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Cryptic species, biogeographic complexity and the evolutionary history of the *Ectemnorhinus* group in the sub-Antarctic, including a description of *Bothrometopus huntleyi*, n. sp.

G.C. GROBLER[1], A.D.S. BASTOS[1], A.M. TREASURE[2] and S.L. CHOWN[2]*

[1]*Department of Zoology and Entomology, University of Pretoria, Pretoria 0001, South Africa*
[2]*Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*
**Corresponding author: slchown@sun.ac.za*

Abstract: The biogeography of the South Indian Ocean Province (SIP) biotas has long been controversial. Much of the discussion has been based on interpretation of species distributions, based on morphological or anatomical delimitations. However, molecular phylogenetic approaches elsewhere have recently shown that interpretations based solely on morphological data may be misleading. Nonetheless, few studies have employed molecular phylogenetic approaches to understand the biogeography of the SIP biotas. We do so here for the *Ectemnorhinus* group of genera, a monophyletic unit of weevils endemic to the region. We use mitochondrial cytochrome oxidase I DNA sequence data to reconstruct relationships among 13 species and 22 populations in the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus*. On the basis of this analysis we find little support for separating the genus *Palirhoeus* from *Bothrometopus*, and little support for the morphologically-based species groups currently recognized within *Bothrometopus*. Using a molecular clock we show that dispersal among islands probably took place against the prevailing wind direction. These data also support a previous hypothesis of radiation of the epilithic genera *Bothrometopus* and *Palirhoeus* during the Pliocene/early Pleistocene, but reject the hypothesis that the genus *Ectemnorhinus* radiated following the last glacial maximum. We show that *Bothrometopus parvulus* (C.O. Waterhouse) on the Prince Edward Islands comprises two species that are not sister taxa. We name the second species *Bothrometopus huntleyi* n. sp. and provide a description thereof.

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Introduction

The evolutionary history and biogeography of the sub-Antarctic islands have long been the topics of both interest and controversy. Since the biotas of the region were first described in the 1800s, many hypotheses have been proposed concerning the origins thereof and the biogeographic relationships among the various islands in the region (e.g. Gressitt 1970, Chown 1990, 1994, Michaux & Leschen 2005, Van der Putten *et al*. 2010). More generally, the geological history of the Kerguelen Plateau and the role it might have played in influencing distributions among the continents has also featured prominently in debates about the biogeographic history of the Southern Hemisphere.

Much of the discussion of sub-Antarctic biogeography has, to date, centred on assessments of species distributions based primarily on either phylogenetic analyses or presence/absence data using morphological or anatomical species delimitations (e.g. Gressitt 1970, Kuschel & Chown 1995). Indeed, even the most recent assessments, though clearly providing modern geological interpretations and contexts (e.g. Craig *et al*. 2003, Michaux & Leschen 2005, Van der Putten *et al*. 2010) still rely heavily on such approaches. Whilst these works have provided a range of important insights (Chown *et al*. 1998, Craig *et al*. 2003) they are also limited, and modern, molecular approaches have shown how misleading interpretations, founded solely on morphologically-based distributional data, may be. In particular, they have demonstrated that dispersal across the Southern Hemisphere has been much more common than previously thought (e.g. De Queiroz 2005). In addition to providing a means for dating significant biogeographic events, molecular studies also bring additional data to bear on hypotheses of relationships among taxa and areas. Such information is particularly useful where analyses of morphological variation might be confounded by cryptic species or substantial environmental influences (see De Wever *et al*. 2009, Torricelli *et al*. 2010).

Despite the benefits that molecular approaches bring to investigations of biogeography and evolutionary history of any region and its biota, few such investigations have focussed on terrestrial taxa. The most common investigations are those of relationships among marine species and

populations across the region (Thornhill *et al*. 2008, Fraser *et al*. 2009, Wilson *et al*. 2009), and for terrestrial groups among plant taxa from New Zealand and its sub-Antarctic islands (see Michaux & Leschen 2005). Several studies have also sought to explore the phylogeography of particular species typically on a single island or archipelago (Grobler *et al*. 2006, Myburgh *et al*. 2007, McGaughran *et al*. 2010a) or relationships among populations or species on the Antarctic Peninsula and Scotia Arc islands (Allegrucci *et al*. 2006, McGaughran *et al*. 2010b). By contrast, investigations of terrestrial taxa across one or more sub-Antarctic archipelagos are limited to springtails (Stevens *et al*. 2006), ameronothroid mites (Mortimer *et al*. 2010), and the Antarctic hair grass (Van de Wouw *et al*. 2007). This situation is particularly concerning given the considerable change in perspective on the evolution and biogeography of both Antarctic and sub-Antarctic groups that has resulted from molecular approaches (reviewed in Chown & Convey 2007), and the controversy surrounding the origins of many of the groups endemic to the sub-Antarctic islands (Jeannel 1964, Chown 1994, Van der Putten *et al*. 2010).

Such controversy about origins and species relationships has been a feature of investigations of the *Ectemnorhinus* group of genera, a monophyletic unit of weevils (Kuschel & Chown 1995) restricted to the South Indian Ocean Province (or Kerguelen Biogeographic Province) of the sub-Antarctic (reviewed in Chown 1992, 1994). Although the group is small by comparison with other taxa in the Curculionidae, it is one of the most speciose monophyletic taxa in the South Indian Ocean Province (Chown 1989), providing an ideal group with which to investigate biogeographic hypotheses in the region. Thus, we provide an analysis of phylogenetic relationships among species from the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus*, based on the material available from Heard Island in the east to the Prince Edward Islands in the west. Whilst this study does not comprise a complete analysis of the six genera and 36 species of the group (= Ectemnorhinini (Kuschel & Chown 1995, Alonso-Zarazaga & Lyal 1999, Grobler *et al*. 2006)), it does provide a strong argument for reconsideration of the species in the group and its evolution, and, as a consequence the need for additional molecular-based investigations of taxa endemic to the sub-Antarctic.

Materials and methods

Study animals and sites

The *Ectemnorhinus* group of genera (Kuschel & Chown 1995) is confined to the South Indian Ocean Province Islands, and is thought to be most closely related to the genera *Oclandius* and *Heterexis* from the New Zealand sub-Antarctic islands (Kuschel & Chown 1995). The systematics of the group has been controversial, especially the status of species within the genera, the genera that are valid, and the evolutionary and biogeographic relationships among these taxa (Kuschel 1971, Dreux & Voisin 1987, 1989, Kuschel & Chown 1995). All of this work has been based on morphological assignments of individuals to species and subsequent assessments of the ecological characteristics and geographic distributions of these species (reviewed in Chown 1994). However, the systematic complexity of the group given its morphological variability suggests that interpretations of the systematic, biogeography and evolutionary history of the group would benefit considerably from, and likely be substantially altered by, the inclusion of molecular data.

One recent approach of this kind has shown that this is indeed the case, demonstrating that the genus *Ectemnorhinus* on the Prince Edward Islands does indeed comprise two species, though not as originally envisaged (cf. Kuschel 1971). *Ectemnorhinus similis* (= *E. marioni* junior synonym) is found on both islands, whereas *E. kuscheli* Grobler *et al*. is found on Prince Edward Island only (Grobler *et al*. 2006). Such complexity is perhaps not unexpected given the extent of variation within the genus *Ectemnorhinus*, and the intricacy of the ecological situation on the Prince Edward Islands, where individuals of the genus *Ectemnorhinus* are a preferred prey item of introduced house mice present on Marion, but not on Prince Edward Island (Chown & Smith 1993). However, both a revision of the *Bothrometopus* species on Possession Island (Chown & Kuschel 1994) and a recent assessment of the phylogeography of the species found on the Prince Edward Islands (Grobler *et al*. 2006, 2011) suggested that cryptic species and complicated evolutionary relationships may also be a feature of other genera in the *Ectemnorhinus* group. We explore this question here.

