Research

Ecography 33: 343–354, 2010
doi: 10.1111/j.1600-0587.2010.06281.x
© 2010 The Authors. Journal compilation © 2010 Ecography
Subject Editor: Ken Kozak. Accepted 6 April 2010
*The first two authors contributed equally.

Using regional comparative phylogeographic data from snake lineages to infer historical processes in Middle America

Juan M. Daza*, Todd A. Castoe* and Christopher L. Parkinson

J. M. Daza and C. L. Parkinson (cparkins@mail.ucf.edu), Dept of Biology, Univ. of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, USA. – T. A. Castoe, Dept of Biochemistry and Molecular Genetics, Univ. of Colorado School of Medicine, Aurora, CO 80045, USA.

Understanding how historical processes have either similarly, or differentially, shaped the evolution of lineages or biotic assemblages is important for a broad spectrum of fields. Gaining such understanding can be particularly challenging, however, especially for regions that have a complex geologic and biological history. In this study we apply a broad comparative approach to distill such regional biogeographic perspectives, by characterizing sets of divergence times for major biogeographic boundaries estimated from multiple codistributed lineages of snakes. We use a large combined (mitochondrial gene sequence) phylogeographic/phylogenetic dataset containing several clades of snakes that range across Middle America – the tropical region between Mexico and northwestern South America. This region is known for its complex tectonic history, and poorly understood historical biogeography. Based on our results, we highlight how phylogeographic transition zones between Middle and South America and the Nicaragua Depression appear to have undergone multiple episodes of diversification in different lineages. This is in contrast to other examples we find where apparently a single vicariant period is shared across multiple lineages. We specifically evaluate the distributions of divergence time estimates across multiple lineages and estimate the number of temporal periods of lineage diversification per biogeographic break. Overall, our results highlight a great deal of shared temporal divergence, and provide important hypotheses for yet unstudied lineages. These multi-lineage comparisons across multiple spatial and temporal scales provide excellent predictive power for identifying the roles of geology, climate, ecology and natural history in shaping regional biodiversity.

Historical biogeography, conservation biology, evolutionary ecology, and global climate change biology all require information about how historical patterns and processes have shaped lineage diversification at various spatial and temporal scales. It is important to understand how specific historical processes, and specific biogeographic boundaries, may have differentially impacted lineages or various components of biotic assemblages. The convergence of molecular phylogeographic datasets with robust approaches for estimating lineage divergence times has enabled an outgrowth of comparative phylogeographic research that may address such questions about differential biological responses of lineages. It is becoming increasingly clear that large comparative phylogeographic datasets may provide an excellent way to use multiple independent lineages simultaneously to infer models of historical divergence across landscapes (Bermingham and Moritz 1998, Arbogast and Kenagy 2001, Hickerson and Meyer 2008). These, in turn, may represent broad and generalizable models for projection onto other unstudied taxonomic groups, and even larger biotic assemblages. This insight from comparative analyses are particularly important for areas with either vague geological or tectonic information, or where little historical consensus is available (Riddle et al. 2008, Castoe et al. 2009).

In historical biogeography, vicariance and dispersal are considered the major forces that determine the divergence and geographic distribution of lineages (Nelson and Platnick 1981, Ree and Smith 2008, Ree and Sanmartín 2009). Neither of these two processes are, however, easily extracted from any single phylogeographic or phylogenetic pattern. Using coalescent models and the geographic structure of genetic data, it is possible to test the data against specific historical demographic scenarios that invoke vicariance or dispersal (Knowles and Carstens 2007, Richards et al. 2007, Hickerson and Meyer 2008). Such statistical approaches, however, are designed to address data associated with shallow phylogenetic trees, mostly at the intraspecific level. For deeper evolutionary events, different biogeographic methods are preferred. The most commonly used methods for such deep historical inferences search for evidence of congruence among different lineages and then explain this congruence (or lack of congruence) with vicariance/dispersal scenarios (Nelson and Platnick 1981, Ronquist 1997, Ree and Smith 2008, Ree and Sanmartín 2009).

Here we explore the application of comparative phylogeography beyond the intraspecific level to interpret regional historical processes in Middle America, and formulate new hypotheses to describe spatial–temporal lineage diversification on this broad regional scale. The core concept is that a biogeographic boundary may represent a spatial context over which a large number of lineage divergences may be temporally mapped (Leaché et al. 2007). For a given area, or axis of vicariance, the distribution of divergence times across lineages holds important biological information which can be used to interpret historical scenarios, and also predict the breadth of impact of historical processes on other components of biological communities (Hickerson et al. 2006a, b, Hickerson and Meyer 2008). Given the overlap of divergence time estimates for multiple related lineages, common patterns can be identified which may represent deep-reaching historical processes. These can be contrasted with patterns unique to particular lineages or groups of lineages. Using related lineages, such that a single phylogenetic tree can be used for the entire analysis (as in the current study), allow the predictions of temporal congruence to be largely independent of errors in calibration points (required for absolute time estimation). This is because estimates of relative time within a single dated tree are particularly robust, making such systems particularly ideal for testing for temporal correspondence of events among lineages (regardless of the accuracy of calibration points).

We applied this comparative approach to patterns of lineage diversification in snakes of Middle America – the tropical region between Mexico and northwestern South America. A fairly large number of lineages of snakes that range throughout Middle America have been sampled for the same mitochondrial loci, making them a good system for the current study. The exaggerated relief, diversity of habitats, and the dynamic tectonic and climatic history of the Middle America have all contributed to its high endemicity and diversity (Whitmore and Prance 1987, Jackson et al. 1996). Middle America has experienced a complex tectonic and geological history, and lies at the active junction of four major tectonic plates and several tectonic blocks (Iturralde-Vinent 2006, Marshall 2007). Deciphering the events that have historically shaped present-day biological diversity is complicated due to the continual physiographical reshaping of the region since the Cretaceous. Details of most of the tectonic history of Middle America still remain fragmentary and controversial (Coney 1982, Iturralde-Vinent 2006, Mann et al. 2007). This region has been the subject of intense biogeographic study for >40 yr, although the geological and climatic complexities of the region have precluded any clear consensus model describing the historical processes that generated its high taxonomic diversity (Savage 1982, Campbell 1999). For this reason, Middle America is an ideal setting for applying comparative phylogeographic data to infer patterns of lineage diversification, and the degree to which divergences are temporally coincident.

While many previous studies largely agree in identifying major biogeographic boundaries across Middle America (Marshall and Liebherr 2000, Perdices et al. 2005, Devitt 2006, Crawford et al. 2007, Castoe et al. 2009), there is no consensus of when these boundaries may have been relevant in splitting lineages. Furthermore, there is even less resolution on how many times, through history, these boundaries were effective in dividing lineages. Thus, our two aims were to 1) determine the degree to which these ecologically diverse lineages appear to share overlapping divergence times over the same biogeographic break, and 2) to estimate the number of discrete times in history each boundary may have led to lineage diversification. To address these questions, we examined Bayesian posterior distributions of divergence time estimates for a total of five major biogeographic boundaries across Middle America that are shared by multiple snake lineages, totaling 28 individual phylogeographic breaks. We also used an approximate Bayesian computation approach, using a hierarchical coalescent model, to infer the discrete number of divergence episodes for the same biogeographic breaks (Hickerson et al. 2006b, 2007). We use these results to infer how the distributions of divergence times may be related to an interpretation of historical biogeographic events that have broadly impacted the fauna in the region.

