PHYLOGENETIC RELATIONSHIPS AMONG POISON FROGS OF THE GENUS *DENDROBATES* (DENDROBATIDAE): A MOLECULAR PERSPECTIVE FROM INCREASED TAXON SAMPLING

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Despite many taxonomic revisions, systematic relationships among members of the genus *Dendrobates* remain poorly understood, particularly the connections between taxa in Amazonia and those in northern South America and Central America. We combine new mitochondrial sequence data with data from previous analyses in order to investigate the relationships among *Dendrobates* from each major biogeographic region. We address the phylogenetic position of taxa not included in previous molecular systematic analyses, including *Dendrobates flavovittatus, D. duellmani, D. galactonotus, D. mysteriosus,* and a new *Dendrobates* species from Brazil. We attempt to resolve relationships among former members of the genus *"Minyobates,"* and we consider the biogeographic and behavioural implications of the overall tree topology.

Key words: Amazonia, *Minyobates*, Neotropics, systematics

INTRODUCTION

Neotropical poison frogs of the genus *Dendrobates* are well known for their bright coloration and potent skin toxins (e.g. Myers & Daly, 1983). Despite many taxonomic revisions (e.g. Silverstone, 1975; Myers, 1982; Caldwell & Myers, 1990), systematic relationships among the members of this genus remain poorly understood. Recent studies employing molecular characters (Summers *et al.*, 1999; Vences *et al.*, 2000, 2003; Symula *et al.*, 2001, 2003; Santos *et al.* 2003) have resolved relationships among species living in Central America and northern South America, as well as among the majority of species from western and central Amazonia. However, the connections between the taxa in Amazonia and those in northern South America and Central America remain poorly resolved. In this paper we combine mitochondrial DNA (mtDNA) sequences from previous analyses with sequences from species within the genus *Dendrobates* that previously have not been sampled in order to provide a more complete analysis of systematic relationships within the genus. Thorough taxon sampling enhances the probability of accurately reconstructing phylogenetic relationships among the members of a clade (Zwickl & Hillis, 2002). In this analysis we have included the majority of taxa from each of the three major biogeographic regions in which members of the genus *Dendrobates* occur: Central America, northern South America, and Amazonia.

The major goals of this study are: (1) to carry out a comprehensive molecular systematic study of the genus *Dendrobates*; (2) to investigate the relationships among members of the genus *Dendrobates* in Amazonia, northern South America, and Central America; (3) to investigate the biogeographic implications of the evolutionary relationships within *Dendrobates*; and (4) to resolve relationships among former members of the genus *Minyobates*, some of which are now considered members of the genus *Dendrobates* (Vences *et al.* 2003).

Myers (1987), suspecting that *Dendrobates* was not monophyletic, defined the genus *Minyobates* to include eight species of miniature dendrobatids, most of which formerly belonged to Silverstone's (1975) *D. minutus* species group (*M. abditus, M. altobueyensis, M. bombetes, M. fulguritus, M. minutus, M. opisthomelas, M. steyermarki,* and *M. viridis*). Clough & Summers (2000) and Vences *et al.* (2000) showed that at least some members of the genus *Minyobates* (*M. minutus* and *M. fulguritus*, respectively) fall within the clade formed by the members of the genus *Dendrobates* and suggested that *Minyobates* may be synonymous with *Dendrobates*. Vences *et al.* (2003) and Santos *et al.* (2003) corroborated the placement of *D. minutus* and *D. fulguritus* within *Dendrobates,* but Vences *et al.* (2003) noted the isolated position of *M. steyermarki*, the type species of *Minyobates*, at the base of the *Dendrobates* clade and suggested that *Minyobates* may be a

