Morphological Evolution of the Cave-, Spring-, and Swampfishes of the Amblyopsidae (Percopsiformes)

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The Amblyopsidae is a small family of fishes from North America in which most of the species occur in caves. Despite considerable interest and study by biologists, a comprehensive morphological phylogenetic analysis of the family has not been conducted to date. We examined the skeletal morphology of all six genera and recognized species, which included 66 characters. The resulting phylogeny was compared to morphological- and molecular-based phylogenies of previous studies. Results showed a progression of cave adaptation that was significantly different from previous phylogenetic studies. Amblyopsidae was supported by 34 synapomorphies of the skeleton, but relationships within the Amblyopsidae were comparatively weak. The relationships of amblyopsids are likely influenced by morphological convergence as well as changes in the timing of development of some characters. Heterochrony is most visible in the unfused bones of the dorsal portion of the skull. The sister group to Amblyopsidae is Aphredoderidae (pirate perches), and the main character that supports this relationship is the presence of a unique set of upper jaw bones termed here lateromaxillae. This relationship is also supported by an anterior position of the vent, which is used for expelling gametes in *Aphredoderus* and for moving eggs to the gill chamber in *Amblyopsis*. It is more likely that *Amblyopsis* is the only branchial brooding amblyopsid and all other species likely exhibit transbranchioral spawning.

THE Amblyopsidae is a small family of fishes from eastern North America that have intrigued biologists since the 1840s. At present, six genera and 7–9 species are recognized in the family (Page and Burr, 2011; Niemiller et al., 2012, 2013a; Chakrabarty et al., 2014). Although cave adaptation in fishes is quite common (Soares and Niemiller, 2013; Niemiller and Soares, 2015), the Amblyopsidae is unusual in that nearly all species in the family are stygobiotic (obligate subterranean). *Chologaster cornuta* (Swampfish) is a small, pigmented species that lives in swamps and sloughs of the Atlantic Coastal Plain, but all other recognized species occur in the Interior Highlands (Interior Low Plateau and Ozark Plateau) and are at least partially cave-adapted. *Forbesichthys agassizii* (Spring Cavefish) is a stygophile occupying karst regions of the Eastern Highlands where they generally occur in spring-fed streams and springs; they feed at night and retreat inside the springs or within dense vegetation during the day (Hill, 1969). *Amblyopsis spelaea* (Northern Cavefish), *A. hoosieri* (Hoosier Cavefish), *Speoplatyrhinus poulsoni* (Alabama Cavefish), *Troglichtys rosae* (Ozark Cavefish), and *Typhlichthys subterraneus* (Southern Cavefish) are stygobionts found in caves in the Eastern and Western Highlands of the eastern United States. These species are obligate inhabitants of caves and have evolved a suite of troglomorphic characters, most notably non-functional, degenerate eyes and almost no pigmentation (Fig. 1; Soares and Niemiller, 2013; Niemiller and Soares, 2015). In addition to these species, Niemiller et al. (2012) recognized *Ty. eigenmanni* for populations of *Typhlichthys* west of the Mississippi River and suggested that many additional populations of *Typhlichthys* east of the Mississippi River may be recognized as species in the future. Niemiller et al. (2013a) also recognized *Forbesichthys papilliferus* as a valid species. *Forbesichthys papilliferus* was placed in the synonymy of *F. agassizii* by Woods and Inger (1957). *Amblyopsis hoosieri* was recently described as a new species by Chakrabarty et al. (2014), and it is distinguished from *A. spelaea* by morpho-

metric differences, genetic differences, and geographical range (north vs. south of the Ohio River).

Amblyopsid phylogenetic relationships.—The relationships of the Amblyopsidae to other fishes have been the subject of considerable debate. Amblyopsids are currently part of Percopsiformes, an order that also includes Percopsidae (two extant species, *Percopsis omiscomaycus* and *P. transmontana*) and Aphredoderidae (one extant species, *Aphredoderus sayanus*, although Niemiller et al. [2013a] recognized a second species, *A. gibbosus*). Gobioids and *Ophidion* + carapids have been suggested as the sister group to Amblyopsidae (McAllister, 1968; Murray and Wilson, 1999; Poly and Proudlove, 2004), but these relationships are unlikely given that these taxa are mainly marine, and *Percopsis* and *Aphredoderus* occur in North American freshwaters. Murray and Wilson (1999) found that Percopsiformes was monophyletic only after the exclusion of Amblyopsidae, but more recent morphological studies find support for the monophyly of Percopsiformes (Springer and Johnson, 2004; Borden et al., 2013; Grande et al., 2013). Springer and Orrell (2004) found support for the monophyly of Percopsiformes, but recovered Percopsidae and Amblyopsidae as sister taxa. Recent broad-scale molecular phylogenies (Betancur-R. et al., 2013; Near et al., 2012; Grande et al., 2013) support the monophyly of Percopsiformes and also have resolved Aphredoderidae and Amblyopsidae as sister taxa, as originally proposed by Rosen (1962).

An early, pre-cladistic phylogeny for species of the Amblyopsidae was provided by Woods and Inger (1957), who hypothesized the five species described and recognized at that time belonged to three lineages (Fig. 2A): 1. *Typhlichthys*, 2. *Chologaster* and *Forbesichthys* (then in *Chologaster*), and 3. *Amblyopsis* and *Troglichtys* (considered congeneric at that time). However, if you treat the seven characters provided by Woods and Inger (1957) cladistically, the family would be a basal polytomy with three branches: 1)

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Fig. 1. Lateral views of (A) epigean *Chologaster cornuta* (by David Neely), (B) stygophilic *Forbesichthys agassizii* (by Dante B. Fenolio), and (C) stygobiotic *Typhlichthys subterraneus* (by Jonathan W. Armbruster).

Chologaster, 2) *Forbesichthys*, and 3) the stygobionts (with *Typhlichthys* sister to *Amblyopsis* + *Troglichthys*).

Swofford (1982) used allozymes to examine population differences and phylogeny in the amblyopsids (Fig. 2C). He found there to be considerable variation within the then two recognized species of *Chologaster* and recommended elevating *Forbesichthys*, which was accomplished in Page and Burr (1991). He additionally suggested that there might be several

species within *Chologaster, Forbesichthys,* and *Typhlichthys*, and that populations of *Typhlichthys* showed evidence of independent colonization of caves. The phylogeny recovered a trichotomy of *Chologaster, Forbesichthys,* and *Typhlichthys* and alternatively found *Amblyopsis* and *Troglichthys* (considered congeneric at the time) as a monophyletic group, or *Troglichthys* as sister to the trichotomy of *Chologaster, Forbesichthys*, and *Typhlichthys*.
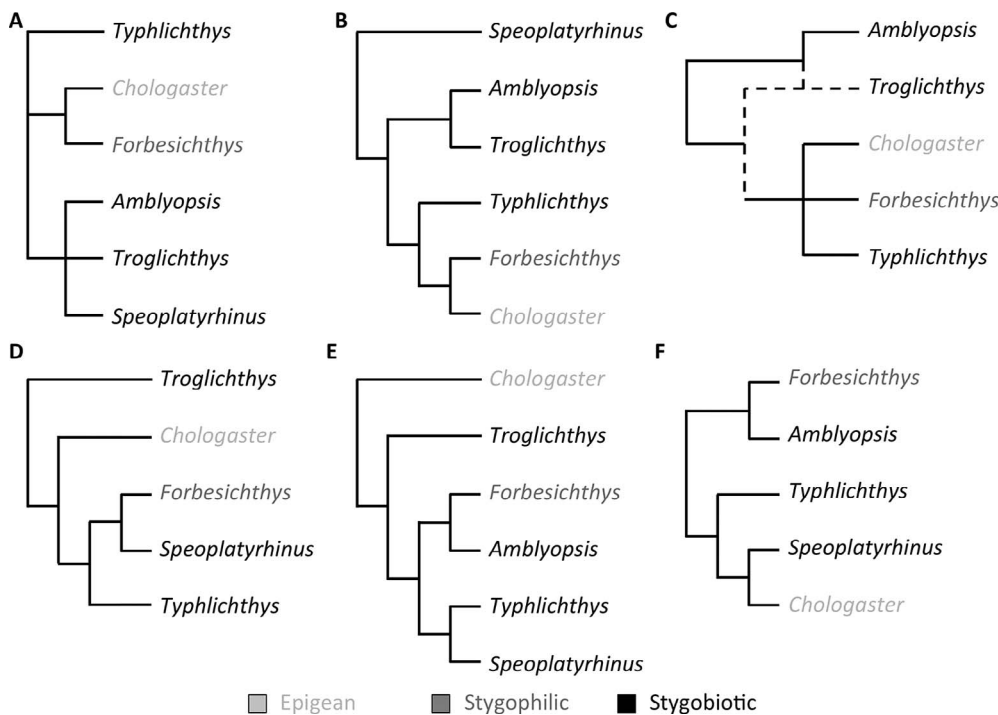

Fig. 2. Previous phylogenies of the Amblyopsidae. (A) Woods and Inger (1957) morphological, pre-cladistic phylogeny, *Speoplatyrhinus* placed based on listed character states, (B) implied phylogeny of Poulson (1963) based on relative amounts of cave adaptation indicating the amount of time spent in caves, (C) Swofford (1982) phylogeny based on allozymes, dotted lines show the uncertain placement of *Troglichthys* as either sister to *Amblyopsis* or the trichotomy of *Chologaster, Forbesichthys,* and *Typhlichthys*, (D) Dillman et al. (2011) molecular phylogeny, (E) Niemiller et al. (2013a) molecular phylogeny, and (F) Grande et al. (2013) molecular phylogeny. Taxa colored according to habitat.

