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Molecular systematics and undescribed diversity of Madagascan scolecophidian snakes (Squamata: Serpentes)

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Abstract

We provide an updated molecular phylogenetic analysis of global diversity of typhlopid and xenotyphlopid blindsnakes, adding a set of Madagascan samples and sequences of an additional mitochondrial gene to an existing supermatrix of nuclear and mitochondrial gene segments. Our data suggest monophyly of Madagascan typhlopids, exclusive of introduced *Indotyphlops braminus*. The Madagascar-endemic typhlopid clade includes two species previously assigned to the genus *Lemuriatyphlops* (in the subfamily Asiatyphlopinae), which were not each others closest relatives. This contradicts a previous study that described *Lemuriatyphlops* based on a sequence of the cytochrome oxidase subunit 1 gene from a single species and found this species not forming a clade with the other Malagasy species included. Based on our novel phylogenetic assessment we include all species in this endemic typhlopid clade in the genus *Madatyphlops* and in the subfamily Madatyphlopinae and consider *Lemuriatyphlops* as junior synonym. Within *Madatyphlops*, we identify several candidate species. For some of these (those in the *M. arenarius* complex), our preliminary data suggest sympatric occurrence and morphological differentiation, thus the existence of undescribed species. We also comment on the genus-level classification of several non-Madagascan typhlopids. We suggest that African species included in *Madatyphlops* (*Afrotyphlops calabresii, A. cuneirostris, A. platyrhynchus,* and *Rhinotyphlops leucocephalus*) should not be included in this genus. We furthermore argue that recent claims of *Sundatyphlops, Antillotyphlops,* and *Cubatyphlops* being "undiagnosable" or "not monophyletic" were based on errors in tree reconstruction and failure to notice diagnostic characters, and thus regard these three genera as valid.

Key words: Madagascar, mitochondrial DNA, taxonomy, Typhlopidae, *Indotyphlops, Madatyphlops, Lemuriatyphlops* **syn. nov.**, Xenotyphlopidae, *Xenotyphlops*

Introduction

Scolecophidians are small to medium sized, fossorial ophidians devoid of external eyes and with simplified scalation. Due to their secretive life and small number of external characters, they are among the least known snakes despite their almost cosmopolitan distribution with 417 species worldwide, distributed among the families Anomalepididae (18 species), Gerrhopilidae (18 species), Typhlopidae (261 species), Leptotyphlopidae (119 species), and Xenotyphlopidae (1 species) (Uetz & Hošek 2015). Recent molecular work has led to a renewed interest in scolecophidian systematics and revised the alpha taxonomy and higher classification of these snakes in numerous geographical regions (Vidal *et al.* 2010; Marin *et al.* 2013a,b; Kornilios *et al.* 2013; Hedges *et al.* 2014; Pyron & Wallach 2014).

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One scolecophidian fauna never subjected to a modern comprehensive systematic revision is that of Madagascar. This island according to current knowledge (Uetz & Hošek 2015) harbors two scolecophidian families, the Typhlopidae (12 species, with 11 endemic species of *Madatyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014, and the introduced *Indotyphlops braminus* (Daudin, 1803)) and the Xenotyphlopidae (with the single endemic genus *Xenotyphlops* Wallach & Ineich, 1996). Except for *I. braminus,* all Madagascan scolecophidians are endemic to the island (Guibé 1958). The relatively low species diversity of Madagascan scolecophidians contrasts with the otherwise extraordinarily rich squamate fauna of the island, with almost 400 species described to date and one fourth of these being snakes (Glaw & Vences 2007; Uetz & Hošek 2015). The vast majority of Madagascan snakes belong to a single radiation, that of the Pseudoxyrhophiinae (Lamprophiidae; Nagy *et al.* 2003), plus Madagascan boas (Boidae, four species), the monotypic psammophiine genus *Mimophis*, and the 13 species of scolecophidians.

It is obvious that the systematics of Madagascan scolecophidians is not satisfyingly established, and even the anecdotal knowledge on their distribution ranges and habits (Glaw & Vences 2007) often refer to incidental findings with only preliminary morphological identification. Recent alpha-taxonomic work was limited to descriptions of three taxa (*Madatyphlops andasibensis* (Wallach & Glaw, 2009), *M. rajeryi* (Renoult & Raselimanana, 2009), and *Xenotyphlops mocquardi* Wallach, Mercurio & Andreone, 2007), resurrection of one synonym (*M. boettgeri* (Boulenger, 1893)) (Wallach & Glaw 2007), and synonymization of *Xenotyphlops mocquardi* with *X. grandidieri* (Mocquard, 1905) (Wegener *et al.* 2013).

Furthermore, the genus-level classification of some of these snakes is disputed. In their recent global assessment of scolecophidians, Hedges *et al.* (2014) found most of the Malagasy species to be part of an endemic radiation and assigned 11 species to a subfamily Madatyphlopinae in the single genus *Madatyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014. *Onychocephalus arenarius* Grandidier, 1872, now *Madatyphlops arenarius*, was assigned as the type species of this genus. Soon thereafter, this view was partly challenged by Pyron & Wallach (2014). According to their phylogenetic analysis, the species *Madatyphlops microcephalus* (Werner, 1909) constituted its own endemic lineage, being sister to an Asian assemblage of typhlopids in the subfamily Asiatyphlopinae. Consequently, they created *Lemuriatyphlops* Pyron & Wallach 2014 to include the species *M. microcephalus* and three further, apparently closely related species. Following Pyron & Wallach (2014), *Lemuriatyphlops* contains *L. albanalis* (Rendahl, 1918) (previously a synonym of *Madatyphlops ocularis* (Parker, 1927)), *L. domerguei* (Roux-Estève, 1980), *L. microcephalus* (type species of *Lemuriatyphlops*) and *L. reuteri* (Boettger, 1881). Their genetic assessment of *Lemuriatyphlops*, however, relied on a single DNA sequence of the mitochondrial cytochrome *c* oxidase I (COI) gene. This sequence of *Madatyphlops/Lemuriatyphlops microcephalus* came from the DNA barcoding study of Nagy *et al.* (2012), along with further barcode sequences of the Madagascan snake fauna. Also, the morphological diagnosis of *Lemuriatyphlops* is cumbersome as a complex combination of several morphological traits is needed to unambiguously identify the genus.

Here we provide a re-assessment of the relationships of Madagascan scolecophidians based on newly collected materials and new DNA sequences. Our scope is not to provide a taxonomic revision of these snakes, but we instead focus on testing the monophyly of Madagascan typhlopids and discussing their genus-level classification. We furthermore provide evidence for undiscovered diversity in Madagascan typhlopids, exemplified by *Madatyphlops arenarius* where our data suggest the existence of genetically divergent lineages concordantly differing in morphological characters.

Material and methods

Newly determined sequences. For the current study, 39 new samples of Madagascan scolecophidians were sequenced and combined with other scolecophidian samples, four of which also originated from Madagascar (from the study of Vidal *et al.* 2010) (Table 1). Total genomic DNA was extracted using commercial products (e.g., NucleoSpin Tissue kit, Macherey-Nagel) and DNA was quantified with a Nanodrop ND-1000 spectrophotometer. Three mitochondrial markers were amplified in PCRs: a fragment of cytochrome *b* (CYTB) either with the primers L14910 or L14919 & H16064 (Burbrink *et al.* 2000) or with the newly designed primers CBMADL (GTAAACTCAGAYWCAGAYAAAAT) and CBMADH (TACDGGYTTTGTTGCTACYCAGGT), the standard DNA barcoding fragment of cytochrome *c* oxidase I with the primers RepCOI-F and RepCOI-R (Nagy *et al.* 2012) and a fragment of the 12S rRNA gene with the primers 12SAL and 12SBH (Kocher *et al.* 1989). We purified

positive PCR products on NucleoFast 96 PCR plates (Macherey-Nagel). DNA sequencing was performed in both directions. We used the BigDye v1.1 chemistry and an ABI 3130xl capillary sequencer (Life Technologies) to obtain DNA sequences. DNA sequences were assembled in CodonCode Aligner v5 (CodonCode Corp.). We checked and corrected all DNA sequences manually, and removed low-quality data. Newly determined sequences were submitted to Genbank (accession numbers KT316428–KT316555; see Table 1 for a complete list of accession numbers of sequences used).

