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## Molecular systematics of caeciliid caecilians (Amphibia: Gymnophiona) of the Western Ghats, India

David J. Gower<sup>a,\*</sup>, Diego San Mauro<sup>a</sup>, Varad Giri<sup>b</sup>, Gopalakrishna Bhatta<sup>c</sup>, Venu Govindappa<sup>d</sup>, Ramachandran Kotharambath<sup>e</sup>, Oommen V. Oommen<sup>e</sup>, Farrah A. Fatih<sup>a</sup>, Jacqueline A. Mackenzie-Dodds<sup>a</sup>, Ronald A. Nussbaum<sup>f</sup>, S.D. Biju<sup>g</sup>, Yogesh S. Shouche<sup>h</sup>, Mark Wilkinson<sup>a</sup><sup>a</sup> Department of Zoology, The Natural History Museum, London SW7 5BD, UK<sup>b</sup> Bombay Natural History Society, Mumbai, India<sup>c</sup> Department of Biology, BASE Educational Services Pvt. Ltd., 27 Bull Temple Road, Basavanagudi, Bengaluru 560 004, India<sup>d</sup> Centre for Applied Genetics, Department of Zoology, Bangalore University, Bengaluru 560 056, India<sup>e</sup> Department of Zoology, University of Kerala, Kariavattom 695 581, Thiruvananthapuram, Kerala, India<sup>f</sup> Museum of Zoology and Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109-1079, USA<sup>g</sup> Systematics Lab, School of Environmental Studies, University of Delhi, Delhi 110 007, India<sup>h</sup> National Centre for Cell Science, Pune University Campus, Pune, India

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## ABSTRACT

Together, Indian plus Seychelles caeciliid caecilian amphibians (Gymnophiona) constitute approximately 10% of the extant species of this order. A molecular phylogenetic analysis of all but one (or two) nominal species (16, in five genera) is presented based on mitochondrial (12S, 16S, cytb, cox1) and nuclear (RAG1) sequence data. Results strongly support monophyly of both Seychelles and peninsular Indian caeciliids, and their sister-group status. Within the Indian caeciliids, *Indotyphlus* and *Gegeneophis* are monophyletic sister genera. The phylogenetic position of *Gegeneophis ramsawamii*, *Gegeneophis seshachari*, and *Gegeneophis carnosus* are not well resolved, but all lie outside a well-supported clade of most northern Western Ghats *Gegeneophis* (*madhavai*, *mhadeiensis*, *goensis*, *danieli/nadkarnii*). Most nominal species of Indian caeciliid are diagnosed by robust haplotype clades, though the systematics of *G. carnosus*-like forms in northern Kerala and southern Karnataka requires substantial further investigation. For the most part, Indian caeciliid species comprise narrowly distributed, allopatric taxa with low genetic diversity. Much greater geographic genetic diversity exists among populations referred to *G. seshachari*, such that some populations likely represent undescribed species. This, the first phylogenetic analysis of Indian caeciliids, generally provides additional support for recent increases in described species (eight since 1999), and a framework for ongoing taxonomic revision.

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## 1. Introduction

The Indian subcontinent is a major center of caecilian amphibian (Gymnophiona) diversity, with the region's fauna including three of the six families recognized in the latest taxonomic treatment (Wilkinson and Nussbaum, 2006), the endemic Uraeotyphlidae, south and southeast Asian Ichthyophiidae, and cosmopolitan Caeciliidae. The latter is the largest, most diverse and widely distributed family of caecilians. Caeciliidae is also the most systematically confused caecilian family because the exact nature of its undoubted paraphyly has yet to be clarified (e.g., Hedges et al., 1993; Wilkinson et al., 2002, 2003; San Mauro et al., 2004, 2009; Frost et al., 2006; Wilkinson and Nussbaum, 2006; Loader et al., 2007; Gower et al., 2008; Zhang and Wake, 2009). With a single exception (Frost

et al., 2006), two uncontradicted and well-supported results from previous molecular phylogenetic analyses of caecilians are the monophyly of the caeciliids from the Seychelles and from the Seychelles + the Western Ghats region of peninsular India (Hedges et al., 1993; Wilkinson et al., 2002, 2003; Loader et al., 2007; Roelants et al., 2007; Gower et al., 2008; Zhang and Wake, 2009). Although the Seychelles caeciliids have been sampled comprehensively at the species level in these studies, the analyses have been based mostly on only partial mitochondrial (mt) 12S and 16S rRNA sequences, and have included only two (at most) of the 12 nominate species (and one of the two genera) of Indian caeciliids.

Comprising currently five genera and 18 nominal species, the Indian and Seychelles caeciliids together form some 10% of the approximately 180 extant caecilians (Frost, 2010). Resolution of the phylogenetic relationships of Indo-Seychelles caeciliids would thus make a substantial contribution toward completing the caecilian tree. Until 1999, there were only three caeciliid species known

\* Corresponding author.

E-mail address: [d.gower@nhm.ac.uk](mailto:d.gower@nhm.ac.uk) (D.J. Gower).

from the Western Ghats of peninsular India (and a fourth from northeast India), but the last decade has seen eight additional Western Ghats species described. This inflation has reinforced the understanding that the Western Ghats of India is an important center of caecilian diversity as well as an incompletely surveyed and studied biodiversity hotspot (Gower et al., 2004; Dinesh et al., 2009). Most of these newly discovered and described species are known from small to very small samples, often from single point localities. For example, the descriptions of *Gegeneophis krishni*, *G. danieli*, *G. seshachari*, *G. nadkarnii*, *G. madhavai*, *G. goaensis*, and *G. mhadeiensis* were based on a grand total of 14 specimens (although a few additional specimens of some of these species have subsequently been reported; Gower et al., 2007, 2008; Bhatta et al., 2007a, 2010). Given that much of *Gegeneophis* and *Indotyphlus* taxonomy is based currently on annular counts, these small to very small type series raise the spectre that poor knowledge of variation in key characters has allowed an artificial inflation in species numbers—something that could be tested with molecular data as well as additional morphological material.

The aim of this paper is to present new mtDNA sequence data for almost all Indian caeciliids, and to present results of analyses of these data that test the monophyly of *Gegeneophis*, *Indotyphlus*, Indian, and Indian + Seychelles caeciliids. These data and analyses also provide new molecular insights into the lower-level systematics of Western Ghats caeciliids.

## 2. Materials and methods

### 2.1. Taxon sampling and laboratory protocols

The taxon sampling and new sequences generated for this study are summarized in Tables 1 and 2, with localities for Indian samples indicated in Fig. 1. We built on the mt 12S and 16S rRNA sequences of Seychelles caecilians generated by Hedges et al. (1993 see Table 2) by sequencing cytochrome oxidase subunit 1 (cox1) and cytochrome b (cytb) for these same six samples (three genera; six species). Previous sequencing of Indian caeciliids has been limited to 12S and 16S data for *Gegeneophis ramaswamii* (Wilkinson et al., 2002) and *G. seshachari* (Gower et al., 2008), and complete mt genome and data for four nuclear genes for *G. ramaswamii* (San Mauro et al., 2004; Roelants et al., 2007). Our new sequencing of four mt genes for Indian caeciliids covers all Western Ghats species except *G. nadkarnii*. Sampling does not include the northeast Indian *Gegeneophis fulleri*, which is known from a single reported specimen collected more than 100 years ago (Alcock, 1904; Taylor, 1968; Pillai and Ravichandran, 1999; Ohler et al., 2004). Following preliminary analyses of mt sequence data, we also generated nuclear RAG1 sequences for 13 Indian caeciliid individuals (Table 2) with the aim of including nuclear data for each of the main lineages. We also include the first DNA sequence data for the genus *Indotyphlus*, sampling multiple individuals of both described species.

