Coalescent Species Tree Inference of *Coluber* and *Masticophis*

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The genus-level taxonomy of the New World racers and whipsnakes (*Coluber* and *Masticophis*) has long been contentious regarding whether the two genera are mutually exclusive clades. This argument is based on morphological characters and largely single-locus analyses. Herein we examine the phylogenetic history of this group using multi-locus data in a coalescent framework, where paraphyly of *Masticophis* would result in support for the recognition of only a single genus (*Coluber*) for these species. We sample all currently recognized species and incorporate broad geographic sampling for the more widespread species groups to explore biogeographic patterns across North America. Our analyses suggest that *Masticophis* is monophyletic with respect to *Coluber constrictor*, albeit with low support. These results also demonstrate that there is undescribed cryptic diversity in this group, and we underscore additional avenues of study to further delimit unrecognized species in this clade. The biogeography of the island endemic, *Masticophis anthonyi*, is discussed with respect to what is known about other codistributed vertebrates. Lastly we provide an overview of the history of the arguments for or against the use of the generic name *Masticophis* and suggest its continued use.

IT is widely accepted that higher-level taxonomy should reflect evolutionary history, and while most modern researchers agree on this general principle, often current taxonomy still uses traditional arrangements that remain untested with respect to phylogenetic history. Molecular phylogenetic hypotheses can explicitly test taxonomic arrangements based on traditional classifications for extant taxa (e.g., D'Erchia et al., 1996; Sullivan and Swofford, 1997) where nodal support for a group can be used to examine taxonomic hypotheses under a parsimony, maximum likelihood, or Bayesian inference framework (e.g., Frost et al., 2006; Reyes-Velasco et al., 2013; Betancur-R. and Ortí, 2014). If strong posterior probability (PP) or bootstrap support (BS) exists for a node that renders a group paraphyletic, then the current taxonomy of that group is considered incorrect under the principle of monophyly (de Queiroz and Gauthier, 1990).

While it is well established that molecular data are important for generating phylogenies and are imperative for testing taxonomy (Bergsten et al., 2013), the tree itself must be a reliable estimate of evolutionary history, which brings up a few issues that should be considered. First, broad taxonomic sampling of the group in question can improve the accuracy of phylogeny estimation (Zwickl and Hillis, 2002; Heath et al., 2008). This extends to within-species population level sampling where gene flow may be occurring inconsistently across the ranges of parapatrically distributed populations; relationships among species may change given the geographic sampling of individuals used in an analysis (Leaché, 2009; Spinks et al., 2013). Importantly, the accuracy of phylogeny estimation has been shown to improve as more alleles and more individuals are sampled (Maddison and Knowles, 2006; Bergsten et al., 2013; Hovmöller et al., 2013). Therefore, complete sampling of species and thorough geographic sampling should be a priority for taxonomic research. Second, gene trees generated using a single locus or multi-gene concatenation can result in a tree that is incompatible with the species history due to incomplete lineage sorting (Edwards, 2009), a situation often exacerbated by rapid species diversification (e.g., Koblmüller et al., 2010). Even at deep phylogenetic time scales, coalescent processes can be a contributing factor to error in phylogeny estimation (Lanier and Knowles, 2014); therefore, species tree approaches that accommodate incomplete lineage sorting are preferred to concatenation approaches (Maddison and Knowles, 2006; Edwards et al., 2016). However, it should be noted that neither concatenation nor species tree methods account for gene flow and reticulation, which could bias phylogeny estimation (Eckert and Carstens, 2008). Ultimately, coalescent-based, species tree approaches rather than gene tree topologies should be used to test any classification based on the principle of monophyly, particularly in rapid radiations where branch lengths are expected to be short (Edwards et al., 2016).

