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Journal of Natural History

ISSN: 0022-2933 (Print) 1464-5262 (Online) Journal homepage: https://www.tandfonline.com/loi/tnah20

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To cite this article: V. Deepak, Sara Ruane & David J. Gower (2018) A new subfamily of fossorial colubroid snakes from the Western Ghats of peninsular India, Journal of Natural History, 52:45-46, 2919-2934, DOI: <u>10.1080/00222933.2018.1557756</u>

To link to this article: https://doi.org/10.1080/00222933.2018.1557756

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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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ARTICLE HISTORY

Received 25 October 2018 Accepted 26 November 2018

KEYWORDS

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;

B Supplemental material for this article can be accessed here

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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).

I able	able 1. Genbank accession and voucher	ucher numbers for gene sequences used in molecular dating analysis.	s used in mole	cular dating ar	ialysis.			
	Species	Family (subfamily)	cytb	165	nd4	cmos	bdnf	rag1
-	Acrochordus javanicus	Acrochordidae	I	AF512745	HM234055	HM234058	AY988036	HM234061
2	Afrotyphlops punctatus	Typhlopidae (Afrotyphlopinae)	I	I	I	I	GU902395	I
m	Agkistrodon contortrix	Viperidae (Crotalinae)	EU483383	AF156566	AF156577	Ι	EU402623	EU402833
4	Ahaetulla pulverulenta	Colubridae (Ahaetuliinae)	KC347454	KC347339	KC347512	KC347378	I	KC347416
Ŝ	Anilius scytale	Aniliidae	U69738	FJ755180	FJ755180	AF544722	EU402625	AY988072
9	Anomochilus leonardi	Cylindrophiidae+Anomochiliidae	I	AY953431	I	I	I	I
7	Aparallactus capensis	Lamprophiidae (Aparallactinae)	AY188006	AY188045	FJ404331	AY187967	I	I
8	Aplopeltura boa	Pareidae (Pareinae)	JF827673	AF544787	JF827650	JF827696	FJ433984	I
6	Aspidura ceylonensis	Colubridae (Natricinae)	KC347477	KC347361	KC347527	KC347400	I	KC347438
10	Asthenodipsas malaccanus	Pareidae (Pareinae)	KX660469	KX660197	KX660597	KX660336	I	I
11	Azemiops feae	Viperidae (Azemiopinae)	AY352747	AF057234	AY352808	AF544695	EU402628	EU402836
12	Bitis nasicornis	Viperidae (Viperinae)	DQ305457	AY188048	DQ305475	AY187970	I	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	I	I
16	Brachyophidium rhodogaster	Uropeltidae	I	AY701023	I	I	I	I
17	Buhoma depressiceps	Lamprophiidae <i>incertae sedis</i>	AY612042	AY611860	I	AY611951	I	I
18	Buhoma procterae	Lamprophiidae <i>incertae sedis</i>	AY612001	AY611818	DQ486328	AY611910	I	I
19	Bungarus fasciatus	Elapidae	EU579523	EU579523	EU579523	AY058924	FJ433989	I
20	Calabaria reinhardtii	Calabariidae	AY099985	Z46494	I	AF544682	EU402631	EU402839
21	Calamaria pavimentata	Colubridae (Calamariinae)	AF471081	KX694624	I	AF471103	FJ434005	I
22	Candoia carinata	Candoiidae	AY099984	EU419850	I	AY099961	FJ433974	AY988065
23	Cantoria violacea	Homalopsidae	EF395897	KX694627	EF395922	I	I	I
24	Casarea dussumieri	Bolyeridae	U69755	AF544827	I	AF544731	EU402632	EU402840
25	Charina bottae	Charinidae (Charininae)	AY099986	AF544816	AF302959	AY099971	FJ433978	AY988076
26	Chilabothrus striatus	Boidae	I	I	KC329966	KC329991	KC330056	KC330027
27	Contia tenuis	Colubridae (Dipsadinae)	GU112384	AY577030	GU112419	AF471134	GU112346	I
28	Corallus annulatus	Boidae	KC750012	I	KC750018	KC750007	JX576167	KC750047
29	Cyclocorus nuchalis	Lamprophiidae (Cyclocorinae)	MG458754	I	I	MG458764	I	I
30	Cyclocorus lineatus	Lamprophiidae (Cyclocorinae)	MG458750	I	I	MG458759	I	I
31	Cylindrophis maculatus	Cylindrophiidae+Anomochilidae	KC347460	KC347355	KC347494	KC347395	I	KC347433
								(Continued)

