**Tectonic vicariance versus Messinian dispersal in western
Mediterranean ground beetles (Carabidae Trechini and
Pterostichini Molopina)**

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ABSTRACT

The complex geological history of the western Mediterranean region complicates the interpretation of the evolutionary history of its current fauna, as similar distribution patterns may have very different temporal and geographical origins. Particularly intriguing are some subterranean species in islands, which origin is usually difficult to interpret as their strongly modified morphologies obscure their relationships. We studied subterranean taxa and their likely relatives of two groups of ground beetles in the western Mediterranean: the *Duvalius* lineage ("isotopic" Trechini) and Molopina (Pterostichini). We included specimens from the islands of Mallorca, Sardinia and Sicily, plus mainland Europe and north Africa. Phylogenetic relationships were reconstructed with a combination of mitochondrial and nuclear data, and divergence dates were estimated with Bayesian methods using the same a priori molecular evolutionary rates for the same gene fragments in the two groups. The subgenus *Trechopsis*, which includes all the highly modified cave or nivicolous species, was found to be polyphyletic: the species from Mallorca was found to be of Pleistocene origin and sister to the less modified species of subgenus *Duvalius* from the same island, whereas the Algerian species of *Trechopsis* were, on the contrary, related to the Sicilian *Duvalius*, indicating a northern colonization route during the late Pliocene. Molopina was divided in three main lineages: the genera *Abax*, *Percus*, and the *Molops* groups of genera. The basal diversification of the latter was dated within a temporal window (35-25 Ma) fully congruent with the tectonic opening of the western Mediterranean basin, and included six main lineages with uncertain relationships among them: the epigean genera (1) *Molops* and (2) *Tanythrix*; and the subterranean (3) *Typhlochoromus* (Eastern Alps), (4) *Speomolops* (Sardinia), (5) *Henrotius* (Mallorca) and (6) a strongly supported clade including the Pyrenean genera *Zariquieya*, *Oscadytes* and *Molopidius*. Despite sharing a similar distribution of some of the subterranean taxa, the two studied groups show thus a strongly contrasting origin and mode of diversification. While the *Duvalius* lineage had a recent origin, with complex colonization patterns and widespread morphological convergence among the subterranean species, the subterranean Molopina had an ancient vicariant origin resulting from the tectonic opening of the western Mediterranean basin.

Keywords: Western Mediterranean region, dispersal, tectonic vicariance, Messinian, subterranean Coleoptera, Carabidae, *Duvalius*, Trechini, Pterostichini, Molopina

1. Introduction

The western Mediterranean fauna has a long tradition of phylogenetic and biogeographic studies trying to understand the complex relationships between the Balearic and Tyrrhenian islands with the neighbouring mainland. Corsica and Sardinia are a classical model (e.g. Ketmaier and Caccione, 2013 and references therein), sharing some endemics with a Tyrrhenian distribution (Médail and Quézel, 1999; Brullo et al., 2001; Ketmaier et al., 2006; Cicconardi et al., 2009), but still with very different faunas in many groups, something that is often overlooked (see e.g. Casale and Vigna Taglianti, 1996 for groundbeetles, or Caccione & Sbordoni 2001, Martinsen et al 2009 among others for other subterranean fauna). The fauna and flora of Balearic Islands are in turn composed by numerous vicariant species from the Iberian Peninsula (Moreno Sáiz et al., 1998), some of them with close relationships with southern France endemics (e.g. Gielly et al., 2001) or Corso-Sardinian species (Contandriopoulos and Cardona, 1984). There are also some well documented relationships of the fauna of Sicily and Calabria with that of Algeria and Tunisia (Chapman and Abbott, 2005; Cosson et al., 2005; Stöck et al., 2008b; Habel et al., 2009, 2010, 2011; Pfenninger et al., 2010; Pabijan et al., 2012; Marrone et al., 2013), as well as relationships of western North Africa with southeast Iberia (the Baetic-Rifean area, e.g. Dobson and Wright, 2000; Stöck et al., 2008a; Molina-Venegas et al., 2013; Faille et al., 2014). However, despite the biogeographic relationships of both Sicily and Sardinia with north Africa, they do not share many endemic species, and their faunas are overall very different (see e.g. Vigna Taglianti et al., 2002 for groundbeetles). The origin of the Kabylean biota has been much less studied, and is still poorly understood.

Many of the relationships within the western Mediterranean basin have been interpreted as the results of vicariant splits due to its tectonic opening during the Oligocene and early Miocene (Rosenbaum, 2002; Meulenkamp and Sissing, 2003; Schettino and Turco, 2006). However, due to lack of adequate fossil record this was often done without strong evidence neither of the relationships between the species nor their age (see e.g. the surprising resolution of the paradigmatic case of *Euproctus* salamanders in Carranza and Amat, 2005). The interpretation of the origin of the species is complicated by the possibility of dispersal during the salinity crisis of the Messinian ending 5.3 Ma (Hsü et al., 1973; Roveri et al., 2014), through land bridges that in many cases paralleled the tectonic connections of the Oligocene plates. The wide temporal interval between the two potential connections of emerged landmasses in the western Mediterranean (original tectonic vicariance, between 35-

25 Ma, and Messinian dispersal, between 7-5 Ma) allows the use of molecular data to discriminate between the two scenarios, despite all the uncertainties associated with molecular clock approaches (Kumar, 2005). This has allowed to reliably reconstruct the origin of some groups as either having an old, vicariant origin (e.g. Ribera et al., 2010; Bidegaray-Batista and Arnedo, 2011) or through dispersal during the salinity crisis (e.g. Carranza et al., 2008; Mora et al., 2017). There are, however, still many groups for which their evolutionary origin is largely unknown.

A particularly interesting case are groups with a general poor dispersal ability but with species in some of the islands, as they have often been assumed to have an ancient, tectonic origin. These notoriously include subterranean species, though to being unable to disperse even through the land connections during the Messinian due to their general reduced mobility and the lack of suitable habitat (e.g. Ribera et al., 2010). Different groups of insects contain species with subterranean habits, but only a few of them have a marked tendency for repeated colonisations of the underground. In the western Mediterranean this is the case of two groups of terrestrial Coleoptera within the Carabidae ground beetles, the Trechini and Pterostichini Molopina.

1.1. Trechini

Within the western Mediterranean, the Pyrenean *Aphaenops* lineage, shown to be monophyletic by Faille et al. (2010), is the most species rich among the strictly subterranean Trechini. Other genera of western Mediterranean Trechini with aphaenopsoid shape, e.g. *Sardaphaenops* Cerruti & Henrot, 1956 (two species endemic to Sardinia) and *Paraphaenops* Jeannel, 1916 (two species endemic to southern Spain), were excluded from the *Aphaenops* lineage by Faille et al. (2011a). In addition to these radiations, belonging to the “anisotropic” Trechini (i.e. with the male genitalia with an asymmetric copulatory piece laying in lateral position, Jeannel, 1928), there is another group of genera with mostly subterranean species: the *Duvalius* lineage, or “isotopic” Trechini (i.e. with the male genitalia with a symmetric copulatory piece not twisted with respect to the body axis, Jeannel, 1926). In Faille et al. (2011a, 2013) this “isotopic” lineage was shown to be monophyletic and not directly related to the “anisotropic” lineages.

