



# The role of wet-zone fragmentation in shaping biodiversity patterns in peninsular India: insights from the caecilian amphibian *Gegeneophis*

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## ABSTRACT

**Aim** Indian biodiversity is concentrated in the wet zone, which is disjunctly distributed in the north-east and in the peninsular Western and Eastern Ghats. The Eastern Ghats region is smaller and less well explored biologically and the affinities and origins of its biota poorly understood. Our aim was to assess whether divergence between east and west lineages might have been caused by fragmentation of the wet zone during Pleistocene climatic fluctuations, by Late Miocene wet-zone contraction or by more ancient events. We present the first dated phylogenetic test of these alternatives by inferring relationships and dating divergences within a wet-zone-restricted lineage endemic to the Eastern and Western Ghats.

**Location** The Eastern and Western Ghats regions of peninsular India.

**Methods** Molecular genetic data (one nuclear and four mitochondrial genes) were newly generated for the only known Eastern Ghats teresomatan caecilian amphibian (*Gegeneophis orientalis*) and the only Western Ghats congener (*G. pareshi*) for which molecular data were not previously available. Phylogenetic relationships were inferred for Indian indotyphlids using maximum likelihood and Bayesian inference methods. Divergence times within the inferred phylogeny were estimated using a Bayesian relaxed clock method, with the Seychelles versus Indian indotyphlid divergence calibrated based on the geological separation of their respective continental land masses.

**Results** The single Eastern Ghats species of *Gegeneophis* is sister to all other (Western Ghats) *Gegeneophis*. The basalmost (and east–west) split within *Gegeneophis* likely occurred > 35 Ma.

**Main conclusions** Divergence between Eastern and Western Ghats *Gegeneophis* is too ancient to have been caused by wet-zone contraction in the Miocene or by Pleistocene climatic fluctuations. Our results are consistent with a relatively ancient origin of wet-zone lineages in the Eastern Ghats and a lack of gene flow between Eastern and Western Ghats *Gegeneophis* for tens of millions of years.

## Keywords

biogeography, caecilian, *Gegeneophis orientalis*, Miocene, molecular dating, phylogeny, Pleistocene, rain forest, Seychelles, wet zone

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## INTRODUCTION

India has an exceptionally species-rich and notably endemic fauna and flora that results, in part, from its distinctive abiotic history (e.g. Mani, 1974, 1995; Ripley & Beehler, 1990;

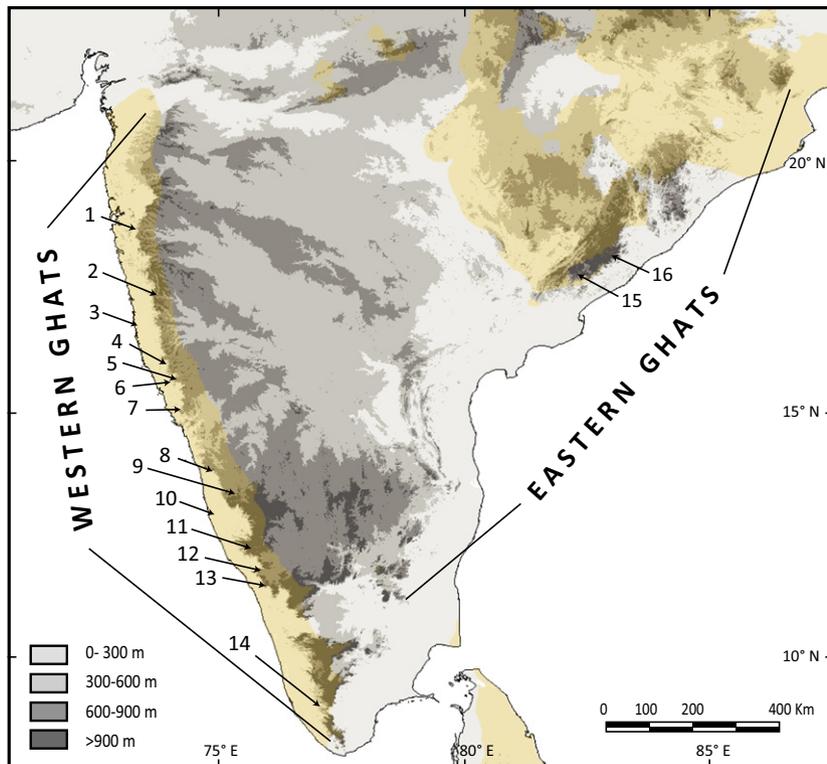
Chatterjee *et al.*, 2013). Much of India's biodiversity is concentrated in moist habitats, especially evergreen broadleaf forests (e.g. Schwartzberg, 1992; Nayar, 1996; Barthlott *et al.*, 2005). The Indian wet-zone (e.g. Karanth, 2003) and its biota are markedly patchy in their current spatial

distribution. The two major centres are north-east India and the Western Ghats region of the peninsula, with these two regions separated by a c. 1500 km wide expanse of less mesic terrain (< 100 cm rainfall per year) (e.g. Schwartzberg, 1992). Within this mostly drier region lie some isolated, less substantial upland areas with mesic habitats, most notably parts of the Eastern Ghats (Fig. 1).

By the Late Cretaceous (99.6–65.5 Ma), the drifting Indian plate had separated from Africa, Australia and Antarctica and was undergoing separation from Madagascar. Through the K–Pg boundary (65.5 Ma) and the temporally associated Deccan Traps volcanism (Keller *et al.*, 2008), and beyond its separation from the Seychelles (c. 62 Ma) and collision with Asia (c. 55 Ma), vegetation in India was dominated by evergreen, broadleaf, dense and closed-canopy tropical rain forest (e.g. Morley, 2000). The environment and climate of the Indian subcontinent changed substantially during especially the second half of the Cenozoic (e.g. Tiwari *et al.*, 2012; Shukla *et al.*, 2013). Indications of Asian monsoonal seasonality extend back to 40 Ma (Licht *et al.*, 2014). In the Late Eocene (37.2–33.9 Ma) and Oligocene (33.9–23.03 Ma), palynomorph diversity is notably lower and more regionally variable than during the warm and very wet Early Eocene (55.8–48.6 Ma) (Morley, 2000). From a climatic optimum at the Palaeocene–Eocene boundary (55.8 Ma) temperatures decreased substantially through the Eocene and remained low into the Oligocene (e.g. Morley, 2007), with a particularly pronounced cooling and drying at c. 34 Ma (Zachos *et al.*, 2001; Morley, 2007). The Indian climate returned again to a warmer and more seasonal, monsoon-dominated

