SMITHSON, T. R. 1982. The cranial morphology of *Greererpeton burkemorani* Romer (Amphibia: Temnospondyli). Zool. J. Linn. Soc. 76:29–90.

———. 1985. The morphology and relationships of the Carboniferous amphibian *Eoherpeton watsoni* Panchen. Zool. J. Linn. Soc. 85:317–410.

SZARSKI, H. 1962. The origin of the Amphibia. Quart. Rev. Biol. 37:189–241.

THOMSON, K. S., AND K. H. BOSSY. 1970. Adaptive trends and relationships in early Amphibia. Forma et Functio 3:7–31.

TRUEB, L., AND R. CLOUTIER. 1991. A phylogenetic investigation of the inter- and intra-relationships of the Lissamphibia (Amphibia: Temnospondyli). Pp. 223–313. *In* H.-P. Schultze and L. Trueb (Eds.), Origins of the Higher Groups of Tetrapods: Controversy and Consensus. Cornell Univ. Press, Ithaca and London.

WAKE, D. B. 1970. Aspects of vertebral evolution in the modern Amphibia. Forma et Functio 3:33–60.

WAKE, M. H., AND J. HANKEN. 1982. Development of the skull of *Dermophis mexicanus* (Amphibia: Gymnophiona), with comments on skull kinesis and amphibian relationships. J. Morphol. 173:203–223.

WATSON, D. M. S. 1940. The origin of frogs. Trans. Roy. Soc. Edin. 60:195–231.

WERNEBURG, R. 1989. Labyrinthodontier (Amphibia) aus dem Oberkarbon und Unterperm Mitteleuropas—Systematik, phylogenie und biostratigraphie. Freiburg. Forschungs. Ser. C 436:7–57.

———. 1991. Die Branchiosaurier aus dem Unterrotliegend des Döhlener Beckens bei Dresden. Veröff. Naturhist. Mus. Schleusingen 6:75–99.

Accepted: 28 February 1993
Associate Editor: David Cannatella

Herpetological Monographs, 7, 1993, 27–42

A MOLECULAR PERSPECTIVE ON LISSAMPHIBIAN PHYLOGENY

S. BLAIR HEDGES AND LINDA R. MAXSON

Department of Biology and Institute of Molecular Evolutionary Genetics, 208 Mueller Laboratory, Pennsylvania State University, University Park, PA 16802, USA

ABSTRACT: Relationships among the three extant orders of the Class Amphibia were examined using new DNA sequence data from the mitochondrial 12S ribosomal RNA gene. A total of 33 families of amphibians was compared, including representatives of nine of the 10 salamander families, four of the six caecilian families, and 20 of the 22 frog families. Approximately 380 nucleotides were sequenced in each taxon. Of the 333 sites that were aligned and compared for all taxa, 227 were variable. Phylogenetic analyses of these data support the monophyly of the Lissamphibia and the monophyly of each of the three orders, with the Caudata and the Gymnophiona as sister groups. Within the Anura, these molecular data also support the monophyly of the two suborders Archaeobatrachia and Neobatrachia. Although this study represents the broadest sampling of amphibian families at the molecular level, statistical confidence in the relationships must await additional sequence data.

Key words: Molecular systematics; Ribosomal RNA; Lissamphibia; Anura; Caudata; Gymnophiona; Phylogeny

THE nature of the phylogenetic relationships among the three living orders of amphibians has received much attention in recent years. However, aside from the demonstration of the monophyly of the Lissamphibia by molecular data (Hedges et al., 1990), sister group relationships among the Anura (frogs), the Caudata (salamanders), and the Gymnophiona (caecilians) have not been resolved definitively. Moreover, there is still considerable discussion regarding the familial branching relationships within each of the three orders (Duellman and Trueb, 1986).

The first temnospondyls appeared in the Paleozoic, and the earliest known frogs and salamanders are from the Jurassic (Bolt, 1991). Due to their great age and relatively sparse fossil record, it was apparent to us that molecular data were needed to examine questions concerning phylogenetic relationships of living amphibians objectively. Our earlier work using sequence data, primarily from the nuclear 18S ri-

bosomal RNA (rRNA) gene (Hedges et al., 1990), supported the monophyly of the Lissamphibia, but demonstrated that this ribosomal gene evolved too slowly to resolve relationships within each of the three major amphibian orders. Accordingly, we sought a conserved gene that evolved more rapidly than the 18S rRNA gene, but not so rapidly that relationships would be obscured by multiple substitutions at each site.

Mitochondrial 12S rRNA gene sequences have been used to examine phylogenetic relationships extending back over 300 million years (e.g., Meyer and Wilson, 1990), and therefore this gene appeared to be useful for addressing lissamphibian phylogeny. In this study, we used representatives of all of the amphibian families we could obtain (including nine salamander families, four caecilian families and 20 frog families—87% of all extant lissamphibian families).

Materials and Methods

DNA was extracted from small amounts (less than 50 mg) of fresh or frozen liver, muscle, or plasma samples from 28 species of amphibians as follows: *Bufo valliceps* (Bufonidae), *Centrolene geckoideum* (Centrolenidae), *Dendrobates speciosus* (Dendrobatidae), *Discoglossus pictus* (Discoglossidae), *Heleophryne natalensis* (Heleophrynidae), *Hyla cinerea* (Hylidae), *Hyperolius argus* (Hyperoliidae), *Leiopelma hamiltoni* (Leiopelmatidae), *Ascaphus truei* (Ascaphidae), *Eleutherodactylus cuneatus* (Leptodactylidae), *Gastrophryne carolinensis* (Microhylidae), *Neobatrachus pelobatoides* (Myobatrachidae), *Scaphiopus holbrookii* (Pelobatidae, *Pelodytes punctatus*(Pelodytidae), *Rana pipiens* (Ranidae), *Rhacophorus pardalis* (Rhacophoridae), *Rhinoderma darwinii* (Rhinodermatidae), *Rhinophrynus dorsalis* (Rhinophrynidae), *Nesomantis thomasseti* (Sooglossidae), *Ambystoma mexicanum* (Ambystomatidae), *Amphiuma tridactylum* (Amphiumidae), *Cryptobranchus alleganiensis* (Cryptobranchidae), *Dicamptodon ensatus* (Dicamptodontidae), *Plethodon yonahlossee* (Plethodontidae), *Necturus lewisi* (Proteidae), *Notophthalmus viridescens* (Salamandridae), *Rhyacotriton olympicus* (Rhyacotritonidae), *Siren intermedia* (Sirenidae), and the sarcopterygian fish *Latimeria chalumnae* (coelacanth). Locality information and museum voucher numbers are presented in Appendix I. Sequence data from several taxa were available from other studies in our lab as well as in the literature. Previously published sequences used in this analysis included the caecilians *Caecilia* sp. (Caeciliaidae), *Epicrionops* sp. (Rhinatrematidae), *Ichthyophis bannanicus* (Ichthyophiidae), and *Typhlonectes natans* (Typhlonectidae), reported in Hedges et al. (1993); *Homo sapiens* (Anderson et al., 1981); and the anuran *Xenopus laevis* (Roe et al., 1985).

The methods used to extract, amplify, and sequence the DNA are described in detail elsewhere (Hedges et al., 1991, 1992). Portions of the mitochondrial 12S ribosomal RNA (rRNA) gene were amplified using the polymerase chain reaction (PCR) and an approximately 400 bp region of this gene was sequenced. The 12S rRNA primers are 5′-AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT-3′, and 5′-TGACTGCAGAGGGTGACGGGCGGTGTGT-3′ (Kocher et al., 1989). Both complementary strands were sequenced using these PCR primers. Sequence data were read from autoradiograms using a digitizing program (GELIN, S. W. Schaeffer, Pennsylvania State University), and alignments were done by eye using the multisequence editing program ESEE (Cabot and Beckenbach, 1989).

To analyze nucleotide sequence and length variation and construct a phylogeny, we selected the neighbor-joining algorithm (Saitou and Nei, 1987) using the computer programs of T. S. Whittam (NJOIN and NJBOOT; Pennsylvania State University). 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 four-state Jukes-Cantor formula (Jukes and Cantor, 1969) for nucleotides plus a two-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]. The programs NJOIN and NJBOOT were modified by the senior author for use with gap data. To obtain precise bootstrap confidence estimates (Felsenstein, 1985) for nodes in the tree, 2000 replications were performed (Hedges, 1992). The bootstrap P-value is the proportion (0–100%) of resampled trees in which a particular group is defined. The statistical meaning of the bootstrap P-value was discussed by Felsenstein (1985, 1988).

Results

Approximately 380 nucleotide sites were sequenced for all 35 taxa (Fig. 1), but only 333 of these could be aligned with confidence. Sites in regions of uncertain alignment (indicated in Fig. 1) were excluded from the analyses. There are 227 variable sites, 188 "informative" under the conditions of parsimony, and 19 gap sites. The numbers of pairwise transitions and transversions are shown in Table 1.

Sequence divergence within the Lissamphibia is moderately high (Table 2). It is important to correct for multiple hits in data sets where most distances exceed 10% (Nei, 1991), as in this study. Multiple-hit corrections are not possible in parsimony analysis. The alternative is to down-weight or entirely eliminate transitions, which normally reach saturation before transversions, from the parsimony analysis. There are several problems with that approach. First, elimination of transitions often results in reducing the data set by 50% or more. For small data sets such as this one (333 total sites), the resulting loss of information is too great to provide adequate resolution. Second, discarding all transitions ignores the possibility that some (or many) such sites have not undergone multiple substitution, and therefore useful data are being discarded. Third, transversions also undergo multiple substitution and accordingly, they also should be corrected.

Differential weighting of transitions and transversions in parsimony analysis suffers for the same reasons, as discussed in Hedges and Maxson (1992). Many informative transitions, such as those defining terminal nodes in the tree, are weighted equally to transitions defining basal nodes which may be convergent due to multiple substitutions. Distance corrections, on the other hand, are scaled. For these reasons, we believe the most appropriate method of analysis for our 12S rRNA sequence data is a neighbor-joining analysis of corrected distances. This method also has been shown to be efficient in computer simulations when compared with other methods (Nei, 1991). The tree resulting from this analysis is presented in Fig. 2.

