PHYLOGENETIC RELATIONSHIPS WITHIN THE LOWLAND TROPICAL SALAMANDERS OF THE *BOLITOGLOSSA MEXICANA* COMPLEX (AMPHIBIA: PLETHODONTIDAE)

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1. INTRODUCTION

This is one of a projected series of papers dealing with phylogenetic relationships in the genus *Bolitoglossa*, the largest and most widely distributed genus in the Order Caudata and one that includes about 20% of recognized salamander species. Our strategy is to investigate primary taxa in proposed subgroups of the genus with the goal of discovering monophyletic groups. When such groups are found, representatives are selected to investigate species-level relationships in other groups, with the goal of establishing a robust phylogenetic hypothesis for the genus. The monophyly of the supergenus *Bolitoglossa* is well supported on both morphological and molecular grounds (Jackman et al., 1997; Lombard and Wake, 1986), and the genus *Bolitoglossa* is supported on morphological characters (Wake and Elias, 1983) but lineages within both taxa are less well established.

Discerning phylogenetic structure within the genus *Bolitoglossa* has proven to be difficult because of extensive homoplasy, a feature of tropical salamanders in general (Wake, 1991; Wake and Elias, 1983). Wake and Lynch (1976) recognized two large sections, termed *alpha* and *beta*, and within each identified several mainly undiagnosed species groups. The *Bolitoglossa mexicana* group (*alpha* section), sometimes also known as the *Bolitoglossa platydactyla* group (Stuart, 1943), is an assemblage of large-bodied,

long-tailed species with large, nearly fully-webbed hands and feet, usually having striking color patterns of tan to bright yellow, orange, or reddish spots, bands, and stripes on a black ground color. The species occur mainly in the lowlands from San Luis Potosí in northeastern México to eastern and southern Honduras, but in Chiapas, México, they extend upwards to approximately 1500 m. Included taxa are: *Bolitoglossa mexicana* Duméril, Bibron, and Duméril 1854, *Bolitoglossa platydactyla* (Gray 1831), *Bolitoglossa mulleri* (Brocchi 1883), *Bolitoglossa salvinii* (Gray 1868), *Bolitoglossa flaviventris* (Schmidt 1936), *Bolitoglossa odonnelli* (Stuart 1943), and *Bolitoglossa jacksoni* Elias 1984 (Frost, 1985; Wake and Elias, 1983). This list includes what generally are considered to be the core members of the group, but some authors have suggested inclusion of additional species: *Bolitoglossa lignicolor* (Peters 1873), *Bolitoglossa yucatana* (Peters 1882) (Brame and Wake, 1963; Dunn, 1926; Taylor, 1952), and *Bolitoglossa dofleini* (Werner 1903) (Schmidt, 1936). In this paper we focus attention on the *B. mexicana* group. Because the group is informal and undiagnosed we selected as many potential members as were available and we also examined members of other species groups of *Bolitoglossa*. We used two remotely related outgroups.

2. MATERIALS AND METHODS

The following members of the *Bolitoglossa mexicana* group (as recognized in this paper) were studied: *B. mexicana* (one to three individuals from each of 11 populations), *B. odonnelli* (two individuals from one population), *B. lignicolor* (one individual), *B. yucatana* (two individuals from one population), *B. platydactyla* (one individual), *Bolitoglossa striatula* (Noble 1918) (one individual), and *B. flaviventris* (three individuals from two populations). Other taxa studied were: *Bolitoglossa dofleini*, *Bolitoglossa adspersa* (Peters 1863), *Bolitoglossa marmorea* (Tanner and Brame 1961), *Bolitoglossa morio* (Cope 1869), and *Bolitoglossa engelhardti* (Schmidt 1936). Outgroups include *Oedipina uniformis* (Keferstein 1868), representing another genus in the supergenus *Bolitoglossa*, and *Batrachoseps campi* Marlow, Brode, and Wake 1979, representing a member of the sister taxon of the supergenus *Bolitoglossa*. All analyses were rooted using *Batrachoseps* as the outgroup. Collection localities, museum collection numbers, and GenBank accession numbers are given in the material examined paragraph (see below) and Table 1. Collection localities of *Bolitoglossa mexicana* analyzed in this study are shown in Fig. 1. Populations of *B. mexicana* and closely associated taxa are referenced by locality number.

Genomic DNA was extracted from small amounts of frozen tissue or protein extracts using NaCl following a protocol modified from Miller et al. (1988). Partial sequences of the first portion of the cytochrome b gene (cyt b) of the mtDNA and of the large (16S) subunit ribosomal mtDNA gene were obtained. Those regions of the mtDNA genome were selected in order to recover a maximum of phylogenetic information both at the terminal nodes and at the base of the tree. Note that we were able to obtain cyt b sequences from only a subset of the total sample (gaps in Table 1).

Fragments of 214 to 647 base pairs, corresponding to codons 7 (part)-223 (part) of the *Xenopus* cyt b gene (Roe et al., 1985), and of approximately 520 bp of the 16S rDNA corresponding to positions 2510–3059 in the human mitochondrial genome (Anderson et al., 1981), were amplified via the polymerase chain reaction (Saiki et al., 1988) using the primers MVZ 15 and MVZ 18 (Moritz et al., 1992) for cyt b, and 16Sar and 16Sbr (Palumbi et al., 1991) for 16S. PCR reactions consisted of 38 cycles with a

Table 1. Samples used in this study, localities, voucher specimen numbers (or field collector number, where voucher not yet available), and GenBank accession numbers for the sequences obtained.

