Frequency of independent origins of viviparity among caecilians (Gymnophiona): evidence from the first 'live-bearing' Asian amphibian

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Abstract

Viviparity is reported for *Gegeneophis seshachari* (Gymnophiona: Caeciliidae) from a gravid female containing four oviductal foetuses. The oviducts are highly vascularized and contain patches of thickened, layered tissue similar to foetal gut contents. *Gegeneophis seshachari* probably resemble other viviparous caecilians in having foetuses that ingest thickened oviduct lining using specialized deciduous teeth. This is the first report of viviparity in Asian amphibians and Indo-Seychellean caeciliids. *Gegeneophis* is the only caecilian genus known to include oviparous and viviparous species, and *G. seshachari* is the smallest known viviparous caecilian. Phylogenetic analysis of mitochondrial DNA sequences supports assignment of *G. seshachari* to a monophyletic *Gegeneophis*. Character optimization indicates that viviparity has evolved independently at least four times within Gymnophiona – a rate of incidence relative to the number of extant species that is higher than for other vertebrate groups except squamate reptiles. Our findings strengthen the proposal that caecilian reproduction demands further attention.

Introduction

The evolution of viviparity in multiple lineages of vertebrates has attracted attention as a fascinating study system in comparative reproductive biology. Most work has focused on amniotes. Studies of the relatively few instances of viviparity in amphibians have lacked a complete, robust evolutionary context because of the absence of basic reproductive data for many species and incomplete phylogenetic frameworks. Most viviparous amphibians are caecilians (order Gymnophiona), an understudied group for which biological knowledge has progressed slowly (e.g. Gower & Wilkinson, 2005).

Recent work has highlighted that the approximately 170 extant species (Wilkinson & Nussbaum, 2006) of

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caecilian amphibians exhibit an impressive diversity of reproductive biology. As far as is known, all species have internal fertilization via copulation (e.g. Gower & Wilkinson, 2002), and reproductive modes include oviparity with a larval stage (presumably the ancestral condition), oviparity with direct development and viviparity (*sensu* Blackburn, 1992, 2000). Furthermore, there are several variations within each major mode (e.g. Loader *et al.*, 2003; Kupfer *et al.*, 2006; Wilkinson & Nussbaum, 2006). Parental care is extensive and also diverse within Gymnophiona. To the best of our knowledge, all oviparous caecilians practice egg guarding, and the newborn of some (but not all) direct developing and perhaps viviparous species receive post-paritive maternal care, sometimes including provision of nutrition (e.g. Kupfer *et al.*, 2004, 2006; Wilkinson *et al.*, 2008). Where known, the foetuses of all viviparous caecilians and the neonates of some oviparous species have a specialized, deciduous ('foetal' or 'foetal-like') dentition (e.g. Parker, 1936, 1956; Parker & Dunn, 1964) used for scraping/peeling

and ingesting the hypertrophied lining of the oviduct or skin, respectively, of the mother (e.g. Wake, 1993; Kupfer *et al.*, 2006).

Caecilians are clearly a fascinating system, but the reproductive biology of most species remains unknown and knowledge of their phylogenetic relationships has only recently begun to improve (Gower & Wilkinson, 2005; Wilkinson & Nussbaum, 2006). Lack of a well-founded caecilian phylogeny has previously limited evolutionary insights because the number and timing of transitions between even the major modes, including viviparity, has remained obscure (Reynolds *et al.*, 2002). Lack of data on reproductive biology has also contributed to the majority of caecilian species being of 'data deficient' conservation status (Gower & Wilkinson, 2005). Here, we provide the first report of viviparity in the Indo-Seychellean clade of caeciliid caecilians (the first report of viviparity for any Asian amphibian) and demonstrate that it represents a previously unrecognized and independent (at least the fourth) evolutionary origin of viviparity for caecilians. Our report increases the knowledge of caecilian reproductive biology and underlines the suitability of the group for comparative studies of reproductive evolution. We use the taxonomic classification of Wilkinson & Nussbaum (2006).

