

Molecular phylogeny and systematics of *Calathus* Bonelli (Coleoptera: Carabidae: Sphodrini) based on mitochondrial *cox1*–*cox2* sequences

Carlos Ruiz & José Serrano

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The current subgeneric taxonomy of the genus *Calathus* is revised according to *cox1*–*cox2* mitochondrial sequences of 44 species representing six subgenera. The subgenus *Calathus s. str.* was found to be monophyletic, but the subgenus *Neocalathus* is clearly polyphyletic and should be split into two or more subgenera. The monotypic subgenus *Bedelinus* appears in an isolated clade. The recent placement of *C. rotundatus* within the new subgenus *Iberocalathus* is supported by molecular data, what makes the subgenus *Amphyginus* to include only the type species *C. rotundicollis*. The species of the *C. granatensis* group are within the sister clade of the subgenus *Calathus* and should be included in a separate subgenus. Finally, the Canarian subgenera *Lauricalathus* and *Trichocalathus* are not well differentiated and do not seem to be monophyletic taxa, which suggests the need for a detailed revision.

C. Ruiz & J. Serrano, Departamento de Zoología y Antropología Física, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, 30100, Murcia, Spain; E-mail: caruiz@um.es, jserrano@um.es

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1. Introduction

The genus *Calathus* Bonelli, 1810 belongs to the tribe Sphodrini and includes many cave, anthropophilic and forest-dwelling specialists in temperate areas. The genus has mainly a Holarctic distribution (a few species are known from Afrotropical Ethiopia and from Neotropical Mexico) and its species are currently separated into several subgenera based on morphological characters (Hovorka & Sciaky 2003, Lorenz 2005). Most of these subgenera are found in the mainland of the western Palaearctic Region. *Calathus s. str.* and *Neocalathus* Ball and Nègre, 1972 are the most species rich. The monotypic

subgenus *Bedelinus* Ragusa, 1885 was ignored by some authors (e.g., Antoine 1957; Zaballos & Jeanne 1994), and the subgenus *Amphyginus* Haliday, 1841 was included within *Neocalathus* by others (e.g., Jeannel 1942). *Iberocalathus* has been recently proposed by Toribio (2006) to include *C. rotundatus*, an Iberian endemism formerly placed within *Amphyginus*. Furthermore, some species of uncertain affinities show intermediate characteristics between two or more subgenera. Thus, *C. granatensis* (Iberian Peninsula) was not included in the subgenus *Calathus* in the revision of Nègre (1969) but was included in this subgenus by Hovorka & Sciaky (2003). In the Canary Islands there are two additional subgen-

era, *Lauricalathus* and *Trichocalathus*, which are endemic to the archipelago and coexist with species of *Neocalathus* (Machado 1992). The pioneering studies of Emerson *et al.* (1999, 2000) yielded interesting clues about the systematic problems of the genus but the major focus of these studies was on the phylogeography of the Macaronesian taxa.

We undertook a phylogenetic study of the genus based on mitochondrial and nuclear sequences. Our first aim was to examine the agreement between conclusions derived from the analysis of cytochrome-oxidase I–II sequences (cox1–cox2) and the current subgeneric arrangement of the *Calathus (sensu lato)* for the western Palaearctic taxa. These classifications (i.e., the grouping of particular taxa into subgenera or species-groups) are here considered as initial hypotheses of relationships that can be tested on molecular grounds.

2. Material and methods

We have sequenced a cox1–cox2 fragment that included part of the cox1 gene and the complete tRNA^{Leu} and cox2 genes. Eighty-four individuals from 44 species of *Calathus* (Table 1) were included in the analysis. The nucleotide sequences of some species have been taken from Emerson *et al.* (1999, 2000), mostly corresponding to Macaronesian taxa (EMBL–Nucleotide Sequence Database, accession numbers AJ236944–AJ236980 and AJ404973–AJ405007). The related genera *Calathidius*, *Eutrichopus* and *Paraeutrichopus* were used as an out-group. The species set thus includes representatives of seven recognized *Calathus* subgenera: *Calathus s. str.*, *Neocalathus*, *Bedelinus*, *Amphigynus*, *Iberocalathus*, *Lauricalathus* and *Trichocalathus*.