Combined sequence matrix. The dataset built for this study includes many previously analyzed samples as well as new samples, thus we have included previously published sequences along with new sequences. We have included 97 ingroup (Typhlopidae and Xenotyphlopidae) samples from the global dataset A of Hedges *et al.* (2014), most with expanded gene coverage; seven outgroup samples (Anomalepididae, Gerrhopilidae, and Leptotyphlopidae) also from the global dataset A of Hedges *et al.* (2014), again with expanded gene coverage; six ingroup (Typhlopidae) samples from Kornilios *et al.* (2013); and 39 new ingroup (Typhlopidae and Xenotyphlopidae) Malagasy samples for a total of 149 samples (see Table 1).

Our final concatenated alignment comprises five nuclear and three mitochondrial genes: amelogenin (AMEL), brain-derived neurotrophic factor (BDNF), bone morphogenetic protein 2 (BMP2), neurotrophin 3 (NT3), recombination-activating gene 1 (RAG1), 12S ribosomal RNA (12S), cytochrome oxidase subunit I (COI), and cytochrome *b* (CYTB) for a total of 4742 aligned sites. Gene coverage and number of ingroup (Typhlopidae and Xenotyphlopidae) parsimony informative (PI) sites are as follows: AMEL (106 sequences, 375 aligned sites, 108 PI); BDNF (109 sequences, 630 aligned sites, 120 PI); BMP2 (102 sequences, 588 aligned sites, 148 PI); NT3 (106 sequences, 639 aligned sites, 148 PI); RAG1 (98 sequences, 516 aligned sites, 122 PI); 12S (48 sequences, 306 aligned sites, 116 PI); COI (21 sequences, 664 aligned sites, 217 PI); and CYTB (136 sequences, 1024 aligned sites; 584 PI) (see Table 1). For our 12S alignment, we identified and excluded poorly conserved regions using Gblocks v0.91b (Castresana 2000) under the following parameters: maximum number of sequences for a conserved position (25); minimum number of sequences for a flanking position (40); maximum number of contiguous non-conserved positions (4); minimum length of a block (4); allowed gap positions (with half). Thus our original 12S alignment of 397 aligned sites was reduced to 306 sites.

Phylogenetic reconstruction. Before performing phylogenetic analyses, we used PartitionFinder v1.1.1 (Lanfear *et al.* 2012) to determine the best partitioning strategy and molecular models under the Bayesian information criterion (BIC) and the "greedy" search scheme. Following the results of this analysis, we broke our dataset into two partitions: (1) the third codon position of CYTB, and (2) all remaining sites (12S, first and second codon positions of CYTB, and all three codon positions of every remaining gene). For the former, GTR+G was the best model, and GTR+I+G was the best model for the latter.

Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed on the final concatenated dataset. RAxML 8.1.11 (Stamatakis 2014) was implemented on the CIPRES Science Gateway (Miller *et al.* 2010). For the ML analysis, both partitions were analyzed using the GTRGAMMA model (the maximized available model in RAxML; modeling invariant sites is explicitly not recommended). All parameters for the ML analysis were estimated by the program during the run. Branch support in the trees was provided by rapid bootstrap analysis (1,000 replicates). MrBayes 3.2.3 (Ronquist *et al.* 2012) was also implemented on the CIPRES Science Gateway (Miller *et al.* 2010). For the BI analysis, the dataset was partitioned again as suggested by the PartitionFinder results, with the first partition being analyzed under GTR+G and the second under GTR+I+G. Two parallel runs of 10,000,000 generations were performed, sampling every 100 generations. Convergence was assessed by the standard deviation of split frequencies (< 0.01) and potential scale reduction factors (approaching 1.000 for all parameters). The first 25% of samples were discarded as burnin. Branch support was assessed with posterior probabilities.

Results and discussion

Phylogenetic analysis and major clades of scolecophidians. The DNA sequence alignment used for analysis (Fig. 1; Table 1) expands previous global datasets (Vidal *et al.* 2010; Hedges *et al.* 2014) by adding numerous terminals from Madagascar, and by complementing one additional gene (CYTB) for numerous Asian taxa. The phylogenetic tree agrees with these previous studies and provides significant support from Bayesian posterior probabilities (PP) and ML bootstrap proportions (BS) for the majority of genera and many intergeneric

relationships. Exceptions are *Ramphotyphlops* Fitzinger, 1843 and *Rhinotyphlops* Fitzinger, 1843, which are recovered as clades albeit without support. As in previous studies (Vidal *et al.* 2010; Hedges *et al.* 2014; Pyron & Wallach 2014) we find high support for four main clades, distributed predominantly or exclusively in Eurasia, Africa, the Caribbean, and South America, but relationships among these clades remain elusive.

Comments on several taxonomic changes proposed by Pyron & Wallach (2014). Some comments are needed regarding other aspects of the generic-level taxonomy of typhlopid snakes before addressing the focus of this study, the species in Madagascar (*Madatyphlops*). Three molecular phylogenetic studies have been published in recent years on typhlopid snakes at the global level. The study by Vidal *et al.* (2010) presented new data on 96 species of scolecophidians using five nuclear protein-coding genes, resolving the deep biogeographic history of the group. Two new families were named, but major taxonomic changes within Typhlopidae (261 species) were set aside until additional molecular data (~500 new DNA sequences) could be gathered and a comprehensive morphological assessment could be completed (Hedges *et al.* 2014). As a result, four subfamilies and 18 genera were recognized. More recently, Pyron & Wallach (2014) reanalyzed these molecular data and presented additional morphological data on typhlopid snakes. Although they largely concurred with the taxonomy of Hedges *et al.* (2014), they proposed several changes. We here follow some of these proposals, e.g. the placement of *Asiatyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014 in the synonymy of *Argyrophis* Gray, 1845 (Fig. 1, Table 1) and the transfer of *Afrotyphlops comorensis* (Boulenger, 1889) into the genus *Madatyphlops*. However, the following proposals require comments: (i) Synonymization of *Sundatyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014 with *Anilios* Gray, 1845; (ii) synonymization of *Antillotyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014 and *Cubatyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014 with *Typhlops* Oppel, 1811; (iii) inclusion of several African blindsnakes in the genus *Madatyphlops*; (iv) description of a new genus (*Lemuriatyphlops*) in the subfamily Asiatyphlopinae for some species in Madagascar which will be discussed in the context of our phylogenetic results.

