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RIBOSOMAL DNA AND THE PHYLOGENY OF FROGS

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ABSTRACT: Phylogenetic analysis of 1656 aligned sites in the 28S ribosomal RNA gene of frogs supports some of the recently recognized higher groups of anurans but provides counter-support for others. The 28S rDNA data support the monophyly of the recently recognized Pipanura (mesobatrachians plus neobatrachians), which in turn indicates paraphyly of archaeobatrachians. Mesobatrachians (pelobatoids plus pipoids), which are either considered paraphyletic or weakly supported as monophyletic in morphological analyses, also receive support as a monophyletic group from the 28S rDNA data. Hyloidea (= Bufonoidea), which is widely recognized but lacks morphological support, receives some molecular support as being monophyletic. However, Ranoidea, which is supported by morphology, is counter-supported by ribosomal DNA. In particular, dendrobatids do not group with ranids (but sometimes group with hyloids). A combined analysis of the molecular data with the morphological data of Duellman and Trueb (1986:Biology of Amphibians) supports Pipanura, Mesobatrachia, Neobatrachia, and Hyloidea, but shows the ranoids as paraphyletic (with Dendrobatidae related to Hyloidea). The agreement between molecular and morphological data in several regions of the anuran tree indicates an approaching stabilization of traditionally labile higher frog classification.

Key words: Anura; Frogs; Phylogeny; Systematics; Ribosomal DNA

THE higher phylogenetic relationships of anurans are so poorly resolved that the major competing hypotheses share little common ground. Twenty years ago, the major subdivisions within frogs were the subject of considerable debate (e.g., compare the classifications of Starrett, 1973, to those of Lynch, 1973). Today, although some progress toward stabilization of frog classification has occurred, there still appears to be little consensus among systematists about relationships among the major groups of frogs (e.g., compare Hedges and Maxson, 1993, to Ford and Cannatella, 1993). Although a few major groups of anuran families are widely recognized, some families (such as Dendrobatidae, Sooglossidae, and Pelobatidae) are regularly shifted back and forth among the higher categories by the various authorities. In short, there is no widely accepted classification of anurans because the inferred phylogenies have shown few signs of stabilizing as new data have been brought to bear on the problem.

To date, most of the relevant data have come from morphological analyses of adult and larval frogs (summarized in Duellman and Trueb, 1986; Ford and Cannatella, 1993). Contributions from cytogenetics and molecular biology have been comparatively minor (see Hedges and Maxson, 1993; Hillis, 1991*a*; Morescalchi, 1973). The reasons that frog phylogeny has been such a difficult problem probably include all of the following:

(1) The major lineages of frogs probably diversified over a relatively short span of time in the Mesozoic (Milner, 1988), so the frog tree is one of long terminal branches leading back to small internodes. This shape of tree is the most difficult type to reconstruct correctly, and is the most likely to lead to misleading or ambiguous results (see Swofford and Olsen, 1990).

(2) Most phylogenetic studies of frogs (and especially molecular studies) have tended to include single exemplars to represent major monophyletic groups, which compounds the problem identified in (1) above. Unlike (1), however, this problem can be corrected by expanding published

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databases to include more taxa. As more taxa are added to the analyses, the long, unbroken branches will be divided and thereby shortened. Hopefully, such approaches will gradually result in better estimates of relationships.

(3) Morphological and cytogenetic variation in frogs is surprisingly slight compared to other vertebrate groups of a similar age and species diversity. This leaves systematists with relatively few morphological or cytogenetic characters that are informative about higher frog relationships, despite the fact that the taxa have been sampled far more intensively for morphological and cytogenetic studies than for molecular studies.

(4) Although there is considerable molecular variation among major groups of frogs, molecular studies of frog relationships have tended to focus on far too few potentially informative characters to achieve any kind of robust support for or against a particular phylogenetic hypothesis. For instance, Hillis and Davis (1987) examined restriction site and length variation in the 28S rRNA gene of 54 species representing 17 families, but were unable to make any robust conclusions about higher frog phylogeny because of the small number of changes. More recently, Hedges and Maxson (1993) examined 333 aligned sites in the mitochondrial 12S rRNA gene among 20 frogs, and found no nodes that they considered significantly supported. A major problem with molecular studies continues to be the tradeoff between sampling intensity among taxa and sampling intensity among sites in the genome. Examination of few taxa for many characters can lead to the problem noted in (2) above, whereas examination of many taxa for few characters produces poor resolution. Hopefully, this problem will also be temporary as more complete gene sequences accumulate for larger numbers of taxa.

Because of these limitations, there are no strongly supported phylogenies that relate most of the families of frogs. The purpose of this paper was to examine a relatively long and evolutionarily conservative gene in enough frogs to determine its potential for estimating higher anuran relationships. Although we are aware of the need to add additional taxa, our sample of species includes enough diversity to test some of the widely recognized (although poorly supported) anuran groups. While previous studies of frog phylogeny have varied considerably in their conclusions, the following higher taxa have been recognized the most consistently:

Archaeobatrachia.—This name is applied by different authors to several different groups of taxa. However, the group usually includes Ascaphidae, Bombinatoridae, Discoglossidae, Leiopelmatidae, and the Mesobatrachia (see below) (cf. Cannatella, 1985; Duellman, 1975; Hedges and Maxson, 1993; cf. Laurent, 1979, 1986; Reig, 1958). When it has been considered to be a monophyletic group, Archaeobatrachia usually has been viewed as the sister taxon of the remaining anurans (e.g., Hedges and Maxson, 1993). However, the monophyly of this group appears highly doubtful (Cannatella, 1985; Ford and Cannatella, 1993); in fact, the part of the anuran tree that shows the strongest resolution from previous morphological analyses indicates the paraphyly of Archaeobatrachia (Hillis, 1991*a*).

Pipanura.—The grouping of mesobatrachians plus neobatrachians has been recognized by several recent authors (e.g., Cannatella, 1985; Duellman and Trueb, 1986; Ford and Cannatella, 1993; Hillis, 1991*a*; Sokol, 1975, 1977). Ford and Cannatella (1993) explicitly defined this group and named it Pipanura, although they noted that the name Ranoidei had been proposed for this clade by Sokol (1977). The latter name is usually used in a more restricted sense (see Dubois, 1984). Recognition of the Pipanura is obviously in conflict with the recognition of Archaeobatrachia, if the latter group is considered to include Mesobatrachia (i.e., in the sense of Duellman, 1975; Hedges and Maxson, 1993; or Reig, 1958). Among those who have recognized the Pipanura as a monophyletic group, opinion is divided as to whether the remaining taxa (discoglossoids) form a monophyletic sister group (e.g., Duellman and Trueb, 1986; Sokol,

1975) or are paraphyletic with respect to Pipanura (e.g., Cannatella, 1985; Ford and Cannatella, 1993; Hillis, 1991*a*; Lynch, 1973).

