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XENOPUS LAEVIS AS A MODEL ORGANISM

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Abstract.—Model organisms are often assumed to be representative of some more inclusive taxon of which the species is a part. This assumption leads to mistaken generalizations about the evolutionary and comparative significance of the data gathered. This paper reviews comparative and evolutionary studies of *Xenopus laevis* and its relatives. Phylogenetic analysis of data from DNA sequences and morphology indicate that *Xenopus* is monophyletic and that *Silurana* is its sister group. The most basal lineages of Pipidae diverged prior to the breakup of Gondwana. The bizarre morphology of *Xenopus* is in part due to changes in the mode of metamorphosis. Speciation in *Xenopus* is unique among Anura in being associated with various levels of polyploidy owing to allopolyploidy. Several kinds of molecular studies indicate substantial divergence between *Xenopus* and *Silurana*. The contribution of data from model studies of *Xenopus* would be greatly enhanced if comparable data were available from a more basally placed lineage such as *Bombina*. [*Xenopus*; *Silurana*; Pipidae; model organisms; morphology; historical biogeography; fossils; molecular systematics; ribosomal DNA; mitochondrial DNA; phylogeny; development; heterochrony; caenogenesis; functional anatomy; behavior; polyploidy; karyotypes; molecular biology.]

Model organisms are exemplars of a particular biological system under investigation. In comparative or evolutionary research, a tacit assumption is that the model organism is representative at some level of a more inclusive taxon. In particular, *Xenopus laevis* is touted as a primitive (but specialized) representative of Anura or Amphibia. This misconception has been fostered in part by systematists in their use of the terms such as primitive. For example, "primitive" has described at least three attributes of a taxon: (1) possession of a large number of plesiomorphic character states, (2) a basal position in a given phylogeny, and (3) a fossil record of early age.

In this paper, we review aspects of the biology of *Xenopus laevis* that are relevant to both its phylogenetic relationships and its use as a model organism. The evidence demonstrates that *Xenopus* is highly derived (apomorphic) in most aspects of its biology in comparison with other frogs and that *Xenopus* is not among the more basally placed lineages (Cannatella, 1985; Ford and Cannatella, 1993). We briefly treat the areas in which large amounts of data have accumulated, focusing on comparative or evolutionary studies. A review of *X. laevis* biology, including life history, physiology, early development, and tissue interactions, was provided by Deuchar (1975). Although incomplete in the areas of morphology, systematics, and comparative biology in general, it is a useful reference to the earlier literature.

Xenopus is an excellent organismal system for laboratory studies because its species are easily bred and maintained (Dawid and Sargent, 1988). Much of the early use of *X. laevis* in the 1930s and 1940s was for physiological research, including the diagnosis of pregnancy. More recently, the species has become a primary model system for molecular biology. In this context, it is the best-known amphibian and, together with *Mus musculus* and *Gallus domesticus*, one of the best-known vertebrates.

Xenopus species are bizarre-looking frogs. They are among the most aquatic anurans and have flattened, pyriform bodies with small heads. The eyes point dorsally, eyelids are absent, and lateral line organs are retained through metamorphosis. The hind limbs are muscular, and the toes are fully webbed. The inner toes are capped with keratinous claws, hence the common name

African clawed frog. In South Africa, the popular name for *Xenopus* is the Platanna (Platie, for short), from *Plathander*, meaning flat hands (Wager, 1965; Deuchar, 1975). The tongue is completely absent, and the middle ear is well developed but concealed by undifferentiated skin. Vocalizations consist of a series of clicks or buzzes, but there are no vocal cords or vocal sacs. The tadpoles are open-water, highly specialized filter feeders that sport a pair of sensory barbels. They form social aggregates and orient their heads downward while rapidly fluttering the tip of the tail to maintain position.

The range of *X. laevis* occupies a large part of subsaharan Africa, from the Republic of South Africa north to Zaire and Uganda and west to Cameroon, generally occupying cooler upland areas (Tinsley, 1981a). Feral populations are established in southern California (McCoid and Fritts, 1980, 1989). Several subspecies are recognized (Frost, 1985).

Phylogenetic Relationships of *Xenopus*

Pipidae

Pipidae is the node-based name (de Queiroz and Gauthier, 1992) for the most recent ancestor of the living taxa *Xenopus, Silurana, Hymenochirus, Pseudhymenochirus,* and *Pipa* and all of its descendants (Ford and Cannatella, 1993). This definition potentially excludes several fossil taxa from Pipidae; these are included in a more inclusive stem-based clade, Pipimorpha (Ford and Cannatella, 1993). *Pipa* (7 species) is found in South America and Panama, and *Xenopus* (12), *Silurana* (2; formerly included in *Xenopus*), *Hymenochirus* (4), and *Pseudhymenochirus* (1) are African (Frost, 1985). The species-level systematics and phylogenetic relationships of *Pipa* were discussed by Trueb and Cannatella (1986), and the monophyly of *Hymenochirus* and *Pseudhymenochirus* was established by Cannatella and Trueb (1988b). The species-level systematics of *Hymenochirus* has not been reviewed recently. Relationships among species of *Xenopus* (excluding *Silurana*) were discussed by Carr et al. (1987). The morphology and species-level systematics of *Xenopus* and *Silurana* are being studied by Linda Trueb. Relationships among the genera were discussed by Cannatella and Trueb (1988a, 1988b) and de Sá and Hillis (1990).

Relationship of Xenopus to Other Genera of Pipidae

Noble (1931) and Dunn (1948) considered the African taxa (*Xenopus, Hymenochirus,* and *Pseudhymenochirus*) to be each others' closest relatives, separate from the New World taxa (now all included in *Pipa*). Estes et al. (1978) suggested instead that *Hymenochirus* and *Pipa* might be closest relatives, and Báez (1981) concluded the same from a cladistic analysis of osteology.

Cannatella and Trueb (1988a) also concluded on the basis of morphological characters that *Hymenochirus* and *Pipa* were closest relatives and called this clade Pipinae. Moreover, they found nine synapomorphies placing *X. tropicalis* and *X. epitropicalis* closer to Pipinae than to other *Xenopus* and resurrected the name *Silurana* for these two species (Fig. 1). However, two characters related to the pectoral girdle are ambiguous because they are reversed in the ancestor of *Pipa*. The other seven characters were not homoplastic within Pipidae: absence of vomers, anterolateral processes of frontoparietal, fusion of first two vertebrae, enlarged sternal plate, loss of pyriformis muscle, reduction of palpebral membrane, and mating behavior. Subsequent study of *Pseudhymenochirus* (Cannatella and Trueb, 1988b) made delineation of the palpebral membrane character states less certain. The observations of mating behavior in *Silurana tropicalis* (Swisher, 1969) are possibly in error. This reduces the unambiguous support for the *Silurana* + Pipinae clade to five synapomorphies (Fig. 1).

Cannatella and Trueb (1988a) also listed four derived features shared by *Xenopus* and *Silurana*: elongate zygomatic process of squamosal, presence of epipubis cartilage, partial fusion of the tendons of the sartorius and semitendinosus muscles, and presence of a subocular tentacle. To these

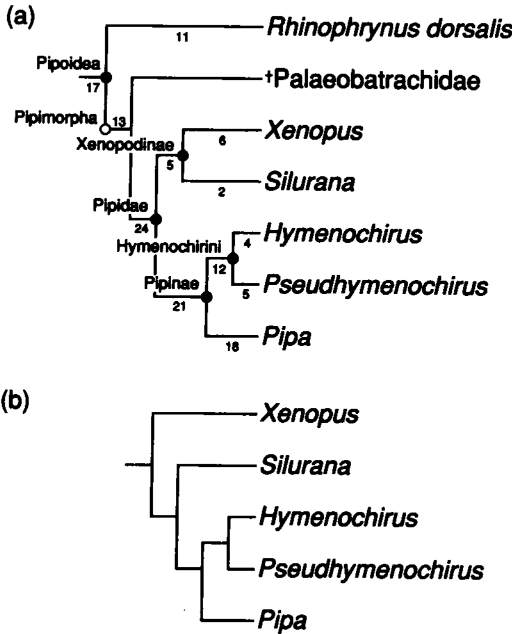

FIGURE 1. (a) Phylogeny and taxonomy of Pipoidea as discussed in this paper. ● = node-based names; ○ = stem-based name (sensu de Queiroz and Gauthier, 1992). The numbers of unambiguous morphological synapomorphies are given for each branch, based on Cannatella (1985, 1986) and Cannatella and Trueb (1988a, 1988b); *Pseudhymenochirus* has only one living species, *merlini*. Relationships among the genera of †Palaeobatrachidae were not analyzed; the family is represented here by data from †*Palaeobatrachus* (Spinar, 1972). (b) Relationships of *Silurana* as proposed by Cannatella and Trueb (1988a).

can be added the presence of cartilaginous endoskeletal supports for the larval barbels, for a total of five synapomorphies. Thus, the support from morphological data for the relationships of *Silurana* to other pipids is ambiguous, but not for the monophyly and distinctiveness of *Silurana* as compared with *Xenopus*.

De Sá and Hillis (1990) provided data on 1,486 nucleotide positions (np) from 12S and 28S genes from the nuclear ribosomal DNA array (as well as part of an internal transcribed spacer region) for *Xenopus, Hymenochirus, Silurana,* and an outgroup, the pelobatid spadefoot toad *Spea*. Their analysis strongly supports *Silurana* and *Xenopus* as sister groups, rather than *Silurana* and *Hymenochirus,* as would be predicted from Cannatella and Trueb (1988a). De Sá and Hillis (1990) included morphological data from Cannatella and Trueb (1988a) in their analysis, but the resulting phylogeny did not change. Of necessity, some data from the latter paper were omitted because of the difference in numbers of taxa between the two analyses. Thus, de Sá and Hillis (1990) concluded that the recognition of *Silurana* was not demanded by their phylogeny but was consistent with it. We continue to use *Silurana* because it conveys more information about relationships.

For the present paper, sequences from an additional 1,162 np were obtained, for a total of 2,648 (Fig. 2). Materials and methods for producing the sequence data were generally as described in de Sá and Hillis (1990). The data were analyzed using PAUP 3.0s (Swofford, 1991). Given the four taxa, there are three possible topologies, using *Spea* to root the tree (Fig. 3). Two primary analyses were done, one omitting gaps in alignment and the other including gaps. Sites with gaps were omitted by setting the gap symbol equal to missing data; for the four-taxon case at hand, this rendered such sites uninformative. The data were then analyzed using both transitions and transversions and using transversions only (Table 1). For the analysis including gap data, the data set was modified in two ways. First, the data were screened for informative sites using PAUP, and three clusters of two or more informative sites with adjacent gaps were identified. For each cluster, the number of informative characters was reduced to one by excluding characters. This was done to reduce bias in the data on the assumption that adjacent gaps are nonindependent. Second, informative insertion/deletion events in which the nucleotide bases were uninformative, such as the state vector [A, G, gap, gap], were identified using PAUP with an EQUATE statement to set all nucleotide states except gaps to the same arbitrary state. A binary dummy character (12 total) was added for each such informative insertion/deletion event, excluding adjacent gaps that were considered nonindependent (Fig. 2). The modified data set was analyzed using transitions and transversions, and the process was repeated using transversions only (Table 1).