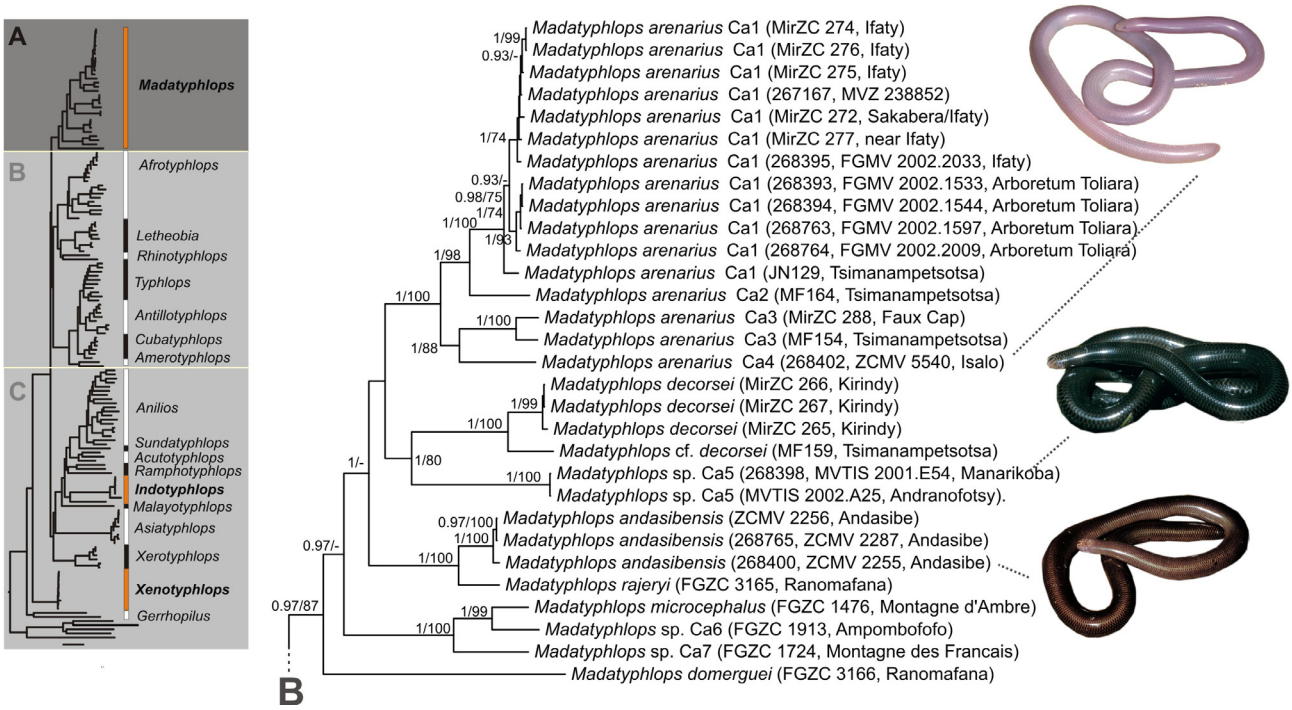

FIGURE 1. Phylogenetic tree (continued on the following pages) obtained by partitioned Bayesian Inference (BI) analysis based on the supermatrix of up to five nuclear and three mitochondrial genes (Table 1). The tree focuses on the families Typhlopidae and Xenotyphlopidae (occurring in Madagascar) and includes only a limited number of representatives of the remaining scolecophidian families (Anomalepididae, Gerrhopilidae, and Leptotyphlopidae) as hierarchical outgroups. The tree was rooted with an anomalepidid following the more inclusive phylogenies of Vidal *et al.* (2010) and Hedges *et al.* (2014). Values at nodes are posterior probabilities from BI followed by bootstrap proportions in percent from ML. Genera present in Madagascar (*Madatyphlops, Indotyphlops*, and *Xenotyphlops*) are marked with inset photos of Madagascan representatives. The inset picture shows the entire tree, with genera present in Madagascar marked with orange bars. Sample numbers without acronym refer to S.B. Hedges tissue collection numbers (SBH in Table 1). For other acronyms see caption to Table 1.

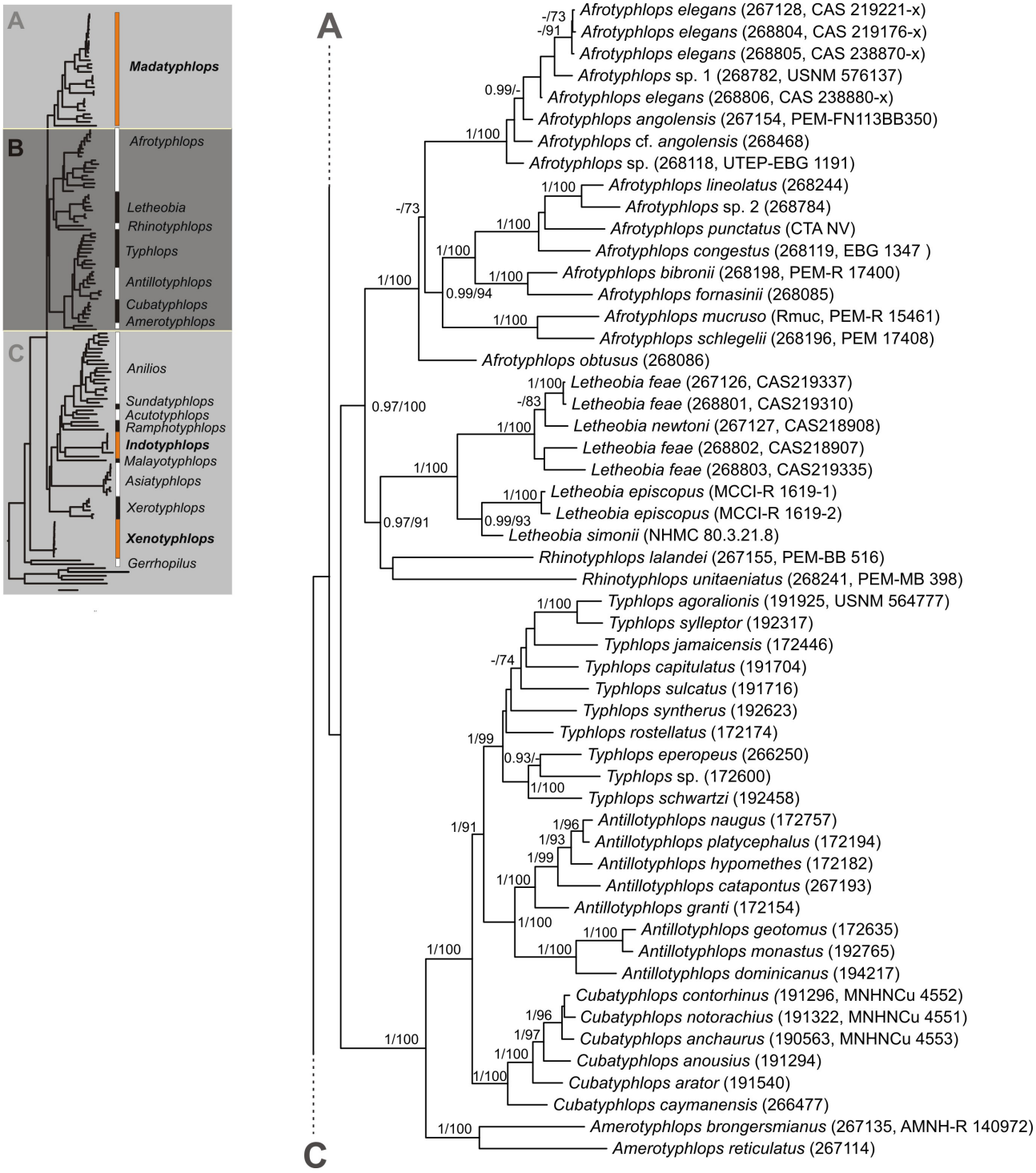

FIGURE 1. (Continued)

(i) *Sundatyphlops* is a genus of Indonesian typhlopid snakes described by Hedges *et al.* (2014) as being a clade (in their molecular phylogeny) distinct from the monophyletic genus *Anilios* (>95% bootstrap support) and occurring in a different biogeographic region (Indonesia versus Australia). This phylogenetic position (sister to *Anilios*) is also corroborated by the tree obtained in the present study (Fig. 1). Hedges *et al.* (2014) further diagnosed *Sundatyphlops* from other genera, morphologically, using external characters, including scale counts. As expected in any taxonomic study, the fewest diagnostic characters are typically with the closest relatives of a taxon, in this case *Anilios* (44 species). Nonetheless, *Sundatyphlops* can be unambiguously diagnosed from *Anilios* by a

combination of just two characters: midbody and middorsal scale rows. For example, of the ten species of *Anilios* having the same number of midbody scale rows (22) as *Sundatyphlops*, none has middorsal scale row counts that overlap with those of *Sundatyphlops* (453–496), as shown in Table 2 of Pyron & Wallach (2014). Thus, *Sundatyphlops* is morphologically diagnosable, phylogenetically distinct and should be considered as a valid genus.

