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Molecular diversification of salamanders of the tropical American genus *Bolitoglossa* (Caudata: Plethodontidae) and its evolutionary and biogeographical implications

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The largest genus of salamanders, *Bolitoglossa* (Plethodontidae), is widespread in tropical America, where it occurs in diverse habitats and elevations, from high elevation grasslands to lowland rain forest. It has the most extensive geographical range of any salamander genus. While most species occur in Middle America, it ranges throughout most of tropical South America as well. Phylogenetic analysis of 1196 bp of two mitochondrial genes (cytochrome *b*, 16S RNA) from 55 species offers strong support for the monophyly of the genus and sorts the species into a number of clades. Taking into account morphology, distribution, general ecology, and prior systematic and taxonomic studies, we recognize seven subgenera, four of them new: *Bolitoglossa* Duméril, Bibron et Duméril, 1854, *Eladinea* Miranda Ribeiro, 1937, *Magnadigita* Taylor, 1944, ***Mayamandra***, ***Nanotriton***, ***Oaxakia*** and ***Pachymandra***. All South American and some lower Middle American species are included in a single well -supported clade, *Eladinea*. At the species level our analyses uncover the existence of large genetic diversity within morphologically homogeneous taxa. We propose the new combination: ***B. (Eladinea) paraensis*** (Unterstein, 1930) **stat. nov.**, for Brazilian salamanders previously included under *B. altamazonica*. We evaluate evidence for the multiple colonization of the tropical lowlands by morphologically derived species groups. South America was invaded by members of one clade, *Eladinea*, which we infer to have dispersed to South America prior to closure of the Panamanian Portal. Despite the relatively long history of salamanders in South America, that continent now accounts for a relatively small proportion of the lineages and species of neotropical salamanders. *Biological Journal of the Linnean Society,* 2004, **81**, 325–346.

ADDITIONAL KEYWORDS: biogeography – Middle America – mtDNA – new taxa – phylogeny – South America – systematics.

INTRODUCTION

Bolitoglossa is the largest and most widely distributed genus in the Order Caudata and includes more than 80 species, or about 16% of the recognized salamander species (Wake & Lynch, 1976; García-París, Parra-Olea & Wake, 2000). The enormous geographical range of *Bolitoglossa*, greater than that of any other genus of salamanders, extends from the Atlantic region of south-western North America (San Luis Potosí, México) to the Amazon basin (Brazil) and the mountains of central Bolivia in South America. Species occur in habitats ranging from alpine grasslands at the tops of volcanoes (over 4000 m) to sea level lowland forests, and from cool, humid cloud forests to Mediterranean-like mesic formations.

These salamanders have maintained the generalized morphology of a plethodontid salamander, with the exception of the extraordinarily long and fast tongue and the unusual limbs, characterized by tarsal reductions and extensive interdigital webbing. Hands and feet have diversified greatly in the genus, with many species having digits that approach complete webbing. Extensive webbing is usually associated with climbing behaviour and semiarboreal to arboreal

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habitats. Species in the genus also display diverse structural changes in characters involving tail autotomy (Wake & Lynch, 1976). A dramatic reduction in size combined with exceptionally large cells (the direct result of very large nuclear volume; Sessions & Kezer, 1991) has imposed morphological constraints resulting in the juvenile-like morphologies of some species (Alberch & Alberch, 1981).

Bolitoglossa differs from the remaining tropical salamanders in having a unique hyobranchial apparatus (which functions importantly in use of the long, fast tongue), in lacking a sublingual fold (a distinctive feature that is found in all other tropical genera and many North American species with freely projectile tongues), and in having hands and feet that are broad with relatively short, stubby digits that are moderately to fully webbed (Wake & Elias, 1983).

While the monophyly of *Bolitoglossa* is supported morphologically (Wake & Elias, 1983), relationships among its members are poorly understood. Wake & Lynch (1976) sorted species into two large and informal sections, termed *alpha* and *beta*, and within each identified several informal groups, based in part on earlier studies. Species composition of these groups has undergone changes from author to author (Taylor, 1941; Stuart, 1943, 1952; Wake & Brame, 1969; Wake & Lynch, 1976; Elias, 1984), based on which morphological characters were emphasized. More recent phylogenetic hypotheses are based on molecular data; such data have been used to analyse relationships of some species and as a basis for discussion of lowland invasions (Larson, 1983a), to examine patterns of species formation (García-París *et al*., 2000), and to support recognition of some species groups (García-París, Parra-Olea & Wake, 2000; Parra-Olea, García-París & Wake, 2002).

The derived morphology and behaviour (all have extensive or complete webbing of the digits, typically associated with climbing) shown by the species of *Bolitoglossa* inhabiting the tropical lowlands is postulated to have been independently achieved in different lineages by different evolutionary processes (Wake & Lynch, 1976; Larson, 1983b). Upland species are more generalized in morphology and ancestors of the lowland species are thought to have been more terrestrial and to have lived at moderate to high elevations in cool environments. Consequently, the colonization of the lowlands was not the result of a single dispersal event (Brame & Wake, 1963; Larson, 1983a). An alternative scenario is that ancestral *Bolitoglossa* were arboreal members of lowland environments that dispersed upward, into highland habitats, in different parts of the enormous geographical range (Jäkel, 2002).

The morphological similarity shown among the species involved in these colonizations is one more exemplar of the extensive homoplasy shown by tropical bolitoglossine salamanders (Wake, 1966, 1991; Parra-Olea & Wake, 2001). For example, large lowland species are thought to have evolved webbing as an adaptation for arboreal existence (production of suction for clinging to leaves, Alberch, 1981), whereas miniaturized species of the *B. rufescens* species group are hypothesized to have evolved webbing nonadaptively, as a by-product of a progenetic developmental pathway (Alberch, 1981). Alternatively, the upland species may have lost webbing secondarily.

The invasion of South America by salamanders with low dispersal capability has been discussed extensively (Dunn, 1926; Darlington, 1957; Brame & Wake, 1963; Wake, 1966; Wake & Lynch, 1976; Hanken & Wake, 1982). A study based on allozyme data (Hanken & Wake, 1982) found that South American species of *Bolitoglossa* are so deeply differentiated genetically that an early origin of the South American salamander fauna was probable, prior to the closure of the Panamanian Portal. Alternatively, lineages that had already differentiated in lower Central America entered South America separately once the portal was closed.

Herein we evaluate the evolutionary and biogeographical consequences of the tropical radiation of *Bolitoglossa*, based on a phylogenetic hypothesis generated from analysis of mitochondrial DNA from a majority of the species and from representatives of all formerly recognized species groups. We focus on two main issues, the multiple colonization of the tropical lowlands by morphologically derived species groups including the problematic case of *B. dofleini*, and the invasion of South America by one or multiple lineages, members of which might have displayed both morphologically derived and ancestral traits. Tests of previous hypotheses are based on the congruence among previous allozyme data and our newly generated mtDNA data sets.

MATERIAL AND METHODS

Sampling design

We obtained partial sequences of 16S (548 bp) and cyt *b* (543–647 bp) genes for 37 specimens of the following 25 taxa: *Bolitoglossa altamazonica, B. biseriata, B. carri, B. celaque, B. colonnea, B. conanti, B. decora, B. diaphora, B. dunni, B. franklini, B. lincolni, B. longissima, B. medemi, B. morio, B. occidentalis, B. palmata, B. paraensis* (see Results)*, B. peruviana, B. porrasorum, B. rufescens, B. schizodactyla, B. synoria, B.* sp. 1 (aff. *B. adspersa*)*, B.* sp. 2 (aff. *B. nigrescens*), and *B.* sp. 3 (aff. *B. celaque*).

Sequences of 16S (548 bp) and a short fragment of cyt *b* (216–354 bp) were obtained for five specimens representing the following four taxa: *B. alvaradoi,*

B. flavimembris, B. rostrata, and *B. sima*. We also obtained additional sequences of 16S (548 bp) for six specimens of five taxa: *B. gracilis, B. pesrubra, B. epimela, B. minutula,* and *B.* sp. 4 (aff. *B. subpalmata*) for which cyt *b* data were published previously (García-París *et al*., 2000; for complete locality and voucher information see Table 1). All of the sequences obtained for this study were added to previously published sequences of 16S and cyt *b* mtDNA genes corresponding to 27 samples from an additional 21 species of *Bolitoglossa* (Jackman, Applebaum & Wake, 1997; García-París & Wake, 2000; García-París, Parra-Olea & Wake, 2000; García-París *et al*., 2003; Parra-Olea *et al*., 2002). The combination of all of these data sets forms the data base for the present paper: 1196 bp for two genes derived from 48 described species plus seven unnamed taxa.

Representatives of three distantly related bolitoglossine genera – *Batrachoseps, Thorius*, and *Pseudoeurycea* – were used as sequential outgroups for all phylogenetic analyses.

Amplification and sequencing

Tissues for this study were obtained through various sources, including recent field collections and donations of several researchers and institutions (see Acknowledgements). A large proportion of the samples was obtained from the frozen tissue collection of the Museum of Vertebrate Zoology, University of California, Berkeley. Such samples are extracts of ground tissues prepared for protein electrophoresis. Many of these samples are extremely valuable because they come from localities where salamanders are no longer present (Parra-Olea, García-París & Wake, 1999).

Whole genomic DNA was extracted from small amounts of frozen or ethanol preserved tissues, or protein extracts using NaCl following a protocol modified from Miller, Dykes & Polesky (1988). Overall, we sequenced 548 base pairs of the large 16S subunit ribosomal mtDNA gene corresponding roughly to positions 2510–3059 in the human mitochondrial genome (Anderson *et al*., 1981) and 216–647 base pairs of cyt *b*, expanding from codon 7 of the *Xenopus* cyt *b* gene (Roe *et al*., 1985). These genes were selected in order to recover maximum phylogenetic information both at the terminal nodes and at the base of the tree. Amplification was done via the polymerase chain reaction (PCR) (Saiki *et al*., 1988), using the primers MVZ15, MVZ16 (Moritz, Schneider & Wake, 1992) and cyt b2 (Kocher *et al*., 1989) for cyt *b*, and the primers 16Sar and 16Sbr (Palumbi *et al*., 1991) for 16S. PCR reactions consisted of 38 cycles with a 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. Obtaining longer sequences is unlikely from ground extracts due to DNA fragmentation.

Double strand templates were cleaned using QIAquick PCR purification kit (QIAgen). We used 5.5 μL of PCR product as the template for cycle sequencing reactions in a 10 μL total volume with the Perkin-Elmer Ready Reaction Kit to incorporate dye-labelled 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).

The short-fragment cyt *b* sequences were obtained using dideoxy chain termination sequencing reactions (Sanger, Nicklen & Coulson, 1977), performed with U.S. Biochemicals Sequenase version 2.0 kit and S^{35}-labelled dATP. All extractions and double- and single-strand PCR reactions included negative controls to check for a possible contamination of reagents with DNA.

Sequence alignment and analyses

All sequences were compiled using Sequence Navigator ver.1.0.1 (Applied Biosystems). 16S sequences were aligned using Clustal X (Aladdin Systems, Heidelberg, Germany) with default gap costs and then refined manually by comparing them to published secondary structure models for 16S (Ortí & Meyer, 1997).

Pairwise comparisons of observed proportional sequence divergence (p-distance) and corrected sequence divergence (Kimura 2-parameter; Kimura, 1980), and numbers of transitions and transversions were obtained using PAUP*4.0b10 (Swofford, 2002). Corrected sequence divergences were estimated using the Kimura 2-parameter distance (K2p) in order to correct for multiple hits.

To test for the possibility that some types of nucleotide substitutions have become saturated, we plotted p-distance (y) vs. corrected (K2p) estimates of proportional sequence divergence (x) for first, second, and third codon positions, and for transitions and transversions separately (Figs 1, 2).

