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Molecular Systematics of the Leptodeirini (Colubroidea: Dipsadidae) Revisited: Species-tree Analyses and Multi-locus Data

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Cat-eyed snakes (*Leptodeira*) were thought to be closely related to nightsnakes (*Hypsiglena* and *Pseudoleptodeira*) based on morphology and immunological data, which allied these genera with blunt-headed vine snakes (*Imantodes*) and the Cloud Forest Snake (*Cryophis hallbergi*). We collected sequence data from six protein-encoding nuclear loci (*SLC30A1, ZEB2, FSHR, NTF3, DNAH3,* and *PNN*; 4149 bp) and additional mtDNA data (*nad5*; 955 bp) added to published *cob* and *nad4* (total 2387 bp mtDNA) from these and other rear-fanged, mildly venomous snakes that prey on vertebrates (frogs and lizards) and from several other dipsadine genera (*Dipsas, Sibon,* and *Atractus*) that prey on invertebrates (goo-eaters). We analyzed relationships using concatenation and a coalescent species-tree method. When analyzed separately, using either concatenation or coalescent methods, nuclear data support a different overall topology from the mtDNA data. Like the mtDNA data, the nuclear data support the *Leptodeira* + *Imantodes* relationship, but instead place this clade more closely to the goo-eaters, with the nightsnakes as the basal divergence in the group. When the data are combined in concatenation analyses, the more variable mtDNA data appear to overwhelm the nuclear data, but not under the coalescent model.

SNAKES of the Neotropical family Dipsadidae are poorly known from both ecological and phylogenetic perspectives, despite unique feeding adaptations and specialized diets. Some genera (e.g., *Hypsiglena, Leptodeira,* and *Imantodes*) have enlarged rear fangs, are mildly venomous, and prey on ectothermal vertebrates such as frogs (including their eggs) and lizards. Others (e.g., *Atractus, Dipsas,* and *Sibon*) prey on invertebrates such as earthworms, slugs, and snails (referred to as ''goo-eaters'' *sensu* Cadle and Greene, 1993). Such goo-eaters lack enlarged rear fangs and associated venoms from Duvernoy's (parotid) glands (Taub, 1967), but some contain toxins in the infralabial glands (de Oliveira et al., 2008), and some goo-eaters possess unique dentitions with an anterior jaw structure that permits them to extract gastropods from their shells (Peters, 1960; Sazima, 1989; Savage, 2002). Previous studies based on morphology have long allied *Hypsiglena* and *Leptodeira* (Tanner, 1946; Duellman, 1958a), and analyses based on albumin immunological data (Cadle, 1984) showed that several of the rear-fanged dipsadines formed a group, the 'Leptodeirini' (Dowling et al., 1983). This group included the cat-eyed snakes (*Leptodeira*), blunt-headed vine snakes (*Imantodes*), the nightsnakes (*Hypsiglena* + *Pseudoleptodeira latifasciata*), and the Cloud Forest Snake (*Cryophis hallbergi*). Dowling and Jenner (1987) reanalyzed immunological data (Cadle and Sarich, 1981; Cadle, 1984), added a few morphological characters (such as head and eye shape, dentition, scalation, and hemipenial morphology), and found support for the tribe Leptodeirini, which also included the rear-fanged *Coniophanes*.

Mulcahy (2007) used two mitochondrial DNA (mtDNA) gene regions (*nad4* + *cob*) to provide a phylogenetic hypothesis for this group and found that the 'Leptodeirini' was not monophyletic. Bayesian and likelihood analyses placed *Rhadinaea* and *Coniophanes* at the base of the tree, followed by an *Imantodes* + *Leptodeira* clade, then a nightsnake *Hypsiglena* + *Pseudoleptodeira* clade, and with *Cryophis* sister to a goo-eater clade (*Atractus, Sibon,* and *Dipsas*). The placement of the nightsnake clade sister to *Cryophis* + goo-eaters was weakly supported, but strong evidence was presented to reject a close relationship between the nightsnakes and *Imantodes* + *Leptodeira*. The overall results depict a rear-fanged, mildly venomous ancestral condition in dipsadines leading to a more derived goo-eater condition. Mulcahy (2007) suggested that the goo-eaters were able to exploit a new niche, preying on invertebrates, and may have undergone an adaptive radiation because those genera are much more speciose (>250 spp.) than the more basal, rear-fanged genera (<100 spp.), yet this hypothesis needs further testing.

More recently, in an expanded analysis of the genus *Leptodeira* with mtDNA data (*cob* and *nad4*), Daza et al. (2009) included two nuclear loci (*DNAH3* and *NT3* [= *NTF3*]) at the generic level of their study. The nuclear data supported a strong relationship between *Imantodes* and *Leptodeira*, a goo-eater clade, but the basal relationships among the dipsadines were unresolved, and nightsnakes were not included in the generic-level analysis (Daza et al., 2009). Mulcahy and Macey (2009) explored the use of these and other similar protein-encoding nuclear loci to test the relationships among closely related species of nightsnakes. These data recovered strong support for the nightsnakes (*Pseudoleptodeira* + *Hypsiglena*) and a monophyletic *Hypsiglena* (Mulcahy and Macey, 2009).

Higher-level studies of xenodontines and/or dipsadines (Vidal et al., 2000; Pinou et al., 2004; Zaher et al., 2009; Vidal et al., 2010) have not included nightsnakes and typically have only included *Leptodeira* and *Imantodes* to represent the Leptodeirini. However, Vidal et al. (2010) added the enigmatic taxon *Nothopsis rugosus* to the 12S–16S data of Vidal et al. (2000) and found it placed sister to *Leptodeira*. Though no Bayesian (>90%) or likelihood (>50%) support was found for its placement, they recommended adding *N. rugosus* to the Leptodeirini. We note that Zaher et al. (2009) recently revised caenophidian taxonomy, with an emphasis on the New World xenodontines, and found the name Dipsadidae to take priority for this family of snakes, which includes the subfamilies Dipsadinae, Carphophiinae, and Xenodontinae; we follow this taxonomy.

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Table 1. Primers Used to Generate Data for This Study.

Gene	Primer 5′–3′
nad5	Leu2f1-TGGTGCAAATCCAAGTGGTA
	nad5r1-AGGAGCCKGAGCATAGRAATA
SLC30A1	F3-AGTKTTTCTGCAYGTYYTTGGAGA
	R4-GCTTCAARGCACACTGRGTTCTGC
ZEB2	B3-GGTTCTTCTCCTAATTCTGTATCTTCCTC
	R-GGCTWGTCATTCCTTTCTCRGAAAGTAC
FSHR	F1-CCDGATGCCTTCAACCCVTGTGA
	R4-MCAGRAGGATCTTTGANTTGGACAC
NTF3	F1-ATGTCCATCTTGTTTTATGTGATATTT
	R5-CGTTTCATAAAAATATTGTTT
DNAH3	F1-GGTAAAATGATAGAAGAYTACTG
	R6-CTKGAGTTRGAHACAATKATGCCAT
PNN	F3-ATGGAGACAGGTAATCAGAGY
	R4-AATCAGAGYAATGAYRTAGAM