The geological and glacial histories of the South Indian Ocean Province islands have been summarized (e.g. Hall 2002, Boelhouwers *et al*. 2008, Van der Putten *et al*. 2010) and their contemporary climatic characteristics (generally cool and oceanic) and nature of their ecosystems have also been reviewed in a range of studies (e.g. Chown *et al*. 1998). The islands vary in age from 0.5 million years (m.y.) for Marion Island to *c*. 40 m.y. for the Kerguelen archipelago, with substantial variation within archipelagos in terms of age, history and extent of glaciation. Perhaps the most enigmatic of the groups in terms of its biogeography is the Crozet archipelago (Jeannel 1964, Chown 1994, Van der Putten *et al*. 2010), owing to a complex geological history.

Taxon sampling, genetic characterization and phylogenetic analysis

For this study we focussed on the genera *Palirhoeus* Kuschel, *Bothrometopus* Jeannel, and *Ectemnorhinus* G.R. Waterhouse. Whilst material of the genera *Canonopsis* C.O. Waterhouse and *Christensenia* Brinck were available, we were unable to obtain DNA in condition that was suitable for sequencing. We obtained sequence data from approximately half of the total number of species in the three

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Table I. Summary of the sampling localities from which the genetically characterized specimens included in this study were collected.

Species	Sampling locality (a.s.l.)	Geographic coordinates		Number of specimens per locality
B. parvulus	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	5
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	Goodhope Bay MI (0 m)	46°57'55.9"S	37°42'04.4"E	2
	Cape Davis MI (0 m)	46°49'41.2"S	37°41'83.3"E	3
	Kildalkey Bay MI (0 m)	46°57'38.3"S	37°51'22.2"E	3
	McAll Coast PEI (0 m)	NA		3
B. randi	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	1
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	Water Tunnel MI (0 m)	46°57'49.2"S	37°44'50.44"E	1
	Goodhope Bay MI (0 m)	46°57'55.9"S	37°42'04.4"E	1
	Long Ridge South MI (450 m)	46°52'45"S	37°47'00"E	1
	Katedraalkrans MI (800 m)	46°53'89.6"S	37°46'48.2"E	1
	Tafelberg MI (250 m)	46°53'03.5"S	37°48'20.1"E	1
	Feldmark Plateau MI (600 m)	46°56'35"S	37°46'10"E	1
	Pyroxene Kop MI (600 m)	46°56'43.4"S	37°41'40.5"E	2
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	1
B. huntleyi	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	2
	Kildalkey Bay MI (0 m)	46°57'38.3"S	37°51'22.2"E	1
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	First Red Hill MI (400 m)	46°53'41.2"S	37°48'21"E	2
	Junior's Kop MI (200 m)	46°52'79.4"S	37°50'08.3"E	1
	Feldmark Plateau MI (600 m)	46°56'35"S	37°46'10"E	1
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	4
	PEI (400 m)	46°38'21.1"S	37°57'48.2"E	1
	Top of VZB PEI (672 m)	46°37'59"S	37°55'89.1"E	1
B. elongatus	Tafelberg MI (250 m)	46°53'03.5"S	37°48'20.1"E	1
	Stony Ridge MI (150 m)	46°54'88.1"S	37°51'48.4"E	1
	Long Ridge South MI (450 m)	46°52'45"S	37°47'00"E	1
	PEI (600 m)	46°37'53.3"S	37°55'98.5"E	2
B. fasciatus	Possession Island*	46°25'33.9"S	51°51'38.2"E	2
B. gracilipes	Heard Island*	53°01'09.4"S	73°23'30.5"E	2
B. angusticollis	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	2
B. sulcatus	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	5
B. brevis	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	7
	Heard Island*	53°01'09.4"S	73°23'30.5"E	2
E. similis	Junior's Kop MI (200 m)	46°52'79.4"S	37°50'08.3"E	1
	Ice Plateau MI (1000 m)	46°54'29"S	37°45'37.5"E	1
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	2
E. kuscheli	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	1
	PEI (400 m)	46°38'21.1"S	37°57'48.2"E	1
E. viridis	Heard Island*	53°01'09.4"S	73°23'30.5"E	2
	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	1
P. eatoni	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	3
	Kildalkey Bay MI (0 m)	46°57'38.3"S	37°51'22.2"E	1
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	2
	Heard Island*	53°01'09.4"S	73°23'30.5"E	1

VZB = Van Zinderen Bakker Peak, * = geographic coordinates given for the scientific stations on Kerguelen and Possession Islands, and for Atlas Cove on Heard Island.

genera and what we thought initially was 12 species and 20 populations representing all of the major archipelagos, but which following analysis turned out to be 13 species from 22 populations (Table I). The most comprehensive sampling was undertaken on the most readily accessible Prince Edward Islands (see also Grobler *et al*. 2006, 2011). For an outgroup, we used two COI gene sequences from *Sitona discoideus* (Curculionidae: Etiminae; Genbank accession numbers EF118292 and EF118299) from Norfolk Island, Australia (Vink & Phillips 2007).

DNA from each individual was extracted from a leg which, following removal from ethanol was washed and rehydrated in distilled water for ten minutes prior to being frozen in liquid nitrogen and ground in individual Eppendorf tubes using an Eppendorf pestle. DNA was extracted using the High Pure PCR Template Preparation

