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The Phylogenetic Relationships of Lampridiform Fishes (Teleostei: Acanthomorpha), Based on a Total-Evidence Analysis of Morphological and Molecular Data

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We investigated the phylogenetic relationships among five species of lampridiform fishes, three basal outgroup species (two aulopiforms and one myctophiform), and two species of non-lampridiform acanthomorphs (*Polymixia* and *Percopsis*) using a combined parsimony analysis of morphological and molecular data. Morphological characters included 28 transformation series obtained from the literature. Molecular characters included 223 informative transformation series from an aligned 854-base pair fragment of 12S mtDNA and 139 informative transformation series from an aligned 561-base pair fragment of 16S mtDNA. A total-evidence analysis using the aulopiforms *Synodus* and *Aulopus* and the myctophiform *Hygophum* as outgroups corroborates the monophyly of Lampridiformes and unites *Polymixia* with *Percopsis*. Among the lampridiform fishes we examined, *Metavelifer* is basal, followed in ascending order by *Lampris, Lophotus, Regalecus,* and *Trachipterus*. This hypothesis is congruent with the most recent morphological analysis of the Lampridiformes and rejects a diphyletic origin of elongate body form within the clade. Analysis of a combined matrix of 12S and 16S mtDNA data yielded a phylogenetic hypothesis isomorphic with the total-evidence phylogeny. Analyses of partitioned DNA data sets reveals that single gene regions are poor predictors of the total-evidence phylogeny while combined analyses of both DNA data sets are good predictors of the total-evidence phylogeny. © 1998 Academic Press

INTRODUCTION

The Lampridiformes include some of the most colorful and bizarre species of higher teleost fishes. The oar fish, *Regalecus glesne,* for example, has been reported to reach 17 m in length and has been offered as the source of sea serpent legends since the first recorded specimen was washed ashore in Bermuda in the 1860's (Olney *et al.,* 1993). Most species of the group are rare, and specimens in museums are uncommon.

The Lampridiformes comprise 21 species in 12 genera and 7 families. The relatively small (40 cm) veliferids and much larger (1.8 m) opahs (Lamprididae) are deep bodied. The remaining families are elongate and range in body size from the small tube-eyes (Stylephoridae, 31 cm) to the very large *Regalecus* (17 m). All but the near shore veliferids are oceanic fishes, ranging from the epipelagic realm to abyssal depths. Lampridiform fishes are characterized by a uniquely protusible upper jaw apparatus and have been considered a natural group since Regan (1907) named them. Prior to 1992, most acanthomorph classifications placed the Lampridiformes within the Percomorpha, a group that traditionally included the Beryciformes, Gasterosteiformes, Perciformes, Pleuronectiformes, Scorpaeniformes, Tetraodontiformes, and Zeiformes. Stiassny and Moore (1992) presented two hypotheses, alternatively placing lampridiforms as basal acanthomorphs or basal percomorphs. Olney *et al.* (1993) concluded that lampridiforms are basal acanthomorphs that diverged before the percomorphs, and Johnson and Patterson (1993) placed them as the sister group to all other acanthomorphs, a position occupied by *Polymixia* in several previous hypotheses (Rosen, 1985; Stiassny, 1986; Patterson and Rosen, 1989; Stiassny and Moore, 1992). Prior to the study of Olney *et al.* (1993), the most recent analysis of lampridiform intrarelationships was that of Oelscahägler (1983). He placed the lampridids as the sister group of the lophotids and hypothesized that elongate body form evolved twice. In contrast, Olney *et al.* (1993) placed the deep bodied veliferids and lampridids as sequential sister groups to the remaining families, thus forming a monophyletic group of species with an elongate body shape (see Fig. 1).

Our study has two major objectives: (1) to report on DNA variation from parts of two mitochondrial ribosomal gene regions and determine the relevance of this variation for addressing the relationships of available lampridiform genera in a total evidence context and (2) to test the two recent hypotheses of relationships