Our results show that the Lissamphibia is monophyletic (67% bootstrap P-value) and is a sister-lineage to the Amniota (as represented by humans)—a result identical to our earlier study of nuclear rRNA (Hedges et al., 1991). All four caecilians form a well-supported monophyletic cluster with an 85% bootstrap value. The nine salamander families also form a monophyletic group (47% bootstrap value) and constitute the sister-group of the caecilians (37%). The monophyletic Anura (49%) consists of two major lineages corresponding to the suborders Archaeobatrachia (49%) and Neobatrachia (94%). Within the Neobatrachia, two clusters of families loosely corresponding to the superfamilies Bufonoidea and Ranoidea (Laurent, 1967)

→

FIG. 1.—Mitochondrial DNA sequences of portions of the 12S rRNA gene (corresponding to sites 1092–1477 in human); in a fish (coelacanth, *Latimeria chalumnae*), an amniote (human), 20 frogs, nine salamanders and four caecilian species. The human (Anderson et al., 1981) and the caecilian (Hedges et al., 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.

```
                  ---------+---------+---------+---------+---------+---------+---------+------ 80
 1 Latimeria      TCGCCAGGGAA-CTACAAGCGCCAGC-TTCAAACCCAAAGGACTTGGCGGCACTTCAAACCCACCTAGAGGAGCCTGTTC
 2 Homo           .......AAC......G...CA.......A....T.........C.......TG.....T.T..CT..............
 3 Ascaphidae     C..................G...CT.......A..........A..........TG.CC..C....C..............
 4 Leiopelmatidae C.........T......G...TA.......A.....................TG.CC..C..................
 5 Discoglossidae C.................G...CT.......A.....................TG.CCN..................
 6 Pipidae        C.................G...CT.......A.....................TG..C...................
 7 Rhinophrynidae C....T.....A....G...CTA.......A.....................TG.CC...................
 8 Pelodytidae    ...NN....T......G....TT......A.....................TG.CC-..................
 9 Pelobatidae    C....T...........G.............A.....................TG.CC-..................
10 Sooglossidae   G.A..T..............AAA......G.....T..........A...TG.CC...................
11 Hyperoliidae   A.....AA.........G.....A......A....TT...........A...TGTCC..TCT--..............
12 Microhylidae   C....C......T.......C.A......A..................A...TGTCC..CC.--..............
13 Ranidae        G................G...AAT-.....A.............T...A...TGTCC..CC.-.-.G.............
14 Heleophrynidae .........T..T...G...C.A.....A..................A...TG.CC...T.-.C.............
15 Myobatrachidae .....C..........G...C.A-..C..A..................A...TG.CC..C.T..C.............C.
16 Leptodactylidae ...T.........A.......AAA-.....T..................A...T.T.C..C.T..C.............C.
17 Centrolenidae   .................G...AAA-.....A..................A...T..CC..C.T..............C.
18 Rhinodermatidae .....C..........G...AAA-.....A..................A...TG.CC..T.T..C.............C.
19 Dendrobatidae  .....T..........G...TAA-.....A..................A...T..CC..T.T..C.............C.
20 Bufonidae      .................G...TAA-.....A..................A...T..CC..T.T..C.............C.
21 Rhacophoridae  .........G......G...AAA-.....A..................A...T..CC..T.T..............C.
22 Hylidae        .................G...AAA-.....A..................A...T..CC..T.T..............C.
23 Typhlonectidae C......A.C......G...AG.......A....T..............TG-CC......T..T.............
24 Caeciliaidae   CT.....A.T......G...A........A....T............A...TG.CC..........C.............
25 Ichthyophiidae C......AAC......G..TAAACA....A...GT............C...TG.CC....T.....C.............
26 Rhinatrematidae .........T......G...AA.......G..................TGTCC....T....TC..............
27 Sirenidae      C......A.C......G...AAT......A....T..............TG..C..C.....................
28 Cryptobranchidae C......A........G...TA.......A....T..............TG..CT.C...................
29 Ambystomatidae C......A.T......G...AAT......A....T..............TG..CT.C...................
30 Amphiumidae    C......A.T......G...CA.......A....T...........A...TG..CT.C...................
31 Rhyacotritonidae C......A.T......GGAGC.A......A....T..............TG.CCT.C.TA..............
32 Plethodontidae CG.....A.T......G...CA.......A....T.....A.........TG..CT.C...................
33 Proteidae      .....C.A.T......G...AA.......A....T..............TG...T.T.T.................
34 Dicamptodontidae C......A.T......G...AA.......A....T...........A...TGT..T.T....G.............
35 Salamandridae  C......A.T......N...AA.......A....T..............TG..CT.T....C.............

                  ---------+---------+---------+---------+---------+---------+---------+------ 160
 1 Latimeria      TAAAACTGACAACCCCCACCTAACCTCACCATCCCTAGCCATTAAACCAGCCTATATACCGCCGTCGCCAGCCCACCCTG
 2 Homo           .GT..TC..T..A....GATC..........C.T..T..-------T.............A..TT....AA......
 3 Ascaphidae     ..T..TC..TT.T....G.TG..........TT..T...C.----T.C...........A...................A
 4 Leiopelmatidae ..T..TC..T.......G.TA.........CTTA.T....A.---A.C...........A...................
 5 Discoglossidae ..T..TC..T.......G.T.........CTT..T....AA---A.C...........A..................C.
 6 Pipidae        .GT..TC..T.C...T.G.TA.........CTT..T....AA----..C...........A................TC.
 7 Rhinophrynidae ..T..TC..T..T....G.T.T.......CTT.......A.---A.C...........A......G............
 8 Pelodytidae    ..T..TC..T.C.....G.T.T.......CTT..T.T..A.---A.C....G......T.....A...A..T....CT
 9 Pelobatidae    ..T..TC..T..T..A.GATC.........CTT..T....A.---T.C.....................TT.....T
10 Sooglossidae   ..T...C....CTA...GATA.........C.A........AC--.......CG......T......T....TT.T.ACT
11 Hyperoliidae   ..T..TC..T..T..T.GTTAT.T......T.TTT....TTA----T...T..G.....TT......TA...TT...A.A
12 Microhylidae   ..T..TC..TTC.....GATAC...C....CCTT.......C----T...T..G.....T.......A...TT...A..
13 Ranidae        .TT..TC..TG.T....G.TAC....G.....TT..T..TCA----T...T..G.....T......AA...TT...A..
14 Heleophrynidae ..T..TC..TG.T....GTTA.........CTT..C...CA----T.C....G......T.................GCA
15 Myobatrachidae ..T..TC..TG.T..A.GTT.T........TCTT..T....A-----..C....G......T......T....T....GCA
16 Leptodactylidae A.T..TC..T.....T.G.T.T.......CAT..T..TTA----T......G.....TT......TA..TAAG..ACA
17 Centrolenidae  ..T..TC..T.C.....G.TA.........C.T..C...CAC---T......G......T......T....TT...ACA
18 Rhinodermatidae ..C...C..T.......GAT.........TTT...TT..----T......G......T......T....TT....C.
19 Dendrobatidae  ..T...C..T..T....GTT.........TT.....T.AG---A......G......T......T....T....GC.
20 Bufonidae      ..T..TC..T..T..A.GTT.........TT....TTTA----T......G......T.....T....TT...AC.
21 Rhacophoridae  ..T..TC..T.......GTT.........TTT.....TC----T......G......T......T....TT...AC.
22 Hylidae        ..T..TC..T..T....G.TA.........TT.....T.G----T......G......T......T....TT...AC.
23 Typhlonectidae ..T..TC..T.....A.GTTA........TT.T.ATCC----T.............A...........TAG..T.T
24 Caeciliaidae   ..T..TC..T.....A.GTT.T.......TT.C..A.-----T.............A...........A...TCT
25 Ichthyophiidae ..T..TC..T.....A.G.TA.........CT.T.T....AA---A.............A.....TG....T.....CA
26 Rhinatrematidae .GT..TC..T.C.....G.TA.........T.....T..T.A.---T.............A.....ATGTCAG.CTA.CT
27 Sirenidae      ..T..TC..T.CT....GAT.C.........TT.T..T.....----.C...........A......T....TT.....T
28 Cryptobranchidae ..T..TC..T...A...GATA.........TTA.T....A.---A.C.....................A...T......T
29 Ambystomatidae ..T..T...T.........ATA.........C..A.T..A.A---A.................C..T....TT.....T
30 Amphiumidae    ..T..TC..T..T..A.GATA.........C.AA.T..A.GC---A.............A.....C.....TT...TCA
31 Rhyacotritonidae ..T..TC..T.......GATA.........C.AA.T....A.---A....T.........A...C.CT...T.T.....T
32 Plethodontidae .GT..TC..T.CT....GATA.........C.T..G....A.---A.............A.....CT.T...T.T.-.T
33 Proteidae      ..T..TC..T.CT...A.GATA.........TT.T..T.-----A.............A.....CT-...T......T
34 Dicamptodontidae ..T..TC..T.TT....GATA.........A.TA.T..A.-----T...A.........A.....CT....T....T.T
35 Salamandridae  ..T..TC..T..T..A.GATA.........TA.T..T.A.---A.............A.....C--...........T
```

```
                 ---------+---------+---------+---------+---------+---------+---------+------ 240
 1 Latimeria     -TGAAGGAAATAC----AATGGGCAAAAATAAA--------------AAAATTAAAAACGTCAGGTCGAGGTGTAGCAA
 2 Homo          A......CT.C.A.....G.AA..GC..G..C-..............CC.CG....G....T.....A.........CC
 3 Ascaphidae    .........CAC-.....G.A....T..TGGT-..............CTTCACC.............A..........T
 4 Leiopelmatidae ........CT.CA.....G.A....C..TGGT-..............C.CCACC.............A..........C
 5 Discoglossidae ....G..G...G......G.A.......TGGC-..............CCCCGCC.G...........A.........GT
 6 Pipidae       ....GA..TTCTT.....G.A...TT..TG.TT..............TTTCA.C..C..........A..........T
 7 Rhinophrynidae ....G.CTG..T-.....G.A...TC..TG.C-..............CC-A.C..C..........A.........GT
 8 Pelodytidae   ....G.A...A--.....G.AA.T.C..TG.T...............TCTTA.CT............A.....C...GT
