Remarkable genetic homogeneity in unstriped, long-tailed *Ichthyophis* along 1500 km of the Western Ghats, India

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Abstract

The mountainous Western Ghats are part of a biodiversity hotspot and extend for about 1600 km, lying close and subparallel to the west coast of peninsular India. The region is a centre of diversity for amphibians, and recent preliminary work on some components of both caecilian (Gymnophiona) and frog (Anura) fauna is indicative of a high degree of local endemism. We investigated diversity in mitochondrial rRNA 12S and 16S sequences for long-tailed, unstriped *Ichthyophis* (Amphibia: Gymnophiona: Ichthyophiidae) that potentially represent four taxonomically confused and poorly known endemic caecilian species. Data were analysed for 18 individuals from along *c*. 1500 km of the Western Ghats region. Genetic diversity is remarkably low, with a maximum uncorrected *p*-distance of 0.5%. These DNA sequences and new morphological data do not allow us to reject the null hypothesis that the sample comprises only a single, widely distributed, highly interconnected species. The phylogenetic signal among the data is extremely low. However, population genetic analyses reveal that the Palghat Gap, a *c*. 30 km discontinuity in the Western Ghats considered to be significant in the biogeography of other organisms occurring in this region, corresponds to a significant subdivision of long-tailed, unstriped *Ichthyophis* into two groups.

Introduction

The Western Ghats, a *c*. 1600-km-long mountain chain lying close to the west coast of peninsular India (e.g. Dahanukar, Raut & Bhat, 2004), are part of a global biodiversity hotspot (Myers *et al*., 2000; Bossuyt *et al*., 2004). The region is a recognized centre of diversity for the limbless, superficially snake-like, caecilian amphibians (Gymnophiona: Taylor, 1961; Gower *et al*., 2004; Ravichandran, 2004). Recently, renewed interest in the systematics of Indian caecilians has led to the recognition of several new species from the Western Ghats (Pillai & Ravichandran, 1999; Giri, Wilkinson & Gower, 2003; Ravichandran, Gower & Wilkinson, 2003; Bhatta & Prasanth, 2004; Bhatta & Srinivasa, 2004; Giri, Gower & Wilkinson, 2004), in agreement with a theoretical prediction (Bhatta, 1997) and with recent progress in the systematics of anuran amphibians of the same region (Biju, 2001; see Gower *et al*., 2004). The recognition of new species of caecilians from the Western Ghats has

been based on traditional taxonomic data, and the impact of molecular techniques has yet to be assessed (Gower *et al*., 2004). Limited work to date has indicated that for Western Ghats uraeotyphlids (Gower, Wilkinson & Oommen, 2001) and striped ichthyophiids (Gower *et al*., 2002), mitochondrial (mt) DNA sequence data provide evidence of previously unrecognized diversity characterized by high levels of local endemism.

Until 1960, only three species of unstriped ichthyophiid were recognized, with most unstriped specimens from throughout south and south-east Asia identified as *Ichthyophis monochrous* (Bleeker, 1858). Taylor (1960) proposed a radically different concept of *I. monochrous* that restricted its distribution to Borneo, and he described no fewer than four new species of unstriped *Ichthyophis* (*Ichthyophis bombayensis, Ichthyophis malabarensis, Ichthyophis peninsularis, Ichthyophis subterrestris*) based on just seven specimens from peninsular India, all of which have a relatively long tail compared with Sri Lankan and south-east Asian

