The origin of the giant ground beetle *Aplothorax burchelli* on St Helena Island

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Abstract

Highly isolated oceanic islands harbor endemic ground beetles that have lost the ability to fly. Here, we investigate the origin of the possibly extinct flightless giant ground beetle *Aplothorax burchelli* on St Helena Island in the South Atlantic. *A. burchelli* was considered to be a member of the subtribe Calosomina (=genus *Calosoma*) of the subfamily Carabinae (Coleoptera: Carabidae) closely related to the genus *Ctenosta* (=*Calosoma* subgenus *Ctenosta*), but this proposition was questioned due to its unique external and genital morphology. We conducted a phylogenetic analysis of mitogenome sequences using historical specimens of *A. burchelli* and samples of representative species of Carabinae. Our analysis of 13 protein coding gene sequences revealed that *A. burchelli* is definitely a member of Calosomina, most closely related to a species of *Ctenosta*. Further analysis using NADH dehydrogenase subunit 5 gene sequences from most groups in Calosomina showed that *A. burchelli* formed a monophyletic group with *Ctenosta* species from Africa and Madagascar. Our results suggest that the ancestor of *A. burchelli*, which had the ability to fly, colonized St Helena from Africa after the emergence of the island 14 million years ago, and has since undergone evolutionary changes in conjunction with loss of flight.

ADDITIONAL KEYWORDS: Biogeography – *Calosoma* – colonization – dispersal – flightlessness – oceanic island – mitogenome – molecular phylogeny.

INTRODUCTION

Flightless ground beetle species are known to predominate on highly isolated oceanic islands (Darlington, 1943). Such flightless species are likely the descendants of flight-capable species with hind wings, and selection for winglessness has been associated with successful establishment of populations, and subsequent evolution and radiation of endemic species. Precise determination of the origin and evolutionary process of flightless ground beetle species on remote islands requires contemporary molecular phylogenetic analyses, but such studies are limited (e.g., Liebherr & Maddison, 2013; Maddison, Sproul, & Mendel, 2019).

St Helena is one of the most isolated oceanic islands in the South Atlantic. The nearest continental land is the western African coast, at a distance of ~1,900 km. St Helena was formed by volcanic activity occurring during the late Miocene; it emerged above the sea surface 14 Ma ago (Ashmole & Ashmole, 2000)(Basilewsky, 1985). The flora and fauna of St Helena include endemic species that often exhibit remarkable characteristics, probably resulting from evolutionary changes since the colonization by ancestral species. The endemic St Helena species are closest to taxonomic groups in Africa, South America, etc. (Ashmole & Ashmole, 2000), but molecular phylogenetic analyses of the origins of endemic St Helena insect species have so far been limited. Unfortunately, extensive habitat destruction and invasion of non-native animals and plants since the discovery of St Helena in the early 1500s have resulted in the extinction of many native animals and plants (Ashmole & Ashmole, 2000), making their study more difficult.

The giant ground beetle *Aplothorax burchelli* Waterhouse is a remarkable, large wingless beetle of the subfamily Carabinae (Coleoptera: Carabidae), representative of

the unique fauna of St Helena. This species was initially considered to belong to the genus *Carabus* (subtribe Carabina) of the tribe Carabini, and *Aplothorax* was proposed as a subgenus (Waterhouse, 1842). However, subsequent taxonomists considered it a distinct group closer to the genus *Calosoma*. Jeannel (1940) proposed the Calosomatina (= Calosomina) which contained 20 genera including *Aplothorax* (note that the latest revision by Bruschi (2013) treats all groups of Calosomina in the genus *Calosoma*). Jeannel (1940) recognized that *A. burchelli* is close to *Ctenosta* (= the subgenus *Ctenosta*; Bruschi, 2013), based on the characteristics of the male genitalia, and included the genus *Aplothorax* in Calosomina. However, Basilewsky (1972) disagreed, instead considering *Aplothorax* a distinct tribe (Aplothoraxini) of the subfamily Carabinae. Prüser & Mossakowski (1998) conducted a parsimony analysis of morphological characters in the subfamily Carabinae and found that *Calosoma* (Calosomina) and *Aplothorax* were sister groups, but they did not analyze the relationships among *Aplothorax* and various subgenera of *Calosoma*. In the review by Bruschi (2013), which we follow in this paper, all groups in Calosomina were treated as subgenera in the genus *Calosoma*, but *Aplothorax* was left out of *Calosoma*. A molecular analysis of the phylogenetic position of *A. burchelli* is thus warranted. Unfortunately, however, live *A. burchelli* have not been found since 1966–1967, when researchers from the Museum of Central Africa led by P. Basilewsky collected a number of specimens (Basilewsky, 1972). Currently, *A. burchelli* is considered to be extinct due to the extensive habitat destruction and predation by invasive animals since the discovery of the island in the 16th century (Ashmole & Ashmole, 2000; Gray *et al.*, 2019).

Historical specimens preserved dry for 50 or more years are frequently used for

molecular phylogenetic studies of insects including carabid beetles. Maddison *et al.* (2019) studied the origin of St Helena's endemic Bembidiini species (Carabidae) by extracting genomic DNA from museum specimens. They showed that the studied species were all placed in the genus *Bembidion* and formed a monophyletic group with a species from La Rèunion; this clade was sister to the African subgenus *Omotaphus*, suggesting African origins of St Helena and La Rèunion species.

