Phylogenetic inferences on West Mediterranean Ditomina (Coleoptera: Carabidae: Harpalini) based on molecular data

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Abstract. Ditomina is a subtribe of ground beetles included in the large tribe Harpalini, with most species restricted to the Mediterranean basin. To date, affinities within Harpalini have mainly been derived from morphological data, and the placement of several genera within Ditomina has been subject to discussion. In this study we provide a regional molecular phylogeny for Ditomina with representatives from seven of the eight known genera from the West Mediterranean region, the Australian *Phorticosomus* Schaum, 1862, and selected Harpalini outgroup taxa. DNA sequences from two different markers, the mitochondrial protein coding gene *cox1* and the nuclear gene *ITS2*, were sequenced and analysed from 19 taxa. Molecular phylogenetic analyses consistently support (i) the sister relationship of genera *Carterus* Dejean, 1830 and *Eocarterus* Stichel, 1923; (ii) a close relationship among *Ditomus* Bonelli, 1810, *Tschitscherinellus* Csiki, 1906 and *Odontocarus* Solier, 1835, which form a lineage well separated from *Dixus* Billberg, 1820; and (iii) the exclusion of *Graniger* Motschulsky, 1864 and the Australian *Phorticosomus* from the subtribe Ditomina. In addition, phylogenetic trees indicate a vicariance event for the brachypterous genus *Eocarterus* on both sides of the Strait of Gibraltar, followed by further geographical differentiation in southern Iberia and northern Morocco. This pattern contrasts with the distribution of various fully winged species of ground beetles on both sides of the same barrier, suggesting the role of dispersal limitation in speciation. Finally, a new key to supraspecific taxa of the Ditomina of the West Mediterranean region is provided.

Key words. Molecular phylogeny, *cox1*, *ITS2*, systematic, vicariance, taxonomic keys.

1. Introduction

The Ditomici group of Bonelli (1810) is currently considered to make up the subtribe Ditomina within the tribe Harpalini (Lorenz 2005; Wrase & Kataev 2017). The group comprises 19 genera and about 98 species worldwide (Wrase & Kataev 2017). In total, 18 genera and 80 of these species are found in the Palearctic region, out of which 8 genera and 21 species are known to occur in the West Mediterranean (Wrase & Kataev 2017). The Ditomina are characterized by a dull and black (or dark brown) dorsal coloration, absence of basal border of elytra, male foretarsi that are not or poorly dilated, and male genitalia relatively small in comparison to other harpalines. Furthermore, many species have a hairy dorsum with coarse punctures. A number of taxa have been shown to be granivorous (seed eaters) (Brandmayr et al. 1990; Kulkarni et al. 2015), in agreement with the morphological design of mouth appendages and a hypertrophic head that harbours the strong muscles needed for crushing hard seeds. Some species are notable by showing presocial behavior (Brandmayr & Brandmayr 1987).

Phylogenetic relationships among members of Ditomina have been analyzed by Stichel (1923) and particularly by Wrase (1993, 1994, 1999), who put forward a number of hypotheses about the primitive or derived

status of morphological characters found in the genera *Eocarterus* Stichel, 1923, *Carterus* Dejean, 1830, and *Oedesis* Motschulsky, 1850. Likewise, a number of phylogenetic hints were derived from the study carried out by Martínez-Navarro et al. (2005) on the whole tribe Harpalini, based on DNA sequences of the mitochondrial *cox1* gene (3' region) and including 10 species of Ditomina. During the last years it was possible to solve some of the major taxonomic problems of this subtribe. For example, various taxa that were wrongly included in the past (e.g., *Bottchrus* Jedlicka, 1935) (Lorenz 2005), are now recognized as members of other harpaline subtribes. Beside this, the hypothesis of a close relationship between "ditomines" and the genus *Ophonus* Dejean, 1821 discussed by Antoine (1959) has been rejected (Martínez-Navarro et al. 2005). Despite these advances in the knowledge of the group, the position of the Palaearctic genera *Graniger* Motschulsky, 1864, *Eucarterus* Stichel, 1923, *Oedesis* Motschulsky, 1850, and the Australian genus *Phorticosomus* Schaum, 1862 are still controversial (Noonan 1976; Wrase 1999), and a comprehensive phylogeny of the whole subtribe has not been yet developed. Here we provide a time-calibrated regional molecular phylogeny for the Ditomina with representatives from seven of the eight known genera from the West Mediterranean region as well as data of three Australian *Phorticosomus* species The main aims of the study are (i) to contribute to a better understanding of relationships among Ditomina occurring in the West Mediterranean, (ii) to test the placement of *Graniger* and *Phorticosomus* within Ditomina, and (iii) to provide a timeframe for the evolution of the group. Results on the molecular phylogeny are discussed considering its congruence with morphological characters and geographic distribution. In addition, we provide a new key to West Mediterranean supraspecific taxa of the Ditomina.

