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Evolutionary history of frogfishes (Teleostei: Lophiiformes: Antennariidae): A molecular approach

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ABSTRACT

Fishes of the family Antennariidae (order Lophiiformes) are primarily shallow-water benthic forms found in nearly all tropical and subtropical oceans and seas of the world, with some taxa extending into temperate waters. Despite an earlier attempt based on morphology, no previous hypothesis of intergeneric relationships of the Antennariidae exists. To resolve phylogenetic relationships within the Antennariidae, and to test the validity of species groups within *Antennarius*, DNA sequences from the mitochondrial 16S and cytochrome oxidase *c* subunit 1 (COI) genes, and nuclear recombination activating gene 2 (RAG2), for 25 described and four undescribed antennariid species, representing 10 of 12 known genera and one undescribed genus, were unambiguously aligned and analyzed using Bayesian and maximum likelihood methods. The markers were partitioned and analyzed for substitution saturation and only the third codon position of COI (COI-3) was found to have reached saturation. However, analysis of both datasets, one with the saturated data and one without, differed only slightly. All molecular analyses recovered two major clades, one comprised of *Fowlerichthys*, *Antennarius*, *Histrio*, and *Antennatus*; and another containing *Rhycherus,* Antennariidae gen. et sp. nov., *Kuiterichthys*, *Phyllophryne*, *Echinophryne*, *Tathicarpus*, *Lophiocharon*, and *Histiophryne*. Evidence is presented to illustrate a correlation between phylogeny, geographic distribution, and reproductive life history. The results of these analyses provide the first hypothesis of evolutionary relationships within the Antennariidae.

1. Introduction

The lophiiform suborder Antennarioidei constitutes a morphologically diverse assemblage of taxa, nearly all of which are laterally compressed, shallow to moderately deepwater, benthic forms with a host of common names including frogfishes, seamice, sea-toads, warty anglerfishes, and handfishes. The 20 genera and approximately 63 extant species are distributed among four families (Pietsch and Grobecker, 1987; Pietsch et al., 2009b): the Brachionichthyidae, containing five genera and 14 species (Last and Gledhill, 2009; Carnevale and Pietsch, 2010); the monotypic Lophichthyidae (Pietsch, 1981); the Tetrabrachiidae, containing two genera and two species (Pietsch, 1981; Pietsch et al., 2009b); and the Antennariidae, containing 12 genera (Figs. 1 and 2) and approximately 46 extant species (Pietsch and Grobecker, 1987). They are part of a much larger assemblage of teleost fishes called the Lophiiformes, characterized most strikingly by the structure of the first dorsal-fin spine (illicium), placed on or near the tip of the snout and modified to serve as a luring apparatus for attracting prey, and gill openings narrowly constricted to form tube-like structures that open posteriorly behind the base of the pectoral fin that facilitate jet-propulsive locomotion.

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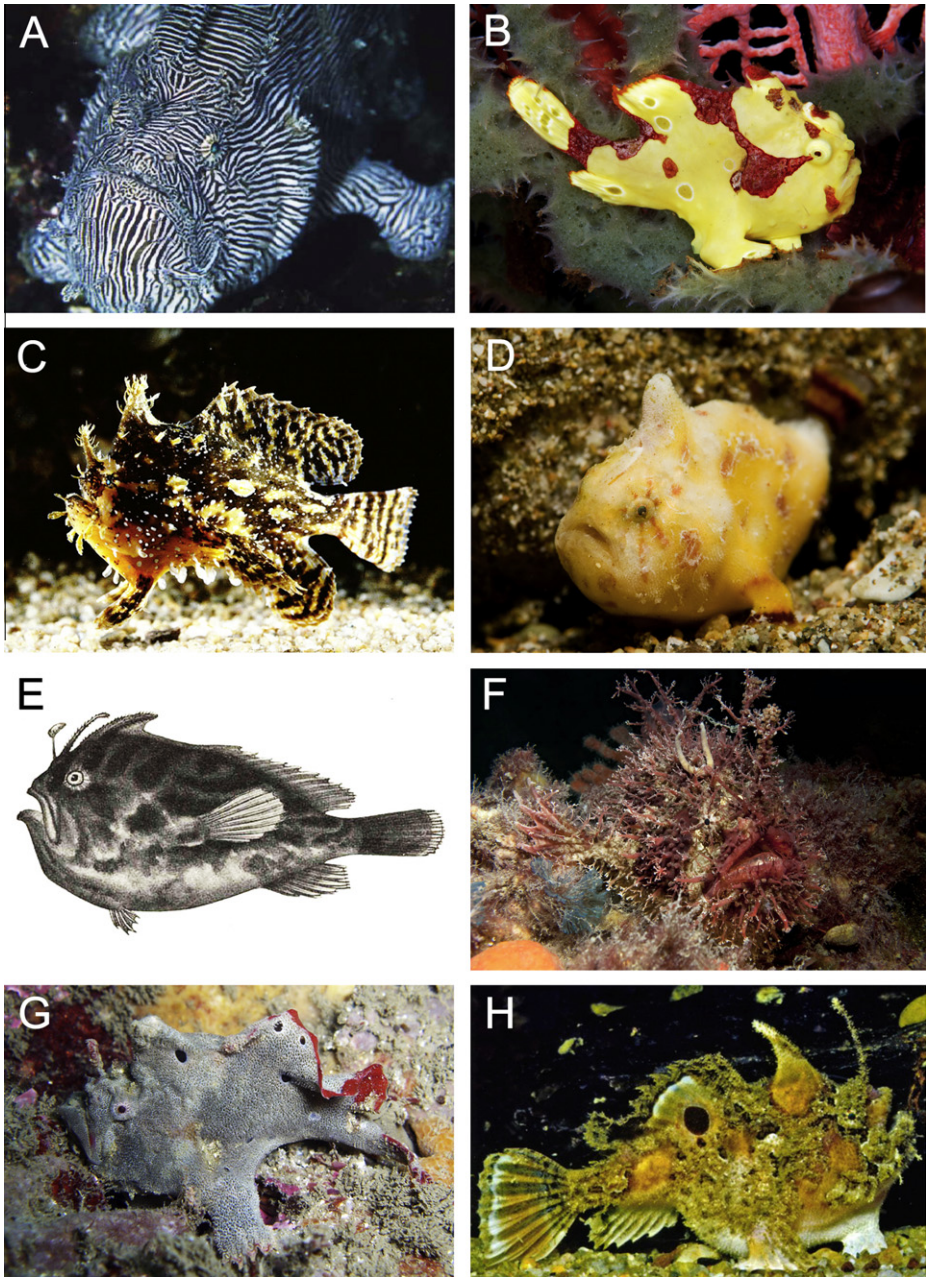

Fig. 1. Representative taxa of the Antennariidae: (A) *Fowlerichthys scriptissimus* (photo by Y. Susaki); (B) *Antennarius maculatus* (photo by D. Harasti); (C) *Histrio histrio* (photo by D. Cook); (D) *Antennatus tuberosus* (photo by D. Harasti); (E) *Nudiantennarius subteres* (after Smith and Radcliffe, in Radcliffe, 1912); (F) *Rhycherus filamentosus* (photo by J. Peake); (G) Gen. et sp. nov. (photo by R. Arnold) and (H) *Kuiterichthys furcipilis* (photo by R. Kuiter).

Fishes of the family Antennariidae are found in nearly all tropical and subtropical oceans and seas of the world, with some taxa extending into temperate waters. They are distinguished from species of other antennarioid families by the presence of an enlarged third dorsal-fin spine (and associated pterygiophore), as well as a shortened body and a sigmoid vertebral column (see Pietsch, 1981, figs. 12, 33–35, 41, 1984; Pietsch and Grobecker, 1987). Antennariids are primarily benthic, sit-and-wait, cryptic predators that blend in nearly perfectly with almost any combination of substrate and structure. Several species appear to mimic sponges (e.g., *Antennarius pictus* and *Antennarius commerson*; Wickler, 1968; Pietsch and Grobecker, 1978) while others look nearly identical to sea urchins (e.g., *Astropyga radiata*; Schneidewind, 2005). Although generally sedentary, frogfishes employ several modes of locomotion. One such mode, a unique tetrapod-like locomotion, is at the heart of myths surrounding the amphibious nature of these animals, which date back three centuries (Pietsch and Grobecker, 1987). The ability to remain out of water for an extended period of time (as many as 3 days) and to move about the substrate was first reported by Renard (1719) and later accepted as truth by a number of prominent naturalists (e.g., Valenciennes, 1837; Swainson, 1838; see also Pietsch, 1984; Pietsch and Grobecker, 1987). Frogfishes use their jointed, arm- and hand-like pectoral fins in conjunction with the pelvic fins to accomplish a kind of locomotion that resembles a "walk" in typical tetrapod-like fashion. However, the ability to remain out of water for any length of time, let alone move about without the buoyancy provided by seawater, is severely restricted. "A living frogfish removed from