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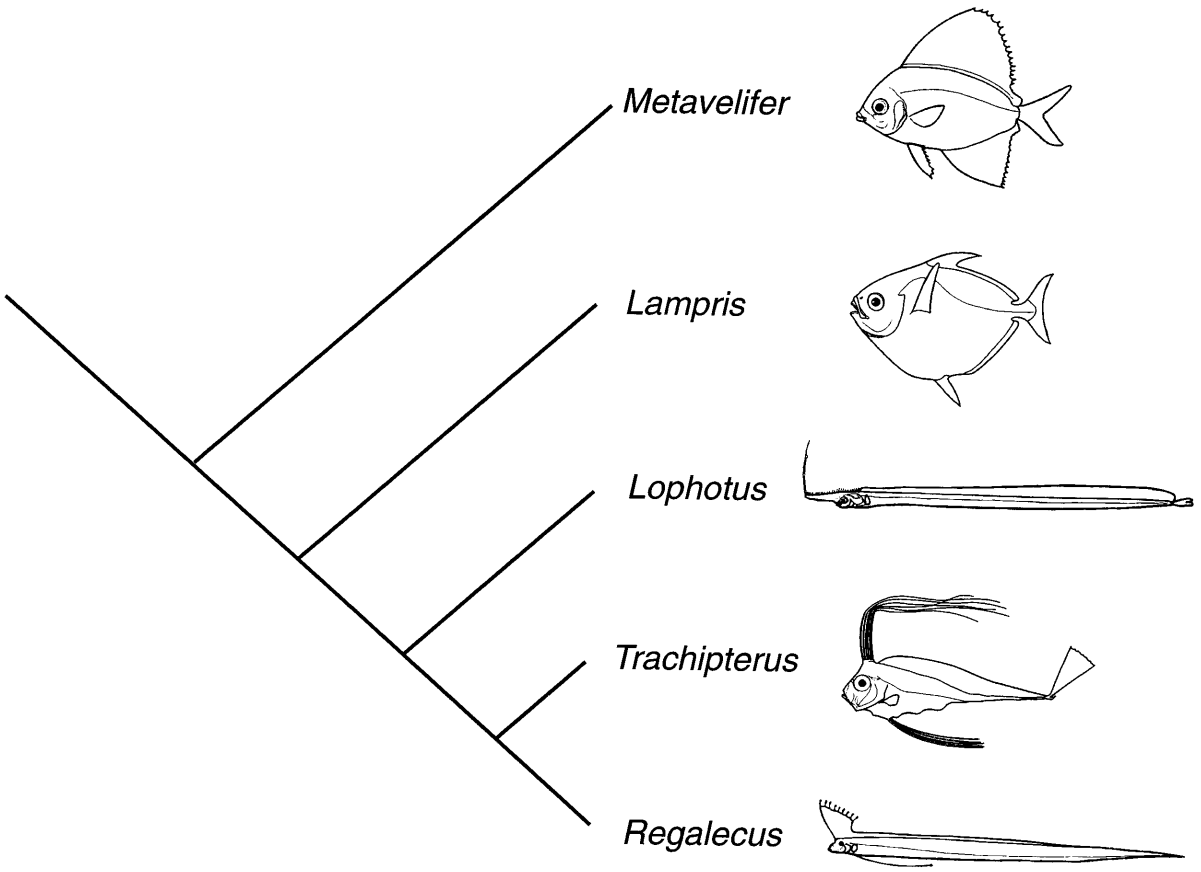

FIG. 1. Relationships among five genera of lampridiform fishes as hypothesized by Olney *et al.* (1993) with outline drawings of representative species. *Trachipterus* has an elongate body form although it is not as extreme in this drawing because the specimen illustrated is a juvenile. (Drawings from J. S. Nelson, 1994, "Fishes of the World," Wiley-Interscience, New York. Copyright John Wiley & Sons, 1994. Used with permission.)

among the lampridiform species for which tissue samples are available.

METHODS AND MATERIALS

Most lampridiforms are rarely collected, and even traditionally curated specimens are few. With the help of colleagues, we were able to obtain tissue samples from five species, each representing a different family. These and the non-lampridiforms used in the study are listed in Table 1.

Approximately 25 mg of tissue was dissected and DNA was extracted using QIAamp (Qiagen) tissue protocols. We used the polymerase chain reaction (Saiki, 1990) to amplify selected gene regions from genomic extractions. The two ribosomal mitochondrial gene regions were amplified using amplitaq DNA polymerase from the Perkin–Elmer Cetus Corp. Palumbi (1996) reviews primer systems and provides a map of the 12S and 16S ribosomal gene regions. Table 2 details the primers used in this study.

Amplification products were separated by electrophoresis on NuSieve (FMC) agarose gels. The band containing the amplified DNA was excised from the gel and melted, and the DNA was recovered with QiaQuick (Qiagen) affinity columns. The purified PCR product was sequenced with an Applied Biosystems 310 automatic sequencer using ABI Prism dye terminator sequencing kits and the primers indicated in Table 2. All sequences were deposited in Genbank (Accession Nos. AF049722–AF049741).

DNA sequences were inspected individually for quality and spliced, and a consensus sequence was produced by comparing heavy and light strand sequences. An initial alignment was made using CLUSTAL. Sequence variation between species was compared against the original electropherograms as a further check on sequence quality. The aligned data were then exported to a NEXUS file and organized into stem and loop regions (Kjer *et al.*, 1994) using models presented by Van der Peer *et al.* (1994) and de Rijk *et al.* (1994) for 12S and 16S mt RNA, respectively. We then visually examined each stem for base-pair complementarity and adjusted alignments in loops where needed. Stem and loop regions were examined for possible site saturation by plotting the number of mutations between pairs of

taxa against the Tamura–Nei genetic distance using MEGA (Kumar *et al.*, 1993).

Morphological data (28 transformation series) used here are those of Olney *et al.* (1993). Specimens examined by us that were not included in Olney *et al.* (1993) are listed in Table 1.

