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Multilocus phylogeny clarifies relationships and diversity within the *Micrurus lemniscatus* complex (Serpentes: Elapidae)

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Abstract. The New World genus *Micrurus* contains more than 80 currently recognized species of venomous coral snakes. The taxonomy of the South American *M. lemniscatus* complex is controversial. Within this group, *M. lemniscatus*, *M. carvalhoi*, *M. diutius*, *M. frontifasciatus,* and *M. helleri* have been treated either as distinct species or subspecies of *M. lemniscatus*. Additional species (*M. filiformis*, *M. isozonus*, *M. potyguara*, *M. serranus*) have also been proposed to belong to the *M. lemniscatus* complex but never included in a molecular phylogeny. In the present work, we sequenced four mitochondrial (12S, 16S, cyt *b*, ND4) and one nuclear (Cmos) genes using specimens of *M. helleri* from the Andean foothills of Colombia and Peru and *M. filiformis* from the Colombian Llanos. Supplemented by previously published sequences, we inferred the phylogeny of the *M. lemniscatus* complex using Bayesian and Maximum Likelihood approaches and estimated divergence times based on fossil-calibrated nodes. Our results strongly support the monophyly of the *M. lemniscatus* complex. Furthermore, populations traditionally assigned to *M. helleri* represent two non-sister lineages, one occurring along the Andean foothills and the other in lowland Amazonia. As a consequence, we restrict the name *M. helleri* to the populations of the Andean foothills. According to our results, the *M. lemniscatus* complex diverged from *M. surinamensis* during the late Miocene and diversified during the Plio-Pleistocene.

Key words. Biogeography, Elapidae, Serpentes, South America, Squamata, systematics.

Introduction

The coral snake genus *Micrurus* Wagler, 1824 is a speciose group of highly venomous snakes belonging to the Elapidae family. Currently, more than 80 species are recognized that occur mainly in Central and South America, with two species encroaching on the southern USA (Silva et al. 2016a, West et al. 2019, Uetz et al. 2020). Two major groups have been distinguished within *Micrurus*: The first one includes species with a monadal coloration pattern (color pattern with one black ring between two red rings), and the second group comprises mainly species with a triadal pattern (three black rings between two red rings; Roze 1996, Campbell & Lamar 2004, Zaher et al. 2016, Jowers et al. 2019). One additional group, corresponding to the former genus *Leptomicrurus* Schmidt, 1937, has been included within *Micrurus*, but the monophyly of the four species formerly assigned to *Leptomicrurus* has not been examined yet (Renjifo et al. 2012, Zaher et al. 2016, Jowers et al. 2019). These three groups are supported by morphological characters, such as hemipenial morphology, relative tail size, and scalation (Slowinski 1995, Roze 1996, Campbell & Lamar 2004). Individual *Micrurus* species are generally diagnosed by scale counts, body proportions, and in particular by color pattern (Roze 1996, Campbell & Lamar 2004, Silva et al. 2016b). However, for some groups, these characters have proven to be insufficient, both for delimitating species and supraspecific groups (Roze 1996, Harvey et al. 2003, Silva et al. 2016b).

One challenging group within *Micrurus* is the *M. lemniscatus* complex. It includes the taxa traditionally identified as subspecies of *M. lemniscatus*, i.e., *M. lemniscatus* (Lin-

naeus, 1758), *M. carvalhoi* Roze, 1967, *M. diutius* Burger, 1955, *M. frontifasciatus* (Werner, 1927), and *M. helleri* Schmidt & Schmidt, 1925 (Roze 1996, Campbell & Lamar 2004, Silva et al. 2016b, Floriano et al. 2019). This group has an entangled taxonomic history. Pires (2011), using morphological data and the largest sampling so far, suggested in his unpublished Ph.D. thesis to recognize *M. carvalhoi*, *M. diutius*, *M. frontifasciatus*, and *M. lemniscatus* as full species, and to treat *M. helleri* as a synonym of *M. lemniscatus*. Additionally, Pires (2011) suggested the existence of an undescribed species, which was later described as *M. potyguara* Pires, Silva, Feitosa, Prudente, Pereira-Filho & Zaher, 2014. Independently from Pires (2011), the recognition of *M. diutius* as a full species was also proposed by Starace (2013), based on a morphological analysis of populations from French Guiana. Later, without explanation, Wallach et al. (2014) listed *M. carvalhoi* and *M. frontifasciatus* as full species, and *M. diutius* and *M. helleri* as junior synonyms of *M. lemniscatus*. Based mainly on molecular evidence, Valencia et al. (2016) elevated *M. helleri* to full species level but kept all other taxa as subspecies of *M. lemniscatus*. Recently, also using molecular data, Jowers et al. (2019) concluded that all subspecies of *M. lemniscatus*, perhaps except *M. l. frontifasciatus*, should be recognized as full species and that there is still some cryptic diversity to be described within this group. Nevertheless, several recent publications (Pires et al. 2014, Silva et al. 2016b, Terribile et al. 2018, Floriano et al. 2019) recognized only *M. diutius* and *M. potyguara*, besides a polytypic *M. lemniscatus* with the three subspecies *M. l. carvalhoi*, *M. l. helleri*, and *M. l. lemniscatus*. As a starting point, we will treat all taxa in the *M. lemniscatus* complex as full species, recognizing the results of Pires (2011), Pires et al. (2014), and Valencia et al. (2016).

