**SUPPLEMENTARY MATERIAL**

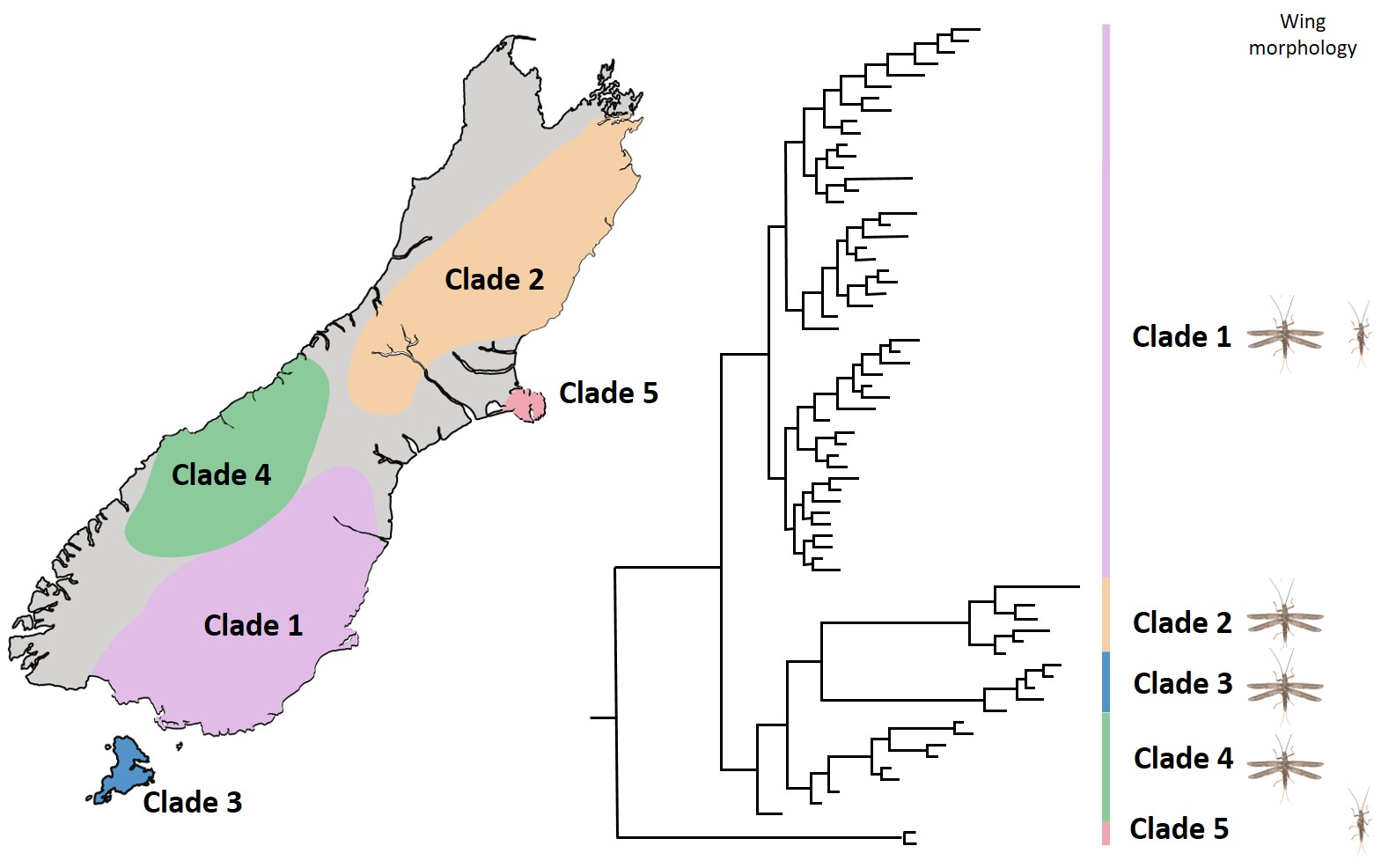
**Genomics Reveals Widespread Ecological Speciation in Flightless Insects**

Graham A. McCulloch1\*, Brodie J. Foster1, Ludovic Dutoit1, Thomas W. R. Harrop2, Joseph Guhlin2, Peter K. Dearden2, Jonathan M. Waters1

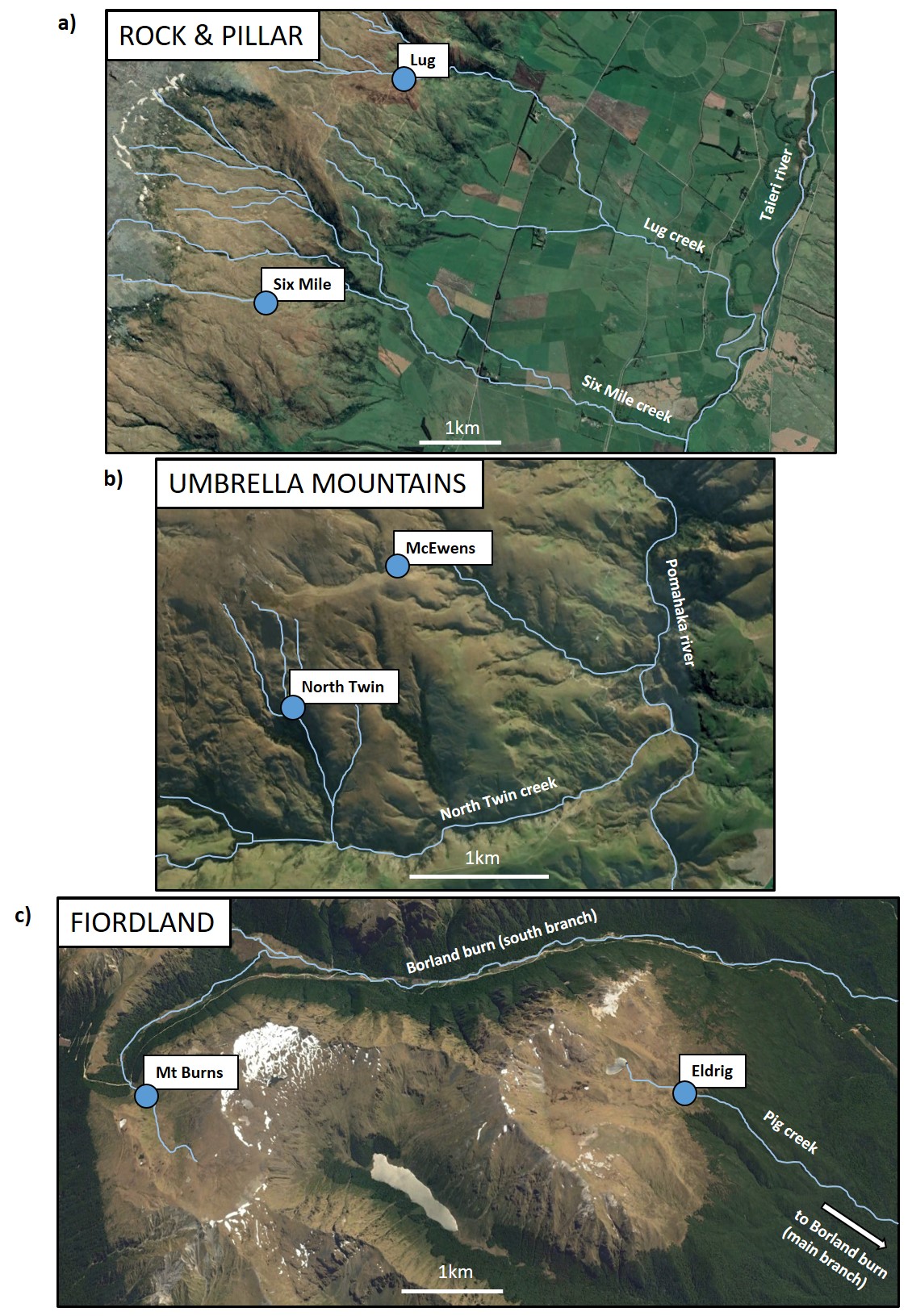
1Department of Zoology, University of Otago, PO Box 56, Dunedin, 9054, New Zealand

