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Brain size evolution in pipefishes and seahorses: the role of feeding ecology, life history and sexual selection

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Abstract

Brain size varies greatly at all taxonomic levels. Feeding ecology, life history and sexual selection have been proposed as key components in generating contemporary diversity in brain size across vertebrates. Analyses of brain size evolution have, however, been limited to lineages where males predominantly compete for mating and females choose mates. Here, we present the first original data set of brain sizes in pipefishes and seahorses (Syngnathidae) a group in which intense female mating competition occurs in many species. After controlling for the effect of shared ancestry and overall body size, brain size was positively correlated with relative snout length. Moreover, we found that females, on average, had 4.3% heavier brains than males and that polyandrous species demonstrated more pronounced (11.7%) female-biased brain size dimorphism. Our results suggest that adaptations for feeding on mobile prey items and sexual selection in females are important factors in brain size evolution of pipefishes and seahorses. Most importantly, our study supports the idea that sexual selection plays a major role in brain size evolution, regardless of on which sex sexual selection acts stronger.

Introduction

Brain size is remarkably variable among vertebrates (Jerison, 1973). This variation has been proposed to be formed through adaptations to different ecological and social environments (Jerison, 1973; Striedter, 2005) and the costs of developing and maintaining a larger brain (Aiello & Wheeler, 1995). Phylogenetic comparative studies have revealed three particular aspects that are related to brain size evolution (Striedter, 2005). First, various aspects of feeding ecology have been shown to be associated with relative brain size (i.e.

brain size after controlling for body size), such as consumption of food items that are cognitively challenging to find or catch (Cluttonbrock & Harvey, 1980; Garamszegi *et al.*, 2002; Hutcheon *et al.*, 2002), wide diet breadth (MacLean *et al.*, 2014) and propensity for consuming novel food items (Garamszegi *et al.*, 2005; Lefebvre *et al.*, 2016). These studies indicate that selection for the ability to explore and exploit food resources may be a common underlying force for adaptive brain size evolution (Striedter, 2005; MacLean *et al.*, 2014). However, the advantages of having large brains come with increased energetic costs of brain development and maintenance (Mink *et al.*, 1981). An increase in brain size over evolutionary time (encephalization) should therefore be accompanied by modifications in energy allocation (Isler & van Schaik, 2009; Tsuboi *et al.*, 2015, 2016) and/or acquisition of more energy rich food resources (Aiello & Wheeler, 1995). Second,

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life-history traits have been found to be related with brain size across vertebrates (Isler & van Schaik, 2006; Gonzalez-Voyer *et al.*, 2016). Increased investment into single offspring (Isler & van Schaik, 2006, 2009; Tsuboi *et al.*, 2015), reduction in litter size (Isler & van Schaik, 2009; Weisbecker & Goswami, 2010; Kotschal *et al.*, 2013), prolongation of gestation/parental care duration (Iwaniuk & Nelson, 2003; Isler & van Schaik, 2006, 2009; Weisbecker & Goswami, 2010; Barton & Capellini, 2011; Isler, 2011; Tsuboi *et al.*, 2015) and overall slower pace of reproduction (Isler & van Schaik, 2006, 2009; Isler, 2011) are all related with encephalization, proposing that the energetic costs of developing and maintaining brain tissue may have played an important role in brain size evolution (Isler & van Schaik, 2009).

The third aspect concerns sexual selection. Since Darwin (1871), it has been an enduring idea that mate choice may be directly based on the cognitive ability of potential partners (Miller, 2000; Boogert *et al.*, 2011; Fusani *et al.*, 2014). Furthermore, intrasexual competition over mating opportunities often involves cognitive challenges (Andersson, 1994), indicating that sexual selection also in this context may favour increased brain sizes (Francis, 1995; Jacobs, 1996; Kolm *et al.*, 2009; Kotschal *et al.*, 2014). However, the role of sexual selection in brain size evolution remains elusive. One line of argument comes from studies that showed male-biased sexual size dimorphism of brain size in species where males experience stronger sexual selection than females (Francis, 1995; Iwaniuk, 2001; Kotschal *et al.*, 2012; Samuk *et al.*, 2014). However, the link between sexual selection and brain size evolution in these studies may be confounded by the influence of parental care on brain size evolution (Gittleman, 1994; Gonzalez-Voyer *et al.*, 2009; Garcia-Pena *et al.*, 2013), because the intensity of sexual selection is usually associated with patterns of parental care. The other line of empirical evidence comes from studies that established a relationship between relative brain size and proxies of sexual selection (Pitnick *et al.*, 2006; Gonzalez-Voyer & Kolm, 2010; Fitzpatrick *et al.*, 2012; Zeng *et al.*, 2016). However, several conflicting results have been also reported (Schillaci, 2006; Lemaitre *et al.*, 2009), making it difficult to gain insights beyond the case-by-case explanations. Consequently, we still have limited understanding for *if* and *how* sexual selection influences brain size evolution. The main aim of our study was thus to investigate the role of sexual selection in brain size evolution using a novel data set of brain size in pipefishes and seahorses (family Syngnathidae), an important model lineage for testing theories in sexual selection.