The *Micrurus lemniscatus* complex is distributed throughout most of the cis-Andean region of South America, including the eastern Andean foothills, Amazonia, the Cerrado and the Atlantic forest, also including the island of Trinidad (Fig. 1; Roze 1996, Feitosa et al. 2007, Pires et al. 2014, Silva et al. 2016b, Terribile et al. 2018, Jowers et al. 2019). Initially it was thought that all taxa within the *M. lemniscatus* complex had allopatric distributions (Figs 1A–B; Roze 1996, Campbell & Lamar 2004). However, recent data suggest that the ranges of several species do overlap. *Micrurus diutius* and *M. lemniscatus* seem to occur sympatrically in the eastern and western Guiana region, as do *M. helleri* and *M. lemniscatus* in central Amazonia, and *M. carvalhoi* and *M. lemniscatus* in the Brazilian Cerrado (Fig. 1C). In addition, a probable overlap between the ranges of *M. carvalhoi* and *M. potyguara* was suggested for western Brazil (Pires 2011, Pires et al. 2014, Silva et al. 2016b, Terribile et al. 2018, Floriano et al. 2019).

In addition to the above-mentioned taxa, three further species have been proposed to belong to the *M. lemniscatus* complex by Feitosa et al. (2007) and Terribile et al. (2018): *Micrurus filiformis* (Günther, 1859), *M. isozonus* (Cope, 1860), and *M. serranus* Harvey, Aparicio & Gonzalez, 2003. *Micrurus filiformis* is distributed throughout most of the Amazon Basin and the surrounding Andean foothills, displaying a widely overlapping distribution with most other taxa belonging to the *M. lemniscatus* complex (Schmidt & Walker 1943, Roze 1996, Harvey et al. 2003, Campbell & Lamar 2004, Feitosa et al. 2007). *Micrurus isozonus* occurs in the dry forests and savannahs of the Guiana Shield, where it could be locally sympatric with *M. diutius*, *M. filiformis*, *M. helleri*, and *M. lemniscatus* (Roze 1996, Kok et al. 2003, Campbell & Lamar 2004). Without explicit evidence, Feitosa et al. (2007) suggested that *M. isozonus* belongs to the *M. lemniscatus* complex, while other authors suggested that this species is more closely related to the *M. frontalis* complex (Roze 1996, Campbell & Lamar 2004). Finally, *M. serranus* occurs in the eastern Andes of Bolivia and apparently is not sympatric with any other species of the *M. lemniscatus* complex (Harvey et al. 2003). Terribile et al. (2018) proposed the inclusion of *M. serranus* in the *M. lemniscatus* complex, but the original description (Harvey et al. 2003) suggested that *M. serranus* most likely belongs to the *M. frontalis* complex, despite some morphological characters resembling the *M. lemniscatus* complex.

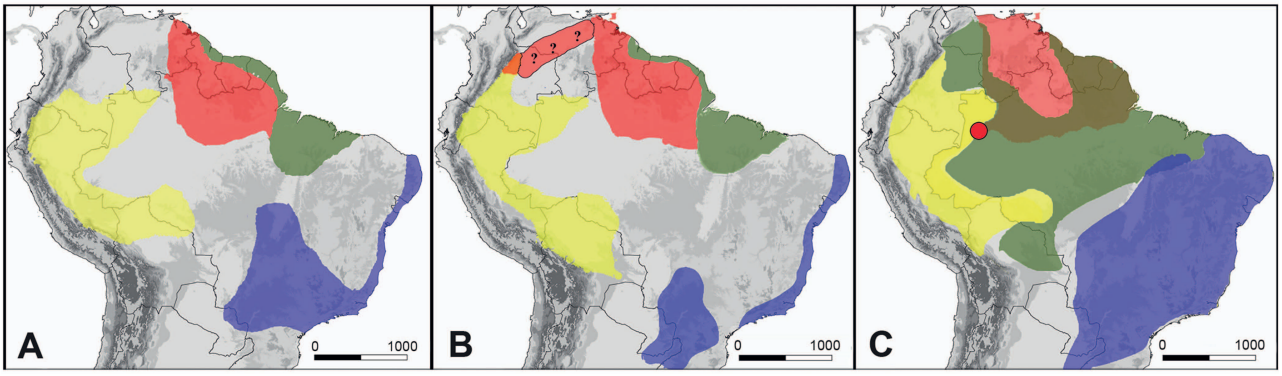

Figure 1. Proposed distribution ranges of taxa of the *Micrurus lemniscatus* complex according to (A) Roze (1996), (B) Campbell & Lamar (2004), and (C) Floriano et al. (2019). Blue: *M. carvalhoi*, red: *M. diutius*, yellow: *M. helleri*, green: *M. lemniscatus*. Red circle: putative sympatric occurrence of *M. helleri* and *M. lemniscatus*.

Until now, molecular phylogenetic analyses included only up to four of the six taxa of *M. lemniscatus* sensu lato (*M. carvalhoi*, *M. diutius*, *M. helleri, M. lemniscatus*), and none of the additional species suggested to belong to the complex (Silva & Sites 2001, Renjifo et al. 2012, Jowers et al. 2019). These studies found the studied taxa monophyletic. However, all analyses were based only on the mitochondrial ND4 gene, even when more genes were sequenced (e.g., Jowers et al. 2019 sequenced five genes but used only ND4).

In order to better understand the genetic variation within the *M. lemniscatus* complex, we present herein a dated multilocus analysis. To this end, we used four mitochondrial genes (12S, 16S, ND4, cyt *b*) and one nuclear locus (Cmos). We also included, for the first time, samples of *M. helleri* from the eastern foothills of the Colombian and Peruvian Andes and samples of *M. filiformis* from the Colombian Llanos.

