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Cladistics (2017) 1–25

Cladistics

10.1111/cla.12208

Hidden biodiversity: total evidence phylogenetics and evolution of morphological traits in a highly diverse lineage of endogean ground beetles, *Typhlocharis* Dieck, 1869 (Carabidae, Trechinae, Anillini)

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Accepted 13 April 2017

Abstract

Typhlocharis is the most diverse eyeless endogean ground beetle genus known to date, with 62 species all endemic to the West Mediterranean region. The lineage is characterized by a conservative and singular body plan within Carabidae that contrasts with a high morphological diversity in many traits. We provide an exhaustive phylogeny of the lineage through the study of 92 morphological characters from all 62 described species and 45 potential new species from 70 additional populations, and the combination of morphological and available molecular data, in the first total evidence phylogenetic approach for a highly diverse endogean lineage. We tracked the evolution of morphological traits over the obtained phylogenies. Results suggest eight morphologically distinct clades, which do not correspond to the species groups proposed formerly. Ancestral state reconstructions and phylogenetic signal analyses of morphological traits revealed that some of the previously key characters to the classification of *Typhlocharis*, such as the umbilicate series or the apical denticles of elytra, are highly homoplasic, whereas other characters show stronger phylogenetic signal, including structures in the antennae, gula, pronotum and last abdominal ventrite. This evidence supports the split of *Typhlocharis* into three genera: *Lusotyphlus* **gen. nov.**; *Typhlocharis* Dieck, 1869 and *Microcharidius* Coiffait, 1969 (revalidated), forming the subtribe Typhlocharina Jeanne, 1973.

Introduction

The tribe Anillini (Coleoptera, Carabidae, Trechinae), with roughly 530 species of litter-, soil- and cave-dwelling beetles (Zaballos, 2003; Lorenz, 2005; Sokolov, 2015), is the richest carabid lineage exclusively composed of belowground species. Anillini are adapted to the absence of light, buffered temperature fluctuations, high humidity and spatial limitations imposed by the soil environment and, consequently, show typical morphological features of the endogean fauna: small body size (from 0.9 mm in some species of *Argiloborus* Jeannel, 1937 and *Typhlocharis* Dieck, 1869 to 4.5 mm in *Perucharidius* Mateu and Etonti, 2002; Pérez-González and Zaballos, 2013c), depigmentation, apterism, absence of eyes (except *Nesamblyops* Jeannel, 1937; *Cryptorites* Jeannel, 1950 and *Microdipnodes* Basilewsky, 1960; with small eyes; Jeannel, 1937; Basilewsky, 1960) and a well-developed system of long sensorial setae in the lateral margins of elytra (the umbilicate series, Jeannel, 1937), expressed in different patterns (Giachino and Vailati, 2011). Within Anillini, the eyeless genus *Typhlocharis* display these features in an exclusive way.

Typhlocharis has a conservative body plan with stout, depressed rectangular bodies, relatively short limbs and square-shaped pronotum that gives the genus an appearance distinct from any other Anillini. This unusual facies within Carabidae led Dieck

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(1869a,b) to describe *Typhlocharis* as a member of Silvanini (Coleoptera, Cucujoidea) and suggests a convergent solution for movement through narrow spaces.

In contrast, *Typhlocharis* is the most diverse genus of the tribe and shows a high variation in many morphological traits over the 62 described species (Serrano and Aguiar, 2014; Zaballos et al., 2016). It is endemic to the Mediterranean region (Western Palaearctic), distributed through the Iberian Peninsula (Spain and Portugal) and North Africa (Morocco and Tunisia). All the species are endogean (*sensu* Giachino and Vailati, 2010), inhabiting soil horizons A and B (Ortuño, 2000), usually in regions with Mediterranean forest formations (holm oak, cork oak or olive trees) or grassland-like open areas (pastures or thistle prairies). The genus has been considered a proper phyletic lineage within Anillini (subtribe Typhlocharina; Jeanne, 1973), a criterion supported by recent molecular data, recovering *Typhlocharis* as an early divergent monophyletic lineage of Anillini (Andújar et al., 2016). Its evolutionary origin was suggested to date back to the Palaeocene (Jeannel, 1963), an estimation recently confirmed by molecular clock methods (Andújar et al., 2016), and molecular data support a reduced spatial scale of speciation related with dispersal limitations, in agreement with the observed pattern of microendemicity (C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted).

Beyond its unique body design among Anillini, *Typhlocharis* shows some morphological traits that are exclusive or shared only by a few other taxa within the family Carabidae, such as the tubular shape of gonocoxites (Vigna-Taglianti, 1972), the reduction of the number of tarsomeres in some species (Pérez-González and Zaballos, 2013c) or the presence of a stridulatory organ in the posterior region of the head (Zaballos and Pérez-González, 2011). The atypical female genitalia of *Typhlocharis* are characterized by tubular and weakly sclerotized gonocoxites (Vigna-Taglianti, 1972), instead of the unguiform gonocoxites present in practically all other Anillini (except *Anillotarsus tetramerus* Mateu, 1980). However, four species of *Typhlocharis* show unguiform gonocoxites and one species possesses intermediate, unguiform-like gonocoxites, raising questions of how this character evolved within the genus (Zaballos and Wrase, 1998; Ortuño and Gilgado, 2011; Pérez-González and Zaballos, 2012). Tarsal tetramery has been related to small size and virtually all of the exceptions to five-articled tarsi in Carabidae occur in the Anillini (Pérez-González and Zaballos, 2013c). Cranioprothoracic stridulatory organs are unknown in any other Anillini and not frequent in Carabidae (Forsythe, 1979). In *Typhlocharis* this structure is widespread, but not present in all the species, and has been associated with the shape of antennomeres, implying a potential role in communication methods (Pérez-González and Zaballos, 2013b). The life cycle is still unknown, with no information about lifespan, eggs or pupae; only one larval stage has been registered (Arndt et al., 1999; Andújar et al., 2010).

The increasing interest in this genus over the last 20 years has resulted in an unprecedented discovery rate of new species (Zaballos and Pérez-González, 2010); and morphological descriptions have progressively become more detailed, adding previously ignored characters (e.g. Zaballos and Ruíz-Tapiador, 1997; Zaballos and Wrase, 1998; Zaballos and Banda, 2001; Serrano and Aguiar, 2006). Some of these characters, such as antennae or tarsomeres, turned out to be good taxonomic tools (Pérez-González and Zaballos, 2013b,c); others are probably diagnostic, too, but this remains to be evaluated. This complexity is reaching a critical point that makes a phylogenetic approach necessary. A recent work conducted on the molecular phylogeny of the genus (C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted) provides the opportunity for a total evidence approach to the phylogeny of *Typhlocharis* based on the combination of morphological and molecular data (as successfully applied to other groups; e.g. Condamine et al., 2013; Basso et al., 2016) and allows us to evaluate for the first time the intrinsic phylogenetic signal of morphological traits over a highly diversified lineage strongly adapted to deep soil conditions.

We have studied 92 morphological characters from all 62 known species and 70 new additional populations to generate exhaustive phylogenetic analyses for morphological data sets only and combined with DNA sequences, available for 70% of the studied species. The phylogenetic proposals for *Typhlocharis* are used: (i) to evaluate the phylogenetic signal and evolutionary patterns of the morphological characters; (ii) to test the validity of previous taxonomic proposals about the internal classification of the group, in particular the case of *Microcharidius* Coiffait, 1969 (Coiffait, 1969; Zaballos, 1983) and species groups (Zaballos and Ruíz-Tapiador, 1997; Zaballos and Wrase, 1998; Ortuño and Gilgado, 2011); and (iii) to build a new proposal for the internal systematics of the genus.

Material and methods

Taxon sampling and collection of specimens

We studied more than 3000 specimens with representatives of the 62 species of *Typhlocharis* considered valid up-to-date, and 70 additional populations, covering the range of distribution of the lineage and all proposed species groups. Type specimens were studied for 56 of the 62 species (see Table S1). *Typhlocharis simoni*

Ganglbauer, 1900, considered *incertae sedis* (Zaballos, 2003), was excluded. Specimens were newly collected or gathered from the following collections (Table S1): ARS, Coll. A.R.M. Serrano, Universidade de Lisboa; CA, Coll. C. Andújar; CZULE, Coll. Zoología de la Universidad de León; DEI, Deutsche Entomologisches Institut; DW, Coll. D.Wrase; JLL, Coll. J.L. Lencina; JPZ, Coll. J.P. Zaballos, Universidad Complutense de Madrid; MFN, Museum für Naturkunde, Berlin; MHNG, Muséum d'Histoire Naturelle, Genéve; 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; SPG, Coll. S. Pérez-González.

New specimens included 29 known species and 45 potential new species, collected from 116 localities (Table S1). Sampling was done by direct observation under deeply buried boulders or through processing soil samples (20–50 L of soil) by soil washing and Berlese apparatus (Berlese, 1905; Normand, 1911; Tullgren, 1918). Specimens were separated by hand and preserved in absolute ethanol, deposited in collections JPZ, SPG (UCM) and CA. Of these specimens, 402 were DNA sequenced (hologenophores, *sensu* Astrin et al., 2013) for two mitochondrial and two nuclear genes (C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted), thus linking morphological and molecular data sets for a total evidence approach in *Typhlocharis* (see details below). Delimitation of "species-level" entities from the sequenced material was based on GMYC (Pons et al., 2006) and bPTP (Zhang et al., 2013) methods and contrasted with detailed morphological study of the hologenophores (C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted).

Three species of *Pseudanillus* (*P. pastorum* Zaballos and Banda, 2000; *P. elegantulus* (Normand, 1915) and *P. magdalenae* (Abeille de Perrin, 1894)), one species of *Anillinus* (*A. steevesi* Barr, 1995) and four species of *Geocharis* (*G. liberorum* Zaballos, 2005; *G. iborensis* Zaballos, 1990; *G. cf. fenestrata* and *G. sp. indet.*) were used as outgroups (Table S1).

Morphological data

Whenever possible, several males and females were studied for every species, to evaluate and minimize the effect of intraspecific variation. Specimens from collections were de-attached from the original cards, hydrated and rinsed in lactic acid to clear the cuticle. Male genitalia were extracted when necessary (if there was no original mounting), by pulling the aedeagus out with a thin hook-shaped needle. Female genitalia were studied *in situ*, by transparence, to avoid damage during manipulation. For species with enough material, some specimens were dissected by separation of head, pronotum, elytra, abdominal pieces and genitalia. After observation, they were re-mounted and stored back in the collections. Dissected specimens and genitalia were mounted embedded in D.M.H.F. resin (Bameul, 1990) in new cards with glass window, keeping the original card in the same pin. Manipulation of loaned material was restricted to ensure observations without damage. The same treatment was applied to newly collected specimens, but dissections were restricted to extraction of male genitalia. Hologenophores were vouchered and digested with proteinase K during DNA extraction, with no observable damage after the procedure, finally mounted or stored in fluid-preserved collections.

Morphological information was obtained from direct observation by light microscopy, using a Zeiss 474620-9900 microscope (Jena, Germany). Incongruities between published and observed data were solved favouring the new observations. For three species where we could only study one of the sexes, data were complemented from literature. Measurements were taken in a Wild Heerbrugg M8 stereomicroscope (Switzerland). Examination of morphological characters followed a systematized protocol. The body was divided in regions (head, prothorax, elytra, abdomen, limbs and genitalia) and data were recorded in standardized datasheets. All of the features described in the literature and used in the systematics of the genus were recorded and studied in detail, in addition to characters rarely used or previously undescribed. A complete list of the studied characters is provided in Appendix S1. Nomenclature of morphological structures (antennal features, sensilla, chaetotaxy of last ventrite) follows Pérez-González and Zaballos (2012, 2013b,c).

Identification criteria and taxonomic decisions

Identifications were mainly performed by one of the authors (SPG), to ensure the systematization of the observations. Newly collected specimens were identified by direct comparison with previously known, described species. This led to four situations, where we follow the "open nomenclature" criteria (Bengston, 1988):

1. The specimen clearly belongs to an already described species. It is labelled with a voucher code followed by the name of the species, such as "BMNH-1046040-*Typhlocharis lunai* Serrano and Aguiar, 2006".
2. The specimen *probably* belongs to a known species but cannot be totally verified with the available data. It is labelled as "*cf.*" (from latin "*confer*" – compare) plus the name of the

presumed species, for example "BMNH-1041970-*Typhlocharis cf. deferreri* Zaballos and Pérez-González, 2011".

3. The specimen has clear affinities to a known species, but shows certain differences and likely represents a different taxon. These species have been numerated and treated as "new" species, labelled as "sp." and a number, followed by "*aff.*" (from latin "*affinis*" – akin) plus the name of the related species, such as "BMNH-1424404-*Typhlocharis* sp.42 *aff. crespoi* Serrano and Aguiar, 2008".
4. The specimen clearly represents a new, undescribed taxon. It is labelled as "sp." plus a number, such as "BMNH-1424387-*Typhlocharis* sp. 20". The full list of taxa considered in this work is detailed in Table S1.

Data matrixes

A set of 92 morphological characters from adult specimens was selected and coded (17 from head, nine from prothorax, 22 from elytra, nine from abdomen, 19 from limbs, eight from male genitalia and eight from female genitalia; Fig. 1). No larval characters were used. All the characters were treated as discrete (continuous characters were adjusted to categories for character states), unordered and with equal weights: 27 were coded as binary and 65 as multistate. Missing data were coded as "?", inapplicable data were coded as "-". Detailed description of characters and character states are provided in Appendix S1.