Recently, there is an increasing trend in systematic biology for studies using multi-locus datasets to replace analyses of concatenated data with coalescent-based methods that incorporate individual gene-tree information to reconstruct an overall species tree (Degnan and Rosenberg, 2009; Edwards, 2009; Knowles and Kubatko, 2010). This shift in methodology recognizes the fact that different unlinked genes often depict different topologies even within the same species tree (Slowinski and Page, 1999), either because of incomplete lineage sorting, horizontal gene transfer, and/or hybridization and introgression (Maddison, 1997). New methods (Liu, 2008; Kubatko et al., 2009) employ coalescent-based algorithms to account for incomplete lineage sorting among individual gene trees to generate hypotheses for an overall species tree. Such methods, however, usually accommodate only coalescent processes and do not deal with other potentially important processes such as horizontal gene transfer or gene flow through hybridization (Liu and Pearl, 2007; but see Kubatko and Meng, 2010). Though these methods are more typically applied to datasets of recently diverged lineages with multiple samples within species (Maddison and Knowles, 2006; Carstens and Knowles, 2007; Degnan and Rosenberg, 2009; Leaché, 2009; Spinks and Shaffer, 2009), the problem of deep coalescence and phylogeny reconstruction at higher taxonomic levels still exists (Slowinski et al., 1997; Slowinski and Page, 1999), and under some conditions coalescent methods can be successfully applied at higher taxonomic levels based on sampling single individuals per species (Liu and Pearl, 2007; Castillo-Ramírez et al., 2010). Thus, there is currently a need to explore the use of coalescent-based methods with a diversity of phylogenetic questions at various taxonomic levels (i.e., species, genera, etc.).

In this study, we re-examine the monophyly of the Leptodeirini by adding new data from six protein-encoding nuclear loci and an additional mitochondrial gene to a subset of taxa from Mulcahy (2007), but including the same genera. In addition to the *cob* and *nad4* data of Mulcahy (2007), we add the mtDNA gene *nad5* and the nuclear loci *SLC30A1, ZEB2, FSHR, NTF3, DNAH3,* and *PNN*. These markers were initially screened and selected for deep-level squamate relationships and are thought to be single copy, unlinked loci (Townsend et al., 2008). We explore phylogenetic relationships among genera of dipsadine snakes using multi-locus concatenation methods and the coalescent-based method Bayesian Estimation of Species Trees (BEST; Liu, 2008).

MATERIALS AND METHODS

We sampled a subset of taxa from Mulcahy (2007), including two representatives of the cat-eyed (*Leptodeira*) and blunt-headed vines snakes (*Imantodes*), and eight species of nightsnakes (*Pseudoleptodeira latifasciata* and all species of *Hypsiglena,* save *H. tanzeri* [Mulcahy, 2008]). The genus *Pseudoleptodeira* is now monotypic, with ''*P. uribei*'' being placed in the genus *Leptodeira* (Reyes-Velasco and Mulcahy, 2010). Previous molecular studies have recovered *Imantodes* and *Leptodeira* as paraphyletic with respect to each other (Mulcahy, 2007; Daza et al., 2009), primarily because of the placement of the presumed most basal taxa of each genus (*Leptodeira nigrofasciata* and *Imantodes inornatus*). Mulcahy (2007) attributed the lack of monophyly of these genera (*Leptodeira* and *Imantodes*) as a result of long-branch attraction. Daza et al. (2009) recovered *Leptodeira* as monophyletic with mtDNA and nuclear combined (but not with nuclear data alone) and recovered a monophyletic *Imantodes* with mtDNA and nuclear combined (but not with mtDNA data alone). Therefore, in this study we included one each of the presumed basal members (*L. nigrofasciata* and *I. inornatus*) and a more derived representative from both genera (*L. septentrionalis* and *I. cenchoa*). We included one each of the goo-eater genera (*Atractus, Dipsas*, and *Sibon*), one each of the other presumed 'Leptodeirini' genera (*Cryophis*, *Rhadinaea*, and *Coniophanes*). *Tantalophis*, *Diadophis,* and *Heterodon* were used as outgroup taxa, and we used *Heterodon* to root the trees (Zaher et al., 2009).

Tantalophis, originally described as a species of *Leptodeira*, later placed in *Hypsiglena*, then its own genus (Duellman, 1958b), has since been thought to be more distantly related to any of these taxa based on hemipenial morphology and is typically placed as ''Dipsadidae incertae sedis'' (Zaher, 1999; Zaher et al., 2009). Mulcahy (2007) found *Tantalophis* to be outside of all the sampled genera and we include it here, but note that our study does not contain all genera within the subfamily Dipsadinae. Rather, our sampling strategy was designed to re-examine the monophyly of the 'Leptodeirini,' (*sensu* Cadle, 1984; Dowling and Jenner, 1987; Mulcahy, 2007). Samples of the enigmatic *Nothopsis rugosus* remain unavailable to us (Vidal et al., 2010).

We collected sequence data from six independent nuclear loci (*SLC30A1, ZEB2, FSHR, NTF3, DNAH3,* and *PNN*), ranging in length from approx. 500–1000 base pairs (bp). The loci are from Townsend et al. (2008), and five (all but *PNN*) were previously explored among species of *Hypsiglena* (Mulcahy and Macey, 2009). We also collected additional mtDNA data from the gene encoding *nad5,* the most informative mt-region found for this group in a study using complete mt-genome data (Mulcahy, 2006; Mulcahy and Macey, 2009). A complete list of primers used in this study is shown in Table 1. Extractions of DNA were taken from previous studies (Mulcahy, 2007; Mulcahy and Macey, 2009) and PCR conditions followed Reyes-Velasco and Mulcahy (2010). The *cob* and *nad4* + trnH, trnS mtDNA are from Mulcahy (2007), and *nad5* (primers in Table 1) are from Mulcahy and Macey (2009) and added for additional taxa using the same PCR protocols described above. Sequence reactions were performed using both forward and reverse PCR primers and BigDye™ Terminator Cycle Sequencing

Table 2. Information for Genes Used in This Study. Size in base-pairs (bp), parsimony-informative (pars.-info.) characters, and substitution models (mtDNA gene models are for codon positions 1–3).

Gene	Size (bp)	Variable	Pars.-info.	Model
SLC030A1	521	46	13	GTR+G
ZEB2	765	66	26	GTR+I
FSHR	746	88	32	HKY+I
PNN	905	109	32	GTR+G
NTF3	515	99	39	K80+I
DNAH3	692	95	47	GTR+I+G
mtDNA (combined)	2,387	1,215	906	GTR+I+G
nad5	957	527	406	GTR+I+G (1,2), GTR+G (3)
cob	636	305	237	GTR+I+G (1), GTR+I (2), GTR+I+G (3)
nad4 + tRNAs	794	383	263	GTR+G (1,3), HKY+I+G (2), SYM+G (tRNAs)

chemistry (v2.0), in 12 μl reactions, purified with Sephadex (Sigma), and run out on an ABI 3730 DNA Analyzer. Complementary strands were examined in Sequencher (v4.7) and consensus sequences were aligned by amino acid translation in MacClade 4.08 (Maddison and Maddison, 2005) and tRNA secondary structure following Mulcahy (2007).

We conducted maximum likelihood (ML; Felsenstein, 1981) phylogenetic analyses on the nuclear loci separately to determine the signal within each locus using RAxML v7.0.4 (Stamatakis et al., 2005) with the rapid hill-climbing algorithm (Stamatakis et al., 2008). Searches were based on single partitions for each locus under the GTR-CAT (which uses a GTR approximation to initiate the search, and final GTR + Γ model) model of nucleotide substitutions, 1000 bootstrap inferences, and 25 discrete GAMMA rate categories. Bootstrap values ≥70% were considered strongly supported (Hillis and Bull, 1993; but see caveats therein).

