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Latitudinal distribution and mitochondrial DNA (COI) variability of *Stereotydeus* spp. (Acari: Prostigmata) in Victoria Land and the central Transantarctic Mountains

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Abstract: We examined mitochondrial DNA (COI) variability and distribution of *Stereotydeus* spp. in Victoria Land and the Transantarctic Mountains, and constructed Neighbour Joining (NJ) and Maximum Likelihood (ML) phylogenetic trees using all publicly available COI sequences for the three *Stereotydeus* species present (*S. belli*, *S. mollis* and *S. shoupi*). We also included new COI sequences from Miers, Marshall and Garwood valleys in southern Victoria Land (78°S), as well as from the Darwin (79°S) and Beardmore Glacier (83°S) regions. Both NJ and ML methods produced trees which were similar in topology differing only in the placement of the single available *S. belli* sequence from Cape Hallett (72°S) and a *S. mollis* haplotype from Miers Valley. Pairwise sequence divergences among species ranged from 9.5–18.1%. NJ and ML grouped *S. shoupi* from the Beardmore Glacier region as sister to those from the Darwin with pairwise divergences of 8%. These individuals formed a monophyletic clade with high bootstrap support basal to *S. mollis* and *S. belli*. Based on these new data, we suggest that the distributional range of *S. shoupi* extends northward to Darwin Glacier and that a barrier to dispersal for *Stereotydeus*, and possibly other arthropods, exists immediately to the north of this area.

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Key words: Antarctica, Arthropoda, dispersal, environmental gradients, mites, Ross Dependency

Introduction

The free-living soil mite genus *Stereotydeus* Berlese, 1901 (Acari: Prostigmata) comprises a circumpolar group with a broad southern hemisphere distribution of ancient Gondwanan origin (Womersley & Strandtmann 1963, Fittkau *et al*. 1969, Spain & Luxton 1971, Olivier 2006). Within the continental and maritime Antarctic, *Stereotydeus* is represented by eight species, seven of which are endemic (Marshall & Pugh 1996). Of the eight species, three (*S. belli*, *S. mollis*, *S. shoupi*) are known from Victoria Land and the central Transantarctic Mountains (Womersley & Strandtmann 1963, Strandtmann 1967), where they exist in mostly non-overlapping succession from north to south (Fig. 1).

Stereotydeus belli Trouessart 1902 is common in northern Victoria Land where Caruso & Bargagli (2007) report that in, and near, Springtail Valley (*c*. 74°42'S), the southern limit of *S. belli* overlaps with the northern limit of *Stereotydeus mollis* Womersley & Strandtmann 1963. South of the Drygalski Ice Tongue (75°24'S), *S. mollis* becomes the dominant acarine inhabiting ice-free areas of the continent and the offshore islands in the McMurdo Sound region, including Ross and Beaufort islands (Womersley & Strandtmann 1963). The southern distributional limit of *S. mollis* is somewhat uncertain, due mostly to limited sampling. However, the species has been reported to occur as far south as Minna Bluff (*c*. 78°40'S) (Gressitt *et al*. 1963, Strandtmann 1967). Immediately to the south of Minna Bluff is an extensively ice-covered region (hatched area Fig. 1), and information on the occurrence of mites beyond this area is limited. Spain (1971) carried out the first comprehensive arthropod surveys of the ice-free regions in proximity to Darwin Glacier (*c*. 80°S) and reported a complete absence of both Collembola and Acari. However, during the summers of 2004 and 2007, individuals of *Stereotydeus* were collected from ice-free areas adjacent to the Darwin Glacier (79°49'S, 159°26'W).

South of the Darwin glacier, *Stereotydeus shoupi* Strandtmann 1967 is known from the ice free areas of the Queen Maud Mountains near the Beardmore and Shackleton glaciers (*c*. 83°–85°S) (Strandtmann 1967, Stevens & Hogg 2006). Owing to the limited field surveys of invertebrates south of Minna Bluff, the distributional limits and/or any phylogeographic breaks for *S. mollis* and *S. shoupi* were previously unknown.