Das (2006) argued that the specific epithet *G. madhavai* Bhatta and Srinivasa (2004) included an incorrect suffix because Bhatta and Srinivasa (2004) named the species in honor of several people named Madhava, not one person, and so Das provided the emendation *Gegeneophis madhavaorum*. This emendation has been followed by at least some studies (e.g., Bhatta et al., 2007a,b) but following Brandon-Jones et al. (2007) and Dubois (2007) it is unjustified (as well as counter to nomenclatural stability), and so we here retain the original name and authority. The same nomenclatural decision has been reached by Frost (2010).

Protocols for DNA extraction and the amplification of 12S, 16S and cytb are those reported by Gower et al. (2002). Amplification

and sequencing of cox1 and RAG1 used the primers reported by San Mauro et al. (2004).

### 2.2. Sequence alignment and phylogenetics

Four datasets were produced, each with a different composition of taxa and genes. First, we built on the partial 12S and 16S dataset analysed by Gower et al. (2008) by adding San Mauro et al.'s (2004) data for the rhinatrematid *Rhinatrema bivittatum* plus new sequences for nine *Gegeneophis* and *Indotyphlus* individuals, each representing a major lineage of Indian caeciliid found among our total sampling (Dataset 1; Tables 1 and 2). This dataset includes representatives of all caecilian families, and it was employed to test the hypotheses that the Seychelles caeciliids and Western Ghats caeciliids are each monophyletic and sister groups, as indicated by previous studies (e.g., Wilkinson et al., 2002, 2003; Roelants et al., 2007; Gower et al., 2008; Zhang and Wake, 2009), albeit with much less dense sampling of Indian caeciliids. Second, we assembled a dataset (Dataset 2) of sequences for four mitochondrial genes (partial sequences of 12S, 16S, cox1, and cytb) for 45 Indian caeciliids using the Seychelles clade as sole outgroup. Third, and to check for local rooting problems, we analysed a reduced version of the second dataset (Dataset 3) including only the 45 Indian caeciliids, excluding Seychelles taxa. Fourth, we analysed an expanded version of the third dataset (Dataset 4) by including RAG1 data for thirteen of the Indian caeciliids (Table 2). Properties of the four datasets are reported in Table 3, and PAUP nexus files for each are available as Supplementary material.

For all datasets, nucleotide sequences were aligned using MAFFT version 6.708 (Katoh et al., 2002; Katoh and Toh, 2008) with default settings, and altered manually to correct for obvious misalignments. Alignment ambiguities were excluded using GBLOCKS version 0.19b (Castresana, 2000) with default parameter settings for block selection and the “With Half” option for gap allowance. For each molecular marker, independent alignments were prepared (available from the corresponding author), and the best-fit models of nucleotide substitution (for each individual marker and for combined datasets) were identified using the Akaike information criterion (AIC; Akaike, 1973) as implemented in Modeltest version 3.7 (Posada and Crandall, 1998). We also identified best-fit models for each codon position of each protein-coding gene (cox1, cytb, RAG1) separately. In all cases, the resulting best-fit model was GTR (Rodríguez et al., 1990) +  $\Gamma$  (Yang, 1994) + I (Reeves, 1992).

The datasets were analysed using some or all of maximum parsimony (MP; Fitch, 1971), neighbor-joining (NJ; Saitou and Nei, 1987), maximum likelihood (ML; Felsenstein, 1981), and Bayesian inference (BI; Huelsenbeck et al., 2001), as indicated in Table 4. MP and NJ analyses were performed with PAUP\* version 4.0b10 (Swofford, 1998). MP analyses used heuristic searches with 10 random stepwise addition sequences of taxa and Tree Bisection and Reconnection branch swapping. NJ analyses assumed ML distances based on the best-fit model of sequence evolution. ML analyses were conducted with RAxML version 7.0.4 (Stamatakis, 2006) using the rapid hill-climbing algorithm (Stamatakis et al., 2007). BI used MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) running four simultaneous Markov chains for 10 million generations, sampling every 1000 generations, and discarding the first one million generations as burn-in (as judged by plots of ML scores and low standard deviation of split frequencies) to prevent sampling before reaching stationarity. Two independent BI runs were performed to increase the chance of adequate mixing of the Markov chains, and to give some chance of spotting failure to converge. For both ML and BI analyses, model parameters were independently optimized for each partition (“unlink” option in effect). For the mt-only datasets including cox1 and

**Table 1**  
Voucher and locality data for the 51 Indian and Seychelles caeciliid samples used in the phylogenetic analyses. Indian localities given as: State, District, locality. Indian states (except Goa) abbreviated as follows: KA (Karnataka), KE (Kerala), MH (Maharashtra), TN (Tamil Nadu). Collection abbreviations as follows: BNHS (Bombay Natural History Society, Mumbai, India), BUB (Bangalore University, Bangalore, India), DU (Delhi University), UK (University of Kerala, Thiruvananthapuram, India), UMMZ (University of Michigan Museum of Zoology, Ann Arbor, USA). Topotypic (or likely within 10 km) samples for the Indian species are indicated by an asterisk. See Fig. 1 for distribution of Indian localities.

