Molecular systematics and life history evolution of anglerfishes (Teleostei: Lophiiformes): Evidence from mitochondrial DNA

ANDREW M. SHEDLOCK, THEODORE W. PIETSCH, MARGO G. HAYGOOD, PAUL BENTZEN & MASAMI HASEGAWA

To compare morphological hypotheses of relationship among anglerfish families based on molecular evidence, and to better understand the complex life history of this group of vertebrates, a region of the mitochondrial 16S ribosomal gene for sixteen lophiiform genera, representing eleven families and all anglerfish suborders, plus two batrachoidid outgroups, was amplified via the polymerase chain reaction (PCR), sequenced, aligned and analyzed using parsimony and likelihood methods of phylogenetic inference. The same was done with a region of mitochondrial cytochrome b for a subset of these taxa. Molecular results differed in several aspects from those of previous studies based on morphology, most notably in the position of *Lophius* relative to antennariids. Mitochondrial DNA indicates that chaunacids are the sister of a diverse monophyletic group of deep-sea forms. An mtDNA hypothesis for six deep-sea families provides preliminary evidence that sexual parasitism is not monophyletic and may have emerged rapidly in basal members of the suborder during ceratioid radiation. A density-dependent model for the evolution of parasitism in ceratioids is presented. Lack of informative characters, multiple substitutions at some nucleotide sites, and short branch lengths between the divergence of several deep-sea lineages appear to be limiting statistical confidence at corresponding nodes in the mtDNA tree. More complete taxonomic sampling and data from independent markers in the nuclear genome should provide a better understanding of anglerfish adaptive radiation into the deep ocean.

Keywords: anglerfishes, Lophiiformes, sexual parasitism, mitochondrial DNA

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DEDICATION

Dedicated to the memory of the late Erik Bertelsen (1912–1993), former Curator of Fishes at the Zoological Museum of the University of Copenhagen and pioneer of anglerfish biology.

INTRODUCTION

The order Lophiiformes as currently recognized includes all anglerfishes and is comprised of 18 families, 65 genera and more than 300 living species. All members are marine, inhabiting...
nearshore, benthic, and deep-water pelagic environments. The modification of the first dorsal-fin spine into a luring apparatus is a remarkable adaptation present in nearly all species. Representatives include the goosefishes (Lophioidei), frogfishes, warty anglers, and handfishes (Antennarioidae), sea toads (Chaunacoidae), batfishes (Ogcocephaloidae), and the deep-sea devilfishes, dreamers, and footballfishes (Ceratioidei).

Regan (1912) initially combined anglerfishes and batrachoidids into a single order called the "Pediculati", based largely on the structure of the pectoral arch, but later separated the Lophioidei as a distinct taxon upon consideration of additional characters (e.g., Regan 1926). This distinction has been upheld by the work of subsequent authors (Regan & Trewavas 1932; Gregory 1933; Gregory & Conrad 1936; Eaton et al. 1954; Greenwood et al. 1966; Rosen & Patterson 1969; Pietsch 1981, 1984; Pietsch & Grobecker 1987; Patterson & Rosen 1989).

In his review of early life history and development of lophiiforms, Pietsch (1984) used cladistic methods to establish a systematic hypothesis for family relationships, based on 20 adult osteological and two egg- and larval characters (Fig. 1). He concluded that the Lophioidei is basal to all other members of the order; that the Antennarioidae is the sister group of the Chaunacoidae, Ogcocephaloidae, and Ceratioidei; that the Chaunacoidae is the sister group of the Ogcocephaloidae and Ceratioidei; and that the Ogcocephaloidae is the sister group of the Ceratioidei. Convergence or reversal of several characters used in the analysis was noted (i.e., conditions of the posterior gill arch, gill teeth, and illicial bone). Intra-familial relationships invite further study, particularly within the ceratioid families and the Antennariidae, where polarizing characters for cladistic analyses has remained problematical (Bertelsen 1984; Pietsch 1984; Pietsch & Grobecker 1987, p. 275). In addition, variability of meristic data in some groups, such as members of the Lophiidae, has been observed by investiga-

Fig. 1. Relationships among lophiiform suborders and families, after the morphological studies of Pietsch (1984) and Pietsch & Grobecker (1987).
tors examining both morphological and biochemical traits (Caruso 1981, 1983, 1985; Grant & Leslie 1993; Leslie & Grant 1994).

Eleven families, 35 genera, and about 155 species of deep-sea ceratioids are presently recognized, following revisions by Regan (1912, 1926), Regan & Trewavas (1932), Bertelsen (1951, 1984), and Pietsch (1972, 1976, 1979). Monophyly of the suborder is supported by a dramatic sexual dimorphism in which males are dwarfed, often an order of magnitude smaller than conspecific females (e.g., the largest recorded male of *Ceratias holboelli* is 73 mm (SL) whereas females have been recorded at 770 mm (SL), Pietsch 1976, 1986). In addition, males lack the characteristic illicium and esca that form the external luring apparatus of females, and most have large eyes, large olfactory organs and small pincer-like teeth on the tips of their jaws—a suite of adaptations for locating and attaching to females (Regan 1925; Fig. 2). In addition to pronounced sexual dimor-

Fig. 2. Deep-sea anglerfishes of the suborder Ceratioidei. Top: Female *Linophryne brevibarbata* with parasitic male attached to her venter (after Bertelsen 1980); middle: dwarf male of the *Linophryne arborifera*-group and bottom: *Haplophryne mollis* (both after Munk & Bertelsen 1983).
phism, in which males in particular display a large number of unique and derived character states, monophyly of the Ceratioidei is supported by two other synapomorphies: absence of pelvic fins (except in larval caulophrynids) and absence of teeth on the fifth ceratobranchial (Pietsch 1984; Pietsch & Grobecker 1987; the pelagic life-style of ceratioids might also be considered a unifying feature of the suborder). Thus, while the suborder as a whole is well defined, relationships among ceratioid families are poorly understood, as described below.