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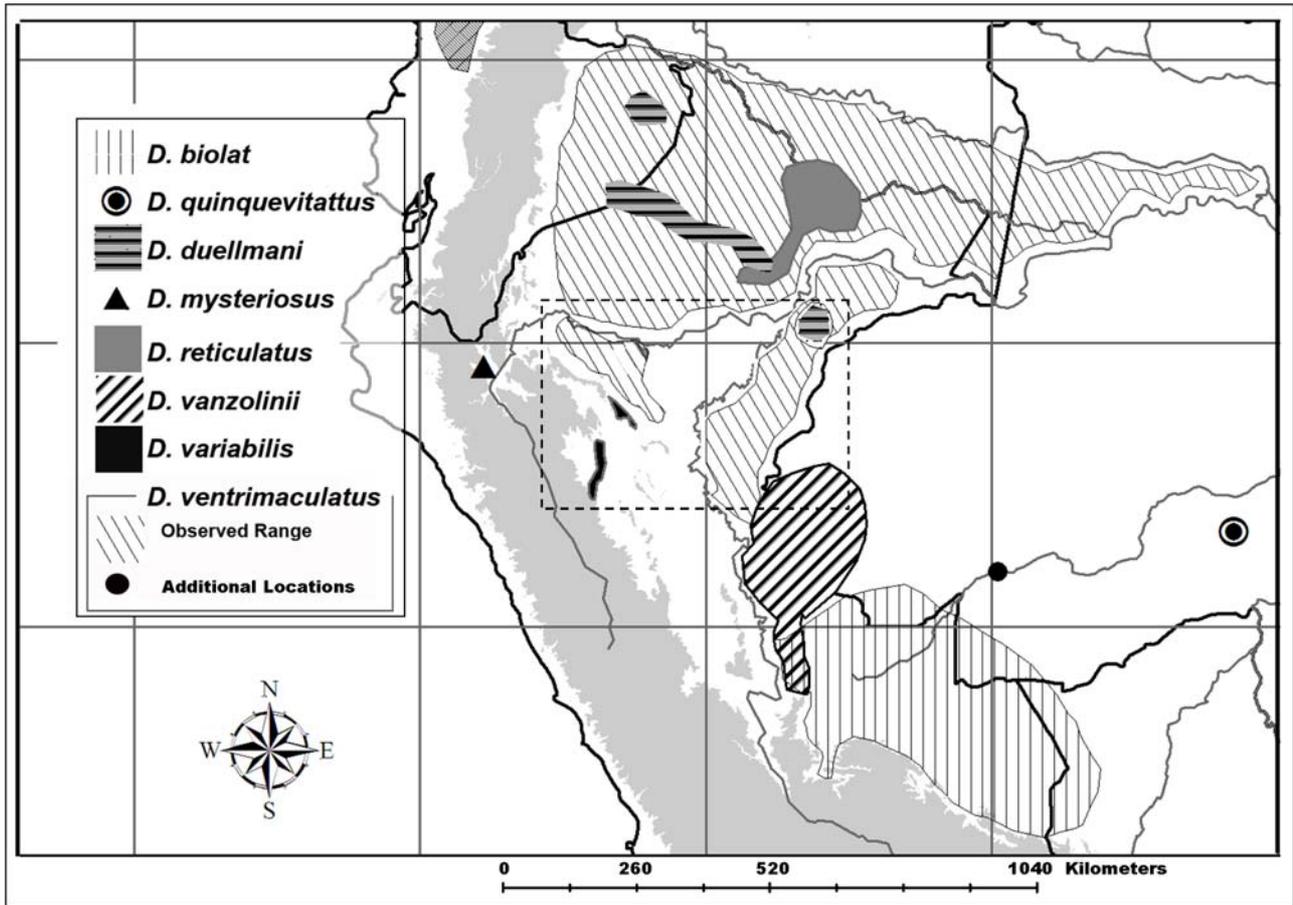
FIG. 1. Distribution of western Amazonian *Dendrobates*. Areas above 1000 m elevation shaded. The dashed box depicts the area covered in Fig. 2.
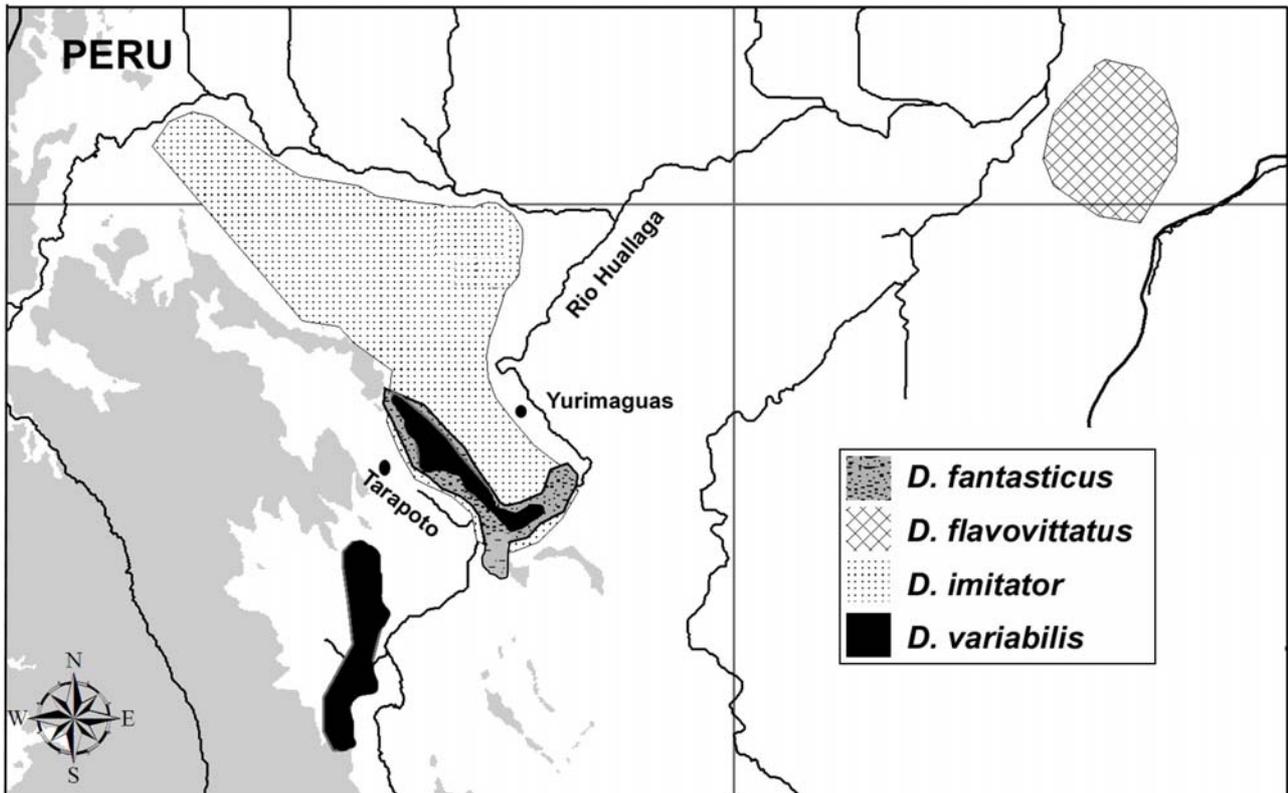

