

Short Communication

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Finding arboreal snakes in an evolutionary tree: phylogenetic placement and systematic revision of the Neotropical birdsnakes

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Abstract

The genus *Pseustes* Fitzinger, 1843 is composed of three recognized species, *Pseustes poecilonotus*, *P. shropshirei* and *P. sulphureus*, which may be the largest sized colubrid snake in the New World. The group has a complex systematic history that has yet to be untangled using modern molecular phylogenetic approaches. The systematic position, within-group diversity and distribution are therefore uncertain. We obtained samples of four species from multiple specimens across their distribution and analysed one nuclear and two mitochondrial genes to determine the phylogenetic placement of the genus and infer relationships among *Pseustes* lineages. We find strong support for the paraphyly of *Pseustes* with respect to the monotypic genus *Spilotes*, both of which are nested within a clade of at least 23 other New World Colubrinae genera. Based on our results, we formally revise the taxonomy of *P. poecilonotus* and *P. sulphureus*, resurrecting the taxon *P. polylepis* for populations of *P. poecilonotus* from South America and allocating *P. sulphureus* to the genus *Spilotes* which renders both genera monophyletic. Additionally, we identify two lineages that are putatively new and currently unrecognized species. Finally, the placement of *P. sulphureus*, the type species of *Pseustes*, in the genus *Spilotes*, requires the allocation of the senior synonym *Phrynonax* be considered for the remaining *Pseustes* taxa.

Key words: Colubridae – phylogeny – *Phrynonax* – *Pseustes* – Serpentes – *Spilotes*

Introduction

Origin and evolution of the Neotropical fauna remains of great interest, especially given the complex geological history of the area, habitat heterogeneity and high levels of endemism of the region (Cracraft and Prum 1988; Duellman 1999; Daza et al. 2009). However, well-resolved phylogenies are still lacking for many organisms, and thus, the evolutionary histories as well as spatial and ecological distributions remain poorly known for a majority of taxa (Daza et al. 2009; Schargel et al. 2010). Despite recent advancements in our understanding of evolutionary relationships of Neotropical colubrid snakes, which represent a majority of snake taxa in the New World (e.g. Pyron et al. 2011), most taxa, including several genera, have yet to be examined using molecular phylogenetic approaches. Here, we begin to assemble one piece of that puzzle by focusing on the Neotropical snake genus *Pseustes*, commonly referred to as ‘birdsnares’ or ‘puffing snakes’. Three species are currently recognized in the genus (Rivas et al. 2012): *Pseustes poecilonotus* Günther 1858; *P. shropshirei* Barbour and Amaral 1924; and *P. sulphureus* Wagler 1824; which is among the largest sized colubrid snake in the New World (Pérez-Santos and Moreno 1988). Other species of *Pseustes* mentioned in Uetz (2013), such as *P. cinnamomeus* Wagler 1824 and *P. sexcarinatus* Wagler 1824; are considered in the synonymy of *Chironius scurrulus* Wagler, 1924 and *C. quadricarinatus* Boie 1827; respectively (Dixon et al. 1993; Giraud and Scrocchi 2002).

To date, evolutionary relationships within this genus have not been assessed, rendering their evolutionary relationships and species boundaries poorly understood. Additionally, accurate taxonomy of *Pseustes* remains questionable as species in the genus have been classified as belonging to numerous other genera, including *Ahaetulla*, *Chironius*, *Coluber*, *Dipsas*, *Herpetodryas*, *Natrix*, *Phrynonax*, *Spilotes*, *Synchalinus*, *Thamnobius* and *Tropidodipsas* (Uetz, 2013). Recently, Pyron et al. (2013) found support that *Pseustes sulphureus* is the sister taxon to *Spilotes pullatus*, both included within the Colubrinae. However, the estimated phylogenetic position of this species was based on a single 12S gene fragment as part of a large, multigene concatenated phylogenetic analysis of 4161 species of squamate reptiles. Therefore, increased individual, taxonomic and gene sampling is warranted to confirm this finding.

We utilize samples collected from multiple individuals of *Pseustes* across Central and South America in order to conduct a molecular phylogenetic analysis of this genus to (i) infer the phylogenetic position of *Pseustes* within the subfamily Colubrinae, (ii) assess the relationship between *Spilotes* and *Pseustes*, (iii) determine whether species of the genus *Pseustes* form a monophyletic group, (iv) infer phylogenetic relationships within *Pseustes* and (v) assess species-level diversity to resolve historical taxonomic debates. We provide a solid foundation for understanding the evolutionary history of the genus *Pseustes*, which will aid future work on this and other understudied groups of Neotropical snakes.

Methods

Molecular sampling

Genomic DNA was isolated from muscle tissue of 17 specimens of *Pseustes* and *Spilotes* using a Qiagen DNeasy extraction kit and protocol.

