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Molecular Phylogenetics and Evolution 55 (2010) 358-371

Contents lists available at ScienceDirect







journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny and Holarctic diversification of the subtribe Calathina (Coleoptera: Carabidae: Sphodrini)

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ARTICLE INFO

Article history: Received 25 November 2008 Revised 16 October 2009 Accepted 19 October 2009 Available online 10 November 2009

Keywords: Calathus Calathina Carabidae Sphodrini Phylogeny Holarctic region Macaronesian archipelagos Nearctic region Speciation cox1-cox2 Nuclear markers

1. Introduction

ABSTRACT

A molecular phylogeny of the subtribe Calathina was inferred from DNA sequence data from the mitochondrial cox1-cox2 region and the nuclear genes 28S and EF-1 α . All lineages within Calathina from the Holarctic region were represented except for the monotypic subgenus *Tachalus*. Maximum Parsimony and Bayesian analyses of the combined data set showed that the subtribe is a monophyletic lineage that includes a single genus *Calathus*, where other taxa currently ranked as independent genera (*Lindrothius*, *Synuchidius*, *Thermoscelis* and *Acalathus*) are nested within this genus. *Neocalathus* and *Lauricalathus*, both subgenera of *Calathus*, were found to be polyphyletic and in need of taxonomic revision. The subtribe appears to have originated in the Mediterranean Basin and thereafter expanded into most parts of the Palearctic region, the Macaronesian archipelagos (at least five independent colonisation events), the Ethiopian highlands and the Nearctic region (at least two independent events).

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According to the last Palearctic catalog (Hovorka and Sciaky, 2003), the subtribe Calathina includes four genera, the Holarctic *Calathus* Bonelli 1810, the monotypic genera *Thermoscelis* (Caucasus) and *Synuchidius* (Balkan Peninsula), and the Caucasian genus *Lindrothius*, (14 species). *Calathus* is the richest in species number (about 164), particularly in the Euromediterranean subregion. There are 19 species in the Nearctic region, 3 species in the Oriental region and 17 in the Afrotropical region. Members of *Calathus* are common in most temperate areas. Some lineages are composed of forest-dwelling and often flightless species, with low dispersal power and a restricted distribution. Other lineages include winged species with high dispersal power that are broadly distributed and often found in open and mesic to xeric habitats.

The systematics of the subtribe is based on morphological characters (Putzeys, 1873; Schatzmayr, 1937; Lindroth, 1956; Ball and Nègre, 1972; Perrault, 1977). However, characters commonly used to define species and to rank supraspecific taxa (pronotum shape,

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elytral vestiture, etc.) are variable (e.g., Ball and Nègre, 1972, p. 518) and thus less suitable for defining evolutionary lineages. A previous molecular study of the tribe Sphodrini (Ruiz et al., 2009) found Calathina to be a monophyletic clade on the basis of mitochondrial and nuclear markers and estimated its origin to be between 10 and 26 Mya (million years ago), thus suggesting that it is a relatively young and species rich group. In the present study we aim to reconstruct the phylogenetic history of this subtribe, to investigate evolutionary relationships among species, diversification rates, geographical origins of main lineages, and the colonisation of the Holarctic, with emphasis on the West-Palearctic region. Hypotheses to be investigated in this study are derived from taxonomical uncertainties. Acalathus is a genus that was treated as a subgenus of Calathus by Lindroth (1956) and Perrault (1977), as a genus closely related to Calathus (Casale, 1988), or more distantly related and placed in the subtribe Dolochina (Hovorka and Sciaky, 2003; Lorenz, 2005). Likewise Lindroth (1956) considered Dolichus as a species group within an inclusive genus Calathus, while other authors have placed this genus separate from Calathus in the subtribe Dolichina (Hovorka and Sciaky, 2003; Lorenz, 2005). The status and limits of certain taxa within Calathina is controversial and we assess hypotheses related to the taxonomic status of proposed

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genera that may be separate from *Calathus* (e.g., *Thermoscelis*, *Lindrothius*) or well-delimited subgenera within this genus (*Amphyginus*, *Bedelinus*, *Neocalathus*, *Lauricalathus*).

Several lineages are particularly species rich on the Macaronesian Islands west of the African coast. Previous studies on these lineages based solely on mitochondrial data have indicated that multiple colonisations have occurred on those islands (Emerson et al., 1999, 2000, see also Ruiz and Serrano, 2006). We further assess this hypothesis with a much broader selection of continental taxa. We have included representative species from nearly the entire geographical range of the subtribe Calathina, and analyzed their phylogenetic relationship using a mitochondrial fragment comprising cox1-tRNAleu-cox2 and fragments of the nuclear genes 28S and EF-1a. Because rDNA and intron sequences frequently include insertions and deletions in multiple alignments, we have explored the effects of different alignment strategies on the reconstruction of the phylogenetic relationships. Finally, we have explored various factors potentially causing a lack of resolution in species rich clades of late Mesozoic age.

2. Material and methods

2.1. Taxon sampling

Molecular sequence data were collected for 102 individuals of 64 species. All genera and subgenera within the subtribe Calathina have been sampled with the exception of the Mexican monotypic subgenus *Tachalus* (Table 1). Related subtribes Dolichina, Synuchina and Pristosiina were used as outgroups based on previous phylogenetic information (Ruiz et al., 2009). For a few species only dry pinned material was available, resulting in a partial sequence of the mitochondrial cox1-cox2 fragment (950–1300 bp).

2.2. PCR and sequencing

DNA was extracted with the Qiagen DNeasy tissue kit (Qiagen, Hilden, Germany). We sequenced a fragment of the mitochondrial cytochrome oxidase I and II (cox1-cox2) genes (1630 bp), which includes part of cox1 gene (865 bp), the complete tRNAleu (63 bp), and cox2 (702 bp). Within the ribosomal 28S rDNA we studied a partial sequence (D2–D4 region) of about 885 bp length. The amplified protein-coding Elongation Factor 1 α gene (EF-1 α C0) had no introns and consisted of 773 bp. Primers and PCR conditions used are described in Ruiz et al. (2009).

PCR products were purified with isopropanol and 5 M ammonium acetate, and sequencing was performed in both directions using the standard protocol for ABI BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Some polymorphic sites were found in a few sequences of EF-1 α CO, and nucleotide variation from these sites was named according to International Union of Pure and Applied Chemistry (IUPAC) symbols.

2.3. Sequence alignment

Sequences of cox1-cox2 and EF-1 α were unambiguously aligned, with insertions of 3 bp at the 3' end of cox2 aligned after translation to amino acids. To align the 28S rDNA sequences five different strategies were followed: (i) A computer-based alignment with Clustal W version 1.6 (Thompson et al., 1994) in MEGA version 4 (Tamura et al., 2007) for the entire sequence (named here as CW). Transitions were weighted 0.5 related to transversions. Different weighting regimes of the gap opening (GOP) and gap extension penalties (GEP) were assayed, namely GOP/GEP: 1/0.25, 1/0.5, 2/0.25, 2/0.5, 2/1, 4/0.5, 4/2, 8/1, 8/4, and the default 15/ 6.6 often cited in many papers, resulting in 10 different matrices for each rDNA matrix. (ii) A manual alignment based on secondary structure (Kjer, 1995) following the model of Gillespie et al. (2004) (abbreviated as SS). To characterize the expansion segments we used the Mfold software (http://frontend.bioinfo.rpi.edu/applications/ mfold/cgi-bin/rna-form1.cgi) which folds RNA sequences based on minimum free energy (Zuker, 2003). Secondary structure was used to infer unambiguous or conserved regions (28Sc) and ambiguous or variable regions (28Sv), and ambiguous regions were thereafter excluded from analyses. (iii) A combination of the first two approaches (mixed approach or MX), in which the unambiguous and ambiguous regions were first defined based on secondary structure and the ambiguous regions (28Sv) were subsequently aligned in Clustal W using the parameters listed above. (iv) The ClustalW alignment of the ambiguous regions was also performed by anchoring to an unambiguously aligned flank of 10-15 bp on each side (named here as MXan). (v) A manual alignment using ClustalW as the starting point (abbreviated as MA).

Sensitivity analyses were carried out to evaluate different alignment parameters and strategies. Congruence of 28S alignments using different GOP/GEP and unambiguously aligned protein-coding genes (cox1-cox2, EF-1 α) was selected as the optimality criterion for further combined analyses. Congruence was measured as the contribution of the gene partition to the Bremer Support (BS) (Bremer, 1994) of the combined analysis (Baker and DeSalle, 1997). This contribution was calculated with BS and Partitioned Bremer Support (PBS) in TreeRot v.3 (Sorenson and Franzosa, 2007). PBS values were standardized for partitions of different length by dividing the PBS value by the minimum possible length of each branch (DeBry, 2001). The relative PBS support for each node of each gene partition was also measured and these measures were related to the accumulated length from the root of the tree to the actual node in the combined data topology.

2.4. Phylogenetic inference

Each data partition was analyzed separately and in combination under Maximum Parsimony (MP), as implemented in PAUP b4.0b10 (Swofford, 2002), and Bayesian analysis (BA) with Mr. Bayes v3.1. (Ronquist and Huelsenbeck, 2003). All data sets were analyzed with Modeltest (v.3.6) (Posada and Crandall, 1998) to find the best-fit model of sequence evolution. The Akaike information criterion (AIC) (Akaike, 1973) was preferred over hLRTs to test the various models as recommended by Posada and Buckley (2004). Parsimony analyses were conducted with 10,000 random addition replicates of heuristic searches and TBR branch swapping. Confidence in each node was assessed by bootstrapping 500 heuristic searches replicates with 20 random additions per replicate. Bayesian analyses were performed with 1,500,000 generations for separate analyses, and 3,000,000 generations for combined analyses, sampling trees every 100 generations. Likelihood values were observed with Tracer v1.4 (Rambaut and Drummond, 2005), discarding all trees before stability in likelihood values as a burn-in (first 3000 trees in separate analyses and 6500 trees in the combined ones). Stationarity was also reassessed using the convergence diagnostic: the average standard deviation of split frequencies and the potential scale reduction factor (PSRF).

To explore long-branch attraction (LBA) artifacts a number of strategies indicated by Bergsten (2005) were followed: analyses of unlinked markers under various tree-reconstruction methods with different sensitivity to LBA, different partition strategies under different models of molecular evolution, removal of a putatively wrongly placed taxon with a re-run of the analysis to check whether the remaining long branched taxa change position, and inclusion of all available sequences of main lineages of *Calathina* and *Dolichina* from GenBank.

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Table 1

Accession numbers and locality for the taxa of the subtribe Calathina included in the study. AG: Algeria, BU: Bulgaria, CI: Canary Islands, ET: Ethiopia, GG: Georgia, IT: Italy, JA: Japan, MC: Macedonia, MEX: Mexico, MO: Morocco, MR: Madeira, NR: Norway, PL: Poland, PT: Portugal, SP: Spain, TR: Turkey, UK: United Kingdom, USA: United States of America.

