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CAECILIAN PHYLOGENY AND BIOGEOGRAPHY INFERRED FROM MITOCHONDRIAL DNA SEQUENCES OF THE 12S rRNA AND 16S rRNA GENES (AMPHIBIA: GYMNOPHIONA)

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Abstract: The classification and phylogeny of the caecilians has received much attention in the past decade, yet no consensus has been reached. We present new mitochondrial ribosomal gene sequence data from 13 caecilian species representing 10 genera from four of the six recognized families. Out of 1208 total sites, 890 were aligned, 475 were variable, and 329 were informative under the parsimony criterion. A phylogeny for these taxa supports the Caudata (as represented by *Amphiuma*) as the sister lineage to the Gymnophiona, the basal position of the Rhinatrematidae and Ichthyophiidae, the paraphyly of the Caeciliaidae, and the monophyly of the Seychellean caecilians.

These sequence data support previous morphological studies suggesting that the typhlonectids were derived from Neotropical caeciliaids, and therefore the Typhlonectidae is synonymized within the family Caeciliaidae. Species previously placed in the Typhlonectidae are assigned to a separate subfamily (Typhlonectinae) of the Caeciliaidae. Resolution of relationships within the largest family, the Caeciliaidae, remains the major task of caecilian systematics.

Key words: Molecular systematics; Ribosomal RNA; Mitochondrial genes; 16S rRNA Molecular clock; Plate tectonics; Seychelles

Of the three living orders of Amphibians, the caecilians (Gymnophiona) are the least understood in terms of both their evolution and phylogenetic relationships. This is in large part attributable to their secretive burrowing habit which makes them difficult to collect and also leaves a relative paucity of morphological characters suitable for phylogenetic analysis. Consequently, these animals are relatively rare in research collections and their systematic arrangements are tentative. Caecilians are tropical in distribution, found only in Central and South America, Africa and the Seychelles Islands, and India and Southeast Asia. This distribution is believed to reflect a Gondwanan origin in the Jurassic and early Cretaceous (Duellman and Trueb, 1986).

Previous studies of caecilian phylogeny have involved both morphological (Duellman and Trueb, 1986; Lescure et al., 1986; Nussbaum, 1979; Nussbaum and Wilkinson, 1989; Wake, 1986) and molecular (Case and Wake, 1977; Hass et al., 1993) analyses. Although a robust phylogeny for the caecilians is still lacking, an interim scheme with six families, 34 genera, and 154 recognized species has been proposed (Nussbaum and Wilkinson, 1989). We have sequenced portions of two mitochondrial genes from representatives of four of the six described families of caecilians (Table 1) in order to examine their phylogenetic relationships. These DNA sequence data support the findings of a companion molecular study of Seychellean caecilians (Hass et al., 1993), allow speculation on the timing and historical biogeography of living caecilians, and support growing evidence that the Gymnophiona and the Caudata are sister taxa (Hedges et al., 1990; Hedges and Maxson, 1993; Larson and Wilson, 1989; Van der Horst et al., 1991).

Materials and Methods

Animals were returned to the laboratory, anesthetized with tricaine methanesulfonate, bled, and tissue removed. The animals then were preserved and cataloged in the University of Michigan Museum of Zoology (UMMZ).

DNA was extracted from small amounts (less than 50 mg) of fresh or frozen liver from 13 species of caecilians as follows: *Caecilia* sp., *Dermophis mexicanus, Epicrionops* sp., *Grandisonia alternans, G. brevis, G. larvata, G. sechellensis, Hypogeophis rostratus, Ichthyophis bannanicus, Praslinia cooperi, Schistometopum thomense, Siphonops annulatus,* and *Typhlonectes natans.* Locality information and museum voucher numbers are presented in Appendix I. Sequences used for outgroups include *Homo sapiens* (Anderson et al., 1981) and the two amphibians *Xenopus laevis* (Roe et al., 1985) and *Amphiuma tridactylum* (Hedges and Maxson, 1993).

The methods used to extract, amplify, and sequence the DNA are described in detail elsewhere (Hedges et al., 1991), with the only exception being that 30,000 molecular-weight (cut-off) filters were used to purify the DNA template rather than 100,000 molecular-weight filters.

Portions of two mitochondrial genes were amplified using the polymerase chain reaction (PCR) and sequenced: (1) an approximately 400-bp region of the 12S rRNA gene (corresponding to sites 1092–1477 in the human sequence; Anderson et al., 1981), and (2) approximately 900 bp of the 16S rRNA gene (corresponding to sites 2205–3055 in the human sequence). To design the oligonucleotide primers we used conserved regions of each of the two genes from several published sequences. (*Xenopus laevis,* Roe et al., 1985; *Mus musculus,* Bibb et al., 1981; *Bos taurus,* Anderson et al., 1982; and *Homo sapiens,* Anderson et al., 1981). The 12S rRNA primers are 5′-AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT-3′ and 5′-TGACTGCAGAGGGTGACGGGCGGTGTGT-3′ (Kocher et al., 1989), and the 16S rRNA primers are 5′-GGCCTAAAAGCAGCCACCTGTAAAGACAGCGT-3′ and 5′-CTCCGGTCTGAACTCAGATCACGTAGG-3′ (presented here). Both complementary strands were sequenced using these four PCR primers, and in the case of the 16S rRNA fragment, three internal primers also were used: 5′-CTGACCGTGCAAAGGTAGCGTAATCACT-3′,5′-AGTGATTACGCTACCTTTGCACGGTCAG-3′, and 5′-GAGGATTTTTATTCTCCGTGGTCGCCCCA-3′ (presented here).

TABLE 1.—Caecilian taxonomy (Nussbaum and Wilkinson, 1989) and species examined.

- Rhinatrematidae
  - *Epicrionops* sp.
- Ichthyophiidae
  - *Ichthyophis bannanicus*
- Uraeotyphlidae
- Scolecomorphidae
- Typhlonectidae
  - *Typhlonectes natans*
- Caeciliaidae
  - *Caecilia* sp.
  - *Dermophis mexicanus*
  - *Grandisonia alternans*
  - *G. brevis*
  - *G. larvata*
  - *G. sechellensis*
  - *Hypogeophis rostratus*
  - *Praslinia cooperi*
  - *Schistometopum thomense*
  - *Siphonops annulatus*

Sequence data were read from autoradiograms using a digitizing program (S. W. Schaeffer, Pennsylvania State University), and alignments were done by eye using the multisequence editing program ESEE (Cabot and Beckenbach, 1989).

Two phylogenetic methods were used to analyze nucleotide sequence and length variation: neighbor-joining (Saitou and Nei, 1987) and maximum parsimony, using the computer programs of T. S. Whittam (NJOIN and NJBOOT; Pennsylvania State University) and D. L. Swofford (PAUP 3.0; Illinois Natural History Survey), respectively. Every length difference of one or more bases was scored as a single event with two states (insertion or deletion), and these gap sites were added to the data set. Sequence variation within inserted regions was analyzed (simultaneously) by treating the gaps as ambiguities, and including the inserted region in the data matrix. The pairwise distances used in the neighbor-joining analysis were corrected for multiple hits using the standard 4-state Jukes–Cantor formula (Jukes and Cantor, 1969) for nucleotides plus a 2-state correction for gap differences ($-1/2 \ln [1 - 2p]$, where p is the proportion of gap differences out of the total number of sites). Because p for gaps usually is very small, it is often identical to the 2-state correction. The programs NJOIN and NJBOOT were modified by the senior author for use with gap data. For the PAUP analysis, the heuristic search option was used with ACCTRAN optimization, simple addition, TBR branch-swapping, and MULPARS. For both analyses, 2000 bootstrap replications were performed to obtain accurate confidence estimates (P-values) for nodes in the trees (Hedges, 1992).

