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A new subfamily of fossorial colubroid snakes from the Western Ghats of peninsular India

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ABSTRACT
We report molecular phylogenetic and dating analyses of snakes that include new mitochondrial and nuclear DNA sequence data for three species of the peninsular Indian endemic *Xylophis*. The results provide the first molecular genetic test of and support for the monophyly of *Xylophis*. Our phylogenetic results support the findings of a previous, taxonomically restricted phylogenomic analysis of ultraconserved nuclear sequences in recovering the fossorial *Xylophis* as the sister taxon of a clade comprising all three recognised extant genera of the molluscivoran and typically arboreal pareids. The split between *Xylophis* and 'pareids' is estimated to have occurred on a similar timescale to that between most (sub)families of extant snakes. Based on phylogenetic relationships, depth of molecular genetic and estimated temporal divergence, and on the external morphological and ecological distinctiveness of the two lineages, we classify *Xylophis* in a newly erected subfamily (Xylophiinae subfam. nov.) within Pareidae.

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Asia; classification; Pareidae; Pareinae; phylogenetics; *Xylophis*; taxonomy

Introduction

The caenophidian snake genus *Xylophis* Beddome, 1878 contains three currently recognised species of small fossorial snakes endemic to the southern part of the Western Ghats of peninsular India (Gower and Winkler 2007; Srinivasulu et al. 2014; Wallach et al. 2014). Morphological systematists have not settled on the phylogenetic relationships of *Xylophis* or of its corresponding suprageneric classification. For example, Underwood (1967) included *Xylophis* in his concept of Dipsadidae, a group comprising xenodermines, pareines, calamarines, sibynophiines, lycodontines, xenodontines and some then enigmatic Asian natricines (including the Sri Lankan *Aspidura* – to which at least superficial similarities to *Xylophis* were noted by Gans and Fetcho 1982; Dowling and Pinou 2003; Gower and Winkler 2007; Simões et al. 2016). Since Underwood's (1967) work, *Xylophis* has been considered to be a xenodermid (or xenodermatid/xenodematine/xenodermine depending on authority) (McDowell 1987; Wallach 1998; Zaher 1999;

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Vidal 2002; Lawson et al. 2005; Cundall and Irish 2008, p. 556, 573; Zaher et al. 2009; Pyron et al. 2013; Dowling and Pinou 2003, see also Underwood 1967, p. 98), an elapoid *incertae sedis* (Wallach et al. 2014), and a colubrine (Cundall and Irish 2008, p. 645).

Until recently, there were no molecular systematic data available for *Xylophis*. Simões et al. (2016) published sequences of three visual opsin genes for *X. captaini*. Although noted as not being neutral phylogenetic markers, Simões et al. (2016) reported various phylogenetic results for *X. captaini* for each of these three genes in isolation: for locus *rh1, X. captaini* was recovered as sister to the pareid *Pareas monticola*, with this clade being sister to all other sampled non-viperid colubroids; for locus *sws1, X. captaini* was recovered as sister to all other sampled colubroids (*P. monticola* was not sampled for this gene); for locus *lws, X. captaini* was recovered as sister to *Amphiesma stolata* within natricine colubrids (again, *P. monticola* was not sampled for this gene). Although the sister relationships with *P. monticola* (for *rh1*) and with *A. stolata* (for *lws*) were well supported, most of the deeper internal branches throughout these trees were not well resolved.

Ruane and Austin (2017) sampled one historical museum specimen of *Xylophis stenorhynchus* in an application of ultraconserved element loci in snake phylogenomics, combining their historical sampling with modern snake sample data from Streicher and Wiens (2016). Ruane and Austin's sampling was sparse (17 species of caenophidians, including one xenodermid and no natricines) but *X. stenorhynchus* was recovered as the well-supported sister taxon to the single sampled pareid, *Pareas hamptoni*, and the number of ultraconserved elements generated for *X. stenorhynchus* (2546 loci) was on par with modern samples (see Table 1, Ruane and Austin 2017). As currently conceived, pareids comprise ca. 20 nocturnal, molluscivorous, non-fossorial species (classified in three genera: *Pareas* Wagler, 1830; *Aplopeltura* Duméril, 1853; *Asthenodipsas* Peters, 1864) restricted to east and south-east Asia, with two species (*P. monticola* (Cantor, 1839) and *P. margaritophorus* (Jan, 1866)) extending into north-east India (Whitaker and Captain 2004; Uetz et al. 2018). Commenting on their somewhat unexpected phylogenetic result for *Xylophis*, Ruane and Austin (2017, p. 5) suggested that the phylogenetic relationships of this genus could be investigated more thoroughly by analysing a wider sample of snakes, including more species of *Xylophis*.

Here we report sequence data for 'standard' mitochondrial (mt) and nuclear (nu) phylogenetic markers for snakes for three species of *Xylophis* and include them in broadly taxonomically sampled phylogenetic analyses of extant snakes. These analyses provide the first molecular test of the monophyly of the genus, and the results support classification of *Xylophis* in a newly erected subfamily within Pareidae.

Material and methods

Classification and institutional abbreviations

We followed the family and subfamily classification used by Uetz et al. (2018), including the recently described subfamilies Ahaetuliinae Figueroa et al., 2016 (within Colubridae) and Cyclocorinae Weinell and Brown, 2018 (within Lamprophiidae). *Xylophis* tissues were sampled from vouchers deposited in the Bombay Natural History Society, Mumbai, India (BNHS), California Academy of Sciences, San Francisco, CA, USA (CAS), and Centre for Ecological Sciences, IISc, Bengaluru, India (CES).

Table 1. GenBank accession and voucher numbers for gene sequences used in molecular dating analysis.