Characters were coded for 513 terminal taxa (505 ingroup terminal and eight outgroups). Of these, 62 terminals corresponded to described species, treated as one terminal per species, summarizing data gathered from specimens of collections. *Typhlocharis zaballosi* Serrano and Aguiar, 2014 was split into two terminal taxa to explore the implications of certain morphological characters that differ significantly between two populations (Serrano and Aguiar, 2014). Newly collected specimens used for DNA sequencing (hologenophores) were vouchered and coded individually within the initial morphological matrix (adding 443 terminals). For 379 of these, sequencing was successful (C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted), and DNA data on the same specimens have been used for the total evidence approach, whereas for the remaining 64 specimens sequencing failed and only morphological data are available. In addition, the molecular data set in the total evidence approach includes 23 specimens that were unavailable to morphological studies. The morphological matrix is detailed in Appendix S2.

The *complete morphological* (CM) data set described above (513 terminal taxa: 443 hologenophores, 62 "described species" and eight outgroups) was the basis to generate three additional data sets: (i) a *reduced morphological* (RM; 133 ingroup taxa and eight outgroups) data set including 62 terminals for "described species" plus 71 of 74 terminals representing the species delimitation hypothesis obtained from molecular data (C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted); the remaining three entities clustered specimens not available for morphological studies, and were not considered in RM; (ii) a *total evidence* (TE; 74 ingroup taxa and two outgroups) data set combining molecular and morphological data for the 74 species level entities from C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera (submitted), with three entities only represented by molecular data; and (iii) *extended total evidence* (e-TE; 136 ingroup taxa and two outgroups), adding the 62 terminals for "described species" (only morphological data) to TE data set.

When coding morphological information to species level, the next considerations were followed: intraspecific polymorphism associated with individual variability (i.e. not all the terminals from a single species/population have the same character state) was coded following the *majority* or *modal coding* criteria (Wiens, 1999), assigning to the "species" the most common character state observed within the studied specimens. Sexually dimorphic characters were coded using the *any instance coding* criteria (Wiens, 1999), assigning to the "species" the outstanding character state (i.e. if males show hypertrophied mandibles but females do not, the character state for the "species" will be coded as hypertrophied mandibles).

Phylogenetic analysis

All data sets were analysed by maximum parsimony (MP), maximum-likelihood (ML) and Bayesian inference (BI).

MP analyses were done using TNT 1.1 (Goloboff et al., 2008). Heuristic searches were performed with the "*New Technology search*" tool, with a *Max. Trees* value of 10 000, using the options *sectorial searches*, *tree ratchet*, *tree drifting* and *tree fusing*, specifying 1000 random addition replicates. For each analysis, 1000 bootstrap replications (Felsenstein, 1985) (standard sample with replacement) were done, considering nodes with values over 80% as well supported. *Anillinus steevesi* was used to root the tree in the morphological data sets; *Geocharis cf. fenestrata* (CA91) was used as root in the combined data sets.

ML analyses were carried out with RAxML 8.1.11 (Stamatakis, 2006) using the CIPRES Science Gateway (Miller et al., 2010). Morphological data sets were not partitioned and the Mkv model was applied.

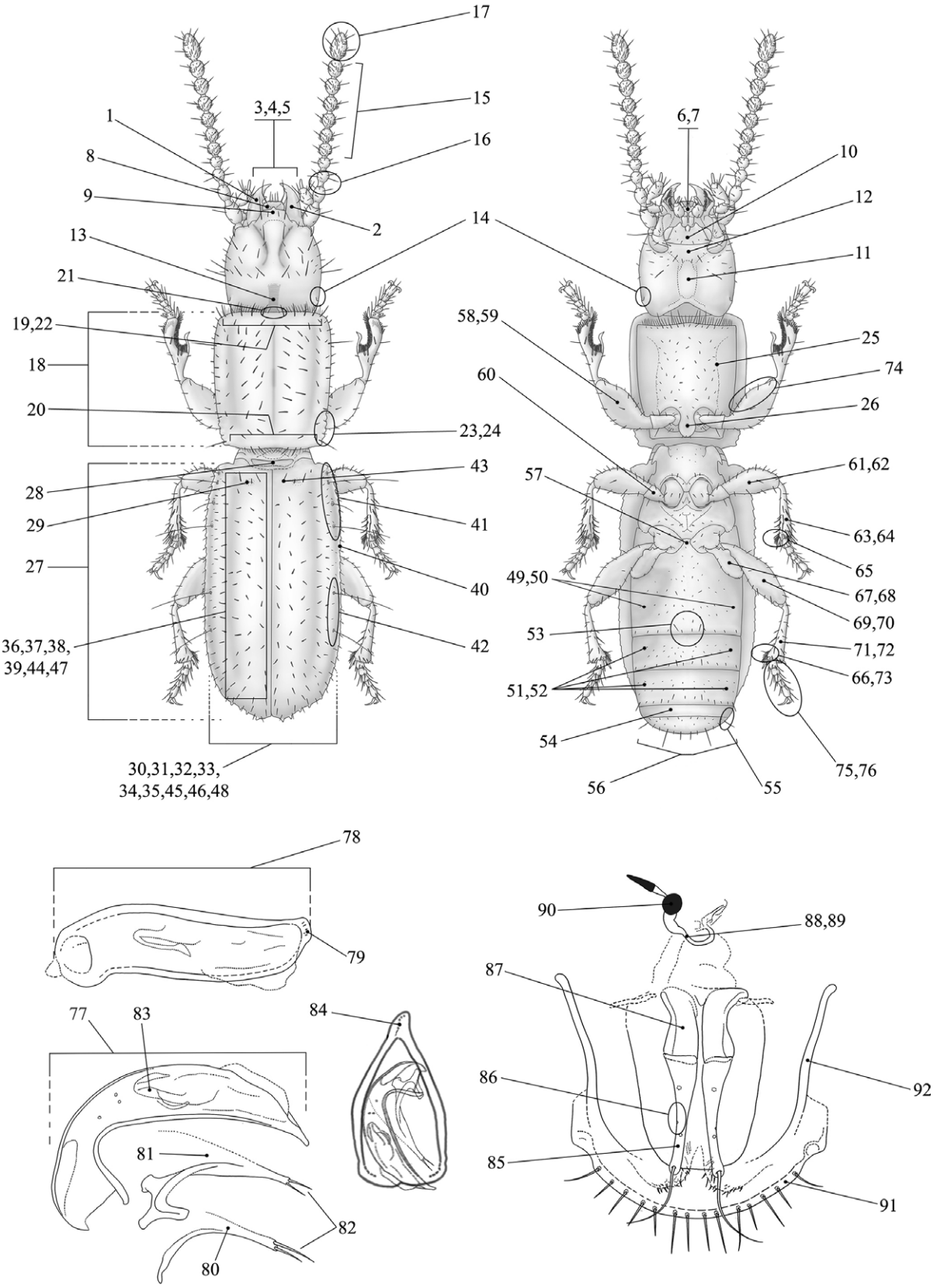

Fig. 1. Morphology of *Typhlocharis*, showing the characters selected and coded in this work (numeration according Appendix S1). Full list and character states are detailed in Appendices S1 and S3.

Combined data sets were partitioned by gene, including morphological data as an independent partition. The protein-coding genes (*cox1-a* and *cox1-b* fragments) were additionally divided into two codon partitions, with first and second codon positions together (Andújar et al., 2012). An independent GTR + CAT model was applied to each partition. Support values were obtained from 1000 bootstrap replicates (Felsenstein, 1985). To further assess the position of terminal taxa represented only by morphological data, the extended total evidence data set (e-TE) was re-analyzed under the same parameters and using the *binary backbone* option of RAxML (Stamatakis, 2006), which allowed to constrain the tree topology as obtained from molecular data only in C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera (submitted) using Bayesian methods.

BI was run in MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) from CIPRES (Miller et al., 2010). For the combined data set, partitions were defined as in ML analyses. The best-fit models were selected using the Akaike information criterion in jModelTest 2.1.7 (Darriba et al., 2012). The *Invgamma* model was used for the morphological partition. The analyses consisted of two independent runs, each with four chains (three hot and one cold), sampling every 1000 generations. To explore the best convergence of the results, analyses were run for 10, 30 and 50 million generations, changing *temperature* parameter between 0.2, 0.4 and 0.6. The standard deviation of split frequencies was checked to assess the convergence of results, as well as the mean and effective sampled size (ESS) of likelihood values computed with TRACER 1.6 (Rambaut et al., 2014). The 50% majority rule and strict consensus trees were calculated excluding 25% of the initial trees as a conservative burn-in ensuring that the plateau in tree likelihood values was reached. Node posterior probabilities were interpreted as support values. All of the trees were visualized using FigTree 1.4.2 (Rambaut, 2012).

Character evolution: phylogenetic signal and ancestral state reconstrucion

The phylogenetic signal of morphological characters was checked using the RM data set (excluding the 62 terminals of described species) over an ultrametric BI tree (74 terminals) obtained with molecular data using BEAST (Drummond et al., 2012). This tree is highly consistent with topologies obtained with BI and ML analyses (see C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted) and with the total evidence analyses performed in this work. Such an approach has the advantage of characters tested over a topology independent from the morphological matrix, but information for taxa with only morphological data available is lost (hence some character states might be under-represented). To avoid this, an additional test of phylogenetic signal was carried out over the BI tree obtained from data set e-TE (138 terminals). To test the level of homoplasy and the relative performance of the characters, we obtained the consistency (CI) and retention (RI) indexes with Mesquite 3.06 (Maddison and Maddison, 2007) for the same data sets. Values per character are specified in Table S2.

Ancestral state reconstruction was conducted under parsimony methods in Mesquite 3.06 (Maddison and Maddison, 2007) using the same topologies and data sets stated for the phylogenetic signal analyses. The 92 characters of the matrix were mapped over the ultrametric molecular BI tree (74 terminals) and over the total evidence BI tree (138 terminals). Ancestral state reconstruction of each character is provided in Appendix S3.

Results

Phylogeny based in morphological data

Results from the MP, ML and BI analyses over the RM data set showed similar topologies (Fig. 2). MP provided 12 equally parsimonious trees (length 1281, CI: 0.182; RI: 0.717), with *Bootstrap* values generally low and few nodes with support > 80% (Fig. 2). Among BI analyses, the best convergence was achieved specifying 50 million generations and $t = 0.4$. The monophyly of *Typhlocharis* was well supported with all methods (1/96/87; BI/ML/MP) and six monophyletic clades were consistently recovered (Fig. 2). Clades "*algarvensis*" (0.99/98/92) and "*belenae*" (1/100/98) always showed high support. Clades "*gomezi*" (0.96/83/-), "*outereloi*" (0.87/-/-) and "*monastica*" (0.99/-/-) were consistently recovered but not supported in all the analyses. Clade "*diecki*" was recovered without support. Species from clades "*diecki*", "*gomezi*", "*outereloi*", "*monastica*" and "*belenae*" were grouped in a large clade (0.99/91/-) with high support in BI and ML analyses. Trees obtained for CM data set (MP: two equally parsimonious trees, length 2063, CI: 0.108, RI: 0.828; BI: best convergence at 30 million generations, $t = 0.4$, see Fig. S1) did not show substantial differences, except clade "*outereloi*", which was split in two polyphyletic lineages under ML. The coding of individual hologenophores instead of species-level terminals does not seem to affect the general topology.

