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Origin and adaptive radiation of the exceptional and threatened bembidiine beetle fauna of St Helena (Coleoptera: Carabidae)

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The central peaks of the isolated island of St Helena (south Atlantic Ocean) are home to an extraordinary set of ground beetles of the tribe Bembidiini, which belong to three endemic genus-group taxa. These beetles are strikingly different in overall body form from the many bembidiines found elsewhere in the world. At least some of the St Helena species are likely to be extinct, and all are threatened by habitat destruction and invasive species. Through next-generation sequencing of historical museum specimens, we examine the phylogenetic relationships of the St Helena fauna. We find that, in spite of their morphological disparities, the endemic bembidiines of St Helena form a clade of genetically similar species, with their sister group being *Bembidion alsium* from the Indian Ocean island of La Réunion, and the sister group of this pair being the African subgenus *Omotaphus*. We propose that the St Helena Peaks *Bembidion* are an adaptive radiation that arose from a single dispersal event to St Helena from a now-extinct African lineage (sister to *Omotaphus*) and that this extinct lineage also served as the ancestral source of *B. alsium*. Given that the St Helena Peaks *Bembidion* are deeply nested in the genus *Bembidion*, we move the three taxa back in that genus as subgenera and provide a new name (*Bembidion shepherdae*) for the now-homonymous *Bembidion wollastoni*.

ADDITIONAL KEYWORDS: adaptive radiation – ancient DNA – Bembidiini, museum genomics – oceanic islands – Trechinae.

INTRODUCTION

The island of St Helena (a British Overseas Territory) is one of the most isolated terrestrial environments on Earth, positioned in the south Atlantic Ocean ~1300 km from the nearest island (Ascension), > 1800 km from the coast of Africa (Fig. 1) and 3260 km from the coast of South America. The island is small, only 122 km^2, and rises to 818 m a.s.l. at the top of Diana's Peak. When St Helena was discovered in 1502, the higher areas were covered by a unique forest of cabbage trees of the family Asteraceae and endemic tree ferns (*Dicksonia arborescens* l'Hér., Cyatheaceae); this forest was home to many other endemic plants and animals (Ashmole & Ashmole, 2000). Almost all of this forest is now gone, devastated through past clearing of native vegetation and conversion into agricultural lands and through damage inflicted by plants and animals introduced onto the island by humans (Cronk, 1989; Ashmole & Ashmole, 2000). Only ~16 ha of native forest remains (Fig. 2), most of which is now infiltrated with non-native plants (Mendel *et al.*, 2008).

In 1875–1876, in and around decaying fern and cabbage tree logs on Diana's Peak, High Peak and elsewhere on the Central Ridge of the island, Thomas Vernon Wollaston found 11 species of extraordinary ground beetles of the tribe Bembidiini (Wollaston, 1877). Wollaston created three new subgenera of the large, cosmopolitan genus *Bembidion* to house the 11 species: *Apteromimus*, *Endosomatium* and

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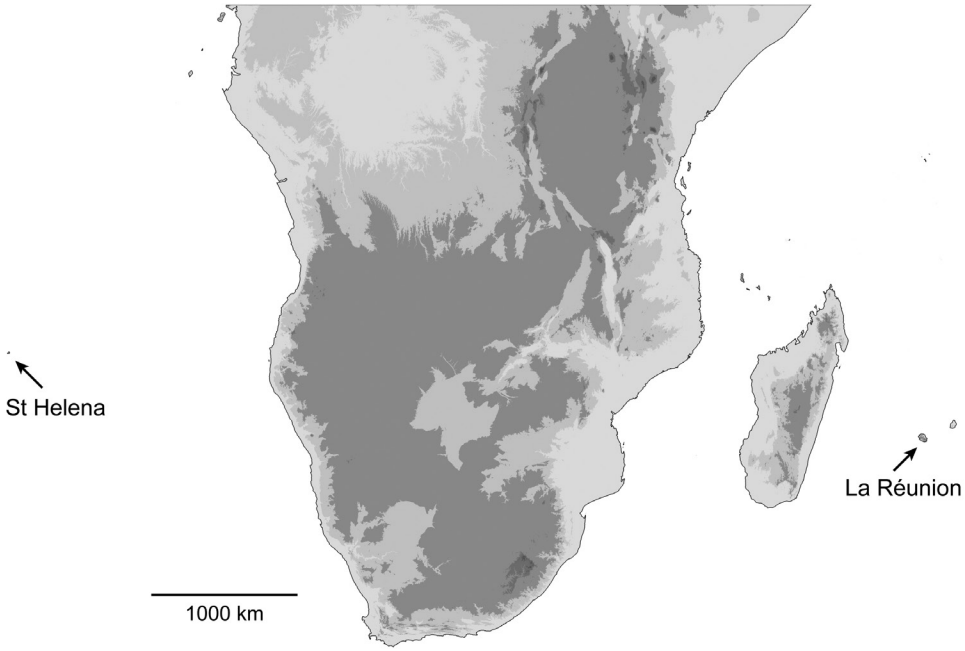

Figure 1. Map showing the locations of St Helena and La Réunion Island relative to Africa and Madagascar.

Pseudophilochthus. Basilewsky (1972) treated these as separate genera because of their unique features and because he followed the French school led by René Jeannel, in which many of the subgenera of *Bembidion* were elevated to separate genera (Lindroth, 1969).

These beetles on the Peaks of St Helena are strikingly different from their relatives elsewhere in the world. They possess body forms (Figs 3, 4) unique within Bembidiini and different from the forms found throughout the genus *Bembidion* (Fig. 5), a large, widespread genus with > 1200 species (Lorenz, 2005; Maddison, 2012b). Within St Helena, *Pseudophilochthus nubigena* (Wollaston, 1877) (Fig. 3A) looks more like some platynines in the subfamily Harpalinae than it does other bembidiines; other *Pseudophilochthus* (e.g. Figs 3B, 4E–G) look more like tachyine carabids; and *Apteromimus platyderoides* (Wollaston, 1877) (Fig. 3C) looks similar to some members of the tribe Pterostichini (Harpalinae). In head and prothoracic shape, *Endosomatium* (Fig. 3D) is the most distinctive, with a body form unique within the entire family.

The St Helena Bembidiini are also unique in their way of life. Rather than living along bodies of water as do most bembidiines, many St Helena species live inside decaying logs, with some living in the galleries of xylophagous weevil larvae, on which they feed (Basilewsky, 1972). As Wollaston (1877) notes, 'the *modus vivendi* of at least half of them, within the damp and rotting stems of the dead tree-ferns on the most elevated ridges of the island, invests them with a significance which it is hardly possible to overrate'.

A few expeditions have sought these beetles since Wollaston's discovery. Howland Roberts collected four of the species between 1884 and 1886. In 1965–1967, a Belgian team led by Pierre Basilewsky found a total of 178 specimens representing six of Wollaston's species, in addition to two specimens of a 12th species. Although this new species confirmed Wollaston's (1877) prediction that 'there can be little doubt that a continued research in the higher regions of the island would yet bring to light others (though perhaps only a few) which we failed to secure', the lack of capture of five of the species during the thorough expedition, the finding of only one new species and the demise of the native vegetation led Basilewsky (1985) to state that 'extinction of all three genera is near at hand'. Another expedition was conducted by Howard Mendel, Philip Ashmole and Myrtle Ashmole in 2005–2006, with additional surveys in 2017 and 2018. Given that these more recent extensive efforts yielded only a single specimen of these beetles (Mendel *et al.*, 2008), it appears that the unique endemic bembidiine fauna of St Helena is certainly diminished, if not at least partly gone.

The bembidiines of St Helena suggest a history common to the endemic flora and fauna of oceanic islands (i.e. those not connected to a continent via a continental shelf): a lineage or lineages arrive from a distant land and, through time, radiate into exceptional species. Some better-known examples of such adaptive radiations include Darwin's finches and tortoises of the

Figure 2. Photographs of habitats. A, St Helena's Peaks Ridge, showing Diana's Peak, February 2013. B, interior of the forest at High Peak Dell, April 2011, showing cabbage trees. St Helena Peaks *Bembidion* would presumably live in enclosed habitats like this, with tree ferns. C, a tree fern with a moss-covered rotting log at its base, at Cuckold's Point, August 2019. This partly cleared forest is not natural, because it is more open and drier than the few remaining undisturbed habitats, but with the rotting logs it is a potential habitat for *Bembidion*. A, B, copyright Roger S. Key; C, copyright Liza Fowler.

Galápagos Islands (Román-Palacios & Wiens, 2018) and the silverswords (Baldwin & Sanderson, 1998) and *Drosophila* of Hawai'i (Magnacca & Price, 2015). Many of these island radiations, including those on St Helena, have species now threatened with extinction because of loss of habitat and invasive species.

Bembidiines have also made their way to other oceanic islands, with endemic species in the Canary Islands (Machado, 1992), Tahiti (Liebherr & Maddison, 2013) and the Galápagos Islands (Desender & Maelfait, 1989), among others. On some islands they radiated into multiple species (e.g. into 23 species on the Hawaiian islands; Liebherr, 2008); in other cases, an island contains only a single endemic species, such as *Bembidion alsium* Coquerel (Fig. 6) on the island of La Réunion (Bonavita *et al.*, 2016). Although the radiation on St Helena is not the most species rich on islands, it contains the most distinctive bembidiine fauna, and one of unknown relationships.

Through next-generation DNA sequencing of pinned museum specimens, we sought to answer three primary questions about the St Helena bembidiines. First, do they represent one or several dispersals onto St Helena? Second, what are they related to? Second, what is the likely source area of the ancestral propagule or propagules? We also examine the relationships of *B. alsium* of La Réunion, because preliminary data indicated that it might play a role in deciphering the origins of the St Helena species.

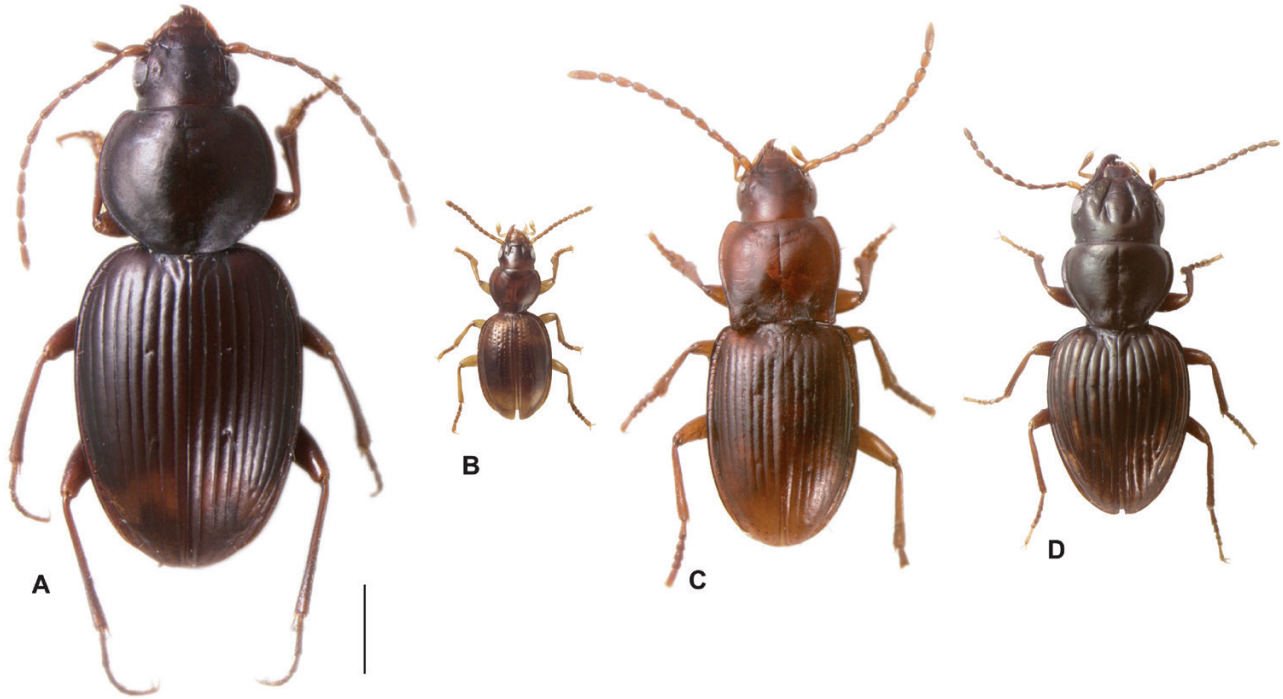

Figure 3. The four species of *Bembidion* sequenced from the endemic St Helena Peaks *Bembidion*, all to the same scale. B–D, the specimen that was sequenced; A, another specimen from the same series as the sequenced specimen. A, *Bembidion (Pseudophilochthus) nubigena*. B, *Bembidion (Pseudophilochthus) rufosuffusum*. C, *Bembidion (Apteromimus) platyderoides*. D, *Bembidion (Endosomatium) megalops*. Images copyright David R. Maddison 2019, released under a Creative Commons Attribution 4.0 International License (CC-BY 4.0). Scale bar: 1 mm.

