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SPECIES LIMITS BASED ON MTDNA AND MORPHOLOGICAL DATA IN THE POLYTYPIC SPECIES *PLESTIODON BREVIROSTRIS* (SQUAMATA: SCINCIDAE)

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ABSTRACT: The Mexican *Plestiodon brevirostris* species group (Squamata: Scincidae) is composed of seven nominal species. The wide-ranging *P. brevirostris* is a polytypic species composed of five subspecies: *P. b. brevirostris*, *P. b. bilineatus*, *P. b. dicei*, *P. b. indubitus*, and *P. b. pineus*. A tree-based approach for species delimitation with mtDNA data was used to test the traditional species-level taxonomy of *P. brevirostris* preliminarily. A haplotype phylogeny for all of the species and subspecies in the *P. brevirostris* group, except *P. colimensis*, was inferred. The mtDNA data consisted of sequences encompassing the genes encoding 16S rRNA (part), ND1, and associated tRNAs (1355 base pairs), which were analyzed with Bayesian methods. Then, a search for diagnostic morphological characters for the putative species delimited by this approach was performed. The results indicate that the *P. brevirostris* group is paraphyletic with respect to *P. lynxe*, and that *P. brevirostris* actually is composed of at least five distinct lineages disguised by traditional taxonomy: *P. b. brevirostris*, *P. b. bilineatus*, *P. b. dicei*, and the eastern populations of *P. b indubitus* (from Morelos, Guerrero, and México) represent distinct species, whereas the western populations of *P. b. indubitus* (from Colima and Jalisco) represent an undescribed species. The data cannot resolve whether *P. b. pineus* is conspecific with *P. b. dicei* or *P. b. dicei* is a paraphyletic (=nonexclusive) species relative to an exclusive *P. b. pineus*. Thus, the status of *P. b. pineus* remains uncertain. The haplotype phylogeny also suggests that *P. b. brevirostris* may represent more than one species.

RESUMEN: El grupo mexicano de especies *Plestiodon brevirostris* (Squamata: Scincidae) está compuesto por siete especies nominales. La especie politípica *P. brevirostris*, de amplia distribución geográfica, está compuesta por cinco subespecies: *P. b. brevirostris*, *P. b. bilineatus*, *P. b. dicei*, *P. b. indubitus*, and *P. b. pineus*. Se utilizó una aproximación a la delimitación de especies basada en árboles construidos a partir de secuencias de DNAmt para probar, de manera preliminar, la taxonomía tradicional a nivel de especie de *P. brevirostris*. Se infirió una filogenia de haplotipos para todas las especies y subespecies en el grupo *P. brevirostris*, excepto *P. colimensis*. Los datos de DNAmt consistieron de secuencias que abarcaron los genes que codifican 16S rRNA (parte), ND1, y los tRNAs asociados (1355 bp), las cuales fueron analizadas con métodos Bayesianos. Después se realizó una búsqueda para encontrar caracteres morfológicos diagnósticos para las especies putativas delimitadas usando esta aproximación. Los resultados indican que el grupo *P. brevirostris* es parafilético con respecto a *P. lynxe*, y que *P. brevirostris* está compuesta en realidad por al menos cinco linajes distintos encubiertos por la taxonomía tradicional: *P. b. brevirostris*, *P. b. bilineatus*, *P. b. dicei*, y las poblaciones orientales de *P. b indubitus* (de Morelos, Guerrero, y México) representan especies distintas, mientras que las poblaciones occidentales de *P. b. indubitus* (de Colima y Jalisco) representan una especie no descrita. Los datos no pueden resolver si *P. b. pineus* es conespecífica con *P. b. dicei* ó *P. b. dicei* es una especie parafilética (=no exclusiva) con respecto a una *P. b. pineus* exclusiva. Por lo tanto, el estado taxonómico de *P. b. pineus* permanece incierto. La filogenia de haplotipos también sugiere que *P. b. brevirostris* puede representar más de una especie.

Key words: Mexico; Phylogeny; *Plestiodon*; *Plestiodon brevirostris*; Scincidae; Species delimitation; Systematics

THE SCINCID lizard genus *Plestiodon* comprises approximately 40 species in eastern Asia and North and Middle America (Brandley et al., 2005; Smith, 2005). The *P. brevirostris* group is composed of seven nominal species (*P. brevirostris*, *P. colimensis*, *P. copei*, *P. dugesii*, *P. ochoterenae*, *P. parviauriculatus*, and *P. parvulus*), which range collectively from Sonora, Chihuahua, Coahuila, Nuevo León, and Tamaulipas south and east to Oaxaca, Mexico (Dixon, 1969; Robinson, 1979). Although Taylor (1935) and Dixon

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(1969) defined the group, Robinson (1979) pointed out that many of the characters used by these workers in their definitions were not exclusive, and therefore their definitions were too broad. Robinson (1979) also stated that only one unequivocal character unites skinks of the *P. brevirostris* group: the scale that medially borders the postgenial is wider than long in the species of the *P. brevirostris* group, and longer than wide in the skinks of the *multivirgatus* and *brevilineatus* groups. In addition, Robinson listed three characters shared by the members of the *P. brevirostris* group, but occasionally present in species of the *multivirgatus* and *brevilineatus* groups: the lack of both a postnasal scale and a median dorsal light stripe, and the presence of a single postmental.

Plestiodon brevirostris is the only polytypic species in the *P. brevirostris* group, with five subspecies: *P. b. brevirostris*, *P. b. bilineatus*, *P. b. dicei*, *P. b. indubitus*, and *P. b. pineus* (Dixon, 1969). However, the traditional species-level taxonomy of *P. brevirostris* is problematic. This taxonomy and its problems are described below.

Historical Resumé

Although *Plestiodon brevirostris* was not recognized as a polytypic species composed of five subspecies until the taxonomic review of the *P. brevirostris* group by Dixon (1969), other workers previously suggested a close, likely infraspecific relationship between these subspecies. Tanner (1958) described *P. b. bilineatus* and conceived it as closely related to *P. indubitus* and *P. dugesii* (recognized as distinct species at the time) and typical *P. brevirostris*, and suggested the existence of a widespread species (*P. brevirostris*) with four subspecies (*bilineatus*, *brevirostris*, *dugesii*, and *indubitus*) and "perhaps others when the data is more complete." Axtell (1960) described *P. dicei pineus* and suggested that *P. dicei* was closely related to the other species of the *P. brevirostris* group, composed of six allopatric species at the time (*P. brevirostris*, *P. colimensis*, *P. dicei*, *P. dugesii*, *P. indubitus*, and *P. ochoterenai*), and noted that, of the diagnostic characters of *P. dicei* pointed out by Taylor (1935), "none appear to be entirely restricted to *P. dicei*, as all of them occur singly or in different combination in other members of the group." Axtell (1960) also suggested that the relationships among the species of the *P. brevirostris* group recognized at the time were very close and that future work will no doubt show "some of them to be infraspecific in rank."

Dixon (1969) noted that the characters used to define *P. dicei* and its subspecies (number of dorsal, longitudinal, and transverse scale rows, subdigital lamellae on fourth toe, superciliary scales, and postsuboculars) overlapped to a large extent with the characters of *P. b. brevirostris* and *P. b. bilineatus*, and hence that *P. d. dicei* and *P. d. pineus* differed from the latter subspecies only in the absence or frequency of an enclosed interparietal and primary temporal and "minor features of color pattern" (width and length of the dorsolateral and lateral light lines and the lateral dark field, and the number and position of the dark, dorsal longitudinal lines). Thus, Dixon (1969) considered that *P. d. dicei* and *P. d. pineus* shared a combination of a number of characters found in *P. b. brevirostris* and *P. b. bilineatus*, and therefore were members of *P. brevirostris*. Similarly, Dixon (1969) noticed that *P. indubitus* was essentially similar to *P. brevirostris* in all characters of squamation, and that they could only be distinguished by the presence or absence of a lateral light line extending from the ear to the hind limb, and the frequency of an enclosed interparietal. He also reported several putative intergrades between these two taxa in northwestern Morelos (see below), and therefore considered *P. indubitus* as conspecific with *P. brevirostris*.

Traditional Species-Level Taxonomy of Plestiodon brevirostris

Plestiodon brevirostris can be distinguished from the other species of the *P. brevirostris* group by having usually four supraoculars, primary temporal scale separated from lower secondary temporal, longitudinal scale rows 20–26, transverse dorsal scale rows 50–68, subdigital lamellae on fourth toe 10–16, toes separated by five or more body scales when adpressed along side of body, a dorsolateral light line on second and third or second, third, and fourth scale rows from middle of neck to

midbody or hind limb, and dorsolateral light lines separated from each other by three and two half-scale rows or four scale rows at midbody (Dixon, 1969).

The subspecies of *P. brevirostris* have been distinguished from one another mainly on the basis of the following characters (Dixon, 1969): A lateral light line on the neck is always present in *P. b. brevirostris* (extending from the ear to the hind limb in Guerrero and Oaxaca, and from the ear to the arm in Distrito Federal, Mexico, Morelos, Puebla, and Veracruz); replaced by "a series of scales that have white anterior edges and centers with black posterior borders, on longitudinal scale rows five, six, seven, eight, and nine, or any combination of these rows in sequence" (hereafter referred to as a modified lateral light line on the neck) in *P. b. indubitus*; and usually "obscure" or absent in *P. b. bilineatus*, *P. b. dicei*, and *P. b. pineus*. The interparietal scale is always enclosed posteriorly by the parietals in *P. b. bilineatus*; usually enclosed in *P. b. indubitus* (in 92%–100% of the specimens); and usually not enclosed in *P. b. brevirostris*, *P. b. dicei*, and *P. b. pineus* (in 63%–97%, 100%, and 98% of the specimens, respectively). The primary temporal scale is usually present in *P. b. brevirostris* and *P. b. indubitus* (in 91%–100% and 96%–99% of the specimens, respectively), present in slightly less than half of the specimens in *P. b. bilineatus* (40%–44%), and usually absent in *P. b. dicei* and *P. b. pineus* (in 100% and 93% of the specimens, respectively). Two additional characters distinguish *P. b. bilineatus* from *P. b. indubitus* (see also Robinson, 1979): the whitish color of the fifth or sixth scale row of the neck grades into the light color of the belly in *P. b. bilineatus*, whereas the dark color below the lateral light line on the neck extends laterally to the belly in *P. b. indubitus*; and the dorsolateral light line may extend to the tail or beyond (distinct to the arm, midbody, or tail; obscure in some old adults) in *P. b. bilineatus*, whereas it is usually faint at the shoulder and beyond (obscure at midbody) in *P. b. indubitus*. Finally, one additional character separates *P. b. dicei* from *P. b. pineus*: The dorsal margin of the broad lateral dark stripe reaches the fourth scale row of the body in *P. b. dicei*, whereas it reaches the third scale row of the body in *P. b. pineus*.

The subspecies of *P. brevirostris* are distributed as follows (morphotectonic provinces sensu Ferrusquía-Villafranca, 1993; Fig. 1): *P. b. brevirostris* is widely distributed in Guerrero and Oaxaca, in the Sierra Madre del Sur province, and in Mexico, Morelos, Puebla, Tlaxcala, and Veracruz in the Mexican Transvolcanic Belt province (Dixon, 1969; Fernández et al., 2006). *Plestiodon b. bilineatus* is known from southwestern Chihuahua to southern Durango in the Sierra Madre Occidental province (Dixon, 1969; Robinson, 1979; Tanner, 1958). *Plestiodon b. dicei* occurs in west-central Nuevo León and western Tamaulipas in the Sierra Madre Oriental province (Axtell, 1960; Dixon, 1969). *Plestiodon b. indubitus* is known from northern Morelos, several localities in Mexico west of Distrito Federal, central-north Guerrero, and central Michoacán in the central portion of the Mexican Transvolcanic Belt province (Auth et al., 1997; Dixon, 1969; Flores-Villela and Hernández-García, 2006), and from central-west and southern Jalisco in the western portion of the same province (Dixon, 1969; Medica et al., 1975; Robinson, 1979). Finally, *P. b. pineus* occurs in southeastern Coahuila and the southern half of Nuevo León in the Sierra Madre Oriental province (Axtell, 1960; Dixon, 1969).

Putative intergrades between some of the above subspecies have been reported. According to these reports, *Plestiodon b. dicei* and *P. b. pineus* intergrade in the Gómez Farías region and "Chihue," Tamaulipas (Axtell, 1960; Dixon, 1969) and possibly Santiago, Nuevo León (Dixon, 1969); *P. b. brevirostris* and *P. b. indubitus* intergrade "between Cuernavaca and Tepoztlán," Morelos, and other localities in Morelos and Mexico (Dixon, 1969); and *P. b. indubitus* and *P. b. bilineatus* intergrade in a wide zone of unknown limits between southern Durango and northwestern Jalisco (Robinson, 1979). Thus, only *P. b. dicei* and *P. b. pineus* are definitively allopatric with respect to the other subspecies of *P. brevirostris*.