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C (Increate adds) actiniziona 115 Cabezo de Manzaneda, Orense (SP) AVM 10887 GU25437 GU25437 GU25437 GU25437 GU254373 GU254368 GU254373 GU254368 GU254373 GU254368 GU254373 GU254368 GU254373 GU254363 GU254373 GU254363 GU254353 GU254353 GU254353 GU254353 GU254373 GU254363 GU254373 GU254363 GU254373 GU254363 GU254373 GU254373 </td <td>C. (Iberocalathus) rotundatus rotundatus</td> <td>21</td> <td>Braganza (PT)</td> <td>AM410886</td> <td>GU254351</td> <td>GU254439</td>	C. (Iberocalathus) rotundatus rotundatus	21	Braganza (PT)	AM410886	GU254351	GU254439
C (Incrette sedis) continuita 253 Mount. Wachacha/ET) CU2544329	C. (Iberocalathus) rotundatus rotundatus	115	Cabezo de Manzaneda, Orense (SP)	AM410887	GU254387	GU254472
C (<i>Increta sedis</i>) constinuaria 84 Chao de Ribiera Seikal (MR) Apl404998 CU254373 CU254374 C (<i>Increta sedis</i>) virbitas 86 Rebecal (MR) Apl404002 CU254375 CU254436 C (<i>Inarcicalmus</i>) abbacides 69 Tenerife (CI) Apl36975 CU254358 CU254435 C (<i>Iauricalmus</i>) amgutatis 95 Tenerife (CI) Apl404973 CU254358 CU254435 C (<i>Iauricalmus</i>) amgutatis 95 Tenerife (CI) Apl404981 GU254378 CU254436 C (<i>Iauricalmus</i>) accendens 93 Tenerife (CI) Apl404981 GU254378 CU254436 C (<i>Iauricalmus</i>) accendens 93 Tenerife (CI) Apl404983 GU254378 CU254436 C (<i>Iauricalmus</i>) carantaris 87 Tenerife (CI) Apl404983 GU254377 CU254437 C (<i>Iauricalmus</i>) carantaris 87 Tenerife (CI) Apl404977 GU254377 CU254432 C (<i>Iauricalmus</i>) generics 55 La Gomera (CI) Apl36968 GU254373 GU254457 C (<i>Iauricalmus</i>) generics 71 <td< td=""><td>C. (Incertae sedis) aethiopicus</td><td>253</td><td>Mount. Wachacha.(ET)</td><td>GU254329</td><td></td><td>GU254495</td></td<>	C. (Incertae sedis) aethiopicus	253	Mount. Wachacha.(ET)	GU254329		GU254495
C (<i>incerta</i> sedi) compliandus 83 Rebecal (MR) A)40909 CU25473 CU25473 CU254742 C (<i>incerta</i> sedi) windus 66 Rebecal (MR) A)405002 CU25475 CU254762 C (<i>inaricalathus</i>) onguitaris 69 Tenerife (O) A)236975 CU254785 CU254747 C (<i>inaricalathus</i>) ongenificialus 95 Tenerife (O) A)404973 CU254785 CU254747 C (<i>inaricalathus</i>) ongenificialus 93 Tenerife (O) A)404980 CU254783 CU254746 C (<i>inaricalathus</i>) cornariss 84 Gran Canaria (O) A)236956 CU254767 C (<i>inaricalathus</i>) cornariss 87 Tenerife (O) A)404983 CU254767 C (<i>inaricalathus</i>) cognatus 52 La Gomera (O) A)404978 CU254777 CU254475 C (<i>inaricalathus</i>) gomerniss 55 La Gomera (O) A)236968 CU254737 CU254476 C (<i>inaricalathus</i>) gennerniss 71 Tenerife (O) A)246979 CU254476 CU254462 C (<i>inaricalathus</i>) gennerniss 7	C. (Incertae sedis) colasianus	84	Chao de Ribiera Seixal (MR)	AJ404998	GU254374	GU254461
C (Increte sets) violats 86 Rebecal (MR) AJ26072 CU254375 CU254475 C (Inurcialathus) abaxilies 69 Tenerife (C1) AJ269475 CU254378 CU254456 C (Inurcialathus) angustulus 95 Tenerife (C1) AJ404973 CU254378 CU254456 C (Inurcialathus) accenders 93 Tenerife (C1) AJ404980 CU254378 CU254464 C (Inurcialathus) accusulus 97 Tenerife (C1) AJ269655 CU254381 CU254448 C (Inurcialathus) cannerus 87 Tenerife (C1) AJ269666 CU254376 CU2544450 C (Inurcialathus) cannerus 87 Tenerife (C1) AJ269666 CU254377 CU254450 C (Inurcialathus) cognerus 52 La Comera (C1) AJ269676 CU254377 CU254450 C (Inurcialathus) freyi 94 Tenerife (C1) AJ269678 CU254361 CU254450 C (Inurcialathus) generemsis 15 La Gomera (C1) AJ269678 CU254361 CU254449 C (Inurcialathus) merculus 71 Tenerife (C1) AJ264	C. (Incertae sedis) complanatus	83	Rebecal (MR)	AJ404999	GU254373	GU254460
C. (<i>Lanicalathus</i>) angularis 44 Gen Canaria (1) A)226975 GU254457 C. (<i>Lanicalathus</i>) angustulus 95 Tenerife (1) A)246947 GU254359 GU254447 C. (<i>Lanicalathus</i>) appendiculus 40 Gen Canaria (1) A)246956 GU254378 GU254447 C. (<i>Lanicalathus</i>) acentars 93 Tenerife (1) A)404980 GU254378 GU254466 C. (<i>Lanicalathus</i>) acutra 97 Tenerife (1) A)404981 GU254378 GU254466 C. (<i>Lanicalathus</i>) acutra 87 Tenerife (1) A)404983 GU254362 GU254451 C. (<i>Lanicalathus</i>) compartus 52 La Gomera (1) A)246966 GU254363 GU254451 C. (<i>Lanicalathus</i>) forpi 94 Tenerife (1) A)246961 GU254363 GU254451 C. (<i>Lanicalathus</i>) morelica 61 La Gomera (1) A)236961 GU254366 GU254454 C. (<i>Lanicalathus</i>) moreliza 71 Tenerife (1) A)249695 GU254366 GU254454 C. (<i>Lanicalathus</i>) moreliza 71 Tenerife (1) A)249695	C. (Incertae sedis) vividus	86	Rebecal (MR)	AJ405002	GU254375	GU254462
C. (<i>Lauricalathus</i>) <i>angustuis</i> 44 Gran Canaria (C) AJ248947 CU254479 CU254479 C. (<i>Lauricalathus</i>) <i>angustuis</i> 95 Tenerife (C) AJ404973 CU254480 CU254464 C. (<i>Lauricalathus</i>) <i>accendens</i> 93 Tenerife (C) AJ404980 CU254478 CU254464 C. (<i>Lauricalathus</i>) <i>accendens</i> 97 Tenerife (C) AJ404980 CU254378 CU254462 C. (<i>Lauricalathus</i>) <i>canarins</i> 87 Tenerife (C) AJ4049805 CU2544760 CU2544480 C. (<i>Lauricalathus</i>) <i>canarins</i> 87 Tenerife (C) AJ4049877 CU2544762 CU2544762 C. (<i>Lauricalathus</i>) <i>capretus</i> 52 La Comera (C) AJ404977 CU2544780 CU2544762 C. (<i>Lauricalathus</i>) <i>freyi</i> 94 Tenerife (C) AJ404977 CU2544762 CU2544762 C. (<i>Lauricalathus</i>) <i>stress</i> 71 Tenerife (C) AJ236966 CU254366 CU254476 C. (<i>Lauricalathus</i>) <i>ambiguus</i> muticolis 175 Gauera (T) AJ236970 CU254476 CU254476 C. (<i>Lauricalathus</i>) <i>ambiguus</i> muticoli	C. (Lauricalathus) abaxoides	69	Tenerife (CI)	AJ236975	GU254368	GU254456
C. (<i>Auricalathus</i>) appendicultus 95 Tenerife (C) A 404973 GU254480	C. (Lauricalathus) angularis	44	Gran Canaria (CI)	AJ236947	GU254359	GU254447
C. (Lauricalathus) ascenders) 93 Tenerife (C1) A 404980 CU254378 CU254446 C. (Lauricalathus) acutus) 97 Tenerife (C1) A 404980 CU254378 CU254466 C. (Lauricalathus) containts) 87 Tenerife (C1) A 236955 CU254378 CU254466 C. (Lauricalathus) containts) 87 Tenerife (C1) A 240985 CU254376 CU254445 C. (Lauricalathus) cognitus) 52 La Gomera (C1) A 240977 CU254377 CU254463 C. (Lauricalathus) foryi 94 Tenerife (C1) A 240977 CU254463 CU254463 C. (Lauricalathus) generensis 55 La Gomera (C1) A 236961 CU254366 CU254445 C. (Lauricalathus) generensis 55 La Gomera (C1) A 236970 CU254366 CU254445 C. (Lauricalathus) ambiguus 10 Gran Saso (T1) A 240992 CU254366 CU254444 C. (Auricalathus) ambiguus migiolitis 172 Skaltutan Pass (TR) CU254307 CU254377 CU254445 C. (Nococalathus) antitrinsis 16 <t< td=""><td>C. (Lauricalathus) angustulus</td><td>95</td><td>Tenerife (CI)</td><td>AJ404973</td><td>GU254380</td><td></td></t<>	C. (Lauricalathus) angustulus	95	Tenerife (CI)	AJ404973	GU254380	
C (<i>Lauricalathus</i>) auctus 93 Tenerife (C) AJ404980 GL254378 GL254465 C (<i>Lauricalathus</i>) canariesis 48 Gran Canaria (C) AJ404983 GL254366 CL254465 C (<i>Lauricalathus</i>) canariesis 87 Tenerife (C) AJ404983 GL254376 CL254463 C (<i>Lauricalathus</i>) corpartus 52 La Comera (C) AJ404977 GL254377 GL254463 C (<i>Lauricalathus</i>) depressus 89 Tenerife (C) AJ404977 GL254377 GL254463 C (<i>Lauricalathus</i>) gomernsis 55 La Gomera (C) AJ236961 GL254361 GL254451 C (<i>Lauricalathus</i>) gomernsis 51 La Gomera (C) AJ236950 GL254366 GL254454 C (<i>Lauricalathus</i>) ambiguus sumbiguus 28 Sora (SP) GL254366 GL254456 GL2544456 GL2544456 GL2544461 GL254456 GL2544456 GL2544461 GL2544626 GL2544462 GL/Recolathus ambiguus migiolitis 122 Skaltutan Pas (TR) GL254307 GL254466 GL254442 GL254442 GL254442 GL254442 GL254442 <td< td=""><td>C. (Lauricalathus) appendiculatus</td><td>40</td><td>Gran Canaria (CI)</td><td>AJ236956</td><td>GU254358</td><td>GU254446</td></td<>	C. (Lauricalathus) appendiculatus	40	Gran Canaria (CI)	AJ236956	GU254358	GU254446
C. (Lauricalathus) cancins 97 Tenerife (C) AJ404981 CL254460 C. (Lauricalathus) carinatus 87 Tenerife (C) AJ404983 GU254360 C. (Lauricalathus) cognitatus 52 La Gomera (C) AJ259656 GU254362 GU254426 C. (Lauricalathus) cognitatus 52 La Gomera (C) AJ404977 GU254377 GU254465 C. (Lauricalathus) foryi 94 Tenerife (C) AJ404977 GU254379 GU254456 C. (Lauricalathus) foryi 94 La Gomera (C) AJ2369616 GU254366 GU254456 C. (Lauricalathus) morellae 61 La Gomera (C) AJ236959 GU254366 GU254456 C. (Incocalathus) ambiguus 28 Soria (SP) GU254371 GU254431 GU254437 C. (Noccalathus) ambiguus rugicolits 175 Güven (TR) GU254390 GU2544747 GU2544747 C. (Noccalathus) asturiensis 16 Galacia (SP) AJ256945 GU254477 GU254437 C. (Noccalathus) asturiensis 16 Galacia (SP) AJ2549422 GU254437	C. (Lauricalathus) ascendens	93	Tenerife (CI)	AJ404980	GU254378	GU254464
C (Lauricalathus) canneriesis 48 Gran Canaria (CI) AJ236955 GL22544360 GL2254436 C (Lauricalathus) cognatus 52 La Comera (CI) AJ404983 GL254377 GL2254463 C (Lauricalathus) cognatus 52 La Comera (CI) AJ404977 GL2254477 GL2254473 C (Lauricalathus) feryi 94 Tenerife (CI) AJ404977 GL2254473 GL2254451 C (Lauricalathus) gomerensis 55 La Comera (CI) AJ256970 GL2254436 GL2254451 C (Lauricalathus) marcillae 61 La Gomera (CI) AJ256970 GL254366 GL254454 C (Lauricalathus) marcillae 61 La Gomera (CI) AJ236978 GL254341 GL254436 C (Invocalathus) ambiguus anbiguus 28 Soria (SP) GL254306 GL254436 GL254436 C (Neocalathus) ambiguus rugiolitis 172 Sakalutan Pass (TR) GL254306 GL254447 GL254436 C (Neocalathus) asturiensis 16 Galicia (SP) GL254300 GL254430 GL254447 C (Neocalathus) asturiensis 17	C. (Lauricalathus) auctus	97	Tenerife (CI)	AJ404981	GU254381	GU254466
C. (Lauricalathus) corinatus 87 Tenerife (C) AJ404983 GU254376 C. (Lauricalathus) copressus 89 Tenerife (C) AJ404977 GU254372 GU254450 C. (Lauricalathus) feryi 94 Tenerife (C) AJ404977 GU254379 GU254451 C. (Lauricalathus) prevensis 55 La Comera (C) AJ236966 GU254361 GU2544451 C. (Lauricalathus) amerensis 55 La Comera (C) AJ236968 GU254366 GU2544451 C. (Lauricalathus) ameretus 71 Tenerife (CI) AJ4049792 GU254341 GU2544457 C. (Neccelathus) ambiguus miguus 10 Gran Saso (TT) AJ404992 GU254341 GU254437 C. (Neccelathus) ambiguus rugicollis 175 Given (TR) GU254308 GU254347 GU254437 C. (Neccelathus) asturiensis 16 Galcici (SP) AJ236945 GU254347 GU254437 C. (Neccelathus) asturiensis 16 Galcici (SP) GU254308 GU254347 GU254432 C. (Neccelathus) asturiensis 16 Galcici (SP)<	C. (Lauricalathus) canariensis	48	Gran Canaria (CI)	AJ236955	GU254360	GU254448
C (Lauricalathus) cignatus 52 La Gomera (CI) AJ263966 CU254362 CU254452 C (Lauricalathus) feyi 94 Tenerife (CI) AJ404977 CU254377 CU254453 C (Lauricalathus) gomernsis 55 La Comera (CI) AJ236961 CU254363 CU254451 C (Lauricalathus) metricola 49 La Comera (CI) AJ236959 CU254366 CU254454 C (Lauricalathus) metricola 61 La Comera (CI) AJ236959 CU254366 CU254454 C (Louricalathus) metricola 91 Gara Sasso (IT) AJ26959 CU254366 CU254457 C (Neocolathus) ambiguus antiguus 10 Gran Sasso (IT) AJ404992 CU254346 CU254442 C (Neocolathus) ambiguus surgicollis 172 Sakaltutan Pass (TR) CU254307 CU254442 CU254442 C (Neocolathus) asturiensis 16 Galicia (SP) AJ236945 CU254437 CU254432 C (Neocolathus) asturiensis 12 Parque ed Avao (PT) CU254300 CU254474 CU254432 C (Neocolathus) asturiensis 13 Galicia (SP) AJ263954 CU254432 CU254432	C. (Lauricalathus) carinatus	87	Tenerife (CI)	AJ404983	GU254376	
C. (Laurcalathus) depressus 89 Tenerife (CI) AJ404977 CU254377 CU2544363 C. (Laurcialathus) generensis 55 La Gomera (CI) AJ236961 CU254363 CU254464 C. (Laurcialathus) guarcitola 49 La Gomera (CI) AJ236968 CU254366 CU254454 C. (Laurcialathus) marcellae 61 La Comera (CI) AJ236959 CU254366 CU254454 C. (Neccalathus) ambiguus ambiguus 10 Gran Sasso (IT) AJ404992 CU254366 CU254454 C. (Neccalathus) ambiguus suigicollis 172 Sakaltutan Pass (TR) CU254296 CU254374 CU254437 C. (Neccalathus) ambiguus suigicollis 175 Güven (TR) CU254308 CU254304 CU254437 C. (Neccalathus) asturiensis 127 Parque de Alvao (PT) CU254300 CU254390 CU254437 C. (Neccalathus) asturiensis 127 Parque de Alvao (PT) CU254303 CU254437 CU254436 C. (Neccalathus) asturiensis 127 Parque (MEX) CU254333 CU254437 CU254436 CU254437 CU254437 <	C. (Lauricalathus) cognatus	52	La Gomera (CI)	AJ236966	GU254362	GU254450
L. [Lunraduttus) rey 94 Tenente (CI) AJ236961 GU254379 GU254379 GU2543763 C. [Lunraduthus) gomerensis 55 La Gomera (CI) AJ236966 GU254361 GU254431 C. [Lunraduthus) mareticola 49 La Gomera (CI) AJ236970 GU254366 GU2544344 C. [Lunraduthus) metus 71 Tenerife (CI) AJ236959 GU254364 GU2544344 C. (Noccalathus) ambiguus ambiguus 28 Soria (SP) GU254376 GU2544376 GU2544376 C. (Noccalathus) ambiguus rugicollis 172 Sakaluttan Pass (TR) GU254307 GU254402 CU2544421 C. (Noccalathus) astriensis 16 Galicia (SP) AJ236945 GU254477 CU254432 C. (Noccalathus) astriensis 17 Parque de Alvao (PT) GU254300 GU254437 CU254432 C. (Noccalathus) astriensis 127 Parque de Alvao (PT) GU254303 GU254417 CU254430 C. (Noccalathus) astriensis 127 Parque de Alvao (PT) GU254333 GU254417 CU254430 C. (Noccalathus) astriensis </td <td>C. (Lauricalathus) depressus</td> <td>89</td> <td>Tenerife (CI)</td> <td>AJ404977</td> <td>GU254377</td> <td>GU254463</td>	C. (Lauricalathus) depressus	89	Tenerife (CI)	AJ404977	GU254377	GU254463
