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Cryptic species diversity reveals biogeographic support for the 'mountain passes are higher in the tropics' hypothesis

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The 'mountain passes are higher in the tropics' (MPHT) hypothesis posits that reduced climate variability at low latitudes should select for narrower thermal tolerances, lower dispersal and smaller elevational ranges compared with higher latitudes. These latitudinal differences could increase species richness at low latitudes, but that increase may be largely cryptic, because physiological and dispersal traits isolating populations might not correspond to morphological differences. Yet previous tests of the MPHT hypothesis have not addressed cryptic diversity. We use integrative taxonomy, combining morphology (6136 specimens) and DNA barcoding (1832 specimens) to compare the species richness, cryptic diversity and elevational ranges of mayflies (Ephemeroptera) in the Rocky Mountains (Colorado; approx. 40°N) and the Andes (Ecuador; approx. 0°). We find higher species richness and smaller elevational ranges in Ecuador than Colorado, but only after quantifying and accounting for cryptic diversity. The opposite pattern is found when comparing diversity based on morphology alone, underscoring the importance of uncovering cryptic species to understand global biodiversity patterns.

1. Background

Understanding patterns of diversity and distributions of species is a cornerstone of ecology. Recently, ecologists have recognized the importance of identifying cryptic species in studies ranging from assessing the dynamics of interspecific interactions [1] to accurately predicting biodiversity losses from climate change [2]. Cryptic species are taxa that are morphologically indistinguishable and consequently often incorrectly considered as a single nominal species when in fact constituent taxa are genetically divergent and reproductively isolated from each other [3]. While the identification of cryptic species is crucial for understanding global biodiversity patterns [3], cryptic species are seldom addressed in studies of large-scale trends in species richness and ranges. Failure to distinguish cryptic species underestimates species richness and distorts our perception of trends in diversity [4–6]. Additionally, pooling together cryptic species with distinct characteristics can obscure our understanding of each taxon's individual niche and function [1,7–12]. These problems are further exacerbated by our incomplete understanding of the geographical distribution

of cryptic diversity [3], which is probably non-random and thus a potential bias for inferences of species diversity and distributions at large scales. Here, we provide evidence that quantifying and accounting for cryptic diversity illuminates biodiversity patterns driven by latitudinal differences in climate variability that would otherwise be missed.

The 'climate variability' hypothesis (CVH) [13,14] posits that the breadth of a species's thermal tolerance and geographical range size should be proportional to the degree of climate variability it has experienced over evolutionary time. Climate (temperature) variability generally increases with latitude [13,15–18], resulting in temperate organisms experiencing broader temperature ranges than tropical species due to pronounced seasonality. The CVH thus predicts selection for broad thermal tolerances of temperate species and narrower thermal tolerances of tropical species. Narrow thermal tolerance will in turn select against dispersal into inhospitable climates, resulting in smaller geographical ranges [13,14,19,20].

Janzen [19] extended the CVH to elevational gradients by proposing the 'mountain passes are higher in the tropics' (MPHT) hypothesis as a mechanism to explain high species turnover and smaller elevational ranges at lower latitudes. The MPHT hypothesis proposes that mountains are more effective physiological barriers for tropical than temperate species, because along elevational transects, the annual thermal regimes of sites in the tropics have less overlap than those in the temperate zone [19]. Consequently, the broad thermal tolerances of temperate-zone species should allow them to disperse more broadly across elevational gradients, whereas narrow thermal tolerances of tropical species should restrict their distributions to relatively narrow elevational bands [19,20].

By extension, the MPHT hypothesis provides a mechanistic explanation for latitudinal differences in species richness [20]. The MPHT predicts that across tropical elevational gradients, populations with narrow thermal tolerances and limited dispersal ability will have increased isolation, genetic divergence and speciation, ultimately leading to higher species richness at low latitudes. Moreover, divergence in the traits functioning as the isolating mechanism proposed by the MPHT hypothesis (i.e. narrow thermal tolerance and low dispersal ability) may not necessarily be correlated with obvious morphological differences among species at different elevational zones. Consequently, in the tropics, we would expect not only more species, but also more morphologically similar, cryptic species.