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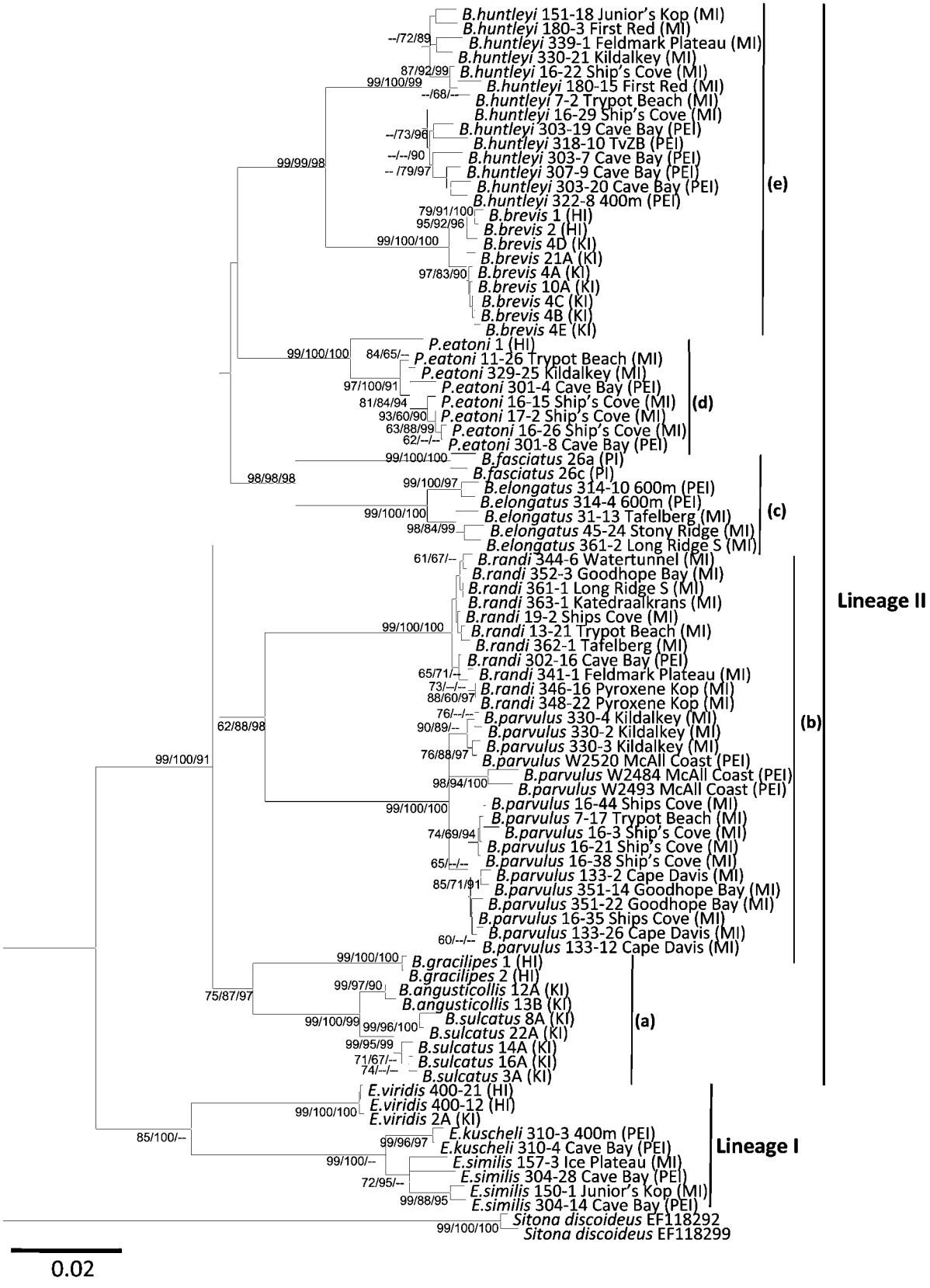

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Kit (Roche Applied Science) using the supplier's procedure for isolation of nucleic acids from mammalian tissue with modification to the proteinase K tissue lysis incubation step which was performed for 24 h instead of the recommended 1 h for mammalian tissue.

Taxon-specific COI primers, GF5-1940 and GR5-2935 (Grobler *et al*. 2006), were used to amplify a 996 bp PCR product under previously described reaction conditions (Grobler *et al*. 2006) using a thermal cycling profile comprising an initial denaturation step at 94°C for 90 s, followed by 40 cycles of 94°C for 22 s, 46°C for 30 s and 72°C for 1 min and concluding with a final extension step of 1 min at 72°C. PCR products of the correct size were purified directly from the tube using a Roche High Pure PCR Product Purification Kit. DNA sequences were determined by automated cycle sequencing reactions run on an ABI PRISM^{TM} 3100 Analyser and generated using the ABI PRISM Big Dye^{TM} Terminator V3.0 sequencing standard (Applied Biosystems). The sequences were viewed, edited and aligned using the alignment explorer function incorporated within the MEGA4 programme (Tamura *et al*. 2007).

Neighbour-Joining (NJ) and Minimum Evolution (ME) algorithms in MEGA4 (Tamura *et al*. 2007) were used to construct distance trees. Bayesian inference (BI) using MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) was performed with the model and parameters estimated in jModelTest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) under the Akaike Information Criterion (AIC). The analysis was initiated with random starting trees with four parallel runs for 10 000 000 generations using one cold and three heated Markov chains using the default heating setting. The Markov chains were sampled every 1000 generations. Tracer plots were visually inspected and tracer diagnostics (standard deviation of split frequencies, effective sample size), as implemented in MrBayes and Tracer v1.4 (Drummond & Rambaut 2007) were checked to ensure that the Markov chain had reached stationarity. Of the 10 000 trees obtained 2000 were discarded as "burn-in" and the trees were summarized using an 'all-compatible' consensus. Maximum parsimony (MP) analyses were performed in PAUP* (Swofford 2003). Starting trees were obtained by closest stepwise addition and heuristic searches were performed using the tree-bisection reconnection (TBR) branch swapping algorithm. Characters were unordered and assigned equal weights in the initial analysis, and subsequently reweighted using the rescaled consistency (RC) index as detailed previously by Farris (1969). Nodal support was assessed by 100 bootstrap replicates.

Haplotype (h) and nucleotide diversities (π) were estimated in DNASP 5.00.07 (Librado & Rozas 2009). To obtain more accurate divergence estimates for the older splits, the standard 2.3% nucleotide sequence divergence per million years estimate (Brower 1994) was used in combination with a model of sequence evolution that corrects for multiple hits and accounts for rate heterogeneity (Papadopoulou *et al*. 2010). We therefore retained and imposed the original 2.3% estimate as it was shown to correspond well with the mean mtDNA divergence rate obtained for Aegean tenebrionids (2.23% and 2.39% m.y^{-1}) when using the GTR+Γ+I model under a strict and relaxed clock, respectively (Papadopoulou *et al*. 2010). BEAST 1.5.3 (Drummond & Rambaut 2007) was used to obtain an ultrametric tree using Bayesian MCMC analysis orientated towards rooted, time-measured phylogenetics. Well supported nodes identified following NJ, ME, MP and BI analyses were constrained to be monophyletic and the HKY+I+Γ model identified in jModelTest 0.1.1 (Posada 2008, Guindon & Gascuel 2003) under the AIC was enforced using a strict molecular clock model. The results of two independent runs were merged and analysed with Tracer v1.4 and TreeAnnotator v1.4.7 (Drummond & Rambaut 2007).

Results

Genetic characterization and phylogenetic analyses

All sequences used in our final dataset were 885 bp in length and correspond to nucleotide positions 514 to 1399 of the COI gene. All novel sequences have been deposited in the Genbank database under accession numbers: GQ856478-80, GQ856482-8, GQ856490-1, GQ856493–GQ856500 and GU947664–GU947703, and were complemented with nucleotide sequence entries from two other studies, *viz.* AY762278, AY762285, AY762298-9, AY762317-20 (Grobler *et al*. 2006) GQ131943, GQ131946, GQ131952, GQ131954-5, GQ131961, GQ131967, GQ131979, GQ131997, GQ131999, GQ132004, GQ132006, GQ132009, GQ132012-4 (Grobler *et al*. 2011).

Of the 885 sequenced sites 592 were conserved across all 86 specimens in the dataset. Of the 293 variable sites 277 sites were parsimony informative and 159 of the latter were assigned weights other than one after rescaled consistency index (RCI) character reweighting. Parsimony

Fig. 1. Minimum Evolution (ME) tree of 13 species from the *Ectemnorhinus* group of genera based on 885 nucleotides of the mitochondrial cytochrome oxidase I (COI) gene. Each taxon label contains the species designation, sample number, sampling locality, and island of origin. Nodal support values obtained from 10 000 bootstrap replications (ME), 100 bootstrap replications from Maximum Parsimony (MP) and posterior support from Bayesian Inference (BI) analyses, expressed as percentages and denoted ME/MP/BI on each node. '- -' indicates support values < 65 (for ME and MP) and < 90 (for BI). The scale indicates the number of nucleotide substitutions. Islands are abbreviated as follows: Marion Island (MI), Prince Edward Island (PEI), The Prince Edward Island Archipelago (PEIA), Heard Island (HI), Kerguelen Island (KI) and Possession Island (PI).