L (Lauricalatius) guereinsis 55 La Comera (CI) AJ25061 GU254363 GU254436 C. (Lauricalathus) narcellae 61 La Gomera (CI) AJ250670 GU254366 GU254436 C. (Lauricalathus) nercellae 61 La Gomera (CI) AJ260970 GU254366 GU254437 C. (Lauricalathus) renzellae 61 La Gomera (CI) AJ260959 GU254366 GU254436 C. (Neocalathus) ambiguus miguolis 172 Sakaltutan Pass (TR) GU254307 GU2544301 GU254437 C. (Neocalathus) astriensis 16 Galicia (SP) AJ263045 GU254430 GU254432 C. (Neocalathus) astriensis 127 Parque ed Alvao (PT) GU254300 GU254410 CU254432 C. (Neocalathus) astriensis 127 Parque ed (MEX) GU254304 GU254417 GU254435 C. (Neocalathus) astriensis 127 Parque ed (MEX) GU254320 GU254417 GU254435 C. (Neocalathus) astriensis 137 Tamacas. Pope-Iztaccihuat (MEX) GU254333 GU254445 GU254436 C. (Neocalathus) cinctus	C. (Lauricalathus) freyi	94	Tenerife (CI)	AJ404978	GU254379	GU254465
C. (Lauricalatus) anteritoria 49 La Comera (CI) AJ25668 GU254451 GU254451 C. (Lauricalathus) marcellae 61 La Comera (CI) AJ266570 GU254366 GU254454 C. (Lauricalathus) ambiguus ambiguus 10 Gran Sasso (TI) AJ404992 GU254356 GU254437 C. (Neocalathus) ambiguus rugicollis 175 Given (TR) GU254308 GU254401	C. (Lauricalathus) gomerensis	55	La Gomera (CI)	AJ236961	GU254363	GU254451
L. (Luinriculatus) induceniae o L. La Goineri (L) Al.2 309/30 GU234396 GU234436 C. (Lauriculatus) ambiguus 10 Gran Sasso (IT) Al404992 GU254366 GU254457 C. (Neocalathus) ambiguus ambiguus zugicollis 172 Sakalutan Pass (TR) GU254307 GU254401	$C_{\rm c}$ (Lauricalathus) laureticola	49	La Gomera (CI)	AJ236968	GU254361	GU254449
C. (Uauriculatus) rectus 71 Tenter (C1) Al2 50539 GU34365 GU34365 C. (Neocalathus) ambiguus ambiguus 28 Soria (SP) GU254307 GU254316 GU254317 GU254430 C. (Neocalathus) ambiguus rugicollis 175 Güven (TR) GU254307 GU254402 GU254432 C. (Neocalathus) asturiensis 127 Parque de Alvao (PT) GU254305 GU254474 GU254447 C. (Neocalathus) asturiensis 127 Parque de Alvao (PT) GU254320 GU254437 GU254474 C. (Neocalathus) astrec 222 La Marquesa (MEX) GU254320 GU254417 GU254430 C. (Neocalathus) aztec 2267 La Marquesa (MEX) GU254323 GU254425 C. (Neocalathus) aztec 257 Tlamacas. Popo-Iztaccihuat (MEX) GU254333 GU254438 GU254436 C. (Neocalathus) cinctus 117 Norloh Brecklands (UK) Al404997 GU254334 GU254436 C. (Neocalathus) cinctus 113 Kizildag Pass (TR) GU254304 GU254377 GU254436 C. (Neocalathus) c	C. (Lauricalathus) marcellae	01	La Gomera (CI)	AJ236970	GU254300	GU254454
L (Necodiathus) ambiguus 10 Chan Jasso (11) Apto 392 GG2 34341 GG2 34340 GG2 34340 GG2 34340 GG2 34401 GG2 34340 GG2 34340 GG2 34340 GG2 34341 GG2 34415 GG2 34415 GG2 34415 GG2 34417 GG2 34415 GG2 34443 GG2 34433 GG2 34343 GG2 34343 GG2 34343 GG2 34343 <td>C. (Lauricalathus) rectus</td> <td>/1</td> <td>Crap Sacco (IT)</td> <td>AJ250959</td> <td>GU254509</td> <td>GU254457</td>	C. (Lauricalathus) rectus	/1	Crap Sacco (IT)	AJ250959	GU254509	GU254457
C. (Neocalathus) ambiguis urgicollis 172 Sakaltutan Pass (TR) GU254230 GU254401	C. (Neocalathus) ambiguus	10	Gidii Sasso (II) Soria (SD)	AJ404992 CU254206	GU254541 CU254256	GU254450 CU254444
C. (Neocalathus) ambiguar rugicollis 172 Gawan (TR) GU254308 GU254401 GU254432 C. (Neocalathus) asturiensis 16 Galicia (SP) AJ236945 GU254330 GU254437 GU254432 C. (Neocalathus) asturiensis 127 Parque de Alvao (PT) GU254300 GU254430 GU254430 GU254430 GU254430 GU254430 GU254430 GU254430 GU254432 GU254445 GU254422 GU254425 GU254425 GU254425 GU254425 GU254433 GU254425 GU254433 GU254425 GU254433 GU254425 GU254425 GU254425 GU254425 GU254425 GU254425 GU254425 GU254435 GU254425 GU254435 GU254445 GU254445 GU254455 GU254445 GU254455 GU254445 GU254455 GU254445 GU2544455 GU254445 GU254455 GU2544455 GU254445 GU254455 GU254455 GU254456 GU254456 GU254456 GU254456 GU254456 GU254456 GU254456 GU254456<	C. (Neocalathus) ambiguus ambiguus	172	Solid (Sr) Sakaltutan Dass (TR)	GU254290 CU254307	GU254350 CU254401	G02J4444
C. (Neocalathus) asturiensis 116 Galicia (SP) Al236945 GU254330 GU254435 C. (Neocalathus) asturiensis 127 Parque de Alvao (PT) GU254300 GU254436 GU254435 C. (Neocalathus) asturiensis 127 Parque de Alvao (PT) GU254320 GU254417 GU254438 C. (Neocalathus) asturiensis 127 La Marquesa (MEX) GU254320 GU254417 GU254488 C. (Neocalathus) aster 222 La Marquesa (MEX) GU254333 GU254422 GU254496 C. (Neocalathus) olivori 257 Tlamacas. Popo-Iztaccihuat (MEX) GU254330 GU254426 GU254496 C. (Neocalathus) cinctus 107 Sierra de Urbión, Soria (SP) AM410899 GU254335 GU254468 C. (Neocalathus) cinctus 107 Sierra de Urbión, Soria (SP) AM410890 GU254396 GU254478 C. (Neocalathus) cinctus 113 Dzyanori (Ilage, Diolo (BU) GU254304 GU254436 GU254471 C. (Neocalathus) gergarius 179 Pennsylvania (USA) GU254321 GU254436 GU254473 C. (Neocalathus) gergarius 179 Pennsylvania (USA) GU254323 G	C (Neocalathus) ambiguus rugicollis	172	Given (TR)	GU254308	GU254402	GU254482
C. (Neocalathus) instruientsis 127 Parque de Alvao (PT) GU254320 GU254330 GU254474 C. (Neocalathus) astre 220 La Marquesa (MEX) GU254320 GU254415 GU2544490 C. (Neocalathus) aztec 222 La Marquesa (MEX) GU254322 GU254415 GU254425 C. (Neocalathus) aztec 267 La Marquesa (MEX) GU254330 GU254422 GU254425 C. (Neocalathus) cinctus 17 Norfolk Brecklands (UK) AJ404997 GU254357 GU254435 C. (Neocalathus) cinctus 31 Kizidag Pass (TR) GU254297 GU254356 GU254478 C. (Neocalathus) cinctus 107 Sierra de Urbión, Soria (SP) AM410889 GU254355 GU254478 C. (Neocalathus) cinctus 141 Aguelmam Afenourir Lake (MO) GU254304 GU254356 GU254478 C. (Neocalathus) erratus 27 Rila Mountains (BU) AM410889 GU254372 GU254478 C. (Neocalathus) graprius 179 Pennsylvania (USA) GU254321 GU254472 GU254475 C. (Neocalathus) graprius <	C (Neocalathus) asturiensis	16	Galicia (SP)	AI236945	GU254347	GU254435
C. (Neocalathus) aztec 220 La Marquesa (MEX) GU254320 GU254415 GU254488 C. (Neocalathus) aztec 222 La Marquesa (MEX) GU254322 GU254417 GU254490 C. (Neocalathus) aztec 267 La Marquesa (MEX) GU254333 GU254422 GU254422 GU254436 C. (Neocalathus) bolivari 257 Tlamacas. Popo-Iztaccihuat (MEX) GU25437 GU25437 GU2543436 GU254436 GU25437 GU254376 GU254436 GU254436 GU254436 GU254436 GU254436 GU254436 GU254436 GU254436 GU254376 GU254436 GU254436 GU254376 GU254476 GU254376 GU254436 GU2544	C (Neocalathus) asturiensis	127	Parque de Alvao (PT)	GU254300	GU254390	GU254474
C. (Neocalathus) aztec 222 La Marquesa (MEX) GU254322 GU254417 GU254490 C. (Neocalathus) aztec 267 La Marquesa (MEX) GU254333 GU254425 C. (Neocalathus) bolivari 257 Tlamacas. Popo-Iztaccihuat (MEX) GU254330 GU254422 GU254436 C. (Neocalathus) cinctus 17 Norfolk Brecklands (UK) AJ404997 GU254337 GU254436 C. (Neocalathus) cinctus 107 Sierra de Urbíon, Soria (SP) AM410889 GU254336 GU254448 C. (Neocalathus) cinctus 107 Sierra de Urbíon, Soria (SP) AM410889 GU254336 GU254448 C. (Neocalathus) cinctus 141 Aguelmam Afenourir Lake (MO) GU254304 GU254372 GU254478 C. (Neocalathus) erratus 113 Dzyanon village, Dolno (BU) GU254306 GU254471 GU254443 C. (Neocalathus) gregarius 179 Pennsylvania (USA) GU254321 GU254438 GU254483 C. (Neocalathus) leechi 224 Nevado de Toluca (MEX) GU254323 GU254416 GU254492 C. (Neo	C. (Neocalathus) aztec	220	La Marguesa (MEX)	GU254320	GU254415	GU254488
C. (Neocalathus) aztec 267 La Marquesa (MEX) GU254333 GU254425 C. (Neocalathus) bolivari 257 Tlamacas. Popo-Iztaccihuat (MEX) GU254330 GU254422 GU254436 C. (Neocalathus) cinctus 17 Norfolk Brecklands (UK) AJ404997 GU254375 GU254436 C. (Neocalathus) cinctus 31 Kizildag Pass (TR) GU254297 GU254333 GU254445 C. (Neocalathus) cinctus 107 Sierra de Urbión, Soria (SP) AM410889 GU254336 GU254478 C. (Neocalathus) cinctus 141 Aguelmam Afenourir Lake (MO) GU254304 GU254355 GU254478 C. (Neocalathus) erratus 27 Rila Mountains (BU) AM410890 GU254355 GU254473 C. (Neocalathus) gonzalezi 77 Lanarote (CI) AJ404986 GU254372 GU2544433 C. (Neocalathus) leechi 221 La Marquesa (MEX) GU254323 GU254416 GU254493 C. (Neocalathus) leechi 224 Nevado de Toluca (MEX) GU254325 GU254492 GU254492 C. (Neocalathus) leechi	C. (Neocalathus) aztec	222	La Marguesa (MEX)	GU254322	GU254417	GU254490
C. (Neocalathus) bolivari 257 Tlamacas. Popo-Iztaccihuat (MEX) GU254330 GU254422 GU254496 C. (Neocalathus) cinctus 17 Norfolk Brecklands (UK) AJ404997 GU254348 GU254436 C. (Neocalathus) cinctus 31 Kizildag Pass (TR) GU254297 GU254383 GU254445 C. (Neocalathus) cinctus 107 Sierra de Urbión, Soria (SP) AM410889 GU254383 GU254478 C. (Neocalathus) cinctus 141 Aguelmam Afenourir Lake (MO) GU254304 GU254355 GU254478 C. (Neocalathus) erratus 27 Rila Mountains (BU) AM410890 GU254375 GU254471 C. (Neocalathus) gregarius 179 Pennsylvania (USA) GU254321 GU254410 GU254489 C. (Neocalathus) leechi 221 La Marquesa (MEX) GU254323 GU254418 GU254492 C. (Neocalathus) leechi 226 Tlamacas. Popo-Iztaccihuat (MEX) GU254321 GU254420 GU254493 C. (Neocalathus) leechi 226 Tlamacas. Popo-Iztaccihuat (MEX) GU254323 GU254418 GU254492 <td< td=""><td>C. (Neocalathus) aztec</td><td>267</td><td>La Marquesa (MEX)</td><td>GU254333</td><td>GU254425</td><td></td></td<>	C. (Neocalathus) aztec	267	La Marquesa (MEX)	GU254333	GU254425	
C(Norfolk Brecklands (UK)AJ404997GU254348GU254346C(Neocalathus) cinctus31Kizildag Pass (TR)GU254297GU254357GU254435C(Neocalathus) cinctus107Sierra de Urbión, Soria (SP)AM410889GU254383GU254445C(Neocalathus) cinctus141Aguelmam Afenourir Lake (MO)GU254304GU254396GU254478C(Neocalathus) erratus27Rila Mountains (BU)AM410890GU254355GU254443C(Neocalathus) gonzalezi77Lanzarote (CI)AJ404986GU254372GU254459C(Neocalathus) gregarius179Pennsylvania (USA)GU254321GU254403GU254489C(Neocalathus) gregarius179Pennsylvania (USA)GU254323GU254416GU254489C(Neocalathus) leechi221La Marquesa (MEX)GU254323GU254418GU254491C(Neocalathus) leechi224Nevado de Toluca (MEX)GU254325GU254420GU254492C(Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254321GU254421GU254492C(Neocalathus) leechi240Nevado de Toluca (MEX)GU254331GU254423GU254494C(Neocalathus) melanocephalus11Gran Sasso (TT)AJ236944GU254342GU254432C(Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254343GU254432C(Neocalathus) melanocephalus11Gran Sasso	C. (Neocalathus) bolivari	257	Tlamacas. Popo-Iztaccihuat (MEX)	GU254330	GU254422	GU254496
C. (Neocalathus) cinctus31Kizildag Pass (TR)GU254297GU254357GU254445C. (Neocalathus) cinctus107Siera de Urbión, Soria (SP)AM410889GU254383GU254486C. (Neocalathus) cinctus141Aguelmam Afenourir Lake (MO)GU254304GU254396GU254478C. (Neocalathus) erratus27Rila Mountains (BU)AM410890GU254355GU254443C. (Neocalathus) erratus113Dzyanon village, Dolno (BU)GU254299GU254386GU254471C. (Neocalathus) gonzalezi77Lanzarote (CI)AJ404986GU254372GU254483C. (Neocalathus) gregarius179Pennsylvania (USA)GU254321GU254416GU254489C. (Neocalathus) leechi221La Marquesa (MEX)GU254323GU254416GU254489C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254324GU254419GU254492C. (Neocalathus) leechi224Nevado de Toluca (MEX)GU254325GU254420GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254327GU254421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254331GU254432GU254494C. (Neocalathus) melanocephalus11Gran Sasso (TT)AJ26944GU254313GU254432C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254333GU254432C. (Neocalathus) melanocephalus11Gran Sasso (TT)AJ236944GU254342GU254432<	C. (Neocalathus) cinctus	17	Norfolk Brecklands (UK)	AJ404997	GU254348	GU254436
C. (Neocalathus) cinctus107Sierra de Urbión, Soria (SP)AM410889GU254383GU254468C. (Neocalathus) cinctus141Aguelmam Afenourir Lake (MO)GU254304GU254396GU254478C. (Neocalathus) erratus27Rila Mountains (BU)AM410890GU254355GU254443C. (Neocalathus) erratus113Dzyanon village, Dolno (BU)GU254299GU254366GU254471C. (Neocalathus) gonzalezi77Lanzarote (CI)AJ404986GU254372GU254485C. (Neocalathus) gregarius179Pennsylvania (USA)GU254321GU254416GU254483C. (Neocalathus) leechi221La Marquesa (MEX)GU254323GU254418GU254491C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254324GU254418GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254420GU254493C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254327GU254421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254331GU254423GU254497C. (Neocalathus) leechi258La Marquesa (MEX)GU254318GU254432GU254432C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254432GU254432C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254333GU254432C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ246994GU254353GU254432	C. (Neocalathus) cinctus	31	Kizildag Pass (TR)	GU254297	GU254357	GU254445
C. (Neocalathus) cinctus141Aguelmam Afenourir Lake (MO)GU254304GU254396GU254478C. (Neocalathus) erratus27Rila Mountains (BU)AM410890GU254355GU254443C. (Neocalathus) erratus113Dzyanon village, Dolno (BU)GU254299GU254386GU254471C. (Neocalathus) gonzalezi77Lanzarote (CI)AJ404986GU254302GU254403C. (Neocalathus) gregarius179Pennsylvania (USA)GU254309GU254403GU254483C. (Neocalathus) leechi221La Marquesa (MEX)GU254323GU254418GU254491C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254323GU254419GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254327GU254420GU254493C. (Neocalathus) leechi240Nevado de Toluca (MEX)GU254327GU254421GU254493C. (Neocalathus) leechi258La Marquesa (MEX)GU254311GU254423GU254497C. (Neocalathus) leechi258La Marquesa (MEX)GU254318GU254413GU254497C. (Neocalathus) leechi218Mineral del Chico (MEX)GU254318GU254433GU254431C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ26944GU254333GU254433GU254432C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ26944GU254353GU254434GU254434C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ26944GU254354G	C. (Neocalathus) cinctus	107	Sierra de Urbión, Soria (SP)	AM410889	GU254383	GU254468