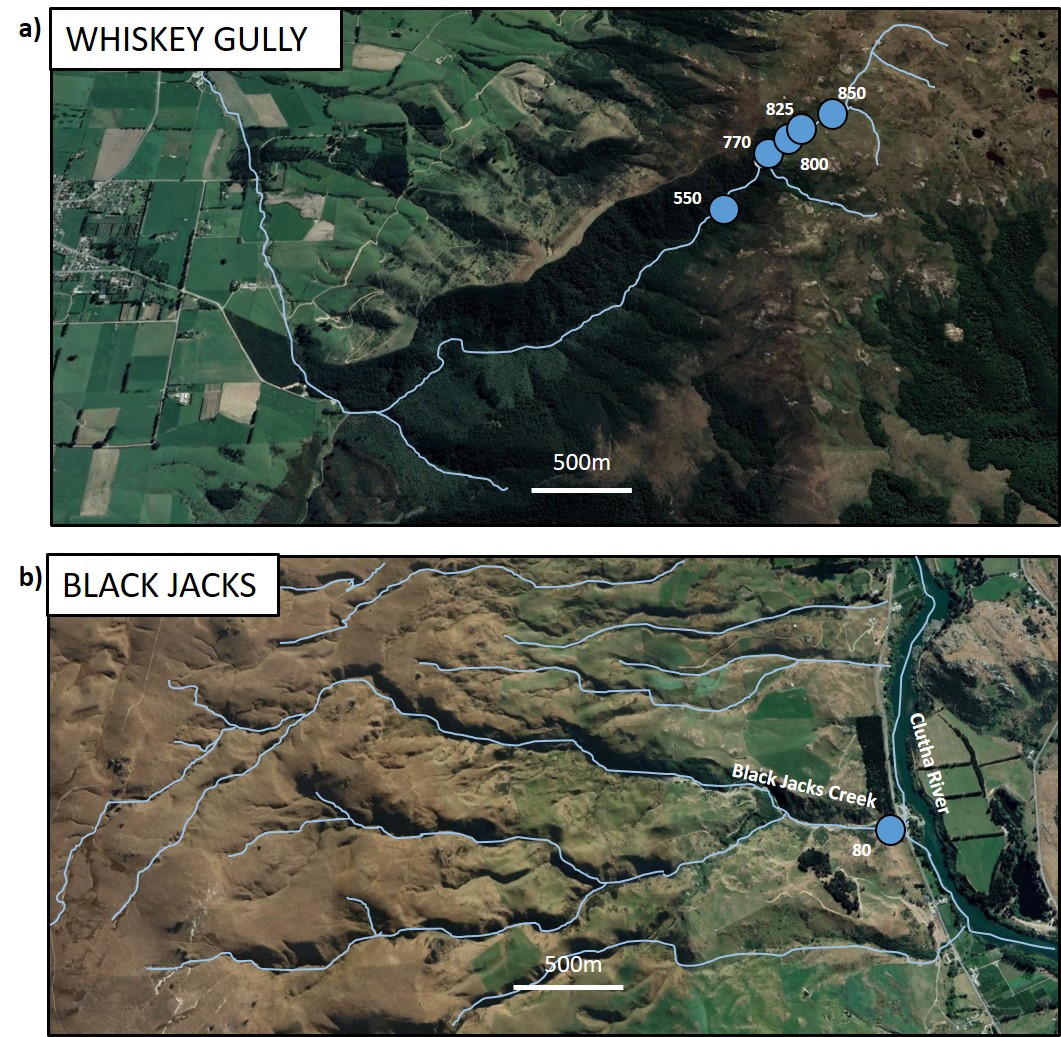
2 Genomics Aotearoa and Department of Biochemistry, University of Otago, PO Box 56, Dunedin, 9054, New Zealand

