Ecography 33: 251–259, 2010
doi: 10.1111/j.1600-0587.2010.06306.x

Subject Editor: Catherine Graham. Accepted 15 May 2010

Patterns and timing of diversification in a tropical montane butterfly genus, *Lymanopoda* (Nymphalidae, Satyrinae)

Kayce L. Casner and Tomasz W. Pyrcz

K. L. Casner (kaycelu@gmail.com), Dept of Evolution and Ecology and Center for Population Biology, Univ. of California, Davis, CA 95616, USA. – T. W. Pyrcz, Zoological Museum, Jagiellonian Univ., 30–060 Krakow, Poland.

Species distributions are a product of contemporary and historical forces. Using phylogenetic and geographic data, we explore the timing of and barriers to the diversification of the Andean butterfly genus *Lymanopoda* (Nymphalidae, Satyrinae). Clade and species level diversification is coincident with Andean orogeny and Pleistocene glaciation cycles. *Lymanopoda* has primarily diversified within elevational bands, radiating horizontally throughout the Andes with occasional speciation across elevational boundaries, often associated with ecotones. Narrow elevational ranges and infrequent speciation into adjacent elevational strata suggest that expansion across elevational gradients is relatively difficult. These results are similar to those found in studies of other Andean taxa.

As anthropogenic forces act to alter species' natural distributions, it is more important than ever to understand "why species are where they are". MacArthur (1972) wrote in the first lines of his commanding work, Geographical ecology, "To do science is to search for repeated patterns, not simply accumulate facts and to do the science of geographical ecology is to search for patterns of plant and animal life that can be put on a map". Contemporary patterns in plant and animal distributions are the product of existing abiotic and biotic forces limiting ranges, and a history of adaptation and speciation in response to past forces. Previous studies have documented and tested existing biotic and abiotic pressures associated with species boundaries (Heller 1971, Terborgh 1971, Heller and Poulson 1972, Brown et al. 1996, Holt and Keitt 2005). In this paper, we explore the historical responses of species to physical and ecological boundaries through patterns of speciation in a montane environment.

Environmental gradients are effective backgrounds for studying species distributions and speciation. Abiotic factors impeding range expansion, such as temperature, aridity and salinity, are apparent and easy to measure along gradients. Additionally, distributional patterns are relatively obvious with respect to the environment (Terborgh 1971). Often, changes in abiotic factors along gradients are salient and conspicuously co-vary with biotic richness and composition, making biotic and abiotic barriers more detectable. Over evolutionary time, species' responses to environmental barriers become apparent through range maintenance, range expansion (Kirkpatrick and Barton 1997), or speciation (Schluter 1996).

Mountain ranges are ideal environments for studying speciation and species distributions. They provide high peaks and low valleys, which provide insurmountable topographic barriers that prevent gene flow and facilitate geographic isolation. Intricate topography and clines in environmental variables such as temperature, aridity, and solar insolence provide opportunity for ecological speciation (Chapman 1917, Elias et al. 2009). The South American Andes in particular are considered a "species pump" (Fjeldsa and Lovett 1997) in the Neotropics, but the mechanistic role that mountains might play in contributing to species richness and diversity patterns remains unclear.

Butterflies of the subtribe Pronophilina (Nymphalidae, Satyrinae) are an excellent group to study patterns of diversification. It is the most speciose group of butterflies in the Andean cloud forest with >400 described and 200 undescribed species (Adams 1985, 1986, Pyrcz and Wojtusiak 2002). This species richness is especially impressive in light of the young age of the Andes, which did not reach their present height until 10–6 Ma in the central section (Garzione et al. 2006) and ca 2.7 Ma in the north (Gregory-Wodzicki 2000). Sets of pronophilines are allopatric along the Andes where deep valleys and high passes act as dispersal barriers. Within each part of the Andes, lower-elevation species with broad geographic ranges are replaced by higher-elevation, narrowly distributed congeners. The result is a stair-step distribution up mountain sides with unique species composition in adjacent regions of an extended mountain chain or in nearby cordilleras (Adams 1985, Pyrcz et al. 1999).

This study focuses on the pronophiline genus *Lymanopoda*, which displays the aforementioned distributional traits of the subtribe. The genus is apparently monophyletic as it shows several synapomorphies in the adult morphology: 1) the ocelli in forewing cells Cu1 and Cu2 are displaced basally, 2) the hindwing ventral surface median band is broken and displaced in the discal cell, 3) male genitalia possess a superuncus, 4) the female genitalia possess a sclerotised lamella on the distal part of the posterior apophysis (Pyrcz et al. 1999, Pyrcz 2004). Currently, 66 species are recognized (including 5 undescribed) (Lamas 2004). The species of *Lymanopoda* are exclusively montane and found between 1000–1200 and 3800–4000 m. Two species occur in Central America, and the other 64 species are found in the Andes and its peripheral cordilleras (Sierra Nevada de Santa Marta, Sierra de Turimiquire). The highest species richness occurs at a latitude of 10°S on the eastern slopes of the Andes in central Peru (Pasco) where 14 species occur along an elevation gradient. Species richness decreases gradually north- and southwards reaching its distributional limit at latitude 17°N and 27°S (Fig. 1). The larvae are oligophagous on montane bamboo, primarily of the widespread Andean genus *Chusquea* in the cloud forests (Adams and Bernard 1981) and *Swallenchloa* in the paramo (Pyrcz et al. 1999). Adults exhibit low vagility, usually staying in close proximity to their bamboo host plants.

Here we explore the evolutionary patterns for 40 species of *Lymanopoda* along elevational gradients and a north–south transect in the South American Andes. Specifically, we test two hypotheses revised from Moritz et al. (2000). 1) Diversification was vertical. This hypothesis is similar to the gradient model whereby speciation is based on divergent selection along an elevational gradient. By this scenario, species along an elevational gradient are more closely related to one another than to species at similar elevations in neighboring regions. 2) Diversification was horizontal. This is similar to the ''refuge'' hypothesis proposed by Moritz et al. (2000) whereby speciation is based on allopatry. In this case, closely related species occupy similar elevational bands in adjacent regions. Each hypothesis produces a unique phylogenetic pattern with respect to the geographic ranges of closely related taxa, which we test using a multilocus data set.