The teleost family Syngnathidae consists of at least 200 pipefish species (Dawson, 1985) and approximately 50 seahorse species (Lourie *et al.*, 1999). Pipefishes and seahorses commonly live in sea grass and coral reef habitats in shallow water (Foster & Vincent, 2004; Lim

et al., 2011) where they feed on mobile prey such as small free-swimming crustaceans (Kendrick & Hyndes, 2005; de Lussanet & Muller, 2007; Van Wassenbergh *et al.*, 2011). Life-history traits are highly diverse among syngnathid species (Foster & Vincent, 2004). The family Syngnathidae thus provides a rich opportunity to test the hypothesis concerning the life-history correlates of brain size evolution (e.g. Isler & van Schaik, 2009). Syngnathid fishes are characterized by a unique form of parental care, where males provide all post-zygotic care (Ripley *et al.*, 2010; Sagebakken *et al.*, 2010; Kvarnemo *et al.*, 2011; Braga Goncalves *et al.*, 2015). This 'reversed' direction in sexual selection has made the syngnathids a crucial model system to test hypotheses related to evolution of sex differences (Jones & Avise, 2001), and our study now applies this framework to brain size evolution. Furthermore, during brooding, males of pipefishes and seahorses reduce social and feeding activities considerably (Svensson, 1988; Cunha *et al.*, 2016), which makes for a stark contrast with the cognitively challenging parental care in the other groups of vertebrates that have been studied in the context of sexual selection and brain size evolution (carnivores: Gittleman, 1994; primates: Schillaci, 2008; cichlids: Gonzalez-Voyer *et al.*, 2009; stickleback: Kotschal *et al.*, 2012; shorebirds: Garcia-Pena *et al.*, 2013; Samuk *et al.*, 2014). Therefore, with substantial among-species variation in the intensity of sexual selection (Jones & Avise, 2001; Rosenqvist & Berglund, 2011) and cognitively passive paternal care (e.g. Svensson, 1988), the family Syngnathidae provides a unique opportunity to test the role of sexual selection in brain size evolution while minimizing the effect of parental care.

We propose a set of predictions in relation to the targeted three key aspects concerning brain size evolution (Table 1). First, we predict that larger snout to head length (HL) ratio, a strong predictor of prey mobility in pipefishes and seahorses (Kendrick & Hyndes, 2005), is associated with larger brain size as capturing evasive prey items requires increased visual acuity and elaborated motor control (Higham, 2007; de Lussanet & Muller, 2007; Van Wassenbergh *et al.*, 2011). Second, based on the energy trade-off hypothesis (Isler & van Schaik, 2006), we predict that large relative brain sizes are associated with large egg size and longer brood care duration (Table 1). Third, if sexual selection is an important factor in brain size evolution, we predict that females should have larger brains than males in this family (Table 1). Furthermore, given that intense female courtship and competition is more commonly observed in polyandrous and polygynandrous pipefishes than monogamous pipefishes and seahorses (Jones & Avise, 2001; Rosenqvist & Berglund, 2011), we predict female-biased sexual dimorphism in brain size to be more pronounced among polyandrous and polygynandrous pipefishes compared to monogamous species (Table 1).

Table 1 List of hypotheses tested in the present study and predictions following from each hypothesis.

Hypothesis	Variables tested	Predicted link to brain size	Rationale for predictions
(a) Feeding adaptation	SnL/HL ratio	Positive	Species that consume evasive prey are selected for increased visual acuity and better motor control for feeding activities
(b) Energy trade-off	Egg size	Positive	Embryos of larger brain species require a larger investment per egg due to increased energetic requirements for brain development
	Brood duration	Positive	Offspring with larger brains (and larger eggs) require longer developmental time
(c) Sexual selection	Sex	Female > Male	Sexual selection, predominantly in females, favours larger brains in mating competition and mate choice
	Sex × Mating pattern	More pronounced female-biased brain size dimorphism in polyandrous than monogamous species	Females of species that experience stronger sexual selection are selected for higher degree of sexual dimorphism in brain size

