

Exceptionally Steep Brain-Body Evolutionary Allometry Underlies the Unique Encephalization of Osteoglossiformes

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Evolutionary constraints · Static allometry · Mormyrid · Measurement error model · Evolutionary allometry

Abstract

Brain-body static allometry, which is the relationship between brain size and body size within species, is thought to reflect developmental and genetic constraints. Existing evidence suggests that the evolution of large brain size without accompanying changes in body size (that is, encephalization) may occur when this constraint is relaxed. Teleost fish species are generally characterized by having close-fitting brain-body static allometries, leading to strong allometric constraints and small relative brain sizes. However, one order of teleost, Osteoglossiformes, underwent extreme encephalization, and its mechanistic bases are unknown. Here, I used a dataset and phylogeny encompassing 859 teleost species to demonstrate that the encephalization of Osteoglossiformes occurred through an increase in the slope of evolutionary (among-species) brain-body allometry. The slope is virtually isometric (1.03 ± 0.09 SE), making it one of the steepest evolutionary brain-body allometric slopes reported to date, and it deviates significantly from the evolutionary brain-body allometric slopes of other clades of tele-

ost. Examination of the relationship between static allometric parameters (intercepts and slopes) and evolutionary allometry revealed that the dramatic steepening of the evolutionary allometric slope in Osteoglossiformes was a combined result of evolution in the slopes and intercepts of static allometry. These results suggest that the evolution of static allometry, which likely has been driven by evolutionary changes in the rate and timing of brain development, has facilitated the unique encephalization of Osteoglossiformes.

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Introduction

The close association between brain and body size is one of the most persistent features of the vertebrate body plan [Jerison, 1973; Tsuboi et al., 2018b]. The scaling relationship between brain size and body size is commonly described by power functions in the form of $Y = aX^b$, where Y is brain size, X is body size, and b is the scaling exponent. This relationship is usually expressed in the logarithmic scale, yielding the standard linear allometric equation: $\log(\text{brain size}) = \log(a) + b \log(\text{body size})$. The theoretical context of this equation is that if brain and body sizes are under common growth regulation, then it

approximates a linear relationship between the two traits [Huxley, 1932; Voje et al., 2014]. The slope “ b ” of this relationship at the within-species level, either across individuals of different developmental stages (ontogenetic allometry) or across adult individuals (static allometry), is of substantial interest to evolutionary biologists because it is considered to characterize the strength and direction of genetic and developmental constraints [Lande, 1979; Riska and Atchley, 1985; Grabowski, 2016; Tsuboi et al., 2018b]. Brain-body allometry across species, which is referred to as evolutionary allometry, is an emergent property of the slope and the intercept of ontogenetic and static allometry and the covariance of these parameters with body size [Pélabon et al., 2013; Voje et al., 2014]. The universal and close fit of brain-body allometry to observations within and among species has triggered rigorous attempts to understand the causes of brain-body allometry and its consequences for brain size evolution [Dubois, 1897; Lapique, 1907; Jerison, 1973; Gould, 1975; Martin, 1981; Striedter, 2005; Boddy et al., 2012; Montgomery et al., 2016; Tsuboi et al., 2018b; Mitov et al., 2019; Ksepka et al., 2020; Smaers et al., 2021].

Currently, most phylogenetic comparative studies of brain size focus on explaining the fraction of variation in brain size that is uncorrelated with body size, which is commonly referred to as relative brain size [Jerison, 1973; Gonzalez-Voyer et al., 2009; Liao et al., 2016; DeCasien et al., 2017; Fox et al., 2017; Fristoe et al., 2017; Tsuboi et al., 2017]. These studies regress log-transformed data on brain size against log-transformed data on body size and a set of explanatory variables in a multiple regression, and the partial correlation coefficients of this model are used to test for hypotheses about adaptation and coevolution. The core premise of this framework is that there is variation in relative brain size within a population. However, this assumption may not always be met. In many vertebrate taxa, brain size and body size are closely correlated within populations [Dubois, 1897; Lapique, 1907; Tsuboi et al., 2016, 2018b; Sukhum et al., 2019], implying that variation in relative brain size (e.g., conditional variance [Hansen and Houle, 2008; Voje et al., 2014]) may often be limited. Birds and mammals are an exception because variational dependence of brain size and body size appears to be decoupled in these taxa, allowing for brain size to respond to selection independently of body size [Boddy et al., 2012; Holekamp et al., 2013; Tsuboi et al., 2018b]. This helps explain the paradox of encephalization under allometric constraints in these lineages, but birds and mammals are not the only lineages with large relative brain sizes.

The fish order Osteoglossiformes, which is also known as boney tongues, is a group of freshwater fish species that inhabit lakes and rivers in Africa, Australia, South America, and southern Asia. One family of Osteoglossiformes, Mormyridae, has long been famous for its extremely large relative brain sizes [Nilsson, 1996; Chapman and Hulen, 2001; Kaufman et al., 2003], and it has received considerable attention from neurobiologists [Striedter, 2005]. For instance, in an emblematic species, *Gnathonemus petersii*, the brain mass constitutes about 3% of the total body mass, which is extremely large for teleost fishes and even higher than the corresponding value for humans, which is about 2% [Nilsson, 1996]. More recently, a growing number of reports on brain and body size in other members of Osteoglossiformes revealed that a large relative brain size is not specific to Mormyridae, but it is a characteristic shared by the whole order [Bauchot et al., 1995; Chapman and Hulen, 2001; Sukhum et al., 2016]. To date, studies on the evolution of brain size in Osteoglossiformes have focused exclusively on the ecological causes and physiological consequences of encephalization [Bauchot et al., 1995; Nilsson, 1996; Chapman and Hulen, 2001; Kaufman et al., 2003; Carlson et al., 2011; Sukhum et al., 2016, 2019], and the mechanism Osteoglossiformes have used to reduce allometric constraints is unknown. One hypothesis is that Osteoglossiformes evolved their large brains by reducing the association between brain and body sizes [Tsuboi et al., 2018b]. However, currently available data suggest that this is unlikely. Chapman and Hulen [2001] reported the brain-body static allometric slopes of four species of mormyrids that ranged between 0.36 and 0.63 with r^2 values of 69% to 99%. Another three mormyrids examined by Sukhum et al. [2019] revealed static slopes between 0.43 and 0.63 with r^2 values of 85%–98%. These ranges of static allometric slopes and high r^2 values are typical in teleost fishes [Tsuboi et al., 2016, 2018b], suggesting that the evolutionary path for encephalization in Osteoglossiformes might be fundamentally different from that in birds and mammals.