unstriped ichthyophiids. Recent molecular work has supported the distinctiveness of this morphotype, has indicated that the presence or absence of a stripe does not differentiate monophyletic groups of *Ichthyophis*, and that at least one of the long-tailed, unstriped species of peninsular India is more closely related to uraeotyphlid caecilians than to other ichthyophiids (Gower *et al.*, 2002; Frost *et al.*, 2006). Generally small sample sizes and little understanding or concern for intraspecific variation in morphology has meant that Taylor's (1960, 1968) taxonomy of ichthyophiids has proven controversial (e.g. Inger, 1954; Nussbaum & Gans, 1980; Gower *et al.*, 2002). Published keys are mostly inadequate for identifying many of the *Ichthyophis* that Taylor described, and diagnoses often do not provide a compelling basis for the recognition of distinct species. Consequently, considerable confusion and taxonomic uncertainty exists over Taylor's four nominate species of Western Ghats long-tailed, unstriped *Ichthyophis* (e.g. Pillai & Ravichandran, 1999; Ravichandran & Krishnamurthy, 2001; Dutta, 2002; Vyas, 2004). For example, a population from Palod in southern Kerala has been referred to as both *I.* cf. *peninsularis* (Oommen *et al.*, 2000; Frost *et al.*, 2006) and *I.* cf. *malabarensis* (Gower *et al.*, 2002; Bossuyt *et al.*, 2004), and it remains very uncertain as to whether *I. bombayensis* is restricted to Gujarat or is more widespread in the Western Ghats (Ravichandran & Krishnamurthy, 2001; Vyas, 2004). Resolution of this taxonomic confusion is considered a priority for accurate conservation assessment (IUCN, Conservation International & NatureServe, 2004; Vyas, 2004).

Study of a single population of long-tailed unstriped *Ichthyophis* from the vicinity of the type locality of *I. bombayensis* (Vyas, 2003, 2004) indicates that the morphological variation among only 26 specimens almost subsumes that of Taylor's (1960) original seven specimens that he ascribed to four different species. This suggests the null hypothesis that the four nominate forms that seem to lack distinguishing morphological characters are actually conspecific. Here we use mtDNA sequence data to investigate haplotype, nucleotide and possible specific diversity in long-tailed, unstriped *Ichthyophis* from along most of the length of the Western Ghats.
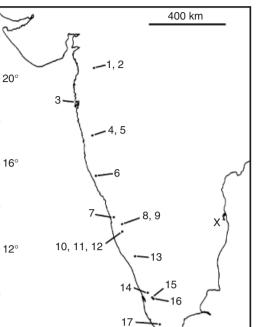
Materials and methods

Sampling

A total of 18 adult long-tailed, unstriped *Ichthyophis* were collected from 13 Western Ghats localities in six states, spanning a total distance of *c.* 1500 km, from the Dangs region of Gujarat in the north to Shonlode in Tamil Nadu in the south (Table 1, Fig. 1). Terrestrial caecilians burrow in the soil for much of their adult lives, so that most of our specimens had to be collected by digging. All but one sample (see Gower *et al.*, 2002) was newly collected for this study. The samples were taken from every population encountered in fieldwork conducted since 1998. Sampling was not influenced by attempts to identify species a priori, although it did cover populations considered by local workers to probably or possibly represent Taylor's (1960) four species. As far as can be ascertained, our localities spanned the entire geographic range of Taylor's (1960) original material. Of the type localities given by Taylor (1960), only one has reasonably precise data (Surat, *I. bombayensis*); the others are at a place we have failed to locate (Maduvangard, *I. malabarensis*) or

Table 1 Details of Western Ghats long-tailed, unstriped *Ichthyophis* sampled for this study

	Voucher	Locality	District	State	GenBank accessions
1	BNHS 4338	Near Bardipada	Dangs	Gujarat	DQ919025, 40
2	BNHS 4344	Near Bardipada	Dangs	Gujarat	DQ919026, 41
3	BNHS 4830	Near Sanjay Gandhi National Park	Mumbai	Maharashtra	DQ919027, 42
4	BNHS 4226	Near Chiplun	Ratnagiri	Maharashtra	DQ919020, 43
5	BNHS 4199	Near Chiplun	Ratnagiri	Maharashtra	DQ919029, 44
6	BNHS 4516	Dhave, near Sanquelim	North Goa	Goa	DQ919030, 45
7	BNHS 4517	Mudur	Udupi	Karnataka	DQ919031, 46
8	BNHS 4518	Near Sringeri	Chikmagalur	Karnataka	DQ919032, 47
9	BNHS 4519	Near Sringeri	Chikmagalur	Karnataka	DQ919033, 48
10	BNHS 4520	Charmadi	Dakshina Kannada	Karnataka	DQ919034, 49
11	BNHS 4521	Charmadi	Dakshina Kannada	Karnataka	DQ919035, 50
12	BNHS 4522	Charmadi	Dakshina Kannada	Karnataka	DQ919036, 51
13	BNHS 4523	Peria	Wayanad	Kerala	DQ919037, 52
14	BNHS 4549	Vettalapara	Ernakulam	Kerala	DQ919038, 53
15	MW 1464	Peralamattayam	Idukki	Kerala	AY700954*, 972*
16	MW 433	Thodupuzha	Idukki	Kerala	AY700953*, 971*
17	MW 198	Palode	Thiruvanathapuram	Kerala	AY101205*, 225*
18	MW 442	Shonlode	Kanyakumari	Tamil Nadu	DQ919039, 54