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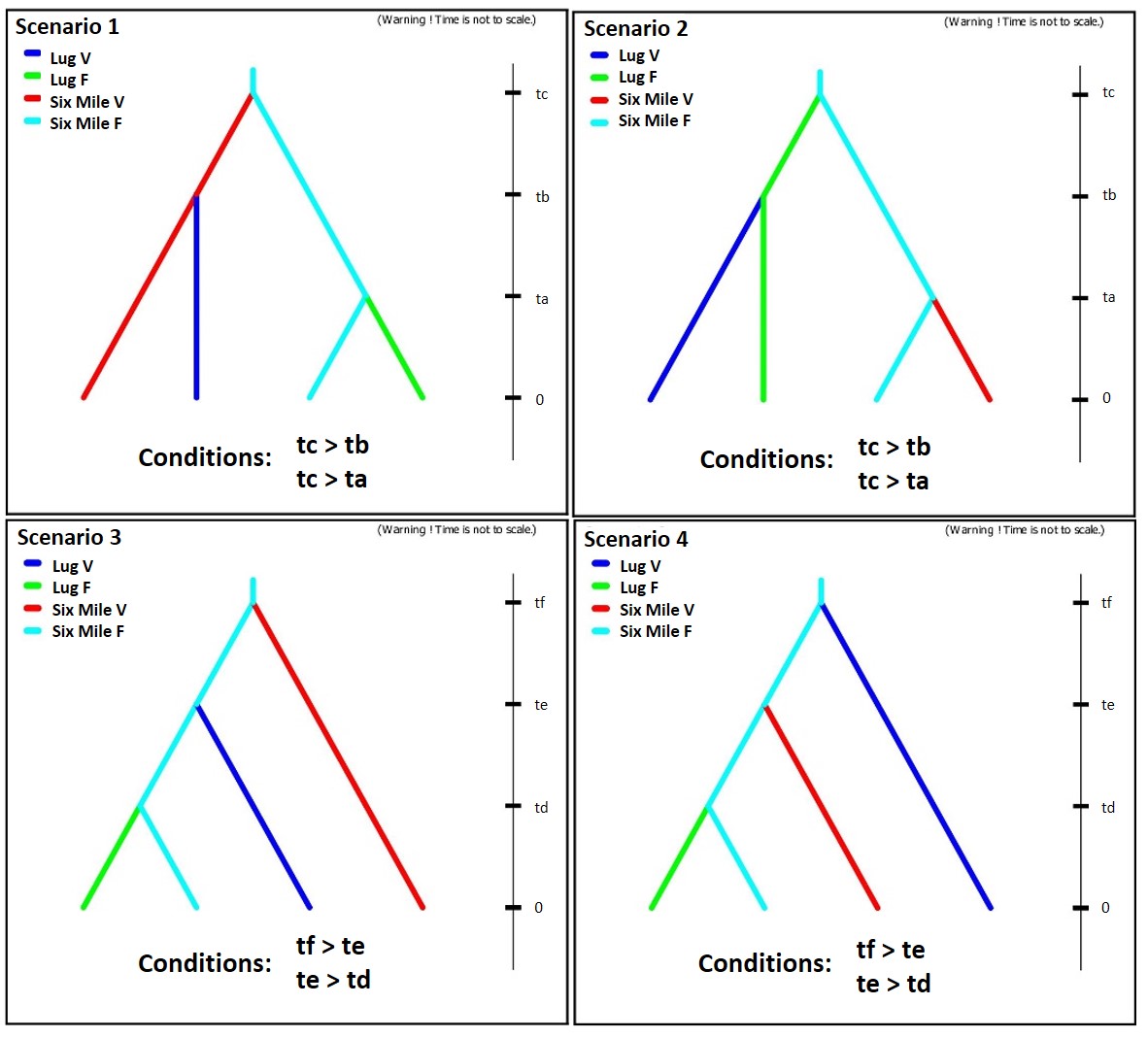


**Supplementary Figure S1.** COI phylogeny of the *Zelandoperla fenestrata* species group illustrating geographic distributions of the five mitochondrial clades (modified from McCulloch et al. 2009, Veale et al. 2018). The wing morphologies found in each clade are also indicated.

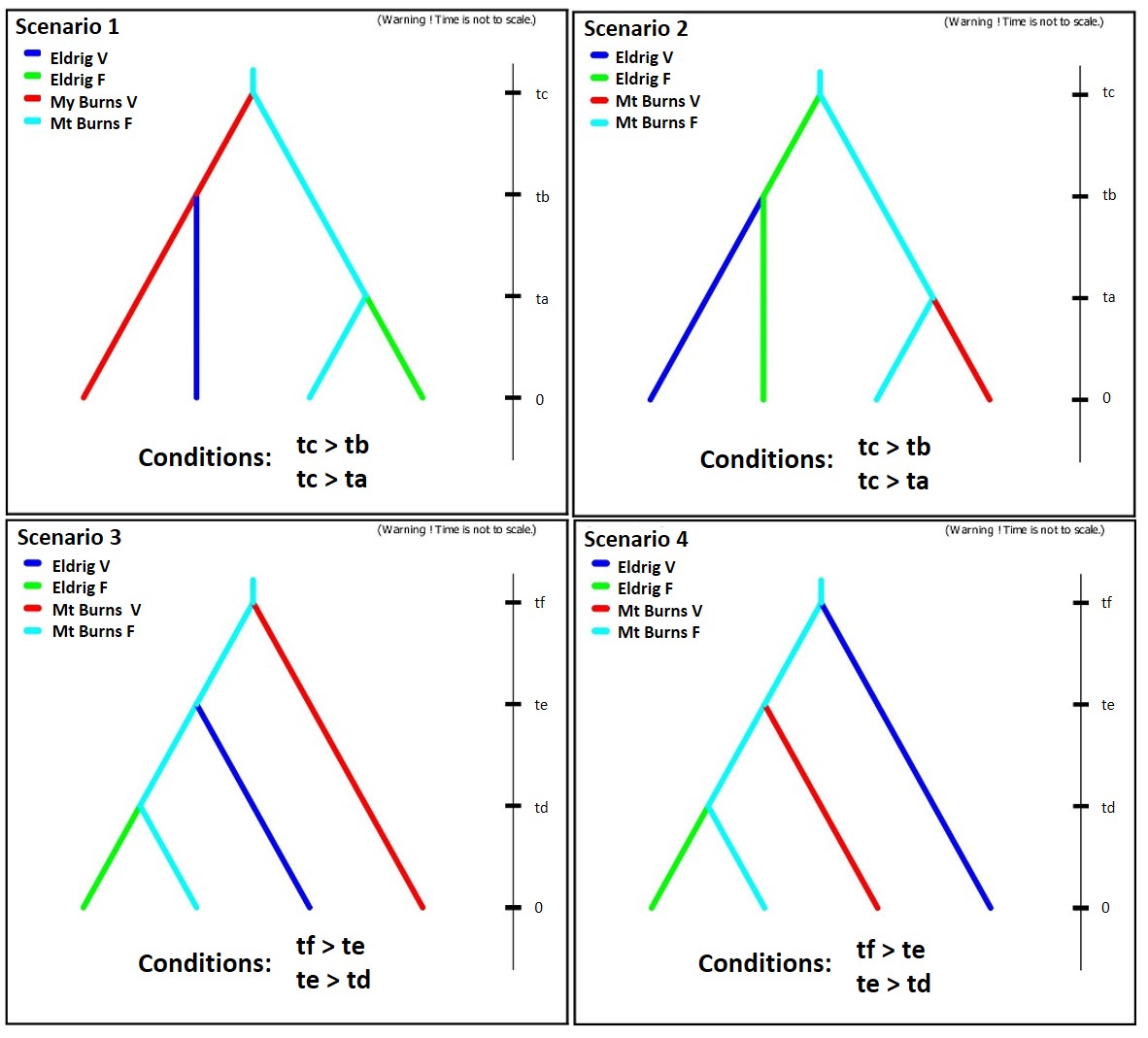
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**Supplementary Figure S2.** Maps showing *Zelandoperla fenestrata* samplinglocalities from a) Rock and Pillar, b) Umbrella mountains, and c) Fiordland.****

