

Allopatric speciation in the flightless *Phoberus capensis* (Coleoptera: Trogidae) group, with description of two new species

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Abstract

The name *Phoberus capensis* (Scholtz) is applied to a small flightless, keratinophagous beetle endemic to the Cape Floristic Region of South Africa. Its gross distribution stretches from roughly 1000 km from the Cederberg (S32°24'22" E19°04'50") to Grahamstown (S33°20'07" E26°32'50"). The populations are spatially discrete, restricted to relict forests of the southern Cape and disjunct high montane refugia of the Cape Fold Mountains. We test the hypothesis that there is more than one distinct species nested within the name *P. capensis*. Phylogenetic relationships among populations were inferred using molecular sequence data. The results support three distinct evolutionary lineages, which were also supported by morphological

characters. Divergence time estimates suggest Pliocene-Pleistocene diversification. Based on these results, it is suggested that the *P. capensis* lineage experienced climatically-driven allopatric speciation with sheltered Afrotropical forests and high mountain peaks serving as important refugia in response to climatic ameliorations. The *P. capensis* complex thus represents a speciation process in which flight-restricted populations evolved in close allopatry, possibly as recently as the Pleistocene. Two divergent and geographically distinct lineages are described as novel species: The new species, *P. disjunctus* sp. n. and *P. herminae* sp. n., are illustrated by photographs of habitus and male aedeagi.

Keywords

Cape Fold Mountains; keratin; *Phoberus*; relict groups; Scarabaeoidea; southern Africa

Introduction

The Cape Floristic Region (CFR) of the southern-western Cape region of South Africa is recognised as one of the world's six Floral Kingdoms (Myers et al. 2000; Mucina & Rutherford 2006; Cowling 2009). The region exhibits exceptional floral diversity and high levels of biotic endemism (Linder 2003, 2005). It is characterised by a Mediterranean (temperate) type climate and a unique sclerophyllous, fire-prone shrubland known as Cape 'fynbos' (Linder 2003, 2005; Galley & Linder 2006; Mucina & Rutherford 2006). The development of the drought-resistant (pyrophytic) vegetation of the CFR is attributed to dramatic climate shifts from warm, tropical conditions to drier, more seasonal conditions during the Miocene/Pliocene (Cowling et al. 2009; Dupont et al. 2011; McDonald & Daniels 2012). Various factors, including the development of the cold Benguela Current along the west coast of southern Africa during the late Miocene (Siesser 1980; Pickford & Senut 1999), and tectonic uplift during the early Miocene (18 Mya) and the Pliocene (5 Mya), caused

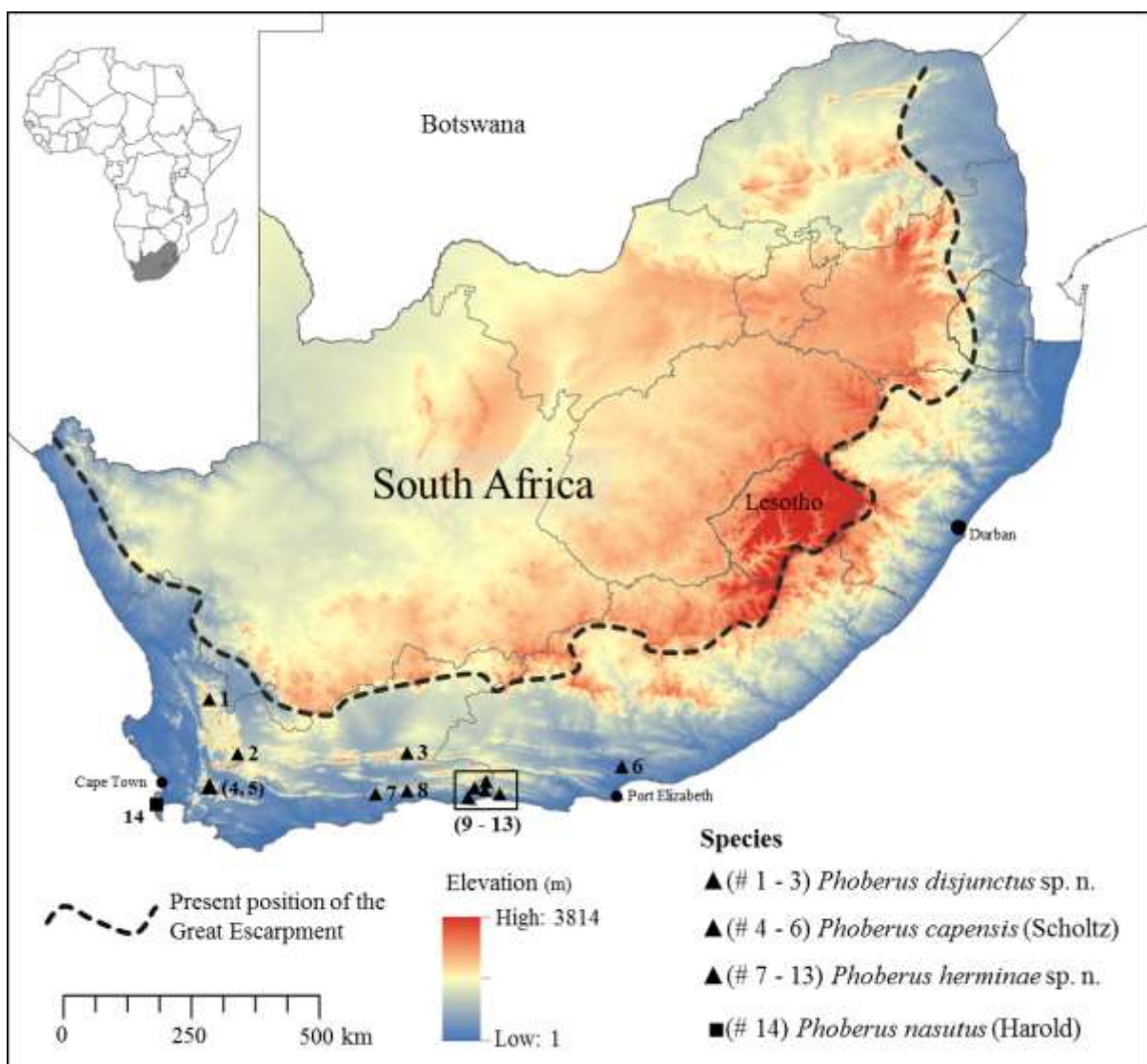
progressive aridification of the sub-continent. The combination of climate fluctuations, tectonic uplift and marine transgression and regression associated with the Pleistocene glacial cycle resulted in considerable habitat fragmentation, extinction and expansion events within the biome, thus providing an important stimulus for speciation and diversification (Partridge et al. 1999; Linder 2005; Tolley et al. 2006; Cowling et al. 2009). Climatically-driven speciation has been shown for a variety of taxa in the CFR (Tolley et al. 2006; Price et al. 2007, 2010; Swart et al. 2009; Linder et al. 2010; McDonald & Daniels 2012; Sole et al. 2013).

The CFR is dominated by the ancient Cape Fold Mountains (CFM) which can be divided into several mountain blocks: (1) a single western mountain range running parallel to the Atlantic Ocean coast, and (2) a double mountain range running parallel to the Indian Ocean coast (Linder 2003). The mountain ranges are separated by arid, low-lying valleys and plains, sheltered ravines and deeply incised gorges (Cowling et al. 2009). The CFM consists of highly erosion-resistant sandstone and most of these mountains reach altitudes higher than 1500 m above sea level, contributing to the heterogeneity of the region. These high mountains act as barriers to maritime moisture moving inland from the Atlantic and Indian Oceans, which has a profound effect on the local climate because seaward-facing slopes are moister and inland plains in the rain shadow of these mountains are very arid (Linder 2003).

The CFR and the CFM specifically provide suitable habitat and refugia for a number of relict flightless Scarabaeoidea (Coleoptera) (Scarabaeidae: Medina & Scholtz 2005; Sole et al. 2005; Deschodt et al. 2007, 2011; Deschodt & Scholtz 2008; Sole & Scholtz 2013; Lucanidae: Endrödy-Younga 1988, Switala et al. 2014; Trogidae: Scholtz 1979, 1980). The flightless species generally occupy the summits, forested palaeo-refugia on the seaward-

facing slopes and the coastal plains. These cool, temperate environments may have acted as buffer zones and long-term refugia for many organisms in response to the effects of changing climate (Stuckenberg 1962; Endrödy-Younga 1978; Linder & Vlok 1991; Geldenhuys 1997; Midgley et al. 2001; Daniels et al. 2013).