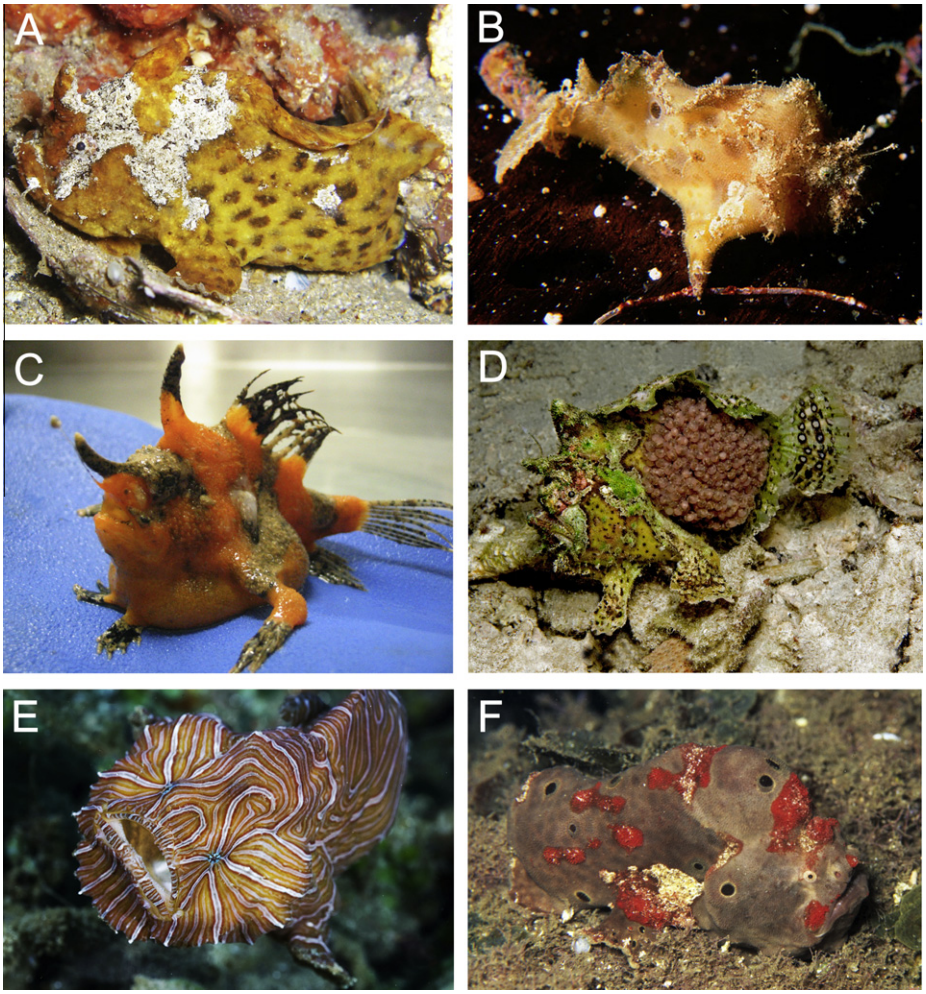

Fig. 2. Representative taxa of the Antennariidae: (A) *Phyllophryne scortea* (photo by R. Arnold); (B) *Echinophryne crassispina* (photo by J. Lewis); (C) *Tathicarpus butleri* (photo by M. Maddern); (D) *Lophiocharon lithinostomus* (photo by N. DeLoach); (E) *Histiophryne psychedelica* (photo by D. Hall) and (F) *Allenichthys glauerti* (photo by A. Storrie).

an aquarium and placed on a flat surface cuts a rather poor figure, its body more or less immobile and spreading out pancake-like under its own weight" (Pietsch and Grobecker, 1987, p. 345).

The Antennariidae has had a long and complex taxonomic history, beginning with the first illustration of a frogfish supplied to Johannes de Laet by an unknown Dutchman dating sometime prior to the year 1630. The first notable contribution to frogfish biology was that of Albertus Seba, published in the first volume of his *Locupletissimus Rerum Naturalium Thesaurus* of 1734. Seba, like many of his contemporaries, believed that antennariids were anuran amphibians and classified them with true frogs, referring to them as *Rana Piscatrix*, or "fishing frog."

The first serious study of antennariid fishes was undertaken by the French naturalist Philibert Commerson. His manuscripts and drawings were incorporated by Lacepède in the latter's *Histoire Naturelle des Poissons* (1798). A full treatment of the group was published by Valenciennes (1837) in the twelfth volume of the *Histoire Naturelle des Poissons*. Although Günther (1861) first recognized the problems inherent in frogfish systematics, an attempt to revise the family on a worldwide basis did not appear until the monograph of Schultz in 1957, and then another monograph by Le Danois (1964). However, until Pietsch (1984) reviewed the family, there were 165 nominal species placed in some 31 nominal genera and subgenera, the descriptive proliferation resulting in an extremely confusing nomenclature that involved well over 350 different combinations of generic, subgeneric, specific, and varietal names for what was then recognized as 40 valid species. Pietsch (1984) split the most diverse antennariid genus, *Antennarius*, into six species groups (Fig. 3): the *A. biocellatus* group, *A. nummifer* group, *A. ocellatus* group, *A. pauciradiatus* group, *A. pictus* group, and *A. striatus* group. In addition, Pietsch (1984) provided geographic distributions, depth ranges, and the first taxonomic keys. His cladistic analysis of morphological characters, however, did not produce a fully resolved hypothesis of relationships within the family. In particular, monophyly for the 24 species of *Antennarius* was not established and no group of two or more genera was found to possess any convincing synapomorphy that did not also occur within lophiiform taxa lying outside the group in question.

The lack of phylogenetic insight drawn from morphological data required that some other dataset, namely molecular sequence data, be analyzed to address the evolutionary relationships of the Antennariidae. To this end, sequences were assembled from 25 described and four undescribed antennariid species representing 10 of 12 known genera and one undescribed genus. A dataset of 1709 bps from the genes 16S, cytochrome oxidase *c* subunit 1 (COI), and recombination activation gene 2 (RAG2) were analyzed using Bayesian and maximum likelihood methods to address the intergeneric relationships of the family and to test the validity of the species groups of *Antennarius* hypothesized by Pietsch (1984). The results of this study provide the first hypothesis of evolutionary relationships within the Antennariidae.

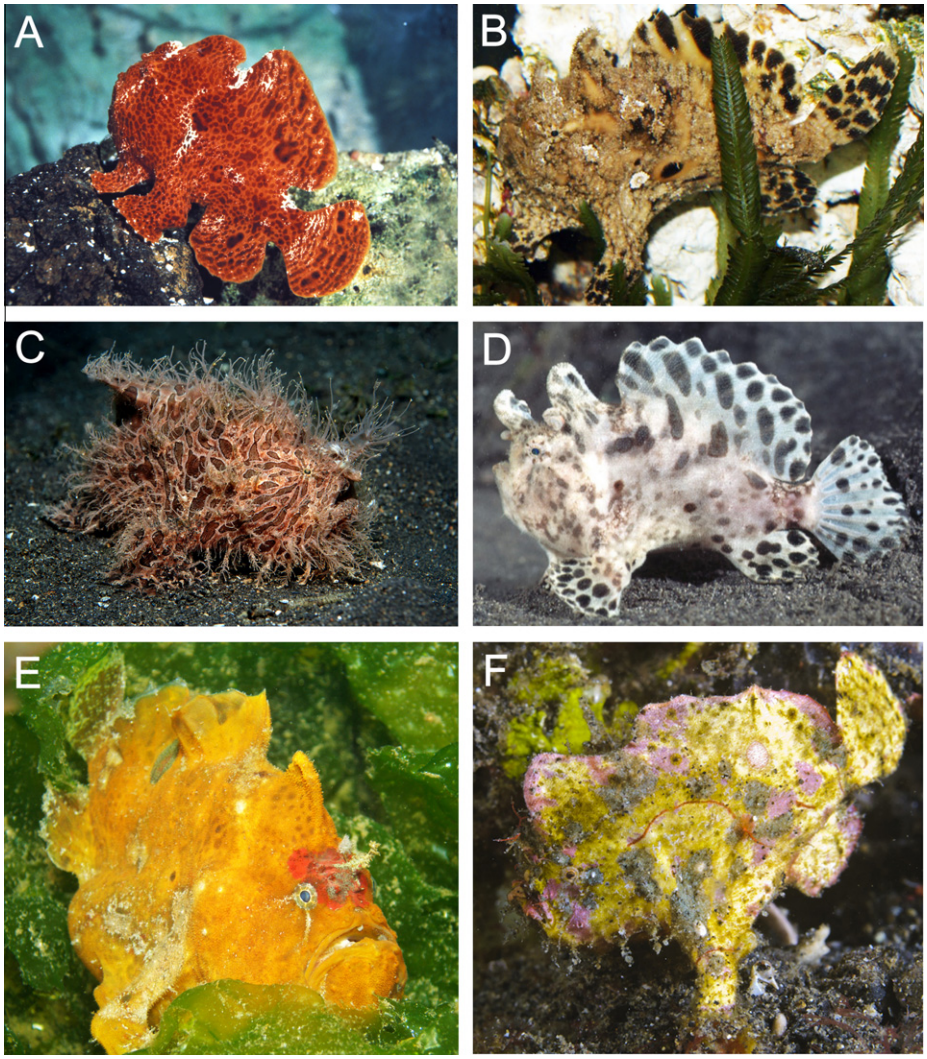

Fig. 3. Representative taxa of *Antennarius* species groups: (A) *Antennarius commerson* (photo by D. Grobecker); (B) *Antennarius indicus* (photo by J. Groomer); (C) *Antennarius striatus* (photo by D. Harasti); (D) *Antennarius striatus* (photo by Y. Otsuka); (E) *Antennarius biocellatus* (photo by J. Gawron) and (F) *Antennarius pauciradiatus* (photo by M. Oldfield).