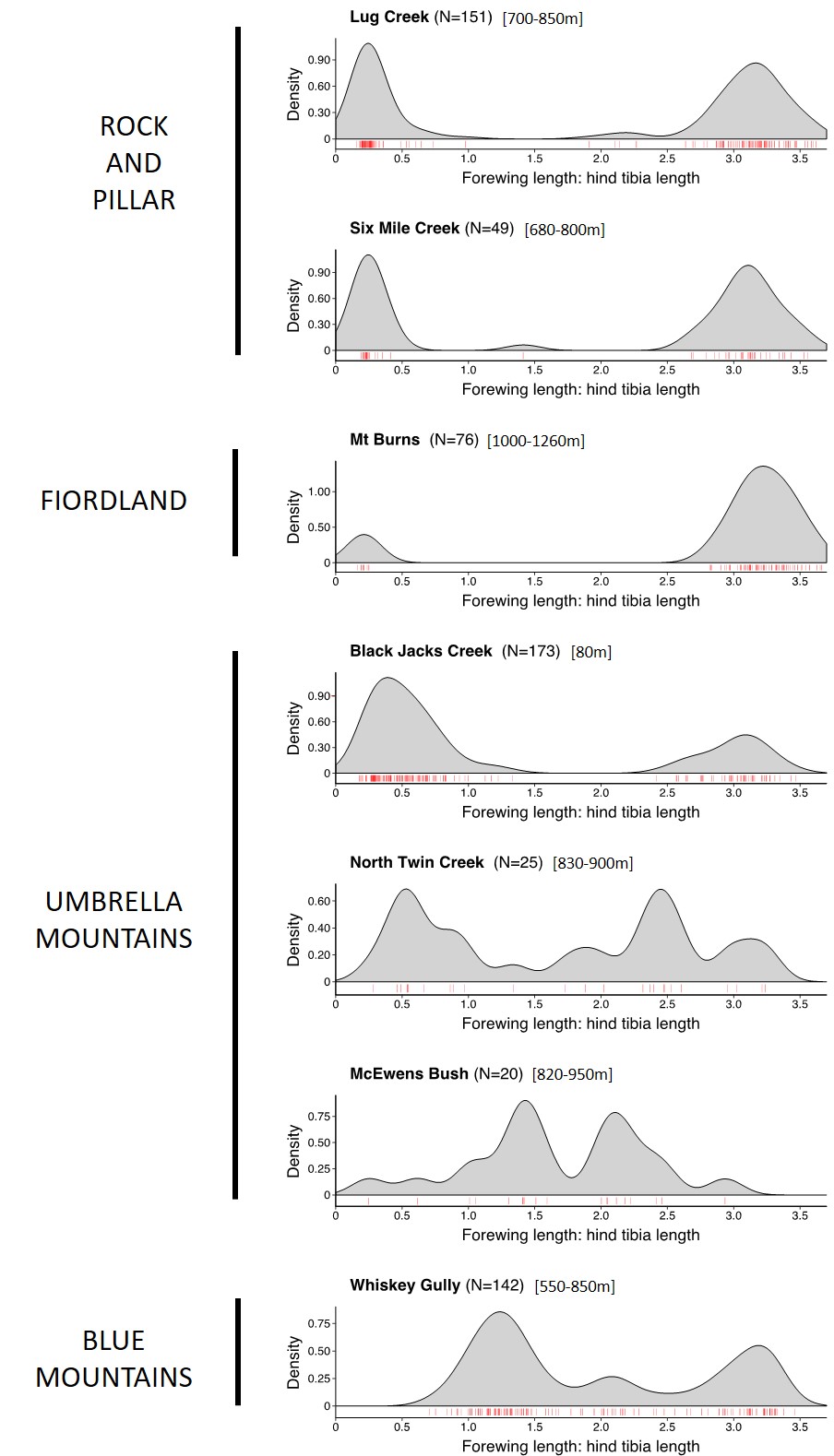
**Supplementary Figure S3.** Maps showing *Zelandoperla fenestrata* samplinglocalities from a) Whiskey Gully (Blue Mountains), and b) Black Jacks (Umbrella mountains)

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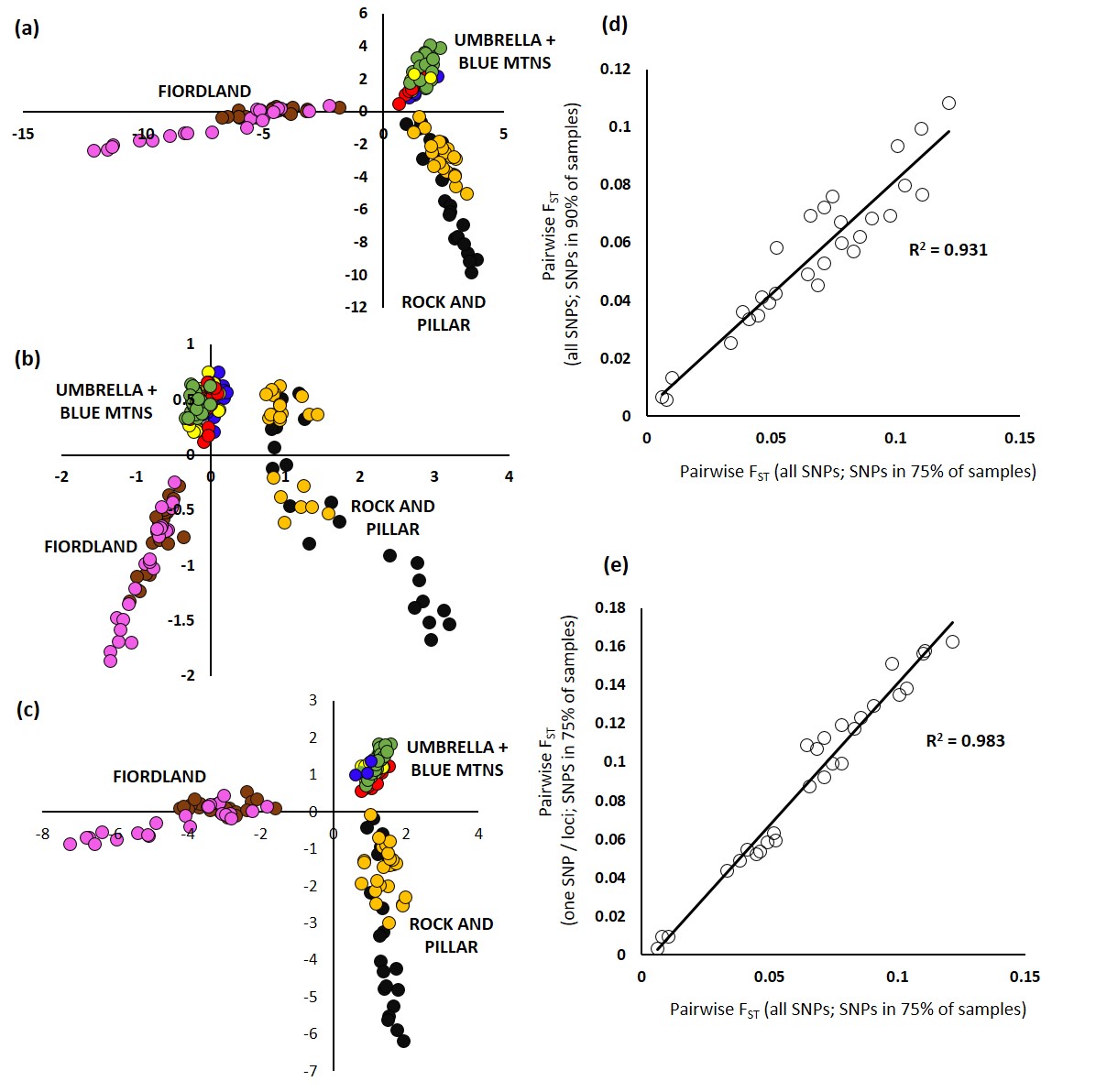
**Supplementary Figure S4.** Demographic scenarios implemented in DIYABC to explored the demographic histories of genomically-divergent *Zelandoperla fenestrata* ecotype pairs within the Rock and Pillar Range.



