Syst. Biol. 61(6):1001–1027, 2012

DOI:10.1093/sysbio/sys060
Advance Access publication on June 27, 2012

The Evolution of Pharyngognathy: A Phylogenetic and Functional Appraisal of the Pharyngeal Jaw Key Innovation in Labroid Fishes and Beyond

PETER C. WAINWRIGHT[1,*], W. LEO SMITH[2], SAMANTHA A. PRICE[1], KEVIN L. TANG[3], JOHN S. SPARKS[4], LARA A. FERRY[5], KRISTEN L. KUHN[6,7], RON I. EYTAN[6], AND THOMAS J. NEAR[6]

[1]*Department of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616;* [2]*Department of Zoology, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605;* [3]*Department of Biology, University of Michigan-Flint, Flint, MI 48502;* [4]*Department of Ichthyology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024;* [5]*Division of Mathematical and Natural Sciences, Arizona State University, Phoenix, AZ 85069;* [6]*Department of Ecology and Evolution, Peabody Museum of Natural History, Yale University, New Haven, CT 06520; and* [7]*USDA-ARS, Beneficial Insects Introduction Research Unit, 501 South Chapel Street, Newark, DE 19713, USA;*
**Correspondence to be sent to: Department of Evolution & Ecology, University of California, One Shields Avenue, Davis, CA 95616, USA;*
E-mail: pcwainwright@ucdavis.edu.

Received 22 September 2011; reviews returned 30 November 2011; accepted 22 June 2012
Associate Editor: Luke Harmon

Abstract.—The perciform group Labroidei includes approximately 2600 species and comprises some of the most diverse and successful lineages of teleost fishes. Composed of four major clades, Cichlidae, Labridae (wrasses, parrotfishes, and weed whitings), Pomacentridae (damselfishes), and Embiotocidae (surfperches); labroids have been an icon for studies of biodiversity, adaptive radiation, and sexual selection. The success and diversification of labroids have been largely attributed to the presence of a major innovation in the pharyngeal jaw apparatus, pharyngognathy, which is hypothesized to increase feeding capacity and versatility. We present results of large-scale phylogenetic analyses and a survey of pharyngeal jaw functional morphology that allow us to examine the evolution of pharyngognathy in a historical context. Phylogenetic analyses were based on a sample of 188 acanthomorph (spiny-rayed fish) species, primarily percomorphs (perch-like fishes), and DNA sequence data collected from 10 nuclear loci that have been previously used to resolve higher level ray-finned fish relationships. Phylogenies inferred from this dataset using maximum likelihood, Bayesian, and species tree analyses indicate polyphyly of the traditional Labroidei and clearly separate Labridae from the remainder of the traditional labroid lineages (Cichlidae, Embiotocidae, and Pomacentridae). These three "chromide" families grouped within a newly discovered clade of 40 families and more than 4800 species (>27% of percomorphs and >16% of all ray-finned fishes), which we name Ovalentaria for its characteristic demersal, adhesive eggs with chorionic filaments. This fantastically diverse clade includes some of the most species-rich lineages of marine and freshwater fishes, including all representatives of the Cichlidae, Embiotocidae, Pomacentridae, Ambassidae, Gobiesocidae, Grammatidae, Mugilidae, Opistognathidae, Pholidichthyidae, Plesiopidae (including *Notograptus*), Polycentridae, Pseudochromidae, Atherinomorpha, and Blennioidei. Beyond the discovery of Ovalentaria, this study provides a surprising, but well-supported, hypothesis for a convict-blenny (*Pholidichthys*) sister group to the charismatic cichlids and new insights into the evolution of pharyngognathy. Bayesian stochastic mapping ancestral state reconstructions indicate that pharyngognathy has evolved at least six times in percomorphs, including four separate origins in members of the former Labroidei, one origin in the Centrogenyidae, and one origin within Beloniformes. Our analyses indicate that all pharyngognathous fishes have a mechanically efficient biting mechanism enabled by the muscular sling and a single lower jaw element. However, a major distinction exists between Labridae, which lacks the widespread, generalized percomorph pharyngeal biting mechanism, and all other pharyngognathous clades, which possess this generalized biting mechanism in addition to pharyngognathy. Our results reveal a remarkable history of pharyngognathy: far from a single origin, it appears to have evolved at least six times, and its status as a major evolutionary innovation is reinforced by it being a synapomorphy for several independent major radiations, including some of the most species rich and ecologically diverse percomorph clades of coral reef and tropical freshwater fishes, Labridae and Cichlidae. [Acanthomorpha; Beloniformes; Centrogenyidae; key innovation; Labroidei; Ovalentaria; pharyngeal jaws; Perciformes.]

Major innovations in organismal design have periodically fueled bursts of diversification throughout the history of life. Breakthroughs in design can facilitate the invasion of unused niche space, previously unattainable functional designs, and novel life history patterns. One widely recognized example of a major innovation is the pharyngeal jaw apparatus found in labroid fishes (cichlids, wrasses, parrotfishes, weed whitings, damselfishes, and surfperches). Originally recognized as a characteristic that grouped together members of Müller's (1843) Pharyngonathi acanthopterygii (modern beloniforms, cichlids, labrids, and pomacentrids), but later disregarded as a systematic feature (e.g., Regan 1913), "pharyngognathy" (Fig. 1) was examined, functionally explored, and described in detail in a widely cited series of papers on cichlids (Liem 1973; Liem and Osse 1975; Liem 1980). Pharyngognathy was proposed to be a major advance in feeding mechanism design that enhanced functional capacity and versatility in prey processing, freeing the oral jaws from these functions, and making possible an extensive trophic radiation. Liem (1980) made the explicit prediction that innovations in the pharyngeal jaw system played a significant role in the remarkable diversification of cichlids. Although this case also became a focal point for critiques of key innovation biology (Lauder 1982; Stiassny 1987; Lauder 2001), pharyngognathy has become one of the most widely cited examples of a key innovation and has maintained a prominent place in the discussions of cichlid diversity and adaptive radiation (Galis and Metz 1998; Futuyma 2005, p. 685).

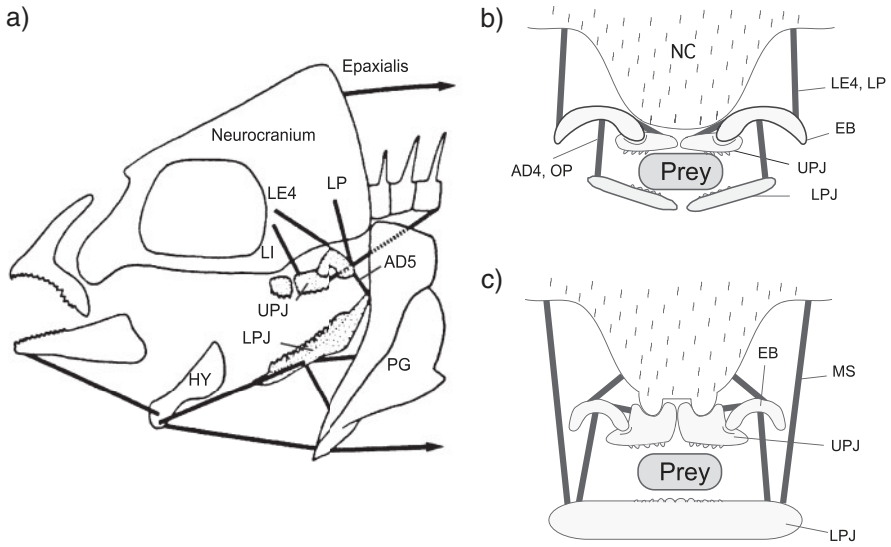

FIGURE 1. a) Lateral view diagram of a generalized spiny-rayed fish head showing the anterior position of the oral jaws and the posterior position of the pharyngeal jaws. The pharyngeal jaws are derived from gill arch bones and muscles and are used during prey processing behaviors. Muscles are shown as thick black lines connecting their attachment sites. b) Diagram of the posterior view of the pharngeal jaw apparatus in a generalized spiny-rayed fish. Note that the left and right lower jaws are separate bones, and the upper jaw is formed by two paired bones, the toothed pharyngobranchials and an epibranchial. The main mechanism of motion in these jaws is by flexion of the joint between the epibranchial and pharyngobranchial, that causes depression of the upper jaw. c) Diagram of the posterior view of a pharyngognathous pharyngeal jaw. Note that the paired lower jaw bones are united into a single lower jaw bone, the lower jaw is suspended from the neurocranium by a muscular sling, and there is a well-developed joint between the upper jaw and the neurocranium that stabilizes the upper jaw during biting. The main mechanism of motion in these jaws is elevation of the lower jaw by the musclular sling. AD4, fourth branchial adductor muscle; AD5, fifth branchial adductor muscle; EB, epibranchial bone; HY, hyoid bar; LE4, fourth *levator externus* muscle; LI, *levator internus* muscle; LP, *levator posterior* muscle; LPJ, lower pharyngeal jaw; MS, muscular sling; OP, *obliquus posterior* muscle; PG, pectoral girdle; UPJ, upper pharyngeal jaw.

As currently recognized, pharyngognathy involves three prominent modifications to the typical percomorph pharyngeal jaw apparatus (Fig. 1c): (i) a single lower pharyngeal jaw bone formed by the fusion or intimate suturing of the left and right fifth ceratobranchial bones; (ii) a muscular sling that directly connects the underside of the neurocranium with the lower pharyngeal jaw; and (iii) a diarthrosis between the dorsal surface of the upper pharyngeal jaw bones and a raised protuberance on the underside of the neurocranium. These morphological and functional characters, as seen in cichlids, were proposed as synapomorphies uniting an expanded Labroidei (Liem and Greenwood 1981) that included Embiotocidae (surfperches), Labridae (wrasses), Odacidae (weed whitings), and Scaridae (parrotfishes). Shortly thereafter, Kaufman and Liem (1982) added Pomacentridae (damselfishes) to the Labroidei and provided additional morphological support for its monophyly. The hypothesis that all of these spectacularly successful lineages, totaling more than 2600 described species or ∼5% of all vertebrates (Eschmeyer and Fricke 2012), shared common ancestry raised the status of pharyngognathy to increased prominence as its evolution became intimately associated with their success. Stiassny and Jensen (1987) further refined the limits and relationships of this group and noted that a remarkably similar series of modifications to the pharyngeal jaws were found in some beloniforms and had been reported in *Pholidichthys* by Springer and Freihofer (1976).

Phylogenetic analyses using morphological characters have repeatedly corroborated labroid monophyly (Kaufman and Liem 1982; Stiassny and Jensen 1987), occasionally including the enigmatic Pholidichthyidae (Springer and Orrell 2004), but, always with the disquieting observation that essentially all labroid synapomorphies are features of the modified pharyngeal jaw apparatus (see comments in Stiassny 1987; Johnson 1993; Springer and Orrell 2004; Wiley and Johnson 2010). No morphological evidence exists, independent of the pharyngeal jaws and gill arches, to corroborate labroid monophyly. Although subsequent morphological studies have questioned the morphological evidence supporting labroid monophyly (e.g., Rosen and Patterson 1990; Johnson 1993), they have not suggested alternative phylogenetic relationships. However, it should be noted that an original Rosen manuscript on labroid polyphyly (available from the authors), which was greatly reduced posthumously by Patterson and published as Rosen and Patterson (1990), does provide some suggestions and evidence for novel labroid alignments that include possible relationships with percomorph groups such as Anabantoidei, Gerreidae, Haemulidae, Kyphosidae, Sparoidea, and the "Squamipennes."

Supporting the cautionary observations presented in these later morphological studies, molecular phylogenetic analyses have substantially eroded

support for a monophyletic Labroidei. All published percomorph molecular studies that have touched on this issue have refuted labroid monophyly (Streelman and Karl 1997; Sparks 2004; Sparks and Smith 2004; Smith and Wheeler 2004; Dettai and Lecointre 2005; Westneat and Alfaro 2005; Smith and Wheeler 2006; Chen et al. 2007; Mabuchi et al. 2007; Setiamarga et al. 2008; Li et al. 2009). However, these molecular studies have emphasized labroid polyphyly and did not focus on resolving the phylogenetic relationships of constituent "labroid" clades. Although the monophyly of Labroidei is no longer viewed favorably, alternative phylogenetic relationships for the constituent clades remain contentious and confused. For example, Smith and Wheeler (2004) suggested that nonlabrid labroids or "chromides" may be part of a larger group of percomorphs that are characterized by demersal, adhesive eggs (see also Smith and Craig 2007; Setiamarga et al. 2008; Li et al. 2009), but this result was one of several recent molecular hypotheses criticized by Mooi and Gill (2010). This lack of a crucial phylogenetic framework for the former "labroid" clades requires resolution before it will be possible to investigate the origins and macroevolutionary implications of pharyngognathy.

The growing evidence that "labroids" are polyphyletic and the presence of all core "labroid" pharyngeal modifications in some beloniforms (Stiassny and Jensen 1987) and the monotypic Centrogenyidae (Springer and Johnson 2004; personal communication) argues for multiple origins of pharyngognathy within percomorphs. Resolution of some basic features of the phylogenetics of percomorphs, broadly, and "labroids" specifically, coupled with a revision of pharyngeal jaw functional morphology would provide a basis for investigating several questions regarding the evolutionary origin and significance of this specialization in the diversification of teleost fishes.

In this study, we combined an analysis of functional diversity among pharyngognathous perch-like fishes with a phylogenetic analyses of 188 species based on DNA sequence data collected from 10 protein-coding nuclear genes. Our goals were to (i) test the monophyly and hypothesize the interrelationships of pharyngognathous percomorphs, generally, and "labroids" specifically; (ii) determine by dissection and specimen manipulation whether or not each origin of pharyngognathy resulted in similar mechanisms of jaw action and whether this mechanism differs from what is found in nonpharyngognathous spiny-rayed fishes; and (iii) estimate the number of independent origins of pharyngognathy among teleosts.

Materials and Methods

Molecular Phylogenetic Analyses and Estimation of Relative Divergence Times

The phylogenetic analyses were rooted with two sampled species of *Polymixia* and the percopsiform *Percopsis omiscomaycus*, following the results of recent analyses that placed polymixiids (independently or with some "paracanthopterygian" and zeiform groups) sister to nonlampriform acanthomorphs (Johnson and Patterson 1993; Miya et al. 2003; Smith and Wheeler 2006). The 188 species sampled for this study are listed in Table 1 and were purposefully selected to include a dense sampling of all four labroid lineages as well as all groups noted by Stiassny and Jensen (1987) and Springer and Johnson (2004) to have at least one of the three "labroid" modifications to the pharyngeal jaw apparatus. Eight species were sampled with more than one specimen, including *Pholidichthys leucotaenia*, to verify accuracy of laboratory methods used in the collection of DNA sequences (Table 1). In addition, we included a broad diversity of acanthomorph, primarily percomorph, lineages that have previously been suggested to exhibit phylogenetic affinities with the constituent labroid clades in recent molecular (Sparks and Smith 2004; Dettai and Lecointre 2005; Smith and Wheeler 2006; Chen et al. 2007) and morphological phylogenetic studies (e.g., Stiassny 1981; Rosen and Patterson 1990; Springer and Orrell 2004).

Fish tissues used in DNA extractions were preserved in 70–95% ethanol or were obtained from museum collections. Genomic DNA was extracted from muscle or fin clips using a DNeasy Tissue Extraction Kit (Qiagen, Valencia, CA). The polymerase chain reaction (PCR) was used to amplify 10 PCR fragments using primers provided in López et al. (2004) for RAG1 exon 3 and Li et al. (2007) for ENC1, Glyt, myh6, plagl2, Ptr, SH3PX3, sreb2, tbr1, and zic1. Double-stranded amplifications were performed in a 25-μL volume containing 1 μL 25 mM $MgCl_2$ (Qiagen, Valencia, CA), 2.5 μL 10× CL PCR buffer (Qiagen, Valencia, CA), 2.5 μL Q solution (Qiagen, Valencia, CA), 0.5 μL 10 mM dNTP mix, 0.5 μL of each primer at 10 μM, 0.3 μL Taq DNA polymerase (Invitrogen Co., Carlsbad, CA), and 2.0 μL of DNA template.

The double-stranded amplification products were desalted and concentrated using AMPure (Agencourt Biosciences, Beverly, MA). Both strands of the purified PCR fragments were used as templates for cycle sequencing and were read using a 3730xL DNA analyzer (Applied Biosystems, Foster City, CA). Contiguous sequences were built using Sequencher (Gene Codes, Ann Arbor, MI) from DNA sequences of the complementary heavy and light strands. All new DNA sequences were submitted to GenBank and assigned accession numbers JX188676-JX190242.