Specimens were collected in the localities indicated in Table 1. Beetles were kept alive in the field, immersed in absolute ethanol in the lab and preserved at +4 °C. DNA was extracted with QIAGEN DNeasy tissue kit (Qiagen, Hilden, Germany) following manufacturer's recommendations. To amplify the mitochondrial COI–COII fragment we used the primers C1-J-2160 5'TAACAGATCGAAATTTAAATACT 3' (designed by Brent Emerson) and TK-N-3782

(“Eva”, Harrison Laboratory) 5'GAGACCATTA CTTGCTTTCAGTCATCT 3'. These primers amplified a mtDNA fragment of 1706 bp. The PCR was carried out in 40 cycles at 94°C for 1 min, annealing at 47 °C for 1 min and extension of 72 °C for 2 min. PCR products were purified with isopropanol and 5M ammonium acetate. Sequencing was performed on an ABI 377 automated sequencer (Applied Biosystems). Internal primers were used to obtain the whole sequence UEA9CAL 5' GTAAATTTAACATTTTTTCCT CAACA 3' and CALCOII 5'TTAAAATCTATT GGTCATCAATGATA 3' (designed by Brent Emerson). Sequences were aligned by eye and incomplete ends were removed from the analysis. The new sequences have been sent to EMBL (accession numbers will be provided within the final version).

The data matrix was subjected to parsimony (MP), Neighbor-Joining (NJ) and Bayesian analyses. The data set was analysed with Modeltest (v. 3.6) (Posada & Crandall 1998) and the generalized time reversible (GTR) model with gamma distributed among site variation and a proportion of invariable sites was selected as best fit to the data. NJ was performed with this model in PAUP version 4.0b10 (Swofford 2002) with 1,000 bootstrap replicates. MP was run in PAUP using heuristic searches with the TBR option. Bayesian analysis was performed with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) consisting in 750,000 generations, sampling trees every 100th generation.

The log-likelihood scores of sample points were plotted against generation time and stationarity of Markov chains was assumed when the log-likelihood values reached a stable equilibrium. The stationarity of the chains was confirmed by plotting the remaining log-likelihood values (*sump* command). The equilibrium was attained before the first 1500 trees (150,000 generations; burn-in value). The model used was GTR setting among-site rate variation I + gamma.

3. Results

The analysed a cox1–cox2 fragment had 1631 bp, of which 603 bp were variable (37%) and 482 bp were parsimony informative (80% of the variable

Table 1. Species of *Calathus* included in the molecular analyses; the asterisks show the samples sequenced in this work. BU = Bulgaria; CI = Canary Islands; GB = Great Britain; IT = Italy; MO = Morocco; PL = Poland; PT = Portugal; SP = Spain.