Within the “isotopic” Trechini, the genus *Duvalius* Delarouzée is the most species rich, with more than 300 species described so far (Moravec et al., 2003). The highest diversity is in the French and Italian Alps, Italian and Balkan peninsulas and the Carpathian area, but the genus reaches its western limit in the western Mediterranean, the westernmost species

being an endemic from Catalonia (Jeannel, 1926, 1928). Other species are found in north Algeria (Kabylia, four species), the Balearic Islands (Mallorca, two species), Sardinia (one species), and Sicily (nine species). All of them are endogean or cave-dwelling (Jeannel, 1928; Henrot, 1964; Lagar, 1976; Bellès 1987; Vigna Taglianti et al., 2002; Magrini et al., 2006, 2016) (Table S1). A previous molecular study on Alpine Trechini showed that some locally restricted and highly modified troglobitic genera were nested within the wider genus *Duvalius* (Faille et al., 2013).

In the current literature four species also with marked troglobiomorphic features are included in *Duvalius* subgenus *Trechopsis* Peyerimhoff (Jeannel, 1928; Moravec et al., 2003), leaving in *Duvalius* s.str. the less troglobiomorphic species. The relationships of the four species of *Trechopsis* are so far unknown. It was originally described as a genus for a species endemic to the Djurdjura, in Algeria (*D. (T.) lapiei* (Peyerimhoff)). This species occurs in high altitude caves or pits with snow ("tesserefts", Peyerimhoff, 1908). The second described species, *D. (T.) iblis* (Peyerimhoff), was found in a cave, and although originally included in a different genus (*Aphaenops*) its affinities with *D. (T.) lapiei* were already noted by its descriptor (Peyerimhoff, 1910) and later confirmed by Jeannel (1928), who also relegated *Trechopsis* to a subgenus of *Duvalius*. A third endogean species was later described from the Babor range (*D. (T.) baborensis* Bruneau de Miré, 1955). Subsequently, *D. (T.) ferreresi* Lagar was described from caves of Mallorca and considered to be very close to the Algerian *D. (T.) iblis* (Lagar, 1976). Jeannel (1928) hypothesised that the north African *Trechopsis* are old relicts from a period when the Kabylia massif was attached to the Tyrrhenian plate (the "Tyrrhenis"). He also suggested that they might be related to some *Duvalius* from the eastern Mediterranean (the "Égée"), and that the ancestors of *Trechopsis* might have colonized Africa from Sicily during the "Nummulitique" (Paleogene) (Jeannel, 1928).

The relationships of the species of *Duvalius* s.str. from the western Mediterranean islands are also unknown. The only *Duvalius* s.str. from Mallorca, *D. balearicus* Henrot, was considered to be close to the only Iberian *Duvalius* s.str. (*D. berthae* Jeannel) (Henrot, 1964). *Duvalius berthae* has been shown to be related to other *Duvalius* from southeast France (Faille et al., 2013), but its potential relationships with the Mallorcan *D. balearicus* have never been tested. Jeannel (1928) noted that the only species of *Duvalius* s.str. from North Africa (*D. jurjurae* Peyerimhoff) had strong morphological similarities with the Sicilian species of *Duvalius*. Following Jeannel (1928), Vigna Taglianti (1982) suggested that the Sicilian *Duvalius* species have a close relationships with some species from the central Apennine. Finally, the only species from Sardinia, *Duvalius sardous* (Dodero), is endemic to

a small massif in the central-eastern part of the island. Jeannel (1928) hypothesised a close relationship of this peculiar, isolated species with the *D. brujasi* Sainte-Claire Deville species group from the Alpes Maritimes, whereas Vigna Taglianti (1982), hypothesized some possible affinities with the Iberian *D. berthae*.

1.2. *Molopina*

Species of *Molopina* (Carabidae Pterostichini) have an Euro-Mediterranean distribution, and include a number of epigean genera, but also some species with subterranean habits, with different degree of troglomorphism. The taxonomic and geographic limits of *Molopina* have been contentious. Jeannel (1948) proposed a close relationship between the Euro-Mediterranean taxa attributed to “Pterostichidae Molopini” and some genera of South Africa, Madagascar, Australia and New Zealand. However, Casale and Ribera (2010) showed that the clade “*Molopina*” with the exclusion of the austral taxa (represented in this study by several Madagascan genera) was strongly supported. *Molopina* thus included the genera *Molops* Bonelli, *Tanythrix* Schaum (treated here as a distinct genus: see Vigna Taglianti, 2005), *Percus* Bonelli and *Abax* Bonelli, but excluding *Styracoderus* Chaudoir (a genus currently attributed to this subtribe, e.g. Mateu, 1955).

Molopina are treated as a valid and distinct subtribe of Pterostichini (or tribe when Pterostichinae is treated as subfamily) in some recent contributions and catalogues (Brandmayr and Zetto Brandmayr, 1979, 1994; Vigna Taglianti, 2005; Serrano, 2013), but was ignored by both Bousquet (2003) and Lorenz (2005). *Molopina* are mainly characterised by the lack of discal setae on elytra, by the presence of a keel at the base of the seventh stria (reduced in some species) and by a membranous band at the base of the first antennal joint of the larvae. In some genera all species show developed parental care and pre-social behaviour. Casale and Ribera (2010) did not study any subterranean species of *Molopina*, and neither did any of the other published phylogenies of the group (Brückner, 2002, 2004a,b; Brückner and Mossakowski, 2006; Sasakawa & Kubota, 2007). These subterranean taxa include in the Western Mediterranean the genera *Speomolops* Patrizi (Sardinia), *Henrotius* Jeannel (Mallorca), and *Oscadytes* Lagar, *Molopidius* Jeannel and *Zariquieya* Jeannel (central and eastern Pyrenees), all of them monospecific except for *Zariquieya*, with two species (Faille et al., 2011b) (Table S1). Three further taxa of *Molopina* from the eastern Alps and the Balkans are treated either as subgenera of *Molops* (e.g. Lorenz, 2005) or as distinct genera (e.g. Bousquet, 2003), including the subterranean *Typhlochoromus* Moczarski with two species in the eastern Alps.

In this contribution we present the first estimation of the phylogenetic relationships of the subterranean species of the *Duvalius* lineage and of *Molopina* in the western Mediterranean. For the *Duvalius* lineage we include species from Mallorca, Sicily and Sardinia, plus species from the European and North African mainland. For *Molopina* we include a sample of all epigean genera plus all subterranean taxa from the western Mediterranean and one from the eastern Alps. In addition to establish the phylogenetic relationships of the subterranean taxa, we provide an approximate time framework for their diversification, and hypothesize a scenario for their biogeographical origin.

2. Materials and methods

2.1. Taxon sampling and DNA sequencing

We sampled representatives of species of the genus *Duvalius* from the westernmost part of its distribution area, including Spain, France, Italy, Algeria and Western Mediterranean Islands: Mallorca, Sardinia and Sicily (Fig. 1; Table S2). As outgroups we included 41 species of Western European *Duvalius* of different species groups (Faille et al., 2013) as well as representatives of other genera of Western European Trechini, both isotopic (*Agostinia*, *Anophthalmus* Sturm, *Luraphaenops*, *Trichaphaenops*) and anisotopic (*Aphaenops* Bonvouloir, *Geotrechus* Jeannel, *Trechus* Clairville). Trees were rooted on the split between the anisotopic and isotopic genera, following previous works on the group (e.g. Faille et al., 2013).

For *Molopina*, we included examples of all the western Mediterranean genera of the subtribe (Jeannel, 1948; Mateu, 1955; Casale and Ribera, 2010), as well as one species of *Typhlochoromus*, a subgenus of *Molops* (Bousquet, 2003) considered to be a valid genus by some authors (e.g. Vigna Taglianti, 2005; Lorenz, 2005; Casale and Vigna Taglianti, 2005) (Fig. 2; Table S2). As outgroups we included a number of genera of Pterostichini, including some Madagascan genera and *Styracoderus*, formerly included in *Molopina*. Trees were rooted in these Madagascan genera, following Casale and Ribera (2010).

