Mediterranean diversification of the grass-feeding Anisopliina beetles (Scarabaeidae, Rutelinae, Anomalini) as inferred by bootstrap-averaged dispersal–vicariance analysis

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ABSTRACT

Aim The circum-Mediterranean region is one of the most complex regions of the Earth in terms of geography and natural history. The Old World species of the beetle subtribe Anisopliina (Scarabaeidae) feed almost exclusively on the pollen of grasses (Poaceae). Within this group, the ‘anisopliine clade’ forms a monophyletic group distributed mainly in the circum-Mediterranean region. Here, we reconstruct the biogeographical history of the anisopliine beetles in relation to the diversification of grasses, and compare this reconstruction with previous hypotheses concerning the evolution of the Mediterranean fauna and with palaeogeographical accounts of the history of this region.

Location The Mediterranean region, including North Africa, the Western Mediterranean, Balkans–Anatolia, Middle East and Caucasus.

Methods Dispersal–vicariance analysis (DIVA) was used to reconstruct ancestral distributions based on the morphological phylogeny and to infer the biogeographical processes that have shaped the observed distribution patterns. To account for phylogenetic uncertainty in the biogeographical reconstruction, we ran alternative ancestral distributions derived by DIVA over a sample of trees obtained by bootstrapping the original data set, reflecting the relative confidence of the ancestral areas on the various clades in the phylogeny.

Results The Eastern Mediterranean region and the Caucasus are inferred as the ancestral area of most of the anisopliine lineages. The Eastern Mediterranean region is also reconstructed as the source area of the majority of dispersal events, in particular towards North Africa and the Western Mediterranean. The Iberian Peninsula is inferred as part of the ancestral distribution of the anisopliine clade but also as the setting of several independent colonization events via both the North African platform (*Anthoplia*) and a European dispersal route (*Anisoplia*).

Main conclusions Our results confirm the role played by the Eastern Mediterranean as an evolutionary cradle of diversity for Mediterranean lineages. This can be explained by a recent and intense orogenic activity that might have promoted isolation and allopatric speciation within lineages. Both the Anomalini fossil record and the close association of anisopliine beetles with grasses suggest that the anisopliine clade originated in the Late Tertiary and that its spatial and temporal evolution within the Mediterranean Basin coincided with that of its major food source, the Mediterranean Poaceae.

Keywords

Biogeography, Coleoptera, dispersal–vicariance, diversification, grain beetles, grasses, Mediterranean Basin, phylogeography.

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INTRODUCTION

The Mediterranean Basin is considered one of 25 'hotspots' worldwide, defined as areas that possess a minimum of 0.5% of the world's plant species as endemics and that have experienced the loss of 70% or more of their primary vegetation (Myers *et al.*, 2000). Species endemism is very high: of about 25,000 known plant species, 13,000 are endemics (Ramos *et al.*, 2001, 2002). Three factors could be responsible for this endemism: an old and complex geological history (Krijgsman, 2002) leading to allopatric speciation and geographic vicariance; the role of the Mediterranean Basin as a place of climatic refugia during Pleistocene glaciations (Hewitt, 2004); and human activity leading to range fragmentation and diversification (Ehrhardt & Thomas, 1991; Phipps, 1991; Galante, 2005). In addition, palaeogeographical reconstructions indicate that this region conforms to what has been termed a 'reticulate' biogeographical scenario, in which the constituent landmasses have repeatedly split, collided, and split again in different configurations over time (Sanmartín *et al.*, 2001). For instance, 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; see Fig. 1), which has resulted in repeated episodes of vicariance and dispersal affecting the same areas at different times (Oosterbroek & Arntzen, 1992; Sanmartín, 2003). This complex palaeogeographical history is probably responsible for the high endemism and level of diversification of the Mediterranean region. Many Mediterranean taxa exhibit east/west (amphi) Mediterranean disjunct distributions, whereas a high number of endemics can be found in Northwest Africa, Iberia, Balkans–Anatolia, the Middle East, and the trans-Caucasus (Oosterbroek & Arntzen, 1992; Ribera & Blasco-Zumeta, 1998; Martín-Piera & Sanmartín, 1999; Veith *et al.*, 2003).

The beetle subtribe Anisopliina (Scarabaeidae: Rutelinae: Anomalini) comprises approximately 100 species and nine genera distributed in the Palaearctic, Oriental, Ethiopian,

Nearctic and Neotropical biogeographical regions. Recently, Jameson *et al.* (2007) reviewed the taxonomy and systematics of the Anisopliina and concluded that the subtribe is not monophyletic but that there is strong phylogenetic support for an 'anisopliine clade' comprising mainly Mediterranean taxa. This clade is composed of species of the genera *Anisoplia* Schönherr, *Anthoplia* Medvedev, *Brancoplia* Baraud, *Chaetopteropia* Medvedev and *Hemichaetoplia* Baraud, with the oriental genus *Tropiorhynchus* Blanchard as the basal sister taxon to the anisopliine clade (Jameson *et al.*, 2007).

One of the most important features of the biology of anisopliines is their close association with grasses. Commonly known as 'grain beetles' or 'grass-feeding beetles', the anisopliines feed almost exclusively on grass pollen and maturing grass seeds as adults, and on grass roots as larvae (Machatschke, 1957; Hurpin, 1962; Micó, 2001). Adults are reported from a wide variety of non-cultivated and also cultivated grass species (see Table 1). Like other phytophagous scarab beetles (Sanmartín & Martín-Piera, 2003), species of Anisopliina are poor flyers (Micó & Galante, 2000; Micó, 2001) and have small geographical ranges, which make them a suitable group through which to study the relative importance of palaeogeography in the shaping of biogeographical patterns (Sanmartín, 2003).