Phylogenetic analyses were performed using the combined data set, which included 70 samples of *Bolitoglossa* and three outgroups for two genes: 16S and cyt *b* (Table 1). Phylogenetic inference was based primarily on maximum parsimony analyses (MP: Swofford, 2002). MP phylogenies were estimated using the heuristic search algorithm for each tree-building methodology. We used 20 repeated randomized input order of taxa for all MP analyses to minimize the effect of entry sequence on the topology of the resulting cladograms. MP analyses were conducted with

Table 1. Localities, voucher information, and GenBank accession numbers for the sequences and specimens used in this study. Number in brackets correspond to sequences used in previous studies: (1) Jackman *et al*. (1997) (2) García-París & Wake (2000) (3) García-París *et al*. (2000) (4) García-París, Parra-Olea & Wake (2000) (5) Parra-Olea *et al*. (2002) (6) García-París *et al*. (2003). Abbreviations: DBW: D. B. Wake field number; GP: G. Parra field number; IBH: Instituto de Biología, Universidad Nacional Autónoma de México herpetology collection (México); INPA: Instituto Nacional de Pesquisas da Amazônia (Brazil); KU: Natural History Museum Univ. Kansas; MVZ: Museum of Vertebrate Zoology collection (USA); S: uncatalogued specimens in MVZ; SMF: Senckenberg Museum collection (Germany); UCR: Universidad de Costa Rica collection (Costa Rica); USNM: United States National Museum collection (USA)

Species	Locality	Museum no.	Cyt *b*	16S
B. (*B.*) *flaviventris*	México: Chiapas	MVZ 194288	AF212983 (4)	AF218489 (4)
B. (*B.*) *mexicana*	Belize: Toledo: Blue Creek	MVZ 191635	AF212099 (3)	AF177588 (4)
B. (*B.*) *mexicana*	Honduras: Atlántida	USNM 343451	AF212975 (4)	AF218468 (4)
B. (*B.*) *mexicana*	México: Chiapas	(photo voucher Bo71)	AF212976 (4)	AF218470 (4)
B. (*B.*) *mombachoensis*	Nicaragua: Granada	SMF 78718	AY133485 (6)	AY133488 (6)
B. (*B.*) *mombachoensis*	Nicaragua: Granada	SMF 78725	AY133486 (6)	AY133489 (6)
B. (*B.*) *odonnelli*	Honduras: Olancho	MVZ 229068	AF212977 (4)	AF218476 (4)
B. (*B.*) *platydactyla*	México: Veracruz	GP 108	AF212981 (4)	AF218487 (4)
B. (*B.*) *platydactyla*	México: Veracruz	GP 587	AY133484 (6)	AY133487 (6)
B. (*B.*) *alberchi*	México: Chiapas	MVZ 194293	AF212979 (4)	AF218480 (4)
B. (*B.*) *alberchi*	México: Veracruz	MVZ 163959	AF212978 (4)	AF218478 (4)
B. (*B.*) *striatula*	Costa Rica: Cartago	MVZ 181280	AF212982 (4)	AF218488 (4)
B. (*B.*) *yucatana*	México: Quintana Roo	MVZ 197507	AF212980 (4)	AF218485 (4)
B. (*N.*) *occidentalis*	México: Chiapas: Berriozabal	MVZ 194254	AY526158	AY526115
B. (*N.*) *rufescens*	Belize: Toledo: Blue Creek National Park	MVZ 194333	AY526159	AY526116
B. (*M.*) *hartwegi*	México: Chiapas	MVZ (DBW945)	AF212985 (4)	AF218494 (4)
B. (*E.*) *adspersa*	Colombia: Cundinamarca	MVZ 158485	AF212984 (4)	AF218492 (4)
B. (*E.*) *altamazonica*	Perú: Loreto: 1.5 km N Teniente López	KU 222111	AY526160	AY526117
B. (*E.*) *biseriata*	Panamá: Nusagandi: Kuna Yala	S13236	AY526161	AY526118
B. (*E.*) *cerroensis*	Costa Rica: San José: Cuericí, 5 km E Villa Mills	DBW5123	AF199195 (2)	AF199233 (2)
B. (*E.*) *colonnea*	Panamá: Chiriquí: Reserva Forestal Fortuna	No voucher	AY526162	AY526119
B. (*E.*) *epimela*	Costa Rica: Cartago: Turrialba	MVZ 181260	AF212097 (3)	AY526120
B. (*E.*) *gracilis*	Costa Rica: Cartago: Reserva Tapantí	MVZ 229170	AF212067 (3)	AY526121
B. (*E.*) *gracilis*	Costa Rica: Cartago: Reserva Tapantí	MVZ 229171	AF212068 (3)	AY526122
B. (*E.*) *marmorea*	Panamá: Chiriquí	MVZ 210286	U89631 (1)	AF218493 (4)
B. (*E.*) *medemi*	Panamá: Nusagandi: Kuna Yala	S13237	AY526163	AY526123
B. (*E.*) *minutula*	Costa Rica: Puntarenas: Las Tablas, Cerro Pando	MVZ 225870	AF212098 (3)	AY526124
B. (*E.*) *palmata*	Ecuador: Napo: Cordillera de Guacamayos a 31 km de Baeza	KU 217422	AY526164	AY526125
B. (*E.*) *palmata*	Ecuador: Napo: Cordillera de Guacamayos a 31 km de Baeza	KU 217423	AY526165	AY526126
B. (*E.*) *paraensis*	Brazil: Amazonas: Rio Juruá	INPA 3098	AY526166	AY526127
B. (*E.*) *paraensis*	Brazil: Amazonas: Rio Ituxi at the Madeireira Scheffer	LSUMZ H-3086	AY526167	AY526128
B. (*E.*) *paraensis*	Brazil: Acre: 5 km N Porto Walter	LSUMZ H-13735	AY526168	AY526129
B. (*E.*) *peruviana*	Ecuador: Sucumbios: Estación Científica University Católica, Cuyabeno	LSUMZ H-12838	AY526169	AY526130
B. (*E.*) *peruviana*	Ecuador: Napo: Jatún Sacha	KU 217421	AY526170	AY526131
B. (*E.*) *pesrubra*	Costa Rica: Cartago: Salsipuedes, 19 km S El Empalme	UCR12068	AF212069 (3)	AY526132
B. (*E.*) *schizodactyla*	Panamá: Coclé: Parque Nacional El Copé	No voucher	AY526171	AY526133
B. (*E.*) *sima*	Colombia: Valle del Cauca	MVZ 163575	AY526172	AY526134

Table 1. *Continued*

Species	Locality	Museum no.	Cyt *b*	16S
B. (*E.*) sp. 1	Colombia: Cundinamarca: El Soche	MVZ 167947	AY526173	AY526135
B. (*E.*) sp. 2	Costa Rica: Puntarenas	MVZ 225871	AY526174	AY526136
B. (*E.*) sp. 4	Costa Rica: Cartago: Macho Gaff	UCR 12066	AF212088 (3)	AY526137
B. (*E.*) *subpalmata*	Costa Rica: Puntarenas: Monteverde Cloud Forest Preserve	MVZ 229172	AF212094 (3)	AF416697 (5)
B. (*M.*) *carri*	Honduras: Cerro Cantagallo	USNM 523276	AY526175	AY526138
B. (*M.*) *carri*	Honduras: Cerro Cantagallo	USNM 523277	AY526176	AY526139
B. (*M.*) *celaque*	Honduras: Lempira	SMF 78087	AY526177	AY526140
B. (*M.*) *celaque*	Honduras: Lempira	SMF 78088	AY526178	AY526141
B. (*M.*) *conanti*	Honduras: Cortés: El Cusuco	MVZ 225843	AY526179	AY526142
B. (*M.*) *decora*	Honduras: Olancho: Monte Escondido	USNM 497533	AY526180	AY526143
B. (*M.*) *diaphora*	Honduras: Cortés: El Cusuco	MVZ 225847	AY526181	AY526144
B. (*M.*) *dunni*	Honduras: Cortés: San Pedro Sula	USNM 523280	AY526182	AY526145
B. (*M.*) *engelhardti*	Guatemala: San Marcos	MVZ 167789	AF212987 (4)	AF218496 (4)
B. (*M.*) *flavimembris*	Guatemala: San Marcos	MVZ 143698	AY526183	AY526146
B. (*M.*) *franklini*	Mexico: Chiapas: Volcán Tacaná	MVZ 185991	AY526184	AY526147
B. (*M.*) *lincolni*	Guatemala: San Marcos	MVZ 143564	AY526185	AY526148
B. (*M.*) *longissima*	Honduras: Olancho: Pico La Picucha	USNM 523285	AY526186	AY526149
B. (*M.*) *morio*	Guatemala: San Marcos	MVZ 143677	AF212986 (4)	AF218495 (4)
B. (*M.*) *morio*	Guatemala: San Marcos	MVZ 232970	AY526187	AY526150
B. (*M.*) *porrasorum*	Honduras: Atlántida: Cerro Búfalo	MVZ 225852	AY526188	AY526151
B. (*M.*) *rostrata*	Guatemala: Huehuetenango	MVZ 163683	AY526189	AY526152
B. (*M.*) *rostrata*	Guatemala: Huehuetenango	MVZ 163930	AY526190	AY526153
B. (*M.*) sp. 3	El Salvador: Santa Ana: Metapán	MVZ 233028	AY526191	AY526154
B. (*M.*) sp. 3	El Salvador: Santa Ana: Metapán	MVZ 200535	AY526192	AY526155
B. (*M.*) *synoria*	Honduras: Ocotepeque: Cerro El Pital	SMF 78084	AY526193	AY526156
B. (*O.*) *hermosa*	México: Guerrero: 11.3 mi NE Atoyac	MVZ 163690	AF416678 (5)	AF416686 (5)
B. (*O.*) *macrinii*	México: Oaxaca: San Gabriel Mixtepec	GP 384	AF416680 (5)	AF416689 (5)
B. (*O.*) *oaxacensis*	México: Oaxaca: 40 km N San Gabriel Mixtepec	IBH 13374	AF416681 (5)	AF416690 (5)
B. (*O.*) *riletti*	México: Oaxaca: 20.9 km NE Putla	MVZ 194328	AF416682 (5)	AF416696 (5)
B. (*O.*) *zapoteca*	México: Oaxaca: Santa María Ecatepec	IBH 13375	AF416683 (5)	AF416698 (5)
B. (*O.*) *zapoteca*	México: Oaxaca: Santa María Ecatepec	IBH 13376	AF416684 (5)	AF416699 (5)
B. (*P.*) *alvaradoi*	Costa Rica: Heredia: El Plástico	MVZ 215735	AY526194	AY526157
B. (*P.*) *dofleini*	Guatemala: Alta Verapaz	MVZ 161607	AF212988 (4)	AF218497 (4)

accelerated character transformation (ACCTRAN) optimization, tree bisection-reconnection (TBR) branch swapping, and zero-length branches collapsed to yield polytomies. We used non-parametric bootstrapping (1000 pseudoreplicates) and decay indices to assess the stability of internal branches in the resulting topologies (Felsenstein, 1985; Felsenstein & Kishino, 1993; Bremer, 1994). Nonparametric bootstrap values (bs) and decay indices (decay) generally are a conservative measure of the probability that a recovered group represents a true clade (Zharkikh & Li, 1992; Hillis & Bull, 1993; Li, 1997). Each base position was treated as an unordered character with four alternative states. Gaps were treated as missing data. Analyses were performed both including all positions and excluding third position transitions.

Modeltest 3.06 (Posada & Crandall, 1998) was used to find the best model of evolution that fit our data for Maximum Likelihood analyses (ML: Felsenstein, 1981, 1993). The GTR model of evolution with gamma parameter and proportion of invariable positions was used for ML analyses (Yang, 1994; Gu, Fu & Li, 1995; Swofford *et al*., 1996). ML analyses with empirical base frequencies were performed using PAUP*. Shimodaira–Hasegawa parametric tests (Shimodaira & Hasegawa, 1999) using bootstrap with full optimization (1000 bs replicates) were used to test for the monophyly of selected taxa (Leaché & Reeder, 2002).

