Wilkinson, M. and Loader, S. P. and Gower, D. J. and Sheps, J. A. and Cohen, B. L. (2003) Phylogenetic relationships of African Caecilians (Amphibia: Gymnophiona): insights from mitochondrial rRNA gene sequences. *African Journal of Herpetology* 52(2):pp. 83-92.

http://eprints.gla.ac.uk/2980/

Phylogenetic relationships of African caecilians (Amphibia: Gymnophiona): insights from mitochondrial rRNA gene sequences

MARK WILKINSON[1], SIMON P. LOADER[1,2], DAVID J. GOWER[1], JONATHAN A. SHEPS[2,*] AND BERNARD L. COHEN[2]

[1]*Department of Zoology, The Natural History Museum, London SW7 5BD, UK*
marw@nhm.ac.uk
[2]*University of Glasgow, Institute of Biomedical and Life Sciences, Division of Molecular Genetics, Pontecorvo Building, 56 Dumbarton Rd, Glasgow G11 6NU, Scotland, UK.*
[*]*present address: Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada*

Abstract.—Africa (excluding the Seychelles) has a diverse caecilian fauna, including the endemic family Scolecomorphidae and six endemic genera of the more cosmopolitan Caeciliidae. Previous molecular phylogenetic studies have not included any caecilians from the African mainland. Partial 12S and 16S mitochondrial gene sequences were obtained for two species of the endemic African Scolecomorphidae and five species and four genera of African caeciliids, aligned against previously reported sequences for 16 caecilian species, and analysed using parsimony, maximum likelihood, Bayesian and distance methods. Results are in agreement with traditional taxonomy in providing support for the monophyly of the African caeciliid genera *Boulengerula* and *Schistometopum*, and for the Scolecomorphidae. They disagree in indicating that the Caeciliidae is paraphyletic with respect to the Scolecomorphidae. Although more data from morphology and/or molecules will be required to resolve details of the interrelationships of the African caecilian genera, the data provide strong support for at least two origins of caecilians in which the eye is reduced and covered with bone, and do not support the hypotheses that the caecilian assemblages of Africa, and of East and of West Africa are monophyletic.

Key words.—Amphibians, biogeography, evolution, eyes, phylogeny, vertebrates, viviparity.

Caecilians (Gymnophiona) are one of the three extant orders of amphibians. The caecilian fauna of Africa, taken as excluding the Seychelles, includes the endemic family Scolecomorphidae (six species in two genera) and six genera and 16 species of the more cosmopolitan Caeciliidae, which also has representatives in the Seychelles, India, and Central and South America. African caecilians make up approximately 13% and 25% of the recognised caecilian species and genera respectively, and thus constitute a substantial proportion of known gymnophionan diversity. Previous molecular phylogenetic analyses (Hedges *et al.* 1993; Gower *et al.* 2002; Wilkinson *et al.* 2002) have included, at most, only a single African caecilian, the insular caeciliid *Schistometopum thomense*. Apart from an unconfirmed report from Central Africa (Nussbaum & Pfrender 1998), this caeciliid is known only from Sao Thome in the Gulf of Guinea. Thus we have no molecular phylogenetic insight into the relationships of any mainland African caecilians. Of the six currently recognised caecilian families (Nussbaum & Wilkinson 1989) only the Scolecomorphidae remains unstudied with regards to molecular data.

Table 1. Voucher specimens deposited in the collections of the Department of Zoology, Natural History Museum, London (BMNH) and the National Museums of Kenya, Nairobi (NMK).

Taxon	Voucher	Provenance
Boulengerula boulengeri	BMNH 2002.95	East Africa, Tanzania, East Usambara, Amani
Boulengerula taitanus	NMK A/3112*	East Africa, Kenya, Taita, Wundanyi
Geotrypetes seraphini	BMNH 2002.96	West Africa, Cameroon (pet trade)
Herpele squalostoma	BMNH 2002.97	West Africa, Cameroon (pet trade)
Schistometopum gregorii	BMNH 2002.98	East Africa, Tanzania, Bagamoyo
Scolecomorphus uluguruensis	BMNH 2002.99	East Africa, Tanzania, Uluguru, Uluguru North
Scolecomorphus vittatus	BMNH 2002.100	East Africa, Tanzania, East Usambara, Amani

*field tag MW 512

Building on the foundations provided by Hedges *et al*. (1993), Wilkinson *et al*. (2002) used partial 12S and 16S SSU mt DNA sequence data to provide well supported resolution of the phylogenetic relationships of representatives of the three families of caecilians present in India, and suggested that expanding the sampling of African caecilians was a priority for caecilian molecular phylogenetics.