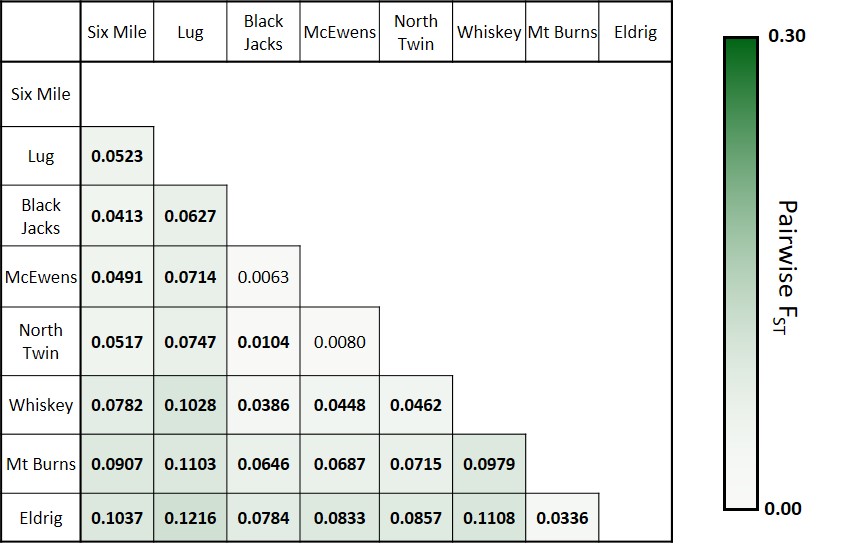
**Supplementary Figure S5.** Demographic scenarios implemented in DIYABC to explored the demographic histories of genomically-divergent *Zelandoperla fenestrata* ecotype pairs within Fiordland.



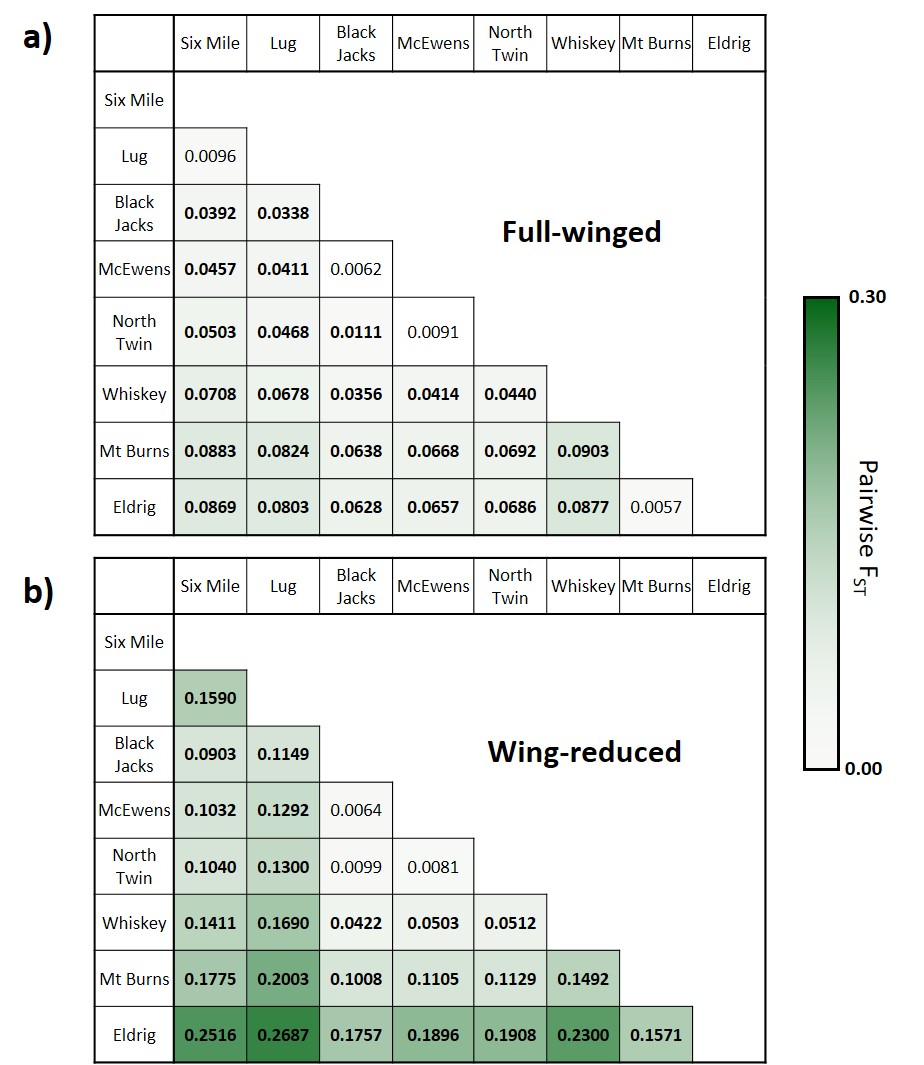
**Supplementary Figure S6.** Density plots offorewing length:hind tibia ratio for adult *Zelandoperla fenestrata* specimens for seven of the eight streams sampled. These plots demonstrate that wing length is broadly dimorphic in each population, and show how the extent of wing reduction varies across streams.