pattern by at least the Early Miocene (c. 23 Ma) (Harris, 2006; Clift *et al.*, 2008; Guo *et al.*, 2008). Following rain forest re-expansion during the Middle Miocene Climatic Optimum (c. 17–15 Ma) a general and pronounced drying and cooling occurred throughout the Late (11.61–5.3 Ma) Miocene, associated with a fragmentation of rain forests and spreading of other biomes including savanna-like grassland habitats (Morley, 2000; Patnaik *et al.*, 2012; Pound *et al.*, 2012). A patchy distribution of moist forest habitats has likely dominated post-Miocene (since 5 Ma) India, although glaciation-driven climatic cycles during the Pleistocene (1.81–0.01 Ma) might have caused periodic contraction and expansion (and possible reconnection of isolated patches) of rain forest. These great environmental and vegetational changes can be expected to have substantially impacted faunal diversification and biogeography, but thorough (including molecular phylogenetic) tests of generalities and detection of spatial, temporal, and taxonomic exceptions have yet to be carried out as highlighted by, for example, Raheem *et al.* (2014). Here, we use the potential of molecular phylogenetics and dating to focus on a single case study in order to determine the extent to which current spatial patterns of Indian biodiversity were shaped by the recent past versus long-lasting consequences of environmental changes in deep history.

Many Indian wet-zone organisms, especially animals, currently occur in either the north-east or the Western Ghats, with few common to both regions. The Eastern Ghats are less well studied biologically than the Western Ghats and their biota remains poorly documented (e.g. Agarwal *et al.*,



**Figure 1** Map of peninsular India showing localities of sampled indotyphlids. Numbers correspond to samples listed in Table 1; relief is shown in greyscale; the approximate extent of the Western and Eastern Ghats is marked by text and lines; the translucent tan-coloured fill shows areas with a mean annual rainfall > 1300 mm (1300 mm being the approximate rainfall at *Gegeneophis orientalis* localities 15 and 16). All Western Ghats *Gegeneophis* are found in areas of rainfall > 1800 mm. Rainfall in north-eastern India (where indotyphlids are absent but the caecilian family Chikilidae occurs, see Kamei *et al.*, 2012: Fig. 1) is > 1300 mm and rainfall elsewhere on the mainland of the Indian subcontinent is < 1000 mm.

**Table 1** Taxon and character sampling for Indian and Seychelles indotyphlids. Newly generated sequences underlined. BNHS, Bombay Natural History Society, Mumbai; *cox1*, cytochrome *c* oxidase subunit 1; *cytb*, cytochrome *b*; DU, Systematics Laboratory, Department of Environmental Science, Delhi University; *rag1*, recombination activating gene 1; UMMZ, University of Michigan Museum of Zoology; UK, University of Kerala, Department of Zoology. Numbers in parentheses correspond to peninsular Indian localities in Fig. 1. See text and Gower *et al.* (2011) for additional locality data. The locality of origin of BNHS 4232 (*Indotyphlus battersbyi*) is Matheran and not Amba Valley as reported by Gower *et al.* (2011: table 1) – who accidentally reported the locality for BNHS 4232 and the conspecific BNHS 5350 the wrong way around.

Taxon	Voucher	GenBank accession number				
		<i>12S</i>	<i>16S</i>	<i>cytb</i>	<i>cox1</i>	<i>rag1</i>
<i>Indotyphlus battersbyi</i> (1)	BNHS 4232	HQ443973	HQ444016	HQ444058	HQ444104	HQ444125
<i>Indotyphlus maharashtraensis</i> (2)	BNHS 4223	HQ443976	HQ444019	HQ444061	HQ444106	HQ444126
<i>Gegeneophis danieli</i> (4)	BNHS 4230	HQ443948	HQ443991	HQ444033	HQ444080	HQ444121
<i>Gegeneophis mhadeiensis</i> (5)	BNHS 5351	HQ443940	HQ443983	HQ444026	HQ444074	HQ444118
<i>Gegeneophis cf. mhadeiensis</i> (4)	BNHS 4233	HQ443945	HQ443988	HQ444031	HQ444079	HQ444120
<i>Gegeneophis goaensis</i> (6)	No voucher	HQ443943	HQ443986	HQ444029	HQ444077	HQ444119
<i>Gegeneophis madhavai</i> (8)	BNHS 5349	HQ443956	HQ443999	HQ444041	HQ444087	HQ444123
<i>Gegeneophis pareshi</i> (7)	BNHS 5289	<u>KP400600</u>	<u>KP400604</u>	<u>KP400608</u>	<u>KP400610</u>	<u>KP400612</u>
<i>Gegeneophis seshachari</i> (3)	BNHS 4231	HQ443970	HQ444013	HQ444055	HQ444101	HQ444124
<i>Gegeneophis krishni</i> (10)	BNHS 4176	HQ443954	HQ443997	HQ444039	HQ444085	HQ444122
<i>Gegeneophis sp.</i> (11)	BNHS 4177	HQ443937	HQ443980	HQ444023	HQ444071	HQ444116
<i>Gegeneophis sp.</i> (9)	BNHS 5348	HQ443938	HQ443981	HQ444024	HQ444072	HQ444117
<i>Gegeneophis carnosus</i> (12)	UK MW295	HQ443936	HQ443979	HQ444022	HQ444070	HQ444115
<i>Gegeneophis primus</i> (13)	DU 1206	HQ443939	HQ443982	HQ444025	HQ444073	–
<i>Gegeneophis ramaswamii</i> (14)	UK MW331	AY456250	AY456250	AY456250	AY456250	AY456255
<i>Gegeneophis orientalis</i> (16)	BNHS 541	<u>KP400601</u>	<u>KP400605</u>	<u>KP400609</u>	<u>KP400611</u>	<u>KP400613</u>
<i>Gegeneophis orientalis</i> (16)	BNHS 5409	<u>KP400602</u>	<u>KP400606</u>	–	–	–
<i>Gegeneophis orientalis</i> (15)	No voucher	<u>KP400603</u>	<u>KP400607</u>	–	–	–
<i>Praslinia cooperi</i>	UMMZ 192933	EF219347	EF219348	HQ444069	HQ444114	<u>KP400614</u>
<i>Hypogeophis rostratus</i>	UMMZ 189115	EF219349	EF219350	HQ444068	HQ444113	<u>KP400615</u>
<i>Hypogeophis brevis</i>	UMMZ 192977	EF219353	EF219354	HQ444065	HQ444110	<u>KP400616</u>
<i>Grandisonia alternans</i>	UMMZ 189118	EF219351	EF219352	HQ444064	HQ444109	<u>KP400617</u>
<i>Grandisonia larvata</i>	UMMZ 193061	EF219355	EF219356	HQ444066	HQ444111	<u>KP400618</u>
<i>Grandisonia sechellensis</i>	UMMZ 193076	EF219357	EF219358	HQ444067	HQ444112	<u>KP400619</u>