Specimens were collected by hand and immediately killed in 96% ethanol, or by means of pitfall traps containing propylene glycol as preserving agent. DNA was extracted non-destructively from whole specimens using a standard phenol chloroform extraction or commercial extraction kits (mostly Qiagen, Hilden, Germany). Voucher specimens have been deposited in IBE (Barcelona), ZSM (Munich) and MNHN (Paris); DNA aliquots are kept in the DNA collections of ZSM (Munich) and IBE (Barcelona).

We sequenced two mitochondrial fragments, including four genes (3'end of partial cytochrome c oxidase subunit I, COI; and a continuous fragment, rrnL+trnL+nad1, including the 3' end of the gene for the large ribosomal RNA subunit, the leucine transfer RNA gene, and the 5' end of the NADH dehydrogenase subunit 1 gene) and two nuclear genes (5' end of the small ribosomal RNA subunit gene, SSU; and an internal fragment of the large ribosomal unit, LSU). For Molopina we also sequenced the mitochondrial protein coding genes NADH5 and an additional fragment of NADH1, and the ribosomal 12S (see Table S3 for the primers used). Sequences were assembled and edited with Bioedit 7 (Hall, 1999), Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) or Geneious v.6 (Biomatters Ltd, Auckland, New Zealand). Some of the sequences were obtained from Faille et al. (2010, 2011a, 2012, 2013) for Trechini and from Düring and Brückner (2000), Brückner (2002, 2004a,b), Brückner and Mossakowski (2006) and Casale and Ribera (2010) for Molopina. New sequences (242) have been deposited in EMBL databases (see Table S1 for Accession Numbers).

Phylogenetic analyses

Protein-coding and ribosomal genes were aligned with the online version of MAFFT v.6 (Kato and Toh, 2008) using the EINS-i and QINS-i algorithms, respectively, with other parameters set to their defaults.

Phylogenetic analyses were conducted with maximum-likelihood (ML) and Bayesian inference (BI) using the CIPRES Science Gateway (Miller et al., 2010). ML trees were obtained using RAxML v.7.2 (Stamatakis, 2006). Data sets were partitioned by gene, with the 16S divided in two fragments in Molopina as for some taxa the sequence data were incomplete. We applied an independent GTR+G evolutionary model to each partition, and obtained node support values with 1000 bootstrap replicates.

For the Bayesian analyses we used BEAST 1.8 (Drummond et al., 2012), initially with the same partitions and evolutionary models as in the ML analyses. In the *Duvalius* phylogeny we deleted duplicated terminals, combining the two specimens of *D. berthae* in a single chimera. In all analyses we used a Yule speciation model as tree prior and established an arbitrary age for the root. We set a lognormal relaxed clock with flat priors for all partitions, and run 100 MY generations sampling every 5,000, assessing convergence with TRACER v1.6 (Rambaut et al., 2014).

For the calibration analyses in BEAST we excluded outgroups and rooted the trees according to the topologies obtained in the ML and BEAST analyses with outgroups. There

are no known fossils of Molopina, *Duvalius* or other isotopic genera, and the available fossils of *Trechus sensu lato* are too distant to be used reliably here (see e.g. Schmidt and Faille, 2015; Schmidt et al., 2016a,b for some recent fossils described from Baltic amber). To calibrate the trees we thus used the rates estimated for some of the same genes for the related ground beetle genus *Carabus* Linnaeus by Andújar et al. (2012, 2014) using a combination of fossils and biogeographic events. We used a normal prior distribution with values 0.0145 subs/branch/MY for the 3' end of COI (Molopina) or 0.0130 for a fragment combining the 5' and 3' ends (*Duvalius*), 0.0016 for 16S, 0.0159 for NADH5 (only in Molopina) and 0.0013 for 28S, all with a standard deviation of 0.0001. Preliminary results (not shown) demonstrated that the exclusion of NADH5 in the calibration did not had any substantial effect in the estimated age of the root of the Molopina tree. We use flat priors for the rates of other genes. For the mitochondrial genes we compared a strict or a lognormal relaxed clock using AICM in TRACER, and used a relaxed clock for all nuclear genes. The use of the same evolutionary rates and models for the same genes in the two groups allows for a direct comparison between the estimated ages.

3. Results

3.1. *Duvalius* phyletic lineage

a) General topology (analyses with outgroups)

The BEAST analysis using a partition by gene failed to converge, so we used two partitions, one for the mitochondrial genes with a GTR+G+I evolutionary model and one for the nuclear, with a HKY+G+I evolutionary model (a GTR model also failed to converge adequately).

The topologies of the ML (RAxML) and Bayesian analyses (BEAST) were very similar, with the same well-supported nodes (Figs 3, S1). The monophyly of the group of western *Duvalius* species plus the genera *Anophthalmus*, *Trichaphaenops*, *Luraphaenops* and *Agostinia* (the "isotopics") was well supported, although neither *Duvalius* nor any of its two subgenera (*Duvalius* and *Trechopsis*) was recovered as monophyletic. The species *D. delphinensis* was sister to the species of *Anophthalmus*, and all the other subterranean genera were nested within *Duvalius* (forming what we call *Duvalius sensu lato* from now on), in agreement with Faille et al. (2013). Within *Duvalius* there were some well defined species groups. First, a clade of *Duvalius* species from the Maritime and Ligurian Alps including *Agostinia*, confirmed to be closer to the species of the *gentilei* species group than to the

carantii group. *Duvalius sicardi* Fagniez, *D. cailloli* (Sainte-Claire Deville) and *D. perrinae* Giordan form a well-supported clade.

b) Calibrated Bayesian analyses

The run with a strict clock for the mitochondrial partitions had a better AICM than that using a relaxed clock (Table 1), so we used a partitioned model by genes, all with a GTR+G+I evolutionary model and a strict clock. For the nuclear genes we used a HKY+G+I model, as a GTR model failed to converge adequately, with a relaxed lognormal clock and flat priors.

The root of the tree (crown diversification of the sampled *Duvalius sensu lato* + *Anophthalmus*) was estimated to have occurred at the end of the Miocene, ca. 6 Ma (95% confidence interval 7.5-4.8 Ma), with the origin of the two species from Mallorca (*D. (Trechopsis) ferreresi* and *Duvalius (s.str.) balearicus*) at ca. 4.2 Ma (c.i. 5.2-3.2). Surprisingly, and despite their strongly different degree of troglomorphy, these two species were sisters, with a very recent separation dating from the Pleistocene (0.7-0.01 Ma) (Fig. 4). All sampled Algerian, Sicilian and Sardinian species formed a well supported clade, with an uncertain sister group (Fig. 4). The Algerian species (*D. (Trechopsis) lapiei* and *D. (T.) iblis*) were sister, with a separation dating from ca. 2.5 Ma, and in turn sister to the two studied Sicilian species (*D. aliciae* Magrini, Baviera and Petrioli and *D. ribaudi* Magrini, Petrioli and Degiovanni). The separation between Algerian and Sicilian species was estimated to have occurred during the Pliocene, ca. 3.2 Ma (c.i. 4.2-2.5). In turn, the separation of the Sicilian+Algerian species from the Sardinian *D. sardous* was estimated to have occurred ca. 4.2 Ma (c.i. 5.3-3.2), with a stem age for the whole clade of ca. 5 Ma (Fig. 4).