The New World racers and whipsnakes (*Coluber* and *Masticophis*) present a challenging taxonomic problem unresolved for nearly a century. While these snakes are conspicuous members of the vertebrate fauna of North America and have been well studied with respect to ecology (Camper and Dixon, 2000; Halstead et al., 2008; Steen et al., 2013), physiology (van Doorn and Sivak, 2013), and behavior (Bealor and Saviola, 2007), there has been considerable controversy regarding recognition of the genus *Masticophis*. Authors have interpreted the same morphological evidence as either support for or against lumping *Masticophis* and *Coluber* (e.g., maxillary teeth number, scale-row formula and method of scale-row reduction, and hemipenial morphology; Ortenburger, 1928 vs. Bogert and Oliver, 1945). Ortenburger (1928) conducted the most thorough taxonomic revision for this group using morphological data and yet several authors have debated this taxonomy (e.g., Bogert and Oliver, 1945; Wilson, 1973; Schätti, 1986; Camper and Dixon, 1994). Recent molecular phylogenetic

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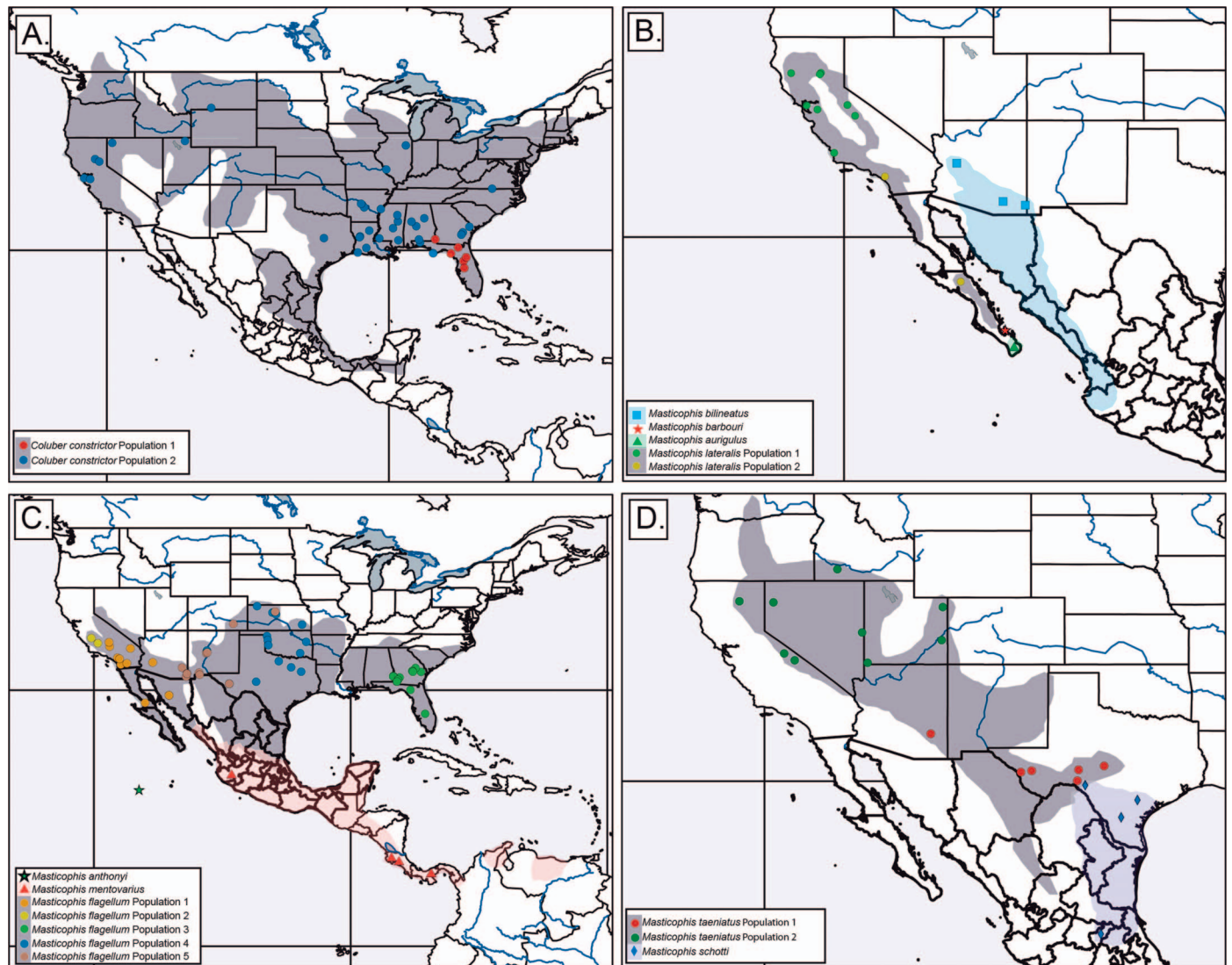
Fig. 1. Collecting localities and approximate distribution of each species, based on Stebbins (2003) and Johnson (1977). (A) *Coluber constrictor* (distribution is in gray); (B) *Masticophis barbouri*, *M. aurigulus*, *M. lateralis* (distribution in gray), and *M. bilineatus* (distribution in blue); (C) *Masticophis anthonyi*, *M. mentovarius* (distribution in red), and *M. flagellum* (distribution in gray); (D) *Masticophis taeniatus* (distribution in gray) and *M. schotti* (distribution in purple).