Material and methods

Target taxa

Our phylogenetic sampling includes multiple clades of snakes, including viperids and elapids, as well as non-venomous colubrids, that contain lineages distributed throughout Middle America. Previously, we had conducted a more restricted comparative study including three lineages of mesic highland-inhabiting viperid snakes in Middle America, and found evidence for shared divergences across three biogeographic boundaries in Middle America (Castoe et al. 2009). The current study includes expanded sampling of a greater ecological diversity of lineages, such as lowland groups (e.g. *Micrurus*, *Bothriechis schlegelli*, *Porthidium*, *Leptodeira*), habitat or dietary specialists (*Micrurus* spp., *Leptodeira nigrofasciata*) and habitat or dietary generalists (*Bothrops asper*, *Leptodeira septentrionalis*). Despite all lineages being snakes and thus sharing somewhat similar dispersal characteristics and life history traits, the lineages sampled do contain a diverse sampling of ecological groupings, and should be capable of providing a much broader perspective on co-diversification and speciation in Middle America than the previous study (Castoe et al. 2009).

We assembled a single combined data set, incorporating 28 nodes that correspond to clear phylogeographic breaks across Middle America (Fig. 1; Devitt 2006, Castoe et al. 2007a, 2009, Daza et al. 2009). The first major lineage comprises the subfamily Crotalinae. This group of venomous snakes is particularly diverse in the Neotropical region, and their phylogenetic relationships have been studied extensively (Parkinson et al. 2000, 2002, Castoe et al. 2005, 2009, Castoe and Parkinson 2006). Sequences for all relevant nodes of pitvipers were obtained from several published trees: *Agkistrodon*, (Parkinson et al. 2000), *Bothriechis schlegelii* (Wüster et al. 2002), *Crotalus durissus* (Wüster et al. 2005); *Lachesis* (Zamudio and Greene 1997), and highland pitvipers (Castoe et al. 2005, 2009). The second lineage includes members of the family Elapidae, and specifically includes representatives of the monadal and triadal coralsnake lineages (Castoe et al. 2007a). Finally, we

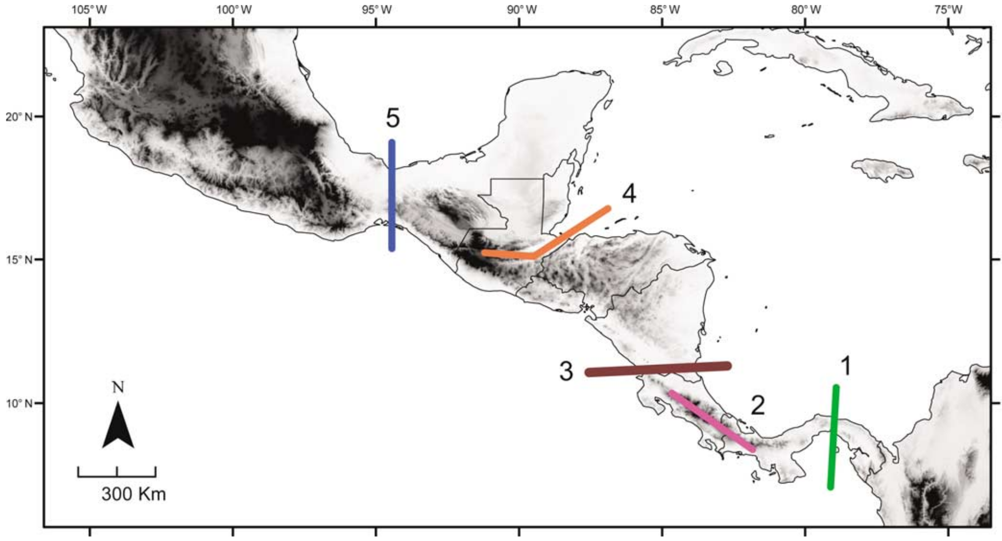

Figure 1. Map of Middle America showing the five major biogeographic boundaries analyzed in this study. [1] Middle–South America transition, [2] Talamanca Cordillera, [3] Nicaraguan Depression, [4] Motagua–Polochic Faults, [5] Isthmus of Tehuantepec.

compiled phylogenetic results of Neotropical colubrids from two sources: Devitt (2006) and Daza et al. (2009). The first includes the major lineages of the genus *Trimorphodon* (Colubrinae) and the second includes the major lineages of the genus *Leptodeira* (Dipsadinae).

Phylogenetic reconstruction

We assembled a molecular dataset that includes two mitochondrial protein-coding genes sequences from cytochrome b and NADH dehydrogenase subunit 4 (Supplementary material). Alignment of each gene was accomplished using Clustal W (Larkin et al. 2007) and corrected manually using GeneDoc 2.6 (Nicholas and Nicholas 1997). The dataset was partitioned by gene and codon position, and a different GTRΓI model for each partition was implemented (as selected by MrModeltest 2.3 using AIC, Nylander 2004). We used the package Beast 1.4.8, a Bayesian approach to estimate simultaneously the phylogeny and both relative and absolute divergence times (Drummond and Rambaut 2007).

Divergence time estimation

We estimated divergence times using two different approaches. First, we estimated relative divergence times (RT analysis thereafter) so that we could examine temporal congruence among nodes regardless of absolute time (and the calibration assumptions that accompany absolute time estimation). Second, we calibrated the molecular phylogenies using fossil and other calibrations to obtain absolute estimates of divergence dates (AT analysis). The strength of this two step approach is that we can first optimize rates using a Bayesian approach and obtain an ultrametric tree that relies only on the evolutionary process (and fitting of the relaxed clock model) and is unaffected by the uncertainty of the fossil record and other calibrations (Graur and Martin 2004, Heads 2005). This non-calibrated tree can be used to infer congruence in divergence time among lineages even when no nodal calibrations exist, and further used to evaluate the impact of adding calibration points on the correspondence of divergence time across nodes. Once inferences of temporal congruence are made, calibration points can then be added to estimate the absolute time scale of divergence events.

We implemented the Bayesian relaxed molecular clock method with uncorrelated lognormal rates among branches (Drummond et al. 2006), assuming a birth–death process for the speciation model. For the RT analysis we set the treeModel.rootHeight parameter to be 1 using a normal distribution with a mean = 1.0 and SD = 0.1 and used the program's default priors. For the AT analysis we used a lognormal prior for the treeModel.rootHeight parameter with a mean = 3.7 and SD = 0.3, and the following additional constraints: for the tMRCA of *Crotalus atrox* and *C. ruber* we used a uniform prior between 2.5 and 4.5 Ma; for the tMRCA of *Sistrurus* + *Crotalus* we used a uniform prior between 9.0 and 32.0; for the tMRCA of *Agkistrodon contortrix* we used a uniform prior between 5.0 and 32.0. The remaining priors were set to the program defaults for the AT analysis.

To ensure convergence of our estimates, we initiated four independent runs in Beast with random starting trees, and ran each for 10 million generations. Chains were sampled every 1000 generations, and convergence and stationarity were verified by examining likelihood scores and parameter estimates using Tracer 1.4 (Rambaut and Drummond 2007). Based on examination of trial runs in Tracer (which burned in prior to 2 million generations), the conservative burnin period of three million generations was used for final runs, and we combined the posterior samples from all four runs, and report the results of this combined posterior sample. We used the program TreeStat 1.2 (Rambaut and Drummond 2008) to summarize the Markov chain results for posterior divergence date estimates, and used an R script to create posterior density plots for nodes of interest.

