Rekindling Jeannel's Gondwanan vision? Phylogenetics and evolution of Carabinae with a focus on *Calosoma* caterpillar hunter beetles

EMMANUEL F. A. TOUSSAINT FLS[1*] and CONRAD P. D. T. GILLETT[2]

[1]*Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA*
[2]*University of Hawai'i Insect Museum, College of Tropical Agriculture and Human Resources, University of Hawai'i at Mānoa Honolulu, HI 96822, USA*

The subfamily Carabinae is a diverse clade distributed across all biogeographical regions except Antarctica. In a seminal work, René Jeannel hypothesized a Gondwanan origin for this group, but this has hitherto remained untested with molecular data. We test this hypothesis by using a supermatrix approach. We also infer the most comprehensive phylogeny of the genus *Calosoma*, the only lineage within Carabinae comprising predominantly flying species. We use a recent timetree of Coleoptera to infer divergence time estimates in Carabinae. Our results identify four main lineages within *Calosoma* and reject the monophyly of several species groups erected by Jeannel. The subfamily Carabinae is estimated to have arisen in the Jurassic as suggested by Jeannel, and this dating is congruent, to some extent, with a vicariant hypothesis linked to the timing of the fragmentation of Gondwana. The main lineages of *Calosoma* are suggested to have diverged from each other in the Palaeogene, suggesting a dynamic biogeography, possibly shaped by dispersal rather than vicariance. This pattern could have resulted from the unique morphological evolution in *Calosoma*, allowing certain lineages to actively fly. Our divergence times within Carabinae are markedly inconsistent with previous studies, therefore reiterating the need for a fine-scale, fossil-based timetree of Adephaga.

ADDITIONAL KEYWORDS: Bayesian relaxed clocks – Carabinae phylogenetics – divergence times – Jurassic – ground beetle evolution – supermatrix approach.

INTRODUCTION

Beetles are a comparatively ancient group of insects whose origin probably arose during the Triassic (McKenna *et al.*, 2015; Toussaint *et al.*, 2017a). Because of their great age, some beetle lineages have been hypothesized to represent vicariant relicts that resulted from the fragmentation of the supercontinent Gondwana. Since the advent of molecular clock estimation of divergence times, testing such hypotheses in a statistical framework has become possible. Although the timing of diversification in some beetle lineages has been shown to concur with that of Gondwanan fragmentation (e.g. Bukontaite, Miller & Bergsten, 2014; Kim & Farrell, 2015; Andújar *et al.*, 2016; Günter *et al.*, 2016; Toussaint *et al.,* 2017c; Toussaint, Fikáček & Short, 2016b; Toussaint, Bloom & Short, 2017b; Eberle *et al.*, 2017), the biogeographical origins of other beetle groups with Gondwana-like distributions have been demonstrated to post-date this major tectonic reassembly (e.g. Toussaint & Short, 2017).

The subfamily Carabinae is a monophyletic species-rich group of ground beetles (*c.* 1500 species; Osawa, Su & Imura, 2004), distributed in all major biogeographical regions except Antarctica (Jeannel, 1940; Osawa *et al.*, 2004). Interestingly, some lineages within the subfamily are restricted in distribution to a single region, while others are more widespread. For instance, the charismatic genus *Carabus*, consisting of *c.* 1000 described species (Deuve 2004), is mostly Holarctic (overwhelmingly Palearctic) in distribution (Sota *et al.*, 2004; Deuve *et al.*, 2012). The same is true of the tribe Cychrini, comprising the Palearctic genera *Cychrus* and *Cychropsis*, and the Nearctic genera *Scaphinotus* and *Sphaeroderus* (Gidaspow, 1973;

*Corresponding author. E-mail: emmanuel.touss1@gmail.com

Su *et al.*, 1996, 2004). In the austral part of the planet, the genera *Ceroglossus*, *Maoripamborus* and *Pamborus* are respectively endemic to Chile and Argentina, New Zealand and Australia (Sota *et al.*, 2005). By contrast, the genus *Calosoma* has a much more widespread geographical range, which encompasses all the biogeographical regions, and includes the Galapagos islands, Madagascar and New Caledonia. The distribution of Carabinae was hypothesized by the famous French entomologist René Jeannel (1940) to potentially reflect the fragmentation of the supercontinent Gondwana. However, this hypothesis has never been properly tested so far.

The only studies to date that have aimed at inferring divergence times within Carabinae are based on a unique late Miocene fossil (†*Carabus cancellatus*; Deuve, 1998) and/or several biogeographical calibrations (e.g. Su *et al.*, 1998, 2004; Osawa *et al.*, 1999; Su, Imura & Osawa, 2001; Andújar *et al.*, 2012a, 2014; Andújar Serrano & Gómez-Zurita, 2012b). These studies assumed that the divergence of flightless carabine lineages resulted from geological vicariance events. In several articles aiming at dating the origin of Japanese *Carabus* ground beetles, Su *et al.* (1998, 2001) dated the split between *Cychrus* and *Carabus* to within the Eocene *c.* 40–50 Ma. More recently, Andújar *et al.* (2014) tested the congruence and compatibility of multiple calibrations to estimate the origin of the genus *Carabus*. They concluded that older vicariant calibrations (e.g. between New Zealand *Maoripamborus* and Australian *Pamborus*; Sota *et al.*, 2005) were inconsistent with gene fragment substitution rates. As a result, their divergence time estimates placed the origin of the subfamily Carabinae in the Eocene *c.* 40 Ma, and the split between *Calosoma* and *Carabus* in the early Miocene *c.* 23 Ma (Andújar *et al.*, 2014). In an independent study of the genus *Carabus*, Deuve *et al.* (2012) estimated an origin of Carabinae, as represented by only the genera *Carabus*, *Cychrus* and *Pamborus*, in the late Oligocene *c.* 25 Ma. This last study, and those of Andújar *et al.* (2012a, b, 2014), used the only known Carabinae fossil found in Messinian deposits of Cantal in France (*c.* 5 Ma; Deuve 1998), in combination with biogeographical calibrations. However, the use of a very young fossil and/or of biogeographical calibrations in these studies might underestimate the ages of the different lineages, especially if the divergences of clades constrained with biogeographical calibrations do not, in fact, correspond to the presumed geological events.

Therefore, there is a great deal of uncertainty surrounding the origin of Carabinae, although little doubt that it represents an early divergent lineage within Carabidae (Maddison *et al.*, 2009; McKenna *et al.*, 2015). The subfamily has been recovered either as sister to all other carabid subfamilies (Maddison *et al.*, 2009; López-López *et al.*, 2017) or as sister to Trachypachidae (Maddison *et al.*, 2009; McKenna *et*

al., 2015). However, it is difficult to predict the age of extant lineages within Carabinae, because extinction events might obscure inferences into cladogenetic events. The fossil record of Carabidae is far from extensive, and the oldest known fossils, from the Cretaceous, do not belong to Carabinae, but rather to other subfamilies (e.g. Cicindelinae: Cassola & Werner 2004; Licininae: Liu *et al.*, 2015; Protorabinae: Wang & Zhang 2011). Therefore, only broader taxon sampling across Carabidae will allow for disentanglement of the timeline of Carabinae ground beetle evolution in the future. There is a substantial taphonomic bias within the fossil record of this group, and it is possible that external fossil-based calibrations recover a more ancient origin of lineages than the one suggested by biogeographically informed dating exercises (e.g. Su *et al.*, 2001, 2004, 2005; Deuve *et al.*, 2012; Andújar *et al.*, 2012a, b, 2014). The most recent attempts to date the beetle tree of life have offered some promising insights with respect to the origin of Carabinae. The large-scale study by McKenna *et al.* (2015) suggested an age for the origin of Carabidae of *c.* 170 Ma, in the middle Jurassic. In their study, McKenna *et al.* (2015) recovered Carabinae (as represented by the genus *Calosoma*) as sister to Trachypachidae, and the latter clade as sister to all other ground beetles. However, Toussaint *et al.* (2017a), using the same dataset but a denser fossil selection for calibration, recovered an origin for the family of *c.* 220 Ma, in the late Triassic, with the split between *Calosoma* and Trachypachidae dated to the early Jurassic *c.* 200 Ma. To date, this is the latest, fossil-based, divergence time estimate for the split between Carabinae and its supposed sister taxon.

