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Complex evolution in the Neotropics: The origin and diversification of the widespread genus *Leptodeira* (Serpentes: Colubridae)

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ABSTRACT

Lineage diversification in the Neotropics is an interesting topic in evolutionary biology but is also one of the least understood. The abiotic and biotic complexity of the region precludes generalizations that can be drawn regarding the historical evolutionary processes responsible for the diversity observed. The snake genus *Leptodeira* provides an excellent opportunity to investigate such processes because it spans the entire Neotropical region. In this study, we infer the phylogenetic position of *Leptodeira* within Dipsadinae, estimate evolutionary relationships among and within *Leptodeira* species, and estimate the diversification time and biogeography of the genus. Three mitochondrial gene regions were sequenced for individuals representing all the *Leptodeira* species and most subspecies currently recognized. Additionally, two nuclear protein-coding gene regions were sequenced for representatives of each species and several genera within the Dipsadinae. We infer that several *Leptodeira* species are either paraphyletic or polyphyletic as currently recognized, and that most recognized subspecies are not monophyletic lineages. Despite the taxonomic discordance with evolutionary relationships, clades appear to correspond very well to major biogeographic regions of Mexico, Central America and South America. Our results thus highlight the important role of the Miocene and Pliocene for lineage diversification in the Neotropics. Additionally, our time estimates suggest that recent intraspecific phylogeographic structure is likely the result of habitat and climatic fluctuations during the Pleistocene. Cumulatively, our inferences of lineage diversification within *Leptodeira* suggest a complex evolutionary scenario in the Mexican transition zone and a north to south expansion with a final colonization of the tropics in South America.

1. Introduction

Inferring patterns of species diversification is among the most interesting topics in evolutionary biology because it may provide key insight into the processes that have led to current biodiversity. This is especially true in the Neotropics, given the extreme geological complexity and the high diversity and endemicity in this region (Prance, 1982; Cracraft and Prum, 1988; Graham, 1997; Burnham and Graham, 1999). This extreme intricacy of historical processes, however, has hampered a consensus regarding the historical and ecological processes responsible for the observed diversity. One particularly important means of developing a strong hypothesis for broad and general biogeographic patterns is the simultaneous analysis and comparison of multiple independent lineages that are codistributed throughout a region (Nelson and Platnick, 1981; Lomolino et al., 2006; Castoe et al., 2009). This approach is particularly difficult to apply in the Neotropical region because the spatial and temporal dimensions of a majority of lineages in this area remain poorly known. To overcome this problem, a more realistic approach is to investigate phylogenetic patterns of independent lineages and then to test specific hypotheses regarding the historical and ecological processes that have shaped the species diversity (Beheregaray, 2008; Riddle et al., 2008). The cat-eyed snakes, *Leptodeira,* range through nearly the entire Neotropical region, making this group excellent to investigate the effects of historical and ecological processes across different biogeographic provinces on lineage diversification.

The genus *Leptodeira* is a member of the subfamily Dipsadinae, a group that originated in Middle America but now inhabits Tropical and Subtropical America (Duellman, 1958a; Cadle, 1985; Zaher, 1999). It is the most widely distributed genus of the subfamily, ranging from the southern USA to northern Argentina and Paraguay, the east coast of Brazil and the islands of Aruba, Margarita, Tobago and Trinidad (Duellman, 1958a). Several hypotheses regarding the diversification in the Mexican transition

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zone (*sensu* Halffter, 1987), in lower Central America and the interchange between Central and South America can be explored through the phylogeography of different lineages of *Leptodeira*. Nevertheless, several recognized species are morphologically similar and the overlapping in color patterns makes distinction among species difficult. Thus, comprehensive molecular phylogenetic analyses of these morphologically complex groups are necessary to elucidate their evolutionary and biogeographic history. Lastly, *Leptodeira* ranges from very dry areas in Mexico and northern South America to mesic and evergreen humid forests in Middle America and the Amazon basin. This extraordinary ecological distribution provides further insight into the environmental factors that may affect gene flow, diversification and geographic distribution of the lineages within the genus.

Phylogenetic hypotheses regarding the genus *Leptodeira* have not been addressed comprehensively. Duellman (1958a) proposed that the genus *Hypsiglena* was the sister group to *Leptodeira*. Dowling and Jenner (1987) inferred the phylogenetic relationships among several Xenodontines (Dipsadines) related to *Leptodeira*, but were unable to resolve which lineages are the closest relatives of *Leptodeira*. Vidal et al. (2000) placed *Leptodeira* within the subfamily Dipsadinae but again they provided no insight into what taxon may be its sister lineage. Recent molecular phylogenetic analyses have hypothesized the genus *Imantodes* as the sister taxon to *Leptodeira* (Pinou et al., 2004; Mulcahy, 2007). Mulcahy (2007) examined the phylogenetic relationships among *Leptodeira* and tested the monophyly of the Leptodeirini (*sensu* Cadle, 1984). The monophyly of *Leptodeira* was not supported under his parsimony analysis but received moderate support using maximum likelihood and Bayesian inference. The only comprehensive taxonomic study within *Leptodeira* was conducted five decades ago by Duellman (1958a). Four species groups were recognized and one species, *Leptodeira discolor*, was considered *incertae sedis*. Few taxonomic changes have been made since Duellman (1958a), except that *L. discolor* and *L. latifasciata* have been allocated to the monotypic genera *Tantalophis* and *Pseudoleptodeira,* respectively (Duellman, 1958b; Smith and Smith, 1976). Taylor (1951) recognized *L. rubricata* as a separate species, but it was synonymized with *L. annulata* by Duellman (1958a). Currently, *L. rubricata* is considered a valid species, although no quantitative evidence has been shown to support this (Savage, 2002). In general, the subspecies proposed by Duellman (1958a) are still recognized today (e.g., Savage, 2002; Köhler, 2008).

The spatial and temporal diversification of *Leptodeira* has not been addressed comprehensively. Duellman (1958a) proposed a tentative biogeographic scenario from which phylogenetic relationships and the spatial and temporal diversification may be extracted (Fig. 1). His reconstruction placed the origin of *Leptodeira* in the Miocene, followed by a diversification into the different species and subspecies throughout the Miocene and Pliocene with some subspecies originating during the Pleistocene. Dowling and Jenner (1987) also suggested a Miocene origin. Duellman (1958a) and Mulcahy (2007) both hypothesized that *Leptodeira* originated in Mexico with at least two dispersal events into South America directly after the closure of the Isthmus of Panama in the Late Pliocene. These dispersal events involved the independent colonization of South America by the species *L. annulata* and *L. septentrionalis*.

In this study, we use sequences from mitochondrial and nuclear genes and extensive taxon sampling to investigate the following questions surrounding the evolution and biogeography of *Leptodeira*: (1) Do nuclear and mitochondrial sequence data yield congruent phylogenetic inferences for the relationships among the dipsadines and the inter- and intra-relationships within *Leptodeira?* (2) Is the monophyly of the genus *Leptodeira* supported? (3) Is the current morphological classification consistent with the molecular phylogenetic estimates? and (4) Is the spatial and temporal diversification of *Leptodeira* congruent with Duellman's hypotheses? In addition to these questions, we apply our phylogenetic and phylogeographic data, together with estimates of divergence times, to develop hypotheses for the historical patterns and processes that have shaped lineage diversity in *Leptodeira* and which may be broadly informative about patterns of Neotropical diversification in general.

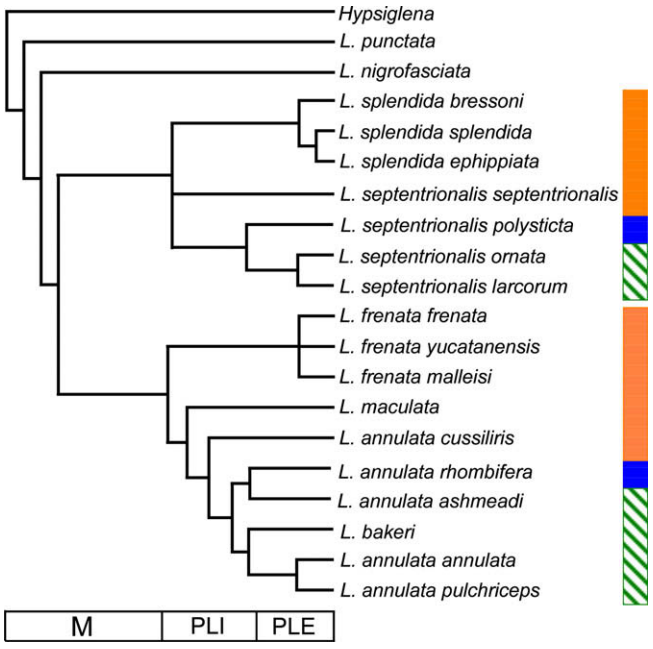

Fig. 1. Hypothesis for the spatio-temporal diversification of *Leptodeira* in the Neotropics based on Duellman (1958a). Time periods (not drawn to scale) as follows: M = Miocene, PLI = Pliocene, PLE = Pleistocene.

2. Material and methods

2.1. Taxon sampling

We combined previously published DNA sequences with new sequences from this study to create a matrix with a total of 135 terminals including taxa outside *Leptodeira* (Table 1). We followed the taxonomic classification of Duellman (1958a) except for *L. latifasciata* and *L. discolor*, which are considered *Pseudoleoptodeira latifasciata* and *Tantalophis discolor* respectively. Although *L. rubricata* was synonymized with *L. a. rhombifera* (Duellman, 1958a), we sequenced one specimen to explore its phylogenetic position and species status (see Savage, 2002). Within the genus *Leptodeira*, our dataset included 89 individuals representing all nine species, and nine of the 15 subspecies. Our geographic sampling spanned the entire known distribution for the genus (Fig. 2). Outgroups were chosen based on two criteria. First, we included 27 members from the subfamilies Dipsadinae, Xenodontinae, Natricinae and Colubrinae to determine the phylogenetic position of *Leptodeira* within Dipsadinae and to gain further insight into the relationships within the subfamily Dipsadinae. Second, because Mulcahy (2007) did not recover *Leptodeira* as a well-supported clade (86% posterior probability), we included 16 samples of the genus *Imantodes* (inferred as the sister taxon to *Leptodeira* by Mulcahy, 2007) to test the monophyly of *Leptodeira*. Finally, to estimate divergence times, we included three representatives of the family Viperidae for calibration purposes.

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Table 1
Sequences used in this study, with GenBank numbers and voucher information. Sequences added specifically in this study are indicated in bold.