In this study, we conducted a molecular phylogenetic analysis focusing on mitogenomes of representative species of the subfamily Carabinae (Coleoptera: Carabidae), including *A. burchelli*, to reveal its phylogenetic origin. We used specimens collected in 1967 and successfully obtained mitochondrial protein coding gene sequence data. Our results indicate that *A. burchelli* is a member of the genus *Calosoma* and closely related to the subgenus *Ctenosta*, as was suggested by Jeannel (1940).

MATERIAL AND METHODS

DNA EXTRACTION AND SEQUENCING

For the mitogenomic analysis, we used 24 species from all five groups of the subfamily Carabinae, together with *A. burchelli* (Table 1). *Trachypachus slevini* (Trachypachidae) was used as the outgroup taxon (Table 1). Of the sample specimens, two *A. burchelli* and one *Calosoma chlorostictum* were pinned, dry specimens collected on St Helena Island in 1967 by the Belgian group and given to Professor Emeritus Ryosuke Ishikawa at Tokyo Metropolitan University by P. Basilewsky. These specimens are due to be deposited at the University Museum of the University of Tokyo. Specimens of the other species were preserved in 99% ethanol or RNAlater solution (Invitrogen, Carlsbad, CA,

USA). Total genomic DNA was extracted from tissues of ethanol or RNAlater-fixed specimens using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). For pinned specimens, total genomic DNA was extracted from legs without noticeable destruction using the DNeasy Micro Kit (Qiagen) following the manufacturer's protocol. The quality and quantity of extracted DNA were assessed using a TapeStation High Sensitivity D1000 kit (Agilent Technologies, Santa Clara, CA, USA), NanoPhotometer (Implen, München, Germany), and Qubit (Thermo Fisher Scientific, Waltham, MA, USA). Shotgun libraries were prepared using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) and sequenced on an Illumina HiSeq X Ten platform (Illumina, San Diego, CA, USA) at Macrogen Co. Ltd. (Kyoto, Japan).

SEQUENCE DATA ANALYSIS

Raw Illumina reads were filtered with fastp (version 0.20.0; Chen *et al.*, 2018) to remove low-quality and adaptor-containing reads. To assemble mitogenomes directly from the clean reads, we used NOVOplasty (Dierckxsens, Mardulyn, & Smits, 2017) with a seed sequence of the *Carabus granulatus* mitogenome (GenBank accession no.: NC044759). If the NOVOplasty assembly failed, we assembled scaffolds from the sequence reads using IDBA-UD (Peng *et al.*, 2012) and searched for mitochondrial DNA sequences in the scaffolds against the *C. granulatus* complete mitochondrion sequence (GenBank accession no.: NC044759) using BLASTn (version 2.2.31+; (Altschul *et al.*, 1997). To obtain mitogenomic sequences for phylogenetic analysis of specimens that failed NOVOplasty assembly, the raw reads were mapped to the reference mitogenome sequence of *C. granulatus* using Geneious Prime 2020.0 5

(Biomatters Ltd., Auckland, New Zealand), and a consensus sequence was obtained for each specimen with IUPAC ambiguity codes or Ns for undetermined or low-coverage sites. All mitogenome sequences thus obtained were aligned to the reference mitogenomes of *C. granulatus* (GenBank accession no.: NC044759) and a *Calosoma* sp. (possibly *Calosoma scrutator*; GenBank accession no.: GU176340) using MEGA version X (version 10; (Kumar *et al.*, 2018), and sequences for 13 protein coding genes were extracted for use in subsequent phylogenetic analyses. The cleaned raw reads were deposited at the DDBI Sequence Read Archive (DRA) of the DNA Data Bank of Japan (DDBJ). Aligned sequence matrices are archived at figshare (doi: 10.6084/m9.figshare.12355844).

PHYLOGENETIC ANALYSIS

We performed a maximum likelihood (ML) analysis with the sequence matrix of 13 protein coding genes using RAxML (version 8.2; Stamatakis, 2014). An optimal partitioning scheme with 24 partitions was obtained from a total of 39 partitions (13 genes × 3 codon positions) using PartitionFinder2 (version 2.1.1; Lanfear *et al.*, 2017), with the GTR+G substitution model used for all partitions. A rapid bootstrap analysis with 1,000 replications was used to obtain the best tree and node support values.

Based on the results of the above analysis, we searched for species closely related to *A. burchelli* using a collection of NADH dehydrogenase subunit 5 (ND5) gene sequences retrieved from GenBank, together with the ND5 sequences of several *Calosoma* species obtained in the present study (see Table S2 for the list of species and sequence accession numbers). Most ND5 gene sequences of *Calosoma* species were published by Su, Imura, & Osawa (2005). The ND5 dataset contained two sequences of

A. burchelli, 46 sequences from 17 of 19 subgenera of *Calosoma*, and 25 sequences from *Carabus* (as the outgroup). In total, 73 sequences were used in the phylogenetic analysis of ND5 gene genealogy. With this dataset, a ML analysis was conducted using RAxML (version 8.2). A rapid bootstrap analysis with 1,000 replications was performed with partitioning of three codon positions, using the GTR+G substitution model for each partition.