Materials and methods

Sampling

We studied six samples of *Micrurus helleri* from the Andean foothills of Colombia, one from the Amazonian slopes of Peruvian Andes, and two samples of *M. filiformis* (Fig. 2; Supplementary Table S1). Tissue samples came from the Banco de ADN y Tejidos de la Biodiversidad (BTBC) of the Instituto de Genética (IGUN), Universidad Nacional de Colombia, corresponding to specimens deposited in Colección de Reptiles, Instituto de Ciencias Naturales, Universidad Nacional de Colombia (ICN), the Instituto Nacional de Salud de Colombia (INSZ), Bogotá, Colombia, and the Laboratoire Évolution et Diversité Biologique, Université Toulouse, France. We obtained sequences for five genetic markers, one nuclear locus (Cmos) and four mitochondrial genes (12S, 16S, cyt *b*, ND4). These genes were chosen to match the dataset of Jowers et al. (2019). Some previously published sequences of the triad group were excluded since they appeared in exploratory analyses in unexpected positions (i.e., deeply divergent from conspecifics) or were unstable between analyses, destabilizing branching patterns (Supplementary Table S2), suggestive of misidentification or substandard sequence quality. Our final alignment included 17 specimens belonging to five taxa of the *M. lemniscatus* complex (*M. carvalhoi*, *M. diutius*, *M. filiformis*, *M. helleri*, *M. lemniscatus*) plus 20 additional *Micrurus* species. Since there has been some confusion regarding the identity of sequences published by Silva & Sites (2001), we follow Renjifo et al. (2012) and Jowers et al. (2019), who resolved this issue and presented trustworthy taxonomic identifications (Supplementary Table S3). For dating purposes, we used as outgroups sequences of 62 species of

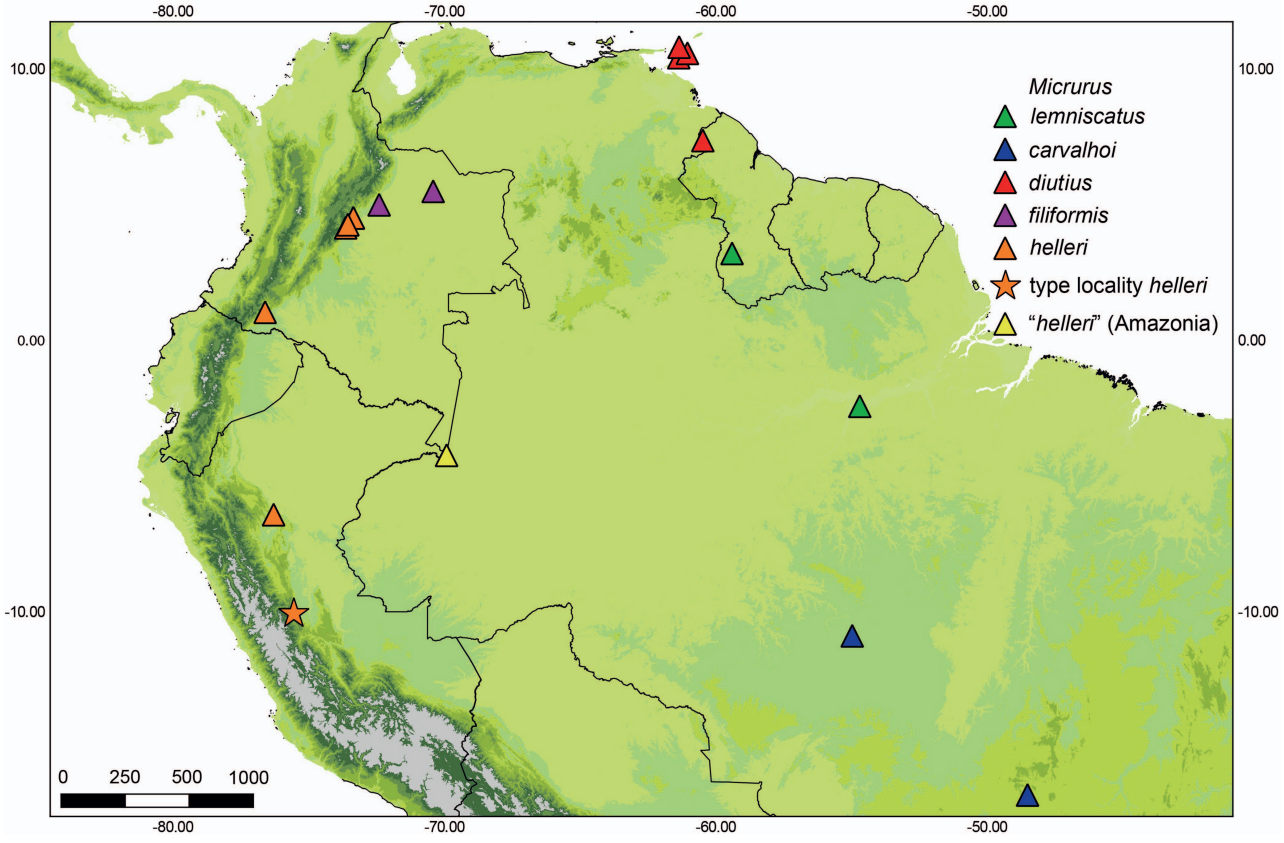

Figure 2. Localities of the studied samples of the *Micrurus lemniscatus* complex and type locality of *M. helleri*.

