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Global dispersal and diversification in ground beetles of the subfamily Carabinae

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ABSTRACT

The origin and diversification process of lineages of organisms that are currently widely distributed among continents is an interesting subject for exploring the evolutionary history of global species diversity. Ground beetles of the subfamily Carabinae are flightless except for one lineage, but nevertheless occur on all continents except Antarctica. Here, we used sequence data from ultraconserved elements to reconstruct the phylogeny, divergence time, biogeographical history, ancestral state of hind wings and changes in the speciation rate of Carabinae. Our results show that Carabinae originated in the Americas and diversified into four tribes during the period from the late Jurassic to the late Cretaceous, with two in South America (Celoglossini) and Australasia (Pamborini) and two in Laurasia (Cychrini and Carabini). The ancestral Carabinae were inferred to be winged; three of four tribes (Cychrini, Celoglossini and Pamborini) have completely lost their hind wings and flight capability. The remaining tribe, Carabini, diverged into the subtribes Carabina (wingless) and Calosomina (winged) in the Oligocene. Carabina originated in Europe, spread over Eurasia and diversified into approximately 1000 species, accounting for around 60% of all Carabinae species. Calosomina that were flight-capable dispersed from North America or Eurasia to South America, Australia, and Africa, and then flightless lineages evolved on oceanic islands and continental highlands. The speciation rate increased in the Cychrini and Carabini clades in Eurasia. Within Carabini, the speciation rate was higher for wingless than winged states. Our study showed that the global distribution of Carabinae resulted from ancient dispersal before the breakup of Gondwana and more recent dispersal through flight around the world. These patterns consequently illustrate the causal relationships of geographical history, evolution of flightlessness, and the global distribution and species diversity of Carabinae.

1. Introduction

How terrestrial organisms have diversified and become globally distributed has long been a pivotal question in biogeography (e.g., [Darlington Jr., 1965](#); [Lomolino et al., 2018](#); [Wallace, 1876](#)). Of particular interest is the global distribution of animal groups with weak or no flight capability. The distribution of related species in one lineage on widely separated landmasses may be explained by vicariance events associated with the fragmentation of ancient supercontinents or more

recent transoceanic dispersal events (e.g., [Sanmartín and Ronquist, 2004](#)). Some recent historical biogeographic studies on insects of the continents and islands of the southern hemisphere revealed different patterns for their distribution processes. For example, the breakup of Gondwana during the Cretaceous drove the disjunct distribution in mostly flightless moss bugs across the separate areas of the southern hemisphere ([Ye et al., 2019](#)). On the other hand, both vicariance associated with the fragmentation of Gondwana and more recent transoceanic dispersal among the major southern landmasses explained the

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trans-Antarctic distribution patterns among orthoclaidiine chironomids (Krosch et al., 2011). Various other studies inferred vicariance or long-distance transoceanic dispersal, or both (Baca and Short, 2020; Bukontaite et al., 2014; McCulloch et al., 2016; Toussaint et al., 2017a, 2017b; Toussaint and Short, 2017). Because insects often show variation in flight capability among related species or lineages due to loss of flight

muscles and/or wings (Roff, 1986, 1990), evolutionary changes in flight capability among lineages within an insect group may have affected the distribution and diversification patterns of the insect group. However, historical biogeography of insects has rarely taken into account the evolution of traits associated with flight capability such as wing morphology.

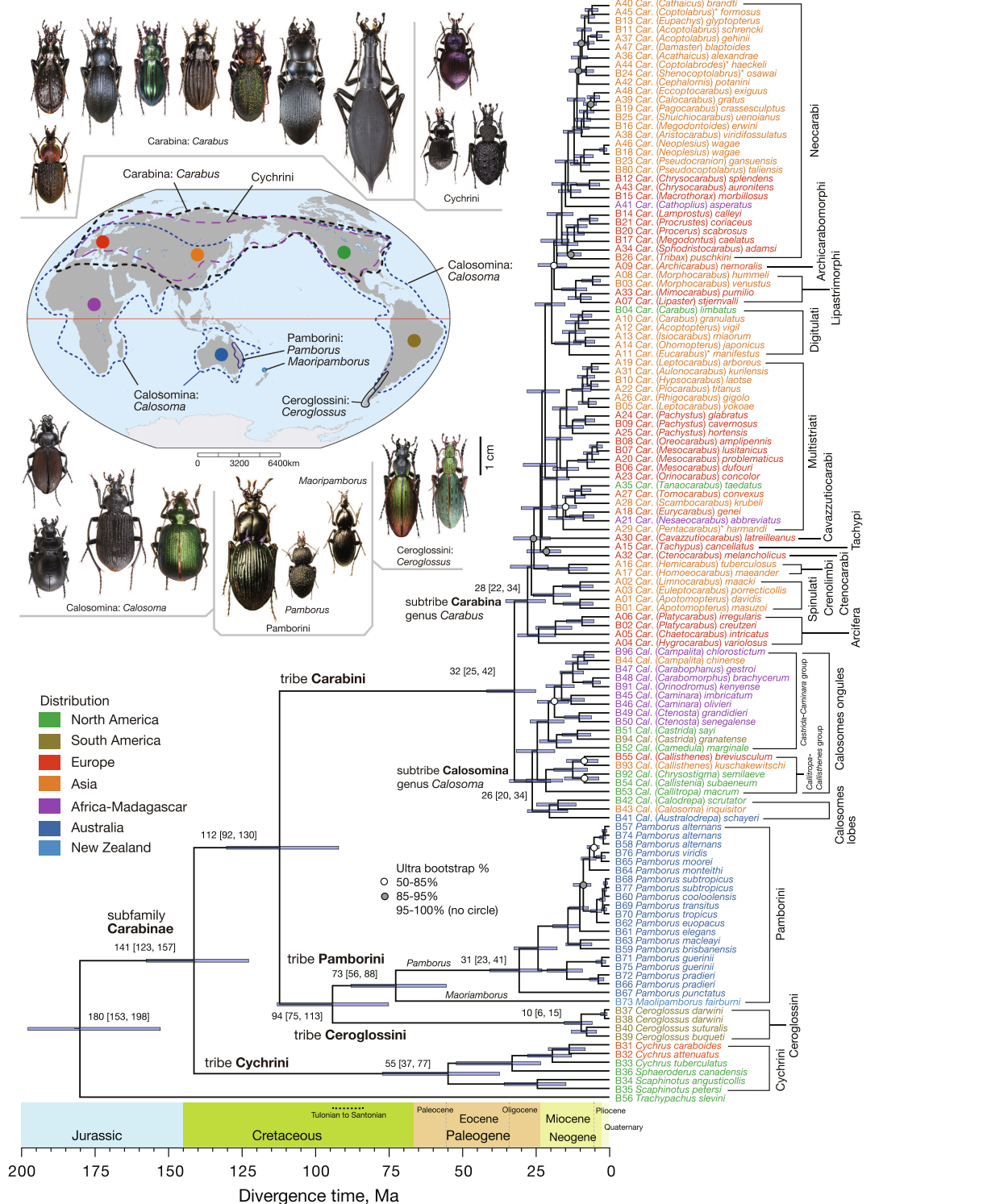


Fig. 1. Phylogeny and divergence times of the subfamily Carabinae. A maximum-likelihood tree derived from IQ-TREE analysis with all 70p data was converted to a clocked tree using the MCMCTree program. Bars at nodes represent 95% CIs for divergence time. Open and gray circles at nodes indicate the ultrafast bootstrap values of 50–85% and 85–95%, respectively; other nodes have values of 100%. The distribution areas of sampled species are indicated by colors. The distributions and example species of the six tribes and subtribes are also shown.