The Taxonomic Problems of Plestiodon brevirostris

In a recent debate on species concepts, Wheeler and Platnick (2000, p. 60) maintained

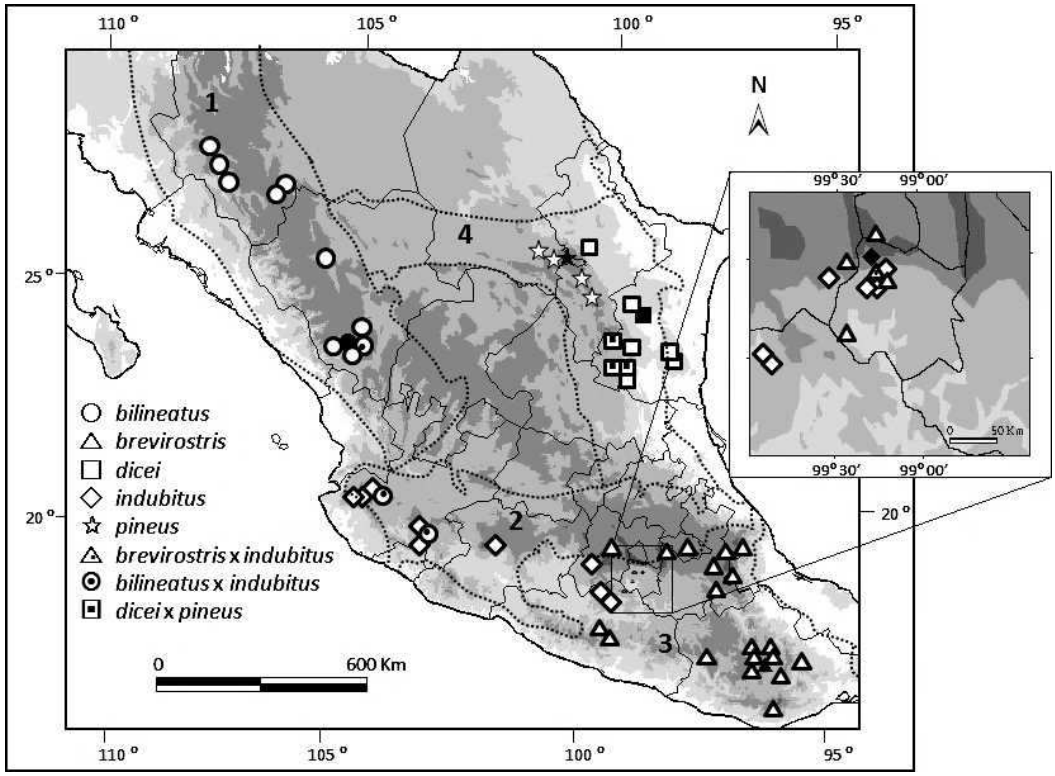

FIG. 1.—Geographic distribution of the subspecies of *Plestiodon brevirostris*. Closed symbols represent type localities. Shaded areas represent elevational bands. Numbered areas demarcated by dotted lines correspond to the morphotectonic provinces of Ferrusquía-Villafranca (1993): 1 = Sierra Madre Occidental Province; 2 = Mexican Transvolcanic Belt Province; 3 = Sierra Madre del Sur Province; 4 = Sierra Madre Oriental Province.

that "the biological species concept has underestimated the number of end products of evolutionary history by permitting subjective decisions about the inclusivity of polytypic species," and predicted that, with the application of phylogenetic species concepts, "many well-substantiated subspecies will no doubt be elevated to species status." Several facts suggest that this might be the case of *P. brevirostris*. The monophyly of *P. brevirostris* has not been investigated. In addition, *P. b. dicei* and *P. b. pineus* are morphologically differentiated and geographically isolated from the other subspecies of *P. brevirostris* (Axtell, 1960; Dixon, 1969; Robinson, 1979); *P. b. brevirostris* and *P. b. indubitus* appear to have parapatric distributions with only a narrow contact zone between them (Dixon, 1969); *P. b. indubitus* is composed of fairly differentiated populations in disjunct areas in central and west-central Mexico, even though the habitat of this taxon is continuous between these areas (Dixon, 1969); and evidence for intergradation between *P. b. bilineatus* and *P. b. indubitus* (otherwise allopatric and morphologically distinct) is not conclusive (see below). Furthermore, Richmond (2006) cited unpublished data showing that the *P. brevirostris* group does not form a clade, and that the presumed subspecies in the group constitute deeply divergent lineages consistent with species-level genetic differentiation.

Herein, we first use the tree-based approach for species delimitation with mtDNA data of Wiens and Penkrot (2002) to test the traditional species-level taxonomy of *P. brevirostris* preliminarily, and then evaluate whether the putative species delimited with the use of this approach are supported by external morphology. We show that *P. brevirostris* is actually composed of at least five distinct lineages disguised by traditional taxonomy.

Materials and Methods

Taxon Sampling

Mitochondrial DNA.—We sampled broadly from the currently recognized *P. brevirostris* group, including (1) representatives of each of the subspecies of *P. brevirostris*; and (2) representatives of *P. copei*, *P. dugesii*, *P. ochoterenae*, *P. parviauriculatus*, and *P. parvulus*. Samples were unavailable for *P. colimensis*. Where possible, multiple representatives of each species and subspecies were used, especially within the polytypic species *P. brevirostris* (in total, 38 individuals from 27 localities were surveyed, covering most of the distribution of *P. brevirostris*; Table 1; Fig. 2). Unfortunately, only one representative of *P. b. pineus* was available.

Recent molecular systematic studies (Richmond, 2006; Richmond and Reeder, 2002; Brandley et al., 2010*a*) have provided growing evidence that a large clade, including all of the North American *Plestiodon* species groups, is the sister taxon to the *P. brevirostris* group. Thus, we included a representative each of this North American clade (*P. skiltonianus*) and the Middle American *P. lynxe* species group (*P. lynxe*) as close outgroups, and used a more distant outgroup taxon, the lygosomine *Scincella silvicola caudaequinae*, to root the *Plestiodon* tree.

External morphology.—We examined 277 specimens representing all of the subspecies of *P. brevirostris* and putative intergrades among them (Appendix I) for most of the characters that have been used to distinguish among the subspecies (see below). However, in order to test the traditional species-level taxonomy of *P. brevirostris*, the samples were first grouped as per the putative species delimited by the Wiens and Penkrot (2002) tree-based approach for species delimitation with the use of mtDNA data regardless of their previous taxonomic designation, and character distributions were then compared among these putative species to determine whether they were supported by diagnostic differences (see below). We considered as putative species populations or sets of populations of the focal species whose haplotypes formed strongly supported lineages that were intermingled in the haplotype phylogeny with the other species of the *P. brevirostris* group, or (if they were of different taxonomic designation) were mutually exclusive and congruent with geography.

Data

Mitochondrial DNA.—Genomic DNA was extracted from small portions (0.5–1.0 g) of liver or muscle with the use of the standard phenol-chloroform method (Hillis et al., 1996). The following regions of the mitochondrial genome were amplified: a portion of the ribosomal gene encoding 16S, the adjacent gene encoding $tRNA^{Leu}$, the gene encoding the first unit of the NADH dehydrogenase (ND1), and the genes encoding $tRNA^{Ile}$, $tRNA^{Gln}$, and $tRNA^{Met}$ (in part). These regions, or parts of them, have been successfully employed to elucidate phylogenetic relationships in other groups of lizards, including anguids (Macey et al., 1999), phrynosomatids (Leaché and Reeder, 2002), and scincids (Brandley et al., 2005). Polymerase chain reaction (PCR) cycle parameters were: an initial denaturation cycle at 94°C for 2 min, followed by 38–40 cycles at 94°C for 30 s, 45–52°C for 30 s, and 72°C for 90 s, and a final extension at 72°C for 5 min. PCR products were purified with PEG precipitation (Lis, 1980). DNA templates were sequenced with the Big Dye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Inc.) and an ABI 3100 automated DNA sequencer (Applied Biosystems, Inc.). The primers used for DNA amplification and sequencing are in Table 2.

Sequences were assembled and edited with the use of the computer software program Staden Package v. 1.6.0 (Whitwham and Bonfield, 2005). Alignment of the structural rRNA and tRNA gene sequences was aided by the secondary structural models of Gutell and Fox (1988) and Kumazawa and Nishida (1993), respectively, and adjusted visually with MacClade version 4.08 (Maddison and Maddison, 2005). Nucleotide positions that could not be unambiguously aligned were excluded from phylogenetic analysis (Gatesy et al., 1993). All DNA sequences are deposited in GenBank (accession numbers HQ655821–HQ655875).

External morphology.—Six of the characters previously used in the taxonomy of *P. brevirostris* (Dixon, 1969) were recorded in all

TABLE 1.—Species used in this study, their collecting municipalities, museum or field numbers, and GenBank accession numbers. Institutional abbreviations follow Leviton et al. (1985) except in the case of the Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC).

Taxon	State	Municipality	Coordinates	Museum or field number	GenBank accession number
Plestiodon b. bilineatus	Chihuahua	Balleza	26°29′23.8″N; 106°21′48.7″W	MZFC 25536 (1) MZFC 25341 (2)	HQ655821 HQ655822
P. b. bilineatus	Durango	Santiago Papasquiaro	25°4′10.8″N; 105°37′43.3″W	MZFC 25342 (3)	HQ655823
P. b. bilineatus	Durango	Pueblo Nuevo	23°42′54.7″N; 105°29′12.3″W	MZFC 25338 (4) MZFC 25340 (5)	HQ655824 HQ655825
P. b. bilineatus	Zacatecas	Valparaíso	22°56′15.0″N; 103°32′16.0″W	MZFC 25537 (6)	HQ655826
P. b. bilineatus	Jalisco	Bolaños	24°55′3.8″N; 103°52′38.0″W	UTA 53300 (7) UTA 52706 (8)	HQ655827 HQ655828
P. b. brevirostris	Puebla	San Salvador el Seco	19°8′10.0″N; 97°38′26.0″W	MZFC 25346 (9)	HQ655829
P. b. brevirostris	Tlaxcala	Huamantla	19°23′28.4″N; 97°55′17.9″W	MZFC 25343 (10) MZFC 25344 (11)	HQ655830 HQ655831
P. b. brevirostris	Oaxaca	Teotitlán del Valle	17°6′12.0″N; 96°29′54.0″W	MZFC 25390 (12)	HQ655832
P. b. brevirostris	Oaxaca	Santa Lucía Monteverde	16°59′41.1″N; 97°42′3.9″W	MZFC 15568 (13)	HQ655833
P. b. brevirostris	Oaxaca	San Pablo Macultianguis	17°32′0.9″N; 96°33′13.0″W	MZFC 25385 (14) MZFC 25386 (15)	HQ655834 HQ655835
P. b. brevirostris	Oaxaca	Cuyamecalco Villa de Zaragoza	17°56′17.0″N; 96°51′55.0″W	MZFC 25538 (16)	HQ655836
P. b. brevirostris	Oaxaca	Villa de Zaachila	16°55′58.0″N; 96°51′59.0″W	MZFC 25539 (17)	HQ655837
P. b. brevirostris	Guerrero	Chilpancingo de los Bravo	17°33′18.0″N; 99°38′29.9″W	MZFC 25382 (18)	HQ655838
P. b. brevirostris	Guerrero	Chilpancingo de los Bravo	17°24′55.1″N; 99°28′13.6″W	MZFC 25384 (19)	HQ655839
P. b. brevirostris	Guerrero	Chilpancingo de los Bravo	17°33′15.8″N; 99°30′58.6″W	MZFC 25383 (20)	HQ655840
P. b. dicei	Tamaulipas	San Carlos	24°37′19.8″N; 99°1′55.0″W	MZFC 18767 (22) MZFC 18768 (23)	HQ655841 HQ655842
P. b. dicei[1]	Nuevo León	Santiago	25°21′8.4″N; 100°11′36.0″W	MZFC 25356 (24) MZFC 25359 (25)	HQ655843 HQ655844
P. b. dicei[1]	Nuevo León	San Pedro Garza García	25°36′50.5″N; 100°21′15.0″W	MZFC 25353 (26)	HQ655845
P. b. dicei × *P. b. pineus*	Tamaulipas	Gómez Farías	23°3′29.3″N; 99°11′37.5″W	MZFC 18764 (21)	HQ655846
P. b. indubitus	Morelos	Tepoztlán	18°59′15.0″N; 99°6′9.0″W	MZFC 25443 (27)	HQ655847
P. b. indubitus	Guerrero	Taxco de Alarcón	18°33′35.0″N; 99°37′44.0″W	MZFC 25471 (35)	HQ655848
P. b. indubitus	Jalisco	Zapotlán El Grande	19°37′0.8″N; 103°33′37.1″W	MZFC 25449 (36) MZFC 25451 (37)	HQ655849 HQ655850
P. b. indubitus × *P. b. brevirostris*	Morelos	Huitzilac	19°1′45.0″N; 99°13′11.0″W	MZFC 25469 (28) MZFC 25460 (29)	HQ655851 HQ655852
P. b. indubitus × *P. b. brevirostris*	Morelos	Huitzilac	19°0′42.0″N; 99°14′14.0″W	MZFC 25461 (30) MZFC 25470 (31)	HQ655853 HQ655854
P. b. indubitus × *P. b. brevirostris*	Morelos	Cuernavaca	18°56′1.9″N; 99°13′53.3″W	MZFC 25472 (32) MZFC 25473 (33)	HQ655855 HQ655856
P. b. indubitus	Morelos	Huitzilac	19°1′25.6″N; 99°16′49.7″W	MZFC 25468 (34)	HQ655857
P. b. pineus	Coahuila	Arteaga	25°22′32.5″N; 100°30′38.9″W	MZFC 25366 (38)	HQ655858
P. copei	Puebla	Chiautzingo	19°12′11.4″N; 98°27′53.9″W	MZFC 14113 (39)	HQ655859
P. copei	D.F.	Cuajimalpa de Morelos	19°16′2.4″N; 99°19′18.6″W	MZFC 25462 (40)	HQ655860
P. dugesii	Michoacán	Nuevo Parangaricutiro	19°23′29.7″N; 102°10′25.3″W	MZFC 25327 (41) MZFC 25328 (42)	HQ655861 HQ655862
P. dugesii	Jalisco	Atemajac de Brizuela	20°7′6.7″N; 103°43′36.9″W	MZFC 25331 (43)	HQ655863
P. ochoterenae	Guerrero	Chilpancingo de los Bravo	17°19′18.9″N; 99°28′12.2″W	MZFC 25454 (44) MZFC 25467 (45)	HQ655864 HQ655865
P. parviauriculatus	Sonora	Alamos	26°59′36.3″N; 108°58′31.4″W	MZFC 25394 (46) MZFC 25395 (47)	HQ655866 HQ655867

Table 1.—Continued.

