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Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America

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Abstract

We constructed phylogenetic hypotheses for Mesoamerican *Rhamdia*, the only genus of primary freshwater fish represented by sympatric species across Central America. Phylogenetic relationships were inferred from analysis of 1990 base pairs (bp) of mitochondrial DNA (mtDNA), represented by the complete nucleotide sequences of the cytochrome *b* (cyt *b*) and the ATP synthase 8 and 6 (ATPase 8/6) genes. We sequenced 120 individuals from 53 drainages to provide a comprehensive geographic picture of Central American *Rhamdia* systematics and phylogeography. Phylogeographic analysis distinguished multiple *Rhamdia* mtDNA lineages, and the geographic congruence across evolutionarily independent *Rhamdia* clades indicated that vicariance has played a strong role in the Mesoamerican diversification of this genus. Phylogenetic analyses of species-level relationships provide strong support for the monophyly of a trans-Andean clade of three evolutionarily equivalent *Rhamdia* taxa: *R. guatemalensis*, *R. laticauda*, and *R. cinerascens*. Application of fish-based mitochondrial DNA clocks ticking at 1.3–1.5% sequence divergence per million years (Ma), suggests that the split between cis- and trans-Andean *Rhamdia* extends back about 8 Ma, and the three distinct *trans*-Andean *Rhamdia* clades split about 6 Ma ago. Thus the mtDNA divergence observed between cis- and trans-Andean *Rhamdia* species is too low to support an ancient colonization of Central America in the Late Cretaceous or Paleocene as had been hypothesized in one colonization model for Mesoamerican fishes. Rather the mtDNA data indicate that *Rhamdia* most likely colonized Central America in the late Miocene or Pliocene, promoting a strong role for the Isthmus of Panamá in the Mesoamerican expansion of this genus. Basal polytomies suggest that both the *R. laticauda* and *R. guatemalensis* clades spread rapidly across the Central American landscape, but differences in the average mtDNA genetic distances among clades comprising the two species, indicate that the *R. laticauda* spread and diversified across Mesoamerica about 1 million years before *R. guatemalensis*.

Keywords: Catfishes; Neotropical freshwater fishes; Mitochondrial DNA; ATP synthase; Cytochrome *b*; Phylogeography; Molecular systematics

1. Introduction

The primary freshwater fish fauna of Central America stands in stark contrast to the South American fauna owing to the lack of sympatrically distributed congeneric taxa. The generally depauperate nature of the Mesoamerican fish fauna indicates an absence of adaptive radiation, perhaps owing to the relatively short period of time since primary Neotropical fishes have colonized Central America (Bermingham and Martin, 1998; Miller, 1966; but see Bussing, 1976, 1975). The only primary freshwater fish genus having sympatrically distributed species north of Panamá is *Rhamdia*, but in the absence of a phylogeny for this group one cannot determine if these species represent an autochthonous radiation or independent colonizations of Central America from South America. Thus, a principal aim of this paper is to provide a phylogenetic hypothesis for *Rhamdia* to infer its history in Central America. Furthermore, because *Rhamdia* is comprised of multiple evolutionary lineages, the genus offers an unusual opportunity to study the timing of colonization, and the subsequent rate of dispersal and diversification across the Central American landscape.

The present study draws upon Silfvergrip's (1996) revision of *Rhamdia* in which the roughly 100 nominal

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species included in the genus were examined and reallocated to 11 species representing the genera's broad Neotropical distribution, extending from México to Argentina. Silfvergrip touched on points that hold general interest for systematists and particular relevance for our more confined study of *Rhamdia* molecular systematics. First, in his morphological analysis of *Rhamdia quelen*, resulting in the synonymy of roughly 40 nominal species, Silfvergrip noted "that different populations can be diagnosed successfully if, but only if, a small number of specimens from two sufficiently widespread populations are compared. Once a larger material with few 'geographic gaps' is filled in, apparent differences between distant populations disappear and a wide range of character state overlaps emerge." The lack of suitable reference material across 'geographic gaps,' or the absence of systematic analysis of available specimens, is a general problem when considering the systematics of tropical organisms (see, for example, Weitzman and Palmer, 1997). In other words, whether one compares such things as the behaviors or geographic distributions of tropical taxa, one needs to question how well current taxonomy describes the evolutionary distinctiveness and relationships of the taxa studied.

Second, Silfvergrip noted that traditional *Rhamdia* systematics is more efficient than phylogenetic systematics in constructing a classification owing to a much less restrictive use of characters in the former. Of course, efficiency does not replace veracity, which led Silfvergrip to emphasize the fact that most *Rhamdia* species had been diagnosed using elimination methods based on dichotomous identification keys. Thus, in his reappraisal of the genus he eliminated emphasis on characters that he determined were not useful cladistic characters, although he made no subsequent attempt to phylogenetically analyze *Rhamdia* species relationships. Here again, *Rhamdia* systematics identifies a general problem confronting Neotropical ichthyology. The prevailing taxonomy of Neotropical fishes relies to a large extent on morphometric and meristic characters that overlap in value between named species. Although there is a strong body of careful cladistic analysis of Neotropical fishes, this is generally carried out at higher taxonomic levels (e.g., Bockmann, 1994; Buckup, 1993; Vari, 1995; Weitzman et al., 1988). At present there are very few morphological studies at the level of the genus and below that provide a sufficient number of characters for thorough interpretation of species identity and relationship. Conflict arises when subjective taxonomic description, often intending to focus attention on populations of particular biological or conservation interest, fails to identify characters that satisfactorily diagnose a species. In this light it is interesting to note the recent description of a new *Rhamdia* species, *R. macuspanensis* from México (Weber and Wilkens, 1998), which specifically ignored Silfvergrip's revision of the genus including his well supported statements concerning conspecific morphological variation, in favor of drawing attention to the population's cave-dwelling natural history.

An apparently simple resolution to the nomenclatural conflict resulting from the lack of sufficient diagnostic characters is the collection of more data. In the case of *Rhamdia*, as we show beyond, mitochondrial DNA (mtDNA) data permit an objective and character-rich analysis of the evolutionary distinctiveness of Central American species and geographic populations. The classification dilemma, however, is less easily dispatched. Thus we utilize our phylogeny of *Rhamdia* to touch upon the classification of phylogenetic lineages across Central America, and the importance of classification for synthetic studies of the evolutionary and ecological processes that underlie patterns of diversity.

1.1. Systematic background

Silfvergrip's (1996) revision of the genus *Rhamdia* (family Pimelodidae) identifies three Central American species, *R. laticauda*, *R. nicaraguensis*, and *R. quelen* (= *R. guatemalensis* and related species of all previous Central American descriptions), whereas other treatments have recognized more than 13 Mesoamerican species (Behre, 1928; Bussing, 1975, 1998; Dahl, 1971; Espinosa et al., 1993; Greenfield and Thomerson, 1997; Hildebrand, 1938; Loftin, 1965; Martin, 1969; Meek and Hildebrand, 1916; Miles, 1947; Miller, 1984; Weber and Wilkens, 1998). Silfvergrip's *R. laticauda* and *R. quelen* are widespread along both the Pacific and Caribbean slopes of Central America, although the former is restricted to Central America, while the latter is found throughout South America (Silfvergrip, 1996, Figs. 19 and 23). *R. nicaraguensis* is known only from Nicaragua and Costa Rica (Bussing, 1998, Map 11; Silfvergrip, 1996, Fig. 20). *R. laticauda* and *R. nicaraguensis* are often associated with highland or more swiftly running streams, whereas *R. quelen* is the more abundant lowland form (Bussing, 1998; Silfvergrip, 1996; Villa, 1975).

Included in our phylogenetic analyses are five species that have been reassigned by Silfvergrip (1996) to *R. laticauda*, at least four species that he considers junior synonyms of *R. quelen* (including *R. guatemalensis*), and *R. nicaraguensis* (Appendix A, Fig. 1). Mexican *Rhamdia* represent some of the best-studied members of the group and exemplify the different taxonomic viewpoints among authors. In México, *R. quelen* (= *guatemalensis*) is found in the lowland reaches of rivers on both the Atlantic and Pacific slopes, including some troglophilic forms not accorded specific status (Espinosa et al., 1993; Miller, 1984; Weber and Wilkens, 1998). Thus authors are in general agreement regarding the evolutionary connectedness of geographic populations representing