MATERIAL AND METHODS

TAXON SAMPLING FOR DNA STUDIES

We obtained sequence data from one specimen of each of four species of Bembidiini from St Helena [*Apteromimus platyderoides*, *Endosomatium megalops* (Wollaston, 1877), *Pseudophilochthus nubigena* and *Pseudophilochthus rufosuffusum* (Wollaston, 1877)] and one specimen of *B. alsium* from La Réunion (Table 1). The species sequenced are shown in Figures 3 and 6. Three of the St Helena specimens were collected in 1967 by the Belgian expedition; the fourth was part of the collection of Major Howland Roberts RA that was donated to the Natural History Museum, London, in 1926. According to Maurice Paul Evans, Historical Projects Officer of the Army Museum Ogilby Trust, Major Roberts was on St Helena from January 1884 to March 1886 (M. P. Evans, personal communication 2019); his specimen of *E. megalops* would have been collected during this time period. The *E. megalops* specimen was borrowed from the Natural History Museum, London (specimen NHMUK 013881416, DRMDNA4945), the *B. alsium* specimen from the Zoologische Staatssammlung München (ZSM, DRMDNA5209) and the remainder (*B. nubigena*, specimen RMCA ENT 000013123, DRMDNA3957; *B. rufosuffusum*, specimen RMCA ENT 000013124, DRMDNA4641; and *B. platyderoides*, specimen RMCA ENT 000013125, DRMDNA4940) from the Royal Museum for Central Africa, Tervuren, Belgium (RMCA).

For sake of simplicity of the text, and in anticipation of our conclusions, we treat all of these species throughout the text as belonging to the genus *Bembidion*. We will collectively call the bembidiine species found in the higher-elevation, forested areas of the island the 'St Helena Peaks *Bembidion*'. This name serves to distinguish them from *Bembidion (Omotaphus) mellissii* Wollaston, 1877, an apparently native but non-endemic species found at lower elevations along stream shores (Basilewsky, 1972).

To the data gathered from these four species we added sequences of 292 other species of Bembidiini and 11 outgroups, with data from Maddison (2012b), Maddison & Maruyama (2019), Bonavita *et al.* (2016) and references therein.

DNA SEQUENCING

Genes studied, and abbreviations used in this paper, are as follows: 28S, 28S ribosomal DNA (D1–D3 domains); 18S, 18S ribosomal DNA (near full-length); *ArgK*, arginine kinase; *CAD*, carbamoyl phosphate

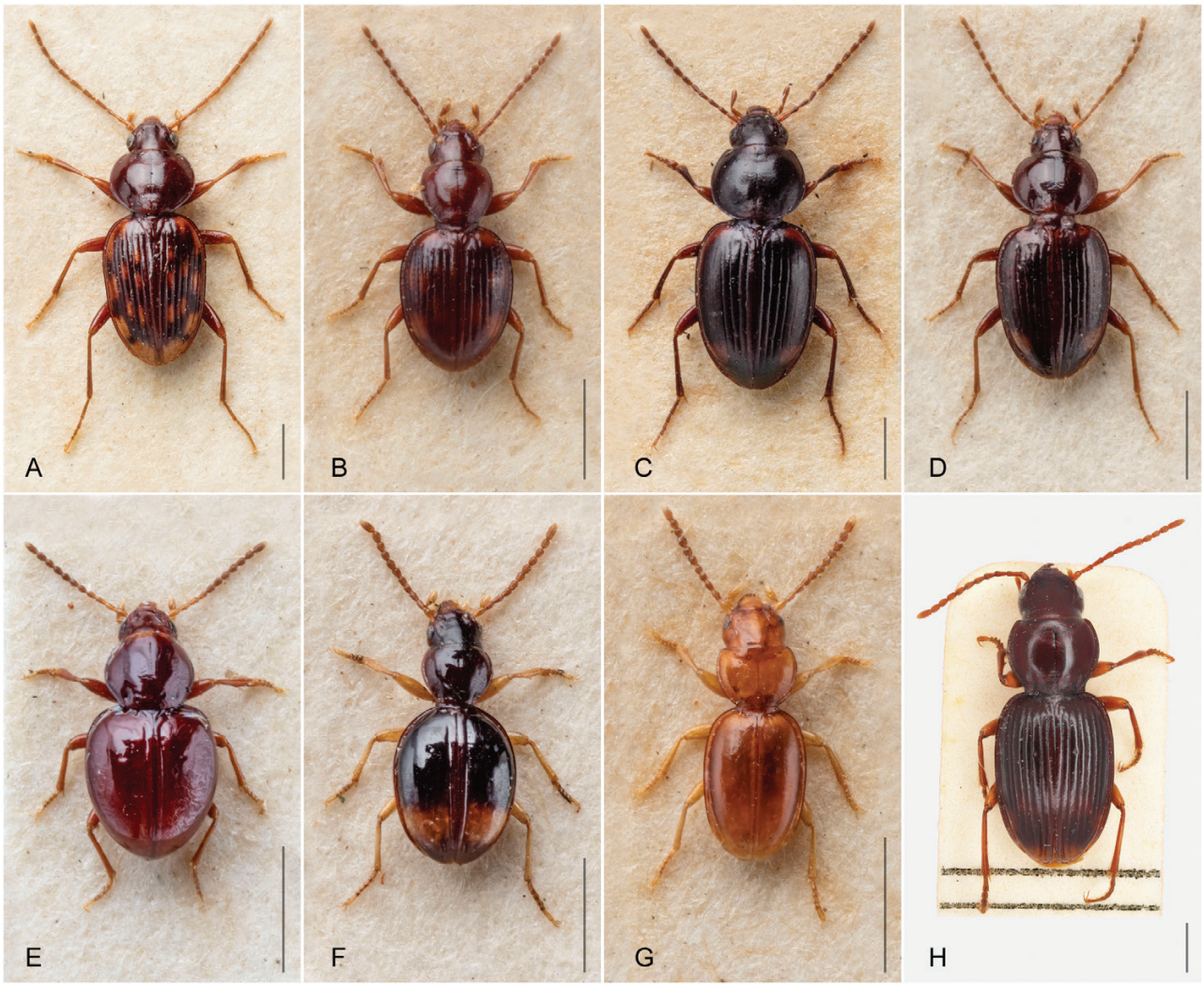

Figure 4. Eight species of St Helena Peaks *Bembidion*; these depict species that have not been sequenced. A–G, specimens collected by Wollaston, now housed in the Natural History Museum, London, and are members of the subgenus *Pseudophilochthus*. H, a member of subgenus *Apteromimus*. A, *Bembidion grayanum*. B, *Bembidion dicksoniae*. C, *Bembidion sublimbatum*. D, *Bembidion trechoides*. E, *Bembidion fossor*. F, *Bembidion gemmulipenne*. G, *Bembidion evanescens*. H, *Bembidion shepherdae*. A–G, copyright The Trustees of the Natural History Museum, London, some rights reserved, distributed under a Creative Commons Attribution 4.0 International License (CC-BY 4.0). Scale bars: 1 mm.

synthetase domain of the *rudimentary* gene; *COI*, cytochrome *c* oxidase I; *Topo*, topoisomerase I; and *wg*, *wingless*.

Details about DNA extraction and sequencing of the five dried specimens are provided by Kanda *et al.* (2015; specimen 3957) and Sproul & Maddison (2017; specimens 4641, 4940, 4945 and 5209). DNA extraction and sequencing of dried specimens followed the protocols of those papers. In brief, DNA in specimen 3957 was extracted using the Qiagen DNeasy Blood & Tissue Kit, with a single-index library prepared using a TruSeq ChIP Sample Prep Kit (Illumina), which was then sequenced on an Illumina HiSeq 2000, multiplexed on a 100 base, paired-end lane. The remaining four specimens were extracted using the Qiagen QIAmp Micro Kit (using the standard protocol with RNA carrier added), with dual-index libraries prepared using the NEBNext DNA Ultra II kit (New England BioLabs), which were then sequenced on an Illumina HiSeq 2500 (specimen 5209) or 3000 (specimens 4641, 4940 and 4945), multiplexed on either a 100 base or 150 base paired-end run. All five libraries were sequenced on separate HiSeq lanes and flow cells, except for *B. megalops* and *B. platyderoides*, which were sequenced on the same lane. No other members of the *Bembidion* complex (*sensu* Maddison, 2012) were included on any of these lanes or flow cells. The number of reads produced per specimen varied