Since its inception, Jeannel's hypothesis of carabine beetle Gondwanan origins has never been revisited per se, because (1) of the difficulty in estimating divergence times within Carabinae without relying on biogeographical calibrations, (ii) the systematics among genera of Carabinae have rarely been studied in a single study (but see Osawa *et al.*, 2004), and (3) some carabine genera lack a comprehensive molecular phylogenetic evolutionary hypothesis. One such group is the caterpillar hunters of the genus *Calosoma* (Fig. 1), which contain species that have been well known to entomologists for centuries owing to their large size, bright colours, and eponymous predatory habits, and oscillating populations, which closely track those of their preferred caterpillar prey (Bruschi, 2013). Although Jeannel (1940), in the last detailed morphological revision of the genus *Calosoma*, divided it into four 'phyletic series', which can be interpreted today as clades, the phylogenetic relationships among its constituent subgenera (Fig. 1) remain largely unknown. A study by Su *et al.* (2005) inferred relationships within *Calosoma* using a unique mitochondrial marker (ND5, see below). The authors inferred a moderately
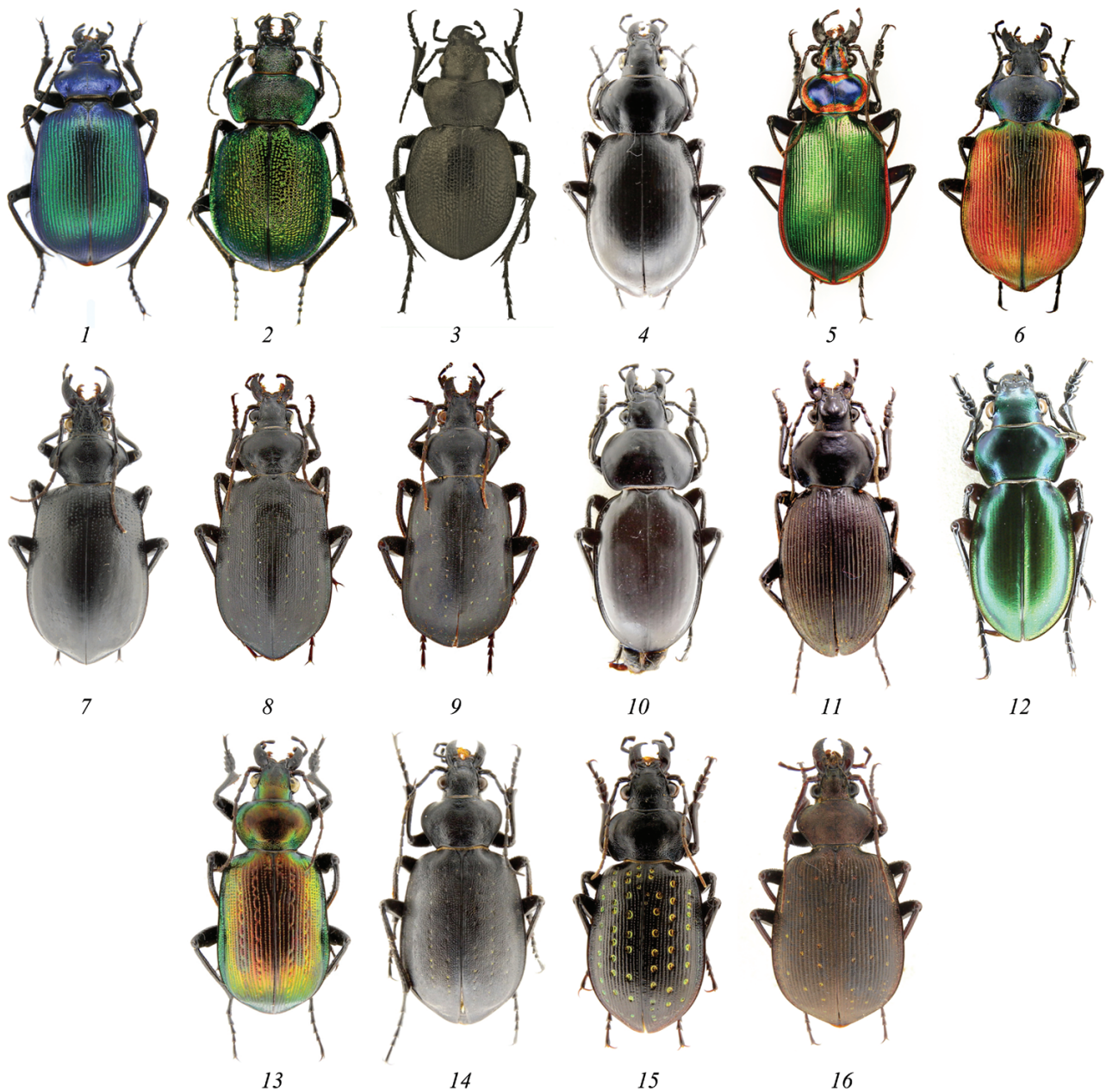
1. C. (Australodrepa) oceanicum	5. C. (Calodrepa) scrutator	9. C. (Campalita) maderae	13. C. (Castrida) fulgens
2. C. (Callisthenes) reticulatum	6. C. (Calosoma) sycophanta	10. C. (Carabomimus) striatulum	14. C. (Charmosta) lugens
3. C. (Callistenia) luxatum	7. C. (Camedula) prominens	11. C. (Carabomorphus) masaicum	15. C. (Chrysostigma) calidum
4. C. (Callitropa) haydeni	8. C. (Caminara) olivieri	12. C. (Carabophanus) arrowi	16. C. (Ctenosta) grandidieri

Figure 1. Morphological diversity in the genus *Calosoma*. Images of *Calosoma* species, represnting the subgenera sampled in this study with subgeneric classification following Jeannel (1940). All photographs by Sandro Bruschi (http://www.calo-somas.com/; last accessed date 31 October 2017).

supported phylogenetic hypothesis for the genus, but their findings shed light on the likely paraphyly or polyphyly of most subgenera currently recognized (Bruschi, 2013; Häckel, 2013). More than a decade later, Ray, Seidel & Husemann (2017) produced a new molecular matrix to investigate the placement of aberrant *Calosoma wilcoxi* specimens in a reduced phylogenetic framework. Their results substantiate those of Su *et al*. (2005), confirming the non-monophyly of some subgenera. Although these two attempts shed light on some systematic issues, the phylogenetic relationships among *Calosoma* lineages remain rather obscure, despite a large amount of molecular data having been generated in ground beetle projects where species of *Calosoma* are often sequenced 'collaterally'. In the present study, we generate a molecular matrix combining all available molecular data for *Calosoma*, to investigate the phylogenetic relationships among major lineages within this genus. We also aim to infer the placement of *Calosoma* within the subfamily Carabinae, and to infer divergence time estimates to test the hypothesis of Gondwanan vicariance in the subfamily Carabinae, as hypothesized by Jeannel (1940).

MATERIAL AND METHODS

TAXON SAMPLING

The taxon sampling was designed to tackle the two main questions of this study, (1) does the molecular phylogeny of the genus *Calosoma* reflect the historical groupings of Jeannel (1940) based on morphology? and (2) is the origin of Carabinae consistent with the timeline of Gondwana fragmentation? To do so, we sampled the major lineages within Carabinae, and sampled as many species of the genus *Calosoma* as possible. We did not include additional Carabidae lineages in this study, because it is not our objective to test *de novo* the placement of Carabinae within Adephaga. Recent studies have proven that an accurate resolution of phylogenetic relationships among adephagan lineages would probably require a phylogenomic approach coupled with a large taxon sampling (Maddison *et al.*, 2009; Toussaint *et al.*, 2016a; Baca *et al.*, 2017; López-López & Vogler, 2017). We based our taxon sampling on the most recent phylogenetic study of Coleoptera, which recovered a sister relationship between Trachypachidae and Carabinae (McKenna *et al.*, 2015). Since the monophyly of Carabinae is well established (Maddison *et al.*, 1999, 2009; Osawa *et al.*, 2004; López-López & Vogler, 2017), we only sampled *Trachypachus holmbergi* to root the phylogeny, following McKenna *et al.* (2015). All available DNA sequence data from GenBank were gathered and imported in Geneious R 8.1.8 (Biomatters, USA). The

downloaded sequences were checked for taxonomic accuracy and aligned by locus. Alignments included sequences from multiple specimens per species when available. We also included unpublished sequences of the species *Calosoma calidum*, *C. luxatum*, *C. moniliatum*, *C. obsoletum* and *C. tepidum* provided by the Canadian National Collection of Insects, Arachnids and Nematodes; Agriculture and Agri-Food Canada (Ottawa, Canada), as well as of *C. reticulatum* provided by the Finnish Barcode of Life project and the University of Oulu (Finland). All alignments were conducted with MAFFT 7.017 (Katoh & Standley, 2013) using the G-INS-I algorithm. Individual locus alignments were then checked for indels, stop codons or obvious poor quality sequences. All loci that were represented by sequences from fewer than four species of *Calosoma* were not considered for further analyses. The retained gene fragments were the following: ribosomal 16S (817 bp), ribosomal 18S (2075 bp), ribosomal 28S (1070 bp), cytochrome oxidase c subunit 1 (CO1, 1448 bp), cytochrome b (CYTB, 702 bp), NADH dehydrogenase 5 (ND5, 1047 bp), phosphoenolpyruvate carboxykinase (PEPCK, 627 bp) and wingless (438 bp). A gene tree was inferred for each aligned gene fragment using FastTree 2.1.5 (Price, Dehal & Arkin, 2010) as implemented in Geneious R8. Based on locus-specific topologies, contamination and taxonomy were checked a second time.

The gene alignments were pruned to retain only a single representative of each species, and all gene alignments were then concatenated. We selected multiple species, representing lineage diversity in all genera of the subfamily Carabinae, except the Saint Helena endemic and monotypic *Aplothorax burchelli*, for which no sequence data were available (it is possibly now extinct; Ashmole & Ashmole 2004). We sampled some of the main lineages within *Carabus*, according to the molecular phylogeny of Deuve *et al.* (2012). The final alignment comprised 70 taxa, including 46 species of *Calosoma*, out of *c.* 130 described species (Bruschi, 2013; Häckel, 2013). Details of the composition of the final molecular matrix are shown in Table 1.

PHYLOGENETIC INFERENCE

The phylogenetic relationships among selected taxa were inferred using IQ-TREE 1.5.4 (Nguyen *et al.*, 2015). The best partitioning scheme was selected using the greedy algorithm implemented in PartitionFinder 2.1.1 (Lanfear *et al.*, 2017), based on a priori delimitation of individual ribosomal loci, and codon positions of protein-coding loci. The fit of all nucleotide substitution models implemented in PartitionFinder 2.1.1 was assessed using the Bayesian Information Criterion (BIC). The partitions selected in PartitionFinder 2.1.1

Table 1. Taxon sampling, indicating the nuclear and mitochondrial loci analysed for each taxon

