	Yes	Eastern Mediterranean
Ochthebius	*quadricollis*	***O. urbanelliae***	Audisio, Trizzino & De Biase, 2010	Yes	Italy

[1] Ochthebius anzar habitat was not properly defined and may be linked with sand as stated by the specimen from Lanzarote. It is also possible that the specimen was drifted and collected in the sand by chance. We refrain to provide a clear status for this species

Table II. Works for species identification of rockpools, with information of habitus and genitalia (drawings or photos).

Tabla II. Artículos para la identificación de las especies que viven en las charcas rocosas, con información del habitus y aparato genital (dibujos o fotos).

Species	References	Habitus	Genitalia
O. rugulosus	JÄCH, 1998; GUTIÉRREZ *et al.* 2011	Yes	Yes
O. anzar	VILLASTRIGO *et al.*, 2020	Yes	Yes
O. balfourbrownei	JÄCH, 1989a; VILLASTRIGO *et al.*, 2020; SABATELLI *et al.* 2021b	Yes	Yes
O. freyi	JÄCH, 1989a	No	Yes
O. gorgadensis	VILLASTRIGO *et al.*, 2020	Yes	Yes
O. lanthanus	RIBERA & FOSTER, 2018	Yes	Yes
O. biltoni	JÄCH & DELGADO, 2017	Yes	Yes
O. cortomaltese[1]	VILLASTRIGO *et al.* 2020	Yes	No
O. evae	VILLASTRIGO *et al.* 2020	Yes	Yes
O. adriaticus adriaticus	JÄCH, 1989a	No	Yes
O. adriaticus moreanus	JÄCH, 1989a	No	Yes
O. adriaticus pleuralis	JÄCH, 1989a	No	Yes
O. algicola	JÄCH, 1989a	No	No
O. asper	JÄCH, 1989a, VILLASTRIGO *et al.* 2020	No	No
O. celatus[2]	JÄCH, 1989a	No	Yes
O. lejolisii	JÄCH, 1989a; VALLADARES *et al.*, 2018	Yes	Yes
O. subinteger	JÄCH, 1989a; VALLADARES *et al.*, 2018	Yes	Yes
O. minoicus	HERNANDO *et al.*, 2017	Yes	Yes
O. mediterraneus	VALLADARES *et al.*, 2018	Yes	Yes
O. lapidicola	JÄCH *et al.* 1998; GUTIÉRREZ *et al.* 2011	Yes	Yes
O. lobicollis	JÄCH *et al.* 1998; VALLADARES *et al.*, 2018	Yes	Yes
O. deletus	VALLADARES *et al.*, 2018	Yes	Yes
O. subpictus	VALLADARES *et al.*, 2018	Yes	Yes
O. javieri	JÄCH, 2000; VALLADARES *et al.*, 2018	Yes	Yes
O. poweri	JÄCH, 1989b; VALLADARES *et al.*, 2018	Yes	Yes
O. scopuli	KÖHLER *et al.* 2016	Yes	Yes
O. semisericeus	VALLADARES *et al.*, 2018	Yes	Yes
O. brevicollis	JÄCH, 1993	Yes	Yes
O. heeri[3]	JÄCH, 1993	No	Yes
O. quadricollis	JÄCH, 1993; VALLADARES *et al.*, 2018	Yes	Yes
O. steinbuehleri[4]	JÄCH, 1993; AUDISIO *et al.*, 2010	No	Yes
O. urbanelliaea	AUDISIO *et al.* 2010	Yes	Yes

1. The species was described only from a damaged female.
2. A photograph of its habitus can be found at: https://www.flickr.com/photos/coleoptera-us/49110511582
3. A photograph of its habitus can be found at: https://www.flickr.com/photos/coleoptera-us/49929075341
4. A photograph of its habitus can be found at: https://commons.wikimedia.org/wiki/File:Ochthebius_brevicollis_steinbuehleri_(Reitter,_1886).png

All the above pages were visited first December 2021.

more complicated due to high intraspecific variability and should rely on a combination of morphology, mainly using male genitalia (see references in Table II for more details) and distribution.

Regarding the knowledge of larvae for the true water beetles from supratidal rockpools, there is considerably less information and they are very difficult to identify to species level, this relying deeply on chaetotaxy of the head and thorax. There are very few studies describing larvae of *Ochthebius* species, even less considering just supratidal rockpool species within the western Palearctic coast: *Ochthebius* (*Cobalius*) *lejolisii* Mulsant & Rey, 1861 (D'ORCHYMONT, 1913), *Ochthebius* (*Cobalius*) *subinteger* Mulsant 6 Rey, 1861 (DELGADO & SOLER, 1995), *O. quadricollis* (DELGADO & SOLER, 1997), and *Ochthebius balfourbrownei* Jäch, 1989 (SABATELLI *et al.*, 2021b).

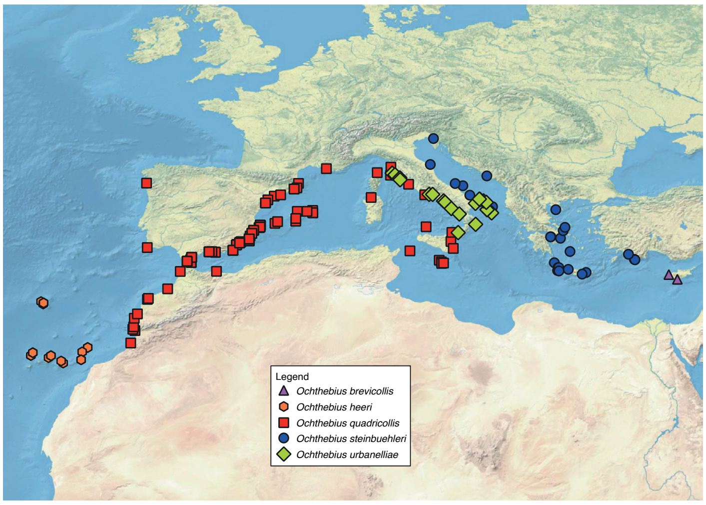

Fig. 1. Distribution of the species belonging to *quadricollis* lineage in the western Palaearctic.

Fig. 1. Mapa de distribución de las especies del grupo *quadricollis* en el Paleártico occidental.

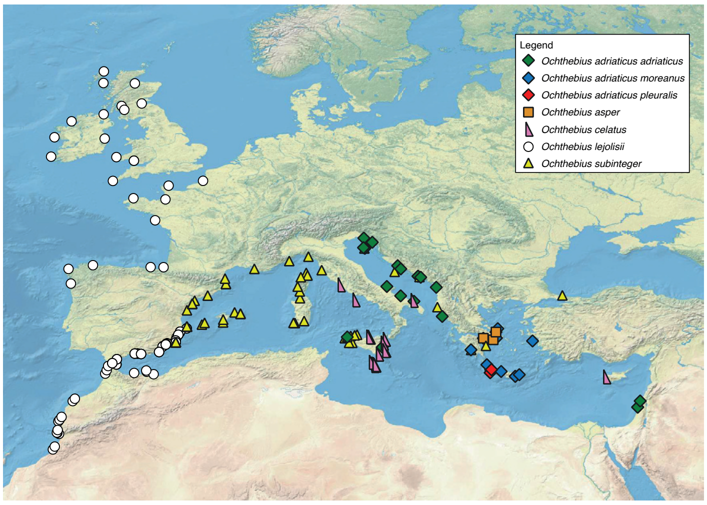

Fig. 2. Distribution of the species belonging to *lejolisii* gr. within the *Cobalius* subgenus in the western Palaearctic.

Fig. 2. Mapa de distribución de las especies del grupo *lejolisii* (subgénero *Cobalius*) en el Paleártico occidental.

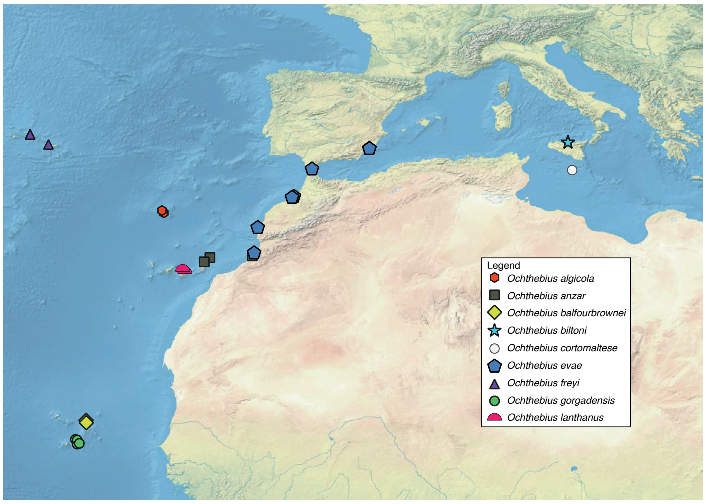

Fig. 3. Distribution of *Ochthebius* (*Cobalius*) *anzar* and the species belonging to *biltoni* gr., all within the *Cobalius* subgenus in the western Palaearctic.

Fig. 3. Mapa de distribución de *Ochthebius* (*Cobalius*) *anzar* y de las especies del grupo *biltoni* (subgénero *Cobalius*) en el Paleártico occidental.

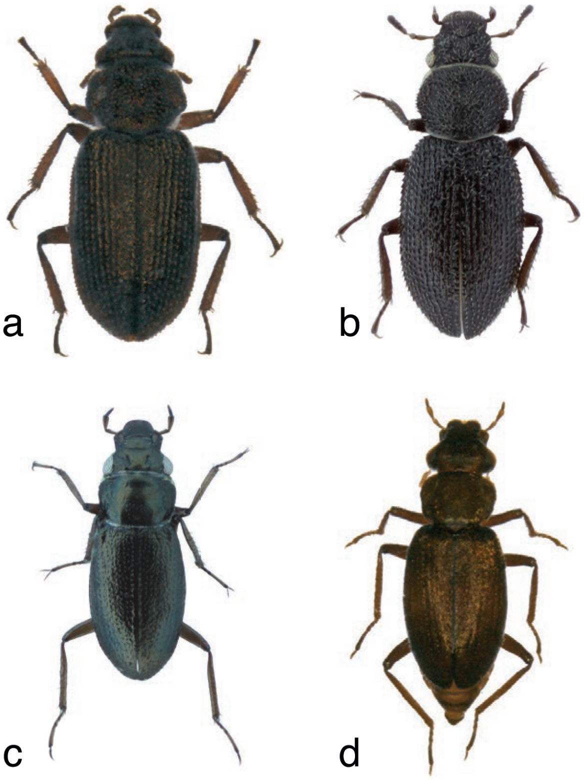

Fig. 4. Main body shapes of true rockpools *Ochthebius*: a) *O.* (*Cobalius*) *lejolisii*, *lejolisii* gr.; b) *O.* (*Cobalius*) *evae*, *biltoni* gr.; c) *O.* (*Cobalius*) *gorgadensis*, *algicola* gr.; d) *O.* (*Ochthebius*) *quadricollis*, *quadricollis* gr.

Fig. 4. Principales formas corporales de los auténticos *Ochthebius* de rockpools: a) *O.* (*Cobalius*) *lejolisii*, *lejolisii* gr.; b) *O.* (*Cobalius*) *evae*, *biltoni* gr.; c) *O.* (*Cobalius*) *gorgadensis*, *algicola* gr.; d) *O.* (*Ochthebius*) *quadricollis*, *quadricollis* gr.

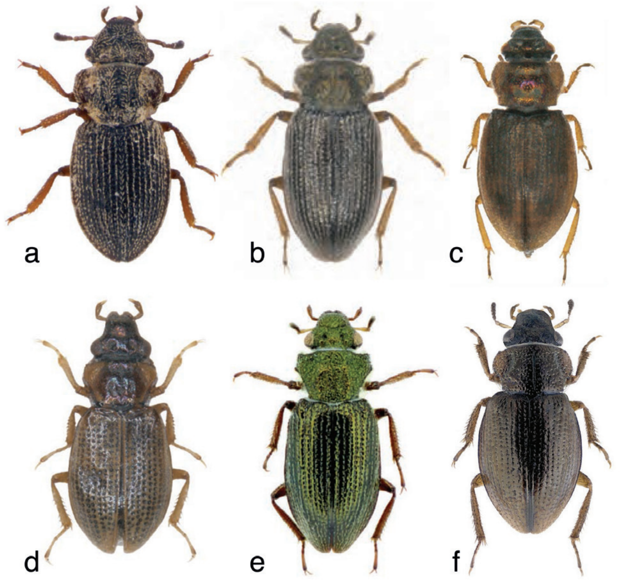

Fig. 5. Main body shapes of sporadic rockpools *Ochthebius*: a) *O.* (*Micragasma*) *minoicus*, *Micragasma* subgenus; b) *O.* (*Ochthebius*) *semisericeus*, *metallescens* gr.; c) *O.* (*Ochthebius*) *subpictus*, *marinus* gr.; d) *O.* (*Ochthebius*) *mediterraneus*, *fossulatus* gr.; e) *O.* (*Ochthebius*) *lobicollis*, *lobicollis* gr.; f) *O.* (*Asiobates*) *rugulosus**, *Asiobates* subgenus. *Photo by U. Schmidt under license Creative Commons Attribution-Share Alike 4.0 International (https://creativecommons.org/licenses/by-sa/4.0/deed.en).

Fig. 5. Principales formas corporales de las especies de *Ochthebius* que se encuentran en rockpools de forma esporádica: a) *O.* (*Micragasma*) *minoicus*, *Micragasma* subgenus; b) *O.* (*Ochthebius*) *semisericeus*, *metallescens* gr.; c) *O.* (*Ochthebius*) *subpictus*, *marinus* gr.; d) *O.* (*Ochthebius*) *mediterraneus*, *fossulatus* gr.; e) *O.* (*Ochthebius*) *lobicollis*, *lobicollis* gr.; f) *O.* (*Asiobates*) *rugulosus**, *Asiobates* subgenus. *Photo by U. Schmidt under license Creative Commons Attribution-Share Alike 4.0 International (https://creativecommons.org/licenses/by-sa/4.0/deed.en).

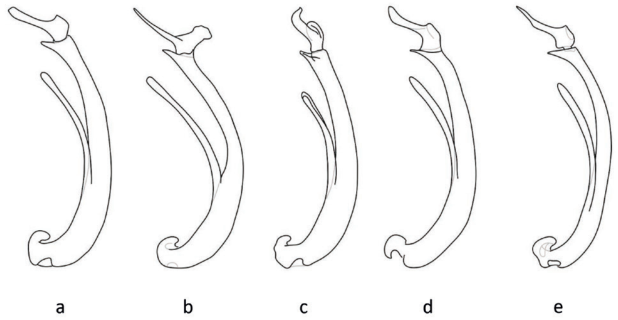

Fig. 6. Aedeagus outline of a) *O.* (*Ochthebius*) *brevicollis*; b) *O.* (*Ochthebius*) *heeri*; c) *O.* (*Ochthebius*) *quadricollis*; d) *O.* (*Ochthebius*) *steinbuenhleri*; e) *O.* (*Ochthebius*) *urbanelliae*. Figures were made redrawing aedeagus from references listed in Table II.

Fig. 6. Silueta del edeago de a) O. (Ochthebius) brevicollis; b) O. (Ochthebius) heeri; c) O. (Ochthebius) quadricollis; d) O. (Ochthebius) steinbuenhleri; e) O. (Ochthebius) urbanelliae. Los esquemas se realizaron a partir de las ilustraciones de los edeagos que aparecen en la lista de referencias de la Tabla 2.

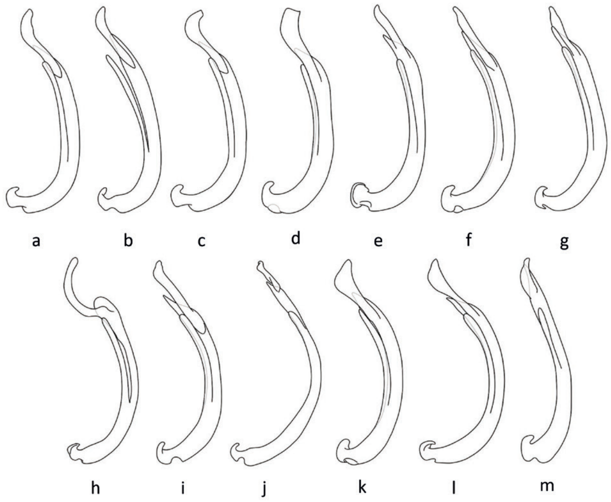

Fig. 7. Aedeagus outline of a) *O.* (*Cobalius*) *adriaticus*; b) *O.* (*Cobalius*) *morenae*; c) *O.* (*Cobalius*) *pleuralis*; d) *O.* (*Cobalius*) *lanthanus*; e) *O.* (*Cobalius*) *lejolisii*; f) *O.* (*Cobalius*) *subinteger*; g) *O.* (*Cobalius*) *celatus*; h) *O.* (*Cobalius*) *gorgadensis*; i) *O.* (*Cobalius*) *anzar*; j) *O.* (*Cobalius*) *balfourbrownei*; k) *O.* (*Cobalius*) *biltoni*; l) *O.* (*Cobalius*) *evae*; m) *O.* (*Cobalius*) *freyi*. Figures were made redrawing aedeagus from references listed in Table II.

Fig. 7. Silueta del edeago de a) *O.* (*Cobalius*) *adriaticus*; b) *O.* (*Cobalius*) *morenae*; c) *O.* (*Cobalius*) *pleuralis*; d) *O.* (*Cobalius*) *lanthanus*; e) *O.* (*Cobalius*) *lejolisii*; f) *O.* (*Cobalius*) *subinteger*; g) *O.* (*Cobalius*) *celatus*; h) *O.* (*Cobalius*) *gorgadensis*; i) *O.* (*Cobalius*) *anzar*; j) *O.* (*Cobalius*) *balfourbrownei*; k) *O.* (*Cobalius*) *biltoni*; l) *O.* (*Cobalius*) *evae*; m) *O.* (*Cobalius*) *freyi*. Los esquemas se realizaron a partir de los edeagos que aparecen en la lista de referencias de la Tabla 2.

DISCUSSION

Our study provides the first comprehensive checklist of *Ochthebius* found in coastal zones, highlighting the 21 species found exclusive in supratidal rockpools. These ecosystems support more *Ochthebius*' diversity than previously expected considering the extreme conditions of rockpools and the apparent lack of physical barriers for isolation (possibly true for other taxa with cryptic diversity, see VECCHIONI *et al.*, 2021 as an example).

The aggressive dynamics of supratidal rockpools, with continuous and swift changes, appears to favour their heterogeneity and consequently, may facilitate a high number of species in the western Mediterranean region (THERRIAULT & KOLASA, 2000). Similarly, niche differentiation may promote the frequent species coexistence observed in most localities (VILLASTRIGO *et al.*, 2020a). Sympatry was first reported by BALFOUR-BROWNE (1958) referring to the different pools in which *Ochthebius heeri* Wollaston, 1854 and *Ochthebius lanthanus* Ribera & Foster, 2018 (named as *Ochthebius subinteger* var *lejolisii*) were found living in the same localities: "The *O. heeri* were in the more salt pools nearer the sea where but few *lejolisii* were found. The latter were in abundance in the stale water pools behind the rocks". Indeed, we have found similar differences in habitat preference for other sympatric species such as *O. lejolisii* and *O. quadricollis*, *O. subinteger* and *O. quadricollis*, or *Ochthebius adriaticus* Reitter, 1886 and *Ochthebius steinbuheleri* Reitter, 1886.