Here we report new 12S and 16S SSU rDNA partial sequences for seven species of African caecilians, including the first sequences for representatives of the Scolecomorphidae, and the first sequences for any East African caeciliid. At the generic level, our sampling of African caecilians is incomplete only in the omission of the monotypic caeciliids *Sylvacaecilia* from Ethiopia and *Idiocranium* from Cameroon, and of the West African scolecomorphid *Crotaphatrema* (three species). The new sequences increase the diversity of caecilians for which these comparative mitochondrial sequence data are available, from 16 to 23 of the approximately 160 currently recognised caecilian species, and from 11 to 15 of the 33 genera. The new sequences allow the first molecular tests of the monophyly of *Scolecomorphus* and the Scolecomorphidae, of *Boulengerula* and of *Schistometopum*, and investigation of the relationships of the caeciliid assemblages of East and West Africa to each other and a range of non-African caecilians.

MATERIALS AND METHODS

Details of voucher specimens are presented in Table 1. Sequencing methods are as given in Wilkinson *et al*. (2002). Sequences have been deposited in GenBank (Benson *et al*. 1998) with accession numbers A7450612 - A7450625.

The newly determined sequences were added to an alignment of concatenated partial 12S and 16S caecilian sequences (Wilkinson *et al*. 2002) and the alignment adjusted manually. Regions in which positional homology could not be assessed with confidence due to length variation were excluded, except where length variation was concentrated in a minority of taxa. In the latter case, regions of uncertainty in the alignment were represented by replacing the sequence data for the minority of taxa with missing entries. This increases the available data for the remaining majority of taxa and reduces the amount of useful information that is discarded. Following Wilkinson *et al*. (2002), the sequence of the rhinatrematid caecilian *Epicrionops marmoratus* was designated as a single outgroup and used to root trees.

Parsimony, maximum likelihood (ML) and distance analyses were performed with PAUP* 4.0b10 (Swofford 1998). LogDet and Maximum Likelihood distance (MLD) analyses used the minimum evolution objective function. ML and MLD analyses used models

of evolution selected by Modeltest (Posada & Crandall 1998) and the corresponding estimated proportion of invariant sites was used in the LogDet analyses. Alignment gaps were treated as missing data. Tree searches were heuristic with 100 (parsimony and distance analyses) or 10 (ML) random addition sequences and TBR branch swapping. A Bayesian analysis was performed using MrBayes 2.01 (Huelsenbeck & Ronquist 2001) using a general time reversible (GTR) model, with rate variation across sites modelled with a discrete gamma distribution (G) and proportion of invariant sites (I). The Metropolis coupled, Markov chain Monte Carlo analysis was run with four chains for 1,000,000 generations. Trees were sampled every 1000 generations, with the first 1000 generations discarded as "burn in".

A parsimony PTP test (Faith & Cranston 1991) was used to test the null hypothesis that the alignment has no more hierarchical structure than expected by chance alone (99 random permutations). Support for clades was measured with bootstrap proportions (Felsenstein 1985) (100 pseudoreplicates) and Bayesian posterior probabilities. Leaf stabilities based on the bootstrap difference measure (Thorley & Wilkinson 1999) were determined using RadCon (Thorley & Page 2000) from sets of bootstrap trees. For these measures, trees were treated as unrooted to allow the stability of the rooting on *Epicrionops marmoratus* to be assessed (Wilkinson *et al.* 2002). Relative rates tests were performed using the program RRTree (Robinson *et al.* 1998). Suboptimal ML trees, conforming to various *a priori* hypotheses, were found through searches enforcing user-defined topological constraints.

Differences between optimal and suboptimal ML trees were assessed using the Kishino-Hasegawa (KH) test (Kishino & Hasegawa 1989) using RELL with 1000 bootstrap replicates. The KH test is biased when, as here, the trees are not selected *a priori* because we are more likely to wrongly reject the null hypothesis than we would like at our selected Type I error rate, with the strength of this liberal bias unknown. Thus, whereas failing the test does allow us to accept the null hypothesis, passing the test does not fully justify rejecting the null hypothesis (Goldman *et al.* 2000). Here a significant result is taken to support the tentative rejection of the null hypothesis, and we used the conservative two-tailed version of the KH test to compensate to some uncertain extent for the liberal bias due to inappropriate tree selection. Although the Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999) is unbiased, it requires that all plausible trees are included. The identification of this set is problematic for trees with more than only a few taxa, and we have not used this test here.