There are two non-mutually exclusive scenarios for encephalization under strong allometric constraints. First, the intercept of the static allometry could evolve even if brain and body sizes remain closely correlated. An artificial selection experiment in the guppy, *Poecilia reticulata*, demonstrated that the static brain-body allometric intercept has additive genetic variance and can evolve in response to artificial selection on relative brain size [Kotrschal et al., 2013]. Additionally, the evolution of relative brain size in Lake Tanganyika cichlids is predominantly driven by evolutionary changes in the static allometric intercept [Tsuboi et al., 2016]. Alternatively, rela-

tive brain size can evolve through coevolution of the static allometric slope and body size [Pélabon et al., 2013]. A positive association between among-species divergence in relative brain size and the variance of the static allometric slope in boney fish corroborates this hypothesis [Tsuboi et al., 2018b]. In this study, I first document the encephalization of Osteoglossiformes using an updated dataset of brain and body sizes in teleost fishes. Then, I evaluate static brain-body allometry from 103 species, including 17 species of Osteoglossiformes, to determine the mechanistic basis of their unique encephalization.

Materials and Methods

Data and Phylogeny

I combined a recently curated dataset of vertebrate brain and body masses [Tsuboi et al., 2018a] with brain and body mass data for Osteoglossiformes reported in Sukhum et al. [2016]. Bauchot et al. [1995] was consulted but not included in this study because they only reported juvenile individuals. My primary dataset consisted of brain mass (g) and body mass (g) of 3,632 adult individuals from 859 species, including 21 species of Osteoglossiformes and 838 species of other teleost fishes. A phylogeny including all of the 859 species was obtained from Rabosky et al. [2018] and used to conduct phylogenetic comparative analyses. Additionally, despite their unknown phylogenetic positions, 19 species of Osteoglossiformes (online suppl. Data S1; for all online suppl. material, see www.karger.com/doi/10.1159/000519067) were included in a subset of my analyses to increase the sample size of this clade. Brain-body static allometries were estimated by fitting ordinary least-squares regressions of the natural log of brain mass against the natural log of body mass for all species with 10 or more observations available. The static allometric intercept of the untransformed data is often considered a parameter of little biological significance due to the inherent negative association between slopes and intercepts [White and Gould, 1965], and mean-centering is recommended before estimating the static allometric intercepts to dissociate the relationship [Voje et al., 2014]. However, the static allometric intercepts and slopes were weakly correlated for the data compiled in this study ($r^2 = 2.0\%$, online suppl. Fig. S1). Additionally, among animals that exhibit indeterminate growth, the static allometric slope is typically constant over a wide size range [Tsuboi et al., 2018b]. Empirical data of fish ontogenetic brain-body allometry suggest that the initially near-isometric slope shifts to species-specific static allometry at 0.03–3.00 g of body mass [Bauchot et al., 1979; Oikawa and Itazawa, 1984; Oikawa et al., 1992]. This suggests that the static allometric intercept, which is the predicted brain mass at 1 g of body mass [Halley, 2016], roughly corresponds to the brain mass at which static allometry starts. Assuming that this ontogenetic model generally applies to teleosts, I used the static allometric intercept in the natural logarithm of uncentered data in my analyses.

Comparative Framework

I modeled trait evolution on a phylogenetic tree based on an Ornstein-Uhlenbeck (OU) model. I first evaluated the phylogenetic signal of each trait, including log brain mass, log body mass,

brain-body static allometric slope, and intercept, by estimating the phylogenetic half-life ($t_{1/2}$) of an OU process [Hansen, 1997]. $t_{1/2}$ describes the tendency of traits to evolve toward an adaptive optimum (θ), which is the phylogenetic weighted mean when OU is fitted to a single trait. The unit of $t_{1/2}$ is the tree length (millions of years), allowing an intuitive interpretation of the phylogenetic signal. Additionally, the stationary variance (v_{st}) characterizes the variance of a fitted trait when they evolve under a constant adaptive regime for a long time.

Evolutionary correlations between variables were assessed using a generalized least-squares regression model in which the residual variance structure was modeled based on an OU process [Hansen et al., 2008]. As brain mass, body mass, static allometric slope, and static allometric intercept are hypothesized to be connected through development, I used a direct-effect model that considers that the evolution of predictors has an immediate effect on the response [Grabowski et al., 2016]. This model has the following form:

$$dy = -\alpha(y - \theta) dt + bdx + \sigma dW,$$

where dy is the change in trait y over an infinitesimal time step dt , α is the strength of pull toward θ , which characterizes the model intercept, σdW describes a white noise with independent, normally distributed random changes with a mean of zero and variance σ^2 , and the parameter b describes the scales of change in log brain mass with the change in predictor variables, dx , which follows an independent white-noise process. This model converges to the phylogenetic generalized least squares with residual structure modeled as Brownian motion (BM) as the α parameter asymptotes toward zero. Based on this model, I estimated the evolutionary brain-body allometry of eight orders represented by 20 or more species: Anguiformes ($n = 20$), Beryciformes ($n = 21$), Gadiformes ($n = 35$), Osteoglossiformes ($n = 21$), Perciformes ($n = 576$), Scorpaeniformes ($n = 34$), Syngnathiformes ($n = 22$), and Tetraodontiformes ($n = 35$), as well as all species ($n = 859$) except for one of the eight examined orders. In the dataset of Beryciformes, *Anoplogaster cornuta* was a clear outlier (online suppl. Fig. S2). Previous studies of brain size in marine fishes have shown that the transition from mesopelagic to bathypelagic habitat is associated with a significant decrease in brain size [Fine et al., 1987; Kotrschal et al., 1998; Iglesias et al., 2015], which likely reflects the lower levels of ambient light and energy sources in bathypelagic zones than mesopelagic zones [Kotrschal et al., 1998]. In the presented data, *A. cornuta* was the only bathypelagic species within Beryciformes and had a small relative brain size. Considering these observations as indications that the brain size of *A. cornuta* is evolving under a selective regime that is distinct from other members of Beryciformes in the data, I removed *A. cornuta* from the analysis of Beryciformes.

Using a subset of data of estimated static allometric slopes (hereafter referred to as static data, $n = 87$ species), I fitted four models. The first model was an allometry-only model in which the log of brain mass was regressed against the log of body mass. The log of body mass was included as a covariate in all other models. In the second model, I regressed the log of brain mass against the static allometric slope. In the third model, the log of brain mass was regressed against the static allometric intercept, and the fourth model regressed the log of brain mass against the static allometric slope and intercept. Model fit was evaluated based on the sample