C. (Neocalathus) erratus27Rila Mountains (BU)AM410890GU254355GU254443C. (Neocalathus) erratus113Dzyanon village, Dolno (BU)GU254299GU254386GU254471C. (Neocalathus) gonzalezi77Lanzarote (CI)AJ404986GU254372GU254459C. (Neocalathus) gregarius179Pennsylvania (USA)GU254309GU254401GU254483C. (Neocalathus) leechi221La Marquesa (MEX)GU254321GU254416GU254489C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254323GU254419GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254420GU254493C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254327GU254421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254318GU254423GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254318GU254413GU254494C. (Neocalathus) leechi218Mineral del Chico (MEX)GU254318GU254413GU254486C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ26944GU254353GU254432GU254432C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254353GU254432C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU25435	C. (Neocalathus) cinctus	141	Aguelmam Afenourir Lake (MO)	GU254304	GU254396	GU254478
C. (Neocalathus) erratus113Dzyanon village, Dolno (BU)GU254299GU254386GU254471C. (Neocalathus) gonzalezi77Lanzarote (CI)AJ404986GU254372GU254459C. (Neocalathus) gregarius179Pennsylvania (USA)GU254321GU254403GU254483C. (Neocalathus) leechi221La Marquesa (MEX)GU254323GU254416GU254491C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254324GU254419GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254420GU254493C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254327GU254421GU254493C. (Neocalathus) leechi240Nevado de Toluca (MEX)GU254327GU254421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254318GU254423GU254494C. (Neocalathus) leechi218Mineral del Chico (MEX)GU254318GU254431GU254483C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254324GU254431C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254353GU254432C. (Neocalathus) melanocephalus11Formigal, Huesca (SP)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254384GU254449C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354 </td <td>C. (Neocalathus) erratus</td> <td>27</td> <td>Rila Mountains (BU)</td> <td>AM410890</td> <td>GU254355</td> <td>GU254443</td>	C. (Neocalathus) erratus	27	Rila Mountains (BU)	AM410890	GU254355	GU254443
C. (Neocalathus) gonzalezi77Lanzarote (Cl)AJ404986GU254372GU254459C. (Neocalathus) gregarius179Pennsylvania (USA)GU254309GU254403GU254483C. (Neocalathus) leechi221La Marquesa (MEX)GU254321GU254416GU254489C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254323GU254419GU254491C. (Neocalathus) leechi224Nevado de Toluca (MEX)GU254324GU254420GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254421GU254493C. (Neocalathus) leechi240Nevado de Toluca (MEX)GU254327GU254423GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254311GU254423GU254497C. (Neocalathus) marmoreus218Mineral del Chico (MEX)GU254318GU254432GU254431C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254323GU254433GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254432C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254494C. (Neocalathus) melanocephalus26Pirin Mountains (BU)GU254295GU254354GU254424C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254424C. (Neocalathus) melanocephalus26Pirin Mountains (BU)AM410893GU2543	C. (Neocalathus) erratus	113	Dzyanon village, Dolno (BU)	GU254299	GU254386	GU254471
C. (Neocalathus) gregarius179Pennsylvania (USA)GU254309GU254403GU254483C. (Neocalathus) leechi221La Marquesa (MEX)GU254321GU254416GU254489C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254323GU254418GU254491C. (Neocalathus) leechi224Nevado de Toluca (MEX)GU254324GU254419GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254421GU254493C. (Neocalathus) leechi240Nevado de Toluca (MEX)GU254327GU254421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254331GU254423GU254497C. (Neocalathus) neechi258La Marquesa (MEX)GU254318GU254432GU254497C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254324GU254431C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254353GU254431C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254494C. (Neocalathus) melanocephalus26Pirin Mountains (BU)AM410893GU254354GU254442C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254424C. (Neocalathus) melanocephalus26Pirin Mountains (BU)AM410893GU254354G	C. (Neocalathus) gonzalezi	77	Lanzarote (CI)	AJ404986	GU254372	GU254459
C. (Neocalathus) leechi221La Marquesa (MEX)GU254321GU254416GU254489C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254323GU254418GU254491C. (Neocalathus) leechi224Nevado de Toluca (MEX)GU254324GU254419GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254420GU254494C. (Neocalathus) leechi240Nevado de Toluca (MEX)GU254327GU254421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254311GU254423GU254497C. (Neocalathus) menoreus218Mineral del Chico (MEX)GU254318GU254324GU254431C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254342GU254431C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254431C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254442C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254494C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254442C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295GU254354GU254424C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254295 <td< td=""><td>C. (Neocalathus) gregarius</td><td>179</td><td>Pennsylvania (USA)</td><td>GU254309</td><td>GU254403</td><td>GU254483</td></td<>	C. (Neocalathus) gregarius	179	Pennsylvania (USA)	GU254309	GU254403	GU254483
C. (Neocalathus) leechi223Nevado de Toluca (MEX)GU254323GU254418GU254491C. (Neocalathus) leechi224Nevado de Toluca (MEX)GU254324GU254419GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254420GU254493C. (Neocalathus) leechi240Nevado de Toluca (MEX)GU254327GU254232GU254421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254331GU254423GU254497C. (Neocalathus) melanocephalus218Mineral del Chico (MEX)GU254318GU254342GU254431C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254342GU254432C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254353GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU254442C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU254442C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254398GU254354GU254442C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU254442C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU254424C. (Neocalathus) melanocephalus111Formigal,	C. (Neocalathus) leechi	221	La Marquesa (MEX)	GU254321	GU254416	GU254489
C. (Neocalathus) leechi224Nevado de Toluca (MEX)GU254324GU254419GU254492C. (Neocalathus) leechi226Tlamacas. Popo-Iztaccihuat (MEX)GU254325GU254420GU254493C. (Neocalathus) leechi240Nevado de Toluca (MEX)GU254327GU25421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254331GU254423GU254497C. (Neocalathus) memoreus218Mineral del Chico (MEX)GU254318GU254413GU254486C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254342GU254431C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254353GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254384GU2544424C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU2544424C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU2544424C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254398GU254354GU2544424C. (Neocalathus) melanocephalus26Pirin Mountains (BU)AM410893GU254354GU2544424C. (Neocalathus) melanicus219La Malinche (MEX)GU254319GU254434GU2544424	C. (Neocalathus) leechi	223	Nevado de Toluca (MEX)	GU254323	GU254418	GU254491
C. (Neocalathus) leecht226Hamacas. Popo-Iztacchuat (MEX)GU254325GU254420GU254493C. (Neocalathus) leecht240Nevado de Toluca (MEX)GU254327GU25421GU254494C. (Neocalathus) leechi258La Marquesa (MEX)GU254311GU254233GU254421GU254497C. (Neocalathus) marmoreus218Mineral del Chico (MEX)GU254318GU254423GU254486C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254342GU254431C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254353GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254384GU2544426C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU2544424C. (Neocalathus) melanocephalus26Pirin Mountains (BU)AM410893GU254354GU2544424C. (Neocalathus) metalicus219La Malinche (MEX)GU254319GU254414GU2544424	C. (Neocalathus) leechi	224	Nevado de Toluca (MEX)	GU254324	GU254419	GU254492
C. (reoculating) recmt 240 Nevado de lotuca (MEX) GU254327 GU254421 GU254494 C. (Neocalathus) leechi 258 La Marquesa (MEX) GU254331 GU254423 GU254497 C. (Neocalathus) marmoreus 218 Mineral del Chico (MEX) GU254318 GU254423 GU254436 C. (Neocalathus) melanocephalus 11 Gran Sasso (IT) AJ236944 GU254333 GU254432 GU254432 C. (Neocalathus) melanocephalus 12 Norfolk Brecklands (UK) AJ404993 GU254353 GU254432 C. (Neocalathus) melanocephalus 25 Rila Mountains (BU) GU254295 GU254353 GU254441 C. (Neocalathus) melanocephalus 111 Formigal, Huesca (SP) GU254295 GU254384 GU2544494 C. (Neocalathus) melanocephalus 111 Formigal, Huesca (SP) GU254298 GU254384 GU2544494 C. (Neocalathus) melanocephalus 26 Pirin Mountains (BU) AM410893 GU254354 GU2544424 C. (Neocalathus) mexicanus 219 La Malinche (MEX) GU254319 GU254444 GU2544442 <td>C. (Neocalatnus) leechi</td> <td>226</td> <td>Navada da Telever (MEX)</td> <td>GU254325</td> <td>GU254420</td> <td>GU254493</td>	C. (Neocalatnus) leechi	226	Navada da Telever (MEX)	GU254325	GU254420	GU254493
C. (Neocalathus) netcht258La Marquesa (MEX)GU254331GU254423GU254497C. (Neocalathus) marmoreus218Mineral del Chico (MEX)GU254318GU254413GU254486C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254342GU254431C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254343GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254384GU254469C. (Neocalathus) metallicus26Pirin Mountains (BU)AM410893GU254354GU254442C. (Neocalathus) metallicus219La Malinche (MEX)GU254319GU254414GU254487	C. (Neocalathus) leechi	240	Nevado de Toluca (MEX)	GU254327	GU254421	GU254494
C. (Neoculatius) maniforeus218Mineral der Chico (MEX)GU254318GU254413GU254486C. (Neocalathus) melanocephalus11Gran Sasso (IT)AJ236944GU254342GU254431C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)AJ404993GU254343GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU254469C. (Neocalathus) metallicus26Pirin Mountains (BU)AM410893GU254354GU254442C. (Neocalathus) metallicus219La Malinche (MEX)GU254319GU254414GU254487	C. (Neocalathus) neerin	258	La Marquesa (MEX)	GU254331	GU254423	GU254497
C. (Neocalathus) melanocephalus11Gran Sasso (n)Aj250944GU254342GU254431C. (Neocalathus) melanocephalus12Norfolk Brecklands (UK)Aj404993GU254343GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) melanocephalus111Formigal, Huesca (SP)GU254298GU254354GU254469C. (Neocalathus) metallicus26Pirin Mountains (BU)AM410893GU254354GU254442C. (Neocalathus) mexicanus219La Malinche (MEX)GU254319GU254414GU254487	C. (Neocalathus) malanoconhalus	21ð 11	(Tran Sasso (IT)	GU234318 AI226044	GUZ54413 CU254242	GUZ34480
C. (Neocalathus) metanocephalus12Notork Diecklands (UK)Aj404993GU254343GU254432C. (Neocalathus) melanocephalus25Rila Mountains (BU)GU254295GU254353GU254441C. (Neocalathus) metanocephalus111Formigal, Huesca (SP)GU254298GU254354GU254469C. (Neocalathus) metallicus26Pirin Mountains (BU)AM410893GU254354GU254442C. (Neocalathus) mexicanus219La Malinche (MEX)GU254319GU254414GU254484	C. (Neocalathus) melanocombalus	11	Morfolk Procklands (UV)	AJZ20944	GUZ3434Z	GUZ34431
C. (Neocalathus) metanocephalus 2.5 Nita Modultatis (BD) GU254255 GU254353 GU254441 C. (Neocalathus) metallicus 111 Formigal, Huesca (SP) GU254298 GU254384 GU254469 C. (Neocalathus) metallicus 26 Pirin Mountains (BU) AM410893 GU254354 GU254442 C. (Neocalathus) mexicanus 219 La Malinche (MEX) GU254319 GU254414 GU254487	C. (Neocalathus) melanoconhalus	12	Rila Mountains (BU)	AJ404993 CU254205	GU254343 CU254252	GUZ54432 CU254441
C. (Neocalathus) metallicus 26 Pirin Mountains (BU) AM410893 GU254354 GU254449 C. (Neocalathus) metallicus 26 Pirin Mountains (BU) AM410893 GU254354 GU254442 C. (Neocalathus) metallicus 219 La Malinche (MEX) GU254319 GU254414 GU254484	C. (Neocalathus) melanocephalus	25	Formigal Huesca (SD)	GU254295 CU254295	GU254555 GU254384	GU254441 CU254460
C. (Neocalathus) mexicanus 219 La Malinche (MEX) GU254319 GU254414 GU254487	C (Neocalathus) metallicus	26	Pirin Mountains (BII)	AM410803	GU254364 GU254354	GU254409
	C. (Neocalathus) mexicanus	219	La Malinche (MEX)	GU254319	GU254414	GU254487