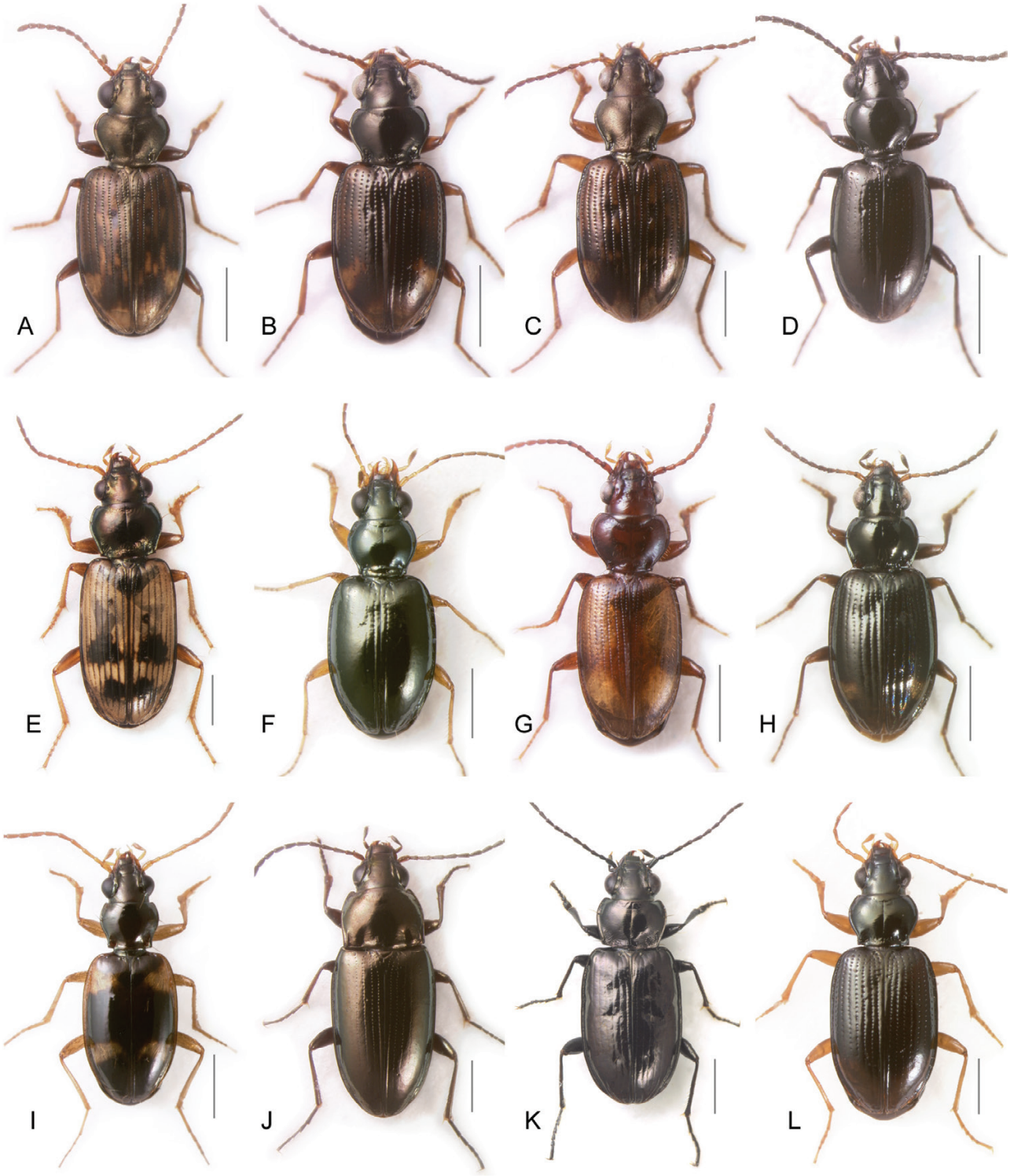

Figure 5. A sampling of *Bembidion*, showing morphologically typical representatives from around the world and across the phylogeny. A–C, members of the African subgenus *Omotaphus*. D, another member of the *Bembidion* complex. E–G, other members of the *Bembidion* series. H–L, *Bembidion* outside the *Bembidion* series. A, *Bembidion (Omotaphus) mellissii*. B, *Bembidion (Omotaphus) scotti*. C, *Bembidion (Omotaphus) gorilla*. D, *Bembidion (Bembidion) crurale*. E, *Bembidion (Notaphus) flohri*. F, *Bembidion (Ecuadion) walterrossii*. G, *Bembidion (Neobembidion) constricticolle*. H, *Bembidion (Taiwanobembidion) aliense*. I, *Bembidion (Liocosmius) darlingtonielum*. J, *Bembidion (Hydrium) nitidum*. K, *Bembidion (Plataphus) lividulum*. L, *Bembidion* (*Ocydromus* complex) *plagiatum*. Images copyright David R. Maddison 2019, released under a Creative Commons Attribution 4.0 International License (CC-BY 4.0). Scale bar: 1 mm.

from 65.6 million (specimen 3957) to 81.4 million (specimen 4945).

Assembly and recovery of genes

Reads were processed in CLC Genomics Workbench v.11.0.1. We trimmed reads to eliminate low-quality ends (limit = 0.05) and to remove adapter sequences. *De novo* assemblies were generated using CLC Genomics Workbench from paired, trimmed reads using an automatic word and bubble size, with the minimum contig length set to 200. The *de novo* assemblies were converted to BLASTable databases using NCBI's makeblastdb tool and they were BLASTed using Mesquite's (Maddison & Maddison, 2018b) local BLAST tool (with 1×10^{40} as the e-value cut-off for nuclear protein-coding genes and 1×10^{100} for *COI* and ribosomal genes, allowing up to 30 hits) using the sequences of *Asaphidion yukonense* Wickham, 1919 as query sequences. This yielded hits for all specimens for all genes except for *B. megalops* and *B. nubigena* for *ArgK*. Of the 33 remaining queries, 25 yielded a single contig as the only hit, six yielded two hits, one three hits and one eight hits. If a single contig was found, it was accepted as the valid sequence. If two or three contigs were found, and the shorter contig or contigs were fully contained within the longest and the longest was at least eight times longer, then only the longer contig was kept. If two contigs were found, and they overlapped by < 20 bases, then the two contigs were merged after alignment. The only query that could not be accommodated with these rules was 18S for *B. platyderoides*, which yielded eight contigs, all of which, when used as a query sequence to GenBank, BLASTed to *Bembidion*. 18S for *B. platyderoides* was synthesized by taking the union of all eight contigs after alignment of the entire 18S matrix. With this process complete, 40 bases were trimmed from either end of all sequences (Sproul & Maddison, 2017).

Figure 6. *Bembidion alsium*, from La Réunion. Image copyright David R. Maddison 2019, released under a Creative Commons Attribution 4.0 International License (CC-BY 4.0). Scale bar: 1 mm.

Sequence alignment

Alignment was not difficult for any of the protein-coding genes. There were no insertion or deletions (indels) evident in the sampled *ArgK*, *Topo* or *COI* sequences. In *CAD*, there was an isolated three-base insertion in *Bembidion rufosuffusum*. In *wingless*, there was a six-base insertion in three species within subgenus *Odontium*, a separate three-base insertion within the New Zealand species *Bembidion parviceps* Bates, 1878, and two separate three-base insertions within the outgroup *Typhlocharis*, in addition to a three-base insertion shared by subgenus *Omotaphus*,

Table 1. Specimens sequenced in this study

Species	Number	Repository	Locality	Collection date
Bembidion alsium	5209	ZSM	Île de La Réunion, near Cilaos, 1900 m a.s.l.	2002
Bembidion megalops	4945	NHMUK	St Helena	1884–1886
Bembidion nubigena	3957	RMCA	St Helena: Centre High Central Ridge, Cabbage Tree Road, 760 m a.s.l.	1967
Bembidion platyderoides	4940	RMCA	St Helena: Centre High Central Ridge, Cabbage Tree Road, 760 m a.s.l.	1967
Bembidion rufosuffusum	4641	RMCA	St Helena: Centre High Central Ridge, ledge SE Diana's Peak, 760 m a.s.l.	1967

The number is the D. R. Maddison DNA voucher number.

B. alsium and the St Helena species. Thus, the protein-coding genes could be aligned manually.

An alignment of 28S and 18S was performed by MAFFT v.7.130b (Katoh & Standley, 2013), using the L-INS-i search option and otherwise default parameter values.

Sites in 28S and 18S were chosen to be excluded from consideration using the modified GBLOCKS analysis present in Mesquite with the following options: minimum fraction of identical residues for a conserved position = 0.2; minimum fraction of identical residues for a highly conserved position = 0.4; counting fraction within only those taxa that have non-gaps at that position, maximum number of contiguous non-conserved positions = 4; minimum length of a block = 4; and allowed fraction of gaps within a position = 0.5.

Molecular phylogenetic analysis

Maximum likelihood analyses were conducted on each gene individually using IQ-TREE v.1.6.7.1 (Nguyen *et al.*, 2015), as orchestrated by Mesquite's Zephyr package (Maddison & Maddison, 2018a, b). The ModelFinder feature within IQ-TREE (Kalyaanamoorthy *et al.*, 2017) was used to find the optimal character evolution models. The MFP model option was used for 28S and 18S, and the TESTMERGE option was used for the protein-coding genes. The TESTMERGE option sought the optimal partition of sites, beginning with the codon positions in different parts. In addition, analyses with the concatenated data were conducted, with the TESTMERGE option also being used, beginning with each codon position for each gene as a separate part; thus, the analysis began allowing for ≤ 17 parts (three for each of the five protein-coding genes, and one for each of 28S and 18S). One hundred searches were conducted for the maximum-likelihood tree for each matrix; for bootstrap analyses, 500 replicates were used.

We used Mesquite's Clade Frequencies in Trees tools to calculate the percentage of bootstrap trees that contain a clade, and the Maximum Frequency of Contradictory Clade option to determine the percentage among the bootstrap trees of the contradictory clade with the highest frequency.

Morphological methods

Basic methods for studying adult structures, and terms used, are given by Maddison (1993). Genitalia were mounted in Euparal between two small coverslips attached to archival-quality heavyweight watercolour paper in advance of photography.

Photographs of beetles in Figures 3, 4 and 5 were taken with a Leica Z6Apo lens and DMC4500 camera, and of male genitalia with a Leica DM5500B compound microscope and DMC425C camera, with the Leica Application Suite v.4.9 software capturing each image. A stack of images from different focal positions was merged using the PMax procedure in Zerene Systems's Zerene Stacker. Photographs in Figure 4A–G were taken with a Canon MPG 65mm macro lens on a Canon 5DS R camera, with stacks shot using Zerene Stacker, and stack elements merged using Helicon Focus. The photograph of *Bembidion shepherdae* (Fig. 4H) was taken using a Laowa 25 mm F2.8 2.5–5× Ultra Macro lens on a Canon 2000D camera, using Helicon Focus v.7 and Helicon Remote v.3 for merging the elements of the photographic stack. The final images thus potentially have some artefacts caused by the algorithms used to merge the elements of the photographic stack.

RESULTS

DNA sequences

We successfully obtained sequences of most gene fragments from the pinned specimens of the five studied species (Table 2). In particular, we obtained most of the ribosomal cistron from *B. alsium*, *B. nubigena* and *B. rufosuffusum*, with the entire fragments of 28S and 18S examined from other bembidiines obtained for all five St Helena and La Réunion species. We sequenced most of the mitochondrial genome from *B. alsium*, *B. megalops* and *B. rufosuffusum*, with the entire 'barcode' region of *COI* recovered for all species except for *B. platyderoides*, for which only 572 of the 658 bases were sequenced. We obtained ≥ 671 bases of the studied *CAD* fragment from all species, ≥ 254 bases of *wingless* and ≥ 163 bases of *Topo* for all five species. We recovered *ArgK* in only three of the species (Table 2), with only 550 bases being obtained for *B. platyderoides* and the entire fragment in the other two.

Phylogenetic results

The DNA sequence data contained within the seven genes studied indicates that the St Helena Peaks *Bembidion* (*Pseudophilochthus* + *Apteromimus* + *Endosomatium*) are derived from a unique common ancestor, that their closest relative is *B. alsium* from La Réunion, that this clade of island endemics is most closely related to *Bembidion* subgenus *Omotaphus*, and that the trio is embedded in the *Bembidion* complex of subgenera within the genus *Bembidion* (Figs 7–9; Table 3).

The genes sequenced provide strong support that the St Helena Peaks *Bembidion* form a clade (Figs 7–9), with a bootstrap percentage for the concatenated matrix of 97 (Table 3). This clade is supported by

Table 2. Number of bases obtained in sequences containing the studied gene fragments

Species	28S	18S	*COI*	*CAD*	*wg*	*Topo*	*ArgK*
Bembidion alsium	11 115	11 115	13 360	2607	1872	2091	1490
Bembidion megalops	5289	4173	14 642	710*	271*	408*	0
Bembidion nubigena	13 129	13 129	4222	1371	336*	854*	0
Bembidion platyderoides	5411	3233	865*	699*	675	413*	772*
Bembidion rufosuffusum	11 747	11 747	14 988	6570	2376	2307	949

*We did not obtain the entire fragment that was studied in other species of bembidiines.

five of the seven genes (Fig. 9), with some bootstrap support provided by 28S, 18S, *CAD* and *ArgK* (Table 3). Although bootstrap support > 60% provided by each of the four genes is not high individually (with bootstrap percentages ranging from 61 to 82; Table 3), the independent signals provided by multiple linkage groups and the high bootstrap support value for the concatenated matrix clearly indicate monophyly of *Pseudophilochthus* + *Apteromimus* + *Endosomatium*.

A sister-group relationship of *B. alsium* and the St Helena Peaks *Bembidion* is also indicated by our data (Figs 7–9), with support for this relationship provided weakly by *ArgK*, moderately by 28S and *CAD* and strongly by *Topo* (Table 3). The 18S gene, *COI* and *wingless* all support alternative relationships of *B. alsium*, but there is no consistency among these three genes regarding the sister group of *B. alsium* (Fig. 9). Given the support provided by the other four genes, and bootstrap support of 100 for the concatenated matrix (Table 3), we consider a sister-group relationship of *B. alsium* and the St Helena Peaks *Bembidion* to be strongly supported.