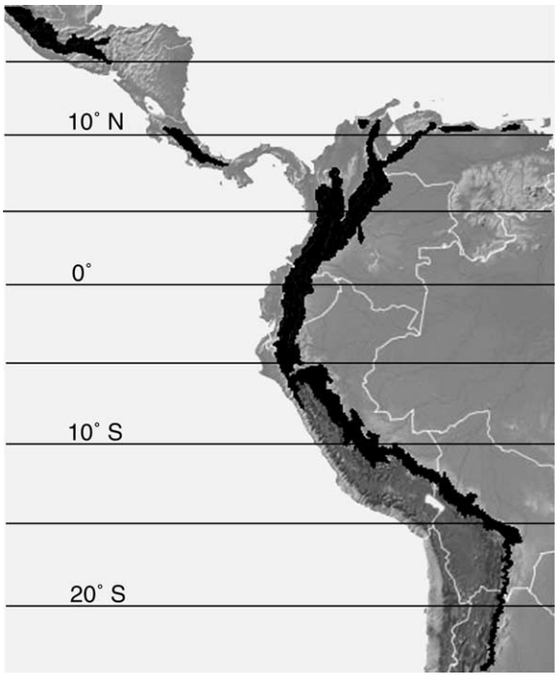

Figure 1. Andean distribution of genus *Lymanopoda.*

Materials and methods

Sample collection

Most of the 228 specimens were collected by the first author between 2006–2008 and second author between 2004–2008. Other specimens were received as donations from museum and personal collections. We collected specimens from ca 75 locations throughout the northern and central Andes (Bolivia, Peru, Ecuador, Colombia, Venezuela) and Costa Rica. Where possible, multiple samples were obtained, although some species, particularly higher elevation species, are rare and only a single individual was collected. Samples of the sister genus *Ianussiusa* (Peña et al. 2006) were collected from its range in Colombia and Ecuador and used as the outgroup.

We determined elevational and latitudinal range limits through widespread sampling between 1991 and 2009. Elevational ranges were established by sampling along altitudinal gradients at 1200–4200 m. When field work at a single locality lasted >5 d, Van Someren-Rydon baited traps (DeVries 1987) were placed every 50 m in elevation and baited with excrement of carnivorous animals. We checked traps and added fresh bait daily (Pyrcz 2004). When <5 d were available to sample a locality, we used standard entomological nets to collect specimens.

To confirm taxonomy of samples, the second author carried out morphological studies of adults at the Museum of Zoology of the Jagiellonian Univ. Samples were also compared to type specimens housed in other major European collections. Systematic arrangement of the genus *Lymanopoda* follows Lamas (2004). Species were defined using the biological species concept in which species recognition is based on morphological traits that facilitate reproductive isolation, primarily genitalic features and differences in wing pattern. Adult Satyrinae communicate visually, and therefore wing color and markings are considered important to intraspecific recognition.

Molecular methods

QIagen's DNEasy extraction kit was used for in-house extractions. DNA was extracted from abdomens for most samples, although in a few cases only legs were available. Purified DNA was resuspended in elution buffer and stored at -20°C. We amplified and sequenced 1458 bp of Cytochrome Oxidase subunit I (COI) of the mitochondrion and 4 nuclear genes – 403 bp of wingless (wg), 606 bp of ribosomal protein S5 (RpS5), 691 bp of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), and 751 bp of elongation factor-1 alpha (EF-1 alpha) – following the

PCR protocols, with minor variations, laid out by Peña et al. (2006) and Wahlberg and Wheat (2008). Primer sequences and PCR cycling protocols, are also available at <http://nymphalidae.utu.fi/Nymphalidae/Molecular.htm>. All PCR products were sequenced in both directions, and in most cases there was complete overlap of fragments. All sequencing was done on the Univ. of California at Davis campus in the College of Agriculture and Environmental Sciences Genomics Facility. For a table of specimens, geographic data and accession numbers, see Supplementary material Table S1.

Phylogenetic analyses

When developing biogeographic hypotheses based on phylogenies, it is critical that species relationships be robust. No single analytical tool available today is ideal, therefore we used a multi-step process relying on several analytical techniques to determine and evaluate the best species tree. First, we analyzed each gene independently and in combination using Bayesian analyses with the program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). One combination included all genes, mitochondrial and nuclear, and a second analysis included only concatenated nuclear genes. Because concatenation methods have been shown to produce species trees inconsistent with the ''true'' phylogeny under certain circumstances (Degnan and Rosenberg 2006, Pollard et al. 2006, Kubatko and Degnan 2007), we used the program Bayesian Untangling of Concordance Knots (BUCKy) (Ane et al. 2007) to identify clades with the greatest concordance among gene trees and assess the validity of concatenated analyses.

Sequences were aligned using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI) and codon positions were defined using MacClade 4.06 (Maddison and Maddison 2000). We used the CIPRES portal (Miller et al. 2009) to perform Bayesian analyses on individual sequences using MrBayes. Each gene was partitioned by codon position, and the GTR (generalized time reversible) model with gamma-distributed rate variation across sites and a proportion of invariable sites for sequence evolution was specified. We allowed for partition-specific rates by setting the rate parameter to variable and unlinked the model parameters for gene partitions. We performed 2 replicates with 4 chains for 2×10^7 generations. The temperature was set to 0.10 to enhance mixing, and chains were sampled every 1000 generations. Combined datasets were partitioned by gene and codon position. Again, two replicates and 4 chains were run for 1×10^7 generations at a temperature of 0.10, and chains were sampled every 1000 generations. To assess convergence of parameters we checked the standard deviation of split frequencies across the independent runs. Using the online software Are We There Yet (AWTY) (Wilgenbusch et al. 2004, Nylander et al. 2007), we assessed convergence of topologies after a 25% burn-in. We visually inspected the 4 diagnostics AWTY provides: posterior probabilities of clades for non-overlapping samples of trees, pair wise split frequencies for independent MCMC runs, cumulative frequencies for selected splits, and the symmetric tree-difference score between and within runs.