RESULTS

All PCR amplifications from genomic DNA yielded products of the expected size, which, on sequencing, contained negligible levels of site ambiguity. Some taxa are relatively unstable in the phylogenetic analyses (see below) but there is no obvious reason to suspect that any of the data could have been derived from nuclear copies of mitochondrial sequences.

After incorporation of the new sequences and the exclusion of regions that could not be aligned with confidence, the alignment comprised 914 sites. Of these, 448 were invariant and 123 were parsimony uninformative, leaving 343 parsimony informative sites. There is no significant variation in base composition across the alignment as a whole (χ^2 tests for homogeneity, $P = 0.858$, d.f. = 66). In contrast, extensive and significant ($P = 0.001$) variation in base composition is evident in the variable sites. Remarkably, if sequences are ranked by their combined GC content, the eight African taxa have the eight highest GC contents (Table 2).

Table 2. Base composition and leaf stabilities. Sequences are ranked according to the proportion of guanine and cytosine in the variable sites included in the alignment (GC). Leaf stabilities are from parsimony (MP) and distance (MLD) bootstrap analyses.

	GC	MP	MLD
Epicrionops marmoratus	0.3231	0.6915	0.7597
Ichthyophis tricolor	0.3297	0.7386	0.8122
Ichthyophis bannanicus	0.3414	0.7386	0.8122
Uraeotyphlus sp.	0.3480	0.738	0.8122
Typhlonectes natans	0.3489	0.6271	0.7444
Caecilia sp.	0.3575	0.6273	0.7458
Siphonops annulatus	0.3616	0.5604	0.6171
Hypogeophis rostratus	0.3742	0.7608	0.8322
Praslinia cooperi	0.3767	0.8048	0.8762
Grandisonia alternans	0.3995	0.7672	0.8396
Average	0.4005	0.6949	0.78
Grandisonia brevis	0.4062	0.7778	0.8482
Grandisonia larvata	0.4093	0.7643	0.839
Grandisonia seychellensis	0.4100	0.7644	0.8339
Gegeneophis ramaswamii	0.4150	0.7604	0.8352
Dermophis mexicanus	0.4158	0.7051	0.8297
Schistometopum thomense	0.4260	0.7166	0.8301
Schistometopum gregorii	0.4318	0.7166	0.8301
Boulengerula boulengeri	0.4427	0.6558	0.7648
Boulengerula taitanus	0.4439	0.654	0.7642
Herpele squalastoma	0.4538	0.6284	0.7351
Geotrypetes seraphini	0.4560	0.5476	0.6042
Scolecomorphus vittatus	0.4593	0.6182	0.6869
Scolecomorphus uluguruensis	0.4820	0.6182	0.6869

Transition - transversion ratios, based on uncorrected pairwise differences, range from 0.79 to 4.0 (Fig. 1). The many very low ratios occuring in taxa with high total pairwise differences suggest that saturation and/or lineage specific relative rate variation may be a problem in this data set. The four lowest transition - transversion ratios, and nine of the 18 ratios that are less than one, involve the African caciliid *Geotrypetes seraphini* (Fig. 1), suggesting that saturation or lineage specific rate variation could be a particular problem in accurately placing this taxon. Relative rates tests revealed no significant differences in evolutionary rates in any taxa, with the exceptions that *G. seraphini* and *Typhlonectes natans* were both significantly faster than *Ichthyophis tricolor* ($P = 0.045$ and $P = 0.033$, respectively).

The data have a parsimony PTP of 0.01, allowing rejection of the null hypothesis that they contain no more structure than expected by chance alone. Using the likelihood ratio test and the Akaike information criterion, Modeltest selected TrN (Tamura & Nei 1993) + I + G, and GTR (Rodriguez *et al.* 1990) + I + G models, respectively. We used the simpler TrN + I + G model, and this yielded a single ML tree (Fig. 2).