The MPHT hypothesis provides a rich framework with which hypotheses about latitudinal differences in species richness, levels of cryptic diversity and elevational ranges are tested [20]. To date, several studies have supported predictions from the MPHT hypothesis [20]. For example, many groups of organisms show increases in species richness from the poles towards the equator (the latitudinal diversity gradient) [21–26]; across elevations, faunal similarity of communities increases with latitude [27], tropical faunas generally have high levels of cryptic species diversity [1,4,5], tropical species often display small elevational ranges [28] and the elevational ranges of many species increase with latitude [29]. By contrast, findings from several other studies appear to contradict the predictions from the MPHT hypothesis. For example, exceptions to the latitudinal diversity gradient exist [30]; the majority of cryptic species found to date are temperate [3], and some taxonomic groups do not show differences in the elevational ranges of tropical and temperate species [31,32].

Equivocal support for predictions of the MPHT hypothesis could arise from several important limitations that potentially affect the conclusions of previous studies. First, most cross-latitude comparisons of species richness and elevational ranges lack comparable taxonomic resolution across latitudes and do not address cryptic diversity. Tropical and temperate sites vary significantly not only in terms of diversity, but also in taxonomic resolution, with less explored and highly diverse tropical biota potentially including high levels of cryptic diversity [1,4,5]. Second, all previous studies comparing latitudinal changes in elevational ranges were based on museum collection records, published literature, regional field guides, surveys and/or online distributional databases [29,31,32]. Such measures of species' elevational ranges can provide biased estimates if species occupy different elevations in different parts of their range or through time [32]. Third, when data are combined from studies using different sampling designs, any associated sampling biases can only be corrected for post hoc. Last, previous studies [29,31,32] have not used comparative phylogenetic approaches to distinguish effects of shared phylogenetic history on elevational range size values. The ideal test of patterns predicted by the MPHT hypothesis would use consistent criteria for species delimitation, identify cryptic species *a priori*, estimate ranges empirically using standardized sampling methods and control for phylogeny.

Here, we test the MPHT hypothesis by comparing species richness, levels of cryptic diversity and elevational ranges of stream-dwelling mayflies (Ephemeroptera) from north temperate (the Rocky Mountains, Colorado, USA) and tropical (the Andes, Napo, Ecuador; figure 1) latitudes. Using standardized methods, we conducted a broad-scale sampling effort along multiple large elevational transects (approx. 2000 m) in both Colorado and Ecuador. Because many tropical mayfly species remain undescribed [33–35] and have not previously been represented with species-level taxonomy in cross-latitudinal comparisons, we used an integrative taxonomic approach, combining morphological taxonomy and DNA barcoding [36], to delimit species and detect cryptic diversity. We then used a comparative phylogenetic approach to control for phylogenetic signal while testing for a relationship between elevational ranges and latitude. Our study extends the MPHT hypothesis by linking latitudinal differences in climate variability to cryptic diversity and finding support for several key predictions of the MPHT hypothesis, thus demonstrating the importance of accounting for cryptic diversity to make correct inferences about global biodiversity patterns.

2. Material and methods

(a) Study area and collection

In the Colorado Rockies, we established elevational transects in three distinct watersheds all draining into the South Platte River and spanning an elevational gradient from 1556 to 3478 m.a.s.l. (1922 m total). In the Andes in Ecuador, we established two transects in watersheds draining into the Napo River and spanning an elevational gradient from 1664 to 4248 m.a.s.l. (2584 m total). Within each transect, we selected