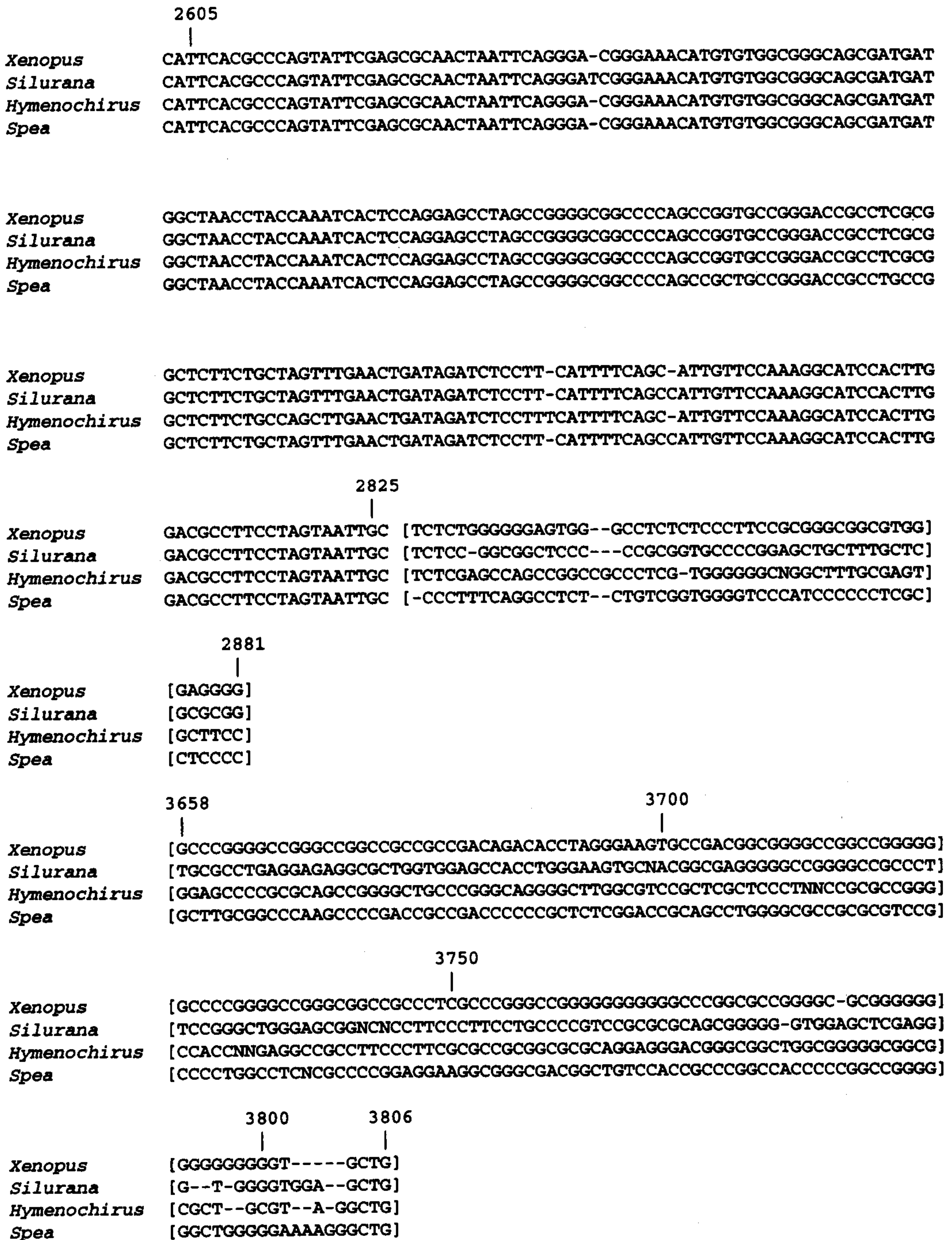

FIGURE 2. Aligned rDNA sequences. The reference numbers correspond to the positions of the *Xenopus laevis* sequence. Nucleotides 2,605–2,825 correspond to positions in the 18S gene; all other numbers (except those of sequences in brackets) correspond to positions in the 28S gene (see references in text). Sequences in brackets correspond to internal transcribed spacer (ITS) regions and were not analyzed because of questionable alignment. The 0's and 1's at the end of the matrix represent the dummy gap characters that were added. Taxa are *Xenopus laevis*, *Silurana tropicalis*, *Hymenochirus curtipes*, and *Spea bombifrons*.

```
              1                         27
              |                         |
Xenopus       AGTCTGGAGTCTAGTCTGCGCCGCTGG
Silurana      AGTCTGGAGTCTAGTCTGCACCGCTGG
Hymenochirus  GCGCTGGAGTCTAGTCTGCGCCGCTGG
Spea          AGTCTGGAGTCTAGTCTGCACCGCTGG

              143                                                            200
              |                                                                |
Xenopus       CTGCA--CACCGCATGCCCTCTGGCCTGG-G-GGGGCCGCGCCGAG-----CCCCCGGGTTCAGGAAGAC
Silurana      CTTTA--CACCGCATGCCCTCTGGCCTGG-G-GGGGCCGCGCCGAG-----CCCCCGGGTTCAGGAAGAC
Hymenochirus  CTTTAAACACCGCATGCCCTCT----TGGAGAGGGGCCGNGGCGAGGGCACCCCCCGGGTTCAGGAAGAC
Spea          CTTTA--CACCGCATTCCCTCTGGCCTGG-G-GGGGTGACTGTGAG-----CCCTTGGGTTCAGGAAGAC

Xenopus       TAGCTCCGGGTCGGGCGCCTGCCACAATCCGGCCACCCGCCGGGGGCCGCGCCGCCC-TGGGCCAGAGGA
Silurana      TAGCTCCGGGTCGGGCGCCTGCCACAATCCGGCCACC-GCCGGGG-CCGNGCCGGCCCTGGGCCAGAGGA
Hymenochirus  TAGCTCCTGTGCGGGCACCTGCCACACTCCGGCCATCCGCCGGGG-CCGNN--GGCC-TGGCCAAGAAGA
Spea          TAGCTCTGGGTCGGGCGCCTGCCACAATCCGGCCATC-GTTGGGN--TTNGTCGCCC-TGGGCCAGATGT

                                          300
                                          |
Xenopus       GCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGGTAGATT-CCGATTTA-TGGCCG
Silurana      GCCTCAGCCCAACAAACACTTACGTCGGGTTTCGCCCACCATTTGAGGTAGATTGCCGATTTA-TGGCCG
Hymenochirus  GCCTCAGCCCAACGAACCCTTACGTCGGGTTTCGCCCACCATTTGAGGTAGATT--CGATTTAATGGCCG
Spea          GCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGGTAGATT-CCGATTTA-TGGCCG

                                                                         400
                                                                         |
Xenopus       TGCTCTGGCTATCGCCT-GTTCATGGCATTCCTTTCAACTTTTCTTGAAACTTCTCTCTCAAGTT-CTCC
Silurana      TGCTC-GG-TATCAGTTGGTTCATGGCGTTCCTTTCAACTTTTCTTGAAACTTCTCTCTCAAGTT-CTCC
Hymenochirus  TGCTCTGGCTATCAGGTTGTTCATGGCATTCCTTTCAACTTT-CTTGAAACTTCTCTCTCAAGTT-CTCC
Spea          TGCTCTGGCTATCAGCT-GTTCATGGCATTTCGGTCAACTTT--TCGAAACTTCTCTCTCAAGTTTCTCC

Xenopus       CGCAC-TTTGGCAATTCTCCATTTGCCCA-CCCC---GGCAC--GCCA-GGCGGGCCTCCTAAGTTGGGC
Silurana      CGCACGTTTGGCAATTGTCCATTTGCCCAACCCCATTGGCACATGGCATGGCGGGCCTC-TAAGTTGGGC
Hymenochirus  CGCAC-TTTGGCAATTCTCCATTTGNNCA-NCCCA--GGNNNNNNGCA-GGCGNGCCTCCTAAGTTGGGC
Spea          CGCAC-TTTGGCAATTCTCCATTTGCCCA-CCCCA--GGCAC--GCCA-GGCGGGCCTCCTAAGTTGGGC

                                             500
                                             |
Xenopus       CGCCCAGTCGCGGC-GGCCCTGGCCCGGTGAGCCGCCTGGGGGGGCGGCCGGGGGAGGGGAGCGGCCCTC
Silurana      CGCCGGGTC-CGGCCGGC--TA--C-GN--GGGCGCCTG---CGGCGGC---GGGAGGGGAG--GCATAC
Hymenochirus  CGCCGTGNC-CAGCNGGC------C-----GGGCCCCT-----------------AGGGGAG-GGAGGGG
Spea          CGCCCGGAGCCGGCCGGC--AA--CGGCACGGGCGCCTG---CGGCGGC---GGGAGGGGAG-GGCGG--

                                     561
                                     |
Xenopus       CCGCCAGCGCGCCCCCCCTGCG
Silurana      GGGAATCAGGCCCCGCGCTGCC
Hymenochirus  GGNNGGGACCTCCCGAGCCCTC
Spea          -GGAAGTGCCCCNGCGCCTCCC
```

FIGURE 2. Continued.

All four treatments yielded the same result as the analyses of de Sá and Hillis (1990): *Silurana* is more closely related to *Xenopus* than to *Hymenochirus*. The *Xenopus* + *Silurana* clade was present in >98% of 1,000 bootstrap replicates in each of the four analyses.

The ambiguous morphological data (five

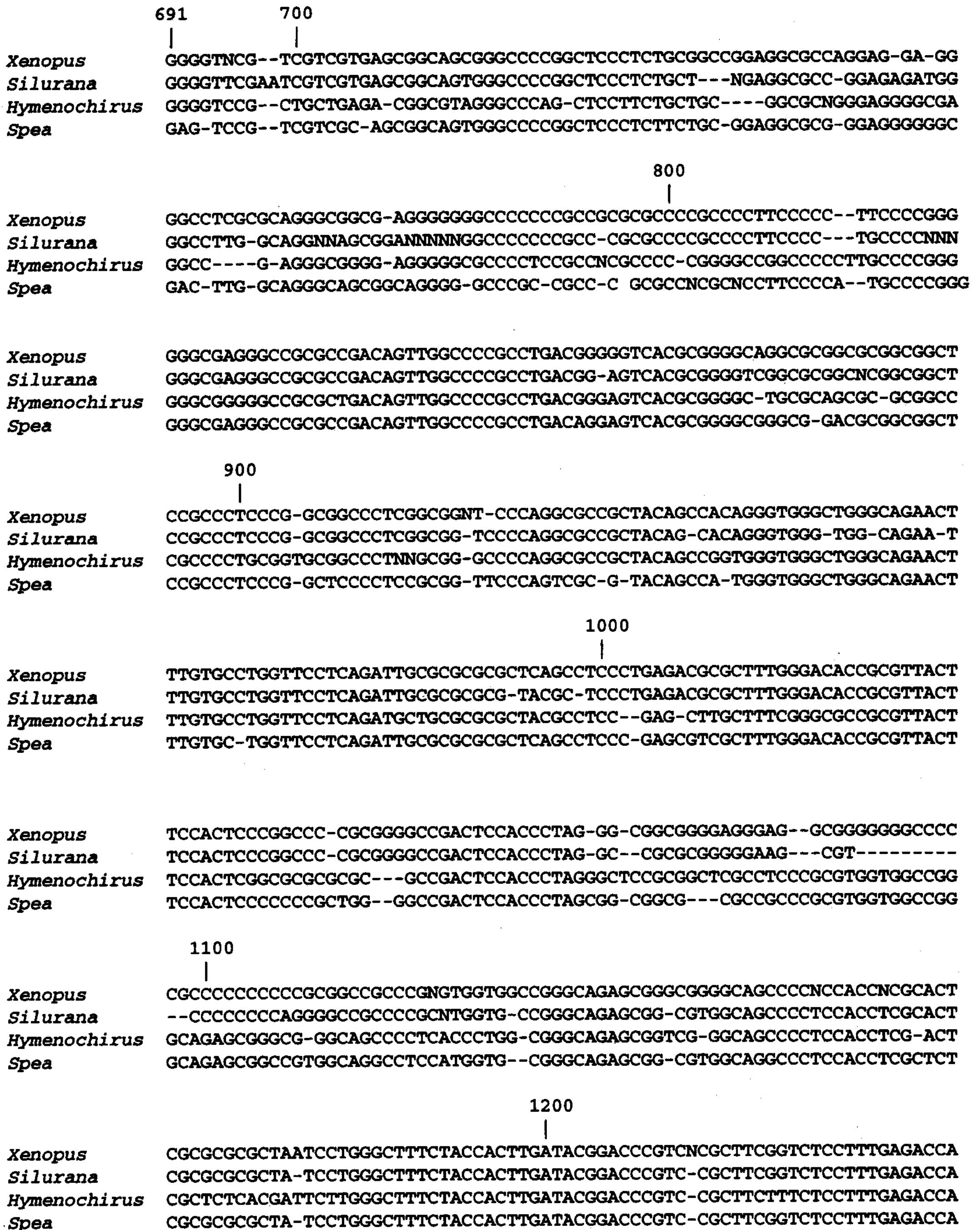

FIGURE 2. Continued.

