Diversity and taxonomic status of some New Zealand grasshoppers

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ABSTRACT

Little is known about the taxonomic and conservation status of low-altitude populations of New Zealand grasshoppers (Acrididae). We examined the extent of differences between populations belonging to three groups: *Sigaus piliferus*, the *Brachaspis nivalis* complex and the *Sigaus australis* complex. There is evidence that the North Island species *S. piliferus* falls into two groups: individuals in the Tararua Ranges and those north of the Manawatu Gorge. In the South Island, the *B. nivalis* complex is made up of two subgroups: from central Canterbury to Marlborough, and from south Canterbury to north Otago. There are four subgroups within the *S. australis* complex: one in central Canterbury and three in Otago. There are also several morphologically distinct populations within the *B. nivalis* and *S. australis* complexes, but further work is needed to fully describe these forms. When managing these grasshoppers, it is important that this geographic and morphological variation is considered and represented within management units.

Keywords: Orthoptera, management units, mitochondrial DNA, hybridisation, grasshoppers, *Sigaus*, *Brachaspis*, Acrididae, New Zealand

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1. Introduction

The taxonomy of New Zealand endemic grasshoppers (Insecta, Acrididae) was substantially revised by Bigelow (1967), drawing on museum collections and the publications of Hutton (1897, 1898) and Salmon (1950), in addition to his own observations and collections. More recent contributions on the taxonomy of endemic genera include papers by Jamieson (1999), Morris (2002a, b, c, 2003) and Trewick (2001a).

In New Zealand, there are some 16 species of grasshopper belonging to four endemic genera (*Sigaus*, *Brachaspis*, *Alpinacris*, *Paprides*). These are largely restricted to montane areas above the natural treeline. Where grasshoppers occur below the treeline, this is in association with non-forest habitats, such as exposed river beds and the semiarid grassland environments of Central Otago and Canterbury. Species within and among genera have a range of allopatric[1] and sympatric distributions, often with some degree of altitudinal or microhabitat segregation among taxa. For instance, *Sigaus villosus* occurs at the highest altitudes (c. 2000 m a.s.l.) for New Zealand grasshoppers, while *S. campestris* is found at the lower extreme (800–1200 m a.s.l., and some specimens have been recorded close to sea level; Bigelow 1967). *Brachaspis collinus* favours areas of tussock grass, whereas *B. nivalis* is found almost entirely in habitats with a high proportion of rocky substrate. In many South Island locations, it is not uncommon for three or four species to be found in sympatry, but only one species (*Sigaus piliferus*) is described from the North Island. Three taxon groups are currently of particular conservation concern by virtue of their scarcity and/or taxonomic uncertainty. These are *Sigaus piliferus*, *Brachaspis nivalis* complex and *Sigaus australis* complex.

The following subsections summarise the history and current taxonomic situation of New Zealand grasshoppers, the background and rationale to the methods chosen for this study, and the objectives of this study.

1.1 HISTORY AND TAXONOMIC STATUS OF NEW ZEALAND GRASSHOPPERS

1.1.1 *Sigaus piliferus*

Sigaus piliferus is currently the only representative of the endemic grasshopper genera found in the North Island, New Zealand. It was described by Hutton (1897) from a single specimen (now missing) collected at 'Auckland'. The species has never been reported from this location since, but Hutton's description agrees, according to Bigelow (1967), with specimens collected at other locations in the North Island. Bigelow (1967) proposed a 'neoallotype' for the species, distinguishing a specimen from the Pohangina Saddle, eastern Ruahine Range. Although the species has been reported from subalpine areas throughout the North Island, it has never been found on Mt Taranaki. According to Bigelow (1967), the main locations for *S. piliferus* were the Kaimanawa, Tararua, Ruahine

[1] See glossary for definitions of technical terms.

and Kaweka Ranges, Central Plateau, and Mt Hikurangi (East Cape), but he also reported specimens from Coromandel (Kauaeranga Valley), Cambridge (Maungatautari), Rotorua area, and near Lake Taupo.

Bigelow (1967) considered that *S. piliferus* could be subdivided into three spatial groups (northern, central and southern) on morphological grounds (size and colour), but a great deal of variation exists within these broad groupings.

The conservation status of *S. piliferus* is of concern, with McGuiness (2001) expressing the view that it has already disappeared from much of its former range except the East Cape.

1.1.2 *Brachaspis nivalis* complex

Hutton (1897) described four species of *Pezotettix* Burmeister (a northern hemisphere genus) from New Zealand. At the time, he was hesitant to put these new species into a new genus due to the absence of northern hemisphere material for comparison. *Pezotettix nivalis* was named from specimens from the Mount Cook area; *Pezotettix collina* was named from specimens collected from Mount Arthur; *Pezotettix petriocola* was named from a female collected at a low-altitude site (Dee River) in Marlborough; and *Pezotettix terrestris* was named from a female specimen stated as collected in Wellington.

These species were later removed from *Pezotettix* and placed in a new genus *Brachaspis* (Hutton 1898). Hutton named a second low-altitude species of *Brachaspis*, *B. petricolus* from 'Kowai River'. Unfortunately, there are two Kowai Rivers in the South Island, one on the east coast, north of Rangiora, the other south of the Torlesse Range. This second location is far more likely to be the type location. Bigelow (1967) suggested that the single specimen of *P. petriocola* and the males under the name of *B. petricolus* represent the same taxon, and he concluded that the original specimen of *P. petriocola* be named the 'type' of the 'low altitude' species. Further confusion arose with regard to *B. terrestris*, as the type location was Wellington, yet no other *Brachaspis* have ever been collected or recorded from the North Island. Bigelow (1967) assumed a labelling error had occurred, and confusion about the provenance of Hutton's type material remains.

Bigelow (1967) revised *Brachaspis* and distinguished three species: *B. collinus* (Nelson area), *B. robustus* (the large and rare Mackenzie Basin grasshopper, which he described) and *B. nivalis* for all other populations (including the small, low-altitude forms). Bigelow concluded, on the basis of the few individuals available, that the small, low-altitude *Brachaspis* were geographic variants that did not warrant species status. However, Bigelow (1967) did suggest that further study of the low-altitude populations of *B. nivalis* from Dee River, Cora Lynn and the Wilberforce was required. He understood that these specimens appeared to be well-isolated from the nearest high-altitude populations, as well as from one another, and that their very small body size suggested that they may be living under adverse conditions (marginal habitats) relative to those optimal for the species as a whole. It is likely that his interpretation may have put undue emphasis on the 'typical' forms because the *B. nivalis* material examined by Bigelow (1967) was dominated by specimens from the central part of the range (Mt Cook, Mt Hutt, Cameron Valley), with few from the south and north of the range as it is now known (Fig. 1). Bigelow (1967) did, however, examine three individuals of the low-altitude morph from Dee Stream (= Hutton's Dee River), all of which were females.

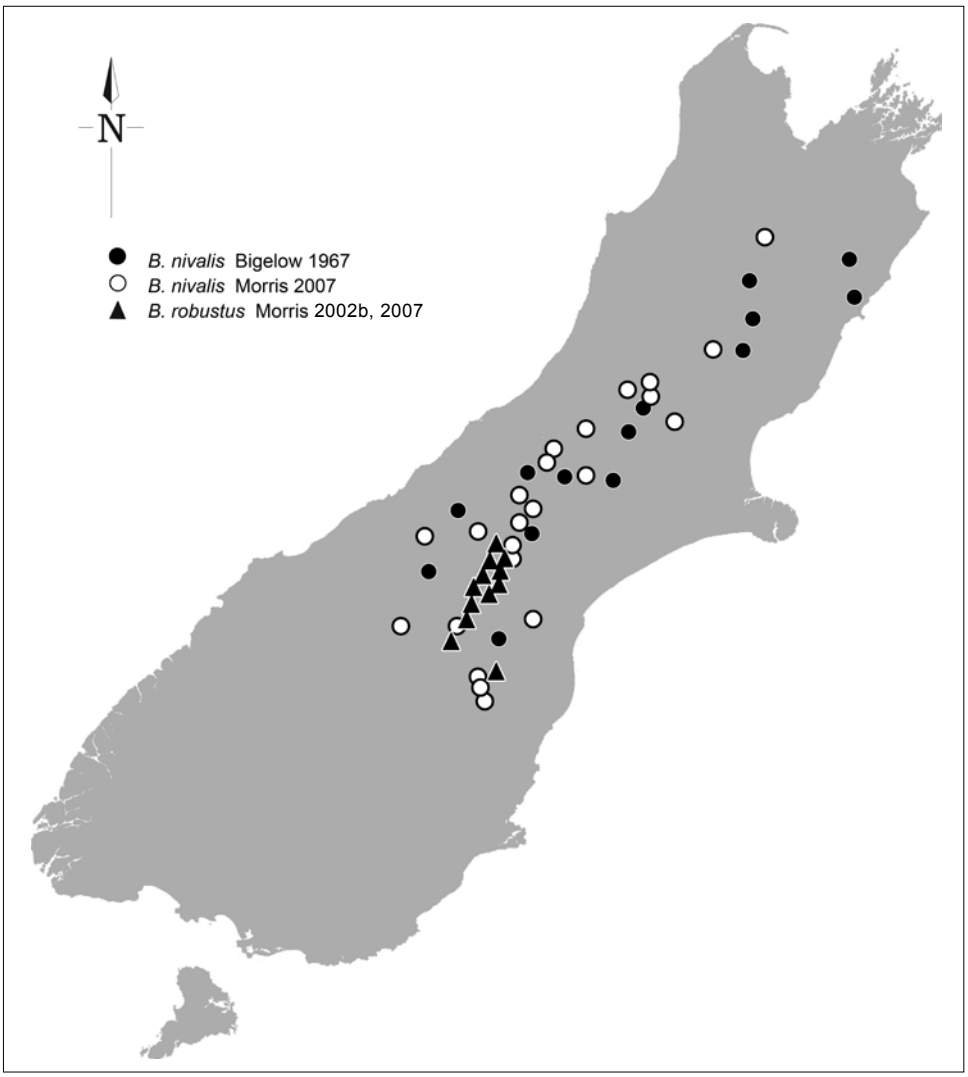

Figure 1. The known distribution of *Brachaspis nivalis* and *B. robustus*. Taxonomy follows Bigelow (1967) and therefore includes putative taxa, such as the small, low-altitude morphs at Dee Stream.

Preliminary re-examination by one of the authors (SM) indicated that several characters do distinguish individuals of the low-altitude form from other *Brachaspis* species, including shape of the male epipallus lophi; overall small size; grey colouration without any gold, purple or orange tinge; and number of spines on the hind tibiae.

In 2001, A.M. Evans (Canterbury Conservancy, Department of Conservation (DOC)) collected a single male grasshopper belonging to a low-altitude population of *Brachaspis* from the Cam River near Blenheim (Marlborough). This grasshopper was collected within 10 km of the original Hudson specimen (examined by Hutton). More recently, Steve Trewick collected specimens from Dee Stream (Clarence Valley, Marlborough). A southern low-altitude population was also recently found at Porter River by Simon Morris, who also collected specimens from the Hakatere, Manuka Point, Glenfalloch and Mesopotamia Pastoral Leases during DOC High Country Tenure Review surveys.

The phylogenetics of the low-altitude species *B. robustus*, montane *B. collinus* and populations of the montane species *B. nivalis* across its spatial range (which extends further south than recognised by Bigelow (1967)) was examined by Trewick (2001a). However, the relationship between the small, low-altitude forms and the above species has not previously been addressed.

1.1.3 *Sigaus australis* complex

Bigelow (1967) examined spatial and altitudinal variation in *S. australis* using pronotum and femur dimensions. More recently, one of the authors (SM) examined morphological variation of *S. australis* and allied taxa in the southern South Island, including an appraisal of male internal genitalia. These structures promise to be the most stable and informative morphological features for determining species within the *S. australis* complex. At present, none of the females in the *S. australis* complex can be distinguished by morphological characters. Bigelow (1967) noted that there was extensive size variation in *S. australis*, but much of the colour polymorphism that exists in living grasshoppers was not evident in the pinned material he examined. The species is distributed in the South Island from Otago north to the Waimakariri River (Fig. 2).

Jamieson (1999) named a new species of *Sigaus* from the Alexandra area as *Sigaus childi*, with a type locality at Graveyard Gully; this was named after Peter Child, who first collected the species in 1967. In an unpublished report in 1999, Colleen Jamieson suggested that another new species of grasshopper, *Sigaus* species A, might exist in the Alexandra area (Morris 2002a). Morris (2002a) agreed that this entity was related to, but a separate species from, *S. childi*, but there is considerable morphological variation among and within *S. childi*, *S.* species A and *S. australis* (Fig. 3).