Throughout their respective geographic ranges, *S. belli*, *S. mollis*, and *S. shoupi* share morphological characters that overlap (Strandtmann 1967). Furthermore, several developmental stages with variable morphological characters may be present at a particular site or at differing sites due to environmental conditions which can present a challenge when

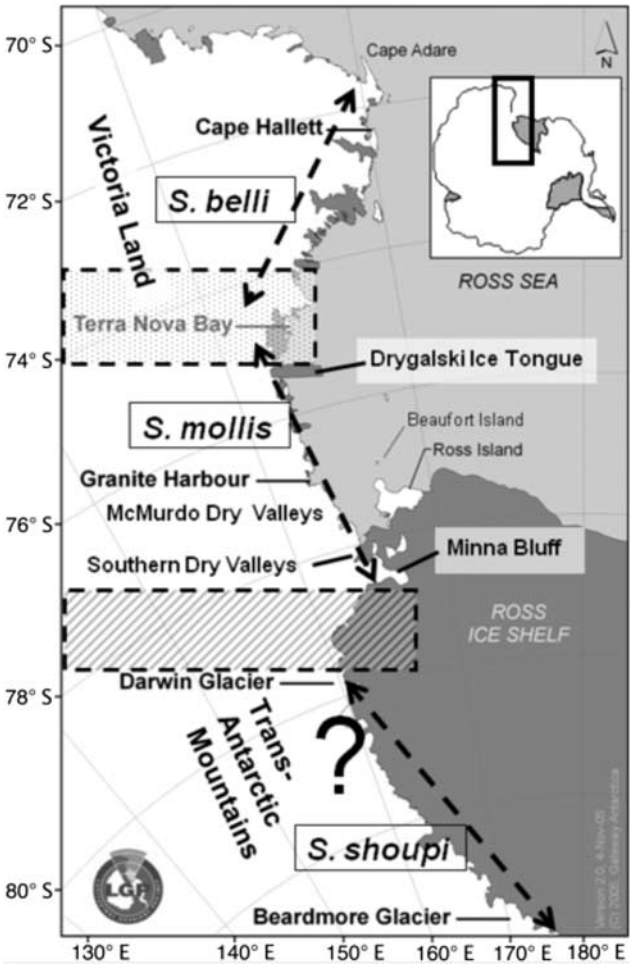

Fig. 1. Location of the Victoria Land Transantarctic latitudinal gradient (inset) showing the known, approximate distributions for three species of *Stereotydeus* (*S. belli*, *S. mollis*, *S. shoupi*). Place names are referred to in the text. McMurdo Dry Valleys include Victoria, Wright and Taylor valleys; Southern Dry Valleys include Garwood, Marshall and Miers valleys. The stippled area indicates an area of overlap in the distributions of *S. belli* and *S. mollis*. The hatched area indicates an ice-covered area that occurs between the ranges of *S. mollis* and *S. shoupi.* The question mark ("?") indicates an area where limited information is currently available on the distribution of *S. shoupi*.

using morphological characters to distinguish between species (Gressitt *et al*. 1964). For example, one of the few defining characters between *S. mollis* and *S. shoupi* is the number of microscopic setae present on the genital cover, six for *S. mollis* and seven for *S. shoupi*. However, the number of setae on each genital cover is different during each of the five developmental stages of *S. mollis* (Strandtmann 1967, Pittard 1971). Although no comparable studies for *S. shoupi* or *S. belli* have been conducted to date, it is reasonable to assume that similar characters are also likely to vary for these species, thus complicating taxonomic assignments, particularly for immature individuals.

Table I. *Stereotydeus mollis* haplotype codes used in this manuscript with cross reference to previous studies and GenBank accession numbers.