Although ceratioids are the most diverse vertebrate taxon in the meso- and bathypelagic marine ecosystem, they are not commonly collected and representatives of some families remain extremely rare. Males are known for only 22 of the 35 recognized genera; consequently present classification of the families and genera is based almost exclusively on characters that define females. While osteological characters of female ceratioids allow separation into more or less well-defined families, the phylogenetic relationships among these families has remained impossible to infer with any confidence. In his detailed 1984 review of the ontogeny and systematics of the suborder, Bertelsen listed 30 morphological characters that he used to propose the hypothesis shown in Fig. 3, which he states “should be regarded only as a very schematic compilation of expressed views” (i.e., those of Regan 1912, 1926; Regan & Trewavas 1932; Bertelsen 1951; Pietsch 1972, 1979). He summed up the difficulty of working on the systematics of the group as follows: “The phylogenetic relationships between the families of the Ceratioidei are still uncertain. The main reason for this is that most of the derived osteological characters shared by two or more families are reduction states or loss of parts... and similarities in such characters in many cases represent convergent developments. At the same time most of the diagnostic family characters which represent new structures or specialization of organs are autapomorphic.”

Fig. 3. Ceratioid relationships as proposed by Bertelsen (1984). Sexual parasitism indicated by O for obligate, F for facultative, and – for non-parasitic. Parentheses indicate poorly understood status.
Male parasitism

One of the life history traits of ceratioids that warrants special consideration is male sexual parasitism, unique among vertebrates. Although this trait is often popularly associated with all deep-sea anglerfishes, only ten genera among five of the 11 ceratioid families exhibit evidence for permanent male attachment to females (Fig. 3). Originally discovered 80 years ago by Sæmundsson (1922) and Regan (1925), the ecological and physiological facts surrounding sexual parasitism in these enigmatic animals remain unclear, despite the important comparative work of Bertelsen (1951), the histological studies of Munk & Bertelsen (1983), a comprehensive review by Pietsch (1976), and DNA typing of a parasitized female by Saruwatari et al. (2001). Dwarf males of some taxa bite onto and become permanently and parasitically attached to females and presumably are completely dependent on host females for sustenance. Subsequent tissue fusion between partners can be extensive as the male degenerates into what forms a small functional appendage of the female. Pietsch (1976) added substantial insight into the variable nature of the phenomenon by examining all available museum material and presenting evidence in support of three different possible modes of reproduction among ceratioids: obligate parasitism, facultative parasitism, and non-parasitism. However, because of the unknown phylogeny among deep-sea families, the evolutionary pattern of this unique life history trait is difficult to understand. For example, the polarity of the transition between non-parasitism and obligatory parasitism cannot be clearly interpreted from available evidence. Pietsch initially proposed a monophyletic origin of sexual parasitism from some oneirodid-like ancestor (1976), and based on a subsequent analysis (1979) considered the trait to be diphyletic. The most recent hypothesis proposed by Bertelsen (1984) implies that the character has arisen independently at least three times.

MATERIALS AND METHODS

DNA extraction and sequencing

Live, frozen, ethanol- and formalin-fixed anglerfish samples were assembled and archived with associated documentation over several years at the University of Washington Fish Collection (see Appendix 1). Symbolic codes for all institutions cited are as follows: AM: Australian Museum, Sydney, Australia; BMNH: British Museum (Natural History), London, England; IOAN: Institute of Oceanography, Russian Academy of Sciences, Moscow, Russia; ISH: Institut fur Seefischerei, Hamburg, Germany; LACM: Natural History Museum of Los Angeles County, Los Angeles, California, USA; MCZ: Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; OSUO: Oregon State University, Department of Oceanography, Corvallis, Oregon, USA; ROM: Royal Ontario Museum, Toronto, Canada; SIO: Scripps Institution of Oceanography, University of California, La Jolla, California, USA; USNM: National Museum of Natural History, Washington, D.C., USA; UW: School of Aquatic and Fishery Sciences, University of Washington, Seattle, Washington, USA; ZMUC: Zoological Museum, University of Copenhagen, Denmark. Representatives for five rare deep-sea families could not be included in the present molecular study, although all lophiiform suborders are represented.

Table 1 shows the taxa, number of specimens, and genes analyzed in the present study, along with Genbank accession and museum voucher numbers. DNA was extracted and amplified using the polymerase chain reaction (PCR; Saiki et al. 1988) from frozen or ethanol-preserved muscle or liver tissues using phenol and chloroform methods and PCR conditions outlined in detail by Shedlock et al. (1992, 1997). Primers for 16S rRNA were obtained from Palumbi et al. (1991): 16SaL 5’-cgc ctg ttt atc aaa aac at-3’ and 16SbH 5’-ccg gtc tga act cag atc acg t-3’. Cytochrome b primers were obtained from Kocher et al. (1989) and Irwin et al. (1991): H15148 5’-aaa ctg cag ccc ttc act tga act cag atc acg t-3’. These two different functional regions of the mtDNA genome were chosen because of their systematic informativeness across a range of divergence times and previous characterization in fishes (Wilson et al. 1985; Thomas & Beckenbach 1989; Smith 1989; Meyer et al. 1990; Meyer &
Wilson 1990; Martin & Palumbi 1992; Block et al. 1993; Alvez-Gomes et al. 1995; Kocher & Stepien 1997; Wiley et al. 1998; Tang et al. 1999). Outgroup species were chosen to minimize artifacts of mutational saturation from excessive levels mtDNA sequence divergence. Two species were selected from the order Batrachoideiformes, *Opsanus tau* and *Porichthys notatus*, based on the affinity of “pediculate” fishes initially classified by Regan (1912, 1926) and subsequent revisions by others (Regan & Trewavas 1932; Gregory 1933; Gregory & Conrad 1936; Eaton et al. 1954; Greenwood et al. 1966; Rosen & Patterson 1969; Pietsch 1981, 1984; Pietsch & Grobecker 1987; Patterson & Rosen 1989) that all underscore the close phylogenetic relationship between the Batrachoideiformes and Lophiiformes.