DIVERGENCE TIME ESTIMATION

To infer the divergence time of *A. burchelli* from its closest species, we performed a Bayesian relaxed clock analysis based on a lognormal clock model, using the MCMCTree program in the PAML package (version 4.8; Yang, 2007). This analysis used the topology of the best tree obtained from the ML analysis of the 13 protein coding gene sequences. Authentic fossil records of Carabinae are limited to those from the Miocene or later (e.g., Penev, Casale, & Turin, 2003) and not readily used for calibrating internal nodes in divergence time estimation. Therefore, we referred to the clock tree of McKenna *et al.* (2019) for the split of *Trachypachus* and Carabinae (170 Ma; 150–197 Ma) and that of Andújar, Serrano, & Gómez-Zurita (2012) for the split of Carabina and Calosomina (the Carabini stem node) (31 Ma; 24–38 Ma). The age of the Carabini stem is controversial as it was estimated to be ancient as 104 Ma (the Cretaceous) when the tree of Carabina taxa was constrained only at the split of *Trachypachus* and Carabinae (Toussaint & Gillett, 2017); however, such a calibration strategy leads to unrealistically ancient divergence of *Carabus* or *Calosoma* taxa. The estimated divergence time of 24–38 Ma (the Eocene–Oligocene) is more probable for the biogeographic pattern of the Carabini (Andújar, Serrano, & Gómez-Zurita, 2012). In

addition to the above two calibration points, we assumed that divergence of *Maoripamborus* in New Zealand and *Pamborus* in Australia occurred >85 Ma ago (Sota *et al.*, 2005). In the divergence time estimation, first, an approximate substitution rate was estimated assuming a putative root age of 170 Ma. Soft bound constraints were used for the three node: (1) divergence between Trachypachidae and Carabinae, 150–197 Ma; (2) divergence between Carabina and Calosomina, 24–38 Ma; and (3) divergence between *Pamborus* and *Maoripamborus*, >85 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.

RESULTS

We obtained 151-bp Illumina paired-end sequence data with 18–106 million reads and total lengths of 2.1–12.6 Gbp for the 25 specimens (Table 1). The NOVOplasty assembly was successful for all but three specimens, including two 52-year-old *A. burchelli* specimens. We blasted the assembled scaffolds from the two *A. burchelli* specimens (B89, B90) against the reference mitogenome of *C. granulatus* (Table S2). We found that 14 and 10 of B89 and B90 scaffolds with total lengths of 13,141 bp and 8,549 bp, respectively, reliably matched portions of the 16,918-bp *C. granulatus* mitogenome sequence (Table S2). We then mapped the *A. burchelli* sequence reads to the *C. granulatus* mitogenome sequence, and obtained the consensus sequence for each specimen used in the mitogenome alignment with other specimens. In contrast to the *A. burchelli* specimens, the sequence reads from the St Helena *Calosoma chlorostictum*

specimen (also aged 52 years) could be assembled into a circularized mitogenome sequence. The mitogenome sequences from 25 specimens were aligned with reference sequences of *Carabus* and *Calosoma* species, and 13 conserved protein coding gene sequences were extracted for phylogenetic analysis.

The ML tree (Fig. 1) resulting from alignment of the 13 protein coding gene sequences (11,201 bp) clearly showed the relationships among the tribes and subtribes of Carabinae, in which Cychrini is sister to all other tribes of Carabinae, and Pamborini and Ceroglossini are sister to each other and to Carabini (subtribes Carabini and Calosomina). Two *A. burchelli* sequences were located within the subtribe Calosomina (genus *Calosoma*) and sister to *Calosoma* (*Ctenosta*) *senegalense*, with 100% bootstrap support.

Among the partial ND5 sequences from 17 of 19 subgenera of the genus *Calosoma* (Fig. 2), *A. burchelli* sequences were grouped with three *Ctenosta* species (*C. senegalense*, *Calosoma grandidieri*, *Calosoma planicolle*), with 94% bootstrap support. *A. burchelli* was closest to *C. senegalense* among the three *Ctenosta* species, but the relationships among these species were not supported by the bootstrap analysis. The clade of *Ctenosta* and *Aplothorax* was included in a well-supported clade (bootstrap value, 95%) with *Campalita*, three flightless subgenera (*Orinodromus*, *Carabomorphus*, and *Carabophanus*), *Caminara*, and a North American *Camedula* species *Calosoma* (*Camedula*) *marginale*. *Calosoma* (*Campalita*) *chlorostictum* from St Helena was close to *Calosoma* (*Campalita*) *maderae* and *Calosoma* (*Campalita*) *auropuncutatum* (= *C.* (*C.*) *maderae*; Bruschi, 2013).

In our divergence time estimation using the three calibration points (Fig. 3), the divergence time of *A. burchelli* and *C. senegalense* was 14 Ma (95% CI, 7–21 Ma).