Bayesian phylogenetic analyses were conducted with MrBayes 2.0 (Huelsenbeck & Ronquist, 2001). The GTR model of evolution with gamma parameter and proportion of invariable positions was used also

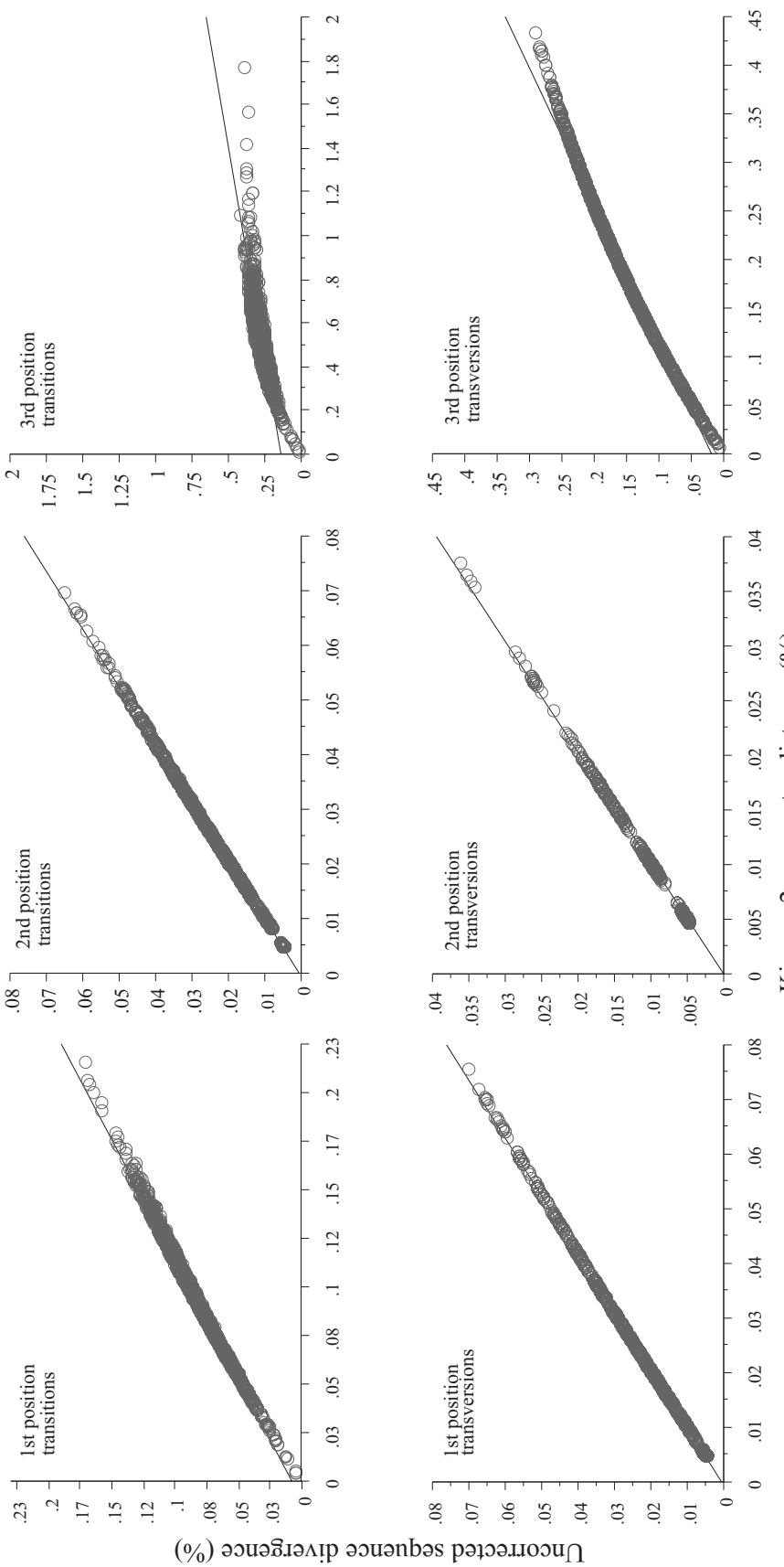


Figure 1. Saturation plots for cyt *b* DNA sequences. Uncorrected sequence divergence vs. Kimura 2-parameter distance plotted by codon position and type of change.

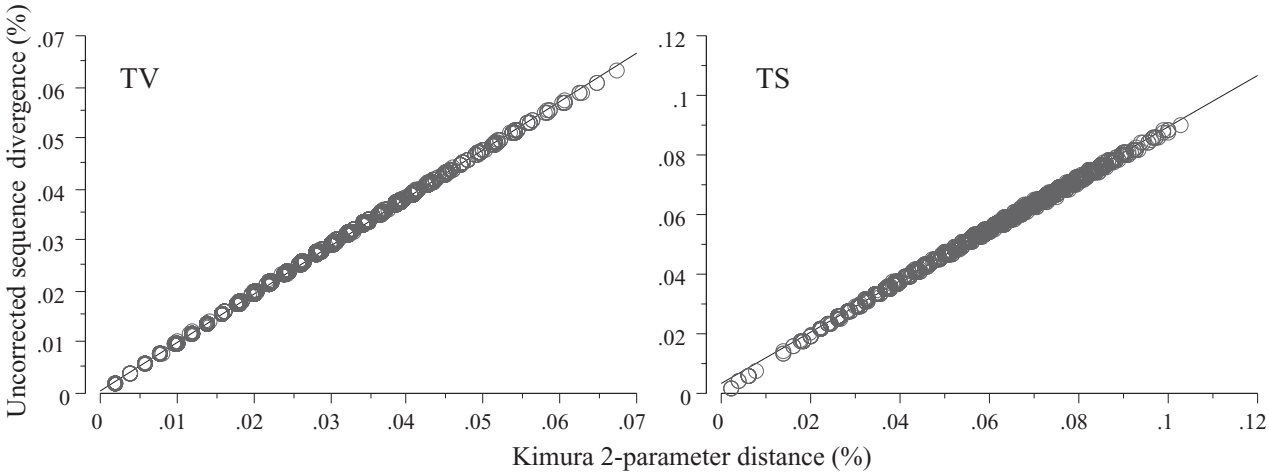


Figure 2. Saturation plots for 16S DNA sequences. Uncorrected sequence divergence vs. Kimura 2-parameter distance plotted by type of change.

for this analysis. Analyses were initiated with random starting trees and run for 500 000 generations. The Markov chains were sampled each 100 generations. Of the resulting 5000 trees, 2000 were discarded as 'burn-in'. All values of posterior probabilities in excess of 95 are considered significant (Leaché & Reeder, 2002).

RESULTS

Saturation plots of uncorrected sequence divergence against corrected sequence divergence for cyt *b* positions are shown in Figure 1. Polynomial regression curves are shown for heuristic purposes. The plots indicate saturation at third position transitions. The saturation plots for 16S sequences show no evidence of saturation of transitions or transversions (Fig. 2).

The g1 statistic indicated that significant phylogenetic signal was present in the data set: cyt *b*: g1 = –0.31; $P < 0.01$; mean ± SD tree length = 4583.69 ± 62.50; 16S: g1 = –0.33; $P < 0.01$; mean ± SD tree length = 1947.65 ± 32.15.

Corrected cyt *b* sequence divergence (K2p) within the ingroup was as high as 32% (*B. subpalmata* compared to *B. striatula*). The smallest divergence between two species was between *B. mombachoensis* to *B. striatula* (2.9%). Base composition was slightly A + T biased (61%). There was an excess of thymine for first codon and second codon positions when the data set was divided according to codon positions. For third codon position adenine was present in high amounts (37%), cytosine and thymine were present in similar amounts (25 and 32%), and guanine was rare (15%). Transitions accounted for 65% of all substitutions, and TC transitions outnumber AG transitions by about 3 : 1. The ratio of transitions to transversion was 4.79.

Corrected 16S sequence divergence (K2p) within the ingroup was as high as 14% (between *B.* sp. 4 and *B. dofleini*). The smallest divergence for two species is 0.5% between *B. mombachoensis* and *B. striatula*. Base composition was also A + T-biased (59%). Transitions account for 60% of all substitutions, and TC transitions outnumbered AG transitions by about 1.6 : 1. The mean ratio of transitions to transversions for all pairwise species comparisons was 2.54.

Phylogenetic relationships

MP analysis of all sequences combined (16S + cyt *b*: 1196 bp) using equal weighting produced three equally parsimonious trees (L = 3791 steps; 475 characters were parsimony informative; CI = 0.255, RI = 0.589). The sequences of all samples of *Bolitoglossa* form a well- supported clade (bs 94, decay 12, Fig. 3). While there is substantial structure within the consensus tree there is little basal resolution. When third position transitions are removed, 191 equally parsimonious trees (L = 1637 steps; 270 characters were parsimony informative; CI = 0.335, RI = 0.641) were recovered. The sequences of all samples of *Bolitoglossa* also form a monophyletic clade (bs 97). The topology obtained (not shown) differs from that displayed in Figure 3 in the resolution of some terminal groupings.

The ML analysis yielded a tree (–ln L = 17125.390) with a topology very similar to that obtained in the MP analyses (Fig. 4). The seven main clades obtained in the latter were also recovered in ML. The length of branches supporting basal relationships among these clades is short.

The results of the Bayesian analyses yielded a consensus tree (50% majority rule) with nodes corre-