Phylogenetic analyses were carried out using PAUP 3.1.1 (Swofford, 1993) using the branch-and-bound option on the total evidence matrix because we find Kluge's (1989) argument for combining all data to estimate phylogenetic relationships to be compelling. Nevertheless, we also analyzed partitions of our matrix in order to identify sources of support for different nodes outside the context of the total evidence matrix. Contrary to the recommendations of Bull *et al.* (1993), we did not conduct tests for homogeneity before assemblying the total-evidence matrix because we do not accept the proposition that such tests are, apriori, a necessary precursor to combining data. However, we have not ignored the details of differential evolutionary rates. In particular, we demonstrate that certain sites are saturated for transitions and we have conducted our analyses in a manner to accommodate this observation.

Aulopus, *Synodus*, and *Hygophum* were designated as outgroups for all phylogenetic analyses following current corroborated phylogenetic analyses of the higher relationships of eurypterygian fishes (Johnson, 1992). This allowed us to test for lampridiform monophyly relative to *Polymixia* and *Percopsis*, but not for the monophyly of Acanthomorpha.

Quality of the phylogenetic trees was evaluated using summary values reported by PAUP (e.g., tree length, ensemble consistency index). Support for internodes (monophyletic groups) was evaluated by calculating branch support values (Bremer, 1988, 1994) using Tree Rot (Sorensen, 1996). Bootstrap values (Felsenstein, 1985) were also calculated using a heuristic search and 100 bootstrap replications.

TABLE 1

Specimens Examined

Aulopiformes
- *Synodus variegatus*, USNM 315318*
- *Synodus saurus*, USNM uncat. (KU tissue 311)
- *Aulopus purpurissatus*, AMS 35007-001 (tissue NI1222)

Myctophiformes
- *Hygophum macrochir*, AMNH 25019*
- *Hygophum hygomii*, KU uncat. (KU tissue 263)

Acanthomorpha
- Lampridiformes
  - *Metavelifer multiradiatus*, AMS 1251-2 (tissue NI1251-2)
  - *Lampris guttatus*, USNM uncat. (KU tissue 397)
  - *Lophotus capellei*, AMS 34438-001 (tissue NI1115)
  - *Regalecus glesne*, AMS 31207-001 (tissue NI1246)
  - *Trachipterus jacksoniensis*, AMS 31208-002 (tissue NI1244)
- Euacanthomorpha
  - Polymixiiformes
    - *Polymixia lowei*, USNM 308378*
    - *Polymixia japonica*, USNM uncat. (KU tissue 258)
  - Percopsiformes
    - *Percopsis omiscomaycus* USNM 179711*
    - *Percopsis omiscomaycus* UAIC 11218.07

Note. Morphological data were collected from specimens denoted with an asterix. Tissue numbers follow specimen voucher number for sequenced specimens where appropriate. Lampridiform morphological data were taken from Olney *et al.* (1993) who provide a list of specimens examined for their work. Acronyms: AMNH, American Museum of Natural History; KU, Natural History Museum, University of Kansas; AMS, Australian Museum, Sidney; UAIC, Museum of Natural History, University of Alabama; USNM, U.S. National Museum of Natural History.

TABLE 2

Sequencing (S) and Amplification (A) Primers Used in this Study

Name	Sequence	Strand	Use
	Mitochondrial 12S Gene		
Phe2-L[a]	5′ AAAGCATAACACTGAAGATGTTAAGATG 3′	Light	A, S
12Sa[b]	5′ AAACTGGGATTAGATACCCCACTA 3′	Light	S
12Sb[b]	5′ AGGAGGGTGACGGGCGGTGTGT 3′	Heavy	A, S
12Sd[c]	5′ GGGTTGGTAAATCTCGTGC 3′	Light	S
	Mitochondrial 16S Gene		
16Sa-L[d]	5′ CGCCTGTTTACCAAAAACATCGCCT 3′	Light	A, S
16SB-H[d]	5′ CCGGTCTGAACTCAGATCACGT 3′	Heavy	A, S

[a] *Oncorhynchus mykiss* position 946-965.
[b] Modified from Kocher *et al.* (1989).
[c] Modified 503 primer of John Patton, Washington University. *Oncorhynchus mykiss* position 1216-1233.
[d] See Palumbi (1996).

RESULTS

Alignment of the 12S mtDNA region resulted in a total of 854 presumed homologous base positions (= column of data) of which 411 columns were variable and 223 columns contained potentially informative variation. Alignment of the 16S mtDNA region resulted in 561 columns of which 256 were variable and 139 were potentially informative. Alignments are shown in Fig. 2.