Mesobatrachia.—Mesobatrachia (Cannatella, 1985; Laurent, 1979) or Pipoidei (Dubois, 1984) has been less consistently recognized, and the support of this group from morphological data is weak (Ford and Cannatella, 1993; Hillis, 1991*a*). Mesobatrachia consists of the last common ancestor of Pipidae, Rhinophrynidae, Pelodytidae, Pelobatidae, and Megophryidae (the latter two families are often combined into one) and all of its descendants (Ford and Cannatella, 1993). The monophyly of the pipids and rhinophrynids (usually grouped together with the extinct Palaeobatrachidae as the Pipoidea) is well supported by both adult and larval morphology (Cannatella, 1985; Ford and Cannatella, 1993), although other relationships have been suggested (Maxson and Daugherty, 1980). The remaining families are often grouped together in the Pelobatoidea, but the support for the monophyly of this taxon is not strong (Hillis, 1991*a*).

Neobatrachia.—This is the most consistently recognized group of frogs, and is supported by five morphological synapomorphies (see Ford and Cannatella, 1993). It is defined by Ford and Cannatella (1993) as "the most recent common ancestor of living hyloids (myobatrachids, leptodactylids, bufonids, hylids, centrolenids, pseudids, sooglossids, *Heleophryne*, brachycephalids, *Rhinoderma*, and *Allophryne*) and Ranoidea . . . and all of its descendants." Among recent classifications, only that of Starrett (1973), which was based on Orton's (1953, 1957) tadpole types, has not recognized a monophyletic Neobatrachia.

Hyloidea.—This group, for which the junior synonym Bufonoidea was formerly used (Dubois, 1986), has been widely recognized but is unsupported by morphological synapomorphies (Ford and Cannatella, 1993). Despite the widespread recognition of a primary division in Neobatrachia between hyloids and ranoids in anuran classifications, there are several families that have been shifted between these two groups by various authors. In particular, the families Dendrobatidae and Sooglossidae have been the most problematic (see Ford, 1989; Nussbaum, 1980). Sooglossids have been placed within the ranoids (e.g., Duellman, 1975; Griffiths, 1959), within the hyloids (see Ford and Cannatella, 1993), or in the sister group to hyloids plus ranoids (e.g., Duellman and Trueb, 1986; Lynch, 1973). Dendrobatids have been considered hyloids by many (e.g., Laurent, 1979, 1986; Lynch, 1971, 1973; Noble, 1922, 1931), despite the fact that they seem to have a full suite of ranoid synapomorphies (e.g., Duellman and Trueb, 1986; Ford, 1989, in press; Ford and Cannatella, 1993; Griffiths, 1963).

Ranoidea.—This group of neobatrachians traditionally has been united on the basis of a firmisternal pectoral girdle [but see Ford and Cannatella (1993) for additional synapomorphies]. Firmisterny is thus viewed as the derived condition, with an arciferal girdle seen as the ancestral condition. Firmisternal girdles are also found in some pipids, where the condition is widely regarded as convergent. Dendrobatids also have firmisternal pectoral girdles, which is part of the evidence used to place Dendrobatidae in this group (Ford, in press). As defined by Ford and Cannatella (1993), Ranoidea includes "the common ancestor of hyperoliids, rhacophorids, ranids, dendrobatids, *Hemisus*, arthroleptids, and microhlids, and all of its descendants."

We chose at least two of what are considered to be among the most divergent taxa from each of these groups to have minimal tests of monophyly of the widely recognized clades of frogs. We examined the large subunit, nuclear ribosomal RNA gene (encoding 28S rRNA) because this gene shows considerable promise for examining phylogenetic relationships across the Mesozoic (Hillis and Dixon, 1991), when the major groups of anurans presumably diverged.

Materials and Methods

High-molecular-weight DNA was isolated from muscle tissue from *Latimeria chalumnae* (Actinistia), *Leiopelma hamiltoni* (Leiopelmatidae), *Spea multiplicata* (Pelobatidae), *Rana catesbeiana* (Ranidae), *Allobates femoralis* (Dendrobatidae), *Ceratophrys ornata* (Leptodactylidae), *Gastrotheca pseustes* (Hylidae), and *Nesomantis thomasetti* (Sooglossidae) following the protocol of Hillis and Davis (1986). Each sample of DNA was cleaved with the restriction enzyme *Eco* RI and ligated into a lambda vector (λgt10 for *Rana* and *Gastrotheca*, Lambda Zap II [Stratagene] for the others) to produce a subgenomic library (Hillis et al., 1990). The *Rana* and *Gastrotheca* libraries were screened by filterlift hybridization with a cloned mammalian 28S rRNA gene (see Hillis and Davis, 1987); the remaining libraries were screened with the isolated 28S rRNA gene of *Rana catesbeiana* (pE2528). Positive plaques were selected and the inserts were subcloned into the vector pBluescript (Strategene). Subclones were verified by restriction digestion, Southern blotting, and sequencing.

Plasmid DNA was purified using the protocols described by Hillis et al. (1990), denatured in alkali, and sequenced by the base-specific dideoxynucleotide chain termination method (Sanger et al., 1977) using modified T7 DNA polymerase (Tabor and Richardson, 1987). Sequencing primers and their locations are given in Hillis et al. (1991) or Hillis and Dixon (1991). Reaction products were separated on 4–6% polyacrylamide gels and visualized by autoradiography. DNA sequences were aligned using the alignment subroutines described by Pustell and Kafatos (1982, 1984, 1986). In addition to the taxa listed above, we aligned the published 28S rDNA sequences of *Xenopus laevis* (Pipidae; Ware et al., 1983; as corrected by Ajuh et al., 1991) and *Mus musculus* (Amniota; Hassouna et al., 1984). Regions of questionable alignment were excluded from phylogenetic analyses.

To compare the results from the 28S rDNA data to morphology, we re-analyzed the data of Duellman and Trueb (1986) for the same families that we examined. We also combined the molecular and morphological data to evaluate the relative strength of phylogenetic support from the two data sets.

All possible tree topologies were evaluated under the parsimony criterion using Swofford's (1990) Phylogenetic Analysis Using Parsimony (PAUP) program, version 3.0s. The amniote (*Mus*) and coelacanth (*Latimeria*) sequences were treated as outgroups. All changes among character states were weighted equally, and gaps were treated as a fifth character state. Regions of the gene that pair during secondary structural folding were not weighted by one-half as suggested by Wheeler and Honeycutt (1988) because this overcompensates for non-independence of the data (Dixon and Hillis, 1993). However, we recognize that equal weighting could introduce bias resulting from the weak interdependence among paired sites. The presence of phylogenetic signal in the sequences was evaluated by examining the skewness of the resulting tree-length distributions (Hillis, 1991*b*; Hillis and Huelsenbeck, 1992). The skewness statistic g_1 can be used to evaluate whether or not a data matrix contains more structure than is expected from variation that is random with respect to phylogenetic history. We did not use non-parametric bootstrapping (Felsenstein, 1985) because interpretation of bootstrapping results is not straightforward and bootstrap proportions are not comparable among branches on a tree or among studies (Hillis and Bull, in press).