Materials and methods

Reproductive biology

The Indian caeciliid caecilian *Gegeneophis seshachari* Ravichandran, Gower & Wilkinson was described in 2003 from a single specimen collected in 1967. Subsequently, a second specimen was reported from the type locality (Gower *et al.*, 2007a), but little remained known of its biology, and nothing of its reproduction. The gravid female reported here was dug from soil in a smallholding on 13 June 2006 (at the beginning of the rainy season, Fig. S1) in Baraki, Kolhapur District, Maharashtra (16°48′N, 73°52′E), representing a range extension of some 55 km East. The foetuses were discovered when the coelom of the gravid female was opened to take a liver sample during preservation. The adult and foetuses are catalogued as BNHS 4782 in the Bombay Natural History Society, Mumbai, India. The specimens were examined under a binocular dissecting microscope (×48–400 magnification) and measured with a ruler and dial calliper. The anteriormost foetuses in the left and right oviducts were removed from the female for closer inspection.

Molecular phylogenetics and character evolution

Partial sequences (GenBank accessions EU364649–EU364650) for mitochondrial 12S and 16S rRNA genes were determined for one specimen (BNHS 4609) of *G. seshachari* from the same locality (Baraki) as the gravid female, using methods and primers reported by Gower

et al. (2002), and added to the alignment analysed by Loader *et al.* (2007). The overlapping regions were aligned manually to produce separate liberal and conservative alignments. For the conservative alignment, all ambiguously aligned regions were excised. For the liberal alignment, sites for which alignment ambiguity was caused by a minority of taxa were modified as reported by Wilkinson *et al.* (2003:84).

Best-fit models of nucleotide substitution were identified using the Akaike information criterion (AIC; Akaike, 1973) as implemented in MODELTEST version 3.7 (Posada & Crandall, 1998). Phylogeny was estimated using maximum likelihood (ML; Felsenstein, 1981) and Bayesian inference (BI; Huelsenbeck *et al.*, 2001). ML analysis was performed with PAUP* version 4.0b10 (Swofford, 1998), using heuristic searches with 10 random stepwise additions of taxa and Tree Bisection and Reconnection branch swapping. BI analysis used MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) running four simultaneous Markov chains, for one million generations, sampling every 100 generations after discarding the first 10 000 generations. Two independent BI runs were performed to increase the chance of adequate mixing of the Markov chains.

Quantitative support for the ML tree was evaluated by bootstrapping with 1000 pseudoreplicates, implemented with GARLI 0.951 (Zwickl, 2006) using program defaults except for the model specification (as for ML analyses) and 5000 generations. Support for clades obtained by BI was measured by Bayesian posterior probability.

Concern has been voiced over the default use of neighbour-joining guide trees in MODELTEST (Marjanovic & Laurin, 2007) and so we also determined best-fit models for ML analysis by supplying MODELTEST analyses with most parsimonious guide trees, as well as carrying out separate maximum parsimony phylogenetic analyses (gaps treated as missing data, heuristic searches with 10 random stepwise additions of taxa and Tree Bisection and Reconnection branch swapping).

MACCLADE 3.08a (Maddison & Maddison, 1999) was used to reconstruct most parsimonious character optimizations onto a summary caecilian phylogeny assembled manually by combining the new phylogenetic results presented here for *G. seshachari* with the trees of Roelants *et al.* (2007) and Wilkinson & Nussbaum (2006).

Results

Morphology and reproductive biology (Fig. 1)

The gravid female is 165 mm in length and 4.9 mm wide at mid-body, and is referable to *G. seshachari* on the basis of its unsegmented terminal 'shield' and 122 primary and zero secondary annuli (see Giri *et al.*, 2003). The female contains four very similarly sized foetuses 53–54 mm long (32–33% of the adult) and 2.3–2.5 mm wide at mid-body, none of which is folded or coiled. There are two

Fig. 1 Ventral view of a gravid female (total length 165 mm) *Gegeneophis seshachari* containing four near-term foetuses (BNHS 4782). The female's head is to the upper left, and her coelom has been opened via a mid-ventral incision from just anterior to the heart to just anterior to the anus.

foetuses in each oviduct. Both foetuses in the right oviduct and the anterior one in the left are disposed with their heads towards the adult's posterior, but the posterior foetus in the left oviduct faces the other way, so that the heads of the two foetuses in the left oviduct are in close proximity. Considering each individual to be a simple cylinder, the foetuses are each approximately 7–9% and in total 31% of the adult's volume.