Voucher specimens are in the collections of the Bombay Natural History Society (BNHS) , Mumbai and the University of Kerala (MW field tags). Localities are indicated in Fig. 1. Horizontal line between samples 13 and 14 represents the Palghat Gap between 10°35′N and 10°50′N.
*Signifies data obtained from a previous study (Gower *et al.*, 2002).

Figure 1 Map of western peninsular India showing provenance of the 18 Western Ghats samples of long-tailed, unstriped *Ichthyophis* listed in Table 1. The X near the south-eastern coast of the peninsula indicates the location of the Eastern Ghats population reported by Ramaswami (1947). The Western Ghats run subparallel and close to the west coast of the peninsula, and are broken by the Palghat Gap, a potentially significant biogeographic barrier between 10°35′N and 10°50′N.

at large historical regions (Malabar, *I. peninsularis*; Travancore, *I. subterrestris*).

Genomic DNA was extracted from liver samples stored in absolute ethanol using a mixture of standard phenol–chloroform techniques (Sambrook, Fritsch & Maniatis, 1989) and Qiagen DNAeasy kits. We selected one fragment each of the mt 12S and 16S rRNA genes for analysis. These markers were selected on the basis of ease of amplification and sequencing, and existing comparative data for long-tailed, unstriped *Ichthyophis*, other ichthyophiids and other caecilians (Gower *et al.*, 2002, 2005; Wilkinson *et al.*, 2002*b*, 2003), as well as evidence that these genes have a blend of faster and more conservatively evolving sites and evolve at an overall rate informative for caecilian generic, specific and subspecific taxonomy (Gower *et al.*, 2002, 2005). Primers were as reported by Gower *et al.* (2002).

Data analysis

Nucleotide diversity and a minimum spanning tree were calculated using ARLEQUIN (Schneider, Roessli & Excoffier, 2000) with uncorrected *p*-distances. ARLEQUIN was also used to test the significance of hierarchical structure using an analysis of molecular variance (AMOVA; Excof-

fier, Smouse & Quattro, 1992) based on 1000 permutations of *p*-distances and an a priori division of the sample into two populations, from north (samples 1–13, Table 1) and south (14–18) of the Palghat Gap, a potentially important biogeographic barrier. PAUP* (Swofford, 2001) was used to infer most parsimonious trees (MPTs) and their bootstrap (Felsenstein, 1985) support, as well as to determine the permutation tail probability (PTP) of the data in order to test for significant hierarchical structure (Faith & Cranston, 1991). All PAUP searches were heuristic using tree bisection–reconnection and ten random addition sequences. A limit of 10 000 saved trees was set for the main search for MPTs, and of 1000 trees for the 1000 bootstrap replicates. Mantel tests of correlation between geographic distances (km) and genetic (uncorrected *p*-) distances were executed using MANTEL (Cavalcanti, 2005) with 100 000 permutations.
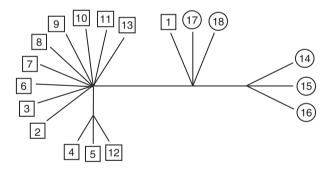
Results