Calibration points

We used four calibration points to obtain absolute date estimates for the molecular phylogeny. We constrained the origin of *Sistrurus* to be at least 9.0 Ma (Parmley and Holman 2007), and the origin of *Agkistrodon contortrix* to be at least 5.0 Ma (Holman 2000). We also constrained the divergence between the species *Crotalus ruber* and *C. atrox* to be between 2.5 and 4.5 Ma based on phylogeographic information on the vicariance between mainland and Baja California peninsula desert regions (Castoe et al. 2007b, 2009). Finally, based on the oldest colubrid fossil known, the root of the tree (the tMRCA of Colubroidea) was set to have occurred before 40 Ma (Rage et al. 1992, Head et al. 2005).

Shared divergence

To make inferences about the degree to which lineage divergences were coordinated in time we used msBayes (Hickerson et al. 2006a) to estimate the number of independent/discrete lineage divergence times per biogeographic break. MsBayes implements an approximate Bayesian computation approach using a hierarchical coalescent model where hyper-parameter estimation is utilized to discriminate the differences between time of divergence among pairs of taxa and variance in coalescent times (Hickerson et al. 2006b, 2007). For these analyses we included only the nodes that had more than two samples per taxon pair, based on the requirements of the program. For each analysis (corresponding to each break) we drew one million samples from the hyper-prior and, using the hierarchical approximate Bayesian computation acceptance/rejection algorithm, constructed the hyper-posterior from 2000 samples (tolerance = 0.002).

We contrasted the results obtained with msBayes and those based on posterior distributions of divergence dates and 95% credibility intervals obtained with Beast. Additionally, from posterior densities of individual lineage divergence times (from the Beast divergence dating analyses), we assemble pooled posterior densities for divergence times by combining data from multiple lineages (for a particular biogeographic break). For these pooled posterior densities, we summed the lineage-specific posterior density per unit time, across all lineages for each break. These distributions can be interpreted as the probability of divergence pooled over all lineages examined, and we discuss in the text how these may be useful particularly as informed priors for future studies. For interpreting co-divergence, however, these pooled posteriors may be somewhat misleading in that they may obscure multi-modal divergence posteriors of different lineages.

Results

Our estimate of phylogeny is consistent with recent studies that have specifically analyzed phylogenetic relationships among the taxa included here (Fig. 2, Wüster et al. 2002, 2005, Devitt 2006, Castoe et al. 2007a, 2009, Daza et al. 2009). The ultrametric trees we obtained with the RT and AT analyses yielded similar results (Supplementary material). When standardizing the root of the RT tree to be the absolute date obtained with the AT analysis, we did not find any difference in the relative timing of phylogenetic events between the two trees. In other words, adding calibration points did not affect our inferences of relative divergence times, as compared between lineages/nodes of codistributed lineages.

The AT analysis resulted in a tree with an overall depth of 41.8 Ma (95% credibility interval = 30.9–55.69). The divergence between Colubridae and Elapidae was estimated to be 38.8 Ma and the split between Old World and New World Elapids was inferred at 21.5 Ma and the same divergence but within crotalines was estimated at 19.4 Ma. Divergence times were consistent with those from Sanders and Lee (2008), Castoe et al. (2009), Daza et al. (2009) and Kelly et al. (2009). In contrast, our estimated divergence times were younger than those from Devitt (2006), Burbrink and Pyron (2008), Wüster et al. (2008), and Vidal et al. (2009).

The eight splits identified in the Middle–South America transition spanned from the early Miocene to the Pleistocene ($CI_{95\%} = 0.8–22.8$ Ma). The three lineage divergences across north and south areas of the Talamanca Cordillera occurred between 2.5 and 3.9 Ma ($CI_{95\%} = 1.4–5.4$). The divergences across the Nicaraguan Depression spanned from 4.1 to 8.8 Ma ($CI_{95\%} = 2.4–11.9$). The divergences across the Motagua–Polochic faults were estimated to have occurred between 3.8 and 6.8 Ma ($CI_{95\%} = 2.4–9.9$). Lastly, the five cladogenetic events identified across the Isthmus of Tehuantepec were estimated to be between 2.8 and 7.35 Ma ($CI_{95\%} = 1.5–10.1$). Out of the five phylogeographic breaks analyzed, three of them showed a strong correspondence in divergence times among multiple lineages (Fig. 3). Across the Isthmus of Tehuantepec break, with the exception of a single divergence estimate (for *Porthidium* species), the lineages appeared to have diverged around the same time. The cladogenetic events occurring at the other biogeographic breaks were not entirely coincident in time, although as we discuss in detail below, a number of strong patterns of congruence are evident.

The summary of estimated parameters using the Approximate Bayesian Computation algorithm is shown in Table 1. According to the msBayes results, the Talamanca Cordillera and the Motagua–Polochic Faults have likely undergone a single vicariant event. The pooled posterior distributions in these two breaks also showed a single peak, and the widely overlapping 95% CIs further supports a shared divergence (Fig. 3, 4). Small values of Ω, a parameter that measures the incongruence among divergence times along the same barrier, were found for these two biogeographic boundaries. In contrast, Ω value was highest for the Middle American–South American transition ($\Omega = 3.46$), followed by the divergences along the Nicaraguan Depression and the Isthmus of Tehuantepec (Table 1). Similarly, non-overlapping 95% CIs and multimodal pooled posterior distribution of dates were observed in these three phylogeographic breaks (Fig. 3).