Species	Population	Locality	Museum no.	Cyt b	16S
B. mexicana	1	Belize: Toledo	MVZ 191635	* * *	AF177588
B. mexicana	1	Belize: Toledo	MVZ 191631		AF177589
B. mexicana	1	Belize: Toledo	MVZ 191632		AF218467
B. mexicana	2	Honduras: Atlántida	USNM 343451	* * *	AF218468
B. mexicana	3	Honduras: El Paraíso	UTA (ENS 8675)		AF218469
B. mexicana	4	México: Chiapas	(photo voucher)	* * *	AF218470
B. mexicana	5	Honduras: Cortés	MVZ 163794		AF218471
B. mexicana	5	Honduras: Cortés	MVZ 163795		AF218472
B. mexicana	5	Honduras: Cortés	MVZ 163793		AF218473
B. mexicana	6	Guatemala: Izabal	UTA (MEA 446)		AF218474
B. mexicana	6	Guatemala: Izabal	UTA (ENS7862)		AF218475
B. mexicana	7	Honduras: Olancho	MVZ 229068	* * *	AF218476
B. mexicana	8	Honduras: Copán	MVZ 163797		AF218477
B. mexicana	9	México: Veracruz	MVZ 163959	* * *	AF218478
B. mexicana	9	México: Veracruz	MVZ 172667		AF218479
B. mexicana	10	México: Chiapas	MVZ 194293	* * *	AF218480
B. mexicana	11	México: Chiapas	MVZ 138658		AF218481
B. odonnelli	12	Guatemala: Alta Verapaz	MVZ 161046		AF218482
B. odonnelli	12	Guatemala: Alta Verapaz	MVZ 161039		AF218483
B. lignicolor		Costa Rica: Puntarenas	MVZ (S11132)		AF218484
B. yucatana		México: Quintana Roo	MVZ 197507	* * *	AF218485
B. yucatana		México: Quintana Roo	MVZ 197508		AF218486
B. platydactyla		México: Veracruz	MVZ (GP108)	* * *	AF218487
B. striatula		Costa Rica: Cartago	MVZ 181280	* * *	AF218488
B. flaviventris		México: Chiapas	MVZ 194288	* * *	AF218489
B. flaviventris		México: Chiapas	MVZ 194287		AF218490
B. flaviventris		México: Chiapas	MVZ 163963		AF218491
B. adspersa		Colombia: Cundinamarca	MVZ 158485	* * *	AF218492
B. marmorea		Panama: Chiriquí	MVZ 210286	* * *	AF218493
B. hartwegi		México: Chiapas	MVZ 177790	* * *	AF218494
B. rufescens		México: Chiapas	MVZ 194254	* * *	
B. morio		Guatemala: San Marcos	MVZ 143677	* * *	AF218495
B. engelhardti		Guatemala: San Marcos	MVZ 167789	* * *	AF218496
B. dofleini		Guatemala: Alta Verapaz	MVZ 161607	* * *	AF218497

denaturing temperature of 92 C (1 min), annealing at 48–50 C (1 min), and extension at 72 C (1 min) in a Techne PHC-1 thermocycler. PCR reactions were run in a total volume of 25 µl, using 0.5 pmol of each primer.

Double strand templates were cleaned using MicroSpin S-300 HR columns (Pharmacia Biotech). We used 5.5 µl of double strand as the template for cycle sequencing reactions in 10 µl total volume with the Perkin Elmer Ready Reaction Kit™ to incorporate dye-labeled dideoxy terminators. Thermal cycling was performed using standard conditions. Cycle sequencing products were purified using ethanol precipitation and separated on a 6% polyacrylamide gel using an ABI 377 DNA sequencer (Applied Biosystems). Partial sequences of cyt b and 16S were read from both strands and aligned by eye to each other and to the outgroups in the program Sequence Navigator™ version 1.0.1 (Applied Biosystems). Sequence divergences were estimated
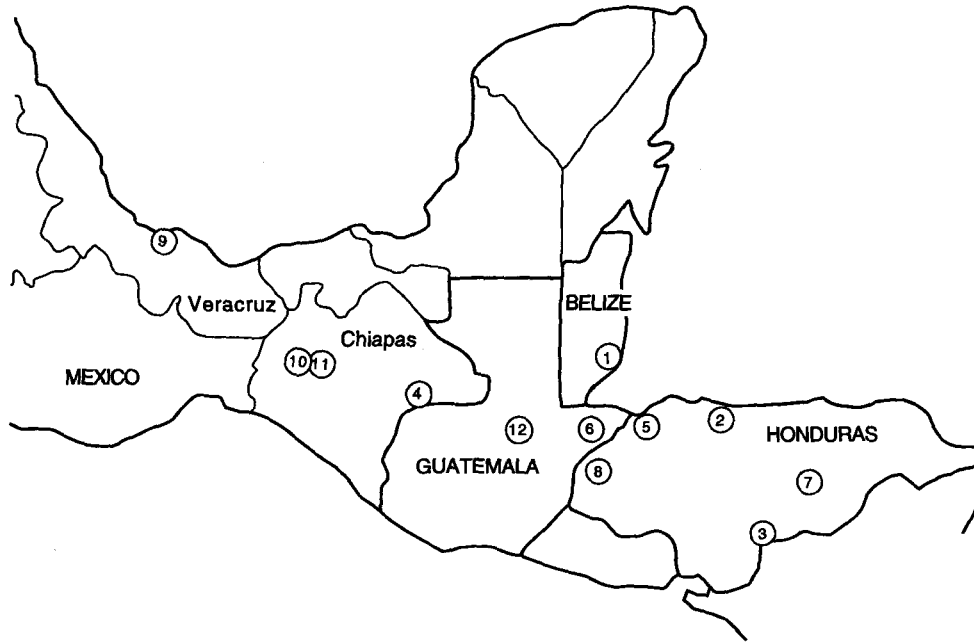
Figure 1. Map of portion of Mesoamerica showing the localities from which samples of the *Bolitoglossa mexicana* complex used for mtDNA sequence studies were obtained. Population numbers correspond to Table 1.

using the Kimura 2-parameter distance (K2p, Kimura, 1980) in order to correct for multiple hits. Corrected sequence divergence between taxa is shown in Table 2.

Phylogenetic inference was based primarily on maximum parsimony analyses (MP: Swofford, 1997) in combination with various weighting schemes, and also on maximum likelihood (ML: Felsenstein, 1981, 1993), and neighbor-joining methods (NJ: Saitou and Nei, 1987). Each base position was treated as an unordered character with four alternative states. Positions with gaps were excluded from the analyses or, alternatively, gaps were treated as missing data. Trees were rooted by outgroup comparisons with sequences of *Oedipina* and *Batrachoseps*. Maximum parsimony phylogenies were

Table 2. Corrected sequence divergence (Kimura 2-parameter distance) between species of *Bolitoglossa* for cyt b (above diagonal) and 16S rDNA (below diagonal).