Results

We aligned 1656 base-positions across the ten taxa (Fig. 1), of which 336 positions were variable. Our sequences spanned three sections of the 28S gene: *Mus* positions 110–425, 1132–1789, and 3342–4134. Parsimony analysis of this data matrix resulted in three most parsimonious trees, which differed only in the placement of *Allobates* (Fig. 2). These three trees were 375 steps long, with a consistency index of 0.622 (excluding uninformative characters).

The skewness analysis indicated a significant amount of phylogenetic signal in the 28S rDNA data matrix ($g_1 = -1.34$; $P < 0.01$). Not surprisingly, the best supported internal branch separated the ingroup and outgroup taxa (Fig. 2). The tree-length distribution of the possible resolutions within frogs was also strongly left skewed ($g_1 = -0.57$; $P < 0.01$), indicating

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            01|20                   01|40                   01|60                   01|80
Mus GGGTCGCGGCTTAGGGGCGGCGCGCAGCGCCGCACCCTTTACACCGCATGCCTTCTGGGTGAGGGGCCGCGG
Xen GGGTCGCGGCTTAGGGGCGGGCGGGCCGCCCGCGCCCTGCACACCGCATGCCCTCTGGCCTGGGGGGGCCGC
Spe GGGTCGCGGCTTANGGGCCGGCGGGCCC-CGCAGCCCTTTACACCGCATTCCCTCTGGCCTGGGGGGGTGAC
Ran GGGTCGCGGCTTAGGGGCANGCAGGCCGCCCGCGCCCTTTACACCGCATGCCCTCCGGCCTGGGTGGGCCGC
Gas GGGTCGCGGCTTANGGGCGNGCGGGC-----NNNCCCTTTACACCGNATGNCCTCTGGNNNGGGTGGGGCCG
Lei GGGTCGCGGCTTAGGGGCGGGCTGGCCG--CGCGCCCTTTACACCGCATGCCCTCTGGCCTGG-TGGGGCCG
Nes GGGTCGCGGCTTAGGGGCGGGCGGGC------CGCCCTCTACACCGCATGCCCTCTGGCCTGGGTGGGGCCG
Den NNNNNNNNGCTTAGGGGCAGGCGGGCCG-CCGAGCCCTGCACACCGCATGCCCTCTGGCCTGG-TGGGGCCG
Cer GGGTCGCGGCTTAGGGGCGAGCGGGCCG-CCGCGCCCTTTACACCGCATGCCCTCTGGCCTGGATGGGGCCG
Lat GGGTCGCGGCTTAGGGGCGAGCAGACCG-CCGCGCCCTTTACACCGCATGTCTTCTGGG-TGGAGGGGCC-G

                    02|00                   02|20                   02|40
Mus CGAGCACCCCCCGGGTTCAGGAAGACTAGCTCCGGG-TCGGGCACCTGCCACACTCCGGCCATCGCCGGGGG
Xen GCCGAGCCCCC-GGGTTCAGGAAGACTAGCTCCGGG-TCGGGCGCCTGCCACAATCCGGCCACCGCCGGGGG
Spe TGTGAGCCCTT-GGGTTCAGGAAGACTAGCTCTGGG-TCGGGCGCCTGCCACAATCCGGCCATCGTTGGGNT
Ran AGCGAGCCCCCNGGGTTCAGGAAGACTAGCTCCGGG-TCGGGCGCCTGCCACAATCCGGCCATCGCCGGGGG
Gas NANCNAGNNNNNGGGTTCAGGAAGACTAGCTCCGGG-TCGCGCGCCTGCCACAATCCGGCCATCGCCGGGGG
Lei CAGCGAGCCCCCGGGTTCAGGAAGACTAGCTCCGGG-TCGGGCACCTGCCACAATCCGGCCATCGCCGGGGG
Nes CAGCGAGCCCC-GGGTTCAGGAAGACTAGCTCCGGG-TCGGGCGCCTGCCACAATCCGGCCANCCGCCGGGG
Den CAGCGAGCCCCCGGGCTCAGGAAGATTATCACCGGGGTCGGGCGCCTGCCACCATCCGGCCATCGCCGGGGG
Cer CAGCGAGCCCCCGGGCTCAGGAAGACTAGCTCCGGG-TCGGGCGCCTGCCACAATCCGGCCATCGCCGGGG-
Lat CGGCGAGCCCCCGGGTTCAGGAAGACTAACTCCGAA-TCGGGTGCCTGCCACGCTCCGGCCATCGCCGGGGG

         02|60                   02|80                   03|00                   03|20
Mus CCG-CGCGGCCCGAGCCCAGAAGGGCCTCAGCCCAACGAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Xen CCG-CGCCGCCCTGGGCCAGAGGAGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Spe TNGTCGCCNCCCTGGGCCAGATGTGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Ran GCCGCGCCGCCCTGGGCCGGAGGGGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Gas CCG-CGCCGCCCTGGGCCAGAGGGGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Lei NCG-CGCCGCCCTGGGCCAGAGGAGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Nes GCCGCGCCGCCCTGGGCCAGAGGGGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Den NCCGCGGCGCCCTNGGCCAGANGGGCCTCAGCCCAACAAACACTTACGTCGGGTTTCTCCCACCATTTGAGG
Cer -CCGCGCCGCCCTGGGCCAGAGGGGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG
Lat G--CCCCCGCCCTGGCCCAGAAGAGCCTCAGCCCAACAAACCCTTACGTCGGGTTTCGCCCACCATTTGAGG

                03|40                   03|60                   03|80
Mus TAGATTCCGATTTATGGCCGTGCT-CTGGCTATCAGTTGTTCATGGCATTCCCTTTCAACTTTTCTTGAAAC
Xen TAGATTCCGATTTATGGCCGTGCT-CTGGCTATCGCCTGTTCATGGCATTCCCTTTCAACTTTTCTTGAAAC
Spe TAGATTCCGATTTATGGCCGTGCT-CTGGCTATCAGCTGTTCATGGCATTTC-GGTCAACTTTTC--GAAAC
Ran TAGATTCCGATTTGTGCCCGCGCT-CTGGCTATCGCCTGTTCATGGCATTCCCTTTCAANNNNNNNNNNNNN
Gas TAGATTCCGATTTATGGCCGCGCT-CTGGCTATCGCCTGTTCATGGCATTCC-TTTCAACTTTTCTTGAAAC
Lei TAGATTCCGATTTATGGCCGTGCT-CTGGCTATCAGCTGTTCATGGCATTCCCTTTCAACTTTTCTTGAAAC
Nes TAGATTCCGATTTATGCCCGCGCT-CTNGCTATCGCCTGTTCANGGCATTCCCTTTCAACTTTTCTTGAAAC
Den TAGATTCCGATTTATGGCCGCGCT-CTGGCTATCGCCTGTTCATGGCACTCCCTTTCAACTTTTCTTGAAAC
Cer TAGATTCCGATTTATGGCCGNGCT-CTGGCTATCGTCTGTTCATGGCATTCC-TTTCAACTTTTCTTGAAAC
Lat TAGATTCCGATTTATGGCC-TGCTGCTGGCTATCAGCTGTTCATGGCATTCC-TTTCAACTTTTCTTGAAAC

