Mosaic Evolution of Craniofacial Morphologies in Ghost Electric Fishes (Gymnotiformes: Apteronotidae)

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Kassandra L. Ford[1,2], Maxwell J. Bernt[2,3], Adam P. Summers[4], and James S. Albert[2]

Ghost electric knifefishes (Gymnotiformes: Apteronotidae) are a dominant component of the species diversity and biomass of large lowland rivers in Greater Amazonia, including 77 species that exhibit diverse craniofacial morphologies associated with trophic and secondary sexual traits. Here we use open-source computed microtomography (μCT) scans to generate 3D reconstructions for a majority of apteronotid species and almost all valid genera, and geometric morphometric and phylogenetic analyses to explore patterns of skull evolution. As with most vertebrates, principal component 1 (PC1) summarizes variance from brachycephalic to dolichocephalic morphologies, previously described as heterocephaly, and PC2 summarizes variance from recurved (upturned) to decurved (downturned) snout morphologies, described here as heterorhynchy. Phylomorphospace and traitogram analyses found instances of both convergent and divergent evolution along both of the first two PC axes, as well as a preponderance of clades characterized by morphological stasis or phylogenetic conservatism. Certain phenotypic combinations predominate among species and clades, including elongated-downturned snouts and foreshortened-upturned snouts, while other phenotypic combinations are not observed, including elongated-upturned snouts and foreshortened-downturned snouts. These results highlight the power of 3D geometric morphometrics in the study of craniofacial evolution and indicate developmental or functional constraints in the evolution of craniofacial phenotypes in an ecologically dominant clade of riverine Amazonian fishes.

FORM and function interact in complex ways to constrain the processes of evolutionary adaptation and phenotypic diversification (Russell, 1916; Lauder, 1981). While most morphological structures are used in the performance of multiple functions, most functions and behaviors are recruited from the coordinated actions of many morphological parts, integrated through complex developmental and biomechanical networks (Albertson et al., 2005; Alfaro et al., 2005; Wainwright et al., 2005; Kane and Higham, 2015). These regulatory networks are especially complex in the ontogeny and phylogeny of the vertebrate head, a multifarious suite of morphofunctional phenotypes and tissues used continually in breathing (e.g., gill ventilation) and feeding behaviors, and episodically in social (e.g., aggressive, communicative) behaviors (Klingenberg, 2008; Hallgrímsson et al., 2009; Collar et al., 2014). Vertebrates experience a range of environmental and organismal constraints that may influence evolutionary changes in head and mouth morphologies, from the effects of habitat on locomotion and diet (Westneat, 2005; Cooper and Westneat, 2009; Cooper et al., 2011) to developmental limits on the production of phenotypic variation (Hallgrímsson et al., 2007; Chapman et al., 2008; Losos, 2011; Ord and Summers, 2015; Stayton, 2015).

Phylogenetic analysis of geometric morphometric data has increasingly been used as a quantitative framework in which to assess the contributions of environmental and organismal constraints on the evolution of skull morphologies within and among evolutionary lineages (Sidlauskas, 2008; Buser et al., 2018; Evans et al., 2019a). Recent work has focused on the independent evolution of phenotypically similar craniofacial phenotypes in different clades that inhabit similar environments or utilize similar trophic resources, coined "convergent evolution" (Sidlauskas, 2008). By contrast, mosaic evolution refers to the mixture of ancestral and derived traits observed in most organismal phenotypes and has been hypothesized to arise from the influences of multiple external pressures (Felice and Goswami, 2018) and internal compartmentalization (Young et al., 2010) of morphological traits. Characterizing skull evolution as convergent, divergent, or unchanging is challenging because most skull traits exhibit multiple aspects of phenotypic similarity (e.g., structural, compositional, positional, functional, developmental), and these traits often involve components that may be assessed at several levels in the integrated hierarchy of organismal design (e.g., genetic, developmental, morphological, functional, behavioral; Losos, 2011; Ord and Summers, 2015; Stayton, 2015).

Gymnotiform electric fishes are a clade of approximately 262 species that inhabit most lowland aquatic habitats across tropical South and Central America (Albert, 2001, 2003; Albert and Crampton, 2006, 2009; Ivanyisky and Albert, 2014; Evans et al., 2017a; Bernt et al., 2018, 2020; Dagosta and de Pinna, 2019; Albert et al., 2020). The family of ghost electric knifefishes (Apteronotidae) is a dominant component of the species diversity and biomass in large and deep (to 100 m) lowland river channels of Greater Amazonia (Lundberg et al., 1987; Crampton et al., 2004; Correa et al., 2006; Duarte et al., 2019). Apteronotidae is represented by

[1] Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland; Email: kassy159@gmail.com; ORCID: 0000-0002-4413-2633. Send reprint requests to this address.

[2] Department of Biology, University of Louisiana at Lafayette, 410 E. St. Mary Blvd., Billeaud Hall, Room 108, Lafayette, Louisiana 70503; Email: (JSA) jalbert@louisiana.edu; ORCID: (JSA) 0000-0001-5477-1749.

[3] Department of Ichthyology, American Museum of Natural History, 79th Street and Central Park West, New York, New York 10024; Email: mbernt@amnh.org; ORCID: 0000-0002-3322-2828.

[4] Department of Biology and SAFS, University of Washington, 620 University Drive, Friday Harbor, Washington 98250; Email: fishguy@uw.edu; ORCID: 0000-0003-1930-9748.

97 valid species, including 77 species in Greater Amazonia (van der Sleen and Albert, 2017; Bernt et al., 2018, 2020), where they exhibit high phenotypic disparity of head and skull shapes (Fig. 1) and a relatively more-conserved posterior body region (Albert, 2001; Evans et al., 2017b). Salient diagnostic characters of apteronotids among gymnotiforms include: a caudal fin with a hypural plate and segmented fin rays, a fleshy electroreceptor organ along the dorsal body margin (Albert, 2001; Bernt et al., 2019), and a neurogenic electric organ that generates a high-frequency wave-type electric signal (Bennett, 1966; Albert and Crampton, 2005).

Most apteronotid species inhabit the deep channels and broad floodplains of large lowland Amazonian rivers, habitats hypothesized to impose selective pressures on the evolution of head and mouth morphologies in electric fishes (Marrero and Winemiller, 1993; Winemiller and Adite, 1997). Head and mouth diversity of riverine apteronotids could be constrained by either the diversity or breadth of dietary contents, including specialized trophic traits for piscivory, planktivory, benthic foraging, or lepidophagy (Albert and Crampton, 2005; Albert and Reis, 2011; Evans et al., 2019b). Head and mouth morphologies could also be specialized for male–male aggressive behaviors, which can include jaw-locking and tail nipping of conspecifics to attenuate their electric signal (Lundberg et al., 1996; Albert and Crampton, 2009; Evans et al., 2018). Analyzing craniofacial diversity in a phylogenetic context has the potential to reveal patterns that may point us towards the selective drivers of shape variation. Yet the deep-channel fauna of Amazonian apteronotids is not monophyletic, having become assembled by multiple evolutionary processes of adaptation, dispersal, speciation, and extinction, over the course of millions of years, and from an evolutionary radiation distributed across millions of square kilometers of northern South America (Albert et al., 2011a, 2011b, 2018).

The goal of our study was to use quantitative methods to analyze shape differences among species in an incredibly diverse clade of electric fishes. Previous studies assessed morphological diversity and craniofacial evolution in Gymnotiformes using two-dimensional geometric morphometric analyses of the neurocranium across Gymnotiformes (Evans et al., 2017b), a comparison of evolutionary rates of skull modules between carnivores and Gymnotiformes (Evans et al., 2017c), analysis of integration and modularity and evolutionary rates of skull modules in the Apteronotidae (Evans et al., 2019a). In this study, we examined 13 of 16 genera and 43 of 97 apteronotid species, using 3D geometric morphometrics of the entire skull, including the neurocranium and the lower jaw, to accurately assess shape-change (Buser et al., 2018). We analyzed these morphological data with macroevolutionary and phylogenetic comparative methods to compare degrees of morphological similarities in the Apteronotidae. The four aims of this study were to 1) gather, and make publicly available, computed microtomography (μCT) scan data representing the diverse craniofacial morphology of this group; 2) use geometric morphometrics to quantify shape change in the skull; 3) use updated evolutionary relationships to reveal the existence and number of convergently evolved skull shapes; and 4) reconstruct the ancestral phenotype for the apteronotid skull.