Taxon[a]	Locality	Voucher[b]	*Cyt-b*	ND4	DNAH3	NT 3
Alsophis portoricensis	Unknown	No voucher	AF471085	U49308		
Amastridium sapperi	**Guatemala, Izabal**	**UTA R-46905**	**GQ334479**	**GQ334580**	**GQ334557**	**GQ334663**
Arrhyton exiguum	USA, Puerto Rico	CAS 200732	AF471071			
Atractus wagleri	**Colombia, Antioquia**	**MHUA 14368**	**GQ334480**	**GQ334581**	**GQ334558**	**GQ334664**
Carphophis amoenus	USA, Illiniois	CAS 160710	AF471067			
Coluber constrictor	USA, California	CAS 212760, SDSU 3929	EU180467	AY487041	EU402743	EU390914
Coniophanes fissidens	El Salvador, San Salvador	KU 289798	EF078586,	EF078538		
Contia tenuis	Unknown	No voucher	AF471095	DQ364666		
Crtotalus tigris	**USA, Arizona, Pima Co.**	**CLP 169**	**AY223606**	**AF156574**		**GQ334665**
Cryophis hallbergi	**Mexico, Oaxaca**	**UTA R-12272**	**GQ334481**	**GQ334582**	**GQ334559**	**GQ334666**
Diadophis punctatus	Unknown	No voucher	AF471094	DQ364667		
Dipsas catesbyi	Peru, Madre de Dios	KU 214851	EF078585,	EF078537		
Dipsas pratti	**Colombia, Antioquia**	**MHUA 14278**	**GQ334482**	**GQ334583**	**GQ334560**	**GQ334667**
Eridiphas slevini	Mexico, Baja California	MVZ 234613	EF078547,	EF078499		
Farancia abacura	USA, Florida	CAS 184359	U69832	DQ902307		
Gloydius shedaoensis	China, Liaoning	ROM-20468	AY223566	AY223623		
Gonyosoma frenatum	Unknown	No voucher	DQ902110	DQ902290		
Helicops angulatus	Trinidad, Tobago	LSUMZ 3346	AF471037	U49310		
Heterodon simus	USA, Florida	CAS 195598	AF217840	DQ902310		
Hydrops triangularis	Peru, Loreto	LSUMZ 3105	AF471039			
Hypsiglena torquata	**USA, California**	**CAS 206502**	**GQ334483**	**GQ334584**		
Imantodes cenchoa	Brazil, Para	MPEGLJV 5763	EF078556,	EF078508		
Imantodes cenchoa	**Colombia, Antioquia**	**MHUA 14290**	**GQ334484**	**GQ334585**	**GQ334561**	**GQ334668**
Imantodes cenchoa	**Colombia, Antioquia**	**MHUA 14500**	**GQ334485**	**GQ334586**		
Imantodes cenchoa	**Colombia, Chocó**	**JMD 1616**	**GQ334486**	**GQ334587**		
Imantodes cenchoa	Costa Rica, Limon	MVZ 149878	EF078553,	EF078505		
Imantodes cenchoa	Guatemala, Izabal	UTA R-42360	EF078554,	EF078506		
Imantodes cenchoa	Panama, Cocle	SIUC R-03724	EF078555,	EF078507		
Imantodes gemmistratus	**Guatemala, San Marcos**	**UTA R-45922**	**GQ334487**	**GQ334588**		
Imantodes gemnistratus	Mexico, Sinaloa	UTA R-51979	EF078557,	EF078509		
Imantodes gemnistratus	Mexico, Sonora	LSUMZ 39541	EF078558,	EF078510		
Imantodes inornatus	**Colombia, Antioquia**	**MHUA 14540**	**GQ334488**	**GQ334589**	**GQ334562**	**GQ334669**
Imantodes inornatus	**Costa Rica**	**ASL 307**	**GQ334489**	**GQ334590**		
Imantodes inornatus	Costa Rica, Cartago	MVZ 204109	EF078559,	EF078511		
Imantodes inornatus	Costa Rica, Heredia	MVZ 204110	EF078560,	EF078512		
Imantodes lentiferus	Brazil, Amazonas	MPEGLJV 6880	EF078561,	EF078513		
Imantodes lentiferus	Brazil, Para	MPEGLJV 5581	EF078562,	EF078514		
Leptodeira annulata annulata	**Brazil, Amazonas**	**LSU-H 14016**	**GQ334494**	**GQ334595**		
L. annulata annulata	**Brazil, Goias**	**No voucher**		**GQ334599**		
L. annulata annulata	Brazil, Para	LSU-H 14438	EF078564	EF078516		
L. annulata annulata	**Brazil, Roraima**	**LSU-H 12442**	**GQ334495**	**GQ334596**		
L. annulata annulata	**Colombia, Meta**	**UTA T-55-G5**	**GQ334490**	**GQ334591**		
L. annulata annulata	**Colombia, Meta**	**UTA T-55-G6**	**GQ334491**	**GQ334592**		
L. annulata annulata	**Colombia, Meta**	**UTA T-55-G7**	**GQ334492**	**GQ334593**		
L. annulata annulata	**Ecuador, Sucumbios**	**LSU-H 12755**	**GQ334496**	**GQ334597**		
L. annulata annulata	**French Guyana**	**Vidal et al. (2000)**	**GQ334497**	**GQ334598**		
L. annulata annulata	Peru, Madre de Dios	KU 214878	EF078563	EF078515		
L. annulata annulata	**Suriname, Para**	**BPN 963**	**GQ334493**	**GQ334594**	**GQ334563**	**GQ334670**
L. annulata ashmeadi	Trinidad, St. Patrick	USNM 314700	EF078565	EF078517		
L. annulata ashmeadi	**Venezuela, Barinas**	**MHNLS-X516**	**GQ334498**	**GQ334600**		
L. annulata cussiliris	**Guatemala, Huehuetenango**	**UTA R-42220**	**GQ334499**	**GQ334601**		
L. annulata cussiliris	**Guatemala, San Marcos**	**UTA R-53305**	**GQ334501**	**GQ334603**	**GQ334564**	**GQ334671**
L. annulata cussiliris	Mexico, Guerrero	JAC 21939	EF078568	EF078520		
L. annulata cussiliris	Mexico, Hidalgo	ITAH 912	EF078566	EF078518		
L. annulata cussiliris	Mexico, Hidalgo	ITAH 913	EF078567	EF078519		
L. annulata cussiliris	**Mexico, Oaxaca**	**ENEPI 6546**	**GQ334500**	**GQ334602**		
L. annulata cussiliris	**Mexico, Oaxaca**	**UTA R-52630**	**GQ334502**	**GQ334604**		
L. annulata cussiliris	**Mexico, Veracruz**	**EBUAP UOGV 188**	**GQ334503**	**GQ334605**		
L. annulata rhombifera	**Costa Rica**	**ICP 1280**	**GQ334505**	**GQ334607**		
L. annulata rhombifera	**Costa Rica, San Jose**	**MSM 130**	**GQ334514**	**GQ334616**		
L. annulata rhombifera	**El Salvador, San Salvador**	**MUHNES C-30-1351**	**GQ334506**	**GQ334608**		
L. annulata rhombifera	**El Salvador, Usulutan**	**KU 289913**	**GQ334507**	**GQ334609**		
L. annulata rhombifera	**Guatemala, Baja Verapaz**	**UTA R-42456**	**GQ334508**	**GQ334610**		
L. annulata rhombifera	**Guatemala, Baja Verapaz**	**MSM 705**		**GQ334617**		
L. annulata rhombifera	**Guatemala, Escuintla**	**UTA R-44713**	**GQ334513**	**GQ334615**		
L. annulata rhombifera	**Guatemala, Zacapa**	**UTA R-42393**	**GQ334512**	**GQ334614**		
L. annulata rhombifera	**Honduras, Comayagua**	**UNAH-MSM 456**	**GQ334511**	**GQ334613**		
L. annulata rhombifera	**Honduras, El Paraiso**	**UTA R-41255**	**GQ334509**	**GQ334611**	**GQ334565**	**GQ334672**
L. annulata rhombifera	**Honduras, Francisco Morazan**	**JHT 2004**	**GQ334504**	**GQ334606**		
L. annulata rhombifera	**Honduras, Olancho**	**UNAH-ENS 8766**	**GQ334510**	**GQ334612**		
L. bakeri	**Aruba**	**Avid 023783888**	**GQ334516**	**GQ334619**		
L. bakeri	**Aruba**	**Avid 023851115**	**GQ334517**	**GQ334620**		
L. bakeri	**Aruba**	**Avid 023858355**	**GQ334515**	**GQ334618**	**GQ334566**	**GQ334673**
L. bakeri	**Aruba**	**Avid D**	**GQ334518**	**GQ334621**		

(*continued on next page*)

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Table 1 (*continued*)