2012) though, in general, elements seem to be shared more with the Western Ghats (and elsewhere in peninsular India) than the north-east (e.g. Bansal & Karanth, 2010; Bauer *et al.*, 2010; Agarwal & Karanth, 2015). One example is the caecilian amphibians (Gymnophiona) of the clade Teresomata (Wilkinson & Nussbaum, 2006), which are represented in India by two families. The Chikilidae comprises one genus and four species endemic to north-east India (Kamei *et al.*, 2012, 2013). The Indotyphlidae has eight species in five genera in Africa and Seychelles (Wilkinson *et al.*, 2011) with the majority of the 22 species occurring in peninsular India, where there are two indotyphlid genera and 14 species, all endemic. The two nominal species of *Indotyphlus* Taylor, 1960 are both endemic to the Western Ghats, as are all but one of the 12 species of *Gegeneophis* Peters, 1880. The other species, *G. orientalis* Agarwal, Wilkinson, Mohapatra, Dutta, Giri & Gower, 2013 is one of the most recently described, and is the only teresomatan caecilian reported from the Eastern Ghats. The recent discovery of *G. orientalis* supports the view that the Eastern Ghats is less well explored biologically than the Western Ghats.

Gower *et al.* (2011) presented a molecular phylogeny (without estimated divergence dates) for Indian indotyphlids,

but their sampling lacked *G. orientalis* as well as another recently described species of *Gegeneophis*, *G. pareshi* Giri, Gower, Gaikwad & Wilkinson, 2011. Here, we present results of phylogenetic analyses including these two species. We focus on the relationships of *G. orientalis* and the date of the divergence of this species from its closest known (Western Ghats) living relative(s), and consider the implications for Indian biogeography, in particular for the potential role of wet-zone fragmentation in shaping spatial biodiversity patterns. Caecilians are generally restricted to wet tropical regions, often to moist forest habitats (e.g. Gower & Wilkinson, 2005; Kamei *et al.*, 2012). Their modern distribution indicates that species of *Gegeneophis* are intolerant of dry regions, and being limbless and generally soil dwelling amphibians with relatively small distributions they can be assumed to have relatively low vagility (*sensu* Kodandaramiah, 2009). Thus, *Gegeneophis* species are likely to be good subjects for examining the historical biogeography of the Indian wet zone.

This study aims to determine what Indian indotyphlids can tell us about the biotic impact of Indian Cenozoic aridification and fragmentation of the wet zone. In particular, we test the following alternative explanations for the disjunct

distribution of *Gegeneophis* in the Western and Eastern Ghats: (1) the disjunction is spurious because the Eastern Ghats *G. orientalis* is incorrectly classified (see Mani, 1974; Karanth, 2003), (2) the divergence between *G. orientalis* and its closest living Western Ghats relative is more ancient than (and was not causally associated with) the onset of Indian aridification in the Cenozoic, (3) the divergence between *G. orientalis* and its closest living Western Ghats relative is possibly causally associated (contemporaneous) with cooling and aridification towards the end of the Eocene (*c.* 34 Ma) or more pronounced aridification during the Late Miocene (*c.* 11–5 Ma), (4) the divergence between *G. orientalis* and its closest living Western Ghats relative is shallow, consistent with a causal relationship with Western-Eastern Ghats wet-zone (dis)connectivity during Pleistocene (1.81–0.01 Ma) climatic fluctuations (and/or the present-day moist forest disjunction is at least partly an artefact resulting from human disturbance).

## MATERIALS AND METHODS

### Taxon and character sampling

For *G. orientalis*, we sampled two paratopotypes in the Bombay Natural History Society, Mumbai (BNHS 5409, 5410) from near Beespuram, Andhra Pradesh and one unvouchered specimen from Kalingakonda, Andhra Pradesh, *c.* 90 km south-west of the type locality. Details of these specimens and localities are reported by Agarwal *et al.* (2013: fig. 1, p. 542). For *G. pateshi* we sampled paratopotype BNHS 5289. We did not use all of the samples analysed by Gower *et al.* (2011) but to increase computational efficiency we instead selected one specimen from each major lineage. Our sampling included all nominal species of Indian indotyphlid except *G. tejaswini* Kotharambath, Wilkinson, Oommen & Gower, 2015, for which only *12s* and *16S* rRNA sequences are available (this species is more closely related to *G. carnosus* than any other nominal species: Gower *et al.*, 2011). Following Gower *et al.* (2013), we consider *G. nadkarnii* Bhatta & Prashanth, 2004 to be a junior synonym of *G. danieli* Giri, Wilkinson & Gower, 2003.

We generated sequence data for one nuclear (nu) and four mitochondrial (mt) genes. The mt genes are *12S* and *16S* rRNA, cytochrome *c* oxidase subunit 1 (*cox1*) and cytochrome *b* (*cytb*), and the nu marker is the recombination activating gene 1 (*rag1*). DNA extraction and amplification methods are as reported by Gower *et al.* (2011). Samples and GenBank accession numbers are given in Table 1. For *G. orientalis*, only specimen BNHS 5410 was sequenced for all five markers and included in the phylogenetic and dating analyses. The other two specimens of *G. orientalis* were sequenced for *12S* and *16S* only and these data were used to calculate intraspecific genetic distances. The six nominal caecilian species of the Seychelles, all indotyphlids, were used as a monophyletic outgroup for the dating analyses (see

below). For all Seychelles species we newly sequenced nu *rag1* to increase completeness of the dataset used for dating. For the phylogenetic analyses, *Indotyphlus* was used to root trees. Outgroup choice was informed by results of previous molecular phylogenetic analyses (Gower *et al.*, 2011; San Mauro *et al.*, 2014).