MATERIALS AND METHODS

Specimen preparation.—A majority of specimens analyzed in this study were collected from the vicinity of Iquitos, Peru from 2016–2017 with seine-net sampling in both the whitewater Amazon and blackwater Nanay Rivers. Additional samples were obtained from museum collections (AMNH). Specimens were selected to include a range of sizes and maturities, with a majority being adults or sub-adults as determined by relative extent of ossification (Albert, 2001; Figs. 1, 2). We based our interpretations on the phenotypes of larger specimens and had very few individuals we would describe as "subadult." Although limited by the size of the μCT scanner, most specimens were between 100 and 200 mm TL, at which size most apteronotids have attained mature phenotypes. Comparisons with cleared and stained specimens show that all species studied except *O. tamandua* are morphologically mature based on the extent of ossification (Albert, 2001; Figs. 1, 2) and their near isometric head growth (Hulen et al., 2005; Albert and Crampton, 2009). We sampled 184 specimens from 44 species (13 genera) in Gymnotiformes, primarily focused on the family Apteronotidae (Supplemental Table 1; see Data Accessibility). The fishes were scanned using μCT at the University of Washington's Friday Harbor Laboratories using a Bruker SkyScan 1173 (USA). Specimens were scanned in groups of up to eight individuals of similar size with parameters ranging from 60–70 kv and 114–133 μA with radio-opaque labels for identification. We used the open-source software 3D Slicer and R to create 3D renderings of fish, segmentations of the skull, and generation of a surface for geometric morphometrics (Fedorov et al., 2012; RStudio Team, 2021).

Phylogenetic tree.—The tree used in the phylogenetic comparative methods is based on Bernt et al. (2019). This phylogeny was inferred using three mitochondrial and four nuclear genes under both maximum-likelihood and Bayesian methods. The tree was pruned to include only the species used in the morphological analyses.

Geometric morphometrics.—Three-dimensional geometric morphometrics were performed using a 22-point homologous landmark scheme across the entirety of the skull using the program 3D Slicer (Fedorov et al., 2012; Fig. 2; Table 1). A generalized Procrustes superimposition was completed in the R package *geomorph* using Procrustes distances to reduce the effects of size, rotation, and translation from the analysis of shape (Goodall, 1991; Collyer and Adams, 2018, 2021; Adams et al., 2021; RStudio Team, 2021). This process translates specimens to a common location in coordinate space, scales them to a unit centroid size, and rotates them to reduce the distances between homologous landmarks.

Principal component analyses.—A principal component analysis (PCA) was performed in *geomorph* (version 3.3.1) from a covariance matrix of Procrustes coordinates to show the major proportions of variance associated with shape change (Collyer and Adams, 2018, 2021; Adams et al., 2021). We used Procrustes ANOVAs to compare Procrustes distances and relative shape and completed a *post hoc* test to determine which genera had significant differences in shape. The values of the first two major axes of shape variation in the principal component analysis (PC1 and PC2) were used for subsequent analyses because they contained the most shape variation
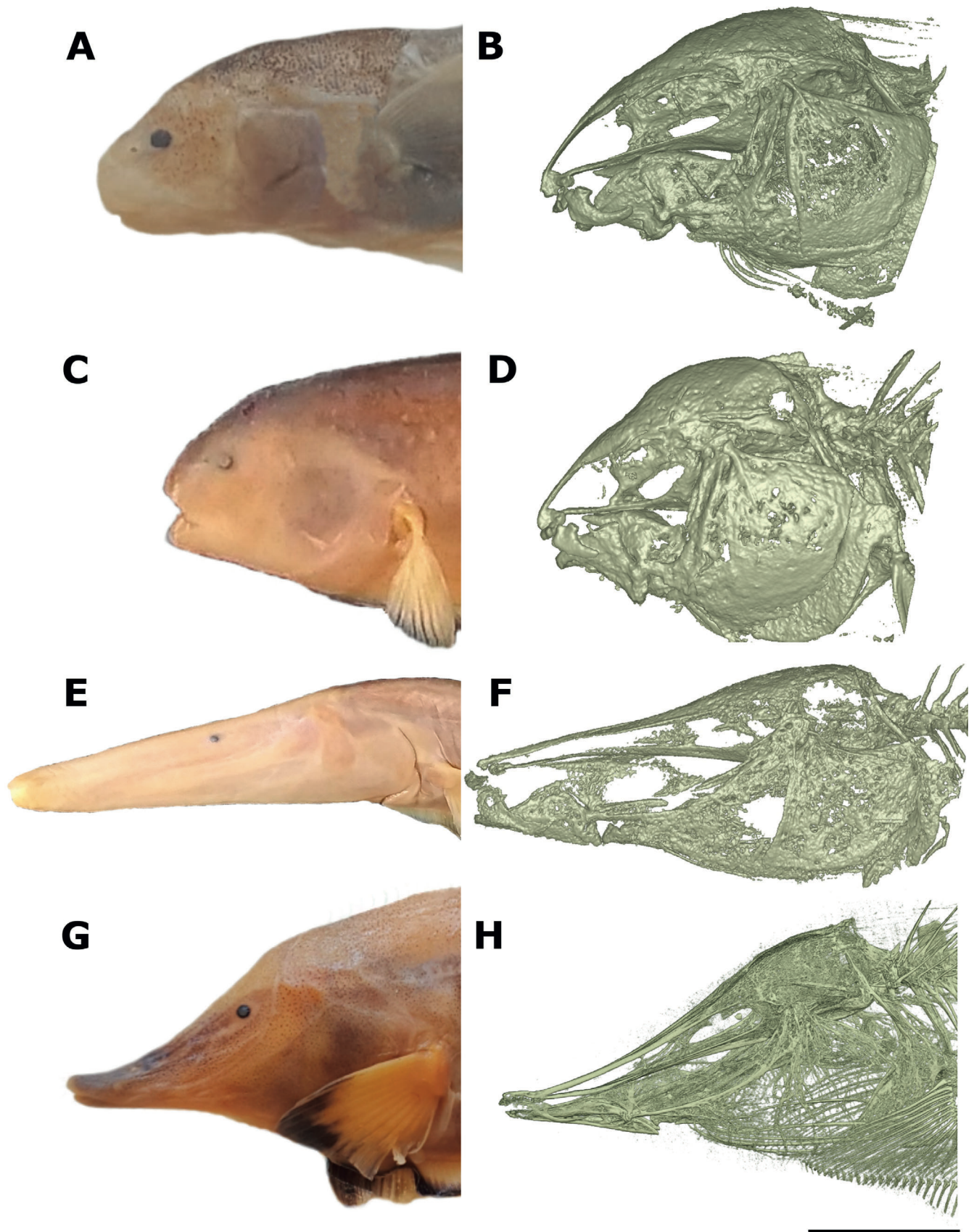
Fig. 1. Diversity of head and skull shape in the family Apteronotidae (Teleostei: Gymnotiformes), with an instance of convergence (*Porotergus duende* [A–B] and *Sternarchogiton nattereri* [C–D]) and an example of skull shape diversity within a lineage (*Orthosternarchus tamandua* [E–F] and *Sternarchorhamphus muelleri* [G–H]). Black scale bar is 5 mm.
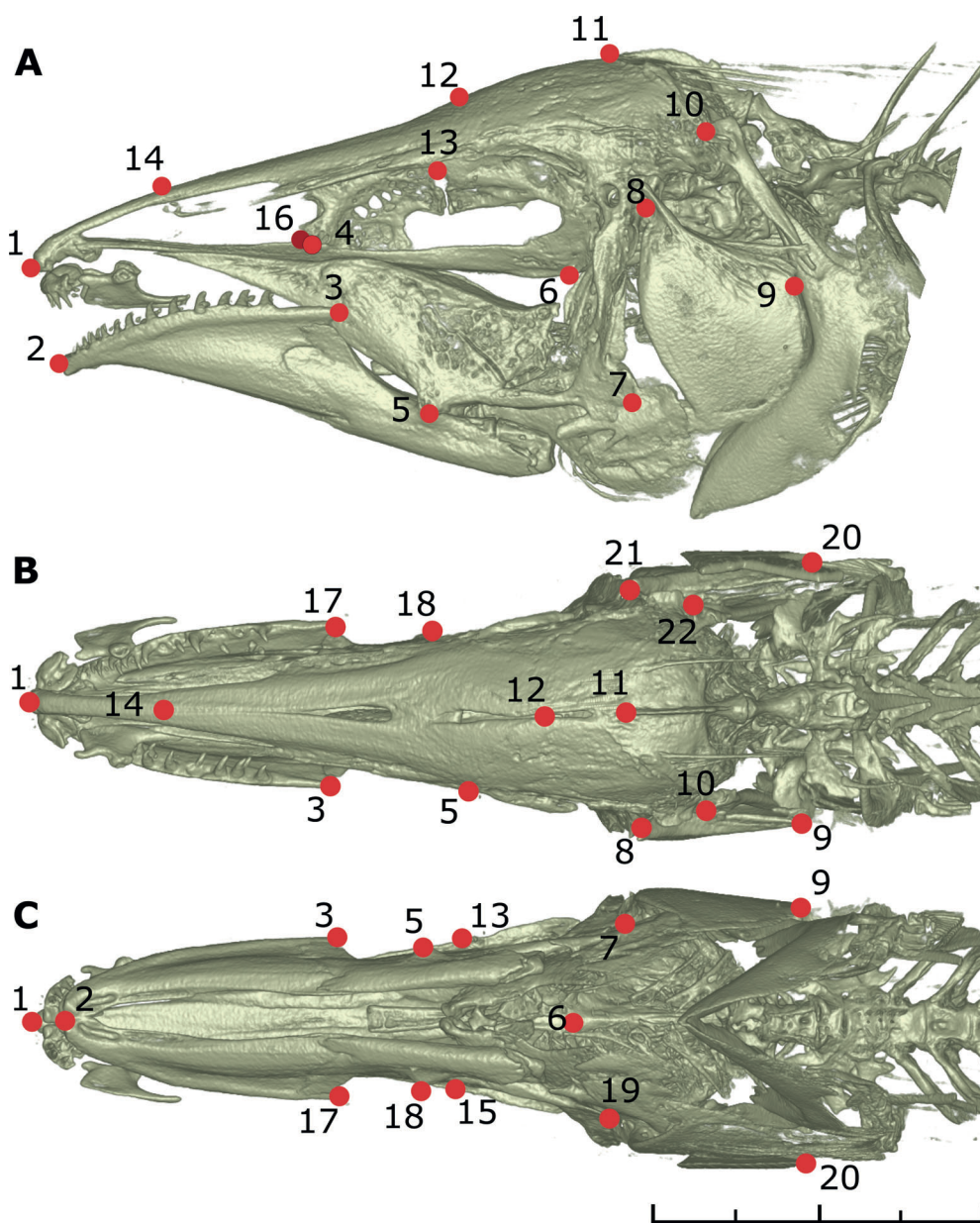
Fig. 2. Three-dimensional (3D) model of an *Apteronotus rostratus* skull with the 22-point landmark scheme used for geometric morphometric analyses. (A) Lateral, (B) dorsal, and (C) ventral views of the 3D model. See Table 1 for landmark definitions. Black scale bar is 10 mm.