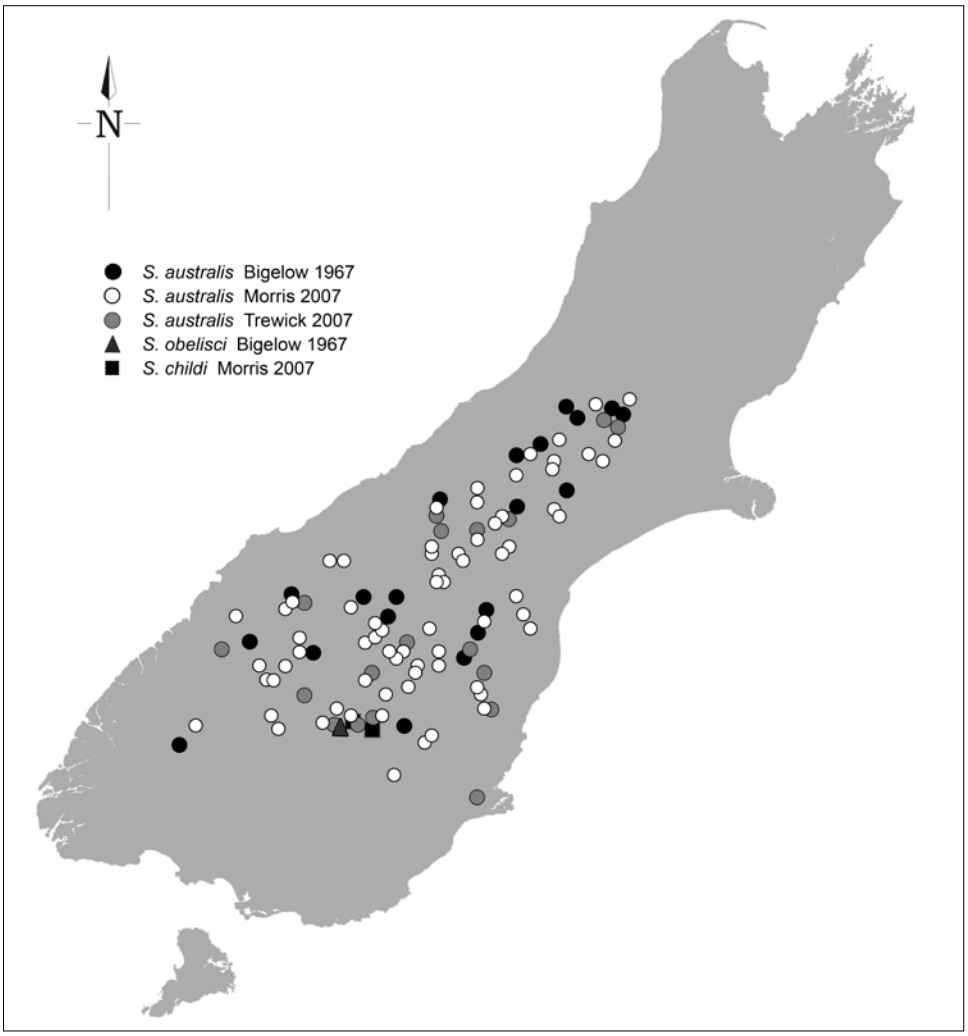

Figure 2. The known distribution of *Sigaus australis*, *S. obelisci* and *S. childi*. *Sigaus australis* here is the species as given by Bigelow (1967) and therefore includes putative species such as *S.* species A.

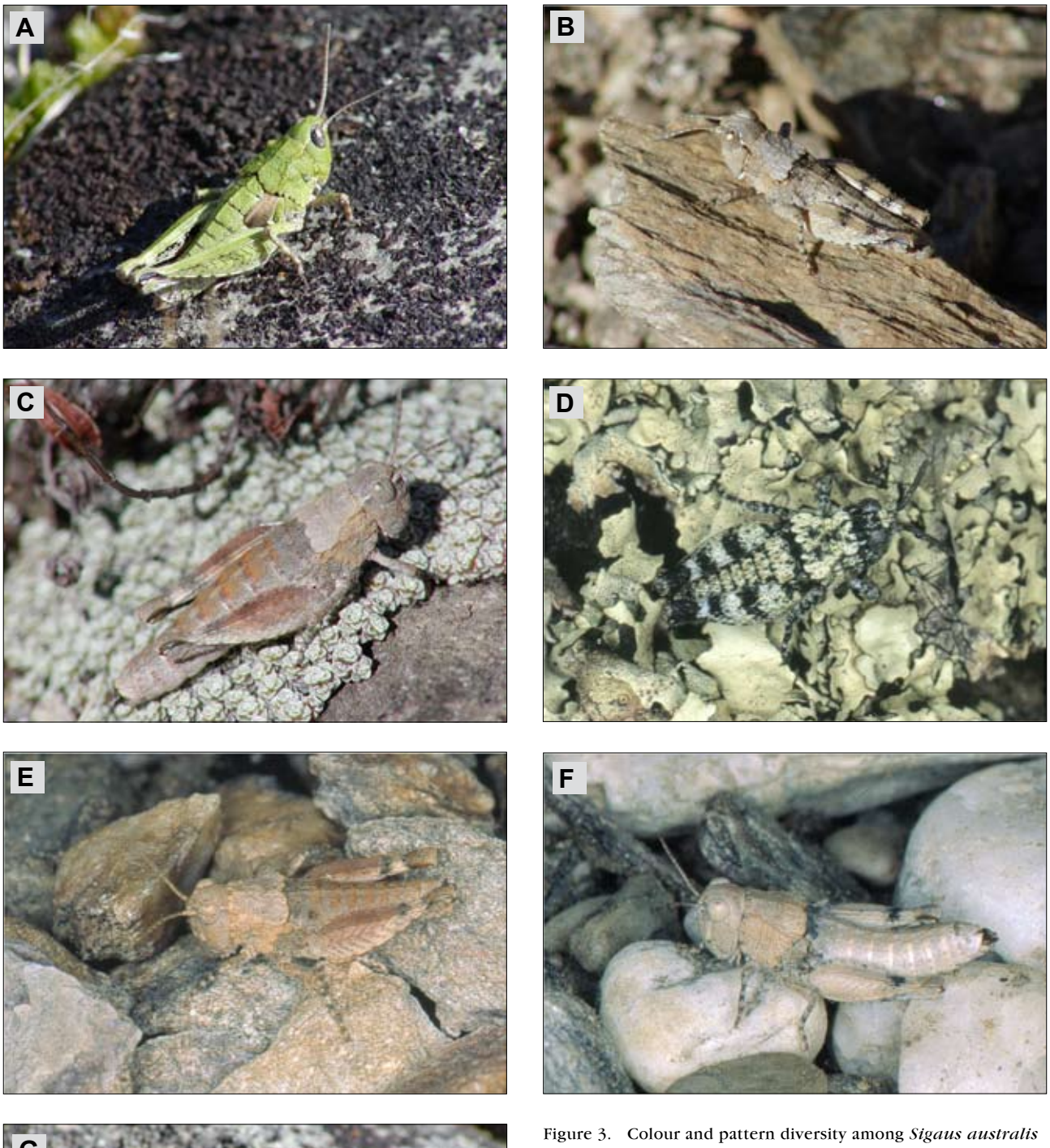

Figure 3. Colour and pattern diversity among *Sigaus australis* complex grasshoppers. A. Unusual *S. australis* from the gold tailing of Northburn (SM); B. unusual *Sigaus australis* from the gold tailings between Alexandra and Northburn (SM); C. *Sigaus childi*, Earnscleugh Tailings, Alexandra (ST); D. male *Sigaus childi* (SM); E. female *Sigaus childi* (SM); F. *Sigaus* "undescribed", juvenile cryptic on lichen, Alexandra (ST); G. *Sigaus childi*, Little Valley Rd, Alexandra (ST).

Bigelow (1967) described *Sigaus obelisci* from specimens collected on the Old Man Range in Otago, but it has proved difficult to distinguish *S. obelisci* from the other grasshoppers in the *S. australis* complex. Five colour morphs are known for *S. obelisci*, all of which are subtly different from other *S. australis* forms. In addition, the habitat that *S. obelisci* prefers is slightly different from the other grasshoppers in the *S. australis* complex. *Sigaus obelisci* appears to prefer cushionfields and herbfields on or near the ridge tops, and is restricted, as far as is known, to the Old Man Range, Central Otago.

Two new species have recently been added to the *S. australis* complex: *Sigaus homerensis* Morris and *Sigaus takahe* Morris (Morris 2003). Both are allopatric at the southern periphery of the *S. australis* complex range. The grasshopper *Sigaus* "Remarkables" (Morris 2002c) is currently undescribed and is very similar externally to *S. obelisci*; however, initial data suggest that the epiproct is consistently longer and broader on *S.* "Remarkables" than on *S. obelisci*.

The *S. australis* group presents the greatest taxonomic and conservation challenge. The status of local allopatric morphs is uncertain and particular confusion exists about species boundaries among the diverse forms of the Central Otago area centred on Alexandra.

1.2 MITOCHONDRIAL SEQUENCE DATA AND TAXONOMY

For the purposes of conservation, where maintenance of biodiversity is the goal, mitochondrial sequence data can provide an effective means of revealing populations/taxa with distinct genealogical histories; genealogical separation between morphologically similar but spatially separate entities; and introgressed taxa resulting from recent speciation and/or hybridisation. Phylogenetic analyses of mitochondrial DNA sequences were developed in the early 1990s and have proved highly informative for conservation (Avise 1989, 1992).

The random, neutral effects of genetic drift (lineage sorting; Avise 2004), whereby some DNA sequence lineages are lost from populations whilst novel mutations are accumulated in those that remain, result in a pattern of inherited sequences that can be used to infer the genealogy of populations. In general, where splits between clusters of similar DNA sequences are correlated with the spatial distribution of the animals from which the DNA came, some barrier to gene flow (movement of grasshoppers in this instance) is indicated. The degree of difference (genetic distance) between clusters of DNA sequences provides a useful indicator of how long ago an existing barrier may have first emerged; the more genetically divergent, the older the event causing the split is likely to be. One can also expect that different species will be genetically distinct from one another even when they exist in sympatry.

For many species that have overlapping ranges, the barrier to gene flow is often behavioural (e.g. differences in microhabitat preference, or the operation of mate recognition systems). However, sister species often exist in allopatry, and therefore express phylogeographic signatures similar to that among spatially separate populations of a single species. Indeed, many species as defined by taxonomy are the descendents of allopatric populations that have accumulated

morphological or behavioural attributes that now distinguish them (in the taxonomist's eye) from one another. Whether or not all allopatric forms that are named as distinct species are biological species (i.e. reproductively isolated should they meet) is a moot point, as the emergence of mate recognition systems would normally require selection against hybrid offspring, i.e. nature does the experiment in such cases.

The application of neutral haplotypic data (i.e. the frequency of alternative mitochondrial DNA sequence variants) for the recognition of species can be very effective. However, it is difficult to detect hybridisation using just mitochondrial sequence data (which are inherited only on the maternal line). It is also difficult to differentiate species that have evolved over a short and recent time frame, i.e. shorter that the time required for lineage sorting to result in the retention and inheritance of mitochondrial lineages by distinct species (which is dependent on effective population size). Although the existence of these processes (recent speciation and hybridisation) can be relatively easy to infer, distinguishing between them is more difficult.

The mitochondrial genes chosen for use in this study—12S and COI (cytochrome oxidase subunit one gene)—have been widely applied to species-level studies of insects. The COI gene has proved useful in both revealing hitherto cryptic taxa to explore the spatial partitioning of phylogenetic structure of species (phylogeography) and demonstrating close genealogical histories of morphologically or ecologically distinct taxa (e.g. Funk et al. 1995; Szymura et al. 1996; Trewick 2000; Buckley et al. 2001).

1.3 OBJECTIVES

The objectives of this study were to:

- Use phylogenetic analyses of mitochondrial DNA sequence data to identify the extent and pattern of genetic diversity within and among *Sigaus piliferus*, the *Brachaspis nivalis* complex and the *Sigaus australis* complex
- Examine the taxonomic, and thus conservation, status of these three grasshopper taxonomic groups

2. Methods

2.1 SAMPLING

Grasshoppers were collected by hand at sites with suitable habitat (Figs 4 & 5). Taxonomic identification primarily followed Bigelow (1967). Most material is held in ethanol at Massey University, although voucher specimens will ultimately be deposited at Museum of New Zealand Te Papa Tongarewa on completion of the research.

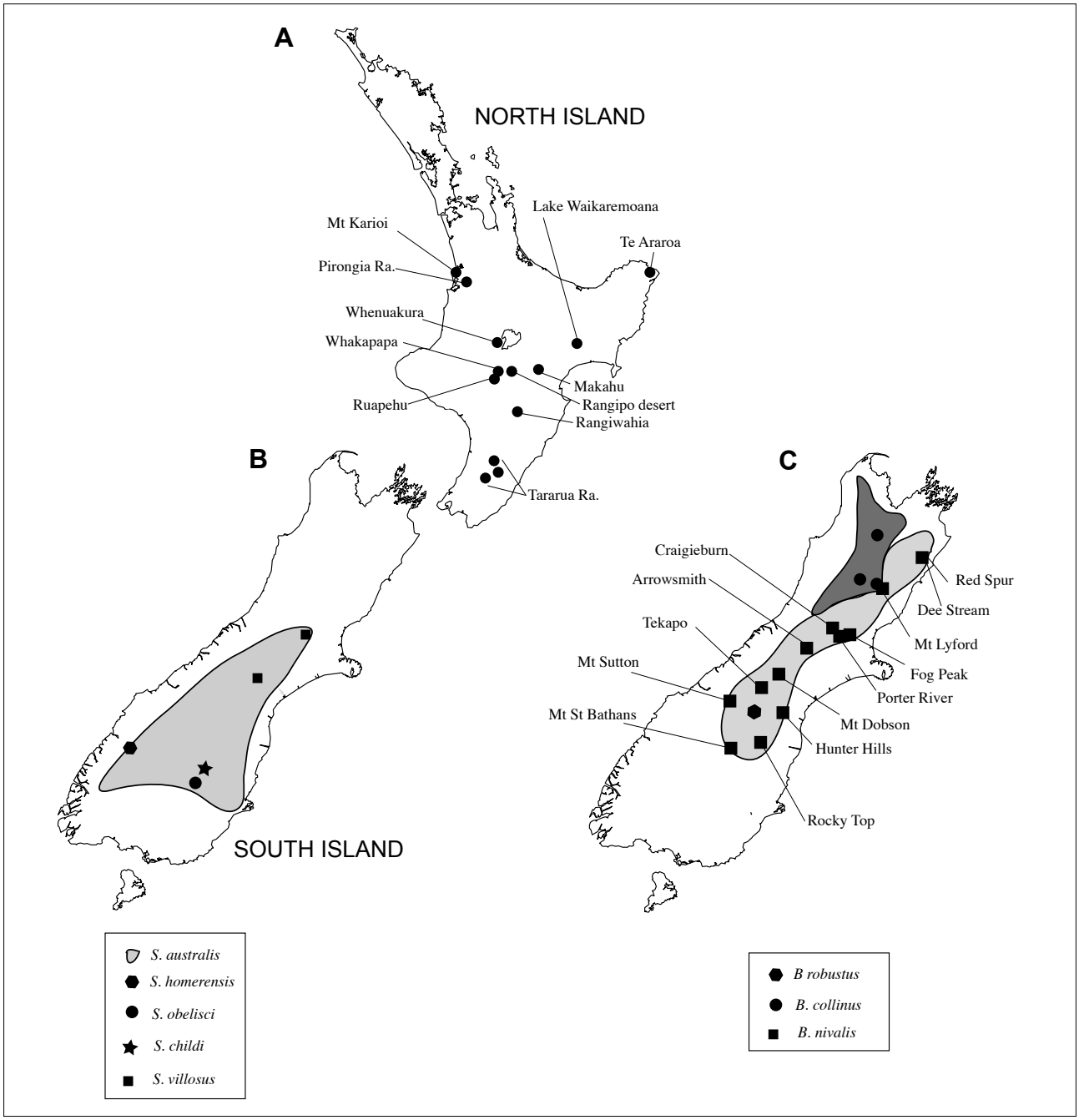

Figure 4. Ranges and sampling locations for grasshoppers. A. Locations in North Island sampled for *Sigaus piliferus*; B. approximate taxon ranges of South Island *Sigaus*; C. ranges and sampling for *Brachaspsis* taxa.