Based on our combined field experience over the last decade, the general pattern found in most Mediterranean rockpools prospected was "one *Cobalius* and one 'quadricollis gr.'" species in each locality. Overall, the abundance of *Cobalius* gradually increases away from the shoreline, where the influence of marine water is undoubtedly less pronounced and the water availability is more associated with precipitation. The opposite occurred with species of the '*quadricollis* gr'. Thus, each species tends to experience different kinds of stresses (MIRÓN-GATÓN *et al.*, 2022; unpublished data), probably to minimise competition. An exception applies with *Cobalius* species in the *biltoni* species group, whose presence is uncommon and always as a third species. They display a completely different pattern than the other true rockpool water beetles as they are the only apterous rockpool species and have a very small body size that makes them easily go unnoticed (VILLASTRIGO *et al.*, 2020a). Their abundance is very low in comparison with the other species, being hundreds of times less abundant even in the same localities (e.g., *Ochthebius cortomaltese* Villastrigo, Hernando, Millán & Ribera, 2020, Villastrigo *et al.*, 2020a). It is reasonable to think that their specific habitat is not yet well characterised, as most of our field experience associates these species with rockpools full of small pores and crevices instead of regular pools (VILLASTRIGO *et al.*, 2020a). This may imply a more terrestrial lifestyle similar to that in other coastal *Ochthebius* from East Asia and western North America (*vandykei* species group, JÄCH & DELGADO, 2014). Particularly, *Ochthebius anzar* may be directly associated with intertidal habitats, being found by washing sand (VILLASTRIGO *et al.*, 2020a) and rocks in the Canary Islands.

The sporadic presence of other species in supratidal rockpools is presumably linked with erratic flights, particularly in *Ochthebius mediterraneus* and *Ochthebius rugulosus* Wollaston, 1857, which are relatively common in freshwater and slightly mineralised water bodies other than rockpools in the coastal zone (GUTIÉRREZ *et al.*, 2011; MILLÁN *et al.*, 2014). Species such as *Ochthebius deletus* Rey, 1886 or *Ochthebius subpictus* Wollaston, 1857 are related to the close presence of saline wetlands (MILLÁN *et al.*, 2014; VALLADARES *et al.*, 2018), whereas *Ochthebius semisericeus* Sainte-Claire Deville, 1914*, Ochthebius poweri* Rye, 1869*, Ochthebius scopuli* Köhler, Jäch & Delgado, 2016*, Ochthebius javieri* Jäch, 2000, *Ochthebius lapidicola* Wollaston, 1864 and *Ochthebius lobicollis* Rey, 1885*,* probably arrive from small freshwater streams or hygropetric habitats (mainly water runoff from sandstone cliffs) on rocky shores (GUTIÉRREZ *et al.*, 2011; MILLÁN *et al.*, 2014; VALLADARES *et al.*, 2018; RIBERA & HERNANDO, 2019). Regarding *Ochthebius* (*Micragasma) minoicus* Hernando, Villastrigo & Ribera, 2017, its preferred habitat is not well known but was found within the vegetation in the surroundings of small coastal springs or pools, sometimes even in supratidal rockpools (HERNANDO *et al.*, 2017).

Sympatry of species within a lineage is less common, and mostly associated with range edges. URBANELLI *et al.* (1996) demonstrated for the first time the coexistence of two species within the *quadricollis* gr. using isozymes, leading to the description of the cryptic species *Ochthebius urbanelliae* Audisio, Trizzino & De Biase, 2010 (AUDISIO *et al.*, 2010), which overlap in distribution with *O. quadricollis*. The absence of any clear physical barrier that prevented gene flow within *O. quadricollis* and *O. urbanelliae* may suggest that similar occurrences may happen in other regions or in other species groups inhabiting supratidal rockpools. In fact, *O. subinteger* and *O. lejolisii* have recently been found to overlap in distribution in southern Spain (MIRÓN-GATÓN *et al.*, 2022; VILLASTRIGO *et al.*, unpublished data). Furthermore, species of the *biltoni* gr. strongly overlap with other species of the subgenus *Cobalius* (VILLASTRIGO *et al.*, 2020a), inhabiting the same pools, which could be considered as some kind of microsympatry or niche segregation (for an extreme example of niche segregation in water beetles, see WATTS & HUMPHREYS, 2009). However, our understanding of how species coexist may be limited by the presence of cryptic lineages such as the ones recently found (SABATELLI *et al.*, 2021a; VILLASTRIGO *et al.*, unpublished data). Certainly, crypticity processes are recurrent across the coastal ecotone for other arthropods such as acari (PFINGSTL, 2021), copepods (VECCHIONI *et al.*, 2021) or isopods (SANTAMARIA *et al.*, 2016; MBONGWA *et al.*, 2019). The underlying mechanisms driving crypticity may be due to the constraints imposed by the above-mentioned highly dynamic and extreme environmental characteristics of rockpools, shrinking phenotypic evolution (BICKFORD *et al.*, 2007), causing morphological stasis or leading to convergence evolution (STRUCK *et al.*, 2018). Current evidence of new cryptic lineages within *Ochthebius* is still limited by the use of a small number of molecular markers instead of comprehensive integrative methodologies combining morphology and next-generation sequencing techniques.

Additional research on ecological and biological traits are also required to enhance understanding of the evolutionary trajectories of true rockpool species and their responses to predicted environmental change scenarios, their tolerance to environmental stresses such as temperature, salinity or desiccation (MIRÓN-GATÓN *et al.*, 2022), life cycles characterisation (VELASCO *et al.*, 2022), metapopulation and metacommunity dynamics, and species dispersal (VELASCO, unpublished data).

In conclusion, the western Palaearctic supratidal rockpools holds, at least, 32 different species, 21 of them exclusive of these environments. The number of species found have increased during the last decade, and currently it is expected to grow due to its likely cryptic species, pointing out the great value of the still poorly unknow biodiversity that these habitats gather.

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When morphology meets genetics: the case of the hypogean *Laemostenus cazorlensis* (Coleoptera, Carabidae)

Cuando la morfología y la genética coinciden: el caso de *Laemostenus cazorlensis* (Coleoptera, Carabidae)

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ABSTRACT

The patchy distribution of the hypogean environment and the adaptations required to thrive in this special habitat have been proposed to constrain the dispersal capacity of hypogean organisms, resulting in processes of diversification even at small geographical scales. Counterintuitively, a recent detailed morphological study on the variability of the hypogean ground beetle *Laemostenus (Antisphodrus) cazorlensis* (Mateu, 1953) by MUÑOZ-SANTIAGO & ORTUÑO (2020) concluded that the three described subspecies are not supported, and their synonymization was proposed. Here we use sequence data of three DNA fragments of two genes (nuclear ITS2 and two fragments of the mitochondrial *cox1*) to study the intraspecific variation of *L. (A.) cazorlensis,* including specimens from the type localities of the three subspecies. Intraspecific variation of *L. cazorlensis* is analysed in the context of a molecular phylogeny of 6 species and 5 subgenera of *Laemostenus*. The maximum genetic distances within *L. (A.) cazorlensis* is 0.016, much lower than the observed values for *L. (P.) terricola* (0.056) and *L. (P.) algerinus* (0.04). The low genetic variation within *L. (A.) cazorlensis*, the mixed relationships between *L. (A.) cazorlensis cazorlensis* and *L. (A.) cazorlensis divergens,* and the limited differentiation *of L. (A.) cazorlensis seguranus* suggest the existence of a single species with well-connected populations. Thus, our results based on molecular information are in full agreement with morphological information, and supports the synonymisation of all subspecies within *L. (A.) cazorlensis.*

Keywords: Subterranean Coleoptera, taxonomy; subspecies; Cazorla; Segura; Calar del Mundo; synonymy

RESUMEN

La distribución discontinua del ambiente hipogeo y las adaptaciones necesarias para sobrevivir en este singular hábitat han sido propuestos como factores que restringen la capacidad de dispersión de los organismos hipogeos. De forma contra intuitiva, en un detallado estudio de la variabilidad morfológica del carábido *Laemostenus (Antisphodrus) cazorlensis* (Mateu, 1953) llevado a cabo por MUÑOZ-SANTIAGO & ORTUÑO (2020), se concluye que las tres subespecies descritas no presentan diferenciación morfológica y por tanto se propone su sinonimización. Aquí usamos datos moleculares de tres fragmentos de ADN pertenecientes a dos genes (un fragmento del gen nuclear ITS2 y dos fragmentos del gen mitocondrial *cox1*) para estudiar la variación intraespecífica de *L. (A.) cazorlensis*, incluyendo especímenes de la localidad típica de las tres subespecies. Su variación intraespecífica es analizada en el contexto de una filogenia molecular que incluye 6 especies y 5 subgéneros de *Laemostenus*. La distancia genética máxima obtenida entre especímenes de *L. (A.) cazorlensis* es de 0.016, mucho menor que la obtenida para *L. (P.) terricola* (0.056) y *L. (P.) algerinus* (0.04). Esta baja variación genética, la ausencia de estructura filogeográfica entre *L. (A.) cazorlensis cazorlensis* y *L. (A.) cazorlensis divergens,* y la reducida diferenciación de *L. (A) cazorlensis seguranus* sugieren la existencia de una sola especie con poblaciones conectadas. De esta manera, nuestros resultados moleculares son consistentes con la información morfológica, y apoyan la sinonimización de todas las subespecies dentro de *L. (A.) cazorlensis.*

Palabras clave: Coleópteros subterráneos, taxonomía; subespecies; Cazorla; Segura; Calar del Mundo; sinonimia.

INTRODUCTION

Hypogean species (*sensu* ORTUÑO & GILGADO, 2010), i.e., species that are adapted to live in the caves, the network of cracks and crevices of the bedrock, and the Mesovoid Shallow Substratum (JUBERTHIE *et al.*, 1980) have in most cases small ranges resulting in high levels of local and regional endemism (e.g, FRESNEDA *et al.*, 2011; CIESLAK *et al.*, 2014; FAILLE *et al.*, 2015). The adaptation to the conditions of relatively constant temperature, high humidity, and darkness of the hypogean environment, together with the patchy distribution of hypogean habitats, have been proposed to constraint the dispersal capacity of hypogean taxa, resulting in processes of diversification even at small geographical scales (e.g., FRESNEDA & SALGADO, 2022). This is the case of many species within the carabid genus *Laemostenus* Bonelli, 1810.

The genus *Laemostenus* includes 262 taxa (species and subspecies) according to CASALE (2017) distributed in the Palearctic region and its boundaries with the Oriental region (CASALE, 1988, 2017). In the Iberian Peninsula, *Laemostenus* is represented by 20 species (MUÑOZ-SANTIAGO & ORTUÑO, 2020; SERRANO, 2020). Most *Laemostenus* species exhibit geophilic and lucifugous behaviour (CASALE, 1988). Eleven Iberian species are included in the subgenus *Antisphodrus* Schaufuss, 1865, all of them hypogean endemics with restricted distributions across the karstic arc of Iberia (the Betic-Periplateu karstic arc *sensu* ORTUÑO & GILGADO, 2010). One of these species, *Laemostenus (Antisphodrus) cazorlensis* (Mateu, 1953), is located in the Betic System, in the sierras of Cazorla, Segura, and Las Villas. *L. (A) cazorlensis* was divided into three subspecies (MATEU, 1953; VIVES & VIVES, 1982; SERRANO, 2013): *L. (A.) cazorlensis cazorlensis* (Mateu, 1953); *L. (A.) cazorlensis divergens* (Mateu, 1953) and *L. (A.) cazorlensis seguranus* (Vives & Vives, 1982). The type locality of the species and the homonym subspecies is the cave Cueva Secreta del Sagreo, located in the Sierra de Cazorla (Jaén, Andalusia) (MATEU, 1953). The type locality of *L.* (*A.*) *c. divergens* is the cave Cueva de Navilla de Fuente Acero (MATEU, 1953), also in Sierra de Cazorla, and located only 25 km away from Cueva Secreta del Sagreo. The third subspecies, *L.* (*A.*) *c. seguranus*, was found under big rocks in the Calar del Mundo (Albacete, Castilla-La Mancha), and was described by VIVES & VIVES (1982). This subspecies has also been collected in additional localities within the mountains of Sierra de Segura, including numerous caves (ANDÚJAR *et al.*, 2000; PÉREZ & TINAUT, 2005, 2009; PÉREZ, 2006, 2008).

Differences among the three subspecies were mainly based on the shape of the pronotum, together with slight differences in the male genitalia and pigmentation of the tegument (MATEU, 1953; VIVES & VIVES, 1982; MUÑOZ-SANTIAGO & ORTUÑO, 2020). Recently, MUÑOZ-SANTIAGO & ORTUÑO (2020) conducted a detailed morphological study of specimens from multiple localities, including representatives from the type localities of the three subspecies. They concluded that the variation of the shape of pronotum and other morphological characters was not geographically structured and proposed the synonymy of the three subspecies.

Here we study mitochondrial and nuclear DNA sequences of specimens collected in the three type localities to check for geographically structured molecular variation. We also aim to compare the intraspecific variation of *L. (A) cazorlensis* relative to other congeneric species. Our principal hypothesis is that, if the lack of structured morphological variation among populations is because evolutionary divergence has not occurred, then DNA variability should be low and not clearly structured according to the geography. If corroborated, it will be in agreement with the proposal of high connectivity of the karstic system in the study area (MUÑOZ-SANTIAGO & ORTUÑO, 2020) and/or with the observed capacity of *L. (A) cazorlensis* to disperse above ground (ANDÚJAR, com. pers., 2010). Alternatively, DNA variation might reveal a geographical structuring suggestive of an ongoing process of speciation or even the existence of well-isolated evolutionary units without morphological change. This alternative would lead to assuming limited dispersal capacity of *L. (A) cazorlensis*, similar to recent reports of high genetic diversity and geographic structure of morphologically cryptic species in the subterranean environment (e.g. JUAN *et al.*, 2010; ZHANG & LI, 2014; DELIĆ *et al.*, 2017) in which it was found a high genetic diversity and geographical structuring within morphologically cryptic species in the subterranean environment.

MATERIAL AND METHODS

Sampling, DNA extraction and sequencing

Specimens were obtained from the Department of Zoology and Physical Anthropology collection at the University of Murcia (ZAFUMU col.) and from the personal collections of Jose Luis Lencina, Vicente M. Ortuño, and Carmelo Andújar. Overall, 37 specimens were DNA extracted and sequenced, and sequences from three additional specimens were retrieved from public nucleotide sequence databases (Appendix I). Selected specimens included 15 *L. (A) cazorlensis* with representatives from the three type localities where subspecies were described (Fig. 1), together with 11 *Laemostenus (Pristonychus) terricola* (Herbst, 1783), five *Laemostenus (Pristonychus) algerinus* (Gory, 1833), four *Laemostenus (Laemostenus) complanatus* (Dejean, 1828), two *Laemostenus (Actenipus) oblongus* (Dejean, 1828), one *Laemostenus (Ceuthostenes) mauritanicus* (Dejean, 1828), and one *Sphodrus leucophthalmus* (Linnaeus, 1758) (Appendix I). All specimens were collected by direct sampling or by means of pitfall traps. DNA extractions were conducted from whole specimens or from legs, using non-destructive methods and DNA extraction kits based on magnetic beads (DNeasy Blood&-Tissue Kit, Qiagen, Hilden, Germany; Mag-Bind® Blood & Tissue DNA Kit, Omega Bio-tek, Norcross, USA). After DNA extraction, specimens were preserved and curated in their source collections.

Three DNA fragments were sequenced, including: (i) the 5' end of cytochrome c oxidase subunit 1 (*cox1-a*, barcode fragment), (ii) the 3' end of the same gene (*cox1-b*), and (iii) a nuclear fragment of the Internal Transcribed Spacer 2 (ITS2). Primers used are provided in Table I. PCR reactions were made using Biotaq Polymerase with 39 cycles at 48-54°C for primer annealing. Sequencing was performed by Macrogen Inc. (Seoul, Korea). Sequence accession numbers are given in Appendix I.

Sequence alignment and molecular phylogenetic analyses

Sequences were viewed and edited in GENEIOUS PRIME v. 2020.0.3 (2020). Sequences from the protein-coding *cox1* gene were aligned using MAFFT 7.450 (KATOH *et al.*, 2002) with the L-INS-i method, while sequences from the *ITS2* gene were aligned using Q-INS-i (KATOH & TOH, 2008), a structural-aided alignment algorithm known to outperform non-structural methods (LETSCH *et al.*, 2010). A concatenated matrix was obtained by combining the three DNA fragments.

The concatenated datasets (with partitions by gene and also by codon position in the case of *cox1*) were analysed with maximum likelihood (ML) and Bayesian inference (BI) phylogenetic methods. ML trees were obtained using RAxML 8.2.12 (STAMATAKIS, 2014) on the CIPRES Science Gateway (MILLER *et al.*, 2010), with gene and codon partitions and applying an independent GTR+G+I model to each data partition. The best scoring ML tree was selected among 100 searches on the original alignment with different randomised parsimony starting trees. Support values were obtained with 1000 bootstrap replicates (FELSENSTEIN, 1985). BI analyses were conducted with MrBayes 3.2.2 (RONQUIST & HUELSENBECK, 2003) on the CIPRES Science Gateway, consisting of two independent runs for 10 million generations, sampling trees every 200 generations. We discarded the first 25% of trees as burn-in and calculated the standard deviation of split frequencies and mean and effective sample size (ESS) of likelihood values, computed with TRACER 1.5 (DRUMMOND & RAMBAUT, 2007), and these were then assessed for parameter mixing and convergence of estimates. A strict consensus tree (*allcompat* option) was obtained. Trees were visualised using FIGTREE 1.4.3 (RAMBAUT, 2012), and node posterior probabilities were interpreted as support values. In addition, an UPGMA tree using HKY corrected distances was computed in Geneious Prime using only sequences from the *cox1-a* gene fragment (barcode fragment). Divergence thresholds of 0.03 and 0.08 where marked over the obtained UPGMA tree. The 0.03 (3%) threshold has been frequently used to delimit species in the absence of further information, and 0.08 has been found as a conservative threshold not splitting known species in Coleoptera with the same DNA fragment

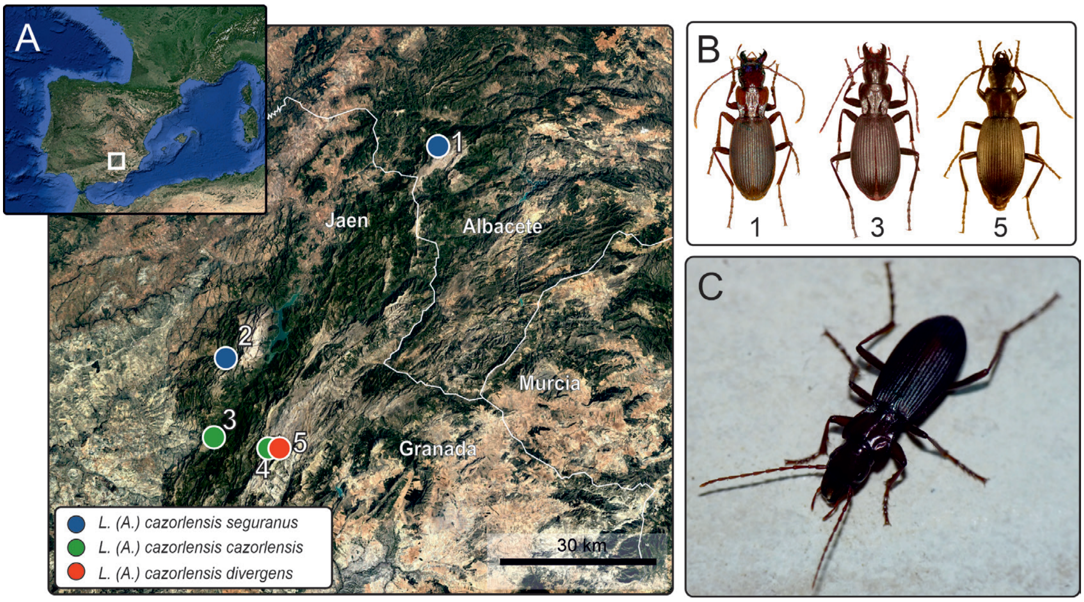

Fig. 1. A. Map of Sierra de Cazorla, Segura, and Las Villas with the collection localities of the studied *L. (A.) cazorlensis* specimens. Numbers: **1**. Cueva del Farallón, Calar del Mundo, Albacete [Type locality of *L. (A.) cazorlensis seguranus*]; **2.** La Fresnedilla, Villanueva del Arzobispo, Jaén; **3**. Cueva Secreta del Sagreo, La Iruela, Jaén [Type locality of *L. (A.) cazorlensis cazorlensis*]; **4**. Cueva Secreta del Poyo Manquillo, Cazorla, Jaén; **5**. Cueva Secreta de Navilla de Fuente Acero, Cazorla, Jaén [Type locality of *L. (A.) cazorlensis divergens*]. **B.** Images in dorsal view of *L. (A.) cazorlensis* subspecies. Numbers indicate localities as in A. **C.** Alive specimen of *L. (A.) cazorlensis* from Pino del Toril, Molinicos, Albacete. Image from Landsat/Copernicus - Google Earth.