RESULTS

For the 12S rRNA and 16S rRNA fragments combined (1208 sites), there are 890 aligned sites (Fig. 1), 475 of which are variable (329 are "informative" under the conditions of parsimony), and 35 "gap sites". The initial 20 nucleotide sites in the 12S rRNA fragment (adjacent to the primer) and about 40 nucleotide sites at the beginning and end of the 16S rRNA fragment could not be obtained in all of the taxa and therefore they are not included in Fig. 1 or in the analyses. There are several highly variable regions where nucleotide sequence and/or length variation have resulted in uncertain alignment. These regions, indicated in Fig. 1, also were excluded from the phylogenetic analyses.

Both phylogenetic methods produced nearly identical trees (Figs. 2 and 3) having very similar bootstrap P-values. All 13 caecilians form a monophyletic cluster, and the salamander (*Amphiuma*) is identified as the sister group to the caecilians. The rhinatrematid (*Epicrionops*) represents the basal lineage within the caecilians, followed by the ichthyophiid (*Ichthyophis*). The Neotropical aquatic family Typhlonectidae (*Typhlonectes*) clusters with our Neotropical caeciliaid representative (*Caecilia*) and that lineage is the next to diverge. All of the remaining species form a significant cluster and are all members of the same subfamily, the Dermophiinae (sensu Taylor, 1969) of the family Caeciliaidae. Within this group, *Siphonops* is the most basal lineage, followed by the lineage leading to *Dermophis* and *Schistometopum*. All of the remaining species occur in the Seychelles and form a significant group. Within this Seychellean group, *Praslinia* is the sister taxon to all other species (which also form a significant cluster). Both analyses join *Grandisonia alternans* and *G. sechellensis* as sister taxa and place *G. brevis* as the most basal lineage of *Grandisonia*. The only difference between the two trees is the position of the Seychellean genus *Hypogeophis*. The neighbor-joining analysis (Fig. 2) places this lineage as the sister group to the four species of *Grandisonia* while the parsimony analysis (Fig. 3) places *Hypogeophis* within *Grandisonia*, as the sister species to *G. larvata*. Bootstrap values for the neighbor-joining arrangement of *Grandisonia* species are higher than the PAUP arrangement of those species, but none is statistically significant.

DISCUSSION

Molecular Evolution

The pattern of nucleotide substitution in both the mitochondrial 12S and 16S rRNA genes shows a relatively high frequency of transitions relative to transversions (Tables 2, 3) as observed in numerous other studies (e.g., De Salle et al., 1987; Hedges et al., 1991). This transition bias is interpreted as evidence that the data are not saturated with respect to multiple substitutions, because the percentage of transitions usually decreases to a plateau around 40–45% when multiple substitutions begin occurring at the same site (Brown et al., 1982; Hedges et al., 1991).

Because of the poor fossil record of caecilians, it would be useful to estimate times of divergence within this group from the DNA sequence data. An excellent calibration point for such a clock is the separation of India and the Seychelles from Africa at 64 million years ago (mya) (Dickin et al., 1986; Mart, 1988). The mean albumin immunological distance between the Seychellean species and other caeciliaids is 106 (Hass et al., 1993), which corresponds exactly to that date (64 mya) based on the vertebrate albumin clock (Maxson, 1992).

In most cases, nucleotide differences between sequences need to be corrected for multiple hits, unequal rates of substitution, and unequal base composition frequencies. The latter correction is not needed for this data set because the four bases are relatively close in frequency, with only a slight overabundance of adenine. The means for all sequences are: A (34%), C (24%), G (19%), T (23%). The effect of multiple substitutions can be corrected by the Jukes–Cantor formula (Jukes and Cantor, 1969). However, to avoid the transition bias, it is sometimes necessary to simply ignore transitions altogether. If the proportion of transversions is above about 10%, they too may exhibit the effects of multiple hits in which case the formula proposed by Kimura (1980) can be used as a correction. In this data set, the highest proportion of transversions is 9% (human versus *Epicrionops*) and therefore such a correction is unnecessary.

The mean number of transversions separating the Seychellean species from their closest relatives among the caeciliaids (*Dermophis*, *Siphonops*, and *Schistometopum*) is 62 (Table 2). Use of the above geologic date (64 mya) gives a calibration of about one transversion per million years pairwise divergence. With that calibration, the divergence of *Praslinia* from the other Seychellean species occurred 25 mya (mean number of transversions = 25.4), and the radiation of *Hypogeophis* and the species of *Grandisonia* began at 15 mya (mean = 15.0 transversions separating all five species). Those dates are quite similar to the independent estimates obtained with albumin immunological data, 30 and 14 mya, respectively (Hass et al., 1993). However, the rate of change before our calibration point (64 mya) appears to be slower, as evidenced by the underestimate of the Lissamphibian/amniote divergence of 98 mya (Table 2, mean of human versus amphibians) which is known to be about 350 mya (Carroll, 1988).

This apparent difference in rate could be the result of (1) a real increase in rate during the evolution of caecilians, or (2) unequal rates of transversions among sites, resulting in saturation at selected sites. We are unable to distinguish between those two alternatives and therefore refrain from making any Mesozoic divergence time estimates from the sequence data. However, some information can be obtained from the immunological data (Hass et al., 1993). Two independent albumin immunological distances were obtained between *Ichthyophis* and a caeciliaid: 210 using an antiserum to *Dermophis* (Case and Wake, 1977), and 215 using an antiserum to *Typhlonectes* (Hass et al., 1993). These values are above the limit of accuracy for micro-complement fixation in estimating divergence time (Maxson, 1992), and suggest that divergence occurred by the early Cretaceous. No cross reactions were obtained between *Epicrionops* and four of the five caecilian antisera used (Hass et al., 1993), suggesting an equally old divergence, and the caecilian relationships obtained in this study (Figs. 2, 3) would indicate that the rhinatrematids must have diverged before the ichthyophiids (early Cretaceous or late Jurassic).

These DNA sequence data support the recognition of the Rhinatrematidae as a family distinct from the Ichthyophiidae (Nussbaum, 1977) in contrast to Taylor's (1968) association of the two taxa. The position of both of these lineages in the sequence phylogeny (Figs. 2, 3) agrees with the morphological evidence (e.g., possession of a true tail) for their basal position among caecilians. Recent evidence from

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FIG. 1.—Mitochondrial DNA sequences of portions of the 12S rRNA gene (corresponding to sites 1132–1477 in human; Anderson et al., 1981) and the 16S rRNA gene (corresponding to sites 2246–3015 in human) in an amniote (human), a frog (*Xenopus*), a salamander (*Amphiuma*), and 13 species of caecilians. The human, the frog (Roe et al., 1985), and the salamander (Hedges and Maxson, 1993) sequences have been published; all others are new to this study. A solid dot (·) denotes identity with the first sequence; a dash (–) denotes a gap, and an "N" denotes an ambiguity. Highly variable regions where alignment is uncertain were not used in any of the analyses and are indicated by a solid line above the alignment.

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               12S>-----+---------+---------+---------+---------+---------+---------+------ 80
 1 Human       TCGCCAGAACACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTCATATCCCTCTAGAGGAGCCTGTTCTG
 2 Xenopus     C.........--........TA..........C........T..........C..A.C..AC..................
 3 Amphiuma    C.......GT...............................T..A.......CT.C.C..AC.................A
 4 Epicrionop  .......GGT.........A.......G....C........T........TCC..A....ACTC................
 5 I.bannanic  C.................TA.ACA.......G.........T..C......CC..A....AC.C...............A
 6 Typhlonect  C.......G..........AG....................T........-CC..A.C.TACT................A
 7 Caecilia    CT......GT.........AC....................T..A......CC..A.C..AC.C...............A
 8 Dermophis   CT......GT.....A...A...........T.....N...T.......ATCC..A.A...C.C...............A
 9 Siphonops   C.A.....GT...............................T........-CC..A....AC.C...............A
10 Schistomet  ........GT.........A.T...................T........TCC..A....ACTC........T......A
11 Praslinia   C.......G..T.......A.....................T........-CC..C.......C...............A
12 Hypogeophi  C.......GT.T.......A.T...................T........-CC..A.C.....C...............A
13 G.alternan  C.......GT.T...N...A.....................T........-CC..A.......C...............A
14 G.brevis    C.......GT.T.......A.T...................T........-CCT.A.C.....C...............A
15 G.larvata   C.......GT.T.......A.T...................T........-C...A....A..C...............A
16 G.sechelle  C.......G..T.......A.T...................T........-CC..A.......C...............A