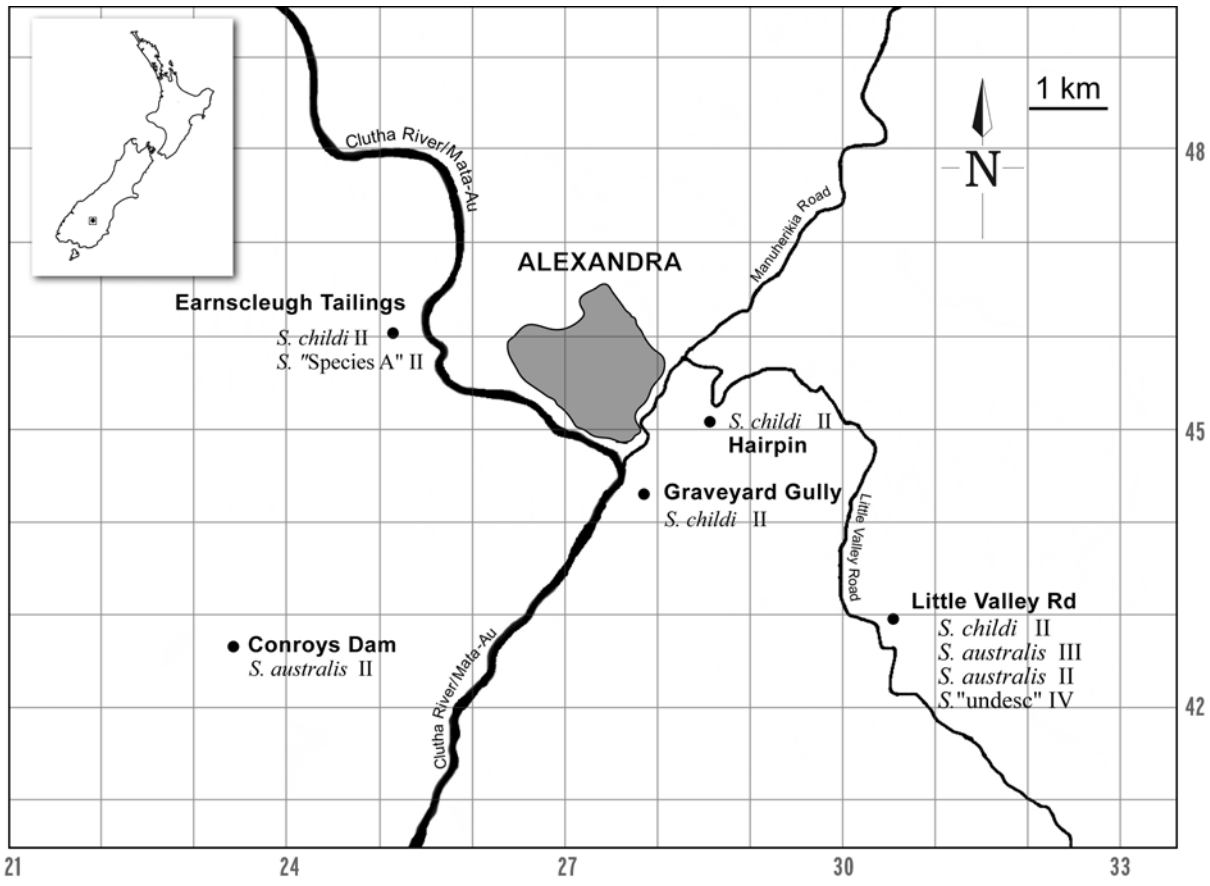

Figure 5. Sampling locations around Alexandra, and details of morphospecies found and DNA sequence haplogroup.

2.2 MOLECULAR METHODS

Single stranded conformational polymorphism (SSCP) was used to screen for variant haplotypes (combinations of alleles) prior to DNA sequencing (Trewick et al. 2000). For this purpose, the primers SR-J-14233 and SR-N-14588 (Simon et al. 1994) were used to amplify a c. 380 bp fragment of the 3' end of mitochondrial 12S rRNA. PCR (polymerase chain reaction) products were labelled with a radio isotope by incorporation of αdATP ^{33}P. Amplification products were denatured for 5 min at 95°C in the presence of an equal volume (10 μL) of 95% formamide loading buffer. These were loaded from ice into vertical, non-denaturing polyacrylamide gels consisting of 6% 37.5:1 bis/acrylamide, 5% glycerol and 0.5 × TBE. Gels were electrophoresed at 4°C for 200 W/h at approximately 13 W and then lifted on blotting paper, dried and exposed with Biomax (Kodak) film for 24–48 h. Individuals were scored for haplotype by comparison of re-natured single strand DNA migration patterns (Sunnucks et al. 2000).

Representatives of each haplotype that was resolved by SSCP were subjected to further PCR to amplify and sequence a larger fragment comprising the 3' end of the 12S rRNA, the tRNA valine and the 5' end of the 16S rRNA, using primers LR-J-13417 and SR-N-14588 (Simon et al. 1994). The 12S-16S fragment of at least one individual of each population presenting a particular SSCP pattern was sequenced to confirm that sequences matched. In addition, a fragment from the 3' end of the mitochondrial COI was amplified and sequenced using primers C1-N-2195 and C1-J-3014 (Simon et al. 1994). PCR reactions for sequencing were

performed in 25 µL volumes using the same conditions as for SSCP. Products were purified using High Pure purification columns (Roche). Cycle sequencing used Perkin Elmer Bigdye chemistry following the manufacturer's protocols and were analysed on a Prism 377 DNA sequencer (Applied Biosystems, Inc., Foster City, California). Sequences were checked against the ABI trace file and aligned manually using SeqEd v1.0.3 (Applied Biosystems, Inc., Foster City, California), Sequencher v4.1 (Applied Biosystems, Inc., Foster City, California) and SeAL v2.0 (Rambaut 1996).

2.3 ANALYSIS

Two types of haplotype data were obtained (as reported in Trewick 2001a): initially, multiple individuals of the *B. nivalis* and *S. australis* complexes were screened using SSCP, which provides a rapid means of identifying DNA sequence variants; secondly, individuals representing the sequence diversity indicated by SSCP were sequenced to provide DNA nucleotide data for phylogenetic reconstruction. For *S. piliferus*, all individuals surveyed were sequenced for the COI mtDNA gene without prior screening. Distance estimation and phylogenetic analyses (maximum parsimony (MP), neighbor-joining (NJ), and maximum likelihood (ML)) were performed using PAUP*4.0b10 (Swofford 2002). Character evolution was assessed using McClade version 3.07 (Maddison & Maddison 1997). Further details of the analyses undertaken are reported in Trewick (2008).

3. Results and discussion

DNA sequences representing those obtained for each of the taxon groups detailed below were deposited on GenBank (accession numbers AY42370-AY42390, EF544487-EF544562). Pairwise genetic distances among sequence variants (haplotypes) are given in Appendix 1.

All phylogenetic analyses resulted in similar trees, and there was consistent support for the existence of the three taxon groups in question (*Sigaus piliferus*, the *Brachaspis nivalis* complex and the *Sigaus australis* complex), with each forming a separate clade. The overall level of genetic diversity within each taxon group is within a range that, for these genes, allows confidence in phylogenetic reconstruction, i.e. exhibits sufficient sequence variation to be sensitive enough to reveal within-species variation, yet does not reach a point of mutational substitution that would mask a deeper phylogenetic signal (> 13% in COI; Szymura et al. 1996). This confidence is reflected in the high statistical support from bootstrap resampling for each of the three target groups (see Trewick 2001, 2008).

The analyses reported here used COI mtDNA sequence alignments of between 540 and 780 nucleotides in length depending on the samples involved. The use of fairly short gene fragments was the result of a compromise between the number

of individuals surveyed and the quantity of data per individual; however, these fragments are sufficient to provide the necessary haplotypic data for our study.

The extent of genetic divergence among sequences from individuals within each group was in the typical range for insect species. In several instances, genetic distances (expressed here as percentage difference using Kimura two parameter correction) within existing grasshopper species complexes were higher than those found in even the most highly diverse New Zealand orthopterans (c. 8% in scree weta *Deinacrida connectens* (Trewick et al. 2000); and 9.5% in Auckland tree weta *Hemideina thoracica* (Morgan-Richards et al. 2001)). This degree of mtDNA sequence diversity within a species is unusual, and other studies of insects report divergences of as little as 2% between species (e.g. Langor & Sperling 1997). For convenience, we present trees generated using the neighbor-joining clustering method, which utilises the pairwise genetic distances determined from mtDNA sequence data (Appendix 1). Phylogenetic trees were inferred for each of the taxon groups in question, as this is the simplest means of expressing the distribution of haplotypes among sampling locations, morphospecies and the overall phylogeny.

A pattern of spatial structuring of genetic diversity was evident in all three taxon groups examined. Not surprisingly, where total genetic diversity was lowest (*Sigaus piliferus*), spatial structuring was least pronounced. An approximate indication of the likely time since the last common ancestor of a set of sequences can be inferred using a standard rate calibration of 2–2.3% per million years (Brower 1994; Juan et al. 1995; Fleischer et al. 1998). Such rates are generalised for a number of genes and taxa, and variation of gene and taxon specific rates is known.

The results for each taxon group are presented below, together with a discussion of any conservation implications or considerations. Table 1 summarises the combined spatial, morphological (current taxonomic) and haplotype (mtDNA) evidence for the grasshopper populations examined here. A set of management units have also been identified, based upon the available information. Note that this should be viewed as a working evolutionary/taxonomic hypothesis.

3.1 *Sigaus piliferus*

3.1.1 Genetic structure

Analysis of *S. piliferus* diversity used an alignment of 780 bp for a total of 51 grasshoppers from 14 locations in the North Island (Fig. 4). Two clades are evident among the data. One group (Sp.I; see Fig. 6) includes sequences from grasshoppers collected from the Ruahine Ranges northwards, including the isolated locations at Pirongia, Mt Karioi, Te Araroa and Lake Waikaremoana. The second group (Sp.II) is restricted to the Tararua Ranges. The maximum genetic divergence among samples of this species was 6.5%, and the mean divergence between the two clades was 5.4% (Table A1.1, Appendix 1). This is consistent with, but not proof of, these two groups having species status, and implies a common ancestor for the lineages during the Pliocene (2–5 mya). Within the main northern group (Sp.I), genetic diversity was distributed unevenly. The numerous samples from the Central Plateau area showed almost no DNA sequence variation,

TABLE 1. DIVERSITY AMONG *Sigaus piliferus*, *Brachaspis nivalis* COMPLEX AND *Sigaus australis* COMPLEX.

Clade code refers to the designations made in Figs 6 and 7. Locations are those given in Figs 4 and 6 for *S. piliferus* and *B. nivalis* complex, and Fig. 7 for *Sigaus australis* complex.