	1	2	3	4	5	6	7	8	9	10	11
1 *B. mexicana* (1)	—	0.0918	0.1666	0.1661	0.2411	0.2244	0.2527	0.1871	0.1703	0.2351	0.2355
2 *B. yucatana*	0.0323	—	0.1725	0.1480	0.2465	0.2484	0.2179	0.1571	0.1605	0.2363	0.2420
3 *B. platydactyla*	0.0573	0.0403	—	0.1598	0.2485	0.2470	0.2681	0.1740	0.1987	0.2150	0.2695
4 *B. flaviventris*	0.0953	0.0679	0.0844	—	0.2366	0.2108	0.2338	0.2035	0.1622	0.2243	0.2674
5 *B. adspersa*	0.0902	0.0815	0.0917	0.1334	—	0.2401	0.2515	0.1926	0.2092	0.1768	0.2475
6 *B. morio*	0.0792	0.0706	0.0806	0.1453	0.0972	—	0.2388	0.2077	0.1735	0.2108	0.3006
7 *B. dofleini*	0.1172	0.1199	0.1332	0.1893	0.1265	0.1149	—	0.2255	0.2080	0.2253	0.3020
8 *B. hartwegi*	0.0857	0.0813	0.0874	0.1550	0.1011	0.0973	0.1218	—	0.1654	0.1881	0.1994
9 *B. engelhardti*	0.1070	0.1047	0.1200	0.1560	0.1279	0.0773	0.1250	0.1161	—	0.1903	0.2410
10 *B. marmorea*	0.0795	0.0887	0.1018	0.1512	0.0773	0.1023	0.1207	0.0818	0.1027	—	0.2351
11 *B. rufescens*	0.0771	0.0707	0.0830	0.1313	0.1023	0.0817	0.1320	0.0925	0.1095	0.1003	—

estimated using the heuristic algorithm. Heuristic searches were done by stepwise random addition of taxa, with 10 replicates in PAUP 4.0b1a (D. Swofford, Smithsonian Institution). We searched for the most parsimonious trees by using three weighting schemes: one assuming equal weights for every codon position and for third position downweighted 1:4 and 1:10, and two differential transition/transversion weighting schemes (ts/tv = 1/4, and ts/tv = 1/10). We report decay indices (decay) and bootstrap (bs) values in excess of 50% (100 replicates for MP, 1000 for NJ analyses). We used Templeton tests (Larson, 1994;Templeton, 1983) to evaluate the monophyly of *B. mexicana* with respect to other species of the group.

Localities of the specimens included in the DNA study (MVZ: Museum of Vertebrate Zoology; UTA: University of Texas at Arlington; MGP: M. García-París photographic collection; GP: G. Parra-Olea field number; LDW: L. D. Wilson field number): *B. adspersa*: Colombia: Cundinamarca: Ubate, MVZ 158485. *B. dofleini*: Guatemala: Alta Verapaz: Finca El Volcán, 25 km (road) NW Senahú, MVZ 161607. *B. engelhardti*: Guatemala: San Marcos: Ruta Nacional 1; 15.5 km (by road) W San Marcos, MVZ 167789. *B. flaviventris*: México: Chiapas: 3.2–10.5 km N Tapachula on road to Nueva Alemania, MVZ 194287–194288; Finca La Esperanza, 5 km (by road) E Acocoyagua, MVZ 163963. *B. hartwegi*: México: Chiapas: Cerro Xontehuitz, 22–23.7 km (by road) NNE PanAmerican Highway. E San Cristóbal de las Casas, 2700–2800 m of elevation, MVZ 177790. *B. lignicolor*: Costa Rica: Puntarenas: Finca La Dibujada, Buenos Aires—Osa, MVZ (S11132). *B. marmorea*: Panamá: Chiriquí: Crater 0.5 km S and 1.5 km E Volcán Barú, MVZ 210286. *B. mexicana*: Belize: Toledo: vicinity of Indian Village of Blue Creek, MVZ 191631–191632, 191635. Guatemala: Izabal: Morales: Sierra de Caral: Finca la Firmeza, UTA (MEA 446); Morales: Sierra de Caral: Camino Finca Quebradas—Cerro Pozo de Agua, UTA (ENS 7862). Honduras: Atlántida: Quebrada de Oro, 15 38 N 86 47 W, 600 m elev., USNM 343451; Copán: 2 kmN Santa Rosa de Copán, MVZ 163797; Cortés: Cafetal, 8 km (road) W Peña Blanca, MVZ 163794–163795; 3.1 km (by road) S Peña Blanca, MVZ 163793; El Paraíso: Las Manos, 13°48'N – 86°34'W, UTA (ENS 8675); Olancho: Río Catacamas, near Catacamas, 480 m elev., LDW 11130. México: Chiapas: Cafetal, 26.5 kmN Ocozocuautla on road to Apicpac, MVZ 194293; 12 km N of Berriozabal, MVZ 138658; Lagos de Montebello, (MGP photographic voucher); Veracruz: Playa Escondida, E of Catemaco (30 km NNE), MVZ 163959; Playa Escondida, N of Catemaco, MVZ 172667. *B. morio*: Guatemala: San Marcos: Between Palo Gordo and La Fraternidad; just S summit on W facing ridge, MVZ 143677. *B. odonnelli*: Guatemala: Alta Verapaz: Finca El Volcán, 25 km (road) NW Senahú, MVZ 161039, 161046. *B. platydactyla*: México: Veracruz: vic. of Cuautlapan, GP 108. *B. rufescens*: México: Chiapas: Cafetal, 12.4 km (road) N Berriozábal on road to Cairo, MVZ 194254. *B. striatula*: Costa Rica: Cartago: I.I.C.A., 4.5 km ESE Turrialba, MVZ 181280. *B. yucatana*: México: Quintana Roo: 13–19 km (road) Coba, MVZ 197507–197508.

3. RESULTS

We obtained sequences of cyt b from 17 samples of *Bolitoglossa* representing 12 nominal species. Sequence divergence among the ingroup taxa was great, largely exceeding K2p values of 0.20 (Tables 2, 3, and 4). Parsimony analysis produced a single most parsimonious tree (L = 904 steps; 229 characters were parsimony informative; CI = 0.499; RI = 0.434) with a monophyletic *Bolitoglossa* clade (decay > 7; bs 83%)

Table 3. Corrected sequence divergence (Kimura 2-parameter distance) between species of the *Bolitoglossa mexicana* species group for cyt b (above diagonal) and 16S rDNA (below diagonal). The sample of *B. odonnelli* used for the cyt b comparisons corresponds to the only representative obtained from the Clade 3 of *B. mexicana* (see Table 4).