Bergstrom et al. (1995) and Bergstrom (1997) were the first phylogenetic studies of amblyopsids using DNA sequence data. These studies examined a 536 bp section of the mitochondrial NADH-dehydrogenase subunit-2 (*nd2*) gene for all species recognized at the time but *S. poulsoni*. *Troglichthys* (as *A. rosae*) was resolved as sister to all other amblyopsids, but low levels of genetic differentiation were found between the other amblyopsid species. Niemiller and Poulson (2010) noted that some of the samples used in these two studies were extracted from formalin-preserved tissues and were likely contaminated. However, intraspecific relationships within *Troglichthys* and western *Typhlichthys* were based on fresh material and are believed valid.

Niemiller and Fitzpatrick (2008) presented a phylogenetic analysis for amblyopsids using both mitochondrial (*nd2*) and nuclear (ribosomal *s7*) loci. The study was centered on the relationships of the populations of *Typhlichthys*, but also included *Amblyopsis, Chologaster,* and *Troglichthys* (as *A. rosae*). They found *Chologaster* sister to the remainder of the amblyopsids examined and *Amblyopsis* either sister to *Typhlichthys* (*nd2*) or *Troglichthys* (*s7*). Niemiller et al. (2012) expanded this study to multiple loci to examine the relationships of the populations of *Typhlichthys* and concluded that *Typhlichthys* is a cryptic species complex comprised of multiple species, and they advocated resurrection of *T. eigenmanni* for populations west of the Mississippi River in the Salem Plateau of the Ozark Highlands.

Dillman et al. (2011) included the *nd2* dataset of Bergstrom et al. (1995) and Bergstrom (1997) and was the first molecular study to include *Speoplatyrhinus*. *Troglichthys* was sister to all other amblyopsids, followed by divergence of *Chologaster*, and then *Forbesichthys* was either sister to *Speoplatyrhinus* or *Typhlichthys* (Fig. 2D). Romero and Green (2005) suggest an intellectual conceit in the study of cave life, in which regressive evolution is invoked to explain the loss of characters normally associated with life in caves and expansionism for other nonregressive traits that exhibit increases or gains, such as lifespan, age of maturity, and offspring size. These latter traits have also been referred to as constructive traits in subterranean fauna (Soares and Niemiller, 2013; Niemiller and Soares, 2015). The idea (progressive regressionism) Romero and Green (2005) posit is that scientists often look for particular patterns in the evolution of cave organisms, but this may not necessarily reflect the phylogeny of the organisms. Dillman et al. (2011) mentioned progressive regressionism, but the Romero and Green paper was not cited, and the idea of a progressive loss of characters was never fully discussed. Dillman et al. (2011) tabulated the change of various morphological states, but the only morphological characters discussed are eye evolution and brain size. The authors proposed two possible ways that genera with eyes could be sister to those without eyes: 1) eye loss occurred separately in stygobiotic taxa or 2) the epigean and stygophilic genera re-evolved eyes from an eyeless ancestor. The authors argued for the latter; however, they conceded that likelihood reconstructions based on a single trait may be misleading. In opposition to Dillman et al. (2011), Eigenmann (1909) believed that eye loss was not homologous in stygobiotic amblyopsids because of differing patterns of the loss of elements of the eye.

Niemiller et al. (2013a) provided the first molecular phylogeny for all amblyopsids using one mitochondrial and eight nuclear genes. In contrast to Dillman et al. (2011), *Chologaster* was resolved as the most basal amblyopsid sister to a clade that contained all stygobiotic taxa +

Forbesichthys (Fig. 2E). *Forbesichthys* was sister to *Amblyopsis* and deeply nested within this clade of subterranean taxa. *Speoplatyrhinus* was resolved as sister to *Typhlichthys*, which may comprise 10–15 morphologically cryptic lineages (Niemiller et al., 2012). Niemiller et al. (2013a) found no support for monophyly of *Amblyopsis* (*A. spelaea* + *A. rosae*) and advocated resurrection of the genus *Troglichthys* for *A. rosae*. As in Dillman et al. (2011), the placement of *Forbesichthys* within a subterranean clade suggests the potential for re-evolution of eye functionality and recolonization of surface habitats in this lineage. Niemiller et al. (2013a) reconstructed eye evolution on their species tree and discussed the results in terms of Dollo (irreversible) characters. A model that allows the eye to re-evolve was significantly better than a model treating eye degeneration as a Dollo character (i.e., the irreversible loss of eye functionality); however, loss-of-function mutations and rates of non-synonymous substitutions in the *rhodopsin* gene (a photoreceptive pigment in the eye) support at least three independent eye degeneration events rather than re-evolution of eye functionality in *Forbesichthys*. Niemiller et al. (2013a) also argued that biogeographical and eye histological evidence support independent evolution of cave lineages rather than re-evolution of eye functionality, and did so in part based on Eigenmann's previous observations on eye morphology. Eye histological data in Niemiller et al. (2013a) included seven characters based primarily on Eigenmann (1897, 1899a, 1899b, 1909): ocular muscles (extrinsic eye musculature), cones, lens, optic nerve connection, pupil, scleral cartilages, and ciliary muscles (actually more likely to be a protractor lentis muscle as fishes do not have ciliary muscles); however, these characters need to be reexamined with more modern techniques.

Grande et al. (2013) provided an analysis of three mitochondrial and four nuclear loci for the Paracanthopterygii, and they and Borden et al. (2013) analyzed some morphological characters (26 in each study) using this phylogeny. Included in the phylogeny were all amblyopsids except *Troglichthys*. The results of the analysis in Grande et al. (2013) were that *Forbesichthys* and *Amblyopsis* were sister taxa as in Niemiller et al. (2013a), but *Typhlichthys* was supported as sister to a clade of *Speoplatyrhinus* + *Chologaster* (Fig. 2F).

Although Poulson (1963) did not provide a phylogeny, he hypothesized that cave forms with the most adaptations for living in caves (i.e., troglomorphies) have been in caves for the longest duration. This could be interpreted as there having been a widespread epigean species that gave rise to all of the other species of cavefishes and subsequently went extinct in the Mississippi River Basin. This would imply that the species with the most characters associated with cave life (*Speoplatyrhinus*) split from the ancestral stock first, followed by *Amblyopsis* + *Troglichthys*, then *Typhlichthys*, then *Forbesichthys*, and finally *Chologaster* as a remnant of this widespread epigean form. This hypothesis would imply the phylogeny in Figure 2B.

The fact that many traits have evolved repeatedly in response to similar environmental conditions strongly suggests that they are adaptive and shaped by natural selection (Losos, 2011; Wake et al., 2011). It has been argued that several morphological characters have a strong likelihood to be convergent in subterranean fauna because adaptation to the cave environment appears to occur along similar pathways in different organisms (Poulson, 1963; Culver and Wilkens, 2000; Culver and Pipan, 2009; Soares and Niemiller, 2013). However, species may also adapt in

different ways under similar selective regimes for various reasons, including differences in phenotypic variation, underlying genetic variation, and constraints in ancestral populations or through random change (Arnold, 1994; Donoghue, 2005; Losos, 2011). Therefore, determining convergent evolution in closely related species requires a comprehensive study of morphological variation when contrasting phylogenetic perspectives exist. To date, the only family-wide morphological phylogeny proposed for Amblyopsidae was that of Woods and Inger (1957), which relied only on subjective placement of eight characters. This hypothesis, however, has not been examined, and to state that morphology would have no use in discerning phylogeny without testing it first would be inappropriate.

Objectives.—The Amblyopsidae is an excellent group to examine character-state evolution in cave species; however, no robust phylogenetic examination of cavefish morphology has been performed. The skeletal anatomy of amblyopsids was examined for this study with the objective to provide a more robust morphological phylogeny for the amblyopsids and to diagnose Amblyopsidae. Additionally, evidence for heterochrony in Amblyopsidae is examined, and the evolution of branchial brooding is discussed.

MATERIALS AND METHODS

Institutional abbreviations follow Sabaj Pérez (2014). Specimens were cleared and stained based on the methods in Taylor and Van Dyke (1985). Branchial baskets were removed in some individuals, but due to the paucity of specimens, they were left intact in some so that future researchers will have the same material and characters to examine. Sixty-six skeletal characters were coded and are described in Results (Table 1). Most characters were visible without dissection. Only one specimen of *Speoplatyrhinus poulsoni* was available for examination, and it was partially cleared but unstained; most of the characters (53 of 66) were observable on the specimen. Other than the replacement of the postcranial lateral line with a series of columns of exposed neuromasts, characters were restricted to elements of the skeleton because there did not appear to be any characters that seemed to be directly associated with living in caves (e.g., the number of infraorbital bones was the same in all of the amblyopsids, and there was no apparent variation in the shapes of the infraorbitals).

The ingroup included each of the six amblyopsid genera, specimens for several lineages of *Typhlichthys* identified by Niemiller et al. (2012), and several individuals of the two nominal species of *Forbesichthys*. Genera were not split into species because of lack of relevant skeletal variation at this level of analysis. The outgroup included all other species of Percopsiformes: *Percopsis omiscomaycus, P. transmontana*, and *Aphredoderus sayanus*. The species of *Percopsis* were combined in the phylogenetic analysis because no relevant variation in their skeletons was found. Characters were coded numerically with the condition in *Percopsis* considered state 0.

