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Supplementary Information for

Genetic diversity of soil invertebrates corroborates timing estimates for past collapses of the West Antarctic Ice Sheet

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## Supplementary Information Text

### Extended methodology

DNA sequencing was undertaken either at the University of Waikato (UoW), New Zealand or the Canadian Centre for DNA barcoding (CCDB), Canada. For total DNA extraction, a REDEExtract-N-Amp kit (Sigma-Aldrich) was used at the UoW while a glass fibre plate method (AcroPrep) was used at the CCDB [1]. The DNA barcoding region (658 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') [2] at the UoW, and the primers LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAATCA-3') [3] at CCDB. At the UoW, PCR amplifications for each specimen were carried out in 15 µL volumes containing 7.5 µL PCR master mix solution (i-Taq) (Intron Biotechnology), 0.2 µM (0.3 µL) of each primer and 1 µL of DNA extract (unquantified). Thermal cycling conditions were: 94 °C for 5 min followed by 5 cycles (94 °C for 1 min, 48 °C for 1.5 min and 72 °C for 1 min) then 35 cycles (94 °C for 1 min, 52 °C for 1.5 min and 72 °C for 1 min) of denaturation and polymerase amplification, with a final elongation at 72 °C for 5 min. At CCDB, thermal cycling conditions were: 94 °C for 1 min followed by 5 cycles (94 °C for 1 min, 45 °C for 1.5 min and 72 °C for 1.5 min) then 35 cycles (94 °C for 1 min, 50 °C for 1.5 min and 72 °C for 1 min) of denaturation and polymerase amplification, with a final elongation at 72 °C for 5 min. Successful amplification products were cleaned with 0.1 µL Exonuclease I (EXO) (10 U/µL) and 0.2 µL Shrimp Alkaline Phosphate (SAP) (1 U/µL) (Illustra from GE Healthcare) at 37°C for 30 min then 80°C for 15 min at the UoW, or Sephadex at CCDB. Sequencing was carried out in forward and reverse directions using an ABI 3130 at the UoW, or an ABI 3730x1 sequencer at CCDB. Sequences uploaded to the Barcode of Life Datasystems (BOLD) database and GenBank remain untrimmed, and for 126 of the 132 specimens generated in this study the full 658 bp COI gene region is covered.

All existing sequences have been labelled here according to previous haplotype assignment [4]. One individual from haplotype GHOD-076 (KX232741) had an additional substitution at site 82 which had been trimmed in the shorter (422 bp) alignment that was presented in [4], and we have accordingly renamed this individual as belonging to a new haplotype; GHOD-089.

**Table S1.** Collection locations for the four endemic species of Collembola now known from the Queen Alexandra Range (QAR) and the Queen Maud Mountains (QMM). The number of COI sequences (560 bp) for each of the four Collembola species (*Antarctophorus subpolaris*, ASUB; *Biscoia sudpolaris*, BSUD; *Gomphiocephalus hodgsoni*, GHOD; *Tullbergia mediantarctica*, TMED) are provided, of which those previously available in GenBank are shown in parentheses.

Locality Site	Latitude	Longitude	Elevation (m a.s.l.)	ASUB	BSUD	GHOD	TMED
<i>North Beardmore Gl. (QAR)</i>							
Gateway Ridge	-83.491	170.778	201		10	12	
<i>South Beardmore Gl. (QMM)</i>							
Ebony Ridge	-83.761	172.745	775	8(6)	11		
Mount Kyffin	-83.774	171.835	735		1		
Harcourt Spur	-83.806	172.268	762		13(1)		
<i>West Shackleton Gl. (QMM)</i>							
Mount Franke	-84.617	-176.700	500	3			9
Mount Wasko	-84.560	-176.820	387	1			8
Mount Speed	-84.467	-176.467	300				15
Mount Speed Spur	-84.450	-177.150	410				9
<i>East Shackleton Gl. (QMM)</i>							
Massam Glacier	-84.559	-175.009	368	5			
Garden Spur	-84.536	-174.944	387	25			
Nielsen Peak	-84.533	-175.500	471				9
<i>Negative Sites</i>							
Darwin Glacier	-79.870	159.360	475				
Cloudmaker	-84.264	169.313	1842				
Meyer Desert	-85.254	167.275	2290				
Waldron Spur	-84.542	-174.780	607				
Thanksgiving Valley	-84.919	-177.060	1298				
Taylor Nunatak	-84.900	-176.000	1517				
Kitching Ridge	-85.198	-177.102	1808				
Bennett Platform	-85.207	-177.389	1646				
Mount Augusta	-85.227	-174.353	2939				
Schroeder Hill	-85.383	-175.200	2699				
Roberts Massif	-85.533	-177.083	2200				
Durham Point	-85.539	-151.150	406				
Mount Howe	-87.345	-149.252	2602				
TOTAL				42(6)	35(1)	12	50