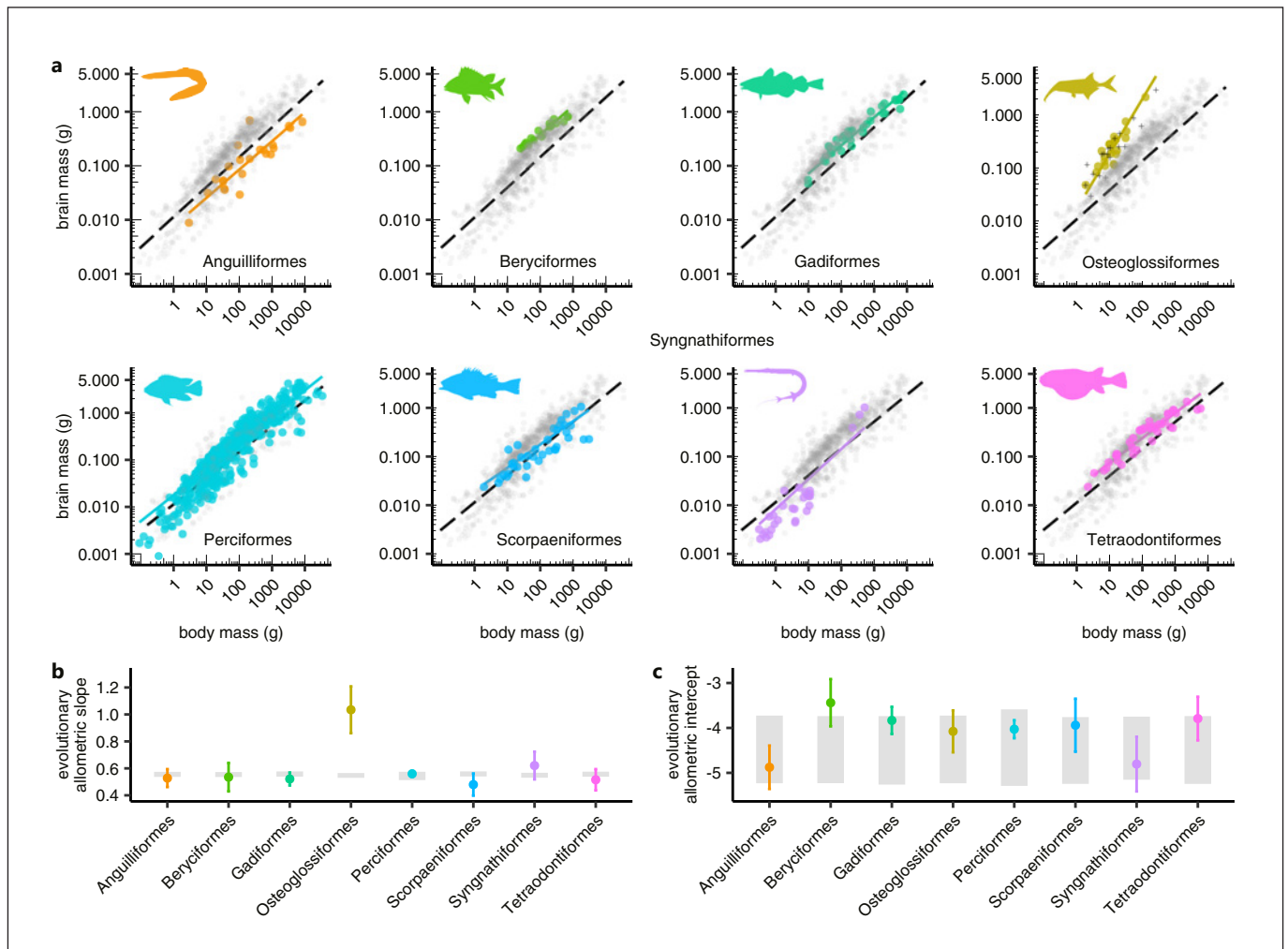


Fig. 1. Comparison of evolutionary allometry among teleost fish species. **a** Relationships between brain mass (g) and body mass (g) are presented. Eight orders with sufficient sample sizes are highlighted in each panel. Colored circles represent mean values of species belonging to one of the eight orders and gray circles indicate the rest. Colored solid lines show evolutionary allometry of each order, and black dashed lines show evolutionary allometry of all taxa except for the compared order (estimates are shown in online suppl. Table S1). Crosses (+) represent Osteoglossiformes whose data were available but not included in SLOUCH analyses due to the lack of phylogenetic information ($n = 21$ species are included

in the analyses and $n = 40$ are plotted). Note that \log_{10} scales are only used for graphical representation, while all analyses were conducted using natural logarithms. Silhouettes were obtained from phylopic.org. See Supplementary Material for individual image credits. **b** Comparison of evolutionary allometric slopes across eight orders of teleost. **c** Comparison of evolutionary allometric intercepts across eight orders of teleost. **b, c** Error bars indicate ± 2 standard errors, and thick gray bars indicate ranges of estimates ± 2 standard errors for evolutionary allometries including all taxa except for the focal order.

size-corrected Akaike Information Criterion (AICc) and the model r^2 . A full model with a nested model without a focal predictor was compared to assess the effect of each predictor on relative brain mass. I repeated the analyses using the subset of data for Osteoglossiformes. Due to the limited sample size in this clade, I included all species with at least two observations ($n = 17$). This caused some estimates of the static allometric slopes to be unreliable. The reliability of static allometric slopes and intercepts was accounted for by including measurement variances of the estimates in the comparative analyses to allow robust statistical infer-

ence. I used SLOUCH version 2.1.2 [Kopperud, 2017] to perform phylogenetic comparative analyses.

Modeling Measurement Variance

Currently, most comparative studies are based on species means. When species means are imprecise or the total variance is low relative to the imprecision of species means, the imprecision in species means must be modeled to obtain unbiased estimates of regression parameters [Riska, 1991; Ives et al., 2007; Hansen and Bartoszek, 2012]. Measurement imprecisions can be evaluated in

Table 1. Evolutionary brain-body allometry estimated by direct-effect SLOUCH model in 8 orders of Teleost

Order	$t_{1/2}$	v_{st}	Intercept \pm SE	Slope \pm SE	r^2	p value (intercept)	p value (slope)
Anguilliformes	–*	0*	–4.876 \pm 0.241 (–4.694 \pm 0.253)	0.528 \pm 0.033 (0.502 \pm 0.035)	90.0%	0.53	0.51
Beryciformes [†]	–*	0*	–3.439 \pm 0.263 (–2.838 \pm 0.339)	0.535 \pm 0.053 (0.409 \pm 0.069)	92.3%	0.10	0.77
Gadiformes	∞ [0.000– ∞]	–	–3.832 \pm 0.151 (–3.746 \pm 0.155)	0.521 \pm 0.024 (0.505 \pm 0.025)	94.9%	0.21	0.28
Osteoglossiformes	0.128 [0.000– ∞]	0.072 [0.022– ∞]	–4.077 \pm 0.233 (–3.610 \pm 0.277)	1.034 \pm 0.087 (0.819 \pm 0.110)	80.3%	0.46	<0.001
Perciformes	∞ [7.020– ∞]	–	–4.029 \pm 0.100 (–0.390 \pm 0.102)	0.560 \pm 0.011 (0.527 \pm 0.012)	86.4%	0.45	0.55
Scorpaeniformes	∞ [0.313– ∞]	–	–3.940 \pm 0.295 (–3.708 \pm 0.309)	0.480 \pm 0.041 (0.432 \pm 0.046)	78.8%	0.41	0.11
Syngnathiformes	∞ [0.000– ∞]	–	–4.805 \pm 0.304 (–4.803 \pm 0.304)	0.621 \pm 0.051 (0.607 \pm 0.053)	85.2%	0.60	0.23
Tetraodontiformes	∞ [0.313– ∞]	–	–3.792 \pm 0.243 (–3.438 \pm 0.270)	0.516 \pm 0.039 (0.450 \pm 0.045)	88.5%	0.26	0.40

