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## Phylogenetic relationships of *Dalyat mirabilis* Mateu, 2002, with a revised molecular phylogeny of ground beetles (Coleoptera, Carabidae)

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### Abstract

*Dalyat mirabilis* Mateu 2002 (Coleoptera: Carabidae) is a cave species recently described from SE Spain, which, based on morphological analyses, has been related to the Promecognathinae (with one genus in western North America and four genera in South Africa). In this paper, we investigated the phylogenetic relationships of the main lineages of family Carabidae, and the placement of *Dalyat* among them, with the 18S rRNA full sequence and a fragment of *wingless* with the use of parsimony, a fast maximum likelihood algorithm (implemented in the program PHYML), and Bayesian posterior probabilities. Although with *wingless* alone the relationships of *Dalyat* were not robustly supported, both with 18S rRNA and in a combined analysis there was a strong support for a sister relationship between *Promecognathus* and *Dalyat* with the three methods used. Using a molecular-clock approach the two lineages were estimated to have diverged at a similar (or slightly earlier) age than the origin of Harpalinae, known to have radiated in the Cretaceous. This is compatible with a vicariant origin of the lineage leading to *Dalyat* because of the isolation of the Iberian plate from Pangea in the late Jurassic to early Cretaceous. Other robust relationships within the Carabidae are the monophyly of Harpalinae (including Morionini, Peleciini and Pseudomorphini), its sister relationship with Brachininae, and the inclusion of these two subfamilies together with Scaritini and the austral Psydrinae in a strongly supported clade (the 'higher' Carabidae).

**Key words:** Coleoptera – Carabidae – phylogeny – caves – vicariance – biogeography – Pangea

### Introduction

Among the hyper-diverse groups of insects, the ground beetles (Coleoptera: Carabidae) are one of the relatively better studied, particularly in the western Palaearctic region. This attention has resulted in the infrequent discovery of species which could not be easily placed in existing supra-generic taxa. According to the recent catalogue of Löbl and Smetana (2003), only one of these supra-generic taxa was proposed to accommodate a genus and species described from Europe in the 20th century: the subtribe Italodytina Jeannel, 1957 (Scaritinae: Clivinini), proposed for *Italodytes stammeri* J. Müller, 1938, an Italian cave species. In the wider Palaearctic region, only two species and genera described since 1950 were placed in previously undescribed tribes or subtribes (*Sinozolus* and *Sugimotoa*, both from the eastern Palaearctic) (Löbl and Smetana 2003).

The discovery of *Dalyat mirabilis* Mateu 2002, a large-sized species found in three caves in Sierra de Gador (province of Almería, SE Spain) (Mateu 2002; Mateu and Bellés 2003), was thus really unexpected. Its deviating morphology (partly because of the presumed adaptations to the cave environment) did not allow an immediate recognition of its systematic placement, and only after preliminary analysis using the 18S rRNA sequence and a more detailed analyses of some morphological structures it could be tentatively placed among the Promecognathidae (*sensu* Deuve 1993, Promecognathinae according to other authors) (Mateu and Bellés 2003). Promecognathidae is a small group of five genera with a widely disjoint distribution: one genus (*Promecognathus*) with two species in western North America, and four closely related genera with six species in the Cape region of South Africa (*Axinidium*, *Paraxinidium*, *Metaxinidium* and *Holaxinidium*) (Lindroth 1961; Basilewsky 1963). A Cretaceous fossil from Botswana (*Palaeoaxinidium*) has been tentatively related to this group (McKay 1991), although the paucity of preserved

characters makes difficult its taxonomic and phylogenetic placement (Mateu and Bellés 2003).

Characters supporting the relationship of *D. mirabilis* with Promecognathidae are the insertion of the defensive gland close to the base of the gonopod; lack of rim on the base of the elytra, which also lack scutellar striae and basal pore; lack of mandibular setae; lack of sexual dimorphism in the protarsi; and presence of an empodium among the claws (Deuve 1993; Mateu and Bellés 2003). However, *D. mirabilis* differs from the known Promecognathidae in several key characters, such (among others) the lack of pubescence in the pronotum and elytra, the structure of the feeding apparatus (including the lack of galea in *D. mirabilis*), and the pattern of the pores and setae on the pronotum and elytra (see Mateu and Bellés 2003 for a detailed account of the morphology of *D. mirabilis* and its comparison with Promecognathidae and Scaritinae). These differences, together with some morphological characters that suggested a possible relationship with Scaritinae (such the size and structure of the mandibles and the lacinia) (Mateu and Bellés 2003), warrants the need of a more detailed study of the phylogenetic placement of this enigmatic species using a different set of characters.

In this paper, we attempt to establish the phylogenetic relationships of *D. mirabilis* using molecular information. For that purpose the available data of two nuclear genes for a good representation of ground beetle diversity was used (complete 18S rRNA and a fragment of *wingless*, Maddison et al. 1998, 1999; Ober 2002), with the addition of the equivalent sequences obtained from *D. mirabilis*. The opportunity is taken to re-evaluate the phylogeny of the main lineages of Carabidae using different analytical methods and taxon sampling to those used originally by Maddison et al. (1998, 1999) and Ober (2002). Finally, using a molecular clock approach, we provide a temporal framework for the diversification of the basal lineages of Carabidae, including the separation between *Dalyat* and their

closest relatives (among the taxa included), with the aim to test the biogeographical scenario proposed by Mateu and Bellés (2003) to explain the diversification of the group.

## Materials and Methods

### Nomenclature

Although there is a more or less general consensus on the composition of the main lineages within ground beetles, the taxonomic rank that different authors give to these lineages is diverse. Two main tendencies can be characterized: a 'top-down' approach giving the rank of family to Carabidae, as a well defined unit within Adephaga similar to other units traditionally considered at the level of family (e.g. Dytiscidae, Gyrinidae, etc.); and a 'bottom-up' approach, giving the rank of tribe to well-defined group of genera within the ground beetles. In the first case, the main lineages within Carabidae are treated as subfamilies (e.g. Ball 1979; Lawrence and Newton 1995). In the second, as the level of tribe is defined well down in the hierarchy, the same (or additional) subdivisions are treated as families, and Caraboidea as superfamily (e.g. Deuve 1993). As noted above, there is general agreement in the composition of the different taxa whatever their rank, so the problem would be restricted to one of nomenclature. Due to the recent publication of the important reference work of Löbl and Smetana (2003), in the present paper we have followed their nomenclature and treat Carabidae as a family, with their main primary divisions as subfamilies. Therefore, Promecognathidae *sensu* Deuve (1993) and Dalyatinae *sensu* Mateu and Bellés (2003) would become Promecognathinae and Dalyatini, respectively. Following this hierarchy, the Promecognathinae would include the tribes Promecognathini, Axiniidiini and Dalyatini. The fossil species *Palaeoaxinidium orapensis* McKay, 1991 would belong to a fourth tribe, Palaeoaxiniidiini, although (as noted above) the taxonomic status of this species is most uncertain.

### DNA extraction and sequencing

Genomic DNA was obtained through a standard phenol–chloroform extraction using abdominal tissue of a specimen collected by J.G. Pardo, M. Piquer, J.G. Mayoral and P. Barranco in the cave 'Simarrón II' (Sierra de Gador, province of Almería – see Mateu and Bellés 2003 for details of the locality) on 1 May 2000, and directly preserved in absolute ethanol (voucher specimen kept at the tissue collection of the Museo Nacional de Ciencias Naturales, Madrid, ref. no. 6408). The 18S rRNA sequence was obtained by direct sequencing of six overlapping fragments amplified with internal primers (see Shull et al. 2001 for details of the primers and PCR conditions used). Both forward and reverse sequences were obtained for each fragment, which were contig and edited using Sequencher 3.1 (GeneCodes corporation).

The *wingless* sequence was obtained using the primers and the PCR protocol described in Ober (2002). Accession numbers for the sequences are AY926478 (18S rRNA) and AJ888887 (*wingless*) (Table 1).

### Other molecular data

18S rRNA sequences of Carabidae were obtained from Maddison et al. (1998) (GenBank accession numbers AF002772–AF002808), Maddison et al. (1999) (AF012471–AF012522) and Ober (2002) (AF398715–AF398722). *wingless* sequences were obtained from Ober (2002) (AF398563–AF398636, AF437989) and Sota and Vogler (2001) (AF219564) (Table 1).

Due to the unequal composition of the data matrices for the two genes, two data sets were initially used in the analyses (Table 1). For the 18S rRNA gene, the initial data set included 95 full sequences plus *D. mirabilis*. Outgroups included three examples of Trachypachidae (two species of *Trachypachus* and one *Systolosoma*, obtained from Maddison et al. 1999, Table 1). In previous analyses of 18S rRNA data, Trachypachidae was found to be the sister group of Caraboidea (Shull et al. 2001).