Figure 1. Map of the distribution of *Phoberus capensis* (Scholtz), *P. disjunctus* sp. n., *P. herminae* sp. n. and *P. nasutus* (Harold). Sample locality numbers follow Table 1.



Phoberus capensis (Scholtz) (Scarabaeoidea: Trogidae) is a small (4-7 mm) flightless, keratinophagous beetle endemic to the CFR of South Africa. Its gross distribution stretches roughly 1000 km from the Cederberg (S32°24'22" E19°04'50") to Grahamstown (S33°20'07"

E26°32'50"). However, its populations are clearly spatially discrete, restricted to relict forests of the southern Cape and disjunct high montane refugia of the CFM (Fig 1). Its habitat specificity, disjunct population distributions and low vagility (due to flightlessness) make *P. capensis* an ideal species for answering questions regarding speciation and diversification events. In this study we test the hypothesis that there is more than one distinct species nested within what is currently recognised as *P. capensis*. To do this we analyse mitochondrial DNA sequences and also estimate the divergence times and ages of the populations.

Material & Methods

Sampling and laboratory protocols

Phoberus capensis was sampled from localities across its known distribution (Table 1 and Table A1 in the Appendix). *Phoberus nasutus* (Harold), the sister species (Strümpher et al., 2014), was used as the outgroup. The latter is restricted to the Cape Peninsula – an isolated mountainous area surrounded by the Atlantic Ocean in the west and the Cape Flats to the east (Macdonald & Daniels 2012). Collected specimens were deposited at the Department of Zoology and Entomology, University of Pretoria, South Africa (UPSA).

Total genomic DNA was extracted from a leg of each of 53 individuals and partially sequenced for the protein-coding mitochondrial Cytochrome Oxidase I subunit (COI) gene and the mitochondrial 16S small subunit ribosomal RNA (16S) gene. The primer pairs used for amplification and sequencing were COI: C1-J-1718 with TL2-N-3014 (Simon et al. 1994); and 16S: 16sf with 16sr (Orsini et al. 2007). DNA fragments for the two gene regions were amplified using the polymerase chain reaction (PCR) performed in a final volume of 25 µl made up of 20 pmol of each primer, Emerald Amp®MAX HS PCRMasternmix (Takara Bio Inc., Otsu, Shiga, Japan), and 50-100 ng of genomic DNA template.

Table 1. Specimens of *Phoberus capensis* group collected from the Western Cape and Eastern Cape provinces, South Africa.

<i>N</i>	Sample locality	Altitude (m asl)	Coordinates	<i>n</i>
1	Cederberg Mountains	1547 m	S32°24'22.43" E19°04'50.37"	1
2	Hexrivier Mountains, Matroosberg	1880 m	S33°22'22.23" E19°39'34.77"	4
3	Swartberg Mountains, Swartberg Pass	1510 m	S33°20'59.27" E22°15'51.90"	6
4	Hottentots-Holland, Sneekop	1406 m	S34°02'21.08" E18°59'24.94"	13
5	Hottentots-Holland, Moordenaarskop	940 m	S34°05'53.12" E18°57'52.74"	2
6	Grahamstown, Signal Hill	622 m	S33°20'07.54" E26°32'50.23"	2
7	Langeberge, Ruiterbos	800 m	S33°52'31.37" E22°01'22.00"	3
8	Outeniqua Mountains, Outeniqua Pass	668 m	S33°54'02.05" E22°24'04.09"	3
9	Tsitsikama Forest, Nature's Valley	38 m	S33°58'02.00" E23°33'36.80"	2
10	Knysna, Diepwalle Forest	420 m	S33°57'40.92" E23°09'22.79"	3
11	Knysna, Buffalo Valley	81 m	S33°59'29.51" E23°17'55.34"	4
12	Knysna, Harkerville Forest	276 m	S34°03'00.53" E23°12'00.34"	9
13	Stormsrivier	102 m	S33°58'18.01" E23°53'53.00"	1
14	Cape Peninsula, nr Simonstown	98 m	S34°13'19.20" E18°24'38.48"	3

N, population number; *n*, number of individuals collected from each locality.

Thermal cycling parameters for COI comprised an initial denaturation for 90 s at 95°C followed by 35 cycles at 94°C for 22 s, annealing at 50°C for 30 s and 72°C for 90 s with a final elongation step at 72°C for 1 min; and for 16S an initial denaturation at 94°C for 90 s

followed by 35 cycles at 94°C for 60 s, annealing at 48°C for 90 s and 72°C for 90 s with a final elongation step at 72°C for 1 min.

Successful amplifications were purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel) following the manufacturer's instructions. To obtain DNA sequences, the cycle sequencing reactions were carried out in both directions using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Cycle sequencing products were precipitated using a standard sodium acetate/ethanol precipitation protocol. All sequences were viewed, edited and assembled in CLC Bio 5.6 (<http://www.clcbio.com/>). New sequences were submitted to GenBank (see Tables A2 and A3 in the Appendix).

Alignment

The sequences were aligned using the program package MAFFT (Kato & Toh 2008) with default settings. The alignments for the two gene regions (COI, 16S) were concatenated into a supermatrix using the program FASconCAT version 1.0 (Kück & Meusemann 2010)

Phylogenetic analysis

jModel Test (Posada 2008) was used to select the appropriate model of sequence evolution under the Akaike information criterion (Akaike 1974). Phylogenetic trees were inferred using Maximum Parsimony (MP), Bayesian Inference (BI) and Maximum Likelihood (ML) approaches.

A parsimony analysis was implemented in PAUP*4.010b (Swofford 2003) with the following heuristic search setting: all characters were equally weighted and unordered, gaps were treated as missing data and uninformative sites were excluded; starting tree obtained via stepwise addition with random addition of sequences with 10 replicates; branch-swapping =

tree-bisection-reconnection; initial ‘maxtrees’ set to 200 with automatic increase by 100 and with ‘MulTrees’ option in effect. Bootstrap values were calculated based on 1000 replicates. A strict consensus tree was calculated from all of the most parsimonious trees obtained.

Bayesian analysis was performed for individual genes and the combined gene regions using the program MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The data set was partitioned into two gene regions. Analysis was performed using GTR+G+I model for COI, a GTR+G model for 16S. Parameters for the different partitions were unlinked to obtain separate parameter estimates for each gene and the rate prior was set to variable. Flat Dirichlet priors were used in all analyses. Bayesian analyses were conducted by simultaneously running two Monte Carlo Markov chains, with one cold and three heated chains, for 5 million iterations. Trees were sampled every 200 iterations. Tracer v1.5 (Rambaut & Drummond 2007) was used to monitor parameter stabilisation (via inspection of estimated sample size (ESS) and graphical plots of parameter sampling). The first 20% of trees sampled were consequently discarded as burn-in.

Maximum Likelihood analysis was conducted in RAxML (Randomized Axelerated Maximum Likelihood) version 8.20 (Stamatakis 2014) on the concatenated dataset under a GTR+G model. A single run was conducted with 1000 bootstrap inferences, followed by a thorough ML search. Each inference was initiated with a random starting tree, and employed the rapid hill-climbing algorithm (Stamatakis et al. 2007). FigTree v1.3.1 (Rambaut 2009) was used to view all tree topologies.

Haplotype phylogenetic relationships were assessed by a split-decomposition neighbour-net analysis on individual and combined dataset using SplitsTrees 4 (Huson & Bryant 2006).

Pairwise genetic distances (p -distances) were calculated between groups using Mega 6 (Tamura et al. 2013) for COI and 16S. Groups were based on the well-supported lineages recovered within the phylogenetic analysis.

Molecular diversity, molecular structure and historical demography

Haplotype (h) and nucleotide diversity (π) for each gene region were assessed using DnaSP v. 5 (Librado & Rozas 2009). Analysis of molecular variance (AMOVA), calculated in Arlequin version 3.5 (Excoffier & Lischer 2010), was performed on the COI mtDNA locus to explore population structure within the genetic variation. Groupings tested within the AMOVA were based on the well-supported lineages recovered within the phylogenetic and neighbor-network analyses. A population was defined as all individuals coming from a single geographic locality.