DISCUSSION

PHYLOGENETIC POSITION

Our results indicated that *A. burchelli* is a member of the genus *Calosoma* (tribe Carabini: subtribe Calosomina) related to the subgenus *Ctenosta* (Figs. 1, 2). This finding corroborates Jeannel's (1940) view of the phylogenetic position of *Aplothorax*, although Jeannel (1940) treated *Ctenosta* and *Aplothorax* as genera of Calosomina. In the analysis of ND5 gene sequences, the relationships among *A. burchelli* and four *Ctenosta* species were not resolved, though they formed a monophyletic group with high bootstrap support (Fig. 3). Thus, the exact relationships between *A. burchelli* and *Ctenosta* species should be explored in a future analysis including all *Ctenosta* species and additional genomic sequence data. *Ctenosta* comprises nine species (Bruschi, 2013), of which four occur in Africa (*Calosoma roeschkei*, *Calosoma guineense*, *Calosoma strandi*, and *Calosoma scabrosum*), two in Africa and Madagascar (*C. senegalense* and *C. planicolle*), two in Madagascar only (*C. grandidieri* and *Calosoma bastardi*), and one in South Asia (*Calosoma orientale*). Of these, *C. senegalense* has the widest distribution range and is a candidate for the closest extant species, as in our study. Other African species such as *C. scabrosum* and *C. roeschkei*, which were not included in our analysis, are also candidates because they show elytral patterns similar to those of *A. burchelli*.

Another *Calosoma* species, *C.* (*Campalita*) *chlorostictum*, inhabits St Helena; this species retains its hind wings and can fly. Its distribution range encompasses Africa, Madagascar, the Middle East, and the isolated South Atlantic islands of St Helena,

Ascension, and Tristan da Cunha (Bruschi, 2013). Although we have no ND5 sequence data for African *C. chlorostictum* specimens, the specimen from St Helena was closely related to *C*. (*Campalita*) *maderae* from Spain and Saudi Arabia. *C*. *maderae* has a wide distribution range including West Asia, Europe, North Africa, the Middle East, and the Canary Islands. The genetic distance between *C. maderae* and *C. chlorostictum* (0.0159, uncorrected p) is much smaller than that between *Calosoma chinense* from East Asia and *C. maderae* (0.045–0.046), and that between *C. senegalense* and *A. burchelli* (0.062–0.065). These facts suggest a recent divergence of *C*. *maderae* and *C*. *chlorostictum*, and a relatively recent colonization of *C. chlorostictum* on St Helena (Basilewsky, 1972).

CHARACTER EVOLUTION

Our results suggest that the ancestral *A. burchelli* was a *Ctenosta*-like *Calosoma* that was able to fly, with marked morphological evolution subsequently occurring on the isolated island. *Calosoma* species with hind wings, including *Ctenosta* species, typically exhibit elytral parts with broad shoulders (Fig. 4). In *Calosoma*, degeneration of the hind wings and wing muscles (i.e., the evolution of flightlessness) has occurred in a few lineages in different continental areas (Africa, Eurasia, and the Americas), but on an island this phenomenon is limited to *A. burchelli*. The degeneration of flight muscles and hind wings is associated with a slender body shape with narrow shoulders, which allows allocation of more resources to reproduction. Thus, flightlessness may have been favored in highly isolated habitats where dispersal by flight confers little fitness advantage within a small oceanic island and dispersal out from the island would have no future return. Many endemic beetle species on St Helena Island are similarly flightless

(Darlington, 1943; Basilewsky, 1985).

The most conspicuous male genital character of *Calosoma* species, compared to its sister group *Carabus*, is the presence of a well-developed ligula in the endophallus and a much less developed aedeagus, which is associated with a less developed endophallus housed in the aedeagus (Ishikawa, 1978). Although the modification of the aedeagus is remarkable in *A. burchelli*, making this species unlike *Calosoma*, its endophallus possesses a sclerotized ligula with a curved hook point, similar to that of *Ctenosta* species (Jeannel, 1940; Fig. 4). In the subfamily Carabinae, development of the endophallus and the associated enlargement of the aedeagus is seen only in *Carabus* and some *Pamborus* (Sota *et al.*, 2005; Takami & Sota, 2006). Other groups, including *Calosoma*, have a slender aedeagus with an undeveloped endophallus. The function of the modified aedeagus in *A. burchelli* and its evolutionary factors are interesting subject for future study.

COLONIZATION OF ST HELENA BY CARABID BEETLES

St Helena Island is a volcanic island that emerged above the sea ~14 Ma ago, and habitable conditions for terrestrial animals may have first occurred 11 Ma ago (Basilewsky, 1985). In our divergence time estimation, the estimated divergence time of *A. burchelli* and *C. senegalense* was 14 Ma, coinciding with the age of St Helena Island, with the confidence interval of 7–21 Ma. These results may imply that *A. burchelli* diverged from its sister species (not necessarily *C. senegalense*) between 7–21 Ma and then colonized on St Helena Island between 7–14 Ma. However, these results were strongly affected by the node-age constraint for the Carabini stem node (see Materials and Methods) and should be interpreted cautiously. We need well-evidenced calibration

points for estimation of divergence times in the Calosomina.