Phylogenetic half-life ($t_{1/2}$, unit: total tree height) and stationary variance (v_{st}) are shown with 2 maximum-likelihood support range from the maximum likelihood estimate in squared brackets. Estimates within parentheses indicate parameters estimated without correcting for measurement errors. Asterisk (*) indicates that the model had no residual variance ($v_{st} = 0$) after measurement errors are accounted for, in which case $t_{1/2}$ is not estimable. Dagger (†) indicates that *Anoplogaster cornuta* was excluded from the analysis. The p values represent tests against the null hypothesis that presented estimates are the same as estimates of overall evolutionary allometry for all teleost excluding the order to be compared (estimates are shown in online suppl. Table S1).

the form of measurement variance (σ^2_m), which is calculated as the squared standard error of the species means. However, this approach cannot be used when species are represented by a single observation, and it provides an unreliable estimate of the true σ^2_m when the sample size is low. Therefore, I used a method developed by Grabowski et al. [2016], which models the sample variance (s^2) as the weighted sum of the species-specific s^2 (s^2_i) and the global s^2 (s^2_{global}). The weighting is determined by the fraction of variation in the s^2 due to true among-species difference in s^2_i over the imprecision of s^2 itself [see Appendix A of Grabowski et al., 2016]. I iteratively fitted SLOUCH with s^2_i as a response variable in an intercept-only model in which $t_{1/2}$ was set to zero (i.e., phylogeny is not considered) to estimate s^2_{global} and variance in s^2_i . In the first iteration, the measurement variance of s^2_i was modeled as $s^4_i/(n_i - 1)$, where n_i was the sample size of the species. In subsequent iterations, the measurement variance of s^2_i was modeled as the square of the estimated mean of s^2_i in the previous iteration divided by $(n_i - 1)$. This procedure was repeated until estimates converged, and the converged v_{st} and mean were obtained as the estimate of variance in s^2 and estimate of s^2_{global} , respectively. I applied this procedure for the log of body mass, log of brain mass, static allometric slopes, and static allometric intercepts, and I used the formula provided in Grabowski et al. [2016] to model σ^2_m in these traits for each species. The estimated measurement variances were included in all statistical analyses performed using R version 3.6.0 [R Core Team, 2019].

Results

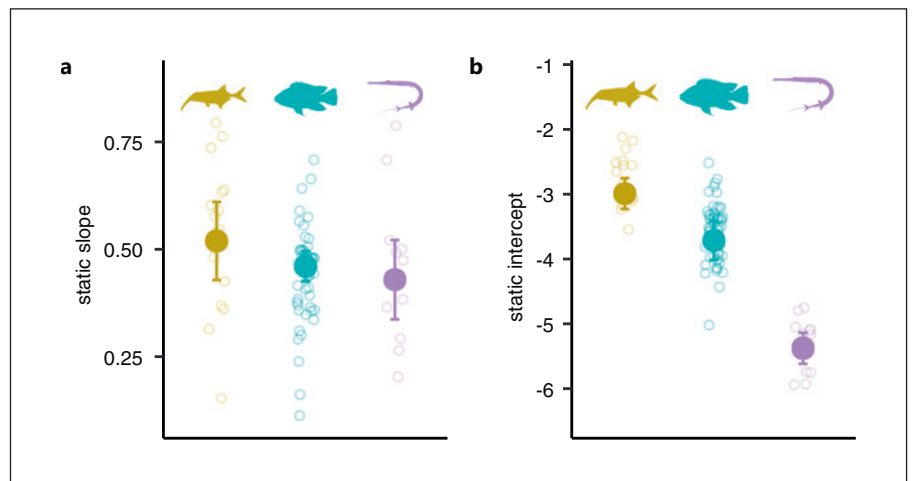
An updated dataset of teleost brain mass and body mass showed an evident deviation of Osteoglossiformes from general teleost brain-body evolutionary allometry (Fig. 1). Visual inspection of evolutionary allometry in eight orders of teleost indicated that evolutionary brain-body allometry in teleost orders is remarkably conserved in terms of both slope and intercept, except for Osteoglossiformes, which clearly showed a steeper evolutionary allometry than the other seven teleost orders (Fig. 1a). A closer examination of the evolutionary allometric slope confirmed that only Osteoglossiformes deviate significantly from the evolutionary allometric slope of all but the focal order (Fig. 1b, $p < 0.001$; Table 1; online suppl. Table S1) with the allometric relationship of $\log(\text{brain mass}) = 1.03 \times \log(\text{body mass}) - 4.08$. In contrast, I found no evidence that the intercepts of evolutionary allometry in any of the eight examined orders deviated from the intercepts estimated for the rest of the data (Fig. 1c; Table 1; online suppl. Table S1). These results provide strong

Table 2. Phylogenetic half-life ($t_{1/2}$), stationary variance (v_{st}), and optimum (θ) of an OU process fitted to four examined traits in the entire static data and in three subsets of the data

Trait	$t_{1/2}$	v_{st}	$\theta \pm SE$
<i>Full static data, n = 87</i>			
Log (brain mass)	0.552 [0.279–5.046]	2.467 [1.546–17.638]	-2.014±0.503
Log (body mass)	0.296 [0.164–0.800]	4.744 [3.212–9.929]	3.791±0.548
Static slope	0.120 [0.007–0.566]	0.005 [0.003–0.010]	0.457±0.014
Static intercept	∞ [0.657– ∞]	–	-3.641±0.330
<i>Perciformes, n = 57</i>			
Log (brain mass)	∞ [0.637– ∞]	–	-1.864±0.404
Log (body mass)	∞ [0.345– ∞]	–	3.989±0.571
Static slope	0.200 [0.025– ∞]	0.005 [0.002– ∞]	0.461±0.017
Static intercept	1.621 [0.253– ∞]	0.420 [0.130– ∞]	-3.713±0.152
<i>Syngnathiformes, n = 12</i>			
Log (brain mass)	0 [0–0.094]	0.533 [0.259–1.390]	-4.772±0.212
Log (body mass)	0 [0–0.081]	1.500 [0.727–3.863]	1.236±0.356
Static slope	0.098 [0.051– ∞]	0.012 [0.002– ∞]	0.430±0.046
Static intercept	0.018 [0.051– ∞]	0.103 [0.030– ∞]	-5.376±0.118
<i>Osteoglossiformes, n = 17</i>			
Log (brain mass)	0.095 [0– ∞]	0.768 [0.424– ∞]	-1.579±0.272
Log (body mass)	0.100 [0– ∞]	0.734 [0.363– ∞]	2.440±0.233
Static slope	0.010 [0.051– ∞]	0.014 [0.004– ∞]	0.520±0.045
Static intercept	–	0*	-2.991±0.119

Phylogenetic half-life ($t_{1/2}$, unit: total tree height) and stationary variance (v_{st}) are shown with 2 maximum-likelihood support range from the maximum likelihood estimate in squared brackets. Asterisk (*) indicates that the model had no residual variance ($v_{st} = 0$) after measurement errors are accounted for, in which case $t_{1/2}$ is not estimable.