The most decisively supported relationship is that between the St Helena plus La Réunion endemics and *Bembidion* subgenus *Omotaphus*, which is supported by all seven genes individually (Fig. 9; Table 3) and with bootstrap support of 100 from analyses of the concatenated data (Fig. 8). This result is further supported by one aspect of the *wingless* gene not considered in the maximum likelihood analyses: *B. alsium* + *Pseudophilochthus* + *Apteromimus* + *Endosomatium* + *Omotaphus* all share a three-nucleotide insertion in *wingless*, corresponding to the addition of a glutamine in the protein. This insertion is unique within at least the subfamily Trechinae.

Finally, the island endemics plus subgenus *Omotaphus* are deeply embedded in the genus *Bembidion* (Figs 7, 8). They belong to the *Bembidion* complex (*sensu* Maddison, 2012b), a clade supported by a concatenated bootstrap value of 100 (Fig. 8; Table 3). This clade contains subgenera *Bembidion* and *Cyclolopha* from the Northern Hemisphere, in addition to species from Australia, Africa and Asia (Maddison, 2012b). More broadly, they belong to a clade (with bootstrap support of 100 in the concatenated matrix) including the *Bembidion* complex, the *Furcacampa* complex, a New Zealand–Australia–Pacific Island radiation and a few small Holarctic lineages. This, in turn, is nested within the well-supported *Bembidion* series (bootstrap support of 100), and that within a large clade containing the bulk of *Bembidion* (bootstrap support of 93). There are thus four well-supported nodes that indicate that the island endemics plus *Omotaphus* belong well within the genus *Bembidion*.

DISCUSSION

The island of St Helena was formed from two coalescent shield volcanoes arising from the ocean floor, some 4 km below sea level. The island arose above the sea ~14.6 Mya (Geraldes *et al.*, 2013), with the active volcanoes altering the structure of parts of the island until ~8.5 Mya. Over the 14 Myr since its emergence, St Helena has been populated by multiple lineages of invertebrates, some of which radiated on the island, in all resulting in 469 native invertebrate species, of which 450 are endemic (Gray *et al.*, 2019). Of the endemics, 157 species are beetles, with the dominant group being the weevils (Curculionidae), with 77 endemic species, of which 57 are members of the subfamily Cossoninae (Basilewsky, 1985).

Although the bembidiines form a small portion of the beetle fauna, they are among its most distinctive elements. As Wollaston (1877: 5–6) noted:

> The *Bembidia* of St Helena are all of them most characteristic and manifestly aboriginal, forming a little geographical assemblage of the utmost interest. In point of importance, indeed, they are scarcely inferior to the members of even the Cossonideous and Anthribideous groups.

The timing of the arrival of bembidiine lineages onto St Helena is not known, but we can presume it was within the last 14 Myr. *Bembidion mellissii* (Fig. 5A), found on both Africa and St Helena, presumably arrived in relatively recent times, but the arrival time

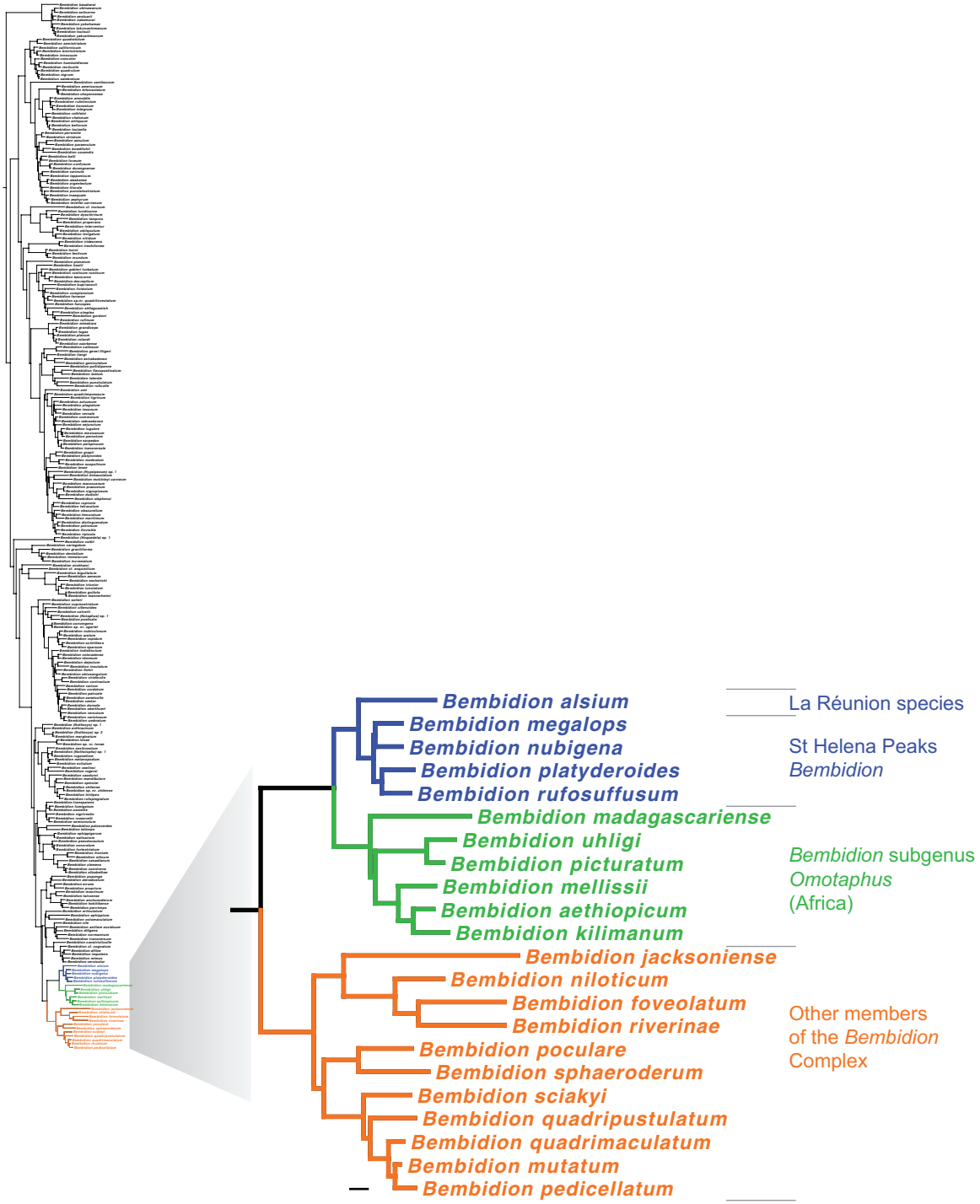

Figure 7. Maximum likelihood tree for the concatenated, seven-gene matrix. Branch length is shown proportional to relative divergence, as estimated by IQ-TREE; scale bar (to the left of *Bembidion pedicellatum* LeConte) indicates 0.01 units.

of the propagule of the St Helena Peaks *Bembidion* is not clear, although some bounds could be provided by a future analysis dating the splits of the phylogenetic tree of *Bembidion*.

An adaptive radiation on St Helena

Given the extreme diversity in overall body form (Fig. 3) and the lack of observed shared, derived morphological characters, it would not be surprising if *Apteromimus*,

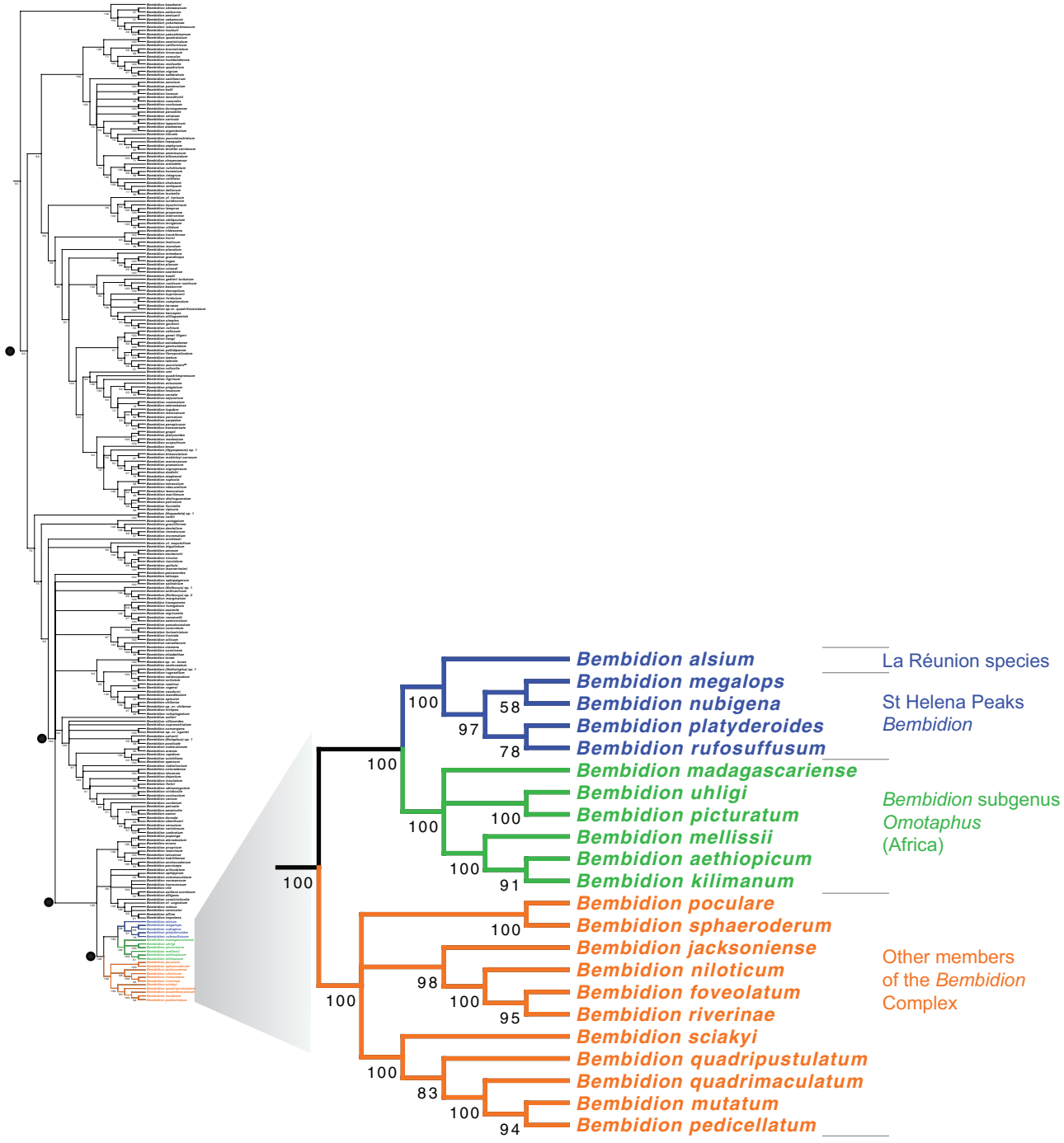

Figure 8. Majority rule consensus tree of 500 maximum likelihood bootstrap replicates for the concatenated, seven-gene matrix. Black dots indicate those branches between the root of *Bembidion* and the St Helena + *Bembidion alsium* + *Omotaphus* clade that are supported by bootstrap percentages > 90 for the concatenated matrix.

