**Mitogenomic phylogenetics of *Diochus occultus* n. sp., a
palaeoendemic endogean species within the tribe Diochini
(Coleoptera: Staphylinidae: Staphylininae)**

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22 ABSTRACT

23 The tribe Diochini has a worldwide distribution, with 2 and 74 epigeal species within the
24 genera *Antarctothius* and *Diochus* respectively. Recent phylogenetic studies suggest a sister
25 relationship of Diochini and a lineage formed by Xantholinini, Maorothiini, and Othiini,
26 within the subfamily Staphylininae. Here we describe the first known endogean
27 representative of Diochini, *Diochus occultus* n. sp., and provide the first two complete
28 mitogenomes for the tribe, corresponding to the two European *Diochus* species: *Diochus*
29 *occultus* n. sp. and *Diochus staudingeri*. These sequences were combined with 40 additional
30 mitogenomes from representatives within Staphylininae, Paederinae, Silphidae, and
31 Aleocharinae, and *COI* sequences from 5 additional species of *Diochus* to conduct a series
32 of mitogenomic phylogenetic and dating analyses. The estimated molecular phylogeny is
33 fully consistent with previous studies based on morphology and molecular data, finding a
34 sister relationship of Diochini with a clade formed by Xantholinini and Othiini (Maorothiini
35 not sampled). Dating analyses inferred an early split of the tribe Diochini at 140-156 Mya.
36 Morphology shows clear differences in the aedeagal and external morphology of *D. occultus*
37 n. sp. and *D. staudingeri*, whereas a sister relationship of these taxa is found in the
38 phylogenetic analyses, with the split dated at 48-61 Mya. Although the study of additional
39 Palaearctic *Diochus* species will be required to conclusively establish that *D. occultus* n. sp.
40 is a palaeoendemic taxon sister to *D. staudingeri*, associated to forests of *Abies pinsapo* in
41 the south of the Iberian Peninsula, this conclusion is consistent with the ancient estimated
42 age of speciation, endogean habitat specificity, low dispersal capacity (flightless species),
43 and microendemism of *D. occultus*. This is also consistent with the continued emersion of
44 the Betic sub-plate along its tectonic evolution. The estimated ages of diversification of the
45 Paederinae-Staphylininae lineage are also discussed.

1 | INTRODUCTION

Difficulties in sampling and cataloguing the variety of life forms inside soil have led to soil being proposed as the third biotic frontier (André *et al.* 1994), after deep sea and forest canopy. A high proportion of edaphic biodiversity corresponds to the phylum Arthropoda (Decaëns 2010), a mega-diverse lineage for which important knowledge gaps still exist with regard to the magnitude and structure of biodiversity in soil, even in well studied European regions (Cameron *et al.* 2018). The combination of specific sampling methods to collect endogean arthropods (such as the Flotation-Berlese-Flotation protocol, FBF; Arribas *et al.* 2016) and the adoption of next generation sequencing methodologies have the potential to alleviate this gap (Andújar *et al.* 2015). These methodologies are already contributing to elucidate general biodiversity patterns from local to continental scales (Arribas *et al.* 2016; Wu *et al.* 2011), increasing our knowledge on poorly known lineages, and leading to the discovery of new species and even higher taxa (e.g., Andújar *et al.* 2017; Bocak *et al.* 2015).

As a part of a soil sampling campaign for a phylogenetic community analysis of the Coleoptera of three mountain regions in southern Spain (Andújar *et al.* 2015), we applied FBF sampling for 3 m² of leaf litter and 60 litres soil (collected down to 40 cm) from the *Abies pinsapo* forest (Pinsapar) of Sierra del Pinar (within the Natural Park of Sierra de Grazalema), a forest formation globally restricted to a few locations in southern Spain. Sampling from deep strata yielded two specimens of the genus *Diochus* Erichson, 1840 with several morphological traits associated with an endogean way of life, including the reduction of eyes (with only 21 ommatidia), loss of wings, and depigmentation. Detailed analyses of these specimens showed that they belong to a new species, the first endogean species recorded within the tribe Diochini.

The tribe Diochini includes two genera, *Antarctothius* Coiffait and Saiz, 1969 with two known species, and *Diochus* with 74 species distributed across all zoogeographic regions: 15 species in the Ethiopian region, 38 in the Neotropical region (recently revised by Irmeler 2017), 10 in the Oriental region, five in the Australian region, five in the Palaearctic region, one in the Nearctic region, and one in the Oceanic region (Schülke & Smetana 2015; Zhou & Zhou 2016). In addition, there is a fossil species described from Baltic amber (Chatzimanolis & Engel 2011). The four West Palaearctic species have been recently revised (Assing 2003), and of these, only *Diochus staudingeri* Kraatz, 1860 is present in Europe.

Diochus staudingeri has an Ibero-Maghrebian distribution, inhabiting in the west and south of the Iberian Peninsula (Spain and Portugal) and extending through a narrow area along the Mediterranean coast of North Africa (Morocco, Algeria, and Tunisia) (Assing 2003; Fig. 1). Regarding phylogenetic affinities, morphologically based studies (Kypke *et al.* 2019; Solodovnikov *et al.* 2013; Solodovnikov & Newton 2005) have placed Diochini within a lineage formed by Xantholinini, Maorothiini, and Othiini, with Diochini as a sister lineage to remaining taxa. This hypothesis is in strong disagreement with findings from molecular studies that included representatives of the tribe (Brunke *et al.* 2016; Chatzimanolis *et al.* 2010), where Diochini was recovered as an early derived lineage within the subfamily Staphylininae. On the contrary, more recent molecular phylogenetic studies, based on larger multilocus datasets, are fully consistent with the relationships derived from morphological data (Tihelka *et al.* 2020; Żyła & Solodovnikov 2020), and led to the recent proposal of the subfamily rank for the lineage including Xantholinini, Maorothiini, Othiini, and Diochini (subfamily Xantholininae sensu Żyła & Solodovnikov, 2020; see also Tihelka *et al.* 2020 for arguments against the new classification proposed by Żyła & Solodovnikov, 2020).