      04|00                   04|20          11|40                   11|60
Mus TTCTCTCTCAAGTT-CTCCCGCACTTTGGCAA AACTTTGTGCCTGGTTCCTCAGATTGCGCACGCGCTCA
Xen TTCTCTCTCAAGTT-CTCCCGCACTTTGGCAA AACTTTGTGCCTGGTTCCTCAGATTGCGCGCGCGCTCA
Spe TTCTCTCTCAAGTTTCTCCCGCACTTTGGCAA AACTTTGTGC-TGGTTCCTCAGATTGCGCGCGCGCTCA
Ran NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Gas TTCTCTCTCAAGTT-CTCCCGCACTTTGGCAA AACTTTGTGCCTGGTTCCTCAGATTGCGCGTCGCGTCA
Lei TTCTCTCTCAAGTT-CTCCCGCACTTTGGCAA AACTTTGTGCCTGGTTCCTCAGATTGTGCGCGCGCTCA
Nes TTCTCTCTCAAGTT-CTCCCGCACTTTGGCAG NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Den TTCTCTCTCAAGTT-CTCNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCA
Cer TTCTCTCTCAAGTT-CTCCCGCACTTTGGCGA AACTTTGTGCCTGGTTCCTCAGATTGCGCGCGCGCTCA
Lat TTCTCTCTCAAGTT-CTCCCGCACTTTGGCAA AACTTTGTGCCTGGTTCCTCAGATTGCGCACGCGCTCA
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FIG. 1.—Aligned DNA sequences from *Mus* (Mus), *Xenopus* (Xen), *Spea* (Spe), *Rana* (Ran), *Gastrotheca* (Gas), *Leiopelma* (Lei), *Nesomantis* (Nes), *Allobates* (Den), *Ceratophrys* (Cer), and *Latimeria* (Lat). Numbers refer to the position in the mouse gene as reported by Hassouna et al. (1984). Sequences enclosed in square brackets were not aligned and were not used in the phylogenetic analysis.

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              11|80               12|00                 12|20
Mus G--TCCCCGAGCAG-GCTTTCGGCGGCACCGCGTTACTTCCACT [TCCCGGGGCGGGCCCCCG]
Xen GCCTCCCTGAGACGCGCTTTGGG--ACACCGCGTTACTTCCACT [CCCGGCCCCGCGGGGCC]
Spe GCCTCCC-GAGCGTCGCTTTGGG--ACACCGCGTTACTTCCACT [CCCCCCCGCTGGGCC]
Ran NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN [ ]
Gas GCCTCCC-GAGCGTNGCTTGGGG--ACACCGCGTTACTTCCACT [ACCCGGGTCTGGGCC]
Lei GCCTCCCGGAGA---GCTTTGGG--ACACCGCGTTACTTCCACT [CCGCCCCNCGGGGCC]
Nes NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN [ ]
Den NCCTCCNNNNNNGT-GCTTTCGGGCGNNNNNNGTTACTTCCACT [CCCGGCCGNC]
Cer GCCTCCC-GAGCGT-GCTTTGGG--ACACCGCGTTACTTCCACT [CCCGGCCCCGCGGGGC]
Lat GTCTCCCGTCNNNTCGCTTTCGGG-GTACCGCGTTACTTTCACT [CCGCGCGCCCGGCC]

             12|40                12|60
Mus GGCTCCACCCTAGGG [CTCCGGAGAGGTCAGGCGGCTCCCG]
Xen GACTCCACCCTAGGG [CGGCGGGGAGGGAGGCGGGGGGCCCCCGCCCCCCCCCGCGGCCGCCCG]
Spe GACTCCACCCTAGCG [GCGGCGCGCCGCCCGCGTGGTGGCCGGGCAGAGCGGCCGTGGCAGGCCTCCAT]
Ran NNNNNNNNNNNNNNN [CGGCCGCCCG]
Gas GACTCCACCCTAGGG [CCGAGCAAGCCNGGCAGGCGCG]
Lei GACTCCACCCTAGGG [CGCGGCGCGCCGCCTG]
Nes NNNNNNNNNNNNNNN [ ]
Den GACTCCACCCTAGGG [CTCCGCGGCTNNTGCCTCCCG]
Cer GACTCCACCCTAGCG [CGGCGGGCAGGCGCCAGTNNTGCCGCCCG]
Lat GACTCCACCCTAGGG [GTGCGGAGCACGCCCCCCG]

             12|80               13|00                13|20                13|40
Mus CGTGGTGGCCGGGCAGAGCGGGCGGCGCGGCCCCTCCACCTCGTGCTCGCATGCGCAATCCTGGGCTTTCTA
Xen CGTGGTGGCCGGGCAGAGCGGGCGGGGCAGCCCCTCCACCTCGCACTCGCGCGCGCTATCCTGGGCTTTCTA
Spe GGTG-----CGGGCAGAGCGG-CGTGGCAGGCCCTCCACCTCGCTCTCGCGCGCGCTATCCTGGGCTTTCTA
Ran CGTGGTGGCCGGGCAGAGCGGGCG--GCGGCCCCTCCACCTCGTACTCGCGCGCACCGTCCTGGGCTTTCTA
Gas CGTGGTGGCCGGGCAGAGCGG-CGAGGCAGCCCCTCCACCTCGTACTCGCGCGCGCNATCCTGGGCTTTCTA
Lei CGTGGTGGCCGGGCAGAGCGGGCGAGGCAGCCCCTCCACCTCGTACTCGCACGCACTATCCTGGGCTTTCTA
Nes CGTGGTGGCCGGGCAGAGCGGGCG-GGCGGCCCCTCCACCTCGTACTCGCACGCGCTATCCTGGGCTTTCTA
Den CGTGGTGGCCGGGCAGAGCGGGCG-GGCAGCCCCTCCACCTCGTACTCGCGCGCACGATCCTGGGCTTTCTA
Cer CGTGGTGGCCGGGCAGAGCGGGCG--GCAGCCCCTCCACCTCGTACTCGCGCGCGCTATCCTGGGCTTTCTA
Lat CGTGGTGGTCGGGCAGAGTGGGCGTCGCAGCCCCTCCACCTCGTACTCGCACGCACTATCCTGGGCTTTCTA