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Two mitochondrial [NADH dehydrogenase subunit 4 (ND4) and cytochrome *b* (cyt *b*)] and one nuclear oocyte maturation factor Mos (*c-mos*) gene fragments were independently PCR-amplified using GoTaq[®] Green master mix by Promega, Madison, WI, USA. Protocols for amplification were carried out as described in Arévalo et al. (1994), Burbrink et al. (2000), de Queiroz et al. (2002) and Lawson et al. (2005) incorporating the primer pairs ND4 + LEU, L14910 + H16064 and S77 + S78 and annealing temperatures 48, 46 and 55°C, respectively. Sequencing was performed in both forward and reverse directions using the PCR primers on a Beckman Coulter automated capillary sequencer, and sequence chromatographs were edited using Sequencher 4.2, Gene Codes Corporation, Ann Arbor, MI, USA. Sequences for each gene fragment were aligned separately, first automatically using the program MUSCLE (Edgar 2004), and then manually rechecked using Se-AL v2.0a11 (Rambaut 2002). No internal stop codons were found in these protein-coding gene fragments. Previously published sequences of snakes within the family Colubridae were downloaded from GenBank (see Appendix 1) and were combined with new sequence data generated in this study (GenBank accession numbers KF669664–KF669710, Table 1).

Our preliminary analyses based on taxa throughout the family Colubridae found *Pseustes* to have a close affinity to members the subfamily Colubrinae, and we therefore incorporated taxa in that subfamily in our final analyses. *Grayia tholloni*, *Pseudorabdion oxycephalum*, *Scaphiodontophis annulatus* and *Storeria dekayi* were used as outgroup taxa to root our Colubrinae phylogenetic tree of 95 species.

Phylogenetic analyses

We conducted mixed-model analyses on a concatenated data set (2340 bp in total), partitioned by gene as well as by codon, resulting in a total of nine partitions. We used Akaike information criterion (AIC) to identify the best-fit models of nucleotide substitution for both Bayesian inference (BI) and maximum-likelihood (ML) analyses. For this, we implemented the program MrModeltest v2.2 (Nylander 2004), run in PAUP* v4.0b10

(Swofford 2002), which recovered the GTR + I + Γ model for all three codon positions of ND4 and cyt *b* while finding models HKY + Γ for the first and second position of *c-mos* and GTR + Γ for the third position.

We inferred phylogenetic relationships using Bayesian inference criterion implementing MrBayes v3.0b4 (Ronquist and Huelsenbeck 2003). Two simultaneous runs were conducted (with the default Markov chain Monte Carlo [MCMC] settings), for a total of 10.0×10^6 generations per run, sampling trees and parameters every 100 generations. We used potential scale reduction factor values (output by MrBayes), together with plots of cold-chain likelihood values and parameter estimates visualized in Tracer v1.5.4 (Rambaut and Drummond 2009) to confirm stationarity and convergence of MCMC runs. Based on this evaluation, the first 2.5×10^6 generations from each run were discarded as burn-in.

Using the same partitioning scheme described above, we inferred the ML tree using RAxML 7.2.8 under the GTRCAT model and assessed tree support with the rapid-bootstrapping algorithm using 1000 nonparametric bootstraps (Stamatakis 2006; Stamatakis et al. 2008). Additionally, we performed the SHL test (Shimodaira and Hasegawa 1999; Anisimova and Gascuel 2006) in RAxML 7.2.8 to provide another maximum-likelihood measure of support. Support for the SHL test is measured as $1 - p$, where p is equivalent to the probability of obtaining a particular test statistic under the null hypothesis that the maximum-likelihood estimate of the branch is not significantly more likely than any nearest-neighbour rearrangements of that branch.

Results

Our phylogenetic analyses are very similar to recent molecular phylogenies of the Colubrinae (e.g. Pyron et al. 2011, 2013). Our analyses recovered the genus *Pseustes* nested within a clade of 23 other New World Colubrinae genera (Fig. 1), and paraphyletic with respect to the closely related genus *Spilotes* (Fig. 2). Unfortunately, our phylogenetic analyses do not resolve the sister

Table 1. GenBank numbers for DNA sequences generated in this study (KF669664–KF669710)