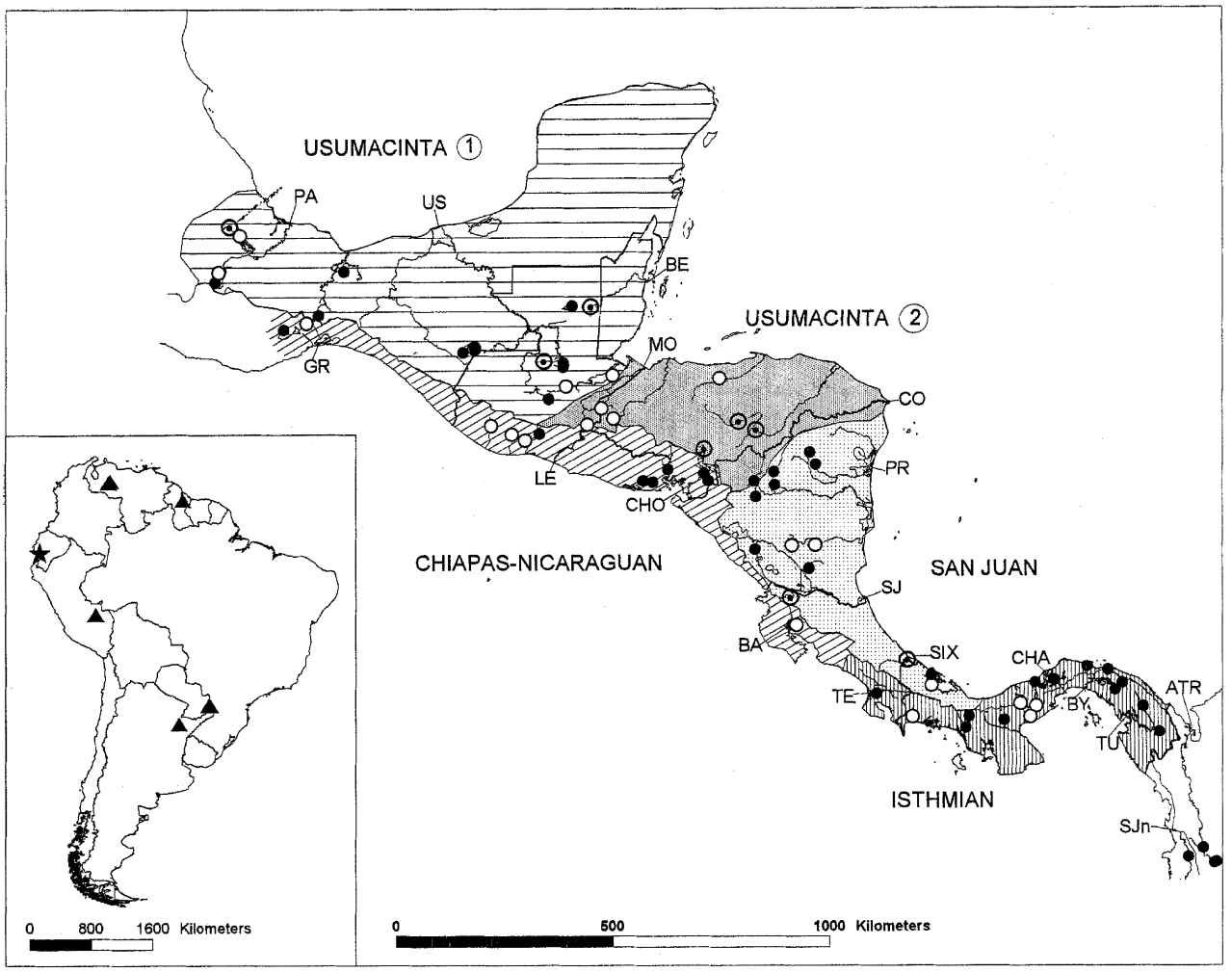

Fig. 1. Sample localities of the *Rhamdia* specimens analyzed. (⊙) symbolizes localities where *R. laticauda* and *R. guatemalensis* were collected in sympatry. Localities marking single species collections are noted as follows: (●) *R. guatemalensis*, (○) *R. laticauda*, (★) *R. cinerascens*, (▲) *R. quelen*. Ichthyological provinces follow Bussing (1976). River codes, Atlantic: PA, Papaloapan; US, Usumacinta; BE, Belize; MO, Motagua; CO, Coco; PR, Prinzapolka; SJ, San Juan; SIX, Sixaola; CHA, Chagres; ATR, Atrato; Pacific: GR, Grande; LE, Lempa; CHO, Choluteca; BA, Barranca; TE, Terraba; BY, Bayano; TU, Tuira; and SJn, San Juan.

the *R. quelen* (= *guatemalensis*) clade. This accord falls apart with respect to *R. laticauda* as defined by Silfvergrip (1996). All cave members of the *R. laticauda* group (*R. reddelli*, *R. zongolicensis*, and *R. macuspanensis*) are considered valid species by Weber and Wilkens (1998). Furthermore, *R. parryi* is recognized as a distinct species (Espinosa et al., 1993; Miller, 1984) with a widespread distribution in rocky stream habitats on the Pacific slope of México, whereas *R. laticauda* is widespread on the Atlantic versant.

Our molecular systematic analysis of *Rhamdia* includes the three Central America (CA) species recognized by Silfvergrip (1996), collected from 53 drainages across Central America (Fig. 1, Appendix A). Measured by the taxonomic indicators presented in Appendix A, the number of sampled Central American species increases to eight. Geographic outgroups for our analysis of CA *Rhamdia* were provided by specimens of *R. quelen* from four cis-Andean drainages in South America and the trans-Andean *R. cinerascens* (also assigned to *R. quelen* by Silfvergrip) from the Pacific slope of Ecuador. Taxonomic outgroups included specimens representing four additional genera in the family Pimelodidae: *Imparales*, *Nanchagresdia*, *Pimelodella*, and *Heptapterus*.

2. Materials and methods

Rhamdia and outgroup individuals were collected by electrofishing or seining. Fig. 1 summarizes sample locations, while details concerning the rivers sampled and their drainage locations are presented in Appendix A. Specimens were individually tagged with unique identification labels and small pieces of gill or muscle tissue were frozen in liquid nitrogen or were preserved in DMSO/EDTA buffer (Seutin et al., 1991) or 95% EtOH.

DNA voucher specimens and their associated lots were subsequently preserved in buffered formalin and have been deposited in the permanent collection at the Smithsonian Tropical Research Institute (STRI: Bermingham et al., 1997a) or in the Museo Nacional de Ciencias Naturales of Madrid, Spain (MNCN).

DNA was extracted from a small piece of tissue using standard CTAB/phenol/chloroform techniques (Palumbi et al., 1991; Sambrook et al., 1989). The entire ATP synthase 6 and 8 (ATPase 8/6) genes were PCR amplified using primers L8331 (5′-AAA GCR TYR GCC TTT TAA GC-3′) and H9236 (5′-GTT AGT GGT CAK GGG CTT GGR TC-3′) (http://nmg.si.edu/bermlab.htm) which bind to conserved tRNA sequences that flank the gene complex. In addition, the entire cytochrome *b* (cyt *b*) gene was PCR amplified with the primers GluDG.L (5′-TGA CCT GAA RAA CCA YCG TTG-3′) (Palumbi, 1996) and H16460 (5′-CGA YCT TCG GAT TAC AAG ACC G-3′) (http://nmg.si.edu/bermlab.htm) located in the glutamine and threonine tRNA flanking regions. In both genes double-stranded DNA was PCR synthesized in 25 μl reactions (2.5 μl 10 mM Tris–HCl buffer, 2 μl 2.0 mM $MgCl_2$, 1.5 μl 10 mM of each primer, 2.5 μl dNTP 200 nM of each dinucleotide, 14.25 μl ddH20, 1 μl template DNA, and 0.25 μl [1U] Amplitaq polymerase [Perkin–Elmer]). The following thermocycler conditions were used: initial preheat at 94 °C for 120 s, denaturation 94 °C for 45 s, annealing 53 °C for 45 s, extension 72 °C for 90 s, repeated for 5 cycles, followed by 29 cycles at 94 °C for 45 s, 58 °C for 45 s and 72 °C for 90 s. The PCR products were electrophoresed in 1.5% low melting point agarose gels using a Tris–acetate buffer (pH 7.8) containing 1 μg/ml of ethidium bromide. The single amplification product was visualized with UV light, cut from the gel, and digested with 1 μl Gelase (Epicentre Technologies) at 70 °C for 120 s, followed by overnight incubation at 45 °C. Five μl of a purified PCR product was used as template in a 10 μl cycle sequencing reaction using a dRhodamine terminator cycle sequencing kit (PE Applied Biosystems). In most cases, each PCR product was sequenced using the two amplification primers and one internal primer, Cb3H (5′ GGCAA ATAGG AARTA TCATT C 3′; Palumbi, 1996) for the cyt *b* gene, and 8.3 (5′ TGATAK GCRTG TGCTT GGTG 3′) (http://nmg.si.edu/bermlab.htm) for the ATPase 8/6 gene. Cycle sequencing conditions were as follows: initial preheat at 96 °C, 96 °C for 15 s, 50 °C for 1 s, and 60 °C for 4 min repeated for 25 cycles. The volume of the cycle sequencing products was doubled by the addition of 10 μl ddH20 and purified using Centrisep columns with 750 μl G-50 Sephadex. Samples were dried and resuspended with 1:5 blue dextran/EDTA (pH 8.0) and formamide.

In some cases, PCR products were cloned using the pGEM-T vector (Promega) into *Escherichia coli* JM109, and were sequenced using the FS-Taq Dye Deoxy Terminator cycle-sequencing kit (Applied Biosystems). DNA sequences of both strands were obtained using M13 universal (forward and reverse) sequencing primers.

All samples were sequenced on an Applied Biosystems 377 DNA sequencer following the manufacturer's instructions. Sequence analysis was performed with Sequencer ver. 3.0 (Gene Codes). Chromatograms and alignments were visually checked and verified and there were no gaps in the resulting DNA sequences.

2.1. Data analysis

Nucleotide composition was examined for variable sites with Sequencer 6.1 (http://nmg/si.edu), and the X^2 homogeneity test of base frequencies utilized PAUP* v.4.0b8 (Swofford, 2001). Nucleotide saturation was analyzed by plotting absolute number of transitions (Ti) and transversions (Tv) against absolute distance values. The aligned data for cyt *b* and ATPase 8/6 were analyzed independently using neighbor-joining (NJ) HKY 85 (Hasegawa et al., 1985), maximum likelihood (ML), and maximum parsimony (MP) methods. MP analysis was performed using heuristic searches with TBR branch swapping with Tv six times the weight of Ti. Different weighting schemes were tried (10:1, 8:1, 6:1, or equal weight). We used the program Model test 3.0 (Posada and Crandall, 1998) to find the best model of nucleotide substitution that fits our data for ML analyses. ML analyses based on empirical base frequencies and Ti/Tv ratio were done in PAUP* (quartet puzzling; Strimmer and von Haesler, 1996). All phylogenetic analyses were implemented using PAUP*. Bootstrap analysis (100 replicates) was used to assess the relative robustness of branches.

Congruence among tree topologies generated with the three mitochondrial genes was tested with the partition homogeneity test in PAUP* (Farris et al., 1994; Mickevich and Farris, 1981) for all possible combinations of the mitochondrial genes analysed (100 replicates).

Nucleotide substitution rate constancy for *Rhamdia* cyt *b* and ATPase 8/6 was evaluated using a X^2 test of the ML log-likelihood ratio of a clock-enforced and a non-enforced tree (Page and Holmes, 1998). Under the assumption of a constant rate of nucleotide substitution, we converted genetic distances calculated from the clock-enforced tree to absolute time using two different calibrations of fish mtDNA clocks. The first is a mtDNA cyt *b* calibration of 1.5% sequence divergence per million years (calculated using HKY 85 genetic distances) for freshwater fishes in the family Cyprinidae based on two well-dated earth history events: the closure of the Gibraltar Strait (5 Ma) and the formation of the Korintho Strait (2.5 Ma) (Zardoya and Doadrio, 1999). The second is a mtDNA ATPase6 calibration of 1.3%

sequence divergence per million years (calculated using K2P genetic distances) for marine fishes separated by the Pliocene closure of the Isthmus of Panamá (Bermingham et al., 1997b).

3. Results

The complete mitochondrial cytochrome *b* and ATPase 8/6 gene sequences were determined for 120 *Rhamdia* and seven individuals representing four additional pimelodid genera (*Heptapterus*, *Imparales*, *Nanchagresdia*, and *Pimelodella*) and have been deposited in GenBank (cyt *b*: AY036625-721; ATPase 8/6: AY036752-876). Cis-Andean South American specimens representing *R. quelen* demonstrated high levels of mtDNA sequence divergence from their trans-Andean counterparts when the *Rhamdia* tree is rooted using any of the other pimelodid genera, alone or in combination (Fig. 2). Thus, *R. quelen* sensu Silfvergrip (1996) is polyphyletic from a mtDNA perspective, and to simplify the ensuing results and discussion we will reserve *R. quelen* for the cis-Andean South American forms and use *R. guatemalensis* (Günther, 1864) for the Central American lowland *Rhamdia* and closely related trans-Andean forms. We refer to the distinctive trans-Andean species of Pacific Ecuador as *R. cinerascens* (Günther, 1860), species also synonymized under *R. quelen* by Silfvergrip (1996).