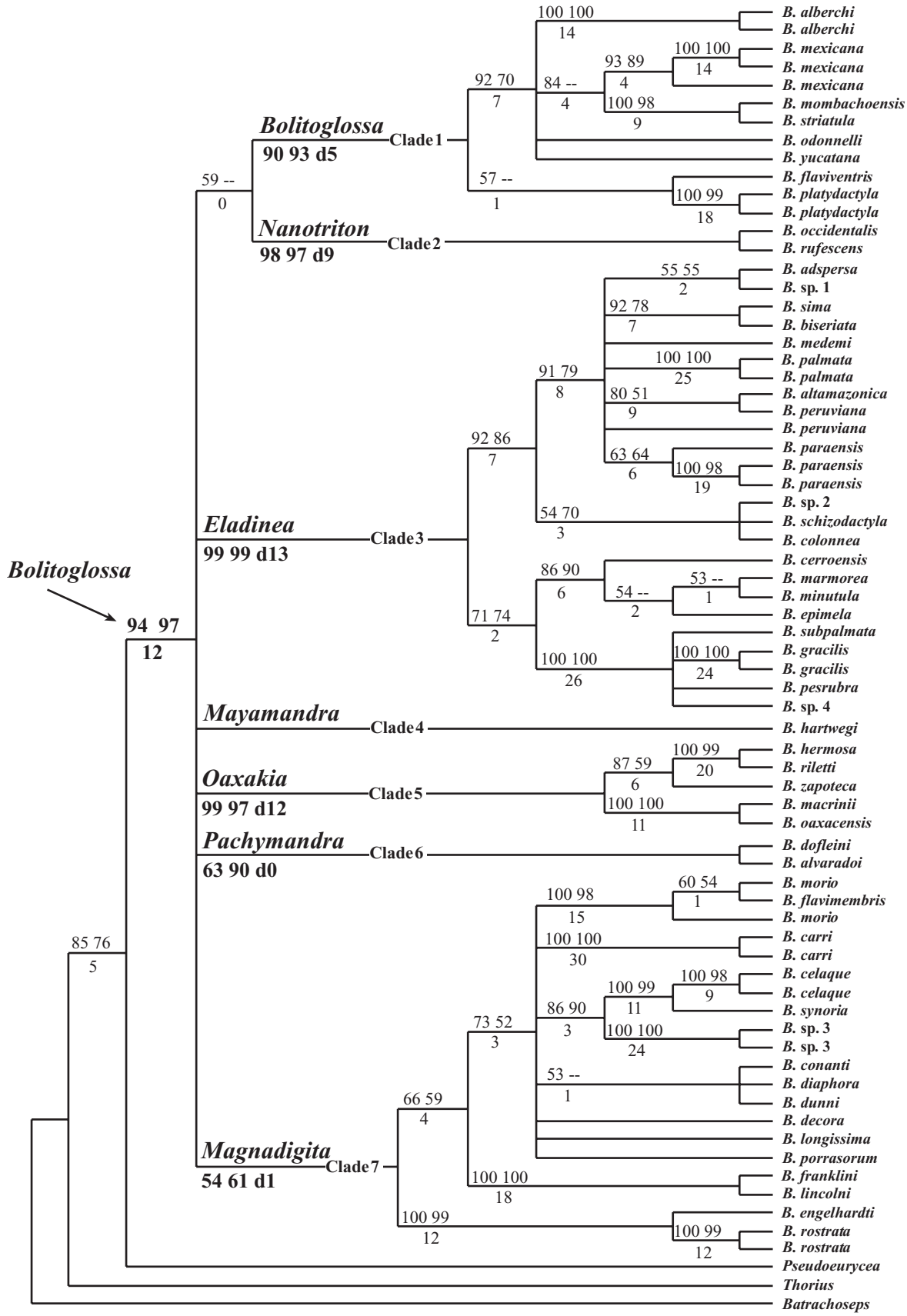

Bolitoglossa
94 97
12
Bolitoglossa
90 93 d5
Clade 1
Nanotriton
98 97 d9
Clade 2
Eladinea
99 99 d13
Clade 3
Mayamandra
Clade 4
Oaxakia
99 97 d12
Clade 5
Pachymandra
63 90 d0
Clade 6
Magnadigita
54 61 d1
Clade 7
B. alberchi
B. alberchi
B. mexicana
B. mexicana
B. mexicana
B. mombachoensis
B. striatula
B. odonnelli
B. yucatana
B. flaviventris
B. platydactyla
B. platydactyla
B. occidentalis
B. rufescens
B. adspersa
B. sp. 1
B. sima
B. biseriata
B. medemi
B. palmata
B. palmata
B. altamazonica
B. peruviana
B. peruviana
B. paraensis
B. paraensis
B. paraensis
B. sp. 2
B. schizodactyla
B. colonnea
B. cerroensis
B. marmorea
B. minutula
B. epimela
B. subpalmata
B. gracilis
B. gracilis
B. pesrubra
B. sp. 4
B. hartwegi
B. hermosa
B. riletti
B. zapoteca
B. macrinii
B. oaxacensis
B. dofleini
B. alvaradoi
B. morio
B. flavimembris
B. morio
B. carri
B. carri
B. celaque
B. celaque
B. synoria
B. sp. 3
B. sp. 3
B. conanti
B. diaphora
B. dunni
B. decora
B. longissima
B. porrasorum
B. franklini
B. lincolni
B. engelhardti
B. rostrata
B. rostrata
Pseudoeurycea
Thorius
Batrachoseps

Figure 3. Strict consensus of the three equally parsimonious trees (L = 3791 steps; 475 characters parsimony informative; CI = 0.255, RI = 0.589) obtained using maximum parsimony analysis of the combined data set (16S + cyt *b*: 1196 bp). Numbers above branches are non-parametric bootstrap values. The first number corresponds to the analysis with all positions included, and the second to the analysis with third position transitions excluded (1000 pseudoreplicates, only values greater than 50% are shown). Numbers below branches or those preceded by a 'd' correspond to decay index values.

sponding to the seven main clades highly supported (Bayesian posterior clade probabilities 99–100). Bayesian posterior clade probabilities (pp) are shown in Figure 4.

For analytical purposes we focus on seven groups of taxa (Figs 3, 4), which are consistently found in all analyses and which correspond well with previously recognized groups. All of the multispecies groups show high posterior clade probabilities in the Bayesian analysis, and only one is not well supported in the parsimony analysis.

The first group (Clade 1) includes eight taxa (12 samples) (bs 90, decay 5, pp 100) and corresponds to the *mexicana* group (García-París *et al*., 2003). This widely distributed clade ranges from north-eastern México to western Panamá, mainly in the lowlands, but reaching elevations as high as *c*.1500 m.

A second cluster (Clade 2) corresponds to the *rufescens* group of Wake & Lynch (1976) and includes two taxa (two samples) (bs 98, decay 9, pp 100). This clade is widely distributed in the lowlands (below *c*.1500 m but typically much lower) throughout southern México, Belize, Honduras, and Guatemala.

A third group (Clade 3) includes 19 taxa (25 samples) (bs 99, decay 13, pp 100); it is ecologically diverse and ranges from sea level to high elevations (over 4000 m). It ranges widely from Costa Rica to the southern limits of the distribution of the entire Caudata, including all of the South American taxa. This cluster corresponds to the informal group identified as *Bolitoglossa* alpha by Wake & Lynch (1976), minus the *mexicana* group (Clade 1). This is the largest clade and it comprises two major subclades, a mainly South American clade of 11 taxa (16 samples) (bs 92, decay 7, pp 100), and a group of eight taxa (nine samples) (bs 71, decay 2, pp 100) that occurs mainly in Costa Rica and western Panamá. Included in this latter cluster is the relatively well-known *subpalmata* group (García-París *et al*., 2000).

The only member of the morphologically distinctive *veracrucis* group (Elias, 1984) in our sample is *B. hartwegi* (Clade 4, one sample). This group occurs in a restricted, mainly montane area in Chiapas, México, and neighbouring Guatemala.

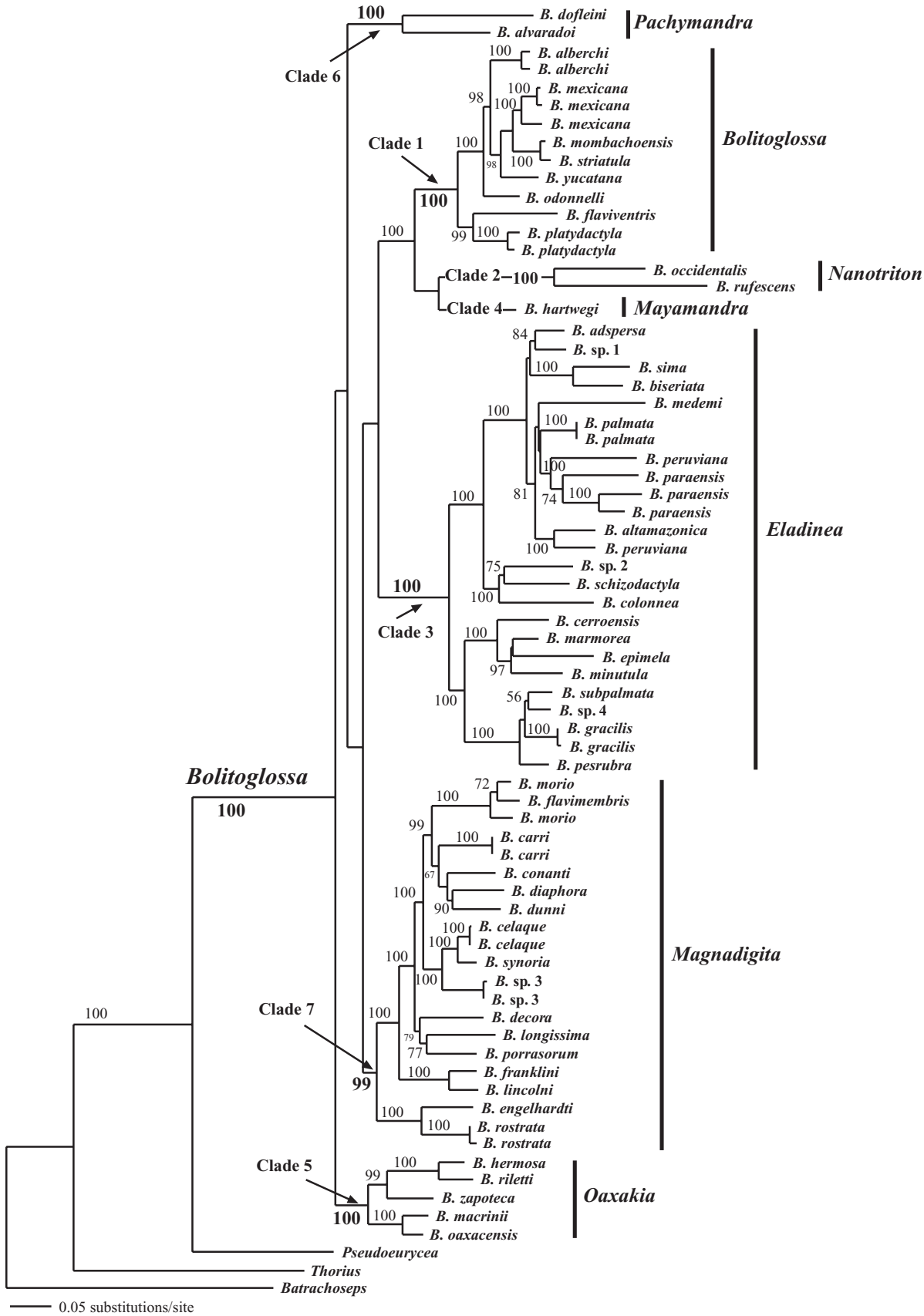
A fifth group (Clade 5) includes five taxa (five samples) (bs 99, decay 12, pp 100) and corresponds to the *macrinii* group of Wake & Lynch (1976). Species (all represented in our sample) are mainly low montane forms, restricted to México north and west of the Isthmus of Tehuantepec in areas of Pacific drainage.

Two species of problematic assignment in previous work – *B. dofleini* from lowlands of the eastern Yucatan Peninsula, Belize, Guatemala and north-eastern Honduras, and *B. alvaradoi* from Costa Rica – cluster together in a weakly supported clade (Clade 6) (two samples of two taxa, bs 63, pp 100). These two species have never been postulated to have any close relatives and were placed in different groups within *Bolitoglossa* beta by Wake & Lynch (1976).

A final large group of 15 taxa (21 samples) (bs 54, decay 1, pp 99) occurs in Nuclear Central America (Clade 7). It corresponds to much of *Bolitoglossa* beta of Wake & Lynch (1976) and is divided into three subclades, one large and including species of the *morio* and *dunni* groups (16 samples) (bs 73, decay 3, pp 100), and smaller clades including species of the *franklini* group (two samples) (bs 100, decay 18, pp 100) and *rostrata* group (three samples) (bs 100, decay 12, pp 100). All of these species are montane, and several occur at elevations above 3000 m. These three subclades might be considered as separate entities, but they are treated as a unit for analytical reasons because of general morphological similarity and because collectively they represent much of the old genus *Magnadigita*, as described by Taylor (1944).

TAXONOMY

Although *Bolitoglossa* contains many more species (80+) than any other genus of Caudata we recommend maintaining the generic taxonomy. Monophyly is well supported by molecular as well as morphological data. The genus is well characterized morphologically, having unique hand and foot morphology, and unique features of the tongue and associated structures. We have identified a number of potential clades; most are well supported by both morphological and molecular data. Clade 6, which has not previously been identified as a phylogenetic unit, includes species that are both relatively large and fully webbed. Clade 7, which has previously been recognized at the generic level based largely on morphological evidence, has the weakest support from our molecular analysis (although both clades 6 and 7 show high posterior clade probabilities in the Bayesian analyses). In our opinion, none of the clades is sufficiently distinctive to warrant assignment to a different genus. However, we believe that


B. dofleini
B. alvaradoi
Pachymandra
Clade 6
B. alberchi
B. alberchi
B. mexicana
B. mexicana
B. mexicana
B. mombachoensis
B. striatula
B. yucatana
Clade 1
B. odonnelli
B. flaviventris
B. platydactyla
B. platydactyla
Bolitoglossa
B. occidentalis
B. rufescens
Nanotriton
Clade 2
Clade 4
B. hartwegi
Mayamandra
B. adspersa
B. sp. 1
B. sima
B. biseriata
B. medemi
B. palmata
B. palmata
B. peruviana
B. paraensis
B. paraensis
B. paraensis
B. altamazonica
B. peruviana
B. sp. 2
B. schizodactyla
B. colonnea
Clade 3
B. cerroensis
B. marmorea
B. epimela
B. minutula
B. subpalmata
B. sp. 4
B. gracilis
B. gracilis
B. pesrubra
Eladinea
Bolitoglossa
B. morio
B. flavimembris
B. morio
B. carri
B. carri
B. conanti
B. diaphora
B. dunni
B. celaque
B. celaque
B. synoria
B. sp. 3
B. sp. 3
B. decora
B. longissima
B. porrasorum
B. franklini
B. lincolni
B. engelhardti
B. rostrata
B. rostrata
Magnadigita
Clade 7
Clade 5
B. hermosa
B. riletti
B. zapoteca
B. macrinii
B. oaxacensis
Oaxakia
Pseudoeurycea
Thorius
Batrachoseps
0.05 substitutions/site

some taxonomic subdivision would be convenient and accordingly we treat the clades as subgenera. This new taxonomy will facilitate analyses and discussion of evolutionary and phylogenetic patterns within the largest species assemblage of Caudata. The revised taxonomy is as follows:

SUBGENUS *BOLITOGLOSSA* DUMÉRIL *ET AL.*, 1854

Type species: Bolitoglossa mexicana Duméril, Bibron et Duméril, 1854; by monotypy.

