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Supplementary Information for

Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains

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Other supplementary materials for this manuscript include the following:

Datasets S1 to S3

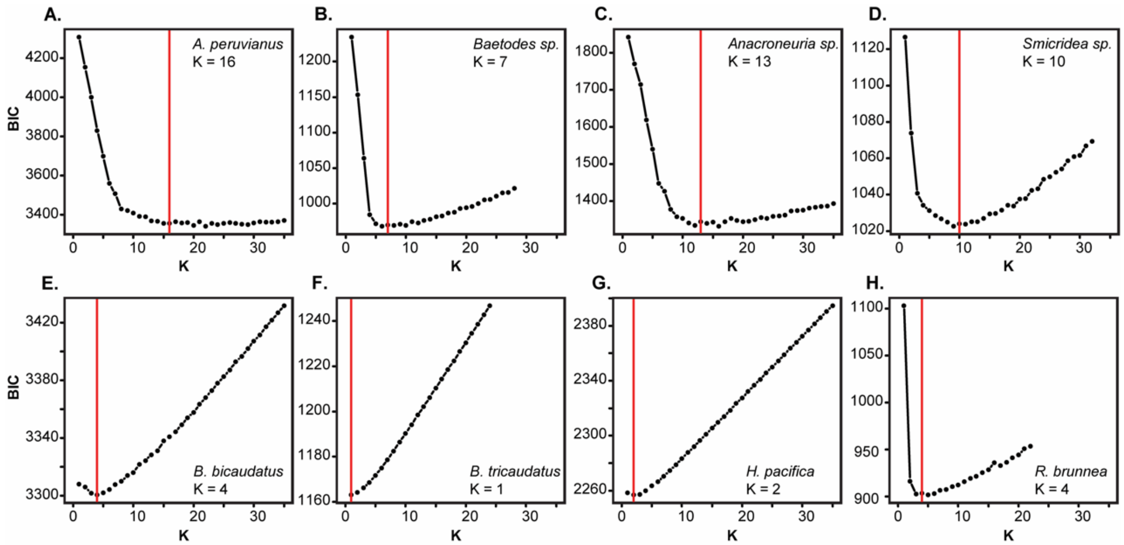

Fig. S1

Plots of Bayesian information content (BIC) for each proposed number of population subclusters (K) for each MTU from Ecuador (**A-D**) and Colorado (**E-H**). Best values of K (indicated by red lines) were consistently higher in tropical MTUs, indicating greater population structure and divergence in tropical montane habitats.