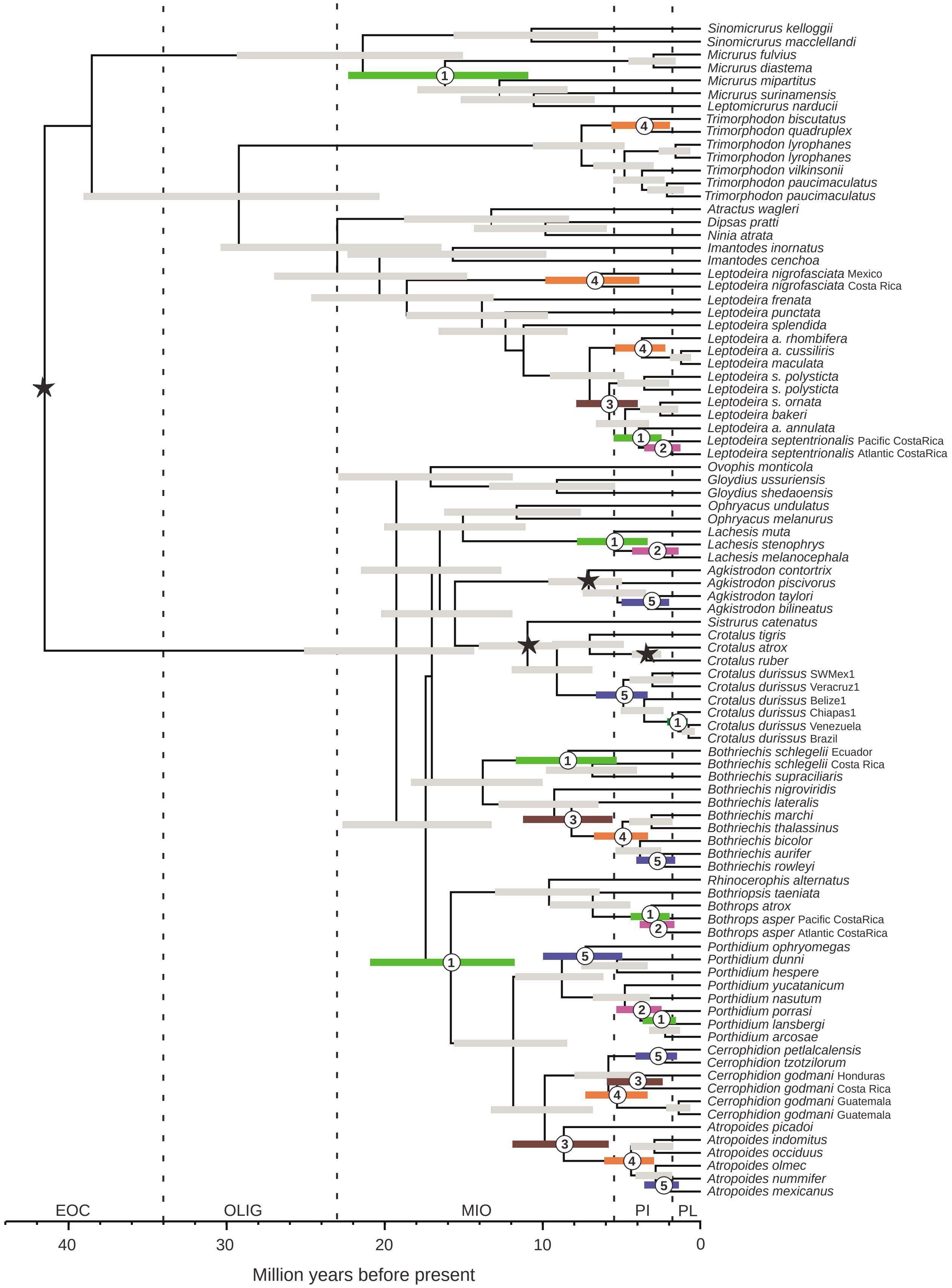

Figure 2. Dated tree obtained using the relaxed molecular clock method implemented in Beast. Node heights represent mean node ages (based on the combined posterior of four independent runs). Bars on nodes represent the 95% credibility interval of divergence times. Stars depict calibration points and numbers indicate nodes (Fig. 1) utilized in the congruence tests.

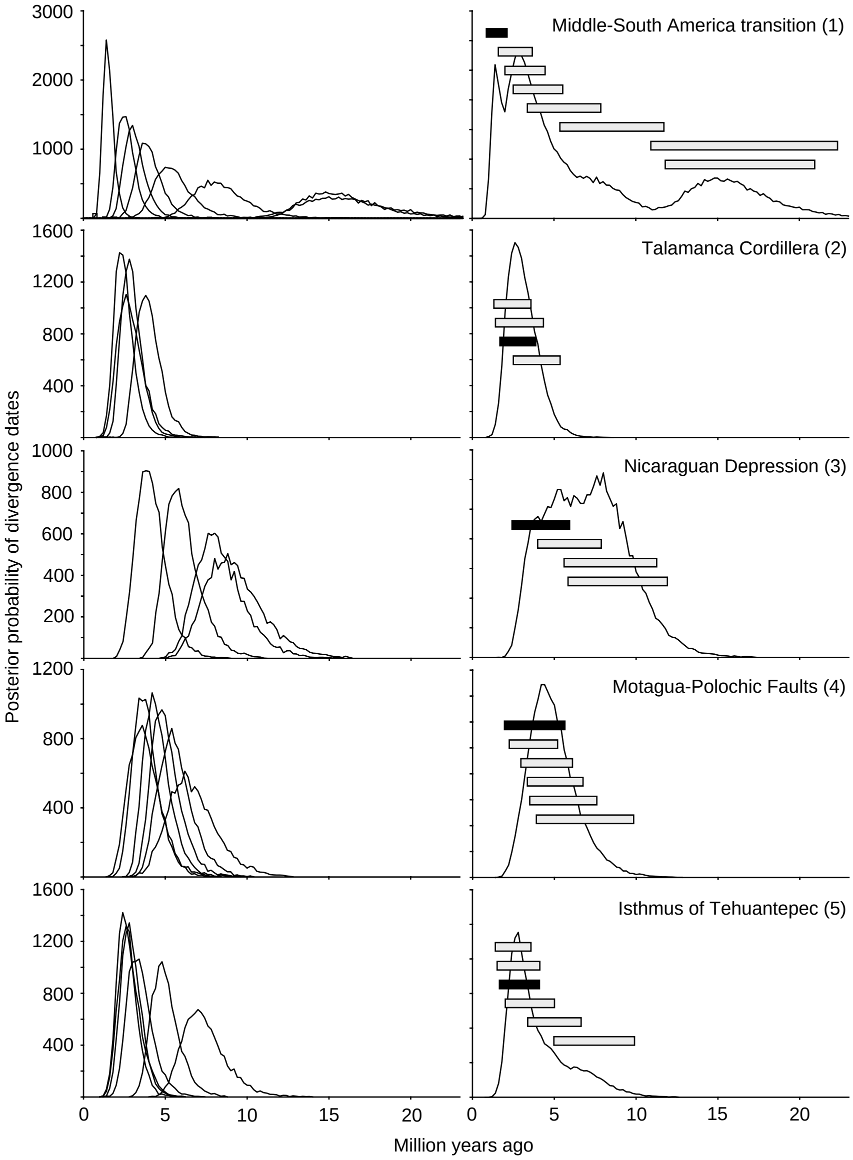

Figure 3. Left: posterior density plots of divergence times for snake lineages across five biogeographic boundaries in Middle America. Right: line represents the pooled posterior distribution of divergence times for each biogeographic barrier and the bars are the 95% credibility intervals for each lineage pair. Black bars indicate the lineage pairs not included in the msBayes analysis.

Discussion

Emerging hypotheses for Middle American speciation patterns

Despite consensus in the identification of major biogeographic boundaries that have shaped Middle America's biodiversity (Savage 1982, Marshall and Liebherr 2000, Morrone 2001), there has been little quantitative insight as to when these barriers may have led to diversification, in what temporal order, and especially the degree to which divergences were temporally coordinated. In total, our dataset included 28 individual cladogenetic events that span five biogeographic boundaries, bringing a fair amount

Table 1. Statistics summary from the msBayes runs. n = number of lineage pairs, Ψ = number of possible divergence times, Ω = parameter indicating the degree of discordance among divergence times.

Phylogeographic break	n	Ψ mode	Ψ mean	Ψ $CI_{95\%}$	Ω mean
(1) Middle–South America transition	7	1.87	2.03	1.00–3.88	3.46
(2) Talamanca Cordillera	3	1.27	1.39	1.00–2.36	0.12
(3) Nicaraguan Depression	3	1.58	1.91	1.07–3.00	0.59
(4) Motagua–Polochic Faults	5	1.01	1.49	1.00–3.29	0.13
(5) Isthmus of Tehuantepec	5	2.23	2.62	1.12–4.56	1.15