Exhaustive analyses were performed in PAUP* ver.4.0a146 (Swofford, 2015). Bootstrap analyses were performed with 10,000 replicates of a branch and bound search. Bremer decay analyses (Bremer, 1988) were performed by successively performing exhaustive searches and saving trees one-step higher than the most parsimonious/previous analysis until clades collapsed. Bremer support values were calculated as

the number of steps where a clade became a polytomy minus the number of steps in the most parsimonious tree.

The resulting phylogeny was compared with the phylogenies of Woods and Inger (1957), the implied phylogeny of Poulson (1963), Swofford (1982), Dillman et al. (2011), Niemiller et al. (2013a), and Grande et al. (2013) with Kishino-Hasegawa and Templeton tests implemented in PAUP*. In order to correct for multiple comparisons, a sequential Bonferroni technique was used (Rice, 1989) with the KH and Templeton tests treated separately. *Speoplatyrhinus* was placed onto the Woods and Inger (1957) phylogeny based on the distribution of the characters in Woods and Inger. The morphological phylogeny was pruned of *Amblyopsis* in the comparison with the Dillman et al. (2011) phylogeny and of *Troglichthys* in the comparison with the Grande et al. (2013) phylogeny, as these taxa were not included in these studies.

RESULTS

Characters.—66 skeletal characters were coded for the skeletons of the Percopsiformes (Table 1).

1. Epipleurals—0: just on a couple anterior vertebrae (outgroup), 1: on all or all but one trunk vertebra (Amblyopsidae).
2. Free rib that stabilizes pelvic girdle when present—0: present, 1: absent (*Amblyopsis, Troglichthys*). This appears to be the postcleithrum of Woods and Inger (1957), but it is not a postcleithrum. A single, large postcleithrum is present in all Percopsiformes.
3. Anterior projections of first vertebra—0: to about same width as posterior projections, 1: much narrower than posterior projections (*Troglichthys* and *Typhlichthys*). The first vertebra has two projections, posterior ones that overlap the second vertebra and anterior ones that attach to a ligament homologous with the ventral ribs.
4. Basibranchial 4—0: partially ossified (Fig. 3A, B), 1: unossifed (*Typhlichthys,* probably also *Speoplatyrhinus;* Fig. 3C). *Troglichthys* is polymorphic.
5. Basihyal—0: rod shaped, slightly wider anteriorly than posteriorly (outgroup, *Amblyopsis;* Fig. 3A, B), 1: triangular, very wide anteriorly (rest of Amblyopsidae, one specimen of *Forbesichthys* had a rod-shaped basihyal; Fig. 3C).
6. Shape of autogenous tooth bones along ceratobranchials—0: thin, roughly rectangular or linear (*Chologaster, Forbesichthys;* Fig. 3E), 1: oval to round (*Amblyopsis, Speoplatyrhinus, Troglichthys, Typhlichthys;* Fig. 3D).
7. Epibranchial 1 forked—0: yes (outgroup), 1: no (Amblyopsidae).
8. Gill rakers present on epibranchials—0: yes (outgroup), 1: no or limited to proximal edge (Amblyopsidae).
9. Epihyal—0: without narrow distal projection (outgroup), 1: with narrow distal projection (Amblyopsidae).
10. Epihyal—0: straight, 1: curved distally (*Amblyopsis*).
11. Hyohyal—0: without distal projection posteriorly (Percopsidae), 1: with distal projection posteriorly (Aphredoderidae and Amblyopsidae).
12. Hypobranchials with toothplates—0: present (outgroup), 1: absent (Amblyopsidae).
13. Pharyngobranchial 1—0: present (outgroup), 1: absent (Amblyopsidae).

Table 1. Character state data for the 66 coded characters.

Species	1	2	3	4	5	6	7	8	9	10
Percopsis omiscomaycus	0	0	0	0	0	0	0	0	0	0
Aphredoderus sayanus	0	0	0	0	0	0	1	0	0	0
Amblyopsis hoosieri	1	1	0	0	0	1	1	1	1	1
Chologaster cornuta	1	0	0	0	1	0	1	1	1	0
Forbesichthys agassizii	1	0	0	0	1	0	1	1	1	0
Speoplatyrhinus poulsoni	?	0	1	1	1	1	?	1	1	0
Troglichthys rosae	1	1	0	(0&1)	1	1	1	1	1	0
Typhlichthys subterraneus	1	0	1	1	1	1	1	1	1	0

Species	11	12	13	14	15	16	17	18	19	20
Percopsis omiscomaycus	0	0	0	0	0	0	0	0	0	0
Aphredoderus sayanus	1	0	0	0	0	0	0	0	1	0
Amblyopsis hoosieri	1	1	1	0	0	1	1	0	1	1
Chologaster cornuta	1	1	1	1	1	1	1	0	1	0
Forbesichthys agassizii	1	1	1	1	1	1	1	0	1	0
Speoplatyrhinus poulsoni	1	?	?	?	?	1	1	0	1	0
Troglichthys rosae	1	1	1	0	0	1	1	0	1	0
Typhlichthys subterraneus	1	1	1	0	1	1	1	1	1	0

Species	21	22	23	24	25	26	27	28	29	30
Percopsis omiscomaycus	0	0	0	0	0	0	0	0	0	0
Aphredoderus sayanus	0	0	0	1	0	0	0	0	0	0
Amblyopsis hoosieri	1	0	1	1	1	0	1	1	1	1
Chologaster cornuta	1	0	1	1	1	0	1	1	1	1
Forbesichthys agassizii	1	0	1	1	1	0	1	1	1	1
Speoplatyrhinus poulsoni	1	1	1	1	1	?	1	1	1	1
Troglichthys rosae	1	0	1	1	1	1	1	1	1	1
Typhlichthys subterraneus	1	1	1	1	1	1	1	1	1	1

Species	31	32	33	34	35	36	37	38	39	40
Percopsis omiscomaycus	0	0	0	0	0	0	0	0	0	0
Aphredoderus sayanus	0	0	1	0	0	0	0	0	0	0
Amblyopsis hoosieri	0	1	1	0	0	1	1	0	1	1
Chologaster cornuta	0	1	1	0	0	1	1	0	1	0
Forbesichthys agassizii	0	1	1	0	0	1	1	0	1	1
Speoplatyrhinus poulsoni	1	1	1	1	1	1	1	?	1	1
Troglichthys rosae	0	1	1	1	0	1	1	1	1	1
Typhlichthys subterraneus	1	1	1	1	1	1	1	1	1	1

Species	41	42	43	44	45	46	47	48	49	50
Percopsis omiscomaycus	0	0	0	0	0	0	0	0	0	0
Aphredoderus sayanus	0	0	0	0	1	1	1	0	0	0
Amblyopsis hoosieri	0	1	1	1	1	2	1	0	1	1
Chologaster cornuta	0	1	0	0	1	2	0	1	0	1
Forbesichthys agassizii	1	1	0	0	1	2	0	1	1	1
Speoplatyrhinus poulsoni	0	1	1	0	1	2	1	1	?	1
Troglichthys rosae	0	1	1	0	1	2	1	1	1	1
Typhlichthys subterraneus	0	1	1	0	1	2	1	1	1	1

Species	51	52	53	54	55	56	57	58	59	60
Percopsis omiscomaycus	0	0	0	0	0	0	0	0	0	0
Aphredoderus sayanus	0	0	0	1	0	0	0	0	0	0
Amblyopsis hoosieri	1	1	1	1	1	1	1	1	1	1
Chologaster cornuta	1	1	1	1	1	1	1	1	1	1
Forbesichthys agassizii	1	1	1	1	1	1	1	1	1	1
Speoplatyrhinus poulsoni	?	1	1	1	1	1	2	1	?	?
Troglichthys rosae	1	1	1	1	1	1	2	1	1	1
Typhlichthys subterraneus	1	1	1	1	1	1	2	1	1	1

Table 1. Continued.

Species	61	62	63	64	65	66
Percopsis omiscomaycus	0	0	0	0	0	0
Aphredoderus sayanus	0	1	0	0	0	0
Amblyopsis hoosieri	1	1	1	1	1	(0&1)
Chologaster cornuta	1	1	1	0	0	1
Forbesichthys agassizii	1	1	0	1	0	1
Speoplatyrhinus poulsoni	?	1	1	1	0	1
Troglichthys rosae	1	1	1	1	0	1
Typhlichthys subterraneus	1	1	1	1	0	1

14. Pharyngobranchial 2—0: *T*-shaped, posterior part wide (outgroup, *Amblyopsis*, *Troglichthys*, *Typhlichthys*), 1: *T*-shaped, all limbs about same width.

15. Pharyngobranchial 2—0: toothed (outgroup, *Amblyopsis*), 1: untoothed (rest of Amblyopsidae). *Troglichthys* is polymorphic.

16. Pharyngobranchial 4 (anterior pharyngeal tooth plate)—0: roughly triangular (outgroup), 1: roughly rectangular (anterior as wide or wider than posterior, Amblyopsidae).

17. Orbital process of anguloarticular—0: wide, square (outgroup; Fig. 4A), 1: narrow, pointed (Amblyopsidae; Fig. 4B).

18. Orbital process of anguloarticular—0: at front edge of mesial process or posterior (Percopsidae, Aphredoderidae, Amblyopsidae except *Typhlichthys*), 1: anterior to mesial process (*Typhlichthys*).

19. Mesial process of anguloarticular—0: narrow (Percopsidae; Fig. 4A), 1: two times or more wider than retroarticular (Aphredoderidae, Amblyopsidae; especially long in *Speoplatyrhinus*, which has the medial edge wider than the lateral edge; Fig. 4B).