We infer that the ancestor of *A. burchelli* could fly as do *Ctenosta* species, and that the ancestral *A. burchelli* may have achieved colonization by flight and wind-borne dispersal. In fact, some *Calosoma* beetles appear to have achieved long distance dispersal by flight, such as from North Africa to Europe (Bruschi 2013). Other ways of colonization, such as rafting on a large log, etc., may not be ruled out completely, but this possibility would be small given the long journey in the sea water until reaching the oceanic island. On the other hand, *C.* (*Campalita*) *chlorostictum* appears to be a much more recent colonizer of St Helena, given the minimal genetic divergence from its related species. For this species, even the possibility of accidental introduction by ships after the discovery of the island (in 1502) cannot be ruled out.

The endemic carabid fauna of St Helena includes flightless species of the tribe Bembidiini, which are called "St Helena Peaks *Bembidion*" and the sister group of the subgenus *Omotaphs* in Africa (Maddison et al. 2020). *Bembidion* beetles are generally small and active flyers, and long distance (probably wind-borne) dispersal from the southwest Pacific to the Society and Hawaiian Islands has been proposed (Liebherr & Maddison, 2013). The ancestral *Bembidion* species might also have colonized St Helena from Africa by wind-borne dispersal. It is interesting to estimate the divergence time of St Helena Peaks *Bembidion* species from their sister group for understanding the colonization history of carabids on St Helena Island.

CONCLUSION

Using mitogenome data, our study resolved the origin of *A. burchelli* and confirmed that

it is a member of the genus *Calosoma*, closely related to the subgenus *Ctenosta*. The ancestral species was probably winged, lost its flight muscles and hind wings, and evolved a modified aedeagus during adaptation to the island habitat. Thus, *A. burchelli* represents a good example of the evolution of flightless beetles in an isolated island habitat. Genomic comparison of *A. burchelli* and its sister species in the subgenus *Ctenosta* may reveal the genetic changes that contributed to the evolution of flightlessness and subsequent morphological evolution, including of the genitalia. Our results demonstrate the utility of DNA extracted from historical specimens of *A. burchelli*, and this line of genomic study could be extended to nuclear genomic DNA. On the other hand, it may be too early to conclude that this beetle is extinct. *A. burchelli* is nocturnal and hides underground during daytime; the beetles are active only after substantial rainfall and may be completely inactive during the dry period of the year (Basilewsky, 1985). The chance of encountering or collecting this species is thus limited; indeed, since the time of Napoleon's exile in the early 1800s, very few specimens had been collected before the expedition of the Belgian team in 1966–1967; thus, there is a faint possibility that it has survived.