2. Materials and methods

2.1. Taxon sampling

Because the monophyletic status of the Antennariidae has been demonstrated previously (Pietsch, 1981), and because the purpose of this study was to determine intergeneric relationships and not to test monophyly of the family, only two genera of the purported sister family Tetrabrachiidae (Pietsch, 1981; Pietsch et al., 2009b), *Dibrachichthys* and *Tetrabrachium*, were included as outgroup taxa. This strategy minimizes the potential occurrence of substitution saturation present in datasets representing highly divergent taxa.

DNA sequences were analyzed from 31 species including two outgroup taxa and 47 individual antennariids from 10 of the 12 currently recognized genera (Table 1). An undescribed species from New South Wales, Australia, which represents a new genus, was also included. *Nudiantennarius*, a monotypic genus represented by only four specimens in collections worldwide, and *Allenichthys*, a monotypic genus endemic to Western Australia, were not included because tissues were unavailable. All polytypic genera, except *Antennarius*, were assumed to be monophyletic. Representatives of four of the six species groups of *Antennarius* (Pietsch, 1984), the *A. nummifer* group, *A. ocellatus* group, *A. pictus* group, and *A. striatus* group, were included in this study. The *A. biocellatus* group, consisting of one species, and the *A. pauciradiatus* group, comprising two species, were not included because tissues were unavailable.

For the following species, data were generated from tissue samples from more than one individual: *A. pictus* (Shaw), *A. striatus* (Shaw), *Antennatus tuberosus* (Cuvier), *Histrio histrio* (Linnaeus), *Histiophryne cryptacanthus* (Weber), *Kuiterichthys furcipilis* (Cuvier), *Lophiocharon lithinostomus* (Jordan and Richardson), *Phyllophryne scortea* (McCulloch and Waite), *Rhycherus filamentosus* (Castelnau), and *Tathicarpus butleri* Ogilby. For all other species, one individual was included in the data set. Voucher specimens have been retained for all samples. A complete list of taxa, catalog numbers for vouchers and tissues, and GenBank accession numbers are presented in Table 1.

2.2. DNA extractions, PCR amplification, and sequencing

Genomic DNA was extracted from approximately 25 mg of ethanol-fixed skeletal muscle or fin tissue using a Qiagen DNeasy tissue extraction kit. Both mitochondrial and nuclear DNA were used

Table 1
Tissue and voucher specimens of tetrabrachiids and antennariids used to generate sequence data, with corresponding GenBank accession numbers for each gene: 16S, COI, and RAG2.

Taxon		Voucher	16S	COI	RAG2
Genus	Species	Catalog number			
Dibrachichthys	*melanurus*	QM I.38226	GQ981534	FJ224367	GU188519
Tetrabrachium	*ocellatum*	UW 049710-1	GQ981536	FJ224370	GU188565
	ocellatum	UW 049710-2	GQ981535	FJ224371	GU188564
Antennariidae	*gen. et sp. nov*	AMS I.43749	GQ981549	GU188506	GU188561
Fowlerichthys	*radiosus*	UW 112080	GQ981539	GU188481	GU188542
	scriptissimus	UW 112642	GQ981538	GU188480	GU188541
Antennatus	*nummifer*	RUSI 65251	GQ981573	GU188492	GU188520
	rosaceus	QM I.38177	GQ981572	GU188493	GU188524
	sanguineus	UW 118813	GQ981561	GU188487	GU188526
	coccineus	AMS I.33711.038	GQ981571	GU188486	GU188525
	tuberosus	UW 115750	GQ981562	GU188518	GU188527
	tuberosus	UW 118814	GQ981563	GU188517	GU188528
Antennarius	*striatus*	UW 112081	FJ219602	FJ219609	FJ219616
	striatus	UW 112082	GQ981564	GU188496	GU188535
	striatus	UW 118815	GQ981565	GU188497	GU188539
	striatus	UW 117695-3	GQ981568	GU188498	GU188540
	striatus	WAM 32906.001	GQ981569	GU188500	GU188538
	striatus	WAM 32906.001	GQ981570	GU188499	GU188537
	hispidus	UW 117828	GQ981567	GU188495	GU188536
	indicus	UW 118818	GQ981566	GU188494	GU188534
	commerson	UW 117686	GQ981579	GU188488	GU188533
	multiocellatus	UW 117826	GQ981578	GU188482	GU188532
	pictus	NMV I.41297	GQ981581	GU188484	GU188531
	pictus	UW 115878	GQ981580	GU188485	GU188530
	maculatus	UW 117687	GQ981577	GU188483	GU188529
Histrio	*histrio*	KU 29308	GQ981576	GU188490	GU188522
	histrio	NMV I.40230	GQ981575	GU188491	GU188523
Tathicarpus	*butleri*	WAM 32904.001	GQ981547	GU188502	GU188551
	butleri	WAM 32903.001	GQ981546	GU188503	GU188550
	butleri	QM I.38191	GQ981548	GU188501	GU188549
Lophiocharon	*lithinostomus*	UW 115749	FJ219604	FJ219611	FJ219618
	trisignatus	UW 115748	FJ219603	FJ219610	FJ219617
	trisignatus	ASIZ P0070179	GQ981540	GU188511	GU188543
Histiophryne	*cryptacanthus*	UW 117821	GQ981542	GU188512	GU188545
	cryptacanthus	UW 117818	GQ981541	GU188513	GU188546
	psychedelica	NCIP 6377	FJ219601	FJ219608	FJ219615
	sp. 1	UW 118820	GQ981543	GU188514	GU188544
	sp. 2	SAM F11719	GQ981545	GU188515	GU188547
	sp. 3	QM I.38176	GQ981544	GU188516	GU188548
Rhycherus	*filamentosus*	AMS I.41560.001	GQ981552	GU188505	GU188558
	filamentosus	NMV A29238.011	GQ981553	GU188476	GU188560
	filamentosus	NMV A22333	GQ981554	GU188478	GU188559
Kuiterichthys	*furcipilis*	AMS I.41007.001	GQ981550	GU188477	GU188563
	furcipilis	SAM 10476	GQ981551	GU188504	GU188562
Phyllophryne	*scortea*	WAM 32905.001	GQ981555	GU188507	GU188555
	scortea	SAM F11721	GQ981557	FJ224369	GU188556
	scortea	SAM F11722	GQ981556	GU188508	GU188557
	scortea	NMV 29226	GQ981558	GU188509	GU188554
Echinophryne	*crassispina*	SAM F11544	GQ981559	GU188510	GU188552
	mitchellii	NMV A25508.002	GQ981560	FJ224368	GU188553

because, ultimately, the mitochondrial genome is a single linked locus that can provide just one perspective on the evolutionary history of species (Zhang and Hewitt, 2003; Ballard and Whitlock, 2004) and is often inadequate for phylogenetic analyses (Chan and Levin, 2005). Aliquots (2.0 μl) of genomic DNA were used as template in all polymerase chain reactions (PCR) to amplify double-stranded DNA product from two mitochondrial genes, 16S and cytochrome oxidase *c* subunit 1 (COI), and one nuclear gene, recombination activating gene 2 (RAG2). All PCR reactions were carried out using a Peltier Thermal Cycler (PT-225), each with a total volume of 25 μl with the following concentrations of reagents: 2 μl of genomic DNA isolate, 1.25 μl of each 10 μM primer, 2.5 μl of 10X buffer, 1.25 μl of 25 mM $MgCl_2$, 0.625 μl of 8 mM premixed deoxynucleotide triphosphates, and 1.0 U of Biolase Taq Polymerase (Bioline).

The universal primer pair 16Sar and 16Sbr (Palumbi, 1996) was used to PCR amplify an approximately 650 base pair (bp) fragment of the mitochondrial rDNA gene 16S using the following protocol: initial denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 45 s. After a final extension step at 72 °C for 10 min, all samples were held at 4 °C.