Here, we reconstruct the biogeographical history of the Mediterranean anisopliine clade using an analytical method, dispersal–vicariance analysis (DIVA), which allows the reconstruction of reticulate biogeographical history (Ronquist, 1997). Because phylogenetic uncertainty is likely to affect biogeographical reconstructions (Ronquist, 2002; Nylander *et al.*, 2008), we inferred anisopliine ancestral ranges and related biogeographical events using a bootstrap sample of trees, reflecting the relative confidence of the alternative ancestral distributions derived by DIVA on the various clades in the phylogeny. In particular, the aims of the study were: (1) to reconstruct the biogeographical history of the monophyletic anisopliine clade; (2) to compare our reconstruction with previous biogeographical hypotheses concerning the Mediterranean Region; and (3) to interpret the origin and diversifi-

Figure 1 Areas of distribution of the subtribe Anisopliina in the Mediterranean region as defined in this study. A, North Africa; B, Iberian Peninsula; C, Central Mediterranean; D, Eastern Mediterranean; E, Middle East; F, Caucasus; G, Central Europe; H, Eastern Europe and Western Siberia. Dashed arrow lines show the main vicariance/palaeogeographical events in the region, based on Dercourt *et al.* (1986) and Oosterbroek & Arntzen (1992).

Table 1 Summary of the known Anisopliina–plant associations, showing the affinities of anisopliine taxa for Poaceae species.

Anisopliine taxa	Plant host	Reference
<i>Anisoplia</i> (Au.) <i>austriaca</i> (Herbst)	POACEAE: <i>Triticum</i> sp.	Özder, 2002
<i>Anisoplia</i> (A.) <i>agricola</i> (Poda)	‘Grasses’	Hurpin, 1962
<i>Anisoplia</i> (A.) <i>baetica</i> Erichson	POACEAE: <i>Avena barbata</i> Pott ex Link, <i>Agropyron repens</i> (L.) Beauv., <i>Bromus hordeaceus</i> L., <i>Cynosorus cristatus</i> L., <i>Dactylis glomerata</i> L., <i>Festuca ovina</i> L., <i>Festuca elegans</i> Boiss., <i>Festuca indigesta</i> Boiss., <i>Holcus mollis</i> L., <i>Holcus lanatus</i> L., <i>Koeleria caudata</i> (Link) Steudel, <i>Lolium perenne</i> L., <i>Stipa gigantea</i> Link, <i>Brachypodium phoenicoides</i> (L.) Roemer et Schultes, <i>Hordeum murinum</i> L.	De los Mozos Pascual & Martin Cano, 1988 De los Mozos Pascual, 1989 Galante, 1981 Micó, 2001 Tomás-Biosca & Galante, 1978
<i>Anisoplia</i> (A.) <i>dispar</i> Erichson	FABACEAE: <i>Psoralea bituminosa</i> L.	
<i>Anisoplia</i> (A.) <i>flavipennis</i> Brullé	non-cultivated grasses	Simandl, 2002
<i>Anisoplia</i> (A.) <i>remota</i> Reitter	POACEAE: <i>Triticum</i> sp.	Özder, 2002
	POACEAE: <i>Brachypodium retusum</i> (Pers.) Beauv., <i>Piptaterum miliaceum</i> (L.) Cosson, <i>Poa angustifolia</i> L., <i>Dactylis hispanica</i> Roth	Micó, 2001
	UMBELLIFERAE: <i>Foeniculum vulgare</i> Mill.	
<i>Anisoplia</i> (A.) <i>villosa</i> (Goeze)	POACEAE: <i>Bromus</i> sp.	Janssens, 1960
<i>Anthoplia</i> <i>floricola</i> (Fabricius)	POACEAE: <i>Alopecurus pratensis</i> L., <i>Avena</i> sp., <i>Bromus hordeaceus</i> L., <i>Bromus rigidus</i> Roth, <i>Dactylis glomerata</i> , <i>Festuca ovina</i> , <i>Gaudinia fragilis</i> , <i>Hordeum stigmum</i> L., <i>Vulpia myuros</i> (L.) C. C. Gmelin, <i>Hordeum murinum</i> L., <i>Brachypodium retusum</i> (Pers.) Beauv., <i>Dactylis hispanica</i> Roth	Micó, 2001 De los Mozos Pascual, 1989
	UMBELLIFERAE: <i>Foeniculum vulgare</i> Mill.	
	BORAGINACEAE: <i>Echium humile</i> Desf.	
<i>Brancoplia</i> <i>leucaspis</i> (Laporte)	POACEAE: <i>Triticum</i> sp.	Hurpin, 1962
<i>Chaetopteropia</i> <i>segetum</i> (Herbst)	POACEAE: non-cultivated grasses and <i>Triticum</i> sp., <i>Secale</i> sp. and <i>Zea</i> sp.	Hurpin, 1962
<i>Chaetopteropia</i> <i>syriaca</i> Burmeister	POACEAE: <i>Triticum</i> sp., <i>Haynaldia villosa</i> Schur, <i>Aegilops cylindrica</i> Schur, <i>Festuca orientalis</i> Kern. ex Hack., <i>Koeleria</i> sp., <i>Dactylis glomerata</i> L., <i>Bromopsis riparia</i> (Rehm.) Holub, <i>Elytrigia repens</i> Desv.	Apostolov & Maltzev, 1986 Özder, 2002

cation of the Mediterranean Anisopliina in the light of the diversification of grasses within this region.

MATERIALS AND METHODS

Taxa and areas

The anisopliine clade comprises 70 species distributed in five genera (Jameson *et al.*, 2007). *Anisoplia* is the most species-rich and most widespread genus in the clade, with over 50 species; this mainly Eurasian genus is absent from North Africa (Machatschke, 1961) (see Appendix S1 in the Supporting Information). The monospecific genus *Anthoplia* is known from North Africa (Morocco, Algeria, Tunisia and north-western Libya) and the Iberian Peninsula (Baraud, 1992) (see Appendix S2). The last three genera (*Brancoplia*, *Chaetopteropia* and *Hemichaetoplia*) comprise 20 species in total (four, twelve and four, respectively) and are distributed mainly in the Middle East region (*Brancoplia* and *Chaetopteropia*) and in the Eastern Mediterranean region and North Africa (all three genera), with the exception of *Chaetopteropia sege-*

tum (Herbst), which is widely distributed in Eurasia, and *Brancoplia leucaspis* Laporte, which is present also in the Caucasian region (Baraud, 1986) (see Appendix S2).