Taxon[a]	Locality	Voucher[b]	*Cyt-b*	ND4	DNAH3	NT 3
L. bakeri	**Aruba**	**Avid E**	**GQ334519**	**GQ334622**		
L. frenata	Mexico, Campeche	LSUMZ 38200	EF078580	EF078532		
L. frenata	Mexico, Guerrero	LSUMZ 39524	EF078579	EF078531		
L. maculata	**Mexico, Guerrero**	**MZFC 19477**	**GQ334520**	**GQ334623**		
L. maculata	**Mexico, Jalisco**	**MZFC 17434**	**GQ334523**	**GQ334626**		
L. maculata	**Mexico, Jalisco**	**UTA R-53323**	**GQ334521**	**GQ334624**	**GQ334567**	**GQ334674**
L. maculata	**Mexico, Jalisco**	**UTA R-53324**	**GQ334522**	**GQ334625**		
L. maculata	**Mexico, Jalisco**	**UTA R-53322**	**GQ334524**	**GQ334627**		
L. nigrofasciata	**Costa Rica**	**ASL 190**	**GQ334525**	**GQ334628**	**GQ334569**	
L. nigrofasciata	**Costa Rica**	**MSM 706**	**GQ334526**	**GQ334629**		
L. nigrofasciata	Mexico, Guerrero	MVZ 241573	EF078581	EF078533		
L. nigrofasciata	**Mexico, Oaxaca**	**UTA R-52634**		**GQ334630**	**GQ334568**	**GQ334681**
L. punctata	Mexico, Sinaloa	UTA R-51974	EF078577	EF078529		
L. punctata	Mexico, Sinaloa	UTA R-51976	EF078578	EF078530		
L. punctata		**UTA R-53503**			**GQ334571**	**GQ334682**
L. rubricata	**Costa Rica**	**ASL 304**	**GQ334527**	**GQ334631**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14291**	**GQ334530**	**GQ334634**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14292**	**GQ334531**	**GQ334635**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14403**	**GQ334528**	**GQ334632**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14404**	**GQ334529**	**GQ334633**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14419**	**GQ334535**	**GQ334639**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14423**	**GQ334532**	**GQ334636**	**GQ334572**	**GQ334676**
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14449**	**GQ334537**	**GQ334642**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14476**	**GQ334534**	**GQ334638**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14495**		**GQ334640**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14541**	**GQ334533**	**GQ334637**		
L. septentrionalis ornata	**Colombia, Antioquia**	**MHUA 14653**	**GQ334536**	**GQ334641**		
L. septentrionalis ornata	**Colombia, Caldas**	**JMD-T 44**	**GQ334538**			
L. septentrionalis ornata	**Costa Rica**	**ASL 308**	**GQ334541**	**GQ334646**	**GQ334574**	**GQ334678**
L. septentrionalis ornata	**Costa Rica, Limon**	**ICP 1089**	**GQ334540**	**GQ334645**		
L. septentrionalis ornata	**Costa Rica, Punta Arenas**	**ICP 1108**		**GQ334643**		
L. septentrionalis ornata	**Costa Rica, Punta Arenas**	**MSM PH 90**	**GQ334539**	**GQ334644**	**GQ334573**	**GQ334677**
L. septentrionalis ornata	Ecuador, Manabi	KU 218419	EF078576	EF078528		
L. septentrionalis ornata	Panama, Bocas del Toro	USNM 347357	EF078575	EF078527		
L. septentrionalis polysticta	**El Salvador, Ahuachapan**	**MUHNES C-30-1352**	**GQ334544**	**GQ334649**		
L. septentrionalis polysticta	**Guatemala, Escuintla**	**UTA R-46878**	**GQ334545**	**GQ334650**	**GQ334570**	**GQ334675**
L. septentrionalis polysticta	**Guatemala, Guatemala**	**UTA R-45878**	**GQ334546**	**GQ334651**		
L. septentrionalis polysticta	**Guatemala, Izabal**	**UTA R-39558**	**GQ334542**	**GQ334647**		
L. septentrionalis polysticta	**Guatemala, Peten**	**UTA R-46125**	**GQ334547**	**GQ334652**	**GQ334575**	**GQ334679**
L. septentrionalis polysticta	Guatemala, Peten	UTA R-50312	EF078572	EF078524		
L. septentrionalis polysticta	Guatemala, Suchitepequez	UTA R-52284	EF078571	EF078523		
L. septentrionalis polysticta	Mexico, Guerrero	MVZ 164942	EF078570	EF078522		
L. septentrionalis polysticta	**Mexico, Oaxaca**	**ENEPI 6819**	**GQ334543**	**GQ334648**		
L. septentrionalis polysticta	**Mexico, Oaxaca**	**MZFC 16548**		**GQ334653**		
L. septentrionalis polysticta	Mexico, Oaxaca	MZFC 16915	EF078574	EF078526		
L. septentrionalis polysticta	Mexico, Sinaloa	UTA R-51978	EF078573	EF078525		
L. splendida bressoni	**Mexico, Jalisco**	**MZFC 17240**	**GQ334548**	**GQ334654**	**GQ334576**	**GQ334680**
L. splendida bressoni	**Mexico, Jalisco**	**UTA R-53409**	**GQ334550**	**GQ334656**		
L. splendida bressoni	**Mexico, Jalisco**	**UTA R-53410**	**GQ334551**	**GQ334657**		
L. splendida bressoni	**Mexico, Nayarite**	**UTA R-53595**	**GQ334549**	**GQ334655**		
L. splendida splendida	**Mexico, Morelos**	**UTA R-51738**	**GQ334552**	**GQ334658**		
L. splendida splendida	Mexico, Puebla	EBUAP 2060	EF078569	EF078521		
Micrurus fulvius	USA, Florida	CAS 21347, YPM 14096	EF137413	EF137405	EU402760	EU390929
Natrix natrix	Spain, Catalonia	MVZ 200534	AY487756	AY487800	EU402762	EU390931
Ninia atrata	**Colombia, Caldas**	**MHUA 14452**	**GQ334553**	**GQ334659**	**GQ334577**	**GQ334683**
Oxyrhopus petola	**Guatemala, Izabal**	**UTA R-46698**	**GQ334554**	**GQ334660**	**GQ334578**	**GQ334684**
Pseudoleptodeira latifasciata	**Mexico**	**EBUAP ENS 10549**	**GQ334555**	**GQ334661**		
Rhadinaea fulvivittis	Mexico, Veracruz	MVZ 231852	EF078539	EF078587		
Sibon nebulatus	**Colombia, Antioquia**	**MHUA 14511**	**GQ334556**	**GQ334662**	**GQ334579**	**GQ334685**
Sistrurus catenatus	**USA, Texas, Haskel Co.**	**Moody-502**	AY223610	AY223648		**GQ334686**
Tantalophis discolor	Mexico, Oaxaca	EBUAP 1853	EF078589	EF078541		

[a] Taxonomy of *Leptodeira* based on Duellman (1958a).

[b] Voucher information: ASL = Alejandro Solórzano (private collection, Serpentario Nacional, Costa Rica); Avid = Pieter Barendsen (private collection); BPN = Brice P. Noonan (field number, UTA); CAS = California Academy of Sciences, Herpetological Collection, USA; CLP = Christopher L. Parkinson (field number, UCF); EBUAP = Escuela de Biología de la Universidad Autónoma de Puebla, Mexico; ENEPI = Escuela Nacional de Estudios Profesionales Ixtacala, Distrito Federal, Mexico; ENS = Eric N. Smith (field number, UTA); ICP = Instituto Clodomiro Picado, Costa Rica; ITAH = Instituto Technológico Agropecuario de Hidalgo, Mexico; JAC = Jonathan A. Campbell (field number, UTA); JHT = Joshua H. Townsend (field number, UF); JMD = Juan M. Daza (field number, MHUA); KU = University of Kansas, Museum of Natural History, Division of Herpetology, USA; LJV = Laurie J. Vitt (field number, OU); LSU H = Lousiana State University Tissue Collection, USA; LSUMZ = Lousiana State University, Museum of Zoology, USA; MHNLS = Museo de Historia Natural La Salle, Caracas, Venezuela; MHUA = Museo de Herpetología, Universidad de Antioquia, Colombia; Moody: Scott Moody (field number, OU); MPEG = Museu Paraense Emilio Goeldi; MSM = Mahmood Sasa Marin (private collection); MUHNES = Museo de Historia Natural de El Salvador, San Salvador; MVZ = Museum of Vertebrate Zoology, University of California, USA; MZFC = Museo de Zoología Facultad de Ciencias, UNAM, Mexico; ROM = Royal Ontario Museum, Canada; SDSU = San Diego State University Museum, USA; SIUC = Southern Illinois University Carbondale, USA; UNAH = Universidad Nacional Autónoma de Honduras, Tegucigalpa; USNM = Smithsonian Institution National Museum of Natural History, USA; UTA = University of Texas at Arlington, Amphibian and Reptile Diversity Research Center, USA; YPM = Yale Peabody Museum, USA.

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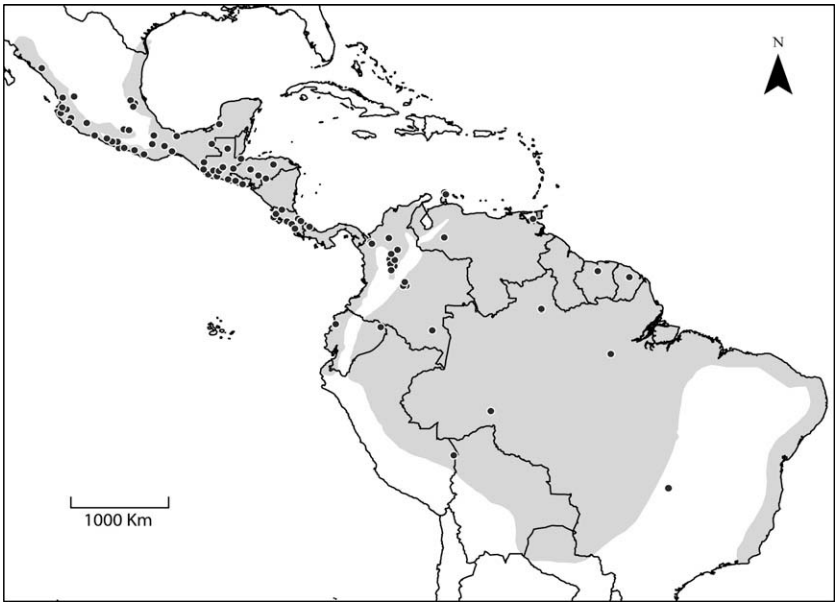

Fig. 2. Geographic distribution of the genus *Leptodeira* based on Duellman (1958a). Dots represent localities sampled in this study.

2.2. Laboratory protocols

Total genomic DNA was extracted from tissue samples (liver, muscle or skin shed) using the Qiagen DNeasy kit (QIAGEN). Two regions of the mitochondrial genome, including genes encoding Cytochrome b (*cyt-b*), NADH dehydrogenase subunit 4 (*ND4*) and the tRNA's His, Ser and Leu were amplified via PCR. Additionally, we amplified 24 terminals for the nuclear protein-coding genes neurotrophin 3 (*NT3*) and dynein, axonemal, heavy chain 3 (*DNAH3*). These terminals represent the main clades recovered with the mitochondrial dataset. *Cyt-b* was amplified using the primers Gludg, AtrCB3, and H16064 (Burbrink et al., 2000; Parkinson et al., 2002). *ND4* plus the adjacent tRNA region was amplified using the primers ND4 and LEU (Arévalo et al., 1994). *NT3* was amplified with the primers NT3-F3 and NT3-R4 (Noonan and Chippindale, 2006a,b), and *DNAH3* was amplified using the primers DNAH3-f1 and DNAH3-r6 (Townsend et al., 2008). All PCR products were sequenced directly in both directions using the amplification primers on an ABI 3730 DNA Analyzer. Raw sequence chromatographs were edited using Sequencher 4.7 (Gene Codes) and aligned manually using GeneDoc 2.6 (Nicholas and Nicholas, 1997). All sequences generated in this study were deposited in GenBank (Table 1).

2.3. Phylogenetic reconstruction

Maximum likelihood (ML) and Bayesian Inference using Metropolis-Hasting coupled Markov chain Monte Carlo methods (BI) were used to infer phylogenies. For the phylogenetic analyses, we used two different datasets, one that was entirely mitochondrial and included all terminals. The second, including both mitochondrial and nuclear genes, was a reduced dataset with only the well-supported haplotype clades inferred in the prior analysis. First we inferred phylogenetic relationships using 130 terminals with the two mitochondrial genes. This extensive sampling included, in many cases, intraspecific sampling for several *Leptodeira* subspecies. By using model-based phylogenetic reconstruction methods, we assumed that mtDNA would have a strong phylogenetic signal to determine relationships both at the intra and interspecific level. To avoid potential problems in phylogenetic reconstruction with only mtDNA (i.e. saturation or introgression), we added two slow evolving genes from the nuclear genome that have been suggested as good candidates for phylogenetic reconstruction (Townsend et al., 2008). Therefore, for the second strategy of analyses, we reduced the dataset to 24 terminals representing the well-supported clades recovered in the first analysis. This dataset included several outgroup species and one representative from each clade within *Leptodeira* recovered with the large mitochondrial dataset. The reduced dataset was analyzed in two ways: using the nuclear gene dataset exclusively, and including the mtDNA sequences in a combined analysis.