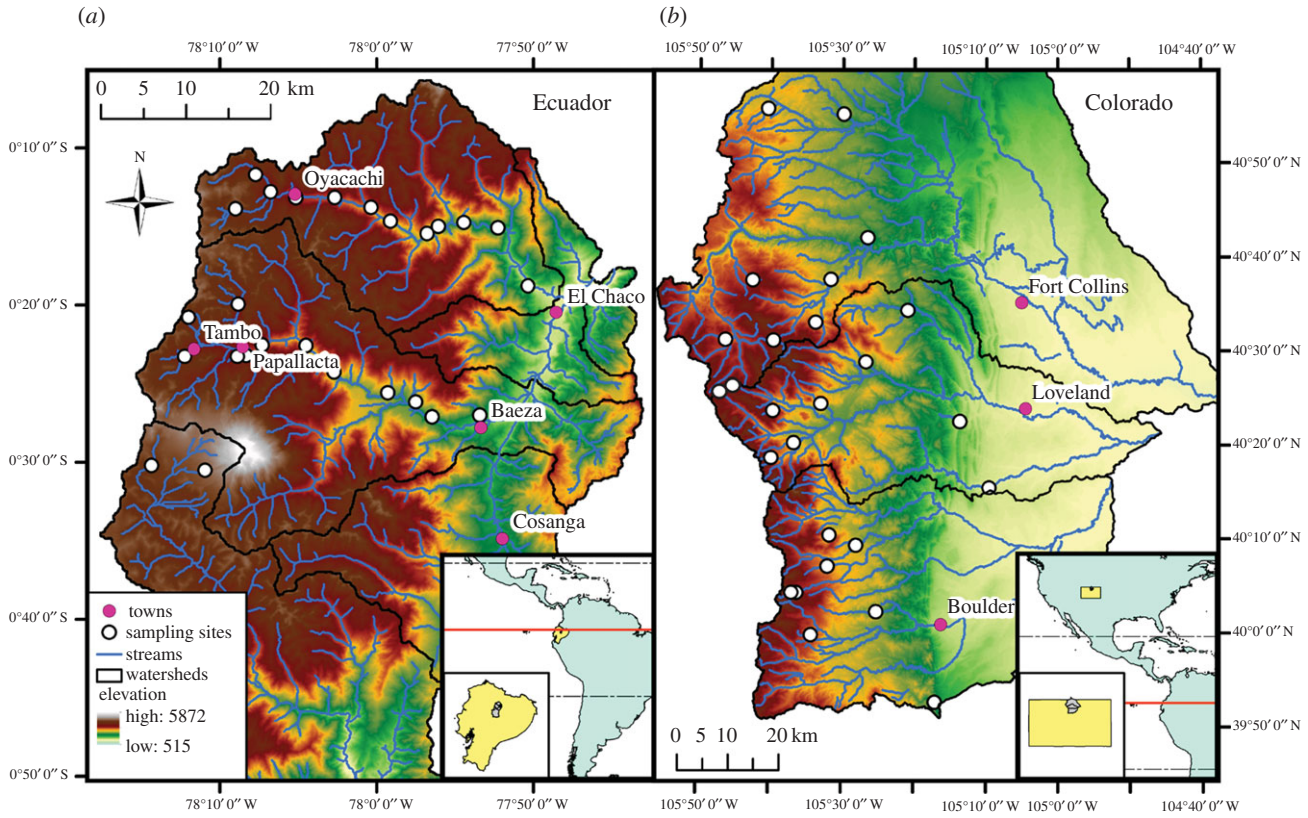

Figure 1. Site maps for Ecuador (*a*; approx. 0°) and Colorado (*b*; approx. 40°N). Sites were selected every 200 m of elevational gain starting at 1500 m.a.s.l. Smallest insets show positions of study area within country of Ecuador or state of Colorado. Larger insets show latitudinal position of study areas relative to equator (red line).

distinct wadeable tributaries to the river main stem every 200 m of elevational gain for sampling.

In Colorado, we sampled a total of 26 sites from 27 June to 10 August 2011. The following year in Ecuador, we sampled 26 sites from 17 January to 25 February 2012. At each site, we collected mayfly immatures using a standard D-frame kick-net (500-µm mesh) for approximately 2 h within a 100 m reach of the stream. We emptied the contents of kick-nets into pans and sorted specimens of numerically abundant taxa in the field to the lowest taxonomic level possible. On the same date at each site, we collected adult mayfly specimens from the riparian area using an aerial net and a beating sheet until no new taxa were found. We preserved all specimens immediately in ≥95% ethanol, which was replaced with fresh ethanol within 24 h [37].

(b) Morphological and molecular species identification

We identified 6136 specimens morphologically (Colorado: 4035; Ecuador: 2101) to the lowest taxonomic level possible, morphological taxonomic units (MTUs), using available literature [34,38–40]. Fewer specimens were examined in Ecuador than Colorado not because of differences in sampling effort, but rather because mayfly densities were naturally lower in Ecuador than Colorado, a potential consequence of latitudinal differences in resource availability [41], the relative strengths of direct [42–44], and indirect interspecific interactions [45] and/or predictability of the hydrologic regime [43,46]. The level of taxonomic resolution achieved for MTUs depended on latitude, life stage and sex, and collections were consequently classified at various taxonomic levels. Expertly identified material was available for comparison for Colorado taxa at the C. P. Gillette Museum of Arthropod Diversity, Colorado State University. For taxa from Ecuador, a regional collection of immatures was available for comparison at the Aquatic Ecology Laboratory at the Universidad San Francisco de Quito.