2. Material and methods

2.1. DNA extraction, amplification, and dataset assembly

Taxa sequenced in the study comprised 44 individuals from 16 species of 7 genera, listed in Table 1. The following Harpalini genera were used as outgroup taxa: *Cryptophonus* Brandmayr & Zetto-Brandmayr, 1982; *Harpalus* Latreille, 1802; *Ophonus* Dejean, 1821; and *Acupalpus* Latreille, 1829. All beetles were captured in the field, immersed in pure ethanol and kept at −20°C until DNA extraction. The right hind leg was usually detached from the animal and used to extract total genomic DNA with a standard glass fibre extraction protocol (Ivanova et al. 2006). Voucher specimens (voucher numbers available on Table 1) and DNA aliquots are deposited in the collection of the Department of Zoology

and Physics Anthropology of the University of Murcia (ZAFUMU col.).

A 658 base pair (bp) fragment of the mitochondrial *cox1* gene (barcode region) was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGA TATTGG) and HCO2198 (5'-TAAACTTCAGGGT GACCAAAAAATCA) (Folmer et al. 1994). PCRs consisted of an initial activation at 95°C for 3 min, followed by 35 cycles of 60 s at 94°C, 60 s at 45°C, and 90 s at 72°C, and then a final extension of 5 min at 72°C, and were performed using BioTAQ™ DNA Polimerase (Bioline Inc.). PCR product purification and sequencing in both directions was performed by Macrogen Inc. (Korea) using the standard protocol for ABI BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Additional available barcoding sequences of the Ditomina genus *Phorticosomus* were retrieved from GenBank resulting in the *cox1-bc* dataset. This dataset was aligned with MAFFT (Katoh et al. 2017) and the L-INS-I method with default parameters.

A second nuclear gene fragment, a part of the internal transcribed spacer 2 (*ITS2*) located between the 18S and 28S rRNA gene, was amplified using the primers 5.8sF (5'-GTGAATTCTGTGAACTGCAGGACACAT GAAC) and 28sR (5'-ATGCTTAAATTTAGGGGGTA) (Porter & Collins 1991). For this marker, PCR conditions included an initial activation at 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 30 s at 54°C, and 30 s at 72°C, with a final extension of 10 min at 72°C, and were performed using BioTAQ™ DNA Polimerase (Bioline Inc.) as before. Final *ITS2* dataset was aligned with MAFFT using L-INS-I method with default parameters, and included 24 sequences from 11 species and 4 genera.

Sequences from an additional mitochondrial fragment of 837 bp from the 3' end of the *cox1* gene were obtained from GenBank from available Ditomina species (*cox1-3P* dataset; 16 sequences from 12 species).

A combined dataset was obtained by concatenating the two mitochondrial fragments (*cox1-bc* and *cox1-3P*) with the nuclear *ITS2*. Missing mitochondrial or nuclear data of particular individuals were concatenated with the complementary data (nuclear or mitochondrial) of individuals of the same species, only in the case of *Acupalpus* generating an interspecific chimera. All newly generated DNA sequences were deposited in GenBank (Table 1).

2.2. Phylogenetic analyses

The program jModelTest2 (Darriba et al. 2012) was used to select the best-fitted model of sequence evolution for each dataset using the Akaike Information Criterion (GTR+I+G for all the datasets). Phylogenetic analyses for the *cox1-bc*, *ITS2*, and combined datasets, the latter partitioning by gene, were conducted with Maximum likelihood (ML) and Bayesian inference (BI) through the online platform CIPRES Science Gateway (Miller et al. 2010). ML analyses were performed using RAXML

Table 1. Specimens included in this study, indicating species distribution, specimen locality and Genbank sequence accession numbers. In-group taxa are highlighted with names in bold. Accession number has been used as "voucher code" for sequences retrieved from Genbank database. * marks a pair of specimens within a species for which sequences were concatenated in the combined dataset. Wings (Yes/No) indicates the presence or absence of fully developed hindwings for the ingroup taxa; IB: Presence on Iberian Peninsula; NF: Presence in northern Africa; IBNF: Presence on Iberian Peninsula and northern Africa (only shown for ingroup taxa).