For the *wingless* gene, the initial data set included 45 sequences obtained from Ober (2002) and Sota and Vogler (2001), plus *D. mirabilis* and two outgroups. As *wingless* sequences for Trachypa-

chidae were not available, two sequences of related families of Adephaga were used (Dytiscidae and Hygrobiidae, Table 1), obtained from Miller (2003).

The combined data set included 41 complete 18S rRNA plus *wingless* sequences of Carabidae, with the addition of *D. mirabilis* and two outgroups (Dytiscidae and Hygrobiidae, 18S rRNA sequences obtained from Ribera et al. 2002). An extended data set was built including 11 taxa for which there were no *wingless* sequence (Table 1), but represented major lineages for which no complete combined data was available (including the Trachypachidae outgroups). The initial data sets were subsequently modified according to the results of the different analyses (see Results).

### Phylogenetic analyses

We followed a two-step procedure for the alignment of length variable regions, with an initial hypothesis of character homology (i.e. an alignment) and a subsequent, independent step of tree search (Phillips et al. 2000; Simmons 2004).

The hypervariable regions of the 18S rRNA gene V4 and V6 (Tautz et al. 1988) were excluded from the analyses, because of the difficulty in establishing homologies among nucleotides. The remaining sequence was aligned by hand, following the secondary structure according to the template providing by Kjer (2004). To align the *wingless* gene we translated the nucleotide sequence with McClade 4.0 (Maddison and Maddison 2000), and aligned the aminoacid sequence manually. The nucleotide sequence was thus reconstructed using the aminoacid sequence as a template.

Congruence between genes was tested with the partition homogeneity test (PHT) (Swofford 2002), which is the implementation of the incongruence length difference test (Mickevich and Farris 1981; Farris et al. 1994), with the only aim of exploring the data.

The two sets of molecular data were analysed separately and in a combined analysis. Parsimony analysis was conducted under equal weights in PAUP\* version 4.0b10 (Swofford 2002), using TBR heuristic searches with 1000 random addition sequences and without saving multiple trees. An heuristic search was subsequently conducted on the most parsimonious trees found, with the option 'save multiple trees' activated, until the end of the search, or until more than 5000 trees were found. In all searches gaps were coded as a fifth character state (Giribet and Wheeler 1999). Node support was measured with Bremer (1994) and Partitioned Bremer Support (PBS) values (Baker and DeSalle 1997), searching on constraint trees generated with TreeRot (Sorenson 1996); and with non-parametric bootstrapping with 1000 pseudoreplicates of 50 random additions each (Felsenstein 1985).

Previous analyses with one of the data sets used in this study (Maddison et al. 1998, 1999) revealed the existence of clades likely to be artefactual because of the distorting effect of long branches. As one of them included Scaritinae (one of the potential lineages to which *Dalyat* could be associated, see above), we conducted additional analyses using model-based phylogenetic methods, which should in principle be less sensitive than parsimony to the biases introduced by highly saturated or homoplasious data (e.g. Swofford et al. 1996; Felsenstein 2004).

The optimal model of nucleotide substitution was determined with MODELTEST 3.6 (Posada and Crandall 1998), for both genes separately and for the combined data set. In all cases a generalized time reversible (GTR) model (Tavaré 1986) with gamma distributed among site rate variation and estimating the proportion of invariable sites (Yang 1993) was selected as the best fit to the data. Maximum likelihood searches were conducted with the program PHYML (Guindon and Gascuel 2003), estimating all the parameters of the GRT + I + G model and starting with a neighbour joining tree. Node support was measured with 100 bootstrap replicates.

We also analysed the combined data set using Bayesian probabilities (Rannala and Yang 1996) as implemented in the computer program MRBAYES 3.0b4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), as it allows the estimation of different evolutionary models for the user-defined data partitions (which at the moment is not possible in PAUP or PHYML). The parameters of the two partitions (i.e. genes) were estimated independently. Searches were conducted using the default priors (uniform probabilities) starting with random trees,

with three heated and one cold Markov chains for 1 000 000 generations, sampled at intervals of 100 generations. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time, and visually determined when the log-likelihood values reached a stable equilibrium. The parameter estimations (including tree topologies) obtained before reaching the stationarity are discarded as a 'burnin', and only the trees sampled after that point are considered (Huelsenbeck and Ronquist 2001).

Posterior probabilities were used to assess node stability. Although generally higher than bootstrap support values, posterior probabilities above the standard 95% threshold can be taken as indicative of strong node stability (Suzuki et al. 2002; Alfaro et al. 2003; Douady et al. 2003; Simmons et al. 2004). According to a recent simulation study, posterior probabilities can be taken as the probability that a node is true provided that the data evolves according to the model used (Huelsenbeck and Rannala 2004).

### Estimation of rates of variation

The historical scenario proposed by Mateu and Bellés (2003) was tested through a molecular clock approach. For that purpose, the topology and branch lengths of the tree obtained using the combined data in MRBAYES was used (see Results). The possibility of directly enforcing a molecular clock to the data was tested using likelihood ratios in PAUP: the likelihood of the tree under an optimal GRT + I + G model (as estimated with MODELTEST, see Results) using the parameters estimated in MRBAYES was computed, and compared with the likelihood enforcing a molecular clock. As the clock-behaviour of the data was significantly rejected, we used the penalized likelihood (PL) method of Sanderson (2002) to obtain an ultrametric tree, as implemented in the software program r8s (<http://ginger.ucdavis.edu/r8s/>). The smoothing parameter was determined by a process of cross-validation in a preliminary analysis using the truncated Newton (TN) method, and a second analysis was run with the smoothing parameter set to the optimal value obtained previously (Sanderson 2002). Two sets of analyses were run, one without any temporal constraint, and a second one with the constraint of the age of the origin of Harpalinae (which radiated in the Cretaceous, Ponomarenko 1977) and Carabidae (unequivocally present in the Triassic, Ponomarenko 1977). It must be noted that the whole molecular-clock approach is subject to a large number of uncertainties (see e.g. Bromham and Penny 2003, for a review), but our purpose was only to check the viability of the hypothesis advanced by Mateu and Bellés (2003), namely a middle to late Jurassic origin for the separation between the lineage eventually leading to *D. mirabilis* and the rest of promecognathids (150–180 MY), due to a vicariant process in the early fragmentation of Pangea. In Mateu and Bellés (2003) a sister relationship between *Promecognathus* and the South African Axinidiini was suggested based on a morphological phylogeny of Promecognathinae, but the lack of molecular data for any member of Axinidiini does not allow the study of the relationships within promecognathids.

## Results

### Phylogenetic position of *Dalyat mirabilis*

#### 18S rRNA

The aligned matrix of the 98 taxa included in the analyses of the 18S rRNA sequences (Table 1) had 1843 characters, 506 of them parsimony informative (available in 'treebase' at <http://www.treebase.org>). The parsimony search strategy outlined in the methods resulted in 378 trees of 2909 steps (retention index RI = 0.64, consistency index CI = 0.37), the strict consensus of which placed *Dalyat* as sister to *Promecognathus*, and both in a large unresolved polytomy at the base of the ground beetles. The phylogram shows a monophyletic clade of species with an accumulation of long branches, including seven of the 10 longest path lengths (Fig. 1). This clade included the

examples of Cicindelinae (*Cicindela* and *Omus*), Rhyssodidae (*Clinidium* and *Omoglymmius*), Paussinae (*Metrius*, *Arthropterus* and *Pachiteles*) and Scaritini (*Pasimachus*, *Scarites* and *Carenum*). The grouping of these four clades was also found by Maddison et al. (1999) (their 'CRPS quartet') who, despite its support, suggested it was the result of long branch attraction. With the exception of Scaritini, to which *Promecognathus* has been related (Lindroth 1961, see Discussion), and to which *Dalyat* has some morphological resemblance (Mateu and Bellés 2003), the other three groups (Cicindelinae, Paussinae and Rhyssodidae) have very characteristic morphologies, and there is no reasonable doubt that *Dalyat* does not belong to them. As the purpose of this paper was not to solve the phylogenetic position of these problematic lineages (for which our data is clearly insufficient, see Maddison et al. 1999), we repeated the analyses after deleting the representatives of the three lineages (Cicindelinae, Rhyssodidae and Paussinae).

The new data matrix had 91 taxa (Table 1) and the same 1843 characters, of which 407 were parsimony informative. The search resulted in more than 5000 trees of 2436 steps (RI = 0.65, CI = 0.38), the strict consensus of which included *Dalyat* sister to *Promecognathus* with very high support (bootstrap 99%), both in a basal polytomy including most of the carabid lineages (Fig. 2).