**Table S2.** Tests of nucleotide substitution saturation performed separately for each of the four divergent species of Collembola (*Antarctophorus subpolaris*, ASUB; *Gomphiocephalus hodgsoni*, GHOD; *Antarctocinella monoculata*, AMON; *Cryptopygus nivicolus*, CNIV). The numbers of sequences ( $n$ ) included are only those longer than 560 bp. Unique haplotypes ( $h$ ) are also shown, along with the best-fit model of sequence evolution according to ModelFinder Plus [5] as implemented in IQ-Tree [6]. Sequence alignments for the four species were each 560 bp in length, utilized for the test by [7] as implemented in DAMBE 7.0.48 [8], showing substitution saturation is unlikely for all codon positions tested ( $P < 0.01$ ).

Species	$n$	$h$	best-fit model (IQ-tree)	Codon position 1 and 2, combined				Codon position 3		
				P(inv)	lss	lss.C	$P$	lss	lss.C	$P$
ASUB	42	9	HKY+F+G4	0.1441	0.1301	0.7428	<0.01	0.3281	0.7174	<0.01
GHOD	674	75	HKY+F+I	0.5779	0.1553	0.7577	<0.01	0.2177	0.7409	<0.01
AMON	27	4	HKY+F	0.8660	0.6630	0.8059	<0.01	0.2387	0.7761	<0.01
CNIV	37	13	HKY+F	0.6358	0.0728	0.7270	<0.01	0.0706	0.6662	<0.01

**Table S3.** All sequences included in analyses for the four species of Collembola now known from the Queen Alexandra Range and the Queen Maud Mountains.

Species	Haplotype code	BOLD sequence ID	Sample	Site name	Source
<i>Antarctophorus subpolaris</i>					
	ASUB-001	GBCO390-09		Ebony Ridge	[9]
	ASUB-002	GBCO389-09		Ebony Ridge	[9]
	ASUB-003	GBCO0098-06		Ebony Ridge	[10]
	ASUB-002	ANTSP1362-18*	KYBJA6	Ebony Ridge	
	ASUB-002	ANTSP106-11	KYUN5	Ebony Ridge	
	ASUB-005	ANTSP001-11*	GS5	Garden Spur	
	ASUB-006	ANTSP002-11	GS5	Garden Spur	
	ASUB-005	ANTSP003-11	GS5	Garden Spur	
	ASUB-007	ANTSP004-11	GS13	Garden Spur	
	ASUB-007	ANTSP005-11	GS13	Garden Spur	
	ASUB-007	ANTSP006-11	GS13	Garden Spur	
	ASUB-007	ANTSP007-11	GS13	Garden Spur	
	ASUB-007	ANTSP011-11	MG2	Massam Glacier	
	ASUB-005	ANTSP012-11	MG2	Massam Glacier	
	ASUB-004	ANTSP013-11	MG3	Massam Glacier	
	ASUB-005	ANTSP022-11	GS5	Garden Spur	
	ASUB-005	ANTSP023-11	GS5	Garden Spur	
	ASUB-005	ANTSP024-11	GS5	Garden Spur	
	ASUB-007	ANTSP025-11	GS6	Garden Spur	
	ASUB-007	ANTSP026-11	GS6	Garden Spur	
	ASUB-007	ANTSP027-11	GS6	Garden Spur	
	ASUB-007	ANTSP028-11	GS6	Garden Spur	
	ASUB-007	ANTSP029-11	GS6	Garden Spur	
	ASUB-005	ANTSP032-11	MG1	Massam Glacier	
	ASUB-007	ANTSP033-11	MG1	Massam Glacier	
	ASUB-005	ANTSP077-11	GS16	Garden Spur	
	ASUB-005	ANTSP078-11	GS16	Garden Spur	
	ASUB-007	ANTSP079-11	GS16	Garden Spur	
	ASUB-005	ANTSP082-11	GS14	Garden Spur	
	ASUB-005	ANTSP084-11	GS14	Garden Spur	
	ASUB-005	ANTSP085-11	GS13	Garden Spur	
	ASUB-005	ANTSP086-11	GS13	Garden Spur	
	ASUB-007	ANTSP087-11	GS13	Garden Spur	
	ASUB-007	ANTSP088-11	GS13	Garden Spur	
	ASUB-007	ANTSP089-11	GS13	Garden Spur	
	ASUB-008	ANTSP1101-18	MW4-3	Mount Wasiko	
	ASUB-009	ANTSP1127-18*	MFX1	Mount Franke	
	ASUB-008	ANTSP1128-18	MFX1	Mount Franke	
	ASUB-008	ANTSP1129-18	MFX1	Mount Franke	
<i>Biscoia sudpolaris</i>					
	BSUD-001	GBCO0083-06		Harcourt Spur	[11]
	BSUD-002	ANTSP051-11	HS8	Harcourt Spur	
	BSUD-003	ANTSP052-11	GR1	Gateway Ridge	
	BSUD-003	ANTSP053-11	GR1	Gateway Ridge	
	BSUD-003	ANTSP055-11	GR1	Gateway Ridge	
	BSUD-003	ANTSP056-11	GR9	Gateway Ridge	
	BSUD-003	ANTSP057-11	GR9	Gateway Ridge	
	BSUD-003	ANTSP058-11	GR9	Gateway Ridge	
	BSUD-003	ANTSP059-11	GR9	Gateway Ridge	