Table 1 (continued)

Species	Cod	Locality	cox1-cox2	285	Ef-1α CO
C. (Neocalathus) micropterus	20	Poznan (PL)	GU254293	GU254350	GU254438
C. (Neocalathus) micropterus	146	Trondheim (NR)	GU254305	GU254398	
C. (Neocalathus) mollis	22	Málaga (SP)	GU254294	GU254352	GU254440
C. (Neocalathus) mollis	120	La Cuenca, Soria (SP)	F[173193	F]173071	FJ173133
C. (Neocalathus) opaculus	190	Tallahasse, Florida (USA)	GU254315	GU254410	
C. (Neocalathus) peropacus	188	Tumacacori, Arizona (USA)	GU254313		
C. (Neocalathus) ruficollis ruficollis	181	Marin Co., California (USA)	GU254310	GU254404	
C. (Neocalathus) ruficollis ruficollis	182	Marin Co., California (USA)		GU254405	GU254484
C. (Neocalathus) ruficollis ruficollis	183	Marin Co., California (USA)		GU254406	
C. (Neocalathus) ruficollis ruficollis	185	Marin Co., California (USA)	GU254311	GU254407	
C. (Neocalathus) ruficollis ruficollis	189	Sta Barbara Co., California (USA)	GU254314	GU254409	
C. (Neocalathus) ruficollis ruficollis	193	Sta Barbara Co., California (USA)	GU254316	GU254411	
C. (Neocalathus) semisericeus	131	Rif Mountains (MO)	GU254301	GU254391	
C. (Neocalathus) semisericeus	139	Rif Mountains (MO)	AM410892	GU254395	GU254477
C. (Neocalathus) simplicicollis	75	Fuerteventura (CI)	AJ404985	GU254371	
C. (Neocalathus) solieri	272	Parc National Chréa (AG)	GU254334		
C. (Neocalathus) sp	262	Nevado de Toluca (MEX)	GU254332	GU254424	GU254498
C. (Neocalathus) subfuscus	72	Pico Ariens (MR)	AJ405003	GU254370	GU254458
C. (Trichocalathus) obliteratus	58	La Gomera (CI)	AJ236971	GU254364	GU254452
C. (Trichocalathus) pilosipennis	62	La Gomera (CI)	AJ236964	GU254367	GU254455
C. (Trichocalathus) refleximargo	60	La Gomera (CI)	AJ236974	GU254365	GU254453
Lindrothius caucasicus orbicollis	271	Martveli district, Lebarde (GG)	FJ173220	FJ173100	FJ173157
Lindrothius pseudopraestans	296	Mount Fish (GG)	FJ173235		FJ173171
Lindrothius sp.	297	Aibja, Abkhazia (GG)	FJ173236	FJ173115	FJ173172
Thermoscelis insignis (Calathina)	304	Summelas, Trabzon (TR)	GU254335		
Synuchidius ganglbaueri (Calathina)	306	Pelister Mount. Veternica (MC)	GU254336		
Acalathus (Procalathus) advena (Dolichina)	186	San Bernardino Co., California (USA)	GU254312	GU254408	
Acalathus (Procalathus) advena (Dolichina)	242	Great Basin N.P., Nevada (USA)	GU254328		
Anchomenidius astur (Dolichina)	277	León (SP)	FJ173221	FJ173101	FJ173158
Dolichus halensis (Dolichina)	282	Nishino, Noichi, Kōchi (JA)	FJ173226	FJ173105	FJ173162
Laemostenus complanatus (Sphodrina)	256	Murcia (SP)	FJ173217	FJ173097	FJ173154
Pristosia aeneola (Pristosiina)	285	Chino, Nagano (JA)	FJ173228	FJ173108	FJ173164
Synuchus nitudus (Synuchina)	289	Jôza, Sakura, Chiba (JA)	FJ173230	FJ173110	FJ173166
			102 ind.	100 ind.	85 ind.