Clades from concatenated trees were assessed using BUCKy. BUCKy estimates concordance among sets of genes based on the assumption that the number of distinct topologies among a survey of genes is small compared to all possible topologies (Ane et al. 2007). BUCKy employs a 2-stage Markov chain Monte Carlo (MCMC) method in which the posterior probability distributions of independent gene trees, derived from the Bayesian analyses above, are input to a second MCMC procedure that estimates a posterior distribution of gene-to-tree maps. A summary of the posterior probabilities of the gene-to-tree maps provides revised posterior probability distributions for each gene, accounting for concordance, and an estimate of the proportion of sampled genes for which given clades are true. Additionally, a primary concordance tree is created from clades with the greatest proportion of support from the revised posterior distributions of individual gene trees. BUCKy analyses only accommodate 31 species so we created 13 pruned sub-trees from the original, full-taxon gene trees, with various combinations of species and gene compositions, using the ape package 2.3-2 (Paradis et al. 2004) for R 2.9.1.

We used sequenced data from two closely related genera and two more distantly related genera, to confirm monophyly of *Lymanopoda*. According to Wahlberg et al. (2009), *Lymanopoda* is sister to genus *Ianussiusa* or part of a polytomy with *Ianussiusa* and *Idioneurula*. Sequences for *Idioneurula eremita*, *Lethe minerva*, and *Neope bremeri* were acquired from GenBank (accession numbers are in Supplementary material Table S1) and included in a Bayesian analysis with similar parameters to the *Lymanopoda* only analysis run above.

Dating the tree

To estimate divergence times for *Lymanopoda*, we used a fossil of butterfly *Lethe carbieri* (Nymphalidae, Satyrinae) from the Late Oligocene (ca 25 Ma) (Nel et al. 1993). Butterfly fossils are exceedingly rare, and the specimen of *Lethe carbieri* is the closest known fossil record to the genus *Lymanopoda*, both of which are members of the Satyrini tribe (Peña and Wahlberg 2008, Wahlberg et al. 2009).

To estimate the timescale of diversification for *Lymanopoda* we used the program BEAST 1.5.2 (Drummond and Rambaut 2007) run through the Computational Biology Suite for High Performance Computing (BioHPC) portal housed at Cornell Univ. MODELTEST v3.7 (Posada and Crandall 1998) was used to identify the optimal model of substitution based on the hierarchical likelihood ratio tests (hLRTs) for each gene (EF1 alpha: TrNef+G+I; GAPDH: TrN+G+I; RpS5: TrNef+G; wg: HKY+G+I; COI: GTR+G+I). The BEAST analysis was partitioned by gene and included the model of evolution, unique gamma distribution shape parameter (G), and proportion of invariable sites (I). We used a normal prior for the age of the fossil calibration, which was set to the age of divergence between *Lethe* and *Neope* ($\mu = 25$ Ma and $\sigma = 1.0$). The topology and branch lengths from the Bayesian analysis including *Lethe* and *Neope* were used as a starting tree, and a Yule model (Yule 1924, Aldous 2001) was assigned to the tree prior. To account

for lineage-specific rate heterogeneity, branch lengths were allowed to vary under a relaxed clock model with an uncorrelated lognormal distribution. We ran five independent chains of ten million generations each, sampling every 1000 generations and combined results with LogCombiner v 1.5.3 (Drummond and Rambaut 2007).

Species range overlap

The degree of overlap in latitudinal and elevational ranges was calculated at each node using methods described in Fitzpatrick and Turrelli (2006). The purpose of this exercise was not to reconstruct ancestral ranges, but to estimate the average overlap between species ranges after a certain time since speciation. Because ranges closely follow the north–south transect of the Andes and there is very little longitudinal variation, we used the latitudinal coordinates as range boundaries. For terminal species pairs, range overlap was calculated by dividing the area of overlap by the area of the smaller species' range. For internal nodes, we found the nested averages of pairwise overlaps between species' ranges in each clade. We also calculated the relative difference in the upper elevational limit for subtending clades at each node. For terminal species, the difference in the maximum elevational range was calculated and divided by the full elevational range of the higher species. Elevational range differences for internal nodes were calculated using nested averages as above. We only calculated range overlap and difference for pairwise relationships supported by the majority of gene trees and BUCKy analyses. Range overlap between species varies between 0 (for no overlap) and 1 (for complete overlap). Range difference values may vary between 0 and ∞ and exceed 1 if the difference in elevational ranges is greater than the species' or clade's full elevational range.

Statistical test of clade distributions

Additionally, we compared elevational and latitudinal distributions among clades. We demarcated the clades as above based on deep divisions in the tree, which were supported by differences in morphology, gene trees and BUCKy analyses. The median of each species' elevational and latitudinal range was determined from the distribution data, and each clade was assigned a numerical dummy variable. Using Student's t-test we compared mean median distributions for each clade pair. We used the Fligner-Killeen test and Fisher's F-test to confirm homogeneity of variances.

Phylogenetic signal in ranges

We tested phylogenetic signal in latitudinal and elevational ranges using Blomberg et al.'s (2003) K in the R package picante (Kembel et al. 2009). We used the same clade demarcations as above to look for phylogenetic signal in latitudinal (northern limit, southern limit, median) and elevational (upper limit, lower limit, median) ranges.

Results

Lymanopoda is monophyletic relative to sister genus *Ianussiusa* and the more distantly-related *Idioneurula* (Supplementary material Fig. S1). Membership of well-defined clades agreed based on individual analysis of gene trees with the exception of a few taxa, primarily *L. eubagioides* and *L. inde* (see below) (see Supplementary material Fig. S2–S6 for gene trees). Because clades produced in individual gene trees were broadly concordant, we used results based on all evidence (i.e. concatenation of all genes). BUCKy's primary concordance trees, using pruned gene trees, supported clades rendered in the all-evidence tree. However, relationships among major clades varied by gene and analysis, preventing inference of deeper relationships.

Placement of *L. eubagioides* and *L. inde* differed among gene trees and analyses. Nuclear genes consistently placed *L. inde* in the *L. caracara*/*L.melia* clade, and the placement of *L. eubagioides* varied by gene. The mtDNA segment COI, and all primary concordance trees from BUCKy placed *L. eubagioides* and *L. inde* as sister taxa sister to the *L. vivientenil L. rana* clade. *Lymanopoda eubagioides* and *L. inde* are sister taxa and sister to the *L. caracara*/*L. melia* clade in the concatenated analysis (Fig. 2).