Total evidence phylogeny

Results from data sets TE and e-TE, combining molecular and morphological data, were very similar and recovered eight main clades. Focusing on data set

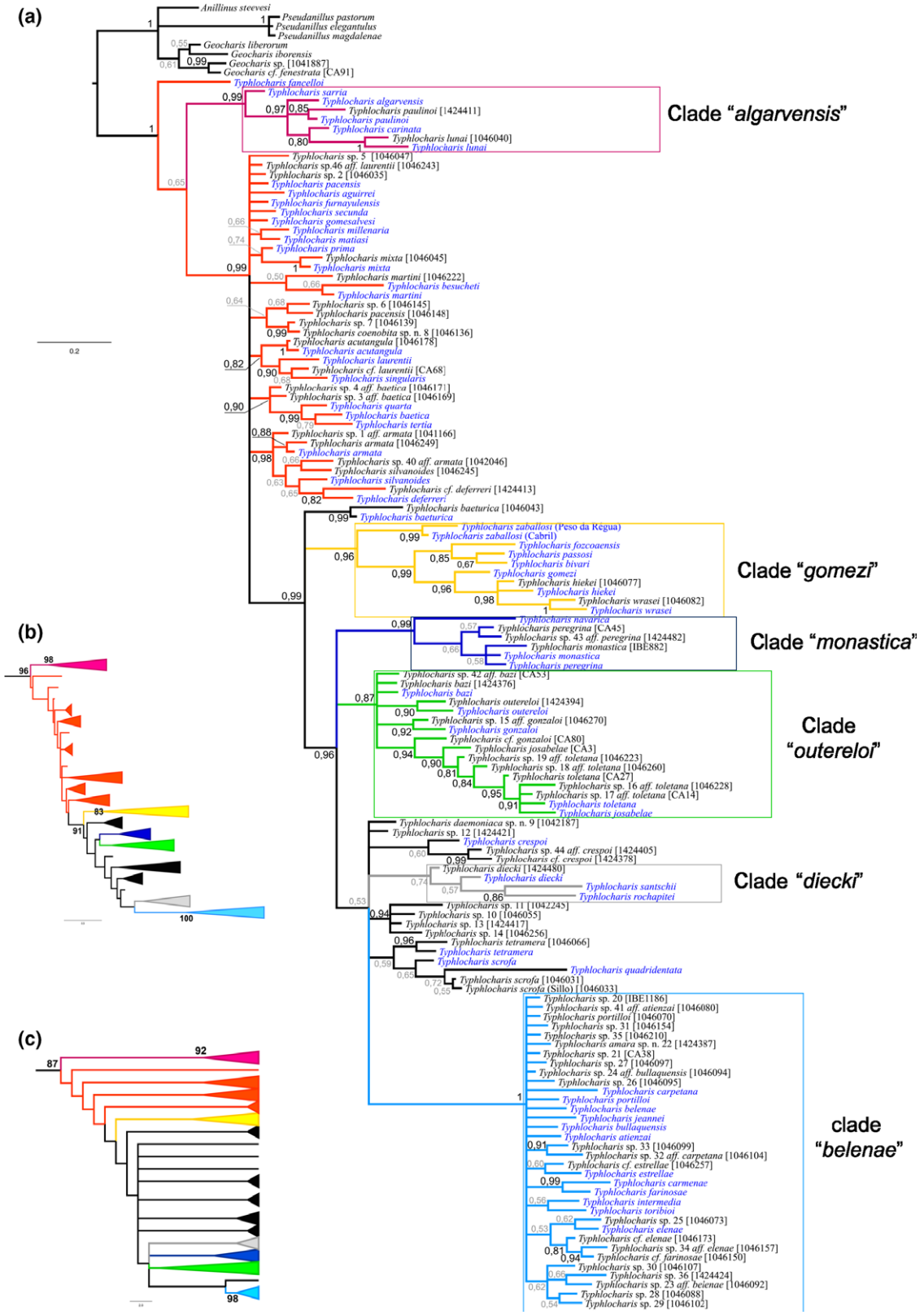

Fig. 2. Phylogenetic relationships of *Typhlocharis* obtained from morphological data (data set RM, 133 ingroup terminals, eight outgroups). (a) BI tree (50% majority rule consensus). Terminals in black refer to hologenophores, in blue to described species. (b) ML tree. (c) MP tree (strict consensus of 12 trees, length 1281, CI: 0.182, RI: 0.717). Support in MP and ML trees are only indicated in nodes > 80. [Colour figure can be viewed at wileyonlinelibrary.com]

e-TE (Figs 3 and 4), analyses under MP (80 trees, length 9158, CI: 0.251; RI: 0.602), BI, standard ML and ML with *binary backbone* were consistent, with BI showing the higher support on nodes for the eight main clades and relationships between them. BI analyses showed a basal split in two large lineages: the first one (*Lineage I*; posterior probability 0.96) splits in a cluster (0.96) including *T. fancelloi* plus **clade "*algarvensis*"** (0.99) and a cluster with **clade "*silvanoides*"** (0.98). The second large lineage (*Lineage II*; 0.98) is subdivided in two highly supported clusters: the first (0.99) with clades "*diecki*" (1), "*gomezi*" (0.83) and "*quadridentata*" (0.81) and the second (1) that includes a cluster for clade "*outereloi*" (1) and a cluster (1) for clades "*monastica*" (0.99) and "*belenae*" (1).

Terminals represented by morphological data only in data set e-TE were consistenly placed within the same clades in most cases, independently of the phylogenetic methodology used, despite showing unstable positions within its clade in some cases (*T. besucheti*, *T. quadridentata*, *T. navarica* and *T. carpetana*). The only exceptions were: (i) *T. fancelloi*, recovered as an early split within *Typhlocharis* (MP analyses; Fig. 2), close to clade "*algarvensis*" (BI; Fig. 3) or nested within clade "*silvanoides*" (ML, ML with *binary backbone*; Fig. 4); and (ii) *T. santschii* plus *T. rochapitei*, both clustered together in a clade recovered as part of clade "*diecki*" (BI, MP; Fig. 3) or as an independent clade of uncertain position (ML, ML with *binary backbone*; Fig. 4). This instability was reflected in the lower support values for ML and MP, and could have originated from long-branch attraction effects. The two populations of *T. zaballosi* were always recovered as sister taxa.

Phylogenetic signal and ancestral state reconstruction

Analyses of phylogenetic signal and ancestral state reconstruction of morphological characters yielded similar patterns over the two analysed trees. Results for individual characters over the ultrametric tree are represented in Appendix S3. Consistency (CI) and retention (RI) indexes for each character are specified in Table S2. CI of characters was generally lower over the total evidence BI tree topology than over the ultrametric molecular tree. Overall, the results pointed to a high level of homoplasy in the selected characters, and many of them reflect "morphological trends" instead of proper apomorphies. However, some traits were informative despite showing certain levels of homoplasy and we distinguish between the following situations: (i) strict apomorphies—diagnostic traits of a clade, shared by all their species and no other species outside the clade; (ii) nonstrict apomorphies—diagnostic or typical of a clade, not present in any other clade but not shared by all the species within a clade; and (iii) homoplasic apomorphies—those typical of a clade, but that could appear in species outside the clade. The main character transformations are represented in Fig. 4.

Description of clades

The structure of clades obtained with the three sources of data: molecular (from C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted), morphology and total evidence is summarized in Fig. 5. Topologies from all analyses are coherent and a series of well-supported clades are consistently obtained (Fig. 5): eight clades are recovered in molecular and total evidence analyses, six of them also found in morphological analyses. Clades "*silvanoides*" and "*quadridentata*" are not recovered as monophyletic in the morphology-based analyses. Basal relationships between clades were not consistent over all data sets and analyses; however, molecular and total evidence analyses point to a basal split in two large lineages (hence named *Lineage I* and *Lineage II*).

Lineage I

Comprises clades "*algarvensis*" and "*silvanoides*" recovered as monophyletic in molecular and total evidence analyses (Figs 3, 4 and 5). This relationship is well supported and both clades share the same shape of pronotum, with sinuous posterior margin (characters 18 and 20, homoplasic apomorphies), long and slender lateral apophyses in tergite VIII of females (character 92, strict apomorphy), and lack of lateral notches in the last ventrite (character 55) (Fig. 4).

Clade "*algarvensis*"

It groups five described species (*T. sarria*, *T. lunai*, *T. paulinoi*, *T. algarvensis* and *T. carinata*), three of them represented only by morphological data. All of the analyses recovered this clade as well supported (Figs 2 3, 4 and 5). The inner relationships between the species, however, are not resolved. Morphologically, the clade is supported by tongue-shaped prosternal apophysis (character 26, strict apomorphy), presence of microdenticles in the apex of elytra (character 34, nonstrict apomorphy), absence of the scutellar pair of setae (character 43, homoplasic apomorphy), presence of three or four pairs of discal setae (character 44, homoplasic apomorphy), presence of apical groove (character 48, strict apomorphy), lack of abdominal belt (character 54, strict apomorphy), a pattern of chaetotaxy in the last ventrite as **m-m-s-s-l-s-m/m-s-l-s-s-m-m** (character 56, strict apomorphy), presence in males of a mesotibial long seta (character 65, strict apomorphy), limb proportions, with slender metatibiae (characters 71 and 72, strict apomorphies), strong asymmetry between the distal setae of left paramere (character 82, strict apomorphy) and subtriangular shape of gonosubcoxites (character 87, homoplasic apomorphy) (Fig. 4).

The overall shape of aedeagus is also characteristic of the clade: robust and slightly arched, with distal

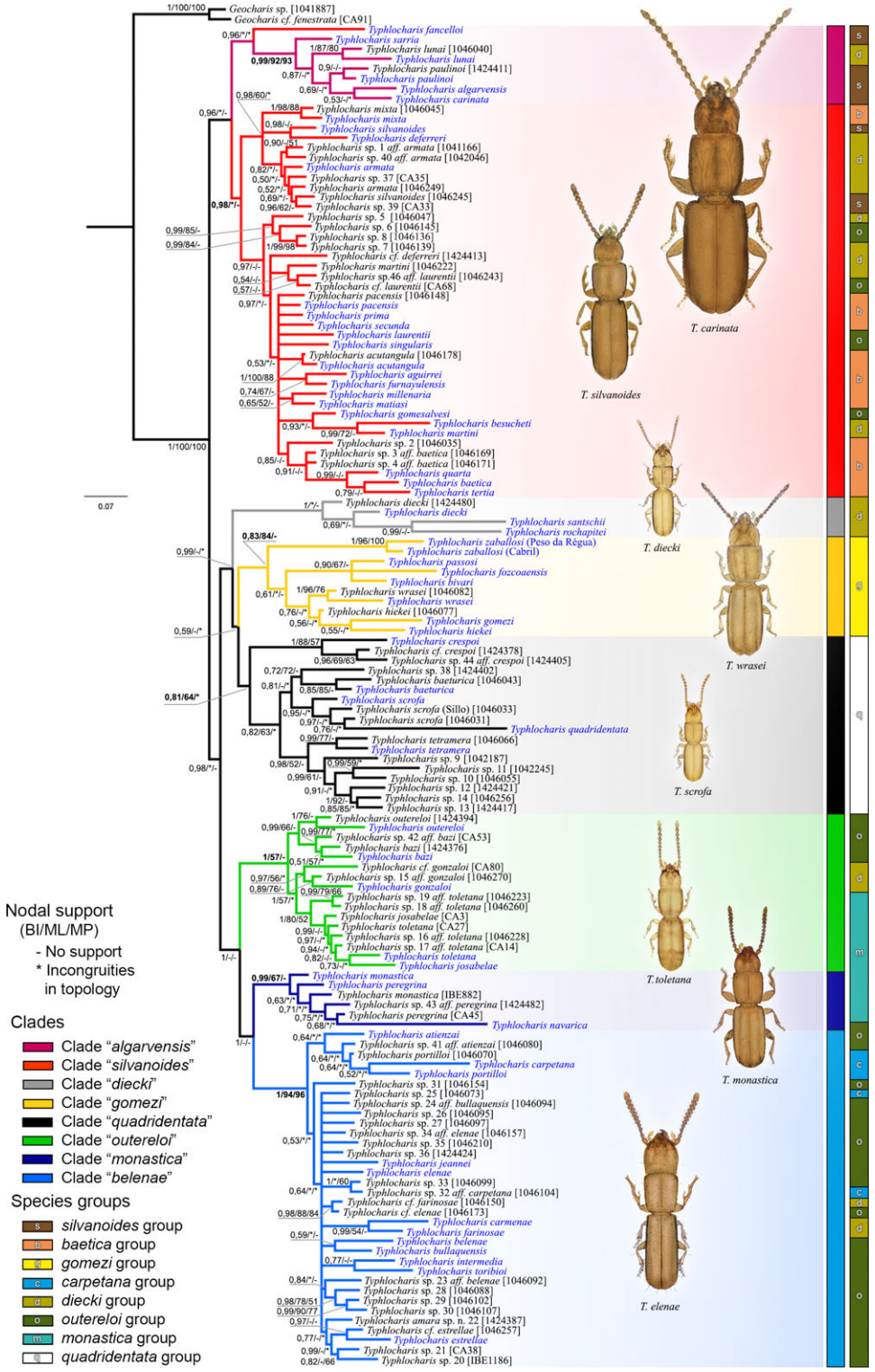

Fig. 3. Phylogenetic relationships of *Typhlocharis* from total evidence analyses (data set e-TE, 136 ingroup terminals, two outgroups). (a) BI tree (50% majority rule consensus). Nodal support indicated as BI/ML/MP (in bold, support of the eight main clades, "-" no support, "*" incongruities in topologies). Terminals in black refer to hologenophores (molecular and morphological data), in blue to described species (only morphological data). Photographs include representatives from the eight main clades (from top to bottom): *T. carinata*, *T. silvanoides*, *T. diecki*, *T. wrasei*, *T. scrofa*, *T. toletana*, *T. monastica* and *T. elenae*. Vertical bars represent clades obtained in this work (left bar) and species groups (right bar) previously proposed for the genus (*sensu* Zaballos and Ruíz-Tapiador, 1997; Zaballos and Wrase, 1998; Ortuño and Gilgado, 2011 and Pérez-González and Zaballos, 2013c). [Colour figure can be viewed at wileyonlinelibrary.com]