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Fig. 1

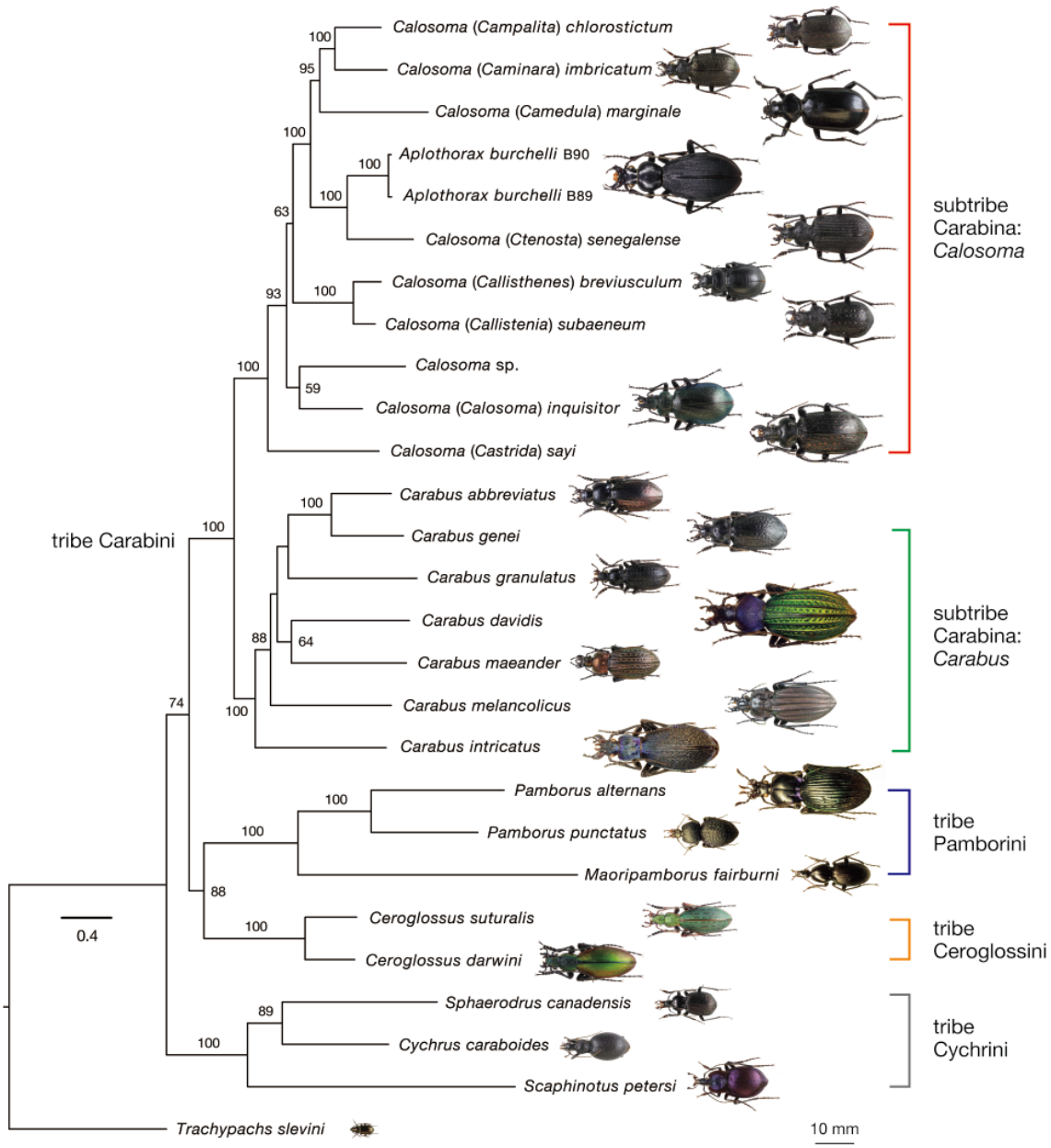

Fig. 1. Phylogenetic relationships among the groups (tribes) of the subfamily Carabinae and the position of *Aplothorax burchelli*. Photo credit: T. Sota.

Fig. 2

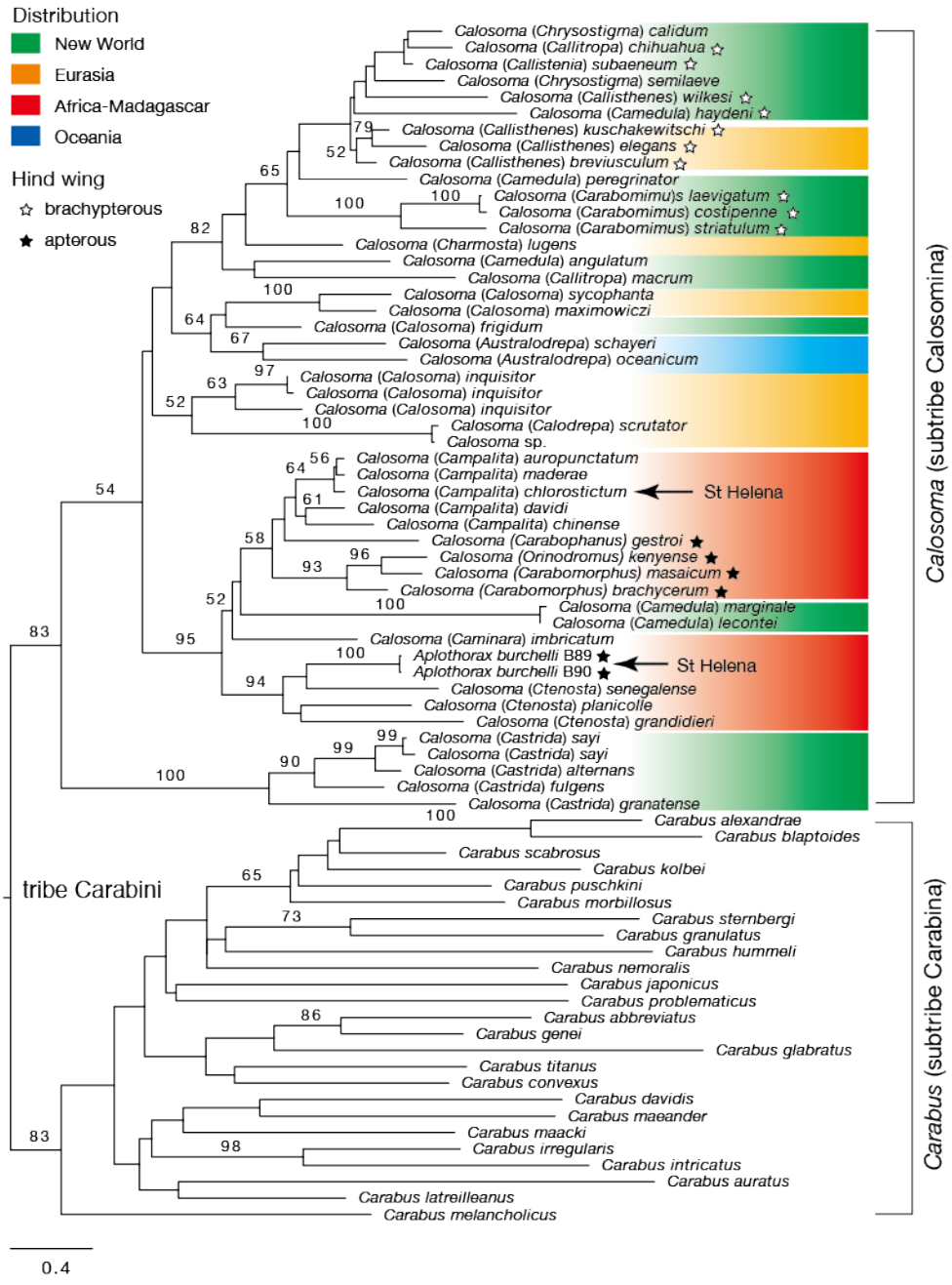

Fig. 2. Phylogenetic tree based on mitochondrial ND5 gene sequences of *Calosoma* (subtribe Calosomina) species and species from the sister group *Carabus* (subtribe Carabina). The hind wing condition follows the description in Bruschi (2013).