Following Jameson *et al.*'s (2007) original analysis, a total of 28 species were included in the analysis, representing all five anisopliine genera, as well as the subgenera *Anisoplia*, *Autanisoplia* Medvedev and *Pilleriana* Baraud (Baraud, 1986, 1991) (subgenus *Pilleriana* has recently been rejected by Jameson *et al.*, 2007) and all species-groups within *Anisoplia* (the ‘agricola’, ‘zwicki’, ‘deserticola’, ‘tempestiva’, ‘signata’, ‘monticola’ and ‘villosa’ groups), with the exception of the ‘lodosi’ group for which no specimens were available. Within each genus, subgenus and species-group, species were selected to include representatives of all areas of distribution where the group is present. The only exception was the genus *Chaetopteropia*, for which no representatives from the Middle East could be sampled (see Appendix S2). Detailed distribution maps of each genus, subgenus and species-group are given in Appendices S1 and S2.

Following Sanmartín (2003), we defined the areas of endemism used in the analysis as congruent distributional ranges (sympatric distributions) shared by two or more species

but also delimited by geological features/vicariance events that historically could have acted as barriers to dispersal; for example, the Iberian Peninsula (area B, Fig. 1) is delimited by the Pyrenees mountain barrier in the north and the Strait of Gibraltar in the south. In addition, because anisopliine species are phytophagous beetles closely associated with grasses, Takhtajan's (1986) floristic regions of the world were considered in the definition of area boundaries.

A total of eight areas (A–H) were included in the analysis (Fig. 1), as follows.

North Africa (A)

This area includes Morocco, northern Algeria, most of Tunisia, and north-western Libya. There are three endemic anisopliine species occurring in this region [*Hemichaetoptelia lanata* (Escalera), *H. peyerimorhoffi* (Baraud) and *H. pallidipennis* (Gyllenhal); see Appendix S2].

Iberian Peninsula (B)

This region is separated from the rest of Europe by the Pyrenees (Fig. 1). This mountain chain, which formed 10 Ma, has been shown to be an important dispersal barrier for many groups of plants and animals (see Takhtajan, 1986; Oosterbroek & Arntzen, 1992; Martín-Piera & Sanmartín, 1999; and references therein). There are two species of *Anisoplia* endemic to the Iberian region: *Anisoplia depressa* Erichson and *Anisoplia baetica* Erichson. They exhibit the pattern of east/west vicariant disjunction (see Appendix S1) observed in several other Mediterranean beetle lineages (Sanmartín, 2003).

Central Mediterranean (C)

This area extends from Italy (including the islands of Corsica, Sardinia and Sicily) to the Iberian Peninsula, with the Pyrenees as the western boundary (Takhtajan, 1986) and the Balkans as the eastern boundary. During the Early Miocene, 20–15 Ma (Rögl & Steininger, 1983), a marine transgression isolated this area from the Balkans and Asia Minor (Fig. 1). Very few species occur in this area and none of them is endemic (see Appendix S1). This seems to be a common pattern in Mediterranean scarabs (Sanmartín, 2003).

Eastern Mediterranean (D)

This area includes the Balkan Peninsula, the Aegean Islands, Crete, Rhodes, the western shores of Anatolia, and the Mediterranean shores of Syria, Lebanon, Israel and Jordan (Fig. 1). The genus *Anisoplia* shows its highest species diversity in this region: six of the seven 'species-groups' defined by Baraud (1991) are represented in the Eastern Mediterranean region, together accounting for more than 50% of all *Anisoplia* species. Some species-groups, such as 'zwicky', 'deserticola' and 'agricola', are entirely restricted to the western part of this region (the Balkans and Aegean islands; see Appendix S1),

whereas the Mediterranean shores of Syria to Jordan harbour the largest number of anisopliine genera: *Chaetopteroptelia*, *Brancoplia*, *Hemichaetoptelia* and *Anisoplia* are all represented here (see Appendices S1 and S2).

The Balkans and European Turkey are considered here as one region because the Bosphorus Strait was not opened until the end of the Pliocene, and the two areas share many species of Anisopliina (see Appendices S1 and S2) and in general a high faunistic similarity (Cheylan, 1995; Sanmartín, 2003). By contrast, the Central Anatolian province (i.e. inner arid and semiarid parts of Anatolia, including the Anatolian Plateau) was excluded from this area because it shares most of its anisopliine species with the Middle East region, within which it was included (Fig. 1: E, see below). Moreover, Takhtajan (1986) considered the floristic composition of the Central Anatolian province as transitional between the Eastern Mediterranean and the typical 'Irano-Turanian' flora.

Middle East (E)

This area includes the northern part of the Arabian plate, Iraq, northern Saudi Arabia, the inner and southern parts of Anatolia (Central Anatolian Province, see above), and the Armeno-Iranian Province (Takhtajan, 1986). There are two species of *Chaetopteroptelia* endemic to this region (see Appendix S2), which coincides with the western part of the Irano-Turanian floristic region defined by Takhtajan (1986). The Mediterranean shores of Syria to Jordan form the western boundary of this region.

Caucasus (F)

This area comprises the mountain systems of the Greater and Lesser Caucasus, the Euxine Province – embracing the western Caucasus and northern Anatolia and the Black Sea shores of European Turkey – and the Hyrcanian Province (i.e. the south-western and southern Caspian coastal plains of Lenkoran in south-eastern Transcaucasia, and the coastal plains of Gilan and Mazandaran in Iran), as defined by Takhtajan (1986). Within the Anisopliina, the Caucasus region shares at least three endemics with the Hyrcanian province and one endemic with the Euxine province. This is the second most important area for the genus *Anisoplia* in terms of species diversity, with four species-groups occurring in this region, which represent 30% of all *Anisoplia* species. All eight species of the 'signata' group, with the exception of *A. pubipennis* Blanchard (see Appendix S1), are endemic to this region. In contrast, the 'tempestiva', 'monticola' and 'villosa' groups have few endemics in this region (see Appendix S1), and the genera *Brancoplia* and *Chaetopteroptelia* are represented here by one species each (see Appendix S2).

Central Europe (G)

This is defined as the area located above the Central Mediterranean and Balkan regions (modified from Takhtajan,

1986). There are no endemic species in this area, but *Chaetopteroplia segetum* and some species of *Anisoplia* extend their ranges into this region.

Eastern Europe and Western Siberia (H)

This is defined as the area from the eastern part of the Baltic Republics to broad western Siberia and extending to the east up to the Yenisey River (Fig. 1). In the south, this region stretches to the northern shores of the Black Sea and the semi-deserts of Kazakhstan. Species of *Anisoplia*, such as *A. zwicki* Fischer and *A. campicola* Ménétériès, are nearly endemic to this region, and other species of *Anisoplia* (*A. deserticola* Fischer, *A. agricola* Poda) and *C. segetum* extend their ranges into this area (see Appendices S1 and S2).