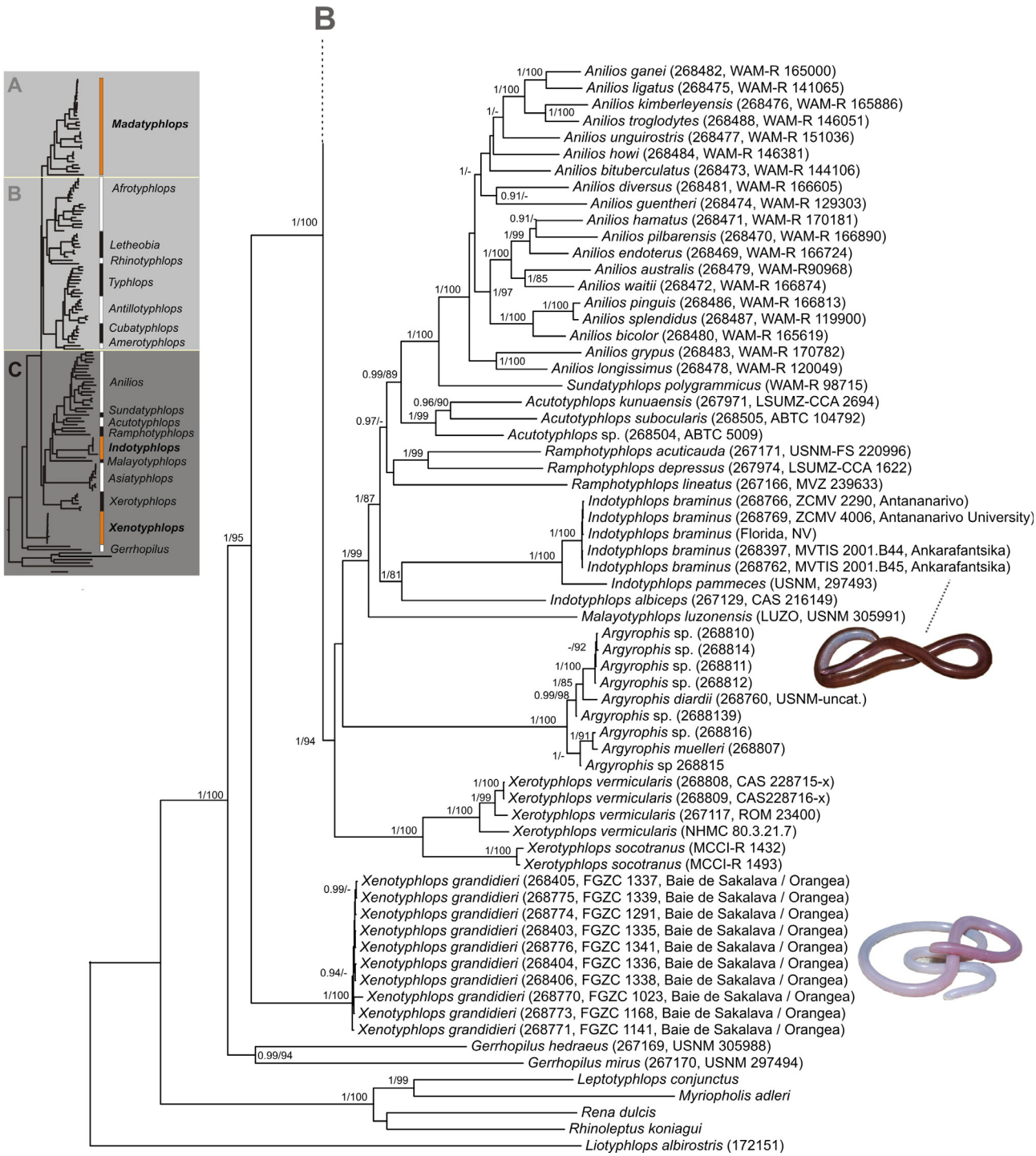

FIGURE 1. (Continued)

TABLE 1. Voucher information and GenBank accession numbers. Taxa marked with an asterisk are from Kornilios *et al.* (2013). Taxa marked with a dagger include 12S sequence data from a second SBH voucher specimen, collected at or nearby the same locality on the same or similar date. Acronyms used in the voucher column are as follows: ABTC (Australian Biological Tissue Collection, Adelaide, Australia), AMNH (American Museum of Natural History, USA), CAS (California Academy of Sciences, USA), CTA (tissue collections, Nicolas Vidal), LSUMZ (Louisiana State University, Museum of Zoology, USA), MCCI (Museo Civico di Storia Naturale di Carmagnola, Italy), MNHNCu (Museo Nacional de Historia Natural de Cuba), NHMC (Natural History Museum of Crete), PEM (Port Elizabeth Museum, South Africa), ROM (Royal Ontario Museum, Toronto, Canada), UADBA (Université d'Antananarivo, Département de Biologie Animale, Madagascar), USNM (National Museum of Natural History, Washington, D.C., USA), UTEP (University of Texas at El Paso, USA), WAM (Western Australian Museum, Perth, Australia), ZSM (Zoologische Staatssammlung München, Germany). FGMV, FGZC, MirZC, MV, and ZCMV are field numbers of F. Glaw, A. Miralles and M. Vences (specimens deposited in UADBA or ZSM). JN and MF are field numbers of J. Nopper. Column SBH refers to numbers in S.B. Hedges tissue collection. Further field numbers are EBG and ELI (E.B. Greenbaum), NV (N. Vidal), RT (R. Thomas), Z (Skip Lazell).

Taxon	SBH	voucher	AMEL	BDNF	BMP2	NT3	RAG1	12S	COI	CYTB
Afrotyphlops angolensis	267154	PEM-FN113BB350	GU902312	GU902389	GU902469	GU902562	GU902639			KT316468
Afrotyphlops bibronii	268198	PEM-R17400	GU902370	GU902450	GU902528	GU902620	GU902696			KT316469
Afrotyphlops cf. *angolensis*	268468	EBG 2220	KF992860	KF992881	KF992902	KF992923	KF992944			KT316470
Afrotyphlops congestus	268119	EBG 1347	GU902368	GU902448	GU902526	GU902618	GU902694			KT316471
Afrotyphlops elegans	267128	CAS 219221-x	GU902314	GU902391	GU902471	GU902564	GU902641			KT316472
Afrotyphlops elegans	268804	CAS 219176-x	KF992861	KF992882	KF992903	KF992924	KF992945			KT316473
Afrotyphlops elegans	268805	CAS 238870-x	KF992862	KF992883	KF992904	KF992925	KF992946			KT316474
Afrotyphlops elegans	268806	CAS 238880-x	KF992863	KF992884	KF992905	KF992926	KF992947			KT316475
Afrotyphlops fornasinii	268085	USNM FS268085	GU902367	GU902447		GU902617	GU902693			KT316476
Afrotyphlops lineolatus	268244	USNM FS268244	GU902371	GU902451	GU902529	GU902621	GU902697			KT316477
Afrotyphlops mucruso		PEM-R15461	GU902310	GU902387	GU902467	GU902560	GU902637			KT316478
Afrotyphlops obtusus	268086	PEM FN1436	GU902375		GU902548	GU902625	GU902700			
Afrotyphlops punctatus		CTA	GU902318	GU902395	GU902475	GU902567	GU902645			KT316479
Afrotyphlops schlegelii	268196	PEM 17408	GU902369	GU902449	GU902527	GU902619	GU902695			KT316480
Afrotyphlops sp.	268118	UTEP-EBG1191	GU902380	GU902460	GU902553	GU902630	GU902705			KT316481
Afrotyphlops sp. 1	268782	USNM 576137	KF992858	KF992879	KF992900	KF992921	KF992942			
Afrotyphlops sp. 2	268784	ELI 158	KF992859	KF992880	KF992901	KF992922	KF992943			KT316482
*Letheobia episcopus**		MCCI-R1619(1)	KC848445	KC848449		KC848457				
*Letheobia episcopus**		MCCI-R1619(2)	KC848446	KC848450		KC848458				
Letheobia feae	267126	CAS 219337	GU902308	GU902385	GU902465	GU902558	GU902635			KT316510
Letheobia feae	268801	CAS 219310	KF992846	KF992867	KF992888	KF992909	KF992930			KT316511
Letheobia feae	268802	CAS 218907	KF992847	KF992868	KF992889	KF992910	KF992931			KT316512
Letheobia feae	268803	CAS 219335	KF992848	KF992869	KF992890	KF992911	KF992932			KT316513