Species	Wings	Distribution	Voucher-code	Locality	cox1-bc	cox1-3P	ITS2
Carterus (*Carterus*) *dama* (Rossi 1792)	Yes	IBNF	381(10)	Zahara de los Atunes (Cádiz, Spain)	MK510477	NA	MK510449
Carterus (*Carterus*) *fulvipes* (Latreille 1817)	Yes	IBNF	25(04)	Laguna del Arquillo, Masegoso (Albacete, Spain)	MK510478	NA	MK510450
			93(02)	El Bonillo 11 km N (Albacete, Spain)	MK510479	NA	MK510451
			96(11)	Arroyo de Santiago, Nerpio (Albacete, Spain)	MK510480	NA	MK510452
			195(10)	Sierra de La Pandera (Jaén, Spain)	MK510481	NA*	NA
			203(07)	Sierra Espuña (Murcia, Spain)	MK510482	NA	NA
			AJ583283	El Burgo (Málaga, Spain)	NA	AJ583283*	NA
Carterus (*Carterus*) *gilvipes* Piochard de la Brûlerie 1873	Yes	IBNF	413(07)	Embalse de Almodovar (Cádiz, Spain)	MK510483	NA	MK510453
Carterus (*Carterus*) *interceptus* Dejean 1829	Yes	IBNF	356(10)	Mers el Hadjadj (Wilaya Oran, Algeria)	MK510486	NA	NA
Carterus (*Carterus*) *rotundicollis* (Rambur 1842)	Yes	IBNF	52(11)	Mulay Abdessalam (Rif region, Morocco)	MK510488	NA*	MK510456
			579(07)	Mulay Adessalam (Rif region, Morocco)	MK510489	NA	MK510457
			57(11)	Mulay Abdessalam (Rif region, Morocco)	MK510490	NA	MK510458
			60(11)	Mulay Abdessalam (Rif region, Morocco)	MK510491	NA	MK510459
			598(07)	Mulay Abdessalam (Rif region, Morocco)	MK510492	NA	MK510460
			AJ583284	Salinas de Pinilla (Albacete, Spain)	NA	AJ583284*	NA
Carterus (*Microcarterus*) *gracilis* (Rambur 1842)	Yes	IBNF	71(11)	Zahara de los Atunes (Cádiz, Spain)	MK510484	NA*	MK510454
			670(10)	Zahara de los Atunes (Cádiz, Spain)	MK510485	NA	MK510455
			AJ583285	Khenichet (Morocco)	NA	AJ583285*	NA
Carterus (*Microcarterus*) *microcephalus* (Rambur 1842)	Yes	IBNF	597(07)	Mulay Abdessalam (Rif region, Morocco)	MK510487	NA	NA
Ditomus tricuspidatus (Fabricius 1792)	Yes	IBNF	38(11)	Mohammedia (Morocco)	MK510493	NA	NA
			113(09)	Menzel-Burguiba- Mateur (Bizerte, Tunisia)	MK510494	NA	NA
			264(10)	Barrage El Fakia, Ouizert (Wilaya Mascara, Algeria)	MK510495	NA	MK510461
			412(07)	Embalse de Almodovar (Cádiz, Spain)	MK510496	NA*	NA
			KJ825786		NA	KJ825786*	NA
Dixus capito capito (Audinet-Serville 1821)	Yes	IBNF	30(09)	Cañada de los Mojones (Albacete, Spain)	MK510497	NA*	MK510462
			AJ583279	Villaverde (Albacete, Spain)	NA	AJ583279*	NA
Dixus clypeatus (Rossi 1790)	Yes	IBNF	47(10)	Sa Negreta, Ibiza (Spain)	MK510498	NA	NA
			74(11)	Sa Negreta, Ibiza (Spain)	MK510499	NA	NA
			97(11)	Sierra Espuña (Murcia, Spain)	MK510500	NA	MK510463
			109(05)	Las Negras (Almería, Spain)	MK510501	NA	MK510464
			218(07)	Sierra de Aitana (Almería, Spain)	MK510502	NA	NA
Dixus sphaerocephalus (Olivier 1795)	Yes	IBNF	35(11)	Loulad (prov. Settat, Morocco)	MK510503	NA	MK510465
			70(11)	Guardamar (Málaga, Spain)	MK510504	NA	NA
			81(10)	Col de Zarifete, N22 (Algeria)	MK510505	NA	NA
			202(07)	Sierra Espuña (Murcia, Spain)	MK510506	NA*	NA

Table 1 continued.

