A PHYLOGENETIC PERSPECTIVE ON ELEVATIONAL SPECIES RICHNESS PATTERNS IN MIDDLE AMERICAN TREEFROGS: WHY SO FEW SPECIES IN LOWLAND TROPICAL RAINFORESTS?

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Differences in species richness at different elevations are widespread and important for conservation, but the causes of these patterns remain poorly understood. Here, we use a phylogenetic perspective to address the evolutionary and biogeographic processes that underlie elevational diversity patterns within a region. We focus on a diverse but well-studied fauna of tropical amphibians, the hylid frogs of Middle America. Middle American treefrogs show a "hump-shaped" pattern of species richness (common in many organisms and regions), with the highest regional diversity at intermediate elevations. We reconstructed phylogenetic relationships among 138 species by combining new and published sequence data from 10 genes and then used this phylogeny to infer evolutionary rates and patterns. The high species richness of intermediate elevations seems to result from two factors. First, a tendency for montane clades to have higher rates of diversification. Second, the early colonization of montane regions, leaving less time for speciation to build up species richness in lowland regions (including tropical rainforests) that have been colonized more recently. This "time-for-speciation" effect may explain many diversity patterns and has important implications for conservation. The results also imply that local-scale environmental factors alone may be insufficient to explain the high species richness of lowland tropical rainforests, and that diversification rates are lower in earth's most species-rich biome.

KEY WORDS: **Amphibians, biogeography, diversity, elevation, phylogeny, speciation, species richness.**

Identifying the underlying causes of species richness patterns in tropical regions is critical, as biodiversity is increasingly threatened due to human impacts in these regions. Considerable focus has been placed on the incredible local species richness of lowland tropical rainforests in many groups of organisms (e.g., Wilson 1992, 2002). Montane regions are also thought to be important

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centers of diversity and endemism in tropical regions, possibly even more important than lowland rainforests (e.g., Rahbek and Graves 2001; Jetz and Rahbek 2002). Nevertheless, the causes of elevational patterns of species richness remain poorly studied for both temperate and tropical regions (e.g., Rahbek 1995; Brown and Lomolino 1998).

Illuminating the underlying causes of diversity patterns (rather than just their correlations) requires an evolutionary perspective. Species richness patterns are ultimately caused by the evolutionary and biogeographic processes of speciation, extinction, and dispersal; these are the only processes that directly change the number of species within a community or region (e.g., Wiens and Donoghue 2004). Other factors are also important (e.g., area, climate, and competition), but their importance ultimately depends on how they influence these evolutionary and biogeographic processes. Elucidating the evolutionary causes of species richness patterns requires consideration of the phylogenetic relationships of the species that make up the diversity gradient, to infer patterns of speciation and dispersal (Wiens and Donoghue 2004). Phylogeny-based studies of species richness patterns are relatively uncommon (e.g., Losos and Schluter 2000; Stephens and Wiens 2003; Cardillo et al. 2005; Davies et al. 2005a; Smith et al. 2005), and almost none have addressed elevational patterns (but see Wiens et al. 2007). Some studies have used phylogenies to address the origins of montane and lowland species (e.g., Bates and Zink 1994; Fjeldsa and Lovett 1997; Roy 1997; Hall 2005), but have not directly related these patterns to elevational species richness gradients.

Surprisingly, the general relationship between elevation and species richness is not well established. Some authors have suggested that regional species richness generally declines with increasing elevation and decreasing productivity, mirroring the latitudinal diversity gradient (e.g., MacArthur 1972; Stevens 1992). However, numerous studies have now shown that many taxonomic groups instead have their highest species richness at intermediate elevations, including studies of amphibians, birds, mammals, and plants (e.g., Rahbek 1995, 1997; Campbell 1999; McCain 2005; Oomen and Shanker 2005).

Why should there be a mid-elevational hump in species richness? Some authors have suggested that montane regions are important sources of new species because topographic heterogeneity increases opportunities for parapatric and allopatric speciation (e.g., Moritz et al. 2000; Rahbek and Graves 2001). Thus, montane regions are a "species pump" within a continent. Another possibility is that the groups in question originated in montane regions (or in ecological conditions that are presently found only at mid-elevations) and have spread to lower and higher elevations more recently. This biogeographic scenario might lead to higher species richness at intermediate elevations through the simple accumulation of species over time in these habitats (i.e., the "time-for-

speciation" effect; Stephens and Wiens 2003), without the need for postulating higher rates of speciation at certain elevations. We dub this the "montane museum" hypothesis, in parallel to a similar explanation postulated to explain the latitudinal diversity gradient. Alternatively, intermediate elevations might represent "sink habitats" for both low and high elevation specialists, and might be expected to have high species richness simply given random distributions of elevational ranges under a stochastic model (i.e., the mid-domain effect; Colwell and Hurtt 1994; Rahbek 1997).

These explanations for hump-shaped diversity patterns make implicit predictions that can be tested with phylogenetic comparative tools. Under the "montane species pump" hypothesis, clades that occur predominately at intermediate elevations should have higher rates of diversification (rate of speciation – rate of extinction; e.g., Magallón and Sanderson 2001) than those at lower or higher elevations. Under the "montane museum" hypothesis, the group in question is expected to originate at intermediate elevations, and there should be a positive relationship between the inferred time when an elevational zone was first colonized by the group and the number of species there presently. Finally, under the mid-domain model, patterns of species richness arise stochastically, and no relationship is expected between elevation and rates diversification or between elevation and colonization times.

In this article, we test these hypotheses in Middle American treefrogs of the family Hylidae. This group is a promising study system because it has been relatively well studied in the region (México to Panama, inclusive) from both a taxonomic and phylogenetic perspective. The species-level taxonomy has been reviewed recently by Duellman (2001) in his comprehensive monograph. Recent molecular analyses have allowed almost all species in the region to be assigned to major clades (Faivovich et al. 2005; Wiens et al. 2005, 2006), at least tentatively (i.e., representatives of all genera and species groups have been included in recent molecular phylogenetic analyses, even though not every single species has). Most species belong to a largely endemic "Middle American clade," so it is possible to address the relevant questions by focusing primarily on one group (Faivovich et al. 2005; Wiens et al. 2005, 2006). Geographic range maps and elevational ranges are available for almost all species, and several local sites in various regions and at various elevations have been well sampled (Duellman 2001). Thus, the Middle American treefrogs offer a diverse, tropical study system where it is possible to reconstruct the evolutionary history of nearly all the species that make up the patterns of species richness, at least at a coarse phylogenetic level.

In this study, we generate new molecular data and combine this information with molecular and distributional data from the literature to address the following questions: (1) How are local and regional species richness of hylid frogs in Middle America related to elevation? (2) How are rates of diversification of clades related to elevation? (3) How are patterns of species richness (with

regard to elevation) related to the timing at which different elevations were colonized?

Materials and Methods
TAXONOMY AND ELEVATIONAL TERMINOLOGY

Throughout this article, we generally adopt the generic-level taxonomy proposed by Faivovich et al. (2005). Most importantly, their taxonomy recognizes many distinct genera for many Middle American species formerly placed in *Hyla*. These genera include *Bromeliohyla*, *Charadrahyla*, *Ecnomiohyla*, *Exerodonta*, *Isthmohyla*, *Megastomatohyla*, and *Tlalocohyla*. However, we favor recognition of *Phrynohyas* as a distinct genus until relationships within the *Phrynohyas* clade (or Lophiohylini) are better resolved.

Throughout this article, we refer to elevations of 0–1000 m as lowland or low elevation, and elevations > 1000 m as montane. We further distinguish between intermediate elevations (1000–2000 m) and high elevations (> 2000 m).

PHYLOGENY

Taxon and gene sampling

Two recent phylogenetic analyses of hylid frogs based on nuclear and mitochondrial DNA sequences and morphology (Faivovich et al. 2005; Wiens et al. 2005) have confirmed that there is a "Middle American clade" of hyline frogs that contains all of the endemic hyline genera of Middle America, as well as all species occurring in temperate North America, Europe, and Asia. These two datasets were only partially overlapping in their sampling of genes and taxa. We combined these two datasets for relevant Middle American taxa and outgroups, added data from three other studies (Darst and Cannatella 2004; Moriarty and Cannatella 2004; Smith et al. 2005), sequenced and included 13 additional species of the Middle American clade not included in previous analyses, and generated additional sequences for nine taxa represented by a limited number of genes in prior studies. In addition to including 94 putative species of the Middle American clade, we also included 31 hylid species outside the Middle American clade (representing the major clades found by Faivovich et al. 2005; Wiens et al. 2005), and 13 species outside Hylidae. The latter were included to facilitate estimating divergence dates (see below), and included species of the Allophrynidae, Bufonidae, Centrolenidae, Leptodactylidae, Microhylidae, Myobatrachidae, Pipidae, and Ranidae. Our phylogenetic analyses included a total of 138 taxa.

We included DNA sequence data from four mitochondrial and six nuclear genes, including the mitochondrial genes cytochrome *b* (cyt *b*; 385 base pairs, bp), NADH dehydrogenase subunit 1 (ND1; 1218 bp including up to 372 bp of the adjacent tRNA genes), mitochondrial ribosomal large subunit (16S; 1646 bp), and the ribosomal small subunit (12S; 1088 bp); and

nuclear loci including the proopiomelanocortin A gene (POMC; 550 bp), portions of exons 2 and 3 of the v-myc myelocytomatosis viral oncogene homolog (MYC, often referred to as *c-myc*; 832 bp total), recombination activating gene 1 (RAG1; 428 bp), tyrosinase (TYR; 530 bp), sevenin absentia homolog 1 (SIAH1, 397 bp), and rhodopsin (RHO; 316 bp). For nuclear genes, we use the official symbols and names for the human genome. Our analysis included a total of 1049 gene sequences, 81 generated for this study (GenBank numbers DQ388685–DQ388763 and DQ394284–DQ394285), 484 from Faivovich et al. (2005), 113 from Smith et al. (2005), 337 from Wiens et al. (2005), 25 from Moriarty and Cannatella (2004), seven from Darst and Cannatella (2004), one from Dunon-Bluteau and Brun (1986), and an unpublished sequence from GenBank (Jiang and Zhou, October 2000). GenBank numbers and original sources for all sequences are reported in Appendix S1 (to be published online).