……*continued on the next page*

TABLE 1. (Continued)

Taxon	SBH	voucher	AMEL	BDNF	BMP2	NT3	RAG1	12S	COI	CYTB
Letheobia newtoni	267127	CAS 218908	GU902311	GU902388	GU902468	GU902561	GU902638			KT316514
*Letheobia simonii**		NHMC80.3.21.8	KC848447	KC848448		KC848459				
Rhinotyphlops lalandei	267155	PEM-BB516	GU902309	GU902386	GU902466	GU902559	GU902636			
Rhinotyphlops unitaeniatus	268241	PEM-MB398	GU902372	GU902452	GU902530					
Acutotyphlops kunuaensis	267971	LSUMZ-CCA2694	GU902339	GU902419	GU902499	GU902590	GU902669			KT316466
Acutotyphlops sp.	268504	ABTC5009	GU902379	GU902459	GU902552	GU902629	GU902704			KT316467
Acutotyphlops subocularis	268505	ABTC104792	GU902338	GU902418	GU902498	GU902589	GU902668	KF993037		JQ910524
Anilios australis	268479	WAM-R90968	GU902331	GU902409	GU902489	GU902580	GU902659			KT316484
Anilios bicolor	268480	WAM-R165619	GU902332	GU902410	GU902490	GU902581	GU902660			KT316485
Anilios bituberculatus	268473	WAM-R144106	GU902325	GU902403	GU902483	GU902574	GU902653			KT316486
Anilios diversus	268481	WAM-R166605	GU902333	GU902411	GU902491	GU902582	GU902661			KT316487
Anilios endoterus	268469	WAM-R166724		GU902399	GU902479	GU902570	GU902649			KT316488
Anilios ganei	268482	WAM-R165000	GU902334	GU902412	GU902492	GU902583	GU902662			KT316489
Anilios grypus	268483	WAM-R170782		GU902413	GU902493	GU902584	GU902663			KT316490
Anilios guentheri	268474	WAM-R129303	GU902326	GU902404	GU902484	GU902575	GU902654			KT316491
Anilios hamatus	268471	WAM-R170181	GU902323	GU902401	GU902481	GU902572	GU902651			KT316492
Anilios howi	268484	WAM-R146381	GU902335	GU902414	GU902494	GU902585	GU902664			KT316493
Anilios kimberleyensis	268476	WAM-R165886	GU902328	GU902406	GU902486	GU902577	GU902656			KT316494
Anilios ligatus	268475	WAM-R141065	GU902327	GU902405	GU902485	GU902576	GU902655			KT316495
Anilios longissimus	268478	WAM-R120049	GU902330	GU902408	GU902488	GU902579	GU902658			JQ910525
Anilios pilbarensis	268470	WAM-R166890	GU902322	GU902400	GU902480	GU902571	GU902650			KT316496
Anilios pinguis	268486	WAM-R166813	GU902336	GU902415	GU902495	GU902586	GU902665			
Anilios splendidus	268487	WAM-R119900	GU902337	GU902416	GU902496	GU902587	GU902666			KT316497
Anilios troglodytes	268488	WAM-R146051		GU902417	GU902497	GU902588	GU902667			KT316498
Anilios unguirostris	268477	WAM-R151036	GU902329	GU902407	GU902487	GU902578	GU902657			KC490399
Anilios waitii	268472	WAM-R166874	GU902324	GU902402	GU902482	GU902573	GU902652			KT316499
Argyrophis diardii	268760	USNM-uncat	KF992856	KF992877	KF992898	KF992919	KF992940			KT316507
Argyrophis muelleri	268807	CAS 222410	KF992857	KF992878	KF992899	KF992920	KF992941			KT316508
Argyrophis sp.	268810	CAS 224653	KF992849	KF992870	KF992891	KF992912	KF992933			KT316500
Argyrophis sp.	268811	CAS 224658	KF992850	KF992871	KF992892	KF992913	KF992934			KT316501
Argyrophis sp.	268812	CAS 224750	KF992851	KF992872	KF992893	KF992914	KF992935			KT316502
Argyrophis sp.	268813	CAS 225173	KF992852	KF992873	KF992894	KF992915	KF992936			KT316503

……*continued on the next page*

TABLE 1. (Continued)

Taxon	SBH	voucher	AMEL	BDNF	BMP2	NT3	RAG1	12S	COI	CYTB
Argyrophis sp.	268814	CAS 230225	KF992853	KF992874	KF992895	KF992916	KF992937			KT316504
Argyrophis sp.	268815	CAS 235322	KF992854	KF992875	KF992896	KF992917	KF992938			KT316505
Argyrophis sp.	268816	CAS 235378	KF992855	KF992876	KF992897	KF992918	KF992939			KT316506
Indotyphlops albiceps	267129	CAS 216149	GU902305	GU902382	GU902462	GU902555	GU902632			KT316509
Indotyphlops braminus		NV	GU902306	GU902383	GU902463	GU902556	GU902633			JQ910548
Indotyphlops braminus	268397	MVTIS 2001.B44						KT316428		KT316545
Indotyphlops braminus	268762	MVTIS 2001.B45						KT316429		KT316546
Indotyphlops braminus	268766	ZCMV 2290						KT316430	JQ909572	KT316547
Indotyphlops braminus	268769	ZCMV 4006								KT316548
Indotyphlops pammeces		USNM 297493	GU902378	GU902458	GU902551	GU902628	GU902703			
Madatyphlops andasibensis	268400	ZCMV 2255	GU902373	GU902453	GU902545	GU902622	GU902698			KT316516
Madatyphlops andasibensis		ZCMV 2256						KT316431	JQ909605	KT316517
Madatyphlops andasibensis	268765	ZCMV 2287								KT316518
Madatyphlops arenarius	267167	MVZ 238852	GU902374	GU902455	GU902547	GU902624	GU902699			KT316515
Madatyphlops arenarius	268395	FGMV 2002.2033		GU902454	GU902546	GU902623				KT316522
Madatyphlops arenarius	268393	FGMV 2002.1533						KT316432		KT316519
Madatyphlops arenarius	268394	FGMV 2002.1544								KT316520
Madatyphlops arenarius	268763	FGMV 2002.1597						KT316434		KT316521
Madatyphlops arenarius	268764	FGMV 2002.2009								KT316523
Madatyphlops arenarius		MirZC 272						KT316435	KT316451	KT316524
Madatyphlops arenarius		MirZC 274						KT316436		KT316525
Madatyphlops arenarius		MirZC 275						KT316437	KT316452	KT316526
Madatyphlops arenarius		MirZC 276						KT316438		KT316527
Madatyphlops arenarius		MirZC 277						KT316439		KT316528
Madatyphlops arenarius		MirZC 288						KT316440	KT316453	KT316529
Madatyphlops arenarius		JN129							KT316454	KT316530
Madatyphlops arenarius		MF154							KT316455	KT316531
Madatyphlops arenarius		MF164							KT316457	KT316532
Madatyphlops arenarius	268402	ZCMV 5540						KT316433	JQ909606	KT316533
Madatyphlops cf. *decorsei*		MF159							KT316456	
Madatyphlops decorsei		MirZC 265							KT316458	KT316534
Madatyphlops decorsei		MirZC 266						KT316441	KT316459	KT316535

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TABLE 1. (Continued)