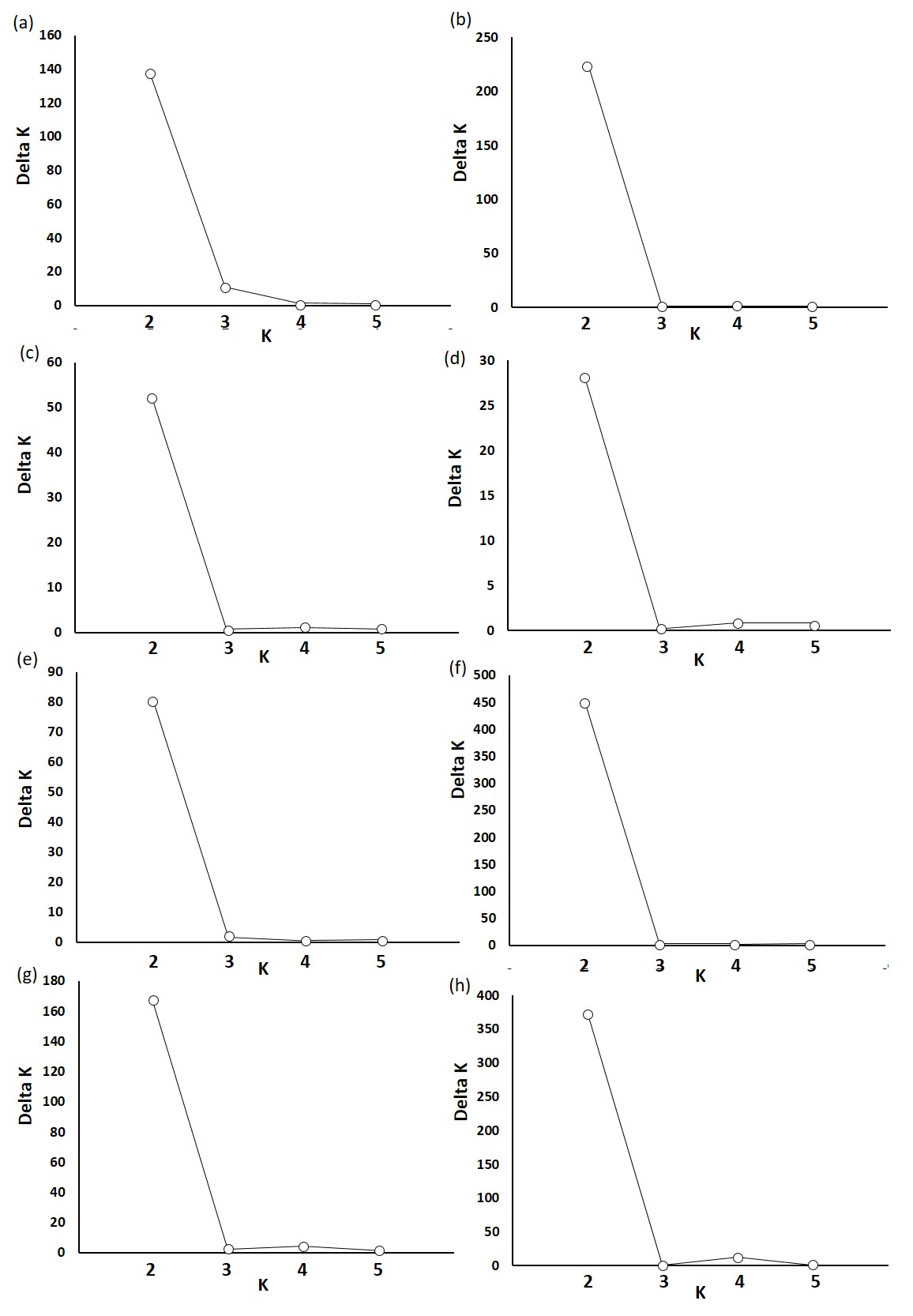
**Supplementary Figure S7.** Assessment of the impact of SNP filtering parameters on the genetic structuring of *Zelandoperla fenestrata* populations from eight independent streams. (a) PCA plot using the dataset III (includes only one SNP / locus; 18,365 SNPs). (b) PCA plot using dataset II (includes only SNPs found in 90% of samples; 2,698 SNPs). (c) PCA plot using dataset III (50, 706 SNPs) (d) Association between population pairwise FST values from dataset I with those from dataset II. (e) Association between population pairwise FST values from dataset I with those from dataset III. These plots indicate that the SNP-calling protocol used is not noticeably impacting downstream analyses.