Sequencing was done using Applied Biosystems Inc. (ABI) model 373A Automated DNA Sequencers operated at the University of Washington Marine Molecular Biotechnology Laboratory, Seattle, and at the Marine Biological Resources Division of the Scripps Institution of Oceanography, University of California, San Diego. Successful PCR products amplified from 50–100 ng of template in 25 mL reactions were isolated and cleaned using Microcon microcentrator filters (Amicon, Inc., Beverly, MA) or Qiaquick PCR cleanup columns (Qiagen, Inc., Chatsworth, CA) and used as templates for automated fluorescent sequencing with ABI Prism Cycle Sequencing *Taq* dye terminator kits according to manufacturer’s specifications.

**Data analysis**

Both strands of PCR fragments were sequenced and aligned visually in conjunction with Clustal

<table>
<thead>
<tr>
<th>Family</th>
<th>Species (Number of Specimens) Voucher Ref.</th>
<th>mtDNA Sequence (Genbank Accession)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batrachoididae</td>
<td><em>Porichthys notatus</em> (1) UW 21590</td>
<td>16S (AY292598)</td>
</tr>
<tr>
<td></td>
<td><em>Opsanus tau</em> (2) UW 48066, UW 48067</td>
<td>16S (AY292597)</td>
</tr>
<tr>
<td>Lophiidae</td>
<td><em>Lophius americanus</em> (3) UW 48064, UW 48065, UW uncatalogued tissues</td>
<td>16S (AY292589), Cyt b (AY292608)</td>
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<tr>
<td>Antennariidae</td>
<td><em>Antennarius nummifer</em> (1) UW 48070</td>
<td>16S (AY292595), Cyt b (AY292606)</td>
</tr>
<tr>
<td></td>
<td><em>Antennarius avalonis</em> (1) UW 20766</td>
<td></td>
</tr>
<tr>
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<td><em>Antennarius maculatus</em> (1) UW 20767</td>
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<tr>
<td></td>
<td><em>Antennarius radiosus</em> (1) MCZ 144916</td>
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<tr>
<td></td>
<td><em>Antennarius striatus</em> (1) UW 20768</td>
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<tr>
<td></td>
<td><em>Histrio histrio</em> (1) UW 48052</td>
<td>16S (AY292596)</td>
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<td></td>
<td><em>Tachycarpus butleri</em> (1) AM IB.3043</td>
<td>16S (AY292591)</td>
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<td>Tetrabrachiidae</td>
<td><em>Tetrabrachium ocellatum</em> (1) AM IB.7178</td>
<td>16S (AY292592)</td>
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<td>Ogcocephalidae</td>
<td><em>Halieutichthys aculeatus</em> (1) UW 21629</td>
<td>16S (AY292593), Cyt b (AY292607)</td>
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<td><em>Dibranchus atlanticus</em> (1) MCZ 51257</td>
<td>16S (AY292594)</td>
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<td>Chaunaciidae</td>
<td><em>Chaunax pictus</em> (1) UW 20770</td>
<td>16S (AY292590)</td>
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<td>Melanocetidae</td>
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<td>16S (AY292587), Cyt b (AY292605)</td>
</tr>
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<td>Himantolophidae</td>
<td><em>Himantolophus appellii</em> (1) UW 22179</td>
<td>16S (AY292586)</td>
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<td>Oneirodidae</td>
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<td>16S (AY292583), Cyt b (AY292601)</td>
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<td><em>Oneirodes thompsonii</em> (1) UW 22083</td>
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<td><em>Bertella idiomorpha</em> (1) UW 42301</td>
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<td>Ceratiidae</td>
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<td>Gigantactinidae</td>
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<td>Linophrynidae</td>
<td><em>Linophryne bicorns</em> (1) MCZ 138063</td>
<td>16S (AY292588), Cyt b (AY292603)</td>
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</table>
MOLECULAR SYSTEMATICS AND LIFE HISTORY EVOLUTION OF ANGLERFISHES

V, as included in Sequence Navigator v. 2.0 (Applied Biosystems, Foster City, CA). The relatively conserved regions employed in this study did not create significant alignment difficulties. Amino acid translation (cytochrome b) and secondary structural considerations (16S rRNA) of published fish sequences available for these mtDNA regions in the literature and in Genbank (Block et al. 1993; Alvez-Gomes et al. 1995; Wiley et al. 1998; Tang et al. 1998) aided alignment, particularly for regions in the 16S matrix that are noted for length polymorphisms in single stranded conformations of this ribosomal gene. The complete, aligned data matrix is available from the corresponding author upon request. DNA sequences for taxa investigated are available from Genbank under the accession numbers listed in Table 1.