3.1. Molecular characterization of the Rhamdia mitochondrial cytochrome b and ATP synthase 8 and 6 genes

The molecular characterization and phylogenetic information content of each gene in the combined data set are presented in Table 1. The majority of variable and informative sites are third position substitutions, with somewhat higher proportions of change at ATPase 6 (25.3%) and cyt *b* (25.3%), in comparison to ATPase 8 (16.1%). Base frequencies were homogeneous across taxa ($X^2 = 51.6$ and 62.5 for ATPase 8/6 and cyt *b*, respectively, df = 378, $p = 1.000$). None of the genes exhibited stop codons when translated into their amino acid sequences. However, in comparison to the salmonid *Oncorhynchus mykiss* (Salmonidae; GenBank NC 001717) or the cyprinid *Cyprinus carpio* (Cyprinidae; GenBank NC 001606), the *Rhamdia* cyt *b* gene is one codon shorter.

Plots of transitions and transversions against uncorrected genetic distance indicated an absence of nucleotide saturation (data not shown). All plots were linear whether the data were plotted by position (first, second, and third codon positions), or by the full sequence of each gene. As expected the slopes of the plots differed, with third position transitions accumulating most rapidly, and cyt *b* and ATPase 6 demonstrating somewhat higher substitution rates than ATPase 8. Thus, all nucleotide positions were employed in the ensuing phylogenetic analysis of *Rhamdia*.

3.2. Molecular systematics of Central American Rhamdia

Independent analyses of the partitioned mitochondrial genes and of the combined data produced congruent phylogenies whether undertaken with NJ, MP, or ML methods. MP trees obtained using 10:1, 8:1, 6:1, or equal Ti/Tv weights were also very similar, and we base the following MP results on a 6:1 Ti/Tv weight, following the empirically determined Ti/Tv ratio for *Rhamdia*. The HKY 85 model of molecular evolution with empirical base frequencies, invariant sites (0.5), and gamma equal to 0.9 were used for ML analyses based on a ModelTest analysis of 56 alternative nucleotide substitution models. Because the partition homogeneity test revealed no significant differences among any of the compared character partitions (ATPase 8 vs ATPase 6 $p = 0.2$; ATPase 8 vs cyt *b* $p = 0.7$; ATPase 6 vs cyt *b* $p = 0.4$; ATPase 8/6 vs cyt *b* $p = 0.9$), our results and the subsequent analyses are based on the combined cyt *b* and ATPase 8/6 data. The combined DNA sequence data for all taxa contained 865 variable characters, of which 711 were phylogenetically informative; excluding outgroups, there were 658 variables, and 531 informative characters.

Our phylogenetic analyses identified four major mtDNA clades representing South and Central American *Rhamdia*. Sequential outgroup analyses identified *Pimelodella* as a near outgroup to *Rhamdia*, and indicated that *Heptapterus*, *Imparales*, and *Nanchagresdia* outgroups were all more distantly related (Fig. 2). *Rhamdia* individuals ($n = 8$) distributed from the Orinoco drainage in Venezuela to the Paraná drainage in Argentina comprised the 'South American' *Rhamdia* clade, which we refer to as the *R. quelen* mtDNA clade or cis-Andean *Rhamdia*. Within this major mtDNA clade, we observed considerable mtDNA divergence between *R. quelen* inhabiting different drainages along the Atlantic slope of South America. For example *R. quelen* from the Río Orinoco ($n = 2$) was 8.3% diverged from conspecifics collected in the Río Manu ($n = 2$).

All other *Rhamdia* grouped in three monophyletic groups that we refer to as the *R. guatemalensis*, *R. laticauda*, and *R. cinerascens* mtDNA clades. Although all analyses suggested a sister group relationship between *R. cinerascens* and *R. laticauda*, we treat the relationship between these species and *R. guatemalensis* as unresolved because bootstrap support for the resolved MP tree was low (66%; but compare to ML, 97%, and NJ, 89%). *Rhamdia cinerascens* mtDNA, on average, was 7.9% diverged from *R. laticauda*, and 7.3% from *R. guatemalensis*. All individuals collected from México through Colombia and Lago Maracaibo, Venezuela fell

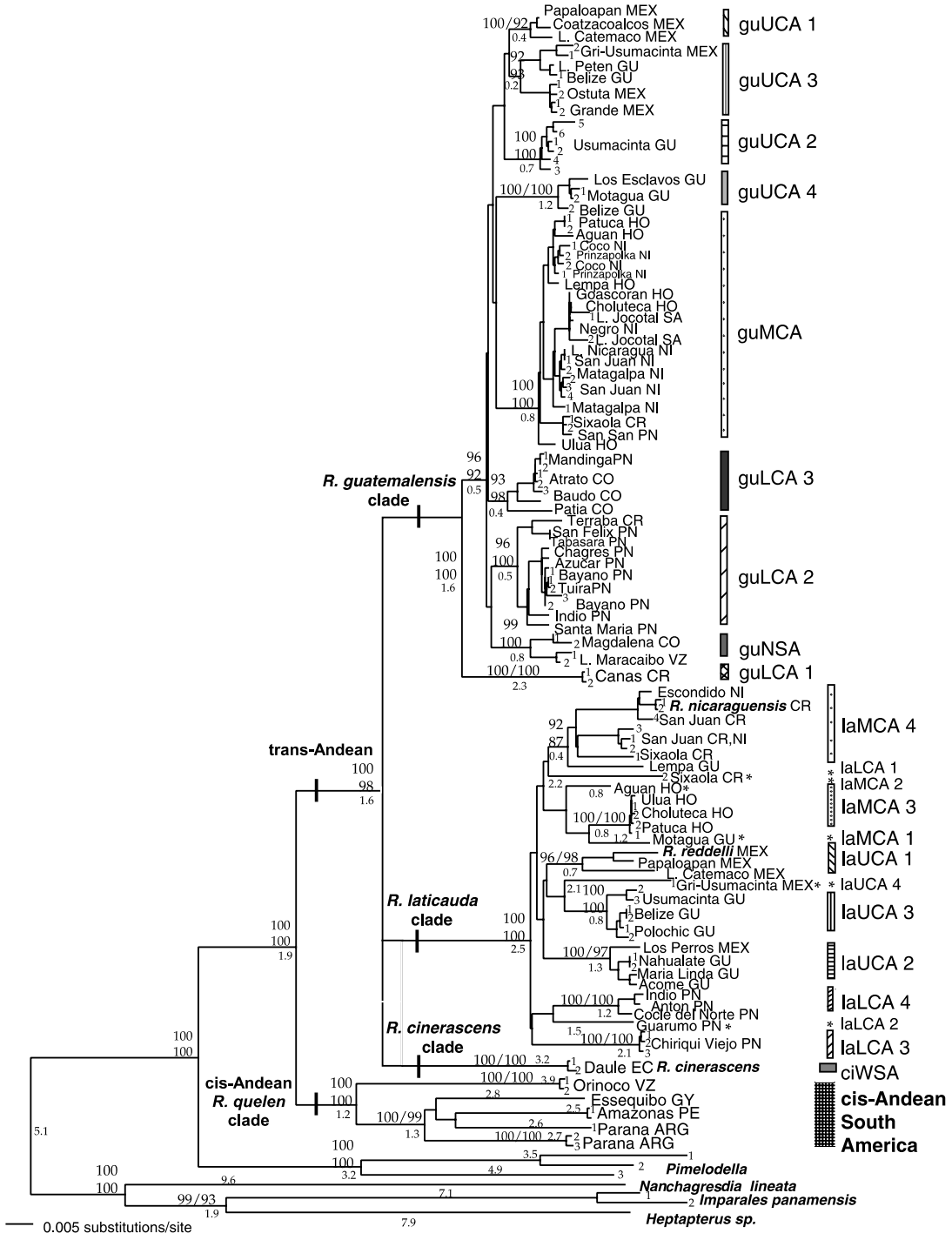

Fig. 2. *Rhamdia* phylogenetic relationships based on the combined mitochondrial DNA ATPase 8/6 and cyt *b* genes. The phylogeny is the single tree recovered using NJ analysis (HKY 85, empirical base frequencies). The weighted parsimony tree (6Ti:1Tv, TL = 5762, CI = 0.531) was highly congruent and identified the same named mtDNA lineages. Upper values on the branches represent NJ bootstrap estimates, and lower values represent MP estimates, based on 100 replicates in both cases. Only bootstrap values > 80% representing named mtDNA lineages are shown. HKY 85 genetic distance values are provided below branches. Named mitochondrial lineages referred to in the text are listed to the right: gu = *guatemalensis*, la = *laticauda*, and ci = *cinerascens*. Single individuals representing a named mtDNA lineage are marked with an *.

into the *R. laticauda* or *R. guatemalensis* mtDNA clades, separated by an average mtDNA distance of 8.4%. Both the *R. laticauda* and the *R. guatemalensis* mtDNA clades demonstrated significant phylogeographic structure, although there was a general lack of phylogenetic resolution among conspecific mtDNA lineages (Fig. 2). Nucleotide saturation plots (results not shown) indicated that unresolved relationships did not result from

Table 1
Molecular characterization of the mitochondrial cytochrome *b* and ATP synthase 8 and 6 genes in the genus *Rhamdia*

	ATPase 8	ATPase 6	Cyt *b*
Nucleotide composition			
% G	11.5	12.5	13.5
% A	35.5	30.5	27.7
% T	25.5	26.6	27.6
% C	27.5	30.4	31.2
Nucleotide bias			
First position	12.1	21.1	5.3
Second position	18.8	32.2	22.9
Third position	33.2	30.4	39.3
Fourfold sites	40.6	37.4	44.1
Total	18.0	16.7	15.3
% Variable sites			
First position	8.3 (14)	9.2 (63)	7.1 (81)
Second position	6.5 (11)	3.1 (21)	2.5 (29)
Third position	16.1 (27)	25.3 (173)	25.3 (288)
Fourfold sites	5.4 (9)	11.5 (74)	10.8 (123)
Total	30.9 (52)	37.6 (257)	35.0 (398)
% Phylogenetically informative sites			
First position	5.4 (9)	6.0 (41)	4.0 (46)
Second position	1.2 (2)	1.0 (7)	0.6 (7)
Third position	10.7 (18)	23.2 (159)	21.7 (242)
Fourfold sites	2.4 (4)	10.8 (74)	9.1 (104)
Total	17.3 (29)	30.1 (207)	25.9 (295)
Ti/Tv ratio	4.5	7.4	8.4
α value	0.3	0.2	0.2

Values represent percentages with absolute numbers in parentheses. Ti = transitions, Tv = transversions.

nucleotide saturation. Thus, the short internodes connecting conspecific, but divergent, mtDNA lineages indicate that the expansion and diversification of *R. guatemalensis* and *R. laticauda* across Central America occurred over a generally shorter time period than that required for the appearance and fixation of a novel mutation.