The evolutionary model that best explained the data was a complex general time reversible model with a proportion of invariable sites and rate heterogeneity (GRT + I + G), as selected by MODELTEST under the Akaike criterion (Posada and Crandall 1998). The search in PHYML resulted in a tree placing *Dalyat* as sister to *Promecognathus*, and both sister to a large clade including Harpalinae and the long-branch 'CRPS quartet' (Cicindelinae, Rhyssodidae, Paussinae and Scaritini), although not monophyletic (Paussinae was paraphyletic with respect to the other three). After deletion of the three groups with the longest branches (Cicindelinae, Rhyssodidae and Paussinae), *Dalyat* was still found sister to *Promecognathus* with strong support (bootstrap value 100%), and both of them sister to a large clade including Harpalinae and Scaritini, although with bootstrap value lower than 50%.

The nodes with bootstrap support higher than 50% were almost identical between the tree obtained with parsimony and the tree obtained with maximum likelihood, with a slight better resolution with parsimony (Fig. 2). The only incompatible node defined the relationships within the Scaritini: in the parsimony tree *Scarites* was sister to *Pasimachus* (80% bootstrap), while in the maximum likelihood tree *Carenum* was sister to *Pasimachus* (53% bootstrap) (Fig. 2).

#### Wingless

The aligned matrix of the 48 taxa included in the analyses of the wingless sequences had 508 characters, of which 270 were informative (available in 'treebase' at <http://www.treebase.org>). The parsimony search strategy outlined in the methods resulted in 16 trees of 3035 steps (CI = 0.23, RI = 0.37), the strict consensus of which placed *Dalyat* as sister to (*Patrobis* + *Diplous*), although with bootstrap support lower than 50% (Fig. 3). The four groups of the 'CRPS quartet' were not monophyletic, and had branches not longer than the rest (the path lengths of the four species included were below the average, data not shown). Most of the nodes of the tree had a bootstrap support lower than 50%, with only one major group

Table 1. Taxa included in the different analyses, with accession numbers. Classification and nomenclature follows Löbl and Smetana (2003) (for the Palearctic species)

No	Subfamily	Species	Acc. no.		18S		wg		18S + wg	
			18S	wingless	Fu	Re	Fu	Re	Fu	Re
1	Apotominae	<i>Apotomus rufithorax</i> Pecchiolo	AF012497		+	+			m	
2	Brachiniinae	<i>Aptinus alpinus</i> DeJean & Boisduval		AF398568			+	+	1/21/2	1/2
3		<i>Aptinus displosor</i> Dufour	AF012480		+	+				1/2
4		<i>Brachinus armiger</i> Dejean	AF012479		+	+				
5		<i>Brachinus hirsutus</i> Bates	AF012478	AF398572	+	+	+	+	+	+
6		<i>Pheropsophus aequinoctialis</i> Linnaeus	AF012477	AF398619	+	+	+	+	+	+
7		<i>Styphlodromus</i> sp.	AF398718	AF398628	+	+	+	+	+	+
8	Broscinae	<i>Broscosoma relictum</i> Weissmandl	AF012502		+	+				
9		<i>Creobius eydouxii</i> (Guérin-Ménéville)	AF012498		+	+				
10		<i>Mecodema fulgidum</i> Broun	AF012501		+	+				
11		<i>Oregus aereus</i> White	AF012500		+	+				
12		<i>Promecoderus</i> nr. <i>brunnicornis</i> Dejean	AF012499		+	+				
13	Carabinae									
14	Carabini	<i>Calosoma scrutator</i> (Fabricius)	AF002800		+	+				
15		<i>Carabus granulatus</i> Linnaeus		AF219564			+	+	1/2	1/2
16		<i>Carabus nemoralis</i> O.F. Müller	AF012507		+	+			1/2	1/2
17	Cychrini	<i>Ceroglossus chilensis</i> Eschscholtz	AF012509		+	+				
18		<i>Cychnus italicus</i> Bonelli	AF012510		+	+				
19	Pamborini	<i>Scaphinotus petersi catalinae</i> Van Dyke	AF002801		+	+				
20	Cicindelinae	<i>Pamborus guerinii</i> Gory	AF012508		+	+				
21		<i>Cicindela sedecimpunctata</i> Klug	AF012518	AF398579	+		+			
22	Elaphrinae	<i>Omus californicus</i> Eschscholtz	AF012519		+					
23		<i>Blethisa multipunctata</i> (Linnaeus)	AF002803		+	+				
24		<i>Elaphrus californicus</i> Mannerheim	AF012514	AF398563	+	+	+	+	+	+
25		<i>Elaphrus clairvillei</i> Kirby	AF002802		+	+				
26	Harpalinae									
27	Catapiiesini	<i>Catapiesis brasiliensis</i> Gray	AF012476	AF398577	+	+	+	+	+	+
28	Chlaeniini	<i>Chlaenius ruficauda</i> Chaudoir	AF002777		+	+				
29		<i>Chlaenius ruficauda</i> Chaudoir	AF012473	AF398578	+	+	+	+	+	+
30	Cnemalobini	<i>Cnemalobus sulciferus</i> Philippi	AF012474	AF398580	+	+	+	+	+	+
31	Cyclosomini	<i>Tetragonoderus latipennis</i> LeConte	AF012471	AF398631	+	+	+	+	+	+
32	Galeritini	<i>Galerita lecontei</i> Dejean	AF002780	AF398590	+	+	+	+	+	+
33	Harpalini	<i>Discoderus cordicollis</i> Horn	AF012472		+	+				
34		<i>Discoderus cordicollis</i> Horn	AF002776	AF398588	+	+	+	+	+	+
35		<i>Pelmatellus</i> sp.	AF398720	AF398615	+	+	+	+	+	+
36	Lachnophorini	<i>Calybe laetula</i> LeConte	AF002772	AF398576	+	+	+	+	+	+
37	Lebiini	<i>Cymindis puntigera</i> LeConte	AF002773	AF398583	+	+	+	+	+	+
38	Loxandriini	<i>Loxandrus</i> n.sp. nr. <i>amplithorax</i>	AF002778	AF398600	+	+	+	+	+	+
39	Morionini	<i>Morion aridus</i> Allen	AF002783	AF398606	+	+	+	+	+	+
40		<i>Moriosomus seticollis</i> MacLeay	AF398721	AF398607	+	+	+	+	+	+
41	Orthogoniini	<i>Orthogonius</i> sp.	AF398719	AF398611	+	+	+	+	+	+
42	Peleciini	<i>Eripus nitidus</i> Chaudoir	AF398716	AF398589	+	+	+	+	+	+
43		<i>Pelecium</i> n.sp. nr. <i>sulcipenne</i>	AF398715	AF398614	+	+	+	+	+	+
44	Platynini	<i>Agonum extensicolle</i> Say	AF002775	AF398564	+	+	+	+	+	+
45	Pseudomorphae	<i>Pseudomorpha</i> sp.	AF002782	AF398622	+	+	+	+	+	+
46		<i>Sphallomorpha</i> sp.	AF398717	AF398636	+	+	+	+	+	+
47	Pterostichini	<i>Pterostichus melanarius</i> Illiger	AF002779	AF398623	+	+	+	+	+	+
48	Zabrini	<i>Amara apricaria</i> Paykull	AF002774	AF398565	+	+	+	+	+	+
49	Zuphiini	<i>Pseudaptinus</i> cf. <i>rufulus</i> LeConte	AF002781		+	+			+	
50		<i>Pseudaptinus lecontei</i> (Dejean)		AF437989			+	+		+
51	Loricarinae	<i>Loricera foveata</i> LeConte	AF012503		+	+			m	
52		<i>Loricera pilicornis</i> (Fabricius)	AF002799		+	+				
53	Melaeninae	<i>Cymbionotum pictulum</i> H.W. Bates	AF012496		+	+				
54		<i>Cymbionotum semeleleri</i> (Chaudoir)	AF012495		+	+			m	
55	Migadopinae	<i>Antarctonomus complanatus</i> (Blanchard)	AF012504		+	+				
56		<i>Monolobus ovalipennis</i> Straneo	AF012505		+	+			m	
57	Nebriinae									
58	Nebriini	<i>Leistus ferruginosus</i> Mannerheim	AF002806		+	+				
59		<i>Nebria hudsonica</i> LeConte	AF002805	AF398608	+	+	+	+	+	+
60	Notiophilini	<i>Notiophilus semiopacus</i> Eschscholtz	AF002804		+	+				
61	Opisthiini	<i>Opisthius richardsoni</i> Kirby	AF012511		+	+				
62	Omophroninae	<i>Omophron obliteratum</i> G.H. Horn	AF012513		+	+			m	
63	Paussinae	<i>Arthropterus</i> sp.	AF012516	AF398570	+		+			
64		<i>Metrius contractus</i> Eschscholtz	AF012515	AF398605	+		+			
65		<i>Pachyteles striola</i> emplx	AF012517		+					
66	Promecognathinae									

Table 1. (Continued)