Endosomatium and *Pseudophilochthus* were unrelated, each representing a separate dispersal event onto St Helena by different lineages of bembidiines. However, *Pseudophilochthus* + *Apteromimus* + *Endosomatium* form a clade (Figs 7, 8; Table 3). A unique common ancestor of the Peaks *Bembidion* is supported by individual analyses of five of the seven genes examined and is strongly supported by the concatenated analyses (Table 3). Thus, it appears that the extraordinary diversity of body forms of St Helena Peaks *Bembidion*

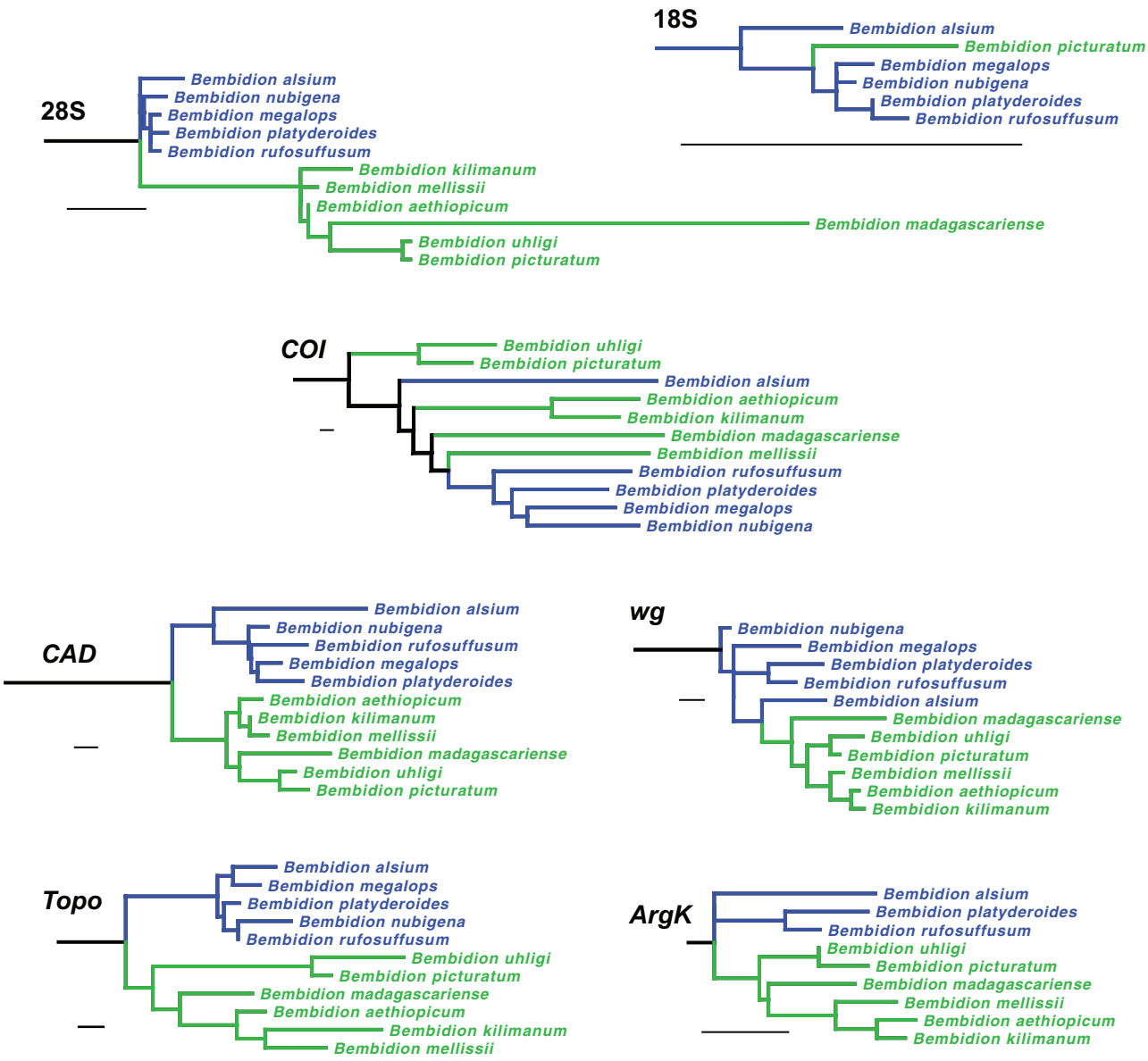

Figure 9. Maximum likelihood trees each of the seven studied genes, showing only the clade containing the subgenus *Omotaphus* (green), in addition to the species from St Helena and La Réunion (blue). Branch length is shown proportional to relative divergence, as estimated by IQ-TREE; scale bars indicate 0.01 units.

arose from a single ancestor that arrived within the last 14 Myr and diversified into the 12 endemic species known today.

In spite of their morphological disparities, the genetic divergence of the four species whose DNA was sequenced is not at all remarkable, showing no more diversity (Figs 7, 9) than within the morphologically fairly uniform subgenus, *Omotaphus* (Fig. 5A–C). This pattern of extreme phenotypic divergence but limited genetic divergence matches that in some other island radiations. For example, the plant genus *Bidens* (Asteraceae) shows more morphological diversity among the 19 species in Hawai'i than it does throughout the entirety of the Americas, and yet the total genetic diversity in Hawai'i is comparable to that found within individual species of mainland *Bidens* (Helenurm & Ganders, 1985).

The St Helena Peaks *Bembidion* appears to have undergone a classical adaptive radiation (Schluter, 2000; Gavrilets & Losos, 2009). The ecological opportunity provided by arrival on a depauperate island with few small predators, and perhaps none

Table 3. Support for various clades, measured with bootstrap support percentages from maximum likelihood phylogenetic analyses

Clade	7g	28S	18S	*COI*	*CAD*	*Topo*	*wg*	*ArgK*
St Helena Peaks *Bembidion*	97	61, –13	71, –16	43, –12	65, –20	2, –58	49, –12	82, –11
Island *Bembidion*	100	69, –13	24, –71	7, –21	86, –9	99	29, –26	19, –28
Island *Bembidion* + *Omotaphus*	100	76, –6	71, –11	6, –12	100	74, –6	72, –8	27, –7
Bembidion complex	100	1, –13	10, –48	0, –12	92	17, –15	66, –5	0, –11

'Island *Bembidion*' indicates St Helena Peaks *Bembidion* plus *Bembidion alsium*; the '*Bembidion* complex' includes those species included in that complex by Maddison (2012) plus the St Helena Peaks *Bembidion* and *B. alsium*. One or two values are given in each cell. If the bootstrap support percentage is > 90, only that value is listed. If bootstrap support is < 90%, two values are listed: the bootstrap support for the clade, followed by a negative value, which is the bootstrap support against the clade, as measured by the bootstrap value for the contradictory clade with the highest support value.

Black cells indicate bootstrap support for the clade of ≥ 90%.
Grey cells indicate bootstrap support for the clade of 50–89%.
White cells indicate that ML tree has a clade, with bootstrap support of 0–49%.
Pink cells indicate that ML tree has a contradictory clade, with bootstrap support of 0–49%.
Red cells indicate bootstrap support against the clade of 50–100%.

that consumed log-inhabiting beetle larvae, enabled the ancestral lineage of the St Helena *Bembidion* to invade niches that are unavailable to bembidiines elsewhere. Through time, the body forms of the beetles adapted to these new environments, resulting in *Bembidion* unlike any elsewhere in the world.

The first steps in this radiation might have followed the same paths as *Bembidion* in mid-elevation habitats in other low-latitude, montane regions: movement in evolutionary time away from stream shores onto the forest floor or open ground distant from water. Most species of the large subgenus *Ecuadion*, for example, are now found in cloud forest litter and high-elevation grasslands in the Andes of South America (Moret & Toledano, 2002; Maddison, 2014), having been derived most probably from a riparian ancestor. A similar transition occurred for the radiation in Hawai'i of subgenus *Nesocidium* (Liebherr, 2008) and for a mostly undescribed radiation (including *Bembidion nahuala* Erwin) in the cloud forests of southern Mexico and Guatemala (D. R. Maddison, unpublished observation). The body forms of many of these forest-inhabiting *Bembidion* have converged upon that found in *B. alsium*: a convex pronotum and hindbody, with a prothorax having rounded lateral margins and a narrow posterior margin; with sloped elytral shoulders and without full flight wings. Some of the body forms of the St Helena species [e.g. *B. (Pseudophilochthus) rufosuffusum*] are somewhat similar to those cloud forest *Bembidion* found elsewhere, whereas others could be imagined to be exaggerated versions of the same form [e.g. *B. (Pseudophilochthus) nubigena* and *B. (Pseudophilochthus) fossor*]. However, the body forms of the subgenera *Apteromimus* and *Endosomatium* are unique and unparalleled within bembidiines.

In archipelagos such as the Hawaiian Islands, island hopping and subsequent allopatric speciation can help to yield adaptive radiations with hundreds of species, as has happened in Hawai'i with *Hyposmocoma* moths (Haines *et al.*, 2014) and *Drosophila* (Magnacca & Price, 2015). However, St Helena is small (1.2% of the land area of the single island of Hawai'i) and currently has relatively simple topography. Speciation within a simple, small island suggests either low vagility of the bembidiines that would allow for allopatric or parapatric speciation over small areas (Paulay, 1994) or sympatric speciation through ecological differentiation (e.g. Schliewen *et al.*, 1994).

Relationships of the St Helena bembidiines

Our result that *B. alsium* from La Réunion is the sister group of the St Helena clade, and subgenus *Omotaphus* sister to this pair, is consistent with the internal structures of the male genitalia, which are expected to be among the most phylogenetically informative morphological character systems (Lindroth, 1963; Maddison, 2012b). For example, the sclerites in the internal sac of the aedeagus in *B. (Pseudophilochthus) nubigena* (Fig. 10C) are similar to those in subgenus *Omotaphus* (Fig. 10E, F), with a long, gently curved

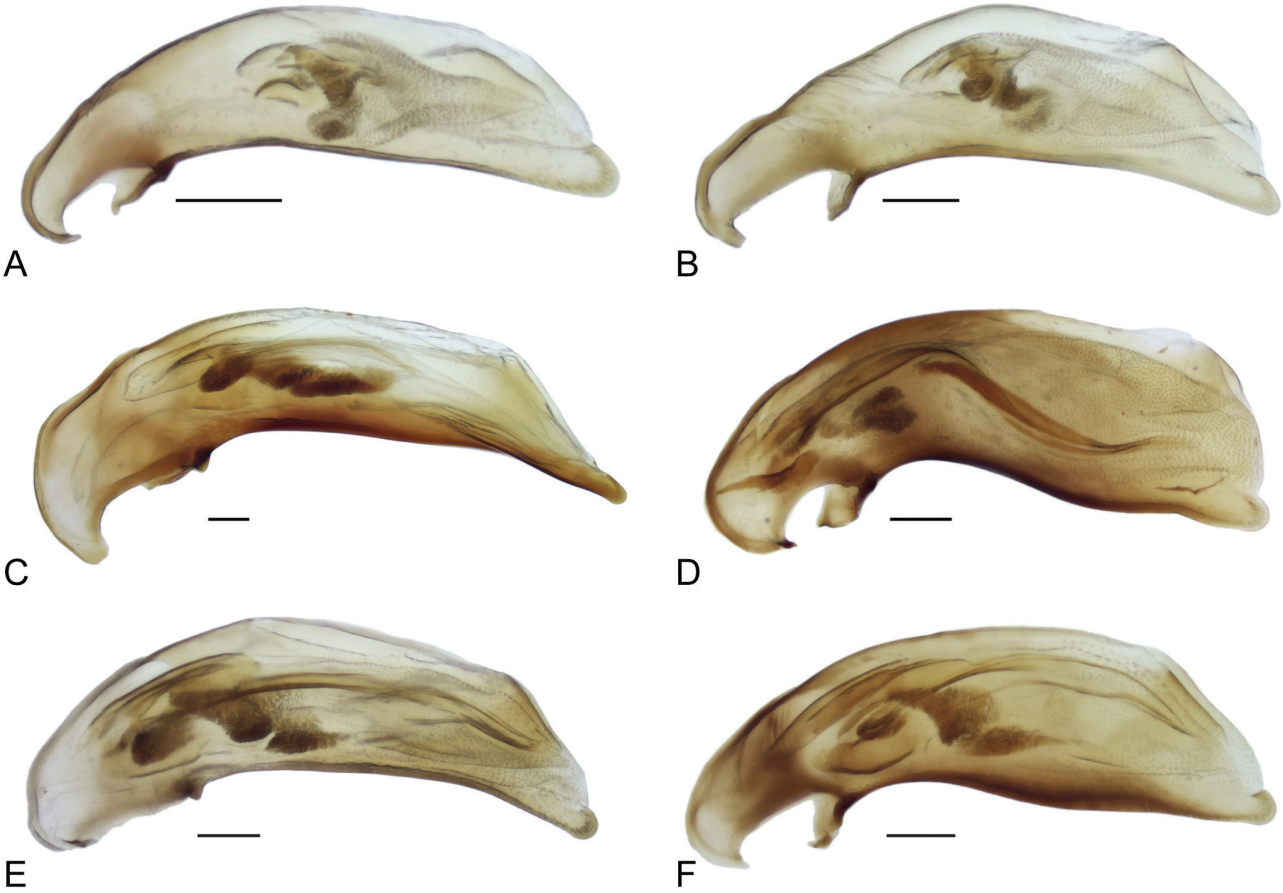

Figure 10. Male genitalia of the three species of the endemic St Helena clade (A–C), *Bembidion alsium* (D) and subgenus *Omotaphus* (D, E). A, *Bembidion rufosuffusum*. B, *Bembidion platyderoides*. C, *Bembidion nubigena*. D, *Bembidion alsium*. E, *Bembidion picturatum*. F, *Bembidion aethiopicum*. Scale bars: 0.1 mm.

