

# Structure of male genitalia in a lineage of minute endogean ground beetles: how four new species of *Microcharidius* Coiffait, 1969 (Coleoptera: Carabidae: Anillini) reveal gradual change and convergent evolution

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**Abstract.** Typhlocharina is a diverse lineage of minute endogean Anillini (Coleoptera: Carabidae: Trechinae) formed by three genera endemic to the Mediterranean region. Four new species of *Microcharidius* Coiffait, 1969 from Extremadura (Spain) are described in this work, all of them sharing unusual features of the male genitalia: *M. andujari* sp.n., *M. lencinai* sp.n., *M. serranoi* sp.n., and *M. aguiari* sp.n. Male genitalia are very important in insect systematics, often bearing diagnostic features involved in prezygotic isolation processes. In Anillini, male genitalia comprise the aedeagus (median lobe and parameres) and ring sclerite, and the aedeagus has been used as a major taxonomic tool to discriminate species. Here we provide an in-depth revision of these structures in Typhlocharina bringing a comparative approach to evaluate their taxonomic potential. The morphological diversity of male genitalia is described in detail, including poorly studied or overlooked characters like ring sclerites, parameres, endophallic sclerites, and apical laminae of the median lobe. The results show phylogenetic patterns and diagnostic differences in male genitalia between *Lusotyphlus* Pérez-González, Andújar & Zaballos, 2017, *Typhlocharis* Dieck, 1869, and *Microcharidius* Coiffait, 1969. Male genitalic anatomy is found to be an efficient taxonomic tool for genus or clade-level recognition, but not for species-specific discrimination, except in a few cases of clear autapomorphies. Also, observed diversity in male genitalia does not have any obvious morphological correlates with the known diversity in female genitalia, but the prevalence of some types of aedeagi in species with unguiform gonocoxites suggests certain parallelisms. The affinities and novel features of the new species are discussed, highlighting the first cases of gradual transition and convergent evolution in acquisition of distinct male genital traits, probably associated with sexual isolation processes. Finally, we use the available data to explore the effect of size reduction in the proportions of male genitalia for first time in a whole lineage of endogean beetles.

**Key words.** Carabidae, Anillini, Typhlocharina, *Microcharidius*, new species, endogean, male genitalia, aedeagus.

## 1. Introduction

Recently, the internal phylogenetic relationships of a unique lineage of endogean beetles, Typhlocharina (Coleoptera: Carabidae: Trechinae: Anillini), have been unraveled using morphological and molecular data (ANDÚJAR et al. 2017; PÉREZ-GONZÁLEZ et al. 2017). This provided the basis for a revised classification, since the species groups proposed by ZABALLOS & RUIZ-TAPIADOR (1997) and ZABALLOS & WRASE (1998) do not fully correspond to natural clades, and the former genus *Typhlocharis* Dieck,

1869 was divided into three distinct genera: *Lusotyphlus* Pérez-González, Andújar & Zaballos, 2017; *Typhlocharis* Dieck, 1869, and *Microcharidius* Coiffait, 1969.

These apparently dull, tiny brown beetles hide an attractive, complex and still poorly known evolutionary history. Endemic to the western Mediterranean region (Spain, Portugal, Morocco, and Tunisia), Typhlocharina is a monophyletic lineage dated back to Paleocene and linked to diverse palaeogeographical events, such as the

Messinian salinity crisis during Miocene (JEANNEL 1963; ZABALLOS & PÉREZ-GONZÁLEZ 2010, 2011; SERRANO & AGUIAR 2014; ANDÚJAR et al. 2016; PÉREZ-GONZÁLEZ et al. 2017). All the Anillini are well adapted to below-ground lifestyles, being found in diverse aphotic habitats, from leaf litter to deep soil and caves (e.g. ESPAÑOL & COMAS 1985; ZABALLOS & BANDA 2000; SOKOLOV & KAVANAUGH 2014). Typhlocharina are specialized soil-dwellers, inhabiting deep soil layers in a wide variety of Mediterranean habitats (e.g. NORMAND 1915; ZABALLOS & RUIZ-TAPIADOR 1997; ANDÚJAR et al. 2008) and, with more than 65 species described, they have become one of the most diversified Anillini lineages known to date.

The sampling efforts towards the preparation for phylogenetic studies of the group led to the discovery of a large amount of potential new species and new taxonomic tools (PÉREZ-GONZÁLEZ et al. 2017). These specimens are currently undergoing more detailed study to clarify their status and provide proper descriptions (ZABALLOS et al. 2016; PÉREZ-GONZÁLEZ et al. 2018; PÉREZ-GONZÁLEZ & ZABALLOS 2018). In this work, we describe four new species of *Microcharidius* found in this context from fieldwork in the west of Extremadura province, Spain (corresponding to “*M. sp. 28*”, “*M. sp. 29*”, “*M. sp. 30*”, and “*M. sp. 31*” in PÉREZ-GONZÁLEZ et al. 2017), that share unusual features in the male genitalia.

Given their role in reproduction, male genitalia are considered very important systematic features for insects (JEANNEL 1955; TUXEN 1970; SONG & BUCHELI 2010), often bearing diagnostic species-specific characteristics (EBERHARD 1985) that could act as mechanisms of prezygotic isolation. In Anillini, the aedeagus is a major taxonomic tool for discriminating species in many genera (e.g. *Geocharis* Ehlers, 1883 or *Anillinus* Casey, 1918; ZABALLOS 2005; SOKOLOV et al. 2004, 2007). But, how informative are these structures in Typhlocharina? Are they diagnostic for species-specific recognition?

The male genital complex of the group fits the model described by JEANNEL (1955) for Anillini and is formed by the aedeagus, with a median lobe or *phallus* and two parameres, and the ring sclerite. Illustrated for the first time by JEANNEL (1937), the aedeagus has been one of the best described structures in the studies of the group, but the available information for each species is often limited to a general lateral view of median lobe and parameres (e.g. JEANNEL 1963; COIFFAIT 1969; ANDÚJAR et al. 2008). Less frequently, the dorsal view is given (e.g. VIGNA-TAGLIANTI 1972; SERRANO & AGUIAR 2014). Structures like the ring sclerite have received little attention (e.g. ORTUÑO & GILGADO 2011; ZABALLOS et al. 2016) yet are known to vary in other Anillini (e.g. SOKOLOV 2013, 2015). Could male genitalia bring more information to the systematics of Typhlocharina? Do male genitalia show any diagnostic differences between *Lusotyphlus*, *Typhlocharis*, and *Microcharidius*? Are there any overlooked morphological features that support the current phylogenetic hypothesis? Is there any evidence of any other evolutionary processes driving morphological changes in this structure? Research on the phylogeny of the group suggests

a positive answer to some of these questions (PÉREZ-GONZÁLEZ et al. 2017), but a comparative study of the aedeagus of Typhlocharina as a whole is still needed to understand these issues.

Also, it has been suggested that, in insects (including Coleoptera), the reproductive system does not decrease in size at the same rate as the body in lineages with a strong trend toward miniaturization (POLILOV & MAKAROVA 2017). Compared to body size, male genitalia tend to show negative allometry (i.e. the rate of size reduction for genitalia is slower than that for the body, EBERHARD 2009) thus smaller insects have proportionally larger genitalia. Does Typhlocharina follow the same trend? The subtribe is well known by their tiny size and extreme miniaturization of some lineages (PÉREZ-GONZÁLEZ & ZABALLOS 2013b; PÉREZ-GONZÁLEZ et al. 2017) which include some of the smallest known Carabidae. The availability of data for the more than 65 species makes Typhlocharina a good candidate to test the effect of size reduction in the proportions of male genitalia for first time in a whole lineage of endogean beetles.

Here, we explore the raised questions through an in-depth revision of the morphology of the male genital complex in the whole tribe Typhlocharina. We present and examine the data for all the species and discuss the similarities and differences between *Lusotyphlus*, *Typhlocharis*, and *Microcharidius*, as well as explore the proportions of the aedeagus with respect to body length. The novel features of the new species (particularly in the apex of the aedeagus) also give new hints of how divergence in male genitalia may have occurred and highlight the first documented case for the group of convergent evolution possibly associated with processes of sexual isolation.

## 2. Material and methods

### 2.1. Collecting

The new species were collected during spring and autumn of 2013 in five localities of Extremadura, in the southwest of Spain (Fig. 1): Zarza la Mayor, Pozuelo de Zarzón, Santa Cruz de Paniagua (two sites), and Valdecaballeros.

Specimens were obtained by indirect sampling, processing soil (about 20–50 L per sample, taken up to 30–50 cm deep) using an optimized version of soil washing technique (NORMAND 1911). The fauna was extracted putting the residue of the washing into a Berlese apparatus (BERLESE 1905), then separating the specimens manually in the laboratory. A total of 41 specimens were finally obtained and stored in absolute ethanol. Of them, 17 were labeled with voucher numbers and correspond to the specimens selected for DNA extraction procedures (detailed in ANDÚJAR et al. 2017 and PÉREZ-GONZÁLEZ et al. 2017).



**Fig. 1.** Type localities. **A:** *Microcharidius andujari* sp.n. – open meadow with scattered small granite boulders crossed by a stream, 4 km S of the village of Zarza la Mayor (Cáceres, SPAIN). **B:** *Microcharidius lencinai* sp.n. – open grass field near a patch of laudanum shrubs in Arroyo Mirabella, 3.5 km S of Pozuelo de Zarzón (Cáceres, SPAIN). **C,D:** *Microcharidius serranoi* sp.n. – Santa Cruz de Paniagua (Cáceres, SPAIN), cork oak “dehesa” 3.6 km E of the village (C) and open low hill close to a small patch of oak trees, 4.2 km SW of the village (D). **E:** *Microcharidius aguiari* sp.n. – open “dehesa” with scattered holm oaks, brooms, and pasture land, 4.8 km SW of Valdecaballeros (Badajoz, SPAIN).

## 2.2. Morphological study

Before morphological studies, the specimens were treated by rinsing in lactic acid to clear the cuticle. Vouchered specimens were dissected by separation of body parts (head, prothorax, elytra, and abdomen) and extraction of male genitalia for detailed observation. Female genitalia were observed *in situ* to avoid damage during manipulation. The rest of the specimens were cleared and observed without dissection to ensure the identification.

Observations and photographs of the specimens were done using a Zeiss 474620-9900 light microscope (Germany). Measurements were made with a Wild Heerbrugg M8 stereomicroscope (Switzerland) with ocular micrometer, registering length of cephalic capsule (LC), from clypeus to vertex; maximum width of cephalic capsule (WC); length of pronotum (LP); maximum width of pronotum (WP); length of elytra (LE), from humeral angle to elytral apex, and maximum width of elytra (WE). Total length is considered as LC+LP+LE and given as “LT of smallest specimen” – “LT of the largest specimen” for males and females, the other measurements are given as “minimum-maximum” for all specimens. Illustrations were made from the obtained photographs, processed and outlined with Adobe Photoshop CS6 13.0.

After observations, dissected specimens and extracted genitalia were mounted on entomological cards with glass window using dimethyl hydantoin formaldehyde resin (BAMEUL 1990). Untreated specimens were mounted on regular entomological cards. The type specimens are deposited in coll. J.P. Zaballos and coll. S. Pérez-González, Universidad Complutense de Madrid (UCM, Madrid),

Natural History Museum (NHM, London), and Museo Nacional de Ciencias Naturales (MNCN, Madrid).

Nomenclature used in the descriptions follows ZABALLOS (2005) for cephalic chaetotaxy, PÉREZ-GONZÁLEZ & ZABALLOS (2012, 2013b) for the rows of setae, and PÉREZ-GONZÁLEZ & ZABALLOS (2013a) for antennal features. Terminology for the sclerite of sternum IX of males, hence named ring sclerite, follows SOKOLOV & KAVANAUGH (2014). Other morphological features (e.g. rail, metatibial spur) are named as in PÉREZ-GONZÁLEZ et al. (2017). The results are discussed within the phylogenetic framework of Typhlocharina proposed in PÉREZ-GONZÁLEZ et al. (2017).

## 2.3. Revision of male genitalia

Data on male genitalia comes from the study of original specimens gathered from the following collections: **ARS** – coll. A.R.M. Serrano, Universidade de Lisboa; **CA** – coll. C. Andújar; **CZULE** – coll. Zoología, Universidad de León; **SDEI** – Senckenberg Deutsches Entomologisches Institut; **DW** – coll. D. Wrase; **JLL** – coll. J.L. Lencina; **JPZ** – coll. J.P. Zaballos, Universidad Complutense de Madrid; **MFNB** – Museum für Naturkunde, Berlin; **MHNG** – Muséum d’histoire naturelle, Genève; **MNCN** – Museo Nacional de Ciencias Naturales de Madrid; **MNHNP** – Muséum national d’Histoire Naturelle de Paris; **NHM** – Natural History Museum, London; **OJ** – coll. Olegario del Junco; **RT** – coll. I. Ruiz-Tapiador and **SPG** – coll. S. Pérez-González, Universidad Complutense de Madrid. This material included more

than 150 male specimens, representing 64 of the 67 described species of Typhlocharina (observations of type specimens for 59 of them). Data were obtained from literature for *Microcharidius rochapiteae* (Serrano & Aguiar, 2008) and *M. fozcoaensis* (Serrano & Aguiar, 2005). Males of *M. gonzaloi* (Ortuño, 2005) are unknown.

When dry-mounted, specimens from collections were detached from the original cards and given the same treatment explained above. Aedeagi were extracted by softly pulling them out with a minute hooked pin and then rinsing in lactic acid to allow tridimensional manipulation. Ring sclerites were studied both in situ (without extraction) and extracted. Position and shape of the intact apodemal ring were registered as informative. No dyes were used; all the structures were studied under light microscopy by changing light and contrast. Aedeagi were observed and photographed in lateral (right), dorsal, and frontal view, ensuring that the position was equivalent and comparable in all the specimens. Sclerites of endophallus were observed in repose (invaginated), with the aedeagus in lateral view. Measurements of aedeagi were obtained using a graduated microscale in a Zeiss 474620-9900 microscope (Germany), as the length from basal bulb to apex. To compare the proportions between body and aedeagus length, studied males were measured following the same procedures described before.

### 3. Results

#### 3.1. Description of the new species

##### 3.1.1. *Microcharidius andujari* sp.n.

(Fig. 2)

**Locus typicus:** Zarza la Mayor, Cáceres, SPAIN.

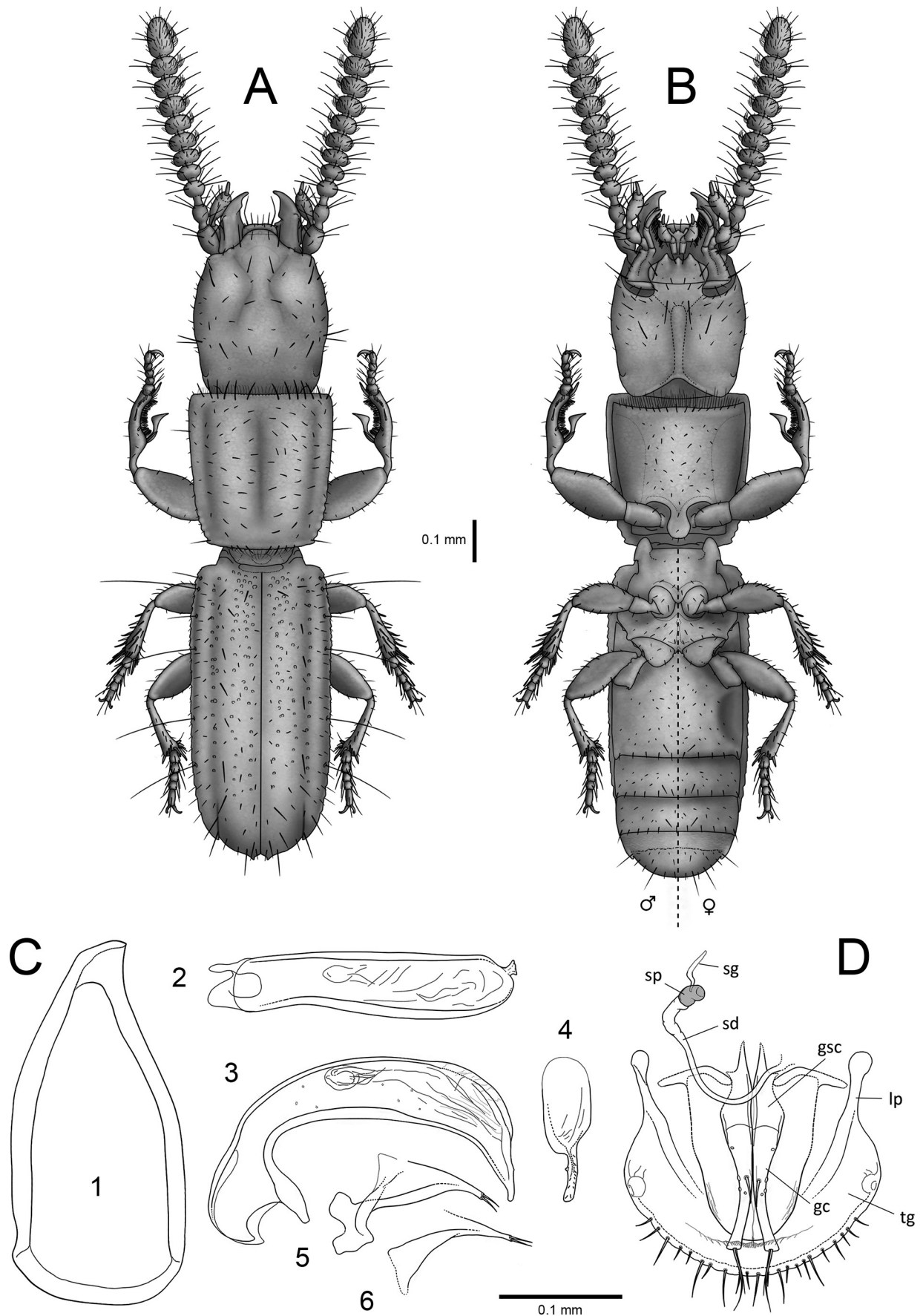
**Material examined. Type series:** Holotype: 1♂ (BMNH-1046089) SPAIN, Cáceres, Zarza la Mayor, 4 km S (39°50'N 06°50'W), 20-04-2013, 326 m, J.P. Zaballos, S. Pérez-González, J.L. Lencina & C. Andújar leg. (coll. J.P. Zaballos, UCM). Paratypes: 18 ex.: 4♂♂, 9♀♀ same data as the holotype (coll. J.P. Zaballos and coll. S. Pérez-González, UCM), 2♂♂ (BMNH-1046088, BMNH-1046208), 3♀♀ (BMNH-1046087, BMNH-1046209, BMNH-1046315) same data as the holotype (coll. J.P. Zaballos and coll. S. Pérez-González, UCM). DNA aliquots are deposited in the Natural History Museum of London (voucher # BMNH-1046088, BMNH-1046089, BMNH-1046208, BMNH-1046087, BMNH-1046209, and BMNH-1046315).