We used partitioned model analyses for all datasets because numerous studies have shown that partitioning models based on gene and codon position may be important for obtaining precise phylogenetic inferences (Brandley et al., 2005; Castoe and Parkinson, 2006), even at interspecific levels of divergence (Castoe et al., 2005). We determined the best partition scheme by calculating the Bayes factor between two competing partition strategies (Nylander, 2004; results not shown). The mitochondrial dataset was partitioned by gene and codon position while the nuclear dataset was partitioned by gene and each gene was partitioned in two: one partition for first and second codon positions, and a second partition for third codon positions. The best substitution model for each partition was determined using the Akaike Information Criterion (AIC) with the programs Modeltest 3.7 (Posada and Crandall, 1998) for the ML analyses and MrModeltest 2.3 (Nylander, 2004) for the BI analyses (Table S1, Supplementary material). The model likelihood values for each partition were calculated with PAUP* 4.0b10 (Swofford, 2003) and then AIC scores were determined in Modeltest and MrModeltest.

Maximum likelihood analyses were conducted in Treefinder (Jobb, 2008). Model parameters for each partition are described in Table S1 (Supplementary material). We allowed the program to estimate the best rate for each data partition. To estimate the relative support of nodes for the ML analysis, we conducted 500 non-parametric bootstrap pseudoreplicates in Treefinder. Bayesian analyses were conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Two independent MCMC runs were initiated with random starting trees and using one cold and three heated chains for 20×10^6 genera-

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tions, sampling every 1000 steps. Model parameters were estimated independently for each partition using the *unlink* option in MrBayes. Stationarity of chains was verified for each analysis by plotting the chain likelihoods against generations using Tracer 1.4 (Rambaut and Drummond, 2007). Three million generations were discarded as burn-in as the remaining samples resulted in ESS values larger than 1000 for all parameters. A consensus phylogram with posterior probabilities was determined by combining the remaining posterior samples from the two independent runs.

2.4. Divergence time estimation

We inferred divergence times among lineages using the combined reduced dataset (nDNA + mtDNA). Relaxed clock methods for divergence time estimation are preferred when the assumption of rate constancy is violated (Arbogast et al., 2002). Using the log likelihood ratio test, we rejected the null hypothesis of rate constancy ($p < 0.001$). Therefore, we used a stochastic model within a Bayesian approach that allows the estimation of rates and dates without the assumption of a molecular clock. We used two different approaches to check for congruence in the time estimates. First, we used Beast v1.4.7, which estimates the phylogeny and divergence times simultaneously, permitting more complex models of evolution and topological uncertainty during the optimization of divergence times (Drummond et al., 2006; Drummond and Rambaut, 2007). We implemented the lognormal relaxed clock option with a Yule prior for the speciation pattern and again partitioning the dataset in a similar way as used in the ML and BI analyses. Second, we used the topology obtained with Treefinder and MrBayes and estimated divergence times using the package Multidistribute (Thorne et al., 1998; Thorne and Kishino, 2002). For this second analysis, we partitioned the molecular data by gene. Using *baseml* (PAML package; Yang, 1997), model parameters for each partition were estimated under the F84+Γ model. Branch lengths and the variance–covariance matrix were calculated using the program *estbranches*. Divergence times were then estimated using the program *multidivtime*. The priors used for analyses in *multidivtime* included: rttm = 3.9, rttmsd = 0.3, rtrate = 0.3, rtratesd = 0.3, brownmean = 0.7, brownsd = 0.7, and bigtime = 10.0. The remaining priors used in *multidivtime* analyses were set to the program's defaults. For both approaches (Beast and *multidivtime*), we used a reduced data set for three reasons. First, we were interested in determining divergence times only at the interspecific level and among the main clades in the *Leptodeira annulata*/*septentrionalis* group. Second, intraspecific relationships do not correspond to a Yule process of speciation, which was the prior utilized in Beast. Third, intraspecific divergences show very short internodes, affecting the performance of branch length optimization in the program *estbranches* and, thus, producing unrealistic divergence time estimates.

2.5. Calibration points

The earliest fossil record of the Dipsadinae is very limited and difficult to interpret based only on osteology (Holman, 2000), making the inferred placement of fossils onto a tree very imprecise (Graur and Martin, 2004). In addition, most well-confirmed records for Dipsadinae come from very recent geological layers, obscuring the deeper origins of lineages (see Holman, 2000). Therefore, we added three viperid species and one representative of Elapidae, Natricinae and Colubrinae to the dataset to constrain the root of the tree. Based on the oldest colubrid fossil found, the split between Viperidae and Colubridae is estimated to have occurred before 40 Ma (Head et al., 2005; Rage et al., 1992). We used a value of 40 ± 16 Ma for the program *multidivtime* and a lognormal prior of the root height of the tree with a lognormal mean = 3.7 and lognormal SD = 0.3 for the program Beast. We used wide uniform priors and constrained the divergence between the New World and Old World Crotalinae to be older than 16 Ma and less than 32 Ma (Holman, 2000; Castoe et al., 2009; Guiher and Burbrink, 2008) and the origin of *Sistrurus* to be older than 9 Ma and less than 32 Ma (Parmley and Holman, 2007). Finally, we constrained the origin of Natricinae to be older than 30 Ma (Rage, 1988) and used a lognormal mean = 3.42 and a lognormal SD = 0.3.

2.6. Ancestral area reconstruction

We tested the biogeographic hypothesis of Duellman (1958a) that states that the genus *Leptodeira* originated in Mexico with a directional north-to-south expansion. We reconstructed the ancestral distribution within *Leptodeira* using DIVA (Ronquist, 1997). This event-based method does not require information about the area relationships and instead optimizes ancestral areas for nodes in a phylogenetic tree using a parsimony algorithm giving costs to dispersal and extinction scenarios. Even though taxon sampling may affect the ancestral area reconstruction (Ronquist, 1997), our inferred ancestral areas for *Leptodeira* were not affected by the areas we used for the tips outside *Leptodeira* and *Imantodes* (results not shown). We assigned lineages to the three main biogeographic regions found in the Neotropics: Mexico that includes the tropical and subtropical region west of the Isthmus of Tehuantepec, Middle America that goes from the Isthmus of Tehuantepec to the Isthmus of Panama and South America that goes from eastern Panama to Brazil.

3. Results

3.1. Alignment and sequence variation

The total alignment for the mitochondrial dataset comprised 1933 bp (*Cyt-b* = 1083 bp, *ND4* = 681 bp, and *tRNA*'s = 169 bp). For the nuclear dataset, it was 1266 bp (*DNAH3* = 741 bp and *NT 3* = 525 bp). The alignment was straightforward for protein-coding genes, as no internal stop codons were detected. The mitochondrial dataset had 916 parsimony-informative sites (47.4%) for the large dataset and 659 parsimony-informative sites (34.1%) for the reduced dataset. On the other hand, the nuclear dataset had 70 (5.5%) parsimony-informative sites. The largest uncorrected percent genetic distance (P), using the mitochondrial dataset, was found between *Oxyrhopus petola* and *Leptodeira nigrofasciata* (23.7%). Similarly, the largest P distance, using the nuclear dataset, was found between *Leptodeira septentrionalis* and *Oxyrhopus petola* (6.7%). Within *Leptodeira*, the largest genetic distance was found between *L. nigrofasciata* and *L. septentrionalis* for both the mitochondrial and the nuclear datasets (20.5% and 3.6%, respectively).

3.2. Phylogenetic reconstruction

Both the ML and BI analyses recovered well-supported clades and nearly identical topologies with some minor differences in nodal support, regardless of the dataset analyzed (Figs. 3 and 4). The genera *Pseudoleptodeira, Hypsiglena*, and *Eridiphas* formed a well-supported clade, as did a cluster of other genera including *Cryophis*, *Atractus*, *Sibon*, *Ninia* and *Dipsas*; the sister-group relationship between these two clades was not well supported, however. *Leptodeira* and *Imantodes* formed a clade with 100% support in both ML and BI analyses. *Leptodeira* was inferred to be monophyletic, with relatively high support (bootstrap = 81% PP = 92%, Fig. 3). In contrast, *Imantodes* was found to be paraphyletic, with a clade containing

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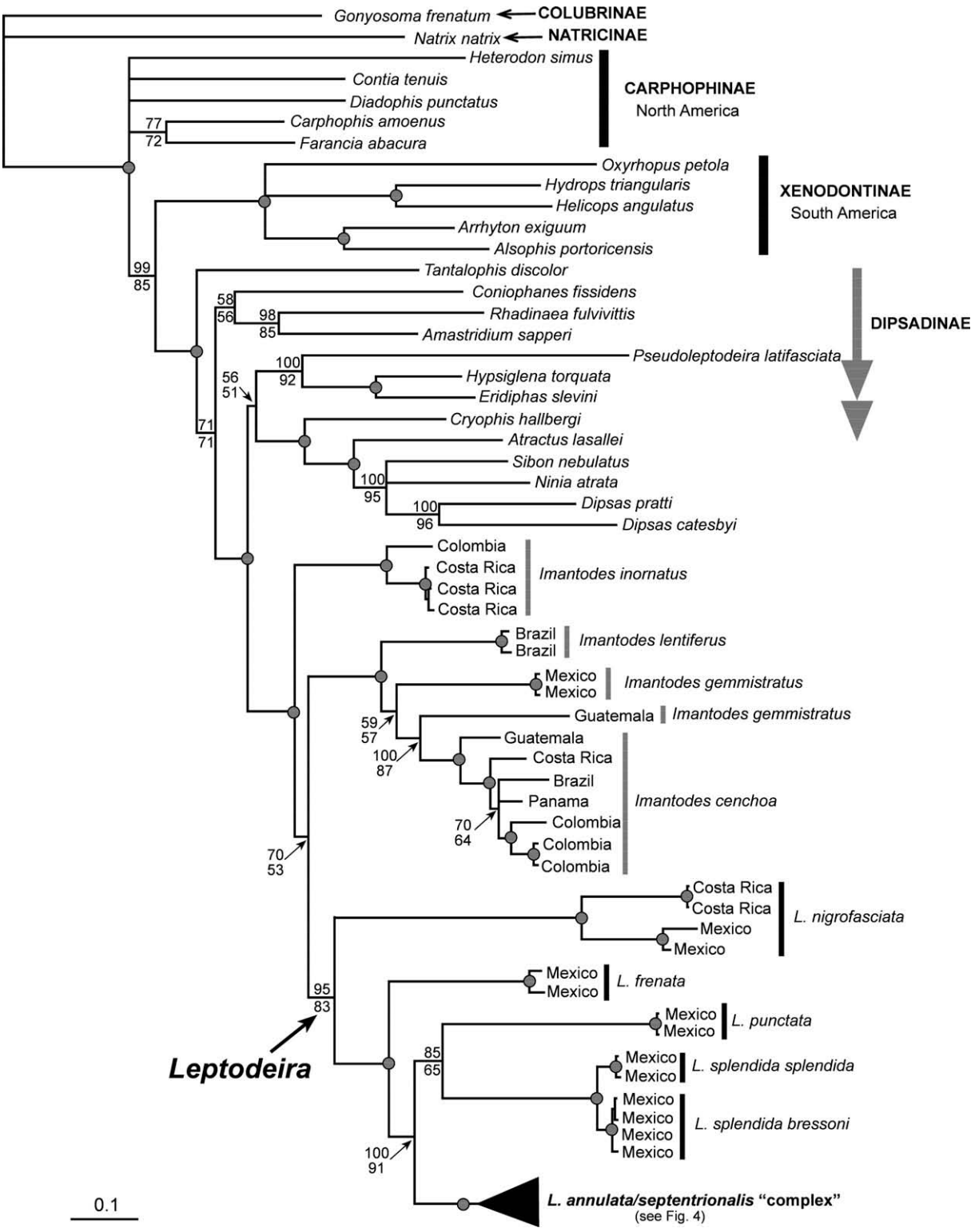