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BSUD-003	ANTSP060-11	GR9	Gateway Ridge
BSUD-003	ANTSP061-11	GR9	Gateway Ridge
BSUD-003	ANTSP109-11	KYDW1	Ebony Ridge
BSUD-003	ANTSP110-11	KYDW1	Ebony Ridge
BSUD-003	ANTSP111-11	KYDW2	Ebony Ridge
BSUD-003	ANTSP724-15	KYUN12	Ebony Ridge
BSUD-003	ANTSP099-11	KYUN4	Ebony Ridge
BSUD-003	ANTSP101-11	KYUN4	Ebony Ridge
BSUD-003	ANTSP102-11	KYUN4	Ebony Ridge
BSUD-003	ANTSP1354-18	KYUN4	Ebony Ridge
BSUD-003	ANTSP105-11	KYUN5	Ebony Ridge
BSUD-003	ANTSP107-11	KYUN5	Ebony Ridge
BSUD-003	ANTSP1360-18*	KYUN8	Ebony Ridge
BSUD-003	ANTSP066-11	HS6	Harcourt Spur
BSUD-003	ANTSP049-11	HS8	Harcourt Spur
BSUD-003	ANTSP050-11	HS8	Harcourt Spur
BSUD-003	ANTSP072-11	HS8	Harcourt Spur
BSUD-003	ANTSP074-11	HS8	Harcourt Spur
BSUD-003	ANTSP094-11	HS8	Harcourt Spur
BSUD-003	ANTSP095-11	HS8	Harcourt Spur
BSUD-003	ANTSP097-11	HS8	Harcourt Spur
BSUD-003	ANTSP073-11	HS8	Harcourt Spur
BSUD-003	ANTSP093-11	HS8	Harcourt Spur
BSUD-004	ANTSP096-11	HS8	Harcourt Spur
BSUD-005	ANTSP041-11	GR4	Gateway Ridge
BSUD-005	ANTSP076-11	MK5	Mt Kyffin

*Tullbergia mediantarctica*

TMED-002	ANTSP1071-18*	MFX3	Mount Franke
TMED-002	ANTSP1072-18	MFX3	Mount Franke
TMED-002	ANTSP1073-18	MFX3	Mount Franke
TMED-002	ANTSP1074-18	MFX3	Mount Franke
TMED-002	ANTSP1075-18	MFX3	Mount Franke
TMED-002	ANTSP1076-18	MFX3	Mount Franke
TMED-002	ANTSP1077-18	MFX3	Mount Franke
TMED-002	ANTSP1078-18	MFX3	Mount Franke
TMED-002	ANTSP1079-18	MFX3	Mount Franke
TMED-002	ANTSP1082-18	MSP4-4	Mount Speed
TMED-002	ANTSP1083-18	MSP4-4	Mount Speed
TMED-002	ANTSP1084-18	MSP4-4	Mount Speed
TMED-002	ANTSP1085-18	MSP4-4	Mount Speed
TMED-002	ANTSP1086-18	MSP4-4	Mount Speed
TMED-002	ANTSP1087-18	MSP4-4	Mount Speed
TMED-002	ANTSP1089-18	MSP4-4	Mount Speed
TMED-002	ANTSP1090-18	MSP4-4	Mount Speed
TMED-002	ANTSP1091-18	MW4-3	Mount Wasko
TMED-002	ANTSP1093-18	MW4-3	Mount Wasko
TMED-002	ANTSP1095-18	MW4-3	Mount Wasko
TMED-002	ANTSP1096-18	MW4-3	Mount Wasko
TMED-002	ANTSP1097-18	MW4-3	Mount Wasko
TMED-002	ANTSP1098-18	MW4-3	Mount Wasko
TMED-003	ANTSP1099-18	MW4-3	Mount Wasko
TMED-002	ANTSP1100-18	MW4-3	Mount Wasko
TMED-002	ANTSP1103-18	MSSpur	Mount Speed
TMED-002	ANTSP1104-18	MSSpur	Mount Speed
TMED-002	ANTSP1105-18	MSSpur	Mount Speed