Materials and methods

Sample assembly

Subject specimens were obtained through our own field sampling in Sweden and Malaysia, sampling of collaborating researchers and access to museum specimens in the National Museum of Nature and Science (NMNS, Tokyo, Japan). In Sweden, field sampling was conducted during May to September in 2011 in the vicinities of Klubban Biological Station (Uppsala University) and Sven Lovén Centre for Marine Sciences, Kristineberg (Gothenburg University), in shallow eelgrass meadows (*Zostera marina*) using a small beam trawl pulled by boat (mesh size 4 mm). Sampling was performed under the ethical approval Dnr 91/2009 Gothenburg. In Malaysia, sampling was conducted during June to July 2014 at various locations in the Malay Peninsula in compliance with Malaysian legislation. We collected specimens through various sources and methods: catching by ourselves, obtaining the by-catch from local fishermen and purchasing individuals from local aquarium fish traders. In addition, we used individuals of *Hippocampus trimaculatus* and *Hippocampus spinosissimus* collected from various locations across Malay Peninsula by Choo and Liew (Choo & Liew, 2003). All individuals collected in our study were fixated in 4% paraformaldehyde in a phosphate buffer (i.e. formalin). However, individuals measured at the NMNS and those obtained by Choo and Liew (Choo & Liew, 2003) were preserved in ethanol after tissue fixation with formalin. Differences in the preservative method should not yield systematic bias in our size measurements (Wetzel *et al.*, 2005). However, to avoid potential biases, we included the preservation method (either formalin or ethanol) as a factor in all analyses. We identified the sex of each individual by examination of the gonads and only sexually mature individuals were included in the analyses. In total, we collected 339 individuals across 23 species of the family Syngnathidae.

We measured the body height, that is the distance from the top of the coronet to the tip of elongated tail (Foster & Vincent, 2004) for seahorses, and the standard length for pipefishes, that is the distance from the anterior tip of the snout to the posterior end of the last vertebrae, as a proxy for body size (precision = 0.1 mm) because the general body plans of pipefishes and seahorses are different from each other. Whole brains were carefully dissected out after tissue fixation. Following a few gentle pats on a piece of paper to remove excess fluid, whole brain weight (precision = 0.1 mg) was measured as a proxy for brain size using MX5 microbalance (Mettler Toledo, Zürich, Switzerland). For geometric morphometric analysis used later to assign phylogenetic relationship for species without molecular data, digital images of the head were taken for a subset of individuals from the left lateral side with a scale. Digital photographing was performed using a Pentax K5 reflex digital camera with a 35 mm 1:2.8 macro lens for large specimens and digital imaging function mounted in a Leica EZ4 microscope for small specimens.

We measured snout length (SnL, length between the dorsorostral tip of the premaxilla and the caudal border of the nostril) and head length (HL, length between the dorsorostral tip of the premaxilla and the posterior most point of operculum) to the nearest 0.1 mm with a digital calliper and calculated the proportion (SnL/HL) as a proxy for prey mobility following Kendrick & Hyndes (2005). Maximum latitude of geographic distribution and the mating pattern, which is categorized into monogamy, polygynandry or polyandry (Jones & Avise, 2001), were obtained from the literature. The relative strength of sexual selection was then scored according to the mating pattern as monogamy = 1, polygynandry = 2, polyandry = 3 ($N_{\text{species}} = 17$). For life-history traits, egg diameter of the major axis (mm, $N_{\text{species}} = 18$) and brooding period (days, $N_{\text{species}} = 14$) were obtained from literature and our own measurements. Information on size measurements, the source

of each specimen, ecological/life-history information, mating pattern as well as their references are provided in the Table S1.

Molecular phylogenetic reconstruction

We reconstructed an mtDNA phylogeny for the 20 syn-gnathid species for which sequence data were available. We used three regions of mitochondrial genes, 12S, 16S and cytochrome *b* downloaded from GenBank (see Table S2 for data accession numbers). Sequences were first aligned using MAFFT (Katoh *et al.*, 2002) and minor adjustments performed manually with SeaView (Gouy *et al.*, 2010), we then used G-blocks (Castresana, 2000) to eliminate poorly aligned sites. The aligned matrices were then concatenated, creating a matrix of 1824 base pairs (12S: 318 bp; 16S: 365 bp; cytochrome *b*: 1141). A general time reversible model with invariable sites and a gamma distribution (GTR + I + Γ) was found to present the best fit for the 16S and cytochrome *b* regions whereas GTR + Γ was favoured for the 12S region based on AIC, AICc and BIC in jModelTest (Posada, 2008). Bayesian inference under MrBayes version 3.2 was used to reconstruct the phylogenetic relationships among species (Ronquist & Huelsenbeck, 2003) with nine-spined stickleback, *Pungitius pungitius*, as an out-group. We ran two independent analyses with four simultaneous chains for 10 000 000 iterations and a sampling period of 1000 with a 50% relative burn-in. We applied the best fitted molecular evolution model for each region of the nucleotide and partitioned the third position of cytochrome *b* because the results of an analysis with DAMBE (Xia & Xie, 2001) suggested this position was saturated. Convergence was assessed based on values of commonly used parameters (average deviation of split frequencies < 0.01, potential scale reduction factor values \approx 1) and visual inspection. A consensus molecular phylogeny was retained for further analyses.