3.3. Phylogeography of Rhamdia

3.3.1. Rhamdia guatemalensis clade

Seventy-one *R. guatemalensis* individuals representing both slopes and 41 river drainages from México (MEX) to Colombia (COL) and Lago Maracaibo in Venezuela (VZ) were analyzed for the complete cyt *b* and ATPase 8/6 genes (Appendix A, Fig. 1). MP, ML, and NJ-based phylogenies identify two *R. guatemalensis* mtDNA sister lineages: a Pacific slope, Costa Rican lineage (guLCA 1), and a second group of mtDNA haplotypes including at least eight other mtDNA lineages (guUCA 1, guUCA 2, guUCA 3, guUCA 4, gu MCA, guLCA 2, guLCA 3, guLCA 4, guNSA) (Fig. 2). Interestingly, the lineages within this second *R. guatemalensis* group ($n = 69$) were structured according to a latitudinal pattern clearly separating the Upper Central American (UCA) and Lower Central American (LCA) mtDNA haplotypes (Figs. 2 and 3). The nine mtDNA lineages in the second *R. guatemalensis* group are supported in all cases by bootstrap values $> 80\%$. Listed from north to south they are: (a) guUCA 1, which includes all individuals ($n = 3$) collected from the Atlantic versant of México north of the Isthmus of Tehuantepec; (b) guUCA 2 ($n = 6$) which includes most of the individuals collected in the Usumacinta drainage; (c) guUCA 3 ($n = 8$) from the Pacific and Atlantic versants of México and Guatemala as far south as the Río Belize; (d) guUCA 4 ($n = 4$), found in rivers draining both slopes of Guatemala; (e) guMCA ($n = 25$), a middle Central America lineage collected from Pacific slope rivers of Honduras, El Salvador and Nicaragua, and Atlantic slope rivers from Honduras south to the Bocas del Toro region of Panamá; (f) guLCA 2, including all *R. guatemalensis* ($n = 12$) collected along the Pacific slope from Río Terraba, Costa Rica south throughout Panamá, and along the Atlantic versant of Panamá from the Río Indio to the Río Azúcar in Kuna Yala; (g) guLCA 3 ($n = 7$), found in the Río Mandinga, Panamá, and the Choco region of Colombia (Ríos Atrato, Baudo, and Patia); (h) guNSA ($n = 4$), a Northern South American lineage collected from the Río Magdalena in the Atlantic versant of Colombia and Lago Maracaibo, Venezuela.

In addition to the named mtDNA clades, the mtDNA data also evidence phylogeographic structure within each named clade, although we will not present these results in detail. Sequence divergence among specimens from the same locality is low or absent in most of cases. One exception is the Río Belize in Guatemala where we collected two *R. guatemalensis* specimens with a sequence difference of 2.9% that fall in different named mtDNA lineages, guUCA 3 and guUCA 4. In most cases, however, nearest genetic neighbors on the trees are also nearest geographic neighbors. The average genetic distance among haplotypes representing a named mtDNA lineage was 0.8%, with guLCA 1 demonstrating the lowest mean intralineage distance (0.05%), and guMCA and guLCA 2 showing the largest mean distance (1.04%). The weighted average distance among named *R. guatemalensis* mtDNA lineages, excluding guLCA 1, was 2.6%; the distance between guLCA 1 and its sister clade was 4.3%.

3.3.2. Rhamdia laticauda clade

The complete cyt *b* and ATPase 8/6 genes were sequenced for 39 *R. laticauda* individuals representing 24 river drainages extending along both Central American slopes from México to Panamá (Appendix A, Fig. 1). MP, ML, and NJ analyses identified 12 lineages

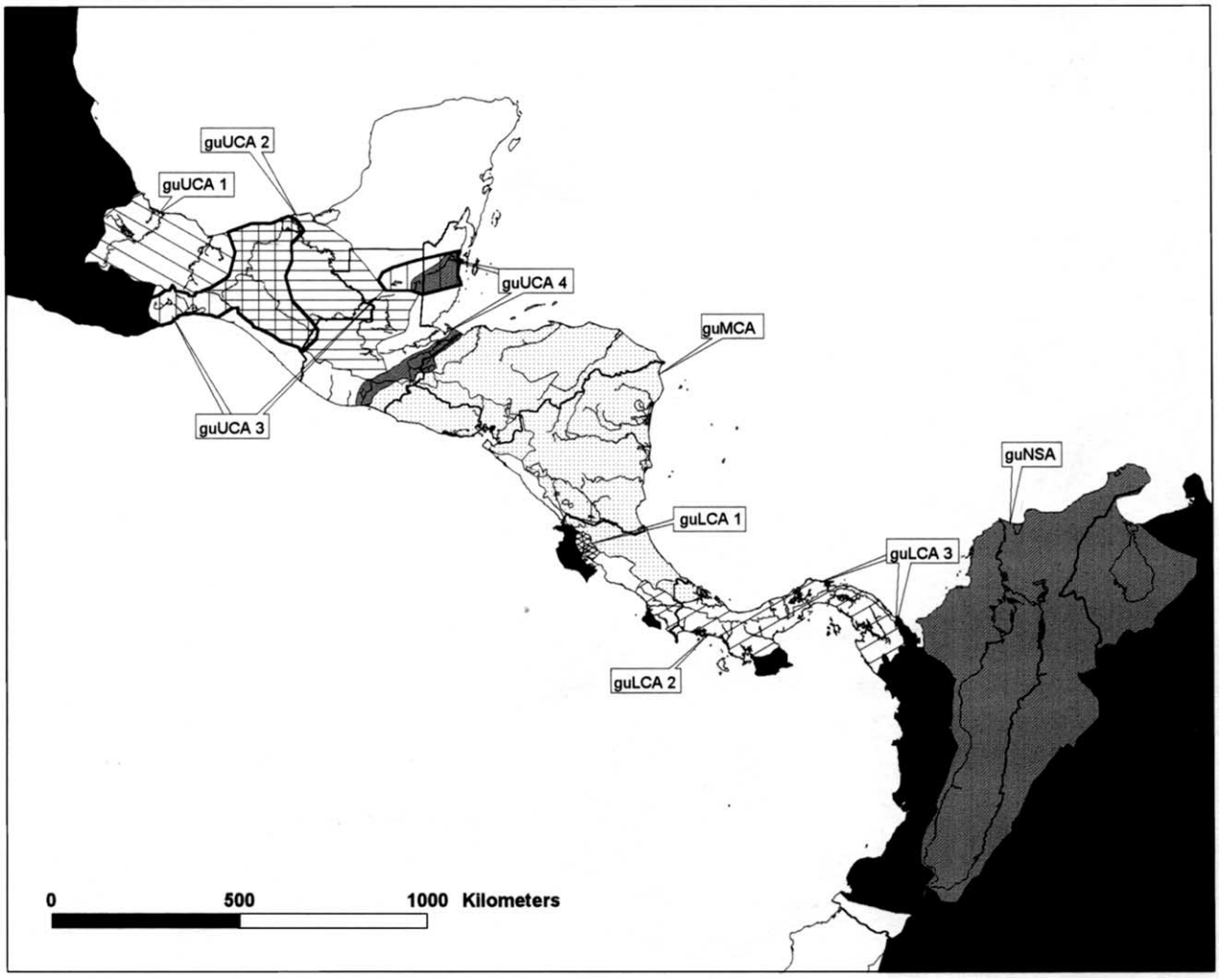

Fig. 3. Map showing the geographical distribution of the *R. guatemalensis* mtDNA lineages. The map fill patterns match the named mtDNA clade patterns presented in Fig. 2. UCA, MCA, LCA abbreviate Upper, Middle, and Lower Central America, respectively and NSA for northwestern South America. Black fill indicates areas outside the putative distribution of *R. guatemalensis*, whereas white fill marks areas included in the distribution of *R. guatemalensis*, but where we failed to collect the species.

supported by bootstrap values exceeding 80%. Similar to *R. guatemalensis*, *R. laticauda* named mtDNA lineages are latitudinally structured. In contrast to *R. guatemalensis*, however, the northern-most lineages of *R. laticauda* exhibit phylogeographic structure on either side of the continental divide (Fig. 4). The 12 named *R. laticauda* lineages are (listed from North to South): (a) laUCA 1 ($n = 3$), includes *R. reddelli*, a cave species from the Papaloapan drainage, epigean forms from the same drainage and the adjacent Lago Catemaco; (b) laUCA 2 ($n = 5$), collected only in Pacific slope drainages from México south to Honduras (Nahualate, Acomé, María Linda, and Los Perros drainages); (c) laUCA 3 ($n = 7$), found only in the Atlantic slope rivers from México to Guatemala (Usumacinta, Belize, and Polochic drainages), (d) laUCA 4 ($n = 1$), represents the Grijalva tributary of the Usumacinta drainage; (e) laMCA 1 ($n = 1$), is represented by a single individual collected from the Río Motagua; (f) laMCA 2 ($n = 1$), found only in the Río Aguan; (g) laMCA 3 ($n = 5$), representing *R. laticauda* collected from the Ríos Ulua and Patuca on the Atlantic slope of Honduras and the Río Choluteca on the Pacific slope; (h) laMCA 4 ($n = 9$) which includes the Río Lempa on the Pacific versant of Honduras, and the Ríos Escondido and San Juan in the Atlantic slope of Nicaragua, and rivers representing both slopes of Costa Rica; (i) laLCA 1 ($n = 1$), representing a unique individual collected from the Río Sixaola on the Atlantic versant of Costa Rica; (j) laLCA 2 ($n = 1$), representing Río Guarumo on the Atlantic slope of Panamá in the Bocas del Toro region; (k) laLCA 3 ($n = 3$) collected only from the Río Chiriquí Viejo on Panamá's Pacific slope; (l) laLCA 4 ($n = 3$), representing individuals collected from the Río Indio and Coclé del Norte, Atlantic slope, and the Río Antón, Pacific slope, in central Panamá (Figs. 2 and 4).