20. Retroarticular shape—0: straight or anterior margin slightly concave, 1: anteroventeral margin almost forming right angle (*Amblyopsis*).

21. Distal end of dentary—0: gradually bent upwards (outgroup; Fig. 4A), 1: nearly forming right angle and could be considered a process that is higher than the teeth (Amblyopsidae; Fig. 4B).

22. Medial lamina of dentary—0: as wide as that of anguloarticular (Fig. 4A), 1: less than half width of anguloarticular (*Speoplatyrhinus*, *Typhlichthys*; Fig. 4B).

23. Maxilla expanded distally—0: present (outgroup), 1: absent (Amblyopsidae).

24. Lateromaxillae—0: absent (Percopsidae), 1: present (Aphredoderidae, Amblyopsidae; Fig. 5).

25. Lateral line—0: present (outgroup), 1: absent, replaced by lines of neuromasts (Amblyopsidae).

26. Basisphenoid anterior process shape—0: pointed, 1: rounded (*Amblyopsis*, *Troglichthys*, *Typhlichthys*).

27. Exoccipital condyles to first vertebra—0: short, length less than width (outgroup), 1: long projections, length approximately twice width (Amblyopsidae).

28. Foramen magnum—0: fully formed (outgroup), 1: open dorsally because exoccipitals do not meet at midline and supraoccipital is shifted anteriorly (Amblyopsidae).

29. Frontals—0: with medial ridges that form a deep, narrow trough (outgroup), 1: flat, without ridges or trough (Amblyopsidae).

30. Lateral ethmoid—0: robust, roughly square (outgroup), 1: reduced to slight, *T*-shaped structure (Amblyopsidae).

31. Lateral ethmoid proportions—0: anterior-posterior aspect about half the size of lateral aspect, 1: anterior-posterior aspect very narrow, that of lateral aspect (*Speoplatyrhinus* and *Typhlichthys*).

32. Mesethmoid—0: with longitudinal ridges (outgroup), 1: without longitudinal ridges (Amblyopsidae).

33. Mesethmoid with transverse ridge—0: absent (Percopsidae), 1: present (Aphredoderidae, Amblyopsidae).

34. Mesethmoid with posterior part (posterior to transverse ridge in amblyopsids) longer than anterior section (anterior to transverse ridge)—0: absent (outgroup, *Amblyopsis*, *Chologaster*, *Forbesichthys*), 1: present (*Speoplatyrhinus*, *Troglichthys*, *Typhlichthys*).

35. Posterior edge of mesethmoid—0: wide, to one half width of anterior edge or greater (outgroup, *Amblyopsis*, *Chologaster*, *Forbesichthys*, *Troglichthys*), 1: not widening much beyond minimum width, to less than width of anterior edge (*Speoplatyrhinus*, *Typhlichthys*).

36. Orbitosphenoid—0: narrower to approximately same width of base of mesethmoid (outgroup), 1: wider than base of mesethmoid (Amblyopsidae).

37. Parasphenoid—0: narrower than base of mesethmoid (outgroup), 1: as wide as base of mesethmoid (Amblyopsidae).

38. Prootic anterior process—0: pointed, leaving small gap with orbitosphenoid, 1: rounded, leaving large gap with orbitosphenoid (*Troglichthys*, *Typhlichthys*).

39. Supraoccipital crest—0: present, tall (outgroup), 1: absent or very short (Amblyopsidae).

40. Transverse crest on supraoccipital—0: absent, 1: present (*Amblyopsis*, *Forbesichthys*, *Speoplatyrhinus*, *Troglichthys*, *Typhlichthys*). Only large *Forbesichthys* appear to have the crest.

41. Ectopterygoid—0: long, fairly straight (through about of palatine or more), 1: short, bent (through a small portion of palatine; *Forbesichthys*).

42. Hyomandibula shape—0: longer or equally dorsoventrally than anteroposteriorly (outgroup; Fig. 6A), 1: longer anteroposteriorly than dorsoventrally (Amblyopsidae; Fig. 6B).

43. Anterior process of hyomandibula—0: short, extending less than half the length of the metapterygoid, 1: long, extending greater than ¾ length of metapterygoid (*Amblyopsis*, *Speoplatyrhinus*, *Troglichthys*, *Typhlichthys*; Fig. 6).

44. Ventral portion of hyomandibula—0: as wide as articulating condyle or with slight lamina less than width of the main ventral shaft of the hyomandibula,
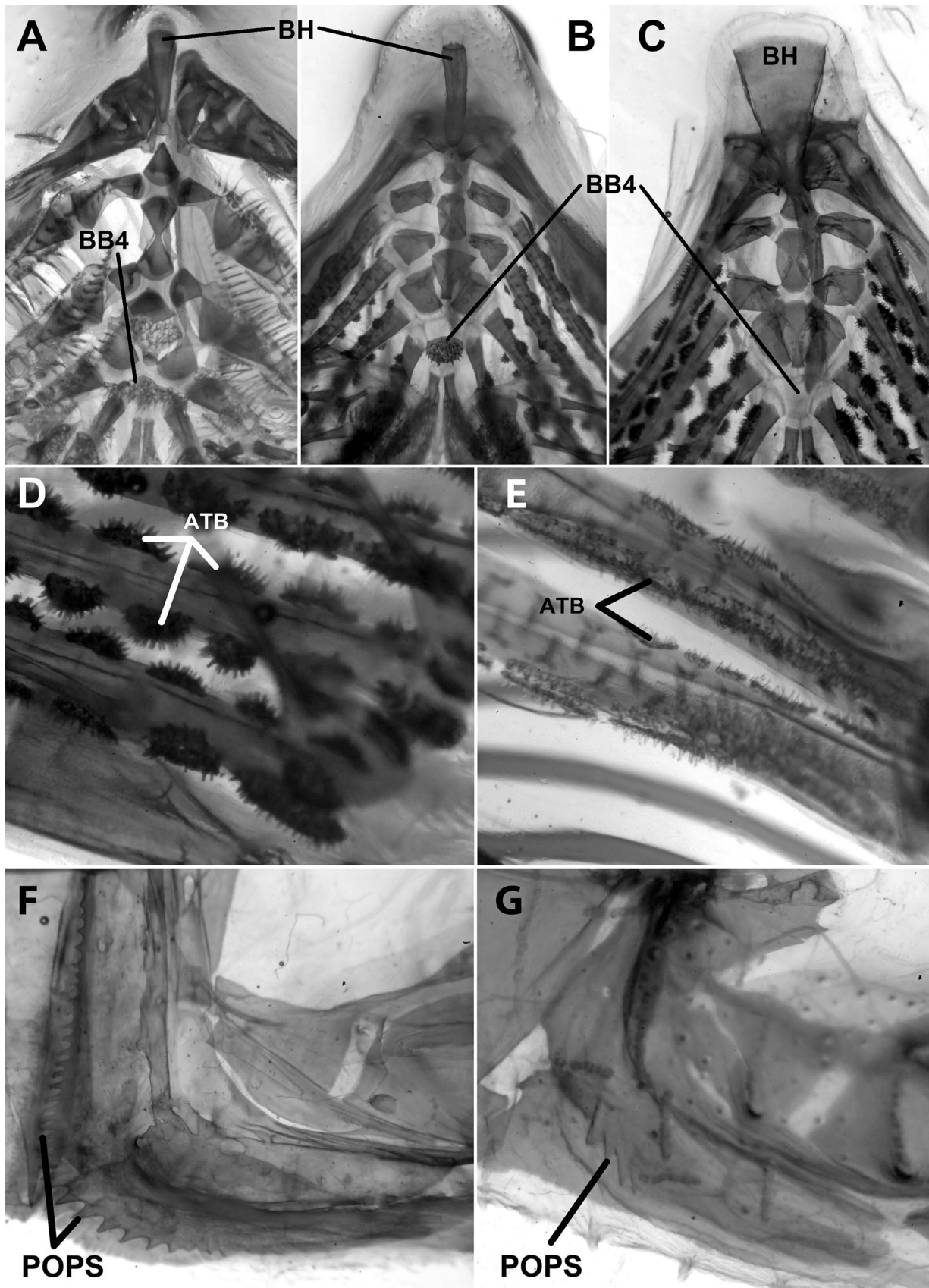
Fig. 3. Important skeletal characters. (A–C) Basihyal and basibranchials, dorsal view. (D–E) Autogenous tooth bones along ceratobranchials, dorsal view. (F–G) Preopercles, lateral view. (A) *Percopsis omiscomaycus*, INHS 88030. (B, G) *Amblyopsis hoosieri*, INHS 42424. (C, D) *Typhlichthys subterraneus*, AUM 16045. (E) *Forbesichthys agassizii*, INHS 37654. (F) *Aphredoderus sayanus,* AUM 26377. Abbreviations: autogenous tooth bones (ATB), basibranchial 4 (BB4), basihyal (BH), preopercular serrae (POPS).
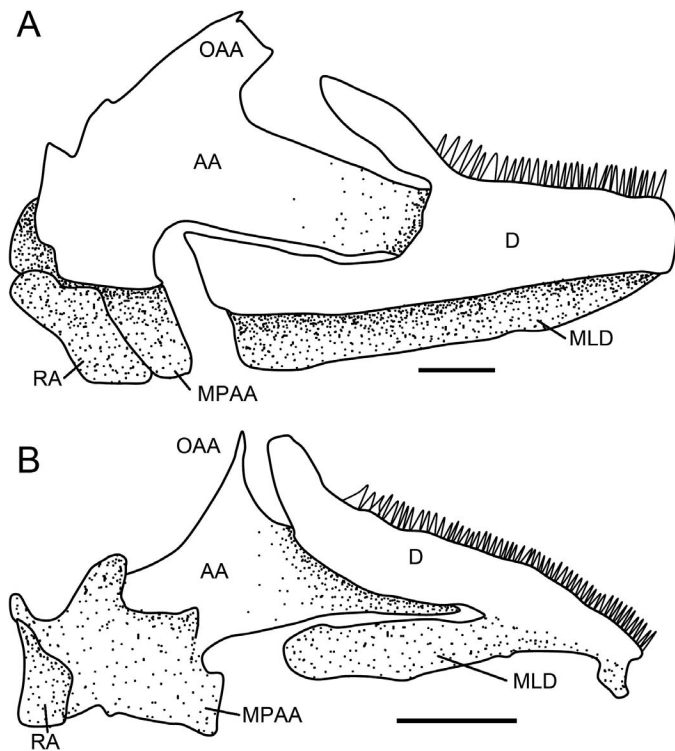
Fig. 4. Ventrolateral view of right mandible. (A) *Percopsis omiscomaycus*, AUM 23034. (B) *Typhlichthys subterraneus*, AUM 16045. Abbreviations: angulo-articular (AA), dentary (D), medial lamina of dentary (MLD), medial process of angulo-articular (MPAA), orbital process of angulo-articular (OAA), retroarticular (RA). Teeth are approximated. Scale = 1 mm.