Relationships among the non-African taxa are mostly those found in previous analyses, with minor differences in the relationships among the Seychellean caeciliids excluding *Praslinia*, and of the Neotropical caeciliid *Siphonops annulatus*, both of which were relatively unstable in previous analyses (Wilkinson *et al.* 2002). Thus there is an Indo-Seychellean caeciliid clade (*Gegeneophis*, *Prasilina*, *Grandisonia*, *Hypogeophis*), that is more closely related to a *Dermophis-Schistometopum* clade than to most other caecilians, there is a *Typhlonectes-Caecilia* clade, and the Uraeotyphlidae and Ichthyophiidae are each other's closest relatives and sister to all other caecilians except the rhinatrematid *Epicrionops*. These core relationships were also recovered in parsimony, distance and Bayesian analyses (trees not shown).

Indications of the support for the relationships recovered in the ML tree are given by Bayesian posterior probabilities and the bootstrap proportions from MLD and parsimony analyses (Fig. 2), as well as from the stability of relationships across the different methods of analysis. Each congeneric pair of African caecilians (*Boulengerula*, *Scolecomorphus* and *Schistometopum*) are recovered as each others' closest relatives in all analyses and with high support, consistent with current taxonomy. Similarly, the *Dermophis-Schistometopum* pairing appears well supported.

Other interrelationships of African caecilians are generally less well supported. All African caecilians fall within Nussbaum's (1991) informal 'higher' caecilians, a group comprising the Caeciliidae, Typhlonectidae and Scolecomorphidae (united by branch H, Fig. 2). Support for this group is not very strong ($P = 0.67$, BP = 60 - 68) but it is recovered in all optimal trees, is independently supported by morphological phylogenetic analyses (Nussbaum 1979; Duellman & Trueb 1986; Hillis 1991; Wilkinson & Nussbaum 1996; Wilkinson 1997) and is accepted here.

The basal splits within the higher caecilians place the African caeciliids *Boulengerula* and *Herpele squalostoma* together (branch X), and these and the scolecomorphids as successive sister groups of the remaining higher caecilians (branches Y and Z). Each of these relationships has unimpressive bootstrap support and poste-
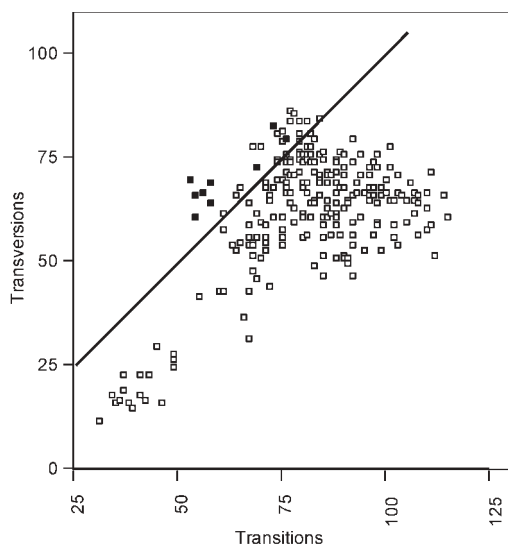

Figure 1. Scatter plot of pairwise uncorrected estimates of transitions and transversions. The straight line indicates a ratio of 1:1, with points above the line representing particularly low transition - transversion ratios. Shaded points are pairwise estimates of transition transversion ratios of less than one that involve *Geotrypetes seraphini*.

rior probabilities. Despite lacking strong quantitative support, branch Y is recovered in the optimal trees from each of the analyses employing different methods, and branch X is contradicted only in two of five most parsimonious trees.

The remaining African caeciliid, *G. seraphini*, is nested within a cosmopolitan group of caeciliids (branch C, Fig. 2) that has low bootstrap support and is not recovered in all optimal trees, but which has a surprisingly high posterior probability (0.93). These relationships must be considered speculative and they are accepted only tentatively.

The precise relationships of *G. seraphini* differ greatly in the optimal trees recovered by the different analyses. It is recovered as sister to the *Dermophis-Schistometopum* clade (ML, MLD, Bayesian), sister to the Indo-Seychellean caeciliids (LogDet) or to *Scolecomorphus* (parsimony) and never with strong support. Apart from its tentative inclusion in clade C, all that can be confidently inferred about the relationships of *Geotrypetes* is that it lies outside the *Dermophis-Schistometopum* clade and the Indo-Seychellean clade. Leaf stabilities (Table 2) calculated from parsimony and MLD bootstrap analyses agreed in the rank order, and both identified *G. seraphini* as the least stable taxon. Leaf stabilities for African taxa except *Schistometopum* are lower than average, indicating that their positions are among the relatively least well supported. Leaf stability of *Epicrionops* is close to the average, indicating no special instability in the root.