We conducted three separate Bayesian phylogenetic analyses: first on the nuclear loci combined, second on the mtDNA data combined, and third on the combined nuclear and mtDNA data, with MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). The analyses of the nuclear data were run with six partitions, one for each locus. The mtDNA analyses were run using ten partitions, one for each codon of each gene (*cob, nad4,* and *nad5*), and one for the tRNA region at the end of *nad4*. The combined nuclear and mtDNA analyses were run with 16 partitions. Models of nucleotide substitution for each partition were selected using the AIC criteria in MrModeltest (Nylander, 2004; see Table 2 for models). The first two analyses (nuclear and mtDNA separately) were run for ten million generations and the combined nuclear and mtDNA analyses for 50 million generations. Each run used four heated chains, sampling trees every 1000 generations, and the first 2500 trees were discarded as the burn-in for the separate analyses, and 12,500 for the combined nuclear and mtDNA dataset. All Bayesian analyses were run twice, all parameters were unlinked, and convergence was checked by comparing the average standard deviation of split frequency (ASDSF) values of <0.01 considered acceptable, and stationarity was assessed by visual plots for each run of $-\ln L$ by generation in the program Tracer v1.4.1 (Rambaut and Drummond, 2004). Clades with posterior probabilities (Pp) ≥0.95 were considered strongly supported (Alfaro et al., 2003; Huelsenbeck and Rannala, 2004).

Coalescent-based analyses were conducted using the program BEST v2.3.1 (Liu, 2008), which simultaneously estimates individual gene trees while also estimating the overall species tree and is implemented as a module in MrBayes, thus using a Bayesian algorithm. This method obtains robust estimates of species trees with fewer loci than other methods (Edwards et al., 2007; Knowles, 2009; Leaché, 2009), and for which performance under various simulated tree topologies (tree shapes and depths) and sampling strategies (loci, individuals, and base pairs) have been studied (Castillo-Ramírez et al., 2010). Our species limits are defined *a priori*, and we included only one representative of each species (Degnan and Salter, 2005), which should alleviate problems of recombination because alleles should have coalesced between terminals (i.e., genera; Belfiore, 2010). This sampling design may preclude optimal performance for closely related species (Maddison and Knowles, 2006), but should not affect the estimation of deeper divergences (Castillo-Ramírez et al., 2010). Our single within-species sampling did not reveal any heterozygotes.

For all BEST analyses the nuclear data were combined in six partitions (one for each locus) and were run for 100 million generations, two runs, sampling every 10,000, with four heated chains, all parameters unlinked, using the same substitution models as Bayesian analyses, with a burn-in value of 5,000 trees. The second analysis included the mtDNA as a seventh partition, with 'ploidy' coded as 'haploid,' (nuclear used default 'diploid') and was run for 200 million generations, two runs, four chains, sampling every 10,000, with a burn-in of 10,000 trees. We ran three analyses for each dataset in BEST with the priors for the effective population size θ (where $\theta = \beta/\alpha - 1$; setting $\alpha = 3$, $\beta = 0.003$, results in $\theta = 0.0015$) set at three different values (0.0015, 0.015, 0.15) for the nuclear and combined nuclear and mtDNA data (Leaché, 2009). We compared the $-\ln L$ harmonic means of analyses with different θ priors using Bayes factors calculated in Tracer. Convergence of runs was checked for by comparing the ASDSF of gene trees, with values <0.1 considered acceptable (program default), by plotting $-\ln L$ by generation, and by examining effective sample size (ESS) estimates for mutation rates across loci (genemu) and tree lengths parameters in Tracer (ESS values above 200 are generally accepted to be above where autocorrelation is minimal in shaping the posterior).

RESULTS

The nuclear loci ranged in size from 515–905 bp and the mtDNA gene-regions from 636–955 bp; the latter contain orders of magnitude more variable and parsimony-informative characters (Table 2). Despite fewer variable characters in

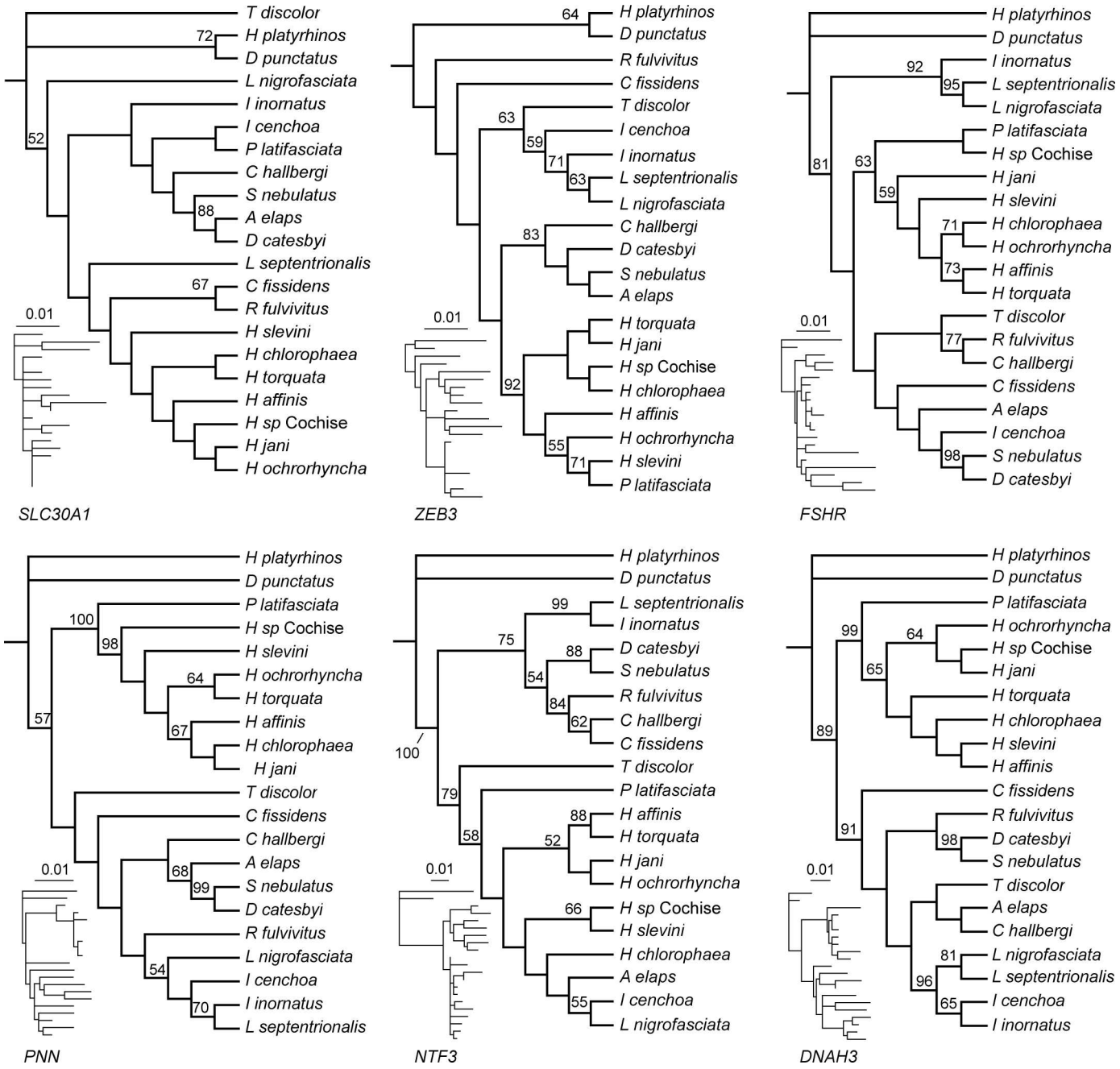

Fig. 1. Likelihood analyses for each nuclear locus. Cladograms are shown and bootstrap support values on nodes >50% are indicated; phylograms are shown in the lower left because short branch lengths obscured visualization of the structure. Using ''ladderise'' right standardized tree order within each tree.