FIG. 2. Distribution of north central Peruvian *Dendrobates* (detail from Fig. 1 to illustrate ranges of *D. imitator, D. fantasticus,* and *D. flavovittatus*). Areas above 1000 m elevation shaded.

monotypic genus. In an analysis of toxin sequestration in dendrobatids, Daly *et al.* (2003) suggested that further molecular analysis is needed to resolve the taxonomic validity of *Minyobates*. To address this question we included in our analysis three members of the *Dendrobates minutus* group, from which the genus *Minyobates* was described (Myers, 1987): *Dendrobates claudiae* Jungfer *et al.* 2000, from the northern limit of the range (Bocas del Toro Archipelago, Panama), *Dendrobates minutus* from southeastern Panama and northen Colombia, at the center of the range, and *Minyobates steyermarki* from Cerro Yapacana in southern Venezuela.

MATERIALS AND METHODS

SAMPLE COLLECTION

The majority of sequences used in this study are derived from previous studies (e.g. Summers *et al.*, 1999; Clough & Summers, 2000; Symula *et al.*, 2003), although some were sequenced for this study. Collection localities and sequence origins for all samples are listed in Table 1. Tissues samples sequenced for this study were taken as toe clips from each frog. Collecting and export permits from Peru were obtained from the Ministry of Natural Resources (INRENA) in Lima, Peru (Authorization No. 061-2003-INRENA-IFFS-DCB, Permit No. 002765-AG-INRENA and CITES Permit No. 4326). Voucher specimens for each species collected in Peru were deposited at the Museo de Historia Natural, Universidad Mayor de San Marcos, Lima, Peru.

Samples from Brazil were collected by J. P. Caldwell and were obtained via a tissue grant to the corresponding author from the Louisiana State University Museum of Natural Sciences Collection of Genetic Resources. Tissues obtained by J. P. Caldwell were collected during expeditions funded by the National Science Foundation (DEB-9200779 and DEB-9505518 to L. J. Vitt and J. P.

Caldwell). Samples of *Dendrobates* sp. from Mato Grosso were obtained from J. Frenkel. The general distributions of each species analyzed in this study are shown in Figs. 1-3.

DNA EXTRACTION, DNA AMPLIFICATION, SEQUENCING

Genomic DNA was extracted from tissue samples preserved in high concentration salt buffer (DMSO/NaCl/EDTA) using the Qiagen DNeasy Tissue Kit. Samples collected by J. P. Caldwell were originally stored in 70% ethanol and then transferred to high concentration salt buffer for storage prior to extraction. The 16S ribosomal RNA (rRNA), 12S rRNA, cytochrome *b*, and cytochrome oxidase I mitochondrial gene regions were amplified using DNA primers and protocols described in Summers *et al.* (1999), Clough & Summers (2000), and Symula *et al.* (2001) for a total of 1591 base pairs in the final dataset. We used the following primer sets: 16S: LGL 381, LGL 286 (Palumbi *et al.*, 1991); 12S: 12SA-L, 12Sb-H (Kocher *et al.*, 1989), Df12SA, Df12SB (Symula *et al.*, 2001); cytochrome *b*: CB1-L, CB2-H (Palumbi *et al.*, 1991), KSCYB1(A)-L, KSCYB(C)L, KSCYB1-H (Clough & Summers, 2000); cytochrome oxidase I: COIA, COIF (Palumbi *et al.*, 1991), DfCOIA, DfCOIB, DiCOIA, DiCOIB (Symula *et al.*, 2001). We were unable to sequence cytochrome oxidase I for *Dendrobates duellmani* Schulte, 1999 from Ecuador, *D. galactonotus* Steindachner, 1864, *D. quinquevittatus* Steindachner, 1864, *D. sylvaticus* Funkhouser, 1956, *D. vanzolinii* Myers, 1982, *D. ventrimaculatus* Shreve, 1935 from Ecuador, *D. ventrimaculatus* from French Guiana, or *D.* sp., the undescribed species from Mato Grosso, Brazil.