                     13|60                13|80                14|00
Mus CCACTTGATACGAACCCGTCCCGCTTCGGTCTCCTTTGAGACCACCTCCAGGCATCGCCAGGACTGCACGTT
Xen CCACTTGATACGGACCCGTCCCGCTTCGGTCTCCTTTGAGACCACCTCCAGGCATCGCCAGGACTGCACGTT
Spe CCACTTGATACGGACCCGTC-CGCTTCGGTCTCCTTTGAGACCACCTCCAGGCATCGCCAGGACTGCACGTT
Ran CCACTTGATACGGACCCGTCCCGCTTCGGTCTCCTTTGAGACCACCTCCAGGCGTCGCCAGGACTGCACGTT
Gas CCACTTGATACGGACCCGTCCNGCTTCGGTCTCCNNTGAGACCACCTCCAGGCGTCGCCAGGANTGCACGTT
Lei CCACTTGATACGGACCCGTCCCGCTTCGGTCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTGCACGTT
Nes CCACTTGATACGGACCCGTNNNNNNNNNNNNNNNNNTGAGAC-ACCTCCAGGCGTNNNNAGGANTGCANGTT
Den CCACTTGATACGGACCCGTCCCGCTTCGGTCTCCTTTGAGACCACCTCCANNNNNCGCCAGGACTGCACGTT
Cer CCACTTGATACGGACCCGTCCCGCTTCGGTCTCCTTTGAGACCACCTCCAGGCGTCGCCAGGACTGCACGTT
Lat CCACTTGATACGGACCCGTCCCGCTTCGGTCTCCTTTGAGACCACCTCCANGCATCGCCAGGACTGCACGTT

         14|20                14|40                14|60                14|80
Mus TAGCCAGCAGGCT-GGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Xen TAGCCAGCAGGCT-GGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Spe TAGCCAGCAGGCT-GGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Ran TAGCCAGCAGGCT-GGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Gas TAGC-AGCAGGGT-GGACCCATATCCCCGNNTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Lei TNNNNNNNAGGCT-GGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Nes TAGC-AGCAGGNT-GGNNNCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACAAGGGAAGGNTTCA
Den TNNNNNNNAGGCT-GGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Cer TAGCCAGCAGGCT-GGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
Lat TAGCCAGCAGGCTTGGACCCATATCCCCGCTTTCTGATTAGCTTGGTAGATCATCGACCAAGGGAGGCTTCA
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FIG. 1.—Continued.

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                    15|00                   15|20                           15|40
Mus AAGGGAGTCCTATCGACCGCGAG [AGCGAGGGCTGCATGC]          GTCAAAATAGGCCATTTCGCT
Xen AAGGGAGTCCTATCGACCGCGAG [CAGGCAGC]                  GTCAAAATAGGCCATTTCGCT
Spe AAGGGAGTCCTATCGACCGCGAG [AGAGGNAGGC]                GTCAAAATAGGCCATTTCGCT
Ran AAGGGAGTCCTATCGACCGCGAG [AGCCGTGC]                  -TCAAAATAGGCCATTTCGCT
Gas AAGGGAGTCCTATGCACCGCGAG [TGAGTTACANAAGTCGCNNC]      GTCAAAATAGGCCATTTCGCT
Lei AAGGGAGTCCTATCGACCGCGAG [TCCGGT]                    GTCAAAATAGGCCATTTCGCT
Nes AAGGGAGTCCTATCGACNNAGCC [NCNCTCCT]                  GTCAAAATAGGCCATTTCGCT
Den AAGGGAGTCCTATCGACCGCGAG [CAGCGTTGGGCTCTTTGGGAGCTC] GTCAAAATAGACCATTTCGCT
Cer AAGGGAGTCCTATCGACCGCGAG [TGGGGGCTTC]                GTCAAAATAGGCCATTTCGCT
Lat AAGGGAGTCCTATCGACCACGAG [CTCGCTTGC]                 GTCAAAATAGACCATTTCGCT

                    15|60                 15|80                 16|00
Mus TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGAG
Xen TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGAG
Spe TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGAG
Ran TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGAG
Gas TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGG-CCGAG
Lei TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTGCGGCCGAG
Nes TACTAATCTCNAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTC-GGCCGAG
Den TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGAG
Cer TACTAATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGAG
Lat TACTGATCTCCAGAACCCCGGCTTTGCTAGAGTTGGATAAGAGTTTGAAATTTACCCATTCTTCGGGCCGAG

      16|20                 16|40                 16|60                 16|80
Mus CGACCGCACCTCGGCCC-GCACCTTACGCTCA--CGGATCACCCGGTGAAAACCATTCGTCTTGACCGCGAC
Xen CGACCGAACCTCGGCCC-GCACCTTACGCTCGTGCGGATCACCCGGTGAAAACCATTCGTCTTGACCGCGAC
Spe CGACCGGACCTCGGCCCCGTACCTTACGCTC--GCGGATCACCCGGTGAAAACCATTCGTCTTGACCGCGAC
Ran CGACCGGACCTCGGCCC-GCACCTTACGCTCNNNNNNNTCACCCGGTGAAAACCATTCGTCTTGACCGCGAC
Gas CGACCGGACCTCGGCCCCGCACCTTACGCTC--GCGG-TCACCCGGTGAAAACCATTCGTCTTGACCGCGAC
Lei CGACCGGACCTCGGCCC-GCACCTTACGCTCNNNNNGATCACCCGGTGNNNNNNNNNNNNNNNNNNNNNNNN
Nes CGACCGGACCTCGGCCC-GCACCTTACGCTC--GCG--TCACCCGGTGAAAACCATTCGTCTTGACCGCGAC
Den CGACCGCACCTCGGCCC-GCACCTTACGCTCT-GCGGATCACCCGGCGAAAACCATTCGTCTTGACCGCGAC
Cer CGACCGGACCTCGGCCC-GCACCTTACGCTCG-GCG--TCACCCGGTGAAAACCATTCGTCTTGACCGCGAC
Lat CGACCGAACCTCGGCCC-GCACCTTACGCTCA--CGGATCACCCGGTGAAAACCATTCGTCTTGACCGCGAC

                    17|00                 17|20                 17|40
Mus GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Xen GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Spe GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Ran GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Gas GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Lei NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Nes GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Den GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Cer GCCCTACTTGGCTTGCGGCCCAATTCCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC
Lat GCCCTACTTGGCTTGCGGCCCAACACCGCGGGCTACGGCTGCGAGTAGTCTGGGGTCTTTTCCACAACCAAC

      17|60                 17|80                         33|60
Mus TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Xen TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Spe TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Ran TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Gas TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Lei TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Nes TATATCTGTCGTCCTGACACCGGTGCCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Den TATATCTGTCGTCCNGCCACCGGTACCTTCAAC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Cer TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
Lat TATATCTGTCGTCCTGCCACCGGTACCTTCAGC  TACACTAAAGACGGGTCACGAGACTTACAGTTTCACT
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FIG. 1.—Continued.