An approximately 700 bp fragment of the 5′ region of the protein-coding mitochondrial COI gene was PCR amplified with primer pairs COI5562f and COI6284r (Pietsch et al., 2009a). Amplification of the COI gene for one species, *A. tuberosus*, was problematic and a new reverse primer was designed using sequences of antennariids amplified with the initial primer pair. These sequences were aligned in MUSCLE (multiple sequence comparison by log-expectation, Edgar, 2004) and 21 bp sequences were copied from a conserved region at the 3′ ends of the gene: COIr 5′-CRG CYT STT CRA ATG TGT GRT-3′. Using these primer pairs, the COI gene was amplified utilizing the same protocol for 16S amplification, except for the annealing temperature that was 52–54 °C.

Primer pair RAG2-f2 and RAG2-r3 (Westneat and Alfaro, 2005) was used to PCR amplify an approximately 490 bp fragment from the protein-coding region of RAG2 using the touchdown thermocycle protocol (Palumbi, 1996). All samples were then held at 4 °C.

PCR products were visualized on 1–2% agarose gels and bands of the target fragment length were cut directly from the gel and cleaned using QIAquick Gel Extraction Kit (Qiagen). Forward and reverse sequences were generated at the High-Throughput Genomics Unit (HTGU), Department of Genome Sciences, University of Washington, Seattle.

2.3. Sequence editing, alignment, and substitution saturation

All forward and reverse chromatographs were aligned in GENEIOUS (Drummond et al., 2007) and checked against each other for base pair calls. Instances of heterozygosity (overlapping peaks of equal strength) for the RAG2 sequences were coded according to the IUPAC ambiguity code. None of the heterozygous sites coded for alternative amino acids.

All sequences were aligned using MUSCLE (Edgar, 2004) as implemented in GENEIOUS (Drummond et al., 2007) using the full gap penalty. 16S fragments were further aligned manually according to the proposed secondary structure of *Polyipnus matsubarai* 16S rRNA, reference sequence GenBank D89739 (Miya and Nishida, 1998). Sequences were trimmed according to the reference. The final length was 521 bps (246 phylogenetically informative sites), with 330 bps of loop region and 191 bps of stem region.

The two protein coding loci, COI and RAG2, were also aligned against reference sequence. To check the reading frame for COI, sequences were aligned against a known sequence of *Antennarius avalonis* (GenBank DQ027984.1), and the sequences were trimmed to a final length of 699 bps (229 phylogenetically informative sites). RAG2 sequences were aligned against a known sequence of *Balistes capriscus* (GenBank DQ874786.1) to confirm the reading frame as well as determine the presence of indels (insertions or deletions). No indels were found. The final length of the RAG2 fragment was 489 bps (130 informative sites).

Sequences of variable lengths introduced cells of missing data in the final matrix. These sites were coded as ambiguous for all analyses. All sequences were deposited in GenBank; their accession numbers and corresponding whole specimen vouchers are listed in Table 1.

Nucleotide substitution saturation was examined in DAMBE (Xia and Xie, 2001) using corrected genetic distances plotted against transitions and transversions for all pairwise comparisons among taxa. 16S stems and loops as well as first, second, and third codon positions of COI and RAG2 were analyzed separately. Second-order polynomial curves were fit to the data, and saturation was established when the curve reached a plateau.

2.4. Phylogenetic analyses

To determine phylogenetic relationships and the effect of the saturated data on topology and clade support, Bayesian and

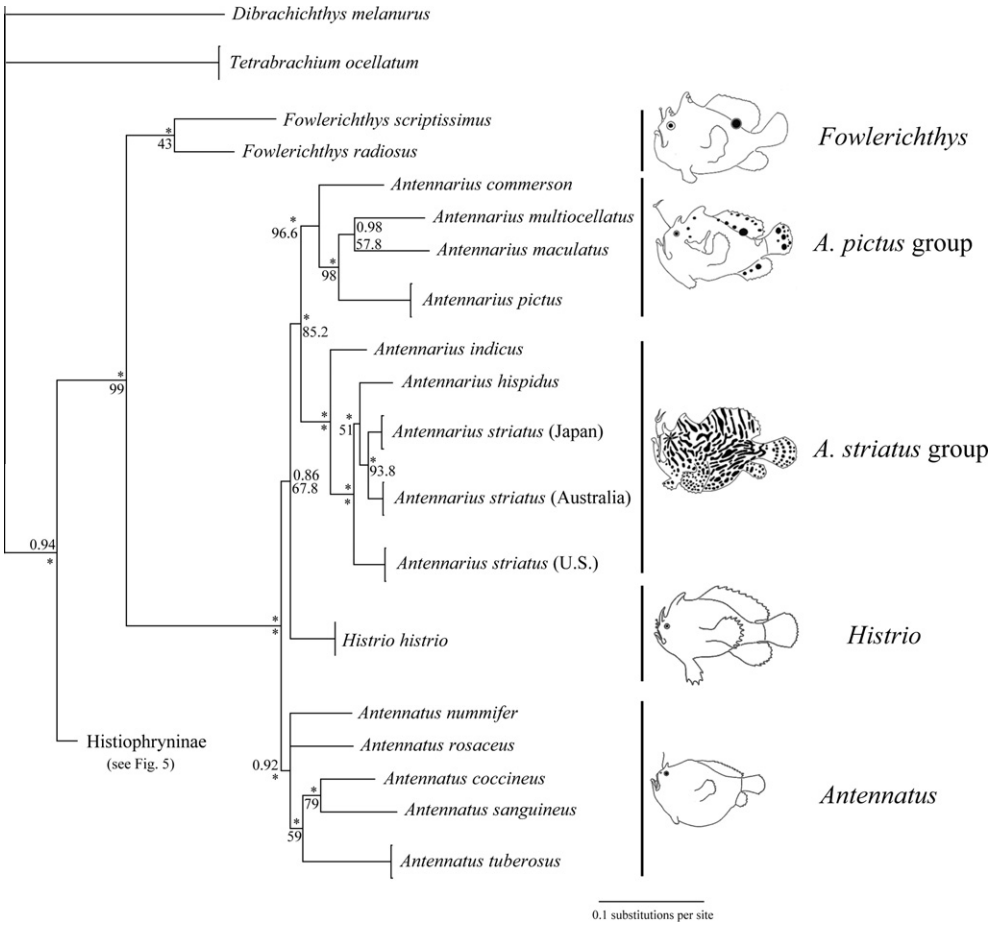

Fig. 4. Fifty percentage majority rule phylogeny of the Antennariinae, from trees sampled in the posterior, generated from Bayesian analyses with the saturated data (third codon position of COI). Branch lengths are measured in expected substitutions per site and are proportional to length. Numbers above nodes are posterior probabilities and numbers below nodes are bootstrap proportions from 500 pseudoreplicates used from maximum likelihood analysis; an asterisk indicates a posterior probability of 1.00 or a bootstrap percentage of 100.

maximum likelihood analyses of two datasets were performed. The first dataset included all sequences produced for 16S stems, 16S loops, COI, and RAG2; the other differed only in the removal of the third codon position of COI, which was saturated with substitutions. To determine which nucleotide substitution model was most appropriate, 16S stems, 16S loops, the first and second codon positions of COI (COI-12), all codon positions of COI (COI-123), and RAG2 were analyzed separately using ModelTest 3.7 (Posada and Crandall, 1998) under the Akaike Information Criterion (AIC). Those models were used in all further phylogenetics analyses. All Bayesian analyses were carried out in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and each dataset was partitioned to accommodate differences among genes and the stem and loop regions of the 16S gene fragment. The partitions are summarized as follows: K80 + I + G for 16S stems, SYM + I + G for 16S loops, TrN + I + G for COI-12, TIM + I + G for COI-123, and TrN + I for RAG2. Sequences were partitioned in Mesquite (Maddison and Maddison, 2009), and all partitions unlinked.

For analyses of both datasets, the default setting of flat priors for the transition/transversion rate ratio, branch length, alpha parameter of the gamma distribution for rate heterogeneity, proportion of invariant sites, base frequencies, and tree topology parameters were all used except for the 16S stems and 16S loops partitions that had fixed nucleotide frequencies. To ensure convergence, 5,000,000 generations were run with two replicates, each with four simultaneous chains, three hot, one cold, with trees sampled every 100 generations. Stationarity was achieved, as indicated by the standard deviation of split frequencies and examination of a graph of posterior probability versus replicates. Of the resulting 50,001 trees, 12,500 were discarded as burn-in. A 50% majority rule consensus tree was constructed of the post burn-in trees.

Concatenated datasets (one with COI-12 and one with COI-123) were used for the maximum likelihood (ML) analyses in PHYML (Guiden and Gascuel, 2003) as implemented in GENEIOUS (Drummond et al., 2007) utilizing the GTR + I + G model of nucleotide substitution. This model was chosen because it is all-inclusive, containing the five parameters estimated by the HKY model. Bootstrapping of the datasets was carried out with 500 pseudoreplicates, and all node values for the ML trees are represented as the proportion of replicates in which that clade was recovered. In all ML and Bayesian analyses, *Tetrabrachium ocellatum* was designated as the outgroup, resulting in rooted phylogenetic trees with a polytomy at the base. Gene trees were also analyzed separately using maximum likelihood analysis with 100 bootstrap pseudoreplicates.