For the concatenated 12S and 16S data, sequences were 918 (11 individuals), 917 (6) or 916 (1) base pairs (bp) long. Most (91%) of the few ambiguous/missing data sites ($<1\%$ of total sites) are confined to the beginning of seven of the 16S sequences. Alignment by hand was trivial and comprised 378 bp 12S and 540 bp 16S. Sequences are remarkably conservative, with only nine positions being variable and six informative under parsimony; these are distributed among at least 10 haplotypes (Table 2). A parsimony PTP test of the six parsimony informative characters for the 18 individuals did not allow the null hypothesis that these have no more hierarchical structure than expected by chance alone to be rejected ($P = 0.018$). Rejection of the PTP null hypothesis can be interpreted as a minimum requirement for data to be analysed phylogenetically (Faith & Cranston, 1991; Wilkinson *et al.*, 2002*a*). Given the lack of phylogenetic structure and very few informative characters relative to the number of taxa, we did not attempt a definitive estimate of phylogenetic relationships. Despite this, the strict consensus of MPTs (Fig. 2) and the minimum spanning tree (not shown) are compatible with a split between those samples from north (samples 1–13) and south (14–18) of the Palghat Gap. There are no fixed nucleotide differences between these two groups, although all northern samples, except one from Gujarat, (sample 1) have adenine at positions 427 and 536 instead of guanine or cytosine, respectively (Table 2). Unsurprisingly, this north–south phylogenetic split receives far from compelling statistical support – a bootstrap value of only 25% under parsimony. However, the mean uncorrected *p*-distance among samples from the north (0.1565%; $n = 78$ comparisons) and south (0.0654%; $n = 10$) is less than that between pairs from the two regions (0.3506%; $n = 65$), and AMOVA found that more than half (53%) of the variance is explained by differences between the groups north and south of the Palghat Gap, and that this structuring is highly significant ($P \approx 0$). The correlation between genetic and geographic distance is positive and significant for the entire dataset (Mantel test, $P = 0.0058$), but most of this is explained by

Table 2 Variable characters in 918 aligned bases of partial 12S (positions 1–378) and 16S (379–918) rRNA mitochondrial gene sequences for long-tailed, unstriped *Ichthyophis* from along the Western Ghats

Sample	Nucleotide position								
	1 1 1	1 8 4	3 1 6	3 4 8	3 9 0	3 9 7	4 2 7	5 3 6	8 6 9
1	C	T	A	A	A	A	G	C	T
2	–	?	G	G	?	–	A	A	–
3	–	–	G	G	–	–	A	A	–
4	–	–	G	G	C	–	A	A	–
5	–	–	G	G	C	–	A	A	–
6	–	–	–	G	?	G	A	A	–
7	–	–	G	G	?	–	A	A	–
8	–	–	G	G	?	?	A	A	–
9	–	–	G	G	?	?	A	A	–
10	A	–	G	G	?	–	A	A	–
11	–	–	G	G	?	?	A	A	C
12	–	–	G	–	C	–	A	A	–
13	–	–	G	G	–	–	A	A	–
14	–	C	G	G	–	–	–	–	–
15	–	C	G	G	–	–	–	–	–
16	–	C	G	G	–	–	–	–	–
17	–	–	G	G	–	–	–	–	–
18	–	–	G	G	–	–	–	–	–

Sample numbers and horizontal line between samples 13 and 14 as in Table 1.

Figure 2 Unrooted strict consensus of 10 000 most parsimonious trees showing inferred relationships among long-tailed, unstriped *Ichthyophis*. The consensus is compatible with (but does not entail) a split between samples from north (squares) and south (circles) of the Palghat Gap. Numbers correspond to samples given in Table 1 and Fig. 1. Tree length is only 11 steps, and the split between north and south of the Palghat Gap receives bootstrap support of only 25%.

differences across the Palghat Gap, because correlations are not significant within the northern ($P = 0.0619$) or southern ($P = 0.0352$) groups.

Uncorrected *p*-distances and nucleotide diversity for the entire sample are similar to those among a smaller sample of *Icthyophis glutinosus* from a much smaller area in Sri Lanka (Table 3). Pairwise genetic distances are much higher for considerably smaller samples of specimens referred to two

striped species of *Ichthyophis* across small geographic distances (<200 km) within the Western Ghats (Table 3).

