UNIVERSITAT DE BARCELONA

Range expansion in the evolutionary history of western palaeartic aquatic coleoptera

Expansiones de rango durante la historia evolutiva de coleópteros acuáticos en el Paleártico occidental

David García Vázquez

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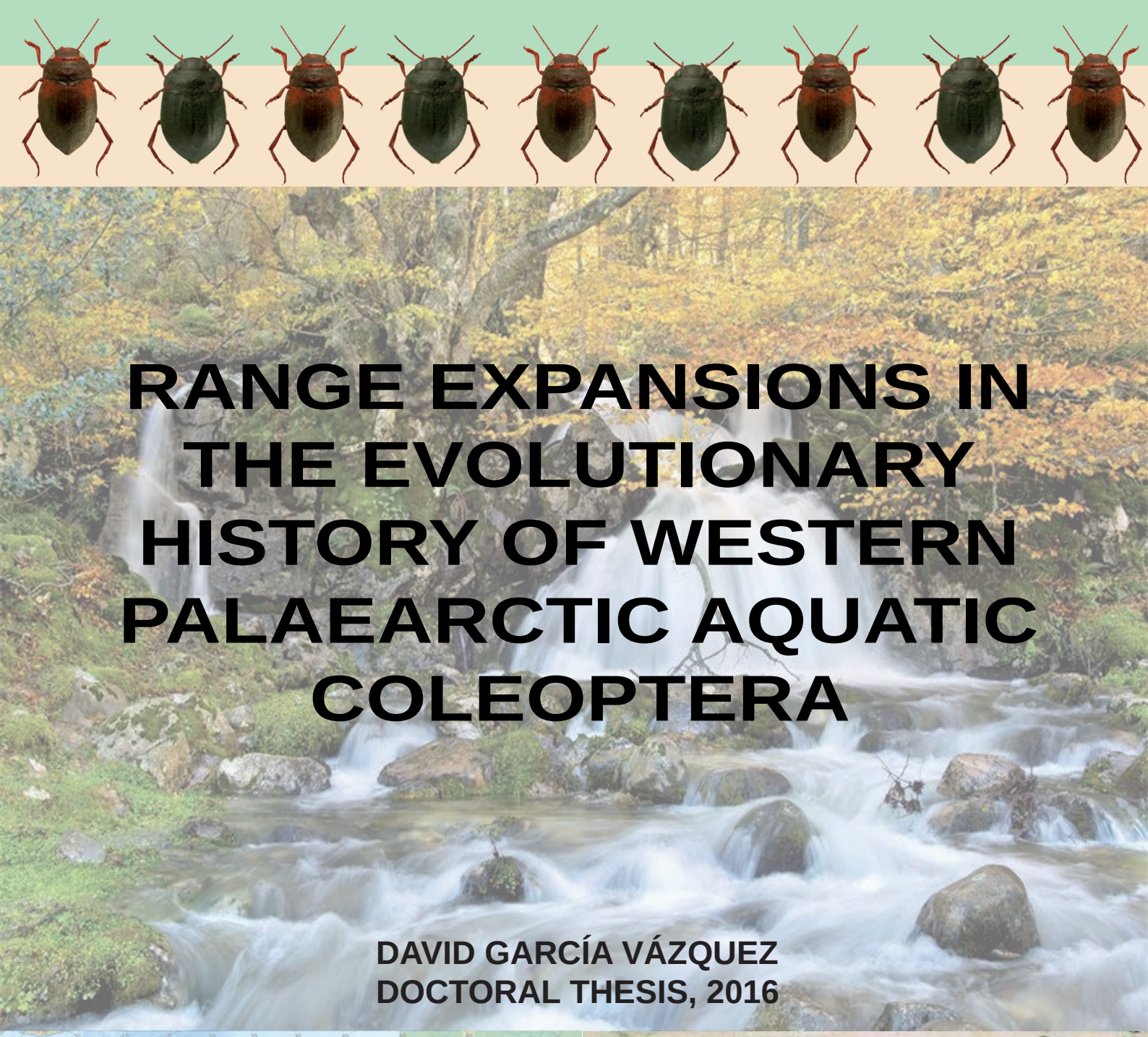
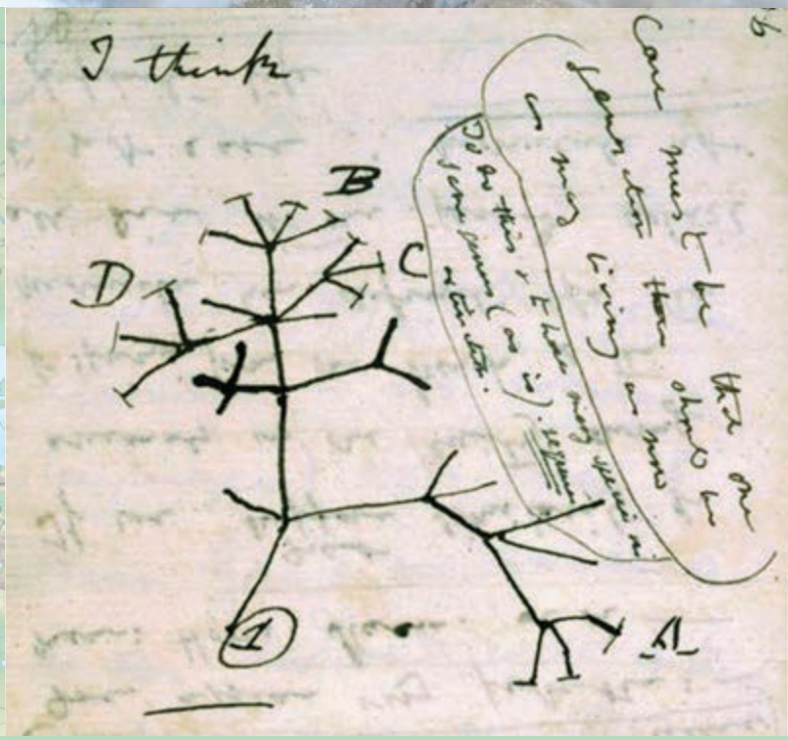


RANGE EXPANSIONS IN THE EVOLUTIONARY HISTORY OF WESTERN PALAEARCTIC AQUATIC COLEOPTERA

DAVID GARCÍA VÁZQUEZ

DOCTORAL THESIS, 2016



RANGE EXPANSIONS IN THE EVOLUTIONARY HISTORY OF WESTERN PALAEARCTIC AQUATIC COLEOPTERA

EXPANSIONES DE RANGO DURANTE LA HISTORIA EVOLUTIVA DE COLEÓPTEROS ACUÁTICOS EN EL PALEÁRTICO OCCIDENTAL

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GENERAL INTRODUCTION

Geographic range of the species

The understanding of the distribution and abundance of organisms is a basic aim in ecology (Elton, 1930; Andrewartha & Birch, 1954). This goal leads to the study of the geographic range of the species, a relevant unit in biogeography (Brown & Lomolino, 1998).

A basic question in geographic range issues is to understand why some species are widespread while others are very restricted geographically, a particularly relevant question in the case of genetically close species with similar biological and ecological requirements due to their common phylogenetic inheritance, but extreme differences in the size of their geographical distribution. It is known that the ecological and biogeographic context in which species arise and evolve determine their range sizes (Böhning-Gaese, 2006). Thus, numerous factors have been proposed to influence the geographic ranges such as resource availability, dispersal ability, population abundance, differences in niche breadth, body size, environmental variability or colonization and extinction dynamics among others (Stevens, 1989; Brown, 1995; Gaston, 2003, Gaston, 2009). However, despite the interest from ecologists, biogeographers or evolutionary biologists (e.g. Brown, 1995; Gaston, 2003), there are still few answers to explain how closely related species might have widely different geographical ranges (Lester *et al*., 2007).

Habitat stability

Dispersal ability of the species is one of the most common studied variables affecting range sizes. Species with greater dispersal ability might have been able to rapidly expand their ranges after changes in their environment and often have larger ranges, as demonstrated for plants and a number of terrestrial and freshwater animal groups (Gaston, 2003). In aquatic invertebrates dispersal ability is known to be strongly associated with habitat stability (Ribera, 2008; Dijkstra *et al*., 2014), that varies according to the main habitat type. Freshwater habitats can broadly be classified into running (lotic) or standing (lentic) waters. Standing water bodies, are generally short-lived and discontinuous in time and space (Hutchinson, 1957) while running water bodies generally persist over longer geological periods and remain connected to other water bodies within the drainage network. Species in more ephemeral lentic water bodies are therefore forced to disperse when the habitat disappears and, due to the

shorter geological duration of their habitats, tend to have better dispersal abilities than species living in the more stable running waters, that do not need to disperse, resulting in smaller range sizes with higher persistence of local populations and reduced interpopulation gene flow. This is in agreement with previous studies across multiple lineages of freshwater invertebrates, which have shown that running water species have on average smaller geographical ranges than the standing water species (e.g. Hof *et al.*, 2006, for Odonates; Ribera & Vogler, 2000 or Ribera *et al*., 2001 for aquatic beetles). Range size is also known to have a strong correlation with extinction probability (Jablonsky, 1987), as higher probabilities of extinction have been linked with narrow geographical ranges in several taxa such as mammals (Purvis *et al*., 2000), marine invertebrates (Hansen, 1980), or freshwater fishes (Rosenfield, 2002). Range size is, therefore, a prevailing indicator for the conservation status of a species (McKinney, 1997). As a consequence of these two general patterns, it could be predicted that due to their smaller range sizes, speciation and extinction rates should be higher in running water species, which should have a higher evolutionary turnover than lentic species (Ribera *et al*., 2001; Ribera, 2008). However, in some circumstances lotic species seem to be able to disperse and reach widespread distributions. Thus, while among standing water groups the frequency of species with small ranges is expected to be low, within running water lineages it is not rare to find widespread species (Ribera & Vogler, 2000). There is, however, few data on what makes a species in a clade of poor dispersers able to expand its range, or in what circumstances.

Historical palaeogeographic events in the Mediterranean Basin

In the case of aquatic beetles, the phylogenetic history of the species and its geographic location are known to be the main factors determining the size of the geographical range (Abellán & Ribera, 2011). Regarding the geographic location of the species, a common pattern among many lineages with a general Western Palaearctic distribution (the main area of study of this thesis) is the presence of one or a few species of widespread European distribution, including areas of central and northern Europe, with various closely related species with restricted distributions in the south, some of them narrow endemics, especially in the Mediterranean peninsulas. This latitudinal gradient in geographic range size has been documented (Rapoport's rule; Stevens, 1989), a local phenomenon being expressed mainly in the Palaearctic and Nearctic in which range sizes increase with latitude above 40°-50°N, that has been found in some groups (Gaston *et al*., 1998). The fact that this correlation between range size and

latitude is found mostly in the Northern Hemisphere above about 40°N suggests that historical environmental factors in this region could explain the geographic variation in range size (Brown, 1995).

For many Western Palaearctic groups of organisms, including freshwater Coleoptera, the Mediterranean peninsulas (Iberia, Balkans and Turkey) contain the highest diversity, with a substantial part of these species forming species radiations of restricted distributions in each of these areas (Oosterbroek & Arntzen, 1992; Crivelli & Maitland, 1995; Sanmartín, 2003). Previous molecular data on aquatic Coleoptera revealed the recent (Pleistocene) origin of most Mediterranean endemics (Ribera & Vogler, 2004), although in other cases the temporal origin of some narrowly distributed Mediterranean species was estimated to be older than the Pleistocene, and even the Pliocene (e.g. Miocene origin of the Iberian endemic species of *Enicocerus*, Ribera *et al*., 2010; and Ibero-Maghrebian endemic species of family Hydrochidae, Hidalgo-Galiana & Ribera, 2011). Diverse palaeogeographic events occurred during the complex geological history in the Mediterranean region are likely to have created suitable conditions for allopatric diversification of organisms with limited dispersal capabilities (Sanmartín, 2003) and shaped the present-day distribution of the Mediterranean fauna.

A series of marine transgressions and regressions has alternately isolated/connected the eastern and western sides and the northern (Tethys) and southern (Paratethys) shores of the Mediterranean (Rögl & Steininger, 1983), resulting in repeated episodes of vicariance and dispersal affecting the same areas at different times (Oosterbroek & Arntzen, 1992; Sanmartin, 2003). Thus, during the Late Oligocene-Early Miocene the formation of a continuous landmass separating the Tethys and Paratethys oceans connected Western Europe with the area roughly corresponding to the Balkans and Turkey, allowing distributions throughout the Mediterranean (Rögl & Steininger, 1983; Oosterbroek & Arntzen, 1992). Subsequent vicariant and distributional events throughout the Mediterranean during the Early and Middle Miocene with re-establishment of marine connections between the Tethys and Paratethys seas followed by successive landmass suture events between the Eastern and Western Mediterranean Basins resulted in the diversification of many Mediterranean groups (Oosterbroek & Arntzen, 1992; Montreuil, 2008).

The Messinian Salinity Crisis (MSC), at the end of the Miocene (5.96-5.33 MY, Krijgsman, 2002) when the isolation of the Mediterranean from the Atlantic Ocean and the Red Sea, combined with a decrease in rainfall, led to a partial drop in Mediterranean

sea level, may also played a key role in biotic dispersal over the Mediterranean Basin. Especially relevant for freshwater fauna was the brief Lago Mare stage of the MSC (Krijgsman *et al*., 1999) in which freshwater from the Sarmatic Sea (Paratethys) drained into the Mediterranean basin, and might have allowed coastal diffusion of freshwater organisms through a more or less contiguous network of river mouths (Bianco, 1990). MSC would have established a land bridge between Northwest Africa and the Iberian Peninsula that allowed dispersal between the two sides of the Gibraltar Strait, being therefore an alternative dispersal route by which Mediterranean lineages could have achieved circum-Mediterranean distributions before the Saharan desert was formed (Sanmartin, 2003).

The role of the Quaternary glacial cycles

The successive glacial–interglacial periods on Milankovitch timescales during the Quaternary have for long been considered key features to understand the origin of most extant central and northern European species. The shifts in ecological conditions and the repeated fragmentation of populations during the glacial cycles lead to the isolation of small populations in areas under new climatic and environmental conditions (Dynesius & Jansson, 2000). Climatic oscillations were especially drastic in northern latitudes of the Palearctic region and might have operated as ecological filters, with only those species displaying broad ecological niches being able to persist in relatively northern regions or re-colonize northern areas from glacial refugia after each glacial cycle. In the Western Palaearctic, as noted above, widespread species tend to have a central and north European distribution, i.e. including the areas most affected by the Quaternary glaciations. Therefore, it is expected that these species are of recent origin as they could not have survived in these areas during the glacial cycles, in which central and northern Europe become inhospitable to temperate species (Dawson, 1992). In this sense some molecular studies have illustrated examples of species that are likely to have originated during the Pleistocene or Holocene, including freshwater beetles (Ribera & Vogler, 2004), although there is little known on what specific factors allow the range expansion of some species and not others, or the evolutionary consequences of these range expansions to southern areas, less affected by the drastic climatic changes of the Quaternary.

The recent origin of widespread species in Europe has been traditionally explained by the classical model of postglacial range expansions from Mediterranean refugia (Hewitt, 2000). According to this model, during the glacial maxima the

populations of most European species were confined to refuge areas in the southern Mediterranean peninsulas, from where they would have re-colonized the continent when the conditions became favourable during the interglacials. The flow of migration would be mostly from south to north, with southern peninsulas acting as a 'melting pots' in which genetic diversity is maximal, and northern populations being a subset of the southern genetic diversity. An alternative hypothesis in which the colonization of central and northern Europe at the end of the Last Glacial is attributed to non-Mediterranean sources has been proposed by Bilton *et al*., (1998). In this model, the isolation during the glacial cycles may have been enough to prevent genetic flow among the peninsulas and mainland Europe, so rather than a source of re-colonizers, the southern peninsulas act as source of endemism. These southern endemics would not recolonize the European mainland, but remain confined as species with restricted distributions in the southern areas. This model is based in the evidence of existence of sheltered refuge areas in central Europe or western Asia along river valleys or some slopes of mountain ranges where moister conditions prevailed and tree cover could develop (Soffer, 1990). The flow of migration in this case would have been mostly east–west rather than north–south, and only the species or populations from these potential refuge areas would be able to expand their ranges to recolonize the newly deglaciated areas. Regarding to freshwater coleoptera, the absence in northern and central Europe of fossil remains from species currently restricted to the Mediterranean Basin among the abundant central and northern European Quaternary records (Abellan *et al*., 2011) would be in agreement with this alternative hypothesis, supporting the view of the Mediterranean peninsulas as an area of endemism, not as a source of postglacial colonisation.

Studied species

The Western Palearctic water beetle fauna is a suitable model to study range size issues, as water beetles are a rich and well-known insect group in both Europe and the Mediterranean Basin, exhibiting a high level of endemism but also with species widely distributed across the Palearctic and Holartic regions (Löbl & Smetana, 2003, 2004; Ribera *et al*., 2003).

Western Palearctic species of five genera from two different families (Family Dytiscidae and Family Hydraenidae) will be analysed in this thesis. All of them are lotic (running water) species (i. e with poor dispersal capabilities), from lineages including at

least one widespread specie plus several narrow range endemics, demonstrating their potential for range expansion:

1) The genus *Deronectes* Sharp (family Dytiscidae), with *ca*. 60 species is the largest clade of Palearctic Dytiscidae entirely confined to running waters, with a predominantly Mediterranean distribution from the Iberian Peninsula and northwestern Africa over large parts of Europe and the Middle East, reaching central Asia in the east. Species are usually restricted to relatively small geographical ranges including some island endemics, frequently in mountain regions, but there are in addition some widespread species with continental-scale distributions, making them suitable for biogeographical reconstructions.

2) The widely distributed genus *Platambus* Thomson (family Dytiscidae) occurring in the Palearctic, Nearctic, Neotropical and Oriental regions, with 64 recognised species (Nilsson, 2015) currently divided in some species-groups, among them the group of *P. maculatus*. I will study the most widespread species of the group, *P. maculatus* (Linnaeus) and its closest relative to which I refer as the *Platambus maculatus* complex.

3) The Holartic genus *Oreodytes* Seidlitz (Family Dytiscidae) typical of cold streams or lakes margins, generally at high altitude or high latitude, with 30 recognised species (Nilsson, 2015), six of them currently present in Europe.

4) The "*Haenydra*" lineage of the genus *Hydraena* Kugelann (Family Hydraenidae) with *ca*. 90 species with a north Mediterranean distribution (Trizzino *et al*., 2013). Many species of this lineage have very restricted distributions, often limited to a single valley or mountain system, but there are also few species with very wide geographical ranges. I will focus in the most widespread species of the "*Haenydra*" lineage, *Hydraena gracilis* Germar and its closest narrowly distributed relatives, named the *Hydraena gracilis* complex *sensu* Jäch (1995).

5) The genus *Meladema* Laporte, one of the largest macroinvertebrates found in Mediterranean streams, that contains three species (Nilsson, 2015), one of them widespread and the other two island endemics. However, in contrast to the rest of studied genus, the widespread species (*M. coriacea* Laporte) is not distributed in central-northern Europe but widespread in the Mediterranean area, while the two narrow range species (*M. imbricata* Wollaston and *M. lanio* Fabricius) are Macaronesian endemics, in the Canary Islands and Madeira respectively.

OBJECTIVES

The main objective of this Thesis is to contribute to the understanding of the factors that lead to the expansion of the geographic range of some species, and to suggest some hypotheses to explain the extreme differences in range size among closely related species. For this aim I reconstructed the evolutionary history of various lineages of aquatic beetles distributed in the Western Palearctic area in the context of the complex geological and climatical history of this area, in order to shed light about the factors that have contributed to the current distribution of species in these groups.

This investigation is structured in four chapters, with the following specific goals:

In the first chapter I reconstruct the evolutionary history of a genus of freshwater beetle with a pan-Mediterranean distribution (genus *Deronectes*) and correlate their phylogenetic patterns with the main historical geological and paleoclimatic events occurred in the Mediterranean Basin. The main objective is to discover which evolutionary processes have shaped the present-day distribution of these species, testing classic hypotheses which proposed a Miocene origin for groups with high biodiversity in the Iberian and Anatolian peninsulas.

In chapter two I study in detail the biogeographic and temporal origin of the widespread species in the same lineage with particularly poor dispersal capabilities, the genus *Deronectes*, including a reconstruction of the geographical history estimated from the distribution of the sampled localities.

In the third chapter I analyse the phylogenetic relationships, the age and the geographical origin of the western Palaearctic species of three wide distributed lineages (*Platambus maculatus* complex, *H. gracilis* complex, and the genus *Oreodytes*), some including species with distributions spanning the whole Palearctic. The main goal here is to shed light about the role of the Pleistocene glaciations in the diversification and current distribution of these species.

And lastly, in chapter four we reconstruct the evolutionary history of the three species of the genus *Meladema*, and use environmental niche modelling with the aim of test for possible differences in climatic preferences among lineages, and reconstruct their ancestral climatic niche.

Advisors report on the publication status of the results and the impact factor of the published papers

Dr Ignacio Ribera, supervisor of the PhD thesis of David García Vázquez, with title "Range expansions in the evolutionary history of Western Palaearctic aquatic Coleoptera", reports that the Thesis is formed by four Chapters consisting in respectively two published papers, one manuscript pending acceptance after "minor revision", and one complete manuscript ready for submission.

Chapter 1

García-Vázquez, D., Bilton, D.T., Alonso, R., Benetti, C.J., Garrido, J., Valladares, L.F. & Ribera, I. 2016. Reconstructing ancient Mediterranean crossroads in *Deronectes* diving beetles. *Journal of Biogeography*, 43: 1533-1545.

Journal of Biogeography has in the latest edition of the Journal of Citations Reports (2015) an impact factor of 3.997. This journal is in the first quartile in the categories "Ecology" (26th of 149) and "Geography, Physical" (5th of 49). *Journal of Biogeography* is edited by the International Biogeography Society, and the leading reference journal in the field of Biogeography.

Chapter 2

García-Vázquez, D. & Ribera, I. 2016. The origin of widespread species in a poor dispersing lineage (diving beetle genus *Deronectes*). *PeerJ*, DOI: 10.7717/peerj.2514 [published date: 27.9.2016]

PeerJ has in the latest edition of the Journal of Citations Reports (2015) an impact factor of 2.183. This journal is in the first quartile in the category "Multidisciplinary sciences" (14th of 63). *PeerJ* is a very recent multidisciplinary open access journal of increasing importance in all biological disciplines.

Chapter 3

García-Vázquez, D., Bilton, D.T., Foster, G.N. & Ribera, I. Pleistocene range expansions in Western Palaearctic aquatic Coleoptera. Complete manuscript, ready to be submitted to an international journal in the fields of Systematics or Zoology.

Chapter 4

Sýkora, V., García-Vázquez, D., Sánchez-Fernández, D. & Ribera, I. Range expansion and ancestral niche reconstruction in the Mediterranean diving beetle genus *Meladema* (Coleoptera, Dytiscidae). *Zoologica Scripta*, revised manuscript submitted after a first review with decision "minor revision" (15.9.2016).

Zoologica Scripta has in the latest edition of the Journal of Citations Reports (2015) an impact factor of 2.733. This journal is in the first quartile in the category "Zoology" (12th of 160), and in the third quartile in the category "Evolutionary Biology" (24th of

45). *Zoological Scripta* is a reference journal in Zoology, published by the Norwegian and Swedish Academy of Sciences.

Barcelona, 25th September 2016

Ignacio Ribera Galán

Advisors report on contribution of the PhD candidate in the papers forming the thesis

Dr Ignacio Ribera, supervisor of the PhD thesis of David García Vázquez, with title "Range expansions in the evolutionary history of Western Palaearctic aquatic Coleoptera", reports that the contribution of the PhD candidate to the publications forming Chapters 1-4 was as follows:

Chapter 1

D.G.-V. and I.R. conceived the work; D.T.B., L.F.V. and I.R. led the specimen collection; D.G.-V., R.A. and I.R. obtained the molecular data; D.G.-V. and I.R. analysed the data and led the writing; all authors contributed to the discussion of results and the writing.

Chapter 2

David García-Vázquez conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
Ignacio Ribera conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Chapter 3

D. García-Vázquez and I. Ribera conceived the work, D.T. Bilton, G.N. Foster and I. Ribera led the specimen collection, D. García-Vázquez and I. Ribera analysed the data and led the writing, all authors contributed to the discussion of results and the writing.

Chapter 4

I. Ribera conceived the work and led the specimen collection, D. García-Vázquez obtained the molecular data, V. Sýkora and D. García-Vázquez obtained distribution and ecological data, D. Sánchez-Fernández obtained and analysed the climatic data, V. Sýkora, D. García-Vázquez and I. Ribera performed the phylogenetic analyses, V. Sýkora and I. Ribera drafter the first version of the manuscript, all authors contributed to the discussion of results and the writing.

I also confirm that none of these publications or the presented data has been used, or will be used, in a different PhD thesis.

Barcelona, 25th September 2016

Ignacio Ribera Galán

ABSTRACT

A basic question in geographic range issues is to understand why some species are widespread while others closely related are very restricted geographically. In this work I want to contribute to the understanding of the factors that lead some species to expand their original geographic range and suggest some hypothesis to explain the extreme differences in range size among closely related species. For this aim I reconstruct the evolutionary history of various lineages of aquatic beetles distributed in the Western Palearctic area, in the context of the complex geological and climatical history of this area, in order to shed light about the factors that have contributed to the current distribution of species in these groups. All these lineages are typical of running waters (i.e. with poor dispersal abilities), and include both narrowly distributed endemics and widespread species, showing their potential for range expansions.

The different biogeographical reconstructions performed suggests the occurrence of several events of range expansions between the eastern and western sides of the Mediterranean Basin under favourable conditions, followed by fragmentation when conditions deteriorate. Range expansions were mainly across the north shore of the Mediterranean as in the south there is virtual absence in the studied groups of species eastern of Tunisia. Despite that it is not possible to establish a common temporal origin for all studied groups, the Miocene, with its various connections and subsequent isolation among the eastern and western sides of the Mediterranean basin, seems to be involved in the origin and east-west disjunction of some groups. However most of the current species were estimated to have originated during the Pleistocene, including those with widespread distributions, being therefore the epoch with a stronger influence in the present-day distribution. The successive rounds of range expansion and fragmentation resulted mainly in the accumulation of narrow range species in the Mediterranean area, especially the Iberian and Anatolian peninsulas. Despite evidence in some cases of speciation with range stasis within the southern Mediterranean area, some species were able to expand their original range reaching widespread distributions in northern areas, likely as a consequence of a privileged geographical position during a narrow window of favourable conditions. However, differences in physiological or ecological tolerances could also have favoured range expansions in some species and limited them in others although further experimental research in the differences in physiological tolerances in these lineages would be necessary to test this hypothesis.

RESUMEN

Una pregunta básica sobre los rangos geográficos es comprender por qué unas especies están ampliamente distribuidas mientras otras, estrechamente emparentadas tienen distribuciones restringidas. En este estudio pretendo comprender los factores que llevaron a algunas especies a expandir su rango original y sugerir posibles hipótesis que expliquen las enormes diferencias de rango entre especies emparentadas. Para ello, reconstruyo la historia evolutiva de varios linajes de coleópteros acuáticos del Paleártico Occidental bajo el contexto de la compleja historia geológica y climática de este área, con el fin de aclarar los procesos que han contribuido a la actual distribución de especies en estos grupos. Estos linajes son típicos de aguas corrientes (es decir, con baja capacidad dispersiva) e incluyen tanto endemismos distribuidos en pequeñas áreas, como especies de amplia distribución, mostrando su potencial de expansión de rango.

Las reconstrucciones biogeográficas llevadas a cabo sugieren la existencia de varios eventos de expansión de rango entre la zona oriental y occidental de la Cuenca Mediterránea bajo condiciones favorables, seguidos de fragmentación cuando las condiciones empeoraban. Estas expansiones debieron producirse principalmente a lo largo de la costa norte del Mediterráneo, ya que por la sur hay ausencia de especies al este de Túnez. A pesar de que no es posible establecer un origen temporal común para los grupos estudiados, el Mioceno con sus múltiples conexiones entre el Mediterráneo occidental y oriental seguidas de aislamiento, parece estar implicado en el origen y disyunción este-oeste en varios grupos. Sin embargo, la mayor parte de las especies actuales se originaron durante el Pleistoceno, incluyendo aquellas que han alcanzado amplias distribuciones, siendo por tanto la época mas influyente en la distribución actual de especies. Las sucesivas rondas de expansión y fragmentación de rango resultaron principalmente en la acumulación de especies restringidas en el área Mediterránea, especialmente en Anatolia y la Península Ibérica. A pesar de la evidencia en muchos casos de especiación con estabilidad de rango dentro del área Mediterránea, algunas especies lograron expandir su rango original hasta alcanzar amplias distribuciones en áreas mas al norte, probablemente aprovechando una posición geográfica ventajosa cuando las condiciones ambientales fueron ventajosas. No obstante, diferencias en tolerancia fisiológica y/o ecológica también pudieron favorecer las expansiones de rango en unas especies y limitarlo en otras, aunque futuras investigaciones experimentales se requieren para poder testar esta hipótesis.

Chapter 1

Reconstructing ancient Mediterranean crossroads in *Deronectes* diving beetles

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This chapter has been published in *Journal of Biogeography*

Journal of Biogeography (J. Biogeogr.) (2016) 43, 1533–1545

ORIGINAL ARTICLE

Reconstructing ancient Mediterranean crossroads in *Deronectes* diving beetles

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ABSTRACT

Aim To reconstruct the evolutionary history of a genus of freshwater beetle with a pan-Mediterranean distribution, to test classic hypotheses which proposed a Miocene origin for groups with high biodiversity in the Iberian and Anatolian peninsulas.

Location Mediterranean basin.

Methods We sequenced four mitochondrial and one nuclear gene from 51 specimens of 30 of the *c.* 60 extant species of *Deronectes* (Dytiscidae), all typical of mid-mountain streams from North Africa and Iberia over most of Europe to the Middle East. We used maximum likelihood, Bayesian probabilities with an *a priori* evolutionary rate and a dispersal–extinction–cladogenesis model to reconstruct their biogeographical history.

Results *Deronectes* has two major lineages which originated in the mid Miocene; one including mostly eastern and another mainly western and central Mediterranean species. From these two areas, range expansions, mainly at the end of the Miocene and beginning of the Pliocene, resulted in the many species groups and some of the extant species of the genus. Most of the current diversity and distributions are, however, of Plio-Pleistocene origin, particularly in widespread European species.

Main conclusions In line with traditional hypotheses, we found an ancient division between eastern and western Mediterranean lineages of *Deronectes*, likely resulting from the isolation of Europe west of the Alps from the Balkans and Anatolia during the early-middle Miocene. The history of the genus was strongly influenced by major geological and climatic events, with successive cycles of fragmentation and subsequent eastward and westward range expansions, resulting in a steady accumulation of species across the basin. Most of these range movements took place through the north side of the Mediterranean, with only local displacements in the south during the Messinian salinity crisis and a recent (Pleistocene) colonization of the Italian Peninsula, which remained largely submerged through most of the genus' evolutionary history.

Keywords
biodiversity hotspot, dispersal, diversification, Dytiscidae, Mediterranean, Messinian salinity crisis, phylogeny

INTRODUCTION

The Mediterranean region, with its complex geological history, is an ideal system to study the effects of palaeogeographical events on evolutionary diversification. The region has had a 'reticulated' biogeographical history, in which the constituent landmasses have repeatedly split, collided and split again in different configurations over time (Rosenbaum *et al.*, 2002; Meulenkamp & Sissingh, 2003; Popov *et al.*, 2004), resulting in repeated episodes of vicariance and dispersal (Oosterbroek & Arntzen, 1992; Sanmartín *et al.*, 2001). While the geological evolution of the basin is

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relatively well understood, the detailed geographical and temporal origins of most Mediterranean organisms remain unknown, especially in diverse groups such as insects.

Traditional hypotheses proposed a Miocene origin for many terrestrial and freshwater Mediterranean lineages, with close relationships between the fauna at the two extreme ends of the basin – the so-called Kiermack disjunction (see e.g. Brehm, 1947 on Iberian and Balkan plants, Banarescu, 1991 on the Mediterranean freshwater fauna, or Ribera & Blasco-Zumeta, 1998 on insects with disjunct distributions between the steppe areas of north-east Spain and those of the eastern Mediterranean and central Asia). Distribution throughout the Mediterranean region became possible during the Late Oligocene–Early Miocene, after the formation of a continuous landmass connecting western Europe with the area roughly corresponding to the Balkans and Turkey, separating the Tethys and Paratethys Oceans (Rögl & Steininger, 1983; Oosterbroek & Arntzen, 1992). During the Miocene, the re-establishment of occasional marine connections between the Tethys and Paratethys and successive landmass suture events between the Eastern and Western Mediterranean Basins likely resulted in the diversification of many Mediterranean groups (Oosterbroek & Arntzen, 1992; Montreuil, 2008). Alternatively, other biogeographical studies consider North Africa and the Gibraltar Strait, which closed during the Messinian salinity crisis (MSC, end Miocene), to be an alternative dispersal route by which lineages could have achieved circum-Mediterranean distributions (e.g. Sanmartín, 2003).

Many of these early hypotheses were based on the presence of the same, or very closely related species, on both sides of the Mediterranean in similar ecological conditions (see Ribera & Blasco-Zumeta, 1998 for a review), but without a wider phylogenetic context. Similarly, and given the lack of fossils in most Mediterranean groups, the estimated temporal origin of these relationships were based on circumstantial evidence alone.

The widespread use of molecular data to obtain reliable, calibrated phylogenies has resulted in a proliferation of studies on Mediterranean lineages (e.g. Levy *et al.*, 2009; Santos-Gally *et al.*, 2012; Condamine *et al.*, 2013). There are, however, very few data on freshwater invertebrates encompassing the entire Mediterranean area, most works to date focussing on only parts of the basin (e.g. Trizzino *et al.*, 2011 for northern Mediterranean freshwater beetles, or Sola *et al.*, 2013 for eastern Mediterranean freshwater planarians).

While there remains a dearth of detailed analyses of lineages with wide Mediterranean distributions, general hypotheses on the origin and composition of the Mediterranean biota as a whole can only be tested by investigating such taxa. In this work we study one of these lineages, the diving beetle genus *Deronectes* Sharp (family Dytiscidae). With *c.* 60 described species, *Deronectes* has a predominantly Mediterranean distribution, ranging from North Africa and the Iberian Peninsula over most parts of Europe and the Middle East, with some species reaching central Asia. *Deronectes* are poor dispersers, with species usually restricted to relatively small geographical ranges particularly in mountain regions, making them eminently suitable for biogeographical reconstructions. There are in addition some widespread species with continental-scale distributions (Abellán & Ribera, 2011), demonstrating their potential for range expansion. *Deronectes* usually live among gravel, stones or submerged tree roots in small streams with sparse vegetation (Fery & Brancucci, 1997).

Previous work based on mitochondrial genes and with incomplete sampling identified two main lineages within the genus, mostly corresponding to species with a western (Iberian Peninsula) or eastern (Anatolia and Middle East) distribution (Ribera *et al.*, 2001; Ribera, 2003; Abellán & Ribera, 2011), but the precise relationships of the species, their geographical origin and the temporal framework of their diversification remained obscure. In this work, we use a comprehensive molecular phylogeny to reconstruct its biogeographical history, the geographical origin of major lineages and the events that led to their current distributions.

MATERIALS AND METHODS

Taxon sampling

Deronectes contains 58 species and four subspecies (Nilsson & Hájek, 2015), most of them revised by Fery & Brancucci (1997) and Fery & Hosseinie (1998). These authors divided the genus into 10 groups based on morphology, to which Hájek *et al.* (2011) added an 11th for a single species from Turkey (*D. ermani* Hájek *et al.*) (see Appendixes S1a,b in Supporting Information for a checklist of the species and subspecies with distributions). We studied 51 specimens of 30 species, with an emphasis on the western clade (20 out of 24 known species) but including representatives of all recognized species groups with the exception of *D. ermani*. We also included three of the four described subspecies (see Appendix S1c). For some species, more than one specimen was included to detect possible unrecognized variation. We used 37 species of closely related genera of Hydroporini as outgroups, following the phylogenies of Dytiscidae in Ribera *et al.* (2008) and Miller & Bergsten (2014).

DNA extraction and sequencing

Specimens were collected and preserved in absolute ethanol directly in the field. We obtained the DNA non-destructively, either with standard phenol-chloroform extraction or commercial kits (mostly DNeasy Tissue Kit; Qiagen GmbH, Hilden, Germany and Charge Switch gDNA Tissue Mini Kit; Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Voucher specimens and DNA extractions are kept in the collections of the Institut de Biología Evolutiva, Barcelona (IBE), Museo Nacional de Ciencias Naturales, Madrid (MNCN) and Natural History Museum, London (NHM).

Six gene fragments from five different genes (four mitochondrial and one nuclear) were obtained in four different

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amplification reactions: (1) 5′ end of the cytochrome *c* oxidase subunit 1 gene (the barcode fragment, Hebert *et al.*, 2003, COI-5′); (2) 3′ end of cytochrome *c* oxidase subunit 1 (COI-3′); (3) 3′ end of 16S rDNA plus tRNA transfer of Leucine plus 5′ end of NADH dehydrogenase subunit 1 (nad1) (16S); (4) an internal fragment of the nuclear gene Histone 3 (H3) (see Appendix S2a for details on primers used and Appendix S2b for polymerase chain reaction conditions).

Phylogenetic analyses

Edited sequences were aligned using MAFFT 6 with the G-INS algorithm and defaults for other parameters (Katoh & Toh, 2008). We used jModeltest 0.1.1 (Posada, 2008) to estimate the evolutionary model that best fitted the data for each gene separately, using AIC (Akaike information criterion) scores as selection criteria and default values for other parameters (Katoh & Toh, 2008). To infer the phylogeny of *Deronectes*, we used Bayesian probabilities (Bp) and maximum likelihood (ML).

For Bayesian analyses, we used MrBayes 3.2 (Ronquist *et al.*, 2012) implementing the most similar evolutionary models to those selected by jModeltest. For the analyses of combined mitochondrial and nuclear data, we used five partitions corresponding to COI-5′, COI-3′, 16S rRNA+tRNA-Leu, nad1 and H3. We also analysed the mitochondrial (with four partitions as above) and nuclear data separately. The program was left running until we obtained a sufficient number of trees after the two independent runs converged, according to the ESS (Effective Sample Size) and PSRF (Potential Scale Reduction Factor) criteria as estimated in Tracer v1.5. (Drummond & Rambaut, 2007) and MrBayes respectively. Convergence and burn-in values were estimated visually after examining a plot of the standard deviation of split frequencies between the two simultaneous runs.

For ML analysis, we used a fast approximate algorithm as implemented in RAxML 7.1 (Stamatakis *et al.*, 2008) using GTR+G as an evolutionary model and the same partitions as in MrBayes. The optimum topology was that of the best likelihood among 100 replicates, and node support was estimated with 1000 bootstrap replicates using the CAT approximation (Stamatakis *et al.*, 2008).

Estimation of ages of divergence

We obtained an estimate of divergence dates among species with beast v1.7 (Drummond & Rambaut, 2007). There are no fossils or unambiguous biogeographical events that could be used to calibrate the phylogeny of *Deronectes*, so we used an *a priori* substitution rate for the combined mitochondrial sequence of 0.01 substitutions/site per Myr (million years) (standard deviation 0.002), similar to that obtained in related beetle groups for the same combination of mitochondrial protein coding and ribosomal genes (Papadopoulou *et al.*, 2010; Ribera *et al.*, 2010b; Andújar *et al.*, 2012). We excluded the nuclear sequence (H3), and to ensure that the topology obtained with mitochondrial sequences was the same as that obtained with the combined matrix, we constrained all well-supported nodes (with a posterior probability in MrBayes > 0.95 and a bootstrap support in RAxML > 70%) after deleting the outgroups. We used a GTR+I+G evolutionary model, an uncorrelated lognormal relaxed clock and a Yule speciation model. We executed two independent runs with the same settings that were allowed to run until they had converged and the number of trees was sufficient according to ESS values, as measured in Tracer v1.5. The consensus tree of the two runs was compiled with Tree Annotator v1.7 (Drummond & Rambaut, 2007).

Diversification

To have an estimation of possible changes in diversification rates through the evolution of the group we used a log-lineage through time approach (LTT) (Barraclough & Nee, 2001). Only the western clade could be studied, with an almost complete taxon sampling (20 out of 24 species). We used the R library 'ape' (Paradis *et al.*, 2004) to compile the LTT plot using the ultrametric tree obtained in beast after deleting duplicated specimens of monophyletic taxa. We used the γ-statistic (Pybus & Harvey, 2000) to test for temporal shifts in the diversification rate. The γ-values of complete reconstructed phylogenies follow a standard normal distribution. If $\gamma < 0$, the internal nodes can be said to be closer to the root than expected under a pure birth process, and vice versa (Pybus & Harvey, 2000).

Ancestral area reconstruction

To estimate ancestral areas of distribution, we used a dispersal–extinction–cladogenesis model implemented in the package Lagrange c++ 0.1, a ML inference model in which parameters are estimated for rates of migratory events between areas (range expansions) and local extinctions within areas (range contraction) (Almeida *et al.*, 2012). Lagrange takes branch lengths into account and allows the definition of a number of areas with an associated probability matrix of dispersal between them (Ree & Smith, 2008). We considered eight geographical areas, based on the current distribution of *Deronectes* species: (A) south-eastern Iberian Peninsula including Mallorca; (B) central and northern Iberian Peninsula; (C) Italy (including Sicily) and south-eastern France; (D) Corsica and Sardinia; (E) Balkan Peninsula; (F) Turkey; (G) northern and central Europe; and (H) Maghreb (see Fig. 1 for the distribution of the main lineages and Appendix S1a and Appendix S1b for the distribution of the species of *Deronectes*). We used the majority rule consensus tree obtained in the beast run after pruning duplicated specimens. In all analyses, a maximum of four possible ancestral areas was allowed for ease of computation.

For the reconstruction of ancestral areas we used three time slices corresponding to the Pleistocene, Pliocene and Miocene, and a different palaeogeographical scenario for each: present,

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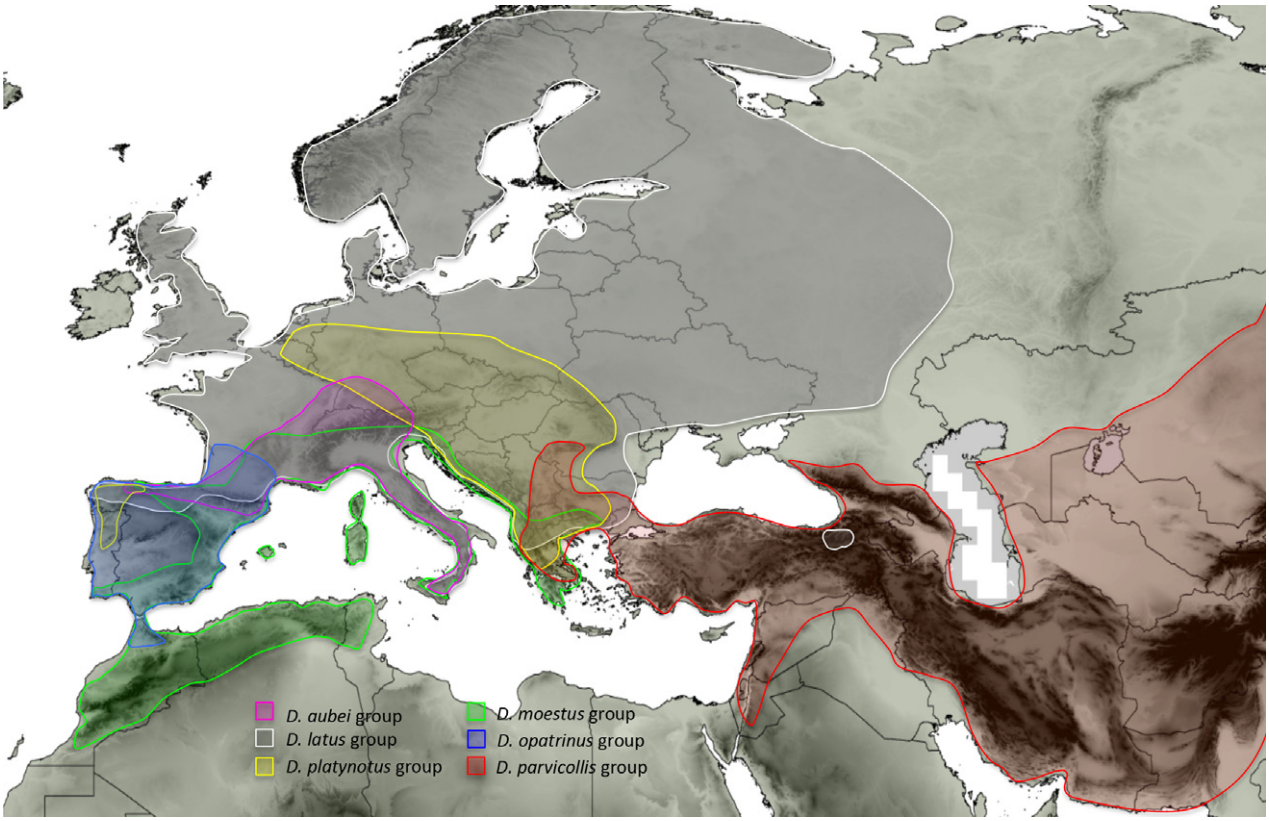

Figure 1 Distribution of the main lineages of *Deronectes* according to our phylogenetic results (see Fig. 2).

Piacenzian/Gelasian (1.8–3.4 Ma) and Late Tortonian (7–8 Ma) respectively, adapted from Meulenkamp & Sissingh (2003). For each scenario, we identified the geographical barriers between our eight pre-defined areas, and assigned probabilities of dispersal to the land or sea barriers in different combinations, including a null model with all probabilities equal to 1 (Table 1). We used the likelihood of the reconstruction to select the model best fitting the current distribution of the species. A difference ≥ 2 log likelihood units was considered significant (Ree *et al.*, 2005; Ree & Smith, 2008).

To account for topological uncertainty we used the best settings as selected above in a Bayes-Lagrange analysis with a selection of 1000 trees from among the last 50,000 trees of the stationary period (post burn-in) of the BEAST analysis. Using a custom script in R and a spreadsheet, we parsed the output and estimated the frequency of each combined area reconstruction for the nodes present in the consensus tree, as well as the individual frequency of each of the eight areas.

RESULTS

Phylogenetic analyses

There were no length differences in protein coding genes, and the length of ribosomal genes ranged 685–693 bp in the ingroup. The MrBayes runs of combined and nuclear H3 analyses reached a standard deviation of split frequencies below 0.01 at 15 and 4 million generations respectively, and below 0.005 at 18.5 million generations in the analysis of the mitochondrial matrix. These were considered the burn-in fractions, after which analyses were left to run until they reached convergence (at 23, 10 and 30 million generations respectively).

Differences between topologies obtained with Bp and ML were minimal, and affected only the degree of resolution and support of some nodes (Fig. 2; Appendix S3a and S3b). The monophyly of *Deronectes* was strongly supported, as well as its separation into two major clades, one including species predominantly distributed in the eastern Mediterranean ('eastern clade'); and a clade of species predominantly distributed in the western and central Mediterranean ('western clade') (Figs 1 & 2).

The eastern clade was further subdivided into two species groups as follows:

1. *D. parvicollis* group, including species from large parts of Asia, Turkey and the Caucasus to southern Siberia and Central Asia. Only one species, *D. parvicollis* (Schaum), extends into Europe (Balkans).
2. *D. latus* group, four species from eastern Turkey to the Iberian Peninsula and throughout central and northern Europe, including the British Isles and Scandinavia. This group included the most widespread species of the genus, *D. latus* (Stephens), ranging over most of Europe north of the Pyrenees and the Apennines.

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Table 1 Dispersal cost schemes used in Lagrange, and likelihood of the different Lagrange models. (a) Dispersal probabilities across sea or land barriers in the six combinations used (#1 to #6; in bold, combination with the best likelihood score). 'Land barrier' refers to one of the pre-defined areas (see Fig. 3). (b) Likelihood of the six dispersal schemes in (a) for the three tested palaeogeographical scenarios. The matrix with the best likelihood score for each scenario is shown in bold, with a asterisk for the best overall scheme. (c) Matrix of dispersal probabilities between our pre-defined geographical areas (A to H) for each palaeogeographical scenario according to the costs of scheme #3 in (a) (see Fig. 3 for the maps used for the reconstruction).

	#1	#2	**#3**	#4	#5	#6
(a) Barrier						
Contiguous land areas	1	1	**1**	1	1	1
Land barrier	0.2	0.2	**0.4**	0.2	0.1	0.2
Sea barrier < 100 km	0.4	0.4	**0.4**	0.2	0.2	0.4
Two land barriers	0	0.1	**0**	0	0	0.2
Sea barrier > 100 km	0	0.1	**0**	0	0	0
> 2 land or 2 land+sea	0	0.1	**0**	0	0	0
(b) Palaeogeographical scenario						
Pleistocene (present)	103.2	104.7	**101.7***	103.2	103.9	103.7
Pliocene	107.5	108.4	**105.1**	107.5	108.4	107.8
Miocene (late Tortonian)	107.6	108.5	**105.5**	107.5	108.5	107.9

	A	B	C	D	E	F	G
(c) Pleistocene							
A: SE Iberian Peninsula and Mallorca	–						
B: Centre and N Iberian Peninsula	1	–					
C: SE France, Italy and Sicily	0.6	1	–				
D: Corsica and Sardinia	0	0	0.6	–			
E: Balkans	0	0.6	1	0	–		
F: Turkey and Middle East	0	0	0.2	0	0.6	–	
G: Northern and central Europe	0.6	1	1	0.2	1	0	–
H: Maghreb	0.6	0.2	0	0	0	0	0
Pliocene							
A: SE Iberian Peninsula and Mallorca	–						
B: Centre and N Iberian Peninsula	1	–					
C: SE France, Italy and Sicily	0.6	1	–				
D: Corsica and Sardinia	0.6	0.6	1	–			
E: Balkans	0	0.6	1	0.6	–		
F: Turkey and Middle East	0	0	0.6	0	1	–	
G: Northern and central Europe	0.6	1	1	0.6	1	0.6	–
H: Maghreb	1	0.6	1	1	0.6	0	0
Miocene (Late Tortonian)							
A: SE Iberian Peninsula and Mallorca	–						
B: Centre and N Iberian Peninsula	1	–					
C: SE France, Italy and Sicily	0.6	1	–				
D: Corsica and Sardinia	0	0.6	1	–			
E: Balkans	0	0.6	1	0.6	–		
F: Turkey and Middle East	0	0	0.6	0	1	–	
G: Northern and central Europe	0.6	1	1	0.6	1	0.6	–
H: Maghreb	0.6	0.2	0.6	0.6	0.2	0	0.2

Within the western clade we recovered four well-supported species groups plus two isolated species (*D. sahlbergi* Zimmermann and *D. doriae* Sharp), but the relationships among them were not well resolved (Fig. 2). These four clades were:

1. *D. opatrinus* group, including mostly species endemic to the Iberian Peninsula, with only one (*D. hispanicus* (Rosenhauer)) reaching northern Morocco and two (*D. hispanicus* and *D. opatrinus* (Germar)) southern France.
2. *D. aubei* group, with three species and one subspecies distributed from the Cantabrian mountains in north-western Spain to Sicily, including the Alps and southern Germany.
3. *D. moestus* group, including species with a predominantly western Mediterranean distribution, from the Maghreb and the Iberian Peninsula to the Balkans through southern France, Italy and Sicily. The two missing North African species from the western clade (*D. perrinae* Fery & Brancucci and *D. peyerimhoffi* (Régimbart)) most likely belong here, as they are morphologically very similar to *D. moestus* and *D. fairmairei* (Leprieur) respectively (Fery & Brancucci, 1997).
4. *D. platynotus* group, including two species and two subspecies from the Balkans, Central Europe and north-west Iberia.

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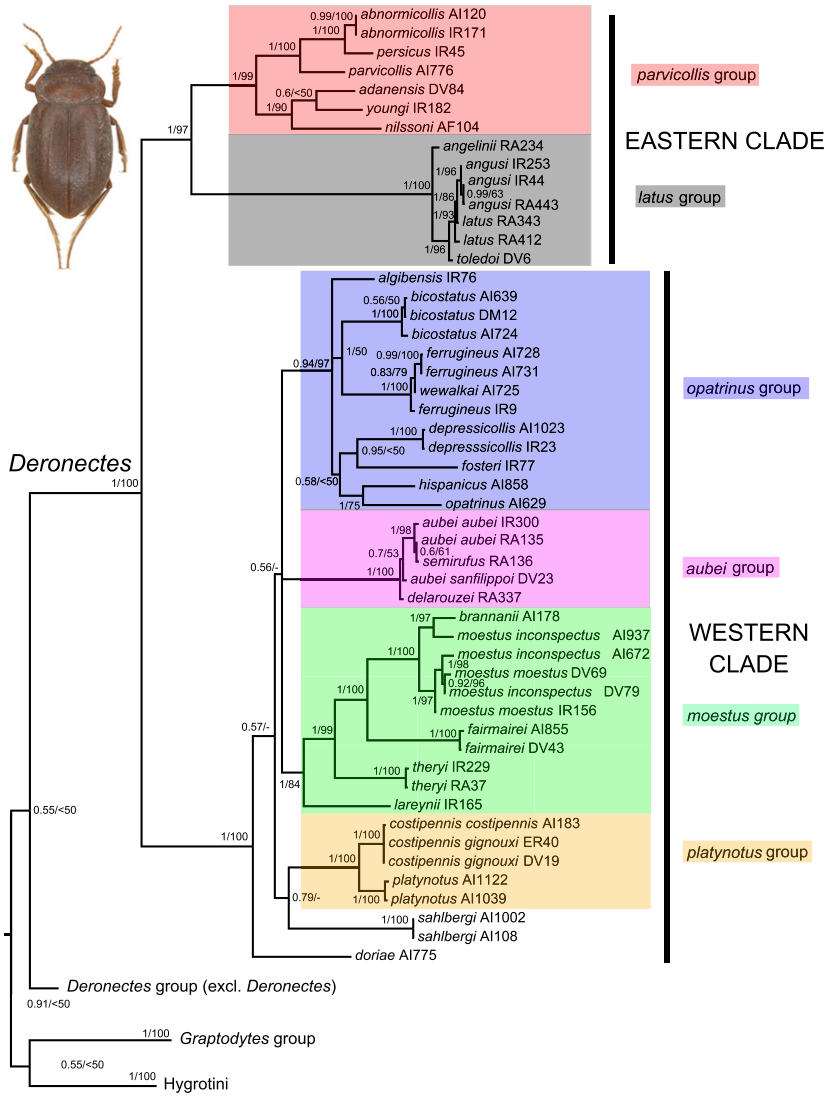

Figure 2 Phylogeny of *Deronectes*, as obtained with MrBayes with the combined nuclear and mitochondrial sequence and a partition by gene. Numbers on nodes, Bayesian posterior probabilities/Bootstrap support values in RAxML. Habitus photograph, *D. fosteri* Aguilera & Ribera (from Millán *et al.*, 2014).

Most species with more than one sequenced specimen were monophyletic, with some exceptions. There were three paraphyletic complexes of closely related species (Fig. 2): (1) the widespread *D. latus*, with the Iberian endemic *D. angusi* Fery & Brancucci nested within it; (2) the *D. aubei* group, with one clade west of the Rhone river, from the French Massif Central to the Cantabrian Mountains including *D. a. sanfilippoi* Fery & Brancucci and *D. delarouzei* (Jacquelin du Val), and another east of the Rhone, from the Alps to Sicily with *D. a. aubei* (Mulsant) and *D. semirufus* (Germar) (see Appendix S1b); and (3) *D. ferrugineus* Fery & Brancucci and *D. wewalkai* Fery & Fresneda, both Iberian endemics. There was also one case (*D. moestus*) with a deep intraspecific divergence, with the specimen from Morocco (MNCN-AI937) sister to *D. brannani* (Schauffus) (a Mallorcan endemic), and both sister to specimens of *D. moestus* from northern Spain to Bulgaria, including the two recognized subspecies (Fig. 2).

The analysis of the nuclear sequence (H3) showed lower resolution and an absence of support at some nodes, with polytomies in some groups (e.g. *D. moestus* or *D. latus*) but with a topology compatible with that obtained from the mitochondrial sequence, with a single exception (see Appendixes S3c and S3d). While with the mitochondrial sequence, the two subspecies of *D. aubei* were recovered as paraphyletic, and respectively sisters to the geographically closest species of the group, the nuclear sequence recovered a monophyletic *D. aubei* as sister to the other two species of the group (*D. delarouzei* and *D. semirufus*).

Estimation of ages of divergence and mode of diversification

The origin of extant species of *Deronectes* and the separation of the eastern and western clades was estimated to have occurred in the Middle Miocene (*c.* 14 Ma, with a 10.0–17.5 95% confidence interval) (Fig. 3; Appendix S3e). The origin of the well-supported species groups was estimated to have occurred over a relatively short time period at the end of the Miocene and beginning of the Pliocene.

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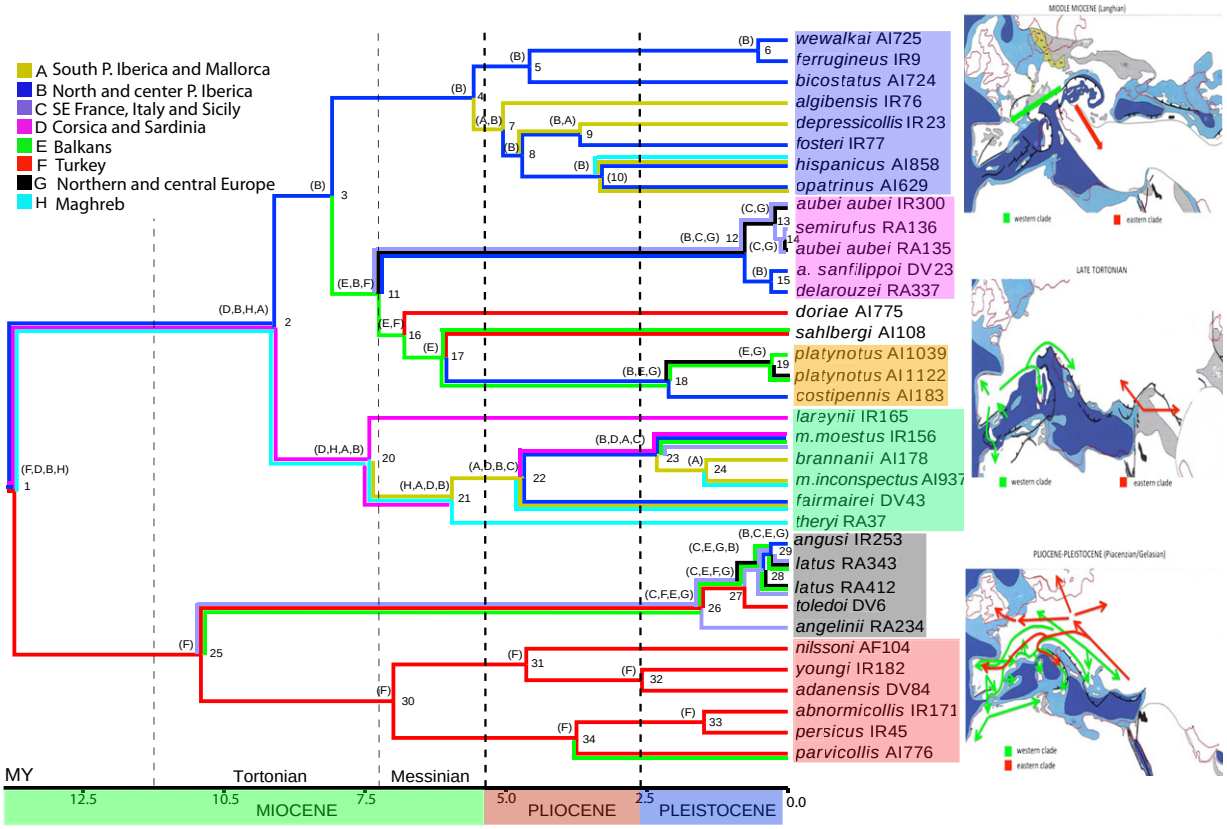

Figure 3 Ultrametric time calibrated tree obtained with BEAST. Coloured branches show ancestral distributions as estimated from the analysis of 1000 post-burn-in trees. Above nodes (in brackets) the most frequent area or combined areas reconstructed as the ancestral area of the node (see Table 2). Numbers inside nodes refer to Table 2.

Some extant species originated during the Pleistocene, particularly within the *D. latus*, *D. aubei* and *D. platynotus* groups, but most species of the Iberian clade (*D. opatrinus* group) and the *D. moestus* group were estimated to be of Pliocene or even late Miocene origin. Observed intraspecific variation was also limited to a Pleistocene origin, except in the case of *D. moestus* (Fig. 3; Appendix S3e).

The LTT plot (Fig. 4) reflecting the temporal pattern of diversification of species of the western clade showed a steady increase in lineages over time. The γ-statistic was negative (-0.96) but not significantly different from zero ($P = 0.33$).

Ancestral area reconstruction

Among models tested, the best likelihood in LAGRANGE was found for the geography of the Pleistocene, assigning the same penalty value for dispersal through one of the predefined land areas or a sea barrier shorter than 100 km, and a zero probability of dispersal over marine barriers longer than 100 km or through two or more land areas (Table 1). These settings were used to reconstruct ancestral areas using the 1000 post-burn-in trees in BEAST. The use of Pliocene or Miocene palaeogeographical scenarios, either alone or in combination, resulted in significantly worse likelihoods (Table 1). Within the same geographical scenario, results were less sensitive to small changes in the values of the cost of dispersal, with differences of less than two units log likelihood, but always significantly better than the null model of all probabilities equal and equal to one (Table 1). In any case, results were very similar for all palaeogeographical scenarios, cost matrixes or topologies, with differences only in the relative proportion of some of the reconstructed areas of the deeper nodes, including a large number of species with wide geographical distributions.

Most of the nodes present in the consensus tree had a well-supported reconstructed ancestral area, with only 5 of 34 lacking at least one area present in more than 90% of reconstructions, and only two (10 and 11 in Fig. 3) where the most likely area was present in fewer than 80% of the 1000 trees (Table 2). Most of the nodes were also well resolved, with 22 (65%) with only two areas with a frequency higher than 90%, and only three with four areas (the maximum number allowed in the settings) with a frequency higher than 90% (Table 2).

The eastern clade of *Deronectes* was unequivocally reconstructed as having an origin in Turkey (region F), with an expansion to Italy and the Balkan Peninsula (areas C and E) at the origin of the *D. latus* group (node 26 in Fig. 3). There was a subsequent expansion to central and northern Europe and the Iberian Peninsula (areas B and G) during the Pleis-

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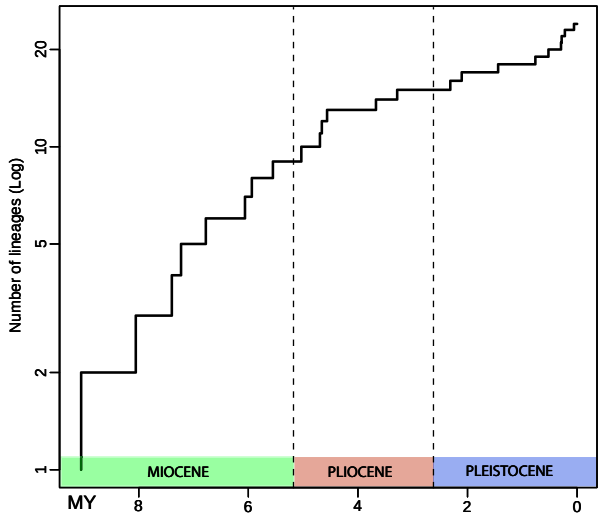

Figure 4 Lineage through time plot (LTT) of the western clade obtained from the ultrametric tree in Fig. 3.

tocene, at the origin of *D. latus* and *D. angusi* respectively (Fig. 3; Table 2).

The reconstructed origin of the western clade was more ambiguous. Although centred in the south-western Mediterranean region, only central and northern Iberia and Corsica and Sardinia (areas B and D) occurred at a frequency higher than 90% in the 1000 trees, but the Maghreb (area H) also had a high frequency (89%, Fig. 3; Table 2). Within the western clade, the *D. opatrinus* group (node 4) had a well-supported central and north Iberian origin (area B), with an expansion to the south-eastern Iberian Peninsula (area A) at the end of the MSC (nodes 7 and 8), at the origin of the endemic species *D. algibensis* Fery & Fresneda and *D. depressicollis* (Rosenhauer) (Fig. 3). This lineage experienced further expansions during the Pliocene to North Africa and within the Iberian Peninsula.

The *D. moestus* group (node 20) was reconstructed as most likely having a Maghrebian or Corso-Sardinian origin (areas H and D), with subsequent expansions to south-east Iberia during the MSC and the rest of the Iberian Peninsula and Mallorca during the Pliocene (Fig. 3; Table 2).

The reconstructed origins of the *D. aubei* (node 12) and *D. platynotus* groups (node 18) were more ambiguous. For the first, three areas had a 100% frequency in the set of 1000 trees: central and north Iberia, central and north Europe and the Italian Peninsula (areas B, C and G; Table 2). The range expansion of this group apparently took place between the late Miocene and middle Pleistocene. Similarly, two areas were reconstructed with a frequency of more than 90% at the origin of the *D. platynotus* group: central and northern Iberia and the Balkan Peninsula (areas B and E) (Fig. 3; Table 2). Two more expansions to the east (Balkan Peninsula and Turkey) were unambiguously reconstructed at the origin of *D. doriae* and *D. sahlbergi*, during the MSC (Fig. 3; Table 2).

DISCUSSION

A Miocene basal split in *Deronectes*

According to our results it seems highly likely that the ancestor of extant *Deronectes* was found on the northern shores of the Mediterranean during the early Miocene. Our estimation of the age of the basal split between eastern and western lineages is in good agreement with the increased isolation of Europe west of the Alps from the Balkans and Anatolia during the middle Miocene. During this time, climate change and tectonic movements associated with Carpathian uplift resulted in a succession of sea level fluctuations in the central and eastern Paratethys basins (Ter Borgh *et al.*, 2014). The extension of the Carpathian Foreland in a narrow deep-sea basin towards the west 20–15 Ma (Dercourt *et al.*, 1985; Meulenkamp & Sissingh, 2003) could have contributed to the isolation of strictly freshwater species in the two areas. The basal split in *Deronectes* agrees with the estimated age of similar western and eastern lineages within the freshwater beetle genus *Hydrochus* (Hidalgo-Galiana & Ribera, 2011), and with many other comparable splits within Mediterranean lineages, although in most cases no age estimates are available (see e.g. examples in Oosterbroek & Arntzen, 1992).

Of the two main lineages of *Deronectes*, the eastern clade was unambiguously reconstructed as having an origin in Anatolia, but the precise origin of the western clade was more uncertain due to the wide geographical ranges of some species within it and the lack of statistical support for the nodes connecting the main groups. During most of the Miocene, the Italian Peninsula was mostly submerged or partly merged with the future Balkan and Anatolian peninsulas (Dercourt *et al.*, 1985; Rosenbaum *et al.*, 2002; Meulenkamp & Sissingh, 2003; Popov *et al.*, 2004), something which could explain the absence of ancient lineages in this area, apparently colonized by *Deronectes* only during the Pleistocene.

Tortonian disaggregation

We traced the origin of the main species groups within *Deronectes* to the late Tortonian and the transition to the Messinian, in most cases with relatively poor topological resolution, suggesting a rapid succession of isolation events. The Tortonian was characterized by strong tectonic activity and changes in sea level in the area between south-eastern Iberia and the Maghreb (Alvinerie *et al.*, 1992; Martín *et al.*, 2009), favouring vicariance events that led to allopatric speciation in a number of groups (e.g. Jolivet *et al.*, 2006; Hidalgo-Galiana & Ribera, 2011; Faille *et al.*, 2014). In agreement with this geological scenario, the diversification of the western clade involved successive splits between the Iberian Peninsula, North Africa and Corsica and Sardinia, resulting in the main species groups recovered in our phylogeny.

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Table 2 Ancestral area reconstruction in the Lagrange analyses of the 1000 post-burn-in trees. For each node present in the consensus tree (see Fig. 3), we give the number of trees in which the node appears, the most frequent area or combined areas and the frequency of the individual areas. In bold and with asterisk, areas with > 90% of frequency; in bold, areas between 70–90% frequency. Area codes: A, south and east of the Iberian Peninsula including Mallorca; B, centre and north of the Iberian Peninsula; C, Italy (including Sicily) and south-eastern France; D, Corsica and Sardinia; E, Balkan peninsula; F, Turkey and Middle East; G, northern and central Europe and H, Maghreb.