To establish species-level taxonomic units from MTUs, we used DNA barcoding [36]. For each MTU defined at each site, we DNA barcoded up to 10 specimens, if available. For Colorado, we used five previously published DNA barcodes per MTU per site if available from Gill *et al*. [6] and when possible sequenced five additional specimens for this analysis (1188 specimens total). For Ecuador, we DNA barcoded 10 individuals per MTU per site if available (644 specimens total). We used standard high-throughput DNA barcoding protocols from the Canadian Center for DNA Barcoding [47–49]. Primers are listed in electronic supplementary material, appendix S1. DNA barcode sequences were trimmed, assembled and checked manually in Sequencher v. 5.3 (Gene Codes, Ann Arbor, MI, USA). Sequences were uploaded to the Barcode of Life Database (BOLD) [50] and made publicly available in the dataset 'Richness and Elevational Ranges of Mayflies' (DS-RERM; doi:dx.doi.org/10.5883/DS-RERM). Sequences were automatically screened by BOLD for common contaminants and stop codons. Refined single linkage (RESL) clustering [51] was used to assign sequences to barcode index numbers (BINs), taxonomic units putatively equivalent to biological species. We examined concordance between MTUs and BINs and re-examined specimens as necessary in instances of conflict. All taxonomic and locality information for both MTU and BIN designations is provided in electronic supplementary material, appendix S2. We used BIN identifications as preliminary units for phylogenetic analysis.

(c) Phylogenetic analyses

(i) Parsimony

We aligned all available DNA barcode sequences representing each BIN from each family independently of the others in

MAFFT v. 7 [52] using strategy G-INS-i with offset value 0.1 and all other options set as default. For each alignment, we included one sequence from each genus from all other mayfly families included in our study as outgroups. We examined alignments for potentially erroneous base calls, gaps and unusually divergent sequences. In cases of potentially erroneous base calls and gaps, we re-examined the original sequencing chromatograms. All alignments are available from the Colorado State University Digital Repository (CSUDR) (http://hdl.handle.net/10217/170247).

We conducted equally weighted parsimony tree searches using each data matrix. Up to 50 trees were held within each of 10 000 random-addition-sequence (RAS) tree-bisection-reconnection (TBR) searches that also implemented 100 ratchet iterations [53], which alternated between equal character weighting and each character having a 10% chance of being upweighted and a 5% chance of being downweighted. A strict consensus tree was then calculated by using TBR-collapsing [54]. Parsimony jackknife [55] analyses were conducted using TNT v. 1.1 [56] with the removal probability set to approximately e^{-1} (0.37). One thousand jackknife pseudoreplicates were performed using the same search strategy, albeit with only 100 RAS + TBR + ratchet searches per pseudoreplicate. Jackknife values were mapped onto the strict consensus trees using SumTrees v. 3.3.1 [57], after which the trees were examined and printed using TreeGraph v. 2.0.54–364 [58].

We reduced well-supported tip clades (≥63% bootstrap [59] or jackknife [55]; support achieved by one uncontradicted synapomorphy) down to a single terminal per species. In many cases, saturation prevented robust resolution of higher-level relationships among the species in our study. Consequently, we constrained higher-level relationships in our tree using previous studies of mayfly systematics. We used decision rules modified from [60] listed in electronic supplementary material, appendix S1 to prioritize which studies to use when multiple sources of information about species relationships were available. Detailed documentation of nodal support based on our phylogenetic analysis of COI and constraints from the literature is provided in electronic supplementary material, appendix S3 and figure S1.

(ii) Bayesian inference

We randomly choose one DNA barcode sequence from among the set of longest sequences for each BIN (supported by our parsimony analysis) and one specimen of *Anacroneuria* Klapálek (Plecoptera) for an outgroup. We aligned these sequences in MAFFT v. 7 [52] using the same settings as described above (alignments available from CSUDR: http://hdl.handle.net/10217/170247). We determined that the HKY + G was the appropriate model of nucleotide substitution using jModelTest2 [61,62] based on the Akiake information criterion. We ran four simultaneous analyses in parallel [63] with four chains for 50 000 000 generations in MrBayes v. 3.2 [64,65] through the CIPRES Science Gateway [66]. We ran this analysis with and without constraints from the literature (described in electronic supplementary material, appendix S3 and figure S1). We ensured that our four simultaneous independent runs converged and reached stationarity by checking that the average standard deviation of split frequencies was less than 0.01, that effective sample sizes for parameters were more than 200, and by plotting the −ln likelihood scores against generation time in Tracer v. 1.6 [67]. We discarded the first 12 500 000 trees (25%) as burn-in and used the remaining trees to construct 50 per cent majority rule consensus trees.