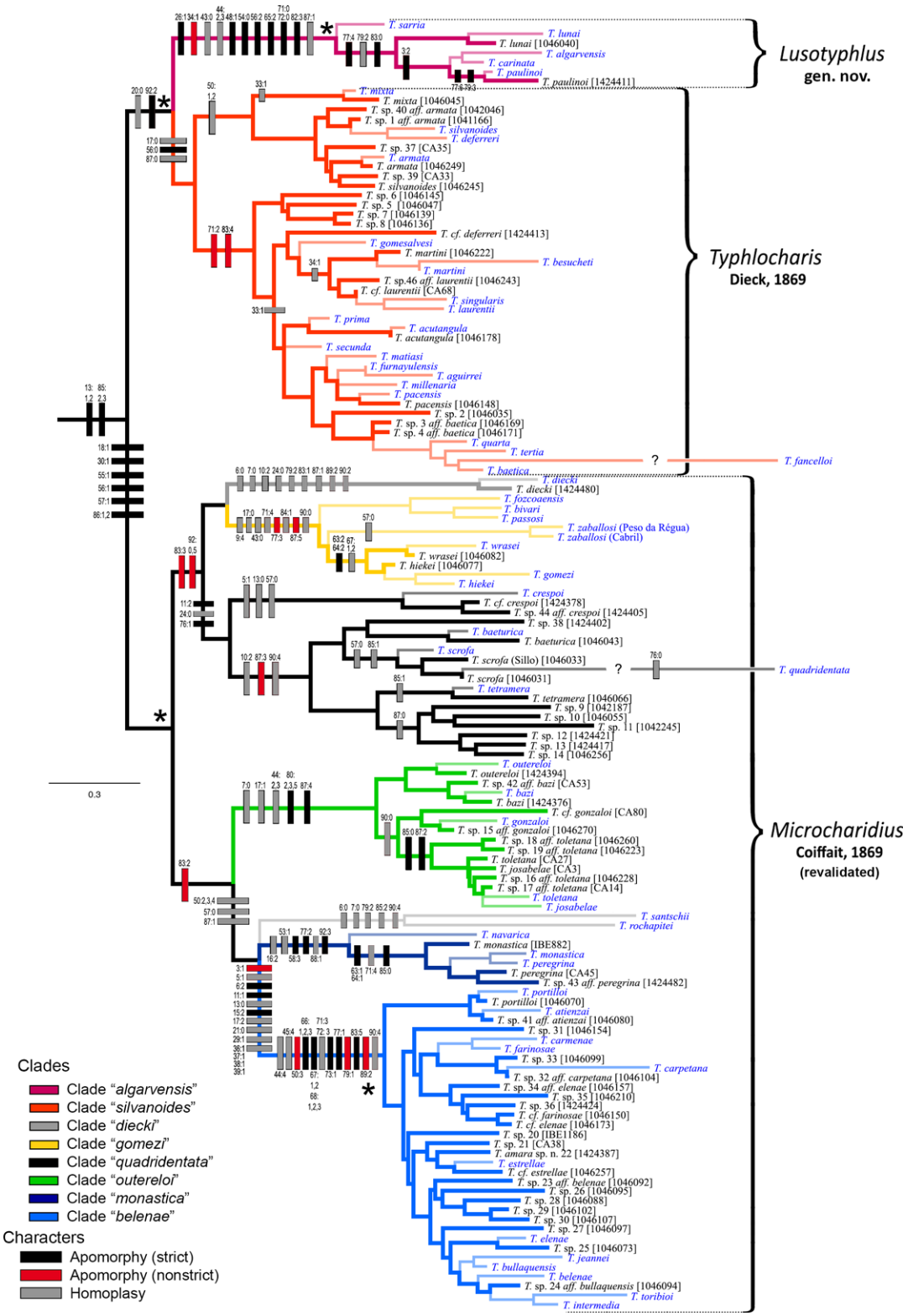

Fig. 4. Reconstruction of characters. *Binary backbone* ML tree (data set e-TE over BI molecular topology). Thick branches with black names represent the constrained topology; thin branches with blue names, added terminals (described species, only morphological data). "*"nodal support > 90. Character evolution: black bars, "strict" apomorphies; red bars, "nonstrict" apomorphies; grey bars, homoplasies. Numbers outsides the bar correspond to "character number: state or states acquired in that clade (as defined in Appendix S1)". Terminal taxa grouped according to the new systematic proposal. [Colour figure can be viewed at wileyonlinelibrary.com]

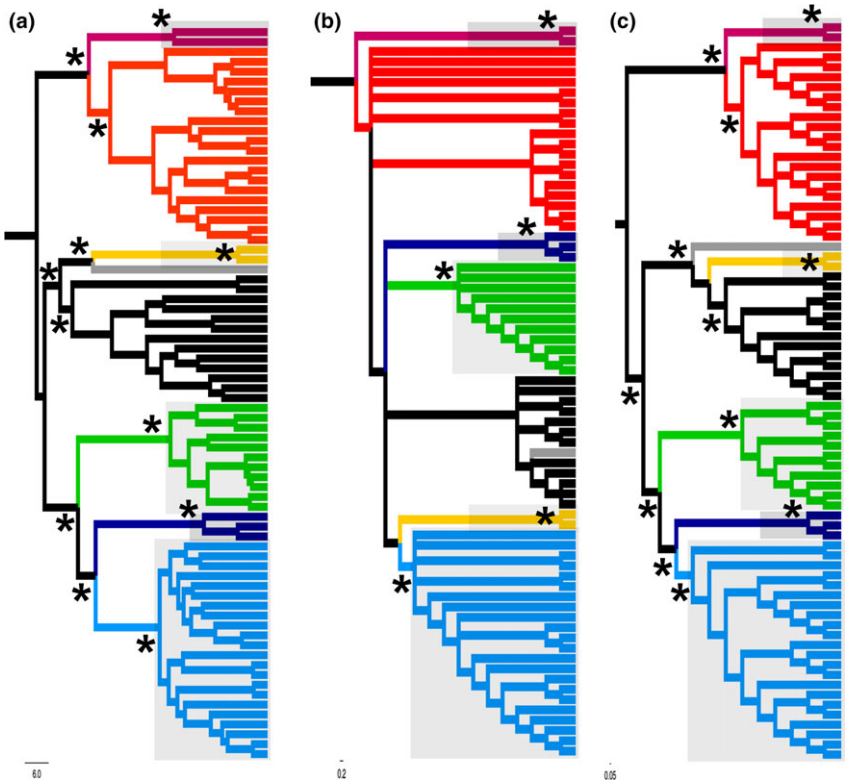

Fig. 5. Summary of the different topologies obtained (BI trees, 50% majority rule consensus). (a) Molecular analyses (modified from C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted); (b) Morphological analyses; (c) Total evidence analyses. "*" indicate nodal support > 0.9. Clades recovered in all the analyses are highlighted in grey. [Colour figure can be viewed at wileyonlinelibrary.com]

lateral crests (character 77, nonstrict apomorphy), broad and rounded apex (character 79, homoplasic apomorphy) and "arrowpoint-shaped" endophallic sclerites (character 83, strict apomorphy); except *T. sarria*, that does not show these features and *T. paulinoi*, that possesses an unique shape of the aedeagus (characters 77 and 79, strict apomorphies) (Fig. 4).

Clade "*silvanoides*"

This clade includes 34 species, 20 already described (*T. mixta*, *T. silvanoides*, *T. deferreri*, *T. armata*, *T. gomesalvesi*, *T. besucheti*, *T. martini*, *T. singularis*, *T. laurentii*, *T. prima*, *T. acutangula*, *T. secunda*, *T. matiasi*, *T. furnayulensis*, *T. aguirrei*, *T. millenaria*, *T. pacensis*, *T. quarta*, *T. tertia* and *T. baetica*; six with molecular data available), and 14 undescribed taxa, represented by both molecular and morphological data.

The clade is supported by the following features: a chaetotaxy pattern in the last ventrite of **l-s-s-l-s-s/s-s-l-s-s-l** (character 56, strict apomorphy), last antenommere with a pattern of three anterior and one posterior *sensilla coeloconica* (character 17, homoplasic apomorphy) and rounded gonosubcoxites (character 87, homoplasic apomorphy) (Fig. 4). The inner relationships of the group are not resolved, apparently affected by the high number of terminals represented uniquely by morphological data, but at a broad level it is possible to distinguish two large subclades: one containing *T. mixta*, *T. armata*, *T. deferreri*, *T. silvanoides* and related taxa (sharing the presence of ventral foveae in females, character 50, homoplasic apomorphy), and another with the rest of the species.

The position of *T. fancelloi* is uncertain. All analyses relate this species to clades "*algarvensis*" or "*silvanoides*", or recover it as sister to the rest of the genus. Represented by a single terminal with only morphological data, *T. fancelloi* shows a singular appearance that probably favours this instability; with autapomorphies, such as the elongated aedeagus (character 77) or the pattern of *sensilla coeloconica* in the last antennomere (character 17).

Lineage II

Lineage II was consistently recovered with morphology and total evidence approaches and most molecular analyses (C. Andújar, S. Pérez-González, P. Arribas, J.P. Zaballos, A. Vogler, and I. Ribera, submitted), clustering all remaining clades (clades "*diecki*", "*gomezi*", "*quadridentata*", "*outereloi*", "*monastica*" and "*belenae*") which shared several morphological features, such as the trapezoidal shape of pronotum

(character 18, strict apomorphy), presence of rail in the apical region of elytra (character 30, strict apomorphy), lateral notches in last ventrite (character 55, strict apomorphy), a pattern of chaetotaxy in last ventrite of **l-s-s-l-s-s/s-l-s-l-s-s-l** (character 56, strict apomorphy) and gonocoxites with lateral setae (character 86, strict apomorphy). A wide intermetacoxal space (character 57, strict apomorphy) is also characteristic of the lineage, but not present in all the clades (Fig. 4).

Within *Lineage II,* clades "*diecki*", "*gomezi*" and "*quadridentata*" are grouped with moderate to high support in molecular and total evidence analyses (Figs 3, 4, 5). These clades share affinities in the shape of endophallic sclerites (character 83, nonstrict apomorphy) and shape of lateral apophyses in tergite VIII of females (character 92, nonstrict apomorphy). Clades "*outereloi*", "*monastica*" and "*belenae*" also form a well-supported group in molecular and total evidence analyses (Figs 3, 4 and 5), but this group is not associated with unequivocal apomorphic features, despite affinities in shape of endophallic sclerites (character 83, nonstrict apomorphy) (Fig. 4). The sister relationship between clades "*monastica*" and "*belenae*" is supported by the constant presence of deep ventral foveae in females (character 50, homoplasic apomorphy), narrow intermetacoxal space (character 57, homoplasic apomorphy) and subtriangular gonosubcoxites (character 87, homoplasic apomorphy) (Fig. 4).

Clade "*diecki*"

It includes three described species: *T. diecki*, *T. santschii* and *T. rochapitei*. Only *T. diecki* is represented by molecular and morphological data. This clade lacks unequivocal apomorphies, but some homoplasic characters can be recognized as typical for this group, such as the ligula, with very sort paraglossae (characters 6 and 7) or the broad and round shape of the apex of aedeagus (character 79) (Fig. 4).

Typhlocharis diecki is characterized by the shape of labium, with pointy epilobes and a very low medial tooth (character 10), posterolateral denticles of pronotum barely insinuated (character 24), rod-shaped endophallic sclerites (character 83), a very long spermathecal duct (character 89) and ovoid spermatheca (character 90) (Fig. 4).

Typhlocharis santschii and *T. rochapitei*, represented only by morphological data, are always recovered as sister taxa with moderate to high support. They have short tubular gonocoxites (character 85) and subcylindrical spermatheca (character 90) (Fig. 4).

The position in the tree of the cluster formed by *T. santschii* and *T. rochapitei* is unstable. The majority of analyses recover it as part of clade "*diecki*", together with *T. diecki* (BI, MP, from morphological data and total evidence); however, some ML analyses (total evidence) recover *T. santschii* and *T. rochapitei* as sister group to clade "*outereloi*", which also have morphological affinities (character 7). Its position remains uncertain and additional data are needed to resolve the relationships of this clade.

Clade "*gomezi*"

This clade covers seven described species (*T. fozcoaensis*, *T. bivari*, *T. pasossi*, *T. zaballosi*, *T. wrasei*, *T. gomezi* and *T. hiekei*), only two of them represented by both molecular and morphological data. Clade "*gomezi*" shows moderate to high support in all analyses and it is well characterized by the presence of a medial tooth in the clypeus (character 9, homoplasic apomorphy), absence of scutellar pair of setae (character 43, homoplasic apomorphy) and shape of metatibiae, dilated and with denticles in the distal area (character 71, homoplasic apomorphy).

Regarding male genitalia, recurved aedeagus (character 77, nonstrict apomorphy) and ring sclerite with a broad and rounded distal end (character 84, homoplasic apomorphy) are predominant. In the female genitalia, gonosubcoxites typically have a bent distal projection (character 87, nonstrict apomorphy) and subsphaeric, large spermathecae (character 90, homoplasic apomorphy) (Fig. 4). Intraclade relationships are not fully resolved, but a structure of three subclades is consistenly recovered: a subclade with the two populations of *T. zaballosi* (Cabril and Peso da Régua); a second with *T. fozcoaensis*, *T. bivari* and *T. pasossi*; and a third one including *T. wrasei*, *T. gomezi* and *T. hiekei*.

Clade "*quadridentata*"

This clade includes five described species (*T. crespoi*, *T. baeturica*, *T. scrofa*, *T. tetramera* and *T. quadridentata*), three of them with molecular and morphological data and two only with morphological data, and nine undescribed taxa represented by molecular and morphological data. It is supported by the diffuse gula (character 11, homoplasic apomorphy), barely insinuated posterolateral denticles of pronotum (character 24, homoplasic apomorphy) and tetramerous tarsi (character 76, strict apomorphy) (Fig. 4). Morphology-based analyses did not recover monophyly for these species, appearing as different polyphyletic lineages of uncertain relationships (Fig. 2). However, molecular and total evidence data recovered this clade with moderate to high support and relationships within the species are also resolved with coherence (Figs 3, 4 and 5). *Typhlocharis crespoi* and two related taxa are separated as a subclade, characterized by sexually dimorphic mandibles (character 5, homoplasic apomorphy), lack of stridulatory organ (character 13, homoplasic apomorphy) and narrow intermetacoxal space (character 57, homoplasic apomorphy). A second subclade, formed by *T. baeturica*, *T. scrofa*, *T. tetramera* and seven related taxa, show affinities in the labium, frequently with pointy epilobes and low or

absent middle lobe (character 10, homoplasic apomorphy) and female genitalia with distally "opened" gonosubcoxites (character 87, nonstrict apomorphy) and subcylindrical spermathecae (character 90, homoplasic apomorphy).