### Sequence alignment and phylogeny reconstruction

Nucleotide sequences of ribosomal genes were aligned using MAFFT 7.130 (Katoh *et al.*, 2002; Katoh & Toh, 2008), and revised by eye to correct any obvious misalignment. Alignment ambiguities were excluded from matrices using GBLOCKS 0.91b (Castresana, 2000) with default parameters for block selection and the 'With Half' option for gap allowance. Protein-coding genes were aligned with TRANSLATORX (Abascal *et al.*, 2010) using MAFFT to compute the protein alignments (on which the nucleotide alignments were subsequently based).

The separate gene alignments were concatenated into a single master alignment (available in TREEBASE – accession S16858). This master alignment excludes the two *G. orientalis* for which only *12S* and *16S* were sequenced – alignment of these sequences with homologous sequences for the third *G. orientalis* (BNHS 5410) was trivial and included no gaps. The master alignment was analysed using maximum likelihood (ML; Felsenstein, 1981) and Bayesian inference (BI; Huelsenbeck *et al.*, 2001), which are currently the most commonly used methods for molecular phylogenetic inference (reviewed in San Mauro & Agorreta, 2010). Analyses were run on the CIPRES Science Gateway (Miller *et al.*, 2010). ML analysis was performed with RAXML 7.6.3 (Stamatakis, 2006) using the rapid hill-climbing algorithm (Stamatakis *et al.*, 2007) and computing 1000 distinct ML trees starting from 1000 distinct randomized maximum-parsimony starting trees. BI was performed with MRBAYES 3.2.2 (Ronquist *et al.*, 2012) conducting two independent MCMC runs (with four chains each) for 10 million generations, sampling every 1000 generations, and discarding the first 25% of samples as burn-in ('relburnin=yes'). Adequate convergence of the BI runs was judged by plots of log likelihood ( $\ln L$ ) scores versus generation time, low standard deviation of split frequencies, as well as convergence diagnostics [Estimated Sample Size (ESS), Potential Scale Reduction Factor (PSRF)], as implemented in MRBAYES.

For each data set, best fit combinations of partitioning schemes and nucleotide substitution models were determined with PARTITIONFINDER 1.1.1 (Lanfear *et al.*, 2012), using the 'greedy' algorithm and the Bayesian information criterion (BIC), with branch lengths of alternative partitions linked and the software set to evaluate specific substitution model sets for either RAXML or MRBAYES independently. Support for internal branches was evaluated by nonparametric bootstrapping with 2000 replicates (RAXML, using the exhaustive bootstrap algorithm) and posterior probabilities (MRBAYES).

## Divergence time estimation

Divergence times were estimated using the Bayesian relaxed clock method (Drummond *et al.*, 2006) implemented in BEAST 1.7.5 (Drummond & Rambaut, 2007), which is currently the most widely used method for dating phylogenies (San Mauro & Agorreta, 2010). We assumed a relaxed uncorrelated clock with rates drawn from a lognormal distribution across branches and the Yule process speciation model. Independent best fit substitution models were used for each data set partition, as determined by PARTITIONFINDER. The Markov chain was run twice for 100 million generations, sampling every 10,000 generations, and burn-in and convergence of the chains were determined with TRACER 1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>). The summary chronogram with time estimates and confidence intervals was generated using the maximum clade credibility tree with mean node heights.

The fossil record of caecilians is notoriously poor and is inadequate for calibrating divergences among extant taxa (Gower & Wilkinson, 2009). Additionally, we did not want to rely on calibrations for phylogenetically distant nodes, and thus we included the Seychelles caecilians (sister group to Indian indotyphlids: e.g. San Mauro *et al.*, 2012, 2014) as sole outgroup and relied on a single calibration point for the split between Indian and Seychelles indotyphlids. We explored 10 calibration strategies (Table 2) applied to the treeModel.rootHeight prior. The first, a mean of 91 Ma (95% range 103–79 Ma), was based on the mean of mean estimates for this divergence from Wilkinson *et al.* (2002: 101 Ma), Roelants *et al.* (2007: 79 Ma) and Zhang & Wake (2009: 103 Ma). The remaining nine calibrations were based on different interpretations and implementations of geological data for the physical

separation of Seychelles and India, given that amphibians are generally poor dispersers across marine barriers (e.g. Nussbaum, 1984).

It is thought that Seychelles-India separation involved two main phases, an initial phase during which the Gop Rift opened and a subsequent phase during which the Carlsberg (mid-ocean) Ridge formed (Collier *et al.*, 2008). The origin of the Gop Rift was previously debated until seismic data showed it to be floored by oceanic crust with an extinct seafloor-spreading ridge at its centre (Minshall *et al.*, 2008). However, the age of the Gop Rift remains disputed (*c.* 69–56 Ma: Yatheesh *et al.*, 2009; *c.* 71–66 Ma: Armitage *et al.*, 2011; or *c.* 71–69 Ma: Eagles & Hoang, 2013). This is because the Rift is only 140 km wide, preventing a unique identification of the prominent but short sequence of linear magnetic anomalies within it. Discriminating among possible age-sequences therefore relies on arguments using other observations, such as the thickness of the oceanic crust produced by the system and comparison with absolute ages of volcanic rocks exposed in the Seychelles (e.g. Ganerød *et al.*, 2011). In contrast, the magnitude of physical separation during the second phase of separation was much more substantial and its timing is not disputed because the seafloor spreading continued to the present-day active Carlsberg Ridge, and so the magnetic anomalies can be readily identified. The onset of this rifting has been dated as 63.4 Ma (Armitage *et al.*, 2011), with the earliest unambiguous indication of separation, a complete mid-ocean ridge, being at Anomaly 27, 62–61.7 Ma (Collier *et al.*, 2008). Our various calibrations stem from alternative approaches to the differing geological interpretations, and are explained in Table 2. Dates for geological time periods are from the GeoWhen Database (<http://www.stratigraphy.org/upload/bak/geowhen/index.html>).