No	Subfamily	Species	Acc. no.		18S		wg		18S + wg		
			18S	wingless	Fu	Re	Fu	Re	Fu	Re	
63	Dalytini	<i>Dalyat mirabilis</i> Mateu	AY926478	AJ888887	+	+	+	+	+	+	
64	Promecognathini	<i>Promecognathus crassus</i> LeConte	AF012492	AF398621	+	+	+	+	+	+	
Psydrinae											
65	Amblytelini	<i>Amblytelus curtus</i> Fabricius	AF012484	AF398566	+	+	+	+	+	+	
66		<i>Tropopterus</i> sp.	AF012483		+	+					
67	Psydrini	<i>Laccocenus ambiguus</i> Sloane	AF012486	AF398596	+	+	+	+	+	+	
68		<i>Psydrus piceus</i> LeConte	AF002784		+	+			m		
69	Mecyclothoracini	<i>Mecyclothorax vulcanus</i> Blackburn	AF012482	AF398601	+	+	+	+	+	+	
70	Melisoderini	<i>Melisodera picipennis</i> Westwood	AF012481	AF398602	+	+	+	+	+	+	
71	Meonidini	<i>Meonis</i> sp.	AF398722	AF398603	+	+	+	+	+	+	
72		<i>Raphetis</i> sp.	AF012485		+	+					
Scaritinae											
73	Clivinini	<i>Clivina ferrea</i> LeConte	AF002796		+	+					
74		<i>Schizogenius falli</i> Whitehead	AF002797		+	+					
75	Dyschiriini	<i>Dyschirius sphaericollis</i> (Say)	AF002798		+	+					
76	Scaritini	<i>Carenum interruptum</i> Macleay	AF012491		+	+					
77		<i>Pasimachus obsoletus atronitens</i> Casey	AF002794		+	+					
78		<i>Scarites subterraneus</i> Fabricius	AF002795	AF398625	+	+	+	+	+	+	
79	Siagoninae	<i>Siagona europaea</i> Dejean	AF012493		+	+			m		
80		<i>Siagona jenissoni</i> Dejean	AF012494		+	+					
Trechinae											
81	Bembidiini	<i>Asaphidion curtum</i> (Heyden)	AF002792		+	+					
82		<i>Batesiana hilaris</i> (Bates)	AF012489		+	+					
83		<i>Bembidion levettei carrianum</i> Casey	AF002791	AF398571	+	+	+	+	+	+	
84		<i>Bembidion mexicanum</i> Dejean	AF012490		+	+					
85		<i>Pericompsus laetulus</i> LeConte	AF002790		+	+					
86	Patrobini	<i>Diplous californicus</i> Motschulsky	AF002785	AF398587	+	+	+	+	+	+	
87		<i>Patrobis longicornis</i> Say	AF002786	AF398613	+	+	+	+	+	+	
88	Pogonini	<i>Diplochaetus planatus</i> G.H. Horn	AF002789		+	+					
89	Trechini	<i>Trechus chalybeus</i> group	AF002793	AF398633	+	+	+	+	+	+	
90	Zolini	<i>Merizodus angusticollis</i> Solier	AF012487		+	+					
91		<i>Oopterus</i> sp.	AF012488		+	+					
92		<i>Sloaneana tasmaniae</i> (Sloane)	AF002788		+	+					
93		<i>Zolus helmsi</i> Sharp	AF002787		+	+					
94	Amarotypini	<i>Amarotypus edwardsi</i> Bates	AF012506		+	+			m		
95	Gehringiini	<i>Gehringia olympica</i> Darlington	AF012512	AF398591	+	+	+	+	+	+	
96	Metiini	<i>Metius</i> sp.	AF012475	AF398604	+	+	+	+	+	+	
97	Rhysodidae	<i>Clinidium calcaratum</i> Leconte	AF012521		+						
98		<i>Omoglymmius hamatus</i> LeConte	AF012520	AF398609	+		+				
OUTGROUPS											
99	Dytiscidae	<i>Agabus heydeni</i> Wehncke	AJ318689								
100		<i>Agabus tristis</i> Aubé		AF392003			+	+	1/2	1/2	
101	Hygrobiidae	<i>Hygrobia hermanni</i> (Fabricius)	AJ318673	AF391997			+	+	+	+	
102	Trachypachidae	<i>Systolosoma lateritium</i> Negre	AF012522		+	+			m		
103		<i>Trachypachus gibbsii</i> LeConte	AF002808		+	+			m		
104		<i>Trachypachus holmbergi</i> Mannerheim	AF002807		+	+			m		
					Totals	98	91	48	44	55	44

Acc. no., Genbank accession number; 18S, analyses of the 18S rRNA sequence; wg, analyses of the *wingless* sequence; 18S + wg, combined analyses of 18S rRNA and *wingless*; Fu, analyses with the full data set; Re, analyses with a reduced data set (excluding taxa with long branches or missing data); m, missing sequence; 1/2, the combined sequence is a chimera of two species of the same genus.

with a high bootstrap (Harpalinae plus Brachininae plus the austral Psydrinae *sensu* Maddison et al. 1999, with 70% bootstrap, Fig. 3).

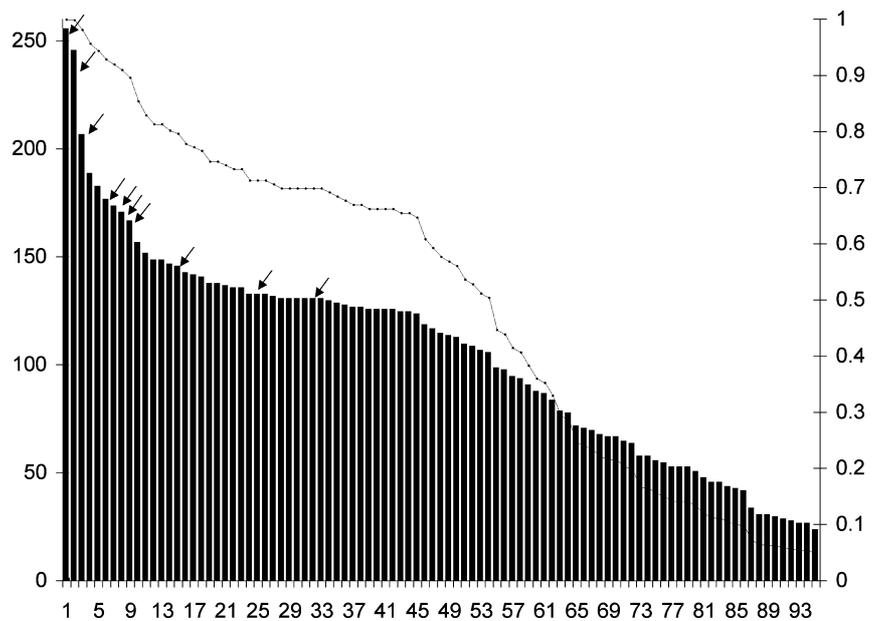
The model selected by MODELTEST as the one best fitting the data was also a GRT + I + G. When implemented in PHYLML, the search resulted in a tree with a clade including *Dalyat* with *Trechus* (with a very long branch), *Diplous* and *Patrobis*. *Promecognathus* was grouped with *Laccocenus* and *Cicindela* in a different section of the tree. However, almost all nodes in the tree had a bootstrap support of less than 50%, with the only exception of several closely related pairs of genera (Fig. 3).

#### Combined analyses of 18S rRNA and *wingless*

The PHT, as implemented in PAUP, suggested a strong incongruence among the phylogenetic signal of the two genes ( $p = 0.009$ ). Given the fact that with only two genes it is not possible to know which one had the 'deviating' signal, and that in examining deep-level relationships we do expect that both gene trees should reflect the true relationship, we opted for a combined analysis in the hope that the possible artefactual support of some nodes by one of the genes could be compensated by the 'true' signal of the other.

A preliminary analysis was conducted including all main lineages with the exclusion of those with long 18SrRNA

Fig. 1. Path lengths of one of the 378 most parsimonious trees obtained from the analysis of the extended 18S rRNA data set (i.e. 95 ingroup taxa, Table 1), sorted from largest to shortest (left scale). Continuous line: cumulative probability of the path length as estimated from a normal distribution with average and standard deviation estimated from the data (right scale). Arrows: path lengths of the species of Cicindelinae, Rhysodidae, Paussinae and Scaritini (the 'CRPS quartet' of Maddison et al. 1999). The three longest pathlengths (*Clinidium*, *Ommoglymmius* and *Pasimachus*) are above the 98% cumulative probability of the distribution



branches (Cicindelinae, Rhysodidae and Paussinae), including eleven with missing *wingless* sequences, to check the possible relationships of *Dalyat* with any of them (Table 1). The data matrix included 55 taxa and 2351 characters, 563 of them parsimony informative.