PCR amplifications were purified with the Qiagen QIAquick PCR Purification Kit. Products were sequenced using Applied Biosystems' (ABI) PRISM (Perkin-Elmer Corporation, Foster City, CA, USA) Sequencing Kit. Samples were then prepared for sequencing as in Clough & Summers (2000).
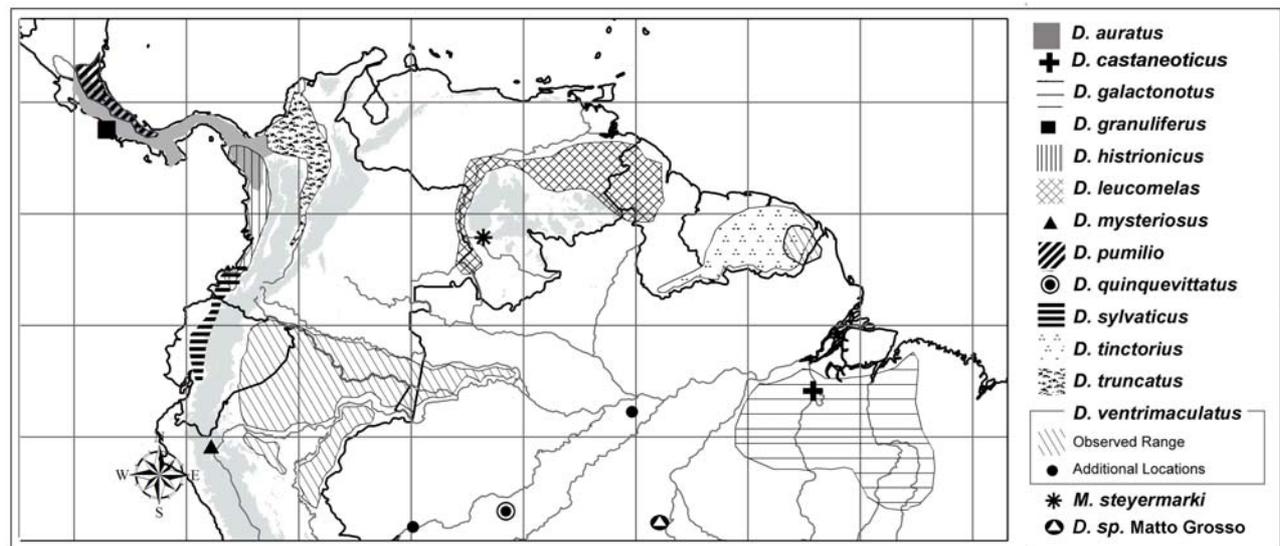

FIG. 3. Distribution of Central American and eastern Amazonian *Dendrobates.* Areas above 1000 m elevation shaded.

TABLE 1. Species names, collection localities, and GenBank accession numbers for taxa included in the analyses.

Species	Location	12S	16S	COI	CytB
Colostethus marchesianus	Peru	AF128584	AF128583	AF128585	NA
Colostethus talamancae	Costa Rica	AF128587	AF128586	AF097496	AF128588
Epipedobates trivittatus	Peru	AF128570	AF128569	AF128571	NA
Dendrobates arboreus	Panama	AF128611	AF128610	AF097504	AF128612
D. amazonicus	Iquitos, Loreto, Peru	AF482770	AF482785	AF482815	AF482800
D. auratus	Panama	AF128602	AF098745	AF097501	AF128603
D. biolat	S. Peru	AF482779	AF482794	AF482823	AF482809
D. castaneoticus 1	E. Brazil	AF482774	AF482789	AF482818	AF482804
D. castaneoticus 2	E. Brazil	AF482775	AF482790	AF482819	AF482805
D. claudiae	Colombia?	DQ371304	DQ371315	DQ371324	DQ371334
D. duellmani E	Napo, Ecuador	AY364566	AY263246	NA	NA
D. duellmani P	Tahuayo, Loreto, Peru	DQ371305	DQ371316	DQ371325	DQ371335
D. fantasticus 1	N. Sauce, San Martin, Peru	AF412444	AF412472	AF412416	AF412500
D. fantasticus 2	Cainarachi, San Martin, Peru	AF412447	AF412475	AF412419	AF412503
D. flavovittatus	Tahuayo, Loreto, Peru	DQ371306	DQ371317	DQ371326	DQ371336
D. galactonotus	E. Brazil	DQ371300	DQ371311	NA	DQ371330
D. granuliferus	Costa Rica	AF128608	AF098749	AF097505	AF128609
D. histrionicus 1	Ecuador	AF128617	AF128616	AF097498	U70154
D. histrionicus 2	Ecuador	AF124098	AF124117	NA	AF173766
D. imitator 1	Huallaga, San Martin, Peru	AF412448	AF412476	AF412420	AF412504
D. imitator 2	Pongo, San Martin, Peru	AF412459	AF412487	AF412431	AF412515
D. lamasi	Tingo Maria, Huanuco, Peru	AF482778	AF482793	AF482822	AF482808
D. leucomelas	Venezuela	AF128593	AF124119	AF097499	AF128594
D. minutus	Panama	AF128590	AF128589	AF128591	MMU70163
D. mysteriosus	N. Peru	DQ371303	DQ371314	DQ371323	DQ371333
D. pumilio	Bocas del Toro, Panama	AF128614	AF128613	AF097500	U70147
D. quinquevittatus	E. Brazil	AF482773	AY263253	NA	AF482803
D. reticulatus 1	Punta Itaya, Loreto, Peru	AF482772	AF482787	AF482817	AF482802
D. reticulatus 2	B. Achille, Loreto, Peru	AF482771	AF482786	AF482816	AF482801
D. sp.	Mato Groso, Brazil	DQ371309	DQ371320	NA	DQ371339
D. speciosus	Panama	AF128596	AF098747	AF097503	AF128597
D. sylvaticus	Ecuador	AY364569	AY364569	NA	AF324041
D. tinctorius	French Guiana	AF128605	AF128604	NA	AF128606
D. vanzolinii	Peru	AF128599	AF128598	NA	AF128600
D. variabilis	Cainarachi, San Martin, Peru	AF412463	AF412491	AF412435	AF412519
D. ventrimaculatus B1	Solimoes, Amazonas, Brazil	DQ371307	DQ371318	DQ371327	DQ371337
D. ventrimaculatus B2	Porto Walter, Acre, Brazil	DQ371301	DQ371312	DQ371322	DQ371331
D. ventrimaculatus B3	Solimoes, Amazonas, Brazil	DQ371308	DQ371319	DQ371328	DQ371338
D. ventrimaculatus E1	Ecuador	AF482780	AF482795	AF482824	AF482810
D. ventrimaculatus E2	Ecuador	AF128620	AF128619	AF097502	AF120013
D. ventrimaculatus FG	French Guiana	DQ371302	DQ371313	NA	DQ371332
D. ventrimaculatus P1	N. Bonilla, San Martin, Peru	AF412466	AF412494	AF412438	AF412522
D. ventrimaculatus P2	Near Rio Napo, Loreto, Peru	AF482781	AF482796	AF482825	AF482811
Minyobates steyermarki	Venezuela	DQ371310	DQ371321	DQ371329	DQ371340
Phyllobates bicolor	Choco, Colombia	AF128578	AF128577		AF128579