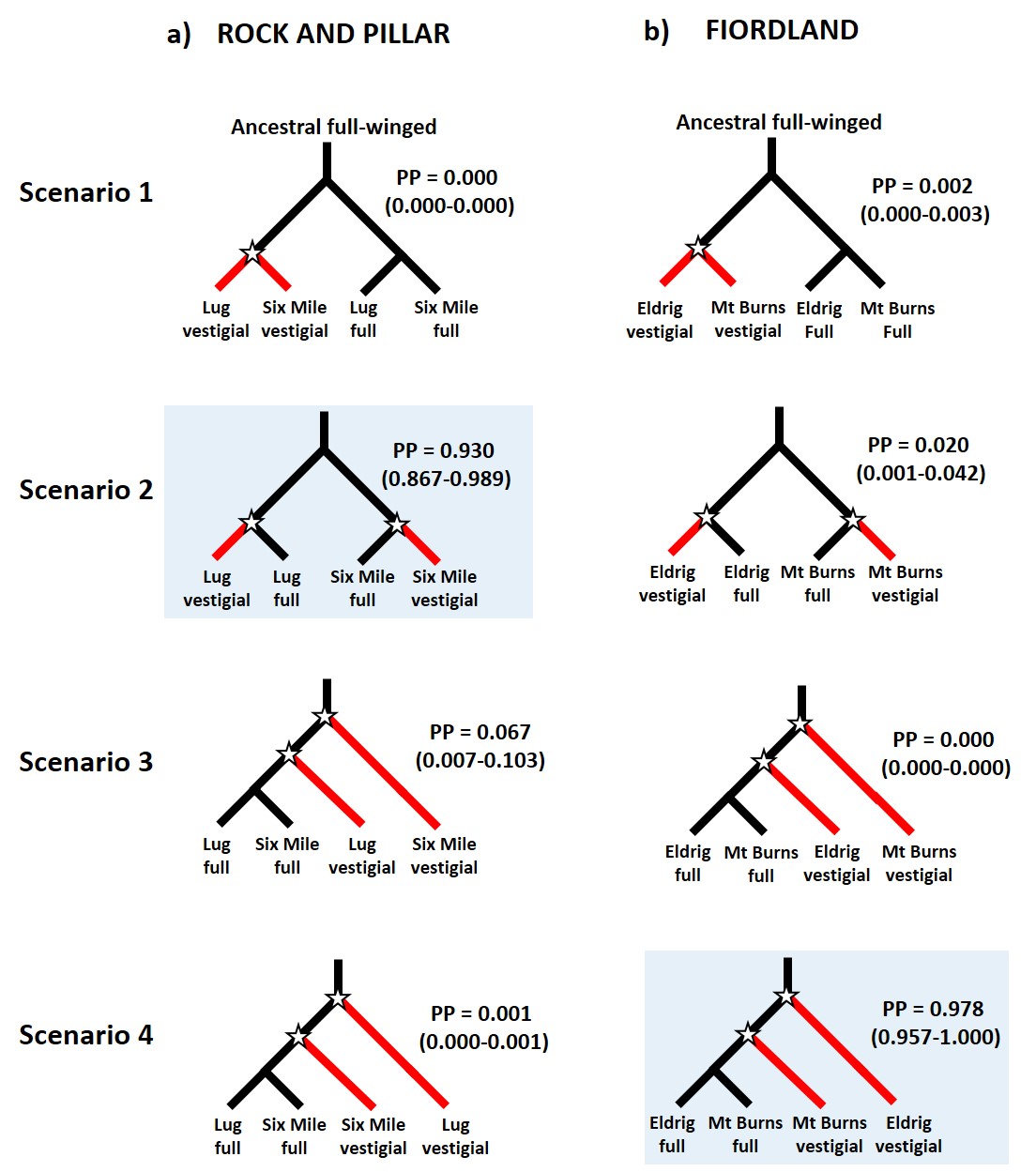


**Supplementary Figure S8.** Pairwise FST values among *Zelandoperla fenestrata* samples across all eight stream populations. Bold values indicate significant *P*-values following Bonferroni correction.

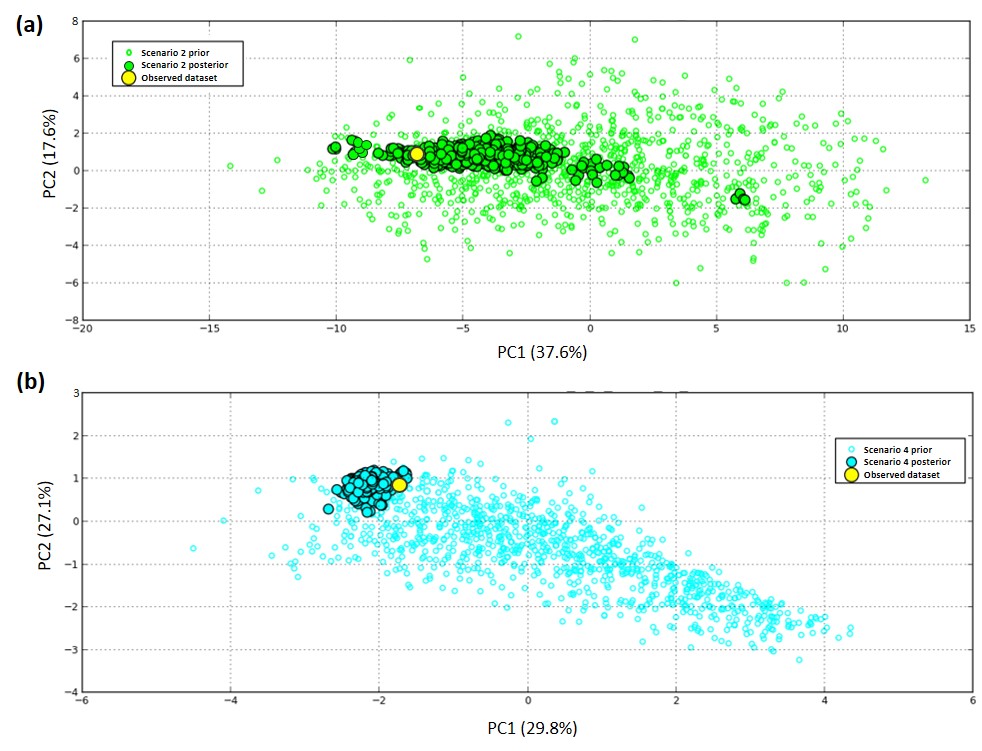


**Supplementary Figure S9.** Wing-reduced ecotypes show substantially stronger population-genetic subdivision than their sympatric winged counterparts. Pairwise FST values among *Zelandoperla fenestrata* collected from eight stream populations, for a) full-winged samples, and b) wing-reduced samples. All values are significant (*P* ≤ 0.05) following Bonferroni correction.

**Supplementary Figure S10.** Rate of change of likelihood (Delta K) across multiple runs of STRUCTURE for *Zelandoperla fenestrata* stoneflies from (a) Lug, (b) Six Mile, (c) Black Jacks, (d) North Twin, (e) McEwens, (f) Whiskey, (g) Mt Burns, and (h) Eldrig.

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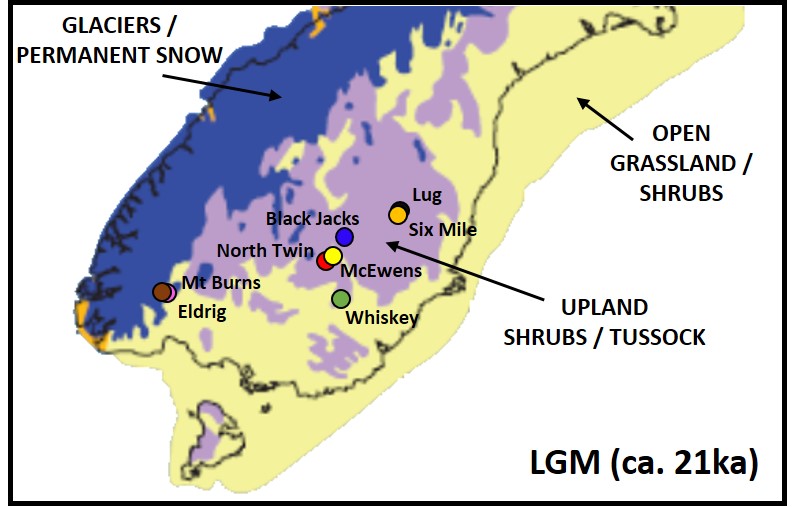
**Supplementary Figure S11.** Coalescent analyses support parallel divergence events linked to independent flight loss in neighbouring stream populations. Schematic of competing evolutionary demographic scenarios for paired *Zelandoperla fenestrata* ecotypes from a) Rock and Pillar, and b) Fiordland mountains. Time is not to scale. Stars at nodes indicate inferred wing-reduction events, assuming a winged ancestor. Posterior probabilities and confidence intervals for each hypothetical scenario were calculated in DIYABC. The scenario with the highest support in each region is highlighted.

