Zootaxa 5383 (2): 225–241
https://www.mapress.com/zt/
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Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5383.2.7
http://zoobank.org/urn:lsid:zoobank.org:pub:2C46E540-54A2-4776-90D4-384BBF330207

Māwhitiwhiti Aotearoa: Phylogeny and synonymy of the silent alpine grasshopper radiation of New Zealand (Orthoptera: Acrididae)

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Abstract

Aotearoa New Zealand has a fauna of endemic alpine grasshoppers, consisting of thirteen species distributed among four genera. The many re-classifications of species within this group and the presence of species complexes highlight the uncertainty that surrounds relationships within and between these genera. High-throughput Next Generation Sequencing was used to assemble the complete mitochondrial genomes, 45S ribosomal cassettes and histone sequences of New Zealand's four endemic alpine genera: *Alpinacris, Brachaspis*, *Paprides* and *Sigaus*. Phylogenetic analysis of these molecular datasets, as individual genes, partitions and combinations returned a consistent topology that is incompatible with the current classification. The genera *Sigaus*, *Alpinacris*, and *Paprides* all exhibit paraphyly. A consideration of the pronotum, epiphallus and terminalia of adult specimens reveals species-specific differences, but fails to provide compelling evidence for species groups justifying distinct genera. In combination with phylogenetic, morphological and spatial evidence we propose a simplified taxonomy consisting of a single genus for the māwhitiwhiti Aotearoa species radiation.

Key words: phylogenetics, grasshopper, Acrididae, *Alpinacris, Brachaspis, Paprides, Sigaus,* mitogenomics

Introduction

Six genera of short-horned grasshopper (Orthoptera: Acrididae (MacLeay 1821)) occur naturally in Aotearoa New Zealand. Two of these are native, comprising two endemic New Zealand species of *Phaulacridium* (Brunner von Wattenwyl 1893) congeneric with Australian species, plus the cosmopolitan *Locusta migratoria* (Linnaeus 1758). Four genera, *Alpinacris* (Bigelow 1967), *Brachaspis* (Hutton 1898), *Paprides* (Hutton 1897) and *Sigaus* (Hutton 1897), are endemic to Aotearoa New Zealand (Figure 1A). The placement of the thirteen species within these four genera is the subject of this revision because recent phylogenetic analysis (Koot *et al.* 2020) resolved a monophyletic New Zealand radiation but questioned current systematic subdivisions. These grasshoppers are referred to as the New Zealand alpine radiation as most of them are restricted to habitat above the elevational treeline. However, some have ranges that extend to lower elevation and three species are found only in lowland habitats (Table 1). All species are flightless and silent (Bigelow 1967; Key 1991) but differ in habit and size with the largest, (*Sigaus villosus* (Salmon 1950)) restricted to high alpine habitat, and the smallest (*Sigaus minutus* (Bigelow 1967)) found only in low elevation semi-arid habitat (Figure 1B).

Globally, the short-horned grasshoppers (Orthoptera: Acrididae) comprise ~6,677 species within a complex and poorly resolved taxonomic classification that includes numerous tribes and subtribes (Cigliano *et al.* 2022). Molecular phylogenetic analyses have improved our understanding of systematic relationships of the acridids, and in doing so reveal morphological homoplasy (Song *et al.* 2018). The four endemic New Zealand genera of the alpine radiation are placed with other 'spur-throated' grasshoppers within the subfamily Catantopinae (Brunner von Wattenwyl 1893) (Cigliano *et al.* 2022; Brunner von Wattenwyl & Fea 1893). The genus *Sigaus* is further classified into the tribe Catantopini (Brunner von Wattenwyl 1893) and subtribe Russalpiina (Key & Colless 1993) (Cigliano *et al.* 2022). Members of Catantopinae and Catantopini are found throughout Australia, Asia, Europe and Africa,

Accepted by R. Mariño-Pérez: 27 Oct. 2023; published: 11 Dec. 2023

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whilst subtribe Russalpiina is limited to New Zealand and the Australian island of Tasmania (Cigliano *et al.* 2022; Johnston 1956, 1968; Key & Colless 1993; Brunner von Wattenwyl & Fea 1893).

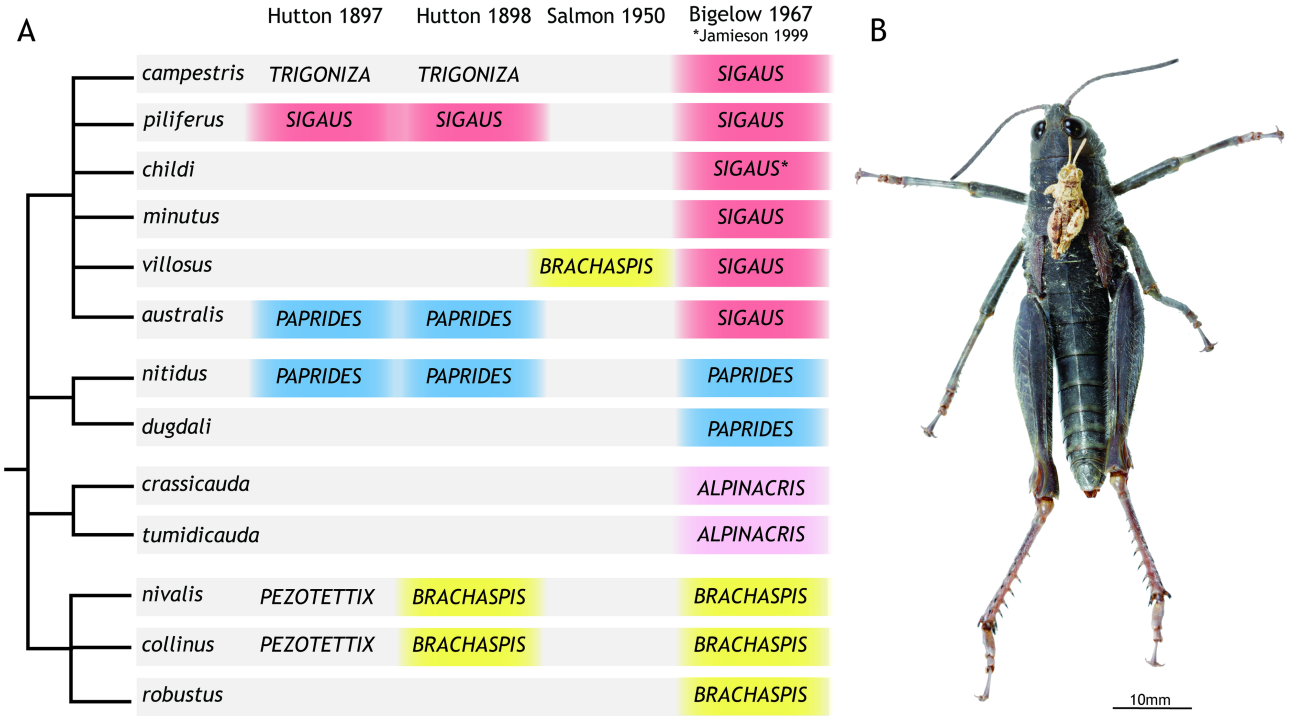

FIGURE 1A. The generic placement of the thirteen species of grasshoppers/māwhitiwhiti from Aotearoa New Zealand since the first species descriptions in 1897 with the phylogenetic relationships implied by the current classification. Figure 1B. Māwhitiwhiti Aotearoa are diverse in form and ecology. An adult male *Sigaus minutus* is shown on top of an adult female *Sigaus villosus* to allow size comparison of smallest and largest species within this radiation. See Table 1.

Taxonomic treatment of Aotearoa New Zealand alpine grasshopper radiation

The current taxonomy of New Zealand grasshoppers is based on Bigelow (1967), with the addition of *Sigaus childi* (Jamieson 1999), *Sigaus takahe* (Morris 2003) and *Sigaus homerensis* (Morris 2003). Analysis of short mitochondrial DNA sequence data (mtDNA partial COI) is largely compatible with current species level taxonomy in that clusters of DNA sequence variants correlate with morphological features, and geographic distribution (Figure 2). However, genetic and geographic diversity of *Sigaus australis* (Hutton 1897) encompasses the morphologically indistinguishable localised population formerly referred to as *S. obelisci* (Bigelow, 1967), and putative local endemics *S. takahe* and *S. homerensis* represent geographic outlier populations nested within the mtDNA lineage of *S. australis* and lack convincing evidence of their distinct taxonomic status (Carmelet-Rescan *et al.* 2021; Trewick 2008; Trewick & Morris 2008; Trewick *et al.* 2022). However, the cryptic and localised taxon *S. childi* maintains distinct morphology in semi-arid lowland habitat despite gene flow with sympatric *S. australis* (Dowle *et al.* 2014). Genetic and morphometric diversity within *S. australis* is mostly distributed along Kā Tiritiri o te Moana Southern Alps, the axial mountain range of South Island, New Zealand (Carmelet *et al.* 2021). Comparison of mitochondrial and nuclear sequences show that three *Brachaspis* lineages do not correspond to the taxonomy; two are within *B. nivalis* (Hutton 1897) (north and south), while the rare, regionally restricted low elevation *B. robustus* (Bigelow 1967) is nested within southern *B. nivalis* (Trewick 2001). Similarly, range-restricted diminutive forms of *Brachaspis* also nest within the genetic diversity of their neighbouring montane *B. nivalis* populations (Trewick & Morris 2008). Inter and intraspecific mtDNA sequence diversity in these lineages is consistent with expectations (Supplementary Table S2).