Typhlocharis quadridentata had an unstable position within the clade. Analyses supporting monophyly for this clade recover it close to *T. scrofa*, a relationship not supported by morphological evidence, more akin to *T. crespoi*.

Clade "*outereloi*"

Represented by five described species (*T. outereloi*, *T. bazi*, *T. gonzaloi*, *T. toletana* and *T. josabelae*) and seven undescribed taxa. Except *T. gonzaloi*, all are represented by molecular and morphological data. The clade is well supported and defined by a ligula with very short paraglossae (character 7, homoplasic apomorphy), a pattern of two anterior and one posterior *sensilla coeloconica* in the last antennomere (character 17, homoplasic apomorphy), presence of three or four pairs of discal setae (character 44, homoplasic apomorphy), elongation of left paramere (character 80, strict apomorphy) and gonosubcoxites with a vermiform distal projection (character 87, nonstrict apomorphy) (Fig. 4).

The internal relationships of this clade are well resolved in molecular and total evidence analyses (Figs 3, 4 and 5), separating a subclade—formed by *T. outereloi*, *T. bazi* and one related nondescribed taxon—from the rest of species which share the sphaeric shape of spermatheca (character 90, homoplasic apomorphy). Within these species, the subclade including *T. toletana*, *T. josabelae* and four related taxa is characterized as well by the female genitalia, with unguiform gonocoxites (character 85, strict apomorphy) and distally recurved gonosubcoxites (character 87, strict apomorphy).

Clade "*monastica*"

This clade groups three described species (*T. navarica*, *T. monastica* and *T. peregrina*) and one undescribed taxon. *Typhlocharis navarica* is the only species represented solely by morphological data.

Well supported in all of the analyses, the clade is characterized by the lengthening of stem in the 3rd antennomere (character 16, homoplasic apomorphy), presence of medial tubercles in first ventrite of males (character 53, homoplasic apomorphy), club-shaped, sexually dimorphic profemora (character 58, strict apomorphy), shape of aedeagus, falciform and robust (character 77, strict apomorphy), a spermathecal duct without clearly different regions (character 88, homoplasic apomorphy) and spatulated lateral apophyses of tergite VIII (character 92, strict apomorphy) (Fig. 4). Moreover, except *T. navarica*, the clade shows a crest of small denticles in mesotibiae (characters 63 and 64, strict apomorphy), dilated metatibiae with denticles (character 71, homoplasic apomorphy) and unguiform gonocoxites (character 85, strict apomorphy). The position of *T. navarica* was unstable within the clade and remains unresolved.

Clade "*belenae*"

This clade comprises 13 described species (*T. portilloi*, *T. atienzai*, *T. carmenae*, *T. farinosae*, *T. carpetana*, *T. estrellae*, *T. elenae*, *T. jeannei*, *T. bullaquensis*, *T. belenae*, *T. toribioi*, *T. intermedia* and *T. amara*), two of them represented by morphological and molecular data, as well as 20 undescribed taxa (with molecular and morphological data). This clade is consistently recovered and well supported in all analyses, but the internal relationships are not resolved. It is the clade with a highest morphological divergence and is supported by many apomorphies, such as sexually dimorphic mandibles, hypertrophied in males (character 3, nonstrict apomorphy; character 5, homoplasic apomorphy), subtriangular ligula (character 6, strict apomorphy), narrow gula (character 11, strict apomorphy), lack of stridulatory organ (character 13, homoplasic apomorphy), reniform flagellomeres (character 15, strict apomorphy), a pattern of one anterior and one posterior *sensilla coeloconica* in the last antennomere (character 17, homoplasic apomorphy), lack of medial hiatus (character 21, homoplasic apomorphy), atrophied elytral buttonholes (character 29, homoplasic apomorphy), strongly marked elytral pores (characters 36, 37, 38 and 39, homoplasic apomorphies), pseudodiscal setae in elytra (character 44, homoplasic apomorphy), one pair of well-developed apical setae (character 45, homoplasic apomorphy), deep ventral foveae in first ventrite of females, frequently double (character 50, nonstrict apomorphy), metatibial spur and metatibial long seta in males (characters 66 and 73, strict apomorphies), sexually dimorphic, angulose metatrochanters (characters 67 and 68, strict apomorphies), nondilated metatibiae (characters 71 and 72, homoplasic apomorphies), strongly curved, falciform aedeagus, (character 77, strict apomorphy) with tipically subtriangular and slightly elongated apex (character 79, nonstrict apomorphy) and "bicycle seat"-shaped endophallic sclerites (character 83, strict apomorphy); very long spermathecal duct (character 89, nonstrict apomorphy) and subcylindrical spermatheca (character 90, homoplasic apomorphy) (Fig. 4).

Discussion

Congruence of the analyses

The structure of clades obtained from morphological data and total evidence was generally coherent, also in agreement with the molecular-based topology (Fig. 5). In general, morphology-based analyses showed lower

support and do not recover monophyly of clades that are well supported when including molecular data (clades *silvanoides* and *quadridentata*). Species from clade *silvanoides* were arranged as a series of basal branches and their relationships were not resolved, species from clade *quadridentata* were recovered as polyphyletic lineages. In addition, the monophyly of clade *diecki* could not be tested, because it is represented by a single species (*T. diecki*) in the molecular data. These three clades are characterized by a lack of autapomorphic features, a combination of several characters regarded as homoplasic, and a high level of morphological diversity within the group. The inclusion of the morphological partition in the total evidence analyses also lowers the support values when compared with the molecular tree, whereas both analyses are largely congruent in their general topology.

However, the integrated study of morphological and molecular data has been proven to be a useful approach; morphological data do not contradict molecular data but instead complement it, even in a group affected by high levels of homoplasic morphological evolution. Building upon the resolution of DNA markers we have unified nondescribed taxa (up to 45 according to our results) and all of the 62 known species of the group into a phylogenetic framework. Most species represented by morphological data only have been unambiguously placed within the phylogeny (e.g. *T. carinata*, *T. besucheti*, *T. carmenae*) and those cases with ambiguous positions (e.g. *T. fancelloi*, *T. santschii*, *T. rochapitei*, *T. quadridentata*) show the direction of future efforts to improve our knowledge on the group. These efforts should contribute towards filling the gaps of molecular data for those species only represented by morphological information and to obtain new molecular markers, such as complete mitochondrial genomes, to improve the phylogenetic resolution in some parts of the tree. Overall, the extensive analyses here conducted for 92 morphological characters and ≈3900 DNA positions from mitochondrial and nuclear genes provided robust results and allowed evaluation of the phylogenetic signal of morphological traits and discussion of character evolution to establish the basis for new internal systematics of the group.

Evolution of morphological characters

Many characters have low values of CI (Table S2), suggesting that morphological traits in *Typhlocharis* are prone to high levels of homoplasy. We cannot fully discard a certain degree of bias in the homoplasy recovered due to character coding and definition. Some structures (e.g. character 83, endophallic sclerites) are problematic because they are difficult to categorize into discrete character states (i.e. coding problems), despite showing recognizable patterns associated to certain species or clades (i.e. potential phylogenetic signal). It is known that decisions during character coding could affect analyses and different criteria have their own advantages and inconveniences (Pleijel, 1995). Nevertheless, the general high level of homoplasy cannot be explained only by coding biases. The strong adaptive pressures to live belowground and the geographical speciation patterns often associated with this environment (Andújar et al., 2016) are usually linked to morphological convergences (including size reduction, depigmentation, shortening of extremities, loss of eyes or flight capacity; Eisenbeis and Wichard, 1987), but less is known about how this may affect interspecific divergence within endogean lineages.

Typhlocharis stands out by its singular and conservative body plan within Anillini as expected from its adaptation to the endogean environment, but at the same time it shows notable morphological variety expressed in many structures that may or may not bear phylogenetic signal. Strict geographic speciation within a lineage adapted to the special conditions of deep soil does not necessarily imply evolutionary pressures towards morphological divergence; otherwise morphological differentiation may be enhanced by reinforcement of speciation after secondary contact (Servedio and Noor, 2003). This seems a likely hypothesis in the case of *Typhlocharis*, where cases of coexistence of two or even three species are well documented (e.g. Pérez-González and Zaballos, 2013a), and characters with high level of convergence and homoplasy include some traits likely associated with species recognition and reproduction, thus potentially contributing to reinforcement of speciation. Among these features we found the umbilicate series, the presence and shape of denticles in the elytral apex and the singular configuration of female genitalia, which have been considered key characters in the study of *Typhlocharis*, and even related to miniaturization and specialized lifestyles within the endogean environment (Jeanne, 1973; Zaballos, 1989b). We discuss their evolutionary patterns from the perspective of the obtained phylogenies.

The umbilicate series

Traditionally, the umbilicate series has been widely used to define systematic groups in Anillini based on the different patterns of the setigerous pores (Jeannel, 1937, 1963). *Typhlocharis* is the only Anillini with reduced umbilicate series (eight or less, instead of nine as in the rest of the tribe, with the exception of *Microdipnites mahnerti* Garetto & Giachino, 1999, where the 8th setigerous pore is lost; Giachino, 2015) showing an unique plasticity in the number and distribution of the setigerous pores between species (an

anterior group of 3–4 setae and a posterior group of 1–4 setae, arranged in combinations from 4 + 4 to 3 + 1; see Appendix S3). This variation and the ease of observation promoted the use of the umbilicate series as a key character through the studies about the genus. However, this character is unstable (Jeanne, 1973; Serrano and Aguiar, 2000, 2002; Zaballos and Pérez-González, 2011) and prone to homoplasy (Appendix S3). Variation in this character has been found at different levels:

1. Individual-level variation. Some specimens show asymmetric umbilicate series (Jeanne, 1973; Zaballos and Pérez-González, 2011) with a different pattern in each side of elytra. The most common alterations are loss of setae in the posterior group (commonly the 6th seta) or loss of short setae in the anterior group (either 1th or 3th seta). Duplications of short setae also occur. The incidence of these anomalies is not very high: only 26 cases have been registered in the more than 3000 specimens studied for this work, in species of clades *silvanoides*, *quadridentata*, *outereloi* and *belenae*. The incidence seems to be higher within clade *outereloi*, with cases of nine aberrant specimens detected in series of about 100 specimens from the same population.
2. Population-level variation. Some specimens show a different pattern to that observed for the majority of a species. This case has been registered for several species of clade *silvanoides* (Serrano and Aguiar, 2000, 2002) and clade *outereloi*.
3. Clade-level variation. Distribution of the different types of umbilicate series in the recovered topologies does not show any clear pattern (Appendix S3). The majority of clades cover a broad spectrum of patterns (for example, only in clade *silvanoides* it goes from 4 + 4 to 4 + 1; Pérez-González and Zaballos, 2013a). However, larger umbilicate series is biased to clade *algarvensis* and clade *silvanoides* (a 4 + 4 pattern is exclusive of these clades and 4 + 3 is predominant) and reduced umbilicate series to the rest of clades (where 4 + 2 and 4 + 1 patterns are predominant). The most reduced umbilicate series (3 + 1) appears in clade *belenae*.

The apical denticles of the elytra

Development of denticles on the apex of elytra is rare within Anillini: some species of *Caecoparvus*, *Iason* and *Pelonomites* have small lateroapical denticles near the pore of the 9th umbilicate seta (Giachino and Vailati, 2011; Giachino, 2015) and there are parasutural denticles in two species of *Anillinus* (Sokolov et al., 2014), but the variation, plasticity and frequency of these structures in *Typhlocharis* is exceptional. The results (Fig. 4) support the hypothesis of independent origins for the different types of denticles in *Typhlocharis* (named supernumerary, parasutural and associated to the 7th stria in Pérez-González and Zaballos, 2013a).

Supernumerary denticles arose independently at least twice (Fig. 4). Unique to species of clade *silvanoides*, its origin is probably related to small excrescences in the epipleural margin (microdenticles) present in several species of this clade. The shape and position of these excrescences suggest homology with the larger denticles, which could derive from hypertrophied microdenticles. Parasutural denticles show the highest level of homoplasy (Fig. 4) and they are widespread in the group, developed in clades *algarvensis*, *silvanoides*, *gomezi*, *quadridentata* and *belenae*. Denticles associated to 7th stria are exclusive of *Lineage II*, formed by clades *diecki*, *gomezi*, *quadridentata*, *outereloi*, *monastica* and *belenae*, directly correlated with the presence of a pair of deep folds of cuticle in the inner surface of the distal region of elytra. We propose the name of *rail* for this structure. The rail has been considered a sulcus where the 7th stria reaches the apex of elytra and forms an area with thicker cuticle where the denticles appear (Pérez-González and Zaballos, 2013a). However, not all the species with rail have denticles (in clades *diecki* and *outereloi* is just a small bump) and the relationships between these clades (Fig. 4) suggest that fully developed denticles associated to 7th stria are a convergence originated at least twice (Appendix S3). In addition, parasutural denticles and denticles of 7th stria frequently appear together (especially in clades *quadridentata* and *belenae*).