Ground beetles of subfamily Carabinae (family Carabidae) are flightless due to the loss (degeneration) of hind wings except for one lineage, but are nevertheless distributed on all continents except Antarctica, suggesting an ancient origin and global dispersal before the breakup of Pangaea and Gondwana (Fig. 1). The flightless members of this subfamily are adapted to temperate and adjacent climatic zones and occur in widely separated areas of the Holarctic region, as well as Africa, South America, Australia, and New Zealand. Subtribe Calosomina is the only group in which 47% of species are winged and is distributed worldwide, including Africa, Madagascar, and some oceanic islands. Jeannel (1940), a pioneering biogeographer, suggested that ancestral Carabinae occurred across Pangaea during the Jurassic, and subsequent vicariance events due to continental drift resulted in the divergence of different tribes. He assumed that flight-capable Calosomina were the ancestor of flightless groups except for tribe Cychrini. Erwin (1979), who referred to the phylogenetic relationships of Carabinae based on larval characteristics (Moore, 1966), assumed a neotropical origin for Carabinae and hypothesized a dispersal history throughout the world during the Jurassic and Cretaceous. For these earlier studies, however, accurate phylogenetic relationships among Carabinae groups were not available. Prüser and Mossakowski (1998) were the first to propose a parsimonious biogeographical scenario for Carabinae based on a mitochondrial gene tree. They hypothesized that Carabinae originated in Laurasia, split between Cychrini and all others, and then Ceroglossini and Pamborini migrated from Laurasia to Gondwana, with the latter reaching Australasia via Antarctica; Carabini in Laurasia diverged into Calosomina and Carabina, and the former dispersed around the world via flight. Recently, Toussaint and Gillett (2018) performed phylogenetic reconstruction and divergence time estimation of Carabinae using a supermatrix approach with several mitochondrial and nuclear gene sequences to test the Jeannel's (1940) hypothesis for the historical biogeography of Calosomina. Their results suggested that the present global distribution of Calosomina was not shaped by Gondwanan vicariance, but rather by more recent dispersal. These and other phylogenetic analyses based on a small number of mitochondrial and nuclear genes or mitogenomes (Imura et al., 2018; Sota et al., 2020) resolved the tribal relationships among Carabinae tribes consistently, but no detailed analysis of the divergence and biogeographical history of Carabinae based on robust phylogenomic data has yet been conducted.

In this study, we analyzed sequence data for ultraconserved elements (UCEs) (Faircloth et al., 2012) to elucidate the phylogenetic relationships among tribes and subtribes of the subfamily Carabinae, as well as among representative subgenera of tribe Carabini (subtribes Calosomina and Carabina). The sequence capture method for UCEs developed for Coleoptera can yield a large sequence data matrix to support the reconstruction of phylogeny for taxa with various relationships, such as among tribes of Carabinae and among species within tribes or subtribes (e.g., Baca et al., 2017; Gustafson et al., 2020; Kobayashi and Sota, 2021; Van Dam et al., 2018). Based on the resulting phylogeny, we performed divergence time estimation and reconstructed biogeographic history to infer the timing and geographic setting of lineage divergence and thereby test or improve the biogeographic hypothesis of Prüser and Mossakowski (1998). In addition, we reconstructed the ancestral state of hind wings (winged-macropterous; wingless-brachypterous or apterous) and studied changes in the speciation rate and the relationship between the hind wing state and the speciation rate. Our study revealed that the present world-wide distribution of Carabinae was achieved through the dispersal of ancestral lineages from somewhere in the Americas to the southern South America and Australasia during the Cretaceous, as well as the dispersal of lineages that diverged in the northern hemisphere into the southern hemisphere during the Paleogene and Neogene.

2. Materials and methods

2.1. Taxon sampling and DNA extraction

We sampled species from all five groups of Carabinae (Table 1; Tables S1-S3). We used *Trachypachus slevini* (Trachypachidae) as the outgroup because Trachypachidae is likely a basal group of Geadephaga (McKenna et al., 2019). Total genomic DNA was extracted from specimens fixed in ethanol or RNAlater using the Promega Wizard DNA Purification Kit (Madison, WI, USA) or Qiagen DNeasy Blood & Tissue Kit (Hilden, Germany) following the manufacturer's protocol. Pinned dry specimens were also used for DNA extraction, in which total genomic DNA was extracted from legs using the Qiagen DNeasy Micro Kit (Hilden, Germany) following the manufacturer's protocol.

2.2. Preparation of UCE-enriched sequencing libraries

Sequencing libraries were prepared using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (E7805) and NEBNext Multiplex Oligos for Illumina (E6609; New England Biolabs, Inc., Ipswich, MA, USA) according to the manufacturer's protocol. For each specimen, approximately 50 ng of genomic DNA was used for library preparation. Target enrichment of the libraries for UCE sequences was performed using myBaits UCE Coleoptera 1.1Kv1 (Arbor Biosciences, Inc., Ann Arbor, MI, USA) following the manufacturer's version 3 protocol. Note that our experiment with this UCE probe set was done before the publication of the Adephaga-specific UCE probe set (Gustafson et al., 2019), which would be more suitable for Carabinae. Target enrichment was performed with pools of 24 indexed libraries. Two final library pools were sequenced using two lanes of an Illumina HiSeqX Ten (paired-end sequences of 151 bases) at Macrogen Japan Corp. (Kyoto, Japan).

2.3. Sequence data processing and phylogenetic tree reconstruction

Sequence data were processed using the *phyluce* pipeline v1.6 (Faircloth, 2016). Adapter trimming and quality control of the raw reads were conducted using *illumiprocessor* software (Faircloth, 2013). Reads were assembled using Trinity v2.1.1 (Grabherr et al., 2011) implemented in *phyluce*. Enriched UCE loci were identified and extracted from

Table 1

Tribal and subtribal composition of subfamily Carabinae, with numbers of known species and species sampled for this study.