2.5. Testing for hard polytomies

In order to explore the lack of resolution at deeper phylogenetic nodes, we evaluated whether such polytomies were 'hard', implying rapid radiation. For all tests we used ultrametric trees recovered with the BEAST software (Drummond and Rambaut, 2007). Lineage through time (LTT) plots were used to visualize changes in diversification rates. Two statistical analyses were used to test for significant departures from the constant speciation rate model (CR), the γ -statistic (Pybus and Harvey, 2000) and the birth-death likelihood (BDL) test (Rabosky, 2006a). Both methods have proven successful in reconstructing diversification rates in various organisms (y-statistic: Barraclough and Vogler, 2002; Kadereit et al., 2004; Turgeon et al., 2005; Kozak et al., 2006; McKenna and Farrell, 2006; Weir, 2006; Roelants et al., 2007; Hines, 2008; Janssens et al., 2009; birth-death models: Rabosky et al., 2007; Egan and Crandall, 2008; Rabosky and Lovette, 2008). As incomplete taxon sampling can result in a spurious decline in diversification rates over time (Pybus and Harvey, 2000), we have explored the effects of missing species on our analysis. We assumed that our sample represents a fraction f (from 0.2 to 0.6) of the true number of lineages, and generated sets of 5000 trees under the null hypothesis of constant rate pure birth, in which taxa were randomly pruned from these trees.

 Γ -statistic analyses were conducted with the Ape Library in R (Paradis, 1997). This analysis indicates whether the diversification rate has increased ($\gamma > 0$), decreased ($\gamma > 0$) or remained constant $(\gamma = 0)$ over time. To take into account incomplete sampling simulations were carried out using different numbers of missing taxa with the MCCR test (Pybus and Harvey, 2000) in LASER (Rabosky, 2006b). BDL analysis has been found to perform as well or better than the popular γ -statistic that only identifies temporal decreases in diversification (Rabosky, 2006a). BDL analyses were conducted using LASER library in R (Rabosky, 2006b) to test the null hypothesis of rate-constancy against rate-variable models using AIC. Significance of the change in AIC scores was determined by creating a distribution of AIC scores. This was done by simulating 5000 trees using yuleSim in LASER (Rabosky, 2006b), considering the same number of taxa and the same speciation rate as those estimated under the pure Birth model. Incomplete taxon sampling was con-

Table 2

Data statistics for each da	ata set. Ti/Tv,	transition/transversion	ratio; Pi,	parsimony	informative sites.
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	А	С	G	Length	Variable	%Variable	Conserved	Pi	Divergence (%)	Ti/Tv
cox 1-cox 2	33.2	13.9	13.4	1630	720	44.2	908	583	7.3	
cox1	31.1	14.3	13.9	865	348	40.2	517	289	4.8	1.0
tRNAleu	36.3	12.2	17.9	63	21	33.3	41	19	16.7	4.1
cox2	35.5	13.6	12.4	702	351	50.0	350	275	11.8	1.0
EF-1α	25.4	27.0	25.0	773	153	19.8	620	102	2.2	3.4
28S CW 15/6.6	26.1	18.0	25.1	885	198	22.4	682	127	2.6	1.4

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Table 3

Data sets and the estimated models of sequence evolution by Akaike information criterion (AIC). I = proportion of invariant sites; G = gamma-distributed rates across sites; 28Sc: conserved region of 28S.

	AIC model	А	С	G	A-C	A–G	A–T	C–G	C-T	Ι	G
cox1-cox2	GTR + I + G	0.3553	0.081	0.1078	7.1325	20.4345	6.1987	4.5081	75.7883	0.4607	0.7057
EF-1α	SYM + I + G	0.25	0.25	0.25	0.8711	3.8231	1.8831	0.6169	11.8965	0.6142	0.9119
28Sc	GTR + I + G	0.2746	0.1844	0.2562	0.5643	7.3847	4.1226	0	3.6522	0.7006	0.802



Fig. 1. Molecular phylogeny of the subtribe Calathina. Sum of the corrected PBS of all nodes for the Sensitivity Analysis of the whole 28S sequence, aligning manually (MA, checked bars, GOP/GEP of ClustalW as starting point indicated) and with 10 different parameter sets (Grey bars, GOP/GEP) for (a) the whole marker with Clustal W (CW approach); (b) considering only variable regions (MX approach); (c) considering only variable regions and anchoring both ends. (MXan approach). Asterisk indicates selected GOP/GEP values. Black bars represent unambiguously aligned regions (28Sc).

sidered by generating trees under a CR model with different levels of incomplete sampling (*f*) in LASER (Rabosky, 2006b).

The possibility that polytomies were caused by insufficient data ('soft' polytomies) was also evaluated. Simulated data were built with Mesquite v.2.01 (Maddison and Maddison, 2007) to explore the predicted increase in node support associated with an increased number of nucleotides (Philippe et al., 2005). Several previous analyses have used simulations to estimate the

amount of data necessary to resolve difficult phylogenetic problems (Berbee et al., 2000; Fishbein et al., 2001; de Queiroz et al., 2002; Rokas et al., 2003; Zou et al., 2008; Spinks et al., 2009). Matrices of increasing size from 500 bp to 20 Kb were constructed taking into account the among-gene-tree variation frequently found in real data sets (Spinks et al., 2009). Two procedures were carried out: (i) simulated data were generated for each partition with a length that was proportional to that of



Fig. 2. The 50% majority consensus tree resulting from the BA of the cox1-cox2 data partitioned by codon (1st, 2nd and 3rd). Number above node shows posterior probability ≥ 0.90 . Bootstrap support values are shown below nodes. Circle numbers are discussed in the text. Name of taxa corresponds to those indicated in Table 1 (C. corresponds to *Calathus*). Capital letters in brackets denote the geographic region: WP, West-Palearctic; EB, Eurosiberian; EU, European; ME, Mediterranean; AF, North Africa; IB, Iberian peninsula; IT, Italy; BK, Balkan peninsula; TR, Turkey; CA, Caucasus; LZ, Lanzarote Island; FV, Fuerteventura Island; TF, Tenerife; LG: La Gomera Island; GC, Gran Canaria; MR, Madeira; NA, North America; MX, Mexico; US, United States of America; ET, Ethiopia.

the original partitioned dataset. Each simulated partition was generated using both the evolutionary model (Table 3) and the tree obtained from the original partition (Figs. 2–4). Polytomies of the trees from each original partition were resolved arbitrarily and the resulting new branches were assigned zero length with Mesquite v.2.01 (Maddison and Maddison, 2007). The simulated

partitions were concatenated and then analyzed with MrBayes; (ii) a non-parametric bootstrap of the combined dataset was performed with Mesquite v.2.01 (Maddison and Maddison, 2007). Pseudoreplicates of variable size were obtained by re-sampling with replacement from the original data and analyzed with MrBayes. Bayesian analyses were carried out for each simulated



Fig. 3. The 50% majority consensus tree resulting from the BA of EF-1 α CO partitioned by codon (1st, 2nd and 3rd). Number above node shows posterior probability \ge 0.90. Bootstrap support values are shown below nodes. Circle numbers are discussed in the text. Name of taxa as in Fig. 2.

matrix and the proportion of nodes with posterior probabilities >0.95 was measured.

3. Results

3.1. Sequence alignment

The secondary structure inferred for 28S had four variablelength regions, comprising 11% of the total length. The CW approach resulted in generally longer alignments (885–933 base pairs) than the MX (886–910 base pairs) and MXan approaches (886–918 base pairs) (Fig. 1, Supplementary material). The unambiguously aligned regions (28Sc) comprised 67 parsimony informative sites across all taxa (8% of the conserved sites), while the remaining ambiguous regions of alignment (28Sv) exhibited 49–60 additional informative characters (37–60% of the variable regions) depending on the alignment approach and parameters.