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

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ss Family (subfamily) orb 15 nd4 cmos ss Cylindrophildae+Anomochilidae AB179619 AB179619 AB179619 AF471135 st Cylindrophildae+Anomochilidae AB179619 AB179619 AF471156 a Dampophildae H03395501 5 KG325975 KG323008 a Boldae Uperidae Upstate U05831 AF47113 AF44716 a Boldae H03395501 5 KG325575 KG323008 AF544684 a Boldae U66831 AF54453 AF54463 AF54463 AF544716 a Boldae U69332 246491 U49307 AF471141 s Colubridae (Grayinae) U69332 246491 U49307 AF47114 s Colubridae (Grayinae) D011707 AF544703 AF54463 AF54463 a Gernholdae Colubridae (Grayinae) D011707 AF544717 AF544726 a Colubridae (Grayinae) D011707 AF54472	able	Table 1. (Continued).							
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Grayia smythiColubridae (Grayinae)DQ112077DQ112080Hologerhum philippinumLamprophilae (Grayinae)MG458758MG458758Homorselaps lacteusLamprophilae (Gyclocorinae)MG458758MG458758Homorselaps lacteusLamprophilae (Ariatyphlopinae II)DQ343649F1404338AY611901Liasis mackoliTyphopidae (Asiatyphlopinae II)DQ343649F544717AF544717Liasis mackoliPythonidaeLamprophilae (Ariatyphlopinae II)U69895AY188063F1404373AY187985Liotyphlops abrinusTyphopidae (Pseudoxyrhophinae)U699993AF544672AF544672AF544727AF544727Lostonenus bicolarLamprophilae (Madatyphlopinae)U69860EF54562AF544675AY144035Lostonenus bicolarLostonenus bicolarUropeltidaeMalayopython eticulatusNY18070AY744035Malayopython reticulatusPythonidae (Aparallactinae)DQ486349AY701024AF544575AF544575Malayopython reticulatusUropeltidaeDQ486349AY701024DQ486173Minophis mahfalensisLamprophilae (Psammophilane)DQ486349AY701024DQ486173Minophis mahfalensisLamprophilae (Psammophilae)DQ486349AY73629AY744035Malayopython reticulatusLamprophilae (Psammophilae)DG486349AY701024DQ486173MinophisMinophisDC486349AY701024DQ48503BU445373AY44035MinophisMinophisDC486349AY701024DQ48503AY44673 <td< td=""><td>41</td><td>Grayia ornata</td><td>Colubridae (Grayiinae)</td><td>I</td><td>AF158503</td><td>AF544663</td><td>AF544684</td><td>FJ434002</td><td>I</td></td<>	41	Grayia ornata	Colubridae (Grayiinae)	I	AF158503	AF544663	AF544684	FJ434002	I
Hologerthum philippinumLamprophildae (Sydocorinae)MG458758MG458758Hologerthum philippinumLamprophildae (Asiatyphilopinae II)D0343649T5404338AY611901Homoroselaps lacteusTypholpidae (Asiatyphilopinae II)D0343649EF55051AF544726ALippsis macklotiPythonidaeNonalea (Asiatyphilopinae II)D0343649EF545051AF544726ALippsis macklotiPythonidaeNonalea (Asiatyphilopinae)U69839EF545051AF544726ALippshologohis sexlineatusLamprophilaeNonalea (Pseudoxyrhophinae)U699998AY188063FJ404373AF544726ALiphyhlops andasibensisTypholpidaeNonadatyphilosAr784636AY641035AF54675AMadaypphon reticulatusPythonidaeNo09993AF5445062AF54675AF54675AMadayphilops andasibensisTypholpidae (Aparallactinae)U69850EF545062AY701024DQ486173Malayopython reticulatusUropetidaeNo09993AF54505AY701024DQ486173Mincelaps bicoloratusLamprophilae (Psammophilae)DQ486461AY187992AY187992Mincelaps bicoloratusLamprophilae (Leptotyphlopinae)DQ486461AY18709AY187992Mincelaps bicoloratusLamprophilae (Leptotyphlopinae)DQ486461AY18679AY187922Malayopython reticulatusPitophilaeColubrinaeEF545098AY18792Malayopython reticulatusLamprophilae (Leptotyphlopinae)DQ486461AY187922M	42	Grayia smythii	Colubridae (Grayiinae)	DQ112077	I	DQ112080	I	I	I