**Table 2** Details of 10 alternative approaches used to calibrate the age of the split between Seychelles and Indian indotyphlids. Calibration (1) is secondary (see Methods), all others are based on different interpretations of geological data pertinent to the physical separation of India and the Seychelles. Approaches (2), (3), (4) and (5) based on lower and upper limit of estimated age of origin of the Gop Rift (excluding Yatheesh *et al.*'s, 2009 lower bound, which is younger than estimates of spreading at the Carlsberg Ridge). (6) based on considering Seychelles-India separation to have occurred sometime between the earliest estimate of the origin of the Gop Rift and of the Carlsberg Ridge. (7) based on the youngest estimate of formation of complete mid-ocean Carlsberg Ridge. (8), (9) and (10) represent alternative calibration probability distributional choices based on same underlying geological ages as used in approaches 2–6. Approaches (2), (3) and (7) employed point calibrations in order to obtain narrower 95% highest posterior density (HPD) ranges for (minimum) divergence estimates; other calibrations attempted to incorporate aspects of geological and biological uncertainty into priors. See text for comments on geological data.

	Distribution	Mean	SD	95% range	Offset	Log(mean)	Log(SD)	Median	Lower	Upper
(1)	Normal	91	6.125	79–103				91		
(2)	Point	66								
(3)	Point	71								
(4)	Normal	68.5	1.275	66–71						
(5)	Exponential	1.67		66–71	66			67.16		
(6)	Normal	67	2.04	63–71				67		
(7)	Point	61.7								
(8)	Exponential	10		66–95.96	66			72.93		
(9)	Truncated N	66	15	66–95.4				76.12	66	150
(10)	Lognormal			63.4–71	63.4	1	0.625	66.12		

## RESULTS

### Molecular systematics

The phylogenetic data set totals 4,010 sites and is complete except for a single marker (*rag1*) for a single taxon (*Gegeneophis primus*: Table 1). There is no significant base composition bias among the Indian indotyphlids. The third codon positions of protein-coding genes did not show substantial saturation, as judged by plots (not shown) of pairwise transition and transversion differences versus corrected (measured as maximum likelihood distance) sequence divergence. In addition, analyses conducted after removing third codon positions resulted only in slightly different arrangements of the weakly supported relationships and slightly different support levels. Thus, we here focus on reporting results of the analyses of the full data set. Best-fitting models for partitions are presented in Table S1 in Supporting Information. Numbers of informative, uninformative and constant sites for each of the five gene alignments are reported in Table S2.

For the *G. orientalis* 12S and 16S sequences, the p-distance between the two Beespuram specimens is 0.2% and between these two and the Kalingakonda specimen is 1.2 and 1.3%. The *G. orientalis* 12S and 16S sequences are 8.5–13.3 and 4.7–8.9% different, respectively, from sampled congeners, and the corresponding values for *G. pareshi* are 7.2–14.6 and 7.0–8.9%, consistent with the recent description of these two forms as distinct species.

For taxa in common, the phylogenetic results (Fig. 2) are very similar to those reported by Gower *et al.* (2011). *Indotyphlus* and *Gegeneophis* are strongly supported monophyla. Within *Gegeneophis* there are nine well supported clades (ML bootstrap values > 75, Bayesian posterior probabilities > 0.97), the only poorly supported relationships (bootstrap values < 50, posterior probabilities < 0.5) are among the following five lineages: *G. carnosus* + *G. primus*, *G. krishni* + two *G. sp.*, *G. seshachari*, *G. ramaswami* and a generally northern Western Ghats clade comprising *G. pareshi*, *G. madhavai*, *G. goaensis*, *G. danieli*, *G. mhaeiensis* and *G. cf. mhadeiensis*. The BI and ML trees (whether or not third codon positions are removed) differ only in the resolution of these relationships, but none of the alternatives receives notable support and these relationships were also not resolved by Gower *et al.* (2011). *Gegeneophis pareshi* is recovered with strong support as sister to all other members of the latter clade and is not sister to the only other species of *Gegeneophis* that has a terminal shield, *G. seshachari*. The Eastern Ghats *G. orientalis* is recovered as sister to a clade comprising all other Western Ghats congeners and this relationship appeared in all of our analyses.

### Dating

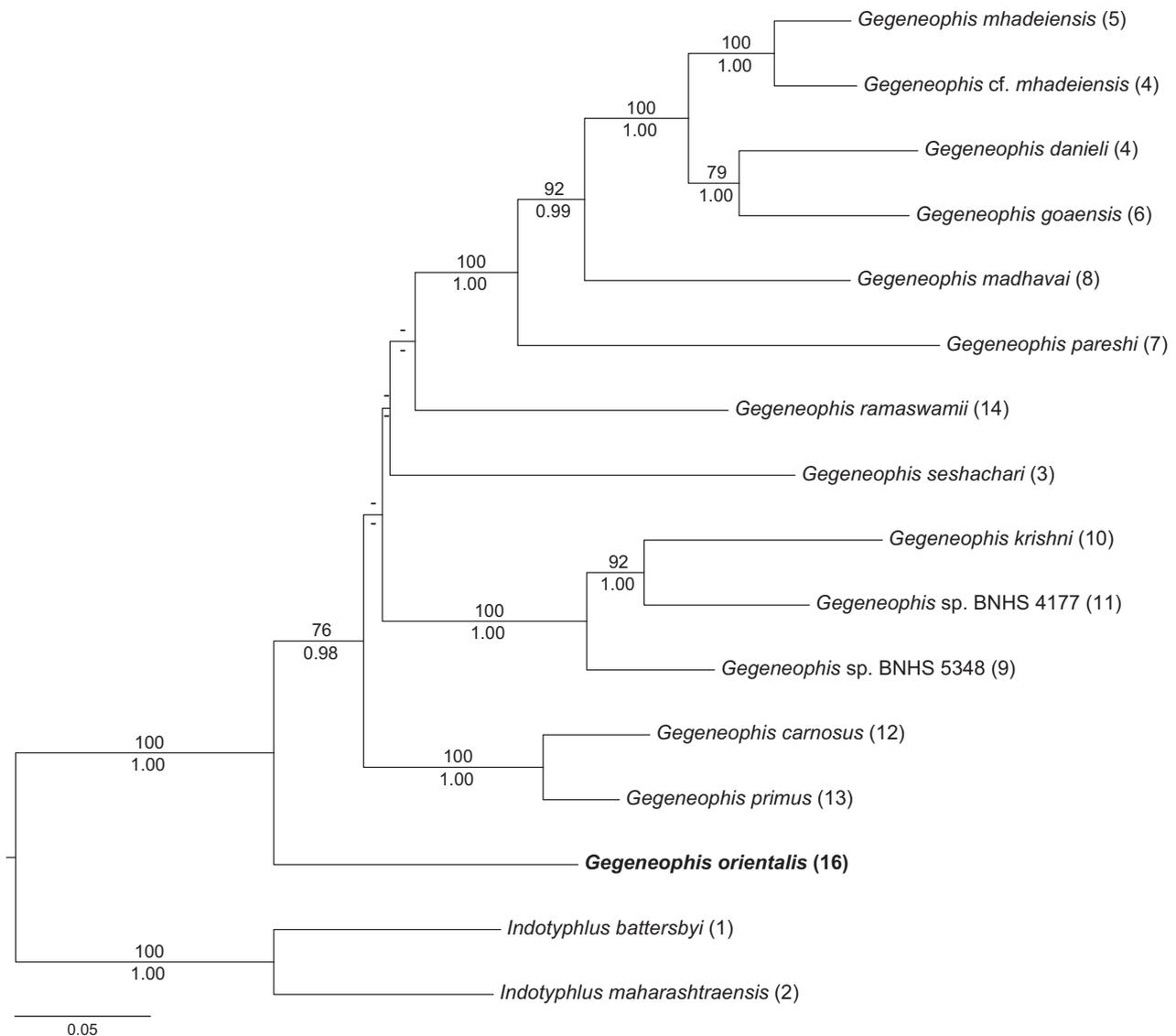
Divergence dating results under the various calibration alternatives are reported in Tables 2 and S3 and Fig. 3. There is variation among the date estimates based on the calibration