There is no visible yolk associated with the foetuses, and no gills or gill scars, neuromast organs or fins. The tentacle lies in the eye–tentacle pale stripe characteristic of adults and in a similar position, marginally closer to the eye than to the naris. The premaxillary–maxillary and dentary dentition of the two removed foetuses each comprises a single row of mostly simple, pointed, fully erupted teeth. However, in both individuals these rows include a few shorter teeth (*c.* two-thirds the length) with a broader, chisel-like tip that bears crenulations. These are very small and difficult to observe, but we are confident that the dentition is at least partly foetal, and consider the teeth perhaps superficially most similar in shape to those in foetal *Typhlonectes* or *Gymnopis* (Wake, 1977) among those taxa for which this dentition has been documented. Head and body pigmentation is well developed and annular grooves clearly visible. Apart from a few foetal teeth, the foetuses generally resemble small adults, and our interpretation that they are near term is supported by the fact that each is only slightly smaller than the smallest (60 mm) post-paritive BNHS specimens of *G. seshachari*.

The orange-yellow fat bodies in the adult's coelom are thin, irregular, tapered structures, perhaps because

their stored energy was used during gestation. Numerous small ova of various shapes (subspherical to elongated ovoid) and sizes (< 0.5–2.5 mm) lie within both the right (20) and left (14) ovaries. The majority are small and whitish, but at least one of the larger, more ovoid ova is pale orange. The oviducts are highly (though unevenly) vascularized, but generally thin walled and translucent to transparent throughout, and not much wider than the foetuses. A small amount of soft, whitish, multi-layered (under magnification) tissue lies between the two foetuses in each oviduct, and posterior to the posterior foetus in the right oviduct. This tissue is partly attached to the oviduct wall in places. The gut of the anterior foetus from the left oviduct was opened, and found to contain similar whitish, multi-layered tissue to that found in the oviduct. More posterior gut contents of this foetus are brown and amorphous. Some small, dark (possibly faecal) flecks lie in the posterior of the left oviduct, close to the anus of the foetus positioned there. The foetal liver is a pale yellow-orange, different to the dark grey-brown of the adult.

Molecular phylogenetics and character evolution

The conservative and liberal alignments and some of their properties are reported in Table S1. The best-fit model for both alignments was general time reversible (GTR; Rodríguez *et al.*, 1990) with gamma-distributed among-site rate heterogeneity approximated with four categories (Γ_4; Yang, 1994) and a proportion of invariant sites (I; Reeves, 1992). In all optimal trees, *G. seshachari*

was recovered as sister to *Gegeneophis ramaswamii* (BPP 100, 99; ML bootstrap 98, 77 for liberal and conservative alignments respectively) within a clade of Indo-Seychellean caeciliids (BPP 100, 99; ML bootstrap 86, 67). Using most parsimonious guide trees in MODELTEST led to the selection of the same models with slightly different parameter values, and ML analyses based on these values recovered identical trees to those using the values determined using neighbour-joining guide trees. Maximum parsimony analyses also recovered *G. seshachari* as sister to *G. ramaswamii* within the Indo-Seychellean caeciliids with good to moderate support (Table S1). Other clades recovered in all optimal trees reflect the best supported hypotheses from other molecular analyses of caecilian phylogeny, including Diatriata and Teresomata (*sensu* Wilkinson & Nussbaum, 2006), Scolecomorphidae, *Caecilia* + *Typhlonectes*, and *Dermophis* + *Schistometopum* (Hedges *et al.*, 1993; Wilkinson *et al.*, 2002a, 2003; Frost *et al.*, 2006; Loader *et al.*, 2007; Roelants *et al.*, 2007) and this, along with morphology (Ravichandran *et al.*, 2003) and support values, lends some confidence to the position of *G. seshachari* inferred here from a smaller character set. All of the less expected relationships recovered in optimal trees (e.g. *Boulengerula* grouping with scolecomorphids) are weakly supported and/or not consistent across all analyses.

Parsimony optimization of a binary oviparity–viviparity character indicates that viviparity has evolved independently at least four times within caecilians (Fig. 2). Viviparity in *G. seshachari* is most parsimoniously interpreted as derived within *Gegeneophis* and the Indo-Seychellean caeciliids. There is no indication that oviparity has re-evolved within caecilians. Optimization of the absence/presence of foetal or foetal-like teeth is partly ambiguous (Fig. S2), with these teeth either having arisen independently in *G. seshachari* and other caecilians or lost twice within the Indo-Seychellean clade.