Homoroselaps lacteusLamprophildae (Atractaspidinae)AY611992AY611809FJ404338AY611901Indotyphlops braminusTyphlopidae (Atractaspidinae)AY611992AY611809FJ404338AY611901Liasis macklotiTyphlopidae (Asiatyphlopinae II)DQ343649EF545051AF544773AF544773Liaptyphlops alprostrisPythonidaeUs9839EF54562AF36562AY187985Liotyphlops albrostrisAnomalepididaeAr5944572AF544572AF544572AF544723Liotyphlops albrostrisLoxocemidaeNyphlopidae (Madatyphlopinae)U69860EF54562AY14035Madatyphlops andasibensisTyphlopidae (Madatyphlopinae)U59860EF545062AY44035A744035Madatyphlops andasibensisTyphlopidae (Madatyphlopinae)U69860EF545062AY701024A744035Madatyphismalayopython reticulatusPythonidaeDQ486461AY701024A744035A744035Mineelaps bicoloratusLamprophilae (Aparallactinae)DQ486461AY701024A77770124A744035Mineelaps bicoloratusLamprophilae (Psammophilinae)DQ486461AY701024A7187992A7187992Mineelaps bicoloratusLamprophilae (Leptotyphlopinae)DQ486349A701024A7187992A7187992Mineelaps bicoloratusElapidaeRe534288GQ469251Lo24209A70592A7187992Mineelaps bicoloratusElapidaeColubridae (Colubrinae)CO469251Lo24209A70592Monohis matrixLamprophilae (Colubrinae)<	43	Hologerrhum philippinum	Lamprophiidae (Cyclocorinae)	MG458758	I	I	MG458766		
Indotyphlops braminus Typhlopidae (Asiatyphlopinae II) DQ343649 EF545051 AF544717 I Liasis mackloti Pythonidae Lamprophildae D0979985 AY188063 FJ404373 AF544726 I Liotyphlops albrostris Lamprophildae Do979985 AY188063 FJ404373 AY187985 Liotyphlops albrostris Loxocemus bicolor Loxocemidae D0979985 AY188063 FJ404373 AY144035 Liotyphlops albrostris Nomalepididae Loxocemidae AY099993 AF54452 AF544725 F Madatyphlops andbrostris Typhlopidae Use860 EF545062 AY144035 AY144035 Madatyphlops andbrostris Uropeltidae Use860 EF545062 AY701024 AY144035 Malayopython reticulturs Uropeltidae Use860 EF545062 AY701024 AY187792 Minophis mahfalensis Dq4863461 AY18870 J AY187922 AY187992 Minophis mathfalensis Pythonidae Lamprophilae Dq4863461 AY18870 AY187922 Morelia viridis Elapidae Ay1464 KC347504 KC347504	4	Homoroselaps lacteus	Lamprophiidae (Atractaspidinae)	AY611992	AY611809	FJ404338	AY611901	JQ599029	I
Liasis macklotiPythonidaeU69839EF545051AF544726ILiopholidophis sextineatusLamprophildaePythonidaePog79985AY188063FJ404373AY187885Liotyphlops albirostrisAnomalepididaeD0979985AY188063FJ404373AY187985Liotyphlops albirostrisLoxocemus bicolorLoxocemidaeAF544672AF544672AF544672AF544675Loxocemus bicolorLoxocemidaeAr7009993AF544672AF544675AY440355Madatyphlops andasibensisTyphopidae (Madatyphlopiae)U69860EF545662AY744035AY444035Malayopython reticulatusPythonidaeU69860EF545062AY701024AY744035AMinophis mahfalensisD0486349AY78070AY78070AY187992AMinophis mahfalensisPythonidaePythonidaeEF545048AY187992AMorelia viridisPythonidaeEF545098EF545048AAY187922Morelia viridisPythonidaeColubrinae)DQ486461AY188070AY187992Morelia viridisPythonidaeEF545098EF545048AANamibiana occidentalisLeptotyphlopinae)ColubrinaeColubrinaeColubrinaeOsynthabdium leporinumLamprophilidae (Colubrinae)KC347464KC347504KC347404Osynthabdium leporinumLamprophilaeColubrinaeDO489251EU547791EU547794Osynthabdium leporinumLapotyphlopinae)ColubrinaeColubrinaeCO469251CA469	45	Indotyphlops braminus	Typhlopidae (Asiatyphlopinae II)	DQ343649	I	I	AF544717	FJ433959	I
Liopholidophis sextineatusLamprophidae(Pseudoxyrhophime)DQ979985AY183063FJ404373AY187985Liotyphlops albirostrisAnomalepididaeAnomalepididaeAr544672AF544727AF544727AF544727AF544727Liotyphlops albirostrisTypholidaeMadatyphlops and sizensisTypholidaeAr909993AF544672AF544675AMadatyphlops andasibensisTypholidaeU60860EF545062A744035AMalayopython reticulatusPythonidaeU69860EF545062AAMialoyopython reticulatusUropeltidaeU69860EF545062AAMinophis mahfalensisDQ486349AY18070AAY187922AMinophis mahfalensisPythonidaePythonidaeEF545048AY187923AY187922AMorelia viridisElapidaeLamprophilae (Psammophinae)DQ486349AY188700AY187922AMorelia viridisPythonidaeR693728G0359757EU624209AY058938ANamibiana occidentalisLeptotyphlopinae)KC347464KC347305KC347404COligodon armensisColubridae (Colubrinae)DC489251EU547734EU547734DOxyrhabdium leporinumLamprophildae (Colubrinae)KC347464KC347504KC347404COxyrhabdium leporinumLamprophildaeEU547051EU547734EU547734EU547734EU546916Oxyrhabdium leporinumLapotyphlopinaeEU5477051EU547761EU547764E0246916	46	Liasis mackloti	Pythonidae	U69839	EF545051	I	AF544726	FJ433970	I