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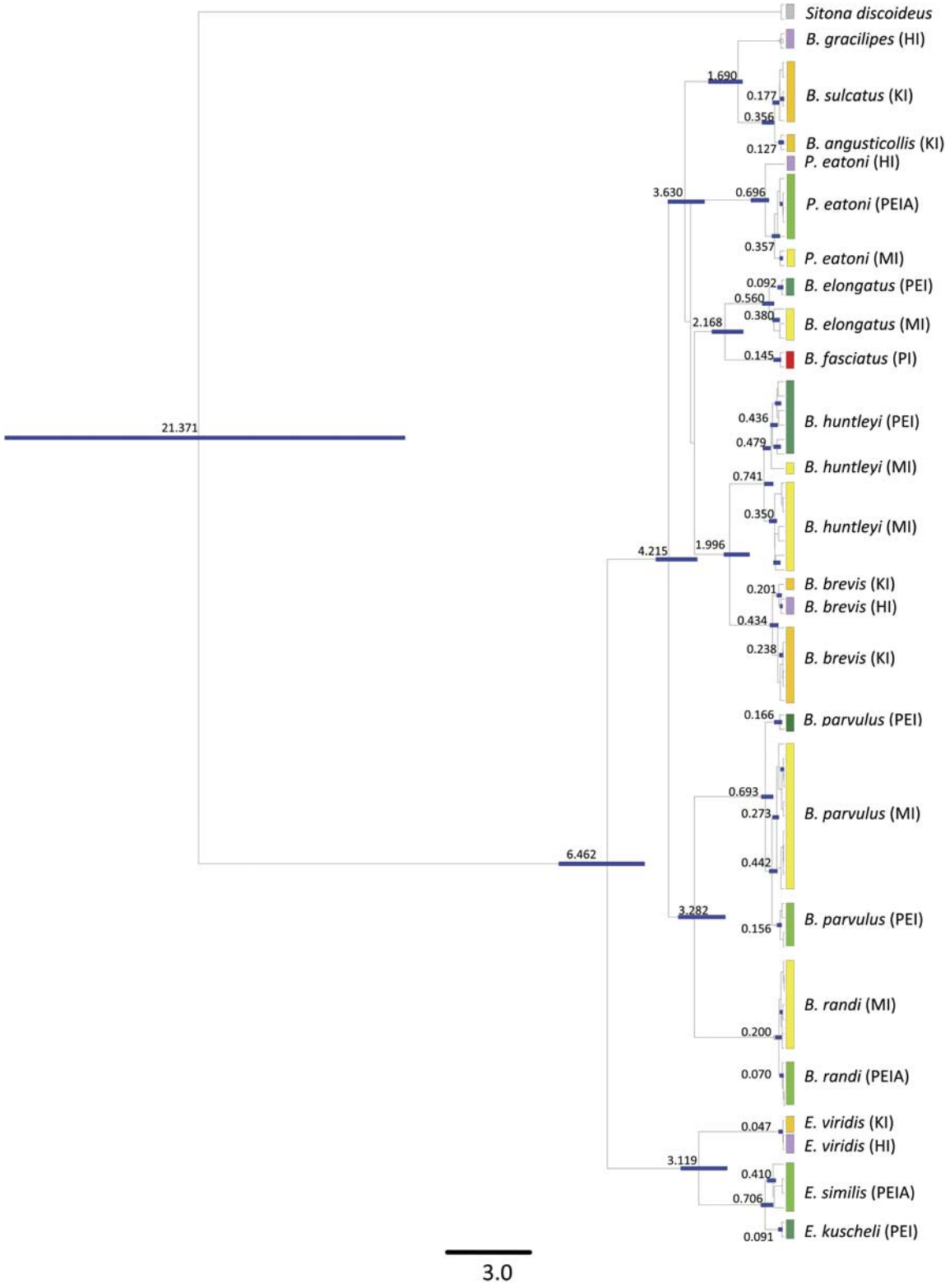

Fig. 2. Ultrametric tree obtained with BEAST with a clock rate of 2.3% sequence divergence per million years. The topology was constrained to retain monophyletic lineages recovered across all methods of inference (i.e. NJ, MP and BI). The numbers in the nodes correspond to the estimated age in million years, and the blue bars to the 95% confidence interval. The scale indicates change in million years. Islands are abbreviated as follows: Marion Island (MI), Prince Edward Island (PEI), The Prince Edward Island Archipelago (PEIA), Heard Island (HI), Kerguelen Island (KI) and Possession Island (PI).

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analyses with equal weighted characters recovered 92 trees with a length of 779 and homoplasy indexes of: CI = 0.485, RI = 0.898 and RCI = 0.435. The analysis in which characters were RCI reweighted also recovered 92 trees, all 342.97 in length, with homoplasy indexes of: CI = 0.672, RI = 0.935 and RCI = 0.629.

The HKY+I+Γ model of sequence evolution selected under the AIC in jModelTest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) recovered a transition transversion ratio of 4.4317, a gamma distribution shape parameter (Γ) of 1.000, proportion of invariable sites (I) = 0.6020 and base frequencies of A = 0.3462, C = 0.1528, G = 0.1012 and T = 0.3998 (% AT = 74.60%). The molecular phylogenies obtained with the different inference methods were topologically similar and recovered two main evolutionary lineages (denoted I and II in Fig. 1) for the *Ectemnorhinus* group of genera. Pairwise uncorrected p-distance comparisons of each monophyletic lineage/species within these lineages revealed mean inter-specific sequence divergence values of between 1.8 and 13.1%, and mean intra-specific diversity values ranging from 0.1 to 1.2% (see supplementary table S1 at www.journals.cambridge.org/jid_ANS). Lineage I (85% bootstrap support from ME and 100% from MP) which contains all of the *Ectemnorhinus* species characterized in this study is basal to the lineage II (99% and 100% bootstrap support from ME and MP, respectively) containing representatives of the genera *Palirhoeus* and *Bothrometopus*. Of the three *Ectemnorhinus* species characterized, *E. viridis* is basal to *E. similis* and *E. kuscheli* and intra-specific divergence for this species is low despite the fact that the *E. viridis* individuals are from different (Heard and Kerguelen) islands. According to the age estimates in Fig. 2, *E. viridis* last shared a common ancestor with the *Ectemnorhinus* species from the Prince Edward Archipelago approximately 3.12 million years ago (m.y.a.). *Ectemnorhinus kuscheli* from Prince Edward Island is basal to *E. similis* that occurs on both Marion Island and Prince Edward Island, and they shared their last common ancestor *c*. 0.71 m.y.a. (Fig. 2).

Lineage II comprises five monophyletic lineages (labelled a–e in Fig. 1) that coalesced approximately 4.22 m.y.a. These clades contain all nine *Bothrometopus* species characterized in this study as well as *Palirhoeus eatoni* (Fig. 1, clade d) suggesting that the monotypic genus *Palirhoeus* should be synonymized with *Bothrometopus* pending confirmation from nuclear gene analyses. Within the *Palirhoeus* lineage, which is estimated to have arisen *c.* 0.696 m.y.a., the *P. eatoni* specimen from eastern Heard Island, is basal to the western Prince Edward Islands' specimens. *Bothrometopus gracilipes*, *B. angusticollis* and *B. sulcatus* group together in a monophyletic clade (Fig. 1, clade a) with 75–91% nodal support. The Heard Island *B. gracilipes* lineage is estimated to have diverged from the remaining species approximately 1.69 m.y.a. The sister taxa *B. angusticollis* and *B. sulcatus*, represented by specimens from Ile Kergeulen, diverged *c*. 0.356 m.y.a.