Haplotype code	Location	Stevens & Hogg (2006)	McGaughran *et al*. (2008)	GenBank accession no.
Sm1	DV	A		DQ305386
Sm2	DV	B		DQ305389
Sm3	DV,	D		DQ305391
Sm4	DV, SV,	E		DQ305398
Sm5	DV, SV	F		DQ305392
Sm6	DV, SV, RI	G		DQ305396
Sm7	DV, SV, RI	H		DQ305368
Sm8	DV, SV,	I		DQ305387
Sm9	DV, SV, RI	J		DQ305397
Sm10	DV, SV	K		DQ305385
Sm11	DV, RI	L		DQ305390
Sm12	GH	M		DQ305394
Sm13	DV	N		DQ305393
Sm14	SV, BI	O		DQ309572
Sm15	DV, BI	P		DQ305395
Sm16	DV	Q		DQ309573
Sm17	BI	R		DQ309574
Sm18	DV		S1	DQ305361
Sm19	DV		S2	DQ305362
Sm20	DV		S3	DQ305363
Sm21	DV		S4	DQ305364
Sm22	DV		S5	DQ305365
Sm23	DV		S6	DQ305367
Sm24	DV, SV		S7	DQ305369
Sm25	DV, RI		S8	DQ305370
Sm26	DV		S9	DQ305371
Sm27	DV		S10	DQ305372
Sm28	DV		S11	DQ305373
Sm29	DV		S12	DQ305374
Sm30	DV		S13	DQ305375
Sm31	DV		S14	DQ305376
Sm32	DV		S15	DQ305377
Sm33	DV		S16	DQ305378
Sm34	DV		S17	DQ305379
Sm35	DV		S18	DQ305380
Sm36	DV		S19	DQ305381
Sm37	DV		S20	DQ305382
Sm38	DV		S21	DQ305383
Sm39	DV		S22	DQ305384
Sm40	SV			HM537082
Sm41	SV			HM537083
Sm42	SV			HM537084
Sm43	SV			HM537085
Sm44	SV			HM537086
Sm45	SV			HM537087
Sm46	SV			HM537088
Sm47	SV			HM537089
Sm48	SV			HM537090
Sm49	SV			HM537091
Sm50	SV			HM537092

Locations where haplotypes were found: DV = McMurdo Dry Valleys (Taylor, Wright, and Victoria valleys and vicinity), SV = Southern Dry Valleys (Garwood, Marshall, and Miers valleys and vicinity), BI = Beaufort Island; RI = Ross Island, and GH = Granite Harbour.

To compound the subtle inter and intra-specific morphological variation in Antarctic *Stereotydeus* spp., several recent studies have found high levels of intra-specific

mtDNA (COI) variation in otherwise morphologically similar populations of *S. mollis* (Stevens & Hogg 2006, McGaughran *et al*. 2008). Pairwise COI sequence divergences of up to 17.5% have been reported between individuals of *S. mollis* from the McMurdo Dry Valleys in southern Victoria Land suggesting the possibility of cryptic species (Stevens & Hogg 2006, McGaughran *et al*. 2008). A preliminary phylogenetic analysis of *Stereotydeus* spp. by Stevens & Hogg (2006) using a 504 base pair (bp) portion of the COI gene found that *S. mollis* from the McMurdo Dry Valleys formed a polyphyletic group with *S. shoupi* from near the Beardmore Glacier. However, since their preliminary study, several more COI haplotypes of *S. mollis* have been identified which may improve the phylogenetic resolution among *Stereotydeus* spp. from Victoria Land and the Transantarctic Mountains.

Here, we more fully examine the geographic distributions and phylogenetic relationships among *Stereotydeus* spp. from Victoria Land and the Transantarctic Mountains using an analysis of all known COI sequences for *S. mollis*, *S. belli* and *S. shoupi*. To assess the phylogenetic affinities of *Stereotydeus* from Darwin Glacier we include new sequence data from the Darwin and Beardmore Glacier regions as well as from the southernmost Dry Valleys (Garwood, Marshall and Miers).

Material and methods

Sample collection

This study includes 39 previously published unique mtDNA COI haplotypes for *Stereotydeus mollis* collected from southern Victoria Land and deposited in GenBank (Stevens & Hogg 2006, GenBank accession numbers DQ305385-87, DQ305389-98, DQ309572-74; McGaughran *et al*. 2008, GenBank accession numbers DQ305361-65, DQ30567, DQ30569-84) (Table I). In addition, 12 previously unpublished *S. mollis* COI haplotypes were identified from specimens collected from the southern Dry Valleys (Garwood, Marshall and Miers) in January 2009 (Fig. 1). Due to the differing haplotype nomenclature used in previous studies, and to aid in interpretation, these data were consolidated and a more simplified nomenclature assigned. Haplotypes were aligned and renamed using the generic prefix "Sm" followed by a unique numerical character (i.e. Sm1–Sm50). Table I lists all of the unique *S. mollis* haplotypes used in this study and cross references them with those identified by both Stevens & Hogg (2006) and McGaughran *et al*. (2008).