Tribal/subtribe/genus [Reference]	Distribution	No. of species	No. of species sampled
Tribe Cychrini ¹			
<i>Cychrus</i>	Holarctic (Palearctic, Nearctic)	184 (182, 2)	3 (2, 1)
<i>Scaphinotus</i>	Nearctic	55	2
<i>Sphaeroderus</i>	Nearctic	5	1
Total		244	6
Tribe Ceroglossini ²			
<i>Ceroglossus</i>	South America (Chile)	8	3
Tribe Pamborini ³			
<i>Pamborus</i>	Australia	17	16
<i>Maoripamborus</i>	New Zealand	1	1
Tribe Carabini			
Subtribe Carabina ⁴			
<i>Carabus</i>	Holarctic (Palearctic, Nearctic)	945 (933, 12)	73 (71, 2)
Subtribe Calosomina ⁵			
<i>Calosoma</i>	Worldwide	128	20

References: [1] Erwin (2007); Löbl and Löbl (2017); [2] Jiroux (2006); [3] Takami and Sota (2006); [4] Deuve (2019); Erwin (2007); [5] Bruschi (2013); Sota et al. (2020).

the assembled contigs through matching against probes with minimum coverage and minimum identity values of 80%. UCE sequences were aligned using MAFFT (Kato and Standley, 2013) and trimmed with Gblocks (Talavera and Castresana, 2007). Subsequently, we created sequence datasets with UCEs recovered from at least 50%, 60%, and 70% of the specimens. Such datasets were created for both Carabinae (all Carabinae specimens including the outgroup *Trachypachus slevini*) and *Carabus* (all *Carabus* specimens and two outgroup species of *Calosoma*). The datasets including all Carabinae were named all_50p, all_60p, and all_70p, and those for *Carabus* were named *Carabus_50p*, *Carabus_60p*, and *Carabus_70p*.

For each of those datasets with initial data partitions by UCE loci, we performed maximum-likelihood (ML) analysis with optimal data partitioning and substitution model selection using IQ-TREE version 2.0.3 (Minh et al., 2020) with the MFP + MERGE option (find the best partition scheme followed by tree inference). Default settings were used for the tree search. Node supports were evaluated using the ultrafast bootstrap method (UFBoot2; Hoang et al., 2018) with 1000 replications.

2.4. Divergence time estimation

We performed Bayesian relaxed clock analysis with all_70p data based on an uncorrelated lognormal clock model using the MCMCTree program in the PAML package (version 4.8; Yang, 2007). This analysis employed the topology of the best tree obtained from the ML analysis. Because fossils of early Carabinae are lacking, we referred to the clock tree of all Coleoptera reported by McKenna et al. (2019) to estimate when the split between *Trachypachus* and Carabinae occurred (170 Ma; 150–197 Ma). They also estimated the timing of the split between *Elaphrus* (the closest taxon to Carabinae in their dataset) and Carabinae as 121 Ma (143–97 Ma), suggesting that the crown age of Carabinae is < 143 Ma. In addition, we assumed that the divergence of *Maoripamborus* in New Zealand and *Pamborus* in Australia occurred at > 85 Ma, because terrestrial connections between New Zealand and the remainder of Gondwanaland dissolved in the late Cretaceous at approximately 85 Ma (Stevens, 1989) and long-distance dispersal across the sea by flight or any other means was unlikely for Pamborini (Sota et al., 2005). Fossils of the subtribe Carabini have been reported from the Jurassic and Eocene but are considered cases of misidentification (Nel, 1988; Deuve, 2004; Penev et al., 2003). The oldest fossils assigned to Calosomina are from the upper Oligocene (Nel, 1988). Fossils of Carabina (*Carabus*) have been found from the Miocene (8.5 Ma) (Deuve, 2004; Penev et al., 2003); an Oligocene fossil was also found, but its identification is questionable (Penev et al., 2003). If we assume that the ancestral form of Carabini, from which Carabina (*Carabus*) is derived, is *Calosoma*-like, then the divergence of Carabina occurred earlier than the late Oligocene (>23 Ma). This assumption is concordant with the divergence time estimate of Carabini stem age (31 Ma; 95% CI, 24–38 Ma) reported by Andújar, Serrano, & Gómez-Zurita (2012) based on a different calibration method.

For divergence time estimation with MCMCTree, a GTR + Γ substitution model was used for the whole sequence data without partitioning, and an approximate substitution rate was estimated by assuming a putative root age of 170 Ma following McKenna et al. (2019) as is mentioned above. Soft bound constraints were used for the four nodes, including the divergence of Trachypachidae and Carabinae (150–197 Ma), the crown age of Carabinae (<143 Ma), the divergence of *Pamborus* and *Maoripamborus* (>85 Ma), and the divergence of Carabina and Calosomina (>23 Ma). We performed two independent Markov chain Monte Carlo runs of 50,000 burn-in generations and 500,000 generations, with sampling every 50 generations, and confirmed convergence of the estimated node ages in the two runs.

2.5. Ancestral state reconstruction for hind wings

Detailed accounts for the hind wing conditions in Carabinae are

provided by Imura et al. (2018). All species of Cychrini, Ceroglossini and Pamborini, and most species of Carabina are apterous with only vestigial hind wings. In Carabina, a few species exhibit both brachypterous and macropterous hind wings. In Calosomina, 47% of species are macropterous, and the others are brachypterous or apterous (Table S2). We reconstructed the ancestral states of hind wings through an ML method using the R package diversitree version 0.9–13 (Fitzjohn, 2012) with ape version 5.3 (Paradis and Schliep, 2018) in R version 3.5.3 (R Core Team, 2019). This analysis employed the clocked ML tree obtained from all_70p data. The hind wing state was categorized as winged (macropterous, 0) or wingless (brachypterous or apterous, 1), and polymorphic *Carabus* species (*C. maacki*, *C. tuberculatus*, *C. granulatus*) were categorized as wingless as they are mostly brachypterous and flightless. We performed ML ancestral state reconstruction with the Mk2 model (continuous-time Markov model of character evolution for a binary character; Lewis, 2001; Pagel, 1994) using functions in diversitree. We ran models with equal and unequal rates for the reciprocal transitions between the character states (i.e., from a winged to a wingless condition, and vice versa) and compared the results from these two models using a likelihood ratio test to select the best-fit model. In addition to the analysis with diversitree, we used the function ace in the R package ape (Paradis and Schliep, 2018) to repeat the ancestral state reconstruction and validate the results of diversitree analysis.

2.6. Historical biogeography

We performed ancestral range reconstruction for major lineages of Carabinae in six regions around the world (Eastern Palearctic, Western Palearctic, Australasian, Afrotropics, Nearctic, Neotropics; following zoogeographical region names) using BioGeoBEARS (Matzke, 2013) implemented in RASP version 4.2 (Yu et al., 2015). We used a clocked tree modified from the tree obtained through divergence time estimation, in which operational taxonomic units (OTUs) were pruned to the genus (Cychrini, Pamborini, Ceroglossini), subgenus (Calosomina) or division level (Carabina). ML analysis was performed using six biogeographical models, namely, dispersal–extinction cladogenesis (DEC; Re and Smith, 2008), likelihood-based dispersal–vicariance analysis (DIVALIKE; Ronquist, 1997), the likelihood-based model in the program BayArea (BAYAREALIKE; Landis et al., 2013), and three models with an additional parameter for jump dispersal (i.e., founder event speciation; DEC + J, DIVALIKE + J, and BAYAREALIKE + J). The fit of each model to the data was compared using the Akaike information criterion corrected for a small sample size (AICc). For the constraint on dispersal among areas, we assumed that migration to Australasia from other regions was only possible from South America via Antarctica between 141.9 and 94 Ma and from the Eastern Palearctic via the Oriental region after 26 Ma.