the nuclear data, several individual loci show substantial structure under ML analyses, although the gene trees show several regions of both topological concordance and discordance (Fig. 1). For example, the nightsnake clade (*Hypsiglena* + *Pseudoleptodeira latifasciata*) is recovered in almost all gene trees, though not always with strong support (*FSHR*) and sometimes paraphyletic (*SLC30A1, NTF3*). As another example, the clade (*Dipsas catesbyi* + *Sibon nebulatus*) is recovered with strong support (bootstraps = 88–99%) in four of the six gene trees, and these are in significant conflict only with the (*Atractus elaps* + *D. catesbyi*) clade recovered in the *SLC30A1* gene tree (bootstrap = 88%).

Bayesian analyses of the concatenated nuclear data converged with ASDSF = 0.003, and an average $-\ln L$ = 19722.05 (post burn-in). The resulting consensus tree (Fig. 2A) recovered the nightsnake, Leptodeirini (*Leptodeira* + *Imantodes*), and goo-eater clades (N, L, and G, respectively) with strong support (Pp = 1.0 for all) and showed moderate support (Pp = 0.92) for the Leptodeirini in a clade with the goo-eaters (solid circle in Fig. 2A). However, other rear-fanged taxa (*Cryophis, Coniophanes,* and *Rhadinaea*) were also in this clade, *Tantalophis* was sister to this clade, and the nightsnake clade was the sister group to all other taxa, but some of these relationships were only moderately supported (Pp < 0.90 in some; Fig. 2A).

Coalescent analyses (BEST) of the nuclear data with θ prior set to 0.0015 did not converge until over 60 million generations, with ESS values for genemu (loci 1–6) <40

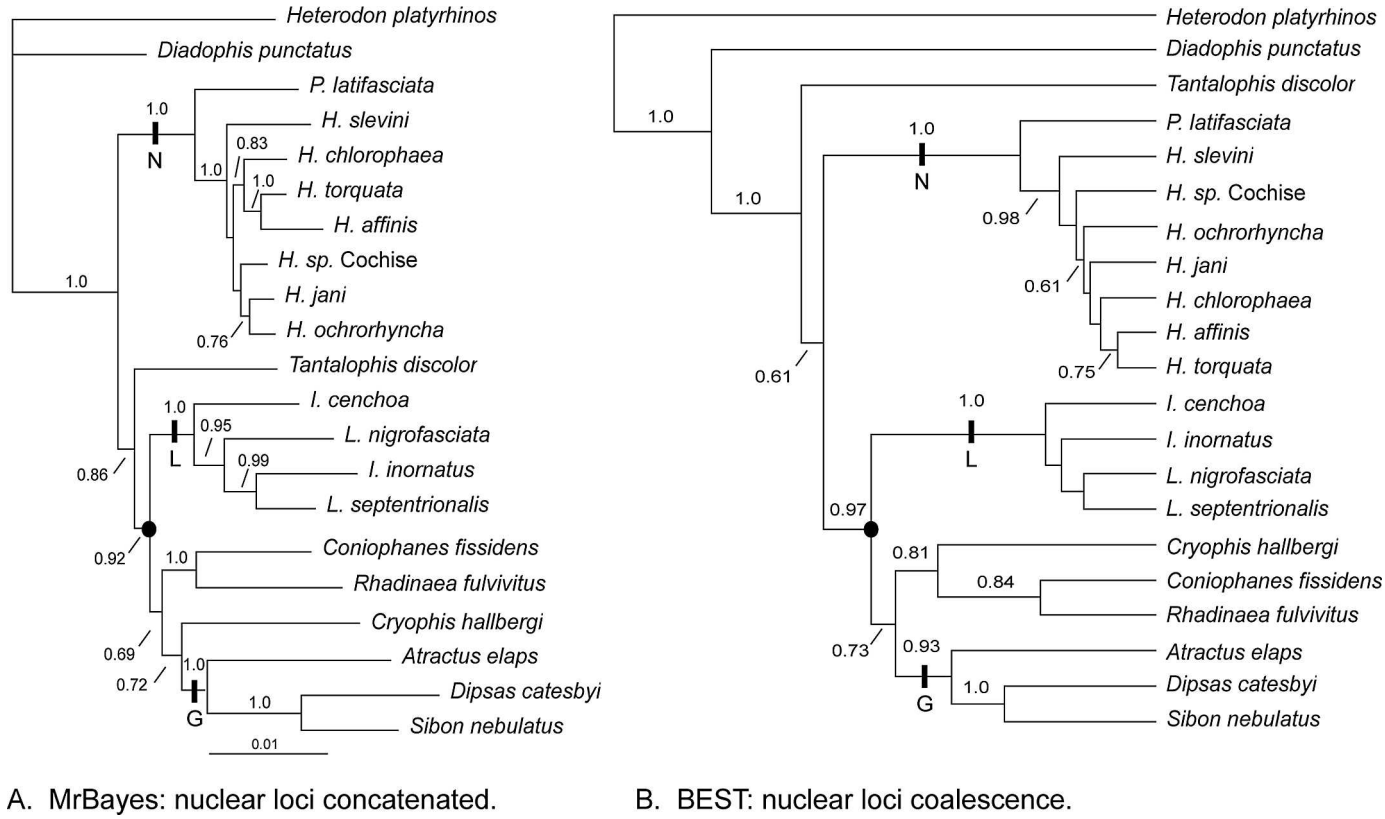

Fig. 2. Phylogenies based on nuclear loci (*SLC30A1, ZEB2, FSHR, NT3, DNAH3,* and *PNN*; 2387 bp) using: (A) Concatenated analyses with MrBayes; and (B) Coalescent analyses with BEST. Clade names: L = Leptodeirini, N = nightsnakes, G = goo-eaters. Posterior probability (Pp) values >0.50 are shown above the node/bar. Node with solid circle is referred to in text.

and tree lengths <10, while runs with the θ priors set to 0.015 and 0.15 converged within ten million generations. The ASDSF for these runs ranged from 0.003–0.005 and 0.003–0.004, ESS values for genemu ranged from 208–732 and 184–834, and tree lengths ranged from 297–1351 and 234–1618, respectively. The Bayes factor calculated from the analyses using θ priors set to 0.015 (harmonic mean = −10146.09) and 0.15 (harmonic mean = −10158.07) was 11.982. The resulting consensus topology (Fig. 2B) from the analyses with θ prior set to 0.015 was similar to the Bayesian concatenated topology for the same data (Fig. 2A). There was strong support for the nightsnake (N) and Leptodeirini (L) clades (Pp = 1.0), but the goo-eater clade (G) received less support (Pp = 0.93). There was increased support of *Imantodes* and *Leptodeira* in a clade with the goo-eaters (solid circle in Fig. 2B; Pp = 0.97), with *Coniophanes* + *Rhadinaea,* and *Cryophis* in this clade, and the nightsnakes were sister to this clade. However, *Tantalophis* was outside all of these clades, but with poor support (Pp = 0.61).