Genus	Subgenus	Species	Authority	Distribution*	16S	18S	28S	CO1	CYTB	ND5	PEPCK	WGL
Calosoma	Castrida	alternans	Fabricius (1792)	CAR/NEO	–	–	–	–	–	×	–	–
Calosoma	Camedula	angulatum	Chevrolat, 1834	NEA/NEO	–	–	–	×	–	×	–	–
Calosoma	Carabophanus	arrowi	Breuning, 1928	AFR	–	×	–	–	–	–	–	–
Calosoma	Campalita	auropunctatum	(Herbst, 1784)	PAL	×	×	×	×	×	×	–	–
Calosoma	Callisthenes	breviusculum	(Mannerheim, 1830)	PAL	×	×	×	×	×	×	–	–
Calosoma	Chrysostigma	calidum	(Fabricius, 1775)	CAR/NEA	–	–	–	×	–	×	–	–
Calosoma	Callitropa	laeve (chihuahua)	Dejean, 1826	NEA	–	–	–	–	–	×	–	–
Calosoma	Campalita	chinense	Kirby, 1818	OR?/PAL	–	×	×	–	–	×	×	×
Calosoma	Carabomimus	costipenne	Chaudoir, 1869	NEA	–	–	–	–	–	×	–	–
Calosoma	Caminara	davidis	Géhin, 1885	OR	–	–	–	–	–	×	–	–
Calosoma	Callisthenes	elegans	Kirsh, 1859	PAL	–	–	–	–	–	×	–	–
Calosoma	Calosoma	frigidum	Kirby, 1873	NEA	–	–	–	×	–	×	–	–
Calosoma	Castrida	fulgens	Chaudoir, 1869	NEO	–	–	–	–	–	×	–	–
Calosoma	Castrida	galapageium	Hope, 1838	GAL	×	–	–	×	×	–	–	×
Calosoma	Castrida	granatense	Géhin, 1885	GAL	×	–	–	×	×	–	–	×
Calosoma	Ctenosta	grandidieri	Maindron, 1900	MAD	–	–	–	–	–	–	×	×
Calosoma	Callitropa	haydeni	Horn, 1870	NEA	–	–	–	–	–	×	–	–
Calosoma	Calosoma	inquisitor	Linnaeus, 1758	PAL	×	×	×	×	–	×	×	×
Calosoma	Callisthenes	kuschakewitschi	Ballion, 1870	PAL	–	–	–	–	–	×	–	–
Calosoma	Carabomimus	laevigatum	Chaudoir, 1869	NEA	–	–	–	–	–	×	–	–
Calosoma	Castrida	leleuporum	Basilewsky, 1968	GAL	×	–	–	×	×	–	–	×
Calosoma	Castrida	linelli	Mutchler, 1925	GAL	×	–	–	×	×	–	–	×
Calosoma	Charmosta	lugens	Chaudoir, 1869	PAL	–	–	–	–	–	×	–	–
Calosoma	Callistenia	luxatum	Say, 1823	NEA	–	–	–	×	–	–	–	–
Calosoma	Callitropa	macrum	LeConte, 1853	NEA	×	–	–	×	×	×	–	–
Calosoma	Campalita	maderae (lepidum)	(Fabricius, 1775)	PAL	×	–	–	×	×	×	–	–
Calosoma	Camedula	marginale	Casey, 1897	NEA	×	–	–	×	–	×	–	–
Calosoma	Carabomorphus	masaicum	Alluaud, 1912	AFR	–	–	–	–	–	×	–	–
Calosoma	Calosoma	maximoviczi	Morawitz, 1863	OR/PAL	–	–	–	–	–	×	×	×
Calosoma	Callistenia	moniliatum	LeConte, 1851	NEA	–	–	–	×	–	–	–	–
Calosoma	Chrysostigma	obsoletum	Say, 1823	NEA	–	–	–	×	–	–	–	–
Calosoma	Australodrepa	oceanicum	Perroud & Montrouzier, 1864	AUS	–	–	–	–	–	×	–	–
Calosoma	Caminara	olivieri	Dejean, 1831	AFR/OR/PAL	–	–	–	×	–	–	–	–
Calosoma	Camedula	peregrinator	Guérin-Méneville, 1844	NEA	–	–	–	×	–	×	–	–
Calosoma	Camedula	prominens	LeConte, 1853	NEA	–	–	–	×	–	–	–	–
Calosoma	Callisthenes	reticulatum	Fabricius, 1787	PAL	–	–	–	×	–	–	–	–
Calosoma	Castrida	sayi	Dejean, 1826	CAR/NEA/NEO	×	–	–	×	×	×	–	–

Table 1. *Continued*

Genus	Subgenus	Species	Authority	Distribution*	16S	18S	28S	CO1	CYTB	ND5	PEPCK	WGL
Calosoma	*Australodrepa*	*schayeri*	Erichson, 1842	AUS	x	x	x	–	–	x	–	–
Calosoma	*Calodrepa*	*scrutator*	(Fabricius, 1775)	NEA, NEO?	–	x	x	x	x	x	x	x
Calosoma	*Chrysostigma*	*semilaeve*	LeConte, 1852	NEA	–	–	–	x	–	–	–	–
Calosoma	*Camedula*	*sponsa (eremicola)*	Casey, 1897	NEA	–	–	–	x	–	–	–	–
Calosoma	*Carabomimus*	*striatulum*	Chevrolat, 1835	NEA	–	–	–	–	–	x	–	–
Calosoma	*Calosoma*	*sycophanta*	Linné, 1758	PAL	x	x	x	x	x	x	–	–
Calosoma	*Chrysostigma*	*tepidum*	LeConte, 1852	NEA	–	–	–	x	x	–	–	–
Calosoma	*Callodrepa*	*wilcoxi*	LeConte, 1848	NEA	x	–	–	x	x	–	–	–
Calosoma	*Callistenia*	*wilkesi*	LeConte, 1851	NEA	–	–	–	x	–	x	–	–
Carabus	*Leptocarabus*	*arboreus*	Lewis, 1882	–	–	x	x	x	–	x	x	x
Carabus	*Carabus*	*arvensis*	Herbst, 1784	–	–	x	x	x	x	x	–	x
Carabus	*Tachypus*	*auratus*	Linnaeus, 1761	–	–	x	x	x	x	x	x	x
Carabus	*Damaster*	*blaptoides*	(Kollar, 1836)	–	–	–	x	x	–	x	x	x
Carabus	*Procrustes*	*coriaceus*	Linnaeus, 1758	–	x	x	x	x	x	x	x	x
Carabus	*Mesocarabus*	*dufouri*	Dejean, 1829	–	x	x	x	x	x	x	–	x
Carabus	*Mesocarabus*	*lusitanicus*	Fabricius, 1801	–	x	x	x	x	x	x	x	x
Carabus	*Hygrocarabus*	*nodulosus*	Creutzer, 1799	–	x	x	x	x	x	x	x	x
Carabus	*Chrysocarabus*	*olympiae*	Sella, 1855	–	–	–	–	–	–	x	x	x
Carabus	*Iniopachus*	*pyrenaeus*	Audinet-Serville, 1821	–	x	–	x	x	x	x	x	x
Carabus	*Megodontus*	*violaceus*	Linnaeus, 1758	–	–	–	x	x	x	x	x	x
Ceroglossus	-	*buqueti*	Laporte de Castelnau, 1834	–	–	–	x	x	–	x	–	–
Ceroglossus	-	*darwini*	Hope, 1837	–	–	x	x	x	–	x	–	–
Cychropsis	*Cychropsis*	*draconis*	Deuve, 1990	–	–	x	x	x	x	x	–	–
Cychrus	*Cychrus*	*attenuatus*	Fabricius, 1792	–	–	–	x	x	x	–	–	–
Cychrus	*Cychrus*	*caraboides*	Linnaeus, 1758	–	x	x	x	x	–	–	–	–
Maoripamborus	–	*fairburni*	Brookes, 1944	–	x	–	–			x	x	–
Pamborus	–	*brisbanensis*	Laporte de Castelnau, 1867	–	x	–	–			x	x	–
Pamborus	–	*tropicus*	Darlington, 1961	–	x	–	–			x	x	–
Scaphinotus	*Stenocantarus*	*angusticollis*	Gistel, 1834	–	–	x	x	x	–	x	–	–
Scaphinotus	*Scaphinotus*	*petersi*	Roeschke, 1907	–	–	x	x	x	x	x	x	x
Sphaeroderus	-	*lecontei*	Dejean, 1826	–	–	–	–	x	–	–	–	–
Sphaeroderus	-	*stenostomus*	Weber, 1801	–	–	–	–	x	–	x	–	–
Trachypachus	-	*holmbergi*	Mannerheim, 1853	–	x	x	x	x	–	x	–	x

AFR, Afrotropics; AUS, Australia; CAR, Carribean Islands; GAL, Galapagos Islands; MAD, Madagascar; NEA, Nearctic; NEO, Neotropics; OR, Oriental; PAL, Palearctic.

were used for the IQ-TREE analyses, but the corresponding models of nucleotide substitution were estimated *de novo* in IQ-TREE using the *Auto* function, across all available models, including the FreeRate model (+R; Soubrier *et al.*, 2012), allowing relaxation of the assumption of gamma-distributed rates. We also performed non-partitioned analyses, in addition to analyses partitioned by locus. All analyses were conducted on the IQ-TREE web cluster (Trifinopoulos *et al.*, 2016), using three different analytical settings, with variable perturbation strength for randomized nearest neighbor interchange (NNI) and stopping rule [number of unsuccessful iterations to stop the maximum-likelihood (ML) search] parameters, of respectively, 0.1/0.3/0.5 and 100/200/500. To assess nodal support, we performed 1000 ultrafast bootstrap replicates (UFBoot). We also performed an SH-aLRT test (Guindon *et al.*, 2010), with 1000 replicates. The UFBoot has been shown to be largely unbiased compared to standard or alternative bootstrap strategies, and the SH-aLRT test has been shown to be as conservative as standard bootstrapping (Minh, Nguyen & von Haeseler, 2013).

DIVERGENCE TIME ESTIMATES

Since there is no fossil known for the subfamily Carabinae that would allow for testing of a different timeline to the one inferred using the unique *Carabus* fossil and biogeographical calibrations (e.g. Deuve *et al.*, 2012; Andújar *et al.*, 2014), we relied on secondary calibrations to date the phylogeny. Specifically, we used the divergence time estimate between *Calosoma* and Trachypachidae as recovered by Toussaint *et al.* (2017a). Since we sampled all but one genus of the subfamily Carabinae, and because Trachypachidae is supposedly the sister clade or a closely related clade to Carabinae (e.g. Maddison *et al.*, 2009; McKenna *et al.*, 2015; López-López & Vogler, 2017), we believe that the use of this secondary calibration is the best approach to obtain absolute divergence time estimates for the group, without enforcing biogeographical calibrations. This dating strategy allows for the testing of the suggested synchronicity between lineage splits and biogeographical vicariant events in Carabinae (Jeannel, 1940; Sota *et al.*, 2005).