The underlying question of generic status of the endemic New Zealand alpine grasshopper radiation remains. Genera are usually assigned to sets of species to indicate similarity and in an evolutionary framework we expect that similarity to reflect phylogenetic relationships. Hence a genus is expected to represent a monophyletic clade or cluster. Taxonomic hierarchy can result in paraphyletic taxa if traits used for classification do not correctly resolve underlying evolutionary relationships (e.g., Stebbins 1956; Boudinet *et al.* 2022). Failure to reconcile taxonomy with evolution leads to confusion

TABLE 1. Summary of taxonomic, ecological and morphological data for Aotearoa New Zealand's endemic alpine grasshoppers. For each genus the currently recognised species are listed with: their synonyms; approximate elevational range occupied in meters above sea level (m.a.s.l.); most common habitat types in which they are found; known range of body lengths for adults in millimetres (mm); colour variants recorded; how many instars male and females undergo to become adults. * indicates measurements are from one individual.

Genus	Species	Synonyms	Elevation (m.a.s.l.)	Habitat type	Body length (mm)		Colour	Instars male/female	Source
					Male	Female			
Alpinacris (Bigelow 1967)	*A. crassicauda* (Bigelow 1967)	-	975–1680	Tussocks, scree	18–22	25–35	Pale brown, olive-grey, reddish brown, dark brown, green, bright green with pale bands	5 / 6	Bigelow 1967 Hudson 1970
	A. tumidicauda (Bigelow 1967)	-	600–1830	Tussocks	15.4*	24*	Olive green or pale brown with distinct pale and black bands	5 / 6	Bigelow 1967 Hudson 1970
Brachaspis (Hutton 1898)	*B. collinus* (Hutton 1897)	*Pezotettix collina* (Hutton 1897)	1000–2000	Tussocks, scree, bare soil, grass	31–32	28–45	Slate grey, grey, grey-brown, green, olive-green with yellow stripes, and flash-display colours in reddish brown	6 / 7	Bigelow 1967 Hudson 1970 Hutton 1897
	B. nivalis (Hutton 1897)	*Pezotettix nivalis* (Hutton 1897) *Pezotettix petricola* (Hutton 1897) *Brachaspis petricolus* (Hutton 1898) *Pezotettix terrestris* (Hutton 1897) *Brachaspis terristris* (Hutton 1898)	600–2000	Rocks, scree	15–24	16–40	Grey, grey mottled brown, with flash-display colours scarlet, purple, indigo	6 / 7	Batcheler 1967 Bigelow 1967 Hudson 1970 Hutton 1897 Hutton 1898
	B. robustus (Bigelow 1967)	-	500–600	Rocks, scree, bare soil, grass	-	26–29	Grey, grey mottled brown. Flash-display colours scarlet, purple, indigo	-	Bigelow 1967
Paprides (Hutton 1897)	*P. dugdali* (Bigelow 1967)	-	400–1160	Tussocks	15–20	21–27	Green, grey, brown, with yellow-brown longitudinal bands	5 / 6	Bigelow 1967 Hudson 1970 Hutton 1897
	P. nitidus (Hutton 1897)	*Paprides furcifer* (Hutton 1898)	600–1830	Tussocks, scree,	14–20	22–30	Bright or olive green, bright red hind tibia, pale dorsal bands	5 / 6	Bigelow 1967 Hudson 1970 Hutton 1897 Hutton 1898

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TABLE 1. (Continued)

Genus	Species	Synonyms	Elevation (m.a.s.l.)	Habitat type	Body length (mm)		Colour	Instars male/ female	Source
					Male	Female			
Sigaus (Hutton 1897)	*S. australis* (Hutton 1897)	*Paprides australis* (Hutton 1897) *Paprides torquatus* (Hutton 1898) *Paprides armillatus* (Hutton 1898) *Sigaus homerensis* (Morris 2003) *Sigaus obelisci* (Bigelow 1967) *Sigaus takahe* (Morris 2003) *Paprides furcifer* (Hutton 1898)	285–2020	Tussocks, scree, bare soil, grass,	12–20	17–40	Dark brown, grey, olive-green, light brown, light olive-green.	5 / 6	Bigelow 1967 Hudson 1970 Hutton 1897 Hutton 1898 Morris 2003
	S. campestris (Hutton 1897)	*Trigoniza campestris* (Hutton 1897) *Trigoniza directa* (Hutton, 1897) *Trigoniza rugosa* (Hutton 1897)	0–1550	Tussocks, grass	13–17	20–32	Light brown, dark brown, green, bright green	-	Bigelow 1967 Hutton 1898
	S. childi (Jamieson 1999)	*Sigaus minutus* (Bigelow 1967)	160–420	Grass, bare soil	11–13	21–22	Light brown, dark grey mottled with brown	5 / 6	Bigelow 1967 Jamieson 1999
	S. minutus (Bigelow 1967)	-	500–1180	Stony riverbeds, bare soil, grass	9–10	14–16	Dark grey mottled with brown	-	Bigelow 1967
	S. piliferus (Hutton 1897)	-	725–1400	Tussocks, scree	15–22	25–40	Dark to light brown, reddish brown, grey, olive-green, green with pale bands	-	Bigelow 1967 Hutton 1897
	S. villosus (Salmon 1950)	*Brachaspis villosa* (Salmon 1950)	1370–2130	Tussocks, rocks, scree	24–32	37–48	Brown, dark grey mottled with brown	6 / 7	Bigelow 1967 Hudson 1970 Salmon 1950

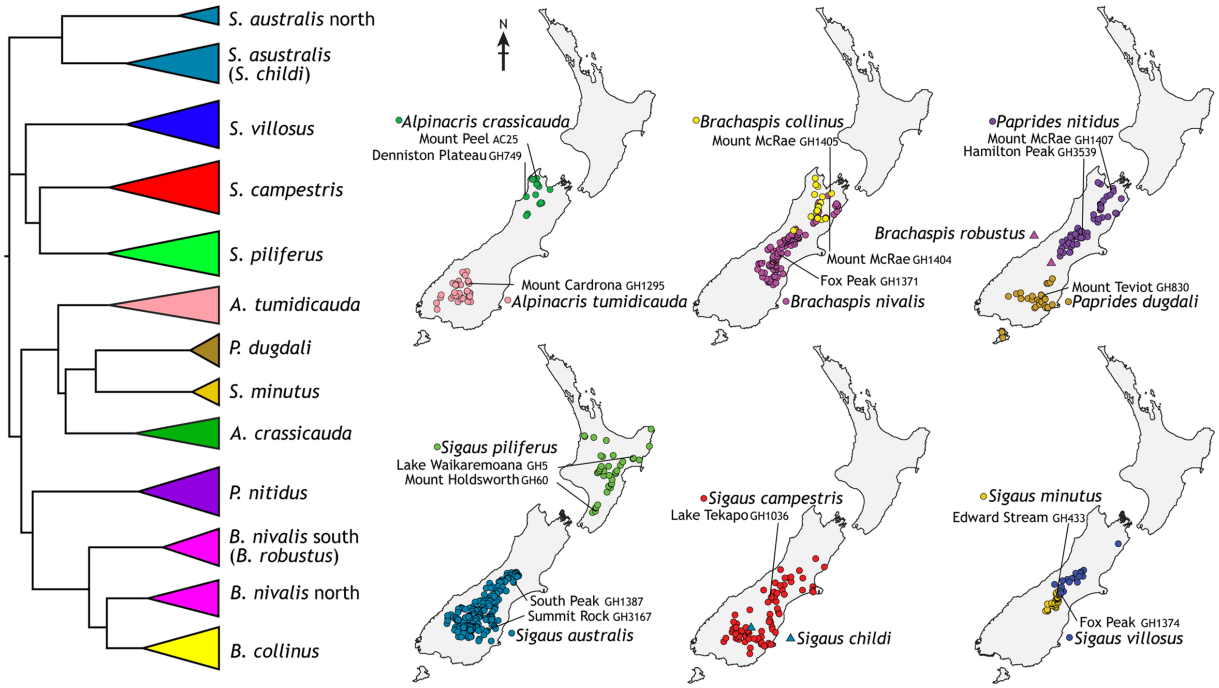

FIGURE 2. Schematic representation of published mtDNA COI sequence variation within species lineages of endemic Aotearoa New Zealand Acrididae in the alpine radiation (Supplementary Table S1 for data details). Recorded locations of each taxon are coloured as shown in the phylogeny (left). Triangles on maps indicate two rare, localised species that are recognised by morphology, but are phylogenetically nested within other more widespread lineages (with corresponding colour). The sampling locations of specimens used in the current study are indicated on the maps with their unique identifiers (see Table 2).

and hinders progress in almost all areas of biology (e.g., Hernández *et al.* 2020; Forni *et al.* 2023; Wilkerson *et al.* 2015). Here we examine the current taxonomic grouping of the alpine radiation of New Zealand grasshoppers into four genera. We used a DNA sequence dataset consisting of 13 protein coding mitochondrial genes, nuclear 45S ribosomal RNA cassettes and two histones from representatives of the New Zealand alpine genera *Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus* to test whether their evolutionary history is consistent with their taxonomy. We consider morphological traits used in the original species descriptions and propose genus level synonymy.