Within the 12S mtDNA region we found stems to be unsaturated for both transitions and transversions, while loops were unsaturated for transversions but saturated for transitions (Figs. 3a and 3b). Both loops and stems were found to be saturated for transitions but unsaturated for transversions in the 16S mtDNA region (Figs. 3c and 3d). Transitions that occurred in stems were excluded from all analyses. We performed analyses on: (1) the total-evidence matrix and (2) partitions of the total-evidence matrix. Our results are shown in tree form in Fig. 4 (total-evidence analysis), Figs. 5–7 (partitioned analyses), and Fig. 8 (combined

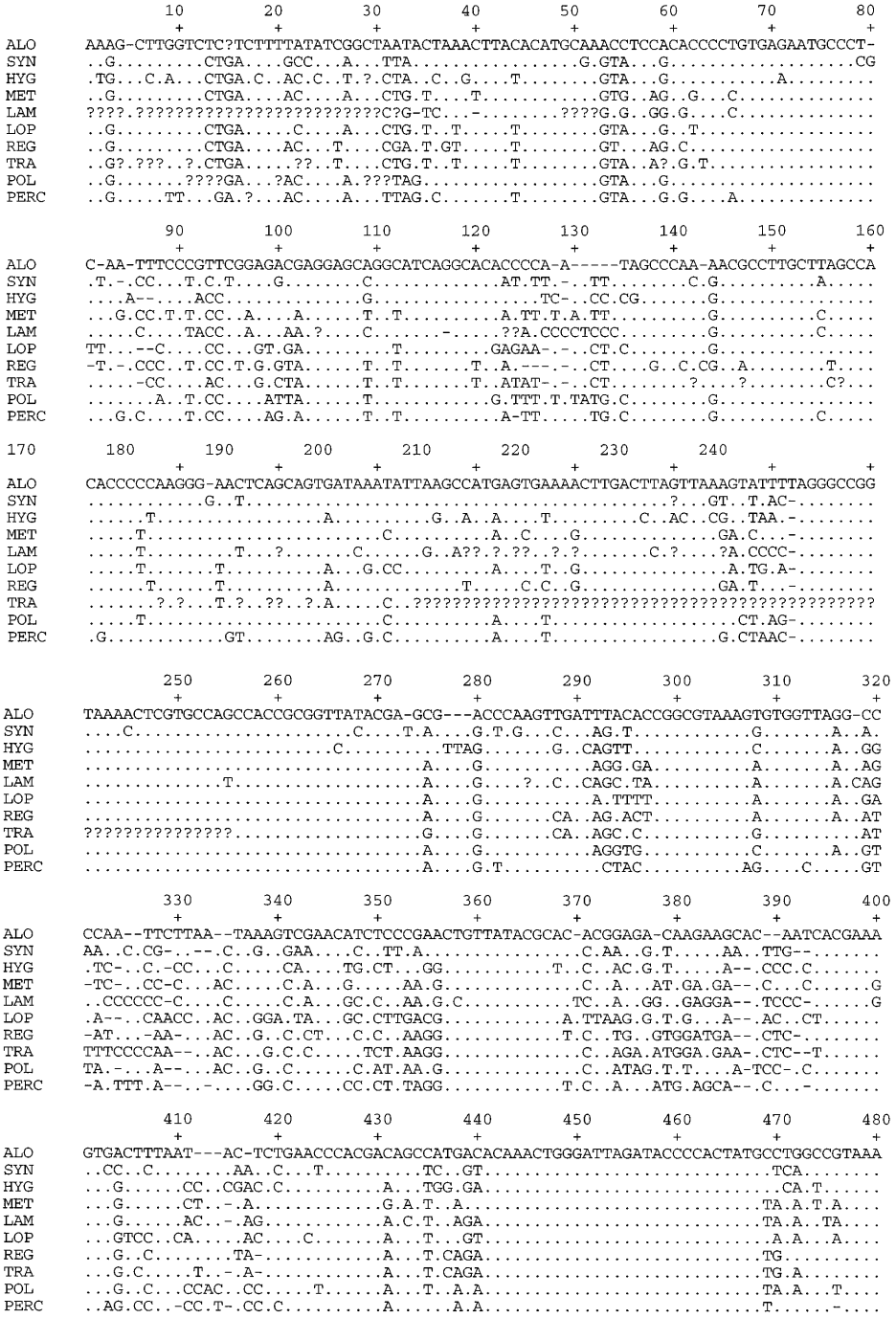

FIG. 2. A matrix of aligned sequence and morphological data used in this study. Columns 1–854, 12S mtDNA. Columns 855–1415, 16S mtDNA. Columns 1416–1443, morphology. ALO, *Alopus;* SYN, *Synodus;* HYG, *Hygophum;* MET, *Metavelifer;* LAM, *Lampris;* LOP, *Lophotus;* REG, *Regalecus;* TRA, *Trachipterus;* POL, *Polymixia;* PERC, *Percopsis.*

DNA analysis). Numerical summary data are shown in each figure caption.

DISCUSSION

The single total-evidence tree (Fig. 4; TL = 954 steps) is associated with high branch support values at all nodes and we conclude that this tree is a robust estimate of phylogeny for the taxa sampled. Lampridiformes appear as a monophyletic group and the sister group to *Polymixia* plus *Percopsis.* Thus the total-evidence analysis corroborates a monophyletic Lampridiformes for the taxa sampled. Within Lampridiformes, this tree recovers the pattern of relationships