associated with relative skull length. Although there are caveats associated with these methods in phylogenetic comparisons, this is the best method for assessing shape differences across and within lineages. We included landmarks covering the neurocranium, suspensorium, and lower jaw in these analyses because of the potential implications of lower jaw morphology on overall shape and dietary correlations. We performed two analyses of morphological disparity using the *morphol.disparity* function in *geomorph*, comparing pairwise differences across genera containing more than one specimen. In one analysis we did not account for changes in allometry (by including the log of centroid size as a factor), and in the other we included that correction.

Phylogenetic comparative methods.—MorphoJ (version 1.07a) was used to generate a phylomorphospace with the "map onto phylogeny" function, based on the Bernt et al. (2019) phylogeny, the PCA values, and the Procrustes coordinates (Klingenberg, 2011). The species included in this analysis (*n* = 31) were subsampled from the original morphological dataset

to include only those species in the apteronotid phylogeny. The R package *phytools* (package version 0.7-70) was used to perform a maximum-likelihood ancestral-state reconstruction and traitogram on species with morphological data that were also in the apteronotid phylogeny (*n* = 36; Revell, 2012; RStudio Team, 2021). This program uses continuous characters and maps them onto a phylogeny. It then estimates states at internal nodes using maximum likelihood with "fastANC," then interpolates the states along the edge using equation two of Felsenstein (1985). The *phytools* traitogram maps continuous characters (PC1 values) onto the phylogeny to estimate confidence levels in ancestral phenotypes.

RESULTS

Principal component analysis.—The first two PC axes account for 67.8% of shape variation with Apteronotidae (Wickham, 2016; Fig. 3). PC1 (49.3%) summarizes a suite of shape changes called "heterocephaly" that is commonly observed in gnathostome lineages (Evans et al., 2017c). Heterocephaly

Table 1. Locations of homologous landmarks in the 22-point landmark scheme used in 3D geometric morphometric analyses.

LM #	Definition
1	Most anterior point of the mesethmoid (nasal septum)
2	Most anterior point of dentary
3	Most posterior point of dentary (L)
4	Articulation point between parasphenoid and prefrontal (L)
5	Articulation point between articular and quadrate (L)
6	Most posterior point of parasphenoid within jaw
7	Most posterior point of metapterygoid (L)
8	Articulation point between opercle and hyomandibular bone (L)
9	Most posterior point of opercle (L)
10	Most anterior point of posttemporal bone (L)
11	Most anterior point of supraoccipital crest
12	Articulation point between parietal and frontal bone
13	Articulation point between alisphenoid and frontal (L)
14	Articulation point between frontal and premaxilla
15	Articulation point between alisphenoid and frontal (R)
16	Articulation point between parasphenoid and prefrontal (R)
17	Most posterior point of dentary (R)
18	Articulation point between articular and quadrate (R)
19	Most posterior point of metapterygoid (R)
20	Most posterior point of opercle (R)
21	Articulation point between opercle and hyomandibular bone (R)
22	Most anterior point of posttemporal bone (R)

refs to ontogenetic changes in the relative shapes of the snout (the pre-orbital portion of the skull) and braincase, characterized by relative elongation (or foreshortening) of the snout (preorbital) region of the neurocranium, and relative contraction (or expansion) of the braincase region of the neurocranium. The extreme morphologies of PC1 include species of *Sternarchorhynchus* with the most dolichocephalic (elongate) snout, and *Adontosternarchus* with the most brachycephalic (short) snout.

PC2 (18.5%) summarizes a suite of shape changes that we herein term "heterorhynchy," referring to allometric changes in the relative dorso-ventral flexion of the snout region of the neurocranium. Negative heterorhynchy refers to a concave ventral snout margin, typically associated with a ventral mouth position and benthic invertebrate feeding behaviors in apteronotid fishes. Positive heterorhynchy refers to a convex ventral snout margin, a terminal or superior mouth, and surface or piscivorous feeding behaviors in apteronotids. The extreme morphologies for PC2 include *Sternarchella duccis* with the most convex ventral snout margin and *Sternarchorhynchus mormyrus* with a concave snout margin. PC3 only accounted for 7.5% of the total shape variation. We could not easily find associations between shape and PCs 3 or 4, so we did not include them in our interpretations.

The Procrustes ANOVAs show a statistically significant relationship between shape and size, shape and genera, and shape and species (Table 2). Further, the *post hoc* test found significant differences between 33 of 78 genera pairs (Supplemental Table 2; see Data Accessibility). In the morphological disparity analysis with size correction, we found significant differences between *Apteronotus* and *Compsaraia*, *Sternarchella*, and *Sternarchogiton* (Supplemental Table 3; see Data Accessibility). When we did not account for the size correction, we also found significant differences between those genera listed above, along with between *Sternarchorhynchus* and *Compsaraia*, *Sternarchella*, and *Sternarchogiton* (Supplemental Table 3; see Data Accessibility).

Phylogenetic comparative methods.—The phylomorphospace shows the main PC axes (PC1 and PC2) with phylogenetic relationships between species in the Apteronotidae and two species representing the outgroup taxon Sternopygidae (represented by *Rhabdolichops* and *Sternopygus*) to quantify morphological convergence within the family (Fig. 4). There are several examples of homoplasy, where morphologies of unrelated taxa converge on similar areas of the morphospace. Multiple areas of the morphospace remain empty, indicating a potential evolutionary bias against evolution towards specific phenotypes.

The ancestral-state estimation shows multiple instances of evolution towards brachycephalic phenotypes and at least one instance of evolution towards more dolichocephalic phenotypes in the Apteronotidae (Fig. 5). The estimated ancestral state is an intermediate-length snout phenotype, with a terminal mouth. The phylogenetic traitogram shows high levels of uncertainty regarding this ancestral state

Table 2. ANOVA statistics of 43 gymnotiform species based on Procrustes distances among specimens using the *procD.lm* function in *geomorph* and resampling permutations. Significant differences were found not only between apteronotid species (A), but at the genus level as well (B). *Post hoc* pairwise comparisons were completed, and 33 genus pairs were significantly different (Supplemental Table 2; see Data Accessibility).