Methods

Sixteen individuals representing eleven major lineages of New Zealand endemic grasshopper were collected: *Alpinacris crassicauda* (Bigelow 1967), *Alpinacris tumidicauda* (Bigelow 1967), *Brachaspis collinus* (Hutton 1897), *Brachaspis nivalis* (Hutton 1897), *Paprides dugdali* (Bigelow 1967), *Paprides nitidus* (Hutton 1897), *Sigaus australis, Sigaus campestris* (Hutton 1897), *Sigaus minutus, Sigaus piliferus* (Hutton 1897) and *Sigaus villosus* (Table 2, Figure 2). Of these, *B. nivalis*, *S. australis*, *A. crassicauda* and *S. piliferus* were each sampled from two locations to better encompass geographic intraspecific diversity indicated by preliminary data (Trewick 2000; Trewick & Morris; and see Results below). This sampling encompasses phylogenetic diversity within *B. nivalis* that includes the endangered, ecologically distinct low-elevation species *B. robustus*, and diversity within *S. australis* that includes *S. childi.* Species were identified using morphological traits proposed by Bigelow (1967) including the texture and outline and shape of the pronotum, subgenital plate and epiproct of males. For an outgroup we use homologous data from two Tasmanian alpine grasshopper species, *Russalpia albertisi* and *Tasmaniacris tasmaniensis*, and two *Phaulacridium* species (Bigelow 1967; Key 1991; Koot *et al.* 2020). All grasshoppers were initially euthanised by freezing then stored in 95% ethanol to preserve DNA. We assembled a rich DNA sequence database including entire mtDNA genome sequences which have been shown to contain phylogenetic signal capable of resolution of lineages that diverged up to 300 Mya (Fenn *et al.* 2008; Song *et al.* 2015). To do this we used a Next Generation Sequencing (NGS) and bioinformatics approach.

TABLE 2. The Aotearoa New Zealand and Tasmanian grasshopper (Acrididae, Catantopinae) individuals for which mitochondrial genomes, 45S rRNA cassettes and histone H3 and H4 sequences were acquired, summarised and used in the phylogenetic analyses of this study. Specimen codes are linked with voucher specimens in the Phoenix Collection at Massey University, Palmerston North (MPN). Data used for constrained topology analysis (Con) indicated by c. Collection locations are indicated in Figure 2.

MPN code	Species	Con	Location	45S	Histones	Mitochondrial genome	Latitude/Longitude
AC25	*Alpinacris crassicauda*	c	Mount Peel, Peel Range	MT072989	MT070974	MN253105	-41.142015/172.593412
GH749	*Alpinacris crassicauda*		Denniston Plateau, Westport	MT072988	MT070973	MN253103	-41.749319/171.792526
GH1295	*Alpinacris tumidicauda*	c	Mount Cardrona, Crown Range	MT072994	MT070975	MN253104	-44.871808/168.943634
GH1405	*Brachaspis collinus*	c	Mount McRae, St Arnaud Range	MT072990	MT070976	MN253106	-41.867995/172.859745
GH1404	*Brachaspis nivalis*		Mount McRae, St Arnaud Range	MT072991	MT070978	MN253108	-41.867995/172.859745
GH1371	*Brachaspis nivalis*	c	Fox Peak, Sherwood Range	MT072992	MT070979	MN253107	-43.850189/170.802212
GH830	*Paprides dugdali*	c	Mount Teviot, Lammerlaw Range	MT072993	MT070980	MN253110	-45.590618/169.536037
GH1407	*Paprides nitidus*		Mount McRae, St Arnaud Range	MT072985	MT070981	MN253111	-41.867995/172.859745
GH3539	*Paprides nitidus*	c	Hamilton Peak, Craigieburn Range	OR670576, 670578	OR670573, R670575	OR677402	-43.109192/171.698112
GH1387	*Sigaus australis*	c	South Peak, Mount Hutt Range	MT072995	MT070983	MN253117	-43.490292/171.539154
GH3167	*Sigaus australis*		Summit Rock, Rock & Pillar Range	OR670577, 670579	OR670572, R670574	OR605599	-45.425480/170.070860
GH1036	*Sigaus campestris*	c	Lake Tekapo, Canterbury	MT072996	MT070984	MN253118	-43.964156/170.525665
GH433	*Sigaus minutus*	c	Edward Stream, Tekapo	MT072984	MT070985	MN253116	-44.043180/170.522747
GH5	*Sigaus piliferus*	c	Awaroa Ridge, Lake Waikaremoana	MT072986	MT070986	MN253120	-38.746252/177.161965
GH60	*Sigaus piliferus*		Mount Holdsworth, Tararua Range	MT072987	MT070987	MN253119	-40.871079/175.423594
GH1374	*Sigaus villosus*	c	Fox Peak, Sherwood Range	MT072997	MT070977	MN253121	-43.850189/170.802212
TAZ3	*Russalpia albertisi*		Mount Wellington, Tasmania	MT072999	MT070982	MN253115	-42.889884/147.229976
TAZ4	*Tasmaniacris tasmaniensis*	c	Mount Wellington, Tasmania	MT072998	MT070988	MN253122	-42.889884/147.229976
GH762	*Phaulacridium marginale*		Whararangi Road, Ahimanawa Range	MT072982	MT070992	MN253112	-39.086555/176.579603
GH1576	*Phaulacridium otagoense*		Quartz Reef Point, Lake Dunstan	MT072983	MT070989	MN253113	-44.998241/169.246026

The natural enrichment of eukaryote cells with the relatively small mtDNA genome makes NGS high-throughput approaches to generate numerous short anonymous sequences an effective way to assemble them. Similarly, the nuclear 45S ribosomal RNA cassette is highly replicated in the genome, with many tandem repeats and copies on multiple chromosomes (Richard *et al.* 2008). The cassette of three rRNAs (18S, 5.8S and 28S) and two Internal Transcribed Spacers (ITS1 and ITS2) is thus amenable to assembly from high-throughput NGS data (Vaux *et al.* 2017). Despite biparental inheritance the 45S ribosomal cassette tends to be homogenised via concerted evolution (Nei & Rooney 2005). The rRNA regions of the cassette are highly conserved and so show a low rate of nucleotide substitution that has been used to study deep phylogenetic relationships (Raué *et al.* 1988). In contrast, the ITS regions are not functionally constrained in the same way, and so have high substitution rates. As a result ITS sequences can vary greatly even within species, and have been used alongside mtDNA in species and population analyses (Álvarez &Wendel 2003; Richard *et al.* 2008; Trewick 2001). In addition we generated contigs for representatives of the histone nuclear gene family targeting the adjacent H3 and H4 exons.

Genomic DNA was extracted from leg muscle using a solvent-free salting-out method (Sunnucks & Hales 1996) and quantified using Qubit fluorometry (Life Technologies, Thermo Fisher Scientific Inc.). Genomic DNA samples were paired-end sequenced through massive parallel, high-throughput sequencing on an Illumina HiSeq 2500 following fragmentation and indexing using the Illumina TruSeq Nano DNA kit. Resulting 100bp paired-end reads were sorted and edited using 'Cutadapt' v 1.11 (Martin, 2011) to remove sample barcodes, and were paired and assembled in Geneious versions 9.1.4–Prime2023 (Kearse *et al*. 2012).

Mitochondrial genomes were assembled from each sample DNA using an iterative reference mapping approach. The first of the New Zealand grasshopper genome assemblies used the published annotated mtDNA genome of *Locusta migratoria* in Oedipodinae for initial mapping. Paired reads were iteratively mapped to the reference sequence in Geneious generating a novel consensus sequence, which was then used as a reference to remap the raw sequence reads (See Sivyer *et al.* 2018; Vaux *et al*. 2017; Koot *et al*. 2020). Sequences were uploaded as raw fasta files to MITOS (Bernt *et al.* 2013) to initially identify protein coding regions, rRNAs and tRNAs. Annotations were transferred and individually checked by comparison of reading frames, amino acid translation and RNA structure.

A similar approach was used to assemble, align and edit 45S ribosomal cassettes and histones H3 and H4 sequences for the same grasshopper samples. Preliminary assembly of 45S used the 5.8S sequence of *Locusta migratoria* (Genbank: KM853191) and for the histones we started with *L. migratoria* H3 (Genbank: AF370817) as the reference, but subsequent mapping used the results from other New Zealand Catantopinae.

DNA sequence alignments were obtained using Geneious v9.1.4–Prime2023 and were checked and edited manually using translated sequences of coding genes. DNA sequence alignments representing 20 specimens were generated for the mitochondrial genomes, two histone genes and the partial 45S cassette. Thirteen mtDNA protein coding genes were extracted from the mitochondrial genomes, aligned and concatenated. These were supplemented by the two histone coding exons H3 and H4 and the 45S sequences (partial 18S, ITS1, 5.8S, ITS2 and partial 28S), resulting in an alignment of 18,874 bp. INDEL regions were removed from alignments, primarily 45S, as homology could not be confirmed, and this resulted in an alignment of 16,565 bp. These data were analysed together applying suitable models to partitions.

Phylogenetic analysis

Alignments were analysed using Maximum Likelihood (ML) implemented in IQ-Tree v2.2 through IQ-Tree tools (Trifinopoulos *et al.* 2016; Minh *et al*. 2020) utilising model selection (Kalyaanamoorthy *et al.* 2017) and ultrafast bootstrapping (Hoang *et al*. 2018). Partition models (Chernomor *et al.* 2016) were applied in ML analyses that identified coding genes, rRNA genes, and codon position of protein coding genes. Bayesian analysis was implemented with MrBayes (Ronquist & Huelsenbeck 2003) in Geneious Prime using an HKY85 model with four rate categories and gamma rate variation and run on 4 chains for 5 million generations and burnin of 100,000 generations.