NODE	No. trees	Areas	A	B	C	D	E	F	G	H
1	1000	BDFH	5	**93***	–	**100***	9	**100***	–	**93***
2	1000	ABDH	56	**93***	1	**100***	18	41	–	**89**
3	488	B	–	**100***	–	–	11	6	–	–
4	1000	B	48	**100***	–	–	–	–	–	–
5	530	B	–	**100***	–	–	–	–	–	–
6	1000	B	–	**100***	–	–	–	–	–	–
7	272	AB	**85**	**75**	–	–	–	–	–	–
8	634	B	57	**85**	–	–	–	–	–	–
9	805	AB	**99***	**100***	–	–	–	–	–	–
10	1000	B	55	64	–	–	–	–	–	–
11	636	BEF	–	**71**	7	–	**72**	43	–	–
12	1000	BCG	–	**100***	**100***	–	–	–	**100***	–
13	1000	CG	–	–	**100***	–	–	–	**100***	–
14	999	CG	–	–	**100***	–	–	–	**81**	–
15	970	B	–	**100***	–	–	–	–	–	–
16	418	EF	–	34	–	–	**89**	**77**	–	–
17	849	E	–	11	–	–	**100***	28	–	–
18	1000	BEG	–	**100***	–	–	**100***	–	**72**	–
19	1000	EG	–	–	–	–	**100***	–	**100***	–
20	1000	ABDH	62	53	4	**100***	–	–	–	**95***
21	1000	ABDH	**91***	**73**	14	**87**	–	–	–	**100***
22	1000	ABCD	**98***	**80**	54	**81**	1	–	–	21
23	1000	ABCD	**100***	**100***	**100***	**100***	–	–	–	–
24	1000	A	**100***	–	–	–	–	–	–	1
25	1000	F	–	–	–	–	–	**100***	–	–
26	1000	CEFG	–	30	**100***	–	**100***	**100***	**70**	–
27	998	CEFG	–	34	**100***	–	**100***	**100***	66	–
28	1000	BCEG	–	**89**	**100***	–	**100***	–	**98***	–
29	907	BCEG	–	**100***	**100***	–	**100***	–	**100***	–
30	1000	F	–	–	–	–	–	**100***	–	–
31	1000	F	–	–	–	–	–	**100***	–	–
32	1000	F	–	–	–	–	–	**100***	–	–
33	1000	F	–	–	–	–	–	**100***	–	–
34	1000	F	–	–	–	–	–	**100***	–	–

These species groups were mostly coincident with those obtained with previous molecular (Ribera *et al.*, 2001; Ribera, 2003; Abellán & Ribera, 2011) and morphological analyses (Fery & Brancucci, 1997; Fery & Hosseinie, 1998). The main differences in our study were the recognition of an Iberian clade, divided into several groups of species not previously thought to be closely related (Fery & Brancucci, 1997), and the composition of the *D. moestus* group (see Appendix S1a).

The origin of species of the western group in the Balkans, east of the Paratethys basin, is more uncertain. In the Lagrange analyses, they were reconstructed as having a western origin, requiring subsequent range expansion towards the east. However, the relative lack of support allowed an alternative scenario (as seen in the topology of Fig. 2), with the eastern-most species within the western clade (*D. platynotus* group plus the isolated *D. doriae* and *D. sahlbergi*) sister to the remaining western lineages, something which would not require a range expansion from the west, but instead a western migration of the Iberian member of the group (*D. costipennis*) in the Plio-Pleistocene.

Messinian crossroads

The onset of the MSC 5.96–5.33 Ma and the establishment of new land corridors seem to have facilitated the expansion of some species of *Deronectes*, although these movements were relatively local and mostly centred in the south-west of the Mediterranean basin. After the closing of the Tortonian sea corridors between mainland Iberia, the Betic-Rifean area and mainland North Africa (Martín *et al.*, 2009), there were expansions of the Iberian lineages towards the south-east (*D. opatrinus* group) and of the *D. moestus* group towards the north-west and the Balearic islands, both likely crisscrossing

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the Gibraltar area. Both expansions continued during the Pliocene, some species of the Iberian clade towards North Africa, and species of the *D. moestus* group towards southern Europe.

In the east, range movements associated with the MSC likely include the crossing of the Bosphorus strait by *D. doriae*, currently known only from Turkey, Armenia and Iran (Fery & Brancucci, 1997; Nilsson & Hájek, 2015), and possibly *D. sahlbergi*, known from Turkey but also from Greece (Nilsson & Hájek, 2015), meaning that Asian populations may have a relatively recent origin.

We did not find any evidence of large scale range movements during the MSC in the south or central Mediterranean basin, or along the coast of the Sarmatic Sea (the Paratethys) which could correspond to the 'lago mare' dispersal routes proposed by, for example Bianco (1990). During the Messinian, changes in climate or vegetation on the northern side of the Mediterranean were not very pronounced (Favre *et al.*, 2007), but it is possible that the newly formed land corridors did not have the ecological conditions to allow the dispersal of species restricted to fast flowing freshwater streams (Roveri *et al.*, 2014).

The establishment of current distributions in the Plio-Pleistocene

The best model for the ancestral reconstruction in Lagrange was that reflecting present geography, suggesting that current distribution patterns within the genus are largely dominated by range movements since the Messinian. This is in contrast with the results obtained with other groups of very poorly dispersing species, such as *Trechus fulvus* group ground beetles (Carabidae), which include many subterranean taxa and have a distribution still dominated by their late Miocene biogeography (Faille *et al.*, 2014). Also supporting the importance of the Plio-Pleistocene in the evolutionary history of *Deronectes* is the high number of species estimated to have originated during this period, reflected by the constancy of the diversification rate estimated from the LTT plot. Exceptions are an island endemic (*D. lareynii* from Corsica), the Moroccan *D. theryi* and the isolated eastern species *D. doriae* and *D. sahlbergi*, all apparently of late Miocene origin. Most of the geographically restricted species in the Iberian clade also have a relatively ancient (Pliocene) origin, most likely driven by vicariance between the main mountain systems (Ribera, 2003). There are other known examples of freshwater Coleoptera with similar biogeographical patterns – for example, in the Hydraenidae (subgenus *Enicocerus*) and Hydrochidae (genus *Hydrochus*) Iberian endemics are mostly of late Miocene origin (Ribera *et al.*, 2010a; Hidalgo-Galiana & Ribera, 2011).

Such an ancient origin is, however, not a generalized pattern, as in other groups of freshwater Coleoptera most Iberian endemics, many of them restricted to the same mountain systems as endemic *Deronectes*, are apparently of Pleistocene origin. This is the case for most species of the '*Haenydra*' lineage (Ribera *et al.*, 2011; Trizzino *et al.*, 2011), some species groups of *Limnebius* (Abellán & Ribera, 2011) (both Hydraenidae) and several Iberian endemic diving beetles from different genera (Dytiscidae, Ribera, 2003; Ribera & Vogler, 2004). Similarly, all speciation events within some groups of *Deronectes* are of Pleistocene origin (*D. aubei*, *D. platynotus* and *D. latus* groups), which are also the groups including most non-monophyletic species in our analyses. Most of these can be explained either by incomplete lineage sorting due to their recent divergence (*D. ferrugineus-wewalkai*, *D. latus-angusi*) or the presence of previously unrecognized species-level diversity (*D. moestus* complex), except for the discordance between mitochondrial and nuclear data within the *D. aubei* group. Incomplete lineage sorting is not expected to leave any predictable biogeographical pattern (Funk & Omland, 2003), so is unlikely to be the reason for the grouping of the mitochondrial haplotypes in two clusters, west and east of the Rhone river (the later including the Pleistocene expansion of *D. semirufus* to peninsular Italy and Sicily). This clear geographical pattern is more consistent with introgressive hybridization between closely related taxa sharing the same geographical range, a pattern seen commonly in areas hypothesized to be glacial refugia (e.g. Berthier *et al.*, 2006; Schmidt & Sperling, 2008 or Nichols *et al.*, 2012). An alternative possibility could be *Wolbachia* infection, known to alter patterns of mtDNA variability (Jiggins, 2003). Our data do not allow further interpretations of this pattern, which may require a more comprehensive taxon sampling in potential refugial areas (e.g. Massif Central or Black Forest) and the sequencing of additional molecular markers.

Another western lineage which apparently diversified in the Pleistocene is the *D. platynotus* group, which was reconstructed to have expanded westwards from the Balkans, giving rise to the Iberian endemic *D. costipennis*. In Fery & Brancucci (1997) another species (*D. hakkariensis* Wewalka) known from a single specimen from south-eastern Turkey was tentatively included in the *D. platynotus* group, although because of its deviating morphology and geographical distribution, this relationship was considered doubtful. Unfortunately, we could not obtain material of this rare species for our analyses, but if shown to genuinely belong to the *D. platynotus* group, *D. hakkariensis* would represent a further expansion to the east, most likely during the Pliocene, constrained by the stem (Messinian) and crown (lower Pleistocene) ages of the group.

In the eastern clade, the diversification and expansion of the *D. parvicollis* group, to occupy large areas of the Middle East and central Asia, with one species (*D. parvicollis*) expanding westward into the Balkans, most likely took place during the Plio-Pleistocene. The other main lineage within the eastern clade, the *D. latus* group, also expanded during the Pleistocene, but in this case towards the west, first to give rise to the only Italian endemic of the genus (*D. angelinii* Fery & Brancucci) and then to reach Iberia and most of Europe north to Scotland and Scandinavia, as testified by Holocene remains of *D. latus* in Britain and Sweden (Abellán *et al.*, 2011).

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CONCLUDING REMARKS

Our reconstruction of the evolutionary and biogeographical history of *Deronectes* shows that its diversification has been shaped by geological and climatic changes around the Mediterranean since the Miocene. These have produced successive rounds of fragmentation and subsequent range expansion leading again to further fragmentation, the overall result of which has been a steady accumulation of species. This pattern of range expansions under favourable conditions followed by fragmentation when conditions deteriorate has been described for other groups of lotic Coleoptera (Ribera *et al.*, 2011), and may be a more general pattern contributing substantially to the overall richness of the Mediterranean biodiversity hotspot.

Within *Deronectes*, most of these eastward and westward range expansions involved overland dispersal through the north side of the Mediterranean basin, with a limited influence of MSC land corridors and the total absence of these beetles in North Africa from Libya to Egypt. This could be expected given their ecological requirements, but what is more surprising is the irrelevance of the Italian Peninsula during most of the evolutionary history of the group. Most of the Italian Peninsula remained submerged until the Pliocene (Rosenbaum *et al.*, 2002; Meulenkamp & Sissingh, 2003; Popov *et al.*, 2004), and all species currently found in mainland Italy south of the Alps are of Pleistocene origin. This absence of ancient Italian species is paralleled in freshwater Coleoptera which have Iberian endemics of Miocene or Pliocene origin (*Enicocerus*, *Hydrochus*, Ribera *et al.*, 2010a; Hidalgo-Galiana & Ribera, 2011), but not by groups with an abundance of Pleistocene species, which have also Italian endemics ('*Haenydra*' and *Limnebius*; Trizzino *et al.*, 2011; Abellán & Ribera, 2011).

Our results clearly show that the timing of key diversification events may differ between taxa even when sharing the same habitat and geographical distribution, differences that have shaped the current distribution of diversity in the Mediterranean hotspot.

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SUPPORTING INFORMATION

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

Appendix S1 Additional materials: checklist and distribution of species of *Deronectes* and studied material.
Appendix S2 Additional methods: primers used and standard PCR conditions.
Appendix S3 Additional phylogenetic results.

BIOSKETCH

David García-Vázquez is a PhD student in the Institute of Evolutionary Biology in Barcelona. This paper is part of his thesis dissertation, focussed on the origin of widespread European species of lotic water beetles. This work is also part of a long-term collaboration between the authors on the evolutionary history of Mediterranean water beetles.

Author contributions: D.G.-V. and I.R. conceived the work; D.T.B., L.F.V. and I.R. led the specimen collection; D.G.-V., R.A. and I.R. obtained the molecular data; D.G.-V. and I.R. analysed the data and led the writing; all authors contributed to the discussion of results and the writing.

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Reconstructing ancient Mediterranean crossroads in *Deronectes* diving beetles

David García-Vázquez, David T. Bilton, Rocío Alonso, Cesar J. Benetti, Josefina Garrido, Luis F. Valladares and Ignacio Ribera

SUPPORTING INFORMATION

Appendix S1 Additional materials.

(a) Checklist of species of *Deronectes*, including geographical distribution, species group according to Fery & Brancucci (1997) and clade in which the species was included according to our results. Nomenclature follows Nilsson & Hájek (2015). In grey, species studied in this work.

(b) Distribution maps of the species of *Deronectes*.

(c) List of the specimens included in the phylogeny, with specimen voucher, locality, collector and Genbank accession numbers (in bold, new sequences). In grey, Hydroporinae outgroups, with genus-groups according to Ribera *et al.* (2008).

Appendix S2 Additional methods.

(a) Primers used for the amplification and sequencing. In brackets, length of the amplified fragment.

(b) Standard PCR conditions for the amplification of the studied fragments.

Appendix S3 Additional results.

(a) Phylogenetic tree obtained with MrBayes with the combined nuclear and mitochondrial sequences and a partition by gene, including all outgroups. Numbers in nodes: Bayesian posterior probabilities.

(b) Phylogenetic tree obtained with RAxML with the combined nuclear and mitochondrial sequences and a partition by gene, including all outgroups. Numbers in nodes: Bootstrap support values.

(c) Phylogenetic tree obtained with MrBayes using only the mitochondrial sequence data. Numbers in nodes: Bayesian posterior probabilities.

(d) Phylogenetic tree obtained with MrBayes using only the nuclear sequence data (H3). Numbers in nodes: Bayesian posterior probabilities.

(e) Calibrated ultrametric tree obtained in BEAST, with estimated ages (in Ma) and 95% confidence intervals (blue bars).

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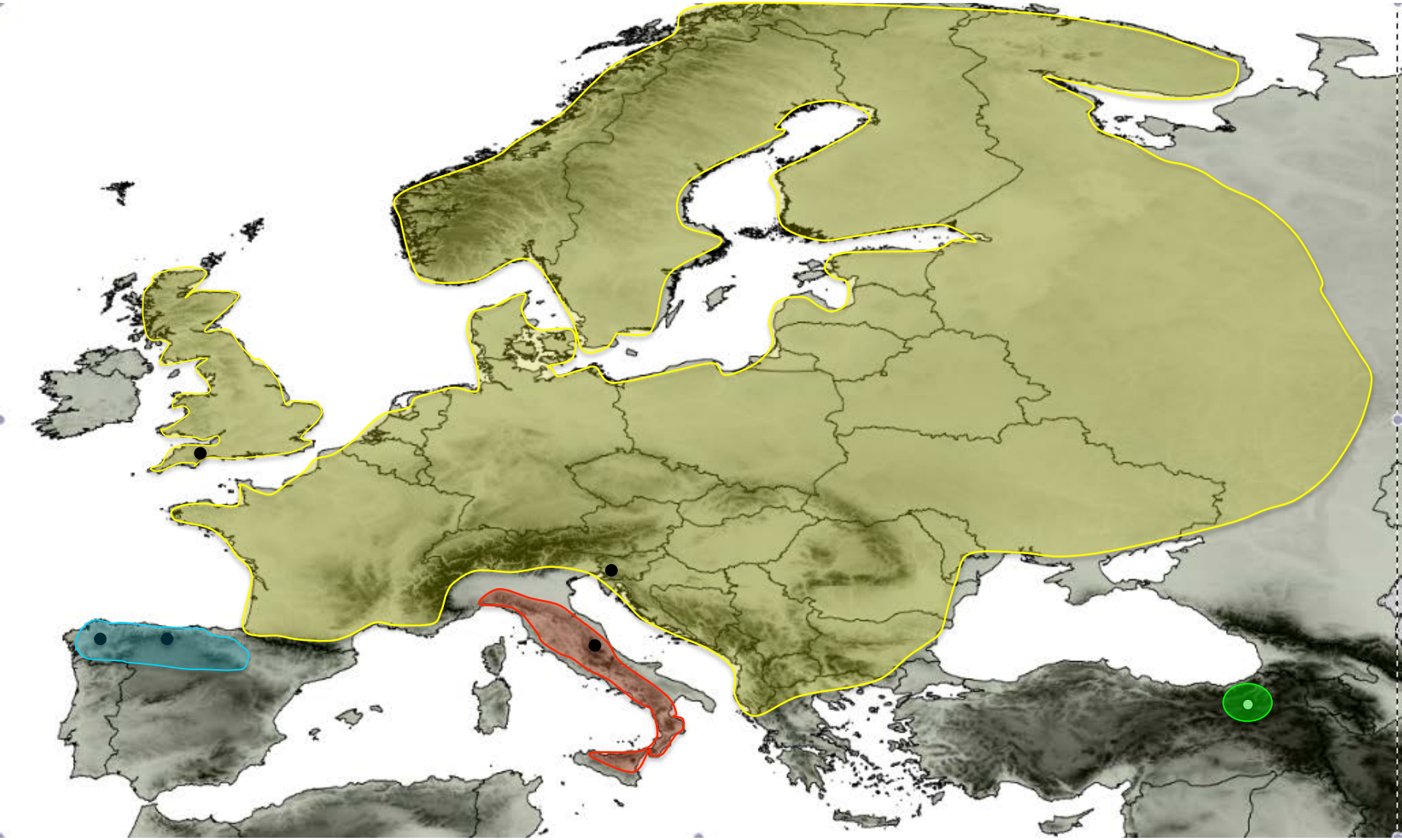
Appendix S1 Additional materials.

(a) Checklist of species of *Deronectes*, including geographical distribution, species group according to Fery & Brancucci (1997) and clade in which the species was included according to our results. Nomenclature follows Nilsson & Hájek (2015). In grey, species studied in this work.

No	Species	Author	Distribution	Areas	clade	Fery & Brancucci (1997)	This work
1	*D. abnormicollis*	Semenow, 1900	Central Asia, including China	F	E	parvicollis	parvicollis
2	*D. adanensis*	Hájek, Šťastný, Boukal & Fery, 2011	Turkey (Adana province)	F	E	parvicollis	parvicollis
3	*D. afghanicus*	Wewalka, 1971	Northeastern Afghanistan and northern Pakistan	F	E	parvicollis	[parvicollis]
4	*D. algibensis*	Fery & Fresneda, 1988	Southeastern Spain (Cádiz and Málaga provinces)	A	W	theryi	opatrinus
5	*D. angelinii*	Fery & Brancucci, 1997	Italian peninsula south of the Alps, Sicily and Elba.	C	E	latus	latus
6	*D. angulipennis*	(Peyron, 1858)	Turkey (Taurus mountains)	F	E	parvicollis	[parvicollis]
7	*D. angusi*	Fery & Brancucci, 1990	North Spain	B	E	latus	latus
8	*D. aubei aubei*	(Mulsant, 1843)	France (East of the Rhone), Northern Italy, Switzerland and Southwest Germany	C G	W	aubei	aubei
9	*D. aubei sanfilippoi*	Fery & Brancucci, 1997	North Spain and South France (Cantabrian mountains, Pyrenees and Massif Central)	B C	W	aubei	aubei
10	*D. balkei*	Fery & Hosseinie, 1998	Southwestern Iran	F	E	parvicollis	[parvicollis]
11	*D. bameuli*	Fery & Hosseinie, 1998	Northern Pakistan	F	E	parvicollis	[parvicollis]
12	*D. bicostatus*	(Schaum, 1864)	Central and Northern regions of Spain and Portugal	B	W	bicostatus	opatrinus
13	*D. biltoni*	Fery & Hosseinie, 1998	Northeastern Iran	F	E	parvicollis	[parvicollis]
14	*D. brancuccii*	Fery & Hosseinie, 1998	Iran (Kerman and Esfahan provinces)	F	E	parvicollis	[parvicollis]
15	*D. brannanii*	(Schauffus, 1869)	Mallorca	A	W	moestus	moestus
16	*D. costipennis costipennis*	Brancucci, 1983	Northwest Iberian peninsula	B	W	platynotus	platynotus
17	*D. costipennis gignouxi*	Fery & Brancucci, 1989	Northwestern Spain	B	W	platynotus	platynotus
18	*D. danielssoni*	Fery & Hosseinie, 1998	Northwestern Afghanistan	F	E	parvicollis	[parvicollis]
19	*D. delarouzei*	(Jacquelin du Val, 1857)	Pyrenees	B	W	aubei	aubei
20	*D. depressicollis*	(Rosenhauer, 1856)	Southeastern Spain	A	W	bicostatus	opatrinus
21	*D. doriae*	Sharp, 1882	Caucasus, Turkey	F	W	doriae	doriae
22	*D. elburs*	Fery, Erman & Hosseinie, 2001	Iran (Elburz mountains)	F	E	parvicollis	[parvicollis]
23	*D. elmii*	Fery & Hosseinie, 1998	Southern Iran	F	E	parvicollis	[parvicollis]
24	*D. ermani*	Hájek, Šťastný, Boukal & Fery, 2011	Turkey (Adana province)	F	E?	ermani	?
25	*D. evelynae*	Fery & Hosseinie, 1998	Southeastern Turkey	F	E	parvicollis	[parvicollis]
26	*D. fairmairei*	(Leprieur, 1876)	Western Mediterranean (Spanish and French coast and Maghreb)	A B C H	W	fairmairei	moestus
27	*D. ferrugineus*	Fery & Brancucci, 1987	Northwest Iberian peninsula	B	W	bicostatus	opatrinus
28	*D. fosteri*	Aguilera & Ribera, 1996	Pyrenees	B	W	opatrinus	opatrinus
29	*D. hakkariensis*	Wewalka. 1989	Southeastern Turkey	F	E?	platynotus	?
30	*D. hebaueri*	Fery & Hosseinie, 1998	Southern Turkey	F	E	parvicollis	[parvicollis]
31	*D. hendrichi*	Fery & Hosseinie, 1998	Southern and southeastern Iran	F	E	parvicollis	[parvicollis]
32	*D. hispanicus*	(Rosenhauer, 1856)	South and eastern Spain, Northern Morocco and Southern France (West of Rhone)	A B C H	W	opatrinus	opatrinus
33	*D. jaechi*	Wewalka. 1989	Southeastern Turkey	F	E	parvicollis	[parvicollis]
34	*D. kinzelbachi*	Fery & Hosseinie, 1998	Southern Turkey and northwestern Syria	F	E	parvicollis	[parvicollis]
35	*D. lareynii*	(Fairmaire, 1858)	Corsica	D	W	opatrinus	moestus
36	*D. latus*	(Stephens, 1829)	Europe from south France to Russia, including British Islands and Scandinavia	E G	E	latus	latus
37	*D. longipes*	Wewalka. 1989	Southwestern Iran (Zagros mountains)	F	E	parvicollis	[parvicollis]
38	*D. moestus inconspectus*	(Leprieur, 1876)	Western Mediterranean and Balkans	A B C E H(1)	W	moestus	moestus
39	*D. moestus moestus*	(Fairmaire, 1858)	Corsica and Sardinia	D(1)	W	moestus	moestus
40	*D. nilssoni*	Fery & Wewalka, 1992	Turkmenistan and South East Iran	F	E	parvicollis	parvicollis
41	*D. opatrinus*	(Germar, 1824)	Spain, Portugal and South France	A B C	W	opatrinus	opatrinus
42	*D. palaestinensis*	Fery & Hosseinie, 1999	Syria (Golan heights)	F	E	parvicollis	[parvicollis]
43	*D. parvicollis*	(Schaum, 1864)	Middle East, Turkey and Balkans	E F	E	parvicollis	parvicollis
44	*D. perrinae*	Fery & Brancucci, 1997	Algeria, Tunisia	H	W	moestus	[moestus]
45	*D. persicus*	Peschet, 1914	South West of Iran	F	E	parvicollis	parvicollis
46	*D. peyerimhoffi*	(Régimbart, 1906)	Algeria	H	W	fairmairei	[moestus]
47	*D. platynotus mazzoldii*	Fery & Brancucci, 1997	Greece	E	W	platynotus	[platynotus]
48	*D. platynotus platynotus*	(Germar, 1834)	Central Europe (from Netherlands to Poland), Balkans and Northern Greece	E G(1)	W	platynotus	platynotus
49	*D. riberai*	Fery & Hosseinie, 1998	Southeastern Turkey and northern Iran	F	E	parvicollis	[parvicollis]
50	*D. roberti*	Fery & Hosseinie, 1998	Central Afghanistan	F	E	parvicollis	[parvicollis]
51	*D. sahlbergi*	Zimmermann, 1932	West and Southwest Turkey, Northeast Greece and Aegean Islands	E F	W	doriae	sahlbergi
52	*D. schuberti*	Wewalka, 1971	Southern Turkey	F	E	parvicollis	[parvicollis]
53	*D. semirufus*	(Germar, 1845)	Western Alps and Sicily	C	W	aubei	aubei
54	*D. syriacus*	Wewalka, 1971	Southeastern Turkey	F	E	parvicollis	[parvicollis]
55	*D. tashk*	Fery, Hasanshahi & Abbasipour, 2014	Iran (Fars Province)	F	E	parvicollis	[parvicollis]
56	*D. theryi*	(Peyerimhoff in Bedel, 1925)	Morocco (Atlas)	H	W	theryi	moestus
57	*D. toledoi*	Fery, Erman & Hosseinie, 2001	Turkey (Erzurum province)	F	E	latus	latus
58	*D. vestitus*	(Gebler, 1848)	Russia (south-western Siberia), Kazakhstan, Uzbekistan and Tajikistan	F	E	parvicollis	[parvicollis]
59	*D. wewalkai*	Fery & Fresneda, 1988	Central Spain	B	W	bicostatus	opatrinus
60	*D. wittmeri*	Wewalka, 1971	Southern Turkey	F	W	doriae	[doriae?]
61	*D. witzgalli*	Fery & Brancucci, 1997	Turkey (Antalya province)	F	W	doriae	[sahlbergi?]
62	*D. youngi*	Fery & Hosseinie, 1998	Southwestern Iran (Zagros mountains)	F	E	parvicollis	parvicollis

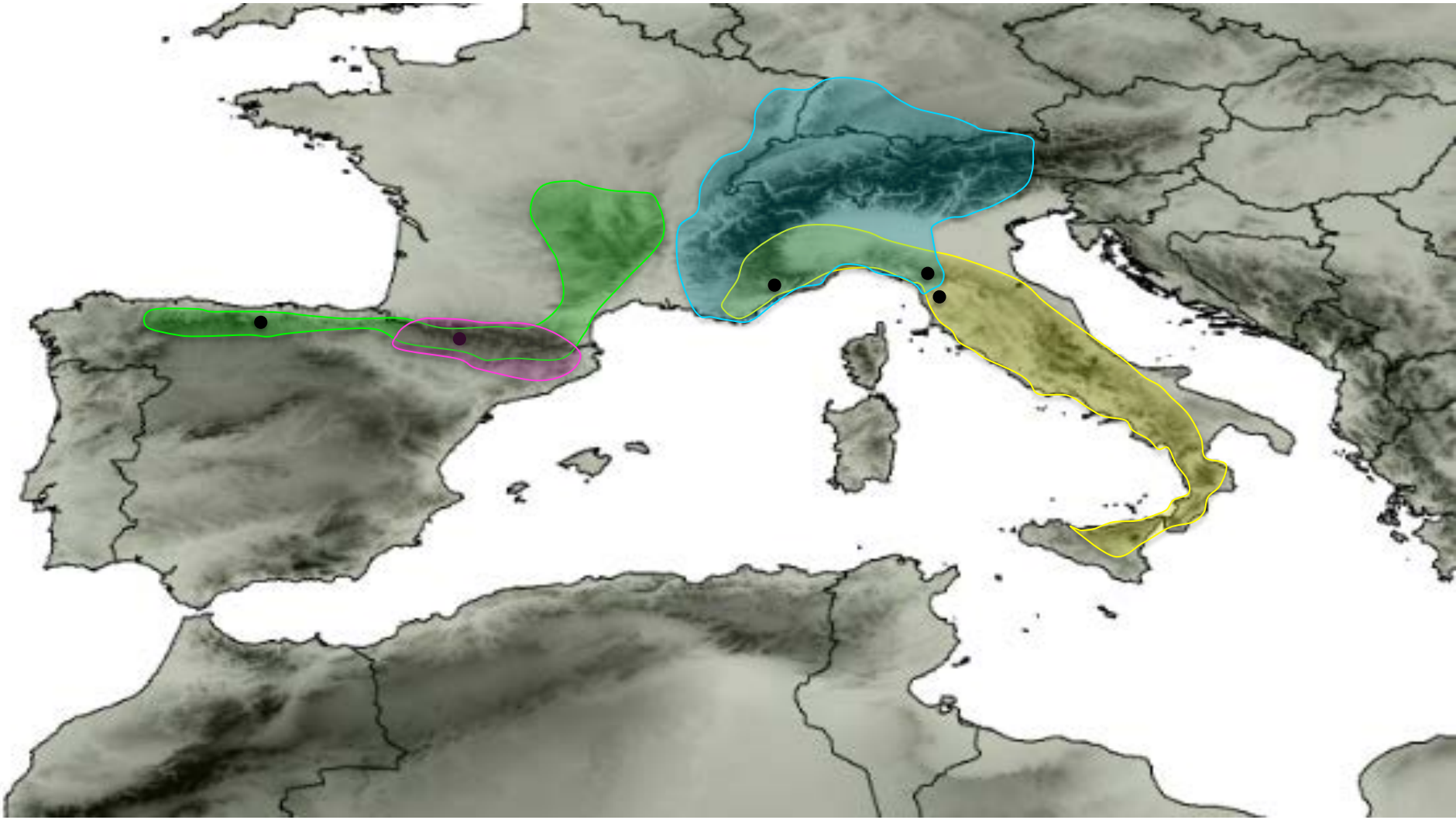
(1) In the biogeographic reconstructuion we considered the species as a whole, including the distribution of both subspecies

D. latus group



latus
angelinii
toledoi
angusi

D. aubei group



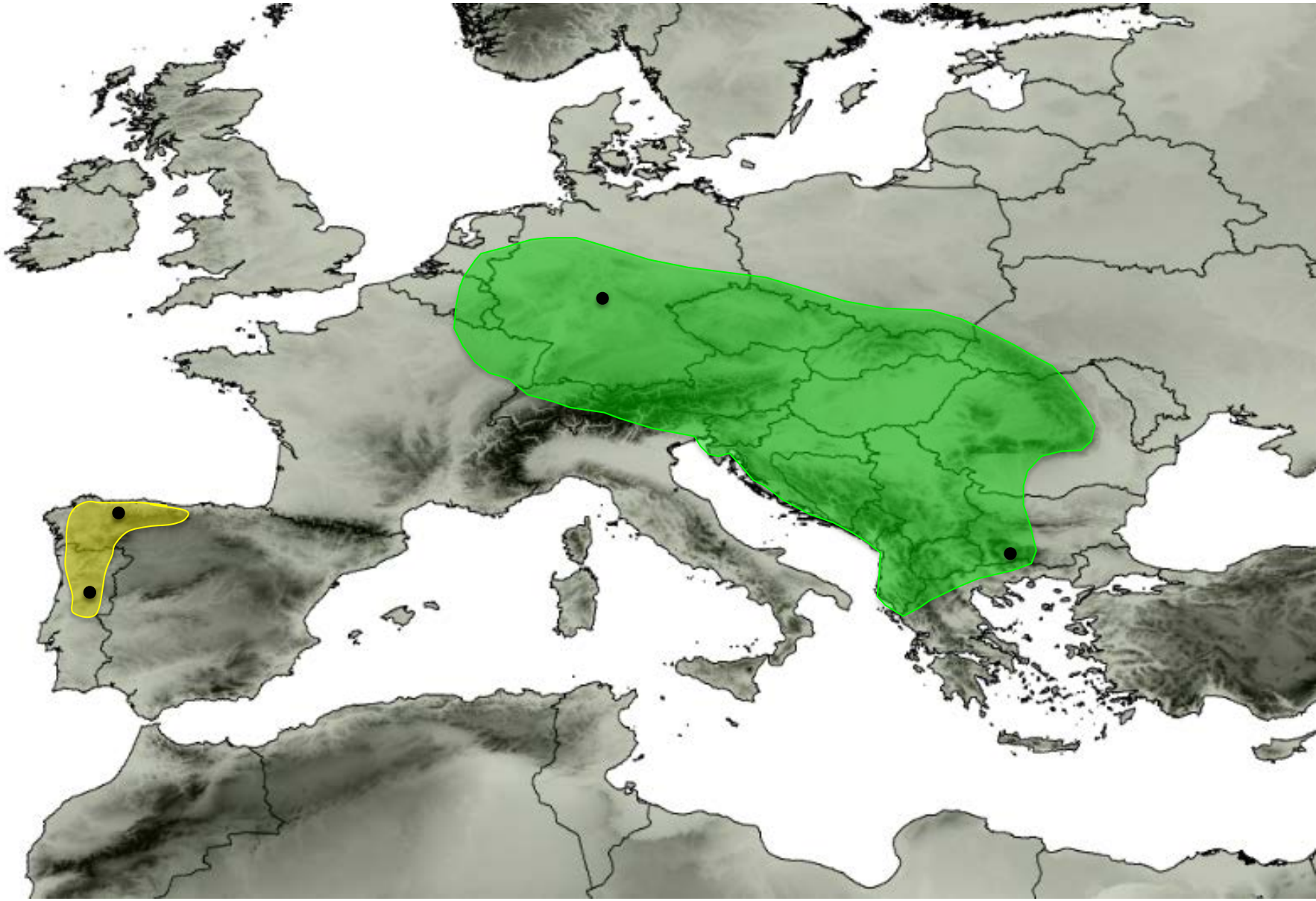
aubei sanfilippoi

delarouzei

aubei aubei

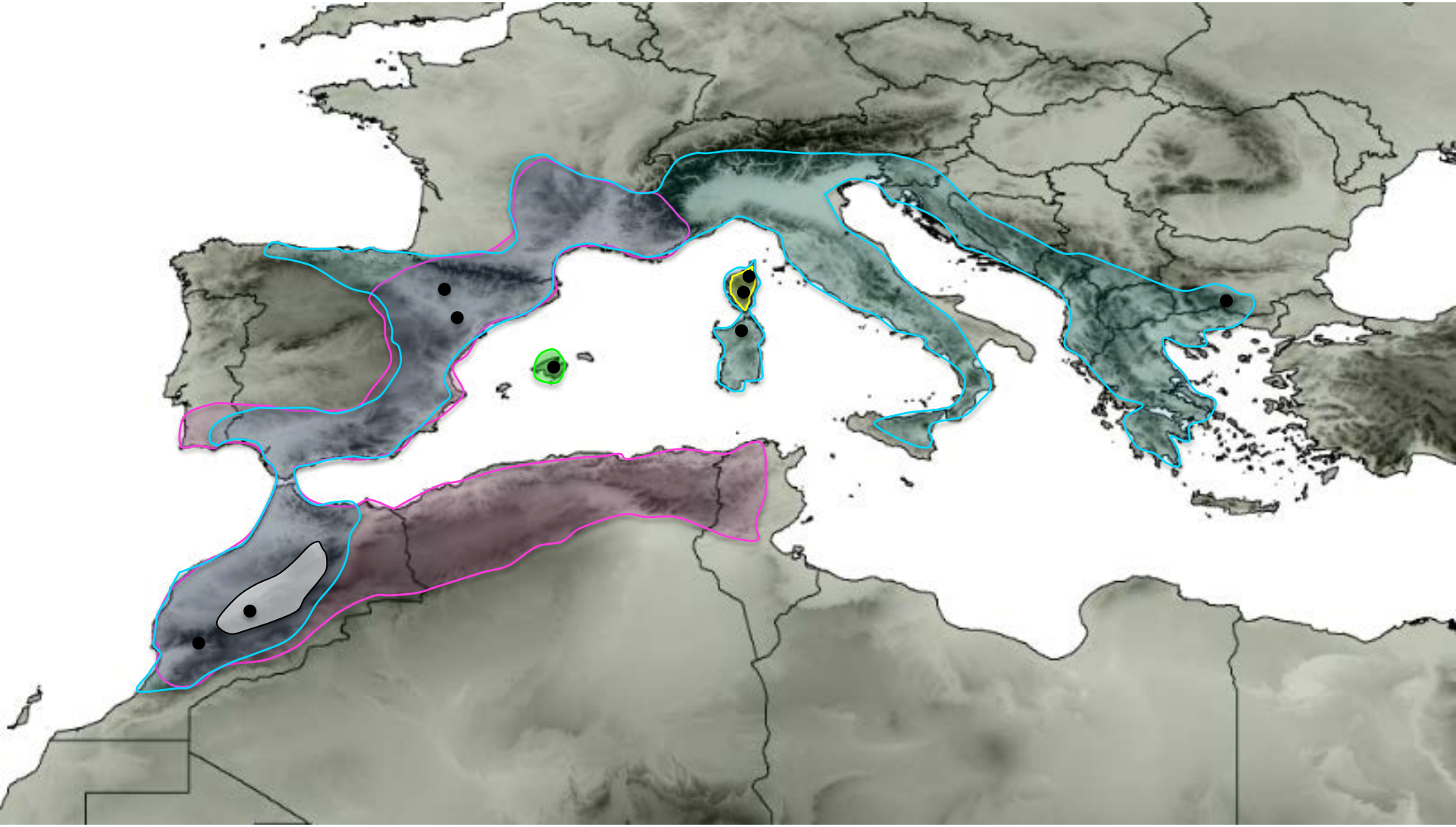
semirufus

D. platynotus group



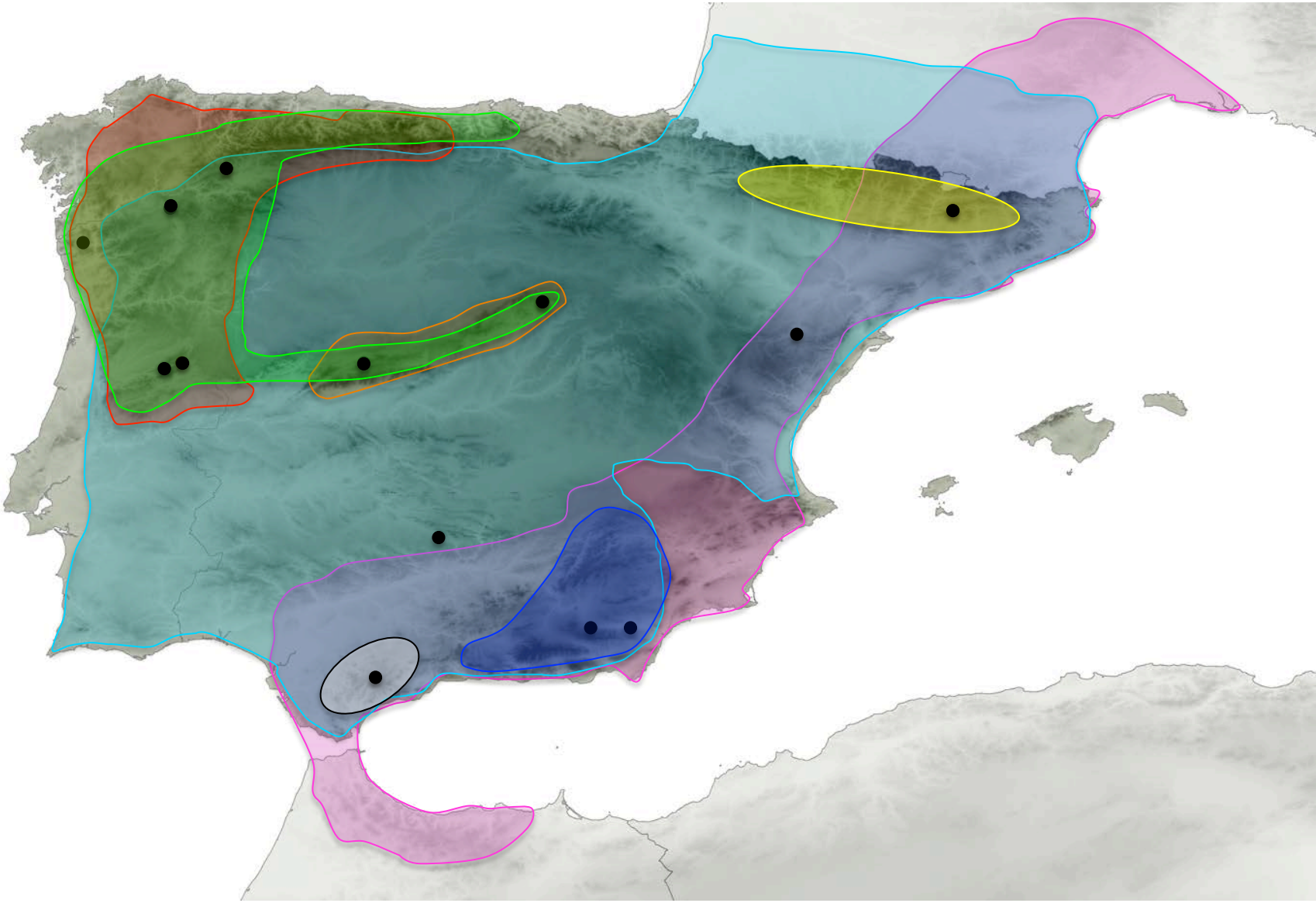
costipennis platynotus

D. moestus group



moestus
fairmairei
lareynii
theryi
brannanii

D. opatrinus group (Iberian clade)



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Appendix S1 Additional materials.

(c) List of the specimens included in the phylogeny, with specimen voucher, locality, collector and Genbank accession numbers (in bold, new sequences). In grey, Hydroporinae outgroups, with genus-groups according to Ribera *et al.* (2008).

										Accesion numbers			
No	Species	Voucher			Country (Island)	Locality	Coordinates	Collector	Date	COI-5'	COI-3'	16SrRNA+tRNA-Leu+NAD1	H3
1	*D. abnormicollis*	MNCN-AI120	AI	120	Uzbekistan	Tashkent province, Yakkatut	41º38'N 70º03'E	L. Hendrich	2004	**LN995086**	**LN995059**	**LN995161**	**LN995126**
2	*D. abnormicollis*	NHM-IR171	IR	171	Kazakhstan	South Kazakhstan region, Chimkent, Dzhabagly	ca. 42°25'32"N 70°32'6"E	J. Cooter	1999		AF309307	AF309250	EF670134
3	*D. adanensis*	IBE-DV84	DV	84	Turkey	Adana province, Yakapinar	36º56'25.8"N 35º 39'38.3"E	M. Boukal	2012		**LN995060**		
4	*D. algibensis*	NHM-JH/IR76	IR	76	Spain	Andalucia, Cádiz province, Puerto de Gáliz	ca. 36°33'35"N 5°36'4"W	I. Ribera	1998		AF309318	AF309261	
5	*D. angelinii*	IBE-RA234	RA	234	Italy	Marche, Ascoli-Piceno province, Quintodecimo	ca. 42°45'48"N 13°23'13"E	M. Toledo	2009	**LN995087**	**LN995061**	**LN995162**	**LN995127**
6	*D. angusi*	IBE-RA443	RA	443	Spain	Galicia, Lugo province, Quintá	ca. 42°58'18"N 7°13'14"W	I. Ribera	1998	**LN995088**	**LN995063**	**LN995163**	
7	*D. angusi*	NHM-IR253	IR	253	Spain	Castilla y León, Burgos province, Pineda de la Sierra	ca. 42°13'40"N 3°18'15"W	I. Ribera	1998		**LN995062**	AF309253	
8	*D. angusi*	NHM-JH/IR44	IR	44	Spain	Galicia, Lugo province, Quintá	ca. 42°58'18"N 7°13'14"W	I. Ribera	1998		AF309310	AF309253	EF670135
9	*D. aubei aubei*	IBE-RA135	RA	135	Italy	Emilia-Romagna, Modena province, Fanano	ca. 44°10'29"N 10°47'56"E	M. Toledo	2009	**LN995089**	**LN995064**	**LN995164**	**LN995128**
10	*D. aubei aubei*	NHM-IR300	IR	300	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Moulinet	ca. 43°58'34"N 7°24'44" E	I. Ribera & A. Cieslak	2000		AF309326	AF309269	EF670136
11	*D. aubei sanfilippoi*	IBE-DV23	DV	23	Spain	Cantabria, Bárago	ca. 43° 4'23"N 4°37'06"W	L.F. Valladares	2011	**LN995090**	**LN995065**	**LN995165**	**LN995129**
12	*D. bicostatus*	MNCN-AI639	AI	639	Portugal	Guarda district, Manteigas (Serra da Estrela)	40º19'57"N 7º37'03"W	I. Ribera	2005	**LN995091**	HE610179	**LN995166**	**LN995130**
13	*D. bicostatus*	MNCN-AI724	AI	724	Portugal	Viana do Castelo district, Sao Lourenço de Montaria (Serra do Minho)	ca. 41°47'48"N 8°43'59"W	I. Ribera	1998	**LN995092**	**LN995066**	**LN995167**	**LN995131**
14	*D. bicostatus*	MNCN-DM12	DM	12	Spain	Castilla y León, Ávila province, Sierra de Gredos	ca. 40°16'25"N 5°13'60"W	H. Fery	2005	**LN995093**	**LN995067**	**LN995168**	**LN995132**
15	*D. brannanii*	MNCN-AI178	AI	178	Spain (Mallorca)	Ternelles	39º53'37.2"N 3º00'14.9"E	I. Ribera & A. Cieslak	2004	**LN995094**	HE610180	HF931404	**LN995133**
16	*D. costipennis costipennis*	MNCN-AI183	AI	183	Portugal	Guarda district, Manteigas (Serra da Estrela)	ca. 40º19'57"N 7º37'03"W	I. Ribera	2005	**LN995095**	HE610181	**LN995169**	**LN995134**
17	*D. costipennis gignouxi*	IBE-DV19	DV	19	Spain	Castilla y León, León province, Valverdín	ca. 42°56'53"N 5°32'10"W	L.F. Valladares	2011	**LN995096**	**LN995068**	**LN995170**	**LN995135**
18	*D. costipennis gignouxi*	NHM-ER40	ER	40	Spain	Castilla y León, León province, Puerto de San Glorio	ca. 43° 3'57"N 4°45'31"W	D.T. Bilton	1999		AF309324	AY250951	
19	*D. delarouzei*	IBE-RA337	RA	337	Spain	Aragón, Huesca province, Aragués del Puerto	ca. 42°45'17"N 0°37'59"W	I. Esteban	2008	**LN995097**	**LN995069**	**LN995171**	**LN995136**
20	*D. depressicollis*	MNCN-AI1023	AI	1023	Spain	Andalucia, Almeria province, Abrucena	ca. 37°8'20"N 2°46'50"W	A. Castro	2006	**LN995098**	HE610182	**LN995172**	**LN995137**
21	*D. depressicollis*	NHM-JH/IR23	IR	23	Spain	Andalucia, Granada province, Puerto de la Rágua	ca. 37°1'14"N 3°0'27"W	I. Ribera	1998		AF309321	AF309264	EF670137
22	*D. doriae*	MNCN-AI775	AI	775	Turkey	Bolu province, Kartalkaya	40º39'20"N 31º47'8.5"E	I. Ribera, P. Aguilera & C. Hernando	2006	**LN995099**	HE610183	**LN995173**	**LN995138**
23	*D. fairmairei*	IBE-DV43	DV	43	Morocco	Sus-Masa-Draa, Ouarzazate province, Tachokchte	ca. 30°47'36"N 7°31'27"W	I. Ribera & A. Cieslak	2001	**LN995101**	**LN995070**	**LN995175**	**LN995140**
24	*D. fairmairei*	MNCN-AI855	AI	855	Spain	Aragón,Teruel province, Beceite	40º47'12.5"N 0º12'13.5"E	I. Ribera	2006	**LN995100**	HE610184	**LN995174**	**LN995139**
25	*D. ferrugineus*	MNCN-AI728	AI	728	Spain	Galicia, Ourense province, Serra de Queixa	42º14'39.3"N 7º10'57.2"W	I. Ribera & A. Cieslak	2005	**LN995102**	**LN995071**		**LN995141**
26	*D. ferrugineus*	MNCN-AI731	AI	731	Portugal	Guarda district, Sabugueiro (Serra da Estrela)	40º24'20"N 7º37'43"W	I. Ribera	2005	**LN995103**	**LN995072**	**LN995176**	**LN995142**
27	*D. ferrugineus*	NHM-JH/IR9	IR	9	Spain	Castilla y León, León province, Puente de Rey	ca. 42°37'42"N 6°48'7"W	I. Ribera	1998		AF309322	AF309265	EF670138
28	*D. fosteri*	NHM-JH/IR77	IR	77	Spain	Cataluña, Barcelona province, Saldes	ca. 42°13'36"N 1°44'30"E	P. Aguilera	1998		AF309317	AF309260	
29	*D. hispanicus*	MNCN-AI858	AI	858	Spain	Comunidad Valenciana, Castellón province, Ballestar	40º41'41"N 0º13'25.5"E	I. Ribera	2006	**LN995104**	**LN995073**	**LN995177**	**LN995143**
30	*D. lareynii*	NHM-IR165	IR	165	France (Corsica)	Haute-Corse department, Vizzavona	ca. 42°6'59"N 9°6'40"E	I. Ribera & A. Cieslak	1999	**LN995105**	AF309316	AF309259	
31	*D. latus*	IBE-RA343	RA	343	Slovenia	Slovene Istria, Dernarnik	N45º29'13.8"N 13º50'01.7"E	I. Ribera, C. Hernando & A. Cieslak	2007	**LN995106**	**LN995074**	**LN995178**	**LN995144**
32	*D. latus*	IBE-RA412	RA	412	England	South East England, Hampshire county, New Forest	ca. 50°50'24"N 1°37'20"W	I. Ribera	1999	**LN995107**	**LN995075**	**LN995179**	**LN995145**
33	*D. moestus inconspectus*	IBE-DV79	DV	79	Spain	Aragón, Zaragoza province, El Frago	42º17'44"N 0º53'45"W	I. Esteban	2008	**LN995111**	**LN995079**	**LN995183**	**LN995149**
34	*D. moestus inconspectus*	MNCN-AI672	AI	672	Bulgaria	Blagoevgrad province, Filipovo (Rhodope Mountains)	ca. 41°45'53"N 23°41'22"E	D.T. Bilton	2005	**LN995108**	**LN995076**	**LN995180**	**LN995146**
35	*D. moestus inconspectus*	MNCN-AI937	AI	937	Morocco	Sus-Masa-Draa, Ouarzazate province, Tachokchte	ca. 30°47'36"N 7°31'27"W	I. Ribera & A. Cieslak	2001	**LN995109**	**LN995077**	**LN995181**	**LN995147**
36	*D. moestus moestus*	IBE-DV69	DV	69	Italy (Sardinia)	Olbia-Tempio province, Monte Limbara	40°51'30.18"N 9° 7'20.81"E	H. Fery & M. Toledo	2009	**LN995110**	**LN995078**	**LN995182**	**LN995148**
37	*D. moestus moestus*	NHM-IR156	IR	156	France (Corsica)	Haute-Corse department, Cassamozza	ca. 41°58'33"N 9°23'58"E	I. Ribera & A. Cieslak	1999		AF309313	AF309256	EF670139
38	*D. nilssoni*	IBE-AF104	AF	104	Iran	Khorasan Shamali province, Eshq Abad	37º 48.2'N 56º55.5'E	J. Hájek & P. Chvojka	2006		**LN995080**	**LN995184**	**LN995150**
39	*D. opatrinus*	MNCN-AI629	AI	629	Spain	Andalucia, Córdoba province, Sierra Morena,	38° 5'51.79"N 4°53'34.37"W	A. Castro	2005	**LN995112**	HE610188	**LN995185**	**LN995151**
40	*D. parvicollis*	MNCN-AI776	AI	776	Turkey	Bolu province, Kartalkaya	40º39'20"N 31º47'8.5"E	I. Ribera, P. Aguilera & C. Hernando	2006	**LN995113**	HF931225	HF931454	**LN995152**
41	*D. persicus*	NHM-JH/IR45	IR	45	Iran	Fars province, Sepidan	ca. 30°17'6"N 51°56'54"E	H. Fery	1998		AF309308	AF309251	EF670140
42	*D. platynotus platynotus*	MNCN-AI1039	AI	1039	Bulgaria	Kyustendil province, Rila (Rila Mountains)	ca. 42° 7'54.80"N 23° 8'40.14"E	D.T. Bilton	2006	**LN995115**	HE610190	**LN995186**	**LN995154**
43	*D. platynotus platynotus*	MNCN-AI1122	AI	1122	Germany	Saxonia, Dresden region, Pöbelbach,	50°48'57.96"N 13°39'44.14"E	L. Hendrich	2006	**LN995114**	HF931162	HF931381	**LN995153**
44	*D. sahlbergi*	MNCN-AI1002	AI	1002	Bulgaria	Haskovo province, Madjarovo (Eastern Rhodope mountains)	ca. 41°38'23"N 25°51'43"E	V. Pesic	2006	**LN995116**	HE610191	HF931361	**LN995155**
45	*D. sahlbergi*	MNCN-AI108	AI	108	Greece (Chios)	Kardamila	ca. 38°31'37"N 26° 5'30"E)	G.N. Foster	2004	**LN995117**	**LN995081**	**LN995187**	**LN995156**
46	*D. semirufus*	IBE-RA136	RA	136	Italy	Emilia-Romagna, Modena province, Fanano	ca. 44°8'4"N 10°46'55"E	M. Toledo	2009	**LN995118**	**LN995082**	**LN995188**	**LN995157**
47	*D. theryi*	IBE-RA37	RA	37	Morocco	Taza-Al Hoceima-Taounate,Taza province, Tazzeka National Park	34º08'56.8"N 4º00'25.5"W	I. Ribera, P. Aguilera & C. Hernando	2008	**LN995119**	**LN995083**	**LN995189**	**LN995158**
48	*D. theryi*	NHM-IR229	IR	229	Morocco	Souss-Massa-Draa, Tizi n´test pass (High Atlas mountains)	ca. 30°51'45"N 8°22'38"W	I. Ribera, P. Aguilera & C. Hernando	2000		AF309319	AF309262	EF670141
49	*D. toledoi*	IBE-DV6	DV	6	Turkey	Erzurum province, Toprakkaleköyü	40°14'22.90"N 40°59'16.70"E	I. Ribera	2011	**LN995120**	**LN995084**	**LN995190**	**LN995159**
50	*D. wewalkai*	MNCN-AI725	AI	725	Spain	Castilla-La Mancha, Guadalajara province, Cardoso de la Sierra,	41º05'34.3"N 3º25'32.1"W	I. Ribera & A. Cieslak	2005	**LN995121**	**LN995085**	**LN995191**	**LN995160**
51	*D. youngi*	NHM-IR182	IR	182	Iran	Kohkiluyeh and Boyer Ahmad province, Gachsaran	ca. 30°24'20.78"N 50°50'7.75"E	H. Fery	1999		AF309306	AF309249	EF670142
52	*Boreonectes ibericus*	NHM-IR22	IR	22	Deronectes gr						EF670064	EF670030	EF670157
53	*Nebrioporus baeticus*	NHM-IR10	IR	10	Deronectes gr					**LN995122**	AF309302	AF309245	EF670143
54	*Nebrioporus carinatus*	NHM-IR17	IR	17	Deronectes gr					**LN995123**	AF309303	AF309246	EF670144
55	*Nebrioporus clarkii*	NHM-IR46	IR	46	Deronectes gr						EF056623	AY250924	EF056585
56	*Nebrioporus stearinus stearinus*	NHM-IR134	IR	134	Deronectes gr						AY250972	AY250932	EF670145
57	*Oreodytes congruus*	NHM-IR440	IR	440	Deronectes gr						AJ850599	AJ850347	EF670146
58	*Oreodytes crassulus*	NHM-IR451	IR	451	Deronectes gr						AJ850600	AJ850348	EF670147
59	*Oreodytes davisii rhianae*	NHM-ER33	ER	33	Deronectes gr						AF309301	AF309244	EF670148

60	*Oreodytes natrix*	NHM-IR611	IR	611	Deronectes gr						AJ850601	AJ850349	EF670149
61	*Oreodytes quadrimaculatus*	NHM-IR366	IR	366	Deronectes gr						AJ850603	AJ850351	EF670151
62	*Oreodytes rhyacophilus*	NHM-IR367	IR	367	Deronectes gr						AJ850604	AJ850352	EF670152
63	*Scarodytes halensis*	NHM-ER35	ER	35	Deronectes gr						AF309305	AF309248	EF670153
64	*Stictotarsus bertrandi*	NHM-IR30	IR	30	Deronectes gr					**LN995125**	AY250984	AY250946	EF670154
65	*Stictotarsus duodecimpustulatus*	NHM-IR42	IR	42	Deronectes gr						AF309304	AF309247	EF670156
66	*Stictotarsus falli*	NHM-IR334	IR	334	Deronectes gr						EF670063	EF670029	EF670155
67	*Stictotarsus roffii*	NHM-IR335	IR	335	Deronectes gr						AJ850607	AJ850355	EF670158
68	*Trichonectes otini*	NHM-IR29	IR	29	Deronectes gr						AJ850608	AJ850356	EF670159
69	*Graptodytes aequalis*	NHM-IR206	IR	206	Graptodytes gr						AY250953/HM588	AY250910	EF670184
70	*Graptodytes flavipes*	NHM-IR40	IR	40	Graptodytes gr						EF056604/HM588	AY250914	EF056561
71	*Graptodytes ignotus*	NHM-IR531	IR	531	Graptodytes gr						AY250956/HM588	AY250915	EF670185
72	*Iberoporus cermenius*	NHM-IR276	IR	276	Graptodytes gr						AY250958	AY250918	EF670186
73	*Metaporus meridionalis*	NHM-IR34	IR	34	Graptodytes gr						AY250959/HM588	AY250919	EF670187
74	*Porhydrus lineatus*	NHM-IR24	IR	24	Graptodytes gr						AY250973	AY250933	EF670188
75	*Rhithrodytes bimaculatus*	NHM-ER39	ER	39	Graptodytes gr					**LN995124**	AY250974	AY250934	EF670189
76	*Rhithrodytes sexguttatus*	NHM-IR183	IR	183	Graptodytes gr						AY250975	AY250936	EF670190
77	*Stictonectes epipleuricus*	NHM-IR41	IR	41	Graptodytes gr						AF518285	AF518255	EF670191
78	*Stictonectes optatus*	NHM-MsC00C	Ms	C00C	Graptodytes gr						AY250981	AY250943	EF670192
79	*Herophydrus musicus*	NHM-IR36	IR	36	Hygrotini						AJ850634	AJ850384	EF670206
80	*Herophydrus nodieri*	NHM-IR242	IR	242	Hygrotini						AJ850632	AJ850382	EF670204
81	*Herophydrus obscurus*	NHM-IR622	IR	622	Hygrotini						AJ850633	AJ850383	EF670205
82	*Hygrotus caspius*	NHM-IR688	IR	688	Hygrotini						AJ850635	AJ850385	EF670207
83	*Hygrotus confluens*	NHM-IR59	IR	59	Hygrotini						AY250964	AJ850386	EF670208
84	*Hygrotus corpulentus*	NHM-IR687	IR	687	Hygrotini						AJ850637	AJ850387	EF670209
85	*Hygrotus impressopunctatus*	NHM-IR603	IR	603	Hygrotini						AJ850653	AJ850405	EF670210
86	*Hygrotus inaequalis*	NHM-IR19	IR	19	Hygrotini						EF056611	EF056681	EF056569
87	*Hygrotus saginatus*	NHM-IR684	IR	684	Hygrotini						AJ850639	AJ850389	EF670211
88	*Hygrotus turbidus*	NHM-IR482	IR	482	Hygrotini						AJ850640	AJ850390	EF670212

Appendix S2: Additional methods

(a) Primers used for the amplification and sequencing. In brackets, length of the amplified fragment. F, forward; R, reverse. (b) Standard PCR conditions for the amplification of the studied fragments.

