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Investigating the diversity of the terrestrial invertebrate fauna of Antarctica: a closer look at the *Stereotydeus* (Acari: Prostigmata) genus

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Abstract: In the extreme Antarctic ecosystems, plants and invertebrates are confined to sparse and isolated ice-free refugia where they survived for millions of years and where they undergo differentiation and population divergence, potentially resulting in speciation. In invertebrates' populations, the gene flow is strongly affected and reduced by their limited dispersal abilities, their specific habitat requirements and the substantial geographical barriers. And thus, results in high genetic differentiation between clusters of individuals.

Mites are surely the predominant invertebrate group of Continental Antarctica and the free-living genus *Stereotydeus* Berlese, 1901 (Acari: Prostigmata) is one of the most abundant along the coastal zones of Victoria Land and the Transantarctic Mountains.

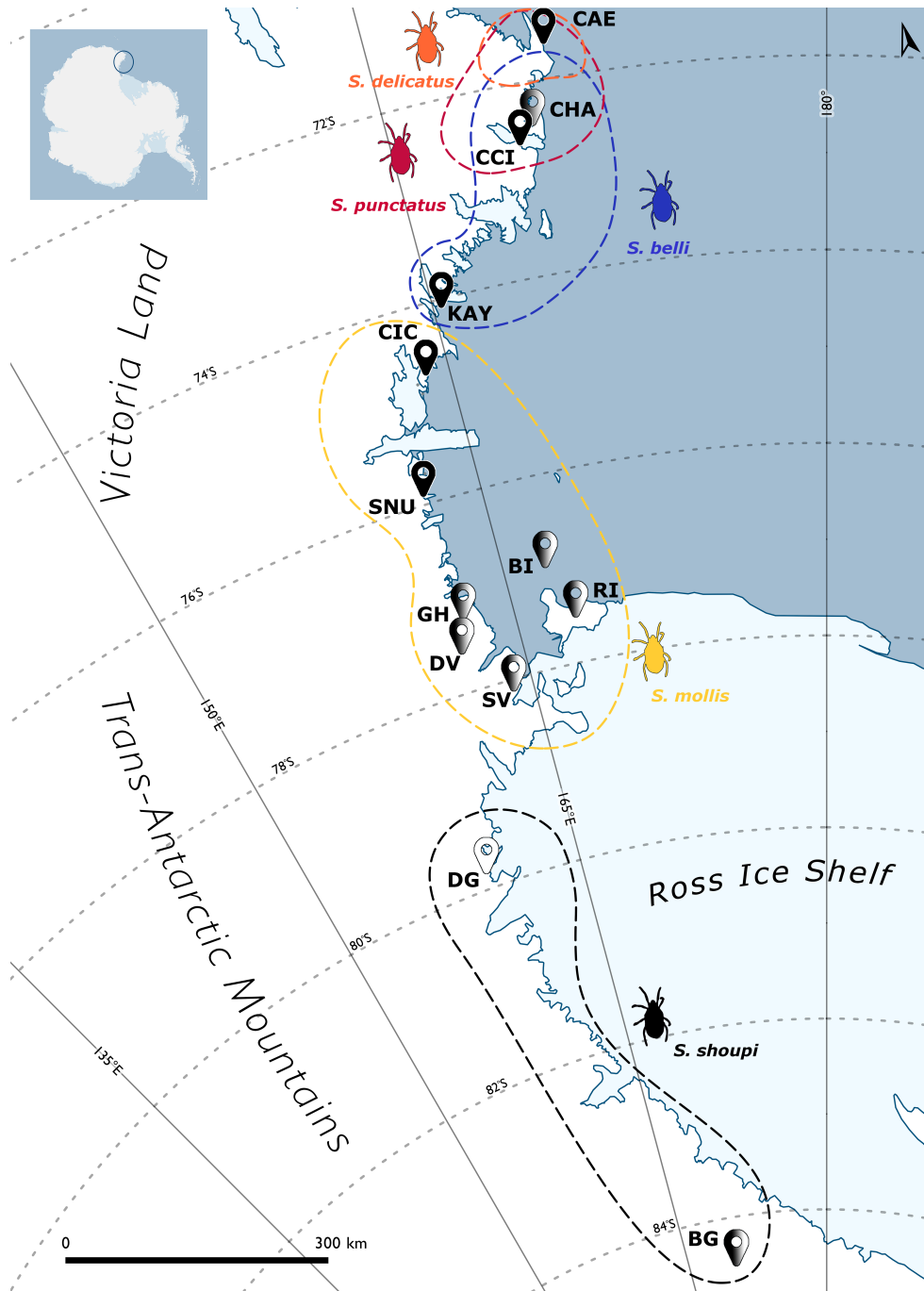
To examine the biodiversity and the phylogeographic distribution ranges of *Stereotydeus* spp. across Victoria Land, we conducted an integrated analysis of the genus through morphological, phylogenetic and population genetics studies. The results suggest a possible common evolutionary history in several isolated glacial refugia, with scarce gene flow even within populations probably resulting from inter/intra-specific events influenced by several abiotic/biotic factors.

