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Molecular phylogenetics and taxonomy of leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) inhabiting the peninsula of Baja California

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Abstract

Herein we assess the phylogenetic relationships and taxonomy of geckos of the genus *Phyllodactylus* inhabiting the peninsula of Baja California, Mexico using five mitochondrial and two nuclear genes. Phylogenetic analysis using maximum parsimony (MP) and Bayesian inference (BI) recovered three distinct peninsular clades with high statistical support. Sequence divergence estimates between peninsular taxa approached 13%. Two of the species, *P. unctus* and *P. xanti* are Cape Region endemics, whereas *P. nocticolus* is widespread throughout much of the peninsula and extreme southern California. Monophyly of the peninsular taxa was strongly supported. In the MP analysis, *P. unctus* rooted at the base of the peninsular clade, resolving *P. xanti* and *P. nocticolus* as sister taxa. Conversely, BI placed *P. nocticolus* and *P. unctus* as sister taxa. These data provide further evidence for a trans-peninsular seaway near the Isthmus of La Paz, severing the Cape Region from the rest of the peninsula. The analysis also supports the validity of *P. nocticolus* as a distinct species and suggests a single invasion to the peninsula from mainland Mexico, presumably during tectonic activity during the Miocene.

Key words: biogeography, cryptic species, Gekkota, phylogeny, speciation

Introduction

The complex geological history of Baja California and the Gulf of California has contributed significantly to the evolution of their regional biota. One hypothesis on the formation of the peninsula and the origin of its flora and fauna suggests a trans-gulfian vicariance model. This posits that tectonic activity during the Miocene led to rifting partitions of western Mexico off the North American Plate (Murphy 1983a,b; Hausback 1984; Lonsdale 1989; Riddle *et al.* 2000). This biogeographic model, in part, explains the patterns of evolutionary relationships observed between several taxa distributed on opposite sides of the Gulf of California (Murphy 1983b).

The dynamic paleogeographic history of the peninsula has, in turn, led to numerous taxonomic and phylogenetic studies conducted throughout Baja California, islands in the Gulf of California, and mainland northwestern Mexico. Several of these studies evaluated genetic breaks in mitochondrial DNA (mtDNA) lineages that coincide with trans-peninsular seaways, one mid-way on the peninsula and one across the Isthmus of La Paz, just north of the Cape Region (Upton & Murphy 1997; Riddle *et al.* 2000; Lindell *et al.* 2005, 2006, 2008).

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The entire peninsula of Baja California is home to three species of leaf-toed geckos: *Phyllodactylus unctus, P. xanti* and *P. nocticolus*. The latter species has been considered by some authorities to be a subspecies of *P. xanti* based on morphological data (Dixon 1964, 1966; Grismer 2002). Analyses based on morphology and allozymes suggested that *P. nocticolus* warrants full species status (Murphy 1983b). Disjunct populations of these and other species of *Phyllodactylus* also occur on islands of the Gulf of California (Dixon 1966; Murphy & Aguirre León 2002). These populations and species are not reported from mainland Mexico and, thus, it is suggested that island colonization resulted from over-water dispersal from the peninsula of Baja California (Savage 1960, Murphy 1983a,b), translocation by Seri Indians (Murphy & Aguirre León 2002) and/or paleotectonic activity (Murphy 1983a; Lonsdale 1989; Carreño & Helenes 2002).

The taxonomy of *Phyllodactylus* remains controversial, mainly due to significant morphological variation within species (Dixon 1964). Several subspecies of *Phyllodactylus* were erected to assist in the classification of intraspecific morphological variability (Dixon 1964, 1966). However, many insular subspecies distributed throughout the Gulf of California were synonymized with their mainland counterparts due to the lack of diagnosable characters differentiating them from the latter (Grismer 1999). Molecular data for *Phyllodactylus* are also generally lacking, making phylogenetic relationships and taxonomic designations difficult to ascertain.

There are two main objectives of the present study. First, we provide further molecular data indicating the validity of *P. nocticolus* and second, we determine the phylogenetic relationships of peninsular and representative mainland forms and compare our results with previously published data to infer and discuss concordant biogeographic patterns throughout the peninsula of Baja California.

Methods

Tissue Collection

Specimens and tissues were collected from populations of each species throughout the peninsula of Baja California (Table 1; Fig. 1). Taxon identification follows Murphy (1983b). Fieldwork was conducted in 1978, 1985, and 2000. Geographic coordinates were obtained for the latter year only, although additional geographic coordinates were obtained using Google Earth. Voucher specimens were deposited in collections housed at the National Autonomous University of Mexico (UNAM), California Academy of Sciences (CAS), Los Angeles County Museum (LACM) and the Royal Ontario Museum (ROM). Additional sequences were obtained from *P. lanei, P. paucituberculatus, P. duellmani,* and *P. davisi* from mainland Mexico as primary outgroup taxa. GenBank sequences were also obtained for *Gekko gecko* (AY282753) to root all networks.

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was digested and extracted from liver or muscle tissue using standard phenol–chloroform protocols (Sambrook *et al*. 1989; Hillis *et al*. 1996; Palumbi 1996). Tissue samples were first mixed with Proteinase K and Laird's buffer and digested overnight prior to extraction with phenol–chloroform. Following extraction, samples were re-suspended in ddH_2O prior to amplification.

Approximately 4300 bp encompassing two nuclear genes and five mitochondrial genes were amplified using the polymerase chain reaction (PCR; Saiki *et al*. 1988). Nuclear genes amplified included brain-derived neurotrophic factor (BDNF, 670 bp) and proto-oncogene C-*mos* (380 bp). Mitochondrial loci included cytochrome *c* oxidase subunit I (COI, 420 bp), and NADH dehydrogenase subunit 4 (ND4, 690 bp). In addition, two ribosomal RNA (rRNA) fragments and the intervening transfer RNA tValine ($tRNA^{Val}$) were amplified, encompassing 1600 bp of 16S rRNA and 900 bp of 12S rRNA. Primers used for amplification and sequencing, their corresponding sequences and references are presented in Appendix I.

PCR reactions (25 µl) were composed of the following reaction mix: 18.55 µl ddH_20, 1 µl 10 mM of each primer, 2.5 µl 1.5 mM $MgCl_2$ buffer, 0.8 µl 10mM dNTPs, 0.15 µl 5 U *Taq* DNA Polymerase (Boehringer Mannheim), and 1 µl template DNA. Amplification was performed on a Perkin Elmer GeneAmp 9700