Table 1. Primers used in this study. * Primers modified by Zaher et al. (2009)

Gene	Primer	Sequence	Reference
12S	L1091mod	5' CAAACTAGGATTAGATACCCTACTAT 3'	Kocher et al. (1989)
	H1557mod*	5' GTACRCTTACCWTGTTACGACTT 3'	Knight & Mindell (1994)
16S	L2510mod (16Sar)*	5' CCGACTGTTTAMCAAAAACA 3'	Palumbi et al. (1991)
	H3056mod (16Sbr)*	5' CTCCGGTCTGAACTCAGATCACGTRGG 3'	Palumbi et al. (1991)
cyt *b*	703Botp.mod*	5' TCAAAYATCTCAACCTGATGAAAYTTYGG 3'	Pook et al. (2000)
	MVZ16p.mod*	5' GGCAAATAGGAAGTATCAYTCTGGYTT 3'	Pook et al. (2000)
ND4	ND4ab	5' CACCTATGACTACCAAAAGCTCATGTAGAAGC 3'	Arevalo et al. (1994)
	H-Leu	5' ATTACTTTTACTTGGATTTGCACCA 3'	Stuart & Parham (2004)
Cmos	S77	5' CATGGACTGGGATCAGTTATG 3'	Lawson et al. (2005)
	S78	5' CCTTGGGTGTGATTTTCTCACCT 3'	Lawson et al. (2005)

the booid and caenophidian radiations, obtained from Zaher et al. (2019).

Laboratory procedures

Genomic DNA was extracted using the innuPREP DNA Micro Kit (Analytik Jena GmbH, Jena, Germany), following the manufacturer's protocol. Primers are listed in Table 1. PCR reactions contained in a final volume of 20 µL 10–100 ng of genomic DNA, 1 unit of *Taq* polymerase (Bioron, Ludwigshafen, Germany), 2 µL buffer (as recommended by the supplier), 0.5 µM of each primer, and 0.2 mM of each dNTP (Fermentas, St. Leon-Rot, Germany). For all genes, the same PCR protocol was used. It had an initial denaturation step at 94°C for 5 min, followed by 35 cycles that included denaturation at 95°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min. The cycle ended with an extension step at 72°C for 10 min. Purification and sequencing protocols followed the conditions described in Fritz et al. (2012).

Molecular analyses

Sequences were aligned and inspected using GENEIOUS 9.1.8 (Kearse et al. 2012) and the implemented MUSCLE algorithm (Edgar 2004) under default conditions. Individual gene alignments were concatenated using SEQUENCE MATRIX 1.8 (Vaidya et al. 2011). For phylogenetic analyses, three different partitioning schemes were examined using PARTITIONFINDER 2 (Lanfear et al. 2017) and the implemented Bayesian Information Criterion: (1) unpartitioned, (2) partitioned by gene and (3) complete partition (i.e., partitioned by gene and for protein-coding genes by codon position), resulting in the selection of the complete partition scheme (Table 2).

Using the selected partition scheme and the appropriate evolutionary models, a phylogenetic tree was built with the Maximum Likelihood (ML) approach as implemented in IQ-TREE 2.0.6 (Nguyen et al. 2015). Node support was estimated using 5000 pseudoreplicates of ultrafast bootstrap, considering values of 95% as high support (Minh et al. 2013).

Table 2. Partitions and evolutionary models for the concatenated alignment obtained using PartitionFinder 2 (PF) and bModelTest (bMT).

Partition	Position	PF	bMT
12S	1-540	GTR+I+G	121343
16S	541-1101	GTR+I+G	
cyt *b*_1	1102-2219\3	GTR+I+G	123345
cyt *b*_2	1103-2219\3	TVM+I+G	
cyt *b*_3	1104-2219\3	TIM+I+G	
ND4_1	2220-3082\3	GTR+I+G	
ND4_2	2221-3082\3	TVM+I+G	
ND4_3	2222-3082\3	K81UF+G	
Cmos_1	3083-3652\3	HKY+G	121121
Cmos_2	3084-3652\3	K80+G	
Cmos_3	3085-3652\3	HKY+G	

Bayesian Inference (BI) and the Relaxed Molecular Clock analysis were run simultaneously in BEAST 2.6.1 (Bouckaert et al. 2019). For BI, we used the best partitioning scheme from PARTITIONFINDER 2. However, evolutionary models were inferred using bMODELTEST 1.2 (Bouckaert & Drummond 2017), exploring all available models. In exploratory analyses some parameters were stable and effective sample sizes were below 200. Therefore, we linked for the software BEAUTi, included in the BEAST package, site and clock models to avoid overparametrization. Site models were linked (i) for non-protein-coding mitochondrial markers (12S and 16S), (ii) for the protein-coding mitochondrial markers (ND4, cyt *b*), and (iii) the protein-coding nuclear marker (Cmos) was treated separately (Table 2). The clock was linked on the one hand for the mitochondrial partitions and on the other for the nuclear partition (Cmos). Finally, all partitions were linked for the tree model using the Birth–Death Model; the log-normal relaxed clock was set as clock model. We used nine fossils as node calibration points (Table 3), following Za-

Table 3. Fossils used for dating. Dates are given in million years.