All the genes used in our phylogenetic analyses are protein coding, therefore the DNA sequence alignments were constructed from alignments of the translated amino acid sequences constructed using the computer program MUSCLE (Edgar 2004). The combined 10-gene dataset contained 8439 aligned base pairs. Thirty data partitions were designated that corresponded to three separate codon positions for each of the 10 sampled protein-coding genes. Partitioned maximum likelihood analysis was executed using the computer

TABLE 1. Rank-free classification of species sampled for molecular phylogenetic analysis (species sampled with two specimens are marked with an asterisk)

- Polymixiidae
  - *Polymixia lowei* (outgroup taxon)
  - *Polymixia japonic*a (outgroup taxon)
- Percopsiformes
  - Percopsidae
    - *Percopsis omiscomaycus* (outgroup taxon)
- Acanthopterygii
  - Beryciformes
    - Berycidae
      - *Beryx decadactylus*
    - Trachichthyidae
      - *Hoplostethus atlanticus*
- Percomorpha
  - Ovalentaria
    - Ambassidae
      - *Ambassis urotaenia*
    - Atherinomorpha
      - Atheriniformes
        - Atherinopsidae
          - *Labidesthes sicculus**
        - Atherinidae
          - *Atherinomorus lacunosus*
        - Melanotaeniidae
          - *Melanotaenia* sp.
        - Bedotiidae
          - *Rheocles wrightaee*
      - Beloniformes
        - Adrianichthyidae
          - *Oryzias latipes*
        - Belonidae
          - *Platybelone argala*
          - *Scomberesox saurus*
          - *Strongylura marina*
          - *Xenentodon cancila*
        - Exocoetidae
          - *Cheilopogon pinnatibarbatus*
          - *Cheilopogon melanurus*
        - Zenarchopteridae
          - *Dermogenys collettei*
        - Hemiramphidae
          - *Arrhamphus sclerolepis*
      - Cyrpinodontiformes
        - Fundulidae
          - *Fundulus heteroclitus*
          - *Lucania goodei*
        - Poeciliidae
          - *Gambusia affinis**
    - Blennioidei
      - Blenniidae
        - *Meiacanthus grammistes*
        - *Ophioblennius atlanticus*
    - Chaenopsidae
      - *Chaenopsis alepidota*
    - Dactyloscopidae
      - *Gillellus semicinctus*
    - Labrisomidae
      - *Labrisomus multiporosus*
    - Cichlidae
      - *Cichla temensis*
      - *Etroplus maculatus*
      - *Herichthys cyanoguttatus*
      - *Heros efasciatus*
      - *Heterochromis multidens*
      - *Oreochromis niloticus*
      - *Paratilapia polleni*
      - *Paretroplus maculatus*
      - *Ptychochromis grandidieri*
    - Embiotocidae
      - *Cymatogaster aggregata*
      - *Rhacochilus vacca*
      - *Embiotoca jacksoni*
      - *Embiotoca lateralis*
      - *Hyperprosopon argenteum*
      - *Phanerodon furcatus*
    - Gobiesocidae
      - *Diademichthys lineatus*
      - *Gobiesox maeandricus*
    - Grammatidae
      - *Gramma loreto*
    - Mugilidae
      - *Mugil cephalus*
      - *Mugil curema*
    - Opistognathidae
      - *Opistognathus aurifrons*
    - Pholidichthyidae
      - *Pholidichthys leucotaenia**
    - Plesiopidae
      - *Plesiops coeruleolineatus*
    - Polycentridae
      - *Monocirrhus polyacanthus*
      - *Polycentrus schomburgkii*
    - Pomacentridae
      - *Abudefduf saxatilis*
      - *Chromis cyanea*
      - *Microspathodon bairdii*
      - *Stegastes leucostictus*
    - Pseudochromidae
      - *Congrogadus subducens*
      - *Pseudochromis fridmani*
      - *Labracinus cyclophthalmus*
      - *Ogilbyina novaehollandiae*
      - *Pholidochromis cerasina*
    - Ophidiiformes
      - Ophidiidae
        - *Brotula multibarbata**
    - Syngnathiformes
      - Aulostomidae
        - *Aulostomus maculatus*
    - Centriscidae
      - *Aeoliscus strigatus*
    - Gasterosteiformes
    - Aulorhynchidae
      - *Aulorhynchus flavidus*
    - Gasterosteidae
      - *Gasterosteus aculeatus**
    - Synbranchiformes
    - Mastacembelidae
      - *Macrognathus siamensis*
    - Synbranchidae
      - *Monopterus albus**
    - Gobiiformes
      - Gobioidei
        - Eleotridae
          - *Eleotris pisonis*
        - Odontobutidae
          - *Odontobutis potamophila*
        - *Perccottus glenii*
        - Apogonidae
          - *Ostorhinchus lateralis*
          - *Cheilodipterus quinquelineatus*
    - Anabantoidei
      - Anabantidae
        - *Microctenopoma nanum*
        - *Ctenopoma kingsleyae*

(*Continued*)

TABLE 1. Continued

- Osphronemidae
  - *Betta splendens*
- Labridae
  - *Bodianus rufus*
  - *Cetoscarus bicolor*
  - *Chlorurus sordidus*
  - *Clepticus parrae*
  - *Coris batuensis*
  - *Coris gaimard*
  - *Diproctacanthus xanthurus*
  - *Epibulus brevis*
  - *Epibulus insidiator*
  - *Gomphosus varius*
  - *Haletta semifasciata*
  - *Halichoeres bivittatus*
  - *Halichoeres margaritaceus*
  - *Labrichthys unilineatus*
  - *Labropsis australis*
  - *Lachnolaimus maximus**
  - *Oxycheilinus celebicus*
  - *Oxyjulis californica*
  - *Pteragogus enneacanthus*
  - *Tautoga onitis*
  - *Tautogolabrus adspersus*
  - *Xyrichtys martinicensis*
- Notothenioidei
  - Channichthyidae
    - *Chionobathyscus dewitti*
- Nototheniidae
  - *Dissostichus eleginoides*
- Acanthuroidei
  - Acanthuridae
    - *Acanthurus bahianus*
    - *Ctenochaetus strigosus*
  - Scatophagidae
    - *Scatophagus argus*
- Bembropidae
  - *Bembrops anatirostris*
  - *Bembrops gobioides*
- Carangidae
  - *Seriola dumerili*
  - *Trachinotus carolinus*
- Centrarchidae
  - *Ambloplites rupestris*
- Centrogenyidae
  - *Centrogenys vaigiensis*
- Centropomidae
  - *Centropomus undecimalis*
- Chaetodontidae
  - *Chaetodon ornatissimus*
  - *Forcipiger flavissimus*
- Channidae
  - *Channa striata*
- Cirrhitidae
  - *Paracirrhites arcatus*
- Cheilodactylidae
  - *Cheilodactylus variegatus*
- Cottiformes
  - Anarhichadidae
    - *Anarhichas lupus*
  - Anoplopomatidae
    - *Anoplopoma fimbria*
  - Cottidae
    - *Cottus carolinae*
  - Cyclopteridae
    - *Cyclopterus lumpus*
  - Hexagrammidae
    - *Hexagrammos otakii*

(*Continued*)

TABLE 1. Continued

- Pholidae
  - *Pholis crassispina*
- Zoarcidae
  - *Lycodes terraenovae*
- Epinephelidae
  - *Cephalopholis argus*
  - *Mycteroperca microlepis*
  - *Rypticus saponaceus*
- Gempylidae
  - *Ruvettus pretiosus*
- Gerreidae
  - *Ulaema lefroyi*
  - *Eugerres plumieri*
- Haemulidae
  - *Haemulon sciurus*
  - *Haemulon vittatum*
- Icosteidae
  - *Icosteus aenigmaticus*
- Kuhliidae
  - *Kuhlia marginata*
- Kyphosidae
  - *Kyphosus elegans*
- Leiognathidae
  - *Gazza minuta*
  - *Leiognathus equulus*
- Lethrinidae
  - *Lethrinus erythropterus*
  - *Monotaxis grandoculis*
- Lutjanidae
  - *Lutjanus biguttatus*
  - *Lutjanus mahogoni*
  - *Ocyurus chrysurus*
- Malacanthidae
  - *Caulolatilus princeps*
  - *Malacanthus plumieri*
- Moronidae
  - *Morone chrysops**
- Nemipteridae
  - *Pentapodus caninus*
  - *Scolopsis bilineata*
  - *Scolopsis margaritifer*
- Nomeidae
  - *Cubiceps baxteri*
- Percichthyidae
  - *Maccullochella peelii*
  - *Gadopsis marmoratus*
- Percidae
  - *Etheostoma atripinne*
  - *Percina caprodes*
  - *Perca flavescens*
- Pinguipedidae
  - *Parapercis clathrata*
- Pleuronectiformes
  - Paralichthyidae
    - *Paralichthys dentatus*
  - Pleuronectidae
    - *Pleuronectes platessa*
    - *Pseudopleuronectes americanus*
  - Scophthalmidae
    - *Scophthalmus aquosus*
- Polyprionidae
  - *Stereolepis gigas*
- Pomacanthidae
  - *Chaetodontoplus melanosoma*
  - *Holacanthus passer*
  - *Pomacanthus zonipectus*
- Sciaenidae
  - *Aplodinotus grunniens*

(*Continued*)

TABLE 1. Continued

Leiostomus xanthurus
Menticirrhus littoralis
Scombridae
Sarda sarda
Sebastidae
Sebastes fasciatus
Sebastes ruberrimus
Sebastolobus alascanus
Serranidae
Hypoplectrus puella
Paralabrax nebulifer
Pseudanthias pascalus
Serranus tigrinus
Hemanthias aurorubens
Sparidae
Lagodon rhomboides
Stenotomus chrysops
Toxotidae
Toxotes jaculatrix
Trichiuridae
Trichiurus lepturus
Uranoscopidae
Astroscopus y-graecum
Kathetostoma averruncus
Lophiiformes
Chaunacidae
Chaunax sp.
Gigantactinidae
Gigantactis vanhoeffeni
Lophiidae
Lophius americanus
Lophius gastrophysus
Tetraodontiformes
Diodontidae
Chilomycterus schoepfi
Diodon holocanthus
Ostraciidae
Ostracion cubicus
Rhinesomus triqueter
Triacanthodidae
Triacanthodes anomalus

program RAxML 7.2.6 with the default GTR+G model for each of the 30 data partitions (Stamatakis 2006). In RAxML, we used the -D option, which stops the ML searches when they have reached the asymptotic convergence phase. The criterion for stopping the searches is based on computing the Robinson–Foulds (RF) distance (Robinson and Foulds 1981) between two consecutive intermediate trees. If the RF distance between two consecutive trees is smaller than 1%, the ML search is stopped (Stamatakis 2011). A single specimen for each species was used in the maximum likelihood phylogenetic analyses. Support for nodes in the RAxML inferred tree was assessed using a thorough bootstrap analysis (option -f i) with 500 replicates. These analyses were repeated for each of the 10 individual genes, where three data partitions were designated for each codon position in each gene.

A species tree was estimated using gene tree parsimony implemented in iGTP (Chaudhary et al. 2010). The 10 RAxML inferred gene trees were used as input files. The trees were rooted before use in iGTP and several rooting strategies were used. The individual gene trees were rooted using *Polymixia lowei* and *P. japonica*; however, *Beryx decadactylus* and *Hoplostethus atlanticus* were used to root two gene trees where both species of *Polymixia* were missing. A heuristic search using randomized hill climbing was performed to find the species tree that minimized the reconciliation cost for deep coalescence. This search was bootstrapped with 100 replicates and bootstrap proportions were calculated using SumTrees in the DendroPy package (Sukumaran and Holder 2010).

Relative divergence times of the sampled species were estimated using an uncorrelated lognormal (UCLN) model of molecular evolutionary rate heterogeneity implemented in the computer program BEAST v. 1.6.0 (Drummond et al. 2006; Drummond and Rambaut 2007). As in the maximum likelihood analysis using RAxML described above, only a single specimen of each sampled species was used in the UCLN analyses. The same 30 data partitions and molecular evolutionary models used in the RAxML analysis were implemented in BEAST to estimate the posterior density of relative divergence times. A birth–death speciation prior was used for branching rates in the phylogeny and each sampled locus was assigned a separate molecular clock model. An age prior with a normal distribution and a mean of 100.0 and a standard deviation equal to 3.0 was applied to the root node of the phylogeny. Using the software TreeEdit (http://tree.bio.ed.ac.uk/software/treeedit/), the RAxML phylogeny inferred from the 10-gene data was converted into an ultrametric tree with a root age that was within the distribution of the prior and was used as a starting tree. The BEAST analyses were run three times with each run consisting of 6.0×10^7 generations. The resulting trees and log files from each of the three runs were combined using the computer program LogCombiner v. 1.5.3 (http://beast.bio.ed.ac.uk/LogCombiner). Convergence of model parameter values and estimated node-heights to their optimal posterior distributions was assessed by plotting the marginal posterior probabilities versus the generation state in the computer program Tracer v. 1.5 (http://beast.bio.ed.ac.uk/Tracer). The posterior probability density of the combined tree and log files was summarized as a maximum clade credibility tree using TreeAnnotator v. 1.5.3 (http://beast.bio.ed.ac.uk/TreeAnnotator). The mean and 95% highest posterior density estimates of divergence times and the posterior probabilities of inferred clades were visualized using the computer program FigTree v. 1.2.3 (http://beast.bio.ed.ac.uk/FigTree).

Pharyngeal Jaw Functional Morphology

We focused on two issues in reviewing pharyngeal jaw functional morphology. First, we wanted to estimate how many times pharyngognathy has evolved in perch-like fishes. Second, we asked whether the functional

morphology of the pharyngeal jaws is similar in the clades that exhibit pharyngognathy.

We studied the pharyngeal jaw apparatus in generalized percomorph taxa, representatives of all four "labroid" clades, and representatives of other percomorph clades known to exhibit some form of pharyngognathous features, or known to have well-developed pharyngeal jaws (e.g., Beloniformes, Centrogenyidae). The generalized percomorphs included Centrarchidae (*Micropterus salmoides* and *Lepomis macrochirus*), Hexagrammidae (*Ophiodon elongatus*), Cottidae (*Scorpaenichthys marmoratus*), Centropomidae (*Centropomus undecimalis*), Sciaenidae (*Sciaenops ocellatus*), Lutjanidae (*Lutjanus griseus*), and Carangidae (*Caranx crysos*). From the "labroid" clades, we examined Labridae (*Bodianus axillaris*, *Cheilinus chlorourus*, and *Halichoeres bivittatus*), Cichlidae (*Cichla ocellaris*, *Herichthys minckleyi*, and *Paratilapia polleni*), Pomacentridae (*Hypsypops rubicundus*, *Chromis punctipinnis*, and *Abudefduf saxatilis*), and Embiotocidae (*Cymatogaster aggregata* and *Embiotoca jacksoni*). Other percomorphs with robust pharyngeal regions were studied, including Centrarchidae (*Lepomis microlophus*), Carangidae (*Trachinotus carolinus*), Sciaenidae (*Pogonias cromis*), Centrogenyidae (*Centrogenys vaigiensis*), Pholidichthyidae (*Pholidichthys leucotaenia*), and several beloniform species (*Strongylura marina, Xenentodon cancila, Hemiramphus brasiliensis*, and *Cypselurus melanurus*). We treated parrotfishes (Scaridae) and weed-whitings (Odacidae) as clades nested within Labridae (Clements et al. 2004; Westneat and Alfaro 2005), and we do not discuss their modified pharyngeal jaw apparatus that we hypothesize to be derived relative to the ancestral condition observed in Labridae.

Detailed observations were made on specimens that had been cleared and stained for bone and cartilage (Dingerkus and Uhler 1977), as well as fresh or formalin-preserved specimens. For each species, our aim was to determine the primary mechanisms of movement in the pharyngeal jaws. This was evaluated by manipulation of fresh and cleared and stained specimens, observing the orientation of joints and muscles, and noting the position of muscles that attach to the pharyngeal jaw elements.

Evolutionary History of Pharyngognathy

To infer the number of transitions to pharyngognathy, while taking into account uncertainty in the tree topology, branch lengths, and the character mapping, we used Bayesian stochastic character mapping (Huelsenbeck et al. 2003 and references therein). Analyses were implemented in the program SIMMAP v1.0 (Bollback 2006) and used the relative time-calibrated ultrametric trees from the BEAST analyses of 188 acanthomorph species. No prior on the rate parameter was used, as we wanted to use the branch lengths as a direct estimate of rate. SIMMAP uses a symmetrical β prior on the symmetry of the transition rate matrix i.e., the extent to which transitions favor one state (0) over the other (1). The shape of the β distribution is described by the α parameter and discretized into κ categories; as α becomes larger the distribution becomes narrower around the state frequency of 0.5. We explored whether highly biased transition models affected the number of estimated transitions to pharyngognathy using three different β distributions: $\alpha = 1$ which is an uninformative prior whereby all possible biases are given equal prior probability, $\alpha = 0.1$ is a strong bias favoring either state 1 or 0 and $\alpha = 10$ adds a strong bias for not favoring either state i.e., equally probable (as illustrated in Fig. 8). κ, the number of categories used to discretize the β distribution, was set at 41 for all analyses as there is a trade-off between increasing the number of categories to more finely describe the distribution and runtime. A random sample of 5000 trees was drawn from the posterior distribution of trees generated by BEAST. We then sampled 1000 possible character histories in proportion to their Bayesian posterior probability for each topology. Combining these results gave us a distribution of the number of possible transitions to pharyngognathy.