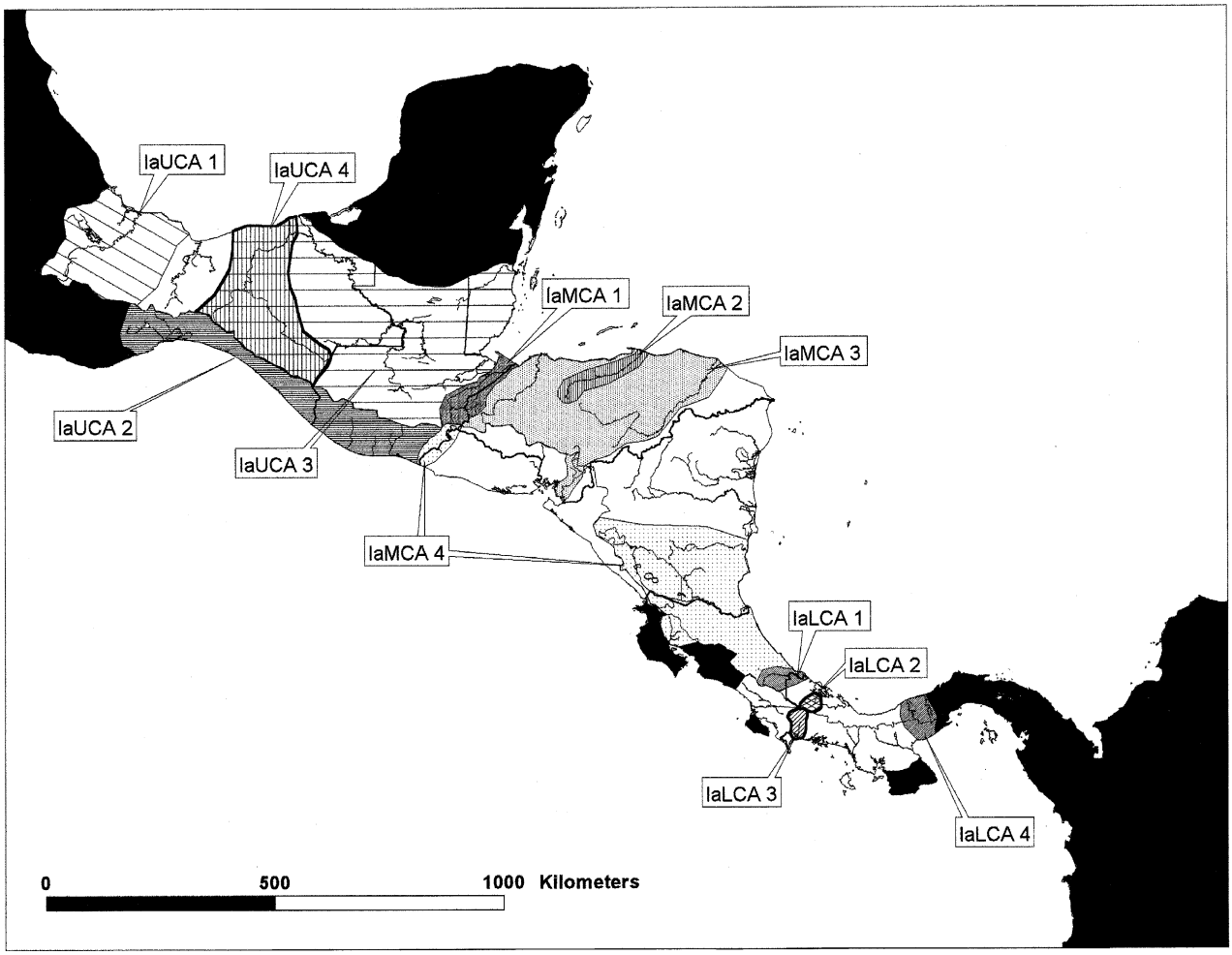

Fig. 4. Map showing the geographical representation of the *R. laticauda* mtDNA lineages. The map fill patterns match the named mtDNA clade patterns presented in Fig. 2. UCA, MCA, LCA abbreviate Upper, Middle, and Lower Central America, respectively and NSA for northwestern South America. Black fill indicates areas outside the putative distribution of *R. laticauda*, whereas white fill marks areas included in the distribution of *R. laticauda*, but where we failed to collect the species.

As was the case with *R. guatemalensis*, sequence divergence among specimens from the same locality is generally low or absent. The single exception is the Río Sixaola, forming the Atlantic slope border between Costa Rica and Panamá, where we collected two *R. laticauda* that differ by 3.6% in their mitochondrial sequence and represent two named mtDNA lineages, laMCA 4 and laLCA 1. The average genetic distance among haplotypes representing a named mtDNA lineage was 1.5%, with laUCA 1 and laMCA 4 showing the largest distance (2.1%) and laLCA 3 the lowest (0.7%). The weighted average distance among named *R. laticauda* mtDNA lineages was 3.8%.

3.4. Rate constancy and time of evolution

A log-likelihood test based on the 37 taxa presented in Fig. 5, or two individuals representing the extreme of intra-clade variation from each of the principal mtDNA clades (except *R. cinerascens* for which we used only a single representative) failed to reject the null hypothesis of rate constancy ($-\ln = 11466.01$ not enforced tree and 11490.43 enforced tree, $\chi^2 = 48.84$, $df = 35$, $p > 0.05$). Fig. 5 represents a ML tree constructed under the assumption of a molecular clock and the HKY85 + G + I model of molecular evolution.

4. Discussion

The lack of phylogenetic hypotheses for species relationships across most Neotropical primary freshwater fishes limits our ability to evaluate alternative models for the diversification of this incredibly species rich vertebrate fauna (Weitzman and Malabarba, 1998). Furthermore, the small number of studies that have used genetic data to interpret the phylogenetic history of these fishes have indicated that the taxonomy of Neotropical freshwater fishes often distorts our view of species-level relationships (Bermingham and Martin,

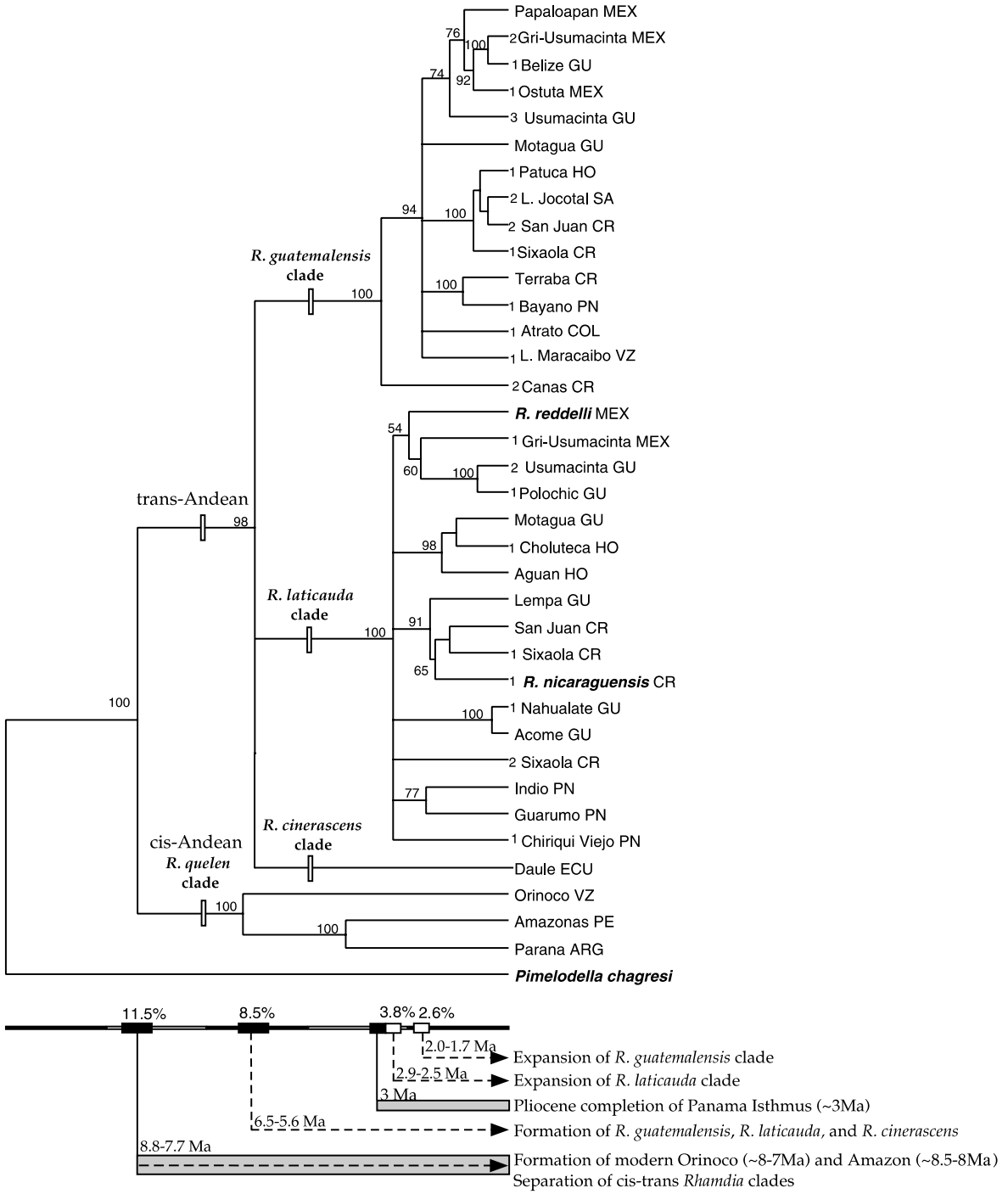

Fig. 5. *Rhamdia* phylogenetic relationships based on a molecular clock-constrained maximum likelihood analysis of the combined mitochondrial DNA ATPase 8/6 and cyt *b* genes. The values on the branches represent bootstrap estimates based on 100 replications. The scale bars present HKY 85 genetic distances (%) (above) and time (Ma) (below) for the major cladogenetic events in the history of Central America *Rhamdia*. The time scale is based on fish mtDNA clock calibrations from the literature (Bermingham et al., 1997b; Zardoya and Doadrio, 1999). Geologically dated times for the formation of the modern Orinoco and Amazon rivers (and putative completion of the Andean drainage divide) and the Pliocene completion of the Isthmus of Panama are also identified on the scale bar.

1998; Bermingham et al., 1997b; Breden et al., 1999; Martin and Bermingham, 1998; Murphy et al., 1999; Reeves and Bermingham, submitted; Sivasundar et al., 2001). Molecule-based phylogenies, in general, have provided more objective assessments of the evolutionary history of closely related Neotropical freshwater fish lineages and their geographic distribution. Within this framework, the study of *Rhamdia* is particularly interesting because it represents the only phyletic lineage of primary freshwater fish that all authors agree is

represented by two or more sympatric clades across the breadth of Central America (Bussing, 1975; Silfvergrip, 1996). The *Rhamdia* phylogeny presented here provides the means to evaluate models of freshwater fish diversification across Central America, including the colonization models of Myers (1966), Bussing (1976, 1975), and Bermingham and Martin (1998).