	Species	Family (subfamily)	*cytb*	*16s*	*nd4*	*cmos*	*bdnf*	*rag1*
1	*Acrochordus javanicus*	Acrochordidae	–	AF512745	HM234055	HM234058	AY988036	HM234061
2	*Afrotyphlops punctatus*	Typhlopidae (Afrotyphlopinae)	–	–	–	–	GU902395	–
3	*Agkistrodon contortrix*	Viperidae (Crotalinae)	EU483383	AF156566	AF156577	–	EU402623	EU402833
4	*Ahaetulla pulverulenta*	Colubridae (Ahaetuliinae)	KC347454	KC347339	KC347512	KC347378	–	KC347416
5	*Anilius scytale*	Aniliidae	U69738	FJ755180	FJ755180	AF544722	EU402625	AY988072
6	*Anomochilus leonardi*	Cylindrophiidae+Anomochiliidae	–	AY953431	–	–	–	–
7	*Aparallactus capensis*	Lamprophiidae (Aparallactinae)	AY188006	AY188045	FJ404331	AY187967	–	–
8	*Aplopeltura boa*	Pareidae (Pareinae)	JF827673	AF544787	JF827650	JF827696	FJ433984	–
9	*Aspidura ceylonensis*	Colubridae (Natricinae)	KC347477	KC347361	KC347527	KC347400	–	KC347438
10	*Asthenodipsas malaccanus*	Pareidae (Pareinae)	KX660469	KX660197	KX660597	KX660336	–	–
11	*Azemiops feae*	Viperidae (Azemiopinae)	AY352747	AF057234	AY352808	AF544695	EU402628	EU402836
12	*Bitis nasicornis*	Viperidae (Viperinae)	DQ305457	AY188048	DQ305475	AY187970	–	KC330012
13	*Boa constrictor*	Boidae	AB177354	AB177354	AB177354	AF544676	KC330044	KC347423
14	*Boaedon fuliginosus*	Lamprophiidae (Lamprophiinae)	AF471060	AY188079	FJ404365	FJ404270	EU402646	EU402849
15	*Bothrolycus ater*	Lamprophiidae (Lamprophiinae)	AY612041	AY611859	AY611950	FJ404347	–	–
16	*Brachyophidium rhodogaster*	Uropeltidae	–	AY701023	–	–	–	–
17	*Buhoma depressiceps*	Lamprophiidae *incertae sedis*	AY612042	AY611860	–	AY611951	–	–
18	*Buhoma procterae*	Lamprophiidae *incertae sedis*	AY612001	AY611818	DQ486328	AY611910	–	–
19	*Bungarus fasciatus*	Elapidae	EU579523	EU579523	EU579523	AY058924	FJ433989	–
20	*Calabaria reinhardtii*	Calabariidae	AY099985	Z46494	–	AF544682	EU402631	EU402839
21	*Calamaria pavimentata*	Colubridae (Calamariinae)	AF471081	KX694624	–	AF471103	FJ434005	–
22	*Candoia carinata*	Candoiidae	AY099984	EU419850	–	AY099961	FJ433974	AY988065
23	*Cantoria violacea*	Homalopsidae	EF395897	KX694627	EF395922	–	–	–
24	*Casarea dussumieri*	Bolyeridae	U69755	AF544827	–	AF544731	EU402632	EU402840
25	*Charina bottae*	Charinidae (Charininae)	AY099986	AF544816	AF302959	AY099971	FJ433978	AY988076
26	*Chilabothrus striatus*	Boidae	–	–	KC329966	KC329991	KC330056	KC330027
27	*Contia tenuis*	Colubridae (Dipsadinae)	GU112384	AY577030	GU112419	AF471134	GU112346	–
28	*Corallus annulatus*	Boidae	KC750012	–	KC750018	KC750007	JX576167	KC750047
29	*Cyclocorus nuchalis*	Lamprophiidae (Cyclocorinae)	MG458754	–	–	MG458764	–	–
30	*Cyclocorus lineatus*	Lamprophiidae (Cyclocorinae)	MG458750	–	–	MG458759	–	–
31	*Cylindrophis maculatus*	Cylindrophiidae+Anomochilidae	KC347460	KC347355	KC347494	KC347395	–	KC347433

(*Continued*)

Table 1. (Continued).