Taxon	SBH	voucher	AMEL	BDNF	BMP2	NT3	RAG1	12S	COI	CYTB
Madatyphlops decorsei		MirZC 267						KT316442	KT316460	KT316536
Madatyphlops domerguei		FGZC 3166						KT316443	KT316461	KT316537
Madatyphlops microcephalus		FGZC 1476						KT316444	JQ909607	KT316538
Madatyphlops rajeryi		FGZC 3165						KT316445	JQ909608	KT316539
Madatyphlops sp.		FGZC 1724						KT316447	KT316462	KT316540
Madatyphlops sp.		FGZC 1913						KT316448	KT316463	KT316541
Madatyphlops sp.	268398	MVTIS 2001.E54						KT316446	KT316464	KT316542
Madatyphlops sp.		MVTIS 2002.A25							KT316465	KT316543
Malayotyphlops luzonensis		USNM 305991	GU902316	GU902393	GU902473		GU902643			KT316544
Ramphotyphlops acuticaudus	267171	USNM-FS220996	GU902304	GU902381	GU902461	GU902554	GU902631			JQ910543
Ramphotyphlops depressus	267974	LSUMZ-CCA1622	GU902340	GU902420	GU902500		GU902670			KT316549
Ramphotyphlops lineatus	267166	MVZ 239633	GU902307	GU902384	GU902464	GU902557	GU902634			KT316550
Sundatyphlops polygrammicus		WAM-R98715	GU902341	GU902421	GU902501	GU902591	GU902671	KF993038		KT316551
*Xerotyphlops socotranus**		MCCI-R1432	KC848443	KC848452		KC848455				
*Xerotyphlops socotranus**		MCCI-R1493	KC848444	KC848453		KC848456				
Xerotyphlops vermicularis	267117	ROM 23400	GU902320	GU902397	GU902477	GU902569	GU902647			JQ910544
Xerotyphlops vermicularis	268808	CAS 228715-x	KF992864	KF992885	KF992906	KF992927	KF992948			KT316552
Xerotyphlops vermicularis	268809	CAS 228716-x	KF992865	KF992886	KF992907	KF992928	KF992949			KT316553
*Xerotyphlops vermicularis**		NHMC80.3.21.7	KC848442	KC848451		KC848454				
Amerotyphlops brongersmianus	267135	AMNH-R140972	GU902313	GU902390	GU902470	GU902563	GU902640	KF993138		KF993239
Amerotyphlops reticulatus	267114	ROM 28368	GU902319	GU902396	GU902476	GU902568	GU902646			KT316483
Antillotyphlops catapontus	267193	Z39342	GU902346	GU902426	GU902506	GU902596	GU902676	KF993142		KF993243
Antillotyphlops dominicanus	194217	USNM FS194217	GU902348	GU902428	GU902508	GU902598		KF993148		KF993249
Antillotyphlops geotomus†	172635	USNM 336089	GU902349	GU902429	GU902509	GU902599		KF993151		KF993252
Antillotyphlops granti†	172154	RT9838	GU902350	GU902430	GU902510	GU902600	GU902678	KF993152		KF993253
Antillotyphlops hypomethes†	172182	USNM 300584	GU902351	GU902431	GU902511	GU902601	GU902679	KF993157		KF993258
Antillotyphlops monastus	192765	USNM FS192765	GU902354	GU902434	GU902514	GU902604		KF993161		KF993262
Antillotyphlops naugus†	172757	USNM FS172757	GU902355	GU902435	GU902515	GU902605	GU902681	KF993162		KF993263
Antillotyphlops platycephalus	172194	USNM FS172194	GU902357	GU902437	GU902517	GU902607	GU902683			KF993269
Cubatyphlops anchaurus	190563	MNHNCu4553	GU902343	GU902423	GU902503	GU902593	GU902673	KF993134		KF993235
Cubatyphlops anousius	191294	USNM 564783	GU902365	GU902445	GU902524	GU902615	GU902691	KF993135		KF993236
Cubatyphlops arator	191540	USNM 564784	GU902344	GU902424	GU902504	GU902594	GU902674	KF993136		KF993237

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TABLE 1. (Continued)

Taxon	SBH	voucher	AMEL	BDNF	BMP2	NT3	RAG1	12S	COI	CYTB
Cubatyphlops caymanensis	266477	USNM FS266477	GU902347	GU902427	GU902507	GU902597	GU902677	KF993143		KF993244
Cubatyphlops contorhinus	191296	MNHNCu4552	GU902366	GU902446	GU902525	GU902616	GU902692	KF993145		KF993246
Cubatyphlops notorachius	191322	MNHNCu4551	GU902356	GU902436	GU902516	GU902606	GU902682	KF993163		KF993264
Typhlops agoralionis	191925	USNM 564777	GU902342	GU902422	GU902502	GU902592	GU902672	KF993133		KF993234
Typhlops capitulatus	191704	USNM FS191704	GU902345	GU902425	GU902505	GU902595	GU902675	KF993141		KF993242
Typhlops eperopeus	266250	USNM 564785	GU902364	GU902444	GU902523	GU902614	GU902690	KF993150		KF993251
Typhlops jamaicensis†	172446	USNM 328408	GU902352	GU902432	GU902512	GU902602		KF993158		KF993259
Typhlops rostellatus	172174	USNM FS172174	GU902359	GU902439		GU902609	GU902685	KF993177		KF993278
Typhlops schwartzi	192458	USNM FS192458	GU902360	GU902440	GU902519	GU902610	GU902686	KF993178		KF993279
Typhlops sp.	172600	USNM FS172600	GU902353	GU902433	GU902513	GU902603	GU902680	KF993166		KF993267
Typhlops sulcatus	191716	USNM FS191716	GU902361	GU902441	GU902520	GU902611	GU902687	KF993179		KF993280
Typhlops sylleptor	192317	USNM 564804	GU902362	GU902442	GU902521	GU902612	GU902688	KF993180		KF993281
Typhlops syntherus	192623	USNM FS192623	GU902363	GU902443	GU902522	GU902613	GU902689			KF993282
Xenotyphlops grandidieri	268403	FGZC 1335, ZSM 2213/2007	GU902376	GU902456	GU902549	GU902626	GU902701		JQ909623	KF770842
Xenotyphlops grandidieri	268771	FGZC 1141, ZSM 2125/2007	GU902377	GU902457	GU902550	GU902627	GU902702	KT316450		KF770847
Xenotyphlops grandidieri	268774	FGZC 1291, ZSM 2193/2007								KF770849
Xenotyphlops grandidieri	268770	FGZC 1023, ZSM 2075/2007								KF770846
Xenotyphlops grandidieri	268773	FGZC 1168, UADBA uncat.								KF770848
Xenotyphlops grandidieri	268404	FGZC 1336, UADBA-R 70142	ERROR!!!	ERROR!!!	ERROR!!!	ERROR!!!	ERROR!!!	KT316449		KF770843
Xenotyphlops grandidieri	268405	FGZC 1337, ZSM 2214/2007								KF770844
Xenotyphlops grandidieri	268406	FGZC 1338, UADBA-R 70144								KF770845
Xenotyphlops grandidieri	268775	FGZC 1339, UADBA-R 70141								KF770850
Xenotyphlops grandidieri	268776	FGZC 1341, UADBA-R 70143								KF770851
Liotyphlops albirostris	172151	USNM FS172151	FJ434039	FJ433960	EU402705		FJ433886			AF544672
Gerrhopilus hedraeus	267169	USNM 305988	GU902315	GU902392	GU902472	GU902565	GU902642			KT316554
Gerrhopilus mirus	267170	USNM 297494	GU902317	GU902394	GU902474	GU902566	GU902644			KT316555
Rena dulcis	267165	MVZ 230602	GQ468999	GQ469182	GU902538	GQ469022	GQ469045			GQ469105
Rhinoleptus koniagui	268182	USNM FS268182	GQ469010	GQ469193	GU902531	GQ469032	GQ469055			GQ469158
Leptotyphlops conjunctus	268183	PEM R17410	GQ468996	GQ469179	GU902544	GQ469019	GQ469042			GQ469159
Myriopholis adleri	268179	USNM FS268179	GQ468989	GQ469172	GU902539	GQ469013	GQ469035			GQ469155

(ii) The synonymization by Pyron & Wallach (2014) of *Antillotyphlops* and *Cubatyphlops* with *Typhlops* was based on an error involving taxon identification and another involving tree reconstruction. First, they selected (from GenBank) two mtDNA sequences (AF366743, AF366812) of a snake from Cuba identified as "*Cubatyphlops biminiensis*" to represent that species. However, *C. biminiensis* (Richmond, 1955) only occurs in the Bahamas, and the Cuban populations are known to belong to eight other species and not *C. biminiensis* (Thomas & Hedges 2007; Dominguez & Moreno 2009): *C. anchaurus* (Thomas & Hedges, 2007), *C. anousius* (Thomas & Hedges, 2007), *C. arator* (Thomas & Hedges, 2007), *C. contorhinus* (Thomas & Hedges, 2007), *C. golyathi* (Dominguez & Moreno, 2009), *C. notorachius* (Thomas & Hedges, 2007), *C. perimychus* (Thomas & Hedges, 2007), and *C. satelles* (Thomas & Hedges, 2007). The sequences they chose to represent the Bahamian species "*C. biminiensis*" are actually of the Cuban species *C. perimychus*, which was otherwise not included in their tree despite the availability of correctly labeled sequences of mitochondrial and nuclear genes of *C. perimychus* collected and analyzed by Hedges *et al.* (2014).