Fig. 1. A. Mapa de la Sierra de Cazorla, Segura, y Las Villas con las localidades de colecta de *L. (A.) cazorlensis* marcadas. Números: **1**. Cueva del Farallón, Calar del Mundo, Albacete [Localidad típica de *L. (A.) cazorlensis seguranus*]; **2.** La Fresnedilla, Villanueva del Arzobispo, Jaén; **3**. Cueva Secreta del Sagreo, La Iruela, Jaén [Localidad típica de *L. (A.) cazorlensis cazorlensis*]; **4**. Cueva Secreta del Poyo Manquillo, Cazorla, Jaén; **5**. Cueva Secreta de Navilla de Fuente Acero, Cazorla, Jaén [Localidad típica de *L. (A.) cazorlensis divergens*]. **B.** Imágenes en visión dorsal de ejemplares representativos de las subesepcies de *L. (A.) cazorlensis*. Los números indican las localidades según A. **C.** Ejemplar vivo de *L. (A.) cazorlensis* de Pino del Toril, Molinicos, Albacete.

(SALCÉS-CASTELLANO *et al*., 2020). Haplotype trees of the *cox1-b* and ITS2 fragments were generated by a median joining network using PopArt software (LEIGH & BRYANT, 2015) to explore the phylogeographic structure of the populations.

RESULTS AND DISCUSSION

Phylogenetic trees obtained with different methods supported the monophyly of *L. (A) cazorlensis* and were widely consistent for interspecific relationships (Fig. 2), with only some instability in the position of *L. (C.) mauritanicus* that received low nodal support and showed inconsistent results between Bayesian and ML inferences. Maximum genetic distance (HKY corrected) within *L. (A) cazorlensis* was 0.016 (*cox1-a* gene; barcode fragment). This value was much lower than in other species, such as *L. (P) terricola* (0.056) and *L. (P.) algerinus* (0.04), but higher than in *L. (L.) complanatus* (0.004). Specimens of *L. (A) cazorlensis* from Cueva del Farallón (Calar del Mundo), the type locality of *L. (A) cazorlensis seguranus*, tended to cluster together, whereas specimens from the type localities of the other two subspecies– i.e., *L. (A) cazorlensis cazorlensis* and *L. (A) cazorlensis divergens*– appeared mixed and with very reduced genetic distances (Fig. 2). The haplotype trees showed a certain spatial structure, with *L. (A) cazorlensis seguranus* clearly separated from specimens of the other two subspecies, that even shared one haplotype (Fig. 3). The low genetic variation within *L. (A) cazorlensis* (below the 3% divergence threshold), the low differentiation *L. (A) cazorlensis seguranus*, and the mixed relationships among the specimens of the other two proposed subspecies did not support the three subspecific divisions within *L. (A) cazorlensis*, suggesting the existence of a single species with well-connected populations. Molecular data here presented revealed a pattern of moderate geographical structuring of the low intraspecific variation within the species. Altogether, these results are in full agreement with those based on morphological information reported by MUÑOZ-SANTIAGO & ORTUÑO (2020), and supports the synonymisation of all subspecies within *L. (A) cazorlensis.*

Results on the whole group of investigated taxa indicated that populations of *Laemostenus (Pristonychus) terricola* are notably differentiated at the molecular level, whereas *L. (Laemostenus) complanatus* shows scarce variability. As both taxa have a large distribution area (CASALE 2017 consider them as cosmopolitan elements), it is inferred that the dispersal ability of these species is different. *L. complanatus* is a winged species and flying may help dispersal and maintaining gene flow among populations. Conversely, *L. terricola* is wingless and shows higher genetic diversity, suggesting that anthropochory may be responsible for its cosmopolitan distribution.

Although the number of investigated taxa is low, we have found that each main branch of the Bayesian and ML trees corresponds to a distinct subgenus of *Laemostenus*. However, their relationships differ notably from the suggestion of CASALE (1988) based on morphological grounds. These initial findings highlight the importance of increasing the representation of species for further molecular phylogenetic analyses.

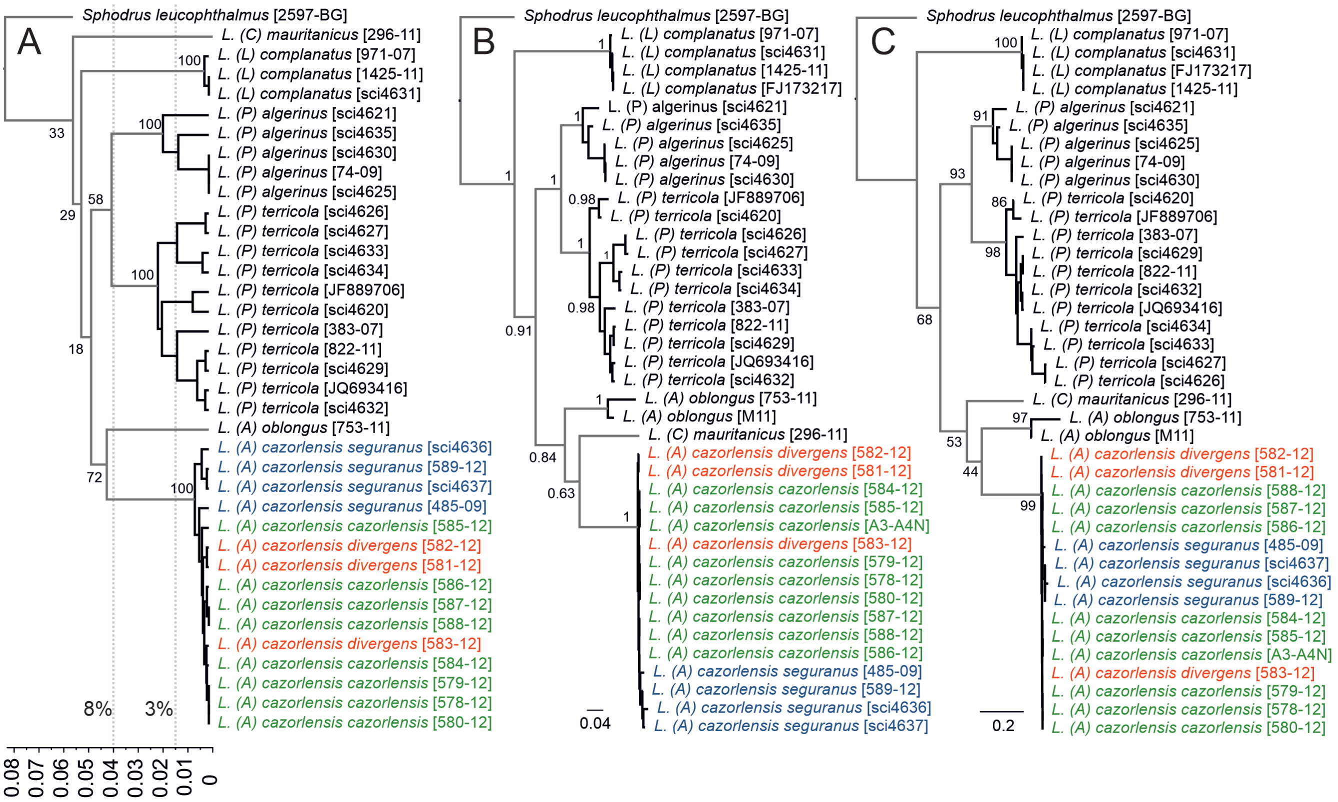

Fig. 2. Phylogenetic trees for *L. (A.) cazorlensis* and other *Laemostenus* taxa. **A.** UPGMA tree obtained with HKY corrected distances estimated for the *cox1-a* gene fragment (barcode fragment). Dotted lines mark the 0.03 (3%) and 0.08 (8%) divergence similarity thresholds; **B.** Strict consensus tree obtained from Bayesian analyses conducted on MrBayes with the concatenated dataset. Nodal support is given as posterior probabilities; **C.** Maximum likelihood tree estimated in RAxML with the concatenated dataset and bootstrap values as nodal support.

Fig. 2. Filogenia molecular obtenida para *L. (A.) cazorlensis* y otros *Laemostenus*. **A.** Árbol UPGMA obtenido con distancias corregidas (HKY) estimadas para el fragmento *cox1-a* ("barcode" estándar). Las líneas punteadas marcan los límites de divergencia al 0.03 (3%) y 0.08 (8%). **B.** Árbol consenso estricto obtenido de los análisis Bayesianos hechos en MrBayes con el set de datos concatenados. Las probabilidades posteriores se indican como valor de soporte de los nodos; **C.** Árbol de máxima verosimilitud obtenido con RAxML con el set de datos concatenado, con los valores de bootstrap indicados como soporte de los nodos.

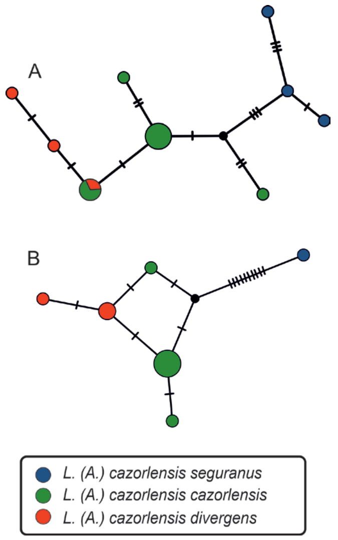

Fig. 3. Haplotype trees of *L. (A.) cazorlensis* generated by a median joining network algorithm using PopArt software. **A.** Haplotype tree for the *cox1-a* gene fragment. B. Haplotype tree for the ITS2 gene fragment.

Fig. 3. Árboles de haplotipos de *L. (A.) cazorlensis* obtenidos con el algoritmo "median joining network" con el programa PopArt. **A.** Árbol de haplotipos para el fragmento *cox1-a* ("barcode" estándar). **B.** Árbol de haplotipos para el fragmento ITS2.

Table I. Primers used in the study.

Tabla I. Cebadores usados en el estudio.

Gene	Primer name	Direction*	Primer sequence	Reference
cox1-a	FoldF	F	TCNACNAAYCAYAARRAYATYGG	(Yu *et al.*, 2012)
cox1-a	FoldR	R	TANACYTCNGGRTGNCCRAARAAYCA	(Yu *et al.*, 2012)
cox1-b	Jerry (M202)	F	CAACATTTATTTTGATTTTTTGG	(Simons *et al.*, 1994)
cox1-b	Pat (M70)	R	TCCA(A)TGCACTAATCTGCCATATTA	(Simons *et al.*, 1994)
ITS	5,8SF	F	GTGAATTCTGTGAACTGCAGGACACATGAAC	(Porter & Collins, 1991)
ITS	28SR	R	ATGCTTAAATTTAGGGGGTA	(Porter & Collins, 1991)

*F, forward; R, reverse.

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Appendix I. Studied specimens with data on localities and Genbank sequence accession numbers

Apendice I. Especímenes estudiados con datos de la localidad de colecta y números de acceso de las secuencias de ADN en Genbank.

Species	Subspecies (according to locality)	CODE	Locality	Cox1-a	Cox1-b	ITS2
L. (P) algerinus		74-09 ZAFUMU	Khetmin, Road C-151, Tunicia	ON206695	n.a.	n.a.
L. (P) algerinus		sci4621 (85-06 ZAFUMU)	Chréa National Park, Blida, Algeria	ON206717	n.a.	n.a.
L. (P) algerinus		sci4625 (20-09 ZAFUMU)	Station Nifza, Algeria	ON206718	n.a.	n.a.
L. (P) algerinus		sci4630 (73-09 ZAFUMU)	Khetmin, Road C-151, Tunicia	ON206722	n.a.	n.a.
L. (P) algerinus		sci4635 (6-13 ZAFUMU)	Chréa National Park, Blida, Algeria	ON206727	n.a.	n.a.
L. (A) cazorlensis	cazorlensis	578-12 ZAFUMU	Cueva Secreta del Poyo Manquillo, Cazorla, Jaen, Spain	ON206699	ON206678	ON229055
L. (A) cazorlensis	cazorlensis	579-12 ZAFUMU	Cueva Secreta del Poyo Manquillo, Cazorla, Jaen, Spain	ON206700	ON206679	ON229056
L. (A) cazorlensis	cazorlensis	580-12 ZAFUMU	Cueva Secreta del Poyo Manquillo, Cazorla, Jaen, Spain	ON206701	ON206680	ON229057
L. (A) cazorlensis	cazorlensis*	584-12 ZAFUMU	Cueva Secreta del Sagreo, La Iruela, Jaen, Spain	ON206705	ON206684	ON229061
L. (A) cazorlensis	cazorlensis*	585-12 ZAFUMU	Cueva Secreta del Sagreo, La Iruela, Jaen, Spain	ON206706	ON206685	ON229062
L. (A) cazorlensis	cazorlensis*	586-12 ZAFUMU	Cueva Secreta del Sagreo, La Iruela, Jaen, Spain	ON206707	ON206686	ON229063
L. (A) cazorlensis	cazorlensis*	587-12 ZAFUMU	Cueva Secreta del Sagreo, La Iruela, Jaen, Spain	ON206708	ON206687	ON229064
L. (A) cazorlensis	cazorlensis*	588-12 ZAFUMU	Cueva Secreta del Sagreo, La Iruela, Jaen, Spain	ON206709	n.a.	ON229065
L. (A) cazorlensis	cazorlensis*	A3-A4N	Cueva Secreta del Sagreo, La Iruela, Jaen, Spain	n.a.	ON206692	n.a.
L. (A) cazorlensis	divergens*	581-12 ZAFUMU	Cueva Secreta de Navilla de Fuente Acero, Cazorla, Jaen, Spain	ON206702	ON206681	ON229058
L. (A) cazorlensis	divergens*	582-12 ZAFUMU	Cueva Secreta de Navilla de Fuente Acero, Cazorla, Jaen, Spain	ON206703	ON206682	ON229059
L. (A) cazorlensis	divergens*	583-12 ZAFUMU	Cueva Secreta de Navilla de Fuente Acero, Cazorla, Jaen, Spain	ON206704	ON206683	ON229060
L. (A) cazorlensis	seguranus	485-09 ZAFUMU	La Fresnedilla, Villanueva del Arzobispo, Jaen, Spain	ON206698	n.a.	n.a.

Species	**Subspecies (according to locality)**	**CODE**	**Locality**	**Cox1-a**	**Cox1-b**	**ITS2**
L. (A) cazorlensis	seguranus*	589-12 ZAFUMU	Cueva del Farallón, Riópar, ALbacete, Spain	ON206710	n.a.	ON229066
L. (A) cazorlensis	seguranus*	sci4636	Cueva del Farallón, Riópar, Albacete, Spain	ON206728	n.a.	n.a.
L. (A) cazorlensis	seguranus*	sci4637	Cueva del Farallón, Riópar, Albacete, Spain	ON206729	n.a.	n.a.
L. (L) complanatus		1425-11 ZAFUMU	Praia da Vitória, Terceira, Azores, Portugal	ON206714	ON206690	ON229069
L. (L) complanatus		971-07 ZAFUMU	North Azemmour, Morocco	ON206713	n.a.	n.a.
L. (L) complanatus		FJ173217	Murcia, Spain	n.a.	**FJ173217**	n.a.
L. (L) complanatus		sci4631 (337-03 ZAFUMU)	Escombreras, Murcia, Spain	ON206723	n.a.	n.a.
L. (C) mauritanicus		296-11 ZAFUMU	Road Larache-Tanger, Morocco	ON206696	ON206677	ON229054
L. (A) oblongus		753-11 ZAFUMU	Puerto de Monteviejo, Leon, Spain	ON206711	ON206688	ON229067
L. (A) oblongus		M11	Spain	n.a.	ON206693	n.a.
L. (P) terricola		822-11 ZAFUMU	Laguna de Medina, Cádiz, Spain	ON206712	ON206689	ON229068
L. (P) terricola		383-07 ZAFUMU	Perales de Tajuña, Madrid, Spain	ON206697	n.a.	n.a.
L. (P) terricola		JF889706	Germany	**JF889706**	n.a.	n.a.
L. (P) terricola		JQ693416	Alicante, Spain	**JQ693416**	n.a.	n.a.
L. (P) terricola		sci4620 (1774-11 ZAFUMU)	Montseny, Barcelona, Spain	ON206716	n.a.	n.a.
L. (P) terricola		sci4626 (1034-06 ZAFUMU)	Benabarre, Huesca, Spain	ON206719	n.a.	n.a.
L. (P) terricola		sci4627 (1034-06 ZAFUMU)	Benabarre, Huesca, Spain	ON206720	n.a.	n.a.
L. (P) terricola		sci4629 (251-07 ZAFUMU)	Puerto de los Alazores, Madrid, Spain	ON206721	n.a.	n.a.
L. (P) terricola		sci4632 (1948-11 ZAFUMU)	Férez, Albacete, Spain	ON206724	n.a.	n.a.
L. (P) terricola		sci4633 (226-08 ZAFUMU)	Aldeia da Serra, Portugal	ON206725	n.a.	n.a.
L. (P) terricola		sci4634 (226-08 ZAFUMU)	Aldeia da Serra, Portugal	ON206726	n.a.	n.a.
Sphodrus leucophtalmus		2597-BG ZAFUMU	Tinerhir, Morocco	ON206715	ON206691	ON229070

What triggers metamorphosis in cave insects with contracted life cycle? A work program

¿Que desencadena la metamorfosis en insectos cavernícolas con ciclo contraído? Un programa de trabajo

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ABSTRACT

Usually, the time of metamorphosis in insects is determined when the individual reaches a critical weight (CW) in the preimaginal stage. However, there are highly specialized cave-dwelling beetles that have only a single larval instar, which, instead of gaining weight during development until metamorphosis, loses it. We propose several mechanisms by which these species could reach the CW and metamorphose. For example, the CW could be reached not through an increase, but the other way around, a decrease in weight, or a related parameter, after which the mechanisms that trigger metamorphosis would be displayed. Another possibility is that the larva would have the CW when emerging, thus the metamorphosis mechanisms would gradually unfold. Finally, it is also possible that there is a specific signaling factor that increases with time; then, mechanisms would be similar to those of epigean insects.