(d) Determination of elevational ranges

For each species supported by our phylogenetic analysis, elevational ranges were interpolated between the highest and lowest elevation collection localities. Species found at only one locality were assigned a value of zero. The elevational ranges of Ecuadorian species were truncated at an upper limit of 3500 m.a.s.l., allowing us to compare the same elevational interval from approximately 1500–3500 m in both Colorado and Ecuador [29]. Species found only above 3500 m.a.s.l. were excluded from further analyses. Because this truncated sampling range could artificially reduce estimates of tropical ranges, we ran analyses with and without truncating Ecuadorian elevational ranges and removing taxa found over 3500 m.a.s.l. Our results were qualitatively the same with and without truncation and the removal of high elevation taxa (for a summary of non-truncated results, see electronic supplementary material, table S1).

(e) Trees for comparative phylogenetic analysis

We trimmed our parsimony supertree to include only species found within the desired 1500–3500 m.a.s.l. elevational interval. Because the parsimony supertree had numerous soft polytomies and no branch lengths, we randomly resolved polytomies 1 000 000 times resulting in 1 000 000 alternative topologies. Arbitrary branch lengths were assigned to each tree using one of two common methods. For half of the alternative topologies (500 000), we set all branch lengths equal to one. For the remaining alternative topologies (500 000), branch lengths were set according to Grafen's [68] method where lengths are set equal to the number of descendant tips minus one.

We trimmed our Bayesian 50% majority rule consensus trees to include only species found within the desired 1500–3500 m.a.s.l. elevational interval. We left polytomies unresolved. We used estimates of relative divergence for branch lengths. All trees are available from CSUDR (http://hdl.handle.net/10217/170247).

(f) Comparative phylogenetic analysis

We used PGLS [68] fitted with an Ornstein–Uhlenbeck (OU) model [69,70] of trait evolution to control for phylogenetic signal while comparing the elevational ranges of species from Colorado and Ecuador using the gls function in the R [71] package nlme [72]. We used maximum-likelihood estimation to determine the appropriate value of the model parameter α. We dummy coded the explanatory variable latitude '0' for Ecuador and '1' for Colorado, and the response variable was elevational range (m). Consequently, significantly positive regression slopes would support the one-sided hypothesis that Colorado species have larger ranges than Ecuador species. For non-ultrametric trees, variance heterogeneity was modelled with the option 'weights' in gls [73]. To summarize regression results (available from CSUDR: http://hdl.handle.net/10217/170247), we provide regression parameter estimates and summary statistics for one-sided hypothesis tests in table 1. For parsimony trees, we model averaged [74] regression parameters (slope, intercept and standard errors).

3. Results

(a) Latitudinal differences in species richness

The use of integrative taxonomy, combining morphological study, DNA barcoding and phylogenetic analyses of DNA barcodes, revealed higher species richness in Ecuador than Colorado streams (figure 2*a*). However, based on morphology alone, we identified more MTUs in Colorado than Ecuador, a disparity caused by high levels of cryptic tropical diversity. DNA barcoding and subsequent phylogenetic analyses of DNA barcodes for a subset of morphologically

Table 1. Summary of results from phylogenetic generalized least-squares regression (PGLS) fitted with an Ornstein–Uhlenbeck model of trait evolution (maximum-likelihood used to determine α). PGLS was used to control for shared evolutionary history while comparing elevational range breadths across latitude. The explanatory variable 'latitude' was dummy coded '0' for Ecuador and '1' for Colorado, and the response variable was elevational range breadth (m). Consequently, significantly positive regression slopes support the one-sided hypothesis that Colorado species have larger elevational ranges than species from Ecuador. For parsimony trees, regression parameters (slope, intercept, standard errors) are model averaged, and values of α and hypothesis tests are presented as medians. Constraints on phylogenies are detailed in electronic supplementary material, appendix S3 and figure S1.

phylogenetic method	constraints	branch lengths	slope (±s.e.m.)	intercept (±s.e.m.)	α (median)	one-sided p CO > EC (median)	$p < 0.05$ (%)	$p < 0.10$ (%)
parsimony	Y	equal	195 (±82)	329 (±54)	(8.39)	(0.008)	99.99	100.00
parsimony	Y	Grafen's	191 (±84)	333 (±56)	(112.10)	(0.010)	99.72	99.99
Bayesian	Y	relative divergence	179	326	17.93	0.018	100.00	100.00
Bayesian	N	relative divergence	179	327	20.42	0.018	100.00	100.00

identified specimens from each MTU and locality resulted in the recognition of 95 distinct species (Ecuador: 54; Colorado: 41), an increase in the number of taxa identified in both locations, but a disproportionate increase in Ecuador (350%) compared with Colorado (46%) (figure 2*a*; MTUs versus species).