	Species	Family (subfamily)	*cytb*	*16s*	*nd4*	*cmos*	*bdnf*	*rag1*
32	*Cylindrophis ruffus*	Cylindrophiidae+Anomochilidae	AB179619	AB179619	AB179619	AF471133	AY988037	AY988071
33	*Daboia russellii*	Viperidae (Viperinae)	EU913478	EU913478	EU913478	AF471156	EU402636	EU402843
34	*Ditypophis* sp.	Lamprophiidae	_	_	_	_	JQ073079	JQ073200
35	*Epicrates cenchria*	Boidae	HQ399501	_	KC329975	KC330008	KC330073	_
36	*Eryx colubrinus*	Erycidae	U69811	AF544819	_	AF544716	EU402639	DQ465571
37	*Eryx conicus*	Erycidae	GQ225658	AF512743	GQ225672	_	_	AY988074
38	*Eunectes notaeus*	Boidae	HQ399499	AM236347	KC329978	HQ399536	KC330076	HQ399516
39	*Farancia abacura*	Colubridae (Dipsadinae)	U69832	Z46491	U49307	AF471141	_	KR814740
40	*Gerrhopilus mirus*	Gerrhopilidae	AM236345	AM236345	AM236345	_	GU902394	_
41	*Grayia ornata*	Colubridae (Grayiinae)	_	AF158503	AF544663	AF544684	FJ434002	_
42	*Grayia smythii*	Colubridae (Grayiinae)	DQ112077	_	DQ112080	_	_	_
43	*Hologerrhum philippinum*	Lamprophiidae (Cyclocorinae)	MG458758	_	_	MG458766	_	_
44	*Homoroselaps lacteus*	Lamprophiidae (Atractaspidinae)	AY611992	AY611809	FJ404338	AY611901	JQ599029	_
45	*Indotyphlops braminus*	Typhlopidae (Asiatyphlopinae II)	DQ343649	_	_	AF544717	FJ433959	_
46	*Liasis mackloti*	Pythonidae	U69839	EF545051	_	AF544726	FJ433970	_
47	*Liopholidophis sexlineatus*	Lamprophiidae (Pseudoxyrhophiinae)	DQ979985	AY188063	FJ404373	AY187985	_	_
48	*Liotyphlops albirostris*	Anomalepididae	AF544672	AF366762	_	AF544727	EU402650	EU402853
49	*Loxocemus bicolor*	Loxocemidae	AY099993	AF544828	_	AY444035	EU402651	_
50	*Madatyphlops andasibensis*	Typhlopidae (Madatyphlopinae)	_	_	_	_	GU902453	JQ073249
51	*Malayopython reticulatus*	Pythonidae	U69860	EF545062	_	AF544675	FJ433969	EU624119
52	*Melanophidium punctatum*	Uropeltidae	_	AY701024	_	_	_	_
53	*Micrelaps bicoloratus*	Lamprophiidae (Aparallactinae)	DQ486349	_	_	DQ486173	_	_
54	*Mimophis mahfalensis*	Lamprophiidae (Psammophiinae)	DQ486461	AY188070	_	AY187992	JQ073081	_
55	*Morelia viridis*	Pythonidae	EF545098	EF545048	_	_	_	_
56	*Naja kaouthia*	Elapidae	FR693728	GQ359757	EU624209	AY058938	EU402654	EU402857
57	*Namibiana occidentalis*	Leptotyphlopidae (Leptotyphlopinae)	_	GQ469251	_	GQ469074	GQ469189	_
58	*Oligodon arnensis*	Colubridae (Colubrinae)	KC347464	KC347365	KC347504	KC347404	_	KC347442
59	*Oxyrhabdium leporinum*	Lamprophiidae (Cyclocorinae)	AF471029	_	_	DQ112081	_	_
60	*Oxyuranus scutellatus*	Elapidae	EU547051	EU547149	EF210827	EU546916	_	_
61	*Pareas carinatus*	Pareidae (Pareinae)	JF827677	AF544802	JF827653	JF827702	FJ433985	_

(*Continued*)

Table 1. (Continued).

	Species	Family (subfamily)	*cytb*	*16s*	*nd4*	*cmos*	*bdnf*	*rag1*
62	*Prosymna janii*	Lamprophiidae (Prosymninae)	FJ404319	FJ404222	FJ404389	FJ404293	_	_
63	*Prosymna visseri*	Lamprophiidae (Prosymninae)	AY188033	AY188072	_	AY187994	_	_
64	*Pseudaspis cana*	Lamprophiidae (Pseudaspidinae)	AY612080	AY611898	DQ486319	DQ486167	_	_
65	*Pseudoxenodon karlschmidti*	Colubridae (Pseudoxenodontinae)	AF471080	JF697330	_	AF471102	JQ599045	_
66	*Python bivittatus*	Pythonidae	JX401131	KF010492	_	AF435016	XM7433022	_
67	*Rena humilis*	Leptotyphlopidae (Epictinae)	AY099991	AB079597	AB079597	AY099979	_	_
68	*Rhinophis drummondhayi*	Uropeltidae	AF544673	AY701028	_	AF544719	FJ433966	_
69	*Sanzinia madagascariensis*	Sanziniidae	U69866	AY336066	_	EU403580	AY988033	AY988067
70	*Sibynophis subpunctatus*	Colubridae (Sibynophiinae)	KC347471	KC347373	KC347516	KC347411	_	KC347449
71	*Tropidophis feicki*	Tropidophiidae	KF811124	AF512733	_	KF811110	KF811074	_
72	*Typhlops jamaicensis*	Typhlopidae (Typhlopinae)	KF993259	AF366764	_	AF544733	EU402664	EU402866
73	*Ungaliophis continentalis*	Charinidae (Ungaliophiinae)	U69870	AF544833	_	AF544724	EU402665	EU402867
74	*Xenodermus javanicus*	Xenodermidae	_	AF544810	U49320	AF544711	EU402667	EU402869
75	*Xenopeltis unicolor*	Xenopeltidae	AB179620	AB179620	AB179620	AF544689	EU402668	DQ465564
76	*Xenophidion schaeferi*	Xenophidiidae	AY574279	_	_	_	_	_
77	*Xenotyphlops grandidieri*	Xenotyphlopidae	KF770844	_	_	_	GU902457	_
78	*Xerotyphlops vermicularis*	Typhlopidae (Asiatyphlopinae I)	JQ910544	_	_	_	GU902397	_
79	*Xylophis perroteti*	Pareidae (Xylophiinae subfam. nov.)	_	MK340908*	MK340910*	MK344193*	MK344197*	MK340913*
80	*Xylophis stenorhynchus*	Pareidae (Xylophiinae subfam. nov.)	MK340915	MK340907	MK340911	MK344194	MK344198	_
81	*Xylophis captaini*	Pareidae (Xylophiinae subfam. nov.)	MK340914*	MK340909*	MK340912	MK344195	MK344196	_

*indicates data used in the ‘map to reference’ analyses to identify homologous sequences from UCE data for the historical sample of *X. stenorhynchus*.

Molecular data and phylogenetic analysis

We generated DNA sequence data for two specimens from freshly collected tissue, a *Xylophis perroteti* from the Nilgiris (CESG 2016b) and a *X. captaini* from the type locality Kannam, Kottayam District (BNHS 3376). Genomic DNA was extracted from liver tissue samples stored in absolute ethanol at −20°C. DNeasy (Qiagen™, Valencia, California, USA) blood and tissue kits were used to extract DNA. We amplified partial sequences of three mitochondrial (mt) genes and three nuclear (nu) genes. The mt genes are 16S rRNA (*16s*), cytochrome b (*cytb*) and NADH dehydrogenase subunit 4 (*nd4*), and the nu markers are the recombination activating gene 1 (*rag1*), oocyte maturation factor (*cmos*), and brain-derived neurotrophic factor (*bdnf*). DNA PCR amplification and Sanger sequencing used previously reported primers (Palumbi et al. 1991; Arévalo et al. 1994; Palumbi 1996; Parkinson et al. 2000; Noonan and Chippindale 2006; Wiens et al. 2008).