Liotyphlops albirostris Anomalepididae AF544572 AF544727 AF544727 A Loxocemus bicolor Loxocemus bicolor Loxocemus bicolor Arya4035 AF544727 A Madaryphlops andosibensis Typhlopidae Madaryphlops AF54452 AF544672 AF544675 A Madaryphlops andosibensis Typhlopidae Wadaryphlops Ary101024 A Malayopython reticulatus Pythonidae Uropeltidae Uo9860 EF545062 AF544575 1 Malayopython reticulatus Uropeltidae Uropeltidae Uo9860 EF545062 AY18792 Minophi Minophi Minophi Ay18661 AY18600 AY18792 Morelia viridis Pythonidae Pythonidae R693728 G0359757 EU624209 AY058938	47	Liopholidophis sexlineatus	Lamprophiidae (Pseudoxyrhophiinae)	DQ979985	AY188063	FJ404373	AY187985	Ι	I
Loxocemus bicolor Loxocemidae AY09993 AF54428 AY44035 I Madatyphlops andasibensis Typhlopidae (Madatyphlopinae) U69860 EF545062 AY44035 I Malayopython reticulatus Pythonidae UsoB60 EF545062 AY701024 A Malayopython reticulatus Uropeltidae UsoB60 EF545062 AY701024 A Malayopitiam punctatum Uropeltidae UsoB6173 DQ486349 AY701024 A A Micrelaps bicoloratus Lamprophidae (Psammophiinae) DQ486349 AY701024 AY187992 AY187992 A Mimophis morelia viridis Pythonidae Psamophiinae) DQ486461 AY187923 A AY187923 A Morelia viridis Pythonidae Psamophiinae) DQ486461 AY187923 A <t< td=""><td>48</td><td>Liotyphlops albirostris</td><td>Anomalepididae</td><td>AF544672</td><td>AF366762</td><td>I</td><td>AF544727</td><td>EU402650</td><td>EU402853</td></t<>	48	Liotyphlops albirostris	Anomalepididae	AF544672	AF366762	I	AF544727	EU402650	EU402853
Madatyphlops andasibensis Typhlopidae (Madatyphlopinae) U69860 EF545062 AF544675 I Malayopython reticulatus Pythonidae Uropeltidae U69860 EF545062 AF544675 I Malayopython reticulatus Pythonidae Uropeltidae Usopstition Uropeltidae DQ486349 AY701024 DQ486173 Micrelaps bicolocatus Lamprophidae Ramophinae) DQ486349 AY187902 AY187992 Mimophis Pythonidae Psthonidae Pstanlactinae) DQ486461 AY188700 AY187992 Morein Pythonidae Elapidae Psthonidae Elapidae AY18709 AY187092 AY187992 Morein Elapidae R693728 GQ469251 AY18792 AY18792 AY187922 Morein Elapidae Pythonidae Elapidae KC347464 KC347365 KC347404 C Oligodon armensis Colubridae (Colubrinae) KC347464 KC347305 EU547149 EP510827 EU546916 Oxyrhabdium leporinum Elapidae EU547169 EU547149 EP510827 EU546916 C Oxyu	49	Loxocemus bicolor	Loxocemidae	AY099993	AF544828	I	AY444035	EU402651	I
Malayopython reticulatus Pythonidae U69860 EF545062 AF544675 I Melanophidium punctatum Uropeltidae Usettidae AY701024 AY70792 AY70792 AY707024 AY707025 AY707025 AY707025 AY707025 AY707025 AY707024 AY707024 AY707024 AY707024 AY707025 AY70725 AY70726 AY70726 AY70726 AY707404 AY70726 AY707404 AY707404 AY707404 AY707404 AY707404 AY707404 AY707404 AY707404 AY70404 AY70	50	Madatyphlops andasibensis	Typhlopidae (Madatyphlopinae)	I	I	I	I	GU902453	JQ073249
Melanophidium punctatum Uropeltidae Ay701024 Ay701024 Micrelaps bicoloratus Lamprophidae (Aparallactinae) DQ486349 - Ay7187992 Mircelaps bicoloratus Lamprophidae (Psammophinae) DQ486461 Ay188070 - Ay187992 Mircelaps bicoloratus Lamprophidae (Psammophinae) DQ486461 Ay188070 - Ay187992 Morelia viridis Pythonidae Ef545098 Ef545098 Ef545048 - Ay058938 - Naja kaouthia Elapidae Leptotyphlopinae) FR693728 GQ359757 EU624209 AY058938 - Numibiana occidentalis Leptotyphlopinae) KC347464 KC347365 KC347504 KC347404 Oligodon amensis Colubrinae) Af471029 -	51	Malayopython reticulatus	Pythonidae	U69860	EF545062	I	AF544675	FJ433969	EU624119