Results

Molecular Phylogenetic Analysis and Estimation of Relative Divergence Time

The coverage among the 188 species for the 10-gene dataset was high with only 8.4% of the data cells (species and genes) missing (Supplementary Table 1, doi:10.5061/dryad.5h951h04). The coverage among the individual genes ranged from 96.3% complete with only 7 missing species for Ptr to 82.4% complete with 33 missing species for sreb2. The optimal molecular evolutionary model for each codon position selected using Akaike information criterion (AIC) was GTR+I+G; however, we did not use the invariant sites parameter in our analyses due to the reasons outlined by Yang (2006, p. 113–114) and the fact that GTR+G is the most complex model available in RAxML (Stamatikis 2006). Data matrices and trees are available at TreeBASE (http://purl.org./phylo/treebase/phylows/study/TBS:S12889).

The RAxML phylogeny inferred from the 10-gene dataset is presented in Figure 2 and a summary of clades resolved in each of the 10 gene trees is illustrated in Figure 3. In the phylogeny, 76% of the nodes were supported with a bootstrap support greater than 70%. The phylogeny showed a wide separation of Labridae from the remainder of the traditional labroids or "chromides" (Cichlidae, Embiotocidae, and Pomacentridae). Labridae was resolved as the sister lineage of Gerreidae (mojarras) with very weak bootstrap support (Fig. 2a). Among the individual gene trees, this labrid-gerreid clade was only supported with *RAG1* (Fig. 3b). The three "chromide" lineages were resolved as part of a species-rich clade, representing approximately 4875 species and 40 families of teleost fishes or 27% of

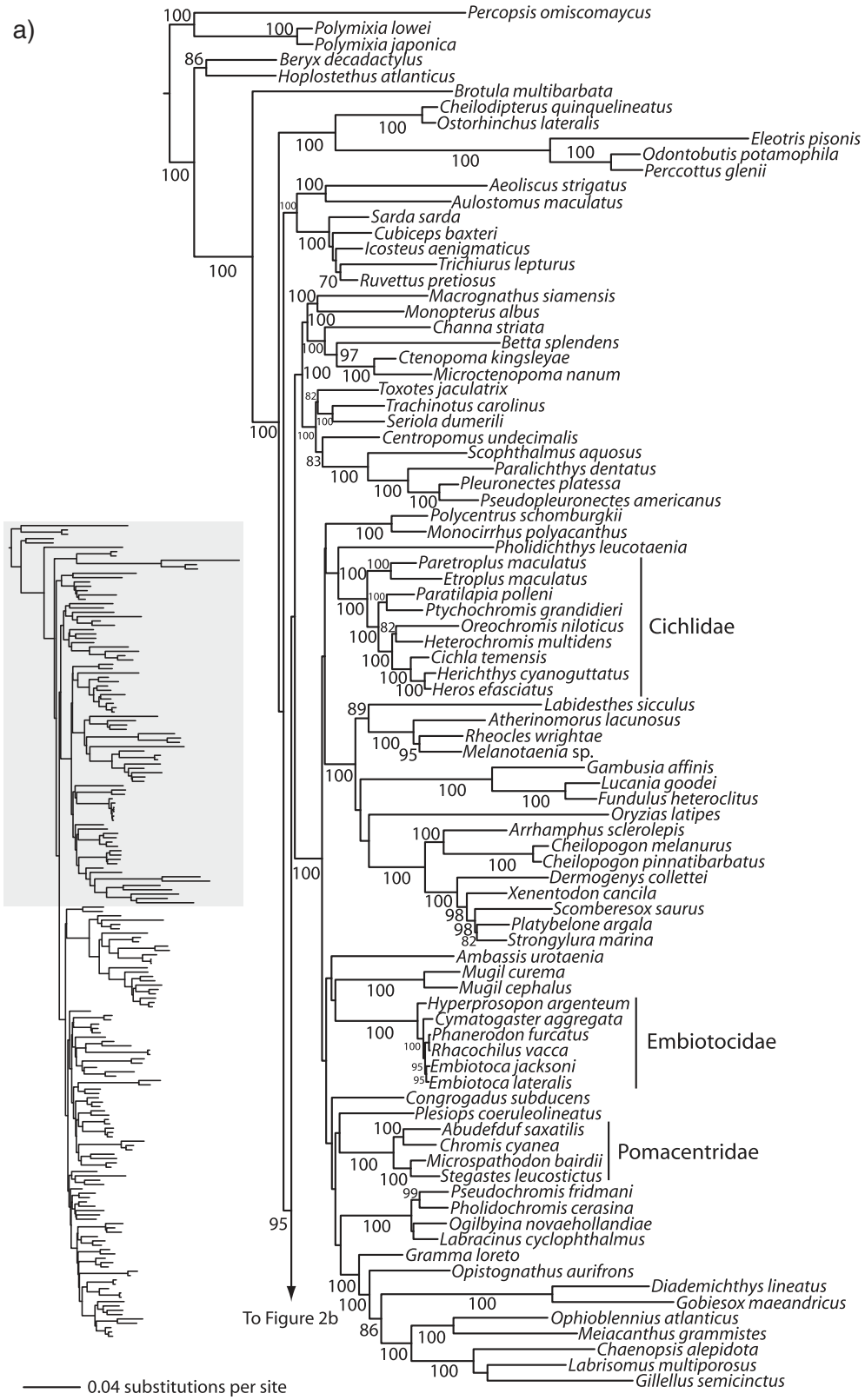

FIGURE 2. Phylogeny of 188 species of Acanthomorpha inferred from a partitioned maximum likelihood analysis of a 10-gene dataset using RAxML. Clade names identify lineages that were previously classified as Labroidei (Cichlidae, Embiotocidae, Pomacentridae, and Labridae). Numbers at nodes report percent presence in a bootstrap analysis with 500 replicates. Bootstrap values less than 70% are not shown. The shaded portion of the phylogeny on the left side of the figure indicates placement of the expanded region of the acanthomorph phylogeny. Arrows connect disconnected branches in the phylogeny. The phylogeny is presented in two parts, labeled a) and b).

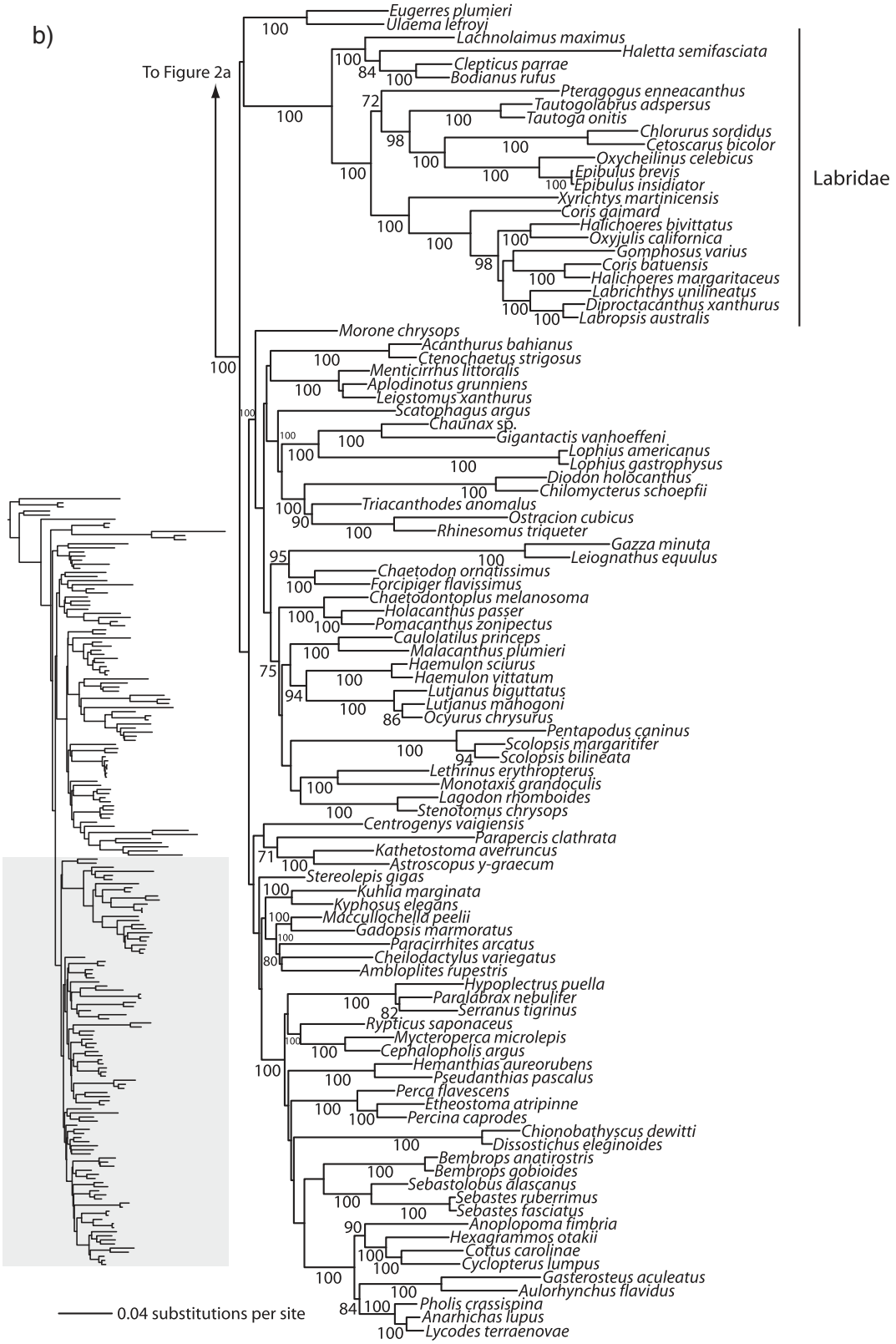

FIGURE 2. Continued.

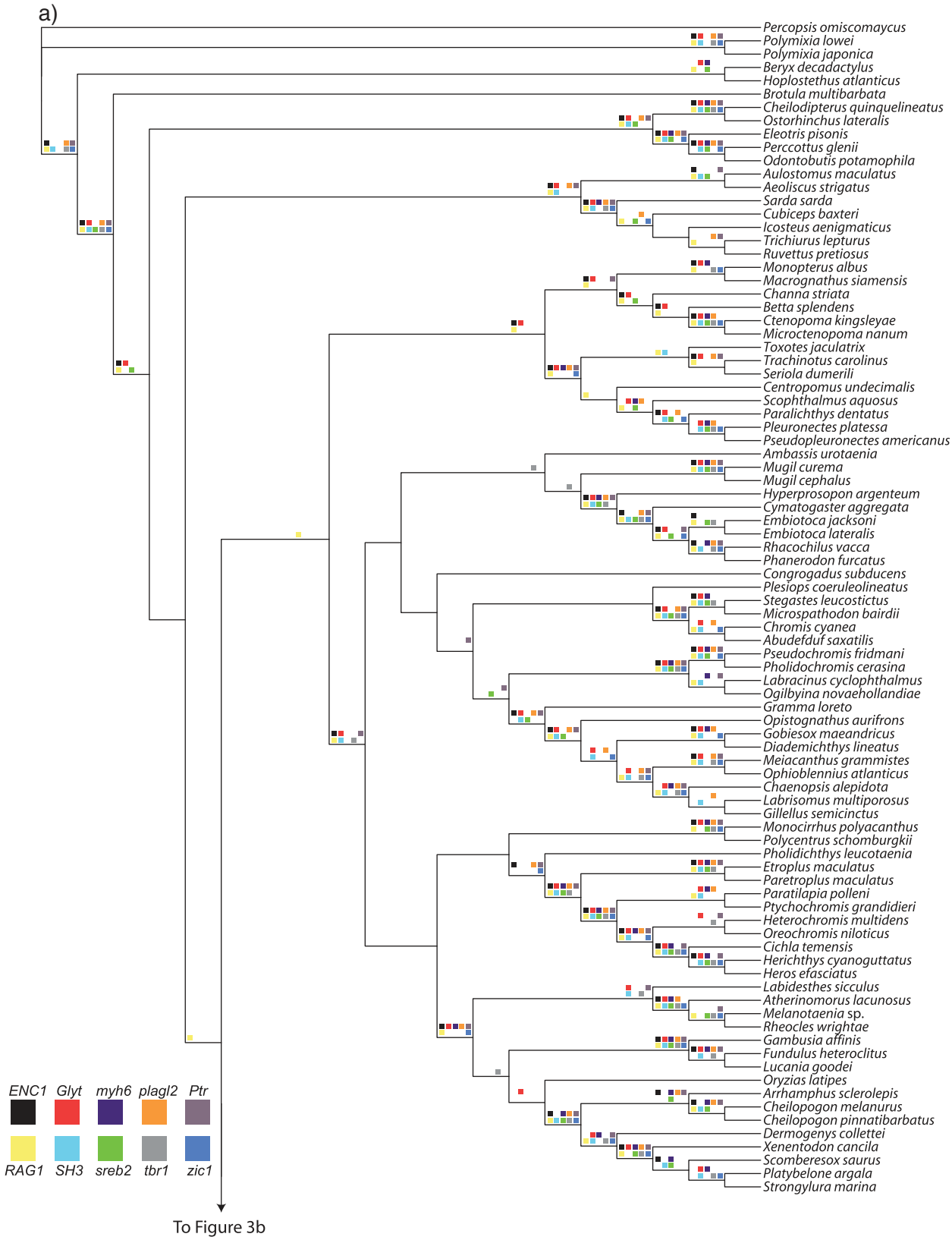

FIGURE 3. Phylogeny of Acanthomorpha that illustrates the congruence between the RAxML phylogenies inferred from the 10-gene dataset and the 10 individual gene trees. Clades that were resolved in the individual gene trees, with the exception of nested missing taxa, are identified with the appropriately colored square above the node. For clades where the earliest diverging taxa were missing, the node was treated as missing (i.e., no colored square present). Nodes where even a single terminal taxon was resolved outside of the clade in the individual gene tree resulted in the clade being scored as not present. The phylogeny is presented in two parts, labeled a) and b).

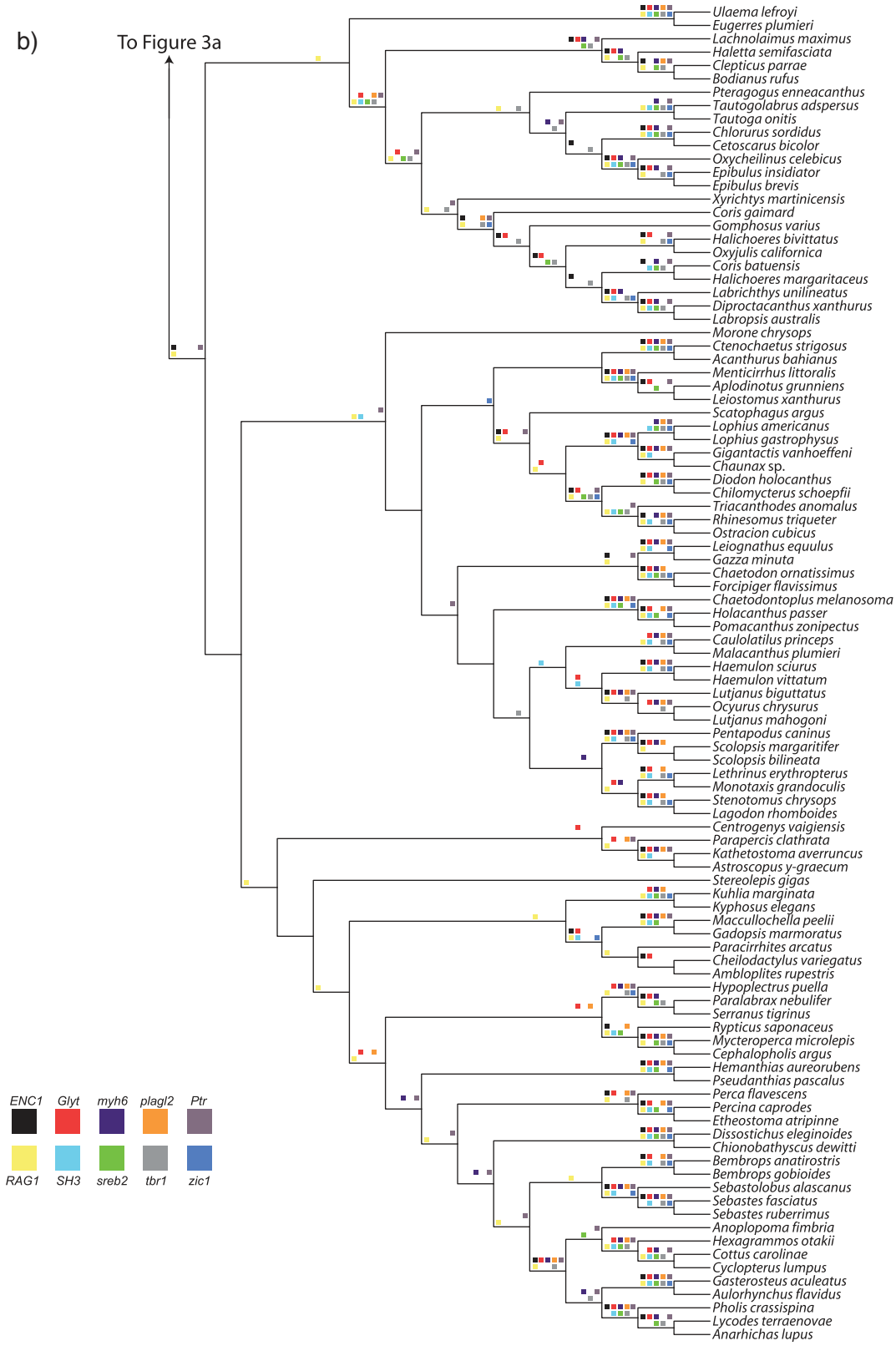

FIGURE 3. Continued.

all percomorph species (Eschmeyer and Fricke 2012). In addition to the "chromides," this clade includes the Ambassidae, Congrogadinae, Gobiesocidae, Grammatidae, Mugilidae, Opistognathidae, Pholidi chthyidae, Plesiopidae, Polycentridae, Pseudo-chrominae, Atheriniformes (e.g., Atherinidae, Atherinopsidae, Bedotiidae, and Melanotaeniidae), Beloniformes (e.g., Adrianichthyidae, Belonidae, Exocoetidae, Hemiramphidae, and Zenarchopteridae), Cyprinodontiformes (e.g., Cyprinodontidae, Fundu lidae, and Poeciliidae), and Blennioidei (e.g., Blenniidae, Chaenopsidae, Dactyloscopidae, and Labrisomidae) (Fig. 2a). This diverse clade is supported with a high bootstrap value (Fig. 2a), is present in six of the gene trees (Fig. 3a), and is named and diagnosed herein as Ovalentaria (Appendix).