CURRENT TAXONOMY	PRINCIPLE CLADE CODE	TAXON/SPATIAL GROUP	MANAGEMENT UNITS	LOCATIONS SAMPLED	CURRENT EVIDENCE
Sigaus piliferus	Sp.II	*S. piliferus* Tararuas		Tararua Range	MtDNA split and morphology (Bigelow 1967)
North Island	Sp.I	*S. piliferus* northern	1 Peripheral	Mt Karioi, Pirongia Range, Whenuakura frost flats, Te Araroa	Localised, unique mtDNA and morphology (Bigelow 1967)
			2 Central	Kawekas, Central Plateau, Ruahines	Shared mtDNA and morphology (Bigelow 1967)
Brachaspis nivalis (complex)	B.II	*B. nivalis* northern	3 Marlborough—subalpine	Red Spur, Mt Lyford	MtDNA split, habitat, size and morphology (Hutton1897)
South Island			—lowland	Dee Stream	MtDNA split, habitat and size
			4 Canterbury —subalpine	Fog Peak, Craigieburn, Arrowsmith	MtDNA split, habitat and size
			—lowland	Porter River	Mt DNA split, habitat and size
	B.III	*B. nivalis* southern ("Hunter")	5 Sub-alpine	Mt Dobson, Hunter Hills, Tekapo, Mt Sutton, Mt St Bathans, Rocky Top	MtDNA split, habitat and morphology (Morris 2001a)
			6 Lowland (*B. robustus*)	Mackenzie Basin	Habitat and morphology (Bigelow 1967)
Sigaus australis (complex)	Sa.I	*S. australis* northern	7 Typical form	Mt Sutton, Mt Dobson, Sealy Tarns, Craigieburn, Fog Peak, Mt St John	MtDNA split
South Island	Sa.II	*S. australis* south central	8 Typical form	Alexandra, Mt St Bathans, Mt Sutton, Lindis Pass, Dunstan Mountains	MtDNA split, morphology
			9 *S.* species A	Alexandra	Morphology (Jamieson 1999)
			10 *S. childi*	Alexandra	Morphology (Jamieson 1999)
	Sa.III	*S. australis* southwest	11 Typical form	Rob Roy, Harris Saddle, Remarkables, Old Woman Range, Alexandra	MtDNA split (additional morphs likely; Morris 2003)
			12 *S. obelisci*	Old Man Range	Isolation, morphology (Bigelow 1967)
			13 *S. homerensis*	Earl Mountains	Isolation, morphology (Morris 2003)
	Sa.IV	*S. australis* southeast	14 Typical form	Danseys Pass, Rock & Pillar Range, Flagstaff Hill, Mt St Bathans, Kakanui Mountains, Rocky Top	MtDNA split
			15 *S.* "undescribed"	Alexandra	Novel morphotyptes, crypsis

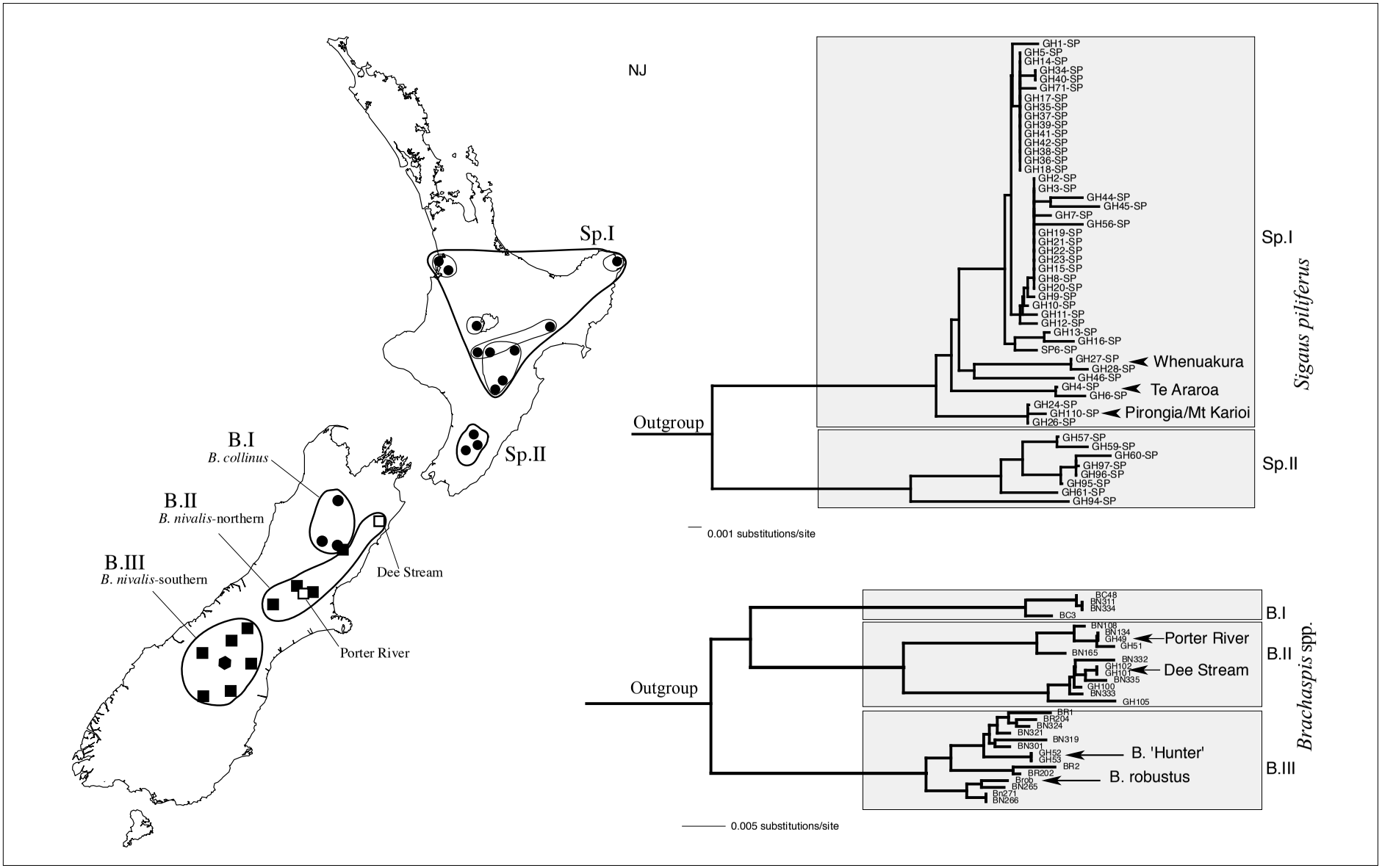

Figure 6. Neighbor-joining trees of mtDNA COI sequences from *Sigaus piliferus* (top), and *Brachaspis* spp. (bottom). Clades inferred from phylogenetic analysis are labelled Sp.I–II and B.I–III, respectively. Contour plots on the map indicate the geographic distribution of grasshopper clades.

and haplotypes found there were also present in the Ruahines, Kaweka and Lake Waikaremoana samples. The few individuals from isolated sites at Te Araroa, Whenuakura (Lake Taupo) and Pirongia area showed a comparatively high level of sequence difference; each location had unique haplotypes that differed by c. 2.5% from those at other locations.

3.1.2 Conservation considerations

In his examination of *Sigaus piliferus*, Bigelow (1967: 29) identified 'three morphological groups, corresponding with three broad geographical areas; a northern group from the Rotorua area and East cape Peninsula, a central group from Tongariro National Park and the Kaimanawa and Kaweka Ranges, and a southern group from the Tararua Range'. The present genetic analysis is broadly consistent with this, allowing for small differences in the locations sampled.

Two features of these genetic data are particularly significant for conservation. First, there is a distinct split between *S. piliferus* collected on the Tararuas and those collected from all other locations, which represents the minimum number of taxa deserving of conservation effort. Second, some populations outside the Tararuas and the central North Island area are probably very small, isolated and dependent on a vegetation type that may not be self-sustaining (see below). Furthermore, grasshoppers at several of these (northern) sites have distinct genetic identities (i.e. Te Araroa, Pirongia and Whenuakura). Further work to determine the status of these populations and their habitats should be considered. Two locations (Kaueranga Valley on the Coromandel Peninsula, and Mt Maungatautari) reported as having *S. piliferus* by Bigelow (1967) were not searched explicitly for the present work, but no grasshoppers have recently been reported from them. Given that Kaueranga Valley is transected by a road and is fairly accessible, it is reasonable to assume that grasshoppers might have been found there if present. No new information is available for Mt Maungatautari.

Many of the sites from which *S. piliferus* was collected for the present study did not have typical subalpine vegetation. North Kaweka, Ruahine, Whakapapa and Tararua locations were above the treeline in tussock grasslands, whereas Pirongia, Mt Karioi, Lake Waikaremoana, Te Araroa, Rangipo Desert and Whenuakura sites were in areas where the combination of low altitude and low latitude would not normally support subalpine vegetation. However, grasshoppers were typically collected from seral tussock grassland or flax/manuka shrubland habitats. The flax shrublands appear to have developed in exposed areas of poor or thin soil. Whether or not such habitats are natural and permanent or products of past habitat modification by humans is not clear, but Rogers (1994, and references therein) concluded that seral grasslands in central North Island are unlikely to have existed in pre-human times. From the perspective of conservation, some active management role may be required to maintain grasshopper habitat at some of these small but widely spaced sites.

3.2 *Brachaspis nivalis* COMPLEX

3.2.1 Genetic structure

The genetic structure of the *Brachaspis nivalis* complex has previously been reported, with an emphasis on the status of the protected species *B. robustus* (Trewick 2001a). Here we used the same DNA sequence data with the addition of sequences from individuals representing three additional locations and forms. Trewick (2001a) reported a prominent split among sequences from individuals of *B. nivalis*, which corresponds with a spatial (north-south) split of populations in the South Island (see Fig. 6, B.II versus B.III; B.I corresponds to the species *B. collinus*, which is not a subject of this report). COI haplotypes from *B. robustus*, the rare, low-altitude species of the Mackenzie Basin area, are very closely related to haplotypes of *B. nivalis* (B.III) from montane locations in the southern part of the *Brachaspis* range. DNA sequence divergence in the B.III group is a maximum of 2.8% (Table A1.2, Appendix 1). Samples from the Hunter Hills that were added in the present study yielded haplotypes that also fell in this southern B.III clade. Sequence divergence between these two *B. nivalis* clades is relatively high (maximum 10.6%), and at a level more typical of interspecific divergence between insect species.

Haplotypes from both samples of small, low-altitude *Brachaspsis* fell in the northern *B. nivalis* clade (B.II), which is consistent with their geographic position. However, a further split within the B.II clade is evident, which also shows a north-south geographic structure. Instead of the two small, low-altitude forms falling together on the tree, as might be predicted from their similar morphology, they fall into separate clades with sequences from individuals from montane sites that they are each geographically close to. Hence, haplotypes from the low-altitude Porter River *Brachaspsis* are genetically most similar to Craigieburn and Fog Peak montane *Brachaspis*, and those from Dee Stream are genetically most similar to alpine *Brachaspis* from Mt Lyford and Red Spur (a montane location close to Dee Stream).

3.2.2 Conservation considerations

Three low-altitude *Brachaspsis* populations were included in this study: two populations of small forms from Porter River and Dee Stream, and the large form *B. robustus* from Mackenzie Basin area. In all three cases, DNA sequences from these low-altitude forms indicate close genealogical relationships with typical nearby *B. nivalis* from montane habitats. This implies that the low-altitude forms have evolved recently under selective pressure that is specific to these habitats, as Bigelow (1967) suggested. The fact that the two small forms do not share a common ancestor indicates that the small form cannot be treated as a single separate species, and that Bigelow (1967) was, considering the information available to him, correct to group them with *B. nivalis*. However, when the genetic and morphological evidence are considered together, it is evident that this approach has clearly underestimated diversity within the group. *Brachaspis robustus* is accepted as a distinct taxon on the grounds of gross external morphological (male genitalia of this species have yet to be characterised) and habitat differences, despite the lack of neutral mtDNA sequence evidence to support it. There may be justification in similarly treating the small, low-altitude forms as distinct taxa (conservation units) as well, given that they are isolated

from one another and may be isolated from their nearest montane relatives, and occupy narrow and atypical habitat. Bigelow (1967) noted that the shape of the subgenital plate of females from low-altitude populations tended to differ from that of other populations and that 'this may raise the question of a possible specific distinction' (Bigelow 1967: 70). Further population genetic research would be required to determine if this is, in fact, the case and what feature of the environment results in the reduced body size.

The southern *B. nivalis* clade (B.III) (*B. robustus** in Trewick 2001a) corresponds with the range delineated by Morris (2003) for *Brachaspis* "Hunter". Preliminary examination of leg spines, colouration and epiphalus indicates that the southern and 'Hunter' group are one and the same, and formal delineation of this taxon is required.

Certainly, the possibility that the low-altitude forms have a greater susceptibility to extinction has to be considered. Low-altitude populations occupy extremely restricted habitats in braided rivers (which are themselves narrowly circumscribed). Flooding events, land development, weed invasion and introduced predators could, quite plausibly, extinguish a population rapidly.

3.3 *Sigaus australis* COMPLEX

3.3.1 Genetic structure

The genetic diversity of *Sigaus australis* complex grasshoppers was initially surveyed using SSCP with the 12S gene fragment. Shared banding patterns indicated a shared mtDNA nucleotide sequence. The alternative haplotypes (banding patterns) were coded alphabetically and their distribution is summarised in Table 2.

Populations of *S. australis* complex tend to have unique mtDNA haplotypes. The general pattern of low diversity at sites that was inferred from SSCP haplotyping was confirmed by sequence data. Most locations have a single and usually unique haplotype, although three closely related haplotypes are evident in the Mt Dobson sample (Table 2, Fig. 7). In contrast, three haplotypes (n, o, j) at Mt St Bathans correspond to two clades (Sa.II and Sa.IV), and five SSCP haplotypes (a, c, i, s, L) at Alexandra correspond to two groups (Sa.II and Sa.III), with the addition of the sequence from *S.* "undescribed" falling into Sa.IV.

Individuals that yielded Sa.I DNA sequences came from the northernmost extent of the *S. australis* complex in the central South Island (Fig. 7). Genetic distances between Sa.I and other *S. australis* complex haplotypes are relatively high (mean 10%) and above typical values for interspecific distances in insects (Table A1.3, Appendix 1). For further discussion, see Trewick (2008).