Size reduction: Jeanne's (1973) hypothesis

The soil is characterized by space limitations that challenge displacement in this environment. Miniaturization is recurrent in endogean lineages as a solution to these constraints. In *Typhlocharis*, reduction of body size has been related to loss of setae in the umbilicate series and appearance of denticles in the apex of the elytra (Jeanne, 1973), a trend interpreted as an increasing adaptation to endogean lifestyles (Zaballos, 1989b). Larger, less rectangular species (presumably less adapted to deep soil), would likely roam in more open spaces, such as the underside of stones, whereas smaller species with more parallel, rectangular or tubular body shapes would roam in narrower spaces, fissures or pores of the soil.

A relationship between miniaturization and umbilicate series reduction (Jeanne, 1973) is broadly coherent with the results, despite several exceptions (large species with reduced pattern: e.g. *T. elenae*, 4 + 1; small species with broad pattern: e.g. *T. baeturica*, 4 + 3). In

fact, 4 + 1 pattern is predominant in clade *quadridentata* (Appendix S3) which shows the strongest trend to miniaturization and includes the smallest species in the group (Pérez-González and Zaballos, 2013a). The fact that this reduction does not occur in any other minute Anillini (e.g. *Argiloborus* Jeannel, 1937) suggests that plasticity in this feature is inherent to the evolution of *Typhlocharis*.

Jeanne (1973) also linked the origin of apical denticles with a hypothetical shortening of the elytral apex associated with miniaturization. Denticles tend to develop in reinforced areas of the apical margin, enhancing the shape of the sutural angle and the end of the 7th stria (Pérez-González and Zaballos, 2013a). Nevertheless, there is not a true shortening of elytra in *Typhlocharis* and the abdomen is always covered by elytra. Other Anillini with truly shortened elytra (e.g. *Winklerites*, *Hypotyphlus*), show weakly sclerotized, nonreinforced apical regions (e.g. Giachino and Vailati, 2011; Magrini, 2013). Also, there is no clear association between small body size and presence of denticles. Although it is true that all species less than 1.10 mm in length show some kind of denticle (e.g. *T. scrofa*, *T. prima*, *T. carpetana*, etc.), there are many exceptions to this trend and denticles are also frequent in medium- to large-sized species. Multiple appearances of different types of denticles and their extended presence over the group suggest an important role, probably related to recognition, social or sexual signaling, or excavation, as reinforcement against abrasion caused by displacement of soil particles (A.R.M. Serrano pers. comm.).

The shape of gonocoxites

The great variation in the shape of gonocoxites in *Typhlocharis* has no equivalent in other Anillini (Pérez-González and Zaballos, 2012, 2013c), being considered a relevant character to the systematics of the genus (Vigna-Taglianti, 1972; Zaballos and Ruíz-Tapiador, 1997; Zaballos and Wrase, 1998). Unguiform gonocoxites are present in all other Anillini (except *Anillotarsus tetramerus*) and thus, it has been assumed to be the plesiomorphic state for *Typhlocharis* (Zaballos and Wrase, 1998), although alternative hypotheses including the possibility of a character reversion have been postulated (Ortuño and Gilgado, 2011). Our results are coherent with unguiform gonocoxites as a reversion occurred independently twice in species from clades *outereloi* and *monastica*. Character reversion scenarios assume that tubular gonocoxites appeared once and do not take into account the possibility of multiple origins for the tubular shape. However, variation within tubular shapes (Pérez-González and Zaballos, 2012, 2013c) shows phylogenetic signal (Fig. 4, Appendix S3); gonocoxites in clades *algarvensis* and *silvanoides* are more slender and lack lateral setae, whereas all other clades (*Lineage II*) share the presence of lateral setae and frequently, more stout shapes. Clade *quadridentata* shows an astounding diversity that covers a whole spectrum from unguiform-like (*T. quadridentata*) or club-shaped (*T. scrofa* and *T. tetramera*) to short tubular gonocoxites (*T. crespoi*) demonstrating that a gradual transition between unguiform and tubular morphologies can occur within a single lineage (Pérez-González and Zaballos, 2013c). Transitional morphologies are not known in clades *outereloi* and *monastica* but both clades include subclades of species with unguiform morphologies as sister taxa to species with short tubular gonocoxites. That at least another genus of Anillini (*Anillotarsus*, from Perú) developed a tubular-like shape of gonocoxites (Mateu, 1980) suggests convergent evolution under certain evolutionary pressures. Gonocoxites are used to lay the eggs (Casale et al., 1982) and different morphologies would likely reflect different strategies of oviposition. Unguiform gonocoxites, which are robust, curved and strongly sclerotized, seem adequate to facilitate removal of soil (digging), creating spaces to lay the eggs. Tubular gonocoxites, which are slender and weakly sclerotized, with sensorial setae concentrated distally, are inadequate for digging and are likely adapted to a tactile function, laying the eggs in natural fissures and spaces of soft soils (Casale et al., 1982; Ortuño and Gilgado, 2011).

Characters with higher phylogenetic signal

Despite the high levels of homoplasy within the array of studied characters, several morphological structures show a notable phylogenetic signal. The lineages and clades obtained from the analyses express clear morphological trends (Pérez-González and Zaballos, 2012) correlated to certain characters that become useful taxonomic tools in the study of the group.

Cephalic structures

Mandibles, clypeus, stridulatory organ and ligula show character states vinculated to certain clades (Appendix S3), but the most relevant cephalic features are the antennae and the gula. Antennae show a progressive gradient from oval to subsphaeric, subsquare and reniform flagellomeres (Pérez-González and Zaballos, 2013b), with a phylogenetic pattern in coherence with the hypotheses of Jeanne (1973) and Zaballos (1989b): rounded flagellomeres in larger species with broad umbilicate series (*Lineage I*: clades *algarvensis* and *silvanoides*), subsquare and reniform flagellomeres in smaller species, generally with reduced umbilicate series (*Lineage II*: clades *diecki*, *gomezi*, *quadridentata*, *outereloi*, *monastica* and *belenae*). There is a clear association between flagellomere shape and stridulatory organ (Appendix S3), absent precisely in the species

with reniform flagellomeres. Square and reniform shapes maximize the concentration on the ventral surface of the flagellomere of a type of *sensilla trichodea* (st3 *sensu* Pérez-González and Zaballos, 2013b), considered olfactory and probably receptors for aggregation pheromones (Pérez-González and Zaballos, 2013b). Thus, these traits could imply different communication mechanisms, ranging from sound emission/reception to a specialization in chemical stimuli (pheromone caption) (Zaballos and Pérez-González, 2011). Aggregation pheromones are documented in other carabids (e.g. Merivee et al., 2000) and would explain why some species of *Typhlocharis* are captured in large numbers (Pérez-González and Zaballos, 2013a). Distribution of *sensilla coeloconica* (sc) in the last antennomere shows a similar pattern (Appendix S3). Despite some exceptions, species from *Lineage II* show a reduced number of sensilla. These sensilla have been regarded as termo-, hygro- or chemoreceptors (Pérez-González and Zaballos, 2013b) and reduction of these sensorial traits could be interpreted as an adaptation to the more stable conditions in deeper layers of the soil.

The gula shows unequivocal phylogenetic signal (CI: 1.00; RI: 1.00) and appears as a diagnostic character for clades *quadridentata* and *belenae* (Appendix S3). A wide gula has been found to be plesiomorphic, in agreement with the morphological trends proposed by Pérez-González and Zaballos (2012). Derived morphologies of the gula appear in clade *quadridentata*, with diffuse gula, and clade *belenae*, with narrow gula associated to the general enlargement of the cephalic capsule typical of this clade.

Thoracic structures

The shape of the pronotum shows notable phylogenetic signal (CI: 0.50; RI: 0:50), with characteristic shapes associated to the main clades (Appendix S3). In spite of the intraspecific variations known for this structure (Pérez-González et al., 2013), subsquare pronotum are typical of clades *algarvensis* and *silvanoides* and subtrapezoidal pronotum are typical of the remaining clades (*Lineage II*). Prosternal apophysis, never used before as taxonomic tool in *Typhlocharis*, is recovered with very high phylogenetic signal (CI: 1.00; RI: 1.00; Appendix S3); tongue-shaped apophysis characterizes very well the species of clade *algarvensis*.

Regarding the elytra, the two most widely used characters, umbilicate series and apical denticles, have been shown ineffective to distinguish the recovered clades (Appendix S3). However, other variations in the elytral apex are among the best features to recognize the clades. The elytra of clade *algarvensis* are characterized by the apical groove, a structure resembling the apical striole of other Bembidiini (Jeannel, 1937) and whose origin could be related to the sulcus that exist near the 9° setigerous pore in other Anillini (Giachino and Vailati, 2011). Clade *silvanoides* lacks apical structures apart from denticles in some species. All other clades (*Lineage II*) share the presence of the aforementioned rail. This structure has not been described in any other Anillini, but *Anillus petriolii* Magrini, 2014 (fig. 12 in Magrini, 2014), *Geocharidius minimus* Sokolov and Kavanaugh, 2014 and *G. jalapensis* Sokolov and Kavanaugh, 2014 (fig. 3 in Sokolov and Kavanaugh, 2014), and *Geocharis iborensis* Zaballos, 1990; *G. julianae* Zaballos, 1989a,b and *G. sp.* (figs 1 in Zaballos, 1989a, 1990; Pérez-González, pers. obs.) show similar folds or keels in the inner surface of the elytral apex.

The limbs are highly prone to developing homoplasic structures. Ornaments such as denticles, tubercles and angular shapes in femora and tibiae are widespread in the group. They are also frequent in other Anillini (femoral denticles in *Serratotyphlus*, *Anillinus*, *Illaphanus* and *Geocharis*, tubercles and rugosities on the inner femora in *Anillinus* or tibial denticles in *Geocharis*; Zaballos, 1990; Sokolov et al., 2004; Giachino, 2005, 2008; Serrano and Aguiar, 2012). The shape of profemora, mesotibiae and metatibiae show character states associated to certain clades (Appendix S3). Some of the structures with stronger phylogenetic signal are sexually dimorphic and could reflect different mating behaviours in different clades. Long tibial setae (for which we propose the term *hair*) in males may be sensory mechanisms to ensure correct position during copulation. Two different types arised independently: in clade *algarvensis*, males of all species show a *long mesotibial hair*; in clade *belenae*, the long hair appears in male metabiae and is paired with the *metatibial spur*, a short, bullet-like seta. Metatrochanters are strongly modified in males of clade *belenae*, where females have deep ventral foveae (Appendix S3) probably allowing a firmer grip during copulation (Zaballos et al., 2016).

The tarsi have strong phylogenetic signal (CI: 1.00; RI: 1.00) and a tetrameric condition is characteristic of the species in clade *quadridentata* (Appendix S3), with sizes ranging from 0.9 to 1.3 mm (Pérez-González and Zaballos, 2013c). Reduction of tarsomere number has been proposed as related to limb shortening and miniaturization in endogean beetles (Coiffait, 1958). All of the cases of strict tetramery confirmed in Anillini (*Pseudanillus*, *Stylulus*, *Anillotarsus* and *Argiloborus*) appear in very small (0.9–1.1 mm) species and the results in *Typhlocharis* are coherent with this trend.

Abdominal structures

Abdominal structures with phylogenetic coherence are the intermetacoxal space, medial tubercles in males and ventral foveae (Appendix S3) (Zaballos and Pérez-

González, 2011; Pérez-González and Zaballos, 2012; Zaballos et al., 2016). But the most informative characters are in the last ventrite: the abdominal belt, presence of lateral notches and the chaetotaxy of posterior margin characterize the main lineages allowing an easy identification (Appendix S3).

The abdominal belt (CI: 0.40; RI: 0.57) is not known in other Anillini (Pérez-González, pers. obs. in *Anillinus*, *Geocharis* and *Pseudanillus*) and is absent in all species of clade *algarvensis*. In the other clades it is highly uniform. Its function remains unknown, but the presence/absence could be related with mechanical differences in invagination/evagination movements of last ventrite (Serrano and Aguiar, 2014).

The chaetotaxy of posterior margin and presence of lateral notches (Pérez-González and Zaballos, 2012) convey the same information (CI: 1.00; RI: 1.00; Appendix S3). Clades *algarvensis* and *silvanoides* show distinctive patterns, in both cases without sexual dimorphism in the long setae and with a continuous posterior margin lacking notches. Clades *diecki*, *gomezi*, *quadridentata*, *outereloi*, *monastica* and *belenae* (*Lineage II*) show sexual dimorphism in the long setae and lateral notches in the posterior margin.

The presence of rail in the elytra matches the presence of lateral notches in last ventrite (Pérez-González and Zaballos, 2012). Both structures fit together physically, fastening elytra and abdomen and, at the same time, allowing invagination/evagination of ventrites in a sliding motion. This ability to seal the abdominal cavity, could be related with the need to resist abrupt changes in humidity, even immersion in water. When closed, the chamber created between the elytra and the membranous dorsal surface of the abdomen could store air or water to regulate gas interchanges or water absorption. The elytral pores could be implied in these processes communicating the outer and inner sides of elytra.

As for the rail, the notches have not been described before in Anillini, but similar lateral grooves or notches can be seen in *Geocharidius integripennis* (Bates, 1882), *G. minimus* Sokolov and Kavanaugh, 2014 and *G. jalapensis* Sokolov and Kavanaugh, 2014 (fig. 2 in Vigna-Taglianti, 1973; fig. 3 in Sokolov and Kavanaugh, 2014), *Honduranillus balli* Zaballos, 1997 (fig. 4 in Zaballos, 1997) and *Geocharis* sp. (Pérez-González, pers. obs.). At least in *Geocharidius* and *Geocharis* these grooves are also associated with a rail-like structure, suggesting that this closing mechanism may not be unique to *Typhlocharis*.