Within Ovalentaria, Cichlidae, Embiotocidae, and Pomacentridae were not resolved as a clade or particularly closely related to each other; however, most of the nodes relating major named clades within the Ovalentaria were not well supported or present in more than one or two gene trees. One interesting exception was the sister-group relationship between Pholidichthyidae and Cichlidae, which was supported with a bootstrap value of 100% and resolved in four gene trees (Figs. 2a and 3a). Two additional well-supported clades within Ovalentaria are noteworthy: monophyly of the traditional Atherinomorpha (and its constituent groups) and the clade (Grammatidae (Opistognathidae (Gobiesocidae (Blennioidei)))), which were both supported by a bootstrap value of 100% and present in seven and six gene trees, respectively (Figs. 2a and 3a). The former corroborates the monophyly and ordinal interrelationships of one of the long-recognized clades of acanthomorphs (Rosen and Parenti 1981). The latter relationship resolves a longstanding question regarding the origin of blennies and will aid future research exploring the evolution of elongation in perch-like fishes.

Labridae was resolved as monophyletic with strong bootstrap support, but the weakly supported Labridae-Gerreidae clade was resolved as the sister lineage of a large clade that included Bembropidae, Centrogenyidae, Chaetodontidae, Epinephelidae, Haemulidae, Leiognathidae, Lethrinidae, Lutjanidae, Malacanthidae, Moronidae, Nemipteridae, Percidae, Pinguipedidae, Polyprionidae, Pomacanthidae, Sciaenidae, Scorpae nidae, Serranidae, Sparidae, Uranoscopidae, Acant huroidei, Gasterosteioidei, Notothenioidei, Cottiformes, Lophiiformes, Tetraodontiformes and Near et al.'s (2012) "centrarchiforms". The monotypic lineage Centrogenyidae, comprising *Centrogenys vaigiensis* (False Scorpionfish), was not resolved as being closely related to Labridae or any member of Ovalentaria, but in a clade containing Pinguipedidae and Uranoscopidae that was not well supported (Fig. 2b) and present only in the *Glyt* gene tree (Fig. 2b). Figure 4 depicts the phylogenetic results of the RAxML analysis, with taxa collapsed to more inclusive taxonomic group designations.

Phylogenetic inferences from the gene tree parsimony "species tree" analysis were largely congruent with those from the 10-gene RAxML analyses (Fig. 2). Specifically, Ovalentaria, Atherinomorpha, the clade containing Grammatidae, Opistognathidae, Gobiesocidae, and Blennioidei, and the monophyly of *Pholidichthys* and Cichlidae were supported in the gene tree parsimony phylogeny and supported with high bootstrap values (Fig. 5). As in the RAxML analysis of the 10-gene dataset, relationships among the major ovalentarian clades were unresolved. Labridae was resolved in a poorly supported clade that also contained Gerreidae, *Centrogenys*, Polyprionidae, Pinguipedidae, and Uranoscopidae.

The combined postburn-in trees and parameter values from three independent BEAST analyses totaled 1.62×10^8 samples. Large effective sample size values ($>10^3$) for all parameters indicated that adequate sampling intensity was achieved. The posterior density of the likelihood score was −232 178.66 (95% highest posterior density [HPD]: [−232 257.30, −232 101.74]). The maximum clade credibility tree estimated from the posterior density of the BEAST analyses was characterized by a large number of nodes supported with significant Bayesian posterior probabilities (Fig. 6). This phylogeny was very similar to the tree estimated using RAxML (Figs. 2 and 4), and relationships of the four labroid clades, Labridae, Cichlidae, Embiotocidae, and Pomacentridae were congruent among the phylogenetic hypotheses; however, the weakly supported Labridae-Gerreidae clade was not present in the BEAST majority rule topology (Fig. 6b). Cichlids and *Pholidichthys* were resolved as a clade nested in Ovalentaria and supported with a Bayesian posterior probability of 1.0 (Fig. 6a).

Pharyngognathy

Pharyngognathy (Fig. 1) involves three character states that have been treated in slightly different ways by previous authors (Liem 1973; Liem and Greenwood 1981; Kaufman and Liem 1982; Stiassny and Jensen 1987; Springer and Johnson 2004): (i) a single lower pharyngeal jaw bone formed by the fusion or intimate suturing of the left and right fifth ceratobranchials; (ii) a muscular sling that directly connects the underside of the neurocranium with the lower pharyngeal jaw; and (iii) a diarthrosis between the dorsal surface of the upper pharyngeal jaw bones and a protuberance on the underside of the neurocranium (Figs. 1 and 7). In our survey, we departed from previous treatments that defined the muscle sling character as a muscular attachment of the fourth *levator externus* and/or *levator posterior* muscle on the fifth ceratobranchial, involving fusion with the fourth *adductor branchialis* muscle and/or the *obliquus posterior* muscle. Instead, the coding used in this study combines separate character states that had been identified by previous researchers that used a comparative anatomical perspective (Stiassny and Jensen 1987; Springer and

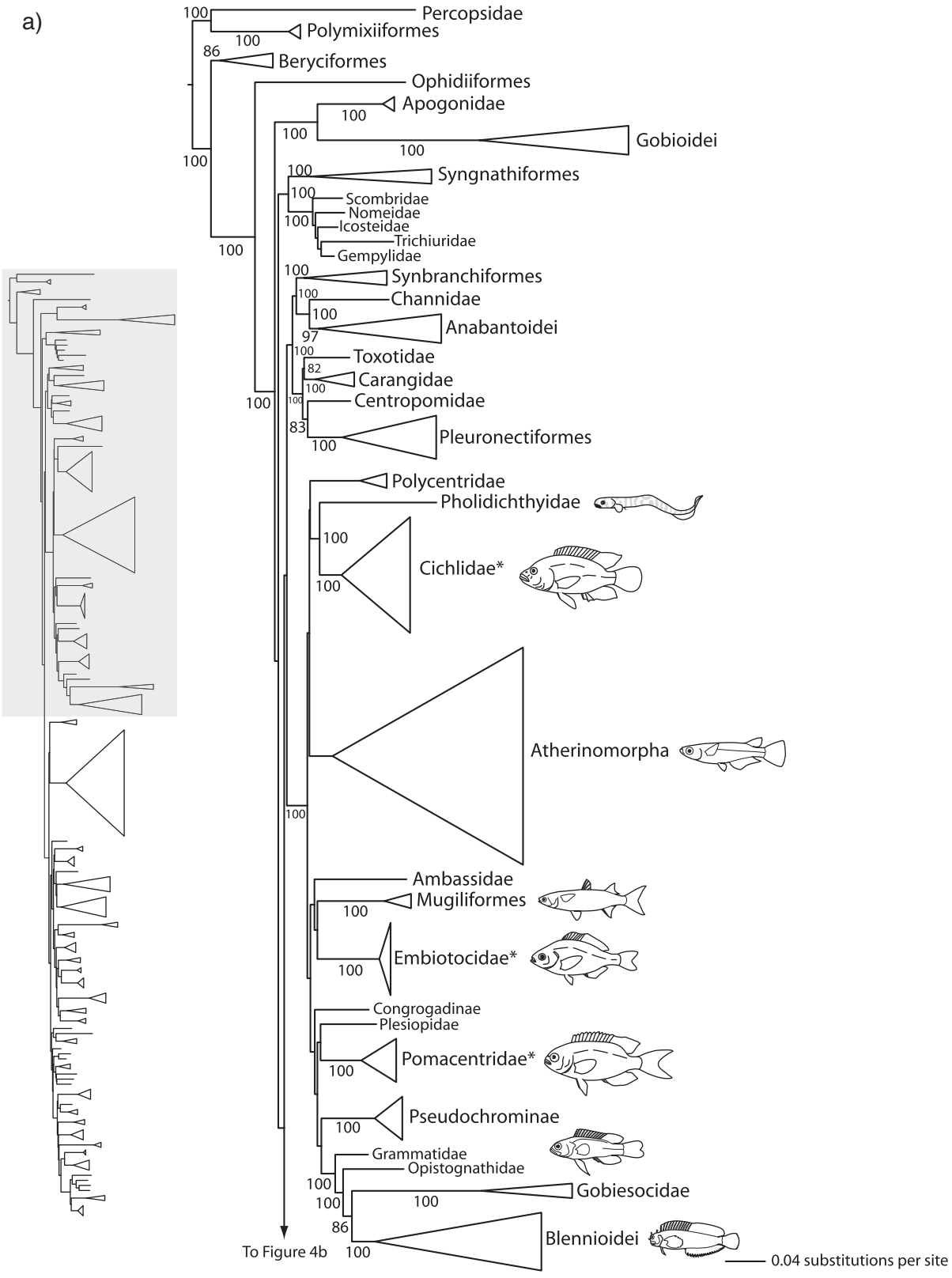

FIGURE 4. Phylogeny of 188 species of Acanthomorpha collapsed to more inclusive taxonomic groups inferred from a partitioned maximum likelihood analysis of a 10-gene dataset using RAxML. Numbers at nodes report percent presence in a bootstrap analysis with 500 replicates. Bootstrap values less than 70% are not shown. The widths of the triangles are proportional to the numbers of species sampled for the named clade. Clades previously classified as Labroidei (Cichlidae, Embiotocidae, Pomacentridae, and Labridae) marked with an asterisk. Line drawings of species from all "labroid" and exemplar Ovalentaria lineages are shown. The shaded portion of the phylogeny on the left side of the figure indicates placement of the expanded region of the acanthomorph phylogeny. Arrows connect disconnected branches in the phylogeny. The phylogeny is presented in two parts, labeled a) and b).

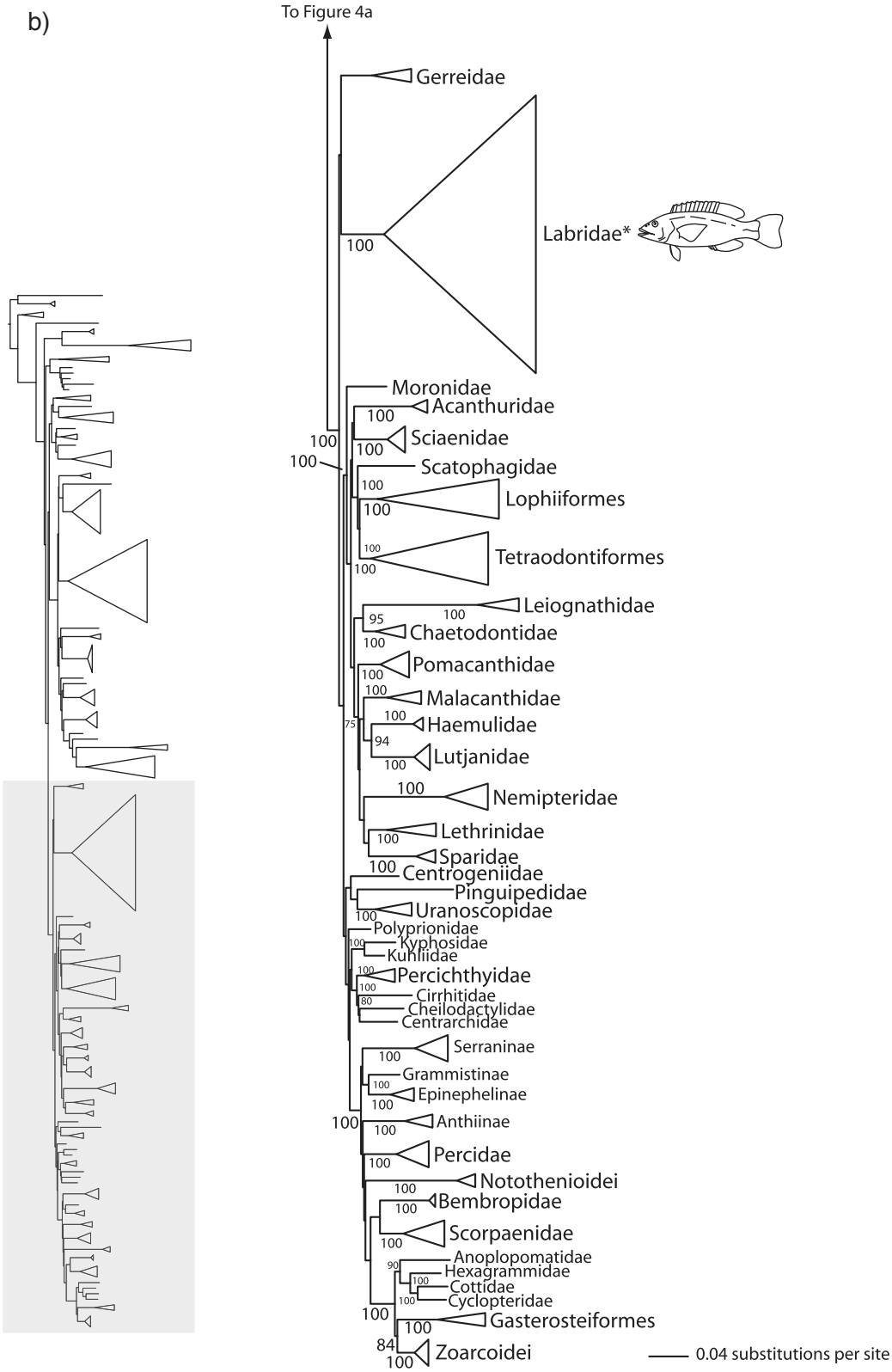

FIGURE 4. Continued.