Assignment of species without molecular data based on head morphology

Three species obtained in the field, *Doryichthys boaja*, *D. martensii* and *Nerophis lumbriciformis*, lacked molecular data. We assigned their position in our molecular phylogeny using maximum likelihood (ML) (Revell *et al.*, 2015) based on head morphology quantified by geometric morphometrics. Following Leysen *et al.* (2011), we digitized 12 homologous landmarks (Fig. 1) using tpsDig2 version 2.16 (Rohlf, 2010a). Description of the 12 landmarks (LMs) were as follows: (LM1) the dorso-rostral tip of the premaxilla; (LM2) the ventrocaudal point of the lower jaw above the suspensorial articulation; (LM3) the dorsal and (LM4) the ventral point where the snout is dorsoventrally most narrow; (LM5) the base of mesethmoidal curvature; (LM6) the caudal border of the nostril; (LM7) the dorsal and (LM8) the ventral point of the eye with respect to the line connecting LM1 and LM6; (LM9) the most dorsal point of the skull at the level of the braincase with respect to the line connecting LM1 and LM6; (LM10) the ventrocaudal tip of the preopercular bone; (LM11) the base of the cleithral curvature where the gill slit is situated; and (LM12) the base of the preoperculum curvature. Generalized procrustes analysis (GPA) was performed for each species to obtain species average consensus configuration using tpsRelw version 1.49 (Rohlf, 2010b). Subsequently, another GPA was performed for the average shape of 23 species and aligned data were retained for downstream analyses.

With morphological data of *N* species and the time-calibrated molecular phylogeny of *N*-1 species, the method devised by Revell *et al.* (2015) searches for the optimal location from which the terminal node of the *N*th species branches out from a molecular phylogeny based on the ML criterion (Felsenstein, 1981). One can also specify a clade within which the ML search is performed. This feature is particularly useful when *a priori*

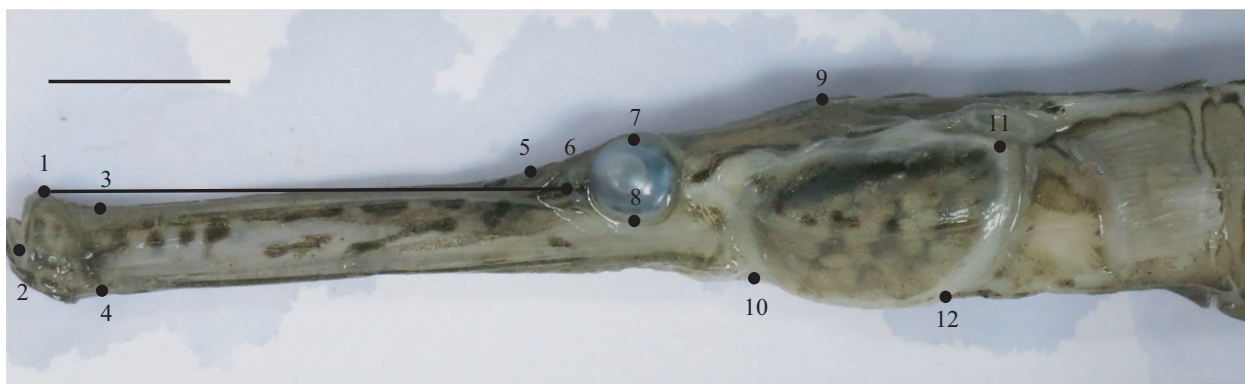


Fig. 1 Left lateral side of the head of *Doryichthys boaja* showing the 12 landmarks used for the geometric morphometrics analysis. The solid black line connecting landmark 1 and landmark 6 was used to measure snout length and to define landmarks 7, 8, and 9. See text for an anatomical description of the landmarks. Scale bar indicates 10 mm.

knowledge about phylogenetically conserved traits is available. In our case, male brood pouch location (either on tail or abdomen) is known to be a well-conserved trait within the family Syngnathidae (Wilson *et al.*, 2001, 2003). As *D. boaja*, *D. martensii* and *N. lumbriciformis* are all abdominal brooding species (Dawson, 1985), we constrained our ML computation to search species positions only within abdominal brooding lineages. Prior to the analysis, we assessed the phylogenetic signal of head shape using the geomorph package (Adams & Otárola-Castillo, 2013) version 2.1.5 and confirmed that the head shape of abdominal brooders represented high phylogenetic signal ($\lambda = 0.94$). Therefore, head morphology was a suitable trait to assess phylogenetic relationship of abdominally brooding species. Using the ape package version 3.3 (Paradis *et al.*, 2004), we first time-calibrated our molecular phylogeny using a penalized likelihood method (Sanderson, 2002) with a smoothing parameter of zero that provided the best fit to our phylogeny according to penalized log-likelihood calculated with *chronos* function in the ape package. To enable ML computation given our sample size ($N_{\text{species}} = 23$), we performed principal component analysis in the geomorph package (Adams & Otárola-Castillo, 2013) and retained the first six PCs that cumulatively explained 97% of the total shape variance. The placement of *D. boaja*, *D. martensii* and *N. lumbriciformis* was then assigned based on information of six PCs of head morphology for all 23 species and molecular phylogeny for 20 species using the locate.yeti function in the phytools package version 0.4.56 (Revell, 2012) in the R statistical environment version 3.2.1 (R Development Core Team, 2011).