Sequences were aligned following Wiens et al. (2005). Faivovich et al. (2005) did not provide an alignment for each gene, and their data were therefore realigned using these same methods. In general, gene regions that differed in alignment under a limited set of different gap opening penalties (12.5, 15, 17.5) were considered to be ambiguously aligned and were therefore excluded from the phylogenetic analyses (using Clustal X.1.81; Thompson et al. 1994).

We included only a single individual or terminal taxon per species, given that our study does not focus on the details of the species-level relationships within genera. In several cases, we combined data from different genes that had been sequenced for different individuals of the same species (e.g., we sampled one set of genes for one individual of a given species, whereas Faivovich et al. [2005] sampled a different set of genes for a different individual). For some distant outgroup taxa, we combined genes for different species of higher taxa that clearly are monophyletic (see online Supplementary Appendix S1).

We did not include data from the 28S gene from Faivovich et al. (2005) or the morphological dataset of Wiens et al. (2005). The nuclear 28S gene contains very few informative characters and suggests several relationships that are highly inconsistent with the other datasets (Wiens, unpubl data). Extensive morphological data were available for a relatively small subset of the taxa included in this study, and these data are not very informative within the Middle American clade (Wiens et al. 2005).

Because of the combination of partially overlapping datasets from different studies, many taxa lacked data for one or more genes. On average, each hylid taxon had data for 7.2 of 10 genes (range 1–10 genes), and lacked data for 29.2% of their characters (range 0–94.6%). Although some taxa had data from only one or two genes (see online Supplementary Appendix S1), recent simulations (Wiens 2003; Phillipe et al. 2004) and analyses of empirical datasets (Driskell et al. 2004; Phillipe et al. 2004; Wiens

et al. 2005) suggest that incomplete taxa can be accurately placed in phylogenetic analyses if the overall number of characters is high (i.e., thousands of characters, as in this study). Furthermore, simulations (Wiens 1998) and empirical analyses (Wiens et al. 2005) suggest that including characters with incomplete taxon sampling can improve phylogenetic accuracy, relative to excluding these sets of characters entirely. In theory, we could have collected more data to fill in the gaps in the combined dataset. However, we did not have access to samples used by Faivovich et al. (2005), and the set of nuclear and mitochondrial genes that we sequenced (ND1, 12S, POMC, MYC) provides strong phylogenetic signal.

Phylogenetic analysis

Data from all 10 genes were combined and analyzed using parsimony and Bayesian methods. Although individual genes can potentially produce misleading phylogenetic signals for some clades (e.g., Maddison 1997), we expect these effects to be overwhelmed by the large number of independent nuclear loci in the combined analysis. Furthermore, previous studies (Smith et al. 2005; Wiens et al. 2005) found very little strongly supported incongruence between trees from nuclear and mitochondrial data or between trees from nuclear genes in this group. In general, we prefer the use of explicitly model-based methods over parsimony. Therefore, we primarily present and use the results of the Bayesian analyses in this study, but we confirmed the major phylogenetic results of these analyses with parsimony analyses.

Parsimony analyses were implemented in PAUP* 4.0b10 (Swofford 2002), using a heuristic search with tree-bisection-reconnection (TBR) branch swapping and 1000 random taxon-addition-sequence replicates per search. All characters were equally weighted. Support for clades was evaluated using nonparametric bootstrapping (Felsenstein 1985a), and clades with bootstrap values $\geq$ 70% were interpreted as strongly supported (following Hillis and Bull 1993; but see their extensive caveats). Bootstrap analyses used 500 pseudoreplicates each with TBR branch swapping and 10 random taxon-addition-sequence replicates per bootstrap pseudoreplicate.

Bayesian analyses were implemented using MrBayes, version 3.0b4 (Huelsenbeck and Ronquist 2001). Bayesian analyses of combined genes requires selection of models for each gene and decisions about whether different genes (or partitions within genes) should have separate or linked models. Analyses of Bayes factors (Nylander et al. 2004; Brandley et al. 2005) by Wiens et al. (2005; for 12S, ND1, POMC, and MYC) and Wiens et al.(2006 analysis for all other genes) support a partitioning strategy wherein each codon or structural region (i.e., stems vs. loops) of each gene is treated as a separate partition. Furthermore, separate analyses using MrModeltest (Nylander 2004) selected the GTR $+$ I $+$ Γ model for each gene region (general time reversible [Rodriguez et al. 1990] with a proportion of sites invariable [Gu et al. 1995]

and rates at other sites varying according to a gamma distribution [Yang 1994]). Three replicate searches were initially run, with 5.0×10^6 generations each and sampling every 1000 generations. Comparisons of log-likelihood plots and harmonic means of the log likelihoods of the putative post burn-in trees from each analysis (using the *sump* command in MrBayes) indicated that one run converged on an island of trees with a lower mean log likelihood than the other two, and this run was not used. To confirm that an adequate number of generations were run, we also ran two analyses for 10×10^6 generations (one sampling every 1000 generations and the other sampling every 5000 generations). These analyses reached stationarity after between 0.5×10^6 and 1.35×10^6 generations, and recovered trees with topology and likelihoods consistent with the two shorter searches. The estimated phylogeny was the majority-rule consensus tree of the 19,551 post burn-in trees taken from these four searches. Each analysis used four chains and default priors (i.e., Dirichlet for substitution rates and state frequencies; uniform for the gamma shape parameter and proportion of invariable sites; all topologies equally likely a priori; branch lengths unconstrained: exponential). Individual clades with Bayesian posterior probabilities (Pp) $\geq$ 0.95 were considered to be strongly supported (for justification see Wilcox et al. 2002; Alfaro et al. 2003; Erixon et al. 2003; Huelsenbeck and Rannala 2004).

ESTIMATING DIVERGENCE DATES

Divergence dates for clades were inferred to estimate rates of diversification for clades (e.g., Magallón and Sanderson 2001) and the relative timing of colonization of different elevations. We used penalized likelihood (PL; Sanderson 2002) to combine molecular branch lengths and ages of fossil taxa to estimate the absolute age of lineages within the Middle American clade. The topology and branch lengths from the Bayesian analysis of the combined data were used, along with the following fossil dates (which were used to constrain the minimum ages of extant clades): (1) Most recent common ancestor (MRCA) of Ranoidea, at least 36 million years old (Myo); Carroll (1988) and Holman (1998) mention ranid fossils in Middle Eocene (about 36–45 Myo). (2) MRCA of Bufonidae, at least 55 Myo; Duellman and Trueb (1986) mention *Bufo* in the Paleocene (55–65 Myo). (3) *Caudiverbera*-Myobatrachidae clade, at least 45 Myo; Carroll (1988) mentions fossils of *Caudiverbera* in the Lower Eocene (minimum 45 Myo). (4) MRCA of Hylidae and sister group, at least 55 Myo; according to Duellman and Trueb (1986), fossils of hylids date back to the Paleocene (55–65 million years ago [Mya]). Because it is uncertain if these fossils can be assigned to a clade within extant hylids, we used these fossils to date the age of the common ancestor of Hylidae and its putative sister group (the clade including hemiphractids and some leptodactylids). (5) MRCA of the Pelodryadinae-Phyllomedusinae clade, at least 28 Myo; Sanmartín and Ronquist (2004) summarize

evidence suggesting that the last terrestrial connection between Australia and South America is at least 28 Myo. Thus, the split between the Australasian pelodryadines and South American phyllomedusines must be at least 28 Myo. (6) *Acris-Pseudacris* clade, at least 15 Myo; Holman (2003) suggested that the extinct fossil taxon *Acris barbouri* is likely the sister group to extant *Acris* species and is at least 15 Myo (end of Miocene Hemingfordian North American Land Mammal Age; NALMA). Thus, the split between *Acris* and *Pseudacris* is at least 15 mya. Various *Pseudacris* fossils are known from the middle Miocene Barstovian of North America (about 12–15 Myo; Holman 2003), but given that these fossils cannot be assigned confidently to clades within *Pseudacris* we did not use this information (and given that the *Acris* fossils already show that the MRCA of the *Acris-Pseudacris* clade is at least 15 Myo). (7) MRCA of Asian and European *Hyla*, at least 16 Myo; Sanchiz (1998a) noted fossil *Hyla* similar to extant *H. arborea* and *H. meridionalis* in the Lower Miocene of Austria (about 16 Myo). We assume that these *Hyla* are closely related to the *Hyla* presently extant in Europe. However, we cannot assume that these fossils are younger than the MRCA of the extant European species. We assume instead that the MRCA of the clade of European and Asian *Hyla* is at least 16 Myo based on these European fossils. (8) *H. squirella-H. cinerea* clade, at least 15 Myo; *H. goini* is a fossil species from the Miocene Hemingfordian NALMA (15–19 Mya) thought to be closely related to, if not actually conspecific with, extant *H. squirella* (Holman 2003). Thus, we assume that the split between *H. squirella* and its sister species (*H. cinerea*) is at least 15 Myo. (9) *H. gratiosa-H. versicolor* clade; *H. miocenica* is thought to be closely related to *H. chrysocelis* and *H. versicolor* and occurs in the early Miocene Barstovian (14–16 Mya; Holman 2003). *Hyla miofloridana* (Miocene, Hemingfordian NALMA; 15–19 Mya) is similar to *H. gratiosa* (Holman 2003). Among the species included in the r8s analysis, *H. avivoca*, *H. gratiosa*, and *H. versicolor* form a clade. Thus, we assume that the MRCA of this clade is at least 15 Myo. We differ from the PL analyses of Smith et al. (2005) in that we exclude *H. swanstoni* (which suggests that North American *Hyla* are at least 33 Myo), given that Sanchiz (1998b) questioned the assignment of this taxon to *Hyla* (Faivovich et al. 2005).