Constrained analyses produced a number of suboptimal ML trees consistent with various hypotheses of taxonomic, biogeographic and biological interest that were tested against the unconstrained ML tree using the KH test (Table 3). Despite apparent strong support for the monophyly of *Boulengerula*, the best tree in which *Boulengerula* is not monophyletic does

Table 3. Kishino-Hasegawa tests comparing the fit of the data to the unconstrained ML tree (Fig. 2) and to a range of suboptimal trees, each constrained to make a particular set of taxa monophyletic or not monophyletic. D = difference in log likelihood between optimal and suboptimal trees; *P* = probability under the null hypothesis that the differences in fit are no greater than expected from random sampling error (noise).

Suboptimal hypothesis	D	P
Boulengerula is not monophyletic	9.026	0.086
Schistometopum is not monophyletic	25.229	0.040
Scolecomorphus is not monophyletic	77.902	<0.001
African caecilians are monophyletic	38.623	0.002
African caeciliids (*Boulengerula* + *Geotrypetes* + *Herpele* + *Schistometopum*) are monophyletic	37.431	0.006
East African caeciliids (*Boulengerula* + *Schistometopum gregorii*) are monophyletic	68.212	<0.001
West African caeciliids (*Herpele* + *S. thomense*) are monophyletic	102.00	<0.001
Caecilians with rudimentary eyes (*Boulengerula* + *Herpele* + *Gegeneophis*) are monophyletic	31.596	0.005
Viviparous caecilians (*Typhlonectes* + *Dermophis* + *Geotrypetes* + *S. thomense* + *Scolecomorphus*) are monophyletic	17.896	0.111

not provide a significantly worse fit to the data. In contrast, trees in which the other individual African genera are not monophyletic have a significantly worse fit to the data, as do trees in which African caecilians, African caeciliids, and East and West African caeciliids are monophyletic. Optimal trees in which caecilians with rudimentary eyes are monophyletic can also be tentatively rejected. In contrast, the data do not allow rejection of the hypothesis that viviparity arose only once within caecilians.

DISCUSSION

Although greatly increasing the taxonomic sampling of African caecilians, our analyses offer incomplete and mostly tentative insights into their phylogenetic relationships. The molecular data support traditional taxonomy in being consistent with the monophyly of the three African genera, *Boulengerula*,

Schistometopum and *Scolecomorphus* (and of the Scolecomorphidae). *Scolecomorphus* and *Schistometopum* are also characterised by unique morphological synapomorphies (e.g., Nussbaum 1985; Nussbaum & Pfrender 1998; Wake 1998; Gower & Wilkinson 2002; Loader *et al.* 2003). Nussbaum (1985: 47) reported that "Studies in progress indicate that *Herpele* and *Idiocranium* are distinctive western forms with no close relationship to other African caecilians" whereas our data provide tentative support for the pairing of *Herpele* with *Boulengerula*.

The data also support, albeit weakly, Nussbaum's (1991) 'higher' caecilian clade, as does morphology (Wilkinson & Nussbaum 1996; Wilkinson 1997). The results suggest that the Caeciliidae is paraphyletic, not only with respect to the Typhlonectidae (e.g., Hedges *et al*. 1993), but also with respect to the Scolecomorphidae, emphasising the need for more comprehensive taxonomic revision. Needless to say, any future revision intended to remove this paraphyly will require greater sampling of caeciliid taxa.

The parallel disjunct distributions of *Schistometopum gregorii* and *Schistometopum thomense*, and of *Scolecomorphus* and *Crotaphatrema* in East and West Africa has been noted previously (e.g., Nussbaum 1985; Nussbaum & Pfrender 1998). The tentative hypothesis that the East African *Boulengerula* and West African *Herpele* are sister taxa, adds a third potential component to this biogeographic parallelism. It is not clear whether the absence of caecilians from Central Africa is real or reflects lack of sampling (Nussbaum & Hinkel 1994), and thus whether the biogeographic pattern is real or apparent. However, this study demonstrates that the caecilian and caeciliid faunas of Africa, and those of East Africa and of West Africa are not monophyletic.

Schistometopum nests within a diverse group of caeciliids as the sister group of *Dermophis*, with which it was considered congeneric until Parker (1941). In contrast, the Scolecomorphidae and *Herpele-Boulengerula* clades appear to represent relatively deep branches in the higher caecilian clade, with a deep split between the species of *Boulengerula* as judged by branch lengths. If the split between *Schistometopum* and *Dermophis* corresponds to the vicariant separation of Africa from the Neotropics, then this suggests that the origins of some of the current diversity of African caecilians may predate the break-up of Gondwana.