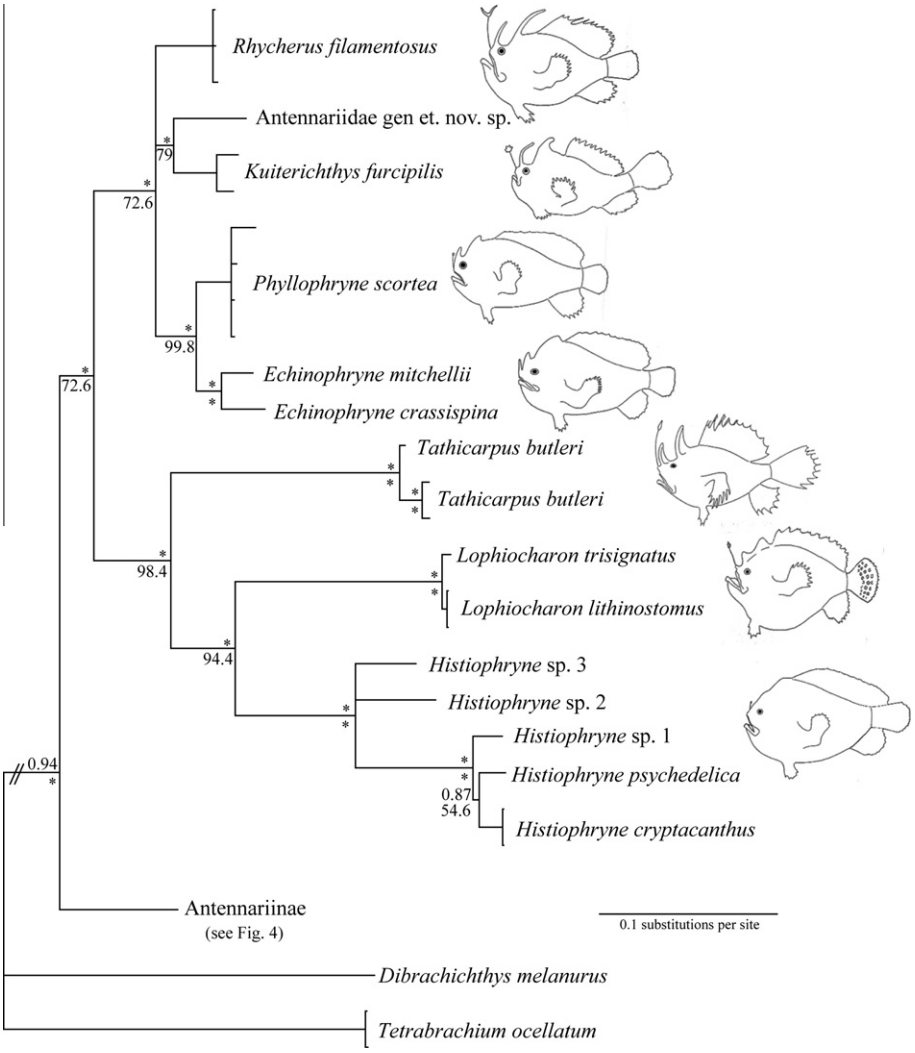

Fig. 5. Fifty percentage majority rule phylogeny of the Histiophryninae, from trees sampled in the posterior, generated from Bayesian analyses with the saturated data (third codon position of COI). Branch lengths are measured in expected substitutions per site and are proportional to length. Numbers above nodes are posterior probabilities and numbers below nodes are bootstrap proportions from 500 pseudoreplicates used from maximum likelihood analysis; an asterisk indicates a posterior probability of 1.00 or a bootstrap percentage of 100.

Nucleotide substitution models for the gene trees are summarized as follows: GTR + I + G for 16S, TIM + I + G for COI, and TrN + I for RAG2.

3. Results

3.1. The effects of substitution saturation on clade support and topology

As expected, loop mutations and synonymous changes were in the greatest abundance; however, only the third codon position of COI reached saturation. To evaluate the effect of the saturated data on tree topologies and clade support, two datasets (one with the saturated data and one without) were analyzed and compared.

Of the 1709 sites included in the total evidence data set, 662 were variable, with the saturated sites providing 35% of the informative sites. With the exception of the *A. pictus* group, the exclusion of the saturated data from further phylogenetic analysis did not change tree topology. In the Bayesian analysis (BA) tree utilizing saturated data (Figs. 4 and 5), *A. maculatus* is sister to *A. multiocellatus* (Posterior Probability $P = 0.98$), and they as a group are sister to *A. pictus* ($P = 1.00$). In the BA tree excluding the saturated data (Figs. 6 and 7), *A. maculatus* is sister to *A. pictus* ($P = 1.00$), and they as a group are sister to *A. multiocellatus* ($P = 1.00$). Clade support values were affected only slightly by the removal of the saturated data; in six instances, decreased posterior probabilities (PPs) were found in the Bayesian tree that excluded the saturated data, but usually from 1.00 to $\geqslant 0.93$, and in three instances PPs increased in support $\leqslant 0.09$.

It is evident that PPs and bootstrap values at some nodes are discordant. It is possible that this is caused by the concatenation of the data. Partitioning the genes, including the stems and loops regions of 16S, with their own gamma distribution and alpha parameter allows for a better fit. It is likely that an appropriate partitioning scheme for the ML analyses would reduce the effects of the saturation and noise in the reconstruction of those nodes with low bootstrap supports.

3.2. Phylogenetic relationships within the family Antennariidae

Two major clades were recovered in both analyses (Figs. 4–7), the first composed of the subfamily Antennariinae, containing genera *Fowlerichthys* (resurrected from the synonymy of *Antennarius*), *Antennarius*, *Histrio*, and *Antennatus*; and a sister clade, subfamily Histiophryninae, containing the remaining genera *Rhycherus,* Antennariidae gen. et sp. nov., *Kuiterichthys, Phyllophryne, Echinophryne*, *Tathicarpus, Lophiocharon,* and *Histiophryne*. The Histiophryninae is defined as including all members of the Antennariidae that have lost the mesopterygoid and epural. Within the Antennariinae (Figs. 4 and 6), *Antennarius* was rendered paraphyletic (requiring resurrection and recognition of *Fowlerichthys* Barbour, 1941, to contain the former members of the *Antennarius ocellatus* group *sensu* Pietsch and Grobecker, 1987:111). At the same time,

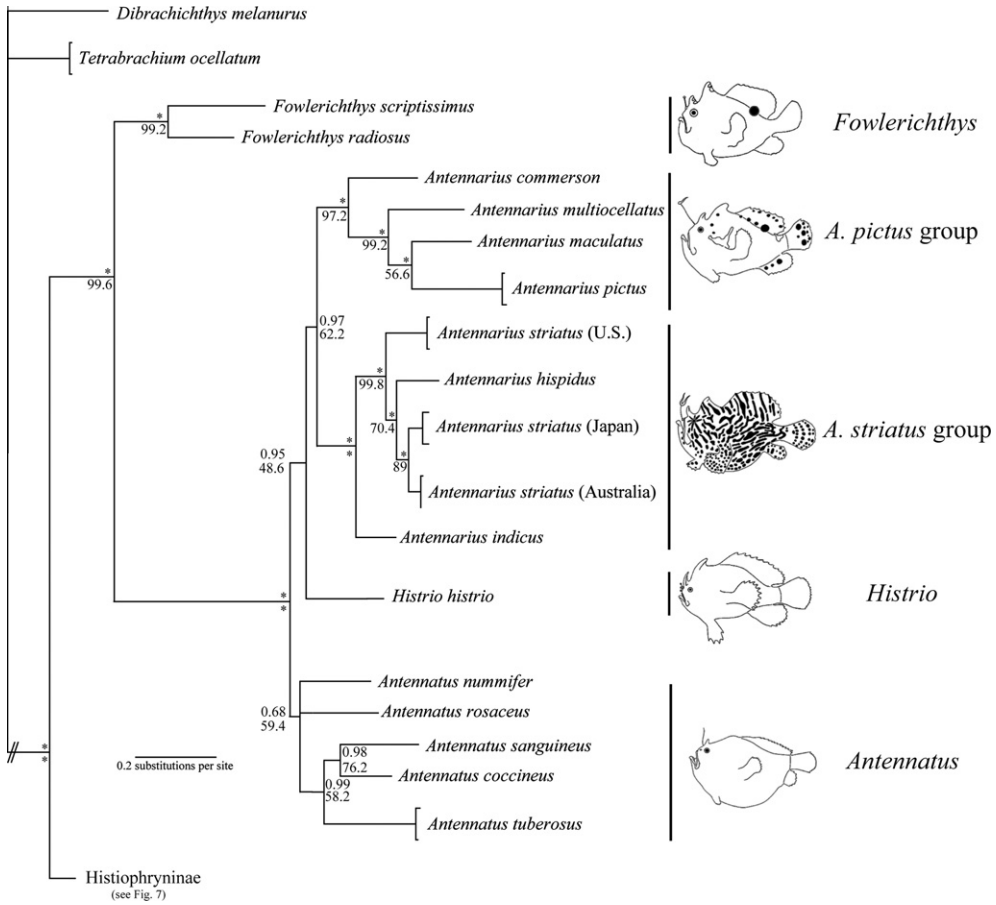

Fig. 6. Fifty percentage majority rule phylogeny of the Antennariinae, from trees sampled in the posterior, generated from Bayesian analyses without the saturated data (third codon position of COI). Branch lengths are measured in expected substitutions per site and are proportional to length. Numbers above nodes are posterior probabilities and numbers below nodes are bootstrap proportions from 500 pseudoreplicates used from maximum likelihood analysis; an asterisk indicates a posterior probability of 1.00 or a bootstrap percentage of 100.