	Taxon	Voucher	Country	Locality
1	<i>Praslinia cooperi</i>	UMMZ 192933	Seychelles	Silhouette
2	<i>Hypogeophis rostratus</i>	UMMZ 189115	Seychelles	Silhouette
3	<i>Grandisonia alternans</i>	UMMZ 189118	Seychelles	Silhouette
4	<i>Grandisonia brevis</i>	UMMZ 192977	Seychelles	Mahé
5	<i>Grandisonia larvata</i>	UMMZ 193061	Seychelles	Silhouette
6	<i>Grandisonia sechellensis</i>	UMMZ 193076	Seychelles	Silhouette
7	<i>Indotyphlus battersbyi</i>	BNHS 5350	India	MH, Raigad, Matheran
8	<i>Indotyphlus battersbyi</i>	BNHS 4232	India	MH, Pune, Amba Valley*
9	<i>Indotyphlus maharashtraensis</i>	BNHS 4223	India	MH, Satara, Humbarli nr. Koyna*
10	<i>Indotyphlus maharashtraensis</i>	BNHS 4224	India	MH, Satara, Humbarli nr. Koyna*
11	<i>Indotyphlus maharashtraensis</i>	BNHS 4200	India	MH, Satara, Humbarli nr. Koyna*
12	<i>Indotyphlus maharashtraensis</i>	BNHS 4333	India	MH, Satara, Humbarli nr. Koyna*
13	<i>Gegeneophis danieli</i>	BNHS 4230	India	MH, Sundhudurg, nr. Amboli*
14	<i>Gegeneophis danieli</i>	BNHS 4803	India	MH, Sundhudurg, nr. Amboli*
15	<i>Gegeneophis danieli</i>	BNHS 4804	India	MH, Sundhudurg, nr. Amboli*
16	<i>Gegeneophis cf. danieli</i>	BNHS 5352	India	KA, Belgaum, Chorla Ghat
17	<i>Gegeneophis cf. danieli</i>	BNHS 5353	India	KA, Belgaum, Chorla Ghat
18	<i>Gegeneophis cf. danieli</i>	BUB 5281	India	KA, Belgaum, Chorla Ghat
19	<i>Gegeneophis cf. mhadeiensis</i>	BNHS 4233	India	MH, Sundhudurg, nr. Amboli
20	<i>Gegeneophis cf. mhadeiensis</i>	BNHS 5170	India	MH, Sundhudurg, nr. Amboli
21	<i>Gegeneophis cf. mhadeiensis</i>	BNHS 5171	India	MH, Sundhudurg, nr. Amboli
22	<i>Gegeneophis mhadeiensis</i>	BNHS 5351	India	KA, Belgaum, Chorla nr. Castle Rock*
23	<i>Gegeneophis mhadeiensis</i>	BNHS 5354	India	KA, Belgaum, Chorla nr. Castle Rock*
24	<i>Gegeneophis goensis</i>	No voucher	India	GOA, North Goa, Keri*
25	<i>Gegeneophis goensis</i>	No voucher	India	GOA, North Goa, Keri*
26	<i>Gegeneophis seshachari</i>	BNHS 4231	India	MH, Ratnagiri, Dorle*
27	<i>Gegeneophis seshachari</i>	BNHS 4602	India	MH, Ratnagiri, nr. Pawas
28	<i>Gegeneophis seshachari</i>	BNHS 4601	India	MH, Ratnagiri, nr. Morewadi
29	<i>Gegeneophis seshachari</i>	BNHS 4609	India	MH, Kolhapur, Baraki
30	<i>Gegeneophis seshachari</i>	BNHS 4610	India	MH, Kolhapur, Baraki
31	<i>Gegeneophis seshachari</i>	BNHS 4596	India	MH, Kolhapur, nr. Dajipur
32	<i>Gegeneophis seshachari</i>	BNHS 4599	India	MH, Kolhapur, Patacha Dang nr. Dajipur
33	<i>Gegeneophis madhavai</i>	BNHS 5349	India	KA, Udipi, Mudur
34	<i>Gegeneophis madhavai</i>	BUB 2840	India	KA, Udipi, Mudur
35	<i>Gegeneophis krishni</i>	BNHS 4176	India	KA, Dakshina Kannada, Bondantheil nr. Mangalore
36	<i>Gegeneophis krishni</i>	BUB 2790	India	KA, Dakshina Kannada, Bondantheil nr. Mangalore
37	<i>Gegeneophis krishni</i>	BUB 2785	India	KA, Dakshina Kannada, Bondantheil nr. Mangalore
38	<i>Gegeneophis krishni</i>	BUB 2659	India	KA, Dakshina Kannada, Bondantheil nr. Mangalore
39	<i>Gegeneophis sp.</i>	BNHS 5348	India	KA, Chikmagalur, Basarikatte nr. Kotegehar
40	<i>Gegeneophis sp.</i>	UK MW3421	India	KE, Kasaragod, Kakkadav-Bedoor
41	<i>Gegeneophis sp.</i>	BNHS 4177	India	KA, Kodagu, Madikeri
42	<i>Gegeneophis carnosus</i>	UK MW295	India	KE, Wayanad, Thalapuzha nr. Peria*
43	<i>Gegeneophis carnosus</i>	DU 1206	India	KE, Wayanad, Suganthagiri
44	<i>Gegeneophis ramaswamii</i>	UK MW2488	India	KE, Kottayam, Chotty nr. Kanjirappalli
45	<i>Gegeneophis ramaswamii</i>	UK MW669	India	KE, Kollam, nr. Punalur
46	<i>Gegeneophis ramaswamii</i>	UK MW708	India	KE, Kollam, nr. Punalur
47	<i>Gegeneophis ramaswamii</i>	UK MW163	India	KE, Kollam, nr. Punalur
48	<i>Gegeneophis ramaswamii</i>	UK MW331	India	KE, Kollam, Thenmalai*
49	<i>Gegeneophis ramaswamii</i>	UK MW1292	India	TN, Kanyakumari, Shonlode
50	<i>Gegeneophis ramaswamii</i>	UK MW1294	India	TN, Kanyakumari, Shonlode
51	<i>Gegeneophis ramaswamii</i>	UK MW436	India	TN, Kanyakumari, Shonlode

cytb (Datasets 2 and 3), two partition schemes were analysed, one in which each gene was a separate partition (four partitions), and one in which each codon position of each gene was separate (plus separate models for 12S and for 16S; eight partitions). The single dataset including RAG1 (Dataset 4) was partitioned by gene and by codon position (11 partitions), a seemingly reasonable partitioning scheme given San Mauro et al.'s (2009) results for caecilian mt and RAG1 data. Support for internal branches was evaluated by non-parametric bootstrapping (Felsenstein, 1985) with 1000 replicates performed with PAUP\* (MP and NJ) and RAxML (ML), and by posterior probabilities (PP, for BI).

### 3. Results

None of the datasets showed severe saturation, as judged by plots (not shown) of pairwise transition and transversion differ-

ences versus corrected (measured as maximum likelihood distance) sequence divergence. Separate analyses excluding cytb and cox1 third codon positions did not produce notably different results (no well-supported relationships overturned; no additional well-supported clades recovered) and are not reported here. All analyses of Dataset 1 recovered a clade of Indo-Seychelles caeciliids (bootstrap proportions 99–100; BI PP 1), in which both the Seychelles (bootstrap proportions 100; BI PP 1) and Indian (bootstrap 90–98; BI PP 1) caeciliids are monophyletic, as are both *Gegeneophis* (bootstrap proportions 90–98; BI PP 1) and *Indotyphlus* (bootstrap proportions 99–100; BI PP 1) (trees not shown). The recovery of, and quantitative support for, various clades from different analyses of Datasets 2–4 are given in Table 4, and this forms the basis for most of the rest of the Sections 3 and 4.

All analyses recovered best trees that include (with maximal support) the split between *Gegeneophis* and *Indotyphlus*, and are



**Table 2**

GenBank accession numbers for DNA sequences for Indian and Seychelles caeciliids. The samples used in the “all-Gymnophiona” 12S and 16S dataset for preliminary analyses (see Section 2) are indicated by asterisks. All sequences new to this study except those in bold from previously published studies (Hedges et al., 1993; San Mauro et al., 2004; Gower et al., 2008). For locality data and key to abbreviations for voucher specimens, see caption to Table 1.