Fig. 3. Phylogenetic estimate of relationships within the Dipsadinae, and among the major groups of *Leptodeira*. The tree represents the Bayesian 50% majority-rule consensus phylogram from a partitioned analysis of mitochondrial gene sequences (*Cyt-b*, *ND4*, and *tRNA*'s; total of 1933 bp). Grey circles represent nodes with >95% support obtained via maximum likelihood (bootstrap values) and Bayesian (posterior probabilities) analyses. Numbers above nodes are posterior probabilities and numbers below nodes are maximum likelihood bootstrap support.

I. lentiferus, *I. gemmistratus* and *I. cenchoa* being the sister taxon to *Leptodeira*, and *I. inornatus* the sister taxon to both. Within *Leptodeira*, there was a ladderized pattern, with *L. nigrofasciata* diverging the earliest, followed by *L. frenata*. *Leptodeira punctata* formed a clade with *L. splendida*, with moderate support (bootstrap = 69%, PP = 94%) and their sister clade is composed of members of the *L. septentrionalis* and *L. annulata* groups (*sensu* Duellman, 1958a).

Intraspecific sampling recovered all *Leptodeira* species as monophyletic except the species *L. annulata* and *L. septentrionalis*. Samples assigned to *L. septentrionalis* were found in three distantly

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related clades (Fig. 4). Although samples assigned to *L. s. polysticta* formed a monophyletic group, such was not the case for *L. s. ornata*. A similar polyphyletic pattern was observed in *L. annulata*, in which four independent clades were recovered. Only the subspecies *L. a. rhombifera* was found to be monophyletic. Each *L. annulata* clade recovered was the sister taxon to either *L. septentrionalis*, *L. maculata*, or *L. bakeri* (Fig. 4). Overall, sister-taxon relationships were found between geographically contiguous lineages rather than between traditionally recognized subspecies (Fig. 4).

The analysis of the combined dataset (nDNA + mtDNA) produced essentially the same topology as the one recovered with the large mtDNA dataset. The phylogenetic signal of the nuclear dataset alone was sufficient to infer the relationships among the main clades that were obtained with the large mitochondrial dataset (around 50% of the nodes were resolved with high support; Fig. 5). The supports for the ML and BI analyses of the nuclear gene data were relatively high for the intergeneric relationships (bootstrap > 70%, PP > 95%). Again, *Leptodeira* and *Imantodes* clustered

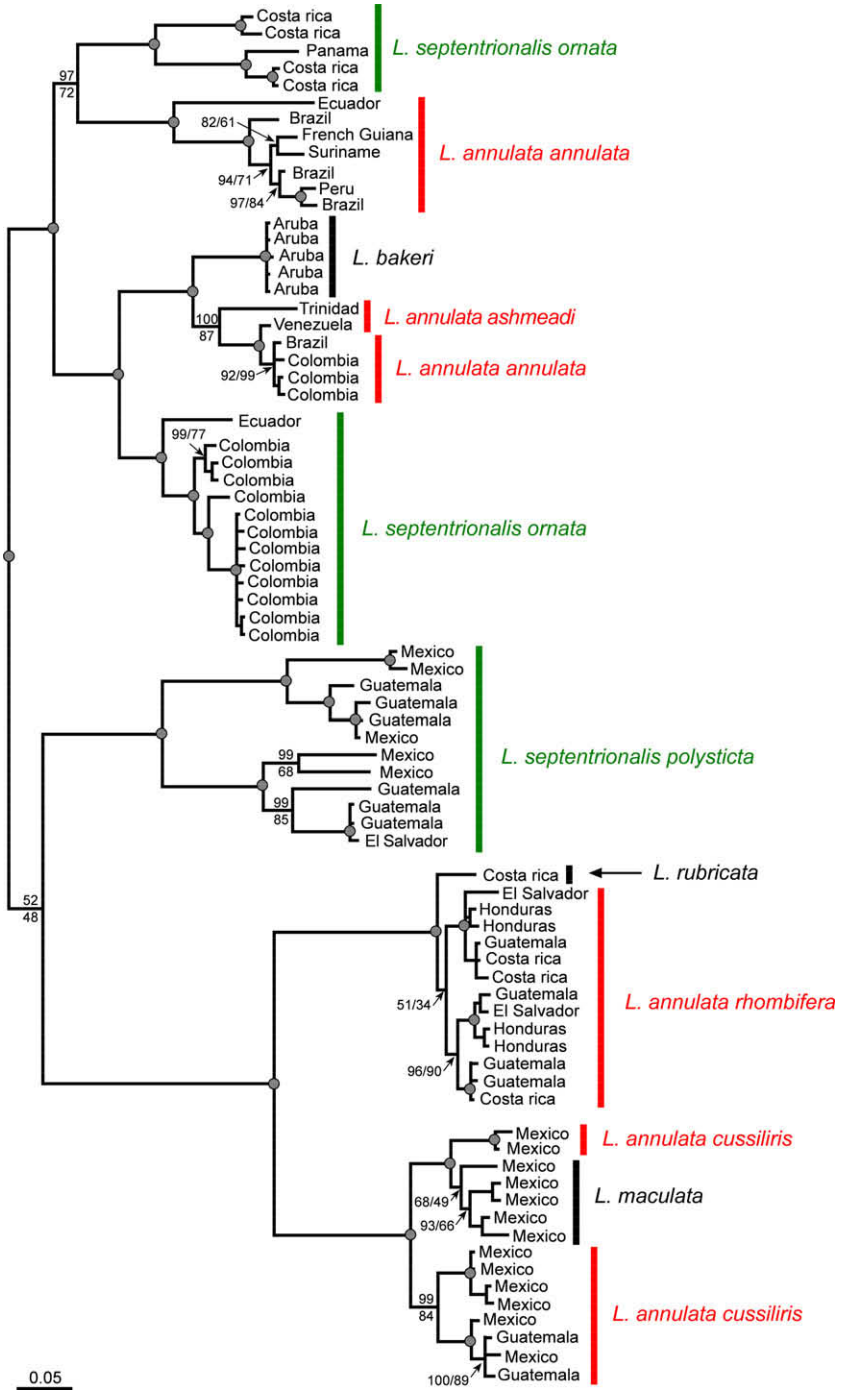

Fig. 4. Expanded view from Fig. 3 depicting the phylogenetic relationships of the *Leptodeira annulata* and *L. septentrionalis* species complex. The tree represents the Bayesian 50% majority-rule consensus phylogram from a partitioned analysis of mitochondrial gene sequences (*Cyt-b*, *ND4*, and *tRNA*'s; total of 1933 bp). Grey circles represent nodes with >95% support obtained via maximum likelihood (bootstrap values) and Bayesian (posterior probabilities) analyses. Numbers above nodes are posterior probabilities and numbers below nodes are maximum likelihood bootstrap support.

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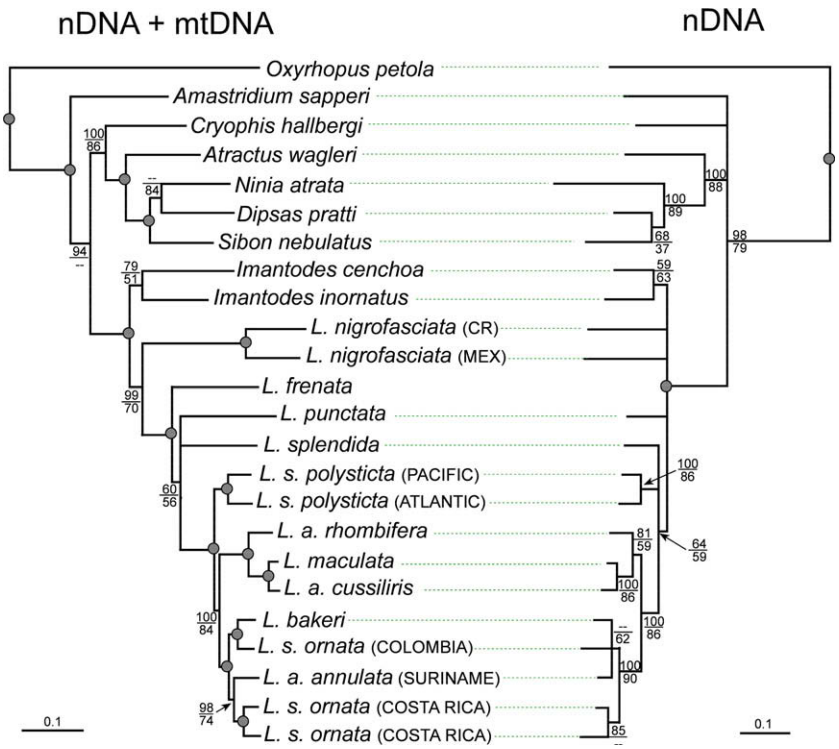

Fig. 5. Phylogenetic relationships of *Leptodeira* and relatives. (A) Bayesian 50% majority-rule consensus phylogram from a partitioned analysis of the mitochondrial and nuclear combined data (total of 3199 bp). (B) Bayesian 50% majority-rule consensus phylogram from a partitioned analysis including only the nuclear genes *DNAH3* and *NT 3* (total of 1266 bp). Grey circles represent nodes with >95% support obtained via maximum likelihood (bootstrap values) and Bayesian (posterior probabilities) analyses. Numbers above nodes are posterior probabilities and numbers below nodes are maximum likelihood bootstrap support. Dashes represent nodes that were not recovered with either Bayesian or maximum likelihood analysis.