Species	Voucher	Locality	ND4	cyt <i>b</i>	<i>c-mos</i>
<i>Pseustes poecilonotus</i>	USNM 564157	Gracias a Dios, Tapalwás, Honduras (14°51'N, 84°32'W)	KF669690	KF669674	KF669708
<i>Pseustes poecilonotus</i>	LSUMZ H-14673	Rio San Juan, Nicaragua: Ca. 15 km S. El Castillo on north bank Rio San Juan at Isla el Diamante	KF669689	KF669673	KF669707
<i>Pseustes poecilonotus</i>	JMR 725	Near La Mica Biological Station, El Copé, Coclé, Panama (8°37'12"N, 80°36'0"W)	KF669687	KF669671	KF669705
<i>Pseustes poecilonotus</i>	JMR 744	Parque Nacional G. D. Omar Torrijos Herrera, Coclé, Panama (8° 40'N, 80° 37'W)	KF669688	KF669672	KF669706
<i>Pseustes polylepis</i>	LSUMZ H-17739	Rondonia, Brazil	KF669693	KF669676	KF669710
<i>Pseustes polylepis</i>	UTA R-55965	Morona-Santiago, Ecuador: Road to Mendez (2.65590°S; 78.20707°W)	KF669692	KF669675	KF669709
<i>Pseustes polylepis</i>	LSUMZ 42718	"Suriname"	KF669691	–	–
<i>Pseustes</i> sp.	UTA R-46140	Alta Verapaz, Guatemala: Cobán, Parque Nacional. Laguna Lachuá. ca. 175 MSNM	–	KF669670	KF669704
<i>Pseustes</i> sp.	LSUMZ 36746	"Honduras"	KF669686	KF669669	KF669703
<i>Pseustes</i> sp.	LSUMZ 39592	North coast of Honduras	KF669685	KF669668	KF669702
<i>Pseustes</i> sp.	LSUMZ H-7806	North coast of Honduras	KF669684	KF669667	KF669701
<i>Spilotes</i> sp.	LSUMZ H-14026	Amazonas, Brazil: Rio Ituxi at the Madeirera Scheffer (8° 20' 47"N, 65° 42' 57.9"W)	KF669679	–	KF669696
<i>Spilotes pullatus</i>	UTA R-52006	Petén, Guatemala: La Libertad, Parque Nacional Sierra Lacandón, Distrito Guayacán	KF669677	–	KF669694
<i>Spilotes pullatus</i>	LSUMZ 36738	"Honduras"	KF669678	–	KF669695
<i>Pseustes sulphureus</i>	LSUMZ H-14023	Amazonas, Brazil	KF669681	KF669665	KF669698
<i>Pseustes sulphureus</i>	LSUMZ 43274	Pasco Department, Peru; 41 km Villa Rica Puerto. Bermudez Hwy, 750 m elev.	KF669680	KF669664	KF669697
<i>Pseustes sulphureus</i>	LSUMZ 42645	"Suriname"	KF669682	KF669666	KF669699
<i>Pseustes sulphureus</i>	EBRG 5107	Bolivar, Venezuela: 25 km W Santa Elena de Uairen via El Paují	KF669683	–	KF669700

Abbreviations of institutions and individuals for voucher specimens are as follows: EBRG (Museo de la Estacion Biologica Rancho Grande, Maracay, Venezuela), JMR (Julie M. Ray field series), LSUMZ (Louisiana State Museum of Natural Science), USNM (Smithsonian Institution, National Museum of Natural History), UTA (Amphibian and Reptile Diversity Research Center, University of Texas, Arlington).