2.7. Diversification rate

We estimated net diversification rates of lineages of Carabinae based on birth–death processes using the R package MEDUSA version 0.954 (Alfaro et al., 2009) in R version 4.0.3 (R Core Team, 2019). Due to incomplete taxon sampling, branches of the clocked ML tree (IQTREE) based on the Carabinae 70p dataset were culled and converted to a tree of higher taxonomic groups. Genus-level grouping was applied to Cychrini, Pamborini, and Ceroglossini. Calosomina was divided into 7 divisions, and Carabina into 16 divisions (Table S4). A mixed model including both birth–death and pure-birth (Yule) models was considered for each possible rate shift position. The best-fit model was determined based on the AICc value.

To test the effect of hind wing degeneration on speciation rate, we performed a likelihood ratio test using a binary-state speciation and extinction (BiSSE) model with the R package diversitree version 0.9.15 (Fitzjohn, 2012), employing the portion of the clocked tree for tribe Carabini (Carabina and Calosomina). We applied diversitree analysis to

only Carabini to support proper computation by the program, as the program could not run with the entire dataset of Carabinae. The tree for this analysis contained 15 of 20 subgenera (104 of 128 species) and 62 of 93 subgenera (738 of 945 species) for Calosomina and Carabina, respectively, and unsampled subgenera were not considered for this tree (Table S4). We used models with equal and unequal speciation rates between the winged and wingless states, with the extinction rates set as equal and the transition rates between the two hind wing states estimated with no constraints. The best-fit model was determined through the likelihood ratio test.

3. Results

3.1. Phylogenetic relationships

Based on all specimens, we obtained a UCE dataset for 126 OTUs containing a total of 53,558 bp of aligned sites in 153 partitions (UCE loci) with > 70% taxon coverage for each locus (all_70p dataset; Fig. 1; Fig. S1). We also obtained two datasets with taxon coverages of > 50% (123,020 bp, 412 loci; all_50p) and > 60% (98,567 bp, 314 loci; all_60p). The ML analyses conducted with these datasets all produced similar results. However, tree construction from these data resulted in obviously wrong placement of one OTU (B92, *Calosoma semilaeve*; pinned specimen collected in 1957) for which too few loci were recovered. Therefore, we repeated the ML analysis excluding the problematic OTU for the all_50p and all_60p datasets (Fig. S2).

Robust tribal relationships were revealed as Cychrini, ((Ceroglossini, Pamborini), Carabini) (Fig. 1). Taxon sampling was nearly complete for Pamborini. The species relationships within this tribe were generally consistent with those reported by Sota et al. (2005), with improved resolution of the derived groups. The relationships between the three genera of Cychrini, *Scaphinotus*, (*Sphaeroderus*, *Cychnus*) were consistent with those reported by Su et al. (2004).

In tribe Carabini, the reciprocal monophyly of the subtribes Carabina and Calosomina was clear. For Calosomina (genus *Calosoma*), Jeannel's (1940) distinction between "Calosomes lobes" and "Calosomes ongules" (Table S2) was supported (Fig. 1), although this relationship was not recovered in previous molecular phylogenetic studies based on mitochondrial genes (Sota et al., 2020; Su et al., 2005; Toussaint and Gillett, 2018). Calosomes ongules was divided into two clades, corresponding to the *Castrida-Caminara* group and a combined group containing the *Callisthenes* and *Callitropa* groups of Jeannel (1940). In subtribe Carabina (genus *Carabus*), which is the most species-rich group, we constructed separate UCE datasets with locus recovery rates of 70%, 60%, and 50%, which contained 235 loci (108,643 sites), 374 loci (157,193 sites), and 453 loci (178,488 sites), respectively. ML analysis of these data resulted in robust topologies (Fig. S3) that were generally consistent with those derived from datasets containing all Carabinae. All trees (Fig. 1, S1–S3) consistently resolved the relationships among intra-subtribal groups proposed by Deuve (2019) except for a few points (Table S3). Arcifera is the earliest-diverged group sister to all other Carabina groups, and Neocarabi is the most derived group containing a large number of species. Between these groups, Spinulati, Crenolimbi, Ctenocarabi, Tachypi, Cavazzutiocarabi, Multistriati, Lipastrimorphi, Digitulati, and Archicarabomorphi were placed. Details of the phylogenetic relationships among groups, including some minor differences among results obtained from different datasets, are described in the Supplementary results.

3.2. Divergence time

Our divergence time estimates (Fig. 1) indicate that the divergence of Cychrini from other groups of Carabinae occurred at 141 Ma (95% CI, 122–157 Ma), around the Jurassic/Cretaceous boundary. The subsequent divergence of Ceroglossini + Pamborini from Carabini occurred at 112 Ma (90–131 Ma). The divergence time of Ceroglossini and

Pamborini was 94 Ma (74–112 Ma), and that of *Maoripamborus* and *Pamborus* 73 Ma (55–88 Ma). Thus, the initial divergence events of these major groups occurred during the Cretaceous. The subsequent divergence of groups within tribes occurred during the Paleogene. Cychrini diverged into *Scaphinotus* and other genera at 54 Ma (38–78 Ma), whereas *Cychnus* and *Sphaeroderus* diverged at 19 Ma (23–53 Ma) in the Miocene. The most recent common ancestor (MRCA) age of *Pamborus* was 31 Ma (23–41 Ma), and the divergence of Carabini into Calosomina and Carabina occurred at 32 Ma (25–40 Ma), both in the Oligocene. The MRCA ages of Calosomina and Carabina were 26 Ma (20–33 Ma) and 28 Ma (22–34 Ma), respectively. The MRCA age of *Ceroglossus* was much more recent, 10 Ma (6–15 Ma) in the Miocene.

3.3. Ancestral state of hind wings

Ancestral state reconstruction assuming different transition rates between winged and wingless states provided a better fit to the data than the model assuming equal rates (likelihood ratio test, $df = 1$, $\chi^2 = 10.535$, $P = 0.00171$). The estimated transition rates were 1.96 per 100 Myr for the winged-to-wingless transition and 4.07e-11 per 100 Myr (approximately 0) for the wingless-to-winged transition. The ancestral state of Carabinae hind wings was winged with approximately 100% probability (Fig. 2). The MRCAs of Cychrini, Ceroglossini, and *Pamborus* were wingless with > 95% probability, whereas the MRCA of Carabini was winged with approximately 100% probability. Within the Carabini, the MRCA of Calosomina was winged with approximately 100% probability and the MRCA of Carabina was wingless with 96.1% probability.

We repeated the ancestral state reconstruction using the function ace in the R package ape because the results obtained using diversitree appeared extreme. The root state was found to be winged with 62% probability, with a transition rate from winged to wingless of 2.0577 per 100 Myr and from wingless to winged of 0.0617 per 100 Myr.