Different alignments were evaluated based on their relative contribution (PBS) to the combined analysis of all data. The results

of changing alignment parameters are shown in Fig. 1. The Clustal alignment of full length sequences (CW) showed two peaks of higher PBS values under GOP/GEP values 4/0.5 and 8/4. The gap penalties 2/1-0.5-0.25, 4/2 and 8/1 were found to be most incongruent with the combined tree topology. For the MX alignment, the highest PBS value was obtained with gap penalties set to 15/6.6 for the ambiguous regions. When the flanking sides of variable regions were anchored (MXan) the highest PBS value was also found with parameters set to 15/6.6, but the overall contribution using different GOP/GEP values was almost the same for each alignment (Fig. 1b and c). Manual correction for each of the alignment strategies showed different values of PBS (Fig. 1a-c).

After measuring the congruence between the variable regions of 28S and unambiguously aligned regions (mitochondrial plus EF-1 α and conserved 28S), we decided to exclude the variable regions from the analysis because the different alignment strategies affected the resolution of the deepest nodes, even in the combined analysis (see below; e.g., relationships between clades 2 and 7 in Fig. 5, and Figs. 3–5 of Supplementary material). This could be due to alignment artefacts that become particularly relevant in cer-

tain clades defined by short internal branches (Kumar and Filipski, 2007).

3.2. Phylogenetic inference

3.2.1. Separate analyses

Data statistics and models selected for the data partitions used in this study are shown in Tables 2 and 3. The three independent markers resulted in similar topologies for the subtribe Calathina. The mtDNA fragment generated a tree with high support for the main clades (Fig. 2). However, relationships between these clades were poorly supported in most cases, but slightly increased when each codon position was modelled independently in MrBayes. The subtribe Calathina includes seven clades (following the notation by Ruiz and Serrano, 2006), which stand out as particularly stable and well supported:

- Clade 1 includes the endemic Iberian species Calathus (Iberocalathus) rotundatus and the North American species Acalathus (Procalathus) advena; this last genus is currently classified in a different subtribe (Dolichina sensu Hovorka and Sciaky, 2003 and Lorenz, 2005).
- Clade 2 is constituted by the Balkan Synuchidius ganglbaueri, three species of Calathus sometimes considered as members of the subgenus Neocalathus (C. granatensis, C. opacus, and C. metallicus), and all species of the subgenus Calathus.



Fig. 4. The 50% majority consensus tree resulting from the BA of 28S aligned manually (MA). Number above node shows posterior probability ≥0.90. Bootstrap support values are shown below nodes. Circle numbers are discussed in the text. Name of taxa as in Fig. 2.

- Clade 3 comprises the West-Palearctic *Calathus* (*Amphyginus*) *rotundicollis* and the Tenerifean *depressus* group (subgenus *Lauricalathus* in part).
- Clade 4 includes *Calathus circumseptus*, a Circummediterranean species of the monotypic subgenus *Bedelinus*.
- Clade 5 includes part of the subgenus *Neocalathus* (the Palearctic *melanocephalus* group plus all the Nearctic species of the subgenus), the North African *C. semisericeus*, and some taxa of uncertain affinity: the Madeiran *vividus* group and the Afrotropical *C. aethiopicus*.
- Clade 6 includes the remaining species of the subgenus *Neocalathus* (the *ambiguus* group) together with species from the Canary Islands (the species of the subgenus *Lauricalathus* other than those of clade 3, plus all *Trichocalathus*).
- Clade 7 comprises the Caucasian genera *Thermoscelis* and *Lindrothius*, which were considered independent genera in the last Palearctic catalog (Hovorka and Sciaky, 2003).

Clade 1 is the sister group of a well-supported clade made up by all other *Calathus* plus the related genera *Synuchidius*, *Thermoscelis* and *Lindrothius* (Fig. 2). Clades 2 and 7 grouped together with maximum support in the Bayesian analysis, and together with clade 6 and the North African *C. solieri* made up a relatively well-supported clade (pp: 0.95).

Nuclear EF-1 α resulted in a topology that generally agreed with the mtDNA results (Fig. 3). The same main clades were recovered although the support for some of the deeper nodes was low. *Calathus circumseptus* (clade 4) was recovered with *C. semisericeus* with



Fig. 5. The 50% majority consensus tree resulting from the BA of the combined data without variable region (SS approach). Number above node shows posterior probability \geq 0.90. Bootstrap support values are shown below nodes. Circle numbers are discussed in the text. Asterisk represents species belonging to subgenus *Trichocalathus*. Name of taxa and acronyms of geographic regions as in Fig. 2.

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support (pp: 0.96). Clades 3 and 7 were recovered together but this group received varying support depending upon the partitioning strategy (with or without considering codon position, pp: 0.86–0.97).

Ribosomal 28S showed less resolution irrespective of the alignment selected (Fig. 4 and Figs. 3–5 of Supplementary material). Only the monophyly of *Calathus* was supported, while all other clade relationships were sensitive to alignment strategy.

3.2.2. Combined analysis

Combined analyses showed very similar results to separate analyses, in particular to the mtDNA analysis, but with increased nodal support. Clades 1-7 recovered by the mtDNA analyses were present in all combined analyses. Clade 1 formed the sister clade of clades 2-7, that showed unstable relationships sensitive to the 28S alignment strategy and reconstruction method. When only unambiguously aligned regions of 28S were used (28Sc), clades 2 and 7 grouped together but only with moderate support (0.94/56)(Fig. 5). When variable regions (28Sv) were included and aligned manually (MA), the same topology was recovered but with higher support (clade 2 and 7, pp: 1.00/57, in Fig. 3, Supplementary material). The other lineages of Calathus (clades 3-6) received moderate support (pp: 0.96). When the CW or MX approaches were used without manual correction, the position of clade 2 changed to become the sister group to a well-supported group of clades 3-7, irrespective of selected alignment gap penalties. Among these clades, only the sister relationship between clade 3 and 7 was resolved with support, albeit low (>0.90) (Figs. 4 and 5, Supplementary material).

PBS values were positive indicating moderate, but generally present, congruence between gene partitions (Fig. 6). These values also indicated that mtDNA data contributed more to the combined tree topology than the other molecular markers (about 4 times more than 28S and about 9 times more than EF-1 α) (Fig. 7). Variable regions of the 28S data revealed twice as much contribution to the combined tree topology compared to the unambiguously aligned regions, but this contribution varied considerably according to the alignment strategy (Fig. 1). The PBS contribution from each partition in the phylogeny is shown in Fig. 6. No support was found for some of the deepest nodes (from a distance of 0 to 0.03 from the root, Fig. 6). MtDNA showed the highest contribution to the combined analysis in regions from median depth to the tips



Fig. 6. Distribution of corrected Partitioned Bremer Support values of the different markers in relation to the distance of each node from the root (molecular clock enforced). Line at value 0 shows the transition between positive and negative contributions to the combined topology.

of the tree (distances from approximately 0.04-0.06). EF-1 α contributed information at median depths (0.035-0.04) and showed low contribution at the tips of the combined data tree. Conserved regions of 28S (28Sc) showed contribution from median depth to the tips, and variable regions of 28S (28Sv) exhibited a wide range of PBS values depending upon gap penalties (mainly positive values, data not shown).

3.3. Testing for hard polytomies

The LTT plot exhibited a relatively constant slope. To test whether this observation indicates a constant diversification rate (CR), we evaluated our results with two diversification tests. The γ -statistics showed a positive value ($\gamma = 3.131573$) but failed to reject the model of a constant diversification rate (CR; p = 0.999). This result is robust to assumptions about missing taxa; the MCCR test of γ -statistics is not significant (p = 1.0) using different numbers of missing taxa. The BDL test (Table 4) showed a positive value of Δ AIC_{RC} (1.4166), which indicates that rate-variable models have the best fit to the data; however, the distribution of simulations with N = 97 was not significant and the null hypothesis of a constant diversification model at $\alpha = 0.05$ could not be rejected (p = 0.2561; Rabosky, 2006a). Simulations based on incomplete taxon sampling also failed to reject the null hypothesis under different levels of taxon inclusion (p = 0.81-0.99).

Matrix length analyses showed that the proportion of supported nodes increased with increasing matrix length both for new simulated data sets and for replicates of the original data set (Fig. 8). Plotting distance to the root against node support revealed that



Fig. 7. Total corrected PBS contribution of each partition to the combined tree. Each bar represents the contribution of each marker: 28Sc (=conserved region of 28S), 28Sv 15/6 (=variable regions of 28S aligned with ClustalW with default GOP/GEP).

Results	of	fitting	diversification	models	to the	subtribe	Calathina	using	birth-deat	th
likelihoo	ha									

Table 4

Model	Pure birth	bd	DDL	DDX	yule2rate
Parameters	r1 = 56.45	r1 = 28.87 a = 0.70	r1 = 56.45 k = 1477084	r1 = 17.96 x = -0.32	r1 = 47.12 r2 = 118.80 st = 0.0026
Ln (L) AIC ΔAIC	633.5398 -1265.08 -8.375	638.7274 -1273.455 0	633.5393 -1263.079 -10.376	636.5 -1269 -4.455	640.4358 -1274.872 1.417

bd, birth-death model; DDL and DDX, logistic and exponential density-dependent speciation model; yule2rate, multi-rate variant of the Yule model; r1 - r2, net diversification rate (speciation event per million year); *a*, extinction fraction; st, time of rate shift (Mya); *k*, parameter in the logistic density-dependent model; *x*, parameter in the density-dependent exponential model; Ln (L), log-likelihood; AlC, Akaike information criterion ; Δ AlC, change in AlC between model and the overall best-fit model.



Fig. 8. Relationship between sequence length of simulated datasets and proportion of supported nodes (posterior probability). Simulated data sets were built up using new data sets (green line) or pseudoreplicates of the original data set (blue line). Red circle indicates proportion of supported nodes in the combined analysis of the original data set.

low support was randomly distributed within the tree, from deeper to shallower nodes (data not shown).

4. Discussion

4.1. Phylogenetic relationships within Calathina

Our analyses corroborated the monophyly of the subtribe Calathina and revealed seven well-supported clades that are inferred to represent main lineages within the subtribe. However, low support was obtained for some of the deepest nodes that were associated with short internal branches, and thus the relationships between these lineages could not be fully resolved. Lack of deep nodal resolution is frequently observed in molecular phylogenies (Whitfield and Lockhart, 2007) and may be due to a number of factors such as (i) inappropriate molecular data; (ii) inappropriate phylogenetic methods or substitution models; (iii) insufficient data (Walsh et al., 1999; Whitfield and Lockhart, 2007); (iv) incongruence between data partitions (Baker and DeSalle, 1997; Cognato and Vogler, 2001); or (v) rapid radiation (Rabosky and Lovette, 2008; Whitfield and Kjer, 2008) resulting in 'hard' polytomies (Maddison, 1989).

The first two factors are unlikely to explain our lack of basal resolution because we have used 812 parsimony informative characters from three independent markers that encompass a range of substitution rates, implementing different substitution models for each data partition together with phylogenetic methods that differ in their analytical strengths. The PBS analyses (Fig. 6) revealed that the low support associated with the deepest nodes was not due to incongruence, and a rapid radiation following the origin of Calathina is unlikely because there were no significant departures from a constant speciation rate model in the γ -statistic and the BDL tests. Although these tests lack power to detect increased diversification rates, they are more effective at detecting a decrease in diversification rates (Rabosky, 2006a), which is expected after rapid radiation events (Rabosky and Lovette, 2008). With regard to insufficient data, both simulations and length-variable bootstrap re-sampling showed that the proportion of supported nodes (pp > 0.95) increased with the size of the data

matrix (Fig. 8), which makes this hypothesis the most likely explanation for the low resolution of the deepest nodes. More data, preferably from other genes, are therefore needed to resolve the relationship between main clades in Calathina.