synapomorphies for *Xenopus* + *Silurana* and five synapomorphies for *Silurana* + Pipinae) were combined with the sequence data, and the analyses were repeated. As would be predicted, for each treatment the length of the shortest tree (tree A) increased by 5, and the length of the two longer trees (B and C) increased by 10, but

```
                                                                                 1300
                                                                                    |
Xenopus        CCTCCAGGC-ATCGCCAGGACTGCACGTTTAGCCAG-CAGGCTGGANCCATATCCCCGCTTTCTGCTTAG
Silurana       CCTCCAGGCTATCGCCAGGACTGCACGTTTAGCCAGTCAGGCTGGA-CCATATCC----NTTCTGATTAG
Hymenochirus   CCTCCAGGCCATCGCCAGGACTGCACGTTTAGCCAG-CAGGCTGGACCCATATCCCCGCTTTCTGATTAG
Spea           CCTCCAGGC-ATCGCCAGGACTGCACGTTTAGCCAG-CAGGCTGGACCCATATCCCCGCTTTCTGATTAG

Xenopus        CTTGGTAGATCATCGACCAAGGGAGGCTTCAAAGGGAGTCCTATCGACCGCGAN-CAG-GCAG-------
Silurana       CTTGGTAGATCATCGACCAAGGGAGGCTTCAAAGGGAGTCCTAT-GACCGCGACGCAGAGCAG-------
Hymenochirus   CTTGGTAGATCATCGACCAAGGGAGGCTTCAAAGGGAGTCCTATCGACCGCGAG-CAGCGTTGGGCTCTT
Spea           CTTGGTAGATCATCGACCAAGGGAGGCTTCAAAGGGAGTCCTATCGACCGCGAGAGAG-GNAGG------

                                                   1400
                                                     |
Xenopus        --------CGTCAAAATAGGCCATTTCGCTTACTAATCTCCAGAACCCCG
Silurana       --------CGTCAAAATAGGCCATTTCGCTTACTAATCTCCAGAACCCCG
Hymenochirus   TGGGAGCTCGTCAAAATAGACCATTTCGCTTACTAATCTCCAGAACCCCG
Spea           --------CGTCAAAATAGGCCATTTCGCTTACTAATCTCCAGAACCCCG

Xenopus        GCTTTAGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGA-GCGACCGAACCTCGGCC
Silurana       GCTTT-GCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGA-GCGACCGAACCTCGGCC
Hymenochirus   GCTTT-GCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGA-GCGACCGCACCTCGGCC
Spea           GCTTT-GCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGACGCGACCGGACCTCCGGC

                                   1500
                                     |
Xenopus        CCGCACCTTACGCNNCGTGCGGTATCACCCG-GTGAAAACCATTCGTCT-TGACCGCGACGCCCTACTTG
Silurana       CCGCACCTTACGCTC-GTGCGG-ATCACCCG-GTGAAAACCATTCGTCT-TGACCGCGACGCCCTACTTG
Hymenochirus   CCGCACCTTACGCTC--TGCGG-ATCACCCG-GCGAAAACCATTCGTCT-TGACCGCGACGCCCTACTTG
Spea           CCGTACCTTACGCTC---GCGG-ATCACACGTGTGAAA-CCATTCGTCTGTGACCGCGACGCCCTACTTG

                                                                         1600
                                                                           |
Xenopus        GCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTC-TTTTCCAC-AACCAACTATATC
Silurana       GCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTC-TTTTCCAC-AACCAACTATATC
Hymenochirus   GCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCCTTTTCCAC-AACCAACTATATC
Spea           GCTTGCGGCCCAATTCCNCGGGCTACGGCTGCGAGTAGTCTGGGGTC-TTTTCCACCAACCAACTATATC

Xenopus        TGTCGTCCTGCCACCGGTACCTTCAGC
Silurana       TGTCGTCCTGCCACCGGTACCTTCAGC
Hymenochirus   TGTCGTCCTGCCACCGGTACCTTCAAC
Spea           TGTCGTCCTGCCACCGGTACCTTCAGC

             1836                                                              1900
               |                                                                 |
Xenopus        CACGTCTAGAACCACCATCATCGTTTATAAGTTTGCTCTTGAAACTTCCGGCTTCACCTCTTCCCAAGGT
Silurana       CACGTCTAGAACCACCATCATCGTTTATAAGTTTGCTCTTGAAACTTCCGGCTTCACCTCTTCCCAAGGT
Hymenochirus   CACGTCTAGAACCACCATCATCGTTTATAAGTTTGCTCTTGAAACTTCCGG-TTCACCTCTTCCCAAGGT
Spea           CACGTCTAGAACCACCATCATCGTTTATAAGTTTGCTCTTGAAACTTCCGGCTTCACCTCTTCCCAAGGT
```

FIGURE 2. Continued.

our conclusions regarding the monophyly of *Silurana* + *Xenopus* were not changed. Given this relationship, we define Xenopodinae as the node-based name for the most recent common ancestor of living *Xenopus* and *Silurana*, and all of its descendants. This usage differs from that of Cannatella and Trueb (1988a), who did not de-

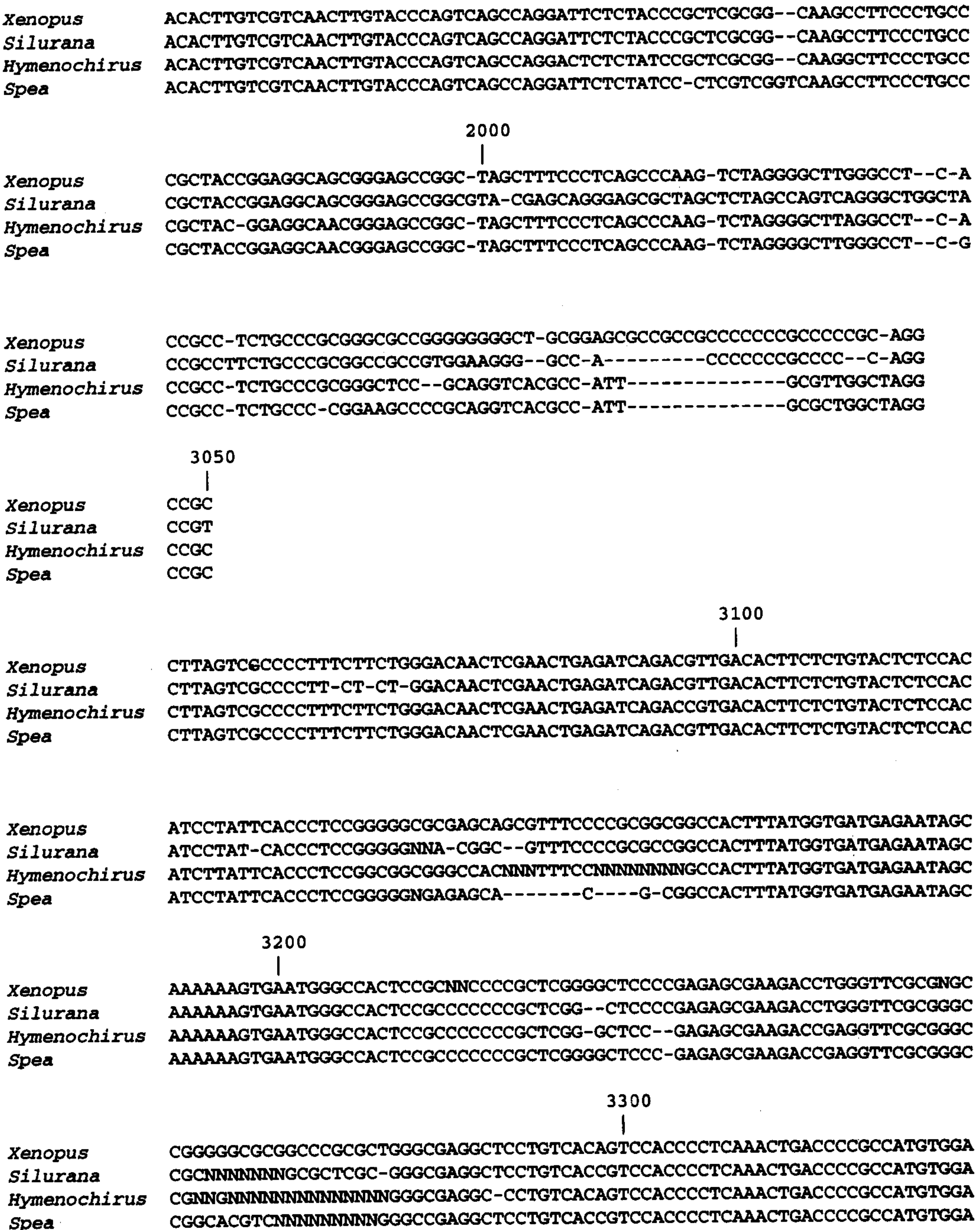

FIGURE 2. Continued.

fine Xenopodinae formally and included only *Xenopus* within the group.

Microcomplement fixation studies by Bisbee et al. (1977) used one-way distances (no reciprocal comparisons were made) of taxa to *Xenopus laevis laevis* and showed differences of 0–19 IDUs (immunological distance units) among species of *Xenopus*, 57 IDUs to *Silurana tropicalis*, and 180 and >200 IDUs to *Hymenochirus* sp. and *Pipa pipa*, re-

```
Xenopus        CAGTTTGGCATTGCGTCCACAGGATTCCGCTC-GAGTCCGCTCGATGTCTTTGGAGGGCACCTCGTCTTC
Silurana       CAGTTTG-CATTGCGTCCACAGGATTCCGCTC-GAGTGCCCTCC-TGTCTTTGGAGGGCA-CTCGTCTTC
Hymenochirus   CAGTT-GGCATTGCGTCCACAGGATTCCG-TCTGAGTCC--TCC-TGTCTTTGGAGGGCACCTCGTCTTC
Spea           CAGTTTGGCATTGCGTCCACAG-ATTCCGCTC-GAGTCC-CTCC-TGTCTTTGGA-GGCACCTCGTCTTC

              3400
               |
Xenopus        CCGTTTTC-GAGCGAACTAGAACTAAAAGTCATACTTATGTCTGGCACTTTGCGCCCNCGGAGTGCTAGG
Silurana       CNNTTTTC-GAGCGAACTAGAACTAAAAGTCATACTTATGTCTGGCACTTT-CGCCC-CGGAGTGCTAGG
Hymenochirus   CCGTTTTCTGAGCGAACTAGAACTAAAAGTCATACTTATGTCTGGCACTTT-CGCCCTCGGAGTGCTAGG
Spea           CCGTTTTC-GAGCGAACTAGAACTAAAAGTCATACTTATGTTTGGCACTTTTGGCCC--GGAGTGCTAGG

                                                    3500
                                                     |
Xenopus        AAGACTGAAAAACCCAAAATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGGCC
Silurana       AAGACTGAAAAACCCAAAATTCGTC-T--A-AGTTTTTT-----GTGTCCCTATTGACCGAACACCG-CC
Hymenochirus   AAGACTGAAAAACCCAAAATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCG-CC
Spea           AAGAATGCAAAACCCAAAATTTGTCCTTCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCG-CC

                        3550
                          |
Xenopus        GGTTCGCAAGTATCG          011111000101
Silurana       GGTTCGCAAGTATCG          100000111001
Hymenochirus   GGTTCGCAAGTATCG          100111101010
Spea           GGTTCGCAAGTATCG          011000010110
```

FIGURE 2. Continued.

spectively. Their tree placed *Silurana* closer to *Xenopus* than to *Hymenochirus*, but that relationship depends on midpoint rooting of the dendrogram; the unrooted tree is consistent with either arrangement.