Discussion

Gegeneophis seshachari is a member of the Indo-Seychellean caeciliids, a radiation of about 18 known species in five genera (assuming *Indotyphlus* is a member), comprising some 10% of known extant caecilian species (IUCN, Conservation International & NatureServe, 2006; Wilkinson & Nussbaum, 2006). Reproductive mode has been reported for all but one (*Praslinia cooperii*) of the Seychelles species (oviparous, based on observation of eggs and/or larvae; Nussbaum, 1984), but was known previously for only a single Indian caeciliid, the oviparous, direct-developing *G. ramaswamii*, which lacks prolonged egg retention (Seshachar, 1942; Wilkinson *et al.*, 2002b; Müller *et al.*, 2005). *Gegeneophis seshachari* provides the only known instance of viviparity among the Indo-Seychellean caeciliids, and *Gegeneophis* is the only caecilian genus that is known to include oviparous and viviparous species. *Gegeneophis seshachari* is the smallest known viviparous caecilian. As in other viviparous caecilians, foetal *G. seshachari* have a specialized, deciduous dentition (e.g. Parker, 1936, 1956; Parker & Dunn, 1964) apparently used to feed on the maternal oviduct lining (e.g. Wake, 1977, 1993; Welsch *et al.*, 1977).

Comparisons without considering phylogeny are crude, but the four known instances of the evolution of viviparity within caecilians (*c.* 170 extant species) are a greater proportion of extant standing diversity than for actinopterygians (*c.* 11 times; > 25 000 species), chondrichthyans (*c.* 10; *c.* 1000), salamanders (< 5; > 540), frogs (< 10; > 5200), crocodilians (0; 23), testudines (0; *c.* 300), birds (0; *c.* 10 000) and mammals (1 or 2; *c.* 5400). Only for squamates (> 100; > 7000) is the proportion comparable (Dulvy & Reynolds, 1997). Approximate frequencies of viviparous species in these groups are < 5% for actinopterygians, 40% for chondrichthyans, < 2% for salamanders and frogs, 19% for caecilians, 20% for squamates, 0% for crocodilians, testudines and birds, and 99% for mammals (data from Wake, 1993; Dulvy & Reynolds, 1997; Wilkinson & Nussbaum, 1998; Blackburn, 2006). There is no indication that oviparity has ever re-evolved from viviparity among caecilians, supporting the interpretation that this is a rare event in vertebrate evolution (e.g. Lee & Shine, 1998; Goodwin *et al.*, 2002). Viviparity has evolved at least four times in caecilians, yet each instance is characterized by foetuses feeding on oviduct lining with a specialized, deciduous dentition. The similarity of the 'foetal' teeth in viviparous and oviparous caecilians and their distribution on the
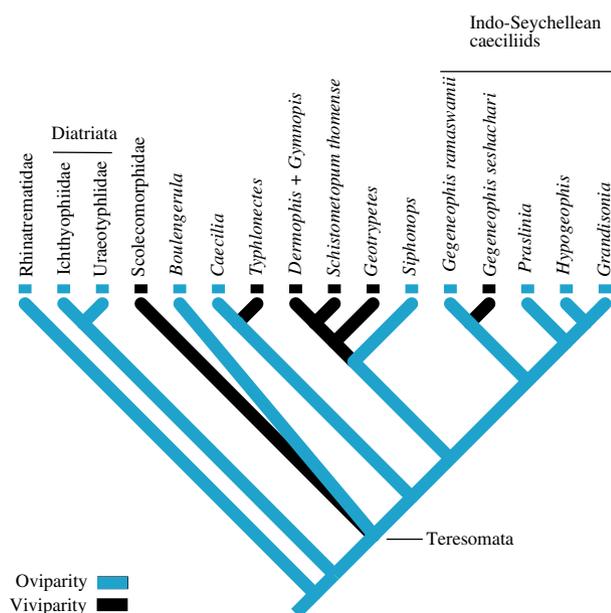

Fig. 2 Parsimony optimization of binary oviparity–viviparity character mapped onto summary caecilian phylogeny.

phylogeny (Fig. S2) lend support to the hypothesis that this dentition is at least partly homologous and originated in a dermatotrophic, oviparous ancestor (Kupfer *et al.*, 2006; Wilkinson *et al.*, 2008).