Fig. 2. Comparison of static allometric parameters. Osteoglossiformes (yellow), Perciformes (blue), and Syngnathiformes (purple) are compared. Solid circles represent the central state of an Ornstein-Uhlenbeck process (θ) fitted to static slopes and intercepts and shown with ± 2 standard errors (estimates are shown in Table 2). Open circles are observations.



evidence that the exceptional encephalization of Osteoglossiformes predominantly occurred through evolutionary change in the slope of evolutionary allometry. Regarding these findings as validation for a single scaling

relationship across non-osteoglossomorph teleosts, I evaluated the brain mass of Osteoglossiformes relative to expected values based on the evolutionary brain-body allometry of $\log(\text{brain mass}) = 0.55 \times \log(\text{body mass}) -$

Table 3. Phylogenetic associations among brain mass, body mass, static slope, and static intercept

Response	Predictor	$t_{1/2}$	v_{st}	Intercept (θ)	Slope	r^2	AICc
<i>Perciformes, n = 57</i>							
Log (brain mass)	log (body mass)	∞ (0.991– ∞)	–	-4.019 ± 0.194	0.536 ± 0.031	83.1%	10.3
Log (brain mass)	log (body mass) + static slope	∞ (0.867– ∞)	–	-4.331 ± 0.211	0.544 ± 0.032 0.604 ± 0.113	83.4%	11.9
Log (brain mass)	log (body mass) + static intercept	0.441 (0– ∞)	–	-2.304 ± 0.310	0.496 ± 0.035 0.421 ± 0.049	85.2%	6.0
Log (brain mass)	log (body mass) + static intercept + static slope	–	0*	0.148 ± 0.260	0.440 ± 0.027 1.590 ± 0.074 4.314 ± 0.227	96.3%	–10.5
<i>Syngnathiformes, n = 12</i>							
Log (brain mass)	log (body mass)	∞ (1.242– ∞)	0.131 (0.061– ∞)	-5.429 ± 0.162	0.559 ± 0.061	87.1%	22.1
Log (brain mass)	log (body mass) + static slope	∞ (0– ∞)	–	-6.431 ± 0.166	0.595 ± 0.059 2.346 ± 0.141	90.5%	24.2
Log (brain mass)	log (body mass) + static intercept	∞ (0– ∞)	–	1.195 ± 0.619	0.447 ± 0.061 1.200 ± 0.112	93.3%	16.4
Log (brain mass)	log (body mass) + static intercept + static slope	∞ (0– ∞)	–	0.806 ± 0.638	0.465 ± 0.055 1.231 ± 0.111 1.198 ± 0.130	94.6%	21.6
<i>Osteoglossiformes, n = 17</i>							
Log (brain mass)	log (body mass)	0.121 (0– ∞)	0.085 (0.035– ∞)	-3.956 ± 0.257	0.989 ± 0.097	82.2%	27.3
Log (brain mass)	log (body mass) + static slope	0.105 (0– ∞)	0.041 (0– ∞)	-5.356 ± 0.299	0.911 ± 0.090 3.099 ± 0.290	87.6%	27.0
Log (brain mass)	log (body mass) + static intercept	0.118 (0– ∞)	0.058 (0.008– ∞)	10.546 ± 0.248	0.652 ± 0.096 4.839 ± 0.014	83.1%	29.8
Log (brain mass)	log (body mass) + static intercept + static slope	–	0*	-1.411 ± 0.510	0.555 ± 0.062 0.935 ± 0.149 2.160 ± 0.299	96.2%	11.9

Phylogenetic half-life ($t_{1/2}$, unit: total tree height) and stationary variance (v_{st}) are presented with lower–upper 2 maximum-likelihood units support interval and intercept (θ) and slope are presented with standard errors. Stationary variance (v_{st}) is not shown in models where the best estimates of phylogenetic half-lives are infinity because stationary phases are never reached in such cases. Asterisk (*) denotes that the model had no residual variance after measurement error was accounted for. $t_{1/2}$ is not estimable in this case.

4.52, and I found that species of Osteoglossiformes have on average $566\% \pm 43\%$ (SE, $n = 40$) heavier brain masses than typical teleost fish species with similar body masses.

As preliminary analyses using the static data revealed that the relationships between static allometry and brain size were order specific, I primarily report results from three orders represented by 10 or more species (Osteoglossiformes, $n = 17$; Perciformes, $n = 57$; Syngnathiformes, $n = 12$). Comparing static allometric slopes and intercepts across these three orders revealed substantial variation in static allometric parameters (Fig. 2). Generally, Osteoglossiformes were found to be evolving toward steeper slopes (OU central state, $\theta \pm SE = 0.52 \pm 0.05$) and higher intercepts (-2.99 ± 0.12) than Perciformes (slope: 0.46 ± 0.01 , intercept: -3.61 ± 0.33), while Syngnathiformes were found to be evolving toward shallower slopes

(0.43 ± 0.05) and lower intercepts (-5.38 ± 0.12) than Perciformes (Table 2). In a hypothetical fish with a body mass of 1 g, intercept estimates indicate that Osteoglossiformes are predicted to have a brain mass (0.050 g) that is twice as large as in Perciformes (0.026 g) and 10 times as large as in Syngnathiformes (0.0046 g). In the complete static data, the four examined traits showed moderate to long $t_{1/2}$ (0.120– ∞ ; Table 2). Notably, even a relatively short $t_{1/2}$ of 0.120 (lower–upper 2 ML units support intervals: 0.007–0.566) for static allometric slope corresponds to 23.1 (1.9–109.9) millions of years (myr) given the total tree height of 192.8 myr. The $t_{1/2}$ of the static intercept was infinity ($0.657-\infty$), meaning that the model converges to BM. BM is a model in which divergence of a trait is proportional to the length of branches of a phylogeny that are not shared among species. Therefore,