A total of 513 and 318 aligned nucleotide sites were analyzed for 16S (18 taxa) and cytochrome b (10 taxa), respectively. Sequences of multiple individuals for the same taxa in Table 1 were identical or nearly identical and the consensus was included in the data set. Analysis of the 16S sequence matrix was completed both with and without insertions and deletions (indels) coded by gaps in the alignment. A combined analysis including data from both genes was also completed (10 taxa). The percentage of sequence divergence (Mindell & Honeycutt 1990) was calculated for all possible binary comparisons of the data. Maximum parsimony (MP) was employed using PAUP*, v. 4.0 (Swofford 1996). Branch and bound (Hendy & Penny 1982) searches for most parsimonious trees were conducted for each analysis with five hundred bootstrap replicates (Felsenstein 1985). One thousand random trees were generated from each search to evaluate relative phylogenetic information content as indicated by the g statistic of tree-length distribution skewness (Huelsenbeck 1991). Using step matrices, transversions (tv) were weighted twice that of transitions (ti) in order to account for observed levels of saturation (multiple substitutions at a single site) of ti at high divergence and at 3rd codon positions in cytochrome b.

We also employed maximum likelihood (ML) with the program NucML in the MOLPHY package (v. 2.3) (Adachi & Hasegawa 1996) using the HKY85 substitution model (Hasegawa et al. 1985). The NucML analysis was completed with the local rearrangement (nearest-neighbor branch interchange) option starting from a neighbor-joining tree. For each internal branch, the percentage probability was estimated with the Resampling of Estimated Log-Likelihoods (RELL) method subject to $10^4$ replications. In the local rearrangement procedure, local bootstrap probabilities were estimated after fixing the relationships within subtrees in each step (Adachi & Hasegawa 1996). A 5% chi-square test was conducted to compare the empirical nucleotide composition of each sequence to the frequency distribution assumed in the ML model by using the TREE-PUZZLE program (Strimmer & von Haeseler 1996).

RESULTS

Observed substitution profiles

The 16S sequence divergences for ingroup taxa (see Table 1 for families containing genera listed below) range from 3.3% for inter-generic comparisons (Bertella vs. Oneirodes) to 25.3% between distant suborders (Melanocetus vs. Tetra-brachium); cytochrome b sequence divergences ranged from 12.8% (Bertella vs. Oneirodes) to 26.7% (Ceratias vs. Halieutichthys). The average ratio of ti/tv was 1.9 for 16S comparisons and 2.1 for cytochrome b. The number of constant sites in the alignment was 183/513 (35.7%) and 171/318 (53.8%) for 16S and cytochrome b, respectively. The number of substitutions per site in the 16S analysis was distributed unevenly, as expected for a ribosomal gene, with some highly conserved regions adjacent to highly variable positions. With respect to secondary structure of 16S rRNA, the most variable sites were associated with single-stranded loops; however, neither stem nor loop sites exhibited a consistent pattern of substitution (e.g., portions of loops were conserved; portions of some stems were variable). Clear amino acid codon position effects were evident for cytochrome b, with approximately five times the number of substitutions at 3rd codon positions than at either position 1 or 2.

Phylogenetics

Both MP and ML analyses provided consistent
tree topologies among suborders and differed only in the relative position of *Gigantactis* and *Ceratias* within the Ceratioidei. Excluding indels from the 16S analysis also changed the relative position of these two taxa. For both methods of inference, 16S rRNA data was more phylogenetically informative than the smaller cytochrome b region analyzed for a subset of taxa. The optimal NucML tree for the 16S data with local bootstrap percentage probabilities is shown in Figure 4. This tree diagram is the best supported hypothesis of lophiiform phylogeny obtained from available sequences. MtDNA results support the relationships proposed by Pietsch (1981, 1984) in the placement of ceratioids as the most derived clade in the Lophiiformes, but place *Chaunax* as the sister of the ceratioid families and nest *Lophius* deeply within the order in contrast to the basal position in morphological studies. Antennarioid families are basal in the ML tree to the remaining lophiiform suborders. RELL local bootstrap probabilities were highest (93–100%) for the relationships among the antennarioid families and ogcocephalid genera examined. The placement of *Tetrabrachium* as a distinct lineage basal to other lophiiform families contrasts with expectations from morphology and biogeography. P-values of the chi-square tests for nucleotide composition variance in the 16S sequence alignment for *Tetrabrachium* and *Tathicarpus* were extremely
low: 0.58% and 7.74%, respectively, with Tetra-
branchium failing the test at the 5% threshhold. 
These results indicate a significant difference in 
base composition for these species relative to all 
ingroup taxa investigated. Branch lengths are 
relatively short between ceratioid families and 
shortest between oneirodid genera. The molecu-
lar hypothesis is congruent with some of the 
relationships diagrammed by Bertelsen (1984), 
but is markedly different in other aspects. In 
particular, linophrynids are indicated as the sister 
of remaining ceratioid families examined but are 
placed much deeper within the suborder based on 
morphological considerations. Relative positions 
of Gigantactis and Ceratias remain ambiguous 
(42% bootstrap), but are basal to the ((himant-
tolophid, melanocetid) oneirodid) group (73% 
bootstrap) in contrast to their position in Ber-
telsen’s tree. Monophyly of Himantolophus and 
Melanocetus is strongly supported (92% boot-
strap) and together these two taxa form the sister 
group of the Oneirodidae.

Trees from other analyses were less well sup-
ported and are not shown. The MP analysis of 16S 
sequences yielded 2 equally parsimonious trees 
(1308 steps, g = -1.24) identical in topology to the 
ML tree in Fig. 4 except for internal branching 
patterns among oneirodid genera and the relative 
positions of Ceratias and Gigantactis. Removal 
of indels from the MP analyses under the same 
search parameters also gave 2 equally optimal 
trees (1033 steps, g = -1.23) identical in topolo-
gies to those yielded by the full 16S data set, but 
did not improve support among ceratioid fami-
lies. MP analyses of cytochrome b sequence in-
formation by itself gave poor resolution of taxa (1 
tree at 453 steps; g = -0.4), with bootstrap values 
of this treatment well below 50% for all nodes. 
The cytochrome b NucML hypothesis (318 sites/ 
10 taxa) provided support for a monophyletic 
Ceratioidei (64% bootstrap). This result was 
strengthened by the MP analysis of the combined 
matrix (831 bp, 10 taxa) which yielded a single 
most parsimonious tree (1105 steps; g1 = -0.51) 
with the strongest statistical support (81% boot-
strap) separating ogcocephalids from the cer-
atioid suborder in the absence of data for Chaunax.