Micrelaps bicoloratus Lamprophildae (Aparallactinae) DQ486349 DQ486173 Mimophis mahfalensis Lamprophildae (Psammophinae) DQ486461 AY188070 AY187992 Morelia viridis Pythonidae Pythonidae EF545098 EF545048 AY187992 Morelia viridis Pythonidae Eapidae E7545098 EF545048 AY058938 Naja kaouthia Elapidae Eapidae Leptotyphlopidae (Leptotyphlopinae) GQ469074 0 Oligodon amensis Colubridae (Colubrinae) AF471029 KC347464 KC347365 KC347404 Oxytrabdium lepoinum Lamprophildae (Cyclocorinae) AF471029 EU547051 EU547149 ED54012081 Oxytrabutis Elapidae EU547051 EU547149 FF210827 EU546916	52	Melanophidium punctatum	Uropeltidae	I	AY701024	I	I	I	I
Mimophis mahfalensis Lamprophilae (Psammophinae) DQ486461 AY188070 AY187992 AY187992 Morelia viridis Pythonidae Pythonidae EF545098 EF545048 AY187992 AY187992 Morelia viridis Pythonidae Exposition EF545098 EF545048 AY187992 AY187992 Naja kaouthia Elapidae Elapidae Esptotyphlopinae) E7645098 EF545048 E764209 AY058938 I Namibiana occidentalis Leptotyphlopidae (Leptotyphlopinae) CQ469251 EU624209 AY058938 I Oligodon armensis Colubridae (Colubrinae) KC347404 KC347305 KC347504 KC347404 Oxyrrhabdium leporinum Lamprophilae (Cyclocorinae) AF471029 D_5477149 EF210827 EU546916 Oxyranse scutellatus Elapidae EU547051 EU547751 EU546916 D<20112081	23	Micrelaps bicoloratus	Lamprophiidae (Aparallactinae)	DQ486349	I	I	DQ486173	I	I
Morelia viridis Pythonidae EF545098 EF545048 EF545048 EF54209 AY058938 I Naja kaouthia Elapidae Elapidae Elapidae Equityphlopinae) 50359757 EU624209 AY058938 I Namibiana occidentalis Leptotyphlopidae (Leptotyphlopinae) 50469251 602469074 6 Oligodon armensis Colubridae (Colubrinae) KC347464 KC347504 KC347404 03112081 Oxythabdium leporinum Lamprophildae (Cyclocorinae) AF471029 50547149 E1510827 EU546916 Docorrectionation EU5477051 EU5477149 EF210827 EU546916 2056016	54	Mimophis mahfalensis	Lamprophiidae (Psammophiinae)	DQ486461	AY188070	I	AY187992	JQ073081	I
Naja kaouthia Elapidae FR693728 GQ359757 EU624209 AY058938 Namibiana occidentalis Leptotyphlopidae (Leptotyphlopinae) GQ469251 GQ469074 6 Oligodon armensis Colubridae (Colubrinae) KC347464 KC347504 KC347404 0 Oxythabdium leporinum Lamprophildae (Cyclocorinae) AF471029 EU547149 FF210827 EU546916 Doxycoracio consistent Elapidae EU547051 EU547149 FF210827 EU546916	55	Morelia viridis	Pythonidae	EF545098	EF545048	I	I	I	I
Namibiana occidentalis Leptotyphlopidae (Leptotyphlopinae) GQ469251 GQ469074 0 Oligodon armensis Colubridae (Colubrinae) KC347464 KC347504 KC347404 Oxyrhabdium leporinum Lamprophildae (Cyclocorinae) AF471029 D07112081 Oxyrinaus EU547751 EU547749 FF210827 EU546916 Domons Contractor EU547751 EU547749 FF210827 EU546916	56	Naja kaouthia	Elapidae	FR693728	GQ359757	EU624209	AY058938	EU402654	EU402857
Oligodon arrensis Colubridae (Colubrinae) KC347464 KC347365 KC347504 KC34704 Oxyrhabdium leporinum Lamprophildae (Cyclocorinae) AF471029 D0112081 Oxyrunus currellatus Elapidae Elapidae EU547051 EU547149 EF210827 EU546916 Doctrost Eucration Lamprophildae Location Location Location Location	57	Namibiana occidentalis	Leptotyphlopidae (Leptotyphlopinae)	I	GQ469251	I	GQ469074	GQ469189	I
0 Oxyrhabdium leporinum Lamprophiidae (Cyclocorinae) AF471029 DQ112081 0 Oxyuranus scutellatus Elapidae EU547051 EU547149 EF210827 EU546916	58	Oligodon arnensis	Colubridae (Colubrinae)	KC347464	KC347365	KC347504	KC347404	I	KC347442
) Oxyuranus scutellatus Elapidae EU547051 EU547149 EF210827 EU546916	59	Oxyrhabdium leporinum	Lamprophiidae (Cyclocorinae)	AF471029	I	I	DQ112081	I	I
	60	Oxyuranus scutellatus	Elapidae	EU547051	EU547149	EF210827	EU546916	I	I
Pareidae (Pareinae) JF82/6// AF544802 JF82/053 JF82//02	51	Pareas carinatus	Pareidae (Pareinae)	JF827677	AF544802	JF827653	JF827702	FJ433985	I