The available *G. seshachari* foetuses are very late term, and we predict that younger foetuses have only foetal teeth, that adult-like teeth develop later, and that newborns are independent and feed on soil invertebrates. The oviduct lining of gravid viviparous female caecilians generally remains hypertrophied up to parition (Wake, 1977). In *Schistometopum thomense*, near-term foetuses have been found in oviducts that are thin walled in places (Müller & Measey, 2004), presumably where they have been grazed extensively. In BNHS 4782, the thickened oviduct walls seem to have been all but eaten by the late-term foetuses but it might alternatively be the case that the oviduct thickens regionally in response to foetal grazing activity (see also Wake, 1977), especially later in development when movement of larger foetuses within the oviduct is presumably restricted.

Despite indications that newborn *G. seshachari* are precocial, the near-term foetuses reported here are more similar in relative size to those of the more altricial type II (*sensu* Loader *et al.*, 2003) *Geotrypetes* (about one-third of mother's length) than the more precocial type I *Schistometopum, Gymnopis* and *Typhlonectes* (40–60%) viviparous caecilians (data from Wake, 1977). *Gegeneophis seshachari* occurs towards the northern end of the Western Ghats region of peninsular India, where the long dry season (see Gower *et al.*, 2007b) prompted Giri *et al.* (2004) to suggest that some caecilians occurring here might aestivate. Viviparity should allow better maternal control of embryonic water balance (e.g. Shine & Thompson, 2006), and enable young *G. seshachari* to be born at the onset of the main (relatively short) rainy season, as occurs in other viviparous caecilians (e.g. Wake, 1980, 1993). For *G. seshachari*, this might be particularly important given the short duration of the rainy, productive season in the northern Western Ghats. It is unknown under what environmental conditions viviparity evolved in this lineage, but adaptation to shorter productive seasons (an extension of the Lack–Itô hypothesis, see Nussbaum, 2003) seems plausible and might be testable once reproductive mode is discovered for the other caeciliids that together span the length of the Western Ghats and this region's pronounced seasonal clines.

Viviparity has been considered to occur, in general, in larger parents (e.g. Goodwin *et al.*, 2002) and to be associated with fewer and larger newborns (e.g. Wourms & Lombardi, 1992), and this is true to some extent of the available data for viviparous frogs and salamanders (e.g. Wake, 1993). The BNHS 4782 near-term foetuses are not substantially smaller than hatchling *G. ramaswamii* (based on a near-term embryo of 61 mm reported by Müller *et al.*, 2005), even though adults of the latter species reach a substantially greater length and girth (D.J.

Gower, G.J. Measey, O.V. Oommen & M. Wilkinson, unpublished data). Excluding the diminutive *Grandisonia brevis* (adults to < 100 mm), the other Seychelles caeciliids (adult length generally up to 200–300 mm) have hatchlings in the range of 42–65 mm (Brauer, 1899), where known. Detailed data on clutch size for Indo-Seychellean caeciliids is scanty, but clutches of some Seychelles species have been found with 6–30 eggs (Brauer, 1897) and *G. ramaswamii* two to > 20 eggs (D.J. Gower, G.J. Measey, O.V. Oommen & M. Wilkinson, unpublished data).

Anurans have been considered to be the most reproductively diverse amphibians, with caecilians having undergone relatively few evolutionary 'experiments' (e.g. Wake, 1993; Wake & Dickie, 1998). Much of the variation documented for frogs and salamanders is in courtship and oviposition sites, which for caecilians are largely unknown and seemingly not very variable respectively. In terms of modes of parity, caecilians have undergone a great evolutionary experimentation, especially when considering the number of extant species in each of the extant amphibian orders.