Divergence analysis

The node ages for the lineage-splitting events were estimated using the software BEAST v. 1.6.2 (Drummond & Rambaut, 2007). Cytochrome Oxidase I (COI) data for those individuals successfully sequenced was used. As no fossil evidence with which to estimate time of origin for lineages is available for this group, we opted to use published mutation rates of 0.0075 and 0.012 (Brower 1994; Juan et al. 1995; Farrell 2001; Smith & Farrell 2005; Wirta et al. 2008) mutational substitutions per site per million years (s/s/Myr), respectively, to cover the range of rates reported for COI mtDNA. A Bayesian relaxed molecular clock approach under the uncorrelated lognormal model and a Yule speciation was used for divergence time estimation. The ingroup was constrained to be monophyletic. The ML topology was specified as a starting tree for BEAST. Two independent MCMC analyses were run for 20 million generations with parameters sampled every 1000 generations. Default settings were kept for

all other priors and operators. Tracer was used to monitor parameter stabilisation and assess the convergence between runs. LogCombiner v1.6.2 from the BEAST package was used to combine the tree and log output files from the two independent runs. The first 20% of trees sampled from each run was discarded as burn-in. The TreeAnnotator v1.6.2 application from the BEAST package was used to generate a maximum clade credibility tree and calculate the mean ages. Tree topologies were viewed in FigTree. The divergence analysis was run twice to validate consistency of the time estimates between runs.

Morphology

Where possible, male and female specimens from the different sampling localities were examined and male genitalia dissected and photographed. In addition, the holotype and several paratype specimens of *P. capensis* (Scholtz) were examined using Zeiss dissecting microscopes. Images of set habitus specimens were taken with a Canon EOS 550D and 100 mm macro lens. Focus stacking was performed using the software Helicon Focus version 5.3. Male genitalia and components were photographed under a Leica M165 C microscope, using the Leica DMC 2900 digital camera. Morphological terminology follows Scholtz (1980).

Results

Phylogenetic analysis

The final combined molecular dataset consisted of 56 taxa and 1608 base pairs (bp): COI = 1152 bp, 16S \approx 456 bp; 252 characters were parsimony informative, 1327 were constant and 280 were variable. Data characteristics and estimated model parameters for individual and combined datasets are shown in Table 2.

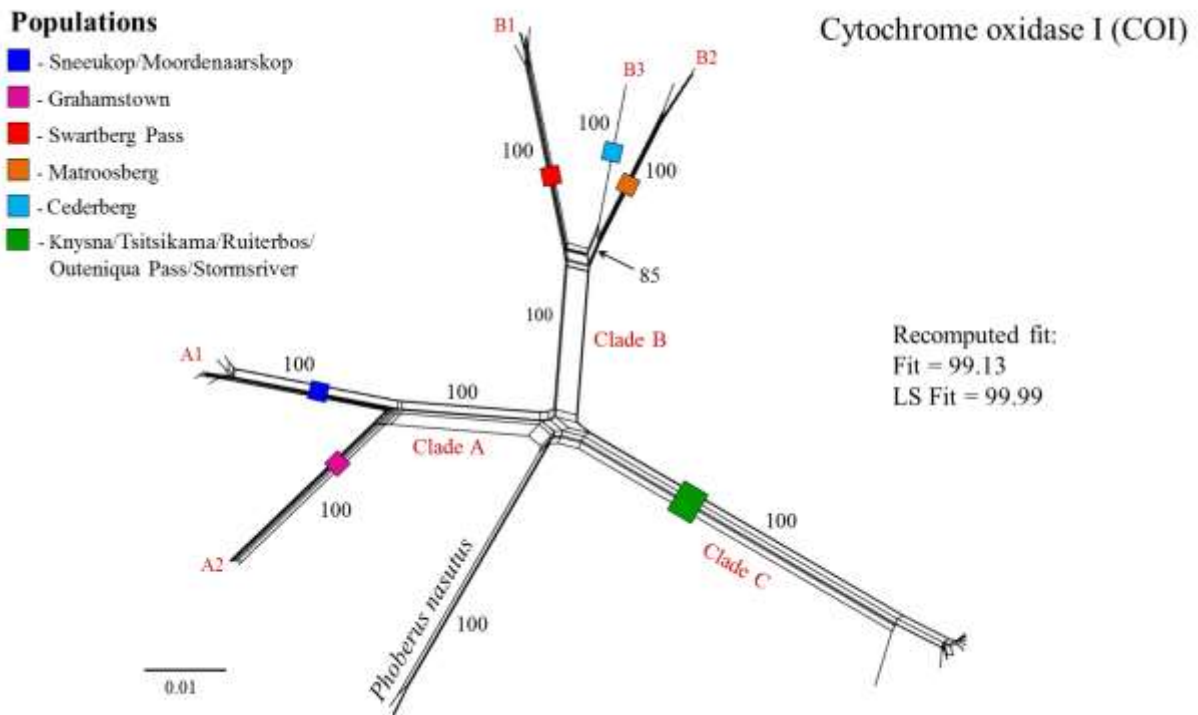
Table 2. Data characteristics and estimated model parameters for COI, 16S and combined datasets.

	COI	16S	Combined
Number of samples	56	40	56
Aligned positions	1152	456	1608
Parsimony informative sites	227	25	252
Tree length (MP)	410	31	444
Number of tree (MP)	616	4	3360
CI/RI	0.680/0.959	0.903/0.986	0.691/0.960
Best fit model (AIC)	GTR+I+G	GTR+G	GTR+G
A frequency	0.3246	0.3899	0.3467
C frequency	0.1583	0.1451	0.1574
G frequency	0.1413	0.0959	0.1260
T frequency	0.3758	0.3691	0.3698
R(a) [AC]	4.6039	2279.2626	5.0453
R(b) [AG]	18.3256	2980.1105	22.6645
R(c) [AT]	3.8626	1718.1791	3.1252
R(d) [CG]	1.0425	1.0000	1.6269
R(e) [CT]	31.2071	11551.1508	31.6934
R(f) [GT]	1.0000	1.0000	1.0000
Gamma (G)	0.4440	0.0150	0.1080
Invariable sites (I)	0.3890	-	-

CI = consistency index, RI = retention index, MP = Maximum Parsimony, AIC = Akaike Information Criterion.

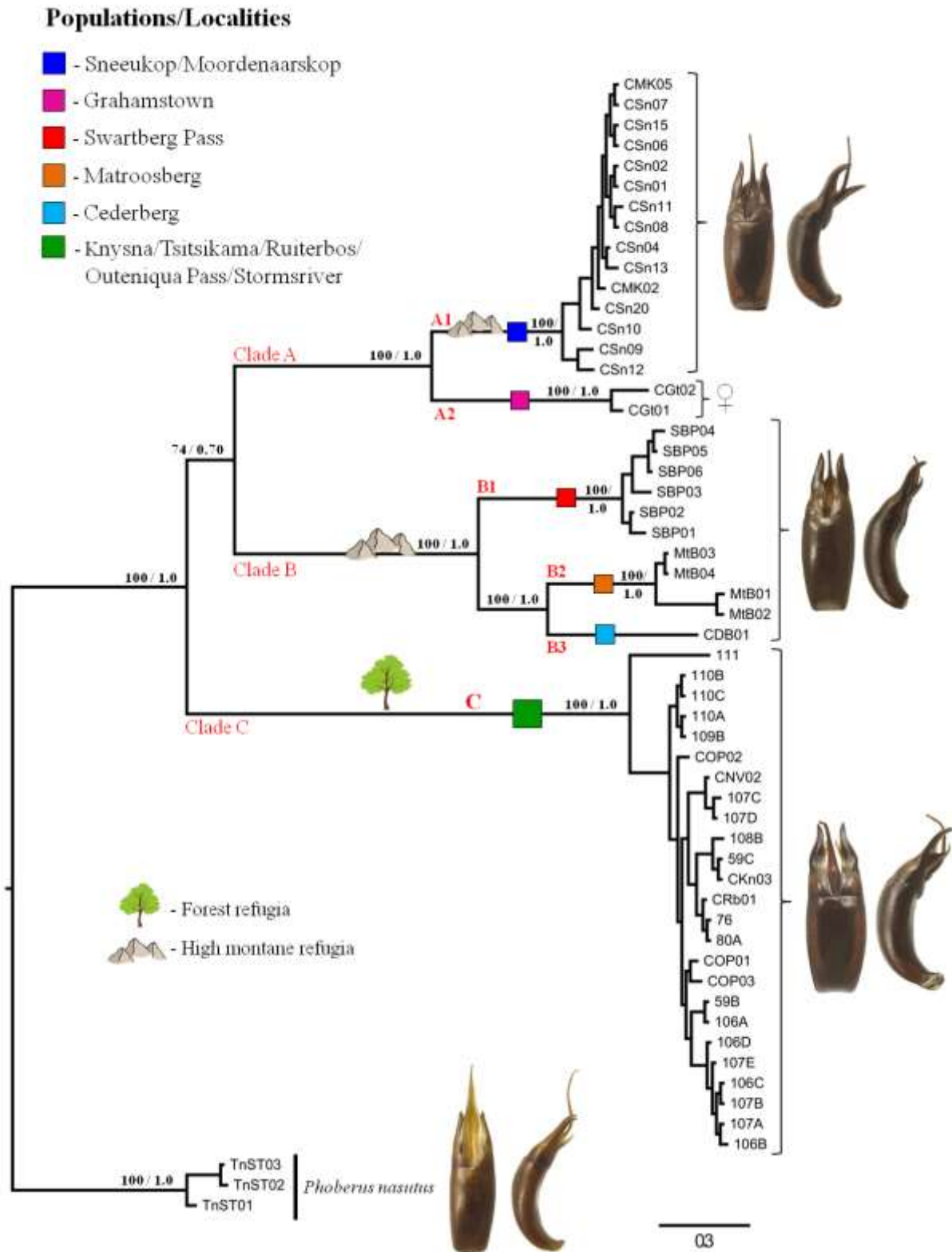
The heuristic search from the parsimony analysis produced 3360 most parsimonious trees, with tree length = 444, consistency index (CI) = 0.691 and retention index (RI) = 0.960. The strict consensus tree, with nodal support, is presented in Fig A1 in the Appendix. Trees obtained from the BI and ML analyses yield very similar topologies, hence only the Bayesian phylogram is presented with Bayesian posterior probability (PP) and ML bootstrap support (BS) for nodes (Fig 3). The resulting neighbour-net splits tree (Fig 2) for the combined dataset had a recomputed fit = 93.01, and LS fit = 99.48. Individual gene trees and splitsgraphs show similar phylogenetic groupings as for the combined dataset and are thus not presented.