A parsimony search in PAUP (as detailed in the Materials and methods section, but with the 'save multiple trees' option activated), resulted in five trees of 4358 steps, CI = 0.32, RI = 0.45 (not shown). *Dalyat* was placed as sister to *Promecognathus*, and both sister to a clade including only one of the species with missing *wingless* (*Omophron*). Five of the 11 species with missing sequences (including the tree Trachypachidae, considered as outgroups) were grouped in a seven-species clade at the base of the tree, suggesting an artefact because of the high proportion of missing data. The analysis was repeated with the exclusion of the species with missing *wingless*, using *Agabus* and *Hygrobia* as the only outgroups (families Dytiscidae and Hygrobiidae respectively) (Table 1).

The reduced matrix had 44 taxa and 520 informative characters. A search in PAUP resulted in nine trees of 3921 steps (CI = 0.33, RI = 0.43). *Dalyat* was again placed as sister to *Promecognathus*, in an unresolved polytomy at the base of the tree. With the aim to increase the resolution of the relationships of Promecognathinae, a successive weighting scheme was applied to the data (Farris 1969). After reweighting the characters according to the rescaled consistency index, a search of the nine most parsimonious trees resulted in a single tree in which *Dalyat* plus *Promecognathus* were sister to *Laccocenus*, and the three sister to the remaining Carabidae with the exclusion of *Carabus* (Fig. 4). However, although the clade *Dalyat* + *Promecognathus* had a bootstrap support of 94%, none of the other relationships had bootstrap support values above 50%, as happened with the equally weighted tree (Fig. 4).

The examination of the PBS showed a high frequency of contradictory support (i.e. partitions with negative support values). This was reflected in a significant negative correlation between the PBS of the two genes, both measured with parametric Pearson ( $r = -0.41$ ) and Spearman's rank correlation

( $s = -0.54$ , both  $p < 0.05$ ). PBS values were, however, significantly and positively correlated with the total Bremer support, with the exception of the PBS of the 18S rRNA partition when measured with the Spearman's rank correlation ( $s = 0.27$ , n.s.).

Preliminary analyses with PHYML suggested the strong influence of the missing *wingless* sequences (not shown), and thus all maximum likelihood analyses were conducted with the reduced data matrix of 44 taxa (Table 1). Using a GRT + I + G evolutionary model (as estimated with MODELTEST), the optimal tree resulted in a topology very similar to that of the parsimony reweighted tree, with *Dalyat* sister to *Promecognathus* with a bootstrap support of 95%, both sister to *Laccocenus*, and the clade formed by these three species sister to the remaining Carabidae (the last two nodes with bootstrap supports lower than 50%, Fig. 5).

The analysis with MRBAYES resulted in a tree with *Dalyat* sister to *Promecognathus* with a posterior probability of 1.0, and both sister to *Laccocenus* (posterior probability of 0.6), in a basal position within the tree (Fig. 6).

#### Other relationships within the Carabidae

Based on the trees obtained with the analyses of the combined full data, and excluding the species with long 18S rRNA branches (i.e. the 44 taxa data matrix, Table 1), there seemed to be a number of robust relationships within the studied Carabidae. All three methods (parsimony implemented in PAUP, maximum likelihood implemented in PHYML, and Bayesian posterior probabilities implemented in MRBAYES) recovered a large clade formed by Harpalinae, Brachininae, Scaritini and the austral Psydrinae (*sensu* Maddison et al. 1999) (what could be called the 'higher' Carabidae), all of them with high support (bootstrap support value of 100 and 94 in PAUP and PHYML respectively, and a posterior probability of 1.0 in MRBAYES: 100/94/1.0 from now on). Within this clade, Harpalinae was monophyletic with high support (100/74/1.0), and sister to Brachininae (100/75/1.0), although the monophyly of Brachininae was not strongly supported (Figs 4–6). In the trees obtained using the GRT + I + G evolutionary

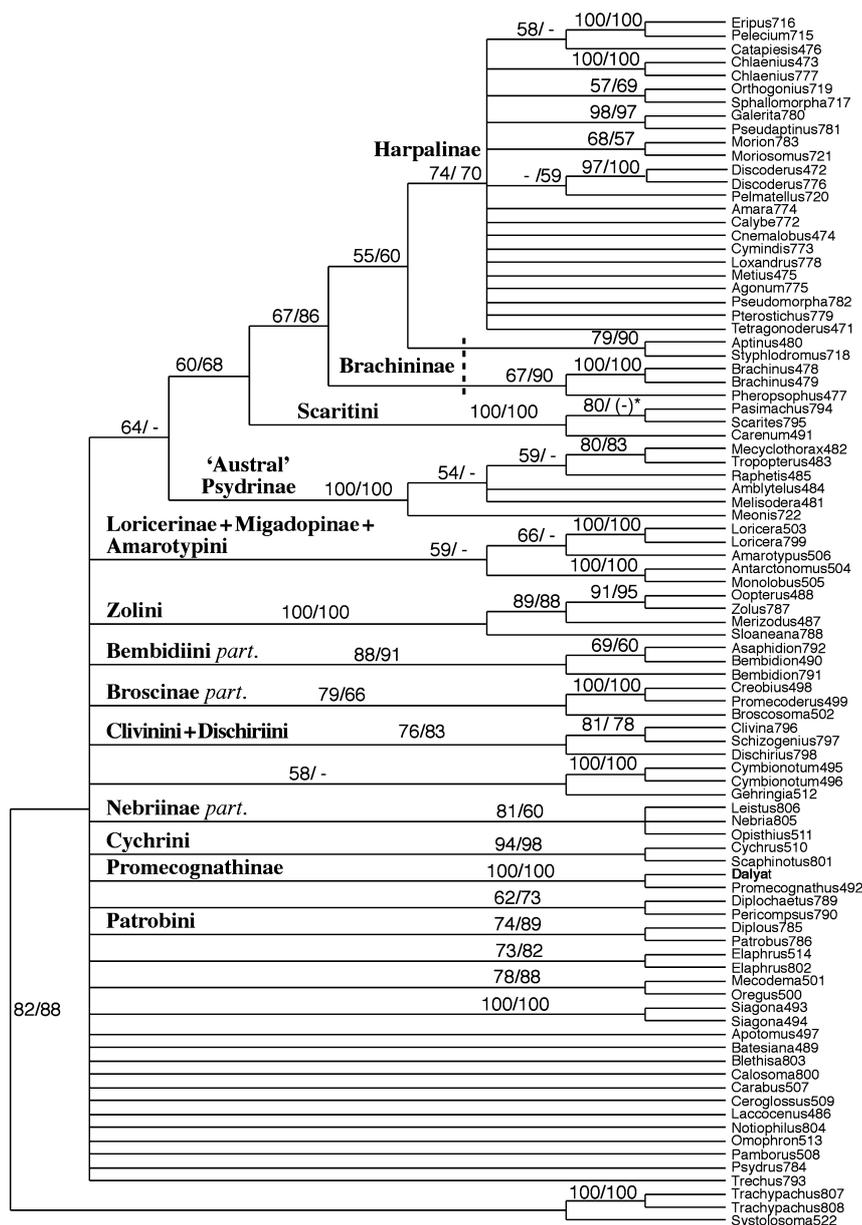


Fig. 2. Phylogenetic relationships of Carabidae and *Dalyat* as recovered from the analyses of the 18S rRNA reduced data matrix (91 taxa, see Table 1). Only nodes with bootstrap support values higher than 50% are shown. Numbers above branches, bootstrap support of the node in the parsimony analysis with PAUP/bootstrap support of the node in the maximum-likelihood analysis in PHYML (see text for details). Numbers after the genus-names refer to the last three figures of the accession number of the 18S rRNA sequence (see Table 1). \*Node not recovered in the ML analysis in PHYML (see text)

model (PHYML and MRBAYES) the clade formed by Harpalinae + Brachininae was sister to Scaritini (*Scarites*) (95/1.0), and then to the austral Psydrinae (Figs 5 and 6); while in the tree obtained with parsimony (PAUP) the order was reversed: first sister to the austral Psydrinae (100) and then to Scaritini (Fig. 4). The monophyly of the austral Psydrinae was highly supported with the three methods used (100/100/1.0). The relationships within subfamily Brachininae and the austral Psydrinae were identical with the three methods used, but those within Harpalinae were markedly different (Figs 4–6).

Among the basal part of the tree (the 'lower' Carabidae, i.e. Promecognathinae, Carabinae, Trechinae, Nebriinae, Elaphrinae and Gehringia) the relationships were much less robust across the different methods used. In the three trees, Promecognathinae (including *Dalyat*) was sister to *Laccocenus* (which was never grouped with the austral Psydrinae), although with low support. The subfamily Trechinae was recovered as monophyletic in PAUP and PHYML (84/-), but in MRBAYES it appeared intermixed with *Nebria* (1.0). On the

contrary, both in PAUP and PHYML *Nebria* was placed as sister to *Gehringia*, although with low support (85/-). The three methods recovered a monophyletic Trechinae formed by the sister groups Trechini + Bembidiini and Patrobiini (including *Nebria* in MRBAYES), but with low support (74/-/0.75). Other basal relationships differ among the trees obtained with the three methods employed, and have in general low support (Figs 4–6).