3.2. Molopina

a) General topology (analyses with outgroups)

In the ML and Bayesian analyses with outgroups Molopina was strongly supported and divided in two equally well supported clades, the genus *Percus* and the remaining genera. Within the latter *Abax* was sister to a group of genera including *Molops*, *Tanythrix* and all the subterranean taxa (the *Molops* group) (Figs 5, S2). Within the *Molops* group only two intergeneric nodes were well supported in the ML analysis: the sister relationship between the Mallorcan *Henrotius* and the genus *Molops*, and the monophyly of all the central and eastern Pyrenean subterranean genera: *Molopidius*, *Oscadytes* and *Zariquieya* (Fig. 5). In the Bayesian analysis, however, most relationships were well supported, with the two epigeal genera (*Molops* and *Tanythrix*) sisters and sister to all the subterranean genera. Within the

later, the relationships of the two island endemics (*Speomolops* and *Henrotius*) with a clade including all the mainland genera were unresolved (Fig. S2).

Within the genus *Percus*, with only epigeal species, relationships were in general very well supported. *Percus villae* Kraatz (French and Italian Maritime Alps) was sister to the rest of the genus, which was divided in an Iberobaleare lineage and a lineage including Corsica, Sardinia, mainland Italy and north Africa. The Balearic endemic *P. plicatus* Dejean was sister to all the Iberian species (traditionally included in a separate subgenus, *Pseudopercus* Motschulsky). The sampled species present in Corsica and Sardinia (*P. strictus* Dejean and *P. grandicollis* Audinet-Serville) were sister to *P. lineatus* Solier, from Sicily, Algeria and Tunisia (Figs 5, S2).

b) Calibrated Bayesian analyses

In the BEAST runs we constrained the separation between *Percus* and the rest of Molopina, according to the topology with outgroups. The best AICM corresponded to the combination of a GTR+G+I evolutionary model for all partitions and a relaxed molecular clock (Table 1). The topology of the ingroup was very similar to that obtained for the analyses with outgroups, with only some internal nodes of the *Molops* group showing a different topology (Fig. 6). Topological changes affected specially the placement of the two island endemics, *Speomolops* and *Henrotius*.

The basal diversification of Molopina was estimated to have occurred in the upper Eocene (ca. 41 Ma, Fig. 6). Within the *Molops* group of genera, the diversification (with the exception of the Pyrenean lineage) was estimated to have occurred in a remarkably narrow temporal window, starting at 35 Ma (c.i. 41-28) and ending in the origin of the six main lineages between 30-28 Ma (Fig. 6). The diversification within the Pyrenean lineage of subterranean Molopina was posterior, during the Miocene, between 17 Ma for the origin of *Molopidius* and ca. 9 Ma for the separation between the two species of *Zariquieya* (Fig. 6).

Within the genus *Percus*, the basal separation between *P. villae* and the rest of species was also dated at ca. 28.6 Ma (c.i. 35-23), simultaneous with the basal diversification of the *Molops* group. The estimated age for the separation of the Balearic endemic (*P. plicatus*) from the Iberian species was ca. 19 Ma (c.i. 24-15), and that of the group of species from the Tyrrhenian islands, mainland Italy and north Africa 14.5 Ma (c.i. 19-11) (Fig. 6).

4. Discussion

Our results clearly establish two contrasting temporal and geologic scenarios for the origin of the subterranean taxa of the *Duvalius* lineage and Molopina in the western Mediterranean islands. Despite the lack of fossil record or other unambiguous calibration points, the use of the same evolutionary rates in the same genes in both lineages allows for a clear discrimination between an Oligocene origin associated to the tectonic opening of the western Mediterranean basin in Molopina, and a Messinian or post-Messinian diversification in the *Duvalius* lineage.

4.1. *Duvalius* phyletic lineage

The reconstructed topology of the western *Duvalius* group plus the genus *Anophthalmus* is consistent with previous results (Faille et al., 2013), including the position of *D. delphinensis* as sister species of *Anophthalmus*, supported also by some morphological characters (shape of male genitalia and pubescence of the head).

The westernmost Mediterranean species of the *Duvalius* lineage, *D. berthae* from the Catalan coastal range, was previously considered to be close to *D. lespesi* (Fairmaire) from southern France (Jeannel, 1928), the Sardinian *D. sardous* (Vigna Taglianti, 1982), or the Mallorcan *D. balearicus*. It was, however, confirmed to be related to *D. raymondi* (Delarouzée) from Provence (Southern France) with strong support, with an estimated separation in the Late Pliocene, a relationship already suggested in a recent work (Faille et al., 2013). The two species of *Duvalius* from Mallorca were recovered as sister with strong support despite their remarkable morphological differences. The relationships of these two species are however uncertain, as the sister relationship with the Ibero-Provençal *D. berthae*-*D. raymondi* clade in the ML and uncalibrated BEAST analyses was poorly supported, and not recovered in the calibrated BEAST analysis. In any case, the estimated age of the Mallorcan lineage is clearly posterior to the tectonic separation of the Balearic Islands from continental Spain during the Oligocene, and more compatible with a Messinian origin as suggested for other terrestrial and low dispersers groups (Chueca et al., 2017). The separation between the two Mallorcan species was estimated to be of Pleistocene origin, which would imply that *D. (Trechopsis) ferreresi* developed its troglomorphic features in a very short time - and in parallel to that of the other species of *Trechopsis* from North Africa (see below).

Among the other island species, *Duvalius sardous*, traditionally considered to be close to *D. brujasi* (south France), was found sister to a clade including the Algerian *Trechopsis* and Sicilian *Duvalius*. The paraphyly of the island species with respect to the Algerian implies that the separation between Sardinian and Sicilian species occurred prior to the

colonization of Africa by *Duvalius*. The northern origin of the Algerian *Duvalius* is in agreement with the complete lack of species belonging to this group in other African regions, which is rich in Trechini of other species groups (genus *Trechus* in the widest sense), especially in the Moroccan, Ethiopian and East African mountains (Jeannel, 1927). A colonization of north Africa from Sicily is also known in other organisms (e.g. firs of the genus *Abies*, Sánchez-Robles et al., 2014), although the contrary pattern - i.e. a southern origin of Sicilian fauna - is also found in e.g. some reptiles (Carranza et al., 2008) and amphibians (e.g. Stöck et al., 2008a,b). The connection between Sicily and Tunisia might have been facilitated by the lower sea level during the glacial phases, reducing the distance between the two continents and causing the emergence of potential stepping-stone islands (Husemann et al., 2014).

The separation between *D. sardous* and the Sicilian-Algerian species was estimated to date from the early Pliocene, approximately at the same age than the two Mallorcan species. Sicily does not belong to the Corso-Sardinian tectonic plate, but during the Tortonian and Messinian it was linked to north Africa, Calabria and the southern Apennines, which in turn were closely linked to Corsica, Sardinia and the Tuscan area (Rosenbaum et al., 2002). The connection between Sicily and north Africa remained until the Pliocene (Rosenbaum et al., 2002; Lo Presti and Oberprieler, 2011), in agreement with our estimations, but the Sicily strait is considered to have been a strong barrier during the Pleistocene, separating the European and African biota much more efficiently than the Gibraltar strait (Lo Presti and Oberprieler, 2011; Husemann et al., 2014).