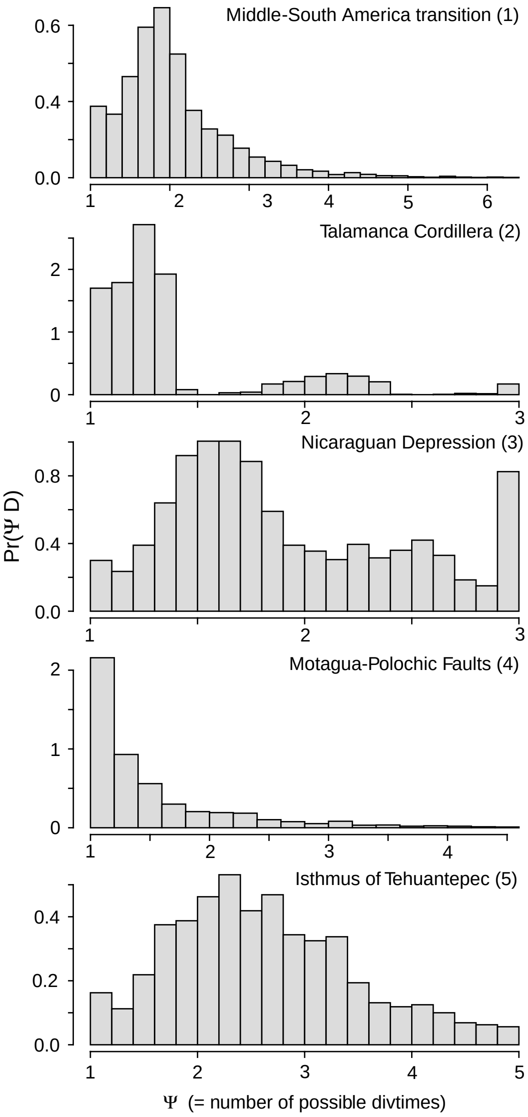

Figure 4. Posterior distribution of the number of divergence times for snake lineages across five biogeographic boundaries in Middle America.

of evidence to bear on inferences of regional diversification. Analysis of this dataset contributes new findings that appear to reject previous hypotheses of temporal diversification and further clarify historical biogeographic patterns in Middle American taxa. It thus presents an example of how such a comparative spatio–temporal approach may yield insight into the historical processes that have shaped a previously well studied yet poorly understood region.

Our results show that a majority of divergences across these diverse snake lineages appeared to be essentially coincident in time and space (Fig. 3). These findings suggest coordinated vicariance as a dominating force in speciation in the Middle American snake lineages studied. We found that some boundaries show great synchrony among diverse lineages (breaks in Talamanca, Motagua-Polochic and Tehuantepec). Other biogeographic breaks show evidence of multiple divergence time periods, evidenced by comparisons of credibility intervals and from the Approximate Bayesian Computation analyses; these multi-modal periods of divergences appear to characterize the breaks in Panama and Nicaragua.

Since the Miocene, Middle America has continually endured extensive terrain dynamics powered by tectonic activity, and we interpret our results as indicating that this dynamic process has been the dominant force in lineage diversification, and that such tectonically-driven vicariance explains the remarkably high degree of synchronization among such ecologically distinct lineages. In some cases, however, we do find evidence that intrinsic factors (e.g. dispersal and ecological features) may have also played roles in lineage divergence times, rather than purely extrinsic (e.g. tectonic) forces. Examples of this include divergences along the Isthmus of Panama, the divergence of *Bothriechis* across the Nicaraguan Depression, and the divergence of *Porthidium* along the Isthmus of Tehuantepec.

Estimates of relative and absolute divergence times

Comparative phylogeographic data coupled with divergence time estimates can illuminate much about a region's history. When divergence time estimates from independent studies are compared, however, we expect that substantial error in absolute divergence time estimates may often exist, due largely to differences in dating approaches and interpretations of the fossil and geological record (Heads 2005). In such comparative studies the precise absolute divergence times are often much less important than the estimates of the relative coordination of divergence events across lineages. This is particularly the case when inferring the number of discrete temporal windows of divergence, such as in the current study. To circumvent this issue here, we assembled multiple related lineages into a single dataset, and use this large combined dataset for jointly estimating divergence times and instances of co-divergence. Because the same calibration assumptions are applied to the entire tree, and also because relative divergence time estimates are highly robust within a tree, this approach can provide more precise estimates of the relative timing of divergence across lineages.

Our absolute dates are consistent with our previous work with these snakes (Castoe et al. 2009, Daza et al. 2009), most likely because of the very similar divergence dating strategies and calibrations, and they are also consistent with other independent studies (Sanders and Lee 2008, Kelly et al. 2009). A few studies, however, on particular lineages we included in our dataset have estimated older node ages than we have here, particularly for deeper nodes. We interpret these discrepancies in two ways. First, fossil snakes are extremely scarce for certain taxonomic groups and usually the available and non-ambiguous ones are used as calibrations for fairly recent cladogenetic events since most of the fossils come from the Pliocene and Pleistocene (Holman 2000); using recent calibrations points to estimate older nodes has been identified as a potential source of error (Ho et al. 2008). Second, discrepancies are likely to occur when different calibrations points are used. For example Wüster et al. (2005, 2008) and Devitt (2006) incorporated geological information (the emergence of the Mexican transvolcanic axis and the Isthmus of Panama, respectively) instead of fossil data (as in our case) for dating *Trimorphodon* and *Crotalus* divergences, respectively. Given the uncertainty in the fossil and geological record we would not necessarily expect multiple studies converge to the same dates (given the use of different calibrations). Because of the potential biases that different choices of calibrations may impose on estimates of shared divergence, our combination of all data into a single dataset, and our ability to rely on highly accurate inferences of relative divergence time across lineages (rather than calibration points), we expect our results of shared divergence to be particularly robust.

Divergence across the Middle America–South America transition (1)

The area between southern Honduras and northwestern Colombia is biogeographically important because it represents the intermediate land connection between the two main continental landmasses of the western Hemisphere, as well as the division between two oceans. The details of the dynamic connections between these landmasses from the Miocene onward, however, remain controversial. Recent phylogenetic and biogeographic evidence has uncovered complex patterns that suggest that biotic interchange between terrestrial fauna may have entailed multiple dispersal and vicariant events that occurred across a fairly broad time scale, far broader than the time surrounding the final closure of the Isthmus of Panama around 3.5 Ma (Collins et al. 1996, Bermingham and Martin 1998, Pennington and Dick 2004, Koepfli et al. 2007).

Our analyses indicate that recurrent diversification has occurred since the middle Miocene (Fig. 3, 4). MsBayes suggests two main episodes of diversification, although there is no strong demarcation between these two episodes based on the 95% CIs of divergence times. Although this study is limited in taxonomic scope, it is the first to include explicit temporal evidence across multiple terrestrial lineages, showing evidence (independent of assumptions of fossil calibrations, etc.) for multiple episodes of lineage divergence among the continents. A similar disparate pattern has been recently found for divergences between marine geminate species on either side of the isthmus (Marko 2002, Hurt et al. 2009), suggesting that both terrestrial and marine species responded in a similar broad temporal fashion. Collectively, our data and others' raise the question of whether pre-final closure dispersal/vicariant events of terrestrial lineages were all based on overwater dispersal, or instead, multiple transient land-connections joined parts of Lower Central America and South America prior to the final isthmus closure. Given the number of Pliocene and Miocene divergences associated with this region, the early transient land bridges hypothesis seems more likely, and warrants further evaluation with additional comparative data.

It is notable that the final closure date for the Panamanian Isthmus at ∼3.5 Ma has been commonly used as a regional calibration point for previous marine and terrestrial biogeographic studies (Bermingham et al. 1997, Wüster et al. 2002, 2005, 2008). In the case of terrestrial studies, this practice is unsound because this time period probably represents a period of dispersal, rather than having any direct relevance to vicariance (and is thus not particularly useful in applying to divergence time estimates). More importantly, based on our results, we find evidence from multiple lineages that divergence times across this boundary appear almost completely independent of this 3.5 Ma closure date (Fig. 3). Therefore, of all the biogeographic breaks we have examined here, this event represents one of the most problematic choices for use as a calibration point. Furthermore, recent evidence has shown that marine geminate species across both sides of the Isthmus diverged in a temporally staggered manner since the Miocene (Hurt et al. 2009), suggesting that this region represents a poor calibration point for both marine and terrestrial divergence times estimates.