	1	2	3	4	5	6	7	8
1 *B. mexicana* (1)	—	0.0940	0.1126	—	0.0918	0.1666	0.1137	0.1661
2 *B. mexicana* (9)	0.0240	—	0.0809	—	0.0632	0.1389	0.1138	0.1107
3 *B. odonnelli*	0.0407	0.0240	—	—	0.1136	0.1503	0.1278	0.1506
4 *B. lignicolor*	0.0426	0.0300	0.0343	—	—	—	—	—
5 *B. yucatana*	0.0323	0.0322	0.0408	0.0384	—	0.1725	0.1191	0.1480
6 *B. platydactyla*	0.0573	0.0423	0.0486	0.0402	0.0403	—	0.2023	0.1598
7 *B. striatula*	0.0240	0.0240	0.0323	0.0343	0.0282	0.0509	—	0.1796
8 *B. flaviventris*	0.0953	0.0755	0.0826	0.0785	0.0679	0.0844	0.0865	—

(Fig. 2A). The most basal lineage in *Bolitoglossa* is *B. hartwegi*, followed by a clade (decay 2) consisting of *B. rufescens* plus a clade including *B. adspersa* and *B. marmorea* (decay 2), and then by a clade with *B. engelhardti* and *B. dofleini* plus *B. morio* (decay 3). The *B. mexicana* group is monophyletic (decay 6; bs 75%), including in addition to the core species *B. yucatana* and *B. striatula. Bolitoglossa flaviventris* and *B. platydactyla* form a clade (decay 4; bs 63%) that is the sister group to the remaining samples, which form a large clade (decay 5; bs 82%). Samples of *B. mexicana* from Honduras (2), Belize (1), and southern Chiapas (4) form a subclade ("Clade 1") (decay 3; bs 93%), with *B. striatula* and *B. yucatana* as sequentially more basal taxa. Two samples of *B. mexicana* from east-central Veracruz (9) and western Chiapas (10) form a subclade ("Clade 2") (decay 3; bs 69%) that is the sister group to the one above. A sample of *B. mexicana* from Honduras (7) is basal ("Clade 3") to these two subclades within the large, main clade.

All differential weighting schemes resulted in two equally most parsimonious trees that varied in length: L = 1304 (for 3rd positions downweighted 1:4) and L = 2636 (1:10). The only topological effect of weighting transversions 4 to 10 times over transitions is that *B. platydactyla* and *B. flaviventris* are separate basal lineages in the *B. mexicana* complex. In the ML analysis of the cyt b data set we obtained a tree with −Ln = 4897.7 (Fig. 2B). The ingroup topology is identical to that in the parsimony analysis, but that of the other species of *Bolitoglossa* differs slightly. The first sister taxon of the *B. mexicana* group is *B. engelhardti*, followed by *B. morio*, then by *B. dofleini*. This in turn is followed by the more basal lineages including a clade consisting of *B. rufescens* and *B. adspersa* plus *B. marmorea*, and *B. hartwegi*.

Table 4. Corrected sequence divergence (Kimura 2-parameter distance) within and between clades of the *Bolitoglossa mexicana—B. odonnelli* complex for 16S rDNA (above diagonal), and cyt b (below diagonal).

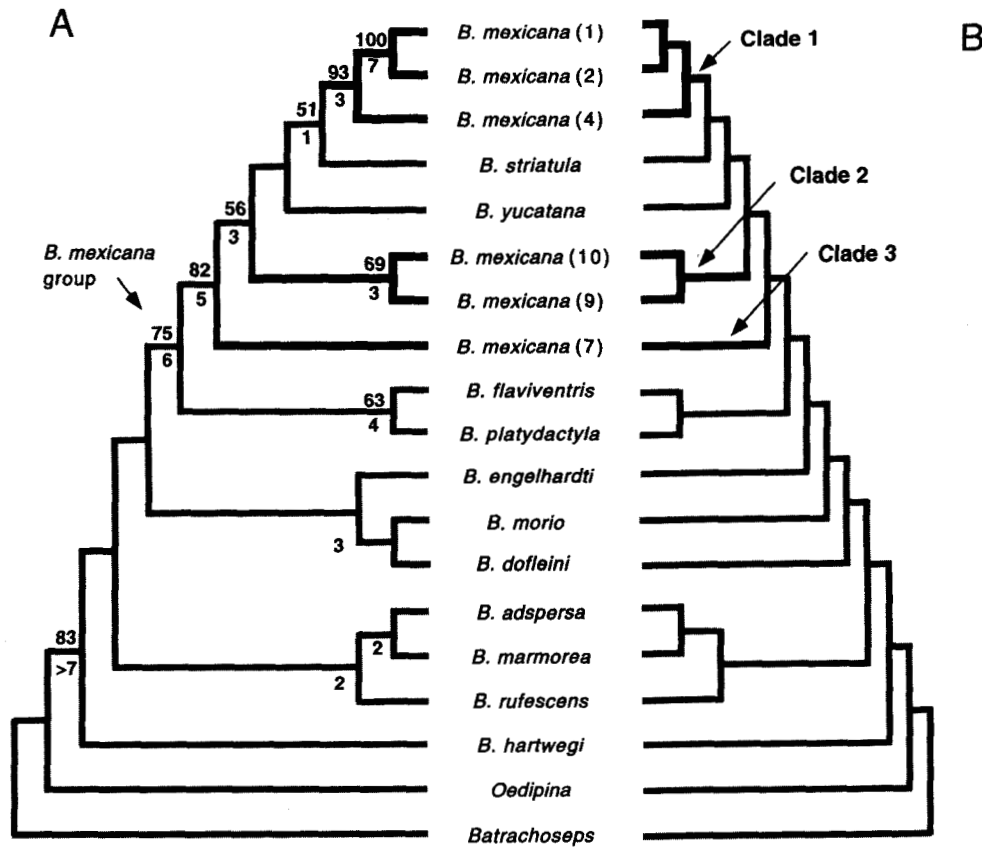|	Within		Between		
	16S	Cyt b	Clade 1	Clade 2	Clade 3
Clade 1	0.0059–0.0240	0.0094–0.0553	—	0.0343–0.0471	0.0240–0.0323
Clade 2	0.0059–0.0059	0.0840	0.0940–0.1178	—	0.0179–0.0240
Clade 3	0.0019–0.0059	—	0.1126–0.1175	0.0809–0.1105	—