Figure 2: Neighbour-net graph for the mtDNA COI dataset with bootstrap support, based on uncorrected p -distances.



Three distinct evolutionary lineages (nodes A, B and C), each with strong nodal support (PP = 1.0, BS = 100; Fig 2 and 3), were recovered across all methods of analysis. Clade A is divided into two well-supported (PP = 1.0, BS = 100; Fig 2 and 3), geographically separated populations, Sneekop/Moordenaarskop (node A1) and Grahamstown (node A2). Clade B split into three well-supported (PP = 1.0, BS = 100) geographically discrete populations, representing the Swartberg Pass (node B1), Matroosberg (node B2) and Cederberg (node B3) sampling localities. Clade C (PP = 1.0, BS = 100; Fig 2 and 3) comprises taxa from several localities: Knysna, Tsitsikama, the Outeniqua Pass, Ruiterbos and Stormsriver. Phylogenetic relationships in this clade are not well-resolved and taxa are interspersed on the tree, despite obvious geographical separation among the populations.

Figure 3: Bayesian topology from the MrBayes analysis for the combined dataset. Numbers next to each node are (ML) bootstrap support and the (BI) posterior probabilities. Images depict the male genitalia type for the respective lineages.



For COI, pairwise genetic distances between defined groups (Table 3) range from 5.4% to 11.9%, and for 16S between 0.4% and 2.6%. The percentage pairwise genetic distance, for COI within clades (A,B,C) was low overall, with 3.93% for clade A, 1.4% for clade B and 0.5% for clade C, and for 16S it was 0.5% for clade A, 0.1% for clade B and 0.1% for clade C.

Table 3. Percentage pairwise genetic distances between and within clades for COI (below the diagonal) and 16S (above the diagonal).

	A1	A2	B1	B2	B3	C
A1		0.7%	1.9%			2.3%
A2	5.4%		1.9%			2.3%
B1	10.4%			0.7%	0.4%	2.6%
B2	10.4%		5.7%		0.2%	
B3	10.4%		6.0%	4.9%		
C	11.7%		11.9%			

Genetic structure and population genetics

Among the 53 individuals of *P. capensis* sequenced for COI, 32 different haplotypes were identified while only 11 haplotypes were identified from the 38 individuals sequenced for 16S. Haplotype diversity for COI was high (0.962 ± 0.014) and somewhat lower but more variable for 16S (0.83 ± 0.031). Nucleotide diversity for COI was 0.069 ± 0.0035 with the average nucleotide differences being 79.827, while 16S had a nucleotide diversity of 0.01834 ± 0.00142 and an average nucleotide difference of 8.215. Statistics of general nucleotide diversity are summarised in Table 4. There were no shared haplotypes among the three clades (A, B, C) and between populations within clades, indicating no gene flow between these populations or possible sampling artefacts, e.g. small sample sizes. The fixation value (F_{st}) of 0.8570 ($p < 0.001$) from the AMOVA analysis indicated strong genetic structure between the

three clades. Differences among the three groups accounted for 85.70% of the variance, while 14.30% could be attributed to variation among populations within groups.

Table 4. Summary of F_{st} statistics calculated by AMOVA (Excoffier & Lischer 2010) for *Phoberus capensis*.

Source of Variation	F_{st}	%	P
Among-groups variation		85.7	< 0.001
Within-groups variation		14.3	< 0.001
Fixation index	0.8570		< 0.001

Divergence times

The divergence time analyses indicate that the *P. capensis* complex is 3.77 or 6.03 Myr old, for 0.012 and 0.0075 s/s/Myr, respectively (Table 5). *Phoberus capensis* diverged from its closest relative, *P. nasutus* (outgroup), at 4.3 or 6.85 mya. Divergence within clade B occurred 2.06 or 3.76 mya, while divergence within clade A occurred 1.95 or 3.06 Mya. Clade C diverged around 1.33 or 2.13 mya.

Table 5. Time to most recent common ancestor (in millions of years) for the lineages identified in the phylogenetic tree (Fig 3).

Clade	Substitution rate	
	0.012	0.0075
Clade A	1.95 (0.98, 3.36)	3.06 (1.47, 5.29)
Sneeukop/Moordenaarskop (A1)	0.85 (0.31, 1.78)	1.34 (0.5, 2.79)
Clade A+Clade B	3.3 (1.95, 5.24)	5.26 (3.05, 8.17)
Clade B	2.06 (1.09; 3.41)	3.27 (1.68, 5.44)
Matroosberg (B2) + Cederberg (B3)	1.34 (0.56; 2.36)	2.12 (0.91, 3.80)
Matroosberg (B2)	0.53 (0.15; 1.16)	0.84 (0.23, 1.86)
Swartberg Pass (B1)	0.58 (0.17; 1.33)	0.93 (0.25, 2.18)
Clade C	1.33 (0.54, 2.70)	2.13 (0.81, 4.24)
Ingroup	3.77 (2.19, 5.96)	6.03 (3.47, 9.43)
Root	4.3 (2.46, 6.94)	6.85 (3.85, 11.01)

Upper and lower 95% confidence limits are shown in parentheses.

Morphology

Pronotal and elytral features in *P. capensis* vary greatly with size of the individual. In smaller specimens, many features tend to be more irregular or obscured (for example intercostal punctures and foveae) and/or pronounced (like tubercles and ridges), thus making it difficult to identify characters that can be used to identify clades/groups within *P. capensis*. Despite the lack of diagnostic pronotal and elytral characters, fairly consistent differences exist between specimens of clade B and clades A and C. Specimens from the Cederberg, Matroosberg and Swartberg Pass (Clade B) are generally smaller (4-7mm) and have the discal area of the pronotum more evenly rounded, with a shallow median depression and tubercles that are not distinctly raised. Members of clade A and C are larger (5-10mm) and the discal area of the pronotum is raised, with a deep median depression and distinct tubercles. Examination of the male genitalia shows three major types corresponding to the recovered clades A1, B and C. However, variation in the male genitalia within each clade, the small number of male specimens available for study and the lack of male specimens from Grahamstown (A2), make it difficult to distinguish between all clades on the basis of male genitalia alone.

Discussion

Phylogeny and divergence time estimates

Despite the small sample sizes from some localities, the results of this study indicate there are at least three well-supported evolutionary lineages within *P. capensis* with high (10-12%) sequence divergence between them at the COI locus, which is similar to that found for other flightless scarabaeoids (Sole & Scholtz 2013; Switala et al. 2014). The high sequence divergence between the three clades justifies recognising them as distinct species using previously-cited threshold values recorded between closely related insect species (Herbert et al., 2003; Price et al. 2007; Astrin et al. 2012; for limitations of genetic distance as a criterion

in delineating species see Ferguson (2002) and Brower (2006)). All three clades (A, B and C) are geographically discrete with apparently no dispersal between them (Fig 1).