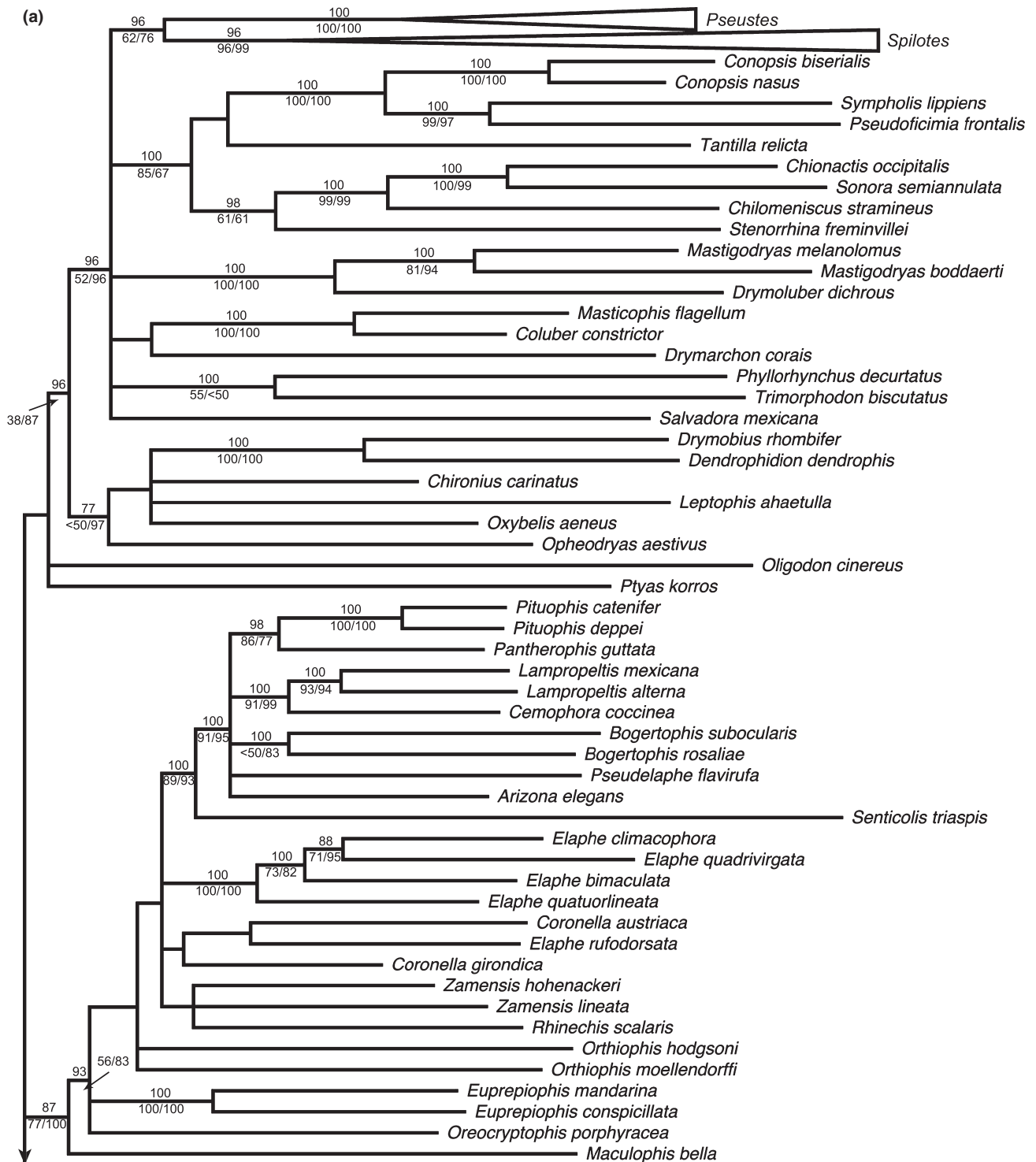


Fig. 1. Phylogenetic estimate of relationships between genera and species within the Colubrinae. The tree was estimated from a Bayesian 50% majority-rule consensus composed from a concatenated multigene data set (ND4, *cyt b* and *c-mos*; total of 2340 bp). Numbers at nodes represent values of Bayesian posterior probabilities (PP, above) and maximum-likelihood bootstraps and SHL tests (BS/SHL, below). Values are provided for nodes supported by $\geq 95\%$ PP and ≥ 70 BS/SHL.

clade to *Pseustes* + *Spilotes*; multiple other lineages of New World Colubrinae snakes form a basal polytomy at this node.

Within the *Pseustes* + *Spilotes* clade, we identify six divergent lineages, three within each of two larger clades (Fig. 2). Our results strongly support *Pseustes sulphureus* being more closely related to *Spilotes pullatus* lineages than other species of *Pseustes*. *Pseustes poecilonotus* as currently recognized is composed of two distinct, geographically separated lineages

based on current sampling, one of which is found in Central America and the other in South America. Sister to this *P. poecilonotus* clade is a lineage that occurs in northern Central America and may be sympatric with Honduran populations of *P. poecilonotus*. Finally, our findings show a deep split between *Spilotes pullatus* from Brazil and other *S. pullatus* populations in Central America, potentially being indicative of cryptic diversity.