**Diagnosis.** Small, eyeless Anillini (Fig. 2) of endogean lifestyle, with subrectangular body covered by microreticulated integument and scattered pubescence. Recognizable by the following combination of characters: Vertex without stridulatory organ. Proportions of cephalic capsule showing sexual dimorphism, larger in males. Clypeus strongly curved outwards (“D” shape). Narrow gula. Robust mandibles, angular in males, smoothly

curved in females, with dorsal and lateral bumps. Subtrapezoidal pronotum, with 4–5 blunt posterolateral denticles. Elytra with 2 pairs of apical denticles (a pair associated with 7<sup>th</sup> stria and a parasutural pair, the latter variable). Transverse scutellar organ straight. Umbilicate series with 6 setae (4+2). Angular metatrochanters, sexually dimorphic (more sharply angulate in males). Males with a medium-short metatibial seta associated with a spur. Females with a pair of deep simple foveae on the 1st ventrite, absent in males. Median lobe of aedeagus sickle-shaped, strongly curved, with apex that is unique within *Microcharidius*, bearing a long, projected lamella, that is very thin and “warped”. “Bicycle seat-shaped” endophallic sclerites, with a short anterior projection. Female genitalia with robust tubular gonocoxites bearing lateral setae, long spermathecal duct, and subcylindrical-reniform spermatheca.

**Description.** Total length 1.18–1.43 mm (males), 1.22–1.42 mm (females). Depigmented, apterous, and anophthalmous, with dark yellow to chestnut brown microreticulated integument, covered by scattered pubescence (Fig. 2A). **Head** (Fig. 2A,B): As long as or slightly longer (LC 0.25–0.35 mm) than wide (WC 0.25–0.32 mm), covered by subhexagonal microreticulation. Sexually dimorphic head proportions: males with proportionally larger cephalic capsules than females. Vertex region without stridulatory organ. Posterolateral semilunar notch at both sides of cephalic capsule. Labrum subrectangular or slightly rounded, with a middle triangular area and small button of thicker cuticle. Clypeus with anterior margin strongly curved outwards in males, less pronounced in females. Antennae moniliform, with 11 antennomeres, the flagellomeres are reniform (**morph 2** as defined in PÉREZ-GONZÁLEZ & ZABALLOS 2013a), except the last one, which is pyriform. Stem of 3<sup>rd</sup> antennomere slightly elongated (proportion length of stem / antennomere body of 0.70). Last antennomere with a pattern of 1 anterodorsal and 1 posterodorsal sensilla coeloconica (**sc**). One ventral **sc** on antennomeres 5 and 6. Mandibles with sexual dimorphism: noticeably more robust and angular in males, with 2 bumps, lateral and dorsal, in the external angle of mandible; less robust and not angular in females, sometimes with 1 slight dorsal bump. In both sexes, right mandible with a large terebral tooth and a sharp, slightly projected edge (smoother in females). Left mandible with projected sharp edge or “flap”. Labium without special features for the genus, with pointed epilobes and a blunt middle tooth. Ligula with triangular middle lobe and medium-sized paraglossae (as long as or slightly longer than ligula). Narrow gula, approximately 4 × longer than wide. **Cephalic chaetotaxy:** 6 pairs of labral setae (**s-s-l-m-s-m/m-s-m-l-s-s**), 2 pairs of clypeal setae (**l-s/s-l**), 1 pair of frontal setae, 2 supraocular pairs (anterior and posterior), 1 supraantennal pair, 1 pair of vertical setae, 1 pair of temporal setae, 2 pairs of occipital setae and 1 pair of genal setae, as well as scattered pubescence. Labium with 1 pair of setae near base of middle tooth, 1 pair of long setae near base of epilobes, 1 pair of very short setae near apex of





**Fig. 2.** Habitus of *Microcharidius andujari* sp.n. **A:** Habitus, dorsal view (male). **B:** Habitus, ventral view (abdominal morphology: left half, male; right half, female). **C:** Male genitalia, (1) ring sclerite, (2) aedeagus (dorsal view), (3) aedeagus (lateral view), (4) apex of aedeagus in frontal view, (5) right paramere, (6) left paramere. **D:** Female genitalia. — **Abbreviations:** gc – gonocoxite, gsc – gonosubcoxite, lp – lateral projection, sd – spermathecal duct, sg – spermathecal gland, sp – spermatheca, tg – tergite VIII.

epilobes and 2 pairs of very short setae near posterior suture. Prebasilar with 2 pairs of setae near anterior margin (lateral pair much longer) and 2 pairs (lateral pair much longer) in posterior region of the prebasilar, with some degree of individual variation over this pattern. **Thorax** (Fig. 2A,B): Pronotum subtrapezoidal, longer (LP 0.31–0.39 mm) than wide (WP 0.27–0.32 mm), slightly narrowed in posterior region. Anterior margin straight, crenulated, without medial hiatus. Posterior margin smoothly curved outwards. Lateral margins with 4 or 5 posterior denticles, low, blunt, irregular. Surface covered by subhexagonal microreticulation. Disc flattened, with a medial line and a pair of faint lateral sulci. **Pronotal chaetotaxy**: 1 pair of long setae in anterior third of lateral margins, 1 pair of long setae near posterior angles, a row of 4–7 pairs of setae [l-(l)-(l)-(l)-l-l-l-l-(l)-(l)-(l)] parallel to anterior margin, 3–4 pairs of setae parallel to posterior margin [s-(s)-l-l-l-(s)-s], a row of small, filiform setae, regularly spaced along anterior and posterior margins, a row of short setae along lateral margins and 4–5 pairs of irregular longitudinal rows of short pubescence on disc. Proepisternal suture visible. Prosternal apophysis rounded. Anterior margin of prosternum with a row of long thin setae and 7–8 pairs of short setae parallel to them. Prosternum covered by scattered pubescence, absent on proepisterna. Mesepisterna barely sunk. Metepisterna depressed near the articulation of hind legs, forming a pair of smooth foveae, more pronounced in females. **Elytra** (Fig. 2A): Subparallel, more than 2 × longer (LE 0.62–0.73 mm) than wide (WE 0.28–0.31 mm). Lateral margins serrated, with 16–23 well defined denticles, progressively less marked towards posterior. Apical margin with 2 pairs of denticles: 1 pair associated with the end of 7<sup>th</sup> stria, and 1 sutural pair, the latter with small differences between individuals. Humeral angle well marked. Disc flattened, with longitudinal lateral carinae associated with the 7<sup>th</sup> stria, reaching apical margin, with rail on underside (Fig. 2B). Surface covered by irregular subhexagonal microreticulation. Strongly marked elytral pits, present in scutellar region, parallel to the suture, on disc and along 7<sup>th</sup> stria. Transverse scutellar organ with straight margin. A pair of small, atrophied “buttonholes” near base of elytra. **Elytral chaetotaxy**: umbilicate series formed by an anterior group of 4 setae and a posterior group of 2 (4+2). One pair of scutellar setae. No discal setae. Discal pubescence arranged in approximately 5 pairs of longitudinal rows of pubescence of irregular length, the 3<sup>rd</sup> row with “pseudo-discal setae”: longer and shorter setae interspersed. One pair of long apical setae and 2 pairs of subapical setae, the inner pair short or medium-sized and the outer pair much longer. Lateral margins with a short seta for every denticle, increasing in length towards posterior. **Legs** (Fig. 2B): Sexually dimorphic. Profemora, protibiae, mesofemora, and mesotibiae without special features. Metacoxal flap smoothly rounded. Metatrochanters markedly angular in both sexes, somewhat stronger in males, showing a slightly hooked inner angle with 1 or 2 small points. Metafemora not or slightly angular, metatibiae with distal region not dilated. Males have a medium-sized perpen-

dicular metatibial long-seta associated with a **spur** in distal inner edge. Perpendicular metatibial seta also present in females, but very short and without spur. Inner margin of femora smooth. Tarsi clearly pentamerous on all legs. Pretarsal claws curved and smooth. **Abdomen** (Fig. 2B): Covered by irregular microsculpture. Intermetacoxal space not widened. Females with a pair of deep foveae (single concavity) in 1<sup>st</sup> ventrite, absent in males. Last ventrite with belt of thin, scaly microsculpture (edge of each scale finely and irregularly serrated in both sexes); posterior margin with a pair of lateral notches and 6–7 pairs of setae, sexually dimorphic: l-s-s-l-s-s-l-s-l-s-s-l. **Male genitalia** (Fig. 2C): Aedeagus with robust, strongly curved sickle-shaped median lobe (length 0.25 mm) (Fig. 2C–3), straight in dorsal view (Fig. 2C–2). Apex with long, projected lamella, very thin and “warped” (Fig. 2C–4). Endophallic sclerites with “bicycle seat” shape (in lateral view) and a very short anterior projection. Subtriangular parameres, with 2 mid-sized apical setae, slightly unequal (Fig. 2C–5,6). Ring sclerite subtriangular, apical margin projected in a subtriangular extension, gently tilted (Fig. 2C–1). **Female genitalia** (Fig. 2D): Tubular gonocoxites with 2 apical setae and 1 lateral seta in middle region, as well as scattered pores, fitting the general model described by VIGNA-TAGLIANTI (1972). Gonosubcoxites narrow and acuminate. Very long spermathecal duct with two well-differentiated regions: a thinner proximal section (diameter 0.004 mm) and a thicker distal section (diameter 0.009 mm). Spermatheca subcylindrical-reniform (length 0.021 mm). Conical spermathecal gland (length 0.020 mm), distally sclerotized. Tergite VIII with posterior margin smoothly curved or slightly subtriangular, covered by a row of thin setae; short lateral projections, broadened distally.

**Variability.** The type series of *M. andujari* includes 19 specimens. It expresses sexual dimorphism, affecting overall proportions (most noticeable in the larger head and strong angular mandibles of males), presence of ventral foveae, and shape of metatrochanters. Also, the studied specimens show a moderate degree of intraspecific differences independent of sex, that are within the range of variation observed in other Typhlocharina (PÉREZ-GONZÁLEZ et al. 2013; PÉREZ-GONZÁLEZ & ZABALLOS 2013b). The shape of labrum (from subrectangular to slightly rounded, very subtly notched in one specimen), clypeus, ligula (subtriangular, but curved in two specimens), development of terebral teeth, chaetotaxy of labium and basilar, general shape and chaetotaxy of pronotum (especially the shape of posterolateral denticles and the rows of setae parallel to anterior and posterior margins), the number of lateral denticles of elytra, and the metatrochanter of males (with one or two small points in the inner angle) are affected. The shape of the sutural pair of apical denticles is also quite labile: from subtriangular denticles with a “U” shaped notch in between (in one specimen) to pointy denticles (in average). One specimen shows asymmetric denticles and some damage on the apex of elytra.

**Derivatio nominis.** The new species is dedicated to our dear colleague and friend, Dr. Carmelo Andújar, one of the major collectors and contributors to the recent advances in the knowledge of Typhlocharina.

**Habitat.** *Microcharidius andujari* sp.n. was found in open prairie environment, a large meadow with scattered small granite boulders crossed by a stream (Fig. 1A). Vegetation was mainly herbaceous, with short grasses, thistles, and scarce brooms (*Retama* sp. Raf.). The sample was taken in the slopes of the stream; the soil was humid and rich in clay.

### 3.1.2. *Microcharidius lencinai* sp.n. (Fig. 3)

**Locus typicus.** Pozuelo de Zarzón, Cáceres, SPAIN.

**Material examined. Type series:** Holotype: 1♂ (BMNH-1046101) SPAIN, Cáceres, Pozuelo de Zarzón (3,5 km S), Arroyo Mirabella (40°07'N 06°26'W), 08-05-2013, 448 m, J.P. Zaballos & S. Pérez-González leg. (coll. J.P. Zaballos, UCM). Paratype: 1♂ (BMNH-1046102) same data as the holotype (coll. J.P. Zaballos, UCM). DNA aliquots are deposited in the Natural History Museum of London (voucher # BMNH-1046101 and BMNH-1046102).

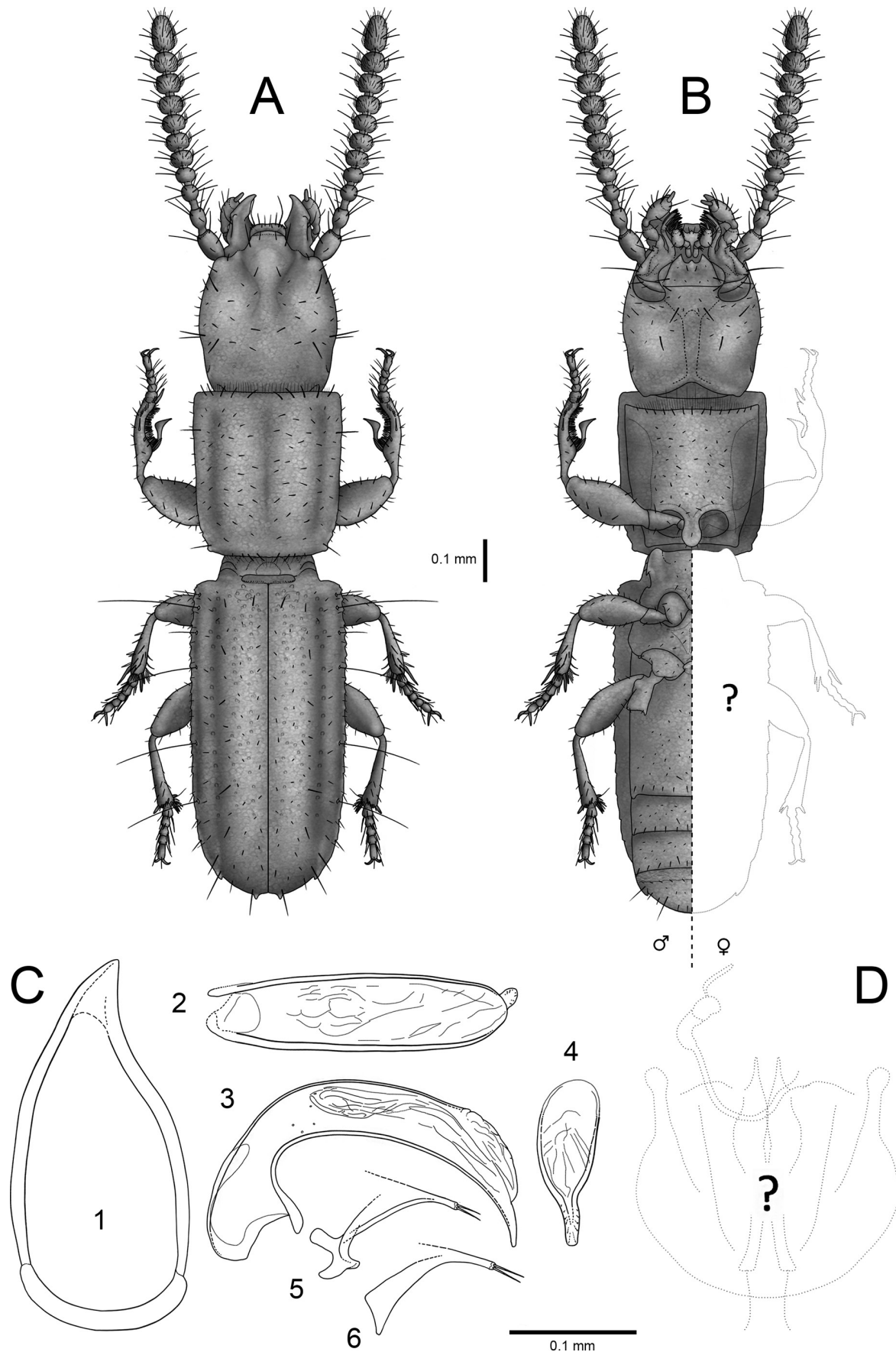
**Diagnosis.** Small, eyeless Anillini (Fig. 3) of endogean lifestyle, with subrectangular body covered by micro-reticulated integument and scattered pubescence. Recognizable by the following combination of features: Vertex without stridulatory organ. Clypeus strongly curved outwards (“D” shape). Narrow gula. Robust, angular mandibles, with dorsal and lateral bumps. Subtrapezoidal pronotum, with 3–4 very low posterolateral denticles. Elytra with 2 pairs of apical denticles (pair associated with 7<sup>th</sup> stria and parasutural pair, the latter acuminate and narrow). Transverse scutellar organ straight or gently curved. Umbilicate series with 6 setae (4+2). Angular metatrochanters. The inner angle of femora could be coarse. Males with a medium-to-short metatibial seta associated with a spur. Sickie-shaped, strongly curved aedeagus, apex with a very long and thin lamella, sinuously curved. “Bicycle seat-shaped” endophallic sclerites, with a gently curved anterior projection. Female unknown.