               ---------+---------+---------+---------+---------+---------+---------+------ 160
 1 Human       TAATCGATAAACCCCGATCAACCTCACCACCTCTTGC-----TCAGCCTATATACCGCCATCTT--CAGCAAACCCTGAT
 2 Xenopus     .........CC..T..C.A...........T......CAAA.C.C...........A..G..GC......CC...TC.-.
 3 Amphiuma    ..........T..A....A............AA....AAGC.A.............A..G..CC......TT...TCA-C
 4 Epicrionop  .........CC.....C.A.........TT.C.....TAAT...............A..G..A.GT....CT...T..-.
 5 I.bannanic  ..........C..A..C.A...........TCT....CAAA.A.............A..G...G......TC....CAT.
 6 Typhlonect  ..........C..A..T.A..........T..T...ATCC................A..G..GC......T.G..T.T-.
 7 Caecilia    ..........C..A..T.TT.........T..T.C...AA................A..G..GC......C....TCT-.
 8 Dermophis   ..........C..A..T.TT....T....TT.T....TAAC....A.............G..A.......CC...T.T-.
 9 Siphonops   ..........C..A..C.A............CT....CTA.....N.T........A..G..A......TTT......-.
10 Schistomet  ..........T..A..T.TT.........TTCT....CC.................A..G..AC......TT...T..-.
11 Praslinia   ..........C..A..C.G............AT...ACAAC.C.............A..G..AC......CTG..T.A-.
12 Hypogeophi  ..........C..A..T.T............AT...ATAAC...............A..G..AC......TTG..T.A-.
13 G.alternan  ..........C..A..C.T............AT.C.ACAACA.....T........A..G..AC.T.C..CTG..T.A-.
14 G.brevis    ..........C..A..C.T............AT...ACAAC.C.............A..G..AC..T...CT...T.A-.
15 G.larvata   ..........T..A..C.T............AT...ATAAT.C.............A..G..AC.......TG..T..-.
16 G.sechelle  ..........C..A..C.T............AT...ACAAC.C.............A..G..AC......CTG..T.A-.

               ---------+---------+---------+---------+---------+---------+---------+------ 240
 1 Human       GAAGGCTACAAAGTAAGCGCAAGTACCCA--CGTAAAGACGTTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGG
 2 Xenopus     ..GA.A.T.TT....G..TT..TG.TTTT.T.A.C..C....C...............AT....A....G..........
 3 Amphiuma    A.GA.AA.A.C.......AA..C..TTA..AAA....A....C...............AT..AT..C..A..........
 4 Epicrionop  .G.AAGATAT..CAGTAA....AATGAAT.C.A.CC.T....C...............A....G.AC..G..........
 5 I.bannanic  .....AA.AT....G...AG..-.GAA.T.TAC....A....C...............A...AGA....A..........
 6 Typhlonect  A..A.AA.T...ACT...CA..CAGGA...G.CC...T....C...............A...A..A...A.-........
 7 Caecilia    ..GA.A.TTGC.A.T...AA..C.GTAGC.C.AC..GC....C.......T.......A...A..A...A..........
 8 Dermophis   ...A.ACTA.T..AC...TA..C.GAA..TTACC...T....C..............TAT....AA...AC.........
 9 Siphonops   ...A.AGT........A..A..T.GATT....TC...T....C...............AT..A...C..G..........
10 Schistomet  ..GA.AACACT...T...AA..A.GAT...TATC...T...AC...............AG..AGAA...AT.........
11 Praslinia   ...A.AACT....C....AA..ACGAAG..TACC...C...CC...............AT..AT.....G..........
12 Hypogeophi  ..GA.AACT.C..C....AA..ACGAAAC.TTCC...T...CC...............AT..AT.....G..........
13 G.alternan  ...A.AAC..T..C....AA..ACGAAAC.TTCC...T....C...............AT...T.....G..........
14 G.brevis    ...A.AACT.T..C....AA..T.GAAAT.TGCC...T....C...............A...AT.....A..........
15 G.larvata   ...A.AAC..C..C....AA..ACGAAAT.CTCC...T...CC...............AT..AT.....G..........
16 G.sechelle  ...A.AATT.T..C....AA..ACGATAT.CTTC...T....C...............A...AT.....G..........

               ---------+---------+---------+---------+---------+---------+---------+------ 320
 1 Human       CTACATTTTCTACCCCAGAAAACT-ACGATAGCCCTTA-TGAAACT-TAAGGGTCGAAGGTGGATTTAGCAGTAAACTAA
 2 Xenopus     ............TA..TT.G..TAA....A..AT..CTA......CAG.TC.A--...AAC...............GAG.
 3 Amphiuma    ............TA.AGA...TA-.CG..C.A..TAA-......TA..T..ACCT.....A.A.............GAG.
 4 Epicrionop  ...........TATAA....T.AC....GATAAG..A.A.....AC.A.CTTT--.....C......GA.......AAG.
 5 I.bannanic  ...........TA.TA....C.A-.....G.AAG.AA.......AA..TCTT.--.....C......G..T.....AAG.
 6 Typhlonect  ...........TTTTA.AG....-....GA.AGT..C.......AC.A.CTCC--A...AC.......A......G.GGG
 7 Caecilia    ...........T..A..AG...T......ATAA.GCA.......AA....TTT--.....C......G.......GAAG.
 8 Dermophis   .........T.TA.AA.A..T.TC.....CTAATA.........AA.AT.TTT--A....C......G.T......A...
 9 Siphonops   ...........TAAAA....T.T.T....A.TTAT.........AC.AC.TTTGAAGG.AG......GAT.....GAGT.
10 Schistomet  ...........TAAAA....T.TC.....ATAA.A.A.......T.TATGTTT--A....C......GA......GA.TG
11 Praslinia   ...........TT.AA....T.T-.....AT.ATAAAC.......C.AC.TCT--.....C......GAT......AATG
12 Hypogeophi  ...........TATAA...CC.T-.....AT.ATAAA........C.AT.TCT--A....C......G.......GAATG
13 G.alternan  ...........TTTAA...CC..-.....AT.G.AAA........C.AT.CCT--.....C......G.......GAGTG
14 G.brevis    ...........TA.AA...TC.T-.....AT.ATA.A.......TC.AC.CCT--.....C......G.......GAACG
15 G.larvata   ...........T.TAA...CC.T-.....AT.A.AAA.......T..AT.TCT--.....C......G.......GGCTG
16 G.sechelle  ...........TTTAA...CC.T-.....ATAATAAA........C.AT.TTT--.....C......G.......GAGTG
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               ---------+---------+---------+-----<12S-||-16S>--+---------+---------+------ 400
 1 Human       GAGTAGAGTGCTT--AGTTGAA-CAGGGCCCTGAAGCGCGTACTGAACTCCTCACAC-CCA-ATTGGACCAATCTATCAC
 2 Xenopus     A.CA....A.T.CCTCT..A...-.C.......G......CT..A...-..C..A...AAT..C..AG.T.T.....A.-
 3 Amphiuma    A.AA....A.T.C.TCT..A.G.-TT...AA.AG....T..T.AA.CT-T.AATACT.AGT...CAAG.........A.-
 4 Epicrionop  A.CATTTACAT.C.TCT..A...-CC.......GG.G...CT.A....-..A.CA.A......AC.AG..C..T...A..
 5 I.bannanic  A.TA.TTA.AT.C.TTT..C.....CA.....T.GCGCT.CT.AA...-..AA.ACA.A....C..AGTT..C....A--
 6 Typhlonect  ACCA.TTA.ATCC.CTC..A...-CCA......GG.GCA.CT.AA.C.-..A.TAG..A....A..AG....C....ACA
 7 Caecilia    A.CC.TTA.AT.C.TTC..A.T.-TT...T....G.....CT.AA.C.-.TAT.A.A......A..AG.T.G.....AGA
 8 Dermophis   ..TC.CCA.A....ATT..A...-CC.......GGAT...CT.AA...-...A.A.A......A..AG.T.CA....A.-
 9 Siphonops   ..CAT.TA.A..-.ACC..A...-CT.......GG.-...CT.AA...-...T.TTAA...A.C..AG.TCCA....A.A
10 Schistomet  A.TA.TCA.AT.A.ACC..A...-CT.......GGA....CT.AA...-.T.A.A.A......ACAAGT..CA....A.A
11 Praslinia   ..TA.T.A...CA.TTTC.A....CA.......GG.....CT.AA...-..ATCA.A...C..A..AGT.CGC....A..
12 Hypogeophi  ..CA.TTA.A.CA.TCC..A.TTTTT.......GG.....CT.AA...-..ATTA.A...T..A..AGTAC......A.A
13 G.alternan  ..TA.T.A...CA.CCC..A.C.T.C.......GG.....CT.AA...-..A.TA.A...T..AC.AGTATGC....A.A
14 G.brevis    ..TA.T.A...CA.CTC..A.C.ATT.......GG.....CT.AA...-..ATTA.A..AT..A..AGTACGC....ATT
15 G.larvata   .GTA.TTA.A.CA.CCC..A.T..TT........G.....CT.AA...-..ATTA.A.A.T..A..AGTATGC....A.T
16 G.sechelle  ..CA.ATA...CA.CCC..A.T..CT........G.....CT.AA...-..A.TA.A.A.T..A..AGTATGC....A.A