DISCUSSION

Inference from mtDNA sequences

The present study represents the first molecular 
investigation of anglerfish phylogeny, and the 
first attempt to resolve ceratioid relationships 
using advanced methods of inference under the 
optimality criteria of parsimony and likelihood. 
Results from the present small heterogeneous 
data matrix suggest that 16S rRNA and cyto-
chrome b sequence comparisons can provide use-
ful systematic information to understand lophii-
form evolutionary history. It is reasonable to ex-
pect that analyzing additional mtDNA sequences 
per taxon for these genes would resolve lophii-
form relationships with better confidence; how-
ever, multiple substitutions are also apparently 
creating noise at the most variable positions given 
high sequence divergences (e.g., > 20%) between 
some suborders. This in part could explain the 
inability to resolve lophiform relationships using 
only a small set of cytochrome b information 
a lone, where high levels of substitution are evi-
dent for 3rd codon positions but not enough 
informative changes at slower sites appear to be 
present to improve inference. This potential 
shortcoming of cytochrome b data has been re-
viewed in detail by Meyer (1994). Rapid diver-
genesis of lophiform lineages within a short time 
frame may also be contributing to weak resolu-
tion with molecular data. This seems apparent 
among ceratioid families and oneirodid genera 
where the shortest MP and ML branch lengths 
between taxa are evident. In this respect the 
grouping of Ceratias and Gigantactis is particu-
larly fragile in the present analysis. Rate variation 
among sites is also less simple to assess in ribos-
omal genes, such as 16S, where differential func-
tional constraints appear to act in complex ways 
related to secondary structure. MP and ML analy-
izes produced the same relationships among all but 
two ceratioid families, indicating a robustness in 
the result in relation to different analytic algo-
rithms. However, ML provided better statistical 
support for most relationships. It should be noted 
that local bootstrap probabilities produced by the 
NucML tree must be interpreted cautiously be-
cause they can be misleading if the topology 
within respective subtrees attached to the branch 
is incorrect. The local bootstrap value can be
considered equivalent to standard bootstrap probability of a particular internal branch when the other parts of the tree are correct. Given the consistent topologies among lophiiform suborders across MP and ML analyses we do not expect this discrepancy to be a prohibitive problem for the hypothesis in Fig. 4.

Taxon sampling and outgroup selection can have a critical influence on the resulting tree topologies obtained from a given dataset. Simulations using real sequence data have confirmed that adding more taxa rather than more data may be the most effective approach to obtaining true phylogenies in certain instances (Hillis 1996). This issue is relevant to the present study in two obvious ways: 1) for practical reasons, rare families and numerous genera could not be included in the dataset; and 2) only a subset of lophiiform taxa in the 16S dataset were available for the cytochrome b analysis. The addition of more taxa and more characters should substantially improve the limited picture available from this initial molecular study of anglerfish evolution. Given the extreme rarity of some forms, the advance of extracting and sequencing DNA from archival specimens is well worth pursuing, as outlined by Shedlock et al. (1997). Based on the present results, adding more mtDNA sequences may not prove more useful than analyzing more slowly evolving nuclear genes for resolving the deepest divergences among lophiiform lineages. This is especially pertinent for resolving relationships of ceratioid families with long-branch lengths that have diverged in short succession in the distant past. A comparison of mitochondrial and nuclear gene data is a promising direction for future molecular systematic study of this diverse order of fishes.

Lophiiform phylogeny

In the present analysis, the Antennarioidei is the sister of all remaining lophiiform suborders recognized by Pietsch (1984, Fig. 1) and is composed of the families Tetrabrachiidae and Antennariidae, with Tetrabrachium placed as the most basal member of the clade. Aspects of antennariid relationships from DNA data are of particular interest because no osteological synapomorphies exist among antennariid genera. Pietsch (in Pietsch & Grobecker 1987, p. 275) states in his review of world antennariids that “Whereas a hypothesis of monophyly for each of the remaining genera can be supported, their phylogenetic relationships as elucidated by cladistics remain largely unknown... Consequently, it appears that new characters, most likely non-osteological ones, must be identified and analyzed before the interrelationships of antennariid genera can be resolved.” The evidence from 16S rRNA sequences suggests with high bootstrap probabilities (93–100%) that Histrio is more closely related to Antennarius than is Tathicarpus, which forms a separate derived lineage relative to other antennariids examined, and is immediately basal to ogcocephalids (Fig. 4). Pietsch (in Pietsch & Grobecker 1987, p. 275) noted in his inconclusive search for synapomorphies among antennariid genera that the pectoral lobe in both Histrio and Tathicarpus is detached from the side of the body as it is also in the more derived ogcocephalids. In the same volume, Tathicarpus is also entered as the most derived genus in the key to known genera of the Antennariidae due to its distinctive morphology relative to other frogfishes (Pietsch & Grobecker 1987). The estimates of ML branch lengths and the amount of rRNA sequence divergence between Tathicarpus and both Histrio and Antennarius are on the same order or greater than those found between members of recognized families in different suborders. These results warrant some consideration of Tathicarpus as a possible candidate for separate family status, although a more comprehensive genetic examination of antennariid genera is badly needed, especially among other Indo-Australian taxa with restricted zoogeographic ranges. This is apparent from placement in the mtDNA hypothesis of Tetrabrachium as the sister lineage of remaining lophiiform families. Pietsch (1981) and Pietsch & Grobecker (1987) recognized Tetrabrachium as the only member of the sister family of the Antennariidae based on the shape and orientation of the vomer and premaxilla and the reduction of the opercle. Although the bootstrap support is 100% for the placement of Tetrabrachium relative to other lophiiform families, it is difficult to reconcile this arrangement with the biogeography and morphology of antennariids. It is worth noting that the p-values of the chi-square tests for nucleotide composition variance in the
16S sequence alignment for *Tetrabrachium* and *Tathicarpus* were extremely low: 0.58% and 7.74%, respectively, with *Tetrabrachium* failing the test at the 5% threshold. It is reasonable to assume from chi-square results that skewed base compositional differences in the sequences of these two relatively unique Indo-Australian species may be creating artifacts for phylogenetic inference under both MP and ML criteria (Collins et al. 1994, Lockhart et al. 1994).