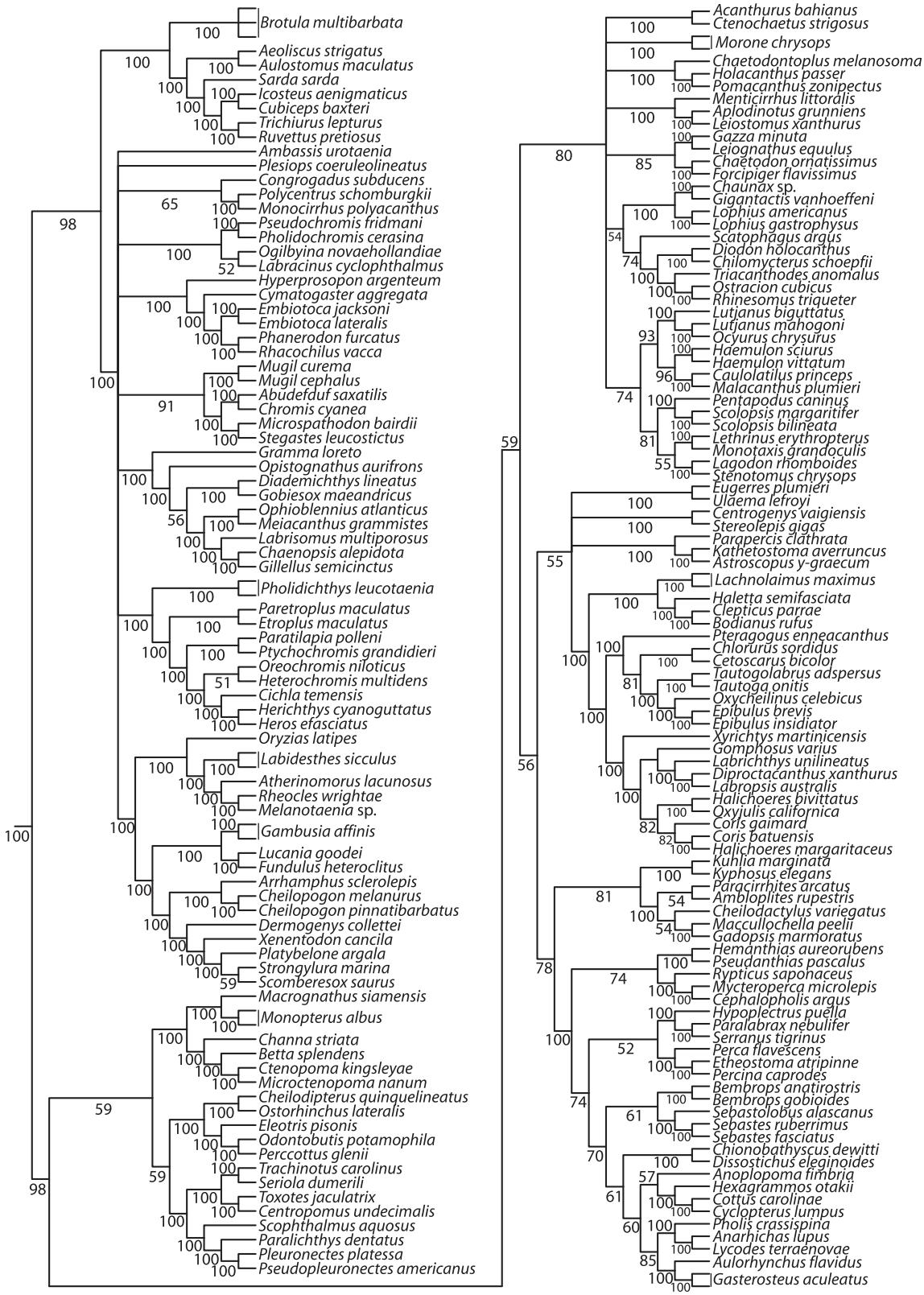

FIGURE 5. Species tree phylogeny of 188 species of Acanthomorpha inferred using gene tree parsimony. Bootstrap values are given at nodes with clades supported in less than 50% of the bootstrap replicates collapsed. Outgroup taxa (*Percopsis*, *Polymixia*, *Beryx*, and *Hoplostethus*) are not shown.

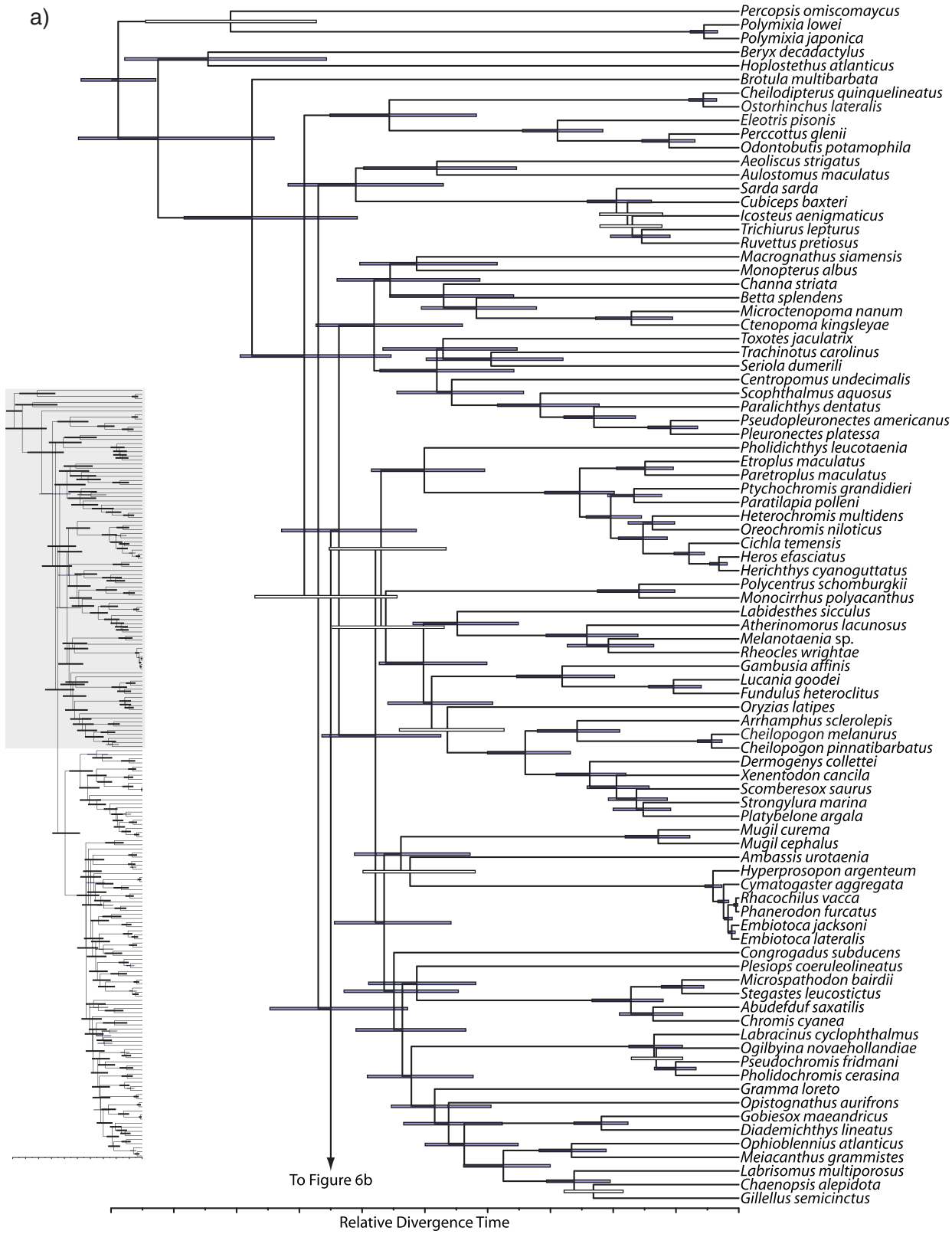

FIGURE 6. Posterior maximum clade credibility relative time tree of 188 species of Acanthomorpha inferred from a relaxed molecular clock analysis of a 10-gene dataset using BEAST. Branches are scaled to relative age estimates. Bars at nodes reflect the 95% highest posterior density of the relative age estimates. Posterior probabilities of clade support 0.95 and greater are labeled with shaded node bars and those with posterior support values less than 0.95 are marked with open node bars. The shaded portion of the phylogeny on the left side of the figure indicates placement of the expanded region of the acanthomorph relative time tree. Arrows connect disconnected branches in the phylogeny. The phylogeny is presented in two parts, labeled a) and b).

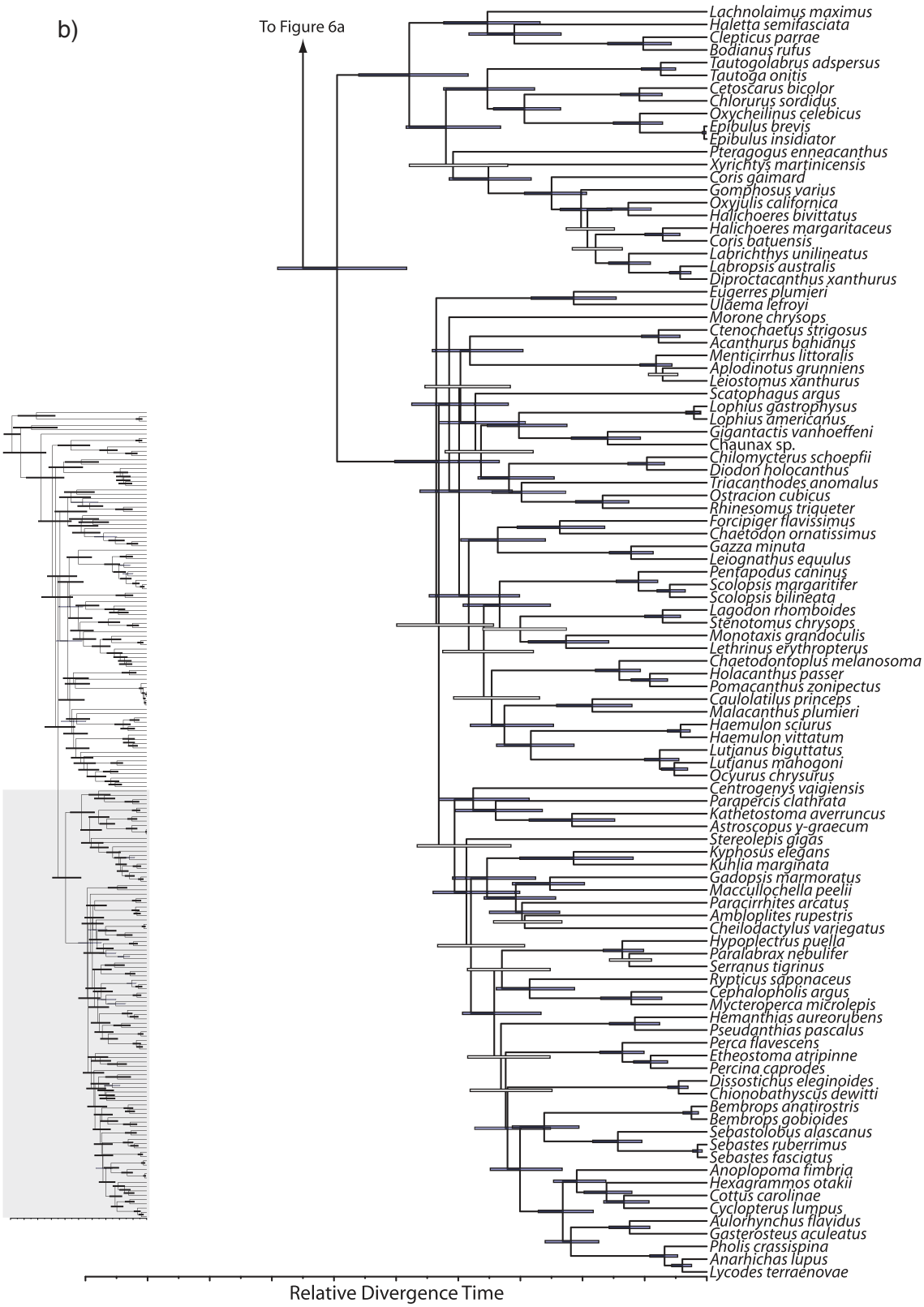

FIGURE 6. Continued.

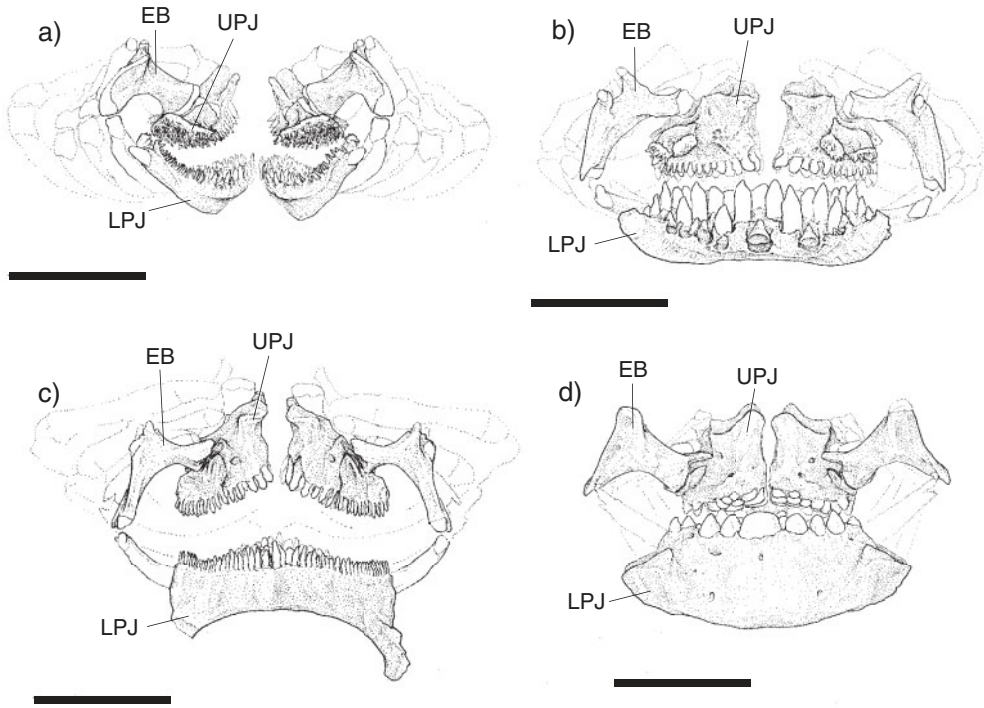

FIGURE 7. Posterior views of the pharyngeal jaws of a generalized percomorph, a) the centrarchid *Lepomis punctatus*, and b) three labroid taxa: the embiotocid *Embiotoca jacksoni*, c) the pomacentrid *Hypsypops rubicundus*, and d) the labrid *Halichoeres garnoti*. Note in b)–d) the single lower pharyngeal jaw element formed by fusion of the left and right fifth ceratobranchials. The relationship between the epibranchial and the upper pharyngeal jaw in *Lepomis* is largely retained in the "labroid" taxa and in *Lepomis*, *Embiotoca* and *Hypsypops*, rotation of the epibranchial causes depression of the upper pharyngeal jaw. EB, fourth epibranchial; LPJ, lower pharyngeal jaw (fifth ceratobranchial); UPJ, upper pharyngeal jaw (third pharyngobranchial). Scale bars are 3 mm.

Johnson 2004). We are studying the evolution of a functional sling—a muscular attachment between the neurocranium and lower pharyngeal jaw. We allow that there may be some diversity in the identity of muscles that contribute to the sling. In the following discussion, we will first treat the distribution of these traits in "labroid" lineages, and then we separately discuss their presence in other percomorphs.

All "labroid" species possess united fifth ceratobranchials. This character state appears to be the ancestral condition in labrids, cichlids, pomacentrids, and embiotocids. In labrids, pomacentrids, and embiotocids, the fifth ceratobranchials are fused with no evidence of a suture, whereas cichlids show a distinct suture connecting the fifth ceratobranchials. A muscular sling was found in all "labroid" species except the labrichthyins, *Labroides* (Springer and Johnson 2004) and *Labropsis*, and the pomacentrid *Chromis* (Stiassny and Jensen 1987). The diarthrosis between the neurocranium and upper pharyngeal jaw was found in all "labroids" and, like the other two character states, appears to be the ancestral condition for each clade.

Some additional taxa possess all three of the pharyngognathous traits. All nonadrianichthyid beloniforms previously described have been shown to exhibit united fifth ceratobranchials. Within this group, all beloniforms, except some belonids (e.g., *Tylosurus* [Springer and Johnson 2004]), possess a diarthrosis with a protuberance on the underside of the neurocranium. *Hemiramphus* and *Cypselurus* have a muscle sling, but this trait is absent in belonids.

We observed all three traits in *Centrogenys*. Springer and Johnson (2004) noted the muscle sling and diarthrosis, but these authors reported that the fifth ceratobranchials were separate. In contrast, our specimens of *Centrogenys* were larger than those of Springer and Johnson (2004) and have strongly united fifth ceratobranchials with a relatively simple suture between the bones. This lower jaw clearly can act as a single mechanical unit. *Pholidichthys* has united fifth ceratobranchials and a diarthrosis on the pharyngobranchial, but there is no muscle sling (see also Springer and Johnson 2004). A muscle sling (Fig. 1), complete or partial, has been previously noted in at least some members of 13 additional percomorph families; species sampled from all 11 of these clades were included in our phylogenetic analyses (Atherinidae, Aulorhynchidae, Badidae, Bedotiidae, Carangidae, Centropomidae, Channidae, Haemulidae, Latidae, Leiognathidae, Ostracoberycidae, Percichthyidae, and Toxotidae). Finally, the Triacanthodidae is the only clade known to exhibit a dorsal diarthrosis while lacking both the muscular sling and the united fifth ceratobranchials.

Pharyngeal Jaw Functional Morphology

If pharyngognathy results in high versatility and performance in prey manipulation and processing by the pharyngeal jaws, it would be important to establish that the three morphological traits result in a similar functional system in the cases where they evolved. In this review of functional morphology, we focused particularly on the "labroids," attempting to estimate mechanisms of action based on muscle presence and orientation and the movement of specific joints in fresh and cleared-and-stained specimens. Our anatomical observations of pharyngognathous beloniforms suggest that this specialization may not be associated with the strong biting action seen in Centrogenyidae and most examined "labroids". The mechanism of pharyngeal jaw action has been described for generalized percomorphs that lack pharyngognathy (Wainwright 1989; Galis and Drucker 1996; Wainwright 2005; Grubich and Westneat 2006). In this mechanism, the primary biting actions are produced by depression of the upper jaw elements that are pushed from their dorsal surface by rotation of the fourth epibranchial bones (Figs. 1 and 7). Biting is produced by the combined actions of levator muscles (the fourth *levator externus* and the *levator posterior*) that connect the neurocranium to the distal arm of the epibranchial and the *obliquus dorsalis* muscle that crosses dorsal to the joint between the epibranchial and the pharyngobranchial (henceforth referred to as the upper jaw bone). Because the epibranchial is stabilized at the midpoint of its shaft, elevation of the distal arm causes depression of the medial arm that presses against the dorsal surface of the upper jaw. In this generalized condition, there is no direct muscular connection from the neurocranium to the lower pharyngeal jaw and the forceful movement of the lower jaw is largely limited to small movements in the anterior–posterior axis (Wainwright 2005). Our survey confirmed the presence of this mechanism in the broad sampling of generalized percomorphs that were inspected. Based on morphological traits and manipulation of specimens as well as literature records (e.g., Springer and Johnson 2004), we infer the presence of this mechanism in all percomorphs sampled in our phylogenetic analyses that lack pharyngognathy.