alternatives, but the various means are not greatly different relative to the bounds of upper and lower 95% highest posterior density (HPD) values. Ignoring the substantially older ages obtained using the secondary calibration (calibration strategy 1 in Table 2), the inferred mean dates of the basal divergence among Indian indotyphlids (*Gegeneophis* versus *Indotyphlus*) using the other nine calibrations range from 63.0 to 51.4 Ma (95% HPD range 80.0–43.4 Ma: Table 3). The corresponding mean dates for the basal split among extant lineages of *Gegeneophis* (= *G. orientalis* versus others, i.e. Western versus Eastern Ghats lineages) range from 45.9 to 37.5 Ma (95% HPD range 60.0–30.2 Ma).

## DISCUSSION

The results of our dating analyses argue against Pleistocene (1.81–0.01 Ma) climate fluctuations as a causative agent in either a recent origin through dispersal of *G. orientalis* to the Eastern Ghats or the vicariance between it and its closest Western Ghats relatives. Pleistocene climate fluctuations have often been proposed as a possible promoter of diversification in the tropics, but a dominant causal role has rarely been demonstrated (e.g. Moritz *et al.*, 2000; Morley, 2007; Gower *et al.*, 2012), in contrast to their obviously substantial impact in temperate regions (e.g. Hewitt, 2004), although we note that Pleistocene glaciation is thought to have had a substantial impact on at least Himalayan bird and mammal diversity patterns in the Indian subcontinent (Srinivasan *et al.*, 2014).

The lower 95% HPD values for estimates of the timing of the split between Eastern and Western Ghats *Gegeneophis* (node 3 in Fig. 3) are all greater than 30 Ma and thus our analyses also argue strongly against this divergence having been caused by Late Miocene (11.61–5.3 Ma) wet-zone contraction. The presence of old caecilian lineages in a region is an indicator of an old and continuous history of wet tropical environments in that region (Kamei *et al.*, 2012). Thus, our results are consistent with a relatively ancient origin of the occurrence of wet-zone species in the Eastern Ghats and a lack of gene flow among Western and Eastern Ghats wet-zone *Gegeneophis* for tens of millions of years. Whether the Eastern versus Western Ghats disjunction was caused by dispersal or vicariance, we cannot rule out that the estimated divergence was coincident with (and possibly caused by) wet-zone fragmentation during the Late Eocene (37.2–33.9 Ma) or Early Oligocene (33.9–28.4 Ma) (Morley, 2000), potentially associated with pronounced cooling and drying at c. 34 Ma (Morley, 2007). Thus, the Eastern Ghats might have served as a moist refugium for a substantial period of time, and wet-zone fragmentation in India approximately during the mid Cenozoic might have resulted in the intervening dry zone forming an effective barrier (generally impermeable to low-vagility taxa). Based on this, we predict that other endemic wet-zone lineages that might be discovered in the Eastern Ghats, particularly among taxa that have a similar age, habitat preference and dispersal ability to indotyphlid caecilians, will be long separated from Western Ghats