The subgenus *Trechopsis*, which originally included some species from Southern Greece and Western Anatolia (Jeannel, 1934a,b; Coiffait, 1973), is currently restricted to four Western Mediterranean species highly specialised to the subterranean environment, with “aphaenopsian” habitus (Casale, 1979; Casale and Laneyrie, 1982; Moravec et al., 2003). Our results clearly demonstrated its polyphyly, with the Mallorcan *D. (T.) ferrerresi* not directly related to the Algerian species, implying that their similarities in body shape are the result of evolutionary convergence. The putative relationships between the oriental and African species formerly included in *Trechopsis* (now treated as *Duvalius huetheri* group sensu Casale and Laneyrie, 1982) remains unexplored, although the polyphyly of *Trechopsis* suggests that their similarity may also be the result of convergence. *Duvalius djurdjurae*, the only *Duvalius* sensu stricto in Africa, is known only from a couple of exemplars from the Djurdjura. Although we failed to find an exemplar for the molecular analysis, the species will most likely be included

in the clade of the Algerian *Trechopsis*+Sicilian *Duvalius*, as its morphological proximity with Sicilian species was already evidenced by Jeannel (1928).

4.2. *Molopina*

The distribution of subterranean Pterostichini *Molopina* presents a classical "Tyrrhenian" pattern, with morphologically specialised species in the Iberian peninsula, Pyrenees, Sardinia and Mallorca. No subterranean species is known so far from Sicily, the Maghreb or the eastern Mediterranean, although the remains of a specimen sampled in a cave in Southern Anatolia were attributed to *Molopina* (Vigna Taglianti, 1980; Casale and Vigna Taglianti, 1999). The ancient origin of *Molopina* and an early differentiation of the genera were already hypothesized by Brandmayr and Zetto Brandmayr (1979), based in part in the study of the peculiar system of parental care in these pre-social groundbeetles. They hypothesised that the pre-social behaviour is ancestral in *Molops*, as all species show strongly reduced ovipositors and the same life specialisation (what they call "aestivation"), considered to be of recent origin. However, what they consider to be the ancient *Molopina*, the stenotopic endogenous or cave-dwelling genera with only one or few species, were thought to be the relics of Oligocene or at least pre-Miocene forms, which were once living on the land masses around the Tethys (Brandmayr and Zetto Brandmayr, 1979). Although the breeding behaviour of the subterranean species is unknown, neither *Speomolops* nor *Typhlochoromus* or *Stenochoromus* show reduced terminalia in the female genitalia, suggesting the lack of pre-social habits. Amongst the troglobitic species, only the larval stages of *Speomolops sardous* are described so far (Casale et al., 2010), from five third instar larvae collected 20–30 cm below the surface of a sandy bank near one of the freshwater subterranean lakes where adults were very abundant. The larvae were never observed walking on the surface. This demonstrates that the larva of this species has obligate underground, fossorial behaviour, in sandy soil periodically flooded by the subterranean river of the cave.

Our results unambiguously show that the subterranean genera (*Molopidius*, *Speomolops*, *Henrotius*, *Zariquieya*, *Oscadytes* and *Typhlochoromus*) are members of this subtribe. They were all also included in the *Molops* group, one of the three lineages within *Molopina* (the other two, the genera *Percus* and *Abax* do not have any known subterranean species). The intergeneric relationships within the *Molops* groups were, however, poorly supported with the exception of the monophyly of the subterranean Pyrenean genera, which were estimated to have diversified during the Miocene, after the opening of the western Mediterranean basin. It is remarkable that all basal lineages of the *Molops* group, with

uncertain relationships between them, were estimated to have originated in a relatively narrow temporal window which fully overlaps with the tectonic opening of the western Mediterranean basin (Rosenbaum et al., 2002; Schettino and Turco, 2006). In the only analysis with some supported basal nodes, the uncalibrated Bayesian (Fig. S2), all subterranean taxa were recovered monophyletic and the cladogenetic order reflected the temporal succession of the separation of the plates: first the Sardinian species (*Speomolops*), then the Mallorcan (*Henrotius*) and, within the continental taxa, first the eastern species (*Typhlochoromus*) and finally the diversification within the Pyrenean group. This scenario would imply an early division between an eastern lineage formed by epigean forest species (*Molops* and *Tanythrix*), with a distribution centred in the central and eastern Alps and the Balkan peninsula, and a mostly western lineage with an ancestor with at least some subterranean habits and some troglomorphic characters, likely similar to the current Pyrenean *Molopidius* or the eastern *Typhlochoromus*. The monophyly of subterranean lineages previously though to have independently colonised the subterranean environment is a recurrent result in multiple groups (e.g. Ribera et al., 2010; Faille et al., 2010, 2011a, 2013), but in the case of Molopina the evidence is still insufficient. Other than the low support of the internal relationships in most analyses, the morphological and geographic evidence supports a closer relationship of *Typhlochoromus* with the eastern lineages of the *Molops* group (Busulini, 1957). There is also no molecular data available of the Balkan-Dinaric subterranean genera *Stenochoromus* and *Henrotiochoromus*, which may provide valuable information on the evolution of the eastern Molopina.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at ...

Table S1: List of species of Isotopic Trechini and Molopina occurring on Western Mediterranean Islands, Iberian Peninsula and North Africa.

Table S2: Material used in the study, with locality data, voucher number and accession numbers of the sequences.

Table S3: Primers used in the amplification and sequencing reactions.

Fig. S1: uncalibrated Bayes tree *Duvalius*

Fig. S2: uncalibrated Bayes tree Molopina

References

- Andújar, C., Serrano, J., Gómez-Zurita, J., 2012. Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. BMC Evol. Biol. 12, 40.
- Andújar, C., Soria-Carrasco, V., Serrano, J., Gómez-Zurita, J., 2012. Congruence test of molecular clock calibration hypotheses based on Bayes factor comparisons. Methods Ecol. Evol. 5, 226–242.
- Bellés, X., 1987. Fauna cavernícola i intersticial de la Península Ibèrica i les illes Balears. Monografies Científiques 4. Consell Superior d'Investigacions Científiques. Editorial Moll, Mallorca.
- Bidegaray-Batista, L., Arnedo, M.A., 2011. Gone with the plate: the opening of the Western Mediterranean basin drove the diversification of ground-dweller spiders. BMC Evol. Biol. 11, 317.
- Bousquet, Y., 2003. Tribe Pterostichini. In: Löbl, I. Smetana, A. (Eds), Catalogue of Palaearctic Coleoptera, Vol. 1, Archostemata, Myxophaga, Adephaga. Apollo Books, Stenstrup, pp. 469–521.