Sequence data for *S. shoupi* from the central Transantarctic Mountains and for *S. belli* from northern Victoria Land were obtained from GenBank. (Stevens & Hogg 2006, GenBank accession numbers DQ309576 and DQ309577, respectively). In addition, mites were collected from the Darwin Glacier region (Diamond Hill) in December 2004 and January 2007 as well as from the Beardmore Glacier region (Ebony Ridge) in January 2010. All individuals were morphologically identified as *S. shoupi* using Strandtmann (1967). Six individuals of *S. shoupi* were sequenced from Darwin Glacier and one from Beardmore Glacier.

mtDNA extraction, amplification and sequencing

Total genomic DNA was extracted from individual animals using the SIGMA REDExtract-N-Amp^{TM} Tissue PCR Kit. Due to the small size of individual animals (0.5–0.75 mm), the manufacturer's recommended volume of extraction buffer was reduced by 90% to concentrate the resulting DNA extract. Following extraction, a 675 bp fragment of the mitochondrial cytochrome *c* oxidase (COI) gene was amplified using the mite specific primers COI-2R and COI-2F (Otto & Wilson 2001). PCR amplification was carried out in a 20 μl reaction containing 4 μl of extracted DNA (unquantified), 1.0 μM of each primer and 10 μl of *i*-Taq^{TM} 2X PCR master mix (iNtRON Biotechnology, Gyeonggi-do, Korea). Thermocycling conditions were: initial denaturation at 94°C for 1.5 min followed by 40 cycles of denaturation and polymerase amplification (94°C for 20 s, 55°C for 30 s and then 1.5 min at 72°C), followed by 5 min at 68°C (McGaughran *et al*. 2008).

All PCR products were purified using SAP/EXO (USB Corp, Cleveland, OH, USA). Sequencing using both forward and reverse primers was performed directly on a capillary electrophoresis ABI 3130XL genetic analyser (Applied Biosystems Inc, Foster City, CA) at the University of Waikato DNA sequencing facility.

Phylogenetic analyses

Individual sequences were confirmed as being derived from applicable taxa using the GenBank BLAST algorithm. A 504 bp (168 codons) portion of unambiguous alignment (no insertions or deletions) of the COI gene was used to match the existing dataset as reported by Stevens & Hogg (2006) and McGaughran *et al*. (2008). All sequences were aligned using Geneious Pro v4.7.6 (Drummond *et al*. 2009), and PAUP* ver.4.0b10 (Swofford 2002) was used to perform neighbour joining (NJ) analysis. Distance matrices of pairwise nucleotide sequence divergences were calculated in PAUP* using all unique sequences. Due to the number of sequences ($n = 58$) and available computing power, the Genetic Algorithm for Rapid Likelihood Inference as implemented in the computer program GARLI ver.0.951 (Zwickl 2006) was used for Maximum Likelihood analysis (ML). Several runs were performed in GARLI in order to obtain the corresponding ML tree of best fit (Zwickl 2006). The prostigmatid mite *Eriorhynchus* sp. (GenBank accession number AF142135; Otto & Wilson 2001) was used as an outgroup as it was the most closely related taxon available on GenBank (Stevens & Hogg 2006). χ^2 tests as employed in PAUP* were used to determine whether the assumption of equal base frequencies among sequences was violated on all sites and third codon positions only. jModeltest