We treated the existing taxonomy as an evolutionary hypothesis (see Figure 1) and tested the predicted clustering of congeneric species using a constrained tree topology in IQ-Tree v2.2, implementing the bootstrap proportion RELL (bp-RELL) approximation (Kishino *et al.* 1990), (KH) Kishino-Hasegawa test (Kishino & Hasegawa 1989), (SH) Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999), (c-ELW) expected likelihood weights (Strimmer & Rambaut 2002), and (AU) approximately unbiased test (Shimodaira 2002). For the purpose of topological comparison, we pruned the data set to include data for one of each of the represented species having already demonstrated the monophyly of respective intraspecific samples. Thus, an alignment of 11 New Zealand

lineages with a Tasmanian taxon to root the tree. For convenience only the species epithets are used for labels. The constrained tree topology grouping congeneric species under the existing taxonomy, in Newick format is: ((*tumidicauda*, *crassicauda*), (*nitidus*, *dugdali*), (*nivalis*, *collinus*), (*australis*, *minutus*, *campestris*, *piliferus*, *villosus*), *tasmaniensis*). Note that this conservative treatment does not constrain the intergeneric topology, or the placement of species within the genus *Sigaus*.

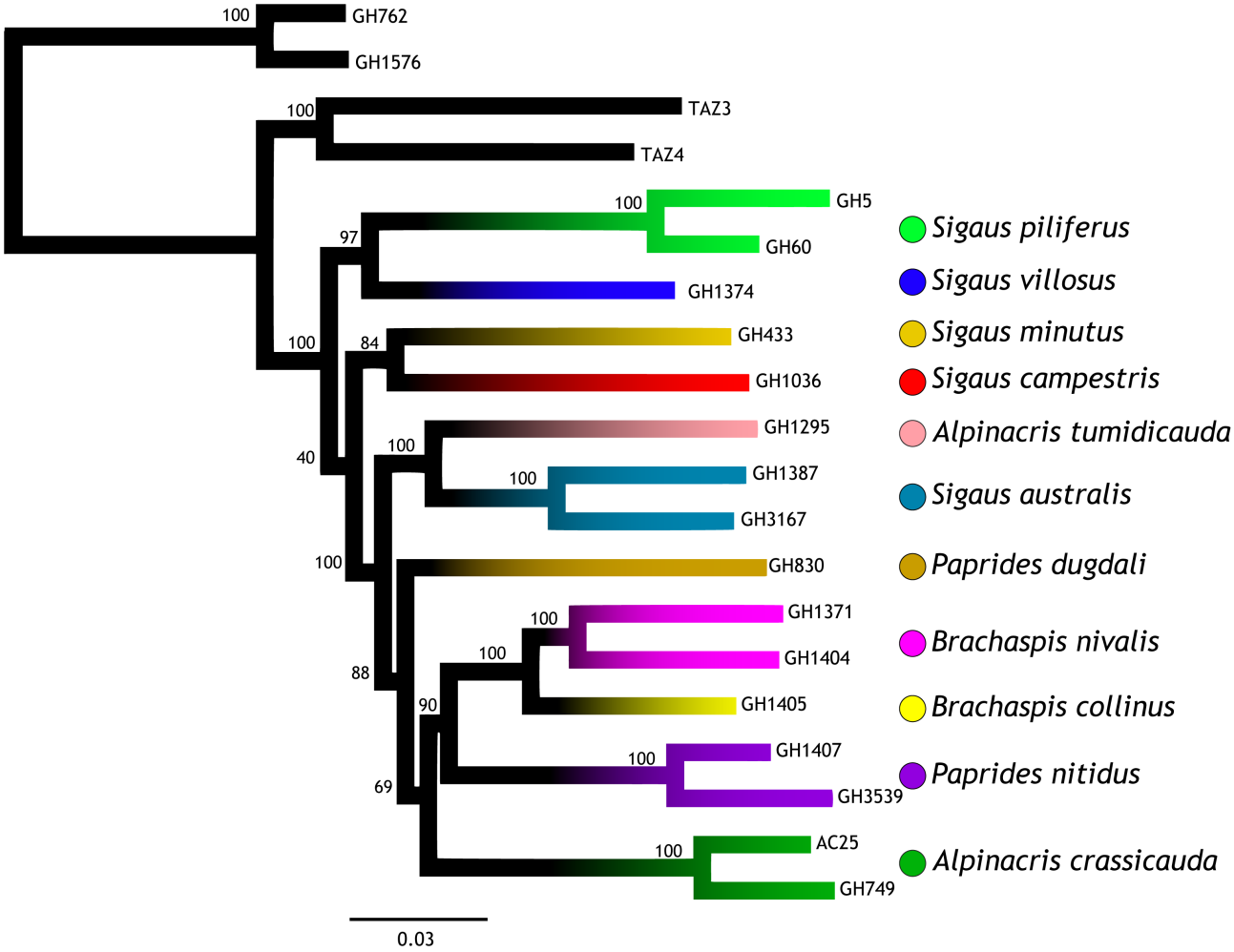

FIGURE 3. Phylogenetic hypothesis for the Aotearoa New Zealand alpine grasshoppers inferred from alignment of 19,778 bp comprising 13 mitochondrial protein coding genes and two rRNAs, nuclear protein coding histones 3 & 4 and 45S cassette (with indels removed). Maximum likelihood analysis performed in IQ-Tree with codon partitioning. Numbers at nodes are results from 10000 bootstrap replicates.

Results

Alignments of data represented 20 individual Catantopinae grasshoppers comprising 11 New Zealand species, 2 Tasmanian species and 2 species of *Phaulacridium*. For details of mitochondrial genome nucleotide composition and gene order see Koot *et al.* (2020). The concatenated alignment of 13 mtDNA protein coding genes spanned 11,196 bp with the typical insect mtDNA AT bias in nucleotide composition (75%), of which 3,847 were variable sites. The two nuclear histone genes comprised 723 bp with 663 invariant sites across the 20 specimens. The alignment of 45S ribosomal RNA genes (18S, ITS1, 5.8S, ITS2, 28S) spanned 5,028 bp, and 4,645 bp after removal of insertion-deletion segments for which alignment homology could not be confirmed. There was a low level of variation within the 45S rRNA with just 71 variable positions, and many (43) in the two ITS regions. The 163 bp 5.8S was invariant. The concatenated alignment of mtDNA CDS, histones and 45S for 20 taxa comprised 16,565 bp after exclusion of all INDELs. Of this, 12,576 sites were invariant and 2,848 were parsimony-informative. Despite the usual alignment limitations for the mitochondrial rRNA genes 12S (small subunit) and 16S (large subunit) we analysed these two

genes in a concatenated alignment of 2,208 bp obtained using the MAFFT E-INS-I algorithm. We then generated a full mitochondrial dataset comprising 13 CDSs and two rRNAs mitochondrial genes (no tRNAs) for 20 specimens that was 13,410 bp, and the addition of the two nuclear CDS gave an alignment of 14,133 bp.