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TMED-001	ANTSP1106-18	MSSpur	Mount Speed
TMED-002	ANTSP1107-18	MSSpur	Mount Speed
TMED-002	ANTSP1108-18	MSSpur	Mount Speed
TMED-002	ANTSP1109-18	MSSpur	Mount Speed
TMED-002	ANTSP1110-18	MSSpur	Mount Speed
TMED-002	ANTSP1111-18	MSSpur	Mount Speed
TMED-002	ANTSP1112-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1113-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1114-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1115-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1116-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1117-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1118-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1120-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1121-18	NP3-4	Nielsen Peak
TMED-002	ANTSP1281-18	MSPX5	Mount Speed
TMED-002	ANTSP1283-18	MSPX5	Mount Speed
TMED-002	ANTSP1284-18	MSPX5	Mount Speed
TMED-002	NZGDW092-18	MSP4-4	Mount Speed
TMED-002	NZGDW093-18	MSP4-4	Mount Speed
TMED-002	NZGDW094-18	MSP4-4	Mount Speed
TMED-002	NZGDW095-18	MSP4-4	Mount Speed
<i>Gomphiocephalus hodgsoni</i>			
GHOD-090	ANTSP039-11*	GR3	Gateway Ridge
GHOD-090	ANTSP040-11	GR3	Gateway Ridge
GHOD-090	ANTSP042-11	GR8	Gateway Ridge
GHOD-090	ANTSP043-11	GR8	Gateway Ridge
GHOD-090	ANTSP046-11	GR3	Gateway Ridge
GHOD-090	ANTSP047-11	GR3	Gateway Ridge
GHOD-090	ANTSP054-11	GR1	Gateway Ridge
GHOD-090	ANTSP062-11	GR9	Gateway Ridge
GHOD-090	ANTSP063-11	GR9	Gateway Ridge
GHOD-090	ANTSP064-11	GR9	Gateway Ridge
GHOD-090	ANTSP065-11	GR9	Gateway Ridge
GHOD-090	ANTSP090-11	GR11	Gateway Ridge

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\* Sequences selected to represent phylogenetic clades