The results show that clades A and B may comprise divergent and geographically distinct populations which can be interpreted as phylogenetically independent lineages. However, these populations are morphologically indiscernible and due to the small number of specimens obtained from the different localities, we refrain from drawing any conclusions regarding the (taxonomic) status of these populations and will not discuss them further here.

Molecular dating hypothesised the diversification of the *P. capensis* group during the Pliocene-Pleistocene. This time period was characterised by habitat and vegetation shifts associated with climatic change in the CFR (Cowling et al. 2009; Swart et al. 2009; Tolley et al. 2006), and the contraction of forested areas and with the establishment of 'fynbos' as the spatially dominant vegetation (~3 to 5 mya; Linder 2003; Galley & Linder 2006). Based on results of this study, it is suggested that *P. capensis* experienced climatically-driven allopatric speciation with sheltered Afrotemperate forest and high mountain peaks serving as important refugia during climatic ameliorations (Stuckenberg 1962; Endrödy-Younga 1978; Picker & Samways 1996; Voje et al. 2009; Daniels et al. 2013). *Phoberus capensis* thus represents a speciation process in which flight-restricted populations evolved in close allopatry, possibly as recently as the Pleistocene.

Taxonomic consideration

These results indicate that the samples represented at least three distinct species according with the phylogenetic species concept (Nixon & Wheeler 1990; Wheeler & Platnick 2000). The Hottentots-Holland clade (clade A) from the Sneekop/Moordenaarskop localities has

morphology identical to the type material of *Phoberus capensis* (Scholtz 1979). The type locality for *P. capensis* is the nearby Jonkershoek Mountain (Scholtz 1980). In stark contrast, situated about 600 km east of the Hottentots-Holland Mountains is the Grahamstown sister population (A2) and the most easterly distribution for *P. capensis*. This population may represent a distinct lineage, but with only two female specimens at our disposal its status remains uncertain and warrants further investigation.

The Swartberg assemblage (clade B), comprises specimens collected from three different mountain ranges, Cederberg, Matroosberg and Swartberg. They are morphologically uniform and can be distinguished from the other two lineages by differences on the pronotum and male genitalia. The different mountain populations are genetically divergent and may represent novel lineages but this requires further investigation. Extensive sampling is needed in the region to delineate distribution boundaries.

The Southern Cape clade (clade C) has a large and fragmented distribution mostly in the Afrotemperate forests along the southern coast (Knysna and Tsitsikama) and in the high-lying forest fragments in the CFM (Outeniqua, Tsitsikama and Langeberg Mountain ranges). The unresolved phylogenetic relationships within the clade, their low divergence, and close association with Afrotemperate forests, suggest that these localities/populations may have been connected in the recent past when forests were widely continuous in southern and eastern Africa (Partridge et al. 1999).

On the basis of the differences discussed above we describe two novel species. *Phoberus capensis* (Scholtz) has had an inconstant taxonomic history. In his original description Scholtz (1979), at the time, treated *P. capensis* as a member of the genus *Trox* Fabricius, and

placed it in the subgenus *Phoberus* MacLeay in his revision of the Afrotropical species (Scholtz 1980), and in his revision of the Trogidae (Scholtz 1986), *T. capensis* was transferred to *Trox* s.str. However, recent molecular evidence suggests that all Afrotropical species of *Trox* form a monophyletic group, falling within *Phoberus* (Strümpher et al. 2014). Strümpher et al. (2014) were explicit that *Phoberus* merits higher taxonomic status and in their paper *Trox capensis* has already been informally placed in *Phoberus* as a genus. Strümpher et al. (2015) subsequently restored *Phoberus* to genus rank to include all of the Afrotropical (including Madagascan endemic) species. From a nomenclatural perspective we thus consider *T. capensis* and the novel species described here as members of the genus *Phoberus* MacLeay. Descriptions of the new species are limited to characters with past diagnostic value. No differences were observed in the external morphology of males and females. Members of the species complex are morphologically similar to *P. nasutus* (Harold) and can best be distinguished on the basis of the male genitalia (Scholtz 1980).

Systematics

Type specimens

Scholtz (1979) in his original generated a large type series (225 specimens) which he deposited in collections around the world. For logistical reasons not all type material was re-examine. Nonetheless, the bulk of the type series (160 specimens), including the holotype, were retained at the Ditsong Museum of Natural History (TMSA) and the Department of Zoology & Entomology, University of Pretoria (UPSA). We deemed the number of primary types available for examination sufficient for this study. All paratypes designated by Scholtz (1979) and the collections housing type material are listed within this paper in the type material section under the description of the each taxon. Type material that could not be examined is marked by an asterisk (*) next to the repository acronym. All label data were

cited verbatim and information on type material examined was copied using “/” between lines, and “//” between labels.

Institutions to which new specimens or type material belongs or in which they have been deposited are abbreviated as follows:

AMNH: American Museum of Natural History, New York, NY, USA

BMNH: The Natural History Museum, London, UK

HMUG: Hunterian Museum, University of Glasgow, Glasgow, UK

HNHM: Hungarian Natural History Museum, Budapest, Hungary

ISNB: Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium

MNHN: Muséum National d’Histoire Naturelle, Paris, France

MZLU: Museum of Zoology, Lund University, Lund, Sweden

NMK: National Museums of Kenya, Nairobi, Kenya

NCSA: National Collection of Insects, Roodeplaat, Pretoria, South Africa

NHRS: Naturhistoriska Riksmuseet, Stockholm, Sweden

RMCA: The Royal Museum for Central Africa, Tervuren, Brussels, Belgium

SAM: Iziko South African Museum, Cape Town, South Africa

SMTD: Staatliches Museum für Tierkunde, Dresden, Germany

SMW: State Museum, Windhoek, Namibia

TMSA: Ditsong Museum of Natural History, Pretoria, South Africa

UPSA: Department of Zoology & Entomology, University of Pretoria, South Africa

ZMUC: Zoological Museum, University of Copenhagen, Copenhagen, Denmark

ZSBS: Zoologische Sammlung des Bayerischen Staates, Munich, Germany

Genus: *Phoberus* MacLeay, 1819

Phoberus MacLeay, 1819: 137-138; Strümpher et al. 2014: 557-558; Strümpher et al. 2015: 15.

Phoberus capensis (Scholtz) (Plate A: 1a, b; Figs 2, 3: Clade A)

Trox capensis Scholtz 1979:174

Trox (Phoberus) capensis Scholtz, 1980:71

Trox (Trox) capensis Scholtz, 1986: 361

Phoberus capensis Strümpher et al., 2014: Table S1 ff.; Strümpher et al., 2015: 27.

Diagnosis

Phoberus capensis is morphologically similar to *P. disjunctus* sp. n., but can be distinguished from the latter by the raised discal area of the pronotum and deep median depression and distinct tubercles. *Phoberus disjunctus* sp. n. has the discal area of the pronotum more evenly rounded, with a shallow median depression and tubercle not distinctly raised. Male genitalia of both species are distinct (Plate A: 1b).