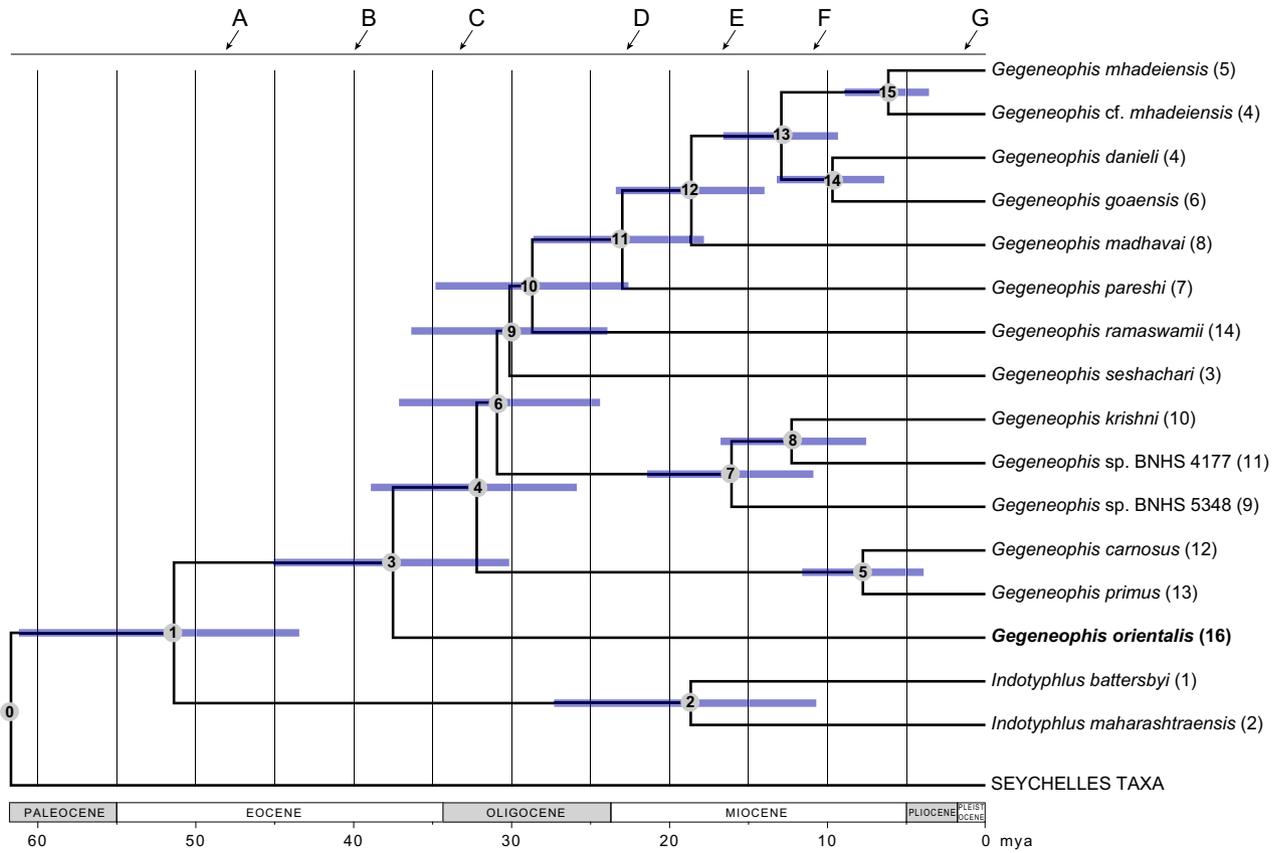
**Figure 2** Maximum likelihood tree of Indian indotyphlid caecilians from analysis of partial sequences of four mitochondrial genes and one nuclear gene (partitioned by gene and by codon position). Quantitative support for internal branches shown as maximum likelihood bootstrap values (above branches) and Bayesian posterior probabilities (below). Values below 50/0.5 not shown. All species displayed are from the Western Ghats of India except for the Eastern Ghats *Gegeneophis orientalis*. Numbers in parentheses correspond to peninsular Indian localities in Fig. 1.

relatives. We are sceptical about reports of low-vagility single species with truly disjunct Western and Eastern Ghats wet-zone distributions. For example, Srinivasulu & Das (2008) reported the Western Ghats frog *Indirana leithii* (Boulenger, 1888) from the Nallamala Hills in the Eastern Ghats but supported this by incorrectly stating that one of us (VG) had identified a specimen as such.

Ideally we would have sampled more loci and for multiple individuals of each species. This additional information would also have made the data set more appropriate for multispecies coalescent analysis. However, we found it difficult to amplify nuclear DNA for some of the available tissue samples. In addition to the not untypical constraints of time, money and permitting, many Indian indotyphlids are known

from only a few specimens each, and we do not have direct access to samples for several of the species. Moreover, our results very strongly indicate that there has not been gene flow between Western and Eastern Ghats *Gegeneophis* for tens of millions of years. Thus, we are confident that additional taxon and character data and application of other methods is unlikely to yield strong support for a much more recent connection (e.g. within the last 10 Ma), and we contend that in some cases it is possible to progress without assembling very large data sets.

The two species of *Gegeneophis* with a terminal shield (*G. pareshi*, *G. seshachari*) are not sister taxa, and neither of these species is sister to the only other indotyphlid species that lacks secondary annular grooves (*G. primus*). Thus, within



**Figure 3** Dated tree estimated by analysis using a point calibration (number 7 in Tables 2 and 3 and in Appendix S2 in Supporting Information) based on the youngest time (61.7 Ma) at which unambiguous separation of India and Seychelles had occurred (see Materials and Methods for details). This dating analysis provided the youngest divergence date estimates among our alternative calibration approaches. Numbers in circles at nodes correspond with divergence dating estimates in Table 3 and Appendix S2 in Supporting Information. Numbers in parentheses correspond to peninsular Indian localities in Fig. 1. The divergence between the Eastern Ghats *Gegeneophis orientalis* and its closest Western Ghats relative is node 3. Seychelles outgroup taxa not shown. Letters and arrows along top of figure indicate approximate timing of following events (see main text for sources): A) *c.* 48 Ma, onset of global cooling and diversification and regionalization of Indian flora following Early Eocene Climatic Optimum; B) *c.* 40 Ma, first indication of monsoonal seasonality in Asia; C) *c.* 34 Ma, pronounced global cooling; D) *c.* 23 Ma, modern Indian monsoonal system; E) *c.* 17–15 Ma, Mid Miocene Climatic Optimum (likely re-expansion of moist tropical forest); F) *c.* 11 Ma, onset of pronounced cooling and aridification; G) *c.* 1.8 Ma onwards, Pleistocene climatic fluctuations.

**Table 3** Results of dating analyses under the 10 calibration alternatives (CA) for five selected nodes (indicated in Fig. 3). Data in each cell are given in the following format: upper 95% highest posterior density (HPD)–mean–lower 95% HPD. Values reported to one decimal place.