Taxon	State	Municipality	Coordinates	Museum or field number	GenBank accession number
P. parvulus	Colima	Manzanillo	21°36′15.3″N; 105°10′19.6″W	MZFC 25370 (48) MZFC 25371 (49)	HQ655868 HQ655869
P. lynxe lynxe	Querétaro	San Joaquín	20°55′59.6″N; 99°35′21.9″W	MZFC 17839 (50) MZFC 17840 (51)	HQ655870 HQ655871
P. skiltonianus	California	Mendocino	39°18′27.5″N; 123°47′57.8″W	MVZ 162314 (52)	HQ655872
P. skiltonianus	California	San Diego	32°42′57.3″N; 117°9′21.8″W	MVZ 162089 (53)	HQ655873
Scincella silvicola caudaequinae	Tamaulipas	Gómez Farías	23°1′49.9″N; 99°8′55.1″W	MZFC 19501 (54) MZFC 647 (55)	HQ655874 HQ655875

[1] Dixon (1969) assigned the population from Santiago, Nuevo León, to *P. b. dicei*. However, he considered that this locality might represent another area of intergradation between *P. b. dicei* and *P. b. pineus*.

of the specimens: (1) snout–vent length (SVL), (2) interparietal (enclosed or not enclosed posteriorly by the parietals), (3) primary temporal (present or absent; recorded on both sides), (4) lateral light line on the sixth and seventh supralabials (present or absent), (5) lateral light line on the neck (present, modified, or absent), and (6) posterior extent of the dorsolateral light line (at the level of the ear, the forelimb, or the hindlimb/tail).

Additionally, nine characters previously used to distinguish *P. b. dicei* from *P. b. pineus* (Axtell, 1960; Dixon, 1969) were examined in all of the specimens of these subspecies and putative intergrades between them: (1) contact between prefrontals (present or absent), (2) size of the first supralabial in relation to that of the second supralabial, (3) number of postsuboculars, (4) number of longitudinal scale rows around midbody, (5) number of subdigital lamellae on the fourth toe, (6) axilla–groin length/SVL ratio, (7) tibia length/SVL ratio, (8) position of the dorsal edge of the lateral dark stripe (on third or fourth scale row), and (9) width of the dorsolateral light line.

None of the above characters showed conspicuous sexual variation. In addition, although the color pattern tended to fade in older specimens, it was nevertheless discernible in all of the specimens examined.

Because fixed differences may indicate an absence of gene flow between putative species and the presence of two or more distinct species, these differences have been required as diagnostic characters to delimit species by some authors (e.g., Davis and Nixon, 1992; Eldredge and Cracraft, 1980; Nixon and Wheeler, 1990). However, it has been suggested that a more realistic approach to species delimitation is to allow some level of polymorphism in the diagnostic characters (Wiens and Servedio, 2000). Herein, we tentatively considered as diagnostic a character that has the alternate state at a frequency below 10%, including fixation (i.e., we assumed that a trait present at a frequency of 90% or higher in one population and 10% or lower in the other population is enough indication of very low levels of gene flow between the putative species).

We identified diagnostic characters as follows: For potentially diagnostic, apparently

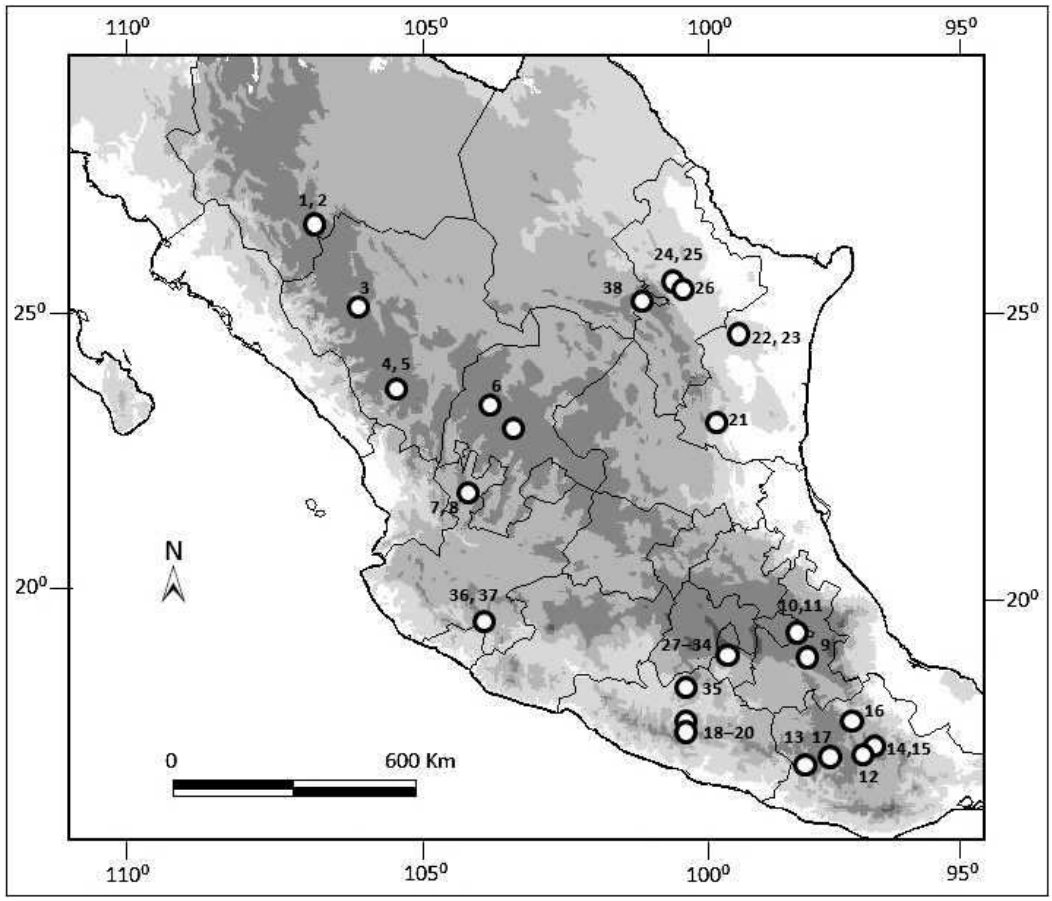

FIG. 2.—Collecting localities for individuals of *Plestiodon brevirostris* surveyed in this study. Individual numbers are those in Table 1 (see table for locality data).

fixed traits, we used formula (3) of Wiens and Servedio (2000) to evaluate whether sample sizes for the putative species were large enough to reject the null hypothesis (with a 5% confidence interval) that the actual frequency of all of the apparently absent traits is above 10%. For potentially diagnostic traits that were present at a frequency >90% (i.e., alternate traits present at a frequency below 10%), we used a confidence interval for proportion calculator (Dimension Research, 2005) to calculate, from the observed frequency of the alternate trait and the sample size, the range for the true population frequency of this trait with a level of confidence of 95%. If the frequencies in this range were below 10%, we considered the character as diagnostic and referred to it as "nearly fixed."

Given that even small differences in trait frequencies might be strong evidence of

TABLE 2.—Primers used in study.

Primer name	Sequence 5′–3′	Source
16DR	CTA CGT GAT CTG AGT TCA GAC CGG ACC GGA G	Leaché and Reeder (2002)
tMET	TCG GGG TAT GGG CCC RAR AGC TT	Leaché and Reeder (2002)
ND1 inter F3	ATA ATR TGR TTY ATY TCN ACN CTA GCA GA	Brandley et al. (2005)
ND1 inter R2	CRA AKG GGC CDG CTG CRT AYT CTA C	Schmitz et al. (2005)
ND1 inter R2A	GGY ICT TTR RTR ADA GTT HAC NCC	This study

reduced or absence of gene flow (Wiens and Servedio, 2000), we additionally reported other large differences in trait frequencies (though not diagnostic) found among the putative species, both because these traits have been previously used to distinguish among subspecies of *P. brevirostris* (Dixon, 1969) and because our frequency cutoff is admittedly arbitrary, and whether it is appropriate or should be modified to include smaller differences in trait frequencies among taxa should be evaluated empirically.

Phylogenetic Analyses

An initial maximum likelihood tree for the entire data set, with the use of the GTR + Γ + I model of evolution, was created in PAUP* version 4.0b10 (Swofford, 2002) with five random addition replicates and tree bisection and reconnection (TBR) branch swapping. The appropriate model of evolution for each partition and combination of partitions (see below) was determined with the use of the Akaike information criterion (AIC) implemented with MrModeltest version 2.3 (Nylander, 2008), and using this same initial tree for all partitions.

Our preferred phylogenetic analyses were conducted with the use of MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Each analysis consisted of 8×10^6 generations with a random starting tree, default priors, the same set of branch lengths for each partition, and four Markov chains (with default heating values) sampled every 1000 generations. Stationarity was estimated by plotting $-\ln L$ against generation time. Burn-in trees were discarded and a 50% majority-rule consensus tree was computed from the sampled trees of the estimated posterior distribution. To decrease the chance of reaching apparent stationarity on local optima, two independent analyses were performed for each partitioning strategy (see below). Frequencies of clades in each consensus tree were interpreted as posterior probabilities (PP). Posterior probability estimates for each clade were then compared between the two analyses and, if posterior probability estimates for clades were similar in both analyses, the results of both analyses were combined. Clades with PP > 0.95 were considered as strongly supported (Alfaro et al., 2003; Huelsenbeck and Rannala, 2004; but see the caveat in Brandley et al., 2005).

To determine the best partitioning strategy, we followed the Brandley et al. (2005) approach of comparing strategies with the use of the Bayes factor. Six partitions were chosen a priori based on gene identity and general biochemical or evolutionary constraints (16S, ND1 codon positions, and tRNAs stems and loops). The tRNA stems and loops were combined because of their small size (ca. 80 base pairs [bp] for each tRNA) and given that they are expected to evolve similarly because of similar functional and evolutionary constraints (Brandley et al., 2005). The 16S stems and loops were combined due to their small size (23 and 124 bp, respectively). Appropriate models of sequence evolution were chosen for each partition with the use of the AIC on the same initial tree (see above). We tested only logical combinations of partitions (Brandley et al., 2005). Partition strategies tested are shown in Table 3. A partitioned Bayesian analysis of the total data set with all the partitions was performed by applying the previously determined models to each data partition. Additional analyses combining data partitions were then performed, and the appropriate models of sequence evolution of the various combined partitions were redetermined with the use of the AIC criterion with the same initial maximum-likelihood tree. Data partitions and combinations of partitions, their estimated models of sequence evolution, AIC values, and the total number of characters of each partition used in the analysis are shown in Table 4. The harmonic mean $-\ln L$ for each partitioning strategy was calculated with the use of the *sump* command in MrBayes, and the results for each partitioning strategy were then compared to the strategy with the best harmonic mean $-\ln L$ with the use of the Bayes factor. The Bayes factor was determined as the difference in harmonic mean $-\ln L$ between the strategy with the best harmonic mean $-\ln L$ (the null hypothesis) and each of the other strategies (the alternative hypotheses), and compared to

TABLE 3.—Partitioning strategies used in this study (see text).

Partitioning strategy	Partition identity
P_1	All data combined
P_4	ND1 codon positions; 16S and tRNAs combined
P_{5A}	ND1 codon positions; one partition each for the combined stems and loops of the 16S and tRNAs
P_{5B}	ND1 codon positions; one partition each for the 16S and tRNAs
P_6	ND1 codon positions; one partition each for the combined stems and loops of the tRNAs; one partition for the 16S

the table provided by Raftery (1996). Based on this table, we considered a 2 ln Bayes factor >10 as significant evidence against the alternative hypothesis. The alternative partitioning strategy that explains the data as well as the best strategy (if any) but with fewer partitions is considered the optimal strategy; that is, the one that best explains the data while incurring the least random error (Brandley et al., 2005; Nylander et al., 2004).