In this study, we: (i) describe the first species of Diochini known to inhabit the endogean environment, *Diochus occultus* n. sp.; (ii) provide the complete mitogenomes of *D. occultus* and its geographically closest relative, *D. staudingeri*, which coexists with *D. occultus* in Sierra de Grazalema, and; (iii) assemble a dataset of 42 mitogenomes, 20 of them newly assembled and annotated for this study (with 7 representatives of the subfamily Staphylininae, 4 of Paederinae, and 9 of Aleocharinae) and 22 retrieved from public repositories (including 15 of Staphylininae, 3 of Paederinae, and 4 of Silphidae). In addition to these mitogenomes, we also included *Cytochrome Oxidase I (COI)* sequences from 5 representatives of *Diochus* from Africa, and from the Oriental and Neotropical regions. This mitogenome dataset is used to perform a series of phylogenetic and dating analyses to confirm and date the split of Diochini within the subfamily Staphylininae, assess the monophyly of the genus *Diochus* and the phylogenetic relationship of the geographically syntopic *D. staudingeri* and *D. occultus*. We discuss estimated ages of divergence for initial diversification of the Paederinae-Staphylininae lineage and the affinities and presumed evolutionary history of *Diochus occultus* in the light of morphological evidence, the known distribution, and dated phylogeny.

2 | MATERIALS AND METHODS

Maximum likelihood and Bayesian phylogenetic analyses were carried out for a set of 42 mitogenomes and five partial *COI* sequences. Of these, 20 mitogenomes have been generated by the authors by re-assembling and annotating raw metagenomic data from Andújar et al. 2015 (Table 1). Nine of these 20 mitogenomes were previously published in Andújar et al. (2019). The remaining 22 mitogenomes were retrieved from GenBank databases.

2.1 | Sampling

Specimens were collected using the Flotation-Berlese-Flotation protocol of Arribas *et al.* (2016). Soil samples comprised 3 m² of leaf litter and 60 litres of soil (collected down to a depth of 40 cm) from the *Abies pinsapo* forest (Pinsapar) of Sierra del Pinar (Natural Park of Sierra de Grazalema). Specimens of *Diochus* were found in the deep strata. Specimens for the other sequenced species were collected using similar procedures in the same and other localities in Sierra de Grazalema, Sierra de Cabra, and Sierra Madrona (south Spain; Table 1).

2.2 | DNA extraction and mitogenome sequencing and assembly

DNA extraction was conducted from whole specimens using non-destructive procedures and Qiagen extraction kits (Hilden, Germany). Genomic DNA from each specimen was shot-gun sequenced using approximately 1% of a run with the Illumina MiSeq platform (Illumina Inc., San Diego, CA) (2x300 bp; 800–950 bp insert size), following the mitochondrial metagenomics approach (Crampton-Platt *et al.* 2015), pooling together genomic DNA from several specimens into TruSeq DNA libraries. Sequencing was conducted within the framework of Andújar et al. (2015), where additional methodological details are provided. Obtained MiSeq reads were processed and *de novo* assembled following the procedures in Andújar et al. (2017). Illumina output was processed with Trimmomatic 0.30 (Bolger *et al.* 2014) for Illumina adapter removal. Reads were subsequently filtered using Blast 2.2.27 (Altschul *et al.* 1990) against a reference database including 245 nearly complete coleopteran mitochondrial genomes from Timmermans *et al.* (2015). Retrieved mitochondrial reads were then assembled using Celera Assembler 7.0 (Myers 2000), Newbler 2.7 (Margulies *et al.* 2006), and IDBA-UD (Peng *et al.* 2012). Celera analyses were done using the RunCa

command with the bogart method for unitigger computations, and Unitig Repeat/Unique
Toggling was conducted from unitigs longer than 500 bp; other parameters were set to default
options. Newbler analyses were run using -mi 95 to -ml 150 and IDBA using -maxk 300 to
-mink 50.

The resulting contigs from the three assemblers were re-assembled in Geneious 7.1.9
(Kearse *et al.* 2012) using the *de novo assembly* function, and showed wide overlap
minimising potential problems associated to the formation of chimeric mitogenome
sequences. Obtained mitogenomes were annotated using gene predictions with MITOS
(Bernt *et al.* 2013) with additional manual editing performed in Geneious. All mitogenomes
were structured following the putatively ancestral gene order for the Coleoptera. Finally,
mitogenomes were assigned a taxonomic identity either by comparison against the *COI*
barcode sequences obtained from the same specimens with PCR-Sanger sequencing
(sequences from Andújar *et al.* 2015), by unambiguous match to the species level on BOLD
Public Data Portal (Ratnasingham and Hebert, 2007, accessed on 20th March 2019), or by
its phylogenetic placement using all available mitogenomes for Staphylinidae within the
nucleotide database of GenBank (Benson *et al.* 2014; Accessed April 2020).

2.3 | Dataset assembly

The 11 mitogenomes newly assembled and annotated include the mitogenome of (i) *Diachus occultus* n. sp., (ii) *D. staudingeri*, (iii) and 9 mitogenomes from a variety of taxa within the subfamilies Staphylininae, Paederinae, and Aleocharinae (Table 1). We included nine additional mitogenomes generated by the authors (Andújar *et al.* 2019), and 22 retrieved from the nucleotide database of GenBank, for specimens within the same subfamilies plus Silphidae. The dataset was complemented with partial *COI* sequences from five species of *Diachus* available in GenBank (accessed on 29-04-2020) (Table 1). Sampling for the dataset was designed according to the availability of fossil information and mitogenome sequences, and finally included representatives of Xantholinini, Othiini, Staphylinini and Diochini (within the subfamily Staphylininae), Paederinae Aleocharinae, and Silphidae (Table 1). The latter was included according to the phylogenetic analyses of Linard *et al.* (2018) who, using a combination of mitogenomes and nuclear genes, found Silphidae to be clustered within Staphylinidae and forming a clade (Aleocharinae (Silphidae + other lineages (Paederinae,

Staphylininae). This relationship is also consistent with the phylogenetic analyses of McKenna *et al.* (2019) using a larger genomic dataset.

Single gene datasets for each of the 13 protein coding genes (PCGs) and the two ribosomal genes were extracted using Geneious and individually aligned using the FFT-G-INS-i algorithm of MAFFT (Kato *et al.* 2002), using the “Translation Align” option of Geneious for PCGs. Individual gene alignments were concatenated, yielding a final dataset of 47 taxa and 12877 bp (Supplementary Data S1).

2.4 | Phylogenetic and dating analyses

Data matrices were analysed with maximum likelihood (ML) and Bayesian phylogenetic methods. ML trees were obtained using RAxML 8.2.12 (Stamatakis 2006) and IQTree (Nguyen *et al.* 2015). RAxML analyses were conducted on the CIPRES Science Gateway (Miller *et al.* 2010), with gene partitions and applying an independent GTR+G+I model to each data partition. The best scoring ML tree was selected among 200 searches on the original alignment with different randomized parsimony starting trees. Support values were obtained with 1000 bootstrap replicates (Felsenstein 1985). IQTree analyses were run on the IQ-TREE web server at <http://iqtree.cibiv.univie.ac.at> (Trifinopoulos *et al.* 2016) using the best fitting substitution model for each gene partition as estimated with ModelFinder (Kalyaanamoorthy *et al.* 2017). Nodal support was obtained by 1000 ultrafast bootstrap (UFBoot) replicates (Minh *et al.* 2013).