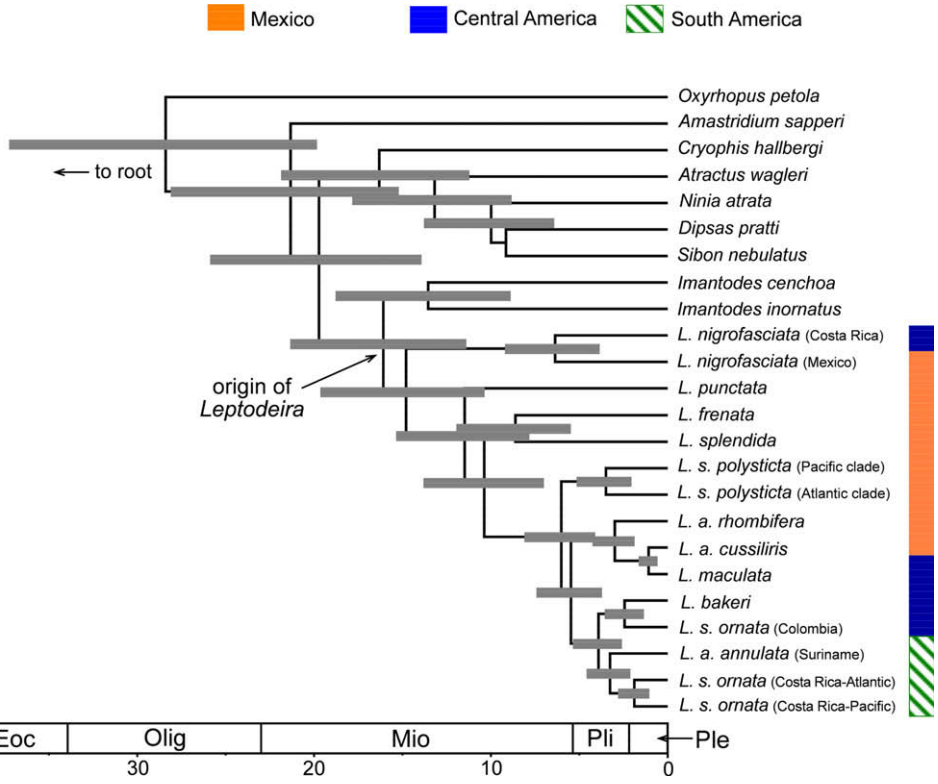

Fig. 6. Divergence time estimates of *Leptodeira* and relatives inferred with Beast 1.4.7. Grey bars represent the 95% credibility intervals for node heights. Time periods as follows: Olig = Oligocene, Mio = Miocene, Pli = Pliocene, Ple = Pleistocene.

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to form a well-supported clade within the Dipsadinae (100% support for both analyses), although there was a polytomy among major lineages of *Imantodes* and *Leptodeira* that rendered the *Leptodeira* monophyly unresolved (Fig. 5). Overall, the resolution of phylogeny estimated from the nuclear data was in excellent agreement with that of the mitochondrial data (Figs. 3–5).

3.3. Divergence times and ancestral area reconstruction

Analyses with Beast and *multidivtime* produced similar divergence time estimates (Table S2, Fig. S1, Supplementary material), and hereafter we refer specifically to the Beast results. Mutation rates varied among branches and between mitochondrial and nuclear markers. The average mutation rate for mitochondrial genes was 1.34% per million years ($CI_{95\%}$ = 0.99–1.70%) and for nuclear genes was 0.14% per million years ($CI_{95\%}$ = 0.10–0.18%). The origin of Dipsadinae was inferred to be approximately 28.4 Ma ($CI_{95\%}$ = 19.9–37.3). Most of the diversification of the Dipsadinae was estimated as having occurred during the first half of the Miocene (~11–20 Ma; Fig 6), while the origin of *Leptodeira* was estimated to be 16.1 Ma ($CI_{95\%}$ = 11.4–21.6 Ma). Speciation within *Leptodeira* appears to be mostly from the second half of the Miocene, although certain lineages originated both during the Pliocene and as recently as the Pleistocene (Fig. 6). Regarding the geographic speciation of *Leptodeira*, lineage diversification in the Mexican transition zone occurred from the Miocene to the Pleistocene, and the diversification of species distributed in Central and South America occurred in a narrower window of time during the Pliocene.

According to DIVA, the geographic origin of *Leptodeira* could not be resolved unambiguously (Fig. S2). The ancestral area for the nodes leading to *Leptodeira* and the first split within *Leptodeira* may have been either Mexico or an area comprising Mexico and Middle America. The ancestral area for the species *L. frenata*, *L. punctata* and *L. splendida* and the subspecies *L. septentrionalis polysticta* was estimated to be Mexico. A general pattern of north-to-south colonization from Mexico to South America was observed within *Leptodeira* (Fig. S2).

4. Discussion

Phylogenetic and biogeographic patterns obtained during this study highlight the spatial and temporal complexity of biological diversification in the Neotropics. Given its broad distribution throughout this region, *Leptodeira* appears to be an excellent model through which to understand the historical patterns of lineage diversification in one of the most biodiverse regions in the world. Our results challenge both the current phylogenetic and taxonomic status of the genus *Leptodeira*, and the traditional use of morphology to delimit evolutionary units in Neotropical snakes. Patterns of lineage diversification within *Leptodeira* also reveal much about the historical processes that have shaped the genus evolution, and probably many other lineages throughout the Neotropics since the Miocene.

4.1. Phylogenetic relationships within Dipsadinae

The subfamily Dipsadinae has been hypothesized to represent a monophyletic group, although roughly 50% of the putative genera have not been analyzed (Zaher, 1999; Vidal et al., 2000; Pinou et al., 2004; Lawson et al., 2005). To increase our understanding of Dipsadinae relationships, we included the genera *Amastridium* and *Ninia*. We found that the two genera should be included within the Dipsadinae (Fig. 3). The monotypic genus *Tantalophis* was previously considered a member of *Leptodeira*, but evidence has repeatedly shown *Tantalophis* to be a very distinct lineage (Duellman, 1958b; Mulcahy, 2007). Our data confirm this idea, as well as the hypothesis of *Tantalophis* as a member of the Dipsadinae, as opposed to Lawson et al. (2005) who defined the genus as *incertae sedis*. The subfamily Dipsadinae has more than 400 extant species and future phylogenetic studies are required to elucidate the patterns and mechanisms by which its fascinating diversity was accomplished.

4.2. Monophyly of Leptodeira

The first species of the genus *Leptodeira* was described by Linnaeus (1758) as *Coluber annulatus*, but Fitzinger (1843) later allocated this species to its own genus, *Leptodeira*. Since then, several species currently in *Leptodeira* have been assigned to other genera of Central American dipsadines (Duellman, 1958a; e.g., *Sibon*, *Hypsiglena*). Mulcahy (2007) examined the monophyly of the genus, and even though he did not include all the species assigned to *Leptodeira*, two main results can be highlighted from his work. First, *Leptodeira* appeared to be non-monophyletic in the parsimony analysis (see his Fig. 4) but monophyletic with moderate support, in the Bayesian analysis (PP = 86%). Second, regardless of the reduced taxon sampling, some species groups and subspecies appeared to be paraphyletic.

Using a combined analysis of four genes, we inferred a strongly supported clade that includes all species of *Leptodeira* (Fig. 5). The nuclear dataset alone, however, did not infer a monophyletic *Leptodeira* but rather a polytomy including *Imantodes* and *Leptodeira* species was recovered. This lack of resolution is likely due to the low numbers of informative characters in the nuclear dataset (see Section 3). The two nuclear genes resolved the relationships among different genera of Dipsadinae and even within *Leptodeira*, but they did not support the monophyly of the genus (Fig. 5). It is also plausible that the divergence between *Imantodes* and *Leptodeira* occurred in a narrow window of time and therefore a high degree of nuclear polymorphism in the ancestor of these genera did not have enough time to coalesce between splitting of population lineages, resulting in a lack of phylogenetic signal (Moore, 1995; Rosenberg, 2002).

The present results suggest *Imantodes* as monophyletic, based on both combined nuclear and mitochondrial data or nuclear alone. In addition to the increased character sampling, including intraspecific sampling of *Imantodes inornatus* and *Imantodes cenchoa* (both from Central America and northern South America) has provided evidence of previously unexpected genetic diversity. This diversity should be further examined to elucidate phylogeographic patterns that might parallel the codistributed genus *Leptodeira*. The paraphyly of *I. gemmistratus*, the uncertain phylogenetic position of *I. tenuissimus* and *I. phantasma* (species not included in this study), and the observed genetic diversity within *I. cenchoa* further justify a broader biogeographic study for this widely distributed group.

4.3. Leptodeira species groups and alpha taxonomy

Current taxonomic classification of *Leptodeira* is based entirely on morphology. Duellman (1958a) defined species groups and alpha taxonomy on hemipenial morphology, color pattern and geographic distribution. Our study, in addition to Mulcahy's (2007) work, supports the idea that current species groups in *Leptodeira* do not represent natural groupings. None of the species groups proposed by Duellman were recovered as monophyletic (Figs 3–6). Consequently, the previously employed species group assignments need to be removed from the systematics of this genus, and species and subspecies status should be reassessed to reflect our new views of the evolutionary history of *Leptodeira*.

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We obtained strong support for *Leptodeira nigrofasciata* being the sister taxon to a clade comprising all other species of the genus. Interestingly, uncorrected genetic distance between *L. nigrofasciata* and the remaining species of *Leptodeira* was as high as that found between *L. nigrofasciata* and *Imantodes* (about 16–17%; see also Mulcahy, 2007). Even though we examined only four individuals of *L. nigrofasciata*, our results present two very divergent allopatric lineages with a fairly ancient divergence; the first lineage includes populations from the pacific coast of Mexico and the second populations from northern Guatemala to northwestern Costa Rica. The deep genetic divergence, the strong morphological difference (Smith and Taylor, 1945; Taylor, 1954; Shannon and Humphrey, 1964), and the allopatric distribution provide evidence for potential species recognition of these two divergent lineages after analyzing samples from the intervening land, El Salvador, Honduras, and Nicaragua.

The sister-taxon relationship between *L. splendida* and *L. punctata*, as suggested by Mulcahy (2007), was not recovered in our combined analysis using nuclear and mitochondrial markers, but it was recovered by the mitochondrial dataset alone. The nuclear dataset, although with low support, suggests that *L. punctata* may be the sister taxon to a clade including *L. splendida* and members of the *L. septentrionalis* and *L. annulata* groups. Whether the mitochondrial or nuclear datasets separately infer the true phylogeny, our results highlight the importance of adding independent phylogenetic markers and more individuals to estimate the species tree from gene trees (Maddison and Knowles, 2006). Regarding the subspecies status within *L. splendida*, we did find reciprocal monophyly between *L. s. splendida* and *L. s. bressoni.* Based on these preliminary results, in addition to the morphological evidence given by Duellman (1958a), we suggest maintaining the subspecies status within *L. splendida* until additional evidence is gathered and phylogeographic boundaries can be discovered (see below).