In all "labroid" clades with a muscular sling, the single lower jaw element is elevated by this muscle, contributing substantially to the pharyngeal jaw biting forces (Fig. 1). However, we also found that the generalized percomorph mechanism of upper jaw depression functioned in most pharyngognathous taxa, including cichlids, pomacentrids, embiotocids, some beloniforms (*Hemiramphus* and *Cypselurus*), and *Centrogenys*. In these taxa, the *obliquus dorsalis* muscle is well developed, and it appears that rotation of the fourth epibranchial by this muscle and the muscle sling, which in many taxa retains partial attachment to the fourth epibranchial, causes depression of the upper jaw.

Functionally, we find that only labrids are characterized by the lack of upper pharyngeal jaw depression. In marked contrast to other pharyngognathous taxa, the *obliquus dorsalis* muscle is weakly developed. Although the muscle sling does attach, in part, to the fourth epibranchial, the bend in the shaft of the fourth epibranchial is mainly oriented anteriorly such that elevation of the distal arm of this bone serves to push the upper jaw medially, against the paired upper jaw from the opposite side (note orientation of the labrid epibranchial-pharyngobranchial joint in Figs. 1 and 7). In labrids, the articulating surface of the fourth epibranchial, at its connection to the upper jaw, is flat and the two bones slide against each other at this articulation. The flattened end of the fourth epibranchial in labrids is in contrast to all other pharyngognathous taxa examined that exhibit a rounded end of the fourth epibranchial that articulates on the dorsal surface on the upper jaw. This sliding joint in labrids appears to facilitate considerable anterior–posterior motion of the upper pharyngeal jaw bones but not dorsal–ventral motion.

The posterior distribution of possible transitions to pharyngognathy, as defined by the presence of the three constituent traits, reveals that there have most likely been between six and 10 independent transitions to pharyngognathy within percomorphs (Fig. 8). This inference is robust as it integrates across uncertainty in tree topology, branch lengths, and character mapping and the result is not altered by placing very different prior expectations on the bias of the transition rate matrix. By looking at a sample of the character mappings upon the phylogeny, as illustrated in Figure 9, we find that pharyngognathy most likely originated on the branches leading to Labridae, Centrogenyidae, Embiotocidae, Pomacentridae, Cichlidae, and once in the common ancestor of the Hemiramphidae + Exocoetidae.

Discussion

Our phylogenetic reconstructions, coupled with an analysis of the nature and occurrence of pharyngognathy in percomorph fishes, suggest a reinterpretation of the evolutionary history of this complex of traits. Several major and novel conclusions about percomorph relationships and the evolution of pharyngognathy emerge from our study. (i) Our results strongly reject the hypothesis of labroid monophyly and suggest many new well-supported relationships within percomorphs. (ii) As many as six to ten separate origins of pharyngognathy have occurred in percomorph fishes. (iii) The functional morphology of pharyngognathy differs among groups, as revealed by the loss of the generalized upper jaw depressing mechanism in Labridae, and the retention of this system in all other pharyngognathous groups. (iv) The multiple independent origins of pharyngognathy will allow for multiple tests of key innovation hypotheses surrounding the consequences of pharyngognathy for lineage diversification and ecological diversification,

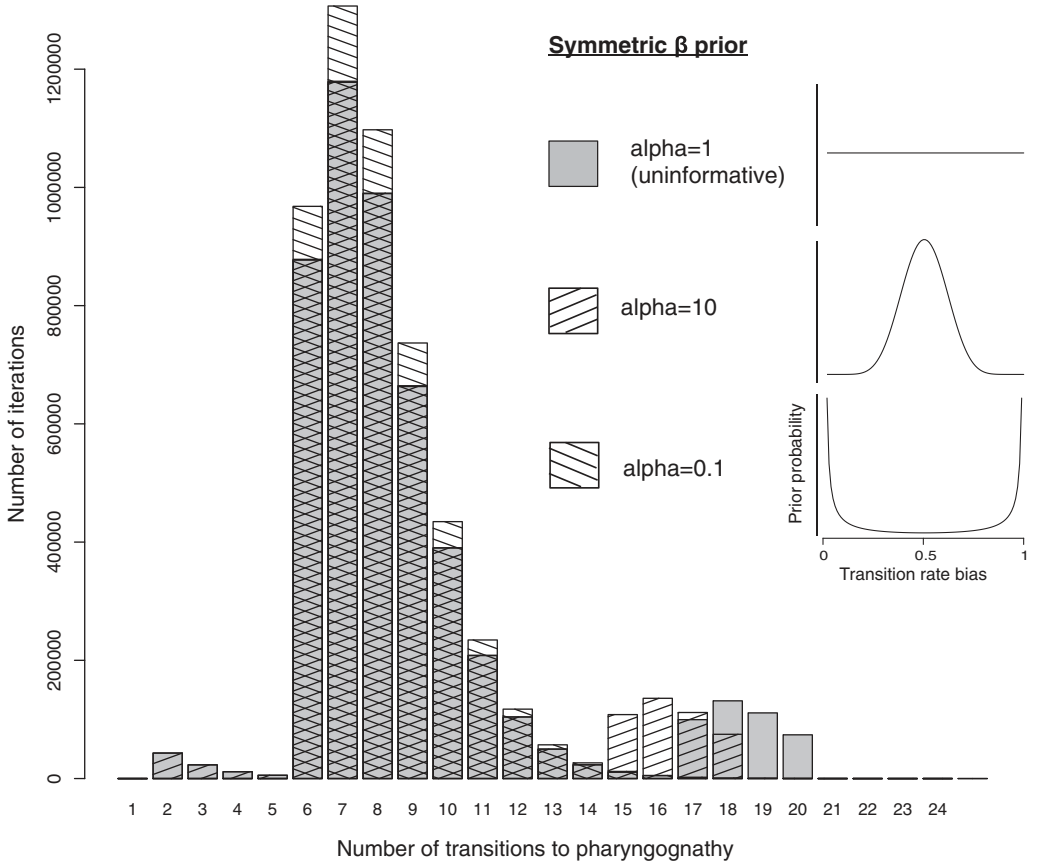

FIGURE 8. Histogram showing the estimated number of transitions to pharyngognathy from 5 million character maps generated in SIMMAP (Bollback 2006) using three very different β distributions on the prior expectation of the symmetry of the transition rate matrix. This illustrates the variability in the number of transitions due to uncertainty in the tree topology, branch lengths and character mapping by using 5000 trees sampled from the posterior distribution generated by BEAST and 1000 samples from the posterior for each map for every prior seperately.

although these analyses must await further clarification of sister-group relationships for most of the identified pharyngognathous clades.

Resolution of Percomorph Phylogeny and the Polyphyly of Labroidei

The 10-gene dataset used in our analyses provided substantial phylogenetic resolution among major lineages of percomorph fishes that has ranked as one of the most difficult long-standing issues in vertebrate phylogeny (e.g., Stiassny et al. 2004; Nelson 2006). Previous efforts at resolving relationships of labroids using whole mtDNA genome sequence data did not have the density of species and lineages sampled in our analyses (e.g., Miya et al. 2005; Setiamarga et al. 2008), and inferences from datasets composed of fewer nuclear genes, or a combination of mitochondrial and nuclear genes (e.g., Chen et al. 2003; Smith and Wheeler 2006; Li et al. 2009), have not provided the degree of resolution observed in our phylogenetic results (Figs. 2–6). Our phylogenetic analyses of percomorphs resulted in several important phylogenetic inferences that are novel to our study, as well as corroborating a number of phylogenetic hypotheses inferred initially from previously published studies based on the analysis of mtDNA, nuclear, or combined mtDNA and nuclear DNA sequences.

One of the most interesting hypotheses involving pharyngognathous fishes is the sister-group relationship between Cichlidae and *Pholidichthys*. The phylogenetic relationships of both lineages within percomorphs have been problematic and inconsistent across recent studies (Smith and Wheeler 2006; Smith and Craig 2007). Compared with earlier studies with less complete taxon sampling for the placement of these lineages, the cichlid–*Pholidichthys* sister-group relationship was supported with a high bootstrap value in the RAxML analysis (Figs. 2 and 4), was present in four of the ten gene trees (Fig. 3), supported with a high bootstrap value as a clade in the species tree analysis (Fig. 5), and was supported with a significant Bayesian posterior probability in the BEAST analysis (Fig. 6). Compared with the species-rich freshwater cichlids, *Pholidichthys* is classified as a monogeneric family that contains two allopatrically distributed species, *Pholidichthys leucotaenia* and *P. anguis*, which occupy shallow marine habitats ranging from northern Australia to the Philippines (Springer and Freihofer

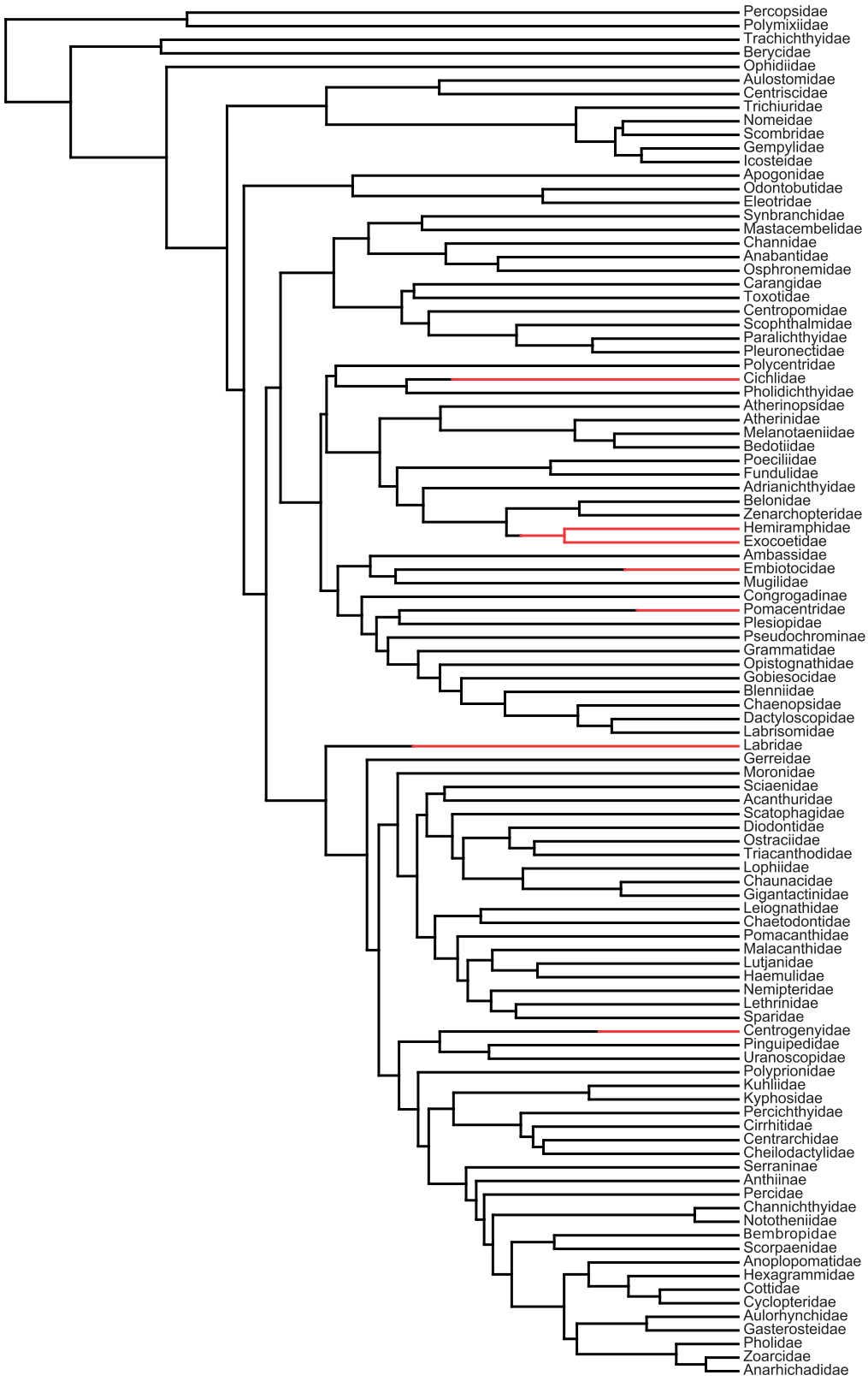

FIGURE 9. Family level phylogeny with one possible evolutionary history of pharyngognathy mapped upon it using stochastic character mapping implemented in the R package Phytools by (Revell 2011). This mapping represents six origins of pharyngognathy in percomorph fishes which is one of the most common SIMMAP reconstructions. For simplicity, the original tree has been pruned to a single representative for each monophyletic subfamily or family and labeled accordingly. All SIMMAP mappings were performed on the complete 188 taxon BEAST tree.

1976; Springer and Larson 1996). Since Springer and Freihofer (1976), many have noted the similarity of the gill arches between pholidichthyids and "labroids," eventually serving as the impetus for the extensive survey of gill-arch musculature in acanthomorphs presented in Springer and Johnson (2004). In the analytical component of the gill-arch study, Springer and Orrell (2004) resolved Pholidichthyidae as nested within the "labroids." *Pholidichthys* was compared with *Notograptus*, where it was noted that the two lineages shared several morphological features observed in other elongate percomorph clades such as blennioids and zoarcoids (Gill and Mooi 1993; Mooi and Gill 2004). Britz (2006) noted that *Pholidichthys* and substrate spawning cichlids share larval attachment organs that are paired and on the forehead, which larvae use to attach themselves to the substrate with mucous threads (Wirtz 1993). In addition to these conclusions from morphological data, molecular studies have provided cursory hypotheses on the relationships of *Pholidichthys*. Mahon (2007), using partial RAG1 DNA sequences inferred a *Pholidichthys*–cirrhitid (hawkfish) relationship. In a study across acanthomorphs that combined mitochondrial and nuclear genes, Smith and Wheeler (2006) suggested that *Pholidichthys* was related to syngnathoids. Later, Smith and Craig (2007) resolved a clade composed of Atherinomorpha, Cichlidae, Pholidichthyidae, and Plesiopidae, where *Pholidichthys* was the sister lineage of their atherinomorphs. As such, the monophyly of a clade containing Cichlidae and *Pholidichthys* resulting from our analysis was intimated in prior morphological and molecular studies, but never explicitly hypothesized. Our new insight on the phylogenetic relationships of *Pholidichthys* is critical because cichlids are extensively studied in the context of adaptive radiation, sexual selection, evolutionary development, speciation, and historical biogeography. The identification of a well-supported cichlid sister lineage provides the fundamentally important context for future studies examining the evolutionary diversification of cichlids and their radiation into freshwater. Possible implications of this relationship are intriguing. *Pholidichthys* possesses two of the three features of pharyngognathy (Springer and Johnson 2004). Although this appears to be a classic example of an exceptionally successful group being sister to a 'depauperon,' we note that fully formed pharyngognathy is diagnostic for Cichlidae.

Our phylogenetic analyses resulted in the polyphyly of Labroidei (Figs. 2–6), corroborating the results of all previous phylogenetic analyses of DNA sequence data that touched on this question (e.g., Streelman and Karl 1997; Sparks and Smith 2004; Dettai and Lecointre 2005; Smith and Wheeler 2006; Chen et al. 2007; Mabuchi et al. 2007; Setiamarga et al. 2008). All "chromides" were nested within Ovalentaria. Several additional species-rich percomorph lineages were also nested within Ovalentaria and this clade was strongly supported in our phylogenetic analyses (Figs. 2–6). Monophyly of components of Ovalentaria have been supported in previous phylogenetic analyses (e.g., Smith and Wheeler 2004; Setiamarga et al. 2008; Li et al. 2009), but no prior studies have included all the major components of this vast lineage: Atherinomorpha, Blennioidei, Ambassidae, Cichlidae, Embiotocidae, Gobiesocidae, Grammatidae, Mugilidae, Opistognathidae, Pholidichthyidae, Plesiopidae, Poly centridae, Pomacentridae, and Pseudochromidae (Figs. 2a, 4a, 5 and 6a). Within Ovalentaria, Cichlidae, Embiotocidae, and Pomacentridae did not comprise a monophyletic group (Figs. 2a, 4a and 6a); however, only nodes with low support, or were resolved in one of the 10 gene trees (Fig. 3A), separated embiotocids and pomacentrids in the phylogenies inferred using RAxML and BEAST.