We used BEAST 1.8.4 (Drummond *et al.*, 2012) to infer absolute divergence time estimates using the concatenated molecular matrix. We set up multiple analyses in BEAUti 1.8.4 (Drummond *et al.*, 2012) to assess the importance of data partitioning, clock partitioning and tree model choice on downstream inferences. The dataset was partitioned in two different ways: (1) in five partitions corresponding to the result of a PartitionFinder 2 analysis based on a priori division of the matrix per locus; and (2)

in ten partitions corresponding to the result of a PartitionFinder 2 analysis based on a priori division of the matrix per ribosomal locus and protein-coding gene codon positions. The models of nucleotide substitution for each partition were selected in PartitionFinder 2 with the BEAST set of models. The number of clocks was set in two different ways: (1) one uncorrelated lognormal relaxed clock (Drummond *et al.*, 2006) for all mitochondrial partitions, and another for all nuclear partitions; and (2) one uncorrelated lognormal relaxed clock for each partition. The Continuous-Time Markov Chain (CTMC) Rate Reference prior (Ferreira & Suchard 2008) was specified for the ucld.mean rates of the different clocks. The tree model was set to birth–death (Gernhard 2008) or Yule (Yule, 1925; Gernhard, 2008) in distinct analyses. The age of the root corresponding to the split between *Calosoma* and Trachypachidae was constrained to the interval [168.8133–219.4772] corresponding to the 95% credibility interval inferred by Toussaint *et al.* (2017a) for the same node. The topology that received the highest likelihood in the IQ-TREE analyses (see Results) was enforced as a fixed topology by manually editing the BEAUti.xml files. All analyses were run twice to detect potential local optima and ensure convergence. The runs consisted of 50 million generations with a tree and parameter sampling every 5000 generations, resulting in 10 000 posterior samples. To compare the different analyses, we calculated the marginal likelihood of each run using the path sampling/stepping-stone sampling (Baele *et al.*, 2013) implemented in BEAST 1.8.4. These analyses were performed with default parameters. Specifically, we used 100 path steps, with chains running for 1 million generations, with a log likelihood sampled every 1000 generations, and with a beta distribution of path steps. All analyses were run on the CIPRES Science Gateway cluster (Miller, Pfeiffer & Schwartz, 2010). The convergence of the runs, mixing of the chains and effective sample size were checked in Tracer 1.6 (http://BEAST. bio.ed.ac.uk/Tracer). The maximum clade credibility chronograms were generated in TreeAnnotator 1.8.4 (Drummond *et al.*, 2012), with the posterior samples of each analysis after applying a conservative burn-in of 25%.

RESULTS AND DISCUSSION

PHYLOGENETIC RELATIONSHIPS WITHIN CARABINAE

The different IQ-TREE analyses resulted in very similar topologies, as summarized in Table 2. The number of partitions, perturbation strength, and stopping rule seem to have little impact on the inferred phylogenetic hypotheses, with only slight discrepancies in derived

Table 2. Comparison of IQ-TREE phylogenetic analyses based on the concatenated molecular matrix

| Analysis | N. Part. | Pert. Str. | Stop. R. | ML Tree Lik. | Cons. Tree Lik. | RFD | Clade | | | | | | |
							CI	CII	CIII	CIV	CV	CVI	CVII
A1	1	0.1	500	--46110.8880	-46110.888824	0	100/100	99/100	88/97	98/100	55/83	71/99	94/98
A2	1	0.3	200	-46109.3933	-46109.391644	0	100/100	99/99	89/90	99/98	56/74	75/75	95/76
A3	1	0.5	100	-46109.3922	-46109.390650	0	100/100	99/99	89/91	99/97	51/76	72/76	94/77
A4	8	0.1	500	-44742.2533	-44745.097559	4	100/100	98/100	67/76	99/99	73/72	73/43	93/42
A5	8	0.3	200	-44765.8116	-44766.007227	4	100/100	99/100	64/72	99/98	80/71	73/45	93/44
A6	8	0.5	100	-44742.2511	-44744.993181	6	100/100	99/99	65/72	99/99	74/71	73/32	93/32
A7*	9	0.1	500	-42568.8758	-42568.876840	2	100/100	97/96	55/62	98/95	77/63	76/69	92/87
A8*	9	0.3	200	-42574.7358	-42574.736825	0	100/100	97/99	56/63	99/96	75/60	75/69	92/87
A9*	9	0.5	100	-42562.7013	-42562.701855	0	100/100	98/97	43/58	99/95	70/54	77/59	93/81

*The PartitionFinder analysis selected a scheme including nine partitions.
N. Part., number of partitions; Pert. Str., perturbation strength; Stop. R., stopping rule; ML, maximum likelihood; Lik., likelihood; RFD, Robinson–Fould distance between the ML and consensus trees.

nodes of the topologies. The resulting phylogenetic tree from the best IQ-TREE analysis (A9, see Table 2) is presented in Figure 2. The overall nodal support across the topology is moderate, with some strongly supported nodes along the backbone and some lower nodal support values in more derived parts of the topology (Fig. 2, Table 2). Within Carabinae, we recovered the four caribine tribes (Carabini, Ceroglossini, Cychrini and Pamborini) as monophyletic, with strong nodal support, with the exception of Pamborini, which was recovered as monophyletic but with moderate support (Fig. 2).

Within Cychrini, we recovered *Cychropsis* as sister to *Cychrus* (but see below), with strong nodal support (SH-aLRT = 93/UFBoot = 98; Fig. 2) although our taxon sampling is very limited. This first clade was recovered with strong support (SH-aLRT = 100/ UFBoot = 100) as sister to a clade consisting of *Scaphinotus* and *Sphaeroderus* as sister genera. The latter relationship was poorly supported in our phylogenetic hypothesis (SH-aLRT = 13/UFBoot = 54). A study by Su *et al.* (2004) on Cychrini recovered slightly different relationships based on an analysis of CO1 sequences. In that study, *Scaphinotus* was recovered as sister to all other genera within Cychrini, and *Sphaeroderus+Cychropsis* (*Cychrus brezinai* was found sister to *Cychropsis draconis*, but the authors argued that the former in fact belongs to *Cychropsis*) were inferred as sister to *Cychrus*. The phylogenetic hypothesis of Su *et al.*, (2004) was supported only by very low nodal support, and therefore the placement of the four genera within Cychrini remains unresolved and in need of further phylogenetic study.

We recovered the three genera *Ceroglossus*, *Maoripamborus,* and *Pamborus* in a clade with moderate support (SH-aLRT = 57/UFBoot = 94), with *Ceroglossus* as sister to *Maoripamborus+Pamborus* (SH-aLRT = 95/UFBoot = 100). This placement is inconsistent with the phylogenetic hypothesis of Osawa *et al.* (2004) and Andújar *et al.* (2012), who inferred *Ceroglossus* as sister to *Carabus+Calosoma*, albeit with low nodal support, but in line with one result from Su *et al.* (2004), based on ND5 sequences, and Deuve & Faille (2013) based on 18S sequences. The exact placement of *Ceroglossus* therefore remains contentious and should be investigated with both additional taxon sampling and sequence data in the future. The placement of the genera *Ceroglossus*, *Maoripamborus,* and *Pamborus* as sister to *Carabus+Calosoma* with maximal nodal support (SH-aLRT = 100/UFBoot = 100) is mostly consistent with previous phylogenetic hypotheses, which recovered either *Pamborus* (Osawa *et al.*, 2004; Su *et al.*, 2004), *Ceroglossus+Pamborus* (Deuve & Faille, 2013) or *Maoripamborus+Pamborus* (Andújar *et al.*, 2012) as sister to *Carabus+Calosoma*. Within Carabini, we recovered *Calosoma* as sister to *Carabus*,
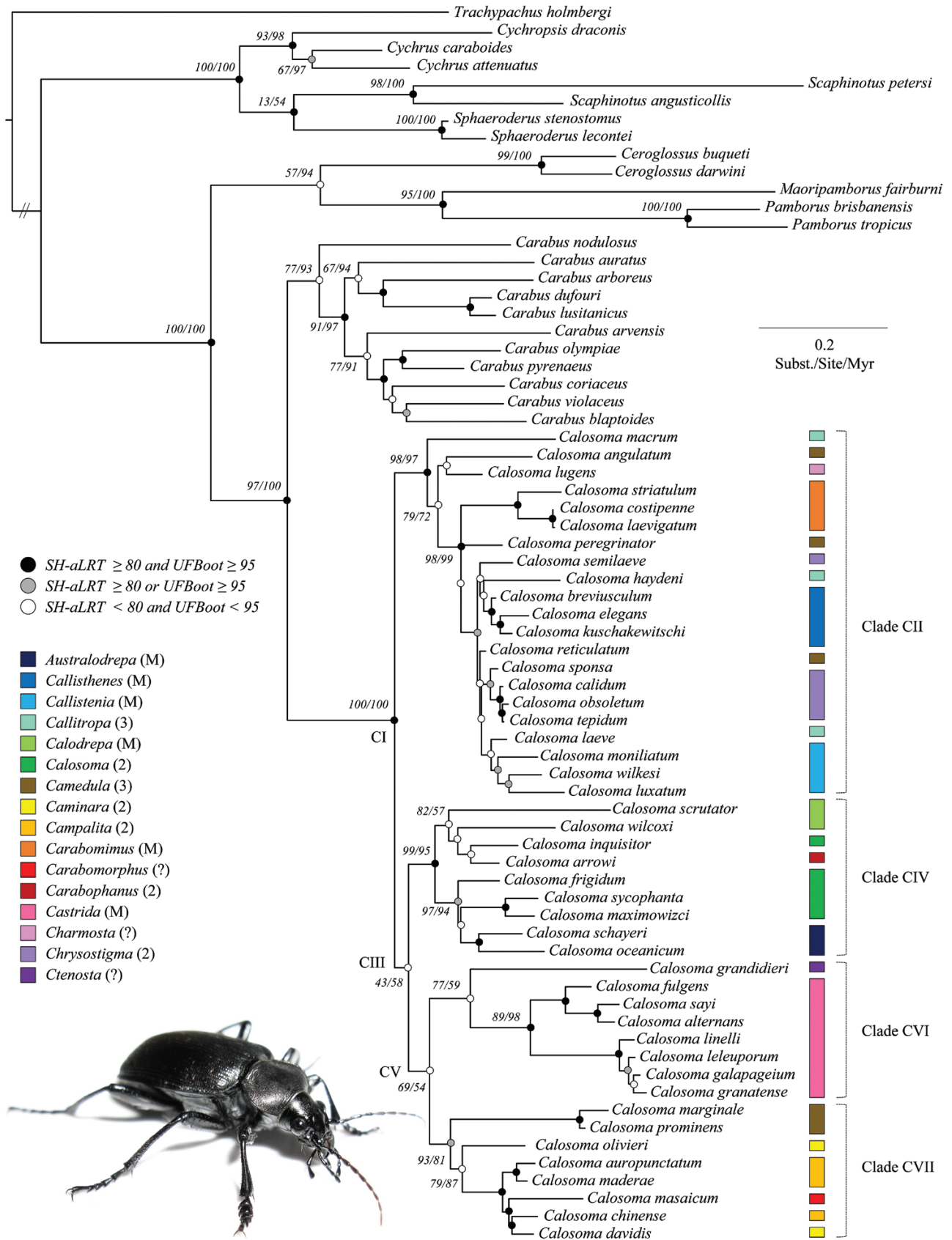
with strong nodal support (SH-aLRT = 97/UFBoot = 100; Fig. 2); as inferred by Osawa *et al.* (2004), Su *et al.* (2004), Andújar *et al.* (2012) and Deuve & Faille (2013).