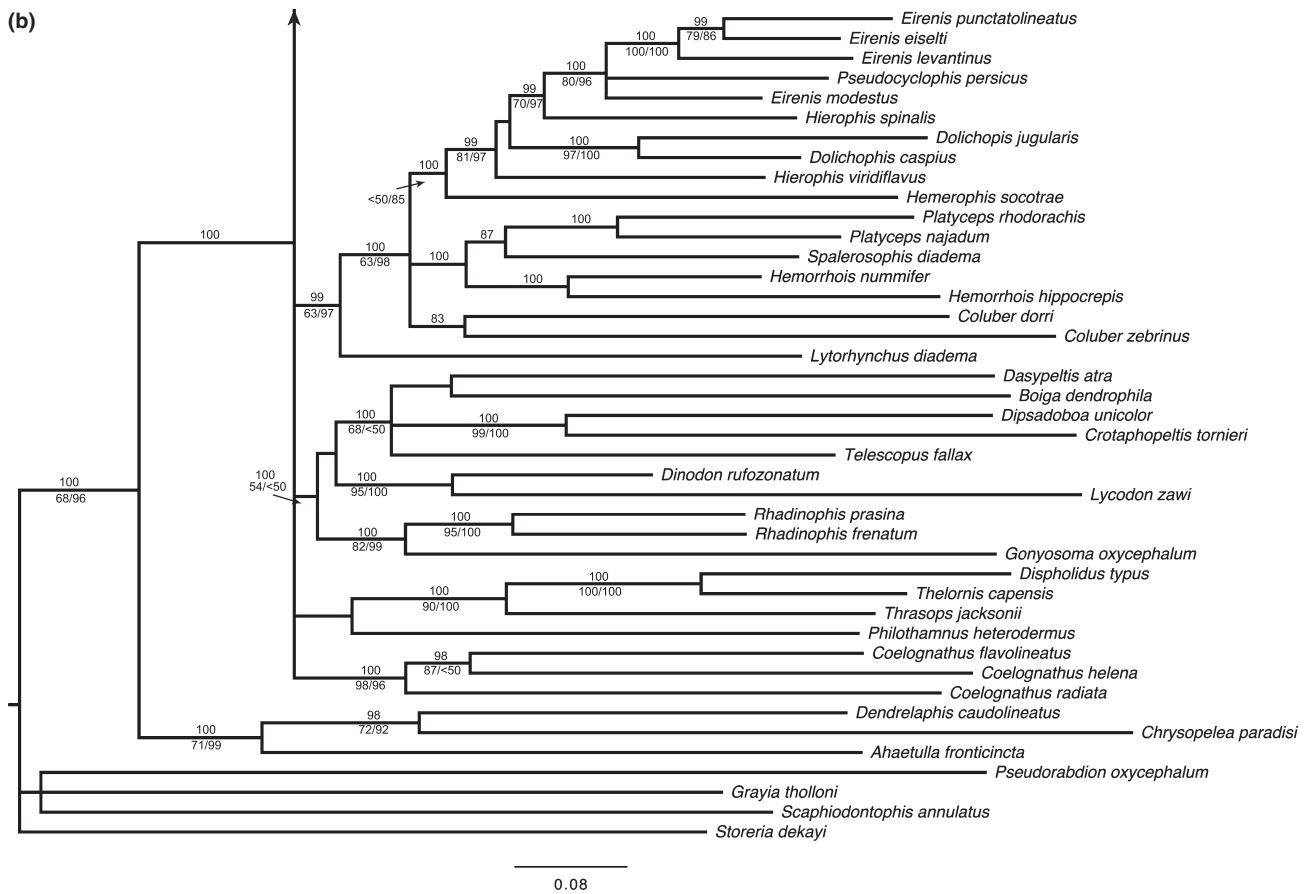


Fig. 1. (continued)

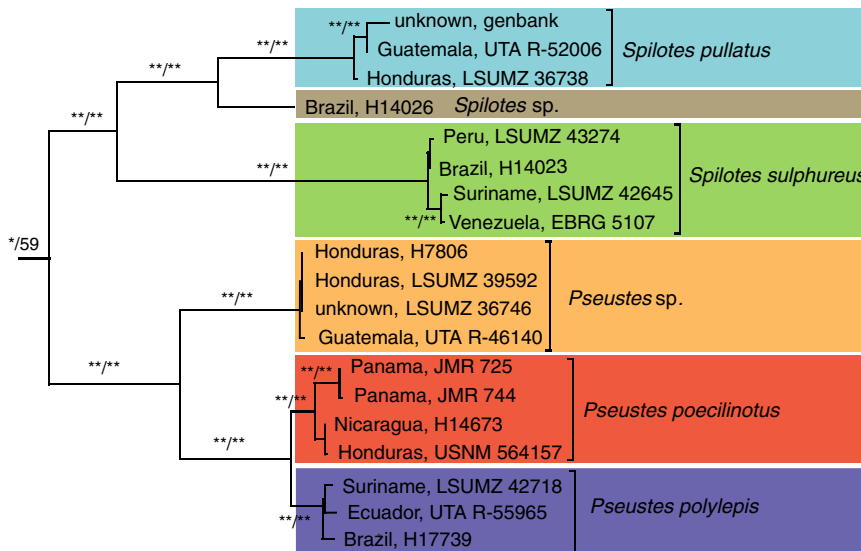


Fig. 2. Phylogenetic estimate of relationships within the *Pseustes* and *Spilotes* clade, resulting from a Bayesian 50% majority-rule consensus phylogram. ** = 100 posterior probability and 95–100 bootstrap/SHL support, respectively

Discussion

Phylogeny, species boundaries and taxonomic recommendations within *Pseustes*

Our study reveals that the genus *Pseustes* can formally be placed inside a New World Colubrinae clade (Fig. 1) and that this genus is paraphyletic with respect to *Spilotes* (Fig. 2). In particu-

lar, we find the species *Pseustes sulphureus* to be the sister taxon of *Spilotes pullatus*, which together are sister to the other *Pseustes* species. Given that the genus *Spilotes* Linnaeus, 1758 is older than *Pseustes* Fitzinger 1843; we recommend changing *Pseustes sulphureus* to *Spilotes sulphureus*. This taxonomic change will resolve the paraphyly of *Pseustes* and render both genera monophyletic. Additionally, this relationship is supported by a unique