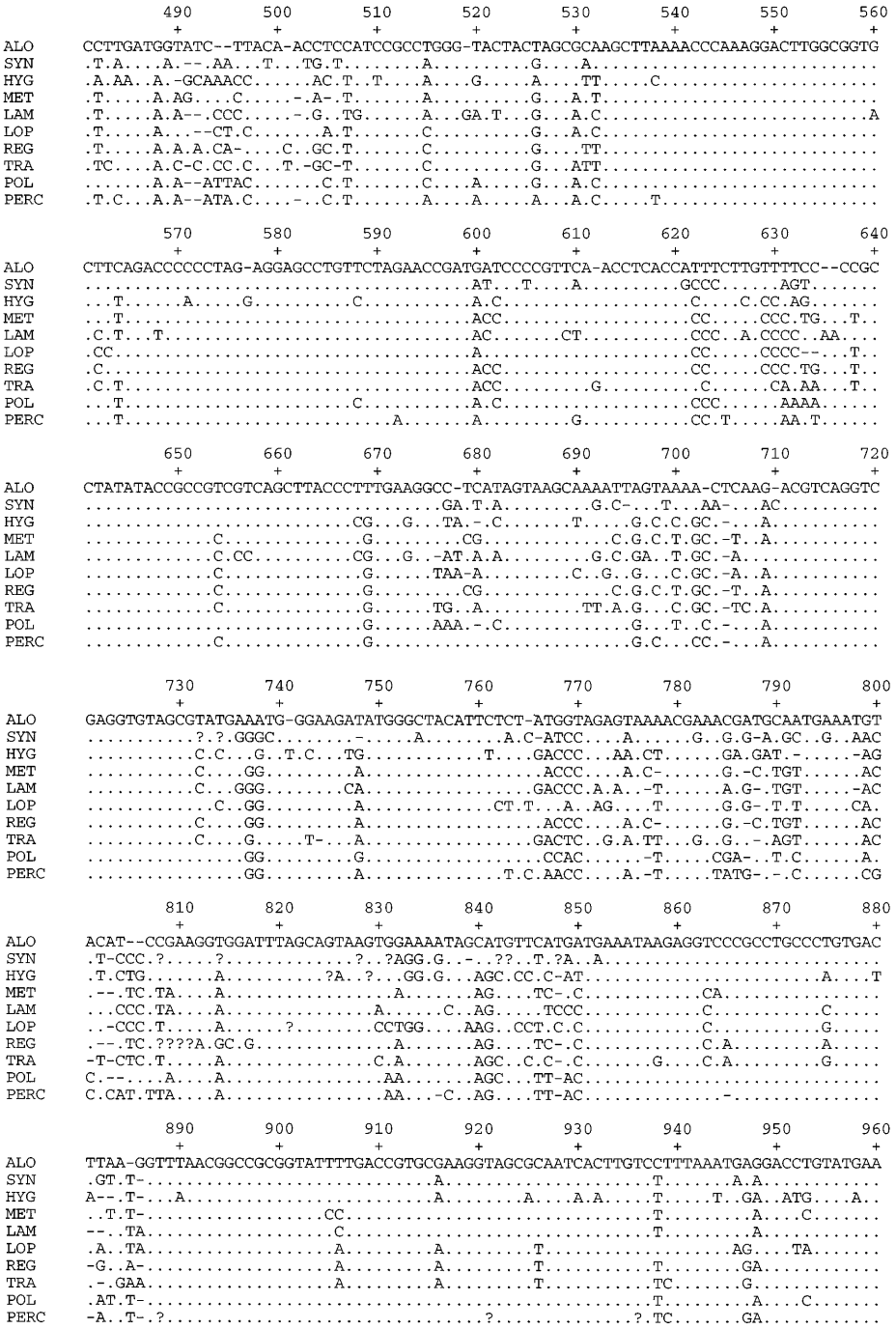

FIG. 2—*Continued*

proposed by Olney *et al.* (1993) and thus refutes Oelschläger's (1983) hypothesis that elongate body form evolved twice within lampridiforms.

We partitioned the total-evidence data set to investigate how different classes of data contributed to our phylogenetic hypothesis. As expected, the morphology-only analysis produced the Olney *et al.* (1993) hypothesis of relationships among Lampridiformes, but did not resolve the position of Lampridiformes relative to *Percopsis* and *Polymixia* (Fig. 5). The 12S mtDNA analysis resolves the basal relationship of Lampridiformes but is characterized by mediocre branch support values and only one "significant" bootstrap value (Fig. 6, corroboration of *Regalecus* plus *Trachipterus*). Thus, the 12S mtDNA data support a tree that is logically consistent with the total-evidence tree if all poorly supported branches are collapsed. The 16S mtDNA analysis resolves the monophyly of lampridiforms, the