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    33|80                    34|00                    34|20                    34|40
Mus TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT
Xen TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT
Spe TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT
Ran TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT
Gas NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Lei TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTNNNNNNNNNN
Nes TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT
Den TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT
Cer TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT
Lat TCTTTAAGTTACTTCGCGCCCATTTGCCGCCCTCATTGATACTGAGAGAATTCCATCGGTTTACGGAGCAGT

             34|60                    34|80                    35|00                    35|20
Mus AGATTAATCACTGCGCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGGTCGCTTTGGTGT
Xen AGATTAATCACTGCGCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Spe AGATTAATCACTGCGCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Ran AGATTAATCACTGCGCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Gas NNNNNNNNNNNNNNNNNNACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Lei NNNNNNNNCACTGCGCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Nes AGATTAATCACTNNNCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Den AGATTAATCACTGNNNNTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Cer AGATTAATCACTGCGCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT
Lat AGATTAATCACTGCGCGTACTTACCTACTTGCTCTAAGGGTGACAGGGATGGATGATAGATCGCTTTGGTGT

                      35|40                    35|60                    35|80
Mus CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACCGTG
Xen CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACGTTG
Spe CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACGTTG
Ran CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCGCAGTCGCCTTTGGGACAACTCGAACTGAGATCAGNNNNNN
Gas CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACGTTG
Lei CGGTTCCCTTGCCCGAACCGCCTTAGTAGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACGTTG
Nes CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACGTTG
Den CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACNNNN
Cer CGGTTCCCTTGCCCGAACCGCCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACGTTG
Lat CGGTTCCCTTGCCCGAACCGTCTTAGTCGCCCCTTTC--TTCTGGGACAACTCGAACTGAGATCAGACCGTG

           36|00                    36|20                         36|40                    36|60
Mus CCACTTCTCTGTACTCTCCACATCTTATTCACCCTCCGGG [GGCCGCGGGCCGGGGCAGGAGCGCAGCCC]
Xen ACACTTCTCTGTACTCTCCACATCCTATTCACCCTCCGGG [GGCGCGAGCAGCGTTTCCCCG]
Spe ACACTTCTCTGTACTCTCCACATCCTATTCACCCTCCGGG [GGNAGCGAGCACGCAC]
Ran NNNNNNNNNNNNNNNNNNNNNNNNCTATTCACC-TC-GGG [CCCTCCGGGTGCGGGAGCGCCACCCCG]
Gas ACACTTCTCTGTACTCTCCACATCCTATTCACCNNNNNNN [ ]
Lei ACACTTCTCTGTACTCTCCACATCCTATTCACCCTCCGGG [GGCAGGCGCCCT]
Nes ACACTTCTCTGTACTCTCCACATCCTATTCACCCTCCGGG [GGCGGGCGCAGGGGCAGTGGGCCCGGGCC]
Den NNNNNNNNNNNNACTCTCCACATCTTATTCACCCTCCGGG [CGGG]
Cer ACACTTCTCTGTACTCTCCACATCCTATTCACCCTCCGGA [GGCGAGTCAGGGGGAGCGCCCAGCCGCTT]
Lat ACACTTCTCTGTACTCTCCACATCCTATTCACCCTCCGGA [GGCAGCNAGGGACCGATCCGACT]

                            36|80                    37|00                    37|20
Mus [CAGCCCCGTGCGGCCGGAGCGCC] CGGCGGCCACTTTATGGTGATGAGAGTAGCAAAAAAGTGACTGGGC
Xen                           CGGCGGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Spe                           GGACGGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Ran                           CGGCGGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Gas                           NNNNNNCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Lei                           CGGCGGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Nes [GTGGCCT]                 CGGCGGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Den                           CNNNNGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Cer [TCCCCG]                  CGGCGGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
Lat                           CGGCGGCCACTTTATGGTGATGAGAATAGCAAAAAAGTGAATGGGC
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FIG. 1.—Continued.

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               37|40                  37|60                  37|80
Mus CACTCCGCCCCCCCGCTCGGGGCTCCCCGAGAGCGAAGACCGCGGTTCGCAG [GCAGGGCGCGCACGC]
Xen CACTCCGCCCCCCCGCTCGGGGCTCCCCGAGAGCGAAGACCTGGGTTCGC-G [GGCCGGGGGCG]
Spe CACTCCGCCCCCCCGCTCGGGGCTCCCCGAGAGCGAAGACCGAGGTTCGC-G [GGCCGGCANNGT]
Ran CACTCCGCCCCCCCGCTCGGGGCTCCCCGAGAGCGAAGACCGAGGTTCGG-G [GCCCCGGGG]
Gas CACTCCGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN [ ]
Lei CACTCCGCCCCCCCGCTCGGCG-TCCCCGAGAGCGAAGACCGAGGTTCGCAG [GCCGCG]
Nes CACTCCGCCCCCCCGCTCGG--CTNNNNGAGAGCGAAGACCGGCGTTCGNNN [ ]
Den CACTCCGCCCCCCCGCTCGG--CTCCCCGAGAGCGAAGACCGAGGTTCGCGG [ ]
Cer CACTCCGCCCCTCCGCTCGGGGCTCCCCGAGAGCGAAGACCAGGGTTCGNGG [ ]
Lat CACTCCGCCCCCCCGCTCAGGGCTCCCCGAGAGCTAAGACCACTGTTCGC-G [GCCGAGC]

    38|00                  38|20                  38|40                  38|60
Mus CCGCCCGCGCTGGGCGAGGCCCCTGTCACGGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGCCA
Xen CGGCCCGCGCTGGGCGAGGCTCCTGTCACCGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGGCA
Spe NNNCGCTCNNNGGGCGAGGCTCCTGTCACCGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGGCA
Ran CAGCCCGCGCTGGGCGAGGCTCCTGTCACCGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGCCA
Gas NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTTGGCA
Lei CACGGCCGGCTGGGCGAGGCTCCTGTCACCGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGCCA
Nes NNNNNNNNNNNNGGCGAGGCTCCTGTCACCGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGGCA
Den NNNNNNNNNNNNGGCGAGGC-CCTGTCACAGTCCACCCATCAAACTGACCCCGCCATGTGGACAGTTTGGCA
Cer NNNNNNNNNNTGGGCGAGGCTCCTGTCACCGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGGCA
Lat CGGCCCACGCTGGGCGAGGCCCCTGTCACCGTCCACCCCTCAAACTGACCCCGCCATGTGGACAGTTTGGCA

            38|80                  39|00                  39|20                  39|40
Mus TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGAGCGAA
Xen TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGAGCGAA
Spe TTGCGTCCACAG-ATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGAGCGAA
Ran TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGAGCGAA
Gas TTGCGTCCACAGGATTCCGCTCGAGTCNCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGAGCGAA
Lei TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGANCGNA
Nes TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGAGCGAA
Den TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGNCACCTCNTCTTCCCGTTTTCGAGCGAA
Cer TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGNNCATCTCGTCTTCCCGTTTTCGAGCGAA
Lat TTGCGTCCACAGGATTCCGCTCGAGTCCCTCCTGTCTTTGGAGGGCACCTCGTCTTCCCGTTTTCGAGCGAA