PL was implemented using r8s (ver. 1.6 for Unix; Sanderson 2003). To estimate a chronogram, the r8s method requires that at least one divergence date on the tree be specified, rather than being a mere constraint on the minimal age of a clade. We considered the root of the tree to be equivalent to the MRCA of Neobatrachia. We used two dates to bracket the age of Neobatrachia, 160 and 100 mya. The sister group of Neobatrachia likely includes Pipoidea and/or Pelobatoidea, based on morphological (Ford and Cannatella 1993) and molecular evidence (Hoegg et al. 2004). Both groups appear in the fossil record in the late Jurassic (minimum about 160 Myo; Zug et al. 2001). Neobatrachian fossils

are absent in the fossil record before the late Cretaceous (65–99 Mya). Nevertheless ranoids are widespread in Africa and Asia (Duellman and Trueb 1986), suggesting that neobatrachians originated before the separation of Africa and South America (about 100 Mya; Brown and Lomolino 1998). The r8s analysis used the truncated Newton method with bound constraints; to avoid local optima, each analysis was started from five different random combinations of divergence times. Smoothing parameters were chosen by cross-validated assessment, using values from 10^0 to $10^{4.5}$ in increments of 0.5 for the exponent. Analyses using both root ages (100 and 160 Myo) selected 3.16 as the optimal smoothing value. Each PL analysis estimated a "chronogram" with branch lengths proportional to time; these chronograms were used in the comparative analyses that require branch length estimates.

To address the sensitivity of the results to the fossil calibration points, we performed a set of PL analyses that did not use any fossil constraints (only the age of the root was specified). We then performed a correlation analysis between the ages estimated with and without fossil constraints for the six clades used in the analyses of diversification rates. To assess the sensitivity of the estimated dates to the particular topology and branch lengths used (i.e., the majority-rule consensus of the post burn-in trees from the combined Bayesian analysis), we estimated these ages on each of 390 trees from the Bayesian analysis, sampling one tree every 50,000 generations from among the 19,551 post burn-in trees pooled from the four replicate searches. We then repeated the PL analysis on each tree using the two root ages (using the best-fitting smoothing parameter for each root age from the original analysis, but not retesting for each replicate), and estimated the standard deviation for the estimated age of each clade using the "profile" command in r8s. Finally, we note that for all of our analyses that use estimates of absolute clade age (i.e., analyses of time of colonization and diversification rate), it is the relative ages of the clades that are actually important, and not the specific ages of individual clades.

LOCAL AND REGIONAL SPECIES RICHNESS

Elevational patterns of species richness were estimated at both local and regional scales, where regional refers to all of Middle America. Estimates of local species richness at 39 sites in Middle America are presented in our (in online Supplementary Appendix S2). Data on species composition were taken mostly from Duellman's (2001) monograph (his table 73 and distributional appendices). However, nine of the communities listed there were excluded (mostly because species data were unavailable). Twelve additional communities were added to increase representation of northern México and Honduras, with nine sites in México (data from Duellman 2001) and three in Honduras (data from McCranie and Wilson 2002). These estimates are based on intensive fieldwork by Duellman (2001) and other researchers at these sites,

prior to major amphibian declines in the region (e.g., Lips 1999; Lips et al. 2004). We acknowledge that there is no strict definition for the exact boundaries or sizes of these local sites. These sites may encompass more than one microhabitat (e.g., forest, stream, pond) but each seemingly occurs within a single elevational zone and general habitat type (e.g., cloud forest).

Following standard practice for elevational studies of species richness (e.g., Rahbek 1997), regional elevational patterns were estimated based on the total number of species occurring in different elevational bands (0–500 m above sea level, 500–1000 m, etc.), considering all of Middle America. The presence of species in each elevational band was estimated from elevational ranges for each species summarized by Duellman (2001; his appendix 4). Despite recent changes in generic-level taxonomy, the number of hylid species recognized in Middle America has remained relatively stable since the publication of Duellman's monograph (i.e., Duellman [2001] recognizes 160 species in Middle America whereas AmphibiaWeb [2006] recognizes 163).

We used the elevational ranges and midpoints for all species in the Middle American clade in the region to test whether empirical patterns of species richness differed from those expected given random distribution of species elevational ranges (i.e., the mid-domain hypothesis; Colwell and Hurtt 1994). We used the program Mid-Domain Null (McCain 2004) to shuffle the elevational ranges of species between the minimum and maximum elevational limits of the Middle American clade, given the empirical elevational range sizes and randomly generated elevational midpoints. One thousand replicates were used to generate upper and lower 95% confidence intervals for the expected values given stochastic placement of elevational ranges. This analysis used default settings, empirical range limits, and sampling of species without replacement.

In addition to examining patterns of species richness for the entire hylid fauna collectively, we also examined patterns for individual clades. We examined patterns of local and regional species richness after dividing species into those of the Middle American clade (about 80% of hylid species in Middle America) and those from other major hylid clades.

RATES OF DIVERSIFICATION

To investigate the possibility that the mid-elevation peak in species richness is caused by increased diversification rates in montane clades (the montane species pump hypothesis), we tested for a relationship between the elevational midpoints and diversification rates of hylid clades in Middle America. Given that diversification rate is calculated for clades rather than species, we used clades as basic units in this analysis. Use of clades as basic units also allowed us to incorporate all species in the Middle American clade even though not all species in the region were included in the phylogeny. Other methods for estimating diversification rates

could be used in theory, but these are potentially compromised by not having all or most of the species represented on the tree. In general, we used genera as terminal units, and our results supported most genera as monophyletic (given the species that were included). However, we treated as a single unit the clade consisting of *Bromeliohyla*, *Duellmanohyla*, and *Ptychohyla*, given that these genera are somewhat interdigitated in our phylogeny (see below). Similarly, we treated *Anotheca* and *Triprion* as a single unit given the apparent nonmonophyly of *Triprion*. We also divided *Plectrohyla* into its two very distinctive subclades (the traditionally recognized *Plectrohyla* and the former *Hyla bistincta* group). We excluded Middle American clade lineages distributed primarily in temperate North America (i.e., the *Acris-Pseudacris* and Holarctic *Hyla* clades) to reduce the confounding of elevational and latitudinal effects. In summary, we used 11 clades as terminal units (Fig. 1).

Although these clades show some variability in their elevational ranges, most clades are predominately montane or lowland, and these elevational distributions are associated with different reproductive modes that are largely consistent within clades (based on data in Duellman 2001). Specifically, species in the montane clades typically breed in streams (e.g., *Charadrahyla*, *Isthmohyla*, *Plectrohyla*, *Ptychohyla*), whereas lowland lineages generally breed in ponds (e.g., *Smilisca*, *Tlalocohyla*).

We excluded other clades of hylids present in Middle America that do not belong to the endemic Middle American clade (i.e., *Dendropsophus*, *Scinax*, *Hypsiboas*, *Hyloscirtus*, *Phrynohyas*, phyllomedusines). These clades collectively represent only 18.5% of the overall hylid species richness in Middle America (30 of 160 species), and they represent relatively little in situ diversification in Middle America. Instead, they seem to represent multiple, recent invasions from South America (Duellman 2001; Faivovich et al. 2005; Wiens et al. 2006). Furthermore, these clades do not contribute to the overall hump-shaped pattern of diversity in Middle American hylids (see Results).

We initially estimated the diversification rate of each clade using the method-of-moments estimator for stem groups (eq. 6 of Magallón and Sanderson 2001). The method-of-moments estimator is advantageous relative to the widely used maximum likelihood estimator (i.e., $\log[n]/t$) because the latter estimator assumes that the extinction rate is negligible. Given that the extinction rate (ε) is unknown, we used 0.90 as an upper limit and 0 as a lower limit, following Magallón and Sanderson (2001). Both values gave similar results, and we present only those results using the lower limit.

The stem group begins where a clade first splits from its sister taxon, whereas the crown group includes only the monophyletic group encompassing all extant members of a clade (see fig. 1 of Magallón and Sanderson 2001). Importantly, use of the crown group assumes that the deepest divergence within each clade has
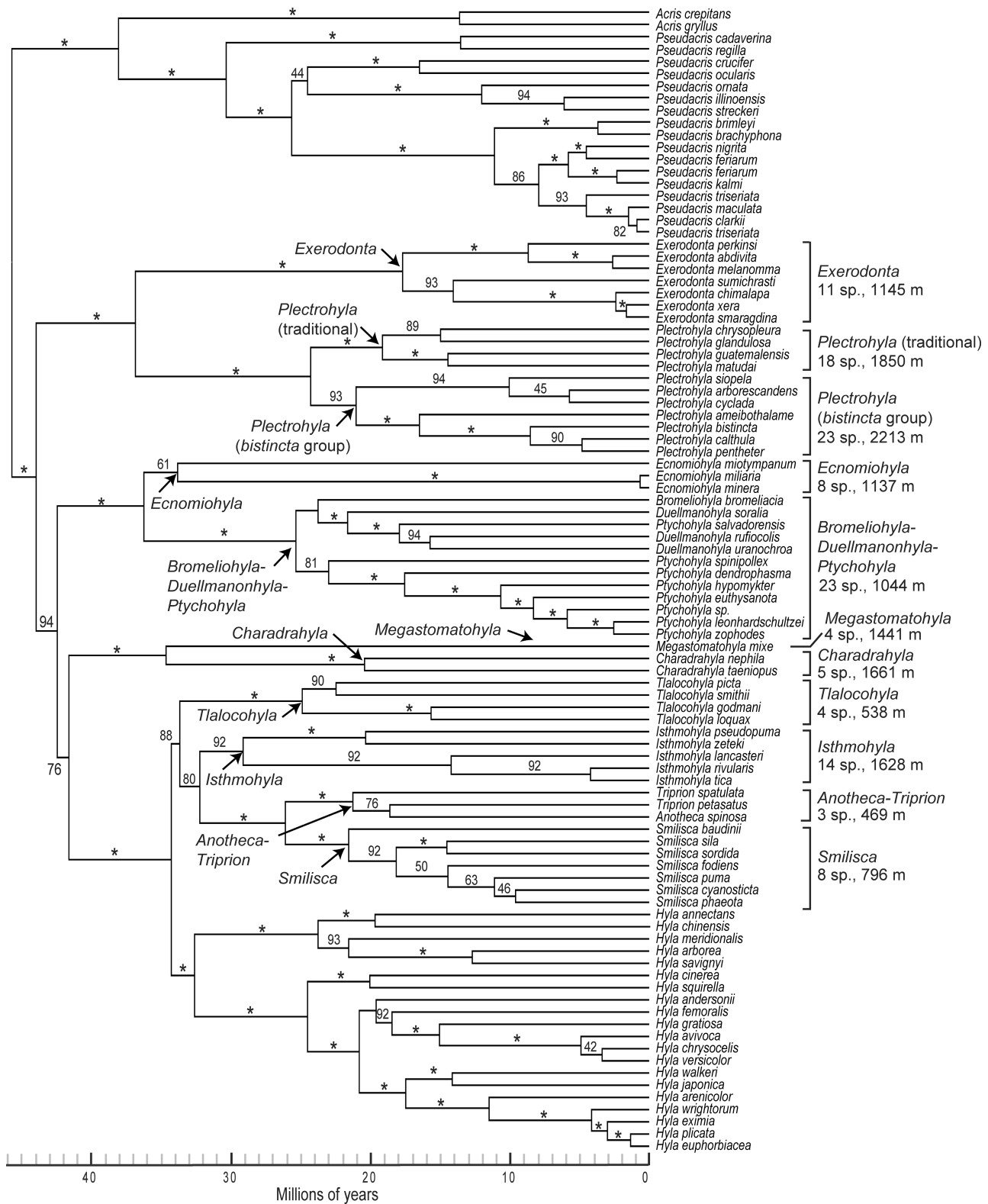
Figure 1. Phylogeny and divergence date estimates (chronogram) for the Middle American clade of hylids (tribe Hylini), based on a combined, partitioned Bayesian analysis of 10 nuclear and mitochondrial genes and a root age of 100 Mya (for Neobatrachia) for the penalized likelihood analyses used to estimate divergence dates. Asterisks adjacent to nodes indicate Bayesian $Pp \geq 0.95$, whereas numbers indicate $Pp < 0.95$ (Pp multiplied by 100). Clades used in analyses of diversification rates are indicated on the tree and at right, along with the total number of described species in each clade and the average of the midpoints of their elevational ranges (see also Table 1). Standard deviations for estimated ages of select clades are provided in Table 1. Outgroup taxa outside the Middle American clade and Hylidae have been excluded for ease of presentation.