Two additional non-Mediterranean areas, the 'Oriental' region (O) and the 'Neotropics' (NT), were used in the analysis to represent the distribution of the outgroup taxa (*Tropiorhynchus* and *Callirhinus* Blanchard, respectively; see below), but they are not considered in the discussion of the results.

Phylogenetic hypothesis

Jameson *et al.* (2007) analysed phylogenetic relationships within the subtribe Anisopliina based on 91 characters derived from the external and genital morphology. The original data set of Jameson *et al.* (2007) included 18 Old and New World outgroup taxa (including the subtribes Popilliina and Anomalina), as well as 34 ingroup taxa from the Anisopliina, representing 34% of the diversity of the group.

To examine the biogeography of the anisopliine clade (as defined in Jameson *et al.*, 2007), we re-analysed the data set of Jameson *et al.* (2007) using the genera *Tropiorhynchus* and *Callirhinus* as outgroup taxa. According to Jameson *et al.* (2007), these taxa are well-supported outgroups of the anisopliine clade. A total of 31 taxa were included in our analysis: 28 ingroup taxa belonging to the anisopliine clade and the three outgroups; with the remaining 21 taxa pruned from Jameson *et al.*'s original character matrix. To reconstruct phylogenetic relationships within the anisopliine clade, we carried out maximum parsimony searches as implemented in PAUP* ver. 4.0b10 (Swofford, 2002) with the following settings: heuristic search 200 random taxon addition replicates and tree bisection–reconnection (TBR) branch swapping (Swofford, 2002). This resulted in 76 equally parsimonious trees [consistency index (CI) = 0.5031; homoplasy index (HI) = 0.4969; retention index (RI) = 0.6836; rescaled consistency index (RC) = 0.3439], whose strict consensus is shown in Fig. 2. An inspection of the character consistency index revealed high levels of homoplasy in several characters. In order to reduce the level of homoplasy in the data set and to increase resolution in the phylogenetic hypothesis, we used a successive weighting strategy (Farris, 1969) based on the maximum value of the rescaled consistency index and a base weight of 1000 in PAUP*. Character weighting in phylogenetic reconstruction has

been criticized for unjustifiable parsimony assumptions (Kluge, 1997) and for decreasing the number of well-supported groups in molecular phylogenetic reconstruction (Källersjö *et al.*, 1999). Recently, however, Goloboff *et al.* (2008) demonstrated that downweighting characters according to their homoplasy – using properly rescaled functions – can actually increase nodal support and improve phylogenetic resolution in morphological (and even molecular) data sets. Moreover, results obtained under homoplasy weighting appear more stable to the addition of new taxa or characters than those obtained under equal weights (Goloboff *et al.*, 2008). In our analysis, stability was reached in the second iteration and resulted in six equally parsimonious trees (CI = 0.8009; HI = 0.1991; RI = 0.8784; RC = 0.7035; see Appendix S3), which were also part of the 76 trees obtained in the unweighted analysis. A strict consensus of the six successive reweighted trees (RWTs) is shown in Fig. 2. We also evaluated nodal support and data consistency using a non-parametric bootstrap approach (Felsenstein, 1985) with 200 bootstrap pseudoreplicates per analysis, each with 10 random taxon addition replicates, 'maxtrees' set at 5000, and characters weighted according to homoplasy levels (see above; Fig. 2).

Dispersal–vicariance analysis

Dispersal–vicariance analysis (Ronquist, 1997), as implemented in DIVA ver. 1.1 (Ronquist, 1996), was used to reconstruct ancestral distributions on the phylogeny of the anisopliine clade. DIVA optimizes distributions for each node of the tree, assuming allopatric speciation by vicariance as the null model and minimizing the number of inferred dispersals and extinction events under a parsimony criterion (Ronquist, 1997). When using DIVA to reconstruct the biogeographical history of a group of organisms, optimizations become less reliable as we approach the root node (Ronquist, 1997). In order to solve this problem, we imposed constraints on the maximum number of unit areas allowed in ancestral distributions, setting this number to the maximum number of areas in the most widespread extant descendant (*C. segetum*) is found (i.e. four). In practice, this means assuming that the dispersal ability of the ancestors was not higher than that of the descendants (Sanmartín, 2003). Because most widespread species of Anisopliina are present in three areas, we repeated the analysis with three areas as the maximum area constraint; the results were very similar to those obtained with the four-area constraint and are not shown here.

Most current methods of biogeographical inference, including DIVA (Ronquist, 1996), reconstruct biogeographical patterns and ancestral distributions on a fixed, fully resolved tree topology (i.e. a binary tree), assuming that phylogenetic relationships are known without error. This is especially problematic in groups where relationships are ambiguous owing to low phylogenetic signal or to incongruity between different character sets, reflected as polytomic relationships. Moreover, some nodes are likely to be more strongly supported than others, and this degree of uncertainty should

Figure 2 Strict consensus tree of six equally parsimonious trees resulting from successive approximation based on Jameson *et al.*'s (2007) data set; bootstrap support is shown above the branches. The dashed lines correspond to phylogenetic relationships recovered in the strict consensus of the 76 trees obtained before reweighting (see text); corresponding bootstrap support is shown within squares below the branches.

be reflected in the biogeographical inference. For example, within the anisopliine clade, phylogenetic uncertainty is highest in the distal part of the tree (Fig. 2), affecting relationships among and within *Anisoplia* species-groups. One way to account for phylogenetic uncertainty in biogeographical analysis is to infer ancestral areas/biogeographical events based on a sample of trees reflecting the relative confidence of the ancestral areas on the various clades in the phylogeny (Ronquist, 2002). Here, we followed a similar approach to the one used by Ronquist & Liljeblad (2001): we used parsimony bootstrapping to generate a set of trees describing the uncertainty in the phylogenetic hypothesis and then reconstructed the biogeographical history of the group in each of these trees. Specifically, we generated a sample of trees by bootstrapping the original data set 10,000 times in PAUP*, sampling characters with equal frequency but weighting them according to the values given by the successive weighting

analysis (Farris, 1969; see above). Only one tree was saved from each bootstrap analysis (i.e. no TBR swapping), using the 'Fast Bootstrap' option in PAUP*, and zero-length branches were not collapsed in order to obtain fully binary trees. The fast bootstrap option is less exact than the heuristic alternative but has been shown to be more efficient in searching between multiple islands of trees (i.e. groups of trees with similar topologies; Felsenstein, 1985). Using scripts kindly provided by Johan Nylander (Nylander *et al.*, 2008), we ran DIVA analyses on each individual tree from the bootstrap pseudoreplicate sample, and summarized/averaged ancestral area reconstructions across all trees for each given node in the reference tree. The reference tree was the majority rule consensus of the six trees obtained by successive weighting. When there were several equally parsimonious reconstructions at a given node (e.g. A/B/AB), these were downweighted by $1/n$, where n was the total number of alternative reconstructions at the node.