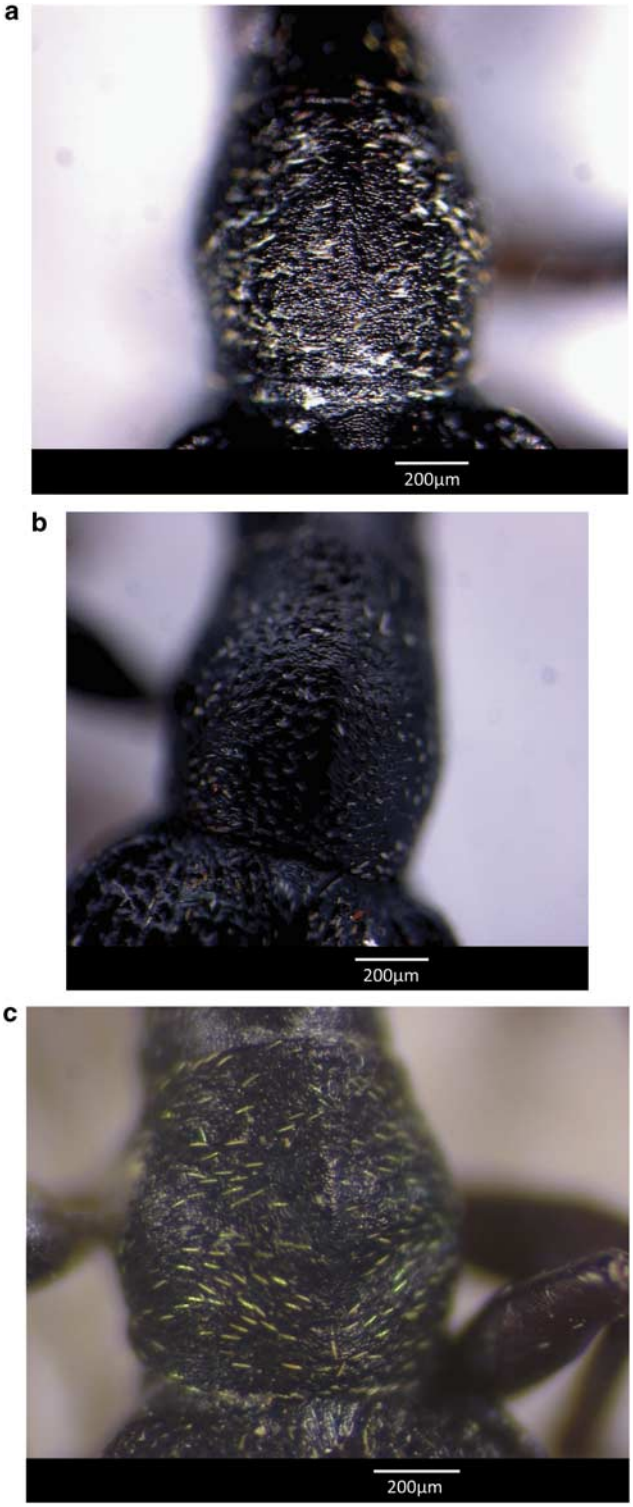

Fig. 3. Light micrographs of the pronota of **a.** *B. parvulus*, **b.** *B. huntleyi*, and **c.** *B. parvulus* type specimen from the National History Museum, London. Both the type specimen and *B. parvulus* show granular microsculpture on the pronotum. The pronotum of *B. huntleyi* is smoother in appearance.

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Bothrometopus fasciatus from Possession Island, groups with, and is basal to, *B. elongatus* from the Prince Edward Islands (Fig. 1, clade c). The estimated time to *B. fasciatus* and *B. elongatus* lineage coalescence is *c*. 2.2 m.y.a. Individuals of *B. elongatus* from Prince Edward Island are distinct from those from Marion Island, diverging *c*. 0.56 m.y.a. Additional *B. elongatus* specimens would need to be examined to determine the extent of gene flow between Prince Edward and Marion Islands.

When examining the remaining two clades (Fig. 1b & e) it became clear that both clades contain individuals from the Prince Edward Islands archipelago, identified morphologically as *B. parvulus*, but which are not sister taxa. One of these clades is sister to *B. randi* from the Prince Edward Islands, having diverged from this sister taxon approximately 3.3 m.y.a., whilst the other morphologically similar counterpart, groups with *B. brevis* from the Kerguelen and Heard islands, constituting a lineage which is estimated to have arisen *c*. 2.0 m.y.a. (Fig. 2).

Detailed external morphological examination of these two species, and comparison with images of the holotype of *B. parvulus* held by the Natural History Museum, London, revealed considerable similarity, with the exception of the microsculpture of the pronotum, which provides a reliable means of distinguishing between them (and also between some species on Possession Island, see Chown & Kuschel 1994). In the case of the holotype of *B. parvulus*, and indeed all material henceforth assigned to that species, the pronotal microscuplture appears pointillistic under a light microscope with granular microsculpture (Fig. 3a & c), and alutaceous when examined using scanning electron microscopy (Fig. 4). By contrast, the other species, which we describe formally below, has a smoother appearance under both light (Fig. 3b) and electron microscopy (Fig. 4), with distinct large punctations. No other completely reliable means exist to distinguish morphologically between these two species, but the characters are 100% reliable, as assessed via two independent approaches. First, morphology-based, in which one of us (SLC) with no advance knowledge of specimen identity, visually matched all specimens to the sequence data determinations with 100% congruence. Second, based on morphology, additional material from Prince Edward

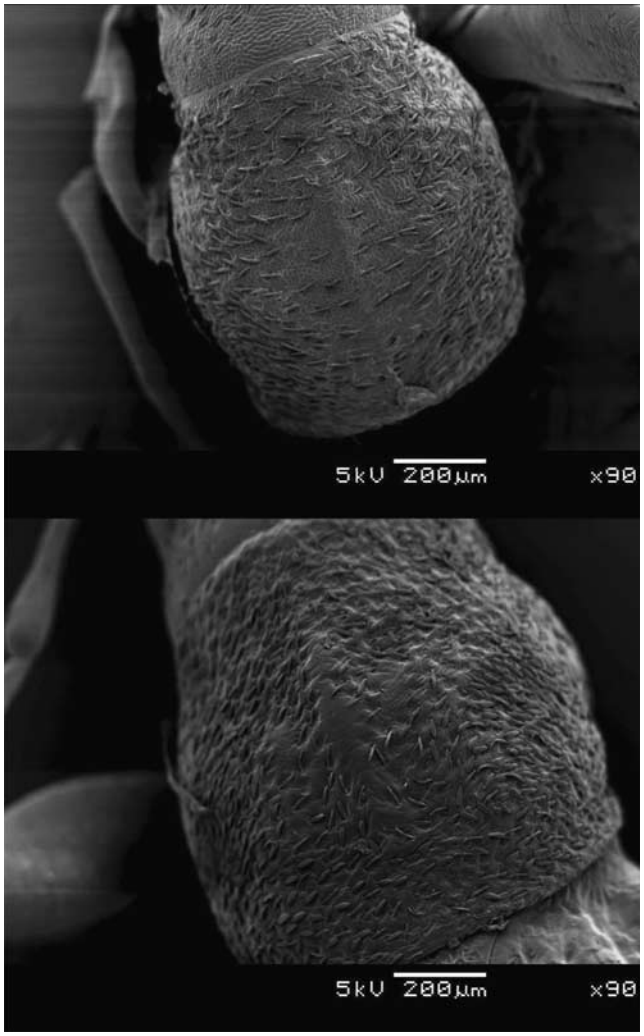

Fig. 4. Scanning electron microscopic comparison of the thorax of *B. parvulus* (top) and that of *B. huntleyi* (bottom) using scanning electron microscopy (SEM). No distinct setal patterning can be discerned, however the *B. parvulus* specimen appears to have a more granular surface and fewer scales than *B. huntleyi.* This feature can be observed with a standard, light microscope and can be used to readily distinguish *B. parvulus* from *B. huntleyi*.