been sampled, whereas use of the stem group does not. We used the stem group estimator exclusively given that our sampling of species within genera may not be sufficient to ensure that the basal divergence within each one has been included.

The age of each clade was based on the estimated chronogram, and analyses were run using both root ages (100 Myo and 160 Myo). The elevational midpoint of each clade was calculated as the average of the elevational midpoints of all species assigned to that clade. We also performed a set of analyses using the reconstructed elevational midpoint of the ancestor of each clade (see below for methods for reconstructing ancestral values). The results of analyses using ancestral elevations were similar to those using average elevations, and are not reported separately. The match between average and ancestral values of elevational midpoint for each clade is close ($r^2 = 0.868$; $P < 0.001$). We also performed a set of analyses in which the ancestral elevation for each was estimated based on reconstructions only including species within the clade (rather than estimates based on including all the species in the study); these local estimates were very similar to the global estimates ($r^2 = 0.925$; $P < 0.001$) and showed a similar relationship between elevation and diversification rates.

The relationship between diversification rate and elevation was evaluated using standard linear regression of the raw data and of phylogenetically independent contrasts (Felsenstein 1985b), given that elevational range and diversification rate might both be shared between clades due to common ancestry. Contrasts were calculated using COMPARE (ver. 4.6, Martins 2004). The phylogeny and branch lengths were obtained from the chronogram, but pruned to include only the MRCA of each clade. Analyses were repeated with root ages of 100 and 160 Myo (note that the same root age used to estimate the diversification rate was used for branch lengths for independent contrasts), and with equal branch lengths. Because COMPARE uses only two decimal places, each diversification rate was multiplied by 1000.

Linear regression analysis of diversification rates could be problematic if the hump-shaped pattern were due to low diversification rates of clades at the highest and lowest elevations (i.e., a hump-shaped pattern of diversification rates with elevation might not be detected). However, we found that the elevational midpoints of clades were either at lower elevations (500–700 m) or intermediate elevations (1000–2000 m), and that there were no clades with elevational midpoints that corresponded to high elevations with relatively low diversity (i.e., 2500–3500 m). Furthermore, the independent contrasts analysis essentially addresses the relationship between changes in elevation and changes in diversification rate, for which a linear relationship is predicted.

TIMING OF COLONIZATION AND SPECIES RICHNESS

To determine whether more species occur at elevations that have been inhabited by hylids for longer periods of time (i.e., the time-for-speciation effect), we estimated the approximate time when each generalized elevational band was first occupied. This required reconstructing the elevational midpoint of each node on the chronogram, and determining the oldest node for each elevational band. However, given that habitats have likely shifted in elevation over time due to climate change, it may be more accurate to think of this analysis as addressing the relative timing of colonization of different elevationally structured habitats and climatic zones, rather than the absolute timing of colonization of specific elevational ranges.

Ancestral values of elevational midpoint were reconstructed for 79 species of the Middle American clade using the linear generalized least squares (GLS) method of Martins and Hansen (1997) implemented with COMPARE (ver. 4.6, Martins 2004). This method is equivalent to maximum likelihood reconstruction for continuous characters (Schluter et al. 1997; Garland et al. 1999; Martins 1999). Ancestral values generated by COMPARE were mapped onto each pruned chronogram and the earliest date in which each elevational band was first colonized in Middle America was estimated. Thus, for the elevational band 1000–1200 m, we reconstructed values of elevational midpoint for each node on the chronogram, and the age of the oldest node with a reconstructed value in that range was considered to be the putative age of first colonization of that band.

In some cases, an extant species appeared to be the earliest inhabitant of an elevational band. Rather than assuming that these species colonized this elevational band as soon as they originated or else only in the past few years, we arbitrarily used half the length of the branch of these species as the estimated time. Alternate approaches seem to have little impact on the results (Smith, unpubl. data).

Analyses were performed using both chronograms. The 79 species that were included represented all sampled species of the Middle American clade but excluding all but five extralimital *Acris* and *Pseudacris* species. Furthermore, species of the *Acris-Pseudacris* clade and Holarctic *Hyla* clade (only six in Middle America) were excluded from estimates of both colonization ages and species richness for these analyses, given that they are extralimital or nested among extralimital species.

The estimated time-since-colonization of each band was regressed against the natural log of the total number of extant species occurring in that band (assuming that species richness increases exponentially over time; Magallón and Sanderson 2001). This procedure was repeated with elevation divided into 200-m and 500-m bands. We present results using 200-m bands; results using 500-m bands are qualitatively similar. We did not use independent contrasts for these analyses given that the basic units in question are elevational bands and not taxa. Nevertheless, we acknowledge that the timing of colonization of different elevational bands need not be fully independent of each other because of the phylogenetic

history of the taxa (i.e., multiple elevational bands may be affected by a single shift in elevational range by a given taxon). Time was also natural-log transformed.

A given elevational band may acquire species through multiple dispersal events. Therefore, we also performed an analysis in which we used the summed estimated ages of each inferred colonization of each elevational band, rather than merely the oldest colonization. This analysis was complicated somewhat by the fact that some inferred elevational changes on the tree span more than one elevational band. In such cases, each band spanned by that transition was counted as a colonization of that band (otherwise one may infer that some bands were never actually colonized).

We also acknowledge that our estimates of ancestral elevations may be highly imprecise and that our estimated ages of when specific elevational bands were first occupied may be somewhat problematic as well. However, the major qualitative results of these analyses should be robust to sources of error in the precise estimates; our main result is simply that, among the extant lineages, the montane regions of Middle America (specifically the intermediate elevations) were colonized prior to the lowlands. Nevertheless, we addressed the potential errors in reconstructing the ancestral elevations in three ways.

First, we used Pagel's λ (phylogenetic correlation) test (Pagel 1999) to determine whether the elevational midpoint data showed significant phylogenetic signal and whether they fit the Brownian motion model assumed by the GLS approach. To test for significant phylogenetic signal, we estimated the log likelihood of the data when λ is at its estimated maximum likelihood value and when λ is set to zero, assuming the null hypothesis that the data are not phylogenetically conserved. We used the likelihood ratio test statistic to assess the difference between these models. We then tested whether the data differ significantly from the Brownian motion model, testing the estimated maximum likelihood value and the value when λ is set to one, assuming the null hypothesis that the data are evolving under a purely Brownian process. Analyses were performed with the program Continuous, version 1.0d13 (M. Pagel, University of Reading, Reading, U.K.), using the chronogram with a root age of 100 Mya.

Second, to address the robustness of the results to an alternate method of ancestral trait reconstruction (which also takes into account error in estimating the topology and branch lengths), we performed a Bayesian analysis of ancestral states (Huelsenbeck et al. 2003). In current versions of MrBayes, this requires constraining a given clade to be present in all topologies, adding the trait of interest as another character to the data matrix, and rerunning the phylogenetic analysis. We constrained the strongly supported basal clade containing all species of the Middle American clade (exclusive of *Acris* and *Pseudacris*) to be present in all estimated trees and evaluated the probabilities of different states being present at this node. We added elevation to our molec-

ular dataset as an ordered multistate character with five states (the maximum allowed), with each state encompassing a 600-m elevational band (i.e., 0–600 m, 601–1200 m), and applied to this character the generalized likelihood model of Lewis (2001). The method then provides an estimate of the posterior probability of each state being reconstructed at that branch, given the uncertainty in trait reconstructions on each tree and the variability in tree topologies and branch lengths among the post burn-in trees.

Third, as an independent line of evidence, we reconstructed the evolution of life-history traits on the tree. Montane and lowland hylids show important differences in their life-history traits; montane species generally deposit their eggs in streams and have stream-adapted larvae (a few deposit their eggs in arboreal sites) whereas lowland species typically deposit their eggs in standing water and have pond-adapted larvae (Duellman 2001). Transitions between highland and lowland habitats may be constrained by these life-history adaptations and by the paucity of ponds on steep mountain slopes and of high-gradient streams in lowlands. If our reconstructions of general elevational habitat are correct, then we expect them to be concordant with our reconstruction of larval habitat (i.e., an ancestral node reconstructed as montane should also be reconstructed as stream breeding). We obtained data on general larval habitat use from Duellman (2001) for each species included in the phylogeny, coded them as breeding in ponds, streams, or arboreal sites, and mapped this character on the tree (as unordered) using parsimony and maximum likelihood with Mesquite, version 1.05 (Maddison and Maddison 2004). Maximum likelihood analyses used branch lengths from the two chronograms.