Species	Wings	Distribution	Voucher-code	Locality	cox1-bc	cox1-3P	ITS2
Dixus sphaerocephalus (Olivier 1795)			208(09)	Ouergech (Wilaya Jendouba, Tunisia)	MK510507	NA	MK510466
			217(10)	Collado la Pandera (Jaén, Spain)	MK510508	NA	MK510467
			316(09)	road Beja-Nefza (Wilaya Beja, Tunisia)	MK510509	NA	NA
			397(09)	Sierra de Baza (Granada, Spain)	MK510510	NA	NA
			1016(07)	Sierra Espuña (Murcia, Spain)	MK510511	NA	MK510468
			AJ583276	San Pedro del Pinatar (Murcia, Spain)	NA	AJ583276*	NA
Eocarterus (*Baeticocarus*) *amicorum* Wrase 1993	No	IB	4(04)	El Torcal de Antequera (Málaga, Spain)	MK510512	NA	MK510469
			180(07)	Villanueva del Trabuco (Málaga, Spain)	MK510513	NA*	MK510470
			AJ583286	Puerto del Viento (Málaga, Spain)	NA	AJ583286*	NA
Eocarterus (*Iberocarterus*) *tazekensis rifensis* Cobos 1961	No	NF	21(11)	Bab Taza (Rif region, Morocco)	MK510514	NA	MK510471
			45(11)	Ketama (Rif region, Morocco)	MK510515	NA	NA
			51(11)	Mulay Abdessalam (Rif region, Morocco)	MK510516	NA	NA
			551(07)	Bab Berred (Rif region, Morocco)	MK510517	NA	NA
Odontocarus cephalotes (Dejean 1826)	Yes	IBNF	411(07)	Embalse de Almodovar (Cádiz, Spain)	MK510519	NA*	NA
			AJ583281	Puerto del Viento (Málaga, Spain)	NA	AJ583281*	NA
Tschitscherinellus cordatus (Dejean 1825)	Yes	IBNF	197(07)	Sierra Espuña Murcia, Spain)	MK510518	AJ583280*	NA
Harpalus attenuatus Stephens 1828	Yes		12(11)	Cubillos el Rojo (Burgos, Spain)	MK510521	NA*	MK510474
			AJ583359	Pto. de Tornavacas (Ávila, Spain)	NA	AJ583359	NA
Graniger femoralis (Coquerel, 1858)	Yes		428(07)	Sierra Monte Figo (Algarve, Portugal)	MK510523	NA	NA
Cryptophonus schaumii (Wollaston 1864)	Yes		18(00)	Arafo (Tenerife, Spain)	MK510520	NA*	MK510473
			AJ583359	Arafo (Tenerife, Spain)	NA	AJ583359*	NA
Ophonus (*Metophonus*) *cordatus* (Duftschmid 1812)	Yes		114(11)	Revilla de Pomar (Palencia, Spain)	MK510522	NA	MK510475
Acupalpus elegans (Dejean, 1829)	Yes		AJ583262	Salinas de Cordovilla (Albacete, Spain)	NA	AJ583262*	NA
Acupalpus cantabricus (Brulerie, 1868)	Yes		162(09)	Babouch (Tunisia)	MK510524	NA*	NA
Acupalpus maculatus (Schaum, 1860)	Yes		713(10)	Santa Eulalia del Río (Ibiza, Spain)	NA	NA	MK510476
Phorticosomus sp.	No		KJ825786	Australia	KJ825786	KJ825786	NA
Phorticosomus sp.	No		AJ583288	Australia	NA	AJ583288	NA
Phorticosomus zabroides Sloane, 1910	No		AJ583287	Australia	NA	AJ583287	NA
Phorticosomus sp.	No		AJ583289	Australia	NA	AJ583289	NA

7.2.7 (STAMATAKIS 2006). The best scoring ML tree was selected among 100 searches and support values were obtained with 1,000 non-parametric bootstrap replicates (FELSENSTEIN 1985). Bayesian inferences were performed with MrBayes 3.2 (RONQUIST & HUELSENBECK 2003). The tree-space of single and concatenated matrices was explored with two independent runs by using four chains over 10 and 30 x 10⁶ generations, respectively, partitioning by gene in the concatenated analysis, sampling trees every 200 generations, and generating 50% majority consensus trees with a burn-in value of 25% of all sampled trees. Convergence between the two runs was used as a parameter to estimate whether the sampling number of

generations was adequate, stopping the analysis when the average value of standard deviation at frequency division fell below 0.01.