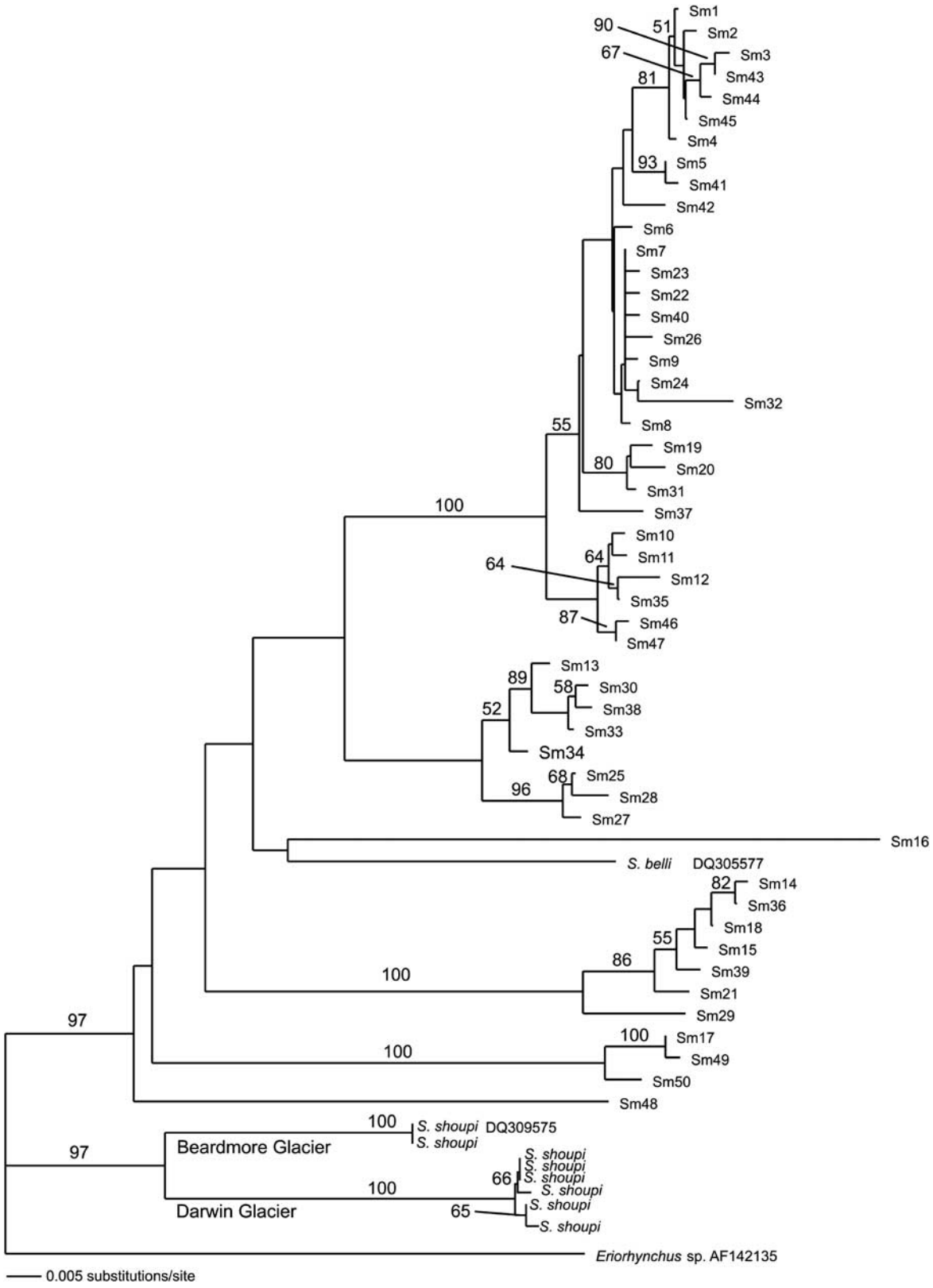

Fig. 2. Neighbour joining phylogram based upon all available *Stereotydeus* sequences from Victoria Land and the central Transantarctic Mountains using a 504 bp fragment of the mtDNA COI gene. Bootstrap confidence limits (1024 replicates) shown above nodes. Haplotype codes refer to those provided in Table I.