	Taxon	Voucher	12S	16S	cytb	cox1	RAG1
1	<i>Praslinia cooperi</i> *	UMMZ 192933	<b>EF219347</b>	<b>EF219348</b>	HQ444069	HQ444114	
2	<i>Hypogeophis rostratus</i> *	UMMZ 189115	<b>EF219349</b>	<b>EF219350</b>	HQ444068	HQ444113	
3	<i>Grandisonia alternans</i> *	UMMZ 189118	<b>EF219351</b>	<b>EF219352</b>	HQ444064	HQ444109	
4	<i>Grandisonia brevis</i> *	UMMZ 192977	<b>EF219353</b>	<b>EF219354</b>	HQ444065	HQ444110	
5	<i>Grandisonia larvata</i> *	UMMZ 193061	<b>EF219355</b>	<b>EF219356</b>	HQ444066	HQ444111	
6	<i>Grandisonia sechellensis</i> *	UMMZ 193076	<b>EF219357</b>	<b>EF219358</b>	HQ444067	HQ444112	
7	<i>Indotyphlus battersbyi</i> *	BNHS 5350	HQ443974	HQ444017	HQ444059		
8	<i>Indotyphlus battersbyi</i>	BNHS 4232	HQ443973	HQ444016	HQ444058	HQ444104	HQ444125
9	<i>Indotyphlus maharashtraensis</i> *	BNHS 4223	HQ443976	HQ444019	HQ444061	HQ444106	HQ444126
10	<i>Indotyphlus maharashtraensis</i>	BNHS 4224	HQ443977	HQ444020	HQ444062	HQ444107	
11	<i>Indotyphlus maharashtraensis</i>	BNHS 4200	HQ443978	HQ444021	HQ444063	HQ444108	
12	<i>Indotyphlus maharashtraensis</i>	BNHS 4333	HQ443975	HQ444018	HQ444060	HQ444105	
13	<i>Gegeneophis danieli</i> *	BNHS 4230	HQ443948	HQ443991	HQ444033	HQ444080	HQ444121
14	<i>Gegeneophis danieli</i>	BNHS 4803	HQ443949	HQ443992	HQ444034		
15	<i>Gegeneophis danieli</i>	BNHS 4804	HQ443950	HQ443993	HQ444035	HQ444081	
16	<i>Gegeneophis cf. danieli</i> *	BNHS 5352	HQ443957	HQ444000	HQ444042	HQ444088	
17	<i>Gegeneophis cf. danieli</i>	BNHS 5353	HQ443958	HQ444001	HQ444043	HQ444089	
18	<i>Gegeneophis cf. danieli</i>	BUB 5281	HQ443959	HQ444002	HQ444044	HQ444090	
19	<i>Gegeneophis cf. mhadeiensis</i>	BNHS 4233	HQ443945	HQ443988	HQ444031	HQ444079	HQ444120
20	<i>Gegeneophis cf. mhadeiensis</i>	BNHS 5170	HQ443947	HQ443990	HQ444032		
21	<i>Gegeneophis cf. mhadeiensis</i>	BNHS 5171	HQ443944	HQ443987	HQ444030	HQ444078	
22	<i>Gegeneophis mhadeiensis</i>	BNHS 5351	HQ443940	HQ443983	HQ444026	HQ444074	HQ444118
23	<i>Gegeneophis mhadeiensis</i>	BNHS 5354	HQ443942	HQ443985	HQ444028	HQ444076	
24	<i>Gegeneophis goaensis</i>		HQ443941	HQ443984	HQ444027	HQ444075	
25	<i>Gegeneophis goaensis</i>		HQ443943	HQ443986	HQ444029	HQ444077	HQ444119
26	<i>Gegeneophis seshachari</i>	BNHS 4231	HQ443970	HQ444013	HQ444055	HQ444101	HQ444124
27	<i>Gegeneophis seshachari</i>	BNHS 4602	HQ443971	HQ444014	HQ444056	HQ444102	
28	<i>Gegeneophis seshachari</i>	BNHS 4601	HQ443972	HQ444015	HQ444057	HQ444103	
29	<i>Gegeneophis seshachari</i> *	BNHS 4609	<b>EU364649</b>	<b>EU364650</b>			
30	<i>Gegeneophis seshachari</i>	BNHS 4610	HQ443967		HQ444052	HQ444098	
31	<i>Gegeneophis seshachari</i>	BNHS 4596	HQ443968	HQ444011	HQ444053	HQ444099	
32	<i>Gegeneophis seshachari</i>	BNHS 4599	HQ443969	HQ444012	HQ444054	HQ444100	
33	<i>Gegeneophis madhavai</i>	BNHS 5349	HQ443956	HQ443999	HQ444041	HQ444087	HQ444123
34	<i>Gegeneophis madhavai</i> *	BUB 2840	HQ443955	HQ443998	HQ444040	HQ444086	
35	<i>Gegeneophis krishni</i>	BNHS 4176	HQ443954	HQ443997	HQ444039	HQ444085	HQ444122
36	<i>Gegeneophis krishni</i>	BUB 2790	HQ443953	HQ443996	HQ444038	HQ444084	
37	<i>Gegeneophis krishni</i>	BUB 2785	HQ443952	HQ443995	HQ444037	HQ444083	
38	<i>Gegeneophis krishni</i> *	BUB 2659	HQ443951	HQ443994	HQ444036	HQ444082	
39	<i>Gegeneophis sp.</i>	BNHS 5348	HQ443938	HQ443981	HQ444024	HQ444072	HQ444117
40	<i>Gegeneophis sp.</i>	UK MW3421	HQ443946	HQ443989			
41	<i>Gegeneophis sp.</i>	BNHS 4177	HQ443937	HQ443980	HQ444023	HQ444071	HQ444116
42	<i>Gegeneophis carnosus</i> *	UK MW295	HQ443936	HQ443979	HQ444022	HQ444070	HQ444115
43	<i>Gegeneophis carnosus</i>	DU 1206	HQ443939	HQ443982	HQ444025	HQ444073	
44	<i>Gegeneophis ramaswamii</i>	UK MW2488	HQ443964	HQ444007	HQ444049	HQ444095	
45	<i>Gegeneophis ramaswamii</i>	UK MW669	HQ443965	HQ444008	HQ444050	HQ444096	
46	<i>Gegeneophis ramaswamii</i>	UK MW708	HQ443966	HQ444009	HQ444051	HQ444097	
47	<i>Gegeneophis ramaswamii</i>	UK MW163	HQ443961	HQ444004	HQ444046	HQ444092	
48	<i>Gegeneophis ramaswamii</i> *	UK MW331	<b>AY456250</b>	<b>AY456250</b>	<b>AY456250</b>	<b>AY456250</b>	<b>AY456255</b>
49	<i>Gegeneophis ramaswamii</i>	UK MW1292	HQ443960	HQ444003	HQ444045	HQ444091	
50	<i>Gegeneophis ramaswamii</i>	UK MW1294	HQ443962	HQ444005	HQ444047	HQ444093	
51	<i>Gegeneophis ramaswamii</i>	UK MW436	HQ443963	HQ444006	HQ444048	HQ444094	

thus consistent with the monophyly of these two genera. All of our analyses recovered *Praslinia* as the sister taxon to all other Seychelles caecilians except for parsimony analysis of the India + Seychelles caeciliid dataset. Support for the monophyly of *Grandisonia* + *Hypogeophis* where this clade appeared was generally high (BI PP 1; ML and NJ bootstrap 88–90), whereas none of the relationships within the Seychelles clade was strongly supported in the incongruent trees from MP analysis (bootstrap <60). Apart from MP and NJ, all other analyses (ML and BI, with four or eight partitions) recovered the same set of relationships among the remaining (non-*Praslinia cooperi*) Seychelles species: (*Grandisonia alternans*, ((*Hypogeophis rostratus* + *Grandisonia brevis*) (*Grandisonia larvata* + *Grandisonia sechellensis*))) although quantitative support is generally unconvincing (Table 4).

Apart from some differences in the resolution of relationships that are not robustly resolved in any analyses, the Indian-caeciliid-only and India + Seychelles datasets yield very similar trees,

so that the in- or exclusion of the Seychelles radiation as an out-group does not substantially affect inferred relationships among Indian caeciliids. The best ML tree for the nine-partition analysis of the Indian + Seychelles dataset (Dataset 2) is shown in Fig. 2. Almost all lettered clades in Fig. 2 are recovered in other analyses of this dataset. Apart from different arrangements of individuals within *G. ramaswamii*, ML and BI analyses of Dataset 4 did not differ from each other. A separate analysis excluding the 32 Indian samples for which RAG1 data were not available was conducted and did not produce notably different results (not reported here) to the 45-leaf dataset (no well-supported relationships overturned; no additional well-supported clades recovered).