Each of the three southern groups comprised sequences from individuals that were collected in geographically distinct (but parapatric) ranges that meet at Alexandra (Fig. 5). Clade Sa.III comprises haplotypes (in brackets) from *S. australis* (c, d, g), *S. obelisci* (p) and *S. homerensis* distributed from Alexandra westwards. Haplotype p was unique to and shared by all 13 *S. obelisci* individuals collected on the Old Man Range (Table 2). In contrast, haplotype c was present in grasshoppers from three locations, including Alexandra. Clade

TABLE 2. MORPHOSPECIES, SAMPLING LOCATIONS, SSCP HAPLOTYPES, SAMPLE SIZES (*n*), NUMBERS OF INDIVIDUALS SEQUENCED FOR THE COI AND 12S mtDNA GENES, AND HAPLOGROUPS FOR *Sigaus australis* COMPLEX GRASSHOPPERS.

		12S-SSCP		SEQUENCE		HAPLOGROUP
SPECIES	LOCATION	HAPLOTYPE	*n**	COI	12S-16S	
S. australis	Mt Sutton	m	3	2	_	+
S. australis	Mt Dobson	e	3	1	1	Sa.I
S. australis	Sealy Tarns	e	7	2	1	Sa.I
S. australis	Mt Dobson	h	5	1	1	Sa.I
S. australis	Craigieburn	k	1	_	_	Sa.I
S. australis	Fog Peak/Torlesse	k	3	_	1	Sa.I
S. australis	Mt Dobson	t	2	_	1	Sa.I
S. australis	Mt Dobson	u	2	_	1	Sa.I
S. australis	Mt John		[1]	_	1	Sa.I
S. australis	Alexandra—Conroy Dam	a	1	_	1	Sa.II
S. childi	Alexandra—Earnscleugh	a	2	1	_	Sa.II
S. childi	Alexandra—Hairpin Little Valley Rd	a	4	1	1	Sa.II
S. childi	Alexandra—Earnscleugh	i	1	1	1	Sa.II
S. childi	Alexandra—Graveyard Gully	i	1	_	_	
S. australis	Alexandra—Little Valley Rd	i	2	2	1	Sa.II
S. australis	Alexandra—Conroy Dam	L	1	1	1	Sa.II
S. australis	Mt St Bathans	n	1	_	1	Sa.II
S. australis	Mt St Bathans	o	3	1	1	Sa.II
S. australis	Mt Sutton	q	1	1	1	Sa.II
S. australis	Dunstan Mountains		[1]	1	_	Sa.II
S. australis	Lindis Pass		[2]	2	_	Sa.II
S. childi	Alexandra—Graveyard Gully	s	3	_	1	Sa.II
S. childi	Alexandra—Little Valley Rd		[1]	_	1	Sa.II
S. species A	Alexandra—Earnscleugh		[1]	1	_	Sa.II
S. australis	Rob Roy		[2]	2	_	Sa.III
S. homerensis	Earl Mountains		[3]	3		Sa.III
S. australis	Harris Saddle	c	9	2	1	Sa.III
S. australis	Alexandra—Little Valley Rd	c	3	_	1	Sa.III
S. australis	Mt Scott	c	5	_	_	Sa.III
S. australis	Remarkables	d	8	1	1	Sa.III
S. australis	Old Woman Ra.	g	8	1	1	Sa.III
S. obelisci	Old Man Ra.	p	13	1	1	Sa.III
S. australis	Danseys Pass	b	15	1	1	Sa.IV
S. australis	Rock and Pillar Ra.	b	13	1	1	Sa.IV
S. australis	Flagstaff Hill	f	8	1	1	Sa.IV
S. australis	Mt St Bathans	j	5	1	1	Sa.IV
S. australis	Kakanui Mnts.	v	1	1	_	Sa.IV
S. australis	Rocky Top	w	8	1	1	Sa.IV
S. australis	Crawford Hills	r	2	1	_	Sa.IV
S. australis	Danseys Pass		[2]	2	1	Sa.IV
S. australis	Rock and Pillar Ra.		[2]	2	_	Sa.IV
S. "undescribed"	Alexandra—Little Valley Rd		[1]	1	1	Sa. IV
Ingroup—Total individuals SSCP screened			144			
—Total individuals inluding non-SSCP			160	40	29	
Outgroup				1	4	
Total sequences			41	33		

* Entries in square brackets [ ] indicate the number of individuals subjected to DNA sequencing but not SSPC haplotyping.

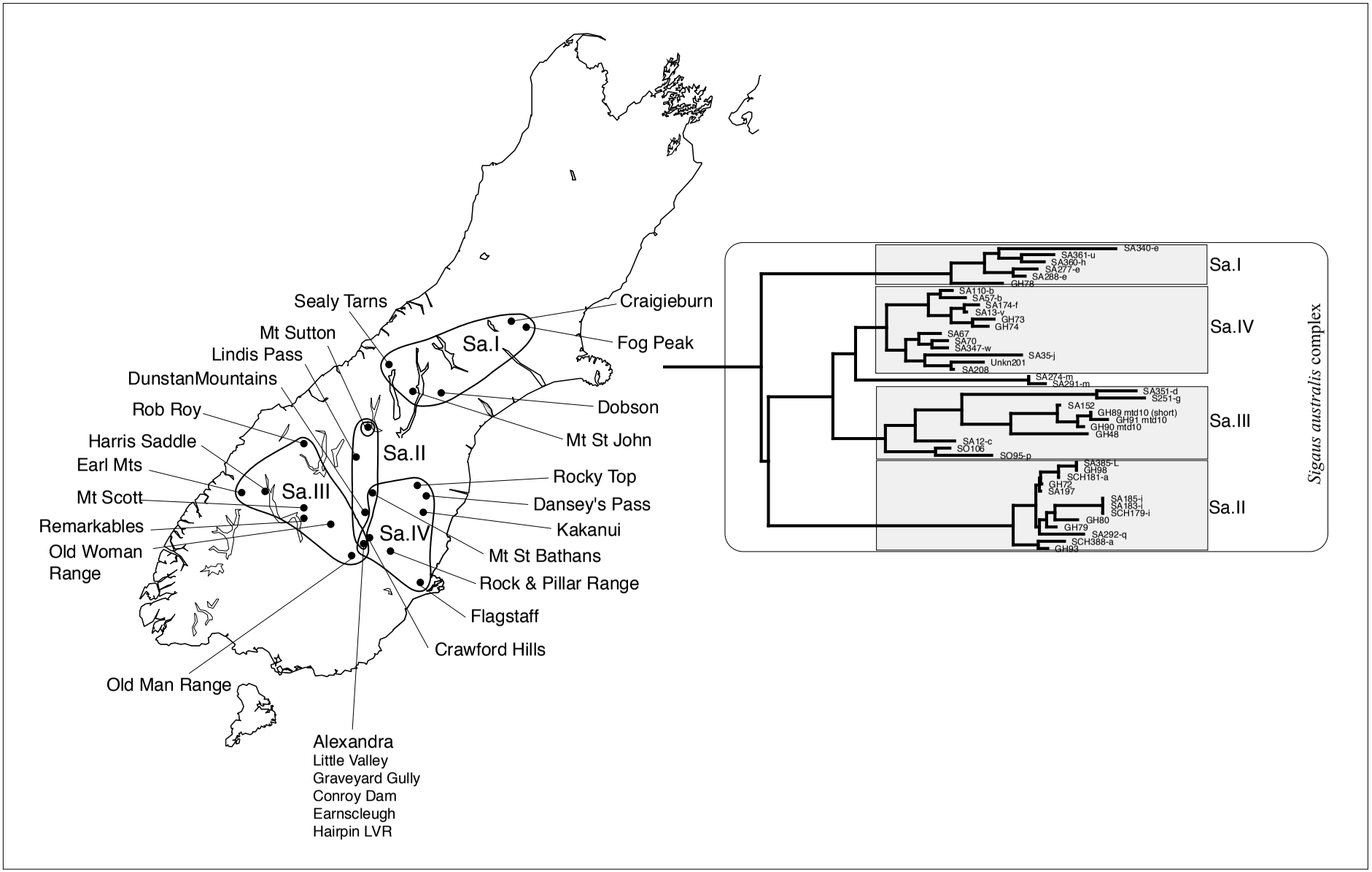

Figure 7. Neighbor-joining tree of mtDNA COI sequences from *Sigaus australis* complex. Clades inferred from phylogenetic analysis are labelled Sa.I–IV. Contour plots on the map indicate the geographic distribution of grasshopper clades. Sampling locations are labelled on the map.

Sa.IV comprised haplotypes from individuals of *S. australis* (b, f, j, v, w, r) and a single individual of *S.* "undescribed" that was collected north and east of Alexandra (see Fig. 3D). Clade Sa.II included all 12 *S. childi* (a, i, s) surveyed, plus *S. australis* (a, i, L, n, o, q) and the single sequence from *S.* species A (Fig. 7). Some individuals of *S. australis* and *S. childi* shared the same putative (SSCP) haplotypes (two *S. australis* and two *S. childi* had putative haplotype i, one *S. australis* and six *S. childi* had haplotype a). Furthermore, two *S. australis* from Little Valley Rd had the same COI sequence haplotype as an *S. childi* from Alexandra (Earnscleugh), and an *S. australis* from the Dunstan Mountains had the same COI sequence haplotype as an *S. childi* from Alexandra (Hairpin, Little Valley Road; see Fig. 5).

3.3.2 Conservation considerations

The *Sigaus australis* complex contains comparatively high genetic and morphological (taxonomic) diversity. Three of the four main mtDNA clades comprise more than one morphotype. This is above and beyond the colour polymorphism that is known from single populations of typical *S. australis* in typical montane habitat. Further morphospecies have been proposed (Morris 2002a). Preliminary indications from ongoing morphological study are that additional diversity may exist. In particular, morphological and behavioural variation among grasshoppers on the Remarkables may mean that there are sympatric species there.

Of the four clades, Sa.I is the most clearly circumscribed spatially and genetically. The presence of a distinct *S. australis* lineage in central South Island suggests a protracted period of isolation throughout many episodes of Pleistocene climate change, rather than colonisation of the area at the end of the Pleistocene. On the basis of estimates of genetic distance between clade Sa.I and other *S. australis* COI haplotypes, this split may date back to the late Pliocene (5 mya). This spatial pattern and estimated time of divergence are similar to those identified for the alpine scree weta (*Deinacrida connectens*) in the same landscape (Trewick et al. 2000; Trewick 2001b).

Clade Sa.IV is also dominated by typical *S. australis* grasshoppers, with a single undescribed form (in our sample) being closely related to these (Fig. 8). Jamieson (1999) recorded a similar form to this undescribed specimen; both are highly cryptic on the tumbling lichen (*Chondropsis semiviridis*) (Fig. 3F). One of the authors (SM) has also observed this form on several occasions within the geographic range encompassed by Sa.IV. Further survey work is required, as there appears to be more morphological variation in this area, and it would be useful to determine whether this represents polymorphism or the existence of independent evolutionary lineages.

Clade Sa.III consists of the southwestern *S. australis* grasshoppers, and includes *S. homerensis* (Morris 2003), *S. obelisci* (Bigelow 1967) and *S.* "Rob Roy" (Morris 2002a). The group also includes specimens from the Remarkables, but whether these represent *S.* "Remarkables" (Morris 2002a) remains to be resolved. All of these taxa are very close in general form to typical *S. australis*. The existence of an additional haplotype at Mt Sutton that is weakly associated with Sa.IV (m; Fig. 8) indicates that this location may deserve further study.