Fig. 5. Dorsal habitus of *B. huntleyi* n. sp. male (length from anterior of eyes to posterior of elytra = 4.7 mm).

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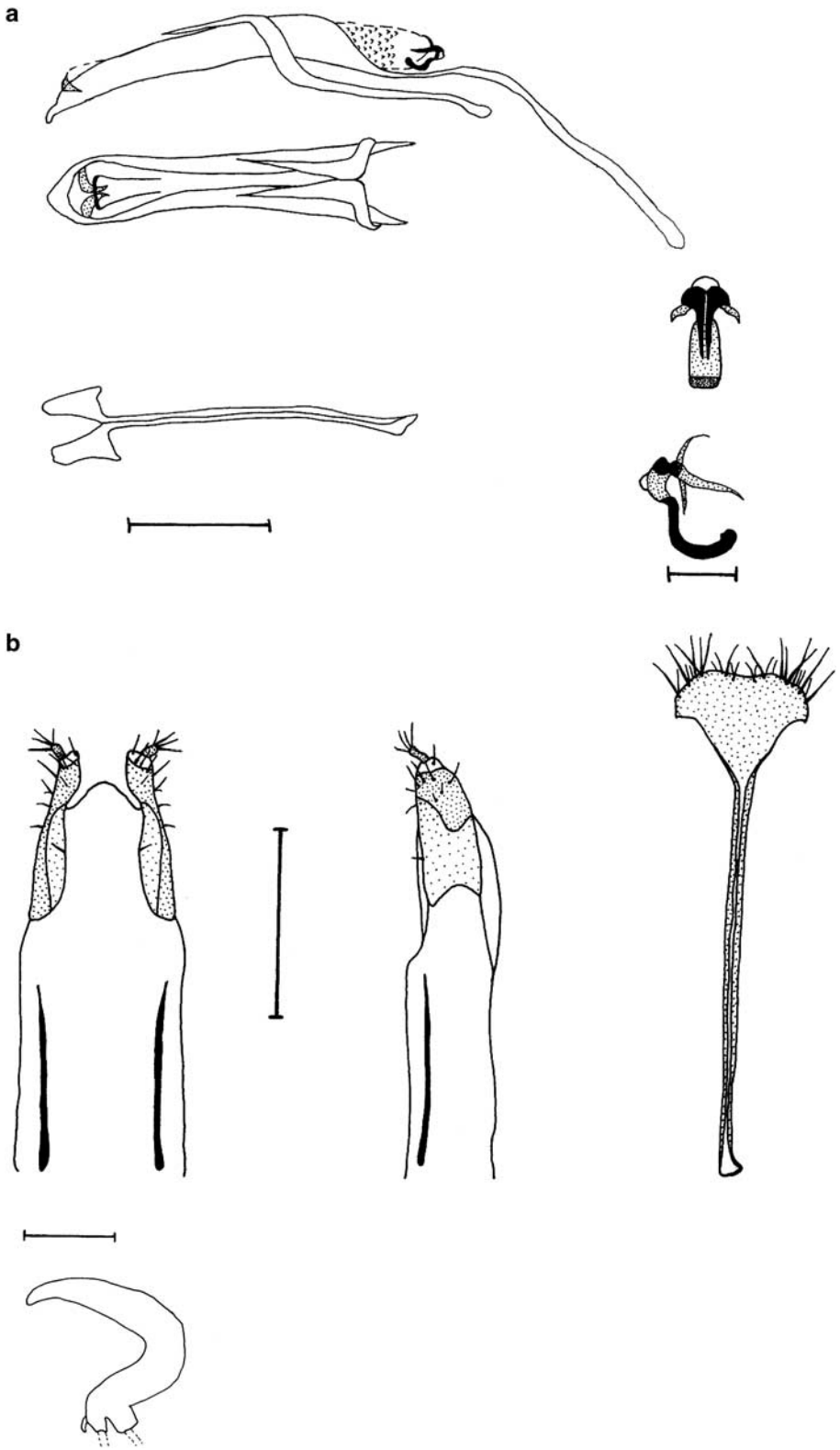

Fig. 6. *Bothrometopus huntleyi* n. sp. **a.** Male genitalia with the aedeagus in lateral and dorsal views (scale bar = 0.5 mm) with the genital armature to the right (scale bar = 0.1 mm). **b.** Female genitalia in dorsal and lateral view (scale bar = 0.5 mm). The spermatheca is shown below (scale bar = 0.2 mm).

Island was identified by one of us (AMT) and then provided to another author (GCG) who sequenced the material without prior knowledge of morphological assignment. The assignment match was 100%. We also noted that the individuals that correspond to *B. parvulus* appear to be restricted to coastal regions whereas the new species is

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distributed island-wide. The new cryptic species, *Bothrometopus huntleyi*, initially identified as *B. parvulus* based on morphology, is formally described below and compared to *B. parvulus*.

Bothrometopus huntleyi n. sp.

Description: Length (anterior of eyes to posterior of elytra): Overall: 3.1–5.5 mm; males: mean ± S.E. = 4.1 ± 0.03 mm (n = 156); females: 4.4 ± 0.04 mm (n = 136). Body dark brown to black with a variable covering of green to blue scales on the dorsal surface; the ventral surface is black (Fig. 5). The density of scales is highest on the elytra, most variable on the prothorax and sparse on the head and femora. The tibiae and tarsi lack scales, with the former having stiff, spine-like setae. On the elytra the scales occasionally form an anchor-shaped pattern, or two spots, one on each of the elytra. Where the scale density is high the scales are not imbricate. Occasionally, on the lateral margins of the elytra, small, fine and transparent to golden-brown to green erect hair-like scales may be present. These do not resemble the stiff, marked erect spines found on the elytra of species in the genus *Ectemnorhinus*. Antennae with light-brown to reddish-brown scape, reddish-brown funicle and dark-brown to almost black club. The first three funicle segments typically have the ratio 0.94:1:0.61 (Fs1:Fs2:Fs3) (n = 10). Epistome symmetric, sometimes with pronounced lobes, but also with a straight margin. Mandibles reddish-brown, each one asymmetric, with the dorsal tooth more pronounced than the ventral tooth, except after substantial wear. Labial palps three-segmented. Ommatidia coarse. Prothorax with an indistinct to distinct dorsal carina which can occasionally be entirely absent; where present it tends not to run the full length of the prothorax. Dorsal surface of the prothorax with pronounced punctations with an otherwise smooth surface between them. No granular microsculpturing is present. Elytra obovate each with a humeral carina which is moderately to well developed. Striations are pronounced as a consequence of deep punctations that are virtually contiguous. Legs reddish-brown to black with lighter colouration towards the base of the femora. Third tarsal segment with a ventral surface of densely packed white setae forming a brush. Tarsal claw segment shorter than the other three segments combined. Aedeagus as in Fig. 6a with a unique basal sclerite. Female genitalia as in Fig. 6b.