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**Supplementary Figure S12.** PCAs evaluating the fit between the observed summary statistics and simulated datasets for the demographic scenarios with the highest support (see Fig. 4). (a) Rock and Pillars, (b) Fiordland.

C:\Users\mccgr18p\Downloads\dmrt2 alignment_tree (1).tiff**Supplementary Figure S13.** Maximum likelihood phylogram of ecdysozoan DMRT1 protein sequences. Accession numbers and identities of sequences are listed in Supplementary file 2. The phylogeny was derived from the alignment in Supplementary file 3. The *Zelandoperla fenestrata* sequence we have identified represents an early diverging branch within a clade (highlighted) of insect double-sex proteins against DMRT1, double-sex and mab-3 sequences from collembolan, crustacean, myriapod, chelicerate, onychophoran and nematode species.

**Fig. S14.** Map of collection sitesshowing the position of the glaciers and different vegetation zones during the peak of the last glacial maximum (ca. 21 ka; modified from Alloway et al. 2007).



**Supplementary Table S1.** GPS coordinates and elevation of collection sites.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Site** | **Latitude** | **Longitude** | **Collection dates** | **Elevation [m] (# full-winged / # wing-reduced)** |
| Lug Creek | -45.418700 | 170.119695 | Jan-Feb 2017 | 700 (10/10), 850 (2/2) |
| Six Mile | -45.443479 | 170.098537 | Jan 2018 | 680 (9/9), 800 (3/3) |
| Black Jacks | -45.574559 | 169.307399 | Nov 2016 | 80 (12/12) |
| McEwens | -45.610065 | 169.153806 | Jan-Mar 2018 | 820 (2/7), 850 (2/1), 870 (2/0), 900 (3/0), 950 (3/4) |
| North Twin | -45.614278 | 169.150057 | Jan-Mar 2018 | 830 (2/1), 860 (2/3), 900 (8/8) |
| Whiskey Gully | -45.943033 | 169.322662 | Nov 2018 | 550 (1/1), 770 (3/3), 800 (3/3), 825 (1/1), 850 (4/4) |
| Mount Burns | -45.749678 | 167.390099 | Feb 2017 | 1000 (3/1), 1050 (3/0), 1100 (6/5), 1260 (0/6) |
| Eldrig Peak | -45.757164 | 167.464085 | Jan 2018 | 960 (9/0), 1000 (3/8), 1160 (0/4) |

**Supplementary Table S2.** Potential outlier loci associated with wing ecotype across all eight populations, calculated using Bayescan.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Site name** | **Probability** | **Log10 (PO)** | | ***q*-value** | **Alpha** | **FST** | **Contig** |
| 396267:11 | 1 | 1000 | 0 | | 2.170 | 0.0941 | 323 |
| 396267:47 | 0.9998 | 3.699 | 0.0001 | | 2.201 | 0.0988 | 323 |
| 410595:44 | 0.9996 | 3.398 | 0.0002 | | 2.233 | 0.1022 | 3362 |
| 374142:36 | 0.9994 | 3.222 | 0.0003 | | 2.194 | 0.0985 | 2998 |
| 546488:46 | 0.9960 | 2.396 | 0.0010 | | 2.009 | 0.0851 | 5132 |
| 396314:26 | 0.9744 | 1.580 | 0.0051 | | 1.757 | 0.0679 | 323 |
| 722125:49 | 0.9540 | 1.317 | 0.0110 | | 1.799 | 0.0731 | 9664 |
| 546489:18 | 0.9298 | 1.122 | 0.0184 | | 1.743 | 0.0701 | 5132 |
| 431147:48 | 0.8658 | 0.810 | 0.0313 | | 1.635 | 0.0667 | 3625 |
| 458809:35 | 0.8368 | 0.710 | 0.0444 | | 1.576 | 0.0649 | 3902 |

**Supplementary Table S3.** Potential outlier loci (*q*-value < 0.05) associated with wing ecotype across two Rock and Pillar populations, calculated using Bayescan.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Site name** | **Probability** | **Log10 (PO)** | | ***q*-value** | **Alpha** | **FST** | **Contig** |
| 396314:26 | 1 | 1000 | 0 | | 2.438 | 0.3662 | 323 |
| 616615:31 | 1 | 1000 | 0 | | 2.281 | 0.3337 | 6498 |
| 616615:41 | 0.9999 | 2.999 | 0.0003 | | 1.846 | 0.2490 | 6498 |
| 396547:34 | 0.6593 | 0.287 | 0.0454 | | 1.182 | 0.1748 | 323 |

**Supplementary Table S4.** Potential outlier loci (*q*-value < 0.05) associated with wing ecotype across two Fiordland mountain populations, calculated using Bayescan.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site name** | **Probability** | **log10 (PO)** | ***q*-value** | **Alpha** | **FST** | **Contig** |
| 374142:36 | 1 | 1000 | 0 | 2.080 | 0.4605 | 2998 |
| 396267:47 | 0.9998 | 3.699 | 0.0001 | 1.985 | 0.4386 | 323 |
| 410595:44 | 0.9992 | 3.097 | 0.0003 | 1.864 | 0.4116 | 3362 |
| 581405:8 | 0.9938 | 2.205 | 0.0018 | 1.788 | 0.3953 | 5846 |
| 546488:46 | 0.9840 | 1.789 | 0.0046 | 1.707 | 0.3786 | 5132 |
| 396218:7 | 0.7133 | 0.532 | 0.0372 | 1.160 | 0.2847 | 323 |
| 559386:37 | 0.7694 | 0.523 | 0.0423 | 1.184 | 0.2830 | 5372 |
| 396267:11 | 0.6971 | 0.486 | 0.0474 | 1.161 | 0.2848 | 323 |