Although relatively neglected, caecilians are reproductively diverse, and an excellent and particularly interesting system for studies of evolutionary reproductive biology (see also Haas, 2007). With some 170 extant species, Gymnophiona is of a size that makes a comprehensive study of reproductive evolution tractable for an entire order that might have undergone > 200 Myr of evolution (Roelants *et al.*, 2007). Unlike the situation with squamates, study of caecilian viviparity does not carry much historical burden, and new work can be conducted at a time when understanding of the group's phylogeny is making good progress (e.g. Wilkinson & Nussbaum, 2006). The ready availability of comparative data and ideas from better studied, non-caecilian groups should provide further impetus to the studies of caecilian reproduction. The Indo-Seychellean caeciliids represent a particularly intriguing system because, in addition to viviparity, there are species with forms of oviparity with direct development and with a larval stage. More primary data are required for thorough analyses of caecilian reproductive evolution, and these same data will be able to inform much needed improvements in conservation assessments (Gower & Wilkinson, 2005).

References

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. In: *Second International Symposium of Information Theory* (B.N. Petrov & F. Csaki, eds), pp. 267–281. Akademiai Kiado, Budapest.

Blackburn, D.G. 1992. Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptiles and other vertebrates. *Am. Zool.* **32**: 313–321.

Blackburn, D.G. 2000. Classification of the reproductive patterns of amniotes. *Herpetol. Monogr.* **14**: 371–377.

Blackburn, D.G. 2006. Squamate reptiles as models organisms for the evolution of viviparity. *Herpetol. Monogr.* **20**: 131–146.

Brauer, A. 1897. Beitrüage zur Kentniss der Entwicklungsgeschichte und Anatomie der Gymnophionen. *Zool. Jahrb. Anat.* **10**: 277–472.

Brauer, A. 1899. Beitrüage zur Kentniss der Entwicklungsgeschichte und Anatomie der Gymnophionen. II. Die Entwicklung der äussern Form. *Zool. Jahrb. Anat.* **10**: 477–508.

Dulvy, N.K. & Reynolds, J.D. 1997. Evolutionary transitions among egg-laying, live-bearing and maternal inputs in sharks and rays. *Proc. R. Soc. B* **264**: 1309–1315.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**: 368–376.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. & Wheeler, W.C. 2006. The amphibian tree of life. *Bull. Am. Mus. Nat. Hist.* **297**: 1–370.

Giri, V., Wilkinson, M. & Gower, D.J. 2003. A new species of *Gegeneophis* Peters (Amphibia: Gymnophiona: Caeciliidae) from the Western Ghats of southern Maharashtra, India, with a key to the species of the genus. *Zootaxa* **351**: 1–10.

Giri, V., Gower, D.J. & Wilkinson, M. 2004. A new species of *Indotyphlus* Taylor (Amphibia: Gymnophiona: Caeciliidae) from the Western Ghats of India. *Zootaxa* **739**: 1–19.

Goodwin, N.B., Dulvy, N.K. & Reynolds, J.D. 2002. Life-history correlates of the evolution of live bearing in fishes. *Philos. Trans. Soc. B* **357**: 259–267.

Gower, D.J. & Wilkinson, M. 2002. Phallus morphology in caecilians and its systematic utility. *Bull. Nat. Hist. Mus. Zool. Ser.* **68**: 143–154.

Gower, D.J. & Wilkinson, M. 2005. Conservation biology of caecilian amphibians. *Conserv. Biol.* **19**: 45–55.

Gower, D.J., Kupfer, A., Oommen, O.V., Himstedt, W., Nussbaum, R.A., Loader, S.P., Presswell, B., Müller, H., Krishna, S.B., Boistel, R. & Wilkinson, M. 2002. A molecular phylogeny of ichthyophiid caecilians (Amphibia: Gymnophiona: Ichthyophiidae): out of India or out of southeast Asia? *Proc. R. Soc. B* **269**: 1563–1569.

Gower, D.J., Giri, V. & Wilkinson, M. 2007a. Rediscovery of *Gegeneophis seshachari* Ravichandran, Gower & Wilkinson (Amphibia: Gymnophiona: Caeciliidae) at the type locality. *Herpetozoa* **19**: 121–127.

Gower, D.J., Dharne, M., Bhatta, G., Giri, V., Vyas, R., Govindappa, V., Oommen, O.V., George, J., Schouche, Y. & Wilkinson, M. 2007b. Remarkable genetic homogeneity in unstriped, long-tailed *Ichthyophis* (Amphibia: Gymnophiona: Ichthyophiidae) along 1500 km of the Western Ghats, India. *J. Zool.* **272**: 266–275.

Haas, A. 2007. Book review of ''Reproductive biology and phylogeny of Gymnophiona (caecilians)''. *J. Zool. Syst. Evol. Res.* **45**: 282–283.