Phylogenetic comparative analyses

We used the phylogenetic mixed model (Hadfield & Nakagawa, 2010) implemented in the MCMCglmm package version 2.21 (Hadfield, 2010) to investigate evolutionary covariates of brain size while taking both phylogenetic nonindependence and within-species variation into consideration. Brain weight, body length, body height and brood size were \log_{10} -transformed prior to analysis. In all analyses, log brain weight was the response variable and log body length or height was included to control for the effect of allometry. Three dichotomous factors that categorize individuals based on the body form (i.e. either pipefish or seahorse), preservation method (i.e. either formalin or ethanol) and sex were entered into each model to statistically control for potential biases introduced by these factors. Prior to the analyses, the degree of multicollinearity in each model was evaluated with the variance inflation factor (VIF) (Zuur *et al.*, 2010). We found that VIF < 2.0 in all but one case, where care duration (VIF = 8.9) and maximum latitude (VIF = 10.2) were highly correlated (Foster & Vincent,

2004; Wilson, 2009). We thus performed the phylogenetic generalized least square regression of care duration against maximum latitude (PGLS: care duration = 0.55(maximum latitude) + 1.18, $r^2 = 0.79$, $P < 0.001$) using the caper package (Orme *et al.*, 2011) version 0.5.2 and retained the residuals of this model (i.e. relative care duration) for analysis in a subsequent phylogenetic mixed model. We constructed five phylogenetic mixed models, each including one of the following main explanatory variable; SnL/HL ratio, egg size, relative care duration, sex or the interaction between sex and mating pattern, to test for its association with brain size. Sample size differed slightly between models due to the heterogeneity in data availability, and our molecular-morphological phylogeny was cropped to match the species included in each model. All continuous variables were scaled to enable comparisons of posterior mean slopes across variables. In all analyses, we used a flat improper prior on the phylogeny. Parameter expansion resulted in a scaled F distribution with numerator and denominator degrees of freedom set to 1 and a scale parameter of 1000. Employment of parameter-expanded priors improved chain mixing. Models were run for 800 000 iterations with 100 000 burnin and a thinning interval of 250. We formally assessed chain mixing using the Heidelberg criteria (Heidelberger & Welch, 1983) and confirmed that all models passed the test (Table S4).

Results

The history of diversification among 23 species of Syngnathidae used in our study is reflected in our molecular-morphological phylogenetic tree (Fig. 2). The phylogeny based on molecular data was in accordance with those previously presented in Wilson *et al.* (2001) and Casey *et al.* (2004). The phylogenetic positions of the three species (*D. boaja*, *D. martensii* and *N. lumbriciformis*) based on head morphology and brood pouch location were largely in agreement with Dawson (1985). Table 2 summarizes the results of phylogenetic mixed models that tested whether feeding ecology, life history or sexual selection is associated with relative brain size. Note that our interpretations of any associations with brain size in this study refer to relative brain size (i.e. brain size after controlling for the effect of body size) as we included body size as a covariate (either body height or body length) in all of our analyses. We found that the SnL/HL ratio was positively associated with relative brain size ($N_{\text{individual/species}} = 339/23$, posterior mean [lower, upper 95% C.I.] = 0.27 [0.04, 0.53], $p\text{MCMC} = 0.03$, Fig. 3a). Among the two life-history traits examined, we found a nonsignificant positive trend between egg size and relative brain size ($N_{\text{individual/species}} = 284/18$, posterior mean [lower, upper 95% C.I.] = 0.21 [-0.03, 0.43], $p\text{MCMC} = 0.09$, Fig. 3b), whereas we found no relationship between