Bayesian analyses of the mtDNA data converged with ASDSF between runs <0.001 and resulted in an average −ln*L* = 19722.36. A post burn-in consensus tree recovered the same N, L, and G clades with strong support (Fig. 3A; Pp = 0.99–1.0), but recovered the nightsnakes sister to the goo-eaters + *Cryophis*, albeit with weak support (Pp = 0.75). The mtDNA data placed *Tantalophis* as the most basal member of the group with strong support (Pp = 1.0; Fig. 3A). Bayesian analyses of the concatenated mtDNA and nuclear sequences converged (ASDSF = 0.001) and resulted in a post burn-in consensus topology nearly identical to the mtDNA-only Bayesian analyses (with an average −ln*L* = 31628.26), but with less support for nightsnake + goo-eater relationship (Pp = 0.60), whereas most other nodes received increased support (Fig. 3A). The mtDNA-only topology placed *H. chlorophaea* sister to *H. ochrorhyncha* (Pp = 0.65), and the combined analyses placed *H. chlorophaea* sister to *H. sp*. Cochise (Pp = 0.51). Therefore, we have collapsed these nodes in Figure 3A for simplicity, but otherwise the mtDNA-only and the combined topologies were identical.

The combined mtDNA and nuclear BEST analyses with θ prior set to 0.0015 ran for 200 million generations and never converged, the ASDSF ranged from 0.074–0.238, with all genemu and tree length ESS values below 25, and final average −ln*L* values from 30993–31040. Analyses with θ prior = 0.015 converged at 60 million generations with the ASDSF ranging from 0.006–0.026, had an average −ln*L* = 30881.47, ESS values for tree length = 26–215, and genemu = 48–4639. One run jumped to a −ln*L* range of 30850 at 25 million generations, then dropped to the 30880 range at 60 million generations. Analyses with θ prior = 0.15 converged within two million generations with ASDSF ranging from 0.002–0.013, and an average −ln*L* = 30828.47, ESS values for tree length = 717–4233 and genemu = 519–16598. The Bayes factor calculated from the analyses using θ priors set to 0.015 (harmonic mean = −30919.12) and 0.15 (harmonic mean = −30864.80) was 54.32. The consensus of the analyses with θ = 0.15 resulted in deep topological structure identical to the nuclear loci coalescent-based analyses (compare Figs. 2B and 3B). The N, L, and G clades were recovered with strong support; the N clade is sister to the (L + (G + (*Cryophis* (*Coniophanes + Rhadinaea*)))) clade; and *Tantalophis* is the most basal (Fig. 3B). For most of these nodes, support values were higher in the ''combined gene

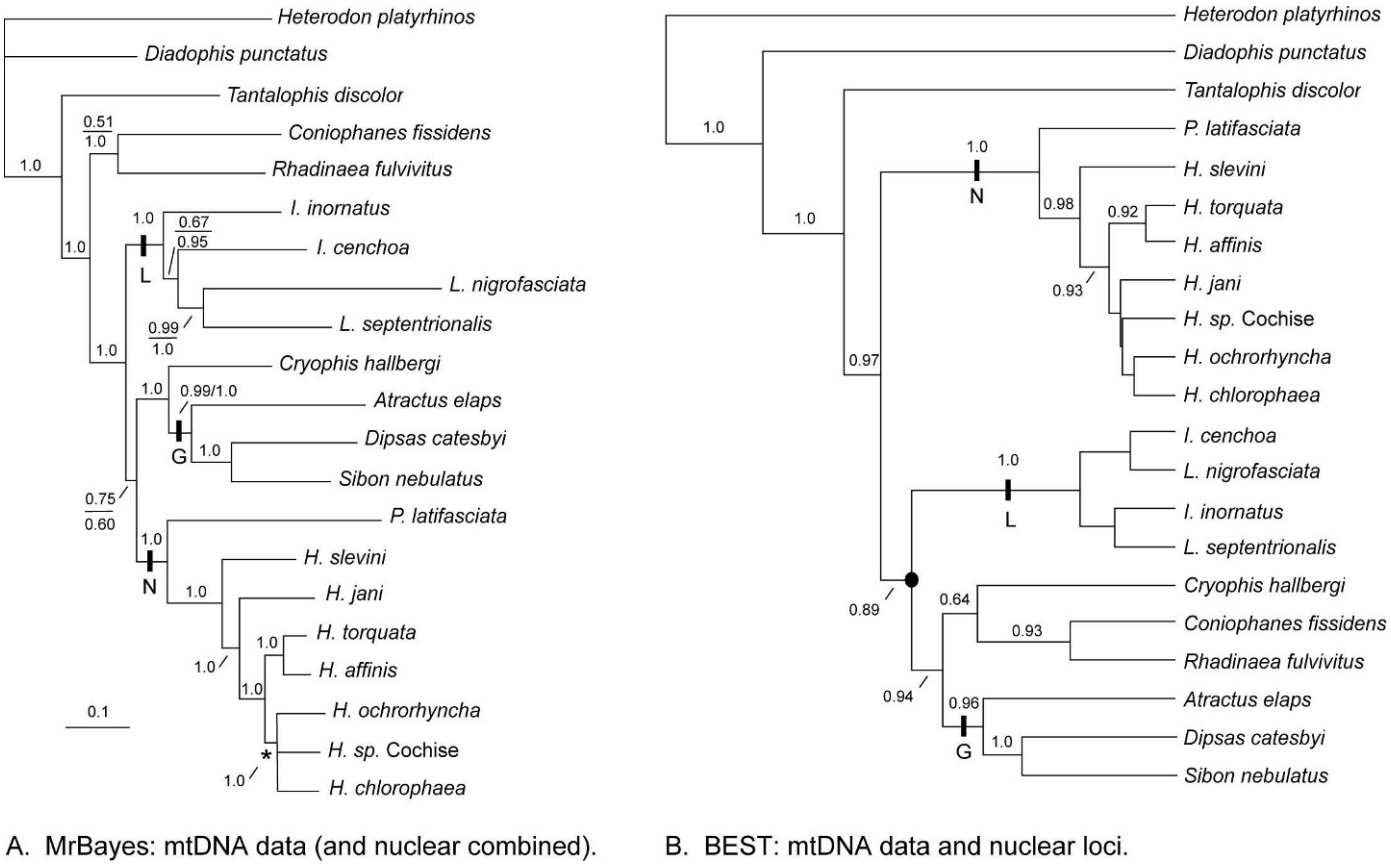

Fig. 3. Phylogenies based on mtDNA and nuclear data combined using: (A) Concatenated Bayesian analyses of the mtDNA data (*cob, nad4, nad5*; 2387 bp) combined with the nuclear loci (*SLC30A1, ZEB2, FSHR, NTF3, DNAH3, PNN*; 4149 bp; ttl. = 6536 bp). When analyzed separately, the mtDNA revealed a nearly identical topology; the asterisk identifies a polytomy where topologies differed. Pp values >0.50 are shown above the node/bar for the mtDNA only, and below the node/bar for the combined analyses; nodes labeled with one value received Pp = 1.0 for both analyses. (B) Coalescent analyses with BEST of the combined mtDNA and nuclear data. Clade names: L = Leptodeirini, N = nightsnakes, G = goo-eaters, and Pp values >0.50 are shown. Solid circle identifies clade referred to in text.

trees'' data set (Fig. 3B) relative to the ''nuclear gene trees only'' data set (Fig. 2B).