Fig. 3

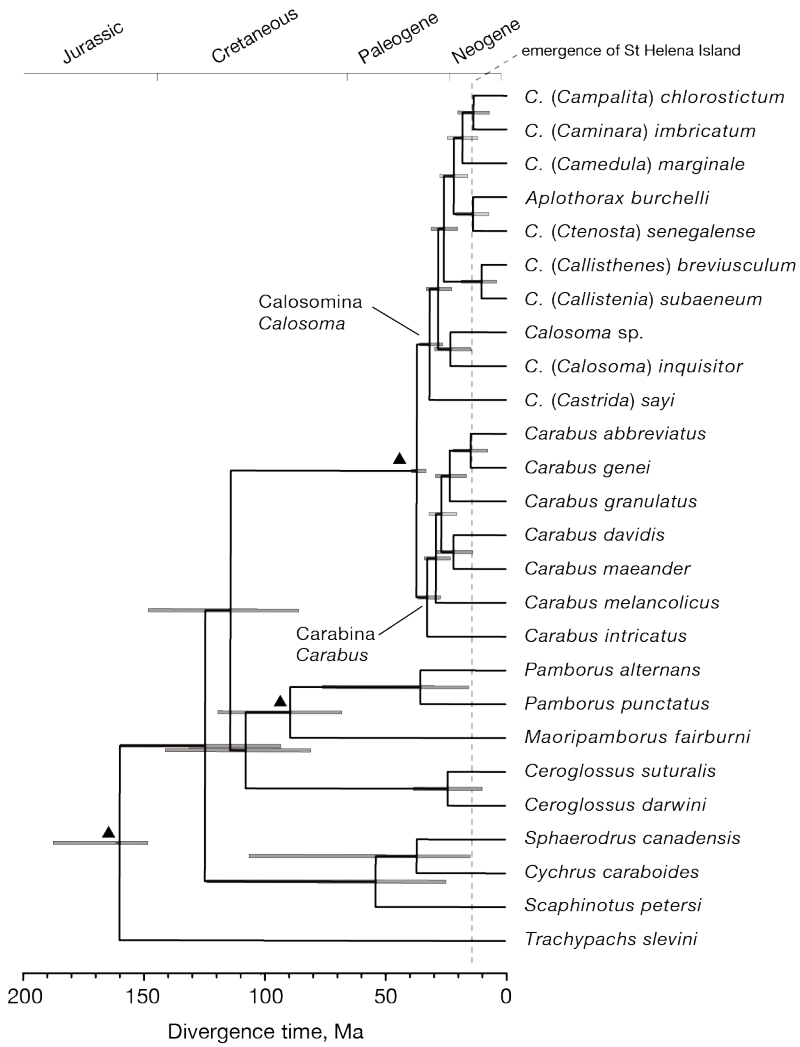

Fig. 3. Divergence time of the Carabinae. Nodes with closed triangles are constrained (see Material and Methods for details). Bars indicate 95% confidence intervals.