Diagnosis: Large, long-tailed salamanders with well developed limbs bearing large hands and feet with extensive interdigital webbing, and a first caudal vertebra that typically bears unbranched transverse processes; males usually have a mental gland; also diagnosed by differences in mtDNA sequences and proteins.

This subgenus contains 12 species previously included in the *mexicana* group (García-París, Parra-Olea & Wake, 2000; García-París *et al*., 2003): *B.* (*Bolitoglossa*) *alberchi* García-París, Parra-Olea, Brame et Wake, 2003; *B.* (*Bolitoglossa*) *flaviventris* (Schmidt, 1936); *B.* (*Bolitoglossa*) *jacksoni* Elias, 1984; *B.* (*Bolitoglossa*) *lignicolor* (Peters, 1873); *B.* (*Bolitoglossa*) *mexicana* Duméril, Bibron et Duméril, 1854; *B.* (*Bolitoglossa*) *mombachoensis* Köhler et McCranie, 1999; *B.* (*Bolitoglossa*) *mulleri* (Brocchi, 1883); *B.* (*Bolitoglossa*) *odonnelli* (Stuart, 1943); *B.* (*Bolitoglossa*) *platydactyla* (Gray, 1831); *B.* (*Bolitoglossa*) *salvinii* (Gray, 1868); *B.* (*Bolitoglossa*) *striatula* (Noble, 1918); and *B.* (*Bolitoglossa*) *yucatana* (Peters, 1882).

NANOTRITON SUBGEN. NOV.

Type species: Oedipus rufescens Cope, 1869.

Diagnosis: Diminutive, short-tailed salamanders with small hands and feet that are pad-like and show phalangeal reduction; usually having reduced dentition and skeletal reductions including loss of prefrontal bones and fusions of mesopodial cartilages, as well as having a first caudal vertebra that bears branched transverse processes; males have a prominent mental gland; also diagnosed by differences in mtDNA sequences and proteins.

At present only two species are recognized in this group, but work in progress in our laboratories indicates that additional species should be recognized. Unpublished molecular data extracted from a large number of additional samples supports results of the present analysis.

The species included in the subgenus *Nanotriton* are those previously included in the *rufescens* species group (Wake & Lynch, 1976; Elias, 1984): *B.* (*Nanotriton*) *occidentalis* Taylor, 1941, and *B.* (*Nanotriton*) *rufescens* (Cope, 1869).

SUBGENUS *ELADINEA* MIRANDA RIBEIRO, 1937

Type species: Eladinea estheri Miranda Ribeiro, 1937 (a junior synonym of *B. paraensis*, which herein is raised from synonymy with *B. altamazonica*); by monotypy.

Diagnosis: A morphologically heterogeneous assemblage of species diagnosed by differences in mtDNA and proteins; all species have a first caudal vertebra that bears unbranched transverse processes, and skeletal reductions are general, especially loss of prefrontal bones; males have a prominent mental gland.

The subgenus *Eladinea* includes two main clades, one corresponding to the *subpalmata* and *epimela* species groups, and the other including the species of the *adspersa*, *altamazonica*, *medemi*, and *sima* species groups, plus a final, morphologically heterogeneous group including *B. colonnea, B. schizodactyla* and species related to *B. nigrescens*. We also consider that the species of the *phalarosoma* group (Wake & Lynch, 1976) are included in *Eladinea* based on morphological grounds, although we did not have material for the molecular analysis.

Many species known from Panamá and South America are not included in this study; all previously recognized species groups except the *phalarosoma* group (the single species is here tentatively placed in the *adspersa* group) are represented. Our limited sampling, especially of South American taxa, restricts us from testing all previous morphological hypotheses for species group assignments in this clade. Although South American species have been placed in species groups based on weakly defined characters (Brame & Wake, 1963, 1972; Wake & Brame, 1966; Wake & Lynch, 1976), we have no resolution in our phylogenetic analysis of these species (Fig. 3) and accordingly have decided to place all species of the South American subclade in a single *adspersa* group.

The species and species groups included in subgenus *Eladinea* are:

subpalmata species group: *B.* (*Eladinea*) *gracilis* Bolaños, Robinson et Wake, 1987; *B.* (*Eladinea*) *pesrubra* (Taylor, 1952); *B.* (*Eladinea*) *subpalmata* (Bou-

Figure 4. Maximum likelihood analysis single tree –ln L = 16725.39, obtained using the GTR +G +I model on the combined data set (16S + cyt *b*: 1196 bp). Bayesian posterior clade probabilities are shown on internodes.

lenger, 1896); plus one undescribed Costa Rican species (*B.* sp. 4; Sp. B of García-París *et al*., 2000).

epimela species group: *B.* (*Eladinea*) *cerroensis* (Taylor, 1952); *B.* (*Eladinea*) *epimela* Wake et Brame, 1963; *B.* (*Eladinea*) *marmorea* (Tanner et Brame, 1961); *B.* (*Eladinea*) *minutula* Wake, Brame et Duellman, 1973; and *B.* (*Eladinea*) *sooyorum* Vial, 1963.

schizodactyla species group: *B.* (*Eladinea*) *anthracina* Brame, Savage, Wake et Hanken, 2001; *B.* (*Eladinea*) *colonnea* (Dunn, 1924); *B.* (*Eladinea*) *diminuta* Robinson, 1976; *B.* (*Eladinea*) *nigrescens* (Taylor, 1949); *B.* (*Eladinea*) *robusta* (Cope, 1894); *B.* (*Eladinea*) *schizodactyla* Wake et Brame, 1966; plus one undescribed species from Costa Rica (*B.* sp. 2).

adspersa species group: *B.* (*Eladinea*) *adspersa* (Peters, 1863); *B.* (*Eladinea*) *altamazonica* (Cope, 1874); *B.* (*Eladinea*) *biseriata* Tanner, 1962; *B.* (*Eladinea*) *borburata* Trapido, 1942; *B.* (*Eladinea*) *capitana* Brame et Wake, 1963; *B.* (*Eladinea*) *chica* Brame et Wake, 1963; *B.* (*Eladinea*) *compacta* Wake, Brame et Duellman, 1973; *B.* (*Eladinea*) *cuna* Wake, Brame et Duellman, 1973; *B.* (*Eladinea*) *digitigrada* Wake, Brame et Thomas, 1982; *B.* (*Eladinea*) *equatoriana* Brame et Wake, 1972; *Bolitoglossa* (*Eladinea*) *guaramacalensis* Schargel, García-Pérez et Smith, 2002; *B.* (*Eladinea*) *hyemalis* Lynch, 2001; *B.* (*Eladinea*) *hypacra* (Brame et Wake, 1962); *B.* (*Eladinea*) *lozanoi* Acosta-Galvis et Restrepo, 2001; *B.* (*Eladinea*) *medemi* Brame et Wake, 1972; *B.* (*Eladinea*) *nicefori* Brame et Wake, 1963; *B.* (*Eladinea*) *orestes* Brame et Wake, 1962; *B.* (*Eladinea*) *palmata* (Werner, 1897); *B.* (*Eladinea*) *pandi* Brame et Wake, 1963; *B.* (*Eladinea*) *paraensis* (Unterstein, 1930) (see below), *B.* (*Eladinea*) *peruviana* (Boulenger, 1883); *B.* (*Eladinea*) *phalarosoma* Wake et Brame, 1962; *B.* (*Eladinea*) *ramosi* Brame et Wake, 1972; *B.* (*Eladinea*) *savagei* Brame et Wake, 1963; *B.* (*Eladinea*) *silverstonei* Brame et Wake, 1972; *B.* (*Eladinea*) *sima* (Vaillant, 1911); *B.* (*Eladinea*) *spongai* Barrio et Fuentes, 1999; *B.* (*Eladinea*) *taylori* Wake, Brame et Myers, 1970; *B.* (*Eladinea*) *vallecula* Brame et Wake, 1963; *B.* (*Eladinea*) *walkeri* Brame et Wake, 1972; and one undescribed species from Colombia (B. sp. 1; 'Soacha' Hanken & Wake, 1982).

SUBGENUS *MAGNADIGITA* TAYLOR, 1944

Type species: Bolitoglossa nigroflavescens Taylor, 1941; by original designation (the name is used now at the subspecific level: *B. franklini nigroflavescens*).

Diagnosis: A morphologically heterogeneous assemblage of species diagnosed by differences in mtDNA and proteins; none of the species has hands and feet that are fully webbed, and most have well defined digits with broad tips; all species have a first caudal vertebra that bears branched transverse processes; males have a prominent mental gland.

The species assigned to this taxon are generalized in structure, being of moderate size and having digits that lack full webbing. They are also localized in Nuclear Central America (Wake & Lynch, 1976).

The species included in subgenus *Magnadigita* are: *rostrata* species group: *B.* (*Magnadigita*) *cuchumatana* (Stuart, 1943); *B.* (*Magnadigita*) *helmrichi* (Schmidt, 1936); *B.* (*Magnadigita*) *engelhardti* (Schmidt, 1936); and *B.* (*Magnadigita*) *rostrata* (Brocchi, 1883).

franklini species group: *B.* (*Magnadigita*) *franklini* (Schmidt, 1936); *B.* (*Magnadigita*) *lincolni* (Stuart, 1943); and *B.* (*Magnadigita*) *meliana* Wake et Lynch, 1982.

dunni species group: *B.* (*Magnadigita*) *carri* McCranie et Wilson, 1993; *B.* (*Magnadigita*) *celaque* McCranie et Wilson, 1993; *B.* (*Magnadigita*) *conanti* McCranie et Wilson, 1993; *B.* (*Magnadigita*) *decora* McCranie et Wilson, 1997; *B.* (*Magnadigita*) *diaphora* McCranie et Wilson, 1995; *B.* (*Magnadigita*) *dunni* (Schmidt, 1933); *B.* (*Magnadigita*) *flavimembris* (Schmidt, 1936); *B.* (*Magnadigita*) *longissima* McCranie et Cruz, 1996; *B.* (*Magnadigita*) *morio* (Cope, 1869); *B.* (*Magnadigita*) *porrasorum* McCranie et Wilson, 1995; *B.* (*Magnadigita*) *synoria* McCranie et Köhler, 1999, plus one undescribed species (*B.* sp. 3; El Salvador).

OAXAKIA SUBGEN. NOV.

Type species: Bolitoglossa macrinii Lafrentz, 1930.

Diagnosis: A group of salamanders of moderate size having little to moderate amounts of interdigital webbing, transverse processes of the first caudal vertebra that are weakly branched or not branched at all, a weakly ossified premaxillary bone, and males that lack mental glands; also diagnosed by differences in mtDNA and proteins.

This morphologically distinctive, geographically localized taxon has been revised recently (Parra-Olea *et al*., 2002). *Oaxakia* includes five species, previously included in the *macrinii* species group: *B.* (*Oaxakia*) *hermosa* Papenfuss, Wake et Adler, 1984; *B.* (*Oaxakia*) *macrinii* (Lafrentz, 1930); *B.* (*Oaxakia*) *oaxacensis* Parra-Olea, García-París et Wake, 2002; *B.* (*Oaxakia*) *riletti* Holman, 1964, and *B.* (*Oaxakia*) *zapoteca* Parra-Olea, García-París et Wake, 2002.

PACHYMANDRA SUBGEN. NOV.

Type species: Spelerpes dofleini Werner, 1903.

Diagnosis: A group of large to very large salamanders with hands and feet that are fully webbed and first caudal vertebrae that have branched transverse processes; males have prominent mental glands; also diagnosed by differences in mtDNA and proteins. *Pachymandra* includes two species, placed in the *dofleini* and *alvaradoi* species groups.

alvaradoi species group: *B.* (*Pachymandra*) *alvaradoi* Taylor, 1954.

dofleini species group: *B.* (*Pachymandra*) *dofleini* (Werner, 1903).

MAYAMANDRA SUBGEN. NOV.