Thus, for each node we showed the phylogenetic uncertainty (bootstrap nodal support) in the rest of the tree and the uncertainty in the biogeographical reconstruction (i.e. the existence of multiple equally parsimonious reconstructions in *drva*) (see Nylander *et al.*, 2008, for a more detailed explanation of this approach).

RESULTS AND DISCUSSION

Anomalini fossil record and the diversification of grasses (Poaceae)

Before discussing the spatial evolution of the anisopliine clade, it is necessary to provide some framework for its temporal evolution. There is no known fossil record of the subtribe Anisopliina. Nonetheless, there are several fossils assigned to the tribe Anomalini (to which the Anisopliina belongs) and to the subfamily Rutelinae (to which Anomalini belongs) that may help us to establish a temporal framework for the evolution of the anisopliine clade (Fig. 3a). In the most complete catalogue of fossil Scarabaeoidea to date, Krell (2007) cites several fossils of Rutelinae from the Early–Late Tertiary. The oldest fossil record corresponds to the extinct genus *Pelidnotites* from the Middle–Early Eocene (50–42 Ma) of England, whose morphology closely resembles that of genus *Pelidnota* L., belonging to the tribe Rutelini. This is the sister-group of tribe Anomalini according to Jameson *et al.*'s (2007) phylogeny (Fig. 3a). In addition, Krell (2000, 2007) cites several fossil species of *Anomala* from the Late Oligocene of Germany, which correspond to extinct taxa (e.g. *Anomala primigenia* Heyden & Heyden); the oldest fossil record of *Anomala* corresponds to the Early Oligocene of the USA (e.g. *Anomala scudderii* Wickham). Based on this fossil evidence, Krell (2006) estimates that subfamily Rutelinae, including the Anomalini tribe, originated not earlier than the Eocene, in contrast to the much older age (Early Cretaceous) of the subfamily Melolonthinae, the other major group of phytophagous Scarabaeinae beetles. Similarly, the minimum age of tribe Anomalini is dated as at least Early Oligocene, 34 Ma (Krell, 2000, 2006; Fig. 3a,b).

In addition, the close association of anisopliine beetles with the 'true grasses', family Poaceae (see Table 1 and Fig. 3a), might further help us to establish a possible origin for the group. There is apparently no specificity between anisopliine species and their Poaceae hosts, as evidenced by the literature and personal observations (summarized in Table 1). Most anisopliine species feed on several grass species, sometimes belonging to different tribes – for example, *Anisoplia baetica* feeds on *Avena* (Aveneae) and *Agropyron* (Triticeae) – or even to different subfamilies of Poaceae – for example *Anisoplia remota* Reitter feeds on *Piptatherum* (Stipoideae) and *Poa* (Pooideae) (Table 1). Host affinity is more probably determined by plant availability and by the ecological (climatic) requirements of the host plant. On the other hand, feeding on grass pollen and seeds seems to be a synapomorphy of the anisopliine clade within the tribe Anomalini (Jameson *et al.*,

2007). Jameson *et al.* (2007) cite several character modifications in mouthpart and leg morphology that are apparently associated with grass pollinivory and grammivory (e.g. the elongated and recurved clypeal apex) and with the grasping of grass stems (e.g. pseudotarsomere associated with protarsomere 5). These character states seem to be derived within the Anomalini tribe and they are generally characteristic of the anisopliine clade (Jameson *et al.*, 2007; see Fig. 3a). Other closely related Anomalini genera, such as *Callirhinus* and *Blitopertha* Reitter, are leaf feeders or general pollen feeders (Fig. 3a). It is thus reasonable to hypothesize that the diversification of the anisopliine clade was at least partly triggered by their evolutionary shift (trophic specialization) to feeding on grass pollen and seeds.

Grass pollen and flowers do not appear in the fossil record until between 60 and 55 Ma (Kellogg, 2000), and it was not until after 24 Ma that grasses became the dominant species in many ecosystems (Vaughan *et al.*, 2005) (see Fig. 3b). Based on new molecular evidence, Inda *et al.* (2008) estimated that grasses began to radiate 60.2 ± 5.2 Ma, and that the separation of Triticeae from Aveneae–Pooeae (three Poaceae tribes exploited by anisopliine species; see Table 1) took place 20.9 ± 4.9 Ma. Anisopliine beetles feed on Poaceae subtribes Cynosurinae Fr., Dactylidinae Stapf. and Poinae Durmot., and on tribes Aveneae Durmot. and Brachypodieae Harz, as well as on the most derived species of subtribe Loliinae, including *Festuca* species (Table 1). Inda *et al.* (2008) showed that the subtribe Loliinae – one of the largest groups of temperate grasses, containing the genus *Festuca* (500 species) – originated in the pan-Mediterranean–Southwest Asian region around the Middle Miocene (13 Ma, Fig. 3b). This is also the area of distribution of the anisopliine clade (Fig. 1). Hence, until additional fossils can provide a more accurate dating, we can date the origin of the anisopliine clade to the period of the Late Tertiary (Miocene), which is close to or at the same time as the expansion of its host plants, the Mediterranean Poaceae (Fig. 3a,b).

Biogeographical history of the Mediterranean Anisopliina

There is considerable uncertainty in the results from the *drva* (Fig. 4), reflecting both phylogenetic uncertainty and uncertainty in the ancestral area reconstruction. Still, some conclusions can be reached.