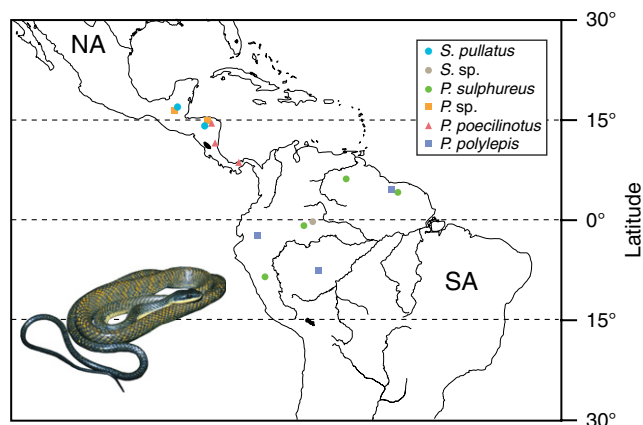


Fig. 3. Locality map showing where tissue samples of *Pseustes* and *Spilotes* used in this study were collected throughout Central and South America. Symbols represent estimations of localities examined for molecular sampling. *Pseustes poecilonotus* from Nicaragua (insert)

defensive behaviour, which includes an exaggerated neck posture display and inflating of the gular region, not found in the remaining species of *Pseustes*. Together, they also share a well-developed tracheal left lung with respect to their snout-vent length (Rossman and Williams 1966). Although the other species of *Pseustes* possesses a similar 'puffing' behaviour (e.g. *P. poecilonotus*), this trait is more similar to typical colubrid snakes that inflate their neck as a defensive behaviour when compared to the more dramatic displays by *Spilotes pullatus* and *S. sulphureus* (Rand and Ortleb 1969). Finally, *S. pullatus* and *S. sulphureus* are notable for being among the largest known New World colubrid snakes (along with *Clelia clelia* and *Drymarchon* spp.), reaching lengths upwards of three metres (Pérez-Santos and Moreno 1988).

Günther (1858) described *Pseustes poecilonotus* from Honduras and Mexico, but Boulenger (1894) later restricted the species to Honduras. Peters (1867) described *P. polylepis* (as *Ahaetulla polylepis*) from Suriname, now considered one of four recognized subspecies of *P. poecilonotus*. In this study, our sampling included specimens from several localities throughout Central and South America, including both Honduras and Suriname, and we find that two lineages of *P. poecilonotus* are separated geographically somewhere between western Panama and northern South America. Therefore, the name *P. poecilonotus* must be applied only to the Mesoamerican populations, while South American populations should be treated as the separate lineage *P. polylepis* (Amazon and the Guianas).

Taxonomic recommendations for the genus *Pseustes*

Spilotes sulphureus is the type species of *Pseustes* Fitzinger 1843; and therefore, the remaining species in the genus *Pseustes* must be allocated to *Phrynonax* Cope 1862 as a replacement. We take this action based on the principle of priority (International Code of Zoological Nomenclature, article 23.9.9.1). In this case, Amaral (1929) was the last author who used *Phrynonax* to refer to a species of *Pseustes*, postdating the 1899 cut-off for such a usage.

Cryptic diversity within *Phrynonax* and *Spilotes*

Our phylogenetic analyses provide a greater understanding of the species composition within the *Phrynonax* and *Spilotes* clade. In particular, our analyses detect two putative species-level lineages

that will likely need to be resurrected from previously described species or elevated from subspecies status based on literature and type material. The first undescribed lineage found within *Phrynonax* occurs in both Honduras and Guatemala, which may be broadly sympatric with *P. poecilonotus* throughout much of its range (Fig. 3, labelled *Pseustes* sp.). The second putative new lineage we detected suggests that populations of *Spilotes pullatus* from Central America are distinct from populations in South America, which may correspond to subspecies that are occasionally recognized for this taxon (Fig. 3, labelled *Spilotes* sp.). At this time however, this inference is based on only one divergent sample found in Brazil. A thorough sampling of specimens throughout their range and an increased sampling of molecular loci for lineages within these genera are needed for a more complete systematic assessment of the diversity, distributions and evolutionary history of these taxa.

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Appendix 1. GenBank numbers for sequence data of New World Colubridae analysed in this study (not including sequences listed in Table 1)