We attempted to extract homologous sequences for our phylogenetic markers from the unfiltered, unassembled, raw sequence reads that were generated during targeted sequencing of ultra-conserved elements (UCEs) for a historical specimen (CAS 17199) of *Xylophis stenorhynchus* from Ruane and Austin (2017). These data comprised 32,236,948 reads each for read 1 and read 2. Although none of the loci used in this study was targeted by the UCE probe kit (MYbaits tetrapod 5K kit, which targets 5060 UCEs from amniotes: Faircloth et al. 2012) used by Ruane and Austin (2017), we considered it possible that the loci of interest were sequenced as 'bycatch' during the high-throughput sequencing, particularly for mtDNA genes due to the high number of copies of these loci in genomic DNA.

Using the program Geneious (Kearse et al. 2012) Ruane and Austin's (2017) unfiltered reads for *X. stenorhynchus* were mapped to each of the newly generated *X. captaini* and *X. perroteti* Sanger sequences for *16s, cytb, nd4, cmos, rag1* and *bdnf* (see Table 1). This was done using the Geneious align/assemble option 'map to reference', with the modern sample serving as the reference for the unfiltered *X. stenorhynchus* reads; sensitivity was set to medium-low with up to five iterations. Where successful, the resulting mapped reads of *X. stenorhynchus* were combined into consensus sequences for each marker to be included in subsequent analyses.

We constructed a molecular dataset for 507 leaves (500 snakes + 7 non-snake squamates; 493 + 7 species, respectively) including 14 of the 20 currently recognised species of pareids. Data coverage for each of the genes in the dataset are as follows: *cytb* 80.9%, *16s* 68.1%, *nd4* 58.8%, *cmos* 71.6%, *bdnf* 30.4% and *rag1* 13.6%. GenBank accession numbers for all sequences included in our phylogenetic and dating analyses are presented in Table S1. Alignments per gene were carried out in MEGA 5 (Tamura et al. 2011) using the ClustalW algorithm with default parameters and are available online from the Natural History Museum data portal (http://data.nhm.ac.uk/dataset/deepak-xylophis). Uncorrected p-distances and Kimura 2-parameter (Kimura 1980) distances were calculated using MEGA 5. Phylogenetic analysis was implemented in the program RAxML v7.4.2 GUI (Stamatakis 2006; Silvestro and Michalak 2012) with the six gene concatenated dataset. This dataset was 4557 bp long and was partitioned by gene and by codon, a total of 11 partitions (see Table S2), determined as the best-fit scheme using ParitionFinder 1.2 (Lanfear et al. 2012). We used GTRGAMMA model in RAxML which is recommended over the GTR +G+ I because the 25 rate categories account for potentially invariant sites (Stamatakis 2006), as was also implemented in other large-scale snake molecular phylogenetic analyses (Pyron et al. 2011; Zaher et al. 2012; Figueroa et al. 2016).

Divergence times were estimated using a subset of taxa for the same genes, with a dataset containing 81 snake species including representatives of all extant subfamilies of alethinophidian snakes and all extant families of scolecophidians (Table 1). These data were newly aligned (using the methods outlined above, alignment available at: http://data.nhm.ac.uk/dataset/deepak-xylophis), producing a dataset 4504 bp. PartitionFinder 1.2 (Lanfear et al. 2012) was used to identify the best-fitting partition scheme and model(s) of sequence evolution according to the Bayesian information criterion (BIC) using the default greedy algorithm with linked branch lengths (see Table S3 for partitions and models). We explored the sensitivity of our phylogenetic results to our selected (ClustalW) alignment method by alternatively aligning the *16s* data also with MUSCLE (Edgar 2004), as well as using Gblocks v0.91b (Castresana 2000) to identify and remove ambiguously aligned sites from the ClustalW alignment using the 'less stringent' option. These alternative approaches to the *16s* data did not notably change the topology or support values in optimal RAxML (data partitioned by gene and by codon) trees for the concatenated data (Figure S1).

Divergence times were estimated using a Bayesian relaxed uncorrelated lognormal clock model implemented in BEAST 1.8.2 (Drummond et al. 2012). We used fossil calibrations recommended by Head (2015) and Head et al. (2016) to date minimum ages of five divergences: (1) oldest divergence within crown Alethinophidia based on *Haasiophis terrasanctus* Tchernov et al. 2000; minimum age 93.9 Ma, soft maximum 100.5 Ma; (2) oldest divergence between non-xenodermid colubroids and their closest living relative (Xenodermidae in our tree), based on *Procerophis sahnii* Rage et al. 2008; minimum 50.5 Ma, soft maximum 72.1 Ma; (3) divergence between Boinae and its sister taxon (Erycinae + Candoiinae in our tree) based on *Titanoboa cerrejonensis* Head et al. 2009; minimum 58 Ma, soft maximum 64 Ma; (4) divergence between Viperinae and Crotalinae based on *Vipera aspis* complex (Szyndlar and Rage 1999); minimum 20.0 Ma, soft maximum 23.8 Ma; and (5) oldest divergence within elapids based on *Naja romani* (Hoffstetter, 1939); minimum age 17 Ma, soft maximum 60 Ma (see Table S4 for exact values applied to each calibration prior). Analyses used random starting trees, with clock and tree models linked across partitions. Two independent analyses were run for 600,000,000 generations sampling every 5000 trees, the effective sample size (ESS) values were evaluated using Tracer 1.6 (Rambaut et al. 2014). The prior distribution for all fossil calibrations was set to lognormal.