Key words: Cave beetle, contracted life cycle, metamorphosis regulation, MEKRE92 pathway, critical weight, Coleoptera Leiodidae, Coleoptera Carabidae.

RESUMEN

En general, el momento de la metamorfosis en los insectos queda determinado cuando el individuo alcanza un peso crítico (CW) en el último estadio larvario. Sin embargo, hay coleópteros cavernícolas altamente especializados que tienen un solo estadio larvario, el cual, en lugar de ganar peso durante el desarrollo hasta la metamorfosis, lo pierde. Proponemos varios mecanismos por los que estas especies podrían alcanzar el CW y metamorfosear. Por ejemplo, el CW podría alcanzarse no a través de un aumento, sino al revés, de una disminución de peso, o un parámetro relacionado, tras lo cual se desplegarían los mecanismos que desencadenan la metamorfosis. Otra posibilidad es que la larva ya tenga el CW al eclosionar, por lo que los mecanismos de metamorfosis se desarrollarían gradualmente. Finalmente, también es posible que exista un factor señalizador específico que aumente con el tiempo. Entonces, los mecanismos serían similares a los de los insectos epigeos.

Palabras clave: Coleóptero cavernícola, ciclo biológico contraído, regulación de la metamorfosis, vía MEKRE92, peso crítico, Coleoptera Leiodidae, Coleoptera Carabidae.

INTRODUCTION

Metamorphosis, by which the insect acquires adult features and stops molting during postembryonic development (BELLÉS, 2020), is a complex and fascinating process. Although many aspects of insect metamorphosis have been elucidated in considerable detail in recent years, particularly the molecular mechanisms that regulate the process itself, there are still issues to be unveiled. One of them is to find out exactly how the insect "decides" that it can metamorphose. The answer, in principle, would be that the insect can metamorphose when it reaches what has been called the critical weight (CW).

The debate on how the insect senses that has reached the CW is still open, as the most diverse hypotheses have been proposed by different authors. The line of reasoning is that the insect reaches the CW through progressive growth during the juvenile period, through feeding. So, the insect seems to sense, either the size, the weight, or some related parameter, such as the amount of accumulated fat or protein, or the limitations in the distribution of oxygen in the body (see MIRTH & RIDDIFORD, 2007; NIJHOUT & CALLIER, 2015; TEXADA *et al.*, 2020). However, this line of reasoning is broken when we consider some beetle species specialized in living in caves that have a post-embryonic development with a single larval instar that does not feed. That larva, instead of gaining weight during development until metamorphosis, loses it (DELEURANCE-GLAÇON, 1963a; STRAMBI, 1970). In this case, as pointed out by the Ignacio Ribera group (CIESLAK *et al.*, 2014), growth and development are decoupled from traditionally recognized strict boundaries in insect ontogeny (see also MINELLI *et al.*, 2006).

It is not known how cave beetles with a single non-feeding larval instar achieve CW. In this essay, we propose some working hypotheses, which, although they are based on considerable amounts of speculation, all of them would be testable, which can stimulate further research on the subject.

THE REGULATION OF INSECT METAMORPHOSIS

Two main types of insect metamorphoses are recognized, hemimetaboly and holometaboly. In hemimetabolans, embryogenesis gives rise to a first instar nymph that shows the essential adult body structure. Then, the nymphs gradually grow, and the final molt to the adult completes the formation of wings and genitalia. In holometabolans, embryogenesis produces a larva with a morphophysiology diverging from that of the adult; then the larva grows through various stages until molting to the pupal stage, which bridges the gap between the form of the larva and that of the winged and reproductively competent adult (BELLÉS, 2020).

In both, hemimetabolans and holometabolans, metamorphosis is essentially regulated by two hormones: the terpenoid juvenile hormone (JH), and the steroid ecdysone, plus its biologically active derivative, 20-hydroxyecdysone (20E) (NIJHOUT, 1994). In turn, the hormonal signal is transduced through a hierarchical pathway of gene activation. The 20E signaling pathway begins with the interaction of the hormone with its receptor, the ecdysone receptor (EcR), which then forms a complex with the coreceptor ultraspiracle (USP). The EcR+20E-USP complex activates a gene cascade that transduces the hormonal signal (see KING-JONES & THUMMEL, 2005; OU & KING-JONES, 2013). Among the genes of the cascade, *Broad complex* (*Br-C*) and *E93* are especially relevant in the regulation of metamorphosis, as Br-C specifies the pupal stage, and E93 the adult (see BELLÉS, 2019). The most important details of the JH signaling pathway have recently been revealed (see JINDRA *et al.*, 2015). It also begins with the interaction of the hormone with its receptor, the Methoprene tolerant (Met) protein, which then forms a complex with the coreceptor Taiman (Tai). The Met+-JH-Tai complex also activates a gene cascade that includes the gene *Krüppel homolog 1* (*Kr-h1*), whose gene product inhibits metamorphosis.

As first observed in the cockroach *Blattella germanica* (BELLÉS & SANTOS, 2014), Kr-h1 represses *E93*, which led to propose the MEKRE93 pathway, the essential axis that regulates metamorphosis in all insects. Accordingly, in nymph-nymph transitions, JH acts through its receptor Met-Tai, inducing the expression of *Kr-h1*, which represses *E93*. In turn, the fall of JH production in the final juvenile stage interrupts *Kr-h1* expression, which induces the expression of *E93* through 20E signaling, thus triggering metamorphosis (Fig. 1). The repressor action of Kr-h1 upon *E93* was later corroborated in the beetle *Tribolium castaneum* (UREÑA *et al.*, 2016), which extended the MEKRE93 pathway to holometabolan metamorphosis. The main peculiarity of the holometabolans is the regulation of pupal morphogenesis, which is triggered by Br-C and requires a specific regulatory loop (Fig. 1). As shown in the beetle *T. castaneum*, E93 is also involved in triggering the pupal stage, as it promotes *Br-C* expression (Fig. 2) (CHAFINO *et al.*, 2019).

THE CRITICAL WEIGHT FOR METAMORPHOSIS IN MODEL INSECTS

The mechanisms that lead to metamorphosis seem clear and universal in insects. But what is not so clear is what triggers these mechanisms, involving a decrease of JH, and the production of a major pulse of 20E. The triggering mechanism is encapsulated in the concept of critical weight (CW), essentially defined by NIJHOUT & WILLIAMS (1974) as the minimal mass at which further growth is not necessary for a normal time course to metamorphose (see also MIRTH & RIDDIFORD, 2007; NIJHOUT & CALLIER, 2015). In the end, the adult size is essentially determined by the rate of growth during juvenile stages, and the duration of this growth period (see REWITZ *et al.*, 2013; BOULAN *et al.*, 2015). The Insulin/IGF Signaling (IIS) and Target of Rapamycin (TOR) pathways, are the main regulators of growth rate, whereas the factor determining growth duration is 20E (see YAMANAKA *et al.*, 2013). In general, energy input is a necessary condition that influences IIS/TOR pathways and 20E signaling.

As CW is likely based on assessment of nutritional status rather than actual body mass per se, a relevant question in this context is how the insect senses the nutritional status to be conveyed to the IIS/TOR pathways. It makes sense that the main nutrient-sensing tissue is the fat body, the organ where the most important metabolic processes reside, including the reception of information from cellular levels of amino acids through TOR, as well as other environmental variables, like oxygen levels, and releases humoral signals that act on insulin-producing cells and insulin like proteins (ILPs) production (see TEXADA *et al.*, 2020).

If the fat body is the sensing organ, then what does it senses? One possibility is that it senses lipids, as proposed by TEXADA *et al.* (2020). The fat body would accumulate lipids, which would be an important resource to successfully undertake metamorphosis and adult development. Another possibility is that fat body sense amino acids. Indeed, amino acids mainly act on two organs, the fat body and the brain, then influencing larval growth, body size and, in the end, pupation. The expression of specific amino acid transporters in fat body cells (and in the brain through specific neurons and glial cells) in response to present amino acid levels, is crucial to activate signaling pathways involving ILPs production, thus larval growth (see MANIÈRE *et al.*, 2020).

THE CONTRACTED LIFE CYCLE OF CAVE BEETLES

Very specialized cave beetles show a postembryonic development characterized by a dramatic reduction in the number of larval instars. Initial observations focused on Leiodidae Leptodirini, and Carabidae Trechini (DELEURANCE-GLAÇON, 1963a, 1963b). Further research fully confirmed these observations and expanded the knowledge with data on ecophysiology and reproduction (DELAY, 1978).

The best-studied group is the Leptodirini, which contains many representatives that live in caves, showing different degrees of specialization. The less specialized species show morphological characters similar to those of the more or less cryptic epigean species, while the most specialized ones show very conspicuous adaptations associated to cave life, like depigmentation, lack of eyes but proliferation of other sensory devices, lack of membranous wings, and elongation of antennae and legs (see VANDEL, 1964). However, these characteristics do not represent a single group category, but there is a continuum between low specialized species that show them very weakly, and highly specialized species, which show all them very conspicuously. The life cycles show also this kind of gradation, from less to more specialized. In the less specialized, which live in mosses, forest litter or in shallow soil, the female produces small oligolecithal eggs and the emerging larvae pass through three larval instars, and then pupate. The larvae feed throughout their life except for the last period of the third instar. This life cycle is similar to that of epigean beetles, except that the larva builds a clay chamber where it is hidden at the time of molting, and during the entire pupal stage. At the other extreme, in the most specialized cave species, the female lays a single large egg at a time. From this macrolecithal egg emerges a larva that has a very short free life, during which it does not feed and builds a clay chamber where it will hide in. The larva remains within the chamber without feeding during five to six months, and then pupates in the same chamber (Fig. 3) (DELEURANCE-GLAÇON, 1963a; DELAY, 1978) (reviewed in VANDEL, 1964; DELAMARE-DEBOUTTEVILLE, 1971; BELLÉS, 1987).

No precise life cycle data are known in cave insects other than those of Leptodirini and Trechini beetles. However, studies comparing the ovaries in cave cockroaches of the genus *Loboptera* from Canary Islands, show an inverse correlation between the number and size of ovarioles, and the degree of adaptation to cave life according to morphological characters (IZQUIERDO *et al.*, 1990). This led to predict that highly specialized cave *Loboptera* with less and bigger ovarioles will have reduced the number of nymphal instars, which would imply the occurrence of contracted cycles also in specialized cave hemimetabolans.

HOW DO CAVE INSECTS REACH THE CRITICAL WEIGHT FOR METAMORPHOSIS?

The case of Leptodirini cave beetles with a single larval instar leads to wonder how do these species reach the CW. In fact, the larva does not feed during the entire instar, having the necessary reserves to reach the pupal

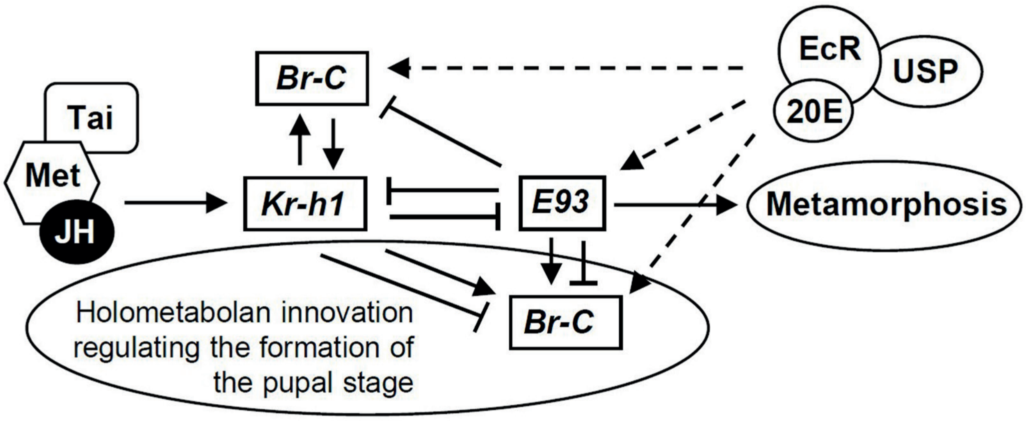

Fig. 1. The MEKRE93 pathway in the hemimetabolan and holometabolan types of metamorphosis. In the nymph-nymph transitions of the hemimetabolans, juvenile hormone (JH), through the receptor complex Methoprene tolerant-Taiman (Met-Tai) induces the expression of *Krüppel homolog 1* (*Kr-h1*), which, in turn, represses the expression of *E93*. In contrast, the decline of JH production in the final nymphal instar interrupts the expression of *Kr-h1*, thus allowing a strong induction of *E93* by 20E signaling, which triggers metamorphosis. The main difference between the hemimetabolan and holometabolan metamorphoses is the regulation (and function) of Broad complex (Br-C). In hemimetabolans, Br-C it is mainly involved in sustaining the growth of wing primordia, whereas in holometabolans, Br-C triggers the formation of the pupa. From BELLÉS (2019), slightly modified.

Fig. 1. La vía MEKRE93 en los tipos de metamorfosis hemimetábola y holometábola. En las transiciones ninfa-ninfa de los hemimetábolos, la hormona juvenil (JH), a través del complejo receptor Methoprene tolerant-Taiman (Met-Tai) induce la expresión de *Krüppel homolog 1* (*Kr-h1*), que a su vez reprime la expresión de *E93*. Por el contrario, la disminución de la producción de JH en el último estadio ninfal interrumpe la expresión de *Kr-h1*, lo que permite una potente inducción de *E93* gracias a la 20E, que desencadena la metamorfosis. La principal diferencia entre la metamorfosis hemimetábola y holometábola es la regulación (y función) de Broad complex (Br-C). En hemimetábolos, Br-C participa principalmente en sustentar el crecimiento de los primordios alares, mientras que, en holometábolos, Br-C desencadena la formación de la pupa. Según BELLÉS (2019), ligeramente modificado.

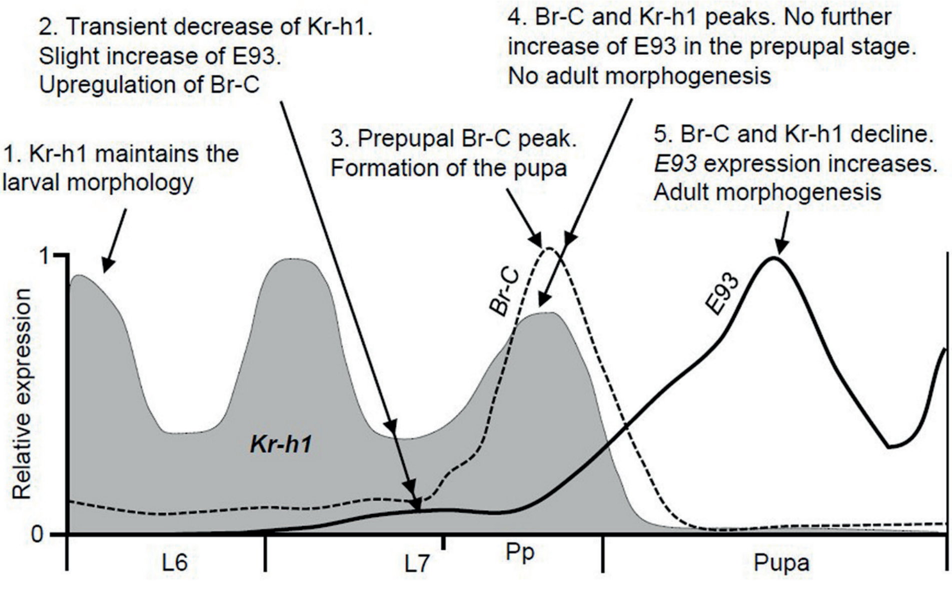

Fig. 2. Expression of *Krüppel homolog 1* (*Kr-h1*), *Broad complex* (*Br-C*) and *E93* during metamorphosis in the beetle *Tribolium castaneum*, indicating the succession of regulatory events. The expression of *Kr-h1* is represented by a gray area, *Br-C* with a dashed line and *E93* with a continuous line. Expression levels are indicated in relation to the maximum value (= 1). In abscissae, the two last larval instars (L6-L7), the prepupal stage (Pp) and the pupa are shown. With data from MINAKUCHI *et al.* (2009) (*Kr-h1*, *Br-C*), and UREÑA *et al.* (2014 and 2016) (*E93*).

Fig. 2. Expresión de *Krüppel homolog 1* (*Kr-h1*), *Broad complex* (*Br-C*) y *E93* durante la metamorfosis en el coleóptero *Tribolium castaneum*, indicando la sucesión de pasos reguladores. La expresión de *Kr-h1* está representada por un área gris, *Br-C* con una línea discontinua y *E93* con una línea continua. Los niveles de expresión se indican en relación al valor máximo (= 1). En abscisas se muestran los dos últimos estadios larvarios (L6-L7), el estadio prepupal (Pp) y la pupa. Con datos de MINAKUCHI *et al.* (2009) (*Kr-h1*, *Br-C*) y UREÑA *et al.* (2014 y 2016) (*E93*).

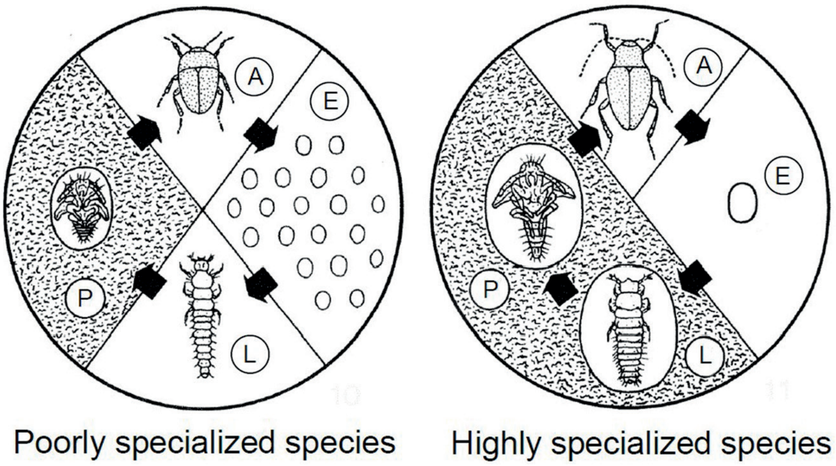

Fig. 3. Extreme models of life cycle in Leptodirini beetles represented by poorly specialized species and highly specialized species. In the first type (left), the adult female (A) lays many small eggs (E), after hatching, a larval period (L) follows, involving three free instar with feeding activity (although the molts occur inside a clay chamber built by the larvae); at the end of the final larval instar, the larva builds again a clay chamber where it will pass the pupal instar (P). The highly specialized species (right), follow the contracted cycle, whereby the adult female (A) lays a single large egg at a time (E), after hatching, the larval period (L) consists of a practically quiescent single instar that elapses in a clay chamber, like the pupal stage (P). From DELAMARE-DEBOUTTEVILLE (1971) and BELLÉS (1987), slightly modified.