CA	Node (see Fig. 3)				
	0	1	2	3	4
(1)	103.4–89.2–74.7	92.3–74.3–66.5	40.6–27.1–15.4	68.1–54.2–40.4	58.6–46.5–34.7
(2)	66	65.7–55.0–46.6	29.4–20.1–11.8	48.1–40.1–32.6	41.4–34.4–27.7
(3)	71	70.2–59.2–49.5	31.3–21.6–12.5	51.8–43.1–34.3	44.9–37.0–29.6
(4)	70.9–68.4–65.9	67.2–56.9–47.0	30.4–20.9–12.1	50.2–41.5–33.3	43.5–35.6–28.7
(5)	70.6–67.6–66.0	67.1–56.6–47.0	30.0–20.6–11.8	49.7–41.1–32.7	43.1–35.3–28.3
(6)	70.1–66.8–62.7	65.8–55.8–45.6	30.0–20.5–11.7	48.8–40.6–32.2	42.1–34.8–27.9
(7)	61.7	61.2–51.4–43.4	27.3–18.7–10.7	45.1–37.5–30.2	38.9–32.2–25.9
(8)	87.9–73.3–66.0	78.4–61.2–46.5	33.4–22.3–12.3	58.0–44.7–33.5	50.0–38.3–28.7
(9)	90.6–75.2–66.0	80.0–63.0–47.0	34.8–23.0–12.6	60.0–45.9–34.4	51.4–39.4–29.2
(10)	70.4–66.5–63.9	66.0–55.6–45.9	30.1–20.4–11.8	49.3–40.1–32.4	42.0–34.8–27.3

*Gegeneophis*, it is most parsimonious to infer that secondary annular grooves have been lost at least three times and an unsegmented terminal shield gained at least twice (assuming that gain and loss are equally probable in both cases). The new molecular sequence data and inferred phylogeny presented here corroborate the descriptions of *G. orientalis* and *G. pareshi* as two distinct species of *Gegeneophis*. This support counters the possibility that, in the case of *Gegeneophis*, the disjunct Eastern-Western Ghats distribution is an artefact (see Karanth, 2003) of incorrect taxonomy. Because our single timetree calibration is based on the physical separation of India and the Seychelles, our estimate for divergence between Eastern and Western Ghats *Gegeneophis* should be interpreted as a minimum. The divergences are unlikely to be much younger than we estimate if the separation of India and Seychelles is correctly calibrated and if indotyphlids did not disperse between these lands as they parted. With some exceptions (e.g. Measey *et al.*, 2007) amphibians are poor dispersers across saltwater barriers (e.g. Nussbaum, 1984) and we know of no evidence for caecilian or frog dispersal between Seychelles and India at any time since their physical separation. Dispersal of terrestrial lower vertebrates to or from the Seychelles after the fragmentation of Gondwana have almost all been from or to Africa and Madagascar (e.g. Agnarsson & Kuntner, 2012).

Our interpretation of an important role for pre-Miocene wet-zone fragmentation in Indian biogeography (at least for lineages as old as *Gegeneophis* and with broadly similar dispersal ability) comes with caveats. For example, extinction could have masked closer phylogenetic links (and more recent divergences) between Eastern and Western Ghats *Gegeneophis*, we have analysed only a single major group (terrestrial caecilians), field surveys of (especially fossorial) amphibians in the Eastern Ghats have been sparse to date, and *Gegeneophis* might have dispersed to the Eastern Ghats much more recently than their phylogenetic divergence from Western Ghats relatives (although there is no evidence of more recent *Gegeneophis* dispersal or gene flow between the two regions). The species discovery curve for Indian caecilians (e.g. Gower *et al.*, 2004) suggests that the fauna remains incompletely documented. Nine of the 12 species of *Gegeneophis* have been described after 1999 and we are optimistic that additional new species from both the Eastern and Western Ghats will allow our conclusions to be further tested.

To the best of our knowledge, the only other dated phylogeny to include Western and Eastern Ghats endemics is for the gecko subgenus *Geckoella* Gray, 1867 (Agarwal & Karanth, 2015). In that study, the species that occurs in a relatively mesic habitat in the Eastern Ghats, *G. jeyporensis* (Beddome, 1877), is inferred to have split from its closest known Western Ghats wet-zone relative approximately 32–24 Ma. However, despite their shared peninsular Indian distribution and general affinity for forest, the comparison between *Geckoella* and *Gegeneophis* is not appropriate in many respects: *Geckoella* is not of Gondwanan origin but is instead the product of an into-India dispersal post-contact

with Asia, it is not restricted to the wet zone, and *G. jeyporensis* is nested within an otherwise dry-zone clade (Agarwal & Karanth, 2015). Thus, although the c. 32–24 Ma Western versus Eastern Ghats wet-zone lineage divergence in *Geckoella* cannot be taken as a proxy for the timing of wet-zone fragmentation, *Geckoella* distribution and phylogeny does, nonetheless, provide an additional example of lack of recent gene flow between wet-zone elements endemic to the Western and Eastern Ghats.

Understanding of the relationships and historical biogeography of the Eastern Ghats biota will benefit from improved spatial and temporal resolution of palaeoenvironmental data as well as more comparative phylogenetic/phylogeographical data for other organisms. This represents a relatively unstudied topic in Indian biodiversity and biogeography. Beyond *Gegeneophis orientalis* and *Geckoella jeyporensis* (Agarwal *et al.*, 2013; Agarwal & Karanth, 2015), other possibly relictual, mesic herpetofauna occur in the Eastern Ghats, including species of the caecilian *Ichthyophis*, the lizard *Sepsophis* and uropeltid snakes (Ramaswami, 1947; Aengals & Ganesh, 2013; Agarwal *et al.*, 2013; Datta-Roy *et al.*, 2013), and these might be targets for comparative molecular analyses in the near future. Interestingly, most of these taxa are also (like *G. orientalis*) fossorial, so if this pattern bears scrutiny it will be worth investigating possible causes, such as a similar propensity for inter-population gene flow shared among soil dwellers.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** PartitionFinder results.

**Appendix S2** Properties of the five gene alignments used in phylogenetic analyses.

**Appendix S3** Divergence dating results showing estimated age of the most recent common ancestor for each node.

## BIOSKETCH

**David Gower** and **Mark Wilkinson** study the systematics and evolution of caecilians. **Ishan Agarwal**, **Anniruddha Datta-Roy** and **Varad Giri** are herpetologists specializing on the Indian fauna; the former two also being biogeographers. **Praveen Karanth** and **Diego San Mauro** are molecular evolutionary biologists with special interest in Indian biogeography and amphibians respectively.

Author contributions: I.A. and A.D.-R. conducted fieldwork; A.D.-R. and D.S.M. generated data; D.S.M. conducted analyses. D.J.G. led the writing; all authors contributed to the concept of the study, interpretation and writing.

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