In addition, we conducted 10 independent maximum-likelihood (ML) searches from different randomly selected trees under the GTR + gamma + PINVAR model (with model parameters estimated during the analysis) in GARLI version 0.96 (Zwickl, 2006). Also, we conducted a nonparametric bootstrap with 100 pseudoreplicates, each consistent in 10 searches from randomly selected trees. Clades with bootstrap proportions ≥70% were considered strongly supported (Hillis and Bull, 1993; but see their caveats).

Testing Alternative Phylogenetic Hypotheses

To test whether particular relationships not present in the Bayesian tree could be statistically rejected by the data (e.g., an alternative hypothesis holding as monophyletic a paraphyletic taxon in the Bayesian tree), a Bayesian approach to hypothesis testing (Brandley et al., 2005; Buckley et al., 2002; Reeder, 2003) was used. First, a 95% set of credible trees was built from the Bayesian analysis output (Ronquist and Huelsenbeck, 2003). Then, a constraint tree with the alternative hypothesis was built in MacClade version 4.08 (Maddison and Maddison, 2005). Finally, the 95% set of credible trees was filtered with the use of PAUP* version 4.0b10 (Swofford, 2002) to determine whether any trees compatible with the specified constraint tree were retained. If not included in the 95% set of credible trees, the alternative hypothesis was rejected.

Species Delimitation

To delimit species preliminarily, we used the tree-based method for delimiting species based on DNA data as proposed by Wiens and Penkrot (2002; hereafter, W-P). This method assumes a phylogeny of nonrecombining DNA haplotypes (of known locality and taxonomic designation) for a set of populations currently classified as a species (the focal species of the

TABLE 4.—Data partitions and combinations of partitions, their estimated models of sequence evolution, Akaike information criterion (AIC) values, and total number of characters of each partition or combination of partitions used in the phylogenetic analyses.

	Model	AIC value	Number of characters in partition or combination of partitions
Partition			
16S	HKY + Γ	1477.0415	147
ND1 first position	HKY + I + Γ	3682.6917	322
ND1 second position	GTR + I + Γ	2049.4365	322
ND1 third position	GTR + I + Γ	10,528.7168	322
All tRNA stems	HKY + I + Γ	1413.4771	130
All tRNA loops	HKY + I + Γ	1125.4965	112
Combination of partitions			
16S and tRNAs	HKY + I + Γ	4388.4507	389
tRNAs	HKY + I + Γ	2591.3047	242
All stems	HKY + I + Γ	1580.5457	153
All loops	HKY + I + Γ	2655.2410	236
All data	GTR + I + Γ	21,778.7344	1355

TABLE 5.—2ln Bayes factors results of comparisons of all partitioning strategies.

	Partitioning strategies				
	P_6	P_{5A}	P_{5B}	P_4	P_1
P_1	781.14	395.2	451.6	−38.2	–
P_4	819.96	434.02	1639.92	–	
P_{5B}	329.58	−56.36	–		
P_{5A}	385.94	–			
P_6	–				

study) and one or more closely related species. The failure of haplotypes from a given locality to cluster together is interpreted as potential evidence for gene flow with other populations, as is the general discordance between haplotype clades and the geographic area from which the haplotypes are found. Thus, this method uses the tree topology to assess whether or not clades are restricted to a set of populations to the exclusion of clades elsewhere (criterion of "exclusivity"). The focal species of the study was *P. brevirostris*. To test the exclusivity of the focal species, representatives of most of the species of the *P. brevirostris* group were included in the analysis (see above).

RESULTS

Mitochondrial DNA

A total of 55 sequences were analyzed, 38 of which corresponded to the subspecies of *P. brevirostris* and 17 to the other taxa included in the analysis. A total of 1355 nucleotide positions were unambiguously aligned and analyzed, and only 6 were excluded from the phylogenetic analysis because of ambiguous alignment. There were no indels or stop codons in the ND1 region.

Partitioning the ND1 by codon position and the tRNAs by the combined stems and loops, in addition to the small 16S partition (strategy P_6), yielded the greatest improvement of mean $-\ln L$ (Table 5). All partitioned Bayesian analyses reached apparent stationarity by 10×10^3 generations (Gelman and Rubin's $r = 1.002$). However, the trees from the first 15×10^3 generations were conservatively eliminated from subsequent analyses.

In the Bayesian tree (Fig. 3), the *P. brevirostris* group was marginally weakly supported relative to *Scincella* and *P. skiltonianus* (PP = 0.93), and strongly supported as paraphyletic with respect to *P. lynxe* (PP = 0.99). Also, *P. brevirostris*, the focal species of this study, was strongly supported as not exclusive with respect to *P. lynxe* and the other species of the *P. brevirostris* group (PP = 0.99), one of which (*P. dugesii*) was itself not exclusive. Thus, the haplotype phylogeny supports the existence of five distinct mtDNA lineages (=putative species) within the polytypic species *P. brevirostris* (Figs. 3 and 4).

The haplotypes of *P. b. dicei*, *P. b. pineus*, and putative *P. b. dicei* × *P. b. pineus* strongly formed the basal-most clade within the *P. brevirostris* group. Within this clade, *P. b. dicei* was strongly supported as paraphyletic relative to the haplotypes of *P. b. pineus* and putative *P. b. dicei* × *P. b. pineus*, which formed a strongly supported subclade. In contrast, the remaining haplotypes of *P. brevirostris* were nested in different clades within the *P. brevirostris* group. The strongly supported sister group to the *P. b. dicei* + *P. b. pineus* clade was composed of *P. parviauriculatus* and a weakly supported polytomy formed by *P. lynxe*, *P. parvulus*, and the two remaining clades in the tree. The strongly supported clade contained two strongly supported basal subclades—one composed of the haplotypes of *P. b. brevirostris*, and the other composed of the haplotypes of *P. b. indubitus* and putative *P. b. brevirostris* × *P. b. indubitus* from Morelos and Guerrero (eastern *P. b. indubitus* hereinafter). In the weakly supported clade, the haplotypes of *P. b. bilineatus* formed a nested, strongly supported clade whose closest relative was a strongly supported clade with the haplotypes of *P. dugesii* from Michoacán, followed by the only haplotype of *P. dugesii* from Jalisco and strongly supported clades with the haplotypes of *P. b. indubitus* from Jalisco (western *P. b. indubitus* hereinafter), *P. copei*, and *P. ochoterenae*, in that order. However, only the clade containing the haplotypes of western

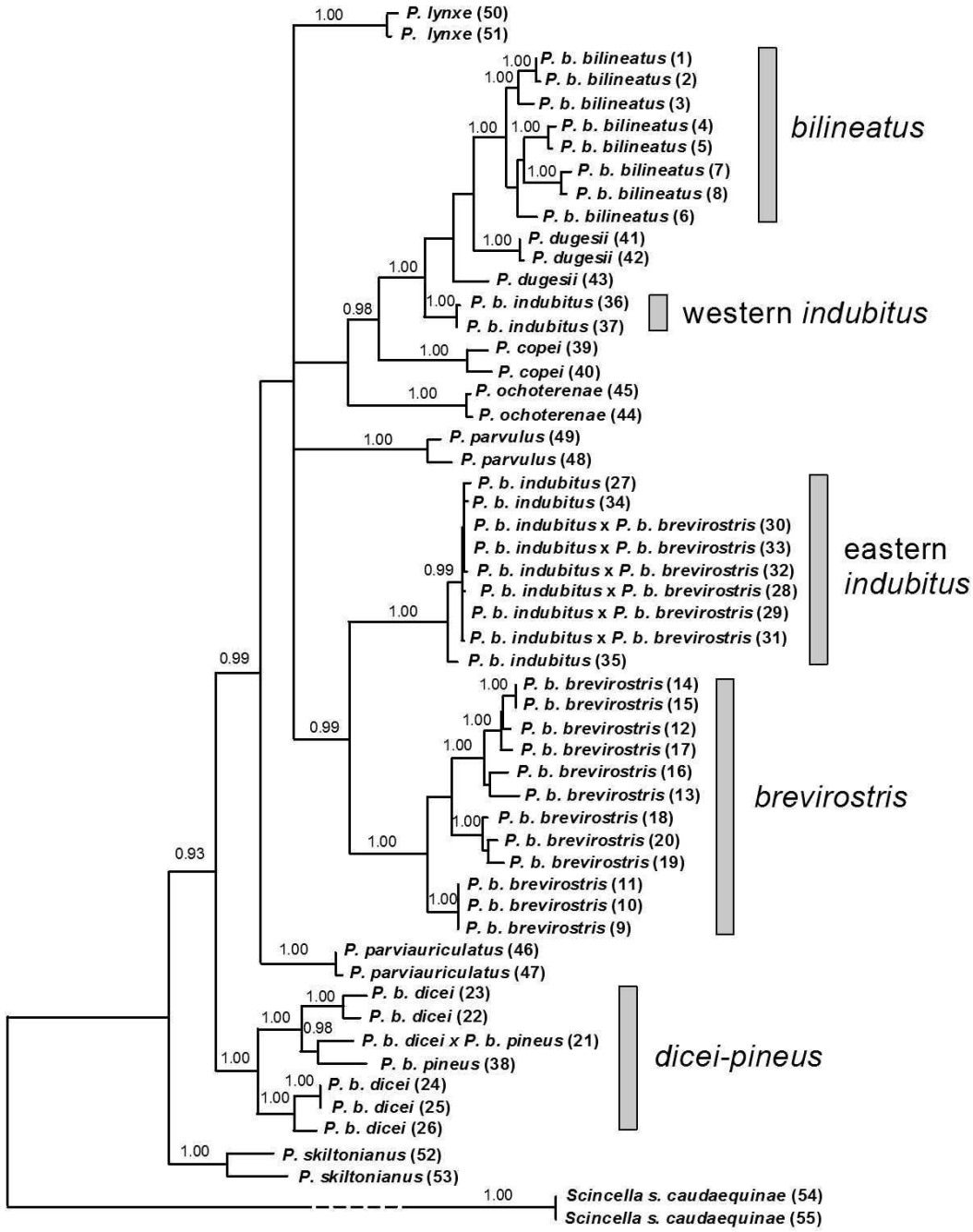

FIG. 3.—Bayesian 50% majority-rule consensus tree obtained from the fully partitioned analysis. Numbers indicate posterior probabilities of clades in the tree. Specimen numbers in the tree are those in Table 1 (see table for locality data).

P. indubitus, *P. dugesii*, and *P. b. bilineatus*, and the sister relationship between this clade and *P. copei*, were strongly supported. The paraphyly of the *P. brevirostris* group with respect to *P. lynxe*, the nonexclusivity of *P. brevirostris*, represented by one haplotype each of *P. b. bilineatus*, *P. b. brevirostris*, *P. b. dicei*, and western *P. b. indubitus*, which fail to cluster with

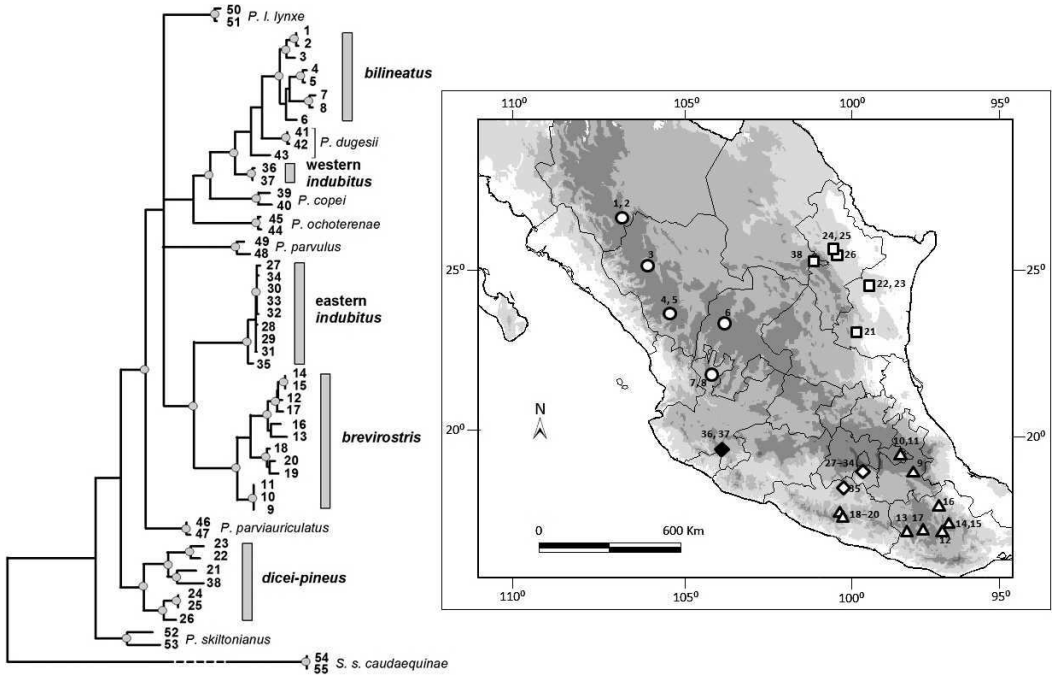

FIG. 4.—The five clades of haplotypes of *Plestiodon brevirostris* in the Bayesian 50% majority-rule consensus tree obtained from the fully partitioned analysis and localities of individuals surveyed in this study. Specimen numbers in the tree and map are those in Table 1 (see table for locality data). The five clades are represented in the tree by brackets (circles on nodes represent clade frequencies ≥95), and in the map as follows: ○ = *P. b. bilineatus*; △ = *P. b. brevirostris*; ◆ = western *P. b. indubitus*; ◇ = eastern *P. b. indubitus*; □ = *P. b. dicei* + *P. b. pineus*.

one another, the basal position of *P. b. dicei*, and the distal group with *P. copei* as sister taxon to a clade composed of *P. b. bilineatus*, western *P. b. indubitus*, and *P. dugesii*, were all recovered in a recent phylogenetic analysis of *Plestiodon* based on essentially the same region of mtDNA and seven nuclear genes (Brandley et al., 2010*b*).