Boulengerula taitanus was transferred to *Afrocaecilia* by Taylor (1968) but *Afrocaecilia* was subsequently synonymised with *Boulengerula* by Nussbaum & Hinkel (1994) based on

phylogenetic analysis of morphological data. Wilkinson *et al.* (in press) argued that phylogenetic signal from the morphological data is weak and that the synonymy might have been premature. The deep divergence of *B. taitanus* and *B. boulengeri* indicated by the molecular data further suggests that a more detailed assessment of the taxonomy of these East African caeciliids is warranted.

Several caecilian genera have closed orbits, and in all of these except the scolecomorphids, the eyes are rudimentary (Wake 1985). It has been argued that rudimentation of the visual system has occurred independently multiple times within caecilians, and that characters of the visual system erroneously group rudimentary-eyed taxa in some morphological phylogenetic analyses (Wilkinson 1997). O'Keefe & Wagner
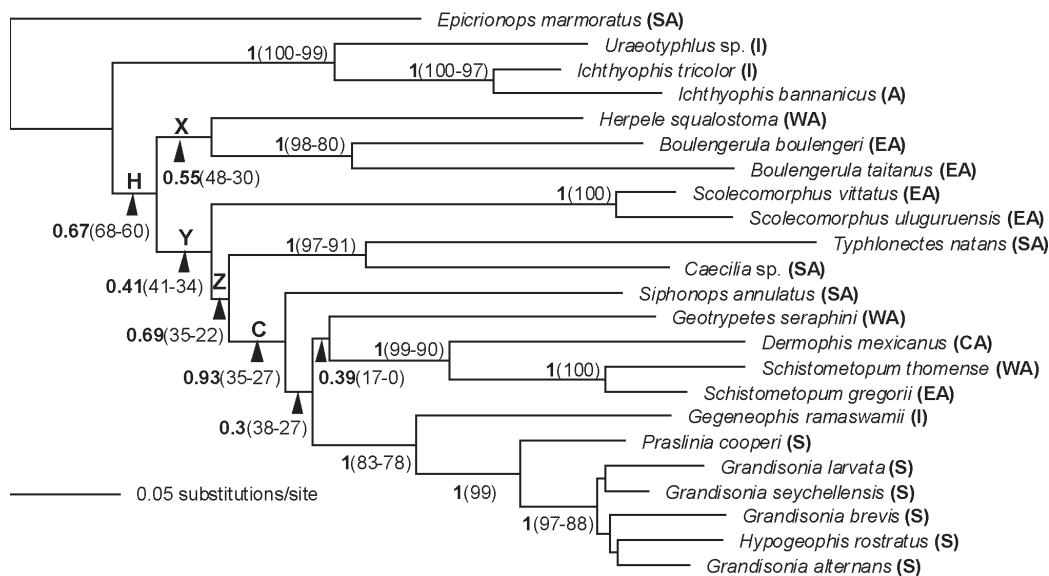

Figure 2. Single maximum likelihood tree (LnL = 7832.49). The chosen model of evolution (TrN + I + G) employed a symmetric rate matrix with AG and CT substitutions set at 3.1738 and 8.9975 respectively, and all other substitution types set at unity; base frequencies estimated at 0.4247, 0.2144, 0.1279 and 0.2330 for A, C, G and T respectively; a four category discrete approximation of a gamma distribution (α = 0.57), and the proportion of invariant sites set at 0.276. Numbers in bold are Bayesian posterior probabilities. Numbers in parenthesis are bootstrap proportions from MLD and (where different) parsimony analyses. Letters in bold after taxon names indicate geographic provenance (A = South East Asia, CA = Central America, EA = East Africa, I = India, SA = South America, S = Seychelles, WA = West Africa). Other bold letters indicate internal branches discussed in the text.

(2001) further rejected the hypothesis that these characters are evolving independently. Our analysis provides the first support from molecular data for the parallel rudimentation of visual systems in caecilians, with this occurring independently in at least two lineages - *Boulengerula* (+ *Herpele*) and *Gegeneophis*.