On the basis of the morphometric and meristic features that were used to discriminate Taylor's four species, specimens referable to *I. bombayensis* [holotype plus Vyas's (2003) topotypic sample plus the very large BNHS 4830] span or exceed the entire range of values for the other three nominate species (Table 4).

Discussion

Before this study, a picture of high genetic diversity among caecilian lineages along the Western Ghats was emerging. Evidence for this includes the recognition of several new species of apparently largely allopatrically distributed caeciliids (e.g. Giri *et al.*, 2003, 2004; Bhatta & Srinivasa, 2004) and multiple previously unrecognized genetic lineages of Western Ghats striped *Ichthyophis* (Gower *et al.*, 2002) and *Uraeotyphlus* (Gower *et al.*, 2001). In strong contrast, the results of this study demonstrate that Western Ghats long-tailed, unstriped *Ichthyophis* probably constitute a single, widely distributed but extremely genetically homogeneous species.

The Western Ghats are almost continuous along their *c.* 1600 km length. The most pronounced break along this range is the *c.* 30 km Palghat Gap (e.g. Daniels, 1992; Biju, 2001; Dahanukar *et al.*, 2004, Biju & Bossuyt, 2005). This feature is a flat, low-lying (to below 100 m asl) region approximately between 10°35′N and 10°50′N, with steep-sided and largely rocky northern and southern margins, that is very dry outside of the monsoon (D. J. Gower, O.V. Oommen & M. Wilkinson, pers. obs.). This gap has been interpreted as an important biogeographic barrier (e.g. Bossuyt *et al.*, 2004), with species and/or genetic lineages of several groups of organisms thought to be restricted to areas either north or south of this (e.g. plants, Subramanyam & Nayar, 1974; birds, Ali & Ripley, 1987; fish, Dahanukar *et al.*, 2004; frogs, Biju & Bossuyt, 2005; elephants, Vidya *et al.*, 2005). Long-tailed, unstriped *Ichthyophis* are on found both sides of the gap, and few, if any, dedicated attempts to find caecilians within the gap itself have been made. We discovered some genetic structure within long-tailed, unstriped *Ichthyophis* across the Palghat Gap, but the signal is not overwhelming and sampling close to the northern edge of the gap is poor.

Our molecular results do not allow us to reject the null hypothesis based on the morphology that Taylor's four species of Western Ghats long-tailed, unstriped *Ichthyophis* represent only a single recognizable taxon. Given the precise, known locality and most detailed low-level study of morphology (Vyas, 2003, 2004), we select *I. bombayensis* Taylor (1960) as the name to be applied to this species. We thus consider *I. malabarensis* Taylor (1960), *I. peninsularis* Taylor (1960) and *I. subterrestris* Taylor (1960) to be junior synonyms of *I. bombayensis* Taylor (1960). We offer the following simple but adequate rediagnosis of the species: an unstriped *Ichthyophis* differing from all other unstriped species, except *Ichthyophis javanicus*, in having a long tail (more than ten annuli behind the vent), and differing from

Table 3 Variation in mitochondrial DNA sequences for samples of six species of Indian and Sri Lankan *Ichthyophis* in the context of a variety of geographic ranges

Taxon	n	Aligned sites	Distance (km)	Haplotypes	Variable characters		p-distance %	Nucleotide diversity
					12S	16S		
Ichthyophis glutinosus	13	910	173	6	3	4	0–0.552	0.00269
Ichthyophis orthoplicatus	2	911	< 5	2	1	0	0.11	
Ichthyophis sp.	3	911	0	1	0	0	0	
Ichthyophis beddomei	3	920	180	2	4	6	0–1.09	
Ichthyophis tricolor	2	924	63	2	4	9	1.408	
Ichthyophis bombayensis	18	918	1500	10	2	7	0–0.561	0.002647

Data for *I. beddomei* and *I. tricolor* from Gower *et al.* (2002); for *I. glutinosus, I. orthoplicatus* and *I.* sp. from Gower *et al.* (2005); for *I. bombayensis* from this study.