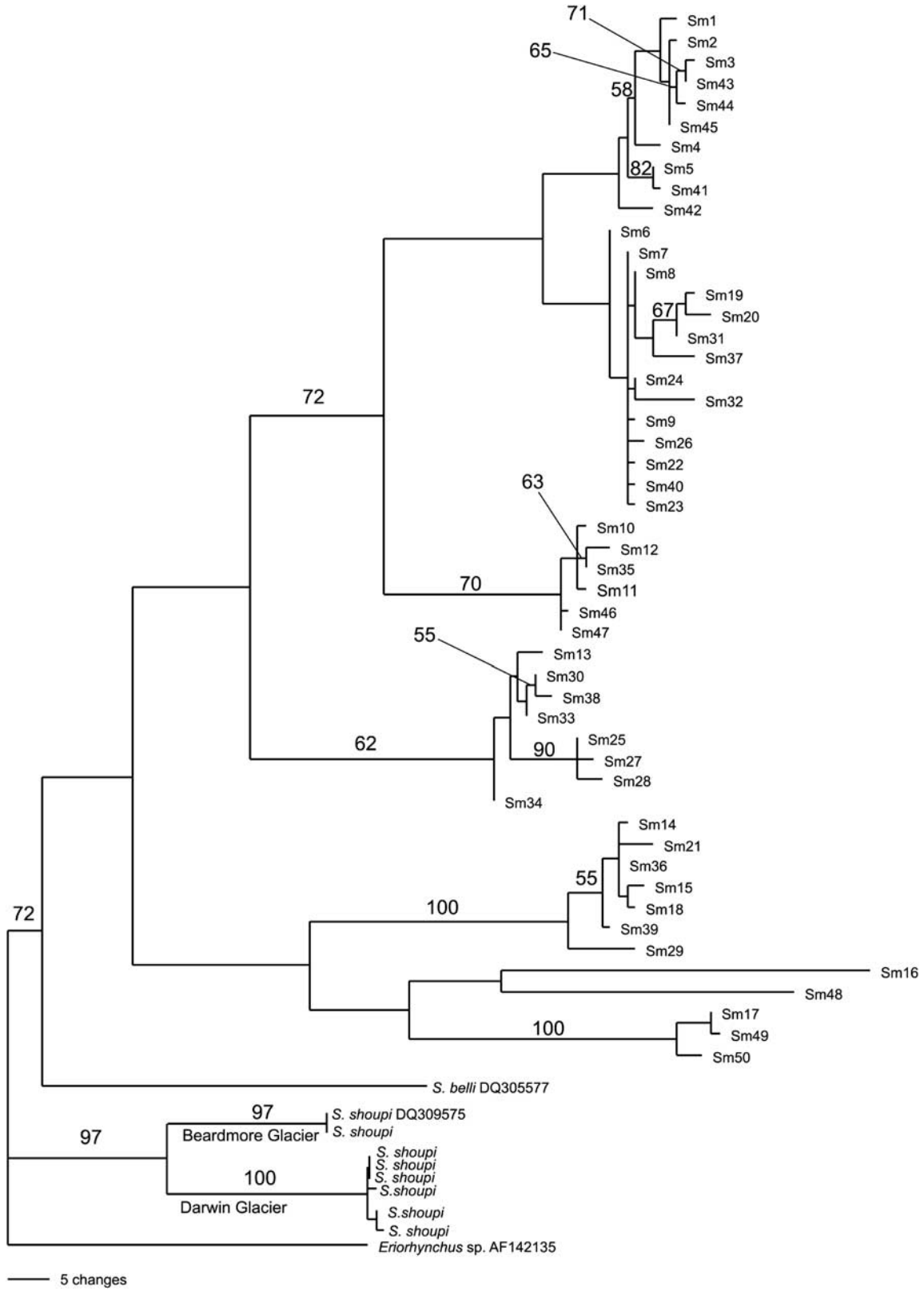

Fig. 3. Maximum likelihood phylogram based upon the substitution model HKY+I+Γ (-lnL = 3158.0623 (AIC); Ti/tv ratio = 2.8729 I = 0.5320 Γ = 0.7250: with base frequencies set to A = 0.4094 C = 0.0919 G = 0.1180 T = 0.3807) derived from jModeltest (see methods), using a 504 bp fragment of the mtDNA (COI) gene from all available *Stereotydeus* sequences from Victoria Land and the central Transantarctic Mountains. Haplotype codes refer to those provided in Table I.