                    39|60                  39|80                  40|00
Mus CTAGAACTAAAAGTCATGCTTATGTCTGGCACTTTCG--CCCCGGAGTGCTAGGAAGACTGGAAAACCCAAA
Xen CTAGAACTAAAAGTCATACTTATGTCTGGCACTTTCG--CCCCGGAGTGCTAGGAAGACTGAAAAACCCAAA
Spe CTAGAACTAAAAGTCATACTTATGTTTGGCACTTTTG--GCCCGGAGTGGTAGGAAGAATGCAAAACCCAAA
Ran CTAGAACTAAAAGTCATACTTATGTCTGGCACTTT-GCGCCCCGGAGTGCTAGGAAGACTGAAAAACCCAAA
Gas CTAGAACTAAAAGTCATACTTATGTCTGGCACTTTCGCCCCNNGGAGTGCTAGGAAGACTGAAAAACCCAAA
Lei CTAGAACTAAAAGTCATACTTATGTCTGGCACTTTCG--CCCCGGAGTGCTAGGAAGACTGAAAAACCCAAA
Nes CTAGAACTAAAAGTCATACTTATGTCTGGCACTTTCG--CCCCGGAGTGCTAGGAAGACTGAAAAACCCAAA
Den CTAGAACTAAAAGTCATACTTATGTCTGGCACTTTCG--CCCCGGANTGCTAGGAAGACTGNAAAACCCAAA
Cer CTAGAACTAAAAGTCATACTTATGTCTGGCACTTTCG--CCCCGGAGTGCTAGGAAGACTGGAAAACCCAAA
Lat CTAGAACTAAAAGTCATACTCATGTCTGGCACTTTCGCGCCCCGGAGTGCTAGGAAGACTGAAAAACCCAAA

          40|20                  40|40                  40|60                  40|80
Mus ATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
Xen ATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
Spe ATTTGTCCTTCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGNNNNNN
Ran ATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
Gas ATTCGTCCTCCACAGTCNNNNNNNNNNNNNNNNNNNNNACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
Lei ATTCGTCCTCCACAGTGTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
Nes ATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGNNNNNNNGCAAGTATNNNNNNNN
Den ATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
Cer ATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
Lat ATTCGTCCTCCACAGTCTTTTCAATGGTGTCCCTATTGACCGAACACCGCCGGTTCGCAAGTATCGCTGCAG
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FIG. 1.—Continued.