Although ancestral area reconstructions are ambiguous, *drva* reconstructs the ancestor of the anisopliine clade as widespread in the Mediterranean region, including the Iberian Peninsula and the Eastern Mediterranean, combined with North Africa or the Caucasian region, with the Eastern Mediterranean as part of the three most frequent reconstructions (node 1, Fig. 4). The first vicariance event in the anisopliines isolated the monotypic genus *Anthoplia* in the North African–Iberian region from the ancestor of the *Anisoplia* lineage in the Eastern Mediterranean region (Fig. 4). How can this first vicariance event be explained, and how did

Figure 3 Temporal framework for the evolution of the anisopline clade, showing: (a) the fossil record of Rutelinae (Krell, 2000, 2007), main vicariance events and feeding habits mapped onto a phylogenetic tree modified from Jameson *et al.* (2007); and (b) a summary of the main evolutionary events within grasses (Poales) based on Inda *et al.* (2008).

Figure 4 Summary of the optimal reconstructions of ancestral distributions of the anisopline clade using dispersal–vicariance analysis (DIVA) while accounting for phylogenetic uncertainty. The phylogenetic hypothesis is the majority rule consensus of the six trees obtained by successive weighting (see Fig. 2). Pie charts at internal nodes represent frequencies of alternative ancestral distributions derived by DIVA over a distribution sample of trees obtained by bootstrapping the original data set. For each node in the tree, the charts represent the phylogenetic uncertainty (bootstrap nodal support) in the rest of clades and the biogeographical uncertainty (multiple equally parsimonious reconstructions) in the node of interest. In each pie chart, colours represent the frequency with which ancestral areas are inferred across the sample of trees: white corresponds to the area inferred with the highest relative frequency; red, blue and grey represent, respectively, the second, third and fourth most frequently inferred areas; black represents the remaining areas, usually with frequencies below 0.01. The area with the highest frequency is also given in bold. The main palaeogeographic/vicariance events discussed in the text are marked with arrows. Area codes for taxa distributions are as in Fig. 1. Abbreviations for *Anisoplia* sections (Baraud, 1986, 1991): (Au), subgenus *Autanisoplia*; (A), subgenus *Anisoplia*; (P), subgenus *Pilleriana*; a-gp, ‘agricola’ species-group; d-gp, ‘deserticola’ species-group; z-gp, ‘zwicki’ species-group; t-gp, ‘tempestiva’ species-group; s-gp, ‘signata’ species-group; v-gp, ‘villosa’ species-group; m-gp, ‘monticola’ species-group.

the ancestor of anisopliines come to occupy such a disjunct distribution? One possibility is that the ancestor of the anisopliine clade was originally distributed in both the northern and southern Peri-Tethys platforms but became extinct in North (Central) Africa following the increasing aridification of the region starting in the Miocene, which led to the formation of the Saharan desert (2.5 Ma) and presumably extirpated many Mediterranean lineages in central North Africa (Sanmartín, 2003; Oberprieler, 2005). Alternatively, palaeogeographical reconstructions (Rögl & Steininger, 1983) indicate the existence of a land connection between the Balkans–Anatolia and the Western Mediterranean region during the Late Oligocene (25 Ma). Given the time frame suggested above for the origin of the anisopliine clade, the first vicariance event postulated by DIVA could be related to the breaking of this connection: the isolation of the Western Mediterranean region from the Balkans and Asia Minor as the result of a marine transgression between the Tethys and Paratethys 20–15 Ma (Oosterbroek & Arntzen, 1992; Gómez-Zurita, 2004) (see Figs 1 & 3). A third possibility is that the ancestor was originally restricted to one or a few areas, such as the Eastern Mediterranean, and that the inferred, relatively vague widespread distribution is the result of the uncertainty in ancestral area reconstruction and the tendency of DIVA to infer widespread distributions at basal nodes (Sanmartín, 2007). Here, the reconstruction of the root node is strongly influenced by the phylogenetic position of the *Anthoplia* lineage and its sole species, *A. floricola* (F.), distributed in Northwest Africa and the Iberian Peninsula (Fig. 4). On the other hand, this position is strongly supported in the phylogeny (100% bootstrap support; see Fig. 2). Moreover, a relatively widespread east–west ancestral distribution has been inferred for other groups of circum-Mediterranean animals (Sanmartín, 2003) and plants (Oberprieler, 2005).

The monotypic genus *Anthoplia* is widespread in Northwest Africa and the Iberian Peninsula (areas A + B). The 'Ibero-Mauritanian' plate has been a fertile matrix for differentiation in many groups of plants and animals (Blondel & Aronson, 1999). The Iberian microplate was part of Africa from the Late Cretaceous until the Eocene, when it began to move northwards, eventually colliding with Eurasia in the Late Eocene (35 Ma) (Sanmartín, 2003). Three palaeogeographical events could explain the disjunct distribution of *Anthoplia*: (1) a collision between southern Iberia and the Maghrib about 15 Ma, which would have caused land emergence, allowing dispersal between the two sides of the Gibraltar Strait (Palmer & Cambefort, 2000); (2) the partial desiccation of the Mediterranean during the Messinian Salinity Crisis at the end of the Miocene (5.96 Ma, Krijgsman, 2002), which would have established a land bridge between Northwest Africa and the Iberian Peninsula, allowing circum-Mediterranean lineages to reach the northern Tethys platform (Sanmartín, 2003; Oberprieler, 2005); and (3) land connections across the Gibraltar Strait during the Late Pliocene and Pleistocene glacial periods, which would have allowed the dispersal of populations between Iberia and Northwest Africa, as shown in

other animal lineages (e.g. Veith *et al.*, 2003). Given that *Anthoplia* populations on both sides of the Gibraltar Strait are currently recognized as a single species (*Anthoplia floricola*), it seems more plausible that the current disjunct distribution of *Anthoplia* is the result of one of the two more recent (Messinian or Pleistocene) connection events across the Gibraltar Strait.

The Eastern Mediterranean (D) region is inferred as the most likely ancestral area of the genus *Anisoplia* (node 2, Fig. 4), with two important centres of diversification, namely the Eastern Mediterranean (Balkans, West Anatolia and Levante) and the Caucasus regions. The Eastern Mediterranean is also inferred as the ancestral area of the subgenus *Autanisoplia* (node 3, Fig. 4), and as the most probable ancestral area of the subgenus *Anisoplia* (node 4, Fig. 4). The *Anisoplia* clade containing the 'agricola', 'deserticola' and 'zwicki' species-groups also apparently originated in the Eastern Mediterranean region (node 5, Fig. 4).