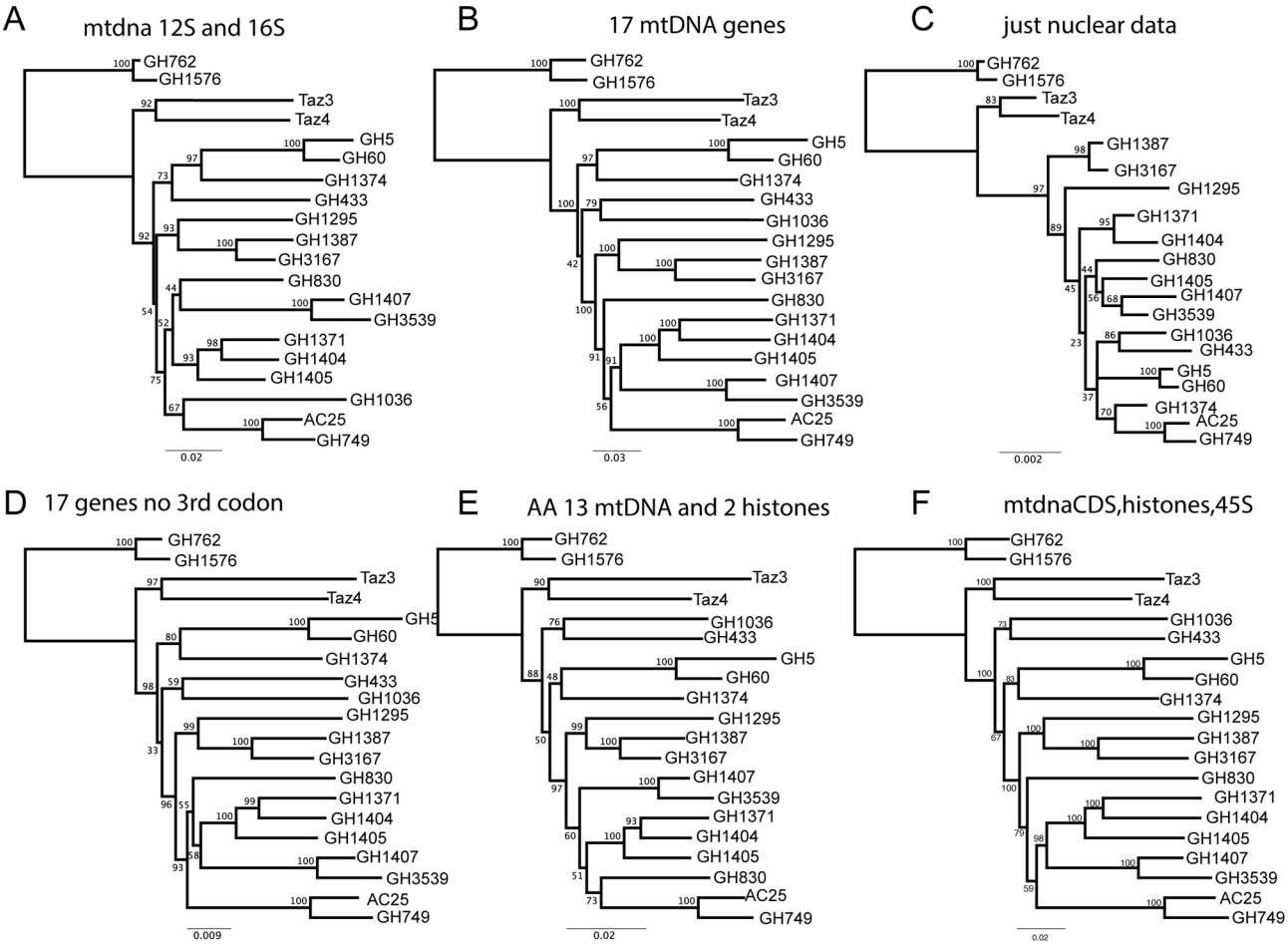

FIGURE 4. Phylogenetic hypothesis for the New Zealand alpine grasshoppers inferred from combinations of mitochondrial and nuclear genes.

We also analysed the concatenated nuclear data (histones and 45S rRNA) that comprised a low level of variation in a separate analysis of two partitions (CDS, rRNA) with GTR+F models selected by BIC and using 10,000 bootstrap replicates. Phylogenetic signal from these partitions was limited but we found support for the pairings: GH5 and GH60 (98%), GH1387 and GH 3167 (99%), GH1371 and GH1404 (98%), AC25 and GH749 (100%), consistent with the results using mitochondrial data (see Table 2 for species details). Maximum likelihood phylogenetic analyses of New Zealand *Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus* grasshoppers using combinations of mtDNA CDS, nuclear histones, and nuclear 45D rDNA cassette, and partitions of these data, returned the same topology (Figure 3). None were consistent with current generic classification. All analyses yielded paraphyly of *Alpinacris*, *Paprides* and *Sigaus* even though *Alpinacris* and *Paprides* consist of just two species. Only the two congeneric species under the current scheme (*Brachaspis*) were consistently grouped as sister to one another in the analysis (Fig. 4).

Exclusion of 3rd codon positions from analysis of protein coding genes (13 mtDNA CDS and 2 histones), which by site saturation might mislead phylogenetic inference, resulted in a tree with the same topology but slightly reduced bootstrap support on one internal node. This suggests that not all 3rd codons were saturated. As an alternative approach we translated the alignment of 13 mtDNA protein coding genes with the invertebrate mitochondrial code yielding 3,960 amino acid residues of which 831 were variable. Analysis of this aa alignment specifying mtMet+R3 model chosen according to BIC, resulted in the same topology as trees built from nucleotide data, with the exception of the position of *P. dugdali* (GH830; Figure 4).

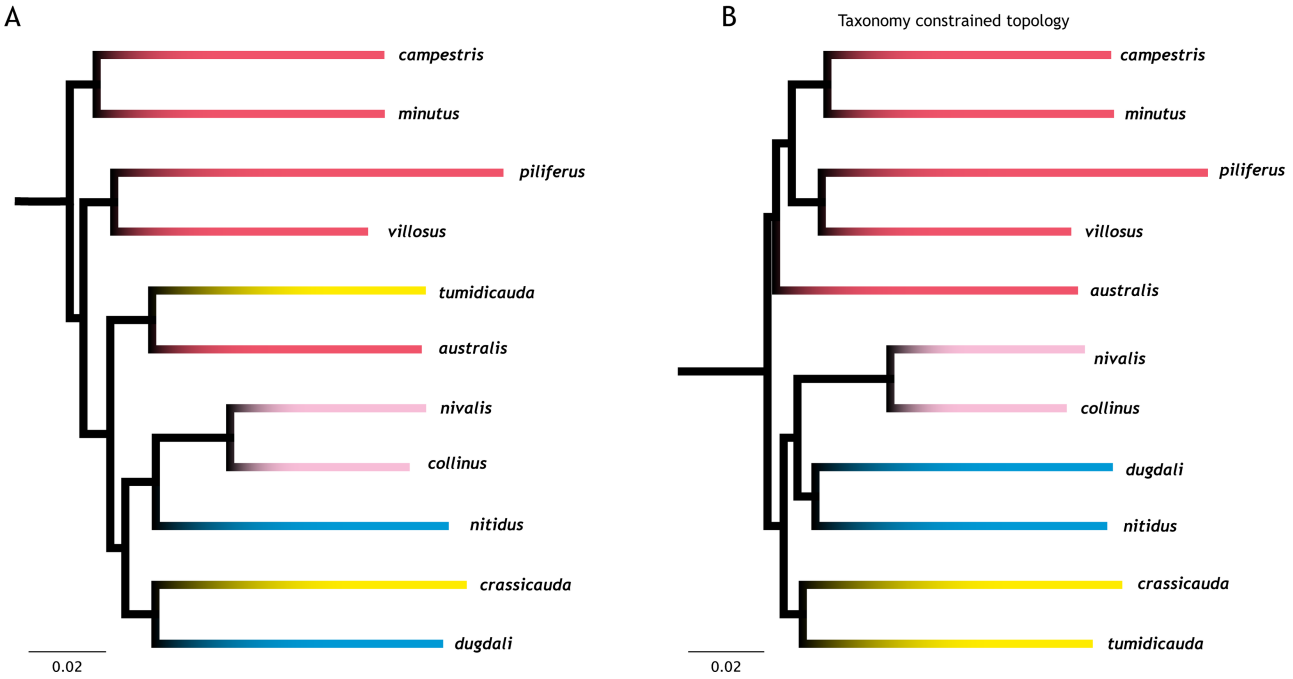

FIGURE 5. Alternative topologies among 11 representative species of Aotearoa New Zealand grasshopper used to test compatibility of existing taxonomic treatment. A) unconstrained ML phylogeny of 15 protein coding genes, and B) the same data with congeneric species constrained to monophyly is a significantly less-likely tree.

We applied a topology test based on the expectation that the current systematic treatment portrays the evolutionary relationships among the grasshopper taxa (Figure 1). Analysis of the alignment of 15 CDS sequences (mtDNA and histone) for 11 ingroup taxa with and without topological constraint (Figure 5) showed a statistically poor fit of the data to monophyly of the current genera (Table 3). The current genus level taxonomy can therefore be rejected as it does not reflect evolutionary relationships.

TABLE 3. Tree topology tests for Aotearoa New Zealand alpine grasshopper species, considering unconstrained topology (Fig.5A) and a topology constrained (Fig.5B) to retained generic groupings. DeltaL is logL difference from the maximal logl in the set; bp-RELL, bootstrap proportion using RELL method (Kishino *et al.* 1990); p-KH, p-value of one sided Kishino-Hasegawa test (1989); p-SH, p-value of Shimodaira-Hasegawa test (1999); c-ELW, Expected Likelihood Weight (Strimmer & Rambaut 2002); p-Au, p-value of approximately unbiased (Au) test (Shimodaira 2002). Plus signs denote the 95% confidence sets and minus signs denote significant exclusion.

Tree	logL	deltaL	Bp-RELL	p-KH	p-SH	c-EL W	p-AU
Constrained	–47823.18779	74.577	0 –	0 –	0 –	4.78^{e-08} –	0.000152 –
unconstrained	–47748.61053	0	1 +	1 +	1 +	1 +	1 +

Discussion

More than a hundred and twenty years ago Captain Frederick Wollaston Hutton published his accounts of New Zealand grasshoppers, seeking to describe the native Acrididae "*...before they vanish*" (Hutton 1897). He recognised that there was extensive undocumented diversity occupying a range of habitats across the country, but noted too, declines of many populations in the period since European settlement. Hutton's predictions are being born out and

threats from climate change, exotic weeds and pests, and intensified land modification are predicted to accelerate this process (Dowle *et al.* 2014; Sivyer *et al.* 2018; Carmelet *et al.* 2021; Morgan-Richards *et al.* 2001; Koot *et al.* 2021; Meza-Joya *et al.* 2023).

Analysis of substantial grasshopper mitochondrial and nuclear DNA sequence data yields a well-resolved phylogenetic hypothesis where the radiation of New Zealand alpine grasshoppers are monophyletic and sister to the two Tasmanian species sampled. The close relationship between Tasmanian Russalpiina and New Zealand *Sigaus* was first suggested by Bigelow (1967), who noted similarities in their ecology and the internal genitalia of males of the Tasmanian *Russalpia* species. Key (1991) suggested a resemblance between the male genitalia of New Zealand *Paprides* and *Brachaspis* with the Tasmanian *Tasmaniacris*, similarity of wing atrophy, and lack of audible communication. More extensive sampling of southern Catantopinae is required to better test this putative sister relationship.