               ---------+---------+---------+---------+---------+---------+---------+------ 480
 1 Human       ---CCTATA--GAAGAACTAATGTTAGTATAAGTAACATGAAAACA----TTCTCCTCC-GCATAAGCCTGCGTCAGAT-
 2 Xenopus     ...A...........C...T...C...A.CT.....TG...-T...CGA........AA.ATG....TG.AAA.......
 3 Amphiuma    ...AA..G...-TGCTG......C..AA..G.......A.G.T---..CCGC.CT..AA.ATGC...TG.AAAC....A.
 4 Epicrionop  ...TA......-CCA.GTCG..AC...A..T.......A.......AGAT..TCT.AAAG..CA.GCTG.AA......C.
 5 I.bannanic  ...TAA..G..A.TA..T.........A........TTA.......CGAT..TCT.CGT.A.GA.CC.G.AAA.......
 6 Typhlonect  CAC...........C..T........AA.CT.......A....G..CCCC..TCT.CA.........TG.AA.CT.T.A.
 7 Caecilia    ....T........CCCGTC.......AA.CT.....T.A.G...T.CAAC...CT..GA....CC..TG.AAT.....C.
 8 Dermophis   ...TA......TT.A..A.T...C...A.CT.....T.A.....T.CAAT..TCT..ATT......A.A.AAA.....A.
 9 Siphonops   ...TA.......CC........AC...A.CT.....T.A....CTCCAAT..TCT........CTCTTA.AAA.....A.
10 Schistomet  ...A........CTAT.T.........A..T.....T.A......TTGAT..TCT..ATAC...CT.TG.ATA....CAT
11 Praslinia   ...A........C.A..T.T...C...A.CT.....T.A....CTTCGAT..TCT.CAT.....CCATG.AAA....CA.
12 Hypogeophi  ...TT.......CCA..T.........A..T.....T.A...GC..CAATC.TCT..ATA.....CATG.AAA....CC.
13 G.alternan  ...TT.......CC...T.........A.CT.....T.A....G..CAAC..TCT....A...CC..TG.AAA...TCC.
14 G.brevis    ..AA.....TA.CC...T.........A.CT.....T.A....CT.CAAT..TCT..AAAA...CT.TA.AAA....C..
15 G.larvata   ...AA.......CC...T.........A..T.....T.A....C..TAAT..TCT..AAA...CCT.TA.AAA....C..
16 G.sechelle  ...A........CT...T.........A.CT.....T.A....T..CAAC..TCT..TTA....CC.TG.AAA....C..

               ---------+---------+---------+---------+---------+---------+---------+------ 560
 1 Human       TAAAACACTGAACTGACAATTAACAGCCCAATATCTAC---------------AATCAACCAACAAGTCATTATTACCCT
 2 Xenopus     CG..TA.A.-C.....T.......GT..TCCCTGAG.TCCTTGCAA.....TA.CAA...A.GA..AC...GCACTT-A.
 3 Amphiuma    CG....G..-CT...GT.......GAA....CC.AA.AGAA..........CT.TAGC..A.TA..AAACC...A.AATA
 4 Epicrionop  CGG.C.CAA-C...A....C..C.GA.....ACGACTATGGCATTAATATTAAC.AACGAG..GCCCA.TAGCC..A..C
 5 I.bannanic  CG..CT.A.-C.....T.....C.GA...TG..AA.GGAAATAAA.CACCAATC..A...A.GA..AA.....AACA.TG
 6 Typhlonect  CGG.C.G.C-C.A.AG......C.GA.T----------......GGCACTACA...A...A.G----CTG.C.AACTAA-
 7 Caecilia    C.G.TATAC-C.....T.....C.GA.GT.T.------......GGCCCAATT..CATTAA.CA.GAAT....CC-TAA.
 8 Dermophis   CGG....AC-C.....T.....C..ATAATGA..A---......TAAACCTTTT.A...GATTT.CAAAC.C.AA-TAA-
 9 Siphonops   CG..TT.A.-C.....T.T.C.C...TAA.-..AAA--.........CAAACT..-ACTAG.TATTA.AC...AA----.
10 Schistomet  CG..C.CA.-C.....T.....C..AA..--..ATA--.......TAAAAACTTT-ACTAGC.ACCCAAT...CA---..
11 Praslinia   CG..CT.A.-C.....T.T...T..A.---.C.GT.CACC.....CAAACAATT.CACTAG.CATTCAACC.CAA---T.
12 Hypogeophi  CG..CT.A.-C.....TG......GA.TA..C.AT.TAACA....TAGACAAT.G.ACTAG.CAT.CAAC..CC.--.TA
13 G.alternan  CG..CT.A.-C.....T.T...T.GAT.GT...AT.TAACC....TAAACAATCCCACCAG.CATTCAAT..T.A---.A
14 G.brevis    CG..C....-C.....T..C.....A..A....AT.CTAT.....TAAAAAATC.CACTAG.CACCCAAT.....TA.TA
15 G.larvata   CG..CT.A.-C.....T.T...T.GA.TA.GC.AT.TAAT....ATAAACAATTCCACTAG.CATTCAAT..TAC--..C
16 G.sechelle  CG..CT.A.-C.....T.C...T.GA..A..C.AT.TAACC...ATTAACAATTC.AC.AG.CATCCAAC..TAC--..A