The monophyly of the named pipid taxa above the species rank is well corroborated by the morphological data (Fig. 1). Wagler (1830) believed pipids to be the most primitive frogs and recognized the suborders Aglossa for pipids and Phaneroglossa for all other frogs. Cope (1865) argued that the dentate (*Xenopus*) and edentate (*Pipa*) members of Aglossa were independently evolved from dentate and edentate ancestors, and that Aglossa was polyphyletic. Since Boulenger's (1896) description of *Hymenochirus*, however, there has been no serious doubt that living pipids are monophyletic.

Cannatella and Trueb (1988a) reported 30 unique synapomorphies for Pipidae, none of which exhibited homoplasy. They also reported 6 unique, nonhomoplastic synapomorphies for *Xenopus*, 2 for *Silurana*, 21 for Pipinae, and 14 for *Pipa*. Twelve unique, nonhomoplastic synapomorphies unite *Pseudhymenochirus* and *Hymenochirus*, and four unite the species of *Hymenochirus* (Cannatella and Trueb, 1988b). Only the relationship of *Silurana* to other pipids is unresolved by the morphological data, and here the DNA sequence data provide clear support.

Relationship of Pipidae to Other Anura

Pipidae is part of a larger clade, Pipoidea, formally defined by Ford and Cannatella (1993). There is abundant evidence that Pipidae and *Rhinophrynus* are sister groups among living taxa (Sokol, 1975; Cannatella, 1985). However, Maxson and Daugherty (1980) found distances of ≈170 IDUs between *Rhinophrynus* and *Ascaphus truei* but >200 IDUs between *Rhinophrynus* and *Xenopus laevis*, suggesting that Pipoidea was not monophyletic. Hedges and Maxson (1993) found Discoglossidae + Pipidae to be a clade, with Rhinophrynidae as the sister group to that clade, based on a neighbor-joining analysis of mitochondrial 12S ribosomal RNA (rRNA) sequences.

In addition to Pipidae and *Rhinophrynus*, Pipoidea includes the exclusively fossil taxon †Palaeobatrachidae, which ranges

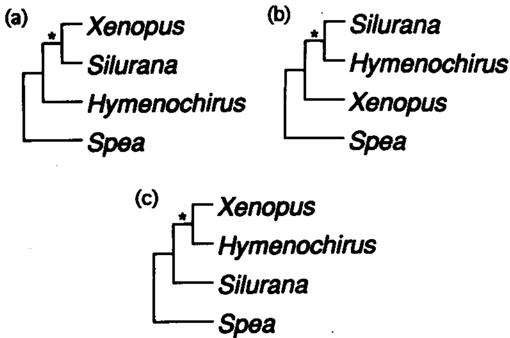

FIGURE 3. The three possible topologies for relationships among *Xenopus, Silurana,* and *Hymenochirus.* The asterisk indicates the internal branch whose length is given in Table 1.

from the Upper Jurassic through the Pliocene of Europe and the Upper Cretaceous of North America (Špinar, 1972; Estes and Sanchíz, 1982; Duellman and Trueb, 1986). Rhinophrynidae includes the extant taxon *Rhinophrynus dorsalis* (also known from late Pleistocene; Holman, 1969) and a fossil species, †*R. canadensis* (Lower Oligocene; Holman, 1963), as well as two extinct genera, †*Eorhinophrynus* (late Paleocene to middle Eocene; Hecht, 1959; Estes, 1975b) and †*Chelomophrynus* (middle Eocene; Henrici, 1991). Relationships among Pipidae, Rhinophrynidae, and †Palaeobatrachidae have been equivocal. Estes and Reig (1973) listed features shared by Pipidae and †Palaeobatrachidae and concluded that they were closest relatives. Špinar (1972) considered †Palaeobatrachidae to be most closely related to the "modern" frogs (=Neobatrachia), largely because procoelous vertebrae are shared by both groups. Lynch (1973) presented Pipidae, Rhinophrynidae, and †Palaeobatrachidae as a trichotomy. Duellman (1975) placed †Palaeobatrachidae as the sister group to Rhinophrynidae but provided no data to support the arrangement. Cannatella (1985, 1986) demonstrated that †Palaeobatrachidae and Pipidae are sister groups and that Rhinophrynidae is the sister group of these (Fig. 1, Appendix).

Relationships of Pipoidea to Other Anura

Pipoids have bizarre tadpoles that lack the keratinous beaks and denticles that most other tadpoles normally use for grazing the substrate and that have paired spiracles rather than a single opening. Combinations of these features were used by Orton (1953, 1957) to recognize four groups, of which pipoid tadpoles were Type I. Starrett (1968) further defined and proposed names for Orton's groups and later (1973) changed some of the names and formalized her views on their evolutionary relationships. Orton's Type I became Xenoanura and included Pipidae and Rhinophryni-

TABLE 1. Summary statistics for the phylogenetic analyses of *Xenopus, Silurana,* and *Hymenochirus* from which the three topologies in Figure 3 were derived. Tree A is the shortest tree in all of the four analyses. Transitions were excluded by setting state A = G and state C = T using an EQUATE statement in the FORMAT paragraph in the PAUP input file. This approach was computationally faster than a user-defined step matrix that weights transitions and transversions differently.

	Tree A[a]				Tree B				Tree C			
	L	BL	CI	RI	L	BL	CI	RI	L	BL	CI	RI
Gaps omitted												
Transitions and transversions	57	25	0.72	0.61	69	13	0.59	0.32	79	3	0.52	0.07
Transversions only	54	24	0.72	0.62	65	13	0.66	0.33	76	2	0.51	0.05
Gaps included												
Transitions and transversions	100	38	0.69	0.55	118	20	0.59	0.29	127	11	0.54	0.16
Transversions only	103	37	0.68	0.53	119	21	0.59	0.30	128	12	0.55	0.17

[a] L = tree length; BL = number of unambiguous synapomorphies on the internal branch (marked by an asterisk on Fig. 3); CI = consistency index; RI = retention index.

dae. The name Scoptanura was applied to Type II larvae and comprised Microhylidae and Phrynomeridae. Leiopelmatidae and Discoglossidae (Type III) were placed in Lemmanura. All other families (Type IV) were placed in Acosmanura. Starrett (1973) considered the xenoanuran larva to be most primitive because it possessed the same characters as her hypothetical primitive tadpole. The scoptanuran type was considered slightly more advanced, and of the two beaked groups, Lemmanura was not as advanced as Acosmanura.

In contrast to Starrett, Sokol (1975, 1977a, 1977b) advanced arguments based on larval characters that pipoid frogs (those with Type I larvae) are derived in most larval characters. He supported the monophyly of Pipoidea and placed it as the sister group to all frogs above the level of the discoglossoids (Leiopelmatidae and Discoglossidae), an arrangement similar to those based on adult characters (Kluge and Farris, 1969; Lynch, 1973).

Most recent workers have regarded the sister group of Pipoidea to be Pelobatoidea + Neobatrachia. However, Cannatella (1985) placed Pelobatoidea as the sister group to Pipoidea in the clade Mesobatrachia, and Mesobatrachia in turn was the sister group to Neobatrachia. This arrangement was not supported in Hillis's (1991) bootstrap analysis of Cannatella's modified data. Hillis et al. (1993) supported the monophyly of Mesobatrachia based on nucleotide sequences from 28S rDNA alone and from a combined analysis of morphological data and nucleotide sequences.

Historical Biogeography

The biogeography of pipids has influenced interpretations of their relationships. Noble (1931) and Dunn (1948) considered that the African species and the American species each formed a distinct lineage. Cracraft (1974) considered Pipidae to be a Gondwanaland faunal element and suggested that the separation of Pipidae from †Palaeobatrachidae may have been correlated with the breakup of Pangaea. This suggestion was supported by Cannatella (1986). Savage (1973) implied that the diversification of pipids in Africa and South America occurred after the split up of the two continents. However, the following synthesis indicates that pipids and fossil taxa of Pipimorpha were well diversified before the separation of the African and South American continental plates in the middle to late Cretaeous (90–100 million years ago [MYA]).

Estes (1975a) placed the Eocene fossil †*Shelania pascuali* from Argentina into *Xenopus*. It differs from †*X. romeri* (see below) but shares with living *Xenopus* the plesiomorphy of unfused first and second vertebrae. Acceptance of Estes' allocation of this taxon, along with the analysis of living taxa, indicates that there are two terminal pairs of sister taxa with transatlantic relations: *Hymenochirus* + *Pipa*, and *Xenopus* + †*X. pascuali.* If the divergence of either of these two pairs occurred after the separation of Africa and South America, then the present distribution would require dispersal across open ocean. Assuming dispersal was not the case, then there were at least two lineages of pipids present in both Africa and South America prior to their separation.

The phylogeny of living taxa indicates that if the divergence of the respective ancestors of Hymenochirini and *Pipa* was prior to or coincident with the breakup of Africa and South America, then the separation of the *Silurana* + *Xenopus* clade (Xenopodinae) from the ancestor of Hymenochirini + *Pipa* (Pipinae) also must have occurred prior to the continental breakup. Thus, a third African lineage, *Silurana*, was present before the breakup (Fig. 4).

Estes (1975a, 1975c) stated that the fossil †*X. romeri* from the Paleocene of Brazil is closely related to *Xenopus* (=*Silurana*) *tropicalis.* Although some of the similarities shared by these two taxa are plesiomorphic, at least one derived feature, the fusion of the first two vertebrae, allies †*X. romeri* with *Silurana.* But this derived feature is also shared with Hymenochirini and *Pipa.* Without more data, we are unable to determine whether †*X. romeri* is closer to *Silu-*

rana or to Pipinae (Fig. 4); in any case, there are no data that suggest that it is the closest relative of *Xenopus*, and we refer to it as †"*Xenopus*" *romeri*. Regardless of its eventual placement on the tree, it represents a third South American lineage present prior to the splitting of the continents. The incorporation of data from Báez's (1981) cladogram indicates that †*Eoxenopoides* and †*Saltenia* were present in Africa and South America, respectively, before the continental division.

Thus, at least four pipimorph lineages were present in both the African (*Xenopus*, *Silurana*, Hymenochirini, and †*Eoxenopoides*) and South American (*Pipa*, †*Xenopus pascuali*, †"*Xenopus*" *romeri*, and †*Saltenia*) land masses at the time of their separation (Fig. 4).

Certain conclusions from this synthesis are contradicted by other evidence. According to Bisbee et al. (1977), *Xenopus* separated from *Silurana* 30–40 MYA, assuming a molecular clock and its correct calibration. However, the split between †*X. pascuali* and (at least some) living *Xenopus* must have occurred at least 90–100 MYA, assuming that it preceded the breakup of Africa and South America. If one accepts these conclusions based on the molecular clock, then the cladogram must be incorrect, or vice versa.