(A)

Procrustes ANOVA	df	SS	MS	Rsq	F	Z	Pr($>$F)
Log(size)	1	0.78	0.78001	0.106	101.317	6.3923	0.001**
Species	42	5.67	0.135	0.77054	17.536	15.2312	0.001**
Residuals	118	0.9084	0.0077	0.12345			
Total	161	7.3584					

(B)

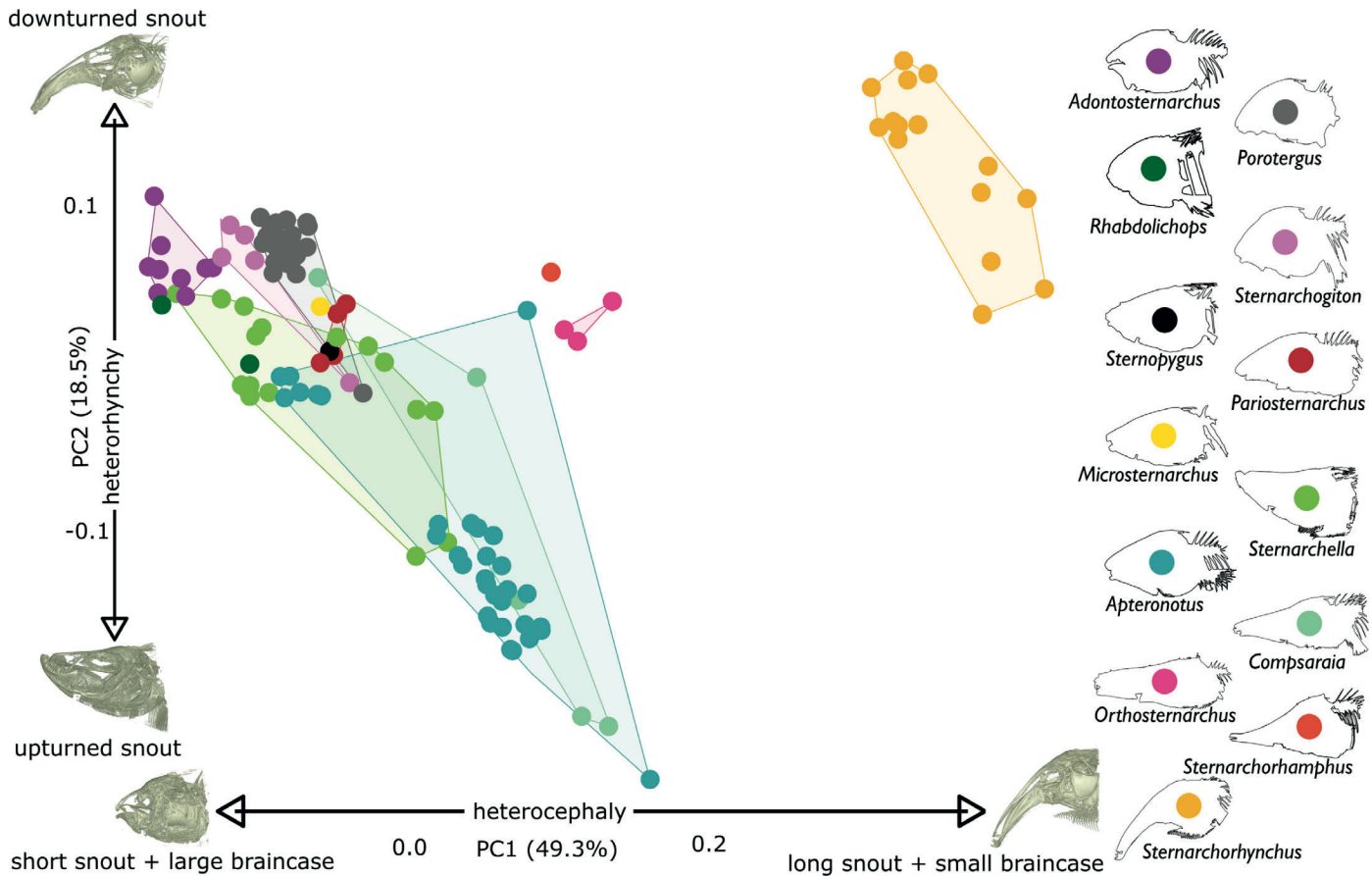| Procrustes ANOVA	df	SS	MS	Rsq	F	Z	Pr($>$F)
Log(size)	1	0.78	0.78001	0.106	55.847	5.7636	0.001**
Genus	12	4.511	0.37595	0.61309	26.918	9.6941	0.001**
Residuals	148	2.0671	0.01397	0.28091			
Total	161	7.3584					

Fig. 3. Principal component analysis with the two major axes of shape variation across 13 genera within Gymnotiformes. PC1 shows heterocephaly, the inverse relationship between the length of the snout and the size of the neurocranium. Extreme morphologies include a relatively short snout with a large braincase and a relatively long snout with a small braincase. PC2 shows heterorhynchy, the relative flexion of the snout along the dorsal-ventral axis. Extreme phenotypes include upturned snouts and downturned snouts. There are morphological similarities across genera, shown by overlap on the morphospace. There is also an inverse relationship between PC1 and PC2 values; the longer the snout the more ventral the snout flexion.

estimation, with higher certainty at recent nodes compared to the first node in the family (Fig. 6). The figure also shows significantly different evolutionary patterns of PC1 and PC2 values, with higher levels of phenotypic diversity occurring deeper on the tree in PC1 and a more recent and rapid expansion of phenotypic diversity in PC2.

DISCUSSION

There are multiple instances of convergent evolution among clades of the Apteronotidae, with brachycephalic phenotypes evolving at least three times and dolichocephalic phenotypes at least twice (Fig. 5). Genera converging on the lowest PC1 values (indicating high levels of brachycephaly), including *Adontosternarchus*, *Sternarchogiton*, and *Porotergus*, are not closely related (Figs. 4–5). There are also high levels of morphological convergence between the planktivorous clades *Adontosternarchus* (Apteronotidae) and *Rhabdolichops* (Sternopygidae; Fig. 4), indicating skull convergence among different gymnotiform families as well. The convergences of species on the phylomorphospace illustrates that relatedness alone does not fully explain phenotypic similarity (Fig. 4). Developmental, functional, and ecological effects contributed to the evolution of head shape.

Evans et al. (2017b, 2019a) showed that for electric fishes there is a difference in modularity between long and short

snouted species. Short snouted electric fishes have more modular skulls and, just as in our data, there is a single way to get a brachycephalic skull—hypoallometric growth of the face module relative to the hyperallometric growth of the braincase module. In contrast, long snouted electric fishes get long snouts with an integration of many potential modules—elongating anterior skull elements, or jaw elements, or both. Our data show the same pattern; the two independent derivations of an elongate skull come from anteroposterior growth of different skull elements. Increased importance of morphological modules in the skull is associated with increased diversity in ecology, diet, and behavior (Evans et al., 2017b, 2017c, 2019a). We propose testing whether this pattern holds true for other lineages of weakly electric fishes, such as the Mormyridae.