Our analyses also suggest that viviparity has evolved at least three times, but using the KH test we are unable to reject trees in which all viviparous caecilians are a monophyletic group. The trees for this test were obtained using a backbone constraint that excluded *Schistometopum gregorii* and *Herpele squalostoma* because the reproductive modes of these species are uncertain (Wilkinson & Nussbaum 1998). Natives informed Loveridge (1936) that *S. gregorii* laid eggs in water, but we concur with Nussbaum & Pfrender (1998) that this is unlikely. The placement of *S. gregorii* and *H. squalostoma* in the optimal trees suggests that they are viviparous and oviparous respectively, predictions that can be tested empirically.

Two caeciliids, the West African *Geotrypetes seraphini* and the South American *Siphonops annulatus* are particularly unstable. Their phylogenetic placement is sensitive to method of analysis, is never well supported, and they have the lowest leaf stabilities. Little can be said of their relationships, other than that they lie somewhere within the higher caecilian clade, probably not basally, and that they lie outside the well-supported groupings of the scolecomorphids, *Typhlonectes* + *Caecilia*, *Dermophis* + *Schistometopum* or the Indo-Seychellean clade.

Although partial sequences of mitochondrial 16S and 12S have provided important insights into the phylogeny of caecilians (Hedges *et al*. 1993; Gower *et al*. 2002; Wilkinson *et al*. 2002), they are not adequate for resolving the relationships of African caecilians. In addition, there are strong base compositional biases and some very low transition - transversion ratios that suggest saturation and/or lineage specific rate variation may hinder accurate phylogenetic inference from these data. These potential problems seem particularly to affect comparisons involving the African caecilian sequences, all of which have below average leaf stabilities (Table 2). African sequences appear to provide disproportionately low estimates of transition - transversion ratios and this is particularly true of *Geotrypetes seraphini*. It is also remarkable that, when taxa are ranked by their GC content, the African caecilians exclusively occupy the highest ranks (Table 2). We would not have predicted any simple correlation between geography and base composition, are unaware of any comparable patterns in the literature, and consider its existence intriguing and worthy of further study.

Given that our alignment includes only 16 of 33 genera and 23 of c.160 caecilian species, partial 16S and 12S data should continue to be useful for placing many of the currently unsampled taxa. However, better resolution of the relationships of the African caecilians is unlikely to be achieved purely through the benefits of denser taxonomic sampling and will probably require data from more genes and morphology.

ACKNOWLEDGEMENTS

LITERATURE CITED

BENSON, D.A., M.S. BOGUSKI, D.J. LIPMAN, J. OSTELL & B.F.F. OULLETTE. 1998. GenBank. Nucleic Acids Res. 26: 1-7.

DUELLMAN, W.E. & L. TRUEB. 1986. Biology of Amphibians. McGraw-Hill, New York

FAITH, D.P. & P.S. CRANSTON. 1991. Could a cladogram this short have arisen by chance alone?: On permutation tests for cladistic structure. Cladistics 7: 1-28.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

GOLDMAN, N., J.P. ANDERSON & A.G. RODRIGO. 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49: 652-670.

GOWER, D.J., A. KUPFER, O.V. OOMMEN, W. HIMSTEDT, R.A. NUSSBAUM, S.P. LOADER, B. PRESSWELL, H. MÜLLER, S.B. KRISHNA, R. BOISTEL & M. WILKINSON. 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. & M. WILKINSON. 2002. Phallus morphology in caecilians (Amphibia: Gymnophiona) and its systematic utilty. Bull. Br. Mus. Nat. Hist. (Zool.) 68: 143-154.

HEDGES, S.B., R.A. NUSSBAUM & L.R. MAXSON. 1993. Caecilian phylogeny and biogeography inferred from mitochondrial DNA sequences of the 12S rRNA and 16S rRNA genes (Amphibia: Gymnophiona). Herpetol. Monogr. 7: 64-76.

HILLIS, D.M. 1991. The phylogeny of amphibians: Current knowledge and the role of cytogenetics. Pp.

7-31 *In* S.K. Sessions & D.M. Green (Eds.), Amphibian Cytogenetics and Evolution. Academic Press, San Diego.

HUELSENBECK, J.P. & F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.

KISHINO, H. & M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29: 170-179.

LOADER, S.P., M. WILKINSON, D.J. GOWER & C. A. MSUYA. 2003. A remarkable young *Scolecomorphus vittatus* (Amphibia: Gymnophiona: Scolecomorphidae) from the North Pare Mountains, Tanzania. J. Zool. 259: 93-101.