Ancestral area reconstructions for the other *Anisoplia* clades are more ambiguous (Fig. 4), and this part of the phylogeny is not well supported in the bootstrap analysis (Fig. 2). The sister-clade to the 'agricola–zwicki' *Anisoplia* clade is reconstructed as of probable Caucasian origin (node 6, Fig. 4). The Caucasus region appears also as the most probable ancestral area for several lineages, including *A. campicola* and the 'signata' species-group (Fig. 4). The isolation of Caucasian lineages from Eastern Mediterranean ancestors could be explained by the restoration of marine conditions between the Tethys and Paratethys in the Middle Miocene (17–13 Ma; see Fig. 1), which isolated East Asia Minor from Western Asia Minor and the Balkans region (Oosterbroek & Arntzen, 1992; Gómez-Zurita, 2004). With the exception of *A. pubipennis*, all species in the 'signata' group (Baraud, 1986, 1991) are endemic to the Caucasus region (see Appendix S1). The internal sac morphology of this group is characterized by the absence of the 'odd sac', whereas *A. pubipennis* presents the odd sac characteristic of the 'villosa' group (Micó & Galante, 2000). Interestingly, our phylogeny includes *A. pubipennis* within the 'villosa' group. Thus, the traditional classification of *A. pubipennis* into the 'signata' group is rejected by internal sac morphology, phylogenetic relationships and geographic distribution.

From this point onwards, the most probable ancestral area reconstruction suggests several instances of dispersal between the Eastern and the Western Mediterranean regions (Iberian Peninsula), although phylogenetic support is low in this part of the tree (Fig. 2). *Anisoplia remota* and *A. baetica* are distributed in the Iberian Peninsula, whereas the geographically heterogeneous clade *A. pubipennis*–*C. syriaca* (66% bootstrap support, Fig. 2) is reconstructed as of probable Eastern Mediterranean origin (node 7, Fig. 4). The well-supported clade *A. bromicola* (Germar) + *A. villosa* Goeze (80% bootstrap support, Fig. 2) suggests a dispersal event from Central Europe (node 8, Fig. 4) to the Central–Western Mediterranean region in *A. villosa*.

Jameson *et al.* (2007) demonstrated that the genera *Hemichaetoptilia*, *Chaetopteroptilia* and *Brancoplia* form an internal

clade within the *Anisoplia* lineage. The ancestor of this well-supported clade (92% bootstrap support) is reconstructed as being widespread in the Eastern Mediterranean region and North Africa (node 9, Fig. 4). The opening of the Red Sea and the Gulf of Aden in the Early Pliocene (5.3 Ma, Fig. 1), now marking the tectonic boundary between the African and Arabian plates (Sanmartín, 2003), could explain the vicariance event that isolated the *Hemichaetoptilia* lineage in Africa (A) from the ancestor of genera *Brancoplia* and *Chaetopteroptilia* in the Levante shores (D). With the exception of *C. segetum*, *C. syriaca* Burmeister, *C. naviauxi* Baraud and *B. leucaspis* (Fig. 1, see Appendix S2), all species of *Chaetopteroptilia* and *Brancoplia* are distributed within the most oriental part of the Eastern Mediterranean region (the Mediterranean shores of Syria, Israel, Jordan and even Egypt).

The Eastern Mediterranean Basin as a cradle of lineage diversification

The Eastern Mediterranean region (Balkans, Anatolia and the Levante shores) is here reconstructed as the main centre of diversification of anisopliine beetles. The subtribe Anisopliina presents its highest species richness in this region (50%), and, according to our reconstructions, this area is also the ancestral area of the genus *Anisoplia* and of the subgenera *Autanisoplia* and *Anisoplia*. The role of the Eastern Mediterranean region as an 'evolutionary cradle' of diversity for Mediterranean lineages has been pointed out in respect of other groups of animals (Sanmartín, 2003) and plants (Torrecilla *et al.*, 2004; Oberprieler, 2005; Inda *et al.*, 2008). In fact, the highest species diversities of Triticeae and Loliinae, two of the most frequent grass hosts of anisopliine beetles (Table 1), are found in this region (Inda *et al.*, 2008).

The Eastern Mediterranean region is younger than the Western Mediterranean, being the result of the collision of the Arabian Plate with a stable Eurasia during the Mid Miocene (16 Ma) (Sanmartín, 2003). It has also been the subject of a recent and intense orogenic activity during the past 16 million years: the Arabian Plate is still colliding with Eurasia, squeezing the Anatolian plate westwards along the Anatolian Fault Zone (Krijgsman, 2002). This has probably favoured vicariance and allopatric speciation events among populations, making the Eastern Mediterranean region and Western Asia a secondary centre of diversification for many groups of Mediterranean animals and plants (Sanmartín, 2003; Oberprieler, 2005; Inda *et al.*, 2008; Mansion *et al.*, 2008).

A similar case can be argued for the Caucasus region, which is the second most species-rich area for anisopliine beetles (see Appendices S1 and S2, Fig. 4). Plant endemism is also high in the Caucasus region (Takhtajan, 1986), especially in the high mountains of the central part of the Greater Caucasus. This high endemism could be explained by vicariance events related to the Miocene split of the Paratethys Sea into smaller parts, now the Black, Caspian and Aral seas, which would have isolated Caucasian lineages from the rest of the trans-Mediterranean lineages (Oosterbroek & Arntzen, 1992). High

diversification rates can also be explained by the intense and recent orogenic activity in this area. The Middle Miocene collision of the Arabian Plate against Eurasia resulted in the uplifting of the Greater and Lesser Caucasus in the Late Miocene (9–8 Ma), with further uplifting during the Pliocene (4–3 Ma, Dercourt *et al.*, 1986), and this could have promoted isolation and allopatric speciation within lineages (Oberprieler, 2005).