Species	ND4	cyt <i>b</i>	c-mos
<i>Ahaetulla fronticincta</i> (Günther, 1858)	–	AF471072	AF471161
<i>Arizona elegans</i> (Kennicott, 1859)	DQ902279	DQ902101	DQ902058
<i>Bogertophis rosaliae</i> (Mocquard, 1899)	DQ902280	DQ902102	DQ902059
<i>Bogertophis subocularis</i> (Brown, 1901)	DQ902281	DQ902103	DQ902060
<i>Boiga dendrophila</i> (Boie, 1827)	U49303	AF471089	AF471128
<i>Cemophora coccinea</i> (Blumenback, 1788)	DQ902282	AF471091	AF471132
<i>Chilomeniscus stramineus</i> (Cope, 1860)	U49305	GQ895856	GQ895800
<i>Chionactis occipitalis</i> (Hallowell, 1854)	–	GQ895857	GQ895801
<i>Chironius carinatus</i> (Linnaeus, 1758)	–	HQ529280	HQ529281
<i>Chrysopelea paradisi</i> (Boie, 1827)	–	GQ895858	GQ895802
<i>Coelognathus flavolineatus</i> (Schlegel, 1837)	U49301	DQ902128	DQ902090
<i>Coelognathus helena</i> (Daudin, 1803)	DQ902292	DQ902112	DQ902071
<i>Coelognathus radiatus</i> (Boie, 1827)	DQ902317	DQ902121	DQ902079
<i>Coluber constrictor</i> (Linnaeus, 1758)	AY487041	AY486914	AY486938
<i>Coluber dorri</i> (Lataste, 1888)	AY487042	AY188040	AY188001
<i>Coluber zebrinus</i> (Broadley and Schätti, 2000)	AY487058	AY188043	AY188004
<i>Conopsis biserialis</i> (Taylor and Smith, 1942)	–	GQ895860	GQ895804
<i>Conopsis nasus</i> Günther, 1858	–	GQ895861	GQ895805
<i>Coronella austriaca</i> (Laurenti, 1768)	AY487065	AY486930	AY486954
<i>Coronella girondica</i> (Daudin, 1803)	AY487066	AF471088	AF471113
<i>Crotaphopeltis tornieri</i> (Werner, 1908)	–	AF471093	AF471112
<i>Dasypteltis atra</i> (Sternfeld, 1912)	–	AF471065	AF471136
<i>Dendrelaphis caudolineatus</i> (Gray, 1834)	–	GQ895864	GQ895808
<i>Dendrophidion dendrophis</i> (Schlegel, 1837)	–	GQ895865	GQ895809
<i>Dinodon rufozonatum</i> (Cantor, 1842)	–	AF471063	AF471163

Appendix 1. (continued)