(a)

Gene	Primer	Sequence	Ref
cox1-3'	F M202 (Jerry)	CAACATTTATTTTGATTTTTTGG	4
826 bp	R M70 (Pat)	TCCAATGCACTAATCTGCCATATTA	4
	F Chy	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	3
	R Tom	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	3
barcode	F LCO 1490	GGTCAACAAATCATAAAGATATTGG	2
658 bp	R HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA	2
rrnL+trnL+nad1	F M14 (16SaR)	CGCCTGTTTAACAAAAACAT	4
685-693 bp	R M223 (ND1A)	GGTCCCTTACGAATTTGAATATATCCT	4
H3	F H3aF	ATGGCTCGTACCAAGCAGACRCG	1
330 bp	R H3aR	ATATCCTTRGGCATRATRGTGAC	1

(b)

Step	Time	Temperature
1	3'	96º
2	30"	94º
3	30"-1'	47-50º *
4	1'	72º
5	Go to step 2 and repeat 34-40 x	
6	10'	72º

* Depending on the annealing temperatures of the primers pair used

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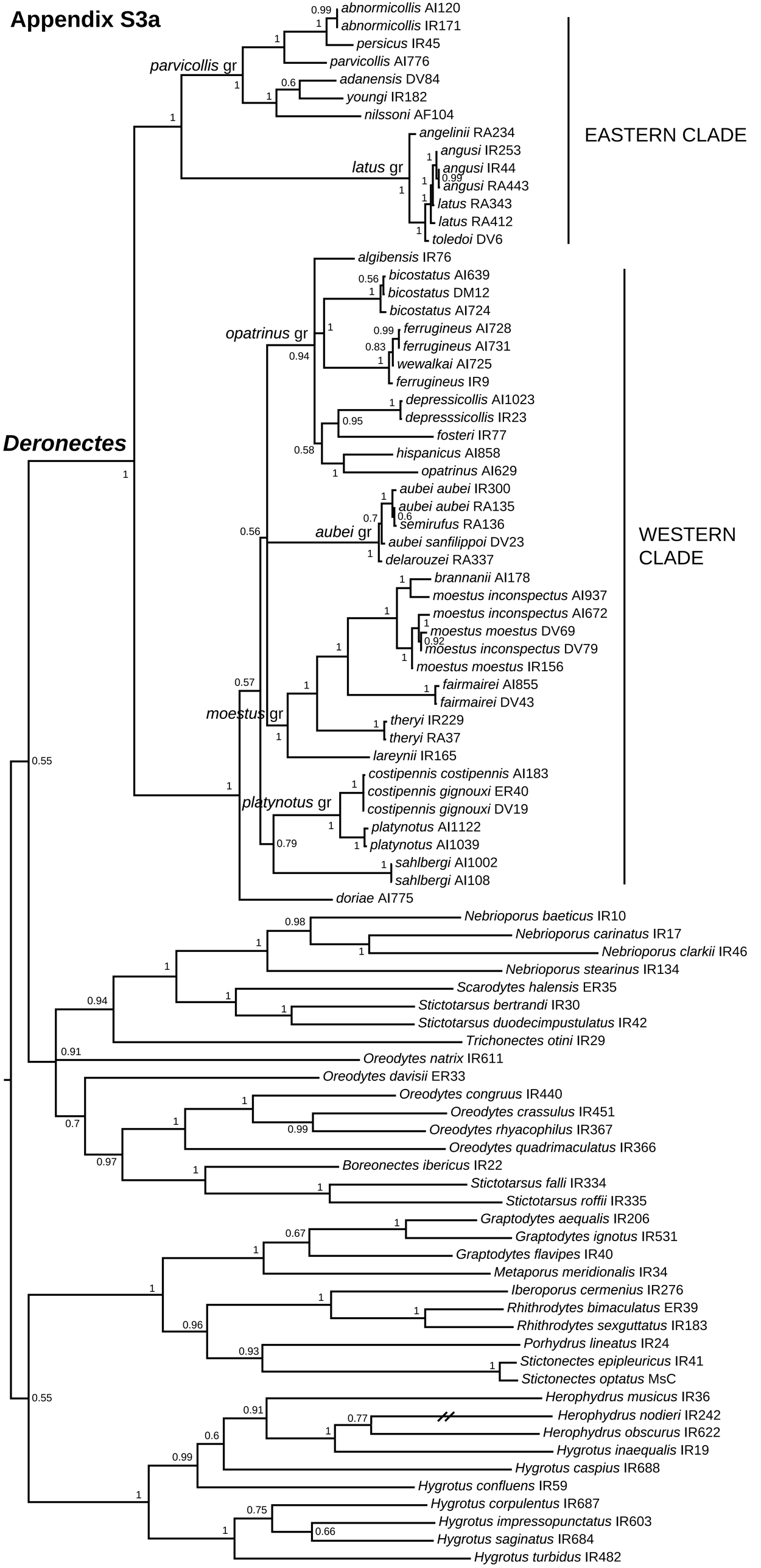
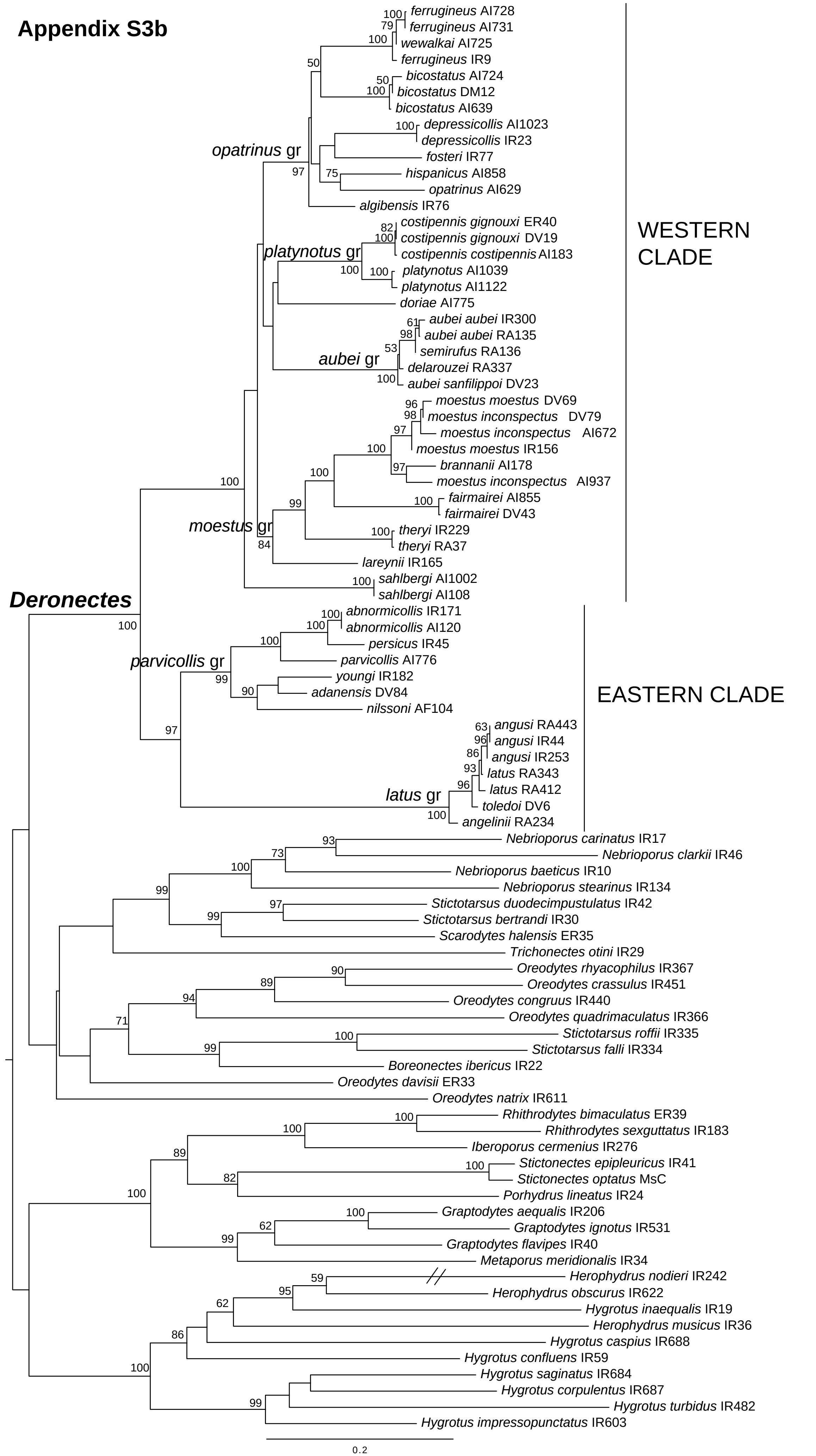
Appendix S3a



Appendix S3b



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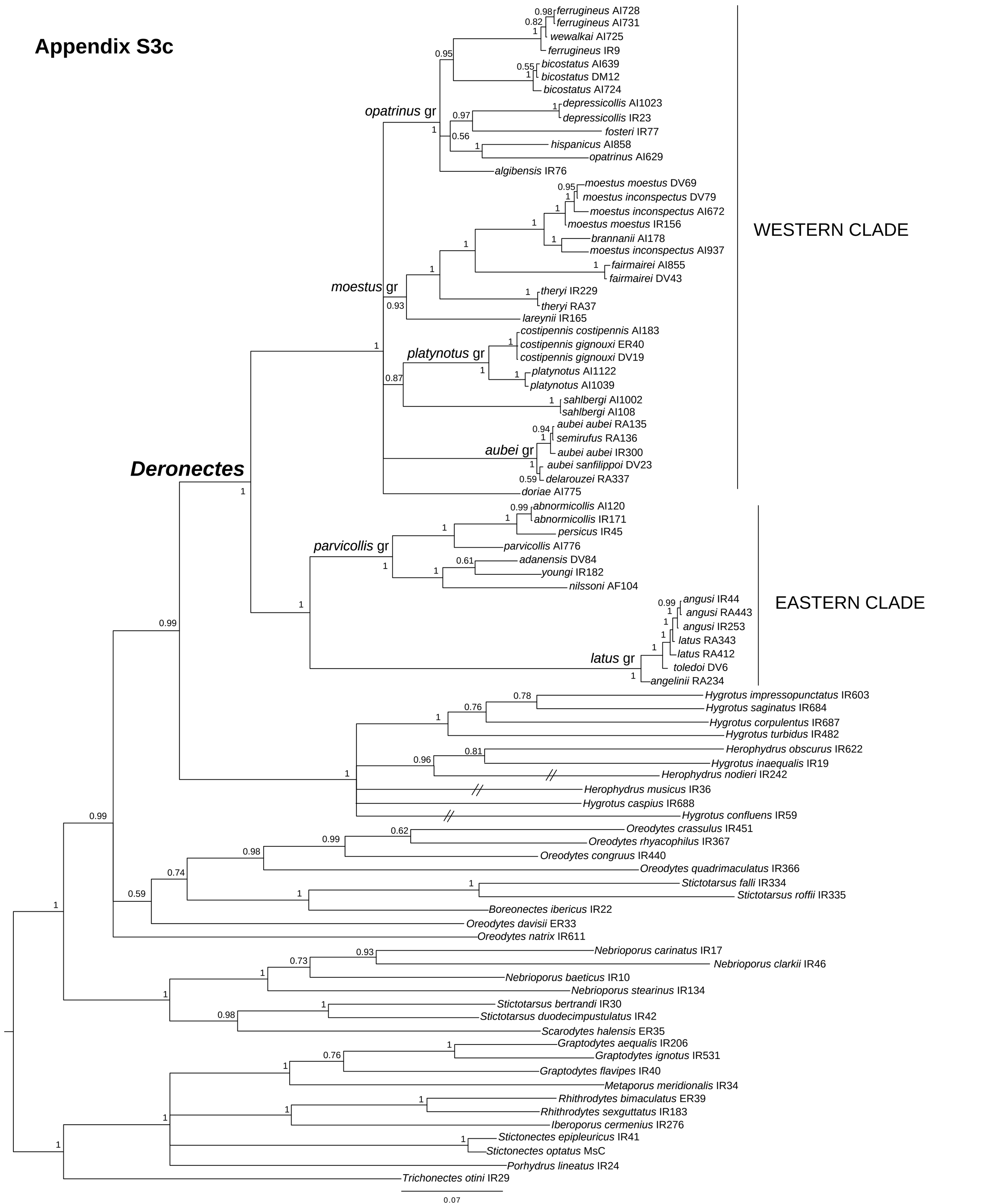
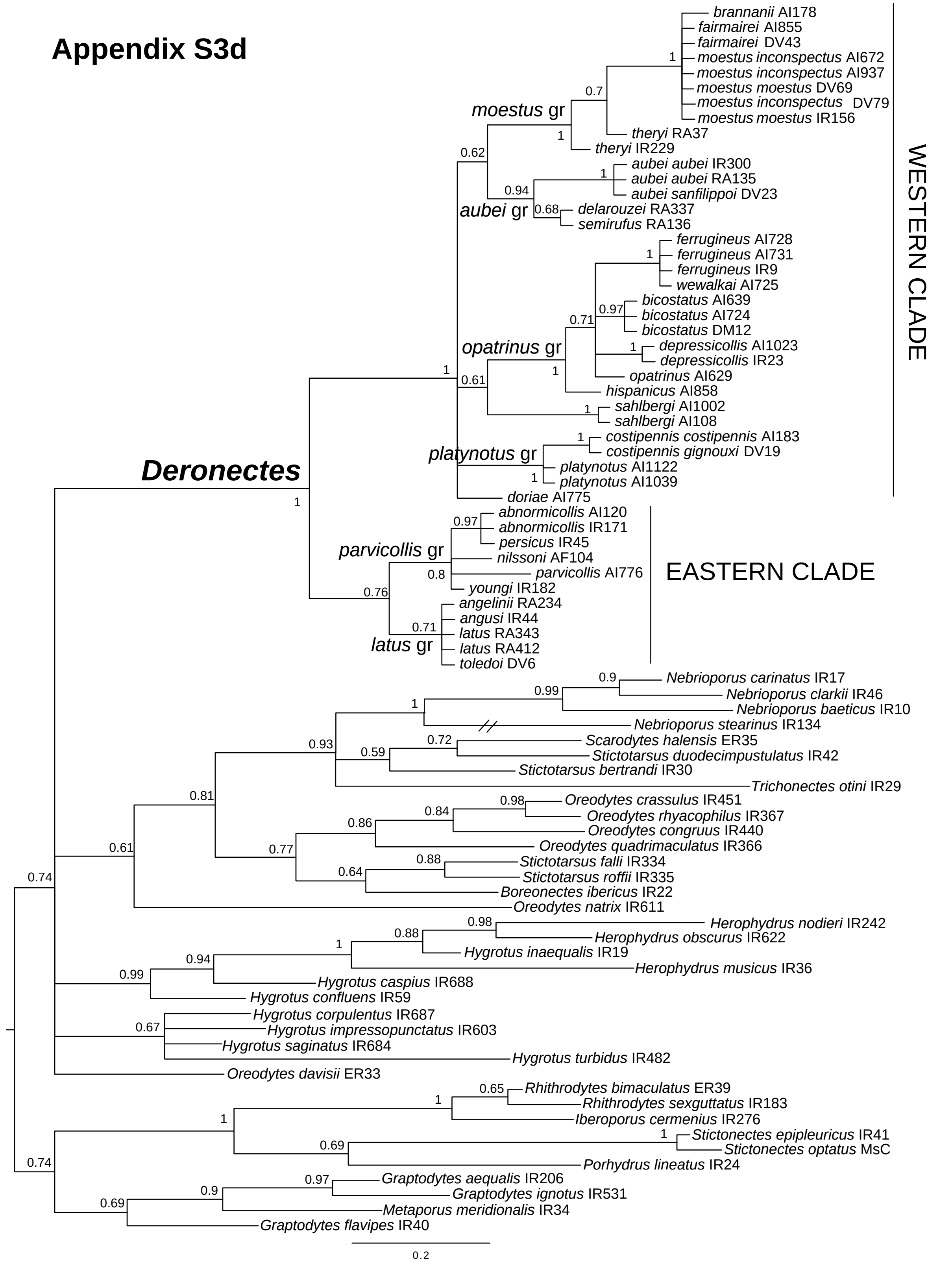
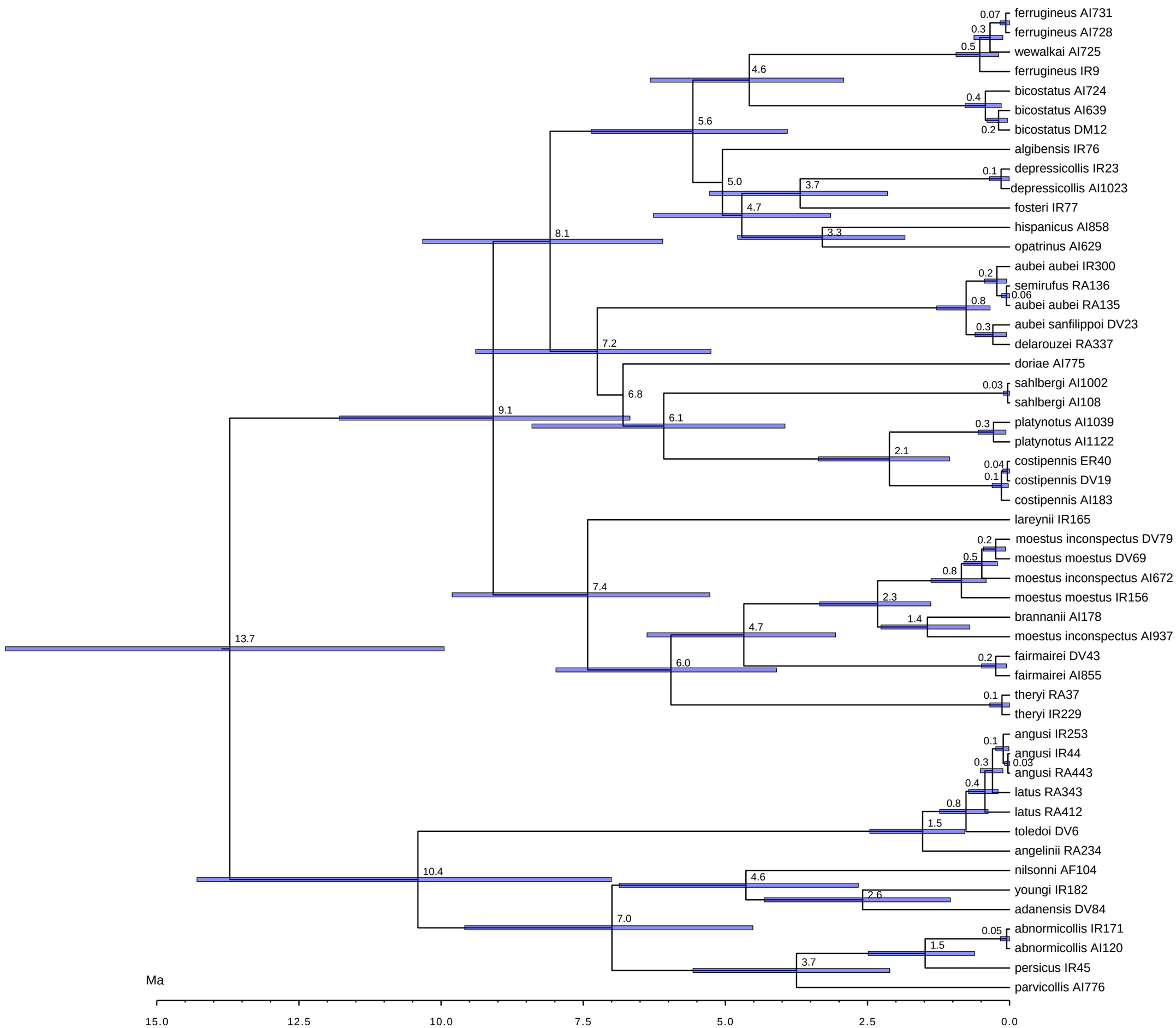


Appendix S3d



Appendix S3e



Chapter 2

The origin of widespread species in a poor dispersing lineage (diving beetle genus *Deronectes*)

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The origin of widespread species in a poor dispersing lineage (diving beetle genus *Deronectes*)

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ABSTRACT

In most lineages, most species have restricted geographic ranges, with only few reaching widespread distributions. How these widespread species reached their current ranges is an intriguing biogeographic and evolutionary question, especially in groups known to be poor dispersers. We reconstructed the biogeographic and temporal origin of the widespread species in a lineage with particularly poor dispersal capabilities, the diving beetle genus *Deronectes* (Dytiscidae). Most of the ca. 60 described species of *Deronectes* have narrow ranges in the Mediterranean area, with only four species with widespread European distributions. We sequenced four mitochondrial and two nuclear genes of 297 specimens of 109 different populations covering the entire distribution of the four lineages of *Deronectes*, including widespread species. Using Bayesian probabilities with an a priori evolutionary rate, we performed (1) a global phylogeny/phylogeography to estimate the relationships of the main lineages within each group and root them, and (2) demographic analyses of the best population coalescent model for each species group, including a reconstruction of the geographical history estimated from the distribution of the sampled localities. We also selected 56 specimens to test for the presence of *Wolbachia*, a maternally transmitted parasite that can alter the patterns of mtDNA variability. All species of the four studied groups originated in the southern Mediterranean peninsulas and were estimated to be of Pleistocene origin. In three of the four widespread species, the central and northern European populations were nested within those in the northern areas of the Anatolian, Balkan and Iberian peninsulas respectively, suggesting a range expansion at the edge of the southern refugia. In the Mediterranean peninsulas the widespread European species were replaced by vicariant taxa of recent origin. The fourth species (*D. moestus*) was proven to be a composite of unrecognised lineages with more restricted distributions around the Western and Central Mediterranean. The analysis of *Wolbachia* showed a high prevalence of infection among *Deronectes*, especially in the *D. aubei* group, where all sequenced populations were infected with the only exception of the Cantabrian Mountains, the westernmost area of distribution of the lineage. In this group there was a phylogenetic incongruence between the mitochondrial and the nuclear sequence, although no clear pattern links this discordance to the *Wolbachia* infection. Our results suggest that, in different glacial cycles, populations that happened to be at the edge of the newly deglaciated areas took advantage of the optimal ecological conditions to expand their ranges to central and northern Europe. Once this favourable ecological window ended populations become isolated, resulting in the presence of closely related but distinct species in the Mediterranean peninsulas.

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Additional Information and Declarations can be found on page 19

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INTRODUCTION

The geographic range of the species is a fundamental trait in ecology and biogeography (*Brown & Lomolino, 1998*). There have been many hypotheses put forward by ecologists, biogeographers or evolutionary biologists to explain geographic ranges (see e.g., *Brown, 1995* or *Gaston, 2003* for reviews), such as differences in niche breadth, body size, population abundance, environmental variability, colonization and extinction dynamics, and dispersal ability among others (*Stevens, 1989*; *Brown, 1995*; *Gaston, 2003*), but there is still a lack of understanding of the evolutionary dynamics of geographical ranges. Thus, although the ecological and biogeographic context in which species evolve are known to determine their range sizes (*Böhning-Gaese et al., 2006*), there are multiple cases of closely related species, sharing a common phylogenetic history and with a similar biology and ecology, that have nonetheless extreme differences in the size of their geographical range (*Lester et al., 2007*).

In freshwater species, one of the most general and robust associations is that of habitat stability and geographic range (*Ribera, 2008*; *Dijkstra, Monaghan & Pauls, 2014*). Species living in standing waters tend to have better dispersal abilities than species living in running waters, due to the shorter geological duration of their habitats. Running water species have on average smaller geographic ranges and a lower gene flow between populations (*Ribera, 2008*; *Abellán, Millán & Ribera, 2009*). Range size is also strongly correlated with extinction probability (*Jablonski, 1987*), and high probabilities of extinction have been linked with narrow geographical ranges in several taxa (see e.g., *Hansen, 1980* for marine invertebrates, *Purvis et al., 2000* for mammals, or *Rosenfield, 2002* for freshwater fishes). This raises the question of how lineages with a predominance of running water species, and thus on average smaller geographic ranges, can persist over long evolutionary periods. It must be noted here that while among standing water species the frequency of species with small ranges is expected to be low, within running water lineages it is not rare to find widespread species (*Ribera & Vogler, 2000*). This is likely due to the asymmetry in the habitat constraints: standing water species have to migrate when their habitat disappears, but the higher stability of running water habitats means that species in them do not need to disperse. Nevertheless, they tend to lose dispersal capabilities, likely due to their associated cost (see *Ribera, 2008* for a review), although in some circumstances running water species seem to be able to disperse and reach widespread distributions. These few widespread species may be of disproportionate importance, as potential sources of new species ("diversity pumps," *Ribera et al., 2011*), but there is very few data on what makes a species in a clade of poor dispersers able to expand its range, or in what circumstances.

A common pattern among many lineages with abundance of species with restricted geographic ranges within the Mediterranean area is the presence of one (or few) species with a widespread distribution including parts of central and northern Europe. These

northern populations should have a recent origin, as most of central and northern Europe was glaciated or with permafrost during the Last Glacial Maximum, when the European ice sheet extended north of 52°N and the permafrost north of 47°N (*Dawson, 1992*). It is thus clear that some species were able to expand their ranges in a short time, as they could not have survived on the ice sheet.

We study the origin and phylogeography of the European widespread species in one of these running water lineages, the diving beetle genus *Deronectes* Sharp (family Dytiscidae). With c. 60 described species (*Nilsson & Hájek, 2015*), *Deronectes* is the largest clade of Palaearctic diving beetle entirely confined to running waters. It has a predominantly Mediterranean distribution, ranging from North Africa and the Iberian Peninsula over most parts of Europe and the Middle East, with some species reaching central Asia (*Nilsson, 2001*). The genus has many narrow range Mediterranean species, including some island endemics, but also a few species widespread in central and northern Europe, showing their potential for range expansion. A recent molecular phylogeny of the genus (*García-Vázquez et al., 2016*) supported the existence of two main lineages, mostly corresponding to species with a western or eastern distribution. The widespread European species were also shown to be of Pleistocene origin, in agreement with other works with the genus (*Ribera, Barraclough & Vogler, 2001*; *Ribera, 2003*; *Ribera & Vogler, 2004*; *Abellán & Ribera, 2011*).

Our main objective here is to reconstruct in detail the temporal and geographic origin of the widespread European species of *Deronectes*, using molecular data from a comprehensive sampling of them and their closest relatives, covering their entire distributions.

MATERIAL AND METHODS

Taxonomic background

Four species of *Deronectes* have widespread European distributions. Three of them are found in central and northern Europe, i.e., areas strongly affected by the last glacial cycle: (1) *D. aubei* (Mulsant), (2) *D. latus* (Stephens) and (3) *D. platynotus* (Germar). The fourth, *D. moestus* (Fairmaire), is distributed along the western Mediterranean from the Maghreb to the Balkans, although with high intraspecific variation, according to the preliminary results of *García-Vázquez et al. (2016)*. All four belong to different species groups within the genus, as defined by *García-Vázquez et al. (2016)*.

(1) The *D. aubei* group includes three species, one of them divided in two subspecies. The widespread *D. aubei* includes *D. a. aubei* from the Alps and surrounding areas, reaching southern Germany (Black forest) in the north, and *D. a. sanfilippoi* Fery & Brancucci distributed from the Cantabrian mountains in NW Spain to southern France (Pyrenees). The other two species are *D. semirufus* (Germar), from the Alps Maritims to Sicily, and *D. delarouzei* (Jacquelin du Val) in the Pyrenees (*Fery & Brancucci, 1997*) (Fig. 1). *García-Vázquez et al. (2016)* found incongruence between mitochondrial and nuclear data within the *D. aubei* group. According to the mitochondrial sequence, the two subspecies of *D. aubei* were recovered as paraphyletic and respectively sisters to the geographically closest species of the group: one clade was distributed west of the Rhone river, including *D. a. sanfilippoi* and *D. delarouzei*, and another east of the Rhone, with *D. a. aubei* and *D. semirufus*. On the

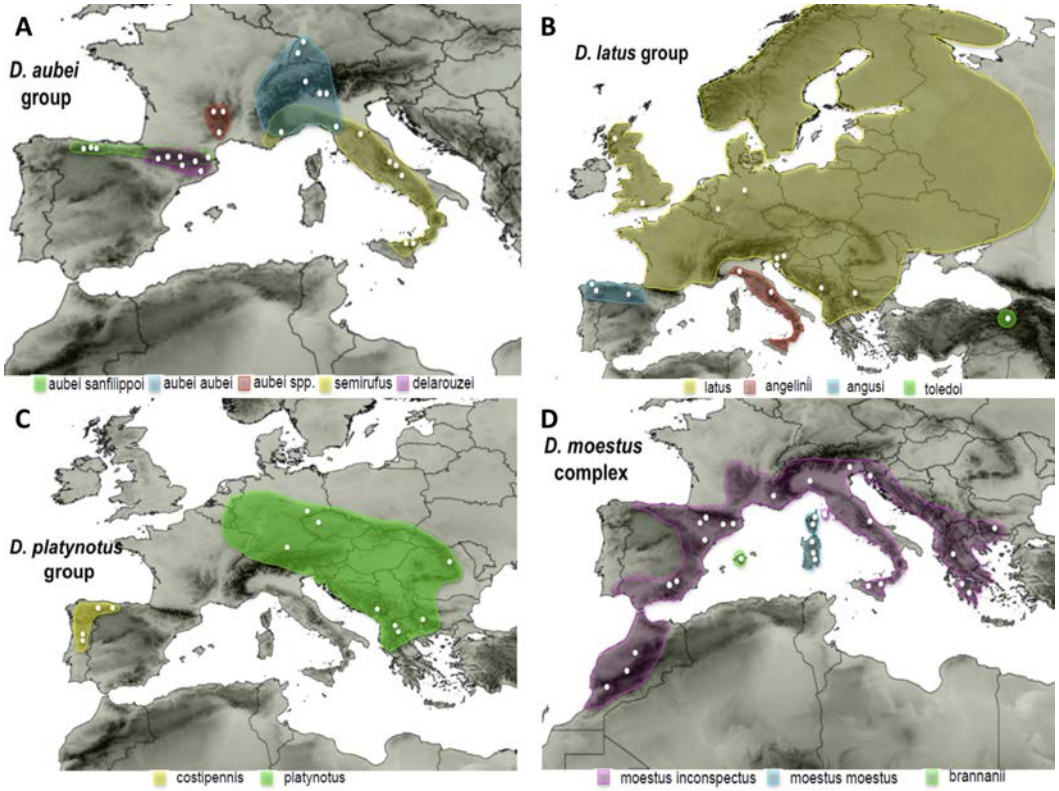

Figure 1 Distribution maps of the studied species. Known distribution of the species of the four studied groups of Deronectes. White circles, sampled localities.

contrary, the nuclear sequence recovered a monophyletic *D. aubei* as sister to the other two species of the group (*D. delarouzei and D. semirufus*).

(2) The *D. latus* group, within the eastern Mediterranean lineage of *Deronectes*, includes four species. *Deronectes latus* is the most widespread species of the genus, occupying large areas of Europe north of the Pyrenees and the Apennines, reaching the British Isles and Scandinavia. On the contrary, the other three species have restricted distributions in the southern peninsulas: *D. toledoi* Fery, Erman & Hosseinie in northeastern Turkey; *D. angusi* Fery & Brancucci in northwestern Spain; and *D. angelinii* Fery & Brancucci, the only Italian endemic of the genus, from the Apennines to Sicily, including the island of Elba (see Fig. 1 for the distribution of each species). The entire group was reconstructed as having an origin in the Anatolian peninsula, from were expanded first to Italy and the Balkan Peninsula and subsequently to the Iberian Peninsula and central and northern Europe (*García-Vázquez et al., 2016*).

(3) The *D. platynotus* group, in the western clade of *Deronectes*, comprises two species, with two subspecies each. *Deronectes platynotus* includes the widespread *D. p. platynotus*, distributed from the Balkans and northern Greece to central Europe, and the subspecies *D. p. mazzoldi* Fery & Brancucci, from northern Greece. The second species, the NW Iberian endemic *D. costipennis* Brancucci, includes *D. c. costipennis* from Portugal and the extreme NW Iberia, and D. c. *gignouxi* *Fery & Brancucci, 1997*, from the Cantabrian region (Fig. 1).

(4) The *D. moestus* group includes the fourth of the widespread European species of the genus, *D. moestus*, distributed from the Maghreb and the Iberian Peninsula to the Balkans through southern France, Italy and Sicily, together with other western Mediterranean species. According to the results of *García-Vázquez et al. (2016)* *D. moestus* as currently understood is paraphyletic, and includes the Mallorcan endemic *D. brannani* (Schauffus). The two recognised subspecies of *D. moestus*, *D. m. moestus* from Corsica and Sardinia and *D. m. inconspectus* (Leprieur) from the continent, were not recovered as respectively monophyletic. Here we studied the species *D. moestus* plus *D. brannani*, what we call the *D. moestus* complex (Fig. 1). The species *D. perrinae* Fery & Brancucci, from Algeria and Tunisia, is morphologically very close to *D. moestus*, and probably should be included in this complex, although no specimens could be obtained for study.

Taxon sampling

We studied a total of 296 specimens of 109 populations (with a maximum of 5 specimens per population) of all species of the four studied lineages of *Deronectes*, of which 23 specimens were previously used by *García-Vázquez et al. (2016)* (Table S1). We aimed to cover the full geographic range of the studied species, in particular potential cryptic refuge areas (in the sense of *Homburg et al., 2013*) at the margins of the Alps and neighbouring mountain ranges (such as the Black Forest), or areas not included in previous works (e.g., Sicily or French Massif Central for the species of the *D. aubei* group).

DNA extraction and sequencing

Specimens were collected and preserved in absolute ethanol directly in the field. We extracted the DNA non-destructively with commercial kits (mostly "DNeasy Tissue Kit"; Qiagen GmbH, Hilden, Germany, and "Charge Switch gDNA Tissue Mini Kit"; Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Specimens and DNA extractions are kept in the collections of the Institut de Biología Evolutiva, Barcelona (IBE), Museo Nacional de Ciencias Naturales, Madrid (MNCN) and Natural History Museum, London (NHM).

We obtained seven gene fragments from six different genes (four mitochondrial and two nuclear) in five different amplification reactions (see Table S2 for primers and typical sequencing reactions): (1) 5′ end of the Cytochrome Oxidase Subunit 1 gene (the barcode fragment, *Hebert, Ratnasingham & De Waard, 2003*, COI-5′); (2) 3′ end of Cytochrome Oxidase Subunit 1 (COI-3′); (3) 5′ end of 16S rRNA plus tRNA transfer of Leucine plus 5′ end of NADH subunit 1 (nad1) (16S); and internal fragments of the nuclear genes (4) Histone 3 (H3) and (5) Wingless (Wg). The nuclear markers were only used in representative specimens according to geographical (one specimen per population) and topological criteria. For each amplification reaction, we obtained both forward and reverse sequences. In some specimens, and due to difficulties of amplification, we used internal primers for the fragment COI-3′, obtaining two fragments of 400 bp each. The obtained products were purified by standard ethanol precipitation and sent to external facilities for sequencing after purification (LMU Genomics Service Unit, Martinsried, Germany). DNA sequences were assembled and edited using the GENEIOUS 6 software (Biomatters Ltd,

Auckland, New Zealand). New *Deronectes* sequences (a total of 791) have been deposited in GenBank with accession numbers LT601818–LT602609, and *Wolbachia* sequences (23) with numbers LT602610–LT602633 (Table S1).

Phylogenetic and phylogeographic analyses

Edited sequences were aligned with MAFFT v.6 using the G-INS algorithm and default values for other parameters (*Katoh & Toh, 2008*). We employed six partitions corresponding to the gene fragments COI-5′, COI-3′, 16S rRNA+tRNA-Leu, nad1, H3 and Wg, and used Partition Finder 1.1.1 (*Lanfear et al., 2012*) to estimate the best partitioning scheme (each gene separately or combining genes in a partition by codons) and the evolutionary model that best fitted the data for each partition separately, using the AIC (Akaike Information Criterion) as selection criteria. We performed two types of analyses using different datasets: (1) a global phylogeny/phylogeography to estimate the relationships of the main lineages within each group and root them; and (2) demographic analyses of the best population coalescent model for each group, including a reconstruction of the geographical history estimated from the distribution of the sampled localities.

For the global phylogeny/phylogeography we first selected one specimen per population to estimate the general topology of the four lineages within the wider genus *Deronectes*, employing Bayesian methods implemented in BEAST 1.8 (*Drummond & Rambaut, 2007*), using as outgroups 18 species of *Deronectes* in other species groups. We rooted the trees in the separation between the western and eastern clades of the genus, following *García-Vázquez et al., (2016)*. For the analysis, we implemented the closer available evolutionary model to those selected by Partition Finder and the best partitioning scheme, including both mitochondrial and nuclear markers. As there are no fossils or unambiguous biogeographic events that could be used to calibrate the phylogeny of *Deronectes* (*García-Vázquez et al., 2016*), we applied an a-priori rate of 0.013 substitutions/site/MY (standard deviation 0.002) for protein coding genes and 0.0016 substitutions/site/MY (standard deviation 0.0002) for 16S, obtained in a related group (family Carabidae) for the same mitochondrial genes (*Andújar, Serrano & Gómez-Zurita, 2012*). As there are no reliable estimations of the evolutionary rate of the nuclear genes, we applied an uniform flat prior (from 0 to infinite). We used an uncorrelated lognormal relaxed clock, a Yule speciation model and executed two independent analyses with the same settings, running 100 million generations (saving trees every 5,000) or until they converged and the number of trees was sufficient according to Effective Sample Size (ESS) values, as measured with TRACER v1.6 (*Drummond & Rambaut, 2007*). The majority rule consensus tree of the two runs was compiled with Tree Annotator v1.8 (*Drummond & Rambaut, 2007*). To test for potential discordances among the different markers, we also analysed separately the mitochondrial and nuclear data. We applied the same settings as the combined analysis but, to estimate the divergence dates among species, we established as a prior for the root of the tree a normal distribution with average 14 Ma and a standard deviation of 0.1, according to the results obtained in *García-Vázquez et al. (2016)*.

For the demographic analyses we used all sequenced specimens from all populations to estimate, for each of the four groups separately, the demographic model (constant, logistic,

expansion or exponential) that best fitted the data. Previously, we tested the adjustment to a strict molecular clock and used the best clock model in the coalescent analyses. We used the mitochondrial sequence only and no outgroups, with a GTR+I+G evolutionary model and the same settings used in the analysis of the topology. For model selection of the molecular clock and coalescent analyses, we used the modified Akaike information criterion (AICM) with moments estimator (*Baele et al., 2012*), as implemented in TRACER v1.6, with 1,000 bootstrap replicates. We also computed Bayesian skyline plots for each group to reconstruct the variation of the effective population sizes with time.

Using the best clock and demographic models as selected above, and after excluding specimens from the same locality with repeated haplotypes, we reconstructed the phylogeographic history of each group in BEAST using the coordinates of each locality as a quantitative trait (*Lemey et al., 2009*). We excluded outgroups, but included the nuclear markers in the analyses. We overlaid the reconstructed coordinates of the nodes of the phylogeography of each group, as obtained with BEAST and SPREAD v1.0.6 (*Bielejec et al., 2011*), in Google Earth (http://earth.google.com), allowing the reconstruction through time of the history of each lineage, from the origin until the current distribution.

Wolbachia detection

The incongruence between the mitochondrial and the nuclear data in the *D. aubei* group noted by *García-Vázquez et al. (2016)* raised the possibility that specimens could be infected with *Wolbachia*, a maternally transmitted parasite that can alter the patterns of mtDNA variability (*Jiggins, 2003*). We thus tested for the presence of *Wolbachia* in 56 selected specimens (see Table S1) of the four studied groups of *Deronectes*, focusing on the species of the *D. aubei* group.

We used specific primers for the wsp gene, amplifying a 632 bp fragment. A 3 μl sample of the PCR reaction mixture was electrophoresed with a 100 bp DNA ladder on 1% agarose gel to determine the presence and size of the amplified DNA bands, that were visualized by Sybr-Safe staining. A selection of positive PCR's were purified by standard ethanol precipitation and sent to external facilities for sequencing after purification. DNA sequences were assembled and edited using GENEIOUS and aligned with MAFFT v.6 using the G-INS algorithm and default values for other parameters (*Katoh & Toh, 2008*). To identify the corresponding supergroup of *Wolbachia*, we compared our sequences with previously classified sequenced according to *Zhou, Rousset & O'Neill (1998)*, obtained from GenBank.

RESULTS

Combined phylogeny/phylogeography

There were no length differences in the protein coding genes with the exception of Wg, with variation of a single AA in the ingroup. Length variation in the ribosomal genes ranged between 691–695 bp for the ingroup. The best partition scheme included a separation by codon position, but the Bayesian analyses did not converge adequately due to insufficient data to estimate some of the parameters, so we used the second best option, a partition

by genes. The best evolutionary models were GTR+I+G for the COI-5′, COI-3′, 16S rRNA+tRNA-Leu and Wingless; GTR+G for nad1, and Trn+I+G for H3.

The ultrametric tree obtained with the combined mitocondrial and nuclear data showed a topology compatible with that obtained in *García-Vázquez et al. (2016)*, with strong support for the monophyly of all studied species groups but poorly resolved relationships within the western clade (Fig. 2). In the *D. aubei* group we recovered the Pyrenean endemic *D. delarouzei* as paraphyletic, at the base of the remaining species of the group, which monophyly was strongly supported (Fig. 2). Within the later, the Sicilian and southern Italian *D. semirufus* were monophyletic and sister to *D. aubei* plus *D. semirufus* from the northern Apennines (Tuscany and Emilia-Romagna) and southeastern France. The Pyrenean *D. aubei* were paraphyletic at the base of a strongly supported clade including first specimens from the Cevennes (geographically closer) and then from the Alps Maritimes, Italian Alps and south Germany (Black Forest), plus the Alpine *D. semirufus* (Fig. 2). The analysis of the mitochondrial data alone divided the *D. aubei* group into two clades well separated geographically: one formed by specimens of *D. aubei aubei* and *D. semirufus* from the Alps, Black Forest, French Massif Central and northern Appenines, and the other clade subdivided into two sister groups, one formed by specimens of *D. delarouzei* and *D. aubei sanfilippoi* from northern Spain and Pyrenees and the other by *D. semirufus* from Sicily and central-southern Apennines (see Fig. S1). Contrary to the results with the combined or the mitochondrial data alone, the nuclear data recovered a monophyletic *D. aubei* within a paraphyletic series including the other two species of the group (Fig. S2).

The species of the *D. latus* group were included in the eastern clade, as sister to the species of the *D. parvicollis* group. Within the *D. latus* group, the Italian *D. angelinii* was sister to the rest, which included the Iberian *D. angusi* and the widespread *D. latus* with the Turkish *D. toledoi* nested within it (Fig. 2). Within the *D. platynotus* group, the two species of the group (*D. platynotus* and *D. costipennis*), and the subespecies *D. c. costipennis*, were respectively monophyletic and well supported (Fig. 2). In the *D. moestus* group there was a deep intraspecific divergence in *D. moestus*. A clade with a predominantly western distribution included the specimens from Morocco, southern Spain and southeastern France as sister to the Mallorcan endemic *D. brannani* (Fig. 2). Its sister clade, with a predominantly eastern distribution, included specimens of *D. moestus* from northern Spain to Bulgaria and with specimens of the two previously recognised morphological subspecies (*D. m. moestus* and *D. moestus inconspectus*).

All species of the four studied groups were estimated to be of Pleistocene origin with the exception of the deepest clades within the *D. moestus* complex, dated to the Pliocene-Pleistocene boundary. Intraspecific variation was very recent (from the Upper Pleistocene), as was the separation between some species, which have still not reached monophyly (e.g., within the *D. latus* or *D. aubei* groups) (Fig. 2).

Demographic models and detailed phylogeography

In all groups a strict molecular clock was preferred over the relaxed lognormal, which was implemented in all analyses of demographic models (see Table 1 for AICM values). The run with a relaxed lognormal clock in the *D. latus* group did not converge adequately despite

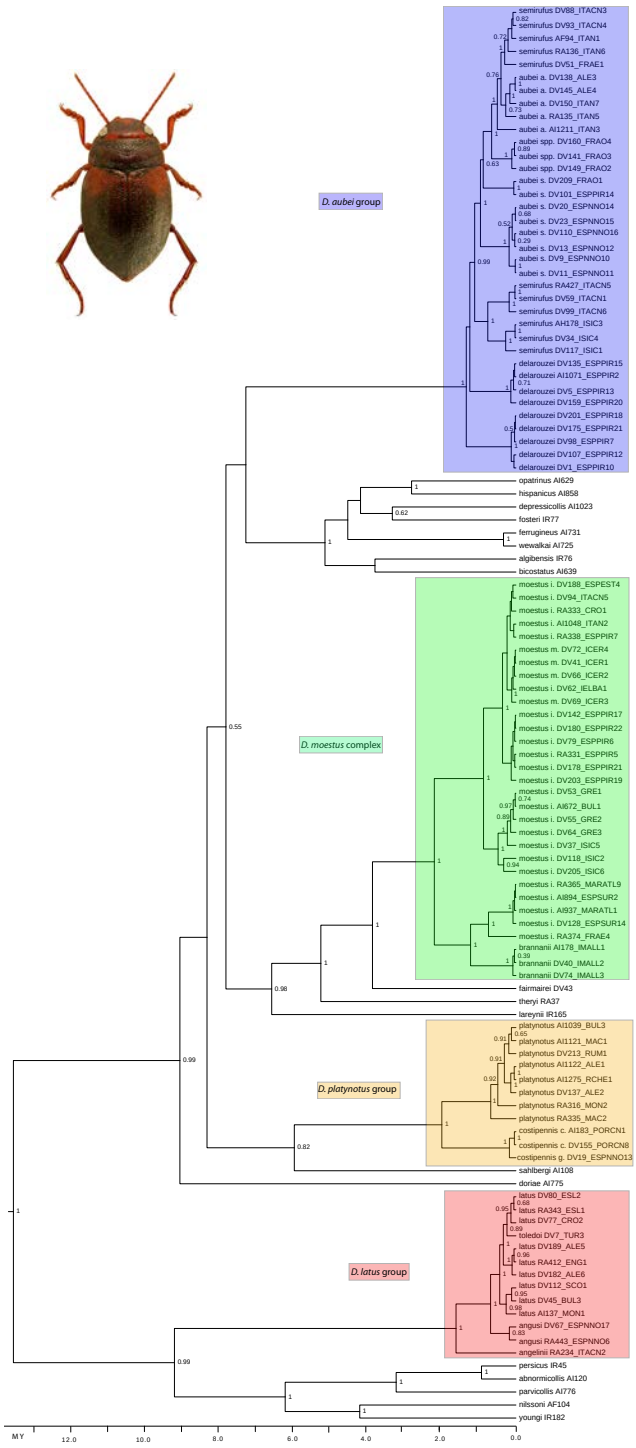

Figure 2 **Phylogenetic tree of the studied groups of *Deronectes*.** Ultrametric tree obtained with BEAST with the combined nuclear and mitochondrial sequence and a partition by gene. The tree had an arbitrary root distance, but the scale is adjusted to the age of the basal node obtained with the mitochondrial tree (Fig. S1). Numbers on nodes, Bayesian posterior probabilities. See Table S1 for details on the specimens and localities. Habitus photograph, *D. latus* (from Lech Borowiec, http://www.colpolon.biol.uni.wroc.pl/index.htm).

Table 1 Analyses of the best molecular clock model and population coalescent model for each group, including AICM values and standard error (SE). In bold, best AICM value for each pair. Differences below 2 were not considered as significant. The relaxed model for *D. latus* failed to converge and was not considered (see 'Results').

Group	Clock model	AICM	SE	Coalescence model	AICM	SE
D. aubei	Relaxed	8235	+/−0.33	Constant	**8196**	+/−0.27
	Strict	**8196**	+/−0.13	Exponential	8199	+/−0.28
D. latus	Relaxed	–	–	Constant	7274	+/−0.09
	Strict	**7274**	+/−0.09	Exponential	7274	+/−0.22
D. moestus	Relaxed	8734	+/−0.30	Constant	8720	+/−0.25
	Strict	**8720**	+/−0.15	Exponential	**8716**	+/−0.16
D. platynotus	Relaxed	7052	+/−0.04	Constant	7049	+/−0.07
	Strict	**7049**	+/−0.18	Exponential	**7046**	+/−0.07

running for 100 million generations, especially for parameters of the 16S gene likely due to insufficient variation. Exploratory analyses of the topology and divergent dates showed no substantial variation with respect to the strict clock model, which was also adopted.

The coalescent analyses with logistic and expansion grow models did not converge adequately in any group. The exponential grow model performed significantly better in the *D. platynotus* group and the *D. moestus* complex, and the constant size model in the *D. aubei* group, while there was no significant difference between both models in the *D. latus* group (Table 1). The Bayesian skyline plot was very similar for the *D. latus* and *D. aubei* groups, with a nearly constant effective population size with a recent, postglacial increase after a slight decline (Fig. 3). In the *D. moestus* complex it also remained constant until recent times, but with a recent sharp decline followed by a fast increase recovering the previous effective population size. On the contrary, in the *D. platynotus* group there was a gradual decrease ending in a sharp decline (Fig. 3).

Due to the absence of outgroups in the phylogeographic analyses in BEAST the origin of each of the individual clades was reconstructed in a position geographically intermediate between the two basal nodes, and was not interpreted.

In the *D. aubei* group there were three main lineages geographically well differentiated. The north of the Iberian peninsula (Pyrenees and Cantabrian mountains) was reconstructed as having been populated from east to the west by *D. delarouzei* and *D. a. sanfilippoi*; Sicily and the central and southern part of the Italian peninsula by the southern *D. semirufus*; and the Alps and nearby areas by *D. a. aubei* and the northern *D. semirufus*. Within the later, the expansion seems to have been from the Alpes Maritimes and the Italian Piamonte to respectively the Massif Central, the north side of the Alps, including the Black Forest, and the northern Apenines (see Fig. 4 for the phylogenetic tree and Fig. 5 for the map with the phylogeographic reconstruction).

The reconstruction of the *D. latus* group showed an initial expansion into Italy at the origin of *D. angelinii*. This initial expansion was followed first by the split between the eastern *D. toledoi* (NE Turkey) and the remaining western lineages, with a second expansion including the colonization of the Iberian peninsula by *D. angusi* and large areas of central

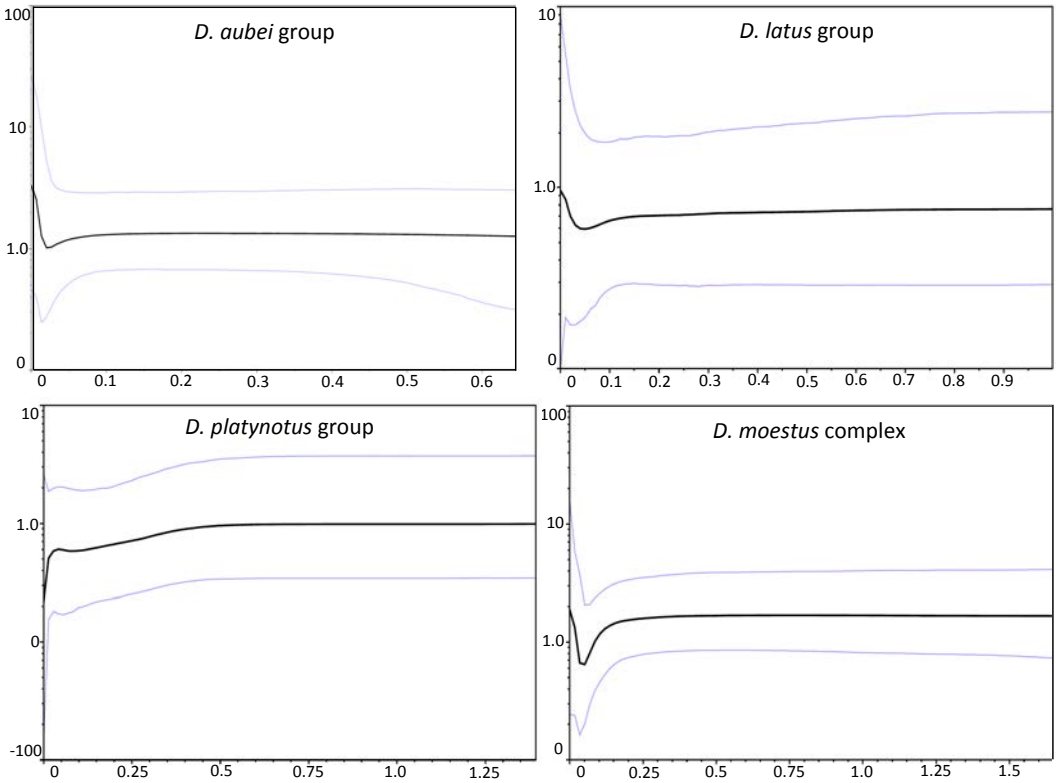

Figure 3 Demographic history of the studied groups of *Deronectes*. Bayesian Skyline plots for each species group of the analyses of the mitochondrial sequence, assuming a strict clock (see 'Material and Methods' for details). Thin lines, 95% confidence intervals; horizontal axis, time (MY); vertical axis, effective population size (NeT).

and northern Europe (including the British Islands) by *D. latus* (Figs. 5 and 6). One of the sequenced specimens of *D. toledoi* was, however, nested within *D. latus* (Fig. 6).

In the *D. platynotus* group the widespread *D. platynotus* originated in the Balkans, from where expanded eastwards towards the Carpathians and northwards to reach central Europe (Figs. 5 and 6).

The western clade of the *D. moestus* complex was reconstructed as having an origin between the Balearic islands (with *D. brannani*) and north Morocco, from were it expanded north to the rest of the Iberian peninsula and southern France. The eastern clade was reconstructed as having an origin between the Tyrrhenian islands and southern Italy, from were one lineage expanded to the north of the Iberian peninsula and north Italy up to Slovenia, and another to Sicily, southern Italy and the Balkan peninsula (Greece and Bulgaria). The islands of Corsica, Elba and Sicily had specimens of different origins within the eastern clade, but the sampled Sardinian haplotypes had a single origin, with a back colonisation to Corsica (Figs. 5 and 7).

Presence of *Wolbachia*

We did not detect *Wolbachia* in any of the eight tested specimens of the *D. platynotus* and *D. latus* groups, with the only exception of one specimen of *D. angelinii*, positive for the supergroup B of *Wolbachia* (Table S1). Within the western clade of the *D. moestus* group

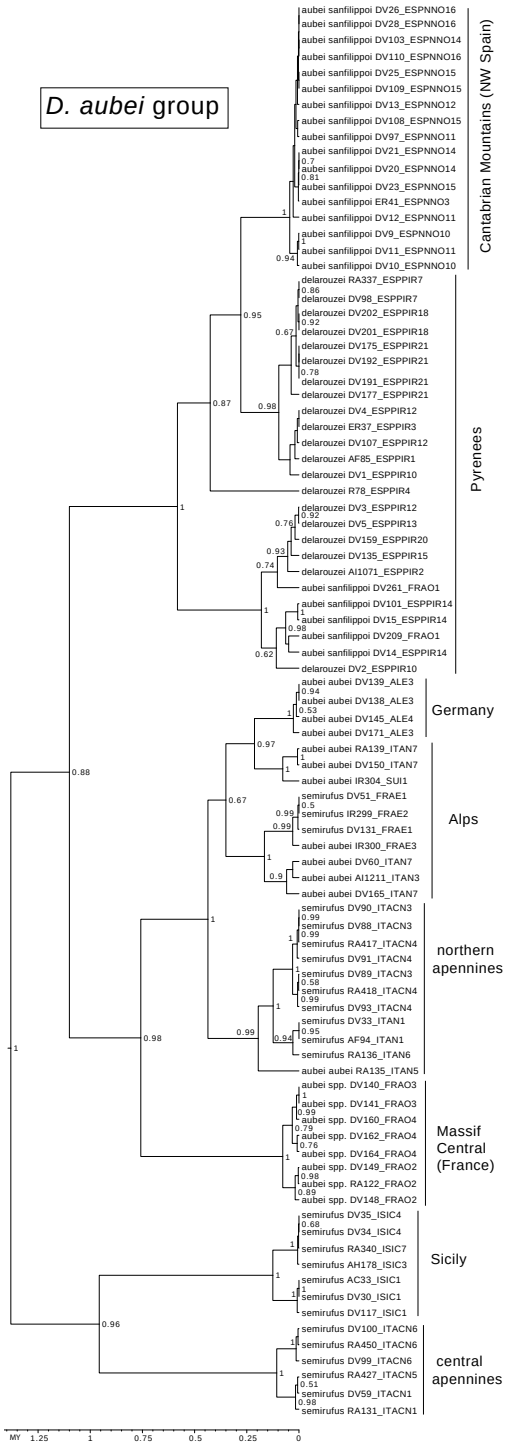

Figure 4 Ultrametric time calibrated tree of the *D. aubei* group. Ultrametric time calibrated tree obtained with BEAST with the combined nuclear and mitochondrial sequence of all sampled specimens of the *D. aubei* group, using the coordinates of each locality as a quantitative trait and the best population coalescent model for each group. Numbers on nodes, Bayesian posterior probabilities. See Fig. 5 for a graphic representation of the reconstructed geographical coordinates, and Table S1 for details on the specimens and localities.

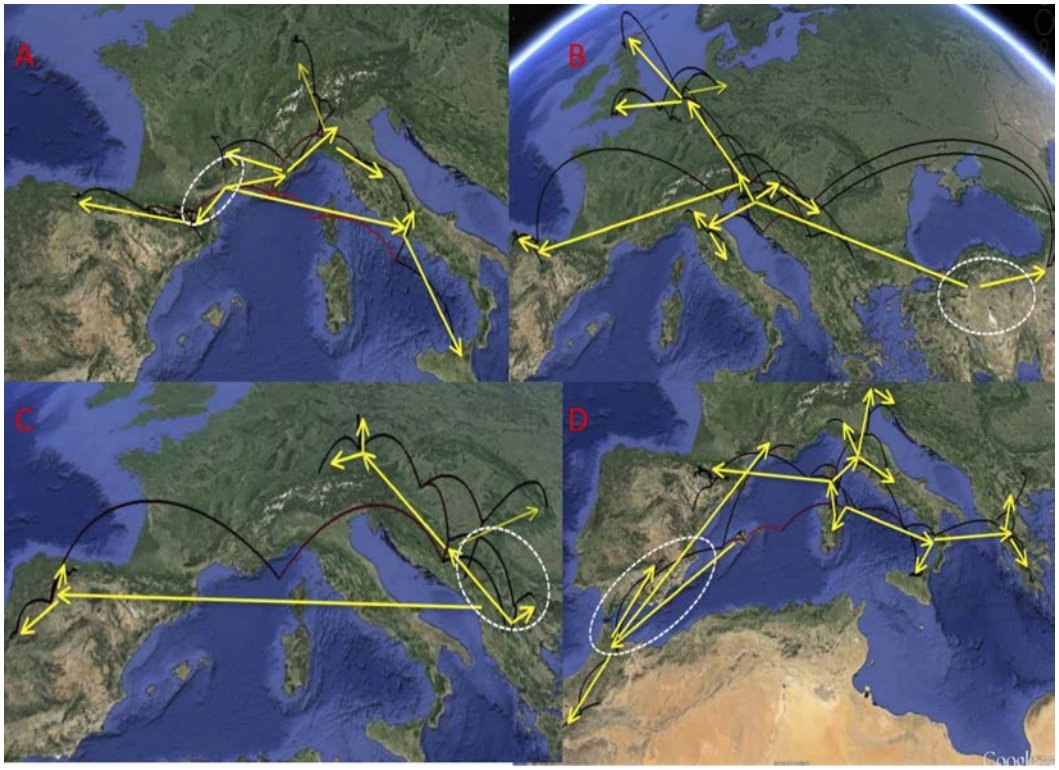

Figure 5 Phylogeographic reconstruction of the history of each lineage. Phylogeographic reconstruction through time in Google Earth (http://earth.google.com) of the history of each lineage, from the origin until the current distribution, based on the results represented in Figs. 4, 6 and 7. Parabolas represent the reconstructed displacements between nodes in the phylogeny (in red, basal nodes). Yellow arrows mark the general direction of the range expansions, and the white dashed line the hypothesized area of origin of each group. (A) *D. aubei* group; (B) *D. latus* group; (C) *D. platynotus* group; (D) *D. moestus* complex. Photo credit: Image Landsat, IBCAO and U.S. Geological Survey. Data SIO, NOAA, US Navy, NGA, GEBCO.

we did not identify *Wolbachia* in any of the six specimens tested from Mallorca, Morocco and southern Spain, but the two tested specimens of *D. moestus* from Southern France were positive for supergroup B. Of the 15 tested specimens of the eastern clade, three were positive for supergroup A in the Pyrenees and northern Italy and four for supergroup B in Sicily and Greece. The three tested specimens from the central Apenines and Elba were negative (Table S1).

Within the 25 tested specimens of the *D. aubei* group all were positive, with the only exception of the six specimens of *D. a. sanfilippoi* from the Cantabrian Mountains, which were negative. All the three tested specimens of *D. a. sanfilippoi* from the Pyrenees were, on the contrary, infected. All infected populations of the group were positive for supergroup A, with the exception of the two specimens from the French Massif Central and one isolated specimen of *D. semirufus* from the Abruzzo, positive for supergroup B (Table S1).

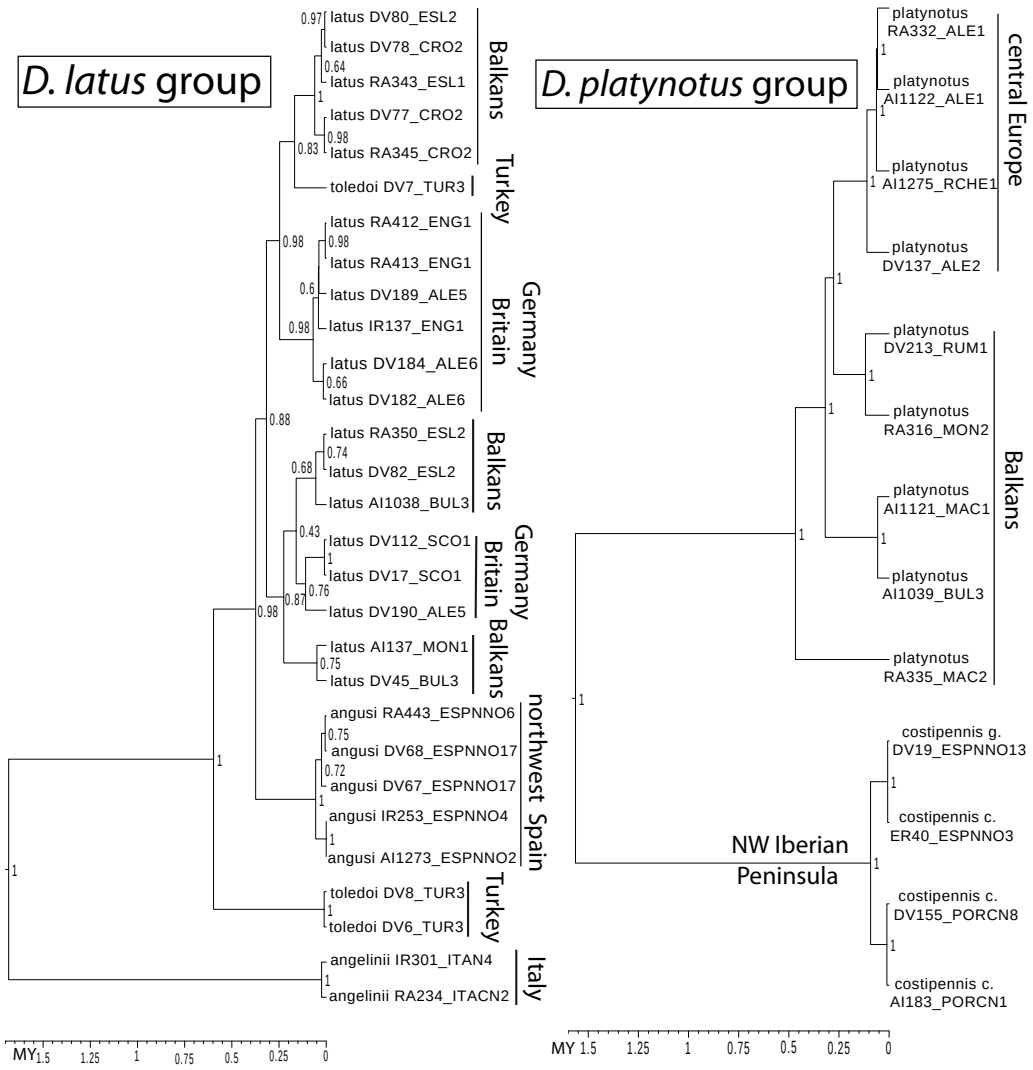

Figure 6 Ultrametric time calibrated trees of the *D. latus* and *D. platynotus* groups. Ultrametric time calibrated trees obtained with BEAST with the combined nuclear and mitochondrial sequence of all sampled specimens of (1) the *D. latus* group and (2) the *D. platynotus* group, using the coordinates of each locality as a quantitative trait and the best population coalescent model for each group. Numbers on nodes, Bayesian posterior probabilities. See Fig. 5 for a graphic representation of the reconstructed geographical coordinates, and Table S1 for details on the specimens and localities.

DISCUSSION

Recolonization process

The species of *Deronectes* with a widespread European distribution have all an origin in the southern Mediterranean peninsulas, in agreement with the common pattern of recolonisation after the glacial cycles (*Hewitt, 2000*). However, there are some fundamental differences with the standard models, as the distribution of the widespread species does not include the southern peninsulas, which are occupied by vicariant taxa of recent origin. Thus, *D. latus* is replaced in northern Anatolia by *D. toledoi*, in the Italian peninsula

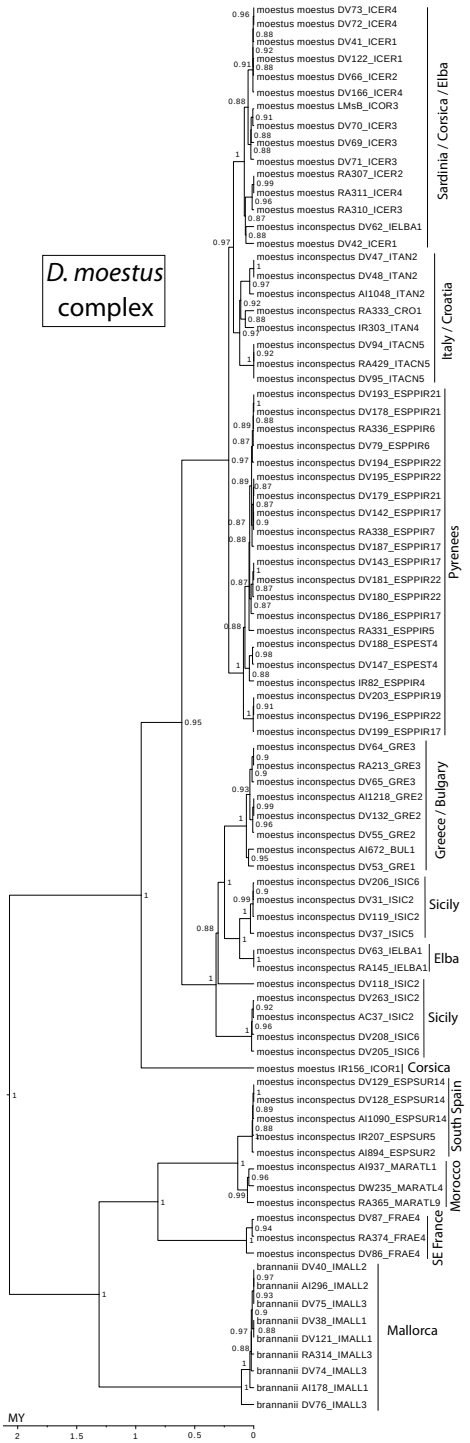

Figure 7 Ultrametric time calibrated tree of the *D. moestus* complex. Ultrametric time calibrated tree obtained with BEAST with the combined nuclear and mitochondrial sequence of all sampled specimens of the *D. moestus* complex, using the coordinates of each locality as a quantitative trait and the best population coalescent model for each group. Numbers on nodes, Bayesian posterior probabilities. See Fig. 5 for a graphic representation of the reconstructed geographical coordinates, and Table S1 for details on the specimens and localities.

by *D. angelini*, and in the Iberian Peninsula by *D. angusi* (Fig. 1). Within *D. aubei* the northern taxa (*D. aubei aubei* and the northern clade of *D. semirrufus*) are restricted to south Germany, the Alps and the Massif Central, being replaced in the north of the Iberian peninsula by *D. aubei sanfilippoi* and in central and south Italy and Sicily by the southern clade of *D. semirrufus*. The situation with *D. platynotus* and *D. moestus* is more complex, in the former due to the disjoint distribution, and in the later due to unrecognised ancient diversity. However, in the Balkans *D. platynotus* only reaches the northern mountain chains, in which populations present some morphological differences that may warrant a taxonomic recognition (H Fery, pers. comm., 2016). The southernmost populations of the species, in north Greece, are considered a distinct subspecies with a very restricted distribution, of which no specimens could be obtained for study (*D. platynotus mazzoldii*, *Fery & Brancucci, 1997*). There is additional evidence that the species currently restricted to Mediterranean peninsulas were never present in central and northern Europe, as there are no known records of Quaternary fossil remains of any of them (*Abellán et al., 2011*).

The existence of these two partly non overlapping species pools (Mediterranean species with ranges never extending to northern Europe, and northern species with limited southern distributions) is consistent with increasing evidence from molecular studies of the role of the Mediterranean peninsulas as a source of endemism (see e.g., *Bilton et al., 1998*; *Petit et al., 2003*; *Ribera & Vogler, 2004*). This suggests that after recolonisation during an ecologically optimal period, northern populations become isolated from their source areas. At the end of each Pleistocene Glacial cycle environmental conditions in the newly deglaciated areas in central and north Europe could have been optimal for running water organisms typical of mountain streams, such as *Deronectes*. The likely vast number of small streams from the thaw of the ice sheet, even in lowland areas, could have represented an ecological opportunity for these species, favouring range expansions to new empty areas without developed communities but with homogeneous favourable conditions (*Ribera et al., 2011*). When ecological conditions changed populations become increasingly isolated, resulting in the currently recognised taxa.

However, only some species in these lineages of poor dispersers expanded their ranges, while the rest remained confined to restricted areas in the southern Peninsulas. The species with widespread distributions do not seem to share any particular phylogenetic pattern. They are not the oldest species in their clades, which could have lead to the hypothesis that their wider ranges were due to a longer time to disperse (*Willis, 1926*; *Gaston, 2003*). On the contrary, they are in most cases nested within southern species or populations. Another possibility is that their physiological or ecological tolerances favoured their range expansion. There is some evidence that differences in the ecological tolerance of the species are related to their geographic range extent (*Addo-Bediako, Chown & Gaston, 2000*; *Gaston & Spicer, 2001*; *Gaston, 2009*). Thus, widespread species would have a higher ecological plasticity while species with restricted distributions may have a limited physiological tolerance (*West-Eberhard, 2003*). Among the many possible ecological or physiological factors, thermal tolerance have been frequently linked with distributional ranges (e.g., *Stillman, 2002*; *Somero, 2005*; *Verdú & Lobo, 2008*). Previous physiological studies with *Deronectes* showed than widespread, more northerly distributed species have broader thermal tolerance

than their restricted southern relatives (*Calosi, Bilton & Spicer, 2008*; *Calosi et al., 2010*). Thus, the most widespread species of the genus, *D. latus*, had also the greatest thermal window (*Calosi et al., 2010*). However, there is a poor adjustment of experimental data with ecological data derived from the localities they currently occupy (*Sánchez-Fernández et al., 2012*). With the available information (with no data on intraspecific variability) it is also not possible to assess if this wider thermal tolerance was previous to the range expansion, making it possible, or if it was developed after the range expansion, as a consequence of being exposed to a wider range of climatic conditions.

An alternative hypothesis is that the widespread species did not have any particular physiological or ecological character favouring their range expansion, but just took advantage of a privileged geographical position. They may have been the ones that happened to be at the edge of the newly deglaciated areas. According to our results, populations in mountain ranges at the northern edge of the southern peninsulas played a key role in the recolonization of glaciated areas. Thus, the *D. latus* group is the westernmost lineage within the eastern *Deronectes* clade, and the central European populations of *D. platynotus* appear nested within those of the northern Balkans. Within the Iberian Peninsula, with numerous species of *Deronectes* with a restricted distribution in most of its mountain systems (*Millán et al., 2014*; *García-Vázquez et al., 2016*), the *D. aubei* group is restricted to the northernmost ranges (Cantabrian mountains and the Pyrenean area, Fig. 1). As already noted, the situation within the *D. moestus* group is more complex, due to the deep divergences between some lineages. Under this hypothesis, historical more than intrinsic factors would determine which species become widespread and may be the origin of further diversification (*Ribera et al., 2011*). The evidence supporting the role of geographic position in facilitating range expansions relies on the assumption that southern species have maintained their geographic ranges through the last glacial cycles, so that it is possible to infer their past distribution from their current location. Although this assumption has been challenged (e.g., *Gaston, 2003*; *Losos & Glor, 2003*), it has been repeatedly shown that in poorly dispersing lineages range movements do not erase completely the geographic signal from the past (*Barraclough & Vogler, 2000*; *Abellán & Ribera, 2011*; *Ribera et al., 2011*).

Demographic history of the recolonization

The dynamics of the range expansions was not the same in all the studied groups, as shown with the reconstructions of the Bayesian skyline plots (Fig. 3). Thus, while in the *D. aubei* and *D. latus* group we estimated a fast expansion through western Europe (*D. aubei*) or the whole continent (*D. latus*), leading to the current continuous distributions, in the *D. platynotus* group there was a sharp decline in the effective population size, which may reflect their current discontinuous distribution, with isolated populations in the east (*D. platynotus*) and the west (*D. costipennis*). More difficult to interpret is the demographic history of *D. moestus*, with a sharp decline corresponding roughly to the last Glacial Maximum (LGM) and a subsequent expansion. In this complex, the late Pleistocene climatic cycles likely resulted in the isolation of the different clades recovered in the phylogeny, some of which experienced subsequent expansions when conditions improved after the LGM.

Wolbachia infections

We found a high prevalence of *Wolbachia* infection among *Deronectes*, with specimens infected in five of the eleven studied species (45%). This percentage is concordant with recent estimations of a 40% of *Wolbachia* prevalence among arthropods (*Zug & Hammerstein, 2012*) and 31% in a group of families of aquatic beetles, among them Dytiscidae (*Sontowski et al., 2015*), thus making it the most successful endosymbiont on earth. As expected, all infected specimens were for supergroups A and B, the most common in arthropods (*Werren, Baldo & Clark, 2008*).

The prevalence of *Wolbachia* was particularly high in the *D. aubei* group, in which all sequenced populations were infected with the only exception of the Cantabrian Mountains, the westernmost area of distribution of the group. It is interesting that this group had also a marked contrast between the results of the mitochondrial and nuclear phylogenetic reconstructions, although with the current data it is not possible to establish a clear link between the *Wolbachia* infection and this discordance. Previous results, with a limited sampling, reported two mitochondrial groups east and west of the Rhone river (*García-Vázquez et al., 2016*). This scenario was modified with the inclusion of additional samples from the margins of the distribution of the group, with the populations of *D. semirrufus* from Sicily and the central and southern Apennines joining the Iberian and Pyrenean clade (including *D. aubei sanfilippoi* and *D. delarouzei*), and the populations of *D. aubei* from the French Massif Central joining the Alpine and German Black Forest clade (including *D. semirrufus* and *D. aubei aubei*). Thus, the mitochondrial data separated the *D. aubei* group in two main lineages north and south of latitude 44°N. On the contrary, nuclear data largely recovered monophyletic species, defined according to their external morphology and the male aedeagus (*Fery & Brancucci, 1997*). In the mitochondrial tree, the two subspecies of *D. aubei* as defined with morphology were recovered in different clades, with the only exception of the populations of the French Massif Central and the Cevennes (west of the River Rhone), morphologically closer to the Pyrenean *D. aubei sanfilippoi* (*Fery & Brancucci, 1997*; H Fery, pers. comm., 2016) but grouped with *D. aubei aubei* from the Alps and south Germany. In the evolutionary history of the *D. aubei* group, there seem thus to be a first event leading to mitochondrial introgression and geographic isolation within *D. aubei* and *D. semirrufus*, separating in the former the recognised subspecies *D. aubei sanfilippoi* and *D. aubei aubei*, and in the later the populations from Sicily and central and Southern Italy and those from the Alps, which although not currently recognised as distinct taxa they have diagnostic morphological differences (D García-Vázquez & I. Ribera, 2016, unpublished observations and H Fery, pers. comm., 2016). Subsequent to this, there may have been a secondary event of introgression between the two subspecies of *D. aubei* in the populations of the Massif Central and the Cevennes. These occupy a geographically intermediate position between the western *D. aubei sanfilippoi* and the eastern *D. aubei aubei*, with the general morphology of the former but a mitochondrial genome related to the later.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- David García-Vázquez conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Ignacio Ribera conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

Sequences were submitted to the EMBL database and are accessible in GenBank with accession numbers LT601818–LT602609 (sequences of specimens of Deronectes) and LT602610–LT602633 (sequences of the Wolbachia endoparasites).

Data Availability

The following information was supplied regarding data availability:

The raw data consists of the sequences (which have been submitted to GenBank) and the geographical coordinates of the localities in which the species were found, which are provided in Table S1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.2514#supplemental-information.

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The origin of widespread species in a poor dispersing lineage (diving beetle genus *Deronectes*)

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ELECTRONIC SUPLEMENTAL INFORMATION

Table S1. List of the specimens included in the phylogeography, with specimen voucher, locality, collector, Genbank accession numbers and specimens tested for infection of *Wolbachia*. In grey, species in other groups of *Deronectes* used as outgroups. In bold, sequences newly obtained for this study.

The origin of widespread species in a generally poor dispersing lineage (diving beetle genus *Deronectes*)

García-Vázquez, D. & Ribera, I.

Appendix S1: Additional materials.

Table S1.List of the specimens included in the phylogeography, with specimen voucher, locality, collector, Genbank accession numbers and specimens tested for infection of Wolbachia. In grey, species in other groups of *Deronectes* used as outgroups. In bold, sequences newly obtained for this study.