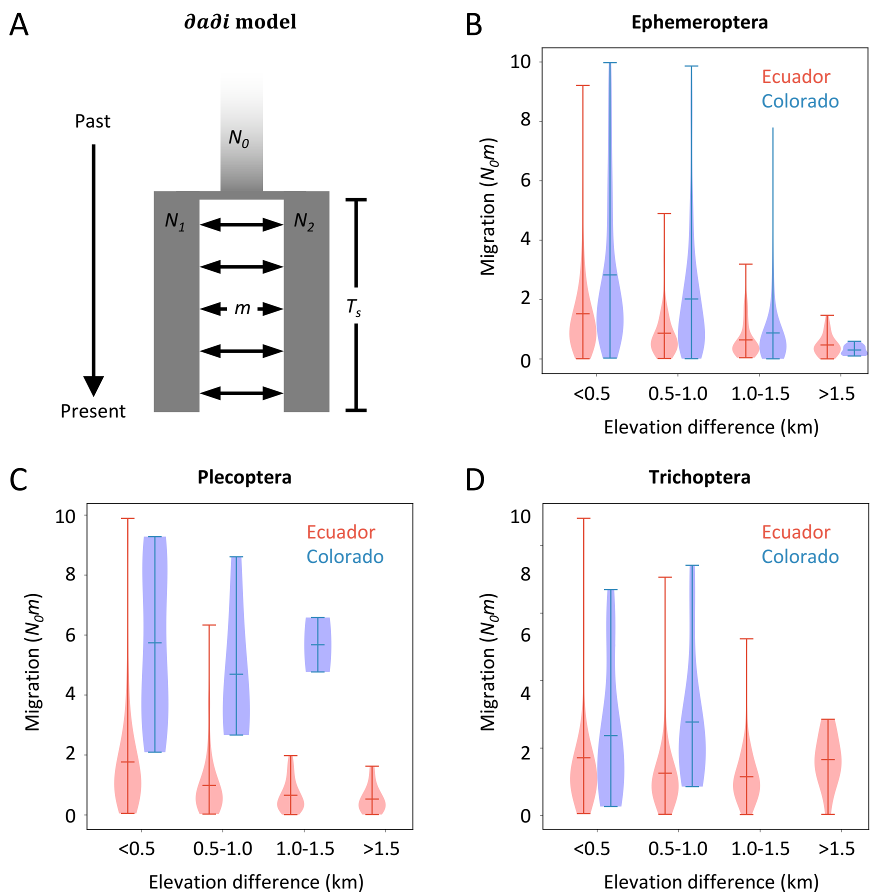

Fig. S2

(**A**) The demographic model used for all *∂a∂i* analyses for estimating effective numbers of migrants per generation between population pairs for eight focal MTUs in Ecuador and Colorado. In this model the ancestral population of constant effective size N_0 splits at time T_s into two subpopulations of constant effective size N_1 and N_2, linked by constant symmetric migration occurring at rate m after the split. (**B-D**) Effective migration rates between population pairs as a function of elevation difference between the populations, shown separately for MTUs within each of the three clades. Migration rates were consistently higher among temperate populations compared to tropical ones.