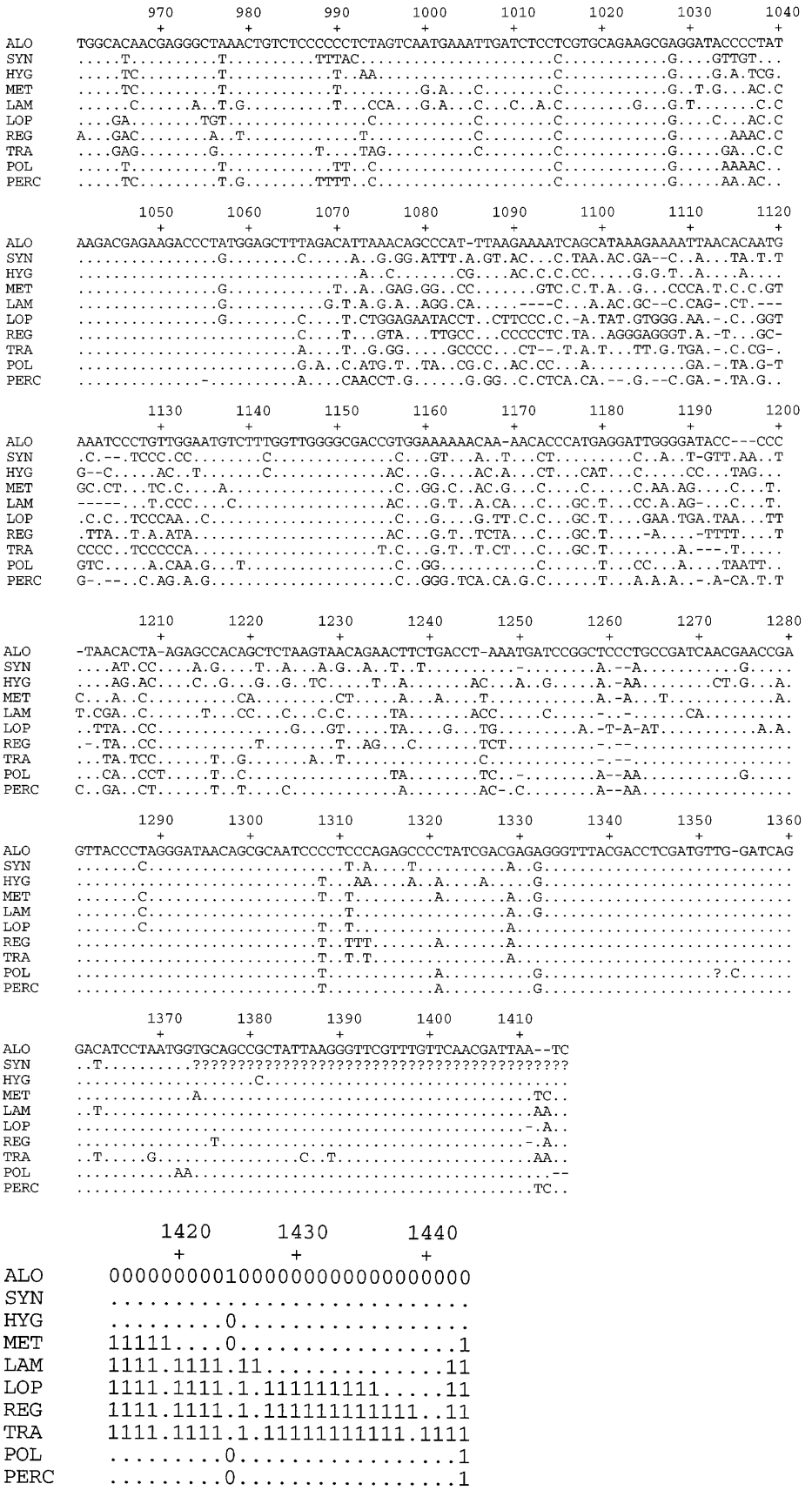

FIG. 2—*Continued*

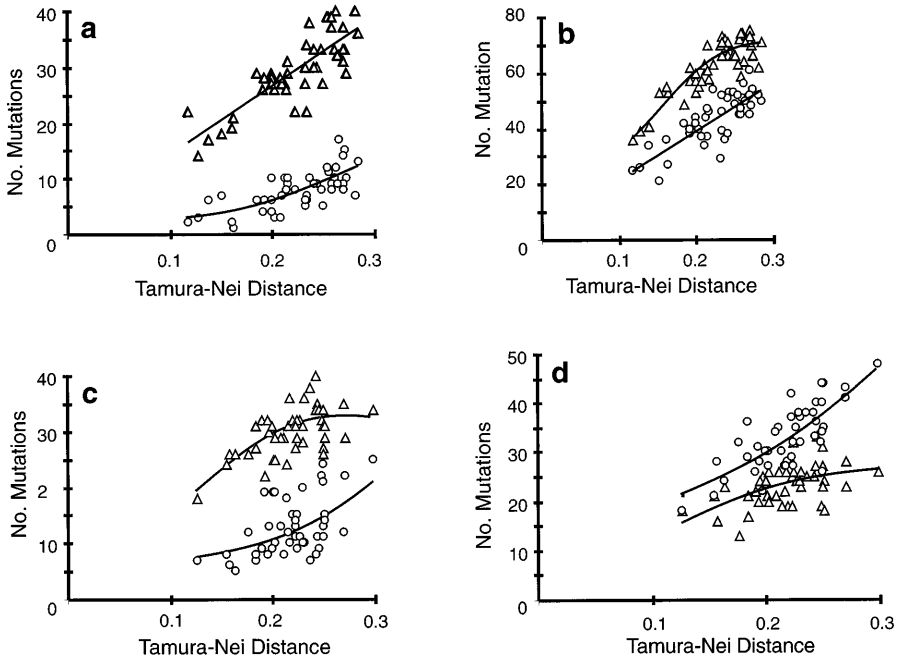

FIG. 3. Scatter-plots of number of mutations over Tamura–Nei genetic distance. (a) 12S mtDNA stem regions. (b) 12S mtDNA loop regions. (c) 16S mtDNA stem regions. (d) 16S mtDNA loop regions. Triangles are transitions and circles are transversions. Second-order polynominals are fitted to each class of data.