								Accesion numbers						Wolbachia	Wolbachia
No	Species	Voucher	Country (Island)	Locality	Locality cod	Coordinates	Collector	**COI-5'**	**COI-3'**	**16SrRNA+tRNA-Leu+NAD1**	**H3**	**Wingless**	**Wsp (wolbachia)**	**Infected**	**Supergroup**
1	*D. angelinii*	IBE-RA234	Italy	Marche, Ascoli-Piceno province, Quintodecimo	ITACN2	ca. 42°45'48"N 13°23'13"E	M. Toledo	LN995087	LN995061	LN995162	LN995127	**LT602418**	**LT602610**	YES	B
2	*D. angelinii*	NHM-IR301	Italy	Lombardia, Pavia province, San Ponzo	ITAN4	ca. 44°50'23"N 9° 6'23"E	I. Ribera & A. Cieslak		**LT601818**	**LT602509**				-	
3	*D. angusi*	IBE-DV67	Spain	Galicia, Lugo province, Viveiro	ESPNNO17	ca. 43°37'17"N 7°38'11"W	C.Benneti	**LT602087**	**LT601819**	**LT602510**	**LT602335**	**LT602419**		NO	
4	*D. angusi*	IBE-DV68	Spain	Galicia, Lugo province, Viveiro	ESPNNO17	ca. 43°37'17"N 7°38'11"W	C.Benneti	**LT602088**	**LT601820**					-	
5	*D. angusi*	IBE-RA442	Spain	Galicia, Lugo province, Quintá	ESPNNO6	ca. 42°58'18"N 7°13'14"W	I. Ribera	**LT602089**	**LT601821**					-	
6	*D. angusi*	IBE-RA443	Spain	Galicia, Lugo province, Quintá	ESPNNO6	ca. 42°58'18"N 7°13'14"W	I. Ribera	LN995088	LN995063	LN995163	**LT602336**	**LT602420**		-	
7	*D. angusi*	MNHN-AI1273	Spain	Castilla y León, Burgos province, Pineda de la Sierra	ESPNNO2	ca. 42°13'40"N 3°18'15"W	D.T. Bilton	**LT602090**	**LT601822**		**LT602337**			-	
8	*D. angusi*	NHM-IR44	Spain	Galicia, Lugo province, Quintá	ESPNNO6	ca. 42°58'18"N 7°13'14"W	I. Ribera		AF309310	AF309253	EF670135			-	
9	*D. angusi*	NHM-IR253	Spain	Castilla y León, Burgos province, Pineda de la Sierra	ESPNNO4	ca. 42°13'40"N 3°18'15"W	I. Ribera		LN995062	AF309253				-	
10	*D. aubei aubei*	IBE-DV60	Italy	Lombardia, Brescia province, Val Palot	ITAN7	ca. 45°47'22"N 10°10'38"E	M. Toledo	**LT602091**	**LT601823**	**LT602511**	**LT602338**	**LT602421**		YES	undetermined
11	*D. aubei aubei*	IBE-DV61	Italy	Lombardia, Brescia province, Val Palot	ITAN7	ca. 45°47'22"N 10°10'38"E	M. Toledo	**LT602092**	**LT601824**					-	
12	*D. aubei aubei*	IBE-DV138	Germany	Baden Württemberg, Karlsruhe region, Eyachmühle	ALE3	48°48'30.8"N 8°33'27.1"E	I. Ribera & A. Cieslak	**LT602093**	**LT601825**	**LT602512**	**LT602339**	**LT602422**		YES	undetermined
13	*D. aubei aubei*	IBE-DV139	Germany	Baden Württemberg, Karlsruhe region, Eyachmühle	ALE3	48°48'30.8"N 8°33'27.1"E	I. Ribera & A. Cieslak	**LT602094**	**LT601826**	**LT602513**	**LT602340**			-	
14	*D. aubei aubei*	IBE-DV144	Germany	Baden Württemberg, Karlsruhe region, Buhlbach	ALE4	48°31'58.9"N 8°16'35.4"E	I. Ribera & A. Cieslak	**LT602095**	**LT601827**	**LT602514**	**LT602341**			-	
15	*D. aubei aubei*	IBE-DV145	Germany	Baden Württemberg, Karlsruhe region, Buhlbach	ALE4	48°31'58.9"N 8°16'35.4"E	I. Ribera & A. Cieslak	**LT602096**	**LT601828**	**LT602515**	**LT602342**	**LT602423**	**LT602611**	YES	A
16	*D. aubei aubei*	IBE-DV150	Italy	Lombardia, Brescia province, Val Palot	ITAN7	ca. 45°47'22"N 10°10'38"E	M. Toledo	**LT602097**	**LT601829**	**LT602516**	**LT602343**	**LT602424**		-	
17	*D. aubei aubei*	IBE-DV165	Italy	Lombardia, Brescia province, Val Palot	ITAN7	ca. 45°47'22"N 10°10'38"E	M. Toledo	**LT602098**						-	
18	*D. aubei aubei*	IBE-DV170	Germany	Baden Württemberg, Karlsruhe region, Eyachmühle	ALE3	48°48'30.8"N 8°33'27.1"E	I. Ribera & A. Cieslak	**LT602099**	**LT601830**					-	
19	*D. aubei aubei*	IBE-DV171	Germany	Baden Württemberg, Karlsruhe region, Eyachmühle	ALE3	48°48'30.8"N 8°33'27.1"E	I. Ribera & A. Cieslak	**LT602100**	**LT601831**					-	
20	*D. aubei aubei*	IBE-DV174	Germany	Baden Württemberg, Karlsruhe region, Buhlbach	ALE4	48°31'58.9"N 8°16'35.4"E	I. Ribera & A. Cieslak	**LT602101**	**LT601832**					-	
21	*D. aubei aubei*	IBE-DV185	Germany	Baden Württemberg, Karlsruhe region, Buhlbach	ALE4	48°31'58.9"N 8°16'35.4"E	I. Ribera & A. Cieslak	**LT602102**						-	
22	*D. aubei aubei*	IBE-RA135	Italy	Emilia-Romagna, Modena province, Fanano	ITAN5	ca. 44°10'29"N 10°47'56"E	M. Toledo	LN995089	LN995064	LN995164	LN995128	**LT602425**	**LT602612**	YES	A
23	*D. aubei aubei*	IBE-RA139	Italy	Lombardia, Brescia province, Val Palot	ITAN7	ca. 45°47'22"N 10°10'38"E	M. Toledo	**LT602103**	**LT601833**					-	
24	*D. aubei aubei*	MNCN-AI299	Italy	Lombardia, Brescia province, Val Trompia	ITAN3	45°49'52"N 10°23'58"E	I. Ribera & A. Cieslak	**LT602104**						-	
25	*D. aubei aubei*	MNCN-AI1211	Italy	Lombardia, Brescia province, Val Trompia	ITAN3	45°49'52"N 10°23'58"E	I. Ribera & A. Cieslak	**LT602105**	**LT601834**	**LT602517**	**LT602344**	**LT602426**		-	
26	*D. aubei aubei*	NHM-IR300	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Moulinet	FRAE3	ca. 43°58'34"N 7°24'44" E	I. Ribera & A. Cieslak		AF309326	AF309269	EF670136			-	
27	*D. aubei aubei*	NHM-IR304	Switzerland	Canton of Ticino, Leventina district, Giornico	SUI1	ca. 46°24'20"N 8°52'16"E	I. Ribera & A. Cieslak		**LT601835**	**LT602518**				-	
28	*D. aubei sanfilippoi*	IBE-DV9	Spain	Cantabria, Urdón river	ESPNNO10	43°16'0.76"N 4°37'59.31"W	A. Millán et col.	**LT602106**	**LT601836**	**LT602519**	**LT602345**	**LT602427**		NO	
29	*D. aubei sanfilippoi*	IBE-DV10	Spain	Cantabria, Urdón river	ESPNNO10	43°16'0.76"N 4°37'59.31"W	A. Millán et col.	**LT602107**	**LT601837**					-	
30	*D. aubei sanfilippoi*	IBE-DV11	Spain	Cantabria, Canal del Valle	ESPNNO11	ca. 43°12'54"N 4°39'15"W	A. Millán et col.	**LT602108**	**LT601838**	**LT602520**	**LT602346**	**LT602428**		NO	
31	*D. aubei sanfilippoi*	IBE-DV12	Spain	Cantabria, Canal del Valle	ESPNNO11	ca. 43°12'54"N 4°39'15"W	A. Millán et col.	**LT602109**	**LT601839**					-	
32	*D. aubei sanfilippoi*	IBE-DV13	Spain	Castilla y León, León province, Picos de Europa	ESPNNO12	ca. 43°6'34"N 4°56'40"W	A. Millán et col.	**LT602110**	**LT601840**	**LT602521**	**LT602347**	**LT602429**		NO	
33	*D. aubei sanfilippoi*	IBE-DV14	Spain	Cataluña, Lleida province, San Juan de Torán	ESPPIR14	42°49'30.09"N 0°46'40.19"E	I. Ribera & A. Cieslak	**LT602111**	**LT601841**	**LT602522**	**LT602348**	**LT602430**		YES	undetermined
34	*D. aubei sanfilippoi*	IBE-DV15	Spain	Cataluña, Lleida province, San Juan de Torán	ESPPIR14	42°49'30.09"N 0°46'40.19"E	I. Ribera & A. Cieslak	**LT602112**	**LT601842**					-	
35	*D. aubei sanfilippoi*	IBE-DV16	Spain	Cataluña, Lleida province, San Juan de Torán	ESPPIR14	42°49'30.09"N 0°46'40.19"E	I. Ribera & A. Cieslak	**LT602113**	**LT601843**					-	
36	*D. aubei sanfilippoi*	IBE-DV20	Spain	Cantabria, Vada (Quiviesa river)	ESPNNO14	43°5'22"N 4°40'30"W	L.F. Valladares	**LT602114**	**LT601844**	**LT602523**	**LT602349**	**LT602431**		NO	
37	*D. aubei sanfilippoi*	IBE-DV21	Spain	Cantabria, Vada (Quiviesa river)	ESPNNO14	43°5'22"N 4°40'30"W	L.F. Valladares	**LT602115**	**LT601845**					-	
38	*D. aubei sanfilippoi*	IBE-DV22	Spain	Cantabria, Vada (Quiviesa river)	ESPNNO14	43°5'22"N 4°40'30"W	L.F. Valladares	**LT602116**	**LT601846**					-	
39	*D. aubei sanfilippoi*	IBE-DV23	Spain	Cantabria, Bárago	ESPNNO15	ca. 43° 4'23"N 4°37'06"W	L.F. Valladares	LN995090	LN995065	LN995165	LN995129	**LT602432**		NO	
40	*D. aubei sanfilippoi*	IBE-DV24	Spain	Cantabria, Bárago	ESPNNO15	ca. 43° 4'23"N 4°37'06"W	L.F. Valladares	**LT602117**	**LT601847**					-	
41	*D. aubei sanfilippoi*	IBE-DV25	Spain	Cantabria, Bárago	ESPNNO15	ca. 43° 4'23"N 4°37'06"W	L.F. Valladares	**LT602118**	**LT601848**					-	
42	*D. aubei sanfilippoi*	IBE-DV26	Spain	Cantabria, Vada (Vejo river)	ESPNNO16	43°5'24"N 4°40'33"W	L.F. Valladares	**LT602119**	**LT601849**	**LT602524**				-	
43	*D. aubei sanfilippoi*	IBE-DV27	Spain	Cantabria, Vada (Vejo river)	ESPNNO16	43°5'24"N 4°40'33"W	L.F. Valladares	**LT602120**	**LT601850**					-	
44	*D. aubei sanfilippoi*	IBE-DV28	Spain	Cantabria, Vada (Vejo river)	ESPNNO16	43°5'24"N 4°40'33"W	L.F. Valladares	**LT602121**	**LT601851**					-	
45	*D. aubei sanfilippoi*	IBE-DV97	Spain	Cantabria, Canal del Valle	ESPNNO11	ca. 43°12'54"N 4°39'15"W	A. Millán et col.	**LT602122**	**LT601852**					-	
46	*D. aubei sanfilippoi*	IBE-DV101	Spain	Cataluña, Lleida province, San Juan de Torán	ESPPIR14	42°49'30.09"N 0°46'40.19"E	I. Ribera & A. Cieslak	**LT602123**	**LT601853**	**LT602525**	**LT602350**	**LT602433**		-	
47	*D. aubei sanfilippoi*	IBE-DV102	Spain	Cataluña, Lleida province, San Juan de Torán	ESPPIR14	42°49'30.09"N 0°46'40.19"E	I. Ribera & A. Cieslak	**LT602124**	**LT601854**					-	
48	*D. aubei sanfilippoi*	IBE-DV103	Spain	Cantabria, Vada (Quiviesa river)	ESPNNO14	43°5'22"N 4°40'30"W	L.F. Valladares	**LT602125**	**LT601855**					-	
49	*D. aubei sanfilippoi*	IBE-DV104	Spain	Cantabria, Vada (Quiviesa river)	ESPNNO14	43°5'22"N 4°40'30"W	L.F. Valladares	**LT602126**	**LT601856**					-	
50	*D. aubei sanfilippoi*	IBE-DV108	Spain	Cantabria, Bárago	ESPNNO15	ca. 43° 4'23"N 4°37'06"W	L.F. Valladares	**LT602127**	**LT601857**					-	
51	*D. aubei sanfilippoi*	IBE-DV109	Spain	Cantabria, Bárago	ESPNNO15	ca. 43° 4'23"N 4°37'06"W	L.F. Valladares	**LT602128**	**LT601858**					-	
52	*D. aubei sanfilippoi*	IBE-DV110	Spain	Cantabria, Vada (Vejo river)	ESPNNO16	43°5'24"N 4°40'33"W	L.F. Valladares	**LT602129**	**LT601859**	**LT602526**	**LT602351**	**LT602434**		NO	
53	*D. aubei sanfilippoi*	IBE-DV111	Spain	Cantabria, Vada (Vejo river)	ESPNNO16	43°5'24"N 4°40'33"W	L.F. Valladares	**LT602130**	**LT601860**					-	
54	*D. aubei sanfilippoi*	IBE-DV209	France	Languedoc-Rousillon, Pyrenees Orientales department, Prats de Mollo	FRAO1	ca. 42°26'36"N 2°27'41"E	H. Fery	**LT602131**	**LT601861**	**LT602527**	**LT602352**	**LT602435**	**LT602613**	YES	A
55	*D. aubei sanfilippoi*	IBE-DV210	France	Languedoc-Rousillon, Pyrenees Orientales department, Prats de Mollo	FRAO1	ca. 42°26'36"N 2°27'41"E	H. Fery	**LT602132**	**LT601862**					-	
56	*D. aubei sanfilippoi*	IBE-DV211	France	Languedoc-Rousillon, Pyrenees Orientales department, Prats de Mollo	FRAO1	ca. 42°26'36"N 2°27'41"E	H. Fery	**LT602133**	**LT601863**					-	
57	*D. aubei sanfilippoi*	IBE-DV212	France	Languedoc-Rousillon, Pyrenees Orientales department, Prats de Mollo	FRAO1	ca. 42°26'36"N 2°27'41"E	H. Fery	**LT602134**	**LT601864**					-	
58	*D. aubei sanfilippoi*	IBE-DV261	France	Languedoc-Rousillon, Pyrenees Orientales department, Prats de Mollo	FRAO1	ca. 42°26'36"N 2°27'41"E	H. Fery	**LT602135**	**LT601865**					-	
59	*D. aubei sanfilippoi*	NHM-ER38	France	Languedoc-Rousillon, Pyrenees Orientales department, Prats de Mollo	FRAO1	ca. 42°26'36"N 2°27'41"E	H. Fery		**LT601866**	**LT602528**				-	
60	*D. aubei sanfilippoi*	NHM-ER41	Spain	Castilla y León, León province, Puerto de San Glorio	ESPNNO3	ca. 43°3'57"N 4°45'31"W	D.T. Bilton		**LT601867**	**LT602529**				-	
61	*D. aubei spp.*	IBE-DV57	France	Languedoc-Rousillon, Gard province, Le Vigan	FRAO2	44º02’14.7”N 3º34’56.8”E	I. Ribera & A. Cieslak	**LT602136**	**LT601868**					-	
62	*D. aubei spp.*	IBE-DV58	France	Languedoc-Rousillon, Gard province, Le Vigan	FRAO2	44º02’14.7”N 3º34’56.8”E	I. Ribera & A. Cieslak	**LT602137**	**LT601869**					-	
63	*D. aubei spp.*	IBE-DV140	France	Auvergne, Cantal department, Vedrines-St. Loup	FRAO3	45°4'0.5"N 3°18'24.6"E	I. Ribera & A. Cieslak	**LT602138**	**LT601870**	**LT602530**	**LT602353**			-	
64	*D. aubei spp.*	IBE-DV141	France	Auvergne, Cantal department, Vedrines-St. Loup	FRAO3	45°4'0.5"N 3°18'24.6"E	I. Ribera & A. Cieslak	**LT602139**	**LT601871**	**LT602531**	**LT602354**	**LT602436**	**LT602614**	YES	B

65	*D. aubei spp.*	IBE-DV148	France	Languedoc-Rousillon, Gard province, Le Vigan	FRAO2	44º02’14.7”N 3º34’56.8”E	I. Ribera & A. Cieslak	**LT602140**	**LT601872**					-	
66	*D. aubei spp.*	IBE-DV149	France	Languedoc-Rousillon, Gard province, Le Vigan	FRAO2	44º02’14.7”N 3º34’56.8”E	I. Ribera & A. Cieslak	**LT602141**	**LT601873**	**LT602532**	**LT602355**	**LT602437**		-	
67	*D. aubei spp.*	IBE-DV160	France	Auvergne, Cantal department, Laveissière	FRAO4	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak	**LT602142**	**LT601874**	**LT602533**	**LT602356**	**LT602438**	**LT602615**	YES	B
68	*D. aubei spp.*	IBE-DV161	France	Auvergne, Cantal department, Laveissière	FRAO4	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak	**LT602143**	**LT601875**					-	
69	*D. aubei spp.*	IBE-DV162	France	Auvergne, Cantal department, Laveissière	FRAO4	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak	**LT602144**	**LT601876**					-	
70	*D. aubei spp.*	IBE-DV163	France	Auvergne, Cantal department, Laveissière	FRAO4	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak	**LT602145**	**LT601877**					-	
71	*D. aubei spp.*	IBE-DV164	France	Auvergne, Cantal department, Laveissière	FRAO4	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak	**LT602146**	**LT601878**					-	
72	*D. aubei spp.*	IBE-DV172	France	Auvergne, Cantal department, Vedrines-St. Loup	FRAO3	45°4'0.5"N 3°18'24.6"E	I. Ribera & A. Cieslak	**LT602147**	**LT601879**					-	
73	*D. aubei spp.*	IBE-DV173	France	Auvergne, Cantal department, Vedrines-St. Loup	FRAO3	45°4'0.5"N 3°18'24.6"E	I. Ribera & A. Cieslak	**LT602148**	**LT601880**					-	
74	*D. aubei spp.*	IBE-RA122	France	Languedoc-Rousillon, Gard province, Le Vigan	FRAO2	44º02’14.7”N 3º34’56.8”E	I. Ribera & A. Cieslak	**LT602149**	**LT601881**	**LT602534**	**LT602357**	**LT602439**		YES	undetermined
75	*D. brannanii*	IBE-DV38	Spain (Mallorca)	Ternelles	IMALL1	39º53’37.2”N 3º00’14.9”E	I. Ribera & A. Cieslak	**LT602150**	**LT601882**					-	
76	*D. brannanii*	IBE-DV39	Spain (Mallorca)	Ternelles	IMALL1	39º53’37.2”N 3º00’14.9”E	I. Ribera & A. Cieslak	**LT602151**	**LT601883**					-	
77	*D. brannanii*	IBE-DV40	Spain (Mallorca)	Sóller	IMALL2	39º45’45.7”N 2º42’39.3”E	I. Ribera & A. Cieslak	**LT602152**	**LT601884**	**LT602535**	**LT602358**	**LT602440**		NO	
78	*D. brannanii*	IBE-DV74	Spain (Mallorca)	Sóller	IMALL3	ca. 39°46'11"N 2°43'60"E	G. Wewalka	**LT602153**	**LT601885**	**LT602536**	**LT602359**	**LT602441**		-	
79	*D. brannanii*	IBE-DV75	Spain (Mallorca)	Sóller	IMALL3	ca. 39°46'11"N 2°43'60"E	G. Wewalka	**LT602154**	**LT601886**					-	
80	*D. brannanii*	IBE-DV76	Spain (Mallorca)	Sóller	IMALL3	ca. 39°46'11"N 2°43'60"E	G. Wewalka	**LT602155**	**LT601887**					-	
81	*D. brannanii*	IBE-DV120	Spain (Mallorca)	Ternelles	IMALL1	39º53’37.2”N 3º00’14.9”E	I. Ribera & A. Cieslak	**LT602156**	**LT601888**					-	
82	*D. brannanii*	IBE-DV121	Spain (Mallorca)	Ternelles	IMALL1	39º53’37.2”N 3º00’14.9”E	I. Ribera & A. Cieslak	**LT602157**	**LT601889**					-	
83	*D. brannanii*	IBE-RA313	Spain (Mallorca)	Sóller	IMALL3	ca. 39°46'11"N 2°43'60"E	G. Wewalka	**LT602158**	**LT601890**					-	
84	*D. brannanii*	IBE-RA314	Spain (Mallorca)	Sóller	IMALL3	ca. 39°46'11"N 2°43'60"E	G. Wewalka	**LT602159**	**LT601891**					-	
85	*D. brannanii*	MNCN-AI178	Spain (Mallorca)	Ternelles	IMALL1	39º53’37.2”N 3º00’14.9”E	I. Ribera & A. Cieslak	LN995094	HE610180	HF931404	LN995133	**LT602442**		NO	
86	*D. brannanii*	MNCN-AI296	Spain (Mallorca)	Sóller	IMALL2	39º45’45.7”N 2º42’39.3”E	I. Ribera & A. Cieslak	**LT602160**	**LT601892**					-	
87	*D. costipennis costipennis*	IBE-DV155	Portugal	Guarda district, Gouveia	PORCN8	40°29'49"N 7°35'56"W	J. Fresneda	**LT602161**	**LT601893**	**LT602537**	**LT602360**	**LT602443**		-	
88	*D. costipennis costipennis*	IBE-DV156	Portugal	Guarda district, Gouveia	PORCN8	40°29'49"N 7°35'56"W	J. Fresneda	**LT602162**	**LT601894**					-	
89	*D. costipennis costipennis*	IBE-DV157	Portugal	Guarda district, Gouveia	PORCN8	40°29'49"N 7°35'56"W	J. Fresneda	**LT602163**	**LT601895**					-	
90	*D. costipennis costipennis*	IBE-DV158	Portugal	Guarda district, Gouveia	PORCN8	40°29'49"N 7°35'56"W	J. Fresneda	**LT602164**	**LT601896**					-	
91	*D. costipennis costipennis*	IBE-DV204	Portugal	Guarda district, Gouveia	PORCN8	40°29'49"N 7°35'56"W	J. Fresneda	**LT602165**	**LT601897**					-	
92	*D. costipennis costipennis*	MNCN-AI183	Portugal	Guarda district, Manteigas (Serra da Estrela)	PORCN1	ca. 40º19’57”N 7º37’03”W	I. Ribera	LN995095	HE610181	LN995169	LN995134	**LT602444**		-	
93	*D. costipennis gignouxi*	IBE-DV19	Spain	Castilla y León, León province, Valverdín	ESPNNO13	ca. 42°56'53"N 5°32'10"W	L.F. Valladares	LN995096	LN995068	LN995170	LN995135	**LT602445**		NO	
94	*D. costipennis gignouxi*	NHM-ER40	Spain	Castilla y León, León province, Puerto de San Glorio	ESPNNO3	ca. 43°3'57"N 4°45'31"W	D.T. Bilton		AF309324	AY250951				-	
95	*D. delarouzei*	IBE-AF85	Spain	Aragón, Huesca province, Valle de Plan	ESPPIR1	ca. 42°34'39"N 0°20'7"E	I. Esteban		**LT601898**					-	
96	*D. delarouzei*	IBE-DV1	Spain	Cataluña, Barcelona province, Bagà	ESPPIR10	42º16’05.3”N 1º48”46.1”E	I. Ribera, P. Aguilera & C. Hernando	**LT602166**	**LT601899**	**LT602538**	**LT602361**			-	
97	*D. delarouzei*	IBE-DV2	Spain	Cataluña, Barcelona province, Bagà	ESPPIR10	42º16’05.3”N 1º48”46.1”E	I. Ribera, P. Aguilera & C. Hernando	**LT602167**	**LT601900**					-	
98	*D. delarouzei*	IBE-DV3	Spain	Cataluña, Lleida province, Llesp	ESPPIR12	42º27’24.5”N 0º44’57”E	I. Ribera, A. Cieslak & J. Fresneda	**LT602168**	**LT601901**	**LT602539**				-	
99	*D. delarouzei*	IBE-DV4	Spain	Cataluña, Lleida province, Llesp	ESPPIR12	42º27’24.5”N 0º44’57”E	I. Ribera, A. Cieslak & J. Fresneda	**LT602169**	**LT601902**					-	
100	*D. delarouzei*	IBE-DV5	Spain	Aragón, Huesca province, Bonansa	ESPPIR13	42º25’26.3”N 0º41’17.6”E	I. Ribera & A. Cieslak	**LT602170**	**LT601903**	**LT602540**	**LT602362**			-	
101	*D. delarouzei*	IBE-DV98	Spain	Aragón, Huesca province, Aragues del Puerto	ESPPIR7	42º45'16.93''N 0º37'59.65''W	I. Esteban	**LT602171**	**LT601904**	**LT602541**	**LT602363**	**LT602446**	**LT602616**	YES	A
102	*D. delarouzei*	IBE-DV105	Spain	Cataluña, Lleida province, Llesp	ESPPIR12	42º27’24.5”N 0º44’57”E	I. Ribera, A. Cieslak & J. Fresneda	**LT602172**	**LT601905**					-	
103	*D. delarouzei*	IBE-DV106	Spain	Cataluña, Lleida province, Llesp	ESPPIR12	42º27’24.5”N 0º44’57”E	I. Ribera, A. Cieslak & J. Fresneda	**LT602173**	**LT601906**					-	
104	*D. delarouzei*	IBE-DV107	Spain	Cataluña, Lleida province, Llesp	ESPPIR12	42º27’24.5”N 0º44’57”E	I. Ribera, A. Cieslak & J. Fresneda	**LT602174**	**LT601907**	**LT602542**	**LT602364**	**LT602447**	**LT602617**	YES	A
105	*D. delarouzei*	IBE-DV135	Spain	Cataluña, Girona province, Collada de Tosas	ESPPIR15	42º20’07.8”N 2º04’22.8E	I. Ribera & A. Cieslak	**LT602175**	**LT601908**	**LT602543**	**LT602365**	**LT602448**		-	
106	*D. delarouzei*	IBE-DV159	Spain	Cataluña, Lleida province, Rubió	ESPPIR20	42°22'42"N 1°13'27"E	I. Ribera & A. Cieslak	**LT602176**	**LT601909**	**LT602544**	**LT602366**	**LT602449**	**LT602618**	YES	A
107	*D. delarouzei*	IBE-DV175	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak		**LT601910**	**LT602545**	**LT602367**	**LT602450**		-	
108	*D. delarouzei*	IBE-DV176	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak	**LT602177**	**LT601911**					-	
109	*D. delarouzei*	IBE-DV177	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak	**LT602178**	**LT601912**					-	
110	*D. delarouzei*	IBE-DV191	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak	**LT602179**	**LT601913**					-	
111	*D. delarouzei*	IBE-DV192	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak	**LT602180**	**LT601914**					-	
112	*D. delarouzei*	IBE-DV201	Spain	Aragón, Huesca province, Astún	ESPPIR18	ca. 42°48'27"N 0°30'47"W	I. Esteban	**LT602181**	**LT601915**	**LT602546**	**LT602368**	**LT602451**		-	
113	*D. delarouzei*	IBE-DV202	Spain	Aragón, Huesca province, Astún	ESPPIR18	ca. 42°48'27"N 0°30'47"W	I. Esteban	**LT602182**	**LT601916**					-	
114	*D. delarouzei*	IBE-RA337	Spain	Aragón, Huesca province, Aragues del Puerto	ESPPIR7	42º45'16.93''N 0º37'59.65''W	I. Esteban	LN995097	LN995069	LN995171	LN995136	**LT602452**		-	
115	*D. delarouzei*	MNCN-AI1071	Spain	Cataluña, Barcelona province, Montseny	ESPPIR2	41º45’23.5”N 2º24’49.8”E	I. Ribera, P. Aguilera & C. Hernando	**LT602183**	**LT601917**	**LT602547**	**LT602369**	**LT602453**	**LT602619**	YES	A
116	*D. delarouzei*	NHM-ER37	Spain	Cataluña, Lleida province, Llesp	ESPPIR3	ca. 42º27’24”N 0º44’57”E	H. Fery		**LT601918**	**LT602548**				-	
117	*D. delarouzei*	NHM-IR78	Spain	Cataluña, Barcelona province, Saldes	ESPPIR4	ca. 42°13'37"N 1°44'32"E	P. Aguilera		**LT601919**	**LT602549**				-	
118	*D. latus*	IBE-DV17	Scotland	Isle of Skye, Broadford river	SCO1	ca. 57°13'57"N 5°56'22"W	G.N. Foster	**LT602184**	**LT601920**	**LT602550**				-	
119	*D. latus*	IBE-DV18	Scotland	Isle of Skye, Broadford river	SCO1	ca. 57°13'57"N 5°56'22"W	G.N. Foster	**LT602185**	**LT601921**					-	
120	*D. latus*	IBE-DV45	Bulgaria	Kyustendil province, Rila	BUL3	ca. 42°7'55"N 23°8'40"E	D.T. Bilton	**LT602186**	**LT601922**	**LT602551**	**LT602370**	**LT602454**		-	
121	*D. latus*	IBE-DV46	Bulgaria	Kyustendil province, Rila	BUL3	ca. 42°7'55"N 23°8'40"E	D.T. Bilton	**LT602187**	**LT601923**					-	
122	*D. latus*	IBE-DV77	Croatia	Istria county, Momjan	CRO2	45º26’30.8”N 13º42’37.8”E	I. Ribera & A. Cieslak	**LT602188**	**LT601924**	**LT602552**	**LT602371**	**LT602455**		-	
123	*D. latus*	IBE-DV78	Croatia	Istria county, Momjan	CRO2	45º26’30.8”N 13º42’37.8”E	I. Ribera & A. Cieslak	**LT602189**	**LT601925**					-	
124	*D. latus*	IBE-DV80	Slovenia	Carniola, Cerkniščica	ESL2	45º45’57.5”N 14º21’40.0”E	I. Ribera & A. Cieslak	**LT602190**	**LT601926**	**LT602553**	**LT602372**	**LT602456**		NO	
125	*D. latus*	IBE-DV81	Slovenia	Carniola, Cerkniščica	ESL2	45º45’57.5”N 14º21’40.0”E	I. Ribera & A. Cieslak	**LT602191**	**LT601927**					-	
126	*D. latus*	IBE-DV82	Slovenia	Carniola, Cerkniščica	ESL2	45º45’57.5”N 14º21’40.0”E	I. Ribera & A. Cieslak	**LT602192**	**LT601928**					-	
127	*D. latus*	IBE-DV112	Scotland	Isle of Skye, Broadford river	SCO1	ca. 57°13'57"N 5°56'22"W	G.N. Foster	**LT602193**	**LT601929**	**LT602554**	**LT602373**	**LT602457**		NO	
128	*D. latus*	IBE-DV113	Scotland	Isle of Skye, Broadford river	SCO1	ca. 57°13'57"N 5°56'22"W	G.N. Foster	**LT602194**	**LT601930**					-	
129	*D. latus*	IBE-DV168	Croatia	Istria county, Momjan	CRO2	45º26’30.8”N 13º42’37.8”E	I. Ribera & A. Cieslak	**LT602195**	**LT601931**					-	
130	*D. latus*	IBE-DV169	Croatia	Istria county, Momjan	CRO2	45º26’30.8”N 13º42’37.8”E	I. Ribera & A. Cieslak		**LT601932**					-	
131	*D. latus*	IBE-DV182	Germany	Rheinland-Pfalz, river Flaunbach	ALE6	50°8'59.5"N 7°16'35.0"E	I. Ribera & A. Cieslak	**LT602196**	**LT601933**	**LT602555**	**LT602374**	**LT602458**		-	
132	*D. latus*	IBE-DV183	Germany	Rheinland-Pfalz, river Flaunbach	ALE6	50°8'59.5"N 7°16'35.0"E	I. Ribera & A. Cieslak	**LT602197**	**LT601934**					-	
133	*D. latus*	IBE-DV184	Germany	Rheinland-Pfalz, river Flaunbach	ALE6	50°8'59.5"N 7°16'35.0"E	I. Ribera & A. Cieslak		**LT601935**					-	
134	*D. latus*	IBE-DV189	Germany	Niedersachsen, Röhrsen	ALE5	52°48'26.1"N 10°37'57.9"E	I. Ribera & A. Cieslak	**LT602198**	**LT601936**	**LT602556**	**LT602375**	**LT602459**		-	
135	*D. latus*	IBE-DV190	Germany	Niedersachsen, Röhrsen	ALE5	52°48'26.1"N 10°37'57.9"E	I. Ribera & A. Cieslak	**LT602199**	**LT601937**					-	

136	*D. latus*	IBE-DV197	Germany	Rheinland-Pfalz, river Flaunbach	ALE6	50°8'59.5"N 7°16'35.0"E	I. Ribera & A. Cieslak	**LT602200**	**LT601938**					-	
137	*D. latus*	IBE-DV198	Germany	Rheinland-Pfalz, river Flaunbach	ALE6	50°8'59.5"N 7°16'35.0"E	I. Ribera & A. Cieslak	**LT602201**	**LT601939**					-	
138	*D. latus*	IBE-DV200	Germany	Niedersachsen, Röhrsen	ALE5	52°48'26.1"N 10°37'57.9"E	I. Ribera & A. Cieslak	**LT602202**	**LT601940**					-	
139	*D. latus*	IBE-RA343	Slovenia	Slovene Istria, Dernarnik	ESL1	45º29’13.8”N 13º50’01.7”E	I. Ribera, C. Hernando & A. Cieslak	LN995106	LN995074	LN995178	LN995144	**LT602460**		-	
140	*D. latus*	IBE-RA345	Croatia	Istria county, Momjan	CRO2	45º26’30.8”N 13º42’37.8”E	I. Ribera & A. Cieslak	**LT602203**	**LT601941**					-	
141	*D. latus*	IBE-RA350	Slovenia	Carniola, Cerkniščica	ESL2	45º45’57.5”N 14º21’40.0”E	I. Ribera & A. Cieslak	**LT602204**	**LT601942**					-	
142	*D. latus*	IBE-RA412	England	South East England, Hampshire county, New Forest	ENG1	ca. 50°50'24"N 1°37'20"W	I. Ribera	LN995107	LN995075	LN995179	LN995145	**LT602461**		-	
143	*D. latus*	IBE-RA413	England	South East England, Hampshire county, New Forest	ENG1	ca. 50°50'24"N 1°37'20"W	I. Ribera	**LT602205**	**LT601943**	**LT602557**				-	
144	*D. latus*	MNCN-AI137	Montenegro	Pljevlja region, river Vodenica	MON1	ca. 43°21'53"N 19°21'48"E	V. Pesic	**LT602206**	**LT601944**	**LT602558**	**LT602376**			-	
145	*D. latus*	MNCN-AI1038	Bulgaria	Kyustendil province, Rila	BUL3	ca. 42°7'55"N 23°8'40"E	D.T. Bilton	**LT602207**	**LT601945**					-	
146	*D. moestus inconspectus*	IBE-DV31	Italy (Sicily)	Mesina province, Cesaro-Randazzo	ISIC2	ca. 37°49'60"N 14°47'37"E	P. Abellán & F. Picazo	**LT602208**	**LT601946**					-	
147	*D. moestus inconspectus*	IBE-DV32	Italy (Sicily)	Mesina province, Cesaro-Randazzo	ISIC2	ca. 37°49'60"N 14°47'37"E	P. Abellán & F. Picazo	**LT602209**	**LT601947**					-	
148	*D. moestus inconspectus*	IBE-DV37	Italy (Sicily)	Catania province, Parco dei Nebrodi, river Flascio	ISIC5	ca. 37°54'13"N 14°52'52"E	P. Abellán & F. Picazo	**LT602210**	**LT601948**	**LT602559**	**LT602377**	**LT602462**		-	
149	*D. moestus inconspectus*	IBE-DV47	Italy	Veneto, Treviso province, Collalto	ITAN2	45º52’20.3”N 12º11’17.3”E	I. Ribera & A. Cieslak	**LT602211**	**LT601949**					-	
150	*D. moestus inconspectus*	IBE-DV48	Italy	Veneto, Treviso province, Collalto	ITAN2	45º52’20.3”N 12º11’17.3”E	I. Ribera & A. Cieslak	**LT602212**	**LT601950**					-	
151	*D. moestus inconspectus*	IBE-DV53	Greece	Epirus region, Kipoi	GRE1	39°51'42.2"N 20°47'10.4"E	P. & V. Ponel	**LT602213**	**LT601951**	**LT602560**	**LT602378**	**LT602463**	**LT602620**	YES	B
152	*D. moestus inconspectus*	IBE-DV54	Greece	Epirus region, Kipoi	GRE1	39°51'42.2"N 20°47'10.4"E	P. & V. Ponel	**LT602214**	**LT601952**					-	
153	*D. moestus inconspectus*	IBE-DV55	Greece	Pelopponese, Laconia region, Mistras	GRE2	ca. 37°4'25"N 22°22'27"E	G. Wewalka	**LT602215**	**LT601953**	**LT602561**	**LT602379**	**LT602464**		-	
154	*D. moestus inconspectus*	IBE-DV56	Greece	Pelopponese, Laconia region, Mistras	GRE2	ca. 37°4'25"N 22°22'27"E	G. Wewalka	**LT602216**	**LT601954**					-	
155	*D. moestus inconspectus*	IBE-DV62	Italy (Elba)	Porto Azurro, Madonna di Monserrato	IELBA1	ca. 42°47'6"N 10°23'30"E	M. Toledo	**LT602217**	**LT601955**	**LT602562**	**LT602380**	**LT602465**		NO	
156	*D. moestus inconspectus*	IBE-DV63	Italy (Elba)	Porto Azurro, Madonna di Monserrato	IELBA1	ca. 42°47'6"N 10°23'30"E	M. Toledo	**LT602218**	**LT601956**					-	
157	*D. moestus inconspectus*	IBE-DV64	Greece	Peloponnese, Achaia region, Erymanthos Mountains	GRE3	ca. 37°55'25"N 21°45'5"E	H. Fery & L. Hendrich	**LT602219**	**LT601957**	**LT602563**	**LT602381**	**LT602466**	**LT602621**	YES	B
158	*D. moestus inconspectus*	IBE-DV65	Greece	Peloponnese, Achaia region, Erymanthos Mountains	GRE3	ca. 37°55'25"N 21°45'5"E	H. Fery & L. Hendrich	**LT602220**	**LT601958**					-	
159	*D. moestus inconspectus*	IBE-DV79	Spain	Aragón, Zaragoza province, El Frago	ESPPIR6	42º 17'43,7''N 0º53'45,5''W	I. Esteban	LN995111	LN995079	LN995183	LN995149	**LT602467**		-	
160	*D. moestus inconspectus*	IBE-DV85	France	Provence-Alpes-Cote D´azur, Var department, Comps	FRAE4	43º42’56.6”N 6º33’03.7”E	I. Ribera & A. Cieslak	**LT602221**	**LT601959**					-	
161	*D. moestus inconspectus*	IBE-DV86	France	Provence-Alpes-Cote D´azur, Var department, Comps	FRAE4	43º42’56.6”N 6º33’03.7”E	I. Ribera & A. Cieslak	**LT602222**	**LT601960**				**LT602622**	YES	B
162	*D. moestus inconspectus*	IBE-DV87	France	Provence-Alpes-Cote D´azur, Var department, Comps	FRAE4	43º42’56.6”N 6º33’03.7”E	I. Ribera & A. Cieslak	**LT602223**	**LT601961**					-	
163	*D. moestus inconspectus*	IBE-DV94	Italy	Abruzzo, L´Aquila province, Mascioni	ITACN5	42º31’57”N 13º20’15”E	I. Ribera & A. Cieslak	**LT602224**	**LT601962**	**LT602564**	**LT602382**	**LT602468**		NO	
164	*D. moestus inconspectus*	IBE-DV95	Italy	Abruzzo, L´Aquila province, Mascioni	ITACN5	42º31’57”N 13º20’15”E	I. Ribera & A. Cieslak	**LT602225**	**LT601963**					-	
165	*D. moestus inconspectus*	IBE-DV96	Italy	Abruzzo, L´Aquila province, Mascioni	ITACN5	42º31’57”N 13º20’15”E	I. Ribera & A. Cieslak	**LT602226**	**LT601964**					-	
166	*D. moestus inconspectus*	IBE-DV118	Italy (Sicily)	Mesina province, Cesaro-Randazzo	ISIC2	ca. 37°49'60"N 14°47'37"E	P. Abellán & F. Picazo	**LT602227**	**LT601965**	**LT602565**	**LT602383**	**LT602469**	**LT602623**	YES	B
167	*D. moestus inconspectus*	IBE-DV119	Italy (Sicily)	Mesina province, Cesaro-Randazzo	ISIC2	ca. 37°49'60"N 14°47'37"E	P. Abellán & F. Picazo	**LT602228**	**LT601966**					-	
168	*D. moestus inconspectus*	IBE-DV126	Italy	Veneto, Treviso province, Collalto	ITAN2	45º52’20.3”N 12º11’17.3”E	I. Ribera & A. Cieslak	**LT602229**	**LT601967**					-	
169	*D. moestus inconspectus*	IBE-DV127	Italy	Veneto, Treviso province, Collalto	ITAN2	45º52’20.3”N 12º11’17.3”E	I. Ribera & A. Cieslak	**LT602230**	**LT601968**					-	
170	*D. moestus inconspectus*	IBE-DV128	Spain	Andalucia, Jaén province, Sierra de Cazorla	ESPSUR14	ca. 38°6'9"N 2°48'46"W	A. Castro	**LT602231**	**LT601969**	**LT602566**	**LT602384**	**LT602470**		NO	
171	*D. moestus inconspectus*	IBE-DV129	Spain	Andalucia, Jaén province, Sierra de Cazorla	ESPSUR14	ca. 38°6'9"N 2°48'46"W	A. Castro	**LT602232**	**LT601970**					-	
172	*D. moestus inconspectus*	IBE-DV132	Greece	Pelopponese, Laconia region, Mistras	GRE2	ca. 37°4'25"N 22°22'27"E	G. Wewalka	**LT602233**	**LT601971**					-	
173	*D. moestus inconspectus*	IBE-DV133	Greece	Pelopponese, Laconia region, Mistras	GRE2	ca. 37°4'25"N 22°22'27"E	G. Wewalka	**LT602234**	**LT601972**					-	
174	*D. moestus inconspectus*	IBE-DV142	Spain	Aragón, Huesca province, Acumuer	ESPPIR17	42º37'34,65''N 0º24'0,62''W	I. Esteban	**LT602235**	**LT601973**	**LT602567**	**LT602385**	**LT602471**		-	
175	*D. moestus inconspectus*	IBE-DV143	Spain	Aragón, Huesca province, Acumuer	ESPPIR17	42º37'34,65''N 0º24'0,62''W	I. Esteban	**LT602236**	**LT601974**					-	
176	*D. moestus inconspectus*	IBE-DV146	Spain	Aragón, Teruel province, Aliaga	ESPEST4	ca. 40°40'53"N 0°41'5"W	I. Esteban	**LT602237**	**LT601975**					-	
177	*D. moestus inconspectus*	IBE-DV147	Spain	Aragón, Teruel province, Aliaga	ESPEST4	ca. 40°40'53"N 0°41'5"W	I. Esteban	**LT602238**	**LT601976**					-	
178	*D. moestus inconspectus*	IBE-DV178	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak	**LT602239**	**LT601977**	**LT602568**	**LT602386**	**LT602472**		-	
179	*D. moestus inconspectus*	IBE-DV179	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak	**LT602240**	**LT601978**					-	
180	*D. moestus inconspectus*	IBE-DV180	Spain	Aragón, Huesca province, Bernués	ESPPIR22	42°26'38"N 0°36'51"W	I. Ribera & A. Cieslak	**LT602241**	**LT601979**	**LT602569**	**LT602387**	**LT602473**		NO	
181	*D. moestus inconspectus*	IBE-DV181	Spain	Aragón, Huesca province, Bernués	ESPPIR22	42°26'38"N 0°36'51"W	I. Ribera & A. Cieslak		**LT601980**					-	
182	*D. moestus inconspectus*	IBE-DV186	Spain	Aragón, Huesca province, Acumuer	ESPPIR17	42º37'34,65''N 0º24'0,62''W	I. Esteban	**LT602242**	**LT601981**					-	
183	*D. moestus inconspectus*	IBE-DV187	Spain	Aragón, Huesca province, Acumuer	ESPPIR17	42º37'34,65''N 0º24'0,62''W	I. Esteban	**LT602243**	**LT601982**					-	
184	*D. moestus inconspectus*	IBE-DV188	Spain	Aragón, Teruel province, Aliaga	ESPEST4	ca. 40°40'53"N 0°41'5"W	I. Esteban	**LT602244**	**LT601983**	**LT602570**	**LT602388**	**LT602474**	**LT602624**	YES	A
185	*D. moestus inconspectus*	IBE-DV193	Spain	Aragón, Huesca province, Santa Cruz de Serós	ESPPIR21	42°31'5.2"N 0°41'36.8"W	I. Ribera & A. Cieslak	**LT602245**	**LT601984**					-	
186	*D. moestus inconspectus*	IBE-DV194	Spain	Aragón, Huesca province, Bernués	ESPPIR22	42°26'38"N 0°36'51"W	I. Ribera & A. Cieslak	**LT602246**	**LT601985**					-	
187	*D. moestus inconspectus*	IBE-DV195	Spain	Aragón, Huesca province, Bernués	ESPPIR22	42°26'38"N 0°36'51"W	I. Ribera & A. Cieslak		**LT601986**					-	
188	*D. moestus inconspectus*	IBE-DV196	Spain	Aragón, Huesca province, Bernués	ESPPIR22	42°26'38"N 0°36'51"W	I. Ribera & A. Cieslak	**LT602247**	**LT601987**					-	
189	*D. moestus inconspectus*	IBE-DV199	Spain	Aragón, Huesca province, Acumuer	ESPPIR17	42º37'34,65''N 0º24'0,62''W	I. Esteban	**LT602248**	**LT601988**					-	
190	*D. moestus inconspectus*	IBE-DV203	Spain	Aragón, Huesca province, Ansó	ESPPIR19	ca. 42°45'39"N 0°49'49"W	I. Esteban	**LT602249**	**LT601989**	**LT602571**	**LT602389**	**LT602475**	**LT602625**	YES	A
191	*D. moestus inconspectus*	IBE-DV205	Italy (Sicily)	Palermo province, Parco naturalle delle Madonie	ISIC6	ca. 37°53'27"N 14°0'1"E	P. Abellán & F. Picazo	**LT602250**	**LT601990**	**LT602572**	**LT602390**	**LT602476**	**LT602626**	YES	B
192	*D. moestus inconspectus*	IBE-DV206	Italy (Sicily)	Palermo province, Parco naturalle delle Madonie	ISIC6	ca. 37°53'27"N 14°0'1"E	P. Abellán & F. Picazo	**LT602251**	**LT601991**					-	
193	*D. moestus inconspectus*	IBE-DV207	Italy (Sicily)	Palermo province, Parco naturalle delle Madonie	ISIC6	ca. 37°53'27"N 14°0'1"E	P. Abellán & F. Picazo	**LT602252**	**LT601992**					-	
194	*D. moestus inconspectus*	IBE-DV208	Italy (Sicily)	Palermo province, Parco naturalle delle Madonie	ISIC6	ca. 37°53'27"N 14°0'1"E	P. Abellán & F. Picazo	**LT602253**	**LT601993**					-	
195	*D. moestus inconspectus*	IBE-DV260	Italy (Sicily)	Palermo province, Parco naturalle delle Madonie	ISIC6	ca. 37°53'27"N 14°0'1"E	P. Abellán & F. Picazo	**LT602254**	**LT601994**					-	
196	*D. moestus inconspectus*	IBE-DV263	Italy (Sicily)	Mesina province, Cesaro-Randazzo	ISIC2	ca. 37°49'60"N 14°47'37"E	P. Abellán & F. Picazo	**LT602255**	**LT601995**					-	
197	*D. moestus inconspectus*	IBE-RA145	Italy (Elba)	Porto Azurro, Madonna di Monserrato	IELBA1	ca. 42°47'6"N 10°23'30"E	M. Toledo	**LT602256**	**LT601996**					-	
198	*D. moestus inconspectus*	IBE-RA213	Greece	Peloponnese, Achaia region, Erymanthos Mountains	GRE3	ca. 37°55'25"N 21°45'5"E	H. Fery & L. Hendrich	**LT602257**	**LT601997**					-	
199	*D. moestus inconspectus*	IBE-RA331	Spain	Cataluña, Lleida province, Llinars	ESPPIR5	ca. 42°6'12"N 1°41'24"E	J. Fresneda	**LT602258**	**LT601998**	**LT602573**	**LT602391**	**LT602477**	**LT602627**	YES	A
200	*D. moestus inconspectus*	IBE-RA333	Croatia	Istria county, Vranja	CRO1	ca. 45°19'7"N 14°8'21"E	H. Fery		**LT601999**	**LT602574**	**LT602392**	**LT602478**		NO	
201	*D. moestus inconspectus*	IBE-RA336	Spain	Aragón, Zaragoza province, El Frago	ESPPIR6	42º 17'43,7''N 0º53'45,5''W	I. Esteban	**LT602259**	**LT602000**					-	
202	*D. moestus inconspectus*	IBE-RA338	Spain	Aragón, Huesca province, Aragues del Puerto	ESPPIR7	42º45'16.93''N 0º37'59.65''W	I. Esteban	**LT602260**	**LT602001**	**LT602575**	**LT602393**	**LT602479**		-	
203	*D. moestus inconspectus*	IBE-RA365	Morocco	Meknès-Tafilalet, Khénifra Province, Kerrouchen (Middle Atlas)	MARATL9	32º48'22.1"N 5º16'13.9"W	I. Ribera, P. Aguilera & C. Hernando	**LT602261**	**LT602002**	**LT602576**	**LT602394**	**LT602480**		NO	
204	*D. moestus inconspectus*	IBE-RA374	France	Provence-Alpes-Cote D´azur, Var department, Comps	FRAE4	43º42’56.6”N 6º33’03.7”E	I. Ribera & A. Cieslak	**LT602262**	**LT602003**	**LT602577**	**LT602395**	**LT602481**	**LT602628**	YES	B
205	*D. moestus inconspectus*	IBE-RA429	Italy	Abruzzo, L´Aquila province, Mascioni	ITACN5	42º31’57”N 13º20’15”E	I. Ribera & A. Cieslak	**LT602263**	**LT602004**					-	
206	*D. moestus inconspectus*	IBE-RA430	Italy	Abruzzo, L´Aquila province, Mascioni	ITACN5	42º31’57”N 13º20’15”E	I. Ribera & A. Cieslak	**LT602264**	**LT602005**					NO	

207	*D. moestus inconspectus*	MNCN-AC37	Italy (Sicily)	Mesina province, Cesaro-Randazzo	ISIC2	ca. 37°49'60"N 14°47'37"E	P. Abellán & F. Picazo		**LT602006**	**LT602578**				-	
208	*D. moestus inconspectus*	MNCN-AH182	Italy (Sicily)	Catania province, Parco dei Nebrodi, river Flascio	ISIC5	ca. 37°54'13"N 14°52'52"E	P. Abellán & F. Picazo	**LT602265**	**LT602007**					-	
209	*D. moestus inconspectus*	MNCN-AI672	Bulgaria	Blagoevgrad province, Filipovo (Rhodope Mountains)	BUL1	ca. 41°45'53"N 23°41'22"E	D.T. Bilton	LN995108	LN995076	LN995180	LN995146	**LT602482**		-	
210	*D. moestus inconspectus*	MNCN-AI894	Spain	Andalucia, Jaén province, Hornos del Segura	ESPSUR2	ca. 38°14'6"N 2°43'42"W	A.G. Valdecasas	**LT602266**	**LT602008**	**LT602579**	**LT602396**	**LT602483**		NO	
211	*D. moestus inconspectus*	MNCN-AI937	Morocco	Sus-Masa-Draa, Ouarzazate province, Tachokchte	MARATL1	ca. 30°47'36"N 7°31'27"W	I. Ribera & A. Cieslak	LN995109	LN995077	LN995181	LN995147	**LT602484**		NO	
212	*D. moestus inconspectus*	MNCN-AI1048	Italy	Veneto, Treviso province, Collalto	ITAN2	45º52'20.3"N 12º11'17.3"E	I. Ribera & A. Cieslak	**LT602267**	**LT602009**	**LT602580**	**LT602397**	**LT602485**	**LT602629**	YES	A
213	*D. moestus inconspectus*	MNCN-AI1090	Spain	Andalucia, Jaén province, Sierra de Cazorla	ESPSUR14	ca. 38°6'9"N 2°48'46"W	A. Castro	**LT602268**	**LT602010**					-	
214	*D. moestus inconspectus*	MNCN-AI1202	Greece	Epirus region, Kipoi	GRE1	39°51'42.2"N 20°47'10.4"E	P. & V. Ponel	**LT602269**	**LT602011**					-	
215	*D. moestus inconspectus*	MNCN-AI1218	Greece	Pelopponese, Laconia region, Mistras	GRE2	ca. 37°4'25"N 22°22'27"E	G. Wewalka	**LT602270**	**LT602012**					-	
216	*D. moestus inconspectus*	NHM-IR82	Spain	Cataluña, Barcelona province, Saldes	ESPPIR4	ca. 42°13'37"N 1°44'32"E	P. Aguilera		**LT602013**	**LT602581**				-	
217	*D. moestus inconspectus*	NHM-IR207	Spain	Castilla La Mancha, Albacete province, river Endrinales	ESPSUR5	ca. 38°33'31"N 2°20'35"W	I. Ribera		**LT602014**					-	
218	*D. moestus inconspectus*	NHM-IR235	Morocco	Sus-Masa-Draa, Chtouka Aït Baha province, Ait-Iftene	MARATL4	ca. 30°4'10"N 9°9'26"W	I. Ribera		**LT602015**	**LT602582**				-	
219	*D. moestus inconspectus*	NHM-IR303	Italy	Lombardia, Pavia province, San Ponzo	ITAN4	ca. 44°50'23"N 9° 6'23"E	I. Ribera & A. Cieslak		**LT602016**	**LT602583**				-	
220	*D. moestus moestus*	IBE-DV41	Italy (Sardinia)	Ogliastra province, Codula di Luna	ICER1	40º10'16.0"N 9º33'36.7"E	P. & V. Ponel	**LT602271**	**LT602017**	**LT602584**	**LT602398**	**LT602486**		YES	undetermined
221	*D. moestus moestus*	IBE-DV42	Italy (Sardinia)	Ogliastra province, Codula di Luna	ICER1	40º10'16.0"N 9º33'36.7"E	P. & V. Ponel	**LT602272**	**LT602018**					-	
222	*D. moestus moestus*	IBE-DV66	Italy (Sardinia)	Ogliastra province, Villagrande Strìsaili	ICER2	39°57'3.17"N 9°31'8.25"E	H. Fery & M. Toledo	**LT602273**	**LT602019**	**LT602585**	**LT602399**	**LT602487**		-	
223	*D. moestus moestus*	IBE-DV69	Italy (Sardinia)	Olbia-Tempio province, Monte Limbara	ICER3	40°51'30.18"N 9° 7'20.81"E	H. Fery & M. Toledo	LN995110	LN995078	LN995182	LN995148	**LT602488**		-	
224	*D. moestus moestus*	IBE-DV70	Italy (Sardinia)	Olbia-Tempio province, Monte Limbara	ICER3	40°51'30.18"N 9° 7'20.81"E	H. Fery & M. Toledo	**LT602274**	**LT602020**					-	
225	*D. moestus moestus*	IBE-DV71	Italy (Sardinia)	Olbia-Tempio province, Monte Limbara	ICER3	40°51'30.18"N 9° 7'20.81"E	H. Fery & M. Toledo	**LT602275**	**LT602021**					-	
226	*D. moestus moestus*	IBE-DV72	Italy (Sardinia)	Ogliastra province, Tortoli	ICER4	ca. 39°56'23"N 9°35'34"E	H. Fery & M. Toledo	**LT602276**	**LT602022**	**LT602586**	**LT602400**	**LT602489**		-	undetermined
227	*D. moestus moestus*	IBE-DV73	Italy (Sardinia)	Ogliastra province, Tortoli	ICER4	ca. 39°56'23"N 9°35'34"E	H. Fery & M. Toledo	**LT602277**	**LT602023**					-	
228	*D. moestus moestus*	IBE-DV122	Italy (Sardinia)	Ogliastra province, Codula di Luna	ICER1	40º10'16.0"N 9º33'36.7"E	P. & V. Ponel	**LT602278**	**LT602024**					-	
229	*D. moestus moestus*	IBE-DV123	Italy (Sardinia)	Ogliastra province, Codula di Luna	ICER1	40º10'16.0"N 9º33'36.7"E	P. & V. Ponel	**LT602279**	**LT602025**					-	
230	*D. moestus moestus*	IBE-DV166	Italy (Sardinia)	Ogliastra province, Tortoli	ICER4	ca. 39°56'23"N 9°35'34"E	H. Fery & M. Toledo	**LT602280**	**LT602026**					-	
231	*D. moestus moestus*	IBE-DV167	Italy (Sardinia)	Ogliastra province, Tortoli	ICER4	ca. 39°56'23"N 9°35'34"E	H. Fery & M. Toledo	**LT602281**	**LT602027**					-	
232	*D. moestus moestus*	IBE-RA307	Italy (Sardinia)	Ogliastra province, Villagrande Strìsaili	ICER2	39°57'3.17"N 9°31'8.25"E	H. Fery & M. Toledo		**LT602028**					-	
233	*D. moestus moestus*	IBE-RA310	Italy (Sardinia)	Olbia-Tempio province, Monte Limbara	ICER3	40°51'30.18"N 9° 7'20.81"E	H. Fery & M. Toledo	**LT602282**	**LT602029**					-	
234	*D. moestus moestus*	IBE-RA311	Italy (Sardinia)	Ogliastra province, Tortoli	ICER4	ca. 39°56'23"N 9°35'34"E	H. Fery & M. Toledo	**LT602283**	**LT602030**					-	
235	*D. moestus moestus*	MNCN-AI886	Italy (Sardinia)	Ogliastra province, Codula di Luna	ICER1	40º10'16.0"N 9º33'36.7"E	P. & V. Ponel	**LT602284**	**LT602031**					-	
236	*D. moestus moestus*	NHM-IR156	France (Corsica)	Haute-Corse department, Vizzavona	ICOR1	ca. 42°6'59"N 9°6'40"E	I. Ribera & A. Cieslak	LN995105	AF309316	AF309259				-	
237	*D. moestus moestus*	NHM-MsC00B	France (Corsica)	Corse-du-Sud department, L´Ospedale	ICOR3	ca. 41°39'14"N 9°12'41"E	I. Ribera & A. Cieslak		**LT602032**	**LT602587**				-	
238	*D. platynotus*	IBE-DV137	Germany	Bayern, Schwaben region, Wertach	ALE2	47°35'9.6"N 10°22'52.2"E	L. Hendrich & R. Müller	**LT602285**	**LT602033**	**LT602588**	**LT602401**	**LT602490**		NO	
239	*D. platynotus*	IBE-DV213	Romania	Bacău county, Scutaru	RUM1	ca. 46°3'54"N 26°31'48"E	H. Fery	**LT602286**	**LT602034**	**LT602589**	**LT602402**	**LT602491**		-	
240	*D. platynotus*	IBE-RA316	Montenegro	Savnik	MON2	ca. 42°57'28"N 19°5'54"E	D.T. Bilton	**LT602287**	**LT602035**	**LT602590**	**LT602403**	**LT602492**		-	
241	*D. platynotus*	IBE-RA332	Germany	Saxonia, Dresden region, Pöbelbach	ALE1	50°48'57.96"N 13°39'44.14"E	L. Hendrich & R. Müller	**LT602288**	**LT602036**	**LT602591**				-	
242	*D. platynotus*	IBE-RA335	Macedonia	Ohrid district, Recica	MAC2	ca. 41°11'52"N 20°55'47"E	D.T. Bilton	**LT602289**	**LT602037**	**LT602592**	**LT602404**	**LT602493**		NO	
243	*D. platynotus*	IBE-RA445	Bulgaria	Kyustendil province, Rila	BUL3	ca. 42°7'55"N 23°8'40"E	D.T. Bilton	**LT602290**	**LT602038**					-	
244	*D. platynotus*	IBE-RA446	Bulgaria	Kyustendil province, Rila	BUL3	ca. 42°7'55"N 23°8'40"E	D.T. Bilton	**LT602291**	**LT602039**					-	
245	*D. platynotus*	IBE-RA447	Czech Republic	Central Bohemia region, Rakovnik district, Skryje	RCHE1	ca. 49°58'21"N 13°45'55"E	J. Statszny	**LT602292**	**LT602040**					-	
246	*D. platynotus*	IBE-RA448	Czech Republic	Central Bohemia region, Rakovnik district, Skryje	RCHE1	ca. 49°58'21"N 13°45'55"E	J. Statszny	**LT602293**	**LT602041**					-	
247	*D. platynotus*	MNCN-AI1039	Bulgaria	Kyustendil province, Rila	BUL3	ca. 42°7'55"N 23°8'40"E	D.T. Bilton	LN995115	HE610190	LN995186	LN995154	**LT602494**		-	
248	*D. platynotus*	MNCN-AI1121	Macedonia	Bitola district, Pelister National Park, Golemo Ezero	MAC1	ca. 40°58'9"N 21°12'19"E	R. B. Angus	**LT602294**	**LT602042**	**LT602593**	**LT602405**	**LT602495**		-	
249	*D. platynotus*	MNCN-AI1122	Germany	Saxonia, Dresden region, Pöbelbach	ALE1	50°48'57.96"N 13°39'44.14"E	L. Hendrich & R. Müller	LN995116	HE610191	HF931361	LN995155	**LT602496**		-	
250	*D. platynotus*	MNCN-AI1123	Germany	Saxonia, Dresden region, Pöbelbach	ALE1	50°48'57.96"N 13°39'44.14"E	L. Hendrich & R. Müller	**LT602295**	**LT602043**					-	
251	*D. platynotus*	MNCN-AI1275	Czech Republic	Central Bohemia region, Rakovnik district, Skryje	RCHE1	ca. 49°58'21"N 13°45'55"E	J. Statszny	**LT602296**	**LT602044**	**LT602594**	**LT602406**			-	
252	*D. platynotus*	NHM-IR246	Czech Republic	Central Bohemia region, Rakovnik district, Skryje	RCHE1	ca. 49°58'21"N 13°45'55"E	J. Statszny			**LT602595**				-	
253	*D. semirufus*	IBE-AF94	Italy	Emilia-Romagna, Modena province, Fanano	ITAN1	ca. 44°9'58"N 10°43'55"E	M. Toledo		**LT602045**	**LT602596**	**LT602407**	**LT602497**		-	
254	*D. semirufus*	IBE-DV29	Italy (Sicily)	Mesina province, Parco dei Nebrodi, Caserma Mafauda	ISIC1	ca. 37°53'42"N 14°30'31"E	P. Abellán & F. Picazo	**LT602297**	**LT602046**					-	
255	*D. semirufus*	IBE-DV30	Italy (Sicily)	Mesina province, Parco dei Nebrodi, Caserma Mafauda	ISIC1	ca. 37°53'42"N 14°30'31"E	P. Abellán & F. Picazo	**LT602298**	**LT602047**					-	
256	*D. semirufus*	IBE-DV33	Italy	Emilia-Romagna, Modena province, Fanano	ITAN1	ca. 44°9'58"N 10°43'55"E	M. Toledo	**LT602299**	**LT602048**					-	
257	*D. semirufus*	IBE-DV34	Italy (Sicily)	Mesina province, Parco dei Nebrodi, river Torti	ISIC4	ca. 37°53'38"N 14°39'6"E	P. Abellán & F. Picazo	**LT602300**	**LT602049**	**LT602597**	**LT602408**	**LT602498**	**LT602630**	YES	A
258	*D. semirufus*	IBE-DV35	Italy (Sicily)	Mesina province, Parco dei Nebrodi, river Torti	ISIC4	ca. 37°53'38"N 14°39'6"E	P. Abellán & F. Picazo	**LT602301**	**LT602050**					-	
259	*D. semirufus*	IBE-DV36	Italy (Sicily)	Mesina province, Parco dei Nebrodi, river Torti	ISIC4	ca. 37°53'38"N 14°39'6"E	P. Abellán & F. Picazo	**LT602302**	**LT602051**					-	
260	*D. semirufus*	IBE-DV51	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Monti	FRAE1	ca. 43°49'28"N 7°28'56"E	D.T. Bilton	**LT602303**	**LT602052**	**LT602598**	**LT602409**	**LT602499**		YES	undetermined
261	*D. semirufus*	IBE-DV52	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Monti	FRAE1	ca. 43°49'28"N 7°28'56"E	D.T. Bilton	**LT602304**	**LT602053**					-	
262	*D. semirufus*	IBE-DV59	Italy	Marche, Ascoli-Piceno province, Acquasanta	ITACN1	ca. 42°43'29"N 13°22'58"E	M. Toledo	**LT602305**	**LT602054**	**LT602599**	**LT602410**	**LT602500**	**LT602631**	YES	A
263	*D. semirufus*	IBE-DV88	Italy	Toscana, Arezzo province, Badia Prataglia	ITACN3	43º47'51"N 11º53'37"E	I. Ribera & A. Cieslak	**LT602306**	**LT602055**	**LT602600**	**LT602411**	**LT602501**		-	
264	*D. semirufus*	IBE-DV89	Italy	Toscana, Arezzo province, Badia Prataglia	ITACN3	43º47'51"N 11º53'37"E	I. Ribera & A. Cieslak	**LT602307**	**LT602056**					-	
265	*D. semirufus*	IBE-DV90	Italy	Toscana, Arezzo province, Badia Prataglia	ITACN3	43º47'51"N 11º53'37"E	I. Ribera & A. Cieslak	**LT602308**	**LT602057**					-	
266	*D. semirufus*	IBE-DV91	Italy	Toscana, Arezzo province, Fosso de Camaldoli	ITACN4	43º49'05"N 11º48'23"E	I. Ribera & A. Cieslak	**LT602309**	**LT602058**	**LT602601**				-	
267	*D. semirufus*	IBE-DV92	Italy	Toscana, Arezzo province, Fosso de Camaldoli	ITACN4	43º49'05"N 11º48'23"E	I. Ribera & A. Cieslak	**LT602310**	**LT602059**					-	
268	*D. semirufus*	IBE-DV93	Italy	Toscana, Arezzo province, Fosso de Camaldoli	ITACN4	43º49'05"N 11º48'23"E	I. Ribera & A. Cieslak	**LT602311**	**LT602060**	**LT602602**	**LT602412**	**LT602502**		YES	undetermined
269	*D. semirufus*	IBE-DV99	Italy	Abruzzo, L´Aquila province, Pescasseroli (Abruzzo N.P)	ITACN6	41°51'4.72"N 13°46'37.24"E	I. Ribera & A. Cieslak	**LT602312**	**LT602061**	**LT602603**	**LT602413**	**LT602503**	**LT602632**	YES	B
270	*D. semirufus*	IBE-DV100	Italy	Abruzzo, L´Aquila province, Pescasseroli (Abruzzo N.P)	ITACN6	41°51'4.72"N 13°46'37.24"E	I. Ribera & A. Cieslak	**LT602313**	**LT602062**					-	
271	*D. semirufus*	IBE-DV117	Italy (Sicily)	Mesina province, Parco dei Nebrodi, Caserma Mafauda	ISIC1	ca. 37°53'42"N 14°30'31"E	P. Abellán & F. Picazo	**LT602314**	**LT602063**	**LT602604**	**LT602414**	**LT602504**	**LT602633**	YES	A
272	*D. semirufus*	IBE-DV130	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Monti	FRAE1	ca. 43°49'28"N 7°28'56"E	D.T. Bilton	**LT602315**	**LT602064**					-	
273	*D. semirufus*	IBE-DV131	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Monti	FRAE1	ca. 43°49'28"N 7°28'56"E	D.T. Bilton	**LT602316**	**LT602065**					-	
274	*D. semirufus*	IBE-DV262	Italy (Sicily)	Mesina province, Parco dei Nebrodi, Caserma Mafauda	ISIC1	ca. 37°53'42"N 14°30'31"E	P. Abellán & F. Picazo	**LT602317**	**LT602066**					-	
275	*D. semirufus*	IBE-RA131	Italy	Marche, Ascoli-Piceno province, Acquasanta	ITACN1	ca. 42°43'29"N 13°22'58"E	M. Toledo	**LT602318**	**LT602067**					-	
276	*D. semirufus*	IBE-RA136	Italy	Emilia-Romagna, Modena province, Passo della Croce Arcana	ITAN6	ca. 44°8'4"N 10°46'55"E	M. Toledo	LN995118	LN995082	LN995188	LN995157	**LT602505**		-	
277	*D. semirufus*	IBE-RA340	Italy (Sicily)	Mesina province, Parco dei Nebrodi, Caserma Mafauda	ISIC7	ca. 37°53'31"N 14°31'35"E	P. Abellán & F. Picazo	**LT602319**	**LT602068**					-	

278	*D. semirufus*	IBE-RA406	Italy	Toscana, Arezzo province, Badia Prataglia	ITACN3	43º47’51”N 11º53’37”E	I. Ribera & A. Cieslak	**LT602320**	**LT602069**					-	
279	*D. semirufus*	IBE-RA407	Italy	Toscana, Arezzo province, Badia Prataglia	ITACN3	43º47’51”N 11º53’37”E	I. Ribera & A. Cieslak	**LT602321**	**LT602070**					-	
280	*D. semirufus*	IBE-RA417	Italy	Toscana, Arezzo province, Fosso de Camaldoli	ITACN4	43º49’05”N 11º48’23”E	I. Ribera & A. Cieslak	**LT602322**	**LT602071**					-	
281	*D. semirufus*	IBE-RA418	Italy	Toscana, Arezzo province, Fosso de Camaldoli	ITACN4	43º49’05”N 11º48’23”E	I. Ribera & A. Cieslak	**LT602323**	**LT602072**					-	
282	*D. semirufus*	IBE-RA427	Italy	Abruzzo, L´Aquila province, Mascioni	ITACN5	42º31’57”N 13º20’15”E	I. Ribera & A. Cieslak	**LT602324**	**LT602073**	**LT602605**	**LT602415**	**LT602506**		-	
283	*D. semirufus*	IBE-RA428	Italy	Abruzzo, L´Aquila province, Mascioni	ITACN5	42º31’57”N 13º20’15”E	I. Ribera & A. Cieslak	**LT602325**	**LT602074**					-	
284	*D. semirufus*	IBE-RA450	Italy	Abruzzo, L´Aquila province, Pescasseroli (Abruzzo N.P)	ITACN6	41°51'4.72"N 13°46'37.24"E	I. Ribera & A. Cieslak	**LT602326**	**LT602075**					-	
285	*D. semirufus*	IBE-RA451	Italy	Abruzzo, L´Aquila province, Pescasseroli (Abruzzo N.P)	ITACN6	41°51'4.72"N 13°46'37.24"E	I. Ribera & A. Cieslak	**LT602327**	**LT602076**					-	
286	*D. semirufus*	IBE-RA452	Italy	Abruzzo, L´Aquila province, Pescasseroli (Abruzzo N.P)	ITACN6	41°51'4.72"N 13°46'37.24"E	I. Ribera & A. Cieslak	**LT602328**	**LT602077**					-	
287	*D. semirufus*	MNCN-AC33	Italy (Sicily)	Mesina province, Parco dei Nebrodi, Caserma Mafauda	ISIC1	ca. 37°53'42"N 14°30'31"E	P. Abellán & F. Picazo		**LT602078**	**LT602606**				-	
288	*D. semirufus*	MNCN-AH178	Italy (Sicily)	Mesina province, Parco dei Nebrodi, lake of Biviere	ISIC3	ca. 37°56'29"N 14°40'15"E	P. Abellán & F. Picazo	**LT602329**	**LT602079**	**LT602607**	**LT602416**	**LT602507**		-	
289	*D. semirufus*	MNCN-AH179	Italy (Sicily)	Mesina province, Parco dei Nebrodi, river Torti	ISIC4	ca. 37°53'38"N 14°39'6"E	P. Abellán & F. Picazo		**LT602080**					-	
290	*D. semirufus*	MNCN-AI1114	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Monti	FRAE1	ca. 43°49'28"N 7°28'56"E	D.T. Bilton	**LT602330**	**LT602081**					-	
291	*D. semirufus*	NHM-IR299	France	Provence-Alpes-Cote D´azur, Alpes-Maritimes department, Monti	FRAE2	ca. 43°49'28"N 7°28'56"E	I. Ribera & A. Cieslak		**LT602082**	**LT602608**				-	
292	*D. toledoi*	IBE-DV6	Turkey	Erzurum province, Toprakkaleköyü	TUR3	40°14'22.90"N 40°59'16.70"E	I. Ribera	LN995120	LN995084	LN995190	LN995159			-	
293	*D. toledoi*	IBE-DV7	Turkey	Erzurum province, Toprakkaleköyü	TUR3	40°14'22.90"N 40°59'16.70"E	I. Ribera	**LT602331**	**LT602083**	**LT602609**	**LT602417**	**LT602508**		NO	
294	*D. toledoi*	IBE-DV8	Turkey	Erzurum province, Toprakkaleköyü	TUR3	40°14'22.90"N 40°59'16.70"E	I. Ribera	**LT602332**	**LT602084**					-	
295	*D. toledoi*	IBE-DV116	Turkey	Erzurum province, Toprakkaleköyü	TUR3	40°14'22.90"N 40°59'16.70"E	I. Ribera	**LT602333**	**LT602085**					-	
296	*D. toledoi*	IBE-DV134	Turkey	Erzurum province, Toprakkaleköyü	TUR3	40°14'22.90"N 40°59'16.70"E	I. Ribera	**LT602334**	**LT602086**					-	
297	*D. abnormicollis*	MNCN-AI120	Uzbekistan	Tashkent province, Yakkatut		41º38'N 70º03'E	L. Hendrich	LN995086	LN995059	LN995161	LN995126				
298	*D. algibensis*	NHM-IR76	Spain	Andalucia, Cádiz province, Puerto de Gáliz		ca. 36°33’35”N 5°36’4”W	I. Ribera		AF309318	AF309261					
299	*D. bicostatus*	MNCN-AI639	Portugal	Guarda district, Manteigas (Serra da Estrela)		40º19’57”N 7º37’03”W	I. Ribera	LN995091	HE610179	LN995166	LN995130				
300	*D. depressicollis*	MNCN-AI1023	Spain	Andalucia, Almeria province, Abrucena		ca. 37°8'20"N 2°46'50"W	A. Castro	LN995098	HE610182	LN995172	LN995137				
301	*D. doriae*	MNCN-AI775	Turkey	Bolu province, Kartalkaya		40º39’20”N 31º47’8.5”E	I. Ribera, P. Aguilera & C. Hernando	LN995099	HE610183	LN995173	LN995138				
302	*D. fairmairei*	IBE-DV43	Morocco	Sus-Masa-Draa, Ouarzazate province, Tachokchte		ca. 30°47'36"N 7°31'27"W	I. Ribera & A. Cieslak	LN995101	LN995070	LN995175	LN995140				
303	*D. ferrugineus*	MNCN-AI731	Portugal	Guarda district, Sabugueiro (Serra da Estrela)		40º24’20”N 7º37’43”W	I. Ribera	LN995103	LN995072	LN995176	LN995142				
304	*D. fosteri*	NHM-IR77	Spain	Cataluña, Barcelona province, Saldes		ca. 42°13'36"N 1°44'30"E	P. Aguilera		AF309317	AF309260					
305	*D. hispanicus*	MNCN-AI858	Spain	Comunidad Valenciana, Castellón province, Ballestar		40º41’41”N 0º13’25.5”E	I. Ribera	LN995104	LN995073	LN995177	LN995143				
306	*D. lareynii*	NHM-IR165	France (Corsica)	Haute-Corse department, Vizzavona		ca. 42°6'59"N 9°6'40"E	I. Ribera & A. Cieslak	LN995105	AF309316	AF309259					
307	*D. nilssoni*	IBE-AF104	Iran	Khorasan Shamali province, Eshq Abad		37º 48.2'N 56º55.5'E	J. Hájek & P. Chvojka		LN995080	LN995184	LN995150				
308	*D. opatrinus*	MNCN-AI629	Spain	Andalucia, Córdoba province, Sierra Morena,		38° 5'51.79"N 4°53'34.37"W	A. Castro	LN995112	HE610188	LN995185	LN995151				
309	*D. parvicollis*	MNCN-AI776	Turkey	Bolu province, Kartalkaya		40º39’20”N 31º47’8.5”E	I. Ribera, P. Aguilera & C. Hernando	LN995113	HF931225	HF931454	LN995152				
310	*D. persicus*	NHM-IR45	Iran	Fars province, Sepidan		ca. 30°17'6"N 51°56'54"E	H. Fery		AF309308	AF309251	EF670140				
311	*D. sahlbergi*	MNCN-AI108	Greece (Chios)	Kardamila		ca. 38°31'37"N 26° 5'30"E)	G.N. Foster	LN995117	LN995081	LN995187	LN995156				
312	*D. theryi*	IBE-RA37	Morocco	Taza-Al Hoceima-Taounate,Taza province, Tazzeka National Park		34º08’56.8”N 4º00’25.5”W	I. Ribera, P. Aguilera & C. Hernando	LN995119	LN995083	LN995189	LN995158				
313	*D. wewalkai*	MNCN-AI725	Spain	Castilla-La Mancha, Guadalajara province, Cardoso de la Sierra,		41º05'34.3"N 3º25'32.1"W	I. Ribera & A. Cieslak	LN995121	LN995085	LN995191	LN995160				
314	*D. youngi*	NHM-IR182	Iran	Kohkiluyeh and Boyer Ahmad province, Gachsaran		ca. 30°24'20.78"N 50°50'7.75"	H. Fery		AF309306	AF309249	EF670142				

Table S2.