Hedges, S.B., Nussbaum, R.A. & Maxson, L.R. 1993. Caecilian phylogeny and biogeography inferred from mitochondrial DNA sequences of the 12SrRNA and 16S rRNA genes (Amphibia: Gymnophiona). *Herpetol. Monogr.* **7**: 64–76.

Huelsenbeck, J.P. & Ronquist, F.R. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Huelsenbeck, J.P., Ronquist, F.R., Nielsen, R. & Bollback, J.P. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**: 2310–2314.

IUCN, Conservation International & NatureServe 2006. *Global Amphibian Assessment*. Available at: http://www.globalamphibians.org. Downloaded on 15 July 2007.

Kupfer, A., Nabhitabhata, J. & Himstedt, W. 2004. Reproductive ecology of female caecilian amphibians (genus *Ichthyophis*): a baseline study. *Biol. J. Linn. Soc.* **83**: 207–217.

Kupfer, A., Müller, H., Jared, C., Antoniazzi, M., Nussbaum, R.A., Greven, H. & Wilkinson, M. 2006. Parental investment by skin feeding in a caecilian amphibian. *Nature* **440**: 926–929.

Lee, M.S.Y. & Shine, R. 1998. Reptilian viviparity and Dollo's Law. *Evolution* **52**: 1441–1460.

Loader, S.P., Wilkinson, M., Gower, D.J. & Msuya, C.A. 2003. A remarkable young *Scolecomorphus vittatus* (Amphibia: Gymnophiona: Scolecomorphidae) from the North Pare Mountains, Tanzania. *J. Zool.* **259**: 93–101.

Loader, S.P., Pisani, D., Cotton, J.A., Day, J.J. & Wilkinson, M. 2007. Relative time scales reveal multiple origins of parallel disjunct distributions of African caecilian amphibians. *Biol. Lett.* **3**: 505–508.

Maddison, D.R. & Maddison, W.P. 1999. MacClade: Analysis of Phylogeny and Character Evolution 3.08. Sinauer, Sunderland, MA.

Marjanovic, D. & Laurin, M. 2007. Fossils, molecules, divergence times, and the origin of lissamphibians. *Syst. Biol.* **56**: 369–388.

Müller, H. & Measey, G.J. 2004. Intraoviductal feeding in embryos of *Schistometopum thomense* (Amphibia: Gymnophiona: Caeciliidae). *J. Morphol.* **260**: 315.

Müller, H., Oommen, O.V. & Bartsch, P. 2005. Skeletal development of the direct-developing caecilian *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae). *Zoomorphology* **124**: 171–188.

Nussbaum, R.A. 1984. Amphibians of the Seychelles. In: *Biogeography and Ecology of the Seychelles Islands* (D.R. Stoddart, ed.), pp. 379–415. Dr W Junk, The Hague.

Nussbaum, R.A. 2003. Parental care. In *Reproductive Biology and Phylogeny of Urodela* (D.M. Sever, ed.), pp. 527–612. Science Publishers Inc., Enfield, NH.

Parker, H.W. 1936. The amphibians of the Mamfe Division, Cameroons. *Proc. Zool. Soc. Lond.* **133**: 135–163.

Parker, H.W. 1956. Viviparous caecilians and amphibian phylogeny. *Nature* **178**: 250–252.

Parker, H.W. & Dunn, E.R. 1964. Dentitional metamorphosis in the Amphibia. *Copeia* **1964**: 75–85.

Posada, D. & Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Ravichandran, M.S., Gower, D.J. & Wilkinson, M. 2003. A new species of *Gegeneophis* Peters (Amphibia: Gymnophiona: Caeciliidae) from Maharashtra, India. *Zootaxa* **350**: 1–8.

Reeves, J.H. 1992. Heterogeneity in the substitution process of amino acid sites of proteins coded for by mitochondrial DNA. *J. Mol. Evol.* **35**: 17–31.

Reynolds, J.D., Goodwin, N.B. & Freckleton, R.P. 2002. Evolutionary transitions in parental care and live bearing in vertebrates. *Philos. Trans. Soc. B* **357**: 269–281.

Rodríguez, F., Oliver, J.F., Marín, A. & Medina, J.R. 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.* **142**: 485–501.