The systematic positions of the ogcocephalid genera *Halieutichthys* and *Dibranchus*, the lophiid genus *Lophius*, and the chaunicid genus *Chaunax* in this analysis represent an interesting departure from that of previous hypotheses. These forms possess a suite of adaptations for living as benthic nearshore and continental slope predators. Pietsch (1984) distinguished the Lophioidei, Chaunacioidae and Ogcocephaloidei from the more inclusive Antennarioidei of Regan (1912). Lophioids have been traditionally considered the sister group of all other lophiiforms (Regan 1912, Pietsch 1984, Pietsch & Grobecker 1987), and in light of this, the more derived position of *Lophius* in the present molecular study is rather striking. The examination of two functionally distinct mtDNA genes and three separate frozen individuals of *Lophius* from different sources makes the possibility of contamination artifacts or inadvertent analysis of mtDNA pseudogenes extremely unlikely in this case. Because of their commercial importance to coastal fisheries, the morphological variation, zoogeography, and genetics among species of lophioids has been considered in relative detail. Caruso (1983, 1985) used meristic and morphometric measurements to review the systematics of the family. Leslie & Grant (1990, 1991, 1994) and Grant & Leslie (1993), elaborating on Caruso’s meristic methods, employed protein electrophoresis to show that meristic and morphometric traits in this family vary with temperature regimes, are convergent among divergent species and do not reflect the population structure of the group inferred from allozyme variation. The extent of such phenotypic plasticity in this and other shallow-water lophiiform families is difficult to assess and invites further examination, but is expected to be less problematic for non-meristic and non-morphometric characters. In this vein, the recent description of the developmental scheme for *Lophius americanus* by Everly (2002) provides a foundation for additional comparative work on the ontogeny of lophiiform suborders. Comparisons of dorsal-fin spine development and the relationship between egg diameter and the timing of gastrulation in *Lophius americanus* and *Antennarius striatus* show potential for providing new phylogenetic information relevant to evaluating suborder relationships presented by Pietsch & Grobecker (1987) as well as those inferred from mtDNA.

The position of *Chaunax* in the molecular hypothesis as sister of the deep-sea ceratioids differs from that of Pietsch & Grobecker’s (1987) hypothesis, in which chaunacids are basal to ogcocephalids plus ceratioids. Members of both these families are deep-water benthic fishes with pelagic larvae. Mead et al. (1964) pointed out that larval and “young specimens” of *Chaunax* are often caught bathypelagically. The rare genus *Bathychaunax* is known from depths up to 2200 meters (Caruso 1989) and the rare ogcocephalid genus *Halieutopsis* has been collected as deep as 4000 meters (Bradbury 1988). Bertelsen (1984, p. 330) hinted at the possibility of either ogcocephalids or chaunacids giving rise to deep-sea anglerfishes: “We may assume an ogcocephalid- or chaunacid-like ancestral ceratioid which, from the benthic and littoral environment of its ancestors, has invaded the bathypelagic zone of the ocean. Probably the evolution has passed through forms in which the adults were benthic, while the juveniles after metamorphosis continued the pelagic life of the larvae during adolescence as for instance found in the family Chaunaciidae and as retained or re-established in the genus *Thaumatichthys*.”

In agreement with the morphological hypothesis of Pietsch & Grobecker (1987), the mtDNA hypothesis shows ceratioids to be the most derived lophiiform suborder. Bertelsen (1984, p. 326) listed 30 characters, ranging from fin-ray counts to cranial osteology to general body shape, and proposed a provisional diagram of relationships for the 11 ceratioid families. However, in the same paper he admitted that “the relationships between the families of the Ceratioidei are still uncertain” because of the paucity of synapomorphies, probable character convergence, reductive
trends in morphology, and a general difficulty in establishing character polarity in the group. To date no previous phylogenetic analysis of ceratioid phylogeny has been attempted using modern analytical approaches.

The relationships among ceratioid families based on DNA sequences are markedly different from those of Bertelsen (1984), although some clear similarities exist. The Oneirodidae remains monophyletic, but is one of the most derived families in this analysis. Also, a relatively close relationship between the Himantolophidae, Melanocetidae, and Oneirodidae is supported by molecular data (73% bootstrap). Linophryne, representing one of the most derived families in Bertelsen’s hypothesis, is basal to all ceratioids examined here (80% bootstrap). The relative positions of Ceratias and Gigantactis are the most fragile relationships inferred from the present study and must remain inconclusive based on the low bootstrap percentage probability of only 42%. Relative branch lengths among ceratioid taxa suggest that the group diverged relatively quickly, presumably in association with adaptive radiation into the deep ocean environment. Incomplete taxon sampling could have an important influence on the ceratioid relationships in this analysis, given that representatives of five deep-sea families are missing from the 16S data matrix. Given the near absence of a fossil record for the Ceratioidei (see Pietsch & Lavenberg 1980), the historical diversity of this suborder also remains completely unknown, and the distribution of extant taxa is extremely uneven, with two monotypic families apparent as well as extremely diverse groups such as the Oneirodidae with 16 genera and 62 species. It is conceivable that entirely new families of extant ceratioids remain to be discovered as more complete exploration and sophisticated sampling of the meso- and bathypelagic ocean are realized. The mtDNA hypothesis presented here offers an important new perspective on the evolutionary history of this enigmatic assemblage of fishes. The molecular phylogeny is used in the following section to consider the evolution of a parasitic mating system that is unique among vertebrates.