A) Primers used for the amplification and sequencing. In brackets, length of the amplified fragment.

gene	primer	sequence	ref.
cox1-3’	Jerry (5')	CAACATTTATTTTGATTTTTTGG	(4)
(826)	Pat (3')	TCCAATGCACTAATCTGCCATATTA	(4)
	Chy (5')	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	(3)
	Tom (3')	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	(3)
barcode	LCO 1490 (5')	GGTCAACAAATCATAAAGATATTGG	(2)
(658)	HCO 2198 (3')	TAAACTTCAGGGTGACCAAAAAATCA	(2)
rrnL+trnL+nad1	16SaR (5')	CGCCTGTTTAACAAAAACAT	(4)
(685-693)	ND1 (3')	GGTCCCTTACGAATTTGAATATATCCT	(4)
H3	H3aF (5')	ATGGCTCGTACCAAGCAGACRCG	(1)
(330)	H3aR (3')	ATATCCTTRGGCATRATRGTGAC	(1)
Wingless	WG550F (5')	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	(5)
(472-475)	WGAbrZ (3')	CACTTNACYTCRCARCACCARTG	(5)

B) Standard PCR conditions for the amplification of the studied fragments.

step	time	temperature
1	3’	96º
2	30”	94º
3	30”-1’	47-50º *
4	1’	72º
5	Go to step 2 and repeat 34-40 x	
6	10’	72º

* Depending on the annealing temperatures of the primers pair used

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Figure S1. Ultrametric time calibrated tree obtained with BEAST with the mitochondrial sequence data of the same specimens used in the analysis of the nuclear sequences (see Fig. S2). Numbers in nodes: Bayesian posterior probabilities. See Table S1 for details on the specimens and localities.

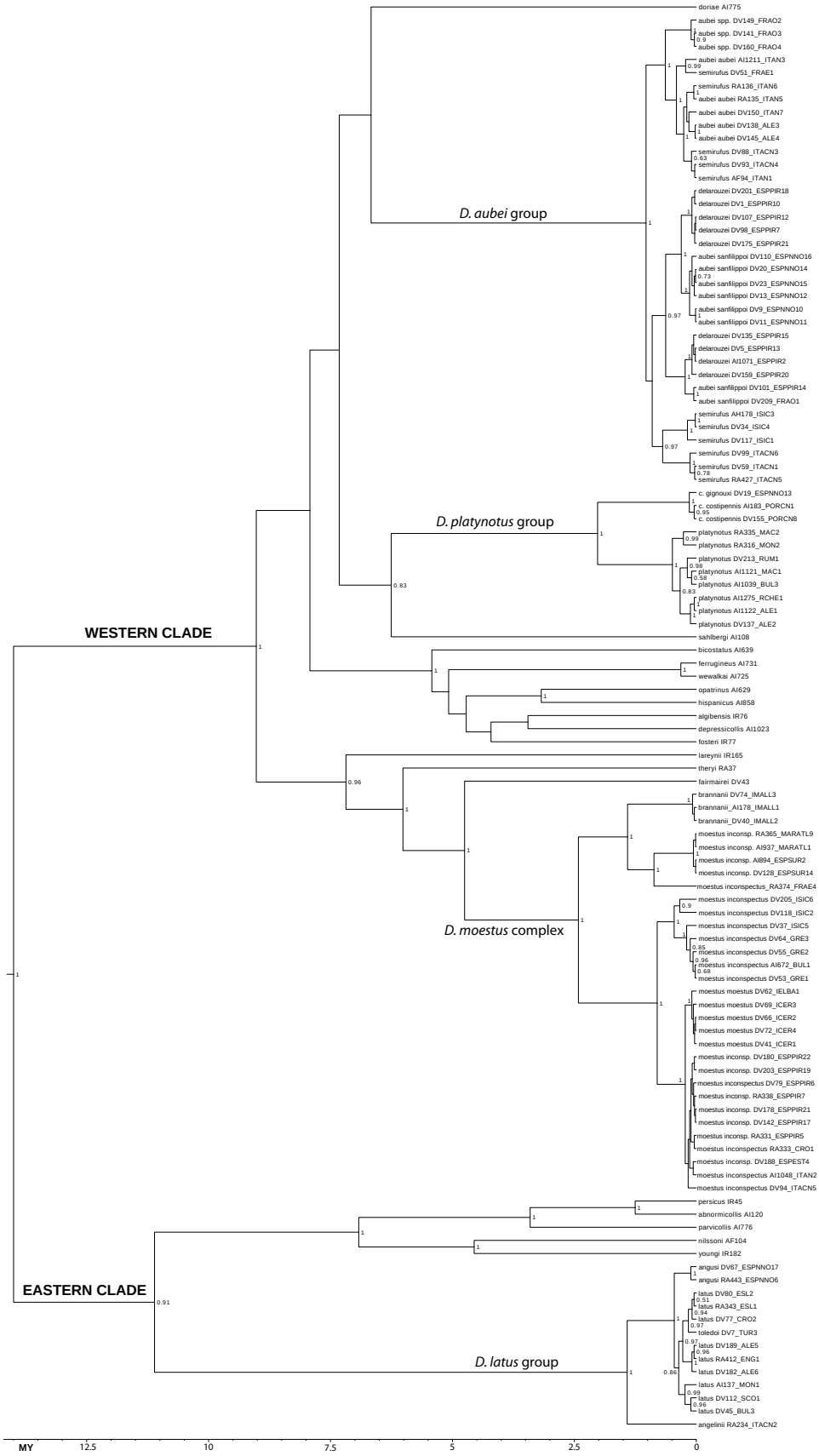
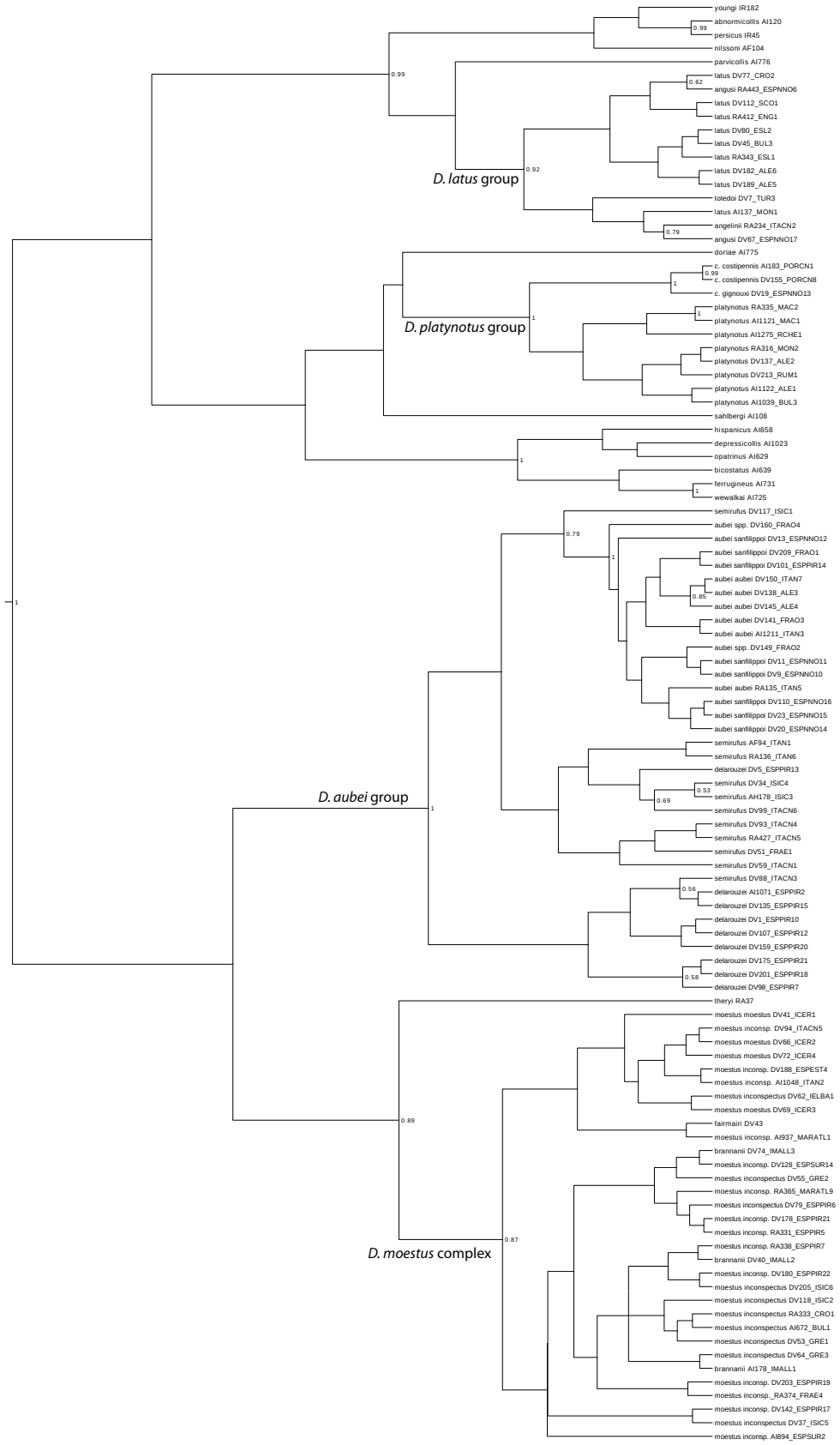


Figure S2. Ultrametric time calibrated tree obtained with BEAST using only the nuclear sequence data of the same specimens used in the analysis of the mitochondrial sequences (see Fig. S1). Numbers in nodes: Bayesian posterior probabilities. See Table S1 for details on the specimens and localities.



Chapter 3

Pleistocene range expansions in Western Palaearctic aquatic Coleoptera

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Pleistocene range expansions in Western Palaearctic aquatic Coleoptera

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ABSTRACT

The Quaternary glacial cycles lead to large changes in the size and location of the geographic distribution of many species. During glacial maxima large areas of central and northern Europe become inhospitable to temperate species, which are generally assumed to have been recolonized by range expansions from Mediterranean refugia in the interglacials. An alternative possibility is that the recolonization was from non-Mediterranean sources, from glacial refugia in central Europe or western Asia, but data are still scarce concerning the origin of the central and north European species with very large geographic distributions, especially for insects. In this work we studied some species of three wide distributed lineages of freshwater beetles (*Platambus maculatus* complex, *H. gracilis* complex, and the genus *Oreodytes*), all typical of running waters and including both narrowly distributed southern endemics and widespread European species, some with distributions spanning the whole Palearctic. The main goal was to understand the role of the Pleistocene glaciations in the diversification and current distribution of these species. For this we sequenced four mitochondrial and two nuclear genes of 212 specimens of 107 different populations of the different lineages and used Bayesian probabilities and Maximum Likelihood to reconstruct the phylogenetic relationships, age and geographical origin of the western Palaearctic species of these three widely distributed lineages. All species of the three studied groups were estimated to be of Pleistocene origin. In the *H. gracilis* complex, a secondary fast range expansion from northern Anatolian populations to western and northern Europe resulted in the

current widespread European *H. gracilis*. However, in the other two groups we found widespread central and northern European species the origin of which was not the Mediterranean area but central Asia, but with peripheral isolated forms in the southern Mediterranean peninsulas. These peripheral forms could have been confined as remnants of earlier diversification cycles or be result of incipient isolation of the most recent post-glacial expansion. The accumulation of narrow endemics of these lineages in the Mediterranean may thus be the result of successive cycles of range expansions with subsequent speciation (and local extinction in glaciated areas) through the multiple Pleistocene glacial cycles.

Keywords: glacial refugia, Dytiscidae, Hydraenidae, Quaternary glaciations, range expansion, Mediterranean Peninsulas, Central Asia

1. Introduction

The Quaternary was a period of drastic cyclic climatic changes, with multiple glacial–interglacial periods driven by variations in the orbit of the earth on Milankovitch timescales. Milankovitch climate oscillations lead to large changes in the size and location of the geographic distribution of many species, in some cases resulting in speciation due to the higher probability of isolation of small populations in areas under new selective regimes (Dynesius & Jansson, 2000). These Pleistocene climatic oscillations and the subsequent shifts in ecological conditions with the repeated fragmentation of populations during the glacials and interglacials have been hypothesised to be the cause of the origin of most extant European species (Rand, 1948; Mayr, 1970).

Some molecular studies have illustrated examples of species that are likely to have originated during the Pleistocene or Holocene in various groups of organisms, among them beetles (e.g. Barraclough & Vogler, 2002; Ribera & Vogler, 2004). Climatic changes were specially drastic in northern latitudes of the Palearctic region, as during the Last Glacial Maximum the European ice sheet extended north of 52° N, with permafrost north of 47° N (Dawson, 1992). Large areas of central and northern Europe become inhospitable to temperate species, while the Mediterranean peninsulas had a more temperate climate and vegetation (e.g. Huntley, 1988; Bennett *et al.*, 1991). However, although most of central and northern Europe and regions of Asia at similar latitudes were exposed to extremely cold conditions (Dawson, 1992), there were sheltered areas on the slopes of mountain ranges and along river valleys where moister conditions prevailed (Soffer, 1990) allowing occupation by temperate fauna and flora.

Two main hypotheses could explain the recent biogeographical origin of the current central and northern European fauna. Firstly, there is the traditional model of postglacial range expansion from Mediterranean refugia (e.g. Hewitt, 2000), in which central and northern Europe were colonized by a northward range expansion from Mediterranean peninsulas at the end of the last glaciation. According to this model the populations of most European species during the glacial maxima were confined to refuge areas in the southern peninsulas, from where they would have re-colonized the continent in the interglacials. Alternatively, a second possibility is that the colonization of central and northern Europe at the end of the Last Glacial was from non-Mediterranean source areas (Bilton *et al*., 1998; Abellán *et al*., 2011). According to this view, the isolation of the Mediterranean peninsulas during the glacial cycles led to

speciation, thus preventing genetic flow with the new colonisers of central and northern Europe during the next interglacial. Rather than being a source of re-colonizers, the southern peninsulas would thus be a source of endemism. These southern endemics remained confined to their restricted distributions in the southern areas, and only the species and/or populations at the edge of the southern peninsulas (e.g. Pyrenees, Alps, Carpathians) or in potential refuge areas in central Europe or western Asia would had been able to expand their ranges to recolonize the newly deglaciated areas in central and north Europe.

The isolation of the Mediterranean peninsulas has been suggested previously for small mammals (Bilton *et al*., 1998) and insects (e.g. Cooper *et al*., 1995). Among aquatic Coleoptera, the absence of fossil remains of southern species among the abundant central and northern European Quaternary subfossil remains (Abellan *et al*, 2011) supports the view of Mediterranean peninsulas as an area of endemism, not as a source of postglacial recolonisation. Data from extant species also showed than current southern endemics did not colonize northern areas (e.g *Hydrochus*, Hidalgo-Galiana & Ribera, 2011; *Enicocerus*, Ribera *et al*., 2010). Some central and northern European species may have their origin in the Mediterranean peninsulas, but in these cases it seems that populations that expanded their ranges were those located in the northernmost areas within the peninsulas, at the edge of the deglaciated area (e.g. Ribera *et al*., 2010 for species of *Enicocerus*, and García-Vázquez & Ribera, 2016 for *Deronectes*).

Despite the increased understanding of the evolution of the European insect fauna, data are still scarce concerning the origin of the central and north European species with very large geographic distributions. In this work we study some of these species, using molecular phylogeographic data to clarify their temporal and geographic origin and to understand the role of the Pleistocene glaciations in their diversification. We studied different species groups in three genera of freshwater beetles of two different families: (1) the *Hydraena gracilis* complex ("*Haenydra*" lineage, family Hydraenidae; (2) the *Platambus maculatus* complex (family Dytiscidae) and (3) the species *Oreodytes sanmarkii* and *O. davisii* (family Dytiscidae). They are all typical of running waters, and include both widespread European and narrowly distributed southern species (Nilsson & Hajek, 2015).

The genus *Hydraena*, with about 900 species widely distributed worlwide (Trizzino *et al.*, 2013) is the largest genus within the water beetle family Hydraenidae

and probably the most diverse among the aquatic Coleoptera (Jäch & Balke, 2008). Within the wider *Hydraena*, the "*Haenydra*" lineage of *Hydranea* s.str. includes *ca*.90 species with a north Mediterranean distribution (Trizzino *et al*., 2012). They are usually found in clean, fast flowing waters, often in mountain streams from the Iberian Peninsula to Iran and the Urals, but absent in North Africa (Jäch 2004; Ribera *et al.*, 2011; Trizzino *et al.*, 2011; Trizzino *et al.*, 2012). Many species of this lineage have very restricted distributions, often limited to a single valley or mountain system, but there are also few species with very wide geographical ranges. In this work we focus in the most widespread species of the "*Haenydra*" lineage, *Hydraena gracilis* Germar and its closest narrowly distributed relatives, the *H. gracilis* complex *sensu* Jäch (1995).

The genus *Platambus* contains 64 recognised species (Nilsson, 2015). The genus has a wide distribution, being present in the Palearctic, Nearctic, Neotropical and Oriental regions, and is currently divided in nine species-groups (Nilsson, 2015). Among them the group of *P. maculatus* as defined by Nilsson (2001) is the largest, with 24 species distributed through Asia and Europe. In a molecular phylogeny of Agabinae Ribera *et al*., (2004) recovered a paraphyletic genus *Platambus*, with the species of the *P. maculatus* group separated of other species of Asian and American *Platambus*. We focus on the most widespread species of the group, *P. maculatus* (Linnaeus) and its closest relative, *P. lunulatus* (Fischer von Waldheim), to which we refer as the *Platambus maculatus* complex.

Finally, the Holarctic genus *Oreodytes* Seidlitz (Family Dytiscidae) contains 30 recognised species, four of them split into two subspecies (Fery, 2015; Nilsson, 2015). *Oreodytes* species live in cool and cold streams or lakes margins, generally at high altitude or high latitude (Balfour-Browne, 1940; Zack, 1992; Nilsson & Holmen, 1995). The genus is distributed in the Palearctic and Nearctic zoogeographic regions, with six species occurring in Europe. In a previous study, Ribera (2003) recovered a paraphyletic genus *Oreodytes*, although with low bootstrap support. *Oreodytes* was divided in two lineages corresponding to the main distinction within the genus in body size and shape, i.e. species larger and more elongated (including *O. davisii*) and species smaller and more round (including *O. sanmarkii*).

Using a combination of mitochondrial and nuclear data we provide here a reconstruction of the phylogenetic relationships, the age and the geographical origin of the western Palaearctic species of these three widely distributed lineages to understand

the effect of the Quaternary glacial cycles in their diversification and current distribution.

2. Material and Methods

2.1. Taxonomic background and taxon sampling

a) *Hydraena gracilis* complex

The *H. gracilis* complex *sensu* Jäch (1995) includes seven recognised species and one subspecies (Trizzino *et al.*, 2013). *Hydraena gracilis* is widely distributed in almost the whole of Europe, ranging from southern France eastwards to Ukraine and northwards to Finland, including the British Isles (Fig. 1). Previous molecular studies (Ribera *et al.*, 2011) showed that despite its widespread distribution, molecular differences among a reduced sample of specimens throughout its geographic range were minimal. Jäch (1995) found morphological differences between specimens from the Balkans and the rest of Europe, supporting the existence of a possible subspecies, *H. gracilis balcanica*, described by d'Orchymont (1930). *Hydraena gracilis* is absent from the Iberian and Anatolian peninsulas, where it is replaced by different species of the complex (Fig. 1). *Hydraena gracilidelphis* Trizzino, Valladares, Garrido & Audisio is the westernmost species, distributed in the Iberian Peninsula (mainly in the north but with some records in the southwest) and the French Pyrenees. The Anatolian Peninsula and adjacent areas are occupied by three species: *H. anatolica* Janssens distributed in northern and eastern Anatolia and probably in areas of the Caucasus and Northwestern Iran; *H. graciloides* Jäch in northern Turkey; and *H. crepidoptera* Jäch known only for two northern Turkish provinces (Kastamonu and Sinop). The other two species of the complex, *H. nike* Jäch and *H. elisabethae* Jäch, are endemic to two Aegean islands, Samothraky and Thassos respectively.

We studied a total of 48 specimens of five of the seven species of the *H. gracilis* complex (we could not obtain fresh specimens of the two Aegean Island endemics) from 37 different localities, covering the full geographical range of the studied species (see Supplementary Information, Table S1). As outgroups we used three sequences of closely related species of the "*Haenydra*" lineage (Trizzino *et al*., 2011).

b) *Platambus maculatus* complex

Platambus maculatus has a wide Palearctic distribution from western Iberian Peninsula to northern Iran and Mongolia, including the Mediterranean peninsulas (Italy,

Balkans and Anatolia), Scandinavia and the British Isles (Nilsson & Hájek, 2015) (Fig. 2). The species has a very variable elytral design, which led to the description of many forms currently all considered as synonyms of *P. maculatus* (Nilsson & Hájek, 2015). Most conspicuous among them is *P. graellsi* (Gemminger & Harold) from the north and centre of the Iberian Peninsula (Millán *et al*., 2014). The other species of the complex, *P. lunulatus*, is distributed from the Anatolian Peninsula and areas of the Caucasus and Middle East to Egypt (Karaman *et al.*, 2008; Nilsson & Hájek, 2015) (Fig. 2).

We sequenced 106 specimens from 67 different localities of the two recognised species of the *P. maculatus* complex, including *P. graellsi* (see Supplementary Information, Table S1). As outgroups we used seven specimens from different species of the *Platambus maculatus* group.

c) European species of the genus *Oreodytes*

Of the six European species of *Oreodytes*, the most widespread is *O. sanmarkii* (C. R. Sahlberg), widely distributed over large parts of the Palearctic, from the Iberian Peninsula to the Russian Far East, reaching the Nearctic in the northern parts of Canada (Larson *et al*., 2000) (Fig. 3). In southern Europe, the species is known from the Iberian Peninsula, northern provinces of Italy and the Balkans until Bulgaria and Macedonia (Fery, 2015; Nilson & Hájek, 2016). The species has a high level of variability over its huge area of distribution (Larson, 1990) but only one subspecies, *O. sanmarkii alienus* (Sharp), endemic to the Iberian Peninsula, is currently recognised (Balke, 1989; Nilson & Hájek, 2015). Also with a wide Palaearctic distribution is *O. davisii* (Curtis), known from the British Isles to Ukraine and the Caucasus, including Scandinavia, the Mediterranean peninsulas and Turkey (Erman & Erman, 2002; Nilsson & Hájek, 2016) (Fig. 3). There is also an Iberian form recognised as subspecies, *O. davisii rhianae* Carr (Nilsson & Hájek, 2016). The other European species of the genus are *O. septentrionalis* (Gyllenhal), distributed from the Iberian Peninsula to eastern Siberia and Mongolia (Erman & Erman, 2002; Nilsson & Hájek, 2016); *O. alpinus* (Paykull), with a northern Palearctic distribution, being present from Scandinavia and some lochs in northern Scotland (Foster, 1992) to Kamchatka in the Russian far East (Nilsson & Kholin, 1994); *O. meridionalis* Binaghi & Sanfilippo, endemic to the southern Apennines (Rocchi 2007, Nilsson & Hájek, 2015); and the recently described *O. angelinii*, from Greece (Fery, 2015).

We studied 58 specimens of 35 different localities of the European species of the genus *Oreodytes* with the exception of the Italian endemic *O. meridionalis* and the newly described *O. angelinii*, with a focus on the species *O. davisii* and *O. sanmarkii* and the two Iberian subspecies (*O. d. rhianae* and *O. s. alienus* respectively). We also included in the analysis 16 specimens of different Asian and American species of the genus as outgroups (see Supplementary Information, Table S1).

2.2. DNA extraction and sequencing

Specimens were collected and preserved in absolute ethanol directly in the field. We extracted the DNA non-destructively with commercial kits (mostly "DNeasy Tissue Kit", Qiagen GmbH, Hilden, Germany and "Charge Switch gDNA Tissue Mini Kit", Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Specimens and DNA extractions are kept in the collections of the Institut de Biología Evolutiva, Barcelona (IBE), Museo Nacional de Ciencias Naturales, Madrid (MNCN) and Natural History Museum, London (NHM). We obtained seven gene fragments from six different genes (four mitochondrial and two nuclear) in five different amplification reactions (see Supplementary Information, Table S2 for primers and typical sequencing reactions): 1) 5´end of the Cytochrome Oxidase Subunit 1 gene (the barcode fragment, Hebert et al., 2003, COI-5'); 2) 3´end of Cytochrome Oxidase Subunit 1 (COI-3´); 3) 5´end of 16S rRNA plus tRNA transfer of Leucine plus 3´end of NADH subunit 1 (nad1) (16S and nad1respectively); and internal fragments of the nuclear genes 4) Histone 3 (H3) and 5) Wingless (Wg). Due to their lower variability, the nuclear markers were only used in representative specimens according to geographical and topological criteria. For each amplification reaction we obtained both forward and reverse sequences. In some specimens, and due to difficulties of amplification, we used internal primers for the fragment COI-3´, obtaining two fragments of 400 bp each (Supplementary Information, Table S2). The obtained products were purified by standard ethanol precipitation and sent to external facilities for sequencing after purification. DNA sequences were assembled and edited using the Geneious 6 software (Biomatters Ltd, Auckland, New Zealand). New sequences (566) have been deposited in GenBank with accession numbers XX-XX (Supplementary Information, Table S1).

2.3. Phylogenetic and divergence time analyses

Edited sequences were aligned with MAFFT v.6 using the G-INS algorithm and default values for other parameters (Katoh & Toh, 2008). We included in some analyses sequences obtained from the literature (GenBank and BOLD (http://www.boldsystems.org/) databases) to increase the geographic coverage and to check for possible variation not covered by our sampling.

In the phylogenetic analyses we used six partitions corresponding to the gene fragments COI-5', COI-3', 16S, nad1, H3 and Wg, and used Partition Finder 1.1.1 (Lanfear *et al*., 2012) to estimate the best-fitting models of nucleotide substitution for each partition separately, using the AIC (Akaike Information Criterion) as selection criteria (Katoh & Toh, 2008). To infer the phylogeny of the three studied groups and estimate the divergence dates among species we used Bayesian methods implemented in Beast 1.8 (Drummond *et al*., 2012). For the analyses we included both mitochondrial and nuclear markers and implemented the closer available evolutionary model to those selected by Partition Finder. We used a Yule speciation model and ran two analyses to test the clock model (strict or lognormal relaxed) that best fitted the data. As there are no fossils or unambiguous biogeographic events that could be used to calibrate the phylogeny of the studied groups we applied published estimations of a-priori rates for the same genes in related groups. For the *Platambus maculatus* complex and the genus *Oreodytes* (both in family Dytiscidae) we used a rate of 0.013 substitutions/site/MY (standard deviation 0.002) for protein coding genes and 0.0016 substitutions/site/MY (standard deviation 0.0002) for 16S, obtained in a related group (family Carabidae) for the same combination of mitochondrial protein coding and ribosomal genes (Andujar *et al*., 2012). For the *Hydraena gracilis* complex we used a rate of 0.015 and 0.006 substitutions/site/MY (standard deviation 0.002 and 0.0002) for the COI-3'+nad1 and 16S partitions respectively, obtained for family Leiodidae (Cieslak *et al*., 2014). Clock rates of *H3* and *Wg* were left with uniform priors due to the absence of any suitable estimations of the evolutionary rate for these nuclear genes. We executed two independent analyses with the same settings, running 100 million generations (saving trees every 5000) or until they converged and the number of trees was sufficient according to Effective Sample Size (ESS) values, as measured with Tracer v1.6 (Rambaut *et al*., 2014). The majority rule consensus tree of the two runs was compiled with Tree Anotator v1.8 (Drummond *et al*., 2012) and visualized with FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). For model selection of the best molecular clock we used the modified Akaike information criterion (AICM) with moments

estimator (Baele *et al*., 2012), as implemented in Tracer v1.6, with 1000 bootstrap replicates.

To test for potential topological discordances among mitochondrial and nuclear data we analysed the nuclear genes only, applying the best clock and the same settings as the combined (mitochondrial and nuclear) analysis.

We also analysed the combined matrix (mitochondrial and nuclear) using maximum likelihood (ML) methods. ML analysis were performed in RAxML v.7.4.2 (Stamatakis *et al*.**,** 2008) as implemented in RAxML GUI v 1.3.1 (Silvestro & Michalak, 2012), executing a ML thorough bootstrap with 100 tree searches, using the GTR+G as an evolutionary model with the same six partitions as in the Bayesian analysis. Node support was estimated with 1000 bootstrap replicates.

3. Results

There were no length differences in protein coding genes, and the length of ribosomal genes in the ingroup ranged between 784-786 bp in the *H. gracilis* complex and 796-797 bp in the *P. maculatus* complex and the genus *Oreodytes*. The best evolutionary models as selected by Partition Finder for each individual partition (Supplementary Information, Table S3) were implemented in the Bayesian analysis for the *H. gracilis* complex and the species of the genus *Oreodytes.* In the *P. maculatus* complex when the more complex models were applied the analyses did not converge adequately for both the relaxed lognormal and strict clocks, so we applied an HKY+I+G model to each partition.

3.1. Hydraena gracilis complex

The nucleotide alignment matrix showed no variability for the two nuclear genes, which were therefore excluded from the subsequent analyses. The analysis using a lognormal relaxed clock did not converge adequately so we used a molecular strict clock.

The ultrametric tree obtained with the combined mitochondrial and nuclear data estimated a recent origin and diversification of the *H. gracilis* complex (ca. 0.3MY) (Fig. 4). Its monophyly was strongly supported, as well as its separation into two major clades. The first included the Iberian *H. gracilidelphis* and the second was formed by two groups, one containing specimens of *H. anatolica*, *H. graciloides* and *H. crepidoptera* from northern Turkey and the other with the widespread European *H.*

gracilis, including the subspecies *H. gracilis balcanica* and two isolated Turkish specimens. Among the later clade, specimens from the southeasternmost populations occupied a basal position, with the more western and northern populations nested within them. We also recovered a lineage including all specimens from the easternmost populations of the current distribution area (eastern Anatolia, the Caucasus and Iran), identified as *H. anatolica* and *H. graciloides*. This eastern lineage had a poorly supported position between the two main clades of the complex, which was variable depending on the method of analysis used. Bayesian analysis (Fig. 4) recovered the eastern lineage as a sister group of the Iberian *H. gracilidelphis*, while ML analysis (Supplementary Information, Fig. S1) linked the eastern lineage to the other clade, as a sister of *H. gracilis* and the westernmost populations of Turkey, with *H. gracilidelphis* sister to the rest of the complex, but always with low support.

3.2. Platambus maculatus complex

The relaxed lognormal was significantly better than the strict molecular clock, and was therefore implemented in the Bayesian analyses (Table 1). The temporal origin of the *P. maculatus* complex and its separation from the rest of the *P. maculatus* groups was estimated to have occurred in the Middle Miocene (ca. 12MY) (Fig. 5), although the split between the two extant species (*P. maculatus* and *P. lunulatus*) occurred in the Messinian (ca. 6.5MY). Current intraspecific variability dates from the Pliocene-Pleistocene boundary (2.5-3.5MY). The ultrametric tree obtained from Bayesian analysis using the combined mitocondrial and nuclear matrix strongly supported the monophyly of the two recognised species (Fig. 5). *Platambus lunulatus* was divided in two clades, one with specimens from northeastern Turkey and other with specimens from the west of Turkey, the Caucasus and the Middle East.

Platambus maculatus was divided in three clades, one with specimens with a predominantly western distribution (northern Spain, France and British Isles, including the specimens of some Scottish lochs in a monophyletic lineage); a second clade with specimens of easterly distribution (central Europe, Scandinavia, Balkans, Anatolia and Middle East); and a third clade including the specimens of the Iberian *P. graellsi* plus the specimens of *P. maculatus* from north Italy and some from one locality in the Pyrenees. The relationships between these lineages were not well supported, and varied depending on the different analyses. In the Bayesian analysis using only nuclear markers (Supplementary Information, Fig. S2) all specimens of *P. graellsi* plus the

north Italian and Pyrenean *P. maculatus* were included within the western clade. In the ML analysis, on the contrary (Supplementary Information, Fig. S3), we recovered the north Italian specimens of *P. maculatus* as sister to the rest of the specimens, which were split in three poorly supported groups (western clade, eastern clade and *P. graellsi* plus some Pyrenean *P.maculatus*).

3.3. European species of the genus Oreodytes

A relaxed lognormal clock was preferred over a strict molecular clock, and was implemented in the Bayesian analyses (Table 1). Preliminary results showed the existence of two group of species, one phylogenetically closely related to *O. sanmarkii* (the *O. sanmarkii* group) and the other group of species related to *O. davisii* (the *O. davisii* group), the later including the European *O. septentrionalis* and *O. alpinus*. These two clades were constrained as monophylethic in subsequent Bayesian analyses without outrgroups. *Oreodytes alpinus* was nested within *O. davisii* in the analysis using the combined mitochondrial and nuclear matrix (Fig. 6), forming a clade with the Mongolian *O. mongolicus* (Brinck). In the analysis using only the nuclear data *O. alpinus* and *O. mongolicus* were also sisters, but both sisters to the rest of *O. davisii* (Supplementary Information, Fig. S4)

Divergence time analysis (Fig. 6) dated the separation between the *O. davisii* and *O. sanmarkii* groups in the Oligocene (ca. 27MY) and the diversification within each group in the Lower Miocene (ca. 22MY) for the species of the *O. sanmarkii* group and the Middle Miocene for the species of the *O. davisii* group (ca. 13MY). The sister species of the clades *O. davisii*+*O. alpinus*+*O. mongolicus* and *O. sanmarkii* were respectively the North American *O. snoqualmie* (Hatch) and *O. obesus* (LeConte). Intraspecific variation in both species, *O. davisii* and *O. sanmarkii*, was of Pleistocene origin (2-1.5MY).

We did not found a clear geographical pattern in *O. davisii* irrespective of the method of analysis (Bayesian or ML). On the contrary, *O. sanmarkii* was divided in two clades with a clear geographic pattern, one including specimens with an eastern distribution, from Mongolia to central Europe, and the other mainly with specimens distributed in the west, in the Iberian Peninsula, Italy and British Isles, although also including some specimens from the Carpathians. Within the western clade, Iberian populations from the Pyrenees were separated of those from Portugal and central and northwestern Spain, the later identified as the subspecies *O. sanmarkii alienus*. This

difference was more pronounced in the ML analysis (Supplementary Information, Fig. S5) (although with poor support) and in the analysis using only nuclear data (Supplementary Information, Fig. S4), in where we recovered the specimens of *O. s. alienus* as a sister of the rest of *O. sanmarkii*.

With respect to the other European species of the group, we found a deep divergence between the Mongolian and European specimens of *O. septentrionalis*, estimated at the late Miocene (Fig. 6).

4. Discussion

4.1. Hydraena gracilis complex

According to our results the *H. gracilis* complex has a recent, Pleistocene origin, with a extreme genetic homogeneity of the widespread *H. gracilis* through its range (Fig. 4). This suggests a very recent expansion, in agreement with preliminary results for this and other species of the *Haenydra* lineage (Ribera *et al*., 2011). The existence of narrow endemics in the periphery of the range of the complex, both in the westernmost (Iberian Peninsula) and easternmost areas (Anatolia, Azerbaijan and Iran) indicates the existence of successive cycles of range expansions within the complex followed by local extinctions, likely during the glacial periods, with remaining populations in refuge areas that resulted in the current *H. gracilidelphis* in the Iberian Peninsula and *H. anatolica* and *H. graciloides* in East Turkey and the Middle East, also in agreement with the model for the diversification of the group proposed in Ribera *et al*., (2011).

With our current data it was not possible to fully resolve the relationships of the eastern Anatolian and Iranian populations, which may be the remnants of earlier diversification cycles (as is the case of *H. gracilidelphis* in the west) or the first isolated lineages of the last range expansion. Our results also suggest that the taxonomic status of *H. anatolica* and *H. graciloides* needs to be reviewed. In any case, the ancestral position of northern and central Turkey populations in the clade of *H. gracilis* indicates a second fast range expansion from these areas to western and northern Europe, resulting in the current widespread European *H. gracilis*.

4.2. Platambus maculatus complex

Although the precise geographic origin of the *P. maculatus* complex is still unknown, their closest relatives are distributed in eastern and central Asia (Nilsson, 2015; Nilsson & Hájek, 2015). After a western range expansion from central Asia one

lineage differentiated in Minor Asia and Anatolia, resulting in *P. lunulatus*, and the other through Europe resulting in *P. maculatus*.

The range expansion in *P. maculatus* followed a clear geographic pattern, with a split between western and eastern populations in two well-supported clades (Fig. 5). The exception is the uncertain position of the specimens of northern Italy and the Iberian variety *P. graellsi*, both with appreciable morphological differences in elytral design and sculpture. North Italian specimens were recognised as amongst the largest, most convex and shiniest of the species (Sharp, 1882), while the populations from central Iberia were originally described as a distinct species (*Agabus glacialis* Graells, subsequently changed to *A. graellsi*), but then reduced to a variety and finally synonymised with *P. maculatus*. They have a more reddish coloration than the typical forms of *P. maculatus*, with a poorly defined design and a very dense and deep microsculpture given their dorsal surface a rough, dull appearance (Sharp, 1882). Both central Iberian and north Italian populations are at the periphery of the main range of the species, in a situation similar to that of *H. gracilidelphis*, as seen above. In this case, there is no conclusive evidence to support an earlier origin of these forms, which could have been confined in the Iberian and Italian Peninsulas as remains of a first expansion, but could also be result of incipient isolation of the most recent expansion.

Within the western clade, specimens from some oligotrophic Scottish lochs, also in the periphery of the main range of the species, formed a monophyletic lineage (Fig. 5). This is most remarkable, as they were, together with *P. graellsi* and the north Italian populations, the only ones to deserve a mention in the monumental revision of Sharp (1882). They were described as being the smallest among the species, with a deeper (less convex) shape, with a dull surface and reduced yellow markings on the elytra (Sharp, 1882). We performed additional analyses including new sequences from databases to search for specimens with the same haplotype as the Scottish specimens, and only found a northern Swedish sequence nested within the Scottish individuals (from Bergsten *et al*., 2012), although the specimen has an external morphology within the typical range of the continental populations.

4.3. European species of the genus Oreodytes

Most species of the genus *Oreodytes*, including the sister species of both *O. davisii* and *O. sanmarkii* (*O. snoqualmie* and *O. obesus* respectively) are distributed along western areas of United States and Canada (Larson, 1990; Larson *et al*., 2000).

The geographic origin of the European species seems thus to be a range expansion through Asia reaching Europe. In the case of *O. davisii* this expansion has not left any apparent relationship between geographic and phylogeographic structure with the studied markers. In the combined analyses (largely driven by mitochondrial data) *O. alpinus* and *O. mongolicus* were nested within *O. davisii*, despite considerable morphological differences among the species (Shaverdo & Fery, 2006; Foster & Friday, 2011) (Fig. 6). On the contrary, the analysis using the nuclear data only separated both species from *O. davisii*, suggesting some process altering the mitochondrial sequence, likely by mitochondrial introgression between populations of closely related species sharing the same geographical range. This is a common pattern in glacial refugia, also observed in other taxa (e.g. Berthier *et al*., 2006; Nichols *et al*., 2012) including aquatic beetles (Hidalgo-Galiana *et al.*, 2014, García-Vázquez & Ribera, 2016).

In *O. sanmarkii* there is a more defined geographic structure, with geographical isolation among a western and eastern extant clades dating from the middle Pleistocene (Fig. 6). This suggests an early origin of the initial European populations with subsequent isolation of different eastern and western refugia. Both species are very cold resistant, likely able to survive in relatively northern latitudes during the glacial cycles, which may have favoured their local persistence. However, recent work with direct sequencing of ancient material has demonstrated unexpected patterns of migration not apparent from the distribution of current haplotypes (Valdiosera *et al*., 2008).

Similarly to what happened with the *P. maculatus* and *H. gracilis* complexes, the only recognised forms within *O. davisii* and *O. sanmarkii* are the Iberian *O. davisii rhianae* and *O. sanmarkii alienus*, west and south of the Ebro valley (Millán *et al*., 2014). In the analysis of the nuclear data, and also in the ML analysis of the combined data, the sequences specimens of *O. sanmarkii alienus* were monophyletic, although in an unsupported position with respect to the rest of the species. Independently of its taxonomic status it seems thus to be isolated from the mainland populations, reinforcing the pattern of the peripheral disaggregation of widespread European species.

4.4. Conclusions

The effect of the Pleistocene glaciations was a key feature in the origin of most extant central and northern European species of lotic water beetles. The general emerging pattern is that central and northern Europe were colonized by range expansions from peripheral refugia at the end of the last glaciation, especially the

northern areas of the Mediterranean peninsulas. Of particular relevance in the studied species were the Iberian and the Anatolian Peninsulas, which were both the source of recolonising populations and the cradle for newly formed narrow endemics. However, in addition to this classic pattern we also found widespread central and northern European species which origin was not the Mediterranean area but central Asia, but also with peripheral isolated forms in the southern Mediterranean peninsulas that showed in some cases different haplotypes from those found in central and northern Europe. The colonization of these species may have occurred from sheltered glacial refugia in central and eastern Europe or western Asia. Some suitable areas may have been refugial populations in the northern Balkans, the southern slopes of the Carpathian and Ural Mountains, northern slopes of the Altai Mountains or the Peninsula of Crimea (Grichuk, 1984; Soffer 1990; Demesure *et al.,* 1996). Range expansions in these cases did not result in mixing and homogenisation of genetic pools, but in isolation and differentiation of new species. The accumulation of narrow endemics of these lineages in the Mediterranean may thus be the result of successive cycles of range expansions with subsequent speciation (and local extinction in glaciated areas) through the multiple Pleistocene glacial cycles.

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Author's contributions

DG-V and IR conceived the work; DTB, GNF and IR led the specimen collection, DG-V and IR analysed the data and led the writing, all authors contributed to the discussion of results and the writing.

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Figure legends

Figure 1. Known distribution of the studied species of the *Hydraena gracilis* complex. White circles, sampled localities.

Figure 2. Known distribution of the studied species of the *Platambus maculatus* complex. White circles, sampled localities.

Figure 3. Known distribution of *Oreodytes sanmarkii* and *Oreodytes davisii* in the Western Palearctic. Coloured circles, sampled localities for each species, including also those from *Oreodytes alpinus* and *Oreodytes septentrionalis*. Localities from Mongolia and Siberia for *O. sanmarkii* and *O. alpinus* respectively are not show.

Figure 4. Phylogenetic tree of the studied species of the *Hydraena gracilis* complex. Ultrametric tree obtained with BEAST with the combined nuclear and mitochondrial sequences and a partition by gene. Numbers on nodes, Bayesian posterior probabilities higher than 0.5. See Supplementary Information, Table S1 for details on the specimens and localities. Habitus photograph, *H. gracilis* (from Lech Borowiec, http://www.colpolon.biol.uni.wroc.pl/index.htm).

Figure 5. Phylogenetic tree of the studied species of the *Platambus maculatus* complex. Ultrametric tree obtained with BEAST with the combined nuclear and mitochondrial sequences and a partition by gene. Numbers on nodes, Bayesian posterior probabilities higher than 0.5. See Supplementary Information, Table S1 for details on the specimens and localities. Habitus photograph, *P. maculatus* (from L. Borowiec, http://www.colpolon.biol.uni.wroc.pl/index.htm).

Figure 6. Phylogenetic tree of the studied species of *Oreodytes*. Ultrametric tree obtained with BEAST with the combined nuclear and mitochondrial sequences and a partition by gene. Numbers on nodes, Bayesian posterior probabilities higher than 0.5. See Supplementary Information, Table S1 for details on the specimens and localities. Habitus photographs, *O. sanmarkii* (from L. Borowiec, http://www.colpolon.biol.uni.wroc.pl/index.htm) and *O. davisii* (from U. Schmidt, https://www.kaefer-der-welt.de/index.htm).

Table 1: Analyses of the best molecular clock model for each group, including AICM values and standard error (SE). In bold, best AICM value for each pair. Differences below 2 were not considered as significant. The relaxed model clock for the *H. gracilis* complex failed to converge and was not considered (see `Results').

GROUP	CLOCK MODEL	AICM	SE
H. gracilis cpx.	relaxed	**9076.7**	+/- 0.109
	strict	9385.8	+/- 0.242
P. maculatus cpx.	relaxed	**18568.9**	+/- 0.525
	strict	18596.7	+/- 0.442
Oreodytes	relaxed	**23948.4**	+/- 0.868
	strict	23952.8	+/- 0.563

Figure 1
H. gracilis complex

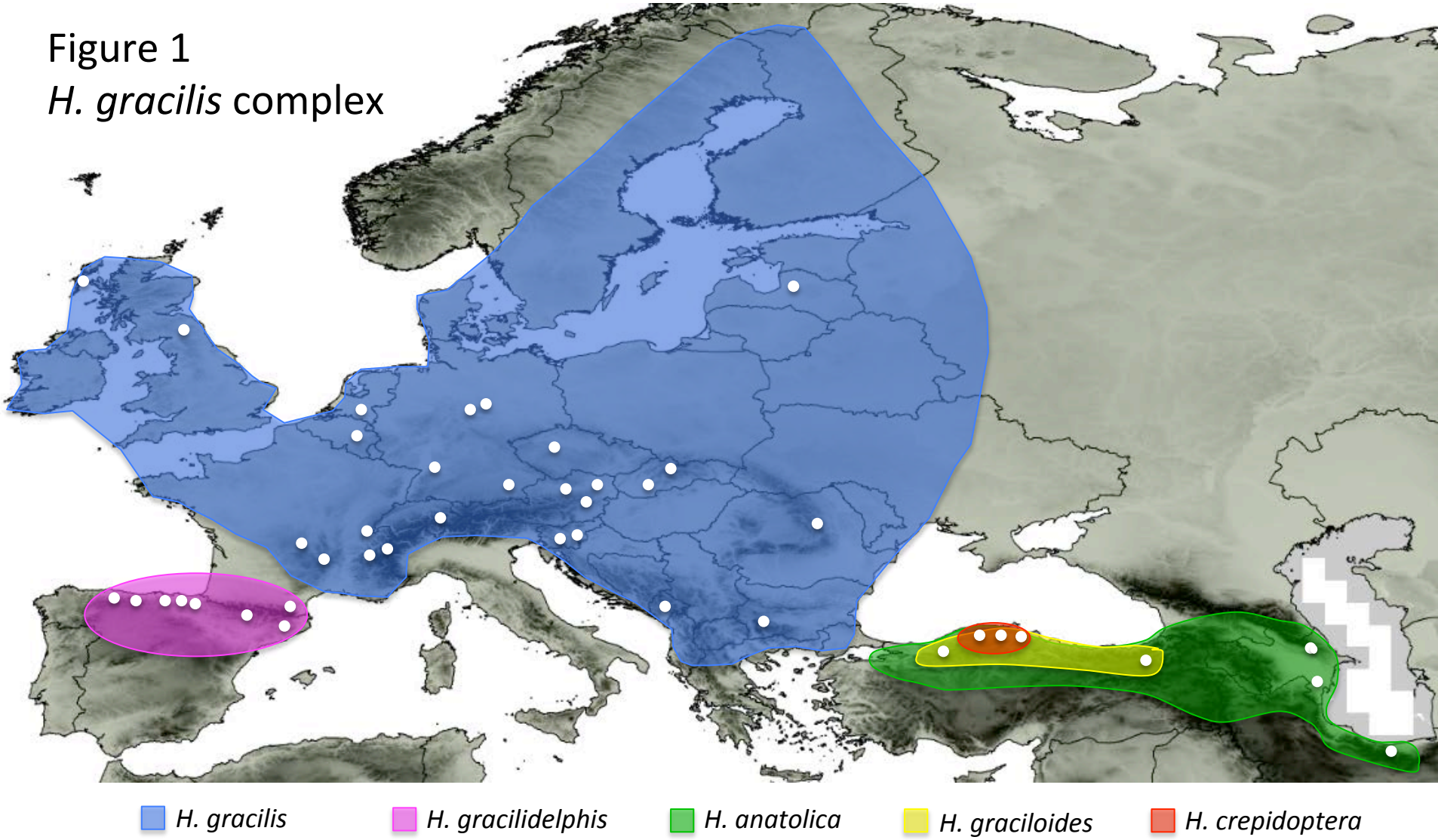
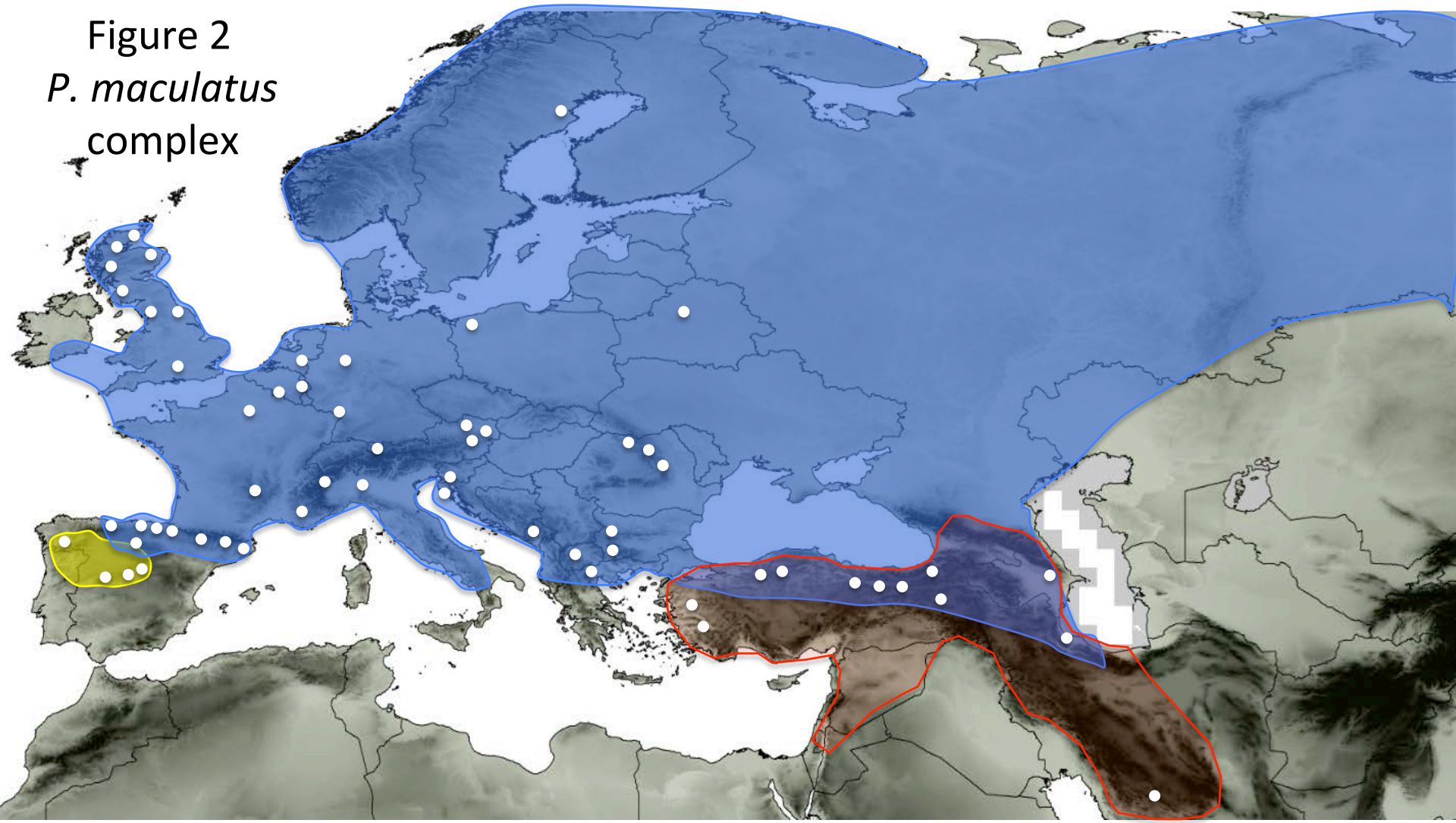
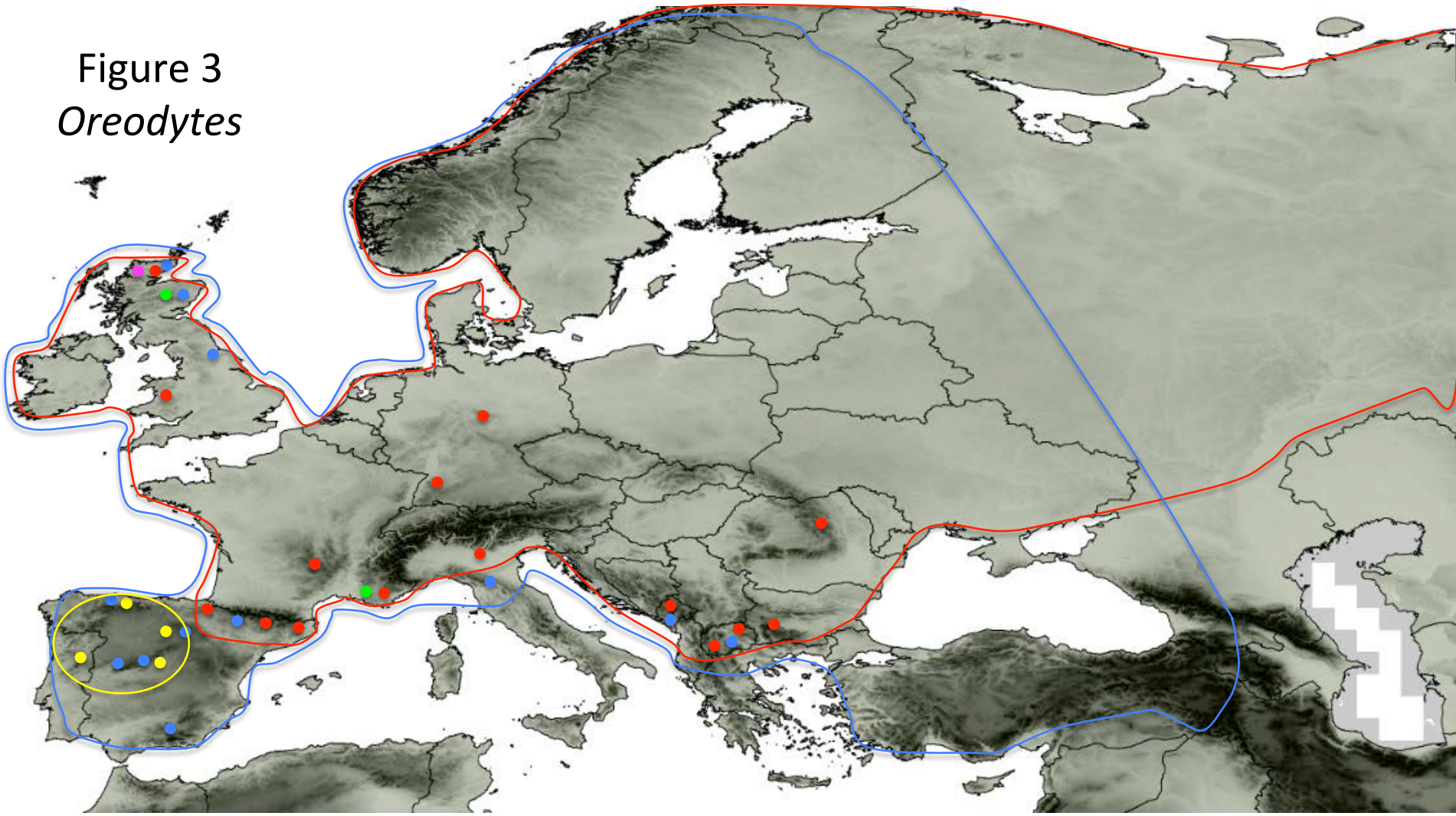


Figure 2
P. maculatus complex



P. maculatus *P. lunulatus* *P. maculatus graellsi*

Figure 3
Oreodytes



O. davisii *O. sanmarkii* *O. sanmarkii alienus* *O. alpinus* *O. septentrionalis*

Figure 4

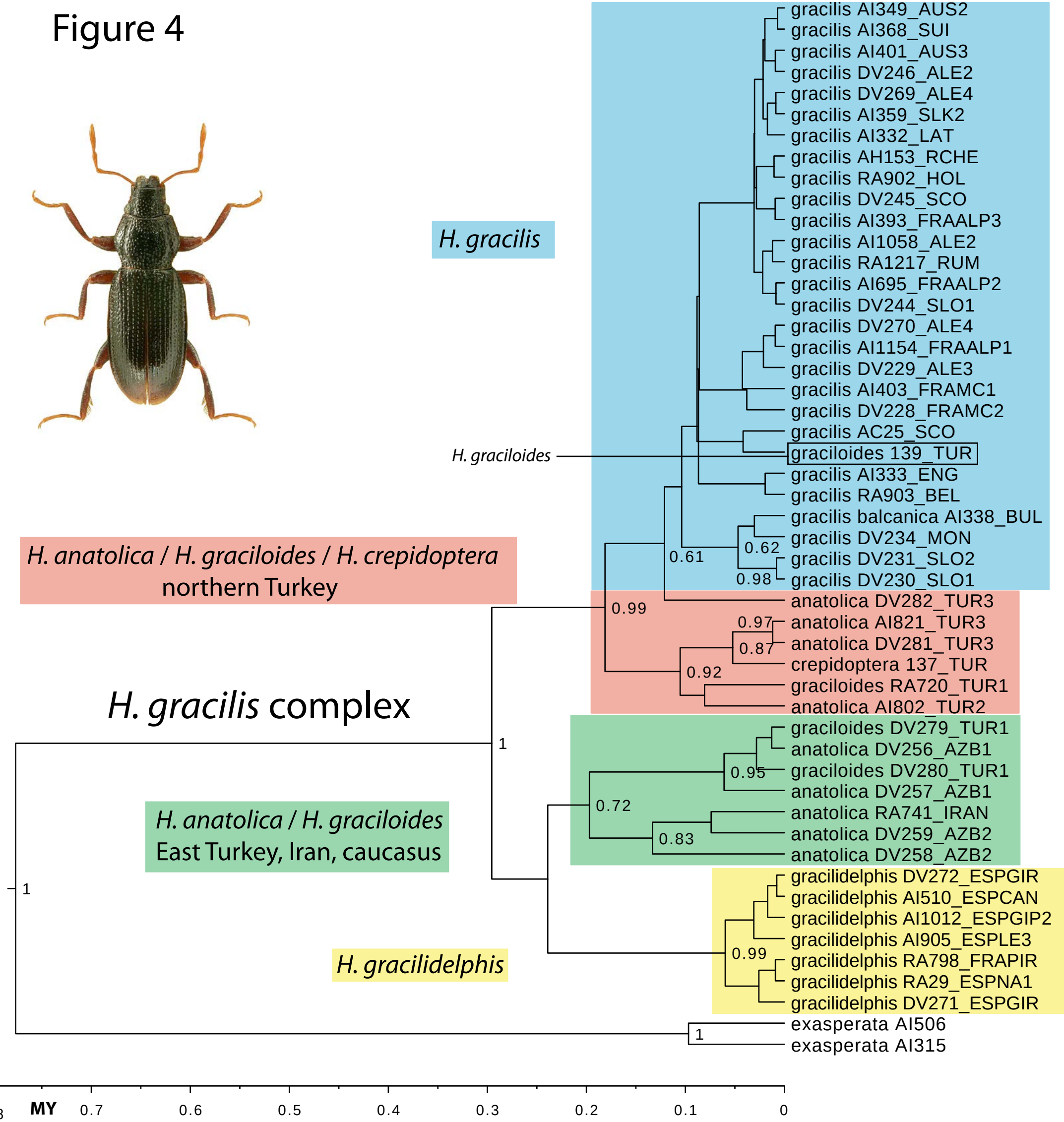

H. gracilis
gracilis AI349_AUS2
gracilis AI368_SUI
gracilis AI401_AUS3
gracilis DV246_ALE2
gracilis DV269_ALE4
gracilis AI359_SLK2
gracilis AI332_LAT
gracilis AH153_RCHE
gracilis RA902_HOL
gracilis DV245_SCO
gracilis AI393_FRAALP3
gracilis AI1058_ALE2
gracilis RA1217_RUM
gracilis AI695_FRAALP2
gracilis DV244_SLO1
gracilis DV270_ALE4
gracilis AI1154_FRAALP1
gracilis DV229_ALE3
gracilis AI403_FRAMC1
gracilis DV228_FRAMC2
gracilis AC25_SCO
H. graciloides
graciloides 139_TUR
gracilis AI333_ENG
gracilis RA903_BEL
gracilis balcanica AI338_BUL
gracilis DV234_MON
gracilis DV231_SLO2
gracilis DV230_SLO1
0.62
0.61
0.98
0.99
H. anatolica / H. graciloides / H. crepidoptera
northern Turkey
anatolica DV282_TUR3
anatolica AI821_TUR3
anatolica DV281_TUR3
crepidoptera 137_TUR
graciloides RA720_TUR1
anatolica AI802_TUR2
0.97
0.87
0.92
H. gracilis complex
1
graciloides DV279_TUR1
anatolica DV256_AZB1
graciloides DV280_TUR1
anatolica DV257_AZB1
anatolica RA741_IRAN
anatolica DV259_AZB2
anatolica DV258_AZB2
0.95
0.72
0.83
H. anatolica / H. graciloides
East Turkey, Iran, caucasus
1
gracilidelphis DV272_ESPGIR
gracilidelphis AI510_ESPCAN
gracilidelphis AI1012_ESPGIP2
gracilidelphis AI905_ESPLE3
gracilidelphis RA798_FRAPIR
gracilidelphis RA29_ESPNA1
gracilidelphis DV271_ESPGIR
0.99
H. gracilidelphis
exasperata AI506
exasperata AI315
1
MY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