Taxon	Sample locality
<i>C. (Amphyginus) rotundicollis</i> Dejean	Soria (SP) *
<i>C. (Bedelinus) circumseptus</i> Germar	Murcia (SP) *
<i>C. (Calathus) baeticus</i> Rambur	Granada (SP) *
<i>C. (Calathus) fracassii</i> Heyden	Gran Sasso (IT)
<i>C. (Calathus) fuscipes</i> Goeze	1 – Soria (SP) (<i>graecus</i>) *
	2 – Rif Mountains (MO) (<i>algericus</i>) *
	3 – Gran Sasso (IT)
<i>C. (Calathus) hispanicus</i> Gautier des Cottés	Barajas (SP)
<i>C. (Calathus) mirei</i> Nègre	Soria (SP) *
<i>C. (Calathus) pirazzolii</i> Putzeys	Gran Sasso (IT)
<i>C. (Calathus?) granatensis</i> Vuillefroy	1 – Cádiz (SP)
	2 – Málaga (SP) *
<i>C. (Calathus?) opacus</i> Lucas	Moyen Atlas (MO)
<i>C. (Iberocalathus) rotundatus</i> Jacquelin du Val	1 – Braganza (PT) (<i>rotundatus</i>) *
	2 – Orense (SP) (<i>rotundatus</i>) *
	3 – Serra da Estrela (PT) (<i>estrelensis</i>) *
<i>C. (Lauricalathus) abaxoides</i> Brullé	Tenerife (CI)
<i>C. (Lauricalathus) angularis</i> Brullé	Gran Canaria (CI)
<i>C. (Lauricalathus) angustulus</i> Wollaston	Tenerife (CI)
<i>C. (Lauricalathus) appendiculatus</i> Wollaston	Gran Canaria (CI)
<i>C. (Lauricalathus) ascendens</i> Wollaston	Tenerife (CI)
<i>C. (Lauricalathus) auctus</i> Wollaston	Tenerife (CI)
<i>C. (Lauricalathus) canariensis</i> Harold	Gran Canaria (CI)
<i>C. (Lauricalathus) carinatus</i> Brullé	Tenerife (CI)
<i>C. (Lauricalathus) cognatus</i> Wollaston	Gomera (CI)
<i>C. (Lauricalathus) depressus</i> Brullé	Tenerife (CI)
<i>C. (Lauricalathus) freyi</i> Colas	Tenerife (CI)
<i>C. (Lauricalathus) gomerensis</i> Colas	Gomera (CI)
<i>C. (Lauricalathus) laureticola</i> Wollaston	Gomera (CI)
<i>C. (Lauricalathus) marcellae</i> Colas	Gomera (CI)
<i>C. (Lauricalathus) rectus</i> Wollaston	Tenerife (CI)
<i>C. (Lauricalathus) spretus</i> Wollaston	El Hierro (CI)
<i>C. (Neocalathus) ambiguus</i> Paykull	Gran Sasso (IT)
<i>C. (Neocalathus) asturiensis</i> Vuillefroy	Vigo (SP)
<i>C. (Neocalathus) cinctus</i> Motschulsky	Soria (SP) *
<i>C. (Neocalathus) erratus</i> C.R. Sahlberg	Rhodopi Mountains (BU) *
<i>C. (Neocalathus) gonzalezi</i> Mateu	Fuerteventura (CI)
<i>C. (Neocalathus) melanocephalus</i> Linnaeus	Norfolk (GB)
<i>C. (Neocalathus) micropterus</i> Duftschmid	Poznan (PL) *
<i>C. (Neocalathus) semisericeus</i> Fairmaire	Rif Mountains (MO) *
<i>C. (Neocalathus) simplicicollis</i> Wollaston	Lanzarote (CI)
<i>C. (Neocalathus) subfuscus</i> Wollaston	Madeira
<i>C. (Neocalathus?) metallicus</i> Dejean	Pirin Mountains (BU) (<i>aeneus</i>) *
<i>C. (Trichocalathus) obliteratus</i> Wollaston	Gomera (CI)
<i>C. (Trichocalathus) pilosipennis</i> Machado	Gomera (CI)
<i>C. (Trichocalathus) refleximargo</i> Machado	Gomera (CI)
<i>C. colasianus</i> Mateu (<i>incertae sedis</i>)	Madeira
<i>C. complanatus</i> Dejean (<i>incertae sedis</i>)	Madeira
<i>C. vividus</i> Fabricius (<i>incertae sedis</i>)	Madeira