approaches to examine taxonomy of this group have suffered from poor taxon sampling, although it should be noted that evidence for paraphyly, despite modest taxon sampling, can still be detected. Previous molecular analyses have only used one nuclear locus and up to four mtDNA genes (Nagy et al., 2004; Utiger et al., 2005). Utiger et al. (2005) inferred that *Masticophis flagellum* was paraphyletic with respect to *Coluber*, though bootstrap values were low (38–68); the authors suggested unifying the two taxa under the name *Coluber*, because this name has priority. Additionally, because of small sample sizes in previous molecular datasets, population structure within species and the biogeography of this group is largely unknown. For example, the phylogenetic relationships of *Masticophis anthonyi*, which is restricted to Isla Clarión, have never been investigated. This is of interest because two other endemic squamates share similar patterns of dispersal to the Revillagigedo Islands (e.g., Feldman et al., 2011; Mulcahy et al., 2014), yet the biogeographic history of *M. anthonyi* and many other terrestrial organisms of this island chain are unknown.

Herein, we test the monophyly of the species of *Masticophis* relative to *Coluber* using all taxa and including a broad geographic sampling of individuals at the population level; our approach accounts for phylogenetic uncertainty given coalescent processes and incomplete lineage sorting (ILS). We also present a review of the history of the taxonomy of this common New World group of snakes. Second, we test for phylogeographic structure in the widespread taxa and identify future areas of study where species show deep phylogeographic structure. Lastly, we discuss the biogeography of the enigmatic *Masticophis anthonyi*, whose distribution is restricted to Isla Clarión.

MATERIALS AND METHODS

Taxon sampling and molecular data.—We acquired tissue samples from 140 individuals representing all currently recognized species of *Masticophis* and *Coluber* from our own fieldwork, museum loans, and from colleagues who did not collect voucher specimens (Supplemental Appendix A, see Data Accessibility; Fig. 1). We used four *Mastigodryas*

melanolomus and three *Salvadora hexalepis* as outgroup taxa given relationships presented in Pyron et al. (2013). Our ingroup sampling includes 39 *C. constrictor*, 56 *Masticophis flagellum*, 5 *M. anthonyi*, 3 *M. bilineatus*, 11 *M. lateralis*, 5 *M. mentovarius*, 4 *M. schotti*, 15 *M. taeniatus*, and 1 each of *M. aurigulus* and *M. barbouri*. Where possible, specimens were sampled across the breadth of species' distributions (Fig. 1). DNA was extracted from tissue samples using Qiagen DNeasy® kits or on an Auto Genprep 965 using phenol extractions (2011 AutoGen, Inc.). We used previously published or optimized preexisting primers for two mitochondrial DNA (cytb and COI) and five nuclear DNA loci (cmos, NT3, R35, DNAH3, and SPTBN1) that have been used previously in systematic studies of squamates (Burbrink et al., 2000; Lawson et al., 2005; Noonan and Chippindale, 2006; Townsend et al., 2008; Leaché, 2009; Pyron and Burbrink, 2009; see Supplemental Appendix B for primer details, see Data Accessibility). These loci were amplified via PCR; products were cleaned using Exo-Sap-IT (USB Corporation) and sequenced in both directions on a Beckman-Coulter CEQ-8000 automated sequencer or on an ABI3730 (Life Technologies). Sequences were edited and aligned in Geneious v7 (Biomatters) using the Muscle (Edgar, 2004) algorithm. No gaps were detected in any of the protein coding genes. The program PHASE v2.1.1 (Stephens and Donnelly, 2003) was used to determine the most probable pair of alleles for all nuclear loci, and the web server interface SeqPhase was used to convert files for this application (Flot, 2010). Because of the diversity of species and large number of sampled individuals, this dataset was phased as four subsets where the species groups follow Ortenburger (1928): 1) *M. flagellum* group (*flagellum, mentovarius, anthonyi*); 2) *M. taeniatus* group (*taeniatus, schotti, bilineatus, lateralis, barbouri, aurigulus*); 3) *C. constrictor* group (*constrictor*, including all lineages from Burbrink et al., 2008); and 4) outgroup taxa. We used default program priors and only accepted haplotypes with a posterior probability >0.9; alleles that could not be resolved remained in the dataset with heterozygosities coded according to the IUPAC ambiguity codes. Current phylogenetic methods assume no recombination within loci; therefore, the signature of this process was tested for in all nuclear loci using the difference of sums of squares (DDS; McGuire and Wright, 1998) method implemented in TOPALi v2 (Milne et al., 2009).