Simulations suggest that estimates of ancestral trait values using GLS are generally quite accurate (Martins 1999). However, these same simulations also suggest that current methods for estimating the error in these reconstructions are not. Therefore, we present only the reconstructed values, and not estimates of error.

Results
PHYLOGENY AND DIVERGENCE TIME ESTIMATES

Analysis of the 10 genes combined included 7390 aligned nucleotide positions (but 988 ambiguously aligned sites were excluded, mostly in the ribosomal genes), of which 3123 were variable and 2452 were parsimony informative. The Bayesian topology (Fig. 1) and parsimony results were generally similar and all nodes strongly supported by parsimony bootstrapping were well supported by Bayesian *Pp* (parsimony results not shown). Our phylogenetic results were largely consistent with previous phylogenies and taxonomies (e.g., Faivovich et al. 2005; Smith et al. 2005; Wiens et al. 2005), but with the addition of several new taxa. Most of the added species were placed within the genera expected by prior taxonomy. However, *Ptychohyla salvadorensis* was

Table 1. Species numbers, elevational midpoints (meters above sea level), estimated ages of the stem group (in millions of years before present, followed by the standard deviation obtained from 390 trees from the Bayesian analysis), and diversification rates for clades of Middle American hylids. The clade ages and diversification rates were calculated using two root ages for Neobatrachia (100 Mya and 160 Mya). For diversification rates, we present values based on the stem group estimates and assuming both a negligible extinction rate ($\varepsilon = 0$) and high extinction rate ($\varepsilon = 0.90$). Sister clades have stem groups of equal age.

Clade	Total species	Elevational midpoint	Age		Diversification rate			
			100 Mya	160 Mya	100 Mya		160 Mya	
					$\varepsilon=0$	$\varepsilon=0.90$	$\varepsilon=0$	$\varepsilon=0.90$
Plectrohyla (traditional)	18	1849.53	24.21±4.08	25.15±7.91	0.119	0.041	0.115	0.039
Plectrohyla (bistincta group*)*	23	2213.61	24.21±4.08	25.15±7.91	0.130	0.048	0.125	0.046
Exerodonta	11	1144.86	36.75±4.08	43.95±9.13	0.065	0.019	0.055	0.016
Ecnomiohyla	8	1136.88	36.16±4.37	48.85±9.06	0.058	0.015	0.043	0.011
Bromeliohyla-Duellmanohyla-Ptychohyla	23	1044.37	36.16±4.37	48.85±9.06	0.087	0.032	0.064	0.024
Megastomatohyla	4	1440.50	34.57±5.47	44.98±8.14	0.040	0.008	0.031	0.006
Charadrahyla	5	1661.10	34.57±5.47	44.98±8.14	0.047	0.010	0.036	0.007
Tlalocohyla	4	537.50	33.61±5.29	46.92±9.73	0.041	0.008	0.030	0.006
Isthmohyla	14	1628.04	32.16±4.80	45.46±9.02	0.082	0.026	0.058	0.018
Smilisca	8	795.63	26.02±3.49	38.16±7.66	0.080	0.020	0.054	0.014
Anotheca-Triprion	3	468.83	26.02±3.49	38.16±7.66	0.042	0.007	0.029	0.005

placed within *Duellmanohyla* and *Anotheca spinosa* was placed within *Triprion*.

The phylogeny and divergence time estimates (Fig. 1; Table 1) suggest that the predominately montane clades (e.g., Charadrahyla, Ecnomiohyla, Exerodonta, Plectrohyla) generally arose and diverged before the predominately lowland clades (e.g., Smilisca, Tlalocohyla). The relative ages of clades seemingly are insensitive to the fossil calibration points (correlation between ages of clades estimated with and without fossil calibration points is 0.996).

LOCAL AND REGIONAL SPECIES RICHNESS

The regional species richness of hylid frogs in Middle America shows the expected hump-shaped pattern (Fig. 2A), with the highest species richness at intermediate and lower elevations (500–1500 m). In contrast, local species richness (Fig. 2B) is highest at low elevations (about 50 m), relatively high at intermediate elevations (about 1000–1300 m), and decreases at higher elevations. These patterns seem to result from the combination of two clade-specific patterns. When considered alone, the species of the Middle American clade show a strong hump-shaped pattern at both the local and regional scales, whereas other hylid clades in Middle America show their highest local and regional species richness at low elevations, and are absent at the highest elevations (Fig. 2C, D). The observed species richness patterns in the Middle American clade do not fit within the upper and lower 95% prediction curve expected under the mid-domain null model (Fig. 3). Instead, the empirical species richness curve is higher at low and intermediate elevations and lower at high elevations than predicted by the

null model. Within the Middle American clade (Fig. 1; Table 1), the hylid fauna consists of eight clades that occur predominately at intermediate elevations, including all of the most species-rich clades and three clades that are relatively species poor that occur at lower elevations (Anotheca-Triprion, Smilisca, Tlalocohyla).

RATES OF DIVERSIFICATION AND SPECIES RICHNESS

There is a significant positive relationship between the elevational midpoint of clades and their rate of diversification (Fig. 4). Results are shown using independent contrasts with a chronogram root age of 100 Mya, assuming negligible extinction, and using estimated branch lengths and the average elevational midpoint for species in the clade ($r^2 = 0.450$, $P = 0.0239$). However, results are similar using both the raw data and the independent contrasts, assuming low and high rates of extinction, using both chronograms (root ages of 100 Mya and 160 Mya), using both equal branch lengths and chronogram branch lengths for calculating independent contrasts, and using both average and reconstructed elevational midpoint values (range among all results $r^2 = 0.398$–0.634; $P = 0.0034$–0.0374).

## TIMING OF COLONIZATION AND SPECIES RICHNESS

Reconstructed elevational midpoints are mapped onto the chronogram in Figure 5 using GLS. This analysis suggests that the intermediate elevations (1000–1500 m) were colonized early in the history of the Middle American clade, and that lower and higher elevations have been colonized more recently, from ancestors that occurred at intermediate elevations. There is a significant relationship between time-since-colonization of each elevational
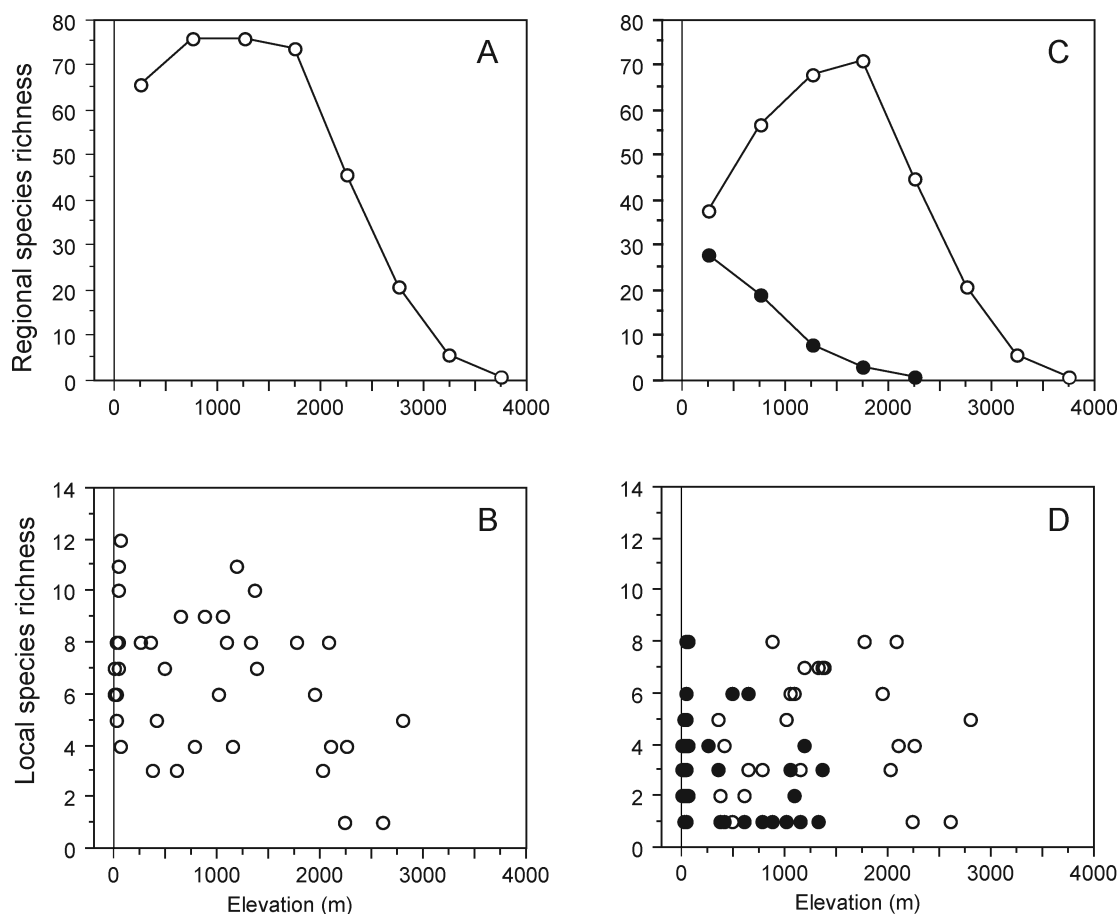
Figure 2. Regional (A) and local (B) species richness for all hylid frogs in Middle America. Regional (C) and local (D) species richness for hylid frogs in Middle America, separated by clade. Most hylid species belong to the Middle American clade of hylines (tribe Hylini; open circles), whereas the other species belong to several more distantly related clades (filled circles).

band and the current regional species richness of that elevation (Fig. 6), regardless of the root age used for the chronogram (100 Myo: $r^2 = 0.705, P < 0.0001$, 160 Myo: $r^2 = 0.740, P < 0.0001$). Results are similar using the summed ages of each inferred colonization of each elevational band (e.g., $r^2 = 0.852, P < 0.0001$ for 100 Myo).