The principal component analysis shows a consistent inverse relationship between PC1 (heterocephaly) and PC2 (heterorhynchy) values, both within apteronotid genera and in the Apteronotidae (Fig. 3). Higher PC1 values correspond to lower PC2 values—longer snouts tend to point ventrally and shorter snouts point dorsally or align with the body's midline. Extremes of this are observed in the brachycephalic genera such as *Adontosternarchus* and *Porotergus*, and in the extremely dolichocephalic genus *Sternarchorhynchus* (Fig. 3). Not only does this pattern appear when looking across the entirety of the Apteronotidae, it is also evident in species
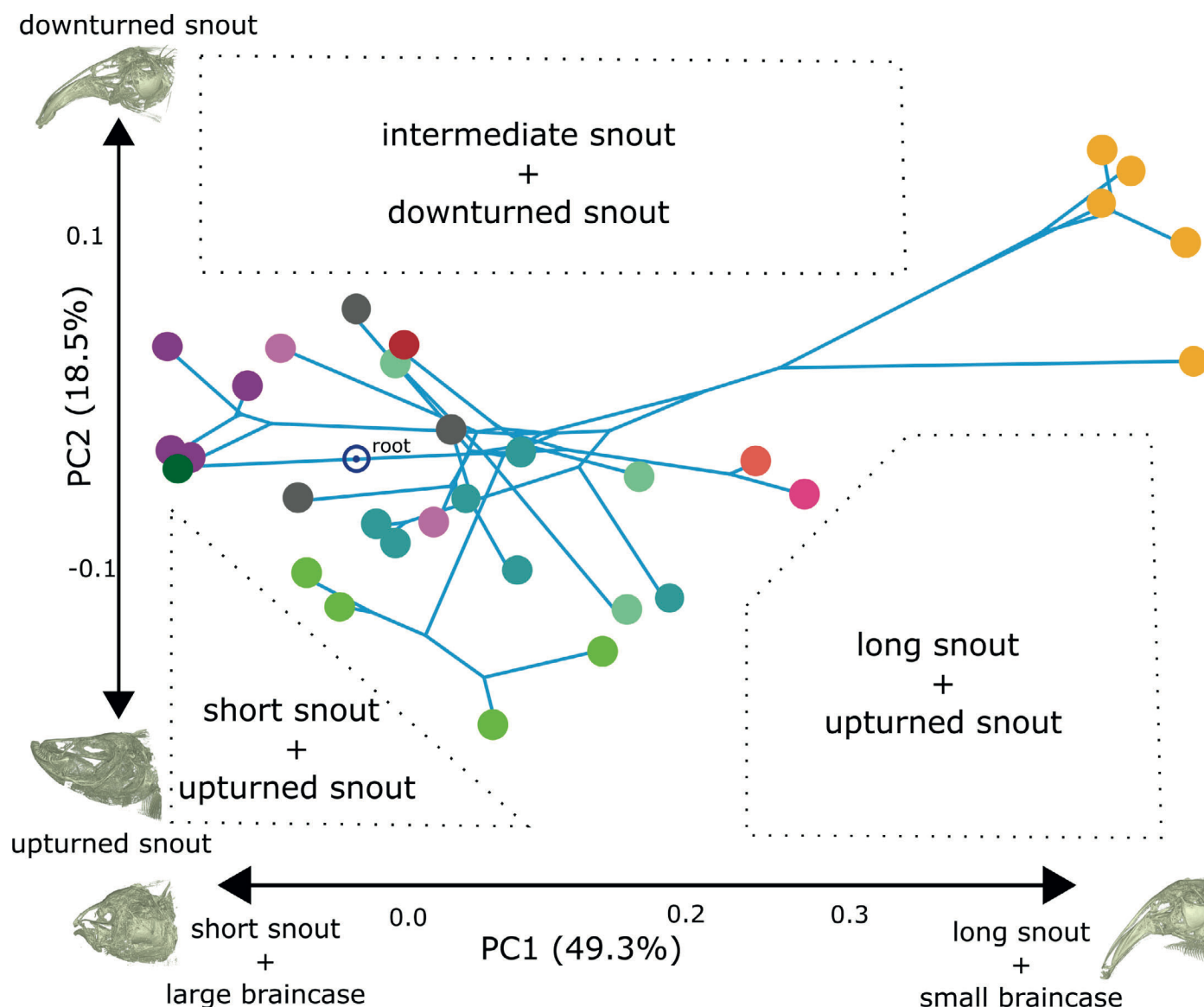
Fig. 4. Phylomorphospace based on the phylogeny of Bernt et al. (2019), colored by genus. Several phenotypes evolved multiple times across the Apteronotidae, including a relatively intermediate-length snout with an upturned snout and a relatively short snout with a terminal mouth. Some phenotypes are not seen in species in this family (visualized by dotted polygons), including relatively long, upturned snouts and relatively intermediate, downturned snouts. Species included in this analysis ($n = 31$) were subsampled from the original morphological dataset to include only those in the apteronotid phylogeny.

such as *C. samueli* and *S. nattereri*, which can be extremely sexual dimorphic (Albert et al., 2009; Cox Fernandes et al., 2010; Keeffe et al., 2019). These listed species in these lines are the ones documented to have sexual dimorphism; there is not known sexual dimorphism in the other species sampled. That said, we did not sample many specimens of extreme morphologies (e.g., the elongated male oral jaws in *C. samueli*) to prevent confounding results. While we are not able to identify specimen sex (sex can only be identified by physically examining reproductive organs within the specimen), we expect sexually dimorphic males to occupy the lower right corner of the morphospace while females and immature animals are in the upper left or center. The majority of species sampled have not been documented as showing sexual dimorphism. The pattern even shows up when looking at a genus like *Apteronotus*, which exhibits substantial interspecific diversity in phenotype (Triques,

2011; Bernt et al., 2019). There are long skulls with ventral snouts, as in *A. rostratus*, and there are short skulls with terminal mouths (*A. albifrons* and *A. cuchillejo*). This implies constraints on the occupation of these portions of the morphospace, either developmental or functional (biomechanical or trophic) in the apteronotid family.

In contrast to potential biases towards certain phenotypes, there are also phenotypic combinations of snout length (PC1) and mouth position (PC2) that are not seen in the apteronotid morphospace (Fig. 4). These "never-seen phenotypes" include brachycephalic-upturned snouts, intermediate-downturned snouts, and dolichocephalic-upturned snouts (Fig. 4). These areas of the morphospace are not exploited by this family but are seen in other teleosts such as syngnathids and characins (Sidlauskas, 2008; Roos et al., 2011; Boyle and Herrel, 2018). We point out these gaps in the phylomorphospace because they may be driven by develop-
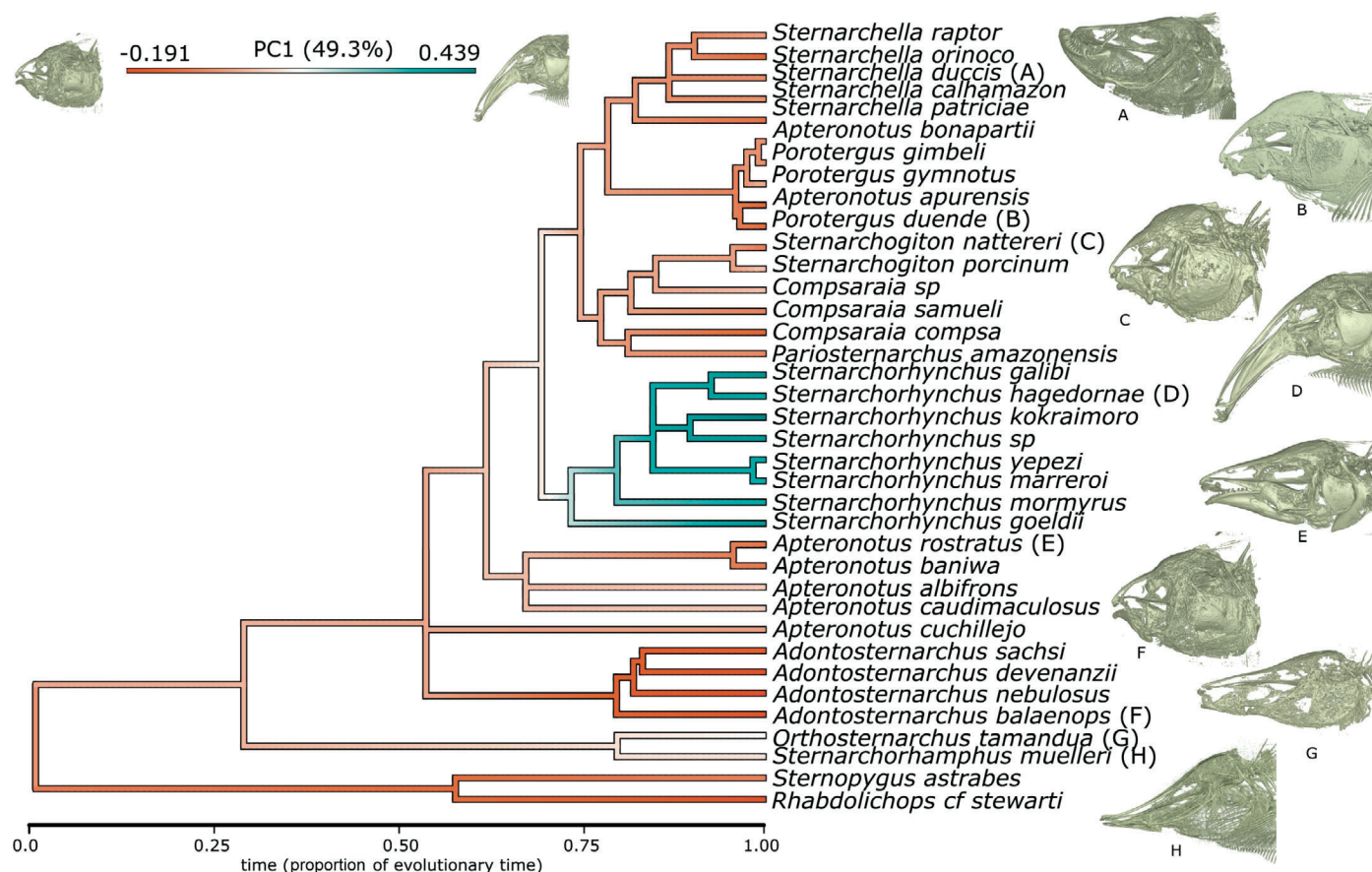
Fig. 5. An ancestral state estimation completed using the *phytools* package, showing the number of times certain phenotypes have evolved within a lineage. Extremely brachycephalic phenotypes (low PC1 values) have evolved multiple times from a hypothesized ancestor with an intermediate-length snout. Dolichocephalic phenotypes (high PC1 values) evolved at least twice within this lineage, in the *Sternarchorhynchus* and Sternarchorhamphinae clades. Species included in this analysis ($n = 36$) were subsampled from the original morphological dataset to include only those in the apteronotid phylogeny.