               ---------+---------+---------+---------+---------+---------+---------+------ 640
 1 Human       CACTGTCAACCCAACACAGGCATGCTCATAAGGAAAGGTTAAAAAAAG-TAAAAGGAACTCGGCAAATCTT-----ACCC
 2 Xenopus     T..C..T..T.T......A.A.CAT.T.-C.......A......G.C..C.G...............CTA....GA....
 3 Amphiuma    A.TC..T.............AGCATGA--C.A.....A......GGTT.A.................CTA-...AGG...
 4 Epicrionop  ...C..T.....G.......AGC..ATT-C.C.....A..T...G.TA.A.G........A......CAA.C.AAGGT..
 5 I.bannanic  ACTC..T.....C........TCTGCA.C..C.....ACA....G.CA.A.G.........A......AA-...AAC.TT
 6 Typhlonect  --.C..T.............AC...A---..C......C.....G.CA.CTG......T.AA.....CA--...AAG.TT
 7 Caecilia    .C.C..T.....T.......AG...C---..CAG...AC..C..G.CA.A.G........A.....CATC-...AAG.TT
 8 Dermophis   ..A...T.....T.......A....ATC-TTA.....A.A...CC.TA.A.G...............ATAAATTAAG.TT
 9 Siphonops   ......T.............AG...AA--C.A.....AC.....G.CA.A................CCT..TG.AA..TT
10 Schistomet  T.T..CT.....T.......A...GGAG-TGAA...--........TA.A........T........CA.-...AAG.TT
11 Praslinia   TT....T.............A....A.C-..A.....ACA.....GTA.A.G..............C.AC-...AAG.TT
12 Hypogeophi  ...C..T.............A.....AT-T.A.....ACAT..T.GTA.A.G..............TCA.-...ACG.TT
13 G.alternan  ...C..T.............AG....AC-C.A.....ACAT..G.GTA.A.G..............TCA.-...AAG.TT
14 G.brevis    ...C..T.............A...T.TT-C.A.....ACAT....GTA.A.G..............C.A.-..TGAG.TT
15 G.larvata   ...C..T..T..........AG....AT.C.A.....ACAC....GTATA.G..............CCA.-...AAG.TT
16 G.sechelle  ...C..T.............A....AAT-C.A.....ACAT....GTA.A.G..............CCAC-...AAG.TT
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               ---------+---------+---------+---------+---------+---------+---------+------ 720
 1 Human       CGCCTGTTTACCAAAAACATCACC-TCTAGCATCACCA-----GTATTAGAGGCACCGCCTGCCCA-GTGACACATGT-T
 2 Xenopus     ....................TCG.C...T..T.A.AA.ACATT....A.....TC.A.........A.....GT..A.G.
 3 Amphiuma    .....................G......T.ATCA..T......A...A.....T..T..................C....
 4 Epicrionop  .....................GT.........AACATCA.CATAC.......AT..T..............T.TTGA...
 5 I.bannanic  .....................G..........AA..T......A.........T..T..............T.A.C-...
 6 Typhlonect  ...............................TAAC..-GACAC........A....T................TTGA...
 7 Caecilia    .....................G.........TAA---.TCCAT..........T..T................T.GA...
 8 Dermophis   ..........................T....TAA-AATTACAAA.....A..C-..T..............T.TTAT...
 9 Siphonops   ...............................CAA-AT.ACCAT.........C-..T..........C...T.TTCT...
10 Schistomet  ...............................TAA-AT.CACATA........C-..T..............ACTT-A...
11 Praslinia   ................................AAC.T.GACAT.........C-..T..............TTTT-A...
12 Hypogeophi  ................................AACAT..ACAC.........C-..T.....N........TTT.-A...
13 G.alternan  ................................AAT.T.AACAC.........C-..T..............TTTT.A...
14 G.brevis    ................................AAC.T.AACAT.........C-..T..........C...TTTT-A...
15 G.larvata   ................................AAC.TTAACAT.........C-..T..............TTTT-A...
16 G.sechelle  ................................AAT-TCAACAT.........C-..T..............TTTT-A...

               ---------+---------+---------+---------+---------+---------+---------+------ 800
 1 Human       TAACGGCCGCGGTACCCTAACCGTGCAAAGGTAGCATAATCACTTGTTCCTTAAATAGGGACCTGTATGAATGGCTCCAC
 2 Xenopus     C.............-T..G................G........----GTC.T....A.A..TG.......C...--...
 3 Amphiuma    ..........A.....A......C..G........GC...........TTC......AA............C...AAA..
 4 Epicrionop  ..............TT..G................G...........ATT.......AA...TA.......C...AAA..
 5 I.bannanic  ..............T.T.G............................CTTC......AA...TA.......C...AAA..
 6 Typhlonect  ........A.....TTT.G.............................TT.......AA...T............AAA..
 7 Caecilia    C.......A.....TTT.G.............................TT.......AA............A...AAG..
 8 Dermophis   .T............TT...............................CTT.......AA...TA.......C...AAG..
 9 Siphonops   ..............TT..G.............................TT.......AA...TA...........AAG..
10 Schistomet  C.............TT.CG............................CTT......CAA...TA.......C...AAA..
11 Praslinia   C.............TT..G............................C.T.......A....TA.......C...AT...
12 Hypogeophi  C.............TT..G............................CTT.......AA...TA...........A....
13 G.alternan  C.............TT..G......NN....................CTT.......AA...TA.......C...A....
14 G.brevis    C.............TT..G............................CTT.......AA...TA.......C...GT...
15 G.larvata   C.............TT..G............................CTT.......AA...TA.......C...A....
16 G.sechelle  C.............TT..G............................CTT.......AA...TA.......C...A....

               ---------+---------+---------+---------+---------+---------+---------+------ 880
 1 Human       GAGGGTTCAGCTGTCTCTTACTTTTAACCAGTGAAATTGACCTGCCCGTGAAGAGGCGGGCATAACACAGCAAGACGAGA
 2 Xenopus     ..A......A.......C.G.A.CC..T.CA.T...C......--.....C.........G...GA..CAT.GA......
 3 Amphiuma    .....CC..A.........TAAAC...T....A.......T..C.T....C...A...A.G...CT.ATAT.........
 4 Epicrionop  ....AC.T.A.......C.TTA.CCT.T............T..TT....AC.A.A....AA.......CA..........
 5 I.bannanic  ..A...CT.A.......C.TTG.C..CT..A.........T.CT..A..AC.A.A..T.TA....A.T.A..........
 6 Typhlonect  ..AA.C.T.A.......CACTG.CC.G.........C...T..T..T..TC.A.A..A..A....A.AT.T.......A.
 7 Caecilia    ..AA.C.T.A.......C.TTG.C..GT.TAC...CC...T..C..T..A..A.A..A..G...CGC.C.T.........
 8 Dermophis   ..AA.C.T.A.......C.TTA...T.T..A.....C...AT.T..T..AC.A.A..A..A....ATT.AT.........
 9 Siphonops   ..AA...T.A.C.....C.TTG.C..GT..A.........T..C.TG..AC.A.A..CA.G......T.ATT........
10 Schistomet  ..AA.C.T.A.......C.TTA..C..TT.A.........A..T.TT..AC.A.A..AA.A..T..CT.AT.........
11 Praslinia   ..AA.CCT.A.......C.TTAC...GT..A.....C...T..C.....AC.A.A.....A...CA.T.A..........
12 Hypogeophi  ..AA.C.T.A.......C.TTAC...GT..A.........T..C.A...AC.A.A...T.A........AT.........
13 G.alternan  ..AA.CCT.A.......C.TTACCC.GT..A.....C...T..T.A...AC.A.A...T.A...CT.A.AT.........
14 G.brevis    ..AA.CCT.A.......C.TTA....GT..A.....C...T..T.A...AC.A.A...T.A...TT.A.AT.........
15 G.larvata   ..AA.CCT.A.......C.TTAC...GT..A.........T..T.....AC.A.A...T.A...CT.A.A..........
16 G.sechelle  ..AA.CCT.A.......C.TTAC...GT..A.....C...TN.T.....AC.A.A...T.A...TT.A.A..........