Divergence across the Talamanca Cordillera (2)

The Talamanca mountain range and associated cordilleras running down the spine of Costa Rica and northwestern Panama represent a composite of Neogene and Quaternary mountains with an active geomorphological history since the Miocene (Marshall et al. 2003, MacMillan et al. 2004, Marshall 2007). Phylogenetically, lineages along the Pacific slope of Costa Rica/Panama and those in northern South America tend to be more closely related than are lineages on either side of the Talamanca ridge (Castoe et al. 2005, Weigt et al. 2005, Crawford et al. 2007, Daza et al. 2009). Combining the results from msBayes and the pooled posterior distributions of divergence times, our results favor a single vicariant event centered around 3.9 Ma (Fig. 3). The timing of this event near the final closure of the isthmus of Panama raises the question of whether this event was driven by the final tectonic uplifts of the Talamancan ridge (MacMillan et al. 2004) or possibly the large-scale changes in habitat distributions brought about through changes in ocean currents and weather patterns accompanying the closure of the isthmus of Panama.

Divergence across the Nicaraguan Depression (3)

The Nicaraguan Depression is a lowland corridor running from the Caribbean to the Pacific near the border between Costa Rica and Nicaragua. Marine sediments indicate that a seaway existed multiple times here during the Pliocene, separating regions to the north and south (Coates and Obando 1996). There is also evidence implying that a continuous peninsular landmass connected Honduras with modern day Costa Rica during the Miocene (Kirby and MacFadden 2005, Kirby et al. 2008), contrasting a hypothesis that this region comprised a set of islands interconnected by shallow waters during the Miocene (Coates and Obando 1996).

Two lineages of highland pitvipers (*Atropoides* and *Bothriechis*) show largely overlapping early divergences over this area, whereas a third highland pitviper lineage (*Cerrophidion*) and the lowland lineage (the colubrid *Leptodeira septentrionalis*) show substantially later divergences. The posterior distributions cluster in a staggered manner that broadly extends from ∼4–10 Ma (Fig. 3), countering a hypothesis of a single coordinated divergence event. This multi-modal pattern of divergence is also evident in the msBayes results that show diffuse posterior density across a broad range of discrete divergence events from one to five, although a majority of posterior density is centered over 2 events (Table 1, Fig. 4). A reasonable a priori expectation for divergences across this boundary may include rapid and highly coordinated divergence across multiple lineages due to the geo-tectonic model including seaway formation in the Pliocene. Instead, our data point to multiple periods (or one long broad period) of vicariance (and probably also dispersal) across the Nicaraguan Depression, rejecting a model centered on a single discrete barrier to gene flow coordinating divergences across lineages. Our data do fit an alternative model, that of Kirby and MacFadden (2005), which suggests a dynamic landmass may have transiently existed across the Nicaraguan Depression during the second half of the Miocene. This particular example highlights the important synergistic role in generating and testing hypotheses that comparative phylogeographic studies can have in conjunction with geological-tectonic data.

Divergence across the Motagua–Polochic Faults (4)

Recent studies have uncovered a sharp phylogeographic break along the axis where the Maya and Chortis tectonic blocks (in northern Middle America) come in contact and form a long NE–SW trending basin along the Motagua–Polochic Fault zone (Perdices et al. 2005, Devitt 2006, Concheiro-Pérez et al. 2007). The continued tectonic activity uplifting highlands on either side of this basin, and its further entrenchment, appears to have generated divergence events in both lowland and highland species. Based on the pooled posterior distribution of divergence times, credibility intervals and msBayes results (Fig. 3, 4), we find a clear pattern of concentrated temporal divergence across multiple species that span this area, suggesting that this zone acted as a barrier to many different lineages over this period from ∼3–8 Ma (Fig. 3). Our phylogeographic analysis suggests the primarily lowland snake genera, *Trimorphodon* and *Leptodeira*, diverged across this barrier in near concert with the highland lineages *Bothriechis*, *Atropoides* and *Cerrophidion* (Fig. 2, 3).

Terrestrial fossil information for Middle America is scarce, therefore the regional calibration for dating purposes needs to rely either on the fossil record from relatively distant lineages, or be based on estimated evolutionary rates. Here, we find evidence that the Motagua–Polochic Fault phylogeographic break may be a reasonably sound calibration point when no other information for regional calibrations is available. For example, the results of our pooled posterior distribution for the shared divergence across this break (Fig. 3) could be readily incorporated as a prior distribution for species divergence times in a Bayesian analysis when other useful calibration points are lacking, or a null hypothesis for other statistical tests in future studies.

Divergence across the Isthmus of Tehuantepec (5)

Mexico's Isthmus of Tehuantepec has long been considered a biogeographic break for both highland and lowland species (Marshall and Liebherr 2000, Parkinson et al. 2000, Morrone and Márquez 2001). Geological evidence suggests that from the late Miocene through late Pliocene, an extensive downdropping of the eastern block along the Tehuantepec fault zone resulted in a considerable reduction of the highlands and probably a marine embayment (Barrier et al. 1998). Given the cumulative evidence of diversification across multiple lineages on both sides of the Isthmus, a broad-reaching vicariant event during the Pliocene has been suggested as being responsible for the divergence of numerous lineages (Marshall and Liebherr 2000, Hasbún et al. 2005, Mulcahy et al. 2006, Castoe et al. 2009).

Our posterior distributions for divergence times strongly support this model, inferring a highly constrained temporal window at the end of the Pliocene when a majority of diversification events (4 of 6) occurred (Fig. 3). This window is consistent with proposals that events during the Pliocene severed gene flow among lineages straddling the isthmus (Hasbún et al. 2005, Mulcahy et al. 2006, León-Paniagua et al. 2007). However, the 95% credibility intervals (Fig. 3) and the msBayes results (Fig. 4) suggest that a second period of divergence also occurred earlier in the Miocene across the isthmus. Two genera, *Crotalus* and *Porthidium*, apparently diverged earlier, suggesting that a different geological/climatic event at the end of the Miocene (e.g. vegetation shifts; Cerling et al. 1997) may have been responsible for divergence in these two arid-adapted groups. Our data are thus consistent with hypotheses of broad vicariance across the isthmus due to Pliocene downdropping and seaway formation across the isthmus, but further suggest a more ancient divergence here affecting at least arid-adapted species.

Conclusion

In this study we investigated Middle American regional historical biogeography by focusing on particular spatial areas known to be major biogeographic boundaries, and characterizing these boundaries by synthesizing information about how multiple lineages temporally diverged across them. The large number of independent lineage diversification events examined provides new data for testing existing hypotheses of regional patterns of lineage diversification, and further evidence for generating new hypotheses of Neotropical diversification.

We expect that our estimates of divergence, and the degree of synchronization, represent sound testable hypotheses for unstudied taxa or communities, certainly in cases where we found divergence to be highly correlated across lineages. Combining ABC statistical methods for inferring the coordination of divergences across lineages (Hickerson et al. 2006b, 2007, Leaché et al. 2007, Hickerson and Meyer 2008) with analyses of posterior distributions of divergence times based on robust probabilistic methods from a combined phylogenetic dataset provided an ideal complementary strategy for dissecting shared divergence patterns. Because the use of pooled posterior distributions may obscure relevant lineage-specific responses to biogeographic boundaries, they should not be used as the only evidence for shared divergence among co-distributed taxa. However, in the absence of any other information about lineage divergence (i.e. when calibration points are scarce), pooled posterior distributions of divergence times are useful as an a priori expectation of divergence time for unstudied species, or even as a Bayesian prior in phylogenetic analyses.