- Brandmayr, P., Zetto Brandmayr, T., 1979. The evolution of parental care phenomena in Pterostichini, with particular reference to the genera *Abax* and *Molops*. In: den Boer P.J., Thiele H.U., Weber F. (Eds.), On the Evolution of Behaviour in Carabid Beetles. Miscellaneous Papers, 18, Landbouwhogeschool Wageningen, pp. 35–49.
- Brandmayr, P., Zetto Brandmayr, T., 1994. The evolutionary history of the genus *Abax* (Coleoptera, Carabidae). In: Desender K., Dufrêne M., Loreau M., Luff M.L., Maelfait J-P. (Eds.), Carabid Beetles. Ecology and Evolution. Kluwer Academic Publishers, series Entomologica, 51, Dordrecht, Boston, London, pp. 19–24.
- Brückner, M., 2002. Phylogenie und biogeographie der Gattung *Percus* Bonelli 1810 (Coleoptera: Carabidae): eine molekularsystematische Analyse. PhD Dissertation, Verlag Mainz.
- Brückner, M., 2004a. Genetic differentiation in *Percus corsicus* (Coleoptera, Carabidae). Mitt. Dtsch. Ges. allg. angew. Ent. 14, 83–86.
- Brückner, M., 2004b. The phylogeny of the subgenus *Pseudopercus* (Coleoptera, Carabidae): conflicts between mitochondrial and nuclear DNA sequences. Mitt. Dtsch. Ges. allg. angew. Ent. 14, 77–82.
- Brückner, M., Mossakowski, D., 2006. Phylogeny of the genus *Percus* (Coleoptera: Carabidae) – nuclear genes and the basal splits. In: Serrano J., Koivula M., Lövei G. (Eds.), Proceedings of the XII European Carabidologists' Meeting. Entomol. Fennica 17, 195–199.
- Brullo, S., Giusso del Galdo, G. Garino, R., 2001. The orophilous communities of the Pino-Juniperetea class in the central and Eastern Mediterranean area. Feddes Repertorium 112, 261–308.
- Bruneau de Miré, P., 1955. Un *Duvalius* nord-Africain nouveau (Col. Trechidae). Rev. Fr. Entomol. 22, 254–256.
- Busulini, E., 1957. Note speleologiche - 1. Diagnosi preliminare di una nuova specie troglobia del genere *Molops* Bon. (Coleoptera-Carab.). B. Mus. Civ. St. Nat. Venezia 9[1956], 10.
- Caccone, A., Sbordoni, V., 2001. Molecular biogeography of cave life: A study using mitochondrial DNA from Bathysciine beetles. Evolution 55, 122–130.
- Carranza, S., Amat, F., 2005. Taxonomy, biogeography and evolution of *Euproctus* (Amphibia: Salamandridae), with the resurrection of the genus *Calotriton* and the description of a new endemic species from the Iberian Peninsula. Zool. J. Linn. Soc. Lond. 145, 555–582.

- 563 Carranza, S., Arnold, E.N., Geniez, P., Roca, J., Mateo, J.A., 2008. Radiation, multiple
564 dispersal and parallelism in the skinks, *Chalcides* and *Sphenops* (Squamata:
565 Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara
566 Desert. Mol. Phylogenet. Evol. 46, 1071–1094.
- 567 Casale, A., 1979. Note su alcuni *Duvalius* del Peloponneso, con descrizione di una nuova
568 specie (Coleoptera, Carabidae, Subf. Trechinae). Entomol. Basil. 4, 173–186.
- 569 Casale, A., Laneyrie, R., 1982. Trechodinae et Trechinae du monde. Tableau des sous-
570 familles, tribus, séries phylétiques, genres, et catalogue général des espèces. Mém.
571 Biospéol. 9, 1–226.
- 572 Casale, A., Ribera, I., 2010. Are Molopina of the Euro-Mediterranean region related to the
573 Madagascan, South African and Australian Pterostichini? (Coleoptera, Carabidae).
574 Biogeographia (n.s.) 29(2008), 33–44.
- 575 Casale, A., Vigna Taglianti, A., 1996. Coleotteri Carabidi di Sardegna e delle piccole isole
576 circumsarde, e loro significato biogeografico (Coleoptera, Carabidae). Biogeographia
577 18, 391–427.
- 578 Casale, A., Vigna Taglianti, A., 1999. Caraboid beetles (excl. Cicindelidae) of Anatolia, and
579 their biogeographical significance (Coleoptera, Caraboidea). Biogeographia (n.s.) 20,
580 277–406.
- 581 Casale, A., Di Giulio, A., Marcia, P., Molinu, A., 2010. The third instar larva of *Speomolops*
582 *sardous* Patrizi, 1955, a cave-dwelling molopine beetle endemic to Eastern Sardinia,
583 with notes on its habitat (Coleoptera, Carabidae). Ital. J. Zool., 77, 159–167.
- 584 Casale, A., Vigna Taglianti, A., 2005. Coleotteri Caraboidei delle Alpi centrali e orientali, e
585 loro significato biogeografico (Coleoptera Caraboidea). Biogeographia, 26, 129–201.
- 586 Chapman, M.A., Abbott, R.J., 2005. The origin of a novel form of *Senecio* (Asteraceae)
587 restricted to sand dunes in southern Sicily. New Phytol. 166, 1051–1062.
- 588 Chueca, L. J., Gómez-Moliner, B. J., Forés, M., Madeira, M. J., 2017. Biogeography and
589 radiation of the land snail genus *Xerocrassa* (Geomitridae) in the Balearic Islands. J.
590 Biogeogr. 44, 760–772.
- 591 Cicconardi, F., Nardi, F., Emerson, B.C., Frati, F., Fanciulli, P.P., 2009. Deep
592 phylogeographic divisions and long-term persistence of forest invertebrates
593 (Hexapoda: Collembola) in the North-Western Mediterranean basin. Mol. Ecol. 19,
594 386–400.
- 595 Coiffait, H., 1973. Contribution à la connaissance des Coléoptères des grottes d'Anatolie.
596 Ann. Spéol. 28, 685–688.

597 Contandriopoulos, J., Cardona, M.A., 1984. Caractère original de la flore endémique des
598 Baléares. Bot. Helv. 94,101–132.

599 Cosson, J.-F., Hutterer, R., Libois, R., Sarà, M., Taberlet, P., Vogel, P., 2005.
600 Phylogeographical footprints of the Strait of Gibraltar and Quaternary climatic
601 fluctuations in the western Mediterranean: a case study with the greater white-toothed
602 shrew, *Crocidura russula* (Mammalia: Soricidae). Mol. Ecol. 14, 1151–1162.

603 Dobson, M., Wright, A., 2000. Faunal relationships and zoogeographical affinities of
604 mammals in north-west Africa. J. Biogeogr. 27, 417–424.

605 Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with
606 BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

607 Duggen, S., Hoernle, K., van den Bogaard, P., Rüpke, L., Morgan, J.P., 2003. Deep roots of
608 the Messinian salinity crisis. Nature 422, 602–606.

609 Düring, A., Brückner, M., 2000. The evolutionary history of the tribe Molopini: a first
610 molecular approach. In: Brandmayr P., Lövei G., Zetto Brandmayr T., Casale A.,
611 Vigna Taglianti A. (Eds.), Natural History and applied ecology of Carabid Beetles.
612 Pensoft, Sofia-Moscow, pp. 1–4.

613 Faille, A., Andújar, C., Fadrique, F., Ribera, I., 2014. Late Miocene origin of a Ibero-
614 Maghrebian clade of ground beetles with multiple colonisations of the subterranean
615 environment. J. Biogeogr. 41, 1979–1990.

616 Faille, A., Bourdeau, C., Fresneda, J., 2012. Molecular phylogeny of the *Trechus brucki*
617 group, with description of two new species from the Pyreneo-Cantabrian area (France,
618 Spain) (Coleoptera, Carabidae, Trechinae). ZooKeys 217, 11–51.

619 Faille, A., Casale, A., Balke, M., Ribera, I., 2013. A molecular phylogeny of Alpine
620 subterranean Trechini. BMC Evol. Biol. 13, 248.

621 Faille, A., Casale, A., Ribera, I., 2011a. Phylogenetic relationships of west Mediterranean
622 troglobitic Trechini ground beetles (Coleoptera: Carabidae). Zool. Scr. 40, 282–295.

623 Faille, A., Fresneda, J., Bourdeau, C., 2011b. Les Molopina hypogés des Pyrénées avec la
624 description d’une nouvelle espèce de *Zariquieya* Jeannel, 1924 d’Espagne
625 (Coleoptera, Carabidae, Pterostichini). Zoosystema 33, 429–441.

626 Faille, A., Ribera, I., Deharveng, L., Bourdeau, C., Garnery, L., Queinnec, E., Deuve, T.,
627 2010. A molecular phylogeny shows the single origin of the Pyrenean subterranean
628 Trechini ground beetles (Coleoptera: Carabidae). Mol. Phylogenet. Evol. 54, 97–106.