flagellum; the structures in *B. alsium* (Fig. 10D) are also similar to *Omotaphus*. Pending analysis of the polarities of character state change in genitalic characters, it is reasonable to presume the genitalia of *B. nubigena* being retained in form from an *Omotaphus*-like ancestor, and those of *B. (Pseudophilochthus) rufosuffusum* and *B. (Apteromimus) platyderoides* (Fig. 10A, B) being derived from *Omotaphus*-like genitalia by reduction. External morphological characters are also consistent with a placement near *Omotaphus*, but some of the key states that are characteristic of the group of *Bembidion* including *Omotaphus* [e.g. presence of a crista clavicularis (Bonavita *et al.*, 2016)] are in a region of the body (posterior portion of the pronotum and anterior portion of the elytra) that is so highly modified in the St Helena Peaks *Bembidion* that the parts that could house those characters are extremely reduced or no longer exist. That said, there does appear to be a 'fossetta tonda' (Bonavita *et al.*, 2016) in *B. platyderoides* and *B. rufosuffusum*, and the discal elytral pores are in the middle of the third interval, as would be expected for a member of the *Bembidion* complex.

Geographical source of the endemic *Bembidion* of St Helena and La Réunion

Although there are no explicit phylogenetic studies of other endemic genera of St Helena insects, botanical phylogenetic studies indicate various sources for the flora. The older elements of the flora of St Helena come from several geographical regions, but with an African bias. St Helena has a relatively low number of plant species (60), but most are endemic, with 49 endemic species and ten endemic genera (Cronk, 1997; Ashmole & Ashmole, 2000) if *Trimeris* is considered a distinct genus or nine if it is considered within the genus *Lobelia* (Lammers, 2011). The phylogenetic studies that have been conducted of nine endemic plant genera suggest that these formed from seven founder lineages, with a mixed pattern regarding the geographical relationships of the St Helena flora (Table 4). Two of

Table 4. Endemic plant genera on St Helena that have been included in phylogenetic studies

Plant lineage	Sister group in study	Distribution of sister group	Reference
Commidendrum + *Melanodendron*	*Felicia aethiopica* (Burm.f.) Grau (Asteraceae)	South Africa	Eastwood *et al.* (2004); Karaman-Castro & Urbatsch (2009)
Lachanodes + *Pladaroxylon*	*Senecio thapsoides* DC. + *Arrh enechthites* + *Dendrocacalia* (if *Jacobaea* plastid ignored) (Asteraceae)	Greece, Australia, Pacific Islands	Pelser *et al.* (2010)
Mellissia	*Withania coagulans* (Stocks) Dunal + *Withania somnifera* (L.) Dunal (Solanaceae)	Afghanistan to India	Olmstead *et al.* (2008); Särkinen *et al.* (2013)
Nesiota	*Noltea africana* (L.) Endl. (Rhamnaceae)	South Africa	Onstein *et al.* (2015)
Nesohedyotis	Multiple possibilities (Rubiaceae)	Varied	Bremer & Eriksson (2009); Groeninckx *et al.* (2009)
Petrobium	Possibly *Oparanthus* + *Fitchia* (Asteraceae)	Marquesas, Pacific Islands	Ryding & Bremer (1992); Kimball & Crawford (2004)
Trochetiopsis	*Dombeya* (Malvaceae)	Madagascar, Africa	Nyffeler *et al.* (2005)

the lineages have South African affinities, and a third has African/Madagascan affinities, with the other four being scattered elsewhere or less certain.

The history of the St Helena bembidiines was probably similar to those plant lineages with African affinities; it is likely that their not-too-distant ancestor lived in Africa. Origin from a propagule from Africa is suggested by the observation that the sister group to the St Helena clade + *B. alsium* is the African subgenus *Omotaphus*, and the sister group to this complex includes several species from Africa, in addition to those from other, diverse geographical regions. It would be natural to presume that an ancestral African lineage of *Omotaphus*-like *Bembidion* made its way to St Helena a few million years ago, established populations along stream shores, and then diversified into the native forests. *Bembidion (Omotaphus) mellissii*, found both in Africa and on St Helena, provides a modern example of dispersal to St Helena.

However, this simple scenario does not account for the presence of *B. alsium* on La Réunion and the apparent sister-group relationship between the St Helena Peaks *Bembidion* and *B. alsium*. Although we could presume that an African lineage made its way to one of the islands (St Helena or La Réunion) and then dispersed to the other island, island species of small carabids typically become flightless and less vagile, as is common, for example, in *Bembidion* and *Mecyclothorax* on Pacific islands (Liebherr & Maddison, 2013), casting some doubt on this proposal.

The bembidiine fauna of St Helena and La Réunion might instead be relict endemics: remainders of a previously more widespread lineage that has gone extinct elsewhere (Cronk, 1997). In this scenario, an ancestral lineage (indicated by the star in Fig. 11), sister to recent *Omotaphus*, lived in Africa. Elements of that lineage independently dispersed to St Helena and La Réunion, and the populations in Africa then became extinct. La Réunion is estimated to be no older than 5 Myr (Gillot *et al.*, 1994), and thus, if the source of lineage of the island endemics has become extinct on Africa, the extinction from the mainland must have happened within the last 5 Myr. Extinctions of source lineages have been observed for other island endemics; for example, the plant genus *Lactoris* (Aristolochiaceae; APG IV, 2009), which is now found only on the Juan Fernández Islands off Chile, was previously more widespread (including, based upon fossil pollen data, South Africa; Zavada & Benson, 1987).

A third scenario that could explain the observed patterns is one in which the source of the island propagules still has descendants in Africa, but they were not sampled in our study; they could be either one of the unsampled species of *Omotaphus* or an uncollected species. We have included in our study six of the 12 known species of the recently revised subgenus *Omotaphus* (Bonavita *et al.*, 2016). Since our analysis

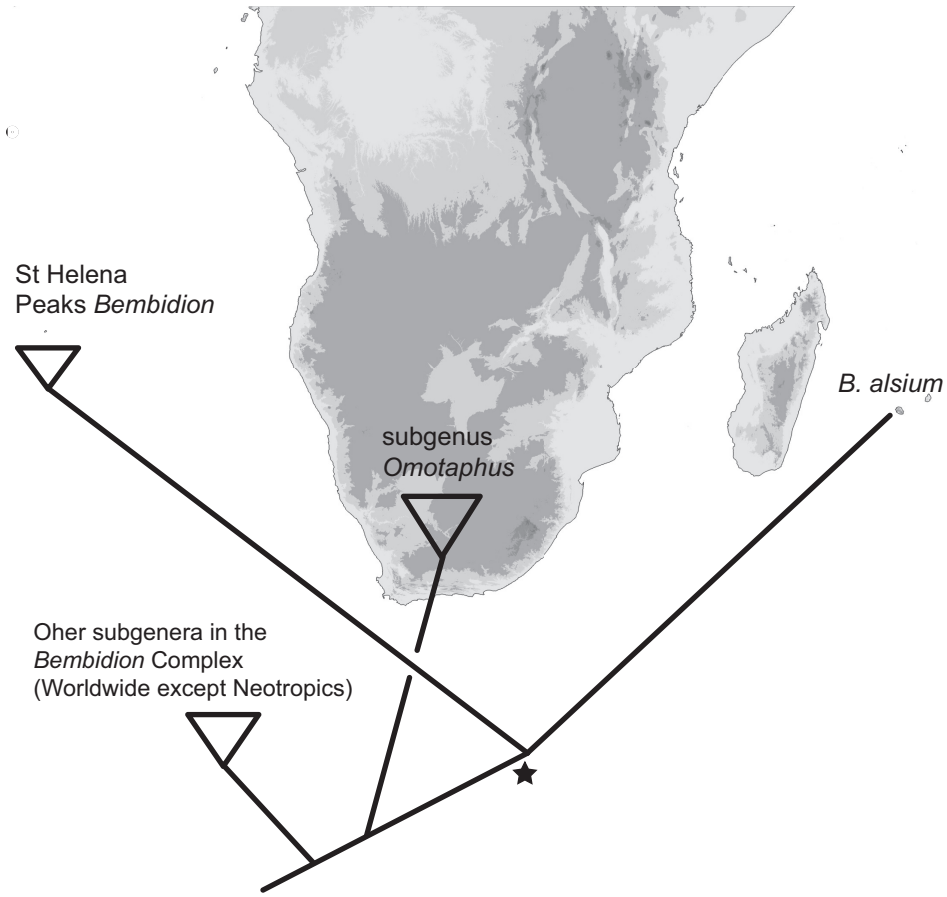

Figure 11. Map showing phylogeny of *Bembidion* related to the St Helena Peaks *Bembidion* overlaid on Africa and neighbouring areas. The dispersal of *Bembidion mellissii* from Africa to St Helena is not depicted.

was completed, we have sequenced two additional species (*Bembidion scotti* Netolitzky and *Bembidion tropicum* Chaudoir), and phylogenetic analysis indicates that they are close to other *Omotaphus* and do not represent the sister species of the island endemics. Although the other four unsampled *Omotaphus* might include the predicted lineage, we expect not, because they are all close morphologically to the sampled *Omotaphus*. There are no other known *Bembidion* complex species in sub-Saharan Africa that we have not sampled. There might be uncollected species in Africa representing the predicted lineage, but given that Africa has been much better collected than St Helena, especially in lowland, riparian areas, we see no reason to prefer that hypothesis over an extinct lineage as the source.

Are any endemic St Helena Bembidiini extinct?

It is difficult to assess Basilewsky's (1985) claim that 'extinction of all three genera is near at hand', because these beetles are small and live in cryptic places. However, it is certainly the case that the native vegetation on which they depend has been devastated since the island was discovered by the Portuguese in 1502. Early Portuguese explorers and visitors deliberately introduced animals and plants in the hope that they would establish and provide sustenance for later generations of travellers, with the most damaging early introductions probably being goats and pigs. By 1588, when the first English ship visited the island, there were literally thousands of goats, and the ship's captain (T. Cavendish) referred to flocks almost a mile long (Gosse, 1938). Goats stripped the vegetation, which in turn led to massive soil erosion.

Settlers arrived (under the aegis of the East India Company) from 1659, and the ever-increasing demands of the growing population for land for cultivation and grazing, for timber and firewood and for economic viability is well documented by Ashmole & Ashmole (2000). The result was the incremental destruction of the natural environment and extinction of species. During the 20th century, the undisturbed native habitat became confined to the steepest cliffs and the ridges of the Peaks of St Helena. Lower-elevation and

mid-level forests were virtually obliterated. Introduced plants have been as problematic as the introduced animals, with the New Zealand flax, *Phormium tenax* J.R.Forst. & G.Forst. (Asphodelaceae) playing a prominent role. The plant was introduced during the first half of the 19th century and later promoted as a valuable crop, supporting a local rope-making industry. The industry collapsed after World War II, but the introduced species naturalized and migrated upwards, reaching the Peaks and altering the native habitat in the process (Ashmole & Ashmole, 2000).