All of the strongly supported relationships present in the best ML and BI trees are also recovered in all other analyses, whereas the less well-supported clades are not universally recovered (Table 4). Thus, Clade Q in Fig. 2, comprising a radiation of all the most northern *Gegeneophis* in peninsular India except *G. seshachari*, and

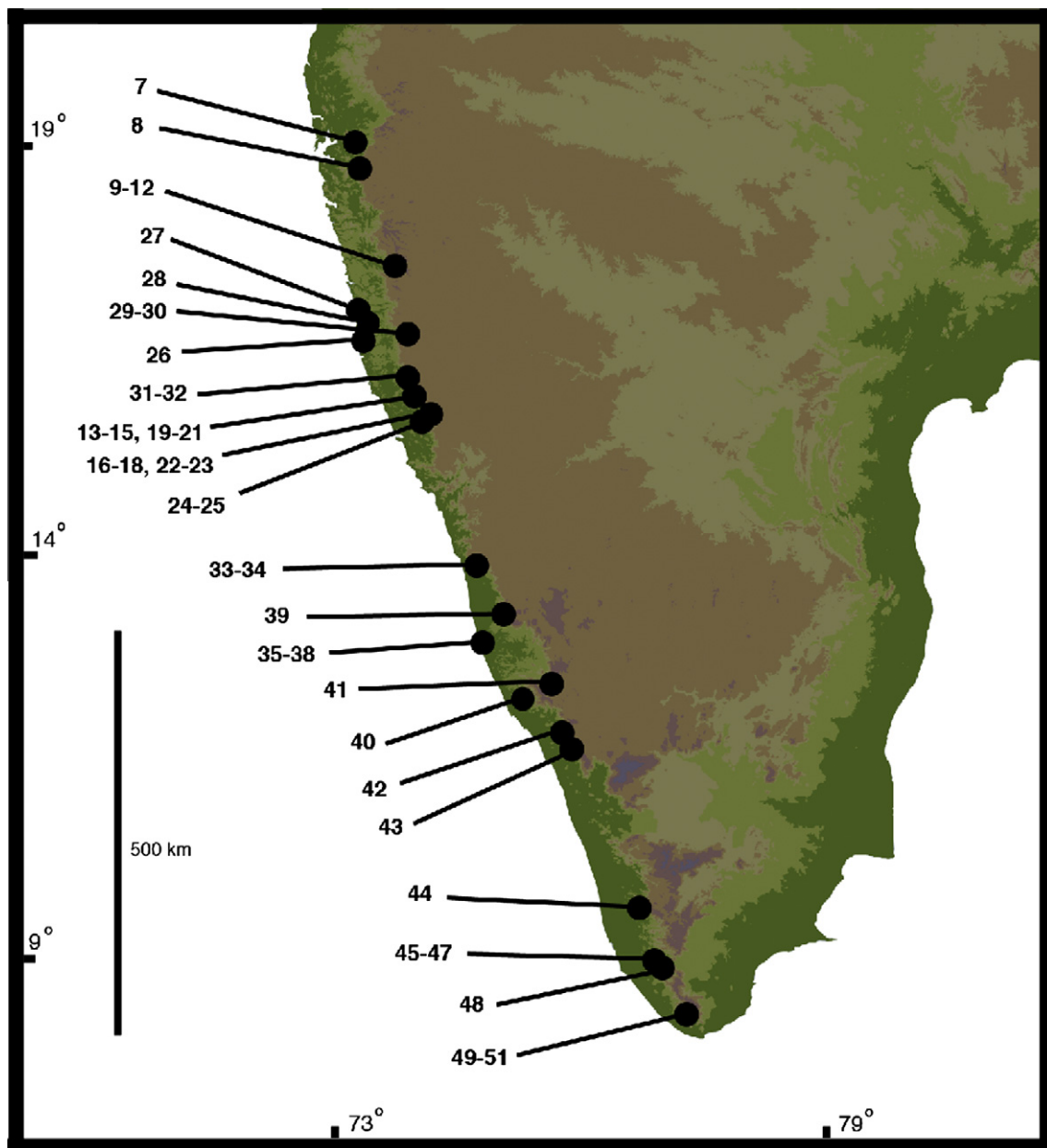


Fig. 1. Map of peninsular India showing position of origin of 45 Indian caeciliid samples. See Table 1 for taxon and locality details.

Table 3

Properties of the four datasets. VUP = variable but parsimony-uninformative; VIP = variable and parsimony-informative.

	1. Gymnophiona	2. India + Seychelles Caeciliidae	3. India Caeciliidae	4. India Caeciliidae
Leaves	34	51	45	45
Markers	12S + 16S	12S, 16S, cytb, cox1	12S, 16S, cytb, cox1	12S, 16S, cytb, cox1, RAG1
Total sites	790 (340 + 450)	2536 (374 + 509 + 813 + 840)	2536 (374 + 509 + 813 + 840)	4045 (374 + 509 + 813 + 840 + 1509)
Constant	342 (137 + 205)	1538 (227 + 358 + 436 + 517)	1632 (255 + 376 + 464 + 537)	3021 (255 + 376 + 464 + 537 + 1389)
VUP	83 (36 + 47)	94 (16 + 15 + 29 + 34)	62 (10 + 9 + 20 + 23)	125 (10 + 9 + 20 + 23 + 63)
VIP	365 (167 + 198)	904 (131 + 136 + 348 + 289)	842 (109 + 124 + 329 + 280)	899 (109 + 124 + 329 + 280 + 57)

all of its constituent relationships are constant and consistently strongly supported. In contrast, the sister group of *G. ramaswamii* is not robustly resolved in any analysis. All analyses except the MP analysis of the India + Seychelles dataset recover a clade of all *Gegeneophis* except *G. seshachari*, but this is never strongly supported. Similarly, most analyses recover a clade comprising *G.*

*krishni*, *Gegeneophis carnosus* and the three samples (39–41 in Table 1) not identified to species level, but this clade is also never strongly supported (Table 4).

Least well resolved (poorly supported) are the interrelationships of five well-supported clades of *Gegeneophis* (D, E, G, Q and V, Fig. 2). Experimental pruning of each of these lineages, or of

**Table 4**

Patterns of support for selected clades recovered in one or more of the different analyses. Support values given are bootstrap proportions for maximum likelihood (ML), maximum parsimony (MP) and neighbor-joining (NJ) and posterior probabilities for Bayesian inference (BI). For ML and BI analyses, support values are given for analyses of data partitioned by gene (ML, BI) and by gene plus codon position (MLc, BIc). BI posterior probabilities multiplied by 100 for ease of comparison with bootstrap values. Letters in left hand column relate to clades indicated in Fig. 2. "\*" indicates maximum support (100).