4.4. *Leptodeira annulata–septentrionalis "complex"*

The most striking result of this study is the polyphyly of the species *L. annulata* and *L. septentrionalis* (Fig. 4). These two groups are the most widely distributed species of the genus, and given the morphological and geographic variation, five subspecies of *L. annulata* and four of *L. septentrionalis* are currently recognized (Duellman, 1958a). Our results detailing excessive polyphyly of these two species, however, are not entirely surprising given the high degree of morphological variability in both species that often overlaps between species. It thus appears that morphological parallelism has likely precluded previous taxonomic efforts to accurately identify evolutionary units in this complex. Sasa-Marin (2000) investigated the phylogeography of *L. annulata* in the dry forests of Central America. His *L. annulata* includes those belonging to *L. a. cussiliris* in the Pacific coast of Oaxaca and western Guatemala and the dry Grijalva Valley of Mexico and Guatemala, and *L. a. rhombifera* from the eastern Pacific coast and interior valleys of Guatemala to northwestern Costa Rica. Both forms represent relatively short and terrestrial forms. Herein we confirm his deep division in Guatemala, between the two subspecies, and find *L. a. rhombifera* also in two main clades located north and south of the Comayagua valley of Honduras.

Several "variants" allied to *L. annulata* have been elevated to species level (*L. rubricata*, *L. maculata*, *L. bakeri*). For instance, Savage (2002) refers to an unpublished work that "convincingly" suggests keeping *L. rubricata* as a distinct species after Duellman (1958a) synonymized it with *L. annulata*. Our analyzed sample of *L. rubricata* was not found to be genetically distinct from members of *L. a. rhombifera* as its sequence divergence was equivalent to that among members of the subspecies (Fig 4.) While genetic distance should not be the sole criterion for species diagnosis (Wiens and Servedio, 2000; Sites and Marshall, 2004; Esselstyn, 2007), this finding warrants further studies to determine if *L. rubricata* is a distinct lineage deserving species status.

As predicted by Duellman (1958a), *L. bakeri* was closely related to the mainland form, *L. a. ashmeadi* (Fig. 4). Given the small geographic distribution of *L. bakeri*, and the monophyly observed we hypothesize that this is most likely the result of a single population lineage that colonized the island of Aruba. In addition to the phylogenetic results, its morphological distinctiveness from the mainland clade and its allopatric distribution (Mijares-Urrutia et al., 1995) support its recognition as a distinct evolutionary unit (*sensu* Wiens and Penkrot, 2002). Based on geographic gradients of the number of dorsal blotches, Duellman (1958a, 1966) also recognized *L. maculata* as a different species from *L. annulata cussiliris* and suggested sympatry as unlikely. One of us (ENS) has reviewed many specimens of *L. annulata* from the Pacific coast of Mexico and has observed that these two species are not easily diagnosable based on the characters given by Duellman (see also Shannon

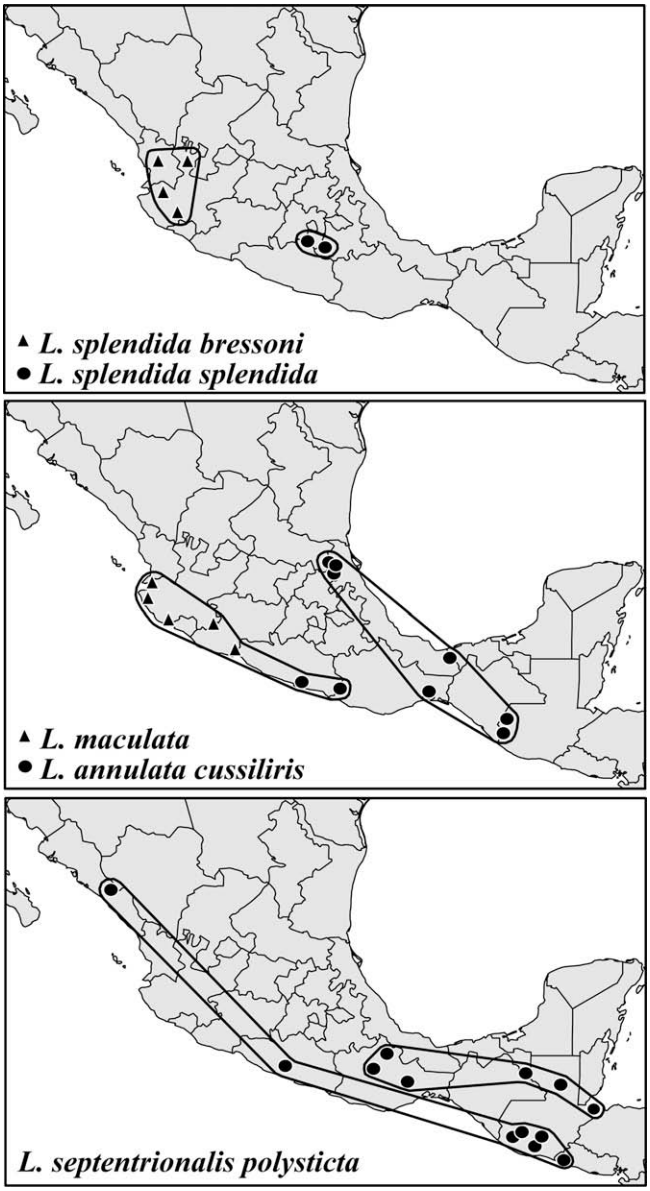

Fig. 7. Intraspecific phylogeographic structure of *Leptodeira* species in the Mexican transition zone. Lines delimit the clades recovered with the mtDNA dataset, and dots represent sampled localities.

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and Humphrey, 1964). Our phylogenetic results (both mitochondrial and nuclear) suggest the same mixed pattern. Individuals from Guerrero and Oaxaca considered *L. a. cussiliris* are phylogenetically nested within *L. maculata*, instead of being nested with the remaining *L.a. cussiliris* (Figs. 4 and 7). This result, in addition to the morphological similarity between the two groups, suggests that *L. maculata* is a geographic variant of the widespread *L. a. cussiliris* and should therefore be synonymized (contra Duellman, 1966).

Leptodeira septentrionalis, as currently recognized, can be distinguished phylogenetically as three distantly related clades: one in northern Central America (Mexico and Guatemala), another clade in lower Central America (Costa Rica and Panama), and a third in northwestern South America (Colombia and Ecuador). Each of these three lineages is the sister group to a clade of *L. annulata*, and all are allopatric except for the presence of sympatric *L. s. polysticta* with *L. a. cussiliris* in Mexico and *L. a. rhombifera* in Central America, from Guatemala to, probably, Costa Rica. Similarly, *L. annulata* consists of five independent clades that intermix with *L. septentrionalis* clades, *L. maculata* or *L. bakeri*. Collectively, these findings underscore the need for numerous taxonomic changes regarding these two species, as well as *L. maculata* and *L. bakeri*. Species delimitation and description is, however, outside the scope of this study, and taxonomic changes will be treated elsewhere using additional lines of evidence, such as morphological and ecological modeling data.

Campbell (1998) elevated *L. septentrionalis polysticta* to species status based on morphological evidence. Our phylogenetic evidence strongly supports his claim as this group represents a monophyletic group, highly divergent from *L. s. ornata* or the other subspecies examined (Fig. 4). More interesting is the fact that *L. s. polysticta* had the greatest within-species genetic structure within the genus. Two divergent clades, which appear candidates for species status, were recovered with high support from both mitochondrial and nuclear datasets (Figs. 4 and 5); one clade represents the humid forests in the Atlantic versant of Mexico and Guatemala while the other clade corresponds to the dry regions of the Pacific coast of Mexico, Guatemala and El Salvador (Fig. 7). Our lack of sampling in Honduras and the Mosquitia region of Nicaragua precludes any further confirmation of the southern extent of *L. s. polysticta* or the northern extent of *L. s. ornata*. According to Duellman (1958a) the first form should occur all the way south to northeastern Costa Rica, and *L. s. ornata* should have its northern limit near de Costa Rica–Panama border.

4.5. Diversification and biogeography

Lineage diversification within *Leptodeira* corresponds largely to the major biogeographic provinces in the Neotropics. Well-recognized biogeographic regions, such as the Mexican transition zone, lower Central America and northwestern South America, played a critical role in shaping the diversity of *Leptodeira*. In contrast, the Amazon basin did not appear to be a major factor for lineage diversification. Understanding the phylogenetic relationships and the time of cladogenetic events within *Leptodeira* will help us to identify the importance of historical events occurring in these provinces and to highlight their contributions to the Neotropical diversity.

4.5.1. The Mexican transition zone

The Mexican transition zone (*sensu* Halffter, 1987) is one of the most complex regions in the Americas, with a dynamic geological evolution since the Cretaceous period (Coney, 1982; Ortega and Arita, 1998). The importance of its *in situ* diversification and the interchange between the Neartic and the Tropical region has been addressed by many authors (Marshall and Liebherr, 2000; Morrone and Marquez, 2001; Escalante et al., 2004; Huidobro et al., 2006; and references therein). It has been hypothesized that the origin of *Leptodeira* occurred in Mexico (Duellman, 1958a; Mulcahy, 2007). This hypothesis is largely based on the observation that the majority of species, many separated by the deepest phylogenetic splits of the genus, occur there. Using an explicit method for ancestral area reconstruction (DIVA), we could not resolve unambiguously the area where the *Leptodeira* + *Imantodes* ancestor may have originated. This lack of resolution is likely due to *Imantodes,* the sister taxon to *Leptodeira*, having a widespread distribution. Instead, we did find evidence that the early and most important lineage diversification of *Leptodeira* occurred in the Mexico (Fig. S2). Using explicit methods to estimate divergence times, we also inferred that this diversification began during the middle Miocene and spanned throughout the Pleistocene. Duellman (1958a) proposed a similar temporal frame, using geological and geographic information (compare Figs. 1 and 6). Most likely, the recurrent orogenic events across the Mexican transvolcanic axis and the Isthmus of Tehuantepec during the Miocene severed gene flow between Atlantic and Pacific populations to give rise to *L. frenata* on the Atlantic and *L. nigrofasciata*, *L. splendida* and *L. punctata* on the Pacific versant. The diversification of lowland species within western Mexico is less obvious but could be related to either the formation of the main river basins or to Miocene climatic changes (Devitt, 2006; Espinosa et al., 2006; Bryson et al., 2008; and references therein). During more recent times, Pleistocene climatic changes and sea level fluctuations in the Isthmus of Tehuantepec might have severed gene flow among Mexican populations, generating the phylogeographic patterns observed at the intraspecific level (Fig. 7).