Results

Phylogenetic inference

Mapping the Sanger sequencing data for *Xylophis captaini* and *X. perroteti* against the unfiltered *X. stenorhynchus* high-throughput sequence reads resulted in potentially homologous consensus sequences for the latter for *16s* (441 bp; 103 reads assembled), *cytb* (513 bp; 60 reads), *nd4* (328 bp; 61 reads), *cmos* (169 bp; 6 reads), and bdnf (91 bp; 4 reads). These consensus sequences are reported in Table 1. These sequences were similar to those of *X. perroteti* (uncorrected p-distances 0.078 for *16s*; 0.204 for *nd4*; 0.037 for *cmos*; 0.045 for *bdnf*) and *X. captaini* (uncorrected p-distances 0.114 for *16s*; 0.221 for *cytb*).

Our ML phylogenetic analysis provides strong support for the monophyly of *Xylophis* and for the sister group relationship between *Xylophis* and a clade comprising the pareids *Pareas, Aplopeltura* and *Asthenodipsas* (Figures 1 and S2). Within the latter clade, *Pareas* and *Asthenodipsas* are strongly supported as monophyletic, with the former being sister to the monotypic *Aplopeltura*. The *Xylophis, Pareas, Aplopeltura* and *Asthenodipsas* clade (here considered to comprise Pareidae) is recovered as a member of a lineage comprising all colubroids except Xenodermidae. Although there is strong signal for pareids lying outside a group comprising most other colubroids, the relationships among Pareidae, Viperidae and all other non-xenodermid colubroids are not clearly resolved by our analyses. Uncorrected p-distances between *Xylophis* and the other three pareid genera for the sampled genes are 0.07–0.12 (*16s*), 0.27–0.38 (*cytb*), 0.21–0.25 (*nd4*), 0.03–0.1 (*cmos*) and 0.03 (*bdnf*). Pairwise distances between recognised colubroid families and between intrafamilial subfamilies for *cmos* and *bdnf* (reported in Tables S5–S8) are summarised in Figure 2. The molecular dating analysis recovers an estimated minimum divergence of 55–35 Ma between *Xylophis* and its sister taxon (*Pareas, Aplopeltura* and *Asthenodipsas*) (Figure 3). Rerunning the dating analysis but excluding third codon positions of the mitochondrial genes *cytb* and *nd4* did not notably alter the results for most divergences (including that for *Xylophis* versus its sister) in terms of relative ages (Figure S3), with estimated divergence dates for the two analytical treatments being strongly correlated (Figure S4).

Systematics

Based on the well-supported inferred phylogenetic relationships of *Xylophis* and divergence from its extant sister lineage, we refer the genus to the family Pareidae, and re-define the latter phylogenetically as all snakes more closely related to *Pareas carinatus* Wagler, 1830 than to *Xenodermus javanicus* Rheinhardt, 1836, *Vipera aspis* (Linnaeus, 1758) or *Homalopsis buccatus* (Linnaeus, 1758). Given the molecular genetic and phenotypic distinctiveness of the two lineages comprising the basal split within Pareidae, we classify *Pareas, Aplopeltura* and *Asthenodipsas* within the subfamily Pareinae (defined phylogenetically as all snakes more closely related to *Pareas carinatus* Wagler, 1830 than to *Xylophis perroteti* Duméril, Bibron and Duméril, 1854) and we erect a new subfamily for *Xylophis*:

DIAPSIDA Osborn, 1903
Superorder **LEPIDOSAURIA** Haeckel, 1866
Order **SQUAMATA** Oppel, 1811
Suborder **SERPENTES** Linnaeus, 1758
Infraorder **CAENOPHIDIA** Hoffstetter, 1939
Superfamily **COLUBROIDEA** Oppel, 1811
Family **PAREIDAE** Romer, 1956
Subfamily **Xylophiinae** subfam. nov.

Type genus

Xylophis Beddome, 1878

Content

A single genus with three currently recognised species: *X. stenorhynchus* (Günther, 1875); *X. perroteti* Duméril, Bibron and Duméril, 1854; *X. captaini* Gower and Winkler, 2007. *Xylophis indicus* Beddome, 1878 has been considered a synonym of *X. stenorhynchus* (e.g. Smith 1943; Wallach et al. 2014) but might also be valid (Gower and Winkler 2007). *Xylophis perroteti* includes the synonyms *Rhabdosoma microcephalum* Günther, 1858 (e.g. Smith 1943; Wallach et al. 2014).