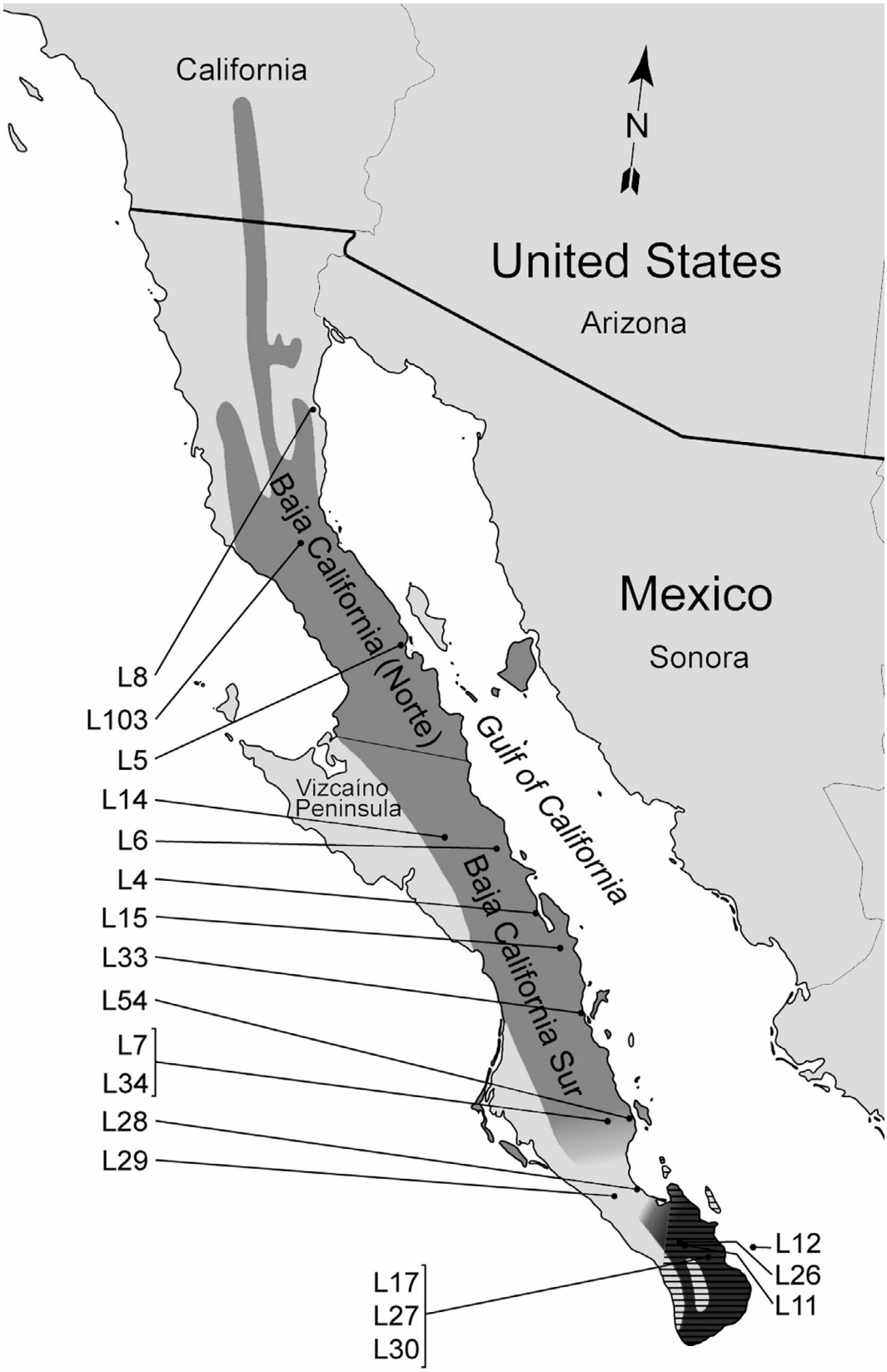

FIGURE 1. Map of Baja California, the Gulf of California, southwestern USA, and northwestern Mexico illustrating the geographic location of samples of *Phyllodactylus* included in this study. Sample numbers correspond to those presented in Table 1. The break in geographic ranges in the Isthmus of La Paz region is due to unsuitable natural habitat (lack of rocky outcrops). Gray area represents the geographic range of *P. nocticolus*, black area represents the geographic range of *P. xanti*, striped area represents the range of *P. unctus*.

TABLE 1. Taxon name, tissue number, sample ID, geographic location, GPS coordinates, and GenBank accession numbers for all species of *Phyllodactylus* sequenced. ROM = Royal Ontario Museum; RWM = Collections of Robert W. Murphy housed at the ROM.

Taxon	Tissue no.	Sample ID	Location	Latitude	Longitude
P. nocticolus	RWM 1166	L8	Mexico; Baja California; San Felipe (near)	30 41' 33" N	114 57' 23" W
P. nocticolus	RWM 1879	L103	Mexico; Baja California; Santa Ines	29 43' 45" N	114 41' 47" W
P. nocticolus	RWM 2164	L5	Mexico; Baja California; Bahia de Los Angeles, 8 km W of	28 58' 44" N	113 38' 13" W
P. nocticolus	RWM 1275	L14	Mexico; Baja California Sur; San Ignacio	27 17' 55" N	112 53' 33" W
P. nocticolus	RWM 2297	L6	Mexico; Baja California Sur; Arroyo Santa Agueda	27 15' 29" N	112 20' 57" W
P. nocticolus	RWM 2347	L4	Mexico; Baja California Sur; Playa Coyote, 4 km S of	26 41' 40" N	111 54' 43" W
P. nocticolus	RWM 1281	L15	Mexico; Baja California Sur; Loreto, 42 km N of	26 23' 20" N	111 27' 48" W
P. nocticolus	RWM 1467	L33	Mexico; Baja California Sur; Juncalito, 1.5 km W of	25 50' 22" N	111 21' 01" W
P. nocticolus	ROM 35407	L54	Mexico; Baja California Sur; 6 km W of San Evaristo	24 52' 23" N	110 44' 27" W
P. nocticolus	RWM 920	L7	Mexico; Baja California Sur; San Pedro la Presa	24 50' 52" N	110 59' 32" W
P. nocticolus	RWM 754	L34	Mexico; Baja California Sur; San Pedro la Presa	24 50' 52" N	110 59' 32" W
P. nocticolus	RWM 34055	L28	Mexico; Baja California Sur; La Paz, 40 km N of	24 17' 45" N	110 39' 45" W
P. nocticolus	RWM 34062	L29	Mexico; Baja California Sur; La Paz, 80 km NW of	24 40' 07" N	110 47' 57" W
P. xanti	RWM 2307	L17	Mexico; Baja California Sur; San Bartolo	23 44' 16" N	109 51' 20" W
P. xanti	ROM 34047	L27	Mexico; Baja California Sur; San Bartolo	23 44' 16" N	109 51' 20" W
P. xanti	ROM 34065	L30	Mexico; Baja California Sur; San Bartolo	23 44' 16" N	109 51' 20" W
P. unctus	ROM 34045	L26	Mexico; Baja California Sur; La Paz, 30 km S of	23 51' 02" N	110 18' 32" W
P. unctus	RWM 633	L11	Mexico; Baja California Sur; Hwy 1, 11 km S of Todos Santos turnoff	23 21' 20" N	110 10' 12" W
P. unctus	RWM 697	L12	Mexico; Baja California Sur; Hwy 13, 2 miles SE of junction with Hwy 1	24 06' 20" N	110 17' 20" W
P. paucituberculatus	ROM 35455	L80	Mexico; Michoacan; Los Olivos vicinity; where Hwy 37 crosses Rio Tepalcatepec	18 50' 56" N	102 08' 05" W
P. davisi	ROM 46935	L97	Mexico; Colima; 2 km NE Santiago	19 08' 12" N	104 20' 18" W
P. duellmani	ROM 35451	L61	Mexico; Michoacan; Los Olivos vicinity; where Hwy 37 crosses Rio Tepalcatepec	18 50' 56" N	102 08' 05" W
P. lanei	ROM 35287	L42	Mexico; Guerrero; Tierra Colorada	17 09' 29" W	099 31" 53" W