(Guindon & Gascuel 2003, Posada 2008) was used to select the appropriate substitution model of evolution for maximum likelihood (ML) heuristic searches (using all unique sequences). The ML model selected was HKY+I+Γ (-lnL = 3158.0623 (AIC); Ti/tv ratio = 2.8729 I = 0.5320 Γ = 0.7250: with base frequencies set to A = 0.4094 C = 0.0919 G = 0.1180 T = 0.3807). All other options in GARLI remained as default. Bootstrap replicates (n = 1024) were performed to assess support for the phylogenies estimated by both NJ and ML analyses.

Results

mtDNA COI sequence variability

We found 11 unique *S. mollis* COI haplotypes from the southern McMurdo Dry Valleys resulting in a total of 50 haplotypes when combined with previously published data. Four haplotypes were obtained from the six *S. shoupi* collected from near the Darwin Glacier and a single haplotype from the *S. shoupi* collected from the Beardmore Glacier region. The nucleotide composition averaged over all sequences showed an A-T bias of 69.0–71.0% for all species (A = 35.8%, T = 33.2%, C = 16.1%, G = 14.9% for *S. mollis*; A = 35.7%, T = 35.5%, C = 16.1%, G = 12.9% for *S. shoupi*; A = 35.9%, T = 34.8%, C = 15.8%, G = 13.5% for *S. shoupi* from the Darwin Glacier; and A = 35.3%, T = 34.1%, C = 15.7%, G = 14.9% for *S. belli*). Base frequencies were homogenous among all sites (χ^2 = 20.6141, p = 1.00, df = 168), and for third codon positions (168 sites, A-T = 82.02%; χ^2 = 21.451, p = 1.00, df = 168), across all taxa.

Among all the *Stereotydeus* sequences there were 326/178 constant/variable sites. Sequence divergence for the 50 *S. mollis* haplotypes ranged from 0.2–17.5% (uncorrected pair wise distances). The six *S. shoupi* sequences from the Darwin Glacier (79.5°S) were 0.2–0.6% divergent from each other and were 8.5–8.7% divergent from the two identical *S. shoupi* sequences from the Beardmore Glacier (83.5°S). *Stereotydeus shoupi* were 13.7–18.1% divergent relative to *S. mollis*. The single *S. belli* sequence from Cape Hallett (71°S) was 13.3–14.5% divergent from *S. shoupi* and 9.1–14.1% divergent in comparison to *S. mollis*.

Sequence divergences (up to 17.5%) within *S. mollis*, resulted in 37 amino acid differences at 25 variable sites. A single amino acid difference separated individuals of *S. shoupi* from the Beardmore and Darwin Glacier regions. There were 10 amino acid differences at nine variable sites for *S. shoupi* relative to *S. mollis* and 14 differences at 13 sites for *S. shoupi* relative to *S. belli*. There was also a single amino acid difference between *S. belli* and the most common amino acid sequences for *S. mollis.*

Phylogenetic analyses

Both the NJ and ML trees were similar and placed 48 of the 50 *S. mollis* haplotypes into five divergent clades with bootstrap support ≥ 50%. There was extremely strong bootstrap support in both the NJ and ML analyses for the placement of *S. shoupi* as basal to all other *Stereotydeus* analysed. In addition, both NJ and ML consistently grouped the four individuals of *S. shoupi* collected from near the Darwin Glacier with *S. shoupi* collected from near the Beardmore Glacier; ≥ 97% bootstrap support (Figs 2 & 3). For both the NJ and ML analyses, there was considerable disagreement in the placement of the highly divergent (up to 17.5%) *S. mollis* haplotype Sm48 and the single available *S. belli* sequence. NJ analysis placed both *S. belli* and the *S. mollis* haplotype Sm16 within a highly divergent clade with low bootstrap support (< 50%) and Sm48 as basal to all other *S. mollis* haplotypes with very strong bootstrap support (97%) (Fig. 2). In contrast, the ML analysis grouped Sm48 and Sm16 together into a similar, highly divergent clade with low bootstrap support (< 50%) while placing *S. belli* as basal to all *S. mollis* haplotypes (bootstrap support 72%) (Fig. 3).