LOVERIDGE, A. 1936. Scientific results of an expedition to rainforest regions in eastern Africa. VII Amphibians. Bull. Mus. Comp. Zool., Harvard 79: 369-430.

NUSSBAUM, R.A. 1979. The taxonomic status of the caecilian genus *Uraeotyphlus* Peters. Occ. Pap. Mus. Zool. Univ. Michigan 687: 1-20.

NUSSBAUM, R.A. 1985. Systematics of Caecilians (Amphibia: Gymnophiona) of the family Scolecomorphidae. Occ. Pap. Mus. Zool. Univ. Michigan, 713, 1-49.

NUSSBAUM, R.A. 1991. Cytotaxonomy of caecilians. Pp. 22-76 *In* S.K. Sessions & D.M. Green (Eds.) Amphibian Cytogenetics and Evolution. Academic Press, San Diego.

NUSSBAUM, R.A. & H. HINKEL. 1994. Revision of East African Caecilians of the genera *Afrocaecilia* Taylor and *Boulengerula* Tornier (Amphibia: Gymnophiona: Caeciliaidae). Copeia 1994: 750-760.

NUSSBAUM, R.A. & M.E. PFRENDER. 1998. Revision of the African Caecilian genus *Schistometopum* Parker (Amphibia: Gymnophiona: Caeciliidae). Misc. Pub. Mus. Zool. Univ. Michigan 187: 1-32.

NUSSBAUM R.A. & M. WILKINSON. 1989. On the classification and phylogeny of caecilians (Amphibia: Gymnophiona), a critical review. Herpetol. Monogr. 3: 1-42.

O'KEEFE, F.R. & P.J. WAGNER . 2001. Inferring and testing hypotheses of cladistic character dependence using character compatibility. Syst. Biol. 50: 657-675.

PARKER, H.W. 1941. The caecilians of the Seychelles. Ann. Mag. nat. Hist. 7: 1-17.

POSADA, D. & K. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817-818.

ROBINSON, M., G. GOUY, C. GAUTIER & D. MOUCHI-

ROUD. 1998. Sensitivity of the relative-rate test to taxonomic sampling. Mol. Biol. Evol. 15: 1091-1098.

RODRIGUEZ, F., J.F. OLIVER, A. MARÍN & J.R. MEDINA. 1990. The general stochastic model of nucleotide substitution. J. Theoret. Biol. 142: 485-501.

SHIMODAIRA, H. & M. HASEGAWA. 1999. Multiple comparisons of log likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16: 1114-1116.

SWOFFORD, D.L. 1998. "PAUP*: phylogenetic analysis using parsimony." Test version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

TAMURA, K. & M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10: 512-526.

TAYLOR, E.H. 1968. The caecilians of the World. University of Kansas Press, Lawrence.

THORLEY, J.L. & R.D.M. PAGE. 2000. RadCon: phylogenetic tree comparison and consensus. Bioinformatics 16: 486-487.

THORLEY, J.L. & M. WILKINSON. 1999. Testing the phylogenetic stability of early tetrapods. J. Theoret. Biol. 200: 343-344.

WAKE, M.H. 1985. The comparative morphology and evolution of the eyes of caecilians (Amphibia: Gymnophiona). Zoomorphology 105: 277-295.

WAKE, M. H. 1998. Cartilage in the cloaca: phallodeal spicules in caecilians (Amphibia: Gymnophiona). J. Morphol. 237: 177-186.

WILKINSON, M. 1997. Characters, congruence and quality: a study of neuroanatomical and traditional data in caecilian phylogeny. Biol. Rev. 72: 423-470.

WILKINSON, M. & R.A. NUSSBAUM. 1996. On the phylogenetic position of the Uraeotyphlidae (Amphibia: Gymnophiona). Copeia 1996: 550-562.

WILKINSON, M. & R.A. NUSSBAUM. 1998. Caecilian viviparity and amniote origins. J. nat. Hist. 32: 1403-1409.

WILKINSON, M., O.V. OOMMEN, J.A. SHEPS & B.L. COHEN. 2002. Phylogenetic relationships of Indian caecilians (Amphibia: Gymnophiona) inferred from mitochondrial rRNA gene sequences. Mol. Phy. Evol. 23: 401-407.

WILKINSON, M., S.P. LOADER, H. MÜLLER & D.J. GOWER. (in press) Taxonomic status and phylogenetic relationships of *Boulengerula denhardti* Nieden, 1912 (Amphibia, Gymnophiona, Caeciliidae). Zool. Reihe.