Table 4 Ranges for meristic and morphometric data for the type series of the four long-tailed, unstriped species of Indian *Ichthyophis* compared with the best-studied population from Gujarat (Vyas, 2003) and the largest known specimen (BNHS 4380, shown in this study to be genetically indistinguishable from topotypic *Ichthyophis bombayensis*)

	Taylor (1960)				Vyas (2003)	This study
	I. bombayensis	*I. malabarensis*	*I. peninsularis*	*I. subterrestris*	*I. bombayensis*	BNHS4830
Sample size	1	1	3	2	19	1
TL (mm)	390	494	222–330	260–295	180–542	*c.* 700
Annuli	386	360	357–366	356–364	352–402	373
Tail folds	14	14	18	16–18	15–18	16
Vent folds	7	6	8	8	6–8	7
Vertebrae	121	111	116	?	112–123	b
TL/tail	*c.* 25	23.5	*c.* 22	22.2	20.1–28.5	40.6
TL/W	26	27	20–22	21	23.7–35.8	20.9
Mx–pmx	45	58	40–49	42–54	38–54[a]	71
VP	48	58	38–51	42–53	36–51[a]	b
D	40	56	30–49	34–40	31–46[a]	58
Spl	18	20	7–8	11–16	6–18[a]	b

'Tail folds' corresponds to the number of annular grooves on the tail, from the anterior of the vent.

TL, total length; W, maximum body width; Mx–pmx, maxillary–premaxillary teeth; VP, vomeropalatine teeth; D, dentary teeth; Spl, splenial teeth.

[a]Data from this study were taken for 10 specimens encompassing the size range of this sample.

[b]Count not made. All specimens are metamorphosed.

I. javanicus in having fewer (<22) splenial teeth, a proportionally longer tail (total length divided by tail length ≪40) and in being less attenuate (total length divided by body width ≪35).

Our taxonomic conclusions provide a solution to the curious, previous reports of up to three extremely similar, sympatric species (e.g. Pillai & Ravichandran, 1999: maps III, V), and Taylor's (1960, pp. 83–84) speculation as to why an animal as large as his *I. malabarensis*, known then from only a single specimen, could be so poorly known. All recent work on the taxonomy of Indian caecilians has resulted only in a description of new species rather than synonymy of existing taxa (e.g. Gower *et al.*, 2004), a situation in contrast to some other regions including Sri Lanka (Nussbaum & Gans, 1980) and the Neotropics (e.g. Wilkinson & Nussbaum, 1992). This first detailed application of molecular techniques to the taxonomy of Indian caecilians supports a dramatic reduction in the recognized diversity of long-tailed, unstriped *Ichthyophis*. However, our additional

unpublished observations suggest that further substantial synonymy is unlikely and that most future taxonomic work will result instead in the recognition of additional Western Ghats species.

Previously, the biology of Taylor's four species was poorly known and shrouded in taxonomic confusion. Our recognition of only a single species pulls together a reasonable body of disparate information that makes *I. bombayensis* a much better known caecilian (Table 5). This is exemplified by conservation assessment data. In the 2004 Global Amphibian Assessment (IUCN *et al.*, 2004), all four of Taylor's species were recognized as 'Data Deficient' – *I. bombayensis* because of 'continuing uncertainties as to its extent of occurrence, status and ecological requirements', and *I. malabarensis, I. peninsularis* and *I. subterrestris* because of 'continuing uncertainty as to its taxonomic status as well as absence of recent information on its extent of occurrence, status and ecological requirements'. Additionally, a range map was presented for only *I. bombayensis*, and

Table 5 Literature on the long-tailed, unstriped species of *Ichthyophis* from the Western Ghats of India