Keywords: Victoria Land; molecular phylogeny; biogeography; Acari; *Stereotydeus* spp.

The logo consists of a dark blue square. Inside the square, the letters 'BDEE' are written in a bold, orange, sans-serif font. Below 'BDEE', the year '2021' is written in a bold, white, sans-serif font.

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Introduction



After the first morphological studies on the *Stereotydeus* spp. during the 1960s, only three genetical studies on the mitochondrial marker *cox1* have been conducted in the past decade only on *S. belli*, *S. mollis* and *S. shoupi* from Victoria Land and the Trans-Antarctic Mountains.

Aims

Investigate the **diversity** of *Stereotydeus* spp. in Victoria Land through morphological and molecular analyses.

-  morphology
-  genetic
-  morphology and genetic

Womersley & Strandtmann 1963; Strandtmann 1967;
Stevens & Hogg 2006; McGaughran et al., 2008; Demetras et al., 2010

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Materials and methods

Table 1. ID and coordinates of the sampling sites for different populations and number of individuals used for the analyses.

ID	Locality	Lat. (S)	Long. (E)	Victoria Land	gen.	morph.	n.
CHA	Cape Hallett	72°26'25"	169°56'32"	North	10	23	-
CCI	Crater Cirque	72°37'52"	169°22'22"	North	14	6	-
CJO	Cape Jones	73°16'38"	169°12'54"	North	10	2	7
KAY	Kay Island	74°04'14"	165°18'60"	North	10	24	
CIC	Campo Icaro	74°42'45"	164°06'21"	Central	32	32	13
VEG	Vegetation Island	74°47'00"	163°37'00"	Central	10	-	-
INE	Inexpressible Island	74°53'39"	163°43'44"	Central	10	16	5
PRI	Prior Island	75°41'31"	162°52'34"	South	15	32	6
SNU	Starr Nunatak	75°53'57"	162°35'08"	South	10	4	7

gen. *Stereotydeus* spp. for molecular analyses only

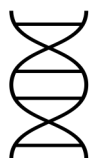
morph. *Stereotydeus* spp. for morphological analyses only

n. *Stereotydeus* spp. for morphological and molecular analyses



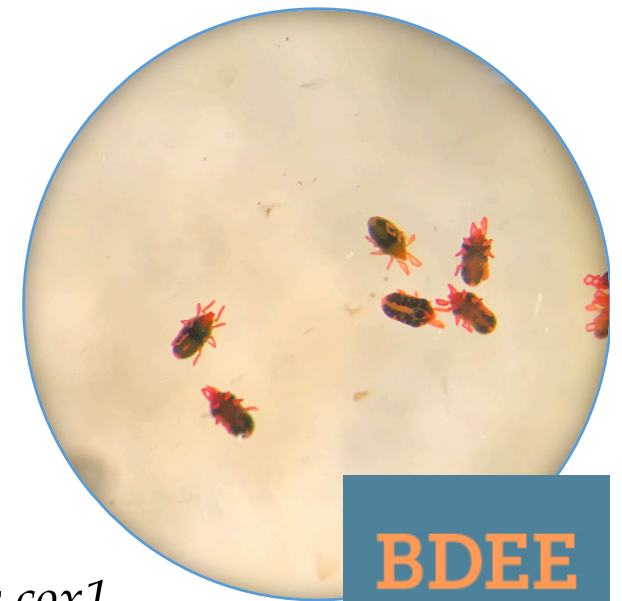
Morphological analyses:

- optical microscope
- scanning electron microscope



Molecular analyses:

- mitochondrial marker *cox1*
- nuclear marker 28S



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Results and Discussion

Table 1. ID of *cox1* haplotypes of five *Stereotydeus* species of Victoria Land

Area	ID	<i>cox1</i>	
Cape Hallett	CHA	MB1(10)	<i>S. belli</i>
Crater Cirque	CCI	MB10(2); MP1(3), MP2(7), MP3(1), MP4(1)	<i>S. belli</i> ; <i>S. punctatus</i>
Cape Jones	CJO	MB2(2), MB3(3), MB4(3), MB5(8), MB6(1)	<i>S. belli</i>
Kay Island	KAY	MB7(1), MB8(3), MB9(6)	<i>S. belli</i>
Campo Icaro	CIC	MD1(18), MD2(1), MD3(2), MD4(1), MD5(6); MI1(6), MI2(1), MI3(1), MI5(4), MI12(4), MI13(1)	<i>S. delicatus</i> ; <i>Stereotydeus</i> n. sp. 2
Vegetation Island	VEG	MD5(9), MD6(1)	<i>S. delicatus</i>
Inexpressible Island	INE	MD6(1); MI6(3), MI7(3), MI8(6), MI12(1), MI14(1)	<i>S. delicatus</i> ; <i>Stereotydeus</i> n. sp. 2
Prior Island	PRI	MN1(2); MI4(15), MI9(1), MI10(1), MI11(2)	<i>Stereotydeus</i> n. sp. 1; <i>Stereotydeus</i> n. sp. 2
Starr Nunatak	SNU	MN1(7), MN2(1); MI4(5), MI9(3), MI11(1)	<i>Stereotydeus</i> n. sp. 1; <i>Stereotydeus</i> n. sp. 2