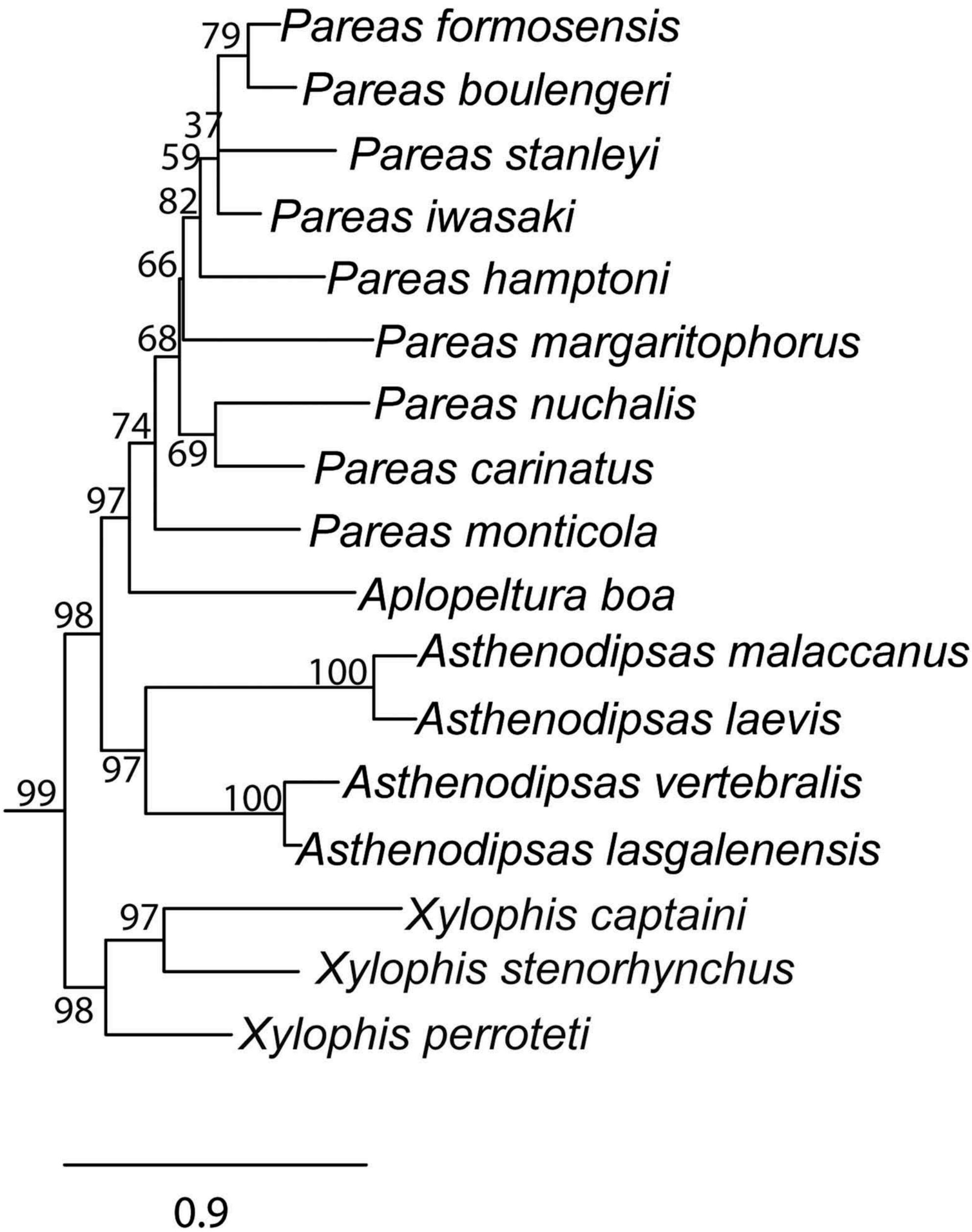

Figure 1. Pruned ML tree showing bootstrap support for the relationships of species in the family Pareidae. See Appendix 4 for the complete ML phylogeny including 507 taxa (493 species of snakes and seven non-snake squamates).

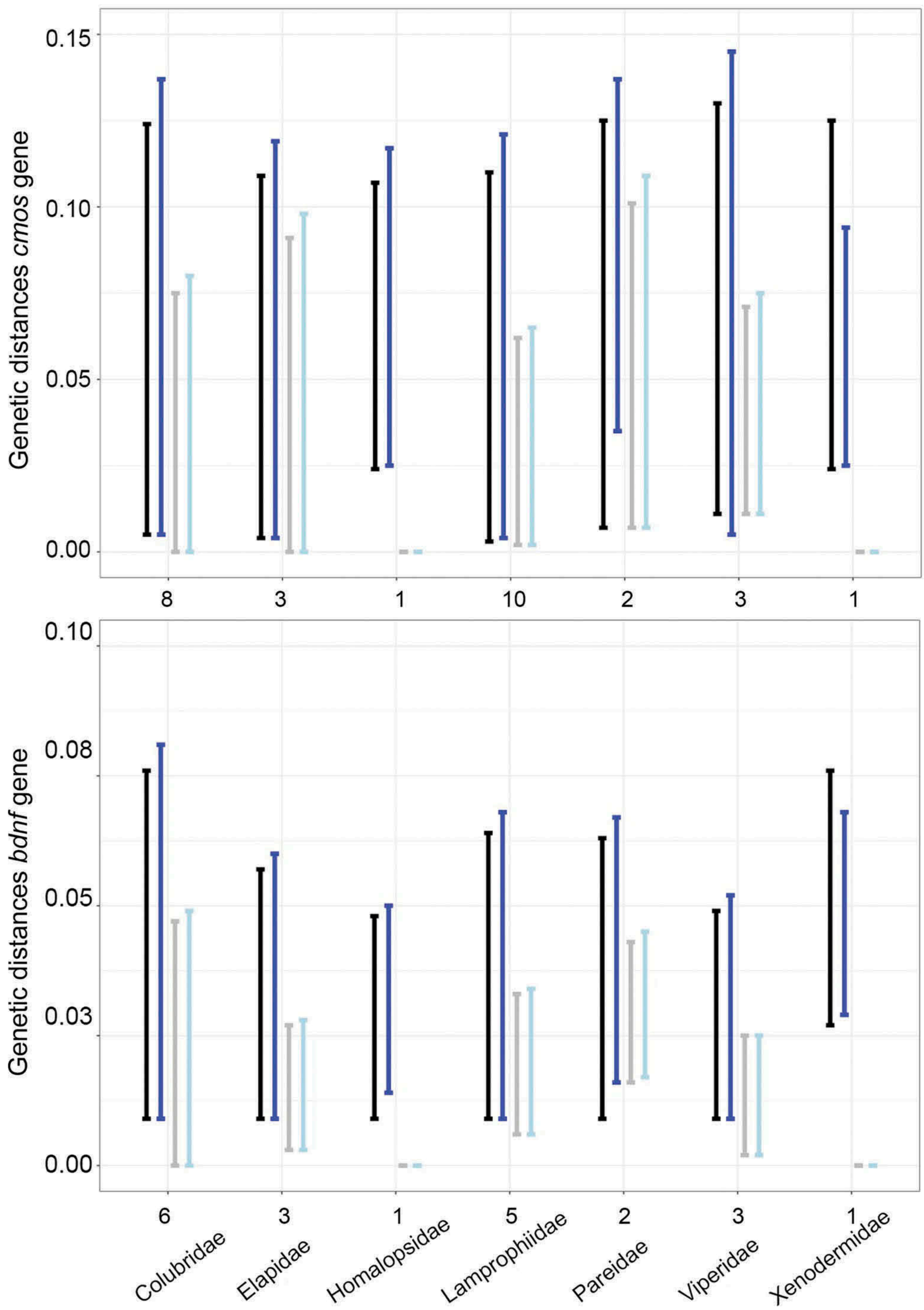

Figure 2. Ranges of uncorrected p-distances (black and grey) and K2P distances (dark blue and light blue) for between-family (dark bars) and within-family (light bars) comparisons of snakes in the superfamily Colubroidea. Pareidae here includes Pareinae and Xylophiinae subfam. nov. Numbers on the x-axis denotes sample size of subfamilies under each family.