Taxon data Character data Method	India + Seychelles Caeciliidae						India Caeciliidae (45 leaves)						mt + RAG1	
	mt (12S, 16S, cytb, cox1)						mt (12S, 16S, cytb, cox1)							
	MLc	BIc	ML	BI	MP	NJ	MLc	BIc	ML	BI	MP	NJ	MLc	BIc
<i>Clade</i>														
A	96	*	*	*	*	*	*	*	*	*	*	*	*	*
B	*	*	*	*	*	94	*	*	*	*	*	91	*	*
C	96	*	*	*	19	92	95	*	96	*	15	92	94	*
D	*	*	*	*	*	*	*	*	*	*	*	*	*	*
E	*	*	*	*	*	*	*	*	*	*	*	*	*	*
F	42	82	42	60	39	65	52	80	41	78	35	*	44	82
G	*	*	*	*	*	*	*	*	*	*	*	*	*	*
H	31	71	37	33	16	16	8	39	11	22	16	16	19	24
I	*	*	*	*	*	*	*	*	*	*	*	*	*	*
J	*	*	*	*	*	*	*	*	*	*	*	*	*	*
K	*	*	*	*	*	86	*	*	*	*	*	86	*	*
L	*	*	*	*	*	*	*	*	*	*	*	*	*	*
M	*	*	*	*	*	*	*	*	*	*	*	*	*	*
N	99	*	99	*	88	56	*	*	*	*	88	56	*	*
O	*	*	*	*	*	98	*	*	*	*	99	98	*	*
P	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Q	*	*	*	*	*	*	*	*	*	*	*	99	*	*
R	*	*	*	*	*	*	*	*	*	*	*	*	*	*
S	*	*	*	*	*	*	*	*	*	*	*	*	*	*
T	98	*	99	*	76	79	*	*	99	*	76	80	99	*
U	*	*	*	*	*	*	*	*	*	*	*	*	*	*
V	*	*	*	*	*	*	*	*	*	*	*	*	*	*
W	53	90	37	54	32	40	20	88	45	75	55	67	39	71
X' and/or X''	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Y	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Z	*	*	*	*	*	*	*	*	*	*	*	*	*	*
F + G	27	21	37	45	14	38	59	55	64	62	21	47	40	64
F + Q	3	<5	3	<5	27	11	3	<5	<5	<5	37	10	40	<5
G + V	1	<5	1	<5	35	19	5	<5	5	<5	19	8	3	<5
1. <i>H. rostratus</i> + <i>G. brevis</i>	77	93	58	69	20	7								
2. <i>G. larvata</i> + <i>G. sechellensis</i>	88	98	79	91	28	13								
3. As above, excluding <i>G. alternans</i>	53	89	49	66	<5	<5								
4. <i>Hypogeophis</i> + <i>Grandisonia</i>	90	*	88	*	21	98								
<i>G. alternans</i> + <i>Praslinia cooperi</i>	3	<5	6	<5	58	<5								

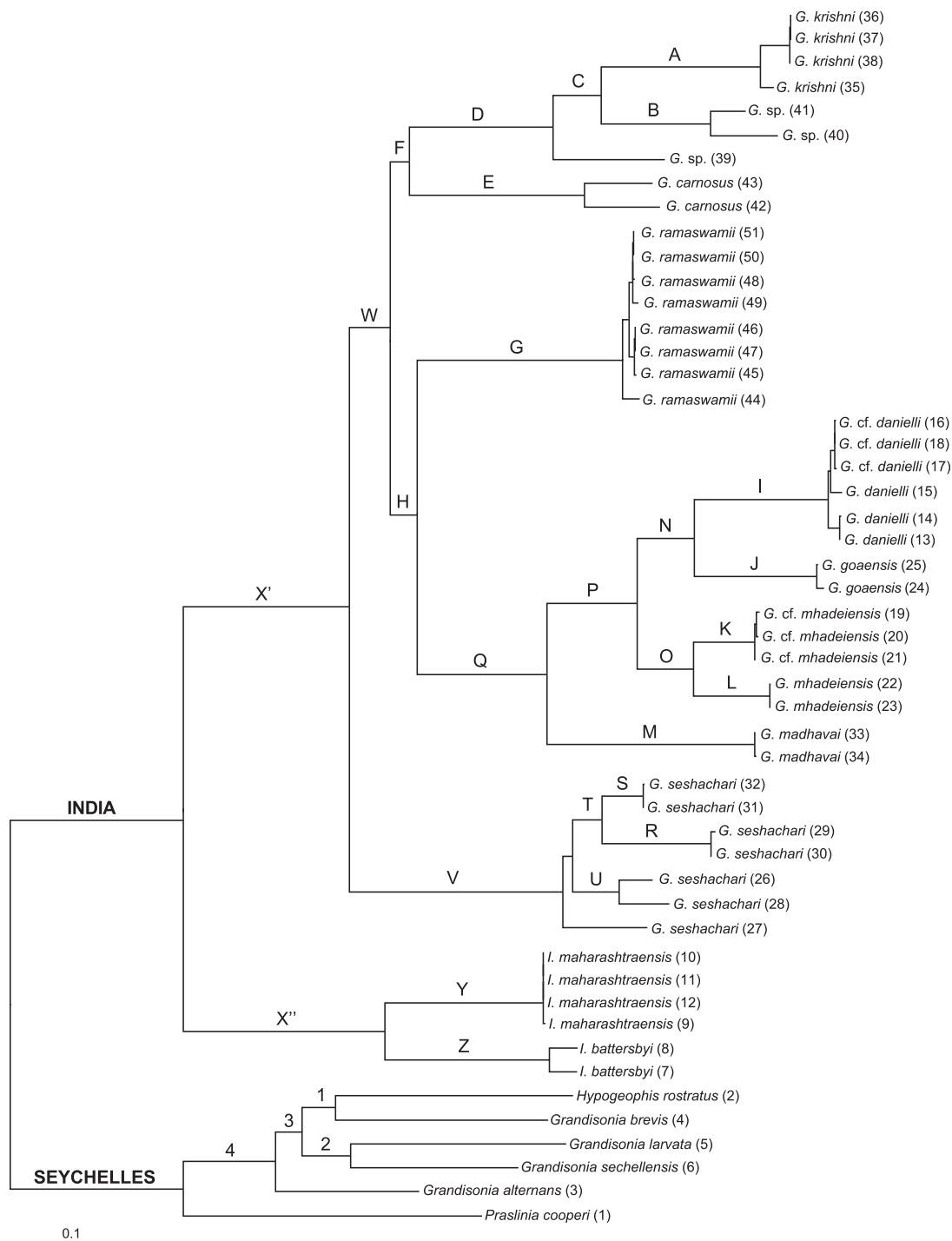
pairs of these lineages, from the ML bootstrap trees from analysis of Dataset 4 and recalculation of consensus trees reveals that not all of the low support for their interrelationships can be attributed to the instability of any one of these clades, but that the three clades (G, V and Q) are the most unstable, with their instability concealing some noteworthy support. For example, pruning only Q reveals bootstrap support of 68% for the grouping of D, E and G to the exclusion of V, and pruning both G and Q reveals more impressive bootstrap support (84%) for the grouping of D and E to the exclusion of V, and pruning both G and V reveals 65% support for the grouping of D and E to the exclusion of Q. Clearly then, the greatest uncertainty pertains to the positions of clades G (*G. ramaswamii*), V (*G. seshachari*), and especially Q (*G. danieli*, *G. goansensis*, *G. mhadeiensis*, *G. madhavai*).

For the most part, haplotypes from specimens identified a priori as conspecific grouped strongly as clades. There is no evidence from our data for more than one species represented by Samples 13–18 (*G. danieli* and *G. cf. danieli*). Genetic *p*-distances between conspecifics are generally low (Table 5), especially for individuals from the same or a nearby locality. The two largest samples present contrasting results, with the eight *G. ramaswamii* exhibiting very little genetic differentiation (mean 0.81%) and the seven *G. seshachari* showing notable structure and high diversity (mean 6.74%) over a similarly sized geographic area (Fig. 1). The allopatric *G. mhadeiensis* and *G. cf. mhadeiensis* samples are reciprocally (and strongly) monophyletic and reasonably distant (mean 5.42%, Table 5).