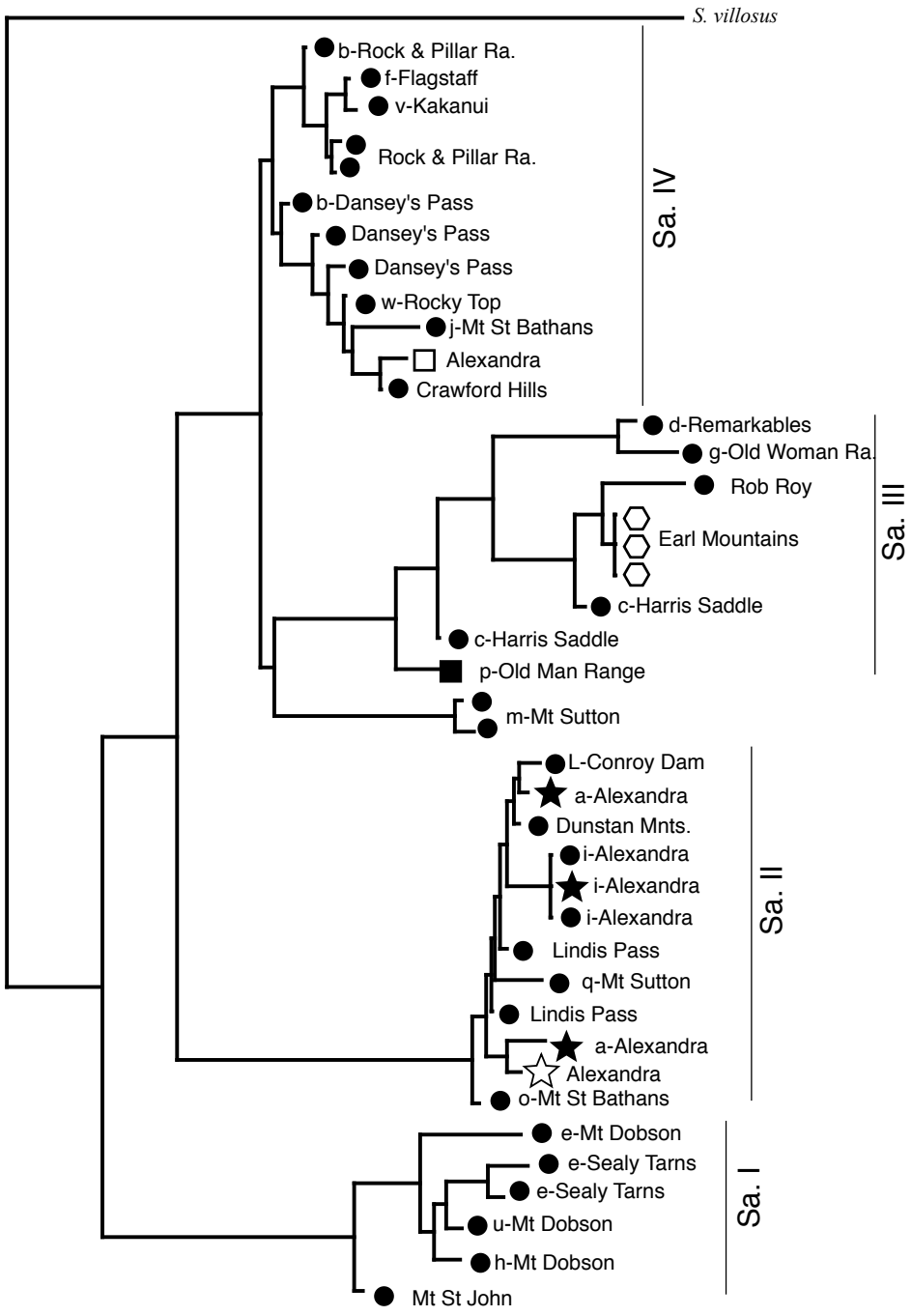

Figure 8. Neighbor-joining tree of mtDNA COI sequences from grasshoppers of the *Sigaus australis* complex. Four clades are indicated: Sa.I–Sa.IV. Symbols at branch tips indicate morphospecies: ● = *S. australis*, ⬡ = *S. homerensis*, ★ = *S. childi*, ■ = *S. obelisci*, ☆ = *S.* species A, □ = *S.* "undescribed".

Clade Sa.II is the south central *S. australis* group, and includes apparently typical *S. australis*, plus *S. childi* and *S.* species A grasshoppers. These taxa have similar or, in some instances, identical haplotypes and a rather narrow geographic range (Fig. 8). The sharing of haplotypes by species, and therefore their paraphyly, can be explained in two contrasting ways: either mitochondria have been exchanged recently via introgression (hybridisation) or they have been retained by incomplete lineage sorting through a recent speciation event (Funk & Omland 2003). In the case of Sa.II, if hybridisation has been involved, it was not restricted to a single ancestral event but rather has been extensive and recent, with multiple similar haplotypes being shared between species. Morris (2002c) noted that some individuals examined had characteristics of both *S. childi* and *S.* species A. Distinguishing these processes is beyond the scope of the present data.

4. Conclusions and recommendations

It is highly likely that some species (e.g. *S. obelisci*) represent small, geographically isolated populations of a more widely distributed taxon that have accumulated subtle morphological differences. Diversity in each of the groups studied has evolved relatively recently and probably during the late Pliocene/Pleistocene at the latest. As noted by Trewick (2001a), *B. robustus* may well have evolved after a population become isolated at the end of the last glacial maximum (LGM). Climate cycling was probably of broad significance in population structuring and speciation in New Zealand grasshoppers. Following each glaciation, the climate warmed and the lowest extent of the alpine zone was raised in altitude. Grasshopper populations presumably tracked this change, maintaining their association with open and predominantly grassland habitat above the treeline. Forest replaced most open country below the montane zone, extirpating grasshoppers. In some instances, successive glacial cycles probably reinforced regional differences. The relatively high genetic distances between *Brachaspis nivalis* groups (B.II versus B.III) and *Sigaus australis* groups (Sa.I versus others) are consistent with this (Appendix 1).

In some instances, it is likely that relict low-altitude populations survived climate and vegetation shifts, finding suitable habitat in braided riverbeds and the semi-arid environments of Central Otago and central Canterbury. Following the LGM, these semiarid environments apparently did support some woodland (Clark et al. 1996; McGlone et al. 1995)—perhaps as much as 80% (Walker et al. 2004)—but it is unlikely that continuous dense forest developed.

The Alexandra area is of particular interest for conservation. It either represents a focus of speciation within the *S. australis* complex or it is an active contact area, where species and geographic populations meet and hybridise. In the same area, two species of *Phaulacridium* grasshoppers have narrowly circumscribed ecological ranges (Westman & Ritchie 1984) and the status of two *Prodontria* beetles has been debated (Emerson & Wallis 1994; Wallis 2001). Because mitochondrial DNA is inherited maternally, the use of mtDNA sequence data alone cannot distinguish between introgression or recent speciation (with incomplete lineage sorting), no matter how many data are collected. Any hope of understanding the state of gene flow among taxa at Alexandra will require the application of sufficiently variable biparentally inherited markers.

Application of a strict phylogenetic approach to the systematics of these grasshoppers would not be consistent with existing taxonomy and would be unhelpful. It is clear that, for the *S. australis* complex in particular, additional genetic markers are required to determine what process has resulted in the mismatch between mtDNA data and morphology. However, for the purposes of biodiversity conservation (as opposed to taxonomic revision), an optimal approach would be to incorporate both morphological and phylolgenetic evidence to maximise the inclusion and retention of diversity. The molecular phylogenetic evidence is an indicator of historic boundaries among populations, while morphological/behavioural evidence may be indicative of adaptive responses to habitat and predators.

4.1 *Sigaus piliferus*

This species almost certainly consists of at least two diagnosable entities deserving species status. These will be referred to as *Sigaus* "Tararuas" for the Tararua lineage and *S. piliferus* for the remainder. However, for the purposes of conservation, additional populations should be accommodated in management policy. From the present survey, these include populations at Whenuakura, Pirongia area and Te Araroa (Table 1). It is likely that other populations exist, and effort needs to be given to find these as soon as possible. Anthropogenic habitat modification, vegetation succession and climate change are expected to impact on these populations in the short, medium and long term.

4.2 *Brachaspis nivalis* COMPLEX

Brachaspis "Hunter" as proposed by Morris (2002a) will, with additional morphological examination, very probably prove to be diagnosable as a separate species from *B. nivalis*. *Brachaspis* "Hunter" corresponds to the southern *B. nivalis* clade (B.III) identified as *B. robustus** by Trewick (2001). In addition, conservation managers need to give special attention to low-altitude populations, all of which are morphologically distinct (on size at least) from alpine populations (White 1994). The two populations of small, low-altitude *Brachaspis* examined in the present study have genealogical relationships in the northern (B.II) clade that are analogous to the pattern observed for *B. robustus* in the southern (B.III) clade. In these cases, morphologically distinct populations (species in the case of *B. robustus*) are allied to nearby montane populations of typical *B. nivalis* (Table 1). Further study will reveal whether there is justification for describing low-altitude forms (e.g. *B.* "low altitude"; Morris 2002a) as distinct species.

4.3 *Sigaus australis* COMPLEX

Recognition needs to be given to the spatial distribution of diversity within this group (Table 1). Further morphological study may support the splitting of one or more of the groups indicated by phylogenetic analysis of mtDNA sequences (i.e. Sa.I, etc.). There is, however, currently little evidence from the mtDNA to support *S.* "Remarkables" and *S.* "Rob Roy" as being distinct, although this may change with further sampling and analysis. Whilst *S. homerensis* and *S. obelisci* show distinct morphological characters, they are closely allied to the above and other Sa.III populations in the geographic area (Table 2). The status of *S. childi* and *S.* species A in Sa.II is of paramount concern in this group. There is little doubt that these and other cryptic forms are geographically localised and deserving of further study to determine to what extent they are threatened ecologically.

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7. Glossary

Allopatric Spatially separate populations or species.

Biogeography The study of the distribution of diversity over space and time.

Haplotypes A set of closely linked alleles (genes or DNA polymorphisms) inherited as a unit. In the case of mitochondrial data, haplotypes are DNA sequence variants identified at mitochondrial gene regions.

Introgression Gene flow between species.

Morphospecies A typological species distinguished solely on the basis of morphology.

Morphotype The morphological form of a species.

Neoallotype The single specimen designated as the name-bearing type of a nominal species or subspecies for which no holotype, etc. is available.

Parapatric Adjacent populations or closely related species.

Paraphyletic A group of organisms that contains its most recent common ancestor but does not contain *all* the descendants of that ancestor.

Phylogenetics The study of the evolutionary relationships of organisms.

Phylogeny The evolutionary relationships of organisms.

Phylogeography Biogeography as revealed by a comparison of estimated phylogenies of populations or species with their geographic distributions.

Sympatric Species inhabiting the same geographic area.

Appendix 1

PAIRWISE GENETIC DISTANCES FOR NEW ZEALAND GRASSHOPPERS

Pairwise genetic distances (Kimura 2 parameter model) among mitochondrial COI DNA sequences from *Sigaus piliferus*, *Brachaspis nivalis* complex and *Sigaus australis* complex. Values indicate genetic distance between pairs of individual grasshoppers (indicated by codes GH1, SP6, etc.); smaller values indicate greater similarity of individuals.

TABLE A1.1. PAIRWISE GENETIC DISTANCES FOR *Sigaus piliferus*. □ = CLADE Sp.I; ■ = Sp.II.

REGION	CLADE	CODE	GH1	SP6	GH2	GH56	GH3	GH5	GH19	GH21	GH22	GH23	GH13	GH14	GH15	GH17	GH18	GH34	GH35	GH36
Central	**Sp.I**	GH1																		
		SP6	0.000																	**Sp.I**
		GH2	0.002	0.002																
		GH56	0.002	0.002	0.000															
		GH3	0.002	0.002	0.000	0.000														
		GH5	0.000	0.000	0.002	0.002	0.002													
		GH19	0.002	0.002	0.000	0.000	0.000	0.002												
		GH21	0.002	0.002	0.000	0.000	0.000	0.002	0.000											
		GH22	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000										
		GH23	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000									
		GH13	0.002	0.002	0.004	0.004	0.004	0.002	0.004	0.004	0.004	0.004								
		GH14	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002							
		GH15	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.004	0.002						
		GH17	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002					
		GH18	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000				
		GH34	0.002	0.002	0.004	0.004	0.004	0.002	0.004	0.004	0.004	0.004	0.004	0.002	0.004	0.002	0.002			
		GH35	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.002		
		GH36	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.002	0.000	
		GH37	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.000
		GH38	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.000
		GH39	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.000
		GH40	0.002	0.002	0.004	0.004	0.004	0.002	0.004	0.004	0.004	0.004	0.004	0.002	0.004	0.002	0.002	0.000	0.002	0.002
		GH41	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.000
		GH42	0.000	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.000
		GH44	0.006	0.006	0.004	0.004	0.004	0.006	0.004	0.004	0.004	0.004	0.008	0.006	0.004	0.006	0.006	0.008	0.006	0.006
		GH45	0.008	0.008	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.010	0.008	0.006	0.008	0.008	0.010	0.008	0.008
		GH10	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.002	0.002	0.004	0.002	0.002
		GH9	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.002	0.002	0.004	0.002	0.002
		GH8	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.002	0.002	0.004	0.002	0.002
		GH7	0.004	0.004	0.002	0.002	0.002	0.004	0.002	0.002	0.002	0.002	0.006	0.004	0.002	0.004	0.004	0.006	0.004	0.004
		GH12	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.002	0.002	0.004	0.002	0.002
		GH11	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.002	0.002	0.004	0.002	0.002
		GH16	0.002	0.002	0.004	0.004	0.004	0.002	0.004	0.004	0.004	0.004	0.000	0.002	0.004	0.002	0.002	0.004	0.002	0.002
		GH20	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.002	0.002	0.004	0.002	0.002
	Pirongia	GH24	0.012	0.012	0.014	0.014	0.014	0.012	0.014	0.014	0.014	0.014	0.014	0.012	0.014	0.012	0.012	0.014	0.012	0.012
	Pirongia	GH110	0.014	0.014	0.016	0.016	0.016	0.014	0.016	0.016	0.016	0.016	0.016	0.014	0.016	0.014	0.014	0.016	0.014	0.014
	Pirongia	GH26	0.012	0.012	0.014	0.014	0.014	0.012	0.014	0.014	0.014	0.014	0.014	0.012	0.014	0.012	0.012	0.014	0.012	0.012
		GH46	0.008	0.008	0.010	0.010	0.010	0.008	0.010	0.010	0.010	0.010	0.010	0.008	0.010	0.008	0.008	0.010	0.008	0.008
	Whenuakura	GH27	0.012	0.012	0.012	0.014	0.014	0.012	0.014	0.014	0.014	0.014	0.014	0.012	0.014	0.012	0.012	0.014	0.012	0.012
	Whenuakura	GH28	0.014	0.014	0.014	0.016	0.016	0.014	0.016	0.016	0.016	0.016	0.016	0.014	0.016	0.014	0.014	0.016	0.014	0.014
	Te Araroa	GH4	0.012	0.012	0.014	0.014	0.014	0.012	0.014	0.014	0.014	0.014	0.010	0.012	0.014	0.012	0.012	0.014	0.012	0.012
	Te Araroa	GH6	0.014	0.014	0.016	0.016	0.016	0.014	0.016	0.016	0.016	0.016	0.012	0.014	0.016	0.014	0.014	0.016	0.014	0.014
Tararuas	**Sp.II**	GH57	0.052	0.052	0.053	0.055	0.055	0.052	0.055	0.055	0.055	0.055	0.050	0.052	0.055	0.052	0.052	0.055	0.052	0.052
		GH59	0.057	0.057	0.058	0.059	0.059	0.057	0.059	0.059	0.059	0.059	0.055	0.057	0.059	0.057	0.057	0.055	0.057	0.057
		GH60	0.061	0.061	0.062	0.063	0.063	0.061	0.063	0.063	0.063	0.063	0.059	0.061	0.063	0.061	0.061	0.059	0.061	0.061
		GH97	0.059	0.059	0.060	0.061	0.061	0.059	0.061	0.061	0.061	0.061	0.057	0.059	0.061	0.059	0.059	0.057	0.059	0.059
		GH95	0.057	0.057	0.058	0.059	0.059	0.057	0.059	0.059	0.059	0.059	0.055	0.057	0.059	0.057	0.057	0.055	0.057	0.057
		GH96	0.059	0.059	0.060	0.061	0.061	0.059	0.061	0.061	0.061	0.061	0.057	0.059	0.061	0.059	0.059	0.057	0.059	0.059
		GH61	0.052	0.052	0.053	0.055	0.055	0.052	0.055	0.055	0.055	0.055	0.050	0.052	0.055	0.052	0.052	0.055	0.052	0.052
		GH94	0.052	0.052	0.055	0.054	0.054	0.052	0.054	0.054	0.054	0.054	0.050	0.052	0.054	0.052	0.052	0.054	0.052	0.052

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Table A1.1—continued.