Figure 5

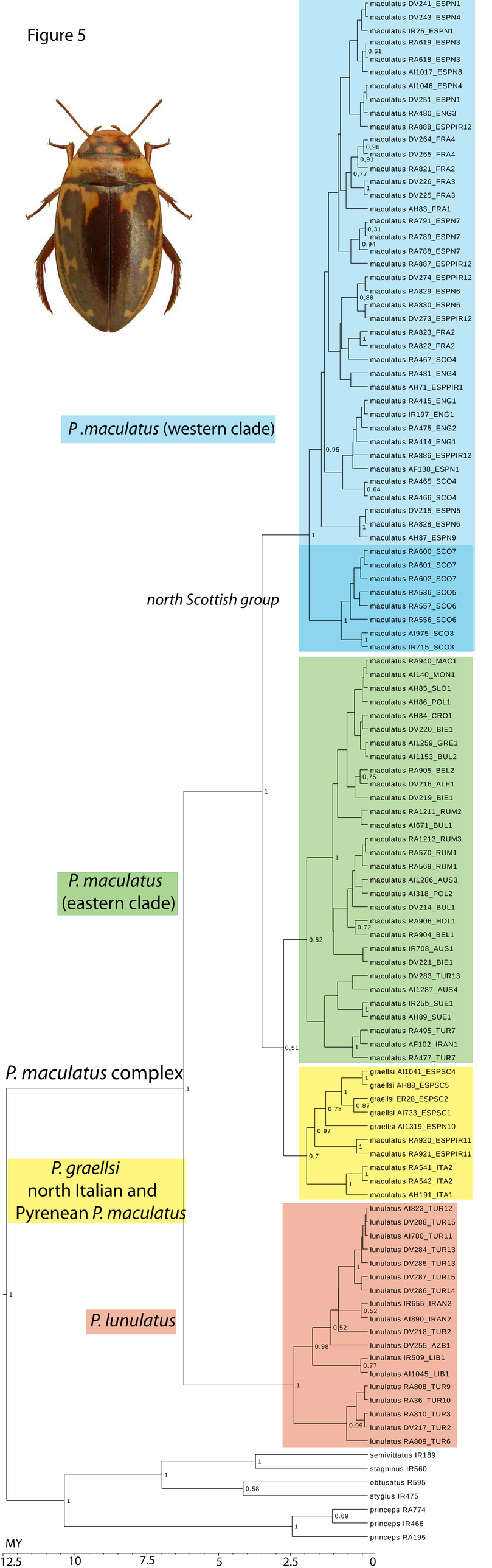
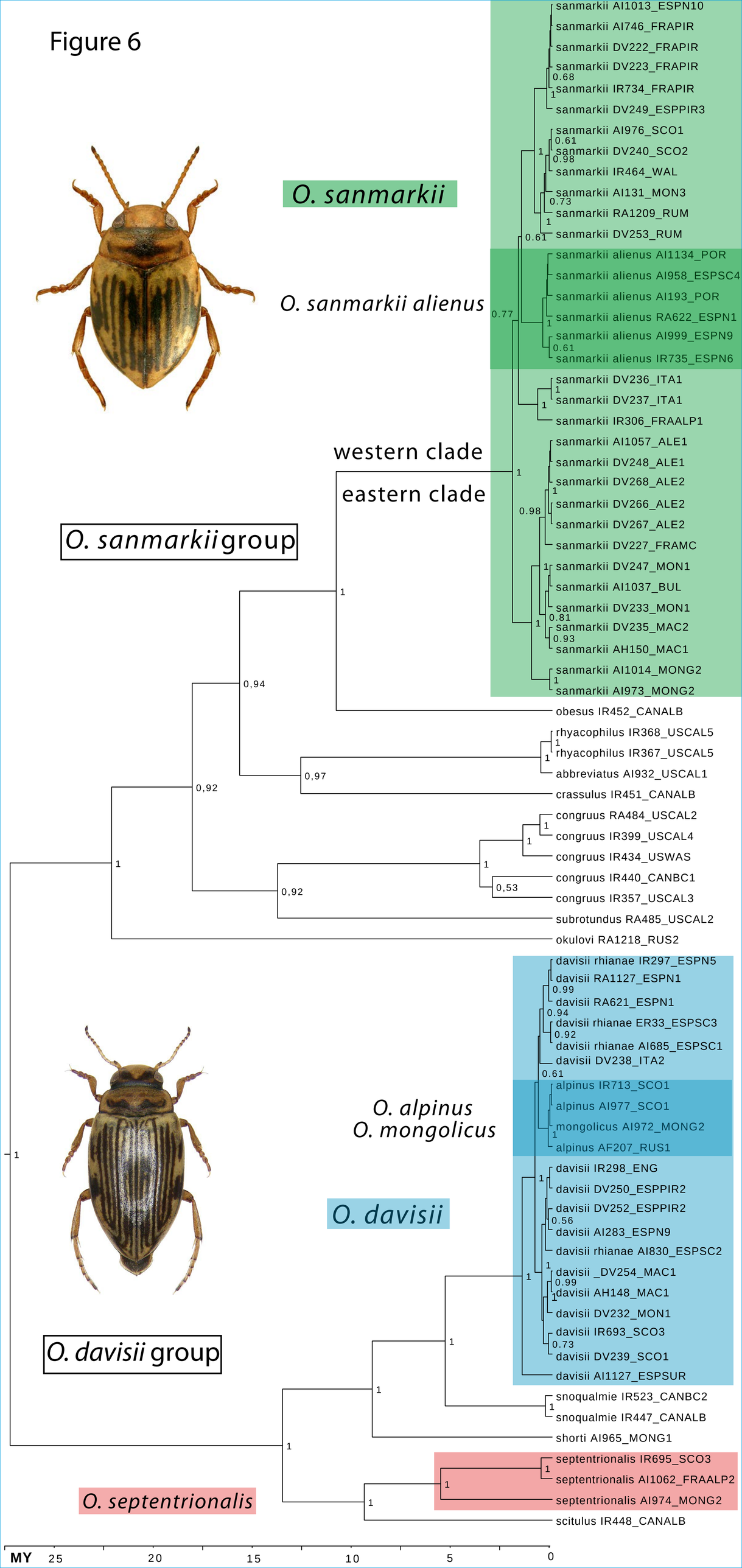

P .maculatus (western clade)
north Scottish group
P. maculatus (eastern clade)
P. maculatus complex
P. graellsi north Italian and Pyrenean P. maculatus
P. lunulatus
maculatus DV241_ESPN1
maculatus DV243_ESPN4
maculatus IR25_ESPN1
maculatus RA619_ESPN3
maculatus RA618_ESPN3
maculatus AI1017_ESPN8
maculatus AI1046_ESPN4
maculatus DV251_ESPN1
maculatus RA480_ENG3
maculatus RA888_ESPPIR12
maculatus DV264_FRA4
maculatus DV265_FRA4
maculatus RA821_FRA2
maculatus DV226_FRA3
maculatus DV225_FRA3
maculatus AH83_FRA1
maculatus RA791_ESPN7
maculatus RA789_ESPN7
maculatus RA788_ESPN7
maculatus RA887_ESPPIR12
maculatus DV274_ESPPIR12
maculatus RA829_ESPN6
maculatus RA830_ESPN6
maculatus DV273_ESPPIR12
maculatus RA823_FRA2
maculatus RA822_FRA2
maculatus RA467_SCO4
maculatus RA481_ENG4
maculatus AH71_ESPPIR1
maculatus RA415_ENG1
maculatus IR197_ENG1
maculatus RA475_ENG2
maculatus RA414_ENG1
maculatus RA886_ESPPIR12
maculatus AF138_ESPN1
maculatus RA465_SCO4
maculatus RA466_SCO4
maculatus DV215_ESPN5
maculatus RA828_ESPN6
maculatus AH87_ESPN9
maculatus RA600_SCO7
maculatus RA601_SCO7
maculatus RA602_SCO7
maculatus RA536_SCO5
maculatus RA557_SCO6
maculatus RA556_SCO6
maculatus AI975_SCO3
maculatus IR715_SCO3
maculatus RA940_MAC1
maculatus AI140_MON1
maculatus AH85_SLO1
maculatus AH86_POL1
maculatus AH84_CRO1
maculatus DV220_BIE1
maculatus AI1259_GRE1
maculatus AI1153_BUL2
maculatus RA905_BEL2
maculatus DV216_ALE1
maculatus DV219_BIE1
maculatus RA1211_RUM2
maculatus AI671_BUL1
maculatus RA1213_RUM3
maculatus RA570_RUM1
maculatus RA569_RUM1
maculatus AI1286_AUS3
maculatus AI318_POL2
maculatus DV214_BUL1
maculatus RA906_HOL1
maculatus RA904_BEL1
maculatus IR708_AUS1
maculatus DV221_BIE1
maculatus DV283_TUR13
maculatus AI1287_AUS4
maculatus IR25b_SUE1
maculatus AH89_SUE1
maculatus RA495_TUR7
maculatus AF102_IRAN1
maculatus RA477_TUR7
graellsi AI1041_ESPSC4
graellsi AH88_ESPSC5
graellsi ER28_ESPSC2
graellsi AI733_ESPSC1
graellsi AI1319_ESPN10
maculatus RA920_ESPPIR11
maculatus RA921_ESPPIR11
maculatus RA541_ITA2
maculatus RA542_ITA2
maculatus AH191_ITA1
lunulatus AI823_TUR12
lunulatus DV288_TUR15
lunulatus AI780_TUR11
lunulatus DV284_TUR13
lunulatus DV285_TUR13
lunulatus DV287_TUR15
lunulatus DV286_TUR14
lunulatus IR655_IRAN2
lunulatus AI890_IRAN2
lunulatus DV218_TUR2
lunulatus DV255_AZB1
lunulatus IR509_LIB1
lunulatus AI1045_LIB1
lunulatus RA808_TUR9
lunulatus RA36_TUR10
lunulatus RA810_TUR3
lunulatus DV217_TUR2
lunulatus RA809_TUR6
semivittatus IR189
stagninus IR560
obtusatus R595
stygius IR475
princeps RA774
princeps IR466
princeps RA195
MY
12.5
10
7.5
5
2.5
0

Figure 6


O. sanmarkii
O. sanmarkii alienus
western clade
eastern clade
O. sanmarkii group
sanmarkii AI1013_ESPN10
sanmarkii AI746_FRAPIR
sanmarkii DV222_FRAPIR
sanmarkii DV223_FRAPIR
sanmarkii IR734_FRAPIR
sanmarkii DV249_ESPPIR3
sanmarkii AI976_SCO1
sanmarkii DV240_SCO2
sanmarkii IR464_WAL
sanmarkii AI131_MON3
sanmarkii RA1209_RUM
sanmarkii DV253_RUM
sanmarkii alienus AI1134_POR
sanmarkii alienus AI958_ESPSC4
sanmarkii alienus AI193_POR
sanmarkii alienus RA622_ESPN1
sanmarkii alienus AI999_ESPN9
sanmarkii alienus IR735_ESPN6
sanmarkii DV236_ITA1
sanmarkii DV237_ITA1
sanmarkii IR306_FRAALP1
sanmarkii AI1057_ALE1
sanmarkii DV248_ALE1
sanmarkii DV268_ALE2
sanmarkii DV266_ALE2
sanmarkii DV267_ALE2
sanmarkii DV227_FRAMC
sanmarkii DV247_MON1
sanmarkii AI1037_BUL
sanmarkii DV233_MON1
sanmarkii DV235_MAC2
sanmarkii AH150_MAC1
sanmarkii AI1014_MONG2
sanmarkii AI973_MONG2
obesus IR452_CANALB
rhyacophilus IR368_USCAL5
rhyacophilus IR367_USCAL5
abbreviatus AI932_USCAL1
crassulus IR451_CANALB
congruus RA484_USCAL2
congruus IR399_USCAL4
congruus IR434_USWAS
congruus IR440_CANBC1
congruus IR357_USCAL3
subrotundus RA485_USCAL2
okulovi RA1218_RUS2
O. alpinus
O. mongolicus
O. davisii
O. davisii group
davisii rhianae IR297_ESPN5
davisii RA1127_ESPN1
davisii RA621_ESPN1
davisii rhianae ER33_ESPSC3
davisii rhianae AI685_ESPSC1
davisii DV238_ITA2
alpinus IR713_SCO1
alpinus AI977_SCO1
mongolicus AI972_MONG2
alpinus AF207_RUS1
davisii IR298_ENG
davisii DV250_ESPPIR2
davisii DV252_ESPPIR2
davisii AI283_ESPN9
davisii rhianae AI830_ESPSC2
davisii _DV254_MAC1
davisii AH148_MAC1
davisii DV232_MON1
davisii IR693_SCO3
davisii DV239_SCO1
davisii AI1127_ESPSUR
snoqualmie IR523_CANBC2
snoqualmie IR447_CANALB
shorti AI965_MONG1
O. septentrionalis
septentrionalis IR695_SCO3
septentrionalis AI1062_FRAALP2
septentrionalis AI974_MONG2
scitulus IR448_CANALB
MY 25 20 15 10 5 0

Supplementary Information

Table S1. List of the specimens included in the study, with specimen voucher, locality, collector and Genbank accession numbers. In grey, species used as outgroups. "XX" represents sequences newly obtained for this study.

Table S2a. Primers used for the amplification and sequencing. In brackets, length of the amplified fragment.

Table S2b. Standard PCR conditions for the amplification of the studied fragments.

Table S3. Best evolutionary model for each gene as selected by Partition Finder. In the *P. maculatus* complex selected models failed to converge and were not performed. Instead, an HKY+I+G model to each partition was applied (see `Results').

Figure S1. Phylogenetic tree of the studied species of the *Hydraena gracilis* complex obtained with RAxML with the combined nuclear and mitochondrial sequences and a partition by gene. Numbers in nodes: Bootstrap support values higher than 50. See Table S1 for details on the specimens and localities.

Figure S2. Ultrametric tree of the studied species of the *Platambus maculatus* complex obtained with BEAST using only the nuclear sequence data. Numbers in nodes: Bayesian posterior probabilities higher than 0.5. See Table S1 for details on the specimens and localities.

FigureS3. Phylogenetic tree of the studied species of the *Platambus maculatus* complex obtained with RAxML with the combined nuclear and mitochondrial sequences and a partition by gene. Numbers in nodes: Bootstrap support values higher than 50. See Table S1 for details on the specimens and localities.

Figure S4. Ultrametric tree of the studied species of *Oreodytes* obtained with BEAST using only the nuclear sequence data. Numbers in nodes: Bayesian posterior probabilities higher than 0.5. See Table S1 for details on the specimens and localities.

Figure S5. Phylogenetic tree of the studied species of the *Oreodytes* obtained with RAxML with the combined nuclear and mitochondrial sequences and a partition by gene. Numbers in nodes: Bootstrap support values higher than 50. See Table S1 for details on the specimens and localities.

Pleistocene range expansions in Western Palaearctic aquatic Coleoptera

David García-Vázquez, David T. Bilton, Garth N. Foster, I. Ribera

Appendix S1: Additional materials.

Table S1.List of the specimens included in the study, with specimen voucher, locality, collector and Genbank accession numbers. In grey, species used as outgroups. XX represents sequences newly obtained for this study.

								Genes				
No	Species	Voucher	Country	Locality	Locality code	Coordinates	Collector	COI-5'	COI-3'	16S	H3	Wingless
1	Hydraena anatolica	IBE-DV256	Azerbaijan	Ismayilli, Green House	AZB1	40°55'49.9"N 48°3'55.1"E	A. Faille, J. Fresneda, I. Ribera& A. Rudoy	XX	XX	XX	XX	XX
2	Hydraena anatolica	IBE-DV257	Azerbaijan	Ismayilli, Green House	AZB1	40°55'49.9"N 48°3'55.1"E	A. Faille, J. Fresneda, I. Ribera& A. Rudoy		XX			
3	Hydraena anatolica	IBE-DV258	Azerbaijan	Lerik	AZB2	38°44'2.05"N 48°37'44.28"E	A. Faille, J. Fresneda, I. Ribera& A. Rudoy	XX	XX	XX	XX	
4	Hydraena anatolica	IBE-DV259	Azerbaijan	Lerik	AZB2	38°44'2.05"N 48°37'44.28"E	A. Faille, J. Fresneda, I. Ribera& A. Rudoy		XX			
5	Hydraena anatolica	IBE-DV281	Turkey	Kastamonu province, Agli	TUR3	N41º42’41.5” E33º30’47”	I.Ribera		XX			
6	Hydraena anatolica	IBE-DV282	Turkey	Kastamonu province, Agli	TUR3	N41º42’41.5” E33º30’47”	I.Ribera		XX			
7	Hydraena anatolica	IBE-RA741	Iran	Mazandaran province, Kheiroud Kenar	IRAN	36º31'38'N, 51º38'46"E	A. Skale	XX	XX	XX	XX	XX
8	Hydraena anatolica	MNCN-AI802	Turkey	Kastamonu province, Senpazar	TUR2	N41º49’38” E33º25’03”	I.Ribera	XX	XX	XX	XX	XX
9	Hydraena anatolica	MNCN-AI821	Turkey	Kastamonu province, Agli	TUR3	N41º42’41.5” E33º30’47”	I.Ribera		XX			
10	Hydraena crepidoptera	MZUR–137	Turkey	Kastamonu province, Arpalikbasi	TUR4	N41º 53’ 24” E34º 05’ 16”	M. Trizzino, A. Biscaccianti & P. Audisio		XX	XX		
11	Hydraena exasperata	MNCN-AI315	Spain	Andalucia, Cádiz province, Facinas			I. Ribera	XX	FR773515	FR773520		
12	Hydraena exasperata	MNCN-AI506	Spain	Casatilla y León, Leon, Puerto de Panderrueda			L.F. Valladares	XX	HM588382	HM588526		
13	Hydraena gracilidelphis	IBE-DV271	Spain	Cataluña, Girona province, St. Hilari	ESPGIR	41°54'55.5"N 2°30'48.1"E	I. Ribera & A. Cieslak	XX	XX	XX	XX	
14	Hydraena gracilidelphis	IBE-DV272	Spain	Cataluña, Girona province, St. Hilari	ESPGIR	41°54'55.5"N 2°30'48.1"E	I. Ribera & A. Cieslak		XX			
15	Hydraena gracilidelphis	IBE-RA29	Spain	Navarra, Goizueta	ESPNA1	43º14'04''N, 1º47'51''W	A. Castro	XX	XX			
16	Hydraena gracilidelphis	IBE-RA798	France	Languedoc-Rousillon, Pyrenees Orientales, L´Albera	FRAPIR	N42º27’53.9” E2º54’02.0”	I. Ribera & A. Cieslak	XX	XX	XX	XX	
17	Hydraena gracilidelphis	MNCN-AI510	Spain	Cantabria, Bejes	ESPCAN	ca. 43°14'28.54"N 4°38'45.02	L. F. Valladares	XX	XX	XX	XX	XX
18	Hydraena gracilidelphis	MNCN-AI905	Spain	Castilla y Leon, León province, Pto. de Panderrueda	ESPLE3	ca. 43°8'22.62"N 4°56'28.17"	L. F. Valladares	XX	XX			
19	Hydraena gracilidelphis	MNCN-AI1012	Spain	Euskadi, Guipuzcoa province, Oiartzun	ESPGIP1	N43º16’10.2” W1º48’20.5”	I. Ribera & A. Cieslak	XX	XX			
20	Hydraena gracilis	IBE-DV228	France	Auvergne, Cantal, Laveissière	FRAMC2	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak		XX			
21	Hydraena gracilis	IBE-DV229	Germany	Sachsen-Anhalt, Harz mountains	ALE3	51º43’59.4”N 11º1’56.7”E	I. Ribera		XX			
22	Hydraena gracilis	IBE-DV230	Slovenia	Carniola	SLO1	N45º45’57.5” E14º21’40.0”	I. Ribera, C. Hernando & A. Cieslak		XX			
23	Hydraena gracilis	IBE-DV231	Slovenia	Gorišča	SLO2	N45º54’14.4” E13º54’43.8”	I. Ribera, C. Hernando & A. Cieslak		XX			
24	Hydraena gracilis	IBE-DV234	Montenegro	Savnik	MON	ca. 42°57'32"N 19°5'31"E	D.T. Bilton		XX	XX	XX	
25	Hydraena gracilis	IBE-DV244	Slovenia	Carniola	SLO1	N45º45’57.5” E14º21’40.0”	I. Ribera, C. Hernando & A. Cieslak	XX	XX	XX	XX	
26	Hydraena gracilis	IBE-DV245	Scotland	Outer hebrides, Barra island	SCO	ca. 57°0'5.00"N 7°25'57"W	D.T. Bilton	XX	XX	XX	XX	XX
27	Hydraena gracilis	IBE-DV246	Germany	Sachsen-Anhalt, Harz mountains	ALE2	N51º48’06.6” E10º43’55.4”	I. Ribera & A. Cieslak		XX			
28	Hydraena gracilis	IBE-DV269	Germany	Baden Württemberg, Eyachmühle	ALE4	48°48'30.8"N 8°33'27.1"E	I. Ribera & A. Cieslak		XX			
29	Hydraena gracilis	IBE-DV270	Germany	Baden Württemberg, Eyachmühle	ALE4	48°48'30.8"N 8°33'27.1"E	I. Ribera & A. Cieslak		XX			
30	Hydraena gracilis	IBE-RA902	Netherlands	Limburg, Vijlen	HOL	ca. 50°47'23"N 5°58'13"E	G.N. Foster		XX	XX	XX	
31	Hydraena gracilis	IBE-RA903	Belgium	Liège province, Heppenbach	BEL	ca. 50°21'45"N6°13'2"E	G.N. Foster		XX			
32	Hydraena gracilis	IBE-RA1217	Romania	Bacău county, Sălătruc	RUM	46.3248N 26.3370E	H. Fery		XX	XX	XX	
33	Hydraena gracilis	MNCN-AC25	Scotland	Outer hebrides, Barra island	SCO	ca. 57°0'5.00"N 7°25'57"W	D.T. Bilton		XX	XX		
34	Hydraena gracilis	MNCN-AH153	Czech Republic	Bohemia, Podmocky	RCHE	ca. 49°56'16"N 13°49'57"E	J. Statszny		XX			
35	Hydraena gracilis	MNCN-AI332	Latvia	Gaujas National Park	LAT	ca. 57°17'56"N 25°1'46"E	D.T. Bilton		XX	XX	XX	XX
36	Hydraena gracilis	MNCN-AI333	England (UK)	Cumbria, river Irthing	ENG	ca. 55°1'41"N 2°38'56"W	D.T. Bilton	XX	XX	XX	XX	
37	Hydraena gracilis	MNCN-AI349	Austria	Nieder Österreich, Schwarzenbach	AUS2	N48º04’42.4” E15º40’42.9”	I. Ribera & A. Cieslak	XX	XX	XX	XX	
38	Hydraena gracilis	MNCN-AI359	Slovakia	Nizke Tatry Mts., Mahnina river	SLK2	ca. 48°57'2"N 19°30'11"E	F. Ciampor	XX	XX	XX	XX	
39	Hydraena gracilis	MNCN-AI368	Switzerland	Oberegg, St. Anton to Wald	SUI	N47º24’32.2” E9º31’20.6”	I. Ribera & A. Cieslak	XX	XX	XX	XX	
40	Hydraena gracilis	MNCN-AI393	France	Rhone-Alps, Isére, Les Valaises	FRAALP3	ca. 45°28'1"N 5° 5'55"E	G.N. Foster		XX			
41	Hydraena gracilis	MNCN-AI401	Austria	Nieder Österreich, Pulkau	AUS3	N4842’25.8” E15º47’11.8”	I. Ribera & A. Cieslak		XX			
42	Hydraena gracilis	MNCN-AI403	France	Limousin, Correze, Ambrugeat	FRAMC1	ca. 45°25'55"N 2°1'11"E	P. Queney	XX	XX	XX	XX	XX
43	Hydraena gracilis	MNCN-AI695	France	Rhone-Alps, Ain, La Valserine	FRAALP2	ca. 46° 7'5"N 5°49'31"E	P. Queney		XX			
44	Hydraena gracilis	MNCN-AI1058	Germany	Sachsen-Anhalt, Harz mountains	ALE2	N51º48’06.6” E10º43’55.4”	I. Ribera & A. Cieslak	XX	XX	XX	XX	
45	Hydraena gracilis	MNCN-AI1154	France	Rhone-Alps, Loire, Pavesin	FRAALP1	ca. 45°28'34"N 4°39'4"E	G.N. Foster		XX			
46	Hydraena gracilis balcanica	MNCN-AI338	Bulgaria	Rhodope Mountains	BUL	ca. 41°34'39"N 23°41'35"E	D.T. Bilton		XX	XX	XX	XX
47	Hydraena graciloides	IBE-DV279	Turkey	Erzincan province, Yurtbaşi	TUR1	N39º52'25" E38º57'5"	I. Ribera		XX			
48	Hydraena graciloides	IBE-DV280	Turkey	Erzincan province, Yurtbaşi	TUR2	N39º52'25" E38º57'5"	I. Ribera		XX			
49	Hydraena graciloides	IBE-RA720	Turkey	Erzincan province, Yurtbaşi	TUR3	N39º52'25" E38º57'5"	I. Ribera	XX	XX	XX	XX	XX
50	Hydraena graciloides	MZUR–139	Turkey	Bolu province, Yedigöller N.P	TUR5	N40º59' 09'' E31º 38'26"	M. Trizzino, A. Biscaccianti & P. Audisio		XX	XX		
51	Oreodytes abbreviatus	MNCN-AI932	California (US)	El Dorado Co., Placerville	USCAL1		A.I. Cognato		HF931243	HF931477	XX	
52	Oreodytes alpinus	MNCN-AI977	Scotland (UK)	Sutherland, Loch Brora	SCO1	ca. 58°1'0"N 3°55'9"W	I. Ribera & G.N. Foster	XX	HF931257	HF931493	XX	XX
53	Oreodytes alpinus	NHM-IR713	Scotland (UK)	Sutherland, Loch Brora	SCO1	ca. 58°1'0"N 3°55'9"W	I. Ribera & G.N. Foster		XX	XX		
54	Oreodytes congruus	NHM-IR357	California (US)	Shasta co., Lake McCumber	USCAL3		I. Ribera & A. Cieslak		XX	XX		
55	Oreodytes congruus	NHM-IR399	California (US)	Marin co., Olema Creek	USCAL4		I. Ribera & A. Cieslak		XX	XX		
56	Oreodytes congruus	NHM-IR434	Washington (US)	Skagit co., Burlington	USWAS		I. Ribera & A. Cieslak		XX	XX		
57	Oreodytes congruus	NHM-IR440	British Columbia (CAN)	Rock Creek	CANBC1		I. Ribera & A. Cieslak		AJ850599	AJ850347	EF670146	
58	Oreodytes crassulus	NHM-IR451	Alberta (CAN)	Lund Breck	CANALB		I. Ribera & A. Cieslak		AJ850600	AJ850348	EF670147	
59	Oreodytes davisii	IBE-DV232	Montenegro	Savnik	MON1	ca. 42°57'32"N 19°5'31"E	D.T. Bilton	XX	XX	XX	XX	
60	Oreodytes davisii	IBE-DV238	Italy	Emilia Romagna, Parma province, Bosco-Mucino	ITA2	N44º27’18.0” E10º02’25.8”	I. Ribera	XX	XX	XX	XX	
61	Oreodytes davisii	IBE-DV239	Scotland (UK)	Sutherland, Loch Brora	SCO1	ca. 58°1'0"N 3°55'9"W	I. Ribera & G.N. Foster	XX	XX	XX	XX	XX
62	Oreodytes davisii	IBE-DV250	Spain	Aragón, Huesca province, Ansó	ESPPIR2	ca. 42°45'46"N 0°44'39"W	I. Esteban	XX	XX	XX	XX	XX
63	Oreodytes davisii	IBE-DV252	Spain	Aragón, Huesca province, Ansó	ESPPIR2	ca. 42°45'46"N 0°44'39"W	I. Esteban		XX			

64	Oreodytes davisii	IBE-DV254	Macedonia	Mavrovo district, Gari	MAC1	ca. 41°29'33"N 20°40'40"E	D.T. Bilton	XX	XX	XX	XX	
65	Oreodytes davisii	IBE-RA621	Spain	Cantabria, Vada	ESPN1	43°5'21"N 4°40'31"W	L.F. Valladares		XX			
66	Oreodytes davisii	IBE-RA1127	Spain	Cantabria, Vada	ESPN1	43°5'21"N 4°40'31"W	L.F. Valladares & D. García	XX	XX	XX	XX	
67	Oreodytes davisii	MNCN-AH148	Macedonia	Mavrovo district, Gari	MAC1	ca. 41°29'33"N 20°40'40"E	D.T. Bilton		XX			
68	Oreodytes davisii	MNCN-AI283	Spain	La Rioja, Posadas	ESPN9	N 42º12’36.0” W 3º4’27.8”	I. Ribera	XX	XX	XX	XX	XX
69	Oreodytes davisii	MNCN-AI1127	Spain	Andalucia, Granada province, Sierra Nevada	ESPSUR	ca. 37°0'30"N 3°15'29"W	F.M. Cabezas	XX	XX	XX	XX	XX
70	Oreodytes davisii	NHM-IR298	England (UK)	Yorkshire, Wheeldale Gill	ENG	ca. 54°22'53"N 0°46'28"W	R. Carr		XX	XX		
71	Oreodytes davisii	NHM-IR693	Scotland (UK)	Easterness, River Druie	SCO3	ca. 57°10'28"N 3°49'45"W	G.N. Foster		XX	XX		
72	Oreodytes davisii rhianae	MNCN-AI685	Spain	Castilla y León, Avila province, Hoyos del Espino	ESPSC1	ca. 40°20'27"N 5°10'18"W	H. Fery	XX	XX	XX	XX	
73	Oreodytes davisii rhianae	MNCN-AI830	Spain	Castilla y León, Avila province, Piedrahita	ESPSC2	ca. 40°26'58"N 5°20'19"W	H. Fery	XX	XX			
74	Oreodytes davisii rhianae	NHM-ER33	Spain	Castilla y León, Avila province, Gredos	ESPSC3	ca. 40°22'6"N 5°6'54"W	H. Fery		AF309301	AF309244	EF670148	
75	Oreodytes davisii rhianae	NHM-IR297	Spain	Castilla y Leon, León province, river Yuso	ESPN5	ca. 43°2'47"N 4°51'1"W	R. Carr		XX	XX		
76	Oreodytes okulovi	IBE-RA1218	Russia	03.09.06 Russia, Tyva, Ungesh River, stream, roiled	RUS2		via H. Fery		XX		XX	
77	Oreodytes mongolicus	MNCN-AI972	Mongolia	Ovorhangay, Orkhon's Waterfall	MONG2	N46.78742 E101.96022	A.E.Z. Short		HF931255	HF931491	XX	
78	Oreodytes rhyacophilus	NHM-IR367	California (US)	Trinity co., Rattlesnake Creek	USCAL5		I. Ribera & A. Cieslak		AJ850604	AJ850352	EF670152	
79	Oreodytes rhyacophilus	NHM-IR368	California (US)	Trinity co., Rattlesnake Creek	USCAL5		I. Ribera & A. Cieslak		XX	XX		
80	Oreodytes obesus cordillerensis	NHM-IR452	Alberta (CAN)	Lund Breck, Crownsnest	CANALB		I. Ribera & A. Cieslak		HF931279	HF931516		
81	Oreodytes sanmarkii	IBE-DV222	France	Pyrenees Orientales, Prats de Molló	FRAPIR	42°24'34.60"N 2°22'52.10"E	Fresneda, Ribera & Cieslak	XX	XX	XX	XX	XX
82	Oreodytes sanmarkii	IBE-DV223	France	Pyrenees Orientales, Prats de Molló	FRAPIR	42°24'34.60"N 2°22'52.10"E	Fresneda, Ribera & Cieslak		XX			
83	Oreodytes sanmarkii	IBE-DV227	France	Auvergne, Cantal, Laveissière	FRAMC	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
84	Oreodytes sanmarkii	IBE-DV233	Montenegro	Savnik	MON1	ca. 42°57'32"N 19°5'31"E	D.T. Bilton		XX			
85	Oreodytes sanmarkii	IBE-DV235	Macedonia	Tetovo district, Vratnica	MAC2	ca. 42°8'1"N 21°7'47"E	D.T. Bilton		XX			
86	Oreodytes sanmarkii	IBE-DV236	Italy	Lombardia, Brescia, val Trompia	ITA1	45°39'34"N 9°46'54"E	I. Ribera, A. Cieslak & M.Toledo	XX	XX	XX	XX	XX
87	Oreodytes sanmarkii	IBE-DV237	Italy	Lombardia, Brescia, val Trompia	ITA1	45°39'34"N 9°46'54"E	I. Ribera, A. Cieslak & M.Toledo		XX			
88	Oreodytes sanmarkii	IBE-DV240	Scotland (UK)	Sutherland, River Brora	SCO2	ca. ca. 58°1'0"N 3°55'9"W	I. Ribera & G.N. Foster		XX			
89	Oreodytes sanmarkii	IBE-DV247	Montenegro	Savnik	MON1	ca. 42°57'32"N 19°5'31"E	D.T. Bilton		XX			
90	Oreodytes sanmarkii	IBE-DV248	Germany	Sachsen-Anhalt, Harz mtn, Wernigerode	ALE1	N51º48’06.6” E10º43’55.4”	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
91	Oreodytes sanmarkii	IBE-DV249	Spain	Cataluña, Lleida province, S.J.Torán	ESPPIR3	N42º49'33.9" E0º46'44.2"	I. Ribera & A. Cieslak		XX			
92	Oreodytes sanmarkii	IBE-DV253	Romania	Bacău county, Mănăstirea Cașin	RUM	46.09319N 26.57051E	H. Fery	XX	XX	XX	XX	XX
93	Oreodytes sanmarkii	IBE-DV266	Germany	Baden Württemberg, Buhlbach	ALE2	48°31'58.9"N 8°16'35.4"E	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
94	Oreodytes sanmarkii	IBE-DV267	Germany	Baden Württemberg, Buhlbach	ALE2	48°31'58.9"N 8°16'35.4"E	I. Ribera & A. Cieslak		XX			
95	Oreodytes sanmarkii	IBE-DV268	Germany	Baden Württemberg, Buhlbach	ALE2	48°31'58.9"N 8°16'35.4"E	I. Ribera & A. Cieslak		XX			
96	Oreodytes sanmarkii	IBE-RA1209	Romania	Bacău county, Mănăstirea Cașin	RUM	46.09319N 26.57051E	H. Fery		XX			
97	Oreodytes sanmarkii	MNCN-AH150	Macedonia	Mavrovo district, Gari	MAC1	ca. 41°29'33"N 20°40'40"E	D.T. Bilton		XX			
98	Oreodytes sanmarkii	MNCN-AI131	Montenegro	Crkvine, stream Bistrica	MON3	ca. 42°47'18"N 19°26'36"E	V. Pesic	XX	XX	XX	XX	XX
99	Oreodytes sanmarkii	MNCN-AI746	France	Pyrenees Orientales, Prats de Molló	FRAPIR	42°24'34.60"N 2°22'52.10"E	H. Fery		XX			
100	Oreodytes sanmarkii	MNCN-AI973	Mongolia	Ovorhangay, Orkhon's Waterfall	MONG2	N46.78742 E101.96022	A.E.Z. Short	XX	XX	XX	XX	XX
101	Oreodytes sanmarkii	MNCN-AI976	Scotland (UK)	Sutherland, Loch Brora	SCO1	ca. 58°1'0"N 3°55'9"W	I. Ribera & G.N. Foster	XX	XX	XX	XX	XX
102	Oreodytes sanmarkii	MNCN-AI1013	Spain	Euskadi, Guipuzkoa province, Oiartzun	ESPN10	N43º16’10.2” W1º48’20.5”	I. Ribera & A. Cieslak		XX			
103	Oreodytes sanmarkii	MNCN-AI1014	Mongolia	Ovorhangay, Orkhon's Waterfall	MONG2	N46.78742 E101.96022	A.E.Z. Short		XX			
104	Oreodytes sanmarkii	MNCN-AI1037	Bulgaria	Rila mts., Rila village.	BUL	ca. 42°6'40"N 23°33'9"E	D.T. Bilton		XX			
105	Oreodytes sanmarkii	MNCN-AI1057	Germany	Sachsen-Anhalt, Harz mtn, Wernigerode	ALE1	N51º48’06.6” E10º43’55.4”	I. Ribera & A. Cieslak		XX			
106	Oreodytes sanmarkii	NHM-IR306	France	Alpes Maritimes, Moulinet	FRAALP1	ca. 43°57'0"N 7°24'58"E	I. Ribera & A. Cieslak		XX	XX		
107	Oreodytes sanmarkii	NHM-IR464	Wales (UK)	Pentre-Llyn-Cymmer	WAL	53°3'58"N 3°31'54"W	I. Ribera		XX	XX		
108	Oreodytes sanmarkii	NHM-IR734	France	Pyrenees Orientales, Prats de Molló	FRAPIR	42°24'34.60"N 2°22'52.10"E	H. Fery		HF931287	HF931524		
109	Oreodytes sanmarkii alienus	IBE-RA622	Spain	Cantabria, Vada	ESPN1	43°5'21"N 4°40'31"W	L.F. Valladares		XX			
110	Oreodytes sanmarkii alienus	MNCN-AI193	Portugal	Manteigas, river Zezere	POR	ca. 40°21'45"N 7°33'17"W	I. Ribera		XX	XX		
111	Oreodytes sanmarkii alienus	MNCN-AI958	Spain	Madrid, Puerto de los Cotos	ESPSC4	N40º49’59” W3º56’09”	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
112	Oreodytes sanmarkii alienus	MNCN-AI999	Spain	La Rioja, Posadas	ESPN9	N 42º12’36.0” W 3º4’27.8”	I. Ribera & G.N. Foster	XX	XX	XX	XX	XX
113	Oreodytes sanmarkii alienus	MNCN-AI1134	Portugal	Manteigas, river Zezere	POR	ca. 40°21'45"N 7°33'17"W	I. Ribera	XX	XX	XX	XX	XX
114	Oreodytes sanmarkii alienus	NHM-IR735	Spain	Asturias, Llanes	ESPN6	ca. 43°23'34"N 4°56'44"W	H. Fery		HG915304	XX		
115	Oreodytes scitulus	NHM-IR448	Alberta (CAN)	Lund Breck, Crownsnest	CANALB		I. Ribera & A. Cieslak		HF931278	HF931515	XX	
116	Oreodytes septentrionalis	MNCN-AI974	Mongolia	Ovorhangay, Orkhon's Waterfall	MONG2	N46.78742 E101.96022	A.E.Z. Short		HF931256	HF931492	XX	
117	Oreodytes septentrionalis	MNCN-AI1062	France	Alpes Maritimes, Le Suquet	FRAALP2	N43º56’29.4”E7º16’57.6”	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
118	Oreodytes septentrionalis	NHM-IR695	Scotland (UK)	Easterness, River Druie	SCO3	ca. 57°10'28"N 3°49'45"W	G.N. Foster		XX	XX		
119	Oreodytes shorti	MNCN-AI965	Mongolia	Arkhangay, Bulgan	MONG1	N47.11192 E101.01048	A.E.Z. Short		HF931252	HF931488	XX	
120	Oreodytes snoqualmie	NHM-IR447	Alberta (CAN)	Lund Breck, Crownsnest	CANALB		I. Ribera & A. Cieslak		XX	XX		
121	Oreodytes snoqualmie	NHM-IR523	British Columbia (CAN)	Hope	CANBC2		I. Ribera & A. Cieslak		HF931282	HF931519		
122	Oreodytes subrotundus	IBE-RA485	California (US)	Mendocino County, Chadbourne	USCAL2		D. Post	XX	XX	XX	XX	
123	Platambus graellsi	MNCN-AH88	Spain	Castilla La Mancha, Guadalajara province, Cardoso de la	ESPSC4	N41º05'34.3" W3º25'32.1"	I. Ribera & A. Cieslak	XX	HF931142/LM	HF931360/LM	LM655142	XX
124	Platambus graellsi	MNCN-AI733	Spain	Castilla y León, Ávila province, Sierra de Gredos	ESPSC2	N40º20’14” W5º00’48”	I. Ribera	XX	XX			
125	Platambus graellsi	MNCN-AI1041	Spain	Madrid, Lozoya	ESPSC3	N40º58'9.3"W3º48'00"	I. Ribera & A. Cieslak	XX	XX			
126	Platambus graellsi	MNCN-AI1319	Spain	Galicia, Ourense province, Reigada	ESPN10	N42º14’39.3” W7º10’57.2”	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
127	Platambus graellsi	NHM-ER28	Spain	Castilla y León, Ávila province, Sierra de Gredos	ESPSC1	ca. 40°21'58"N 5°8'22.48"W	H. Fery		XX			
128	Platambus lunulatus	IBE-DV217	Turkey	Rize province, Ikizdere	TUR2	ca. 40°46'28"N 40°33'45"E	Ö.K. Erman		XX			
129	Platambus lunulatus	IBE-DV218	Turkey	Rize province, Ikizdere	TUR2	ca. 40°46'28"N 40°33'45"E	Ö.K. Erman		XX			
130	Platambus lunulatus	IBE-DV255	Azerbaijan	Ismayilli, Green House	AZB1	40°55'49.9"N 48°3'55.1"E	A. Faille, J. Fresneda, I. Ribera& A. Rudoy	XX	XX	XX	XX	XX
131	Platambus lunulatus	IBE-DV284	Turkey	Balikesir province, Kürendere	TUR13	39°18'46.4"N 28°31'56.7"E	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
132	Platambus lunulatus	IBE-DV285	Turkey	Balikesir province, Kürendere	TUR13	39°18'46.4"N 28°31'56.7"E	I. Ribera & A. Cieslak		XX			
133	Platambus lunulatus	IBE-DV286	Turkey	Balikesir province, Asagigöcek	TUR14	39°23'46.3"N 28°24'19.8"E	I. Ribera & A. Cieslak		XX			

134	Platambus lunulatus	IBE-DV287	Turkey	Afyonkarahisar province, Kiziloren	TUR15	ca. 38°15'39"N 30°7'30"E	D.T. Bilton	XX	XX	XX	XX	XX
135	Platambus lunulatus	IBE-DV288	Turkey	Afyonkarahisar province, Kiziloren	TUR15	ca. 38°15'39"N 30°7'30"E	D.T. Bilton		XX			
136	Platambus lunulatus	IBE-RA36	Turkey	Sivas-Tokat provinces border, Çamlibel pass	TUR10	ca. 39°57'56"N 36°31'35"E	Ö.K. Erman		XX	XX	XX	XX
137	Platambus lunulatus	IBE-RA808	Turkey	Erzincan province, Refahiye	TUR9	ca. 39°53'42"N 38°46'9"E	Ö.K. Erman		XX			
138	Platambus lunulatus	IBE-RA809	Turkey	Erzurum province, Hamzalar village	TUR6	ca. 39°28'2"N 41°15'59"E	Ö.K. Erman		XX			
139	Platambus lunulatus	IBE-RA810	Turkey	Rize province, Rüzgarli village	TUR3	ca. 40°46'28"N 40°33'45"E	Ö.K. Erman		XX	XX		XX
140	Platambus lunulatus	MNCN-AI780	Turkey	Bolu province,Yeniçaga	TUR11	N40º50’49” E32º03’47.5”	I. Ribera	XX	XX	XX	XX	XX
141	Platambus lunulatus	MNCN-AI823	Turkey	Karabuk province, Cayorenguney	TUR12	N40º48’23” E32º16’00.5”	I. Ribera		XX	XX		
142	Platambus lunulatus	MNCN-AI890	Iran	Fars, Sepidan	IRAN2	ca. 30°14'8"N 51°59'46"E	K. Elmi & H. Fery		LM654922	LM654810	LM655143	XX
143	Platambus lunulatus	MNCN-AI1045	Lebanon	Aral Qadichi	LIB1	ca. 33°49'31"N 35°49'2"E	A. Dia		HF931150	HF931369		XX
144	Platambus lunulatus	NHM-IR509	Lebanon	Aral Qadichi	LIB2	ca. 33°49'31"N 35°49'2"E	A. Dia			XX		
145	Platambus lunulatus	NHM-IR655	Iran	Fars province, Sepidan	IRAN2	ca. 30°14'8"N 51°59'46"E	K. Elmi & H. Fery		AY138761	AY138674		
146	Platambus maculatus	IBE-AF102	Iran	Guilan province, Siyahkal	IRAN1	ca. 37°8'47"N 49°38'21"E	Reza Vafaei via H. Fery		XX	XX		
147	Platambus maculatus	IBE-AF138	Spain	Castilla y León, Burgos province, Pineda de la Sierra	ESPN1	ca. 42°13'24"N 3°17'49"W	I. Ribera		XX			
148	Platambus maculatus	IBE-DV214	Bulgaria	Bacevo, Rila mts.	BUL1	ca. 41°58'2"N 23°26'21"E	D.T. Bilton		XX	XX	XX	XX
149	Platambus maculatus	IBE-DV215	Spain	Euskadi, Alava province, Murua	ESPN5	ca. 42°58'1"N 2°44'20"W	G.N. Foster	XX	XX			
150	Platambus maculatus	IBE-DV216	Germany	Nordrhein-Westfalen, Wrexen	ALE1	51º30'42.28"N 8º59'43.50"E	R. Müller	XX	XX	XX	XX	XX
151	Platambus maculatus	IBE-DV219	Belarus	Vitebsk Province, Berezinskiy Biosphere Reserve	BIE1	54°41'54.86"N 28°18'1.01"E	I. Ribera	XX	XX	XX	XX	XX
152	Platambus maculatus	IBE-DV220	Belarus	Vitebsk Province, Berezinskiy Biosphere Reserve	BIE1	54°41'54.86"N 28°18'1.01"E	I. Ribera		XX			
153	Platambus maculatus	IBE-DV221	Belarus	Vitebsk Province, Berezinskiy Biosphere Reserve	BIE1	54°41'54.86"N 28°18'1.01"E	I. Ribera		XX			
154	Platambus maculatus	IBE-DV225	France	Auvergne, Cantal, Laveissière	FRA3	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak	XX	XX	XX		XX
155	Platambus maculatus	IBE-DV226	France	Auvergne, Cantal, Laveissière	FRA3	45°6'55.3"N 2°48'47.4"E	I. Ribera & A. Cieslak		XX			
156	Platambus maculatus	IBE-DV241	Spain	Castilla y León, Burgos province, Pineda de la Sierra	ESPN1	ca. 42°13'24"N 3°17'49"W	I. Ribera		XX	XX	XX	XX
157	Platambus maculatus	IBE-DV243	Spain	La Rioja, Posadas	ESPN4	N 42º12’36.0” W 3º4’27.8”	I. Ribera & G.N. Foster		XX			
158	Platambus maculatus	IBE-DV251	Spain	Castilla y León, Burgos province, Pineda de la Sierra	ESPN1	ca. 42°13'24"N 3°17'49"W	I. Ribera	XX	XX	XX	XX	XX
159	Platambus maculatus	IBE-DV264	France	Lorraine, PN Regional Vosgues, Rolbing	FRA4	49°10'22.7"N 7°26'29.7"E	I. Ribera & A. Cieslak		XX			
160	Platambus maculatus	IBE-DV265	France	Lorraine, PN Regional Vosgues, Rolbing	FRA4	49°10'22.7"N 7°26'29.7"E	I. Ribera & A. Cieslak		XX			
161	Platambus maculatus	IBE-DV273	Spain	Cataluña, Gerona province, St. Hilari	ESPPIR12	41°54'55.5"N 2°30'48.1"E	I. Ribera & A. Cieslak		XX			
162	Platambus maculatus	IBE-DV274	Spain	Cataluña, Gerona province, St. Hilari	ESPPIR12	41°54'55.5"N 2°30'48.1"E	I. Ribera & A. Cieslak		XX			
163	Platambus maculatus	IBE-DV283	Turkey	Balikesir province, Kürendere	TUR13	39°18'46.4"N 28°31'56.7"E	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
164	Platambus maculatus	IBE-RA414	England (UK)	Hampshire, New Forest	ENG1	ca. 51°15'14"N 1°13'8"W	I. Ribera	XX	XX	XX	XX	XX
165	Platambus maculatus	IBE-RA415	England (UK)	Hampshire, New Forest	ENG1	ca. 51°15'14"N 1°13'8"W	I. Ribera		XX	XX		
166	Platambus maculatus	IBE-RA465	Scotland (UK)	Argyll, Loch Eck	SCO4	ca. 56°5'6"N 4°59'5"W	G.N. Foster		XX	XX	XX	XX
167	Platambus maculatus	IBE-RA466	Scotland (UK)	Argyll, Loch Eck	SCO4	ca. 56°5'6"N 4°59'5"W	G.N. Foster		XX	XX		
168	Platambus maculatus	IBE-RA467	Scotland (UK)	Argyll, Loch Eck	SCO4	ca. 56°5'6"N 4°59'5"W	G.N. Foster		XX			
169	Platambus maculatus	IBE-RA475	England (UK)	Yorkshire, River Skell	ENG2	ca. 54°18'18"N 56°5'6"N 4°59	G.N. Foster		XX	XX		
170	Platambus maculatus	IBE-RA477	Turkey	Erzincan province, Yurtbaşi	TUR7	N39 52 25.2 E38 57 04.7	I. Ribera	XX	XX	XX	XX	XX
171	Platambus maculatus	IBE-RA480	England (UK)	Westmorland, Ullswater	ENG3	ca. 54°35'38"N 2°50'32"W	G.N. Foster		XX			
172	Platambus maculatus	IBE-RA481	England (UK)	Cumberland,Crummock Water	ENG4	ca. 54°32'52"N 3°17'28"W	G.N. Foster		XX			
173	Platambus maculatus	IBE-RA495	Turkey	Erzincan province, Yurtbaşi	TUR7	N39 52 25.2 E38 57 04.7	I. Ribera		XX	XX		
174	Platambus maculatus	IBE-RA536	Scotland (UK)	Scotland, Loch Doon	SCO5	ca. 55°15'33"N 4°21'39"W	G.N. Foster	XX	XX	XX	XX	XX
175	Platambus maculatus	IBE-RA541	Italy	Piamonte, Torino, Bianca, forest stream	ITA2	45º30'09"N 7º53'02"E	I. Ribera & A. Cieslak	XX	XX	XX		
176	Platambus maculatus	IBE-RA542	Italy	Piamonte, Torino, Bianca, forest stream	ITA2	45º30'09"N 7º53'02"E	I. Ribera & A. Cieslak	XX	XX	XX		
177	Platambus maculatus	IBE-RA556	Scotland (UK)	Dumfriesshire, Loch Skene	SCO6	ca. 57°11'15"N 2°19'50"W	G.N. Foster	XX	XX	XX	XX	XX
178	Platambus maculatus	IBE-RA557	Scotland (UK)	Dumfriesshire, Loch Skene	SCO6	ca. 57°11'15"N 2°19'50"W	G.N. Foster		XX	XX		
179	Platambus maculatus	IBE-RA569	Romania	Maramures, Calinesti	RUM1	ca. 47°28'12"N 23°34'2"E	J. Fresneda	XX	XX	XX		
180	Platambus maculatus	IBE-RA570	Romania	Maramures, Calinesti	RUM1	ca. 47°28'12"N 23°34'2"E	J. Fresneda	XX	XX	XX	XX	XX
181	Platambus maculatus	IBE-RA600	Scotland (UK)	Easterness, Glen Urquhart	SCO7	ca. 57°18'25"N 4°20'30"W	G.N. Foster	XX	XX			
182	Platambus maculatus	IBE-RA601	Scotland (UK)	Easterness, Glen Urquhart	SCO7	ca. 57°18'25"N 4°20'30"W	G.N. Foster		XX			
183	Platambus maculatus	IBE-RA602	Scotland (UK)	Easterness, Glen Urquhart	SCO7	ca. 57°18'25"N 4°20'30"W	G.N. Foster	XX	XX			
184	Platambus maculatus	IBE-RA618	Spain	Cantabria, Vada	ESPN3	43°5'21"N 4°40'31"W	L.F. Valladares	XX	XX	XX		XX
185	Platambus maculatus	IBE-RA619	Spain	Cantabria, Vada	ESPN3	43°5'21"N 4°40'31"W	L.F. Valladares	XX	XX	XX		
186	Platambus maculatus	IBE-RA788	Spain	Euskadi, Guipuzkoa province, Zaldibia	ESPN7	ca. 43°0'26"N 2° 9'15"W	I. Ribera & C. Hernando	XX	XX			
187	Platambus maculatus	IBE-RA789	Spain	Euskadi, Guipuzkoa province, Zaldibia	ESPN7	ca. 43°0'26"N 2° 9'15"W	I. Ribera & C. Hernando	XX	XX			
188	Platambus maculatus	IBE-RA791	Spain	Euskadi, Guipuzkoa province, Zaldibia	ESPN7	ca. 43°0'26"N 2° 9'15"W	I. Ribera & C. Hernando		XX			
189	Platambus maculatus	IBE-RA821	France	Picardia, Aisne, Venilly-la-Poteria	FRA2	ca. 49°5'14"N 3°12'45"E	P. Queney		XX			
190	Platambus maculatus	IBE-RA822	France	Picardia, Aisne, Venilly-la-Poteria	FRA2	ca. 49°5'14"N 3°12'45"E	P. Queney	XX	XX	XX	XX	XX
191	Platambus maculatus	IBE-RA823	France	Picardia, Aisne, Venilly-la-Poteria	FRA2	ca. 49°5'14"N 3°12'45"E	P. Queney		XX			
192	Platambus maculatus	IBE-RA828	Spain	Euskadi, Guipuzcoa province, Zubialde	ESPN6	ca. 43°15'21"N 2°13'55"W	G.N. Foster		XX			
193	Platambus maculatus	IBE-RA829	Spain	Euskadi, Guipuzcoa province, Zubialde	ESPN6	ca. 43°15'21"N 2°13'55"W	G.N. Foster		XX			
194	Platambus maculatus	IBE-RA830	Spain	Euskadi, Guipuzcoa province, Zubialde	ESPN6	ca. 43°15'21"N 2°13'55"W	G.N. Foster		XX			
195	Platambus maculatus	IBE-RA886	Spain	Aragón, Huesca province, Villanua	ESPPIR12	ca. 42°40'31"N 0°32'11"W	I. Esteban		XX			
196	Platambus maculatus	IBE-RA887	Spain	Aragón, Huesca province, Villanua	ESPPIR12	ca. 42°40'31"N 0°32'11"W	I. Esteban		XX			
197	Platambus maculatus	IBE-RA888	Spain	Aragón, Huesca province, Villanua	ESPPIR12	ca. 42°40'31"N 0°32'11"W	I. Esteban	XX	XX			
198	Platambus maculatus	IBE-RA904	Belgium	Namur province, Le Moulin de Lisogne	BEL1	ca. 50°28'45"N 6°6'16"E	G.N. Foster	XX	XX			
199	Platambus maculatus	IBE-RA905	Belgium	Liège province, Sourbrodt	BEL2	ca. 50°16'24"N 4°57'35"E	G.N. Foster	XX	XX	XX	XX	XX
200	Platambus maculatus	IBE-RA906	Netherlands	Limburg, Vijlen	HOL1	ca. 50°46'57"N 5°57'50"E	G.N. Foster	XX	XX			
201	Platambus maculatus	IBE-RA920	Spain	Cataluña, Lleida province, Llesp	ESPPIR11	42°27'48.2"N 0°46'12.5"E	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
202	Platambus maculatus	IBE-RA921	Spain	Cataluña, Lleida province, Llesp	ESPPIR11	42°27'48.2"N 0°46'12.5"E	I. Ribera & A. Cieslak	XX	XX			
203	Platambus maculatus	IBE-RA940	Macedonia	Mavrovo district, Gari	MAC1	ca. 41°29'33"N 20°40'40"E	D.T. Bilton	XX	XX	XX	XX	XX

204	Platambus maculatus	IBE-RA1211	Romania	Bacău county, Mănăstirea Caşin	RUM2	46.09319N 26.57051E	H. Fery		XX	XX		
205	Platambus maculatus	IBE-RA1213	Romania	Hargita county, Jacobeni	RUM3	46.1885N 26.1405E	H. Fery		XX	XX		
206	Platambus maculatus	MNCN-AH71	Spain	Cataluña, Barcelona province, Guardiola del Berguedà	ESPPIR1	N 42º15’05.9” E1º55”20.1”	I. Ribera & P. Aguilera & C. Hernando		XX	XX		
207	Platambus maculatus	MNCN-AH83	France	Alpes Maritimes, Malamaire, river Artuby	FRA1	N43º47’32.6” E6º39’45.6”	I. Ribera & A. Cieslak		XX	XX		
208	Platambus maculatus	MNCN-AH84	Croatia	Istria, Momjan	CRO1	N45º26’30.8” E13º42’37.8”	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
209	Platambus maculatus	MNCN-AH85	Slovenia	Carniola, river Dula	SLO1	N45º32’07.5” E14º15’01.0”	I. Ribera & A. Cieslak		XX	XX		
210	Platambus maculatus	MNCN-AH86	Poland	Zachodniopomorsky, Parseta river	POL1	N54º05’32” E15º42’48”	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
211	Platambus maculatus	MNCN-AH87	Spain	Navarra, Barindano	ESPN9	42°45’34“N 2°07’04“ W	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
212	Platambus maculatus	MNCN-AH89	Sweden	Ore river	SUE1	ca. 63°43'18"N 20°6'22"E	A.N. Nilsson		XX			
213	Platambus maculatus	MNCN-AH191	Italy	Emilia-Romagna, Vigoleno, Parco dello Stirone	ITA1	N44º48’41.6” E9º54’21.8”	I. Ribera	XX	XX	XX	XX	XX
214	Platambus maculatus	MNCN-AI140	Montenegro	Virpazar, Orahovska river	MON1	ca. 42°14'20"N 19°5'27"E	V. Pesic	XX	XX	XX	XX	XX
215	Platambus maculatus	MNCN-AI318	Poland	Zachodniopomorsky, Pyszka	POL2	N54º07’16.5” E15º46’03”	I. Ribera & A. Cieslak		XX			
216	Platambus maculatus	MNCN-AI671	Bulgaria	Bacevo, Rila mts.	BUL1	ca. 41°58'2"N 23°26'21"E	D.T. Bilton		XX			
217	Platambus maculatus	MNCN-AI975	Scotland (UK)	Sutherland, Loch Brora	SCO3	ca. 58°1'0"N 3°55'9"W	I. Ribera & G.N. Foster	XX	XX	XX	XX	XX
218	Platambus maculatus	MNCN-AI1017	Spain	Euskadi, Guipuzkoa province, Aia	ESPN8	N43º14’50.8” W2º9’11.3”	I. Ribera & A. Cieslak		XX			
219	Platambus maculatus	MNCN-AI1046	Spain	La Rioja, Posadas	ESPN4	N 42º12’36.0” W 3º4’27.8”	I. Ribera & G.N. Foster	XX	XX	XX	XX	XX
220	Platambus maculatus	MNCN-AI1153	Bulgaria	Gorno, Pirin mts.	BUL2	ca. 41°31'30"N 23°31'23"E	D.T. Bilton		XX			
221	Platambus maculatus	MNCN-AI1259	Greece	Makedonia, Mt Vitsi	GRE1	N40°37'21.0", E021°22'20.1"	P. & V. Ponel	XX	XX	XX	XX	XX
222	Platambus maculatus	MNCN-AI1286	Austria	Nieder Österreich, Pressbauch	AUS3	N48º11’42.8” E16º00’50.0”	I. Ribera & A. Cieslak	XX	XX	XX	XX	XX
223	Platambus maculatus	MNCN-AI1287	Austria	Nieder Österreich, Schwarzenbach	AUS4	N48º04’42.4” E15º40’42.9”	I. Ribera & A. Cieslak		XX			
224	Platambus maculatus	NHM-IR25	Spain	Castilla y León, Burgos province, Pineda de la Sierra	ESPN1	ca. 42°13'24"N 3°17'49"W	I. Ribera		AF309330	LM654811	LM655144	
225	Platambus maculatus	NHM-IR25b	Sweden	Ore river	SUE1	ca. 63°43'18"N 20°6'22"E	A.N. Nilsson		XX	XX		
226	Platambus maculatus	NHM-IR197	England (UK)	Hampshire, New Forest	ENG1	ca. 51°15'14"N 1°13'8"W	I. Ribera			XX		
227	Platambus maculatus	NHM-IR708	Austria	Arte Rütenen, Gisingen	AUS1	ca. 47°15'21"N 9°34'54"E	I. Ribera		XX	XX		
228	Platambus maculatus	NHM-IR715	Scotland (UK)	Sutherland, Loch Brora	SCO3	ca. 58°1'0"N 3°55'9"W	I. Ribera		XX	XX		
229	Platambus princeps cplx	IBE-RA195	Laos	Attapeu prov., Annam Highlands Mts.		15º05.9'N 107º25.6'E			XX	XX		
230	Platambus princeps	IBE-RA774	China	Yunnan, Mo Pan shan		N23.95210 E101.95096			XX	XX	XX	
231	Platambus semivittatus	NHM-IR189	New York (US)	New York, Tompkins Co., Ithaca					AY138765	AY138678	XX	
232	Platambus princeps	NHM-IR466	China	Yunnan, Shizeng					AY138764	AY138677		
233	Platambus stygius	NHM-IR475	Japan	Hokkaido, Fujiami, Rubeshibe Town					AY138766	AY138679		
234	Platambus stagninus	NHM-IR560	Maryland (US)	Talbot co., Wittman		38.48N,76.17W			AY138721	AY138631	XX	
235	Platambus obtusatus	NHM-IR595	Pennsylvania (US)	Pennsylvania, Fowlers Hollow					AY138762	AY138675	XX	

Appendix S2: Additional methods

Table S2a. Primers used for the amplification and sequencing. In brackets, length of the amplified fragment.

gene	primer	sequence	ref.
cox1-3'	Jerry (5')	CAACATTTATTTTGATTTTTTGG	(4)
(826)	Pat (3')	TCCAATGCACTAATCTGCCATATTA	(4)
	Chy (5')	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	(3)
	Tom (3')	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	(3)
barcode	LCO 1490 (5')	GGTCAACAAATCATAAAGATATTGG	(2)
(658)	HCO 2198 (3')	TAAACTTCAGGGTGACCAAAAAATCA	(2)
rrnL+trnL+nad1	16SaR (5')	CGCCTGTTTAACAAAAACAT	(4)
(784-797)	ND1 (3')	GGTCCCTTACGAATTTGAATATATCCT	(4)
H3	H3aF (5')	ATGGCTCGTACCAAGCAGACRCG	(1)
(330)	H3aR (3')	ATATCCTTRGGCATRATRGTGAC	(1)
Wingless	WG550F (5')	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	(5)
(472-478)	WGAbrZ (3')	CACTTNACYTCRCARCACCARTG	(5)

Table S2b. Standard PCR conditions for the amplification of the studied fragments.

step	time	temperature
1	3'	96º
2	30"	94º
3	30"-1'	47-50º *
4	1'	72º
5	Go to step 2 and repeat 34-40 x	
6	10'	72º

* Depending on the annealing temperatures of the primers pair used

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Supporting Information. Additional results:

Table S3: Best evolutionary model for each gene as selected by Partition Finder. In the *P. maculatus* complex selected models failed to converge and were not performed. Instead, an HKY+I+G model to each partition was applied (see `Results').

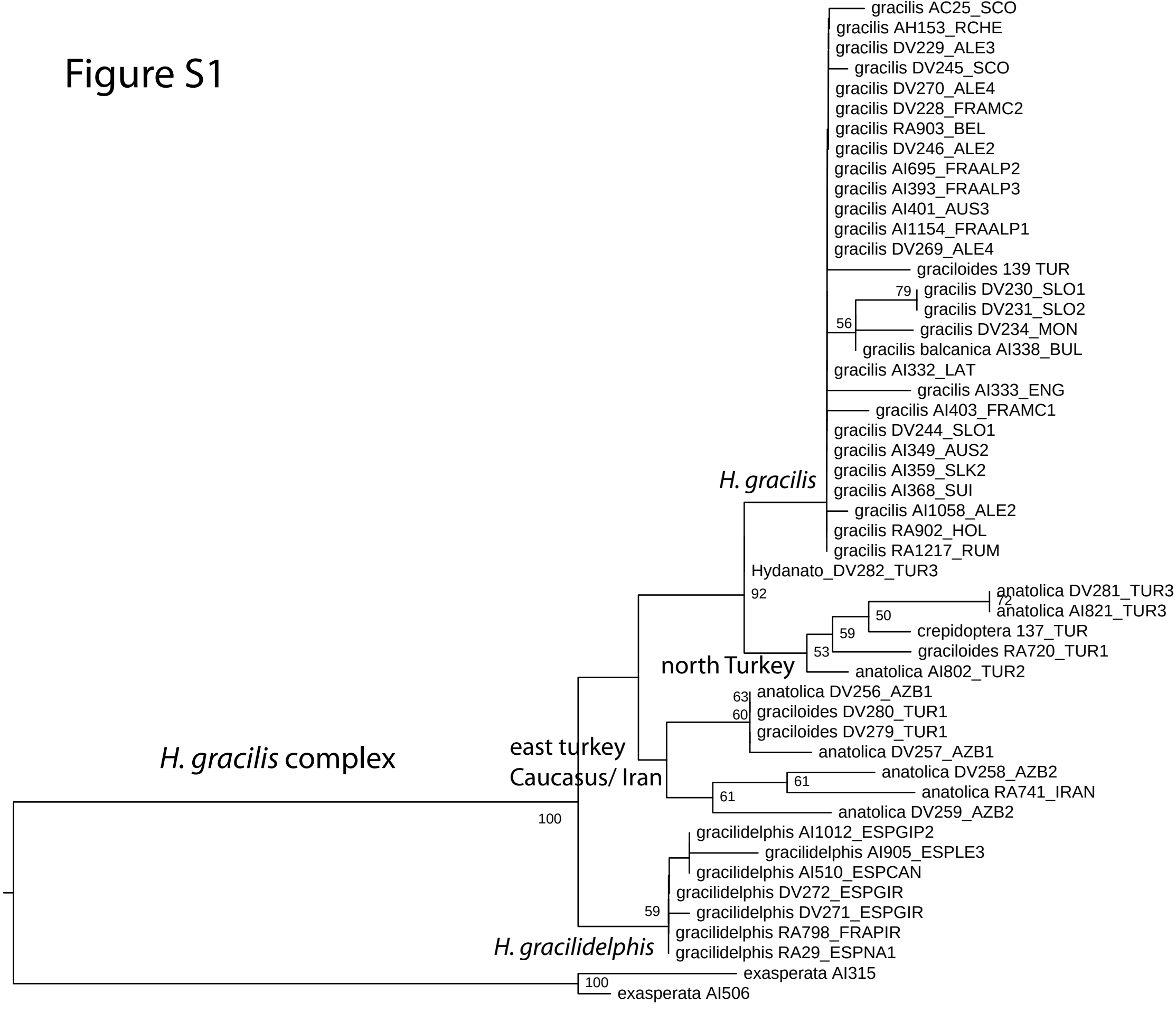
	H. gracilis complex	*P. maculatus* complex	Oreodytes
COI-5'	HKY+I+G (1)	TrN+I+G (2)	GTR+I+G (3)
COI-3'	HKY+I+G (1)	GTR+G (3)	GTR+I+G (3)
rrnL+trnL	TrN (2)	GTR+I+G (3)	GTR+I (3)
Nad1	HKY (1)	GTR+G (3)	HKY (1)
H3	TrN (2)	HKY (1)	TrN+I+G (2)
Wingless	X	TrN+I+G (2)	GTR+G (3)

(1) Hasegawa, M., Kishino, H., & Yano, T. A. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of molecular evolution*, *22*, 160-174.