Roelants, K., Gower, D.J., Wilkinson, M., Loader, S.P., Biju, S.D., Guillaume, K. & Bossuyt, F. 2007. Patterns of diversification in the history of modern amphibians. *Proc. Natl Acad. Sci. USA.* **104**: 887–892.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Seshachar, B.R. 1942. The eggs and embryos of *Gegeneophis carnosus* Bedd. *Curr. Sci.* **11**: 439–441.

Shine, R. & Thompson, M.B. 2006. Did embryonic responses to incubation conditions drive the evolution of reproductive modes in squamate reptiles? *Herpetol. Monogr.* **20**: 159–171.

Swofford, D.L. 1998. PAUP*: Phylogenetic Analysis using Parsimony (*and Other Methods), Version 4.0. Sinauer, Sunderland, MA.

Wake, M.H. 1977. Fetal maintenance and its evolutionary significance in the Amphibia: Gymnophiona. *J. Herpetol.* **11**: 379–386.

Wake, M.H. 1980. Reproduction, growth, and population structure of the central American caecilian *Dermophis mexicanus*. *Herpetologica* **36**: 244–256.

Wake, M.H. 1993. Evolution of oviductal gestation in amphibians. *J. Exp. Zool.* **266**: 394–413.

Wake, M.H. & Dickie, R. 1998. Oviduct structure and function and reproductive modes in amphibians. *J. Exp. Zool.* **282**: 477–506.

Welsch, U., Muller, W. & Schubert, C. 1977. Electronmicroscopical and histochemical observations on the reproductive biology of viviparous caecilians (*Chthonerpeton indistinctum*). *Zool. Jahrb. Anat.* **97**: 532–549.

Wilkinson, M. & Nussbaum, R.A. 1998. Caecilian viviparity and amniote origins. *J. Nat. Hist.* **32**: 1403–1409.

Wilkinson, M. & Nussbaum, R.A. 2006. Caecilian phylogeny and classification. In: *Reproductive Biology and Phylogeny of Gymnophiona* (J.-M. Exbrayat, ed.), pp. 39–78. Science Publishers Inc., Enfield, NH.

Wilkinson, M., Sheps, J.A., Oommen, O.V. & Cohen, B.L. 2002a. Phylogenetic relationships of Indian caecilians (Amphibia: Gymnophiona) inferred from mitochondrial rRNA gene sequences. *Mol. Phylogenet. Evol.* **23**: 401–407.

Wilkinson, M., Richardson, M.K., Gower, D.J. & Oommen, O.V. 2002b. Extended embryo retention, caecilian oviparity and amniote origins. *J. Nat. Hist.* **36**: 2185–2198.

Wilkinson, M., Loader, S.P., Gower, D.J., Sheps, J.A. & Cohen, B.L. 2003. Phylogenetic relationships of African Caecilians (Amphibia: Gymnophiona): Insights from mitochondrial rRNA Gene Sequences. *Afr. J. Herpetol.* **52**: 83–92.

Wilkinson, M., Kupfer, A., Marques-Porto, R., Jeffkins, H., Antoniazzi, M.A. & Jared, C. 2008. One hundred years of skin feeding? Extended parental care in a Neotropical caecilian (Amphibia: Gymnophiona). *Biol. Lett.*, doi: 10.1098/rsbl.2008.0217.

Wourms, J.P. & Lombardi, J. 1992. Reflections on the evolution of piscine viviparity. *Am. Zool.* **32**: 276–293.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Mol. Evol.* **39**: 306–314.

Zwickl, D.J. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Dissertation, The University of Texas at Austin. GARLI 0.951. Available at: http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html.

Supplementary material

The following supplementary material is available for this article:

Figure S1 Total monthly rainfall for Dajipur, near Baraki, showing that the gravid female BNHS 4782 collected on 13 June 2006 was carrying near-term foetuses at the beginning of the short, pronounced rainy season.

Figure S2 Most parsimonious optimization of the absence/presence of foetal or foetal-like dentition mapped onto summary caecilian phylogeny.

Table S1 Properties of liberal and conservative alignments for 26 leaves (*Gegeneophis seshachari* plus the 25 taxa analysed by Loader *et al.*, 2007), and summary of some of the phylogenetic results.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1420-9101.2008.01577.x

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