Advances in estimation and comparison of divergence times, coupled with the growing interest in phylogeographic research, will surely continue to illuminate new understanding of the roles that historical processes have played in generating the planet's biodiversity. We found widespread evidence for a surprisingly high number of lineages showing coordinated divergence, and these divergences often fit previous expectations based on geological and tectonic evidence. In other cases, however, (e.g. Nicaraguan Depression) we found substantial evidence supporting one geological model (dynamic transient land connections) over other models. Overall, our findings are highly encouraging, and strongly implicate the existence of an underlying and unifying model of Middle American biogeography that is tractable to assemble and eventually comprehend. The level of detailed information emerging from comparative phylogeographic studies, augmented with information from the fossil, geological, tectonic, and climatic records, hold great promise for accelerating insight into how biodiversity was established on the planet, and also how it may be shaped by climate change and anthropogenic disturbance.

Acknowledgements – We acknowledge participants of the Boundaries Symposium at the International Biogeography Society Meeting, including B. Riddle, D. Hafner, J. Morrone, T. Pennington, J. Klicka, and R. Whittaker, for stimulating discussion, suggestions, and encouragement. We thank J. Castoe, A. Fenwick, A. P. Jason de Koning, S. Johnson, H. Kalkvik, J. Reece, K. Kozak, R. Tursi, and two anonymous reviewers for constructive comments on the manuscript, and T. Hether for his help with the R script. We acknowledge the support of a National Inst. of Health Training Grant (LM009451) to TAC, and a National Science Foundation (NSF) Collaborative Research grant to CLP (DEB–0416000). Many sequences used in this study were from tissues generously donated by J. A. Campbell and E. N. Smith, who's fieldwork was supported by the NSF (grants DEB–0613802 and DEB–9705277 to J. A. Campbell and DEB–0416160 to E. N. Smith) and the Inst. Bioclon (to E. N. Smith).

References

Arbogast, B. S. and Kenagy, G. J. 2001. Comparative phylogeography as an integrative approach to historical biogeography. – J. Biogeogr. 28: 819–825.

Barrier, E. et al. 1998. Neotectonic evolution of the Isthmus of Tehuantepec (southeastern Mexico). – Tectonophysics 287: 77–96.

Bermingham, E. and Martin, A. P. 1998. Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. – Mol. Ecol. 7: 499–517.

Bermingham, E. and Moritz, C. 1998. Comparative phylogeography: concepts and applications. – Mol. Ecol. 7: 367–369.

Bermingham, E. et al. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. – In: Kocher, T. D. and Stepien, C. A. (eds), Molecular systematics of fishes. Academic Press, pp. 113–128.

Burbrink, F. T. and Pyron, R. A. 2008. The taming of the skew: estimating proper confidence intervals for divergence dates. – Syst. Biol. 57: 317–328.

Campbell, J. A. 1999. Distribution patterns of amphibians in Middle America. – In: Duellman, W. E. (ed.), Patterns of distribution of amphibians: a global perspective. Johns Hopkins Univ. Press, pp. 111–210.

Castoe, T. A. and Parkinson, C. L. 2006. Bayesian mixed models and the phylogeny of pitvipers (Viperidae: Serpentes). – Mol. Phylogenet. Evol. 39: 91–110.

Castoe, T. A. et al. 2005. Modeling nucleotide evolution at the mesoscale: the phylogeny of the Neotropical pitvipers of the *Porthidium* group (Viperidae: *Atropoides*, *Cerrophidion*, *Porthidium*). – Mol. Phylogenet. Evol. 37: 881–898.

Castoe, T. A. et al. 2007a. Higher-level phylogeny of Asian and American coralsnakes, their placement within the Elapidae (Squamata), and the systematic affinities of the enigmatic Asian coralsnake *Hemibungarus calligaster* (Wiegmann, 1834). – Zool. J. Linn. Soc. 151: 809–831.

Castoe, T. A. et al. 2007b. Phylogeographic structure and historical demography of the western diamondback rattlesnake (*Crotalus atrox*): a perspective on North American desert biogeography. – Mol. Phylogenet. Evol. 42: 193–212.

Castoe, T. A. et al. 2009. Comparative phylogeography of pitvipers suggests a consensus of ancient Middle American highland biogeography. – J. Biogeogr. 36: 88–103.

Cerling, T. E. et al. 1997. Global vegetation change through the Miocene/Pliocene boundary. – Nature 389: 153–158.

Coates, A. G. and Obando, J. A. 1996. The geologic evolution of the Central American isthmus. – In: Jackson, J. B. C. et al. (eds), Evolution and environment in tropical America. Univ. of Chicago Press, pp. 21–56.

Collins, L. S. et al. 1996. Earliest evolution associated with closure of the Tropical American Seaway. – Proc. Nat. Acad. Sci. USA 93: 6069–6072.

Concheiro-Pérez, G. A. et al. 2007. Phylogeny and biogeography of 91 species of heroine cichlids (Teleostei: Cichlidae) based on sequences of the cytochrome b gene. – Mol. Phylogenet. Evol. 43: 91–110.

Coney, P. J. 1982. Plate tectonic constraints on the biogeography of Middle America and the Caribbean region. – Ann. Mo. Bot. Gard. 69: 432–443.

Crawford, A. J. et al. 2007. The role of tropical dry forest as a long-term barrier to dispersal: a comparative phylogeographical analysis of dry forest tolerant and intolerant frogs. – Mol. Ecol. 16: 4789–4807.

Daza, J. M. et al. 2009. Complex evolution in the Neotropics: the origin and diversification of the widespread genus *Leptodeira* (Serpentes: Colubridae). – Mol. Phylogenet. Evol. 53: 653–667.

Devitt, T. J. 2006. Phylogeography of the western lyresnake (*Trimorphodon biscutatus*): testing aridland biogeographical hypotheses across the Nearctic-Neotropical transition. – Mol. Ecol. 15: 4387–4407.

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

Drummond, A. J. et al. 2006. Relaxed phylogenetics and dating with confidence. – PLoS Biol. 4: 1–12.

Graur, D. and Martin, W. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. – Trends Genet. 20: 80–86.

Hasbún, C. R. et al. 2005. Mitochondrial DNA phylogeography of the Mesoamerican spiny-tailed lizards (*Ctenosaura quinquecarinata* complex): historical biogeography, species status and conservation. – Mol. Ecol. 14: 3095–3107.

Head, J. J. et al. 2005. First report of snakes (Serpentes) from the Late Middle Eocene Pondaung formation, Myanmar. – J. Vertebr. Paleontol. 25: 246–250.

Heads, M. 2005. Dating nodes on molecular phylogenies: a critique of molecular biogeography. – Cladistics 21: 62–78.

Hickerson, M. J. and Meyer, C. P. 2008. Testing comparative phylogeographic models of marine vicariance and dispersal using a hierarchical Bayesian approach. – BMC Evol. Biol. 8: 322.

Hickerson, M. J. et al. 2006a. Comparative phylogeographic summary statistics for testing simultaneous vicariance. – Mol. Ecol. 15: 209–223.

Hickerson, M. J. et al. 2006b. Test for simultaneous divergence using approximate bayesian computation. – Evolution 60: 2435–2453.

Hickerson, M. J. et al. 2007. msBayes: pipeline for testing comparative phylogeographic histories using hierarchical approximate Bayesian computation. – BMC Bioinform. 8: 268.

Ho, S. Y. W. et al. 2008. The effect of inappropriate calibration: three case studies in molecular ecology. – PLoS One 3: e1615.

Holman, J. A. 2000. Fossil snakes of North America: origin, evolution, distribution, paleoecology. – Indiana Univ. Press.