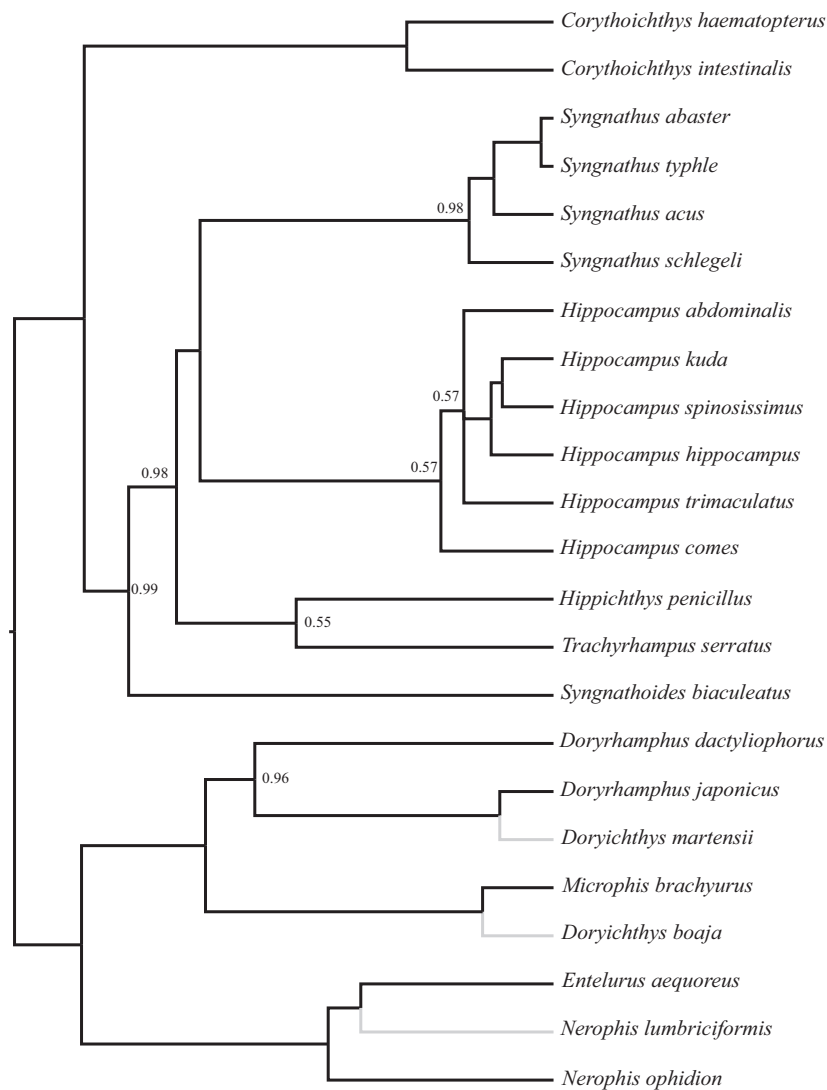


Fig. 2 Molecular-morphological phylogeny of 23 species of Syngnathidae included in this study, based on fragments of three mitochondrial genes, head morphology and brood pouch location (see text for more information). All nodes except for the ones indicated with values have a posterior probability of 1.00. Three grey tips indicate species that were added, based on morphological information, to the consensus molecular phylogeny.

Table 2 Bayesian estimates for multivariate models with phylogenetic correction testing for various hypotheses of brain size evolution in pipefishes and seahorses. The response variable is \log_{10} brain weight. Sample size, effective sample size (Eff. Samp.), posterior mean, 95% lower, followed by upper confidence intervals (C.I.) and p MCMC are presented. Significant P -values are shown in bold text. Note that all models included sex, body form and preservative method as a covariate in addition to the variables shown in this table (please see Table S3 for complete results).

Hypothesis	Main predictor (covariate)	Sample size indiv./species	Eff. Samp.	Posterior mean [95% C.I.]	p MCMC
(a) Feeding adaptation	SnL/HL ratio	339/23	3600	0.271 [0.042, 0.532]	0.034
	(\log_{10} body length)		3813	0.692 [0.617, 0.762]	< 0.001
(b) Energy trade-off	Egg size	284/18	3600	0.208 [-0.033, 0.432]	0.088
	(\log_{10} body length)		3600	0.741 [0.658, 0.819]	< 0.001
	Relative brood duration	250/14	3873	0.135 [-0.113, 0.380]	0.263
(c) Sexual selection	(\log_{10} body length)		3600	0.654 [0.577, 0.723]	< 0.001
	Sex	325/20	3431	-0.078 [-0.134, -0.027]	0.004
	(\log_{10} body length)		3600	0.704 [0.634, 0.784]	< 0.001
	Sex \times Mating pattern	243/12	3600	-0.059 [-0.119, 0.000]	0.054
	(\log_{10} body length)		3600	0.708 [0.619, 0.802]	< 0.001

Fig. 3 Relationship between (a) snout length to head length ratio (SnL/HL ratio) and relative brain size, (b) egg diameter and relative brain size and (c) relative brood duration and relative brain size. Relative brain size on the y-axis is the residuals from a phylogenetic generalized least square analysis of \log_{10} brain weight as a response variable and \log_{10} body length/height as an explanatory variable and body form as a covariate. Each data point represents one species, and error bars are the standard error of relative brain size for each species.