(2) Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution*, *10*, 512-526.

(3) Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on mathematics in the life sciences*, *17*, 57-86.

Figure S1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gracilis AI332_LAT
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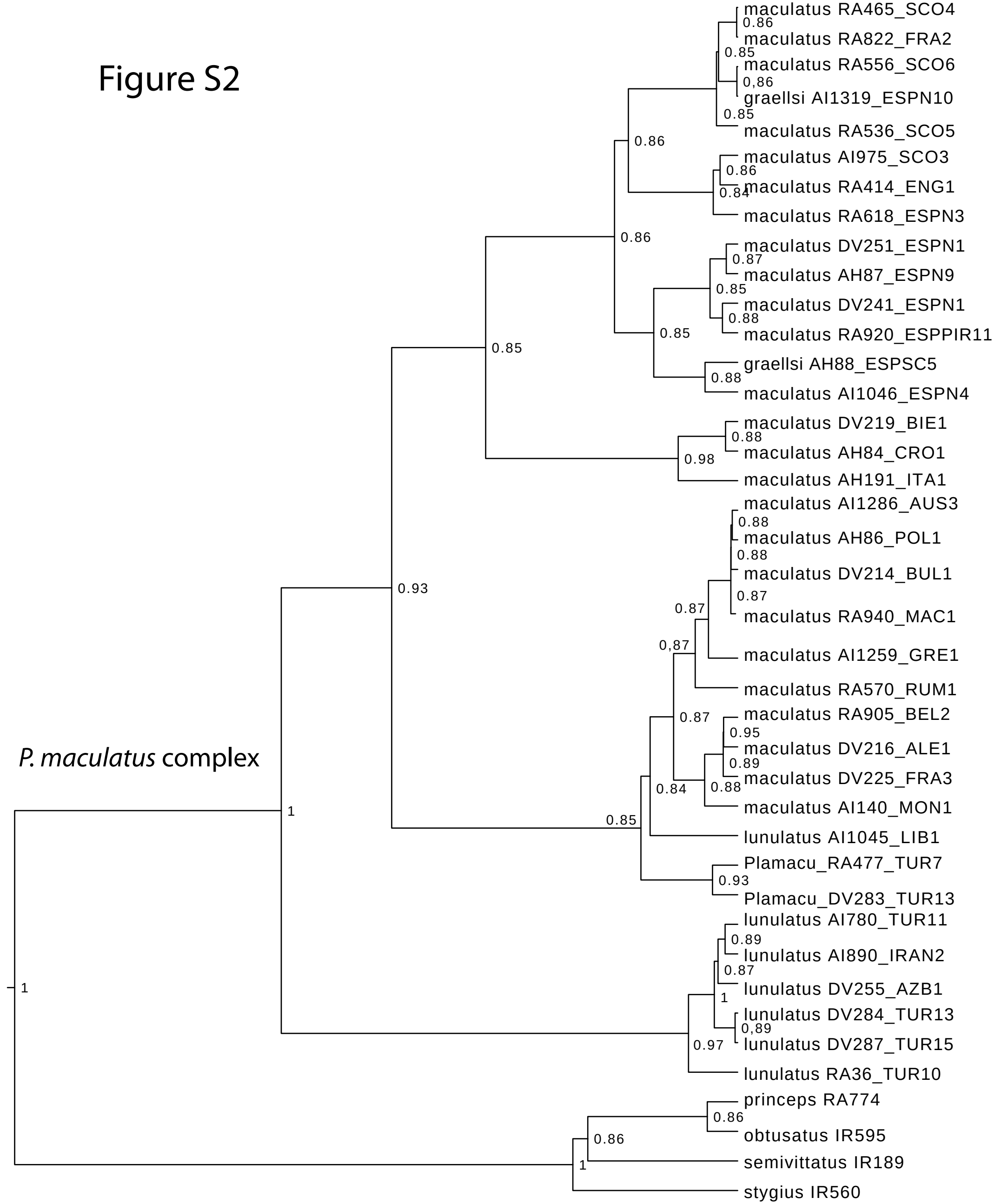
Figure S2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Plamacu_RA477_TUR7
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princeps RA774
obtusatus IR595
semivittatus IR189
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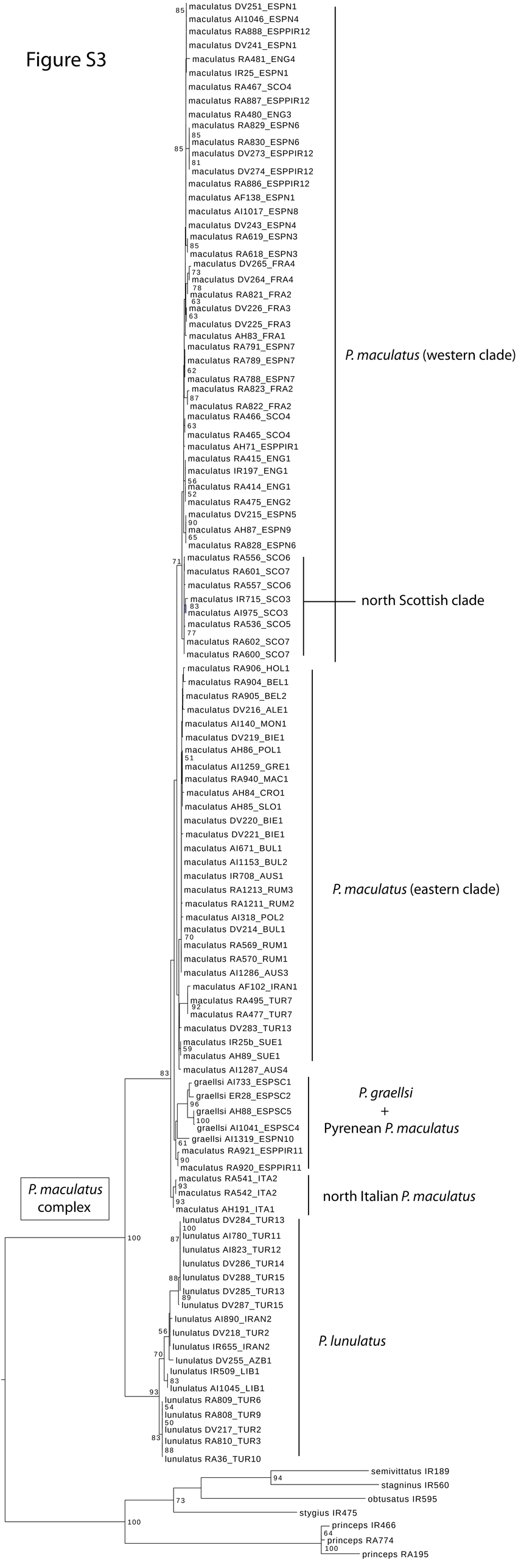
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maculatus AI975_SCO3
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maculatus RA495_TUR7
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P. graellsi
+
Pyrenean P. maculatus
maculatus RA541_ITA2
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maculatus AH191_ITA1
north Italian P. maculatus
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Figure S4

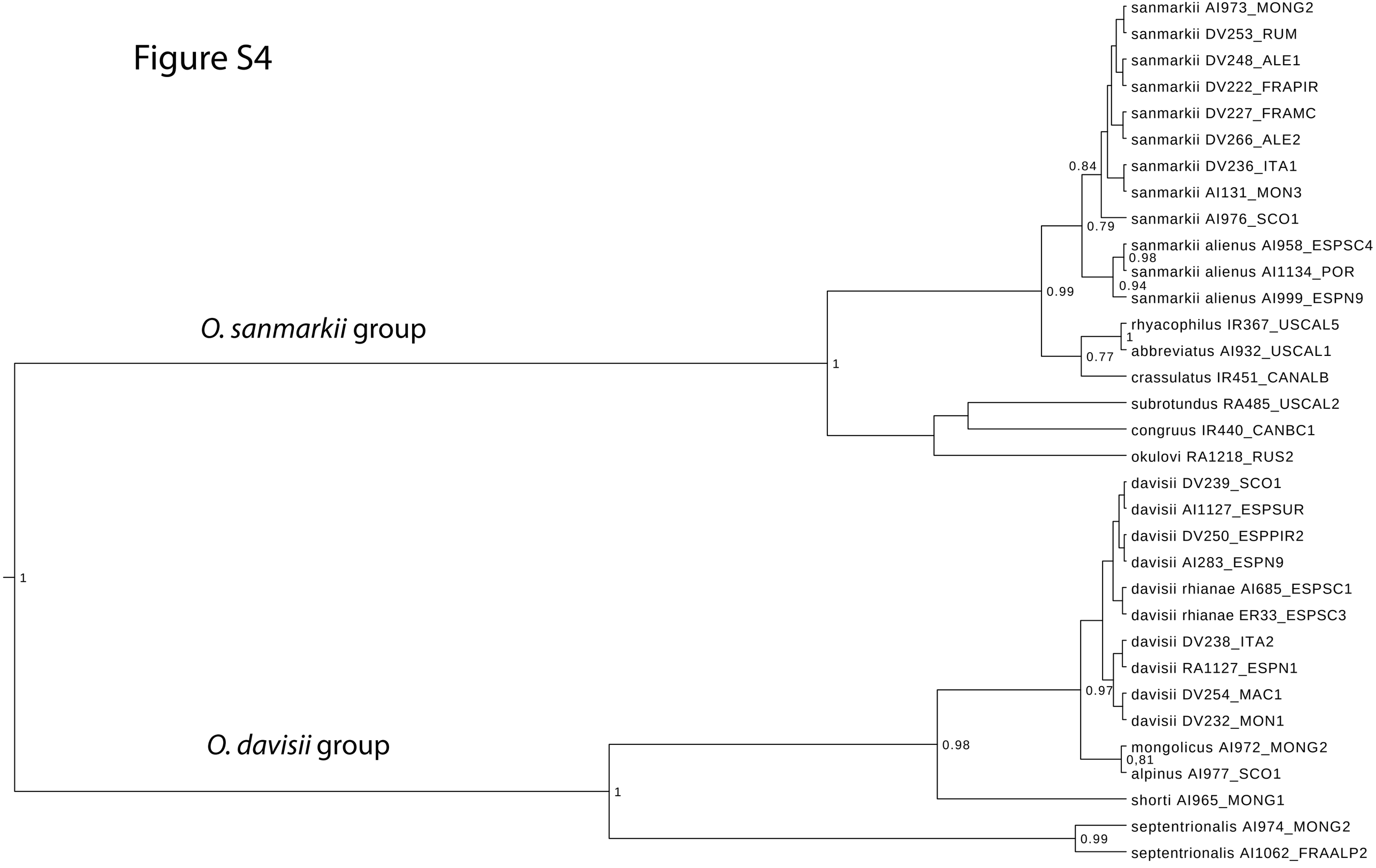
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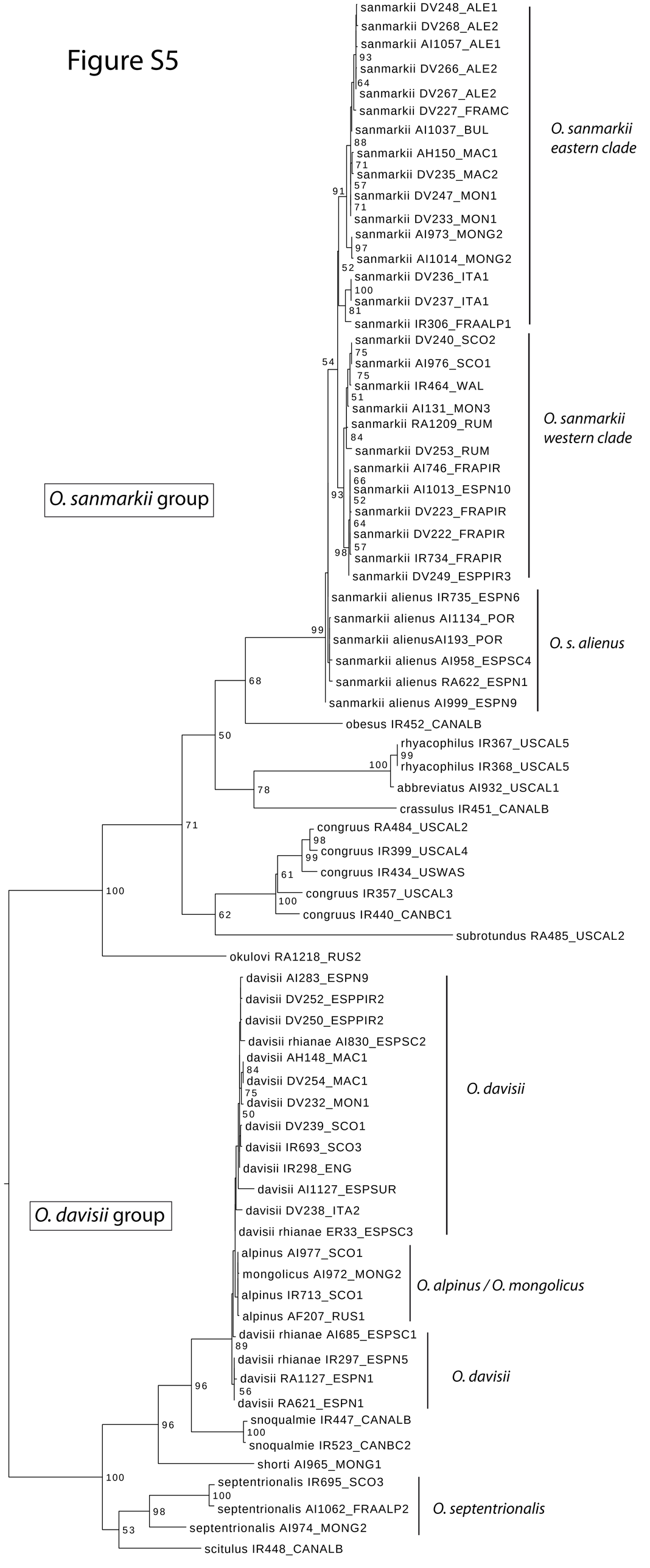
Figure S5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congruus IR440_CANBC1
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O. davisii
O. davisii group
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mongolicus AI972_MONG2
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Chapter 4

Range expansion and ancestral niche reconstruction in the Mediterranean diving beetle genus Meladema (Coleoptera, Dytiscidae)

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David García-Vázquez
David Sánchez-Fernández
Ignacio Ribera

Chapter submitted to *Zoological Scripta* (first review with decision "minor revision")

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Keywords:	Range expansion, climatic niche, ancestral niche reconstruction, cryptic lineages
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AppxS5.kml/a kml file with the phylogeographic reconstruction to be visualised in google earth	

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Range expansion and ancestral niche reconstruction in the Mediterranean diving beetle genus *Meladema* (Coleoptera, Dytiscidae)

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Running title: Range expansion and niche reconstruction in *Meladema*

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Abstract

Species of the genus *Meladema* (Dytiscidae, Colymbetinae) are some of the largest macroinvertebrates in the western Palearctic region, being top predators in fishless streams. Two of the three described species, *M. imbricata* (Wollaston, 1871) and *M. lanio* (Fabricius, 1775) are Macaronesian endemics from the Canary Islands and Madeira respectively, while the third, *M. coriacea* Laporte, 1835, is widely distributed from Morocco and the Iberian Peninsula to Turkey, including the Canary Islands. Previous phylogenetic analysis using only mitochondrial markers revealed the existence of two cryptic lineages within *M. coriacea*, one restricted to Corsica and the other including the rest of sampled populations. We reconstruct here the evolutionary history of the species of *Meladema* using a more comprehensive sampling covering its whole geographical range, adding nuclear markers and Bayesian molecular dating. Using environmental niche modelling we test for possible differences in climatic preferences among lineages, and reconstruct their ancestral climatic niche. Our results strongly supported the existence of four monophyletic lineages represented by the three recognised species plus a fourth cryptic lineage with populations of *M. coriacea* from the Tyrrhenian islands (Corsica, Sardinia and Montecristo). This pattern is not likely to be the result of mitochondrial artefacts due to *Wolbachia* infection, as all 11 tested individuals were negative for this parasite. Dating analysis placed the origin of *Meladema* in the Middle Miocene although diversification among extant *Meladema* lineages started in the early Pleistocene and took place in a relatively short time period. Phylogeographic analysis inferred a continental origin of *Meladema*, with an independent colonization of the Macaronesian and Mediterranean islands. From the southwestern Mediterranean region the continental *M. coriacea* expanded its range up to Bulgaria and Turkey in the northern basin, and to Tunisia in the southern. Results of niche modelling showed that seasonality is the critical factor in shaping the current distribution of *Meladema*. Island lineages (*M. imbricata*, *M. lanio* and the Thyrrenian lineage of *M. coriacea*) occur in sites with low seasonality, within the range of the reconstructed ancestral climatic niche of the genus. On the contrary, continental *M. coriacea* expanded its range to localities outside the ancestral climatic range of the genus, with a higher seasonality and aridity.

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Introduction

The diving beetle genus *Meladema* (Dytiscidae, Colymbetinae) is a common inhabitant of Mediterranean streams, in which it is often one of the largest macroinvertebrates. With a body size ranging from 20-23 mm, in small, fishless streams it may also be a top predator, both in the adult and larval stages. The genus currently includes three species: *M. coriacea* Laporte, 1835, *M. imbricata* (Wollaston, 1871) and *M. lanio* (Fabricius, 1775) (Ribera *et al*. 2003; Alarie & Hughes 2006). *Meladema* is confined to the western Palaearctic region, with one species (*M. coriacea*) distributed from Morocco and the Iberian Peninsula to Turkey and the other two endemic to Macaronesia. The widespread *M. coriacea* is common in the western Mediterranean, including the Iberian Peninsula, Southern France, North Africa and all the western Mediterranean islands (Nilsson & Hajek 2015). It seems to become scarcer towards the east, with few records from the Balkan Peninsula (Zaitsev 1972; Nilsson & Hajek 2015) and a single specimen reported from Turkey in the literature (Guéorguiev 1981; Darilmaz & Kiyak 2009). It is also present in the Canary Islands, likely resulting from a recent colonization (Ribera *et al*. 2003). There are references of the presence of *M. coriacea* in northern France and in Belgium (Gschwendtner 1936), but even when correct, these were undoubtedly occasional records. In contrast, *M. imbricata* and *M. lanio* are endemics to the Canary Islands and Madeira respectively, in where they are of high conservation concern due to their confinement to well preserved streams (Machado 1987; Balke *et al*. 1990; Ribera *et al*. 2003). All three species occupy running waters, mainly deeper pools in streams at intermediate altitudes (Ribera *et al*. 2003).

In a previous study using only mitochondrial DNA sequences, Ribera *et al*. (2003) found that *Meladema* was in fact constituted by four genetically distinct lineages with unresolved relationships between them. The two Macaronesian endemics *M. imbricata* and *M. lanio* were found to be respectively monophyletic, but the analyses suggested that *M. coriacea* was not monophyletic, as it was split into a lineage restricted to Corsica and another including the rest of sampled populations (Morocco, the Iberian peninsula and southern France). Despite uncertainties in the calibration, diversification within the genus was estimated to be of early Pleistocene origin. None of the lineages seem therefore to be a Tertiary relict, as hypothesized for other Macaronesian endemics (Ribera *et al*. 2003).

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In this study we present an update of the phylogeny and biogeography of the species of *Meladema*, with a more comprehensive sampling encompassing their whole geographical range, nuclear markers and a Bayesian molecular clock dating. We also include environmental niche modelling to test for differences in climatic preferences among individual lineages, trying to understand the factors determining the current and past distribution of the species of this emblematic genus of freshwater macroinvertebrate.

Material and methods

Sampling, DNA extraction and sequencing

We sampled all three known species of the genus *Meladema* (*M. coriacea*, *M. imbricata* and *M. lanio*) from 38 localities covering the whole range of all species (Fig. 1; see Appendix S1 for a list of specimens included in the study). Specimens were collected and preserved in absolute ethanol directly in the field. We extracted the DNA non-destructively with commercial kits (mostly "DNeasy Tissue Kit", Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Specimens and DNA extractions are kept in the collections of the Institut de Biologia Evolutiva, Barcelona (IBE) and the Museo Nacional de Ciencias Naturales, Madrid (MNCN). We used the data of Ribera *et al*. (2003), to which we added 17 newly sequenced specimens, especially from areas not previously studied in the Central and Eastern Mediterranean, and nuclear data. Six gene fragments from five different genes (three mitochondrial and two nuclear) were obtained in four different amplification reactions: (1) 3´end of Cytochrome Oxidase Subunit 1 (*COI-3´*); (2) 5´end of 16S rRNA plus tRNA transfer of Leucine plus 3´end of NADH subunit 1 (*16S+tRNA-Leu+nad1*); (3) an internal fragment of the nuclear gene Histone 3 (*H3*); (4) a fragment of the nuclear gene Wingless (*Wg*) (see Appendix S2 for the primers used and general cycling conditions). The new 142 sequences have been deposited in the EMBL database with accession numbers XX-XX. Sequences of outgroup taxa were downloaded from GenBank: a representation of other genera of subfamily Colymbetinae, including the closest known relatives of *Meladema* (Morinière *et al*. 2015, 2016), and one genus of Agabinae (*Agabus*) to root the tree, which is likely the sister subfamily of Colymbetinae (Ribera *et al*. 2008; Miller & Bergsten 2014). DNA sequences were assembled and edited using

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Geneious v1.6 (Biomatters, http://www.geneious.com). Alignments of variable length sequences were obtained with the MAFFT plugin v7.017 (Katoh *et al*. 2002) in Geneious v1.6.

The likely presence of cryptic mitochondrial lineages within *Meladema* (Ribera *et al*. 2003) raised the possibility that specimens could be infected with *Wolbachia*, a maternally transmitted parasite that can alter the patterns of mtDNA variability (Jiggins 2003). We thus tested for the presence of *Wolbachia* in 11 selected specimens of the two lineages of *M. coriacea* (Appendix S1). We used specific primers for the *wsp* gene, trying to amplify a 632 bp fragment. A 3 µl sample of the PCR reaction mixture was electrophoresed with a 100 bp DNA ladder on 1% agarose gel to determine the presence and size of the amplified DNA bands, that were visualized by Sybr-Safe staining. The same methods have yielded positive results, thus detecting the presence of *Wolbachia* infested individuals, in another genus of the same family (*Deronectes*, García-Vázquez & Ribera submitted).

Datasets for molecular analyses

We used three different datasets in our analyses: (1) phylogenetic and divergence time analysis with the complete, combined sequence and with only nuclear data; (2) coalescence analysis with only mitochondrial data; (3) phylogeography and climatic niche reconstruction with mitochondrial and nuclear data.

Phylogenetic and divergence time analysis

For the phylogenetic and divergence time analysis we used only specimens with different haplotypes of the *COI-3´* gene, plus the corresponding sequences of the rest of the genes. The final length of the data matrix was 2439 nucleotides for 52 specimens. Two specimens of *M. coriacea* known to have mtDNA haplotypes of *M. imbricata* and three specimens of *M. imbricata* with mtDNA of *M. coriacea* from Tenerife were also excluded from the analyses. These specimens were identified in preliminary phylogenetic analyses performed with sequences of all available specimens of the genus *Meladema* (see Results below and Ribera *et al*. 2003).

The dataset was divided into five partitions corresponding to each gene, and we used Partition Finder v1.1.1 (Lanfear *et al*. 2012) to estimate the evolutionary model

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that best fitted the data for each partition separately, using BIC (Bayesian Information Criterion) as selection criteria. We analysed the data with BEAST 1.8 (Drummond *et al*. 2012) linking substitution models in two partitions corresponding to the mitochondrial (*COI-3'*, *16S+tRNA-Leu* (*16S* onwards) and *nad1*) and nuclear (*H3+Wg*) markers respectively. Molecular clock models were linked in three partitions corresponding to the mitochondrial protein coding genes (*COI-3'+ nad1*), *16S* and nuclear genes (*H3+Wg*), and we ran two analyses to test the clock model (strict or lognormal relaxed) that best fitted the data. We applied an a-priori rate of 0.0145 substitutions/site/MY (standard deviation 0.002) for the protein coding genes and 0.0016 substitutions/site/MY (standard deviation 0.0002) for *16S*. These are substitution rates estimated for a related group, family Carabidae, based on a combination of fossils and biogeographic events (Andújar *et al*. 2012). Clock rates of *H3* and *Wg* were left with uniform priors due to the absence of any suitable estimations of the evolutionary rate for these nuclear genes. We used a Yule speciation prior for all of the partitions and ran the analysis for 100 million generations, logging parameters every 5000 generations. Unless otherwise stated, we used a conservative burn-in of 10% for combined datasets of both mitochondrial and nuclear DNA, after checking convergence of all parameters in Tracer v1.6.

We performed four runs with different topological constraints: (1) *M. imbricata* + *M. lanio* as monophyletic; (2) *M. coriacea* as monophyletic; (3) *M. lanio*, *M. imbricata* and *M. coriacea* (only specimens from Corsica, Sardinia and Montecristo) as monophyletic; (4) without any constraints. We then compared tree likelihoods in Tracer v1.6 (Rambaut *et al*. 2014). A consensus tree was obtained via Tree Annotator v1.8 (Drummond *et al*. 2012) and visualized using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

We also analysed the nuclear genes only to test for potential incongruence between the nuclear and mitochondrial genomes. The dataset for the nuclear sequence contained 46 specimens and the aligned sequence was 805 nucleotides long, and was analysed with a fast maximum likelihood algorithm implemented in RAxML (Stamatakis *et al*. 2008) with a single partition and a GTR model. Node support was assessed with 500 fast bootstrap replicas.

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Coalescence analysis

We used all available mitochondrial sequences of the continental *M. coriacea* for analyses of coalescence and Bayesian Skyline Plots (Drummond *et al*. 2005) to estimate the population history of this species. A similar methodology has been successfully applied to relate demographic models and geographical expansion of other water beetles species complexes (Hidalgo-Galiana *et al*. 2015). The aligned nucleotide matrix of 35 specimens was 1632 nucleotides long. Both coalescence and Bayesian Skyline Plot analyses were carried out in BEAST 1.8. The dataset was divided into three partitions corresponding to each mitochondrial genes (*COI-3'*, *16S* and *nad1*), with a GTR substitution model. We estimated the best clock model and set an a-priori rate of 0.0134 substitutions/site/MY with a standard deviation of 0.001 for the combined sequence, estimated for the family Carabidae (Andújar *et al*. 2012). Preliminary analyses had shown that if the sequence was split in different partitions there were severe convergence problems, so we used a single concatenated partition. To identify the best demographic model, we ran four analyses, including Constant Size, Exponential Growth, Logistic Growth and Expansion Growth. Models were then compared using modified Akaike information criterion (AICM) with moments estimator (Baele *et al*. 2012) with 100 bootstrap replicates, as implemented in Tracer v1.6. The MCMC chain was set to 100 million generations, logging parameters every 5000 generations. The consensus tree was obtained with Tree Annotator v1.8 and visualized using FigTree v1.4.2. The Bayesian Skyline Plot was calculated in Tracer v1.6.

Phylogeographic analysis and environmental niche modelling

For an estimation of the dynamics of the geographic expansion of the genus *Meladema* we performed a continuous phylogeography analysis in BEAST v1.8. The datasets for both phylogeographic and climatic niche analyses included sequences of all available specimens, excluding the hybrid specimens mentioned above. The aligned nucleotide matrix of 63 specimens was 2436 nucleotide long. The dataset was divided into three partitions, *COI-3'*, *16S+nad1* and *H3+Wg*, with estimated optimal substitution models for each partition. Another partition containing specimen coordinates as a continuous trait was created, with a homogenous Brownian evolutionary model. We set the best clock model for mitochondrial and nuclear partitions, with a-priori clock rates as for

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analyses before. We ran the estimated optimal coalescent model for 100 million generations with parameters logging every 5000 generations. The consensus tree was obtained in the same way as with previous analyses. The geographic range dynamics of *Meladema* was obtained by using Spread v1.0.6 (Bielejec *et al*. 2011) and visualized in Google Earth v7.

We used environmental niche modelling based on large-scale climatic data and known presence localities of all *Meladema* lineages to characterize their environmental niche and to test for niche divergence among them. As a source of climatic data, we used WORLDCLIM v1.4 and obtained 19 climatic variables (http://www.worldclim.org; Hijmans et al. 2005) (Appendix S3). We included localities of all sequenced specimens, plus additional localities for mainland Italy from CKmap v5.4.1 (Latella *et al*. 2007), which were considered separately in some specific analyses. In order to characterize the preferred climatic conditions for each species we represented the location of the occurrence cells of each of them across the climatic space of the study area. Values of all 19 bioclimatic variables were summarized using a principal component analysis (PCA) to obtain uncorrelated environmental factors (Varimax rotation). Values of the first two PCA factors were used to plot a bidimensional climatic space of the study area (e.g. Sánchez-Fernández *et al*. 2013) and to locate sampling localities of each lineage of *Meladema*, including the Italian mainland localities obtained from CKmap (Appendix S3). To test for significant differences in climatic conditions among *Meladema* species we also conducted one-way ANOVA analyses of the first three PCA factors with post-hoc tests using the Bonferroni correction. All statistical analyses were conducted using SPSS 15.0.1 and Statistica v8.0 (www.statsoft.com, 2007).

To compare the climatic niche between lineages we generated ecological niche models using MaxEnt v3.3.3 (Phillips *et al*. 2006). To avoid autocorrelation in the variables we also used the three first factors of the climatic PCA as predictors. We included all known localities for all lineages except for continental *M.coriacea*, for which we included only localities with sequenced specimens to avoid uncertainties. The localities with molecular data are widespread in the climatic space, providing a good geographic and climatic subsample of all known records (Fig. 1; Appendixes S1,S3), minimizing the risk of bias due to unbalanced climatic and spatial sampling

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effort. MaxEnt is a machine-learning method that estimates organisms distribution by finding the probability distribution of maximum entropy (i.e. the most uniform), given the constraint that the expected value of each environmental predictor under this estimated distribution matches the empirical average of sample locations (Phillips *et al.* 2006). MaxEnt simulations of realized distributions produce continuous suitability scores for each cell (from 0 to 1), and we calculated Schoener's D metric (Schoener 1968) using ENMtools (Warren *et al*. 2010) to assess niche similarity among populations, where D (pX, pY) = 1 - ½S (pXi - pYi). This metric assumes probability distributions defined over geographic space, in which pXi (or pYi) denotes the probability assigned by the modelling method to species X (or Y) in the i cell ranging from 0 (niches do not overlap) to 1 (niches completely overlap). The null hypothesis of the background similarity test states that observed niche overlap between taxa is explained by regional similarities in available background environments. This hypothesis involves a two-tailed test, so it is rejected if the observed D between two taxa falls outside the 95% confidence limits of the null distribution. Niche conservatism is supported when the observed value of D is larger than the upper 95% confidence limit of the null distribution, suggesting that niches are more similar than expected based on their background environments (i.e. species are occupying niches that are as similar as possible given what is available). Niche divergence is supported when the observed value of D is smaller than the lower 95% confidence limit of the null distribution, suggesting that niches are more divergent than expected based on background divergence. Because niche differences may be simply result of the spatial autocorrelation of the used explanatory environmental variables (background environmental divergence, Warren *et al*. 2008), strong evidence for niche divergence requires two conditions: (1) that niche characteristics differ between the two considered populations and (2) that these differences are greater than the background environmental divergence (McCormack *et al.* 2010). Niche conservatism, on the other hand, would be supported if niche differences were smaller than the obtained background environmental divergence. Thus, comparison of environmental characteristics from these two classes of data should allow discrimination between differences as a result of simple spatial autocorrelation caused by geographic distance and strong niche divergence that occurs because two species occupy different

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environmental conditions. To test the null hypothesis that niches are similarly divergent in comparison to background environments, we used the 'background similarity test' procedure implemented in ENMtools, estimating 100 niche overlap values generated by comparing model suitability values of one population to those generated from random cells drawn from the geographic range of the other population (Warren *et al*. 2010). The background area of each species should be adjusted to the habitat available, and should be biologically realistic (Warren *et al.* 2010). As three of the lineages within the genus are insular (see Results) the background area was restricted to the islands in which the species appear. For continental *M. coriacea* we defined the background as the whole study area.

We also performed an ancestral character state reconstruction analysis in BEAST v1.8 in order to estimate ancestral climatic preferences of individual *Meladema* lineages. All substitution models and settings were the same as in coalescence analyses above. We created another partition representing the first three best scoring PCA factors used in previous PCA analysis as a continuous trait, with a homogenous Brownian model of evolution. We ran the ancestral character state reconstruction analysis for 100 million generations with parameters logging every 5000 generations. For each of the main nodes we identified the geographic area with climatic conditions encompassed by the 95% interval of the reconstructed value for all three PCA factors.

Results

Datasets for molecular analyses

There were no length differences in protein coding genes. The only length differences in alignment were three insertions in ribosomal genes in outgroup taxa and one single nucleotide deletion in several specimens of *M. coriacea*.

The GTR+I+G (Tavaré 1986) was identified as the best substitution model for the *COI-3'*, *16S* and *nad1* partitions, and the TRN+I+G (Tamura & Nei 1993) for the *H3* partition. For the *Wg* partition, the K80+I (Kimura 1980) was identified as the most suitable, but as it is not implemented in BEAST 1.8, we used the HKY model instead (Hasegawa *et al*. 1985), with equal base frequencies (Drummond *et al*. 2012). The same substitution models were used for each gene in all datasets.

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Phylogenetic and divergence time analysis

The strict clock was significantly better than the lognormal clock when using the combined data (Table 1), so in consequence we used the strict clock in all analyses. The comparison of likelihood scores among trees with topological constraints showed no significant differences between likelihood scores of the tree without any constraints and trees with constrained clades (with less than 5 units of AICM between the most divergent values, Table 2), so we used the topology without any prior constraints.

The analysis of phylogenetic relationships including only specimens with unique haplotypes supported a sister relationship of Corsican, Sardinian and Montecristo specimens of *M. coriacea* (*M. coriacea* CSM onwards) and the rest of the genus (Bayesian posterior probability, pp = 1; Fig. 2). The rest of specimens of *M. coriacea* (continental *M. coriacea* onwards, although including specimens from the Balearic and Canary Islands and Malta) were placed as sister of a clade including *M. imbricata* and *M. lanio* (Fig. 2), but with a low support (pp < 0.50). On the contrary, the monophyly of *M. imbricata* plus *M. lanio* was strongly supported (pp = 0.96), as well as monophyly of respectively *M. lanio* (pp = 1), *M. imbricata* (pp = 1), *M. coriacea* CSM (pp = 1) and the continental *M. coriacea* (pp = 0.98) (Fig. 2).

The dating analysis using an a priori rate obtained for the family Carabidae showed a very long branch between the ingroup and outgroup taxa, with an estimation for the origin of the genus *Meladema* at ~14.4 Ma (million years ago) (95% CI of 9.99 to 20.02 Ma), in the Miocene (Fig. 2). The crown age of the genus *Meladema* was estimated at ~1.49 Ma (95% CI of 1.01 to 2.05 Ma), with the youngest speciation between *M. imbricata* and *M. lanio* dated to ~1.17 Ma (95% CI of 0.77 to 1.69 Ma), thus in a relatively short time period in the Pleistocene (Fig. 2).

For nuclear partitions, the lognormal relaxed clock was considered as a more suitable clock model (Table 1). In the *H3* nuclear protein coding gene sequence there were only two variable positions, one synonym change synapomorphic for *M. imbricata* plus *M. lanio* and another with ambiguous callings in most individuals, without a clear pattern. In the *Wingless* nuclear protein coding gene sequence there were three variable nucleotide positions with changes shared by: (1) *M. imbricata* + *M. lanio*; (2) *M. coriacea* CSM and (3) *M. coriacea* CSM + *M. imbricata* + *M. lanio*. As happened in *H3*, there was more variability among outgroup taxa. The monophyly of *M. coriacea*

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CSM and the sister relationship of *M. imbricata* and *M. lanio* were thus corroborated by nuclear DNA (Appendix S4).

Coalescence analysis

The best demographic model for the continental *M. coriacea* was an expansion growth, although with an AICM almost identical to the constant size model (Table 3). The analysis using the expansion growth model dated the age of the most recent common ancestor (MRCA) of the continental *M. coriacea* lineage to ~145 thousand years before present (Ka) (95% confidence interval 71 to 246 Ka), in the Late Pleistocene (Table 3). All analyses using different demographic models estimated a Late Pleistocene age of the MRCA of continental *M. coriacea* (Table 3).

The Bayesian Skyline Plot showed a continuous increase in the population size of continental *M. coriacea* during the last 56 Ka, with a slight decrease towards the present (Fig. 3).

Phylogeographic analysis

The phylogeographic analysis inferred a continental origin of *Meladema*, although the lack of well defined outgroups did not allow a precise reconstruction. Macaronesian and Mediterranean islands were colonized independently from the continent. There were two main clades within the *M. coriacea* CSM lineage, one with exclusively specimens from Corsica and a second with specimens from Corsica, Sardinia and Montecristo. The basal splitting events within continental *M. coriacea* took place in northwestern Africa, with subsequent expansions through the western Mediterranean and possibly several secondary colonizations of the Canary Islands. The eastern most sampled specimens (Malta and Turkey) were not derived from the northern lineages (Iberian peninsula and southern France), but originated directly from within the north African stock (see Appendix S5 for a phylogeographic reconstruction visualized in Google Earth).

Presence of Wolbachia

None of the 11 tested specimens was positive for the presence of *Wolbachia*.

Environmental niche modelling

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The main two factors of the climatic PCA jointly accounted for a high percentage of the total variance (78.7%; the first three factors 85.8%.). The first factor was positively correlated with annual mean temperature and negatively with annual precipitation, and was interpreted as representing an "aridity" gradient with higher values represent hot and dry sites. The second axis was negatively correlated with temperature seasonality, and was interpreted as representing a "seasonality" gradient. The third factor showed a positive correlation with precipitation of the coldest quarter and negative with mean temperature of the wettest quarter, and its interpretation was less clear. The environmental space of continental *M. coriacea* almost covered that of all other lineages, with the only exception of *M. lanio* (Fig. 4). Among the island lineages, *M. lanio* and *M. imbricata* were closer to each other, although *M. lanio* occupied sites with lower seasonality, while *M. coriacea* CSM occupied relatively more seasonal sites (Fig. 4). The environmental space of the peninsular Italian *M. coriacea* fitted into the space occupied by continental *M. coriacea* and *M. coriacea* CSM, with a higher seasonality than in *M. imbricata* and *M. lanio* (Fig. 4).

Results of the ANOVA analyses showed differences among the four main lineages for the first three main PCA factors (Fig. 5). Post hoc analyses with Bonferroni corrections showed that *M. coriacea* occurs in significantly more arid localities than *M. coriacea* CSM (first factor), and *M. lanio* in significantly less seasonal localities than *M. coriacea* and *M. coriacea* CSM (second factor; Fig. 5).

Results of the MaxEnt analysis estimated by Schoener's D showed a low niche overlap among the four main lineages (Appendix S6), with the highest between continental *M. coriacea* and *M. coriacea* CSM (0.48), and *M. imbricata* and *M. lanio* (0.41). The lowest overlaps were between *M. lanio* and both lineages of *M. coriacea*, continental (0.08) and CSM (0.09) (Fig. 6; Appendix S7). In the background similarity tests the null hypothesis of no niche difference was rejected in both directions in four of the six pairwise comparisons. In the other two, the comparison of *M. imbricata* against *M. coriacea* CSM and of *M. lanio* against *M. coriacea* CSM, only one direction showed niche divergence (Fig. 6).

Reconstruction of ancestral climatic preferences

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We obtained the 95% Highest Posterior Density (HPD) intervals in the BEAST analysis for the first two PCA factors (Appendix S8). The analysis showed that all studied specimens of *M. coriacea* CSM, *M. imbricata* and *M. lanio* occurred in climatic conditions reconstructed as being ancestral for the whole genus *Meladema* (Fig. 7). On the contrary, some current localities of continental *M. coriacea* (mostly in Morocco, Spain and Turkey) did not fit into the 95% HPD interval for at least one reconstructed PCA factor, suggesting occurrence in non-ancestral conditions for the genus (Fig. 7; Appendix S8). When considering the ancestral conditions of each lineage individually, for all insular lineages the climatic conditions of the sampled localities fall within the 95% HPD interval of the ancestral conditions of the same lineage only. This is in contrast to some specimens of continental *M. coriacea*, which fall within the 95% HPD interval of other lineages (Fig. 7; Appendix S8).

Discussion

Phylogeny and age of Meladema

Previous work on the genus *Meladema* had revealed the existence of a fourth, cryptic lineage in addition to the three recognized species (Ribera *et al*. 2003). This lineage was apparently morphologically indistinguishable from the widespread *M. coriacea*, but found only in Corsica. This study had, however, two important shortcomings: it was based on mitochondrial data only, and the geographical sampling was very incomplete. Our results using both mitochondrial and nuclear DNA and a more comprehensive sampling corroborated the existence of this cryptic lineage within the genus. Although we found very small amount of variability in the nuclear genes used in our analyses, the variable nucleotide positions within them supported the existence of a distinct lineage of *M. coriacea* from Corsica, Sardinia and the island of Montecristo, in the Tuscan archipelago (*M. coriacea* CSM). The genus and each of four previously suggested lineages were strongly supported as monophyletic, although the internal relationships within the genus were not resolved reliably except for the sister relationship between the Macaronesian species *M. lanio* and *M. imbricata*.

The origin of *Meladema* was dated back to the Middle Miocene. However, the diversification among extant *Meladema* lineages started much later, in the Early Pleistocene. According to our estimate, these events took place in a relatively short time

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period, suggesting the possibility of a nearly simultaneous speciation. The poor support for the relationships among the lineages with an exception of the sister relationship of *M. lanio* and *M. imbricata* would also imply that the speciation took place in a short sequence of events. These age estimates are very close to those suggested by the analysis of Ribera *et al*. (2003), which used a standard rate of variation for the mitochondrial genes of 2% per million years (Brower 1994). The age estimated for the crown diversification of *Meladema* was also in good agreement with that in the analysis of Colymbetinae phylogeny of Morinière *et al*. (2016), including a wider representation of the subfamily and different calibration schemes. Nevertheless, the lack of internal calibration points for *Meladema* or closely related outgroups still leaves some level of uncertainty in molecular clock estimates in our analyses. This is especially the case for the long stem branch of *Meladema*, estimated here to date from the middle Miocene, but from the late Eocene in Morinière *et al*. (2016).

As was already stated by Ribera *et al*. (2003), *M. coriacea* CSM is morphologically extremely similar to the continental *M. coriacea*, and no differences have been identified so far, not even in male genitalia. In contrast, there are clear differences both in genitalia and external morphology between Macaronesian species and *M. coriacea* (Machado 1987). This could have several possible reasons. One explanation could be a difference in population sizes. It is likely that in the case of *M. coriacea* CSM there was present a large population of *M. coriacea* before the isolation from the mainland, which may have dampen the morphological variation in the CSM lineage. In contrast, if the colonization of Macaronesian islands was only by a small number of ancestral *Meladema* individuals, this might have resulted in a founder effect driven fast morphological evolution. Another explanation could be an occasional gene flow between CSM lineage and continental *M. coriacea* preventing morphological differentiation, while not being enough to leave a clear signal in mtDNA. However, we do not have any clear evidence for either of these two scenarios (which are not mutually exclusive). A third possible explanation for the absence of morphological differences between *M. coriacea* CSM and continental *M. coriacea* could have been the presence of *Wolbachia*, which can cause cytoplasmic incompatibility and thus reproductive isolation (Werren *et al*. 2008). Recent works gave an estimate of 40% of *Wolbachia* prevalence among arthropods (Zug & Hammerstein 2012) and 31% in a group of

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families of aquatic beetles, among them Dytiscidae (Sontowsky *et al*. 2015), a frequency agreeing with recent reports of the same family (García-Vázquez & Ribera submitted). However, none of the specimens of *Meladema* tested was positive for the presence of these endosymbiotic bacteria.

Phylogeography and climatic preferences of Meladema

Our phylogeography analysis suggests the origin of *Meladema* in the western Mediterranean, most probably in southern Iberia or in northwestern Africa, during the Middle Miocene. This implies that for most of their evolutionary history the ancestors of extant *Meladema* lineages evolved under the tropical climate present in southern Europe and North Africa during the time (Ruddiman *et al*. 1989; Griffin 2002; Jiménez-Moreno *et al*. 2010; Köhler *et al*. 2010). The climatic change from wet tropical to Mediterranean, with a stronger seasonality and dry summers, occurred at approximately 3.2 Ma, in the Pliocene (Suc 1984). This could have confined the ancestral *Meladema* to regions with a climate more similar to that in Miocene. Our reconstruction of ancestral climatic preferences implies that *M. lanio*, *M. imbricata* and *M. coriacea* CSM are probably relicts inhabiting sites with conditions closer to the ancestral for the whole genus. In contrast, continental *M. coriacea* was the only lineage able to colonize also sites with climatic conditions non-ancestral for *Meladema*. Environmental niche modelling analyses showed that seasonality is the most important factor in current distribution of *Meladema*. All island lineages (*M. lanio*, *M. imbricata* and *M. coriacea* CSM) inhabit on average less seasonal sites than continental *M. coriacea*, which is the lineage occupying the highest diversity of climatic conditions. Among the island lineages, endemic Macaronesian species inhabit the least seasonal sites, while *Meladema* CSM generally occupies moderately seasonal localities. The estimated time of the colonization of Macaronesia by the ancestors of *M. lanio* and *M. imbricata* clearly excludes the possibility of them being tertiary relicts of laurisilva forests on Madeira or the Canary Islands, as already suggested by Ribera *et al*. (2003).

There are several possible scenarios for the colonization of these archipelagos. One is that both were independently colonised from the continent; the Canary Islands (*M. imbricata*) likely from northwestern Africa, and Madeira (*M. lanio*) from southwestern Iberia. During the Pleistocene there were several islands between Madeira

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and continental Europe, even during the Last Glacial Maximum (Fernández-Palacios *et al*. 2011). The two colonisations could have originated from a common continental stock or they may have previously speciated, reaching their recovered sister relationship through a process of coalescence with the extinction of some of the continental lineages. A second possibility is that there was a single colonisation process from the continent, either to Madeira or the Canary Islands, with subsequent dispersal between islands. However, since *Meladema* is absent on Salvages and no other islands were probably present between Madeira and Canary Islands in the past (Fernández-Palacios *et al*. 2011), the dispersion between both archipelagos seems rather unlikely.

The estimated age of the *M. coriacea* CSM lineage (Pleistocene) indicates that the isolation occurred much later than the end of the Messinian Salinity Crisis, which lasted from 5.93 to 5.33 Ma and was the last time when islands in Tyrrhenian Sea were connected to the continental Europe (Krijgsman *et al*. 1999; Manzi *et al*. 2013; Pérez-Asensio *et al*. 2013). This suggests that ancestral *Meladema* were able to colonize islands across the sea, probably either from France or Italy. Although we sampled only Corsica, Sardinia and Montecristo, it is likely that also other islands such as Pianosa, Capraia or Elba are inhabited by *M. coriacea* CSM (Rocchi *et al*. 2014). On the contrary, Malta (and likely Sicily, from where no specimen could be obtained) were likely colonised through other routes.

After the speciation, continental *M. coriacea* started to spread to northwestern Africa, Iberia, southern France, Turkey and several Mediterranean islands (Malta, Balearic Islands), including one or more secondary recent colonizations of the Canary Islands. The phylogenetic assignment of continental Italian, Sicilian and Balkan *M. coriacea* remains uncertain, as no specimens from these regions could be sequenced. In the case of Greece, *M. coriacea* was reported for the south, in the Attica region and the Cyclades (Oertzen 1886). Since the Turkish specimen from the Izmir province included in our analysis is nested deep within the continental *M. coriacea* clade, it seems that also Bulgaria, Greece and Cyclades are likely inhabited by the same lineage. The phylogenetic assignment of *M. coriacea* from mainland Italy is more problematic. According to the "Checklist of Italian Fauna" database (CKMAP2000; http://www.faunaitalia.it/ckmap/), *M. coriacea* is widely distributed in the Italian peninsula and Sicily, with the northernmost records from Liguria. The fact that *M.*

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coriacea CSM inhabits islands in the Tuscany archipelago very close to the Italian mainland (Montecristo) cannot be taken as evidence that Italian *Meladema* also belongs to the CSM lineage. These islands are known to be inhabited by some Corso-Sardinian endemics not found in mainland Italy (e.g. *Agabus aubei* Perris, 1869, *A. rufulus* Fairmaire, 1859 or *Rhithrodytes sexguttatus* Aubé, 1838 among the Dytiscidae, Rocchi *et al*. 2014). Moreover, *Meladema* has never been found in North Africa east of Tunisia (Touaylia *et al*. 2010), or in the Near East but Turkey. This suggests that *M. coriacea* reached western Turkey and Greece probably from southern Italy or the Balkans, which would thus be inhabited by the same lineage (continental *M. coriacea*). Results of niche similarity test do not provide clear evidences from a climatic niche perspective. The single specimen from Malta belongs to the continental *M. coriacea* lineage, suggesting that the Sicilian populations may also belong to the same lineage. The aquatic Coleoptera fauna of Malta is known to be strongly related to that of Sicily and mainland Italy (e.g. among the Hydraenidae, with a high degree of local endemism, all species known from Malta occur also in Sicily, Mifsud *et al*. 2004), which can also be taken as evidence of the absence of *M. coriacea* CSM outside the northern Tyrrhenian islands.

We can only speculate what is the reason of the wide distribution of continental *M. coriacea* through the Mediterranean, while all other lineages are exclusively restricted to different archipelagos. Similarly to all other *Meladema* lineages, continental *M. coriacea* could have initially been restricted to sites with climate resembling the wet tropical climate of the Miocene, but developed capabilities necessary for survival during dry periods after the climatic change from a wet tropical to Mediterranean climate 3.2 MA (Suc 1984). To test this hypothesis it would be necessary to obtain experimental data on the physiological tolerance of the different species. However, it could also be that continental *Meladema* expanded its range, at least initially, just because it was in the continent, that is, without geographical barriers to impede its displacement, similarly to what is hypothesized to have happened in other groups of aquatic beetles during the interglacials (García-Vázquez et al. submitted). Only at a later stage it may have acquired the dispersal or physiological capabilities necessary to cross the Straits of Gibraltar or to re-colonise the Canary Islands.

Conclusions

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Our results strongly support the existence of a cryptic lineage of *Meladema* on the northern Tyrrhenian islands. Thus, *M. coriacea* as currently understood is not a monophyletic species. In contrast, the Macaronesian species *M. lanio* and *M. imbricata* were found respectively monophyletic and sisters. The speciation among extant lineages likely took place in a very short time period in the Early Pleistocene. This could have been the result of the change from a tropical climate in the Miocene to the typical Mediterranean seasonality in the Pliocene. Climate niche modelling showed that seasonality is the most important factor in the distribution of *Meladema*. All three island lineages inhabit less seasonal sites, likely relicts of climatic conditions ancestral to *Meladema*. On the contrary, continental *M. coriacea* was able to colonize more seasonal sites and expand its range across the Mediterranean.

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Supporting information

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Appendix S7. Pairwise values of observed niche overlap (Schoener's D-value) between the four lineages of *Meladema*.

Appendix S8. Specimens used in the reconstruction of the ancestral niche from the scores of the first two PCA axes. Overlap "YES" indicates that the two PCA scores of the climatic conditions of the locality in which the specimen was found fall within the 95% Highest Posterior Density (HPD) intervals of the reconstructed conditions of the most recent common ancestor of the respective lineage.

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Figure legends

Figure 1. Location of the studied specimens. Black, continental *M. coriacea*; blue, *M. coriacea CSM*; red, *M. imbricata*; green, *M. lanio*. See Appendix S1 for details of the localities.

Figure 2. Reconstructed phylogeny of *Meladema* obtained with BEAST with the combined nuclear and mitochondrial sequence. Numbers at nodes, estimated age (Ma); coloured points, node support. See Appendix S1 for details of the specimens.

Figure 3. Bayesian Skyline Plot of continental *M. coriacea* showing reconstructed changes in its effective population size during the last 60 Ka. Thin blue lines, 95% confidence intervals; horizontal axis, time (thousand years ago); vertical axis, effective population size (NeT).

Figure 4. Pooled climatic space of the lineages of *Meladema* as represented with the two first PCA factors of the climatic variables (Appendix S3). Grey background, climatic space covered by the whole studied area; in colours, climatic space occupied by the different lineages plus continental Italy. White dots indicate the localities of continental *M. coriacea* with molecular data.

Figure 5. ANOVA comparison of the climatic space represented by the three main axes of the climatic PCA of the four lineages of *Meladema*. Letters indicate significant differences between lineages (Bonferroni post-hoc tests, $P < 0.05$).

Figure 6. Background similarity test comparisons between the four lineages of *Meladema*. Observed values of niche overlap (arrows) were tested against null distributions (100 replicates) generated by comparing model suitability values of one lineage with those generated from random cells drawn from the background area of the other lineage. Only in (C) and (D) one of the comparisons was not significant (see text for details). (A) continental *M. coriacea* x *M. coriacea* CSM; (B) continental *M. coriacea* x *M. lanio*; (C) *M. imbricata* x continental *M. coriacea* CSM; (D) *M. coriacea* CSM x *M. lanio*; (E) *M. imbricata* x continental *M. coriacea*; (F) *M. imbricata* x *M. lanio*.

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Figure 7. Map of the areas that currently have climatic conditions similar to the reconstructed ancestral values of each of the *Meladema* lineages, as measured with the 95% confidence interval of the first two axes of the climatic PCA. (A) Genus *Meladema*; (B) continental *M. coriacea*; (C) *M. coriacea* CSM; (D) *M. imbricata*; (E) *M. lanio*.

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Tables

Table 1. Likelihood and AICM scores for the BEAST analyses of the combined data with different clock models, calculated in TRACER. Lower AICM values indicate a better model fit.

Analysis	Clock model	Likelihood score	AICM
Phylogeny & dating	Lognormal clock	7875.6	15994.9
Phylogeny & dating	Strict clock	7889.8	15962.3
Phylogeny (nuclear genes)	Lognormal clock	2218.6	4494.6
Phylogeny (nuclear genes)	Strict clock	2240.5	4560.6

Table 2. Likelihood and AICM scores for the BEAST analyses of the combined data with different topological constraints under a strict clock, calculated in TRACER. Lower AICM values indicate a better model fit.

Topological constraint	Likelihood score	AICM
M. lanio + *M. imbricata*	7890.2	15964.0
M. coriacea	7889.3	15959.5
M. lanio + *M. imbricata* + *M. coriacea* CSM	7889.9	15959.4
No constraints	7889.8	15962.3

Table 3. Ages (in thousands of years before present) of the most recent common ancestor of continental *M. coriacea* estimated by the coalescence analysis, with the 95% confidence intervals of the MRCA ages and likelihood and AICM scores of individual demographic models. Lower AICM values indicate a better model fit.

Demographic model	MRCA age (Ka)	95% CI (Ka)	Likelihood score	AICM
Constant Size	0.170	(0.084 – 0.281)	2327.1	4 692.2
Logistic Growth	0.117	(0.061 – 0.194)	2328.7	4 698.7
Expansion Growth	0.145	(0.071 – 0.246)	2327.2	4 692.1
Exponential Growth	0.123	(0.061 – 0.209)	2327.7	4 697.5

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Figure 1

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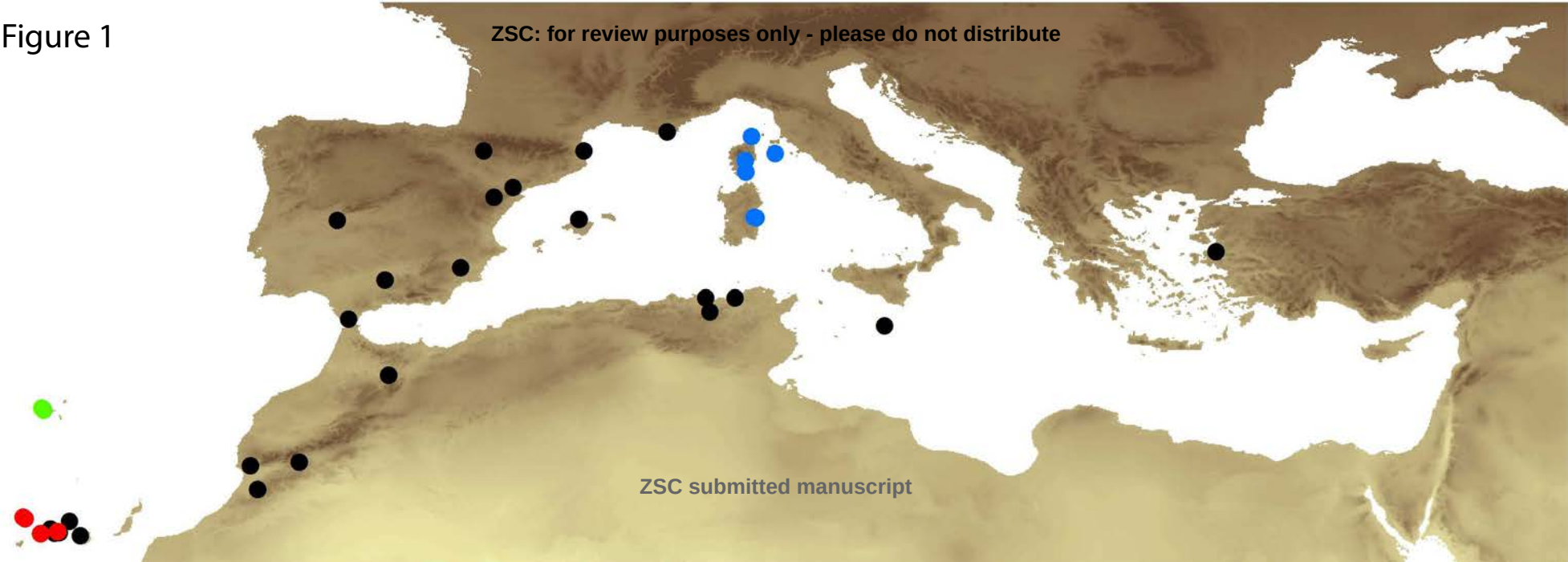

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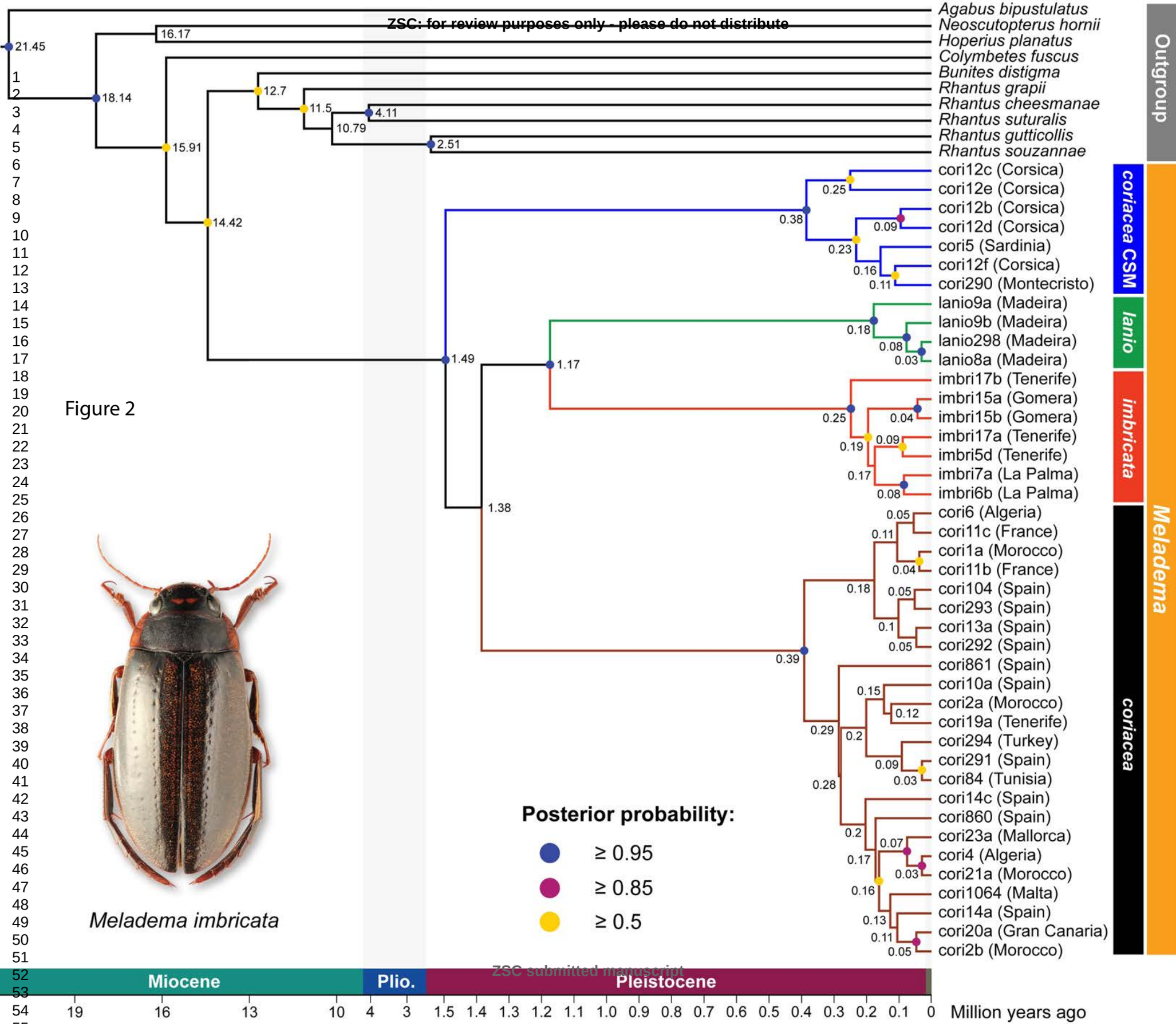

Figure 2

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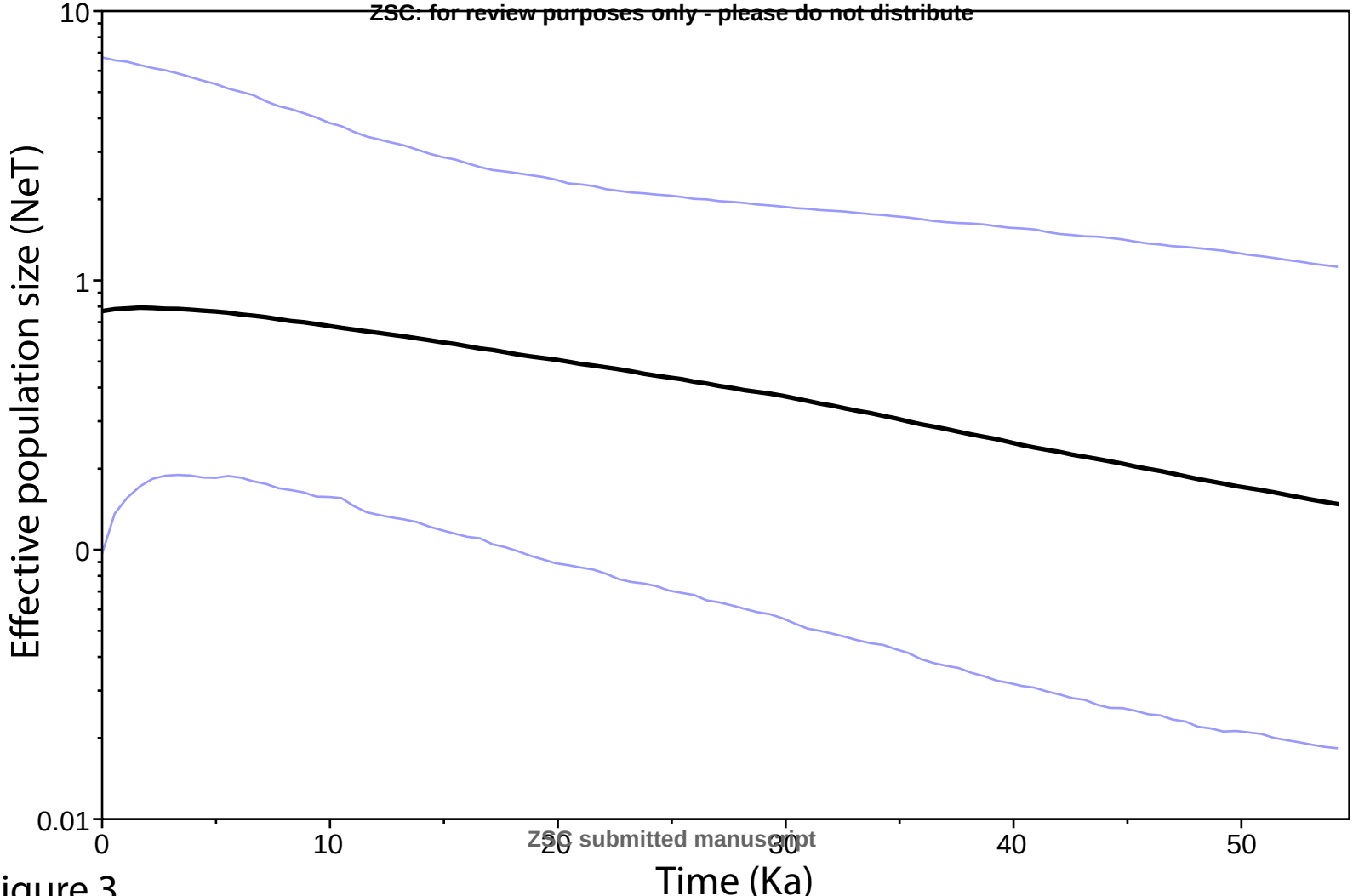

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Figure 3

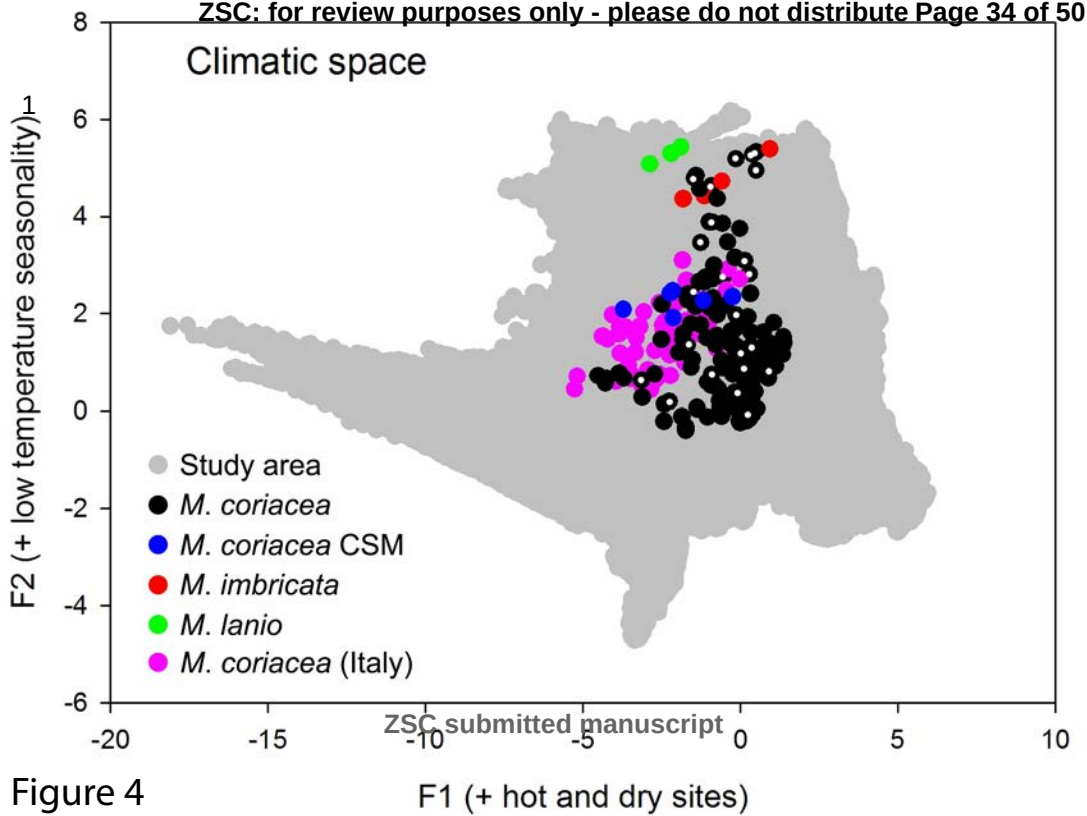

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Figure 4

Figure 5

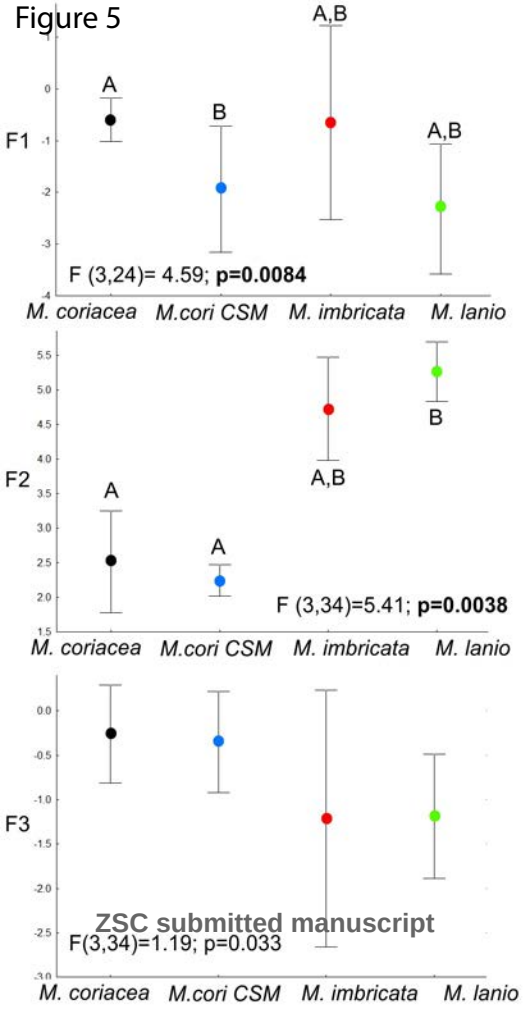

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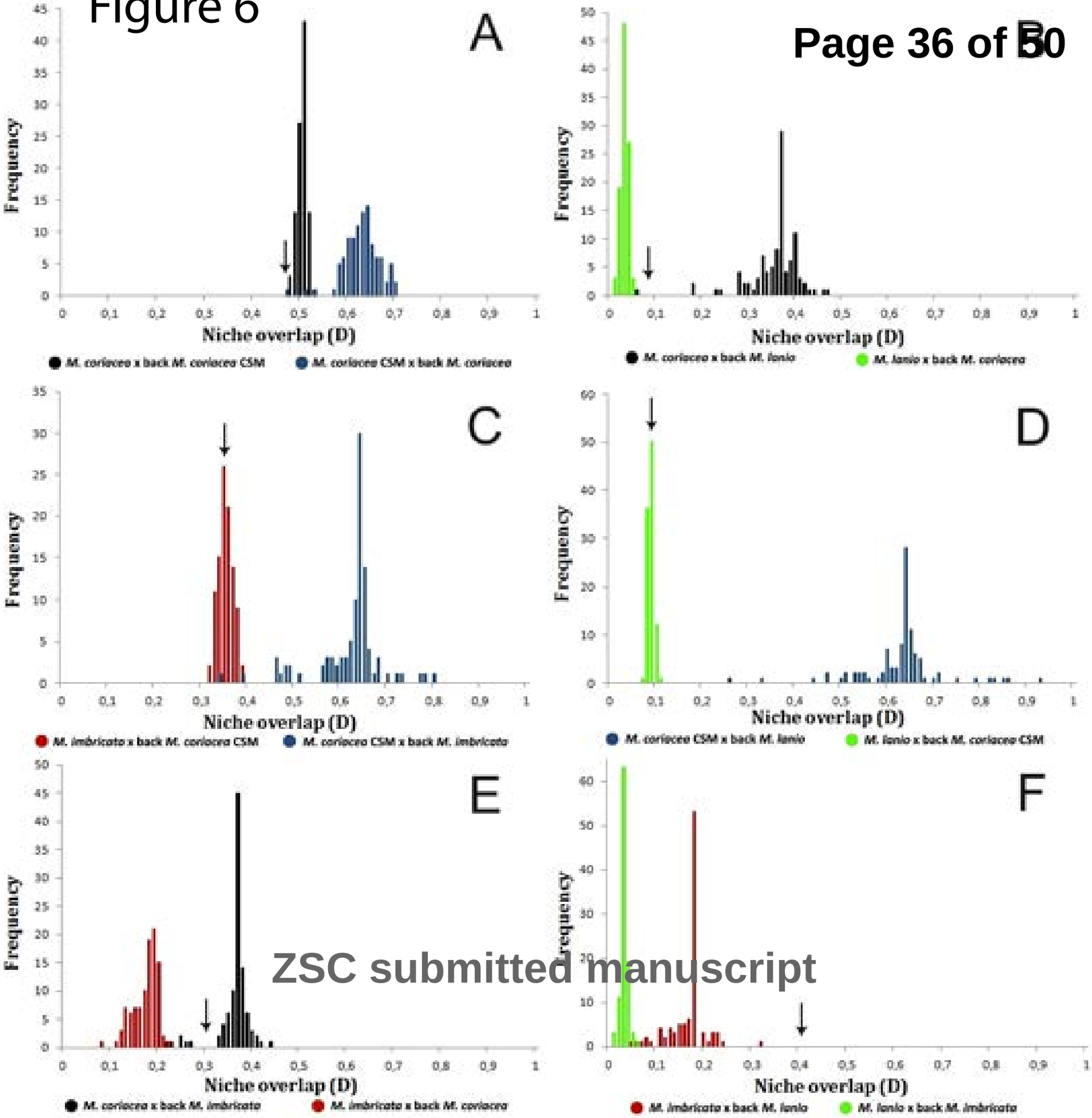
Figure 6



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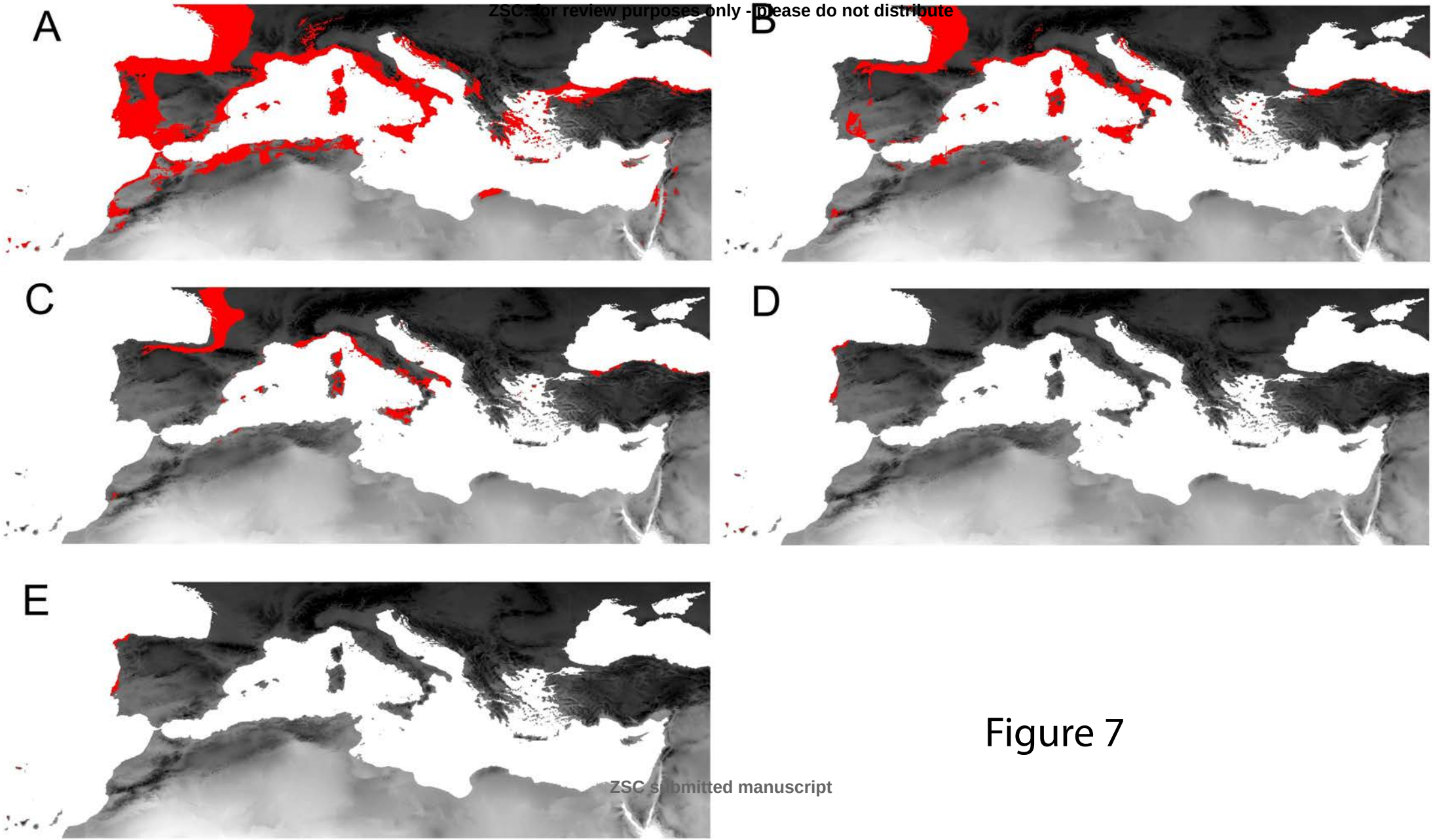

Figure 7

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Range expansion and ancestral niche reconstruction in the Mediterranean diving beetle genus *Meladema* (Coleoptera, Dytiscidae)

Vit Sykora, David García-Vázquez, David Sánchez-Fernández & Ignacio Ribera

Supporting information

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In bold, sequences newly obtained for this study. Specimens with * were tested for the presence of Wolbachia infection.