Population assignment.—Prior knowledge of species or population assignment is required for most species tree analyses (i.e., BEST, *BEAST, but see O'Meara, 2009). It is unlikely that any morphologically described species with a large geographic range is panmictic (Burbrink et al., 2008), and combining alleles from distinct populations to estimate species trees has been shown to overestimate branch lengths while decreasing the overall accuracy of the tree estimation (Carstens and Dewey, 2010).

Where we had samples that spanned the distribution of the range of a species we tested for population subdivision with the program STRUCTURE (Pritchard et al., 2000), which uses a Bayesian clustering algorithm to infer populations that are in both Hardy-Weinberg and linkage equilibrium, while assigning individuals to the inferred populations. Phased nuclear alleles were converted to STRUCTURE input files using xmfa2struct (http://www.xavierdidelot.xtreemhost. com/clonalframe.htm). The locus SPTBN1 was excluded because of large amounts of missing sequence data, as were those individuals that failed to sequence for >75% of the

nuclear loci. STRUCTURE was then run separately on the following species: *M. flagellum*, *M. lateralis*, *M. taeniatus*, and *C. constrictor*. The number of potential populations, *K*, was set from 1–10 and each *K* value was run for five independent iterations, the admixture model was used, and allele frequencies were set to independent. Each MCMC analysis was run for 1,000,000 iterations, with a burn-in of 100,000 iterations. The optimal value of *K* was selected using the method of Evanno et al., which is based on the rate of change in the log probability of the data under successive values of *K* (Evanno et al., 2005), via the StructureHarvester webserver interface (Earl, 2012). Individuals that were excluded from this analysis were assigned back to populations based on geographic locality for species tree estimation.

Species tree estimation.—Species trees were estimated using *BEAST (Heled and Drummond, 2010) in the software Beast v2.4.4 (Bouckaert et al., 2014). *BEAST was used because it has been shown to be robust to missing data (Hovmöller et al., 2013) and mutational rate differences among loci (Lanier et al., 2014). jModeltest2 (Darriba et al., 2012) was used to infer models of sequence evolution for each locus where model fit was assessed via Bayesian information criteria (Schwarz, 1978). Four unconstrained species trees were run to ensure topological convergence. In all *BEAST runs, the two mtDNA loci were linked and a relaxed lognormal clock model prior was used for all loci. A Yule speciation process was selected for the species tree prior, with a piecewise linear and constant root. Each analysis was run for 500 million iterations, which were thinned every 50,000 generations. Gene trees for each locus were estimated in these analyses. The four independent analyses were combined using LogCombiner v2.4.5 (Bouckaert et al., 2014). The post burn-in sample of the combined trees was visualized in DensiTree v2.1.11 (Bouckaert, 2010) to illustrate uncertainty in species relationships.

RESULTS

Molecular data.—Sequences were obtained and aligned for cytb (1117 bp), COI (654 bp), NT3 (541 bp), SPTBN1 (864 bp), DNAH3 (721 bp), R35 (485 bp), and cmos (616 bp). All sequences have been deposited in GenBank (Supplemental Appendix A, see Data Accessibility). The number of variable sites was highest for COI (149 sites), followed by cytb (63 sites), SPTBN1 (43 sites), DNAH3 (37 sites), NT3 (32 sites), cmos (11 sites), and lastly, R35 (7 sites). The number of parsimony informative sites is greatest for COI (95 sites), followed by cytb (37 sites), DNAH3 (34 sites), SPTBN1 (33 sites), NT3 (29 sites), cmos (8 sites), and lastly, R35 (7 sites). Models of sequence evolution determined by jModeltest2 are as follows: cytb—GTR+G+I; COI—GTR+G+I; cmos—HKY +G; DNAH3—GTR+G+I; NT3—GTR+G+I; SPTBN1—HKY +G; R35—HKY + I. Results from TOPALi indicate that only NT3 shows a significant signature of recombination among the ingroup taxa; however, this could be an artifact of high among-site rate variation within this locus (McGuire and Wright, 1998). Further, phylogeny estimation has been shown to be robust to violations of the assumption of nonrecombination within loci, particularly for rapidly radiating groups (Lanier et al., 2014; Lavretsky et al., 2014).