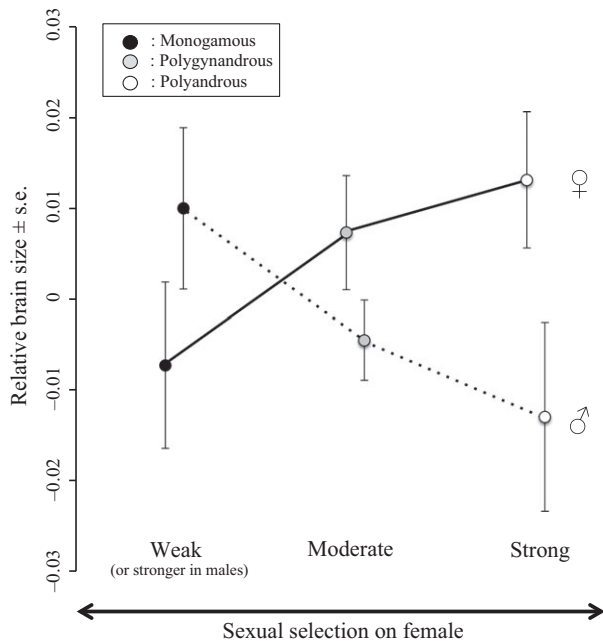
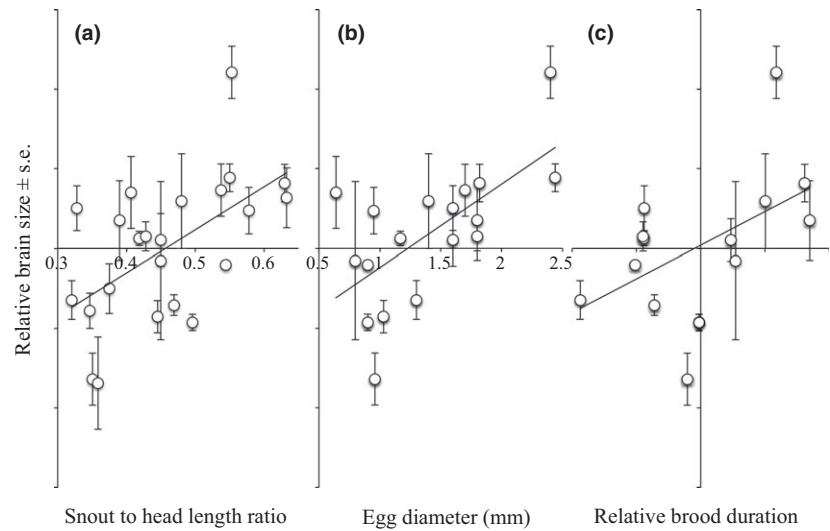


Fig. 4 Comparison of relative brain size between species with different mating patterns: monogamy (black, $N_{\text{individual/species}} = 101/5$), polygynandry (grey, $N_{\text{individual/species}} = 90/3$) and polyandry (open, $N_{\text{individual/species}} = 52/4$). Relative brain size on the x-axis is the residuals from a linear mixed-effects model of \log_{10} brain weight as a response variable and \log_{10} body length/height as a fixed effect and species identity as a random effect. The y-axis represents strength of sexual selection on females in correspondence to mating patterns (Jones & Avise, 2001). Mean observed relative brain size dimorphism \pm standard error is presented for males (connected with dashed lines) and females (connected with solid lines) separately.

relative paternal care duration and relative brain size ($N_{\text{individual/species}} = 250/14$, posterior mean [lower, upper 95% C.I.] = 0.14 [-0.01, 0.38], $p\text{MCMC} = 0.26$,

Fig. 3c). We found a sexual dimorphism in brain size: females had larger brains than males ($N_{\text{individual/species}} = 325/20$, posterior mean [lower, upper 95% C.I.] = -0.08 [-0.13, -0.03], $p\text{MCMC} = 0.004$). The posterior means indicate that, for a syngnathid fish with an average brain weight (6.7 mg), females have 4.3% heavier brains than males. Finally, we found a nonsignificant trend ($p\text{MCMC} = 0.05$) for an interaction between mating pattern and sex (Table 2). A closer visual investigation of this trend showed that sexual dimorphism in brain size was strongly female-biased in polyandrous species, less female-biased in polygynandrous species and male-biased in monogamous species (Fig. 4).

Discussion

Our study investigated three major hypotheses in brain size evolution using a novel data set of pipefishes and seahorses. We employed a Bayesian phylogenetic mixed model approach that enabled us to test our predictions using the full extent of the data (i.e. including both within and among-species variation) while correcting for phylogenetic nonindependence. Due to severe difficulty in collecting samples of this highly inconspicuous group of fishes, our species coverage is relatively low (i.e. 23 species) for phylogenetic comparative studies. We are fully aware of the statistical limitations that our data entail and emphasize that our results are proposals for future scrutiny. With these considerations in mind, we will below discuss our findings in the light of the three hypotheses tested in our study.

Feeding ecology

Natural selection for the ability to explore and exploit food resources has been proposed to be a key factor influencing vertebrate brain size evolution (Garamszegi