## 4. Discussion

### 4.1. Caecilian phylogeny

Although limited by the exclusion of the very poorly known (a single reported specimen) and geographically distant *G. fulleri*, our new data and analyses provide further support for monophyly of Indo-Seychelles caeciliid caecilians, and of the Indian and Seychelles components of this clade. They also provide the first molecular evidence for monophyly of *Indotyphlus*, as well as extended evidence for monophyly of *Gegeneophis*. These are important results that support ongoing morphological efforts to resolve and revise caecilian taxonomy. The monophyly of *Gegeneophis* in particular is a non-trivial result because the great recent expansion in number of recognized species (see Section 1) includes forms with more visible eyes (see Giri et al., 2003), terminal keels (Gower et al., 2007), viviparity (Gower et al., 2008), and with greater numbers (e.g., *G. danieli*, *G. nadkarnii*) or total absence (*G. seshachari*) of secondary annuli – features not present in the first few species described in the genus, and characters widely used in diagnoses of other caecilian genera (Taylor, 1968; Wilkinson and Nussbaum, 2006). Thus, our results provide welcome support for Ravichandran et al.'s (2003) rediagnosis of the genus *Gegeneophis*. Although the two nominate species of *Indotyphlus* are very similar to each other and have been considered likely sister species, none of the diagnostic morphological features of the genus is uniquely derived (Giri et al., 2004; Wilkinson and Nussbaum, 2006). Monophyly of



**Fig. 2.** Maximum likelihood tree of Western Ghats Indian (*G* = *Gegeneophis*; *I* = *Indotyphlus*) and Seychelles caeciliids based on Dataset 2 (see Table 3) comprising sequences of four partial mt genes (partitioned by gene and codon position). Details of samples numbered (1)–(51) are given in Table 1. Quantitative support for nodes A–Z and 1–4 is given in Table 4. Scale indicates substitutions per site.

Indian caeciliids was suggested tentatively by Giri et al. (2004) and Wilkinson and Nussbaum (2006) based on biogeography and overall similarity.

#### 4.2. Seychelles caecilians

All known caecilians in the Seychelles (six species in three genera; Nussbaum, 1984) are members of a single radiation of caeciliids (see also Nussbaum and Ducey, 1988; Hedges et al., 1993). Previous molecular studies based on 12S and 16S failed to resolve

robustly the relationships among the three genera and six species of Seychelles caecilians, other than that the monotypic *Praslinia* is the sister taxon to *Hypogeophis* + *Grandisonia* (Hedges et al., 1993; Wilkinson et al., 2002, 2003; Loader et al., 2007; Gower et al., 2008; see also Roelants et al., 2007; Zhang and Wake, 2009).

Our new data for the six Seychelles species have more than doubled the available characters for comprehensive phylogenetic analysis at the species level, yet little improvement in robust resolution was reached. Although other data and/or approaches are required to resolve this more robustly, we interpret the levels of support



**Table 5**

Mean% *p*-distance (number of pairwise comparisons in parentheses) for 2536 aligned bp of 12S, 16S, cytb and CO1. Taxa as follows: lb (*Indotyphlus battersbyi*); lm (*I. maharashtraensis*); Gd (*Gegeneophis danieli*); Gcn (*G. cf. nadkarnii*); Gcmh (*G. cf. mhadeiensis*); Gmh (*G. mhadeiensis*); Gg (*G. goensis*); Gs (*G. seshachari*); Gma (*G. madhavai*); Gk (*G. krishni*); Gsp (*G. sp.*); Gc (*G. carnosus*); Gr (*G. ramaswamii*). Numbers in parentheses in left hand column and top row refer to sample numbers detailed in Table 1. Values of note (discussed in text) in bold type.

	lb (7–8)	lm (9–12)	Gd (13–15)	Gcn (16–18)	Gcmh (19–21)	Gmh (22–23)	Gg (24–25)	Gs (26–32)	Gma (33–34)	Gk (35–38)	Gsp (39)	Gsp (40)	Gsp (41)	Gc (42–43)	Gr (44–51)
lb (7–8)	<b>2.07</b> (1)														
lm (9–12)	9.43 (8)	0.06 (6)													
Gd (13–15)	13.91 (6)	14.94 (12)	0.8 (3)												
Gcn (16–18)	14.37 (6)	15.46 (12)	<b>0.98</b> (9)	0.14 (3)											
Gcmh (19–21)	14.07 (6)	14.91 (12)	8.35 (9)	8.7 (9)	0.23 (3)										
Gmh (22–23)	14.02 (4)	15.51 (8)	8.86 (6)	9.62 (6)	<b>5.42</b> (6)	0.04 (1)									
Gg (24–25)	14.66 (4)	15.22 (8)	8.48 (6)	8.79 (6)	8.42 (6)	8.88 (3)	0.46 (1)								
Gs (26–32)	14.56 (14)	15.31 (28)	13.42 (21)	14.34 (21)	13.72 (21)	14.03 (14)	14.38 (14)	<b>6.74</b> (21)							
Gma (33–34)	14.3 (4)	14.91 (8)	11.06 (6)	11.56 (6)	9.86 (6)	11.18 (4)	11.91 (4)	14.26 (14)	0.13 (1)						
Gk (35–38)	15.26 (8)	15.54 (16)	13.57 (12)	14.42 (12)	13.29 (12)	14.06 (8)	14.23 (8)	15 (28)	14.07 (6)	1.25 (6)					
Gsp (39)	14.52 (2)	14.86 (4)	12.72 (3)	13.43 (3)	12.45 (3)	13 (2)	13.57 (2)	14.14 (7)	14.03 (2)	9.73 (4)	– (0)				
Gsp (40)	12.53 (2)	12.99 (4)	11.45 (3)	11.51 (3)	10.85 (3)	9.62 (2)	10.18 (2)	12.05 (7)	11.39 (2)	6.1 (4)	6.34 (1)	– (0)			
Gsp (41)	14.88 (2)	15.58 (4)	13.73 (3)	14.36 (3)	13.77 (3)	13.96 (2)	15.08 (2)	14.32 (7)	14.21 (2)	10 (4)	9.16 (1)	2.36 (1)	– (0)		
Gc (42–43)	14.45 (4)	15.78 (8)	12.22 (6)	13.03 (6)	12.15 (6)	13.23 (4)	13.25 (4)	13.67 (14)	13.01 (6)	12.9 (8)	12.27 (2)	10.35 (2)	12.73 (2)	<b>5.68</b> (1)	
Gr (44–51)	14.1 (16)	15.45 (32)	12.6 (24)	13.35 (24)	12.09 (24)	12.2 (16)	13.29 (16)	12.93 (56)	12.91 (16)	13.53 (32)	12.4 (8)	10.66 (8)	13.24 (8)	12.06 (16)	<b>0.81</b> (28)

for the results of the different analyses (Table 4) to indicate (far from compellingly) that the most likely set of relationships is (*P. cooperi* ((*Granisonia alternans* (*Hypogeophis*, *Granisonia brevis*) (*Granisonia larvata*, *Granisonia sechellensis*))), such that *Grandisonia* is paraphyletic with respect to *Hypogeophis*.

### 4.3. Indian caeciliids

No previous phylogenetic hypotheses have been forwarded for Indian caeciliids (other than the suggested monophyly of *Indotyphlus*; Giri et al., 2004). Our sampling in terms of numbers of individuals and species is impressive among molecular phylogenetic studies of any group of caecilians published to date. For the Western Ghats region of peninsular India we sample all (or all but one) nominal (and perhaps some undescribed) species. For most of these species, our samples substantially increase the meagre number of specimens in museum collections. For example, we add six *G. seshachari* to the previously reported three specimens (Gower et al., 2008), four *G. krishni* to the previous two (Pillai and Ravichandran, 1999), two *G. madhavai* to the previous two (Bhatta and Srinivasa, 2004), two *G. goensis* to the previous three (Bhatta et al., 2007b), two *G. mhadeiensis* to the previous six (Bhatta et al., 2007c, 2010), at least two *G. carnosus* to the previous two (Beddome, 1870), and at least three *G. danieli* to the previous one (Giri et al., 2003).