DISCUSSION

Similarities in data and methods.—Despite some heterogeneity among individual gene trees (Fig. 1), all data sets (mtDNA gene tree, nuclear loci, mtDNA + nuclear loci) and methods (concatenation vs. multi-locus coalescent approaches) recovered the following well-supported clades: (1) clade ''N'', the nightsnakes (*Hypsiglena* + *Pseudoleptodeira latifasciata*); (2) clade ''L'', the Leptodeirini (*Imantodes* + *Leptodeira*); (3) clade ''G'', the goo-eaters, all with the following structure (*Atractus* (*Dipsas* + *Sibon*)); and (4) the clade (*Coniophanes* + *Rhadinaea*). All BEST and the nuclear concatenated Bayesian analyses recovered the clade (clade L (clade G + *Cryophis* + *Coniophanes* + *Rhadinaea*)), albeit with varying structures among the three genera and the goo-eater clade (G). These shared nodes across methods and data suggest the presence of an underlying phylogenetic signal inherent in all of the data.

Differences in mitochondrial vs. nuclear data.—The nuclear data support different overall relationships from the mtDNA data by placing the nightsnakes (clade N) sister to all other genera (Fig. 2), as opposed to the mtDNA data, which place the nightsnakes in a clade with *Cryophis* and the goo-eaters (*Dipsas, Sibon,* and *Atractus*; Fig. 3A). The nuclear data placed the Leptodeirini (clade L) and *Cryophis* + *Coniophanes* + *Rhadinaea* and the goo-eaters (clade G) altogether in a clade (solid circles in Fig. 2) to the exclusion of nightsnakes. While relationships within this clade differed when analyzed with traditional concatenation methods versus the coalescent-based method, support for this clade increased using BEST (Pp from 0.92 to 0.97), but support for most relationships within it decreased (Fig. 2). The enigmatic *Tantalophis* was placed within the sampled dipsadines under the concatenation method, whereas it was placed sister to the dipsadines using the coalescent-based method, though either placement of this taxon was poorly supported (Pp = 0.86 and 0.61, respectively). The inclusion of *nad5* to the *cob* + *nad4* mtDNA data placed the nightsnakes (*Hypsiglena* + *Pseudoleptodeira*) sister to the goo-eater clade (Fig. 3A), similar to previous studies based on mtDNA data (Mulcahy, 2007; Daza et al., 2009), and provided strong support for many nodes.

When the mtDNA and nuclear data were concatenated, the overall results were similar to the mtDNA data alone, with the nightsnakes sister to the goo-eaters, and *Tantalophis* outside of the dipsadines (Fig. 3A). However, under the coalescent-based method, the analyses of the mtDNA and nuclear gene trees combined recovered a topology much more similar to the nuclear data alone. This suggests that, when concatenated, the much more variable mtDNA data (Table 2) overwhelm the signal of the nuclear loci, whereas the BEST analyses compensate for the much higher

difference in mitochondrial mutation rates (Haag-Liautard et al., 2008), by using the 'ploidy' prior, and allow for more equally weighted contribution of all of the gene trees. We consider the BEST topology based on the combined data (Fig. 3B) to be our best estimate of relationships among dipsadine genera, because it is based on all available molecular evidence and analyses that accommodate known gene-tree discordance (Fig. 1).

It is important to note that under both concatenated and coalescent-based methods, the overall topology (nightsnakes as basal, or sister to the goo-eaters) received moderate to strong support by the nuclear data (Pp = 0.92 and 0.97, to the exclusion of the nightsnakes; Fig. 2), but was only weakly supported when the mtDNA were added (Pp = 0.75 and 0.60, placing nightsnakes sister to goo-eaters; Fig. 3A). In other words, the mtDNA data contain weakly conflicting signal when compared to the nuclear data; this signal dominates the nuclear data under concatenation methods, but does not when using the coalescent-based method. Most importantly, neither the mtDNA nor the nuclear data placed the nightsnakes with *Leptodeira* (regardless of the methods used [concatenation or coalescent]), as historically thought based on morphology (Tanner, 1946; Duellman, 1958a) or albumin data (Cadle, 1984; Dowling and Jenner, 1987).

Differences in gene trees among lineages can arise through various processes, such as incomplete lineage sorting, horizontal gene transfer, and hybridization (Degnan and Rosenberg, 2009; Meng and Kubatko, 2009). Discrepancies between mitochondrial and nuclear DNA are often commonly attributed to hybridization and introgression of mtDNA across species boundaries, which appears to be common in animals (Funk and Omland, 2003). This has been documented in closely related species of lizards (Leaché, 2009) and turtles (Spinks and Shaffer, 2009), and in a well-studied example of horned lizards (*Phrynosoma*), mtDNA data showed strong support for relationships not supported by nuclear sequence data or morphology (Leaché and McGuire, 2006). Introgression of mtDNA was assumed in the horned lizards because hybridization was suspected between the two species united by the mtDNA (Mulcahy et al., 2006), which were otherwise considered more distantly related based on morphology and nuclear sequence data (Leaché and McGuire, 2006).

In the case of dipsadine snakes, we are less inclined to attribute the discrepancy between mtDNA and nuclear data to hybridization because the level of divergence is much deeper. Hybridization between *Hypsiglena* and the goo-eaters (*Dipsas, Sibon,* and *Atractus*) is highly unlikely because of differences in hemipenial morphology (Mulcahy, 2007; Zaher et al., 2009). Nevertheless, an ancient hybridization event could have occurred between common ancestors of these genera, perpetuating the contrasting phylogenetic signal between the mtDNA and nuclear data. If hybridization were suspected, we would recommend not including the mtDNA in combined analyses. Spinks and Shaffer (2009) suspected ancient hybridization and gene-capture of mtDNA among congeneric species of emydine turtles as the cause of mtDNA–nuclear gene-tree incongruence; their multi-locus data and time-calibrated phylogenies permitted these authors to hypothesize when and where this ancient hybridization event occurred.

Dipsadine taxonomy.—Interestingly, neither the nuclear nor the mtDNA data support the traditional 'Leptodeirini' (groups N + L, *Cryophis*, and perhaps *Coniophanes*; Fig. 4), which was considered a monophyletic group based on albumin immunological data (Cadle, 1984) and combined albumin and morphological data (Dowling and Jenner, 1987). Recent phylogenetic studies based on mtDNA sequence data suggest that Leptodeirini is not monophyletic (Mulcahy, 2007; Daza et al., 2009). Instead, *Leptodeira* and *Imantodes* are strongly supported as a clade in all analyses, but the mtDNA data place the nightsnakes sister to a clade containing *Cryophis* and the goo-eaters (*Atractus, Sibon,* and *Dipsas*), albeit with weak support. Our results provide additional support for the close relationship between *Leptodeira* and *Imantodes;* neither the mtDNA nor nuclear data support *Leptodeira* and *Imantodes* as being closely related to the nightsnakes (*Hypsiglena* + *Pseudoleptodeira*). Additionally, the data differ with respect to which of these groups are more closely related to the goo-eaters and other dipsadine genera (Figs. 2–3). The rearrangement of these genera based on the nuclear data does not increase or decrease the number of evolutionary transitions when the dentition and diet characters are mapped onto the phylogeny (*sensu* Mulcahy, 2007). For instance, the transition from rear-fanged snakes that prey on vertebrates to a non-rear-fanged goo-eater still only requires two character changes (Fig. 4). Thus, the new phylogeny presented here still supports the 'adaptive radiation' hypothesis (Mulcahy, 2007) for the goo-eaters—a transition from less speciose groups of rear-fanged snakes that prey on vertebrates to more speciose groups that are not rear-fanged and prey on invertebrates. This hypothesis requires further testing based on increased taxon sampling and rigorous statistical analyses (Harmon et al., 2003).