Hurt, C. et al. 2009. A multilocus test of simultaneous divergence across the Isthmus of Panama using snapping shrimp in the genus *Alpheus*. – Evolution 63: 514–530.

Iturralde-Vinent, M. A. 2006. Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. – Int. Geol. Rev. 48: 791–827.

Jackson, J. B. C. et al. (eds) 1996. Evolution and environment in tropical America. – Univ. Chicago Press.

Kelly, C. M. R. et al. 2009. Phylogeny, biogeography and classification of the snake superfamily Elapoidea: a rapid radiation in the late Eocene. – Cladistics 25: 38–63.

Kirby, M. X. and MacFadden, B. 2005. Was southern Central America an archipelago or a peninsula in the middle Miocene? A test using land-mammal body size. – Palaeogeogr. Palaeoclimatol. Palaeoecol. 228: 193–202.

Kirby, M. X. et al. 2008. Lower Miocene stratigraphy along the Panama Canal and its bearing on the Central American peninsula. – PLoS One 3: e2791.

Knowles, L. L. and Carstens, B. C. 2007. Estimating a geographically explicit model of population divergence. – Evolution 61: 477–493.

Koepfli, K.-P. et al. 2007. Phylogeny of the Procyonidae (Mammalia: Carnivora): molecules, morphology and the Great American Interchange. – Mol. Phylogenet. Evol. 43: 1076–1095.

Larkin, M. A. et al. 2007. Clustal W and Clustal X version 2.0. – Bioinformatics 23: 2947–2948.

Leaché, A. D. et al. 2007. Two waves of diversification in mammals and reptiles of Baja California revealed by hierarchical Bayesian analysis. – Biol. Lett. 3: 646–650.

León-Paniagua, L. et al. 2007. Diversification of the arboreal mice of the genus *Habromys* (Rodentia: Cricetidae: Neotominae) in the Mesoamerican highlands. – Mol. Phylogenet. Evol. 42: 653–664.

MacMillan, I. et al. 2004. Middle Miocene to present plate tectonic history of the southern Central American Volcanic Arc. – Tectonophysics 392: 325–348.

Mann, P. et al. 2007. Overview of plate tectonic history and its unresolved tectonic problems. – In: Bundschuh, J. and Alvarado, G. E. (eds), Central America: geology, resources, and hazards. Taylor and Francis, pp. 205–241.

Marko, P. B. 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. – Mol. Biol. Evol. 19: 2005–2021.

Marshall, C. J. and Liebherr, J. K. 2000. Cladistic biogeography of the Mexican transition zone. – J. Biogeogr. 27: 203–216.

Marshall, J. S. 2007. The geomorphology and physiographic provinces of Central America. – In: Bundschuh, J. and Alvarado, G. E. (eds), Central America: geology, resources, and hazards. Taylor and Francis, pp. 75–122.

Marshall, J. S. et al. 2003. Landscape evolution within a retreating volcanic arc, Costa Rica, Central America. – Geology 31: 419–422.

Morrone, J. J. 2001. Biogeografía de América Latina y el Caribe. – Manuales y Tesis, SEA.

Morrone, J. J. and Márquez, J. 2001. Halffter's Mexican transition zone, beetle generalized tracks, and geographical homology. – J. Biogeogr. 28: 635–650.

Mulcahy, D. G. et al. 2006. Historical biogeography of lowland species of toads (*Bufo*) across the Trans-Mexican Neovolcanic Belt and the Isthmus of Tehuantepec. – J. Biogeogr. 33: 1889–1904.

Nelson, G. J. and Platnick, N. I. 1981. Systematics and biogeography: cladistics and vicariance. – Columbia Univ. Press.

Nicholas, K. B. and Nicholas, H. B. Jr 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. – Distributed by the authors.

Nylander, J. A. A. 2004. MrModeltest v2. – Distributed by the author.

Parkinson, C. L. et al. 2000. Phylogeography of the pitviper clade *Agkistrodon*: historical ecology, species status, and conservation of cantils. – Mol. Ecol. 9: 411–420.

Parkinson, C. L. et al. 2002. Multigene phylogenetic analysis of pitvipers, with comments on their biogeography. – In: Schuett, G. W. et al. (eds), Biology of the vipers. Eagle Mountain Publ., USA, pp. 93–110.

Parmley, D. and Holman, J. A. 2007. Earliest fossil record of a pigmy rattlesnake (Viperidae: *Sistrurus* Garman). – J. Herpetol. 41: 141–144.

Pennington, R. T. and Dick, C. W. 2004. The role of immigrants in the assembly of the South American rainforest tree flora. – Phil. Trans. R. Soc. B 359: 1611–1622.

Perdices, A. et al. 2005. Evolutionary history of the synbranchid eels (Teleostei: Synbranchidae) in Central America and the Caribbean islands inferred from their molecular phylogeny. – Mol. Phylogenet. Evol. 37: 460–473.

Rage, J.-C. et al. 1992. A colubrid snake in the late Eocene of Thailand: the oldest known Colubridae (Reptilia, Serpentes). – C. R. Acad. Sci. Paris 314: 1085–1089.

Rambaut, A. and Drummond, A. J. 2007. Tracer v1.4. – <http://beast.bio.ed.ac.uk/Tracer>.

Rambaut, A. and Drummond, A. J. 2008. TreeStat v1.2: tree statistic calculation tool. – <http://tree.bio.ed.ac.uk/software/treestat/>.

Ree, R. H. and Smith, S. A. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. – Syst. Biol. 57: 4–14.

Ree, R. H. and Sanmartín, I. 2009. Prospects and challenges for parametric models in historical biogeographical inference. – J. Biogeogr. 36: 1211–1220.

Richards, C. L. et al. 2007. Distribution modelling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. – J. Biogeogr. 34: 1833–1845.

Riddle, B. R. et al. 2008. The role of molecular genetics in sculpting the future of integrative biogeography. – Prog. Phys. Geogr. 32: 173–202.

Ronquist, F. 1997. Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. – Syst. Biol. 46: 195–203.

Sanders, K. L. and Lee, M. S. Y. 2008. Molecular evidence for a rapid late-Miocene radiation of Australasian venomous snakes (Elapidae, Colubroidea). – Mol. Phylogenet. Evol. 46: 1180–1188.

Savage, J. M. 1982. The enigma of the Central American herpetofauna: dispersal or vicariance? – Ann. Mo. Bot. Gard. 69: 464–547.

Vidal, N. et al. 2009. Snakes (Serpentes). – In: Hedges, S. B. and Kumar, S. (eds), The timetree of life. Oxford Univ. Press, pp. 390–397.

Weigt, L. A. et al. 2005. Biogeography of the túngara frog, *Physalaemus pustulosus*: a molecular perspective. – Mol. Ecol. 14: 3857–3876.

Whitmore, T. C. and Prance, G. T. (eds) 1987. Biogeography and Quaternary history in tropical America. – Clarendon Press.

Wüster, W. et al. 2002. Origin and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence analysis. – In: Schuett, G. W. et al. (eds), Biology of the vipers. Eagle Mountain Publ., USA, pp. 111–128.

Wüster, W. et al. 2005. Tracing an invasion: landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). – Mol. Ecol. 14: 1095–1108.

Wüster, W. et al. 2008. A nesting of vipers: phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). – Mol. Phylogenet. Evol. 49: 445–459.

Zamudio, K. R. and Greene, H. W. 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics and conservation. – Biol. J. Linn. Soc. 62: 421–442.

Download the Supplementary material as file E6281 from <www.oikos.ekol.lu.se/appendix>.