Antennatus was recovered nested within the *Antennarius nummifer* group, requiring reallocation of all members of the latter (as recognized by Pietsch and Grobecker, 1987), resulting in a much expanded *Antennatus*. While it is tempting to erect subgenera to separate *Antennatus sensu stricto* from the former members of the *A. nummifer* group, such action is postponed until more detailed analysis of this assemblage is possible.

The *A. pictus* group was recovered as monophyletic, but *Fowlerichthys* (=the *A. ocellatus* group) was found to be monophyletic only to the exclusion of *A. indicus*, a species recovered as sister of the monophyletic *A. striatus* group. The *A. pictus* group was sister to the *A. striatus* group, together forming the sister group of the genus *Histrio*. The *A. pictus* group, *A. striatus* group, and *Histrio* comprise a clade sister of the now expanded *Antennatus*. *Fowlerichthys* was recovered as sister to all species groups of *Antennarius* plus *Antennatus* and *Histrio*, and is the basal lineage of the Antennariinae.

The Histiophryninae was subdivided into two groups (Figs. 5 and 7), the first containing *Tathicarpus, Lophiocharon,* and *Histiophryne*; and the second consisting of *Rhycherus,* Antennariidae gen. et sp. nov., *Kuiterichthys, Phyllophryne*, and *Echinophryne*. *Tathicarpus* was recovered as sister to a clade containing *Lophiocharon* and *Histiophryne*. *Phyllophryne* and *Echinophryne* were recovered as sister taxa, as were *Kuiterichthys* and Antennariidae gen. et sp. nov. These two groups and *Rhycherus* were recovered in a polytomy. *Tathicarpus* is recovered as sister to a clade containing *Lophiocharon* and *Histiophryne*.

3.3. A comparison of gene trees

With the exception of unresolved clades, gene trees did not differ notably in topology. In fact, all intergeneric relationships recovered in the combined data set were in agreement with all gene trees except in the case of RAG2, in which an individual of *K. furcipilis* was recovered as sister of Antennariidae gen. et sp. nov., while a different specimen of *K. furcipilis* was recovered as the sister to them. While unresolved in the combined data set analyses, both the RAG2 and 16S gene trees recovered *Rhycherus* as basal to the sister genera *Phyllophryne* and *Echinophryne*. In the 16S gene tree, with the exception of *A. commerson*, the *A. pictus* group was

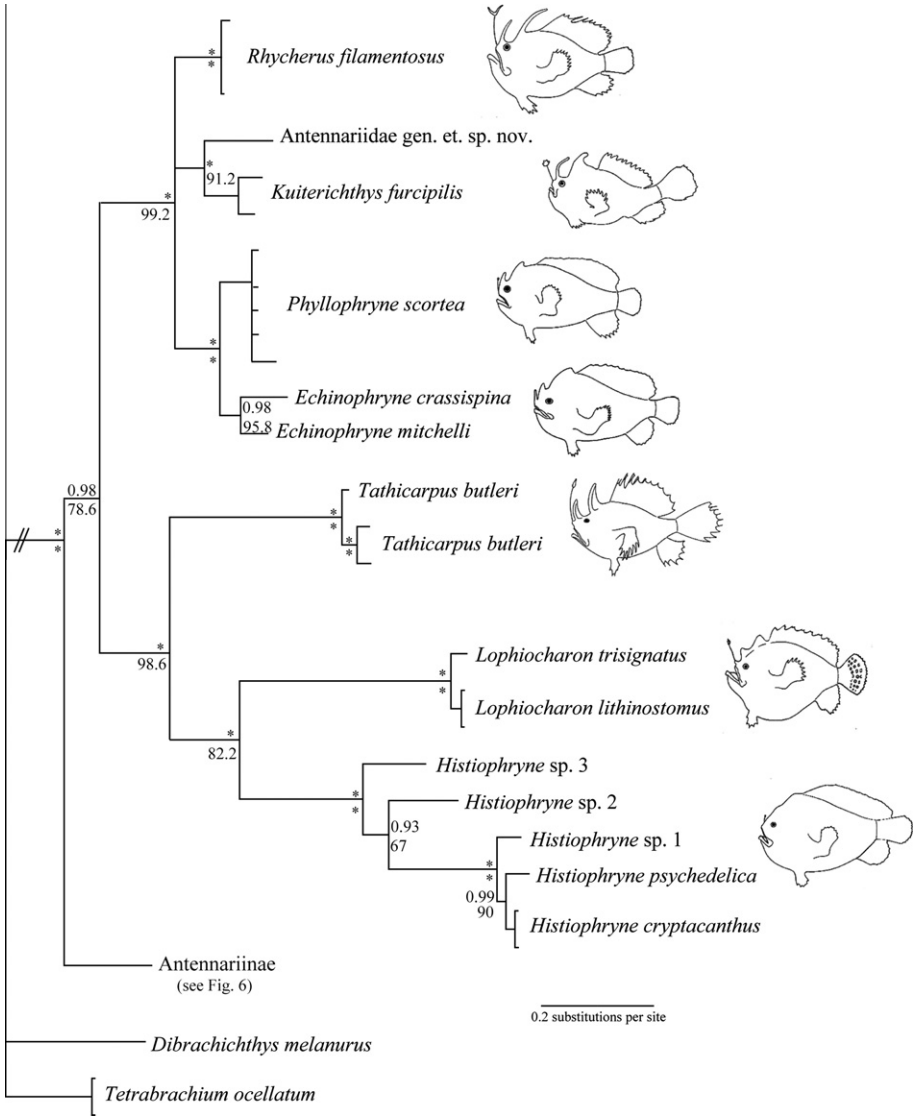

Fig. 7. Fifty percentage majority rule phylogeny of the Histiophryninae, from trees sampled in the posterior, generated from Bayesian analyses without the saturated data (third codon position of COI). Branch lengths are measured in expected substitutions per site and are proportional to length. Numbers above nodes are posterior probabilities and numbers below nodes are bootstrap proportions from 500 pseudoreplicates used from maximum likelihood analysis; an asterisk indicates a posterior probability of 1.00 or a bootstrap percentage of 100.

recovered as sister to a clade containing the remaining species of *Antennarius*, *Antennatus,* and *Histrio*, while in the combined data set it was recovered as sister to the *A. striatus* group, and included *A. commerson*.

4. Discussion

4.1. Morphological evidence

While Pietsch (1984) and Pietsch and Grobecker (1987) were not able to resolve relationships within the Antennariidae, morphological characters found in their studies correspond with clades recovered in this study. For instance, within the Antennariinae, *Fowlerichthys* has a unique combination of 20 vertebrae and five bifurcate pelvic-fin rays, while members of the second clade (*Antennatus*, *Histrio*, *A. pictus* group, and *A. striatus* group) have 19 vertebrae and one bifurcate pelvic-fin ray. Members of the Histiophryninae (Figs. 5 and 7) have lost the mesopterygoid and epural, a point which also led Pietsch (1984) and Pietsch and Grobecker (1987) to conclude that this lineage likely forms a monophyletic group. *Lophiocharon* and *Histiophryne* were recovered as sister groups and are the only antennariids that have lost pharyngobranchial I (this element is also absent in brachionichthyids and some ogcocephalids; Pietsch, 1984; Pietsch and Grobecker, 1987).

4.2. Phylogenetic position of Fowlerichthys

As seen in Figs. 4 and 6, *Fowlerichthys* (containing remnants of the previously recognized *A. ocellatus* group; *sensu* Pietsch and Grobecker, 1987) was recovered as the sister group of the paraphyletic genus *Antennarius*. The members of this species group, *F. avalonis, F. ocellatus, F. radiosus, F. senegalensis*, and *F. scriptissimus* (excluding *A. indicus*), share a character unique among lophiiforms in having all five pelvic-fin rays bifurcate (all other antennariids have four simple and one bifurcate rays). They also have 20 vertebrae while nearly all other antennariids examined within the Antennariinae have 19 (the two exceptions noted by Pietsch (1984) are a single specimen of *A. striatus*, having 18 vertebrae, and a single specimen of *A. commerson*, with 20 vertebrae). Furthermore, *F. radiosus* has an unusual early life-history stage called the "scutatus" prejuvenile form. This stage is characterized by a pair of shield-like bony extensions of the cranium that reach beyond the level of the opercular bones, and an expansion of the anterior margin of the bones of the suspensorium (see Pietsch and Grobecker, 1987, Figs. 43 and 44). This early life-history stage was so strange, in fact, that it was originally described as a new genus and species, *Kanazawaichthys scutatus*, by Schultz (1957), and only later shown by Hubbs (1958) to be the prejuvenile of *F. radiosus* (see Pietsch and Grobecker, 1987). Although a similar morphological adaptation in closely related species was expected, Pietsch (1984) found nothing comparable upon examination of a 19.0-mm SL *F. ocellatus* and 12.0-mm SL *F. avalonis*. However, Adams (1960) found that in *Histrio histrio*, 10.0-mm SL marked the transition from postlarva to juvenile and by 15.5-mm SL the specimens had taken on full adult appearances. While larval antennariids are not rare in collections, rapid metamorphosis from larvae to adult may severely limit the number of available prejuveniles for examination.