**Sexual parasitism among ceratioids**

Pietsch (1976) reviewed the reproductive biology of ceratioid families based on available information in the literature and from a comprehensive survey of preserved museum specimens. He presented data in support of three reproductive modes: permanent or obligate male parasitism among the families Ceratiidae, Linophrynidae, and possibly the Neoceratiidae; no parasitism in the Melanocetidae, Himantolophidae, Gigantactinidae, and three of the better known genera of the Oneirodidae; and facultative or temporary parasitism in the family Caulophrynidae and in the oneirodid genus *Leptacanthichthys*. Pietsch (1976) proposed a scenario whereby an oneirodid-like ancestor with facultative parasitic reproduction could have given rise to a monophyletic assemblage of other families exhibiting parasitism, then subsequently (1979) proposed a diphyletic origin. Bertelsen (1984) later constructed a hypothesis requiring at least three independent losses and gains of male parasitism among eleven families in the suborder.

Mapping the evolution of male parasitism onto the molecular phylogeny suggests yet a different scenario as to how this remarkable trait may have evolved. Evidence from mtDNA places the obligately parasitic linophrynids and ceratiids basal to those with no known parasitism (melanocetid-himantolophilid group). Although the relative positions of gigantactinids and ceratiids remain equivocal, there is no molecular evidence to suggest that they are more derived than the Himantolophidae or Melanocetidae or the non-parasitic oneirodid genera examined. As presented in the mtDNA hypothesis, the position of *Gigantactis* represents a loss of male parasitism over a very short evolutionary period. Within a molecular phylogenetic context, the oneirodid *Leptacanthichthys* exhibits possible secondary gain of an intermediate, facultative mode of this life history trait (note that a female specimen of the oneirodid genus *Bertella* with an attached parasitic male has recently been identified by Pietsch, unpublished data, and may thus be added to those ceratioid taxa displaying a possible facultative mode of sexual parasitism). Adding the rare families Neoceratiidae and Caulophrynidae, both parasitic taxa, to the molecular dataset should provide an even clearer picture of the evolution of this trait. However, the present analysis needs no elaboration to warrant reconsideration of some
existing ideas about the pace and relative plasticity of sexual parasitism through evolutionary time.

Molecular data, both in terms of topology and relative short branch length, do not support the monophyletic condition of sexual parasitism (sensu Pietsch 1976), but rather suggest an abrupt appearance and subsequent loss of sexual parasitism during ceratioid evolution, possibly in the process of being secondarily gained in the Oneirodidae. The apparent plasticity of this life history strategy is more consistent with the multiple gains and losses of male parasitism required by Bertelsen’s (1984) hypothesis.

A dynamic model for the successive gain and loss of sexual parasitism in ceratioids would include a chaunacid-like ancestor colonizing a new adaptive zone in the bathypelagic, where sexual dimorphism could evolve rapidly. It is reasonable to expect that density-dependent factors could drive selection toward or away from male parasitism in these deep-ocean fishes. If available females are rarely encountered in the mesopelagic expanse, then the opportunity for mate choice would be drastically reduced in males. Females would be expected to remain large to accommodate fecundity. Males on the other hand would be expected to shrink to reduce metabolic costs, and to develop acute homing abilities for finding conspecific females. If a male finds a mate in such a low-density population, attaching to her parasitically could improve lifetime fitness relative to the prospect of searching for additional mates as a free-living individual. Higher densities of male-female encounters could relax selection on obligate male parasitism, perhaps through facultative association as described by Pietsch (1976).

Molecular results also promote a cautious consideration of what we actually do and do not know about this intriguing phenomenon. The permanent parasitic attachment of males fused to females is by far the most conclusive and meaningful character we have in studying this system where observation of living animals is impossible. A lack of specimens, especially for the rarest families, namely the Diceratiidae, Thaumatichthyidae, and Centrophrynidae makes it impossible to infer with confidence whether or not male parasitism exists in these taxa. Until more ceratioid specimens become available for both morphological and molecular analyses, our understanding of their unique life history and evolutionary biology will remain provisional.

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Appendix 1. List of material examined.

Note: All measurements in standard length unless indicated by TL (total length).

Batrachoididae: Batrachoides pacifici, MCZ 41805, 153 mm. – Daector dowi, LACM 31310-19, 97 mm. – Op sanus tau, UW 48066, 260 mm; UW 48067, 290 mm. – Porichthys notatus, LACM 22083, 114.5 mm; UW 21590, 140 mm.

Lophiidae: Lophioleodes caulinaris, MCZ 51260, 35.5 mm. – Lophioleodes monodi, MCZ 40928, 92 mm. – Lophius americanus, MCZ 51259, 121 mm; UW 48064, 334 mm; UW 48065, 335 mm.

Antennariidae: Antennarius avalonis, UW 20766, 67 mm. – Antennarius maculatus, UW 20767, 64 mm. – Antennarius nummifer, UW 48070, 55 mm. – Antennarius radiosus, MCZ 144916, 120 mm. – Antennarius striatus, UW 20768, 67 mm. – Histrio histrio, UW 48052, 59 mm. – Tathicarpus butleri, AM IB.3043, 63 mm.