SEQUENCE ANALYSIS

Each sample was sequenced in both directions and complimentary sequences were aligned using Autoassembler version 1.4.0 (ABI, 1995). Consensus sequences were transferred to Gene Jockey (Taylor, 1990) for alignment with a sequence of the same region from a different individual. We translated the protein coding sequences to confirm that they were in the proper reading frame and did not contain stop codons. We aligned the DNA sequences using Clustal X (Thompson *et al.*, 1997). For the cytochrome oxidase I and cytochrome *b* gene regions, alignments were unambiguous and contained no gaps. For the 16S rRNA and 12S rRNA gene regions, regions of ambiguous alignment were removed from the analysis. The resulting dataset included 1591 unambiguous base pairs.

PHYLOGENETIC ANALYSIS

Phylogenetic analyses were carried out using Bayesian inference in MrBayes (Version 3.0b4, Huelsenbeck & Ronquist, 2001) and Maximum Likelihood (ML) in PAUP* version 4.0b10 (Swofford, 2002). We included three species from taxa closely related to *Dendrobates* as outgroups in the analysis: *Epipedobates trivittatus* (Spix, 1824), *Colostethus talamancae* (Cope, 1875), and *Colostethus marchesianus* (Melin, 1941) (Table 1).

We partitioned the dataset into seven partitions as follows: non-coding gene regions (12S + 16S ribosomal RNA), cytochrome oxidase I (COI) 1st position codons, COI 2nd position codons, COI 3rd position codons, cytochrome *b* (cyt *b*) 1st position codons, cyt *b* 2nd position codons, and cyt *b* 3rd position codons, and used MrModeltest version 2.0 (Nylander, 2004) to determine which model of DNA substitution best fit each partition. Data may better be explained by partitioning a dataset than by applying an average model across genes and codon positions, as indicated by higher model likelihood scores in partitioned analyses (Mueller *et al.*, 2004).

We applied the models indicated by MrModeltest and used MrBayes version 3.0b4 (Huelsenbeck & Ronquist, 2001) to infer a tree topology including only those taxa for which a full set of sequence data (12S rRNA, 16S rRNA, cytochrome *b* and cytochrome oxidase I) was available. We ran four simultaneous Markov Chain Monte Carlo (MCMC) chains for one million generations, saving trees every 100 generations. We examined a plot of –ln likelihood scores and discarded all trees before –ln stabilization (burn-in phase). We created a 50% majority rule consensus tree from the remaining trees in PAUP*, then repeated the Bayesian analysis to ensure consistency of topology and posterior clade probabilities for the consensus tree.

The consensus tree derived from the Bayesian analysis was loaded as a backbone constraint topology in PAUP*. We used Modeltest version 3.0.6 (Posada & Crandall, 1998) to determine the appropriate model of DNA substitution for the unpartitioned dataset, implemented the specified model parameters, and conducted a Maximum Likelihood search in PAUP* that included the taxa with incomplete datasets (i.e. those lacking COI sequence data).