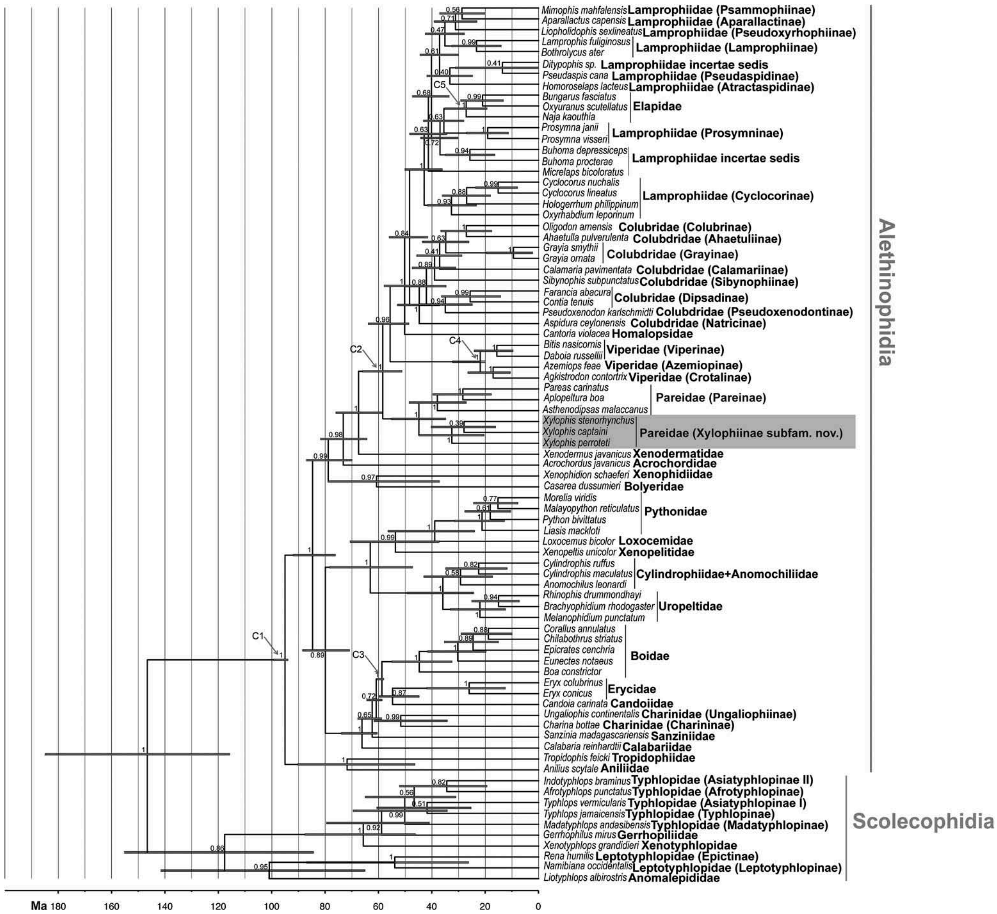

Figure 3. BEAST chronogram generated using concatenated-gene for all families and subfamilies of snakes. Numbers at internal branches indicate posterior probabilities. Error bars indicate 95% highest posterior densities for node ages. Nodes C1–C5 are the five calibrated nodes.

Phylogenetic definition

All snakes more closely related to *Xylophis perroteti* than to *Pareas carinatus* Wagler, 1830.

Diagnosis

Colubroid snakes with first (anteriormost) three pairs of infralabial shields reduced to narrow strips, together much smaller than large pair of anterior chin (genial) shields.

Distribution

The Western Ghats region of peninsular India. *Xylophis* is thus far known only from the southern part of the Western Ghats, in the states of Kerala and Tamil Nadu (Figure 4). Species of the genus have been recorded from close to sea level (*Xylophis captaini*: Gower and Winkler 2007) to at least 2000 m (*X. perroteti*: Srinivasulu et al. 2014).

Discussion

Age of divergence (whether absolute or relative) between sister lineages has sometimes been applied as a secondary criterion in recognition of suprageneric taxa (e.g. Wilkinson et al. 2011), including the formal naming of new families (e.g. Kamei et al. 2012). Although the general application of such a criterion has been cautioned against (e.g. Vences et al. 2013; Frost 2017: see 'comments on taxonomy related to version 5.6'), we see some merit in using estimated divergence age cautiously as an additional guide alongside phylogenetic relationships and extent of phenotypic and raw molecular genetic divergence. In this case, we take some comfort in naming a new subfamily given that the estimated age of divergence of Xylophiinae from Pareinae is comparable to that between sister pairs of other snake (sub)families (Figures 3 and S3).

Although xylophiines and pareines are phenotypically disparate superficially, the anatomy and anatomical diversity of these two lineages (and of other major lineages of colubroids) is insufficiently known to yet rule out the identification of unambiguous synapomorphies for Pareidae. Although classifying *Xylophis* as a xenodermine on the basis of skull, head muscle and hemipenis features, McDowell (1987, p. 35–36) also drew attention between at least *X. perroteti* and pareines (and calamariines) in terms of posteriorly extensive kidneys and a distinct rectal caecum. The morphology of *Xylophis* is poorly studied and further work in the light of the renewed interest in its phylogenetic relationships seems warranted.