Node	Fossil	Minimum	Maximum	Source
Stem Boinae	*Titanoboa cerrejonensisi*	58	93.9	Head et al. (2009)
Stem Colubriformes	*Procerophis sahnii*	54	93.9	Rage et al. (2008)
Stem Viperidae	*Vipera* cf. *antiqua*	22.1	93.9	Szyndlar & Böhme (1993)
Stem Crotalinae	Crotalinae indet.	11.2	54	Ivanov (1999)
Stem Elapidae	Elapid morphotype A	24.9	54	McCartney et al. (2014)
Stem "Oxyuranine"	*Incongruelaps iteratus*	10	54	Scanlon et al. (2003)
Stem Colubroidea	Colubridae indet.	35.2	54	Smith (2013)
Crown Natricidae	Natricidae incertae sedis	13.8	54	Rage & Szyndlar (1986)
Stem Dipsadidae	*Paleoheterodon tiheni*	12.5	54	Holman (1964, 1977)

her et al. (2018, 2019), enforcing monophyly and with log-normal distributions with a mean of 1.0 (parameter M) and a standard deviation of 1.25 (parameter S). Two independent chains of 100 million generations, sampling every 5000th, were run in BEAST, using the CIPRES portal (www.phylo.org/; Miller et al. 2010). Chain convergence and burn-in value (10%) were assessed using TRACER 1.7.1 (Rambaut et al. 2018). A consensus tree was summarized using TREEANNOTATOR as implemented in BEAST 2.6.1 (Bouckaert et al. 2019).

Finally, for the individual species uncorrected p distances were calculated for the gene with the broadest taxonomic coverage (ND4) using MEGA X (Kumar et al. 2018) and the pairwise deletion option.

Results

Both tree building methods revealed the same well-supported general topology. *Micrurus* was found as a highly supported clade (Fig. 3), and *Sinomicrurus maclellandi* was identified as sister taxon of *Micrurus*, albeit with weak support (Supplementary Figs S1 and S2). Within *Micrurus*, two deeply divergent and well-supported clades were recovered: One comprised the monad species and the other comprised *M. narducci* plus the triad species. The monophyly of the triad species clade was only weakly supported. The triad clade comprised two well-supported subclades. One contained *M. dissoleucus*, *M. mipartitus*, *M. obscurus*, and the species of the *M. frontalis* complex; and the other one, *M. ortoni*, *M. surinamensis*, and the *M. lemniscatus* complex.

Both ML and BI approaches revealed the *M. lemniscatus* complex as a well-supported clade, and *M. surinamensis* as its sister taxon (Figs 3 and 4). All evolutionary relationships within the *M. lemniscatus* complex were identical for both analyses and robustly supported (Fig. 3). All taxa represented by more than one sequence were recovered as monophyletic, except for *M. helleri*. The only central Amazonian sample identified as *M. helleri* clustered with *M. carvalhoi*, whereas the Colombian and Peruvian foothill samples constituted the sister clade of *M. diutius*, rendering *M. helleri* polyphyletic.

The *M. lemniscatus* complex consisted of two well-supported clades, one contained *M. carvalhoi*, *M. filiformis*, *M. lemniscatus*, and the Amazonian sample of *M. helleri*; and the other comprised *M. diutius* and the specimens of *M. helleri* from the Andean foothills. Within the first clade, *M. carvalhoi* was sister to the Amazonian *M. helleri*, with *M. lemniscatus* and *M. filiformis* as successive sister taxa (Fig. 3).

Our time-calibrated phylogenetic analysis suggested that the *M. lemniscatus* complex diverged from *M. surinamensis* in the late Miocene (approx. 5.64 million years ago = Mya; Fig. 4; Supplementary Fig. S3). Diversification within the *M. lemniscatus* complex commenced in the Pliocene (approx. 3.86 Mya), while the individual species diverged during the Pleistocene, with *M. filiformis* representing the earliest branch-off (approx. 2.33 Mya). The divergence inferred for the Amazonian *M. helleri* sample and *M. carvalhoi* was about 1.08 Mya; the split between *M. diutius* and the *M. helleri* samples from the Andean foothills was estimated at about 1.78 Mya.

The lowest uncorrected p distance between *Micrurus* species for the mitochondrial ND4 gene was between *M. brasiliensis* and *M. frontalis* (1.58%; Supplementary Table S4). Within the *M. lemniscatus* complex, the lowest divergence occurred between the *M. helleri* samples from the Andean foothills and *M. diutius* (2.41%), and the highest between the two clades of *M. helleri* (8.87%).

Discussion

Taxonomy of the *Micrurus lemniscatus* complex

Our results suggest that the *Micrurus lemniscatus* complex contains at least six species, one of them undescribed. Morphology-based research has previously led to a revised taxonomy of *M. lemniscatus* sensu lato, but mostly focused on whether the individual taxa should be recognized as species or subspecies (Table 4). In an unpublished dissertation, Pires (2011) proposed to recognize four species: *Micrurus carvalhoi*, *M. diutius*, *M. frontifasciatus*, and *M. lemniscatus* (the latter including *M. helleri*). However, in subsequent publications this proposal was not followed. Instead, a more conservative taxonomy was used by several authors, with *M. diutius* as a full species and a poly-

typic *M. lemniscatus* with the subspecies *M. l. carvalhoi*, *M. l. helleri,* and *M. l. lemniscatus* (Pires et al. 2014, Silva et al. 2016b, Terribile et al. 2018, Floriano et al. 2019). Other authors (Wallach et al. 2014, Jowers et al. 2019) distinguished a varying number of distinct species within *M. lemniscatus* sensu lato (Table 4), with *M. helleri* recognized as another species by Jowers et al. (2019).