2.3. Calibration analysis

Estimation of divergence time was performed for the combined dataset using BEAST v 1.8.4 (DRUMMOND et al. 2012) through the online platform CIPRES Science Gateway. The dataset were partitioned by gene, and additionally, by codon ((1+2), 3) for the mitochondrial fragments, using an uncorrelated lognormal (UNL) clock
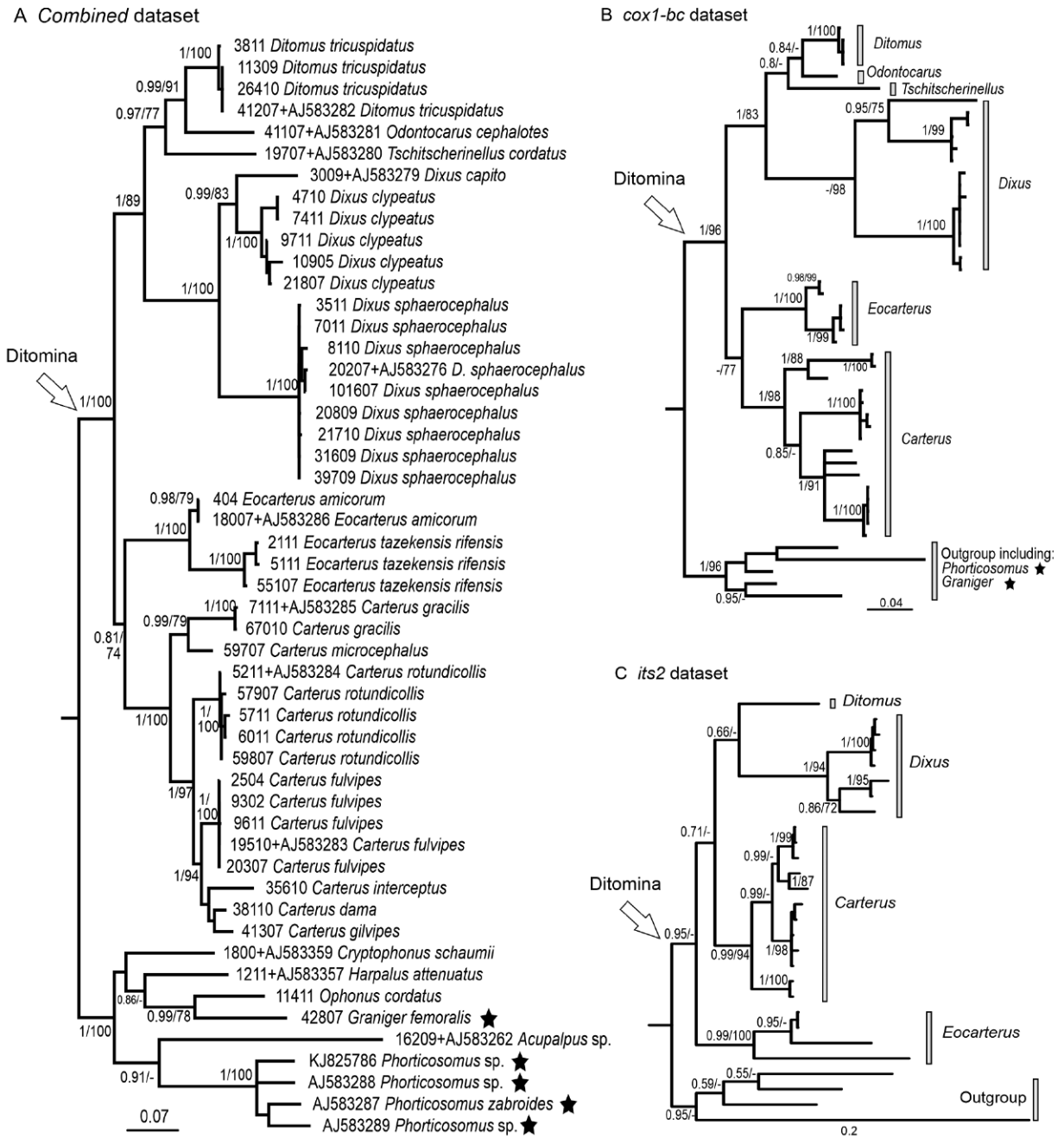
Fig. 1. The 50% majority consensus tree of West Mediterranean Ditomina resulting from the Bayesian inference of the ***combined*** dataset (**A**), the ***cox1-bc*** dataset (**B**) and ***its2*** dataset (**C**). Numbers above nodes show Bayesian posterior probabilities (pp) retrieved from MrBayes and bootstrap values (bv) from Maximum likelihood analyses in RAxML (pp/bv; only shown values higher than 0.7 and 70 respectively). Black stars represent taxa that were previously (but are no longer) considered to be within the subtribe Ditomina.

and GTR+I+G model of substitution. Calibration priors for each gene fragment were used as a uniform function on the mean substitution rate obtained from previous calibration studies on Carabidae for the same fragments (ANDÚJAR et al. 2012a). In detail: *cox1-bc*: initial -0.0113 (min 0.0081, max 0.0147); *cox1-3P:* initial 0.0145 (min 0.01, max 0.0198); ITS2: initial 0.0057 (min 0.0035, max 0.0081). Two independent analyses were run for a total of 10×10^6 generations sampling trees every 1,000 generations. 50% of the trees of each run were discarded as burn-in. Remaining trees were then combined using LogCombiner v. 1.8.4 (http://beast.community/logcombi

ner) and TreeAnnotator v. 1.8.4 (http://beast.community/treeannotator) to build a "maximum clade credibility tree" from this posterior distribution of trees.