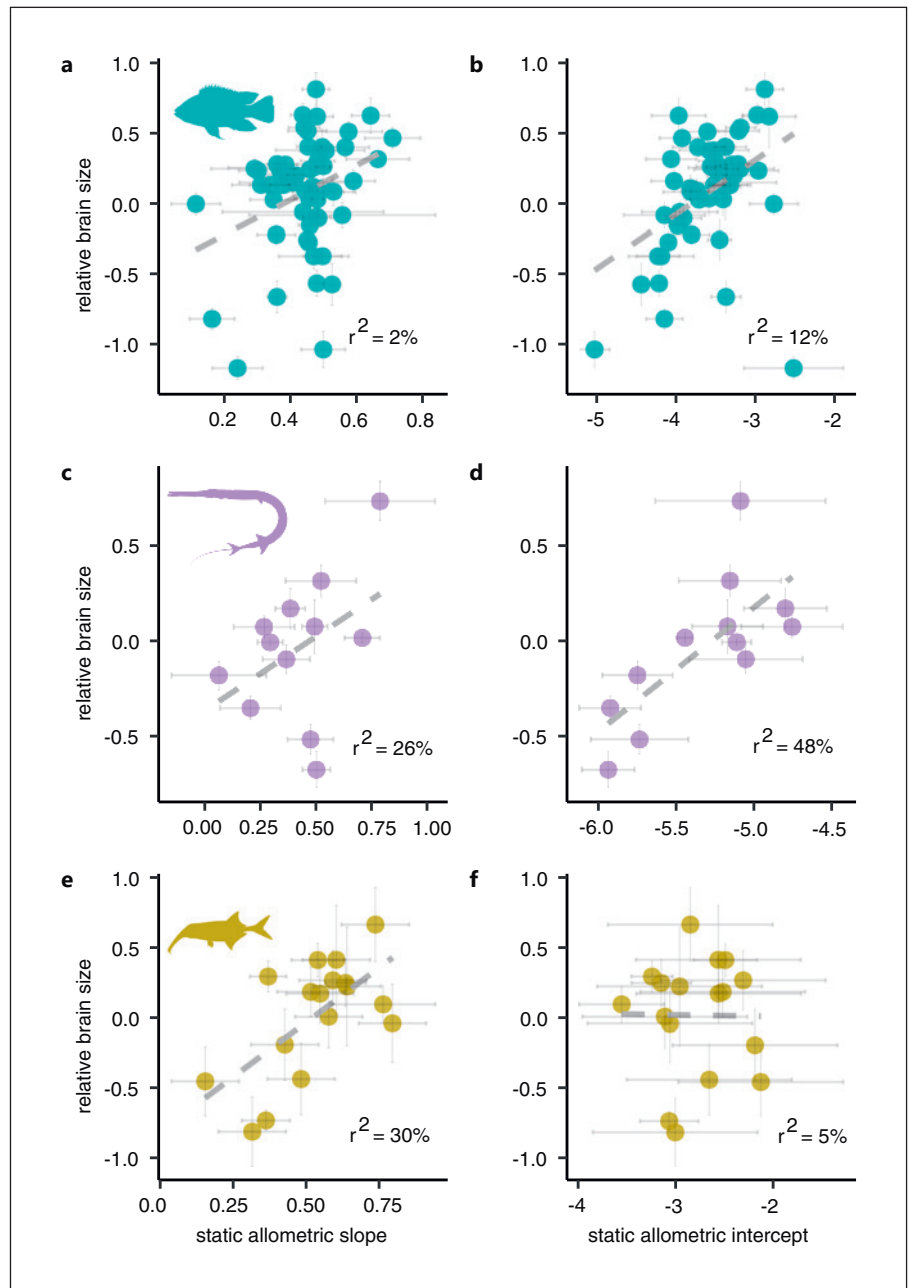


Fig. 3. Relationship between relative brain size and brain-body static allometric slopes and intercepts. The relationship is plotted within Perciformes (**a, b**), Syngnathiformes (**c, d**), and Osteoglossiformes (**e, f**). Relative brain sizes are residuals of a direct-effect SLOUCH model of $\log(\text{brain mass})$ against $\log(\text{body mass})$ in each of the three datasets. Note that these residuals are only used for visualization purposes. Dashed lines are ordinary least-squares regressions. r^2 values were evaluated as the proportional increase in a model r^2 with the focal predictor relative to a reduced model (details of model parameters are presented in Table 3). Error bars indicate standard errors.

the BM fit suggests that the evolution of static intercepts is strongly influenced by shared ancestry. Taken together, although static allometric parameters varied considerably among taxa, their evolution was relatively slow and left substantial room for the static allometry to constrain the direction of brain size evolution over short time scales.

Parameter estimates of the direct-effect OU regressions are presented in Table 3. Static allometric slopes were positively correlated with relative brain size in spe-

cies of Osteoglossiformes ($r^2 = 30\%$; Fig. 3) and Syngnathiformes ($r^2 = 26\%$). The effect of static slope on relative brain size was weak in Perciformes ($r^2 = 4\%$), but there was a positive trend. This finding could be due to a lack of statistical power as the static slope of Perciformes showed considerably lower variance than the other two orders (stationary variance of fitted OU processes [lower–upper 2 ML units support intervals]; Perciformes: 0.005 [0.002– ∞], Osteoglossiformes: 0.014 [0.004– ∞],

Syngnathiformes: 0.012 [0.002–∞]; Table 2). Static allometric intercepts were correlated with relative brain size in Perciformes ($r^2 = 12\%$) and Syngnathiformes ($r^2 = 48\%$) but not in Osteoglossiformes ($r^2 = 5\%$). This finding indicates that both intercepts and slopes are related to relative brain size in fish species, but their relative contributions vary among orders. In Osteoglossiformes, the inclusion of a static intercept in the model resulted in a dramatic drop in the evolutionary allometry (slope \pm SE = 0.65 ± 0.10). Decomposition of the effect of static intercept, static slope, and their interaction on evolutionary allometric slope resulted in 52, 9, and 18% increases in evolutionary allometric slope, respectively. Notably, despite variation in static allometry in the other two examined orders, there was only a minor increase in the slope of evolutionary allometry for Perciformes (21%) and Syngnathiformes (20%). This finding demonstrates that the evolution of static allometry does not necessarily alter evolutionary allometry. These results are all qualitatively equivalent using complete static data (online suppl. Table S2).

Discussion

Explaining the evolution of relative brain size is challenging when allometric constraints are strong. The fish order Osteoglossiformes has long been known for its large relative brain sizes [Nilsson, 1996; Chapman and Hulen, 2001; Kaufman et al., 2003]. However, available data suggest close brain-body static allometry in this clade [Chapman and Hulen, 2001; Sukhum et al., 2019] (online suppl. Table S3). Here, I confirmed a case of exceptional encephalization in Osteoglossiformes using an updated database and a statistical method accounting for measurement errors. Additionally, I demonstrated that the unique encephalization in this order results from evolutionary change in the slope of evolutionary brain-body allometry. I showed that the slope is virtually isometric (1.03 ± 0.09 SE), making it the steepest brain-body evolutionary allometry reported to date [Tsuboi et al., 2018b; Ksepka et al., 2020] along with hominins that appear to exhibit a similarly steep evolutionary allometry [1.10 ± 0.16 ; Smaers et al., 2021]. Furthermore, the dramatic steepening of the evolutionary allometric slope was a combined result of evolution in the slopes and intercepts of static allometry. In teleosts, the static allometric slopes could reflect the rate of brain growth relative to body growth during the adult stage, while static allometric intercepts could approximate brain mass at the transition

between embryonic and juvenile growth phases [Bauchot et al., 1979; Oikawa and Itazawa, 1984; Oikawa et al., 1992]. Therefore, my findings suggest that the evolution of static allometry, which may have been driven by evolutionary changes in the timing of transition between embryonic and juvenile stages and the rate of brain development during the juvenile phase, altered the slope of evolutionary brain-body allometry and facilitated the unique encephalization of Osteoglossiformes.