 9 Pelobatidae   .......TT.A.A.....G.AA.T....TGGT-..............CTCACC.............A.........G.
10 Sooglossidae  .CA.GT...T.TT.......AA..C...TGGC-..............CCCCACC..T..........A.....C....T
11 Hyperoliidae  .....T.T.T.--.....G.AA.TT...TAGT.ATAAC.............TTACC.............A.A...C...C.
12 Microhylidae  .....C......-.....G..A..TT...GGCC..............CCCCGCC..C..........A.....C...TC
13 Ranidae       .....C.TCT.C-.....G.A...TC..TG.TCATAA...........TT.CA.C..T..........A.....C...TT
14 Heleophrynidae ....GC.TG.G.A.....G.....CT...G..C..............CTTT.CC..T..........A.....C....C
15 Myobatrachidae ....TC.GTCA.T.....G..A......TGGCC..............GCGCC.............A.....C....C
16 Leptodactylidae .....T...CCTT.....G.AA..CC..TGTCTACACATAAATGTGTTG..CAAC..T..........A.....C...GG
17 Centrolenidae  ....GC.C...TT.....G..A..TT..TGCCC..............T.CGCC..C..........A.....C.....
18 Rhinodermatidae ....GC......T.....G..A..TT..TGCCT..............TT.CACC..T..........A.....C.....
19 Dendrobatidae ....GC.TC.GTG.....G..A..CT..TGTT..............TTCAACTT...........A.....C.A..T
20 Bufonidae     ....GC.CC.ATT.....G..A..TT..TGTTT..............TTTCACC..C..........A.....C.....
21 Rhacophoridae ....GC.TT..TT.....G..A..TT..TGTCT..............CT.CA.C..T..........A.....C..T..
22 Hylidae       ....GC.TC.CC......G..A..CT..TGTTC..............TT.CACC..C..........A.....C..T..
23 Typhlonectidae ..A..A......AA....C.A.C....C.GG.-..............C.GCCC...T..........A..........C
24 Caeciliaidae  ....GA..TT.G.A....T.A.CA...CTGT.-..............GCCCAC..GC..........A.T........C
25 Ichthyophiidae ..TG.A.G..A.TA....G..A...G..TG..-..............CTT.C..............A..........C
26 Rhinatrematidae ...TG.A..GATATAAC.G.AA......TG.--..............TCCA.CC.T..........A..........C
27 Sirenidae     ..A.......A.T.....G.AA...T..TGGC-..............C.CGCC.............A.........GT
28 Cryptobranchidae .CA.G..T.TA.......G.AA...C..TG.T-..............CA..............A..........T
29 Ambystomatidae ..A.G.....ACA.....G.AA......TG.T-..............CA..............A..........C
30 Amphiumidae   .CA.GA....A.......G.AA......C..TT..................A..............A..........T
31 Rhyacotritonidae ..A.........A.....G.TAA..C..C..TT..................TA.....A.....A..........T
32 Plethodontidae .CA..A...T..T.......AA...T..CA.TT..............T...A......A.C.....A..........C
33 Proteidae     .CA.......A.T.....G..A......TA.T-..................CA.T....A.A.....A.........CT
34 Dicamptodontidae ..A..NN.....A.....G.AA......TG.T-..................CA..............A..........
35 Salamandridae ..A.....T.A.......G.A....C..T..T-..................CA..............A..........T

                 ---------+---------+---------+---------+---------+---------+---------+------ 320
 1 Latimeria     ATGAGATGGGAAGAAATGGGCTACATTTTCTAAATATAGAATATTACGAAAAATACAG--CGAAACCTGTACTTTGAAGG
 2 Homo          .....G...C.....................CCC-C....A.C.....T.GCCCTTA..T.....T.AAGGG.C.....
 3 Ascaphidae    ....A....C.....................CTA-.....A.CA-.....G..TTCA.GT.......AAT.--A.....
 4 Leiopelmatidae ....AG........................CCC-.....C.AA-....CG..CATT.AT.....A..CT.--A.....
 5 Discoglossidae ....AG...A.....................GGC.-C....C.AC......GG.CACT.AT.........AC.--CA....
 6 Pipidae       ....AG.........................GT.A-ACAGCATAA......G.CCACT.AT.........GT.--CA....
 7 Rhinophrynidae ...GAG.............................-.....A.CA...G..G.CCATT.AT.........GT.--......
 8 Pelodytidae   ....AG...A.....................T..-....C..CA-.....G..TACT.AT....T..AAT.--......
 9 Pelobatidae   ....AG...A.....................TA-ACAG.ATA......CG..TACT.AT....AAGAAT.--......
10 Sooglossidae  ...TCG....C.............C.CC..C.CA-ACC.GG.AA-AAC....G..CTAGA.....AAAGT.--AA....
11 Hyperoliidae  .CA.A.A.................A......CT.-A....C..A-....TTTA.GTAACT....A..ACTA--......
12 Microhylidae  .C..AGC..A.............A.C....TC.-.....C.AA-.....G.CCACA..T....AACAGT.A-......
13 Ranidae       .A..A........C...T.......A.......TA-.....C.A--.....GGCTAT...T....T.ATAG.AGC.....
14 Heleophrynidae ....AG...A.............C.C.....CC-.....A.CA-.....G.CTGCC..T.....ACCAGTC-......
15 Myobatrachidae ....ATA...T...G.....................-.....A...-AACGG..G..TCAAT......CAGT.--A.....
16 Leptodactylidae ....TG...A.T.TG..T.......C...T....C.-..A..G.AA-..G..G.C.A.AAAT.....TCTGT.--A.....
17 Centrolenidae  ..............G.........C.C.....TA-.....A.CA-..G..G.CTACTTAT.......AGT.--.A....
18 Rhinodermatidae ..A.A.........G.........C.......CA-.....A..A-.....G.CTACATAT.......AGT.--A.....
19 Dendrobatidae ...T..........G.........C.C.....C.-........A-.....G.CTACTTAT....T..AGT.--AA....
20 Bufonidae     ....A...AA....G.........C.......T.-.....A..A-.....CTACCTAT.......AGTT--A.....
21 Rhacophoridae ..A.A.........G.........C.C.....C.-....G..CA-....TG.CTATTTAT....T..AGT.--A.GG..
22 Hylidae       ....A.........G.........C.......TC-.....C.CA-.....G.CTATTTAT.......AGT.--G.....
23 Typhlonectidae ..A......A.-.....................TTT.-A.AG.A.CA-..G...G.CTCA..T....A.AACT.--CA...A
24 Caeciliaidae  ..A......A.....................TCCA-C.AG.A....-ACG..T.ACGCA.AT....AA.AATT--......
25 Ichthyophiidae ..AGAG...A.....................T.C.-A....C.AA-...G...GCA.A..T....AA.TCTT--G.....
26 Rhinatrematidae ...G..C.......................T.TA-A......AC-ACGG.T.AG.TAAAT....A.AACTT--......
27 Sirenidae     ..A.......T.....................T...TA....A.C.......G..CTTA..T....TTA.G.TC-CA....
28 Cryptobranchidae ..T.A.........................--.-.....A..A-.....T.CTT.A..T....TTAAATA--......
29 Ambystomatidae ...G.G.........................T--.-.....A.AA-.......CCA.A..T....AAAAGTT--G.....
30 Amphiumidae   ..AT.GC..A.....................T.C--.....A..A-..G.C..CCT.A..T....TA.TAGACC......
31 Rhyacotritonidae .....G...A.....................G..G.C....A.CA-.A...G.CTT.A..T....ATATAT.--......
32 Plethodontidae .....GC..A.....................CC..-.....A.CA-..T...G.C.TA..T....AAAAA..--AA....
33 Proteidae     ..A.........................T.T.CC--.A..A..A-.A..TG.A.G.A..T....AA.A.T.--......
34 Dicamptodontidae ..T.........................A.T..G...T..A.CA...GG.T.GC.TTATT....TTAAA..--.A....
35 Salamandridae ..A.............................TC-.....A..A-..G....GT.TA..T....TAG.ACTA-.A....
```

```
                    ---------+---------+---------+---------+---------+------ 380
 1 Latimeria        AGGATTTAGCAGTAAAAGGGGAATA-GAGAGCCCCTCTGAAA-CCGGCCCTGAAATGCGC
 2 Homo             T.............CTAA..G......T..TTAGT....C.AG.........GC...T
 3 Ascaphidae       C.............A.AA.T......TATT.T.T.T..T.TT......A.G-C....
 4 Leiopelmatidae   T.............A..A.CA..A....T..T.T.T..T..........GG-C...T
 5 Discoglossidae   .........T......GAAAA.CA.......TT.TCT.T..C.T.........GG-C....
 6 Pipidae          .........T.....GA.AA.CA.......TT.TCT.T....A.........G.GC....
 7 Rhinophrynidae   C.............A.AA.CAG......TT.T.T.T...GG........TGGGC....
 8 Pelodytidae      C.......A.....GA.AA.C......T.TT.TCT.T.....T.......GG-C....
 9 Pelobatidae      ..............A...TC..TGAT......T.T.....T.......GG-C....
10 Sooglossidae     C........T.....A.AA..C.....T.TT.T.T.T..C.T.........GG.CA..T
11 Hyperoliidae     C........A.....T.AA.....A..T.TT.A.T.T..C.AAT..T...GG.C.T.T
12 Microhylidae     C........T.....A.AA.....A..T.TT.T.T.T..T.TA.......GG.C...T
13 Ranidae          T........T.....A.AA.........T.TT.T.T.T..C......T...GG.....T
14 Heleophrynidae   C........T.....A.AA.CA.T.....TT.T.T.T..C.T.........GGG..T.T
15 Myobatrachidae   C..............AA.CC....A..T..T.T.T..C.A......A.GG-GAT.T
16 Leptodactylidae  C........A.....G.AAAAG....TCATATTTT...T..TAAG...A...G.G.AT.T
17 Centrolenidae    C........A.....AAAA..AT...AT.T..T.T.T..CTGG...A...GGG..T.T
18 Rhinodermatidae  C..............A.AT..C.......TT.T.T.T..T.TT...A...GGG..T.T
19 Dendrobatidae    T........A.....C.TA.CA.....T..T.T.T..C.AT...A...GG-..T.T
20 Bufonidae        C..............A..A.TC...CAT.T..A.T.T..CC.GCA.A...GG-..T.T
21 Rhacophoridae    C........G.....A.A..CA.....T.T.T.T..C.TT...A...GG-..T.T
22 Hylidae          C........A.....AAA..TC..T.A.A.T.T.T.T..C......A...GGG..T.T
23 Typhlonectidae   C.......A......GC...ACCA..TTATAT......TG..A..A......GGGGCA..
24 Caeciliaidae     C......G.......G.A.AA.CC..TTATATT.T...T..T.TT...T....GGC....
25 Ichthyophiidae   C......G..T......A.AA.TA..TTATATT.T.T.TC..CA.A.....T.GCGCT..
26 Rhinatrematidae  C......GA.....A.AA.CAT.TTACATT.TCT.T.............GGGG....
27 Sirenidae        T..............A.AA.CA.....C.TT.TAT.T..T.TA.......G.GC....
28 Cryptobranchidae C........T.....A.AA..........TT.T.T.T....TT..T....G.GC....
29 Ambystomatidae   ..............A.AA.TA.....T.TT.T.T.T...TTT...TA.AG.GC....
30 Amphiumidae      ..A............GA.AA..A.....TT.TCT.T..G.TT...AA.AG.GC.T.T
31 Rhyacotritonidae ..............A.AA.....T.TT.T.T.T.....AATAA.AGGGNT...
32 Plethodontidae   .........A.....A.AA.TA....AT.TT.T.T.T......A.G.A.A..GCA...
33 Proteidae        C..............A.AA.T...TTAT.TT.T.T.T..T.TA...AA.A..GC....
34 Dicamptodontidae ..............A.AA..A.....T.TT.T.T.T....AT..TAA.A...C....
35 Salamandridae    ..............A.AA..A.....T.TT.T.T.T..G......AA..G.GC....
```

FIG. 1.—Continued.

are supported at the 68% and 83% levels, respectively.