The clade with the haplotypes of *P. b. brevirostris* was composed of three strongly supported subclades concordant with geography. One subclade contained all of the haplotypes from Guerrero, and was the sister group to a subclade containing all of the haplotypes from Puebla and Tlaxcala. The remaining subclade was the sister group to these two subclades and comprised all of the haplotypes from Oaxaca (Figs. 3 and 4).

The ML bootstrap tree (not shown) was in general similar to the Bayesian tree. The same five strongly supported clades with haplotypes of *P. brevirostris* in the Bayesian tree also were recovered and strongly supported in the ML tree, and most of the same relationships among these clades and the other species of the *P. brevirostris* group in the Bayesian tree also were recovered in the ML tree. The few exceptions are the tetratomy that forms the sister taxon to *P. parviauriculatus* in the Bayesian tree also includes *P. ochoterenae* and *P. parviauriculatus* in the ML tree; the relationships among *P. b. bilineatus*, *P. dugesii* from Jalisco, *P. dugesii* from Michoacán, and western *P. b. indubitus* are not resolved in the ML tree; and within the *P. b. brevirostris* clade the subclade from Guerrero (individuals 18–20), that grouped with the subclade from Oaxaca in the Bayesian tree, grouped with the subclade from Tlaxcala and Puebla (individuals 9–11) in the ML tree. However, in all cases the differences involved weakly supported relationships in the Bayesian tree.

The Bayesian approach for hypothesis testing demonstrated that alternate topologies enforcing the monophyly of the haplotypes of *P. brevirostris* and the haplotypes of *P. b.*

TABLE 6.—Variation in selected morphological characters among putative species within *Plestiodon brevirostris* (see text). Summary data for a given putative species are total sample size and, for the potential, nonfixed diagnostic character states in that species, numbers are the observed frequency of the state in the sample ± confidence interval, and numbers in parentheses are the range for the true species frequency (confidence level = 95%). Presence of a primary temporal was recorded on both sides of the head; thus sample size = 2*n*.

Taxon/character	N	Snout-to-vent length $\bar{X}$ ± SE (range)	Interparietal enclosed posteriorly (%)	Primary temporal (%)	Light line on supralabials 6 and 7 (%)	Lateral light line on neck (%)			Dorsolateral light line posterior extension (%)		
						Absent	Normal	Modified	Forelimb	Midbody	Hindlimb or tail
P. b. dicei											
Tamaulipas, Cd. Victoria	3	47.0 ± 2.6 (44.0–51.0)	0.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0
P. b. pineus											
Nuevo León	39	51.9 ± 1.3 (26.0–66.0)	2.6	2.6	0.0	100.0	0.0	0.0	20.5	0.0	79.5
P. b. dicei × *P. b. pineus*											
Tamaulipas, Gómez Farías	10	52.8 ± 2.56 (41.0–62.0)	20.0	0.0	0.0	100.0	0.0	0.0	80.0	0.0	20.0
Summary	52		5.8 ± 6.3 (−0.6–12.0)								
P. b. brevirostris											
Morelos	1	68.0	100.0	100.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0
Puebla	21	50.9 ± 2.6 (24.0–66.0)	19.0	100.0	66.6	0.0	100.0	0.0	4.7	23.8	71.5
Guerrero	38[1]	55.0 ± 1.8 (28.0–71.0)	2.6	100.0	12.0	0.0	92.1	7.9	94.7	0.0	5.3
Oaxaca	22[2]	51.8 ± 2.5 (26.5–67.5)	4.5	100.0	57.1	0.0	100.0	0.0	36.4	0.0	73.6
Summary	82		7.3 ± 5.6 (1.7–12.9)				3.7 ± 4.11 (−0.4–7.8)				
Eastern *P. b. indubitus*											
Guerrero	11	49.1 ± 5.1 (28.0–73.0)	100.0	100.0	100.0	0.0	0.0	100.0	100.0	0.0	0.0
México	12	51.1 ± 5.0 (31.0–69.0)	100.0	100.0	100.0	0.0	0.0	100.0	100.0	0.0	0.0
Morelos, Huitzilac (municipality)	22	61.3 ± 1.3 (54.0–68.0)	72.7	100.0	100.0	0.0	9.1	91.9	100.0	0.0	0.0
Morelos, Tepoztlán[3]	7	60.1 ± 1.1 (56.0–65.0)	100.0	100.0	100.0	0.0	0.0	100.0	100.0	0.0	0.0
Morelos, Cuernavaca[3]	8	60.5 ± 3.3 (55.0–66.5)	100.0	100.0	100.0	0.0	0.0	100.0	100.0	0.0	0.0
Summary	60		10.0 ± 7.6 (2.4–17.6)					3.3 ± 4.5 (−1.2–7.8)			
Western *P. b. indubitus*											
Jalisco	14	52.3 ± 3.9 (28.0–67.0)	85.7	100.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0

Table 6.—Continued.

Taxon/character	N	Snout-to-vent length $\bar{X}$ ± SE (range)	Interparietal enclosed posteriorly (%)	Primary temporal[1] (%)	Light line on supralabials 6 and 7 (%)	Lateral light line on neck (%)			Dorsolateral light line posterior extension (%)		
						Absent	Normal	Modified	Forelimb	Midbody	Hindlimb or tail
Colima	21	53.8 ± 2.9 (31.0–69.0)	90.5	100.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0
Summary	35		11.4 ± 10.4 (0.6–21.4)								
P. b. bilineatus											
Chihuahua	30	51.2 ± 1.7 (33.0–66.0)	93.3		0.0	100.0	0.0	0.0	13.3	56.7	30.0
Durango, Suchil				10.0							
Durango, El Salto	12	50.9 ± 3.1 (30.0–62.0)	100.0	20.8	0.0	58.3	41.7	0.0	0.0	0.0	100.0
Jalisco, Bolaños	10	58.8 ± 2.3 (24.0–66.0)	80.0	0.0	0.0	0.0	100.0	0.0	50.0	0.0	50.0
Summary	52		7.7 ± 7.2 (0.4–14.8)	10.6 ± 5.8 (4.2–15.8)							

[1] Sample size for light line on supralabials 6 and 7 = 35.
[2] Sample size for light line on supralabials 6 and 7 = 14; for lateral light line on neck = 21.
[3] Putative intergrades *P. b. indubitus* × *P. b. brevirostris*.

indubitus could each be statistically rejected, because neither alternative set of relationships was present in the 95% credible set of trees. However, the alternative topology constraining the haplotypes of *P. b. bilineatus* and western *P. b. indubitus* to comprise a monophyletic group could not be statistically rejected.

External Morphology

Variation in the morphological characters examined for all of the putative species is summarized in Table 6. Given that the vast majority of the putative intergrades *P. b. brevirostris* × *P. b. indubitus* were similar to eastern *P. b. indubitus* in all of the examined characters (see below), they were grouped with the other samples of the latter taxon. Similarly, putative intergrades *P. b. dicei* × *P. b. pineus* were grouped with the samples of these two subspecies, because they constituted a single putative species in the mtDNA phylogeny. All of the characters were polymorphic in one or more samples with $n > 1$.

The division of *P. brevirostris* into five putative species was generally supported by the morphological data (see below). Given that five characters (excluding SVL) were surveyed in all of the putative species, and that the sample of each species showed two or three potentially diagnostic fixed traits, formula (3) of Wiens and Servedio (2000) indicated that 35 or more individuals constituted sufficient evidence to reject the null hypothesis (with a 5% confidence interval) that the actual frequency of all of the apparently absent traits is above 10%. Thus, our samples of all of the putative species were large enough to infer ($\alpha = 0.05$) that at least one of the putatively fixed diagnostic characters is either fixed or has the alternate trait at a frequency below 10% in the species, although unfortunately the test does not indicate which one.

Variation within putative species.—All of the characters examined showed differences in trait frequencies among the samples of one or more of the putative species represented by more than one individual. Noteworthy variation exists within some putative species. Within *P. b. bilineatus*, the samples from El Salto, Durango, and Bolaños, Jalisco showed a

normal lateral light line on the neck (i.e., the supposedly unusual trait in the subspecies; see above) in 41.7% and 100% of the specimens, respectively. Within *P. b. brevirostris*, the samples from Puebla and Oaxaca appeared relatively homogeneous. However, the sample from Guerrero differed from the two aforementioned samples by having a much lower frequency of specimens with a light line on the sixth and seventh supralabials (12.0%, vs. 57.1%–66.6% in the other samples), and a much higher frequency of specimens with a short dorsolateral light line (94.7%, vs. 4.7% and 36.4% in the samples from Puebla and Oaxaca, respectively). The single individual of *P. b. brevirostris* from Morelos showed an enclosed interparietal and lacked a light line on the sixth and seventh supralabials, traits seen at low frequencies in the other samples of the putative species.

Variation among putative species.—*Plestiodon b. dicei* and *P. b. pineus* differed from *P. b. brevirostris* and western and eastern *P. b. indubitus* by the fixed absence of a lateral light line on the neck (vs. presence of a normal or modified lateral light line on the neck fixed in *P. b. brevirostris* and western and eastern *P. b. indubitus*) and the nearly fixed absence of a primary temporal (alternative state in 2.0% of the specimens; $n = 52$; confidence interval = −0.7% to 4.5%; vs. presence of a primary temporal fixed in *P. b. brevirostris* and western and eastern *P. b. indubitus*); and additionally from eastern *P. b. indubitus* by the fixed absence of a light line on the sixth and seventh supralabials (vs. presence of a light line on the sixth and seventh supralabials fixed in the latter taxon). *Plestiodon b. dicei* and *P. b. pineus* differed from *P. b. bilineatus* by usually lacking an enclosed interparietal (alternative state in 5.8% of the specimens, $n = 52$; confidence interval = −0.6% to 12.0%; vs. enclosed interparietal usually present [alternative state in 7.7% of the specimens, $n = 52$; confidence interval = 0.4%–14.8%] in *P. b. bilineatus*).

Plestiodon b. brevirostris differed from western and eastern *P. b. indubitus* by the nearly fixed presence of a normal lateral light line on the neck (alternative state in 3.7% of the specimens; $n = 82$; confidence interval = −0.4% to 7.8%; vs. presence of a modified lateral light line on the neck fixed in western *P. b. indubitus*, and nearly fixed [alternative state in 3.3%; $n = 60$; confidence interval = −1.2% to 7.8%] in eastern *P. b. indubitus*), and from western and eastern *P. b. indubitus* and *P. b. bilineatus* by usually lacking an enclosed interparietal (alternative state in 7.3% of the specimens, $n = 82$; confidence interval = 1.7%–12.9%; vs. enclosed interparietal usually present in western *P. b. indubitus* [alternative state in 11.4% of the specimens; $n = 35$; confidence interval = 0.6%–21.4%], eastern *P. b. indubitus* [alternative state in 10.0% of specimens; $n = 60$; confidence interval = 2.4%–17.6%], and *P. b. bilineatus* [see above]). *Plestiodon b. brevirostris* differed additionally from *P. b. bilineatus* by the fixed presence of a primary temporal (primary temporal usually absent [alternative state in 10.6% of the specimens; $n = 52$; confidence interval = 4.2%–15.8%] in *P. b. bilineatus*).

Eastern *Plestiodon b. indubitus* differed from western *P. b. indubitus* and *P. b. bilineatus* by the fixed presence of a light line on the sixth and seventh supralabials (absence of a light line on the sixth and seventh supralabials fixed in the latter taxa), and additionally from *P. b. bilineatus* by the nearly fixed presence of a modified lateral light line on the neck (see above; vs. absence of a modified lateral light on the neck fixed in *P. b. bilineatus*) and the fixed presence of a primary temporal (vs. primary temporal usually absent in *P. b. bilineatus*; see above).

Western *P. b. indubitus* differed from *P. b. bilineatus* by the fixed presence of a modified lateral light line on the neck (vs. absence of a modified lateral light on the neck fixed in *P. b. bilineatus*) and by the fixed presence of a primary temporal (vs. primary temporal usually absent in *P. b. bilineatus*; see above).

Putative intergrades P. b. brevirostris × P. b. indubitus.—All of the specimens from Tepoztlán and Cuernavaca, Morelos showed all of the characters of eastern *P. b. indubitus* and no indication of intergradation with *P. b. brevirostris*. However, in the sample from Huitzilac, Morelos ($n = 22$), a nonenclosed interparietal and a normal lateral light line on the neck (traits usually present in *P. b. brevirostris* and absent in the other samples of eastern *P. b. indubitus*)

were present in 27.3% and 9.1% of the specimens, respectively.