Genital structures

The aedeagus is falciform, as typical in Anillini (Jeannel, 1937, 1963), with subtle variations coherent with the clades (Appendix S3). Clade *algarvensis* shows characteristic aedeagi with predominance of stout shapes with lateral crests in the distal region. The aedeagus within species of clade *belenae* is strongly falciform and curved. A recurved shape seems to be independently acquired at least twice, in *T. sanstchii* and in several species of clade *gomezi*, where it is the most frequent shape. Autapomorphies in the aedeagus are not rare: several species show unique features, either in general shape (e.g. *T. fancelloi*, *T. paulinoi*) or in the apical region (e.g. *T. paulinoi*, *T. hiekei*, *T. atienzai*). It should be noted that all of these species coexist with other species of *Typhlocharis* (Zaballos and Farinós, 1995; Magrini, 2000; Serrano and Aguiar, 2006), suggesting that unique features in aedeagus might act as mechanisms of reproductive isolation.

Gonocoxites, gonosubcoxites, spermathecal complex and lateral apophyses of tergite VIII yielded a good delimitation of the main clades (Appendix S3). Subtriangular gonosubcoxites are widespread in the group and it seems to be the plesiomorphic state. In clade *outereloi*, gonosubcoxites show a distal vermiform projection. It is noteworthy that species with unguiform gonocoxites have more robust and stouter gonosubcoxites than species with tubular gonocoxites. This reinforcement of gonosubcoxites is coherent with an active use of unguiform gonocoxites for digging, given that gonocoxites and gonosubcoxites are articulated.

The spermathecal complex is highly plastic yet spermathecal variation can be summarized in two broad categories: spheroid and related morphologies (round, ovoid, irregular) and subcylindrical and related morphologies ("hourglass" or "peanut" shapes, short cylinder, long cylinder). The first category is predominant and likely plesiomorphic. The second category seems to be a derived condition of clades *monastica* and *belenae* (the latter characterized by very long spermathecal ducts and subcylindric spermathecae), with sporadic convergences in species of clades *algarvensis*, *silvanoides*, *diecki* and *quadridentata*. The spermatheca retains the sperm after copulation (Schuler, 1960), but the factors behind the observed morphological changes are unknown.

The lateral apophyses of tergite VIII are informative despite never considered as a taxonomic tool for the group before. Clades *algarvensis* and *silvanoides* have homogeneous morphologies; with very thin and slender apophyses, whereas in the remaining clades the apophyses are highly variable and virtually each clade shows a particular configuration (Appendix S3).

Finally, there are species with very similar external appearances, where only one of the sexes show differential features in the genital structures. For example, males of *Typhlocharis* sp. 15 *aff. gonzaloi* and *T.* sp. 16 *aff. toletana* have nearly identical external morphology and aedeagus, whereas females have tubular and unguiform gonocoxites respectively. On the contrary, females of *T. wrasei* and *T. hiekei* are almost

indistinguishable, whereas males show completely different aedeagi. The morphological changes suffered by male and female genital structures do not seem to be associated.

Taxonomic implications: species groups vs. obtained clades

The first attempt at an internal systematics for the genus defined six species groups (*silvanoides*, *baetica*, *gomezi*, *carpetana*, *diecki* and *outereloi* groups) based in certain morphological key characters, with emphasis in the pattern of the umbilicate series, which diagnose four of the groups (Zaballos and Ruíz-Tapiador, 1997). A seventh group was defined from the discovery of the first species with unguiform gonocoxites (*monastica* group; Zaballos and Wrase, 1998). With the quick increase in the number of described species, the groups suffered some modifications: the diagnosis of *monastica* group was simplified to fit *Typhlocharis josabelae* Ortuño and Gilgado, 2011 and *T. toletana* Lencina and Andújar, 2010, two species with unguiform gonocoxites that do not resemble the rest of the group (Ortuño and Gilgado, 2011), and the last species group to date (*quadridentata* group) was coined to accommodate several new species with tetramerous tarsi and small size, splitting two species of the *outereloi* group in the process (Pérez-González and Zaballos, 2013c).

The validity of the species groups as systematic entities in this genus has been questioned, as they unlikely reflect true monophyletic lineages (Andújar et al., 2010; Ortuño and Gilgado, 2011; Pérez-González and Zaballos, 2012, 2013a). This criticism is supported by our results (Fig. 3) and from the eight-species groups traditionally considered, only *gomezi* group (*sensu* Zaballos and Ruíz-Tapiador, 1997) and *quadridentata* group (*sensu* Pérez-González and Zaballos, 2013c) remain unaltered and reflect true monophyletic entities, equivalent to the clades *gomezi* and *quadridentata* recovered in the analyses (Fig. 3). Within clade *gomezi*, the two populations of *T. zaballosi* cluster together, thus not contradicting their entity as a single species (Serrano and Aguiar, 2014) despite the morphological differences between them.

The *silvanoides* group splits into two clades: clade *algarvensis* including all the species previously assigned to *silvanoides* group except *T. silvanoides*, recovered within clade *silvanoides*. The original diagnosis, based on the possession of a 4 + 4 umbilicate series, is no longer useful to characterize these species. The *baetica* group has been recovered as a polyphyletic entity nested within clade *silvanoides*, where *T. mixta* is separated from the rest of the species of *baetica* group (*sensu* Zaballos and Ruíz-Tapiador, 1997). These species are part of a single cluster, with low support in total evidence analyses (Fig. 3), that also includes *T. laurentii*, *T. singularis*, *T. gomesalvesi*, *T. besucheti* and *T. martini*, and shares many morphological features with the species of *baetica* group (*sensu* Zaballos and Ruíz-Tapiador, 1997) in spite of lacking the characteristic supernumerary denticles in the elytral apex.

The *monastica* group *sensu* Zaballos and Ruíz-Tapiador (1997) is monophyletic and clusters with *T. navarica* forming clade *monastica* (Fig. 3). The redefinition of the group (Ortuño and Gilgado, 2011) represents a polyphyletic entity, given that *T. toletana* and *T. josabelae* are included in clade *outereloi*, and they are not close geographically or morphologically to *T. monastica* and *T. peregrina*. Likewise, the old *diecki* and *outereloi* groups were not recovered as clades. These groups have been repositories for species with umbilicate series patterns of 4 + 3, 4 + 2 and 4 + 1, without any other significant diagnostic trait (Zaballos and Ruíz-Tapiador, 1997). Our results show that the species of these groups (Fig. 3) are barely related as a whole and cluster in several clades diagnosed by traits different to the umbilicate series.

Finally, the *carpetana* group, defined to include species with anterior umbilicate series with three setae, also resulted in a polyphyletic entity. *Typhlocharis carpetana*, *T. portilloi* and two new taxa (*T.* sp. 25 and *T.* sp. 32 *aff. carpetana*), sharing the diagnostic trait for the group, were recovered within clade *belenae* and do not cluster together.

New taxonomic proposal

To sum up, *Typhlocharis* is a complex monophyletic taxon with high morphological diversity, despite their singular and conservative body plan. The previous taxonomic considerations for the group could be tested from the results of this work. *Typhlocharis* has been considered a proper phyletic lineage of Anillini (subtribe Typhlocharina; Jeanne, 1973). The current phylogenetic hypothesis for Anillini supports this criterion, suggesting that *Typhlocharis* is a well-differentiated lineage within the tribe (Andújar et al., 2016), where the internal diversity is phylogenetically structured. Among the most relevant results of this work are the consistency of clade *algarvensis* and a large monophyletic lineage well defined genetically and morphologically (*Lineage II*) clustering clades *diecki*, *gomezi*, *quadridentata*, *outereloi*, *monastica* and *belenae*. Some apomorphies that diagnose these clades (Fig. 4) affect characters, such as shape of pronotum, apex of elytra and in particular the differences in the last ventrite (abdominal belt, shape and chaetotaxy), that, in other Anillini, have led to genus-level distinction (e.g. Jeannel, 1963; Barr, 1995; Giachino, 2005, 2008; Pavesi, 2010; Giachino and Vailati, 2011; Sokolov and Carlton, 2012; Sokolov, 2013).

In 1969, Coiffait described *Microcharidius* as a genus "separated from the lineage of *Typhlocharis* and more evolved", to include *T. quadridentata* (Coiffait, 1969), basing the diagnosis in its tiny size, reduced umbilicate series and elytra fused to the mesothorax. Jeanne (1973) considered these characters to be inadequate for a genus-level split and established *Microcharidius* as a synonym of *Typhlocharis*. However, some authors kept *Microcharidius* as a subgenus to fit *T. outereloi* and *T. belenae* (Novoa, 1978; Zaballos, 1983), a proposal that has since fallen into disuse (Pérez-González and Zaballos, 2012). *Typhlocharis quadridentata*, *T. outereloi* and *T. belenae* are now recovered as part of *Lineage II*, which includes six of the eight obtained clades (Figs 2, 3 and 4). The former diagnosis for *Microcharidius* is inadequate to define this lineage, but is largely identified by other features discussed before (e.g. pronotum, rail and notches and chaetotaxy of the last ventrite) and Coiffait's (1969) criterion is supported by the results.

On this basis, we propose a phylogeny-based restructuring of the internal systematics of the group, with a split of genus *Typhlocharis sensu* Dieck, 1869a,b into three genera: *Lusotyphlus* **gen. nov.** for the species of clade *algarvensis*; *Typhlocharis* Dieck, 1869 for the species of clade *silvanoides*; and *Microcharidius* Coiffait, 1969 (revalidated) for the species of *Lineage II* (Table 1), within the subtribe Typhlocharina Jeanne, 1973 recognized as a distinct entity within the tribe Anillini Jeannel, 1937 (Zaballos, 2003).

Subtribe Typhlocharina Jeanne, 1973

Type genus: *Typhlocharis* Dieck, 1869

Diagnosis (emended from Jeanne, 1973): Anillini from 0.8 mm to 2.9 mm, characterized by genera with the following synapomorphic characters: square-shaped pronotum, reduced umbilicate series (eight or fewer setae) and male protarsi without adhesive phaneres.

Genus composition: *Lusotyphlus* **gen. nov.**, *Typhlocharis* Dieck, 1869 and *Microcharidius* Coiffait, 1969 (revalidated).

Lusotyphlus gen. nov.

Type species: *Lusotyphlus algarvensis* **comb.nov.** (Coiffait, 1969)

Diagnosis: Anillini from 1.4 mm to 2.9 mm in length, anophthalmous, body subparallel covered with microreticulation and scattered pubescence. Vertex with stridulatory organ. Moniliform antennae, ovoid antennomeres and variable pattern of *sensilla coeloconica* in last antennomere, always with five or more sensilla. Large and curved mandibles. Wide gula. Pronotum subsquare, lateral margins smoothly arquate and sinuous posterior margin. Prosternal apophysys tongue-shaped. Elytra without scutellar setae, always with three or four pairs of discal setae and one or two pairs of long subapical setae. Apical region of elytra rounded, with apical grooves; without denticles or with parasutural denticles, commonly with microdenticles. Limbs slender, males with mesotibial long seta. Last ventrite without belt, posterior margin smooth and continuous, without lateral notches, and pattern of chaetotaxy **m-m-s-s-l-s-m/m-s-l-s-s-m-m**. Male genitalia: robust and smoothly arched aedeagus, with distal crests or flaps. Endophallic sclerites arranged in an "arrowpoint-like" fashion. Two distal setae of the parameres asymmetric, with long and sabre-like lower seta in left paramere. Female genitalia: tubular gonocoxites, long and curved without lateral setae; gonosubcoxites subtriangular and apophyses of tergite VIII long and thin.

Etymology: From *Luso-* a prefix referring to Lusitania, the latin name for Portugal, where all of the species of this genus have been found so far, and *-typhlus*, from the Greek τυφλός ("*typhlós*" = blind), a root commonly used for naming Anillini. Masculine name.

Typhlocharis Dieck, 1869

Type species: *Typhlocharis silvanoides* Dieck, 1869

Diagnosis: Anillini from 1.03 mm to 1.8 mm in length, anophthalmous, body subparallel covered with microreticulation and scattered pubescence. Vertex with stridulatory organ. Moniliform antennae, subsphaeric antennomeres and pattern of *sensilla coeloconica* in last antenommere with three anterior and one posterior sensilla. Wide gula. Pronotum subsquare, lateral margins straight or smoothly curved and sinuous posterior margin. Elytra with a pair of scutellar setae, no discal setae and apical row of long or short thin setae, rarely with apical or subapical setae outstanding. Apical region of elytra rounded, without denticles or with parasutural or supernumerary denticles. Last ventrite with belt, posterior margin smooth and continuous, without lateral notches, and pattern of chaetotaxy **l-s-s-l-s-s/s-s-l-s-s-l**. Male genitalia with falciform aedeagus, with apical lamina subtriangular and blunt. Endophallic sclerites arranged as irregular rods of bifurcated structures with lateral projection curved upwards. Female genitalia with long, tubular gonocoxites, without lateral setae; gonosubcoxites blunt and rounded and apophyses of tergite VIII long and thin.