**Description.** Total length 1.44–1.48 mm. Unpigmented, apterous, and anophthalmous, integument dark yellow to brownish, microreticulated, and covered by scattered pubescence (Fig. 3A). Female unknown. **Head** (Fig. 3A,B): Slightly longer (LC 0.33–0.35 mm) than wide (WC 0.31–0.32 mm). Males with large cephalic capsule, seemingly as in other species with sexual dimorphism in cephalic proportions. Labrum subrectangular. Clypeus with anterior margin strongly curved outwards. Antennae moniliform, with 11 antennomeres, the flagellomeres are reniform (**morph 2**), except the last one, which is pyriform. Stem of 3<sup>rd</sup> antennomere not elongated (length of stem / antennomere body proportion of 0.63). Mandibles robust and angular, with 2 bumps, lateral and dorsal in external angle of mandible. Right mandible with 2 ter-

ebraal teeth and a smoothly projected edge. Left mandible with a smoothly projected sharp edge or “flap”. Rest of cephalic features and cephalic chaetotaxy as described for *M. andujari* sp.n., except in prebasilar, where there is an additional pair of short setae in middle region. **Thorax** (Fig. 3A,B): Pronotum subtrapezoidal, slightly longer (LP 0.37–0.39 mm) than wide (WP 0.35–0.36 mm), moderately narrowed in posterior region. Anterior margin faintly crenulated. Lateral margins with 3–4 very low posterior denticles, blunt and irregular. All other features and prosternal chaetotaxy like in *M. andujari* sp.n., but a row of setae parallel to anterior margin with 5 pairs of setae [I-I-I-I-I-I-I-I] and 5 pairs of irregular longitudinal rows of short pubescence on disc. Prosternal and mesosternal features as in *M. andujari* sp.n. Metepisterna softly depressed near the articulation of hind legs. **Elytra** (Fig. 3A): Subparallel, about 2 × as long (LE 0.74 mm) as wide (WE 0.34–0.37 mm). Elytral features as described for *M. andujari* sp.n., but 17–20 denticles in lateral margins, and a pair of acute sutural apical denticles. Elytral pits strongly marked in scutellar region, present but less developed parallel to suture, in disc, and along 7<sup>th</sup> stria. Transverse scutellar organ with margin substraight or very gently curved. Elytral chaetotaxy does not differ from that described for *M. andujari* sp.n., the umbilicate series is formed by an anterior group of 4 setae and a posterior group of 2 (4+2). **Legs** (Fig. 3B): As described for males of *M. andujari* sp.n., except less angular metafemora; metatrochanters markedly angular, with pointy inner angle, and inner margin of femora smooth or slightly coarse. **Abdomen** (Fig. 3B): Abdominal features and chaetotaxy as described for males of *M. andujari* sp.n. Female unknown. **Male genitalia** (Fig. 3C): Aedeagus stout, with strongly curved sickle-shaped median lobe (length 0.24 mm) (Fig. 3C–3), straight in dorsal view (Fig. 3C–2). Apex with long lamella, very thin and gently curved, sinuous (Fig. 3C–4). Endophallic sclerites with “bicycle seat” shape (in lateral view) and a short anterior projection, gently curved. Subtriangular parameres, with slim distal region, ended in 2 medium-sized apical setae, slightly unequal (Fig. 3C–5,6). Ring sclerite subtriangular, apical margin projected in a tilted subtriangular extension (Fig. 3C–1). **Female genitalia** (Fig. 3D): Unknown.

**Variability.** *M. lencinai* sp.n. is only known from 2 male specimens. Both express subtle differences in structures like mandibles, ligula, pronotum, number of lateral denticles of elytra or the transverse scutellar organ, as well as the pattern or position of several small setae. The apical denticles of elytra are virtually identical, but in one of the specimens the sutural pair is slightly asymmetric. Sexual dimorphism is unknown, but may be expected due to the similarity of male proportions in comparison to other dimorphic species, like *M. andujari* sp.n.

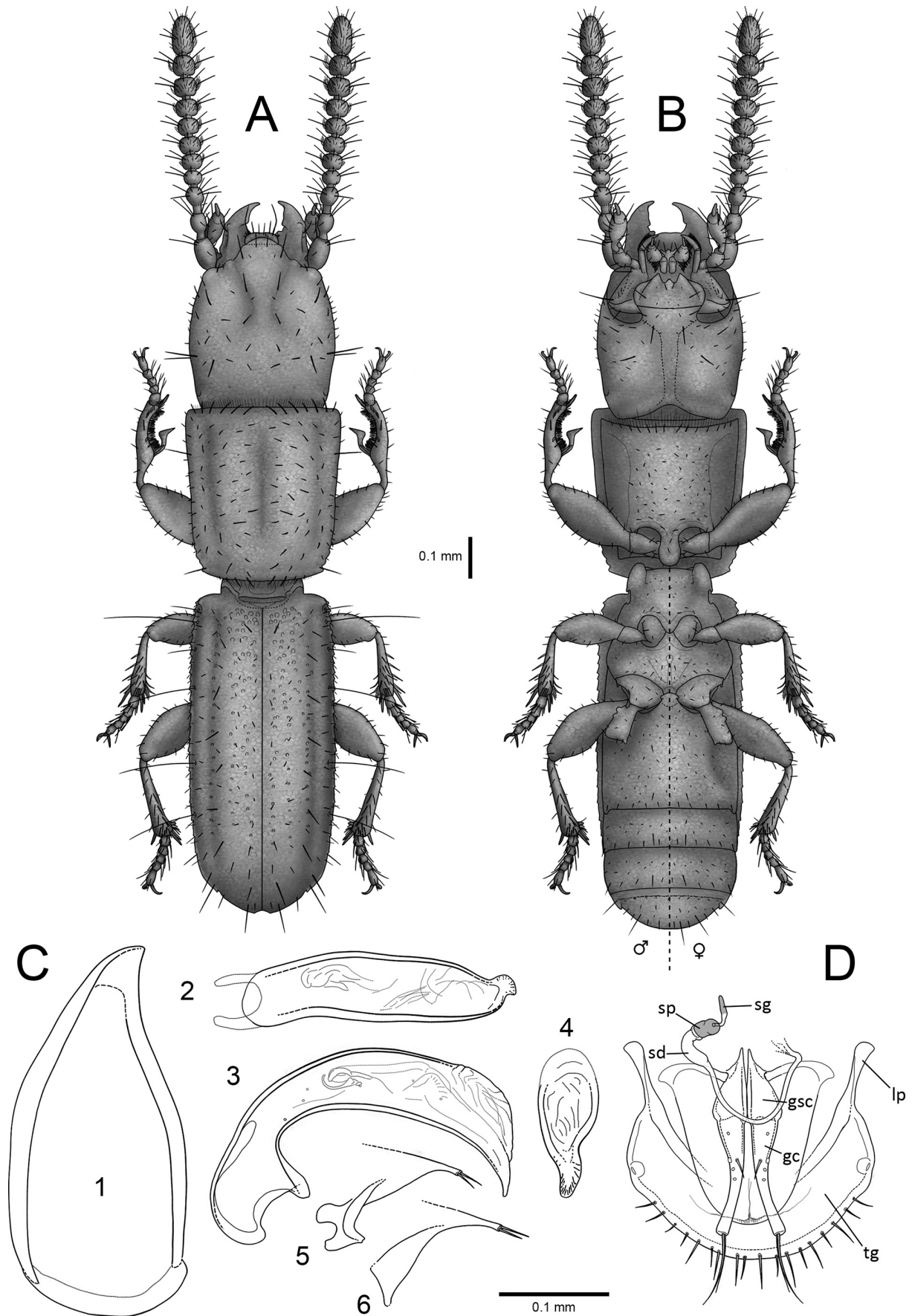
**Derivatio nominis.** This species is dedicated to José Luis Lencina, great naturalist and friend, who has collected many Typhlocharina during his life and actively



**Fig. 3.** Habitus of *Microcharidius lencinai* sp.n. **A:** Habitus, dorsal view (male). **B:** Habitus, ventral view (abdominal morphology: left half, male; right half, female, unknown). **C:** Male genitalia, (1) ring sclerite, (2) aedeagus (dorsal view), (3) aedeagus (lateral view), (4) apex of aedeagus in frontal view, (5) right paramere, (6) left paramere. **D:** Female genitalia, unknown. — **Abbreviations:** gc – gonocoxite, gsc – gonosubcoxite, lp – lateral projection, sd – spermathecal duct, sg – spermathecal gland, sp – spermatheca, tg – tergite VIII.







**Fig. 4.** Habitus of *Microcharidius serranoi* sp.n. **A:** Habitus, dorsal view (male). **B:** Habitus, ventral view (abdominal morphology: left half, male; right half, female). **C:** Male genitalia, (1) ring sclerite, (2) aedeagus (dorsal view), (3) aedeagus (lateral view), (4) apex of aedeagus in frontal view, (5) right paramere, (6) left paramere. **D:** Female genitalia. — **Abbreviations:** gc – gonocoxite, gsc – gonosubcoxite, lp – lateral projection, sd – spermathecal duct, sg – spermathecal gland, sp – spermatheca, tg – tergite VIII.

**Male genitalia** (Fig. 4C): Robust aedeagus, with strongly curved sickle-shaped median lobe (length: 0.27 mm) (Fig. 4C–3), straight in dorsal view (Fig. 4C–2). Apex with projected lamella, sinuously curved (Fig. 4C–4). Endophallic sclerites with “bicycle seat” shape (in lateral view) and a very short anterior projection. Subtriangular parameres, with slim distal regions and 2 mid-sized apical setae (Fig. 4C–5,6). Ring sclerite subtriangular, apical margin projected in a subtriangular extension, gently tilted (Fig. 4C–1). **Female genitalia** (Fig. 4D): Tubular gonocoxites with 2 apical setae and 1 lateral seta in middle region, as well as scattered pores. Gonosubcoxites narrow and acuminate. Moderately long spermathecal duct well-differentiated in a thinner proximal section (diameter 0.004 mm) and a thicker distal section (diameter 0.011 mm). Spermatheca subcylindrical-reniform (length 0.023 mm). Conical spermathecal gland (length 0.017 mm), distally sclerotized. Tergite VIII with posterior margin smoothly curved, covered by a row of thin setae; medium-sized lateral projections, slightly broadened distally.

**Variability.** The studied specimens come from two different populations, one of them represented by a single female and the other represented by 17 specimens. The single female does not show any differences with the females from the other population beyond the expected degree of intraspecific variation. This variation affects the aforementioned features known to vary between individuals of the same species in Typhlocharina. In the larger population, it is clear that *M. serranoi* sp.n. is sexually dimorphic in multiple features, including the proportions of the head (larger in males), mandibles, shape of metatrochanters, and presence/absence of foveae in the first ventrite. One of the specimens has an abnormally wide transverse scutellar organ and another specimen shows damage to the apex of the elytra.

**Derivatio nominis.** Dedicated to our dear colleague and friend Dr. Artur R.M. Serrano, who for many years, alongside Carlos A.S. Aguiar, has shed light on the Typhlocharina of Portugal.

**Habitat.** *Microcharidius serranoi* sp.n. is currently known from two separate localities in the surroundings of Santa Cruz de Paniagua. One of the localities (3.6 km E of the village) was a “dehesa”, with cork oaks (*Quercus suber* L.) and pasture of short herbaceous plants and grasses (Fig. 1C). The sample was taken near the base of a cork oak tree. The other locality (4.2 km SW of the village) was an open low hill close to a small patch of oak trees [*Quercus faginea* Lam. (1785)] (Fig. 1D). The sample was obtained in a small slope of the upper part of the hill. The vegetation in the area was mainly herbaceous, with plenty of grasses and thistles, but also includes scattered bushes like bramble (*Rubus* sp. L.), Spanish broom (*Spartium junceum* L.), and laudanum shrubs (*Cistus ladanifer* L.). In both sites, the sampled soil was humid and rich in clay.

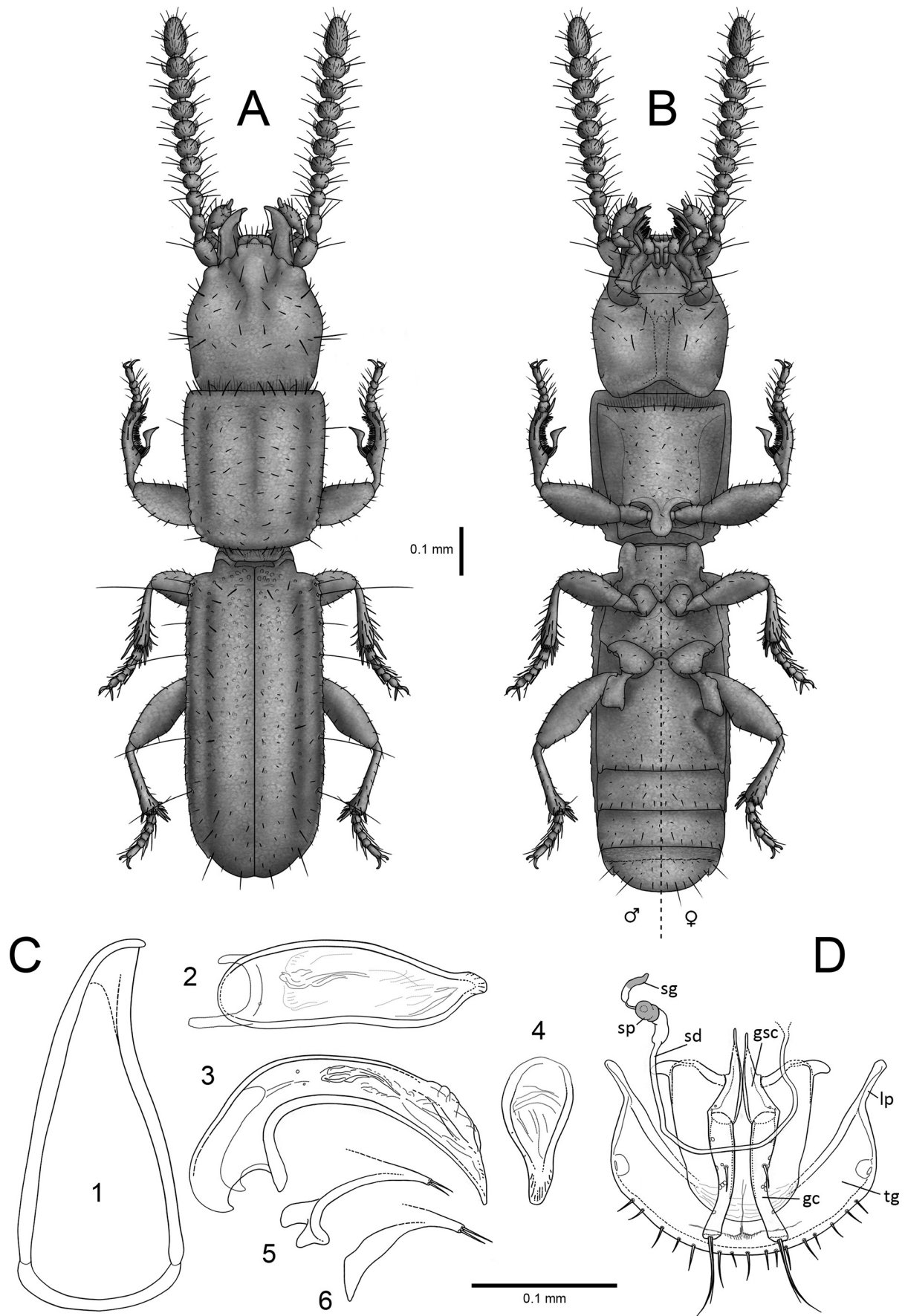
### 3.1.4. *Microcharidius aguiari* sp.n. (Fig. 5)

**Locus typicus.** Valdecaballeros, Badajoz, SPAIN.

**Material examined. Type series:** Holotype: 1♂ (BMNH-1046155) SPAIN, Badajoz, Valdecaballeros, 4.8 km SW (39°12'N 05°12'W), 17-12-2013, 401 m, J.P. Zaballos & S. Pérez-González leg. (coll. J.P. Zaballos, UCM). Paratype: 1♀ (BMNH-1046154) same data as the holotype (coll. J.P. Zaballos, UCM). DNA aliquots are deposited in the Natural History Museum of London (voucher # BMNH-1046154 and BMNH-1046155).

**Diagnosis.** Small, eyeless Anillini (Fig. 5) of endogean lifestyle, with subrectangular body covered by microreticulated integument and scattered pubescence. It is recognizable by the following combination of features: vertex without stridulatory organ. Clypeus with a strong medial subtriangular projection. Narrow gula. Robust mandibles, angular in males, smoothly curved in females, with lateral bumps. Subtrapezoidal pronotum, with 3–4 blunt posterolateral denticles. Elytra with 1 pair of apical denticles (associated with 7<sup>th</sup> stria). Transverse scutellar organ straight or subtly curved. Umbilicate series with 6 setae (4+2). Metatrochanters angulate, sexually dimorphic (more sharply angulate in males). Males with a medium-large metatibial seta associated with a spur. Female with a pair of deep, double foveae in the 1<sup>st</sup> ventrite, absent in male. Sickle-shaped, strongly curved median lobe of aedeagus, apex with a projected, sinuously curved lamella. “Bicycle seat-shaped” endophallic sclerites, with a gently curved anterior projection. Female genitalia with robust tubular gonocoxites, with lateral setae. Long spermathecal duct and very short subcylindrical-reniform spermatheca.