By our estimation, the genus *Lymanopoda* diverged from sister genus *Ianussiusa* ca 27 Ma, which is 5 million years before Wahlberg et al.'s (2009) estimate. In either case, divergence with *Ianussiusa* was very early in Andean orogeny, which we discuss more below. Formation of most major clades has occurred within the last 8–10 million years, and most of the species-level diversification has occurred within the last 6 million years. More than half of the sampled species are of Pleistocene or post-Pleistocene origin (Fig. 2).

Calculations of range overlap are presented in Table 1. Because relationships among the major clades were variable in the above analyses, we only calculated range overlap and differences for nodes within major clades. Of the 32 nodes where overlap was calculated, 17 nodes demonstrate a high degree of elevational overlap, sharing more than 75% of their elevational range with a sister species/clade, while only ten nodes join clades with a similar degree of overlap in their latitudinal range. Likewise, fifteen nodes join species sharing 25% and less of their latitudinal ranges (indicated by nodes with a triangle (▲) in Fig. 2), and only 4 nodes show a correspondingly low level of overlap in elevational range. Only 5 nodes join sister clades with an elevational difference of 75% or greater (indicated with a circle (●) in Fig. 2). The differences in proportions of species overlapping horizontally versus vertically demonstrate a stronger signal of speciation associated with latitudinal rather than elevational shifts. A few cases exist in which sister species segregate one below the other along the forest-paramo ecotone, but because of variation in the elevation of the ecotone due to latitude and aspect, this elevational segregation is not apparent in the data. These cases are based on decades of observation, and have been marked with a square (■) in Fig. 2.

There were significant differences in the mean elevational and latitudinal distributions of clades (Fig. 3). Members of the "*L. caracara*" clade have significantly higher elevational ranges than members of all other clades. The "*L. araneola*" clade also has a significantly higher

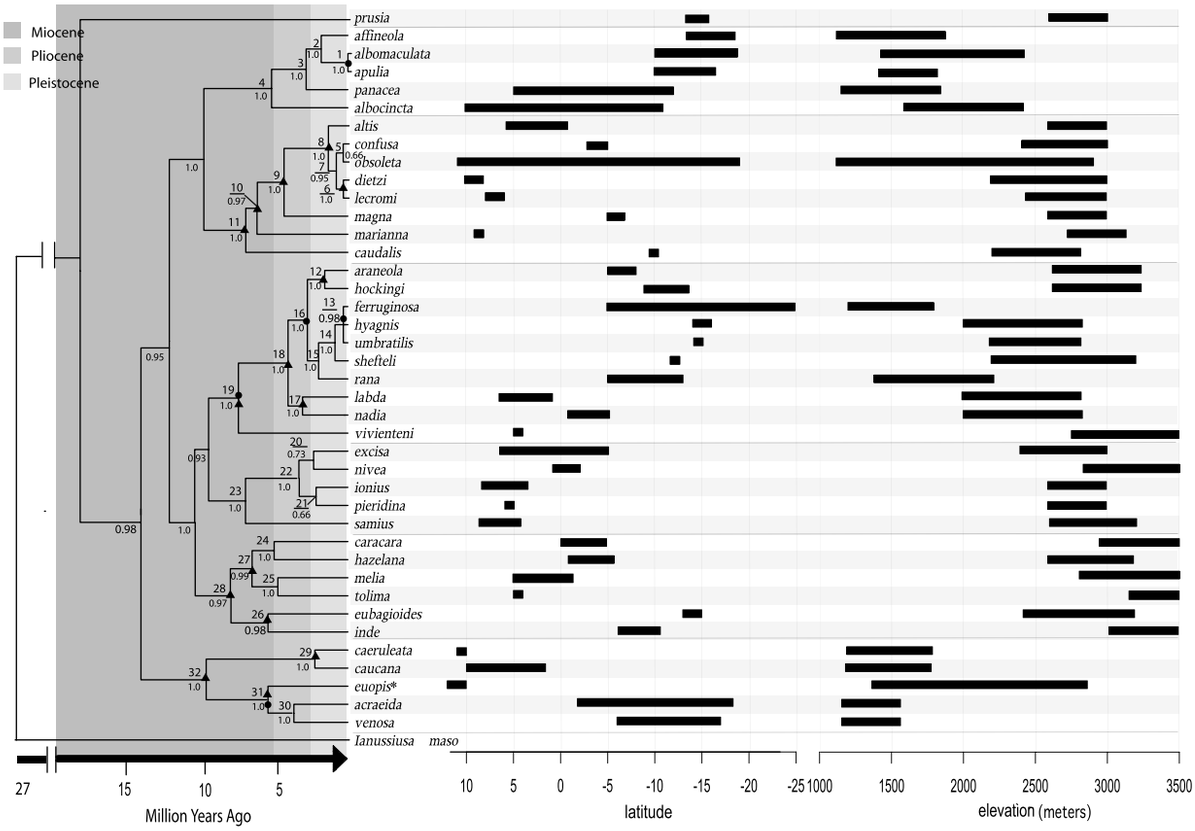

Figure 2. A species tree for genus *Lymanopoda* with associated latitudinal and elevational distributions for each species. Node numbers, left of nodes and above branches, correspond to Table 1. Posterior probabilities are given on the left of nodes below branches. Circles (●) indicate elevational range shift of at least 75% relative to a sister clade. Triangles (▲) indicate nodes that join sister taxa that overlap by no more than 25% in their latitudinal ranges. **Lymanopoda euopis* is one of two species (the other is *L. cinna* of Guatemala) of *Lymanopoda* that occur outside of the Andes.

distribution than the "*L. caeruleata*" and "*L. affineola*" clades, and the "*L. caeruleata*" and "*L. affineola*" clades occupy significantly lower elevations than all other clades. Several clades were also distinct in their latitudinal range. Members of the "*L. excisa*" clade occupy ranges significantly further north than members of the "*L. affineola*" and "*L. araneola*" clades, and members of the "*L. altis*" clade are significantly north of members of the "*L. araneola*" clade.

Significant phylogenetic signal was detected in all six latitudinal and elevational range variables tested (lower elevation limit $K=0.32$, $p=0.015$; upper elevation limit $K=0.29$, $p=0.001$; mid elevation $K=0.32$, $p=0.001$; northern limit $K=0.31$, $p=0.01$; southern limit $K=0.26$, $p=0.028$; mid latitude $K=0.48$, $p=0.001$).