TABLE 1. (continued)

	Genbank Accession Numbers				
Taxon	RNA	COI	ND4	BDNF	C-*mos*
P. nocticolus	FJ662582	FJ662539	FJ662560	FJ662496	FJ662517
P. nocticolus	N/A	FJ662529	FJ662550	N/A	FJ662507
P. nocticolus	FJ662577	FJ662534	FJ662555	FJ662491	FJ662512
P. nocticolus	FJ662583	FJ662540	FJ662561	FJ662497	FJ662518
P. nocticolus	FJ662578	FJ662535	FJ662556	FJ662492	FJ662513
P. nocticolus	FJ662581	FJ662538	FJ662559	FJ662495	FJ662516
P. nocticolus	FJ662580	FJ662537	FJ662558	FJ662494	FJ662515
P. nocticolus	FJ662579	FJ662536	FJ662557	FJ662493	FJ662514
P. nocticolus	FJ662575	FJ662532	FJ662553	FJ662489	FJ662510
P. nocticolus	FJ662576	FJ662533	FJ662554	FJ662490	FJ662511
P. nocticolus	FJ662572	FJ662528	N/A	FJ662486	N/A

P. nocticolus	FJ662573	FJ662530	FJ662551	FJ662487	FJ662508
P. nocticolus	FJ662574	FJ662531	FJ662552	FJ662488	FJ662509
P. xanti	FJ662569	FJ662525	FJ662547	FJ662483	FJ662504
P. xanti	FJ662571	FJ662527	FJ662549	FJ662485	FJ662506
P. xanti	FJ662570	FJ662526	FJ662548	FJ662484	FJ662505
P. unctus	FJ662567	FJ662524	FJ662546	FJ662482	FJ662503
P. unctus	FJ662566	FJ662523	FJ662545	FJ662481	FJ662502
P. unctus	FJ662568	N/A	N/A	N/A	N/A
P. paucituberculatus	FJ662564	FJ662521	FJ662543	FJ662479	FJ662500
P. davisi	FJ662563	FJ662520	FJ662542	FJ662478	FJ662499
P. duellmani	FJ662562	FJ662519	FJ662541	FJ662477	FJ662498
P. lanei	FJ662565	FJ662522	FJ662544	FJ662480	FJ662501

(Applied Biosystems) or a PT200 DNA Engine (MJ Research) thermal cycler. Amplification conditions were as follows: initial denaturation of 94 °C (2 min) followed by 39 cycles of 94 °C (30 sec), 49-50 °C (45 sec), 72 °C (45 sec), with a final extension temperature of 72 °C for 6 min (Lindell *et al*. 2005). Following amplification, DNA products were separated on a 1% agarose gel stained with ethidium bromide under ultraviolet (UV) light. Visible bands were then excised from the gel and centrifuged through a filter pipette tip for 10 min. The resulting solution was used as the template for sequencing reactions.

Sequencing reactions (10 µl) were performed on an Eppendorf AG 5345 thermal cycler with the BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems) using the following recipe: 1 µl BigDye, 2 µl 5x BigDye Terminator Buffer, 2 µl ddH_20, 1 µl of 10 mM primer solution, and 4 µl PCR product. Reactions were run for 25 cycles with the following conditions: initial denaturation at 96 °C (1 min), 96 °C (10 sec), 50 °C (5 sec), 60 °C (4 min), and 4 °C indefinitely. All gene regions were sequenced in both forward and reverse directions. Following sequencing reactions, samples were cleaned and precipitated with sodium acetate and ethanol and visualized on an ABI 3100 Automated Sequencer (Applied Biosystems).

Sequence alignment and phylogenetic analysis

Forward and reverse sequences were initially imported and edited in BIOEDIT v.5.0.6 (Hall 1999) and subsequently combined for manual alignment. Aligned sequences were then imported into MACCLADE v.4.08 (Maddison & Maddison 2005) and checked by eye. The final MACCLADE alignment was then exported into PAUP* v.4.0b10 (Swofford 2002) for phylogenetic inference.

Maximum parsimony analysis (MP) was performed on the concatenated dataset using PAUP* v.4.0b10 (Swofford 2002). Uninformative characters (3373) were excluded from the analysis. All characters were treated as unordered with equal weighting. Gaps were treated as missing data. Because of the relatively small dataset (24 specimens), we used a branch-and-bound search strategy with furthest addition sequences. Branches were collapsed if the maximum branch length equaled zero. Support for nodes was assessed via 10,000 nonparametric bootstrap replicates using "fast" stepwise additions and retaining groups with >50% support (Felsenstein, 1985).

Bayesian inference (BI) was also used to infer the phylogenetic relationships among Baja Californian *Phyllodactylu*s. To better account for heterogeneous rate variation across sites both within and between regions, we employed a partitioned analysis for each gene (Ronquist & Huelsenbeck 2003; Nylander *et al*. 2004; Brandley *et al*. 2005). Theoretical and empirical evidence suggests several benefits to employing a partitioned Bayesian analysis to explain different subsets of data (gene, codon position, etc.) including higher –ln*L* scores and clade credibility values (i.e. posterior probabilities). Combining different genes and/or codon positions into one evolutionary model ignores the fact that different partitions are presumably evolving at very different rates, which will in turn increase the systematic error involved in model fitting (Brandley *et al*. 2005).

MrModeltest v.2.2 (Nylander 2004) was used to obtain evolutionary models for each gene partition using the Akaike Information Criterion (AIC; Akaike 1974, 1979). The AIC was chosen over hierarchical likelihood ratio tests (hLRTs) for model selection because the former tends to penalize more over-parameterized models more strongly than the latter and, thus, minimizes the degree of variance involved in model fitting (Akaike 1974, 1979). Bayesian inference was performed using MrBayes v.3.1 (Huelsenbeck & Ronquist 2001). Two simultaneous runs of six chains were run for 3 X 10^6 generations, sampling every 100 generations. Stationarity was assessed when likelihood scores reached a stable equilibrium. A burn-in value of 7500 was then implemented to discard topologies with low likelihood scores prior to generating a 50% majority rule consensus tree. Node support within the Bayesian consensus tree was assessed from the posterior distribution of topologies (Erixon *et al*. 2003).

In order to assess potentially conflicting phylogenetic signals from nuclear and mtDNA, we performed MP and BI on each dataset independently. For both MP analyses, a heuristic search was run with 100 replicate additions and tree bisection-reconnection (TBR) branch swapping. Nodal support for each MP analysis was also assessed by nonparametric bootstrapping with 10,000 "fast" stepwise addition replicates (Felsenstein 1985). BI was performed on each dataset independently under the same conditions as the concatenated dataset described above. For all nuclear data, *Gekko gecko,* L12, and L103 were removed prior to phylogenetic analysis due to missing data. Trees were thus rooted using *P. lanei*.