At least four lines of evidence suggest that this result is not an artifact of inaccurate ancestral trait reconstructions. First, the estimated maximum likelihood value of phylogenetic correlation (λ) for elevational midpoints is 0.8796, suggesting strong phylogenetic signal. The log likelihood of the data under the hypothesis of no conservatism (λ = 0) is –625.04 and under the alternate hypothesis (estimated λ) is –605.02. The likelihood ratio test statistic is 20.02 ($P = 2.5 \times e^{-10}$). These results indicate that the evolution of elevational ranges does show significant phylogenetic conservatism, and imply that the GLS reconstructions may not be grossly inaccurate. However, we acknowledge that these data do not show a perfect fit to the Brownian motion model either, as there is a significant difference between the log likelihood under the estimated λ (–605.02) and the value under the hypothesis of a
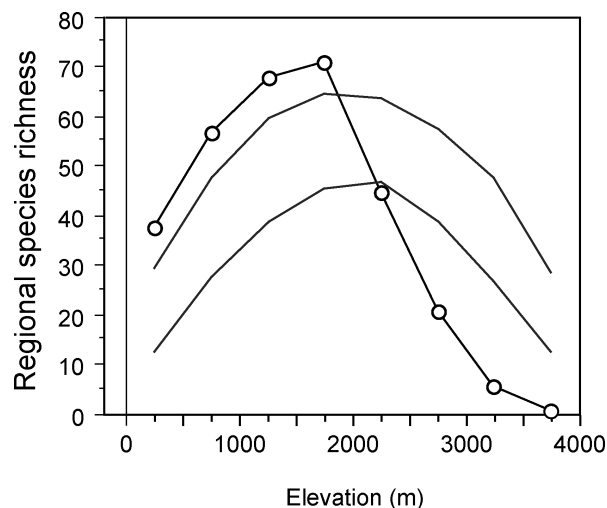
Figure 3. Regional species richness of the Middle American clade, with their predicted range under the mid-domain null model. Thin lines are the upper and lower 95% prediction curves generated from 1000 replicates and randomly shuffling the midpoints of species' elevational ranges.
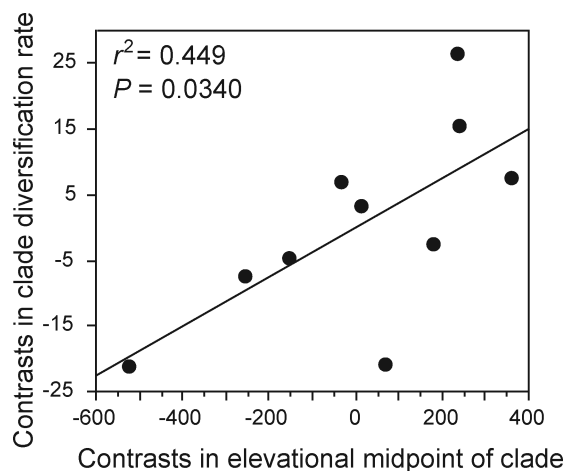
Figure 4. The relationship between independent contrasts of the diversification rate of clades (×1000) and the elevational midpoints of these clades, using the chronogram with a root age of 100 Mya to estimate diversification rates and branch lengths for the independent contrasts. Values are based on negligible extinction rates and diversification rate estimates based on the stem group. Results are similar using a root age of 160 Mya, when the raw data are analyzed without independent contrasts, using equal branch lengths to calculate contrasts, and assuming high extinction rates.

purely Brownian model ($\lambda = 1$; –615.12), with a likelihood ratio test statistic of 10.11 ($P = 6.92537 \times e^{-6}$).

Second, similar results are obtained using Bayesian reconstructions, using a different model of character evolution and incorporating uncertainty in both the topology and branch lengths. Bayesian ancestral state reconstruction suggests that the 600-m elevational band with the highest posterior probability for the common ancestor of the Middle American clade (exclusive of *Acris* and *Pseudacris*) is between 601 and 1200 m ($Pp = 0.951$), consistent with the results from GLS (1041 m; Fig. 5).

Third, our taxon sampling is biased against reconstructing this ancestor as montane, given that the montane clades are sampled poorly relative to the low-elevation clades (e.g., average proportion of species included is 38% for the eight primarily montane clades and 96% for the three primarily lowland clades; Fig. 1), and the numbers of montane and lowland taxa included in this analysis are nearly equal (39 montane and 40 lowland).

Fourth, reconstruction of larval habitat on the phylogeny supports the idea that montane habitats were colonized early in the history of the group in Middle America and that lowland species were derived from montane ancestors. Reconstructions using both parsimony and likelihood are concordant and show an early transition to stream breeding (as in most extant montane species) and a later transition to pond breeding (as in most extant lowland species). Stream breeding was the ancestral state in the *Plectrohyla*, *Ptychohyla*, and *Charadrahyla* clades, and in the common ancestor of the Middle American clade (exclusive of the *Acris*

clade), corresponding to the reconstructions of montane habitat (Fig. 5). There was then a return to pond breeding in the ancestor of the *Hyla, Isthmohyla, Smilisca*, and *Tlalocohyla* clades, corresponding to the reconstructed shift to lowland habitats (Fig. 5).

Geological evidence is also consistent with an early colonization of montane habitats in Middle America by hylids, given that the major mountain ranges where most montane hylid genera occur today (e.g., Sierra Madre Oriental, Sierra Madre del Sur, nuclear Central American highlands) are known to have been present since the Laramide Revolution of the late Cretaceous to early Tertiary (Campbell 1999). Thus, our reconstructions of the timing of montane colonization (Fig. 5; mid-Tertiary) are broadly consistent with an origin of the relevant mountain ranges prior to the origin of the montane clades.

Discussion
### EVOLUTIONARY CAUSES OF ELEVATIONAL SPECIES RICHNESS PATTERNS

Along with the latitudinal gradient, elevational diversity gradients are one of the most widespread patterns of species richness (Brown and Lomolino 1998). In some ways, elevational diversity patterns may be more relevant to conservation than the latitudinal gradient, given that elevational patterns apply to much smaller spatial scales and may be more relevant to the design of reserves within a region. In recent years, there has been renewed interest in documenting and explaining elevational patterns of species richness (e.g., Stevens 1992; Rahbek 1995, 1997; Lomolino 2001; McCain 2005; Oomen and Shanker 2005). However, there has been very little attempt to address the evolutionary causes of this pattern, even though all species richness patterns must ultimately arise through the evolutionary and biogeographic processes of speciation, extinction, and dispersal. In this study, we address the evolutionary causes of elevational species richness patterns, using the relatively well-studied hylid frogs of Middle America (Duellman 2001).

Recent surveys suggest that regional species richness tends to reach its peak at intermediate elevations in many groups of organisms (e.g., Rahbek 1995; McCain 2005; Oomen and Shanker 2005). Middle American hylids show this pattern as well, especially in the largely endemic Middle American clade (Fig. 2).

Our results suggest that this pattern is caused by two factors. First, we find some evidence that rates of diversification are higher in montane clades than lowland clades (the "montane species pump" hypothesis). Although previous studies have suggested that tropical montane regions are important crucibles of recent speciation (e.g., Rahbek and Graves 2001; Hall 2005), none have explicitly compared diversification rates across elevations and related these differences to elevational patterns of species richness. However, Weir (2006) did find evidence for higher diversification rates in higher elevation groups of Neotropical birds.
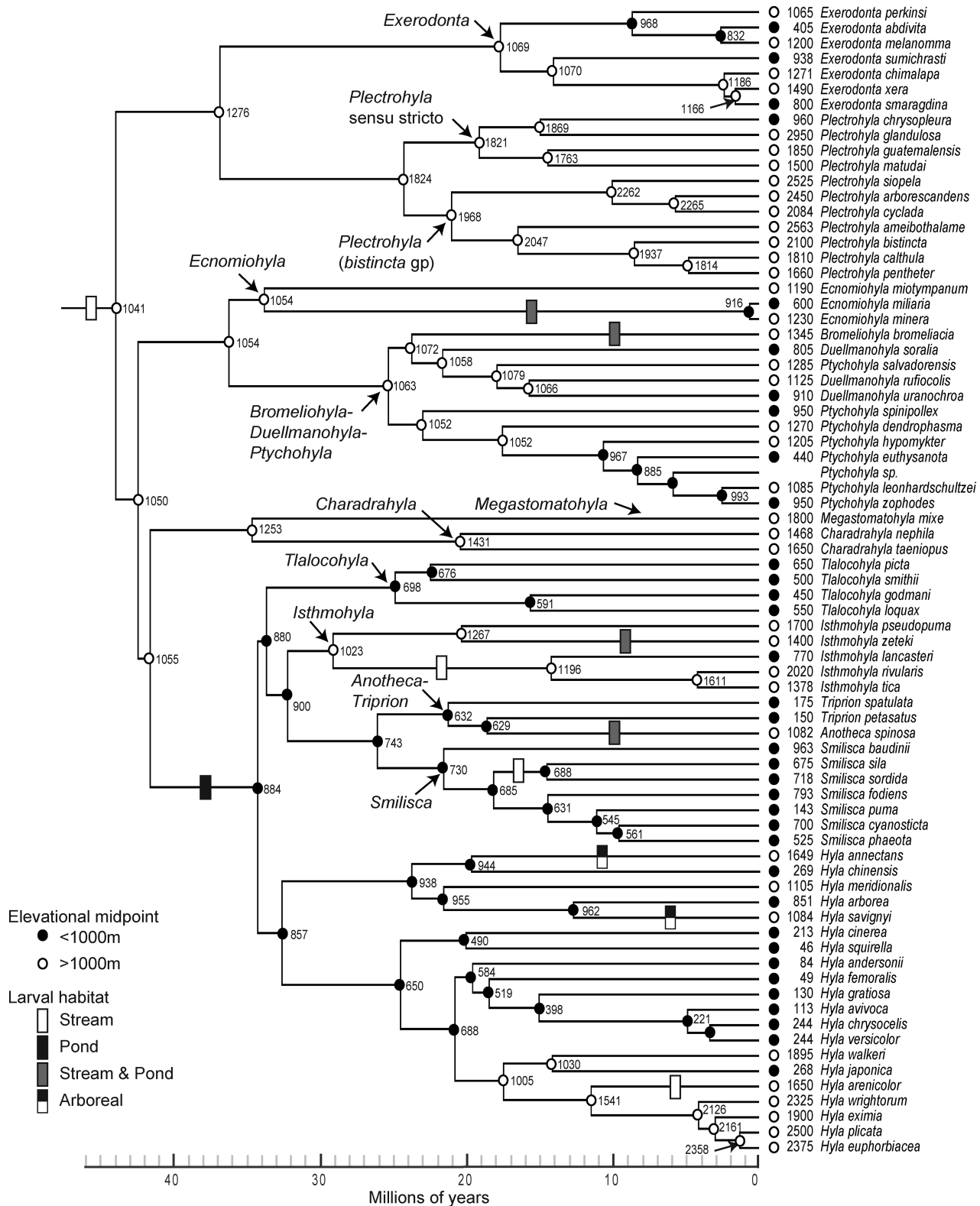
Figure 5. Reconstruction of the elevational midpoint of species ranges on a chronogram for hylid frogs of the Middle American clade (using a root age of 100 Mya), showing an early colonization of intermediate elevations and subsequent spread to lower and higher elevations in some lineages. Filled circles indicate nodes (reconstructed) or species (observed) with elevational midpoints greater than or less than 1000 m, based on reconstruction of elevation as a continuous trait. Note that five species of the basal, extralimital *Acris-Pseudacris* clade were included in this analysis but are not shown here given space constraints (*A. crepitans*, *P. clarkii*, *P. ocularis*, *P. cadaverina*, *P. regilla*); this clade occurs primarily in lowland habitats.
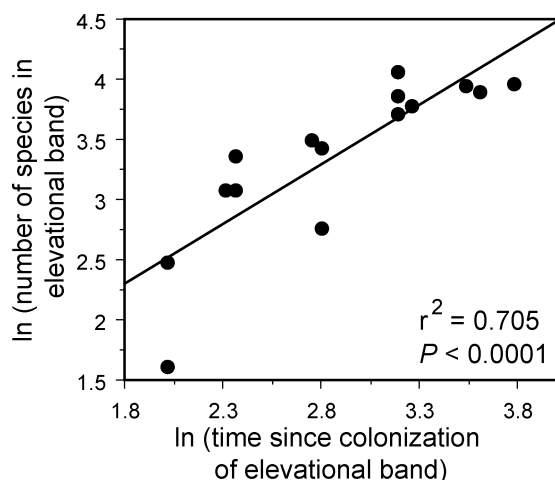
Figure 6. Regression of the time-since-colonization for each 200-m elevational band versus the number of species occurring in that band for hylid frogs of the Middle American clade. The timing of colonization of each band was based on the reconstructions shown in Figure 5 (using a chronogram with a root of 100 Mya), but results are similar using a root age of 160 Mya.