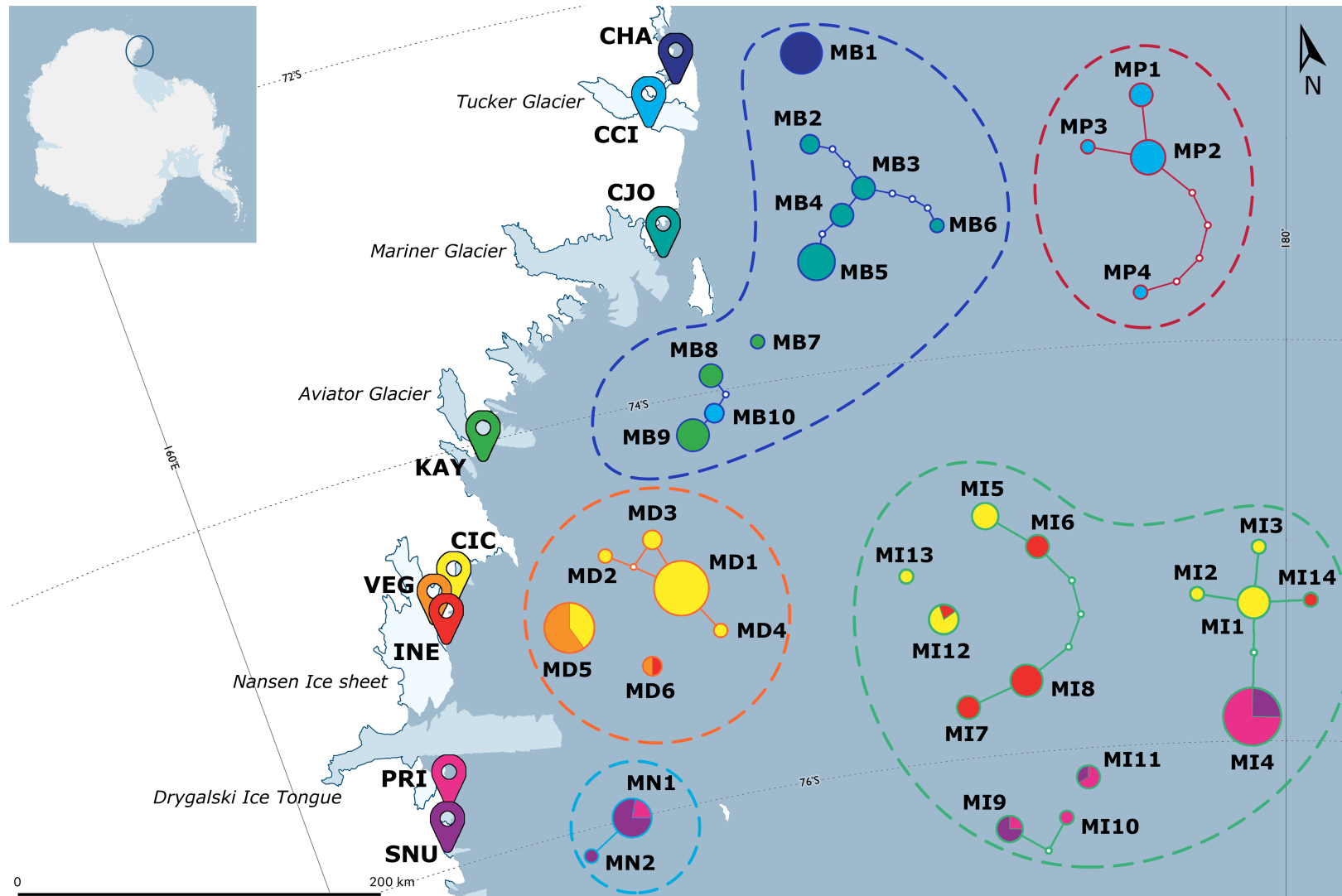


Figure 1. Haplotype networks (*cox1*) of five *Stereotydeus* species.

Collecting sites are indicated by the pie charts colours; the different species are identified by the outlines of the networks together with the haplotype ID and the dashed line around the clusters.

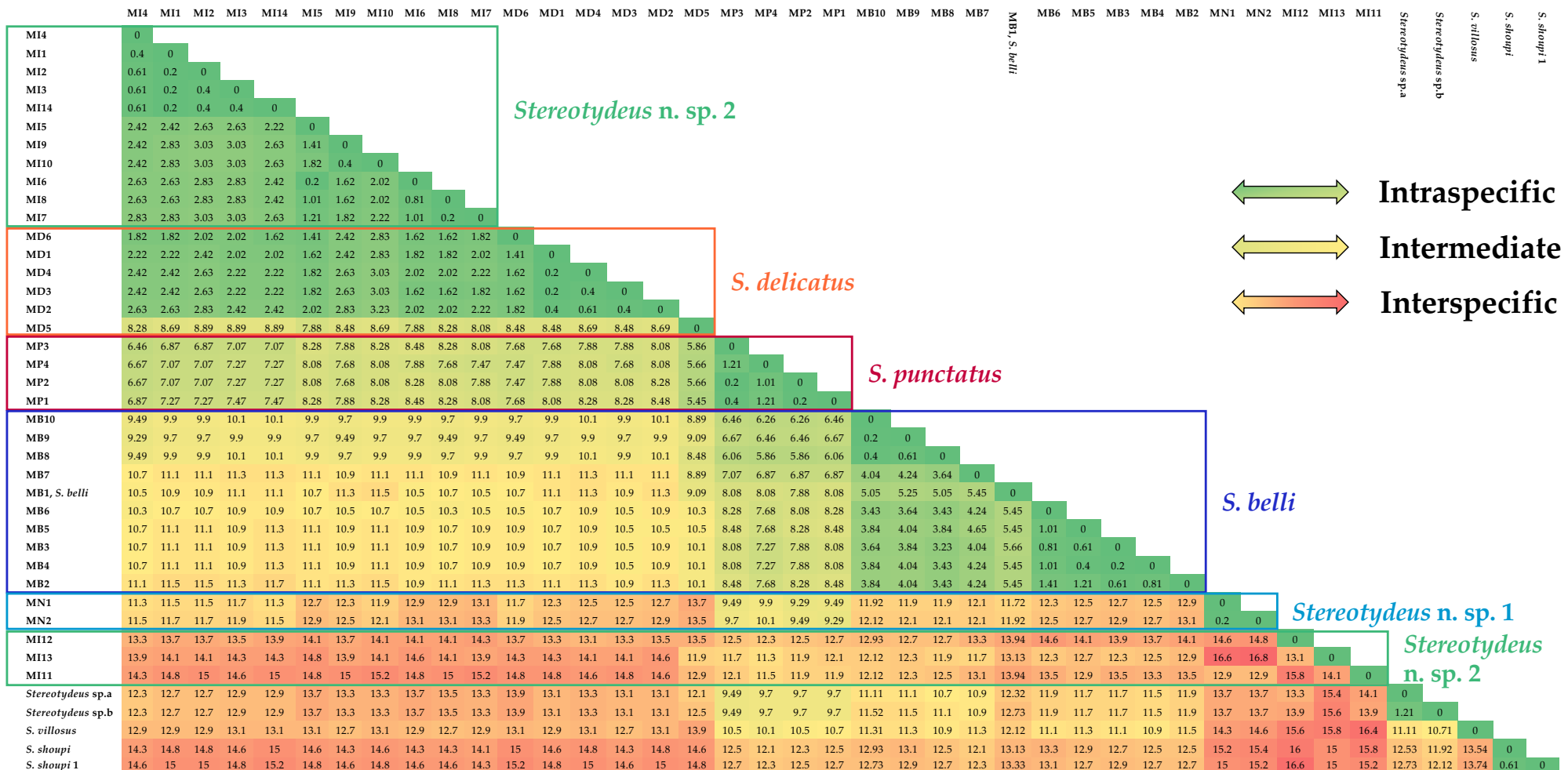


Results and Discussion

Table 1 (right). ID of *cox1* haplotypes of five *Stereotydeus* species.