relationships among lampridiform species, and the relative positions of *Polymixia* and *Percopsis* in a manner identical to the total-evidence analysis (Fig. 7). However, branch support is mediocre to poor for most of the branches and several different alternative topologies are only a single step removed from the most parsimonious tree.

Combining the two DNA data sets produces a fully resolved tree that is isomorphic with the total-evidence phylogeny (Fig. 8). All internodes, except one, are associated with high branch support values and "significant" bootstrap values. The only marginal internode is

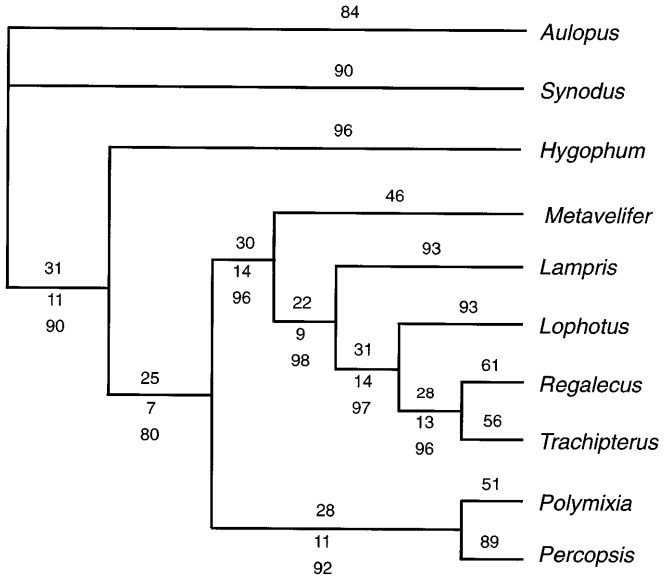

FIG. 4. The single most-parsimonious hypothesis obtained from the total evidence analysis. Numbers above internodes are branch lengths. Numbers below internodes are Bremer branch support values (upper) and bootstrap percentage values (lower). Tree statistics: TL = 954 steps; CI = 0.667; RI = 0.596; RC = 0.398.

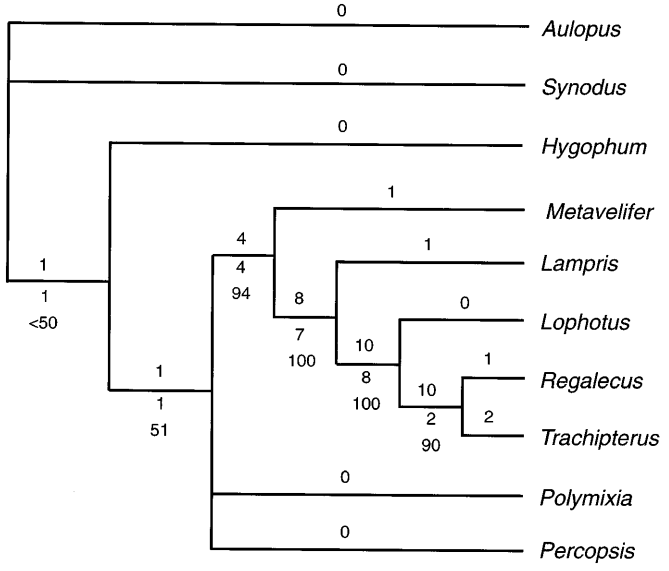

FIG. 5. Analysis of the morphological data. Tree statistics: TL = 29 steps; CI = 0.883; RI = 0.983; RC = 0.949. Values associated with internodes as in Fig. 4.

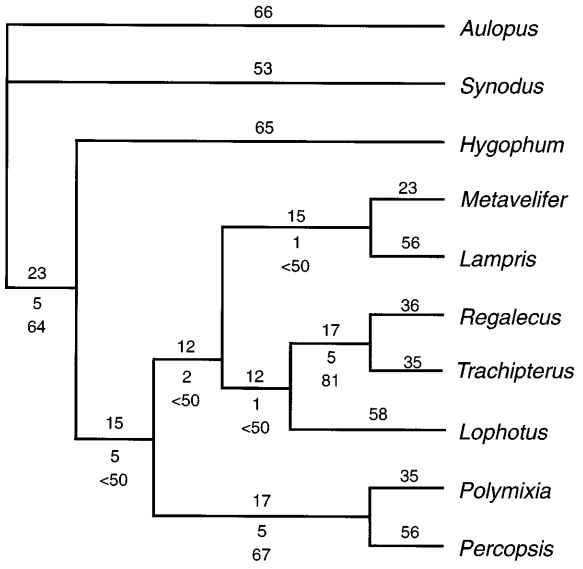

FIG. 6. Analysis of the 12S mtDNA data. Tree statistics: TL = 594 steps; CI = 0.616; RI = 0.383; RC = 0.236. Values associated with internodes as in Fig. 4.