Our phylogenetic analysis of the complete mtDNA cytochrome *b* and ATPase 8/6 genes strongly supports the separation of Central American *Rhamdia* into two monophyletic clades. The evolutionary distinctiveness evidenced by the molecule-based phylogeny for *Rhamdia* appears to perfectly match the ecological separation that investigators have long noted between the highland and lowland forms of this genus in Central America (Bussing, 1975; Miller, 1966; Villa, 1975). In addition, our results correspond well with the morphological distinctiveness of the two clades presented by Silfvergrip (1996) in his revision of the genus. However, our mtDNA results differ in two important respects from Silfvergrip's view of Central American *Rhamdia* systematics.

First, Silfvergrip notes that Central America represents the northern extreme of the widespread *R. quelen*, which is otherwise distributed along both slopes to Argentina in the east and Ecuador in the west. The mtDNA data do not support this view and indicate that Mesoamerican *R. quelen* are phylogenetically distinct from South American individuals considered conspecific by Silfvergrip. Appraisal of the evidence currently available would suggest that Silfvergrip's *R. quelen* has an entirely cis-Andean distribution, and that the trans-Andean representatives of his *R. quelen* are evolutionarily distinct. Because the type specimen was collected in Montevideo (now Uruguay, formerly Brazil), we reserve *R. quelen* for the South American cis-Andean taxon that is represented in our study by specimens from the three principal Atlantic slope drainages of South America, the Orinoco, Amazon, and Paraná. We use *R. guatemalensis* (Günther, 1864) to reference the mtDNA clade that represents the lowland *Rhamdia* species collected from Central America, and trans-Andean Colombian and Lago Maracaibo drainages, and *R. cinerascens* (Günther, 1860) for the *Rhamdia* clade collected from the Pacific slope of Ecuador. These are the oldest synonyms from specimens that can be confidently assigned to Central American and Ecuadorian Pacific slope collecting localities, respectively. Our Colombian trans-Andean collections of *Rhamdia* from the Atlantic slope drainages of Lago Maracaibo, Magdalena, and Atrato and the Pacific slope drainages of Baudo and Patia are clearly part of the *R. guatemalensis* mtDNA clade, suggesting that *R. cinerascens* has a fairly narrow trans-Andean distribution.

Second, the mtDNA data do not support Silfvergrip's view regarding the evolutionary equivalence of *R. nicaraguensis* with respect to *R. laticauda* and *R. guatemalensis*. Rather, our small sample ($n = 2$) of *R. nicaraguensis* clearly falls within the *R. laticauda* mtDNA clade. Furthermore, these two *Rhamdia* specimens collected from the Pacific slope Barranca drainage in northern Costa Rica are very closely related (0.7% mtDNA sequence divergence) to individuals from the Atlantic slope San Juan and Escondido drainages assigned to *R. laticauda* on the basis of adipose fin measurements (results not shown). *Rhamdia nicaraguensis* and *R. laticauda* both lack the serrae on the anterior margin of pectoral fin spine that characterize *R. guatemalensis*, and are distinguished on the basis of head characteristics and the relatively longer barbels and adipose fin of *R. nicaraguensis*. However, Tables 29 and 31 of Silfvergrip (1996) presenting morphometric measures for *R. laticauda* and *R. nicaraguensis*, respectively, demonstrate considerable overlap in all measured characters, except the length of the adipose fin.

Thus, the perspective offered by the mtDNA-based phylogeny indicates that there are two, not three, *Rhamdia* clades of *equivalent* evolutionary status in Central America, and the morphological data do not appear to strongly contradict this view (Silfvergrip, 1996; Villa, 1975). Our emphasis on equivalent should be clearly noted as it refers to the reciprocal monophyly of the mtDNA clades that we refer to as *R. laticauda* and *R. guatemalensis*. Inspection of Fig. 2 identifies a number of additional *Rhamdia* mtDNA lineages whose evolutionary distinctiveness is supported by their genetic divergence and high levels of bootstrap-based confidence estimates ($> 80\%$). But each of these additional *Rhamdia* lineages clearly nests within either the *R. laticauda* or the *R. guatemalensis* clade. For convenience we recognize *R. laticauda* and *R. guatemalensis* as species, and use the pictured phylogenies and distribution maps to convey the geographic and phylogenetic separation among the different evolutionary lineages within the two species. It could equally be argued that these two clades should be elevated to genera. Such a policy might preserve the taxonomic status of some described *Rhamdia* species, thus drawing increased attention to their unique natural histories and geographies. However, and by example, to draw attention to the cave habit of *R. reddelli* by preserving its species status would suggest that more than 20 additional species should be recognized in the *R. laticauda* clade alone if a taxonomic goal is to fairly represent evolutionary history. Ultimately, reconciling our phylogenetic analysis of Central American *Rhamdia* with the systematic classification of these fishes will depend on the objectives of the revised taxonomy.

Most Central America drainages appear to harbor only a single *R. guatemalensis* mtDNA lineage and one *R. laticauda* lineage. For example, guMCA was the only *R. guatemalensis* mtDNA lineage collected from the majority of Honduras and Costa Rican rivers and all

Nicaraguan rivers, and laMCA 4 was the sole representative of *R. laticauda* in many of the same rivers (Figs. 3 and 4). Generally, there was less sympatry of moderately diverged conspecific mtDNA lineages across lower Central America, than we observed in upper Central America. In lower Central America the single case of sympatry was the syntopic collection of the *R. laticauda* lineages from the Rio Sixaola (laLCA 1 and laLCA 2). In upper Central America, the Usumacinta drainage harbored sympatric lineages representing both *R. laticauda* (laUCA 3 and laUCA 4) and *R. guatemalensis* (guUCA 2 and guUCA 3), and we also sampled two *R. guatemalensis* mtDNA lineages from the Rio Belize (guUCA 3 and guUCA 4).

4.1. Historical biogeography

Our discussion of *Rhamdia* biogeography focuses on the mtDNA lineages named in Fig. 2 and mapped in Figs. 3 and 4. Our tests of mtDNA evolutionary rate homogeneity failed to reject a molecular clock for the genus *Rhamdia*, suggesting that mtDNA divergence can be used in a comparative context to evaluate both the pattern and timing of *R. guatemalensis* and *R. laticauda* diversification across Central America.

The geographic distribution of *Rhamdia*'s congeners in the family Pimelodidae indicates that the genus *Rhamdia* originated in South America. Our phylogenetic analysis provides no additional insight regarding the geographic origin of the genus (Fig. 2), owing to the fact that one sister group is widespread in South America (the cis-Andean, *R. quelen*), whereas the other is found throughout Central America (*R. guatemalensis* and *R. laticauda*) and in trans-Andean South American localities (*R. guatemalensis* and *R. cinerascens*). The mtDNA-based phylogeny supports a cis-, trans-Andean split in the genus, and indicates that separation of *R. guatemalensis*, *R. laticauda*, and *R. cinerascens* occurred after that split. The mean level of mtDNA sequence divergence separating cis- and trans-Andean *Rhamdia* is 11.5% (range, 9.8–13.4%), and application of mtDNA molecular clocks determined for fish by Bermingham et al. (1997b) and Zardoya and Doadrio (1999) indicate that the age of this split was between 8.8 and 7.7 (6.5–10.3) Ma. These mtDNA-based dates match geologically based ages for the final uplift of the Andes and the formation of the modern Amazon (~8.5–8 Ma) and Orinoco (~8–7 Ma) (Lundberg et al., 1998; and references therein), suggesting that the completion of the Andes caused the split between cis- and trans-Andean *Rhamdia* clades. Our data indicate that the separation between *R. guatemalensis*, *R. laticauda* and *R. cinerascens* occurred roughly 2 million years later. Mean sequence divergence between these clades is 8.5% (range, 6.3–9.6%), or 6.5–5.6 (4.3–7.4) Ma, according to the two fish-based mtDNA clocks.

The mtDNA divergence between cis- and trans-Andean *Rhamdia* is too low to support Bussing's (1975) model of Late Cretaceous or Paleocene (roughly 75–65 Ma) arrival of "Old Southern" elements such as *Rhamdia* to Central America via the hypothetical proto-Antillean land bridge. Rather the mtDNA data support the models of Myers (1966) and Bermingham and Martin (1998) indicating a Pliocene or late Miocene colonization of Central America by all primary freshwater fish derived from South American ancestors. Although molecular clocks are notoriously difficult to validate, the extreme difference (> 50 Ma) in the temporal predictions of the Bussing vs the Myers and Bermingham/Martin (B/M) models permits confidence that the mtDNA data can reliably discount the Late Cretaceous or Paleocene date of Central American colonization by *Rhamdia*, and thus its inclusion in Bussing's "Old Southern" element.

It also appears that *Rhamdia* colonized Central America considerably after *Rivulus* and the heroine cichlids, two groups of secondary freshwater fishes apparently capable of at least rare dispersal via brackish or marine colonization routes. Recent molecular systematic treatments of *Rivulus* (Murphy et al., 1999) and Central American heroine cichlids (Martin and Bermingham, 1998; Roe et al., 1997) indicate that either the mitochondrial clocks for these groups are ticking considerably faster than the *Rhamdia* mtDNA clock, or that the arrival of the ancestors of contemporary *Rivulus* and heroine cichlids in Central America predated the arrival of *Rhamdia* by roughly 4–8 million years. Application of the same mtDNA clock calibrations used in this study would set the colonization of Central America by heroine cichlids at 11.3–13 Ma, and by *Rivulus* at 15.9–18.4 Ma.

Paleogeographic reconstructions of the lower Central American isthmus published by Coates and Obando (1996) indicate that the deep-water connection between South America shallowed some time between the middle to late Miocene (15–6 Ma), with the emerging Panamá Isthmus forming an island chain across the shallow sea separating northwestern Colombia and nuclear Central America. This landscape might have been suitable for the dispersal of salinity-tolerant secondary freshwater fishes, but not for primary fishes until a complete connection was established between South and Central America. The Coates and Obando (1996) description of the geological evolution of the Panamá Isthmus establishes completion of the terrestrial corridor roughly 3 Ma, whereas Bermingham and Martin's colonization model posits a short-lived corridor during the late Miocene low sea level stand (5.3–5.7 Ma). Slight differences among the landscape models notwithstanding, both are largely consistent with the molecular systematic records regarding the geographical opportunities and relative timing of dispersal into Central America for primary and secondary freshwater fishes.

The widespread Central American distribution of both *R. guatemalensis* and *R. laticauda* mtDNA clades makes it difficult to determine their geographic origin and cause of their cladogenesis. Nonetheless, the phylogenetic analyses presented in Figs. 2 and 5 permit some speculation regarding these themes.