Fig. 4

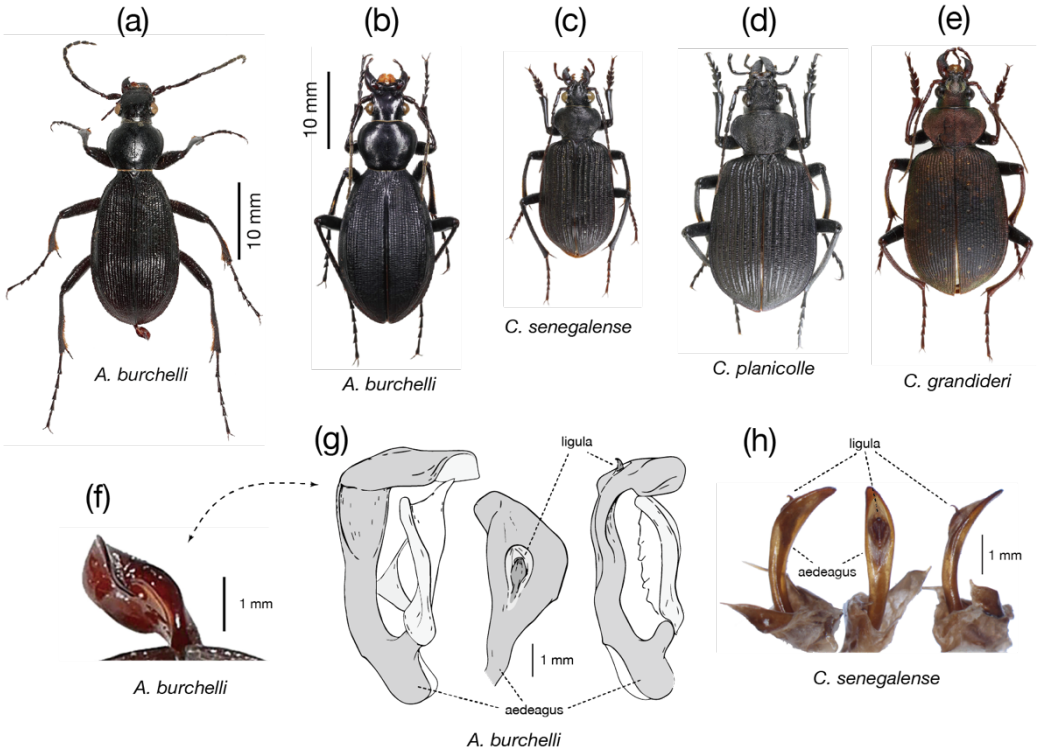

Fig. 4. Habitus and male genitalia. (a) *Aplothorax burchelli*, male; (b) *A. burchelli*, female; (c) *Calosoma* (*Ctenosta*) *senegalense*, male; (d) *Calosoma* (*Ctenosta*) *planicolle*, male; (e) *Calosoma* (*Ctenosta*) *grandidieri*, male; (f) apex of the aedeagus of *A. burchelli* male; enlargement of the abdominal tip of (a); (g) male genitalia of *A. burchelli* (redrawn from Jeannel, 1940; p. 134, fig. 96–98), (h) male genitalia of *C.* (*C.*) *senegalense*. Photo credit: (a) and (f), D. Pryce/the St Helena National Trust; (b)–(e), (g) and (h), T. Sota.

Table 1. List of specimens used in the mitogenome study.

Species	Sample ID	Locality	Year of collection	Sequence reads[1] No. reads, $\times 10^6$	Total length, Gb	Mean length, bp	Accession BioSample ID[2]	Mitogenome Assembled bp[3]
Aplothorax burchelli	B89	St Helena I.	1967	22.9470	2.3203	101	SAMD00208720	(15825)
Aplothorax burchelli	B90	St Helena I.	1967	48.1959	5.6238	116	SAMD00208721	(15834)
Tribe Carabini Subtribe Calosomina								
Calosoma breviusculum	B55	Shirak, Armenia	2013	50.7072	6.2075	122	SAMD00208715	16457
Calosoma chlorostictum	B96	St Helena I.	1967	72.3468	6.9062	95	SAMD00208722	17110
Calosoma imbricatum	B45	Northern Cape, South Africa	2017	27.5755	3.5488	128	SAMD00208708	(16474)
Calosoma inquisitor	B43	Hokkaido, Japan	2016	27.9983	3.5649	127	SAMD00208707	16426
Calosoma marginale	B52	Texas, USA	2012	18.4811	2.1390	115	SAMD00208713	16433
Calosoma sayi	B51	Grenada, Mississippi, USA	2015	28.0091	3.2591	116	SAMD00208712	16482
Calosoma senegalense	B50	Hardap, Namibia	2017	46.7255	5.8125	124	SAMD00208711	16458
Calosoma subaeneum	B54	Washington, USA	2016	105.7605	12.5722	118	SAMD00208714	16456
Tribe Carabini Subtribe Carabina								
Carabus davidis	A01	Jiangxi, China	2006	36.2739	4.8191	132	SAMD00208696	16625
Carabus maeander	A17	Hokkaido, Japan	1998	35.6161	4.3731	122	SAMD00208698	16646
Carabus abbreviatus	A21	Tenerife I., Spain	2017	41.6532	5.4019	129	SAMD00208700	16600
Carabus genei	A18	Sardinia I., Italy	2017	47.2469	6.2459	132	SAMD00208699	16547

Carabus intricatus	A05	Moravia, Czech	2015	47.5110	6.1245	128	SAMD00208697	16574
Carabus melancholicus	A32	Madrid, Spain	1996	59.0931	7.5855	128	SAMD00208701	16802
Tribe Ceroglossini								
Ceroglossus darwini	B38	Puyehue, Chile	2015	44.0739	6.0803	137	SAMD00208705	16467
Ceroglossus suturalis	B40	Mallaganes, Chile	2015	44.5999	5.5543	124	SAMD00208706	16474
Tribe Cychrini								
Cychrus caraboides	B31	Val Sessera, Italy	2000	45.3358	5.8602	129	SAMD00208702	17343
Scaphinotus petersi	B35	Arizona, USA	2016	51.2401	7.2032	140	SAMD00208703	16844
Sphaerodrus canadensis	B36	Virginia, USA	2016	42.1741	5.6247	133	SAMD00208704	16885
Tribe Pamborini								
Maoripamborus fairburni	B73	Waipaua, New Zealand	2002	44.1781	6.2910	142	SAMD00208719	17025
Pamborus alternans	B57	New South Wales, Australia	2001	60.3485	6.9825	115	SAMD00208717	16668
Pamborus punctatus	B67	Mt.Hypipamee, Australia	2001	34.9593	4.5790	130	SAMD00208718	16798
Family Trachypachidae								
Trachypachus slevini	B56	Oregon, USA	2018	42.2076	5.2948	125	SAMD00208716	17173

[1], Sequence reads after filtering.
[2], DDBI/DRA BioSample IDs under BioProject PRJDB9367.
[3], Length of the whole mitogenome resulting from NOVOplasty assembly. Sequence lengths of B89, B90 and B45 obtained by mapping on the reference genome are in parentheses.