Zaher et al. (2009) revised the taxonomy and classification of most Neotropical species based on mtDNA (12S and 16S) and one nuclear locus (*CMOS*). The family Dipsadidae was recognized to include the subfamiles Xenodontinae, Carphophiinae, and Dipsadinae, with the latter containing most of the species examined in our study. Zaher et al. (2009) did not formally recognize any tribes within the Dipsadinae, but suggested that the tribe Leptodeirini could be considered to include at least *Imantodes* and *Leptodeira*. Our study supports this hypothesis and further indicates that future use of Leptodeirini should be constrained to the genera *Imantodes* and *Leptodeira*. As previously noted, Vidal et al. (2010) placed the enigmatic *Nothopsis rugosus* sister to *Leptodeira* in an analysis of Dipsadidae based on 12S–16S, and without statistical support (>50% ML bootstrap or Bayesian Pp > 0.90) for its placement they recommended including this genus in the Leptodeirini. Zaher (1999) and Zaher et al. (2009) recommended placing *Nothopsis* in Dipsadinae *incertae sedis* based on similar hemipenial morphology. We agree with this recommendation and refrain from including *Nothopsis* within Leptodeirini until significant phylogenetic support can be found to verify its placement. During revisions of this manuscript we learned that the validity of the name Leptodeirini is being challenged because of the nature in which it was originally described (Myers, 2011). Alternative to redefining Leptodeirini, a new tribal name was proposed for *Leptodeira* and *Imantodes* (Imantodini Myers 2011).

Cadle (1984) pointed out that *Imantodes* and *Leptodeira* had not been considered closely related prior to his albumin immunological studies, other than both being placed in the Dipsadinae. Mulcahy (2007) found these genera closely

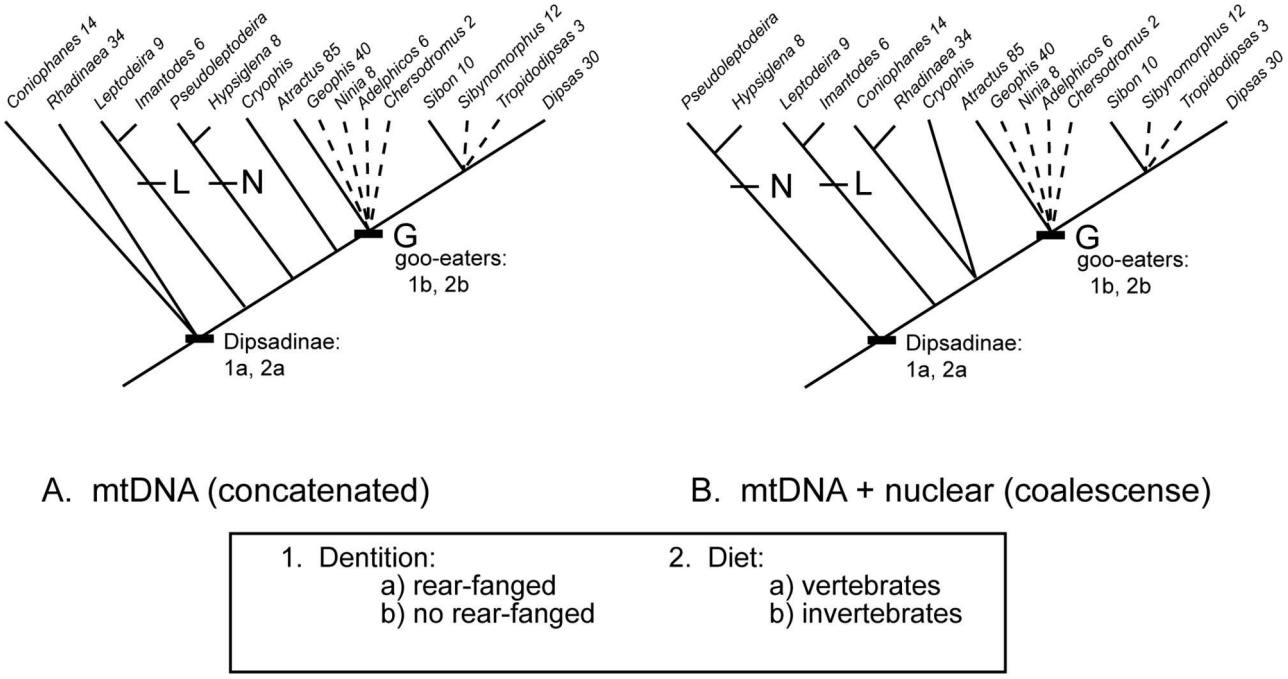

Fig. 4. Simplified cladograms based on mitochondrial data (A), and mtDNA and nuclear data combined (B). Tree B is a strict consensus between the concatenated likelihood and Bayesian analyses, and the BEST analyses. Numbers above generic names indicate estimated number of species if more than one, dashed lines indicate inferred placement for dipsadine genera that were not included in this study, dentition and diet traits are mapped on the trees (following Mulcahy, 2007). Clade names used throughout this study: L = Leptodeirini, N = nightsnakes, G = goo-eaters.

related, but paraphyletic with respect to one another based on parsimony analyses of mtDNA data (*cob* and *nad4*), and Bayesian analyses recovered *Leptodeira* as monophyletic, but not *Imantodes*. The presumed basal members of each group (*L. nigrofasciata* and *I. inornatus*) were placed sister to one another in the parsimony analyses, which was attributed to long-branch attraction (Mulcahy, 2007). With increased taxon sampling in both genera, Daza et al. (2009) found moderate to strong support for a monophyletic *Leptodeira,* but *Imantodes* remained paraphyletic with strong support based mtDNA data (*cob* and *nad4*). In this study, concatenated analyses of the nuclear data recovered *I. inornatus* sister to *L. septentrionalis* with strong support (Pp = 0.99), whereas the coalescent-based method BEST recovered *Leptodeira* as monophyletic, but with no support (Pp < 0.50). Our increased mtDNA dataset (*nad5* added) further supported the monophyly of *Leptodeira*, but also failed to recover a monophyletic *Imantodes* (Fig. 3A). Daza et al. (2009) concluded that both genera were likely monophyletic and attributed this lack of concordance (between mtDNA and nuclear data) to low variation in the nuclear loci and the recent divergence between these genera not allowing enough time for coalescence. This problem should be accommodated in our BEST analyses, and our failure to recover one or both genera as monophyletic (Figs. 2B and 3B, respectively) suggests that additional sampling of individuals, species, and gene regions within and among these genera will be needed to further test their monophyly with coalescent-based analyses.