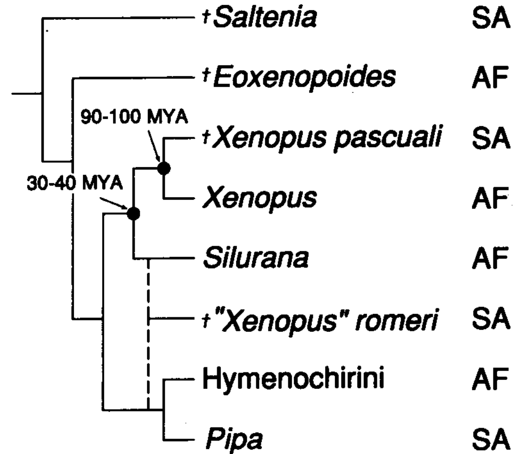

FIGURE 4. Phylogeny of living and some extinct pipimorph taxa as derived from Estes (1975a), Báez (1981), Cannatella and Trueb (1988a, 1988b), and this paper. The estimate of 30–40 MYA for the divergence of *Xenopus* and *Silurana* is from Bisbee et al. (1977). SA = South America; AF = Africa. The uncertain placement of †"*Xenopus*" *romeri* is indicated by dashed lines.

MORPHOLOGY AND DEVELOPMENT

Early Development

Xenopus is commonly used in developmental studies of mesoderm, neural crest (using *borealis/laevis* chimeras; Krotoski et al., 1988), notochord (Keller, 1984, 1986), somites (Youn et al., 1980), somitomeres (Jacobson, 1993), and limbs (Holmgren, 1933; de Saint-Aubain, 1981; Alberch and Gale, 1983, 1985). Several developmental mutants were described by Krotoski et al. (1985) and Droin and Buscaglia (1978); these are potentially useful for the study of the genic control of morphogenesis. There are excellent references for features of ontogeny (Nieuwkoop and Faber, 1967) and morphogenesis (Fox, 1984), but the potential contribution of *Xenopus* to evolutionary developmental studies remains largely untapped.

Nieuwkoop and Sutasurya (1976) claimed that the observed difference in mesoderm formation in *X. laevis* and salamanders supports a polyphyletic origin of Amphibia. In *Xenopus*, the mesoderm is derived only from the deep region, whereas in salamanders (*Triturus*, *Pleurodeles*, and *Ambystoma*) it is derived in part from more superficial cells. However, older studies of *Discoglossus* and *Bombina* (cited in Hanken, 1986) indicated that the mesoderm was derived from superficial cells. Although Nieuwkoop and Sutasurya (1976) dismissed the older studies as being in error, they did so primarily because they expected to see no variation in morphogenetic pattern in anurans.

Anatomy and Heterochrony

With the exception of *Rana esculenta* (Gaupp, 1896–1904), *Xenopus laevis* is the best studied frog in terms of its anatomy. Aspects of the skull were investigated by Ridewood (1897), Paterson (1939a, 1939b, 1949, 1960), Smit (1953), Reumer (1985), and Nikitin (1986); hyoid and larynx by

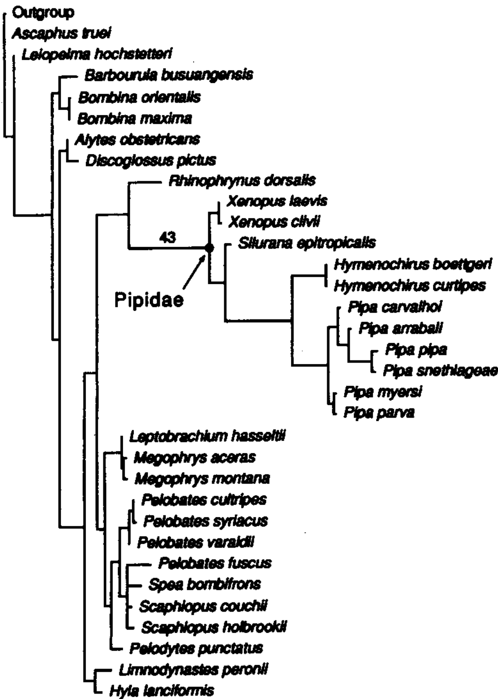

FIGURE 5. Phylogram of Anura derived from morphological data indicating morphological anagenesis in pipids; slightly modified from Cannatella (1985). The branch subtending Pipidae has a length of 43; other branch lengths are proportional to the number of apomorphies as determined using ACCTRAN optimization. The total tree length is 352, with a consistency index of 0.665 and retention index of 0.898.

Ridewood (1897), Blume (1932), Loumont (1981), and Cannatella and Trueb (1988a); teeth by Katow (1979) and Shaw (1979, 1985, 1986); limbs and girdles by Jungersen (1891), de Villiers (1924, 1925, 1929), van Pletzen (1953), Palmer (1960), and Fabrezi (1992); vascular anatomy by Millard (1941); and muscles by Beddard (1896), Grobbelaar (1924), Sedra and Michael (1957), and Cannatella and Trueb (1988a). Larval morphology was examined by Edgeworth (1930), Kotthaus (1933), Weisz (1945a, 1945b), Witschi (1950), Ryke (1953), Nieuwkoop and Faber (1967), Gradwell (1971, 1975), Starrett (1973), Sokol (1975, 1977a), Wassersug and Rosenberg (1979), Vigny (1979b), Viertel (1987), and Trueb and Hanken (1993), among others.

In almost every aspect of their morphology, pipoids are highly derived. In the phylogram (Fig. 5) generated from the morphological data in Cannatella (1985), most of the longest branch lengths are found within Pipoidea. Many states initially coded as plesiomorphies were discovered a posteriori to be reversals; pipoids possess very few truly plesiomorphic states as compared with the outgroup. Many of these reversals are associated with phylogenetic shifts in ontogeny. The distinctiveness of ontogenetic shifts in *Xenopus* has been appreciated by several workers: skull and visceral arch muscles by Sedra and Michael (1957) and Brown (1980), vertebral column by Smit (1953), and overall ossification patterns by Bernasconi (1951) and Trueb and Hanken (1993).

In *Xenopus* and other pipoids, nearly all of the skull bones appear prior to the completion of metamorphosis, whereas in most other frogs several of the skull bones appear after metamorphosis (Trueb, 1985; Trueb and Hanken, 1993). This may be because metamorphosis in pipoids is not as abrupt as in other frogs (Wassersug and Hoff, 1982), and therefore less remodeling of the osteocranium is required in pipoids. With respect to metamorphosis, the timing of appearance of cranial ossifications is accelerated. Acceleration of development is also reflected in the fusion of the frontoparietals to each other, medial fusion of the paired vomers (in *Xenopus*), fusion of the parasphenoid to the sphenethmoid, expansion of the medial ramus of the pterygoid, and the elongate columella (Cannatella and Trueb, 1988a). Thus, pipids have several characters that can be interpreted as peramorphic; i.e., the general process by which the ontogeny of structures in a descendant has proceeded further than that of its ancestor or outgroups (Gould, 1977; Alberch et al., 1979; Fink, 1982). However, paedomorphosis (the retardation or truncation of development in the descendant) exists also: mentomeckelians, quadratojugals, and vomers (except in *Xenopus*) do not develop. Lateral line organs of the larvae are retained in the adult, and eyelids do not develop; these are paedomorphic traits. Moreover, analysis of the data in Canna-

tella (1985) indicates that many of the synapomorphies of pipoid lineages are paedomorphic reversals.

However, nonterminal changes in pipoid ontogeny are also influential, as in some other groups (see Mabee, 1993). The ontogeny of salamanders is relatively gradual from the early larva (Fig. 6, state A) to metamorphosis (state B). Primitively in frogs and in most of the major lineages of frogs, metamorphosis is abrupt (Wassersug and Hoff, 1982). The ancestral tadpole of Anura deviated from a more gradual ancestral amphibian ontogeny via caenogenesis; i.e., tadpoles have larval specializations (nonterminal additions; state Z) in the form of keratinous beaks and denticles and less obvious features such as a highly modified palatoquadrate. These are perhaps an adaptation for exploring primary production in a fluctuating environment (Slade and Wassersug, 1975). The cranium of beaked tadpoles is extensively and abruptly remodeled when these larval specializations are lost during metamorphosis (Orton, 1957). In pipoids, the remodeling is not nearly as extensive, and metamorphosis is more gradual (Wassersug and Hoff, 1982), probably because most pipoids remain aquatic as adults. Ossification begins earlier in pipids, and the prolonged development yields peramorphic features. In abandoning the beaks, denticles, and associated musculoskeletal modifications, pipoids have converged on the more plesiomorphic ontogeny of salamanders by nonterminal deletion, although the extreme degree of filter feeding in Pipoidea (hymenochirines have become carnivorous) is derived in the context of Anura.

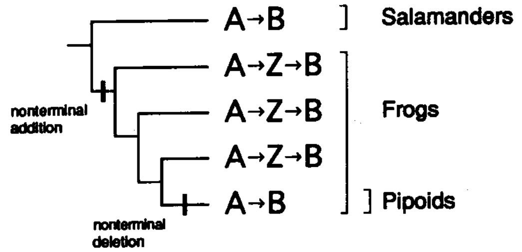

FIGURE 6. Caenogenesis (nonterminal addition) in the ontogeny of frog tadpoles as compared with that of salamander larvae and pipoid tadpoles. State A represents early ontogeny, B is metamorphosis, and Z is the suite of caenogenetic larval characters, such as keratinized beaks and denticles.

Functional Morphology, Neurobiology, and Behavior

Xenopus laevis larvae have been used as models in studies of aquatic locomotion and filter feeding; however, they differ substantially from *Rana* tadpoles in aspects of behavior, kinematics, and neurobiology (Gradwell, 1971, 1975; Hoff and Wassersug, 1986; Nishikawa and Wassersug, 1988, 1989; Wassersug, 1989). Llinás and Precht (1976) listed several hundred references for the neurobiology of *Xenopus*. Also, *Xenopus* larvae are used increasingly for studies of developmental neurobiology at morphological and molecular levels (Nikundiwe and Nieuwenhuys, 1983; van Mier et al., 1985; Nordlander, 1986).

The constraints of an aquatic existence likely account for many of the modifications seen in adult *Xenopus*. Sound production in *Xenopus* (and probably other pipids [Rabb, 1960]) and the structure of the hyoid and larynx (Ridewood, 1897) is radically different from that of other frogs in that there are no vocal cords and a moving air column is not used. Rather, rapid separation of adhering portions of the arytenoid cartilages from each other produces a clicking sound by implosion (Yager, 1992). Vigny (1979a) compared the vocalizations of several taxa, and a vinyl record of the calls of *X. laevis*, *X. muelleri*, and *X. gilli* was included with the book by Passmore and Carruthers (1979). Male larynges are much larger than those of females (Sassoon and Kelly, 1986; Yager, 1992). Aspects of underwater hearing were studied by Christensen-Dalsgaard et al. (1990).

Lacking a tongue and being aquatic, *Xenopus* apparently feed by inertial suction, assisted by forelimb movements that guide prey into the mouth, but the detailed kinematics and functional morphology of feeding (reviewed by Lauder, 1985, and Lauder and Shaffer, 1993) in this secondarily

TABLE 2. Chromosome numbers for taxa of pipoids. References are given in the text.

Taxon	Chromosome number	Ploidy
Xenopus fraseri	36	4n
X. borealis	36	4n
X. clivii	36	4n
X. gilli	36	4n
X. laevis	36	4n
X. muelleri	36	4n
X. amieti	72	8n
X. andrei	72	8n
X. boumbaensis	72	8n
X. vestitus	72	8n
X. wittei	72	8n
X. ruwenzoriensis	108	12n
Silurana tropicalis	20	2n
S. epitropicalis	40	4n
Hymenochirus boettgeri	24	2n
Pipa carvalhoi	20	2n
P. parva	30	? 2n
P. pipa	22	2n
Rhinophrynus dorsalis	22	2n

aquatic tetrapod have not been studied. Sokol (1969) described feeding in *Hymenochirus*. Palmer (1960), Emerson (1979, 1982), Emerson and de Jongh (1980), and Videler and Jorna (1985) called attention to the sliding nature of the joint between the pelvic girdle and vertebral column, which results in changes of body length of about 15% during swimming.