Figure 2. A. Single most parsimonious tree for sequences of the mtDNA cyt b gene (L = 904 steps; 229 parsimony informative characters; CI = 0.499, RI = 0.434). Bootstrap values above 50% are shown above branches (100 replicates). Decay index values are indicated below branches. Population numbers correspond to Fig. 1 and Table 1. B. Maximum likelihood tree for sequences of the mtDNA cyt b gene (–Ln = 4897.7). Bold branches correspond to *B. mexicana* populations.

We obtained sequences of 16S from 36 samples of *Bolitoglossa* representing 14 nominal taxa. Divergence among the ingroup taxa ranged as high as about 0.13, with *B. dofleini* being the most divergent (Tables 2 and 3). We obtained 74 equally most parsimonious trees (L = 452; 105 parsimony informative characters; CI = 0.611; RI = 0.568). The samples of *Bolitoglossa* form a well supported monophyletic clade (decay ≥ 4; bs 96%). The strict consensus tree (Fig. 3A) is unresolved at the base, with support for a *B. mexicana* clade (which includes *B. striatula*, *B. lignicolor*, and *B. yucatana*) (bs 75%), a clade formed by *B. adspersa* plus *B. marmorea* (bs 75%), and a clade consisting of *B. morio* plus *B. engelhardti* (bs 76%). Basal relationships within the *B. mexicana* group are not resolved. Samples of *B. mexicana* are distributed in three well supported clades concordant with those found using cyt b sequences. The first one ("Clade 1") includes three samples of *B. mexicana* from Belize (1), two samples from Honduras (2, 3), and one from southern Chiapas (4) (decay 2; bs 69%). The second ("Clade 2") includes two samples of *B.mexicana* from Los Tuxtlas region of east-central Veracruz (9), and two (10, 11) from Chiapas (decay = 1; bs 71%). The third clade ("Clade 3") includes five samples of *B. mexicana* from Honduras (5, 7, 8), two from southeastern Guatemala (6),
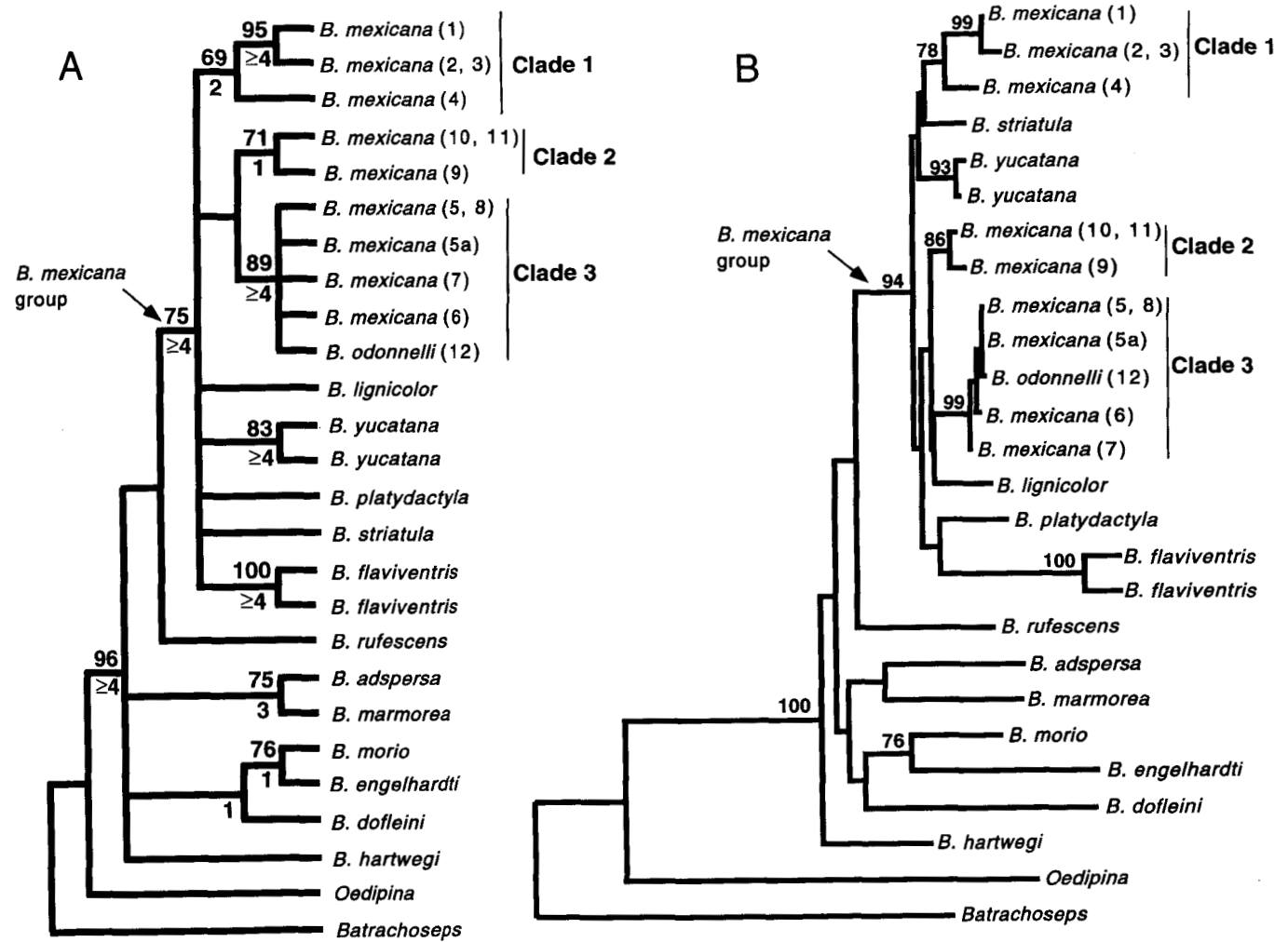
Figure 3. A. Strict consensus of the 74 equally most parsimonious trees for sequences of the 16S rDNA gene (L = 452; 105 parsimony informative characters; CI = 0.611; RI = 0.568). Decay indices and bootstrap values above 60% are shown (100 replicates). B. Neighbor-joining analysis of sequences of the 16S rDNA gene (gapped positions excluded). Bootstrap values above 70% are shown (1000 replicates). Population numbers correspond to Fig. 1 and Table 1.