Area	ID	<i>cox1</i>	Species
Cape Hallett	CHA	MB1(10)	<i>S. belli</i>
Crater Cirque	CCI	MB10(2); MP1(3), MP2(7), MP3(1), MP4(1)	<i>S. belli</i> ; <i>S. punctatus</i>
Cape Jones	CJO	MB2(2), MB3(3), MB4(3), MB5(8), MB6(1)	<i>S. belli</i>
Kay Island	KAY	MB7(1), MB8(3), MB9(6)	<i>S. belli</i>
Campo Icaro	CIC	MD1(18), MD2(1), MD3(2), MD4(1), MD5(6); MI1(6), MI2(1), MI3(1), MI5(4), MI12(4), MI13(1)	<i>S. delicatus</i> ; <i>Stereotydeus</i> n. sp. 2
Vegetation Island	VEG	MD5(9), MD6(1)	<i>S. delicatus</i>
Inexpressible Island	INE	MD6(1); MI6(3), MI7(3), MI8(6), MI12(1), MI14(1)	<i>S. delicatus</i> ; <i>Stereotydeus</i> n. sp. 2
Prior Island	PRI	MN1(2); MI4(15), MI9(1), MI10(1), MI11(2)	<i>Stereotydeus</i> n. sp. 1; <i>Stereotydeus</i> n. sp. 2
Starr Nunatak	SNU	MN1(7), MN2(1); MI4(5), MI9(3), MI11(1)	<i>Stereotydeus</i> n. sp. 1; <i>Stereotydeus</i> n. sp. 2

Table 2 (below). Matrix of the % of genetic distances of the *Stereotydeus cox1* sequences



Results and Discussion


Area	ID	Haplotypes	28S	Combined (cox1+28S)
Cape Hallett	CHA	MB1(10)	RB1(9), RB2(1)	Sb1(9), Sb2(1)
Crater Cirque	CCI	MB10(2); MP1(3), MP2(7), MP3(1), MP4(1)	RB8(1), RB9(1); RP1(12)	Sb12(1), Sb13(1); Sp1(7), Sp2(3), Sp3(1), Sp4(1)
Cape Jones	CJO	MB2(2), MB3(3), MB4(3), MB5(8), MB6(1)	RB3(16), RB4(1)	Sb3(8), Sb4(2), Sb5(3), Sb6(3), Sb7(1)
Kay Island	KAY	MB7(1), MB8(3), MB9(6)	RB5(7), RB6(1), RB7(1), RB8(1)	Sb8(1), Sb9(2), Sb10(5), Sb11(1), Sb14(1)
Campo Icaro	CIC	MD1(18), MD2(1), MD3(2), MD4(1), MD5(6); MI1(6), MI2(1), MI3(1), MI5(4), MI12(4), MI13(1)	RD1(12), RD2(2); RX1(31)	Sd1(1), Sd2(1), Sd3(9), Sd4(2), Sd5(8), Sd6(1), Sd9(1), Sd10(4), Sd11(1); Si1(1), Si2(4), Si5(4), Si11(1), Si12(6), Si13(1)
Vegetation Island	VEG	MD5(9), MD6(1)	RD1(10)	Sd8(1), Sd10(9)
Inexpressible Island	INE	MD6(1); MI6(3), MI7(3), MI8(6), MI12(1), MI14(1)	RD2(1); RI1(14)	Sd7(1); Si3(1), Si6(3), Si7(3), Si8(6), Si14(1)
Prior Island	PRI	MN1(2); MI4(15), MI9(1), MI10(1), MI11(2)	RN1(2); RI3(19)	Sn1(2); Si4(2), Si9(1), Si10(1), Si16(15)
Starr Nunatak	SNU	MN1(7), MN2(1); MI4(5), MI9(3), MI11(1)	RN1(7), RN2(1); RI2(1), RI3(8)	Sn1(6), Sn2(1), Sn3(1); Si4(1), Si9(3), Si15(1), Si16(4)