The slope of evolutionary allometry in Osteoglossiformes was sharply distinct from all the other teleost orders examined in this study. I interpret this as suggesting that species of Osteoglossiformes are evolving under a unique adaptive landscape [Simpson, 1944; Hansen, 1997; Uyeda et al., 2017] that is not shared by other teleost species. An important question to address is whether this shift reflects changes in the pattern of constraints or selective regimes. Based on the partial correlation coefficients of phylogenetic regression models, the isometric evolutionary allometry of Osteoglossiformes was explained 52% by the static intercept, 9% by the static slope, and 18% by the interaction between the static slope and static intercept. Therefore, the steepness of the evolutionary allometric slope has primarily resulted from the evolution of static allometric intercepts. Theoretical work on the relationship between static and evolutionary allometry showed that the static allometric intercept could result in a steep evolutionary allometry when the static intercept is positively correlated with body size [Pélabon et al., 2013; Voje et al., 2014]. This prediction was supported by data in this study (online suppl. Fig. S3), suggesting that the coevolution of body size and static allometric intercept may have played a major role in encephalization of Osteoglossiformes. Furthermore, the static allometric intercepts of Osteoglossiformes were substantially larger than those of other orders of teleosts, strengthening the hypothesis that the evolution of static allometric intercepts could underlie the exceptional encephalization in this clade. The present study corroborates previous findings that the evolution of relative brain size is primarily mediated by evolution in the static allometric intercepts in the guppy [Kotrschal et al., 2013], cichlids [Tsuboi et al., 2016], and sunfishes [Axelrod et al., 2021]. More generally, static allometric intercepts are more variable than static allometric slopes in a variety of traits and taxa [Egset et al., 2012; Voje et al., 2014; Bolstad et al., 2015]. Taken together, the encephalization of Osteoglossiformes appears to have occurred under strong allometric constraints similar to those in other systems, providing limited support for the hypothesis that changes in the pat-

tern of allometric constraints underlies the encephalization of Osteoglossiformes.

An alternative hypothesis is that the brain-body evolutionary allometry of Osteoglossiformes manifests a unique adaptive scaling relationship. Notably, the family Mormyridae, which is the most species-rich clade within Osteoglossiformes, uses its electroreception and active electrolocation ability for foraging, which allows these fishes to detect food efficiently in turbid and murky waters [Von der Emde and Bleckmann, 1998]. It might be possible that the efficient food intake improves metabolic turnover, which enables mormyrid fishes to evolve exceptionally large brains, which are energetically costly to develop and maintain [Nilsson, 1996]. Furthermore, adaptation to oxygen-deficient environments, which is extremely important in protecting brains from hypoxia damage, has been reported for several mormyrid fishes in the form of an enlarged gill surface area [Chapman and Hulen, 2001], increased hemoglobin content in the blood [Fish, 1956], aquatic surface respiration [Chapman and Chapman, 1998], and decreased metabolic rate [Chapman and Chapman, 1998]. These physiological and behavioral adaptations in mormyrids, and possibly Osteoglossiformes in general, could be the foundation of the adaptive brain-body scaling relationships that are dramatically different from other teleost clades.

There was a modest effect of the static allometric slope on the shift in the evolutionary allometric slope of Osteoglossiformes, and relative brain size (i.e., the deviation of brain size from evolutionary allometry) was positively correlated with the static slope in this clade. Together with the finding that the static slopes of Osteoglossiformes were on average slightly steeper than those of other orders of teleost, these results suggest that the evolution of steep static allometric slopes have contributed to the encephalization of Osteoglossiformes. Although brain size evolution driven by the reduction of allometric constraints and the steepening of static allometric slope are equally plausible scenarios toward encephalization, the latter path seems to be exceedingly rare. Based on currently available data, Osteoglossiformes, Aves, and Mammalia are three clades that represent the apex of the vertebrate brain-body morphospace, and Osteoglossiformes is the only clade of the three in which encephalization appears to be partly driven by the steepening of the static allometric slope. Why is it so uncommon? One explanation is the pleiotropic constraint [Houle et al., 2019]. Due to its high metabolic cost [Nilsson, 1996; Sukhum et al., 2016], enlargement of the brain is often accompanied by evolutionary changes in traits that are tightly linked to

energy budgets of organisms, such as fecundity [Kotrschal et al., 2013], egg size [Tsuboi et al., 2015, 2017], or the size of other metabolically expensive organs [Kotrschal et al., 2013; Tsuboi et al., 2015]. It may be plausible that the static allometric slope manifests physiological links among energetically expensive traits, and makes the static slope evolutionarily stable because stabilizing selection in any of the correlated traits would reduce its evolvability. Another concept for consideration is the evolutionary burden [Riedl, 1977]. In mammals, the cerebellum, which is a region of the brain that is most conspicuously expanded in Osteoglossiformes [Bauchot et al., 1995; Nilsson, 1996; Sukhum et al., 2018], develops earlier than the telencephalon [Workman et al., 2013], which is the brain region that is enlarged in birds [Rehkämper et al., 1991] and mammals [Stephan et al., 1981]. If the mammalian model of neurodevelopmental timing is applicable in fish, the evolution of the cerebellum requires changes in the gene regulatory network earlier than those of the telencephalon, and this might be the key event leading to the evolution of, rather than the reduction of, allometric constraints. However, modification in early developmental pathways may influence many other traits. Therefore, it is a “burden” and difficult to realize.

Throughout this study, parameters of static brain-body allometries (intercept and slope) were considered to reflect the rate and timing of neurogenesis, which are poorly understood in teleost fishes. Therefore, an important step toward understanding the developmental and genetic bases of allometric constraints is to elucidate how the simple power function, which is currently used widely in studies of brain size, reflects real neurodevelopmental processes. Available data on the ontogenetic allometry of hatchling to adult stages in teleost fishes [Bauchot et al., 1979; Oikawa and Itazawa, 1984; Brandstätter and Kotrschal, 1989; Oikawa et al., 1992; Tomoda and Uematsu, 1996] suggest that fish brains generally undergo a biphasic mode of growth. The first phase roughly corresponds to the larval period when allometric slopes are close to isometry [Brandstätter and Kotrschal, 1989; Toyoda and Uematsu, 1994]. The rate of brain growth is then slowed down at the onset of the juvenile period, resulting in a shallow allometric slope that continues through the adult stage [Tsuboi et al., 2018b]. Generally, the standard allometric equation fits the data well within each of the two growth phases, suggesting that the exponent of the power function (the allometric slope) may approximate the rate of brain growth in fish species. However, the selection of statistical models should not be determined by statistical fit alone [Smith, 2009; Houle et al.,

2011; Glazier, 2021]. It needs to be augmented by empirical knowledge of the developmental processes that model parameters are supposed to describe. Further research is required to explore alternative models of allometry [Packard, 2009; Echavarría-Heras et al., 2020] in conjunction with additional empirical knowledge of brain development in fish to construct a model of allometry that is biologically more realistic than the standard model of allometry.