Etymology: *Typhlo-* from the Greek τυφλός ("*typhlós*" = blind), and *–charis*, from the Greek χάρις ("*charis*" = grace), both roots commonly used for naming Anillini. Feminine name.

Microcharidius Coiffait, 1969 (revalidated)

Type species: *Microcharidius quadridentatus* Coiffait, 1969

Table 1
New systematic proposal for Typhlocharina Jeanne, 1973 from the results of the present work

Family CARABIDAE Latreille, 1802
Subfamily TRECHINAE Bonelli, 1810
Tribe ANILLINI Jeannel, 1937
Subtribe TYPHLOCHARINA Jeanne, 1973
Genus *Lusotyphlus* gen. nov.
L. algarvensis (Coiffait, 1969) **(comb. nov.)**
L. lunai (Serrano and Aguiar, 2006) **(comb. nov.)**
L. paulinoi (Serrano and Aguiar, 2006) **(comb. nov.)**
L. sarrius (Serrano and Aguiar, 2001) **(comb. nov.)**
Genus *Typhlocharis* Dieck, 1869
T. acutangula Pérez-González et al., 2013
T. aguirrei Zaballos and Banda, 2001
T. armata Coiffait, 1969
T. besucheti Vigna-Taglianti, 1972
T. deferreri Zaballos and Pérez-González, 2011
T. fancelloi Magrini, 2000
T. furnayulensis Zaballos and Banda, 2001
T. gomesalvesi Serrano and Aguiar, 2002
T. laurentii Magrini, 2000
T. martini Andújar, Lencina and Serrano, 2008
T. matiasi Zaballos and Banda, 2001
T. millenaria Zaballos and Banda, 2001
T. mixta Pérez-González et al., 2013
T. pacensis Zaballos and Jeanne, 1987
T. prima Pérez-González and Zaballos, 2013
T. quarta Pérez-González and Zaballos, 2013
T. secunda Pérez-González and Zaballos, 2013
T. silvanoides Dieck, 1869;
T. singularis Serrano and Aguiar, 2000
T. tertia Pérez-González and Zaballos, 2013
Genus *Microcharidius* Coiffait, 1969 (revalidated)
Clade ***diecki***
M. diecki (Ehlers, 1883) **(comb. nov.)**
M. rochapitei (Serrano and Aguiar, 2008) **(comb. nov.)**
M. santschii (Normand, 1911) **(comb. nov.)**
Clade ***gomezi***
M. bivari (Serrano and Aguiar, 2006) **(comb. nov.)**
M. fozcoaensis (Serrano and Aguiar, 2005) **(comb. nov.)**
M. gomezi (Zaballos, 1991) **(comb. nov.)**
M. hiekei (Zaballos and Farinós, 1995) **(comb. nov.)**
M. passosi (Serrano and Aguiar, 2005) **(comb. nov.)**
M. wrasei (Zaballos and Farinós, 1995) **(comb. nov.)**
M. zaballosi (Serrano and Aguiar, 2014) **(comb. nov.)**
Clade ***quadridentata***
M. baeturicus (Pérez-González and Zaballos, 2013) **(comb.nov.)**
M. crespoi (Serrano and Aguiar, 2008) **(comb. nov.)**
M. quadridentatus Coiffait, 1969;
M. scrofa (Pérez-González and Zaballos, 2013) **(comb.nov.)**
M. tetramerus (Pérez-González and Zaballos, 2013) **(comb.nov.)**
Clade ***outereloi***
M. bazi (Ortuño, 2000) **(comb. nov.)**
M. gonzaloi (Ortuño, 2005) **(comb. nov.)**
M. josabelae (Ortuño and Gilgado, 2011) **(comb. nov.)**
M. outereloi (Novoa, 1978) **(comb. nov.)**
M. toletanus (Lencina and Andújar, 2010) **(comb. nov.)**
Clade ***monastica***
M. monasticus (Zaballos and Wrase, 1998) **(comb. nov.)**
M. navaricus (Zaballos and Wrase, 1998) **(comb. nov.)**
M. peregrinus (Zaballos and Wrase, 1998) **(comb. nov.)**
Clade ***belenae***
M. carmenae (Zaballos and Ruíz-Tapiador, 1995) **(comb. nov.)**
M. carpetanus (Zaballos, 1989) **(comb. nov.)**
M. elenae (Serrano and Aguiar, 2002) **(comb. nov.)**
M. estrellae (Zaballos and Ruíz-Tapiador, 1997) **(comb. nov.)**
M. farinosae (Zaballos and Ruíz-Tapiador, 1997) **(comb. nov.)**
M. intermedius (Zaballos, 1986) **(comb. nov.)**
M. jeannei (Zaballos, 1989) **(comb. nov.)**
M. portilloi (Zaballos, 1991) **(comb. nov.)**
M. toribioi (Ortuño, 1988) **(comb. nov.)**
Incertae sedis
T. simoni Ganglbauer, 1900

Diagnosis: Anillini from 0.9 mm to 1.9 mm in length, anophthalmous, body subparallel covered with microreticulation and scattered pubescence. Moniliform antennae, antennomeres subquare to reniform and pattern of *sensilla coeloconica* in last antennomere, with one or two (exceptionally three) anterior and one posterior sensilla. Pronotum subtrapezoidal, narrowed in posterior region, with straight or slightly curved posterior margin. Elytra with variable chaetotaxy, one pair of apical setae and one pair of long and prominent subapical setae. Apical region with rail, frequently with denticles at the end of 7th stria and medial suture. Last ventrite with belt, posterior margin with a pair of lateral notches and pattern of chaetotaxy **l-s-s-l-s-s/s-l-s-l-s-s-l**. Male genitalia variable, aedeagus generally falciform and curved. Female genitalia variable, with unguiform, tubular of club-shaped gonocoxites, always with lateral setae.

Etymology: *Micro-* from the Greek μικρός ("*micrós*" = small), and *–charidius*, derived from the greek χάρις ("*charis*" = grace), a root commonly used for naming Anillini. Masculine name.

Of the three genera, *Lusotyphlus* **gen. nov.**. is the least diverse, with five species (Table 1), and the most restricted geographically, found only in the southwestern part of the Iberian Peninsula. *Typhlocharis* is now restricted to 22 described species (Table 1) plus 12 potential new species, and it spans through the southern half of the Iberian Peninsula and into North Africa. *Microcharidius* Coiffait, 1969 is the most widespread and diverse Typhlocharina, including 35 described species plus 36 potential new species distributed through the southern, central and eastern regions of the Iberian Peninsula, as well as North Africa. This genus includes the highest morphological diversity of the tribe and it is the only one with a clear internal structure, so we propose six species groups within this genus, according to the clades obtained in this work (Table 1).

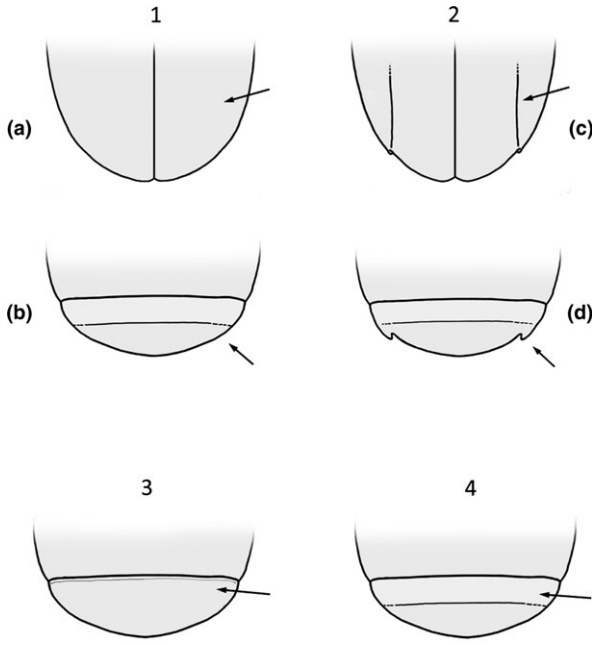

Fig. 6. 1: Apex of elytra (a) and last ventrite (b) of *Typhlocharis silvanoides*. Arrows indicate the 7th stria without rail and posterior margin without lateral notches. 2: Apex of elytra (c) and last ventrite (d) of *Microcharidius diecki*. Arrows indicate the 7th stria with rail and posterior margin with lateral notches. 3: Last ventrite of *Lusotyphlus algarvensis* (without belt). 4: Last ventrite (b) of *Typhlocharis silvanoides*.

Identification key to the genera of Typhlocharina

1. Last ventrite without lateral notches in posterior margin (Fig. 6: 1b). The carina associated with the 7th stria of elytra does not reach the apical margin (Fig. 6: 1a). 2
   Last ventrite with a pair of lateral notches in posterior margin (Fig. 6: 2d). The carina associated with the 7th stria of elytra reaches the apical margin, visible (by transparency) as two deep sulci (rail) (Fig. 6: 2c). . . . . *Microcharidius* (revalidated).
   2. Without abdominal belt (Fig. 6: 3). . . . . *Lusotyphlus* gen.n.
      With abdominal belt (Fig. 6: 4). . . . . *Typhlocharis*.

Conclusions

Typhlocharina represents a complex monophyletic taxon with high morphological diversity, despite their singular and conservative body plan. Of the eight species groups previously established, six do not correspond with natural groups. The total evidence phylogenetic approach allowed definition of two large lineages that include eight natural groups supported by both morphological and DNA data, six of them clustered in the second lineage (*Lineage II*) and well supported. The range of morphological variation between clade *algarvensis*, clade *silvanoides* and *Lineage II* is coherent with genus-level differences, and thus we split *Typhlocharis* into three genera, supported by several apomorphies and consistent with the phylogenetic inferences. The results also suggest that there is a morphological gradient within the group, broadly coherent with the hypotheses of Jeanne (1973) and Zaballos (1989b), with trends towards smaller size and higher morphological specialization that may be associated with ecological differences yet to be determined.

Acknowledgements

The authors want to acknowledge the contribution of all the curators from different institutions that kindly provided access to the specimens, by loan or donation: Beulah Garner and Max Barclay (NHM, London), Thierry Deuve and A. Taghavian (MNHNP, Paris), Fritz Hieke† and Bernd Jaeger (MFNB, Berlin), Lutz Behne (DEI, Müncheberg), Mercedes París (MNCN, Madrid), Karla Schneider and Joachim Händel (ZSMLU, Halle), Gyózo Szél (HNHM, Budapest), Heinrich Schönmann (NM, Viena), Synda Boulahia (INAT, Tunis), Antonio Laborda and Luis Miguel Fernández (CZULE, Leon), Giulio Cuccodoro (MHNG, Geneva) and Dejan Kulijer (NMBH, Bosnia). Thanks, also, to collectors and colleagues who helped in gathering the newly collected specimens of these elusive animals: Jesús Arribas, A. Castro, Arnaud Faille, Carles Hernando, Jose Luis Lencina, Ildefonso Ruiz-Tapiador, Samir Ghannem and Olegario del Junco. Special thanks to Paolo Magrini and Artur R. M. Serrano, who gave access to type specimens of obscure species which have been key to the comprehension of the group. David Wrase, Esther Lantero, Paula Arribas and Jacinto Berzosa provided valuable comments and support during the elaboration of the manuscript. Ignacio Ribera (IBE) and Alfried Vogler (NHM), contributed to the generation of the molecular data. Thanks also to Alfried Vogler for hosting S.P.G in the facilities of the NHM (London). The authors want to express their gratitude to the two anonymous referees that revised the manuscript. This research has been partially funded by projects CGL2010-16944 (Spanish Ministry of Education) and GR35/10-A Research Group 921632 (Santander-UCM). S.P.G. received support from a predoctoral grant of the Universidad Complutense de Madrid and C.A. from a Marie Curie postdoctoral grant (Project SOILBIODIV-705639; Program H2020-MSCA-IF-2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of specimens studied in this work. Abbreviations: Seq., sequences (molecular data); Morph., morphological data; HT, Holotype; PT, Paratype; M, male; F, female; Coll., collection with the specimen deposited: JPZ, MNCN, CA, NHM, CZULE, MT; MHNG, ARS, SPG, DW, RT, MFNB, DEI, MNHNP, OJ. Collectors: AFai, A.Faille; Ag, C.Aguiar; Arr1, P.Arribas; Arr2, J.Arribas; CA, C.Andújar; Cher, C.Hernando; Gh, S.Ghannem; IRT, I.Ruiz-Tapiador; JL, J.Lencina; O, V.Ortuño; PG, S.Pérez-González; R, Reolid; S, A.Serrano; Z, J.P.Zaballos; Var, Various (collections).

Table S2. CI and RI indexes of characters obtained with Mesquite 3.06 over the ultrametric BI molecular tree and the BI total evidence tree (details in main text).

Appendix S1. Character descriptions.

Appendix S2. Morphological matrix.

Appendix S3. Plates I–XII. Ancestral state reconstruction of the morphological characters used in this work, obtained with Mesquite 3.06 over the ultrametric BI molecular tree. Character name and coding as in Appendix S1. Each character state is illustrated. Clade colours as in Figs 3 and 4.

Fig. S1. Phylogenetic relationships based on morphological data, obtained from CM data set (513 terminal taxa (443 hologenophores, 62 "described species" and eight outgroups). (A) MP tree (strict consensus of two trees, length: 2063, CI: 0.108, RI: 0.828); (B) ML tree; (C) BI tree. "*" indicates well supported nodes (MP, ML > 85; BI > 0.85).