Bayesian phylogenetic analyses were conducted using MrBayes 3.2.7 (Ronquist & Huelsenbeck 2003) and BEAST 1.8.4 (Drummond *et al.* 2012). MrBayes analyses were performed with two parallel runs of 20,000,000 generations, using four chains in each, and retaining one tree every 500 generations. The dataset was partitioned by gene and a GTR+G+I model was applied to each partition. A fifty percent majority rule consensus tree was obtained after discarding the first 25% of trees, and posterior probabilities were used as a measure of nodal support.

BEAST analyses were used to estimate simultaneously an ultrametric phylogenetic tree and ages of divergence. Analytical constraints used to date the tree included up to 11 calibration points under the calibration scheme 1 (Table 2). Ten of these corresponded to minimum age hard bounds on stem nodes according to minimum first appearance ages of lineages in the fossil record, and included constraints on: (1) The stem of Aleocharinae

according to fossils of *Cretodeinopsis aenigmatica*, *Mesosymbion compactus*, and
Cretotrichopsenius burmiticus (Cai *et al.* 2017; Cai & Huang 2015; Yamamoto *et al.* 2016)
 from Burmese amber, dated at ≈ 99 Mya (Early Cenomanian) (Shi *et al.* 2012). (2) The stem
 of Athetini based on a fossil of *Atheta (Datomicra) jantarica* (Paśnik 2005) from Baltic
 amber, dated at 33.9-44 Mya (Aleksandrova & Zaporozhets 2008; Perkovsky *et al.* 2007;
 Ritzkowski 1997). Considering uncertainty about the ages of Baltic amber (discussed in
 Bogri *et al.* 2018), we conservatively used the younger age of 33.9 Mya to constrain the
 minimum age for the first appearance of taxa recorded in Baltic amber. (3) The stem of
 Aleocharini based on a fossil of *Aleochara baltica* from Baltic amber (Paśnik & Kubisz
 2002). (4) The stem of Silphidae based on a fossil from the Jurassic Daohugou Formation of
 China, dated at 166.1-157.3 Mya (Callovian-Oxfordian) (Cai *et al.* 2014). (5) The stem for
 the genus *Silpha* based on fossils of *Silpha beutenmuelleri* and *Silpha colorata* (Scudder
 1900; Wickham 1914) from the Tertiary deposits at Florissant, EEUU, dated at 37.2-33.9
 Mya (Chadronian). (6) The stem of Paederinae based on a fossil of *Mesostaphylinus antiquus*
 and other co-described species (Solodovnikov *et al.* 2013) from the Yixian formation of
 China, dated at 126–124.6 Mya (Early Aptian). (7) The stem of the lineage formed by *Medon*,
Rugilus and *Astenus* based on a fossil of *Diminudon schomannae* from Burmese amber and
 its phylogenetic placement within the Paederinae based on morphological data (Żyła *et al.*
 2019). (8) The stem of Staphylinini based on a fossil of *Cretoquedius distinctus* and other
 co-described species from the Yixian formation (Solodovnikov *et al.* 2013). (9) The stem of
 Diochini based on the phylogenetic placement of the fossil taxa *Cretoprosopus*
problematicus from the Yixian formation (Solodovnikov *et al.* 2013). And (10) the stem for
Atrecus based on fossils of *Vetatrecus adelfae* and *Vetatrecus secretum* from Burmese amber
 (Kypke *et al.* 2019). The last age calibration constraint used (11) was a maximum age hard
 bound of 182.4 Mya for the root of the tree, defined by the 95% confidence age interval for
 the first fossil appearance as estimated using the Strauss & Sadler approach (Strauss & Sadler
 1989). Estimations were performed in R with the package CladeAge (Claramunt & Cracraft
 2015) and using the maximum ages for the series of geological strata where fossils have been
 found, with data downloaded from the Paleobiology database on 1st May, 2020, using the
 group names “Staphylininae, Paederinae, Aleocharinae, and Silphidae” and the option
 “geological strata”.

Two additional calibration analyses were performed. Calibration scheme 2 excluded the maximum age constraint on the root (set up in this case to a non-informative maximum of 300 Mya). Calibration scheme 3 included additional hard constraints to the mean substitution rate of each of the 15 mitochondrial genes (Table 3). This analysis (calibration scheme 3) was done to explore the potential age overestimation of more recent splits derived from the use of only deep nodes as calibration points, and associated to the estimation of a low substitution rate. Consequently, in the calibration scheme 3, the mean rate (*uclد.mean* parameter of BEAST) of each gene was constrained using as reference the mean rate obtained for the *COI* gene by Andújar et al. (2012), which is similar to the rates obtained in other studies for Coleoptera (e.g., Papadopoulou *et al.* 2010). Results from analyses under the calibration scheme 1 were used to estimate a coefficient between the rate of each gene and the rate of *COI*, and this coefficient was multiplied by the mean rate of *COI* from Andújar et al. (2012) to obtain the rate values subsequently constrained on each gene in the calibration scheme 3 (Table 3).

BEAST analyses were conducted partitioning by gene and applying a GTR+G+I model to each partition. Reciprocal monophyly of Aleocharinae and remaining taxa was enforced. An uncorrelated lognormal clock and a Birth-Death speciation prior on the tree was applied, and analyses were run for 50 million generations sampling one tree every 5000 generations. The maximum clade credibility tree was estimated with TreeAnnotator (Drummond *et al.* 2012) discarding the first 50% of trees as a burn-in fraction after checking ESS of likelihood, evolutionary rates and root age values, and ensuring that the tree likelihood values had reached a plateau. Posterior probabilities were considered as a measure of node support. All BEAST analyses were conducted on the CIPRES Science Gateway (Miller *et al.* 2010).

2.5 | Taxonomic study

Body pieces and aedeagus were mounted on microscopic slides using Dimetil Hidantoína Formaldehído (DMHF), a hydrosoluble syntetic resin. Measures were taken using an ocular micrometer on a stereomicroscope Euromex KT2. Aedeagus, habitus and head images were taken with a digital camera Canon EOS 50D adapted to a microscope Zeiss Axiostar plus.