Fig. 3. Modelos extremos del ciclo biológico de los coleópteros Leptodirini representados por especies poco especializadas y especies altamente especializadas. En el primer tipo (izquierda), la hembra adulta (A) pone muchos huevos de pequeño tamaño (E), después de la eclosión, sigue un período larvario (L), que implica tres estadios libres con actividad alimentaria (aunque las mudas se dan dentro de una celda de arcilla construida por las larvas); en la etapa larvaria final, la larva construye nuevamente una celda de arcilla donde pasa la etapa de pupa (P). Las especies altamente especializadas (derecha), siguen el ciclo contraído, por el cual la hembra adulta (A) pone un solo huevo de gran tamaño (E), tras la eclosión, el período larvario (L) consiste en un único estadio prácticamente inactivo que transcurre en una celda de arcilla, como la etapa de pupa (P). Según DELAMARE-DEBOUTTEVILLE (1971) y BELLÉS (1987), ligeramente modificado.

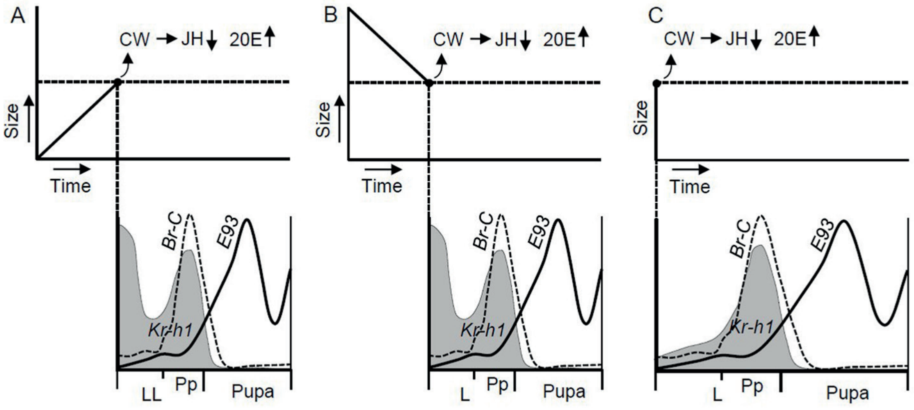

Fig. 4. Possible mechanisms leading to reach the critical weight (CW) that triggers metamorphosis in cave Leptodirini beetles. In poorly specialized species (A), the mechanisms would be similar to those described for epigean species. The CW would be reached in the last larval instar (LL) and the prepupal stage (Pp), involving a decrease of juvenile hormone (JH), and the production of a major pulse of 20-hydroxyecdysone (20E), leading to metamorphosis, with the successive formation of the pupa, determined by Broad complex (Br-C), and the adult, determined by E93. In highly specialized species (B and C), with a single larval instar (L), the CW would be reached from largest to smallest size, after which the same molecular mechanisms would follow (B). It could be also possible that the larva has the CW when hatching, thus the molecular mechanisms triggering metamorphosis would gradually unfold. In this case, the juvenile hormone (JH) and the concomitant expression of *Krüppel homolog 1* (*Kr-h1*) would not be necessary to maintain larval growth, but it would be required to prevent the appearance of adult characters in the pupa. Finally, it is also possible that there is a specific triggering factor that increases its production with time; in this case, the CW reaching would be similar to that of epigean insects (A).

Fig. 4. Posibles mecanismos que llevan a alcanzar el peso crítico (CW) que desencadena la metamorfosis en los coleópteros cavernícolas Leptodirini. En especies poco especializadas (A), los mecanismos serían similares a los descritos para especies epigeas. El CW se alcanzaría en el último estadio larvario (LL) y en la etapa prepupal (Pp), lo que implica una disminución de la hormona juvenil (JH) y la producción de un potente pulso de 20-hidroxiecdisona (20E), que conduce a la metamorfosis, con la sucesiva formación de la pupa, determinada por Broad complex (Br-C), y del adulto, determinada por E93. En especies altamente especializadas (B y C), con un solo estadio larvario (L), el CW se alcanzaría de mayor a menor tamaño, tras lo cual seguirían los mismos mecanismos moleculares (B). También podría ser que la larva tenga el CW al eclosionar, por lo tanto, los mecanismos moleculares que desencadenan la metamorfosis se desarrollarán gradualmente. En este caso, la hormona juvenil (JH) y la expresión concomitante de *Krüppel homolog 1* (*Kr-h1*) no serían necesarias para mantener el crecimiento larvario, pero sí para prevenir la aparición de caracteres adultos en la pupa. Finalmente, también es posible que exista un factor desencadenante específico que aumente su producción con el tiempo; en este caso, el proceso de alcanzar la CW sería similar al de los insectos epigeos (A).

stage. Intriguingly, the size (length) of this single larval instar is similar to the size of the adult (CIESLAK *et al.*, 2014), but the dry weight decreases during larval development. A freshly emerged larva of *Troglodromus bucheti*, a species with a single larval instar, has a dry weight of 41.3%, whereas the dry weight of freshly emerged first larval instar of *Speophyes lucidulus*, with two feeding larval instars, is 18%. During postembryonic development, both species absorb water through the integument in the saturated atmosphere of the cave environment, but the dry weight of *T. bucheti* larvae decreases while that of *S. lucidulus* increases. Thus, just before pupation, the ratio of dry to total weight results to be similar in both species: 18% and 22%, respectively (STRAMBI, 1970). Thus, the dry weight in cave beetles with a single larval instar does not increase, but can even decrease as the reserves are consumed. What kind of reserves accumulated the larvae of these species? According to histological data, the fat body would contain reserves mainly of proteins, but also of lipids (DELEURANCE-GLAÇON, 1963a). Apparently, both would be consumed throughout the larval period, and preliminary data from DELEURANCE-GLAÇON (1963a) indicate that between a mid-stage larva and the prepupa of *Speonomus longicornis*, a species with a single larval instar, the lipid content would drop from 6% to 3%.

The data available would suggest that in the single larva of contracted cycle species, the CW might be reached not through an increase, but the other way around, a decrease in weight, or a correlated parameter, like lipid or protein contents. Then, once reached, the CW would be sensed by the same mechanisms that have been postulated in species with several larval instars (Fig. 4A and B). Another possibility is that, from the moment of hatching, the larva already has the CW, so that the hormonal and molecular mechanisms of metamorphosis progressively unfold (Fig. 4C). It is also possible that there is a signal that increases its production, despite (or thanks to) that the energy parameters decrease. In this sense, we can recall the case of myoglianin, whose increased expression leads to metamorphosis, both in hemimetabolans (ISHIMARU *et al.*, 2016; KAMSOI & BELLÉS, 2019) and in holometabolans (HE *et al.*, 2020). In holometabolans, it has even been suggested that myoglianin is the signal by which body size is sensed, and that triggers the transition between growth and metamorphosis (HE *et al.*, 2020). In this case, the dynamics of reaching CW and subsequent mechanisms leading to metamorphosis would be similar to that of epigean insects (Fig. 4A).

Moreover, in cave beetles with contracted cycle, whose larval (and pupal) instars takes place in a clay cell with limited gas exchange transpiration, the progressive decrease of O_2 and accumulation of CO_2 can be important environmental cues. Indeed, such hypoxic conditions have been observed inside termite nests, in which they appear to be favorable for reproduction (TASAKI *et al.*, 2020). A progressively hypoxic environment in the larva of contracted cycle beetle species may contribute to trigger molting and metamorphosis, as occurs in the lepidopteran *Manduca sexta* (CALLIER & NIJHOUT, 2011). It is also interesting to consider the role of the JH in species with contracted cycle. An important consequence of the repressive action of JH on metamorphosis, and perhaps its most important function in post-embryonic development, is that it allows growth during the juvenile stages, the larval period in holometabolans. It would seem, therefore, that this JH function is not necessary in species with a contracted cycle that have a single larval instar, which does not grow. It is therefore possible that circulating levels of JH are low in larvae of these species, as indicated in figure 4C. However, a burst of JH, and a concomitant pulse of *Kr-h1* expression, should occur at the prepupal stage, when the phasic expression of *Br-C* triggers pupal formation, as Kr-h1 would prevent the development of adult characters in the pupa.

Finally, some observations on the development of fasted insect larvae may help to understand the phenomenon of regulation of contracted cycles. In some Dermestid beetles, the suppression of food in the last larval instar involves the inhibition of molting to pupa and "retrogressive" development through a variable number of larval molts. This has been described in *Trogoderma glabrum* (BECK, 1971) and in *Trogoderma variabile* (ELBERT, 1979), which during retrogressive development, the larva loses weight and dramatically reduces oxygen uptake. The mechanisms that determine the successive retrogressive molts are a mystery, but their elucidation would perhaps illuminate the mechanisms that regulate the development of cave beetles with a contracted cycle. Also interesting in a context of development under nutritional restrictions is the case of the beetle *T. castaneum*, in which part of the larvae reared under nutrient-poor diets (20%-flour diet) from the antepenultimate larval instar, prematurely transformed to pupae after one or two molts (CHAFINO et al. al., 2019). In these larvae, the nutritional restriction promoted a precocious up-regulation of *Br-C* and *E93* expression, which led to premature metamorphosis.

APPROACHES FOR TESTING THE HYPOTHESES

The above postulates about the regulation of metamorphosis of cave beetles on a molecular scale are mere speculations, but they are empirically testable. The first condition would be to have a continuous rearing in the laboratory of cave species from the Leptodirini tribe, which offers the widest range of possibilities. Ideally, the most appropriate approach would be to compare a poorly specialized species, with a cycle of three free larval instars, with a highly specialized species, with a single quiescent larval instar. This is not trivial, since cave species, especially the most specialized, are extremely sensitive to environmental disturbances, which easily prevent the progress of the life cycle, from mating and oviposition. In fact, the studies by Sylvie Deleurance-Glaçon and Bernard Delay were carried out in terrariums installed in natural caves, under very stable conditions (and failures far outweighed successes in many occasions…). Once a continuous rearing has been established, the most obvious approach would be to measure the mRNA levels of *Kr-h1*, *Br-C* and *E93* by Real-Time Quantitative Reverse Transcription PCR (qRT-PCR), at different times in the penultimate and last larval phases of the less specialized species, and in the single larval instar of the more specialized species, and reconstruct the respective expression profiles.

An alternative approach would be to build mRNA libraries at the same time periods in both species, the sequencing of which would provide information similar to that of direct qRT-PCR measurements. Moreover, the analysis of the libraries would provide valuable additional information, for example, on the expression of genes involved in metabolism that would shed more light on the issue of how CW is reached in the two species. Either of the two approaches would lead to know whether the times and molecular mechanisms represented in figure 4A, B or C occur. However, surprises cannot be ruled out. In fact, the very existence of these bizarre contracted life cycles is in itself surprising.

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Ignacio Ribera's Tree of Life

El árbol de la vida de Ignacio Ribera

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ABSTRACT

Ignacio Ribera (1963-2020) was an influential contemporary entomologist and evolutionary biologist and a dear friend. In this article we pay humble homage to a significant part of his research interests and commitment: revealing the details of the Coleoptera Tree of Life using his combined knowledge on the taxonomy of the groups he liked and information provided by DNA sequence data. GenBank contains 17,479 individual entries related to articles published by Ignacio Ribera, most of them beetle *cox1* sequences (7,725). This is not trivial, representing ~ 3.5% of the global production for this genetic marker, the most studied by far in Metazoans. The first beetle *cox1* sequences were deposited in GenBank in the early 90's, with just a handful of sequences, but numbers rocketed to tens of thousands in 2015, reaching 215,962 entries when writing this. Most beetle studies originally amplified by PCR and sequenced the 3'-end of the *cox1* gene using the primer set JERRY-PAT, but later on, with the success of the DNA barcoding agenda, researchers switched to the 5'-end of the gene using the primer pair LCO-HCO, becoming the most common *cox1* fragment in GenBank. Most of Ignacio's *cox1* sequences correspond to the JERRY-PAT fragment (6,217), with the LCO-HCO fragment representing a much smaller fraction (1,508). The majority of sequences for both fragments were obtained from water beetles, most of them, 4,521 and 588 sequences, respectively, from representatives of the family Dytiscidae. The data presented here, highlighting both the phylogenetic focus of Ignacio's studies and the significance of his work, shall illustrate the merit of Ignacio's legacy to the Coleoptera Tree of Life.

Key words: beetles, COI, Phylogenetics, DNA barcode, biodiversity, homage.

RESUMEN

Ignacio Ribera (1963-2020) fue un entomólogo y biólogo evolutivo contemporáneo muy influyente y también un gran amigo. En este artículo humildemente rendimos homenaje a una parte muy importante de sus intereses y compromiso científico: desvelar los detalles del Árbol de la Vida de Coleoptera usando su conocimiento combinado de la taxonomía de los grupos que le gustaban y de información obtenida a partir de secuencias de ADN. La base de datos GenBank contiene 17.479 registros individuales relacionados con artículos publicados por Ignacio Ribera, la mayoría (7.725) secuencias del gen *cox1* de coleópteros. Este detalle no es trivial, pues estas secuencias representan aproximadamente el 3,5% de la producción global para Coleoptera de este marcador genético, el más estudiado en Metazoa con diferencia. Las primeras secuencias de *cox1* de Coleoptera se depositaron en GenBank al inicio de los años 90, que al principio fueron muy pocas, pero las nuevas incorporaciones se dispararon a decenas de miles de secuencias en 2015, alcanzando los 215.962 registros en estos momentos. La mayoría de estudios sobre Coleoptera inicialmente amplificaron por PCR y secuenciaron el extremo 3' del gen *cox1* usando los cebadores JERRY y PAT, pero más adelante, con el éxito de la iniciativa de los códigos de barras de ADN, los investigadores empezaron a estudiar el extremo 5' del gen mediante los cebadores LCO y HCO, pasando a ser el fragmento más común de *cox1* en GenBank. La mayoría de las secuencias *cox1* de Ignacio son del fragmento JERRY-PAT (6.217), y las del fragmento LCO-HCO representan una fracción menor (1.508). La mayoría de secuencias para ambos fragmentos se obtuvieron a partir de coleópteros acuáticos, muchas de ellas, 4.521 y 588 secuencias, respectivamente, de representantes de la familia Dytiscidae. Los datos que presentamos aquí, resaltando tanto el enfoque filogenético de los estudios de Ignacio como la trascendencia de su trabajo, deberían ilustrar el mérito del legado de Ignacio al Árbol de la Vida de Coleoptera.

Palabras clave: escarabajos, COI, filogenética, código de barras ADN, biodiversidad, homenaje.

INTRODUCTION

Last 15th of April, 2020, the entomology, evolutionary biology and ecology research communities in Spain lost one of our most remarkable scientists, Ignacio Ribera Galán, and we lost one of our best friends and most admired colleagues. Ignacio devoted most of his time, professionally and also a great deal of his leisure time to the study of biodiversity, of evolutionary and ecological patterns at different geographic and taxonomic scales, and also applying the knowledge he generated towards the protection of the organisms he knew and understood the most. Throughout his entire career, the study of those questions would specifically focus on different groups of beetles, the organisms he was most passionate about. He used every opportunity he had to do fieldwork to collect them in the five continents, especially around the Mediterranean basin. His first published studies, in the late 80‘s and early 90‘s, were with different groups of water beetles, a non-taxonomic category that groups beetles adapted to aquatic lifestyles, and this would become a constant in his career, becoming one of the most reputed contemporary international specialists in the study of these organisms. Indeed, in Ignacio‘s remarkable scientific production, with well over 270 scientific articles, about 70% of his published research was devoted to different groups of water beetles (Appendix I). Among these, the Dytiscidae represented 25.8% of his target groups, followed by Hydraenidae (17.7%), Limnichidae (14.0%) and Hydrophilidae (8.1%). But Ignacio also generated published knowledge on several other water beetle families, including Elmidae, Hydrochidae, Meruidae, Noteridae, and on an entire new family that he discovered and described, the Aspidytidae (RIBERA *et al*., 2002). A significant proportion (26.3%) of his water beetle studies, including faunistic works, catalogues, but also studies with a focus on ecological or functional traits, were centred on suprafamilial assemblages (e.g., Hydradephaga or the functional assemblage of water beetles across major suborders of Coleoptera). The remainder of his scientific contributions targeted other groups of organisms, but mainly beetles, with some taxa, but also lifestyles that clearly attracted his preferences. Specifically, Ignacio was well versed and genuinely interested in the study of endogean and subterranean diversity and adaptations, where different groups of Coleoptera offered him the opportunity to investigate them in some detail. In particular, 20% of his publications were on beetle groups that often had the added interest of their subterranean lifestyle, mainly Carabidae (47.7%) and Leiodidae (43.2%). Anecdotally (4.5% of publications), Ignacio would also lead or collaborate in studies with other families of beetles, which included the Chrysomelidae, Malachiidae, Ptiliidae, Scarabaeidae, Scirtidae, and others, as well as broader views on the Coleoptera, including his participation and expertise towards the most ambitious molecular phylogeny of beetles of its time (HUNT *et al*., 2007).

Ignacio‘s scientific trajectory is fascinating also in the way it developed and matured, incorporating gradually interests, ideas and tools defining a clear scientific path and specialization, not only because of the organisms he studied, but also according to the way he approached their study. An attempt at classification of Ignacio‘s scientific production (Appendix II; other classifications are possible, since the same article usually covers simultaneously different research topics or approaches), reveals how this specialization developed and how it consolidated in a way of creating and communicating research that contributed to Ignacio being well known and reputed in his area of expertise. In the late 80‘s and first half of the 90‘s, Ignacio‘s interests grew around faunistic and community studies, starting to explore functional aspects of shape and questioning himself about adaptations and ecological, and eventually evolutionary processes. From these early dates started to take shape some of what would become his most celebrated contributions integrating notions of habitat type, adaptations for dispersal and macroevolutionary consequences, for example in terms of diversification (RIBERA, 1993; RIBERA *et al*., 1997; RIBERA & VOGLER, 2000). In the late 90‘s, two important circumstances would definitely shape Ignacio‘s scientific profile, his trademark and definitely his particular preference, which we knew as his friends, but also easily recognizable from the volume of associated scientific production. Specifically, Ignacio‘s knowledge and confidence about the organisms he studied reached a point when it allowed him to start contributing to their taxonomy, and Taxonomy became one of his passions. The first taxonomic article authored by Ignacio appeared in 1996, describing a new Iberian Dytiscid (AGUILERA & RIBERA, 1996) and from that date on, taxonomic articles represented slightly over 22% of his scientific production. The second defining circumstance in Ignacio‘s career was incorporating the study of DNA sequences to infer phylogenies as his preferred tool to investigate the hierarchy of diversity in the beetle groups that attracted his attention and the evolution of traits and adaptations of his interest. Thus, Molecular Systematics became his new passion and, already in the new Century, the bulk of his scientific production, with nearly 30% of his published articles, would use molecular phylogenies to investigate the systematics, phylogeography, diversification and evolution of different groups of beetles.