Specimen				Locality					Sequences					Analyses			
No	Genus	Species	Code	Voucher	Country	Site	Latitude	Longitude	COI-3'	nad1	16S	Wg	H3	Phylogeny	Nuc Phylogeny	Coalescence	Phylogeography
1	*Meladema*	*coriacea*	cori104	MNCN-AI104	Spain	Tarragona, Corbera d'Ebre, R. Gaia	41°4'27.47"N	0°28'32.04"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
2	*Meladema*	*coriacea*	cori1064	IBE-RA1064	Malta	Malta, between Mosta and L-Imtarfa	35°53'60"N	14°24'14"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
3	*Meladema*	*coriacea*	cori1095	MNCN-AI1095	Tenerife	Chamorga, Bco. Roque Bermejo	28°34'43.45"N	16°8'9.36"W	**XX**	**XX**	**XX**	**XX**	**XX**	NO	YES	YES	YES
4	*Meladema*	*coriacea*	cori10a	NHM-IRM10a	Spain	Cadiz, Fancinas	36°9'32.26"N	5°40'34.12"W	AF428215	AF428189	AF428189	-	-	YES	NO	YES	YES
5	*Meladema*	*coriacea*	cori11a	NHM-IRM11a	France	Var, La Londe-les-Maures, rallon de Valcros	43°8'57.17"N	6°15'11.32"E	AF428207	AF428189	AF428189	-	-	NO	NO	YES	YES
6	*Meladema*	*coriacea*	cori11b *	NHM-IRM11b	France	Var, La Londe-les-Maures, rallon de Valcros	43°8'57.17"N	6°15'11.32"E	AF428208	AF428189	AF428189	**XX**	**XX**	YES	YES	YES	YES
7	*Meladema*	*coriacea*	cori11c	NHM-IRM11c	France	Var, La Londe-les-Maures, rallon de Valcros	43°8'57.17"N	6°15'11.32"E	AF428207	AF428189	AF428189	-	-	YES	NO	YES	YES
8	*Meladema*	*coriacea*	cori12a	NHM-IRM12a	Corsica	Porto-Vecchio: l'Ospedale	41°39'13.67"N	9°12'41.02"E	AF428203	-	-	-	-	NO	NO	NO	YES
9	*Meladema*	*coriacea*	cori12b	NHM-IRM12b	Corsica	Ghisoni: rd. to Campannella	42°4'8.68"N	9°11'5.98"E	AF428204	AF428187	AF428187	**XX**	-	YES	YES	NO	YES
10	*Meladema*	*coriacea*	cori12c *	NHM-IRM12c	Corsica	Cap Corse: Bertolacce	42°58'2.39"N	9°24'42.38"E	AF428205	AF428188	AF428188	**XX**	**XX**	YES	YES	NO	YES
11	*Meladema*	*coriacea*	cori12d *	NHM-IRM12d	Corsica	Porto-Vecchio: l'Ospedale	41°39'13.67"N	9°12'41.02"E	AF428203	AF428187	AF428187	**XX**	**XX**	YES	YES	NO	YES
12	*Meladema*	*coriacea*	cori12e	NHM-IRM12e	Corsica	Cap Corse: Bertolacce	42°58'2.39"N	9°24'42.38"E	AF428206	AF428188	AF428188	-	-	YES	NO	NO	YES
13	*Meladema*	*coriacea*	cori12f	NHM-IRM12f	Corsica	Cap Corse: Bertolacce	42°58'2.39"N	9°24'42.38"E	AF428207	AF428187	AF428187	-	-	YES	NO	NO	YES
14	*Meladema*	*coriacea*	cori12g	NHM-IRM12g	Corsica	Porto-Vecchio: l'Ospedale	41°39'13.67"N	9°12'41.02"E	AF428203	-	AF428187	-	-	NO	NO	NO	YES
15	*Meladema*	*coriacea*	cori13a	NHM-IRM13a	Spain	Murcia, Fte. Caputa, R. Mula	38°3'16.95"N	1°28'46.32"W	AF428218	AF428189	AF428189	**XX**	**XX**	YES	YES	YES	YES
16	*Meladema*	*coriacea*	cori14a *	NHM-IRM14a	Spain	Cordoba, Baena, Arroyo de las Beatas	37°36'30.73"N	4°19'13.55"W	AF428216	AF428190	AF428190	**XX**	**XX**	YES	YES	YES	YES
17	*Meladema*	*coriacea*	cori14b	NHM-IRM14b	Spain	Cordoba, Baena, Arroyo de las Beatas	37°36'30.73"N	4°19'13.55"W	AF428216	AF428190	AF428190	-	-	NO	NO	YES	YES
18	*Meladema*	*coriacea*	cori14c	NHM-IRM14c	Spain	Cordoba, Baena, Arroyo de las Beatas	37°36'30.73"N	4°19'13.55"W	AF428217	AF428189	AF428189	-	-	YES	NO	YES	YES
19	*Meladema*	*coriacea*	cori16a	NHM-IRM16a	Tenerife	Bco. Del Rio, 600 m	28°9'14.18"N	16°32'7.09"W	AF428223	AF428192	AF428192	**XX**	**XX**	NO	YES	NO	NO
20	*Meladema*	*coriacea*	cori16b	NHM-IRM16b	Tenerife	Bco. Del Rio, 600 m	28°9'14.18"N	16°32'7.09"W	AF428223	AF428192	AF428192	**XX**	**XX**	NO	YES	NO	NO
21	*Meladema*	*coriacea*	cori18 *	IBE-RA18	Sardinia	Sardinia, Tortoli (Ogliastra historical region)	39°56'23.35"N	9°35'34.08"E	**XX**	**XX**	**XX**	**XX**	**XX**	NO	YES	NO	YES
22	*Meladema*	*coriacea*	cori18a	NHM-IRM18a	Tenerife	Bco. Del Infierno	28°8'37.11"N	16°41'50.37"W	AF428222	AF428189	AF428189	**XX**	**XX**	NO	YES	YES	YES
23	*Meladema*	*coriacea*	cori19a	NHM-IRM19a	Tenerife	Bco. De Masca	28°17'37.20"N	16°50'46.31"W	AF428222	AF428189	AF428189	**XX**	**XX**	YES	YES	YES	YES
24	*Meladema*	*coriacea*	cori19b	NHM-IRM19b	Tenerife	Bco. De Masca	28°17'37.20"N	16°50'46.31"W	AF428222	AF428189	AF428189	**XX**	**XX**	NO	YES	YES	YES
25	*Meladema*	*coriacea*	cori1a *	NHM-IRM1a	Morocco	Moyen Atlas, Tazzeka N.P.	34°3'9.21"N	4°10'27.00"W	AF428212	AF428189	AF428189	**XX**	**XX**	YES	YES	YES	YES
26	*Meladema*	*coriacea*	cori1c	NHM-IRM1c	Morocco	Moyen Atlas, Tazzeka N.P.	34°3'9.21"N	4°10'27.00"W	AF428213	AF428189	AF428189	-	-	NO	NO	YES	YES
27	*Meladema*	*coriacea*	cori20a	NHM-IRM20a	Gran Canaria	Bco. Guguy grande	28°2'28.22"N	15°44'40.64"W	AF428221	AF428189	AF428189	**XX**	**XX**	YES	YES	YES	YES
28	*Meladema*	*coriacea*	cori21a	NHM-IRM21a	Morocco	Agadir	30°39'31.07"N	9°21'21.96"W	AF428214	AF428189	AF428189	**XX**	**XX**	YES	YES	YES	YES
29	*Meladema*	*coriacea*	cori21b	NHM-IRM21b	Morocco	Agadir	30°39'31.07"N	9°21'21.96"W	AF428215	AF428189	AF428189	-	-	NO	NO	YES	YES
30	*Meladema*	*coriacea*	cori22a	NHM-IRM22a	Morocco	Tachokchte, Assif Siroua	30°47'36.36"N	7°31'26.65"W	AF428215	AF428189	AF428189	**XX**	**XX**	NO	YES	YES	YES
31	*Meladema*	*coriacea*	cori22b	NHM-IRM22b	Morocco	Tachokchte, Assif Siroua	30°47'36.36"N	7°31'26.65"W	AF428216	AF428189	AF428189	-	-	NO	NO	YES	YES
32	*Meladema*	*coriacea*	cori23a	NHM-IRM23a	Mallorca	Mortixet, Tributary Te. Son March	39°51'56.43"N	2°56'19.17"E	AF428219	AF428191	AF428191	**XX**	**XX**	YES	YES	YES	YES
33	*Meladema*	*coriacea*	cori23b	NHM-IRM23b	Mallorca	Mortixet, Tributary Te. Son March	39°51'56.43"N	2°56'19.17"E	AF428220	AF428189	AF428189	-	-	NO	NO	YES	YES
34	*Meladema*	*coriacea*	cori24a	NHM-IRM24a	Mallorca	Els Casals, Te. Son March	39°52'6.99"N	2°57'57.73"E	AF428215	AF428189	AF428189	-	-	NO	NO	YES	YES
35	*Meladema*	*coriacea*	cori289	IBE-DV289	Montecristo	Montecristo	42°20'2.41"N	10°18'28.81"E	**XX**	**XX**	**XX**	**XX**	**XX**	NO	YES	NO	YES
36	*Meladema*	*coriacea*	cori290 *	IBE-DV290	Montecristo	Montecristo	42°20'2.41"N	10°18'28.81"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	NO	YES
37	*Meladema*	*coriacea*	cori291	IBE-DV291	Spain	PN Monfragüe / Arroyo de Trasierra, G. del Fraile	39°50'07.2"N	6°06'02.6"W	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
38	*Meladema*	*coriacea*	cori292	IBE-DV292	Spain	ES Huesca, Bernués, Bco. de Bernués	42°26'38"N	0°36'51"W	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
39	*Meladema*	*coriacea*	cori293 *	IBE-DV293	Spain	ES Girona, Port Bou / Cami de la Riera	42°25'34.5"N	3°8'9.3"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
40	*Meladema*	*coriacea*	cori294 *	IBE-DV294	Turkey	Izmir prov., Phoca	38°39'37.3"N	26°49'23.9"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
41	*Meladema*	*coriacea*	cori2a	NHM-IRM2a	Morocco	Anti Atlas, Oued Massa	29°47'24.97"N	9°5'28.73"W	AF428210	AF428189	AF428189	-	-	YES	NO	YES	YES
42	*Meladema*	*coriacea*	cori2b	NHM-IRM2b	Morocco	Anti Atlas, Oued Massa	29°47'24.97"N	9°5'28.73"W	AF428211	-	-	**XX**	**XX**	YES	YES	YES	NO
43	*Meladema*	*coriacea*	cori4 *	MNCN-HI4	Algeria	Oued Bagrat	36°56'43.59"N	7°42'5.61"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
44	*Meladema*	*coriacea*	cori5 *	IBE-RA5	Sardinia	Sardinia, Nuoro prov.(Ogliastra historical region)	39°57'3.79"N	9°31'9.37"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	NO	YES
45	*Meladema*	*coriacea*	cori6	MNCN-HI6	Algeria	Aïn Damous	36°25'22.38"N	7°51'19.56"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
46	*Meladema*	*coriacea*	cori84	MNCN-AI84	Tunisia	Cite el Morjne	36°56'17.1"N	8°47'29.8"E	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
47	*Meladema*	*coriacea*	cori860	MNCN-AI860	Spain	ES Castellón, Ballestar/R. Sénia	40°41'41"N	0°13'25.5"W	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	YES	YES
48	*Meladema*	*coriacea*	cori861	MNCN-AI861	Spain	ES Castellón, Ballestar/R. Sénia	40°41'41"N	0°13'25.5"W	**XX**	**XX**	**XX**	-	-	YES	NO	YES	YES
49	*Meladema*	*imbricata*	imbri15a	NHM-IRM15a	Gomera	El Cedro	28°7'12.18"N	17°13'36.71"W	AF428224	AF428192	AF428192	**XX**	**XX**	YES	YES	NO	YES

50	*Meladema*	*imbricata*	imbri15b	NHM-IRM15b	Gomera	El Cedro	28°7'12.18"N	17°13'36.71"W	AF428225	AF428192	AF428192	**XX**	**XX**	YES	YES	NO	YES
51	*Meladema*	*imbricata*	imbri17a	NHM-IRM17a	Tenerife	Bco. Del Rio, 1600 m	28°12'3.60"N	16°34'47.91"W	AF428228	AF428193	AF428193	-	-	YES	NO	NO	YES
52	*Meladema*	*imbricata*	imbri17b	NHM-IRM17b	Tenerife	Bco. Del Rio, 1600 m	28°12'3.60"N	16°34'47.91"W	AF428230	AF428192	AF428192	**XX**	**XX**	YES	YES	NO	YES
53	*Meladema*	*imbricata*	imbri17c	NHM-IRM17c	Tenerife	Bco. Del Rio, 1600 m	28°12'3.60"N	16°34'47.91"W	AF428222	AF428189	AF428189	**XX**	**XX**	NO	YES	NO	NO
54	*Meladema*	*imbricata*	imbri3a	NHM-IRM3a	Gomera	El Cedro	28°7'12.18"N	17°13'36.71"W	AF428224	AF428192	AF428192	**XX**	**XX**	NO	YES	NO	YES
55	*Meladema*	*imbricata*	imbri4a	NHM-IRM4a	Gomera	El Cedro	28°7'12.18"N	17°13'36.71"W	AF428224	AF428192	AF428192	**XX**	**XX**	NO	YES	NO	YES
56	*Meladema*	*imbricata*	imbri4b	NHM-IRM4b	Gomera	El Cedro	28°7'12.18"N	17°13'36.71"W	AF428224	AF428192	AF428192	**XX**	**XX**	NO	YES	NO	YES
57	*Meladema*	*imbricata*	imbri5a	NHM-IRM5a	Tenerife	Bco. Del Rio, 1600 m	28°12'3.60"N	16°34'47.91"W	AF428230	AF428192	AF428192	**XX**	**XX**	NO	YES	NO	YES
58	*Meladema*	*imbricata*	imbri5b	NHM-IRM5b	Tenerife	Bco. Del Rio, 1600 m	28°12'3.60"N	16°34'47.91"W	AF428222	AF428189	AF428189	-	-	NO	NO	NO	YES
59	*Meladema*	*imbricata*	imbri5c	NHM-IRM5c	Tenerife	Bco. Del Rio, 1600 m	28°12'3.60"N	16°34'47.91"W	AF428222	AF428189	AF428189	**XX**	**XX**	NO	YES	NO	NO
60	*Meladema*	*imbricata*	imbri5d	NHM-IRM5d	Tenerife	Bco. Del Rio, 1600 m	28°12'3.60"N	16°34'47.91"W	AF428229	-	-	-	-	YES	NO	NO	NO
61	*Meladema*	*imbricata*	imbri6a	NHM-IRM6a	La Palma	Barranco del Hoyo Verde	28°44'22.94"N	17°52'43.08"W	AF428227	-	-	-	-	NO	NO	NO	YES
62	*Meladema*	*imbricata*	imbri6b	NHM-IRM6b	La Palma	Barranco del Hoyo Verde	28°44'22.94"N	17°52'43.08"W	AF428227	AF428192	AF428192	**XX**	**XX**	YES	YES	NO	YES
63	*Meladema*	*imbricata*	imbri6c	NHM-IRM6c	La Palma	Barranco del Hoyo Verde	28°44'22.94"N	17°52'43.08"W	AF428227	AF428192	AF428192	**XX**	**XX**	NO	YES	NO	YES
64	*Meladema*	*imbricata*	imbri7a	NHM-IRM7a	La Palma	Barranco del Rio above Santa Cruz	28°41'54.40"N	17°48'23.93"W	AF428226	AF428192	AF428192	-	-	YES	NO	NO	YES
65	*Meladema*	*lanio*	lanio298	IBE-DV298	Madeira	Canhas, lado sur de Paul da Serra	32°44'36"N	17°05'56"W	**XX**	**XX**	**XX**	**XX**	**XX**	YES	YES	NO	YES
66	*Meladema*	*lanio*	lanio8a	NHM-IRM8a	Madeira	Ribera dos Cedros	32°49'17.12"N	17°10'25.39"W	AF428233	AF428194	AF428194	**XX**	**XX**	YES	YES	NO	YES
67	*Meladema*	*lanio*	lanio9a	NHM-IRM9a	Madeira	Levada das Faias	32°46'41.80"N	17°6'53.59"W	AF428231	AF428194	AF428194	-	-	YES	NO	NO	YES
68	*Meladema*	*lanio*	lanio9b	NHM-IRM9b	Madeira	Levada das Faias	32°46'41.80"N	17°6'53.59"W	AF428232	AF428194	AF428194	**XX**	**XX**	YES	YES	NO	YES
69	*Agabus*	*bipustulatus*							AF309332	AF428198	AF428198	-	EF670076	YES	NO	NO	NO
70	*Bunites*	*distigma*							KJ637978	KJ637894	KJ637894	-	KJ638008	YES	NO	NO	NO
71	*Colymbetes*	*fuscus*							KJ637976	KJ637892	KJ637892	KJ638022	KJ638006	YES	NO	NO	NO
72	*Hoperius*	*planatus*							KJ637974	KJ637889	KJ637889	-	KJ638001	YES	NO	NO	NO
73	*Neoscutopterus*	*hornii*							-	KJ637886	KJ637886	KJ638016	KJ637999	YES	NO	NO	NO
74	*Rhantus*	*cheesmanae*							FN263051	FN298824	FN298824	KJ638019	KJ638004	YES	NO	NO	NO
75	*Rhantus*	*grapii*							KJ637979	KJ637897	KJ637897	KJ638027	KJ638010	YES	NO	NO	NO
76	*Rhantus*	*gutticollis*							KJ637973	KJ637884	KJ637884	KJ638015	KJ637998	YES	NO	NO	NO
77	*Rhantus*	*souzannae*							KJ637975	KJ637890	KJ637890	KJ638018	KJ638002	YES	NO	NO	NO
78	*Rhantus*	*suturalis*							-	FN298810	FN298810	FN256293	FN257532	YES	NO	NO	NO

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Appendix S2. DNA extraction and sequencing.

(A) Primers used for the amplification and sequencing. In brackets, length of the amplified fragment (ingroup only).

gene	primer	sequence	ref.
cox1-3'	Jerry (5')	CAACATTTATTTTGATTTTTTGG	(3)
(826)	Pat (3')	TCCAATGCACTAATCTGCCATATTA	(3)
	Chy (5')	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	(2)
	Tom (3')	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	(2)
rrnL+trnL+nad1	16SaR (5')	CGCCTGTTTAACAAAAACAT	(3)
(799-800)	ND1 (3')	GGTCCCTTACGAATTTGAATATATCCT	(3)
H3	H3aF (5')	ATGGCTCGTACCAAGCAGACRCG	(1)
(327)	H3aR (3')	ATATCCTTRGGCATRATRGTGAC	(1)
Wingless	WG550F (5')	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	(4)
(478)	WGAbrZ (3')	CACTTNACYTCRCARCACCARTG	(4)

(B) Standard PCR conditions for the amplification of the studied fragments.

step	time	temperature
1	3'	96º
2	30"	94º
3	30"-1'	47-50º *
4	1'	72º
5	Go to step 2 and repeat 34-40 x	
6	10'	72º

* Depending on the annealing temperatures of the primers pair used

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References

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(2) Ribera, I., Fresneda, J., Bucur, R., Izquierdo, A., Vogler, A.P., Salgado, J.M. & Cieslak, A. (2010). Ancient origin of a Western Mediterranean radiation of subterranean beetles. *BMC Evolutionary Biology*, 10: 29.

(3) Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the entomological Society of America*, 87: 651-701.

(4) Wild, A. L., & Maddison, D. R. (2008). Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution*, 48: 877-891.

Appendix S3.

(A) Bioclimatic variables of each locality obtained in Worldclim (http://www.worldclim.org) used in the PCA (Principal Component Analysis).

Bioclimatic variables	
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

(B) Geographical coordinates and values of the first three axes of the climatic PCA of the four lineages of *Meladema*.

lineage	**longitude**	**latitude**	**Factor 1**	**Factor 2**	**Factor 3**
cont. *M. coriacea*	6.271	43.146	-1.81	2.40	-0.34
cont. *M. coriacea*	-0.604	42.438	-2.99	0.58	-1.27
cont. *M. coriacea*	3.146	42.438	-1.67	1.35	-1.25
cont. *M. coriacea*	0.479	41.063	-0.92	0.74	-1.03
cont. *M. coriacea*	-0.229	40.688	-2.25	0.19	-1.57
cont. *M. coriacea*	-6.104	39.854	0.14	0.88	0.67
cont. *M. coriacea*	2.938	39.854	-1.88	2.37	-0.41
cont. *M. coriacea*	2.979	39.854	-1.55	2.45	-0.46
cont. *M. coriacea*	26.813	38.646	-0.54	2.74	2.11
cont. *M. coriacea*	-1.479	38.063	0.92	0.81	-0.67
cont. *M. coriacea*	-4.313	37.604	0.36	1.33	1.16
cont. *M. coriacea*	7.688	36.938	-1.27	3.46	1.47
cont. *M. coriacea*	8.771	36.938	-0.89	3.88	1.57
cont. *M. coriacea*	7.854	36.438	-0.14	1.96	1.27
cont. *M. coriacea*	-5.688	36.146	-1.46	4.79	0.76
cont. *M. coriacea*	14.396	35.896	0.15	3.09	-0.40
cont. *M. coriacea*	-4.188	34.063	-0.08	0.37	0.53
cont. *M. coriacea*	-7.521	30.813	0.25	-0.07	1.70
cont. *M. coriacea*	-9.354	30.646	0.28	2.82	-1.06
cont. *M. coriacea*	-9.104	29.771	0.03	1.20	0.20

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cont. *M. coriacea*	-16.146	28.563	0.51	5.33	-2.34
cont. *M. coriacea*	-16.854	28.313	-0.14	5.19	-1.67
cont. *M. coriacea*	-16.688	28.146	-0.90	4.63	-1.00
cont. *M. coriacea*	-16.521	28.146	0.40	5.28	-2.11
cont. *M. coriacea*	-15.729	28.021	0.50	4.94	-2.38
cont. *M. coriacea (Italy)*	8.032	43.899	-2.06	2.21	-0.45
cont. *M. coriacea (Italy)*	8.551	44.384	-3.81	1.18	-0.61
cont. *M. coriacea (Italy)*	8.635	44.390	-3.31	1.50	-0.13
cont. *M. coriacea (Italy)*	8.854	44.428	-3.84	1.58	0.15
cont. *M. coriacea (Italy)*	8.878	44.435	-3.64	1.72	1.30
cont. *M. coriacea (Italy)*	9.069	44.385	-4.38	1.53	0.82
cont. *M. coriacea (Italy)*	9.167	44.326	-4.21	1.47	0.81
cont. *M. coriacea (Italy)*	9.188	44.388	-3.82	1.72	1.22
cont. *M. coriacea (Italy)*	9.340	44.315	-3.19	1.72	0.94
cont. *M. coriacea (Italy)*	10.541	43.298	-2.46	1.76	1.42
cont. *M. coriacea (Italy)*	10.895	42.768	-2.40	1.83	1.22
cont. *M. coriacea (Italy)*	11.024	43.211	-2.15	1.93	1.03
cont. *M. coriacea (Italy)*	11.140	42.749	-2.29	1.69	0.69
cont. *M. coriacea (Italy)*	11.139	43.018	-2.22	1.76	1.54
cont. *M. coriacea (Italy)*	11.274	43.105	-2.44	1.66	1.34
cont. *M. coriacea (Italy)*	11.275	43.284	-1.72	1.84	1.43
cont. *M. coriacea (Italy)*	10.322	43.514	-2.49	1.29	0.74
cont. *M. coriacea (Italy)*	10.306	43.751	-2.22	1.53	0.51
cont. *M. coriacea (Italy)*	10.307	43.842	-1.24	1.75	0.64
cont. *M. coriacea (Italy)*	10.420	43.481	-2.24	1.14	0.81
cont. *M. coriacea (Italy)*	10.429	43.569	-2.05	1.19	0.83
cont. *M. coriacea (Italy)*	10.428	43.749	-1.28	1.58	0.36
cont. *M. coriacea (Italy)*	10.550	43.657	-3.08	0.51	1.22
cont. *M. coriacea (Italy)*	10.555	43.839	-2.56	0.73	1.60
cont. *M. coriacea (Italy)*	11.173	43.823	-2.93	0.84	1.59
cont. *M. coriacea (Italy)*	11.194	43.913	-2.13	1.18	1.68
cont. *M. coriacea (Italy)*	11.290	43.555	-2.13	1.17	1.50
cont. *M. coriacea (Italy)*	11.300	43.825	-3.95	0.60	0.56
cont. *M. coriacea (Italy)*	11.304	43.915	-3.13	0.52	1.72
cont. *M. coriacea (Italy)*	11.412	43.643	-3.47	0.75	1.10
cont. *M. coriacea (Italy)*	11.612	42.822	-2.68	0.64	0.54
cont. *M. coriacea (Italy)*	11.858	42.462	-5.18	0.70	1.73
cont. *M. coriacea (Italy)*	11.866	42.642	-2.56	0.74	1.79
cont. *M. coriacea (Italy)*	11.541	43.643	-5.26	0.44	1.54
cont. *M. coriacea (Italy)*	11.545	43.730	-3.49	0.66	0.44
cont. *M. coriacea (Italy)*	11.670	43.635	-2.22	0.72	0.55
cont. *M. coriacea (Italy)*	11.785	43.544	-1.76	0.99	0.38
cont. *M. coriacea (Italy)*	12.044	42.095	-1.77	1.82	0.04
cont. *M. coriacea (Italy)*	12.171	41.919	-1.41	2.09	0.59
cont. *M. coriacea (Italy)*	12.279	42.105	-1.85	1.74	0.41
cont. *M. coriacea (Italy)*	12.428	41.662	-1.60	1.69	1.44
cont. *M. coriacea (Italy)*	12.410	42.019	-1.69	2.67	1.38
cont. *M. coriacea (Italy)*	12.531	41.664	-1.64	2.41	1.67
cont. *M. coriacea (Italy)*	12.785	37.792	-2.01	1.44	1.46
cont. *M. coriacea (Italy)*	12.899	37.794	-0.02	2.71	-0.41
cont. *M. coriacea (Italy)*	13.353	37.796	-0.44	2.47	1.17
cont. *M. coriacea (Italy)*	13.351	37.891	-1.27	1.99	1.21
cont. *M. coriacea (Italy)*	13.239	37.982	-0.39	2.92	1.02
cont. *M. coriacea (Italy)*	13.344	38.161	-1.17	1.96	1.09
cont. *M. coriacea (Italy)*	13.497	41.490	-1.32	1.71	1.61

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cont. *M. coriacea (Italy)*	12.681	41.799	-3.55	0.93	1.16
cont. *M. coriacea (Italy)*	13.379	41.764	-3.07	2.03	1.12
cont. *M. coriacea (Italy)*	13.585	42.852	-2.85	0.43	0.15
cont. *M. coriacea (Italy)*	14.156	37.906	-0.60	2.24	1.25
cont. *M. coriacea (Italy)*	14.004	38.027	-1.01	1.79	0.48
cont. *M. coriacea (Italy)*	14.626	37.989	-1.81	1.50	-0.06
cont. *M. coriacea (Italy)*	14.704	40.690	-2.56	2.22	1.63
cont. *M. coriacea (Italy)*	14.836	40.964	-2.13	1.80	-0.06
cont. *M. coriacea (Italy)*	14.999	41.990	-2.28	1.59	-0.38
cont. *M. coriacea (Italy)*	15.057	37.542	-2.36	1.33	0.60
cont. *M. coriacea (Italy)*	15.159	37.897	-0.69	2.21	0.81
cont. *M. coriacea (Italy)*	15.858	37.988	-1.18	2.29	0.16
cont. *M. coriacea (Italy)*	15.657	40.505	-2.71	1.24	-0.56
cont. *M. coriacea (Italy)*	15.865	40.427	-2.88	0.81	-0.81
cont. *M. coriacea (Italy)*	15.059	40.875	-3.32	1.20	-0.60
cont. *M. coriacea (Italy)*	15.769	40.790	-1.71	2.68	0.30
cont. *M. coriacea (Italy)*	16.201	38.258	-1.84	3.10	0.15
cont. *M. coriacea (Italy)*	16.459	39.354	-2.42	1.35	-0.55
cont. *M. coriacea (Italy)*	16.240	40.428	-4.05	1.97	-0.34
cont. *M. coriacea (Italy)*	17.307	40.673	-1.75	1.19	0.07
cont. *M. coriacea (Italy)*	17.411	40.580	-0.74	1.27	-0.05
M. coriacea CSM	9.396	42.979	-2.15	2.47	-0.30
M. coriacea CSM	10.313	42.354	-1.17	2.27	-1.03
M. coriacea CSM	9.188	42.063	-3.71	2.08	-0.01
M. coriacea CSM	9.229	41.646	-2.22	2.41	-0.42
M. coriacea CSM	9.521	39.938	-2.13	1.91	0.47
M. coriacea CSM	9.604	39.938	-0.25	2.34	-0.81
M. imbricata	-17.896	28.729	-1.82	4.36	-0.38
M. imbricata	-17.771	28.688	0.94	5.39	-2.47
M. imbricata	-16.563	28.188	-1.14	4.43	-0.76
M. imbricata	-17.229	28.104	-0.58	4.72	-1.25
M. lanio	-17.188	32.813	-1.88	5.42	-1.44
M. lanio	-17.104	32.771	-2.87	5.08	-0.88
M. lanio	-17.104	32.729	-2.20	5.29	-1.25

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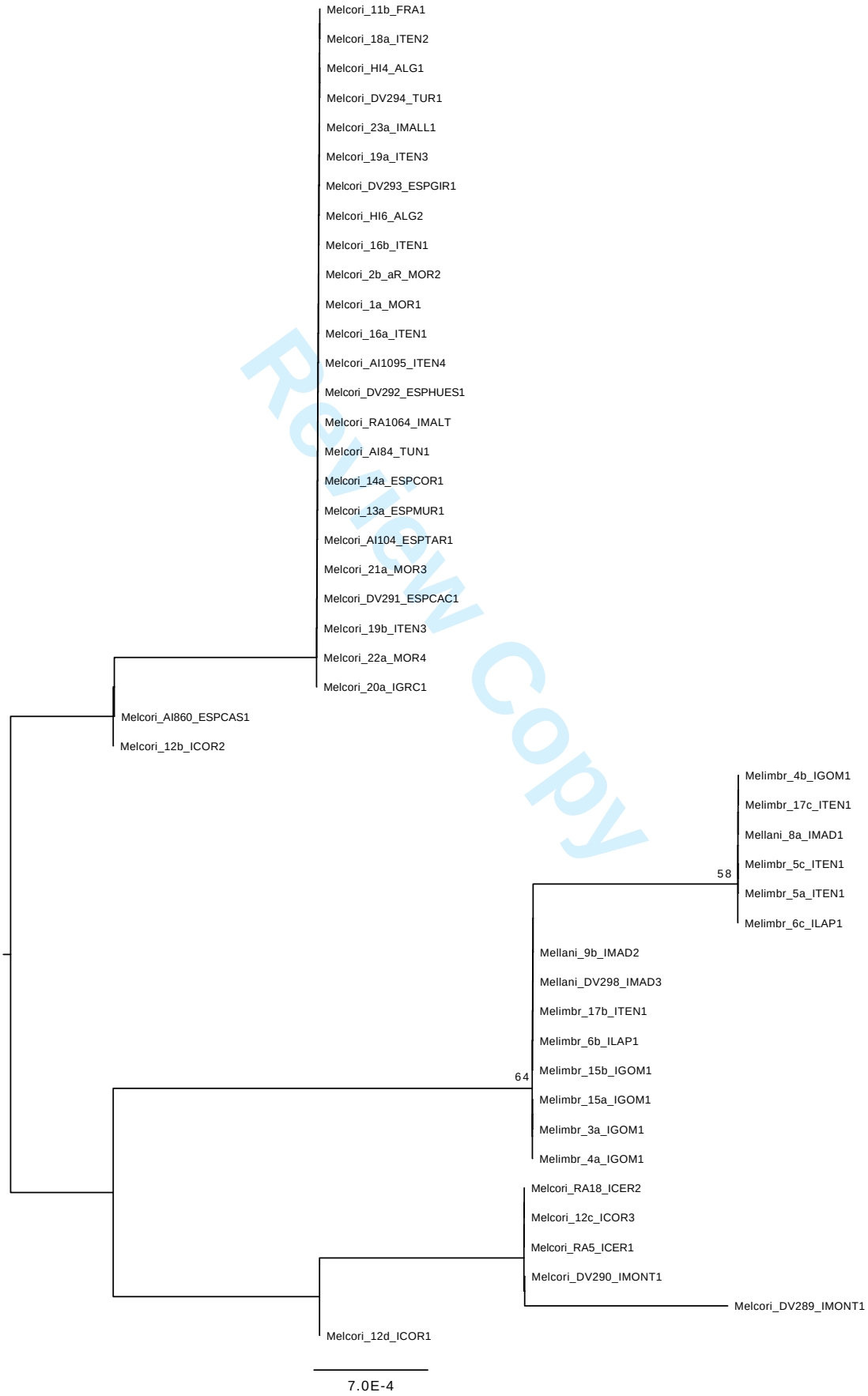
Appendix S4. Ultrametric phylogenetic tree of *Meladema* obtained with RAxML using only the nuclear sequence (*H3+Wg*) (see Methods for details). Numbers in nodes, bootstrap support values.



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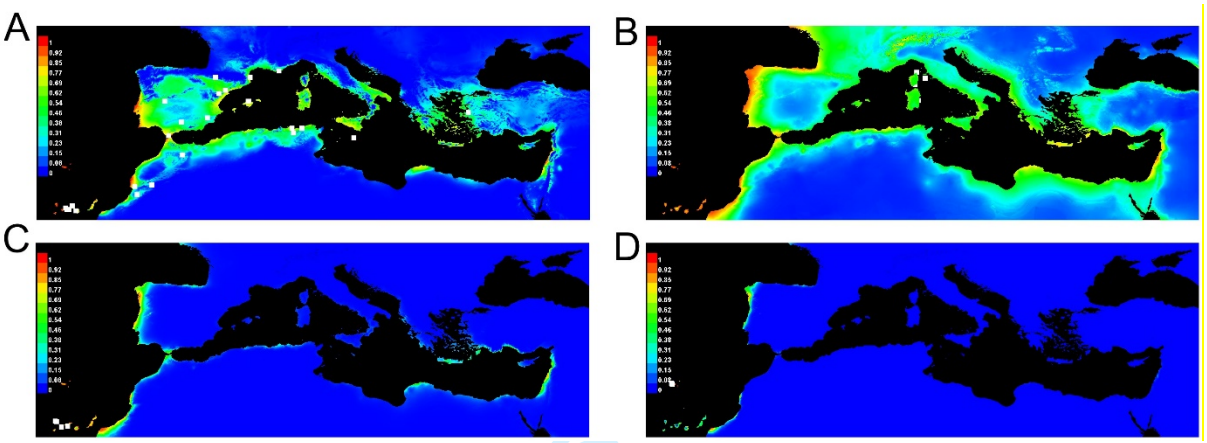
Appendix S6. Environmental niche models (ENMs) using MaxEnt for: (A) continental *M. coriacea*; (B) *M. coriacea* CSM; (C) *M. imbricata* and (D) *M. lanio*. Higher MaxEnt probabilities (red colours) represent areas more suitable for the species according to the MaxEnt models, lower values (blue) represent less suitable areas. These continuous measures of habitat suitability were used in ENM-based tests of niche similarity (see Fig. 6 and Appendix S7).



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Appendix S7. Pairwise values of observed niche overlap (Schoener's *D*-value) between the four lineages of *Meladema*.

lineage	***M. lanio***	**continental *M. coriacea***	***M. coriacea* CSM**	***M. imbricata***
M. lanio	1	0.083	0.093	0.411
continental *M. coriacea*	-	1	0.478	0.310
M. coriacea CSM	-	-	1	0.353
M. imbricata	-	-	-	1

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Range expansion and ancestral niche reconstruction in the Mediterranean diving beetle genus Meladema (Coleoptera, Dytiscidae).

Vit Sykora, David García-Vázquez, David Sánchez-Fernández & Ignacio Ribera

Appendix S8. Specimens used in the reconstruction of the ancestral niche from the scores of the first two PCA axes. Overlap "YES" indicates that the two PCA scores of the climatic conditions of the locality in which the specimen was found fall within the 95% Highest Posterior Density (HPD) intervals of the reconstructed conditions of the most recent common ancestor of the respective lineage.

Specimen			Locality	Coordinates		PCA coordinates		Overlap (with 95% interval at the respective node)				
No	Specimen	Lineage	Country	Latitude	Longitude	Latitude	Longitude	Meladema	cont. coriacea	imbricata	lanio	coriacea CSM
1	cori1a	continental *M. coriacea*	Morocco	34.052558	-4.174167	34.063	-4.188	NO	NO	NO	NO	NO
2	cori1b	continental *M. coriacea*	Morocco	34.052558	-4.174167	34.063	-4.188	NO	NO	NO	NO	NO
3	cori1c	continental *M. coriacea*	Morocco	34.052558	-4.174167	34.063	-4.188	NO	NO	NO	NO	NO
4	cori22a	continental *M. coriacea*	Morocco	30.793433	-7.524069	30.813	-7.521	NO	NO	NO	NO	NO
5	cori22b	continental *M. coriacea*	Morocco	30.793433	-7.524069	30.813	-7.521	NO	NO	NO	NO	NO
6	cori860	continental *M. coriacea*	Spain	40.694722	-0.223750	40.688	-0.229	NO	NO	NO	NO	NO
7	cori861	continental *M. coriacea*	Spain	40.694722	-0.223750	40.688	-0.229	NO	NO	NO	NO	NO
8	cori294	continental *M. coriacea*	Turkey	38.660361	26.823306	38.646	26.813	NO	NO	NO	NO	NO
9	cori10a	continental *M. coriacea*	Spain	36.158961	-5.676144	36.146	-5.688	YES	NO	NO	NO	NO
10	cori13a	continental *M. coriacea*	Spain	38.054708	-1.479533	38.063	-1.479	YES	NO	NO	NO	NO
11	cori14a	continental *M. coriacea*	Spain	37.608536	-4.320431	37.604	-4.313	YES	NO	NO	NO	NO
12	cori14b	continental *M. coriacea*	Spain	37.608536	-4.320431	37.604	-4.313	YES	NO	NO	NO	NO
13	cori14c	continental *M. coriacea*	Spain	37.608536	-4.320431	37.604	-4.313	YES	NO	NO	NO	NO
14	cori20a	continental *M. coriacea*	Gran Canaria	28.041172	-15.744622	28.021	-15.729	YES	NO	NO	NO	NO
15	cori84	continental *M. coriacea*	Tunisia	36.938083	8.791611	36.938	8.771	YES	NO	NO	NO	NO
16	cori104	continental *M. coriacea*	Spain	41.074297	0.475567	41.063	0.479	YES	NO	NO	NO	NO
17	cori1095	continental *M. coriacea*	Tenerife	28.578736	-16.135933	28.563	-16.146	YES	NO	NO	NO	NO
18	cori4	continental *M. coriacea*	Algeria	36.945442	7.701558	36.938	7.688	YES	NO	NO	NO	NO
19	cori6	continental *M. coriacea*	Algeria	36.422883	7.855433	36.438	7.854	YES	NO	NO	NO	NO
20	cori292	continental *M. coriacea*	Spain	42.443889	-0.614167	42.438	-0.604	YES	NO	NO	NO	NO
21	cori293	continental *M. coriacea*	Spain	42.426250	3.135917	42.438	3.146	YES	NO	NO	NO	YES
22	cori18a	continental *M. coriacea*	Tenerife	28.143642	-16.697325	28.146	-16.688	YES	NO	YES	NO	NO
23	cori19a	continental *M. coriacea*	Tenerife	28.293667	-16.846197	28.313	-16.854	YES	NO	YES	NO	NO
24	cori19b	continental *M. coriacea*	Tenerife	28.293667	-16.846197	28.313	-16.854	YES	NO	YES	NO	NO
25	cori2a	continental *M. coriacea*	Morocco	29.790269	-9.091314	29.771	-9.104	YES	YES	NO	NO	NO
26	cori2b	continental *M. coriacea*	Morocco	29.790269	-9.091314	29.771	-9.104	YES	YES	NO	NO	NO
27	cori21a	continental *M. coriacea*	Morocco	30.658631	-9.356100	30.646	-9.354	YES	YES	NO	NO	NO
28	cori21b	continental *M. coriacea*	Morocco	30.658631	-9.356100	30.646	-9.354	YES	YES	NO	NO	NO
29	cori1064	continental *M. coriacea*	Malta	35.900000	14.403889	35.896	14.396	YES	YES	NO	NO	NO

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30	cori291	continental *M. coriacea*	Spain	39.835333	-6.100722	39.854	-6.104	YES	YES	NO	NO	NO
31	cori11a	continental *M. coriacea*	France	43.149214	6.253144	43.146	6.271	YES	YES	NO	NO	YES
32	cori11b	continental *M. coriacea*	France	43.149214	6.253144	43.146	6.271	YES	YES	NO	NO	YES
33	cori11c	continental *M. coriacea*	France	43.149214	6.253144	43.146	6.271	YES	YES	NO	NO	YES
34	cori23a	continental *M. coriacea*	Mallorca	39.865675	2.938658	39.854	2.938	YES	YES	NO	NO	YES
35	cori23b	continental *M. coriacea*	Mallorca	39.865675	2.938658	39.854	2.938	YES	YES	NO	NO	YES
36	cori24a	continental *M. coriacea*	Mallorca	39.868608	2.966036	39.854	2.979	YES	YES	NO	NO	YES
37	cori12b	*M. coriacea* CSM	Corsica	42.069078	9.184994	42.063	9.188	YES	NO	NO	NO	YES
38	cori12a	*M. coriacea* CSM	Corsica	41.653797	9.211394	41.646	9.229	YES	YES	NO	NO	YES
39	cori12c	*M. coriacea* CSM	Corsica	42.967331	9.411772	42.979	9.396	YES	YES	NO	NO	YES
40	cori12d	*M. coriacea* CSM	Corsica	41.653797	9.211394	41.646	9.229	YES	YES	NO	NO	YES
41	cori12e	*M. coriacea* CSM	Corsica	42.967331	9.411772	42.979	9.396	YES	YES	NO	NO	YES
42	cori12f	*M. coriacea* CSM	Corsica	42.967331	9.411772	42.979	9.396	YES	YES	NO	NO	YES
43	cori12g	*M. coriacea* CSM	Corsica	41.653797	9.211394	41.646	9.229	YES	YES	NO	NO	YES
44	cori5	*M. coriacea* CSM	Sardinia	39.950840	9.519120	39.938	9.521	YES	YES	NO	NO	YES
45	cori18	*M. coriacea* CSM	Sardinia	39.939820	9.592800	39.938	9.604	YES	YES	NO	NO	YES
46	cori289	*M. coriacea* CSM	Montecristo	42.334000	10.308000	42.354	10.313	YES	YES	NO	NO	YES
47	cori290	*M. coriacea* CSM	Montecristo	42.334000	10.308000	42.354	10.313	YES	YES	NO	NO	YES
48	imbri6a	*M. imbricata*	La Palma	28.739706	-17.878633	28.688	-17.771	YES	NO	NO	NO	NO
49	imbri6b	*M. imbricata*	La Palma	28.739706	-17.878633	28.688	-17.771	YES	NO	NO	NO	NO
50	imbri6c	*M. imbricata*	La Palma	28.739706	-17.878633	28.688	-17.771	YES	NO	NO	NO	NO
51	imbri3a	*M. imbricata*	Gomera	28.120050	-17.226864	28.104	-17.229	YES	NO	YES	NO	NO
52	imbri4a	*M. imbricata*	Gomera	28.120050	-17.226864	28.104	-17.229	YES	NO	YES	NO	NO
53	imbri4b	*M. imbricata*	Gomera	28.120050	-17.226864	28.104	-17.229	YES	NO	YES	NO	NO
54	imbri15a	*M. imbricata*	Gomera	28.120050	-17.226864	28.104	-17.229	YES	NO	YES	NO	NO
55	imbri15b	*M. imbricata*	Gomera	28.120050	-17.226864	28.104	-17.229	YES	NO	YES	NO	NO
56	imbri5a	*M. imbricata*	Tenerife	28.201000	-16.579975	28.188	-16.563	YES	NO	YES	YES	NO
57	imbri5b	*M. imbricata*	Tenerife	28.201000	-16.579975	28.188	-16.563	YES	NO	YES	YES	NO
58	imbri5c	*M. imbricata*	Tenerife	28.201000	-16.579975	28.188	-16.563	YES	NO	YES	YES	NO
59	imbri5d	*M. imbricata*	Tenerife	28.201000	-16.579975	28.188	-16.563	YES	NO	YES	YES	NO
60	imbri7a	*M. imbricata*	La Palma	28.690411	-17.789239	28.729	-17.896	YES	NO	YES	YES	NO
61	imbri17a	*M. imbricata*	Tenerife	28.201000	-16.579975	28.188	-16.563	YES	NO	YES	YES	NO
62	imbri17b	*M. imbricata*	Tenerife	28.201000	-16.579975	28.188	-16.563	YES	NO	YES	YES	NO
63	lanio9a	*M. lanio*	Madeira	32.778278	-17.114886	32.771	-17.104	YES	NO	NO	YES	NO
64	lanio9b	*M. lanio*	Madeira	32.778278	-17.114886	32.771	-17.104	YES	NO	NO	YES	NO
65	lanio8a	*M. lanio*	Madeira	32.821422	-17.173719	32.813	-17.188	YES	NO	YES	YES	NO
66	lanio298	*M. lanio*	Madeira	32.743333	-17.098889	32.729	-17.104	YES	NO	YES	YES	NO

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GENERAL DISCUSSION

Throughout these four chapters I have explored the main mechanisms of range expansion and their role in the diversification of five Western Palearctic lineages of freshwater beetles with general limited dispersal capabilities, but with large differences in the size of the geographical distribution among genetically closely related species. With that purpose I set several objectives at the beginning of the thesis from which I can extract some generalities.

Historical events driving current distribution of species in the Mediterranean area

In the first Chapter the main objective was to link the evolutionary and biogeographical history of the genus *Deronectes* with the most relevant historical geological and climatic processes to discover how they could have affected the current distribution of the species in this genus, which has a predominantly Mediterranean distribution.

The phylogenetic and dating analysis estimated an ancient split among eastern and western Mediterranean lineages dated from the Middle Miocene, in agreement with the general pattern described by Oosterbroek and Arntzen (1992) for several groups and from other aquatic coleoptera groups (e.g. *Hydrochus*; Hidalgo-Galiana & Ribera, 2011). Vicariance between western and eastern Mediterranean lineages could have occurred during this time likely resulting from the isolation of Europe west of the Alps from the Balkans and Anatolia as a consequence of tectonic movements associated with the Carpathian uplift, with a documented western extension of the Carpathian Foreland 20-15 MY (Dercourt *et al*., 1985; Meulenkamp & Sissingh, 2003) and marine transgressions that restored connections between the Tethys and Paratethys, that may have contributed to the isolation of freshwater species in these two areas (Oosterbroek & Arntzen, 1992; Sanmartin, 2003). The Anatolian peninsula and the southwestern Mediterranean region (probably the Iberian Peninsula) were in the origin of the eastern and western clades of *Deronectes* respectively. From these two areas, range expansions mainly at the end of the Miocene (Late Tortonian) and beginning of the Pliocene, resulted in the main species groups and a few of the extant species of the genus. The Tortonian was a time of strong tectonic activity and sea level changes mainly in the west side of the Mediterranean as shoreline reconstructions on this period based on

coral deposits (Jolivet *et al*., 2006) reflect a succession of islands of different sizes in the Ibero-Maghrebian area that could have favoured multiple vicariant events.

While the Tortonian was a time of possible fragmentation of populations with the subsequent speciation, the new land corridors emerged at the end of the Messinian might have facilitated biotic dispersal. However, we did not found evidence of large scale range movements between both sides of the Mediterranean during the Messinian Salinity Crisis. Instead range movements were relatively local, mostly centred in the southwest of the Mediterranean basin (Ibero-Maghrebian and Corso-Sardinian areas) and in the east, with the closing of the Bosphorus strait. Late Miocene and Early Pliocene were therefore highly relevant in the origin of the main species-groups of the genus, but according to the ancestral reconstruction the present-day distribution of species is largely influenced by range expansions during the Pleistocene, where most of the current species originated, including those with current central and northern European distribution and its closest narrow distributed relatives. The main exception to this pattern are some Iberian endemisms with an older origin (Pliocene), probably favoured by the continuity in the general ecological and climatic conditions in the Iberian Peninsula that allowed the continuous presence of some species throughout all the Pleistocene glaciations (Oosterbroek & Arntzen, 1992).

According to our results, the evolutionary history of this genus was strongly influenced by major geological and climatic events around the Mediterranean since the Miocene, with successive cycles of fragmentation and subsequent eastward and westward range expansions through the north shore of the Mediterranean (with only local displacements in the south during the MSC), that resulted in a steady accumulation of species across the basin.

The role of the Quaternary glaciations

In the second and third Chapters, the biogeographic and temporal origin of several species-groups comprising central and northern European widespread species studied, with the main aim of shed light about the role of the Pleistocene glaciations in the diversification and current distribution of these species.

All widespread European species were, as expected, to be of Pleistocene origin, with a very recent (Upper Pleistocene and even Holocene) intraspecific variation. This is in agreement with some molecular studies that have illustrated examples of species that very probably did arise in the Pleistocene or Holocene such as some mammals

(Lister, 2004), birds (Klicka *et al*., 1999), fishes (Seehausen, 2002) and insects, among them aquatic Coleoptera (Ribera & Vogler, 2004; Ribera *et al*., 2011; Trizzino *et al*., 2013).

Hydraena gracilis and the species of *Deronectes* with a widespread European distribution were reconstructed as having an origin in the southern Mediterranean peninsulas, in agreement with the classic model of recolonisation of Europe after the glacial cycles from the southern Mediterranean area (Hewitt, 2000). Nevertheless, the obtained results do not support exactly this model, as the distribution of the widespread species does not include the southern peninsulas, which are occupied by vicariant taxa of recent origin especially in the Iberian and Anatolian peninsulas (the areas with a high number of extant species in the studied groups), suggesting that after recolonisation northern populations become isolated from their source areas. It seems that the populations that expanded their ranges were those located at the northernmost regions within the peninsulas, just at the edge of the deglaciated areas, taking advantage of their privileged geographical position to expand to the newly available habitat. Data from other taxa of freshwater beetles also showed than southern species did not colonize northern areas (e.g *Hydrochus*, see Hidalgo-Galiana & Ribera, 2011; *Enicocerus*, see Ribera *et al*., 2010). Even more relevant, at least for aquatic Coleoptera, is the total absence in northern and central Europe of Quaternary fossil remains from the species currently restricted to the Mediterranean (Abellán *et al*., 2011), supporting the view of the Mediterranean peninsulas as areas of endemism, not as a source of postglacial colonisation (Bilton *et al*., 1998).

Also in contrast to the classic view of the origin of central and north European current fauna we found that the widespread species in the *P. maculatus* complex and in *Oreodytes* did not had an origin in the Mediterranean area but in central Asia, with peripheral isolated forms that showed in most cases different haplotypes from those found in central and northern Europe, or even evident morphological differences. Most of these peripheral form were in the southern Mediterranean peninsulas, but remarkably also in the north Scottish lochs in the case of *P. maculatus*. In these cases re-colonization of the newly deglaciated areas may have occurred from sheltered glacial refugia at relatively high latitudes in central and eastern Europe or western Asia, a pattern also suggested for trees (Demesure *et al.*, 1996), small mammals (Bilton *et al.*, 1998) and insects (Cooper *et al*., 1995). The accumulation of narrow endemics of these lineages in the periphery of the range of the complex may thus be the result of

successive cycles of range expansions with subsequent speciation (and local extinction in glaciated areas) through the multiple Pleistocene glacial cycles. These peripheral isolated forms could have been confined as remnants of earlier diversification cycles, but also be the result of incipient isolation of the most recent post-glacial expansion.

Climatic preferences driving evolution

In Chapter four the evolutionary history and ancestral climatic niche of the genus *Meladema* was reconstructed, to investigate the range expansion of a widespread species not in recently deglaciated areas (i.e. central and northern Europe) but through the entire Mediterranean basin.

The genus *Meladema* originated in the Middle Miocene (with a similar age of that found in *Deronectes*), although diversification among extant *Meladema* lineages started in the early Pleistocene and took place in a relatively short time period. Phylogeographic analysis inferred a continental origin of *Meladema*, centred in the southwestern Mediterranean area (southern Iberia or in northwestern Africa), from where the continental *M. coriacea* expanded its range eastwards through both shores of the Mediterranean, reaching western Turkey in the northern basin, and Tunisia in the southern. Regarding to the two endemic Atlantic Island taxa, they appear as sister groups, indicating that they arose from a common ancestor, apparently derived from mainland populations of *Meladema* in the Early Pleistocene, a time in which there were several islands between Macaronesia and continental Europe (Fernández-Palacios *et al*., 2011), that might allowed biotic dispersal to these archipelagos. It is interesting that the two Atlantic Island species, with evident morphological differences, have diverged morphologically and ecologically during a time window that is equivalent to the separation of the Corso-Sardinian populations of *M. coriacea* (CSM) from the continental ones, which did not acquire obvious morphological differences. This observation may point to the role of environmental factors in allopatric speciation (Schluter, 1998), because the habitats occupied by endemic Meladema species on the subtropical Atlantic Islands differ in flow regime and climate from those in which members of the genus occur elsewhere, but could also be due to founder effects in the colonization of these two oceanic archipelagos.

Environmental niche modelling analyses showed that seasonality is the most important factor associated to current distribution of *Meladema*. The reconstruction of ancestral climatic preferences suggested that the three island lineages (*M. coriacea*

CSM and the two Macaronesian endemisms) occurred in climatic conditions reconstructed as being ancestral for the whole genus, that is the wet tropical climate of southwestern Mediterranean during the Miocene (Ruddiman *et al*., 1989; Jiménez-Moreno *et al*., 2010). In contrast, continental *M. coriacea* was the only lineage able to colonize also sites with climatic conditions non-ancestral for *Meladema* but typical from the current Mediterranean climate, with a highest aridity and seasonality. This is correlated with the current distribution, as all island lineages inhabit on average less seasonal sites than continental *M. coriacea*, especially the two endemic Macaronesian species, restricted to subtropical pine and laurel forests with high humidity conditions. Thus, developed capabilities necessary for survival during the typical dry periods of the Mediterranean could explain the wide distribution of continental *M. coriacea* through the Mediterranean, while all other lineages are exclusively restricted to different archipelagos, although experimental data on the physiological tolerance of the different species would be necessary to test this hypothesis.

A general wiew and future research

From these four studies it is possible extract some conclusions about the diversification of freshwater running water beetle lineages in the Western Palearctic.

The different biogeographical reconstructions suggests the occurrence of several events of range expansions between the eastern and western sides of the Mediterranean Basin under favourable conditions, followed by fragmentation when conditions deteriorate. Range expansions were mainly across the north shore of the Mediterranean, as in the south there is virtual absence in the studied groups of species eastern of Tunisia, likely as a consequence of the lack of favourable ecological conditions for these freshwater species in Libia and Egypt. Despite that it is not possible to establish a common temporal origin for all studied groups, the Miocene, with its various connections and subsequent isolation among the eastern and western sides of the Mediterranean basin, seems to be involved in the origin and east-west disjunction of some groups. However most of the current species were estimated to have originated during the Pleistocene, including those with widespread distributions, being therefore the epoch with a stronger influence in the present-day distribution.

The successive rounds of range expansion and fragmentation resulted mainly in the accumulation of narrow range species in the Mediterranean area, considered as a hotspot of endemism (Myers *et al*., 2000), a pattern also documented for other taxa (e.g.

mammals, Myers, 1988; plants, Gomez-Campo, 1985; or insects, Balletto & Casale, 1991). Especially relevant in the studied groups are the Iberian and Anatolian peninsulas, involved in the origin of most of these lineages and currently including the highest number of species, some of them with a relatively ancient origin. Less important is the Italian peninsula, with only endemisms of Pleistocene origin, as it remained submerged until the Pliocene (Meulenkamp & Sissingh, 2003; Popov *et al*., 2004).

Despite evidence in some cases of speciation with range stasis within the southern Mediterranean area, some species were able to expand their original range reaching widespread distributions in northern areas. We have hypothesized that these species took advantage of a privileged geographical position during a narrow window of favourable conditions, especially relevant in groups with poor dispersal abilities. However, differences in physiological or ecological tolerances could also have favoured range expansions in some species and limited them in others, as species with broader fundamental niches would survive in a wider range of conditions, and so occupy wider geographical areas than narrow-niched relatives (Gaston & Spicer, 2001). Following this trend, further experimental research in the differences in physiological tolerances in these lineages would be necessary to test this hypothesis. A promising physiological candidate could be the differences in thermal tolerances. In *Deronectes*, previous experimental studies showed that widespread species of the genus have greater thermal tolerance than their restricted southern relatives (Calosi *et al*., 2008; Calosi *et al*., 2010). In fact, thermal tolerance experiments performed by myself in latitudinally restricted populations of the *D. aubei* group from Cantabria and Sicily also showed a narrower thermal widow with respect to other wider populations (unpublished preliminary results, still in analysis).

MAIN CONCLUSIONS

1. The evolutionary history of the genus *Deronectes* was strongly influenced by many geological and climatic events since the Middle Miocene, with successive cycles of fragmentation and subsequent eastward and westward range expansions, resulting in a steady accumulation of species across the basin.

2. Most of these range movements took place through the north side of the Mediterranean, with only local displacements in the south during the Messinian Salinity Crisis.

3. Present-day distribution of species is largely influenced by range movements during the Pleistocene.

4. All widespread European species are of Pleistocene origin, with a very recent (Upper Pleistocene and even Holocene) intraspecific variation.

5. *Hydraena gracilis* and the species of *Deronectes* with a widespread European distribution originated in the southern Mediterranean peninsulas.

6. Populations that expanded their ranges were those located at northern areas within the peninsulas just at the edge of the deglaciated area, taking advantage of a privileged geographical position, while southernmost species did not colonize northern areas.

7. The existence of these two partly non overlapping species pools (Mediterranean species with ranges never extending to northern Europe, and northern species with limited southern expansions during the glacials) is consistent with increasing evidence from molecular studies of the role of the Mediterranean peninsulas as a source of endemism not of re-colonization of northern deglaciated areas.

8. The origin of widespread central and northern European species in the *P. maculatus* complex and in *Oreodytes* was not the Mediterranean area but central Asia, with peripheral isolated forms in the southern Mediterranean peninsulas.

9. These peripheral isolated forms could have been confined as remnants of earlier diversification cycles or also be result of incipient isolation of the most recent post-glacial expansion.

10. Continental origin in *Meladema* centred in the southwestern Mediterranean area from where expanded its range up to Turkey in the northern basin, and to Tunisia in the southern, with an independent colonization of the Macaronesian and Mediterranean islands.

11. Seasonality is the critical factor in shaping the current distribution of *Meladema*. Island lineages, especially the two Macaronesian endemics, occur in sites with low seasonality, within the range of the reconstructed ancestral climatic niche of the genus (the wet tropical climate of southwestern Mediterranean during the Miocene).

12. Continental *M. coriacea* was the only lineage able to expand its range across the Mediterranean, outside the ancestral climatic range of the genus, with a higher seasonality and aridity.

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