3 | RESULTS

3.1 | Molecular phylogenetics and dating

All phylogenetic analyses in RAxML, IQTree, MrBayes, and BEAST resulted in very similar topologies (Figs. 1 and 2, Supplementary Figs. S1-S5). The seven *Diochus* species were always clustered together, with moderate support in RAxML (bootstrap = 76%), and high support in IQTree (bootstrap = 96%), MrBayes (pp = 1), and BEAST (pp = 0.99-1 with the different calibration schemes), with *Diochus occultus* n. sp., and *D. staudingeri* always clustered together with maximum support (Table 4). Staphylininae was always found as monophyletic (RAxML bootstrap = 41%; IQTree bootstrap = 65%; MrBayes pp = 1; BEAST pp = 1) and sister to Paederinae. Diochini was found in all analyses as the sister group to a clade formed by Xantholinini and Othiini, with relationships within Staphylininae as follows: (((Xantholinini, Othiini), Diochini) Staphylinini) (node support from all analyses in Table 4).

The variation on the obtained ages for the analyses under calibration schemes 1-3 are shown in Figure 2 and summarized in table 4. The age for the split between *Diochus occultus* n. sp., and *D. staudingeri* under the calibration scheme 2 resulted in a mean value of 54.6 (95%HPD 47.8-61.3 Mya), and ranged between a median value of 40.95 Mya (95%HPD 34.3-47.4 Mya) in the calibration scheme 3, and 56.4 Mya (95%HPD 48.2-66.3 Mya) with the calibration scheme 2.

3.2 | Taxonomic acts

Diochus occultus n. sp.

<http://zoobank.org/NomenclaturalActs/906ca2f6-cf11-4466-88ac-49c325dcb0f1>

3.2.1 | Type locality

Soil sample from forest “El Pinsapar”, placed in the surroundings of Puerto del Pinar, N 36° 46' 19.92'' W 5° 25' 19.70'', 1115 m, Sierra de Grazalema, Cádiz, Spain.

3.2.2 | Type series

Holotype (coll. C. Hernando, Badalona): 1 male, << SP, Grazalema, Cádiz, 1115m / El Pinsapar, Puerto del Pinar / N 36° 46' 19,92'' W 5° 25' 19,70'' / 24. 11. 2012, Andújar-Arribas leg.>>

Paratype (coll C. Hernando, Badalona): 1 female, same data as holotype.

291 3.2.3 | Description

292 *Holotype measurements* (mm): Total length = 5.1; length of antennae = 1.37; length of the
293 head (from the margin of clypeus to the neck) = 0.67; maximum width of head = 0.52; length
294 of pronotum = 0.80; maximum width of pronotum = 0.52; length of elytra (from the apex of
295 scutellum to the posterior margin) = 0.57; maximum width of elytra = 0.62.

296 *Coloration* reddish testaceous; habitus as in Fig. 3A.

297 *Head* oval, sides subparallel, clearly longer than wide; eyes reduced, formed by 21 ommatidia
298 (Fig. 3B and 3C), not protruding from head margins in dorsal view; punctation strong, sparse,
299 with punctures separated approximately 3x their diameter on vertex, approximately 2x their
300 diameter on marginal areas. Frons smooth; surface micro-reticulated, formed by minute
301 isodiametric mesh, well defined except on disc where it is diffuse; micro-reticulation formed
302 by thin transversal striolae on lateral areas. Anterior margin of labrum bilobed, with small
303 medial dent. Antennae slender; antennomeres 2-3 oblong and of similar size, antennomeres
304 4-10 almost square, slightly transversal, antennomere 11 longer than antennomere 10, apex
305 acute.

306 *Pronotum* oblong, clearly longer than head; lateral margins straight and subparallel; surface
307 smooth and shiny, without micro-reticulation; with four longitudinal rows of punctures (two
308 at each side of central line); two central rows formed by five large punctures; two external
309 rows formed by three large punctures situated on anterior half of the pronotum. (Fig. 3B).

310 *Elytra* clearly shorter than pronotum, lateral margins slightly divergent in dorsal view;
311 surface with shallow reticulation with granular appearance; with two irregular series of light
312 punctures on each elytron; apterous.

313 *Legs* with non-dilated protarsus, narrower than distal part of protibia in both males and
314 females, tarsomeres 1-3 cordiform and of equal size, fourth tarsomere also cordiform but
315 clearly smaller, fifth cylindrical and longer, of similar length to tarsomeres 1-3 together.

316 *Abdomen* thin and densely punctated on whole surface; tergites and sternites finely micro-
317 reticulated, with thin transversal striolae except at base of each segment, where the

318 microsculpture forms a fine isodiametric polygonal mesh; male sternite VIII with posterior
319 margin markedly concave (Fig. 3F); female sternite VIII unarmed, male tergite VIII with
320 posterior margin strongly arcuate (Fig. 3E).

321 *Aedeagus* of distinctive shape (Fig. 3D); bulb membranous, apex strongly acuminate;
322 parameres as long as median lobe, thickened towards apex and with three setae each; median
323 lobe with a large internal piece with base covered by squamiform-like structures; additional
324 spiniform-like structures mainly distributed in apical part (Fig. 3D).

325 **3.2.4 | Distribution**

326 Only known from the type locality, forest of El Pinsapar in Sierra de Grazalema, Cádiz,
327 Spain. The map in Figure 1C shows the distribution of *D. staudingeri* and *D. occultus* n. sp.,
328 the two species of *Diachus* known to occur in the Iberian Península.

329 **3.2.5 | Habitat**

330 Specimens were collected using the Flotation-Berlese-Flotation protocol (Arribas *et al.*
331 2016), which combines soil floating and Berlese extraction with further flotation steps in the
332 lab to separate specimens from vegetation and soil debris. Soil samples were taken from a
333 limestone rocky soil (depth up to 40 cm) from the *Abies pinsapo* forest (Pinsapar) of Sierra
334 del Pinar (Natural Park of Sierra de Grazalema) (Fig. 1B), at 1115 m of altitude. *A. pinsapo*
335 is an endemic tree from the south of the Iberian Peninsula, restricted to a few locations in the
336 Subbetic region with high pluviosity, typically about 2000-3000 mm per year.

337 **3.2.6 | Etymology**

338 The name refers to the way of life of this species, “occulted” deep inside a rocky soil in one
339 of the last remnants of *Abies pinsapo* forest in the south of the Iberian Peninsula.