(b) Latitudinal comparison of elevational ranges

We found strong support for larger elevational ranges in Colorado mayfly species compared with related species from Ecuador, a pattern only apparent after delimiting species and elevational ranges using DNA barcoding (figure 2*b*). Using almost all (more than 99%) parsimony supertree topologies and both constrained and unconstrained Bayesian trees, we consistently found that Colorado elevational ranges were significantly larger (approx. 200 m wider; approx. 61% greater) than those of species from Ecuador (table 1). In most cases, we found limited evidence for phylogenetic signal in elevational range size estimates (table 1 and figure 2*c*).

4. Discussion

The MPHT hypothesis predicts that reduced climate variability in the tropics will result in reduced dispersal and smaller ranges across elevational gradients than in the temperate zone [19,20]. If true, substantial elevational climatic zonation in tropical mountain systems should lead to increased opportunities for speciation and an accumulation of species at low latitudes [20], many of which may be cryptic. Using integrative taxonomy to delimit species and identify cryptic diversity, we found higher mayfly species richness in Ecuador than in Colorado. After controlling for phylogeny, we also found strong evidence that the elevational ranges of mayfly species are smaller in the Andes than in the Rocky Mountains. Collectively, our results indicate that many tropical mayflies previously identified morphologically as taxa with large elevational ranges are in fact collections of different cryptic species with small elevational ranges. Below, we discuss the implications of these results in more detail.

The large increases in the number of mayfly species we detected using integrative taxonomy underscores the need for multipronged approaches in understudied and potentially highly cryptic tropical taxa. To date, large-scale ecological studies of stream insects in the Neotropics have relied primarily on morphological species descriptions and most have limited their analyses to the family or genus levels (e.g. [75]). By applying DNA barcoding methods [36], we standardized our comparisons of diversity and elevational ranges of the mayfly fauna in both Colorado and Ecuador. This approach had two important advantages over identifications based solely on morphology: separating species for which keys do not exist and distinguishing cryptic species. Cryptic species are common in the tropics [1,4,5], and if undetected, pooling of multiple cryptic species will lead to underestimates of species richness and overestimates of species distributions. In our study, overcoming tropical taxonomic limitations using integrative taxonomy allowed for a robust latitudinal comparison of mayfly species richness and distributions.

We found higher mayfly species richness in Ecuador than Colorado (figure 2*a*), supporting the hypothesis that higher levels of elevational climatic zonation along tropical gradients and reduced dispersal of tropical species may promote speciation [20]. Assuming extinction rates are similar across latitude, high rates of tropical speciation should lead to higher species richness at lower latitudes [20]. Mechanistically, this accumulation of species is thought to occur in one of two ways. Populations might become isolated on distinct sides of mountain passes, leading to divergence and eventual speciation (allopatric speciation [20,32]). Alternatively, populations might also adaptively diverge and speciate along a single elevational gradient (parapatric speciation [31,32]). Though a few studies have attempted to draw generalizations about latitudinal differences in modes of speciation [31,32], it seems that both allopatric (e.g. [32]) and parapatric (e.g. [31]) mechanisms operate in the tropics, and that the taxon-specific balance between dispersal and selection [32,76] determines the extent of parapatric speciation along single elevational gradients. Our results provide evidence that high species richness of tropical mayflies may arise not only via allopatric speciation, but also by parapatric speciation along single elevational gradients because limited thermal tolerance restricts the dispersal ability of these species [33,35].