mental or functional constraints in apteronotids, while the same constraints are not present in other clades. These possible constraints point to areas where it would be useful to investigate the developmental mechanisms leading to these morphologies and the functional morphology of the feeding apparatus. For example, most apteronotid species are fast-water, deep-channel specialists, foraging throughout the water column. This could make a long-upturned snout less useful because it would require an erect foraging body posture that exposed the fish to the flow. The tradeoff between the body posture required for feeding in a high-flow environment and the shape of the feeding apparatus is a fertile area for investigation, and the Gymnotiformes may provide additional insights on this topic. We suggest using the morphological diversity of the slow-water specialists in the Rhamphichthyidae as a test of whether these proposed constraints vanish with release on selection for low drag due to high-speed flow.

There have been many hypotheses concerning the meaning of phenotypic constraints during organismal diversification (Waddington, 1968; Monod, 1974; Alberch, 1982; Schluter, 1996). Here we note that constraints can arise from either internal (genetic, developmental) biases in the production of variation, external pressures from the abiotic and biotic environments (functional), and, most commonly, interactions between the two (Striedter and Northcutt,

1991; Wake, 1991). In the case of apteronotid skull evolution, constraints arising from vertebrate skull development (Evans et al., 2017b, 2017c) have interacted with environmental pressures associated with foraging (Marrero and Winemiller, 1993; Winemiller and Adite, 1997) and sexual communication (Evans et al., 2019c) to produce multiple independent evolutionary transitions to elongate (dolichocephalic) and foreshortened (brachycephalic) heads, with elongate or foreshortened oral jaws, in a pattern well-described as "mosaic evolution" (Ellis, 1913; Albert, 2001; Albert and Crampton, 2009).

Maximum-likelihood methods estimate a snout in between a brachycephalic and intermediate-length rostrum (based on PC1 values) as the ancestral state for the Apteronotidae (Figs. 5–6). Considering the evolution of such diverse and extreme phenotypes in the lineage, this is unsurprising but should be considered cautiously. While maximum-likelihood methods are more statistically powerful than parsimony, they can still be biased towards averaging phenotypes to estimate an ancestral condition. Also, the *phytools* traitogram shows low confidence levels in this ancestral phenotype estimate, emphasizing the need for additional sampling and a useful fossil record (Fig. 6). This study included two groups of dolichocephalic genera, but with low levels of sampling. Sampling larger, mature individuals of *Sternarchorhamphus* and *Orthosternarchus*, and
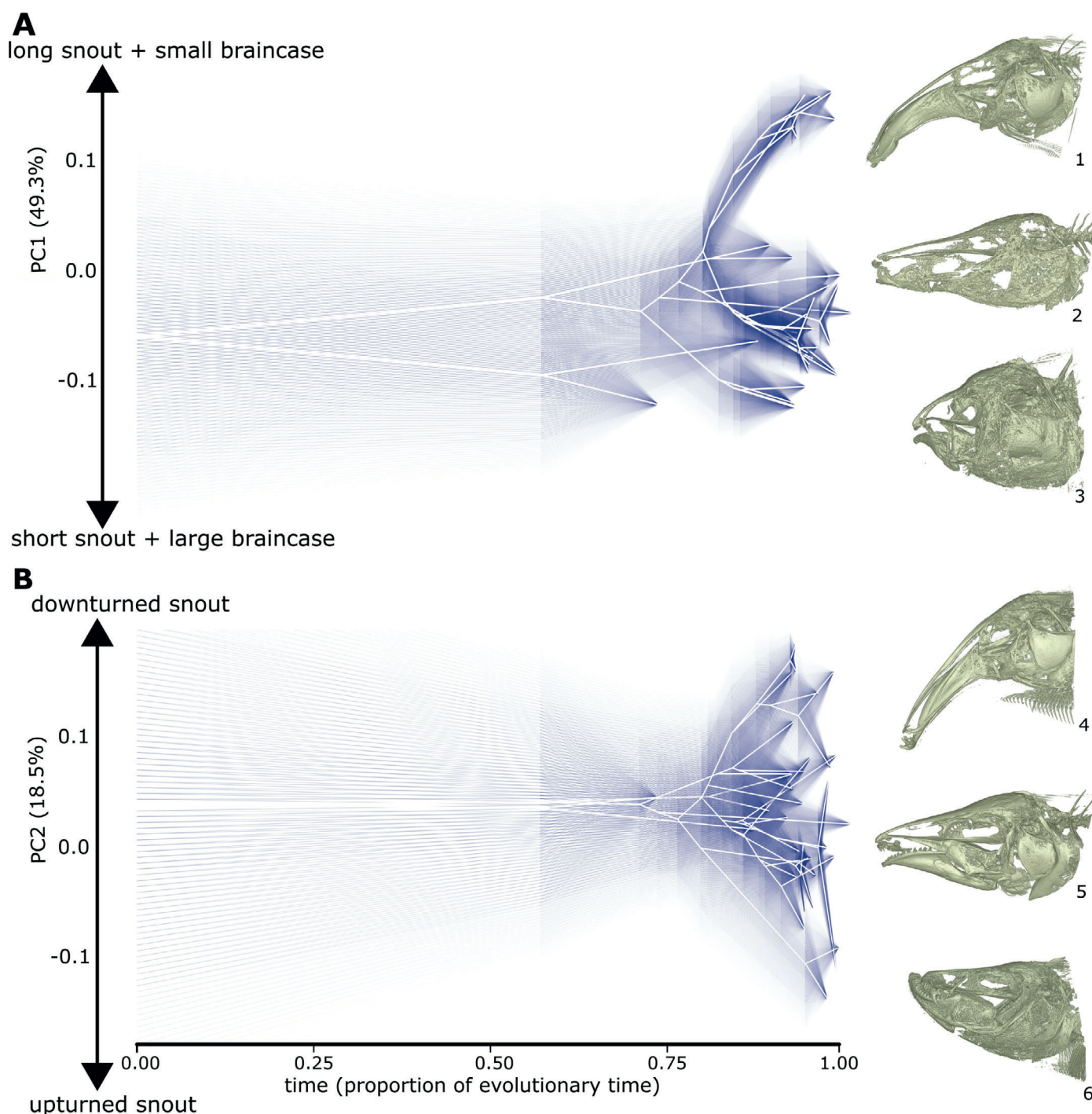
Fig. 6. A traitogram showing the uncertainty about ancestral character states using transparent probability density in *phytools*. The darker nodes indicate a higher confidence in estimated phenotype. These results show an extreme uncertainty about the clade's ancestral phenotype and high confidence in more recent ancestral character states. (A) Traitogram for PC1 values (the relative size of the snout and neurocranium); (B) traitogram for PC2 values (the relative flexion of the snout). 1, *Sternarchorhynchus montanus*; 2, *Orthosternarchus tamandua*; 3, *Adontosternarchus baleanops*; 4, *Sternarchorhynchus hagedornae*; 5, *Apteronotus rostratus*; 6, *Sternarchella duccis*.

other dolichocephalic taxa (*P. hasemani, A. rostratus*), may influence the accuracy of this ancestral state estimation.