Results

Genetic diversity

For the concatenated dataset, 915 characters were potentially phylogenetically informative. Variability within nDNA was low with only eight characters potentially phylogenetically informative. There was a significant difference in average base frequencies (χ^2 = 164.80; *df* = 69; *P* < 0.001) with an adenine/cytosine bias. Percent sequence divergence based on uncorrected *p*-distances revealed significant differentiation between major groups (Table 2). Further, divergence estimates between *P. nocticolus* and *P. xanti* approached 10%, clearly indicating the mtDNA distinctiveness of both species. MrModeltest selected the GTR+I+G model of nucleotide evolution for the COI, ND4, 12S and 16S partitions, the HKY model for C-*mos* and BDNF and GTR+G for $tRNA^{val}$.

Phylogenetic analysis of concatenated dataset

The branch-and-bound search resulted in 2 most parsimonious trees (MPTs) of 2596 steps (CI = 0.5458; RI = 0.6837; RC = 0.3732). The only difference between the two trees involved the relationships among samples L4, L15, and L33 of *P. nocticolus*. In order to illustrate branch lengths we chose one of the MPTs (Fig. 2).

BI and MP analyses of the concatenated dataset resulted in highly compatible topologies with high support from both Bayesian posterior probabilities (BPP) and MP bootstrap proportions (BSP) (Figs. 2,3). Both analyses resulted in three major well-differentiated peninsular clades corresponding to species. *Phyllodactylus lanei* rooted as the sister group of all ingroup taxa and *P. paucituberculatus* was sister to a clade containing *P. davisi* and *P. duellmani*. Both analyses supported the monophyly of peninsular species from mainland Mexican taxa (BSP = 92; BPP = 1.00). *Phyllodactylus unctus* rooted the base of the peninsular clade in the MP analysis, resolving *P. xanti* as sister to a clade containing *P. nocticolus* from across the peninsula. However, support for the sister relationship of *P. xanti* and *P. nocticolus* was relatively weak (BSP <50%). Conversely, BI placed *P. nocticolus* and *P. unctus* as sister taxa with moderate support (0.94). Monophyly of *P. nocticolus* was strongly supported with both methods (BSP = 99; BPP = 1.00).

TABLE 2. Percent sequence divergence (uncorrected *p*-distances from mtDNA) for Baja Californian and mainland Mexican *Phyllodactylus* compared to *Gekko gecko*. N/A = undefined distance between sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	Gekko gecko	-																						
2	P. duellmani (L61)	28.80	-																					
3	P. davisi (L97)	28.60	11.30	-																				
4	P. paucituberculatus (L80)	29.00	12.70	13.00	-																			
5	P. lanei (L42)	28.30	14.50	14.40	14.20	-																		
6	P. unctus (L11)	27.90	11.90	12.10	11.80	13.90	-																	
7	P. unctus (L26)	28.00	11.80	12.10	11.70	13.80	0.90	-																
8	P. unctus (L12)	29.70	14.50	14.90	15.60	18.00	1.00	1.00	-															
9	P. xanti (L17)	28.10	12.00	12.20	12.10	13.70	9.70	9.60	11.70	-														
10	P. xanti (L30)	28.00	11.90	12.50	12.20	13.90	9.90	9.80	12.10	1.50	-													
11	P. xanti (L27)	28.30	12.00	12.40	12.10	13.80	9.70	9.70	11.90	0.56	1.10	-												
12	P. nocticolus (L34)	28.00	11.90	12.80	12.60	14.00	9.80	10.00	12.20	10.10	9.90	10.10	-											
13	P. nocticolus (L103)	27.80	15.80	15.50	14.00	16.20	11.30	11.60	N/A	12.10	12.10	12.00	3.90	-										
14	P. nocticolus (L28)	28.80	12.80	12.90	12.20	14.10	10.10	10.10	12.80	10.20	10.30	10.10	2.10	2.70	-									
15	P. nocticolus (L29)	28.80	12.80	13.00	12.20	14.20	10.10	10.10	12.60	10.20	10.20	10.10	2.00	2.80	0.25	-								
16	P. nocticolus (L54)	28.50	12.60	13.00	12.40	14.10	9.80	10.00	12.50	9.80	9.80	9.90	0.20	3.10	2.00	2.00	-							
17	P. nocticolus (L7)	28.40	12.60	13.00	12.30	14.10	9.80	10.00	12.40	9.70	9.70	9.70	0.34	3.10	2.00	2.00	0.35	-						
18	P. nocticolus (L5)	28.20	13.10	13.00	12.40	14.10	10.20	10.40	12.70	10.00	10.20	9.90	2.60	2.80	2.80	2.80	2.60	2.70	-					
19	P. nocticolus (L6)	28.90	13.00	13.30	12.50	13.90	9.90	10.10	12.80	9.80	10.00	9.70	3.10	2.70	3.10	3.10	3.10	3.20	2.50	-				
20	P. nocticolus (L33)	28.70	13.00	13.20	12.60	14.40	10.30	10.30	12.60	10.30	10.20	10.10	3.20	2.90	2.90	2.90	3.40	3.30	2.70	2.60	-			
21	P. nocticolus (L15)	28.60	13.00	13.00	12.60	14.10	10.30	10.40	13.00	10.00	10.00	9.90	3.20	2.80	3.10	3.10	3.30	3.30	2.60	2.40	1.00	-		
22	P. nocticolus (L4)	28.80	13.00	13.20	12.50	14.20	10.10	10.20	12.50	9.90	9.90	9.90	3.20	2.90	3.00	3.00	3.20	3.20	2.70	2.40	1.10	1.10	-	
23	P. nocticolus (L8)	28.60	13.10	12.90	12.40	14.10	9.90	10.10	12.60	10.00	10.20	10.10	2.90	0.87	2.80	2.80	2.90	2.90	2.30	2.50	2.80	2.70	2.80	-
24	P. nocticolus (L14)	28.00	13.00	13.10	12.30	14.00	9.80	9.90	12.60	9.90	10.10	9.90	2.80	2.40	3.00	3.00	2.90	2.90	2.50	2.70	3.00	2.60	2.80	2.30

Phylogenetic analysis of nDNA

MP analysis of the two nuclear loci resulted In 42 MPTs of 28 steps (CI = 0.9643; RI = 0.9500; RC = 0.9161). Highly compatible topologies were recovered with both MP and BI (Fig. 4). *Phyllodactylus duellmani* and *P. davisi* were again resolved as sister taxa. However, unlike the total evidence analysis and mtDNA analysis (presented below), the relationship of *P. paucituberculatus* to its congeners was unresolved. The phylogenetic relationships of all peninsular species were also ambiguous. However, both MP and BI found strong support for the monophyly of both *P. xanti* and *P. nocticolus.*

Phylogenetic analysis of mtDNA

MP analysis of the mitochondrial genes resulted In 2 MPTs of 3192 steps (CI = 0.6313; RI = 0.6826; RC = 0.4309). The strict consensus mtDNA tree was identical to the total evidence tree with strong support for three monophyletic peninsular clades (Figs. 2,5). As with the total evidence topology, MP of mtDNA placed *P. xanti* and *P. nocticolus* as sister taxa, although support was relatively low (<50%). Monophyly of the three peninsular species was also strongly supported in the Bayesian topology (BPP = 1.0), although the phylogenetic relationships of these taxa were ambiguous (*P. nocticolus* + *P. unctus* = 0.84). Monophyly of *P. nocticolus* and *P. xanti* was strongly supported.