Generic assignment

Although phylogenetic analyses of the mitochondrial and nuclear molecular data gave consistent results, these are not consistent with the grouping of species predicted by current classification using four genera. We do not find four reciprocally monophyletic clades as was expected, and neither of the putatively congeneric species pairs within *Alpinacris* and *Paprides* emerge as sister lineages. Instead *Alpinacris tumidicauda* is sister to *Sigaus australis* rather than *A. crassicauda*. In our phylogenetic analysis *Sigaus villosus* is not found to be closely related to *Brachaspis*, although initially being placed in *Brachaspis* because of similarities in colour and shape (Salmon 1950). Nor is *S. australis* found to be closely related to members of *Paprides*, despite formerly being classified in this genus and displaying confusing similarity of pronotum shape and texture with *P. nitidus*. The phenotypic similarity of *S. childi* (within *S. australis* diversity) and *S. minutus* also demonstrates how morphological convergence among species can obscure taxonomic relatedness and thus phylogenetic relationships. The most parsimonious taxonomic solution is to place all thirteen species currently recognised within this radiation into the genus *Sigaus*.

Hutton was the first to assign endemic New Zealand alpine acridids to multiple genera (Hutton 1897), but the generic placement of species within this radiation has varied since then (Figure 1A). The taxonomic revision of New Zealand Acrididae by Bigelow (1967) focused on male genitalic structures following Dirsh (1956a, b), and appropriately transferred New Zealand species from the Australasian genus *Trigoniza* to *Sigaus*. Bigelow (1967) also established *Alpinacris* with two new species and moved two described species from one genus to another (Figure 1A and Table 1). Despite using male genitalia and suggesting the genus *Sigaus* could be distinguished by features such as the combination of apical tooth on hind femora, and ratio of antennae and hind femur length, Bigelow (1967) also acknowledged that "*Sigaus species share numerous similarities, but few of these are universally present in all species, and many are present also in species of other genera*".

Is the male epiphallus useful for grasshopper systematics?

Bigelow (1967) believed that features of the internal male genitalia were the most useful traits for the classification of New Zealand grasshoppers, stating that the complexity of these structures makes them more evolutionarily informative than external traits that tend to display minor variation within and between species. While the male epiphallus appears to provide some useful information in higher level grasshopper systematics (Song 2010), the failure to identify homologous features, use appropriate levels of sampling for comparison, or undertake any formal analysis limits confidence in the existing treatment of New Zealand grasshoppers (c.f. for example Mariño-Pérez & Song 2017). In some grasshoppers, intraspecific variation in dimensions of epiphallic structures exceeds interspecific variation, and different methods used for preparation of the epiphallus for examination can result in additional inconsistencies (e.g., Hochkirch 2001). Studies that have combined molecular and morphological information have identified inconsistent variation in genitalic structures such as the acridid epiphallus (e.g., Zahid *et al.* 2021), and this appears to be a common source of paraphyly in grasshopper taxonomy reliant on morphological traits (Mariño-Pérez & Song 2017; Song *et al.* 2018; Zahid *et al.* 2021). In the radiation of New Zealand grasshoppers we find no compelling evidence in the shape of the epiphallus to justify any particular groups or even pairs of species that might form the basis of a genus classification (Figure 6).

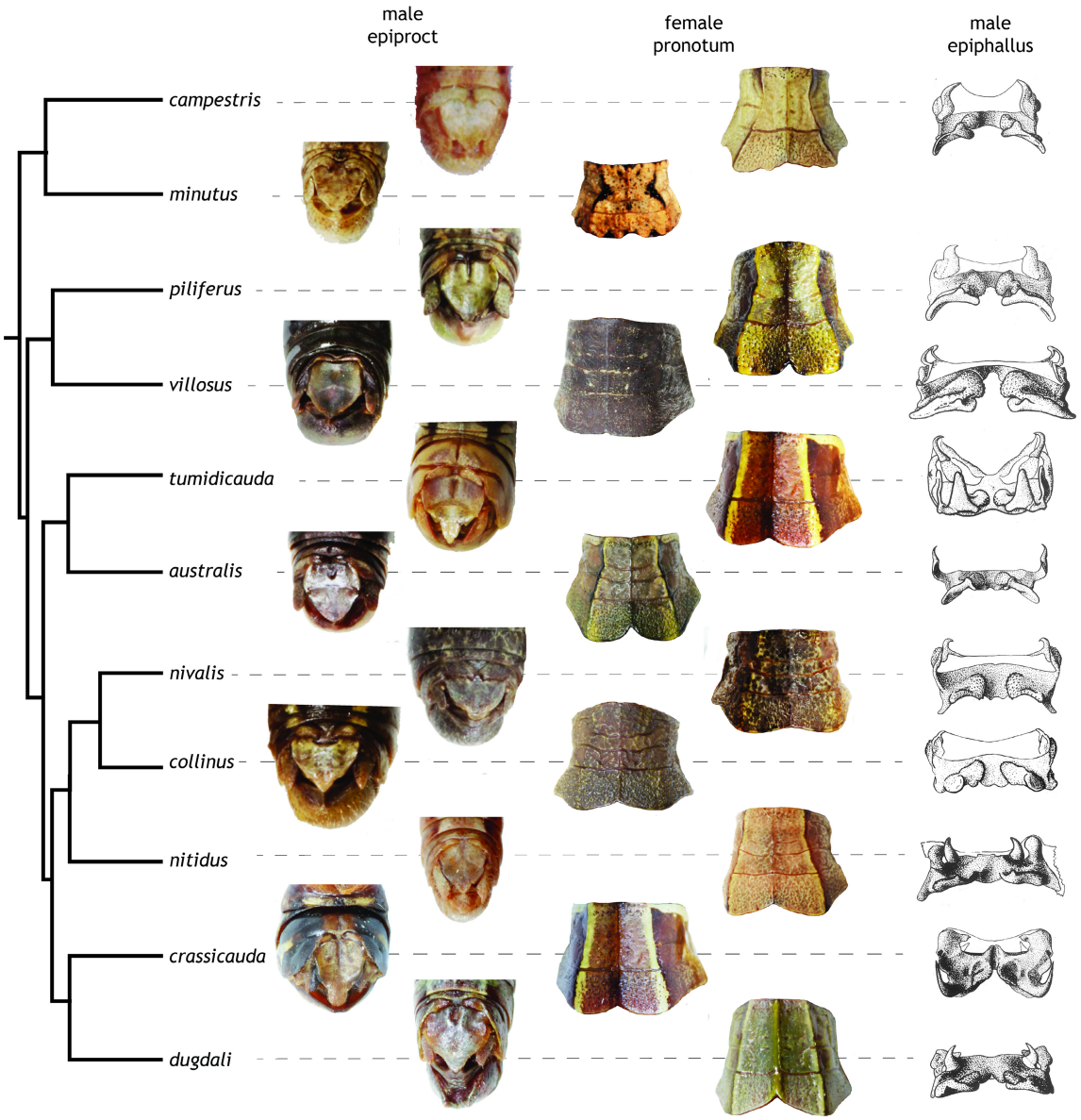

FIGURE 6. Morphological features of adult māwhitiwhiti Aotearoa New Zealand grasshoppers helpful for species identification, mapped to the molecular phylogeny. Drawings of the male internal reproductive structure (epiphallus) from Bigelow (1967) and are not to scale.

Systematics and synonymy of Māwhitiwhiti Aotearoa—New Zealand's alpine grasshoppers

We find the molecular phylogenetic and morphological evidence do not support the current or previous taxonomic treatment. A consideration of the pronotum and terminalia of adult specimens reveals species-specific differences that are valuable for identification at that level, but fails to provide compelling evidence for species groups justifying distinct genera (Figure 6). Well-supported pairings of species lineages do not, in most cases, correspond with current genera and support for some deeper nodes is, despite a large amount of sequence data, poor. This suggests an evolutionary history involving relatively rapid and concurrent lineage diversification. The taxonomic solution we favour is synonymy of all of *Brachaspis*, *Alpinacris* and *Paprides* species into the genus *Sigaus* Hutton 1897 which has precedence. This yields the following new combinations: *Sigaus nitidus* (Hutton 1897), *Sigaus dugdali* (Bigelow 1967), *Sigaus crassicauda* (Bigelow 1967), *Sigaus tumidicauda* (Bigelow 1967), *Sigaus crassicauda* (Bigelow 1967), *Sigaus nivalis* (Hutton 1897), *Sigaus collinus* (Hutton 1897), *Sigaus robustus* (Bigelow 1967). The type species remains, as originally established, *Sigaus piliferus* Hutton 1897. Unfortunately the single female holotype

proposed by Hutton is missing; it was not found by Bigelow (1967) who investigated New Zealand collections. A recent check of the collections at Canterbury Museum in 2023 produced a negative result but confirmed that the neotypes prepared by Bigelow (1967) are in good order (Figure 7). These neotypes therefore also represent the *Sigaus* genus, a monophyletic group of grasshoppers endemic to New Zealand.

FIGURE 7. *Sigaus piliferus* Hutton 1897. Anterior, lateral and dorsal views of neotypes (Bigelow 1967) at Canterbury Museum, New Zealand. A) NEOTYPE, adult male, Pohangina Saddle, East Ruahine Range, 4600 feet (~1400 metres) asl, 27 February 1965, R & A Hilson, CM2007.177.279. B) NEOALLOTYPE, adult female, Pohangina Saddle, East Ruahine Range, 4600 feet (~1400 metres) asl, 27 February 1965, R & A Hilson, CM2007.177.280. Photographs courtesy of Jonathon Ridden.