               ---------+---------+---------+---------+---------+---------+---------+------ 960
 1 Human       AGACCCTATGGAGCTTTAATTTATTAAT--GC-AAACAGTACCTAAC----AAACC--CACAGGTCCTAAACTACCA-AA
 2 Xenopus     ..-................AC..AAG.ACT..C..GTT.A.......CCAT..GGA..A.T.ACAAT.....A.G..G..
 3 Amphiuma    ..................GA--------..C.T....TACCTAATTACCTCT...A..T.T.ATCAT..G...T---.--
 4 Epicrionop  ......C.........C..A-----C.CGCAGTC...TAC.TTAC..AATCT.C.A....A.AACATG--------.AG.
 5 I.bannanic  ................A..A--------..--C.C..TAG.AAGTG.AACCTTTTT..T.ACTTCATG-------..TC.
 6 Typhlonect  ............A.....GA--------..--.-------CA.A..-TCAGCT.TGC.-----CAA.AG.C.GCAT.TGC
 7 Caecilia    ................A.GA--------..--.-------TAAAC.GACAACTTTAC.----CACT.ACCCAC.GT.A..
 8 Dermophis   ............A...A..A--------..--.-------CA.GC.GTCA.CTTTAA.----------GTTA..TT.A..
 9 Siphonops   ............A...A..A--------..--.-------CA.GC.TCCACCC.TAC.--GTTTATAA..CA.CT..T.T
10 Schistomet  ............A...A..C--------..--.-------CA.AC.ACCAACTTAAT.-----TAAT...TAA..T.A.T
11 Praslinia   ............A...A..A--------..--.-------CA.AC.ACCA.CTTTAA.-----TAAAAC.TA.TAA.AT.
12 Hypogeophi  ............A...A..A--------..--.-------CA.GC.ACCA.CCTTAA..CACCACAA..GTAATAT.ACT
13 G.alternan  ............A...A..A--------..--.-------CA.GC.ACCA..TTTAA.TCT.ATGTAAT.G.A.TA.AC.
14 G.brevis    ............A...A..A--------..--.-------CA.GC.GCCA.TTTTAAT.TA.CACAAC.GCAATAATAC.
15 G.larvata   ............A...A..A--------..--.-------CA.GC.ACCA.CTTTAA.TCTTATATAAC.GTA..A.AT.
16 G.sechelle  ............A...A..A--------..--.-------CA.AC.ACCA.CTTTAA..C.TACATAAT..TA.TA.AC.
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               ---------+---------+---------+---------+---------+---------+---------+------1040
 1 Human       CCTGCATTAA--AAATTTCGGTTGGGGCGACCTCGGAGCAGAACCCAACCTCCGAGC-----------------AGTACA
 2 Xenopus     A...ACC......GT.................A.....A.T..AAA.T.....TT.AAGAATAGGGCCTACCACCC.TTC
 3 Amphiuma    ----AGCC....CGTC..A.............A..C..G.A..AAA.T...A....A................TGAACA.
 4 Epicrionop  ....AC.CCC..TGT.................A.......AT.T.A.C........A.........TGATAACC.CCTA.
 5 I.bannanic  AT.C..CAC-..TGT.................A.....A.C.-.TT..........A.................--.GAC
 6 Typhlonect  .A..ATCC.-..C.TC..TA...........TG.....A.C.-AAA.T......T.A.................-TAT.T
 7 Caecilia    .....C...C..T.TC...A...........TA.......C.-.AA.C......C.A................ACTATAT
 8 Dermophis   ....A.CAT.C.TGT...T........T....A.....A.T.-AAA.T........A.................TAACA.
 9 Siphonops   AT..G.CCGC..TGT...T.........A...A.....A.A.-A.A.T.....T..A................G.T..A.
10 Schistomet  T...G.CCCGT.TGG...T.......A.....GT....A.A.-AAA.T.....A..A.................-A.TT.
11 Praslinia   A...G.CAT.T.TGT...T.............G.....A.A.-..T..........A..................AC..T
12 Hypogeophi  T...G.CCTCGCTGT...T.............A.....A.A...AT..........A..................TATAT
13 G.alternan  ....G....TACTGT...T.............A.....T.A...A...........A.................-AC..T
14 G.brevis    ....G...TCGCTGT...T.............A.....T.A..TA...........A..................CAT.T
15 G.larvata   ....G....TGCTGT...T.............A.....A.A...AA..........A.................-AC.TT
16 G.sechelle  ....G.C..TGCTGT...T.............A.....T.A...AA..........A.................-AC.TT

               ---------+---------+---------+---------+---------+---------+---------+------1120
 1 Human       TGCTAAGACT-TCACCAGTCAAAGCGAACTACTAT-ACT-CAATTGATCCAATAA-CTT-GACCAACGGAACAAGTTACC
 2 Xenopus     AC.A.GA..C.A.CATTC.A.GTAAC..AATT...G...AT..........G.CCTAC....T.....A.C.........
 3 Amphiuma    ACAA.GAGA..A..T.TC..T.CCAT..GA.ACT.A.-CAT............GT.T.....T.....A.C.........
 4 Epicrionop  ACTA.G..AA.A.C..CCAA.GTATC.GAA.T.C.G.-NA.......C....ACC.......T.....A.C.........
 5 I.bannanic  ATGCC..GAA.A..ATCC.A..CATC.GAA.A-C.G..A.T......C.....T.A...T..T.....A.C.........
 6 Typhlonect  ACTA..TTAC.A..AA.C.A.GTA.C..GACACT.G.AA...GC...........AA.....T.....A.CT...C....
 7 Caecilia    ACTA..C.AC.A..AATTGACGCATT..AACA-T.A.AA........C..............T.....A.C....C....
 8 Dermophis   .TT..GT.A..A..AA.CAA...TTC..AA-T.T.G.--...CCA..C....A.TAT.....T.....A.C....C....
 9 Siphonops   ATTA.GT.A..A..AT.CAA..TT.C..TA-.C..G---.G.CA..........TT.A....T.....A.C....C....
10 Schistomet  AC.A.GATAA.A..TA.CAA.GTCTC..AA...T-G.A-...C....C......CAT......A....A.C....C....
11 Praslinia   ACTA.GATAA.A...A.CAA.GTA.A..AA...T.G.AC.T.CA...C.......AT.....T.....A.C....C....
12 Hypogeophi  AC.A.GATTG.A..AA.TAAGGTA.T..AA-..T-G.A-...TAA..C.......AT.....T..T..A.C....C....
13 G.alternan  AC...GATTATA...G.CA..GTA.C..AA-..T.G.T-.T.TAA..C.......AT.....T..T..A.C....C....
14 G.brevis    ACTA.GATTA.A..AA.CAACGTA.C..AA-..T.AG.C.TTTAA..C.......A......T..T..A.C....C....
15 G.larvata   AC.A.GAGTG.A.GTA.CAA.GTA.C..AA-.CT.GGA-.TTT.A..C.......AT.....T..T..A.C....C....
16 G.sechelle  AC.C.GATAA.A..TA.CAA.GTATC..AA-.CT.G.A-.TT.AA..C.......AT.....T..T..A.C....C....

               ---------+---------+---------+---------+---------+---------+---------+------1200
 1 Human       CTAGGGATAACAGCGCAATCCTATTCTAGAGTCCATATCAACAATAGGGTTTACGACCTCGATGTTGGA-TCAGGACATC
 2 Xenopus     .....................AT...A.A...T.C....G....AT.......................A.....G....
 3 Amphiuma    ......................T.CTA............G..G.AT................................C.
 4 Epicrionop  ..G.............C.....T.CAA.......CC......G.A....G..........................T.C.
 5 I.bannanic  ......................C..TA....C.......G....G....C..........................T.A.
 6 Typhlonect  ....................T.C..AC....G.......G....G.A.............................T...
 7 Caecilia    ......................C..T........T....GG.G.G.A............................GT..T
 8 Dermophis   ....................T.C.C.A....CA.C....GC...G.A.............................T...
 9 Siphonops   ................T...T.C.C.A............C..G.G.A.............................T...
10 Schistomet  ....................T.C..TA.............CT..G.A.............................T.C.
11 Praslinia   ....................T.C.C.A.......C.....C...G.A.............................T...
12 Hypogeophi  ....................T.C...AC...C..T.....C...G.A.............................T.C.
13 G.alternan  ....................T.C...AC...C..T.....C...G.A.............................T.C.
14 G.brevis    ....................T.C..TC....C..T.....C...G.A.............................TGC.
15 G.larvata   ....................T.C...A....C..T.....C...G.A.....................T.A.....TG..
16 G.sechelle  ....................T.C...A....C..T.....C...G.A.............................T.C.