Report	Taxa	Context
Thurston (1888)	*mo*	Systematics, distribution
Boulenger (1890)	*mo*	Systematics, distribution
Taylor (1960)	*b, ma, p, s*	Systematics
Taylor (1961)	*b, ma, p, s*	Systematics
Daniel (1963)	*b, ma, p, s*	Distribution, natural history
Satyamurti (1967)	*mo*	Systematics
Taylor (1968)	*b, ma, p, s*	Systematics
Wake (1968, 1970*a*, 1972)	*p, s*	Morphology
Wake (1970*b*)	*p*	Morphology
Jaisingh (1978)	*p*	Distribution
Balakrishna *et al.* (1982)	*b*	Taxonomy morphology
Balakrishna *et al.* (1983)	*ma*	Reproduction
Seshachar *et al.* (1982)	*ma*	Reprroduction
Inger & Dutta (1987)	*b, ma, p, s*	Distribution
Nussbaum & Wilkinson (1989)	*b, ma, p, s*	Systematics
Das & Whitaker (1990)	*p*	Distribution, habitat
Andrews & George (1993)	*ma, p, s*	Checklist
Krishnamurthy & Shakuntala (1993)	*b, ma*	Distribution
Krishnamurthy (1996)	*b*	Distribution
Daniels (1992)	*b, ma, p, s*	Distribution
Bhatta (1997)	*ma*	Distribution, habitat
Dutta (1997)	*b, ma, p, s*	Systematics, distribution
Bhatta (1998)	*b, ma, p, s*	Systematics, distribution
Das & Dutta (1998)	*b, ma, p, s*	Checklist
Pillai & Ravichandran (1999)	*b, ma, p, s*	Systematics, distribution
Bhatta (1999)	*m*	Feeding, reproduction
Krishnamurthy & Hussain (2000)	*b*	Distribution
Oommen *et al.* (2000)	cf. *p*	Distribution
Ravichandran & Krishnamurthy (2001)	*b, ma, p*	Systematics, distribution
Chanda (2002)	*b, ma, p, s*	Systematics, distribution
Ghate (2002)	*b, s*	Checklist
Naik & Vinod (2002)	b	Conservation biology
Venkatachalaiah & Venu (2002)	m	Karyology
Gower *et al.* (2002); [12]Bossuyt *et al.* (2004)	cf. *ma*	Molecular phylogenetics
Vyas (2000, 2003, 2004); Singh *et al.* (2000)	b	Distribution, natural history, ecology, conservation, taxonomy
Ravichandran (2004)	*b, ma, p, s*	Distribution
Frost *et al.* (2006)	cf. *p*	Molecular phylogenetics
Wilkinson and Nussbaum (2006)	*b, ma, p, s*	Systematics

Taxon abbreviations: *b, bombayensis*; *ma, malabarensis*; *mo, monochrous*; *p, peninsularis*; *s, subterrestris*.

the taxonomy of all four species was stated to be uncertain and in need of revision. Given that only a single species is valid, we suggest that *I. bombayensis* can now be assessed as 'Least Concern', given that it is distributed throughout the Western Ghats region and occurs in several different disturbed and agricultural habitats (e.g. Bhatta, 1997; Oommen *et al.*, 2000; Vyas, 2003, 2004). This does not mean that the species is not threatened locally (e.g. Vyas, 2003). Our conclusions further underline the importance of taxonomy in conservation biology (e.g. Gower & Wilkinson, 2005).

Excluding the unlikely possibility of some sort of selective sweep, the very low genetic diversity, lack of phylogeographic structure, and uncompelling relation between genetic and geographic distance suggest that *I. bombayensis*

populations along the length of the Western Ghats region are highly genetically interconnected and/or have obtained their distribution through rapid, recent expansion from a geographically and genetically restricted ancestral population. Fossorial limbless vertebrates have been considered to have relatively limited dispersal capabilities (e.g. Cadle *et al.*, 1990; Daniels *et al.*, 2005) that may contribute to reducing interpopulation gene flow (Reid, Ashton & Zamudio, 2004), but this is not apparent in our sampling of *I. bombayensis*. That the genetic diversity of *I. bombayensis* is low across a great geographic distance, and much lower than for other Western Ghats caecilian lineages, begs the question why? However, understanding Western Ghats caecilian biology is too incomplete to draw firm conclusions. For example, very little is known generally about details of