Studies of the behavior of *Xenopus* are few (Deuchar, 1975). The reproductive behavior of *X. laevis* was investigated by Russel (1954, 1960), and phylogenetic aspects of amplectant behavior were discussed by Duellman (1985). Components of feeding behavior were studied by Avila and Frye (1977, 1978). Some aspects of larval social behavior were examined by Wassersug and Hessler (1971) and Wassersug and Feder (1983).

PARASITES

Tinsley (1981a) discussed interactions between sympatric *Xenopus* and *Silurana* and their parasites, pointing out associations between ploidy level in *Xenopus* and the host specificity of parasites such as *Protopolystoma* (monogenean), *Cephalochlamys* (tapeworm), and *Chitwoodchabaudia* (nematode). Tinsley (1981b) also considered the known parasite fauna of *X. laevis* to be highly distinctive and interpreted this as support for the placement of pipids as primitive anurans.

CHROMOSOMAL AND GENOME STUDIES

Polyploidy is rampant in Xenopodinae (*Xenopus* and *Silurana*). Polyploidy has been reported among other anuran families (Bogart and Wasserman, 1972; Bogart and Tandy, 1976), but nowhere else are the increases of ploidy levels as drastic as in *Xenopus* and *Silurana*. The only known truly diploid xenopodine species is *S. tropicalis*, with a chromosome complement of 2n = 20 (Tymowska, 1973). Its chromosome morphology, including constriction-bearing positions, is strikingly different from that of *Xenopus* (Tymowska and Fischberg, 1973).

The chromosome number of *X. laevis* was reported by Wickbom (1945) as 2n = 36; other karyotype characteristics were determined by Mikamo and Witschi (1966), and the lampbrush chromosomes were described by Müller (1974). Subsequently, *X. laevis* has served as the standard to compare and contrast other *Xenopus* karyotypes (Tymowska and Kobel, 1972; Kobel and Du Pasquier, 1986).

There is disagreement in the terminology of the ploidy levels that are applied to xenopodines. *Xenopus laevis* and other 36-chromosome species have generally been considered diploids (e.g., Duellman and Trueb, 1986) based on karyotype morphology, the formation of only bivalents during meiosis, and gene expression. However, the group of 36-chromosome species clearly has undergone genome duplication (Bisbee et al., 1977) and doubling of DNA content relative to *Silurana tropicalis* and is best considered a tetraploid. Virtually all xenopodine species have been karyologically examined, and chromosome numbers range from 20 to 108 (Table 2). Most species are tetraploids, but there are five octoploids, one dodecaploid (*X. ruwenzoriensis* [Kobel et al., 1980]), and undescribed tetraploid, octoploid, and dodecaploid species (Tymowska, 1991).

The second species of *Silurana*, *S. epitropicalis*, has 2n = 40 (Tymowska and Fisch-

berg, 1982) and is tetraploid with respect to *S. tropicalis.* Although these two species differ in chromosome number and length, number of secondary positions, and distribution of heterochromatin, they both have a nucleolus organizing region (NOR) on chromosome 5 as well as similar C-banding patterns (one chromosome complement of *S. epitropicalis* has a pattern identical to that of *S. tropicalis*). These data suggest that *S. epitropicalis* is an allopolyploid (Tymowska, 1991), that *S. tropicalis* is one of the parental species (the other parental species of *S. epitropicalis* has not yet been identified), and that the two species form a clade. The monophyly of *Silurana* is also supported by morphological data (Cannatella and Trueb, 1988a).

By accepting that *Silurana* is monophyletic and that it is the sister group of *Xenopus,* one can then conclude that (1) polyploidy arose independently within *Silurana,* (2) the origin of the entire *Xenopus* clade was by polyploidy, and (3) polyploidy occurred at least twice more within *Xenopus,* producing octoploid (2n = 72) and dodecaploid (2n = 108) species. Thus, within Xenopodinae there were at least four polyploidization events. In this respect, xenopodines are unique among amphibians.

The group has undergone some diploidization; all xenopodines possess only two NORs on a single chromosome pair, suggesting suppression of redundant genetic loci. However, the expression of duplicate globin, lactate dehydrogenase, and creatine kinase isozymes has been reported (Tymowska, 1991). Because almost all xenopodines are polyploids, bichemical and molecular studies on species other than *S. tropicalis* should consider functional or potentially functional paralogous genes.

The origin of polyploid *Xenopus* species is thought to be by allopolyploidy (King, 1990; Tymowska, 1991) because bivalents (instead of multivalents) are formed and segregate normally (Tymowska and Fischberg, 1973). Most other anuran polyploids consist of cryptic species pairs and are thought to be autopolyploids (Tymowska, 1991). Allopolyploidy may have been facilitated by multiple areas of sympatry among otherwise parapatric species that show weak ecological specialization, as suggested by Kobel (1981b). A possible mechanism for the origin of polyploid species via polyploid oocytes was discussed by Tymowska (1991).

The karyotypes of the tetraploid *Xenopus* species are uniform in number, size, and chromosome shape. However, the species can be identified by the type and position of secondary constriction (Tymowska, 1977). The karyotypes of *X. wittei* and *X. vestitus* are very similar to one another (Tymowska and Fischberg, 1980b). Also, the presence of a terminal secondary constriction on chromosome 12 of these octoploids suggests a partial common ancestry of these species with *X. laevis* (Tymowska et al., 1977). The karyotype of *X. ruwenzoriensis* was described as an allopolyploid of triple hybrid origin (Tymowska and Fischberg, 1980a). The two-step hybridization process among three tetraploid species that may have led to this dodecaploid (Fig. 7) is also supported by mitochondrial DNA (mtDNA) data (Carr et al., 1987).

Genome size for *X. laevis* was determined by Dawid (1965), and Thiébaud and Fischberg (1977), using cytofluorometric analysis, reported the nuclear DNA content of 12 *Xenopus* species. These results were corroborated by results from other techniques (Giorgi and Fischberg, 1982). *Silurana tropicalis* (2n = 20) has 3.55 pg/nucleus, a value equivalent to nonxenopodine pipid species (Olmo, 1973). The tetraploid species (2n = 36) have values between 6.35 and 8.45 pg, the octoploids (2n = 72) range from 9.02 to 12.83 pg, and *X. ruwenzoriensis* (2n = 108) has 16.25 pg (Tymowska, 1991).

Triploid and gynogenetic diploid *X. laevis* have been produced in the laboratory (Tompkins, 1978). Interspecific hybrids are commonly produced under laboratory conditions for studies of dominance and gene expression (e.g., Wall and Blackler, 1974; Müller, 1977; Kobel et al., 1979; Mohun et al., 1981; Reeder and Roan, 1984). Knoepffler (1967) reported the occurrence of natural hybrids between *X. fraseri* and *S. tropicalis.* However, subsequent experiments failed to produce viable laboratory

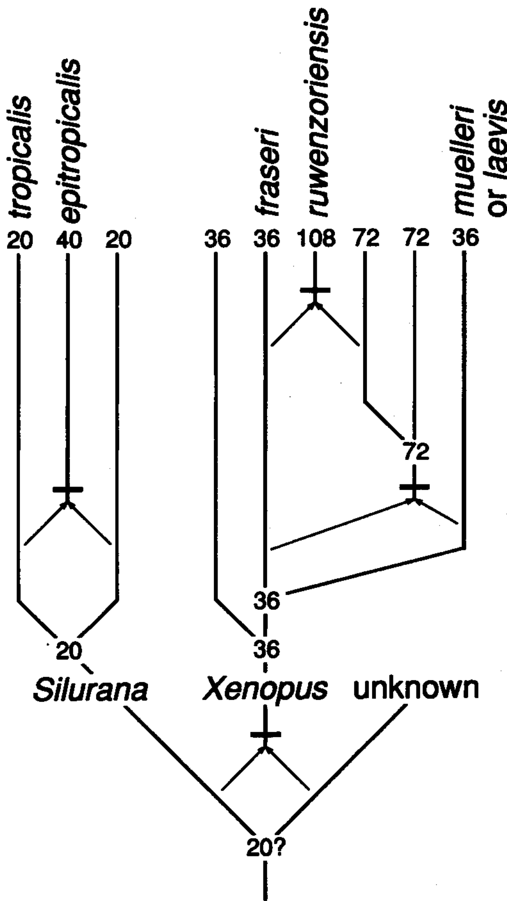

FIGURE 7. Scenario for the evolution of chromosome number in *Xenopus* and *Silurana* by allopolyploidy (Fischberg and Kobel, 1978) based on evidence presented in the text; modified from Carr et al. (1987). The chromosome numbers of other pipoids (Table 2) suggest that the ancestral number for Xenopodinae may have been 20. The arrows indicate the origin of the parental genomes in the polyploid descendant, and the horizontal bars mark the four hypothesized polyploidization events in the evolution of Xenopodinae. Evidence from albumin proteins discussed herein suggests that *Silurana* provided one parental genome to the ancestor of *Xenopus*, but the other parent is labeled as "unknown."

hybrids between *S. tropicalis* and any *Xenopus* species (Blackler, 1970; Tymowska and Fischberg, 1973; Vigny, 1977).

Sex-determining systems have been studied only in *X. laevis* among pipids and were determined by immunological and sex-reversal studies (Zaborski, 1979). The ZW female heterogamety in *Xenopus* and some other groups of frogs is plesiomorphic for Anura, whereas the XY mechanism has evolved independently at least four times in frogs (Hillis and Green, 1990).

MITOCHONDRIAL AND RIBOSOMAL DNA

Mitochondrial DNA

Mitochondrial DNA has become popular among molecular systematists, in part because of the advantageous structural characteristics and mode of maternal inheritance of this molecule (see reviews by Brown et al., 1982; Brown, 1985; Wilson et al., 1985; Moritz et al., 1987). The characteristics of *Xenopus laevis* mtDNA were first studied more than two decades ago (Dawid, 1965). These studies were followed by others on the expression of the coding genes and nucleotide sequences of different segments (Dawid, 1972a, 1972b). A restriction map identified 72 sites using 19 restriction enzymes (Cordonnier et al., 1982). Ramirez and Dawid (1978) compared the restriction site maps of *X. laevis* and *X. borealis* and concluded that the overall organization (then called the functional map) was highly conserved, whereas the primary sequences of these two species differed by approximately 25%. These authors located the mtDNA replication origin for the heavy strand at the displacement loop (D-loop), and this location has been confirmed in microscopy studies (Cordonnier et al., 1982). The D-loop of *Xenopus* has been intensively analyzed to understand the common underlying replication mechanism of animal mtDNA (Callen et al., 1983; Mignotte et al., 1983; Bogenhagen et al., 1985). The nucleotide sequences of the two origins of replication for *X. laevis* mtDNA (one corresponding to the initiation site for the heavy strand and the other one for the light strand) are known (Wong et al., 1983). The complete nucleotide sequence of the *Xenopus* mtDNA is now available (Roe et al., 1985) and encodes 22 transfer RNA, 2 rRNA, and 13 protein genes.