The impact of Ignacio‘s contributions in the fields mentioned above, particularly the study of biodiversity assisted by the use of DNA sequence data, is outstanding. We thought that a fun way to pay him homage that he would have found amusing and also intellectually challenging would be showing precisely how his work, his collaborations, and the data that he helped producing actually represent a massive and objective progress in our understanding of the diversity and phylogeny of beetles. Thus, the aim of this work is compiling DNA sequence data generated by Ignacio‘s research and putting it in the broader context of homologous sequence data generated worldwide, as a way to demonstrate in a phylogenetic context which parts of the tree and to what extent Ignacio is responsible for our current knowledge of that significant branch of the Tree of Life. Specifically, among the sequences deposited in GenBank from all studies published by Ignacio, we will pay special attention to the most common phylogenetic maker, the mitochondrial cytochrome *c* oxidase subunit 1 (gene name *cox1* and product name COI). The study we present below shall illustrate how much of a setback for the development of the field will be Ignacio‘s loss, and also our humble way to show how much we admired and miss him.

MATERIAL AND METHODS

DNA sequence data acquisition

GenBank, the genetic sequence database of the NCBI (https://www.ncbi.nlm.nih.gov/genbank/) was searched on 17th October 2021 with the query “Ribera I[Author]” and matching entries were downloaded in GenBank format. Data were parsed using a custom Python v3.9.1 script built in Biopython v1.79 (Supplementary Data) that parses gene feature qualifiers from each entry matching gene names of the most common phylogenetic markers: *cox1*, and ribosomal RNAs 16S, 18S, and 28S. Parsing also included alternative gene names such as CO1, COI or *rrnL*. Besides, this script also removes sequences not including “Ribera, I.” among authors of first publication record [“references”] and thus verifying the initial search criterion.

GenBank was also searched for *cox1* gene sequences from beetles with the following terms: “Coleoptera[Organism] AND 300:1800[Sequence Length] AND (COI[Gene Name] OR cox1[Gene Name]) NOT (tRNA[-Feature key])”. *Cox1* entries in GenBank format were parsed using a custom Python script to export the sequences in FASTA format with headers including accession number, species name, full taxonomy, submission year, and warranting that “Ribera I.” was included among publication authors (Supplementary Data). The *cox1* dataset was subsequently cleaned to remove nucleotide sequences with more than five consecutive Ns, including stop codons or including insertions or deletions (e.g. causing reading frame shift and hence a very divergent protein).

Phylogenetic matrices

Cox1 sequences retrieved in the previous steps were aligned with *clustalo* in SeaView v4.6.4 (GOUY *et al*., 2010) using as reference the full *cox1* nucleotide sequence parsed from the complete mitochondrial genome of *Scarodytes savinensis* (voucher IBE-ESP-RA219, NC_057553; VILLASTRIGO *et al*., 2021). The 5’- and 3’-ends of the alignment were trimmed to match the length of the reference sequence. Few sequences were complete, e.g. only those from mitochondrial genomes, while most included either the 5'- or the 3'-ends of *cox1* gene sequence without overlap in the alignment. The 5'-end of the gene is generally PCR-amplified with the primer set forward-HCO2198 and reverse-LCO1490 (alias LCO-HCO; FOLMER *et al.*, 1994) or taxon-specific derivatives. On the other hand, the 3'-end is obtained with the primer pair forward-C1-J-2183 and reverse-TL2-N-3014 (alias JERRY-PAT; SIMON *et al.*, 1994), the latter designed on the *trnL2* gene that typically separates the *cox1* and *cox2* genes, hence also amplifying a fragment of this transfer RNA gene. Since the two *cox1* fragments do not overlap, this gene alignment was split into two sub-alignments, one including the region amplified with the LCO-HCO primer set (645 bp), and the other including the region amplified with JERRY-PAT pair (810 bp), both alignments excluding primer regions. On the latter, one sequence of *Ozaena lemoulti* (Carabidae, EF694932), one of *Neochlamisus* sp. (Chrysomelidae, AM283154), and two of *Neochlamisus bebbianae* (JN625283 and JN62528) included an extra triplet in positions 274-276 of the alignment.

Each sequence was identified with the taxonomy extracted from the FASTA header of *cox1* sequences using the grep (https://www.gnu.org/software/grep/manual/grep.html) command "grep -o -P ,(?<=STARTTAX).*(?=ENDTAX)'", taking advantage of the Python script parsing original GenBank entries that added those delimiters to FASTA headers to perform further analyses at different taxonomic ranks. Taxa retrieved from all FASTA sequences were also concatenated in a single list, and rank names counted with a Python 3 script using the library Counter from collections (https://docs.python.org/3/library/collections.html; Supplementary Data). The year of submission was also parsed using a similar Python script (Supplementary Data).

Both *cox1* datasets included a very large number of sequences, and they were simplified by clustering those sequences not belonging to Ignacio's studies using algorithms coded in VSEARCH v2.8.0 (ROGNES *et al.*, 2016). Sequences below a 3% divergence threshold were clustered and the longest one selected as representative (centroid) sequence of the group (vsearch "--cluster_fast --id 0.97"). Final *cox1* datasets included only the so-called centroids and all the sequences from Ignacio's studies. This approach helped speeding up phylogenetic tree searches under Maximum Likelihood (ML) and assisted tree visual navigation.

Phylogenetic analyses

Despite of the applied dataset reduction strategy, phylogenetic matrices were still huge and phylogenetic tree searches in IQTREE (NGUYEN *et al.*, 2015) and RAxML-NG (KOZLOV *et al.*, 2019) did not complete after several days. Therefore, an alternative tree building method was performed using the ML criterion in VeryFastTree v3.0.1 (PIÑEIRO *et al.*, 2020) under the Generalised Time Reversible substitution model (GTR; TAVARÉ *et al.*, 1986). Among-site rate variation (rate heterogeneity) was first modelled with 20 rate categories (CAT model; STAMATAKIS, 2006) but in a final step ML was optimized with Gamma parameter (GTR+G4). VeryFastTree commands were "-threads 12 -double-precision -nosupport -nt -gtr". We ran five independent analyses with different starting seeds to avoid local optima (Table I). Since VeryFastTree is less accurate than RaxML-NG and IQTREE, the output from this approach was subsequently optimised in IQTREE v1.6.8 ("-ninit 2 -ntop 1 -nbest 1 -n 10 -m GTR+G4") using the strict consensus tree from the previous five ML topologies ("sumtrees.py min-clade-freq=1"; SUKUMARAN & HOLDER, 2010) as topological constrain. Using this approach, tree rearrangements focus on incongruent nodes only (mostly basal nodes), thus speeding up the analyses. This hybrid approach represents a compromise between speed and accuracy to find the best ML tree for datasets with large number of sequences. Final trees were uploaded to iTOL v6 to make them available online. In the trees, taxonomic assignments were done automatically by iTOL taking advantage of tips labelled with taxon-ID numbers as parsed from Genbank entries (feature.qualifiers["db_xref"]). Branches were coloured according to taxonomy in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS AND DISCUSSION

Public sequence databases included 17,499 entries for nucleotide sequences deposited from Ignacio's articles. After parsing the taxonomy of these sequences, most of them (17,455) were from beetles, 24 sequences were from other taxonomic groups whereas 20 of the entries did not include Ignacio Ribera as author. The taxonomic exceptions were bacterial *Wolbachia* surface proteins sequences amplified by PCR from full DNA extractions from beetles (GARCÍA-VÁZQUEZ & RIBERA, 2016; LT602610-LT602633). Most entries included *cox1* as the gene feature qualifier (8,322) with lower numbers for the large ribosomal subunit of the mitochondrion (*rrnL* or 16S rRNA, 1,387), mitochondrial NADH dehydrogenase subunit 1 (*nad1*, 1,272), small nuclear ribosomal subunit (SSU or 18S rRNA, 1,069), mitochondrial cytochrome b (*cob*, 900), nuclear Histone 3 subunit a (H3a, 800), large nuclear ribosomal subunit (LSU or 28S rRNA, 650), and the nuclear gene Wingless (296). Gene qualifiers of the remaining entries included mitochondrial identifiers such as 12S rRNA (*rrnS*), NADH dehydrogenase subunit 5 (*nad5*), full mitochondrial genomes, but also partial nuclear protein coding genes such as Arginine Kinase (ARGK), Enolase (ENO), and Elongation Factor 1a (EEF1A).

The most common molecular marker in metazoans, and also in Ignacio's studies, is the *cox1* gene (PORTER & HAJIBABAEI, 2018; MACHIDA *et al.*, 2017) so all nucleotide sequences coding for COI from beetles were downloaded from GenBank with a total of 221,966 entries. Filtering sequences with the quality controls described above, a total of 215,962 sequences remained, with 7,725 belonging to articles published by Ignacio and his colleagues. Of the two independent *cox1* multiple sequence alignments, the alignment of the LCO-HCO region consisted of 163,142 sequences including 1,508 sequences from Ribera's studies while the alignment of JERRY-PAT fragment comprised 52,820 and 6,217 sequences, respectively. Both datasets included about one third of missing data due to shorter ends in some sequences (35.5% in HCO-LCO and 33.0% in JERRY-PAT). As expected, the beetle suborder most represented by Ignacio's data is Adephaga (JERRY-PAT 4,775, and LCO-HCO 1,057 sequences). Polyphaga data included 1,441 and 451 sequences, respectively. There was a single sequence from the suborder Myxophaga contributed by Ignacio's research (Hydroscaphidae sp. BMNH703455, EF517595). None of those datasets included *cox1* sequences obtained with the primer pair RON-TOM used by Ignacio in several studies on Trechini (e.g. FAILLE *et al.*, 2010). Those sequences overlap about 300 bp with HCO-LCO amplicon and by a similar length with JERRY-PAT fragment, so they are shorter than 300 bp after splitting them between datasets and hence removed from both alignments.

Most of Ignacio's sequences in the JERRY-PAT alignment were from water beetles of the Adephagan family Dytiscidae (4,521) with few representatives from the families Noteridae, Hygrobiidae, Haliplidae, and Aspidytidae. This scientific production included DNA sequence data for other Adephaga, represented by 228 sequences of ground beetles in the family Carabidae and a four of whirligig beetles, Gyrinidae (Appendix III). In Polyphaga (1,441), the most commonly represented infraorders were Staphyliniformia (1,161) and Elateriformia (173), with lower numbers in the Cucujiformia (39) and Bostrichiformia (12). Among Staphyliniformia, the superfamily Staphylinoidea was very well represented by Hydraenidae (635), Leiodidae (290) and Ptiliidae (16), and the superfamily Hydrophiloidea by a large number of Hydrophilidae (141). Across the lower taxon ranks studied by Ignacio, some of the most commonly represented subfamilies were Agabinae (2,061), Hydroporinae (1,835), Copelatinae (347), Cholevinae (288), Hydrophilinae (140), Colymbetinae (109), Dytiscinae (81), Laccophilinae (59), and Matinae (19) (Appendix III). In these studies, some genera were studied in depth, providing data from hundreds to thousands of individuals: *Agabus* (1,335), *Ilybius* (477), *Deronectes* (332), *Ochthebius* (327), *Nebrioporus* (258), *Hydraena* (246), *Sternopriscus* (173), *Platambus* (154), *Papuadytes* (147), *Exocelina* (145), *Microcharidius* (144), *Hydroporus* (139), *Enochrus* (138), *Copelatus* (99), and *Platynectes* (98).

The larger LCO-HCO dataset (163,142) included fewer representatives from Ribera's studies (1,508), with two thirds of them corresponding to different groups of water beetles (Appendix III). Most of Ignacio's

Table I. Maximum Likelihood (ML) log values for tree searches with *cox1* datasets.

Tabla I. Valores logarítmicos de Máxima Probabilidad de los árboles filogenéticos obtenidos con los dos fragmentos de las secuencias de *cox1*.

ML program	LCO-HCO	JERRY-PAT
VeryFastTree		
Run 1	-3202857.510	-4402954.905
Run 2	-3202230.441	-4403074.405
Run 3	-3202380.613	-4403008.207
Run 4	-3202445.518	-4402856.409
Run 5	-3202209.481	-4403311.659
IQTREE	-3185363.774	-4389690.674

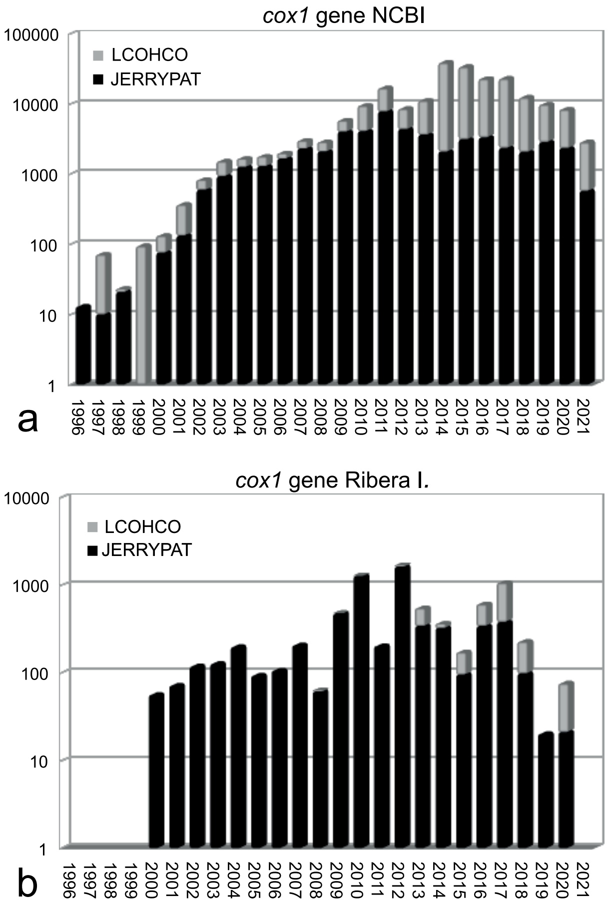

Fig. 1. Number of sequences coding for mitochondrial *cox1* gene deposited at GenBank per year split by 3'- (JERRY-PAT) and 5'-ends (LCO-HCO) in log scale: **a)** total entries in NCBI (GenBank excluding Ignacio's works), and **b)** those submitted by studies published by Ignacio Ribera and colleagues.

Fig. 1. Número de secuencias en escala logarítmica que codifican para el gen mitocondrial *cox1* depositadas por año en GenBank separadas en dos regiones, extremos 3' (JERRY-PAT) y 5' (LCO-HCO): **a)** número total de entradas en NCBI (GenBank excluyendo las secuencias de los trabajos realizados por Ignacio), y **b)** número de aquellas depositadas por estudios de Ignacio Ribera y colaboradores).

LCO-HCO sequences belong to Adephaga (1,057), particularly to the water beetle families Dytiscidae (588), and the subfamilies Hydroporinae (523), Agabinae (48), and Colymbetinae (16) (Appendix III). Among the lower ranks several genera of Dytiscidae were more densely studied, including *Deronectes* (290), *Hygrotus* (49), *Platambus* (45), *Nebrioporus* (37), *Oreodytes* (33), *Scarodytes* (22), and *Graptodytes* (19). Ground beetles are well represented in Ignacio's LCO-HCO sequence data, all of them belonging to the subfamily Trechinae (469), with most sequences obtained from three genera: *Microcharidius* (434), *Typhlocharis* (66) and *Trechus* (26). Polyphaga had fewer sequences (451) mostly from the subfamily Cholevinae (232) and the water beetles Hydraenidae (177) and Hydrophilidae (28), with a particular emphasis on the genus *Hydraena* (Hydraenidae, 119).

We also parsed the date of submission of the 215,962 *cox1* GenBank entries to shed light on the sequencing rate of *cox1* gene for the last decades in Coleoptera (Fig. 1). The *cox1* sequences from the first molecular analyses in beetles were submitted to public nucleotide sequence databases in the mid 90's. In the early years of the first decade of the 21st century, the rate of submission was in the order of several hundreds, reaching several thousands in the second half of the first decade, and exploding in the period 2014-2017, when tens of thousands of new sequences were added to the database, to decline afterwards to few thousands submissions more recently. Since 2012 the rate of submissions of JERRY-PAT and LCO-HCO fragments were very similar, although the latter were particularly higher between 2015 and 2017 (Fig. 1). A closer look to the sequences from Ignacio's publications shows a different trend, where most sequences were amplified using the JERRY-PAT primer combination until 2013, when LCO-HCO fragment took the leading position, but only by a slight margin (Fig. 1). The first DNA-based phylogenetic studies of beetles used the JERRY-PAT primer set to amplify the 3'-end of the mitochondrial *cox1* gene, but after the adoption of the 5'-end of this gene as the standard DNA barcode region for animals (HEBERT, 2003), most studies switched to this marker instead (PORTER & HAJIBABAEI, 2018). Ignacio, as many people working with beetles since the late 90's, still relied on the use of the 3'-end of the *cox1* gene, because they already built a large reference dataset for their studies and because of its higher phylogenetic information content compared to the standard DNA barcode, recalling the legacy problem in computing sciences and how software upgrades have to be compatible with old data formats.

Our results illustrate how the research done by Ignacio Ribera and his co-workers had a major impact in the study of the genetic and phylogenetic diversity of *cox1* in Adephaga, since about a quarter of the sequences of JERRY-PAT fragment for taxa in this group deposited in GenBank were obtained in studies promoted by Ignacio and his colleagues. This contribution was particularly relevant in the case of water beetle groups in this suborder, since most of Ignacio's sequences of Adephaga (95%) belong to these families. On the other hand, the production of LCO-HCO sequences in the suborder Adephaga was much lower relative to those sequenced and archived by other scientists, ~ 5% for whole Adephaga, ~ 10% for Adephagan water beetles, and ~ 3% for ground beetles. In summary, a phylogenetic *cox1* tree of Coleoptera without the contributions of Ignacio would be significantly less informative than what we know today. The ML trees from both *cox1* datasets, JERRY-PAT and LCO-HCO, are available online at https://itol.embl.de/tree/18826219154412191639522288, and https://itol.embl.de/tree/18826219154399771639573888, respectively. Both trees are also supplied in the Supplementary Data in NEXUS and PHYLIP formats to be visualized offline. The samples coming from Ignacio's studies can be highlighted in trees by uploading in iTOL webpage the annotation

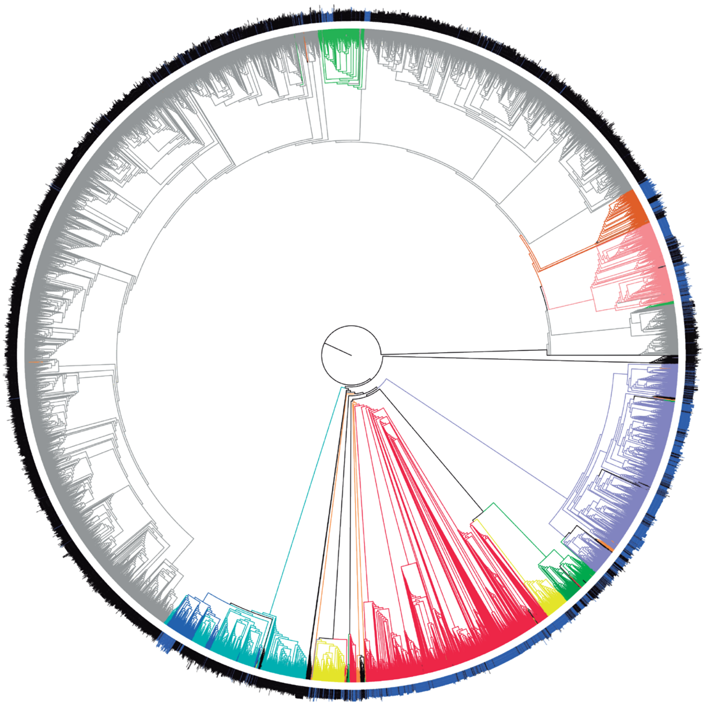

Fig. 2. Circular cladogram based on Maximum Likelihood criterion using sequences of the 3'-end fragment of the mitochondrial *cox1* gene with 6,217 downloaded from GenBank and deposited by Ignacio's studies (names in blue), and 16,167 entries from other studies but using centroid sequences (see Material and Methods). Clades with taxonomic ranks with many sequences from Ignacio's work are highlighted in different colours.