Species	ND4	cyt <i>b</i>	c-mos
<i>Dipsadoboa unicolor</i> Günther, 1858	–	AF471062	AF471139
<i>Dispholidus typus</i> (Smith, 1828)	U49302	AY188012	AY187973
<i>Dolichophis caspius</i> (Gmelin, 1789)	AY487039	AY376739	AY376797
<i>Dolichophis jugularis</i> (Linnaeus, 1758)	AY487046	AY486917	AY486941
<i>Drymarchon corais</i> (Boie, 1827)	DQ902314	AF471064	AF471137
<i>Drymobius rhombifer</i> (Günther, 1860)	–	GQ927320	GQ927313
<i>Drymoluber dichrous</i> (Peters, 1863)	–	GQ895869	GQ895812
<i>Eirenis eiselti</i> (Schmidtler, 1978)	AY487069	AY376747	AY376805
<i>Eirenis levantinus</i> (Schmidtler, 1993)	AY487071	AY376765	AY376823
<i>Eirenis modestus</i> (Martin, 1838)	AY487072	AY486933	AY486957
<i>Eirenis punctatolineatus</i> (Boettger, 1892)	AY487073	AY376755	AY376813
<i>Elaphe bimaculata</i> (Schmidt, 1925)	DQ902283	DQ902104	DQ902062
<i>Elaphe climacophora</i> (Boie, 1826)	DQ902285	DQ902105	DQ902064
<i>Elaphe quadrivirgata</i> (Boie, 1826)	DQ902300	DQ902120	DQ902078
<i>Elaphe quatuorlineata</i> (Bonnaterre, 1790)	AY487067	AY486931	AY486955
<i>Elaphe rufodorsata</i> (Cantor, 1842)	DQ902301	DQ902123	DQ902081
<i>Euprepiophis conspicillata</i> (Boie, 1826)	DQ902286	DQ902106	DQ902065
<i>Euprepiophis mandarinus</i> (Cantor, 1842)	DQ902294	DQ902115	DQ902073
<i>Gonyosoma oxycephalum</i> (Boie, 1827)	DQ902309	AF471084	AF471105
<i>Grayia tholloni</i>	DQ486326	DQ486351	DQ486175
<i>Hemerophis socotrae</i> (Günther, 1881)	AY487055	AY188042	AY188003
<i>Hemorrhois hippocrepis</i> (Linnaeus, 1758)	AY487045	DQ451987	AY486940
<i>Hemorrhois nummifer</i> (Reuss, 1834)	AY487049	AY376742	AY376800
<i>Hierophis spinalis</i> (Peters, 1866)	AY487056	AY486924	AY486948
<i>Hierophis viridiflavus</i> (Lacépède, 1789)	AY487057	AY486925	AY486949
<i>Lampropeltis alterna</i> (Brown, 1901)	AY497307	AF337130	FJ627799
<i>Lampropeltis Mexicana</i> (Garman, 1884)	AY497310	AF337146	FJ627800
<i>Leptophis ahaetulla</i> (Linnaeus, 1758)	–	GQ927321	GQ927316
<i>Lycodon zawi</i> (Slowinski et al., 2001)	–	AF471040	AF471111
<i>Lytorhynchus diadema</i> (Duméril et al., 1854)	–	AY188025	AY187986
<i>Maculophis bella</i> (Stanley, 1917)	DQ902316	DQ902134	DQ902097
<i>Masticophis flagellum</i> (Shaw, 1802)	AY487060	AY486928	AY234228
<i>Mastigodryas boddaerti</i> (Sentzen, 1796)	–	GQ895867	GQ895811
<i>Mastigodryas melanolomus</i> (Cope, 1868)	–	GQ895868	–
<i>Oligodon cinereus</i> (Günther, 1864)	–	AF471033	AF471101
<i>Opheodrys aestivus</i> (Linnaeus, 1766)	–	AF471057	AF471147
<i>Oreocryptophis porphyracea</i> (Cantor, 1839)	DQ902298	DQ902118	DQ902076
<i>Orthriophis hodgsoni</i> (Günther, 1860)	DQ902318	DQ902136	DQ902096
<i>Orthriophis moellendorffi</i> (Boettger, 1886)	DQ902295	DQ902116	DQ902074
<i>Oxybelis aeneus</i> (Wagler, 1824)	–	AF471056	AF471148
<i>Pantherophis guttatus</i> (Linnaeus, 1766)	DQ902291	DQ902111	DQ902070
<i>Philothamnus heterodermus</i> (Hallowell, 1857)	–	AF471055	AF471149
<i>Phyllorhynchus decurtatus</i> (Cope, 1868)	–	AF471083	AF471098
<i>Pituophis catenifer</i> (Blainville, 1835)	AF138764	AF337112	FJ627790
<i>Pituophis deppei</i> (Duméril, 1853)	AF141096	FJ627818	FJ627801
<i>Platyceps florulentus</i> (Geoffroy, 1827)	AY487043	AY486915	AY486939
<i>Platyceps najadum</i> (Eichwald, 1831)	AY487038	AY486912	AY486936
<i>Platyceps rhodorachis</i> (Jan, 1865)	AY487051	AY486921	AY486945
<i>Pseudelaphe flavirufa</i> (Cope, 1867)	DQ902289	DQ902109	DQ902068
<i>Pseudocyclophis persicus</i> (Anderson, 1872)	–	AY376757	AY376815
<i>Pseudoficimia frontalis</i> (Cope, 1864)	–	GQ895886	GQ895827
<i>Pseudorabdion oxycephalum</i> (Boie, 1827)	–	AF471073	DQ112083
<i>Pseustes sulphureus</i> (Wagler, 1824)	–	–	–
<i>Ptyas korros</i> (Schlegel, 1837)	AY487062	AY486929	AY486953
<i>Rhadinophis frenatum</i> (Gray, 1853)	DQ902290	DQ902110	DQ902069
<i>Rhadinophis prasina</i> (Blyth, 1854)	DQ902299	DQ902119	DQ902077
<i>Rhinechis scalaris</i> (Schinz, 1822)	AY487068	AY486932	AY486956
<i>Salvadora mexicana</i> (Duméril et al., 1854)	AY487075	AY486934	AY486958
<i>Scaphiodontophis annulatus</i> (Duméril et al., 1854)	–	GQ927323	GQ927318
<i>Senticolis triaspis</i> (Cope, 1866)	AF138775	DQ902127	DQ902086
<i>Sonora semiannulata</i> (Baird and Girard, 1853)	–	AF471048	AF471164
<i>Spalerosophis diadema</i> (Schlegel, 1837)	AY487059	AF471049	AF471155
<i>Spilotes pullatus</i> (Linnaeus, 1758)	–	AF471041	AF471110
<i>Stenorrhina freminvillei</i> (Duméril et al., 1854)	–	GQ895889	GQ895830
<i>Storeria dekayi</i> (Holbrook, 1839)	EF417365	AF471050	AF471154
<i>Sympholis lippiens</i> (Cope, 1861)	–	GQ895890	GQ895831
<i>Tantilla relicta</i> (Telford, 1966)	–	AF471045	AF471107
<i>Telescopus fallax</i> (Fleischmann, 1831)	–	AF471043	AY188000
<i>Thelotornis capensis</i> (Smith, 1849)	–	AF471042	AF471109

Appendix 1. (continued)

Species	ND4	cyt <i>b</i>	c-mos
<i>Thrasops jacksonii</i> (Günther, 1895)	–	AF471044	DQ112084
<i>Trimorphodon biscutatus</i> (Duméril et al., 1854)	DQ497506	GQ927324	GQ927319
<i>Zamenis hohenackeri</i> (Strauch, 1873)	DQ902320	DQ902137	DQ902098
<i>Zamenis lineata</i> (Camerano, 1891)	DQ902319	AJ277674	DQ902099