Ecography

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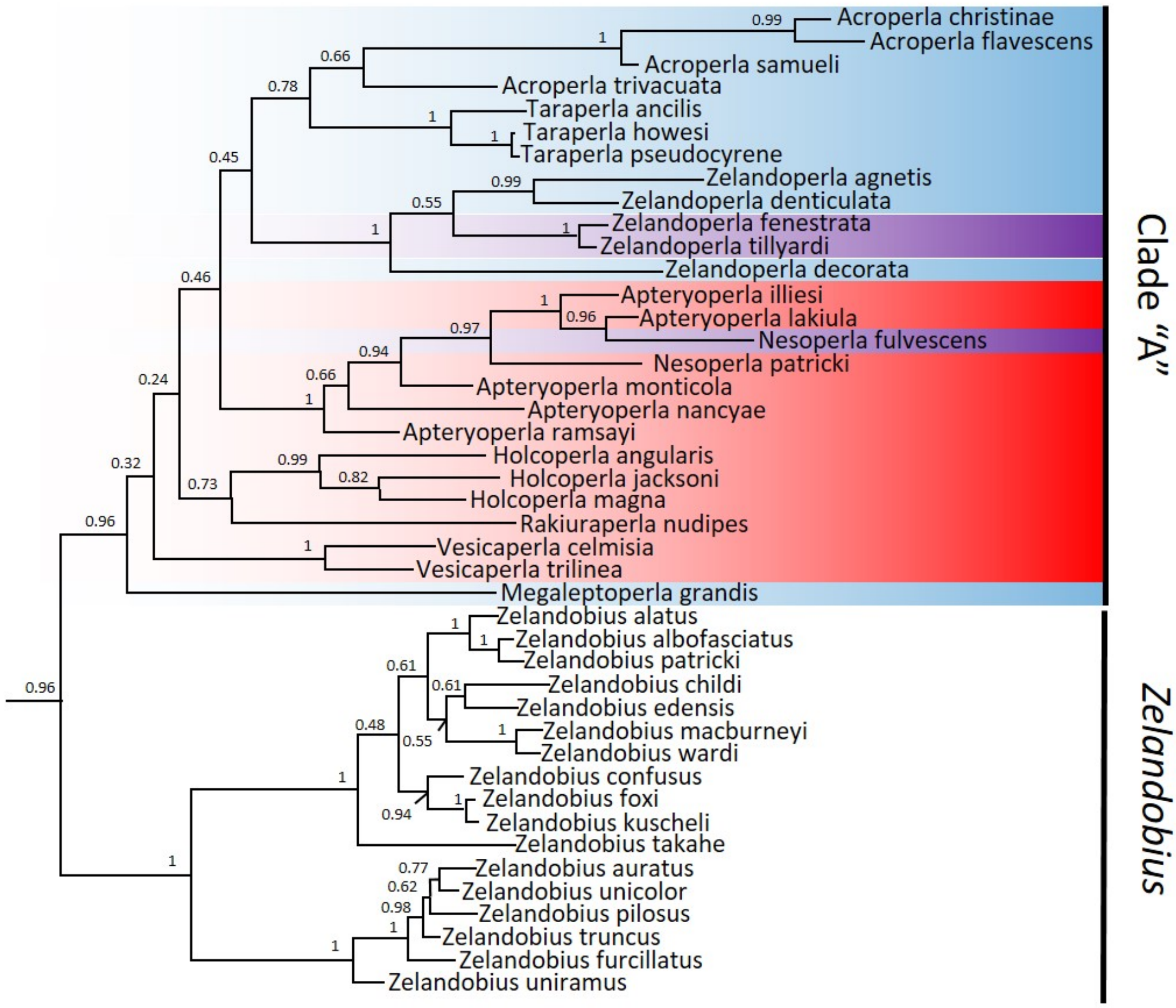
McCulloch, G. A., Foster, B. J., Ingram, T. and Waters, J. M. 2019. Insect wing loss is tightly linked to the treeline: evidence from a diverse stonefly assemblage. – Ecography doi: 10.1111/ecog.04140

Supplementary material

Appendix 1: Phylogenetic relationships among New Zealand Gripopterygidae



Bayesian phylogeny of NZ Gripopterygidae based on three gene regions (18S, H3, COI; modified from McCulloch et al. (2017)). The two divergent Gripopterygidae clades (*Zelandobius* and Clade "A") are indicated, as is the distribution of wingless, fully-winged, and wing dimorphic lineages in Clade "A".