Wiens (1998) suggested that adding characters, despite incomplete taxon sampling, usually increases phylogenetic accuracy, but may be misleading. We compared the tree topology recovered using a backbone constraint of taxa with complete datasets (described above) to a topology recovered by a second Bayesian run of 5 million generations, including taxa with and without complete character sets, using MrBayes version 3.1.2. The tree topologies obtained by the two different methods were consistent; however the inclusion of taxa with incomplete datasets lowered the posterior probabilities at many branches between taxa

with complete datasets. This decrease may be a result of the equivocal placement of taxa with incomplete datasets within the phylogeny. Finally, we used Shimodaira-Hasegawa (1999) tests to assess the validity of certain relationships among taxa by comparing our tree topology to alternative topologies.

RESULTS AND DISCUSSION

The complete dataset included a total of 1591 base pairs, 305 from 12S rRNA, 540 from 16S rRNA, 196 from cytochrome *b,* and 550 from cytochrome oxidase I. Of the 1591 base pairs, 625 were variable, 471 of which were parsimony informative. Fig. 4 shows the tree that resulted from the ML search that added those taxa with incomplete sequence data to the backbone constraint tree derived from those taxa with complete sequence data.

Symula *et al.* (2003) found a division between eastern Amazonian (mainly Brazilian) *Dendrobates* (e.g. *D. castaneoticus* Caldwell & Myers, 1990 and *D. quinquevittatus*) and western Amazonian (mainly Peruvian) *Dendrobates.* Within the western clade there was a well-supported division between southern (i.e. *D. lamasi* Morales, 1992, *D. biolat* Morales, 1992, *D. vanzolinii,* and *D. imitator* Schulte, 1986) and northern (i.e. *D. ventrimaculatus, D. variabilis* Zimmermann & Zimmermann, 1988, *D. amazonicus* Schulte, 1999, *D. reticulatus* Boulenger, 1884, and *D. fantasticus* Boulenger, 1884) taxa, roughly corresponding to the Inambari and Napo refuge regions, respectively (Symula *et al.,* 2003). This division within the western Amazonian clade was also recovered by Santos *et al.* (2003). We recovered a tree topology in overall accordance with the findings of Symula *et al.* (2003) and Santos *et al.* (2003), but our analysis included several new taxa. We consider the placement of these taxa in terms of general biogeography and trends in parental care where notable.

Dendrobates flavovittatus Schulte, 1999 falls within the "southwestern" clade (roughly corresponding to the Inambari refuge region) described by Symula *et al.* (2003), including *D. biolat*, *D. lamasi*, *D. vanzolinii*, and *D. imitator*, and further supports the hypothesis (Symula *et al.*, 2001, 2003) of a northward radiation by southern ancestors in this clade (Fig. 2). All members of the *D. vanzolinii* group (*D. biolat, D. flavovittatus, D. imitator, D. lamasi,* and *D. vanzolinii*) are believed to demonstrate biparental care, though this has not been confirmed in *D. flavovittatus*.

Although their placement within the "northwestern" clade (roughly corresponding to the Napo refuge region) described by Symula *et al.* (2003) supports the findings of Santos *et al.* (2003), two *Dendrobates duellmani* Schulte, 1999 individuals from populations on either side of the Amazon River in northeastern Peru and eastern Ecuador did not fall out together. The individual from the Napo River in eastern Ecuador fell out with two *D. reticulatus* individuals from the same geographic region while a *D. ventrimaculatus* individual
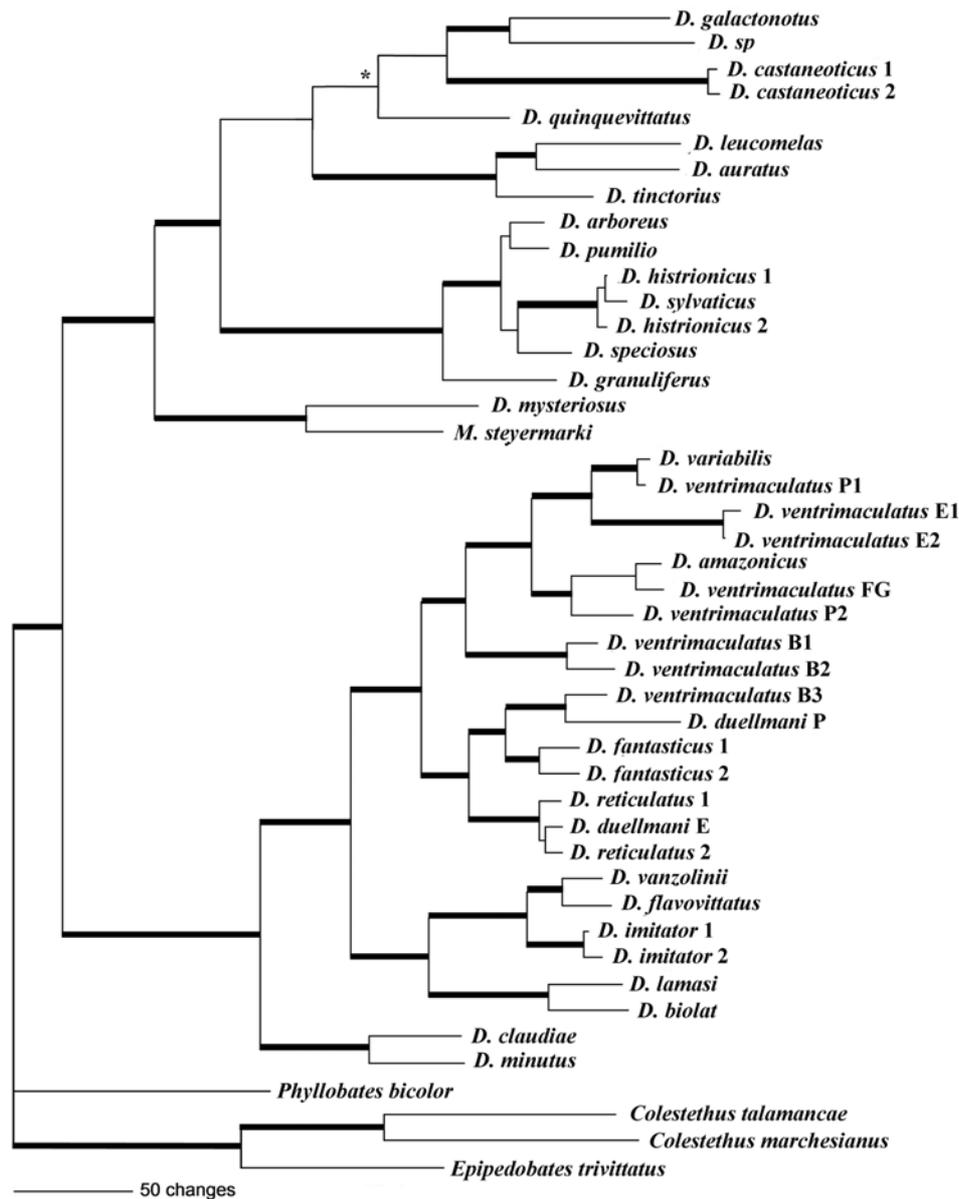
FIG. 4. Maximum Likelihood phylogram derived from a Bayesian backbone constraint consensus tree constructed using only taxa for which 12S, 16S, cytochrome *b* and cytochrome oxidase I sequence data were available (1591 bp). Thick lines indicate Bayesian posterior probabilities greater than 75.