**Description.** Total length 1.27 mm (male), 1.18 mm (female). Unpigmented insect, apterous and anophthalmous, with amber yellow or light brown microreticulated integument, covered by scattered pubescence (Fig. 5A). **Head** (Fig. 5A,B): slightly longer (LC 0.26–0.30 mm) than wide (WC 0.26–0.28 mm), Head proportions without sexual dimorphism. Labrum subrectangular. Clypeus with anterior margin strongly projected in a subtriangular expansion, less pronounced in females. Antennae moniliform (11 antennomeres) with smoothly reniform flagellomeres (**morph 2**), last one pyriform. Stem of 3<sup>rd</sup> antennomere not elongated (proportion length of stem / antennomere body of 0.63). Remaining cephalic features and cephalic chaetotaxy as described for *M. andujari* sp.n., except 1 lateral bump, not 2, in mandibles (external angle) and 1–2 pairs of very short lateral seta in middle region of prebasilar. **Thorax** (Fig. 5A,B): pronotum subtrapezoidal, longer (LP 0.31–0.33 mm) than wide (WP 0.29–0.30 mm), slightly narrowed in posterior region. Like that of *M. andujari* sp.n., but anterior margin slightly crenulated and lateral margins with 3–4 posterior denticles, blunt and irregular. Chaetotaxy, prosternal and mesosternal features as in *M. andujari* sp.n., but with 5–7 pairs of setae [1-(1)-(1)-1-1-1/1-1-1-(1)-(1)-1] in the row of setae parallel to anterior margin, 3 pairs of setae parallel to posterior margin [s-1-1/1-s], and 5 pairs of ir-



**Fig. 5.** Habitus of *Microcharidius aguiari* sp.n. **A:** Habitus, dorsal view (male). **B:** Habitus, ventral view (abdominal morphology: left half, male; right half, female). **C:** Male genitalia, (1) ring sclerite, (2) aedeagus (dorsal view), (3) aedeagus (lateral view), (4) apex of aedeagus in frontal view, (5) right paramere, (6) left paramere. **D:** Female genitalia. — **Abbreviations:** gc – gonocoxite, gsc – gonosubcoxite, lp – lateral projection, sd – spermathecal duct, sg – spermathecal gland, sp – spermatheca, tg – tergite VIII.

regular longitudinal rows of short pubescence on disc. Metepisterna depressed near articulation of hind legs. **Elytra** (Fig. 5A): subparallel, more than  $2 \times$  longer (LE 0.61–0.64 mm) than wide (WE 0.28–0.32 mm). Elytral features as described for *M. andujari* sp.n., but with 18–20 well defined denticles in lateral margins, and only 1 pair of denticles in apical margin, associated with end of 7<sup>th</sup> stria. Elytral pits well marked, present in scutellar region, parallel to the suture, in disc, and along 7<sup>th</sup> stria. Transverse scutellar organ with straight or smoothly curved margin. Elytral chaetotaxy like *M. andujari* sp.n., umbilicate series formed by an anterior group of 4 setae and a posterior group of 2 (4+2). Lateral margins with a short seta for every denticle, of equal length in the entire margin. **Legs** (Fig. 5B): as described for *M. andujari* sp.n., except markedly angular metatrochanters in both sexes but stronger in males, with an acute inner angle; metafemora slightly angular and a long or medium-sized perpendicular metatibial long-seta associated with a **spur** in the distal inner edge of males (a very short perpendicular metatibial seta also appears in females). **Abdomen** (Fig. 5B): as described for *M. andujari* sp.n., except the foveae of first ventrite, deep and with double concavity in females, absent in males. **Male genitalia** (Fig. 5C): Aedeagus with robust, strongly curved sickle-shaped median lobe (length 0.20 mm) (Fig. 5C–3), smoothly curved in dorsal view (Fig. 5C–2). Apex with projected lamella, sinuously curved (Fig. 5C–4). Endophallic sclerites with “bicycle seat” shape (in lateral view) expanded forwards in a gently curved anterior projection. Subtriangular parameres, with 2 medium-sized apical setae, slightly unequal (Fig. 5C–5,6). Ring sclerite subtriangular, apical margin projected in a subtriangular extension, gently tilted (Fig. 5C–1). **Female genitalia** (Fig. 5D): Tubular gonocoxites, thick, with 2 apical setae and 1 lateral seta in middle region, as well as scattered pores. Gonosubcoxites narrow and pointy. Long spermathecal duct differentiated in a thinner proximal section (diameter 0.003 mm) and a thicker distal section (diameter 0.009 mm). Spermatheca subcylindrical-reniform, very short (length 0.017 mm). Conical spermathecal gland (length 0.025 mm), distally sclerotized. Tergite VIII with posterior margin smoothly curved, covered by a row of thin setae; with short lateral projections of uniform thickness.

**Variability.** *Microcharidius aguiari* sp.n. is currently known only from 2 specimens, male and female, so the internal variability of the population cannot be described. However, both specimens show small individual differences in features that are known to vary in other species of Typhlocharina (e.g. PÉREZ-GONZÁLEZ et al. 2013; PÉREZ-GONZÁLEZ & ZABALLOS 2013b), such as the patterning or position of diverse types of small setae. Also, there is clear sexual dimorphism in these specimens, expressed, beyond genitalia, in head (mandibles), legs (metatibial long seta and spur), and abdomen (ventral foveae).

**Derivatio nominis.** Dedicated to our dear colleague and friend, Carlos A.S. Aguiar, who for many years, along-

side Dr. Artur R.M. Serrano, has shed light on the Typhlocharina of Portugal.

**Habitat.** The new species was found in open “dehesa” environment, with scattered holm oaks (*Quercus ilex* L.), brooms (*Retama* sp. Raf.), and pasture land (Fig. 1E). The sample was taken in the slope of a dry temporary watercourse near a holm oak, with brooms, and thorny bushweeds (*Flueggea tinctoria* (L.) G.L. Webster) in the surroundings. The sampled soil was clayey and moist.

### 3.2. Affinities

The four new species are part of **clade belenae** (*sensu* PÉREZ-GONZÁLEZ et al. 2017), a very distinct clade diagnosed by reniform flagellomeres, lack of stridulatory organ, and a narrow gula, among other characters. Within Typhlocharina, this clade is particularly complicated due to their homogeneous morphology, but the combination of diagnostic features in the mandibles, labrum, clypeus, apex of elytra, metatrochanters, ventral foveae, and genitalia are especially useful to discriminate between its species.

All the new species share a 4+2 umbilicate series pattern. Although this feature shows variation at different levels, including intraspecific variation, and it is not reliable as a single differentiating feature (PÉREZ-GONZÁLEZ et al. 2017) it allows a first discrimination. Only six species of **clade belenae** have patterns other than 4+2: *M. carpetanus* (Zaballos, 1989) (3+1), *M. portilloi* (Zaballos, 1991) (3+2), *M. loebli* Pérez-González & Zaballos, 2018 (3+2), *M. carmenae* (Zaballos & Ruiz-Tapiador, 1995) (4+3), *M. farinosae* (Zaballos & Ruiz-Tapiador, 1997) (4+3) and *M. elenae* (Serrano & Aguiar, 2002) (4+1), and the diagnostic traits of the new species readily differentiate them from any of these species.

The 4+2 pattern appears in *M. belenae* (Zaballos, 1983), *M. intermedius* (Zaballos, 1986), *M. toribioi* (Ortuño, 1988), *M. jeannei* (Zaballos, 1989), *M. atienzai* (Zaballos & Ruiz-Tapiador, 1997), *M. bullaquensis* (Zaballos & Ruiz-Tapiador, 1997), *M. estrellae* (Zaballos & Ruiz-Tapiador, 1997), and *M. amara* (Zaballos, Andújar & Pérez-González, 2016). Of them, *M. belenae*, *M. estrellae*, *M. bullaquensis*, and *M. amara* have exaggerated robust angular mandibles as occurs in the four new species. *Microcharidius andujari* sp.n., *M. lencinai* sp.n., and *M. serranoi* sp.n. are very similar to each other and, indeed, molecular and total evidence data suggest they are closely related (Figs. 10, 11; ANDÚJAR et al. 2017; PÉREZ-GONZÁLEZ et al. 2017) but differ in the diverse modifications of the apex of aedeagus (Figs. 2C–4, 3C–4, 4C–4). They share a highly curved, “D-shaped” clypeus with *M. amara* and *M. belenae*, but in both species the apex of aedeagus is typically subtriangular. Also, *M. amara* possesses unique deep double ventral foveae (single in *M. andujari* sp.n. and *M. serranoi* sp.n., unknown in *M. lencinai* sp.n.), while *M. belenae* have a markedly bilobate labrum (subrectangular in the new species).



The combination of robust angular mandibles, strong subtriangular clypeus, lack of sutural pair of apical denticles, double foveae, and the shape of the apex of the aedeagus make *M. aguiari* sp.n. easy to differentiate from any other species. The most similar are *M. estrellae* and *M. bullaquiensis* which shares the same type of mandibles and a similar clypeus (although the subtriangular projection is much less developed). *Microcharidius bullaquiensis* also presents double foveae (single in *M. estrellae*) and some resemblances in the shape of endophallic sclerites and female genitalia, but is well differentiated from *M. aguiari* sp.n. by the presence of sutural apical denticles and a subtriangular apex of the aedeagus. The lack of this pair of denticles is not frequent within clade *belenae* and it's only seen in three species: *M. toribioi*, *M. portilloi* (Zaballos, 1991), and *M. atienzai* (that also possesses double foveae). In *M. portilloi* this feature is sexually dimorphic and only males lack denticles. In *M. toribioi* and *M. atienzai*, as in *M. aguiari* sp.n., the denticles are absent in both sexes.

The affinities with *M. atienzai* are very interesting. Until now, *M. atienzai* was the only species known with such a sinuously curved apex of the aedeagus (Fig. 6C–5vii). However, the same shape is shared by *M. serranoi* sp.n. and *M. aguiari* sp.n. (Figs. 4C–4, 5C–4), species that otherwise show important differences with *M. atienzai*, like the shape of clypeus, labrum, mandibles, and endophallic sclerites. *Microcharidius atienzai* also shows a peculiar thick and short spermathecal duct akin to that of *M. portilloi* that suggest is not closely related to the new species.

### 3.3. Structure of male genitalia

Table 1 shows the variability in male genitalia for every species of Typhlocharina and Fig. 10 illustrates it over the current phylogenetic hypothesis.

#### 3.3.1. Median lobe of aedeagus

The aedeagi of Typhlocharina fit the diagnostic characters given by JEANNEL (1937, 1963) for Anillini. The aedeagus can be interpreted as the set comprising the median lobe and parameres (JEANNEL 1955). The **median**

**lobe**, also called phallus or penis, is formed by: the base – **basal bulb** – open, with two subequal lateral laminae; the central part – **middle lobe** – arched and narrowed through the distal end, and the apex, which is open – **apical opening** or **phallotreme** – and finish in a platform of variable development – **apical lamina** – (Fig. 6A–1). In dorsal view, it could be straight, arched or bent to the right (anatomically oriented) (Fig. 6B).

The median lobe can be grouped in four general morphological models, plus several exceptional cases:

**Morph Ae–1.** Falciform, with an abrupt transition between the basal bulb and the base of middle lobe (Fig. 6A–1). The base can be straight (e.g. *M. diecki*) or smoothly curved (e.g. *T. quarta*). It is the most widespread model within the group.

**Morph Ae–2.** Falciform, robust, and gently curved, with a distal region projected in one or two tall lateral crests [e.g. *L. carinatus* (Serrano & Aguiar, 2006), Fig. 6A–2].

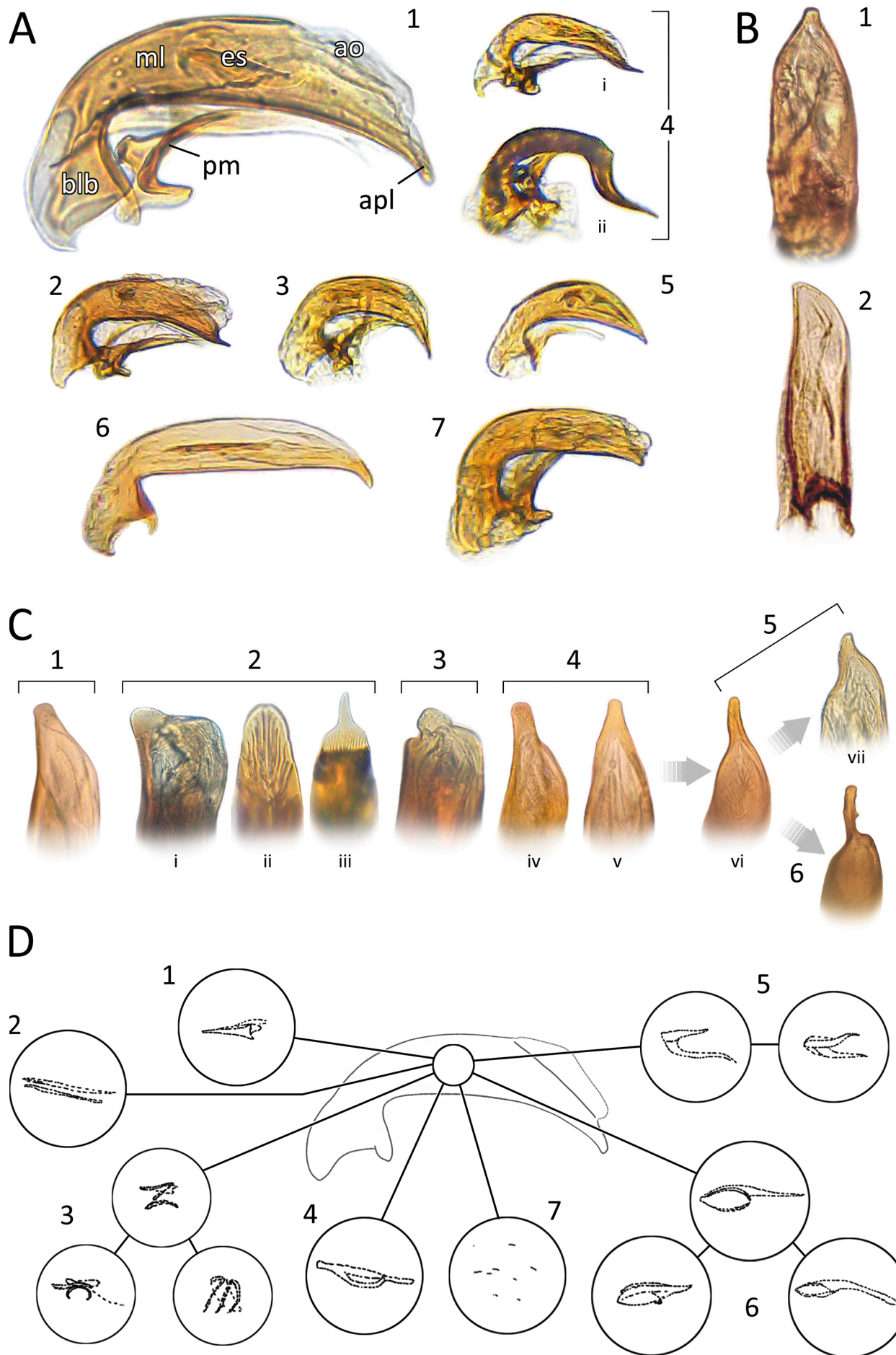
**Morph Ae–3.** Falciform, sickle shaped (Fig. 6A–3), with a highly arched middle lobe, in a smooth transition with the basal bulb (e.g. *M. bullaquiensis*). The apical region of this type is bent downwards.

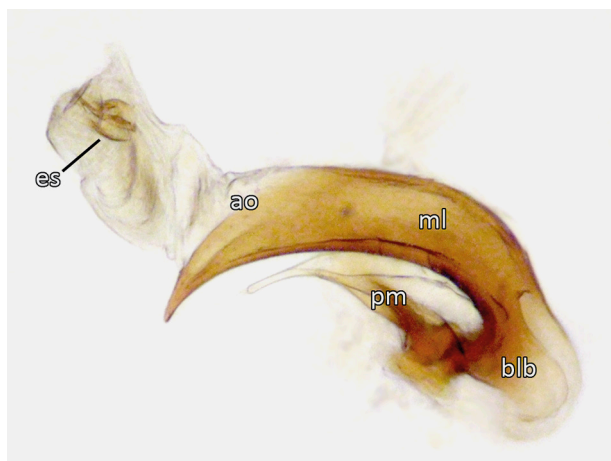
**Morph Ae–4.** Recurved, with a smooth transition between basal bulb and the base of middle lobe, arched in the distal third (Fig. 6A–4). The apical region is curved forward again. This morphology appears in different ways, from soft curves (e.g. *M. santchsi*, Fig. 6A–4i) to extreme shapes [e.g. *M. gomezi* (Zaballos, 1991) or *M. hiecki* (Zaballos & Farinós, 1995), Fig. 6A–4ii].

Some species [e.g. *M. monasticus* (Zaballos & Wrase, 1998)] have a characteristic median lobe, intermediate between morphs Ae–1, Ae–2: stout and high, with the base of middle lobe curved (**morph Ae–5**, Fig. 6A–5). *Typhlocharis fancelloi* Magrini, 2000 possess a unique shape, resembling morph Ae–1 but with a very elongated middle lobe (**morph Ae–6**, Fig. 6A–6). The median lobe of *L. paulinoi* (Serrano & Aguiar, 2006) is also atypical (**morph Ae–7**, Fig. 6A–7), with a basal bulb strongly bent, almost in a straight angle respect to middle lobe, and apical region with two small lateral crests.