The first point of comparison between the Central American *Rhamdia* clades is the apparent restriction of *R. laticauda* to Central America in contrast to the broader distribution of *R. guatemalensis* from México south to Colombia. Despite extensive sampling, no *R. laticauda* have been recorded from eastern Panamá (Loftin, 1965; Meek and Hildebrand, 1916; Miles, 1947; Silfvergrip, 1996) and the absence of this species in our collections from suitable habitats and elevations in eastern Panamá and Colombia provides modestly robust evidence that the southeastern extent of *R. laticauda* lies near El Valle de Antón in Central Panamá. The *R. laticauda* distribution data indicate that this species originated in Central America, and our estimated date of separation between *R. laticauda* and *R. guatemalensis* (6.5–5.6 Ma) would seem to establish a late Miocene *Rhamdia* presence in Central America, conforming to the first wave of freshwater fish colonization hypothesized in the B/M model. Soon thereafter *Rhamdia*'s range would have been sundered by the Pliocene sea level rise (Haq et al., 1997), isolating one group in northwestern South America and the other in Costa Rica's Talamanca region (which would have remained emergent but cut off by marine seaways to the north and south). Under this scenario, allopatric separation led to the formation of *R. laticauda* in the north and *R. guatemalensis* in northern South America. The Central American spread of the *R. guatemalensis* clade includes South American representatives, thus raising the possibility that the expansion of this group initiated in South America.

The mtDNA-based phylogeny provides more information regarding the spread and subsequent diversification of the two Central American *Rhamdia* clades. In each case, mtDNA lineages representing virtually the complete geographic range of *R. laticauda* and *R. guatemalensis* form basal polytomies, indicating that each clade spread rapidly across the Mesoamerican landscape, followed by subsequent diversification in situ. In addition, differences in the average genetic distances among mtDNA lineages representing the two Central American *Rhamdia* clades suggest a slightly earlier spread of *R. laticauda*. On average, *R. laticauda* lineages are about 1.1% more divergent than *R. guatemalensis* lineages (Fig. 5), indicating that this clade might have spread through Central America roughly one million years prior to *R. guatemalensis*. Given the absence of information regarding the effective population sizes of the ancestral species, the accuracy of such a conclusion remains highly uncertain (Edwards and Beerli, 2000) and represents a hypothesis that is difficult to test.

The general picture emerging from the small number of molecular systematic studies of Central American fish is that they have dispersed and diversified rapidly, probably soon after initial colonization. This appears to be the case for earlier colonists such as cichlids (Martin and Bermingham, 1998; Roe et al., 1997) and *Rivulus* (Murphy et al., 1999), and for the later arriving siluriform and characiform fishes (Bermingham and Martin, 1998; Martin and Bermingham, 2000; Reeves and Bermingham, submitted).

Given the different postulated times of colonization and spread of Central American fishes, one might anticipate that each phyletic lineage would interact with a changing landscape in an idiosyncratic fashion, thus diminishing the likelihood of observing common geographic patterns. But this is not completely the case, and the two Central American *Rhamdia* clades share zoogeographic boundaries with one another, and more generally with the regional fauna. *R. laticauda* and *R. guatemalensis* both reach slightly north of the Isthmus of Tehuantepec, México, which represents the northern boundary for many tropical organisms (Bussing, 1975; Hill, 1962; Savage, 1966). Phylogenetic evidence indicates that both *Rhamdia* clades apparently reached this boundary, or close to it, during their initial spread through Central America, indicating that *Rhamdia*'s continued northward dispersion has probably been checked by a climatic barrier (such as temperature) rather than a lack of time.

Our phylogeographic analyses established other points of biogeographic similarity between *R. laticauda*, *R. guatemalensis*, and previously defined ichthyological provinces in Central America (Bussing, 1976, 1975; Miller, 1966; Regan, 1906). For example, we observed a reasonably strong correspondence between the Usumacinta ichthyological province (Bussing, 1976, 1975; Miller, 1966; Regan, 1906) and mtDNA-based phylogeographic breaks observed in both *Rhamdia* clades (Figs. 1, 3, and 4). The Usumacinta province extends along the Atlantic slope from the Río Papaloapan, México to the Río Coco along the Honduras/Nicaragua border, and includes two sub-provinces separated by the Río Motagua in Guatemala (Bussing, 1976). The Usumacinta sub-province 1 encompasses three widespread, and non-overlapping mtDNA lineages with a geographic break between the mtDNA lineages at the Río Coatzacoalcos (Tehuantepec, México), whereas Usumacinta sub-province 2 contains a single widespread lineage (Figs. 1 and 4). The Río Motagua also defines a phylogeographic break between the widespread *R. guatemalensis* mtDNA lineage, guUCA2, from Usumacinta sub-province 1, and guMCA representing Usumacinta sub-province 2. The Atlantic continental shelf in the Motagua region is very narrow, suggesting that this river may be relatively isolated from adjacent drainages even during low sea level stands, thus serving as a more

effective filter barrier to fish dispersal than rivers that drain across a wide coastal plain. In addition to representing a geographic break between widespread mtDNA lineages, the Motagua also harbors the endemic laMCA1 and the narrowly distributed guUCA 4.

The Chiapas-Nicaraguan, San Juan, and Isthmian ichthyological provinces (Bussing, 1976) also have boundaries that correspond with some mtDNA phylogeographic breaks registered for *Rhamdia* (Figs. 1, 3, and 4). Drawing particular attention to the Chiapas–Nicaraguan province, Bussing (1976) noted "that at least 14 of the 45 known species are primarily Atlantic forms that have gained access to the Pacific slope . . .," a pattern also observed for mtDNA lineages. The two CA *Rhamdia* mtDNA clades have each crossed the continental divide into the Chiapas–Nicaraguan province at least three times, in some cases recently (laMCA 3 and guUCA 4, guMCA), and in other cases around the time of clade's initial expansion (laUCA 2, laMCA 4, and guUCA3). Of the three *Rhamdia* lineages that crossed the central cordillera early, only laUCA 2 has established a relatively broad distribution (Fig. 4).

Our discussion has focused on *Rhamdia* historical biogeography, but the extensive northward dispersion of *R. laticauda*, in comparison to its limited spread south and east, indicates that factors other than time and geography are restricting *R. laticauda*'s distribution. Furthermore, given the widespread sympatry between the two CA *Rhamdia* clades, we presume that *R. laticauda*'s restricted distribution in comparison to *R. guatemalensis* does not result from its competitive exclusion by the latter. Thus, *R. laticauda*'s spread may have been stopped by other members of the more diverse ichthyofauna found in the rivers of eastern Panamá, as compared to rivers of nuclear Central America. Another possibility is that central Panamá is the region where *R. laticauda*, back-colonizing south and east from Costa Rica, came into secondary contact with *R. guatemalensis*, moving north and west from Colombia. This scenario would suggest that the sympatry of the two Central American *Rhamdia* clades depends on the order of colonization, with *R. laticauda* succeeding only when it colonizes a river prior to *R. guatemalensis*. Under either scenario it would appear that the colonizing success of *R. laticauda* depended more on ecological context than was probably the case for *R. guatemalensis*.

In conclusion, phylogenetic evidence suggests that the *R. laticauda* and *R. guatemalensis* mtDNA clades dispersed rapidly across Mesoamerica following the Pliocene rise of the Isthmus of Panamá. We argue that rapid dispersion would be facilitated by river anastomosis during low sea level stands, particularly along the Atlantic slope from Lago Nicaragua north to the Isthmus of Tehuantepec. But the cyclical nature of Pliocene/Pleistocene sea level change raises a paradox regarding the extensive phylogeographic structure observed across both *Rhamdia* mtDNA clades. Given that our *Rhamdia* data suggest that each clade probably dispersed across the entirety of its present geographic distribution in its early expansion phase, why has not the history of mtDNA diversification been overwritten by each subsequent low sea level stand? We presume that in many cases the record has been overwritten, thus explaining the extensive geographic distributions of some mtDNA lineages. In other cases, however, the effectiveness of barriers to dispersal may change over time as a function of sea level fluctuation (Bermingham and Avise, 1986), and/or the demographic history of fish populations on either side of a drainage divide (Reeves and Bermingham, submitted).

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Appendix A

Specimens studied for molecular systematic analysis of Central American *Rhamdia*

MtDNA lineages	Species group[a]	Species[b]	Country	Drainage/slope	Coordinates	Specimen identification
guUCA 1	*guatemalensis*	*guatemalensis*	MEX	Lago Catemaco/A	18.62N 96.87W	2215-MEX
guUCA 1	(*quelen*)	*guatemalensis*	MEX	Papaloapan/A	17.48 N 97.17W	29-MEX
guUCA 1		*guatemalensis*	MEX	Coatzacoalcos/A	16.80N 95.02W	452-MEX
guUCA 2		*guatemalensis*	GU	Usumacinta/A	15.83N 00.33W	STRI-7831[3]
guUCA 2		*guatemalensis*	GU	Usumancinta/A	15.81N 89.95W	STRI-7919[4]
guUCA 2		*guatemalensis*	GU	Usumacinta/A	15.72N 89.94W	STRI-7872[5], 7875[6]
guUCA 2		*guatemalensis*	GU	Usumacinta/A	15.05N 90.23W	GU-106[7], 118[8]
guUCA 3		*guatemalensis*	MEX	Gri-Usumacinta/A	16.10N 91.77W	GU-6[1]
guUCA 3		*guatemalensis*	MEX	Gri-Usumacinta/A	16.02N 92.02W	2583[2] -MEX
guUCA 3		*guatemalensis*	GU	Lago Petén/A	16.98N 89.75W	STRI-8136
guUCA 3		*guatemalensis*	GU	Belize/A	16.96N 89.36W	STRI-8107[1]
guUCA 3		*guatemalensis*	MEX	Grande/P	17.70N 94.50W	2121[1], 2122[2]-MEX
guUCA 3		*guatemalensis*	MEX	Ostuta/P	16.50N 95.75W	1430[1], 1431[2]-MEX
guUCA 4	*guatemalensis*	*guatemalensis*	GU	Belize/A	16.96N 89.36W	STRI-8097[2]
guUCA 4	(*quelen*)	*guatemalensis*	HO	Motagua/A	14.86N 89.12W	STRI-8377[1], 8389[2]
guUCA 4		*guatemalensis*	GU	Los Esclavos/P	14.33N 90.40W	GU-10275
guMCA	*guatemalensis*	*guatemalensis*	HO	Ulua/A	14.39N 88.52W	STRI-8354
guMCA	(*quelen*)	*guatemalensis*	HO	Aguán/A	15.29N 86.39W	STRI-8586
guMCA		*guatemalensis*	HO	Patuca/A	14.58N 86.29W	STRI-8799[1]
guMCA		*guatemalensis*	HO	Patuca/A	14.40N 85.93W	STRI-8734[2]
guMCA		*guatemalensis*	NI	Coco/A	13.31N 85.32W	STRI-14061[1]
guMCA		*guatemalensis*	NI	Coco/A	13.20N 85.57W	STRI-13893[2]
guMCA		*guatemalensis*	NI	Prinzapolka/A	13.41N 84.41W	STRI-14249[1]
guMCA		*guatemalensis*	NI	Prinzapolka/A	13.40N 84.33W	STRI-14141[2]
guMCA		*guatemalensis*	NI	Grande de Matagalpa/A	13.15N 85.32W	STRI-14173[1]
guMCA		*guatemalensis*	NI	Grande de Matagalpa/A	13.09N 85.55W	STRI-13843[2]
guMCA		*guatemalensis*	NI	L. Nicaragua/San Juan/A	11.92N 85.94W	STRI-8989
guMCA		*guatemalensis*	NI	San Juan/A	11.30N 84.50W	STRI-14533[1]
guMCA	*guatemalensis*	*guatemalensis*	CR	Sixaola/A	9.60N 82.80W	STRI-220[1], 221[2]
guMCA	(*quelen*)	*wagneri*	PN	San San/A	9.29N 82.31W	1262-IST
guMCA		*guatemalensis*	HO	Goascorán/P	13.59N 87.76W	STRI-8814
guMCA		*guatemalensis*	HO	Lempa/P	14.23N 89.12W	STRI-8328
guMCA		*guatemalensis*	SA	Laguna Jocotal/P	13.32N 88.07W	84[1], 62[2]-SA
guMCA		*guatemalensis*	HO	Choluteca/P	13.50N 87.00W	STRI-8935[1], 8970[2]