Some morphological characters useful for distinguishing *S. delicatus* from *Stereotydeus* n. sp. 2:

 Body length of the adults

 Division of the femora

 Number of aggenital setae

 Position of the anal pore

 Shape of the epirostrum

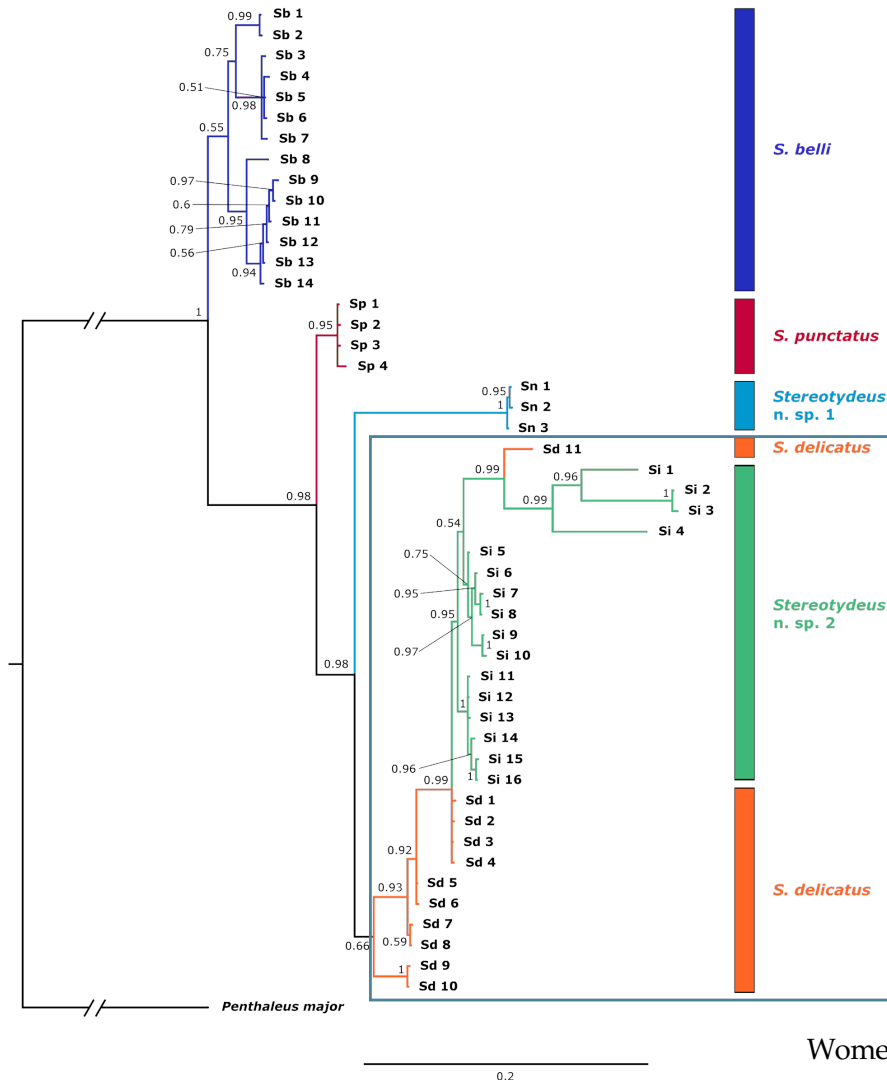
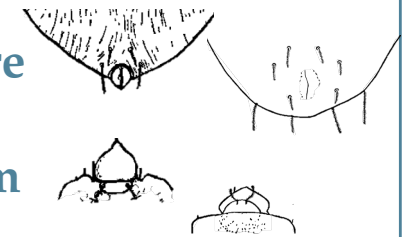


Figure 2. Phylogenetic tree of combined *cox1* and 28S haplotypes of the *Stereotydeus* specimens. Posterior probabilities at the nodes; labels indicate the ID of the haplotypes.



Results and Discussion

Table 3. Population genetic parameters for the *cox1* in *S. belli*, *S. delicatus*, *Stereotydeus n. sp. 1* and *Stereotydeus n. sp. 2* sampled across Victoria Land. *n*, number of individuals; N_H , number of haplotypes within the populations and their frequencies; haplotype (*h*) and nucleotide (π) diversity; mean number of pairwise differences $\theta(\pi)$ and of segregating sites $\theta(S)$.