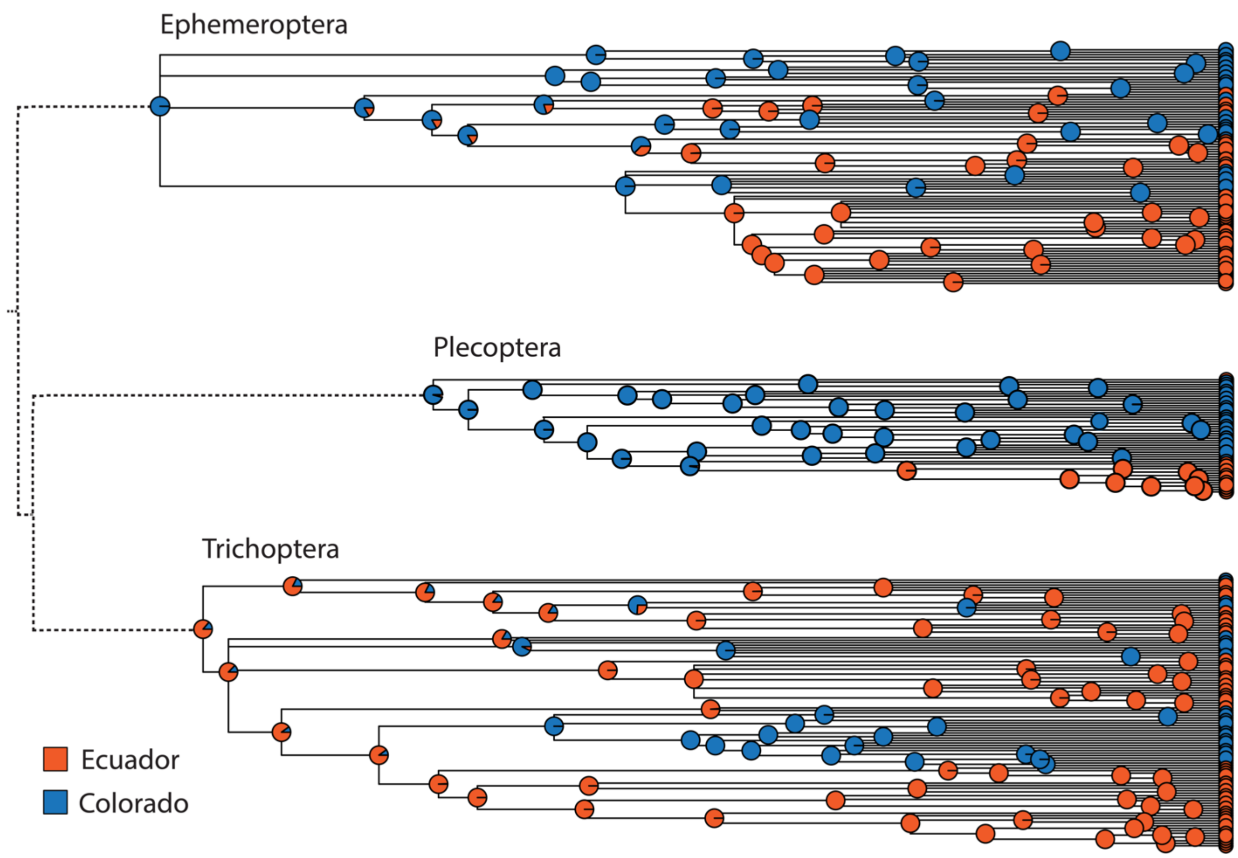

Fig. S3

Stochastic character mapping of latitude estimated independently for barcoded species in each clade. We determined the best-fit model for each character transition matrix using a likelihood ratio test and then estimated 10,000 stochastic character maps for each major clade. To summarize mappings, pie charts on internal nodes indicate posterior probabilities for historical locations of ancestors for all barcoded species from Ecuador and Colorado. Dotted lines connecting major clades show accepted relationships based on the aquatic insect systematics literature.