The results of this morphological investigation of Apteronotidae are similar to those of other studies on evolution within and among gymnotiform and mormyrid electric fishes, in which diversification patterns arise from instances of both convergent and divergent evolution, as well as instances of phylogenetic stasis or conservatism (Albert,

2001; Zakon et al., 2006; Gallant et al., 2014; Evans et al., 2017b, 2017c). We propose that these patterns are driven by a combination of developmental, functional, and ecological constraints that result in a bias towards phenotypic combinations with elongated-downturned snouts and foreshortened-upturned snouts, and against phenotypic combinations with elongated-upturned snouts and foreshortened-downturned snouts. These results can be tested against evolution-

ary patterns in other species-rich groups of riverine fishes such as mormyrids, cichlids, and characiforms. Similar, extreme phenotypes have evolved across teleost fishes and levels of convergence between lineages is an interesting lens to use to study selective and developmental pressures. The breadth and quality of open-source data (including the μCT scans used in this study) and free, open source software lowers barriers to further investigations into these phenomena and groups.

DATA ACCESSIBILITY

Supplemental material is available at https://www. ichthyologyandherpetology.org/i2021016. The image stacks from the μCT scans are made publicly available using the open-source website MorphoSource. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License.

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LITERATURE CITED

Adams, D., M. Collyer, A. Kaliontzopoulou, and E. Baken. 2021. Geomorph: software for geometric morphometric analyses. R package version 3.3.2. https://cran.r-project. org/package=geomorph

Alberch, P. 1982. Developmental constraints in evolutionary processes, p. 313–332. *In*: Evolution and Development. J. T. Bonner (ed.). Springer, Berlin, Heidelberg.

Albert, J. S. 2001. Species diversity and phylogenetic systematics of American Knifefishes (Gymnotiformes, Teleostei). Miscellaneous Publications of the Museum of Zoology, University of Michigan 190:1–127.

Albert, J. S. 2003. Family Apteronotidae, p. 503–508. *In*: Checklist of the Freshwater Fishes of South and Central America. R. E. Reis, S. O. Kullander, and C. J. Ferraris, Jr. (eds.). Edipucrs, Porto Alegre.

Albert, J. S., H. L. Bart Jr., and R. E. Reis. 2011a. Species richness and cladal diversity, p. 89–104. *In*: Historical Biogeography of Neotropical Freshwater Fishes. J. S. Albert and R. E. Reis (eds.). University of California Press, Berkeley.

Albert, J. S., T. P. Carvalho, P. Petry, M. A. Holder, E. L. Maxime, J. Espino, I. Corahua, R. Quispe, B. Rengifo, H. Ortega, and R. E. Reis. 2011b. Aquatic biodiversity in the Amazon: habitat specialization and geographic isolation promote species richness. Animals 1:205–241.

Albert, J. S., and W. G. Crampton. 2005. Diversity and phylogeny of Neotropical electric fishes (Gymnotiformes), p. 360–409. *In*: Electroreception. T. H. Bullock, C. D. Hopkins, A. N. Popper, and R. Fay (eds.). Springer, New York.

Albert, J. S., and W. G. Crampton. 2006. *Pariosternarchus amazonensis*: a new genus and species of Neotropical electric fish (Gymnotiformes: Apteronotidae) from the Amazon River. Ichthyological Exploration of Freshwaters 17:267–274.

Albert, J. S., and W. G. R. Crampton. 2009. A new species of electric knifefish, genus *Compsaraia* (Gymnotiformes: Apteronotidae) from the Amazon River, with extreme sexual dimorphism in snout and jaw length. Systematics and Biodiversity 7:81–92.

Albert, J. S., and R. Reis (Eds.). 2011. Historical Biogeography of Neotropical Freshwater Fishes. University of California Press, Berkeley.

Albert, J. S., V. A. Tagliacollo, and F. Dagosta. 2020. Diversification of Neotropical freshwater fishes. Annual Review of Ecology, Evolution, and Systematics 51:27–53.

Albert, J. S., P. Val, and C. Hoorn. 2018. The changing course of the Amazon River in the Neogene: center stage for Neotropical diversification. Neotropical Ichthyology 16:e180033.

Albertson, R. C., J. T. Streelman, T. D. Kocher, and P. C. Yelick. 2005. Integration and evolution of the cichlid mandible: the molecular basis of alternate feeding strategies. Proceedings of the National Academy of Sciences of the United States of America 102:16287–16292.

Alfaro, M. E., D. I. Bolnick, and P. C. Wainwright. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. The American Naturalist 165:E140–E154.

Bennett, M. V. L. 1966. An electric organ of neural origin. Federation Proceedings 25:569.

Bernt, M. J., W. G. Crampton, A. B. Orfinger, and J. S. Albert. 2018. *Melanosternarchus amaru*, a new genus and species of electric ghost knifefish (Gymnotiformes: Apteronotidae) from the Amazon Basin. Zootaxa 4378:451–479.

Bernt, M. J., A. H. Fronk, K. M. Evans, and J. S. Albert. 2020. A redescription of deep-channel ghost knifefish, *Sternarchogiton preto* (Gymnotiformes: Apteronotidae), with assignment to a new genus. Neotropical Ichthyology 18: e190126.

Bernt, M. J., V. A. Tagliacollo, and J. S. Albert. 2019. Molecular phylogeny of the ghost knifefishes (Gymnotiformes: Apteronotidae). Molecular Phylogenetics and Evolution 135:297–307.

Boyle, K. S., and A. Herrel. 2018. Neurocranium shape variation of piranhas and pacus (Characiformes: Serrasalmidae) in association with ecology and phylogeny. Biological Journal of the Linnean Society 125:93–114.

Buser, T. J., B. L. Sidlauskas, and A. P. Summers. 2018. 2D or Not 2D? Testing the utility of 2D vs. 3D landmark data in geometric morphometrics of the sculpin subfamily Oligocottinae (Pisces; Cottoidea). The Anatomical Record 301:806–818.

Chapman, L., J. Albert, and F. Galis. 2008. Developmental plasticity, genetic differentiation, and hypoxia-induced

trade-offs in an African cichlid fish. The Open Evolution Journal 2:75–88.

Collar, D. C., P. C. Wainwright, M. E. Alfaro, L. J. Revell, and R. S. Mehta. 2014. Biting disrupts integration to spur skull evolution in eels. Nature Communications 5:5055.

Collyer, M. L., and D. C. Adams. 2018. RRPP: an R package for fitting linear models to high-dimensional data using residual randomization. Methods in Ecology and Evolution 9:1772–1779.

Collyer, M. L., and D. C. Adams. 2021. RRPP: linear model evaluation with randomized residuals in a permutation procedure. R package version 0.6.2. https://cran.r-project.org/package=RRPP

Cooper, W. J., J. Wernle, K. Mann, and R. C. Albertson. 2011. Functional and genetic integration in the skulls of Lake Malawi cichlids. Evolutionary Biology 38:316–334.

Cooper, W. J., and M. W. Westneat. 2009. Form and function of damselfish skulls: rapid and repeated evolution into a limited number of trophic niches. BMC Evolutionary Biology 9:24.

Correa, S. B., W. G. R. Crampton, and J. S. Albert. 2006. Three new species of the neotropical electric fish *Rhabdolichops* (Gymnotiformes: Sternopygidae) from the central amazon, with a new diagnosis of the genus. Copeia 2006: 27–42.

Cox Fernandes, C., G. T. Smith, J. Podos, A. Nogueira, L. Inoue, A. Akama, W. W. Ho, and J. A. Alves-Gomes. 2010. Hormonal and behavioral correlates of morphological variation in an Amazonian electric fish (*Sternarchogiton nattereri*: Apteronotidae). Hormones and Behavior 58:660–668.

Crampton, W. G., K. G. Hulen, and J. S. Albert. 2004. Redescription of *Sternopygus obtusirostris* (Gymnotiformes: Sternopygidae) from the Amazon basin, with description of osteology, ecology and electric organ discharges. Ichthyological Exploration of Freshwaters 15:121–134.

Dagosta, F., and M. de Pinna. 2019. The fishes of the Amazon: distribution and biogeographical patterns, with a comprehensive list of species. Bulletin of the American Museum of Natural History 431:1–163.

Duarte, C., A. E. Magurran, J. Zuanon, and C. P. Deus. 2019. Trophic ecology of benthic fish assemblages in a lowland river in the Brazilian Amazon. Aquatic Ecology 53: 707–718.

Ellis, M. M. 1913. The Gymnotid Eels of Tropical America. Carnegie Institute, Pittsburgh.