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	Species	Family (subfamily)	cytb	165	nd4	cmos	bdnf	rag1
62	Prosvmna ianii	Lamprophiidae (Prosvmninae)	FJ404319	FJ404222	FJ404389	FJ404293		
							I	I
ņ	Prosymna visseri	Lamprophiidae (Prosymninae)	AY 188033	AY1880/2	I	AY18/994	I	Ι
64	Pseudaspis cana	Lamprophiidae (Pseudaspidinae)	AY612080	AY611898	DQ486319	DQ486167	I	I
65	Pseudoxenodon karlschmidti	Colubridae (Pseudoxenodontinae)	AF471080	JF697330		AF471102	JQ599045	
99	Python bivittatus	Pythonidae	JX401131	KF010492	I	AF435016	XM7433022	I
67	Rena humilis	Leptotyphlopidae (Epictinae)	AY099991	AB079597	AB079597	AY099979		I
68	Rhinophis drummondhavi	Uropeltidae	AF544673	AY701028		AF544719	FJ433966	I
69	Sanzinia madagascariensis	Sanziniidae	U69866	AY336066		EU403580	AY988033	AY988067
0	Sibynophis subpunctatus	Colubridae (Sibynophiinae)	KC347471	KC347373	KC347516	KC347411	I	KC347449
–	Tropidophis feicki	Tropidophiidae	KF811124	AF512733	I	KF811110	KF811074	
2	Typhlops jamaicensis	Typhlopidae (Typhlopinae)	KF993259	AF366764		AF544733	EU402664	EU402866
73	Ungaliophis continentalis	Charinidae (Ungaliophiinae)	U69870	AF544833		AF544724	EU402665	EU402867
4	Xenodermus javanicus	Xenodermidae	I	AF544810	U49320	AF544711	EU402667	EU402869
5	Xenopeltis unicolor	Xenopeltidae	AB179620	AB179620	AB179620	AF544689	EU402668	DQ465564
9	Xenophidion schaeferi	Xenophidiidae	AY574279	I	I	I	I	ļ
7	Xenotyphlops grandidieri	Xenotyphlopidae	KF770844	I	I	I	GU902457	I
œ	Xerotyphlops vermicularis	Typhlopidae (Asiatyphlopinae I)	JQ910544	1		1	GU902397	
6	Xylophis perroteti	Pareidae (Xylophiinae subfam. nov.)	I	MK340908*	MK340910*	MK344193*	MK344197*	MK340913*
80	Xylophis stenorhynchus	Pareidae (Xylophiinae subfam. nov.)	MK340915	MK340907	MK340911	MK344194	MK344198	I
81	Xylophis captaini	Pareidae (Xylophiinae subfam. nov.)	MK340914*	MK340909*	MK340912	MK344195	MK344196	