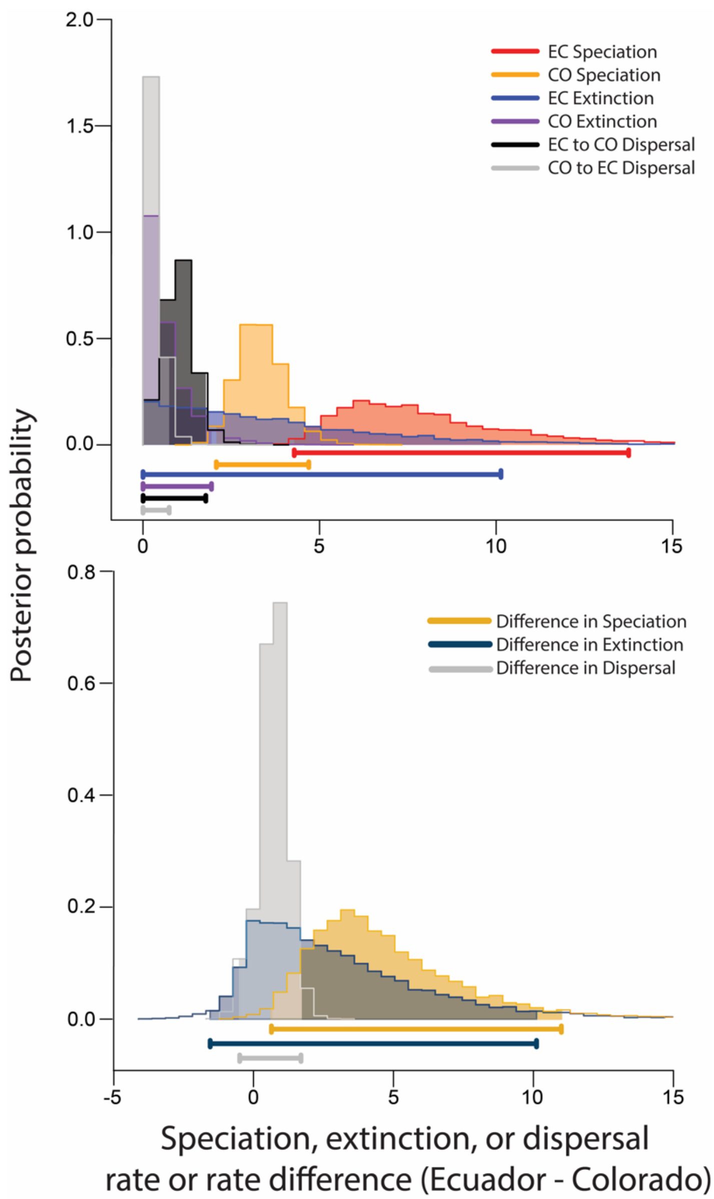

Fig. S4

Posterior probability densities for full BiSSE model parameters and latitudinal difference in parameters (Ecuador - Colorado) estimated based on 10,000 generations of Bayesian MCMC, for all barcoded species analyzed together. Line plots paralleling x-axis indicate 95% credibility intervals for parameter estimates and latitudinal differences in parameter estimates.

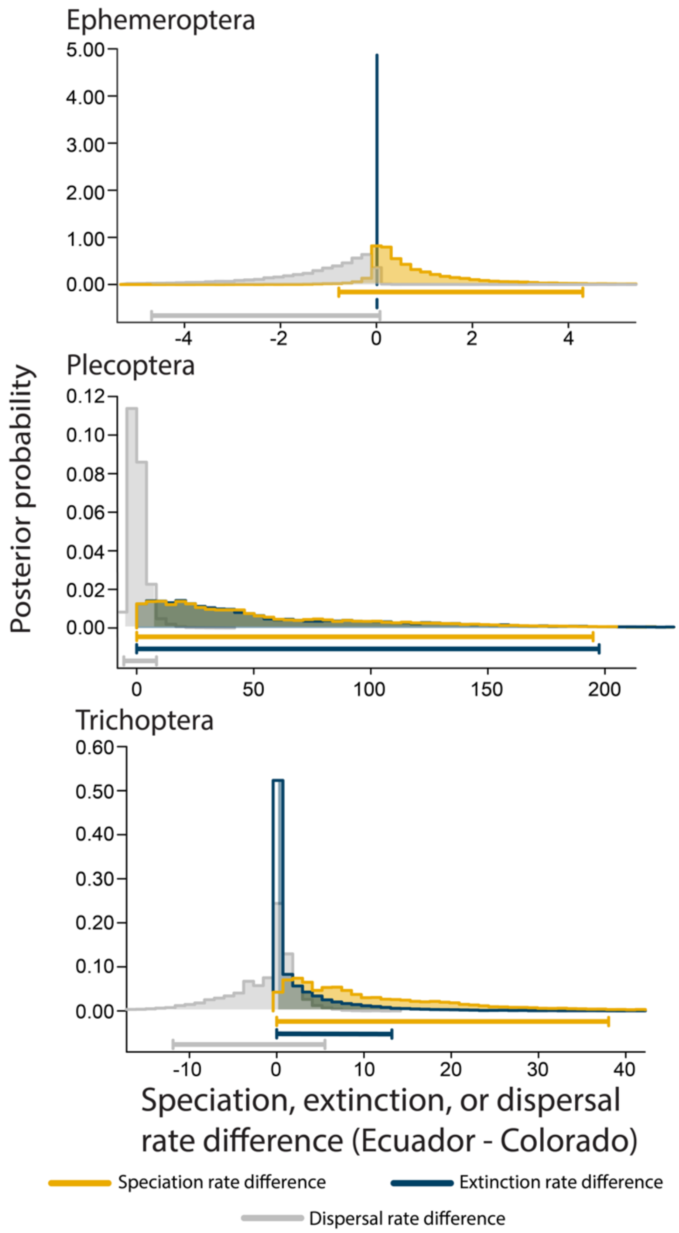

Fig. S5

Posterior probability densities for latitudinal difference in full BISSE model parameters estimated based on the maximum-likelihood fits of 20,000 post burn-in trees, independently for barcoded species in each major clade. Line plots paralleling x-axis indicate 95% credibility intervals for latitudinal differences in parameter estimates.

Table S1. Critical thermal maximum, minimum, and acclimation temperatures for barcoded species in three focal clades (Ephemeroptera, Plecoptera, Trichoptera) of aquatic insects sampled along temperate and tropical elevational gradients.