Discussion

Taxonomy of *Phyllodactylus*

The phylogenetic analyses presented herein provide further genetic evidence for the validity of *P. nocticolus* as a distinct species, separate from *P. xanti*. Our MP analysis suggests that *P. nocticolus* is sister of

P. xanti, which together form a clade sister to *P. unctus*. In contrast, the more strongly supported BI analysis resolved *P. nocticolus* and *P. unctus* as sister taxa. These results are of interest because, historically, *P. nocticolus* was considered a subspecies of *P. xanti* (Dixon 1964; Grismer 1999). Both *P. nocticolus* and *P. xanti* are easily distinguished from *P. unctus* because the latter lacks enlarged dorsal tubercles.

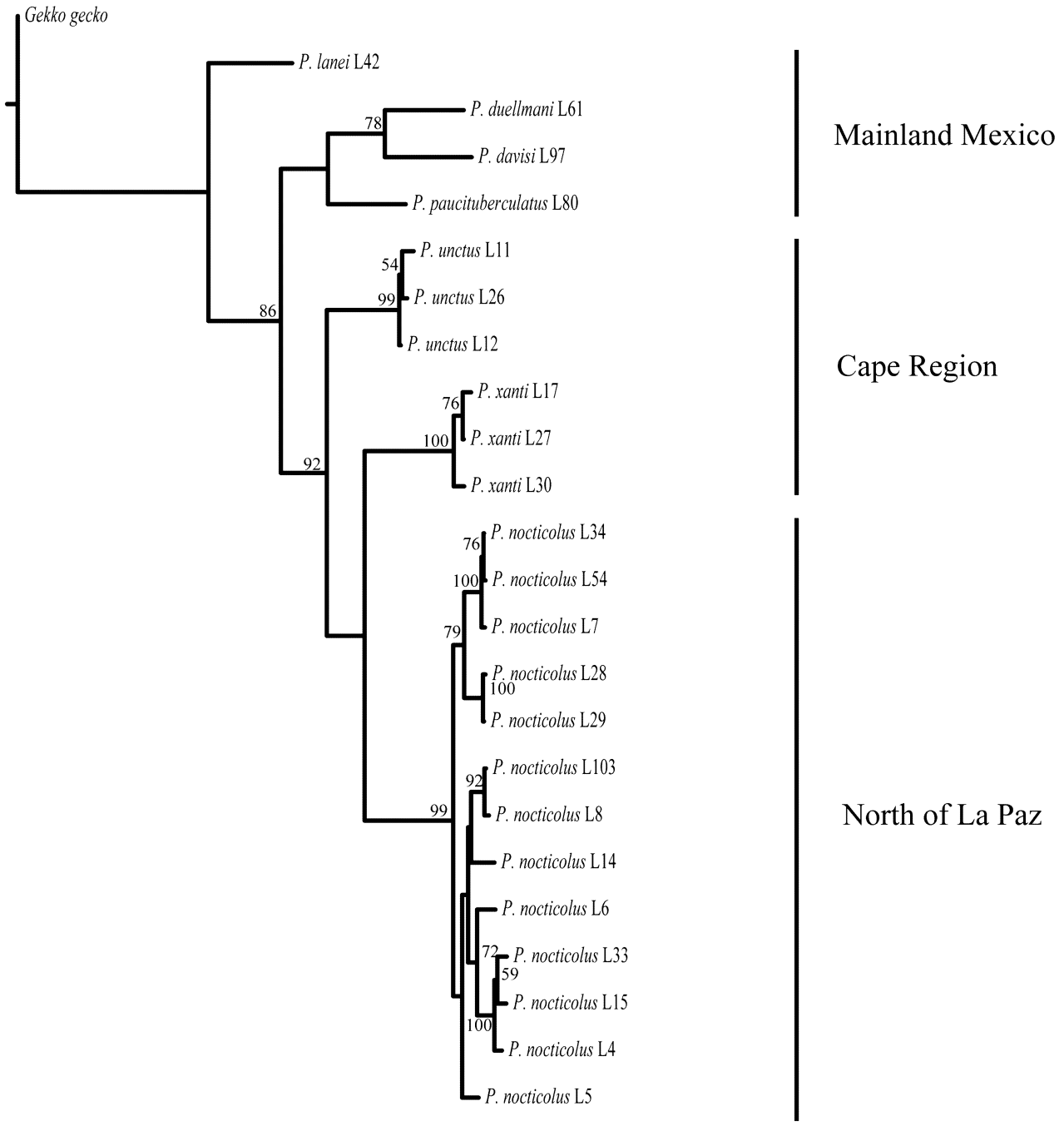

FIGURE 2. Phylogenetic relationships of Mexican *Phyllodactylus* based on a branch-and-bound maximum parsimony (MP) analysis of the concatenated dataset. Numbers above nodes represent MP bootstrap proportions (BSP) resulting from 10,000 pseudoreplicates.

Estimates of pairwise mtDNA divergence approached 13% between peninsular species of *Phyllodactylus*. This is in concordance with previous genetic work based on allozyme data, which showed significant genetic differences between Cape Region geckos and those north of La Paz (Murphy 1983b). Although previous researchers documented morphological variation between *P. xanti* and *P. nocticolus* including color pattern,

absence of thigh tubercles in *P. nocticolus*, and scutellation differences, authors felt that it was not significant enough to warrant full species status (Dixon 1964; Grismer 1994, 2002). Refer to Dixon (1964) for a full list of morphological characters used to differentiate *P. nocticolus* from *P. xanti* and other congeners.