DISCUSSION

When the pattern of nucleotide substitution in the mitochondrial 12S rRNA gene shows a relatively high frequency of transitions relative to transversions, as has been observed in numerous studies (see De Salle et al., 1987 and Hedges et al., 1991, for examples), this transition bias is interpreted as evidence that the data are not saturated with respect to multiple substitutions. The percentage of transitions appears to plateau around 40–45% when multiple substitutions begin occurring at the same site (Brown et al., 1982). In this study, some transition bias is evident across all comparisons (Tables 1 and 2) and therefore saturation does not appear to have occurred. However, the uncorrected percent sequence divergence is moderately high (15–25% for most comparisons) stressing the importance of correcting for multiple hits in the data set (Nei, 1991).

Lissamphibian Relationships

Historically, there has been a lack of consensus regarding the origin of the Lissamphibia, although within this lineage there has been general agreement that the frogs and salamanders are sister groups (see summary in Benton, 1990; Trueb and Cloutier, 1991). This consensus rests upon up to nine shared derived morphological characters (Milner, 1988), although most are absences. However, some authors have interpreted morphological data from both living and fossil amphibians as supporting a salamander–caecilian sister group relationship (Bolt, 1991).

Molecular studies of nuclear ribosomal genes (Hedges et al., 1990; Larson and Wilson, 1989; Larson, 1991) concluded that, contrary to the interpretation of the most commonly accepted morphological evidence, salamanders and caecilians are sister taxa, not salamanders and frogs. Our 12S rRNA data represent the most comprehensive taxonomic sampling of extant amphibian lineages at the molecular level

(87% of all families). A neighbor-joining phylogenetic analysis of this data set (Fig. 2) supports, but only weakly, the close association of salamanders and caecilians suggested by Bolt (1991).

Gymnophiona

Molecular data bearing on phylogenetic relationships within the Gymnophiona are presented elsewhere in this volume (Hass et al., 1993; Hedges et al., 1993). In the more detailed DNA sequence study (Hedges et al., 1993), a larger data set (1208 sites) was obtained from the 12S rRNA and 16S rRNA genes in 13 caecilian species representing ten genera and four of the six families. The Rhinatrematidae was found to be the basal lineage, with the Ichthyophiidae as a sister group to the remaining caecilians. A close relationship was found between the neotropical aquatic family Typhlonectidae and a neotropical caeciliaid (*Caecilia*). For that reason, the Typhlonectidae was synonymized within the Caeciliaidae, and was assigned to a separate subfamily (Typhlonectinae).

Caudata

Familial relationships within the Caudata have been addressed by Larson (1991) who analyzed nuclear ribosomal gene sequences from representatives of nine salamander families using the maximum parsimony method. As part of our survey of the Lissamphibia, we included representatives of nearly all salamander families—missing only the Hynobiidae, for which we were unable to obtain 12S rRNA sequence information. Our work includes three species also studied by Larson (*Amphiuma tridactylum, Cryptobranchus alleganiensis,* and *Siren intermedia*), and the remaining six species differ from those used by Larson (1991).

A major conclusion of Larson's analysis was that *Dicamptodon* and *Rhyacotriton*, which long have been thought to be members of the same family (Fig. 3A; Duellman and Trueb, 1986), are not sister taxa, but rather that *Dicamptodon* and *Ambystoma* are sister taxa and *Rhyacotriton* is a more basal offshoot in this order (Fig. 3B; Larson, 1991). Our sequence data support the independence of *Dicamptodon* and *Rhyacotriton* (Fig. 2) but find *Dicamptodon* and the Salamandridae to be sister lineages (50% bootstrap value), whereas *Rhyacotriton* and *Plethodon* appear to be sister taxa. Thus, although our results disagree with those of Larson as to which lineage is closest to *Dicamptodon*, both agree it is not *Rhyacotriton*. This supports the recent recognition of *Rhyacotriton* as belonging to a separate family, the Rhyacotritonidae (Good and Wake, 1992).

Perhaps most surprising in Larson's phylogeny (Fig. 3B) is the basal position of the Amphiumidae and the Plethodontidae. Although sirens exhibit a mix of primitive and advanced morphological characters (Duellman and Trueb, 1986), the Sirenidae usually has been posited to be the most basal of the salamander lineages (Fig. 3A). Larson noted that the lineage leading to *Siren* had incurred the largest number of changes in the nuclear ribosomal gene sequence and that the lineage leading to *Rhyacotriton* has sustained the fewest changes (Larson, 1991:242). In fact, our neighbor-joining analysis of Larson's (1991) data yields a different tree than his and locates the Sirenidae basally in the tree (Hedges and Maxson, unpublished). Hillis's (1991) reanalysis of an earlier and smaller version of that data set (Larson and Wilson, 1989) plus the morphological data summarized by Duellman and Trueb (1986) found yet a third tree that places the cryptobranchids and hynobiids as the most basal families.

Our data (Fig. 2) indicate that the Sirenidae is a basal lineage but concur with Larson's nuclear data that the three families Rhyacotritonidae, Plethodontidae, and Amphiumidae are more closely related to one another than to other families. Our data suggest [Rhyacotritonidae + Plethodontidae] + Amphiumidae whereas Larson (1991:Fig. 6A) found [Amphiumidae + Plethodontidae] + Rhyacotritonidae. Although our finding that the sirenids are the basal family of salamanders is appealing in that it agrees with morphology, the salamander portion of our tree (Fig. 2) has low bootstrap values and therefore it cannot be interpreted as a robust estimate of relationships.

TABLE 1.—Transitions (above diagonal) and transversions (below diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Latimeria*	0	48	42	35	49	47	41	51	43	51	60	54	49	53	49
2. *Homo*	34	0	42	36	38	39	36	44	35	54	48	50	42	45	46
3. Ascaphidae	26	27	0	24	27	33	21	30	27	45	37	34	30	31	41
4. Leiopelmatidae	29	36	15	0	23	25	15	31	23	41	37	28	26	34	32
5. Discoglossidae	20	35	14	11	0	18	21	28	35	40	48	35	32	33	43
6. Pipidae	28	39	18	19	12	0	23	33	39	41	50	39	36	40	43
7. Rhinophrynidae	30	41	22	21	14	14	0	25	31	39	40	35	29	34	36
8. Pelodytidae	27	38	19	18	13	17	19	0	31	43	41	37	34	36	42
9. Pelobatidae	28	43	24	25	18	20	26	21	0	49	37	34	37	39	38
10. Sooglossidae	43	54	37	40	39	37	39	38	35	0	46	39	41	37	40
11. Hyperoliidae	39	44	40	41	40	44	42	41	44	45	0	36	32	39	36
12. Microhylidae	38	40	27	32	31	31	29	30	35	34	24	0	29	27	35
13. Ranidae	41	45	31	34	33	35	35	32	41	40	32	24	0	34	35
14. Heleophrynidae	31	39	21	20	19	19	21	24	31	36	40	25	29	0	24
15. Myobatrachidae	39	46	25	32	27	33	29	32	35	34	39	36	44	30	0
16. Leptodactylidae	44	46	37	44	43	43	41	42	47	46	41	38	34	38	42
17. Centrolenidae	34	37	24	25	24	20	25	25	32	32	31	23	27	21	34
18. Rhinodermatidae	28	35	20	25	24	26	22	27	28	30	37	23	29	23	30
19. Dendrobatidae	39	42	27	24	23	29	23	28	39	39	40	32	38	26	31
20. Bufonidae	35	36	21	26	25	27	26	28	31	35	34	26	32	22	29
21. Rhacophoridae	29	42	23	18	19	23	21	24	29	33	32	26	28	20	31
22. Hylidae	29	42	21	20	21	23	23	24	29	31	32	24	26	20	31
23. Typhlonectidae	34	43	34	35	34	36	40	35	32	47	55	47	47	37	49
24. Caeciliaidae	37	44	31	34	29	35	35	28	27	42	47	40	44	36	40
25. Ichthyophiidae	40	53	39	32	35	42	39	38	39	53	53	51	50	41	47
26. Rhinatrematidae	41	54	39	38	37	39	45	34	39	44	49	46	48	46	48
27. Sirenidae	31	29	19	22	21	25	27	18	25	34	44	33	33	28	34
28. Cryptobranchidae	28	33	20	21	22	26	28	25	28	39	43	35	35	33	39
29. Ambystomatidae	29	38	23	18	21	27	32	24	21	36	46	36	38	32	42
30. Amphiumidae	28	34	30	23	26	32	36	35	32	45	51	44	45	33	45
31. Rhyacotritonidae	33	38	29	32	31	35	37	32	33	46	51	39	43	37	47
32. Plethodontidae	36	39	28	27	30	32	36	31	32	38	52	38	44	36	46
33. Proteidae	39	42	33	28	33	39	41	32	27	40	41	35	45	42	44
34. Dicamptodontidae	34	39	35	34	33	33	40	34	33	39	48	46	44	40	47
35. Salamandridae	31	31	24	23	26	30	33	27	26	39	48	38	37	34	41

There has been much discussion about the merits of molecular versus morphological data and whether one should analyze each data set separately or combine all data for the closest approximation to the real phylogeny (Hillis, 1987). If molecular and morphological data lead to different phylogenetic conclusions, combining data sets merely allows the larger data set to "swamp" information in the smaller data set. Although there are methods available for distinguishing phylogenetic signal from random noise (Hillis and Huelsenbeck, 1992), there is no objective method for evaluating which data set is inherently "better" (more accurate) when there are strongly supported alternative phylogenetic arrangements. Larson (1991) has reinterpreted and explained morphological data on caudate amphibians in the context of his nuclear ribosomal tree. However, until such time as we derive a robust molecular phylogeny for the salamander families with statistically significant nodes, it seems premature to reanalyze and explain morphological data in the context of any molecular tree. It is clear that more work with greater sampling of nucleotide sites has the potential to provide us with valuable information about caudate phylogeny.