from eastern Brazil was sister to the *D. duellmani* individual from the Tahuayo River. Jukes-Cantor genetic distances between the Napo River *D. duellmani* and the two *D. reticulatus* individuals ranged from 2.09% to 2.71% (compared to 1.32% between the two *D. reticulatus* individuals). The genetic distance between the Tahuayo River *D. duellmani* and its sister, *D. ventrimaculatus* from Amazonas, Brazil, was 5.54%, still closer than the distance of 6.18% between the two *D. duellmani* individuals. Hence, *D. duellmani* may need revision with respect to the specific populations that should be considered members of this species. Given geographic location and morphology, the *D. duellamani* samples from Yasuni, Ecuador are most likely the nominal from.

With respect to the *D. ventrimaculatus* species group, our results support the findings of Symula *et al.*

(2003); *D. ventrimaculatus* itself did not form a monophyletic group. These findings further support the suggestion by Caldwell & Myers (1990) that *D. ventrimaculatus* comprises a complex of species that are distinguishable from formerly synonymous *D. quinquevittatus,* but which share several morphological characters. An individual *D. ventrimaculatus* from western Peru along the Andean slope was sister to *D. variabilis* from the same geographic area; this pair grouped with two other western Amazonian *D. ventrimaculatus* from Ecuador. A second Peruvian individual, from the Rio Napo in eastern Peru, grouped with *D. amazonicus* (also from eastern Peru) and its sister, a *D. ventrimaculatus* from French Guiana. Two Brazilian *D. ventrimaculatus,* one from Porto Walter in the west and one from Amazonas in the east, formed the base of this *D. ventrimaculatus/D. variabilis/D. amazonicus*

clade. The third Brazilian *D. ventrimaculatus*, also from Amazonas, was most closely related to *D. duellmani* from Peru, as discussed above; both of those individuals are part of a larger clade that also includes *D. fantasticus* and *D. reticulatus*. These relationships, which generally were supported by high Bayesian posterior clade probabilities (see Fig. 4), suggest that *D. ventrimaculatus* may need taxonomic revision in order to maintain reciprocally monophyletic species names in *Dendrobates.* Caldwell & Myers (1990) suggest that a species from eastern Ecuador may represent *D. ventrimaculatus* sensu stricto, while other populations may belong to undiagnosed members of a *D. ventrimaculatus* species complex.