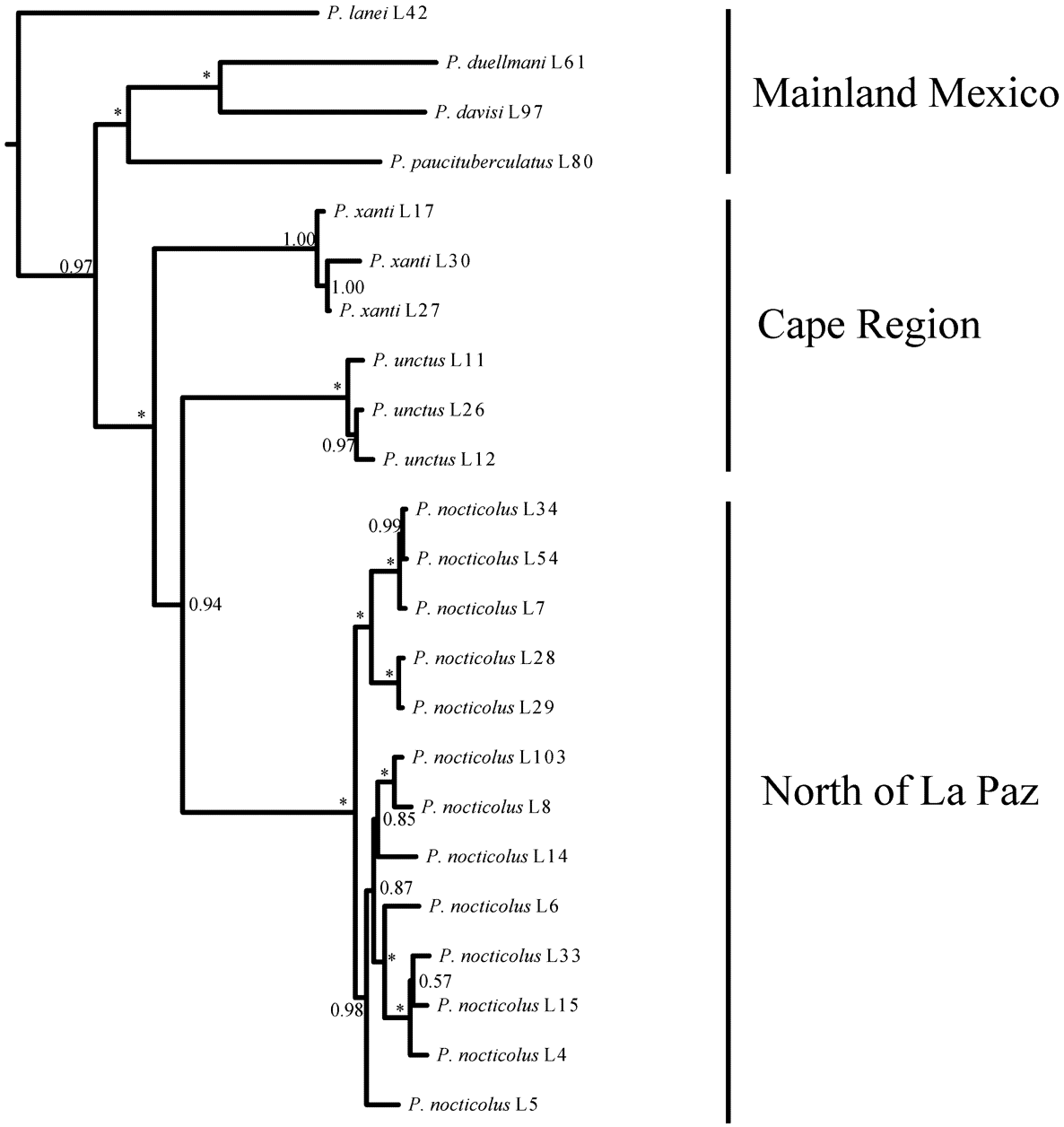

FIGURE 3. Phylogenetic relationships of Mexican *Phyllodactylus* based on Bayesian inference (BI) of the concatenated dataset. Numbers above and adjacent to nodes represent Bayesian posterior probabilities (BPP; * = 1.0) sampled from the posterior distribution of trees. *Gekko gecko* was removed from the tree due to its long branch length.

The taxonomy and delimitation of species ranges in *Phyllodactylus* has been ambiguous due to high intraspecific variability in several morphological characters (Dixon 1964). This variability, in conjunction with discordance between molecular and morphological characters, has hindered the elucidation of accurate phylogenetic hypotheses for the genus (Dixon 1964; Murphy 1983b). However, our molecular data, when taken in conjunction with previous allozyme and morphological investigations, provides comprehensive evidence for the validity of *P. nocticolus* as a distinct species

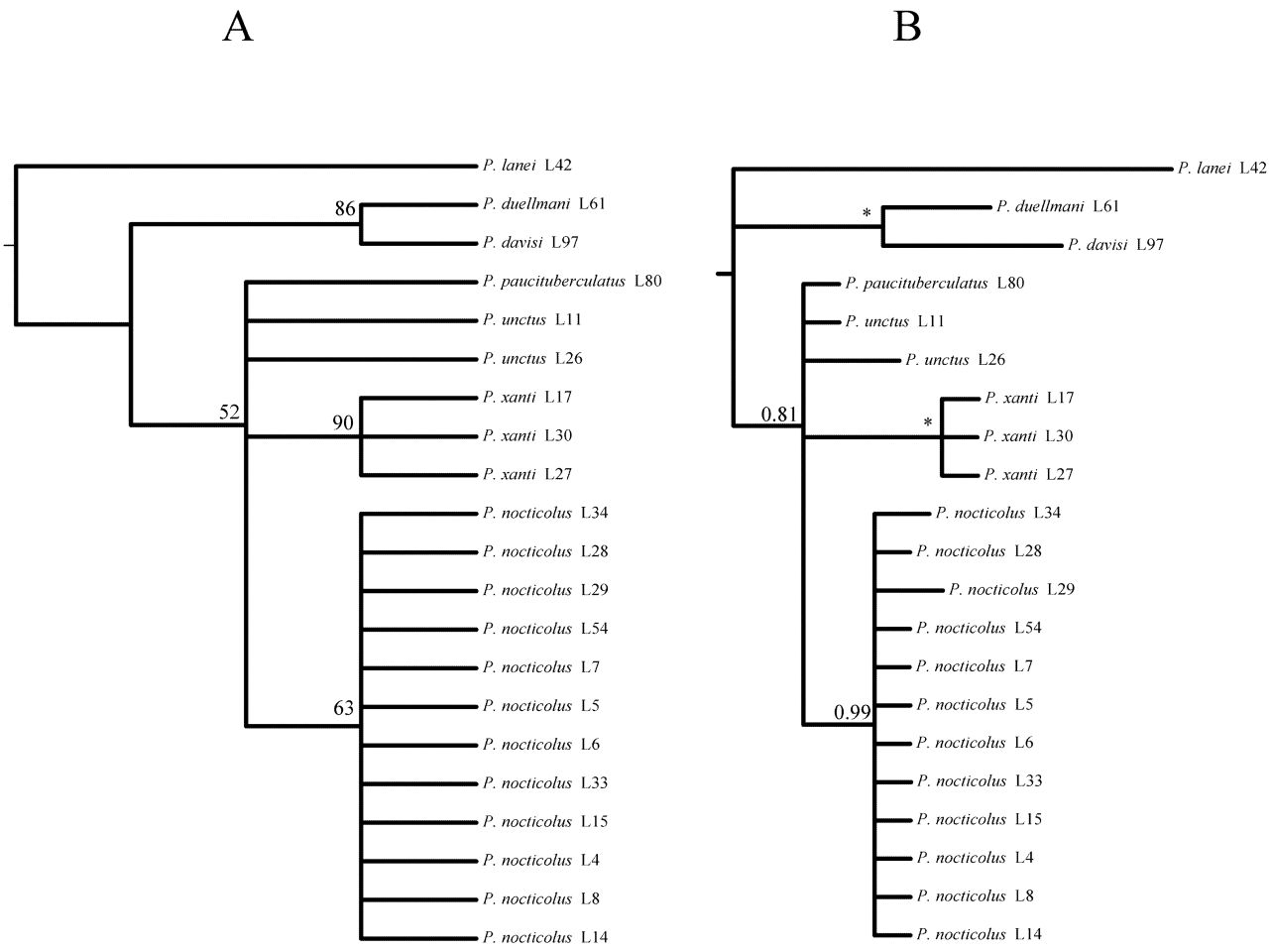

FIGURE 4. Phylogenetic relationships of Mexican *Phyllodactylus* based on maximum parsimony (MP; A) and Bayesian inference (BI; B) of nuclear DNA (nDNA). Numbers above nodes on MP tree represent nonparametric bootstrap proportions (BSP). Numbers above nodes on the Bayesian topology represent Bayesian posterior probabilities (BPP; * = 1.0). Refer to text for a description of loci and total base pairs (bp) sequenced.