SYSTEMATICS OF *CALOSOMA* CATERPILLAR HUNTER BEETLES

Within *Calosoma*, we recovered four main lineages, although our phylogenetic hypothesis is not strongly supported (Fig. 2). Most subgenera delimited by Jeannel (1940) and recognized by Bruschi (2013) are not inferred as monophyletic, although some of these clades are broadly congruent with Jeannel's four 'phyletic lines', as follows: Jeannel's 'calosomes lobés' phyletic line equates to our Clade CIV minus *Carabophanus arrowi*, an African high-elevation highly modified species; his '*Castrida-Caminara*' line is highly congruent to our Clade CV minus the two species of *Camedula*; and finally, his '*Callisthenes*' and '*Callitropa*' phyletic lines combined equate to our Clade CII minus *Charmosta lugens*, but with the aforementioned two species of *Camedula* missing from it. These results are also consistent with two other existing molecular phylogenetic hypotheses for the genus (Su *et al.*, 2005; Ray *et al.*, 2017).

The first main lineage (Clade CII, Fig. 2), comprises the subgenera *Callistenia*, *Callitropa*, *Camedula*, *Carabomimus*, *Charmosta* and *Chrysostigma*. This clade is inferred with strong nodal support (SH-aLRT = 98/UFBoot = 97). The different subgenera belonging to Clade CII are morphologically very heterogeneous, and this grouping does not correspond to the classification of Jeannel (1940; see also Bruschi, 2013), although containing within it both the Palaearctic *Callisthenes* and the Nearctic *Callistenia* subgenera, which had in the past been assembled together (as *Callisthenes*) on morphological grounds (Breuning, 1928). Jeannel was the first to consider that this more inclusive *Callisthenes* belonged to the same lineage as the Nearctic subgenus *Chrysostigma*. Indeed, our analyses support Jeannel's assertion, as the two are grouped together within Clade CII, in a subclade also containing a species of the subgenus *Camedula* (*C. peregrinator*), and two paraphyletic representatives of the subgenus *Callitropa*. Both of the latter subgenera predominantly consist of black,

flight-able beetles (Fig. 3), restricted to the deserts of the south-western United States and northern Mexico, that have apparently, as noted by Jeannel (1940), undergone such a remarkable convergence that they would probably have been classified within a single subgenus, were it not for puncturation and chaetotaxy characters that divide them. Our results indicate that the subgenera are differentiated, although related, and that *Callitropa* as presently defined is a polyphyletic subgenus, especially given the position of *C. macrum*, another of its species included in this analysis, and recovered as sister to the rest of Clade CII (Fig. 2).

Jeannel (1940) also postulated that *Callisthenes* (including *Chrysostigma*) diverged from the Old-World subgenus *Caminara*, subsequently dispersing into North America during the Tertiary. However, our results indicate that the two groups are very distantly related and last shared a common ancestor in the late Cretaceous (Figs 2 and 3).

Similarly, Jeannel (1940) had also hypothesized that *Camedula* and *Callitropa* belonged to a divergent lineage of the predominantly Neotropical subgenus *Castrida*, which had spread northwards during the Cretaceous. Our results cannot support this theory, partly because the subgenus *Camedula* is retrieved as polyphyletic in our analyses, occupying positions in two of the four main clades (CII and CVII). Those members of *Camedula* in Clade CII, together with the also polyphyletic *Callitropa*, are only very distantly related to *Castrida*, which itself belongs to a separate and highly divergent clade (Fig. 2). However, because two species of *Camedula* (*C. marginale* and *C. prominens*) were recovered in Clade CVII, which is sister to Clade CVI, that predominantly contains members of *Castrida*, our results could be more in line with Jeannel's thinking only in the case of a more restrictive *Camedula*. However, in this case, our divergence time estimates date the splitting of the two clades CVI and CVII to approximately 60 Ma, during the Palaeogene (Figs 2 and 3). Interestingly, Jeannel (1940) does highlight chaetotaxy characters that clearly separate the two groups, consequently somewhat contradicting his own theory.

A morphological parallelism, noted by Jeannel (1940), and congruent with our results, is the '*Carabusization*' that has occurred in three disparately

Figure 2. Molecular phylogeny of the genus *Calosoma* and closely related genera within Carabinae maximum-likelihood phylogeny inferred in IQ-TREE. Presented is the topology with the highest likelihood across all analyses (Table 2). Nodal support values for the major nodes are given (SH-aLRT/UFBoot). All other nodes are labelled with a coloured circle corresponding to the embedded caption. The names of all sampled subgenera are given on the left, and coloured rectangles indicate their placement in the phylogeny. The parentheses after the subgenera names indicate whether the lineage is recovered as monophyletic (M) or as paraphyletic/polyphyletic in which case the number of independent lineages is given. A photograph of a caterpillar hunter species is presented (credit: Jon Richfield).
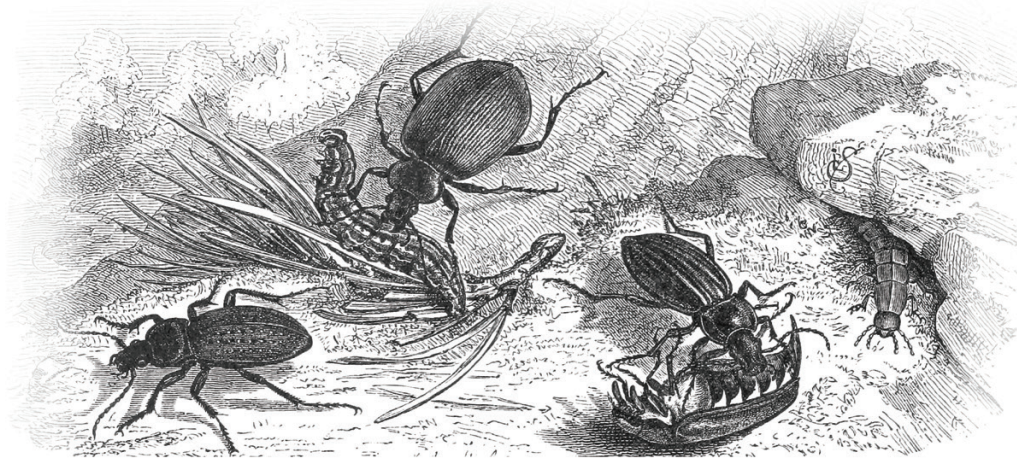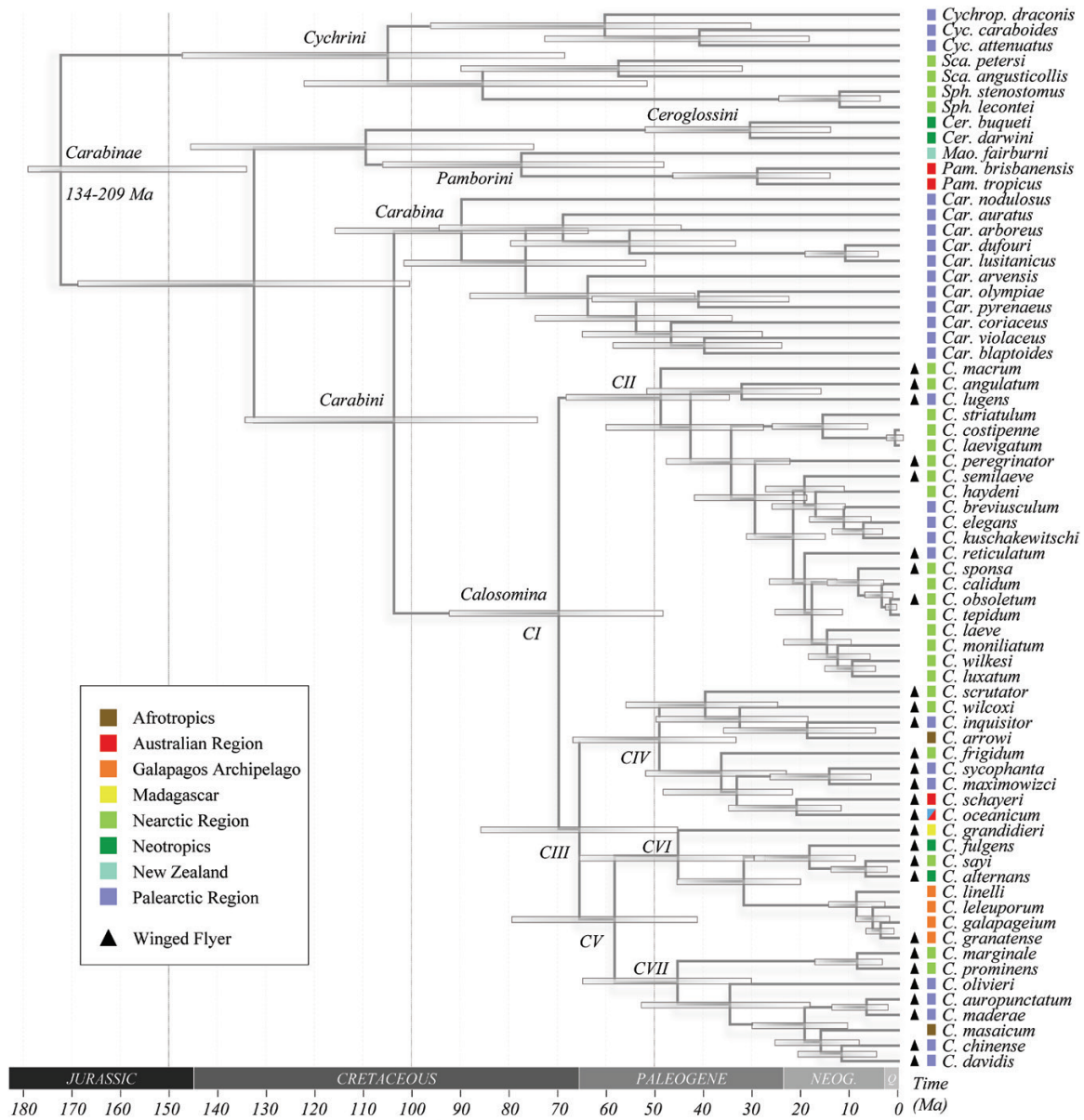
distributed lineages of flightless and mountain-dwelling *Calosoma* (Figs 1–3). This has resulted in their superficial but marked resemblance to members of the genus *Carabus*. The atrophy (or eventual loss) of the hind wings, with its associated muscles, has attenuated the elytral humeri, leading to a more oval elytral shape, as found generally in *Carabus* (Fig. 1). This has been accompanied by a remarkable convergence upon certain elytral surface sculptural patterns commonly present in the latter genus. Of the three subgenera affected, sequences from only two, the Mexican *Carabomimus* (mostly brachypterous) and the East African *Carabomorphus* (all wingless), were available for study. The missing lineage is the former subgenus *Microcallisthenes* from the Balkans, now synonymized with *Callisthenes*, and containing mostly brachypterous species. Our tree clearly indicates that these lineages occupy two of the four main clades, with *Carabomimus* (and *Callisthenes*) belonging to Clade CII, and *Carabomorphus*, which has undergone the more extreme '*Carabus*-ization', occupying a position in Clade CVII, amongst representatives of the Old World subgenera *Caminara* and *Campalita*, which are both recovered as paraphyletic (Fig. 2). It is evident that these aberrant, flightless *Calosoma*, occupying high-elevation mountain massifs on three continents, and having encountered similar environmental and climatic conditions during the course of their history, have converged on an altogether more terrestrial lifestyle. This is in keeping with members of the genus