- Gielly, L., Debussche, M., Thompson, J.D., 2001. Geographic isolation and evolution of the Mediterranean endemic *Cyclamen*: Insights from chloroplast *trnL* (UAA) intron sequence variation. *Plant Syst. Evol.* 230, 75–88.
- Habel, J. C., Dieker, P., Schmitt, T., 2009. Biogeographical connections between the Maghreb and the Mediterranean peninsulas of southern Europe. *Biol. J. Linn. Soc.* 98, 693–703.
- Habel, J. C., Lens, L., Rödder, D., Schmitt, T., 2011. From Africa to Europe and back: refugia and range shifts cause high genetic differentiation in the Marbled White butterfly *Melanargia galathea*. *BMC Evol. Biol.* 11, 215.
- Habel, J.C., Rödder, D., Stefano, S., Meyer, M., Schmitt, T., 2010. Strong genetic cohesiveness between Italy and North Africa in four butterfly species. *Biol. J. Linn. Soc.* 99, 818–830.
- Hall, T.A. 1999., BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.
- Henrot, H., 1964. Un *Duvalius* nouveau de l'île de Majorque (Col. Carabidae). *Bull. Soc. Entomol. Fr.* 69, 15–17.
- Hsü, K.J., Ryan, W.B.F., Cita, M.B., 1973. Late Miocene desiccation of the Mediterranean. *Nature* 242, 240–244.
- Husemann, M., Schmitt, T., Zachos, F.E., Ulrich, W., Habel, J.C., 2014. Palaeartic biogeography revisited: evidence for the existence of a North African refugium for Western Palaeartic biota. *J. Biogeogr.* 41, 81–94.
- Jeannel, R., 1926. Monographie des Trechinae. Morphologie comparée et distribution d'un groupe de Coléoptères. *L'Abeille* 32, 221–550.
- Jeannel, R., 1927. Monographie des Trechinae 2. Morphologie comparée et distribution géographique d'un groupe de coléoptères. *L'Abeille* 33, 1–592.
- Jeannel, R., 1928. Monographie des Trechinae. Morphologie comparée et distribution d'un groupe de Coléoptères. Troisième Livraison: les Trechini cavernicoles. *L'Abeille* 35, 1–808.
- Jeannel, R., 1929. Le sillon transégéen et description de Coléoptères cavernicoles nouveaux de la Grèce. *Bull. Soc. Sci. Cluj* 4, 59 – 84
- Jeannel, R., 1934a. Diagnoses préliminaires de deux coléoptères cavernicoles de l'Asie Mineure. *Bull. Soc. Entomol. Fr.* 39, 20–21.
- Jeannel, R., 1934b. Coléoptères cavernicoles de la grotte de Fersine, en Asie Mineure. *Ann. Soc. Entomol. Fr.* 103, 159–174, 345–346 [rectification].
- Jeannel, R., 1943. Les fossiles vivants des cavernes. Gallimard, Paris.

- Jeannel, R., 1948. Coléoptères Carabiques de la Région Malgache (Deuxième Partie). Faune de l'Empire Français, X, Editions du Muséum, Paris, pp. 373–765.
- Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* 9, 286–298.
- Ketmaier, V., Caccone, A., 2013. Twenty years of molecular biogeography in the West Mediterranean Islands of Corsica and Sardinia: lessons learnt and future prospects. In: Silva-Opps M. (Ed.), *Current Progress in Biological Research*, Chapter 4, Intech, Rijeka, pp.71–93.
- Ketmaier, V., Giusti, F., Caccone, A., 2006. Molecular phylogeny and historical biogeography of the land snail genus *Solatopupa* (Pulmonata) in the peri-Tyrrhenian area. *Mol. Phylogenet. Evol.* 39, 439–451.
- Kumar, S., 2005. Molecular clocks: four decades of evolution. *Nature Rev. Gen.* 6, 654–662
- Lagar, A., 1976. La cueva de Sa Campana y el karst de Castellots (Mallorca). *Biospeleología. Speleon* 22 (1975-1976), 69–72.
- Lo Presti, R.M., Oberprieler, C., 2011. The central Mediterranean as a phytodiversity hotchpotch: phylogeographical patterns of the *Anthemis secundiramea* group (Compositae, Anthemideae) across the Sicilian Channel. *J. Biogeogr.* 38, 1109–1124.
- Lorenz, W., 2005. Systematic list of extant ground beetles of the world (Insecta Coleoptera “Geadephaga”: Trachypachidae and Carabidae. incl. Paussinae, Cicindelinae, Rhysodinae). Lorenz, W., Tutzing.
- Magrini, P., Baviera, C., Vigna Taglianti, A., 2006. Note sul genere *Duvalius* in Sicilia con descrizione di due nuove specie (Coleoptera, Carabidae). *Fragm. entomol.* 38, 33–53.
- Marrone, F., Lo Brutto, S., Hundsdoerfer, A.K., Arculeo, M., 2013. Overlooked cryptic endemism in copepods: Systematics and natural history of the calanoid subgenus *Occidodiaptomus* Borutzky 1991 (Copepoda, Calanoida, Diaptomidae). *Mol. Phylogenet. Evol.* 66, 190–202.
- Martinsen, L., Venanzetti, F., Bachmann, L., 2009. Phylogeography and mitochondrial DNA divergence in Dolichopoda cave crickets (Orthoptera, Rhahidophoridae). *Hereditas* 146, 33–45.
- Mateu, J., 1955. Los Molopini Bon. de la Península Ibérica. *Eos* 31, 297–301.
- Médail, F., Quézel, P., 1999. Biodiversity hotspots in the Mediterranean basin: setting global conservation priorities. *Conserv. Biol.* 13, 1510–1513.
- Meulenkamp, J.E., Sissingh, W., 2003. Tertiary palaeogeography and tectonostratigraphic evolution of the Northern and Southern Peri-Tethys platforms and the intermediate

- domains of the African–Eurasian convergent plate boundary zone. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 196, 209–228.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans, pp 1–8.
- Molina-Venegas, R., Aparicio, A., José Pina, F., Valdés, B. & Arroyo, J., (2013) Disentangling environmental correlates of vascular plant biodiversity in a Mediterranean hotspot. *Ecol. Evol.* 3, 3879–3894.
- Mora, E., Paspati, A., Decae, A.E., Arnedo, M., 2017. Rafting spiders or drifting islands? Origins and diversification of the endemic trap-door spiders from the Balearic Islands, Western Mediterranean. *J. Biogeogr.* 44, 924–936.
- Moravec, P., Uéno, S.-I., Belousov, I.A., 2003. Tribe Trechini. In: Löbl, I., Smetana, A. (Eds.), *Catalogue of Palaearctic Coleoptera*, vol. 1, Archostemata, Myxophaga, Adephaga. Apollo Books, Stenstrup, pp. 288–346.
- Moreno Saiz, J.C., Castro Parga, I., Sainz Ollero, H., 1998. Numerical analyses of distributions of Iberian and Balearic endemic monocotyledons. *J. Biogeogr.* 25, 179–194.
- Pabijan, M., Crottini, A., Reckwell, D., Irisarri, I., Hauswaldt, J.S., Vences, M., 2012. A multigene species tree for Western Mediterranean painted frogs (*Discoglossus*). *Mol. Phylogenet. Evol.* 64, 690–696.
- Peyerimhoff, P. de, 1908. Nouveaux Coléoptères du Nord-Africain (sizième note: faune du Djurdjura). *Bull. Soc. Entomol. Fr.* 8, 117–125.
- Peyerimhoff, P. de, 1910. Nouveaux Coléoptères du Nord-Africain (onzième note: faune cavernicole du Djurdjura). *Bull. Soc. Entomol. Fr.* 8, 149–154.
- Pfenninger, M., Vela, E., Jesse, R., Elejalde M. A., Liberto, F., Magnin, F., Martínez-Ortí, A., 2010. Temporal speciation pattern in the western Mediterranean genus *Tudorella* P. Fischer, 1885 (Gastropoda, Pomatiidae) supports the Tyrrhenian vicariance hypothesis. *Mol. Phylogenet. Evol.* 54, 427–436.
- Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1. 6. 2014. Available at <<http://beast.bio.ed.ac.uk/Tracer>>.
- 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 Evol. Biol.* 10, 29.
- Rosenbaum, G., Lister, G.S. & Duboz, C., 2002. Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. *J. Virtual Explorer* 8, 107–130.