REGION	CLADE	CODE	GH37	GH38	GH39	GH40	GH41	GH42	GH44	GH45	GH10	GH9	GH8	GH7	GH12	GH11	GH16	GH20
Central	**Sp.I**	GH1																
		SP6																**Sp.I**
		GH2																
		GH56																
		GH3																
		GH5																
		GH19																
		GH21																
		GH22																
		GH23																
		GH13																
		GH14																
		GH15																
		GH17																
		GH18																
		GH34																
		GH35																
		GH36																
		GH37																
		GH38	0.000															
		GH39	0.000	0.000														
		GH40	0.002	0.002	0.002													
		GH41	0.000	0.000	0.000	0.002												
		GH42	0.000	0.000	0.000	0.002	0.000											
		GH44	0.006	0.006	0.006	0.008	0.006	0.006										
		GH45	0.008	0.008	0.008	0.010	0.008	0.008	0.006									
		GH10	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.006								
		GH9	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.006	0.000							
		GH8	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.006	0.000	0.000						
		GH7	0.004	0.004	0.004	0.006	0.004	0.004	0.006	0.008	0.002	0.002	0.002					
		GH12	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.006	0.000	0.000	0.000	0.002				
		GH11	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.006	0.000	0.000	0.000	0.002	0.000			
		GH16	0.002	0.002	0.002	0.004	0.002	0.002	0.008	0.010	0.004	0.004	0.004	0.006	0.004	0.004		
		GH20	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.006	0.000	0.000	0.000	0.002	0.000	0.000	0.004	
Pirongia		GH24	0.012	0.012	0.012	0.014	0.012	0.012	0.018	0.020	0.014	0.014	0.014	0.016	0.014	0.014	0.014	0.014
Pirongia		GH110	0.014	0.014	0.014	0.016	0.014	0.014	0.020	0.022	0.016	0.016	0.016	0.018	0.016	0.016	0.016	0.016
Pirongia		GH26	0.012	0.012	0.012	0.014	0.012	0.012	0.018	0.020	0.014	0.014	0.014	0.016	0.014	0.014	0.014	0.014
		GH46	0.008	0.008	0.008	0.010	0.008	0.008	0.014	0.016	0.010	0.010	0.010	0.012	0.010	0.010	0.010	0.010
Whenuakura		GH27	0.012	0.012	0.012	0.014	0.012	0.012	0.018	0.020	0.014	0.014	0.014	0.016	0.014	0.014	0.014	0.014
Whenuakura		GH28	0.014	0.014	0.014	0.016	0.014	0.014	0.016	0.022	0.016	0.016	0.016	0.018	0.016	0.016	0.016	0.016
Te Araroa		GH4	0.012	0.012	0.012	0.014	0.012	0.012	0.018	0.020	0.014	0.014	0.014	0.016	0.014	0.014	0.010	0.014
Te Araroa		GH6	0.014	0.014	0.014	0.016	0.014	0.014	0.020	0.022	0.016	0.016	0.016	0.018	0.016	0.016	0.012	0.016
Tararuas	**Sp.II**	GH57	0.052	0.052	0.052	0.055	0.052	0.053	0.059	0.061	0.055	0.055	0.055	0.057	0.055	0.055	0.050	0.055
		GH59	0.057	0.057	0.057	0.055	0.057	0.057	0.063	0.065	0.059	0.059	0.059	0.061	0.059	0.059	0.055	0.059
		GH60	0.061	0.061	0.061	0.059	0.061	0.061	0.068	0.070	0.063	0.063	0.063	0.065	0.063	0.063	0.059	0.063
		GH97	0.059	0.059	0.059	0.057	0.059	0.059	0.065	0.067	0.061	0.061	0.061	0.063	0.061	0.061	0.057	0.061
		GH95	0.057	0.057	0.057	0.055	0.057	0.057	0.063	0.065	0.059	0.059	0.059	0.061	0.059	0.059	0.055	0.059
		GH96	0.059	0.059	0.059	0.057	0.059	0.059	0.065	0.067	0.061	0.061	0.061	0.063	0.061	0.061	0.057	0.061
		GH61	0.052	0.052	0.052	0.055	0.052	0.053	0.059	0.061	0.055	0.055	0.055	0.057	0.055	0.055	0.050	0.055
		GH94	0.052	0.052	0.052	0.054	0.052	0.053	0.059	0.061	0.054	0.054	0.054	0.057	0.054	0.054	0.050	0.054

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Table A1.1—continued.

REGION	CLADE	CODE	GH24	GH110	GH26	GH46	GH27	GH28	GH4	GH6	GH57	GH59	GH60	GH97	GH95	GH96	GH61	GH94
Central	**Sp.I**	GH1								**Sp.I**								
		SP6																
		GH2																
		GH56																
		GH3																
		GH5																
		GH19																
		GH21																
		GH22																
		GH23																
		GH13																
		GH14																
		GH15																
		GH17																
		GH18																
		GH34																
		GH35																
		GH36																
		GH37																
		GH38																
		GH39																
		GH40																
		GH41																
		GH42																
		GH44																
		GH45																
		GH10																
		GH9																
		GH8																
		GH7																
		GH12																
		GH11																
		GH16																
		GH20																
Pirongia		GH24																
Pirongia		GH110	0.002															
Pirongia		GH26	0.000	0.002														
		GH46	0.008	0.010	0.008													
Whenuakura		GH27	0.016	0.018	0.016	0.012												
Whenuakura		GH28	0.018	0.020	0.018	0.014	0.002											
Te Araroa		GH4	0.016	0.018	0.016	0.012	0.016	0.018										
Te Araroa		GH6	0.018	0.020	0.018	0.010	0.018	0.020	0.002									
Tararuas	**Sp.II**	GH57	0.057	0.059	0.057	0.057	0.053	0.055	0.048	0.050								
		GH59	0.057	0.059	0.057	0.057	0.057	0.059	0.053	0.055	0.004							**Sp.II**
		GH60	0.061	0.064	0.061	0.061	0.061	0.063	0.057	0.059	0.012	0.008						
		GH97	0.059	0.061	0.059	0.059	0.059	0.061	0.055	0.057	0.010	0.006	0.002					
		GH95	0.061	0.064	0.061	0.061	0.057	0.059	0.053	0.055	0.008	0.008	0.004	0.002				
		GH96	0.059	0.061	0.059	0.059	0.059	0.061	0.055	0.057	0.010	0.006	0.002	0.000	0.002			
		GH61	0.057	0.059	0.057	0.057	0.057	0.059	0.053	0.055	0.008	0.008	0.016	0.014	0.016	0.014		
		GH94	0.052	0.055	0.052	0.052	0.057	0.059	0.048	0.050	0.026	0.026	0.030	0.028	0.030	0.028	0.026	

TABLE A1.2. PAIRWISE GENETIC DISTANCES FOR *Brachaspis nivalis* COMPLEX. ☐ = CLADE B.I; ☐ = B.II; ■ = B.III.

TAXON/ REGION	CLADE	CODE	BC48	BN311	BN334	BC3	BN108	BN134	GH49	GH51	BN165	BN332	GH102	GH105	GH100	GH101	BN335	BN333	BR1	BR204
B. collinus	**B.I**	BC48				**B.I**														
		BN311	0.002																	
		BN334	0.002	0.000																
		BC3	0.004	0.004	0.004															
Northern	**B.II**	BN108	0.080	0.083	0.083	0.066												**B.II**		
B. nivalis		BN134	0.080	0.083	0.083	0.066	0.004													
Porter		GH49	0.080	0.083	0.083	0.066	0.004	0.000												
		GH51	0.083	0.085	0.085	0.069	0.006	0.002	0.002											
		BN165	0.083	0.086	0.086	0.069	0.010	0.010	0.010	0.012										
		BN332	0.063	0.065	0.065	0.053	0.044	0.040	0.040	0.042	0.048									
Dee		GH102	0.063	0.065	0.065	0.053	0.044	0.040	0.040	0.042	0.048	0.008								
		GH105	0.069	0.067	0.067	0.057	0.046	0.042	0.042	0.044	0.051	0.010	0.014							
		GH100	0.061	0.063	0.063	0.051	0.042	0.038	0.038	0.040	0.046	0.006	0.002	0.012						
Dee		GH101	0.063	0.065	0.065	0.053	0.044	0.040	0.040	0.042	0.048	0.008	0.000	0.014	0.002					
		BN335	0.067	0.069	0.069	0.058	0.044	0.044	0.044	0.046	0.048	0.008	0.004	0.014	0.006	0.004				
		BN333	0.061	0.063	0.063	0.051	0.044	0.040	0.040	0.042	0.044	0.008	0.004	0.014	0.002	0.004	0.008			
Southern	**B.III**	BR1	0.076	0.074	0.074	0.069	0.082	0.078	0.078	0.080	0.083	0.065	0.065	0.067	0.063	0.065	0.069	0.065	**B.III**	
B. nivalis		BR204	0.078	0.076	0.076	0.066	0.085	0.080	0.080	0.082	0.085	0.067	0.067	0.073	0.065	0.067	0.071	0.067	0.010	
		BN319	0.074	0.072	0.072	0.062	0.080	0.076	0.076	0.078	0.081	0.067	0.067	0.074	0.065	0.067	0.071	0.067	0.014	0.012
		BN321	0.074	0.071	0.071	0.062	0.080	0.076	0.076	0.078	0.081	0.063	0.063	0.069	0.060	0.063	0.067	0.063	0.010	0.004
		BN301	0.074	0.072	0.072	0.062	0.076	0.071	0.071	0.074	0.076	0.063	0.063	0.069	0.061	0.063	0.067	0.063	0.010	0.008
		BN324	0.078	0.076	0.076	0.066	0.080	0.076	0.076	0.078	0.081	0.067	0.067	0.073	0.065	0.067	0.071	0.067	0.006	0.004
"Hunter"		GH52	0.083	0.080	0.080	0.071	0.085	0.080	0.080	0.082	0.085	0.067	0.067	0.074	0.065	0.067	0.071	0.067	0.014	0.012
"Hunter"		GH53	0.083	0.080	0.080	0.071	0.085	0.080	0.080	0.082	0.085	0.067	0.067	0.074	0.065	0.067	0.071	0.067	0.014	0.012
		BR2	0.073	0.071	0.071	0.062	0.078	0.078	0.078	0.080	0.076	0.069	0.073	0.075	0.071	0.073	0.078	0.069	0.026	0.024
		BR202	0.076	0.073	0.073	0.064	0.080	0.080	0.080	0.082	0.078	0.071	0.071	0.078	0.069	0.071	0.075	0.067	0.020	0.018
B. robustus		Brob	0.080	0.078	0.078	0.069	0.082	0.078	0.078	0.080	0.079	0.069	0.069	0.076	0.067	0.069	0.073	0.069	0.024	0.022
		Bn271	0.071	0.069	0.069	0.060	0.074	0.069	0.069	0.071	0.070	0.061	0.065	0.067	0.063	0.065	0.069	0.065	0.024	0.022
		BN265	0.074	0.071	0.071	0.062	0.080	0.076	0.076	0.078	0.076	0.063	0.063	0.069	0.060	0.063	0.067	0.063	0.022	0.024
		BN266	0.071	0.069	0.069	0.060	0.074	0.069	0.069	0.071	0.070	0.061	0.065	0.067	0.063	0.065	0.069	0.065	0.024	0.022

Continued on next page

Table A1.2—continued.