The timing of neurodevelopmental events in teleost species is currently only described in a handful of model species [Ishikawa, 1997; Wullimann and Puelles, 1999]. While a comparative study of brain development across fishes requires more empirical work, it is important to begin testing hypotheses about the consequences of evolutionary changes in the timing of development on brain size evolution. One possible approach is to use life history traits as proxies for the timing of key developmental events. For example, life history traits that are associated with the level of parental investment have been shown to predict relative brain size in birds [Iwaniuk and Nelson, 2003; Isler and van Schaik, 2006] and mammals [Sacher and Staffeldt, 1974; Barton and Capellini, 2011]. Recently, similar patterns have been found in fish, where egg size [Tsuboi et al., 2015, 2017], mode of reproduction [Mull et al., 2011, 2020], or duration of parental care [Tsuboi et al., 2015] were correlated with relative brain size. Based on the aforementioned biphasic model of teleost brain development, it could be hypothesized that these life history traits covary with the timing of the transition between the embryonic and juvenile growth phases so that higher parental investment prolongs the larval growth phase, leading to increased relative brain sizes at adult stages. Testing this idea requires more knowledge of brain development in fishes. Based on the results presented in this paper, obtaining such data in Osteoglossiformes [Haugedé-Carré et al., 1977; Radmilovich et al., 2016] and linking it to data on static allometric slopes and intercepts would be particularly relevant to further advance our understanding of the mechanism of allometric constraints.

In contrast to my findings, Sukhum et al. [2016] reported an evolutionary brain-body allometric slope of 0.79 for 21 species of Osteoglossiformes. The discrepancy between their estimate and mine (1.03) arises primarily from measurement error, as my estimate becomes 0.82 (± 0.11 SE) if measurement errors are not controlled for (Table 1). The necessity of accounting for measurement errors in phylogenetic comparative studies is repeatedly debated [Ives et al., 2007; Garamszegi and Møller, 2010; Hansen, 2014], and methods that account for measure-

ment errors are regularly implemented [Beaulieu et al., 2012; Hansen and Bartoszek, 2012; Pennell et al., 2014; Uyeda and Harmon, 2014; Mitov et al., 2019]. However, the correction of measurement error is not currently standard practice in phylogenetic comparative studies. It has often been argued that measurement error tends to be small compared to the overall effect that comparative studies seek to explore and that the correction will have negligible effect on statistical inferences [Harmon and Losos, 2005]. This was shown to be the case in several subsets of the presented analyses, with the effect of correction on estimates of the slope ranging from 3% to 31% (Table 1). In contrast, Grabowski et al. [2016] reported that the effect of measurement error on brain-body evolutionary allometric slope in 161 primate species is only 0.2%. This example suggests that the effect of measurement errors is data specific, and it is likely to be more conspicuous in lineages with indeterminate growth than lineages with determinate growth due to the wide adult body size range in lineages with indeterminate growth. Therefore, in future studies, the isometric evolutionary brain-body allometry of Osteoglossiformes should be compared with estimates corrected for measurement errors. For example, Smaers et al. [2021] recently reported a steep evolutionary brain-body allometric slope in hominins (1.10 ± 0.16 SE) using a method that does not account for measurement errors. As hominins and Osteoglossiformes are two of the most extreme cases of encephalization in vertebrates, steep evolutionary allometries in these clades may represent an interesting case of convergence associated with exceptional encephalization. These examples illustrate the value of using a measurement error model and prompt its use in phylogenetic comparative studies [Silvestro et al., 2015].

Although my analyses indicated that the evolution of the static brain-body allometric slope is relatively slow and that brain size evolution in fish is largely likely to occur in the direction of static allometric slopes, these results do not exclude the possibility that the organization of brain regions can change freely. Available data suggest that the encephalization of Osteoglossiformes has been driven primarily by the enlargement of the cerebellum [Bauchot et al., 1995; Nilsson, 1996; Sukhum et al., 2018]. Therefore, it could be possible that selection to increase brain size in Osteoglossiformes may have acted specifically on the size of the cerebellum. This leads to the question of whether the observed steepening of static and evolutionary allometry in the relationship between whole brain mass and body mass could reflect changes in the allometry of cerebellum size. Sukhum et al. [2018] studied

the allometry of cerebellum volume in relation to total brain volume in 10 species of Osteoglossiformes and showed that the variation in cerebellum volume relative to total brain volume among species is predominantly driven by differences in the intercept of cerebellum-whole brain allometry [online suppl. Table S1 in Sukhum et al., 2018]. More broadly, the scaling relationship (i.e., slope) between brain region size and whole brain size is highly conserved across vertebrates [Yopak et al., 2010]. Therefore, selection to re-structure brain organization may typically act on the intercept of brain region allometry, which is similar to the pattern in the brain-body static allometry suggested in this and previous studies [Kotrschal et al., 2013; Tsuboi et al., 2016].

The present study leaves us with unanswered questions regarding the mechanism that allowed Osteoglossiformes to evolve steep brain-body static and evolutionary allometric slopes, which seems to be extremely rare among vertebrates. Although there is limited information available on the natural history of Osteoglossiformes and conclusive suggestions are difficult to make, I propose four hypotheses that could be worth exploring in the future. First, the encephalization of Osteoglossiformes occurred through the expansion of the cerebellum, which is a brain region that develops relatively early in mammalian neurogenesis [Workman et al., 2013]. A comparison of early neurogenesis between Osteoglossiformes [Haugedé-Carré et al., 1977; Radmilovich et al., 2016] and other teleost fishes [Oikawa and Itazawa, 1984; Brandstätter and Kotrschal, 1990; Oikawa et al., 1992; Toyoda and Uematsu, 1994; Ishikawa, 1997; Maeyama and Nakayasu, 2000; Sylvester et al., 2010] could allow us to identify the developmental and genetic mechanisms underlying the evolutionary changes in static and evolutionary allometric slopes. Second, similar to birds and mammals, the evolution of static allometric intercepts and slopes in Osteoglossiformes might have been channeled through enhanced parental investment [Iwaniuk and Nelson, 2003; Barton and Capellini, 2011; Tsuboi et al., 2018b]. Available data of life history traits in Osteoglossiformes [Okedi, 1970; Kirschbaum and Schugardt, 2002; Nguyen et al., 2017] suggest that the Arowana family Osteoglossidae has particularly advanced parental investment in the form of mouth brooding [Scott and Fuller, 1976; Merrick and Green, 1982; Verba et al., 2014], feeding of juveniles with skin secretion [Lüling, 1964], and extremely large eggs [Yue et al., 2020]. Therefore, it would be interesting to investigate how parental investments and relative brain sizes are related in Osteoglossiformes. Third, as there is good evidence for unique

physiological adaptations in mormyrid fishes to cope with oxygen-deficient environments [Fish, 1956; Nilsson, 1996; Chapman and Chapman, 1998; Chapman and Hulen, 2001], examinations of these characteristics in other members of Osteoglossiformes could allow us to test adaptive hypotheses concerning their unique brain-body scaling exponent. Finally, selection could act on traits that are more directly relevant for brain function than overall brain mass, such as the number, distribution, and density of neurons [Marhounová et al., 2019], and these traits may determine the uniqueness of brain size evolution in Osteoglossiformes. Elucidating these and other unforeseen alternative hypotheses explaining the mechanistic basis of the evolutionary change of static and evolutionary allometric slope in Osteoglossiformes could shed light on the role of allometric constraints in vertebrate brain size evolution.

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Statement of Ethics

This study is based completely on published resources and thus no ethical permissions are necessary.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

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Author Contributions

M.T. conceived the idea of this paper, assembled and analyzed the data, and wrote the paper.

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