               ----1208
 1 Human       CCGATGGT
 2 Xenopus     ..AG....
 3 Amphiuma    .AA.....
 4 Epicrionop  .AAG....
 5 I.bannanic  .T..CA..
 6 Typhlonect  .A..CA..
 7 Caecilia    A.C.GACA
 8 Dermophis   .A..CA..
 9 Siphonops   .A..C..C
10 Schistomet  .AA.CA..
11 Praslinia   .T..CA..
12 Hypogeophi  .A..CA..
13 G.alternan  .A..CA..
14 G.brevis    .A..CA..
15 G.larvata   .T..CA..
16 G.sechelle  .A..CA..
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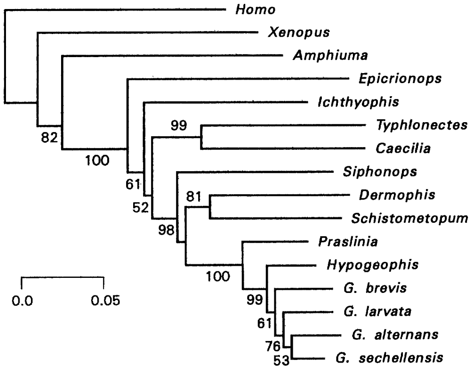

FIG. 2.—Relationships of 13 species of caecilians, a salamander, and a frog obtained by the neighbor-joining method. The tree was rooted with the amniote (human). Numbers on the tree are bootstrap *P*-values (above 50%) based on 2000 replications. Branch lengths represent estimated changes per site (nucleotide changes + insertion/deletion events) corrected for multiple hits (see text).

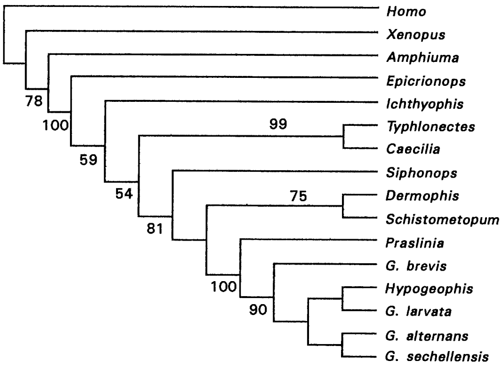

FIG. 3.—Relationships of the same taxa as in Fig. 2, obtained by the maximum parsimony method. Numbers on the tree are bootstrap *P*-values (above 50%) based on 2000 replications. Both nucleotide and length variation were used in this analysis. This is the single most parsimonious tree obtained, with a length of 1309 and a consistency index (CI) of 0.56 (CI = 0.48 excluding characters not informative under parsimony).

the larval lateral line system of caecilians also supports the basal placement of the Rhinatrematidae (Wilkinson, 1992).

The family Typhlonectidae, which is believed to be a monophyletic group (Wilkinson, 1989), is considered to be closely related to the caeciliaids, the primary difference being that its members are aquatic or semi-aquatic (and possess associated morphological traits). Our findings suggest that *Typhlonectes* is the sister lineage to *Caecilia* thus making the Caeciliaidae paraphyletic. This paraphyly has been recognized by many authors for some time, but prior attempts to subdivide the Caeciliaidae have been criticized (Nussbaum and Wilkinson, 1989) primarily due to absence of definitive data such as these sequence analyses are now beginning to provide.

The morphological data (Nussbaum and Naylor, 1982; Nussbaum and Wilkinson, 1989; Wake, 1977) and both molecular analyses (Hass et al., 1993; this study) indicate that the typhlonectids are derived from within the Caeciliaidae, and appear to be most closely related to members of the neotropical subfamily Caeciliainae. We therefore place the Typhlonectidae in the synonymy of the Caeciliaidae and allocate those species to their own subfamily, the Typhlonectinae. This eliminates the obvious paraphyly of the Caeciliaidae, although we recognize that subdivision of this large family almost certainly will occur when detailed studies of relationships of many more species are completed. However, the relatively close association between the typhlonectines (type genus, *Typhlonectes* Peters, 1879) and the type genus of the Caeciliaidae (*Caecilia* Linnaeus 1758, an older name) suggests that it is unlikely that the Typhlonectidae will reappear in the future as a family unless there is considerable subdivision of the present family Caeciliaidae.

Although Nussbaum and Wilkinson (1989) did not recognize the division of the Caeciliaidae, Taylor (1969) placed the genera, *Grandisonia, Hypogeophis, Dermophis, Schistometopum,* and *Siphonops* in the subfamily Dermophiinae, an association also suggested by the immunological analysis (Hass et al., 1993) and the ribosomal sequence data as well (with the addition of *Praslinia*). If the Typhlonectinae proves to be distinct from the Caeciliinae, those two subfamilies almost certainly are sister groups distinct from the Dermophiinae.

Seychellean Species

The sequence data presented here are concordant with the immunological data of Hass et al. (1993) and give clear support

TABLE 2.—Transitions (above diagonal) and transversions (below diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Human	–	98	120	125	115	131	136	123	112	130	121	121	124	124	117	119
2. *Xenopus*	91	–	101	117	99	133	124	120	117	130	112	111	109	108	114	104
3. *Amphiuma*	95	77	–	114	105	124	115	133	118	120	127	113	115	117	119	121
4. *Epicriono*	109	91	93	–	98	120	122	108	108	102	107	107	109	113	115	108
5. *I. bannani*	102	97	87	73	–	99	90	81	92	84	74	72	76	69	70	61
6. *Typhlonec*	96	84	73	72	75	–	86	103	99	95	96	86	84	89	96	80
7. *Caecilia*	102	82	88	74	85	62	–	103	91	107	103	88	92	94	98	90
8. *Dermophis*	107	91	95	76	81	73	65	–	70	78	64	74	73	64	81	68
9. *Siphonops*	96	84	82	65	65	73	82	65	–	91	70	67	67	73	71	76
10. *Schistome*	107	93	86	74	78	78	66	46	53	–	72	65	71	65	71	71
11. *Praslinia*	94	85	78	67	67	71	69	62	52	55	–	41	47	44	45	39
12. *Hypogeoph*	98	91	83	67	71	77	73	61	62	57	26	–	38	43	36	32
13. *G. alterna*	99	96	85	71	80	77	73	69	65	62	25	15	–	41	31	26
14. *G. brevis*	98	93	79	69	81	77	71	69	64	61	28	14	15	–	40	32
15. *G. larvata*	103	92	82	70	76	76	70	68	63	58	25	19	18	19	–	31
16. *G. sechell*	99	92	80	68	76	74	70	66	57	58	23	15	10	15	10	–

to the hypothesis that the Seychellean species represent a single lineage (Nussbaum and Ducey, 1988). All prior molecular data (Hass et al., 1993; Nussbaum and Jones, unpublished data) and these DNA sequence data suggest that *Praslinia* is the sister taxon to the remaining Seychellean caecilians, and this hypothesis is supported by morphological and life history data (Nussbaum, unpublished). While the micro-complement fixation data (Hass et al., 1993) did not allow resolution of branching order within the *Grandisonia* and *Hypogeophis* clade, the neighbor-joining analysis of the DNA sequence data indicate that *Hypogeophis* is the sister taxon to the four species of *Grandisonia* (Fig. 2). However, none of the bootstrap values for nodes involving those five species is above 95% and therefore further resolution of their relationships must await additional sequence data.

Biogeography

Duellman and Trueb (1986) proposed a biogeographic history for the caecilians based on their phylogenetic reanalysis of 21 morphological characters reported by Nussbaum (1977, 1979). They presumed that caecilians originated in Gondwanaland and that their subsequent evolutionary history must have been associated with the breakup of that southern paleo-landmass. With the exception of the Typhlonectidae, which was posited to have arisen more recently from a caeciliaid-like ancestor in South America, the presence of the Caeciliaidae in South America, Africa, Seychelles, and India suggested that the separation of continents occurred after the origin of the families.

One of the major questions in caecilian biogeography is the origin of the Indian and Asian groups. Duellman and Trueb (1986) proposed that during the Cretaceous the ichthyophiids, uraeotyphlids (of which we had no sample), and some caeciliaids drifted on the Indian Plate to their present location. The presence of the ichthyophiids in Southeast Asia was attributed to dispersal from India following the collision of the Indian subcontinent with Asia.