TAXON/ REGION	CLADE	CODE	BN319	BN321	BN301	BN324	GH52	GH53	BR2	BR202	BROB	BN271	BN265	BN266
B. collinus	**B.I**	BC48												
		BN311												
		BN334												
		BC3												
Northern		BN108												
B. nivalis	**B.II**	BN134												
Porter		GH49												
		GH51												
		BN165												
		BN332												
Dee		GH102												
		GH105												
		GH100												
Dee		GH101												
		BN335												
		BN333												
Southern		BR1												
B. nivalis	**B.III**	BR204												**B.III**
		BN319												
		BN321	0.012											
		BN301	0.008	0.004										
		BN324	0.008	0.004	0.004									
"Hunter"		GH52	0.012	0.012	0.008	0.008								
"Hunter"		GH53	0.012	0.012	0.008	0.008	0.000							
		BR2	0.024	0.022	0.018	0.020	0.024	0.024						
		BR202	0.018	0.016	0.012	0.014	0.018	0.018	0.006					
B. robustus		Brob	0.022	0.018	0.014	0.018	0.022	0.022	0.026	0.020				
		Bn271	0.022	0.018	0.014	0.018	0.022	0.022	0.022	0.020	0.008			
		BN265	0.024	0.020	0.016	0.020	0.024	0.024	0.028	0.022	0.006	0.006		
		BN266	0.022	0.018	0.014	0.018	0.022	0.022	0.022	0.020	0.008	0.000	0.006	

TABLE A1.3. PAIRWISE GENETIC DISTANCES FOR *Sigaus australis* COMPLEX. ☐ = CLADE Sa.I; ☐ = Sa.II; ☐ = Sa.III; ☐ = Sa.IV.

REGION	CLADE	CODE	SA340-e	SA277-e	SA288-e	SA361-u	SA360-h	GH78	SA110-b	SA57-b	SA174-f	SA13-v	GH73	GH74	SA67	SA70	SA347-w
Northern	**Sa.I**	SA340-e						**Sa.I**									
		SA277-e	0.022														
		SA288-e	0.020	0.004													
		SA361-u	0.018	0.010	0.010												
		SA360-h	0.020	0.014	0.014	0.006											
		GH78	0.020	0.020	0.018	0.014	0.012										
Southeastern	**Sa.IV**	SA110-b	0.061	0.062	0.055	0.057	0.057	0.047									**Sa.IV**
		SA57-b	0.063	0.068	0.061	0.059	0.059	0.046	0.004								
		SA174-f	0.069	0.065	0.059	0.063	0.063	0.052	0.008	0.012							
		SA13-v	0.067	0.063	0.057	0.060	0.061	0.050	0.010	0.014	0.002						
		GH73	0.073	0.073	0.066	0.064	0.066	0.055	0.008	0.010	0.006	0.008					
		GH74	0.068	0.069	0.062	0.060	0.062	0.051	0.004	0.010	0.006	0.008	0.004				
		SA67	0.063	0.068	0.061	0.063	0.059	0.046	0.008	0.006	0.018	0.020	0.016	0.014			
		SA70	0.066	0.070	0.064	0.066	0.062	0.049	0.010	0.006	0.018	0.020	0.014	0.016	0.000		
		SA347-w	0.072	0.076	0.069	0.072	0.067	0.055	0.018	0.012	0.026	0.028	0.020	0.024	0.008	0.000	
		SA35-j	0.074	0.074	0.067	0.069	0.069	0.057	0.024	0.018	0.028	0.030	0.026	0.030	0.018	0.012	0.014
		Unkn201	0.072	0.076	0.069	0.072	0.067	0.055	0.022	0.016	0.030	0.028	0.024	0.028	0.016	0.010	0.012
		SA208	0.069	0.073	0.066	0.067	0.064	0.051	0.018	0.012	0.026	0.024	0.020	0.024	0.012	0.006	0.008
		SA274-m	0.067	0.071	0.065	0.061	0.063	0.050	0.026	0.026	0.034	0.032	0.036	0.032	0.026	0.030	0.040
		SA291-m	0.069	0.074	0.067	0.063	0.065	0.052	0.028	0.028	0.036	0.034	0.039	0.034	0.028	0.032	0.042
Southwestern	**Sa.III**	SA351-d	0.083	0.085	0.078	0.083	0.085	0.076	0.045	0.042	0.048	0.050	0.049	0.049	0.049	0.047	0.052
		S251-g	0.087	0.092	0.085	0.091	0.092	0.083	0.042	0.040	0.048	0.050	0.049	0.049	0.047	0.045	0.050
		SA152	0.069	0.078	0.076	0.067	0.070	0.061	0.040	0.039	0.050	0.047	0.043	0.043	0.041	0.037	0.041
		GH48	0.083	0.087	0.080	0.081	0.083	0.074	0.047	0.044	0.052	0.050	0.053	0.054	0.049	0.044	0.050
		GH89	0.074	0.083	0.080	0.072	0.074	0.065	0.045	0.044	0.056	0.054	0.049	0.049	0.046	0.042	0.046
		GH90	0.072	0.081	0.078	0.070	0.072	0.063	0.043	0.042	0.054	0.052	0.047	0.047	0.044	0.040	0.044
		GH91	0.076	0.085	0.083	0.074	0.076	0.067	0.047	0.046	0.059	0.056	0.051	0.051	0.048	0.045	0.048
		SA12-c	0.063	0.072	0.065	0.063	0.063	0.055	0.028	0.028	0.040	0.038	0.039	0.035	0.030	0.028	0.034
		SO106	0.066	0.064	0.057	0.054	0.057	0.044	0.018	0.018	0.026	0.028	0.024	0.020	0.020	0.018	0.024
		SO95-p	0.063	0.069	0.063	0.060	0.061	0.052	0.028	0.028	0.036	0.036	0.034	0.030	0.030	0.028	0.034
		SA385-L	0.074	0.071	0.069	0.062	0.067	0.061	0.053	0.053	0.054	0.052	0.051	0.047	0.053	0.057	0.065
		GH98	0.074	0.071	0.069	0.062	0.067	0.061	0.053	0.053	0.054	0.052	0.051	0.047	0.053	0.057	0.065
		SCH181-a	0.072	0.069	0.067	0.060	0.065	0.059	0.051	0.050	0.052	0.050	0.049	0.045	0.051	0.055	0.063
		SA185-i	0.072	0.072	0.069	0.065	0.069	0.067	0.053	0.055	0.054	0.052	0.051	0.047	0.055	0.059	0.067
		SA183-i	0.072	0.072	0.069	0.065	0.069	0.067	0.053	0.055	0.054	0.052	0.051	0.047	0.055	0.059	0.067
		SCH179-i	0.072	0.072	0.069	0.065	0.069	0.067	0.053	0.055	0.054	0.052	0.051	0.047	0.055	0.059	0.067
Central	**Sa.II**	GH79	0.071	0.069	0.066	0.062	0.069	0.058	0.050	0.050	0.051	0.049	0.049	0.045	0.050	0.054	0.062
		GH80	0.075	0.073	0.071	0.067	0.073	0.062	0.050	0.050	0.051	0.049	0.049	0.045	0.050	0.054	0.062
		GH72	0.069	0.066	0.064	0.060	0.067	0.056	0.048	0.047	0.049	0.047	0.047	0.043	0.048	0.052	0.060
		SA197	0.069	0.066	0.064	0.060	0.067	0.056	0.048	0.047	0.049	0.047	0.047	0.043	0.048	0.052	0.060
		SA292-q	0.076	0.074	0.072	0.067	0.074	0.063	0.049	0.050	0.052	0.050	0.049	0.045	0.051	0.055	0.063
		SCH388-a	0.078	0.071	0.069	0.067	0.072	0.065	0.053	0.053	0.054	0.052	0.051	0.047	0.053	0.057	0.065
		GH93	0.074	0.072	0.069	0.065	0.072	0.061	0.049	0.048	0.050	0.048	0.047	0.043	0.048	0.053	0.061
		SV305	0.100	0.084	0.082	0.085	0.087	0.080	0.072	0.078	0.078	0.080	0.081	0.077	0.080	0.081	0.087

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Table A1.3—continued.

REGION	CLADE	CODE	SA35-j	Unkn201	SA208	SA274-m	SA291-m	SA351-d	S251-g	SA152	GH48	GH89	GH90	GH91	SA12-c	SO106	SO95-p
Northern	**Sa.I**	SA340-e															
		SA277-e															
		SA288-e															
		SA361-u															
		SA360-h															
		GH78															
Southeastern	**Sa.IV**	SA110-b			**Sa.IV**												
		SA57-b															
		SA174-f															
		SA13-v															
		GH73															
		GH74															
		SA67															
		SA70															
		SA347-w															
		SA35-j															
		Unkn201	0.018														
		SA208	0.014	0.004													
		SA274-m	0.042	0.040	0.037												
		SA291-m	0.044	0.042	0.039	0.002											
Southwestern	**Sa.III**	SA351-d	0.063	0.061	0.056	0.057	0.059										**Sa.III**
		S251-g	0.061	0.059	0.054	0.059	0.057	0.010									
		SA152	0.052	0.045	0.041	0.043	0.045	0.035	0.037								
		GH48	0.061	0.050	0.049	0.050	0.052	0.030	0.032	0.016							
		GH89	0.056	0.046	0.045	0.050	0.052	0.038	0.040	0.004	0.016						
		GH90	0.054	0.044	0.043	0.048	0.050	0.036	0.038	0.002	0.014	0.002					
		GH91	0.059	0.048	0.047	0.052	0.054	0.040	0.042	0.006	0.018	0.002	0.004				
		SA12-c	0.044	0.038	0.035	0.034	0.036	0.036	0.038	0.010	0.020	0.016	0.014	0.018			
		SO106	0.032	0.032	0.028	0.036	0.038	0.038	0.043	0.031	0.040	0.036	0.034	0.038	0.020		
		SO95-p	0.044	0.040	0.036	0.044	0.046	0.038	0.042	0.026	0.036	0.032	0.030	0.034	0.012	0.008	
Central	**Sa.II**	SA385-L	0.067	0.065	0.060	0.065	0.067	0.085	0.087	0.074	0.087	0.083	0.081	0.085	0.066	0.055	0.063
		GH98	0.067	0.065	0.060	0.065	0.067	0.085	0.087	0.074	0.087	0.083	0.081	0.085	0.066	0.055	0.063
		SCH181-a	0.065	0.063	0.058	0.063	0.065	0.083	0.085	0.072	0.085	0.081	0.078	0.083	0.064	0.053	0.061
		SA185-i	0.069	0.067	0.062	0.067	0.069	0.083	0.085	0.076	0.090	0.085	0.083	0.087	0.068	0.058	0.065
		SA183-i	0.069	0.067	0.062	0.067	0.069	0.083	0.085	0.076	0.090	0.085	0.083	0.087	0.068	0.058	0.065
		SCH179-i	0.069	0.067	0.062	0.067	0.069	0.083	0.085	0.076	0.090	0.085	0.083	0.087	0.068	0.058	0.065
		GH79	0.064	0.062	0.058	0.062	0.064	0.081	0.083	0.072	0.083	0.078	0.076	0.080	0.063	0.052	0.060
		GH80	0.064	0.062	0.058	0.062	0.064	0.081	0.083	0.072	0.083	0.078	0.076	0.080	0.063	0.052	0.060
		GH72	0.062	0.060	0.055	0.060	0.062	0.078	0.081	0.069	0.080	0.076	0.074	0.078	0.061	0.050	0.058
		SA197	0.062	0.060	0.055	0.060	0.062	0.078	0.081	0.069	0.080	0.076	0.074	0.078	0.061	0.050	0.058
		SA292-q	0.065	0.063	0.058	0.063	0.065	0.083	0.085	0.072	0.085	0.081	0.078	0.083	0.064	0.053	0.061
		SCH388-a	0.067	0.065	0.060	0.065	0.067	0.085	0.087	0.074	0.087	0.080	0.081	0.083	0.066	0.055	0.063
		GH93	0.063	0.061	0.055	0.061	0.063	0.081	0.083	0.070	0.083	0.076	0.076	0.078	0.061	0.051	0.059
		SV305	0.075	0.089	0.088	0.089	0.091	0.107	0.109	0.101	0.105	0.102	0.103	0.105	0.089	0.078	0.087

Continued on next page

Table A1.3—continued.

REGION	CLADE	CODE	SA385-L	GH98	SCH181-a	SA185-i	SA183-i	SCH179-i	GH79	GH80	GH72	SA197	SA292-q	SCH388-a	GH93	SV305
Northern	**Sa.I**	SA340-e														
		SA277-e														
		SA288-e														
		SA361-u														
		SA360-h														
		GH78														
Southeastern	**Sa.IV**	SA110-b														
		SA57-b														
		SA174-f														
		SA13-v														
		GH73														
		GH74														
		SA67														
		SA70														
		SA347-w														
		SA35-j														
		Unkn201														
		SA208														
		SA274-m														
		SA291-m														
Southwestern	**Sa.III**	SA351-d														
		S251-g														
		SA152														
		GH48														
		GH89														
		GH90														
		GH91														
		SA12-c														
		SO106														
		SO95-p														
Central	**Sa.II**	SA385-L														**Sa.II**
		GH98	0.000													
		SCH181-a	0.002	0.002												
		SA185-i	0.010	0.010	0.008											
		SA183-i	0.010	0.010	0.008	0.000										
		SCH179-i	0.010	0.010	0.008	0.000	0.000									
		GH79	0.006	0.006	0.004	0.008	0.008	0.008								
		GH80	0.010	0.010	0.008	0.008	0.008	0.008	0.004							
		GH72	0.004	0.004	0.002	0.010	0.010	0.010	0.002	0.006						
		SA197	0.004	0.004	0.002	0.010	0.010	0.010	0.002	0.006	0.000					
		SA292-q	0.010	0.010	0.008	0.012	0.012	0.012	0.008	0.008	0.006	0.006				
		SCH388-a	0.016	0.016	0.014	0.014	0.014	0.014	0.010	0.010	0.012	0.012	0.014			
		GH93	0.012	0.012	0.010	0.010	0.010	0.010	0.006	0.006	0.008	0.008	0.010	0.004		
		SV305	0.095	0.095	0.093	0.100	0.100	0.100	0.092	0.097	0.090	0.090	0.098	0.091	0.091	

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