Many of the lineages within Ovalentaria exhibit a suite of features associated with the spawning of demersal, adhesive eggs with chorionic filaments (Smith and Wheeler 2004; Smith and Craig 2007). In addition, the composition of Ovalentaria inferred in the analyses is remarkably similar to that of "Clade A" (with some representatives from "Clade C") in Springer and Orrell's (2004) phylogenetic analysis of dorsal gill-arch morphology. The new phylogenetic analyses of the 10-gene dataset, previous studies based on DNA sequence data, and gill-arch morphology converge upon a consensus phylogenetic hypothesis of pharyngognathous percomorphs that facilitate a detailed examination of pharyngognathy in the necessary historical context.

Ovalentaria is a major radiation of over 4800 species. The lineages contained within Ovalentaria represent major tropical marine reef radiations (Pomacentridae, Blennioidei), major tropical freshwater radiations (Cichlidae, Cyprinodontiformea) and several lineages with a propensity to cross between freshwater and marine habitats (Ambassidae, Atherinomorpha, Mugilidae). Ovalentaria appears to contain several origins of viviparity (e.g., some atherinomorphs [including both cyprinodontiforms, beloniforms], embiotocids, and labrisomids).

Functional Diversity Among Pharyngognathous Taxa

Our analysis indicates two distinct patterns of pharyngeal jaw functional morphology among pharyngognathous taxa formerly classified in the Labroidei (Nelson 2006). The widespread, generalized percomorph condition, exemplified by *Lepomis* (Centrarchidae) is characterized by a pharyngeal jaw biting mechanism that involves depression of the upper jaw by a rotating fourth epibranchial bone (Fig. 1a,b). Labrids have lost this mechanism, replacing it entirely with biting by elevation of the lower jaw using the muscle sling (Fig. 1c). In labrids, the articulation between the fourth epibranchial and the upper jaw bone (the pharyngobranchial) is usually a flat sliding joint that permits anterior–posterior movement of the upper jaw relative to the fourth epibranchial. This

articulation is oriented in such a way that rotation of the epibranchial results in medially or posteriorly directed force. The third *obliquus dorsalis* muscle that flexes the joint between the epibranchial and the pharyngobranchial is greatly reduced in labrids, compared with generalized percomorphs and other pharyngognathous taxa, indicating a loss of forceful rotation of the epibranchial. The labrid upper jaw moves anteriorly and posteriorly but does not move ventrally, away from the neurocranium (Liem and Sanderson 1986; Wainwright 2005).

Cichlids, pomacentrids, embiotocids, pharyngo gnathous beloniforms, and Centrogenys exhibit both the generalized percomorph biting mechanism and the derived muscle sling mechanism (Fig. 1). In these lineages, two distinct linkages contribute to biting, resulting in a pharyngeal jaw with greater flexibility in movement. By retaining the generalized biting mechanism, these lineages possess the ability to depress the upper jaw as well as elevate the lower jaw. Upper jaw depression can be coupled with retraction (Aerts et al. 1986) and a shearing action of the upper and lower jaws (Liem 1986; Wainwright 2005).

Multiple Origins of Pharyngognathy

Reconstruction of the evolution of pharyngognathy on our phylogeny indicates that this complex of traits has evolved six to ten times among percomorphs (Figs. 8 and 9). Among the members of the former Labroidei, separate origins are inferred for Labridae, Cichlidae, Pomacentridae, and Embiotocidae. The phylogenetic position of labrids, several nodes away from the other pharyngognathous clades, strongly supports the inference of independent origin. Other recent phylogenetic analyses have also found strong support for a labrid position well away from the other labroid taxa (Dettai and Lecointre 2005; Sparks and Smith 2005; Westneat and Alfaro 2005; Smith and Wheeler 2006; Chen et al. 2007; Mabuchi et al. 2007; Setiamarga 2008). The finding that the mechanism of pharyngeal jaw movement in labrids is distinct from that found in other pharyngognaths lends additional support to the interpretation that labrid pharyngognathy is not homologous with the condition seen in the other pharyngognathous lineages.

A better understanding of the relationships among the major groups within Ovalentaria, including cichlids, pomacentrids, beloniforms, and embiotocids may alter the interpretation of the number of independent origins of pharyngognathy among these clades. We note, however, that our stochastic mapping was done on 10 000 trees from the posterior distribution of the BEAST analyses and thus accounts for the phylogenetic uncertainty in the dataset. The relatively close phylogenetic proximity of these four pharyngognathous clades indicates that the pharyngeal jaws have been a site of considerable evolutionary activity within Ovalentaria, but recent analyses vary widely with respect to the number of independent origins that are implied by the inferred phylogenetic relationships among these lineages (Sparks and Smith 2004a, 2004b; Dettai and Lecointre 2005; Mabuchi et al. 2007; Setiamarga et al. 2008). This is an area that will require focused attention and expanded taxon sampling to more conclusively clarify patterns in the transformation of the muscular sling, diarthrosis, and lower pharyngeal jaw.

Consequences of Pharyngognathy

Liem (1973) proposed that pharyngognathy has major implications for feeding performance. A united lower jaw element and muscle sling facilitates mechanically direct biting actions. When the muscular sling contracts, it pulls the lower jaw dorsally against an upper jaw that is stabilized by its intimate articulation with the neurocranium. Because the ceratobranchials are united into a single lower jaw element, the biting force is equal to the combined force of the left and right sling muscles. Hence, pharyngognathy results in a marked enhancement of force delivery to prey held in the pharyngeal jaws. If these muscles have a large physiological cross-sectional area and the lower jaw is robust, then this configuration could be used to generate very large forces. Indeed, this trait combination led to high-performance mollusk crushing in numerous cichlid lineages (Hulsey 2006), at least one group of embiotocids (Liem 1986), and many labrids (Wainwright 1987, 1988; Bellwood et al. 2006). Whereas this is the most obvious functional advantage of pharyngognathy, durophagy is not the most common diet in any of the major groups of pharyngognathous percomorphs. No pomacentrids or beloniforms are molluscivores, most cichlids are not, and the majority of labrids and embiotocids feed primarily on crustaceans. Nevertheless, although the idea has never been formally investigated, it appears likely that durophagy has evolved unusually frequently in both cichlids (Hulsey 2006) and labrids (Wainwright 1988; Bellwood et al. 2006). It may be that efficient biting generally enhances pharyngeal jaw performance with prey that undergo some level of processing prior to swallowing, a pattern that is particularly common among labrids that often feed on shelled invertebrates (Randall 1967; Bellwood et al. 2006).

The presence of the generalized upper jaw depression mechanism in most pharyngognathous taxa, in addition to the derived biting mechanism by lower jaw elevation, combines to produce a level of pharyngeal jaw versatility not previously recognized. Because pharyngognathy and the generalized condition can produce different biting actions, the combination may allow these taxa greater flexibility and dexterity when handling prey items that require some reduction and processing. Functional versatility of the pharyngeal jaws may be reduced in labrids, who have lost the generalized mechanism of biting by upper jaw depression.

In addition to the ways in which it enhances feeding ability, there is one important constraint on feeding performance due to pharyngognathy that has not been widely appreciated. Fusion of the fifth ceratobranchials of the lower jaws results in a restriction of the pharyngeal gape that has been shown to be an important constraint on the size of prey that can be swallowed (Wainwright 1991). Among predatory teleosts, other fishes are among the largest prey that are commonly eaten whole, and the importance of oral gape (Wainwright and Richard 1995) and pharyngeal gape (Lawrence 1957) in limiting piscivory is well documented. We note that few labrids are piscivores, no embiotocids are primarily piscivorous, and no pomacentrids or pharyngognathous beloniforms are known to be piscivores. Among 105 labrid species from the Great Barrier Reef, roughly three have diets dominated by fish (Bellwood et al. 2006). None of 25 labrid species in the Caribbean are categorized as piscivores (Randall 1967). Given the remarkable diversity of labrid diets and the prevalence of piscivory among other groups of predatory coral reef fishes, the scarcity of fish eating in labrids is striking.

In cichlids, piscivory is widespread and has evolved many times independently (Hulsey and De Leon 2005). It is interesting to note, however, that one of the more highly modified piscivorous lineages of neotropical cichlids, *Cichla*, has unsutured, separate fifth ceratobranchials as juveniles (Stiassny and Jensen 1987). It is likely that the separate fifth ceratobranchials are able to spread apart laterally, accommodating the large fish prey eaten by young *Cichla* (Wainwright 2005) although it is not known what mechanisms accommodate large prey in adult *Cichla*. Cichlids are unique among the traditionally recognized pharyngognathous clades in that the fifth ceratobranchials are sutured together, rather than being fused. The same condition was also found in *Centrogenys*. This appears to have left open the possibility of secondary separation of the fifth ceratobranchials, whereas the other pharyngognathous clades appear to have lost this potential. We are not aware of secondary separation of the fifth ceratobranchials in labrids, embiotocids, pomacentrids, or beloniforms.

One aspect of labrid biology may be related to the more specialized functional morphology of their pharyngeal jaw. Whereas some embiotocids and numerous cichlids are durophagous, this trophic tendency is strongly developed in labrids and is found throughout the radiation. High-performance shell-cracking of marine bivalves and gastropods require very high biting forces (Wainwright 1987, 1988) and the loss of the generalized upper jaw depression mechanism in all labrids appears to reflect an early specialization of the labrid pharyngeal jaw system for stabilizing the jaws during strong biting actions, resulting in a jaw system with less mobility.

New Life for a Key Innovation Poster Child?

Although pharyngognathy remains a widely cited example of a key innovation, it has also drawn attention from critics of this hypothesis who have focused on two points. First, several authors noted that the apparently singular origin of this complex of traits—as shared derived traits for "Labroidei"—prevented tests of the generality of the key innovation hypothesis (Lauder 1982; Stiassny and Jensen 1987; Lauder 1996). It is widely appreciated that historical tests of the impact of any trait on subsequent lineage diversification or ecological success should be replicated, because this greatly bolsters the argument that the trait in question is responsible for the predicted consequences. Ironically, the dissolution of "labroids" as a monophyletic group and the implication of multiple independent origins of pharyngognathy may renew the status of this system as a model for key innovation biology, as the opportunity now exists to work toward several separate tests of key innovation hypotheses with these lineages.

Second, a recent analysis of diversification rate shifts in cichlids highlighted the association between increased net diversification rate and living in lakes for the major African cichlid radiations (Seehausen 2006). It was argued in this study that because only haplochromine cichlids living in lakes show significant increases in net diversification rate, whereas all cichlids have pharyngognathy, then this lack of phylogenetic congruence indicates that the derived pharyngeal jaw condition does not account for cichlid diversification. Seehausen (2006) instead emphasized the role of ecological opportunity in young lakes and strong patterns of sexual selection in cichlids. Although the connection between speciation or extinction rates and pharyngognathy is certainly not direct, we disagree with the logic used therein to rule out a role for pharyngognathy in cichlid diversification and adaptive radiations. A major innovation does not guarantee speciation or ecological diversification as many other factors can limit radiation or, in the right circumstances, act synergistically to do so. There are many examples of groups that appear to have possessed a specific innovation for an extended period of time before consequences for diversification emerged (Navarro et al. 2005; Bininda-Emonds et al. 2008; Price et al. 2010).

Indeed, the evidence in support of pharyngognathy as an innovation that has been key to the ecolo gical diversification of pharyngognathous fishes is substantial. Pharyngognathy evolved independently in the common ancestors of labrids and cichlids, two of the most ecologically diverse and successful percomorph clades that live on modern coral reefs and in tropical freshwater habitats, respectively. In both lineages, pharyngeal jaw functional morphology and diversity play a major role in underlying trophic diversity and various forms of durophagy are particularly common in both radiations. Whereas pharyngognathy plays an important role in trophic diversity in embiotocids and pomacentrids, neither lineage shows the ecological or mechanical diversity seen in labrids and cichlids. This is also true for pharyngognathous beloniforms, which primarily eat plankton and drifting plant material, and for *Centrogenys*, which, as a monotypic lineage is not

known to show any ecological diversity. Thus, whereas four groups of pharyngognathous percomorphs appear to have not achieved exceptional species diversity, two have.

Considerable progress has been made in recent years in understanding that labrids, cichlids, embiotocids, and pomacentrids do not comprise a monophyletic group, but much less progress has been made in identifying sister groups for each of these lineages. Future tests of the consequences of pharyngognathy for diversification rate, morphological diversity of the oral jaws, or ecological diversity will benefit from the identification of pharyngognathous clades and their closest relatives. Although it is tempting to imagine that cichlids or labrids represent special radiations of exceptional diversity compared with their closest relatives (Mabuchi et al. 2007), rigorous testing of this hypothesis will ultimately depend on the identification and composition of their sister groups. In the maximum likelihood analysis, we recovered the comparatively species poor pholidichthyids (2 spp.) and gerreids (51 spp.) as sister groups to the Cichlidae and Labridae, respectively. We note that some of our analyses do not refute the idea put forward in several recent analyses (e.g., Dettai and Lecointre 2005; Smith and Wheeler 2006; Chen et al. 2007; Mabuchi et al. 2007) that labrids may be sister to a very large clade which includes lineages such as centarchids, haemulids, lutjanids, percichthyids, sciaenids, serranids, sparids, percoids, cottiforms, lophiiforms, and/or tetraodontiforms. If such a relationship is corroborated in future studies that include greater taxon sampling and enhanced phylogenetic resolution, it appears unlikely that tests will show that labrids outstrip such morphologically and taxonomically disparate groups in ecological, morphological or species diversity. In contrast, a comparison between cichlids and their sister group, *Pholidichthys*, would undoubtedly uncover differences in the net diversification rate and morphological and ecological diversity. Despite the discovery and recognition of Ovalentaria, such tests for all pharyngognaths must await better resolution of percomorph relationships, one of the most compelling and last remaining frontiers in modern vertebrate phylogenetics.

Supplementary Material

Supplementary material, including data files and/or online-only appendices, can be found in the Dryad data repository at http://datadryad.org, doi:10.5061/dryad.5h951h04.

Funding

Funding for this research was provided by the National Science Foundation (DEB-0444842, DEB-0716155, DEB-0717009, DEB-0732642, IOS-0444554, IOS-0749943, ANT-0839007, DEB-1060869, DEB-1061806, and DEB-1061981).

Acknowledgments

For valuable feedback during various phases of this research, we benefited greatly from discussions with Michael Alfaro, David Bellwood, Matthew Davis, David Johnson, Darrin Hulsey, Masaki Miya, Melanie Stiassny, and Tom Waltzek. We thank T.-Y. Cheng and K.-T. Shao of the Biodiversity Research Museum, Academia Sinica, Taiwan, A.C. Bentley and E.O. Wiley of the Biodiversity Institute of the University of Kansas, USA, for generous gifts of tissue specimens.

References

Aerts P., Devree F., Vandewalle P. 1986. Pharyngeal jaw movements in Oreochromis niloticus (Teleostei, Cichlidae)—Preliminary results of a cineradiographic analysis. Ann. Soc. R. Zool. Belg. 116:75–81.

Bellwood D.R., Wainwright P.C., Fulton C.J., Hoey S.A. 2006. Functional versatility supports coral reef biodiversity. Proc. R. Soc. B Biol. Sci. 273:101–107.

Bininda-Emonds O.R.P., Cardillo M., Jones K.E., McPhee R.D.E., Beck R.M.D., Grenyer R., Price S.A., Vos R.A., Gittelman J.L., Purvis A. 2007. The delayed rise of present-day mammals. Nature 456:274.

Bollback J.P. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics 7:88.

Breder C.M. Jr., Rosen D.E. 1966. Modes of reproduction in fishes. Garden City (NJ): The Natural History Press.

Breining T., Britz R. 2000. Egg surface structure of three clingfish species, using scanning electron microscopy. J. Fish Biol. 56: 1129–1137.

Britz R. 1997. Egg surface structure and larval cement glands in nandid and badid fishes, with remarks on phylogeny and biogeography. Amer. Mus. Novit. 3195:1–17.

Britz R. 2006. Review of "Study of the Dorsal Gill-Arch Musculature of Teleostome Fishes, With Special Reference to the Actinopterygii". V. G. Springer and G. D. Johnson with illustrations by Karolyn Darrow and the Appendix, Phylogenetic Analysis of 147 Families of Acanthomorph Fishes Based Primarily on Dorsal Gill-arch Muscles and Skeleton, by V. G. Springer and T. M. Orrell. Copeia 2006: 323–330.

Chen W.J., Bonillo C., Lecointre G. 2003. Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Mol. Phylogenet. Evol. 26:262–288.

Chen W.J., Ruiz-Carus R., Ortí G. 2007. Relationships among four genera of mojarras (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percomorph fishes. J. Fish Biol. 70:202–218.

Chaudhary R., Bansal M.S., Wehe A., Fernandez-Baca D., Eulenstein O. 2010. iGTP: a software package for large-scale gene tree parsimony analysis. BMC Bioinformatics 11:574.

Clements K.D., Alfaro M.E., Fessler J.L., Westneat M.W. 2004. Relationships of the temperate Australian fish tribe Odacini (Perciformes; Teleostei). Mol. Phylogenet. Evol. 32:575–587.