Even without knowing which species pass through the scutatus prejuvenile form, *Fowlerichthys* is markedly different from all other species of *Antennarius*, *Antennatus*, and *Histrio*. For the reasons discussed above, the genus *Fowlerichthys* Barbour (1941), type species *F. floridanus* (a junior synonym of *A. radiosus* Garman, 1896), is applied to the previously recognized *A. ocellatus* group (excluding *A. indicus*, see below).

4.3. Phylogenetic position of Antennarius indicus

With the exception of the placement of *A. indicus*, all species of *Antennarius* included in this study were recovered within the same species groups of *Antennarius* hypothesized by Pietsch (1984). *A. indicus* was recovered as the basal species of *Fowlerichthys* (previously the *A. ocellatus* group) in the morphological study by Pietsch (1984), while it is here recovered as sister to the *A. striatus* group (Figs. 4 and 6).

Some characters of *A. indicus* overlap with the diagnostic features of *Fowlerichthys* and the *A. striatus* group. For example, the membrane behind the second dorsal-fin spine is divided into naked dorsal and ventral portions by a dense cluster of dermal denticles in *Fowlerichthys*, while this division is absent in the *A. striatus* group. Specimens of *A. indicus* usually lack a divided membrane behind the second dorsal-fin spine, or at most, it is only weakly divided (Pietsch, 1984). In *Fowlerichthys*, the ventral portion of the membrane usually extends posteriorly, dividing the depressed area between the bases of the second and third dorsal-fin spines and nearly reaches to the base of the third; the membrane behind the second dorsal-fin spine in *A. indicus* terminates distinctly anterior to the third dorsal-fin spine, as it does in the *A. striatus* group. The anterior end of the pterygiophore of the illicium nearly always extends anteriorly beyond the symphysis of the upper jaw in the *A. striatus* group, a feature thought to be unique among lophiiforms by Pietsch and Grobecker (1987). Despite the fact that specimens of *A. indicus* have never been recognized as having an overhanging pterygiophore, all specimens examined in this study have pterygiophores extending anteriorly beyond the symphysis of the upper jaw.

The pigment pattern of *A. indicus* also overlaps with both species groups. *Fowlerichthys* has 1–3 darkly pigmented ocelli on each side of the body while the *A. striatus* group lacks ocelli; *A. indicus* generally has 2–3 ocelli. The *A. striatus* group usually has darkly pigmented streaks or elongate blotches on the body (Fig. 3C) with similar markings on the soft-dorsal, caudal, and anal fins. However, markings are completely absent in some specimens, while recent unpublished photographs depict specimens that have blotchy, somewhat circular markings (Fig. 3D). Elongate blotches also occur along the posterior margin of the soft-dorsal and anal fins of *A. indicus* (Fig. 3B). Furthermore, *A. striatus*, *A. hispidus*, and *A. indicus* all have numerous darkly pigmented blotchy markings on the pectoral and pelvic fins (Fig. 3B–D). These markings do not occur in any other member of *Fowlerichthys*.

Meristics support inclusion of *A. indicus* in the *A. striatus* group as well. Most or all of the rays of the dorsal fin, and all five rays of the pelvic fin, are bifurcate in *Fowlerichthys* (the latter feature unique among the Lophiiformes), except for *A. indicus*, for which only the posteriormost two or three are bifurcate. Members of the *A. striatus* group have as many as four of the posteriormost dorsal-fin rays bifurcate. All members of *Fowlerichthys* have 20 vertebrae except *A. indicus*, which has only 19 vertebrae. All members of the *A. striatus* group, and all members of other species groups of *Antennarius*, have 19 vertebrae, with the exception of a single specimen of *A. striatus,* noted to have 18 vertebrae, and a single specimen of *A. commerson*, noted to have 20 vertebrae (Pietsch and Grobecker, 1987).

Pietsch (1984) stated that *A. indicus* is the "least specialized member" of his *A. ocellatus* group (=*Fowlerichthys*), and that the remaining five species form a monophyletic assemblage on the basis of having all five rays of the pelvic fin, and most or all of the rays of the dorsal fin, bifurcate, and having relatively high vertebral and dorsal-fin ray counts. Whereas *A. indicus* shares characteristics with both species groups, most characters exclude it from *Fowlerichthys* and support the molecular analyses for inclusion in the *A. striatus* group. These include an overhanging pterygiophore of

the illicium, 19 vertebrae, only the posteriormost pelvic-fin ray bifurcate, elongated blotches on the dorsal and anal fins, and the membrane behind the second dorsal-fin spine lacking a clear division of distinct clusters of dermal spinules. *A. indicus* is, therefore, removed from *Fowlerichthys* and placed within the *A. striatus* group.

4.4. Interrelationships of the A. striatus group

A single specimen of *Antennarius hispidus*, acquired through the aquarium trade, but purportedly collected in Indonesia, rendered *A. striatus* paraphyletic (Figs. 4 and 6). Tissues from *A. striatus* originated from three geographic locations: off the Louisiana coast in the Gulf of Mexico, US; Tosa Bay, Kōchi Prefecture, Japan; and Exmouth Gulf, Western Australia. Until Pietsch and Grobecker (1987) placed 28 nominal forms within the synonymy of *A. striatus*, the Western Atlantic population had been recognized as *A. scaber* and much of the known material from the Western Pacific as *A. tridens*. The molecular analyses suggest that these previously synonymized species do in fact represent distinct species. However, a number of authors have reported an excess of silent substitutions in mtDNA, providing strong evidence of selection on mitochondria or mitochondrial cellular interactions (reviewed by Gerber et al. 2001), and in many instances mtDNA is probably not selectively neutral (Ballard and Kreitman, 1995; Gerber et al., 2001; Rand, 2001; Ballard and Whitlock, 2004; Ballard and Rand, 2005). Because the tips of the tree are based primarily on mtDNA, and although there is some support from the nuDNA for *A. hispidus* rendering *A. striatus* paraphyletic, more evidence is needed from faster evolving nuclear markers, in particular, before these populations can be definitively recognized as distinct.

4.5. Phylogenetic position of Antennatus

Antennatus was introduced by Schultz (1957), but the type species, *Antennatus strigatus*, was originally described as a member of the genus *Antennarius* by Gill (1863). The only characters that differentiate members of *Antennatus* from those of *Antennarius* are the absence of a pseudobranch and the lack of small naked areas between the pores of the acoustico-lateralis system (Pietsch and Grobecker, 1987). However, it shares with members of the *A. nummifer* group a smaller maximum body size compared to most other species groups of *Antennarius* (the *A. pauciradiatus* group being a notable exception), and the second dorsal-fin spine free, not connected to the head by a membrane. Within the Antennariinae, *Antennarius coccineus*, *A. sanguineus*, *A. bermudensis*, and all species of *Antennatus* (as recognized by Pietsch and Grobecker, 1987) lack a caudal peduncle, the membranous posteriormost margin of the soft-dorsal and anal fins being connected to the body at the base of the outermost rays of the caudal fin. Of those species included in the molecular analyses, all form a clade whose members make up the *A. nummifer* group and the genus *Antennatus* (Figs. 4 and 6). All molecular analyses place the *A. nummifer* group and *Antennatus* in a group sister to a clade consisting of *Histrio* and the remaining species groups of *Antennarius*. Therefore, the *A. nummifer* group is hereby removed from the genus *Antennarius*. Because it is already in use, the generic designation *Antennatus* Schultz, 1957, is applied to this group. Members of *Antennatus* fall conveniently into two groups: the *A. tuberosus* group (new name), comprised of *A. tuberosus*, *A. strigatus*, *A. flagellatus*, and *A. linearis*, have relatively thick skin, short close-set dermal denticles, all rays of the caudal fin bifurcate, and a shorter, smaller caudal fin; and members of the *A. nummifer* group, comprised of *A. nummifer*, *A. coccineus*, *A. bermudensis*, *A. sanguineus*, *A. duescus*, *A. analis*, *A. dorehensis*, and *A. rosaceus*, with a darkly pigmented basidorsal spot nearly always present, dorsal fin with as many as six (usually only three) posteriormost rays bifurcate, and the posterior surface of the second dorsal spine usually devoid of dermal denticles (Pietsch and Grobecker, 1987).