340 **3.2.7 | Identification key to the West Palaearctic species**

341 1. Larger species, 4.4 – 6.5 mm. Maximal width of pronotum (PW): > 0.53 mm, length of
342 pronotum along median line (PL): >0.79 mm. Aedeagus with the apex of the median lobe
343 pointed (Figs. 3D and 3J) 2

344 - Smaller species, 3.8 – 4.8 mm. PW:> 0.48 mm, PL: >0.67 mm. Aedeagus with the apex of
345 the median lobe spatuliform (see Assing, 2003: Fig. 17) 4
346

347 2. Pronotum with the dorsal rows of punctures each composed of 3 punctures (Fig 3H).
348 Protarsomeres 1-4 moderately dilated (Fig. 3H) *D. staudingeri* Kraatz
349 - Pronotum with the dorsal rows of punctures each composed of 5 punctures (Fig. 3B).
350 Protarsomeres 1-4 not dilated (Fig. 3B) 3
351

352 3. Eyes strongly reduced, composed only of 21 ommatidia (Fig. 3C); pronotum more slender
353 and parallel, less convex in cross-section (Fig. 3B). Aedeagus with parameres of the same
354 length of the median lobe; internal pieces of the aedeagus composed of a single large and
355 strongly chitinized piece (Fig. 3D) *D. occultus* sp. nov.
356 - Eyes of normal size, composed of more than 90 ommatidia; pronotum less slender and more
357 convex in cross-section. Aedeagus with the parameres shorter than the median lobe; internal
358 pieces of the aedeagus composed of two pieces (see Assing, 2003: Fig.
359 10).....*D. hatayus* Assing
360

361 4. Central dorsal area of head without punctures. Pronotum at least as wide as head, with
362 moderately coarse dorsal punctures, and not sulcate along midline (see Assing, 2003: Figs.
363 3, 16). Elytra shorter, at suture less than 0.7 times the length of pronotum, lateral margins
364 diverging posteriorly (see Assing, 2003: Fig. 3). Protarsomeres 1-4 weakly dilated.
365 Abdominal tergite VII at posterior margin without palisade fringe. Aedeagus with the apex
366 of median lobe spatuliform (see Assing, 2003: Fig. 17).....*D. libanoticus* Fagel
367 - Central dorsal area of head with punctures. Pronotum narrower than head (see Assing, 2003:
368 Fig. 4), with very coarse dorsal punctures, central part of midline (between dorsal punctures)
369 sulcate (see Assing, 2003: Fig. 20). Elytra longer, at the suture approximately 0.8 times the
370 length of pronotum, lateral margins not distinctly diverging posteriorly. Protarsomeres I-IV
371 not dilated. Posterior margin of abdominal tergite VII with palisade fringe. Male unknown
372 *D. sulcatus* Assing
373 Note: Keys modified from Assing 2003
374

4 | DISCUSSION

4.1 | Morphological differences between *D. occultus* n. sp. and *D. staudingeri*

The molecular divergence between the specimens collected in the soil of El Pinsapar, in Sierra de Grazalema, and *D. staudingeri* is in good agreement with the observed morphological differences, altogether supporting the description of the new species. *Diochus occultus* n. sp. has a less elongated head, with more parallel lateral margins, resulting in a more oval aspect. The head has the disc punctated while it is totally smooth in *D. staudingeri*. Eyes are strongly reduced in *D. occultus* n. sp., not protruding in dorsal view and formed by only 21 ommatidia (Fig. 3C), while *D. staudingeri* has fully developed eyes, including between 95 and 99 ommatidia (Fig. 3I). The pronotum is shorter in *D. occultus* n. sp., with dorsal rows including 5 punctures (Fig. 3B), while *D. staudingeri* has a longer pronotum with rows of 3 punctures, occasionally 4 (Fig. 3H). The elytra are shorter and more strongly convergent toward the suture in *D. occultus* n. sp., with punctuation forming irregular series of light punctures; this punctuation is doubled in *D. staudingeri*, including irregular series of thick punctures and a fine and scattered punctuation through all the surface. In *D. occultus* n. sp. male protarsi are not dilated (Fig. 3B), while *D. staudingeri* has strongly dilated protarsus in males, as wide as the distal part of the protibia (Fig. 3H). The male VIII sternite of *D. occultus* n. sp. has a deeper notch posteriorly (Figs. 3F and 3L). The median lobe of the aedeagus of *D. occultus* n. sp. is of the same length as the parameres, including a single large internal piece, while in *D. staudingeri* the median lobe is shorter than the parameres and including several small separate internal pieces (Figs. 3D and 3J).

4.2. The time scale of Staphylininae evolution

Our dating analyses rely on the conservative application of hard constraints on the minimum age for the stem node of ten lineages, based on well-validated fossils according to recent literature (e.g., Cai *et al.* 2014; Chatzimanolis 2018; Solodovnikov *et al.* 2013). Dating analysis under the calibration scheme 1 resulted in a split between Paederinae and Staphylininae estimated at 156.6 Mya (95% HPD 150.4-165.1 Mya) during the late-Jurassic period, with a subsequent split of Staphylininae into main lineages (here represented by Staphylinini, Diochini, Othiini and Xantholinini) estimated between 152.9 and 143.9 Mya (95% HPD 138.8-161.1 Mya) during the late-Jurassic and early Cretaceous. These ages are

fully consistent with the fossil record, particularly with the existence of additional fossils tentatively assigned to Staphylininae and Paederinae from the mid- to late-Jurassic period (ca. 180–145 Mya) as reviewed by Solodovnikov *et al.* (2013). Estimated ages are consistent with those obtained by Brunke *et al.* (2017) for the stem node of Staphylinini ($\approx$ 140-165Mya; Figure 4 in Brunke *et al.* 2017), although in that study a likely wrong sister relationship of Staphylinini and Paederinae is found.

4.3 | Phylogenetic affinities and evolutionary history of *D. occultus* n. sp.

D. occultus n. sp. is the first representative within the tribe Diochini associated to the endogean environment. It has been found only in deep soil samples inside a patch of *Abies pinsapo* forest in south Spain, in soils characterized by high stone content and the presence of an abundant number of roots and hollows, referred to as the Mesovoid Shallow Substratum (described in French as Milieu Souterrain Superficiel; MSS) (Giachino & Vailati 2010; Juberthie *et al.* 1980). The MSS captures the conditions of darkness, temperature stability, and high humidity found in the deeper subterranean environments (caves and crevices on the rock substratum). MSS environments are often situated between soil layers and the rock substratum, and are well known to harbour a notable number of species that share adaptations to these special environmental conditions (Assing 2018; Giachino & Vailati 2010; Juberthie *et al.* 1980; Ortuño *et al.* 2013). In these environments, endogean species known to occur mainly in deep soil layers are commonly found together with subterranean species, which are frequently found in crevices and cavities of the rock substratum. Both subterranean and endogean species typically share some morphological adaptations, such as loss of eyes, loss of wings and depigmentation. In the case of *D. occultus* n. sp., the reduction of eyes to only 21 ommatidia and depigmentation is suggestive of an adaptation to the soil or MSS environment (Fig. 3B).