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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 (QiagenTM, 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 brainderived 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 highthroughput 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 largescale 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

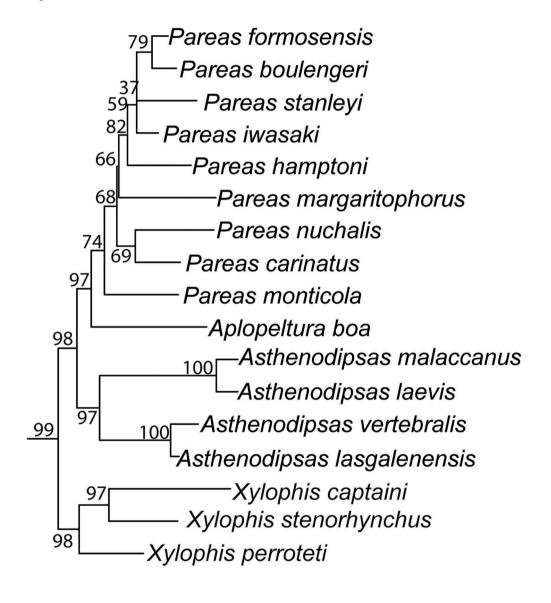
Based on the well-supported inferred phylogenetic relationships of *Xylophis* and divergence from its extant sister lineage, we refer the genus to the family Pareidae, and re-define the latter phylogenetically as all snakes more closely related to *Pareas carinatus* Wagler, 1830 than to *Xenodermus javanicus* 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).



0.9

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).