Population structure.—All species for which range-wide samples were available showed population subdivision (*Masticophis bilineatus, M. mentovarius,* and *M. schotti* not
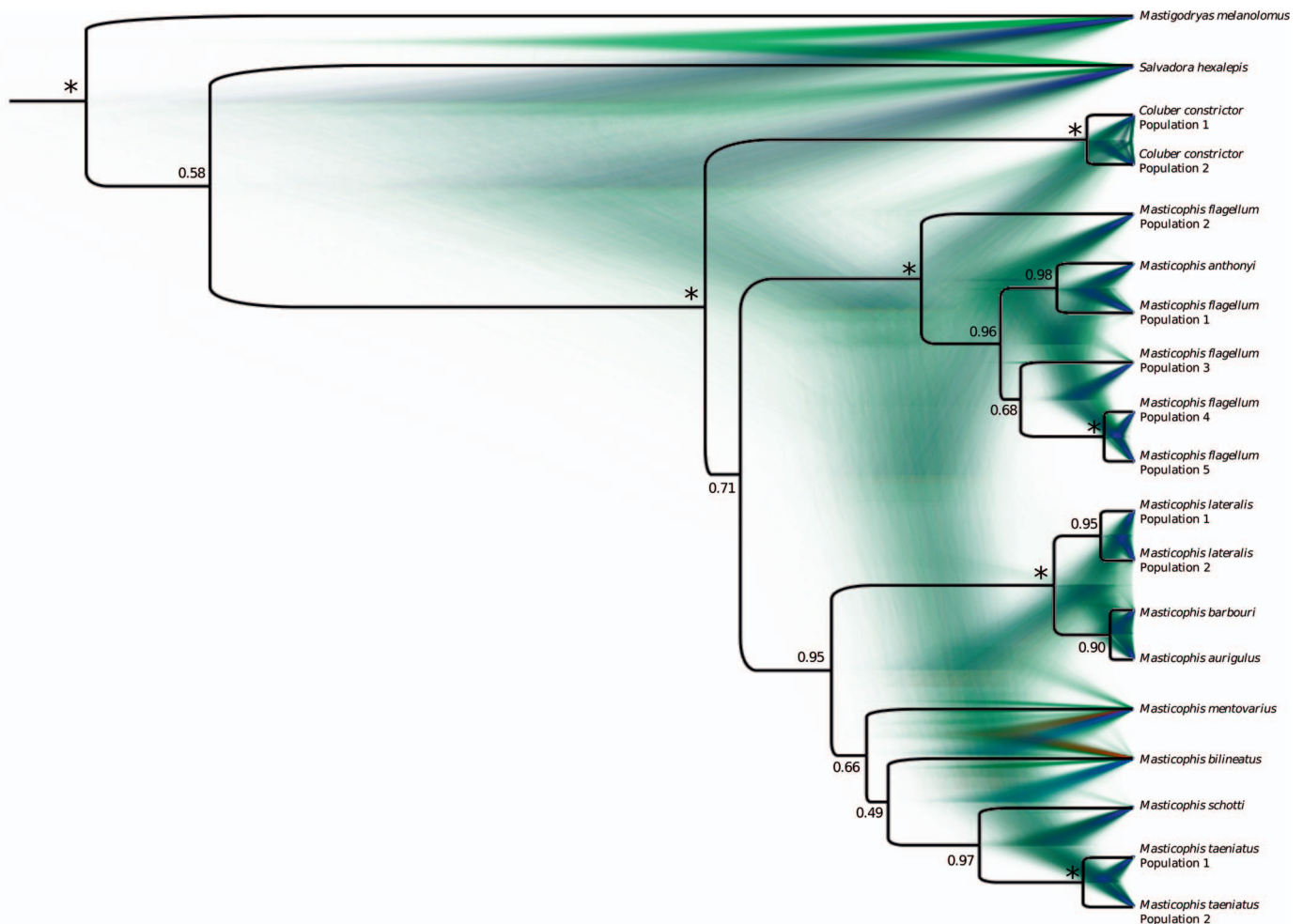
Fig. 2. Topology of the maximum credible clade combined species tree inferred from *BEAST overlaid (in black) on the cloud of trees from the posterior sample. Posterior probability support values are listed at each node, where '*' represents PP support values of 1. Note that the branch lengths of the MCC tree are proportional to time.