and the two samples of *B. odonnelli* (12) (decay ≥ 4; bs 89%). A weakly supported clade includes *B. platydactyla* and *B. flaviventris* (bs 52%). A similar analysis excluding positions affected by gaps produced 52 equally most parsimonious trees (L = 388; 93 parsimony informative characters; CI = 0.616; RI = 0.564). The strict consensus of these 52 trees shows a monophyletic *B. mexicana* clade, including the same samples as in the previous analysis. *Bolitoglossa* remains monophyletic, but relationships among the basal taxa are more resolved, with *B. hartwegi* basal. The monophyly of *Bolitoglossa* is strongly supported (bs 100%) in the NJ analysis (Fig. 3B), and there is strong support (bs 94%) for a *B. mexicana* group. Within the *B. mexicana* clade there is strong support (bs 99%) for Clade 3, including samples of *B. mexicana* and *B. odonnelli* from Honduras, and eastern Guatemala (5, 6, 7, 8, and 12). There is also strong support (bs 99%) for Clade 1, including *B. mexicana* samples from Belize, Honduras, and southern Chiapas (1, 2, and 3), and weaker support (bs 78%) for adding a sample from southern Chiapas (4) to this clade. A well supported Clade 2 (bs 86%) includes samples of *B. mexicana* from western Chiapas and Veracruz (9, 10, 11). Samples assigned to *B. mexicana* never form a monophyletic clade.

A maximum likelihood analysis (not shown) produced two trees (–Ln 2775.1) each supporting a monophyletic *Bolitoglossa* clade and a monophyletic *B. mexicana* group. The samples of *B. mexicana* do not form a monophyletic group, but are clustered in three clades, as outlined above. In the parsimony analysis of the combined data set our sampling is limited to 17 individuals. The level of resolution and bs values of the trees we obtained using the combined data set are generally higher than for single gene analyses, and the topologies obtained using both MP and ML are nearly identical. Therefore, we favor the phylogenetic hypothesis derived from the combined analyses. An heuristic search (stepwise random addition of taxa, with 10 replicates) generated two equally most parsimonious trees (L = 1346; 329 parsimony informative characters; CI = 0.536; RI = 0.447) which differ only in that either *B. yucatana* or *B. striatula* is the most internal sister taxon to Clade 1 of *B. mexicana* (Fig. 4A,B). Samples of *Bolitoglossa* form a strongly supported monophyletic group (decay ≥ 14; bs 100%). Relationships within *Bolitoglossa* are poorly resolved, with strong support only for the recognition of the *B. mexicana* group (decay 14; bs 98%). The *B. mexicana* clade is well structured with a basal clade formed by *B. platydactyla* and *B. flaviventris* (decay 5; bs 71%), and a clade including the samples of *B. mexicana*, *B. striatula*, and *B. yucatana* (decay 7, bs 94%). The samples corresponding to *B. mexicana* are clustered in three clades. Clade 1 includes samples from Belize, Honduras, and southern Chiapas (1, 2, and 4) (decay 4; bs 93%); it is the sister taxon to *B. yucatana* and *B. striatula*. Clade 2 includes samples from western Chiapas and Veracruz (9 and 10) (decay 5; bs 92%). Clade 3 is represented by a single sequence from Honduras (7) that differs only slightly from *B. odonnelli* in the 16S data set (K2p = 0.006). An analysis with tv overweighted 10 times ts produced a single most parsimonious tree (L = 4954; CI = 0.598; RI = 0.530) (not shown) which differs from the equally weighted analysis only with respect to the position of the *B. yucatana* sample, which became basal to a group formed by *B. mexicana* (not monophyletic) and *B. striatula*. The positions of *B. hartwegi* and the other clades within *Bolitoglossa* also change, but both *Bolitoglossa* and *B. mexicana* group are consistently monophyletic.