Putative intergrades P. b. dicei × P. b. pineus.—*Plestiodon b. dicei* differed from *P. b. pineus* by the fixed presence of a short dorsolateral light line (vs. short dorsolateral light line usually absent [in 79.5% of the specimens] in *P. b. pineus*). In the putative intergrades *P. b. dicei* × *P. b. pineus* from Rancho El Cielo, Tamaulipas, a short dorsolateral light line was present in 80.0% of the specimens; that is, at a frequency relatively intermediate between those found in *P. b. dicei* and *P. b. pineus*.

Additional variation between P. b. dicei *and* P. b. pineus.—Variation in the additional characters examined for these subspecies is summarized in Table 7. There were consistent, large differences in the state frequencies of two characters between the two samples of *P. b. dicei*. The prefrontals were usually not in contact (in 75% of the specimens) and the presence of a dorsolateral light line less than one scale wide was fixed in the sample from Marmolejo, whereas the presence of prefrontals in contact and the absence of a dorsolateral light line less than one scale wide were fixed in the sample from Rancho El Manzano and Chipinque. *Plestiodon b. dicei* differed from *P. b. pineus* by the fixed presence of the dorsal margin of the lateral dark stripe on the fourth scale row (vs. dorsal margin of the lateral dark stripe on the fourth scale row usually absent [in 90.4% of the specimens] in *P. b. pineus*), and by having slightly higher numbers of transverse dorsal scale rows between the levels of the axilla and groin (see Table 7). In the putative intergrades *P. b. dicei* × *P. b. pineus* from Rancho El Cielo, Tamaulipas, the dorsal margin of the lateral dark stripe on the fourth scale row was present in 33.3% of the specimens; that is, at a frequency relatively intermediate between the respective frequencies in *P. b. dicei* and *P. b. pineus*. However, the small size of most of the samples makes it difficult to evaluate the significance of these differences.

Discussion

Species Limits

Species delimitation based on mtDNA data.—In the W-P tree-based method for delimiting species based on DNA sequence data, if the haplotypes of the focal species are not exclusive (the focal species is paraphyletic or polyphyletic) with respect to one or more species that are each distinct and exclusive, then the focal species may represent multiple species if there is no evidence of gene flow between the basal lineages (Wiens and Penkrot, 2002).

Phylogenetic analysis of our mtDNA sequence data showed that the haplotypes of the focal species of this study, *P. brevirostris*, are not exclusive, and that they are instead intermingled with the other species of the *P. brevirostris* group. These haplotypes are partitioned among five strongly supported, relatively highly divergent lineages: *P. b. dicei* + *P. b. pineus*, *P. b. brevirostris*, eastern *P. b. indubitus*, western *P. b. indubitus*, and *P. b. bilineatus*, only two of which are sister taxa. Each of these five lineages is allopatric or parapatric and congruent with geography. Furthermore, the mtDNA phylogeny showed no evidence of gene flow between the most basal of these lineages (i.e., *P. b. dicei* + *P. b. pineus* and *P. b. brevirostris* + eastern *P. b. indubitus*), nor between them and the more distal lineages (i.e., western *P. b. indubitus* and *P. b. bilineatus*). Thus, application of the W-P method to our data indicates that *P. brevirostris* is not exclusive and consists of five distinct lineages with no apparent gene flow among them, thus supporting the division of this taxon into five species (Figs. 3 and 4).

Morphological evidence for putative species.—Our morphological data showed that four of the putative species (*P. b. dicei* + *P. b. pineus*, *P. b. brevirostris*, eastern *P. b. indubitus*, and western *P. b. indubitus*) may be distinguished from one another by fixed or nearly fixed differences in the state frequencies of one or more of three characters (lateral light line on the sixth and seventh supralabials, lateral light line on the neck, and primary temporal). In addition, our data showed large (yet not diagnostic) differences in the state frequencies of the interparietal between *P. b. brevirostris* and eastern and western *P. b. indubitus* that were similar to differences previously reported by Dixon (1969).

Plestiodon b. bilineatus may be distinguished from both eastern and western *P. b. indubitus* by fixed or nearly fixed differences in the state frequencies of the lateral light line on the neck, and additionally from eastern *P.*

TABLE 7.—Variation in selected morphological characters in *Plestiodon brevirostris dicei*, *P. b. pineus*, and putative intergrades between them. For the meristic and morphometric characters, numbers are mean ± one standard error; numbers in parentheses are ranges, unless noted otherwise.

Character/taxon	*P. b. dicei* NL, Marmolejo (n = 4)	*P. b. dicei* Nuevo León (NL), El Manzano and Chipinque (n = 9)	*P. b. pineus* Coahuila-NL (n = 21)	*P. b. dicei* × *P. b. pineus* Tams, Rancho El Cielo (n = 6)
Snout to vent length (SVL)	35.7 ± 6.9 (24.0–51.0)	47.7 ± 2.2 (33.3–52.0)	48.7 ± 2.3 (41.0–65.0)	49.2 ± 3.7 (37.5–60.0)
Prefrontals in contact (%)	25.0	100.0	71.4	50.0
Relative size of supralabials (%)				
First = second	50.0	25.0	52.3	80.0
First > second	50.0	75.0	47.6	20.0
Number of postsuboculars (%)	3-3 (50.0) 3-4 (50.0)	2-2 (11.1) 3-2 (11.1) 3-3 (66.6) 3-4 (11.1)	3-3 (80.0) 4-3 (15.0) 4-4 (5.0)	3-3 (50) 4-4 (50)
Transverse dorsal scale rows between levels of axilla and groin	23.0 ± 0.4 (22–24)	24.0 ± 0.2 (23–25)	22.2 ± 0.2 (21–24)	22.5 ± 0.3 (22–24)
Subdigital lamellae on fourth toe	12.3 ± 0.3 (12–13)	13.8 ± 0.4 (12–15)	12.8 ± 0.2 (11–14)	12.3 ± 0.4 (11–13)
Dorsolateral light line width (%)				
Less than one scale	100.0	0.0	4.7	20.0
More than one scale	0.0	100.0	95.3	80.0
Dorsal margin of lateral dark stripe (%)				
On third row	0.0	0.0	90.4	66.6
On fourth row	100.0	100.0	9.6	33.3
Trunk length (axilla–groin length/SVL) × 100	55.0 ± 5.9 (43.7–65.9)	57.9 ± 1.0 (52.2–60.9)	56.6 ± 0.7 (51.8–60.2)	58.6 ± 1.0 (55.8–62.7)
Hindlimb length (tibia length/SVL) × 100	10.6 ± 0.8 (9.8–11.4)	9.6 ± 0.3 (8.6–11.7)	9.2 ± 0.24 (7.7–12.2)	9.6 ± 0.4 (8.0–10.7)

b. indubitus by fixed differences in the state frequencies of the light line on the sixth and seventh supralabials. Also, there were large (yet not diagnostic) differences in the state frequencies of the primary temporal between *P. b. bilineatus* and eastern and western *P. b. indubitus*, which were similar to differences previously reported by Dixon (1969). Although none of the characters examined showed fixed or nearly fixed differences in state frequencies between *P. b. bilineatus* and *P. b. dicei* + *P. b. pineus*, large differences (only marginally not diagnostic) were found in the state frequencies of the interparietal between these putative species (Table 6). Previously, the fixed presence of an enclosed interparietal in *P. b. bilineatus* ($n = 48$) and its usual absence in *P. b. dicei* + *P. b. pineus* (alternative state in approximately 3.8% of the specimens; $n = 53$; confidence interval = −1.4% to 8.8%) were documented by Dixon (1969). Thus, on the basis of Dixon's (1969) data, this character can be regarded as diagnostic to separate *P. b. bilineatus* from *P. b. dicei* + *P. b. pineus*.

None of the characters examined showed fixed or nearly fixed differences in state frequencies between *P. b. brevirostris* and *P. b. bilineatus*. However, large differences exist in the state frequencies of the interparietal between these taxa (Table 6), and the usual absence of an enclosed interparietal in *P. b. brevirostris* (alternative state in 12.1% of the specimens; $n = 240$; confidence interval = 7.89%–16.11%) and its fixed presence in *P. b. bilineatus* (see above) were previously reported by Dixon (1969). In addition, our data showed the fixed presence of a primary temporal in *P. b. brevirostris* and its usual absence in *P. b. bilineatus* (Table 6). The presence of a primary temporal nearly fixed in *P. b. brevirostris* (alternative state in 2.0% of the specimens; $n = 240$; confidence interval = 0.2%–2.8%) was documented by Dixon (1969); however, a primary temporal was present in approximately 37% of his specimens of *P. b. bilineatus*.

In addition to our data, Dixon (1969) documented other morphological differences between eastern and western *P. b. indubitus*. He stated that his sample of *P. b. indubitus* from Jalisco "was similar to the Morelos and Mexico sample, but usually lacks the lateral light line on the supralabials. The upper secondary dark line is longer, extending posteriorly to midbody in most specimens. The lateral light line is represented by light-centered, dark scales on the seventh and eighth scale rows on the neck rather than the fifth, sixth, seventh, eighth, or ninth, or any combination in sequence." Only the first of these characters was recorded in the specimens of *P. b. indubitus* examined herein. However, the presence of a light line on the sixth and seventh supralabials was fixed in our sample of eastern *P. b. indubitus*, whereas its absence was fixed in our sample of western *P. b. indubitus* (Table 6). Thus, not only can western and eastern populations of *P. b. indubitus* be distinguished from each other by at least one diagnostic character, but it is possible that other trait differences exist between them. In addition, there is a large geographic gap between the western and eastern populations, although a recent record for *P. b. indubitus* from central Michoacán (Auth et al., 1997; specimen not examined herein) reduces this gap to some extent. The gap seems the more significant since the habitat of this taxon appears to be continuous across southern Jalisco, Michoacán, Mexico, and northern Puebla (Dixon, 1969).

Intergradation Among Subspecies of Plestiodon brevirostris

Plestiodon b. dicei × P. b. pineus.—Axtell (1960) compared a number of squamation and color-pattern characters in *P. b. dicei*, *P. b. pineus*, and 24 specimens from Tamaulipas (23 from the Gómez Farías region and 1 from Chihue), and concluded that the latter specimens were "meristically and morphologically intermediate" between *P. b. dicei* and *P. b. pineus*, although in pattern and color they "favored *P. d. dicei* more than *P. d. pineus*." Later, Dixon (1969) endorsed Axtell's (1960) view and suggested that Santiago, Nuevo León, might be another area of intergradation between *P. b. dicei* and *P. b. pineus*, based on "intermediate conditions in coloration" in a sample of six specimens from this locality.

Unfortunately, only a single individual of *P. b. pineus* and a single putative intergrade *P. b. dicei* × *P. b. pineus* from the Gómez Farías

region, Tamaulipas (Rancho El Cielo), could be surveyed herein (Table 1). Nonetheless, these two haplotypes were strongly supported as sister taxon to each other and nested within the clade otherwise composed of the haplotypes of *P. b. dicei* (although we assigned the specimens from Chipinque and Rancho El Manzano, Nuevo León, to *P. b. dicei*, Dixon (1969) suggested that the nearby area of Santiago, Nuevo León, might be one of secondary intergradation between *P. b. dicei* and *P. b. pineus*). This suggests that *P. b. pineus* may be actually conspecific with *P. b. dicei*, or that *P. b. dicei* may be a nonexclusive species relative to a distinct *P. b. pineus*. Interestingly, the branch leading to the haplotype of *P. b. pineus* was slightly longer than other branches in the *P. b. dicei* + *P. b. pineus* clade. Also, the haplotypes of *P. b. dicei* from Tamaulipas and Nuevo León formed clades congruent with geography, but whereas the haplotype of the putative intergrade *P. b. dicei* × *P. b. pineus* (from southwestern Tamaulipas) grouped with the geographically closer haplotypes of *P. b. dicei* (from west-central Tamaulipas), the haplotype of *P. b. pineus* (from extreme eastern Coahuila) grouped with the haplotype of the putative intergrade *P. b. dicei* × *P. b. pineus*, rather than with the haplotypes of *P. b. dicei* from the much closer state of Nuevo León (Figs. 3 and 4).

Our morphological data showed large differences in the state frequencies of the position of the dorsal edge of the lateral dark stripe and somewhat smaller differences in the state frequencies of the posterior end of the dorsolateral light line between *P. b. dicei* and *P. b. pineus* (Tables 6 and 7). The putative intergrades between these subspecies from Rancho El Cielo, Tamaulipas, showed state frequencies for these characters that were intermediate between the frequencies in each subspecies. Although these data are consistent with the assessment (based on larger samples) by Axtell (1960) and Dixon (1969) that *P. b. dicei* and *P. b. pineus* are conspecific, the small size of some of our samples makes it difficult to interpret their significance. Clearly, additional molecular and morphological data are needed to establish confidently whether *P. b. dicei* and *P. b. pineus* are conspecific. At any rate, it seems clear that these taxa together are specifically distinct from the other putative species within *P. brevirostris*.