Our results revealed that *M. lemniscatus* sensu lato is paraphyletic with respect to *M. filiformis*. Moreover, the deep divergence between our samples identified as *M. helleri* from the Andean foothills and from the Amazon Basin implies that two distinct species are currently lumped together under this name (Figs 3 and 4). One of these species (from the Andean foothills) is sister to *M. diutius* (Fig. 3), a

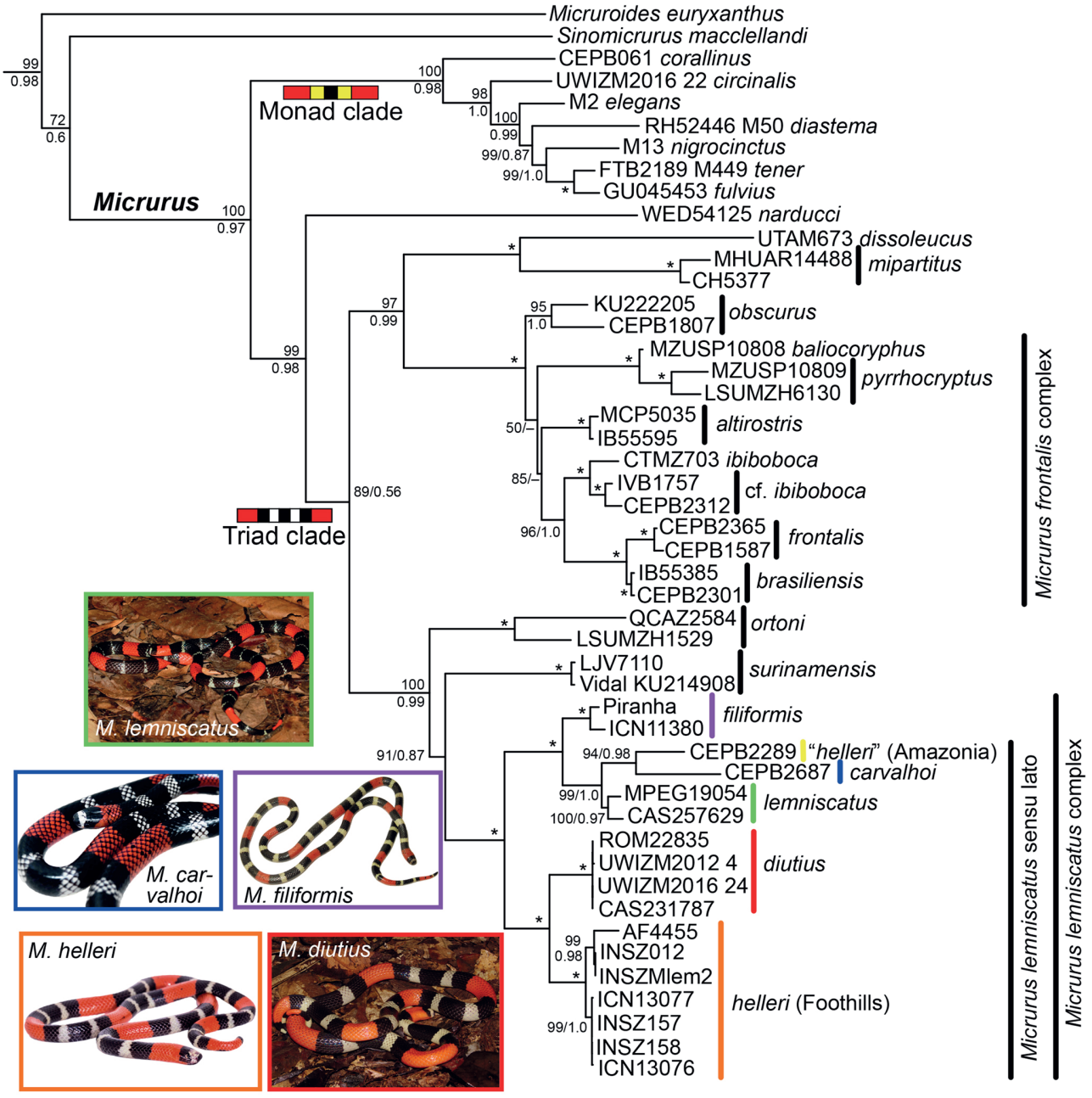

Figure 3. Maximum Likelihood tree for *Micrurus* based on 3,618 base pairs of the concatenated alignment of five genes (12S, 16S, cyt *b*, ND4, Cmos). Values above and below branches correspond to ultrafast bootstrap values and Bayesian posterior probabilities. Asterisks indicate maximum support under both approaches; dashes that those clades were not recovered in the Bayesian 50% majority rule consensus tree. Bar colors correspond to Figure 2 and inset photographs. Photos: Maël Dewynter (*M. lemniscatus,* French Guiana), Lucas Pousa (*M. carvalhoi*, Brazil); Juan Pablo Hurtado Gómez (*M. filiformis*, Colombia), Antoine Fouquet (*M. helleri*, Peru, AF4455 in the tree; *M. diutius*, French Guiana).

Table 4. Classification of *Micrurus lemniscatus* sensu lato according to different authors and the present study.

Pires (2011)	Wallach et al. (2014)	Silva et al. (2016b)	Valencia et al. (2016)	Jowers et al. (2019)	This study
M. carvalhoi	*M. carvalhoi*	*M. l. carvalhoi*	*M. l. carvalhoi*	*M. carvalhoi*	*M. carvalhoi*
M. diutius	*M. lemniscatus*	*M. diutius*	*M. l. diutius*	*M. diutius*	*M. diutius*
M. frontifasciatus	*M. frontifasciatus*	*M. l. lemniscatus*	*M. l. frontifasciatus*	unclear	unclear
M. lemniscatus	*M. lemniscatus*	*M. l. lemniscatus*	*M. l. lemniscatus*	*M. lemniscatus*	*M. lemniscatus*
M. lemniscatus	*M. lemniscatus*	*M. l. helleri*	*M. helleri*	*M. helleri*	*M. helleri*: two species

finding that conflicts with the hypothesis of several authors (Pires 2011, Silva et al. 2016b, Terribile et al. 2018, Floriano et al. 2019) that *M. helleri* is a subspecies of *M. lemniscatus*. The second species (from Amazonia) currently identified as *M. helleri* is the sister species of *M. carvalhoi*.