tested). Results from STRUCTURE suggests that *C. constrictor* is composed of two populations, with $K = 2$ having the largest ΔK (2111.98) as estimated from the rate of change in the log probability of the data under successive values of K (Evanno et al., 2005). These genetic clusters correspond to the most divergent mtDNA lineages found by Burbrink et al. (2008), one distributed in the Florida peninsula and the other occupying the remaining areas of North America (Fig. 1A). Results for *M. flagellum* suggest that this taxon is composed of $K = 5$ populations ($\Delta K = 2.94$). These populations are geographically structured as follows: 1) all individuals west of the Cochise Filter Barrier including Baja California but excluding the San Joaquin Valley of California; 2) individuals found within the San Joaquin Valley ("*M. f. ruddocki*"; Brattstrom and Warren, 1953); 3) east of the Mississippi River; 4) west of the Mississippi River to west-central Texas and north to Kansas; 5) west-central Texas to eastern Arizona and north to Colorado (Fig. 1C). *Masticophis lateralis,* with the greatest ΔK (450.12) at $K = 2$, corresponding to northern and southern clusters, which show a division in the vicinity the Transverse Ranges of southern California (Fig. 1B). *Masticophis taeniatus* also showed subdivision ($K = 2$; $\Delta K = 197.72$) corresponding to a population northwest of the Colorado Plateau and a population south of this physiographic region (Fig. 1D).

Species trees.—Stationarity of the MCMC in all four *BEAST analyses was assessed in Tracer v1.6 (Rambaut et al., 2014). The samples from most parameters across the four analyses have ESS values of greater than 200, suggesting stationarity, and the most credible clade inferred across all runs converged on the same topology (Fig. 2). These species trees consistently show that *Masticophis* is monophyletic with respect to *Coluber*, albeit not with strong support (PP = 0.71). The cloudogram of the posterior sample of species trees suggests considerable uncertainty in the placement of the *M. flagellum* group (Fig. 2). These species trees also place *M. anthonyi* as sister to the western clade of *M. flagellum* found in the Sonoran and Mojave deserts (Population 1). Individual gene trees generated from the *BEAST analysis show considerably different relationships among the ingroup taxa (e.g., *Coluber constrictor* is nested within *Masticophis* in DNAH3, NT3, and R35; Supplemental Appendix C, see Data Accessibility).

DISCUSSION

Taxonomic implications and overview.—Using coalescent-based species-tree methods, we assess the relationships within a widespread group of New World snakes. Here, we find that the widely recognized and studied snake genus *Masticophis* is monophyletic with respect to *Coluber* and suggest that placing *Masticophis* in the synonymy of *Coluber* is unwarranted pending additional data. Applying the names

Masticophis and *Coluber* has typically been based on morphological evidence, where various authors have used the same evidence for opposing arguments (Ortenburger, 1928 vs. Bogert and Oliver, 1945). Here we provide an overview of these opposing arguments:

The genus *Coluber* is attributed to Linnaeus (1758), including *C. constrictor* as the type species, and this was used as the generic name for nearly all colubrid taxa known at the time. *Masticophis* was proposed by Baird and Girard (1853) for the currently recognized species *M. taeniatus* and *M. flagellum*, but also included a subspecies of *Coluber constrictor*, *C. c. mormon* (however, at the time this taxon was described as a species), and *M. ornatus* was designated as the type species; however, this taxon is currently considered a junior synonym of *M. taeniatus*. Baird and Girard (1853) also used the genus name *Bascanion* for *Coluber constrictor*. Stejneger and Barbour (1917) placed both names in the synonymy of the older name, *Coluber*, but did not give an argument for this arrangement. In a monographic revision of the group, Ortenburger (1928) argued that *Masticophis* and *Coluber* should remain as separate genera based largely on maxillary tooth numbers, scale-row formulae and patterns of scale-row reduction, as well as hemipenial morphology. Inger and Clark (1943) supported this division based on scale counts and hemipenial morphology; however, Bogert and Oliver (1945) stated that only the single genus *Coluber* should be used for the group because "no satisfactory basis for partitioning *Coluber* has been offered". Auffenberg (1955) suggested that *Masticophis* be placed in the synonymy of *Coluber* based on scale row reduction, citing scale row counts of *M. taeniatus* that are the same as those of *C. constrictor*. Later authors suggested retaining both genera to keep *Coluber* from being too 'unwieldy' (Wilson, 1970). Therefore, Old World taxa and the New World *C. constrictor* were all placed in *Coluber* (Schätti and Wilson, 1986; but see Wallach et al., 2014), with the remainder of the New World taxa placed in *Masticophis*; placing *Masticophis* in the synonymy of *Coluber* was not considered useful until such a move was shown to be well supported (Wilson, 1973). Schätti (1986) argued for uniting *Masticophis* and *Coluber* because they cannot be easily differentiated based on dentition, vertebral structure (particularly when attempting to refer fossil taxa; Auffenberg, 1963), and hemipenes. Molecular data have been used to address this question; however, these analyses do not agree, and have shown either paraphyly of *M. flagellum* with respect to *C. constrictor* (Utiger et al., 2005) or a sister relationship, and monophyly of both *C. constrictor* and *M. flagellum* (Nagy et al., 2004; note that this study only sampled *C. constrictor* and *M. flagellum*, therefore a sister relationship could be an artifact of taxon sampling); both studies suffered from very limited taxon sampling (only 20% of species diversity). Nagy et al. (2004) did, however, show that the Old World taxa, such as *Bamanophis dorri* and *Mopanveldophis zebrinus* (*Coluber* [s.l.] *dorri* and *Coluber* [s.l.] *zebrinus*), previously referred to *Coluber* were not closely related to *C. constrictor* and therefore concluded that the name *Coluber* be restricted to the New World, following the type designation (see Nagy et al., 2004: p. 231). Supermatrix approaches to squamate systematics using data from Nagy et al. (2004) and Utiger et al. (2005) have also inferred the paraphyly of *Masticophis* with respect to *C. constrictor* (Pyron et al., 2013; Figueroa et al., 2016).

We assessed whether *Coluber* renders *Masticophis* paraphyletic, an issue that cannot be resolved using single locus gene trees, which are likely subject to incomplete lineage sorting or introgressive hybridization. This issue also cannot be addressed using a limited number of morphological characters, several of which are likely to be homoplastic (e.g., the number of scale rows or maxillary teeth). It is very likely that the low support found for the monophyly of *Masticophis* is the result of a recent, rapid radiation of species. This may produce two difficulties for reconstructing phylogenetic history. First, rapid radiations often result in high levels of incomplete lineage sorting making species tree estimation difficult with only a handful of loci (Ogilvie et al., 2016). Secondly, such a radiation could result in high levels of hybridization and introgression, a process not accounted for in species-tree methods but that is being shown to be increasingly prevalent (von Holdt et al., 2016; Zinenko et al., 2016; Kumar et al., 2017). We suggest that future studies on this group would benefit from increased genomic sampling (e.g., the use of restriction-site associated DNA sequencing [RAD-seq] or sequence capture of exomes or conserved regions of the genome; Davey and Blaxter, 2010; Mamanova et al., 2010), coupled with analyses assessing the fit of a strictly bifurcating history. Here we infer that the two genera, *Coluber* and *Masticophis*, are monophyletic with respect to one another (Fig. 2) though not with strong support. Based on the principle of monophyly (Hennig, 1966; de Queiroz and Gauthier, 1990), we suggest the continued recognition of the genus *Coluber* for *C. constrictor* and *Masticophis* for *anthonyi*, *aurigulus*, *barbouri*, *bilineatus*, *flagellum*, *lateralis*, *mentovarius*, *schotti*, and *taeniatus*.