Appendix A (*continued*)

MtDNA lineages	Species group[a]	Species[b]	Country	Drainage/slope	Coordinates	Specimen identification
guMCA		*guatemalensis*	NI	Negro/P	13.13N 86.34W	STRI-13476
guMCA		*guatemalensis*	CR	San Juan/A	10.91N 85.21W	STRI-2163[2], 2164[3], 2165[4]
guLCA 1	*guatemalensis* (*quelen*)	*guatemalensis*	CR	Cañas/P	10.35N85.17W	STRI-1207[1], 1208[2]
guLCA 2	*guatemalensis*	*wagneri*	PN	Indio /A	9.13N 80.17W	STRI-10051
guLCA 2	(*quelen*)	*wagneri*	PN	Chagres/A	9.20N 79.78W	STRI-7569
guLCA 2		*wagneri*	PN	Azúcar/A	9.40N 78.66W	STRI-3775
guLCA 2		*guatemalensis*	CR	Terraba/P	8.90N 83.44W	STRI-2049
guLCA 2		*wagneri*	PN	Tabasará/P	8.20N 81.59W	STRI-87
guLCA 2		*wagneri*	PN	San Félix/P	8.43N 81.52W	774-IST
guLCA 2		*wagneri*	PN	Santa María/P	8.35N 80.80W	STRI-3191
guLCA 2		*wagneri*	PN	Bayano/P	9.12N 78.36W	STRI-4862[1], 4880[2]
guLCA 2		*wagneri*	PN	Bayano/P	8.98N 78.51W	STRI-3638[3]
guLCA 2		*wagneri*	PN	Tuira/P	8.63N 77.93W	STRI-3562[1]
guLCA 2		*wagneri*	PN	Tuira/P	8.11N 77.60W	STRI-4112[2]
guLCA 3	*guatemalensis*	*wagneri*	PN	Mandinga/A	9.47N 79.09W	STRI-1669[1], 1670[2]
guLCA 3	(*quelen*)	*wagneri*	COL	Atrato/A	5.69N 76.67W	STRI-1525[1], 1526[2]
guLCA 3		*wagneri*	COL	Atrato/A	5.38N 76.44W	STRI-1569[3]
guLCA 3		*wagneri*	COL	Baudo/P	5.51N 76.98W	STRI-1419
guLCA 3		*wagneri*	COL	Patia/P	2.04N 77.02W	STRI-9570
guNSA	*guatemalensis*	*wagneri*	COL	Magdalena/A	9.36N 74.72W	STRI-816[1]
guNSA	(*quelen*)	*wagneri*	COL	Magdalena /A	4.46N 76.00W	STRI-12004[1]
guNSA		*wagneri*	VZ	Lago Maracaibo/A	8.84N 71.98W	VZ-1[1], 2[2]
laUCA 1	*laticauda*	*laticauda*	MEX	Lago Catemaco/A	18.62N 96.87W	3499-MEX
laUCA 1	(*laticauda*)	*reddelli*	MEX	Papaloapan/A	18.45N 96.65W	2781-MEX
laUCA 1		*laticauda*	MEX	Papaloapan/A	17.68N 97.09W	653-MEX
laUCA 2	*laticauda*	*parryi*	MEX	Los Perros/P	16.63N 95.25W	25-OAX
laUCA 2	(*laticauda*)	*parryi*	GU	Nahualate/P	14.50N 91.41W	STRI-7723[1], 7724[2]
laUCA 2		*parryi*	GU	Acomé/P	14.32N 90.98W	GU-178
laUCA 2		*parryi*	GU	María Linda/P	14.20N 90.71W	STRI-7776
laUCA 3	*laticauda*	*laticauda*	MEX	Gri-Usumacinta/A	16.10N 91.77W	GU-3[1]
laUCA 3	(*laticauda*)	*laticauda*	GU	Usumacinta/A	15.83N 00.33W	STRI-7821[2], 7822[3]
laUCA 3		*laticauda*	GU	Belize/A	16.96N 89.36W	STRI-8099[1], 8100[2]
laUCA 3		*laticauda*	GU	Polochic/A	15.54N 88.90W	STRI-8199[1]
laUCA 3		*laticauda*	GU	Polochic/A	15.32N 89.56W	STRI-8284[2]
laMCA1	*laticauda*	*laticauda*	NI	Escondido/A	12.00N 84.40W	STRI-13670
laMCA1	(*laticauda*)	*laticauda*	NI	San Juan/A	11.30N 84.50W	STRI-14536[1]
la MCA1		*rogersi*	NI	San Juan/A	12.26N 86.12W	STRI-13805[2]
laMCA 1		*rogersi*	CR	San Juan/A	10.91N 85.21W	STRI-2161[3], 2162[4]
laMCA 1		*rogersi*	CR	Sixaola/A	9.60N 82.80W	STRI-219[1]
laMCA 1		*cabrerai*	GU	Lempa/P	14.53N 89.42W	GU-10266
laMCA 1	*nicaraguensis*	*nicaraguensis*	CR	Barranca/P	10.34N 85.07W	STRI-2121[1]
laMCA 1	(*nicaraguensis*)	*nicaraguensis*	CR	Barranca/P	10.34N 85.07W	STRI-2122[2]
laMCA2	*laticauda*	*cabrerai*	HO	Motagua/A	14.51N 89.7W	STRI-8393
laMCA2	(*laticauda*)	*cabrerai*	HO	Ulua/A	14.39N 88.52W	STRI-8345
laMCA2		*cabrerai*	HO	Aguan/A	15.29N 86.39W	STRI-8585

Appendix A (*continued*)

MtDNA lineages	Species group[a]	Species[b]	Country	Drainage/slope	Coordinates	Specimen identification
laMCA 2		*cabrerai*	HO	Patuca/A	14.58N 86.29W	STRI-8794[1]
laMCA 2		*cabrerai*	HO	Patuca/A	14.40N 85.93W	STRI-8729[2]
laMCA 2		*cabrerai*	HO	Choluteca/P	14.01N 87.00W	STRI-8965[1], 8966[2]
laLCA 1	*laticauda* (*laticauda*)	*rogersi*	CR	Sixaola/A	9.60N 82.80W	STRI-218[2]
laLCA 2	*laticauda*	*rogersi*	PN	ChiriquíViejo/P	8.42N 82.68W	46-IST[1]
laLCA 2	(*laticauda*)	*rogersi*	PN	ChiriquíViejo/P	8.76N 82.83W	STRI-662[2]
laLCA 2		*rogersi*	PN	ChiriquíViejo/P	8.67N 82.85W	STRI-6345[3]
laLCA 3	*laticauda*	*rogersi*	PN	Guarumo/A	9.07N 82.29W	STRI-11527
laLCA 3	(*laticauda*)	*rogersi*	PN	Coclédel Norte/A	8.70N 80.45W	STRI-10085
-laLCA 3		*rogersi*	PN	Indio/A	8.65N 80.12W	STRI-2686
laLCA 3		*rogersi*	PN	Antón/P	8.42N 80.25W	STRI-722
cinWSA	*cinerascens*	*cinerascens*	EC	Daule/P	2.17N 79.86W	STRI-12103[1]
cinWSA	(*quelen*)	*cinerascens*	EC	Daule/P	2.17N 79.86WS	STRI-12104[2]
Q SA	*quelen*	*quelen*	VZ	Orinoco/A	8.25N 69.55W	VZ-54[1]
Q SA	(*quelen*)	*quelen*	VZ	Orinoco/A	8.49N 69.20W	MH-317[2]
Q SA		*quelen*	GY	Essequibo/A	5.59N 58.34W	MH-198
Q SA		*quelen*	PE	Manu-Amazon/A	11.80S 71.46W	STRI-425[1]
Q SA		*quelen*	PE	Manu-Amazon/A	11.80S 71.44W	STRI-517[2]
Q SA		*quelen*	ARG	Paraná/A	28.53S 58.91W	STRI-2308[1]
Q SA		*quelen*	ARG	Paraná/A	25.72S 54.43W	STRI-2224[2]
Q SA		*quelen*	ARG	Paraná/A	25.68S 54.17W	STRI-2458[3]
outgroup	*Imparales*	*panamensis*	PN	Tuira/P	7.75N 77.68W	STRI-11587[1]
outgroup	*Imparales*	*panamensis*	PN	Santa María/P	8.43N 81.05W	STRI-7479[2]
outgroup	*Heptapterus*	*sp.*	ARG	Paraná/A	25.72S 54.43W	STRI-2427
outgroup	*Nanchagresdia*	*lineata*	CR	Coto/P	8.78N 83.25W	STRI-1192
outgroup	*Pimelodella*	*chagresi*	PN	Chagres/A	9.15N 79.73W	STRI-271[1]
outgroup	*Pimelodella*	*chagresi*	PN	San Félix/P	8.17N 81.85W	953-IST[2]
outgroup	*Pimelodella*	*chagresi*	PN	Tuira/P	8.63N 77.93W	STRI-3556[3]

[a] *Rhamdia* species groups based on Bussing (1987) in italics, and Silfvergrip (1996) in parentheses.
[b] Species names sources: Miles (1947); Martin (1969); Villa (1977); Miller (1984); Bussing (1985, 1998) Bussing (1975, 1998); Espinosa et al. (1993); Greenfield and Thomerson (1997).

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