Hutton (1897) did not provide etymology for the names he used; however, it is most likely that the name *Sigaus* derives from the Greek σιγάω (sigáo) meaning to be silent or to keep silent. The name *Sigaus* could be interpreted as 'silent one', which is applicable to all the species of this endemic radiation. All species lack fully developed wings and elytra, and their tegmina are too short to engage with movements of hind legs in the usual stridulatory behaviour of short-horn grasshoppers (Greenfield 1997). Stridulatory pegs found on the hind femora of other Acrididae (e.g., Pitkin 1976) are absent from the New Zealand radiation. The genus *Sigaus* in the classification of Bigelow (1967) had already spanned the most divergent species among the monophyletic group in terms of morphology, habitat, ecology and body size (Figure 1), so its application to additional species is not problematic in that regard. This classification of māwhitiwhiti Aotearoa simplifies the taxonomy, leaving the distinct ecological, behavioural and morphological features (e.g. Schori *et al*. 2020; Meza-Hoya *et al*. 2022; Nakano *et al*. 2022) of each species lineage to be explored without the distraction of relationships implied by artificial and unnecessary partitions that do not reflect their evolutionary ancestry.

Sigaus are flightless, silent acridids with short tegmina; up to five times as long as wide and extending to second abdominal tergite (e.g. *S. campestris*), about twice as long as wide and extending beyond first abdominal tergite (e.g. *S. australis*), or very short and rounded and no longer than metanotum (e.g. *S. robustus* comb. nov.). Adult size is greatest in females but varies considerably among species with smallest (~10mm) individuals being male *S. minutus* (~10mm) and largest (~50mm) being female *S. villosus* (Figure 1B). Prosternal spine blunt or rounded. Profile of antenna (21–25 segments) rounded to flattened dorso-ventrally especially in males of some species (e.g. *S. australis*). Lateral carina on pronota range from more acute to absent. Colouration, pattern and texture varies within and between species. External appearance appears associated with crypsis in principle microhabitats; e.g. *S. villosus* and *S. nivalis* comb. nov. living on rock have rounded pronotal margins, rugose texture and grey or brown tones, whereas, *S. australis* and *S. nitidus* comb. nov. in grassland have more acute pronotal carina, often bold colour patterns with linear markings. Dorsal transverse pronotal sulci vary from three usually distinct and intact (e.g. *S. australis*), two (e.g. *S. nitidus* comb. nov.) or one (e.g. *S. crassicauda* comb. nov.). Posterior margin of pronotum with mesal indentation and variously sinuous (e.g. *S. villosus*), triangular (e.g. *S. dugdali* comb. nov.) or toothed (e.g. *S. campestris*) in profile. All species are endemic to Aotearoa New Zealand.

Acknowledgements

We are grateful to the New Zealand Department of Conservation for granting access and approval to collect from the Conservation Estate (Authorisation 49878-RES). We thank the ski field and managers who allowed us to access field sites. This research was assisted by a grant from the Miss E. L. Hellaby Indigenous Grasslands Research Trust EMK. Johnathon Ridden at Canterbury Museum provided images of *Sigaus australis* neotypes.

References

Álvarez, I. & Wendel, J.F. (2003) Ribosomal ITS sequences and plant phylogenetic inference. *Molecular phylogenetics and evolution*, 29 (3), 417–434.
https://doi.org/10.1016/S1055-7903(03)00208-2

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M. & Stadler, P.F. (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, 69 (2), 313–319.
https://doi.org/10.1016/j.ympev.2012.08.023

Bigelow, R.S. (1967) *The grasshoppers (Acrididae) of New Zealand; their taxonomy and distribution*. University of Canterbury Publications, Christchurch, 109 + (43) pp.

Boudinet, B.E., Borowiec, M.L. & Prebus, M.M. (2022) Phylogeny, evolution, and classification of the ant genus *Lasius*, the tribe Lasiini and the subfamily Formicinae (Hymenoptera: Formicidae). *Systematic Entomology*, 47, 113–151.
https://doi.org/10.1111/syen.12522

Brunner von Wattenwyl, K.B. & Fea, L. (1893) n.k. *In*: *Révision du système des orthoptères et description des espèces rapportées par M. Leonardo Fea de Birmanie. Vol. 33.* Tipografia dei R. Istituto sordo-muti, Genova, pp. 6–230.
https://doi.org/10.5962/bhl.title.5121

Carmelet-Rescan, D., Morgan-Richards, M., Koot, E.M. & Trewick, S.A. (2021) Climate and ice in the last glacial maximum explain patterns of isolation by distance inferred for alpine grasshoppers. *Insect Conservation and Diversity*, 14, 568–581.

https://doi.org/10.1002/ece3.9633
Chernomor, O., von Haeseler, A. & Minh B.Q. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, 65, 997–1008.
https://doi.org/10.1093/sysbio/syw037
Cigliano, M.M., Braun, H., Eades, D.C. & Otte. D. (2022) Family Acrididae MacLeay, 1821.Orthoptera Species File. Version 5.0/5.0. Available from: http://Orthoptera.SpeciesFile.org (accessed 1 November 2023)
Dirsh, V.M. (1956a) Orthoptera Acridoidea. *In*: Hanstrom, B., Brinck, P. & Rudebeck, G. (Eds.), S*outh African Animal Life: Results of the Lund University Expedition in 1950–1951. Vol. 3.* Almqvist & Wiksell, Stockholm, pp. 121–272.
Dirsh, V.M. (1956b) *Preliminary revision of the genus Catantops Schaum and review of the group Catantopini (Orthoptera, Acrididae)*. Companhia de Diamantes de Angola, Serviços Culturais Dundo-Lunda-Angola, Dundo, 151 pp.
Dowle, E.J., Morgan-Richards, M. & Trewick, S.A. (2014) Morphological differentiation despite gene flow in an endangered grasshopper. *BMC Evolutionary Biology*, 14 (1), 216.
https://doi.org/10.1186/s12862-014-0216-x
Fenn, J.D., Song, H., Cameron, S.L. & Whiting, M.F. (2008) A preliminary mitochondrial genome phylogeny of Orthoptera (Insecta) and approaches to maximizing phylogenetic signal found within mitochondrial genome data. *Molecular Phylogenetics and Evolution*, 49 (1), 59–68.
https://doi.org/10.1016/j.ympev.2008.07.004
Forni, G., Cussigh, A., Brock,. PD., Jones, B.R., Nicolini, F., Martelossi, J., Luchetti, A. & Mantovani, B. (2023) Taxonomic revision of the Australian stick insect genus *Candovia* (Phasmida: Necrosciinae): insight from molecular systematics and species-delimitation approaches. *Zoological Journal of the Linnean Society*, 197, 189–210.
https://doi.org/10.1093/zoolinnean/zlac074
Greenfield, M. (1997) Acoustic Communication in Orthoptera. *In*: Gangwere, S.K., Muralirangan, M.C. & Muralirangan M. (Eds.), *The bionomics of grasshoppers, katydids, and their kin.* CAB International, Delémont, pp. 197–230.
Hernández, C., Aristeu da Rosa, J., Vallejo, G.A., Guhl, F. & Ramírez, J.D. (2020) Taxonomy, evolution, and biogeography of the Rhodniini Tribe (Hemiptera: Reduviidae). *Diversity*, 12, 97.
https://doi.org/10.3390/d12030097
Hoang, D.T., Chernomor, O., von Haeseler, A, Minh, B.Q. & Vinh, L.S. (2018) UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522.
https://doi.org/10.1093/molbev/msx281
Hochkirch, A. (2001) *A Phylogenetic Analysis of the East African grasshopper genus Afrophlaeoba* JAGO, 1983 (Orthoptera: Acridoidea: Acridinae). Dissertation zur Erlangung des Doktorgrades, Universität Bremen, Bremen, 192 pp.
Hudson, L. (1970) Identification of the immature stages of New Zealand alpine acridid grasshoppers (Orthoptera). *Transactions of the Royal Society of New Zealand: Biological Sciences*, 12 (17), 185–208.
Hutton, F.W. (1897) The grasshoppers and locusts of New Zealand and the Kermadec Islands. *Proceedings and Transaction of the New Zealand Institute*, 30, 135–150.
Hutton, F.W. (1898) Notes on the New Zealand Acrididae. *Proceedings and Transaction of the New Zealand Institute*, 31, 44–50.
Jamieson, C.D. (1999) A new species of *Sigaus* from Alexandra, New Zealand (Orthoptera: Acrididae). *New Zealand Journal of Zoology*, 26 (1), 43–48.
https://doi.org/10.1080/03014223.1999.9518176
Johnston, H.B. (1956) *Annotated catalogue of African grasshoppers. Vol. Part 1*. Cambridge University Press, New York, 833 pp.
Johnston, H.B. (1968) *Annotated catalogue of African grasshoppers. Supplement. Vol. 2.* Cambridge University Press, New York, New York, 447 pp.
Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F, von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589.
https://doi.org/10.1038/nmeth.4285
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28 (12), 1647–1649.
https://doi.org/10.1093/bioinformatics/bts199
Key, K.H.L. (1991) On four endemic genera of Tasmanian Acrididae (Orthoptera). *Invertebrate Systematics*, 5 (2), 241–288.
https://doi.org/10.1071/IT9910241
Key, K.H.L. & Colless, D.H. (1993) A higher classification of the Australian Acridoidea (Orthoptera). II.* Subfamily Catantopinae. *Invertebrate Systematics*, 7 (1), 89–111.
https://doi.org/10.1071/IT9930089
Kishino, H., Miyata, T. & Hasegawa, M. (1990) Maximum likelihood inference of protein phylogeny and the origin of chloroplasts. *Journal of Molecular Evolution*, 31, 151–160.
https://doi.org/10.1007/BF02109483
Kishion, H. & Hasegawa, M. (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in hominoidea. *Journal of Molecular Evolution*, 29, 170–179.