3.4. Historical biogeography

From the BioGeoBEARS analysis, DIVALIKE + J was the best-fitting model for the phylogeny and geographic distribution of Carabinae (Table S5; Fig. 3). The ancestor of Carabinae occurred in the Americas (Nearctic/Nearctic), and the Cychrini in North America (Nearctic) diverged from all other taxa (i.e., the common ancestor of Carabini, Pamborini, and Ceroglossini). The latter group diverged into tribe Carabini in the Palearctic and the common ancestor of Pamborini and Ceroglossini in South America; the ancestor of Pamborini dispersed from South America to Australasia during the late Cretaceous. The ancestral range of subtribe Carabina in Carabini was the western Palearctic (Europe), and this group dispersed to the Eastern Palearctic (Asia), then to North America (Nearctic). The other subtribe, Calosomina, had an estimated ancestral range encompassing the eastern Palearctic and North America, which were the ancestral areas of the Calosomes lobes and Calosomes ongules groups, respectively. During the late Paleogene and Neogene, Calosomes lobes dispersed to the western Palearctic, North America, and Australasia, whereas Calosomes ongules dispersed from North America to South America, Eurasia, and the Afrotropics. From the Afrotropics, Calosomes ongules dispersed further to the west and east Palearctic regions. The genus *Cychnus* of Cychrini dispersed to the Holarctic during the Paleogene.

Because the comparison between models with and without jump dispersal parameter J using standard statistical methods has been deemed problematic (Ree and Sanmartín, 2018; but see Klaus and Matzke, 2020 for a rebuttal), we also show the reconstruction by DIVALIKE model, which was the second best-fitting model and did not include the jump dispersal parameter (Fig. S4). The results of the ancestral area reconstruction by DIVELIKE and DIVELIKE + J models were similar and almost consistent with each other.

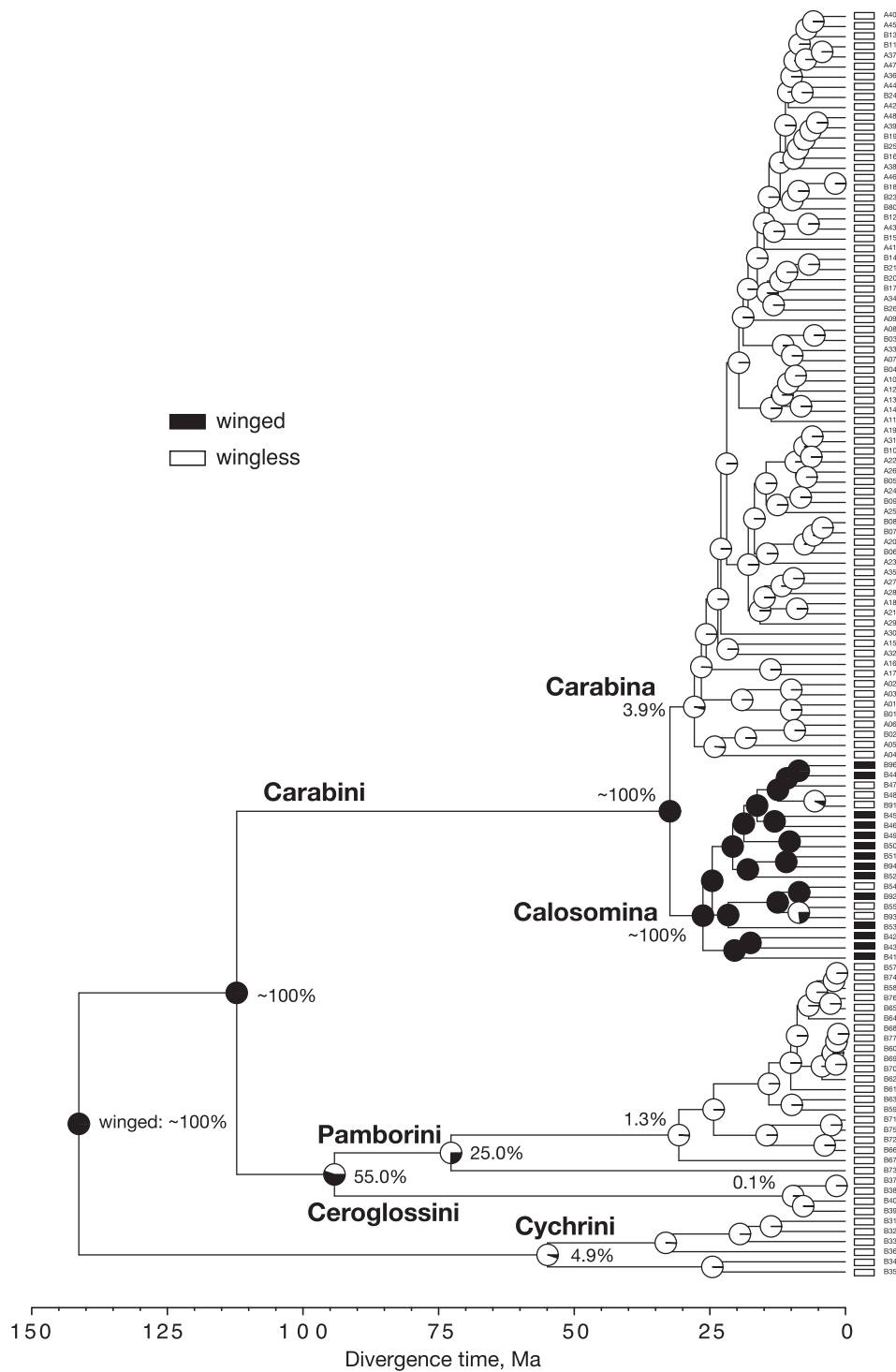


Fig. 2. Ancestral state reconstruction for hind wing state (wingless/winged) based on maximum-likelihood analysis. The pie graph shows the relative probability (%) of winged (black) and wingless (white) states at the ancestral node. The probability of the winged state is noted with numbers beside major ancestral nodes.

3.5. Variation in diversification rate

Analysis of diversification rate changes across the phylogenetic tree resulted in an optimal scheme based on the Yule model (pure-birth process; number of parameters = 9; log-likelihood = -73.13; AICc = 168.09). Marked shifts in the net diversification rate (r , per Myr, where $r = \lambda$ [speciation rate] - μ [extinction rate]) were detected in tribe Carabini, especially from the derived clades in subtribe Carabina (Fig. 4). Compared to the rate at the ancestral node ($r = 0.026$), the rate in

subtribe Calosomina and the two earliest-diverged groups of Carabina (Arcifera, Spinulati) was much higher ($r = 0.16$). This rate rose further in Orthocarabi, a derived group of Carabina ($r = 0.27$); meanwhile, the rate was lower ($r = 0.06$) in Crenolimbi, Ctenocarabi, Tachypi, and Cavazzutiocarabi. In all other tribes, net diversification remained at the ancestral rate, except for *Cychnus* and *Sphaeroderus* in the Cychrini, which exhibited an elevated rate ($r = 0.14$).