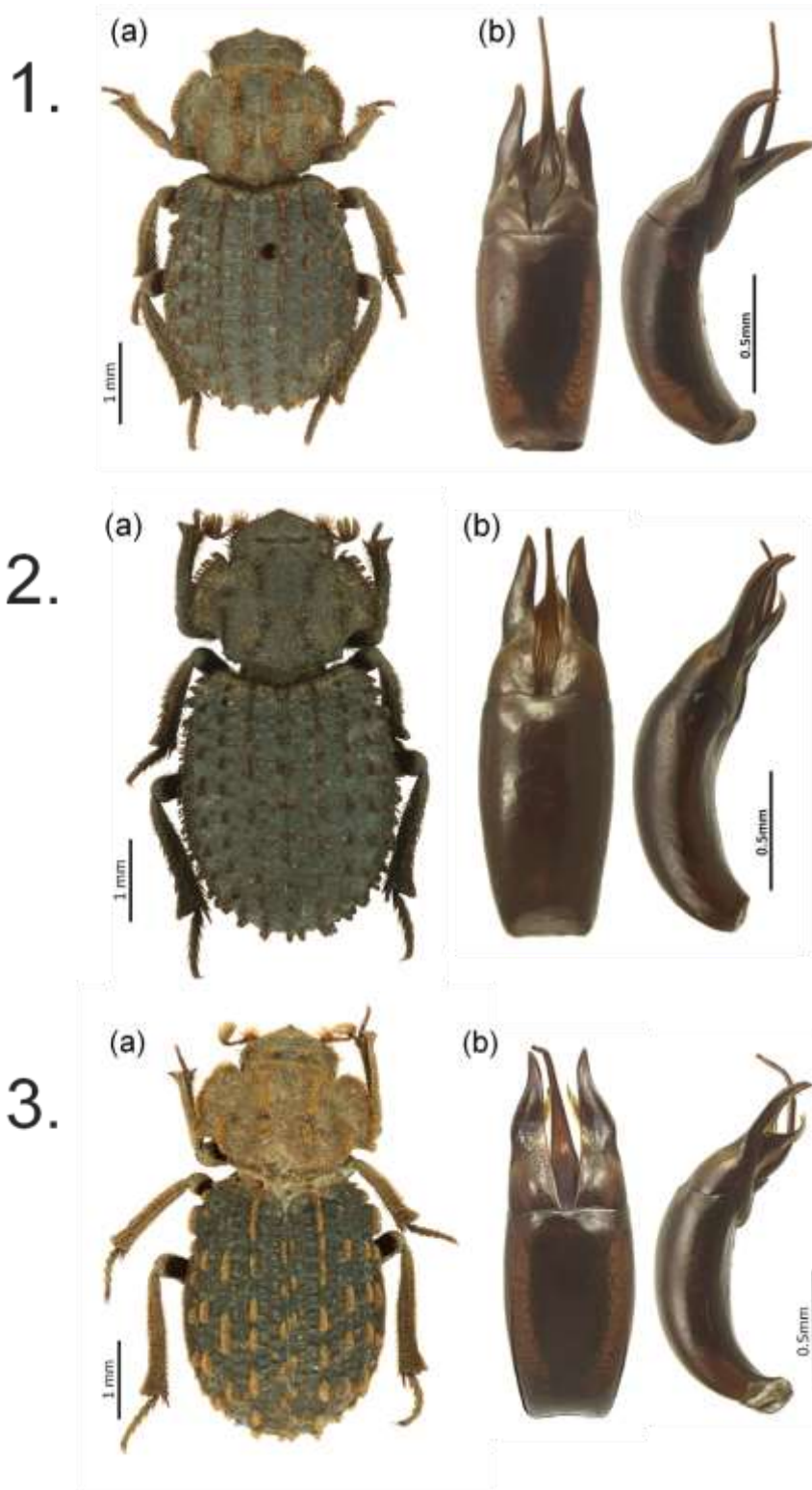
Redescription

Size: 7-8 mm, width: 4-5 mm ($n = 20$).

Head: Clypeus triangular; frons with two oval setose ridges; antennal scape setose, slightly longer than wide, pedicel attached to apex of scape.

Pronotum: Attenuated anteriorly, sides broadly flattened, surface pitted; lateral margins with fringes of short setae; discal area raised, median depression deep divided into two halves by low transverse ridge, anterior half of median depression broad, posterior half narrow; discal ridges high, interrupted approximately in middle; median basal tubercles fused to discal

Plate A: 1(a) Adult habitus of *Phoberus capensis* (Scholtz); **1(b)** Aedeagus of *Phoberus capensis* (Scholtz): dorsal and lateral view. **2(a)** Adult habitus of *Phoberus disjunctus* sp. n.; **2(b)** Aedeagus of *Phoberus disjunctus* sp. n.: dorsal and lateral view. **3(a)** Adult habitus of *Phoberus herminae* sp. n.; **3(b)** Aedeagus of *Phoberus herminae* sp. n.: dorsal and lateral view.



ridges; another ridge is present laterad of, and parallel to, the median ridges, ridges and tubercle setose.

Elytra: Scutellum small and oval, humeral calli absent; sides flattened; lateral margins with fringes of short setae; even numbered costae distinct, tubercles with tufts of setae, tubercles raised, single or partially fused to form distinct ridges; odd numbered costae indistinct; intercostal punctures irregular large and shallow; elytral profile convex, attaining maximum height behind middle.

Male genitalia: parameres symmetrical, attenuated towards the apex, parameres curving distinctly downwards, pars basalis thick, not distinctly c-shaped and slightly longer than parameres, ratio of length of parameres to length of pars basalis about 1:1. Median lobe exposed in dorsal view, flattened basally and attenuated sharply into long, narrow pointed projection (flagellum) extending beyond parameres; pair of blade-like projections ventrally between parameres (Plate A: 1b).

Distribution: *Phoberus capensis* is restricted to the Hottentots-Holland Mountain range in the greater Cape Fold Mountain Range and confined to the high mountain peaks (Fig 1).

Type material: Holotype, ♂: Jonkershoek Mnt. / Forest Reserve / SE 33 19 Cc / 20.XII.1978 / C.H. Scholtz (TMSA); Paratypes: Ditto (10 UPSA*; 3 BMNH*; 3 MNHMS*; 3 NHRS*; 3 ISNB*; 3 RMCA*; 3 ZSBS*; 3 HNHM*; 3 NCSA*; 3 SMW*; 3 SAM*); S. Afr., S.W. Cape / Nuweberg, Sneekop / 34.03S - 18.59E // 16.XI.1973, E-Y: 265 / ground traps, 130 days / leg. Endrödy-Younga (30 TMSA; 1 UPSA; 1 BMNH*; 1; MNHN*; 1 NHRS*; 1 ISNB*; 1 RMCA*; 1 ZSBS*; 1 HNHM*; 1 NCSA*; 1 SMW*; 1 SAM*); S. Afr., W. Cape / Nuweberg Camp / 34.03S 19.04E // 14.XI.1973, E-Y: 244 / groundtrap: fish / leg. Endrödy-Younga (13 TMSA; 1 UPSA; 1BMNH*; 1 ISNB*; 1 RMCA*; 1 ZSBS*); S. Afr.: W. Cape / Hawequas / 33°34'S 19°08'E // 5.XI.1973, E-T: 202 / sifted litter / leg. / Endrödy-Younga (2 TMSA); Stellenbosch / 20.7.1965 / H. Geertsema (1 UPSA).

Type material examined bears the new type label: HOLOTYPE [or PARATYPE] / *Phoberus capensis* (Scholtz) / Strümpher et al. 2015 (printed on white rectangle with red border).

Additional material examined: RSA: Western Cape Prov. / Caledon, Sneekop / Hottentots-Holland Mnts. / S34.05000° E18.98333 / 16.XI.1973, E-Y: 265 / Soil trap, Coll: EY-STR (20 TMSA).

Phoberus disjunctus Strümpher **sp. n.** (Plate A: 2a, b; Figs 2, 3 Clade B)

Diagnosis

Phoberus disjunctus is similar to *P. capensis* but can be distinguished from the latter by an evenly rounded pronotal disc and shallow median depression; *P. capensis* has a high pronotal disc with a deep median depression. Male genitalia of both species are distinctive (Fig 3; Plate A).

Description

Size: 5-6 mm, width: 3-4 mm ($n = 25$).

Pronotum: discal area of pronotum evenly rounded, with shallow median depression, tubercle not distinctly raised.

Male genitalia: parameres symmetrical, attenuated towards the apex, in lateral profile straight and curved apically; pars basalis longer than parameres, the ratio of length of parameres to length of pars basalis about 1:1.5, pars basalis variable in shape and thickness; median lobe exposed in dorsal view, flattened basally and attenuated sharply into short narrow pointed projection (flagellum) only slightly longer than parameres; genitalia in ventral view with a pair of blade-like projections between parameres (Plate A: 2b).

Distribution: This species is distributed (disjunctly) along the Swartberg, Matroosberg and the Cederberg Mountains in the greater Cape Fold Mountain Range and confined to the high mountain peaks (Fig 1).