Carr et al. (1987) compared restriction site maps of mtDNA for nine taxa of *Xenopus* based on 30 phylogenetically informative sites derived from 11 restriction en-

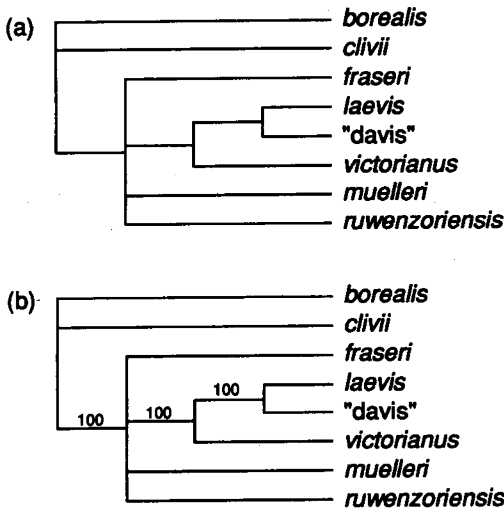

FIGURE 8. Consensus trees obtained from four most-parsimonious trees of 51 steps, consistency index = 0.588, retention index = 0.580, from the unweighted restriction site data of Carr et al. (1987). The name "davis" refers to a laboratory population of *Xenopus laevis* of unknown origin. (a) Strict consensus tree. (b) Semistrict and majority-rule consensus tree.

zymes. Their four shortest, undirected trees suggested that the dodecaploid species (*X. ruwenzoriensis*) represents an allopolyploid between *X. fraseri* and an *X. laevis*-like species (either *X. laevis* or *X. muelleri*). Using PAUP 3.0s (branch-and-bound search, ACCTRAN optimization), we found the same four trees, for which consensus summaries are presented (Fig. 8). Carr et al. (1987) supported Fischberg and Kobel's (1978) hypothesis that the dodecaploid *X. ruwenzoriensis* is an allopolyploid and that the contribution of the cytoplasmic genome was from *X. muelleri* or *X. laevis* and the paternal genome from *X. fraseri*. The multiple doses of the *fraseri* genome account for the morphological similarity of *X. ruwenzoriensis* to *X. fraseri*.

In an attempt to increase the resolution of the consensus tree obtained from Carr's data, we weighted the data so that the loss of a restriction site was five times more likely than the gain of that site. Two most-parsimonious trees of 76 steps were found, which differ only in the arrangement of the *X. laevis* subspecies (Fig. 9a). All of the changes were losses of restriction sites; no

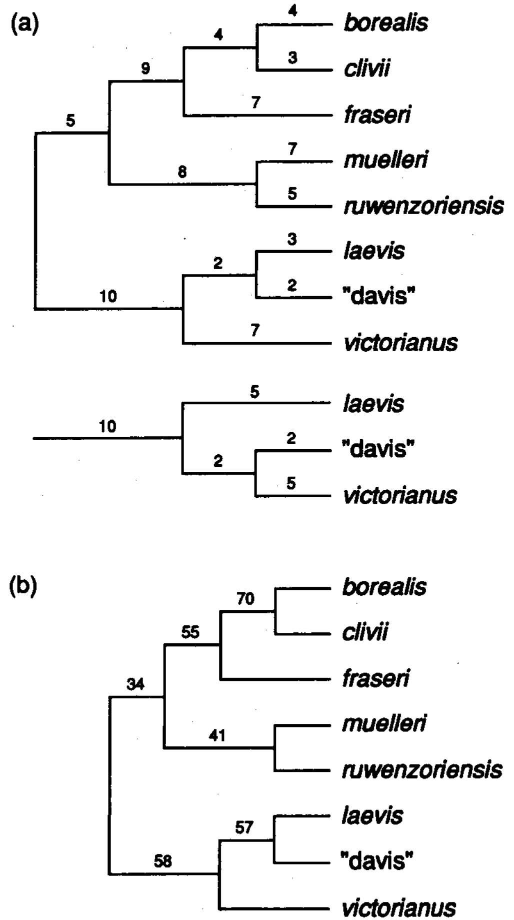

FIGURE 9. (a) Two equally parsimonious undirected trees found using the restriction site data of Carr et al. (1987) with losses weighted as five times as likely as gains. The numbers are branch lengths; all state changes were losses. Weighting losses as only twice as likely as gains yielded one most-parsimonious tree identical to the first topology except that *muelleri* clusters with the *fraseri–clivii–borealis* clade. Tree length = 76, consistency index = 0.395, retention index = 0.610. (b) Majority-rule consensus tree of the weighted data set, showing other compatible clusters of <50%. The values above the branch are the percentages of 1,000 replicates in which a particular clade was found. Because all of the state changes were site losses, the bootstrap analysis was run using the irreversible character type of PAUP; this increased computational efficiency by more than 100-fold.

gains occurred. Through repetition with various weights, we discovered that weighting losses as from 3 to 10 times more likely gave identical results. When losses were weighted as only twice as likely as

gains, we obtained one tree of 70 steps (both gains and losses) that differed from Figure 9a only in that *muelleri* clustered with the *fraseri* + *clivii* + *borealis* clade. Both trees support the hypothesis of Carr et al. (1987) in that *fraseri* and the *ruwenzoriensis* + *muelleri* clade are adjacent to each other. However, we caution against overinterpretation of these results. Because Carr et al. (1987) used no outgroup, the dendrograms are undirected trees; also, the bootstrap values (1,000 replications) for most of the clades from the weighted data set are not very large (Fig. 9b).

Ribosomal DNA

Two structural components of ribosomes have been studied: ribosomal proteins (r-proteins) and rRNA genes. Little is known about the r-proteins, but ribosomes of *Xenopus laevis* may consist of up to 70 r-proteins (Bozzoni et al., 1981). New r-proteins continue to be discovered, isolated, and characterized in *X. laevis* (Loreni et al., 1992). Also, the r-proteins of this species have served as a model to study translational control as a mechanism of gene regulation in eukaryotic cells (Pierandrei-Amaldi et al., 1982, 1985; Preugschat and Wold, 1988).

However, information on rRNA genes is abundant. Among nuclear genes, the organization, characteristics, and evolution of rDNA sequences are well known (Gerbi, 1985; Mindell and Honeycutt, 1990). The unique characteristics of the rDNA sequences have made them a commonly used molecule to study phylogenetic relationships across a considerable period of evolutionary time (Hillis and Davis, 1987; Larson, 1991). However, de Sá and Hillis (1990), Hillis et al. (1993), and the present study are the only ones in which rDNA sequences have been used in phylogenetic analyses of pipids.

Eukaryotic rDNA consists of tandemly arranged repeated units that contain coding and noncoding regions. In *X. laevis*, the primary nucleotide sequences for the different segments of the repeat have been reported: 18S gene (Salim and Maden, 1981), 28S gene (Ware et al., 1983; Schnare and Gray, 1992), internal transcribed spacers (ITS-1 and ITS-2, Hall and Maden, 1980), external transcribed spacer (ETS, Maden et al., 1982), and the 5.8S gene (Boseley et al., 1978). The combined sequence data support a repeat length of approximately 8,628 np for the rDNA unit of *X. laevis.* Of these, 712 np correspond to the ETS region, 2,825 np to the 18S gene, 557 np and 262 np to ITS-1 and ITS-2, respectively, 162 np to the 5.8S gene, and 4,110 to the 28S gene. Length polymorphism of the rDNA repeats results from variation within the nontranscribed spacer region (Junakovic et al., 1978). The ETS and ITS regions are very rich in G + C content (>80%), whereas the three rRNA genes have lower G + C amounts (50–66%). Among the rRNA genes, the 28S gene has the highest content of G + C (66%). Within the 28S gene, the conserved regions have higher A + T content than do the more variable regions, which show a G + C composition of up to 83%. Secondary structure models for the 18S and 28S rRNA genes of *X. laevis* are available (Atmadja and Brimacombe, 1984; Clark et al., 1984). The *X. laevis* model is commonly used when comparing the extent of secondary structure variation among rRNA sequences among organisms (Michot and Bachellerie, 1987; Gutell and Fox, 1988; Gutell et al., 1990).

Few studies have focused on comparing *X. laevis* primary rDNA sequences. Furlong and Maden (1983) and Furlong et al. (1983) compared the spacer regions with those of other *Xenopus* species, whereas Cutruzzola et al. (1986) compared the conserved 28S sequences in *X. laevis* and *Silurana tropicalis.* Regulation of rRNA transcription has also been studied in *X. laevis* (Dawid and Wellauer, 1976; Sollner-Webb and Reeder, 1979; De Winter and Moss, 1986, 1987). The 5S somatic rRNA gene has been intensively studied in *X. laevis* (Brown et al., 1972; Brownlee et al., 1972; Brown and Sugimoto, 1974). These studies have identified interesting molecular characteristics of transcription and gene expression. However, comparative data from other *Xenopus* species are lacking, precluding assessment about how widespread these characteristics are within the genus and among anurans.

Comparative Genic Studies

In addition to mitochondrial and ribosomal genes, data are available on albumin evolution, sperm proteins, and lactate dehydrogenase (LDH) electrophoretic patterns. Bisbee et al. (1977) and Maxson and Daugherty (1980) conducted immunological studies. Graf and Fischberg (1986) reported the electrophoretic pattern and peptide map of serum albumins for 21 xenopodine species and subspecies. The diploid *Silurana tropicalis* has a single albumin of 68 kilodaltons (kDa), *X. laevis* and its subspecies have a phenotype with two albumins of 70 and 74 kDa, and the remaining species of *Xenopus* have one 70-kDa albumin. The 70-kDa estimates of Graf and Fischberg (1986) are possibly in error. Westley et al. (1981) obtained complementary DNA clones with sequences complementary to the albumins of *X. laevis* messenger RNAs (mRNAs) that code for 68 (rather than 70)- and 74-kDa albumins. Westley et al. demonstrated that the two albumins are translational products of two distinct mRNAs encoded by two closely related genes. The two mRNA sequences are mismatched by only 8%; the 74-kDa albumin is glycosylated (Westley and Weber, 1982). The entire albumin mRNA sequence for *X. laevis* was reported by Moskaitis et al. (1989).

Gene mapping techniques have been used to establish the chromosomal location of certain genes. Hybridization procedures have served to locate repeated DNA sequences such as rRNA genes (Pardue, 1973; Pardue et al., 1973). The location of NORs are known and are species specific (Tymowska and Kobel, 1972; Kobel, 1981b). Linkage group studies were used to identify the position of the genes for major histocompatibility and immunoglobin heavy chains (Du Pasquier and Kobel, 1979; Kobel and Du Pasquier, 1979). Based on characteristic linkage groups, two species pairs, *X. laevis–X. gilli*, and *X. borealis–X. muelleri*, have been identified (Kobel, 1981a).

Analyses of spermatid/sperm basic nuclear proteins for 17 xenopodine species and subspecies have suggested that these proteins can serve as molecular markers (Mann et al., 1982). The electrophoretic pattern and stain characteristics and a lower ratio of arginine in the *S. tropicalis* sperm proteins distinguish *S. tropicalis* from *Xenopus.* Lactate dehydrogenase is a well-known tetrameric enzyme composed of two subunits, A and B, which are coded by different genes (Appella and Markert, 1961; Markert, 1963). The similarities of LDH structural components in *X. laevis* to those of mammals have been reported (Lyra et al., 1976). Vonwyl and Fischberg (1980) reported that electrophoretic patterns for the *LDH-A* gene (or at least the mobility of the gene product) are identical in all 13 taxa examined, including *S. tropicalis.* In contrast, the mobility of the LDH-B gene product exhibits high levels of species-specific variability. Surprisingly, a subsequent study (Vonwyl, 1982) reported slight differences between the *LDH-A* genes of *S. tropicalis* and *S. epitropicalis.* Several studies have focused on the different expression of LDH during *X. laevis* development (Kunz and Hearn, 1967; Claycomb and Villee, 1971; Kunz, 1973). The LDH system has also been used to examine paternal and maternal dominance, maternal influence, and time of paternal allele expression in interspecific reciprocal hybrids of *Xenopus* (Wall and Blackler, 1974). Regulation of gene expression in *Xenopus* hybrids has also been studied for NORs (Blackler and Gecking, 1972a, 1972b; Honjo and Reeder, 1973; Cassidy and Blackler, 1974), mitochondrial malate dehydrogenase and tetrazolium oxidase (Wall and Blackler, 1974), glutamate-oxaloacetate transaminase (Johnson and Chapman, 1971), and creatine kinase (Kobel and Wolff, 1985).