We propose an alternative explanation for the origin of the ichthyophiids and uraeotyphlids. There are no representatives of these groups anywhere on the southern continents, Madagascar, or the Seychelles. This presents a problem for the previous hypothesis because the phylogenetic evidence (morphological and molecular) indicates that the Ichthyophiidae and Uraeotyphlidae are old groups whose origin must have predated the breakup of Gondwanaland. For the biogeographic hypothesis of Duellman and Trueb (1986) to be correct, either (1) the ancestors of those groups must have occurred only in the portion of Gondwanaland that later became the Indian Plate (for paleogeography, see

TABLE 3.—Percent transitions (above diagonal), percent sequence divergence (including gap differences) (below diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Human	–	51.9	55.8	53.4	53.0	57.7	57.1	53.5	53.8	54.9	56.3	55.3	55.6	55.9	53.2	54.6
2. *Xenopus*	28.9	–	56.7	56.2	50.5	61.3	60.2	56.9	58.2	58.3	56.9	55.0	53.2	53.7	55.3	53.1
3. *Amphiuma*	31.9	27.0	–	55.1	54.7	62.9	56.7	58.3	59.0	58.3	62.0	57.7	57.5	59.7	59.2	60.2
4. *Epicriono*	35.5	31.8	30.4	–	57.3	62.5	62.2	58.7	62.4	58.0	61.5	61.5	60.6	62.1	62.2	61.4
5. *I. bannani*	32.1	29.9	27.7	24.2	–	56.9	51.4	50.0	58.6	51.9	52.5	50.3	48.7	46.0	47.9	44.5
6. *Typhlonec*	34.2	33.6	28.6	27.6	24.3	–	58.1	58.5	57.6	54.9	57.5	52.8	52.2	53.6	55.8	51.9
7. *Caecilia*	35.9	31.3	29.4	28.0	24.2	20.0	–	61.3	52.6	61.8	59.9	54.7	55.8	57.0	58.3	56.2
8. *Dermophis*	34.8	32.6	34.2	26.1	22.5	24.7	23.1	–	51.9	62.9	50.8	54.8	51.4	48.1	54.4	50.7
9. *Siphonops*	31.3	31.3	29.6	25.0	22.1	24.2	24.4	18.5	–	63.2	57.4	51.9	50.8	53.3	53.0	57.1
10. *Schistome*	36.6	35.2	30.6	25.2	22.9	24.6	24.4	16.6	20.3	–	56.7	53.3	53.4	51.6	55.0	55.0
11. *Praslinia*	32.2	30.3	30.2	24.9	19.4	23.1	23.9	16.9	16.4	17.2	–	61.2	65.3	61.1	64.3	62.9
12. *Hypogeoph*	33.0	31.2	28.7	24.6	19.9	22.8	22.5	18.2	17.7	16.4	8.6	–	71.7	75.4	65.5	68.1
13. *G. alterna*	33.8	31.8	29.4	25.4	22.0	22.5	23.1	19.3	18.2	18.4	9.5	6.8	–	73.2	63.3	72.2
14. *G. brevis*	33.5	31.0	28.6	25.9	21.0	23.2	23.1	17.9	18.9	17.0	9.2	7.0	7.1	–	67.8	68.1
15. *G. larvata*	33.2	32.0	29.6	26.5	20.5	24.3	23.7	20.4	18.6	17.6	9.1	6.9	6.4	7.4	–	75.6
16. *G. sechell*	32.8	30.1	29.5	24.9	19.0	21.4	22.3	18.1	18.3	17.5	7.9	5.8	4.6	5.7	5.1	–

Tarling, 1988), or (2) those ancestors were more widespread in Gondwanaland, but later disappeared from all southern areas. Both of these alternatives seem unlikely.

We believe that a more likely explanation is that the ichthyophiids (and possibly the uraeotyphlids) became isolated in Laurasia either (1) when the Tethys Sea separated Pangaea into Laurasia and Gondwanaland in the Jurassic, or (2) by dispersal from nearby Gondwanaland in the late Jurassic or early Cretaceous. Whichever mechanism was responsible, the molecular data (Case and Wake, 1977; Hass et al., 1993) suggest that this event occurred prior to the mid-Cretaceous (100 mya) and therefore probably was not associated with the movement of the Indian plate. The present distribution of the Indian and Asian caecilians is consistent with this hypothesis. The recent arrivals (the caeciliaids) are found only on the Indian subcontinent whereas the presumably long-term residents (the ichthyophiids) are more widely distributed in southeast Asia and southern China.

Another possible explanation for the origin of the ichthyophiids is that they occupied a small fragment of Gondwanaland ("Sundaland," Tarling, 1988) that now forms part of southeast Asia, although the geologic basis for this scenario is not well established. Our biogeographic hypothesis presumes that (1) ichthyophiids dispersed to India from southeast Asia after the Indian Plate contacted the Asian continent, and (2) the Uraeotyphlidae (found only in India) is the sister group to the Ichthyophiidae. If future evidence shows otherwise, this biogeographic scenario still could be supported, but an association of the uraeotyphlids with the Indian Plate would be more likely. A testable prediction of this hypothesis, that the ichthyophiids are of Laurasian origin, is that the southeast Asian species should show greater phylogenetic structure (i.e., older lineages and more basal branching) than the ichthyophiids of India. Although there is a greater number of species known from southeast Asia, little is known of relationships within this family.

Future Problems in Caecilian Systematics

These new sequence data, combined with previous morphological and molecular analyses, now provide a preliminary phylogenetic framework for the order Gymnophiona. The relatively strong evidence that the Rhinatrematidae is the basal lineage of caecilians will allow future workers to use this family as an outgroup for future phylogenetic studies of the remaining caecilians. The present morphological evidence suggests that the uraeotyphlids occupy a basal position among the remaining caecilians (possibly as a sister group to the ichthyophiids) and the scolecomorphids are associated with the caeciliaids. Clarification of the relationships of those two relatively small and difficult to obtain groups will be an important goal. However, the major task facing caecilian systematics is the resolution of relationships within the largest and most complex group of caecilians, the Caeciliaidae.

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Appendix I

Taxa Examined

Voucher specimens for all taxa are deposited in the University of Michigan Museum, Ann Arbor (UMMZ).

Caecilia sp. (UMMZ 190146)—Ecuador: Cotopaxi; San Francisco de las Pampas; *Dermophis mexicanus* (UMMZ 190145)—Guatemala: Quezaltenango; Finca el Faro, S slope Volcan Santa Maria, 4 km N El Palmar (875 m); *Epicrionops* sp. (UMMZ 190478)—Ecuador: Cotopaxi; San Francisco de las Pampas; *Grandisonia alternans* (UMMZ 189118) Seychelles Islands: Silhouette; Gratte Fesse trail (La Passe side); *Grandisonia brevis* (UMMZ 192977)—Seychelles Islands: Mahé; Grande Bois River at Forêt Noir Road; *Grandisonia larvata* (UMMZ 193061)—Seychelles Islands: Silhouette; Gratte Fesse trail (La Passe side); *Grandisonia sechellensis* (UMMZ 193076)—Seychelles Islands: Silhouette; Gratte Fesse trail (Grande Barbe side); *Hypogeophis rostratus* (UMMZ 189115)—Seychelles Islands: Silhouette; Gratte Fesse trail (La Passe side); *Ichthyophis bannanicus* (UMMZ 189122)—China: Yunnan; Longling, Dai Village near km post 141, N of Mengla (875 m); *Praslinia cooperi* (UMMZ 192933)—Seychelles Islands: Silhouette; Gratte Fesse trail (Grande Barbe side); *Schistometopum thomense* (UMMZ 189119)—São Tomé/Principe: São Tomé Island; Ribeira Peixe; *Siphonops annulatus* (UMMZ 190477)—Ecuador: Napo; Tena; *Typhlonectes natans* (UMMZ [LM2509])—Pet trade.