Fig. 2. Cladograma circular obtenido mediante el criterio de máxima probabilidad utilizando 6217 secuencias del fragmento del extremo 3' del gen mitocondrial *cox1* descargadas de GenBank y depositadas por los estudios de Ignacio (nombres en azul), y otras 16167 secuencias representativas de otros estudios (secuencias centroides ver Material y Métodos). Los clados (rangos taxonómicos) con muchas secuencias obtenidas en estudios realizados por Ignacio y colegas están resaltados en diferentes colores.

files provided as Supplementary Data. In these trees, the sequences not generated by Ignacio are underrepresented for analytical efficiency and ease of visualisation, with a reduction from 161,634 to 39,169 sequences in the case of the LCO-HCO dataset, and from 46,603 to 16,167 in the JERRY-PAT dataset. However, it is expected that, given the divergence threshold used for clustering (above 97% sequence similarity), sequence clustering should mostly occur at the intraspecific level, without loss of species diversity. The tree based on JERRY-PAT sequences (the only one showing the reciprocal monophyly of Adephaga and Polyphaga) was used to represent the gene tree phylogeny, with some families of interest highlighted in different colours (Fig. 2). The trees summarize the same pattern described above in a visual manner, emphasizing the areas of the topology where Ignacio's contributions were more relevant, particularly towards the knowledge of the families Dytiscidae, Hydraenidae, and Limnichidae, the branches that flourished in Ignacio's Tree of Life.

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Despite we do not recall to talk about music with Ignacio ever, the hit song from the 80's "Groenlandia" from Zombies (Bernardo Bonezzi, 1980, https://www.youtube.com/watch?v=He8lS9Vf63Q) is a great epitome of his life. Going around the world collecting beetles from different habitats and ecosystems and then unravelling their natural and evolutionary histories through their sequences. He is now way beyond as lyrics of "Groenlandia" acknowledge.

Supplementary Data is available in Zenodo under de DOI https://doi.org/10.5281/zenodo.6299643

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Appendix I. Taxonomic scope of the publications authored by Ignacio Ribera in chronological order (source: https://www.um.es/ecoaqua/index.php/component/sppagebuilder/?view=page&id=251).

Apéndice I. Enfoque taxonómico de las publicaciones de Ignacio Ribera en orden cronológico (fuente: https://www.um.es/ecoaqua/index.php/component/sppagebuilder/?view=page&id=251).

Year	Hydradephaga	Dytiscidae	Hydrophilidae	water beetles	Carabidae	Coleoptera	Hydraenidae	Chrysomelidae	insects	Leiodidae	Heteroceridae	Elmidae	spiders	Gordiidae	Limnichidae	Noteridae	Aspidytidae	plants	Scirtidae	Staphyliniformia	Adephaga	aquatic organisms	Meruidae	Hydrophiloidea	Scarabaeidae	Hydrochidae	cave beetles	Ptiliidae	Malachiidae	
1988	1																													1
1989	1																													1
1990	1	1																												2
1991		2																												2
1992		1																												1
1993	3		1																											4
1994	2		1																											3
1995	4			3																										7
1996		1	1	4	1																									7
1997	1	1	2	1	1	1	1																							8
1998			3	1			5	1	1	1	1	1	1																	15
1999		1	2	1	3	1	2						1	1	3															15
2000			1	2			1						1		3															8
2001		2	1	1	1		1					1		1	2	1														11
2002	1			1													1													3
2003		3		2											2		1	1	1											10
2004		4													4				1	1										10
2005				1	1		2								2		1				1									8
2006	1				1		1					1			2															6
2007		6				1	1			1												1								10
2008		2		2			1														1	1	1							8
2009		1		2													1							1	1					6
2010		3			2	1	1			1					1		1				1			1						12
2011				2	2		3			1																1				9
2012		3	1	1		1			1	1												1								9
2013				3	1		1			1												1								7
2014		2	1	2	1	1			1	1					2												1			12
2015				1	1					1																				3
2016		3			1		2			2					2		2				1	1								14
2017		4	1	2	1		3			2					1							1					1			16
2018		3			3		2								1												2			11
2019		2		2			3			4																		1	1	13
2020		1					3								1						1									6
2021		2			1					3																				6
	15	**48**	**15**	**34**	**21**	6	**33**	1	3	**19**	1	3	3	2	**26**	1	7	1	2	1	5	6	1	2	1	1	4	1	1	264

Appendix II. One possible ranking for the thematic scope of the publications authored by Ignacio Ribera in chronological order (source: https://www.um.es/ecoaqua/index.php/component/sppagebuilder/?view=page&id=251).

Apéndice II. Clasificación temática de las publicaciones de Ignacio Ribera por orden cronológico (fuente: https://www.um.es/ecoaqua/index.php/component/sppagebuilder/?view=page&id=251).

Year	Faunistics, catalogs and communities	Indicators	Morphology and morphometrics	Ecological strategies	Observations	Biogeography	Opinions	Conservation	Taxonomy	Phylogeny and systematics	Species richness	Phylogeography	Speciation	Molecular evolution	Proteomics and transcriptomics	Development	
1988	1																1
1989	1																1
1990	1	1															2
1991	1		1														2
1992			1														1
1993	2	1		1													4
1994	3																3
1995	2		1	3	1												7
1996	6				1	1	2		1								11
1997	4			2				2									8
1998	8					1			6								15
1999	3		1	1		1		1	7	1							15
2000	2	1		1			1	1	3								9
2001	1			1				1	7	1							11
2002	1						1			2							4
2003						1		1	3	2	2	1					10
2004									5	4		1					10
2005	1							2	1	4							8
2006	2								1	3							6
2007								1	3	5		1					10
2008								2	1	4	1						8
2009							1	1		3		1	1				7
2010						1			3	6	1			1			12
2011						2			2	4		1					9
2012	1			2				1	1	3	1						9
2013	1							3		3							7
2014	3			3					1	3					1	1	12
2015	2			1													3
2016	1		1	2		1			1	7					1		14
2017	1		2	1		1			5	2		2	1				15
2018	1		2	1		1	2		3	1	1						12
2019	3		3	1			1		2	4							14
2020				1					4	1							6
2021				3					1	1					1		6
	52	3	12	24	2	10	8	16	**61**	**64**	6	7	2	1	3	1	272

Appendix III. List of nucleotide entries retrieved from NCBI for mitochondrial *cox1* gene from beetles by taxonomic rank split by 3'- (JERRY-PAT) and 5'-ends (LCO-HCO): total entries in GenBank (excluding Ignacio's works) and those submitted by studies published by Ignacio Ribera and colleagues as well as its percentage. Within ranks sequences were sorted by higher number of sequence submitted by Ignacio in JERRY-PAT fragment. This is a summary of the ranks with more entries in Ignacio's publications.

Apéndice III. Lista de las entradas de secuencias nucleotídicas recuperadas del NCBI/GenBank para el gen mitocondrial *cox1* de coleópteros desglosadas por grupo taxonómico y por fragmentos 3' (JERRY-PAT) y 5' (LCO-HCO). Además las entradas se desglosan en totales de GenBank (excluyendo los trabajos de Ignacio) y los presentados por los estudios publicados por Ignacio Ribera y colaboradores así como su porcentaje. Dentro de los grupos taxonómicos, las secuencias se ordenan según el mayor número de secuencias enviadas por Ignacio para el fragmento JERRY-PAT. Este resumen incluye los taxa más frecuentes en las publicaciones de Ignacio Ribera.

		JERRY-PAT			**LCO-HCO**		
rank name	**rank**	**GenBank**	**Ribera I**	**% Ribera I**	**GenBank**	**Ribera I**	**% Ribera I**
Adephaga	suborder	17,142	4,775	27.856	19,154	1,057	5.518
Dytiscoidea	superfamily	11,177	4,539	40.610	3,986	588	14.752
Dytiscidae	family	11,129	4,521	40.624	4,003	588	14.689
Hygrobiidae	family	19	5	26.316	2	0	0.000
Agabinae	subfamily	3,341	2,061	61.688	1,001	48	4.795
Hydroporinae	subfamily	4,846	1,835	37.866	1,750	523	29.886
Copelatinae	subfamily	888	347	39.077	134	1	0.746
Colymbetinae	subfamily	550	109	19.818	436	16	3.670
Dytiscinae	subfamily	742	81	10.916	420	0	0.000
Laccophilinae	subfamily	408	59	14.461	134	0	0.000
Matinae	subfamily	24	19	79.167	3	0	0.000
Agabus	genus	2,283	1,335	58.476	614	1	0.163
Ilybius	genus	1,183	477	40.321	330	2	0.606
Deronectes	genus	507	332	65.483	293	290	98.976
Ochthebius	genus	822	327	39.781	330	22	6.667
Nebrioporus	genus	434	258	59.447	76	37	48.684
Hydraena	genus	451	246	54.545	276	119	43.116
Sternopriscus	genus	173	173	100.000	0	0	0.000
Platambus	genus	154	154	100.000	84	45	53.571
Papuadytes	genus	148	147	99.324	0	0	0.000
Exocelina	genus	335	145	43.284	2	2	100.000
Hydroporus	genus	2,956	139	4.702	550	21	3.818
Enochrus	genus	229	138	60.262	310	26	8.387
Copelatus	genus	462	99	21.429	69	0	0.000
Platynectes	genus	194	98	50.515	14	0	0.000
Oreodytes	genus	121	81	66.942	78	33	42.308
Hygrotus	genus	423	72	17.021	342	49	14.327
Graptodytes	genus	448	49	10.938	32	19	59.375
Leptolambus	genus	49	42	85.714	195	30	15.385
Scarodytes	genus	65	31	47.692	27	22	81.481
Caraboidea	superfamily	5,335	228	4.274	14,618	469	3.208
Carabidae	family	5,326	228	4.281	14,660	469	3.199
Trechinae	subfamily	897	198	22.074	3,251	469	14.426
Microcharidius	genus	149	144	96.644	434	434	100.000
Typhlocharis	genus	29	29	100.000	66	66	100.000
Trechus	family	315	28	8.889	444	26	5.856
Polyphaga	suborder	35,670	1,441	4.040	142,477	451	0.317
Staphyliniformia	infraorder	5,540	1,161	20.957	23,070	449	1.946
Elateriformia	infraorder	3,079	173	5.619	24,691	2	0.008
Hydraenidae	family	1,339	635	47.423	718	177	24.652
Hydrophilidae	family	894	141	15.772	1,679	28	1.668
Leiodidae	family	741	290	39.136	1,636	232	14.181
Hydrophilinae	subfamily	606	140	23.102	985	27	2.741
Cholevinae	subfamily	539	288	53.432	949	232	24.447
Limnebius	genus	89	74	83.146	86	51	59.302
Troglocharinus	genus	64	64	100.000	155	155	100.000
Hydrochus	genus	63	62	98.413	48	7	14.583
Speonomus	genus	82	29	35.366	11	11	100.000
Bathysciola	genus	40	19	47.500	27	6	22.222

Life cycle differences between two coexisting species of supratidal rockpools: *Ochthebius quadricollis* Mulsant, 1844 and *Ochthebius lejolisii* Mulsant & Rey, 1861 (Coleoptera, Hydraenidae)

Diferencias en el ciclo de vida de dos especies que coexisten en pozas rocosas supralitorales: *Ochthebius quadricollis* Mulsant, 1844 y *Ochthebius lejolisii* Mulsant & Rey, 1861 (Coleoptera, Hydraenidae)

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ABSTRACT

The knowledge of the life cycle of species occurring in extreme environments such as supratidal rockpools is crucial to better understand mechanisms enabling their coexistence and to predict the species responses to climate change. Here, we compared the life cycle of two *Ochthebius* species (*O. lejolisii* and *O. quadricollis*) living on the Iberian Mediterranean coast and highlighted their main differences referred to breeding period, oviposition rate, egg hatching success, duration of life cycle stages through a combination of field and laboratory approaches. The results showed that the breeding period of both species expanded most of the year, showing overlap in spring and autumn. While oviposition and larval development in *O. quadricollis* was limited in winter, it seems not to be limited by low temperatures in *O. lejolisii*. In the laboratory, at 20ºC, no differences between species were found in oviposition rate and larval development time. However, egg development time was shorter in *O. lejolisii* than in *O. quadricollis*. Moreover, the latter had more successful egg hatching, which along with a shorter overall life cycle time gives it higher demographic success and advantage to face climate change.

Key words: breeding period, oviposition rate, egg hatching success, life cycle duration, multivoltine cycle, coexistence.

RESUMEN

Conocer el ciclo de vida de las especies que coexisten en ambientes extremos como las pozas rocosas supralitorales es crucial para comprender los mecanismos que posibilitan la coexistencia y para predecir las respuestas de las especies al cambio climático. Aquí, comparamos el ciclo de vida de dos especies de *Ochthebius* (*O. lejolisii y O. quadricollis*) que viven en la costa mediterránea ibérica y destacamos sus principales diferencias referidas al período de reproducción, tasa de ovoposición, éxito de eclosión de los huevos y duración total y de cada una de las etapas del ciclo de vida a través de una combinación de aproximaciones de campo y laboratorio. Ambas especies extienden el período de reproducción la mayor parte del año, coincidiendo en primavera y otoño. Mientras que la ovoposición y el desarrollo larvario en *O. quadricollis* estuvieron limitados en invierno, en *O. lejolisii* no parecen estar limitados por las bajas temperaturas. En el laboratorio, a 20ºC, no se encontraron diferencias entre especies en la tasa de ovoposición y el tiempo de desarrollo larvario. Sin embargo, el tiempo de desarrollo de los huevos fue más corto en *O. lejolisii* que en *O. quadricollis.* Además, esta última tuvo una eclosión de huevos más exitosa, lo que junto con una duración del ciclo de vida más corta le confieren mayor éxito demográfico y ventaja para hacer frente al cambio climático.

Palabras clave: periodo reproductivo, tasa de ovoposición, éxito de eclosión, duración del ciclo de vida, ciclo multivoltino, coexistencia.

INTRODUCTION

The genus *Ochthebius* Leach, 1815 (Hydraenidae) is one of the most specious water beetles' genera, with more than 500 species. Although they mostly inhabit freshwaters, some have colonized saline or brackish waters (VILLASTRIGO *et al.*, 2019). Among the halophile's species, just a few are known to spend their entire life cycle in hypersaline ephemeral marine rockpool environments. These are found on rocky marine coastal shores worldwide (SABATELLI *et al.*, 2016), particularly in shallow depressions above the high tide line but within the splash zone. Supratidal rockpools are very dynamic environments holding multiple stress levels (VECCHIONI *et al.*, 2021), suffering drastic modifications in their water levels due to flickering cycles of rain and desiccation, and they are also exposed to some extent to the sea level and tides (POWLIK, 1999). Thus, species inhabiting rockpools need to deal with contrasting degrees of desiccation and salinity levels (GAINES & DENNY, 2007; POWLIK & LEWIS, 1996).

Coastal rockpools have been considered good model systems for investigating the climate change impacts and their inhabitants as sentinels of global warming (HELMUTH *et al*, 2006, KAPLANIS *et al.*, 2020). These habitats and their inhabitant species have drawn the attention of different naturalists and biologists since the beginning of the last century. First studies, especially on the Mediterranean species *Ochthebius quadricollis* Mulsant, 1844, were focused on some biological and physiological aspects providing descriptive and fragmentary information about its life cycle at different localities from the Mediterranean Sea (ISELL, 1913; HASE, 1926; D'ORCHYMONT 1913, 1932; JACQUIN, 1956). *Ochthebius* rockpools species are well known in their adult stage, although cryptic species and lineages in this habitat are commonly found (VILLASTRIGO *et al.*, 2020; SABATELLI *et al.*, 2021b), whereas immature phases are considerably less studied. Pupae are poorly known and larvae are described for a very few species: *Ochthebius* (*Cobalius*) *lejolisii* Mulsant & Rey, 1861 (D'ORCHYMONT 1913) from Atlantic coasts of Europe; *Ochthebius* (*Cobalius*) *subinteger* Mulsant and Rey, 1861 (DELGADO & SOLER, 1996) from North Mediterranean coasts; *O. quadricollis* (DELGADO & SOLER, 1997) from South-western Europe, and *Ochthebius balfourbrownei* Jäch 1989 from Cape Verde Islands (SABATELLI *et al.*, 2021a). In the 21st century, these species have received fair attention, including research on their taxonomy, faunistic, systematics, phylogeny, and phylogeography (e.g., ANTONINI *et al.*, 2010; AUDISIO *et al.*, 2010; BILTON, 2021; GARRIDO & VALLADARES, 2001; JÄCH & DELGADO, 2017; PORRETTA & URBANELLI, 2012; RIBERA & FOSTER, 2018; RIBERA & HERNANDO, 2019; SABATELLI *et al.*, 2016; 2021a,b; URBANELLI, 2002; URBANELLI & PORRETTA, 2008; VILLASTRIGO *et al.*, 2019, 2020). However, no study has been made to compare life cycles of congeneric and coexisting species. Although species living in this fluctuating and stressful environment are expected to have similar adaptations and life cycle strategies, recent thermal and salinity tolerance experiments performed by the authors have showed that physiological and behavioral responses differ between *O. quadricollis* and *O. lejolisii* (MIRÓN-GATÓN *et al.*, 2022). These and other potential differences on biological traits (e.g. desiccation resistance, fly capacity, life cycle) could help to understand their spatial and temporal coexistence patterns and to predict their specific responses to climate change.