Phyllodactylus xanti occurs both in the Cape Region of the peninsula as well as on several islands in the Gulf of California (Dixon 1964, 1966; Murphy & Ottley 1984; Grismer 1999, 2002). Historically, the range of *P. xanti* encompassed most of the peninsula of Baja California into extreme southern California (Dixon 1964). Populations residing in areas north of La Paz were designated as *P. xanti nocticolus*, whereas populations south of La Paz were classified as *P. xanti xanti* (Dixon 1964) with potential intergradation zones around Loreto and Comondu (Dixon 1964; Stebbins 2003). Our genetic analysis combined with previous allozyme and morphological comparisons suggests that peninsular populations north of La Paz be elevated to full species status. Thus, we recognize these populations as *P. nocticolus*. Further, the lack of suitable habitat surrounding the La Paz region (Fig. 1) suggests that populations of *P. xanti* and *P. nocticolus* are likely not in contact. Additional molecular studies are needed to ascertain the taxonomic status of insular forms north of La Paz.

Phyllodactylus unctus has long been considered a monotypic species, even though insular populations exhibiting slight morphological variation occur on islands associated with the Cape Region (Banks & Farmer 1962; Grismer 1999). On the peninsular mainland, the species is restricted to the Cape Region, where it occurs sympatrically and syntopically with *P. xanti* (Dixon 1964). Our high statistical support for the monophyly of the *P. unctus* clade are in concordance with previous investigations (Dixon 1964) and suggests that it constitutes a single species.

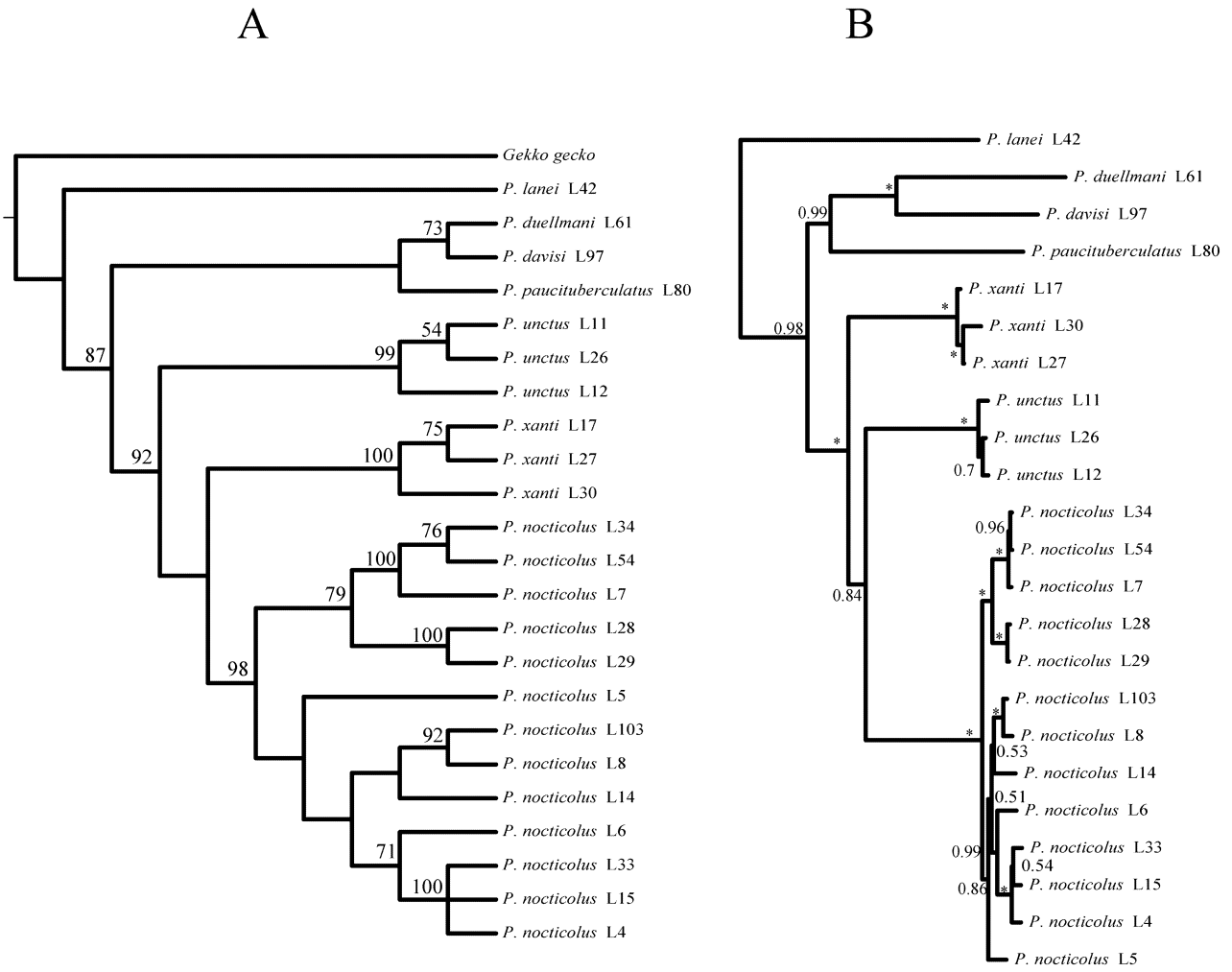

FIGURE 5. Phylogenetic relationships of Mexican *Phyllodactylus* based on maximum parsimony (MP; A) and Bayesian inference (BI; B) of mitochondrial DNA (mtDNA). Numbers above nodes on MP tree represent nonparametric bootstrap proportions (BSP). Numbers above nodes on the Bayesian topology represent Bayesian posterior probabilities (BPP; * = 1.0). *Gekko gecko* was removed from the BI tree due to its long branch length. Refer to text for a description of loci and total base pairs (bp) sequenced.

Biogeography

Several paleobiogeographic hypotheses have been put forth to explain patterns of diversification in *Phyllodactylus* and other Baja Californian taxa (Murphy 1983a,b; Murphy & Aguirre León 2002). Biogeographic and geological evidence suggests that the peninsula of Baja California was continually fragmented into smaller landmasses and islands throughout periods of the Miocene through the Pleistocene (Murphy 1983b; Smith 1991; Aguirre León *et al*. 1999; Riddle *et al*. 2000; Carreño & Helenes 2002; Murphy & Aguirre León 2002). This fragmentation resulted in a trans-peninsular seaway during the late Miocene, which temporarily isolated the Cape Region from the remainder of the peninsula (Lindell *et al*. 2008). Furthermore, somewhat later during the late Miocene, a temporary mid-peninsular seaway separated northern and southern peninsular biotas (Upton & Murphy 1997; Murphy & Aguirre León 2002; Lindell *et al*. 2005, 2006, 2008). Our genetic data support the hypothesis of a trans-peninsular seaway that isolated the Cape Region from the rest of the peninsula (Schwennicke *et al.* 1996; Carreño & Helenes 2002). This is shown in the high mtDNA sequence divergence estimates between the Cape Region's endemic species (*P. xanti* and *P. unctus*) and more widespread species north of La Paz (*P. nocticolus*).