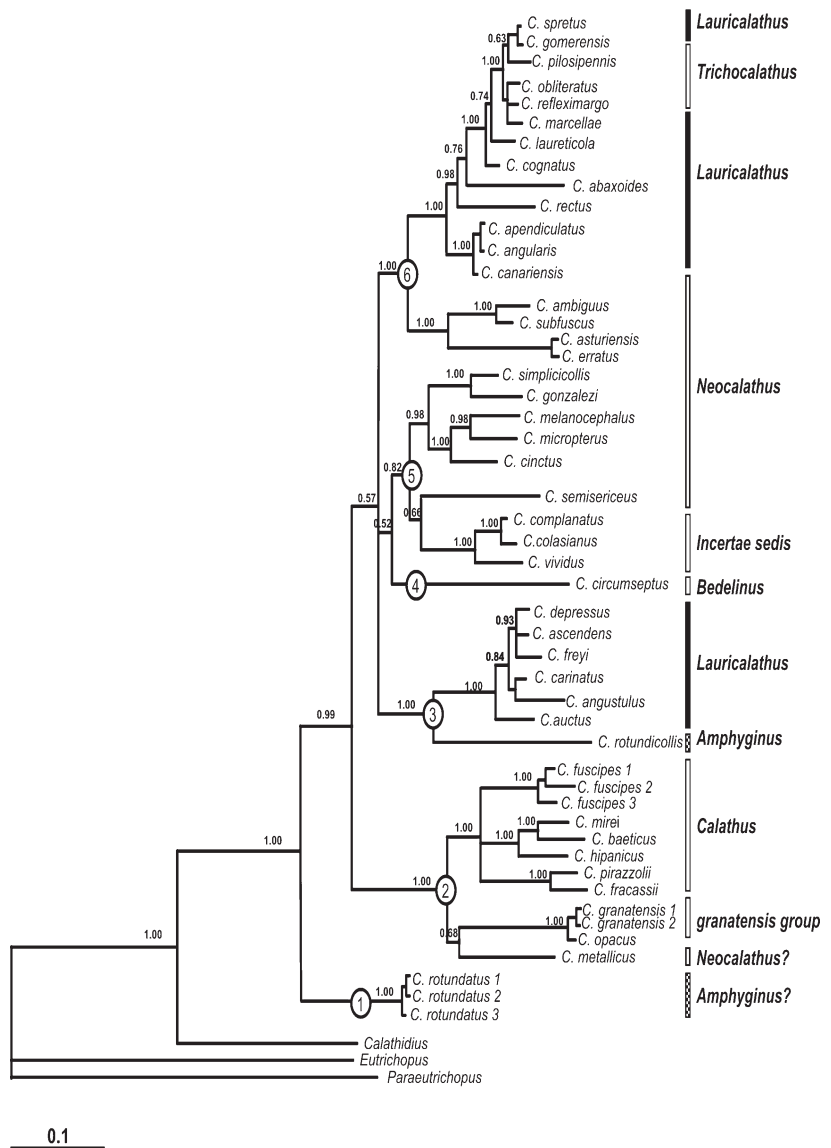
Table 2. Sequence statistics in *Calathus*.

Gen	Sequence length	Variable sites	Informative sites	A	C	G
COI	852	324	256	31.3	14.2	14.1
tRNA ^{Leu}	78	19	14	35.2	12.4	17.0
COII	701	309	243	35.4	13.9	12.3
Total	1631	660	556	33.2	13.9	13.4

sites). The average base composition is shown in Table 2. Trees obtained by Bayesian (Fig. 1), NJ, and MP (Fig. 2) analyses showed the same main clades.

- (a) Clade 1 includes *C. rotundatus*, a species placed in the subgenus *Iberocalathus* recently proposed by Toribio (2006).
- (b) Clade 2 includes in one branch all the species

Fig. 1. The 50% majority consensus tree derived from the Bayesian analysis under a model of GTR+I+X. Posterior probabilities of the main clades are shown above the branches. Circled numbers designate nodes discussed in the text.