1: with a lamina wider than ventral shaft of hyomandibula that extends the length of the anterior arm (*Amblyopsis*).

45. Metapterygoid—0: fan shaped (Percopsidae), 1: with medial constriction (Aphredoderidae, Amblyopsidae).

46. Opercle—0: dorsal and posterior projections at right angle (Percopsidae; Fig. 7A), 1: dorsal slighted projections sloped posteriorly (Aphredoderidae; Fig. 7B), 2: dorsal projection almost parallel to posterior projection (Amblyopsidae; Fig. 7C).

47. Palatine—0: untoothed or with small patch of teeth less than half length (Percopsidae, *Chologaster*, *Forbesichthys*; teeth may be present or absent in *Forbesichthys*), 1: toothed along most of length (Aphredoderidae, *Amblyopsis*, *Troglichthys*, *Typhlichthys*; one of two specimens of *Percopsis transmontana* had palatine teeth, but *Percopsis* was coded as state 0). Rosen and Patterson (1969) show a partially toothed palatine in *Amblyopsis*, but it was fully toothed in the specimens examined here.

48. Preopercle—0: weakly to strongly serrated (outgroup, *Amblyopsis*; Fig. 3F, G), 1: serrae absent (Amblyopsidae except *Amblyopsis*).

49. Symplectic—0: ovoid with medial ventral lamina, widest in center (outgroup, *Chologaster*), 1: triangular, widest at posterior margin (*Amblyopsis*, *Forbesichthys*, *Troglichthys*, *Typhlichthys*).

50. Anal-fin spines—0: present (outgroup), 1: absent (Amblyopsidae).

51. First anal-fin radial—0: longer than second (outgroup), 1: ¾ length of second or less (Amblyopsidae).

Fig. 5. Dorsolateral view of right premaxilla (PM) and lateromaxillae (LM) of *Forbesichthys agassizii*, INHS 37654.

52. Epurals—0: 2 fully developed (outgroup), 1: 1 (Amblyopsidae; occasionally a small first epural is present according to Borden et al., 2013).

53. Uroneural contacting preural + ural 1 centrum—0: yes (outgroup), 1: no (Amblyopsidae).

54. Ural 2 and hypurals 3 and 4—0: separate (Percopsidae), 1: fused.

55. Hypural 6 supporting fin rays—0: yes (outgroup), 1: no (Amblyopsidae). Borden et al. (2013) report no separate hypural 6 in amblyopsids, but there is a small ossification ventral to the uroneural and along the anterodorsal edge of, or within a small indentation of, the fused ural 2 and hypurals 3–5, that is likely homologous to hypural 6.

56. Parhypural—0: contacting preural 1 + ural 1 centrum (outgroup), 1: not contacting preural 1 + ural 1 centrum (Amblyopsidae).

57. Dorsal-fin rays + spines—0: 12+ (outgroup), 1: 10–11 (*Amblyopsis*, *Chologaster*, *Forbesichthys*), 2: 8–9 (*Speoplatyrhinus*, *Troglichthys*, *Typhlichthys*).

58. Dorsal-fin spines—0: present (outgroup), 1: absent (Amblyopsidae).

59. Lamina of anterior dorsal-fin pterygiophores—0: present (outgroup), 1: absent (Amblyopsidae).

60. First dorsal-fin pterygiophore—0: reaching the neural spines (outgroup), 1: not reaching the neural spines (Amblyopsidae).

61. Supraneural—0: present (outgroup), 1: absent (Amblyopsidae).

62. Postcleithrum anterior process—0: gradually tapering to a point (Percopsidae), 1: anterior process abruptly narrowing so that approximately half of the process remains at approximately the same width (Aphredoderidae, Amblyopsidae).

63. Postcleithrum ventroposterior margin—0: strongly concave with posterior margin that extends posterior to posterior edge of anterodorsal portion (outgroup, *Forbesichthys*), 1: weakly concave with posterior margin that does not extend posterior to posterior edge of anterodorsal portion (all other Amblyopsidae).

64. Ligament of ventral process of posttemporal—0: length approximately equal to width or less (outgroup, *Chologaster*; Fig. 8A), 1: at least twice as long as wide (rest of Amblyopsidae; Fig. 8B).

65. Supracleithrum—0: narrow, 1: with wide, pointed lamina (*Amblyopsis*).

66. Pelvic fins—0: present (outgroup), 1: absent. *Amblyopsis* generally has small, rudimentary pelvic fins, but they are occasionally absent, so it was coded as polymorphic.
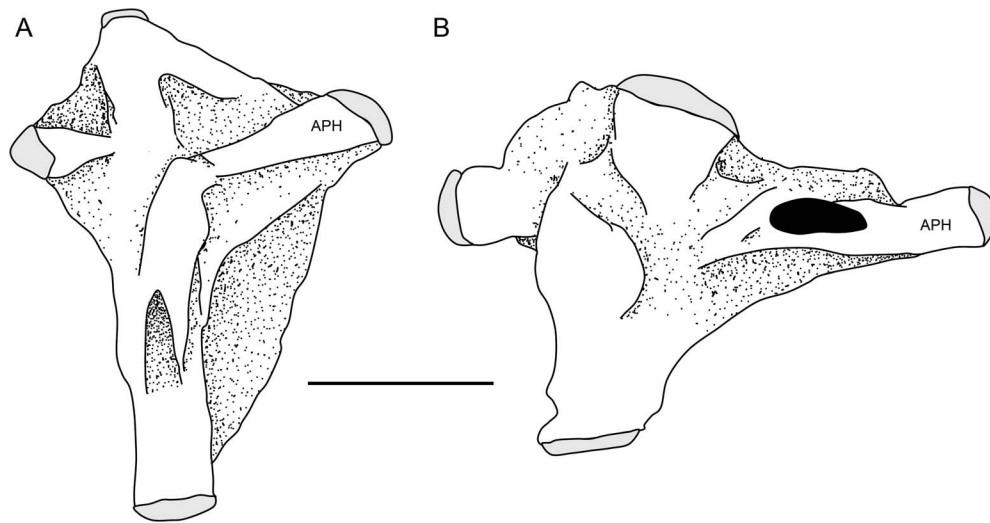
Fig. 6. Lateral view of right hyomandibula. (A) *Aphredoderus sayanus*, AUM 31430. (B) *Typhlichthys subterraneus*, AUM 16045. Abbreviation: anterior process of hyomandibula (APH). Gray areas are cartilage, and black area is a foramen. Scale = 1 mm.
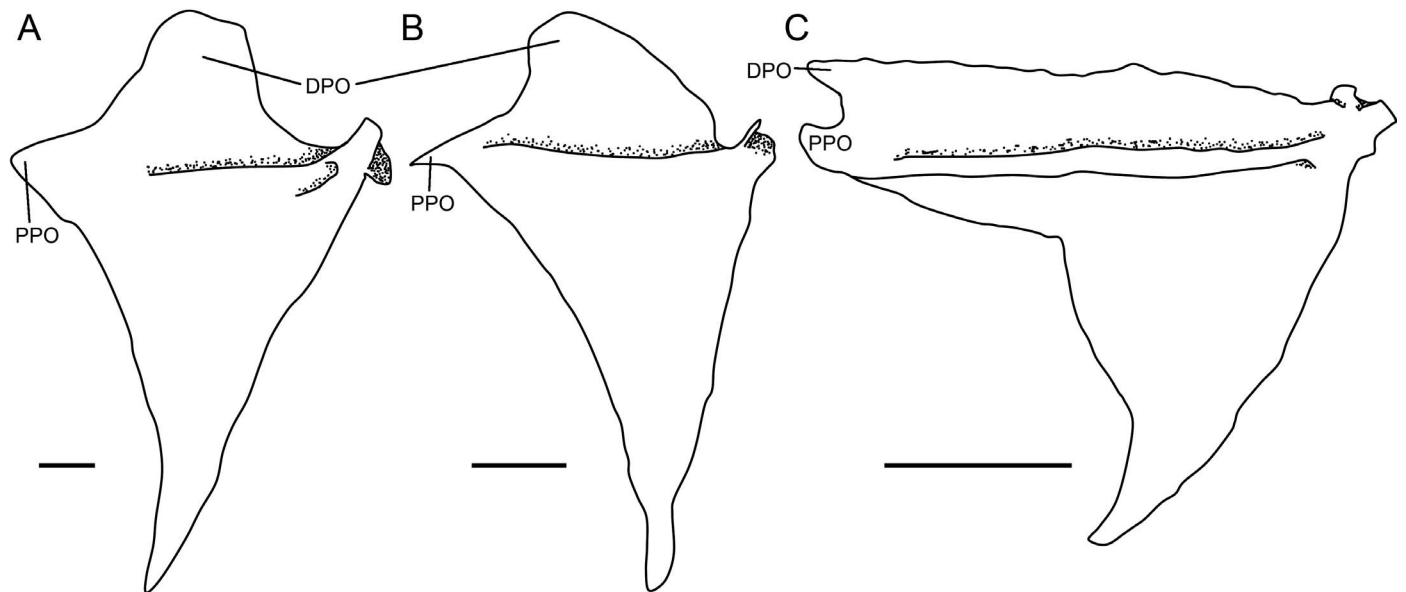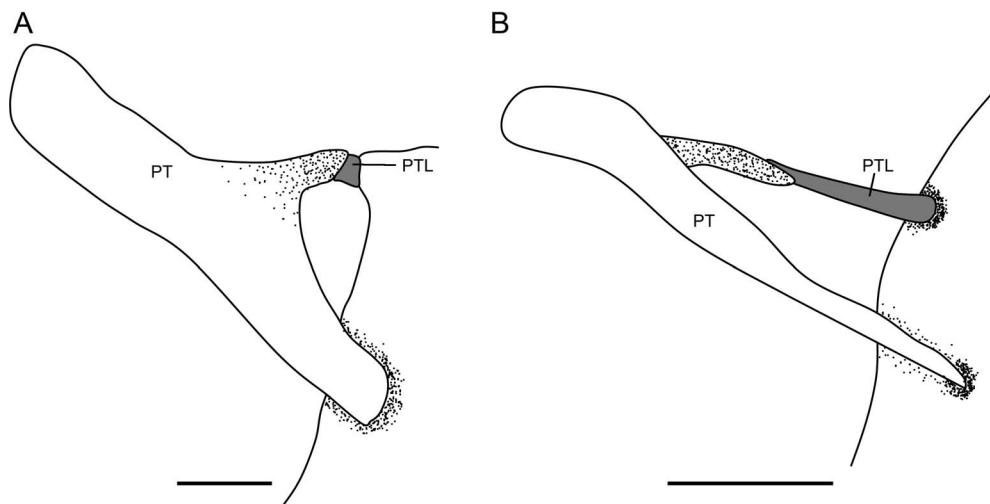