This identification mistake was compounded by a tree reconstruction error. *Cubatyphlops perimychus* was represented in the tree of Pyron & Wallach (2014) only by fast-evolving mtDNA sequences, compared with most other species in their tree which were represented by slow-evolving nuclear DNA sequences. Not unexpectedly, the mtDNA sequences of *C. perimychus* appeared deeper in the tree because of their artificially longer branch, causing the otherwise monophyletic clade (*Cubatyphlops*) to be non-monophyletic.

None of these problems occurred in the analyses of Hedges *et al.* (2014) because the sequences were correctly labeled and nuclear genes were sequenced and included for *C. perimychus*. Also, different alignments were constructed and analyzed separately based on gene composition, to avoid such problems as occurred in Pyron & Wallach (2014). Hedges *et al.* (2014) found that *Antillotyphlops* and *Cubatyphlops* were each unambiguously monophyletic (significantly) in a global alignment (not including *C. perimychus*) that emphasized nuclear genes, and in a separate alignment (including *C. perimychus*) focused on the New World and containing mitochondrial and nuclear genes. The tree shown here (Fig. 1) is an expansion of the global alignments of Hedges *et al*. (2014), with emphasis on Old World species (especially, from Madagascar). Nonetheless, *Antillotyphlops* and *Cubatyphlops* remain monophyletic. Therefore, and considering the morphological diagnoses provided for each genus in Hedges *et al.* (2014), we consider *Antillotyphlops* and *Cubatyphlops* as valid genera.

(iii) We also disagree with the transfer of the African species *Afrotyphlops calabresii* (Gans & Laurent, 1965), *A. cuneirostris* (Peters, 1879), *A. platyrhynchus* (Sternfeld, 1910), and *Rhinotyphlops leucocephalus* (Parker, 1930) to *Madatyphlops* by Pyron & Wallach (2014) based solely on selected morphological affinities and therefore suggest re-allocating these taxa to *Afrotyphlops* Broadley & Wallach, 2009 and *Rhinotyphlops*, respectively, until molecular data become available (thus restoring the classification of Hedges *et al.* 2014 for these taxa). Besides creating a more complex biogeographic history for the species, the action by Pyron & Wallach (2014) also largely disagrees with a defining morphological trait of *Madatyphlops*, rounded snout shape (Hedges *et al.* 2014). Three of those four African species (*A. calabresii*, *A. cuneirostris* and *R. leucocephalus*) have pointed snouts (acuminate or beaked), common among Afrotyphlopinae but absent (in this strong expression of the trait) in *Madatyphlops*.

(iv) Phylogenetic relationships of Madagascan scolecophidians and synonymy of *Lemuriatyphlops* with *Madatyphlops*

Our phylogenetic analysis (Fig. 1) placed all studied samples of Madagascan scolecophidians into three distinct and unrelated clades: (1) All four Madagascan samples of the probably introduced *Indotyphlops braminus* were placed into *Indotyphlops*, and showed no genetic differences to a sample from Florida, USA, in overlapping gene regions, consistent with human introduction and parthenogenetic reproduction (minor differences in tree branch length derive from missing data). The relationships among populations of this species will be studied in more detail elsewhere. (2) All included samples of *Xenotyphlops* (most of which from Wegener *et al.* 2013) were genetically almost identical and formed a clade sister to the Typhlopidae as in previous studies (e.g., Vidal *et al.* 2010), corroborating their inclusion in a distinct family Xenotyphlopidae. (3) All remaining samples formed a single monophyletic group which includes species assigned by Pyron & Wallach (2014) to *Lemuriatyphlops,* and which we here consider as *Madatyphlops* as originally defined by Hedges *et al.* (2014).

In our tree (Fig. 1) the *Madatyphlops* clade receives moderate support (PP 0.97, BS 87%) and all species-level lineages are highly supported. On the contrary, the interrelationships within this clade remain largely unresolved. The two included species that would correspond to *Lemuriatyphlops* sensu Pyron & Wallach (2014) did not form a monophyletic group (*M. domerguei*, *M. microcephalus*); they were placed as successive sister groups of other

Madatyphlops, although their placement received no consistent support among ML and BI analyses. *Madatyphlops andasibensis* and *M. rajeryi* were sister groups with maximum support. Our tree also contains a number of deep genetic lineages of *Madatyphlops* here considered as candidate species (sensu Vieites *et al.* 2009) and named Ca1–Ca7 following the scheme proposed by Padial *et al.* (2010). Two candidate species from northern Madagascar (*M.* sp. Ca6 and Ca7, from Ampombofofo and Montagne des Français) were placed with maximum support in a clade with *M. microcephalus* (also occurring in northern Madagascar), one candidate species (*M.* sp. Ca5 from Manarikoba and Andranofotsy) was placed in a clade with *M. decorsei* (Mocquard, 1901), and the *M. arenarius* clade consisted of four candidate species, here named *M. arenarius* Ca1–Ca4 for convenience (although two of them most likely refer to the two valid species *M. arenarius* and *M. boettgeri*, see below). The apparent northern distribution of several candidate species (data herein) and nominal species (Glaw & Vences 2007) including *Xenotyphlops grandidieri* suggest that northern Madagascar, as with other Madagascan organisms, qualifies as a center of species richness and endemism (Vences *et al.* 2009).

The poor phylogenetic resolution within *Madatyphlops* is likely a consequence of the limited nuclear sequence information available for most of the taxa (Table 1). Because for many specimens, only minute amounts of tissue and poor-quality DNA were available, determining additional sequences and improving the phylogeny will have to await fresh sampling. Despite these limitations the amount of information available in our matrix for the Malagasy species is substantially higher than in Pyron & Wallach (2014) who based the description of *Lemuriatyphlops* on only a single gene fragment (COI) from a single specimen (*M. microcephalus*) obtained from a previous DNA barcoding study (Nagy *et al.* 2012). In their supermatrix analysis, this terminal was placed sister to several Asian typhlopid genera with 76% ML bootstrap support while three other Madagascan taxa (*M. arenarius*, *M. andasibensis*, *M. rajeryi*) formed a strongly supported clade. We here add a second species of "*Lemuriatyphlops*" (*M. domerguei*) and partial DNA sequences of two additional mitochondrial genes (CYTB, 12S) to the analysis. Our data indicate that the two included species of "*Lemuriatyphlops*" might not form a monophyletic group, although the support for their non-monophyly is poor. However, it is relevant that our extended analysis does not provide evidence for a stable clade containing species of "*Lemuriatyphlops*" sensu Pyron & Wallach (2014). This genus therefore does not satisfy the criterion of stable monophyly for supraspecific clades, and because it was defined only by a complex combination of morphological character states, it also does not fulfill the criterion of phenotypic diagnosibility (Vences *et al.* 2013). Our analysis is based on a much extended sampling of Malagasy typhlopids compared to the analysis of Pyron & Wallach (2014), and finds a biogeographically more parsimonious phylogeny. We therefore consider *Lemuriatyphlops* Pyron & Wallach, 2014 as a junior synonym of *Madatyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014.