<i>Stereotydeus belli</i>						
Collection site	<i>n</i>	N_H	$h \pm \sigma$	$\pi \pm \sigma$	$\theta(\pi) \pm \sigma$	$\theta(S) \pm \sigma$
CHA	10	MB1(10)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
CCI	2	MB10(2)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
CJO	17	MB2(2), MB3(3), MB4(3), MB5(8), MB6(1)	0.743 ± 0.086	0.005 ± 0.003	2.559 ± 1.616	2.662 ± 1.247
KAY	10	MB7(1), MB8(3), MB9(6)	0.600 ± 0.130	0.010 ± 0.006	5.200 ± 3.108	7.423 ± 3.330
<i>Stereotydeus delicatus</i>						
Collection site	<i>n</i>	N_H	$h \pm \sigma$	$\pi \pm \sigma$	$\theta(\pi) \pm \sigma$	$\theta(S) \pm \sigma$
CIC	28	MD1(18), MD2(1), MD3(2), MD4(1), MD5(6)	0.553 ± 0.093	0.030 ± 0.015	14.966 ± 7.682	11.307 ± 3.860
VEG	10	MD5(9), MD6(1)	0.200 ± 0.154	0.017 ± 0.010	8.400 ± 4.807	14.846 ± 6.322
INE	1	MD6(1);	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
<i>Stereotydeus n. sp. 2</i>						
Collection site	<i>n</i>	N_H	$h \pm \sigma$	$\pi \pm \sigma$	$\theta(\pi) \pm \sigma$	$\theta(S) \pm \sigma$
CIC	17	MI1(6), MI2(1), MI3(1), MI5(4), MI12(4), MI13(1)	0.801 ± 0.060	0.071 ± 0.037	35.375 ± 18.143	29.579 ± 10.687
INE	14	MI6(3), MI7(3), MI8(6), MI12(1), MI14(1)	0.769 ± 0.083	0.026 ± 0.014	13.121 ± 7.058	24.213 ± 9.242
PRI	19	MI4(15), MI9(1), MI10(1), MI11(2)	0.380 ± 0.134	0.033 ± 0.017	16.316 ± 8.502	22.317 ± 7.940
SNU	9	MI4(5), MI9(3), MI11(1)	0.639 ± 0.126	0.042 ± 0.023	21.028 ± 11.648	28.699 ± 12.242
<i>Stereotydeus n. sp. 1</i>						
Collection site	<i>n</i>	N_H	$h \pm \sigma$	$\pi \pm \sigma$	$\theta(\pi) \pm \sigma$	$\theta(S) \pm \sigma$
PRI	2	MN1(2)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
SNU	8	MN1(7), MN2(1)	0.250 ± 0.180	0.001 ± 0.001	0.250 ± 0.355	0.386 ± 0.386