Discussion

If we assume that there is a relationship between the mode of speciation and the current distribution of species' geographic ranges, we may infer the geography of speciation and look for inherent patterns. The relatively young age of most species of *Lymanopoda* and distinctive allo- and parapatric species ranges indicate minimum levels of dispersal since speciation that might mask speciation patterns (Barraclough and Vogler 2000). The phylogeny and species range data suggests that *Lymanopoda* has diversified both along elevational gradients and horizontally throughout the Andes. This is supported by a strong phylogenetic signal for both elevational and latitudinal range limits. Although there is evidence for both directions of diversification, a prevalence of clades composed of species within the same elevational strata and significant differences in mean elevation among clades suggests that there has been greater diversification within elevation and across latitude than the converse.

Approximately half of all nodes in the phylogeny in which we calculated overlap join sister taxa that have a low level of latitudinal overlap, suggesting speciation associated with a horizontal shift into an adjacent mountain range. A latitudinal shift likely indicates allopatric speciation through either dispersal or vicariance. Both hypotheses are plausible. The incidence of dispersal is difficult to test (Voelker 1999), although it has been shown to play an important role in diversification of other Andean taxa (Remsen 1984, Schulte II et al. 2000).

Adams (1985) suggested that the stair-step pattern of pronophiline butterflies is the consequence of vicariance facilitated through Pleistocene glaciation cycles (2.588 million–12 000 BP), wherein warm periods caused species

Table 1. Latitudinal and elevational range overlap and elevational difference in the upper range limits for clades joined at a shared node. Node numbers correspond to the numbers above branches in Fig. 2.

Node	Latitudinal overlap	Elevational overlap	Diff. in upper range limits
1	1.00	1.00	0.60
2	0.70	0.83	0.30
3	0.70	0.42	0.15
4	0.54	0.69	0.59
5	1.00	1.00	0.33
6	0.00	1.00	0.00
7	0.50	1.00	0.15
8	0.25	1.00	0.13
9	0.13	1.00	0.06
10	0.13	0.44	0.35
11	0.03	0.29	0.57
12	0.00	1.00	0.00
13_{fh}	1.00	0.00	1.25
13_{fu}	1.00	0.00	1.67
13_{hu}	1.00	1.00	0.00
14	0.33	0.58	0.73
15	0.67	0.15	0.71
16	0.71	0.31	1.09
17	0.00	1.00	0.00
18	0.00	0.40	0.10
19	0.25	0.23	0.86
20	1.00	0.33	0.67
21	1.00	1.00	0.00
22	0.45	0.75	0.33
23	0.69	0.83	0.17
24	0.80	0.67	0.67
25	1.00	1.00	0.00
26	0.00	0.33	0.67
27	0.05	0.83	0.25
28	0.00	0.73	0.10
29	0.00	1.00	0.00
30	1.00	1.00	0.00
31	0.00	0.50	0.86
32	0.00	0.59	0.19

to move up-slope, become isolated and diversify, and glacial periods pushed faunas to lower elevations where reinforcement and dispersal into adjacent mountain ranges ensued. He argued that repetitions of this cycle might produce vertical stacking of species' ranges through completely allopatric speciation processes. While it appears that the Pleistocene certainly contributed to the species-level diversity of *Lymanopoda*, dating suggests a much earlier origin for the elevational stratification.

The geologic and climatic events occurring between the formation of the early Andes to today are complex and under debate (Sempere et al. 2008). Andean orogeny was neither uniform nor simultaneous throughout the range, varying in timing and rate of uplift from east to west and north to south (Gregory-Wodzicki 2000). Most studies suggest that Andean uplift began in the Eocene or Oligocene but then halted until the Late Miocene (Sempere et al. 2008). The western Cordillera of the central Andes was at no more than half of its current elevation 25 Ma, while the eastern Cordillera only reached half of its modern elevation ca 10 Ma. High altitudes probably emerged first in the central Andes, ~13°S–28°S, and progressed northward (Picard et al. 2008). Surface uplift on the order of 2000–3500 m has occurred in the eastern Cordillera and Altiplano in the last 10 million years. Uplift of the northern Andes was very recent, estimated to have been at no more than 40% of its current elevation 4 Ma in some regions (Gregory-Wodzicki 2000). Timing of the split with *Ianussiusa* between 20 to 30 Ma corresponds with very early Andean orogeny, and subsequent diversification likely occurred when uplift resumed 10–15 million years later. Dramatic uplift during the Miocene and Pliocene coincides with formation of *Lymanopoda*'s major clades, and continued uplift in the north and intervals of global cooling during the Pleistocene coincide with much of the species-level diversification.

Speciation across elevational clines occurred multiple times in the early stages of diversification of *Lymanopoda* and again more recently, particularly within a few clades. Some shifts in elevation are dramatic and apparent in the data, while other shifts in elevation are associated more with habitat type than strict elevation, and therefore, are not obvious in the range data. Ecotones can occur at different elevations depending on latitude and aspect, and some species' elevational ranges co-vary with specific habitat. Therefore a few species pairs appear to be partially sympatry according to the data, but they are locally parapatric. For example, *L. caracara* is a cloud forest–paramo ecotone specialist, and *L. hazelana* occurs in high montane cloud forest directly below *L. caracara*. The forest–paramo ecotone occurs between 3600 m in the north of Ecuador and 3150 m in southern Ecuador, and ranges of *L. caracara* and *L. hazelana* closely parallel this gradient. A similar pattern is true for *L. excisa* and *L. nivea*, which occur in forest just below the edge. In cases where forests have been logged and the ecotone is lower, ranges similarly extend to lower elevations. Habitat segregation, as demonstrated by these species, could be the result of allopatric speciation followed by secondary contact and subsequent niche partitioning or ecological speciation. In these cases, the ecotone is obvious and the butterfly's association with the ecotone is apparent, but this is the exception. In most cases, elevational ranges are relatively consistent and the biotic or abiotic factors limiting elevational distributions are unknown, which is why we have used elevation rather than microhabitat as a proxy for range boundaries.