It is worth noting that the only species of the genus also found in the west Mediterranean region, *D. staudingeri*, has a distribution that encompasses the type locality *D. occultus* n. sp., being particularly frequent in the Betic region (Fig 1C). Our molecular phylogenetic analyses group together *D. staudingeri* and *D. occultus* n. sp. (but note that only five other *Diochus* species were included in the molecular phylogeny, and the three additional Western Palaearctic representatives are lacking). Regarding the morphological

similarity, it is difficult to establish whether this is higher between *D. occultus* and *D. staudingeri* than between *D. occultus* and the other species from the Palaearctic region. Available molecular information and current geographic distribution suggest a sister relationship between *D. staudingeri* and *D. occultus* n. sp., but this hypothesis needs further corroboration. An extensive study of the genus including wider morphological and molecular data is required.

Considering the hypothesis of a sister relationship between *D. occultus* and *D. staudingeri*, the molecular phylogenetic dating analyses suggest an ancient split of these species. Estimated ages under calibration schemes 1 and 2 indicates a Paleocene-Eocene origin (mean ≈ 55 Mya; 95% HPD 47.8-66.3 Mya). The estimated rate of molecular evolution for the *COI* gene under calibration scheme 1 was 0.00675 substitutions per site per million years per lineage (= 1.45% divergence per million years; rates of other genes shown in Table 3). This rate is notably lower than rates reported in other lineages within the Coleoptera (≈ 2.5 -3.5% divergence/Ma; e.g., Andújar *et al.* 2012; Papadopoulou *et al.* 2010) and further research is needed to evaluate reliability of these values. Considering the possibility of an infra-estimation of the rate of nucleotide change caused by saturation effects, we conducted an additional analysis (calibration scheme 3) where rates of each gene are forced to be close to the mean values obtained in other studies. This showed younger ages for most diversification events (Fig. 2), with the split of *D. occultus* and *D. staudingeri* dated during the Eocene, at 40.95 Mya (95%HPD 34.3-47.4 Mya). Despite this variation, all estimates placed the split of *D. occultus* n. sp. prior to the Miocene, during the early or intermediate stages of the tectonic evolution of the Betic-Riffian plate (Andeweg 2002; Rosenbaum *et al.* 2002), with two main implications. Firstly, this discards a scenario of speciation associated with the geological complexity of the Betic plate in the late Miocene, when repetitive connections and disconnections between the Mediterranean sea and the Atlantic ocean occurred, and the fragmentation of the Betic plate could have contributed to allopatric speciation. Secondly, it is consistent with the continuous presence of at least some emerged parts of the Betic plate along its tectonic evolution during the Miocene (Andeweg 2002). This is also consistent with the presence of other ancient species of insects in the area, such as the genus *Daliat* Mateu 2002 (Mateu & Bellés 2003; Ribera *et al.* 2005), and the family Iberobaenidae (Bocak *et al.* 2016) distributed within the Betic plate and having ancient ages

of divergence from their closest known relatives. These taxa can be considered as palaeoendemics, as they have been demonstrated to be of ancient origin based on molecular phylogenetic evidence and are restricted to a reduced geographical area (Malik 2015; Prentice 1976). There is no need to invoke here a former wider distribution, as these taxa may have been restricted to geologically stable but relatively small areas inside the Betic plate. In the case of *D. occultus* n. sp., the estimated ages of speciation, its endogean habitat specificity, low dispersal capacity (it is a flightless species), and its current microendemism within the Betic-riffian tectonic plate support the consideration of this species as a palaeoendemic taxon linked to the singularity of the *Abies pinsapo* forest in the south of the Iberian Peninsula. This is also consistent with pollen deposits which have been interpreted as evidence of a relict forest formation surviving in limited number of refugia, with a previously wider distribution across the Betic plate (Alba-Sánchez *et al.* 2010).

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FIGURE CAPTIONS AND SUPPORTING INFORMATION

Figure 1. A. Maximum likelihood phylogenetic tree obtained with RAxML for the complete mitogenome dataset including Diochini and a selection of lineages within the Staphylinidae. Branches in red: Staphylinini; in green: Diochini; in blue: Xantholinini; and in violet: Othiini. Yellow spot marks the position of *D. occultus* n. sp. and red spot the position of *D. staudingeri*. Numbers of nodes indicates bootstrap values obtained with RAxML. Nodes with maximum support (pp=1) in MrBayes are marked with black spots. **B.** Image of the soil slope where *D. occultus* n. sp. was collected. **C.** Map of the Iberian Peninsula and North of Africa indicating the distribution of *D. occultus* n. sp. (yellow spot) and *D. staudingeri* (red spots; its distribution reach Tunisia to the East, not represented).

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Figure 2. Time calibrated ultrametric phylogenetic tree obtained in BEAST (“maximum clade credibility tree” with median ages) with the calibration scheme 1. Braches in red: Staphylinini; in green: Diochini; in blue: Xantholinini; and in violet: Othiini. Nodes with maximum support (pp=1) are marked with black spots; nodes with pp = 0.95-0.99 are marked with grey spots. Bars on nodes represent 95% high posterior density interval for obtained ages. Bars in light blue: age interval from calibration scheme 1; bars in light green with dotted contour: age interval from calibration scheme 2 (maximum age on root increased to 300 Mya); bars in light red: age interval from calibration scheme 3 (substitution rates fixed). Nodes used as calibration points are marked with a number, corresponding to numbering of calibration points in table 2. See Material and Methods, and Tables 2 and 3 for details on calibration schemes.

Figure 3. Habitus and morphological details of *D. occultus* n. sp. (left, A-F) and *D. staudingeri* (right, G-L). A. Habitus of *D. occultus*. B. Detail of head and pronotum, dorsal view. C. Detail of head, lateral view. D. Aedeagus. E. Tergite VIII. F. Sternite VIII. G. Habitus of *D. staudingeri*. H. Detail of head and pronotum, dorsal view. I. Detail of head, lateral view. J. Aedeagus. K. Tergite VIII. L. Sternite VIII.

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796 **Supplementary Materials File 1.**

797 1. **Figure S1:** IQTree Maximum Likelihood tree obtained for the dataset including 13
798 protein coding plus two ribosomal mitochondrial genes.

799 2. **Figure S2:** Bayesian 50% majority rule consensus tree as estimated with MrBayes
800 for the dataset including 13 protein coding plus two ribosomal mitochondrial genes

801 3. **Figures S3-S5:** BEAST time calibrated Bayesian trees (Maximum clade credibility
802 trees) obtained under calibration schemes 1, 2, and 3.

803 **Supplementary Materials File 2.**

804 **Data S1.** Alignment of 13 protein coding and two ribosomal mitochondrial genes for
805 the 47 taxa used for phylogenetic analyses, in nexus format.

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809 Table 1. Data on specimens used for the phylogenetic analyses.