Appendix 2: Material and methods

Collection records for NZ stonefly species were obtained from the Stoneflies of New Zealand database (http://stoneflies.org.nz). Over 2,000 records were analysed across 29 species (12 fully-winged, 17 wingless) from Gripopterygidae clade "A" (see McCulloch and Waters 2018). Wing dimorphic species (*Nesoperla fulvescens*, and *Zelandoperla fenestrata*; see McCulloch et al. 2009) were not included in the analysis, as the wing-length data could not be determined for a large proportion of samples (particularly nymphal specimens). Records from duplicate locations were removed. Likewise records where the collection location could not be accurately determined were removed. The altitude of each record was assessed by mapping the collection details onto the NZ Topo Map (http://www.nztopomap.co.nz), and the mean altitude and altitude range for each species calculated. The altitude of the local treeline relative to each record was assessed using the NZ Topo Map, and the relationship between the collection records and the local treeline recorded.

We tested whether altitude or position relative to the treeline better predicted wing reduction, while accounting for non-independence due to species' shared evolutionary history. We used a 26-species phylogenetic tree from McCulloch (2017), made ultrametric using the R function 'chronos' with a smoothing parameter of $\lambda = 1$. We then carried out a phylogenetic logistic regression following Ives & Garland (2014), which iteratively estimates the logistic regression parameters and the degree of phylogenetic signal (α) in the response variable. We used as predictors a species' mean altitude and the proportion of its records that were above the local treeline, both centered and standardized to unit variance. These variables were correlated (Spearman's rank correlation $\rho = 0.71$, $p < 0.001$), but not to the extent that they could not be fit simultaneously (variance inflation factor = 2.28). We fitted the phylogenetic logistic regression with wingless phenotype as the binary response variable, using ‘phyloglm’ function in the ‘phylolm’ R package (Ho & Ané 2014). We tested statistical significance of predictors using the Wald-like χ^2 test for significance and carried out 1000 parametric bootstraps to construct confidence intervals around regression coefficients and the measure of phylogenetic signal $a = -\log(\alpha)$ (Ives and Garland 2014).

The proportion of a species’ occurrence records above the treeline significantly predicted wing reduction ($\beta = 2.84$, $Z = 2.40$, $p = 0.016$, bootstrap 95% confidence interval 1.32 - 3.09). With the treeline predictor in the model, mean altitude did not have a significant effect on wing reduction ($\beta = 0.66$, $Z = 0.57$, $p = 0.56$, bootstrap 95% confidence interval -0.72 - 2.75). The point estimate of phylogenetic signal was very large ($a \approx 6.0$, the upper bound set for the analysis), but a was not estimated precisely with a bootstrap 95% confidence interval of -5.71 - 6.0, likely due to the relatively small sample size.

Appendix 3: Results

Table: The total number of collection localities below and above the treeline for each stonefly species. Mean altitude (and altitudal range) are also shown for each species. *Note *Vesicaperla townsendi* is not completely wingless, but rather has rudimentary vestigial wings.

Wing length	Species	Below treeline	Above treeline	Mean altitude (m) [range]
Fully-winged	*Acroperla christinae*	4	1	763 [200-1300]
	Acroperla flavescens	11	0	558 [100-1000]
	Acroperla samueli	15	0	467 [20-1200]
	Acroperla trivacuata	220	0	321 [0-1200]
	Megaleptoperla diminuta	60	0	347 [0-1400]
	Megaleptoperla grandis	131	0	503 [0-1260]
	Taraperla ancilis	21	5	607 [0-1345]
	Taraperla howesi	41	3	724 [100-1620]
	Taraperla pseudocyrene	6	0	1058 [740-1220]
	Zelandoperla agnetis	154	0	417 [0-1330]
	Zelandoperla decorata	180	8	446 [0-1600]
	Zelandoperla denticulata	20	0	267 [0-1020]
	Total	**863**	**17**	
Wingless	*Apteryoperla illiesi*	1	17	1240 [820-1800]
	Apteryoperla lakiula	0	2	675 [650-700]
	Apteryoperla monticola	0	1	1432 [1432-1432]
	Apteryoperla nancyae	0	1	1400 [1400-1400]
	Apteryoperla tillyardi	0	1	1000 [1000-1000]
	Holcoperla angularis	1	14	1330 [820-1830]
	Holcoperla jacksoni	0	8	1484 [1160-1830]
	Holcoperla magna	0	19	1667 [1120-1950]
	Nesoperla patricki	0	3	1000 [680-1600]
	Rakiuraperla nudipes	0	6	420 [200-650]
	Taraperla johnsi	0	2	830 [800-860]
	Vesicaperla celmisia	0	5	1478 [1250-1600]
	Vesicaperla eylesi	0	1	1300 [1300-1300]
	Vesicaperla kuscheli	0	1	1200 [1200-1200]
	Vesicaperla substirpes	0	2	1144 [1067-1220]
	*Vesicaperla townsendi**	0	2	1244 [1188-1300]
	Vesicaperla trilinea	0	1	1550 [1550-1550]
	Total	**2**	**86**	