Some *Micrurus* species displaying low genetic distances for the mitochondrial ND4 gene (Supplementary Table S4), like *M. brasiliensis* and *M. frontalis* (1.58%) or *M. baliocoryphus* and *M. pyrrhocryptus* (2.00%), have undergone taxonomic revision based on relatively large sampling (Silva & Sites 1999). As a result, their species status has been widely accepted (Harvey et al. 2003, Silva et al. 2016b, Costa & Bérnils 2018, Nogueira et al. 2020). The same is true for *M. fulvius* and *M. tener* (Castoe et al. 2012, Streicher et al. 2016), differing by 2.67%. Some of these divergences are lower than those among taxa belonging to the *M. lemniscatus* complex (2.41–8.87%; Supplementary Table 4), supporting the recognition of *M. carvalhoi*, *M. diutius*, *M. filiformis*, *M. helleri*, and *M. lemniscatus* as full species and that an undescribed species exists that has been confused with *M. helleri*.

Micrurus helleri was described from the Andean foothills of Peru (Schmidt & Schmidt 1925), with a proposed distribution across the Andean foothills of Colombia, Ecuador, Peru, and Bolivia and in westernmost Amazonia (Figs 1 and 2; Roze 1996, Campbell & Lamar 2004, Pires 2011, Silva et al. 2016b, Valencia et al. 2016, Terribile et al. 2018, Floriano et al. 2019). However, our results suggest that "*M. helleri*" is composed of two distinct species. Our sample from Peru (AF4455) comes from a locality approximately 400 km north of the type locality of *M. helleri* (Fig. 2) but in the same ecoregion (Very Humid Premontane Forest; Britto 2017). Therefore, we restrict the name *M. helleri* to the foothill clade. To our knowledge, there are no names available for the Amazonian lineage. Further investigations with expanded sampling and additional lines of evidence, in particular morphology, should be undertaken to properly delimit and name this species.

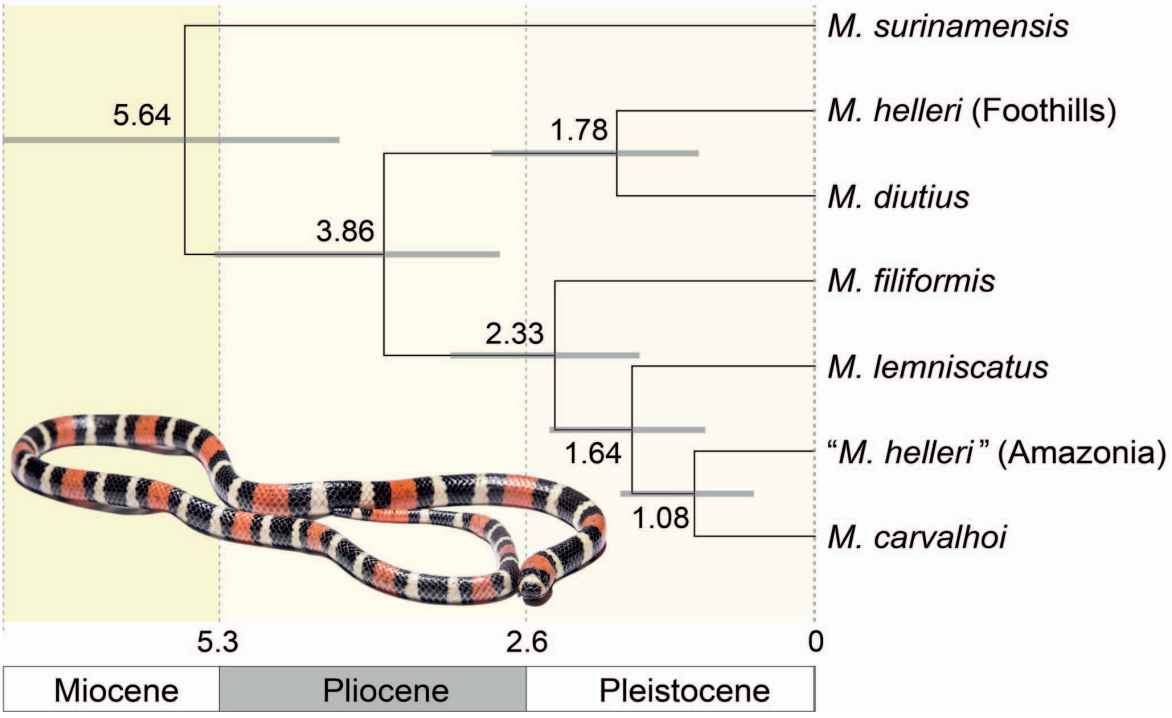

Figure 4. Time tree of the *Micrurus lemniscatus* complex. Node values are mean dates in millions of years; grey bars indicate 95% Highest Posterior Density (HPD) intervals. Inset photo of *M. helleri*: Juan Pablo Hurtado Gómez.

Table 5. Dates for the main clades obtained in the BEAST analysis (95% HPD) and comparison with previous studies. The results of Jowers et al. (2019) were based on mutation rates; those of Lee et al. (2016) and Zaher et al. (2016, 2019) on a fossil-calibrated molecular clock. *Approximate dates because exact values were not given by the authors; NR, not recovered.