Specimen identification	GB Ac. N.	Sequence length	Live stage of specimen	Geographic origin	Source of Specimen identification
Aleocharinae					
<i>Aleochara</i> sp. †	MK692703	16784	Adult	COR	V. Assing det.
Aleocharinae †	MK692598	17194	N.A.	SS	BOLD match 92.8% to genus <i>Oxypoda</i>
Aleocharinae †	MK692706	16780	Larva	COR	BOLD match 90.1% to Aleocharinae; within Aleocharinae based on tree
<i>Atheta</i> sp. ††	MK692597	17418	Adult	COR	V. Assing det.
<i>Geostiba</i> sp. ††	MK692616	12211	Adult	CAD	V. Assing det.
<i>Lomechusa pubicollis</i> ††	MK692638	18381	NA	CR	BOLD match 99.1% to <i>Lomechusa pubicollis</i>
<i>Mocyta fungi</i> ††	MK692702	17526	Adult	COR	BOLD match 99.8% to <i>Mocyta fungi</i> ; Identified as <i>Atheta (Mocyta)</i> sp. by Assing
<i>Oxypoda</i> sp. †	MK692600	16864	Larva	CAD	BOLD match 97.3% to <i>Oxypoda annularis</i>
<i>Oxypoda</i> sp. †	MK692704	16982	Larva	COR	BOLD match 100% to genus <i>Oxypoda</i> ;
Silphidae					
<i>Diames osculans</i>	MN900864	19400	N.A.	China	GenBank
<i>Necrophila americana</i>	GU176343	16902	N.A.	USA: KY	GenBank
<i>Silpha tyrolensis</i>	KX087347	12813	N.A.	NA	GenBank
<i>Thanatophilus sinuatus</i>	KX087354	5853	N.A.	NA	GenBank
Paederinae					
<i>Achenium seditiosum</i> ††	MK692556	17854	Adult	COR	V. Assing det.
<i>Medon</i> sp. ††	MK692601	18239	Adult	CR	V. Assing det.
<i>Micrillus testaceus</i> ††	MK692603	17402	Adult	COR	V. Assing det.
Paederinae †	MK692650	11019	N.A.	SS	Within Paederinae based on tree
<i>Rugilus geniculatus</i>	KT780671	17640	N.A.	N.A.	GenBank
<i>Astenus lyonesius</i>	KT780626	17451	N.A.	N.A.	GenBank
<i>Lathrobium brunnipes</i>	KT780654	18639	N.A.	N.A.	GenBank
Diachini					
<i>Diachus occultus</i> n. sp. †	MK692584	17347	Adult	CAD	C. Hernando det.
<i>Diachus</i> sp. Neotropical region	KT021982	774	N.A.	N.A.	GenBank
<i>Diachus</i> sp. Oriental region	GU377370	773	N.A.	N.A.	GenBank
<i>Diachus</i> sp. Afr.	MN264586	654	N.A.	N.A.	GenBank
<i>Diachus</i> sp. Afr.	MN264587	750	N.A.	N.A.	GenBank
<i>Diachus schaumii</i>	MN264588	807	N.A.	N.A.	GenBank
<i>Diachus staudingeri</i> †	MK692573	17122	Adult	CAD	V. Assing det.
Othiini					
<i>Atrecus affinis</i>	KT780630	16844	N.A.	N.A.	GenBank
<i>Atrecus affinis</i>	KT876882	16842	N.A.	UK: London	GenBank
<i>Othius laeviusculus</i>	KX087327	6793	N.A.	N.A.	GenBank
<i>Othius myrmecophilus</i> ††	MK692560	17910	Larva	CR	BOLD match 99.4% to <i>Othius myrmecophilus</i>
<i>Othius punctulatus</i> †	MK692618	10803	Larva	CAD	BOLD match 98.7% to <i>Othius punctulatus</i>
<i>Othius</i> sp. †	MK692613	14085	Larva	COR	BOLD match 93.8% to genus <i>Othius</i> ; within <i>Othius</i> based on tree
Staphylinini					
<i>Bisnius sordidus</i>	KT780632	18280	N.A.	N.A.	GenBank
<i>Gabronthus thermarum</i>	KT780649	16434	N.A.	N.A.	GenBank
<i>Neobisnius villosulus</i>	KT780662	19494	N.A.	N.A.	GenBank
<i>Ocypus aethiops</i> ††	MK692599	18643	N.A.	COR	BOLD match 100% to <i>Ocypus aethiops</i>
<i>Ocypus brunnipes</i>	KT780665	16620	N.A.	N.A.	GenBank
<i>Ocypus olens</i>	KT876908	19948	N.A.	UK: London	GenBank
<i>Ocypus ophthalmicus</i>	KX087323	16887	N.A.	N.A.	GenBank
<i>Ontholestes tessellatus</i>	KX087324	15691	N.A.	N.A.	GenBank
<i>Philonthus fimetarius</i>	KT780669	18290	N.A.	N.A.	GenBank
<i>Philonthus decorus</i>	MN122841	13929	N.A.	N.A.	GenBank
<i>Tasgius compressus</i>	KT780697	18546	N.A.	N.A.	GenBank
Xantholinini					
<i>Gyrophypnus fracticornis</i>	KT780650	17602	N.A.	N.A.	GenBank
<i>Gyrophypnus</i> sp. †	MK692614	12795	N.A.	SS	BOLD match 99.1% to <i>Gyrophypnus wagneri</i>
<i>Nudobius lentus</i>	KT780664	17657	N.A.	N.A.	GenBank

810 COR: Sierra de Cabra, Córdoba. Spain; CAD: Sierra de Grazalema, Cádiz, Spain; CR: Sierra Madrona, Ciudad
811 Real, Spain; SS: South Spain; GB Ac. N.: GenBank Accession Number; N.A. Information not available. †
812 Mitogenomes assembled and annotated in the course of this study; †† Mitogenomes generated by the authors
813 and published in Andújar *et. al* (2019); remaining mitogenomes where retrieved from GenBank databases.

814 **Table 2.** Summary of calibration priors and calibration schemes analysed in BEAST.