Type species: Bolitoglossa hartwegi Wake et Brame, 1969.

Diagnosis: A group of small to medium-sized salamanders with distinctive hands and feet that are broad, with fully webbed, relatively short digits and a distinctive triangular third toe extending from the centre of the webbed foot; first caudal vertebra bears branched transverse processes; males have prominent mental glands; also diagnosed by differences in mtDNA and proteins.

Mayamandra includes three species, previously placed in the *veracrucis* species group: *B.* (*Mayamandra*) *hartwegi* Wake et Brame, 1969; *B.* (*Mayamandra*) *stuarti* Wake et Brame, 1969, and *B.* (*Mayamandra*) *veracrucis* Taylor, 1951.

Our phylogenetic hypothesis is consistent with current species recognition except in three cases. The first involves samples of the *B. altamazonica* complex of *Eladinea*, three from Brazil and the other from Perú, which are highly divergent and are not sister taxa. We believe at least two species are represented, and the Peruvian sample should retain the name *B. altamazonica* since it is geographically closer to the type locality. There are two available names that can be used for the Brazilian species represented by our sample: *Oedipus paraensis* Unterstein, 1930 and *O. estheri* Miranda Ribeiro, 1937. We think that both names correspond to a single species described from geographically close type localities. Based on the principle of priority we choose Unterstein's name, applied in the combination: ***Bolitoglossa* (*Eladinea*) *paraensis*** (Unterstein, 1930) **stat. nov.** The large divergence found among the three sequences of what we call *B. paraensis* suggests that the taxon should be considered a species complex in need of a detailed study.

The other cases involve *B.* (*M.*) *morio* and *B.* (*E.*) *peruviana*, each a composite species with samples that are not sister. Further study is required, and results will be presented elsewhere.

DISCUSSION

SYSTEMATICS

Our analyses include a comprehensive sampling of nearly all previously recognized species groups within *Bolitoglossa*, including a wide range of the morphological and ecological diversity represented along the entire distribution range of the genus. The monophyly of *Bolitoglossa* is well supported by our analyses of the molecular data (bs 94–100, decay 10, pp 100) and changes in the outgroup (different species of *Pseudoeurycea*, *Parvimolge*, *Lineatriton, Ixalotriton*, *Oedipina* and *Nototriton*, not shown) do not alter this result. *Bolitoglossa* is also well supported as a monophyletic clade by morphological data (Elias & Wake, 1983).

We place the species sampled in seven subgenera in accordance with our analysis of the molecular (Figs 3, 4), as well as the morphological data. The subgenera either constitute previously known species groups, or include more than one of the previously recognized species groups, with the exception of subgenus *Pachymandra*, which is constituted by species previously assigned to separate species groups (*B. dofleini*, the sole member of the *dofleini* group and *B. alvaradoi,* the sole member of the *alvaradoi* group). Although basal relationships within *Bolitoglossa* are not resolved, there is good support in the Bayesian analysis (pp 100) for a clade that includes *Nanotriton* and *Mayamandra* as sister clades, and the combination, in turn, is sister to *Bolitoglossa* (pp 100). However, there is no support in the parsimony analyses for this arrangement.

Species groups previously defined on morphological or molecular grounds are generally monophyletic and typically well supported in our phylogenetic hypothesis. Species composition of the clades does not change with the alternative analytical treatments used, although support for clades is generally stronger when 3rd positions transitions are downweighted or excluded from the analyses (Fig. 3).

Several earlier attempts to organize species of *Bolitoglossa* into subunits have been made. Wake & Lynch (1976) recognized 'alpha' and 'beta' sections of *Bolitoglossa* and found some morphological support for them (osteological reductions in alpha, complex tail base in beta). García-París, Parra-Olea & Wake (2000) revised this organization by transferring *B. yucatana* from the beta section to the *mexicana* group of the alpha section. After such modification the alpha section corresponds to our subgenera *Bolitoglossa* and *Eladinea*. The beta group includes our subgenus *Magnadigita* as the main constituent, but also includes the species we place in *Nanotriton*, *Mayamandra*, *Oaxakia* and *Pachymandra*. The alpha and beta division is not supported by our analysis, and while we cannot reject the

main hypothesis, we do find some support in the parsimony analysis for a sister-group relationship of *Bolitoglossa* and *Nanotriton* (bs 61), which bridges the alpha and beta sections. We performed a Shimodaira–Hasegawa (SH) parametric test (Shimodaira & Hasegawa, 1999) using our molecular data to test the alpha–beta hypothesis. When we forced all the samples to be integrated in monophyletic 'alpha' and 'beta' groups, the tree recovered ($-\ln L = 17141.891$) did not differ significantly ($P = 0.081$) from the ML tree shown (Fig. 4), so reciprocal monophyly of alpha and beta groupings of *Bolitoglossa* cannot be conclusively rejected.

Wake & Lynch (1976) further arranged all species known at that time into 16 species groups, incorporating and modifying previous proposals of Brame & Wake (1963, 1972), Dunn (1926), Taylor (1941), Stuart (1952), Wake & Brame (1969), and Wake, Brame & Duellman (1973). Elias (1984) summarized the informal group names used for the species of the 'beta' section and proposed some changes. Newly described species have been assigned to existing species groups (e.g. McCranie & Wilson, 1993; García-París *et al*., 2003).

Some of our subgenera correspond to previously defined species groups (*Bolitoglossa*, *Mayamandra*, *Oaxakia*), but most of them include two or more members assigned to other species groups. The subgenus *Bolitoglossa* corresponds to the *mexicana* species group of Wake & Lynch (1976), redefined by García-París *et al*. (2000) and García-París *et al*., 2003) to include *B. yucatana*, *B. striatula*, *B. mombachoensis*, and *B. lignicolor*, and the newly described *B. alberchi*. *Bolitoglossa yucatana* was previously placed in the *dofleini* species group (Dunn, 1926; Wake & Lynch, 1976; Larson, 1983a, b; Elias, 1984); *B. striatula* and *B. mombachoensis* were part of the *striatula* species group (Wake & Lynch, 1976; Köhler & McCranie, 1999); and *B. lignicolor* was part of the *lignicolor* species group (Wake & Lynch, 1976), some species of which are now transferred to *Eladinea*. The subgenus *Bolitoglossa* is distributed widely, primarily in México and Nuclear Central America, but because of its ability to live in the lowlands it was able to disperse into more southerly regions (it reaches central Panamá) (Fig. 5). The only species of the genus *Bolitoglossa* other than those assigned to subgenus *Eladinea* that occur south of Honduras-El Salvador are a few representatives of subgenus *Bolitoglossa* and a single species of *Pachymandra* (Figs 5, 6).

Eladinea includes most of the species from Costa Rica and Panamá, and all from South America; it is a

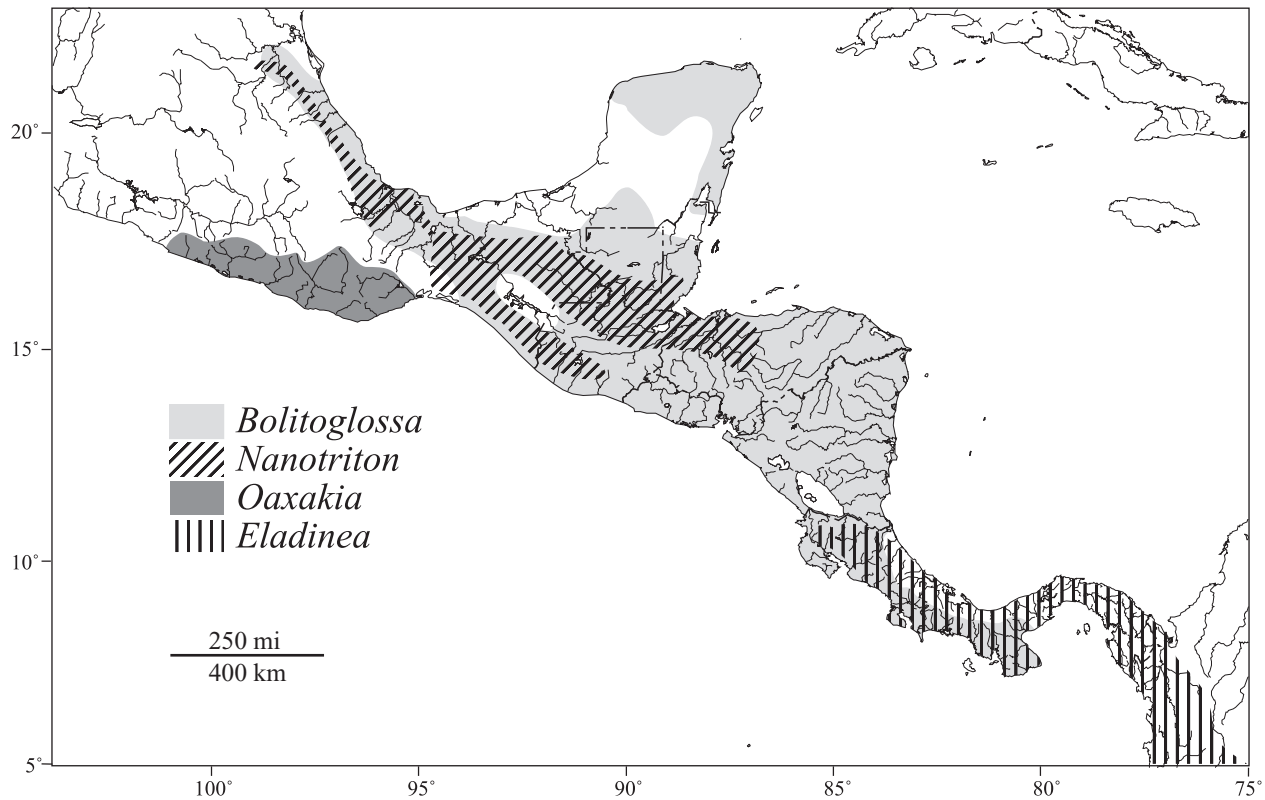


Figure 5. General distribution of four subgenera of *Bolitoglossa* (*Bolitoglossa*, *Nanotriton*, *Oaxakia*, *Eladinea*). Distribution of *Eladinea* continues through much of tropical South America (see Fig. 7).