Carabus, which are ubiquitous in high-mountain regions of much of the northern hemisphere (Deuve *et al.*, 2012).

The latter two subgenera, considered closely related by Jeannel (1940), owing to certain synapomorphic characters of the endophallus, were hypothesized by him to belong to a single phylogenetic lineage also containing the subgenus *Castrida*, which he attributed to a Neotropical 'origin'. This view is congruent with our own results insofar as of the four main clades, Clade CVII containing *Caminara* and *Campalita* is sister to Clade CVI, consisting of a monophyletic *Castrida* plus the Madagascan endemic *Ctenosta* as a sister group (Fig. 3).

In summary, consideration of Jeannel's broader evolutionary interpretations in the light of the molecular data ultimately reveals that several of his conclusions are generally supported by, whilst others are incongruent with, our own results.

DIVERGENCE TIME ESTIMATES AND EVOLUTION OF CARABINAE GROUND BEETLES

The best BEAST analysis based on SS MLE (marginal likelihood calculated using stepping-stone/path sampling estimates) was set up with a birth–death Tree model, ten partitions and two clocks (Table 3). All BEAST analyses recovered very similar absolute divergence time estimates for the major nodes of the phylogeny, with broadly overlapping 95% credibility

Table 3. Comparison of BEAST analyses based on the concatenated molecular matrix

Analysis	N. Part	N. Clocks	Tree model	Posterior	Likelihood	SS MLE
A1	5	2	Yule	−46507.897	−44524.180	−44741.245
A2	5	2	Birth–death	−46288.558	−44524.265	−43865.477
A3	5	5	Yule	−48455.327	−44443.930	−44569.263
A4	5	5	Birth–death	−48237.321	−44443.770	−44826.695
A5*	10	2	Yule	−44752.916	−42515.030	−42910.520
A6*	10	2	Birth–death	−44532.544	−42514.749	−42910.144
A7*	10	10	Yule	−49911.309	−42423.357	−42994.628
A8*	10	10	Birth–death	−49666.514	−42395.946	−42995.262

The PartitionFinder analysis selected a scheme including ten partitions.
N. Part., number of partitions; N. CLOCKS, number of clocks; SS MLE, marginal likelihood calculated using stepping-stone/path sampling estimates; A1, 5 partitions, 2 clocks and Yule model; A2, 5 partitions, 2 clocks and birth–death model; A3, 5 partitions, 5 clocks and Yule model; A4, 5 partitions, 2 clocks and birth–death model; A5, 10 partitions, 2 clocks and Yule model; A6, 10 partitions, 2 clocks and birth–death model; A7, 10 partitions, 10 clocks and Yule model; A8, 10 partitions, 10 clocks and birth–death model.

Figure 3. Bayesian median divergence time estimates of the subfamily Carabinae chronogram derived from the best BEAST analysis as determined using SS MLE (see Table 3). The 95% credibility intervals are given for each node using a horizontal grey bar. The geographical range of each species is indicated by coloured squares. The color-coding follows the inserted caption on the left of the figure. Black triangles indicate a fully winged species known to be a good flyer. All other species are either wingless or brachypterous. An engraving from Alfred Edmund Brehm's book *Brehms Tierleben, Allgemeine kunde des Tierreichs* (Band 9; 1892) is presented under the chronogram, depicting, from left to right, *Carabus hortensis*, *Calosoma sycophanta* and *Carabus auratus* (credit: Wikimedia Commons).

Table 4. Bayesian divergence time estimates (Ma) for major lineages of Carabinae

Analysis	Carabinae	Carabini	Carabus	CI (Calosoma)	Clade CII	CIII	CIV	CV	CVI	CVII
A1	173.0 (135.2–209.0)	135.5 (102.0–171.2)	93.0 (65.8–125.0)	75.9 (54.7–99.7)	52.6 (34.9–72.3)	70.9 (49.9–92.6)	54.7 (37.6–74.5)	63.4 (44.6–84.3)	49.8 (31.2–68.9)	49.2 (29.8–70.3)
A2	171.1 (134.5–212.6)	133.5 (98.5–170.9)	91.2 (64.1–122.6)	74.4 (53.1–99.4)	51.5 (33.3–72.0)	69.3 (48.1–91.8)	53.4 (36.4–73.2)	61.9 (43.1–84.6)	48.3 (30.2–68.3)	48.3 (28.7–70.2)
A3	173.8 (142.3–211.0)	134.7 (104.6–166.8)	97.7 (73.6–123.1)	75.5 (56.9–94.9)	51.4 (36.2–67.8)	71.4 (54.3–90.5)	56.2 (41.0–72.9)	65.3 (48.9–82.9)	52.1 (38.0–69.5)	51.2 (32.8–72.2)
A4	174.0 (140.3–209.9)	135.1 (105.6–166.8)	98.3 (74.1–124.2)	75.0 (56.7–94.9)	51.0 (35.5–66.8)	71.0 (56.7–94.9)	55.9 (41.0–71.7)	65.1 (49.5–84.0)	52.2 (37.8–70.0)	50.7 (32.1–71.3)
A5	172.4 (134.8–211.3)	134.1 (101.2–169.4)	92.2 (65.1–120.3)	72.9 (53.2–96.2)	51.3 (34.5–70.0)	68.6 (50.2–90.7)	51.5 (35.2–69.0)	61.4 (42.6–80.9)	47.7 (30.6–66.3)	48.1 (28.6–68.7)
A6	171.8 (134.1–209.2)	132.2 (99.2–169.2)	89.8 (61.8–117.3)	69.9 (49.3–92.7)	48.9 (32.7–69.0)	65.6 (46.1–87.2)	49.2 (32.4–67.5)	58.4 (41.1–79.9)	45.5 (29.9–65.2)	45.4 (27.3–66.7)
A7	176.1 (145.1–210.6)	134.8 (108.1–165.7)	99.1 (79.1–122.6)	76.9 (60.5–94.8)	52.0 (39.1–67.6)	73.7 (58.5–91.4)	55.9 (43.3–70.3)	67.7 (53.5–85.3)	52.9 (38.8–68.1)	53.7 (35.7–73.0)
A8	172.4 (145.5–202.4)	131.9 (109.1–154.9)	99.0 (80.7–116.8)	77.0 (63.0–90.4)	51.9 (39.5–64.9)	73.9 (60.7–87.4)	56.9 (43.5–65.7)	67.5 (54.2–80.0)	52.7 (41.1–66.4)	53.4 (37.6–70.8)

A1, 5 partitions, 2 clocks and Yule model; A2, 5 partitions, 2 clocks and birth–death model; A3, 5 partitions, 5 clocks and Yule model; A4, 5 partitions, 5 clocks and birth–death model; A5, 10 partitions, 2 clocks and Yule model; A6, 10 partitions, 2 clocks and birth–death model; A7, 10 partitions, 10 clocks and Yule model; A8, 10 partitions, 10 clocks and birth–death model.

interval (CI) ([Table 4](#)). The divergence time estimates from the best analysis recover an origin of Carabinae in the mid-Jurassic *c.* 171.8 Ma (95% CI = 134.1–209.2 Ma). Our estimate of the mitochondrial rate of substitution is 0.0051 substitutions per site/My per lineage (95% CI = 0.0035–0.0067). The divergence time and substitution rate estimates are at odds with previous ones derived from fossil and biogeographical calibrations (e.g. Deuve *et al.*, 2012; Andújar *et al.*, 2012, 2014), which inferred an origin of Carabinae in the Eocene or Oligocene and recovered mitochondrial substitution rates of about 0.0154 substitutions per site/My per lineage (95% CI = 0.0112–0.0198) in *Carabus* (Andújar *et al.*, 2014). We believe the significant difference between these estimates and ours might stem from the use of biogeographical calibrations potentially pulling the origin of Carabinae toward the present. The age of the genus *Carabus* is inferred to be *c.* 89.8 Ma (95% CI = 61.8–117.3 Ma). Here as well, our estimates are much older than previously suggested, and if our estimates are more accurate than previous ones, then the evolutionary history of the genus as inferred by Deuve *et al.* (2012) needs to be revisited to accommodate this substantial shift in the timeframe of *Carabus* ground beetle evolution. However, it is important to remain cautious when interpreting the divergence time estimates inferred in our study, since these are derived from a secondary calibration (see, for example, Graur & Martin 2004 for pitfalls of this approach), and because the phylogenetic relationships among Carabidae subfamilies are still far from being resolved (Maddison *et al.*, 2009; McKenna *et al.*, 2015; López-López & Vogler 2017). Our mitochondrial rate estimates are slower than what has been previously reported for multiple groups of Coleoptera (Pons *et al.*, 2010). However, estimates from Pons *et al.* (2010) assumed the crown of Coleoptera to be 250 My old, an assumption that may no longer hold (Toussaint *et al.*, 2017a). Similarly, Andújar *et al.* (2016) recovered substantially slower rates than Pons *et al.* (2010) for mitochondrial genes in Trechinae using fossil-based calibrations. Their estimate of the substitution rate for the combined cytochrome oxidase c subunits 1 and 2 in their preferred analysis ('ULN_1', cox1_cox2 ≈ 0.005substitutions per site/My per lineage) was in fact equivalent to the one recovered in our study. Nevertheless, our results indicate that additional dating studies, possibly at the family level, are needed to understand the evolutionary timeframe of this clade, using fossil-based divergence time estimates in combination with calibration cross-validation procedures as nicely developed for instance by Andújar *et al.* (2014).