Our analyses recover several well-supported clades of species and indicate that much of the lack of robust resolution of the whole tree lies with the unclear positions of *G. ramaswamii*, *G. seshachari*, and Clade Q. Although more data would be welcome, additional sampling of *G. carnosus*- and *G. krishni*-like animals (Samples 39–41) would also be beneficial given the genetic diversity and lack of phylogenetic cohesion recovered here. Referral of specimens to *G. carnosus* from beyond the type locality in Kerala, and especially of populations from further into Karnataka (e.g., Bhatta, 1998; Malathesh et al., 2002; Taylor, 1968; Venu and Venkatachalaiah, 2006) seems to be unwarranted, and further field effort and morphological and well as molecular data for these and similar populations are a priority in striving for a complete and stable *Gegeneophis* taxonomy and phylogeny.

The sampled *G. danieli* specimens (13–15 in Table 1) are topotypic. The “*G. cf. danieli*” specimens (16–18) were collected from a locality from where the *G. danieli*-like *G. nadkarnii* (but not *G. danieli*) has been reported (Bhatta et al., 2007a) and between the type localities for *danieli* (ca. 24 km) and *nadkarnii* (ca. 35 km). Material referable to *G. nadkarnii* has not been included in our study but *G. danieli* and *G. nadkarnii* are morphologically similar, and we expect them to be sister taxa if both species are valid. *Gegeneophis cf. mhadeiensis* (samples 19–21) might represent a previously undescribed species. It is from a locality ca. 35 km from the type locality of its sister group, *G. mhadeiensis*, from which it differs by a mean *p*-distance (5.42%, Table 5) that is much higher than most of the intraspecific distances of the other species included here (Table 5). Similarly, the geographic structure and high genetic diversity within our *G. seshachari* sample (mean *p*-distance 6.74%, Table 5) suggest that a closer examination of the conspecificity of this material is warranted (V. Giri, unpublished data). Although based on only two specimens, the two sampled populations of *Indotyphlus battersbyi* are also relatively genetically distant, with the taxonomy of the population from the relatively isolated hill station of Matheran worthy of closer examination. Venu and Venkatachalaiah (2005) reported two variants in chromosomal complements for *Gegeneophis* specimens from the type locality for *G. ramaswamii* and suggested that these were indicative of species-level differences. However, Venu and Venkatachalaiah's (2005) voucher specimens all resemble *G. ramaswamii* (at least superficially), and the new DNA sequence data presented here are in accord with morphology (D.J. Gower, M. Wilkinson, pers. obs.; B. Presswell, unpublished data) in supporting the hypothesis that there is only a single species of *Gegeneophis* known from the southern end of the Western Ghats (South of the Palghat Gap).

Other than the close relationship between the northernmost Kerala and southernmost Karnataka samples (samples 35–41 and to a lesser degree 42–43), biogeographic structure is most apparent in the monophyly of all the *Gegeneophis* from the most northerly peninsular samples (Clade Q in Fig. 2), and in the internal relationships of this clade, in which the most southern sample (*G. madhavai*) is the sister taxon to a clade comprising the remaining samples.

The new fieldwork carried out to obtain our tissue samples, along with our results, provide support for a primarily allopatric pattern of distribution for Indian caeciliids. Thus far, the only reported instances of sympatry are *G. danieli* and *G. cf. mhadeiensis* at Amboli, southern Maharashtra (Fig. 1; Table 1), and *G. mhadeiensis*, *G. goaensis* and *G. nadkarnii* in the border area between Goa and northern Karnataka (Bhatta et al., 2007a,c, 2010), though given the number of species in the region, there is potential for further instances especially in southern Maharashtra, Goa and northern Karnataka.

Other than studies of *G. ramaswamii* (e.g., Müller et al., 2005; Ramaswami, 1943, 1948; Scheltinga et al., 2003; Taylor, 1969, 1972, 1977a,b; Wake, 1968, 1994) and a brief description of scales in *G. carnosus* (Taylor, 1972) the anatomy of Indian caeciliids is known only from external morphology. Until more detailed work is done on other taxa, we remain ignorant whether morphological characters exist that support the more robust nodes in the molecular phylogeny presented here, and potentially serve to resolve the other parts of the tree. An exception lies perhaps in the superficial resemblance of the members of clade P in their well-marked (whitish) annular grooves and relatively high numbers of primary annuli that bear secondary grooves (Giri et al., 2003; Bhatta and Prashanth, 2004; Bhatta et al., 2007b,c).

The chromosomal complement of all species of the Seychelles radiation is  $2n = 26$  (Nussbaum and Ducey, 1988), and this is true also for *I. battersbyi*, "*G. carnosus*" (from the same locality as sample 41 in this study) and *G. krishni* (Venkatachalaiah et al., 2006; Venu and Venkatachalaiah, 2006). Among those Indian caeciliid species karyotyped thus far, only *G. ramaswamii* differs, with  $2n = 28$  or  $30$  (Venu and Venkatachalaiah, 2005). Assuming that multiple independent reductions to  $2n = 26$  have not occurred within the India-Seychelles clade, the higher complement in *G. ramaswamii* (and its position, nested within a clade of otherwise  $2n = 26$  species) might be evidence of fission, especially given that the fundamental number across the other Indian and Seychelles species is almost constant (50 or 52).

Interpretation of phenotypic patterns from the phylogeny is further limited by the poorly resolved position of *G. ramaswamii* and *G. seshachari*. *Gegeneophis* is the only caecilian genus known to have both oviparous (*G. ramaswamii*) and viviparous (*G. seshachari*) species (Gower et al., 2008), but the lack of well resolved relationships for these two species, and lack of information on the reproductive mode of other Indian caeciliids precludes insight into reproductive evolution. Additional complication in interpreting reproductive evolution is caused by variation among the Seychelles caecilians, which are all oviparous but include species with direct-development and larvae (Nussbaum, 1984, unpublished data).

Given the relative lack of detailed knowledge on caecilian ecology and natural history (e.g., Gower and Wilkinson, 2005), the IUCN conservation status of most species is dictated currently by the size of species' distributions, being either small (Threatened) or large (Least Concern). Assessing species' ranges depends fundamentally on accurate and precise species-level taxonomy and, beyond that, extensive fieldwork. Many Indian caeciliid species are known only from point localities and were described from small samples. Our molecular (phylogenetic and *p*-distance) results provide confidence for the interpretation that almost all described species are distinct. In the case of *G. ramaswamii*, the low genetic diversity (Table 5) of samples from a relatively wide area (in which this species tolerates agricultural ecosystems; Oommen et al., 2000) supports this species' current taxonomy and Least Concern conservation status (IUCN, 2010). Indeed, *G. ramaswamii* is the only Indian caeciliid that is not Data Deficient. While our results impart some confidence in current taxonomy, more fieldwork is required to boost sample sizes and clarify distributions as well as natural history.

## Note added in proof

Wilkinson et al. (in press) propose a taxonomic revision of caecilians in which the Indian and Seychelles "caeciliids" referred to here are transferred from the Caeciliidae to the Indotyphlidae, and the Seychelles species *Grandisonia brevis* is transferred to *Hypogeophis*.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympmv.2011.03.002.

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