Clade	Present study	Jowers et al. (2019)	Lee et al. (2016)	Zaher et al. (2016)	Zaher et al. (2019)
Micrurus	11.79 (9.56–14.09)	31	18.75*	19*	14.78
Monadal clade	5.28 (3.69–7.79)	7	8.30*	5.3*	4.21
Triadal clade	8.91 (6.96–10.92)	24	14.51*	14*	11.17
M. surinamensis + *M. lemniscatus* complex	5.64 (4.26–7.29)	14.8*	10.15*	NR	NR
M. lemniscatus complex	3.86 (2.82 –5.38)	9.6*	NR	NR	NR
M. diutius + *M. helleri*	1.78 (1.04–2.89)	NR	NR	NR	NR

For *M. carvalhoi*, an additional ND4 sequence is available from GenBank (accession number: AF228435, voucher: IB55598; Supplementary Table S1). It was included in exploratory analyses, and *M. carvalhoi* was found monophyletic with strong support (Supplementary Fig. S4), in agreement with the findings of Renjifo et al. (2012) but contradicting Jowers et al. (2019), who found *M. carvalhoi* polyphyletic. However, in our trees, the sequence AF228435 displayed a long branch. Under ML, the support values for other clades were lower, and the BI topology changed. We assume that the sequence AF228435 shows some base-calling errors, which is why we did not use it, except for the exploratory analysis presented as Supplementary Figure S4.

Given the mislabeling and misidentification of many GenBank sequences associated with the *M. lemniscatus* complex, a few erroneous interpretations about the evolution and diversity of the group have been published (e.g., cryptic diversity in Jowers et al. 2019; evolution of venom composition in Sanz et al. 2019). To avoid future misunderstandings, we summarize misidentifications of GenBank sequences in Supplementary Table S3.

Diversification and biogeography in the *Micrurus lemniscatus* complex

Our fossil calibrated tree (Fig. 4 and Supplementary Fig. 3) retrieved ages for the *M. lemniscatus* complex more recent than the ones reported in previous works (Table 5; Lee et al. 2016, Zaher et al. 2016, 2019, Jowers et al. 2019). This could be associated with the fact that we analyzed a larger number of genes for the ingroup. Nevertheless, our dates were closer to the previously published results using fossil calibrations (Lee et al. 2016, Zaher et al. 2016, 2019) than to the results based on a priori mutation rates (Jowers et al. 2019), which advocates for a careful use of priors for molecular dating.

Species in the *M. lemniscatus* complex are all considered semi-aquatic (Roze 1996, Campbell & Lamar 2004, Feitosa et al. 2007, Almeida et al. 2016, Silva et al. 2016b). These taxa diverged from their fully aquatic sister taxon *M. surinamensis* in the late Miocene (approx. 5.64 Mya; Fig. 4). *Micrurus surinamensis* is co-distributed with the *M. lemniscatus* complex throughout most of its range, and their diversification might have also been driven by ecological differences. According to our results, the radiation of the *M. lemniscatus* complex began in the Pliocene (approx. 3.86 Mya) and could have been triggered by factors like changes in river courses (Albert et al. 2018, 2020) or the Plio-Pleistocene climatic fluctuations and associated forest dynamics, as it has been shown for other vertebrates (Wüster et al. 2005, Quijada-Mascareñas et al. 2007, Hoorn et al. 2010, Vargas-Ramírez et al. 2010).

Further research is needed to clarify the identity and distribution of *M. helleri* sensu stricto and of the unnamed Amazonian species. The distribution of *M. helleri* sensu stricto seems to be continuous across the eastern Andean foothills of Colombia, Ecuador and Peru (Fig. 2), as it has been found for other snakes (Campbell & Lamar 2004, Arteaga et al. 2018), for instance *Bothrocophias microphthalmus* (Cope, 1875), *Dipsas peruana* (Boettger, 1898) sensu lato or *D. vermiculata* (Boulenger, 1885), and several other animal groups endemic to this region (Lynch et al. 1997, Patterson et al. 1998, Kattan et al. 2004, Ron et al. 2011). Additionally, there are records further north in Venezuela, in the Merida foothills, identified as *M. lemniscatus* (Barrio-Amorós & Calcaño 2003, Natera-Mumaw et al. 2015). These records could also refer to *M. helleri* sensu stricto. The distribution of the unnamed Amazonian species is unclear but could be identical to the whole range originally assigned to *M. helleri* in central and western Amazonia (Fig. 1).

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Supplementary data

The following data are available online:

Supplementary Table S1. GenBank/ENA accession numbers for *Micrurus* samples used herein.

Supplementary Table S2. Questionable *Micrurus* sequences excluded from final analyses.

Supplementary Table S3. ND4 sequences of *Micrurus lemniscatus* sensu lato with conflicting identifications in GenBank.

Supplementary Table S4. Uncorrected p distances between *Micrurus* species for the ND4 gene.

Supplementary Figure S1. Complete Maximum Likelihood tree based on 3,618 base pairs of the concatenated alignment of five genes (12S, 16S, cyt *b*, ND4, Cmos).

Supplementary Figure S2. Complete Bayesian tree based on 3,618 base pairs of the concatenated alignment of five genes (12S, 16S, cyt *b*, ND4, Cmos).

Supplementary Figure S3. Complete Bayesian time tree with mean dates above nodes in million years before present.

Supplementary Figure S4. Complete Maximum Likelihood tree including the specimen *Micrurus carvalhoi* IB55598.