Type material: Holotype ♂: S. Afr.: Cape, Cederberg / Jeep track, 1380 m / 32.24S - 19.10E // 01.IX.1981, E-Y: 1878 / ground traps, 63 days / leg. Endrödy-Younga // ground traps with meat bait // *Trox capensis* Scholtz / det. C.H. Scholtz (white rectangle printed) (TMSA);

Paratypes: S. Afr.: Cape, Cederberg / Jeep track, 1380 m / 32.24S - 19.10E // 01.IX.1981, E-Y: 1878 / ground traps, 63 days / leg. Endrödy-Younga // ground traps with meat bait // *Trox capensis* Scholtz / det. C.H. Scholtz (white rectangle printed) (2 TMSA); S. Afr: Swartberge / Blesberg - E[ast], 2000 m / 23[33].25S - 22.41E // 06.XI.1978, E-Y: 1508 / ground traps, 40 days / leg. Endrödy-Younga // ground traps with faeces bait (9 TMSA); S. Afr: Swartberge / Blesberg - E[ast], 2000 m / 23[33].25S - 22.41E // 05.XI.1978, E-Y: 1505 / ground traps, 41 days / leg. Endrödy-Younga // ground traps with faeces bait (10 TMSA); S. Afr: Swartberge / Blesberg - W[est], 1820 m / 33.25S - 22.40E // 06.XI.1978, E-Y: 1512 / ground traps, 41 days / leg. Endrödy-Younga // ground traps with meat bait (1 TMSA); S. Afr: Swartberge / Blesberg - W[est], 1820 m / 33.25S - 22.40E // 06.XI.1978, E-Y: 1513 / on flowers / leg. Endrödy-Younga (1 TMSA); S. Afr: Swartberge / Blesberg - W[est], 1850 m / 33.24S - 22.40E // 07.XI.1978, E-Y: 1514 / ground traps, 40 days / leg. Endrödy-Younga // ground traps with meat bait (4 TMSA); RSA: Western Cape Province / Swartberg Range, Blesberg / 33.25S 22.41E, 1820 m // 16.XII.1987, E-Y: 1532B / leg. Endrödy-Younga, (1 NCSA; 4 TMSA; 2 UPSA); RSA: Western Cape Prov. / Swartberg Pass, nr. Oudtshoorn / 13.III.1979, ground traps (meat) / Coll: Breytenbach (3 UPSA).

Type material examined bears the new type label: HOLOTYPE [or PARATYPE] / *Phoberus disjunctus* sp.n. / Strümpher et al. 2015 (printed on white rectangle with red border).

Etymology: From the Latin *disjunctus*, meaning separated or disconnected, in reference to the disjunct montane distribution of this species.

Phoberus herminae Strümpher **sp. n.** (Plate A: 3a, b; Figs 2, 3 Clade C)

Diagnosis

Phoberus herminae has very similar external morphology to *P. capensis*, and the only reliable means of identification involves the male genitalia. Constant differences exist in the shape of the parameres and the median lobe (Fig 3; Plate A).

Description

Size: 7-10 mm, width: 4-6 mm ($n = 28$).

External morphology as for *P. capensis*.

Male genitalia: parameres symmetrical, attenuated towards the apex, parameres curved, pars basalis thick, c-shaped and longer than parameres, ratio of length of parameres to length of pars basalis about 1:1.5. Median lobe exposed in dorsal view, flattened basally and attenuated broadly into narrow pointed projection (flagellum) extending beyond parameres, often with knob-like structure on apex; genitalia with a single pair of ventral blade-like projections between parameres (Plate A: 3b).

Distribution: This species is distributed (disjunctly) along coastal edge and the Tsitsikama, Outeniqua and Langeberg Mountain Ranges of the south Western Cape Province (Fig 1).

Type material: Holotype ♂: S. Afr., Cape Prov. / Knysna forest / 22km N Knysna // 08-12.III.1992 / leg. J. Klimaszweski (TMSA). Paratypes: S. Afr., Cape Prov. / Knysna Forest / 22km N Knysna // 08-12.III.1992 / leg. J. Klimaszweski (TMSA 2; UPSA 2); Baviaanskloof

Mnts. / Studtis, SE 33 23 Db / 1627m, 09.VIII.1978 / E. Breytenbach (3 UPSA; 1 BMNH*; 1 MNHN*; 1 NHRS*); S. Afr., S. Cape / Harkerville Forest / 34.04.S - 23.10E // 13.12.1976 / E-Y: 1311 / ground traps, 4 days / leg. Endrödy-Younga // ground traps with / faeces bait (6 TMSA; 2 UPSA; 1 MNHN*); Bloukrans Forest / Knysna, SE 34 23 Ab / Jan.1978 / C.H. Scholtz (1 UPSA); Goudveld Forest / Knysna, SE 33 22 Dd / Jan. 1978 / C.H. Scholtz (1 UPSA; 1 SMTD*); Kafferkop Forest / Knysna, SE 34 23 Aa / Jan.1978 / C.H. Scholtz (1 UPSA); Tsitsikama Forest / SE 33 23 Dc, Jan.1978 / C.H. Scholtz (2 UPSA); S. Afr., S. Cape / Keurboomstrand / 34.00S – 23.27.E // 18.12.1976, E-Y: 1301 / ground traps 8 days / leg. Endrödy-Younga (4 TMSA; 1 UPSA); Bergplaas Forest / Knysna, SE 33 22 Dc / Jan.1979 / J.H. Botha (2 UPSA; 2 AMNH*; 2 ZMUC*; 2 HMUG*; 1 ISNB*; 2 NMK*; 1 RMCA*); S. Afr.: S. CapeMt. / Grootberg, W., 1450 m / 33.55S 20.51E // 2.XI.1978, E-Y: 1501 / groundtraps, 35 days / leg. Endrödy-Younga // ground traps with faeces bait (49 TMSA). Ysternek Forest / Knysna, SE 33 23 Cc / Jan.1978 / C.H Scholtz (1 UPSA; 1 RMCA*; 1 ZSBS*); Diepwalle Forest / Knysna, 33 23 Cc / Jan.1978 / C.H. Scholtz (1 MZLU*).

All type material examined bears the new type label: HOLOTYPE [or PARATYPE] / *Phoberus herminae* sp. n. / Strümpher et al. 2015 (printed on white rectangle with red border).

Additional material examined: RSA: Western Cape Province / Outeniqua Pass, 25.XII.2007 / S33.90000° E22.40107°, 668m / Baited pitfall trap, C Deschodt (5 UPSA); S. Afr: Cape Prov. / Lily Vlei Nat. Res. / 33.56S 23.02E // Gouna State Forest, IV.1983, , J. Koen (12 TMSA; 4 UPSA); S. Afr.: Cape Prov. / Diepwalle St. Forest / 33.56S 23.09E // January1984 / dry forest litter / leg. J. Koen (2 UPSA); Diepwalle St. Forest / 33.56S 23.09E // February 1984 / dry forest litter / leg. J. Koen (1 TMSA); Diepwalle St. Forest / 33.56S 23.09E // 02.

1984 / wet forest / leg. J. Koen (1 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // January 1985 / pitfall trap / leg. J. Koen (2 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // February 1985 / pitfall trap / leg. J. Koen (2 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // 7. 1985 / pitfall trap / leg. J. Koen (3 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // 10. 1985 / pitfall trap / leg. J. Koen (3 TMSA); S. Afr.: SE Cape Mts / Baviaanskloofberg / 33.36S - 24.23E // 15.XI.1982, 1035 m. / groundtraps, 30 days / leg. W. Breytenbach // ground traps with meat bait (8 TMSA).

Etymology: It is with great pleasure that I name this species after my wife Hermien Viljoen for her support of my entomological pursuits.

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Appendix

Table A1. List of taxa and collection data of trogid beetles used in this study.

Species	Locality data	Code	16S	CO1
Outgroup				
<i>Phoberus nasutus</i>	RSA: Western Cape Prov., near Simonstown, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt (UPSA)	TnST01	KC801097*	Y
<i>P. nasutus</i>	RSA: Western Cape Prov., near Simons Town, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt (UPSA)	TnST02	KC801096*	Y
<i>P. nasutus</i>	RSA: Western Cape Prov., near Simons Town, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt (UPSA)	TnST03	Y	Y
Ingroup				
<i>Phoberus capensis</i>	RSA: Western Cape Prov., Moordenaarskop, 940m, 15.XII.2007, S34.09809° E18.96465°, WP Strümpher (UPSA)	CMK02	Y	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Moordenaarskop, 940m, 15.XII.2007, S34.09809° E18.96465°, WP Strümpher (UPSA)	CMK05	X	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn01	Y	Y

Species	Locality data	Code	16S	CO1
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn02	Y	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn04	Y	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn06	X	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn07	X	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn08	X	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn09	Y	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn10	Y	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn11	X	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn12	Y	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn13	Y	Y
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpfer (UPSA)	CSn15	X	Y