Etymology: This new species is named in honour of the youngest biologist on the first biological and geological expedition (1965/1966) to the Prince Edward Islands: Brian John Huntley.

Remarks: Bothrometopus huntleyi is a medium-sized *Bothrometopus* species – the genus varies from *c*. 2–10 mm in length (Chown & Kuschel 1994, Kuschel & Chown 1995). It is morphologically very similar to *B. parvulus* (C.O. Waterhouse) from the Prince Edward Islands and *B. brevis* (C.O. Waterhouse) from Kerguelen and Heard islands. Adults of *B. huntleyi* can be separated from *B. parvulus* based on the former species' deep punctations and lack of granular microsculpture on the prothorax, dorsal carina which does not stretch from end to end of the prothorax, and typically lighter funicle segments of the antennae by comparison with the general body colouration. The most reliable distinguishing feature is the difference in microsculpture on the prothorax of the two species (as described above and shown in Figs 3 & 4). No characters have yet been found to distinguish the larvae.

Distribution: Island-wide (coastal rocks and inland areas, see Chown 1989, 1992) on both Marion Island and Prince Edward Island. This contrasts with *B. parvulus*, which thus far has only been found on coastal rocks at both Marion Island and Prince Edward Island. The phylogeography of this new species is discussed in detail in Grobler *et al*. (2011).

Material examined

Holotype:
♂, South Africa, Marion Island, 400 m a.s.l., First Red Hill, 46°53.412'S, 37°48.21'E, Genbank no. GQ131999, voucher no. 180-15, collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

Paratypes:
♀, South Africa, Marion Island, 0 m a.s.l., 'Ship's Cove', 46°51'41"S, 37°50'66"E, Genbank no. GQ132012, voucher no. 16-22, collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.
♂, South Africa, Marion Island, 200 m a.s.l., Junior's Kop, 46°52.794'S, 37°50.083'E, Genbank no. GQ131946, voucher no. 151-18, collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.
♀, South Africa, Marion Island, 600 m a.s.l., Feldmark Plateau, 46°56'35"S, 37°46'10"E, Genbank no. GQ131952, voucher no. 339-1, collected April 2002, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.
♂, South Africa, Marion Island, 400 m a.s.l., First Red Hill, 46°53.412'S, 37°48.21'E, Genbank no. GQ131967, voucher no. 180-3, collected April 2001, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.
♀, South Africa, Marion Island, 0 m a.s.l., 'Ship's Cove', 46°51'41"S, 37°50'66"E, Genbank no. GQ131943, voucher no. 16-29, collected April 2001, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.
♂, South Africa, Prince Edward Island, 0 m a.s.l., Cave Bay, 46°38.752'S, 37°59.780'E, Genbank no. GQ131954, voucher no. 303-19, collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

Table II. Summary of the 43 nucleotide sites in the COI gene region characterized in this study, that are consistently different between *B. parvulus* and *B. huntleyi*.

Nucleotide site	Base position	*B. parvulus*	*B. huntleyi*
9	3rd	T	A
33	3rd	T	C
39	3rd	T	A
42	3rd	A	G
63	3rd	T	A
64	1st	C	T
82	1st	C	T
84	3rd	T	G
87	3rd	A	T
141	3rd	T	C
153	3rd	T	A
195	3rd	A	T
285	3rd	T	A
288	3rd	T	C
321	3rd	A	C
333	3rd	C	T
348	3rd	T	C
360	3rd	A	G
366	3rd	T	C
444	3rd	A	T
468	3rd	T	A
482	2nd	C	A
486	3rd	A	T
492	3rd	C	T
493	1st	C	T
507	3rd	T	A
543	3rd	C	A
547	1st	G	A
585	3rd	T	C
615	3rd	C	T
618	3rd	T	C
648	3rd	A	T
651	3rd	A	T
669	3rd	T	C
672	3rd	C	A
699	3rd	C	T
706	1st	T	C
712	1st	G	A
714	3rd	C	T
750	3rd	G	A
765	3rd	T	C
780	3rd	C	T
876	3rd	C	T

♀, South Africa, Prince Edward Island, 672 m a.s.l., Top of van Zinderen Bakker, 46°37.590'S, 37°55.891'E, Genbank no. GQ131961, voucher no. 318-10, collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♂, South Africa, Prince Edward Island, 0 m a.s.l., Cave Bay, 46°38.752'S, 37°59.780'E, Genbank no. GQ131955, voucher no. 303-20, collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♀, South Africa, Prince Edward Island, 400 m a.s.l., 46°38.211'S, 37°57.482'E, Genbank no. GQ132006, voucher no. 307-9, collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♂, South Africa, Prince Edward Island, 400 m a.s.l., 46°38.211'S, 37°57.482'E, Genbank no. GQ132004, voucher no. 322-8, collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

♀, South Africa, Prince Edward Island, 0 m a.s.l., Cave Bay, 46°38.752'S, 37°59.780'E, Genbank no. GQ131997, voucher no. 303-7, collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

Additional material was examined for the morphometric analysis on which the length measurements used in the description are based (A. Treasure and S.L. Chown, unpublished data).

Molecular comment

DNA barcoding, its recognized flaws notwithstanding (Rubinoff 2006), was considered here as a complementary tool for the unequivocal differentiation of *B. parvulus* from *B. huntleyi*. The 43 nucleotide sites that are conserved within species, and consistently different between the two morphologically indistinct species occurring on PEIA are summarized in Table II. When comparing the partial amino acid COI gene sequences of the thirteen species of the *Ectemnorhinus* group of genera generated in this study, 17 non-synonymous amino acid substitutions were observed in the 84 ingroup taxon dataset. These non-synonymous amino acid substitutions revealed several consistent and therefore possibly diagnostic differences between species and include the following positions in our dataset: Codon 7 (I in *B. elongatus* and M in all other species, except for two *B. sulcatus* specimens which have a V at this position); Codon 19 (V in *E. viridis* and I in all other species); Codon 41 (all species within the genus *Ectemnorinus* have an I at this position, whereas a V is present in all species of the genera *Bothrometopus* and *Palirhoeus*); Codon 161 (T in *B. parvulus* and N in all other species); Codon 183 (V in *B. parvulus* and I in all other species); Codon 241 (M in *B. gracilipes*, and either a V or a L in all other species). As some of the species in this study are only represented by two specimens, additional data will need to be generated to determine the consistency and species-exclusivity of some of these characters.

Discussion

The phylogenetic analyses revealed three major points. First, the monotypic genus *Palirhoeus* is not readily distinguishable, on a mtCOI sequence basis, from the genus *Bothrometopus*, thus questioning the retention of the species *P. eatoni* in a separate genus, *Palirhoeus*, created by Kuschel (1971), and its position in Kuschel & Chown's (1995) phylogeny as basal to the genera *Bothrometopus* and *Ectemnorhinus*. Nonetheless, limited taxon and gene

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sampling means that we refrain from proposing formal generic synonymy. Second, the two species groups in the genus *Bothrometopus* (*fasciatus* group and *gracilipes* group), identified on the basis of absence or presence of dorsal wall vaginal spicules, by Kuschel & Chown (1995) are not supported by the COI gene phylogeny. *Bothrometopus gracilipes* and *B. angusticollis* fall into the *gracilipes* group of *Bothrometopus* species (Kuschel & Chown 1995) while *B. sulcatus* falls in the *fasciatus* group of *Bothrometopus* species (Kuschel & Chown 1995). The sister taxon relationship of *B. elongatus*, which is assigned to the *gracilipes* group, with *B. fasciatus* from the *fasciatus* group of *Bothrometopus* species (Kuschel & Chown 1995) in the COI gene tree also raises questions regarding the phylogenetic utility of these two major groups. Third, what was previously considered a single species on the Prince Edward Islands, *B. parvulus* Jeannel, is clearly two species that are certainly not sister taxa, but rather share relationships with different species from our sample taxa. Identification of this cryptic species increases the number of species within the *Ectemnorhinus* group of genera from 36 to 37.