Several empirical studies have documented genetic breaks in maternal lineages that coincide with a seaway across the mid-peninsular region (Upton & Murphy 1997; Riddle *et al.* 2000; Murphy & Aguirre León 2002; Lindell *et al*. 2005, 2006, 2008; Lindell & Murphy 2008). In contrast, our genetic data for the widespread *P. nocticolus* shows no such break (Figs. 2–5); there was no significant geographic structure throughout the peninsula for this species congruent with a mid-peninsular seaway. However, two reciprocally monophyletic lineages were detected, with a break between San Evaristo and Juncalito (Figs. 2–5). It is possible that this break in maternal lineages corresponds to the intergradation zone described in Dixon (1964).

Few studies have examined intraspecific trans-peninsular variation at nuclear loci. However, Murphy (1983b) found substantial allozyme evidence for the validity of *P. nocticolus* north of the Isthmus of La Paz using allozyme data. Conversely, Adest (1987) found no significant genetic differentiation in the zebra tailed lizard, *Callisaurus draconoides* throughout seven peninsular populations. These data contrast significantly to those utilizing mtDNA as molecular markers. Lindell *et al*. (2005), for example, showed highly divergent maternal lineages for *C. draconoides* distributed across the peninsula of Baja California. A similar discordance between mitochondrial and allozyme data is evident in *Urosaurus nigricaudus* (Aguirre León *et al*. 1999; Lindell *et al*. 2008). This mitochondrial-nuclear discordance clearly illustrates the need to incorporate bi-parentally inherited nuclear loci such as nuclear sequences and/or microsatellite DNA when examining biogeographic patterns of genetic differentiation throughout the peninsula of Baja California. Unfortunately, our nuclear data provided only eight potentially phylogenetically informative characters to the MP analysis.

The peninsular clade was resolved as monophyletic with high support from both MP and BI (Figs. 2,3). These results suggest a single invasion to the peninsula from mainland Mexico, presumably resulting from the rifting of western Mexico off the North American Plate during the increased tectonic activity associated with the Miocene (Murphy 1983b; Lonsdale 1989; Carreño & Helenes 2002; Murphy & Aguirre León 2002; Lindell *et al*. 2005). This rifting isolated peninsular populations from their mainland counterparts through the formation and northward expansion of the Gulf of California.

This study is the first to incorporate molecular data to infer evolutionary relationships among species of *Phyllodactylus*. In our phylogenetic analysis *P. lanei* rooted the base of our ingroup taxa. *Phyllodactylus duellmani* was resolved as sister to *P. davisi*, which together formed a clade sister to *P. paucituberculatus* (Figs. 2,3). However, only seven of the approximately 20 Mexican species were included in the analysis and until a more comprehensive sampling is undertaken, phylogenetic relationships among *Phyllodactylus* inhabiting mainland Mexico should remain tentative. Regardless, the inclusion of these taxa supports a single origin for Baja Californian species with high support from both BPP and BSP in our total evidence and mitochondrial analyses (*P. paucituberculatus* was unresolved with nDNA). Further, these results contrast to those of Dixon (1964), Grismer (1994), and Murphy and Papenfuss (1980) who suggested multiple independent origins to the peninsula.

Researchers have postulated alternative vicariant hypotheses to explain the evolution of Baja Californian biota, suggesting that climatic oscillations during the Quaternary contributed significantly to speciation in the flora and fauna of the region (Hafner & Riddle 1997). However, Lindell *et al*. (2006) suggested that paleogeographic and geologic forces have played a more significant role since the age of the concordant patterns are older than the Quaternary climatic fluctuations. Thus, it is highly probable that the biogeographic patterns observed in *Phyllodactylus* and other taxa distributed throughout the peninsula of Baja California are primarily due to the geological history of the region and not paleoclimatic phenomena.

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Appendix I. Mitochondrial and nuclear loci sequenced for *Phyllodactylus* and their corresponding primer sequences. Different combinations of primers were used to obtain full reads of regions.

Genome	Gene	Primer	Primer Sequence	Source
Mitochondrial	12S rRNA	12sa	5'—AAA CTG GGA TTA GAT ACC CCA CTA T—3'	Palumbi, 1996
		3395H	5'—AGT CTT CCC GTC CTT TTG CCA C—3'	This study
		504L	5'—GAT GAG GCA AGT CGT AAC ATG G—3'	This study
		12s2L	5'—ACA CAC CGC CCG TCA CCC TC—3'	Kocher *et al.*, 1989
		12s2H	5'—AGG GTG ACG GGC GGT GT GT—3'	Kocher *et al.*, 1989
		12S1L	5'—CAA ACT GGG ATT AGA TAC CCC ACT AT—3'	This study
	16S rRNA	1320L	5'—GGG GAA CCA CTG TTA AAA CTA G—3'	This study
		1715H	5'—TAA AGC TCC ACA GGG TCT TCT CGT C—3'	This study
		16s5H	5'—CTA CCT TTG CAC GGT TAG GAT ACC GCG GC—3'	This study
		3985L	5'—TTA CCA AAA ACA TGG CCT TTA GC—3'	This study
		16s1Lm	5'—CCG ACT GTT GAC CAA AAA CAT—3'	This study*
		16s3Lm	5'—CCC GAA CCC AGT CGA GCT AC—3'	This study*
		3456L	5'—GGT GAA ACG CCA AYC GCG CCT GG—3'	This study
		950L	5'—CCC TGT GGC AAA AGG GYG GG—3'	This study
		760L	5'—GTA CCA TAT AGG AAC ACT GAA AG—3'	This study
		990H	5'—CAT TTG ATG AGC AAC CAG CTA TCR C—3'	This study
		690H	5'—GTT CTT TCT CCA ATA CTG C—3'	This study
		16S2HM	5'—CCG GTC TGA ACT CAG ATC ACG—3'	This study*
	COI	COIphyR	5'—CTA TTG ATA GGA CGT AGT GG—3'	This study
		COIphyL	5'—TCC TTC CCG GVT TYG GMA T—3'	This study
		COIphyL1	5'—CTC ATC CTC CCS GGR TTT GG—3'	This study
		COIm72L	5'—TGA TTC TTC GGT CAC CCA GAA GTG TA—3'	Austin *et al.*, 2004
		COIm73H	5'—CCT ATT GAT AGG ACG TAG TGG AAG TG—3'	Austin *et al.*, 2004
	ND4	Nd4phyL	5'—CCT ATC CCC CAC AAC CCA AAC—3'	This study
		Nd4phyH	5'—TTA GTG TTT TGG TTA AAY TAT—3'	This study
		ND412931L	5'—CTA CCA AAA GCT CAT GTA GAA GC—3'	This study
		ND413824H	5'—CAT TAC TTT TAC TTG GAT TTG CAC CA—3'	Arevalo *et al.*, 1994
Nuclear	BDNF	BDNFf	5'—GAC CAT CCT TTT CCT KAC TAT GGT TAT TTC ATA CTT—3'	Leache and McGuire, 2006
		BDNFr	5'—CTA TCT TCC CCT TTT AAT GGT CAG TGT ACA AAC—3'	Leache and McGuire, 2006
	CMOS	CmosG73L	5'—GCG GTA AAG CAG GTG AAG AAA—3'	Saint *et al.*, 1998
		CmosG74H	5'—TGA GCA TCC AAA GTC TCC AAT C—3'	Saint *et al.*, 1998

* Modified after Palumbi, 1996