Finally, as implied by its name, the taxon *Tantalophis discolor* has troubled systematists for nearly a century and a half. This species was initially described as a species of *Leptodeira* (Günther, 1860), later placed in the genus *Hypsiglena* (Cope, 1887; Günther, 1894), then tentatively placed in *Pseudoleptodeira* (Taylor, 1938), all on the basis of scalation, until it was finally placed in its own genus *Tantalophis* (Duellman, 1958b), largely based on hemipenial morphology. The description of two other monotypic genera, *Rhadinophanes* (Myers and Campbell, 1981) and *Chapinophis* (Campbell and Smith, 1998), revealed similar derived hemipenial features among these genera of snakes, different from typical dipsadines. Myers and Campbell (1981) regarded *Tantalophis* and *Rhadinophanes* as sister, but maintained separate genera because of extensive morphological differences beyond hemipenial morphology. They further speculated among sister relationships between this group and the *Rhadinaea* + *Coniophanes* group, as well as the *Leptodeira*, but Zaher (1999) placed *Tantalophis* as Dipsadinae *incertae sedis* based on hemipenial morphology. Recent molecular studies based on mtDNA data (*cob* and *nad4*) placed *Tantalophis* as the sister group to the Dipsadinae (Mulcahy, 2007; Daza et al., 2009). Zaher et al. (2009) recommended placing *Chapinophis* within Dipsadinae, but maintained *Tantalophis* and *Rhadinophanes* as Dipsadidae *incertae sedis*. Our study is the first to include nuclear data for the genus *Tantalophis* and concatenated nuclear sequences recovered nightsnakes at the most basal divergence within the Dipsadinae, with *Tantalophis* weakly placed as sister to all other genera of dipsadines sampled (Fig. 2A; Pp = 0.86). However, the coalescent-based analyses placed it outside of the clade of all other genera, but with weak support (Fig. 2B; Pp = 0.61). The addition of the mtDNA placed *Tantalophis* sister to the dipsadines with much greater support in both concatenated (Fig. 3A; Pp = 1.0) and coalescent-based (Fig. 3B; Pp = 0.97) methods. We recommend keeping *Tantalophis* as Dipsadidae *incertae sedis* (Zaher et al., 2009), but suggest that future studies should include this taxon, and perhaps the more rare *Rhadinophanes* and *Chapinophis,* if possible, when trying to assess the phylogenetic relationships within this group.

MATERIAL EXAMINED

Specimen information is shown in the following order: taxon, voucher number, brief locality, and GenBank numbers follow in the order of: *cob, nad4, nad5*, SLC30A1, ZEB2, FSHR, NTF3, DNAH3, PNN (except *I. inornatus* lacks NTF3). GenBank GU numbers were generated for this study, other numbers are from published data and correspond with: Mulcahy, 2007 (EF078s); Mulcahy, 2008 (EU363055); Mulcahy and Macey, 2009 (EU728s, FJ455s); Reyes-Velasco and Mulcahy, 2010 (FJ810s). *In two outgroup cases (*Heterodon* and *Diadophis*) different vouchers were used for one locus (see GenBank accessions for vouchers and references). Institutional abbreviations follow Leviton et al. (1985) with the addition of MZFC = Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM), EBUAP = Escuela de Biología de la Universidad Autónoma de Puebla, and JRM = Joseph R. Mendelson, III.

Atractus elaps: KU 214837, Peru, Madre de Dios, EF078536, EF078584, GU353263, GU353304, GU353314, GU353253, GU353273, GU353244, GU353283.

Coniophanes fissidens: KU 289798, El Salvador, San Salvador, EF078538, EF078586, GU353264, GU353305, GU353315, GU353254, GU353274, GU353245, GU353284.

Cryophis hallbergi: MZFC-JRM 4778, Mexico, Oaxaca, EF078496, EF078544, GU353266, GU353307, GU353317, GU353256, GU353276, GU353247, GU353286.

Diadophis punctatus: FMNH 259791, USA, Virginia, GU353240, EU194213*, GU353261, GU353302, GU353313, GU353252, GU353272, GU353243, GU353281.

Dipsas catesbyi: KU 214851, Peru, Madre de Dios, EF078537, EF078585, GU353267, GU353308, GU353318, GU353257, GU353277, GU353248, GU353287.

Heterodon platyrhinos: YPM 13421, USA, Connecticut, GU353239, AF402659*, GU353260, GU353301, GU353312, GU353251, GU353271, GU353242, GU353280.

Hypsiglena affinis: LSU 39533, Mexico, Jalisco, GU353241, EU363055, GU353268, GU353309, GU353319, GU353258, GU353278, GU353249, GU353294.

Hypsiglena chlorophaea: MVZ 237359, USA, Arizona, EU728593, EU728593, EU728593, FJ455179, FJ455163, FJ455211, FJ455195, FJ455227, GU353289.

Hypsiglena jani: CAS 228960, USA, Texas, EU728592, EU728592, EU728592, FJ455177, FJ455161, FJ455209, FJ455193, FJ455225, GU353291.

Hypsiglena ochrorhyncha: MVZ 236396, Mexico, Baja Calif. Sur, EU728578, EU728578, EU728578, FJ455186, FJ455170, FJ455218, FJ455202, FJ455234, GU353292.

Hypsiglena slevini: MVZ 234613, Mexico, Baja Calif. Sur, EU728584, EU728584, EU728584, FJ455175, FJ455159, FJ455207, FJ455191, FJ455223, GU353288.

Hypsiglena torquata: MZFC 16926, Mexico, Sinaloa, EU728591, EU728591, EU728591, FJ455176, FJ455160, FJ455208, FJ455192, FJ455224, GU353293.

Hypsiglena sp. Cochise Clade: CAS 228951, USA, Arizona, EU728580, EU728580, EU728580, FJ455178, FJ455162, FJ455210, FJ455194, FJ455226, GU353290.

Imantodes cenchoa: MVZ 149878, Costa Rica, Limon, EU728586, EU728586, EU728586, FJ455171, FJ455155, FJ455203, FJ455187, FJ455219, GU353295.

Imantodes inornatus: MVZ 204110, Costa Rica, Heredia, EF078512, EF078560, GU353269, GU353310, GU353320, GU353259, GU353250, GU353296.

Leptodeira nigrofasciata: MVZ 241573, Mexico, Guerrero, EF078533, EF078581, GU353270, GU353311, FJ810223, FJ810231, FJ810241, FJ810227, GU353297.

Leptodeira septentrionalis: MVZ 164943, Mexico, Guerrero, EU728590, EU728590, EU728590, FJ455172, FJ455156, FJ455204, FJ455188, FJ455220, GU353298.

Pseudoleptodeira latifasciata: LSUMZ 39571, Mexico, Guerrero, EU728579, EU728579, EU728579, FJ455174, FJ455158, FJ455206, FJ455190, FJ455222, GU353299.

Rhadinaea fulvivittis: MVZ 231852, Mexico, Veracruz, EF078539, EF078587, GU353265, GU353306, GU353316, GU353255, GU353275, GU353246, GU353285.

Sibon nebulatus: MVZ 233298, Costa Rica, Limon, EU728583, EU728583, EU728583, FJ455173, FJ455157, FJ455205, FJ455189, FJ455221, GU353300.

Tantalophis discolor: EBUAP 1853, Mexico, Oaxaca, EF078541, EF078589, GU353262, GU353303, FJ810222, FJ810230, FJ810240, FJ810226, GU353282.

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