**Table S4.** Sequences included in analyses for the four species of Collembola from southern Victoria Land.

SpeciesHaplotype code	BOLD sequence ID	GenBank Accession	Site name	Source
<i>Gomphiocephalus hodgsoni</i>				
GHOD-001	ANTSP966-16*	MK609301	SVL widespread	[12]
GHOD-071	ANTSP223-13*	KU876875	Mt Gran	[13]
GHOD-071	ANTSP225-13	KU876815	Mt Gran	[13]
GHOD-075	ANTSP584-15	KX232843	Towle Glacier	[4]
GHOD-075	ANTSP585-15	KX232842	Towle Glacier	[12]
GHOD-076	ANTSP579-15*	KX232814	Towle Glacier	[4]
GHOD-076	ANTSP580-15	KX232799	Towle Glacier	[4]
GHOD-076	ANTSP582-15	KX232729	Towle Glacier	[4]
GHOD-089	ANTSP581-15	KX232741	Towle Glacier	[4]
<i>Antarcticinella monoculata</i>				
AMON-001	ANTSP258-13*	KU876808	Springtail Point	[13]
AMON-003	ANTSP645-15	KX232795	Benson Glacier	[4]
AMON-004	ANTSP639-15	KX232853	Pegtop Mt	[4]
AMON-004	ANTSP641-15	KX232698	Pegtop Mt	[4]
AMON-005	ANTSP575-15*	KX232851	Cliff Nunatak	[4]
AMON-005	ANTSP576-15	KX232789	Cliff Nunatak	[4]
AMON-005	ANTSP577-15	KX232834	Cliff Nunatak	[4]
AMON-005	ANTSP589-15	KX232700	Cliff Nunatak	[4]
AMON-005	ANTSP590-15	KX232782	Cliff Nunatak	[4]
AMON-005	ANTSP591-15	KX232736	Cliff Nunatak	[4]
AMON-005	ANTSP592-15	KX232702	Cliff Nunatak	[4]
AMON-005	ANTSP593-15	KX232705	Cliff Nunatak	[4]
AMON-005	ANTSP602-15	KX232716	Cliff Nunatak	[4]
AMON-005	ANTSP603-15	KX232778	Cliff Nunatak	[4]
AMON-005	ANTSP604-15	KX232722	Cliff Nunatak	[4]
AMON-005	ANTSP605-15	KX232821	Cliff Nunatak	[4]
AMON-005	ANTSP578-15	KX232816	Mt Murray	[4]
AMON-005	ANTSP594-15	KX232811	Mt Murray	[4]
AMON-005	ANTSP595-15	KX232753	Mt Murray	[4]
AMON-005	ANTSP596-15	KX232761	Mt Murray	[4]
AMON-005	ANTSP597-15	KX232727	Mt Murray	[4]
AMON-005	ANTSP598-15	KX232817	Mt Murray	[4]
AMON-005	ANTSP599-15	KX232845	Mt Murray	[4]
AMON-005	ANTSP600-15	KX232687	Mt Murray	[4]
AMON-005	ANTSP601-15	KX232852	Mt Murray	[4]
AMON-005	ANTSP636-15	KX232689	Mt Murray	[4]
AMON-005	ANTSP638-15	KX232806	Mt Murray	[4]
<i>Cryptopygus nivicolus</i>				
CNIV-003	ANTSP118-13	KU876880	Springtail Point	[13]
CNIV-004	ANTSP119-13	KU876872	Springtail Point	[13]
CNIV-004	ANTSP121-13	KU876871	Springtail Point	[13]
CNIV-004	ANTSP211-13	KU876823	Springtail Point	[13]
CNIV-004	ANTSP212-13	KU876800	Springtail Point	[13]
CNIV-004	ANTSP253-13	KU876859	Springtail Point	[13]
CNIV-005	ANTSP559-15	KX232836	Mt Seuss	[4]
CNIV-005	ANTSP566-15	KX232686	Mt Seuss	[4]
CNIV-005	ANTSP567-15	KX232824	Mt Seuss	[4]
CNIV-005	ANTSP571-15	KX232745	Mt Seuss	[4]



CNIV-005	ANTSP632-15	KX232855	Tiger Island	[4]
CNIV-005	ANTSP633-15	KX232822	Tiger Island	[4]
CNIV-005	ANTSP634-15	KX232826	Tiger Island	[4]
CNIV-005	ANTSP642-15	KX232772	Mt Gran	[4]
CNIV-005	ANTSP643-15	KX232743	Mt Gran	[4]
CNIV-005	ANTSP644-15	KX232839	Mt Gran	[4]
CNIV-005	ANTSP698-15	KX232854	Mount Seuss	[4]
CNIV-005	ANTSP700-15	KX232790	Mount Seuss	[4]
CNIV-005	ANTSP701-15	KX232758	Mount Seuss	[4]
CNIV-005	ANTSP728-15	KX232684	Mount Seuss	[4]
CNIV-005	ANTSP124-13	KU876789	Mt Gran	[13]
CNIV-005	ANTSP156-13	KU876834	Mt Seuss	[13]
CNIV-006	ANTSP155-13	KU876857	Mt Seuss	[13]
CNIV-007	ANTSP170-13	KU876811	Mt Seuss	[13]
CNIV-008	ANTSP220-13	KU876836	Mt Gran	[13]
CNIV-008	ANTSP222-13	KU876860	Mt Gran	[13]
CNIV-009	ANTSP249-13	KU876842	Springtail Point	[13]
CNIV-010	ANTSP250-13	KU876877	Springtail Point	[13]
CNIV-011	ANTSP251-13	KU876869	Springtail Point	[13]
CNIV-011	ANTSP257-13	KU876844	Springtail Point	[13]
CNIV-012	ANTSP254-13*	KU876862	Springtail Point	[13]
CNIV-013	ANTSP256-13	KU876813	Mt Gran	[13]
CNIV-014	ANTSP560-15	KX232773	Mt Seuss	[4]
CNIV-014	ANTSP573-15	KX232760	Mt Seuss	[4]
CNIV-015	ANTSP570-15*	KX232840	Mt Seuss	[4]
CNIV-016	ANTSP625-15*	KX232765	Towle Glacier	[4]
CNIV-016	ANTSP626-15	KX232747	Towle Glacier	[4]

\* Sequences selected to represent phylogenetic clades

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