The evolutionary divergence between xylophiines and pareines resulted in sister clades with markedly differing distributions, morphologies and ecologies. Although both lineages comprise small to moderately sized predators of invertebrates, xylophiines are small-headed, small-eyed, fossorial, relative generalist or opportunistic predators (Kumar and Kannan 2017) restricted to the southern part of the Western Ghats of peninsular India, while pareines are relatively larger-headed (head greater in girth than anterior of body), large-eyed, surface dwelling (often arboreal) specialist molluscivores (Cundall and Greene 2000) restricted almost entirely to east and south-east Asia (also extending into north-east India; Figure 4). Given that the Indian subcontinent (part of Gondwana) did not accrete with the rest of Asia until ca. 55 Ma (Patriat and Achache 1984), our estimated divergence between xylophiines and pareines (55–35 Ma) is consistent with dispersal of either a peninsular Indian ancestor into east/south-east Asia or *vice versa*. This hypothesis is a little more parsimonious than one invoking a widespread ancestral pareid lineage followed by spatially exclusive extinctions of xylophiines (in east and south-east Asia) and pareines (in peninsular India). However, that Pareidae, Xenodermidae and Acrochordidae are all Asian and that they might comprise a paraphyletic assemblage lying successively outside of a clade (= Endoglyptodonta of Zaher et al. 2009) comprising Viperidae, Elapidae, Colubridae, and Lamprophiidae (e.g. Vidal et al. 2007; Zaher et al. 2009; Grazziotin et al. 2012; Pyron et al. 2013; Figueroa et al. 2016) is more supportive of an Asian (rather than Gondwanan) origin of Pareidae, and thus of a dispersal of the ancestor of the Xylophiinae lineage into peninsular India from east or south-east Asia rather than *vice versa*. Resolution of the phylogenetic position of the north-east Indian *Pareas moniticola* and *P. margaritophorus* might usefully inform the question of the historical biogeography of Pareidae (or at least of Pareinae).

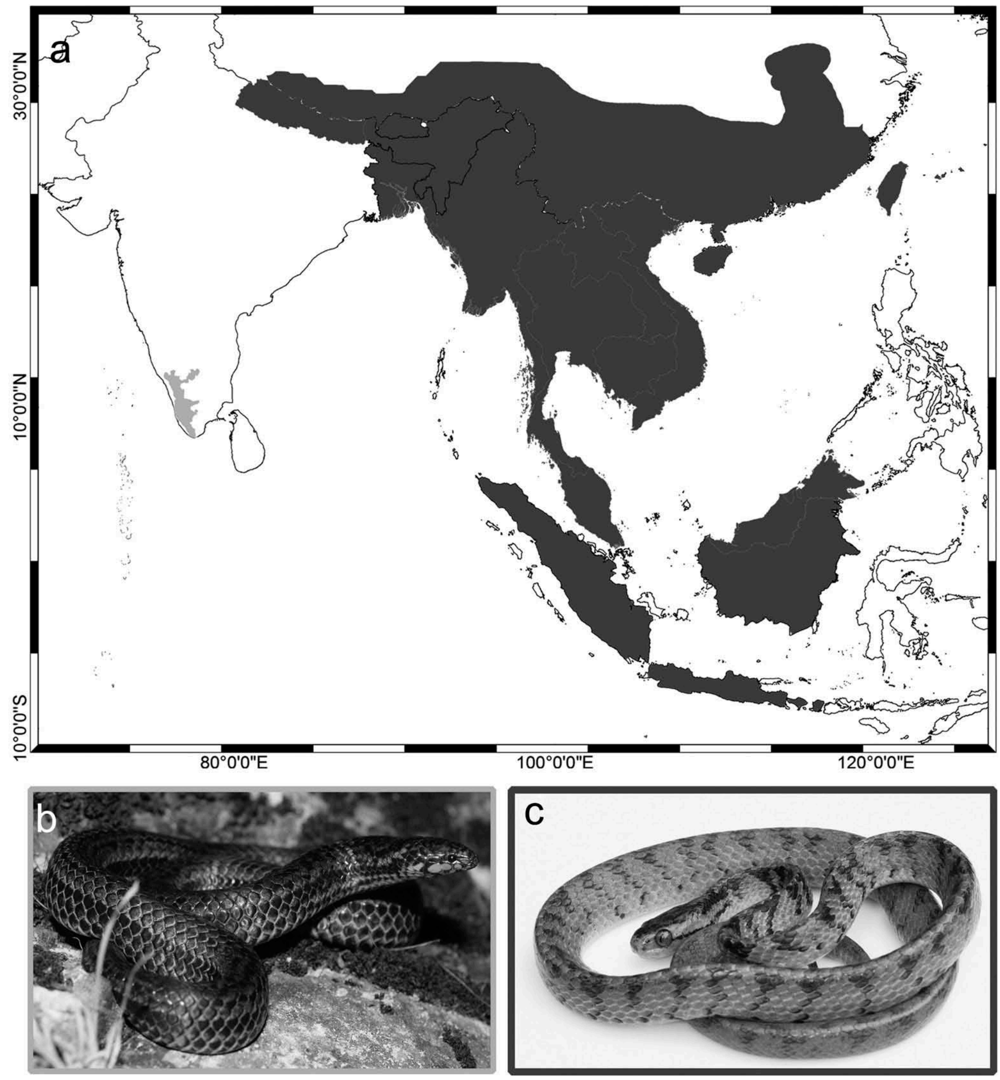

Figure 4. (a) Geographic distribution of Xylophiinae subfam. nov. (green) and approximate distribution of subfamily Pareinae (blue). Photographs show representative taxa of the two subfamilies within Pareidae: (b) *Xylophis perroteti* from Nilgiris, Tamil Nadu, India (Photo: Achyuthan N. Srikanthan); (c) *Pareas monticola* from Barail, Assam, India (Photo: V. Deepak). Approximate distribution drawn based on locations provided in Srinivasulu et al. (2014) and Wallach et al. (2014).

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Disclosure statement

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Geolocation Information

Study Area (box): 8.65000°N, 76.95000°E to 11.31198°N, 76.58653°E

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