The relatively high diversification rates within montane clades may be associated with vicariant fragmentation of cloud forests and similar montane habitats through cycles of climatic cooling and warming. This hypothesis will require further analysis within these montane clades.

Second, we also find evidence supporting a "time-for-speciation" effect on elevational patterns of species richness (the "montane museum" hypothesis), in which regions or habitats that are colonized more recently have fewer species because they have had less time for speciation to occur (Stephens and Wiens 2003). We find that lower montane regions (about 1000–1500 m) were colonized relatively early in the evolutionary history of the Middle American clade, that most montane species are derived from this early colonization of montane regions, and that lowland lineages of the Middle American clade are derived more recently from within this highland clade (Fig. 5). This pattern seemingly has created a strong relationship between the estimated time when each elevational band was first colonized and the number of species presently there (Fig. 6). Stephens and Wiens (2003) tested the time-for-speciation effect among continents, but we confirm that it is also relevant to species richness patterns in different habitats (i.e., elevations) within a continental region (for a very different example see McPeek and Brown 2000). These results also underscore the need for using phylogenies to interpret patterns of species richness. For example, the secondary invasion of the lowlands from the highlands would not be apparent without a phylogenetic analysis.

Our results show support for both the "montane species pump" and "montane museum" hypotheses, but do not necessarily favor one over another. Instead, we suggest that both hypotheses describe factors that combine to create the hump-shaped diversity pattern in Middle American treefrogs. For example, among the 11 subclades that we considered, some of the highest diversification rates were in the highest-elevation clades, and some of the lowest were in the lowest-elevation clades. This factor clearly contributes to the high species richness in montane regions. However, another important factor is that eight of these 11 clades are montane, and seven of these eight montane clades seemingly are derived from a single invasion of highland regions early in the phylogenetic history of the Middle American clade. Furthermore, the eight montane clades are (on average) older than the lowland clades (32.35 vs. 28.55 Myo). Are the observed differences in ages and numbers of clades more important than the differences in diversification rates in explaining high montane species richness? This is a difficult question to resolve, but, as a thought experiment, we can ask: what if we reversed the relative number of lowland and highland clades and their ages, but kept the average diversification rates the same? Would we still observe more species in montane regions? If there were eight lowland clades with the observed average diversification rate (0.054) of the lowland clades but with the average age of the highland taxa (32.35), and only three highland clades with the same average diversification rate (0.078) but each with the younger age of the lowland taxa (28.55), then we expect that the higher montane species richness would be a replaced by a bias toward more lowland species (46 vs. 29 species expected). This thought experiment suggests that the greater age and number of montane clades may be as important as the observed elevational differences in diversification rates in explaining the pattern of high montane species richness (but note that a higher montane diversification rate in the distant past could also contribute to the larger number of montane clades, in addition to the early colonization of montane regions). We anticipate that other studies will find these two factors to be important in other clades and regions, although the importance of each factor may vary in individual cases.

The hump-shaped species richness pattern in Middle America is highly asymmetric, with many more species at lower elevations than higher elevations. In fact, the overall pattern of species richness in hylids seems to reflect the combination of two specific patterns; a strong hump-shaped diversity pattern in the endemic Middle American clade and a decrease in species richness with elevation in several other hylid clades in the region (Fig. 2C, D). Most of these other hylid clades seem to have dispersed into the tropical lowlands of Middle America from South America (Duellman 2001), where they are very species rich (AmphibiaWeb 2006). The clades include *Agalychnis* (subfamily Phyllomedusinae, with 52 species total), *Scinax* and *Dendropsophus* (hyline tribe Dendropsophini, with 189 species), *Phrynohyas* (hyline tribe Lophiohylini, with 62 species), and *Hypsiboas* (hyline tribe Cophomantini, with 145 species). The sole exception to this pattern is the

genus *Hyloscirtus* (tribe Cophomantini), which apparently dispersed from montane regions in South America to montane regions in lower Central America. These clades contribute substantially to species richness at lower elevations (Fig. 2), especially in Costa Rica and Panama. However, most of these clades show very little in situ diversification within the Middle American lowlands (except for phyllomedusines) and some evidence suggests that they have dispersed into Middle America relatively recently. For example, more inclusive phylogenetic analyses by Faivovich et al. (2005) and Wiens et al. (2005, 2006) suggest that most species in these clades in Middle America have their closest relatives in South America, including *Dendropsophus ebraccatus*, *Phrynohyas venulosa*, *Scinax boulengeri*, *Hyloscirtus colymbus*, *Hyloscirtus palmeri*, *Hypsiboas rufitela*, and *Hypsiboas boans*. Many species are also widespread in South America (e.g., *Phrynohyas venulosa*, *Dendropsophus microcephalus*, *Hypsiboas boans*, and *Scinax ruber*), and most of the species in these clades are confined to lower Central America (only nine of the 30 species reach as far north as México; Duellman 2001). Species that do seem likely to have originated within Middle America include *Dendropsophus robertmertensi* and *D. sartori*, *Hypsiboas crepitans* and *H. rosenbergi*, and many species of *Agalychnis* (Faivovich et al. 2005; Wiens et al. 2005). Although divergence date estimates are unavailable for many of these taxa, our PL analyses for all taxa in our tree (results not shown) suggest that the common ancestor of *Agalychnis* and of the *Dendropsophus* species pair are each considerably younger than the two lowland clades of the Middle American clade (i.e., *Agalychnis* ancestor 14–21 Myo; ancestor of *D. robertmertensi*-*D. sartori* 6–8 Myo; vs. 26–38 for the *Smilisca* clade and 25–37 for *Tlalocohyla*; Table 1). In summary, several clades that are very species rich in tropical South America have failed to diversify extensively in lowland Middle America, possibly because of their relatively recent colonization and the corresponding "time-for-speciation" effect. Thus, the hump-shaped diversity pattern in the Middle American clade and the limited species richness of these other clades in the lowlands may both be explained, at least in part, by a common factor (i.e., the time-for-speciation effect).

The recent spread of these predominately South American clades into lowland Middle America may also help explain their failure to disperse into and diversify in the Middle American highlands, and this failure also contributes to the hump-shaped diversity pattern in Middle America. These lineages may have had too little time to adapt to montane climatic regimes and the limited availability of pond-breeding sites at higher elevations (Middle American species of *Dendropsophus*, *Hypsiboas*, *Phrynohyas*, *Scinax*, and Phyllomedusinae generally are pond breeders; Duellman 2001).

How widespread might the evolutionary causes of elevational species richness patterns in Middle American hylids be? Address-ing this question will require similar studies of other groups of organisms. A recent study (Weir 2006) found evidence for higher diversification rates in Neotropical birds in montane regions, but did not relate this finding to elevational patterns of species richness or the relative timing of colonization. A parallel study by Wiens et al. (2007) on Middle American plethodontid salamanders has revealed an early colonization of mid-elevations, a strong relationship between time of colonization and species richness of each elevational zone, and no significant relationship between diversification rates of clades and their average elevational midpoints. If the phylogenetic pattern found in Middle American hylids and plethodontids is widespread, this would suggest even greater need for conservation of montane habitats, as these areas would be expected to harbor substantial phylogenetic and genetic diversity as well as endemism and species richness.

ELEVATION, CLIMATE, AREA, AND THE MID-DOMAIN MODEL

Our study says little regarding climate, area, or the mid-domain effect. We briefly discuss these factors here. By itself, climate is not a direct cause of species richness patterns, even if there are strong correlations between climate and species richness in many cases. Instead, the importance of climate will depend on how it affects speciation, extinction, and dispersal (e.g., Wiens and Donoghue 2004). Specialization of lineages to different climatic regimes may play at least two important roles in our system. First, climatic specialization may limit the dispersal of mid-elevation montane lineages into higher or lower elevations (e.g., Smith et al. 2005), and may be an important causal mechanism underlying the time-for-speciation effect. Second, climatic specialization and climate change may together drive vicariance of montane habitats, leading to speciation and high montane diversification rates. These hypotheses will require further study to test.