In the clade Carabini (Calosomina and Carabina), the hind wing state (winged or wingless) had a significant effect on the speciation rate

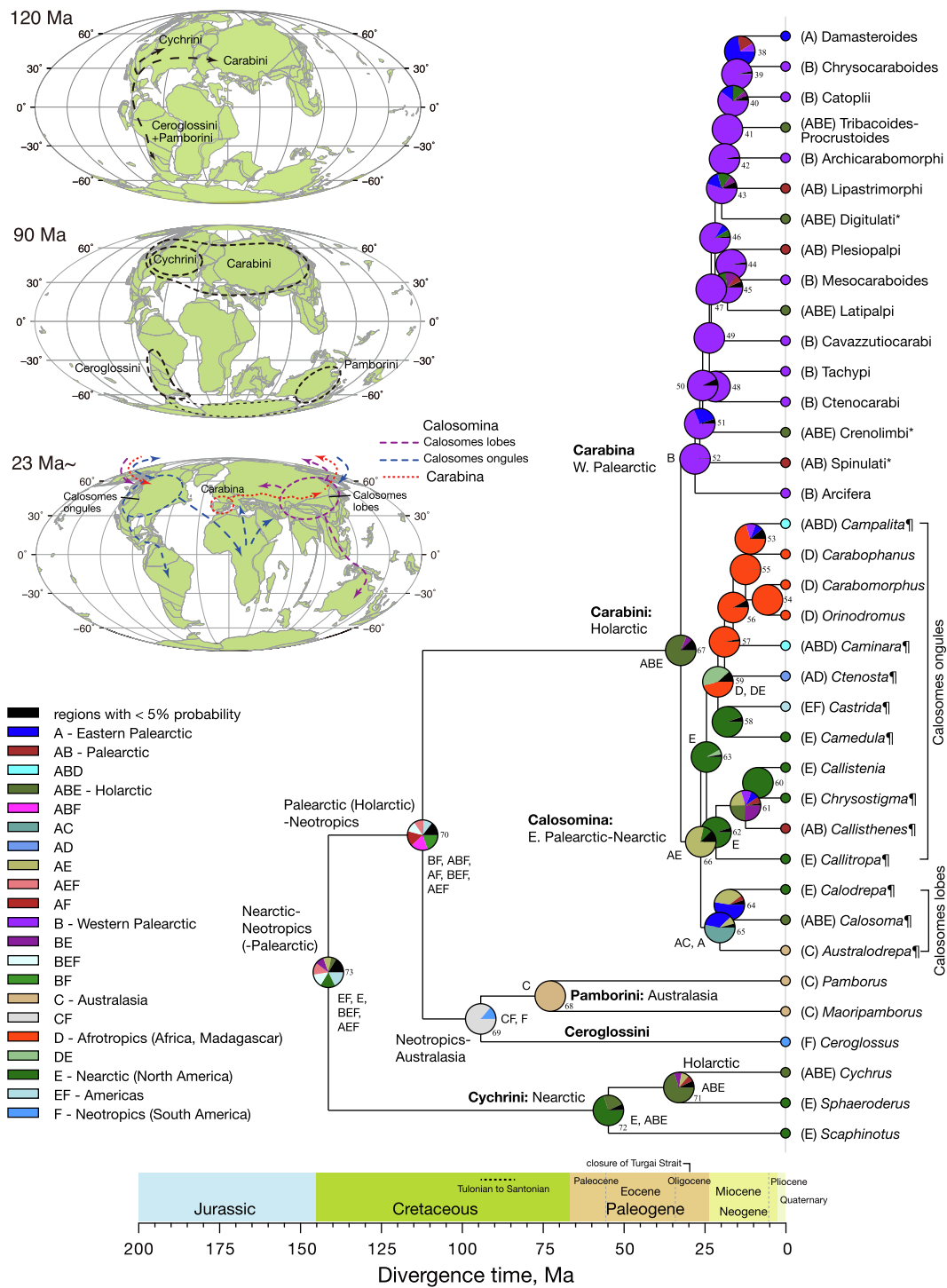


Fig. 3. Reconstructed historical biogeography of subfamily Carabinae based on the DIVALIKE + J model. The pie graph at each node represents probabilities of the ancestral ranges. Node numbers (refer to Table S5) are indicated beside the pie graphs. The global dispersal history of major groups is schematically illustrated on paleogeographic maps of 120, 90, and 23 Ma. The maps were obtained from the ODSN Plate Tectonic Reconstruction Service (<https://www.odsn.de/odsn/services/paleomap/paleomap.html>). In Calosomina, ¶ indicates that all or some species in the group are macropterous; in Carabina, * indicates that one or more species in the group are polymorphically macropterous. All other groups are apterous or brachypterous.

(likelihood ratio test, $df = 1$, $\chi^2 = 6.7649$, $P = 0.009297$, Table S6). The speciation rate in the wingless state (0.6165 per Myr) was faster than that in the winged state (0.5186 per Myr) with an equal extinction rate (0.4670 per Myr) (Table S6).

4. Discussion

4.1. Origin and global dispersal of Carabinae

Our results provide a comprehensive account of the global dispersal and diversification of subfamily Carabinae based on molecular phylogeny, divergence time estimation, and historical biogeographic