Regardless of the weakly resolved basal relationships within Calathina, there was strong support for a paraphyletic *Calathus* that included the current genera *Synuchidius* (clade 2), *Thermoscelis* and *Lindrothius* (clade 7), and *Acalathus* (clade 1). This result agrees well with the ideas of Lindroth (1956) who divided *Calathus* into many different species groups, including the aforementioned genera. Thus, molecular data support the existence of a subtribe Calathina that includes a single inclusive genus *Calathus*, of which *Synuchidius*, *Lindrothius*, *Thermoscelis* and *Acalathus* should be ranked as subgenera. The inclusion of *Acalathus* within the subtribe Calathina instead of Dolichina has also been suggested by Ball and Nègre (1972), Perrault (1977) and Casale (1988).

The close relationship between the North American Acalathus advena and the Iberian species Calathus (Iberocalathus) rotundatus (clade 1) was somewhat surprising because of the geographical disjunction between these taxa. However, this relationship was supported independently by mtDNA as well as 28S data. Also, the relatively short branch lengths observed for Calathus and Acalathus in the 28S data indicate that this relationship is not an artefact due to long-branch attraction (Bergsten, 2005). The Bayesian analyses are particularly suited to handle erroneous relationships that can emerge as a consequence of elevated substitution rates, but none of the implemented evolutionary models that were fitted to different genomic data and different codon positions revealed alternative hypotheses (Figs. 4, 6 and 7, and Fig. 2 of Supplementary material). Spurious inclusion of Acalathus in the ingroup was also tested by sampling a much wider range of Calathina (all genera) and Dolichina (4 out of 7 genera) outgroups, but did not result in topological changes (data not shown). Long-branch attraction was also tested by removing each of these putatively wrongly placed taxa, one at a time, but none of them swapped position in the absence of the other. We are therefore confident that Acalathus belongs within the genus Calathus, a hypothesis that should be further tested by studying the extant species presently found in China. Molecular data also support the inclusion of the genus Dolichus within the subtribe Dolichina, as put forward by Casale (1988) and Ruiz et al. (2009).

Clade 2 showed an unexpected relationship between the Balkan Synuchidius ganglbaueri, Calathus metallicus, the Iberian C. granatensis, the North African C. opacus and all species of the subgenus Calathus. Synuchidius ganglbaueri is usually regarded as a separate genus from Calathus on morphological grounds (Apfelbeck, 1930; Guéorguiev, 2007). Calathus metallicus shows external characters similar to the subgenus Neocalathus, and C. granatensis and C. opacus have been placed either in the subgenera Calathus or Neocalathus because they show intermediate morphological characters between these subgenera. Nevertheless, at least one morphological character supports this clade by the presence of a tooth within the endophallus in all four taxa. A taxonomic reassessment is therefore required.

Clade 3 was made up by most but not all species of the subgenus Lauricalathus inhabiting the island of Tenerife (the depressus group), with the European C. (Amphyginus) rotundicollis as sister group (Fig. 9). Nuclear data added in this study thus corroborate a relationship reported by Emerson et al. (2000) and Ruiz and Serrano (2006) based on mitochondrial data only. Two additional species from Tenerife (C. rectus and C. abaxoides) were on the other hand related to taxa inhabiting other Canary Islands (Gran Canaria, La Gomera, El Hierro, clade 6), including the three La Gomeran species in the subgenus Trichocalathus. These Canarian taxa combined made up the sister group to a clade consisting primarily of western Palearctic species of the ambiguus group in the subgenus Neocalathus, including also one species from Madeira (Fig. 9). The nuclear data added in this study thus corroborate the lack of support for a monophyletic subgenus Trichocalathus (see also Emerson et al., 1999), as species in this subgenus from La Gomera are nested within the subgenus Lauricalathus on the same island. Generally short branches throughout the La Gomeran clade suggest that the ancestor of these species underwent rapid morphological evolution giving rise to the distinctive traits of Trichocalathus. A similar case of fast morphological evolution associated with little molecular divergence has been reported among carabids in the genus Carabus (see Osawa et al., 2004 and further references therein).

The lack of monophyly for species currently included in *Neocal-athus* (clades 5 and 6) corroborates the recently suggested polyphyletic nature of this subgenus (Ruiz and Serrano, 2006). On morphological grounds *Neocalathus* is relatively uniform and does not currently pose any taxonomic controversies (e.g., Ball and Nègre, 1972; Toribio, 2006), but no cladistic analysis has been carried out on the whole subgenus, and a thorough study to corroborate the polyphyly of this subgenus is required. The Nearctic



Fig. 9. Colonisation events of Macaronesia by subtribe Calathina. Roman numbers refer to colonisation events detailed in the text.

Neocalathus (clade 5) formed a monophyletic group divided into Mexican and North American sister clades (or nearly so, depending on the type of analysis), as proposed by Ball and Nègre (1972) based on morphological characters (Fig. 67, p. 519). We can therefore infer a single colonisation of the Nearctic region by *Neocalathus*, and only two independent colonizations when including *Acalathus*. Further molecular studies on Mexican *C*. (*Tachalus*) *ovipennis* and Asian taxa of *Procalathus* and *Acalathus* are nevertheless needed to achieve deeper insight into the evolutionary history of Nearctic *Calathus*.

4.2. Diversification of the subtribe Calathina in the Mediterranean Basin, Caucasus and Europe

The distribution of taxa within the subtribe is concentrated around the Mediterranean Basin and the Caucasus. The most extensive diversification in this area is found within the subgenus Calathus, with about 50 flightless species most of which are endemic to the mountain massifs on the northern side of the Mediterranean Basin. This suggests that isolation in mountains has been a significant factor in driving speciation within this lineage where only the wing dimorphic and eurytopic C. fuscipes has a wide Palearctic distribution. The more distantly related species of this clade, C. granatensis, C. opacus, C. metallicus and Synuchidius ganglbaueri seem to be peripheral isolates around the Mediterranean Basin, apparently relics of an ancestor that once had a wider distribution. The same explanation holds for the Caucasian taxa Lindrothius and Thermoscelis (clade 7, Fig. 2). In contrast to the subgenus Calathus, the subgenera Bedelinus (C. circumseptus, clade 4, full winged) and Amphyginus (C. rotundicollis, clade 3, wing dimorphic) that have similar Mediterranean distributions are much less diverse lineages. Calathus rotundicollis has a discontinuous distribution that includes Atlantic Europe, Italy and Greece. The low sequence divergence between Italian and Iberian individuals (p-distance cox1-cox2: 0.014) supports a recent origin for this disjunction, possibly during the Pleistocene, as postulated for other organisms (e.g., Hewitt, 2001, 2004).

Members of the *melanocephalus* (Fig. 5, clade 5) and the *ambig-uus* (Fig. 5, clade 6) groups in the subgenus *Neocalathus* show a wider geographical distribution, in agreement with their higher dispersal power (they are either winged or wing dimorphic species) and lack of ecological specialisation (they are generalist taxa). These species are not limited to the Mediterranean Basin but occur in temperate and boreal Europe, and have colonised the Macaronesian archipelagos and other areas out of the Palearctic region.

4.3. The colonisation of the Macaronesian subregion

The study of a large number of continental taxa and the use of multiple nuclear and mitochondrial markers allowed reconstruction of colonisation events and subsequent radiations in *Calathus* on the Macaronesian archipelagos. New data have not only corroborated previous conclusions by Emerson et al. (1999, 2000) but added interesting clues about the origin of multiple lineages endemic to these archipelagos. Based on these data, at least four or five independent colonisation events of the Macaronesian archipelagos have taken place (Fig. 9).

(i) The *depressus* group, which includes most *Lauricalathus* of Tenerife except for *C. abaxoides* and *C. rectus*, and the European *C. rotundicollis* share a common ancestor, suggesting a West-Palearctic origin of the Tenerifean clade. However, the genetic divergence between *C. rotundicollis* and the *C. depressus* group is relatively large (*p*-distance cox1-cox2: 0.07), and thus extinction of a more closely related taxa from another geographical region is a possibility.

- (ii) The European *ambiguous* group consisting of *C. ambiguus*, *C.* asturiensis, C. erratus and the Madeiran C. subfuscus formed a sister group to a large clade of Canary island species (clade 6, the canariensis group). Although the sister relationship is not prima facie evidence for an ancestral European distribution, we note that most other distantly related species are also of western Palearctic origin. It therefore seems likely that the canariensis group diversified from a West-Palearctic ancestor. Further diversification within this group possibly occurred in an east to west direction as indicated by the nested structure among Gran Canaria, Tenerife and La Gomera populations and higher taxa. The nested structure is consistent with a stepping-stone model of colonisation from the island closest to the mainland to the more distant islands in the west, as reported for other insect taxa in the Canary Islands (Juan et al., 2000). The most recent colonisation event has possibly occurred between La Gomera and the youngest island of El Hierro (1 Myr old), where the endemic C. spretus is closely related to the La Gomera species C. gomerensis (not included, see Emerson et al., 2000).
- (iii) The eastern Canary Islands lineage (gonzalezi group), represented by *C. gonzalezi* (Fuerteventura) and *C. simplicicollis* (Lanzarote), is paraphyletic with respect to the widespread continental species *C. mollis* that is distributed along North Africa and Europe. The paraphyletic distribution makes inference about their colonisation history equivocal. The nested position of the continental species (*C. mollis*) may indicate back colonisation from the eastern Canary Islands to the mainland, a scenario documented in other Macaronesian beetles (e.g., Jordal and Hewitt, 2004) and many other taxa (Bellemain and Ricklefs, 2008), or it may indicate a double colonisation of the Canary Islands by the continental ancestor (Emerson, 2002).
- (iv) The origin of the vividus group in Madeira (C. complanatus, C. colasianus, C. vividus) is uncertain given its sister relationship with a clade consisting of species from many different geographical areas. The relatively deep genetic divergence of the vividus group points to a potentially ancient origin of this lineage.
- (v) C. subfuscus from Madeira is closely related to the European C. ambiguus, which indicates an independent and recent colonisation of this island.

From this minimum estimate of five independent colonisations of the Macaronesian islands we note that a European mainland to Macaronesian island pattern is the most common pattern. This may appear at odds with the geographically closer location of the African mainland. However, the general biogeography of the Macaronesian islands indicates that many (but not all) groups of insects and other organisms have indeed colonised these islands from the mainland north of the Mediterranean Sea. These patterns are usually explained by way of prevailing winds and sea currents from the Iberian Peninsula (Juan et al., 2000). The inferred colonisation patterns in Calathus could also be an artefact caused by incomplete sampling, although this seems unlikely as we have studied most North African species of Calathus (7 out of 9 species), representing all extant lineages in that region (Antoine, 1957). It is however possible that now extinct ancestors of the present Macaronesian Calathus formerly inhabited North Africa, as has been suggested more generally for the flora and fauna of the region (Emerson, 2002).

Acknowledgments

This work has been supported by projects 00595/PI/04 of the Fundación Séneca (Murcia) and CGL2006-06706/BOS of the Span-

ish Ministerio de Educación y Ciencia. Carlos Ruiz was granted with FPU predoctoral grant of the Ministerio de Educación y Ciencia. We heartily thank the many colleagues that helped us with collecting material, particularly Carmelo Andújar, Jose Fermín Sánchez-Gea, Esteban Jiménez, José Luís Lencina and Javier Ibáñez (University of Murcia), Boby Gueorguiev (University of Sophia). Roy Danielsson helped with loaned material (Museum of Zoology, University of Lund). Irene Muñoz and Lope Lorenzo helped with Fig. 9. George Ball (University of Alberta) helped with the identification of Nearctic material. Ignacio Ribera (Museo Ciencias Naturales, Madrid) helped with valuable bibliography.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.10.026.

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