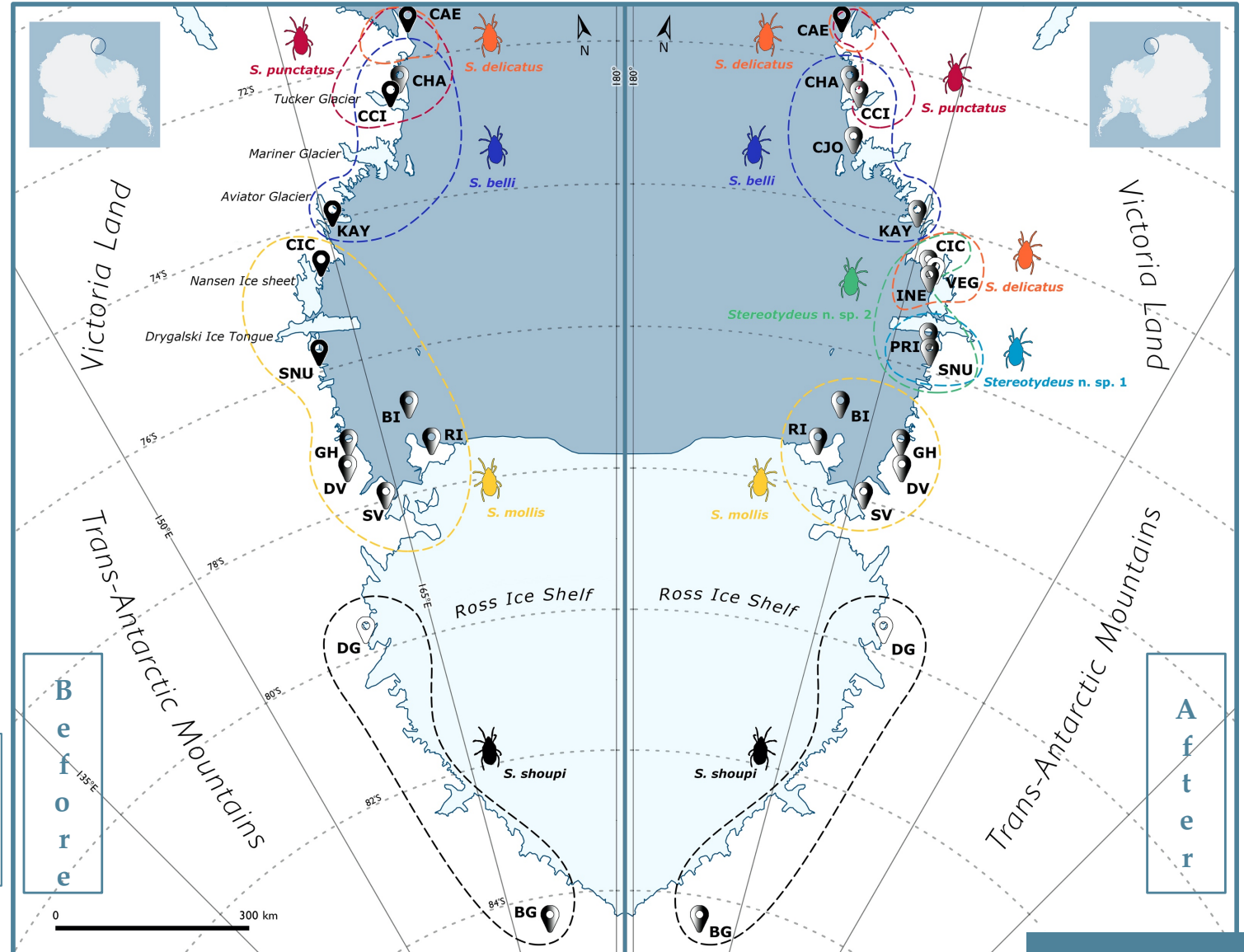
Highest values recorded in *Stereotydeus n. sp. 2* from CIC and SNU

Table 4. Percentage of variation (%) of molecular variance (AMOVA) of different levels of hierarchical population structure for *Stereotydeus sp.* for the *cox1*. Test with structure enforced according to geographical regions (see Table 1).

Species		Among groups Φ_{CT}	Among populations within groups Φ_{SC}	Within populations Φ_{ST}
<i>S. belli</i>	Variance component	10.48068	0.05397	1.25345
	p	(0.16735 ± 0.00273)	(0.45057 ± 0.00422)	(0.00000 ± 0.00000)
	%	88.91	0.46	10.63
<i>S. delicatus</i>	Variance component	9.51162	0.28149	6.66210
	p	(0.33383 ± 0.00347)	(0.24403 ± 0.00340)	(0.0006 ± 0.00006)
	%	57.80	1.71	40.49
<i>Stereotydeus n. sp. 2</i>	Variance component	2.94891	-0.55777	10.89525
	p	(0.16135 ± 0.00259)	(0.62355 ± 0.00382)	(0.00056 ± 0.00018)
	%	22.19	-4.20	82.00

Results and Discussion

- Presence of two new *Stereotydeus* species in Victoria Land;
- First record of *S. delicatus* in Central Victoria Land;
- Close genetic relationship of *S. delicatus* and *Stereotydeus n. sp. 2*;
- High genetic diversity within *Stereotydeus n. sp. 2* Campo Icaro's populations;
- S. mollis* not detected in Central Victoria Land during this study.



Central role of the geographical barriers in delineating species boundaries

Creation of isolated ice-free refugia

High genetic and morphological differentiation

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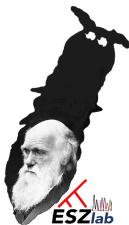
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Conclusions

Thanks to the use of the integrative taxonomy applied to evolutionary studies, it appears clear that the mite biodiversity of Antarctica has been overlooked for a long time. These results suggest a possible common evolutionary history in several isolated glacial refugia, with scarce gene flow even within populations probably resulting from inter/intra-specific events influenced by several abiotic/biotic factors. Recent threats to Antarctic biodiversity like accelerated climate change, pollution, biological invasions and the increase of human activities have caused increased calls for adequate conservation measures. Establishing a new distribution map for the *Stereotydeus* species of Victoria Land may help lay the foundations for future decisions in matters of protection and conservation of the unique terrestrial fauna of Antarctica.

Acknowledgments

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