Consistent with the predictions of the MPHT hypothesis, we found that mayflies from Colorado had significantly larger (approx. 200 m wider; approx. 61% greater) elevational ranges than mayflies from Ecuador (figure 2*b* and table 1). Our results are consistent with those observed for many terrestrial vertebrate species, in which tropical taxa had

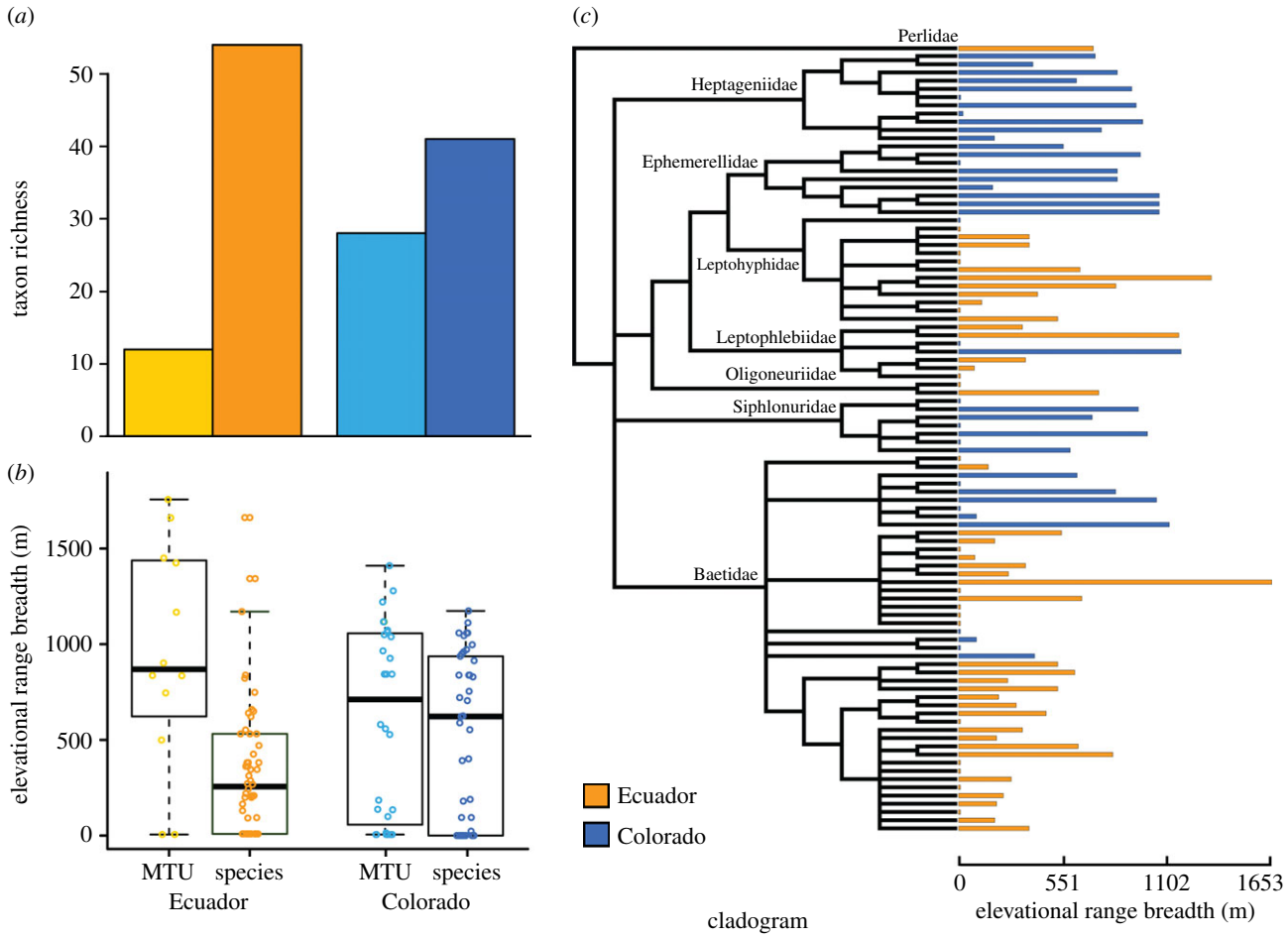

Figure 2. (*a*) Taxon richness by latitude determined for morphological taxonomic units (MTUs) and species defined using integrative taxonomy (Ecuador: MTU $n = 12$, species $n = 54$; Colorado: MTU $n = 28$, species $n = 41$), (*b*) elevational range breadths (m) by latitude determined for MTUs and species and (*c*) cladogram of species relationships (left) with elevational range breadths (right) coloured by latitude.

smaller elevational distributions than temperate species [29]. By contrast, latitudinal differences were not found in two other studies [31,32], which focused more on latitudinal differences in modes of speciation than on comparing elevational ranges. Discrepancies among the aforementioned studies are probably driven by differences in procedures for selection and analysis of elevational range size data. McCain [29] found that many tropical vertebrate species had smaller elevational distributions than related temperate species. In doing so, McCain demonstrated the need to control for strong effects of mountain height, sampling scale (local versus regional) and percentage of height sampled in studies compiling data from multiple published sources. Additionally, none of the aforementioned studies addressed cryptic diversity, and given the high occurrence of cryptic species in the tropics, this limitation is clearly important. Here, we avoided these problems by using standardized protocols across latitude for species delimitation, detection of cryptic diversity and determination of elevational ranges.