The end result of this process is the loss of many species and the reduction in population numbers of others. Of the 450 endemic species of invertebrates on St Helena, 49 are considered extinct, with many of uncertain status and many threatened (Gray *et al.*, 2019). The *Bembidion* were surely affected. In some key areas of the Peaks, the canopy and structure of the cloud forest is much more open than it used to be, leading to drier conditions at ground level, conditions likely to be detrimental to the rotting process of dead wood and therefore the *Bembidion* species. Almost certainly, *Bembidion* species we know nothing about became extinct before they were discovered, and the 12 species we do know about are likely to have suffered at least population declines.

The relative lack of *Bembidion* captured since Wollaston and Roberts's time has led to the suggestion that they are at least in decline, if not extinct (Basilewsky, 1985). The intensive Belgian expeditions in 1965–1967 (Basilewsky, 1972) failed to find five of the species [*B.* (*Endosomatium*) *megalops*, *B. (Pseudophilochthus) dicksoniae*, *B. fossor*, *B. sublimbatus* and *B. trechoides*]; two other species were found in limited numbers (Table 5). The survey conducted in 2005–2006 by H.M. and the Ashmoles yielded only a single *Bembidion* specimen at High Peak (Mendel *et al.*, 2008); this was originally reported as a new species, but subsequent investigation revealed that it is the described *B.* (*Pseudophilochthus*) *evanescens* Wollaston, 1877 (Luca Toledano, personal communication 2018). During the most recent survey (2017, 2018) by H.M. and St Helena conservation staff, no specimens were collected. The surveys since 2005 entailed hand-searching in suitable habitat (taking samples of rotten wood apart on a sheet) and pitfall traps, both methods that could have yielded *Bembidion*.

Although some of the 12 endemic *Bembidion* species on St Helena are likely to be extinct, there is some hope for others. The five species that have not been found since the late 1800s may be gone, but there is insufficient evidence about any of the species to be confident of their status. The collection of only a single specimen since 1967 is not strong evidence of their absence, because the effort to find them has not been nearly as extensive as it could be. We estimate that surveys since 1967 have totalled only ~10% of the time effort of the Belgian researchers. In addition, in all expeditions since the Belgian one, there has been a reluctance to take apart (and thereby destroy) all but a small proportion of the available rotten wood, particularly tree fern trunks. Hand-searching at night with headlamps has been conducted on only a limited basis, and that might be the method most likely to succeed in finding specimens based upon patterns of wood-dwelling Trechinae elsewhere in the world. For example, recent (February 2019) daytime collecting by D.R.M. of the Trechinae *Oopterus suavis* Broun, 1917 (which lives within rotting logs) in southern New Zealand yielded one specimen; during nighttime collecting with a headlamp over the same length of time (2 h) on the same day, 100 specimens were found on the outside of the same logs. Finally, on St Helena,

Table 5. Number of specimens collected by various researchers

Species	Wollaston (1875–1876)	Roberts (1884–1886)	Belgians (1965–1967)	Mendel et al. (2005–2017)
B. nubigena	2	11	64	
B. grayanum	14	9	5	
B. dicksoniae	17			
B. sublimbatum	8	4		
B. trechoides	12	–		
B. rufosuffusum	9	–	54	
B. fossor	1			
B. gemmulipenne	16	–	12	
B. evanescens	22	–	26	1
B. platyderoides	1	–	17	
B. shepherdae	–	–	2	
B. megalops	3	2		

Cells immediately to the left of grey areas indicate the last captures of those species.

a large proportion of the highest-quality habitat of the Peak is virtually inaccessible, on precipitous inclines, little changed since Wollaston's time in the 19th century.

The natural history of the individual St Helena Peaks *Bembidion* species is little known, but there is clearly a general association with endemic Curculionidae associated with the endemic tree fern *Dicksonia arborescens* and endemic cabbage trees (particularly black cabbage trees, *Melanodendron integrifolium* DC.). Presumably, the weevils or their larvae are the prey species. The only associations specifically mentioned by Basilewsky (1972) are between *B. (Pseudophilochthus) evanescens* and treefern weevils *Pseudomesoxenus minutissimus* Wollaston, 1877 and *Microxylobius bissectus* Wollaston, 1877, and between *Bembidion* (*Apterominus*) *wollastoni* and a cabbage tree weevil *Pseudostenocelis sculpturata* Wollaston, 1877. *Pseudomesoxenus minutissimus* and *M. bissectus* were still present on the Peaks in 2006 (Mendel *et al.*, 2008), when indeed, *B. evanescens* was last recorded. *Pseudostenocelis sculpturata* has not been recorded in recent decades. The continued presence of a number of weevil species, including those on which some *Bembidion* prey, suggests that the conditions for survival of at least some of the *Bembidion* still exist.

Of the four species of St Helena Peaks *Bembidion* sequenced, *B. megalops* is the most likely to be extinct, because it has not been found for > 120 years. If *B. megalops* or any of the other species are extinct, then they join the list of unfortunate species for which genomic studies depend upon dead specimens present in natural history museums (Huynen *et al.*, 2012; Kehlmaier *et al.*, 2017; Woods *et al.*, 2018).

Classification of St Helena and La Réunion bembidiines

Active systematic work on the bembidiines of St Helena has not been conducted since Basilewsky's (1972) study, and thus the classification of them has been static since then. Basilewsky's elevation of *Apteromimus*, *Endosomatium* and *Pseudophilochthus* to generic status follows the Jeannelian school of increasing taxonomic rank, which has not been followed outside a few countries for reasons outlined by Lindroth (1969). However, in the absence of a phylogenetic study, these three taxa are so morphologically distinctive that treating them as separate from *Bembidion* was not unreasonable. [We might note that in some online databases, e.g. the Catalogue of Life (Lorenz, 2019), *Apteromimus*, *Endosomatium* and *Pseudophilochthus* are treated as subgenera of *Bembidion*, but that is a result of following the Tree of Life Web Project's (Maddison, 2012a) classification (W. Lorenz, personal communication 2019), which itself was based on the previously unpublished results we are now presenting.]

The St Helena bembidiines are not only well nested within the large genus *Bembidion* as generally delimited in the literature, but they are also closely related to the type species of *Bembidion* (*Bembidion quadrimaculatum* Linnaeus, 1758). To maintain them as separate genera would require either shattering *Bembidion* into many pieces or having *Bembidion* as a paraphyletic assemblage. To maintain monophyly of *Bembidion*, as is preferred, we formally move the St Helena species back into the genus, where they were originally placed by Wollaston (1877), who described all but one of the species.

The one species that Wollaston did not describe, *Apteromimus wollastoni* Basilewsky, 1972, then becomes a junior homonym of *Bembidion wollastoni* (Lindberg, 1953). We propose the name *Bembidion shepherdae* Maddison as a replacement name for *Apteromimus wollastoni* Basilewsky, 1972. The specific epithet is derived from the maiden name of Wollaston's wife, Edith. Edith Wollaston (née Shepherd) was an entomologist herself; she accompanied T. Vernon Wollaston to St Helena in 1875–1876 and joined him on many of his collecting trips, later publishing on the Lepidoptera on the island (Wollaston, 1879; Machado Carrillo, 2006).

We do not place *B. alsium* in any existing subgenus. To place it in *Omotaphus* would render that subgenus paraphyletic; to make the subgenus monophyletic, it would have to be expanded to include all of the St Helena forms. This would both force all of the St Helena species into one subgenus (which would not be untoward given their lack of molecular divergence, but would be unfortunate given the vast morphological disparities within the group) and cause a change in the subgeneric name of the common African species, because the subgeneric name with priority would be one of Wollaston's subgeneric names, not *Omotaphus*. The simplest solution would be a new subgenus to house *B. alsium*, but we do not create one now, awaiting a more thorough morphological characterization of that species.

An updated classification for the bembidiines from St Helena and La Réunion is presented in Table 6.

Should we maintain the generic status of these threatened species?

In moving *Apteromimus*, *Endosomatium* and *Pseudophilochthus* back into the genus *Bembidion*, we transfer three taxa from the list of endemic genera on St Helena to the list of endemic subgenera. This should not have any effect on judgement of the importance of these species, nor efforts to conserve them, because the rank we give to these taxa in no way changes the

Table 6. Revised classification of the Bembidiini of St Helena and La Réunion

Bembidiini of St Helena
Bembidion (Omotaphus) mellissii Wollaston
B. (Pseudophilochthus) nubigena Wollaston
B. grayanum Wollaston
B. dicksoniae Wollaston
B. sublimbatum Wollaston
B. trechoides Wollaston
B. rufosuffusum Wollaston
B. fossor Wollaston
B. gemmulipenne Wollaston
B. evanescens Wollaston
B. (Apteromimus) platyderoides Wollaston
B. shepherdae Maddison
B. (Endosomatium) megalops Wollaston
Bembidiini of La Réunion
Bembidion alsium Coquerel

distinctiveness of their body forms and way of life. At whatever rank we might place them, *Apteromimus*, *Endosomatium* and *Pseudophilochthus* are unique and special within carabid beetles.

Although Stuessy *et al.* (2014) argue for maintenance of generic status for distinct island endemics even if that renders a more widespread genus paraphyletic, we reject this path for the St Helena Peaks *Bembidion* and we follow the vast majority of researchers in the systematic community in recognizing clades whenever possible as the elements of our classification and rejecting demonstrably non-monophyletic groups. We do so for the reasons well argued over the decades (summarized by Schmidt-Lebuhn, 2012), in particular, that classifications are tools for communicating and organizing knowledge and that they will function best in this capacity if they reflect the majority of information that might be discussed about the organisms, both now and in the future, and that the majority of characteristics of the genome and other levels (phenotypic and ecological) will be inherited along the branches of the evolutionary tree and throughout a clade, and the distribution of the majority of traits will be more consistent within clades than within grades.

However, we recognize that if threatened taxa of higher rank are treated as more important than taxa of lower rank, however at odds with biological reality that might be, our change might lead to a diminished sense of urgency to save these beetles. We hope that those involved with conservation efforts on St Helena would recognize the importance of the beetles based upon the distinctiveness and similarities they exhibit: they have unique body forms and ecological relationships, setting them apart from all of the rest of the > 1200 species of *Bembidion*, but based upon our sampling of genes and on other morphological characteristics, such as their genitalia, they otherwise have typical *Bembidion* genomes and phenotypes. This, in a way, makes them even more special, because recognizing by the classification that they are members of *Bembidion* highlights the rapidity of change involved in their unique adaptive radiation.

SHARED DATA

Sequences have been deposited in GenBank with accession numbers MN367919–MN367951. Aligned data and the inferred phylogenies have been deposited in Dryad (data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.n5tb2rbrh).

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REFERENCES

APG IV. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181:** 1–20.

Ashmole P, **Ashmole M. 2000.** *St Helena and Ascension Island: a natural history*. Oswestry: Anthony Nelson.

Baldwin BG, **Sanderson MJ. 1998.** Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences of the United States of America* **95:** 9402–9406.

Basilewsky P. 1972. La faune terrestre de l'Île de Sainte-Hélène (deuxième partie). II. – Insectes. 9. Coleoptera. 1. Fam. Carabidae. *Annales du Musée Royal de l'Afrique Centrale* **8:** 11–84.

Basilewsky P. 1985. The South Atlantic island of Saint Helena and the origin of its beetle fauna. In: Ball GE, ed. *Taxonomy, phylogeny and zoogeography of beetles and ants. A volume dedicated to the memory of Philip Jackson Darlington, Jr. (1904–1983)*. Dordrecht, Boston and Lancaster: Dr W. Junk, 257–275.