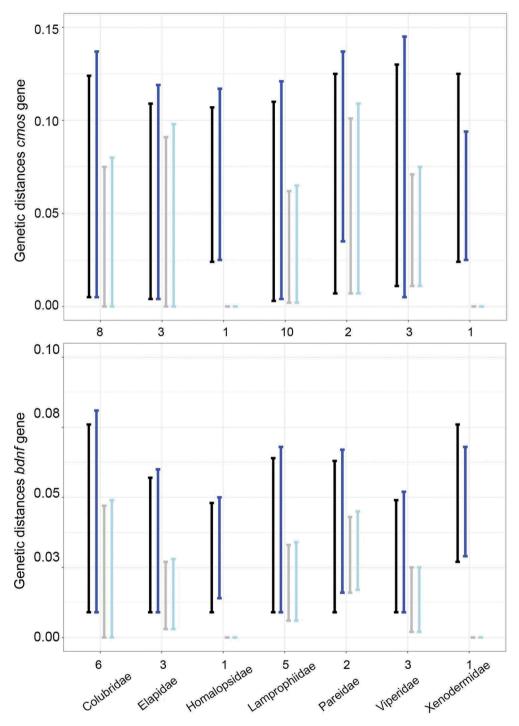


Figure 2. Ranges of uncorrected p-distances (black and grey) and K2P distances (dark blue and light blue) for between-family (dark bars) and within-family (light bars) comparisons of snakes in the superfamily Colubroidea. Pareidae here includes Pareinae and Xylophiinae subfam. nov. Numbers on the x-axis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

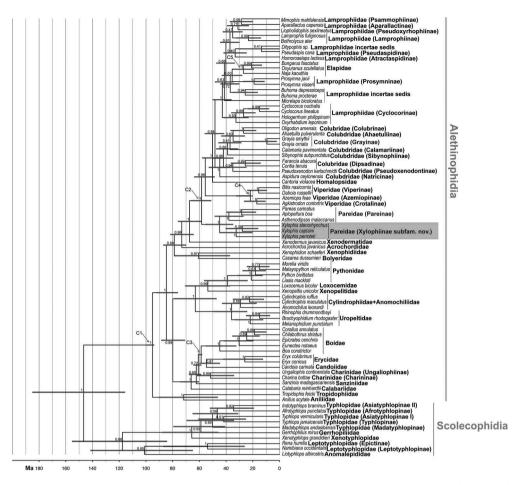


Figure 3. BEAST chronogram generated using concatenated-gene for all families and subfamilies of snakes. Numbers at internal branches indicate posterior probabilities. Error bars indicate 95% highest posterior densities for node ages. Nodes C1–C5 are the five calibrated nodes.

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).

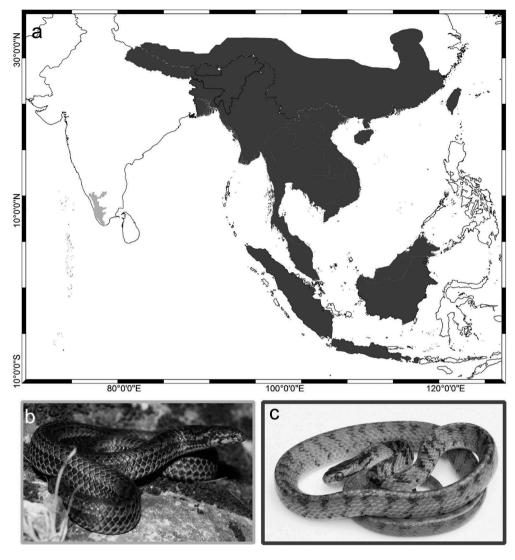


Figure 4. (a) Geographic distribution of Xylophiinae subfam. nov. (green) and approximate distribution of subfamily Pareinae (blue). Photographs show representative taxa of the two subfamilies within Pareidae: (b) *Xylophis perroteti* from Nilgiris, Tamil Nadu, India (Photo: Achyuthan N. Srikanthan); (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).

Acknowledgements

In addition to people acknowledged by Gower and Winkler (2007), we thank Srihari Ananthakrishna and Krishna Chaitanya for their support during fieldwork. Chinta Sidharthan is thanked for help with some of the lab work, and Mark Wilkinson and Natalie Cooper for help with some of the analyses. VD thanks Achyuthan Srikanthan for sharing *Xylophis perroteti* photos. We also further thank those acknowledged in Ruane and Austin (2017), especially Jens Vindum (California Academy of Sciences) for the loan of *Xylophis stenorhynchus* and Jeff Streicher for sharing the dataset from Streicher and Wiens (2016). Part of this project was made possible from

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NSF DEB-1146033 to C.C. Austin. VD's contribution was support, in part, by Marie Skłodowska-Curie Fellowship EU project 751567.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Marie Skłodowska-Curie Fellowship [EU project 751567].

Geolocation Information

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

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