Histrio is retained by the removal of the *A. ocellatus* and *A. nummifer* groups. Several characters, including exceptionally long pelvic fins (length greater than 25% SL), two cutaneous cirri on the mid-dorsal line of the snout between symphysis of premaxilla and base of illicium, and a pseudo-pelagic lifestyle (Pietsch and Grobecker, 1987), require that *Histrio* be recognized as distinct from the *A. pictus*, *A. striatus*, *A. pauciradiatus*, and *A. biocellatus* groups.

4.6. Phylogenetic positions of the A. pauciradiatus and A. biocellatus groups

The *A. pauciradiatus* and *A. biocellatus* groups have characters unique to the Antennariidae: in the monotypic *A. biocellatus* group, the second dorsal spine is free, not connected to the head by membrane, nearly always straight and tapering distally to a point; and, most peculiarly, the ability to live in brackish and even fresh waters (Pietsch and Grobecker, 1987); members of the *A. pauciradiatus* group are paedomorphic forms that reach a maximum length of only 40 mm; in addition, they have a membranous connection of the third dorsal-fin spine to the soft dorsal fin (Pietsch and Grobecker, 1987). There are no known morphological characters that unite either of these groups with any other species group of *Antennarius*, nor to *Fowlerichthys, Histrio*, or *Antennatus* (including the *A. nummifer* group). Vertebral counts (19) along with four simple and one bifurcate pelvic-fin rays suggest their placement somewhere within the lineage containing *Antennarius*, *Antennatus*, and *Histrio* (Figs. 4 and 6), but until adequate morphological and/or molecular data are analyzed, their placement within this lineage remains unknown and designated *incertae sedis*.

4.7. Phylogenetic position of Nudiantennarius

Nudiantennarius was not included in the molecular analyses because tissue was unavailable. A combination of morphological and meristic characters, including presence of the mesopterygoid and epural, 19 vertebrae, and a single bifurcate pelvic-fin ray (Pietsch, 1984) suggests its position in the lineage containing *Antennarius*, *Antennatus*, and *Histrio* within the Antennariinae (Figs. 4 and 6). Beyond this, its placement is unresolved and designated herein as Antennariinae *incertae sedis*.

4.8. Phylogenetic position of Allenichthys

Although *Allenichthys* was not included in the molecular analyses, its ovarian morphology and endemism in Western Australia, usually above about 35°S latitude, suggests its placement within the Histiophryninae (Figs. 5 and 7), in the clade containing *Tathicarpus*, *Lophiocharon*, and *Histiophryne* (see Section 4.10, below). A single record of *Allenichthys* from South Australia, captured near Port Lincoln, on 9 January 1967, is likely a result of dispersal via the Leeuwin and Zeehan currents. The Leeuwin Current flows down and around the Western Australian coast where it meets the Zeehan Current, which flows along the continental slope from the Great Australian Bight to Tasmania. The combination of the Leeuwin and Zeehan currents can bring tropical species well into southern coastal waters (Gomon et al., 2008).

4.9. Revised classification of the Antennariidae

Family Antennariidae Gill, 1863
- Subfamily Antennariinae
  - Genus *Fowlerichthys* Barbour, 1941
    - *Fowlerichthys avalonis* (Jordan and Starks, 1907), new combination
    - *Fowlerichthys ocellatus* (Bloch and Schneider, 1801), new combination
    - *Fowlerichthys radiosus* (Garman, 1896), new combination
    - *Fowlerichthys scriptissimus* (Jordan, 1902), new combination
    - *Fowlerichthys senegalensis* Cadenat, 1959
  - Genus *Antennarius* Daudin, 1816
  - *Antennarius pictus* group Pietsch, 1984
  - *Antennarius commerson* (Latreille, 1804)
  - *Antennarius pardalis* (Valenciennes, 1837)
  - *Antennarius pictus* (Shaw, 1794)
  - *Antennarius maculatus* (Desjardins, 1840)
  - *Antennarius multiocellatus* (Valenciennes, 1837)
  - *Antennarius striatus* group Pietsch, 1984
    - *Antennarius hispidus* (Bloch and Schneider, 1801)
    - *Antennarius indicus* Schultz, 1964
    - *Antennarius striatus* (Shaw, 1794)
  - *Antennarius pauciradiatus* group Pietsch, 1984
    - *Antennarius pauciradiatus* Schultz, 1957
    - *Antennarius randalli* Allen, 1970
  - *Antennarius biocellatus* group Pietsch, 1984
    - *Antennarius biocellatus* (Cuvier, 1817)
  - Genus *Histrio* Fischer, 1813
    - *Histrio histrio* (Linnaeus, 1758)
  - Genus *Antennatus* Schultz, 1957
  - *Antennatus tuberosus* group, new
    - *Antennatus flagellatus* Ohnishi et al., 1997
    - *Antennatus linearis* Randall and Holcom, 2001
    - *Antennatus strigatus* (Gill, 1863)
    - *Antennatus tuberosus* (Cuvier, 1817)
  - *Antennatus nummifer* group, new
    - *Antennatus analis* (Schultz, 1957), new combination
    - *Antennatus bermudensis* (Schultz, 1957), new combination
    - *Antennatus coccineus* (Lesson, 1831), new combination
    - *Antennatus dorehensis* (Bleeker, 1859), new combination
    - *Antennatus duescus* (Snyder, 1904), new combination
    - *Antennatus nummifer* (Cuvier, 1817), new combination
    - *Antennatus rosaceus* (Smith and Radcliffe, 1912), new combination
    - *Antennatus sanguineus* (Gill, 1863), new combination
  - Genus *Nudiantennarius* Schultz, 1957
    - *Nudiantennarius subteres* (Smith and Radcliffe, 1912)
- Subfamily Histiophryninae
  - Genus *Rhycherus* Ogilby, 1907
    - *Rhycherus filamentosus* (Castelnau, 1872)
    - *Rhycherus gloveri* Pietsch, 1984
  - Antennariidae genus and species novum
  - Genus *Kuiterichthys* Pietsch, 1984
    - *Kuiterichthys furcipilis* (Cuvier, 1817)
  - Genus *Phyllophryne* Pietsch, 1984
    - *Phyllophryne scortea* (McCulloch and Waite, 1918)
  - Genus *Echinophryne* McCulloch and Waite, 1918
    - *Echinophryne crassispina* (McCulloch and Waite, 1918)
    - *Echinophryne mitchellii* (Morton, 1897)
    - *Echinophryne reynoldsi* Pietsch and Kuiter, 1984
  - Genus *Tathicarpus* Ogilby, 1907
    - *Tathicarpus butleri* Ogilby, 1907
  - Genus *Lophiocharon* Whitley, 1933
    - *Lophiocharon hutchinsi* Pietsch, 2004
    - *Lophiocharon lithinostomus* (Jordan and Richardson, 1908)
    - *Lophiocharon trisignatus* (Richardson, 1844)
  - Genus *Histiophryne* Gill, 1863
    - *Histiophryne bougainvilli* (Valenciennes, 1837)
    - *Histiophryne cryptacanthus* (Weber, 1913)
    - *Histiophryne maggiewalker* Arnold and Pietsch, 2011
    - *Histiophryne psychedelica* Pietsch et al., 2009a
  - Genus *Allenichthys* Pietsch, 1984
    - *Allenichthys glauerti* (Whitley, 1944)

4.10. Life history and geographic distribution

The Antennariinae, containing *Fowlerichthys*, *Antennarius, Histrio*, and *Antennatus*, has a relatively wide geographic distribution, with all genera found circumglobally throughout the tropics and subtropics. The Histiophryninae, containing *Rhycherus,* Antennariidae gen. et sp. nov., *Kuiterichthys, Phyllophryne, Echinophryne*, *Tathicarpus, Lophiocharon,* and *Histiophryne*, is restricted to the Indo-Australian Archipelago, a region that extends from Taiwan to Tasmania, including all of the inland seas and islands of the Philippines, Indonesia, New Guinea, the Solomons, and the continent of Australia. Within the Histiophryninae, the clade containing *Rhycherus*, *Kuiterichthys*, Antennariidae gen. et sp. nov., *Phyllophryne*, and *Echinophryne* is restricted to temperate waters of Australia, below approximately 30°S latitude. The second clade, containing *Tathicarpus*, *Lophiocharon*, and *Histiophryne*, extends from the Philippines to approximately 35°S latitude, except for *Histiophryne*, which ranges from Taiwan to the temperate waters of southern Australia.

Reproductive life history also differs considerably between the two clades: the Antennariinae has double scroll-shaped ovaries described in detail by Rasquin (1958; see also Pietsch and Grobecker, 1987, pl. 10, Fig. 161), while the Histiophryninae has an entirely different type of ovary. In addition, each ovarian type corresponds to a different life history: members of the Antennariinae are broadcast spawners and go through a distinct larval stage, while those of the Histiophryninae undergo direct development and display various degrees of parental care. The details of ovarian morphology, life history, and associated behavior will be described in a future publication.

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