Assignment of the taxon *comorensis* from *Afrotyphlops* to *Madatyphlops* by Pyron & Wallach (2014) is tentatively confirmed by similarites of COI sequences provided by Hawlitschek *et al.* (2013) and is therefore accepted here. It also has a rounded snout shape, consistent with *Madatyphlops* (Hedges *et al.* 2014). Consequently, the genus *Madatyphlops* now includes the following species: *Madatyphlops andasibensis, M. arenarius, M. boettgeri*, *M. comorensis, M. decorsei, M. domerguei, M. madagascariensis* (Boettger, 1877)*, M. microcephalus, M. mucronatus* (Boettger, 1880)*, M. ocularis, M. rajeryi,* and *M. reuteri*, and tentatively *M. albanalis* (a taxon of dubious origin and previously considered a synonym of *M. ocularis*; see Pyron & Wallach 2014).

Undescribed diversity exemplified by the *Madatyphlops arenarius* clade. Within the genus *Madatyphlops* we observed high genetic diversity, with numerous deep genealogical lineages as known from other Madagascan squamates (Nagy *et al.* 2012). Their divergence as indicated by branch lengths (Fig. 1) is similar to or higher than that between nominal species such as *M. andasibensis* and *M. rajeryi*. As with other Madagascan squamates (e.g., Gehring *et al.* 2012) taxonomic conclusions require more data on the morphology of these lineages, and of their differentiation in nuclear genes. For instance, the divergent sequence of *M.* cf. *decorsei* (MF159) might just reflect intraspecific variation in *M. decorsei* between the populations from Kirindy and Tsimanampetsotsa that are over 400 km apart. As with specimens from Kirindy, MF159 was collected in xerophytic forest with sandy substrate. However, at this moment, we do not have morphological or other evidence for the potential differentiation of *M. decorsei*.

The situation is however different in the *M. arenarius* clade where four deep lineages can be recognized in our tree (Fig. 1), and some of these occur in sympatry with apparent morphological differentiation. The level of genetic distance at cytochrome *b* (13–18%) is as great or greater than that separating recognized species of reptiles (Avise 1998).We here refer to these as candidate species *M. arenarius* Ca1 to Ca4 (naming scheme as proposed by Padial

et al. 2010) because none of them can currently be assigned reliably to *M. arenarius* or *M. boettgeri*. All specimens superficially resemble *M. arenarius,* but this species was described from "Mouroundava" (Grandidier 1872), and we have no samples from this locality, which is over 300 km north of our northernmost localities Ifaty and Isalo. Also, it is unclear which of the candidate species might correspond with *M. boettgeri* (type locality: southwest Madagascar; see Boulenger 1893) which is morphologically similar to *M. arenarius* and has been reported from one of our localities, Tsimanampetsotsa (Wallach & Glaw 2009).

With respect to the molecular samples analysed herein, we have also gathered preliminary morphological data from the voucher specimens of two candidate species (Ca1 and Ca2) occurring in Tsimanampetsotsa National Park and one candidate species (Ca4) occurring in Isalo National Park. According to these data, *M. arenarius* Ca1 (specimens JN129 and FGMV 2002.1597) is characterized by a total length of 140–172 mm, an anterior body diameter of 2.4–2.9 mm, longitudinal scale row counts of 20-20-20, and the presence of a short apical spine. The examined specimens are dorsally beige (coloration in ethanol of FGMV 2002.1597 is darker than of JN129), the intensity of this pigmentation increasing towards the tail. This low scale count and bicolored pattern agrees with *M. boettgeri.* Wallach & Glaw (2009) provide, in addition to scale counts and coloration/pigmentation, two additional characters distinguishing *M. arenarius* from *M. boettgeri*: parietal orientation (transverse in *M. boettgeri* and oblique in *M. arenarius*) and relative size of the occipital (enlarged in *M. boettgeri* and not enlarged in *M. arenarius*). The examined specimens of Ca1 have a transverse parietal orientation (as presumably in *M. boettgeri*) and an occipital that is not enlarged (as presumably in *M. arenarius*) and therefore cannot be readily assigned to any of the two species.

A second candidate species (Ca4; based on specimen ZCMV 5540 from Isalo) is also characterized by low longitudinal scale row counts of 20-20-20 and the presence of an apical spine. Total length of the examined specimen is 149 mm and its anterior body diameter is 3.4 mm. The coloration resembles that of specimen JN129 of *M. arenarius* Ca1 (hence, with a somewhat pigmented dorsum). This specimen has a transverse parietal orientation and an enlarged occipital. *M. arenarius* Ca4 morphologically does not resemble *M. arenarius* but appears to correspond to *M. boettgeri* according to the characters assessed, following Wallach & Glaw (2009).

A third candidate species, *M. arenarius* Ca2 (specimen MF164) has a relatively thicker body (total length 124 mm, anterior body diameter 2.8 mm) and a much higher scale row count of 24-26-24 than both Ca1 and Ca4. This specimen also is bicolored, however, with a uniform dorsal pigmentation. Longitudinal scale row counts are higher than have been reported for both *M. arenarius* and *M. boettgeri*. The only species that have been reported to have a similar amount of scale rows are *M. mucronatus*, *M. andasibensis*, *M. rajeryi* and *M. decorsei* (Wallach & Glaw 2009). The specimen apparently lacks a distinct apical spine; if confirmed, this character state would be a distinction from both *M. arenarius* and *M. boettgeri,* as well as from *M. mucronatus*, *M. andasibensis*, and *M. decorsei*. Additionally, Ca2 differs from *M. decorsei* by a T-V supralabial imbrication pattern (instead of a T-III supralabial imbrication pattern; see Wallach 1993), from *M. mucronatus* and *M. andasibensis* by an oblique (instead of transverse) parietal orientation, and from *M. mucronatus* additionally by a lower number of subcaudal scales (8 instead of 13–18; Wallach & Glaw 2009).

Despite being rather anecdotal, the observed differences suggest the possible presence of morphologically well differentiated species of this clade which partly occur in sympatry and sometimes perhaps in syntopy. The specimen from Isalo National Park corresponds most closely to the morphological description of external characters of *M. boettgeri* provided by Wallach & Glaw (2009) and thus could be this species; in fact, Wallach & Glaw report this species from Zombitse, which is a reserve relatively close to Isalo (ca. 70 km in linear distance). On the other hand, we could not unambiguously assign any specimens to *M. arenarius* (Grandidier, 1872). This might suggest that the distribution of this species is not as large as has previously been assumed (Glaw & Vences 2007); in fact we lack specimens from the type locality of *arenarius*, our nearest sampling localities being at least 300 km away. As a general conclusion, the definition of *M. arenarius* vs. *M. boettgeri* given in Wallach & Glaw (2009) is in need of confirmation, because much of the diversity within the *M. arenarius* group is still undescribed. However, their type localities are located in different bioclimatic regions and separated by a large distance, suggesting that both species might be valid.

The situation seems to be in contrast to that of the burrowing, blind and limb-reduced skink *Grandidierina fierinensis* (formerly placed in the genus *Voeltzkowia*) from the same area in southwestern Madagascar (Miralles *et al.* 2015). In this species, two morphologically well differentiated morphs are genetically poorly differentiated and possibly represent intraspecific variation. In *Madatyphlops arenarius*, there seem to be, instead, genetically well

differentiated lineages which are partly difficult to distinguish morphologically. It is obvious that a thorough integrative taxonomic revision of the *Madatyphlops arenarius* complex and other Madagascan blind snakes is needed. We conclude that simultaneous analysis of molecular, ecological and morphological characters is important to understand the evolution of scolecophidians. However, in such a secretive and morphologically cryptic group, molecular data are key to delimit species and understand evolutionary relationships. In Madagascar and the Comoros, new fieldwork is needed to assemble tissue samples from the many scolecophidian species which remain to be studied from a molecular perspective (*Madatyphlops albanalis*, *M. comorensis* complex, *M. madagascariensis*, *M. mucronatus*, *M. ocularis*, *M. reuteri*).

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