Dettai A., Lecointre G. 2005. Further support for the clades obtained by multiple molecular phylogenies in the acanthomorph bush. C. R. Biol. 328:1031–1032.

Dingerkus G., Uhler C. 1977. Enzyme clearing of alcian-blue stained whole vertebrates for demonstration of cartilage. Stain Tech. 52: 229–232.

Drummond A.J., Ho S.Y.W., Phillips M.J., Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. Plos Biol. 4:699–710.

Drummond A.J., Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Edgar R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

Eschmeyer W.N., Fricke R. (eds) 2012. Catalog of Fishes electronic version (12 Jan 2012), San Francisco: California Academy of Sciences. Available from: http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Futuyma D.J. 2005. Evolution. Sunderland (MA): Sinauer Associates.
Galis F., Drucker E.G. 1996. Pharyngeal biting mechanics in centrarchid and cichlid fishes: insights into a key evolutionary innovation. J. Evol. Biol. 9:641–670.
Galis F., Metz J.A.J. 1998. Why are there so many cichlid species? Trends Ecol. Evol. 13:1–2.
Gill A.C., Mooi R.D. 1993. Monophyly of the Grammatidae and of the Notograptidae, with evidence for their phylogenetic positions among perciforms. Bull. Mar. Sci. 52:327–350.
Grubich J.R. and Westneat M.W. 2006. Four-bar linkage modeling in teleost pharyngeal jaws: computer simulations of bite kinetics. J. Anat. 209:79–92.
Huelsenbeck J.P., Nielsen R., Bollback J.P. 2003. Stochastic mapping of morphological characters. Syst. Biol. 52(2):131–158.
Hulsey C.D. 2006. Function of a key morphological innovation: fusion of the cichlid pharyngeal jaw. Proc. Roy. Soc. Lond. B. Biol. Sci. 273:669–675.
Hulsey C.D., De Leon F.J.G. 2005. Cichlid jaw mechanics: linking morphology to feeding specialization. Funct. Ecol. 19:487–494.
Johnson G.D. 1984. Percoidei: development and relationships. In: Moser H.G., Richards W.J., Cohen D.M., Fahay M.P., Kendall J.A.W., Richardson S.L., editors. Ontogeny and systematic of fishes. Gainesville (FL): American Society of Ichthyologists and Herpetologists, p. 464–498.
Johnson G.D. 1993. Percomorph phylogeny: progress and problems. Bull. Mar. Sci. 52:3–28.
Johnson G.D., Patterson C. 1993. Percomorph phylogeny: a survey of acanthomorphs and a new proposal. Bull. Mar. Sci. 52:554–626.
Kaufman L., Liem K.F. 1982. Fishes of the suborder Labroidei (Pisces: Perciformes): phylogeny, ecology, and evolutionary significance. Breviora 472:1–19.
Lauder G.V. 1982. Historical biology and the problem of design. J. Theor. Biol. 97:57–67.
Lauder G.V. 1996. The argument from design. In: Rose M.R., Lauder G.V., editors. Adaptation. San Diego (CA): Academic Press, p. 55–91.
Lauder G.V. 2001. Evolutionary innovations and constraints: Functional analyses and phylogenetic tests. J. Morph. 248:254.
Lawrence J.M. 1957. Estimated size of various forage fishes largemouth bass can swallow. Proc. Southeast. Assoc. Game Fish Comm. 11: 220–226.
Li B., Dettai A., Cruaud C., Couloux A., Desoutter-Meniger M., Lecointre G. 2009. RNF213, a new nuclear marker for acanthomorph phylogeny. Mol. Phylogenet. Evol. 50:345–363.
Li C.H., Ortí G., Zhang G. and Lu G.Q. 2007. A practical approach to phylogenomics: the phylogeny of ray-finned fish (Acanthopterygii) as a case study. BMC Evol. Biol. 7:44.
Liem K.F. 1973. Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. Syst. Zool. 22:425–441.
Liem K.F. 1980. Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. In Ali M.A., editors. Environmental physiology of fishes. New York: Plenum Press, p. 299–334.
Liem K.F. 1986. The pharyngeal jaw apparatus of the Embiotocidae (Teleostei) - a functional and evolutionary perspective. Copeia 1986:311–323.
Liem K.F., Greenwood P.H. 1981. A functional approach to the phylogeny of the pharyngognath teleosts. Amer. Zool. 21:83–101.
Liem K.F., Osse J.W.M. 1975. Biological versatility, evolution, and food resource exploitation in African cichlid fishes. Amer. Zool. 15: 427–454.
Liem K.F., Sanderson S.L. 1986. The pharyngeal jaw apparatus of labrid fishes - a functional morphological perspective. J. Morph. 187: 143–158.
López J.A., Chen W.J., Ortí G. 2004. Esociform phylogeny. Copeia 2004:449–464.
Mabuchi K., Miya M., Azuma Y., Nishida M. 2007. Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. BMC Evol. Biol. 7:10.
Mahon A.R. 2007. Molecular phylogenetics of perciform fishes using the nuclear recombination activating gene 1 [unpublished dissertation]. [Norfolk (VA)]: Old Dominion University.
Miya M., Satoh T.R., Nishida M. 2005. The phylogenetic position of toadfishes (order Batrachoidiformes) in the higher ray-finned fish as inferred from partitioned Bayesian analysis of 102 whole mitochondrial genome sequences. Biol. J. Linn. Soc. 85:289–306.
Miya M., Takeshima H., Endo H., Ishiguro N.B., Inoue J.G., Mukai T., Satoh T.P., Yamaguchi M., Kawaguchi A., Mabuchi K., Shirai S.M., Nishida M. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Mol. Phylogenet. Evol. 26:121–138.
Mooi R.D. 1990. Egg surface morphology of pseudochromids (Perciformes, Percoidei), with comments on its phylogenetic implications. Copeia 1990:455–475.
Mooi R.D., Gill A.C. 2004. Notograptidae, sister to *Acanthoplesiops* Regan (Teleostei: Plesiopidae: Acanthoclininae), with comments on biogeography, diet and morphological convergence with Congrogadinae (Teleostei: Pseudochromidae). Zool. J. Linn. Soc. 141:179–205.
Mooi R.D., Gill A.C. 2010. Phylogenies without synapomorphies-a crisis in fish systematics: time to show some character. Zootaxa 2450:26–40.
Müller J. 1843. Nachträge zu der Abhandlung über die natürlich Familien der Fische. Archiv für Naturgeschichte 9:381–384.
Navarro N., Neige P., Marchand D. 2005. Faunal invasions as a source of morphological constraints and innovations? The diversification of the early Cardioceratidae (Ammonoidea; Middle Jurassic). Paleobiology 31:98–116.
Near T.J., Sandel M., Kuhn K.L., Unmack P.J., Wainwright P.C., Smith W.L. 2012. Nuclear gene-inferred phylogenies resolve the relationships of the enigmatic Pygmy Sunfishes, *Elassoma* (Teleostei: Percomorpha). Mol. Phylogenet. Evol. 63:388–395.
Nelson J.S. 2006. Fishes of the world. 4th ed. New York: Wiley.
Parenti L.R. 1993. Relationships of atherinomorph fishes (Teleostei). Bull. Mar. Sci. 52:170–196.
Price S.A., Wainwright P.C., Bellwood D.R., Kazancioglu E., Collar D.C. and Near T.J. 2010. Functional innovations and morphological diversification in parrotfish. Evolution 64:3057–3068.
Randall J.E. 1967. Food habits of reef fishes of the West Indies. Stud. Trop. Oceanogr. 5:655–847.
Regan C.T. 1913. The classification of the percoid fishes. Ann. Mag. Nat. Hist. 12(8):111–145.
Revell L.J. 2011. Phytools: Phylogenetic tools or comparative biology (and other things). Available from: http://anolis.oeb.harvard.edu/~liam/R-phylogenetics.
Robinson D.F., Foulds L.R. 1981. Comparison of phylogenetic trees. Math. Biosci. 53:131–147.
Rosen D.E., Parenti L.R. 1981. Relationships of *Oryzias* and the groups of atherinomorph fishes. Amer. Mus. Novit. 2719:1–25.
Rosen D.E., Patterson C. 1990. On Muller's and Cuvier's concept of pharyngognath and labyrinth fishes and the classification of percomorph fishes, with an atlas of percomorph dorsal gill arches. Amer. Mus. Novit. 2983:1–57.
Seehausen O. 2006. African cichlid fish: a model system in adaptive radiation research. Proc. R. Soc. Lond. B Biol. Sci. 273: 1987–1998.
Semple G.P. 1985. Reproductive behavior and development of the Glassperchlet, *Ambassis agrammus* Gunther (Pisces: Ambassidae), from the Alligator Rivers System, Northern Territory. Aust. J. Mar. Freshwater Res. 36:797–805.
Setiamarga D.H.E., Miya M., Yamanoue Y., Mabuchi K., Satoh T.P., Inoue J.G., Nishida M. 2008. Interrelationships of Atherinomorpha (medakas, flyingfishes, killifishes, silversides, and their relatives): the first evidence based on whole mitogenome sequences. Mol. Phylogenet. Evol. 49:598–605.
Smith W.L., Craig M.T. 2007. Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes. Copeia 2007:35–55.
Smith W.L., Wheeler W.C. 2004. Polyphyly of the mail-cheeked fishes (Teleostei : Scorpaeniformes): evidence from mitochondrial and nuclear sequence data. Mol. Phylogenet. Evol. 32:627–646.
Smith W.L. and Wheeler W.C. 2006. Venom evolution widespread in fishes: a phylogenetic roadmap for the bioprospecting of piscine venoms. Heredity 97:206–217.
Sparks J.S. 2004. Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae). Mol. Phylogenet. Evol. 30:599–614.

Sparks J.S., Smith W.L. 2004a. Phylogeny and biogeography of cichlid fishes (Teleostei : Perciformes : Cichlidae). Cladistics 20:501–517.
Sparks J.S. and Smith W.L. 2004b. Phylogeny and biogeography of the Malagasy and Australasian rainbowfishes (Teleostei: Melanotaenioidei): Gondwanan vicariance and evolution in freshwater. Mol. Phylogenet. Evol. 33:719–734.
Springer V.G., Freihofer W.C. 1976. Study of the monotypic fish family Pholidichthyidae (Perciformes). Smithson. Contrib. Zool. 216:1–43.
Springer V.G., Johnson G.D. 2004. Study of the dorsal gill-arch musculature of teleostome fishes, with special reference to the Actinopterygii. Bull. Biol. Soc. Wash. 11:1–260.
Springer V.G., Larson H.K. 1996. *Pholidichthys anguis*, a new species of pholidichthyid fish from Northern Territory and western Australia. Proc. Biol. Soc. Wash. 109:353–365.
Springer V.G., Orrell T.M. 2004. Phylogenetic analysis of 147 families of acanthomorph fishes based primarily on dorsal gill-arch muscles and skeleton. Bull. Biol. Soc. Wash. 11:237–260.
Stamatakis A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.
Stamatakis A. 2011. Phylogenetic search algorithms for maximum likelihood. In: Elloumi M., Zomaya A.Y., editors. Algorithms in computational biology: techniques, approaches and applications. Hoboken (NJ): Wiley, p. 549–577.
Stiassny M.L.J. 1981. The phyletic status of the family Cichlidae (Pisces, Perciformes) - A comparative anatomical investigation. Neth. J. Zool. 31:275–314.
Stiassny M.L.J. 1987. Cichlid familial intrarelationships and the placement of the neotropical genus *Cichla* (Perciformes, Labroidei). J. Nat. Hist. 21:1311–1331
Stiassny M.L.J. and Jensen J. 1987. Labroid interrelationships revisited: morphological complexity, key innovations, and the study of comparative diversity. Bull. Mus. Comp. Zool. 151:269–319.
Stiassny M.L.J., Wiley E.O., Johnson G.D., Carvalho M.R.d. 2004. Gnathostome fishes. In: Cracraft J., Donoghue M.J., editors. Assembling the tree of life. New York: Oxford University Press, p. 410–429.
Streelman J.T. and Karl S.A. 1997. Reconstructing labroid evolution with single-copy nuclear DNA. Proc. R. Soc. Lond. B Biol. Sci. 264:1011–1020.
Sukumaran J., Holder M.T. 2010. DendroPy: a Python library for phylogenetic computing. Bioinformatics 26:1569–1571.
Wainwright P.C. 1987. Biomechanical limits to ecological performance: Mollusc-crushing by the Caribbean hogfish, Lachnolaimus maximus (Labridae). J. Zool. Lond. 213:283–297.
Wainwright P.C. 1988. Morphology and ecology: the functional basis of feeding constraints in Caribbean labrid fishes. Ecology 69:635–645.
Wainwright P.C. 1989. Functional morphology of the pharyngeal jaw apparatus in perciform fishes: an experimental analysis of the Haemulidae. J. Morph. 200:231–245.
Wainwright P.C. 1991. Ecomorphology: experimental functional anatomy for ecological problems. Amer. Zool. 31:680–693.
Wainwright P.C. 2005. Functional morphology of the pharyngeal jaw apparatus. In: Shadwick R. and Lauder G.V., editors. Biomechanics of fishes. San Diego (CA): Academic Press, p. 77–101.
Wainwright P.C., Richard B.A. 1995. Predicting patterns of prey use from morphology of fishes. Environ. Biol. Fishes 44:97–113.
Westneat M.W., Alfaro M.E. 2005. Phylogenetic relationships and evolutionary history of the reef fish family Labridae. Mol. Phylogenet. Evol. 36:370–390.
Wiley E.O. and Johnson G.D. 2010. A teleost classification based on monophyletic groups. In: Nelson J.S., Shultz H.P., Wilson M.V.H., editors. Origin and Phylogenetic Relationships of Teleosts. Munchen (Germany): Verlag, p. 123–182.
Wirtz P. 1993. Does the larva of *Pholidichthys leucotaenia* give a clue to the systematic position of the monotypic fish family Pholidichthyidae? In: Schröder J.H., Bauer J., Schartl M., editors. Trends in ichthyology-an international perspective. Oxford: Blackwell Scientific Publications, p. 237–238.
Yang Z. 2006. Computational molecular evolution. Oxford: Oxford University Press.

Appendix

Phylogenetic Definition of Ovalentaria

Ovalentaria W.L. Smith and T.J. Near, new clade name. Definition (node-based): the least inclusive clade containing *Ambassis urotaenia* Bleeker, *Mugil cephalus* L., *Embiotoca lateralis* Agassiz, *Pseudochromis fridmani* Klausewitz, *Gobiesox maeandricus* Girard, *Gillellus semicinctus* Gilbert, *Polycentrus schomburgkii* Müller and Troschel, *Pholidichthys leucotaenia* Bleeker, *Cichla temensis* Humbolt, *Labidesthes sicculus* Cope, *Gambusia affinis* Baird and Girard, and *Oryzias latipes* Temminck and Schlegel. Etymology: from the Latin words *ovum* meaning egg and *lentae* meaning sticky or tenacious, referring to the diagnostic and characteristic adhesive eggs found in most species in this clade. Reference phylogeny–Figure 2a. Composition: includes the species designated in the definition as well as all species in Ambassidae, Cichlidae, Embiotocidae, Gobiesocidae, Grammatidae, Mugilidae, Opistognathidae, Pholidichthyidae, Pomacentridae, Plesiopidae (including *Notograptus*), Polycentridae, Pseudochromidae, Atherinomorpha (Atheriniformes [Atherinidae, Atherinopsidae, Bedotiidae, Isonidae, Melanotaeniidae, Phallostethidae, and Pseudomug ilidae], Beloniformes [Adrianichthyidae, Belonidae, Exocoetidae, Hemiramphidae, and Zenarchopteridae], Cyprinodontiformes [Anablepidae, Aplocheilidae, Cyprinodontidae, Fundulidae, Goodeidae, Notho branchidae, Poeciliidae, Profundulidae, Rivulidae, and Valenciidae]), and Blennioidei (Blenniidae, Chaenopsidae, Clinidae, Dactyloscopidae, Labrisomidae, and Tripterygiidae). Diagnosis: species in this clade have demersal eggs with adhesive filaments extending from the egg surface (Breder and Rosen 1966; Semple 1985; Mooi 1990; Wirtz 1993; Britz 1997; Breining and Britz 2000). In several lineages, the reproductive mode has been further modified into live bearing (e.g., embiotocids, zenarchopterids, many cyprinodontiforms, and some labrisomids), become secondarily pelagic with adhesive filaments (e.g., exocoetids, some belonids), or become secondarily pelagic without adhesive filaments (e.g., mugilids). Additionally, many species in Ovalentaria share some of the following uncommon features that may prove diagnostic for the whole clade or major lineages within the clade as we continue to resolve relationships within percomorphs: loss of an interarcual cartilage, loss of supraneurals, reduced number of pharyngobranchials, reduced number of branchiostegals, and/or fusion of caudal-fin elements (Johnson 1984; Rosen and Patterson 1990; Johnson 1993; Parenti 1993; Smith and Wheeler 2004; personal communication).