Calibration point	Reference taxa	Calibration prior †	References ††	Calibration Scheme		
				1	2	3
1. Aleocharinae (Stem)	<i>Cretodeinopsis aenigmatica</i> & others	Min 99 (Burmese amber)	a; b; c	✓	✓	✓
2. Athethini (Stem)	<i>Atheta (Datomicra) jantastica</i>	Min 33.9 (Baltic amber)	d	✓	✓	✓
3. Aleocharini (Stem)	<i>Aleochara baltica</i>	Min 33.9 (Baltic amber)	e	✓	✓	✓
4. Silphinae (Stem)	Silphidae indet	Min 157.3 (Daohugou formation)	f	✓	✓	✓
5. <i>Silpha</i> (Stem)	<i>Silpha beutenmuelleri</i> , <i>S. colorata</i>	Min 33.9 (Florissant Tertiary)	g	✓	✓	✓
6. Paederinae (Stem)	<i>Mesostaphylinus antiquus</i> & others	Min 124.6 (Yixian formation)	h	✓	✓	✓
7. <i>Medon-Rugilus-Astenus</i> lineage (Stem)	<i>Diminudon schomannae</i>	Min 99 (Burmese amber)	i	✓	✓	✓
8. Staphylinini (Stem)	<i>Cretoquedius distinctus</i> & others	Min 124.6 (Yixian formation)	h	✓	✓	✓
9. Diochini (Stem)	<i>Cretoprosopus problematicus</i>	Min 124.6 (Yixian formation)	h	✓	✓	✓
10. <i>Atrecus</i> (Stem)	<i>Vetatrecus adelfae</i> , <i>V. secretum</i>	Min 99 (Burmese amber)	j	✓	✓	✓
11. Root	Fossil series (see methods)	Max 182.4	k	✓	X	✓
12. Rate of all genes	Carabidae	mean values (see methods)	l	X	X	✓

815 † All numbers are in Mya; *Min* and *Max* indicate the age used respectively to set up minimum and maximum age calibration
816 priors on corresponding nodes. †† References: a: Cai & Huang 2015; b: Liu *et al.* 2018; c: Yamamoto *et al.* 2016; d: Paśnik
817 2005; e: Paśnik & Kubisz 2002; f: Cai *et al.* 2014; g: Scudder 1900; Wickham 1914; h: Solodovnikov *et al.* 2013; i: Żyła *et*
818 *al.* 2019; j: Kypke *et al.* 2019; k: Paleobiology Database; l: Andújar *et al.* 2012

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821 **Table 3.** Data on mean rates of molecular evolution obtained under calibration scheme 1 (column
822 “mean rate”) and values used to constraint rates in calibration scheme 3. All rates are given in
823 substitutions per site per million years per lineage. Column “Gene ratio against COI” shows the ratio
824 of the rate of each gene against the rate of COI based on estimations from calibration scheme 1. The
825 extrapolation of the mean rate values according to the rate of COI obtained for the genus *Carabus* is
826 shown in the column “Extrapolated mean rate” (calculated as gene ratio*0.0129). *Constrained min*
827 *and max rate* values were used to constrain a uniform function prior for each gene on calibration
828 scheme 3.
829

Gene	Mean rate †	Gene ratio against COI	Extrapolated mean rate	Constrained min rate	Constrained max rate
<i>ATP synthase subunit 6 (ATP6)</i>	0.00478	0.70813	0.00913	0.009	0.010
<i>ATP synthase subunit 8 (ATP8)</i>	0.00550	0.81513	0.01052	0.010	0.011
<i>Cytochrome c oxidase subunit I (COI)</i>	0.00675	1.00000	0.01290††	0.012	0.013
<i>Cytochrome c oxidase subunit II (COII)</i>	0.00772	1.14381	0.01476	0.014	0.015
<i>Cytochrome c oxidase subunit III (COIII)</i>	0.00573	0.84903	0.01095	0.010	0.011
<i>Cytochrome b (CYB)</i>	0.00727	1.07760	0.01390	0.013	0.014
<i>NADH dehydrogenase subunit 1 (ND1)</i>	0.00299	0.44259	0.00571	0.005	0.006
<i>NADH dehydrogenase subunit 2 (ND2)</i>	0.00330	0.48896	0.00631	0.006	0.007
<i>NADH dehydrogenase subunit 3 (ND4)</i>	0.00590	0.87441	0.01128	0.011	0.012
<i>NADH dehydrogenase subunit 4L (ND4L)</i>	0.00284	0.42056	0.00543	0.005	0.006
<i>NADH dehydrogenase subunit 4 (ND4)</i>	0.00261	0.38709	0.00500	0.005	0.006
<i>NADH dehydrogenase subunit 5 (ND5)</i>	0.00326	0.48371	0.00624	0.006	0.007
<i>NADH dehydrogenase subunit 6 (ND6)</i>	0.00565	0.83806	0.01081	0.010	0.011
<i>16S ribosomal RNA (rrnL)</i>	0.00229	0.33956	0.00438	0.004	0.005
<i>12S ribosomal RNA (rrnS)</i>	0.00248	0.36804	0.00475	0.004	0.005

830 † Mean rate of molecular evolution obtained with the uncorrelated log-normal relaxed molecular clock under
831 calibration scheme 1 (*ucl.d.mean* parameter of BEAST)

832 †† rate of molecular evolution of the COI gene obtained for the genus *Carabus* (Andújar et al. 2012)

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Table 4. Summary of support values and ages obtained for a selection of nodes from the phylogenetic analyses conducted on RAxML IQtree, MrBayes and BEAST.

Node	Result†	RAxML	IQTree	MrBayes	BEAST c.s. 1† †	BEAST c.s. 2	BEAST c.s. 3
<i>(D. occultus, D. staudingeri)</i>	Support	100	100	1	1	1	1
Diochini crown	Support	76	96	1	1	0.99	1
(Diochini (Xantholinini, Othiini))	Support	82	97	1	1	1	1
(Xantholinini, Othiini)	Support	57	71	0.73	0.74	0.64	0.5
Staphylininae crown	Support	41	65	1	1	1	1
(Staphylininae, Paederinae)	Support	47	100	1	1	1	1
<i>(D. occultus, D. staudingeri)</i>	Ages	N.a.	N.a.	N.a.	47.8-61.3	48.2-66.3	34.3-47.4
Diochini crown	Ages	N.a.	N.a.	N.a.	106.3-143.2	107.3-155.4	77.0-122.2
(Diochini (Xantholinini, Othiini))	ages	N.a.	N.a.	N.a.	139.9-155.5	138.9-172.5	124.6-132.2
(Xantholinini, Othiini)	Ages	N.a.	N.a.	N.a.	136.5-151.9	135.5-167.8	121.0-130.3
Staphylininae crown	Ages	N.a.	N.a.	N.a.	146.11-161.1	145.9-179.6	127.6-137.2
(Staphylininae, Paederinae)	ages	N.a.	N.a.	N.a.	150.4-165.1	149.4-183.5	129.9-140.0

† Support is shown as bootstrap values for ML analyses on RAxML and IQTree, and posterior probability for MrBayes and BEAST; Ages are given in Mya and represent the 95% high posterior density interval from sampled trees; †† BEAST analyses under calibration schemes 1, 2, and 3, respectively.