Initial studies on *Xenopus* and *Silurana* hemoglobins indicated that no differences could be detected at the species level (Muir, 1981), but Bürki et al. (1984) and Bürki and Fischberg (1985) found that each species examined exhibited specific hemoglobin and globin patterns. However, the subspecies of *X. laevis* could not be unequivocally distinguished. The α and β globin genes of *X. laevis* have been described and intensively studied (Hentschel et al., 1979; Kay

et al., 1980; Patient et al., 1982; Hosbach et al., 1983). Molecular analyses of these globin genes provided strong evidence of a tetraploid origin for *X. laevis*. These studies showed that *S. tropicalis* has a single closely linked pair of α and β globin genes, whereas *X. laevis* exhibits two distinct linkage pairs of α and β globin genes (Jeffreys et al., 1980; Westley et al., 1981; Westley and Weber, 1982). The two pairs of globin genes of *X. laevis* may have arisen either by a tandem duplication of the *S. tropicalis* linkage β genes or by chromosome duplication; however, considering all other available data, the second alternative seems the more likely (Jeffreys et al., 1980). Bending and Williams (1983, 1984) studied the expression of tadpole and adult β globin genes injected into *X. laevis* eggs. Injection of purified genes and molecules into *Xenopus* eggs and oocytes is now routinely done to study gene expression and regulation (reviewed by Gurdon and Melton, 1981). This *Xenopus* system became widely used after the pioneer study of Gurdon et al. (1971), which showed that purified mRNA could be injected and efficiently translated in *X. laevis* eggs. This procedure has been used to study other *Xenopus* (Gurdon and Brown, 1978; Bending, 1981) and other species (Wickens et al., 1980; Etkin et al., 1983).

The structure and composition of *X. laevis* immunoglobins is well known. Originally, these studies examined antibody production and antigenic response (Butler et al., 1962; Elek et al., 1962; Lykakis and Cox, 1968; Lykakis, 1969; Du Pasquier and Wabl, 1978). Subsequently, this research has taken a more descriptive, structural, and comparative approach (Marchalonis et al., 1970; Hadji-Azimi, 1971; Jurd and Stevenson, 1974; Hadji-Azimi et al., 1976; Wabl and Du Pasquier, 1976). Recent studies have focused on molecular structure, evolution, and arrangement of the immunoglobin genes and have provided data on antibody diversity (Du Pasquier and Wabl, 1978; Brandt et al., 1980), nucleotide sequences (Yamawaki-Kataoka and Honjo, 1987; Schwager et al., 1988b; Grossberger et al., 1989), and gene organization and evolution (Schwager et al., 1988a; Hsu et al., 1989). Our current knowledge indicates that the immunoglobin genes of *Xenopus laevis* have an organization similar to that of mammals. The overall structure of the immunoglobin molecule seems to be conserved in vertebrates; however, antibody diversity is lower in amphibians than in mammals (reviewed by Du Pasquier, 1982). However, the conclusions from these immunological studies are based on the assumption that *X. laevis* represents a primitive tetrapod.

The intensive studies on vitellogenin (yolk protein precursor) genes have generated data on nucleotide sequence (Wahli et al., 1978), gene expression (Tata, 1976), gene transcription and translation (Berridge and Lane, 1976; Shapiro and Baker, 1977; Farmer et al., 1978), protein synthesis and composition (Ohlendorf et al., 1977; Wiley and Wallace, 1978), and hormonal regulation (Wallace and Bergink, 1974; Baker and Shapiro, 1977; Ryffel et al., 1977; Skipper and Hamilton, 1977). From a phylogenetic perspective, the presence of four distinct groups of sequences reported by Wahli et al. (1979) for *X. laevis* provides additional support for the tetraploid origin of the species.

Interest in myogenesis in *Xenopus* is related to the biphasic life cycle of amphibians. During metamorphosis, the larva undergoes dramatic changes, providing an ideal system to study muscle restructuring (Kordylewski, 1986), hormonal regulation, and gene regulation. The molecular analysis of these genes has provided information on promoter activity and localization (Mohun et al., 1986, 1989), gene activation (Gurdon et al., 1985; Radice and Malacinski, 1989), and sequence composition and homologies (Mohun et al., 1987; Stutz and Spohr, 1987). Radice et al. (1989) provided an extensive review of aspects of molecular myogenesis.

The present review of the biochemical and molecular studies of *Xenopus laevis* has focused on those areas and those molecules suitable for evolutionary studies. Studies of other genes are just beginning, and in

some cases nucleotide sequences are available, such as for histone genes (Turner and Woodland, 1982), genes involved in neurogenesis (Richter et al., 1988), and the elongation factor gene (EF-1α) (Krieg et al., 1989), even though comparative data for evolutionary studies are lacking. However, studies of the molecular biology of *X. laevis* and use of this organism in understanding molecular processes and mechanisms continue. Examples of the proliferation of molecular research on *Xenopus* are recent papers on insulin genes (Scavo et al., 1991; Shuldiner et al., 1991), histones (Dimitrov et al., 1992), protein expression (Loreni and Amaldi, 1992; Swick et al., 1992), new gastric cell type (Moore et al., 1992), protein receptors (Mathews et al., 1992), RNA processing (Fragapane et al., 1992; Yang et al., 1992), nucleosomal assembly (Sekiguchi and Kmiec, 1992), heat-shock protein genes (Krone et al., 1992), and metabolic pathways (Matsuda et al., 1992). Also, the interest in *Xenopus* is indicated by the continuing appearance of review articles and books (e.g., Woodland, 1989; Hausen and Riebesell, 1991; Kay and Peng, 1991).

Conclusions

Polyploidization seems to have played an important role in the evolution of *Xenopus* species. The chromosomal and available molecular data strongly suggest that the ancestor of *Xenpus* was of alloploid origin and that some descendant species are yet more derived allopolyploids. Studies of *X. laevis* should consider effects of its ploidy level. Genes acting in early development of *X. laevis* are commonly used and compared with those of mammals and *Drosophila* (Melton, 1991). However, future molecular studies, particularly those interested in the underlying mechanisms and processes of gene function instead of evolutionary aspects, would benefit by comparisons with *Silurana tropicalis*, the only known diploid xenopodine. This comparison will eliminate the problems of the existence of paralogous and silent duplicated genes. At the same time, a better understanding of molecular characteristics of the diploid species will facilitate its further comparisons with *X. laevis* and other polyploid species of *Xenopus* to answer phylogenetic questions.

At a broader level, phylogenetic studies on *Xenopus* have indicated that most of the biological systems and processes examined (e.g., morphology, development) are evolutionarily highly derived compared with those in other frogs. Consequently, studies based on the assumption that *Xenopus laevis* is a primitive frog or amphibian may result in conclusions not generally applicable to Anura or Amphibia. Hanken (1986) was concerned that the results of developmental studies based on one or two species unfortunately have been generalized to all Amphibia. If the objective of a research project is to describe a general pattern for frogs (Anura), then data from one species will never suffice. If contrasted with a suitably basal taxon, however, the highly derived aspects of *Xenopus* make it a good choice for comparative studies. Suitable comparisons can be made with *Bombina orientalis* (Fig. 5), a species whose use as a model organism is increasing (e.g., Frost and Robinson, 1984; Hanken and Hall, 1984). A feature observed in both *Xenopus* and *Bombina* might be inferred to be present in the common ancestor of these two taxa (in the absence of contrary data from other taxa). This common ancestor is slightly less inclusive than the common ancestor of all frogs. But the two taxa that are more basal, *Ascaphus* and *Leiopelma*, are difficult and impractical to secure for laboratory studies because of their status as threatened organisms and their more stringent husbandry requirements. From both phylogenetic and practical viewpoints, the common ancestor of *Xenopus* + *Bombina* is reasonably close to the ancestor of Anura. As comparative data become available from *Bombina orientalis*, the value of *Xenopus laevis* as a model organism will increase.

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APPENDIX

†Palaeobatrachidae appears to be the sister group of Pipidae, as supported by the following synapomorphies: (1) columella elongate, directed forward; (2) squamosal with expanded processes that surround the columella; (3) medial ramus of pterygoid covering Eustachian tube ventroposteriorly; (4) parasphenoid long and narrow; (5) quadratojugal absent; (6) maxilla overlapping premaxilla anteriorly; (7) teeth nonpedicellate; (8) pubis ossified; (9) cleithrum greatly expanded and forked; (10) metapodials elongate; (11) vertebrae epichordal; (12) free ribs present in larvae; (13) larvae with oral barbels (see below).

Characters 1, 3, 5, 6, 7, and 8 were listed by Cannatella and Trueb (1988a) as synapomorphies of Pipidae, but after examination of palaeobatrachid fossils it is evident that these are synapomorphies at the more general level of †Palaeobatrachidae + Pipidae. Our comparisons with pipids are limited to specimens of Recent taxa and published literature on fossil pipids, especially Báez (1981). The data for †Palaeobatrachidae were derived from examination of fossil specimens in the care of Dr. Zdenek Špinar, Prague, Czechoslovakia, and from illustrations in his 1972 monograph. Estes and Reig (1973) listed characters 1, 2, 4, 7, and 8 as features shared by the two groups; they also included enlarged otic capsules and azygous frontoparietal in their list. These two features are shared by pipids and palaeobatrachids but also with *Rhinophrynus* and thus are not synapomorphies at the level in question.

We consider the larval barbels reported by Špinar (1972) to be homologues of those found in *Xenopus* and *Silurana*. However, the absence of similar barbels in the fossil pipid larvae of †*Shomronella* (Estes et al., 1978) suggests that either the barbels were lost in this fossil form (as in *Hymenochirus* and *Pipa*) or that the barbels of palaeobatrachids and *Xenopus* + *Silurana* are independently derived. Lacking evidence for the phylogenetic position of †*Shomronella*, we cannot choose between these alternatives.

The alternative hypotheses have little if any support. The only character that might unite †Palaeobatrachidae and Rhinophrynidae to the exclusion of Pipidae is the presence of the parahyoid bone. However, the polarity of this character at this level of the tree is ambiguous because of the unresolved nature of relationships among the clades of pelobatoids (Cannatella, 1985). The only derived character known to us that would unite Pipidae and Rhinophrynidae to the exclusion of †Palaeobatrachidae is the absence of mentomeckelian bones, which are reported to be present in palaeobatrachids (Špinar, 1972). One of us (D.C.C.) has examined presumed mentomeckelian bones in the specimens illustrated in Špinar (1972: plates 25, 29) and is not convinced that these are ossified elements. Mentomeckelian bones are ossified from part of the larval infrarostral cartilages. In *Rhinophrynus* and Pipidae, the infrarostrals do not ossify but are nevertheless evident as blocks of cartilage. The elements identified as mentomeckelians in palaeobatrachids actually may be cartilaginous. If ossified mentomeckelians are indeed present in palaeobatrachids, then consideration of all the evidence indicates that the mentomeckelian bones have been lost independently in *Rhinophrynus* and Pipidae.

A second unresolved issue of homology is the septomaxilla of palaeobatrachids. The elements identified as septomaxillae by Špinar (1972) appear to be vomers; this apparently is the view of Estes and Reig (1973), who stated that paired, toothed vomers are present in palaeobatrachids, but they did not mention septomaxillae. Špinar (1972:35) mentioned the vomer in palaeobatrachids only once in passing, indicating that it may be present, but he did not illustrate it.

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