A similar case of sympatric/parapatric speciation occurred in antbirds (Thamnophilidae, Percnostola) of northern South America (Braun et al. 2005). The roraiman antbird *Percostola saturata*, endemic to tepuis of southeastern Venezuela and northern Brazil, is elevationally parapatric to its more widespread lowland sister, the spotted-winged antbird *Percnostola leucostigma*. Species ranges are structured on environmental variables associated with elevation, such as fast-moving water common in highlands and standing pools common in the lowland, and the borders of the ranges interdigitate according to local habitat. Recent theoretical and empirical studies have shown that parapatric speciation along a gradient and with gene flow, might be more realizable and widespread than previously thought (Schneider et al. 1999, Doebeli and Dieckmann 2003, Doebeli et al. 2005). Results of previous studies and speciation patterns in *Lymanopoda* suggest that ecotones associated with a change

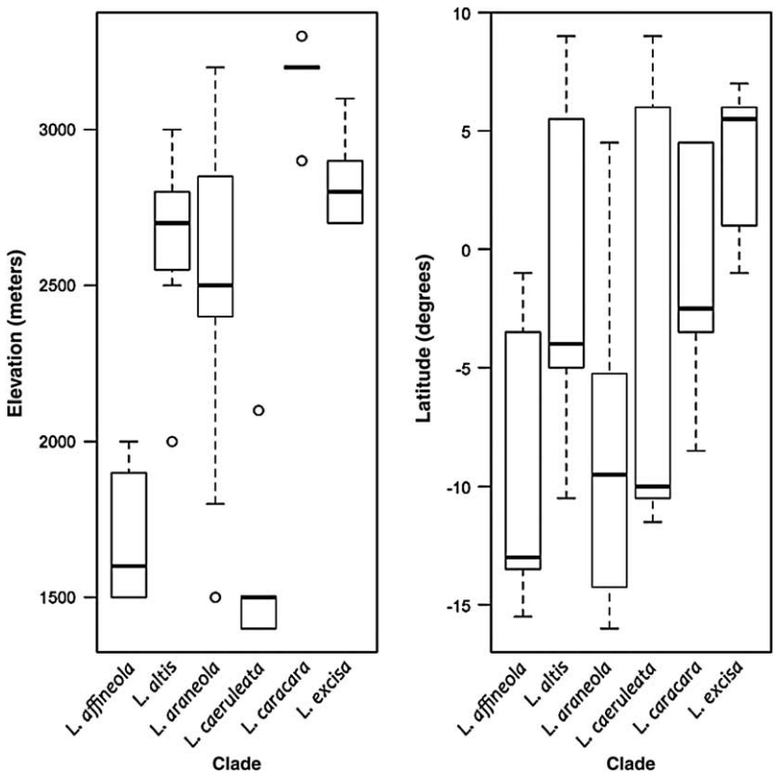

Figure 3. Box and whisker plot of clades versus mean median elevations. Dark horizontal lines show median values. The top and bottom of boxes represent the 25th and 75th percentiles, respectively. Vertical dashed lines show maximum and minimum values or 2 standard deviations, whichever is smaller. Open circles represent outliers.

in elevation may facilitate sympatric/parapatric speciation and further expansion into higher elevations.

Janzen (1967) outlined a possible mechanistic explanation for the relatively common observation of elevationally stacked species ranges in the tropics in his seminal work, ''Why mountain passes are higher in the tropics''. He argued that tropical organisms are exposed to minimal seasonal variation, and therefore are adapted to a narrower range of climatic conditions. Because elevation co-varies with temperature, and the temperature difference rather than absolute height likely determines the efficacy of a barrier, tropical organisms are more likely than their temperate counterparts to encounter temperatures beyond their physiological tolerance, and therefore, dispersal is obstructed by elevational changes. The temperature lapse rate in tropical mountains is 0.5–0.6°C for each 100 m of elevation gain (Grubb 1977). *Lymanopoda* span more than 2000 m of elevation, with low and high elevation species experiencing a temperature difference of ca 10–12°C. Differences in day and nighttime temperature in cloud forest average 8–14°C, which is greater than seasonal differences. This means that a low-elevation species in the highlands or a highland species at lower elevation would certainly experience temperatures outside its normal climate regime. Janzen went on to predict that this should lead to high fidelity to a specific set of abiotic conditions and lead to narrow elevational distributions in tropical-montane species. A study of $>16\,500$ montane species spanning 48 degrees of latitude recently confirmed his prediction (McCain 2009).

Other studies have directly tested the effects of changing environmental conditions on organisms at various elevations. Montane carabid beetles studied along an elevational transect in Wales showed significantly lower optimal body temperatures than widespread, low elevation species (Buse et al. 2001). Similarly, distributions of two congeneric species of jumping plant lice in Norway were restricted elevationally by heat budgets acting on development rates. *Craspedolepta nebulosa* developed more efficiently at low temperatures and was able to inhabit higher elevations than its congener, *C. subpunctata* (Bird and Hodkinson 2005).

In addition to direct physiological effects, increases in elevation influence forest structure and composition. Forests become progressively more stunted and open with elevation, which may have indirect effects on fauna. The bamboo host plant of *Lymanopoda* is abundant up to the forest–paramo edge. At lower elevations it grows tall and is often shaded by an extensive canopy cover, while at higher elevations the forest is stunted. Therefore, at timberline *Chusquea* and *Lymanopoda* are more exposed to variable weather conditions – strong winds, sudden changes in temperature and humidity. Differences in the nutritional value of *Chusquea* at low and high elevations have not been directly tested, but there is a large body of evidence that

suggests that plant nutrient composition changes with elevation (Morecroft and Woodward 1996, Erelli et al. 1998, Cordell et al. 1999).