East–west Mediterranean dispersal and the role of the Iberian Peninsula

Our biogeographical reconstruction suggests the occurrence of several migration events between the eastern and western shores of the Mediterranean Basin within the *Anisoplia* lineage (Fig. 4). Two distinct dispersal routes could have been used for these migration events: a northern dispersal route across Europe (Oosterbroek & Arntzen, 1992), or a dispersal route across the North African platform before the Saharan desert was formed (Sanmartín, 2003). Palaeogeographical reconstructions (Rögl & Steininger, 1983) indicate a continuous landmass connection between the eastern and western Mediterranean basins several times during the Late Oligocene–Miocene, following successive Tethys–Paratethys marine regressions, which would have been followed by east–west Mediterranean dispersal of terrestrial animals and plants (Oosterbroek & Arntzen, 1992). The absence of *Anisoplia* species in North Africa, together with the lack of endemic species in the central Mediterranean region, suggests a colonization of the Western Mediterranean (e.g. *A. baetica*, *A. depressa*) by a European dispersal route. A similar biogeographical scenario of biotic expansion from the east into the western Mediterranean basin, followed by vicariance, has been postulated for other groups of animals and plants (Palmer & Cambefort, 2000; Oberprieler, 2005; Mansion *et al.*, 2008). In contrast, the current distributions of the genus *Anthoplia* in Northwest Africa–Iberian Peninsula and of the *Hemichaetoptilia* lineage in Northwest Africa point to a North African dispersal route. Dispersal between the Eastern Mediterranean and North Africa/Iberian Peninsula could possibly have taken place during the Messinian Salinity Crisis (5.96–5.33 Ma, Krijgsman, 2002), and before increasing aridification in the Saharan desert (5–2.5 Ma) created a barrier between the eastern and western Mediterranean shores across North Africa.

In an influential paper on Mediterranean biogeography, Oosterbroek & Arntzen (1992) concluded that the oldest faunal elements of circum-Mediterranean lineages can be found in the Western Mediterranean region, in particular in the Iberian Peninsula. In contrast, Sanmartín (2003) found that the Iberian Peninsula was colonized relatively late in the history of the scarab subfamily Pachydema, and that the Eastern Mediterranean region was instead the ancestral area for this group of circum-Mediterranean beetles. The Iberian Peninsula is reconstructed as both the ancestral area of the anisopliine clade and the receiving area of several independent colonization events from the east (e.g. *A. baetica*, *A. depressa*;

Fig. 4). Thus, the Iberian Peninsula seems to have acted as both source and sink areas of migration events within this group.

CONCLUSIONS

It is likely that both palaeogeographical and palaeoclimatic factors have contributed to the rapid diversification of the anisopline clade in the Mediterranean region. The complex palaeogeographical history of the Mediterranean Basin is likely to have created suitable conditions for allopatric diversification of organisms with limited dispersal capabilities (Sanmartín, 2003), such as the anisopline beetles. A reticulate Mediterranean palaeogeography – with its splitting and colliding landmasses – has presumably played a major role in promoting species diversification within Mediterranean Poaceae, especially in *Vulpia* and *Festuca* (Torrecilla & Catalán, 2002; Torrecilla *et al.*, 2004), two grass genera frequently visited by anisopline species (see Table 1). Both Mediterranean grasses and anisopline beetles are more diverse in the Eastern Mediterranean region, which seems to have acted as a cradle of lineage diversification within these groups. This is probably related to the recent and intense orogenic activity in this region, which has favoured isolation and allopatric speciation among populations.

On the other hand, some of the most recent diversification events within the genus *Anisoplia*, and within the derived *Brancoplia*, *Hemichaetoplia* and *Chaetopteroplia* lineages, could be associated with climatic/vicariance events during the Quaternary period. Recent molecular studies in vertebrate animals (Veith *et al.*, 2003) indicate that ice-sheets and permafrost did not reach the Mediterranean region during the Pliocene–Pleistocene glacial periods and suggest that the effect of glaciations on the Mediterranean biota must have been a retreat of species' distributional ranges. Dispersal and geographic expansion must have been possible in the comparatively warm phases of climatic stability during the Early Pliocene and the Early Pleistocene (Veith *et al.*, 2003). Opportunities for species to differentiate could thus have arisen during these periods, as colonization of new areas (dispersal) and fragmentation of the initial areas of distribution (vicariance) occurred repeatedly through time (Veith *et al.*, 2003; Hewitt, 2004). A similar biogeographical scenario of population contraction–expansion events during glacial–interglacial periods has been postulated for the most recent speciation events in the Mediterranean plant groups Anthemidae (Oberprieler, 2005) and Loliinae (Inda *et al.*, 2008).

Finally, the evolution, both temporal and biogeographical, of grasses in the Mediterranean region shows striking similarities to the evolution of the anisopline clade in this area (Fig. 3), although strictly speaking they did not co-evolve. Inda *et al.* (2008) date the start of diversification of Mediterranean grasses to the Late Tertiary (Early Oligocene), which is congruent with the first fossil record of *Anomala*. The highest species diversity and endemism of the anisopline clade is found in the Eastern Mediterranean region, followed by the

Caucasus, in agreement with pollen evidence that indicates the initial spread of grassland and steppe communities in the region around the Black Sea, beginning in the late Miocene (5–2.3 Ma) (Jacobs *et al.*, 1999). Thus, it seems reasonable to hypothesize that the Late Neogene expansion of grasses (Poaceae) within the Mediterranean region triggered or at least contributed to the diversification of the anisopline clade in this area.

ACKNOWLEDGEMENTS

We thank M. L. Jameson for her observations and recommendations throughout this study, and two anonymous referees for their valuable comments that helped us to improve the manuscript. We are also very grateful to Johan Nylander for his help with the bootstrap–DIVA analysis. Kate Burke helped with the English grammar in the first stages of the manuscript. This research was supported by the CGL2005-07213/BOS project. I.S. has been supported by the 'Ramon y Cajal' Program of the Spanish Ministry of Education and Science.

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SUPPORTING INFORMATION

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

Appendix S1 Distribution of the *Anisoplia* (*Anisoplia*) species in the Mediterranean region based on Baraud (1986, 1992).

Appendix S2 Distribution of the anisopline clade species in the Mediterranean region based on Baraud (1986, 1992), with the exception of the *Anisoplia* subgenus.

Appendix S3 The six equally parsimonious trees resulting from successive weighting based on Jameson *et al.*'s (2007) data set.

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Editor: Bradford Hawkins