#### Relative age of the separation between *Dalyat* and *Promecognathus*

The preliminary analysis in the program r8s of the tree obtained with the combined matrix of 44 taxa in MRBAYES (Table 1) using the penalized likelihood method of Sanderson (2002) and the TN algorithm indicated an optimal smoothing factor of 1.0 (i.e. the lowest of those tested). The ultrametric tree obtained when the smoothing factor was set to 1.0 and the nodes were unconstrained (i.e. the root of the tree was set to an

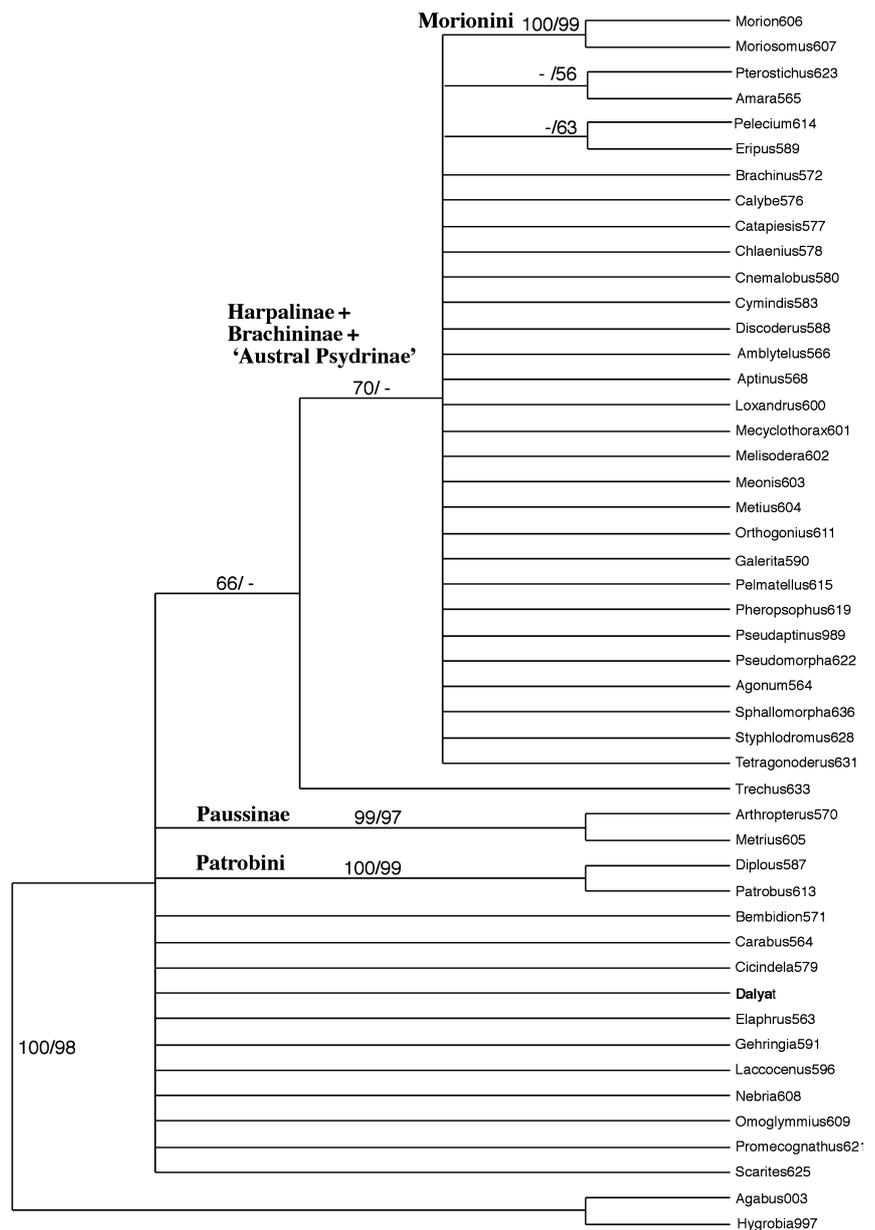


Fig. 3. Phylogenetic relationships of Carabidae and *Dalyat* as recovered from the analyses of the *wingless* reduced data matrix (44 taxa, see Table 1). Only nodes with bootstrap support values higher than 50% are shown. Numbers above branches, bootstrap support of the node in the parsimony analysis with PAUP/bootstrap support of the node in the maximum-likelihood analysis in PHYML (see text for details). Numbers after the genus-names refer to the last three figures of the accession number of the *wingless* sequence (see Table 1)

arbitrary depth of 1.0, Sanderson 2002) is represented in Fig. 6. The node linking *Dalyat* with *Promecognathus* had a relative height of 0.49, very similar to that of the root of Harpalinae (0.48).

Setting different constraint combinations on the origin of Harpalinae and Carabidae resulted in estimated ages for the divergence between the lineages of *Dalyat* and *Promecognathus* of *c.* 140–145 MY (Table 2), corresponding to the Late Jurassic to Early Cretaceous.

## Discussion

### Phylogenetic placement and historical origin of *Dalyat mirabilis*

Although in the tree obtained with *wingless* data the relationships of *D. mirabilis* are resolved with very low support, both with 18S rRNA and in the combined analyses there is strong support for a sister relationship between *Dalyat* and *Promecognathus*, as suggested by Mateu (2002) and Mateu and Bellés (2003) based on morphology. This relationship is most

remarkable, adding a third, widely disjoint taxa to what was already an exceptional distribution – *Promecognathus* in western North America and the four Axinidiini genera in the Cape region of South Africa (Basilewsky 1963).

The *Promecognathinae* have been traditionally related with the Scaritinae (e.g. Lindroth 1961; Kryzhanovskiy 1976), mostly because of similarities in the body form and the structure of the mandibles, although it has also been related to the Siagoninae (Jeannel, in Kryzhanovskiy 1976) or Amaroptyini (Liebherr and Will 1998). There is, however, no clear synapomorphy linking *Promecognathinae* with any related group within the Carabidae, either for adults or the first instar larvae (Bousquet and Smetana 1986); and its distinctiveness has been recognized in recent treatments (e.g. Ball and Bousquet 2001). In our trees, the relationships of *Promecognathinae* (including *Dalyat*) are not well supported, but results point towards a sister relationship with the Australian monotypic genus *Laccocenus*. The genus was included by Moore (1963) in the tribe Psydrini of subfamily Psydrinae, together