Fig. 7. Lateral view of right opercle. (A) *Percopsis omiscomaycus*, AUM 23034. (B) *Aphredoderus sayanus*, AUM 31430. (C) *Typhlichthys subterraneus*, AUM 16045. Abbreviations: dorsal process of opercle (DPO), posterior process of opercle (PPO). Scale = 1 mm.

Fig. 8. Dorsolateral view of left posttemporal. (A) *Aphredoderus sayanus*, AUM 31430. (B) *Typhlichthys subterraneus*, AUM 16045. Abbreviations: posttemporal (PT), ligament of the ventral arm of the posttemporal (PTL). Scale = 1 mm.
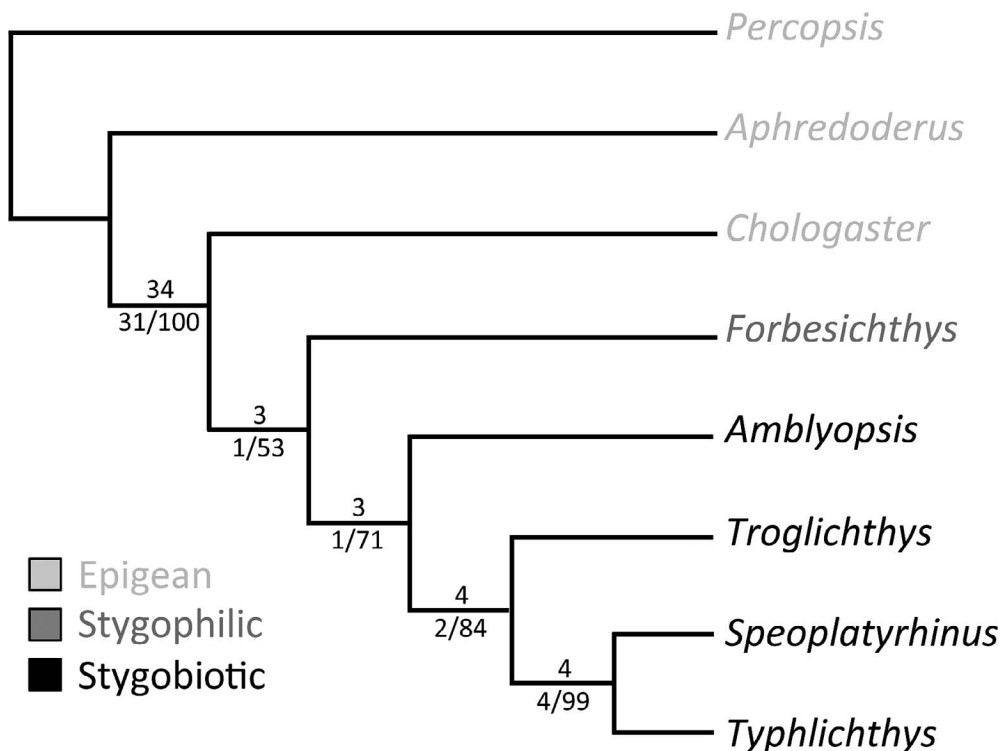
Fig. 9. Single most parsimonious tree (L = 76 steps, CI = 0.895, RI = 0.881). Numbers above the branches are the number of unambiguous changes along the branch. Numbers below the branches are Bremer Decay Index Values/Bootstrap Values. Taxa colored according to habitat.

Analysis.—Analysis of the 66 above skeletal characters yielded a single most parsimonious tree (Fig. 9). Support for Amblyopsidae was very strong, with 34 skeletal character changes supporting the monophyly of the family (Appendix 1). Support was also strong for the monophyly of all stygobiotic species (three character state changes, Appendix 1). *Troglichthys* was supported as the sister to *Typhlichthys* + *Speoplatyrhinus* by four character state changes (Table 2). Kishino-Hasegawa (Kishino and Hasegawa, 1989) and Templeton (Templeton, 1983) tests (Table 2) showed significant differences between the tree found in this study and phylogenies of all other studies except Swofford (1982) and the implied tree of Poulson (1963).

Table 2. Steps and significance values from Kishino-Hasegawa (K-H) and Templeton (T) tests. The Dillman et al. (2011) study did not include *Amblyopsis*, the Swofford (1982) study did not include *Speoplatyrhinus*, and the Grande et al. (2013) study did not include *Troglichthys,* so the length of the pruned most parsimonious tree from this study is in parentheses next to the number of steps observed with the trees in these studies. Swofford (1982)-1 is with *Troglichthys* sister to *Amblyopsis*, and Swofford (1982)-2 is with *Troglichthys* sister to a polytomy of *Chologaster, Forbesichthys,* and *Typhlichthys*. Asterisks indicate that the trees that are not statistically different to the tree in this study. A table level alpha of 0.05 was used and corrected using a sequential Bonferroni technique for multiple comparisons.

	Steps	K-H	T
This study (Fig. 3)	76		
Woods and Inger (1957)	90	0.0022	0.0029
Poulson (1963)	88	0.0174*	0.0210*
Swofford (1982)-1	83 (76)	0.0704*	0.0707*
Swofford (1982)-2	84 (76)	0.0882*	0.0968*
Dillman et al. (2011)	86 (68)	0.0004	0.0005
Niemiller et al. (2013a)	83	0.0072	0.0082
Grande et al. (2013)	89 (74)	0.0004	0.0006

DISCUSSION

Phylogeny.—Kishino-Hasegawa and Templeton tests showed that the phylogeny presented here is significantly different than most previously presented phylogenies. The only trees found similar to the tree in this study are both alternatives in Swofford (1982) and the implied tree of Poulson (1963). The similarities of the present tree to the Swofford trees can be explained, in part by the lack of resolution in the Swofford trees and in part due to the lack of *Speoplatyrhinus* in the analysis. Removing *Speoplatyrhinus* from the analysis causes all of the other phylogenies except Niemiller et al. (2013a) to also not be significantly different from the phylogeny presented here. There are several characters uniting *Typhlichthys* with *Speoplatyrhinus*, and removing *Speoplatyrhinus* from the analysis removes these important characters. The implied tree of Poulson (1963) is essentially a reverse of the tree found here, so the branching patterns are similar, and it is not surprising that it is not significantly different.

The most significant differences with the tree presented here were with the Dillman et al. (2011) and Grande et al. (2013) phylogenies. Dillman et al. (2011) found a clade of *Forbesichthys* and *Speoplatyrhinus*, and Grande et al. (2013) found a sister group relationship between *Chologaster* and *Speoplatyrhinus*. In contrast, a sister group relationship between *Typhlichthys* and *Speoplatyrhinus* was found in this study and in Niemiller et al. (2013a). *Speoplatyrhinus* is a single cave endemic found in Key Cave, Lauderdale Co., Alabama, where it also co-occurs with *T. subterraneus* (Kuhajda and Mayden, 2001; Niemiller and Poulson, 2010). Given that *Typhlichthys* was strongly supported as monophyletic in Niemiller et al. (2013a), *Speoplatyrhinus* is not likely to be descended from *Typhlichthys*, but shares a common ancestor that diverged 6.6 mya (95% CI: 4.9–8.9 mya; Niemiller et al., 2013a). Rather than the result of sympatric speciation, it is more plausible that co-occurrence of *Speoplatyrhinus* and *Typhlichthys* at Key Cave is the result of secondary contact.