Given that we have identified potential evolutionary explanations for the elevational species richness patterns, how do the observed patterns relate to climate? As a crude way of addressing this question, we obtained climatic data from the WORLDCLIM dataset (Hijmans et al. 2004, 2005) for all 39 local communities and performed linear regression of annual mean precipitation and annual mean temperature with local species richness. When considering all the species, local species richness is positively correlated with temperature ($r^2 = 0.148$, $P = 0.0156$) and precipitation ($r^2 = 0.480$, $P < 0.0001$). However, when considering only species in the Middle American clade (which makes up most species in Middle America and drives the hump-shaped regional diversity pattern), species richness shows a weak negative relationship with annual mean temperature ($r^2 = 0.099$, $P = 0.0513$) and no relationship with precipitation ($r^2 = 0.037$, $P = 0.2424$). Thus, the most obvious climatic variables are not strongly correlated with species richness patterns in this group.

Another potentially important factor is the relative areas of lowland and highland habitats in Middle America. Area is a well-established correlate of species richness patterns and has a more intuitive relationship with speciation than climate (e.g., Rosenzweig 1995; Losos and Schluter 2000). Using ArcView GIS 3.3 (Environmental Systems Research Institute, Redlands, CA, 1992), we found that about 44% of Middle America is over 1000 m and about 56% is below 1000 m. Thus, lowland habitats have somewhat greater area than highland habitats in Middle America, despite their lower species richness. Our results imply that time may be a more important factor than area in determining large-scale species richness patterns and that greater area alone does not lead to higher diversification rates, at least in this system.

Many recent studies on elevational species richness have focused on the mid-domain model as an explanation for these patterns (e.g., McCain 2005; Oomen and Shanker 2005). The basic idea of the model is that species ranges (whether latitudinal or elevational) are placed stochastically within a bounded range; for elevational patterns the ranges are bounded by the heights of the tallest mountains and by sea level. According to proponents of this approach, only patterns that deviate from the random model require biological explanation. Recently, Davies et al. (2005b) have argued that the mid-domain effect is an inappropriate null model because deviations from the null model can be generated by phylogenetic effects if closely related species tend to share similar geographic ranges. In other words, a species richness peak may develop stochastically wherever the group originates (i.e., the time-for-speciation effect; Stephens and Wiens 2003). The Middle American clade of hylids seems consistent with this hypothesis, with the highest species richness at intermediate elevations, which appears to be the ancestral elevational range for the group (Fig. 5). Furthermore, individual clades appear to have limited but often distinct elevational ranges (Fig. 5), suggesting phylogenetic effects rather than random distributions, and elevational distributions show significant phylogenetic signal across the tree based on Pagel's λ. Finally, elevational species richness patterns

in Middle American hylids do not seem to match the predictions of the mid-domain model (Fig. 3), even though the mid-domain model also predicts a mid-elevation hump.

WHY SO FEW SPECIES IN THE LOWLAND TROPICAL RAINFORESTS?

A surprising result of our study is that the local and regional species richness in lowland tropical rainforests in Middle America is similar to or less than that of the montane habitats. This result is surprising for several reasons. First, many correlative ecological studies predict the highest species richness in areas with the highest energy and productivity, such as lowland tropical rainforests (e.g., Francis and Currie 2003; Hawkins et al. 2003; Willig et al. 2003). Second, the highest local species richness of hylids overall occurs in lowland tropical rainforests in the Amazon basin (Duellman 1988; Rodriguez and Cadle 1990; Smith et al. 2005; Wiens, unpubl. data), where up to 36 hylid species may occur in sympatry (Santa Cecilia, Ecuador; Duellman 1978). Third, most of the clades that make up the high local species richness in lowland tropical rainforests of South America are also present in the tropical lowland forests of Middle America (including *Dendropsophus*, phyllomedusines, *Phrynohyas* and relatives, *Hypsiboas, Scinax*; Duellman 1978; Rodriguez and Cadle 1990). Fourth, the most hylid-rich lowland rainforest sites in South America (Santa Cecilia, Ecuador and Manu, Peru) have similar (if not lower) annual mean temperature and precipitation to the most hylid-rich lowland rainforest sites in Middle America (Barro Colorado Island Panama and La Selva and Rincon de Osa, Costa Rica), even though the South American sites have roughly three times as many hylid species (Table 2).

Overall, our results for hylids imply that local-scale environmental factors may not be enough to explain the high species richness of tropical rainforests, and that other factors may be involved instead. In support of this idea, it seems likely that much speciation among lower elevation hylids in Middle America is allopatric, given that many extant sister species are largely or

Table 2. Comparison of climatic characteristics and local hylid species richness at the most species-rich tropical lowland sites in Middle America and South America. Climatic data were obtained from the WORLDCLIM dataset (Hijmans et al. 2004, 2005).

Locality	Hylid species	Annual mean temperature (°C)	Annual mean precipitation (mm/year)	Literature source (for species)
South America				
Santa Cecilia, Ecuador	36	25.4	3670	Duellman (1978)
Manu National Park, Peru	35	25.1	3031	Rodriguez and Cadle (1990)
Middle America				
La Selva, Costa Rica	12	26.1	4147	Scott et al. (1983)
Rincon de Osa, Costa Rica	11	26.2	4504	Scott et al. (1983)
Barro Colorado Island, Panama	10	26.7	2757	Rand and Myers (1990)

Table 3. Apparent geographic modes of speciation in hylid frogs in Middle America inferred from geographic overlap of sister species. Sister species pairs were identified from the Bayesian phylogeny (Fig. 1), except for *Dendropsophus robertmertensi* and *D. sartori* (Wiens et al. 2005). We only included species pairs for which all the relevant species in the group are represented in the phylogeny. All species pairs involve species that occur predominately in low and/or intermediate elevations. Geographic overlap was estimated from maps in Duellman (2001); species with no geographic overlap are considered allopatric. Note that not all sister pairs are strongly supported, and that range shifts after speciation can obscure the signature of different geographic modes (e.g., Barraclough and Vogler 2000).

Sister species	Geographic overlap
Dendropsophus robertmertensi-D. sartori	Allopatric
Exerodonta smaragdina-E. xera	Allopatric
Smilisca cyanosticta-S. phaeota	Allopatric
Smilisca sila-S. sordida	Mostly allopatric, partial overlap
Tlalocohyla picta-T. smithii	Allopatric
Tlalocohyla godmani-T. loquax	Allopatric
Anotheca spinosa-Triprion petasatus	Allopatric

entirely allopatric (Table 3). In other words, many new lineages do not appear to be generated at the local scale, but rather at the regional scale. We suspect that the high local species richness of hylids at rainforest sites in the Amazon Basin may be caused (at least in part) by the co-occurrence of several species-rich clades over long time scales, clades that are each of similar age to the Middle American clade (e.g., phyllomedusines, Cophomantini, Lophiohylini, *Dendropsophus*, *Scinax*).

Why does the local species richness of Middle American lowland rainforests fail to converge on that of lowland forests in South America? Several different factors may be involved. Although there has been sufficient time for considerable species richness to develop in Middle America (>160 hylid species), most of this species richness is confined to the highlands. Perhaps the two most important phenomena to explain are (1) why did so few clades invade lowland habitats? and (2) why are there no older clades in the lowlands?

Dispersal between highlands and lowlands may be limited by specialization to montane climatic zones, especially in the tropics (e.g., Janzen 1967). Niche modeling for one of the highland clades generally supports this hypothesis (Smith et al. 2005). The shift between highland and lowland habitats may also be constrained by reproductive modes. Specifically, the major clades of montane hylids in Middle America are stream breeders (e.g., *Charadrahyla*, *Plectrohyla*, *Ptychohyla*), whereas most of the lowland species are pond breeders (e.g., *Smilisca*, *Tlalocohyla*).

Extinction may also have reduced the diversity of the lowland hylid fauna in Middle America. This is difficult to support or reject, given that hylids have little or no fossil record in Middle America. PL analyses show that some lowland clades are old in an absolute sense (about 30 Myo; Table 1, Fig. 1), even if they are young relative to the highland clades, suggesting that at least some of the lowland hylid fauna has been present for relatively long periods of time. Our suspicion is that extinction, possibly associated with fluctuating sea levels, may have eliminated one or more early hylid lineages that first entered Middle America as lowland pond breeders. Outside of the Middle American clade, most of the major hylid clades are predominately pond breeders (implying that pond breeding is the ancestral condition; Duellman 2001) and a basal, lowland pond breeding clade (*Acris-Pseudacris*) is present in North America but not Middle America.

Conclusions

Elevational patterns of species richness are global in extent and important for conservation but remain largely unexplained and unexplored from an evolutionary perspective. In this article, we address elevational diversity patterns from a phylogenetic perspective in a diverse tropical fauna, the hylid frogs of Middle America. We find that Middle American hylids show their highest species richness at mid-elevations, a pattern seen in many groups of organisms in many regions (e.g., mammals, plants). In hylids, this pattern seems to reflect both differences in diversification rate (higher in montane clades) and in the relative timing of colonization into different elevational regimes and the subsequent time available for speciation (the "time-for-speciation effect"; Stephens and Wiens 2003). Our study is among the first to directly relate patterns of diversification rates to elevational patterns of species richness within a clade, and to document an elevational "time-for-speciation" effect (see also Wiens et al. 2007) Both factors may be important in explaining the widespread hump-shaped pattern of elevational species richness in many organisms and regions.

Our results also have implications for explaining the high local species richness of lowland tropical rainforests. Given the modest species richness of hylids in the wet lowland rainforests of Middle America, we suspect that local-scale environmental variables alone may not be sufficient to explain why some rainforest sites do have very high local species richness (e.g., in the Amazon basin). Our results also imply that attempts to understand patterns of diversity in tropical regions might benefit from focusing on broad-scale patterns of historical biogeography in addition to phylogeographic studies within or between closely related species.

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Supplementary Material

The following supplementary material is available for this article:

Appendix S1. GenBank accession numbers for DNA sequences analyzed in this study.

Appendix S2. Estimates of local species richness at 39 sites in Middle America based on data summarized by Duellman (2001) and McCranie and Wilson (2002).

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