Dendrobates sp. from Mato Grosso, Brazil, appears to be the sister taxon to *Dendrobates galactonotus*, with *Dendrobates castaneoticus* sister to the pair. This phylogentic relationship is supported by morphology. *Dendrobates* sp. from Mato Grosso is similar in appearance to *D. galactonotus*, with a yellow-orange dorsum and legs mottled by irregular, barbell- to kidney-shaped blotchy spots, and a black venter. This group of Brazilian species forms a larger clade that includes the eastern Amazonian species *D. leucomelas* Steindachner 1864 and *D. tinctorius* Wagler, 1830, as well as the southern Central American *D. auratus* Dunn, 1931. This topology agrees with the findings of Vences *et al.* (2003), contrary to Silverstone's (1975) suggestion that *D. galactonotus* may be more closely related to the morphologically similar *D. tinctorius* than to the sympatric *D. castaneoticus* or *D. quinquevittatus*. All of the species that have been studied in this group have male parental care (Weygoldt, 1987; Summers & McKeon, 2004). Basal to the male care clade is the southern Central American/ northern South American *D. histrionicus* Berthold, 1845 clade, all of which express female or asymmetric biparental care (Weygoldt, 1987; Summers & McKeon 2004). The topology of the female care clade suggests that this trait evolved in Central America and then spread to northern South America (with *D. arboreus* Myers, Daly & Martínez 1984 and *D. pumilio* Schmidt, 1857 from Central America the most basal and *D. sylvaticus* from Ecuador the most derived species).

Our phylogenetic analysis indicates that the clade from central and eastern Amazonia (*D. castaneoticus, D. galactonotus, D.* sp. and *D. quinquevittatus*) is the sister taxon to the male care clade from northern South America and Central America (including *D. auratus, D. leucomelas,* and *D. tinctorius* in this analysis, as well as *D. truncatus* Cope, 1861) (Fig. 3). This arrangement is plausible biogeographically; the range of *D. tinctorius*, which extends to the Guyana Shield, approaches the range of *D. galactonotus* in northeastern Brazil (Fig. 2). Hence, it seems likely that divergence of a perhaps widespread ancestral population gave rise to the *D. galactonotus* clade, in central and eastern Amazonia, and the *D. auratus* clade, which spread northward and westward from Amazonia. The sister taxon of these two

clades is the female care clade from Central America and northern South America, which includes *D. arboreus, D. speciosus, D. pumilio, D. sylvaticus,* and *D. histrionicus* in this analysis, as well as *D. granuliferus* Taylor, 1958, *D. lehmanni* Myers & Daly 1976*, D. vicentei* Jungfer, Weygoldt & Juraske, 1996 and *D. occultator* Myers & Daly, 1976. The simplest biogeographic scenario would involve the divergence of the ancestor of the female care clade from an ancestral species within the northern male care clade (*D. auratus, D. leucomelas,* and *D. tinctorius*). However, it appears instead that the ancestral species that eventually gave rise to the female care clade diverged from Amazonian stock before the divergence of the *D. auratus* clade and the *D. galactonotus* clade (Fig. 1). We used a Shimodaira-Hasegawa (1999) test to determine that a topology that placed the female care clade as sister to *D. auratus* was significantly less likely than the topology we recovered (*P*<0.01). As an alternative, we also tested (Shimodaira & Hasegawa, 1999) the *D. galactonotus* clade as sister to the female care clade. While the test was not significant, the *D. galactonotus* clade and the female care clade occurred as sister taxa in only 24 of 9502 (0.25%) post burn-in Bayesian trees (a Bayesian analysis including all taxa was conducted in order to examine this percentage).

Dendrobates mysteriosus Myers, 1982 consistently fell out as sister to *Minyobates steyermarki*, which may be the result of long branch attraction. Both species occupy limited, isolated ranges (*D. mysteriosus* in northern Peru and *M. steyermarki* in southern Venezuela) (Fig. 3) and may represent relicts of ancient lineages (Schulte, 1990). Vences *et al.* (2003) noted the position of *M. steyermarki*, basal to *Dendrobates*, and suggested the validity of *Minyobates* as a potentially monotypic genus; however, this suggestion was based on the results of analysis of a single gene (16S). In our analyses, based on analysis of multiple gene regions, *D. mysteriosus* and *M. steyermarki* nearly always fell within *Dendrobates*, leading us once again to question the validity of the genus *Minyobates*. Shimodaira-Hasegawa tests forcing *M. steyermarki* and *D. mysteriosus* outside of the rest of the *Dendrobates*, both separately and together, were not significant, though the test of *D. mysteriosus* alone outside *Dendrobates* yielded a nearly significant p-value of 0.06. Of 9, 502 post burn-in Bayesian trees, 30 placed *D. mysteriosus* alone outside *Dendrobates*, none placed *M. steyermarki* alone outside *Dendrobates,* and 92 placed *D. mysteriosus* and *M. steyermarki* together outside *Dendrobates*. We have no reason to suspect that *D. mysteriosus* and *M. steyermarki* are evolutionarily closely related (i.e. as sister taxa), so we do not advocate retaining *Minyobates* and including *D. mysteriosus* in that genus, however we were not able to accurately resolve the relationships among *M. steyermarki, D. mysteriosus,* and the rest of the *Dendrobates* with the data available to us.

The position of *Dendrobates quinquevittatus* was also poorly resolved by our ML search using the

Bayesian backbone constraint tree. Symula *et al.* (2003) and Vences *et al.* (2003) found *D. quinquevittatus* to be closely related to *D. castaneoticus* and *D. galactonotus*. This relationship was recovered in some of our analyses, but at times we also found *D. quinquevittatus* as sister to *D. mysteriosus* and *M. steyermarki*. More sequence data (we were lacking COI data for *D. quinquevittatus*) may help resolve the position of *D. quinquevittatus* within *Dendrobates*.

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