ML analysis produced four trees (–Ln 7139.7, not shown) differing from the MP trees only in the relative positions of the basal samples of *Bolitoglossa*. The monophyly of *Bolitoglossa* and the *B. mexicana* clade is supported. NJ analysis also provides strong support for the monophyly of *Bolitoglossa* (bs 100%), the *B. mexicana* group (bs 100%),
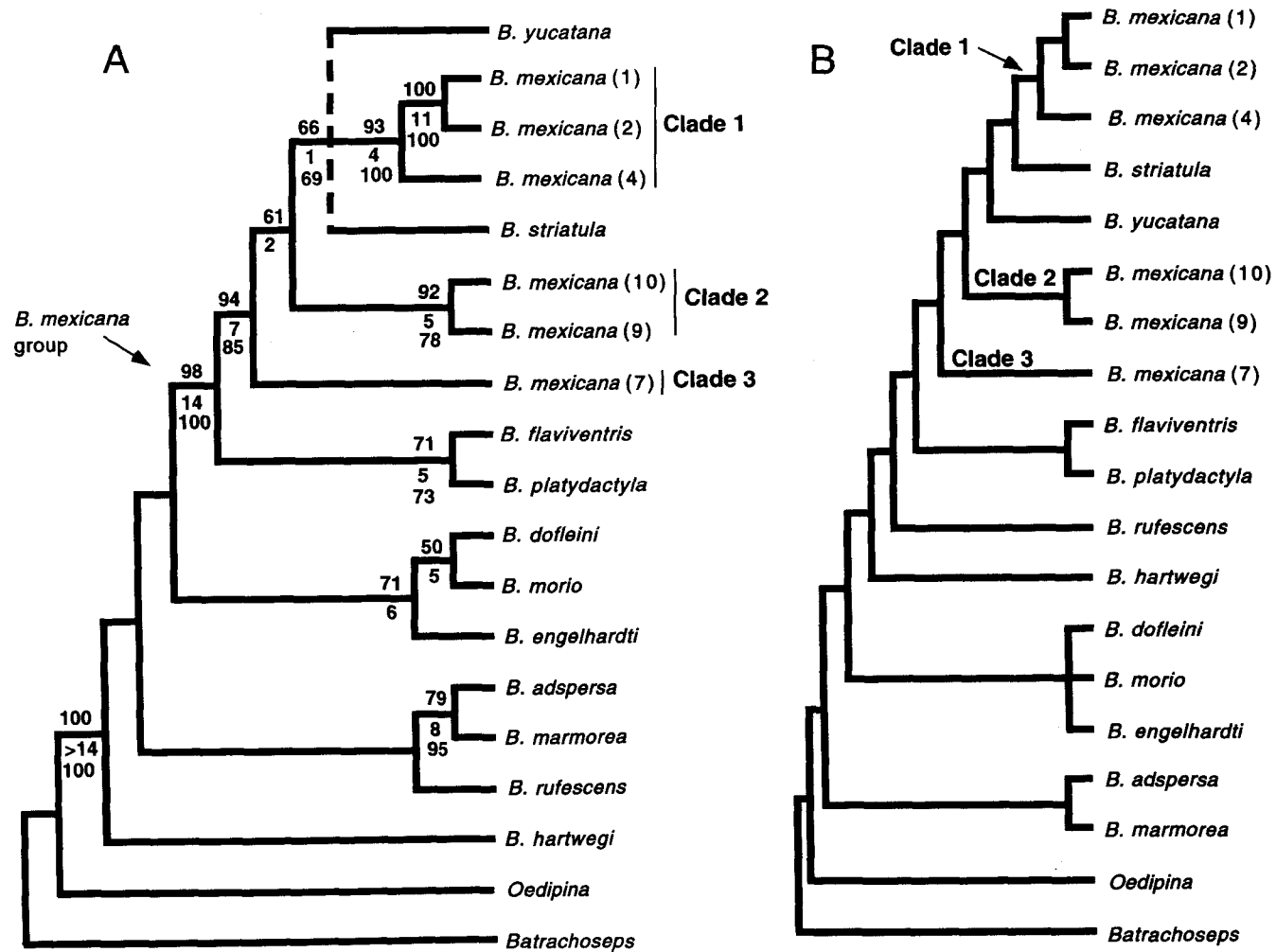
Figure 4. A. The most parsimonious trees (dashed lines indicate alternatives; see text) for the combined (cyt b—16S rDNA) data set (L = 1346; 329 parsimony informative characters; CI = 0.536; RI = 0.447). Bootstrap values above 50% are shown above branches (100 replicates). Decay index values are indicated for the branches with bootstrap values above 50%. Bootstrap values above 60% from the NJ analysis are shown below branches (1000 replicates). Population numbers correspond to Fig. 1 and Table 1. B. Strict consensus of the four maximum likelihood trees (−Ln 7139.7) for the mtDNA combined data set.

and for Clade 1 of *B. mexicana* (bs 100%). Clade 2 is less supported (bs 78%) and appears as the sister taxon of the sample representative of Clade 3 (bs 57%).

4. DISCUSSION

As a first step towards discerning the phylogenetic structure within the genus *Bolitoglossa* we analyzed partial sequences of the mtDNA genes cyt b and 16S rDNA of samples from 13 species representing both *alpha* and *beta* sections of *Bolitoglossa* (Wake and Lynch, 1976), and corresponding to 9 species groups. Basal phylogenetic structure within *Bolitoglossa* is not well resolved. Species included in the *alpha* section of Wake and Lynch (1976) (*B. mexicana* group, *B. lignicolor*, *B. striatula*, *B. adspersa*, and *B. marmorea*) do not form a monophyletic group, nor do the species previously included in the *beta* section (*B. yucatana*, *B. dofleini*, *B. engelhardti*, *B. morio*, *B. rufescens*, and *B. hartwegi*). However, if we exclude *B. yucatana* (see below), there is no strong evidence to reject the sections as clades, since the basal structure of our phylogenetic hypothesis is basically unresolved, and the better supported branches leading to a *B. marmorea—B. adspersa* clade and a *B. morio—B. engelhardti* clade are in agreement with the two sections hypothesis. Sequence divergence between *B. dofleini* and the other taxa is very high, rendering possible the existence of problems of long branch attraction-repulsion within our data set (Felsenstein, 1978; Siddall, 1998). More extensive sampling within both the *alpha* and *beta* sections is thus required to clarify the phylogenetic structure within *Bolitoglossa*. We will deal with this issue in a separate paper.

All taxa previously included within the *B. mexicana* species group (Wake and Elias, 1983) are part of a single clade (decay 14; bs 98% for the combined data set MP analysis). However, this clade includes both *B. yucatana* and *B. striatula*. While *B. striatula* was included in the *alpha* section (in a *B. striatula* group) by Wake and Lynch (1976), *B. yucatana* was included in *beta* (as a member of a *B. dofleini* group); neither has been associated with the *B. mexicana* group in the recent literature. When only the 16S data are considered, *B. lignicolor* is added to the complex (we lack cyt b data for this species). This species was placed in the *alpha* section of *Bolitoglossa* by Wake and Lynch (1976), but in a separate *B. lignicolor* group. In our analyses *B. dofleini* is either a separate lineage in an unresolved basal polytomy, or a member of a clade also including *B. morio* and *B. engelhardti* (both in the *beta* section of *Bolitoglossa*); it is never associated with *B. yucatana*. All three species, *B. striatula*, *B. yucatana*, and *B. lignicolor*, should be considered members of the *B. mexicana* clade. The *B. dofleini* group is now represented exclusively by *B. dofleini*. *Bolitoglossa schizodactyla* (Wake and Brame, 1966), the only other member of the *B. lignicolor* species group, and *Bolitoglossa colonnea* (Dunn, 1924), the only other member of the *B. striatula* species group, were not available for study.