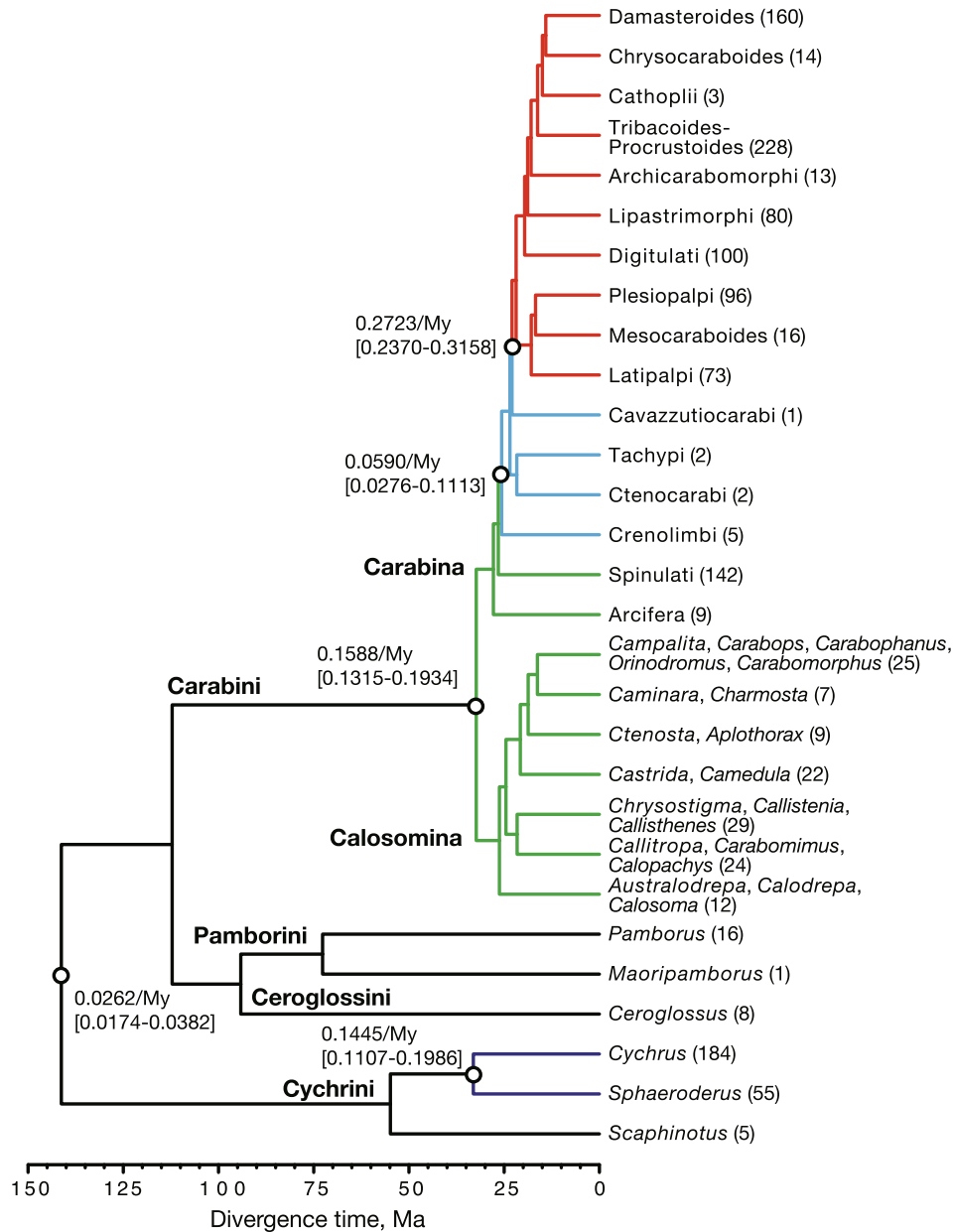


Fig. 4. Changes in the net diversification rate (r) in subfamily Carabinae obtained from Medusa analysis. The net diversification rates for differently colored branches are indicated beside the nodes as open circles with 95% CIs.

inferences. Our results indicate that the ancestral Carabinae originated somewhere in the Americas, diverged into Cychrini and another group containing Pamborini + Ceroglossini and Carabini around the Jurassic/Cretaceous boundary, and then Pamborini + Ceroglossini diverged from Carabini during the Cretaceous (112 Ma; 95% CI, 90–131 Ma). These initial divergence events were likely related to the breakups of Pangaea and Gondwana.

The ancestral range of tribe Cychrini was probably in present North America. The ancestor of Cychrini diverged into *Scaphinotus* and *Sphaeroderus* + *Cychrus* during the Paleogene, and *Cychrus* diverged from *Sphaeroderus*, colonized Eurasia in the Miocene, and then diversified there. The Pamborini + Ceroglossini lineage occupied South America and Australasia across Antarctica in the late Cretaceous; the migration from South America to Australasia occurred around the Turonian–Santonian age of the Cretaceous, when temperate rainforest was present in the Antarctic (Klages et al., 2020). The divergence time of Pamborini and Ceroglossini was 94 Ma (74–112 Ma), and the divergence

time of *Pamborus* in Australia and *Maoripamborus* in New Zealand was 73 Ma (56–88 Ma), around the boundary of Cretaceous and Paleogene. This estimated divergence time resulted from our assumption that the ancestral *Maoripamborus* had colonized before the separation of New Zealand from Australia. However, a recent review of estimated divergence time for terrestrial biota in New Zealand suggested that a large portion of extant lineages colonized after the separation of New Zealand and the median divergence time for sampled terrestrial invertebrates was 34 Ma (Wallis and Jorge, 2018). Although we had excluded the possibility of transoceanic dispersal in Pamborini (see also Sota et al., 2005), we cannot completely exclude the possibility of wind-borne dispersal by flight-capable ancestors, or dispersal by rafting with floating trees, etc. Also, the phylogenetic relationships among the southern hemisphere genera, (*Ceroglossus*, (*Pamborus*, *Maoripamborus*)), are not consistent with the typical area cladogram of the Gondwanan vicariance: (New Zealand, (South America, Australia)) (Sanmartín and Ronquist, 2004). Considering the uncertainty of the node age constraint,

we repeated the divergence time estimation by removing the node age constraint for the *Pamborus-Maoripamborus* divergence, >85 Ma, while retaining the other node constraints (Fig. S5). The results showed that the divergence time of *Pamborus* and *Maoripamborus* was 44 Ma (95% CI, 31–60 Ma) in the Paleogene, which implies that long-distance dispersal across the sea occurred from Australia to New Zealand, or *vice versa*. Therefore, we should reserve the possibility that the divergence of *Pamborus* and *Maoripamborus* occurred in the Paleogene.

4.2. Divergence of tribe Carabini

Although Carabini diverged from the common ancestor of Pamborini and Ceroglossini during the Cretaceous, the split of Carabini into two subtribes was inferred to have occurred more recently, at 32 Ma in the Oligocene (Fig. 1). The ancestral Carabini occurred somewhere in Laurasia (the present Holarctic) and diverged into subtribe Carabina in Europe and subtribe Calosomina in the Eastern Palearctic and Nearctic during the Oligocene (Fig. 3).

The worldwide distribution of Calosomina was previously attributed to their ancestral distribution across Pangaea in the Jurassic by Jeannel (1940). However, we inferred that the radiation of extant Calosomina is much more recent, and the worldwide distribution is likely a result of dispersal from the Holarctic region. This dispersal would have occurred mostly via flight as their ancestor was inferred to be winged and about a half of extant species are winged and flight-capable. Of the two major groups in this subtribe, Calosomes lobes originated in the eastern Palearctic and spread to the western Palearctic, North America, and Australasia. Dispersal from the eastern Palearctic to North America likely occurred via Beringia, and that to Australia occurred through Southeast Asia. The ancestral area of another Calosomina group, Calosomes ongles was North America, from which one lineage dispersed to South America, and the other to Africa then to Eurasia. The dispersal to South America might have been facilitated by the formation of the Isthmus of Panama. There was also dispersal from North America to Eurasia probably via Beringia. Calosomes ongles colonized islands around continents such as Madagascar as well as remote oceanic islands such as St. Helena and the Galapagos Islands, where endemic species evolved.

In contrast to subtribe Calosomina, the dispersal of Carabina is thought to be achieved basically by locomotion as their ancestor was inferred to be wingless. Carabina originated in Europe and spread over the Palearctic region during the Neogene (Fig. 3). Some lineages migrated from Eurasia to North America probably via Beringia. The apparently sudden dispersal and radiation of tribe Carabini during the Neogene might have been facilitated by the disappearance of the Turgai Sea, which had separated Europe from the rest of Eurasia until the Late Oligocene (Sanmartín et al., 2001). Global climatic cooling (Zachos et al., 2001) and the expansion of grasslands (Retallack, 2001) during the Cenozoic era might have provided suitable conditions for the dispersal of Carabini.