Niemiller et al. (2013a) is the only other robust amblyopsid phylogeny with complete taxon sampling. The phylogeny presented here is different from Niemiller et al. in two main aspects. First, Niemiller et al. found *Troglichthys* as the sister to all other amblyopsids except *Chologaster*, and second, *Forbesichthys* was resolved as sister to *Amblyopsis*. In our morphological study, no characters were found to support the clades of *Amblyopsis* + *Forbesichthys* and *Amblyopsis* + *Forbesichthys* + *Speoplatyrhinus* + *Typhlichthys* from Niemiller et al. (2013a); however, one specimen of *Forbesichthys* had a rod-shaped basihyal (5:0, Fig. 3A, B) that is found as a reversal in *Amblyopsis*.

Species sharing similar environments often tend to resemble each other in traits that are adaptive in such environments (Winemiller, 1991; Kocher et al., 1993; Wiens et al., 2003). In extreme environments, such as caves, trait similarity can occur among very disparate taxa strongly implicating convergent evolution (Hobbs, 2001; Protas et al., 2006; Culver and Pipan, 2009; Bilandzija et al., 2012). Morphological convergence may mislead inference of phylogenetic relationships and may be particularly problematic in groups that are morphologically very similar and have colonized and adapted to an extreme environment (Wiens et al., 2003; Niemiller et al., 2013a). For example, Wilcox et al. (2004) found the two species of Mexican cave catfishes of the genus *Prietella* to not be monophyletic under Bayesian and Maximum Likelihood analyses despite the species having several synapomorphies (the genus was, however, monophyletic under maximum parsimony). Wilcox et al. (2004) suggested that the differences between their analyses were due to long branch attraction in the maximum parsimony dataset, and the two species of *Prietella* were similar due to convergence due to cave adaptation. Morphological convergence may account for discrepancies in the relationships between our phylogeny and that of Niemiller et al. (2013a). All stygobiotic amblyopsids share three synapomorphies (6:1, 43:1, 47:1). These characters are likely all related to feeding: change in the shape of the autogenous tooth bones along the ceratobranchials (6:1; Fig. 3D, E), an elongate anterior arm of the hyomandibula (43:1, Fig. 6B), and a fully toothed palatine bone (47:1).

The elongate anterior arm of the hyomandibula in stygobiotic amblyopsids (Fig. 6B) could be the result of elongation and flattening of the skull seen in several other aquatic cave vertebrates (Poulson, 1963; Wiens et al., 2003; Bendik et al., 2013; Ivanovic et al., 2013); however, this relationship is made with reference to the metapterygoid, which would be expected to lengthen in proportion with the hyomandibula if this were simply due to skull lengthening. However, the metapterygoid in stygobiotic amblyopsids is not lengthened. Also, the elongate arm of the hyomandibula is the only character examined in the stygobiotic amblyopsids that could be associated with this head shape change. The palatine bone is fully toothed in *Aphredoderus* but only partially toothed in most *Percopsis* (one of the two *P. transmontana* examined has a fully toothed palatine), suggesting that the character may be plastic, and similar coding could be the result of convergence. All of the characters that support the stygobionts as monophyletic are associated with feeding, indicating possible feeding mechanism changes associated with a purely stygobiotic existence. Thus, these characters may be unneeded and reversed in *Forbesichthys*. However, in his behavioral examination of amblyopsids, Poulson (1963) did not present any significant differences between *Forbesichthys* and the stygo-

bionts with each feeding on the substrate and sometimes in midwater; thus, the skeletal characters may not be related to a stygobiotic existence.

One character that supports *Troglichthys, Typhlichthys,* and *Speoplatyrhinus* as a monophyletic group is the elongation of the posterior part of the mesethmoid (34:1). The mesethmoid forms the snout, and as such is associated with its length. *Speoplatyrhinus* has the most elongate snout, thus the most elongate mesethmoid, so this character could be due to advanced cave adaptation (although the stygobiotic *Amblyopsis* has a short mesethmoid). The lengthening of the mesethmoid is not entirely homologous in *Troglichthys* and *Typhlichthys* + *Speoplatyrhinus*, as the mesethmoids of *Typhlichthys* and *Speoplatyrhinus* are uniquely shaped in that the posterior end does not widen (35:1). There is variation in the skeletons of *Typhlichthys* examined (including the presence of lateral laminas on the mesethmoid in some), suggesting that there may be skeletal support for some of the lineages in Niemiller et al. (2012).

Heterochrony.—Niemiller and Poulson (2010) suggested that neoteny may be a powerful driver in morphology of stygobiotic amblyopsids. Some neotenic characters listed are the absence of bifurcate fin rays in *Speoplatyrhinus* and larger heads. In this study, two potentially neotenic characters were found in common between *Troglichthys* and *Typhlichthys*: a rounded (vs. pointed) anterior process of the basisphenoid complex and a widely separated orbitosphenoid and prootic. In most fishes, the basisphenoid has a long, pointed anterior process that runs down the midline and is surrounded by the parasphenoid, forming what is essentially a long suture. In *Troglichthys* and *Typhlichthys* (the condition was not observable in *Speoplatyrhinus*), there is little or no sutural contact between the basisphenoid and parasphenoid, and such a loss of sutures is likely due to developmental truncation in the formation of these structures. The prootic in *Troglichthys* and *Typhlichthys* (again not observable in *Speoplatyrhinus*) also seems to be incompletely formed, and does not fully contact the orbitosphenoid (38:1).

Chologaster and *Forbesichthys* may show neoteny in feeding characters, including the comparative weakness of the posterior section of pharyngobranchial 4 (15:1) and relative lack of teeth on the palatine (15:1). If neoteny is the causative factor for similarities between *Chologaster* and *Forbesichthys*, the basal position of *Forbesichthys* in the morphological phylogeny could be an artifact of similar development in the two genera and not as a result of common ancestry.

Although relative changes in timing of development within Amblyopsidae probably are a causative factor in morphological evolution of some characters within the family, the greater effect of neoteny is observable between Amblyopsidae and its relatives. *Percopsis* and *Aphredoderus* are robust fishes, similar in body form to other members of the Neoteleostei (for phylogenies see Near et al., 2012; Betancur-R. et al., 2013). The lack of fin spines and the comparatively weak head skeletons of amblyopsids resemble larval characters. In most fishes, the dorsal bones of the skull either meet at the midline or have a suture by maturity. In all amblyopsids, the medial edges of the frontals are crenulate and overlap one another instead of forming a suture (Fig. 10). The failure of the formation of a connection of the frontals is possibly neotenic; perhaps the bones fail to meet and suture early in development (neoteny) and then are free to grow in this overlapping fashion. In addition, loss of serrae on the
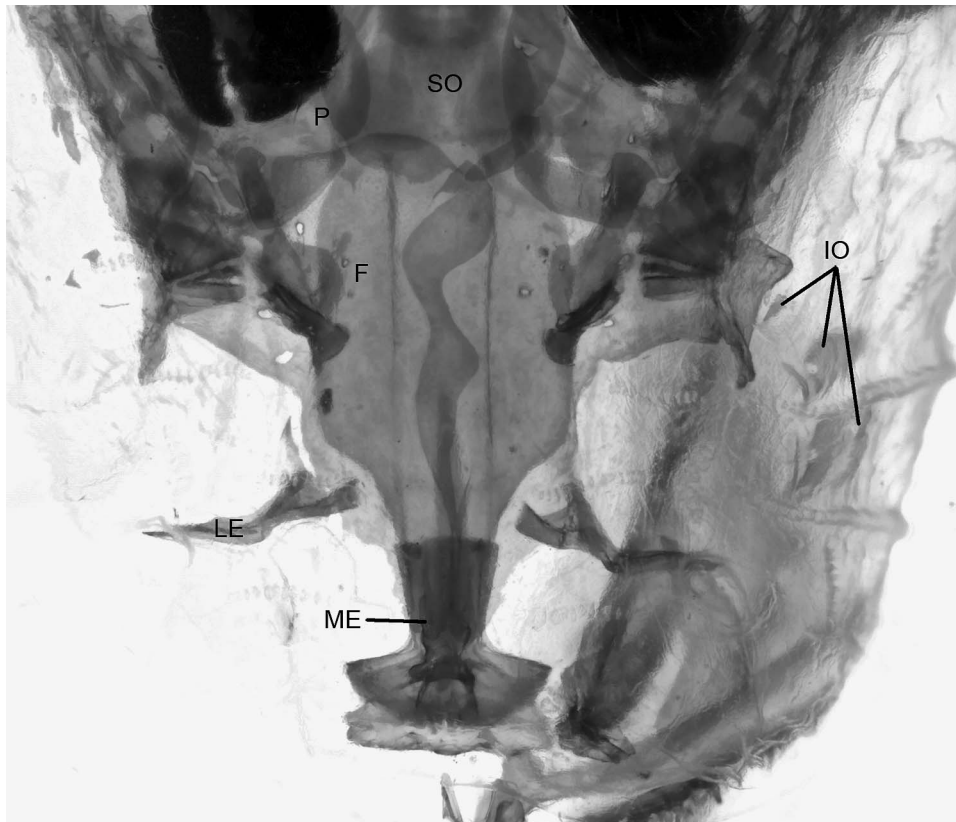
Fig. 10. Dorsal view of the skull of *Typhlichthys subterraneus,* AUM 16045, showing the crenulate, overlapping borders of the frontals (F) as well as the junctions of the frontals, pterotics (P), and supraoccipital (SO). Other indicated bones are the infraorbitals (IO), lateral ethmoid (LE), and mesethmoid (ME). Photo by Pamela B. Hart and Charles D. R. Stephen.

preopercle (48:1) and absence of pelvic fins (66:1) are likely due to developmental truncation. Serrae on the preopercle of *Aphredoderus* appear between about 12–20 mm SL (JWA, pers. obs.), and the pectoral-fin buds of fishes appear in embryos, but pelvic-fin buds do not appear until larvae metamorphose into juveniles (Tanaka, 2011); thus, loss of preopercular serrae and pelvic fins would be a result of neoteny or developmental truncation.