3.   Results

Bayesian and ML phylogenetic inference of West Mediterranean Ditomina produced very similar topologies (Figs. 1, S1–S3). The Bayesian consensus trees and ML
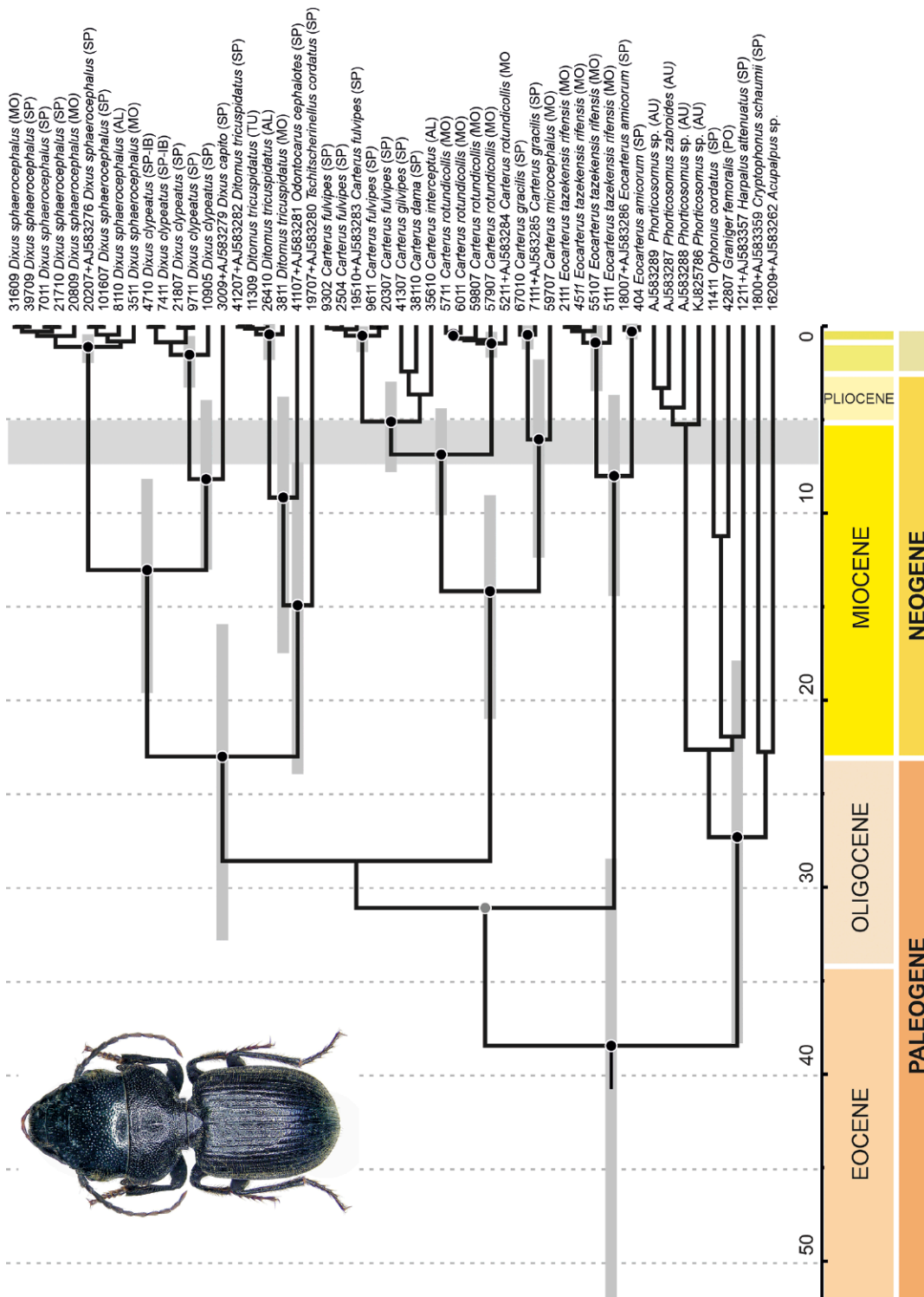
Fig. 2. Ultrametric time-calibrated trees obtained with BEAST for the combined dataset. Circles at nodes represent posterior probabilities (pp): black-filled for pp ≥ 0.9, grey-filled for pp 0.8–0.9 (lower values not included). Bars represent 95% HPD intervals for node ages in Ma. The Messiniense period (latest Miocene) is shaded in grey. Image of *Dixus capito* by U. Schmidt, 2007, licensed under the Creative Commons Attribution-Share Alike 2.0.

trees obtained for the *cox1-bc* dataset (Figs. 1B, S1) and the combined dataset (Figs. 1A, S3) revealed that (i) *Eocarterus* and *Carterus* appeared as closely related genera, with moderate support based on Bayesian posterior probabilities (pp) (pp = 0.94) for the *cox1*-bc dataset and low (pp = 0.81) with the combined dataset (Fig. 1), (ii) the genus *Carterus* is divided in two lineages corresponding to the nominal subgenus *Carterus* and the subgenus *Microcephalus* Antoine, 1959, with two species (*C. microcephalus* and *C. gracilis*); (iii) the second main branch

of the phylogenetic tree includes one clade made up by the three species of *Dixus*, and another clade with representatives of genera *Ditomus* Bonelli, 1810; *Odontocarus* Solier, 1835; and *Tschitscherinellus* Csiki, 1906; (iv) *Graniger* is not closely related to Ditomina; instead, it appears related to a clade in the outgroup that is made up by representatives of other subtribes of Harpalini; (v) *Phorticosomus* is not closely related to Ditomina, and seems to be more related to lineages within the outgroup taxa. Nevertheless, the uncertainties about its position

(low to moderate support on the different analyses) suggest caution when interpreting these results. The combined dataset gave rise to similar results to the *cox1-bc* dataset (Fig. S3), but higher support was found for various lineages (see Fig. 1), e.g., the relationships between *Ditomus*, *Dixus*, *Odontocarus,* and *Tschitscherinellus*. Trees obtained for the *its2* dataset were generally consistent with the *cox1-bc* and *combined* datasets. Phylogenetic relationships among the three main clades (i.e., clade of *Carterus*, clade of *Eocarterus* and clade of *Dixus + Ditomus* and related taxa), however, were not supported (Fig. 1C).

The calibration analyses showed a similar topology; again, relationships among the three main clades (i.e., clade of *Carterus*, clade of *Eocarterus* and clade of *Dixus* and related taxa) were not supported (Fig. 2). The split between the vicariant ibero-maghrebian species of *Eocarterus* is dated back to the Late Miocene (9–6 mya).