While the observed latitudinal differences in elevational ranges provide indirect evidence for some of the predictions of the MPHT hypothesis, the mechanisms responsible for these patterns remain untested. Variation in thermal tolerance is commonly invoked as an explanation for climate-based latitudinal differences in elevational range sizes [19,20,77], but several other mechanisms could also be operating. First, species interactions could modify distributions by elevational niche partitioning [20,29,31,32]. Smaller elevational ranges of tropical species could result from higher levels of tropical interspecific competition [78]. Second, latitudinal differences in rates of growth and development could limit available time for downstream larval drift and upstream adult flight (the recolonization cycle [79]), with shorter-lived and faster-developing tropical species moving less than temperate species [80,81]. More limited dispersal of tropical species would result in smaller tropical elevational ranges. Last, latitudinal differences in mayfly emergence periodicity could limit the dispersal of tropical species. While temperate species generally emerge simultaneously or over short periods (synchronously), tropical mayflies generally emerge year-round (asynchronously [33,35]). Thus, for tropical mayflies, finding a mate at a distant site might be challenging if the timing of emergence among sites is not consistent. This stochasticity could result in selection for philopatry, resulting in smaller tropical elevational ranges.

Consistent with previous surveys [82], we found high within-latitude variability in elevational ranges (three orders of magnitude), even among closely related species (figure 2*c*). Interspecific variation in elevational ranges could result from variation in physiological and dispersal traits, which reflect species-specific differences in levels of genetic variation available for selection to act on and/or genetic constraints on physiological adaptation. Additionally, other ecological factors could act in concert with species-specific physiological and dispersal traits to explain observed within-latitude variation in elevational ranges. Mayflies can occupy multiple trophic

groups including benthic detritivory, suspension feeding, algivory and predation [39]. Consequently, species distributions could be restricted by food availability, which is regulated by an independent suite of factors. Mayfly species also differ significantly in behaviour; the clade includes swimmers, clingers, climbers, burrowers or sprawlers [39], and those modes require specific microhabitats with variable distributions determined by hydro-geomorphic processes. Moreover, some mayfly species may have specific requirements for oviposition sites [83]; consequently, the availability of suitable sites for eggs may influence species distributions. Thus, species-specific differences in traits provide a viable explanation for the observed within-latitude variation in elevational ranges.

In conclusion, using integrative taxonomy, we found higher species richness, higher cryptic diversity and smaller elevational ranges in Ecuador than Colorado, providing strong support for several key predictions of the MPHT hypothesis. Our results implicate climate variability along tropical and temperate elevational gradients as an important selective pressure determining large-scale biogeographic trends for higher cryptic diversity and smaller elevational range sizes in the tropics. Moreover, large increases in the number of species detected and the effects of addressing cryptic diversity on estimates of latitudinal differences in elevational ranges underscore the general importance of uncovering cryptic species in cross-latitudinal and large-scale biogeographic studies.

Data accessibility. DNA barcode sequences are available from the Barcode of Life Database (BOLD) in the project 'Richness and Elevational Ranges of Mayflies' (DS-RERM: doi:dx.doi.org/10.5883/DS-RERM). DNA sequence alignments, phylogenetic trees and PGLS regression results are available from the Colorado State University Digital Repository (CSUDR): http://hdl.handle.net/10217/170247. Some datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. B.C.K., A.C.E., A.S.F., C.K.G., J.M.G., N.L.P., S.A.T., K.R.Z. and W.C.F. designed the study. B.A.G. and K.L.C. conducted the fieldwork. B.A.G. and B.C.K. identified specimens. B.A.G. and D.G.G. performed DNA barcoding. B.A.G. and M.P.S. performed phylogenetic analyses. B.A.G. did comparative phylogenetic analysis. B.A.G. wrote the first draft of the manuscript. All authors contributed substantially to revisions and gave final approval for publication.

Competing interests. The authors declare no competing interests.

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