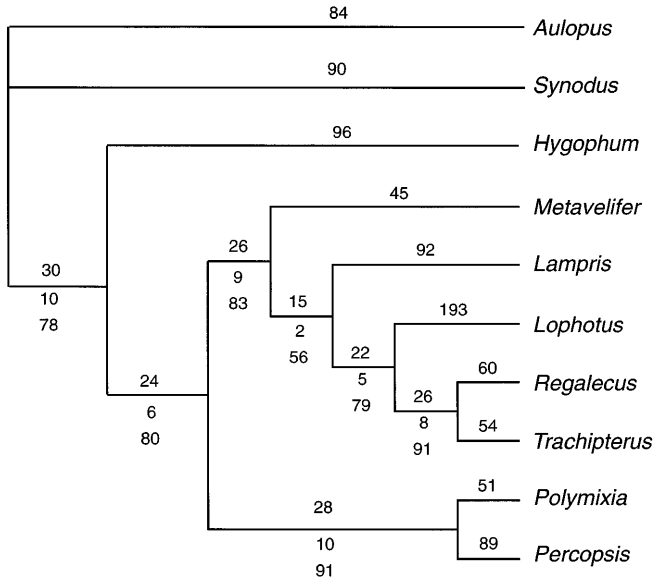

FIG. 8. Analysis of the combined 12S and 16S mtDNA data. Tree statistics: TL = 925 steps; CI = 0.616; RI = 0.383; RC = 0.236. Values associated with internodes as in Fig. 4.

that linking *Lampris* with the higher lampridiforms (branch support = 2, bootstrap = 56%). Tension between the 12S and 16S mtDNA data sets results in the low branch support values for this internode within the context of the DNA-only analysis.

CONCLUSIONS

DNA variation in both 12S and 16S mtDNA regions is relevant to elucidating relationships of lampridiform fishes to other acanthomorphs and to elucidating relationships among genera within Lampridiformes. However, these data must be used in combination and with proper screening for site saturation. Both the total-evidence analysis and the combined-DNA analysis corroborate the monophyly of Lampridiformes. Both analyses also support a monophyletic group composed of *Polymixia* and *Percopsis* (Figs. 4 and 8). This indicates that the 12s and 16s mtDNA gene regions can be used to test the alternate hypotheses of whether Lampridiformes (Johnson and Patterson, 1993; Stiassny and Moore, 1992) or Polymixiiformes (Rosen, 1985; Stiassny, 1986; Patterson and Rosen, 1989; Stiassny and Moore, 1992) is the most basal group of Acanthomorpha. We cannot adequately test these alternative hypotheses until additional putative euacanthomorph groups sensu Johnson and Patterson (1993) are analyzed.

The Olney *et al.* (1993) hypothesis of lampridiform intrarelationships is corroborated by the DNA data independent of the morphological data. Both the 12S and 16S mtDNA data corroborate the hypothesis that *Lophotus* is the sister group of *Regalecus* plus *Trachipterus* and both data sets corroborate the sister group relationship between *Regalecus* and *Trachipterus*. Whereas the 16S mtDNA data set resolved lampridiform relationships in a manner logically consistent with the total-evidence analysis, only the combined DNA data set resolved these relationships with sufficient branch support to form a viable hypothesis. We conclude that the synergism produced by combining DNA data sets is an important ingredient in resolving relationships.

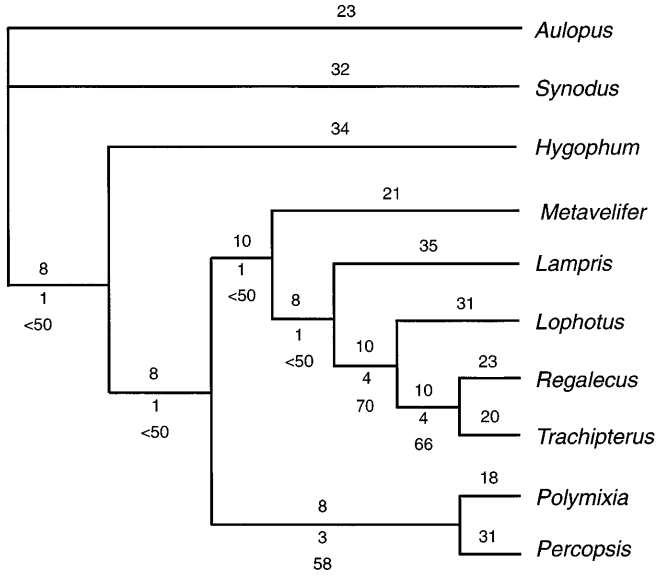

FIG. 7. Analysis of the 16S mtDNA data. Tree statistics: TL = 330 steps; CI = 0.591; RI = 0.384; RC = 0.227. Values associated with internodes as in Fig. 4.

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