Biogeographic patterns.—Species for which range-wide sampling was available showed substantial geographic population subdivision. Many of these populations may represent distinct species, and most of the phylogeographic breaks occur at well-documented barriers that have been important for species formation in disparate taxa (O'Connell et al., 2017). For example, the Florida peninsula versus continental North America disjunction has been well documented in a wide range of taxa (reviewed in Soltis et al., 2006) and is observed here in *Coluber constrictor* as a major subdivision (Figs. 1, 2). This taxon has been shown to have additional phylogeographic breaks in a previous study using a single locus (Burbrink et al., 2008) that are not recovered here using nuclear and additional mtDNA loci. This is not an unusual pattern to find in phylogeographic studies (e.g., Myers et al., 2013) and can be due to the stochastic nature of the coalescent given a single locus or that the information content in the nucDNA loci used in this study do not provide enough information at the intraspecific level to detect fine-scale population differentiation.

Masticophis flagellum has population subdivisions near previously recognized phylogeographic barriers described for other taxa, including the Mississippi River, the transition from prairie to arid-lands in the central US, and at the Cochise filter barrier in the North American arid southwest (e.g., Mulcahy, 2008; Burbrink and Guiher, 2015; McKelvy and Burbrink, 2017; Myers et al., 2017). At least one delimitable phylogeographic lineage coincides with a previously named subspecific taxon, the San Joaquin Valley subspecies *M. f. ruddocki* (Fig. 1; Brattstrom and Warren, 1953). The San Joaquin Valley population is the oldest diverging lineage within *M. flagellum* (Fig. 2). Other squamate taxa are endemic to the San Joaquin Valley, including *Thamnophis gigas* (Rossman and Stewart, 1987), *Gambelia sila* (Jennings, 1995), and *Sceloporus occidentalis biseriatus* (Bell and Price, 1996). A comparative phylogeographic study

on the causes of this shared divergence deserves further investigation. *Masticophis lateralis* shows population subdivision in south central California, a phylogeographic pattern shared with other vertebrates, such as turtles and snakes, within this region (Myers et al., 2013; Spinks et al., 2014). The Datil-Mogollon Section of the Colorado Plateau lies within the discontinuity between northern and southern lineages of *M. taeniatus*, although this is observed with limited geographic sampling. There is substantial cryptic diversity within many of the wide-ranging whipsnakes, suggesting that these patterns warrant further investigation using coalescent species delimitation methods.

Finally, the species found on the remote Isla Clarión, *M. anthonyi*, is nested within *M. flagellum* and sister to a lineage distributed in the Sonoran and Mojave deserts, and Baja California, with high support (PP = 0.98; Fig. 2). This pattern is similar to population structure seen in other squamates found on Isla Clarión. For instance, the closest relatives of the nightsnake *Hypsiglena unaocularus* has been shown to occur on Isla Santa Catalina in the Gulf of California and mainland México, near the Sonoran Desert–Sinaloan thornscrub contact zone (Mulcahy et al., 2014). A similar pattern is seen in the sister relationship between *Urosaurus* lizards on Isla Clarión and Isla Socorro, and those in the Sonoran Desert (Feldman et al., 2011). Isla Clarión, part of the volcanic seamount Revillagigedo Archipelago, is approximately 1,100 km from Manzanillo and 710 km from Cabo San Lucas; thus, the presence of *M. anthonyi* is likely explained by overwater dispersal from the Rio Fuerte region of México (Mulcahy et al., 2014). Further exploration of the biogeographic patterns observed here, for example with genomic scale data and statistical model testing, will likely result in the recognition of several cryptic species and help clarify the process of speciation in North America whipsnakes and racers.

Conclusions.—We account for phylogenetic uncertainty given incomplete lineage sorting to examine the monophyly of *Masticophis* as previously described (i.e., to the exclusion of *Coluber constrictor*) using a coalescent-based framework. Our results show that even with sampling more loci than previous studies and accounting for incomplete lineage sorting, the relationships among these taxa are not conclusively resolved. We suggest that future studies focus on increased genomic sampling, while also assessing whether these taxa have diverged in a strictly bifurcating history. Additionally, many of the currently recognized widespread species may require taxonomic revision as they likely contain multiple, undescribed cryptic species.

DATA ACCESSIBILITY

Supplemental material is available at http://www.copeia journal.org/ch-16-552.

ACKNOWLEDGMENTS

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