- Roveri, M., Flecker, R., Krijgsman, W., Lofi, J., Lugli, S., Manzi, V., Sierro, F.J., Bertini, A., Camerlenghi, A., de Lange, G., Govers, R., Hilgen, F.J., Hübscher, C., Meijer, P.T., Stoica, M., 2014. The Messinian Salinity Crisis: Past and future of a great challenge for marine sciences. *Marine Geol.* 352, 25–58.
- Sánchez-Robles, J.M., Balao, F., Terrab, A., García-Castaño, J.L., Ortiz, M.A., Vela, E., Talavera, S., 2014. Phylogeography of SW Mediterranean firs: Different European origins for the North African *Abies* species. *Mol. Phylogenet. Evol.* 79, 42–53.
- Sasakawa, K., Kubota, K., 2007. Phylogeny and genital evolution of Carabid beetles in the genus *Pterostichus* and its allied genera (Coleoptera: Carabidae) inferred from two nuclear gene sequences. *Ann. Entomol. Soc. Am.* 100, 100–109.
- Schettino, A., Turco, E., 2006. Plate tectonics of the Western Mediterranean region during the Oligocene and Early Miocene. *Geophys. J. Int.* 166, 1398–1423.
- Schmidt, J., Faille, A., 2015. Confirmation of occurrence of *Trechus* Clairville, 1806 in the Baltic amber forests, with description of a flightless edaphic species, and remarks on *Trechoides* Motschulsky, 1856 (Coleoptera: Carabidae: Trechini). *Zootaxa* 4028, 539–550.
- Schmidt, J., Belousov, I., Michalik, P., 2016b. X-ray microscopy reveals endophallic structures in a new species of the ground beetle genus *Trechus* Clairville, 1806 from Baltic amber (Coleoptera: Carabidae: Trechini). *ZooKeys* 614, 113–127.
- Schmidt, J., Hoffmann, H., Michalik, P., 2016a. Blind life in the Baltic amber forests: description of an eyeless species of the ground beetle genus *Trechus* Clairville, 1806 (Coleoptera: Carabidae: Trechini). *Zootaxa* 4083, 431–443.
- Serrano, J., 2013. New Catalogue of the Family Carabidae of the Iberian Península (Coleoptera). Servicio de Publicaciones de la Universidad de Murcia, Murcia.
- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stöck, M., Dubey, S., Klütsch, C., Litvinchuk, S.N., Scheidt, U., Perrin, N., 2008a. Mitochondrial and nuclear phylogeny of circum-Mediterranean tree frogs from the *Hyla arborea* group. *Mol. Phylogenet. Evol.* 49, 1019–1024.
- Stöck, M., Sicilia, A., Belfiore, N.M., Buckley, D., Lo Brutto, S., Lo Valvo, M., Arculeo, M., 2008b. Post-Messinian evolutionary relationships across the Sicilian channel: Mitochondrial and nuclear markers link a new green toad from Sicily to African relatives. *BMC Evol. Biol.* 8, 56.

- 764 Vigna Taglianti, A., 1980. Nouvelles données sur la systématique et la répartition
765 géographique des Coléoptères Carabiques cavernicoles et endogés du Proche-Orient
766 (Coleoptera, Carabidae). Mém. Biospéol. 7, 163–172.
- 767 Vigna Taglianti, A., 1982. Le attuali conoscenze sui Coleotteri Carabidi cavernicoli italiani.
768 Lav. Soc. ital. Biogeogr. (n.s.) 7(1078), 339–430.
- 769 Vigna Taglianti, A., 2005. Checklist e corotipi delle specie di Carabidae della fauna italiana.
770 Appendice B. In: Brandmayr, P., Zetto, T., Pizzolotto, R. (Eds.), I Coleotteri Carabidi
771 per la valutazione ambientale e la conservazione della biodiversità. APAT, Manuale
772 operativo 34/2005.I.G.E.R., Roma , pp. 186-225.
- 773 Vigna Taglianti, A., Casale, A., Fattorini, S., 2002. I carabidi della Sicilia ed il loro
774 significato biogeografico (Coleoptera, Carabidae). Boll. Accad. Gioenia Sci. Nat.
775 Catania35, 435–464.
- 776

Table 1

Evolutionary model and mitochondrial clock comparisons for the *Duvalius* lineage and Molopina, including AICM values. Best AICM values shown in bold, models that failed to converge adequately by a dash.

	evolutionary model nuclear	mitochondrial clock	AICM
<i>Duvalius</i>	GTR	strict	-
	HKY	strict	18283.3
	GTR	relaxed	-
	HKY	relaxed	18378.8
Molopina	GTR	strict	45275.5
	HKY	strict	45033.2
	GTR	relaxed	45006.4
	HKY	relaxed	45108.8

Figure legends

Fig. 1. Distribution of the subterranean species of the *Duvalius* lineage in the western Mediterranean.

Fig. 2. Distribution of the subterranean species of Molopina in the western Mediterranean. (except the epigeal genera *Percus* and *Abax*). 1, *Oscadytes*; 2, *Zariquieya*; 3, *Molopidius*; 4, *Henrotius*; 5, *Speomolops*; 6, *Typhlochoromus*; 7, *Henrotiochoromus*; 8, *Molops* (including *Tanythrix* and *Stenochoromus*). In red, hypogean genera.

Fig. 3. Phylogeny of the western Mediterranean species of the *Duvalius* lineage. Phylogram obtained with ML in RAxML. Numbers in nodes, bootstrap support. In red, highly modified troglomorphic species; in red bold species of the subgenus *Trechopsis*. See Table S1 for details on the specimens.

Fig. 4. Bayesian calibrated phylogeny of the *Duvalius* lineage. Ultrametric tree obtained with BEAST. Number inside nodes, estimated age; numbers above branches, posterior probability of the node. Dotted line marks the end of the Messinian salinity crisis 5.3 Ma. In red, highly modified troglomorphic species; in red bold species of the subgenus *Trechopsis*. See Table S1 for details on the specimens. Habitus photographs, A. Faille.

Fig. 5. Phylogeny of the western Mediterranean species of Molopina. Phylogram obtained with ML in RAxML. Numbers in nodes, bootstrap support. In red, subterranean species. See Table S1 for details on the specimens. Habitus photographs, A-C & H, F. Ciampor; D, R. Panin, E-G & I, A. Faille.

Fig. 6. Bayesian calibrated phylogeny of Molopina. Ultrametric tree obtained with BEAST. Number inside nodes, estimated age; numbers above branches, posterior probability of the node. Dotted line marks the end of the Messinian salinity crisis 5.3 Ma; purple band marks the tectonic opening of the western Mediterranean basin. In red, subterranean species. See Table S1 for details on the specimens.

Duvalius

Trechopsis

Agostinia, *Trichaphaenops*,
Arctaphaenops