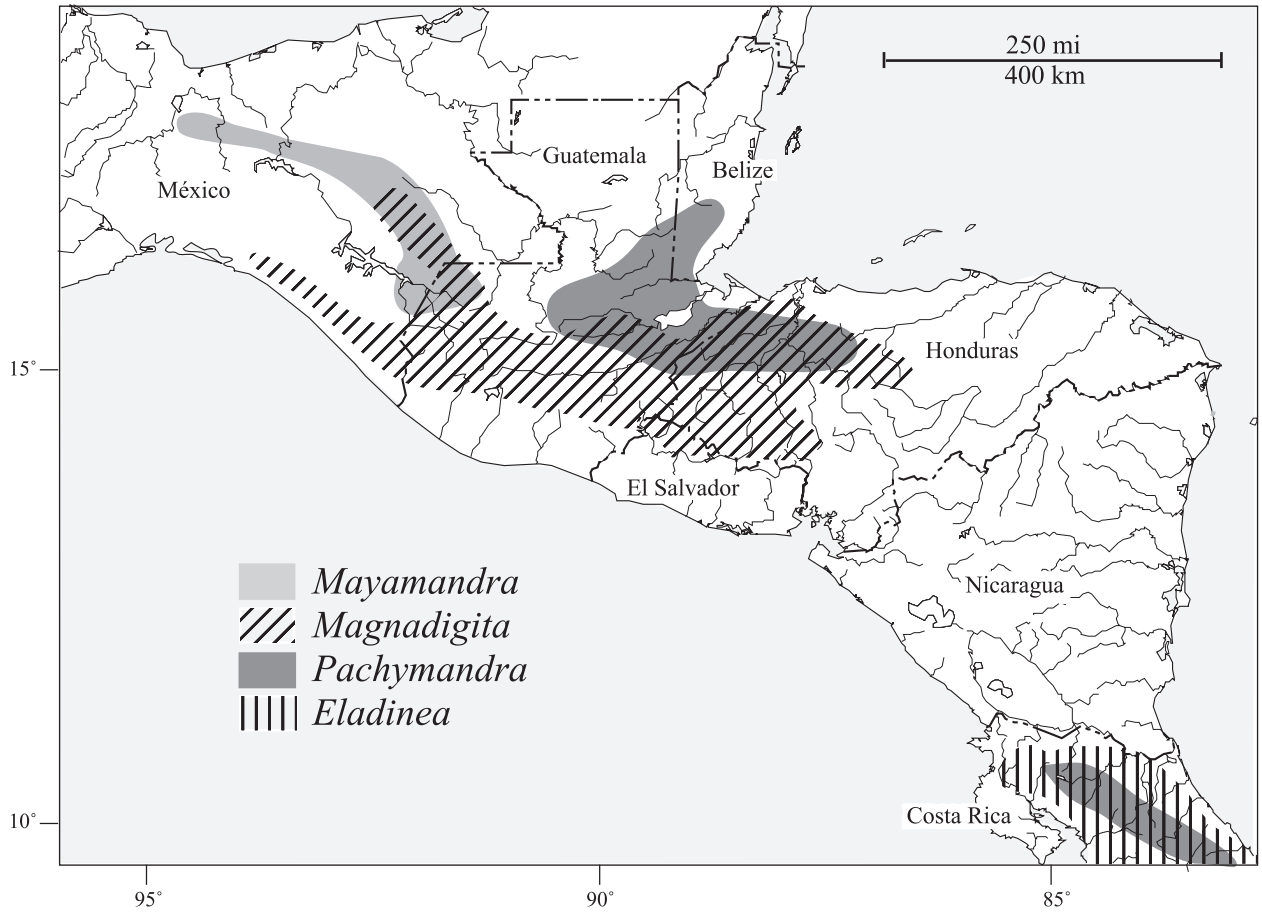


Figure 6. General distribution of four subgenera of *Bolitoglossa* (*Mayamandra*, *Magnadigita*, *Pachymandra*, *Eladinea*). The distribution of *Pachymandra* (i.e. *Bolitoglossa alvaradoi*) continues to the South and East in Costa Rica nearly to the Panamanian border (Savage, 2002), and that of *Eladinea* continues through Panamá (see Fig. 5) and into South America (see Fig. 7).

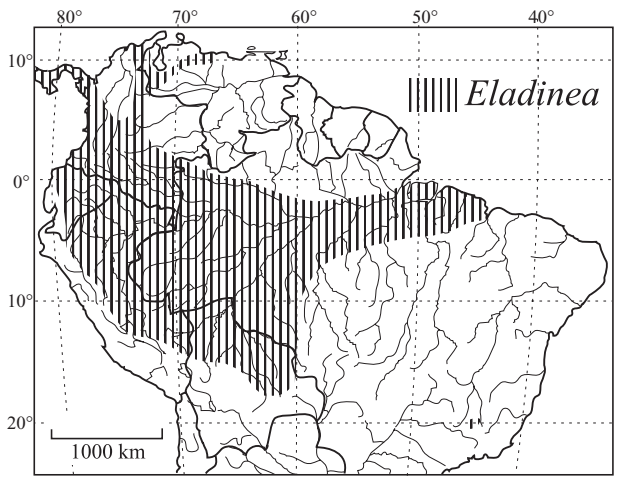


Figure 7. General distribution of the subgenus *Eladinea* in South America. Distribution continues northward through Central America (see Fig. 5).

discrete zoogeographical entity (Figs 6, 7). Within *Eladinea* we recognize two major subclades, one including the *subpalmata* (Wake & Lynch, 1976; modified by García-París, Parra-Olea & Wake, 2000) and *epimela* groups, and the other the *adspersa* and *schizodactyla* groups. The first subclade occurs exclusively north of South America. The second much larger subclade includes all South American species sampled. Our *schizodactyla* group is a heterogeneous assemblage, weakly supported as a clade in MP but well supported in the Bayesian analysis. Species included here in the *schizodactyla* group were placed in three different groups by Wake & Lynch (1976). The large *adspersa* group is principally South American, but includes two species that enter Panamá, one (*B. biseriata*) nearly reaching the Costa Rican border. The *adspersa* group includes taxa representing the *adspersa, altamazonica, medemi,* and *sima* species groups of Wake & Lynch (1976), but our molecular analysis does not support further subdivision at this time.

Eladinea is a morphologically and ecological diverse assemblage of species utilizing the full range of habitats and elevations shown by the entire genus. It includes the smallest (*B. diminuta*, not included in our present sample but assigned to *Eladinea* on the basis of our study of 385 bp of cyt *b* and of allozymic similarities to the *subpalmata* complex; García-París *et al*., 2000) and largest (*B. robusta*, also not included here but assigned to this clade based on partial sequences) members of the genus. Species in this clade range from highland species that have mainly unwebbed hands and feet such as *B. cerroensis,* to fully webbed lowland forms (most of the species in the clade).

The only previous molecular-based study of *Eladinea* is that of Hanken & Wake (1982), who examined proteins of ten taxa (by our current understanding of the taxonomy of their samples). They found two clades, one corresponding to our more northern subclade and the other to our South American subclade; the two studies are in complete agreement. Hanken & Wake (1982) studied one Panamanian species (their *B. nigrescens*, unavailable to us) that clustered with the South American species.

The *macrinii* group of Wake & Lynch (1976), our subgenus *Oaxakia*, is well defined on morphological and molecular grounds (Parra-Olea *et al*., 2002), and it has a distinctive distribution as well. It includes the only species of *Bolitoglossa* found in the Sierra Madre del Sur north and west of the Isthmus of Tehuantepec in southern Oaxaca and Guerrero, México (Fig. 5).

Nanotriton includes two diminutive species with reduced skulls, reduced dentition, short tails, and short legs bearing diminutive, fully webbed hands and feet. It corresponds to the *rufescens* group of previous authors (Taylor, 1941; Wake & Brame, 1969; Wake & Lynch, 1976; Larson, 1983a, b; Elias, 1984). This clade ranges from north-eastern México into north-central Honduras (Fig. 5). Unpublished studies of many additional samples indicate that this taxon is more diverse than currently recognized and revisionary studies are in progress.

Taylor (1944) described the genus *Magnadigita* for species of generalized morphology and relatively reduced interdigital webbing, but his genus also included species we have assigned to *Eladinea*. For nearly 40 years, all members of the genus *Bolitoglossa*, as currently recognized, were divided between *Magnadigita* and *Bolitoglossa*, but Wake & Brame (1963) showed that the morphological characters used by Taylor to diagnose *Magnadigita* did not work and synonymized the two taxa. Our subgenus *Magnadigita* is less inclusive than Taylor's original proposal (which included species we include in the subgenus *Eladinea*) and includes species that have been placed in three or four species groups (Stuart, 1952; Wake & Brame, 1969; Wake & Lynch, 1976; Elias, 1984; McCranie & Wilson, 2002). These species range from the mountains of central and southern Chiapas through Guatemala into western and central Honduras and northern El Salvador (Fig. 6).

Interpretations of relationships have changed through time, reflecting new discoveries and new data, but also different perspectives on evolution. Stuart (1952) placed species of *Magnadigita* in three groups (*dunni, franklini, morio*) based on size, features of the head and feet, coloration, and ecology. Wake & Brame (1969) envisaged parallel evolutionary trends in the direction of increased interdigital webbing associated with arboreality and movement into the lowlands in different lineages. They stressed differences in end members of transformation series, which they interpreted as evidence of independent origin. The members of the hypothesized transformation series were recognized as species groups. Elias (1984), in contrast, returned to Stuart's criteria and stressed phenetic similarity; he expected close relatives to be not only similar in morphology but to have complementary allopatric distributions and similar ecology. We now stress genealogical relationships estimated from our analysis of molecular data.

The species we studied fall into three subclades in our analysis: the *rostrata* group including *B. rostrata* and *B. engelhardti*; the *franklini* group including *B. franklini* and *B. lincolni*, and the *dunni* group, a large, rather heterogeneous assemblage of 11 nominal species and one undescribed species. The first two groups occur only in western Guatemala and adjoining Chiapas, México. The *dunni* group is mostly Honduran (e.g. *B. dunni*), with some species in Guatemala (e.g. *B. morio*) and El Salvador, and one (*B. flavimembris*) that barely enters México. The *franklini* and *rostrata* groups are both small, and both have been recognized previously (although with different constituents). Stuart (1952) recognized both a *morio* and a *dunni* group, but our ML analysis places *B. morio* and *B. dunni* in the same clade; we choose to call this the *dunni* species group because that name has been used extensively in recent work by McCranie and colleagues (work summarized in McCranie & Wilson, 2002; who are responsible for description of several new species, mainly from Honduras, in the past decade). Most of the recently described Honduran species were placed in the *dunni* group, but *B. diaphora*, was thought to be related to *B. cuchumatana* and *B. helmrichi* (McCranie & Wilson, 1995). The latter two species are not included in this study, but unpublished data shows that they are members of the *rostrata* species group. In our analysis, *B. diaphora* is a member of the *dunni* species group.

Elias (1984) attempted the most recent analysis of relationships within *Magnadigita*, and his groupings

were evaluated by Larson (1983a), whose work cited the then unpublished manuscript of Elias. The *morio* group, as designated by Elias (1984) (*B. morio*, *B. flavimembris*) is well supported (100 bs, 100 pp) within the subclade that we designate as the *dunni* group. His *franklini* group (of which we studied only *B. franklini* and *B. lincolni*) is also supported (100 bs, 100 pp). The sister-taxon relationship of two species included in Elias' *dunni* group, *B. rostrata* and *B. engelhardti* (100 bs, 100 pp) is supported. Larson (1983a, b), using data from allozymes, found support for a grouping of *B. morio* and *B. flavimembris*, and for a grouping of *B. rostrata, B. engelhardti* and some species we did not sample (*B. cuchumatana, B. meliana, B. helmrichi*). Larson's comparisons were limited, but he supported the concept of a *dunni* group that included *B. engelhardti* and *B. rostrata* (essentially Elias' *dunni* group). We found some differences from previous work. While *B. dunni* has been associated with *B. engelhardti* since Stuart (1952) first recognized a *dunni* group, an association endorsed by Wake & Brame (1969), Wake & Lynch (1976), and Elias, 1984), our new analysis places *B. dunni* in the large subclade, well separated from *B. engelhardti*. Within that subclade it is associated with two recently described Honduran species (*B. diaphora* and *B. conanti*, the latter previously included in *B. dunni*). Wake & Brame (1963) included *B. morio* and *B. dunni* in the same species group (but in different subgroups), but subsequent authors have separated the species. They are both included in the large subclade of our revised *dunni* group (Figs 3, 4).

The most problematic of our taxa, *Pachymandra*, includes the enigmatic species *B. dofleini* and *B. alvaradoi*. The former was discussed in detail by Larson (1983a), who was unable to resolve its relationships. It was thought to be related to *B. yucatana*, with which it was included in the *dofleini* group by Wake & Lynch (1976), but *B. yucatana* was later transferred to the *mexicana* group (García-París, Parra-Olea & Wake, 2000), our subgenus *Bolitoglossa*, an assignment supported by our current analyses. The second species, *B. alvaradoi*, was placed in its own species group by Wake & Lynch (1976), and it (together with *B. arborescandens*, considered a synonym by Savage, 2002) was thought to be the only member of the beta section that occurred as far south as Costa Rica. Both *B. dofleini* and *B. alvaradoi* are relatively large and have large, fully webbed hands and feet. The species occur at elevations below 1500 m, *B. dofleini* from the Yucatán Peninsula of México into Belize, Guatemala and northern Honduras, and *B. alvaradoi* restricted to northern and eastern Costa Rica (Fig. 6). While these two species form a clade in all treatments, there is strong support in the Bayesian analysis (100 pp), but not in the parsimony analysis (62 bs, 0 decay), except when third positions are excluded (95 bs). The two species are distant relatives at best, with a K2p of 24.5%.