Species	Locality data	Code	16S	CO1
<i>P. capensis</i>	RSA: Western Cape Prov., Sneekop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn20	Y	Y
<i>P. capensis</i>	RSA: Eastern Cape Prov., Grahamstown, Signal Hill, 620m, S33°20'07" E26°32'50", 03.IX.2008, WP. Strümpher (UPSA)	CGt01	X	Y
<i>P. capensis</i>	RSA: Eastern Cape Prov., Grahamstown, Signal Hill, 620m, S33°20'07" E26°32'50", 03.IX.2008, WP. Strümpher (UPSA)	CGt02	Y	Y
<i>Phoberus disjunctus</i> sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB01	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB02	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB03	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB04	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP01	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP02	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP03	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP04	Y	Y

Species	Locality data	Code	16S	CO1
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP05	Y	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP06	X	Y
<i>P. disjunctus</i> sp. n.	RSA: Western Cape Prov., Cederberg Conservancy, 1547m, S32°24'22" E19°04'50", 12.XII.2011, WP. Strümpher & CH. Scholtz (UPSA)	CDB01	Y	Y
<i>Phoberus herminae</i> sp. n.	RSA: Western Cape Prov., Outeniqua Mountains, Outeniqua Pass, 668m, S33.90000° E22.40107°, 25.XII.2007, C Deschodt (UPSA)	COP01	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Outeniqua Mountains, Outeniqua Pass, 668m, S33.90000° E22.40107°, 25.XII.2007, C Deschodt (UPSA)	COP02	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Outeniqua Mountains, Outeniqua Pass, 668m, S33.90000° E22.40107°, 25.XII.2007, C Deschodt (UPSA)	COP03	Y	Y
<i>P. herminae</i> sp. n.	RSA: Eastern Cape Prov., Langeberge, Ruiterbos, 800m, S33°52'31" E22°01'22", 15.XI.2004, C. Deschodt (UPSA)	CRb01	Y	Y
<i>P. herminae</i> sp. n.	RSA: Eastern Cape Prov., Langeberge, Ruiterbos, 800m, S33°52'31" E22°01'22", 15.XI.2004, C. Deschodt (UPSA)	76	Y	Y
<i>P. herminae</i> sp. n.	RSA: Eastern Cape Prov., Langeberge, Ruiterbos, 800m, S33°52'31" E22°01'22", 15.XI.2004, C. Deschodt (UPSA)	80A	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Natures Valley, 38m, S33°57'55" E23°33'36", 07.VIII.2009, WP. Strümpher (UPSA)	CNV02	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Natures Valley, 40m, 33°58'02" E23°33'36", 02.VII.2004, C. Deschodt (UPSA)	108B	Y	Y

Species	Locality data	Code	16S	CO1
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Diepwalle Forest, 4 30m, S33°57'40" E23°09'22", 15.VI.2003, C. Deschodt (UPSA)	CKn03	X	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Diepwalle Forest, 4 30m, S33°57'40" E23°09'22", 15.VI.2003, C. Deschodt (UPSA)	59B	X	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Diepwalle Forest, 4 30m, S33°57'40" E23°09'22", 15.VI.2003, C. Deschodt (UPSA)	59C	X	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Buffalo Valley, 81m, S33°59'29" E 23°17'55", 02.VII.2004, C. Deschodt (UPSA)	109B	X	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Buffalo Valley, 118m, S33°58'56" E 23°18'06", 02.VII.2004, C. Deschodt (UPSA)	110A	X	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Buffalo Valley, 118m, S33°58'56" E 23°18'06", 02.VII.2004, C. Deschodt (UPSA)	110B	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Buffalo Valley, 118m, S33°59'29" E 23°18'06", 02.VII.2004, C. Deschodt (UPSA)	110C	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106A	X	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106B	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106C	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106D	X	Y

Species	Locality data	Code	16S	CO1
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107A	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107B	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107C	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107D	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107E	Y	Y
<i>P. herminae</i> sp. n.	RSA: Western Cape Prov., Stormsrivier. December, 2005 (UPSA)	111	Y	Y

Y indicates PCR amplification successful, X indicates PCR amplification failed. UPSA, depository for molecular voucher. * Sequences generated by Strümpher et al. (2014).

Table A2. GenBank accession numbers for the COI haplotypes of *Phoberus capensis*, *P. disjunctus* sp. n., *P. herminae* sp. n. and *P. nasutus*

Species	Population/Locality	Haplotype	Individuals	GenBank accession No.
<i>Phoberus capensis</i>	Sneeukop / Moordenaarskop	TCAA01	CSn01, CSn02, CSn04, CSn06, CSn07, CSn08, CSn15, CMK05	KT075105
	Moordenaarskop	TCAA02	CMK02	KT075106
	Sneeukop	TCAA03	CSn20	KT075107
	Sneeukop	TCAA04	CSn11	KT075108
	Sneeukop	TCAA05	CSn13	KT075109
	Sneeukop	TCAA06	CSn10	KT075110
	Sneeukop	TCAA07	CSn09	KT075111
	Sneeukop	TCAA08	CSn12	KT075112
	Grahamstown	TCAA09	CGt02	KT075113
	Grahamstown	TCAA10	CGt01	KT075114
<i>Phoberus disjunctus</i> sp. n.	Cederberg	TCBA01	CDB01	KT075115
	Matroosberg	TCBA02	MtB01	KT075116
	Matroosberg	TCBA03	MtB02	KT075117
	Matroosberg	TCBA04	MtB03, MtB04	KT075118
	Swartberg Pass	TCBA05	SBP01	KT075119

	Swartberg Pass	TCBA06	SBP02	KT075120
	Swartberg Pass	TCBA07	SBP04	KT075121
	Swartberg Pass	TCBA08	SBP05, SBP06	KT075122
	Swartberg Pass	TCBA09	SBP03	KT075123
<i>Phoberus herminae</i> sp. n.	Outeniqua Pass	TCCA01	COP01	KT075124
	Ruiterbos	TCCA02	76, 80A, CRb01	KT075125
	Knysna	TCCA03	109B, 110A, 110B, 110C	KT075126
	Outeniqua Pass	TCCA04	COP02	KT075127
	Outeniqua Pass	TCCA05	COP03	KT075128
	Knysna	TCCA06	107A, 107B, 107E, 106C, 106D	KT075129
	Knysna	TCCA07	106B	KT075130
	Knysna	TCCA08	108B	KT075131
	Knysna	TCCA09	59C, CKn03	KT075132
	Knysna	TCCA10	106A, 59B	KT075133
	Knysna	TCCA11	107C, 107D	KT075134
	Tsitsikama	TCCA12	CNV02	KT075135
	Stormsrivier	TCCA12	111	KT075136

Phoberus nasutus

Cape Peninsula

TNA01

TnST01

KT075102

TNA02

TnST02

KT075103

TNA03

TnST03

KT075104

Table A3. GenBank accession number for the 16S haplotypes of *Phoberus capensis*, *P. disjunctus* sp. n. and *P. herminae* sp. n. * = Sequences generated by Strümpher et al. (2014).

Species	Population/Locality	Haplotype	Individuals	GenBank accession #
<i>Phoberus capensis</i>	Sneeukop / Moordenaarskop	TCAB01	CSn01, CSn02, CSn04, CSn10, CSn12, CSn13, CSn20	KT008923
	Grahamstown	TCAB02	CGt02	KT008924
<i>Phoberus disjunctus</i> sp. n.	Cederberg / Matroosberg	TCBB05	CDB01, MtB03, MtB04	KT008929
	Matroosberg	TCBB04	MtB01, MtB02	KT008928
	Swartberg Pass	TCBB01	SBP01, SBP02	KT008925
	Swartberg Pass	TCBB02	SBP03	KT008926
	Swartberg Pass	TCBB03	SBP04, SBP05	KT008927
<i>Phoberus herminae</i> sp. n.	Knysna	TCCB01	107A, 107B, 107C, 107E, 106B, 106C	KT008930
	Outeniqua Pass / Tsitsikama/ Ruiterbos / Knysna	TCCB02	107D, 80A, CRb01, 76, COP01, COP02, COP03, 110B, 110C, 108B, CNV02	KT008931
	Stormsrivier	TCCB03	111	KT008932
<i>Phoberus nasutus</i>	Cape Peninsula		TnST01	KC801097*
			TnST02	KC801096*

Figure A1. The strict consensus Parsimony tree.

Populations

- - Sneekop/Moordenaarskop
- - Grahamstown
- - Swartberg Pass
- - Matroosberg
- - Cederberg
- - Knysna/Tsitsikama/Ruiterbos/
Outeniqua Pass/Stormsriver