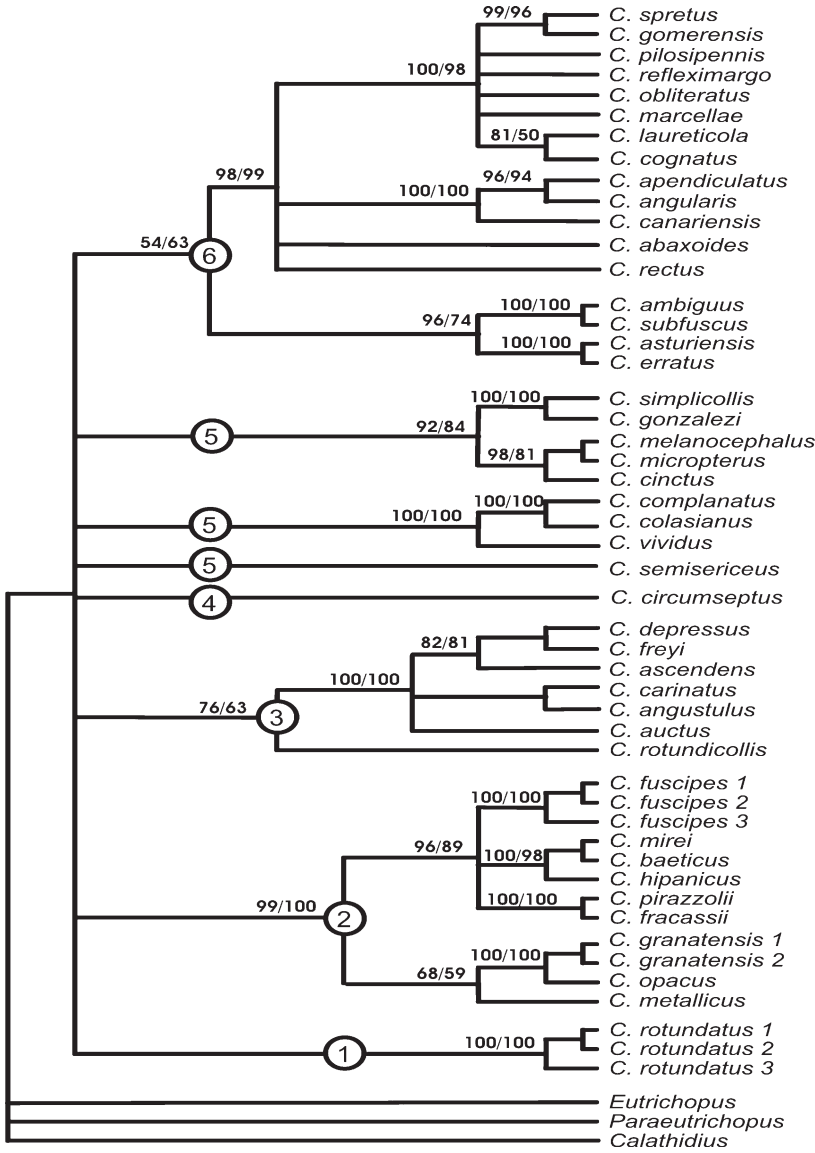


Fig. 2. 50% majority consensus tree obtained from Neighbor-Joining (NJ) and Maximum Parsimony (MP) analyses. Numbers above branches correspond to NJ/MP bootstrap values. Circled numbers designate nodes discussed in the text.

of the subgenus *Calathus* and in a second branch *C. granatensis* + *C. opacus* and *C. metallicus*, although the relationships of this last species to the *granatensis* group is weak.

- (c) Clade 3 includes *C. rotundicollis* (the type species of the subgenus *Amphyginus*) and the Tenerifean species of the subgenus *Lauricalathus* that made up the Clade D in the study of Emerson et al. (2000). This relationship is highly supported in the Bayesian analysis.
- (d) Clade 4 includes the single species of subgenus *Bedelinus*, *C. circumseptus*.