In his monograph, René Jeannel hypothesized that Carabinae originated in Australia, where he thought the ancestral lineages of *Calosoma* first appeared during the Jurassic. Even though our dating for the origin of Carabinae is consistent with his hypothesis, our phylogenetic reconstruction rejects it, because *Calosoma* is a derived lineage in the evolution of Carabinae, as suggested, for instance, by Osawa *et al.* (2004) or Deuve & Faille (2013). However, Jeannel's theory that the biogeographical history of Carabinae has been shaped by the breakup of Gondwana is partly substantiated by our dating analyses. Based on our divergence time estimates, it is more likely that the first stages in the diversification history of Carabinae were shaped by Pangaean vicariance (Seton *et al.*, 2012). However, some branchings potentially represent relics of Gondwanan vicariance. For instance, the divergence times in the clade Ceroglossini+Pamborini match the steps of the breakup of Gondwana. Chilean *Ceroglossus* diverged from Pamborini *c.* 109.4 Ma (95% CI = 74.6–147.2 Ma), when Australia and South America were still connected via Antarctica (Seton *et al.*, 2012). The ancestor of this clade might have been more widespread in the early Cretaceous. The climate in Antarctica at this time was much warmer than at present, and entire biomes were available that could have potentially permitted carabine ground beetles to survive until the major glaciation period began in the Oligocene (Galeotti *et al.*, 2016; McKay *et al.*, 2016). Examples of clades that might have survived/ dispersed in Antarctica before it became an ice-covered continent exist (e.g. Winkworth *et al.*, 2015; Givnish *et al.*, 2016; Toussaint *et al.*, 2017c). The divergence between the New Zealand endemic *Maoripamborus* and its sister taxon, the Australian endemic *Pamborus*, is estimated to *c.* 77.5 Ma (95% CI = 48.9–106.8 Ma). Even though the credibility interval for this event is wide, the median age inferred in our BEAST analysis perfectly matches the split between Australia and New Zealand, in the Cretaceous *c.* 80 Ma (Seton *et al.*, 2012). In agreement with Sota *et al.* (2005), we argue that the divergence between these two austral lineages is probably the result of Gondwanan vicariance, but a proper biogeographical reconstruction will be needed to test this hypothesis in a statistical framework.

The only direct attempt to date the origin of the genus *Calosoma* was made by Su *et al*. (2005). In their study, the authors relied upon the rate of substitution calculated for the gene ND5 in other carabid studies based on biogeographical calibrations (e.g. Su *et al.*, 2001). However, most *Calosoma* species are not flightless, and therefore are unlikely to share similar rates of evolution (Ikeda, Nishikawa & Sota, 2012). The origin of the genus was dated in the study of Su *et al.* (2005) from the Oligocene *c.* 30 Ma, although there was no mention of the divergence time between *Calosoma* and *Carabus*. In this study, we recover an origin of *Calosoma c.* 69.9 Ma (95% CI = 49.3–92.7 Ma) in the late Cretaceous. Although *Calosoma* is a widespread lineage with representatives in most continents ([Fig.](#)

3), our phylogenetic hypothesis and divergence time estimates cannot reconcile the diversification of lineages with geological events (e.g. Gondwanan vicariance). The placement of the Malagasy endemic *C. grandidieri* as sister to a Neotropical clade (Figs 2, 3) is the only topological pattern that could indicate a Gondwanan signature. However, the moderate support for this phylogenetic relationship and our divergence times estimates reject the hypothesis of Gondwanan vicariance. Other examples of beetle lineages with 'Gondwana-like' distributions whose biogeographical evolution cannot be reconciled with Gondwanan vicariance exist (Toussaint & Short, 2017). The uncertain placement and/or missing taxon sampling for key taxa from the Afrotropics and Madagascar hampers a better understanding of biogeographical patterns and processes in *Calosoma*. Based on our phylogenetic hypothesis (Fig. 2), and the likely Holarctic or Palearctic origin of *Carabus*, we hypothesize that *Calosoma* caterpillar hunter beetles originated in Laurasia during the late Cretaceous (Fig. 3). The colonization of the Afrotropics seems to have happened multiple times out of the Palearctic (Fig. 3). An interesting pattern is the placement of the Galápagos endemic clade as sister to a Neotropical clade (Figs 2 and 3), with an origin for the former *c.* 10 Ma, when current islands of the Galápagos archipelago had not yet emerged, but now-submerged ones might have existed (Werner *et al.*, 1999; Geist *et al.*, 2014). In conclusion, based on our phylogenetic tree and divergence time estimates, it seems that the diversification of *Calosoma* was not shaped by Gondwanan vicariance as suggested by Jeannel (1940), but rather by dispersal, possibly linked to the good flying ability of some lineages. However, the biogeographical mechanisms that shaped the current distribution of *Calosoma* across the globe need to be studied using a more comprehensive phylogenetic hypothesis, and using proper models of geographical range evolution.

CONCLUSION

In this study, we present a new phylogenetic hypothesis for *Calosoma* caterpillar hunter beetles (Figs 1 and 2). Our results indicate that the genus should be fully revised to take into account the likely paraphyly/polyphyly of many groups erected about a century ago based on seemingly homoplasious morphological characters. New synapomorphies should be searched for to reconcile the taxonomy and systematics of *Calosoma* with its evolutionary history. On a different note, we infer a comparatively ancient origin for Carabinae in the Jurassic. This result is at odds with the comprehensive corpus of studies looking at the evolution of the genus *Carabus* using modern molecular techniques

and a different calibration strategy (Andújar *et al.*, 2012a, b, 2014; Deuve *et al.*, 2012). We prefer to remain very cautious on the interpretation of our results because a fully-resolved phylogeny of ground beetles is not yet available, and because the use of secondary calibrations is not optimal. However, we believe that this study might represent a paradigm shift in our understanding of the evolutionary history of carabine beetles. Future progress in beetle phylogenomics will hopefully allow the inference of a fine-scale, fossil-based timetree of Carabidae, which in turn will permit the testing of René Jeannel's seminal hypothesis.

REFERENCES

Andújar C, Gomez-Zurita J, Rasplus JY, Serrano J. 2012a. Molecular systematics and evolution of the subgenus *Mesocarabus* Thomson, 1875 (Coleoptera: Carabidae: Carabus), based on mitochondrial and nuclear DNA. *Zoological Journal of the Linnean Society* **166:** 787–804.

Andújar C, Serrano J, Gómez-Zurita J. 2012b. Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. *BMC Evolutionary Biology* **12:** 40.

Andújar C, Soria-Carrasco V, Serrano J, Gómez-Zurita J. 2014. Congruence test of molecular clock calibration hypotheses based on Bayes factor comparisons. *Methods in Ecology and Evolution* **5:** 226–242.

Andújar C, Faille A, Pérez-González S, Zaballos JP, Vogler AP, Ribera I. 2016. Gondwanian relicts and oceanic dispersal in a cosmopolitan radiation of euedaphic ground beetles. *Molecular Phylogenetics and Evolution* **99:** 235–246.

Ashmole P, Ashmole M. 2004. *The invertebrates of Prosperous Bay Plain, St Helena.* Report for St Helena Government.

Baca SM, Alexander A, Gustafson GT, Short AE. 2017. Ultraconserved elements show utility in phylogenetic inference of Adephaga (Coleoptera) and suggest paraphyly of 'Hydradephaga'. *Systematic Entomology* **42:** 786–795.

Baele G, Li WL, Drummond AJ, Suchard MA, Lemey P. 2013. Accurate model selection of relaxed molecular clocks

in bayesian phylogenetics. *Molecular Biology and Evolution* **30:** 239–243.

Breuning S. 1928. Monographie der Gattung Calosoma Web. (Carab.) III. *Koleopterologische Rundschau* **14:** 43–101.

Bruschi S. 2013. *Calosoma of the world: Coleoptera, Carabidae*. Bologna: Natura edizioni scientifiche.

Bukontaite R, Miller KB, Bergsten J. 2014. The utility of CAD in recovering Gondwanan vicariance events and the evolutionary history of Aciliini (Coleoptera: Dytiscidae). *BMC Evolutionary Biology* **14:** 5.

Cassola F, Werner K. 2004. A fossil tiger beetle specimen from the Brazilian Mesozoic: *Oxycheilopsis cretacicus* n. gen., n. sp. *Mitteilungen der Münchner Entomologischen Gesellschaft* **94:** 75–81.

Deuve T. 1998. Trois fossiles remarquablement conservés du Miocène de France, appartenant aux genres *Carabus* L. et *Ledouxnebria* nov. (Coleoptera, Carabidae et Nebriidae). *Bulletin de la Société entomologique de France* **103:** 229–236.

Deuve T. 2004. *Illustrated catalogue of the Genus Carabus of the world (Coleoptera: Carabidae*. Sofia: PENSOFT.

Deuve T, Cruaud A, Genson G, Rasplus JY. 2012. Molecular systematics and evolutionary history of the genus Carabus (Col. Carabidae). *Molecular Phylogenetics and Evolution* **65:** 259–275.