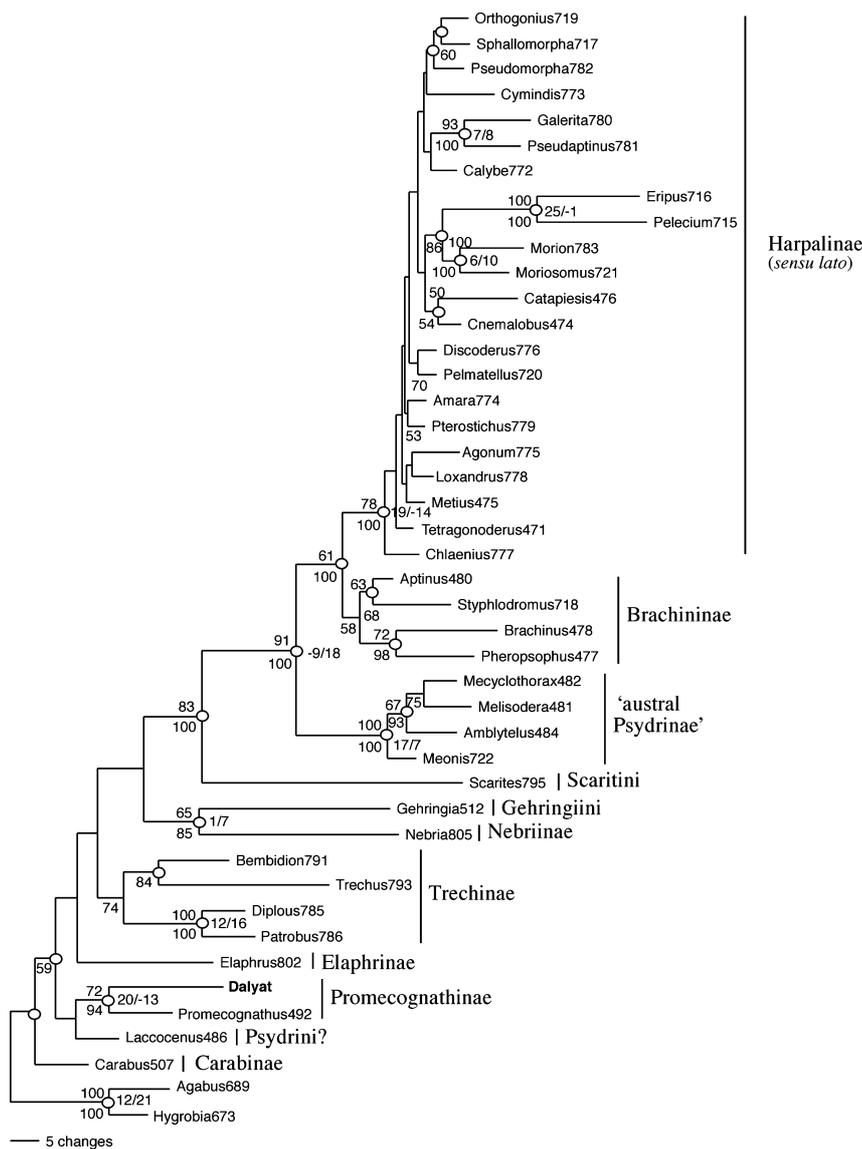


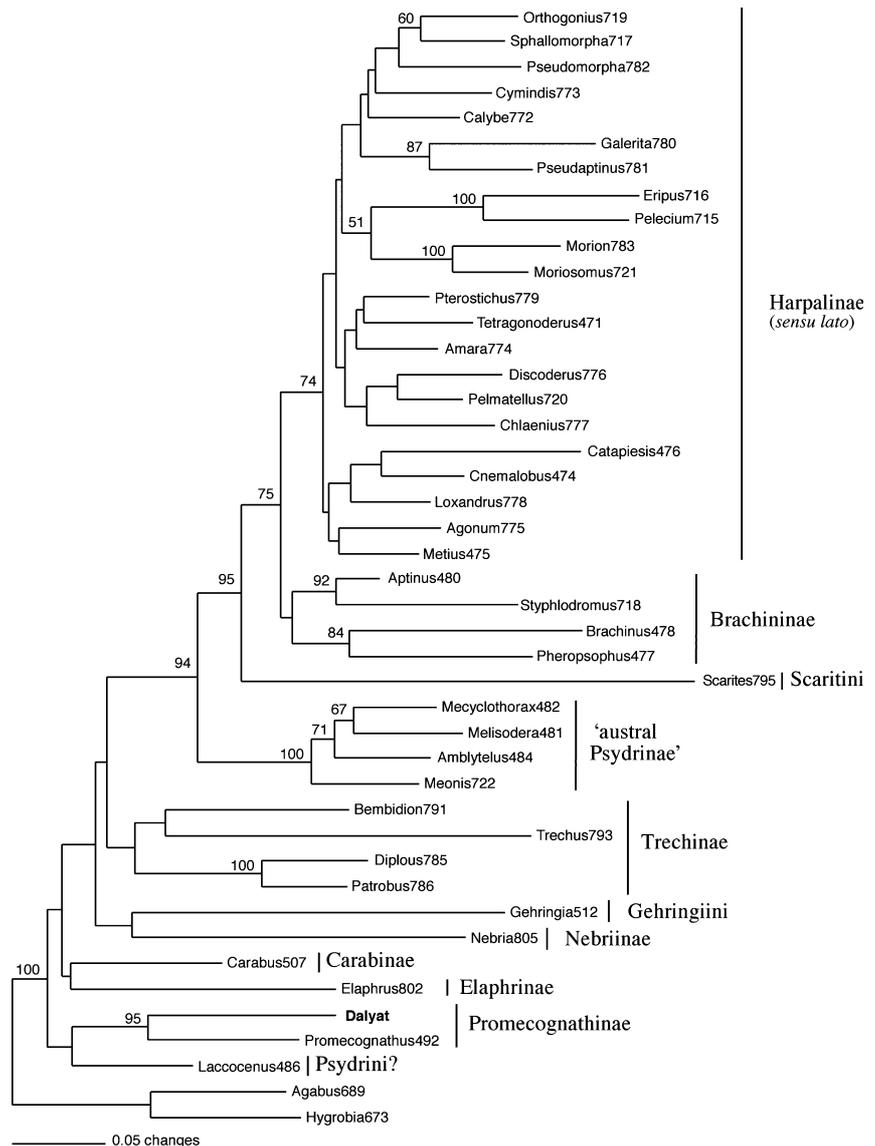
Fig. 4. Single most parsimonious tree obtained with the analysis of the combined reduced data matrix (44 taxa, *wingless* and 18S rRNA), with characters reweighted according to the rescaled consistency index. Numbers above branches, bootstrap support value of the node in the analysis of the equally weighted characters; below branches, bootstrap support value of the node in the analysis of the reweighted characters. Numbers inside nodes, partitioned Bremer support values of the nodes in the analyses with equally weighted characters (18S rRNA/*wingless*). With white circles, nodes present in the strict consensus of the most parsimonious trees obtained with equally weighted characters. Numbers after the genus-names refer to the last three figures of the accession number of the 18S rRNA sequence (see Table 1)

with *Nomius* and *Psydrus*. They were considered to be the most primitive tribe of the subfamily, with many 'archaic' characters (Moore 1963), although in our analyses (as in Maddison et al. 1999; Ober 2002) it was never related with *Psydrus* or the other austral Psydrinae, which form a strongly supported monophyletic group. This raised the possibility of *Laccocenus* being the Australian member of an even more widely distributed Promecognathinae. However, the study of a specimen of *Laccocenus ambiguus* Sloane from South Queensland indicated that it is not closely related to the Promecognathinae, as we could not find any potential character linking both taxa to the exclusion of other basal lineages of Carabidae. In any case, it is worth noting that the morphology of *L. ambiguus* shows important differences with respect to other Psydrinae (*sensu* Moore 1963 and Baehr 1998). These include the presence of a conspicuous crown of flat and well sclerotized spines at the apex of the protibia, suggesting that even if it is related to the Psydrinae (with the exclusion of the 'austral' group *sensu* Maddison et al. 1999), *Laccocenus* should be considered a unique and quite isolated genus both for its morphology and DNA sequence.

The estimated divergence between *Dalyat* and *Promecognathus* is slightly older than the origin of Harpalinae, which are well documented to have radiated in the Cretaceous (Ponomarenko 1977). This gives a minimum age of the separation of the two lineages of *c.* 150 MY, i.e. compatible with a vicariant origin because of the separation of the Iberian plate from the Pangean continent (Barron et al. 1981; Smith et al. 1994; placed at 130–120 MY by the Ocean Drilling Stratigraphic Network 2004, <http://www.odsn.de/odsn/services/paleomap/paleomap.html>), as proposed in Mateu and Bellés (2003). In the same paper, the authors establish the closer morphological relationship between the Nearctic *Promecognathus* and the South African Axiniidiini, which would have diverged at a later age (end of Cretaceous, after the separation of Laurasia and Gondwana, Mateu and Bellés 2003).

Despite the multiple uncertainties surrounding the use of molecular clocks to obtain age estimates (Bromham and Penny 2003), it has proved an extremely valuable approach to confirm – or reject – biogeographical hypotheses which would have been otherwise virtually impossible to test (e.g. Trewick 2000; Raxworthy et al. 2002; Moyle 2004; De Queiroz 2005).

Fig. 5. Tree obtained with the analysis of the combined reduced data matrix (44 taxa, *wingless* and 18S rRNA) in PHYL, using a GRT + I + G model of nucleotide substitution. Numbers above branches, bootstrap support value of the node. Numbers after the genus-names refer to the last three figures of the accession number of the 18S rRNA sequence (see Table 1)



In our case, a closer relationship between *Promecognathus* and *Dalyat* (similar to that of typical genera within a tribe, Table 1 and Fig. 6) would have made very unlikely the vicariant ancient origin proposed by Mateu and Bellés (2003), and would have required an alternative historical explanation for the origin of the lineage leading to *Dalyat*.

#### A reappraisal of Carabidae phylogeny

The analysis of the phylogenetic placement of *D. mirabilis* offered the opportunity to revisit the relationships of the main lineages within Carabidae. With our taxon set and analytical methods, in the combined analyses of the 18S rRNA and *wingless* we obtain very robust relationships among what could be called the 'higher' Carabidae, in particular a strong monophyly of Harpalinae (including Morionini, Peleciini and Pseudomorphini) and of the clade Harpalinae plus Brachininae. Peleciini had been related to Promecognathinae (Bousquet and LaRochelle 1993), but we never found any link between *Eripus* and *Pelecium* and *Dalyat* or *Promecognathus*.

There is somehow less support for the monophyly of Brachininae, although it was recovered in all trees, with the inclusion of *Aptinus* (which position was dubious according to Maddison et al. 1999). The inclusion of the austral Psydrinae and of Scaritini in this highly supported clade is in agreement with Ober (2002), although our results show a general higher node support and concordance among different analytical methods, perhaps because of the exclusion of three of the members of the problematic 'PCRS quartet' (Maddison et al. 1999) (we kept *Scarites* due to its potential relationship with *Dalyat*, Mateu and Bellés 2003). The analyses of the phylogenetic position of these groups is beyond the scope of our work, but the problem of the long branches seems to be restricted to the 18S rRNA sequence, as there is no evidence of unusual levels of divergence in the *wingless* sequence of any of them.