- (e) Clade 5 receives less support in the Bayesian analysis and is not recognized in NJ and MP analyses. It includes part of the subgenus *Neocalathus* (the *melanocephalus*-group), the species from Madeira *C. vividus*, *C. complanatus* and *C. colasianus*, that are placed as *incertae sedis* in the last Palearctic catalogue and the North African *C. semisericeus* (Fig. 1).
- (f) Clade 6 includes the other species of the subgenus *Neocalathus* (the *ambiguus* group) and the Canarian taxa belonging to the subgenera

Lauricalathus and *Trichocalathus* that made up the Clade C of Emerson *et al.* (2000).

4. Discussion

The molecular data corroborate the separation of the subgenus *Iberocalathus* (Toribio 2006), described on the basis of morphological and geographic data. This monotypic subgenus includes *C. rotundatus*, a species formerly included in the subgenus *Amphyginus* by Hovorka & Sciaky (2003). Therefore this last subgenus only includes the European *C. rotundicollis* that is related to the clade D of Emerson *et al.* (2000). This clade is made up by the *depressus*-group inhabiting Tenerife. This finding suggests that the clade D of Emerson *et al.* (2000) has a Continental ancestor related to *C. rotundicollis*.

The subgenus *Calathus*, here represented by the widespread and polytypic *C. fuscipes* and 5 taxa endemic to Iberia and Italy, is a monophyletic taxon that is in accordance with the delimitation of the subgenus put forward by Nègre (1969) on morphological grounds: the number of elytral punctures, the shape of the apical tip of median lobe and the presence of a distinct spine in the internal sac of aedeagus. Its sister taxon is the *granatensis*-group.

The relationship of *C. granatensis* + *C. opacus* to *C. (Neocalathus) metallicus* was not previously suspected and suggests that the affinities of the last species must be assessed by considering different character sets. The uncertain position of the siblings *C. granatensis* and *C. opacus*, formerly assigned to *Calathus s. str.*, has been solved to some extent as the molecular data suggest that they should be better placed in a subgenus of their own. In fact, the diagnostic characters of the subgenus *Calathus s. str.* are not found in the *C. granatensis* group.

The relationships of the monotypic subgenus *Bedelinus* have not satisfactorily solved, as it only appears weakly related to some *Neocalathus* taxa in the Bayesian analysis (clade 5).

The subgenus *Neocalathus* is polyphyletic as some species are related to *Lauricalathus* and *Trichocalathus* (clade 6, Figs. 1–2), whereas other species make a monophyletic group with Continental and Macaronesian taxa (clade 5).

Further, *C. metallicus* is not related to the above mentioned lineages but to the *C. granatensis* group (clade 2). These results suggest that the taxonomy of *Neocalathus* should be thoroughly revised. The species of *Neocalathus* are currently characterised by preference for open habitats and a good dispersal power based on the development of functional wings. These features are congruent with the hypothesis of a close relationship between particular Macaronesian and *Neocalathus* taxa [*C. subfuscus* and *C. ambiguus*; *C. simplicicollis* + *C. gonzalezi* and the *C. melanocephalus* group; Emerson *et al.* (2000)].

Finally, the subgenus *Lauricalathus* is polyphyletic as it is divided between clades 3 and 6 (Figs. 1–2). Clade 3 is made up by the *depressus*-group of Tenerife, whereas clade 6 includes the other species of *Lauricalathus* plus the three species of the subgenus *Trichocalathus*, *C. obliteratus*, *C. pilosipennis* and *C. refleximargo* inhabiting La Gomera. The scarce differentiation of *Trichocalathus* in relation to the species of *Lauricalathus* also present in La Gomera shows the need of further molecular analyses for a better assessment of its taxonomic validity. The species of the *depressus*-group must be kept within *Lauricalathus* (*C. depressus* is the type species of the subgenus), whereas the other species of the subgenus (the clade C of Emerson *et al.* 2000) should be eventually included in a new subgenus.

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