Deuve T, Faille A. 2013. Structure secondaire de l'ARN 18S et phylogénie basale du genre *Carabus* L., 1758 (Coleoptera: Carabidae). *Annales de la Société entomologique de France (NS)* **49:** 430–445.

Drummond AJ, Ho SY, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4:** e88.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Eberle J, Fabrizi S, Lago P, Ahrens D. 2017. A historical biogeography of megadiverse Sericini—another story "out of Africa"?. *Cladistics* **33:** 183–197.

Ferreira MA, Suchard MA. 2008. Bayesian analysis of elapsed times in continuous-time Markov chains. *Canadian Journal of Statistics* **36:** 355–368.

Galeotti S, DeConto R, Naish T, Stocchi P, Florindo F, Pagani M, Barrett P, Bohaty SM, Lanci L, Pollard D, Sandroni S, Talarico FM, Zachos JC. 2016. Antarctic Ice Sheet variability across the Eocene-Oligocene boundary climate transition. *Science (New York, N.Y.)* **352:** 76–80.

Geist D, Snell H, Snell H, Goddard C, Kurz M. 2014. A paleogeographic model of the Galapagos islands and biogeographical and evolutionary implications. In: Harpp K., Mittelstaedt E., D'Ozouville N., Graham D, *eds. The Galapagos: a natural laboratory for the earth sciences*. Hoboken: John Wiley & Sons, Inc.

Gernhard T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* **253:** 769–778.

Gidaspow T. 1973. Revision of ground beetles of American genus *Cychrus* and four subgenera of genus *Scaphinotus* (Coleoptera, Carabidae). *Bulletin of the AMNH* **152:** 2.

Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Doucette A, Caro GG, McDaniel J, Clements MA, Arroyo MT. 2016. Orchid historical biogeography, diversification, Antarctica and the paradox of orchid dispersal. *Journal of Biogeography* **43:** 1905–1916.

Graur D, Martin W. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics* **20:** 80–86.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59:** 307–321.

Gunter NL, Weir TA, Slipinksi A, Bocak L, Cameron SL. 2016. If dung beetles (Scarabaeidae: Scarabaeinae) arose in association with dinosaurs, did they also suffer a mass co-extinction at the k-pg boundary? *PloS One* **11:** e0153570.

Häckel M. 2013. *A critical overview on the recent infrageneric taxonomy of the caterpillar hunter (Calosoma, Carabini, Carabidae)*. Diploma's Work, Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Department of Ecology.

Ikeda H, Nishikawa M, Sota T. 2012. Loss of flight promotes beetle diversification. *Nature Communications* **3:** 648.

Jeannel R. 1940. *Les Calosomes (Coleoptera, Carabidae)*. Éditions du Muséum.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30:** 772–780.

Kim SI, Farrell BD. 2015. Phylogeny of world stag beetles (Coleoptera: Lucanidae) reveals a gondwanan origin of darwin's stag beetle. *Molecular Phylogenetics and Evolution* **86:** 35–48.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34:** 772–773.

Liu Y, Shi H, Cai C, Liang H, Huang D. 2015. The first record of cretaceous ground beetle (Coleoptera: Carabidae: Oodini) from burmese amber. *Cretaceous Research* **52:** 427–430.

López-López A, Vogler AP. 2017. The mitogenome phylogeny of Adephaga (Coleoptera). *Molecular Phylogenetics and Evolution* **114:** 166–174.

Maddison DR, Moore W, Baker MD, Ellis TM, Ober KA, Cannone JJ, Gutell RR. 2009. Monophyly of terrestrial adephagan beetles as indicated by three nuclear genes (Coleoptera: Carabidae and Trachypachidae). *Zoologica Scripta* **38:** 43–62.

McKay RM, Barrett PJ, Levy RS, Naish TR, Golledge NR, Pyne A. 2016. Antarctic Cenozoic climate history from sedimentary records: ANDRILL and beyond. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **374:** 20140301.

McKenna DD, Wild AL, Kanda K, Bellamy CL, Beutel RG, Caterino MS, Farnum CW, Hawks DC, Ivie MA, Jameson ML, Leschen RA. 2015. The beetle tree of life reveals that Coleoptera survived end-permian mass extinction to diversify during the cretaceous terrestrial revolution. *Systematic Entomology* **40:** 835–880.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic

trees. In *Gateway Computing Environments Workshop (GCE), IEEE Xplore Digital Library, 2010*, 1–8.

Minh BQ, Nguyen MA, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* **30:** 1188–1195.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32:** 268–274.

Osawa S, Su ZH, Kim C, Okamoto M, Tominaga O, Imura Y. 1999. Evolution of the carabid ground beetles. *Advances in Biophysics* **36:** 65–106.

Osawa S, Su ZH, Imura Y. 2004. Phylogeny and distribution of the subfamily carabinae. In: Osawa S, Su ZH, Imura Y, eds. *Molecular phylogeny and evolution of carabid ground beetles*. Tokyo: Springer, 25–32.

Pons J, Ribera I, Bertranpetit J, Balke M. 2010. Nucleotide substitution rates for the full set of mitochondrial protein-coding genes in Coleoptera. *Molecular Phylogenetics and Evolution* **56:** 796–807.

Price MN, Dehal PS, Arkin AP. 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. *PloS One* **5:** e9490.

Ray JW, Seidel M, Husemann M. 2017. A new melanistic variant of the caterpillar hunter *Calosoma wilcoxi* LeConte, 1848 from Texas, United States of America and a preliminary phylogeny of the genus *Calosoma* Weber, 1801 (Coleoptera: Carabidae). *The Canadian Entomologist* **149:** 1–7.

Seton M, Müller RD, Zahirovic S, Gaina C, Torsvik T, Shephard G, Talsma A, Gurnis M, Turner M, Maus S, Chandler M. 2012. Global continental and ocean basin reconstructions since 200Ma. *Earth-Science Reviews* **113:** 212–270.

Sota T, Ishikawa R. 2004. Phylogeny and life-history evolution in *Carabus* (subtribe Carabina: Coleoptera, Carabidae) based on sequences of two nuclear genes. *Biological Journal of the Linnean Society* **81:** 135–149.

Sota T, Takami Y, Monteith GB, Moore BP. 2005. Phylogeny and character evolution of endemic Australian carabid beetles of the genus *Pamborus* based on mitochondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution* **36:** 391–404.

Soubrier J, Steel M, Lee MS, Der Sarkissian C, Guindon S, Ho SY, Cooper A. 2012. The influence of rate heterogeneity among sites on the time dependence of molecular rates. *Molecular Biology and Evolution* **29:** 3345–3358.

Su ZH, Ohama T, Okada TS, Nakamura K, Ishikawa R, Osawa S. 1996. Phylogenetic relationships and evolution of the Japanese Carabinae ground beetles based on mitochondrial ND5 gene sequences. *Journal of Molecular Evolution* **42:** 124–129.

Su ZH, Tominaga O, Okamoto M, Osawa S. 1998. Origin and diversification of hindwingless *Damaster* ground beetles within the Japanese islands as deduced from mitochondrial ND5 gene sequences (Coleoptera, Carabidae). *Molecular Biology and Evolution* **15:** 1026–1039.

Su ZH, Imura Y, Osawa S. 2001. Evolutionary discontinuity of the Carabine ground beetles. *Journal of Molecular Evolution* **53:** 517–529.

Su ZH, Imura Y, Okamoto M, Osawa S. 2004. Pattern of phylogenetic diversification of the Cychrini ground beetles in the world as deduced mainly from sequence comparisons of the mitochondrial genes. *Gene* **326:** 43–57.

Su ZH, Imura Y, Osawa S. 2005. Evolutionary history of Calosomina ground beetles (Coleoptera, Carabidae, Carabinae) of the world as deduced from sequence comparisons of the mitochondrial ND5 gene. *Gene*. **360:** 140–150.

Tominaga O, Su ZH, Kim CG, Okamoto M, Imura Y, Osawa S. 2000. Formation of the Japanese *Carabina* fauna inferred from a phylogenetic tree of mitochondrial ND5 gene sequences (Coleoptera, carabidae). *Journal of Molecular Evolution* **50:** 541–549.

Toussaint EFA, Beutel RG, Morinière J, Jia F, Xu S, Michat MC, Zhou X, Bilton DT, Ribera I, Hájek J, Balke M. 2016a. Molecular phylogeny of the highly disjunct cliff water beetles from South Africa and China (Coleoptera: Aspidytidae). *Zoological Journal of the Linnean Society* **176:** 537–546.

Toussaint EFA, Fikáček M, Short AEZ. 2016b. India–Madagascar vicariance explains cascade beetle biogeography. *Biological Journal of the Linnean Society* **118:** 982–991.

Toussaint EFA, Short AEZ. 2017. Biogeographic mirages? Molecular evidence for dispersal-driven evolution in Hydrobiusini water scavenger beetles. *Systematic Entomology* **42:** 692–702.

Toussaint EFA, Seidel M, Arriaga-Varela EM, Hájek J, Král D, Sekerka L, Short AEZ, Fikáček M. 2017a. The peril of dating beetles. *Systematic Entomology* **42:** 1–10.

Toussaint EFA, Bloom D, Short AEZ. 2017b. Cretaceous West Gondwana vicariance shaped giant water scavenger beetle biogeography. *Journal of Biogeography* **44:** 1952–1965.

Toussaint EFA, Hendrich L, Hájek J, Michat MC, Panjaitan R, Short AEZ, Balke M. 2017c. Evolution of Pacific Rim diving beetles sheds light on Amphi-Pacific biogeography. *Ecography* **40:** 500–510.

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* **44:** W232–W235.

Wang B, Zhang H. 2011. A new ground beetle (Carabidae, Protorabinae) from the Lower Cretaceous of Inner Mongolia, China. *ZooKeys* **130:** 229–37.

Werner R, Hoernle K, van den Bogaard P, Ranero C, von Huene R, Korich D. 1999. Drowned 14-my-old Galápagos archipelago off the coast of Costa Rica: implications for tectonic and evolutionary models. *Geology* **27:** 499–502.

Winkworth RC, Hennion F, Prinzing A, Wagstaff SJ. 2015. Explaining the disjunct distributions of austral plants: the roles of Antarctic and direct dispersal routes. *Journal of Biogeography* **42:** 1197–1209.