The relationships among the basal lineages of the family are much less robust, although in general with higher resolution and support than in Maddison et al. (1999) and Ober (2002). It is interesting to note the strong support for the sister relationship between Patrobini and the remaining Trechinae

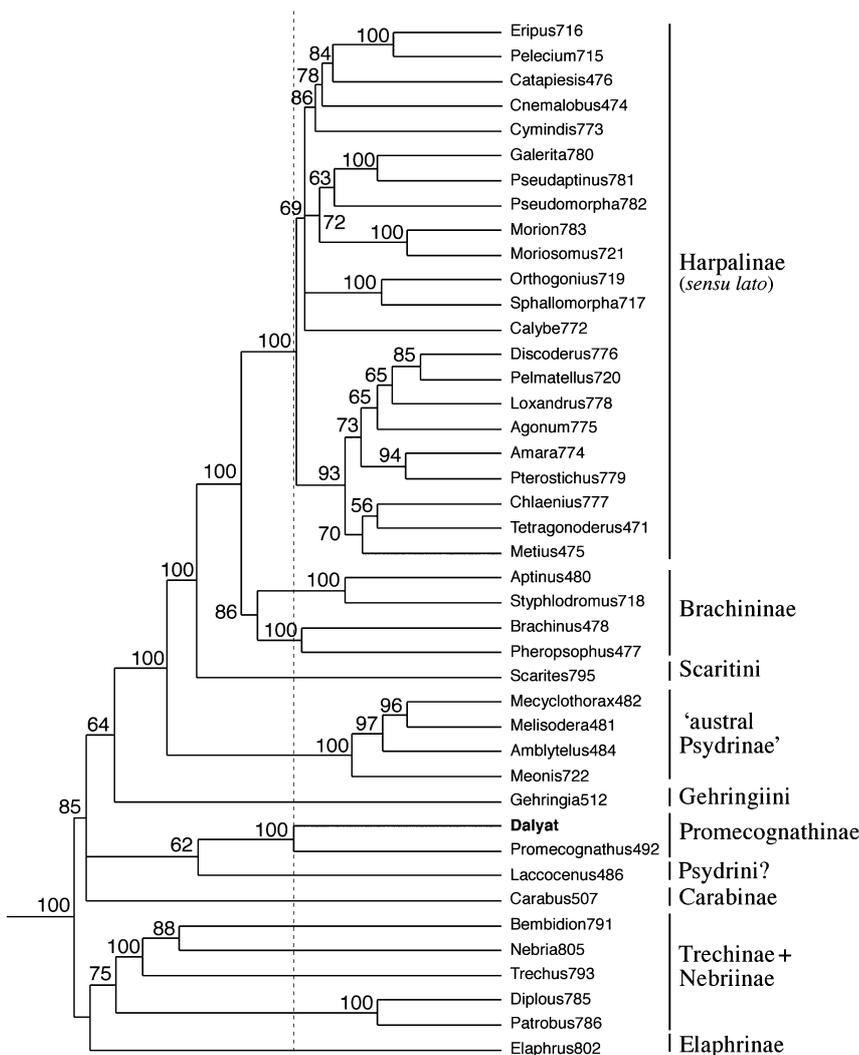


Fig. 6. Tree obtained with the analysis of the combined reduced data matrix (44 taxa, *wingless* and 18S rRNA, outgroups not represented) in MRBAYES, using a GRT + I + G model of nucleotide substitution. Numbers above branches, posterior probability of the node ( $\times 100$ ). Numbers after the genus-names refer to the last three figures of the accession number of the 18S rRNA sequence (see Table 1). Branch lengths have been modified to fit an ultrametric tree with the Penalized Likelihood method of Sanderson (2002) as implemented in the program r8s (see text for details). The discontinuous vertical line shows the relative height of the node at the base of Promecognathinae, which is very close to that at the base of Harpalinae, known to have radiated in the Cretaceous (Ponomarenko 1977)

Table 2. Estimations of the age of the divergence between *Dalyat* and *Promecognathus* using the Penalized Likelihood method of Sanderson (2002) as implemented in the program r8s, assuming different maximum and minimum constraints to the origin of Harpalinae and Carabidae (according to data in Ponomarenko 1977)

Analysis	Harpalinae			Carabidae			Promecognathinae
	Min	Max	Est	Min	Max	Est	
1	100	150	118.2	200	250	250	142.5
2	–	150	150	200	–	Failed	Failed
3	130	–	130	–	250	250	143.7

Min, minimum constrained age (MY); max, maximum constrained age (MY); est, estimated age (MY); failed, the analysis failed to converge.

(with the inclusion of *Nebria* in the Bayesian analysis), for what there was only limited support in the previous analyses, confirming the validity of the wide concept of subfamily Trechinae adopted by Löbl and Smetana (2003).

The low resolution attained with the analysis of the *wingless* sequence alone, together with the significant incongruence among the two genes used (as measured with the PHT), could question the use of a combined analyses to study the data (e.g. Huelsenbeck et al. 1996; Lecointre and Deleporte 2005). However, the combined tree with the reduced data set had a

better resolution and a general stronger support than any of the trees obtained with the separate genes. For example, the monophyly of Brachininae and its sister relationship to a monophyletic Harpalinae is only obtained in the combined analysis. Moreover, the Partition Bremer Support values show that the support of both genes is equally correlated with the combined support, suggesting that the signal of both fragments had a similar role in the final topology of the tree (although among the nodes with higher support only one has negative 18S and positive *wingless*, Fig. 4), clearly favouring a 'total evidence approach' (Grant and Kluge 2003) despite the apparent incongruence of the data partitions (as measured with the PHT).

The results of the analyses were very congruent among the three methods used, and although node support was in general higher for parsimony and Bayesian probabilities (as expected, Huelsenbeck and Rannala 2004 and references therein), it was not exceedingly higher than that obtained with a fast maximum likelihood algorithm (PHYML, Guindon and Gascuel 2003). It is interesting to note the apparent strong effect that the missing data had on the results of the combined analyses (grouping many of the taxa with missing *wingless* sequence in a highly unlikely clade both with parsimony and maximum likelihood). This suggests that, in this case, a complete data set seemed to be preferable than an extended taxon sampling.

## Acknowledgements

We are particularly thankful to the collectors of the specimen of *D. mirabilis* used for the DNA extraction for allowing us to study this most interesting material. We are also thankful to Alfried P. Vogler and Christine Taylor (NHM, the Natural History Museum, London) for the facilities used to obtain the 18S rRNA sequence and sending material of Psydrinae for study respectively. IR was a Marie Curie research fellow in the NHM while doing part of this work, finished as a Ramón y Cajal researcher in the MNCN (Madrid).

## Resumen

*Relaciones filogenéticas de Dalyat mirabilis* Mateu, 2002, con una revisión de la filogenia molecular de los carábidos (Coleoptera, Carabidae)

*Dalyat mirabilis* Mateu, 2002 (Coleoptera: Carabidae) es una especie cavernícola recientemente descrita del sureste de España, y que, en base a un análisis morfológico, se ha relacionado con los Promecognathinae (con un género al oeste de Norteamérica y cuatro géneros en Sudáfrica). En este trabajo estudiamos sus relaciones filogenéticas en base a la secuencia completa del gen 18S rRNA y a un fragmento del gen *wingless*, utilizando parsimonia, un algoritmo rápido de máxima verosimilitud (implementado en el programa PHYLML), y probabilidades a posteriori bayesianas. Aunque cuando se utiliza solamente *wingless* las relaciones de *Dalyat* no están bien definidas, tanto con el gen 18S rRNA como en el análisis conjunto con los tres métodos utilizados se encontró un fuerte soporte a la relación de *Promecognathus* y *Dalyat* como grupos hermanos. Aplicando técnicas de relojes moleculares se estimó que los dos linajes divergieron a una edad similar (o algo anterior) al origen de Harpalinae, de los que se sabe que experimentaron una fuerte radiación evolutiva en el Cretácico. Esta antigüedad es compatible con un origen del linaje que eventualmente daría lugar a *Dalyat* por vicarianza al aislarse la placa Ibérica del resto de Pangea en el Jurásico tardío – Cretácico temprano. Otras relaciones con soporte alto dentro de Carabidae son la monofilia de Harpalinae (con la inclusión de Morionini, Peleciini y Pseudomorphini), su relación de grupo hermano con Brachininae, y la inclusión de estas dos subfamilias, junto a Scaritini y a los Psydrinae australes, en un clado con soporte alto (los Carabidae “superiores”).

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