We obtained samples for approximately two thirds of the species of *Lymanopoda.* Many species lacking from this analysis are endemic to Colombia, which has been the subject of less collecting than other parts of the Andes. Most of these species are localized, rare and live at high elevations. Based on morphology we can place the missing species into existing clades. Five of the missing species – *L. altaselva*, *L. lactea*, *L. labineta*, *L. schmidti* and *L. paisa* – belong to the *L. ionius* clade and resemble each other and *L. ionius* in morphology and genitalia. *Lymanopoda paisa* flies at a similar elevation to *L. ionius* while the other four fly in adjacent regions at elevations just above *L. ionius*. A similar situation exists for the low elevation species *L. obsoleta* and *L. ferruginosa*, which are broadly distributed and replaced by closely related congeners at higher elevation. *Lymanopoda lebbaeã* flies in the eastern Cordillera of Colombia and is most closely aligned with *L. labda*, which occurs in the Colombian western and central Cordilleras and south into Ecuador. *Lymanopoda maletera* is probably most closely related to *L. dietzi* and flies at a similar elevation in Colombia. Similarly, *L. melendeza* appears to be most closely aligned with *L. marianna* and occurs at a similar elevation in Colombia. Without molecular analysis we cannot be certain of sister species relationships, and therefore of the mode of speciation. Morphology, however, suggests that many of the missing species are most closely related to allopatric replacements occurring at similar elevations in adjacent regions.

Whether covariates of elevation limit *Lymanopoda* directly or indirectly is unclear, but relatively narrow ranges and less frequent speciation across elevational boundaries suggest that changing environmental factors along a gradient have played a principal role in the pattern of diversification. Gaining insight into the mechanisms limiting the distribution of species with restricted ranges is of great evolutionary interest and is essential to conservation.

Changes in climate, invasive species and habitat destruction have the potential to drastically alter habitats and shift species' geographic ranges. As we take measures to moderate wide-spread diversity loss, it is important to be equipped with knowledge of why species ''are where they are''. An evolutionary perspective can inform us of types and strengths of barriers that might prevent range expansion or dispersal. Diversification of *Lymanopoda* butterflies and other tropical montane faunas appear to be constrained elevationally, and thus, geographically by high mountains and deep valleys. Highly defined habitat requirements combined with difficult dispersal make tropical montane faunas especially vulnerable to anthropogenic forces and priorities for protection.

Acknowledgements – KLC would like to thank Arthur Shapiro for turning her on to Andean butterflies and PBR, and the National Science Foundation for making the extended South American field trips possible through the aid of a Graduate Research Fellowship. She would also like to thank H. B. Shaffer for use of his molecular lab and Keith Willmott and Rupert Griffiths for their assistance with field work. T. Pyrcz field work was financed by KBN-6P04F06711 and DS-MZ-IZ/UJ-2003-2009 grants. T. Pyrcz would like to thank Janusz Wojtusiak (Jagiellonin Univ., Kraków, Poland), Pierre Boyer (Le Puy Sainte Reparade, France) and Mauro Costa (Caracas, Venezuela). Part of this work was carried out by using the resources of the Computational Biology Service Unit from Cornell Univ., which is partially funded by Microsoft Corporation.

References

Adams, M. J. 1985. Speciation of the pronophiline butterflies (Satyridae) of the northern Andes. – J. Res. Lepid. Suppl. 1: 33–49.

Adams, M. J. 1986. Pronophiline butterflies (Satyridae) of the 3 Andean cordilleras of Colombia. – Zool. J. Linn. Soc. 87: 235–320.

Adams, M. J. and Bernard, G. I. 1981. Pronophiline butterflies (Satyridae) of the Cordillera De Merida, Venezuela. – Zool. J. Linn. Soc. 71: 343–372.

Aldous, D. J. 2001. Stochastic models and descriptive statistics for phylogenetic trees, from Yule to today. – Stat. Sci. 16: 23–34.

Ane, C. et al. 2007. Bayesian estimation of concordance among gene trees. – Mol. Biol. Evol. 24: 412–426.

Barraclough, T. G. and Vogler, A. P. 2000. Detecting the geographical pattern of speciation from species-level phylogenies. – Am. Nat. 155: 419–434.

Bird, J. M. and Hodkinson, I. D. 2005. What limits the altitudinal distribution of *Craspedolepta* species (Sternorrhyncha: Psylloidea) on fireweed? – Ecol. Entomol. 30: 510–520.

Blomberg, S. P. et al. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. – Evolution 57: 717–745.

Braun, M. J. et al. 2005. Avian speciation in the Pantepui: the case of the roraiman antbird (*Percnostola* [*Schistocichla*] ''*Leucostigma*'' *saturata*). – Condor 107: 327–341.

Brown, J. H. et al. 1996. The geographic range: size, shape, boundaries, and internal structure. – Annu. Rev. Ecol. Syst. 27: 597–623.

Buse, A. et al. 2001. Arthropod distribution on an alpine elevational gradient: the relationship with preferred temperature and cold tolerance. – Eur. J. Entomol. 98: 301–309.

Chapman, F. M. 1917. The distributin of bird life in Colombia. – Bull. Am. Mus. Nat. Hist. 31: 1–169.

Cordell, S. et al. 1999. Allocation of nitrogen and carbon in leaves of *Metrosideros polymorpha* regulates carboxylation capacity and delta C-13 along an altitudinal gradient. – Funct. Ecol. 13: 811–818.

Degnan, J. H. and Rosenberg, N. A. 2006. Discordance of species trees with their most likely gene trees. – PLoS Genet. 5: e68.

DeVries, P. J. 1987. The butterflies of Costa Rica and their natural history. Volume 1: Papilionidae, Pieridae, Nymphalidae. – Princeton Univ. Press.

Doebeli, M. and Dieckmann, U. 2003. Speciation along environmental gradients. – Nature 421: 259–264.

Doebeli, M. et al. 2005. What we have also learned: adaptive speciation is theoretically plausible. – Evolution 59: 691–695.

Drummond, A. J. and Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. – BMC Evol. Biol. 7: 214.

Elias, M. et al. 2009. Out of the Andes: patterns of diversification in clearwing butterflies. – Mol. Ecol. 18: 1716–1729.

Erelli, M. C. et al. 1998. Altitudinal patterns in host suitability for forest insects. – Oecologia 117: 133–142.

Fitzpatrick, B. M. and Turrelli, M. 2006. The geography of mammalian speciation: mixed signals from phylogenies and range maps. – Evolution 60: 601–615.