Discussion

The 504 bp portion of the COI gene from the 50 known haplotypes of *S. mollis* used in this study showed very high levels of intra specific divergence (up to 17.5% uncorrected-*p* distance). These levels of divergence exceeded the levels of inter-specific divergence found between the three recognized *Stereotydeus* species of southern Victoria Land suggesting that there may be cryptic species within *S. mollis*. Similar levels of COI divergence were found in morphologically identical specimens of the "pan-Antarctic" springtail *Friesea grisea* (Schäffer) from opposite sides of the continent and Torricelli *et al*. (2009) concluded that this was due to the presence of cryptic species.

Both NJ and ML analyses revealed five well-supported *S. mollis* clades and two discrete *S. shoupi* clades which grouped the sequences from Beardmore Glacier with the four *S. shoupi* sequences from Darwin Glacier. However, both NJ and ML analyses disagreed on the placement of both *S. belli* and the highly divergent *S. mollis* haplotype Sm48. This is possibly an artefact of using only a single *S. belli* sequence in the analyses rather than the choice of the COI gene for estimating species level phylogenies (Linares *et al*. 2009). For example, the inclusion of additional *S. mollis* and *S. shoupi* sequences, along with the four individuals of *S. shoupi* from the Darwin Glacier, increased the resolution of the phylogeny by placing *S. shoupi* as a monophyletic sister taxa with strong bootstrap support basal to all *S. mollis* haplotypes. This is in contrast to the position of *S. shoupi* as a clade within *S. mollis* as reported by Stevens & Hogg (2006) using a more limited dataset.

The mtDNA COI gene has been widely accepted as a suitable molecular marker for the phylogenetic study of mite taxa and for investigating both the intra-specific relationships of populations at the species level as well as

the inter-specific relationships of closely related species (Navajas & Fenton 2002, Cruickshank 2002, Dabert 2006). Boyer *et al*. (2007), found a similar pattern of extremely high COI variability (up to 19.2% uncorrected-*p* distance) in the arachnid *Aoraki denticulata denticulata* (Forster) endemic to the South Island of New Zealand. The inclusion of several sequences from the subspecies *A. denticulata major* (Forster), as well as other closely related sister taxa within the genus *Aoraki*, resulted in a deep branching phylogeny which was well supported by bootstrap analysis and suggested the presence of several cryptic species. These results were in agreement with previous research which has found increased resolution of phylogenies with the inclusion of a representative range of molecular data from closely related taxa (Talavera & Castresana 2007).

Springtails and mites show broadly similar biogeographical patterns along the Victoria Land latitudinal gradient (Frati *et al*. 2000, 2001, Stevens & Hogg 2006, McGaughran *et al*. 2010), although intraspecific and conspecific mite distances are greater in comparison with Collembola. The greater divergence values for mites, compared with springtails, may be due to mites' smaller size, higher activity levels and shorter generation time (Martin & Palumbi 1993). The comparative hardiness of mites (Sjursen & Sinclair 2002), may also have allowed them to survive in additional refugia during past glaciations, resulting in the complex patterns of genetic diversity that we see today. Differences in the eco-physiological behaviour of *G. hodgsoni* observed by McGaughran *et al*. (2009), if present in *Stereotydeus*, could also contribute to increased genetic divergence rates and possibly correlate with the deeper branches within our mite phylogeny.

Based on the COI sequence data, we conclude that the range of *S. shoupi* extends northward towards the Darwin Glacier and that *S. shoupi* is a monophyletic sister taxon of *S. mollis*. The availability of only a single *S. belli* COI sequence severely limited the ability to accurately place it within the phylogeny as evidenced by the low bootstrap support values and differing placements within the NJ and ML trees. The addition of further *S. belli* sequences, from across its distributional range, may help resolve its relationship with that of the highly divergent lineages of *S. mollis*. Sequence data from other *Stereotydeus* sp. from across the Antarctic continent hold the promise of a more comprehensive understanding of the evolutionary history of Antarctic *Stereotydeus*.

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