We find some support for a sister relationship between *Bolitoglossa* and *Nanotriton* in the parsimony analysis (bs 61) and when third position transitions are excluded we also find support for a clade combining *Bolitoglossa–Nanotriton* and *Mayamandra* (bs 62). The arrangement is reversed in the Bayesian analysis, with support for *Nanotriton–Mayamandra* (100 pp) and of these two with *Bolitoglossa* (100 pp). Wake & Brame (1969) recognized a grouping of *B.* (*Mayamandra*) *hartwegi* with some species of *Magnadigita* from southern Mexico and Guatemala in what they called the *helmrichi* group, but they believed that this group was well separated from both *Nanotriton* and *Bolitoglossa*. Elias (1984) further separated what he termed the *veracrucis* group (*Mayamandra*) from other members of the *helmrichi* group.

Our recovery of a possible phylogenetic relationship among the members of clades *Bolitoglossa–Nanotriton* and *Mayamandra* is problematic, in view of the large K2p distances (the lowest K2p of *B. hartwegi* to any species is 14.6%, to *B. striatula* of the *Bolitoglossa* clade) and the morphological differences (see below). Members of the subgenus *Bolitoglossa* are large species with long, prehensile tails and long legs bearing large, extensively webbed hands and feet that are capable of suction, whereas members of *Nanotriton* are diminutive forms with reduced skulls, reduced dentition, short tails, and short legs bearing diminutive hands and feet that while fully webbed have attained that state via a different developmental route than in *Bolitoglossa*, via paedomorphosis (Alberch, 1980; Alberch & Alberch, 1981). In contrast, *Mayamandra* is somewhat intermediate between *Nanotriton* and *Bolitoglossa* in morphology, having broad but nearly fully webbed hands and feet with short digits, and resembling members of *Nanotriton* in having mesopodial fusions (Wake & Brame, 1969).

Character evolution

Taylor (1944) used the degree of webbing and the shapes of the digits and phalanges as the basis for sorting species into two genera: *Magnadigita* and *Bolitoglossa*. His *Magnadigita* included species in our subgenera *Eladinea*, *Magnadigita*, and *Oaxakia*, and his *Bolitoglossa* included species in our subgenera *Bolitoglossa, Nanotriton*, *Eladinea*, and *Pachymandra* (*Mayamandra* was unknown to him but he later described *B. veracrucis* and placed it in *Bolitoglossa*). Thus, by implication, Taylor believed that full webbing had evolved only once. Wake & Brame (1963) thought that webbing had evolved repeatedly and argued that taxa with intermediate limb structure existed. They

reduced Taylor's *Magnadigita* to synonymy with *Bolitoglossa.* Brame & Wake (1963) had earlier argued that increased webbing and digital reduction had evolved repeatedly within *Bolitoglossa*, a point that has been made on several occasions (Wake, 1966; Wake & Brame, 1969; Wake & Lynch, 1976; Alberch & Alberch, 1981).

The lack of basal structure in our tree makes testing this hypothesis difficult. All members of our clades *Bolitoglossa*, *Nanotriton*, *Mayamandra* and *Pachymandra* have extensively, almost fully webbed hands and feet. Each of the remaining clades, *Eladinea*, *Magnadigita*, and *Oaxakia* shows variation from relatively unwebbed to relatively webbed. Based on outgroup analysis the ancestral state for the genus is likely to have been largely unwebbed (all other genera of the supergenus *Bolitoglossa* have relatively short digits showing some degree of basal webbing), and accordingly the most parsimonious explanation of the variation in these three clades is that lack of webbing is ancestral. Accordingly, full webbing has evolved at least four times independently, at least once each in clades *Eladinea*, *Magnadigita*, and *Oaxakia*, and once (if the following clades form a larger clade, not supported in our data) or more in clades *Bolitoglossa*–*Nanotriton*, *Mayamandra*, and *Pachymandra*.

Larson (1983b) postulated parallel trends in the direction of increased interdigital webbing within the 'beta' section of *Bolitoglossa*, once within the *dunni* group, once within the *morio* group (both within the subgenus *Magnadigita*), once within the *veracrucis* group (*Mayamandra*), and within the combination of *dofleini* (*Pachymandra*) and *rufescens* (*Nanotriton*) groups, which he considered to be sister taxa. Our results support the existence of parallel trends in interdigital webbing, but the lack of basal structure in our tree does not permit us to determine how many times interdigital webbing increased with respect to the taxa mentioned. Both *Nanotriton* and *Bolitoglossa* have extensive interdigital webbing, but their close relationship is equivocal and the nature of the webbing differs in the two. The first is paedomorphic and essentially represents retention of an embryonic padlike condition of the hands and feet (Alberch & Alberch, 1981). In contrast, *Bolitoglossa* has large hands and feet with long digits and true interdigital webbing.

Larson presented three alternative hypotheses concerning the origin of the condition in the *rufescens* group (*Nanotriton*), and these apply as well to our phylogeny with the *mexicana* group (*Bolitoglossa*) as a sister taxon, as to his with *B. dofleini* as sister. We favour his third hypothesis, that the diminutive *Nanotriton* was derived via progenetic evolution (see Alberch & Alberch, 1981) from a large-bodied ancestral form that evolved neotenically. We also offer an alternative interpretation of the evolution of *B. dofleini*, a giant species with extensively webbed but paedomorphic hands and feet (Alberch, 1983). The putative sister taxon of *B. dofleini* is the smaller *B. alvaradoi*. The hands and feet of *B. alvaradoi* are large and there is extensive, nearly complete interdigital webbing. We believe that while its morphology is derived relative to the genus as a whole, it is closer to the ancestral state than is that of *B. dofleini*, and we agree with Larson (1983a) and Alberch (1983) that *B. dofleini* represents an extreme derived state that has been attained by the paedomorphic process of neoteny.

A radical alternative interpretation is that extensive webbing is ancestral for the genus (there is no out-group support for such an hypothesis, but extensive webbing is found in at least some species in all seven clades). Furthermore, one must explain the independent reversal to rounded, relatively unwebbed digits with expanded digital tips and subdigital pads within *Eladinea*, *Magnadigita*, and *Oaxakia*. If webbing is ancestral, the relatively unwebbed species of high elevation would be postulated to have been derived from species in lowlands near to them (the reverse of the pattern predicted for South American forms by Brame & Wake, 1963). We are unable to perform a rigorous test of this hypothesis but some of our data are relevant and are counter to the prediction. Within *Oaxakia*, *B. hermosa*, the species with the most webbing, is nested (see also Parra-Olea *et al*., 2002). Within *Eladinea*, the large, southern subclade includes two upland relatively unwebbed species (B. adspersa, *B.* sp. 2) that are not close relatives but there is insufficient phylogenetic structure to say whether they are basal or well nested. In the more northerly subclade, relatively unwebbed species are found both in basal and nested positions. Thus there is little or no support for the webbing-first hypothesis within *Eladinea*. Within *Magnadigita* the only species we studied with relatively extensive webbing are *B. engelhardti*, *B. flavimembris*, and *B. diaphora*. These are not close relatives, falling in different parts of the tree, and the last two are nested, so there is no support for this hypothesis in *Magnadigita* either.

South American invasion

The invasion of South America by representatives of the genus *Bolitoglossa* has been discussed extensively (Dunn, 1926; Darlington, 1957; Brame & Wake, 1963; Wake, 1966; Wake & Lynch, 1976; Hanken & Wake, 1982). Although it is generally accepted that salamanders entered South America from southern Central America, the number and timing of invasions is uncertain. The most recent studies using molecular data (allozymes) (Hanken & Wake, 1982) concluded that South American species of *Bolitoglossa* are deeply

differentiated genetically from one another, suggesting an earlier origin than the establishment of the current land connection between Panamá & Colombia. Hanken & Wake (1982) found no evidence that the South American salamanders form a clade, but their sampling was limited (only five species, whereas we have studied 11). According to the allozyme data, the Panamanian *B. nigrescens* was nested within their South American *adspersa* species group, and closer to the Venezuelan *B. borburata* than to any Central American species. Hanken & Wake (1982) proposed two alternative hypotheses: either salamander lineages already differentiated in Central America entered South America separately once the portal was closed, or diversification occurred in South America from an ancestral stock that reached the Continent prior to the closure of the portal about 3 Mya (Coates & Obando, 1996). Both hypotheses are consistent with the large genetic divergence found among South American species, with genetic distances between species as large as $D_{Nei} = 0.74$. This degree of divergence likely represents more than 10 Myr of lineage independence (Hanken & Wake, 1982).

Our data show large mtDNA sequence divergence among species within South America, thus confirming the antiquity of the differentiation of the South American lineages within *Eladinea*. We compared three taxa from western Panamá (*B. marmorea* and *B. minutula*, large and small montane species, respectively, and *B. schizodactyla*, a large lowland species) with 11 samples from species we consider to be South American (two of the species, *B. medemi* and *B. biseriata*, occur in South America but our samples were from eastern Panamá, so any bias is in favour of shorter times of divergence). The range of K2p distances is between 13.9% and 22.0%, mean 17.9%. If we apply commonly used molecular clock estimates for mtDNA data ranging from 1% divergence per 2 Myr (a general estimate for vertebrates, e.g., Brown *et al*., 1982; Avise, 2000) to 1% divergence per 0.7–0.8 Myr (Tan & Wake, 1995; calibrated for salamanders) we obtain estimates of 35.8 and 12.5 Myr, respectively (using 0.7 for the latter estimate). Even if we use the slowest clock with the least divergence (K2p = 13.9%, between *B. minutula* and *B. medemi*, both from Panamá), the time estimate is 9.7 Myr, more than three times longer than the time since the closure of the Panamanian Portal. To test our hypothesis that the two eastern Panamanian populations were appropriately combined with the South American samples we conducted a non-parametric test comparing a constrained topology with the South American samples forming a monophyletic group with the Panamanian populations as sister, vs. the MP topology (TL = 3699, $n = 114$, $p = 0.38$). The topology where the South American taxa are a monophyletic group by exclusion of *B. medemi* and *B. biseriata* does not differ significantly from the MP topology.

Both hypotheses proposed for the South American invasion require at least two independent, relatively recent dispersals back to Central America (*B. medemi* and *B. biseriata*). Since we do not have Colombian samples of these species we cannot estimate the timing of the dispersal events. When and how many lineages of *Bolitoglossa* entered South America remain uncertain, but we suspect extensive species formation occurred in South America. For example, *B. biseriata* and *B. sima* form a well-supported clade (93 bs, 6 decay, 100 pp), but have a K2p of 12.0 (minimally 8.4 Myr). Another clade includes one of the two samples each of *B. peruviana* and *B. altamazonica* (88 bs, decay 6, 100 pp), with a K2p of 10.3 and minimal divergence of 7.2 Myr. We had two samples attributed to *B. peruviana* (K2p 15.2), but they are not sister taxa (two morphologically similar species are likely represented).

Accordingly, we think it is likely that the radiation of South American salamanders is autochthonous and that the entrance of salamanders into South America probably preceded establishment of the Isthmian link about 3 Mya, perhaps by several to many Myr. This then raises another question: why have salamanders been so successful in Middle America but not in South America? There are profound differences in numbers and diversity of salamanders in lower Middle America as compared with north-western South America. Colombia is vastly larger than Costa Rica and has much greater topographic complexity. It has by far the largest number of species of salamanders of South American countries, but many fewer than in Costa Rica (counting only formally recognized species Colombia has 18 species, whereas Costa Rica has 36 and these occur in many more lineages). Only about 20% of all salamanders in the New World tropics occur in South America, a region that is vastly larger than Middle America. Lowland tropical salamanders are notoriously difficult to observe and it is possible that many South American species have gone undetected. In addition, lowland South American members of *Bolitoglossa* are very similar in morphology and doubtless the number of species recognized on morphological grounds is an underestimate. Even granting these possibilities, it is surprising that there are not more species of salamanders in South America.

The likeliest explanation for this phenomenon is that whatever the time of entry into South America, it was late relative to the occupancy of Middle America (Brame & Wake, 1963; Wake, 1987), as evidenced by the fact that only two clades (one of seven clades within the genus *Bolitoglossa*, this paper, and one of two major clades of the genus *Oedipina*; García-París & Wake, 2000) of the many that are found in Middle

America enter South America. We conclude that the relative scarcity of South American salamanders is primarily attributable to the failure of most lineages of Middle American salamanders to disperse sufficiently far to reach South America. The Middle American salamander fauna required a great deal of time to achieve their present representation. We propose that the lineages that did reach South America have not been there sufficiently long for substantial diversification to have occurred.

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