Anura

Our analysis of 20 of the 22 extant frog families is, taxonomically, the most extensive molecular sequence sampling of the Anura to date, yet the results are only preliminary. We presently are extending our study to include data from 16S rRNA as

TABLE 1.—Continued.

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
61	50	52	43	49	54	51	55	56	57	53	50	45	45	62	53	51	44	46	38
48	46	47	48	50	45	40	53	51	38	44	40	38	33	44	41	39	35	35	38
48	40	34	39	41	45	39	41	34	34	40	32	25	36	37	36	39	29	29	28
42	38	35	38	40	45	38	38	38	35	36	29	28	35	40	33	40	41	33	26
48	39	41	42	48	50	42	43	42	40	43	31	33	41	38	40	41	46	40	32
47	43	41	40	47	46	43	43	40	39	41	33	31	36	34	43	39	40	38	32
46	35	36	38	41	41	35	47	39	35	29	29	26	27	33	32	39	34	31	23
44	39	34	37	39	39	42	53	48	44	36	44	30	39	36	37	47	44	42	38
46	39	42	39	35	48	35	39	43	42	49	42	32	41	43	41	44	42	40	39
47	36	41	34	44	43	42	56	54	49	50	46	41	42	41	48	45	50	46	44
49	43	32	39	36	37	36	41	39	39	50	37	33	40	36	42	46	36	35	33
42	33	34	40	40	39	38	48	46	38	50	40	35	38	39	43	47	49	39	46
46	36	33	30	34	33	30	48	52	45	44	38	31	39	42	40	46	45	33	32
43	29	28	28	31	34	32	53	39	42	43	46	34	43	38	43	49	47	45	37
38	24	26	27	25	33	27	45	48	35	38	42	41	42	40	45	46	42	40	41
0	37	42	43	44	48	38	50	50	46	48	52	46	48	46	48	53	49	46	47
27	0	20	18	21	26	17	48	42	42	39	44	40	39	40	44	49	44	45	43
28	16	0	17	17	15	19	49	40	37	40	38	34	39	41	46	58	37	42	42
35	21	19	0	19	21	22	49	45	46	41	38	35	42	42	47	57	41	36	38
28	15	13	20	0	27	18	47	45	39	44	49	41	44	44	47	54	47	46	43
29	11	11	14	12	0	22	56	47	44	44	43	36	44	44	49	63	42	43	44
27	11	11	16	12	4	0	54	47	38	39	45	40	44	46	48	55	42	45	43
52	41	42	51	42	41	39	0	32	41	49	40	43	49	44	47	42	46	40	39
46	39	37	48	36	38	36	23	0	36	41	41	35	40	32	43	43	37	33	33
53	43	45	45	43	41	40	33	37	0	35	35	32	34	36	36	40	39	35	37
54	42	48	53	49	45	43	31	32	41	0	44	40	39	42	42	44	43	39	37
42	25	27	36	30	28	30	31	30	43	34	0	23	25	30	35	38	27	27	20
45	32	28	37	35	33	31	36	37	44	39	23	0	20	26	30	35	20	22	20
47	31	29	36	34	30	30	28	28	29	34	20	19	0	25	27	35	29	25	28
51	36	34	39	39	35	37	40	41	38	49	31	22	19	0	36	29	30	26	26
50	37	35	44	40	40	38	38	42	40	47	32	23	20	19	0	30	28	32	32
54	35	36	38	38	34	34	36	39	40	39	23	28	15	26	25	0	30	27	29
45	35	35	44	36	38	36	35	30	33	35	28	34	22	29	32	29	0	18	21
53	31	37	46	42	38	38	38	40	44	40	25	26	21	26	29	26	33	0	17
46	30	28	37	33	33	33	32	33	35	38	24	19	13	15	17	20	25	18	0

well as larger portions of the 12S rRNA gene (Hedges and Maxson, unpublished).

Our mitochondrial sequence data (Fig. 2) distinguish the Archaeobatrachia and Neobatrachia (sensu Reig, 1958) as separate monophyletic groups within the Anura. Looking first at the Archaeobatrachia, we see that the Ascaphidae is the most basal lineage in this suborder, followed by the Leiopelmatidae. *Leiopelma* and *Ascaphus* were each accorded separate family status by Savage (1973) based on their geographic isolation and inferred great age, and again by Green et al. (1980) in recognition of their karyotypic dissimilarities. These latter authors argued that these two genera previously had been mistakenly grouped together based on shared primitive characters and were sufficiently unique to warrant separate family recognition. Duellman and Trueb (1986) still placed them in a single family, the Leiopelmatidae. Cannatella (1985) and Hillis's (1991) reanalysis of Cannatella's (1985) morphological data on archaeobatrachians found the Ascaphidae to be the most basal lineage, followed by the Leiopelmatidae. However, Hillis's reanalyses of Duellman and Trueb's (1986) and Cannatella's (1985) morphological data sets did not support the monophyly of the Archaeobatrachia.

In our analysis, the most strongly supported sister group relationship within the Archaeobatrachia is seen between the Pipidae and Discoglossidae, which are next joined by the Rhinophrynidae. Traditionally, pipids and rhinophrynids have been classified in the superfamily Pipoidea, with the discoglossids being grouped with *Leiopelma* and *Ascaphus* in the subfamily Dis-

TABLE 2.—Percent transitions (above diagonal), percent sequence divergence (corrected, including gap differences) (below diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. *Latimeria*	0.0	58.5	61.8	54.7	71.0	62.7	57.7	65.4	60.6	54.3	60.6	58.7	54.4	63.1	55.7	58.1	59.5
2. *Homo*	33.5	0.0	60.9	50.0	52.1	50.0	46.8	53.7	44.9	50.0	52.2	55.6	48.3	53.6	50.0	51.1	55.4
3. Ascaphidae	28.0	28.7	0.0	61.5	65.9	64.7	48.8	61.2	52.9	54.9	48.1	55.7	49.2	59.6	62.1	56.5	62.5
4. Leiopelmatidae	26.1	30.1	14.0	0.0	67.6	56.8	41.7	63.3	47.9	50.6	47.4	46.7	43.3	63.0	50.0	48.8	60.3
5. Discoglossidae	28.6	30.7	14.9	12.1	0.0	60.0	60.0	68.3	66.0	50.6	54.5	53.0	49.2	63.5	61.4	52.7	61.9
6. Pipidae	30.9	32.6	19.2	16.4	10.9	0.0	62.2	66.0	66.1	52.6	53.2	55.7	50.7	67.8	56.6	52.2	68.3
7. Rhinophrynidae	29.7	32.8	16.6	13.8	13.5	13.8	0.0	56.8	54.4	50.0	48.8	54.7	45.3	61.8	55.4	52.9	58.3
8. Pelodytidae	33.6	35.9	18.6	18.6	15.3	19.4	17.5	0.0	59.6	53.1	50.0	55.2	51.5	60.0	56.8	51.2	60.9
9. Pelobatidae	29.9	33.6	19.3	18.1	20.2	23.1	22.9	19.6	0.0	58.3	45.7	49.3	47.4	55.7	52.1	49.5	54.9
10. Sooglossidae	41.2	49.6	34.0	33.4	32.6	31.5	32.1	34.2	35.5	0.0	50.5	53.4	50.6	50.7	54.1	50.5	52.9
11. Hyperoliidae	45.1	41.3	32.4	32.9	38.2	40.9	35.1	35.7	34.9	38.7	0.0	60.0	50.0	49.4	48.0	54.4	58.1
12. Microhylidae	40.3	39.3	24.9	24.4	27.3	28.6	26.5	28.3	29.0	30.4	23.7	0.0	54.7	51.9	49.3	52.5	58.9
13. Ranidae	39.2	37.7	25.7	25.2	27.6	29.9	27.3	28.7	34.3	35.2	26.2	20.6	0.0	54.0	44.3	57.5	57.1
14. Heleophrynidae	35.8	36.0	20.8	21.7	20.9	23.5	22.4	25.0	29.4	30.3	33.4	20.1	25.7	0.0	44.4	53.1	58.0
15. Myobatrachidae	39.3	41.6	26.6	25.7	28.6	31.7	27.2	31.1	30.4	30.3	31.7	30.7	34.7	22.8	0.0	47.5	41.4
16. Leptodactylidae	48.5	42.2	36.3	36.8	39.7	38.5	36.7	37.7	41.2	39.6	39.0	34.7	34.7	35.1	33.3	0.0	57.8
17. Centrolenidae	36.6	36.3	26.0	25.6	25.7	25.2	23.7	26.7	29.8	27.0	30.8	23.3	26.5	20.7	22.9	24.4	0.0
18. Rhinodermatidae	34.2	35.4	21.3	23.9	26.3	26.7	23.3	25.0	29.0	28.1	28.0	23.4	25.7	20.7	21.7	27.6	13.1
19. Dendrobatidae	35.7	40.2	26.3	24.5	25.9	28.0	25.0	26.5	32.5	29.5	33.4	30.8	28.9	22.5	22.3	31.9	14.7
20. Bufonidae	37.1	38.4	24.8	26.6	30.1	30.8	27.3	27.8	27.1	32.8	29.3	28.3	28.3	22.4	20.9	28.6	13.1
21. Rhacophoridae	36.3	38.6	27.2	24.9	27.8	28.0	25.5	25.5	32.0	30.9	28.5	27.5	25.7	22.5	24.9	31.4	13.9
22. Hylidae	34.2	35.4	23.9	23.0	25.3	26.2	23.3	27.3	26.1	29.0	27.6	25.7	23.0	21.2	22.6	25.3	10.1
23. Typhlonectidae	39.1	43.2	31.8	30.9	33.0	33.4	37.4	39.4	30.4	47.0	43.9	42.6	43.5	39.7	43.1	46.4	39.0
24. Caeciliaidae	40.3	41.6	25.4	28.7	28.3	29.7	29.9	31.3	28.2	41.0	36.5	36.7	43.0	30.9	37.9	41.8	33.8
25. Ichthyophiidae	42.6	39.4	29.9	27.1	31.0	33.4	29.9	35.2	34.4	45.3	40.6	38.3	42.5	34.9	35.6	41.5	35.8
26. Rhinatrematidae	41.2	43.6	32.4	30.0	33.1	32.5	30.2	28.7	37.6	39.4	43.2	42.5	41.1	38.4	36.4	44.7	33.3
27. Sirenidae	33.4	27.8	19.7	19.7	20.2	22.3	22.1	25.1	27.2	32.9	34.4	29.6	29.4	30.0	32.2	41.2	28.4
28. Cryptobranchidae	29.6	28.9	17.2	18.8	21.4	21.8	21.2	21.9	24.0	32.9	31.9	28.3	27.2	26.8	34.2	39.5	29.8
29. Ambystomatidae	30.4	29.2	23.5	20.8	24.9	24.8	22.9	25.9	25.2	32.3	37.3	30.5	32.8	30.9	36.6	41.2	28.4
30. Amphiumidae	37.7	31.7	27.1	25.3	25.8	26.2	28.3	29.7	31.4	36.4	37.9	34.9	37.0	28.9	37.2	43.2	32.1
31. Rhyacotritonidae	36.3	32.9	25.9	25.9	28.9	31.7	28.0	28.5	30.7	40.5	41.0	34.4	35.7	33.2	40.8	43.7	34.4
32. Plethodontidae	37.2	32.7	27.2	27.2	29.2	28.6	31.3	33.3	32.0	34.9	44.2	36.3	39.8	36.1	41.1	49.4	36.3
33. Proteidae	35.1	32.2	24.9	28.1	33.2	32.6	31.3	32.3	28.6	38.7	32.8	35.8	39.8	38.3	37.8	41.7	33.7
34. Dicamptodontidae	33.7	30.9	25.1	26.5	29.5	28.0	28.6	31.6	29.8	35.2	35.1	36.4	33.1	36.3	37.5	43.7	31.4
35. Salamandridae	28.6	28.8	21.0	19.7	23.7	25.0	23.0	27.5	27.3	35.6	35.5	36.2	29.5	29.5	36.4	41.9	31.3