distribution, autecology and reproductive biology. However, some characteristics of *I. bombayensis* point toward areas for possible further investigation. *Icthyophis bombayensis* has the largest known clutch size of any caecilian species, the > 100 eggs (Bhatta, 1999) being much greater than the next largest known for other Western Ghats taxa (< 40 for *Icthyophis beddomei*; G. Bhatta, unpubl. data; Kupfer, Nabithabatha & Himstedt, 2004: table 5). *Icthyophis bombayensis* larvae have more strongly developed tail fins than other ichthyophiids, the adults have the longest tails of any Indian caecilian and the species is perhaps more aquatic than other Western Ghats caecilians. Evidence for the latter is extremely scanty, but many of our samples were collected in close proximity to moderately sized streams and/or larger rivers, and adults of the species have been found swimming in water (anecdotal report given to J. George; Pillai & Ravichandran, 1999: 15). In Gujarat, this species can be found in freshwater crab burrows at the margins of water bodies (Vyas, 2004). Finally, the only caecilian species recorded from the Eastern Ghats (Fig. 1) of peninsular India is a long-tailed, unstriped *Ichthyophis* potentially conspecific with *I. bombayensis* (Ramaswami, 1947, 1948; Satyamurti, 1967).

That *I. bombayensis* has a range of at least 1500 km across 12° of latitude prompts other biological considerations. This species is found in a wide variety of habitats under very different climatic regimes. For example, in the northern part of its range, *I. bombayensis* can be found in dry deciduous riverine forest in regions with a yearly rainfall of < 2000 mm and up to 7 or 8 dry months. In contrast, further south in its range, this species can be found in moist, shady, evergreen plantations and forest in regions with a yearly rainfall of up to > 7000 mm and as few as 2 or 3 dry months (D. J. Gower *et al.*, pers. obs.; Daniels, 1992; Gimaret-Carpentier, Dray & Pascal, 2003; Vyas, 2003, 2004; Davidar, Puyravaud & Leigh, 2005).

The taxonomy, distribution and range of most caecilians are poorly circumscribed (Gower & Wilkinson, 2005), but a few species are thought to have ranges comparable to, or greater than, that reported here for *I. bombayensis*. Seven species with potentially very large ranges (*c*. 1 to over 10 million km^2; IUCN *et al.*, 2004) are the south-east Asian *Ichthyophis kohtaoensis*, African *Geotrypetes seraphini*, and South American *Siphonops annulatus, Siphonops paulensis, Caecilia tentaculata, Potomotyphlus kaupii* and *Typhlonectes compressicauda*, but the low-level taxonomy of these species has not yet been subjected to detailed molecular or morphometric analyses.

As we recognize only a single, long-tailed, unstriped species of *Ichthyophis* in the Western Ghats, the onus is now on us and other workers to test this hypothesis with further fieldwork and molecular and morphological analyses. Our preliminary studies of morphology suggest that our hypothesis concerning Taylor (1960) four species will be corroborated (Table 4), but additional taxa may remain undiscovered. Further sampling and analysis might be encouraged, especially at new localities and for individuals that are morphometrically and meristically extralimital. Taxon-

omy may change in the future, and therefore we urge workers reporting aspects of the biology of *I. bombayensis* to collect vouchers and detailed locality information. The intraspecific structure across the Palghat Gap also merits further investigation through additional sampling, analysis of more rapidly evolving genetic markers and more comparative genetic data for other groups (see also Vidya *et al.*, 2005).

Poor knowledge of caecilian biology currently precludes firm conclusions on the explanation for why *I. bombayensis* is unlike most other Western Ghats amphibian species in having such a large range. Pronounced climatic gradients are thought to have had a telling impact on the diversity and distribution of Western Ghats organisms, particularly amphibians (e.g. Daniels, 1992; Gadgil, 1996; Gimaret-Carpentier *et al.*, 2003; Davidar *et al.*, 2005), making the biology of *I. bombayensis* and how it might vary along the Western Ghats an intriguing system worthy of further investigation.

Acknowledgements

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