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          41|00               41|20
Mus CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCGTCTTAAG
Xen CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCGTCTTAAG
Spe NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Ran CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCNTCTTAAG
Gas CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCGTCTTAAG
Lei CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCGTCTTAAG
Nes NNAAAAACTAGGAAGCTACAGNNNAGAAGGNTAGTAACACTTCGTCTTAAG
Den CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCGTCTTAAG
Cer CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCGTCTTAAG
Lat CGAAAAACTAGGAAGCTACAGCCGAGAAGGATAGTAACACTTCGTCTTAAG
```

FIG. 1.—Continued.

the additional presence of phylogenetic signal among the frogs. The data matrix continued to show significant structure as the next four branches were resolved: these branches provided support for the monophyly of the pipanurans, the two mesobatrachians, the ranid plus the two mesobatrachians, and the hylid plus sooglossid.

Of the six commonly recognized higher groups of frogs described in the introduction, our analysis of 28S rDNA provided independent support for three taxa: Mesobatrachia, Pipanura, and Hyloidea. In agreement with recent morphological analyses (see Ford and Cannatella, 1993), our data suggest that Archaeobatrachia (sensu Duellman, 1975) is not monophyletic. However, our data also do not support some of the groups that are supported by morphological analyses, namely Neobatrachia and Ranoidea (if Dendrobatidae is included in the latter group).

The morphological data of Duellman and Trueb (1986) conflict with our results by supporting Neobatrachia and Ranoidea, but not Mesobatrachia or Hyloidea (Fig. 3). The morphological tree is 12 steps long and has a consistency index of 0.917. The two data sets agree that Pipanura is monophyletic and that Archaeobatrachia is not. A combined analysis of our molecular data and the corresponding morphological data from Duellman and Trueb (1986) produces a single most parsimonious tree that supports the monophyly of Pipanura, Mesobatrachia, Neobatrachia, and Hyloidea, but not Archaeobatrachia or Ranoidea (Fig. 3). This tree is 395 steps long and has a consistency index (excluding uninformative characters) of 0.797.

DISCUSSION

The only higher clade of frogs that is strongly supported by both the morphological and molecular data sets is Pipanura. The morphological and our 28S rDNA data therefore agree that Archaeobatrachia (sensu Duellman, 1975) is not monophyletic, in contrast to the analysis of Hedges and Maxson (1993). There is also support of the Mesobatrachia from both the 28S data and some morphological studies (e.g., Cannatella, 1985; Ford and Cannatella, 1993), although other morphological (e.g., the data of Duellman and Trueb, 1986) and molecular (e.g., Hedges and Maxson, 1993) studies do not support this group.

Other comparisons between morphological and molecular studies show little agreement in the relationships among families. The Hyloidea has no known support from morphology, and yet appears to

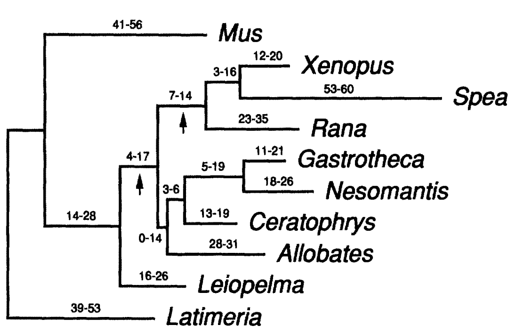

FIG. 2.—One of three most parsimonious trees for the 28S rDNA data. The two arrows indicate the alternative placement of *Allobates* in the other two trees. The numbers along the branches show the minimum and maximum number of changes that can occur across all most parsimonious character reconstructions.

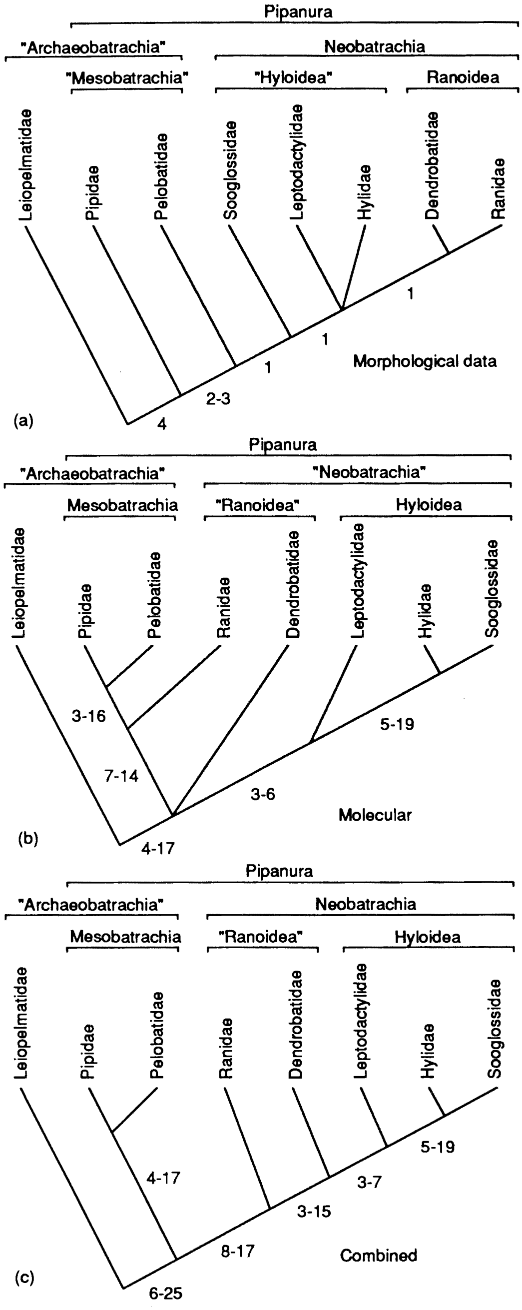

FIG. 3.—Comparison and combination of molecular (28S rDNA) and morphological (from Duellman and Trueb, 1986) data sets. The two outgroup taxa in the molecular study were used to root the trees but are not shown here for the sake of simplicity. For the morphological data, the outgroups were scored as having all ancestral states. The content of the six major groups of frogs follows common usage, and non-monophyletic groups on each tree are enclosed in quotation marks.

receive some support from the ribosomal genes. Hedges and Maxson (1993) also found some support for a modified Hyloidea (their Bufonoidea), but only if Rhacophoridae (which has all the morphological synapomorphies of Ranoidea) was included in the group. Neither the 12S nor the 28S rDNA data support the inclusion of Dendrobatidae in the Ranoidea. If dendrobatids are considered ranoids, then ranoids are either paraphyletic or polyphyletic in our analysis, and in the tree of Hedges and Maxson (1993) Dendrobatidae is embedded within the hyloids. If we limit our analysis to the frog taxa, the shortest tree that would place ranids and dendrobatids together would require six additional steps (although there are fewer steps between the alternatives if various combinations of outgroup taxa are added). Morphologists have long disagreed about the relationships of this family, and have been divided about whether or not Dendrobatidae belonged with ranoids (e.g., Duellman and Trueb, 1986; Ford, 1989; Ford and Cannatella, 1993; Griffiths, 1963) or hyloids (Laurent, 1979, 1986; Lynch, 1971, 1973; Noble, 1922, 1931). We see the molecular data (i.e., this paper and Hedges and Maxson, 1993) as too weak to resolve this controversy satisfactorily, although they do provide some support for a hyloid relationship of dendrobatids.

Perhaps the most surprising relationship suggested by the 28S rDNA data is the connection between the ranid and the mesobatrachians, which suggests that Neobatrachia (as usually recognized) is not monophyletic. For the 28S rDNA data, the shortest ingroup tree that contains a monophyletic group of taxa that are currently considered to be neobatrachians is 6 steps longer than the most parsimonious tree. Of course, if the tree shown in Fig. 2 is correct and the phylogenetic definitions of Neobatrachia, Pipanura, and Ranoidea used by Ford and Cannatella (1993) are followed, then all three of these names would be synonyms. Figure 2 also suggests that the possibility of firmisterny as the ancestral condition of the pipanuran pectoral girdle should be given consideration. However, the monophyly of Neobatrachia appears to be well supported by morphological synapomorphies (Ford and Cannatella, 1993), and also is the group most strongly supported by 18S rDNA sequences (Hedges and Maxson, 1993).

As can be seen in Fig. 3, our tree is considerably different from the tree based on earlier morphological data. For the ingroup taxa, the morphological tree would require 19 additional steps to explain the 28S rDNA data compared to the most parsimonious tree. In cases of conflict between multiple data sets, one option is to combine the data in a joint analysis (Hillis, 1987; Kluge, 1989; Miyamoto, 1985). Minimally, this permits discovery of which data set shows the strongest support for its respective conclusions. It is also possible that weak but compatible signal in the two data sets will reveal underlying historical patterns where none was visible in the separate analyses (Barrett et al., 1991). However, there is also the possibility that a noisy, misleading data set will overwhelm the phylogenetic signal in an otherwise informative data set. Despite these limitations, we believe the results from the combination of the morphological and 28S rDNA data sets are revealing (Fig. 3). The combined analysis shows elements of both the molecular and morphological trees, and is nearly consistent with the classification proposed by Ford and Cannatella (1993). The only deviations are that this tree provides support for the monophyly of the hyloid taxa (which Ford and Cannatella considered to be paraphyletic), and the two included "ranoids" appear to be paraphyletic. If this tree accurately reflects the phylogenetic history of frogs, then it suggests that firmisterny could be ancestral in Neobatrachia. However, a tree that unites *Rana* and *Allobates* is only three steps away from the shortest tree in this analysis, so the additional synapomorphies of Ranoidea discussed by Ford and Cannatella (1993) are probably sufficient to support the monophyly of this group.

Obviously, an expansion of the 28S rDNA data set to include additional taxa would be desirable; it appears that this gene contains information that will be useful in elucidating the relationships of frogs. We are encouraged by the level of independent support by the 28S rDNA data for some groups that were suggested originally by morphological studies, and we expect that a continued parallel development of morphological and molecular studies eventually will result in a well supported phylogenetic hypothesis for frogs.

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SYSTEMATIC STUDIES OF THE COSTA RICAN MOSS SALAMANDERS, GENUS *NOTOTRITON*, WITH DESCRIPTIONS OF THREE NEW SPECIES

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ABSTRACT: Study of allozyme variation, external morphology, and osteology reveals that there are more species of moss salamanders (genus *Nototriton*) in Costa Rica than the two currently recognized. The three species for which names are available are valid, and new diagnoses are presented for them; three additional species are described. The phylogenetic relationships and biogeography of the six species are investigated. The radiation of *Nototriton* in present-day Costa Rica has involved miniaturization accompanied by both morphological and ecological specialization. Costa Rican species inhabit moss-mats and leaf-litter; most of the remaining species in the genus are bromeliad-dwellers. The revised genus *Nototriton* includes two Mexican, one Guatemalan (another, detected in the present study, remains undescribed), two Honduran, and six Costa Rican species. The six Costa Rican species appear to form a monophyletic group, but the phylogenetic relationships of the two northern species groups to each other and to the southern group remain uncertain.

Key words: Salamanders; Plethodontidae; *Nototriton*; Costa Rica; Allozymes; Morphometrics; Systematics; New species

SALAMANDERS of the genus *Nototriton* (commonly known as moss salamanders) are inconspicuous components of cloud forest faunas from Oaxaca, Mexico, to central Costa Rica. Most of the species occur in moss mats hanging in trees or bushes, or in moss covering dirt banks, large boulders, or stumps. Others inhabit bromeliads. In a few places (such as on the northeastern slopes of the Cordillera Central in Costa Rica), they can be found easily, but characteristically they are uncommon. Even species that have been known taxonomically for more than 40 years (e.g., *N. richardi*) are represented by fewer than 25 specimens in the museums of the world. Typically, species of *Nototriton* are small; none exceeds 40 mm in snout–vent length and several species are not known to exceed 30 mm. These salamanders have slender bodies, narrow heads, and long, tapering tails that exceed their snout–vent length. Their eyes are small and oriented anteriorly, and several of the species have

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