Amblyopsis, however, does have a serrate preopercle (though not as strongly serrate as *Aphredoderus* or *Percopsis,* Fig. 3G vs. F) and pelvic fins. We interpret the presence of these characteristics as a relaxation of neoteny in *Amblyopsis*. The preopercle does not develop serrae in other amblyopsids, but the preopercles are thinner near the area where the serrae form in *Amblyopsis*. Moreover, the edge of the preopercle is slightly crenulate, suggesting that the serrae do not complete development in the other amblyopsids. Because of the potential strong influence of neoteny on the morphology of amblyopsids, morphological evolution can be rapid simply by changing the degree of developmental truncation. With significant, demonstrable reversals in *Amblyopsis* indicating probable heterochrony, it is possible that any morphological phylogeny of the group should be interpreted with caution; however, no synapomorphies to link *Amblyopsis* and *Forbesichthys* nor any synapomorphies to support a clade of *Amblyopsis, Forbesichthys, Speoplatyrhinus,* and *Typhlichthys* were found in this study. Only the elongation of the mesethmoid appears directly related to cave adaptation.

The Percopsiformes.—There had been past debate on whether the three families that comprise the order Percopsiformes form a monophyletic group. However, this dialog has been settled by recent, robust molecular analyses of teleosts (Near et al., 2012; Betancur-R. et al., 2013; Grande et al., 2013), which collectively have resolved the order as monophyletic

and the relationships of the families as presented in Figure 9. The monophyly of the Amblyopsidae has not been in question, but only a few skeletal synapomorphies had been presented (Borden et al., 2013; Grande et al., 2013). In this study, 34 skeletal synapomorphies were found for the Amblyopsidae (Table 2), making the family very well diagnosed.

The sister group relationship of Aphredoderidae and Amblyopsidae was also well supported with nine synapomorphies. Of note, aphredoderids and amblyopsids share the presence of a unique premaxilla that has paired, central elements similar to what is found in percopsids and then a distal series of small, toothed plates (Fig. 5). Rosen (1962) called this arrangement of jaw plates a segmented premaxilla, but this terminology does not appear accurate. The premaxilla is a single dermal ossification, and what is found in aphredoderids and amblyopsids is a chain of dermal elements. The medial element is the approximate size and shape of the premaxilla of other fishes and likely the only part of the system that is homologous to the premaxilla of other fishes. Given the way that dermal bones develop, the other bones are likely separate ossifications termed here *lateromaxillae*. Lateromaxillae have only been found in aphredoderids and amblyopsids, and their presence is a strong indication of the close relationship of the two families. It appears that lateromaxillae may be added as the fishes grow, lending credence to the hypothesis that these bones are separate skeletal evidence; however, this was not clear from the specimens available, and the hypothesis requires further testing.

Another interesting synapomorphy for aphredoderids and amblyopsids is the anterior position of the vent, which is located on the throat in adults. In *Amblyopsis*, the anterior vent allows eggs to be more easily transferred into the branchial chamber for brooding (Eigenmann, 1909). Although

it has been speculated that all amblyopsids have branchial brooding (Eigenmann, 1909; Woods and Inger, 1957; Poulson, 1963; Breder and Rosen, 1966; Niemiller and Poulson, 2010), it has only been observed in *Amblyopsis*, and there is not enough space in the branchial chamber of at least *Chologaster* to support a brood (Jenkins and Burkhead, 1994; Niemiller and Poulson, 2010). In a study on the natural history of *Troglichthys*, Adams and Johnson (2001) handled over 200 cavefish during a two-week period during the breeding season, observing females with mature ova as well as juveniles, and never found a female branchial brooding. Likewise, branchial brooding has never been observed in any other amblyopsid species (reviewed in Niemiller and Poulson, 2010).

Branchial brooding was hypothesized to also occur in *Aphredoderus* (Boltz and Stauffer, 1986), but nesting behavior has been observed (Forbes and Richardson, 1920; Katula, 1992). Poly and Wetzel (2003) observed a unique behavior in *Aphredoderus* that they termed *transbranchioral spawning*. Males and females of *Aphredoderus* pass gametes from their vents, through their branchial chambers and out of their mouths. Females eject the eggs into the substrate as males release milt over them. In the most parsimonious scenario, the anterior position of the vent did not evolve for branchial brooding, and branchial brooding in *Amblyopsis* would be considered an exaptation of the anterior vent position. Given the observations by Poly and Wetzel (2003) and the fact that branchial brooding has never been observed outside of *Amblyopsis* among amblyopsids, there is no reason to expect other amblyopsids to exhibit branchial brooding.

Conclusions.—The first morphological phylogeny for the Amblyopsidae could be construed as evidence for a progression from epigean, to stygophile, to stygobiont; however, support for internal nodes within Amblyopsidae are weak, and recent molecular studies suggest that some cavefish lineages are the result of multiple, independent colonization events (Dillman et al., 2011; Gross, 2012; Niemiller et al., 2013a). The apparent smooth transition from epigean, to stygophile, to stygobiont in the morphological phylogeny could be due to convergence in stygobiotic species and morphological conservatism causing failure to find useful synapomorphies exhibited within the Amblyopsidae. A recent molecular phylogeny (Niemiller et al., 2013a, 2013b) strongly found the stygophile *Forbesichthys* to be a reversal to a partially epigean existence. In another recent molecular phylogeny, Grande et al. (2013) found the epigean *Chologaster* nested within stygobionts. Neoteny is likely to be a strong causative factor in the morphological evolution of amblyopsids, and a few of the skeletal characters could be convergent based on neoteny or cave adaptation.

MATERIAL EXAMINED

Only cleared and stained specimens indicated with the exception of the specimen of *Speoplatyrhinus*, which was cleared but not stained.

Percopsidae

Percopsis omiscomaycus: ANSP 79925, 1; AUM 14519, 1; AUM 23084, 2; INHS 25638, 2; INHS 33160, 4; INHS 86885, 1; INHS 88030, 2.

Percopsis transmontana: INHS 41290, 2.

Aphredoderidae

Aphredoderus sayanus: AUM 21433, 2; AUM 26377, 2; AUM 26953, 2; AUM 29036, 2; AUM 31430, 2; AUM 35253, 2.

Amblyopsidae

Amblyopsis hoosieri: INHS 42424, 2.

Chologaster cornuta: AUM 12931, 2; AUM 19382, 1; INHS 38356, 3; INHS 75192, 2; INHS 82327, 1; USNM 317538, 1.

Forbesichthys agassizii: AUM 10706, 1; INHS 37654, 1; INHS 60745, 1; INHS 63500, 3; INHS 68216, 1.

Speoplatyrhinus poulsoni: USNM 204998, 1, paratype.

Troglichthys rosae: FMNH 62032, 1; TU 22675, 2; UMMZ 64947, 1.

Typhlichthys subterraneus: AUM 16045, 1; AUM 35501, 1; INHS 60576, 1; TU 22765, 2; UAIC 3958.01, 1.

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APPENDIX 1

Character state changes along the morphological phylogeny of Tree 1 (Fig. 9). Abbreviations: character changed above (CA, character is changed in one or more taxa within this clade), convergent with another taxon (CO), no unambiguous changes (NUC), polymorphic (P), and reversal (R).

Chologaster: NUC

Forbesichthys: 41:1

Amblyopsis: 5:0(R), 10:1, 20:1, 44:1, 48:0(R), 65:1, 66:0(R, P)

Troglichthys: NUC

Typhlichthys: 18:1

Speoplatyrhinus: NUC

Aphredoderidae + Amblyopsidae: 7:1, 11:1, 19:1, 24:1, 33:1, 45:1, 46:1, 54:1, 62:1

Amblyopsidae: 1:1, 5:1(CA), 8:1, 9:1, 12:1, 13:1, 16:1, 17:1, 21:1, 23:1, 25:1, 27:1, 28:1, 29:1, 30:1, 32:1, 36:1, 37:1, 39:1, 42:1, 46:2, 48:1(CA), 50:1, 51:1, 52:1, 53:1, 55:1, 56:1, 57:1(CA), 58:1, 59:1, 60:1, 61:1, 66:1(CA)

Forbesichthys + Amblyopsis + Troglichthys + Typhlichthys + Speoplatyrhinus: 40:1, 49:1, 64:1

Amblyopsis + Troglichthys + Typhlichthys + Speoplatyrhinus: 6:1, 43:1, 47:1(CO)

Troglichthys + Typhlichthys + Speoplatyrhinus: 26:1, 34:1, 38:1, 57:2

Typhlichthys + Speoplatyrhinus: 3:1, 22:1, 31:1, 35:1