Tetrabrachiidae: Tetrabrachium ocellatum, AM IB.7178, 61 mm.

Brachionichthyidae: Brachionichthys hirsutus, AM L.12148; AM E.2212.

Chaunacidae: Chaunax pictus, UW 20770, 90 mm. – Chaunax stigmaeus, MCZ 144911, 162 mm. – Chaunax sp., ZMUC P922155, 9.8 mm TL; UW 25870, 125 mm.

Ogcocephalidae: Dibranchus atlanticus, MCZ 51257, 105 mm; UW 25869, 85 mm. – Halieutichthys aculeatus, UW 21629, 47 mm. – Zalietes elater, LACM 8824-13, 98 mm.

Caulophyridae: Caulophryne jordani, ZMUC P92198, 9.5 mm TL; MCZ 134496, 36.5 mm. – Caulophryne polynema, BMNH 1932.7.1, 142 mm female with 16 mm parasitic male. – Caulophryne sp., LACM 36025-1, 98 mm female with 12 mm male.

Melanocetidae: Melanocetus eustales, LACM 30037-12, 111 mm. – Melanocetus johnsoni, SIO Haygood Lab no. 18, female; SIO Haygood Lab no. 21, female.

Himantolophidae: Himantolophus appelli, UW 22179, 232 mm; UW 025871, 105 mm. – Himantolophus groenlandicus, BMNH 4.78.1909, 405 mm TL.

Diceratidae: Bifoceraspis wedli, ZMUC P921130, 94 mm; ZMUC P922153, 117 mm. – Diceratias bispinosus, BMNH 1887.12.7.14, 55 mm.

Oneirodidae: Bertella idiomorpha, UW 42301, 66 mm; Natural History Museum and Institute, Chiba, Japan, 77 mm female with 11 mm attached male. – Chaenophryne longiceps, OSUO 13197, 25 mm. – Chaenophryne melanorhabdus, UW 18208, 67 mm. – Dolopichthys longicornis, UW 46115, 34 mm. – Leptacanthichthys gracilispinus, LACM 33625-2, 56 mm female with 7.5 mm parasitic male; ROM 27284, 54 mm female with ovaries containing developing eggs. – Lopholodos indicus, UW 46114, 31.5 mm. – Oneirodes acanthias, UW 21263, 139 mm. – Oneirodes eschrichti, UW 22372, 79 mm. – Oneirodes thompsoni, UW 22083, 65 mm female.

Thaumaticthystidae: Lasigeanthus beebei, ISH 5542/79, 112 mm.

Centroprynidae: Centroprynus spinulosus, LACM 31105-24, 2 females, 168 and 209 mm.

Ceratidae: Ceratias holboelli, ZMUC, 690 mm female with two parasitic males, 80 and 85 mm TL (Sæmundsson 1922); BMNH, 670 mm female with 75 mm parasitic male (Regan 1925); ZMUC, 650 mm female with 105 mm parasitic male (Sæmundsson 1939); MCZ 36042, 770 mm female with 108 mm parasitic male (Barbour & Bigelow 1944); ZMUC, 735 mm female with 75 mm parasitic male (Bertelsen 1951); ZMUC, 740 mm female with 24.5 mm parasitic male; UW 21617, 525 mm; UW 22322, 553 mm female with 46 mm parasitic male; UW 46158, 740 mm. – Ceratias tentaculatus, UW 22321, 460 mm. – Cryptopsaras coesiust, MCZ 29855, 290 mm female with 12 mm parasitic male (Barbour 1941); USNM, 15.5 mm female with 9.8 mm parasitic male (Pietesch 1975); UW 21774, 215 mm female with 20 mm parasitic male; UW 21775, 240 mm female with 34 mm parasitic male.

Gigantactinidae: Gigantactus vanhoefeni, MCZ 101608, 335 mm; UW 22178, 132 mm; UW 025879, tissue only.

Neoceratidae: Neoceratias spinifer, ZMUC P921276, 52 mm female with 15.5 mm parasitic male (Bertelsen 1951); IOAN, 86 mm female with 17.5 mm parasitic male (Pietesch 1976); LACM 34271-1, 42 mm female with 8.5 mm parasitic male (Pietesch 1976); SIO, 108.5 mm female with 18 mm parasitic male (Pietesch 1976); AM 1.20908-02, 77 mm female with 12.5 mm parasitic male; ISH 554679, 74 mm female with 11.5 mm parasitic male; SIO 68-478, 67.5 mm female with 17.5 mm parasitic male; SIO 70-336, 108.5 mm female with 18 mm parasitic male.

Linophrynidae: Borophryne apogon, ZMUC, 50 mm female with 14 mm parasitic male (Regan & Trewavas 1932); BMNH 1932.5.3.38, 45 mm female with 15 mm parasitic male (Regan & Trewavas 1932); LACM 30053-10, 101 mm female with 2 parasitic males, 16 and 22 mm (Pietesch 1976). – Haplophryne mollis, ZMUC, 48 mm female with 10 mm parasitic male (Regan 1925); ZMUC P92138, 34 mm female with 11 mm parasitic male (Regan & Trewavas 1932); BMNH, 50 mm female with 3 parasitic males, all 12 mm (Regan & Trewavas 1932); AM 1.21365-008, 62 mm female with 2 parasitic males, 12 and 15 mm (Munk & Bertelsen 1983). – Linophryne argyresca, ZMUC P92142, 61 mm female with 12 mm parasitic male (Regan & Trewavas 1932). – Linophryne bicornis, MCZ 138063, 101 mm female with 19 mm male. – Photocorynus spiniceps, ZMUC, 46 mm female with 8 mm parasitic male (Regan 1925b); ISH, 50.5 mm female with 7 mm parasitic male; SIO, 47 mm female with 7 mm parasitic male.