In the rockpools of the southeastern Iberian Mediterranean coast inhabit four species of *Ochthebius: O. quadricollis,* two cryptic species of the subgenus *Cobalius*: *O. subinteger* and *O. lejolisii,* and the recently described *O. evae* (VILLASTRIGO *et al.,* 2020). Our study focused in the study of the life cycle of *O. quadricollis* and *O. lejolisii,* the most abundant and frequently co-occurring species in the southeastern Iberian Mediterranean coast and highlight their main differences referred to breeding period, oviposition rate, egg hatching success, and duration of life cycle stages through a combination of field and experimental approaches.

MATERIAL AND METHODS

Field sampling

To study life cycle, two near sampling localities, where each species was dominant, were selected: Cala Reona (30S 701818 4165830) for *O. quadricollis* and Cala de las Pulgas (30S 634182 4144025) for *O. lejolisii*. From each locality, ten pools, chosen randomly along a gradient of distance to sea level, were monthly sampled from October 2018 to September 2019. Specimens were collected from each pool during a five-minute period with soft entomological forceps and brushes, and fixed in ethanol 95%. In the laboratory, adults and larval instars of each species were morphologically identified under a binocular microscope (Leica M165C with a Leica MEB10 fibre optic illuminator) and *O. quadricollis* differentiated from the subgenus *Cobalius* species. We confirmed the identity of *O. lejolisii,* morphologically indistinguishable of *O. subinteger,* by genetic analysis of COI comparing five individuals of the study population with individuals from other localities where exclusively inhabit each species (author's unpublished data).

Additionally, we measured water temperature in one pool of each locality with a data logger (HOBO Pendant® MX Water Temperature Data Logger - MX2201) every five minutes for 24 hours to determine the annual temperature variation and its possible influence in the species' life cycle.

Laboratory experiments

In late winter and spring 2020, we collected twenty reproductive copulating pairs of each species: *O. quadricollis* from Cala Reona and *O. lejolisii* from Cala de las Pulgas. Each pair was introduced in an aerated plastic jar with 40 ml of seawater and filter paper as a substrate, and then transported to the laboratory. All pairs of each species were reared at 20 °C and marine water (35 g L^{-1} of salinity), with a 12:12 h light:dark cycle in a climate chamber (Sanyo MLR-351). Individuals were daily checked and fed *ad libitum* with green microalgae *Tetraselmis chuii* Butcher 1959. Jars were refilled with seawater when necessary to keep the level constant. Finally, we determined the total number of eggs laid, egg development time, and hatching success (number of larval instar I/ number of eggs) during a 28-day period.

After egg hatching, larval instar I were put in independent jars with the same reared conditions as adults. Days elapsed until moulting to instar II and III were recorded from the observation of exuvial records. Full-grown larvae (instar III highly pigmented and shortened) were extracted from the water and placed individually in glass test tubes with a strip of filter paper moistened inside to pupation. A tube rack was placed inside an aquarium with water in the background and externally lined with aluminium foil to maintain humidity and darkness conditions, and maintained at 20ºC in the climatic chamber. Last, we registered time elapsed until pupa formation and adult emergence.

Data analysis

One-way analysis of variance (ANOVA) was applied to analyse the interspecific (species as a fixed factor) variation on oviposition rate, egg development time and hatching success. However, due to the non-normality of the data, a non-parametric test (Kruskal-Wallis test) was also chosen to check the interspecific differences on duration of larval instars I, II and III, pupa stage and overall life cycle duration. Normality and homoscedasticity assumptions were validated on model residuals by graphical inspection. All statistical analyses were performed in R studio version 3.3.1 (2019-07-05, Development Core Team, 2019).

RESULTS

Breeding period

Field data showed that both species were present, as adult and larval stages, in the studied localities almost all the year round (Fig. 1). We also observed copulations of both species throughout the year, it being not rare to see a female with two males. In *O. quadricollis*, breeding seasons were principally in spring and summer, while in *O. lejolisii* were mainly in autumn, winter and spring. Consequently, a great number of larvae, mainly belonging to the instars II and III of *O. quadricollis* were found from March to October 2019, while in *O. lejolisii*, these instars were found from October 2018 to April 2019. Both species presented multivoltine cycles with overlapped cohorts, completing, at least, three (*O. lejolisii*) or four generations per year (*O. quadricollis*) (Fig. 1).

Water temperatures of pools from both localities ranged along the studied annual cycle between the absolute minimum values of 8.49 ºC (Cala Reona) and 7.25 ºC (Cala de las Pulgas) in January 2019, and the maximum values of 36.59 ºC (Cala Reona) and 35.82 ºC (Cala de las Pulgas) in July 2019, being annual mean water temperature 24.8 ºC and 22.1ºC in Cala Reona and Cala de las Pulgas, respectively. Low temperatures were not a constraint for reproduction and development of *O. lejolisii*, although they do seem to be so for *O. quadricollis*. However, we found that summer desiccation of the most distant shallow pools from the coastline considerably diminished the abundance of both larvae and adults of *O. lejolisii*.

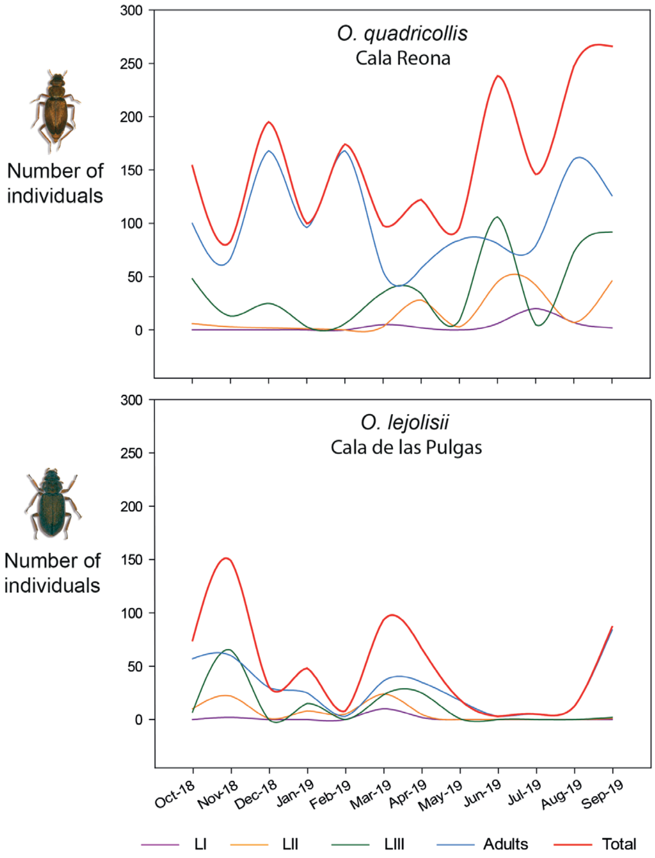

Fig. 1. Monthly variation of the abundance of the different larval instars (LI, LII, LIII) and adults of *O. quadricollis* and *O. lejolisii* in the study localities

Fig. 1. Variación mensual de la abundancia de los diferentes estadios larvarios (LI, LII, LIII) y adultos de *O. quadricollis* y *O. lejolisii* en las localidades de estudio.

Life cycle in the laboratory

Eggs

In the lab, the studied species laid the eggs singly or as a small group (3-5 eggs) attached to filter paper under water. Females of *O. lejolisii* can cover the eggs with a thick adhesive coating of mucilage (Fig. 2a), while *O. quadricollis´s* eggs presented a ring mounting structure (Fig. 2b) to better attach to the substrate.

At 20ºC, the number of eggs produced by a single female in a 28-day period was between 5 and 33 in *O. quadricollis,* and between 7 and 28 in *O. lejolisii*, but the difference in the mean number of eggs was not significant (Table I, Fig. 3). However, we found significant differences between species in egg development time and hatching success (Table I, Fig. 3). Mean egg development time was shorter in *O. lejolisii* (19.4 ± 1 days) than in *O. quadricollis* (23.5 ± 0.75 days), while *O. quadricollis* showed higher hatching success (47.3 ± 7.7 %) than *O. lejolisii* (19.1 ± 3.4 %).

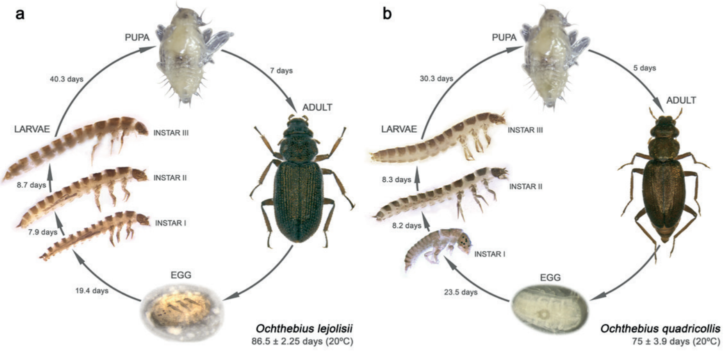

Fig. 2. Mean overall life cycle duration of *O. lejolisii* (a) and *O. quadricollis* (b) and of the different life stages at 20ºC.

Fig. 2. Duración del ciclo de vida de *O. lejolisii* (a) y *O. quadricollis* (b) y de los diferentes estados a 20ºC.

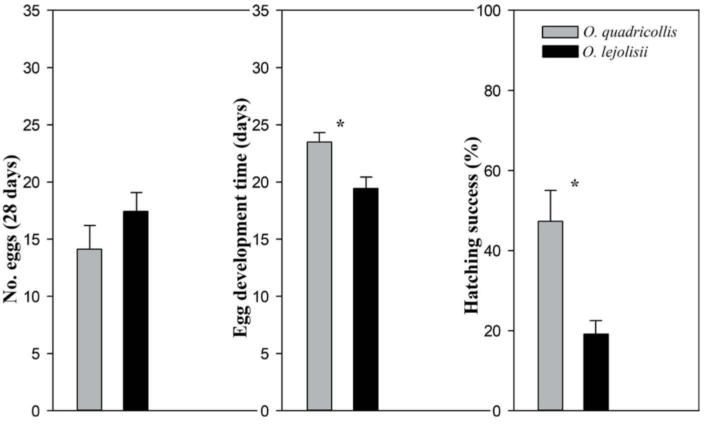

Fig. 3. Mean ± SE of oviposition rate, egg development time and hatching success in the study species. * Significant interspecific differences (P < 0.005).

Fig. 3. Valores medios ± ES de la tasa de ovoposición, duración del desarrollo del huevo y éxito de eclosión de las especies de estudio. *Diferencias significativas entre especies (P < 0.005).

ORIGIN	D.F.	SUM SQ	MEAN SQ	F-VALUE	P-VALUE
NO. EGGS AT 28 DAYS	1	81.5	81.5	1.5	0.232
RESIDUALS	28	1527.2	54.5		
EGG DEVELOPMENT TIME	1	123.8	123.8	10.2	**< 0.005**
RESIDUALS	28	341.8	12.2		
HATCHING SUCCESS	1	5918	5918	10.1	**< 0.005**
RESIDUALS	28	16417	586		

Table I. The ANOVA results of the interspecific differences in oviposition rate, egg development time and hatching success. In bold, significant differences (P < 0.05)

Tabla I: Resultados del ANOVA de las diferencias interespecíficas en la tasa de ovoposición, duración del desarrollo del huevo y éxito de eclosión. En negrita, diferencias significativas (P < 0.05)

ORIGIN	χ^2	D.F.	P-VALUE
INSTAR I	0.001	1	0.972
INSTAR II	0.483	1	0.487
INSTAR III	2.036	1	0.154
PUPA	2.625	1	0.105
TOTAL	4.5	1	< **0.05**

Table II. The Kruskal-Wallis results of the interspecific differences in duration of larval instars I, II and III, pupa stage and total life cycle duration. In bold, significant differences (P < 0.05)

Tabla II. Resultados del análisis Kruskal-Wallis de las diferencias interespecíficas en la duración de los estadios larvarios I, II y III, el estado pupa y el ciclo de vida completo. En negrita, diferencias significativas (P < 0.05)

Larva and pupa duration

The Kruskal-Wallis test showed non-significant interspecific differences in the duration of the different larval instars (Table II, Fig. 4). In *O. quadricollis* instar I spent a mean of 8.2 days, instar II 8.3 days, and instar III 30.3 days, whereas *O. lejolisii* completed each larval phase in 7.9, 8.7, and 40.3 days, respectively (Fig. 4). The pupal stage had a mean duration of 5 days in *O. quadricollis* and 7 days in *O. lejolisii*. Overall life cycle of *O. quadricollis* (75 ± 3.9 days) was significantly shorter than that of *O. lejolisii* (86.5 ± 2.25 days) (Fig.2).

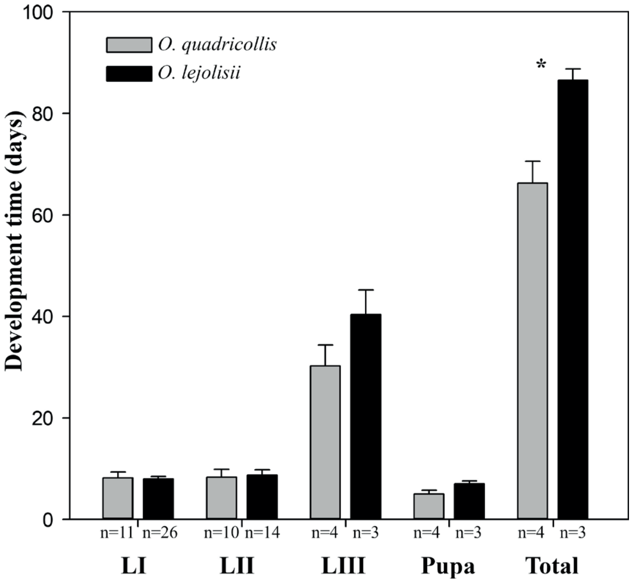

Fig. 4. Mean ± SE of larval instars development time and total life cycle duration of the study species. * Significant interspecific differences (P < 0.05)

Fig. 4. Valores medios ± ES de la duración de los diferentes estadios larvarios y el ciclo de vida completo de las especies de estudio. *Diferencias significativas entre especies (P < 0.05).

DISCUSSION

In general, species of the family Hydraenidae mainly breed in spring and summer, although some species are capable of reproducing in autumn and winter (VALLADARES *et al*., 2018). Reproduction of *O. quadricollis* have been commonly observed in summer in different Mediterranean localities (HASE, 1926; MARGALEF, 1949; JACQUIN, 1956), while ORCHYMONT (1913) found *O. lejolisii* mating earlier at the end of June. Our field results, tracking an annual cycle, shown both study species (*O. quadricollis* and *O. lejolisii*) extend the breeding period most part of the year, showing overlap in spring and autumn. However, *O. lejolisii* was capable of breeding in winter and *O. quadricollis* in summer. While JACQUIN (1956) pointed to an annual generation in *O. quadricollis*, we have found in both species multivoltine cycles with overlapping cohorts, completing, at least, three (*O. lejolisii*) or four (*O. quadricollis*) generations per year.

Temperature appears to be a selective pressure, conferring longer life cycles (ANGILLETTA, 2009) and egg development period (WARM, 1992) in colder environments. At 20ºC we found a mean egg development time of 19.5 and 23.5 days in *O. lejolisii* and *O. quadricollis*, respectively, while JACQUIN (1956) found shorter times in *O. quadricollis* (12-13 days). These differences probably were due to that reproductive specimens used in the laboratory came from different populations and seasons (spring in our study and summer in Jacquin´s study). Although in both studies, specimens were reared in the laboratory to the same temperature, in the field they experienced different acclimatization temperatures. Also, JACQUIN (1956) found that lower temperatures increased the egg development time in *O. quadricollis*: 24-25 days at 15-16ºC, and at temperatures below 15ºC it could take up to two months. Thus, minimum temperatures could explain the absence of larvae of *O. quadricollis* in winter because eggs laid in December may hatch towards the end of February. However, we found oviposition and larval development of *O. lejolisii* to be not limited by the low temperatures experienced during winter in the studied rockpools.

Interspecific differences in reproductive period and number of generations found in our study are congruent with other physiological and behavioural differences (MIRÓN-GATÓN *et al*. 2022) and point to different life history strategies for maintaining populations in these variable extreme habitats that enable the coexistence of species. The interspecific differences observed in reproduction periods could be related with their thermal tolerance. Both study species have a wide thermal tolerance, as expected by the extreme thermal conditions of their habitats, but previous studies showed that the tolerance of adult stage to cold temperature was higher in *O. lejolisii* (supercooling point: -15.45 ± 0.37 °C) than *O. quadricollis* (-12.82 ± 0.31°C), while tolerance to warm temperature was higher in *O. quadricollis* (heat coma: 48.04 ± 0.12 °C) than in *O. lejolisii* (47.36 ± 0.12 °C) (MIRÓN-GATÓN *et al*., 2022). The same interspecific variation pattern in warm tolerance of adults was found in larval instar III of both species (MIRÓN-GATÓN *et al*., 2022). However, experimental studies showed that the water emergence response in *O. lejolisii* started at lower temperature thresholds (35.62 ± 0.81 ºC) than in *O. quadricollis* (38.42 ± 0.67 ºC) at 90 $g.L^{-1}$ salinity (MIRÓN-GATÓN *et al.* 2022), temperature and salinity values that are frequently reached in the studied rockpools in summer.

In *O. lejolisii*, the low abundance and absence of reproduction in summer could be due, in addition to the high temperatures and salinity levels, to the loss of available pools, which are desiccated by strong water evaporation. However, flooded pools in summer that are close to the sea are avoided by this species. Adults and larval instar III of *O. lejolisii* have been observed walking along shorelines, probably looking for cryptic microhabitats, such as crevices, as well as buried below pool sediment covered by a cyanobacterial biofilm, where water loss and temperature are lower than on exposed surfaces in desiccated pools. These behaviour responses allow adults and larvae to resist until the pools are refilled with autumn rains.

Because *O. lejolisii* usually live in a more temporary habitat that dries up frequently, we expected that adult and larvae, and even the egg stages of *O. lejolisii* will be more resistant to desiccation than *O. quadricollis.* In the laboratory, *O. quadricollis* adults and larval instar III had some resistance to desiccation, but eggs development was not viable out of water (JACQUIN, 1956). Further studies on comparative resistance to desiccation in the different life cycle stages of both species are of crucial concern to test this hypothesis.

In summary, reproduction in summer, higher number of generations by year, shorter overall life cycle and more successful egg hatching give more demographic success to *O. quadricollis.* These life cycle traits together with the greater tolerance to high temperatures, suggest that *O. quadricollis* will be less vulnerable to climate change than *O. lejolisii*.

ACKNOWLEDGMENTS

Dedicated to Ignacio Ribera who inspired many studies on the evolution, physiology, biology and ecology of *Ochthebius* rockpool species and with whom we share many rewarding and unforgettable experiences in sampling trips along the Mediterranean and Atlantic coasts looking for beetles. We thank Andrés Millán, Carlos Hernando, Adrián Villastrigo, Susana Pallarés and Simone Guareschi for their field assistance, Juan José Sánchez-Meca for his help in the identification of the different larval instars of the studied species, and graduate student Mario Rodríguez for laboratory assistance. This study was supported by the Excellence Research Project CGL2017-84157P (J.V.) funded by MCIN/ AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe". JM.M-G is supported by a predoctoral grant (FPU Program), AJ.G-M by a predoctoral grant (FPI Program) and M.B.-C.by a pre-doctoral grant (University of Murcia).

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