Plestiodon b. brevirostris × *eastern* P. b. indubitus.—Dixon (1969) reported three individuals from the area where the ranges of these taxa approach each other in Morelos ("between Cuernavaca and Tepoztlán"; actually between Tres Marías and Cuernavaca, according to his list of examined specimens) with "enclosed interparietals and a faint lateral light line on the neck or a non-enclosed interparietal without a lateral light line on the neck." He also reported that 10 of 41 individuals referred to *P. b. indubitus* from Morelos and Mexico had "a faint indication of a lateral light line, represented by a series of light-centered, dark scales on the fifth and sometimes the sixth longitudinal scale rows at midbody." Because in each case the characters found in the reported individuals represented a combination of the diagnostic characters of *P. b. brevirostris* and *P. b. indubitus*, Dixon (1969) considered these taxa as conspecific. However, our mitochondrial data suggest these two taxa are genetically divergent and gene flow between them is absent. Also, most of the specimens of *P. brevirostris* examined by us from Morelos, including those from Tepoztlán and Cuernavaca, exhibited the usual characters of *P. b. indubitus* (Table 6). Thus, they did not support the intergradation between *P. b. brevirostris* and *P. b. indubitus* in the Tepoztlán-Cuernavaca area.

However, 27.3% of the specimens from the region of Huitzilac ($n = 22$) lacked an enclosed interparietal and 9.1% had a normal lateral light line on the neck (i.e., showed character states usually present in *P. b. brevirostris* and usually absent in *P. b. indubitus*). This supports Dixon's (1969) assessment that these taxa intergrade in the area between Tres Marías and Cuernavaca in northwestern Morelos. Unfortunately, although Dixon (1969) reported populations of *P. b. brevirostris* in northwestern Morelos (the Lagunas de Zempoala) and southwestern Distrito Federal (Ajusco mountains), we failed to find them, despite repeated attempts. At any rate, the above area of intergradation is relatively narrow, which suggests that gene

flow between *P. b. brevirostris* and *P. b. indubitus*, if existent, is reduced and the taxa maintain their distinctness in spite of it (Wiley, 1981). In addition, the relatively high mtDNA divergence between these lineages also suggests that they represent distinct species.

Plestiodon b. indubitus × P. b. bilineatus.—Dixon (1969) noted the existence of a geographic gap between populations of *P. b. bilineatus* and *P. b. indubitus* from southern Durango to northwestern Jalisco, and suggested that the valley of the Río Santiago is an effective barrier to gene flow between northwestern Jalisco and southern Nayarit. However, Robinson (1979) documented the presence of the diagnostic or most frequent characters of *P. b. indubitus* (a modified lateral light line on the neck, dark color below this line extending laterally to the belly, and dorsolateral light line becoming faint near the shoulder) in a paratype of *P. b. bilineatus* from the Sierra de Juanacatlán, Jalisco; "patterns typical of *P. b. indubitus*" in two individuals collected near the type locality of *P. b. bilineatus* (approximately 10 miles southwest of El Salto, Durango), and pattern features of both *P. b. bilineatus* and *P. b. indubitus* (lateral light line on the neck absent, dark color of the neck extended onto the venter, and dorsolateral light line extended only to the shoulder) in three specimens from southern Durango and northwestern Jalisco. Hence, Robinson (1979) suggested that the type locality of *P. b. bilineatus* is in a zone of intergradation with *P. b. indubitus* (i.e., western *P. b. indubitus*), and concluded that the limits of the intergradation zone were unknown and "must await additional material from the remote region between southern Durango and northwestern Jalisco."

Our molecular data suggested the absence of gene flow between *P. b. bilineatus* and western *P. b. indubitus*. Also, none of the specimens examined herein from Durango or Jalisco had characters intermediate between those of *P. b. bilineatus* and *P. b. indubitus*. Except for a series from the Sierra de Bolaños, Jalisco, all of the specimens from Chihuahua and Durango ($n = 30$) and Colima and Jalisco ($n = 35$) exhibited mostly the characters of *P. b. bilineatus* or *P. b. indubitus*, respectively (Table 6).

The series from the Sierra de Bolaños, Jalisco ($n = 10$), an area geographically intermediate between the ranges of western *P. b. indubitus* and *P. b. bilineatus fide* Dixon (1969), was similar to *P. b. bilineatus* in the absence of a primary temporal, similar to both *P. b. bilineatus* and *P. b. indubitus* in the posterior extension of the dorsolateral light line, and different from most *P. b. bilineatus* and all *P. b. indubitus* in the presence and nature of the lateral light line on the neck, respectively (Table 6). Thus, these specimens exhibited a combination of characters unlike that of *P. b. indubitus* or *P. b. bilineatus*, and cannot be regarded as intergrades between these taxa, as could be expected from their geographic provenance. In addition, the haplotypes from the Sierra de Bolaños were nested within the clade of *P. b. bilineatus*, and were only slightly more divergent from the remaining haplotypes in the clade than the latter were to one another (Fig. 3). Thus, we tentatively assigned the population from the Sierra de Bolaños to *P. b. bilineatus*.

Thus, our molecular and morphological data did not provide evidence of intergradation between *P. b. bilineatus* and *P. b. indubitus*, in spite of their possible parapatry. More importantly, *P. b. bilineatus* was more closely related to *P. dugesii* than to *P. b. indubitus* (Figs. 3 and 4), which implies that, if there were some gene flow between them, this would be secondary and presumably restricted. On the other hand, the hypothesis that *P. b. bilineatus* and western *P. b. indubitus* are sister taxon to each other could not be rejected statistically (see above). Additional samples from *P. b. bilineatus* and especially *P. b. indubitus* from west-central Mexico (as well as additional samples from localities geographically intermediate between their geographic ranges) are needed to evaluate possible gene flow between these taxa. Whether the population from the Sierra de Bolaños is conspecific with *P. b. bilineatus* cannot be properly evaluated with the current data, and a larger sample from this population also is needed to confidently resolve its taxonomic status and relationships.

Concordance Between mtDNA-Based Species Delimitation and Traditional Taxonomy.—The putative species suggested by the molecular, morphological, and geographic data were

partially concordant with the traditional taxonomy of *P. brevirostris*. *Plestiodon b. brevirostris* and *P. b. bilineatus* were each strongly supported as exclusive with respect to the remaining subspecies of *P. brevirostris* and other species of the *P. brevirostris* group. Although our data (both mtDNA and morphological) cannot resolve whether or not *P. b. pineus* is conspecific with *P. b. dicei*, or *P. b. dicei* is a nonexclusive species relative to a distinct *P. b. pineus*, the mtDNA clade comprising these two subspecies was strongly supported as exclusive with respect to the remaining *P. brevirostris* group taxa. In contrast, *P. b. indubitus* was shown to be nonexclusive, and its haplotypes were intermingled with other species of the *P. brevirostris* group.

Given that all of the new delimited putative species within *P. brevirostris* are mitochondrially exclusive, moderately to strongly divergent genetically, and allopatric or parapatric, a substantial history of genetic isolation between these putative taxa is indicated (Funk and Omland, 2003). This is supported by the morphological diagnosability of the putative species. Thus, we conclude that the polyphyly of *P. brevirostris* results from imperfect taxonomy (i.e., the taxonomic "lumping" of multiple species under a single name), and therefore can be validly eliminated by changing this taxonomy (Funk and Omland, 2003). Alternative causes for species-level polyphyly in *P. brevirostris* include inadequate phylogenetic information, incorrect estimation of the gene tree, recent or ongoing interspecific hybridization, unrecognized parology, and incomplete lineage sorting (Funk and Omland, 2003). However, the first four of these causes seem unlikely, given the good resolution and high support values of our haplotype tree, the absence of sympatric sharing of geographically localized haplotypes between otherwise genetically and morphologically divergent species, and the absence of unusual patterns of molecular evolution that are consistent with reduced functional constraints in the data (e.g., indels, stop codons). Also, given that retention of ancestral polymorphisms is more likely in populations that have split very recently, and the five putative species within *P. brevirostris* do not appear to have split recently. For example, the haplotypes of at least three of the putative species within *P. brevirostris* formed basal clades, and the remaining, more distal clades are distantly related and divergent from the basal ones and each other. Thus, retention of ancestral polymorphism also seems an unlikely cause for the polyphyly of *P. brevirostris*.

Taxonomic Implications

Given that *P. indubitus* Taylor was described from "km 63 (on Mexico-Cuernavaca highway), near Cuernavaca," Morelos (Taylor, 1933), and that *P. dicei* has priority over *P. pineus*, our results support recognition of the populations of *P. b. brevirostris*, *P. b. bilineatus*, *P. b. dicei*, and *P. b. pineus*, and eastern *P. b. indubitus* as four distinct species (*P. brevirostris*, *P. bilineatus*, *P. dicei*, and *P. indubitus*, respectively), and of the western populations of *P. b. indubitus* as an additional, undescribed species. Given that the status of *P. b. pineus* is uncertain, for the time being it is considered as a synonym of *P. dicei*. Characters used by Dixon (1969) to distinguish among several of these taxa, in addition to the diagnostic characters and other large differences in trait frequencies among these taxa identified in this study, should generally make possible the allocation of specimens to each species. Note, however, that because of the high variation within and among these taxa for all of the characters examined, single individuals cannot be unfailingly distinguished from one another. Specific allocations based on series of one or two individuals may be suspect in the absence of geographic information; series of five or more individuals would be necessary for reasonably secure allocations.

Additional Hidden Diversity

It is possible that a basal lineage of a putative species might contain multiple species (Wiens and Penkrot, 2002). The W-P method does not determine when splitting is no longer justified by the available data; however, at these progressively lower taxonomic levels small sample sizes limit the ability to rule out gene flow among potentially distinct lineages confidently (Wiens and Penkrot, 2002). The *P. b. brevirostris* lineage

was composed of three strongly supported subclades that were concordant with geography. A main subclade occurs in Guerrero, another one in Puebla and Tlaxcala, and the other one in Oaxaca (Figs. 3 and 4). Interestingly, populations in these relatively distant areas appear to be geographically isolated from one another (especially populations in the Guerrero and Puebla-Tlaxcala subclades, which were strongly supported as sister taxon to each other), and each of these areas belongs to a different biogeographic region (Morrone et al., 2002). Furthermore, there were some large differences in trait frequencies among these populations (Table 6; also see Dixon, 1969). We suggest that additional data, analyzed with the W-P method and complementary methods such as nested clade analysis (Templeton et al., 1995) or analyses of mtDNA branching rates (Pons et al., 2006), might reveal yet additional species within *P. b. brevirostris*.

Level of Polymorphism Allowed in Diagnostic Characters

Herein, we tentatively used a nonfixed frequency cutoff of 10% to identify diagnostic characters and make species decisions. By using this cutoff, we found enough diagnostic characters so that all the putative species may be distinguished from one another by at least one diagnostic character, with the only exception of *P. b. brevirostris* and *P. b. bilineatus*. However, we also found other large, yet not diagnostic, differences in trait frequencies among the putative species (population frequency for the alternative trait up to 22% in each of the compared lineages; confidence level = 95%; Table 6). Significantly, all of these differences were previously used to distinguish among the subspecies of *P. brevirostris* (Dixon, 1969). Because the specific status of these putative species is indicated by their phylogenetic relationships, genetic distinctness, geography, and (in most cases) one or more diagnostic characters, the large (yet not diagnostic) differences likely result from reduced or absent gene flow between species (Wiens and Servedio, 2000). However, if species delimitation requires fixed or nearly fixed diagnostic characters between species, then taxa that are likely good species on the basis of their phylogenetic relationships, genetic distinctness, geography, and even possession of large, but not diagnostic, differences in trait frequencies would be disregarded as species with the consequent underestimation of biodiversity. Clearly, this would be the case of *P. b. bilineatus* and *P. b. brevirostris*. Although fortunately in this case there is evidence for their specific status other than the large but not diagnostic differences in trait frequencies between them, taxa for which similar evidence is not available might be disregarded as species. This suggests that the level of polymorphism allowed in diagnostic characters should perhaps be considerably higher in at least some taxonomic groups, and therefore should be evaluated on a case-by-case basis.

Patterns of Evolution in North American Plestiodon

Richmond and Reeder (2002) and Richmond (2006) provided valuable insights on the patterns of evolution in two species groups of North American *Plestiodon*. Richmond and Reeder (2002) identified three instances of parallel morphological evolution in the *P. skiltonianus* group, and suggested that this system was consistent with a model of parallel ecological speciation. In each of those instances, a large-bodied and typically uniform-colored morphotype (at the time assigned to *P. gilberti*) was the sister taxon to a small-bodied and striped morphotype (assigned to *P. skiltonianus* or *P. lagunensis*). The large-bodied, typically uniform-colored morphotype generally inhabits lower elevations and warmer and drier environments than the smaller, striped morphotype, which inhabits mesic, cooler conditions, such as higher elevations and coastal regions. Richmond and Reeder (2002) suggested that the repeated, independent evolution of the *gilberti* morphology in similar environments implied that the phenotypic divergence was the result of adaptation to contrasting selection regimes. In addition, they suggested that selection acted directly on body size and that color pattern (presumably result of peramorphosis) evolved as an incidental by-product.