https://doi.org/10.1007/BF02100115
Koot, E.M., Morgan-Richards, M. & Trewick, S.A. (2020) An alpine grasshopper radiation older than the mountains, on Kā Tiritiri o te Moana (Southern Alps) of Aotearoa (New Zealand). *Molecular Phylogenetics and Evolution*, 147, 106783. https://doi.org/10.1016/j.ympev.2020.106783
Koot, E.M., Morgan-Richards, M. & Trewick, S.A. (2022) Climate change and alpine-adapted insects: modelling environmental envelopes of a grasshopper radiation. *Open Science*, 9, 211596. https://doi.org/10.1098/rsos.211596
Linnaeus, C.N. (1758) S*ystema naturae per regna tria naturae :secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. 10th Edition*. Impensis Direct. Laurentii Salvii, Holmiae, 824 pp. https://doi.org/10.5962/bhl.title.542
MacLeay, W.S. (1821) *Horae entomologicae: or essays on the annulose animals. Vol. 1. Pt. 1–2*. Printed for S. Bagster, London, 162 pp. [1819–1821]
Mariño-Pérez, R. & Song, H. (2017) Phylogeny of the grasshopper family Pyrgomorphidae (Caelifera, Orthoptera) based on morphology. *Systematic Entomology*, 43 (1), 90–108. https://doi.org/10.1111/syen.12251
Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17 (1), 10–12. https://doi.org/10.14806/ej.17.1.200
Meza-Joya, F.L., Morgan-Richards, M. & Trewick, S.A. (2022) Relationships among body size components of three flightless New Zealand grasshopper species (Orthoptera, Acrididae) and their ecological applications. *Journal of Orthoptera Research*, 21, 91–103. https://doi.org/10.3897/jor.31.79819
Meza-Joya, F.L., Morgan-Richards, M., Koot, E.M. & Trewick, S.A. (2023) Global warming leads to habitat loss and genetic erosion of alpine biodiversity. *Journal of Biogeography*, 50 (5), 961–975. https://doi.org/10.1111/jbi.14590
Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. & Lanfear, R. (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530–1534. https://doi.org/10.1093/molbev/msaa015
Morgan-Richards, M., Vilcot, M. & Trewick, S.A. (2021) Lack of assortative mating might explain reduced phenotypic differentiation where two grasshopper species meet. *Journal of Evolutionary Biology*, 35 (4), 509–519. https://doi.org/10.1111/jeb.13879
Morris, S.J. (2003) Two new species of *Sigaus* from Fiordland, New Zealand (Orthoptera: Acrididae). *New Zealand Entomologist*, 26 (1), 65–74. https://doi.org/10.1080/00779962.2003.9722110
Nakano, M., Morgan-Richards, M., Clavijo-McCormick, A. & Trewick, S.A. (2022) Abundance and distribution of antennal sensilla on males and females of three sympatric species of alpine grasshopper (Orthoptera: Acrididae: Catantopinae) in Aotearoa New Zealand. *Zoomorphology*, 142, 51–62. https://doi.org/10.1007/s00435-022-00579-z
Nei, M. & Rooney, A.P. (2005) Concerted and birth-and-death evolution of multigene families. *Annual Review of Genetics*, 39, 121–152. https://doi.org/10.1146/annurev.genet.39.073003.112240
Pitkin, L.M. (1976) A comparative study of the stridulatory files of the British Gomphocerinae (Orthoptera: Acrididae). *Journal of Natural History*, 19, 17–28. https://doi.org/10.1080/00222937600770031
Raué, H.A., Klootwijk, J. & Musters, W. (1988) Evolutionary conservation of structure and function of high molecular weight ribosomal RNA. *Progress in Biophysics and Molecular Biology*, 51 (2), 77–129. https://doi.org/10.1016/0079-6107(88)90011-9
Richard, G.F., Kerrest, A. & Dujon, B. (2008) Comparative genomics and molecular dynamics of DNA repeats in eukaryotes. *Microbiology and Molecular Biology Reviews*, 72 (4), 686–727. https://doi.org/10.1128/MMBR.00011-08
Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19 (12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
Salmon, J.T. (1950) A new species of Acrididae (Insecta: Orthoptera) from New Zealand. *Transactions of the Royal Society of New Zealand: Biological Sciences*, 78 (1), 69.
Schori, J.C., Steeves, T.E. & Murray, T.J. (2020) Designing monitoring protocols to measure population trends of threatened insects: A case study of the cryptic, flightless grasshopper *Brachaspis robustus*. *PLoS ONE*, 15 (9), e0238636. https://doi.org/10.1371/journal.pone.0238636
Sivyer, L., Morgan-Richards, M., Koot, E. & Trewick, S.A. (2018) Anthropogenic cause of range shifts and gene flow between two grasshopper species revealed by environmental modelling, geometric morphometrics and population genetics. *Insect Conservation and Diversity*, 11, 425–434.

https://doi.org/10.1111/icad.12289
Song, H. (2010) Grasshopper systematics: past, present and future. *Journal of Orthopteran Research*, 19 (1), 57–68. https://doi.org/10.1665/034.019.0112
Song, H., Amédégnato, C., Cigliano, M.M., Desutter-Grandcolas, L., Heads, S.W., Huang, Y. Otte, D. & Whiting, M.F. (2015) 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. *Cladistics*, 31 (6), 621–651. https://doi.org/10.1111/cla.12116
Song, H., Mariño-Pérez, R., Woller, D.A. & Cigliano, M.M. 2018. Evolution, diversification, and biogeography of grasshoppers (Orthoptera: Acrididae). *Insect Systematics and Diversity*, 2 (4), 3. https://doi.org/10.1093/isd/ixy008
Stebbins, G.L. (1956) Taxonomy and the evolution of genera, with special reference to the family Gramineae. *Evolution*, 10, 235–245. https://doi.org/10.2307/2406009
Strimmer, K. & Rambaut, A. (2002) Inferring confidence sets of possibly misspecified gene trees. *Proceedings of the Royal Society B*, 269, 137–142. https://doi.org/10.1098/rspb.2001.1862
Shimodaira, H. (2002) An Approximately unbiased test of phylogenetic tree selection *Systematic Biology*, 51, 492–508. https://doi.org/10.1080/10635150290069913
Shimodaira, H. & Hasegawa, M. (1999) Multiple Comparisons of Log-Likelihoods with Applications to Phylogenetic Inference. *Molecular Biology and Evolution*, 16, 1114. https://doi.org/10.1093/oxfordjournals.molbev.a026201
Sunnucks, P. & Hales, D.F. (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). *Molecular Biology and Evolution*, 13 (3), 510–524. https://doi.org/10.1093/oxfordjournals.molbev.a025612
Trewick, S.A. (2001) Identity of an endangered grasshopper (Acrididae: *Brachaspis*): taxonomy, molecules and conservation. *Conservation Genetics*, 2 (3), 233–243. https://doi.org/10.1023/A:1012263717279
Trewick, S.A. (2008) DNA Barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). *Cladistics*, 24 (2), 240–254. https://doi.org/10.1111/j.1096-0031.2007.00174.x
Trewick, S.A. & Morris, S.J. (2008) *Diversity and taxonomic status of some New Zealand grasshoppers*. Department of Conservation, Wellington, 40 pp.
Trewick, S.A., Hegg, D., Morgan-Richards, M., Murray, T., Watts, C., Johns, P. & Michel, P. (2022) *Conservation status of Orthoptera (wētā, crickets and grasshoppers) in Aotearoa New Zealand. New Zealand Threat Classification Series 39*. Department of Conservation, Wellington, 28 pp
Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A. & Minh, B.Q. (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis, *Nucleic Acids Research*, 44 (1), W232–W235. https://doi.org/10.1093/nar/gkw256
Vaux, F., Hills, S.F., Marshall, B.A., Trewick, S.A. & Morgan-Richards, M. (2017) A phylogeny of Southern Hemisphere whelks (Gastropoda: Buccinulidae) and concordance with the fossil record. *Molecular Phylogenetics and Evolution*, 114, 367–381. https://doi.org/10.1016/j.ympev.2017.06.018
Wilkerson, R.C., Linton, Y-M., Fonseca, D.M., Schultz, T.R., Price, D.C. & Strickman, D.A. (2015) Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PlosONE*, 10 (7), e0133602. https://doi.org/10.1371/journal.pone.0133602
Zahid, S., Mariño-Pérez, R. & Song, H. (2021) Molecular phylogeny of the grasshopper family Pyrgomorphidae (Caelifera, Orthoptera) reveals rampant paraphyly and convergence of traditionally used taxonomic characters. *Zootaxa*, 4969 (1), 101–118. https://doi.org/10.11646/zootaxa.4969.1.5