Bonavita P, **Toledano L**, **Maddison DR**, **Vigna Taglianti A. 2016.** Bembidiina of the Afrotropical region: *Bembidion* subgenus *Omotaphus* Netolitzky, 1914 (Coleoptera, Carabidae, Bembidiini). *Entomologische Blätter und Coleoptera* **112:** 67–106.

Bremer B, **Eriksson T. 2009.** Time tree of Rubiaceae: phylogeny and dating the family, subfamilies, and tribes. *International Journal of Plant Sciences* **170:** 766–793.

Cronk QCB. 1989. The past and present vegetation of St Helena. *Journal of Biogeography* **16:** 47–64.

Cronk QCB. 1997. Islands: stability, diversity, conservation. *Biodiversity and Conservation* **6:** 477–493.

Desender K, **Maelfait JP. 1989.** Contribution to the knowledge of the Carabid beetles of Galápagos (Ecuador). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie* **58:** 55–65.

Eastwood A, **Gibby M**, **Cronk QCB. 2004.** Evolution of St Helena arborescent Astereae (Asteraceae): relationships of the genera *Commidendrum* and *Melanodendron*. *Botanical Journal of the Linnean Society* **144:** 69–83.

Gavrilets S, **Losos JB. 2009.** Adaptive radiation: contrasting theory with data. *Science* **323:** 732–737.

Geraldes MC, **Motoki A**, **Costa A**, **Mota CE**, **Mohriak WU. 2013.** Geochronology (Ar/Ar and K-Ar) of the South Atlantic post-break-up magmatism. In: Mohriak WU, Danforth A, Post PJ, Brown DE, Tari GC, Nemčok M, Sinha ST, eds. *Conjugate divergent margins*. London: Geological Society, 41–74.

Gillot P-Y, **Lefèvre J-C**, **Nativel P-E. 1994.** Model for the structural evolution of the volcanoes of Réunion Island. *Earth and Planetary Science Letters* **122:** 291–302.

Gosse P. 1938. *St. Helena 1502–1938*. London: Cassell.

Gray A, **Wilkins V**, **Pryce D**, **Fowler L**, **Key RS**, **Mendel H**, **Jervois M**, **Hochkirch A**, **Cairns-Wicks R**, **Dutton A-J**, **Malan L. 2019.** The status of the invertebrate fauna on the South Atlantic island of St Helena: problems, analysis, and recommendations. *Biodiversity and Conservation* **28:** 275–296.

Groeninckx I, **Dessein S**, **Ochoterena H**, **Persson C**, **Motley TJ**, **Kårehed J**, **Bremer B**, **Huysmans S**, **Smets E. 2009.** Phylogeny of the herbaceous tribe Spermacoceae (Rubiaceae) based on plastid DNA data. *Annals of the Missouri Botanical Garden* **96:** 109–132.

Haines WP, **Schmitz P**, **Rubinoff D. 2014.** Ancient diversification of *Hyposmocoma* moths in Hawaii. *Nature Communications* **5:** 3502.

Helenurm K, **Ganders FR. 1985.** Adaptive radiation and genetic differentiation in Hawaiian *Bidens*. *Evolution; International Journal of Organic Evolution* **39:** 753–765.

Huynen L, **Millar CD**, **Lambert DM. 2012.** Resurrecting ancient animal genomes: the extinct moa and more. *Bioessays: News and Reviews in Molecular, Cellular and Developmental Biology* **34:** 661–669.

Kalyaanamoorthy S, **Minh BQ**, **Wong TKF**, **von Haeseler A**, **Jermiin LS. 2017.** ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14:** 587–589.

Kanda K, **Pflug JM**, **Sproul JS**, **Dasenko MA**, **Maddison DR. 2015.** Successful recovery of nuclear protein-coding genes from small insects in museums using Illumina sequencing. *PLoS ONE* **10:** e0143929.

Karaman-Castro V, **Urbatsch LE. 2009.** Phylogeny of *Hinterhubera* group and related genera (Hinterhuberinae: Astereae) based on the nrDNA ITS and ETS sequences. *Systematic Botany* **34:** 805–817.

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

Kehlmaier C, **Barlow A**, **Hastings AK**, **Vamberger M**, **Paijmans JLA**, **Steadman DW**, **Albury NA**, **Franz R**, **Hofreiter M**, **Fritz U. 2017.** Tropical ancient DNA reveals relationships of the extinct Bahamian giant tortoise *Chelonoidis alburyorum*. *Proceedings of the Royal Society B: Biological Sciences* **284:** 20162235.

Kimball RT, **Crawford DJ. 2004.** Phylogeny of Coreopsideae (Asteraceae) using ITS sequences suggests lability in reproductive characters. *Molecular Phylogenetics and Evolution* **33:** 127–139.

Lammers TG. 2011. Revision of the infrageneric classification of *Lobelia* L. (Campanulaceae: Lobelioideae). *Annals of the Missouri Botanical Garden* **98:** 37–62.

Liebherr JK. 2008. Taxonomic revision of Hawaiian *Bembidion* Latreille (Coleoptera: Carabidae: Bembidiini) with a discussion of their reductive and derivative evolutionary specializations. *Annals of Carnegie Museum* **77:** 31–78.

Liebherr JK, **Maddison DR. 2013.** Colonisation of the Pacific by *Bembidion* beetles (Coleoptera: Carabidae), with description of *Bembidion tahitiense*, sp. nov. from Tahiti, French Polynesia. *Invertebrate Systematics* **27:** 439–449.

Lindberg H. 1953. Zweiter Beitrag zur Kenntnis der Käferfauna der Kanarischen Inseln. *Commentationes Biologicae* **13:** 1–18.

Lindroth CH. 1963. The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska. Part 3. *Opuscula Entomologica Supplementum* **XXIV:** 201–408.

Lindroth CH. 1969. The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska. Part 1. *Opuscula Entomologica Supplementum* **XXXV:** i–xlviii.

Lorenz W. 2005. *Systematic list of extant ground beetles of the world (Insecta Coleoptera "Geadephaga": Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae), 2nd edn*. Tutzing: Published by the author.

Lorenz W. 2019. CarabCat: global database of ground beetles (version Oct 2017). In: Roskov Y, Ower G, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, Van Nieukerken E, Zarucchi J, Penev L, eds. *Species 2000 & ITIS Catalogue of Life, 2019 annual checklist*. Leiden: Naturalis. ISSN 2405-884X. Available at: www.catalogueoflife.org/annual-checklist/2019.

Machado A. 1992. *Monografia de los carabidos de las Islas Canarias (Insecta, Coleoptera)*. La Laguna: Instituto de Estudios Canarios.

Machado Carrillo A. 2006. *T. Vernon Wollaston (1822–1878) un entomólogo en la Macaronesia*. Madrid: Fundación César Manrique.

Maddison DR. 1993. Systematics of the Holarctic beetle subgenus *Bracteon* and related *Bembidion* (Coleoptera: Carabidae). *Bulletin of the Museum of Comparative Zoology* **153:** 143–299.

Maddison DR. 2012a. *Bembidion* Latreille 1802. Version 06 January 2012. In: Maddison DR, Schulz K, eds. *The tree of life web project*. Available at: http://tolweb.org/Bembidion

Maddison DR. 2012b. Phylogeny of *Bembidion* and related ground beetles (Coleoptera: Carabidae: Trechinae: Bembidiini: Bembidiina). *Molecular Phylogenetics and Evolution* **63:** 533–576.

Maddison DR. 2014. An unexpected clade of South American ground beetles (Coleoptera, Carabidae, *Bembidion*). *Zookeys* **416:** 113–155.

Maddison DR, Maddison WP. 2018a. *Zephyr: a Mesquite package for interacting with external phylogeny inference programs, Version 3.0*. Available at: http://zephyr.mesquiteproject.org

Maddison WP, Maddison DR. 2018b. *Mesquite: a modular system for evolutionary analysis, version 3.51*. Available at: http://mesquiteproject.org

Maddison DR, Maruyama M. 2019. Phylogenetic relationships and convergent evolution of ocean-shore ground beetles (Coleoptera: Carabidae: Trechinae: *Bembidion* and relatives). *Systematic Entomology* **44:** 39–60.

Magnacca KN, Price DK. 2015. Rapid adaptive radiation and host plant conservation in the Hawaiian picture wing *Drosophila* (Diptera: Drosophilidae). *Molecular Phylogenetics and Evolution* **92:** 226–242.

Mendel H, Ashmole P, Ashmole M. 2008. *Invertebrates of the Central Peaks and Peak Dale, St Helena*. Jamestown, St Helena: St Helena National Trust, Overseas Territories Environment Programme.

Moret P, Toledano L. 2002. *Ecuadion*, nouveau sous-genre de *Bembidion* Latreille, 1802 du páramo équatorien (Coleoptera, Carabidae, Bembidiini). *Bollettino del Museo Civico di Storia Naturale di Venezia* **53:** 155–205.

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.

Nyffeler R, Bayer C, Alverson WS, Yen A, Whitlock BA, Chase MW, Baum DA 2005. Phylogenetic analysis of the Malvadendrina clade (Malvaceae s.l.) based on plastid DNA sequences. *Organisms Diversity & Evolution* **5:** 109–123.

Olmstead RG, Bohs L, Migid HA, Santiago-Valentin E, Garcia VF, Collier SM. 2008. A molecular phylogeny of the Solanaceae. *Taxon* **57:** 1159–1181.

Onstein RE, Carter RJ, Xing Y, Richardson JE, Linder HP. 2015. Do Mediterranean-type ecosystems have a common history?—Insights from the Buckthorn family (Rhamnaceae). *Evolution; International Journal of Organic Evolution* **69:** 756–771.

Paulay G. 1994. Biodiversity on oceanic islands: its origin and extinction. *American Zoologist* **34:** 134–144.

Pelser PB, Kennedy AH, Tepe EJ, Shidler JB, Nordenstam B, Kadereit JW, Watson LE. 2010. Patterns and causes of incongruence between plastid and nuclear Senecioneae (Asteraceae) phylogenies. *American Journal of Botany* **97:** 856–873.

Román-Palacios C, Wiens JJ. 2018. The tortoise and the finch: testing for island effects on diversification using two iconic Galápagos radiations. *Journal of Biogeography* **45:** 1701–1712.

Ryding O, Bremer K. 1992. Phylogeny, distribution, and classification of the Coreopsideae (Asteraceae). *Systematic Botany* **17:** 649–659.

Särkinen T, Bohs L, Olmstead RG, Knapp S. 2013. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): a dated 1000-tip tree. *BMC Evolutionary Biology* **13:** 214.

Schliewen UK, Tautz D, Pääbo S. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature* **368:** 629–632.

Schluter D. 2000. *The ecology of adaptive radiation*. New York: Oxford University Press.

Schmidt-Lebuhn AN. 2012. Fallacies and false premises—a critical assessment of the arguments for the recognition of paraphyletic taxa in botany. *Cladistics* **28:** 174–187.

Sproul JS, Maddison DR. 2017. Sequencing historical specimens: successful preparation of small specimens with low amounts of degraded DNA. *Molecular Ecology Resources* **17:** 1183–1201.

Stuessy TF, Konig C, Sepulveda PL. 2014. Paraphyly and endemic genera of oceanic islands: implications for conservation. *Annals of the Missouri Botanical Garden* **100:** 50–78.

Wollaston TV. 1877. *Coleoptera Sanctae-Helenae*. London: John van Voorst.

Wollaston TV. [Mrs Edith] 1879. Notes on the Lepidoptera of St. Helena, with descriptions of new species. *Annals and Magazine of Natural History (5th Series)* **3:** 219–233, 329–343, 415–441.

Woods R, Turvey ST, Brace S, MacPhee RDE, Barnes I. 2018. Ancient DNA of the extinct Jamaican monkey *Xenothrix* reveals extreme insular change within a morphologically conservative radiation. *Proceedings of the National Academy of Sciences of the United States of America* **115:** 12769–12774.

Zavada MS, Benson JM. 1987. First fossil evidence for the primitive angiosperm family Lactoridaceae. *American Journal of Botany* **74:** 1590–1594.