TABLE 2.—Continued.

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
65.0	52.4	58.3	65.1	63.7	61.8	60.2	58.8	56.4	61.7	61.6	60.8	68.9	61.6	58.6	53.0	57.5	55.1
57.3	53.3	58.1	51.7	48.8	55.2	53.7	41.8	44.9	58.0	53.5	46.5	56.4	51.9	50.0	45.5	47.3	55.1
63.0	59.1	66.1	66.2	65.0	54.7	52.3	46.6	50.6	62.7	55.6	61.0	55.2	55.4	58.2	46.8	45.3	53.8
58.3	61.3	60.6	71.4	65.5	52.1	52.8	52.2	48.6	56.9	57.1	66.0	63.5	50.8	59.7	59.4	49.3	53.1
63.1	64.6	65.8	72.5	66.7	55.8	59.2	53.3	53.7	59.6	60.0	66.1	59.4	56.3	57.7	58.2	54.8	55.2
61.2	58.0	63.5	66.7	65.2	54.4	53.3	48.1	51.2	56.9	54.4	57.1	51.5	55.1	54.9	50.6	53.5	51.6
62.1	62.3	61.2	66.1	60.3	54.0	52.7	47.3	39.2	51.8	48.1	45.8	47.8	46.4	52.0	45.3	43.7	41.1
55.7	56.9	58.2	61.9	63.6	60.2	63.2	53.7	51.4	71.0	54.5	61.9	50.7	53.6	60.3	57.9	55.3	58.5
60.0	50.0	53.0	62.3	54.7	54.9	61.4	51.9	55.7	62.7	53.3	66.1	57.3	55.4	57.9	60.9	54.8	60.0
57.7	46.6	55.7	56.6	57.5	54.4	56.2	48.0	53.2	57.5	51.2	53.8	47.7	51.1	54.2	55.6	54.1	53.0
46.4	49.4	51.4	53.6	52.9	42.7	45.3	42.4	50.5	45.7	43.4	46.5	41.4	45.2	46.9	46.8	42.2	40.7
59.6	55.6	60.6	60.0	61.3	50.5	53.5	42.7	52.1	54.8	50.0	51.4	47.0	52.4	55.3	58.3	45.9	54.8
53.2	44.1	51.5	54.1	53.6	50.5	54.2	47.4	47.8	53.5	47.0	50.6	48.3	48.2	51.1	50.0	42.9	46.4
54.9	51.9	58.5	63.0	61.5	58.9	52.0	50.6	48.3	62.2	50.7	57.3	53.5	53.7	57.6	52.8	52.9	52.1
46.4	46.6	46.3	51.6	46.6	47.9	54.5	42.7	44.2	55.3	51.2	50.0	47.1	48.9	50.0	48.8	46.0	50.0
60.0	55.1	61.1	62.3	58.5	49.0	52.1	46.5	47.1	55.3	50.5	50.5	47.4	49.0	49.5	52.1	46.5	50.5
55.6	46.2	58.3	70.3	60.7	53.9	51.9	49.4	48.1	63.8	55.6	55.7	52.6	54.3	58.3	55.7	59.2	58.9
0.0	47.2	56.7	57.7	63.3	53.8	51.9	45.1	45.5	58.5	54.8	57.4	54.7	56.8	61.7	51.4	53.2	60.0
13.2	0.0	48.7	60.0	57.9	49.0	48.4	50.5	43.6	51.4	48.6	53.8	51.9	51.6	60.0	48.2	43.9	50.7
11.2	14.3	0.0	69.2	60.0	52.8	55.6	47.6	47.3	62.0	53.9	56.4	53.0	54.0	58.7	56.6	52.3	56.6
9.4	12.5	14.3	0.0	84.6	57.7	55.3	51.8	49.4	60.6	52.2	59.5	55.7	55.1	64.9	52.5	53.1	57.1
10.5	14.0	11.2	9.4	0.0	58.1	56.6	48.7	47.6	60.0	56.3	59.5	55.4	55.8	61.8	543.8	54.2	56.6
40.6	46.3	39.5	44.5	41.7	0.0	58.2	55.4	61.2	56.3	54.4	63.6	52.4	55.3	53.8	56.8	51.3	54.9
31.4	40.3	34.3	36.0	34.5	22.1	0.0	49.3	56.2	57.7	48.6	58.8	43.8	50.6	52.4	55.2	45.2	50.0
34.8	40.0	34.8	36.8	32.7	30.1	29.5	0.0	46.1	44.9	42.1	54.0	48.6	47.4	50.0	54.2	44.3	51.4
36.6	40.4	40.1	37.6	33.5	34.2	29.0	31.3	0.0	56.4	50.6	53.4	46.2	47.2	53.0	55.1	49.4	49.3
26.2	30.9	33.7	29.4	30.9	29.1	28.2	31.6	31.9	0.0	50.0	55.6	49.2	52.2	62.3	49.1	51.9	45.5
24.8	29.9	32.2	28.5	29.0	33.0	28.7	30.6	32.4	16.8	0.0	51.3	54.2	56.6	55.6	37.0	45.8	51.3
27.9	33.2	32.7	31.2	30.8	31.6	27.1	24.1	29.8	16.8	14.3	0.0	56.8	57.4	70.0	56.9	54.3	68.3
31.3	34.7	36.1	33.7	35.3	35.9	29.5	30.0	39.0	23.5	18.0	16.7	0.0	65.5	52.7	50.8	50.0	63.4
34.1	39.9	38.0	38.8	36.7	36.3	35.3	30.7	37.8	26.1	19.8	17.6	21.0	0.0	54.5	46.7	52.5	65.3
41.4	42.5	41.1	43.6	38.6	33.0	34.1	33.1	34.9	23.7	24.6	19.2	21.4	21.1	0.0	50.8	50.9	59.2
29.9	37.0	36.3	34.4	32.9	34.5	26.8	29.1	32.4	21.0	20.6	19.7	23.1	23.3	23.2	0.0	35.3	45.7
32.6	34.6	38.1	34.1	34.6	33.1	28.9	32.7	32.1	19.8	18.1	17.6	20.2	23.9	20.7	19.8	0.0	48.6
29.5	32.5	33.3	33.5	32.5	30.1	26.8	29.7	31.5	16.8	14.8	16.0	15.9	19.0	19.4	17.4	13.7	0.0