In regards of the varied shape of the apical lamina (Fig. 6C), it is possible to recognize the following morphs:

→ **Fig. 6.** Aedeagus in Typhlocharina. **A:** Diversity of aedeagus morphotypes (seen in lateral view); (1) **morph Ae–1**, typically falciform aedeagus (represented by *Typhlocharis quarta*); (2) **morph Ae–2**, robust and gently curved falciform aedeagus, with distal crests (represented by *Lusotyphlus carinatus*); (3) **morph Ae–3**, strongly curved, sickle shaped aedeagus (represented by *Microcharidius bullaquiensis*); (4) **morph Ae–4**, recurved aedeagus, expressed as (i) soft shapes (represented by *M. santchsi*) or (ii) extreme shapes (represented by *M. gomezi*); (5) **morph Ae–5**, falciform aedeagus, stout and high, slightly curved (represented by *M. peregrinus*); (6–7) autapomorphic shapes: **morph Ae–6**, *T. fancelloi*, **morph Ae–7**, *L. paulinoi*. **B:** Dorsal view, (1) straight middle lobe (represented by *M. monasticus*), (2) deviated middle lobe (represented by *T. armata*). **C:** Diversity of the apical lamina, (1) **morph Al–1**, subtriangular, short and blunt in the extreme (represented by *T. prima*); (2) wide and rounded, (i) short and tilted as in *L. carinatus*, (ii) long, straight and blunt as in *M. gomezi* or (iii) similar to ii but with an acuminate projection, exclusive of *M. hiecki*; (3) round, constricted in the base, with two lateral crests, exclusive of *L. paulinoi*; (4) projected, long and blunt in the extreme, (iv) subparallel, as in *M. toletanus* or (v) subtriangular, as in *M. elenae*; (5) sinuous, (vii) long and gently curved, exclusive of *Microcharidius lencinai* sp.n. or (viii) short and strongly curved, as in *M. atienzai*; (6) projected, thin and warped, exclusive of *M. andujari* sp.n. **D:** Diversity of the endophallic sclerites, (1) **morph ES–1**, thick, subtriangular spines; (2) long and slender rods; (3) complex array of short irregular rods; (4) thick branched rods, with a lateral projection curved upwards; (5) pieces in shape of “τ” or “V”; (6) pieces in shape of “bicycle seat”, dilated posteriorly with variable anterior projection; (7) diffuse sclerites. — **Abbreviations:** ao – apical opening or phallotreme; apl – apical lamina; blb – basal bulb; es – endophallic sclerites; ml – middle lobe; pm – parameres.





**Fig. 7.** Aedeagus and parameres of *Microcharidius* cf. *elenae* (in left lateral view) showing partially everted (from apical opening) endophallic membranes and sclerites. — **Abbreviations:** ao – apical opening or phallotreme; apl – apical lamina; blb – basal bulb; es – endophallic sclerites; ml – middle lobe; pm – parameres.

**Morph Al–1.** Subtriangular lamella, short or moderately projected, not widened, blunt distally and usually tilted (e.g. *T. prima* Pérez-González & Zaballos, 2013, Fig. 6C–1), quite narrow in some species (e.g. *M. monasticus*). It is the most widespread shape.

**Morph Al–2.** Round and wide lamella, blunt in the extreme (Fig. 6C–2). It could be short, tilted, and accompanied by a lateral crest (e.g. *L. carinatus*, Fig. 6C–2i) or very projected and straight (e.g. *M. gomezi*, Fig. 6C–2ii).

**Morph Al–3.** Round and wide lamella, very projected, and straight, expanded in an acuminate curved projection in the middle. Exclusive of *M. hieki* (Fig. 6C–2iii).

**Morph Al–4.** Round lamella, constricted at the base, with two lateral crests. Exclusive of *L. paulinoi* (Fig. 6C–3).

**Morph Al–5.** Very projected lamella, straight, long, and narrow, blunt in the extreme (Fig. 6C–4). Quite parallel-sided [e.g. *M. toletanus* (Lencina & Andújar, 2010), Fig. 6C–4iv] or subtriangular (e.g. *M. elenae*, Fig. 6C–4v), very narrow in some species (e.g. *M. belenae*).

**Morph Al–6.** Sinuous lamella (Fig. 6C–5). Only in *M. atienzai* (Fig. 6C–5vii), *M. serranoi* sp.n., and *M. aguiari* sp.n. and less marked in *M. lencinai* sp.n. (Fig. 6C–5vi).

**Morph Al–7.** Projected lamella, very thin, and warped (Fig. 6C–6). Similar to the sinuous lamellae, but more extreme. Only known in *M. andujari* sp.n.

The inner part of the phallus – **endophallus** – is a tube bearing a series of membranous structures and sclerotized pieces – **endophallic sclerites** – that expand and project out during copulation (JEANNEL 1955). In other Carabidae is known that the internal membranes are everted in a three dimensional design of taxonomic utility (e.g. RUIZ-TAPIADOR & ANICHTCHENKO 2007; JANOVSKA et al. 2013), but the minuscule size and fragility of the aedeagus in Typhlocharina does not allow applying the methods of BERLOV (1992) or JANOVSKA et al. (2013) to study the

structure of the membranes. However, two or three of the observed specimens naturally had these membranes partially everted (Fig. 7), suggesting these structures act in the same way as in other Carabidae. In resting position, inverted in the median lobe of aedeagus, these sclerites are seen as irregularly shaped pieces, highly variable between species. It is possible to recognize a series of repeated patterns:

**Morph ES–1.** Thick, subtriangular spines (Fig. 6D–1) with the thicker end pointing to the apical region (e.g. *L. carinatus*).

**Morph ES–2.** Long and slender rod-like pieces, more or less straight (Fig. 6D–2) (e.g. *T. armata* Coiffait, 1969).

**Morph 3.** Complex array of short irregular rods, shaped as “M”, “Z” or similar (Fig. 6D–3) [e.g. *M. peregrinus* (Zaballos & Wrase, 1998)].

**Morph ES–4.** Thick branched rods, with a lateral projection curved upwards (Fig. 6D–4). Typical of the species of **baetica group** (*sensu* ZABALLOS & BANDA 2001), (e.g. *T. secunda* Pérez-González & Zaballos, 2013).

**Morph ES–5.** Pieces in shape of “τ” or “V” (Fig. 6D–5) (e.g. *M. gomezi*).

**Morph ES–6.** Pieces in shape of “bicycle seat” (Fig. 6D–6), dilated posteriorly and extended anteriorly in a thin projection. This model has some variations: the anterior projection can be absent or very short (e.g. *M. belenae*), moderately long and straight (e.g. *M. carmenae*) or sinuous (e.g. *M. estrellae*), or very long and well developed (e.g. *M. portilloi*).

**Morph ES–7.** Diffuse sclerites, hardly visible as small, weak scarce scales (Fig. 6D–7). Exclusive of *T. pacensis* Zaballos & Jeanne, 1987.

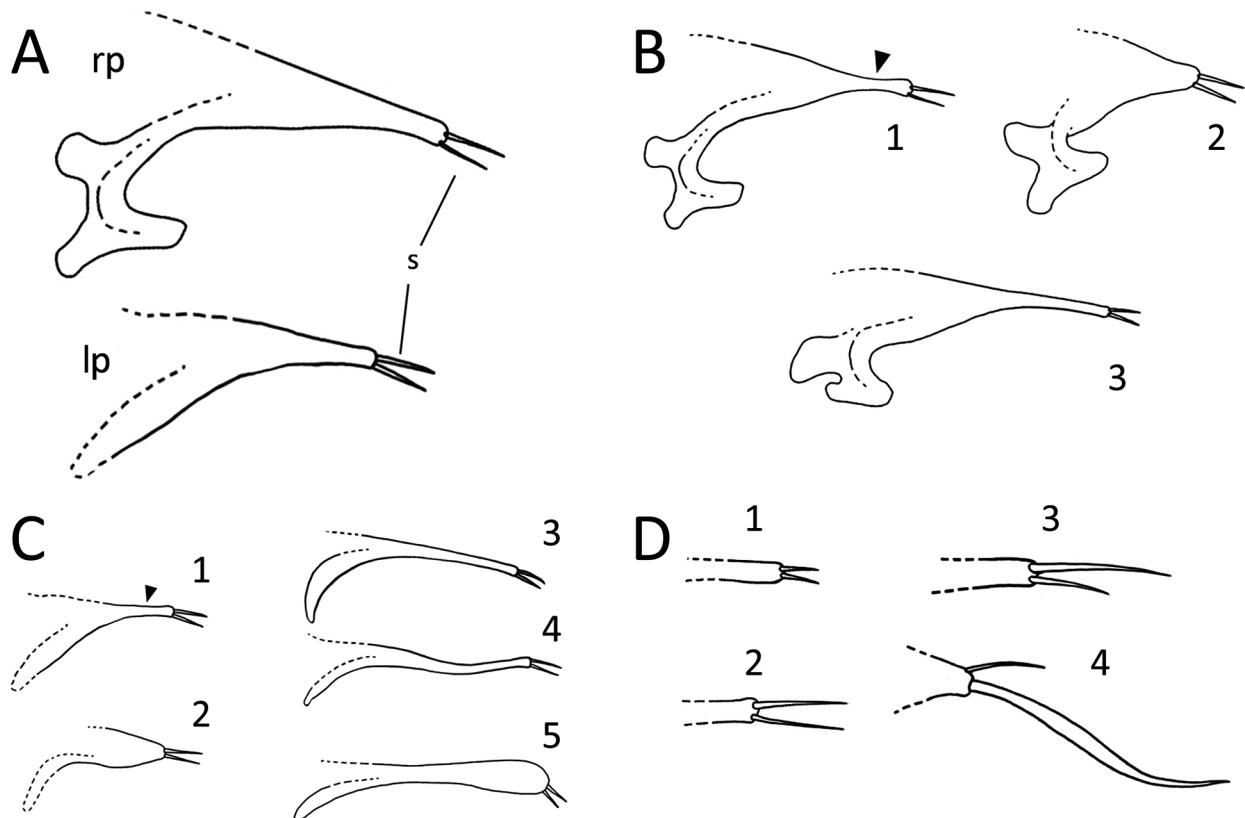
### 3.3.2. Parameres

The parameres are two small pieces located on each side of the base of the median lobe of aedeagus (Fig. 6A–1). Both parameres end in a more or less tubular distal extreme, with two distal setae.

The **right paramere** is broader proximally, with a general subtriangular shape (Fig. 8A, e.g. *T. silvanoides* Dieck, 1869). The distal end can be smoothly narrowed (Fig. 8B–1, e.g. *M. peregrinus*), short and robust (Fig. 8B–2, e.g. *T. tertia* Pérez-González & Zaballos, 2013) or very long (Fig. 8B–3, e.g. *M. santschii*).

The **left paramere** is smaller and narrower than the right paramere (Fig. 8A) and shows a higher diversity of shapes: the subtriangular shape is predominant (Fig. 8A, e.g. *T. silvanoides*), but it can be smoothly narrowed at the distal end (Fig. 8C–1, e.g. *M. peregrinus*), short and robust (Fig. 8C–2, e.g. *T. tertia*), very long and thin, either straight (Fig. 8C–3, e.g. *M. santschii*) or gently curved (Fig. 8C–4, e.g. *M. bazi*) or hyaline and long, club-shaped (Fig. 8C–5, e.g. *M. toletanus*).

The distal setae could be short, long or asymmetric (Fig. 8D). In *Lusotyphlus* they are very characteristic: the inferior seta is long and “saber-like”, while the superior seta is thin and much shorter (Fig. 8D–4).



**Fig. 8.** Diversity of parameres in Typhlocharina. **A:** General morphology in the majority of the species. **B:** Variability of the right paramere, (1) distally narrowed (black arrow); (2) short and stout; (3) long and thin. **C:** Variability of the left paramere, (1) distally narrowed (black arrow); (2) short and stout; (3) long and thin, straight; (4) long and thin, curved or sinuous; (5) long, club-shaped, weakly sclerotized hyaline aspect. **D:** Variability of the distal setae, (1) symmetric short setae; (2) symmetric mid-sized or long setae; (3) slightly asymmetric setae; (4) strongly asymmetric setae, with a very long, “saber-like” inferior seta. — **Abbreviations:** lp – left paramere; rp – right paramere; s – distal setae.

### 3.3.3. Ring sclerite

The **ring sclerite** surrounds the male genital complex. According to some authors, it corresponds to the abdominal sternum IX (e.g. MAGRINI 2014), but DEUVE (1988) interprets it as a result of the fusion of the antecostae of laterotergite IX. It is known that this structure shows interspecific variation in other genera of Anillini (e.g. SOKOLOV & KAVANAUGH 2014), but it has never been considered for Typhlocharina and, for the more than 65 species, it has been only described in *M. josabelae* (Ortuño & Gilgado, 2011) (as “apodemal ring”, ORTUÑO & GILGADO 2011), *M. amara*, *M. loebli*, *T. mendesi* Serrano & Aguiar, 2017, *T. coenobita* Pérez-González, Andújar, Lantero & Zaballos, 2018, *T. anachoreta* Pérez-González, Andújar, Lantero & Zaballos, 2018, *T. eremita* Pérez-González, Andújar, Lantero & Zaballos, 2018 (ZABALLOS et al. 2016; PÉREZ-GONZÁLEZ & ZABALLOS 2018; PÉREZ-GONZÁLEZ et al. 2018).

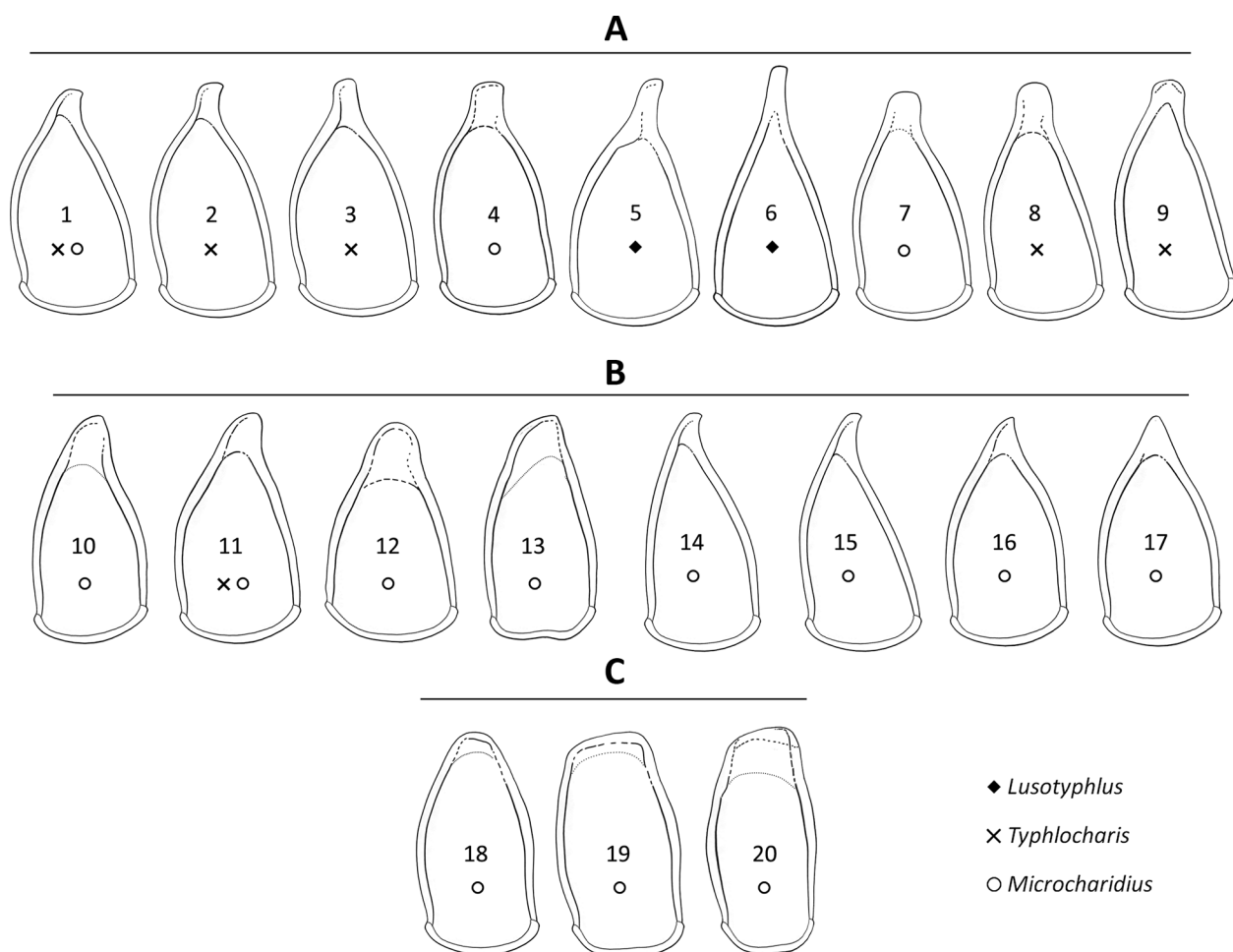
The study of this structure in all Typhlocharina has revealed a high morphological diversity (Fig. 9) and, in the majority of species, the distal end is prolonged in an expansion of variable shape: it could be well differentiated (Fig. 9A) or smoothly integrated in the body of the ring (Fig. 9B). In the first case, the expansion could be subtriangular, ended in a blunt, tilted point (Fig. 9A–1);

tongue-shaped or subrectangular (Fig. 9A–2,3,4); long and narrow, with a blunt point (Fig. 9A–5,6) or “spoon-shaped”, ending in a smooth rounded projection (Fig. 9A–7,8,9). In the second case, the projection can be wide (Fig. 9B–10,11,12,13); narrow (Fig. 9B–14,15), or small and triangular (Fig. 9B–16,17). In some species, the distal end is not or almost not projected, with round and wide apex (Fig. 9C–18,19,20). Frequently, the distal margin is curved backwards creating a “flap”.