4.5.2. The bridge between Central and South America

Lower Central America harbors one of the most diverse biota per square kilometer on the planet (Savage, 2002). The tremendous *in situ* diversification and the role as the final bridge between South America and the Neartic region during the Pliocene allowed multiple lineages to colonize both continents (Marshall et al., 1979; Webb, 1997). Current phylogenetic and biogeographic evidence shows that this interchange occurred several times, even prior to the Pliocene, a time for which evidence of land connection between the two regions is missing (Marshall et al., 1979; Bermingham and Martin, 1998; Pennington and Dick, 2004; Koepfli et al., 2007; and references therein). Our DIVA results show that *Leptodeira* reached South America via the Panama Isthmus in a single colonization. Later on, an event of dispersal from South America back to Lower Central America (Fig.S2) is predicted. If the expansion of *Leptodeira* into South America was gradual and monotonic, we would expect to see sister-taxon relationships between adjacent regions. Instead, *L. septentrionalis* from Costa Rica is the sister taxon to the clade in the Amazon basin, and the Colombia + Ecuador + Venezuela clade is the sister taxon to the Costa Rica + Amazon basin clade. Ancestors of *Leptodeira* colonized northern South America around 4 Ma prior to the closure of the isthmus. We hypothesize that after the closure, around 3.4 Ma, a second colonization event occurred, this time from South America back to Lower Central America. It is interesting to note that cat-eyed snakes from humid forests in Costa Rica resemble the ones in the Amazon basin in their arboreal–semiarboreal habits, whereas *Leptodeira* from Colombia and northern Venezuela are mostly terrestrial (Duellman, 1958a; Savage, 2002; pers. obs.). Given these phylogenetic patterns and the ecological distribution of *Leptodeira* in South America, we hypothesize that fluctuations in vegetation cover allowed range expansion and severed gene flow affecting the arid and mesic clades differently (Crawford et al., 2007; Peterson and Nyari, 2008; Wang et al, 2008). Finally, the divergence between the Chocó-Magdalena clade and the northern Colombia-Venezuela clade during the Pleistocene

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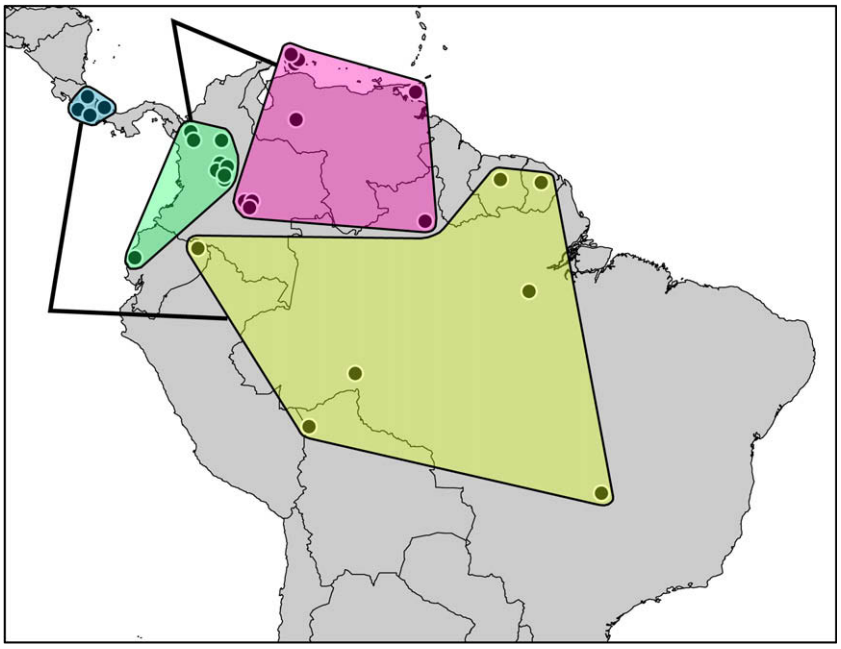

Fig. 8. Phylogeographic structure of *Leptodeira* species in Lower Central America and South America. Lines represent the clades recovered with the mtDNA dataset, and dots represent sampled localities. Lines connecting clades indicate sister relationships.

might have been mediated by climatic fluctuations and eustatic sea level changes, isolating and severing gene flow among the different populations (Nores, 2004).

4.5.3. The Amazon basin

In contrast with other biogeographic provinces where *Leptodeira* is distributed, the Amazon basin clade did not show strong genetic structure, despite having the largest distribution (Fig. 8). Lack of genetic structure in the Amazon basin, attributed to Quaternary expansion, has been observed in other groups (Zamudio and Greene, 1997; Dick et al., 2004; Nyári, 2007; Peterson and Nyári, 2008). It has been documented that climatic fluctuations in the Amazon basin were drastic during the Pleistocene, expanding and contracting dry and humid habitats, which might have led to speciation or intraspecific phylogeographic patterns (Prance, 1982; Hooghiemstra and van der Hammen, 1998; Quijada-Mascareñas et al., 2007; Rull, 2008; but see Colinvaux et al., 2000). Although our results suggest that *Leptodeira* did not respond to these dramatic changes in the Amazon, it is also possible that the Amazonian clade was never fragmented and persisted in a more stable environment (Colinvaux et al., 2000). Sampling from the southernmost part of the *Leptodeira* distribution (*L. annulata annulata* and *L. a. pulchriceps*) and from the Atlantic forests of Brazil might reveal hidden phylogeographic structure, which has been observed in other codistributed species (Wüster et al., 2005; Grazziotin et al., 2006; Martins et al., 2007).

4.6. Conclusion

The present study highlights the complex evolutionary history of the widespread genus *Leptodeira* across the entire Neotropical region. Current species and subspecies recognition is not consistent with our phylogenetic results. Our inferred lineages correspond to biogeographic provinces rather than to previous classifications based solely on morphology. We concur with Duellman (1958a) in recognizing that geological and climatic changes since the Miocene determined the lineage diversification within *Leptodeira*. Such observation regarding spatial and temporal diversification in the Neotropical region, evidenced in the genus *Leptodeira*, should be tested with other widely codistributed lineages. Increasing taxon sampling in some areas (southern USA, northeastern Mexico, eastern Paraguay and southeastern Brazil) might uncover new phylogeographic patterns that, in turn, will provide us with a better picture of lineage diversification of populations inhabiting the limits of the Neotropical region. Finally, current taxonomy of *Leptodeira* warrants dramatic changes so that a new classification will reflect the evolutionary and biogeographic history of the genus.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.07.022.

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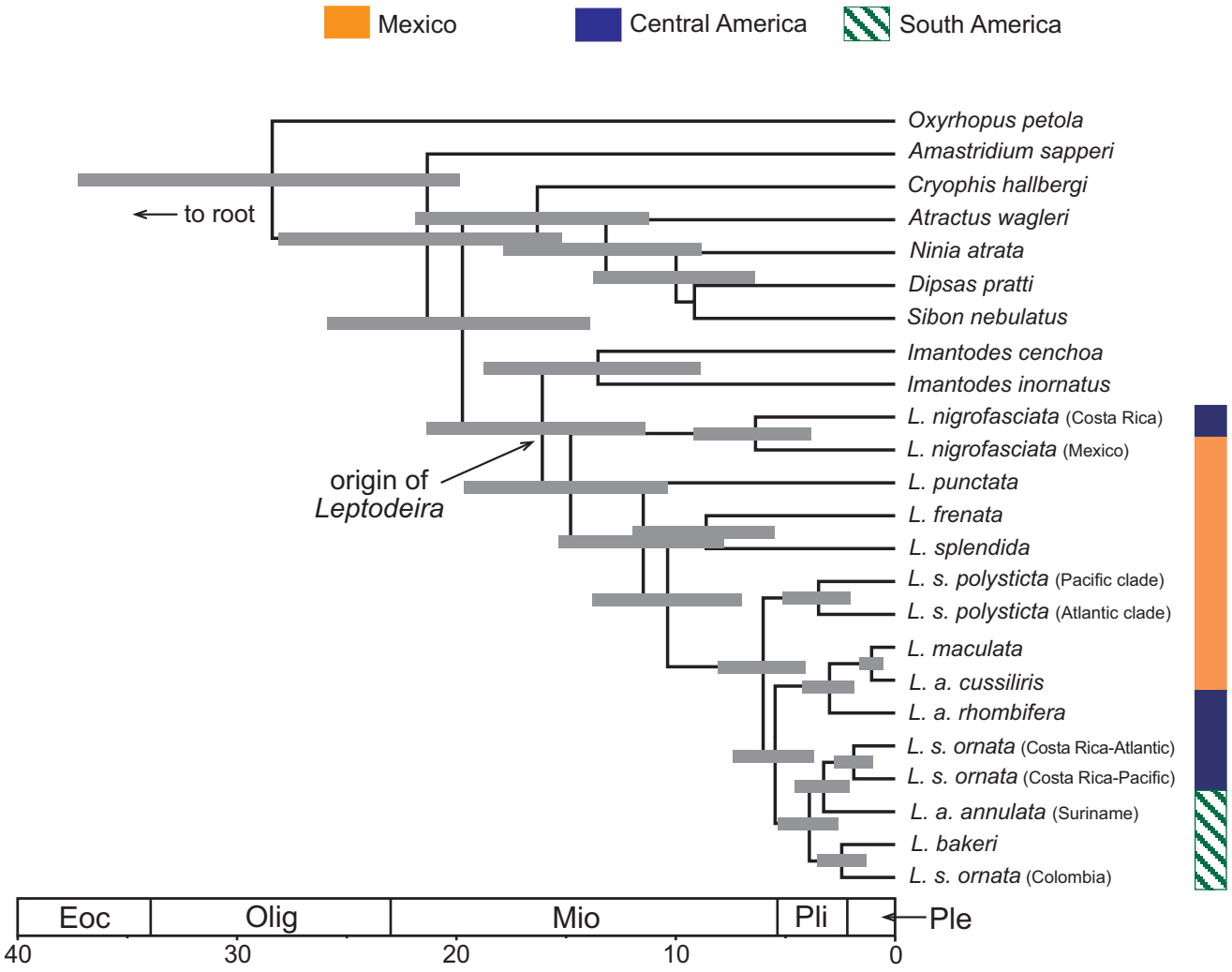
Erratum to

"Complex evolution in the Neotropics: The origin and diversification of the widespread genus *Leptodeira* (Serpentes: Colubridae)"

Juan M. Daza, Eric N. Smith, Vivian P. Páez, Christopher L. Parkinson

Replace Fig 6. in the manuscript for this one below

Explanation: the taxa are in a different order in the published paper



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