LATITUDE	ELEVATION (m)	ELEVATION CLASS	ORDER	GENUS	SPECIES	ACCLIMATION TEMP. (°C)	AVG. CT_{MAX} (°C)	AVG. CT_{MIN} (°C)
Colorado	1992	Low	Ephemeroptera	*Baetis*	*flavistriga*	14	34.1	0.2
Colorado	2212	Low	Ephemeroptera	*Diphetor*	*hageni*	14	33.0	0.4
Colorado	2212	Low	Ephemeroptera	*Baetis*	*bicaudatus*	14	29.4	0.6
Colorado	2212	Low	Ephemeroptera	*Baetis*	*tricaudatus*	14	29.4	0.4
Colorado	2590	Mid	Ephemeroptera	*Baetis*	*tricaudatus*	10	31.6	0.5
Colorado	2590	Mid	Ephemeroptera	*Baetis*	*tricaudatus B*	10	28.1	0.8
Colorado	2798	Mid	Ephemeroptera	*Amaletus*	*celer*	10	29.1	0.3
Colorado	2798	Mid	Ephemeroptera	*Drunella*	*doddsii*	10	28.2	0.5
Colorado	2798	Mid	Ephemeroptera	*Baetis*	*bicaudatus*	10	27.1	0.3
Colorado	3166	High	Ephemeroptera	*Baetis*	*bicaudatus*	6	29.0	0.5
Colorado	1992	Low	Plecoptera	*Hesperoperla*	*pacifica*	14	33.9	0.3
Colorado	2212	Low	Plecoptera	*Hesperoperla*	*pacifica*	14	31.1	0.6
Colorado	2590	Mid	Plecoptera	*Hesperoperla*	*pacifica*	10	33.2	0.9
Colorado	2798	Mid	Plecoptera	*Megarcys*	*signata B*	10	29.8	0.4
Colorado	2798	Mid	Plecoptera	*Kogotus*	*modestus*	10	28.6	0.4
Colorado	3166	High	Plecoptera	*Kogotus*	*modestus*	6	27.7	0.4
Colorado	3166	High	Trichoptera	*Rhyacophila*	*hyalinata*	6	25.1	0.5
Ecuador	2003	Low	Ephemeroptera	*Baetodes*	*sp. E*	14	22.0	6.6
Ecuador	2003	Low	Ephemeroptera	*Leptohyphes*	*sp. G*	14	22.2	5.7
Ecuador	2003	Low	Ephemeroptera	*Baetodes*	*sp. M*	14	19.6	7.8

Ecuador	2694	Mid	Ephemeroptera	*Andesiops*	*sp. A*	10	25.4	2.9
Ecuador	2694	Mid	Ephemeroptera	*Andesiops*	*sp. C*	10	24.6	2.5
Ecuador	2694	Mid	Ephemeroptera	*Andesiops*	*sp. D*	10	26.3	2.7
Ecuador	2694	Mid	Ephemeroptera	*Andesiops*	*sp. G*	10	26.7	2.9
Ecuador	2694	Mid	Ephemeroptera	*Baetodes*	*sp. H*	10	26.2	3.4
Ecuador	2694	Mid	Ephemeroptera	*Prebaetodes*	*sp. B*	10	24.2	3.1
Ecuador	2694	Mid	Ephemeroptera	*Myobaetis*	*sp. D*	10	26.9	2.7
Ecuador	2694	Mid	Ephemeroptera	*Baetodes*	*inconclusive*	10	22.7	3.7
Ecuador	3387	High	Ephemeroptera	*Andesiops*	*sp. A*	6	25.7	0.1
Ecuador	3387	High	Ephemeroptera	*Andesiops*	*sp. C*	6	28.6	-0.2
Ecuador	3387	High	Ephemeroptera	*Andesiops*	*sp. E*	6	28.7	0
Ecuador	1845	Low	Plecoptera	*Anacroneuria*	*guambiana B*	14	28.6	4.6
Ecuador	1845	Low	Plecoptera	*Anacroneuria*	*rawlinsi*	14	30.3	4.7
Ecuador	2003	Low	Plecoptera	*Anacroneuria*	*complex B*	14	28.8	4.6
Ecuador	2003	Low	Plecoptera	*Anacroneuria*	*guambiana C*	14	29.0	4.5
Ecuador	2003	Low	Plecoptera	*Anacroneuria*	*sp. D*	14	29.4	4.7
Ecuador	2694	Mid	Plecoptera	*Anacroneuria*	*cajas A*	10	27.3	1.1
Ecuador	2694	Mid	Plecoptera	*Anacroneuria*	*guambiana A*	10	27.5	1.1
Ecuador	2003	Low	Trichoptera	*Leptonema*	*sp. B*	14	26.5	5.5
Ecuador	2694	Mid	Trichoptera	*Leptonema*	*sp. D*	10	26.7	2.8