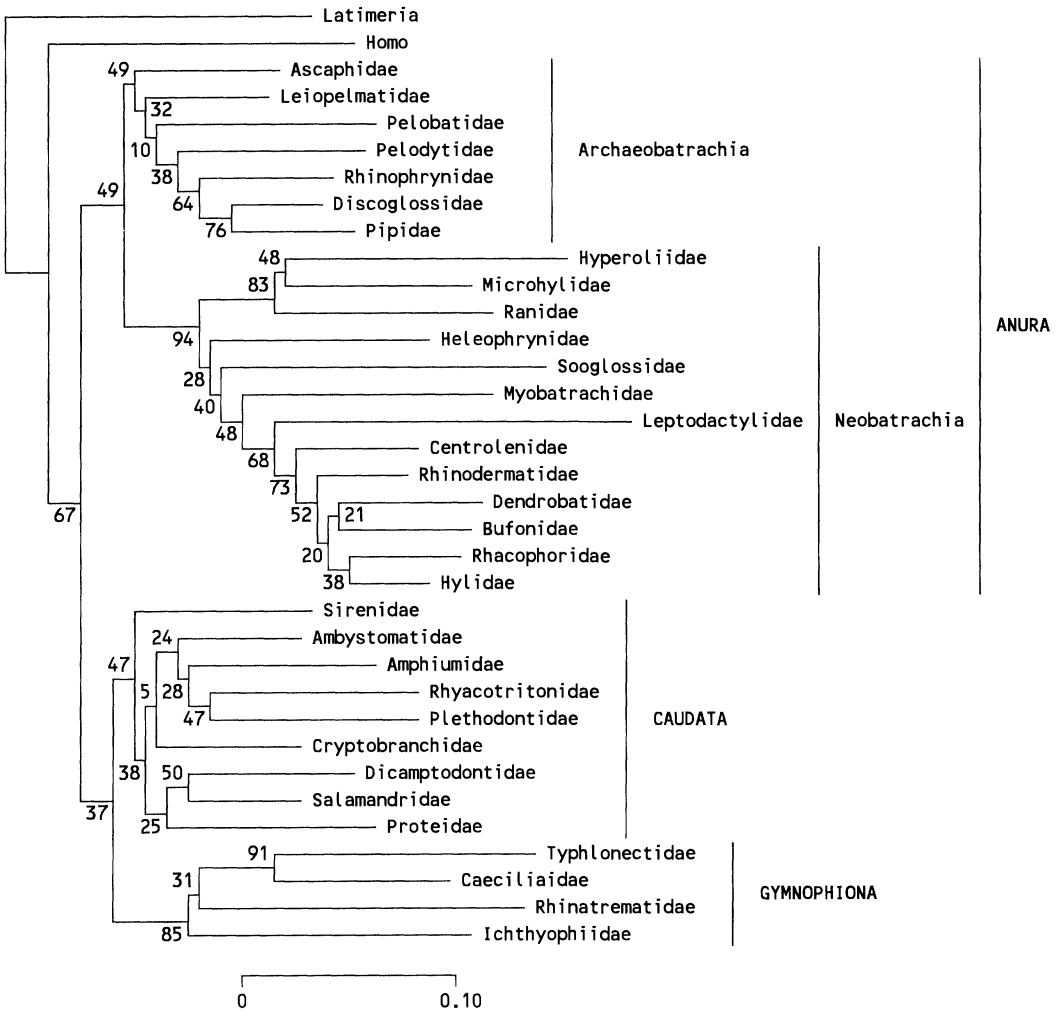

FIG. 2.—Relationships of 33 species of amphibians, human, and coelacanth obtained by the neighbor-joining method. The tree was rooted with the coelacanth (fish). Numbers on the tree are bootstrap *P*-values based on 2000 replications. Branch lengths represent estimated changes (nucleotide changes + insertion/deletion events) corrected for multiple hits (see text).

coglossoidea (Duellman and Trueb, 1986). Hillis's (1991) analyses of Cannatella's (1985) data showed support of this traditional view of pipids and rhinophrynids as sister lineages. As none of the nodes in this portion of our tree are associated with very high bootstrap *P*-values, it is apparent more data will be needed before we can comment further on archaeobatrachian relationships.

Turning to the Neobatrachia, we see two well-supported nodes, one leading to the ranids, microhylids, and hyperoliids, and the other to all remaining frog families included in this study. Most prior workers have placed the microhylid frogs in their own superfamily (Microhyloidea). However, Kluge and Farris (1969) made an example of anuran phylogeny in an early paper on quantitative phylogenetics. The only major difference between their phylogenetic conclusions and those of earlier students of anuran phylogeny (using the morphological data of all earlier workers) was that Kluge and Farris found a "close relationship" between the Microhylidae and the Ranidae. A subsequent study by Lynch (1973) also found strong morphological evidence for a sister group relationship between the Ranidae (in which

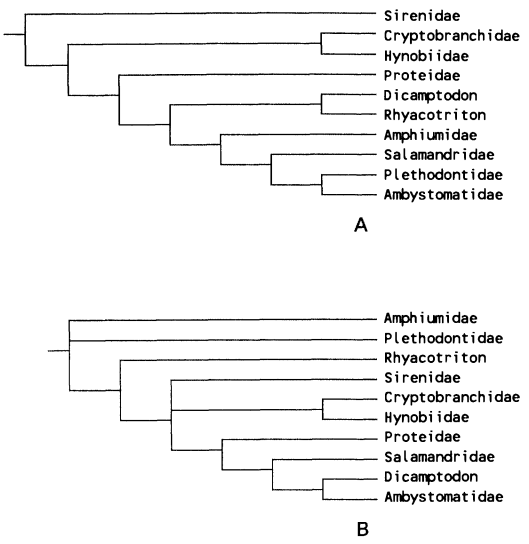

FIG. 3.—Evolutionary relationships among salamander families based on molecular and morphological data. A. Tree based on morphological data (Duellman and Trueb, 1986). B. Tree based on ribosomal RNA sequences from Larson (1991).

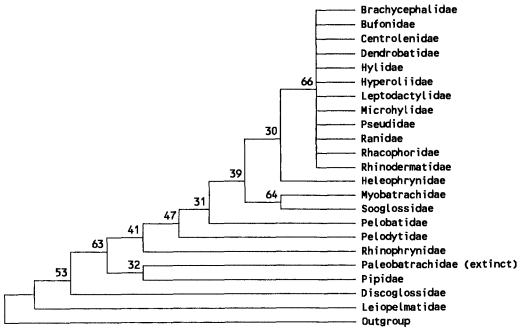

FIG. 4.—Phylogenetic tree of frog families based on the morphological data summarized by Duellman and Trueb (1986), reanalyzed using maximum parsimony (PAUP 3.0, D. L. Swofford, Illinois Natural History Survey, Urbana, Illinois). This is a strict consensus of 18 most parsimonious trees (length = 35); numbers on the tree are bootstrap *P*-values based on 2000 replications.

Lynch included the hyperoliid frogs) and the Microhylidae (= Lynch's Ranoidea). In their analysis of frog family relationships, Duellman and Trueb (1986:473) examined 16 morphological characters for all 22 frog families and indicated the microhylids radiating from a fork leading also to ranids, dendrobatids, hyperoliids, and rhacophorids. However, Hillis's (1991) and our maximum parsimony reanalyses of the same data used by Duellman and Trueb found relatively little resolution within the Neobatrachia. A strict consensus of the 18 most parsimonious trees (Fig. 4) shows a sister group relationship between the Myobatrachidae and Sooglossidae (64% bootstrap) and an unresolved clade of 12 families (66% bootstrap).

Examination of the remainder of our tree (Fig. 2) showed a suggestion of a bufonoid cluster (bootstrap = 68%) containing the Leptodactylidae, Centrolenidae, Rhinodermatidae, Dendrobatidae, Bufonidae, Rhacophoridae, and the Hylidae. This association is highly congruent with that found by Duellman and Trueb (1986:Fig. 17.3), but not in our reanalysis of their data (Fig. 4). Also, in our 12S rRNA tree (Fig. 2), this group includes the Rhacophoridae, a family usually grouped with the ranoid frogs. Lynch (1973) also recognized a superfamily, the Bufonoidea, which corresponds to our grouping except Lynch's study (1) included *Pseudis*, which we were unable to include in this study (although our unpublished 16S rRNA data associate *Pseudis* with this lineage) and (2) excluded the Rhacophoridae, which Lynch (1973) included as a subfamily in the Ranidae. Our "bufonoid" grouping contains two sister groups which, although not well supported statistically, are nonetheless intriguing. One, Dendrobatidae + Bufonidae, is an association that has been suggested previously based on morphology (Lynch, 1973). The other, Hylidae + Rhacophoridae, has not been suggested before, primarily because of skeletal differences in these two families: rhacophorids have a firmisternal pectoral girdle (as in the ranids, for example) whereas hylids have the arciferal type, believed to be more primitive in anurans as a whole (Duellman and Trueb, 1986). However, despite these internal differences, rhacophorids bear a striking resemblance to hylids in external morphology and behavior. Both hylids and rhacophorids are typically arboreal groups and they often have extensive interdigital webbing. Their complementary, and primarily Gondwanan, distributions may reflect a vicariant origin.

The remaining three families, the Heleophrynidae, Sooglossidae, and Myobatrachidae have been placed in the Pelo-

batoidea (Lynch, 1973:Figs. 3–7) with several other families (e.g., Pelobatidae) not generally associated with one another. Duellman and Trueb's (1986) analysis and our reanalysis of their data (Fig. 4) join the sooglossids with the myobatrachids. However, our 12S rRNA data (Fig. 2) do not show support for that grouping.

In summary, our data show some tantalizing patterns which we are pursuing by gathering more extensive data. When studying such large numbers of taxa, more extensive data sets than this one are needed to define statistically significant nodes in the phylogenetic tree. As the number of taxa increases, the number of sites in a data set must also increase in order to resolve the branching pattern. Our work has shown that the mitochondrial rRNA genes are evolving rapidly enough to provide the nucleotide variation needed for investigating familial relationships of amphibians while multiple substitutions at the same site do not appear to have obscured the phylogenetic signal. Our current studies suggest that expansion of the data set presented here will allow us to address lingering questions in amphibian phylogeny more fully.

Acknowledgments.—We thank L. Lierman and L. Tu for laboratory assistance. D. C. Cannatella, C. A. Hass, D. M. Hillis, and an anonymous reviewer offered helpful comments on the manuscript. J. A. Musick, R. W. Chapman, the Explorer's Club, and the Virginia Institute of Marine Science Coelacanth Program generously supplied tissues from *Latimeria*. We also thank the many colleagues who have contributed to our frozen tissue collections over the years, thus making a study such as this one possible. Procedures involving animals were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (approval code 1296). This research was supported by grants from the National Science Foundation (BSR 88-96270 to LRM; BSR 89-18926 to LRM and SBH).

Literature Cited

ANDERSON, S. A., A. T. BANKIER, B. G. BARRELL, M. H. L. DEBRUIJN, A. R. COULSON, J. DROUIN, I. C. EPERSON, D. P. NIERLICH, B. A. ROE, F. SANGER, P. H. SCHREIER, A. J. H. SMITH, R. STADEN, AND I. G. YOUNG. 1981. Sequence and organization of the human mitochondrial genome. Nature 290: 457–465.

BENTON, M. J. 1990. Phylogeny of the major tetrapod groups: Morphological data and divergence dates. J. Molec. Evol. 30:409–424.

BOLT, J. R. 1991. Lissamphibian origins. Pp. 194–222. *In* H-P. Schultze and L. Trueb (Eds.), Origins of the Higher Groups of Tetrapods. Comstock Publ. Assoc., Ithaca, New York.

BROWN, W. M., E. M. PRAGER, A. WANG, AND A. C. WILSON. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. J. Mol. Evol. 18:225–239.

CABOT, E. L., AND A. T. BECKENBACH. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comput. Apl. Biosci. 5:233–234.

CANNATELLA, D. C. 1985. A Phylogeny of Primitive Frogs (Archaeobatrachians). Ph.D. Dissertation, University of Kansas, Lawrence, Kansas.

DESALLE, R., T. FREEDMAN, E. M. PRAGER, AND A. C. WILSON. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. J. Mol. Evol. 26:157–164.

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

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

———. 1988. Phylogenies from molecular sequences: Inference and reliability. Annu. Rev. Genet. 22:521–565.

GOOD, D. A., AND D. B. WAKE. 1992. Geographic variation and speciation in the torrent salamanders of the genus *Rhyacotriton* (Caudata: Rhyacotritonidae). Univ. California Publ. Zool. 126:1–91.