4.3. Evolution of flightlessness and species richness

In general, regaining flight ability from a flightless state with degenerated wings is unlikely in insects, although stick insects might have repeatedly transitioned between winged and wingless forms (Whiting et al., 2003; Zeng et al., 2020; see also Goldberg and Igić, 2008; Stone and French, 2003; Trueman et al., 2004 for a discussion of ancestral state reconstruction). In the Carabinae, all tribes except Carabini are completely flightless due to degeneration of the hind wings. Flightlessness may be caused by the degeneration of flight muscles first, followed by the degeneration of hind wings (Roff, 1986), which ultimately results in changes to body shape (reduction of meso- and metathorax widths as well as basal elytral widths). Multiple back mutations would be needed for the reversal of the evolution of flight ability, and therefore, regaining flight ability appears to be difficult. In our ancestral state reconstruction, the estimated rate of evolution from the wingless to

winged state (reversal) was almost nil and the ancestor of Carabinae was winged. Thus, our results suggest the ancestors of Carabinae were capable of flight and that all tribes except Carabini have lost this ability in parallel since their divergence. The ancestor of Carabini retained flight capability until the divergence of Calosomina and Carabina; the ancestral Calosomina were winged and the degeneration of hind wings occurred in several lineages independently, whereas all Carabina have evolved to become wingless except for a few lineages that have retained a polymorphic state. In accordance with this evolutionary scenario, Imura et al. (2018) showed that, within Carabinae, complete degeneration of the hind wings occurred in Cychrini, Pamborini, and Ceroglossini, whereas the hind wing condition ranges from macropterous to brachypterous in Carabini, suggesting more recent degeneration of the hind wings in Carabini than in the other three groups. In Calosomina, the recent evolution of flightlessness from flight-capable species is undisputed for flightless species found on oceanic islands, including *Calosoma (Aplothorax) burchelli* on St. Helena, which emerged at approximately 14 Ma (Sota et al., 2020), and three flightless species of the subgenus *Castrida* on three islands of the Galapagos, which emerged at 0.8–4.0 Ma (Hendrickx et al., 2015). Contemporary parallel evolution of brachypterous (flightless) populations from macropterous (flight-capable) populations in different habitat types has been documented in the carabid beetle *Pogonus chaldeus*, in which macropterous populations contain brachypterous alleles as a standing genetic variation (Van Bellegem et al., 2018); thus, repeated evolution of flightless species can occur following colonization by flight-capable individuals of multiple isolated habitats, even without novel mutations.

In contrast to Calosomina, almost all species of Carabina are wingless, and only a few species exhibit polymorphism, in which macropterous individuals occur within mostly brachypterous populations. Polymorphism has been reported for species in the subgenera *Hemicarabus* and *Limnocarabus* as well as in *Carabus (Carabus) granulatus* and *Carabus (Eucarabus) italicus*, which occur in wetlands or grasslands (Darlington, 1943; Turin et al., 2003; Yamashita et al., 2006). However, direct observation of flying macropterous individuals has been reported in only a few cases for *C. (Limnocarabus) clathratus* and *C. (Carabus) granulatus* (Turin et al., 2003). In some other groups of Carabidae with hind wing polymorphism, the brachypterous allele is dominant to the macropterous allele, and the macropterous allele is maintained in populations at very low frequencies (Den Boer et al., 1980). A similar condition may exist for hind wing polymorphism in Carabina.

In Calosomina, flight ability may have been retained due to their dependence on lepidopteran larvae, which fluctuate in abundance spatially and temporally (Bruschi, 2013); flight ability would thus be advantageous for using locally and temporarily abundant caterpillars across a wide area. Flightlessness in Calosomina has been selected only in isolated grasslands at high altitudes or on isolated oceanic islands, where removing the cost of unprofitable dispersal and allocating more resources to reproduction and survival may be advantageous (Leihy and Chown, 2020; Roff, 1990; Wagner and Liebherr, 1992). In Carabina, a sedentary lifestyle without flight might have been more strongly selected in their ancestors, likely in association with the change in their dietary preference toward land snails and earthworms (Sota and Ishikawa, 2004), and macropterous alleles may have been retained only in species inhabiting unstable habitats.

The evolutionary loss of flight might have promoted species diversification in beetle lineages through its effects on reducing gene flow and promoting genetic differentiation among geographically isolated populations (Ikeda et al., 2012). Our test of the speciation rate difference between the winged and wingless states in Carabini (Calosomina + Carabina; winged, 6% of all species) indicated that the speciation rate was higher under the flightless condition than under the flight-capable condition. However, among all Carabinae, the speciation rate (net diversification rate) varied greatly among flightless lineages, possibly due to the influences of other factors. Notably, Ceroglossini and Pamborini in the Southern Hemisphere had low speciation rates, which may

be attributed to the more restricted occurrence of temperate habitats suitable for Carabinae beetles in South America and Australasia compared to the Northern Hemisphere, especially Eurasia. Thus, the diversification of flightless lineages of Carabinae might have been restricted by the effect of smaller area, which may include smaller chances of allopatric differentiation and higher extinction rate. However, the flightless groups Carabina and Cychrini in the Holarctic region exhibit a marked difference in species richness, suggesting that habitat area is not the only limiting factor driving differences in species richness. The greater species diversification rate of Carabina may be associated with their divergence in feeding habits, which has resulted in the coexistence of multiple species occupying different ecological niches as well as their occurrence in diverse habitats across latitudes and altitudes; on the other hand, Cychrini are specialized exclusively in feeding on snails (Sota and Ishikawa, 2004), their occurrence is limited to temperate forests at moderate latitudes and altitudes, and the number of sympatric species is low.

4.4. Conclusions

The global distribution of wingless Carabinae lineages has arisen due to ancient dispersal during the Cretaceous and more recent dispersal since the Oligocene. Ancestral state reconstruction for hind wings indicated that the ancestor of Carabinae was likely winged. The ancestral populations might have exhibited polymorphic wing conditions, from which completely wingless lineages might have evolved repeatedly at different times and places. We hypothesized that Cychrini, Ceroglossini, and Pamborini completely lost their hind wings much earlier than did Carabini, among which Carabina have almost completely lost their hind wings, whereas about a half of Calosomina species have retained them. Revealing the timing of hind wing degeneration in various lineages of Carabinae is an interesting topic for future genomic studies focused on the evolution of genes involved in hind wing formation. The loss of flight capability due to the loss of hind wings can promote species diversification; however, species richness of flightless lineage could vary depending on geographic as well as ecological factors. Revealing the relationship between the loss of flight and species richness is also an important topic of future studies.

CRedit authorship contribution statement

Teiji Sota: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Funding acquisition. **Yasuoki Takami:** Formal analysis, Investigation, Writing – review & editing. **Hiroshi Ikeda:** Formal analysis, Investigation, Writing – review & editing. **Hongbin Liang:** Investigation, Resources, Writing – review & editing. **Gayane Karagyan:** Investigation, Resources, Writing – review & editing. **Clarke Scholtz:** Investigation, Resources, Writing – review & editing. **Michio Hori:** Investigation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The raw sequence data obtained were deposited in the DNA Read Archive of the DNA Data Base of Japan (Bioproject number, PRJDB9367).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2021.107355>.

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