What has been called *B. mexicana* is never monophyletic in our analyses, which instead identify three distinct clades that include *B. mexicana* samples: Clade 1, including samples from southern Chiapas (4), Honduras (2, 3), and Belize (1); Clade 2, including samples from central Veracruz (9) and western Chiapas (10, 11); and Clade 3, including samples from eastern Guatemala (6) and Honduras (5, 7). Samples of *B. yucatana* and *B. striatula* are commonly associated with Clade 1. The samples of *B. mexicana* included in Clade 3 do not form a monophyletic group since topotypic *B. odonnelli* is deeply nested within it. Sequences of *B. mexicana* from Clade 3 are

almost identical to 16S (we lack cyt b data for typical *B. odonnelli*) sequences of *B. odonnelli* (K2p distance ranging from 0.002 to 0.006; while K2p distances between *B. mexicana* samples from Clade 1 and Clade 3 range from 0.034 to 0.047) (Table 4).

When we force *B. mexicana* to be monophyletic with respect to *B. striatula* and *B. yucatana* (Fig. 5), the most parsimonious tree (using the combined data set) in which *B. mexicana* is monophyletic is 8 steps longer (L = 1354) than the two most parsimonious trees overall (Figs. 4A, 4B). However a Templeton test (Templeton, 1983)

Figure 5. The most parsimonious tree in which *B. mexicana* is monophyletic with respect to *B. striatula* and *B. yucatana* for the combined (cyt b—16S rDNA) data set. Population numbers correspond to Fig. 1 and Table 1.

comparing those trees does not provide evidence to suggest that they differ significantly ($n = 26$, $P = 0.1167$; $n = 22$, $P = 0.0881$), and therefore the monophyly of *B. mexicana* with respect to *B. yucatana* and *B. striatula* cannot be rejected. The most parsimonious trees including a monophyletic *B. mexicana* (using a reduced 16S data set, consisting of *B. odonnelli*, all of our *B. mexicana* sequences, and *B. platydactyla* as the outgroup) are 5 steps longer than the most parsimonious trees in which *B. odonnelli* is nested within *B. mexicana*. In this case a Templeton test rejects the monophyly of *B. mexicana* ($n = 5$, $P = 0.0253$). We were unable to obtain sequences of cyt b for typical *B. odonnelli*, but given the high congruence between the 16S rDNA and the cyt b phylogenetic estimates we consider that the 16S data provide sufficient evidence to reject the monophyly of *B. mexicana*.

We lack specimens from the Departamento de El Petén, Guatemala, the non-specific type locality of *B. mexicana* (Smith, 1966). Accordingly we tentatively accept the samples that are geographically closest to the type locality (from southern Chiapas and Belize) as representing *B. mexicana*. These samples belong to Clade 1. Clade 2 includes populations of large, strikingly colored salamanders that differ from others that have been assigned to *B. mexicana* in having relatively broad heads and long limbs. We present a description of Clade 2 as a new species elsewhere, together with a more detailed morphological analyses. The name *B. odonnelli* is available for the third clade. However, the morphological traits which distinguish *B. odonnelli* from *B. mexicana* are not expressed in specimens of this clade from several areas where Clade 3 occurs. Most specimens of Clade 3 are morphologically indistinguishable from individuals of Clade 1. This problem is complicated by the fact that Clades 1 and 3 are not geographically segregated; both *B. odonnelli* and *B. mexicana* occur in Honduras. Because we cannot separate them by coloration or morphology we recommend that populations from Honduras be referred to the *B. mexicana* complex.

Monophyly of the mtDNA lineages does not guarantee monophyly of the species involved, and the use of mtDNA sequences to define species boundaries and make taxonomic decisions is not appropriate when hybridization or speciation processes are taking place (Moritz et al., 1992; Patton and Smith, 1994). Mismatches between mitochondrial gene phylogenies and species phylogenies due to stochastic lineage sorting and recent speciation events restrict the usefulness of mtDNA sequences in defining taxonomic units based on phylogenetic grounds (Avise, 1994; Harrison, 1991; Powell, 1991).

Strict sympatry between members of the *B. mexicana* group is not known, but may exist (1) between *B. mexicana* and *B. platydactyla* near Catemaco and elsewhere in the Tuxtlas area of Veracruz, México, (2) between *B. jacksoni*, *B. mulleri*, and *B. mexicana* near the Chiapas-Huehuetenango, Guatemalan border, (3) between *B. mulleri* and *B. odonnelli* in Alta Verapaz, Guatemala, (4) in the Guatemalan lowlands of Depto. San Marcos between *B. flaviventris* and *B. salvinii* (one possible hybrid is known—MVZ collection), (5) between *B. striatula* and *B. lignicolor* in Costa Rica, and (6) between *B. mexicana* and *B. odonnelli* in Honduras.

The possibility of hybridization among the three clades of the *B. mexicana* complex cannot be disregarded considering our limited sampling. We believe that the distinctive morphology of the representatives of Clade 2 justifies its recognition as an independent species. The problem posed by the *B. mexicana—B. odonelli* clades is still far from resolution and the addition of nuclear markers (protein or DNA) will be necessary to clarify the taxonomic and morphological puzzle that they present. It is conceivable that these two clades are conspecific and that we have a reticulating gene

tree. However, given the conservative nature of evolution in the 16S gene (Orti and Meyer, 1997; Orti et al., 1996), we think it unlikely.

The deepest divergence found within the *B. mexicana* group is between the *B. flaviventris—B. platydactyla* clade and the remaining taxa (about 17% sequence divergence for cyt b; Tables 2, 3). Using the Tan and Wake (1995) cyt b calibration for North-American salamandrids, the evolutionary radiation of the group in the lowlands of Mesoamerica could have been the result of an old process which started about 20 My ago. Divergences among the remaining members of the clade are relatively small, suggesting a relatively rapid further radiation.

5. SUMMARY

Sequences of the mitochondrial genes cytochrome b and 16S RNA were used separately and combined to generate phylogenetic hypotheses for the *Bolitoglossa mexicana* group of Mesoamerican plethodontid salamanders. Samples representing the major species groups of *Bolitoglossa* were used to determine the limits of the *B. mexicana* group, which was found to include the species typically associated with the group (*B. mexicana, B. platydactyla, B. flaviventris, B. odonnelli*) as well as members of some species that have been suggested to be members of the group by previous workers (*B. lignicolor, B. striatula, B. yucatana*). The species *B. mexicana* is not monophyletic in our analyses, and is considered to be a composite of three clades, two of which cannot be separated on morphological grounds (*B. mexicana, B. odonnelli*).

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