GREEN, D. M., C. H. DAUGHERTY, AND J. P. BOGART. 1980. Karyology and systematic relationships of the tailed frog *Ascaphus truei*. Herpetologica 36: 346–352.

HASS, C. A., R. A. NUSSBAUM, AND L. R. MAXSON. 1993. A molecular investigation of phylogenetic relationships among the Seychellian caecilians (Amphibia: Gymnophiona) and a preliminary report on family level relationships. Herpetol. Monogr. 7:56–63.

HEDGES, S. B. 1992. The number of replications needed for accurate estimation of the bootstrap *P* value in phylogenetic studies. Mol. Biol. Evol. 9:366–369.

HEDGES, S. B., R. L. BEZY, AND L. R. MAXSON. 1991. Phylogenetic relationships and biogeography of xantusiid lizards inferred from mitochondrial DNA sequences. Mol. Biol. Evol. 8:767–780.

HEDGES, S. B., J. P. BOGART, AND L. R. MAXSON. 1992. Ancestry of unisexual salamanders. Nature 356:708–710.

HEDGES, S. B., AND L. R. MAXSON. 1992. 18S rRNA sequences and amniote phylogeny: Reply to Marshall. Mol. Biol. Evol. 9:374–377.

HEDGES, S. B., K. D. MOBERG, AND L. R. MAXSON. 1990. Tetrapod phylogeny inferred from 18S and 28S ribosomal RNA sequences and a review of the evidence for amniote relationships. Mol. Biol. Evol. 7:607–633.

HEDGES, S. B., R. A. NUSSBAUM, AND 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. 1987. Molecular versus morphological approaches to systematics. Ann. Rev. Ecol. Syst. 18:23–42.

———. 1991. The phylogeny of amphibians: Current knowledge and the role of cytogenetics. Pp. 7–31. *In* D. M. Green and S. K. Sessions (Eds.), Amphibian Cytogenetics and Evolution. Academic Press, Inc., San Diego, California.

HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. J. Hered. 83:189–195.

JULES, T. H., AND C. R. CANTOR. 1969. Evolution of protein molecules. Pp. 21–132. *In* H. N. Munroe (Ed.), Mammalian Protein Metabolism. Academic Press, New York.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18:1–32.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:6196–6200.

LARSON, A. 1991. A molecular perspective on the evolutionary relationships of the salamander families. Evolutionary Biology 25:211–278.

LARSON, A., AND A. C. WILSON. 1989. Patterns of ribosomal RNA evolution in salamanders. Mol. Biol. Evol. 6:131–154.

LAURENT, R. 1967. Taxonomia de los anuros. Acta Zool. Lilloana 22:207–210.

LYNCH, J. D. 1973. The transition from archaic to advanced frogs. Pp. 133–183. *In* J. L. Vial (Ed.), Evolutionary Biology of the Anurans. University of Missouri Press, Columbia.

MEYER, A., AND A. C. WILSON. 1990. Origin of tetrapods inferred from their mitochondrial DNA affiliation to lungfish. J. Mol. Evol. 31:359–364.

MILNER, A. R. 1988. The relationships and origin of living amphibians. Pp. 59–102. *In* M. J. Benton (Ed.), The Phylogeny and Classification of the Tetrapods. Vol. 1. Clarendon, Oxford.

NEI, M. 1991. Relative efficiencies of different tree-making methods for molecular data. Pp. 90–128. *In* M. M. Miyamoto and J. Cracraft (Eds.), Phylogenetic Analysis of DNA Sequences. Oxford University Press, New York.

REIG, O. A. 1958. Proposiciones para una nueva macrosistematica de los anuros. Nota preliminar. Physis 21:109–118.

ROE, B. A., D.-P. MA, R. K. WILSON, AND J. F.-H. WONG. 1985. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. J. Biol. Chem. 260:9759–9774.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

SAVAGE, J. 1973. The geographic distribution of frogs: Patterns and predictions. Pp. 351–446. *In* J. L. Vial (Ed.), Evolutionary Biology of the Anurans. University of Missouri Press, Columbia.

TRUEB, L., AND R. CLOUTIER. 1991. A phylogenetic investigation of the inter- and intrarelationships of the Lissamphibia (Amphibia: Temnospondyli). Pp. 223–313. *In* H-P. Schultze and L. Trueb (Eds.), Origins of the Higher Groups of Tetrapods. Comstock Publ. Assoc., Ithaca, New York.

Accepted: 27 February 1993
Associate Editor: David Cannatella

APPENDIX I

Taxa Examined

Voucher specimens for most taxa are deposited in the following collections: University of Michigan Museum, Ann Arbor (UMMZ), University of Illinois Museum of Natural History (UIMNH), University of California, Berkeley Museum of Vertebrate Zoology (MVZ), Chicago Field Museum of Natural History (FMNH), University of Kansas (KU), Smithsonian National Museum of Natural History (USNM), California Academy of Science (CAS). The abbreviations LM, RH, and SBH refer to specimens in the frozen tissue collections of Linda R. Maxson (Pennsylvania State University), Richard Highton (University of Maryland), and S. Blair Hedges (Pennsylvania State University), respectively. *Caecilia* sp. (UMMZ 190146)—Ecuador: Cotopaxi; San Francisco de las Pampas; *Epicrionops* sp. (UMMZ 190478)—Ecuador: Cotopaxi; San Francisco de las Pampas; *Ichthyophis bannanicus* (UMMZ 189122)—China: Yunnan; Longling, Dai Village near km post 141, N of Mengla (875 m); *Typhlonectes natans* (LM 2509)—Pet trade; *Bufo valliceps* (UIMNH 95424)—Louisiana: Allen Parish, Calcasieu River; *Centrolene geckoideum* (LM 85)—Ecuador; *Dendrobates speciosus* (UIMNH 94442–99)—Panama; *Discoglossus pictus* (LM 2352–53)—Tunisia; *Heleophryne natalensis* (LM 1001)—South Africa: Natal, St. Helier, 495 m; *Hyla cinerea* (RH 57458)—Maryland: Dorchester Co., Smithville; *Hyperolius argus* (CAS 161016)—Kenya: Kilif District, Arabuka Forest; *Leiopelma hamiltoni* (LM 2105)—New Zealand; *Ascaphus truei* (UIMNH 94103)—Oregon: Wallowa Mts.; *Eleutherodactylus cuneatus* (SBH 172809)—Cuba; *Gastrophryne carolinensis* (RH 55501)—South Carolina: Aiken County, Savannah River Plant, Flamingo B; *Neobatrachus pelobatoides* (LM2779)—Western Australia: Beverley; *Scaphiopus holbrookii* (LM 3070)—North America; *Pelodytes punctatus* (LM 731)—Spain: Cadiz; *Rana pipiens* (UIMNH 95421)—dealer, locality unknown; *Rhacophorus pardalis* (FMNH 221728)—Sarawak; *Rhinoderma darwinii* (LM[Jan/77])—Chile; *Rhinophrynus dorsalis* (UIMNH 94144)—Mexico; *Nesomantis thomasseti* (LM 2549)—Seychelles: Silhouette Island; *Ambystoma mexicanum* (UIMNH 95430)—bred at the Indiana University Amphibian Facility; *Amphiuma tridactylum* (LM 2594)—dealer; *Cryptobranchus alleganiensis* (UIMNH 94035)—dealer; *Dicamptodon ensatus* (LM 445)—Washing-

ton: Wahkiakam County, Rock Creek; *Plethodon yonahlossee* (RH 69670–72)—North Carolina: Buncombe Co., Walker Falls; *Necturus lewisi* (UIMNH 94301)—North Carolina: Johnston County; *Notophthalmus viridescens* (LM 2660)—Pennsylvania: Centre County; *Rhyacotriton olympicus* (LM 384)—Washington: Mason County; *Siren intermedia* (LM 2531)—Illinois: Alexander County, Horseshoe Lake Dam; *Latimeria chalumnae* (Virginia Institute of Marine Science 8118).

Herpetological Monographs, 7, 1993, 42–55

NON-TRADITIONAL CHARACTERS IN THE ASSESSMENT OF CAECILIAN PHYLOGENETIC RELATIONSHIPS

MARVALEE H. WAKE

Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720, USA

ABSTRACT: Phylogenetic relationships among caecilians and of caecilians to other amphibians are not well understood. A generally accepted hypothesis of relationships exists for families of caecilians, but relationships among genera and among species remain unresolved for the most part. Current hypotheses of relationships are based largely on morphological characters, primarily external features and some osteology; molecular data are accruing. However, I contend that morphology has not been explored adequately in the search for characters of systematic utility. I present a summary of work in progress on the examination of aspects of the neuroanatomy and reproductive morphology of caecilians and a discussion of the possible contribution of such characters to phylogenetic analysis. I conclude that morphologists should, whenever possible, both place their work in a phylogenetic context, and use their data to contribute to phylogenetic hypotheses.

Key words: Amphibia; Gymnophiona; Caecilians; Neuroanatomy; Sperm

THIS presentation has two purposes: first, to review and comment on the current state of understanding of the phylogenetic relationships of caecilians, and second, to present new information and analysis using characters rarely considered that might be useful in both structuring and analyzing hypotheses of relationships among caecilians. I will be speculative, and I hope provocative, in the latter effort.

Hypotheses of relationships of caecilians, at least at the family level, are becoming refined as more morphological data are collected and analyzed, and as molecular trees are generated. However, I remain concerned that information for the three orders of the extant Amphibia are not often fully parallel. Therefore, assumptions about the condition in caecilians, and especially the nature of characters, in the absence of developmental data and full suites of adult morphological information, too often are inserted into phylogenetic data bases. Hanken (1986) has recently referred to this problem. However, as more workers examine more specimens and taxa, this problem will be alleviated.

The hypothesis of caecilian relationships used by most workers as a baseline for studies is that presented by Duellman and Trueb (1986), and amplified by Nussbaum and Wilkinson (1989). It includes six families, some 36 genera and approximately 175 species. The seminal work of E. H. Taylor, published in his monograph on *The Caecilians of the World: A Taxonomic Review* in 1968, initiated a new era of interest in caecilians. Taylor recognized three families (Ichthyophiidae, Caeciliidae, and Typhlonectidae) and described a number of new genera and species. In 1969, he recognized a fourth family, the Scolecomorphidae. Nussbaum distinguished the Rhinatrematidae from the Ichthyophiidae in 1977, and Duellman and