In contrast, Richmond (2006) documented that, although large-bodied, uniform-colored

and small-bodied, striped species exist in the *P. fasciatus* group, a single major shift seems to have occurred from the large body size in *P. laticeps* (and the successive lineage *P. obsoletus*) to the small body size in the remaining species of the group. Because habitat partitioning is weak among *P. laticeps*, *P. inexpectatus*, and *P. fasciatus*, and they are broadly sympatric throughout their ranges, Richmond (2006) suggested that the increased body size and exaggerated sexual dimorphism in *P. laticeps* was consistent with a model of sexual selection in the common ancestor of the group.

No conspicuous phenotypic divergence (i.e., large-bodied, uniform-colored vs. small-bodied, striped species) exists among species in the *P. brevirostris* group. All of the species and previously recognized subspecies in the group are miniaturized and striped (if the number of stripes varies among them), and differences among them in color pattern and scale characters are relatively slight and extend to the whole ontogeny, although some pattern features tend to fade with age. This may be associated to the fact that most of the species in the group, including the previously recognized subspecies of *P. brevirostris* and their closest relatives, are montane species that dwell in the forest litter. Thus, phenotypic divergence caused by natural selection (i.e., associated with contrasting selection regimes) would not be expected in the group. Similarly, because there is little sexual dimorphism among the *P. brevirostris* group species, no shifts in body size associated with sexual selection would be expected in the group. Therefore, speciation in the group is more likely the result of geographic isolation, founder events, and genetic drift.

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Appendix I

Specimens Examined

Taxa are presented in alphabetical order. Institutional abbreviations follow Leviton et al. (1985), except in the case of the Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC), and the Museo de Zoología, Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México (MZFZ). IDLH and SARCH are abbreviations for field numbers of uncatalogued specimens being deposited in the MZFC and IBH, respectively.

Plestiodon brevirostris brevirostris.—GUERRERO: Zona Cueva del Tigre, 4 km ESE Omiltemi (MZFC 2851); El Cedral, 5 km S Omiltemi (MZFC 2852); road to Las Trincheras, Omiltemi (MZFC 2853, 2863, 2874); 2 km SE Omiltemi (MZFC 2854); road to Agua Fría, 1 km S Omiltemi (MZFC 2855–2856); 2 km ESE Omiltemi (MZFC 2857–2858, 2870); road to La Joya, Omiltemi (MZFC 2859, 2864); 2 km E Omiltemi (MZFC 2860); Palo Hueco, Omiltemi (MZFC 2861, 2866–2867); Laguna Escondida, Omiltemi (MZFC 2862); Plan de Potrerillos, 2.4 km E Omiltemi (MZFC 2865); road to Cedral, 1.5 km S Omiltemi (MZFC 2868); 2 km S Omiltemi (MZFC 2869); 3 km E Omiltemi (MZFC 2871); Potrerillos, Omiltemi (MZFC 2872); Barranca de Potrerillos, Omiltemi (MZFC 2873); Omiltemi, 7000 ft (UTA 4079–4080, 4083, 4100, 4107); Omiltemi, 7349 ft (ENCB 6064); Omiltemi (SARCH 955); Chilpancingo de los Bravo, 7381.9 ft (MZFC 1082–1086); Zumpango del Río (MZFC 642, 691); OAXACA: Sierra de Juárez, highway Mitla-Totontepec (MZFC 5309–5310); 15.9 mi SW of Tlaxiaco, 17°6′20″N, 97°42′22″W (MZFC 7470–7473); Sierra de Juárez, ca. 5 km E Comaltepec (MZFC 11102); On road from Teotitlán del Valle, just downslope from Benito Juárez (MZFC 11161–11162); Santa María Asunción, Tlaxiaco, 2640 m (MZFC 13446); San Andrés Cabecera Nueva, 1250 m (MZFC 13447); Chalcatongo de Hidalgo, 2830–2900 m (MZFC 13448–13449); Tejocotes (IBH 14052); Ixtlán de Juárez, 3.5 mi (by road) N of La Cumbre del Estudiante (IBH 14053); North of Cerro San Felipe (IBH 1054); Santa Lucía Monteverde, 16°59′41.1″N, 97°42′03.9″W (MZFC 15568); Sierra San Felipe (IBH 14058); 14 mi NE Díaz Ordaz, 2900 m (MZFC 25390); Sierra de Juárez, 9 km (by road) from Ixtlán de Juárez to Tuxtepec, 2100 m (MZFC 25391); Cerro San Felipe, 2450 m (MZFC 25392); Carretera Teotitlán del Valle-Benito Juárez, 2750 m (MZFC 25393); PUEBLA: Región norte del Citlaltépetl, 5 km SE de Francisco I. Madero, aproximadamente Cañada de los Berros (IBH 4422 [21 specimens]); MORELOS: Acatlipa (IBH 174).

Plestiodon brevirostris bilineatus.—CHIHUAHUA: Guachochi, km 28 on highway Creel-La Bufa, 2186 m (MZFC 10147, 10149); DURANGO: La Michilía, municipio Súchil (IBH 1796, 2251 [4 specimens], 6868 [3 specimens]); Hacienda los Coyotes (IBH 3837); Cerro Alto, NW Coyotes (IBH 14056); La Peña, 2520–2640 m (ENCB 9294, 9538–9545, 9752–9757, 9993, 10171, 14108); Ejido Chavarría Viejo, municipio Pueblo Nuevo, 23°42.912′N, 105°29.209′W, 2260 m (MZFC 25334–25340); El Salto (MZFC 25401); Ejido La Ciudad, municipio Pueblo Nuevo (MZFC 25400, 25402); Municipio Pueblo Nuevo, cerca del límite con Sinaloa (MZFC 25403); 3 km N of El Salto (MZFC 25399); JALISCO: Highway Bolaños-Tuxpan de Bolaños, municipio de Bolaños (MZFC 17248, 17249, 17253, 17259-61, UTA 52706, 53300).

Plestiodon brevirostris dicei.—TAMAULIPAS: Ciudad Victoria (IBH 4573); Jaumave-Cd Victoria (MZFC 5516 [two specimens]); Marmolejo, Sierra de San Carlos, 24°37′19.8″N, 99°01′55.0″W, 598 m (MZFC 18767–18768).

Plestiodon brevirostris dicei × *P. brevirostris pineus*.—TAMAULIPAS: Rancho Viejo, Gómez Farías, 1300 m (ENCB 8372–8374); Rancho Viejo, Gómez Farías (IBH 14034–14036); Ejido La Gloria, Reserva de la Biósfera Rancho El Cielo, Gómez Farías, 1860 m (MZFC 8511); approximately 4 km SE of Estación Canindo, Rancho El Cielo, Gómez Farías, 1400 m (MZFC 8512); Gómez Farías, Las Palomas, near Ejido La Gloria, Reserva de la Biósfera Rancho El Cielo, 1930 m (MZFC 8513); Sierra de Juárez, Rancho El Cielo (LACM 74306); Ejido La Cima, Reserva de la Biósfera Rancho El Cielo, Gómez Farías, 23°3′29.3″N, 99°11′37.5″W, 904 m (MZFC 18763–

18765); NUEVO LEON: Rancho El Manzano, carretera a Santiago, municipio Santiago, Nuevo León, 25°21′8.4″N, 100°11′36.0″W, 1600 m (MZFC 25354–25359); Parque Ecológico Chipinque, municipio San Pedro Garza García, 25°36′50.5″N, 100°21′15″W (MZFC 18785, 25351–25352).

Plestiodon brevirostris indubitus.—GUERRERO: Ixcateopan de Cuauhtémoc, km 26.5 on highway Taxco-Ixcateopan, 2200 m (MZFC 3920); Ixcateopan de Cuauhtémoc, Barranca W, 1700 m (MZFC 3921); Ixcateopan de Cuauhtémoc (MZFC 3922); 2 km E Ixcateopan de Cuauhtémoc, 1900 m (MZFC 3923); Ixcateopan de Cuauhtémoc, 2 km SW Las Peñas (MZFC 3928); 750 m NE Ixcateopan de Cuauhtémoc (MZFC 3929); 4 km W Ixcateopan de Cuauhtémoc (IBH 6583 [2 specimens]); km 8 on highway Taxco-Ixcateopan (MZFC 3924); Taxco de Alarcón, San Miguel, 2170 m (MZFC 3925); Pedro Ascencio Alquisiras, 1780 m (MZFC 3926); MEXICO: km 27 on highway Santa Martha-Chalma (IBH 3231, 3232 [two specimens]); on gravel road Cuernavaca-Chalma, Ocuilán de Arteaga, 2350 m (MZFC 11796); Rancho "La Mecedora," road to Peña Blanca, Avándaro, Valle de Bravo (MZFC 11798); MORELOS: Tepoztlán, N Pirámide (IBH 4907); Tepoztlán (MZFC 25417–25420, 25433–25434); JALISCO: Ciudad Guzmán, km 86–87 on highway Ciudad Guzmán–El Grullo, Chaparral El Floripondio (IBH 5224, 5976 [11 specimens], 5977); 9.2 mi WNW Ciudad Guzmán (IBH 14051); COLIMA: Minatitlán, El Tapeixtle, 2090–2235 m (MZFC 8249–8250, 8252–8253, 8255–8262, 8266–8268); Minatitlán, km 9.5 on road to Terrero, 1575–1610 m (MZFC 8251, 8263–8265, 8269); Minatitlán, km 9.8 on road to Terrero, 1585 m (MZFC 8254).

Plestiodon brevirostris indubitus × *P. brevirostris brevirostris*.—MORELOS: km 61 de la carretera federal Tres Marías-Cuernavaca, 19°1′45.0″N, 99°13′11.0″W (MZFC 25461, 25470; MZFZ 1550, 1555–1556); approximately 1 km N of Huitzilac, 19°1′25.6″N, 99°16′49.7″W (MZFC 25468; MZFZ 1541, 1544–1545); km 56 de la carrterera federal Tres Marías-Cuernavaca, 19°1′45.0″N, 99°13′11.0″W (MZFC 25460, 25469; MZFZ 1540, 1542–1543, 1548, 1554); 5 km S Tres Marías, 2582–2575 m (IBH 88–89); approximately 1 km N de Biomédicas, Universidad Autónoma de Cuernavaca (MZFC 4553, 25467, 25472; IDLH 322–324); 1 km NW Cuernavaca (IBH 2875); 1.8 km W Huitzilac (by road) on Hwy 95, between Tres Marías and Zempoala (MZFC 11189); Huitzilac (IBH 3230, 3233 [two specimens]).

Plestiodon brevirostris pineus.—NUEVO LEON: Ojo de Agua, Pablillo (ENCB 8303–8304; IBH 14041–14043; LACM 92598–92602); Pablilllo, 24°34′53.0″N, 99°57′56.2″W, 2330 m (MZFC 18769, 18771–18774); Cerro Potosí (IBH 14050); San Antonio Peña Nevada, 2640 m (IBH 751); San Antonio Peña Nevada, 2221 m (IBH 763, 5523); Las Adjuntas, Nuevo León (IBH 14039); Ojo de Agua, Cerro Potosí, 8000 ft (FMNH 30712); near Galeana, Ojo de Agua, 7250 ft (FMNH 30708); east slope Cerro Potosí, 9500 ft (CM 42883–42892); 21.6 mi NW of Galeana on Cerro Potosí (CM 65301); 18 km from Ojo Agua on road to Cerro Potosí (UAZ 38268–38269, 38272); Cerro Potosí, near road to radar tower, 10500–11000 ft (UAZ 23576); just up Cayon Tia Juana from road to Caballada, 3.8 mi (rd) E Hwy 51 (UAZ 46365); 12.2 mi NE San Antonio de Peña Nevada, 2640 m (MZFC 750); COAHUILA: 10.7 km E San Antonio de las Alazanas, 8700 ft (UTA 5931–5932); 8 mi E San Antonio de las Alazanas (LACM 109438–39); 13 mi E de San Antonio (LACM 66392); 14 mi E San Antonio (LACM 66393); 14 km E of San Antonio de las Alazanas (MZFC 18779); Los Lirios, 25°24′6.4″N, 100°34′15.2″W, 2464 m (MZFC 18784–85, 18787–88); Los Lirios, 25°22′32.5″N, 100°30′38.9″W, 2429 m (MZFC 18776, 25361–25362, 25363–25366).

Plestiodon dugesii.—MICHOACAN: 2 km N Nuevo San Juan Parangaricutiro, 2100 m (ENCB 13848); 1 km NW Nuevo San Juan Parangaricutiro (ENCB 13849–13852); 3.5 km N Nuevo San Juan Parangaricutiro, 2400 m (ENCB 13853); 1.5 km N Nuevo San Juan Parangaricutiro, 2250 m (ENCB 13854–13855); Nuevo San Juan Parangaricutiro (ENCB 15665); Rancho Los Lobos, 5 km NE Nuevo San Juan Parangaricutiro, 19°23′29.0″N, 102°10′25.0″W, 2090 m (MZFC 25327–25329); JALISCO: 4 km SE Atemajac de Brizuela, 2380 m (ENCB 14833–14839); 2.5 km S Atemajac de Brizuela, 2350 m (ENCB 14840–14842); approximately 3 km S Atemajac de Brizuela, 20°7′6.7″N, 103°43′36.9″W, 2420 m (MZFC 25330–25333).