Evans, K. M., M. J. Bernt, M. A. Kolmann, K. L. Ford, and J. S. Albert. 2018. Why the long face? Static allometry in the sexually dimorphic phenotypes of Neotropical electric fishes. Zoological Journal of the Linnean Society 186: 633–649.

Evans, K. M., W. G. Crampton, and J. S. Albert. 2017a. Taxonomic revision of the deep channel electric fish genus *Sternarchella* (Teleostei: Gymnotiformes: Apteronotidae), with descriptions of two new species. Neotropical Ichthyology 15:e160168.

Evans, K. M., L. Y. Kim, B. A. Schubert, and J. S. Albert. 2019c. Ecomorphology of Neotropical electric fishes: an integrative approach to testing the relationships between form, function, and trophic ecology. Integrative Organismal Biology 1:obz015.

Evans, K. M., M. Vidal-García, V. A. Tagliacollo, S. J. Taylor, and D. B. Fenolio. 2019a. Bony patchwork: mosaic patterns of evolution in the skull of electric fishes (Apteronotidae: Gymnotiformes). Integrative and Comparative Biology 59:420–431.

Evans, K. M., B. Waltz, V. Tagliacollo, P. Chakrabarty, and J. S. Albert. 2017b. Why the short face? Developmental disintegration of the neurocranium drives convergent evolution in Neotropical electric fishes. Ecology and Evolution 7:1783–1801.

Evans, K. M., B. T. Waltz, V. A. Tagliacollo, B. L. Sidlauskas, and J. S. Albert. 2017c. Fluctuations in evolutionary integration allow for big brains and disparate faces. Scientific Reports 7:40431.

Evans, K. M., K. L. Williams, and M. W. Westneat. 2019b. Do coral reefs promote morphological diversification? Exploration of habitat effects on labrid pharyngeal jaw evolution in the era of big data. Integrative and Comparative Biology 59:696–704.

Fedorov, A., R. Beichel, J. Kalpathy-Cramer, J. Finet, J. C. Fillion-Robin, S. Pujol, C. Bauer, D. Jennings, F. M. Fennessy, M. Sonka, J. Buatti, S. R. Aylward, J. Y. Miller, K. Pieper, and R. Kikinis. 2012. 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magnetic Resonance Imaging 30:1323–1341.

Felice, R. N., and A. Goswami. 2018. Developmental origins of mosaic evolution in the avian cranium. Proceedings of the National Academy of Sciences of the United States of America 115:555–560.

Felsenstein, J. 1985. Phylogenies and the comparative method. The American Naturalist 125:1–15.

Gallant, J. R., L. L. Traeger, J. D. Volkening, H. Moffett, P. Chen, C. D. Novina, G. N. Phillips Jr., R. Anand, G. B. Wells, M. Pinch, R. Güth, G. A. Unguez, J. S. Albert, H. H. Zakon . . . M. R. Sussman. 2014. Genomic basis for the convergent evolution of electric organs. Science 344:1522–1525.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 52:285–339.

Hallgrímsson, B., H. Jamniczky, N. M. Young, C. Rolian, T. E. Parsons, J. C. Boughner, and R. S. Marcucio. 2009. Deciphering the palimpsest: studying the relationship between morphological integration and phenotypic co-variation. Evolutionary Biology 36:355–376.

Hallgrímsson, B., D. E. Lieberman, W. Liu, A. F. Ford-Hutchinson, and F. R. Jirik. 2007. Epigenetic interactions and the structure of phenotypic variation in the cranium. Evolution & Development 9:76–91.

Hulen, K. G., W. G. Crampton, and J. S. Albert. 2005. Phylogenetic systematics and historical biogeography of the Neotropical electric fish *Sternopygus* (Teleostei: Gymnotiformes). Systematics and Biodiversity 3:407–432.

Ivanyisky, S. J., and J. S. Albert. 2014. Systematics and biogeography of Sternarchellini (Gymnotiformes: Apteronotidae): diversification of electric fishes in large Amazonian rivers. Neotropical Ichthyology 12:565–584.

Kane, E. A., and T. E. Higham. 2015. Complex systems are more than the sum of their parts: using integration to understand performance, biomechanics, and diversity. Integrative and Comparative Biology 55:146–165.

Keeffe, R., E. J. Hilton, M. J. F. Thomé de Souza, and C. Cox Fernandes. 2019. Cranial morphology and osteology of the sexually dimorphic electric fish, *Compsaraia samueli* Albert & Crampton (Apteronotidae, Gymnotiformes), with

comparisons to *C. compsa* (Mago-Leccia). Zootaxa 4555: 101–112.

Klingenberg, C. P. 2008. Morphological integration and developmental modularity. Annual Review of Ecology, Evolution, and Systematics 39:115–132.

Klingenberg, C. P. 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11:353–357.

Lauder, G. V. 1981. Form and function: structural analysis in evolutionary morphology. Paleobiology 7:430–442.

Losos, J. B. 2011. Convergence, adaptation, and constraint. Evolution 65:1827–1840.

Lundberg, J. G., C. Cox Fernandes, J. S. Albert, and M. Garcia. 1996. *Magosternarchus*, a new genus with two new species of electric fishes (Gymnotiformes: Apteronotidae) from the Amazon River Basin, South America. Copeia 1996:657–670.

Lundberg, J. G., W. M. Lewis, J. F. Saunders, and F. Mago-Leccia. 1987. A major food web component in the Orinoco River channel: evidence from planktivorous electric fishes. Science 237:81–83.

Marrero, C., and K. O. Winemiller. 1993. Tube-snouted gymnotiform and mormyriform fishes: convergence of a specialized foraging mode in teleosts. Environmental Biology of Fishes 38:299–309.

Monod, J. 1974. On chance and necessity, p. 357–375. *In*: Studies in the Philosophy of Biology. F. J. Ayala and T. Dobzhansky (eds.). Macmillan Education, London.

Ord, T. J., and T. C. Summers. 2015. Repeated evolution and the impact of evolutionary history on adaptation. BMC Evolutionary Biology 15:137.

Revell, L. J. 2012. *phytools*: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3:217–223.

Roos, G., S. Van Wassenbergh, P. Aerts, A. Herrel, and D. Adriaens. 2011. Effects of snout dimensions on the hydrodynamics of suction feeding in juvenile and adult seahorses. Journal of Theoretical Biology 269:307–317.

RStudio Team. 2021. RStudio: integrated development for R. RStudio, PBC, Boston, Massachusetts. https://www.rstudio.com

Russell, E. S. 1916. Form and Function: A Contribution to the History of Animal Morphology. J. Murray, London.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Sidlauskas, B. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. Evolution 62:3135–3156.

Stayton, C. T. 2015. What does convergent evolution mean? The interpretation of convergence and its implications in the search for limits to evolution. Interface Focus 5: 20150039.

Striedter, G. F., and R. G. Northcutt. 1991. Biological hierarchies and the concept of homology. Brain, Behavior and Evolution 38:177–189.

Triques, M. L. 2011. *Apteronotus acidops*, new species of long snouted electric fish (Teleostei: Gymnotiformes: Apteronotidae) from the upper rio Paraná basin in Brazil, with a key to the apteronotid species from the area. Vertebrate Zoology 61:299–306.

van der Sleen, P., and J. S. Albert (Eds.). 2017. Field Guide to the Fishes of the Amazon, Orinoco, and Guianas. Vol. 115. Princeton University Press, Princeton, New Jersey.

Waddington, C. H. 1968. Towards a theoretical biology. Nature 218:525–527.

Wainwright, P. C., M. E. Alfaro, D. I. Bolnick, and C. D. Hulsey. 2005. Many-to-one mapping of form to function: a general principle in organismal design? Integrative and Comparative Biology 45:256–262.

Wake, D. B. 1991. Homoplasy: the result of natural selection, or evidence of design limitations? The American Naturalist 138:543–567.

Westneat, M. W. 2005. Skull biomechanics and suction feeding in fishes. Fish Physiology 23:29–75.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Winemiller, K. O., and A. Adite. 1997. Convergent evolution of weakly electric fishes from floodplain habitats in Africa and South America. Environmental Biology of Fishes 49:175–186.

Young, N. M., G. P. Wagner, and B. Hallgrímsson. 2010. Development and the evolvability of human limbs. Proceedings of the National Academy of Sciences of the United States of America 107:3400–3405.

Zakon, H. H., Y. Lu, D. J. Zwickl, and D. M. Hillis. 2006. Sodium channel genes and the evolution of diversity in communication signals of electric fishes: convergent molecular evolution. Proceedings of the National Academy of Sciences of the United States of America 103:3675–3680.