Table S2. Phylogenetically paired MTUs within three focal clades (Ephemeroptera [E], Plecoptera [P], Trichoptera [T]) of aquatic insects included in genomic comparisons across latitudes. Sample sizes, ddRAD sequencing results, and final loci and individuals retained after bioinformatic filtering for each MTU.

	Taxon (MTU)	Order	N inds	Mean coverage depth	Total N of merged stacks	N loci MAF >0.01	N loci MAF >0.05	% inds retained	N inds retained	N loci retained
Colorado	*Baetis bicaudatus*	E	606	71	7,026	10,058	7,767	99%	603	3,100
	Baetis tricaudatus	E	316	62	5,354	9,141	7,098	77%	245	1,272
	Hesperoperla pacifica	P	404	39	15,717	17,436	13,520	95%	383	4,544
	Rhyacophila brunnea	T	399	46	6,175	8,847	7,366	56%	222	870
Ecuador	*Andesiops peruvianus*	E	990	64	5,447	8,450	6,963	88%	877	1,378
	Baetodes sp	E	401	66	7,164	7,801	6,461	68%	275	848
	Anacroneuria sp	P	598	30	14,104	23,977	21,260	65%	391	1,021
	Smicridea sp	T	614	23	16,027	9,748	7,891	52%	319	419

Table S3.

Linear mixed effects model results using genetic distance (Dc) as the response variable, and geographic distance and elevation difference as predictors. Taxonomic membership (Ephemeroptera, Plecoptera, Trichoptera) was nested within latitude as a random effect. Geographic distance and/or latitude were significant predictors of genetic differences, and the significant interactions between latitude and both geographic distance and elevation difference showed that temperate and tropical populations do not respond equally to elevation differences in terms of genetic differentiation.

		Estimate	Std.Error	t-value	p-value
All Taxa	(Intercept)	0.219	0.011	20.255	**<0.001**
	Geographic Dist.	0.001	0.000	2.542	**0.011**
	Latitude	0.099	0.011	9.331	**<0.001**
	Elevation Diff.	-0.000	0.000	-0.731	0.465
	Geo. Dist. x Elevation Diff.	-0.001	0.001	-2.511	**0.012**
	Latitude x Elev. Diff.	0.000	0.000	5.762	**<0.001**

Dataset S1 (excel file)
Site information for stream temperature measurements, physiology, landscape genomics, and regional diversity surveys. An "X" indicates that data for particular project component were collected at a site.

Dataset S2 (excel file)
Taxonomic identifications by latitude and sampling site determined using morphology (Morphological Taxonomic Units; MTUs) and DNA barcoding (Barcode Species). Taxa lists for both types of classifications are provided. These records, and their associated Barcode Index Numbers (BINs), are from the Barcode of Life Database (http://www.boldsystems.org) dataset “Thermal tolerance and dispersal drive diversification” (DS-TTADDD; dx.doi.org/10.5883/DS-TTADDD).

Dataset S3 (excel file)
Documentation of information used to determine constraints for supertree topology for analysis of diversification. Node reference numbers refer to subsequent tabs within dataset with figures describing constrained nodes within supertree (organized by order). Citations detail sources of phylogenetic information. Data describes the type of information with relevant figure numbers, nodal support type, and nodal support values from the aquatic insect systematics literature.