## 4. Discussion

### 4.1. Morphological diversity and taxonomic implications of male genitalia

Male genital anatomy provides valuable systematic information in Typhlocharina. Although delicate to manipulate, the genitalia are easy to observe and provide diagnostic traits that allow a quick identification. The results show morphological variation in diverse features, and update and standardize the available information from a comparative perspective (Table 1, Fig. 10). It is the first time that the ring sclerite, the endophallic sclerites, and



**Fig. 9.** Diversity of ring sclerites in Typhlocharina. **A:** Upper margin projected, expansion well differentiated from ring body, (1) subtriangular, blunt and tilted; (2–4) “tongue-shaped” or subrectangular; (5–6) long and narrow, with a blunt point; (7–9) “spoon-shaped”, smoothly rounded. **B:** Upper margin projected, expansion smoothly integrated with the ring body, (10–13) wide projection; (14–15) narrow projection; (16–17) small triangular projection. **C:** (18–20) Upper margin not projected, distal end wide and round.

the apical lamina are detailed and described in all the species, yet the variation patterns in these cryptic, previously overlooked characters are consistent with the available phylogenetic information (Fig. 10).

But, to what extent does male genital anatomy reflect the phylogenetic relationships in Typhlocharina? The results show that the three genera, *Lusotyphlus*, *Typhlocharis*, and *Microcharidius*, have characteristic morphologies in the different structures of the male genitalia (Fig. 10) that allow making some diagnostic generalizations.

Morph Ae–1 is the most common model in the group and appears in the three genera (Table 1, Fig. 10), being the most similar to the generic carabid model described by JEANNEL (1955, 1963). Similar morphologies are found in other Anillini genera, like *Anillus* Jacquelin du Val, 1851, *Nothanillus* Jeannel, 1962, *Pelodiaetodes* Moore, 1980, *Geocharis* (JEANNEL 1963; SOKOLOV 2015; ZABALLOS 2005), and the phylogenetic reconstruction of this feature suggest this morph is probably plesiomorphic for Typhlocharina (PÉREZ-GONZÁLEZ et al. 2017).

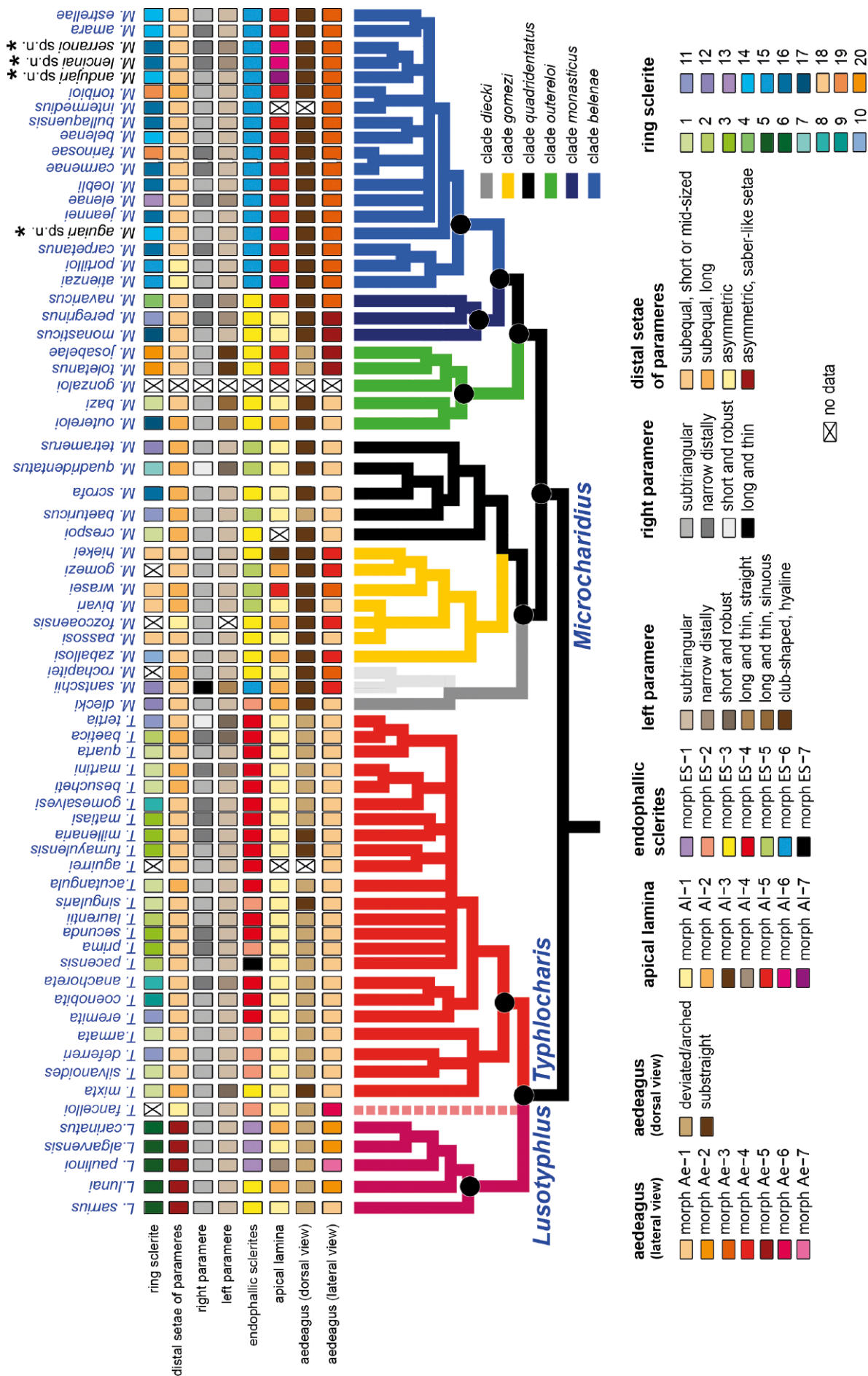
This morph is prevalent in *Typhlocharis*, characterized by generic falciform aedeagi, generally bent in dorsal view, with a short and blunt subtriangular apical

lamina (Fig. 6A–1, 6C–1; Fig. 10). The endophallic sclerites of *Typhlocharis* are arranged as long irregular rods or branched rod-like pieces with lateral projection curved upwards (Fig. 6D–2,4; Fig. 10). The parameres are subtriangular with two subequal setae in the distal ends and the most significant variations are seen in *T. tertia* and *T. mixta* Pérez-González & Zaballos, 2013, which show shorter and more robust parameres than any other species of the genus (Fig. 8B–1,2, 8D–1,2; Fig. 10).

In *Lusotyphlus*, morph Ae–2 is predominant, typically with robust, somewhat recurved middle lobes, and broad, “duckbill” like apical laminae (Fig. 6A–2, 6C–2; Fig. 10). The development of anterolateral crests is common and the main pattern of the endophallic sclerites are subtriangular irregular rods (Fig. 6D–1; Fig. 10). Long, saber-like setae in the parameres are characteristic, usually with an exaggerated asymmetry (Fig. 8D–4; Fig. 10). It seems likely that archetypal morph Ae–2 shape derive from a more basic “morph Ae–1” aedeagus, as suggested by the moderate shape of aedeagus in *L. sarricus* (Serrano & Aguiar, 2001).

*Microcharidius* displays the major diversity in aedeagus shape and each different clade within the genus pre-





**Fig. 10.** Simplified phylogenetic relationships of Typhlocharina (modified from the total evidence Bayesian tree in Pérez-González et al. 2017, undescribed taxa not included) showing the diversity of male genital features discussed in the text. — **Symbols:** \* – the four new species; black circles on nodes – clade support of 0.95–1.

**Table 1.** Variability of male genitalia features in Typhlocharina. Models for shape of middle lobe of aedeagus, apical lamina and endophallic sclerites named as in Fig. 6 and main text; ring sclerite morphologies numbered as in Fig. 9. Species of *Microcharitidius* in bold defined by PÉREZ-GONZÁLEZ et al. (2017). \* Indicates autapomorphic features.

Species	Shape of middle lobe of aedeagus (lateral view): morphs Ae	Shape of middle lobe of aedeagus (dorsal view)	Apical lamina: morphs Al	Endophallic sclerites: morphs ES	Left paramere	Right paramere	Ring sclerite
<b><i>Lusotyphlus</i></b>							
1. <i>L. algarvensis</i>	morph 2	deviated	morph 1 (subtriangular, short and blunt)	morph 1 (thick subtriangular spine)	subtriangular, large, saber-like inferior seta	subtriangular, thick and slightly asymmetric setae	5
2. <i>L. sarrius</i>	morph 1	slightly deviated	morph 1 (subtriangular, short and blunt)	morph 3 (short irregular rod)	subtriangular, large, saber-like inferior seta	subtriangular, large, saber-like inferior seta	5
3. <i>L. lunai</i>	morph 2	deviated	morph 2 (wide and round, with a lateral crest)	morph 3 (short rods, more or less "E-shaped")	subtriangular, two large saber-like setae, inferior longer	subtriangular, two subequal large setae	5
4. <i>L. carinatus</i>	morph 2	deviated	morph 2 (wide and round, tilted, with a lateral crest)	morph 1 (thick subtriangular spine)	subtriangular, large, saber-like inferior seta	subtriangular, large, saber-like inferior seta	6
5. <i>L. paulinoi</i>	morph 7*	deviated, smoothly sinuous	morph 4 (round, strangled in the base, with two lateral crests)	morph 1 (thick subtriangular spine)	subtriangular, large, saber-like inferior seta	subtriangular, large asymmetric setae	5
<b><i>Typhlocharis</i></b>							
6. <i>T. silvanoides</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 2 (irregular long and slender rod-like pieces)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	1
7. <i>T. deferri</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 2 (irregular slender rod)	subtriangular, short subequal setae	subtriangular, short subequal setae	11
8. <i>T. armata</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 2 (long and slender rod-like pieces)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	1
9. <i>T. martini</i>	morph 1	strongly deviated	morph 1 (subtriangular, short and blunt)	morph 4 (thick, branched rod-like pieces with lateral projection curved upwards)	subtriangular, narrow distally, long subequal setae	subtriangular, narrow distally, long subequal setae	1
10. <i>T. hesucheti</i>	morph 1	deviated, smoothly sinuous	morph 1 (subtriangular, short and blunt)	morph 4 (branched rod-like pieces with lateral projection)	subtriangular, mid-sized subequal setae	subtriangular, long subequal setae	1
11. <i>T. laurentii</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (thick, branched rod-like pieces with lateral projection curved upwards)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	2
12. <i>T. gomesalvesi</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (branched rod-like pieces with lateral projection curved upwards)	subtriangular, thin, mid-sized subequal setae	subtriangular, thin and narrow distally, mid-sized subequal setae	8
13. <i>T. singularis</i>	morph 1	substraight	morph 1 (subtriangular, short, blunt and narrow)	morph 2 (slender rod-like pieces in "A" shape)	subtriangular, short subequal setae	subtriangular, short subequal setae	1
14. <i>T. quarta</i>	morph 1	deviated, smoothly sinuous	morph 1 (subtriangular, short and blunt)	morph 4 (thick, "C-shaped" branched rod-like pieces with lateral projection curved upwards)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	1
15. <i>T. baetica</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (thick, long rod-like pieces with lateral projection)	subtriangular, robust, mid-sized subequal setae	subtriangular, slightly narrow distally, long subequal setae	2
16. <i>T. tertia</i>	morph 1, robust	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (thick, branched rod-like pieces with lateral projection curved upwards)	subtriangular short and robust, mid-sized subequal setae	subtriangular short and robust, mid-sized subequal setae	11
17. <i>T. aguirrei</i>	morph 1	no data	no data	morph 4 (branched rod-like pieces with lateral projection curved upwards)	subtriangular long and thin, short subequal setae	subtriangular long and thin, mid-sized subequal setae	no data
18. <i>T. matisi</i>	morph 1	arched / deviated	morph 1 (subtriangular, short and blunt)	morph 4 (thick, branched rod-like pieces with lateral projection curved upwards)	subtriangular, mid-sized subequal setae	subtriangular, subtly narrowed distally, mid-sized subequal setae	3
19. <i>T. mixta</i>	morph 1	substraight	morph 1 (subtriangular, short and blunt)	morph 3 (irregular, "plough-shaped" piece)	subtriangular short and robust, long subequal setae	subtriangular robust, long slightly asymmetric setae	1

Table 1 continued.

Species	Shape of middle lobe of aedeagus (lateral view): morphs Ae	Shape of middle lobe of aedeagus (dorsal view)	Apical lamina: morphs Al	Endophallic sclerites: morphs ES	Left paramere	Right paramere	Ring sclerite
20. <i>T. acutangula</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (thick, branched rod-like pieces with lateral projection curved upwards)	subtriangular, long subequal setae	subtriangular, long subequal setae	1
21. <i>T. secunda</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (thick, branched rod-like pieces with lateral projection curved upwards)	subtriangular and thin, mid-sized subequal setae	subtriangular, narrowed distally, mid-sized subequal setae	3
22. <i>T. pacensis</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 7 (diffuse sclerites*)	subtriangular and thin, short subequal setae	subtriangular, mid-sized subequal setae	2
23. <i>T. fumayulensis</i>	morph 1	substraight	morph 1 (subtriangular, short, blunt and narrow)	morph 4 (branched rod-like pieces with lateral projection curved upwards)	subtriangular and thin, short subequal setae	subtriangular long and thin, mid-sized subequal setae	3
24. <i>T. millenania</i>	morph 1	substraight / slightly arched	morph 1 (subtriangular, short and blunt)	morph 4 (branched rod-like pieces with lateral projection curved upwards)	subtriangular, mid-sized subequal setae	subtriangular subtly narrowed distally, mid-sized subequal setae	3
25. <i>T. prima</i>	morph 1	deviated, smoothly sinuous	morph 1 (subtriangular, short and blunt)	morph 2 (thick rod-like piece)	subtriangular, mid-sized subequal setae	subtriangular subtly narrowed distally, mid-sized subequal setae	3
26. <i>T. mendesi</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (branched rod-like pieces with lateral projection curved upwards)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	8
27. <i>T. coenobita</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (branched rod-like pieces with lateral projection curved upwards)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	9
28. <i>T. eremita</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (branched rod-like pieces with lateral projection)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	11
29. <i>T. anachoreta</i>	morph 1	deviated	morph 1 (subtriangular, short and blunt)	morph 4 (branched rod-like pieces with lateral projection curved upwards)	subtriangular narrowed distally, mid-sized subequal setae	subtriangular narrowed distally, mid-sized subequal setae	8
30. <i>T. fancelloi</i>	morph 6*	deviated	morph 1 (subtriangular, slightly wide, short and blunt)	morph 2 (long and slender rod-like pieces)	subtriangular, long asymmetric setae	subtriangular, long subequal setae	no data
<b><i>Microcharidius</i></b>							
31. <i>M. diecki</i>	morph 1, straight base	straight	morph 2 (wide and round)	morph 2 (long and slender rod-like pieces)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	12
32. <i>M. sanschii</i>	morph 4, soft curve	substraight / arched	morph 2 (wide and round)	morph 6 (irregular "bicycle-seat" shape)	subtriangular very long and thin, short subequal setae	subtriangular very long and thin, short subequal setae	12
33. <i>M. rochapitei</i>	morph 3	substraight / slightly arched	morph 1 (subtriangular, narrow)	morph 3 (irregular, more or less "C-shaped" pieces)	subtriangular, long subequal setae	subtriangular, long subequal setae	no data
34. <i>M. outerelei</i>	morph 1	straight	morph 2 (wide and round)	morph 3 (complex array of short irregular rods, with a "v" shaped piece)	subtriangular, long and thin, slightly asymmetric setae	subtriangular, long subequal setae	17
35. <i>M. bazi</i>	morph 1	substraight / slightly sinuous	morph 1 (subtriangular, short and blunt)	morph 3 (complex array of short irregular rods, with a "v" shaped piece)	subtriangular, long, thin and sinuously curved, mid-sized subequal setae	subtriangular, mid-sized subequal setae	1
36. <i>M. gonzaioi</i>	no data	no data	no data	no data	no data	no data	no data
37. <i>M. toletanus</i>	morph 5	deviated, sinuous	morph 5 (projected, "tongue shaped" and blunt)	morph 3 (complex array of short irregular rods, with a "v" shaped piece)	club-shaped and hyaline, short subequal setae	subtriangular, mid-sized subequal setae	20
38. <i>M. josabetae</i>	morph 5	deviated, smoothly sinuous	morph 5 (projected, "tongue shaped" and blunt)	morph 3 (complex array of short irregular rods, with a "v" shaped piece)	club-shaped and hyaline, short subequal setae	subtriangular, mid-sized subequal setae	20
39. <i>M. monasticus</i>	morph 5	straight	morph 1 (subtriangular, short, narrow and blunt, "bottleneck shape")	morph 3 (complex array of short "Z-shaped" irregular rods)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	17

Table 1 continued.