4.   Discussion

4.1.   Phylogenetic relationships within West Mediterranean Ditomina

Relationships among taxa derived from our phylogenetic analyses match to a large extent with implicit relationships derived from taxonomic studies based on morphological characters (e.g., Antoine 1959; Coulon 2011). The close relationship between *Eocarterus* and *Carterus*, suggested by Antoine (1959), is here confirmed with moderate to high support for the *cox1-bc* dataset (Bayesian analyses: pp = 94; ML analyses: bootstrap = 77) and moderate to low support on the combined dataset (Bayesian analyses: pp = 81; ML analyses: bootstrap = 74). It is also corroborated that the subgenus *Microcarterus*, put forward by Antoine (1959) and here represented by *C. gracilis* (Rambur 1842) and *C. microcephalus* (Rambur, 1842), forms a well differentiated lineage with a sister relationship with the clade including species of the nominal subgenus *Carterus*. Within the latter, it is worth mentioning that *C. fulvipes* (Latreille, 1817) and *C. rotundicollis* (Rambur, 1842) are well discriminated based on molecular data, whereas these species often show problems for its morphological identification as they resemble each other closely.

The molecular affinity between the genera *Ditomus*, *Odontocarus,* and *Tschitscherinellus* provides an interesting clue to the phylogeny of these taxa, as hints derived from previous works are controversial (e.g., Jeannel 1942; Antoine 1959; Coulon 2011). It seems that the lineage made up by these three genera could be characterized by a clypeus armed with teeth and (or) tubercles, and a medium or large body size, among other characters. Wrase (1994) showed that each of these three genera is characterized by particular apomorphies, including: (i) Clypeus with four teeth in *Odontocarus* (plesiomorphic

state: without teeth); (ii) hairy paraglossae and presence of two supraorbital setae in *Tschitscherinellus* (plesiomorphic states: hairless paraglossae; one supraorbital seta); and (iii) clypeus with a horn in middle in *Ditomus* (plesiomorphous: lack of horn). Wrase (1994) indicated the presence of two large supraorbital setae in *Ditomus* but the individuals here studied showed only one. As Wrase (1994) indicated, the evolution of the supraorbital setae character within the group needs further investigation.

Based on molecular data, *D. clypeatus* (Rossi, 1790) is closer to *D. capito* (Audinet-Serville, 1821) than to *D. sphaerocephalus* (Olivier, 1795). This finding suggests that the frontal depressions, the sparse dorsal punctures and the vanished hind pronotal angles of *D. clypeatus* are autapomorphies developed during the differentiation of this species.

The distant position of *Graniger femoralis* (Coquerel, 1858) to Ditomina taxa (clustered with outgroup taxa of the subtribe Harpalina) corroborates the conclusion of Wrase (1999) about a closer relationship between this genus and members of the subtribe Harpalina. Likewise, the Australian genus *Phorticosomus* is distantly related to members of Ditomina, and according to our results where it is clustered within the the outgroup taxa (Figs. 1, 2) and could be placed in a different subtribe of Harpalini rather than within Ditomina. Nevertheless, as indicated above, the uncertainties about its position (low to moderate support on the different analyses) suggest caution when interpreting these results.

4.2.   Vicariance of Ditomina taxa in the West Mediterranean

Most species included in the study are found in open habitats and, according to the presence of functional wings, are presumed a notable dispersal capability. This might be related with the absence of phylogeographical structure on specimens of *Dixus sphaerocephalus* from both sides of the West Mediterranean basin based on our molecular data, and also is reflected in the fact that all studied species but those within the genus *Eocarterus* are distributed at both sides of the Gibraltar strait (Table 1).

On the other hand, the West Mediterranean species of the genus *Eocarterus* are found in forest habitats and are the only micropterus lineage within the group, thus having a presumed lower dispersal capability that fully winged species. In agreement with this low dispersal capacity, this genus includes morphologically close related endemic species restricted to the south of the Iberian Peninsula and to the northwest of Africa. Our dating analyses indicates that the split between *Eocarterus amicorum* Wrase, 1993 and *E. tazekensis* Cobos, 1961 likely occurred during the last part of the Miocene (Fig. 2), before the opening of the strait of Gibraltar, which is dated 5.3 mya. By this time, a large island between North Africa and Iberia became definitely separated from the first and included in the second (Andeweg 2001). This

tectonic movement and associated changes in the Mediterranean region likely favoured a number of lineage splits due to geographic separation (reviewed by OOSTERBROEK & ARNTZEN 1992), as this has been proposed for other wingless carabids including species of the genus *Calathus* (RUIZ et al. 2012), *Carabus* (ANDÚJAR et al. 2012b) and *Typhlocharis* (ZABALLOS & PÉREZ-GONZÁLEZ 2010), and even for winged taxa that are tied to particular habitats (*Cicindela campestris* Linnaeus, 1758; GARCÍA-REINA et al. 2015). Our results are consistent with vicariant speciation of *Eocarterus* related with the tectonic evolution of the Betic-rifean plates during the end of the Miocene leading to the final opening of Gibraltar strait. In addition, after the separation of Iberian and Moroccan *Eocarterus* populations, the first ones probably speciated and gave rise to *E. amicorum* and *E. baeticus* (not sampled), whereas Moroccan populations diverged into *E. tazekensis tazekensis* (Middle Atlas mountains; not sampled) and *E. tazekensis riffensis* (Rif mountains) in North Morocco.