Despite being a partial analysis of this group of weevils endemic to the South Indian Ocean Province Islands, the current study has important implications for interpretation of biogeographic and evolutionary dynamics in the region more generally. Perhaps the most significant point to emerge is that colonization of the Prince Edward Islands is likely to have taken place repeatedly from other islands in the South Indian Ocean Province. Thus, although *B. parvulus* and *B. randi* are sister species in the current tree (Fig. 1), the molecular clock based on a 2.3% nucleotide sequence divergence per million years estimate obtained from an arthropod mtDNA survey of Brower (1994), which has proven useful for studies of this group (see Grobler *et al*. 2006), indicates that divergence must have taken place approximately *c*. 3.3 m.y.a. (Fig. 2). This could not have happened on the Prince Edward Islands because the oldest date for the islands is *c*. 0.5 m.y., and there is no geological evidence to suggest that they are very much older than this (Boelhouwers *et al*. 2008). The date of the divergence between *B. huntleyi* and *B. brevis*, *c*. 2.0 m.y.a., also suggests that an early colonization of the Prince Edward Islands is unlikely. Instead, the dated phylogeny suggests that dispersal to the Prince Edward Islands must have occurred from elsewhere, sometime after the islands emerged, and on at least two separate occasions. Because we were unable to sample all taxa in the genus *Bothrometopus* (see Chown & Kuschel 1994, Kuschel & Chown 1995 for review) it seems likely that the colonization has been from species on the Crozet archipelago. *Bothrometopus randi* (the sister species of *B. parvulus*, based on this analysis) is known from Possession Island and other *Bothrometopus* species are widespread across Iles Crozet (Chown & Kuschel 1994). Such an hypothesis of colonization against the prevailing west wind drift is not new, and was in fact proposed by Dreux and Voisin in a series of works on the group (e.g. Dreux & Voisin 1987, 1989). Thus, unlikely as their hypotheses may have seemed initially, they cannot, on present evidence, be rejected. Indeed, it also appears that *P. eatoni* colonized the Prince Edward Islands relatively recently (Figs 1 & 2) and that dispersal between Marion Island and Prince Edward Island has been quite common since their emergence.

Several independent lines of evidence support this proposal of repeated colonization across the region. Using a molecular phylogenetic approach, Stevens *et al*. (2006) demonstrated that repeated colonizations across the sub-Antarctic islands probably took place from the late Miocene (*c*. 7 m.y.a.) to approximately 0.3 m.y.a. Likewise, recent investigations of the ameronothroid mite genera *Halozetes* and *Alaskozetes* have shown colonization of the islands by species in these genera over the last ten million years (Mortimer *et al*. 2010). These dates also correspond closely with those for dispersals among populations of the springtail *Cryptopygus antarcticus* in the Scotia Arc and Antarctic Peninsula region (McGaughran *et al*. 2010b), and trans-Drake Passage dispersal of the nudibranch *Doris kerguelenensis* (Wilson *et al*. 2009). However, the divergence times differ substantially for those estimated for the bull kelp *Durvillaea antarctica*, which apparently recolonized the South Indian Ocean Province Islands after its removal during the last glacial maximum, *c*. 16 000 years ago (Fraser *et al*. 2009).

These dispersal dates indicate that for the terrestrial species much of the diversification considerably preceded the last glacial maximum and many events date to either the Pliocene–early Pleistocene, or as soon as a particular island group (such as the Prince Edward Islands) emerged. Thus, it appears likely that the groups survived several glacial cycles in refugia on the islands, and are certainly not post-glacial colonists. Such proposals have been made previously for various groups (see discussions in Chown 1990, Van der Putten *et al*. 2010). Indeed for the *Ectemnorhinus* group of genera, Chown (1989, 1994) suggested that the species typical of the epilithic biotope, (i.e. those in the genera *Bothrometopus*, *Palirhoeus* and *Disker*) probably radiated since the end of the Pliocene in the epilithic biotopes that must have come to predominate as a consequence of cooling (for revised climatic histories see Turner *et al*. 2009). The divergence times calculated on the basis of an arthropod mtDNA survey of Brower (1994) certainly support such a proposal. Whether the groups more typical of vegetated areas will show an equally deep history is not clear. However, the deep divergence time, approximately 6.46 m.y.a., found here between *Ectemnorhinus* (a genus in which species are typical of vegetated areas - Chown 1989, 1994) and *Bothrometopus* (restricted to epilithic biotopes) and the fairly substantial divergence dates among species within this genus (see also Grobler *et al*. 2006), suggests that they may well do so. That recent studies have supported the persistence of vascular plants on the South Indian Ocean Province Islands through

several glacial periods (e.g. Van der Putten *et al*. 2010) also suggests that survival during these periods is likely. In consequence, the proposal that the genus *Ectemnorhinus* diversified following the last glacial maximum (Chown 1994) must be rejected. Similar hypotheses of recolonization of terrestrial areas from refugia, such as marine refugia in the case of the ameronothroid mites have also been rejected on the grounds of new molecular evidence (Mortimer *et al*. 2010). However, within particular species it remains clear that volcanic and glacial cycles and refugia on particular islands have played important roles in population structuring. Such structure has thus far been identified for indigenous springtails, mites, and weevils (Grobler *et al*. 2006, Myburgh *et al*. 2007, Grobler *et al*. 2011), and seems also to apply to a vascular plant species and to other insects. Significantly, though, in a sub-Antarctic context such details are available only for the Prince Edward Islands, and to a lesser extent for Macquarie and Heard islands.

These results clearly indicate the need for further comprehensive molecular phylogenetic analyses of the biogeography of the region including a range of taxa. Only in this way will clearer reconstructions of the history and evolutionary relationships of the endemic and frequently enigmatic taxa in the region be established, and the hypotheses concerning the origins of the group (e.g. Jeannel 1964) assessed on a sounder basis. Moreover, they suggest that hypotheses concerning the historical biogeography of the region based solely on distributional data are perhaps no longer as useful as they once were. The distributional data must be accompanied by modern phylogenetic analyses for two reasons. First, the phylogenetic approach can reveal divergence times and relationships more straightforwardly than other approaches (acknowledging that a match with earth history must still be sought), thus helping to resolve biogeographic interpretation. Second, molecular evidence has been instrumental in revealing the presence of cryptic species, the existence of which can change interpretation substantially (Stevens *et al*. 2006, Torricelli *et al*. 2010). Given enhanced scientific cooperation across the Antarctic within a variety of scientific programmes, the development of comprehensive molecular phylogenies is likely to be achieved readily, and will almost certainly change current perspectives on the biogeography and biodiversity of the region, as this initial study has demonstrated.

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Supplemental material

A supplemental table will be found at www.journals.cambridge.org/jid_ANS.

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