Species	Shape of middle lobe of aedeagus (lateral view): morphs Ae	Shape of middle lobe of aedeagus (dorsal view)	Apical lamina: morphs Al	Endophallic sclerites: morphs ES	Left paramere	Right paramere	Ring sclerite
40. <i>M. peregrinus</i>	morph 5	straight	morph 1 (subtriangular, short, narrow and blunt, "bottleneck shape")	morph 3 (complex array of short "Z-shaped" irregular rods)	subtriangular, strongly narrowed distally, mid-sized subequal setae	subtriangular, strongly narrowed distally, mid-sized subequal setae	11
41. <i>M. navaricus</i>	morph 3	substraight	morph 5 (subtriangular)	morph 3 (complex array of short "M-shaped" irregular rods)	subtriangular, thin and narrowed distally, mid-sized subequal setae	subtriangular, thin and narrowed distally, mid-sized subequal setae	4
42. <i>M. gonezi</i>	morph 4, extreme curve	straight	morph 2 (long, wide and round)	morph 5 (irregular "x-shaped" piece)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	no data
43. <i>M. wrasei</i>	morph 3	straight	morph 5 (subtriangular)	morph 5 (irregular "x-shaped" piece)	subtriangular and slender, long subequal setae	subtriangular and slender, long subequal setae	18
44. <i>M. hiekei</i>	morph 4, extreme curve	substraight, smoothly sinuous	morph 3* (long, wide and round, with a medial acuminate projection)	morph 3 (complex array of short irregular pieces)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	18
45. <i>M. passosi</i>	morph 1	straight	morph 1 (subtriangular, short, narrow and blunt, "bottleneck shape")	morph 3 (complex array of short "M-shaped" irregular rods)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	18
46. <i>M. fozzoaensis</i>	morph 4, soft curve	substraight	morph 2 (wide and round)	morph 3 (complex array of short irregular pieces)	no data	subtriangular, mid-sized slightly asymmetric setae	no data
47. <i>M. bivari</i>	morph 1	substraight	morph 1 (subtriangular, slightly wide, short and blunt)	morph 5 (irregular "x-shaped" piece)	subtriangular, long subequal setae	subtriangular, long subequal setae	18
48. <i>M. zaballosi</i>	morph 4, soft curve	straight	morph 2 (wide and round)	morph 3 (complex array of short irregular pieces)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	10
49. <i>M. quadridentatus</i>	morph 1, taller distally	straight	morph 1 (subtriangular, "arrowhead" shape)	morph 5 (irregular, more or less "α-shaped" pieces)	subtriangular, short and stout, long subequal setae	subtriangular and stout, long subequal setae	7
50. <i>M. baeturicus</i>	morph 1, straight base and taller distally	arched	morph 1 (subtriangular, short and blunt)	morph 5 (stout, irregular, "scissor-shaped" pieces)	subtriangular, long subequal setae	subtriangular, long subequal setae	11
51. <i>M. crespai</i>	morph 1	straight	no data	morph 3 (two irregular slender rods, more or less "V" shaped)	subtriangular, long subequal setae	subtriangular, long subequal setae	1
52. <i>M. scrofa</i>	morph 1, straight base and taller distally	substraight	morph 1 (subtriangular, wide, short and blunt)	morph 3 (irregular slender rods, more or less "r" shaped)	subtriangular, long subequal setae	subtriangular, long subequal setae	16
53. <i>M. tetramerus</i>	morph 1, straight base and taller distally	substraight / slightly arched	morph 1 (subtriangular, wide, short and blunt)	morph 5 (two irregular slender rod, more or less "V" shaped)	subtriangular, long subequal setae	subtriangular, long subequal setae	12
54. <i>M. belenae</i>	morph 3	straight	morph 5 (subtriangular, very narrow and projected)	morph 6 ("bicycle seat" shaped pieces with very short anterior projection)	subtriangular, short subequal setae	subtriangular, short subequal setae	14
55. <i>M. portilloi</i>	morph 3	straight	morph 5 (subtriangular)	morph 6 ("bicycle seat" shaped pieces with very long and developed anterior projection)	subtriangular and stout, mid-sized slightly asymmetric setae	subtriangular, mid-sized slightly asymmetric setae	15
56. <i>M. carmenae</i>	morph 3	straight	morph 5 (subtriangular)	morph 6 ("bicycle seat" shaped pieces with long anterior projection)	subtriangular, mid-sized subequal setae	subtriangular and narrowed distally, mid-sized subequal setae	16
57. <i>M. farinosae</i>	morph 3	substraight	morph 5 (subtriangular)	morph 6 ("bicycle seat" shaped pieces with long anterior projection)	subtriangular, short subequal setae	subtriangular and narrowed distally, short subequal setae	19

Table 1 continued.

Species	Shape of middle lobe of aedeagus (lateral view): morphs Ae	Shape of middle lobe of aedeagus (dorsal view)	Apical lamina: morphs Al	Endophallic sclerites: morphs ES	Left paramere	Right paramere	Ring sclerite
58. <i>M. toribioi</i>	morph 3	straight	morph 5 (subtriangular)	morph 6 ("bicycle seat" shaped pieces with very short anterior projection)	subtriangular, long subequal setae	subtriangular, long subequal setae	19
59. <i>M. estrellae</i>	morph 3	straight	morph 5 (subtriangular, slightly broad)	morph 6 ("bicycle seat" shaped pieces with long and sinuous anterior projection)	subtriangular, mid-sized asymmetric setae	subtriangular, mid-sized asymmetric setae	14
60. <i>M. jeannei</i>	morph 3	straight	morph 5 (subtriangular)	morph 6 ("bicycle seat, U-shaped" slender rods)	subtriangular, short subequal setae	subtriangular, short subequal setae	16
61. <i>M. intermedius</i>	morph 3	no data	no data	morph 6 ("bicycle seat" shaped pieces without anterior projection)	subtriangular, short subequal setae	subtriangular, short subequal setae	16
62. <i>M. atenzai</i>	morph 3, very long	straight	morph 6 (sinuous)	morph 6 ("bicycle seat" shaped pieces with very long and developed anterior projection)	subtriangular and slender, mid-sized asymmetric setae	subtriangular and slender, mid-sized asymmetric setae	15
63. <i>M. bullaquensis</i>	morph 3	straight	morph 5 (subtriangular)	morph 6 ("bicycle seat" shaped pieces with stout anterior projection)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	16
64. <i>M. elenae</i>	morph 3	straight	morph 5 (subtriangular)	morph 6 ("bicycle seat" shaped pieces with short anterior projection)	subtriangular and narrowed distally, mid-sized subequal setae	subtriangular and narrowed distally, mid-sized subequal setae	13
65. <i>M. carpetanus</i>	morph 3, soft curve	straight	morph 5 (subtriangular)	morph 6 ("bicycle seat" shaped pieces with very short anterior projection)	subtriangular and thin, mid-sized subequal setae	subtriangular and slightly narrowed, mid-sized subequal setae	16
66. <i>M. amara</i>	morph 3	straight	morph 5 (subtriangular, slightly broad)	morph 6 ("bicycle seat" shaped pieces with long anterior projection)	subtriangular, mid-sized subequal setae	subtriangular and slightly narrowed distally, mid-sized subequal setae	14
67. <i>M. loebli</i>	morph 3	straight	morph 5 (subtriangular, slightly broad)	morph 6 ("bicycle seat" shaped pieces with gently curved anterior projection)	subtriangular, mid-sized asymmetric setae	subtriangular, mid-sized subequal setae	16
68. <i>M. andujari</i> sp. n.	morph 3	straight	morph 7* (projected, thin and warped)	morph 6 ("bicycle seat" shaped pieces with very short anterior projection)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	14
69. <i>M. lenchai</i> sp. n.	morph 3	straight	morph 6 (projected, thin and sinuously curved)	morph 6 ("bicycle seat" shaped pieces with short anterior projection)	subtriangular and slender distally, mid-sized subequal setae	subtriangular and slender, mid-sized subequal setae	16
70. <i>M. serranoi</i> sp. n.	morph 3	substraight	morph 6 (sinuous)	morph 6 ("bicycle seat" shaped pieces with very short anterior projection)	subtriangular and slightly narrowed, mid-sized subequal setae	subtriangular and narrowed distally, mid-sized subequal setae	16
71. <i>M. aguari</i> sp. n.	morph 3	substraight, smoothly sinuous	morph 6 (sinuous)	morph 6 ("bicycle seat" shaped pieces with gently curved anterior projection)	subtriangular, mid-sized subequal setae	subtriangular, mid-sized subequal setae	14



sents recognizable features (PÉREZ-GONZÁLEZ et al. 2017, see Table 1 and Fig. 10). Morph Ae-1 predominates in clade *quadridentatus*, but also appears in clades *diecki*, *gomezi*, and *outereloi* (Fig. 10). According to molecular and total evidence phylogenies (ANDÚJAR et al. 2017; PÉREZ-GONZÁLEZ et al. 2017) these clades are basal within *Microcharidius*, reinforcing the hypothesis of “morph Ae-1” as the non-derived state for the shape of median lobe in Typhlocharina. Wide, rounded apical laminae are common within clades *diecki*, *gomezi*, and *outereloi* (Fig. 6C-2ii; Fig. 10), clades that share other affinities like the presence of morph Ae-4, recurved aedeagi (e.g. in *M. santschii*), in a similar fashion to that of genus *Lusotyphlus*. This pattern is especially common within clade *gomezi* and taken to the extreme in *M. gomezi* and *M. hiecki* (Fig. 6A-4; Fig. 10). Clade *outereloi* does not show a recurved aedeagus but is prone to the elongation of the left paramere, exaggerated in the club-shaped hyaline paramere of *M. toletanus* and *M. josabelae* (Fig. 8C-5; Fig. 10). *Microcharidius santschii* shows elongation in both parameres. Strongly curved median lobe of aedeagi, (morph Ae-3, straight in dorsal view and commonly with narrow subtriangular apical laminae), are characteristic of clade *belenae*, as well as the “bicycle seat” pattern of the endophallic sclerites (Fig. 6A-3, 6C-4v, 6D-6; Fig. 10).

Interpretation of the patterns of the endophallic sclerites is difficult because they are internal structures that most of the time are folded inside the phallus, buried in layers of soft tissue and membranes, and include pieces with different degrees of sclerotization. This complicates the adequate observation and comparison of the pieces. Additional difficulties imply that the position of the sclerites may be slightly altered in different stages of the adult life (e.g. before/after being unfolded during copulation) and the three-dimensional aspect of these pieces remains unknown. However, the results show that the observed patterns of the sclerites represent a good taxonomic tool for clade-level recognition (Fig. 10) and the overall patterns suggest that this is one of the male genital structures with best phylogenetic signal (PÉREZ-GONZÁLEZ et al. 2017).

Regarding the ring sclerites, the variation found in Typhlocharina can be considered quite conservative in comparison with the intrageneric variation reported for other Anillini, such as *Prioniomus* Jeannel, 1937; *Iason* Giachino & Vailati, 2011; *Winklerites* Jeannel, 1937; *Pelodiaetodes*; *Zapotecanillus* Sokolov, 2013; *Geocharidius* Jeannel, 1963 or *Anillinus*, among others (GIACHINO & VAILATI 2011, 2012; SOKOLOV et al. 2004; SOKOLOV & KAVANAUGH 2014; SOKOLOV 2013, 2015). However, there are certain differences in this feature associated with the three genera. The basic shape is an arched, roughly subtriangular structure with a variable distal end. In *Lusotyphlus*, the ring sclerite is more slender, high, and triangular, with a very projected and narrow distal end (Fig. 9A-5,6). In *Typhlocharis*, the distal end is, in general, well differentiated from the body of the sclerite and the expansion is blunt, more or less subtriangular,

subsquare or rounded (Fig. 9A-1,2,3,8,9; Fig. 9B-11). As with the shape of aedeagus, the greatest diversity is found within *Microcharidius* (Fig. 9A-1,4,7; Fig. 9B-10,11,12,13,14,15,16,17,18,19,20), with some morphologies overlapping that of *Typhlocharis*, but in general, characterized by a much smoother transition between the distal expansion and the body of the ring. Acute triangular expansions predominate, especially in clade *belenae*. Wide rounded rings without projected expansions are only known in *Microcharidius* (Fig. 9C-18,19, 20).

The observed diversity in male genitalia does not have any obvious morphological correlates with changes in the female genitalia (PÉREZ-GONZÁLEZ & ZABALLOS 2012, 2013b; PÉREZ-GONZÁLEZ et al. 2017). This implies that the sclerotized structures of female genitalia (such as gonocoxites), do not act as “counterpart” to aedeagus shape in a “lock-and-key mechanism” fashion. In Coleoptera, the aedeagus does not penetrate far in the female genitalia during copulation, but instead the inner membranes evaginate and the endophallic sclerites are unfolded inside the female genital tract, playing an important role in the attachment of the male genitalia (JEANNEL 1955; SIMMONS 2014). A “lock-and-key” mechanism has been suggested between male endophallus and female bursa copulatrix (e.g. DUFFOR 1844), but there are some evidences this is not always the case in some Carabidae (JANOVSKA et al. 2013). In Typhlocharina the bursa copulatrix is not described and this potential correlation between the soft membranous parts of the genitalia remains an open question.

However, it is interesting that “morph 5” median lobes (curved, stout, and higher near the distal region) appear precisely in *M. monasticus*, *M. peregrinus* (clade *monasticus*), *M. toletanus*, and *M. josabelae* (clade *outereloi*). These four species are the only members of Typhlocharina with unguiform gonocoxites, but are not closely related (PÉREZ-GONZÁLEZ et al. 2017). The similarities in aedeagus shape suggest certain degree of convergence that might parallel the presence of unguiform gonocoxites. The only other Typhlocharina where gonocoxites are not tubular are part of clade *quadridentatus*, a group of five species that covers a morphological spectrum between unguiform-like, club-shaped, and tubular gonocoxites (PÉREZ-GONZÁLEZ & ZABALLOS 2013b). This raises a question: What type of aedeagi do they have?

With the exception of *M. crespoi*, all members of clade *quadridentatus* show the same trend to distally higher middle lobes (Table 1), supporting the idea of a certain relation between distally high aedeagus-unguiform gonocoxites within Typhlocharina. However, if we compare with other Anillini, we find a high heterogeneity in aedeagus shape, yet all other genera have unguiform gonocoxites (with exception of *Anillotarsus* Mateu, 1980), so there is not a clear pattern. Similar distally high morphologies occur, among others, in *Geocharis*, *Geocharidius* or *Winklerites* (ZABALLOS 2005; SOKOLOV & KAVANAUGH 2014; GIACHINO & VAILATI 2011) but are not seen in other genera that could feature falciform “morph 1” like shapes, like *Anillus* Jacquelin du Val, 1851 or *Prioniomus* (MAGRINI 2014; GIACHINO & VAILATI 2011), or

varied morphologies unlike any observed in Typhlocharina, like *Anillinus*, *Pseudanillinus* Bedel, 1896 or *Microtyphlus* Linder, 1863 (SOKOLOV et al. 2004; ZABALLOS & BANDA 2000; ORTUÑO & SENDRA 2011).

Taking all this information in account, it seems the different features of male genitalia are very efficient for genus or clade-level recognition. But how about species-specific recognition? In general, within a lineage, the species show very similar aedeagi, lacking enough differences for reliable species-level discrimination. For example, a large portion of species within *Typhlocharis* (including the *baetica* group *sensu* Zaballos & Ruiz-Tapiador, 1997) share morph 1 median lobe of aedeagi with the same type of subtriangular apical laminae, endophallic sclerites (branched with a projection curved upwards), and parameres (Table 1, Fig. 10). The current phylogenies suggest these species are part of a single clade (ANDÚJAR et al. 2017; PÉREZ-GONZÁLEZ et al. 2017) and this morphology readily identifies the clade as a whole, but it is not efficient to discriminate between the species in the absence of other non-genital characters.

Unequivocal species-level autapomorphies in male genitalia are present in a few cases (e.g. *L. paulinoi*, *T. fancelloi*, *M. hiekei*, *M. gomezi*, *M. andujari* sp.n.; see Table 1) but are far less common than synapomorphies. *Microcharidius* is the genus with more cases of specific autapomorphies recorded so far and in particular, clades *gomezi* and *belenae* are especially prone to high modifications of apical lamina and shape of aedeagus.

It seems that aedeagal morphology within lineages can be quite stable without certain evolutionary pressures and these autapomorphies might be the product of other processes instead of phylogenetic signal, acting as efficient elements of prezygotic isolation due to sexual recognition (ZABALLOS & FARINÓS 1995). If so, are there any evidences of these types of evolutionary processes driving the morphological changes of male genitalia? The four new species may highlight this question.