Fjeldsa, J. and Lovett, J. C. 1997. Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. – Biodivers. Conserv. 6: 325–346.
Garzione, C. N. et al. 2006. Rapid late Miocene rise of the Bolivian Altiplano: evidence for removal of mantle lithosphere. – Earth Planetary Sci. Lett. 241: 543–556.
Gregory-Wodzicki, K. M. 2000. Uplift history of the central and northern Andes: a review. – Geol. Soc. Am. Bull. 112: 1091–1105.
Grubb, P. J. 1977. Control of forest growth and distribution on wet tropical mountains: with special reference to mineral nutrition. – Annu. Rev. Ecol. Syst. 8: 83–107.
Heller, H. C. 1971. Altitudinal zonation of chipmunks (*Eutamias*): interspecific aggression. – Ecology 52: 312–319.
Heller, H. C. and Poulson, T. 1972. Altitudinal zonation of chipmunks (*Eutamias*): adaptations to aridity and high temperature. – Am. Midl. Nat. 87: 296–313.
Holt, R. D. and Keitt, T. H. 2005. Species' borders: a unifying theme in ecology. – Oikos 108: 3–6.
Huelsenbeck, J. P. and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. – Bioinformatics 17: 754–755.
Janzen, D. H. 1967. Why mountain passes are higher in the tropics. – Am. Nat. 101: 233–249.
Kembel, S. W. et al. 2009. R tools for integrating phylgenies and ecology. – R package ver. 1.0-0, <http:/picante.r-forge.r-project.org>.
Kirkpatrick, M. and Barton, N. H. 1997. Evolution of a species' range. – Am. Nat. 150: 1–23.
Kubatko, L. and Degnan, J. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. – Syst. Biol. 56: 17–24.
Lamas, G. (ed.) 2004. Checklist: part 4A. Hesperioidea – Papilionoidea. Atlas of Neotropical Lepidoptera. – Association for Tropical Lepidoptera/Scientific Publ.
MacArthur, R. 1972. Geographical ecology: patterns in the distributions of species. – Harper and Rowe.
Maddison, D. R. and Maddison, W. P. 2000. MacClade version 4: analysis of phylogeny and character evolution. – <www.mesquiteproject.org>.
McCain, C. 2009. Vertebrate range sizes indicate that mountains may be 'higher' in the tropics. – Ecol. Lett. 12: 550–560.
Miller, M. et al. 2009. The CIPRES portals. – CIPRES, <www.phylo.org/sub_sections/portal>.
Morecroft, M. D. and Woodward, F. I. 1996. Experiments on the cause of altitudinal differences in the leaf nutrient contents, size and delta C-13 of *Alchemill alpina*. – New Phytol. 132: 471–479.
Moritz, C. et al. 2000. Diversification of rainforest faunas: an integrated molecular approach. – Annu. Rev. Ecol. Syst. 31: 533–563.
Nel, A. et al. 1993. Un nouveau Lepidoptere Satyrinae fossile de l'Oligocene ud sud-est de la France (Insecta, Lepidoptera, Nymphalidae). – Linn. Belg. 14: 20–36.
Nylander, J. A. A. et al. 2007. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. – Bioinformatics 24: 581–583.
Paradis, E. et al. 2004. APE: analyses of phylogenetics and evolution in R language. – Bioinformatics 20: 289–290.
Peña, C. and Wahlberg, N. 2008. Prehistorical climate change increased diversification of a group of butterflies. – Biol. Lett. 4: 274–278.
Peña, C. et al. 2006. Higher level phylogeny of Satyrinae butterflies (Lepidoptera: Nymphalidae) based on DNA sequence data. – Mol. Phylogenet. Evol. 40: 29–49.
Picard, D. et al. 2008. Direction and timing of uplift propagation in the Peruvian Andes deduced from molecular phylogenetics of highland biotaxa. – Earth Planetary Sci. Lett. 271: 326–336.
Pollard, D. A. et al. 2006. Widespread discordance of gene trees with species tree in *Drosophila*: evidence for incomplete lineage sorting. – PLoS Genet. 10: 173.
Posada, D. and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. – Bioinformatics 14: 817–818.
Pyrcz, T. W. 2004. Pronophiline butterflies of the highlands of Chachapoyas in northern Peru: faunal survey, diversity and distribution patterns (Lepidoptera, Nymphalidae, Satyrinae). – Genus 15: 455–622.
Pyrcz, T. W. and Wojtusiak, J. 2002. The vertical distribution of pronophiline butterflies (Nymphalidae, Satyrinae) along an elevational transect in Monte Zerpa (Cordillera de Merida, Venezuela) with remarks on their diversity and parapatric distribution. – Global Ecol. Biogeogr. 11: 211–221.
Pyrcz, T. W. et al. 1999. Contribution to the knowledge of Ecuadorian Pronophilini. Part III. Three new species and five new subspecies of Lymanopoda (Lepidoptera: Nymphalidae: Satyrinae). – Genus 10: 497–522.
Remsen, J. V. J. 1984. High incidence of ''Leapfrog'' pattern of geographic variation in Andean birds: implications for the speciation process. – Science 224: 171–173.
Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics 19: 1572–1574.
Schluter, D. 1996. Ecological causes of adaptive radiation. – Am. Nat. 148: S40–S64.
Schneider, C. J. et al. 1999. A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. – Proc. Nat. Acad. Sci. USA 96: 13869–13873.
Schulte II, J. A. et al. 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. – Biol. J. Linn. Soc. 69: 75–102.
Sempere, T. et al. 2008. New insights into Andean evolution: an introduction to contributions from the 6th ISAG symposium (Barcelona, 2005). – Tectonophysics 459: 1–13.
Terborgh, J. 1971. Distribution on environmental gradients: theory and a preliminary interpretation of distributional patterns in the avifauna of the Cordillera Vilcabamba, Peru. – Ecology 52: 23–40.
Voelker, G. 1999. Dispersal, vicariance, and clocks: historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*: Motacillidae). – Evolution 53: 1536–1552.
Wahlberg, N. and Wheat, C. 2008. Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of Lepidoptera. – Syst. Biol. 57: 231–242.
Wahlberg, N. et al. 2009. Nymphalid butterflies diversify following near demise at the cretaceous/terriary boundary. – Proc. R. Soc. B 276: 4295–4302.
Wilgenbusch, J. C. et al. 2004. AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. – <http://king2.csit.fsu.edu/CEBProjects/awty/awty-start.php>.
Yule, G. U. 1924. A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis. – Phil. Trans. R. Soc. B 213: 21–87.

Download the Supplementary material as file E6306 from <www.oikos.ekol.lu.se/appendix>.