4.3. A proposal of a new taxonomic key to genera of West Mediterranean Ditomina

In addition to the relationships among taxa of Ditomina inferred for the molecular phylogeny discussed above, here we propose a new key to determine supraspecific taxa of Ditomina from the West Mediterranean, where we have highlighted characters that allow defining natural groups. Unfortunately, we have not been able to get individuals of the genus *Oedesis* for the molecular analysis, so the key that follows should be revised in the light of new evidence. In any case, *Oedesis* is morphologically distant from the other taxa of Ditomina, according to WRASE (1999), and it is a good candidate for opening the key.

1 Pronotum cordiform, posterior angle acute and protruding outwards. Lateral side of pronotum slightly serrate, particularly close to posterior angle. Head size normal, eyes protruding. Ligula with two setae. Mentum with medial tooth and two setae. Male protarsi slightly dilated and with two parallel rows of adhesive setae. Bordered basal margin of elytron complete. .... ***Oedesis*** Motschulsky, 1850

1' Pronotum notably transverse or cordiform, but posterior angle not acute and protruding outwards. Lateral side of pronotum smooth, not serrate. Head often hypertrophic and with eyes not protruding; alternatively, head of normal size with protruding eyes. Ligula with many setae. Mentum without setae. Male protarsi not dilated except for *Carterus*; in that case there are adhesive setae underneath without forming rows. Bordered basal margin of elytron incomplete, only visible from humerus to origin of 4th stria. **2**

2 Pronotum clearly cordiform, not transverse. Humeral region rounded, epipleura invading antero-lateral area of elytron and clearly visible in dorsal view. Propleura without a slight carena. .... ***Eocarterus*** Stichel, 1923; subgenus ***Baeticocarus*** Jeanne, 1971.

2' Pronotum transverse and posteriorly forming a peduncle. Humeral region squared, epipleura, at most, slightly invading antero-lateral area of elytron, and not seen on dorsal view. Propleura with a slight carena. **3**

3 Head moderately broadened, eyes protruding, anterior border of clypeus slightly thickened, first antennomere (scape) large, abruptly widened from the first quarter. Male protarsi slightly dilated and with adhesive setae underneath, disorderly arranged. Posterior border of abdominal segments not thickened in the female (except for *C. interceptus*). .... **4** ***Carterus*** Dejean, 1830

3' Head either broad and with non-protruding eyes, or of moderate size with protruding eyes, anterior border of clypeus variable (either normal, toothed or horned), first antennomere gradually broadened from the base, and of variable size. Male protarsi not broadened. Posterior border of female abdominal ventrites thickened. **5**

4 Apex of protibia projected outwards forming a triangular tooth. Punctures of striae arranged in two or more rows. .... subgenus ***Carterus***

4' Apex of protibia simple on external side, not projected forming a triangular tooth. Punctures of striae arranged in a single row. .... subgenus ***Microcarterus*** Antoine, 1959

5 Head hypertrophic, not narrowed behind eye and without forming a neck. Anterior angle of pronotum acute and projecting towards the eye. Medial tooth of mentum large, of the same size as lateral ones. .... ***Dixus*** Billberg, 1820

5' Head of normal size, narrowed after the eye and forming a neck. Anterior angle of pronotum nor protruding forewards. Medial tooth of mentum small, clearly smaller than lateral ones. **6**

6 Clypeus with convex shape and medially thickened. Suture between 2nd and 3rd abdominal sternites visible in middle. Only one large supraorbital seta. .... ***Odontocarus*** Solier, 1835

6' Clypeus either normal or strongly thickened and forming a horn. Suture between 2nd and 3rd abdominal sternites not visible in middle. Two large supraorbital setae. **7**

7 Large body size (15–20 mm). Temporal region of head notably developed, almost perpendicular to head axis. Lateral margin of pronotum well developed (particularly close to anterior angle), posterior margin complete but vanishing close to angle. Paraglossae short-haired. .... ***Tschitscherinellus*** Csiki, 1906

7' Smaller body size (< 15 mm). Temporal region weakly developed and oblique to head axis. Lateral margin of pronotum narrow, basal margin not emarginate. Paraglossae hairless .... ***Ditomus*** Bonelli, 1810

5. Acknowledgements

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Authors' contributions

All authors contributed to the sampling. J.S. identified specimens and did taxonomic keys. All authors collaborated on the molecular lab work. C.A. and C.R. performed phylogenetic analyses. All authors contributed to the writing of the manuscript.

Electronic Supplement File

at http://www.senckenberg.de/arthropod-systematics

File 1: andújar&al-ditominaphylogeny-asp2019-electronicsupplement-1.pdf — Best scoring Maximum Likelihood trees for different datasets of West Mediterranean Ditomina. Each ML tree was selected with 100 independent searches, and bootstrap values estimated with 1000 bootstrap pseudo-replicates. — **Fig. S1.** Tree for combined dataset. — **Fig. S2.** Tree for the *cox1-bc* dataset. — **Fig. S3.** Tree for *its2* dataset. DOI: 10.26049/ASP77-2-2019-01/1