## 4.2. Implications of the new species

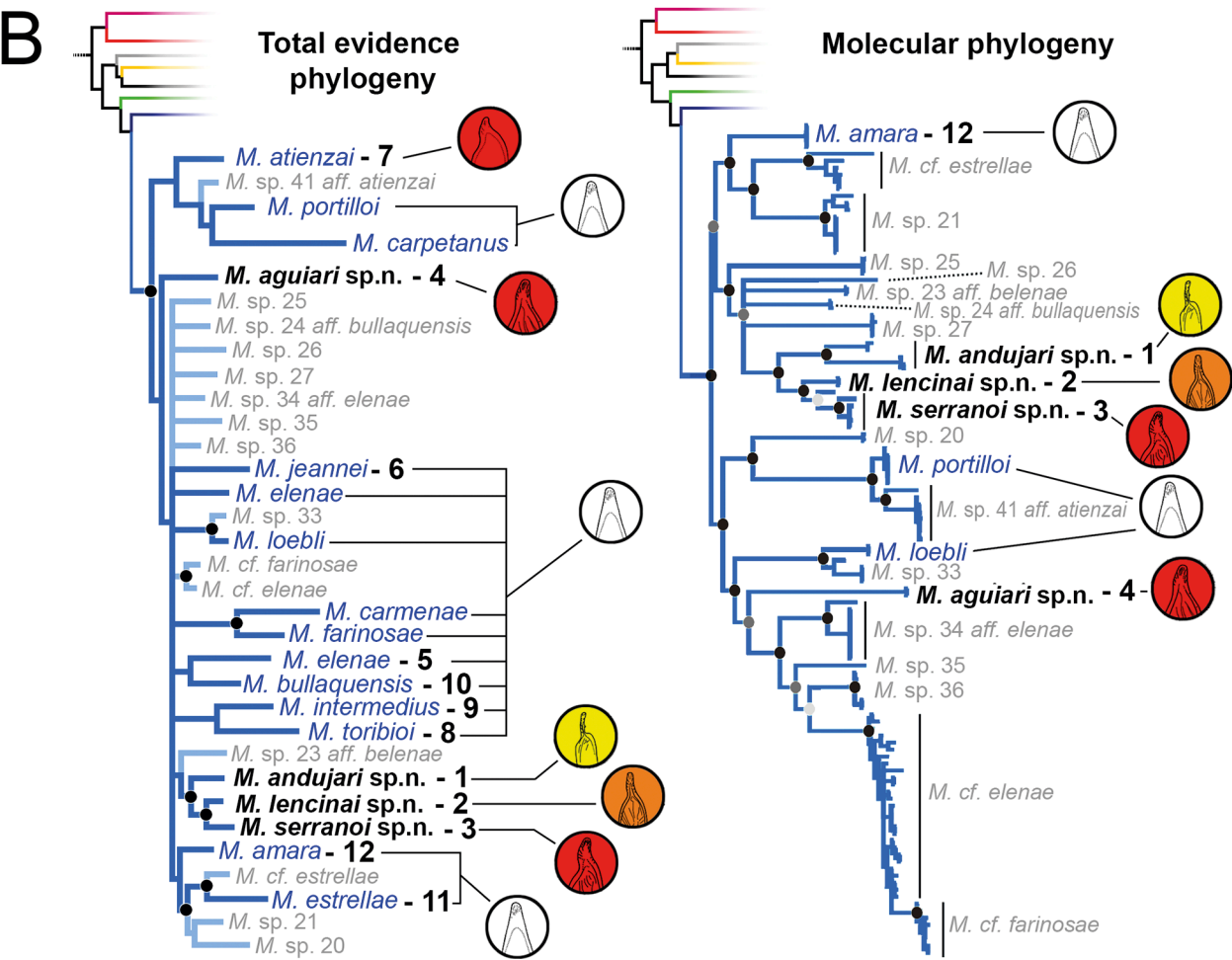
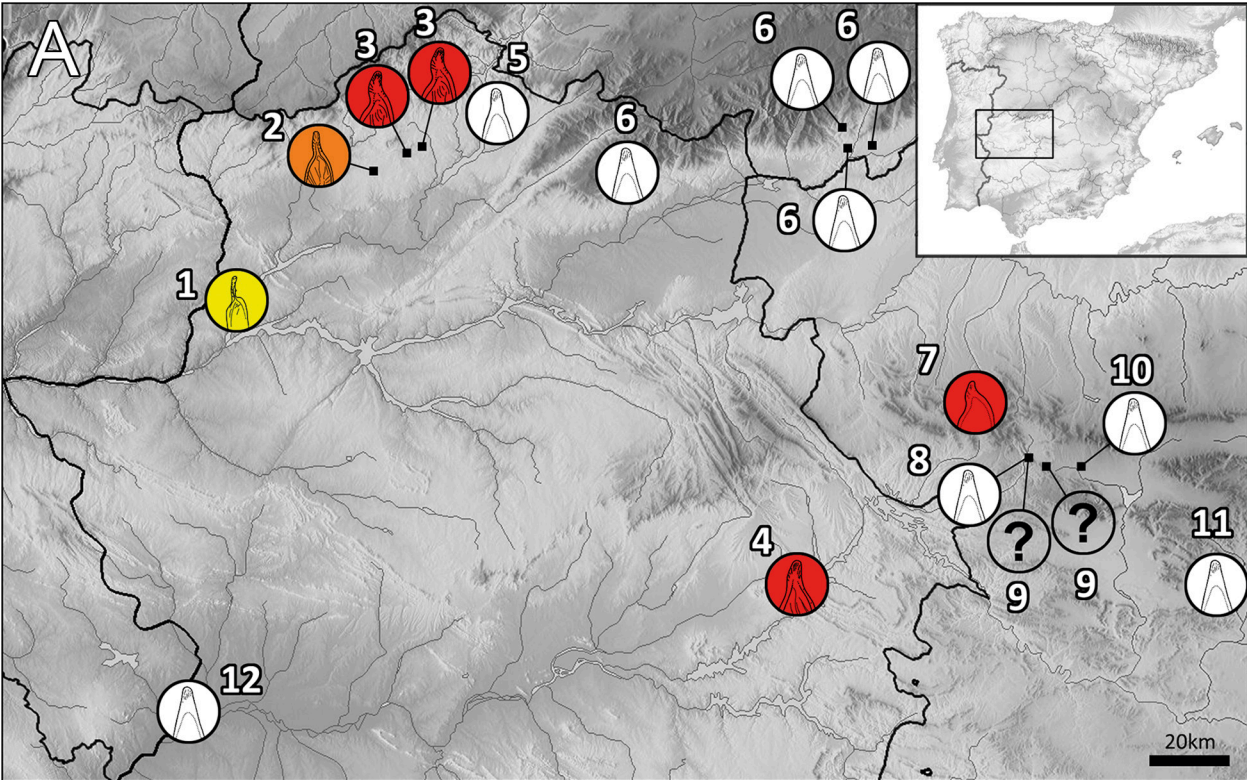
The four new species are unusual within Typhlocharina by their highly modified apical laminae (Figs. 2C–4, 3C–4, 4C–4, 5C–4). Apical laminae show a high tendency for diversification (Fig. 6C) that could be related to mechanisms of sexual isolation that facilitate sexual recognition or mechanically prevent crossbreeding. Unique specializations of apical laminae are unknown in *Typhlocharis*, and infrequent but present in *Lusotyphlus* (in one of the five species, *L. paulinoi*). In *Microcharidius* such a phenomenon was only known in *M. hiekei* and *M. atienzai*. The new species raise this number to six of the 41 species in the genus and, except *M. hiekei*, all of them belong to clade *belenae*, suggesting that this complex and highly homogeneous lineage is especially prone to diversification of the apical laminae.

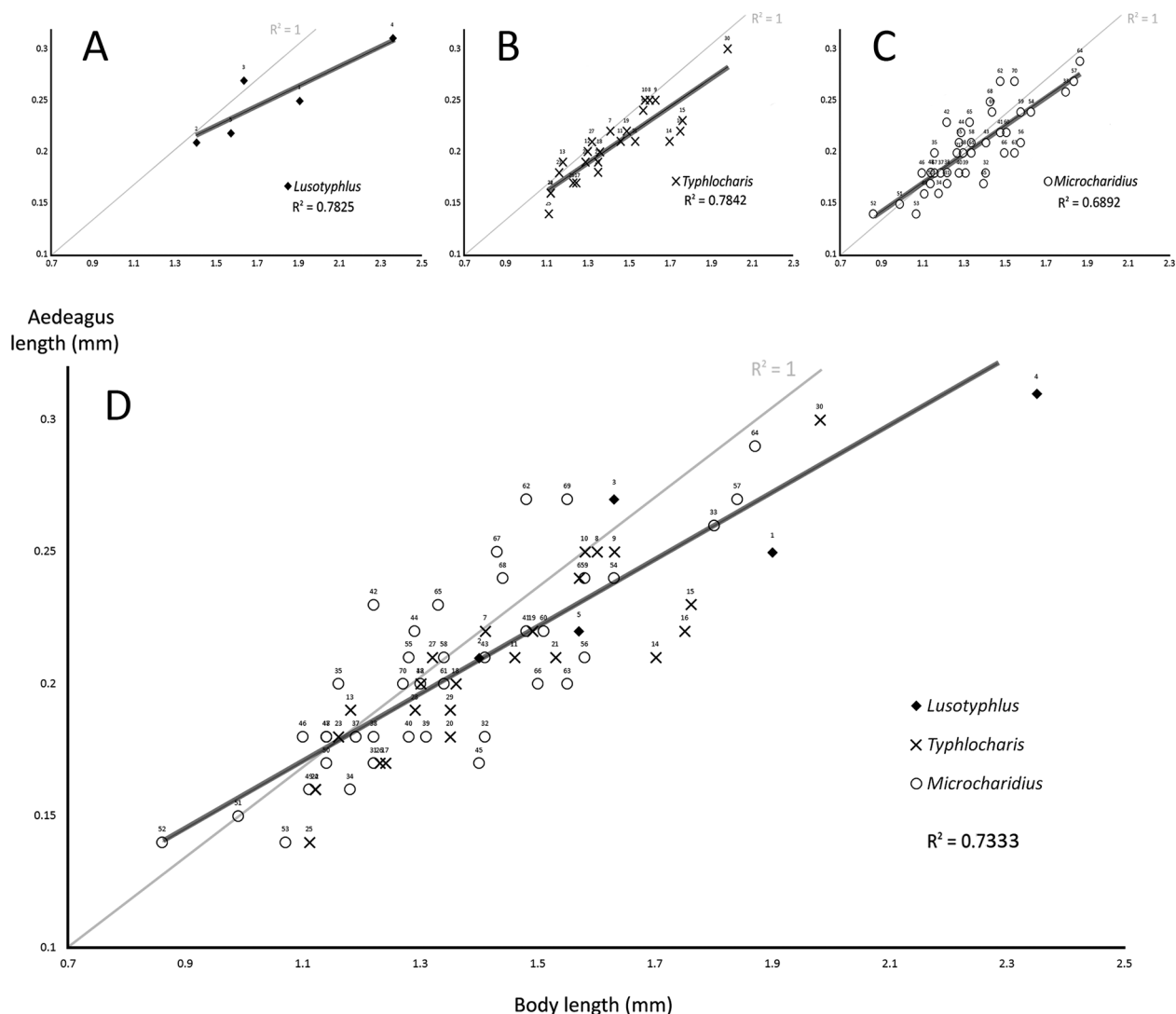
All previously known species with autapomorphies in the aedeagus coexist with other Typhlocharina (PÉREZ-GONZÁLEZ et al. 2017), reinforcing the idea of morphological divergences in male genitalia as a mechanism of sexual isolation. Of the four new species, however, only *M. lencinai* sp.n. is known to coexist with another species of Typhlocharina. The fact that three of the new species apparently do not follow this rule should not be surprising given the “randomness” of the captures in the samples, thus the potential existence of syntopic species cannot be discarded. The new species are distributed within an area of roughly 40,000 km<sup>2</sup> occupied by 12 species of morphologically similar *Microcharidius* of clade *belenae* (Fig. 11A). While the real extent of each species distribution is still unknown, the overlapping of the different populations is highly probable, as documented by other species of clade *belenae* (ZABALLOS & RUIZ-TAPIADOR 1997; ZABALLOS 1989; ORTUÑO 1988).

Then, it is possible to conceive an evolutionary scenario where coincidental populations of these similar species reinforce their isolation mechanisms evolving different types of sexual recognition devices. The new species point to a high potential plasticity of the apical laminae and shed some light on “how” these differences may have occurred.

On one hand, *M. andujari* sp.n., *M. lencinai* sp.n., and *M. serranoi* sp.n. are very similar, yet illustrate strong divergences in the shape of the apical lamina. Molecular and total evidence phylogenies suggest they are closely related species, members of a same clade (Fig. 11B). *Microcharidius andujari* sp.n. (Figs. 2C–4, 6C–6) and *M. serranoi* sp.n. (Figs. 4C–4, 6C–5vii) represent two types of extremely derived shapes, well differentiated from each other and from the widespread subtriangular shape characteristic of clade *belenae* (Fig. 6C–4v). The smoothly sinuous apical lamina seen in *M. lencinai* sp.n. represents an intermediate stage that fills the morphological gap between *both* derived shapes and the subtriangular laminae (Fig. 6C–4,5,6). This suggests that extremely derived autapomorphies in the apical laminae are acquired through a gradual transition of several clinal morphological stages that could lead to heavily different morphologies. This evidence fits well with a model of clinal speciation (e.g. TEMPLETON 1981), that could be an important mechanism in the evolution of diversity in clade *belenae*. In this case, *M. andujari* sp.n., *M. lencinai* sp.n., and *M. serranoi* sp.n. could be parapatric species (Fig. 11), with *M. lencinai* sp.n. in the hybrid zone between the three species. More data would be needed to test parapatry, delimiting the distribution ranges of these species.

On the other hand, the sinuous shape observed in *M. serranoi* sp.n. is shared by *M. aguiari* sp.n. and *M. atienzai*. In this case, the three species had developed virtually identical apical laminae (Figs. 4C–4, 5C–4, 6C–5vii), yet differ in many other morphological features (see Affinities section). Molecular evidence does not support a close relationship between *M. serranoi* sp.n. and *M. aguiari* sp.n. (Fig. 11B) and, while molecular data is not available for *M. atienzai*, total evidence phylogenies do





**Fig. 12.** Graphical comparison of aedeagus length *versus* total body length in all species of Typhlocharina. Numbers above symbols correspond to the species as listed in Table 1.

not place *M. atienzai* close to any of the new species but strongly suggest a close affinity to *M. portilloi*, as evidenced in their morphology by the shape of the clypeus, type of spermathecal duct and features of endophallic sclerites, among other features (Fig. 11B). Also, *M. atienzai* occurs more than 150 km away from *M. serranoi* sp.n. and approximately 65 km away from *M. aguiari* sp.n. (Fig. 11A), which given the morphological differences observed, reinforces the idea that the three species are not closely related.

While phylogenetic relationships within clade *beleanae* cannot be considered fully resolved or stable (ANDÚJAR et al. 2017; PÉREZ-GONZÁLEZ et al. 2017), these lines of evidence imply that the acquisition of such sinuous shape has occurred independently at least three times within clade *beleanae* and becomes the first record of convergent evolution in male genital features within Typhlocharina.

← **Fig. 11. A:** Distribution map of clade *beleanae* species with 4+2 umbilicate series, including the four new species, highlighting the shape of apical laminae. — **Symbols:** white circle – subtriangular morphologies, orange circle – smoothly sinuous lamina, yellow circle – warped lamina, red circle – short, strongly sinuous lamina. 1 – *Microcharidius andujari* sp.n., 2 – *M. lencinai* sp.n., 3 – *M. serranoi* sp.n., 4 – *M. aguiari* sp.n., 5 – *M. beleanae*, 6 – *M. jeannei*, 7 – *M. atienzai*, 8 – *M. toribioi*, 9 – *M. intermedius*, 10 – *M. bullaquensis*, 11 – *M. estrellae*, and 12 – *M. amara*. **B:** Phylogenetic relationships of clade *beleanae* as recovered in PÉREZ-GONZÁLEZ et al. 2017 (total evidence phylogeny) and ANDÚJAR et al. 2017 (molecular phylogeny), showing the shape of apical laminae for described species of the clade. Note the relationships between *Microcharidius atienzai*, *M. andujari* sp.n., *M. lencinai* sp.n., *M. serranoi* sp.n., and *M. aguiari* sp.n. suggesting an independent evolution of the sinuous apical lamina (red and orange circles). — **Symbols:** names in blue – described species, names in black bold – the four new species, names in light grey – undescribed taxa (as appear in PÉREZ-GONZÁLEZ et al. 2017 and ANDÚJAR et al. 2017), black circles on nodes – clade support of 0.95–1. Symbols for apical lamina and numbered species same in Fig. 11A and B.

### 4.3. Does size matter? Body size reduction vs aedeagus size in Typhlocharina

The lineage of Typhlocharina is characterized by their small size, ranging from a body length of 2.9 mm in *L. carinatus* (the biggest known Typhlocharina, SERRANO & AGUIAR 2006) to 0.8 mm of *M. scrofa* (one of the smallest known Carabidae, PÉREZ-GONZÁLEZ & ZABALLOS 2013b). Miniaturization seems to be a common evolutionary trend in the group, and many species of *Typhlocharis* and *Microcharidius* achieve sizes of less than 1 mm. In particular, the lineage of clade *quadridentatus* shows the strongest trend toward miniaturization (PÉREZ-GONZÁLEZ & ZABALLOS 2013b). On the other hand, *Lusotyphlus* is the largest genus of the lineage, with an average size of 2 mm.

It has been proposed that, in insect groups with strong tendencies toward size reduction, the reproductive structures do not follow the same trend (POLILOV & MAKAROVA 2017) and male genitalia show negative allometry (EBERHARD 2009). Correlation of body length and length of aedeagus in Typhlocharina (Fig. 12) do not contradict this hypothesis: the allometric slopes for *Lusotyphlus* ( $R^2 = 0.78$ ; Fig. 12A), *Typhlocharis* ( $R^2 = 0.78$ ; Fig. 12B), *Microcharidius* ( $R^2 = 0.69$ ; Fig. 12C), and for Typhlocharina as whole ( $R^2 = 0.73$ ; Fig. 12D) all suggest low negative allometry.

However, the smaller species do not show significantly larger aedeagi than the bigger species (e.g. the aedeagus of *M. scrofa* represents 16% of the total length, while the aedeagus of *L. carinatus* is the 13%) and species with proportionally larger aedeagi are not especially small (e.g. *M. atienzai*, with an aedeagus of 18% of the total length, 1.48 mm). The proportionally smallest aedeagi represent 12% of the total length and appear in species of varied sizes (e.g. *T. prima*, length 1.11 mm; *M. fozcoensis*, length 1.4 mm; *M. santschii*, length 1.41 mm; *M. bullaquensis*, length 1.55 mm; *T. quarta*, length 1.7 mm; and *T. tertia*, length 1.75 mm). If genitalia size does constrain miniaturization in insects (POLILOV & MAKAROVA 2017) and smaller species have significantly larger genitalia proportionally, Typhlocharina seems out of the limits where these effects are conspicuous. So, the answer might be that no, size does not matter in Typhlocharina, and miniaturization within the lineage does not affect the proportional size of male genitalia.

## 5. Conclusions

This study covers an in-depth revision of the morphology of the male genital complex in a diverse lineage of endogean Carabidae as well as the description of four new taxa. There are phylogenetic patterns in the male genitalia of Typhlocharina and they can serve as an efficient taxonomic tool for genus or clade-level recognition. However, except in a few cases of clear autapomorphies, male genitalia do not perform well for species-specific

discrimination and it should be always complemented with non-genital traits. The newly described morphological models of the aedeagus, parameres, and ring sclerite should be incorporated into future descriptions of Typhlocharina taxa, and applied to other Anillini where this information remains undescribed. It can be expected that male genitalia could express similar phylogenetic information in other Anillini genera and more in-depth studies are still needed for the group. Also, the new species greatly increase the knowledge of an obscure clade of *Microcharidius*, which goes from 14 to 18 described species. They provide the first lines of evidence of gradual evolution and convergence in male genital features (changes of the apical lamina), probably associated with sexual isolation processes.

## 6. Acknowledgements

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## Authors' Contributions

S.P.G. examined the material, performed the morphological observations and did the illustrations. S.P.G. and J.P.Z. wrote the manuscript.