et al., 2002; Hutcheon *et al.*, 2002; MacLean *et al.*, 2014). In this study, we found that syngnathid fish species with relatively longer snouts, compared to the HL, had larger brains than species with relatively shorter snouts. In pipefishes and seahorses, a longer snout is a typical character of a species that consume highly mobile prey with rapid motions such as mysids, shrimps and fish, whereas shorter snout length is typical for species that consume slower moving prey such as amphipods, harpacticoid copepods and polychaetes (Kendrick & Hyndes, 2005). Functional morphological studies indicate that hunting of mobile prey would require better visual acuity and more precise coordination in locomotor movements than species foraging on slow moving food items (Higham, 2007; de Lussanet & Muller, 2007; Van Wassenbergh *et al.*, 2011). Specialization in visual abilities has been shown to be associated with relative brain size in primates (Barton, 1998), carnivores (Kirk, 2006) and birds (Garamszegi *et al.*, 2002). Although evidence for a direct link between relative brain size and visual specialization is still limited in fish (Huber *et al.*, 1997; White & Brown, 2015), we speculate that the ecological adaptation to feed on mobile, evasive prey may have selected for the enhancement of visual information processing (Garamszegi *et al.*, 2002) and thus led to relatively larger brain sizes in the more long snouted species of pipefishes and seahorses. In adding to previous examples of evolutionary covariations between brain size and feeding-related traits, our results provide support for the idea that improved cognition related to resource exploitation is an important factor in forming the diversity of vertebrate brain size (Cluttonbrock & Harvey, 1980; Hutcheon *et al.*, 2002; Garamszegi *et al.*, 2005; MacLean *et al.*, 2014; Lefebvre *et al.*, 2016).

Life history: egg size and parental care duration

In a wide variety of taxa, brain size evolution coincides with evolution in life-history traits (Isler & van Schaik, 2006, 2009; Weisbecker & Goswami, 2010; Barton & Capellini, 2011; Isler, 2011; Tsuboi *et al.*, 2015). It has been argued that the cost of evolving large brains underlies such life-history trade-offs. However, we did not find clear support for the energy trade-off hypothesis in pipefishes and seahorses. Our result revealed that duration of paternal care was not related to relative brain weight and egg diameter showed only a non-significant trend with relative brain weight. The contrast of our results with previously reported associations between life-history traits and relative brain size (birds: Isler & van Schaik, 2006; mammals: Isler & van Schaik, 2009; cichlids: Tsuboi *et al.*, 2015; anurans: Liao *et al.*, 2016) may indicate that pipefishes and seahorses employ energy allocation strategy that is atypical for vertebrates. Indeed, the family Syngnathidae is the only known vertebrate lineage that performs male

pregnancy. Therefore, one interpretation of our found lack of support for the energy trade-off hypothesis could be that the physiological connections of male pipefishes/seahorses with embryo (Ripley *et al.*, 2010; Sagebakken *et al.*, 2010; Kvarnemo *et al.*, 2011; Braga Goncalves *et al.*, 2015) may have changed the underlying life-history trade-offs associated with costly brain development and maintenance. However, given our relatively small sample size, we cannot rule out that we lack the necessary statistical power to detect existing patterns. Our findings highlight the need for future efforts to include wider taxonomic sampling to broaden and consolidate the scope of the energy trade-off hypothesis.

Sexual selection

Sexual selection has long been proposed to have major influence on vertebrate brain size evolution (Darwin, 1871; Francis, 1995; Jacobs, 1996; Miller, 2000; Kolm *et al.*, 2009; Boogert *et al.*, 2011; Kotrschal *et al.*, 2014), but studies supporting this idea remained inconsistent (Schillaci, 2006; Lemaitre *et al.*, 2009). The present study demonstrates that females have significantly larger brains than males in pipefishes and seahorses. The posterior distribution of our phylogenetic mixed model indicates that a female syngnathid has on average a 4.3% heavier brain than a male of the same species and body size. Sexual brain size dimorphism that supports the sexual selection hypothesis was previously reported in some taxa (Francis, 1995; Iwaniuk, 2001; Kotrschal *et al.*, 2012; Samuk *et al.*, 2014). Unlike those groups previously investigated in the context of the role of sexual selection in brain size evolution, offspring care in Syngnathidae involves less cognitively demanding care behaviours, that is, they change to a passive behaviour during brood care (Svensson, 1988; Cunha *et al.*, 2016). Therefore, our results add substantial support to the idea that sexual selection is involved in brain size evolution.

In addition, we found that more polyandrous species tended to have a much higher degree of female-favoured dimorphism in brain size as females had almost 12% heavier brains than males in these species. This pattern cannot be only explained by the allometric relationship between brain size and body size because the brain-body allometric relationship is < 1.0 in our data (PGLS: $\log_{10}(\text{brain mass}) = 0.81\log_{10}(\text{body length})^{3/2} - 1.83$). With female-biased sexual size dimorphism in polyandrous pipefishes, the observed allometric slope should therefore yield male-biased relative brain size dimorphism. Thus, our found female-biased brain size dimorphism indicates that even under the allometric constraints where larger bodied animals tend to have proportionally smaller brain size, sexual selection in females of polyandrous pipefishes is strong enough to generate an overall female-biased brain size dimorphism. Our found trend corroborates the idea that

sexual selection resulted in pronounced degree of female-biased sexual dimorphism in brain size, as females of polyandrous pipefishes experience considerably stronger sexual selection than polygynandrous and monogamous syngnathids (e.g. Rosenqvist & Berglund, 2011). As emphasized earlier, however, this pattern is based on a relatively small number of species per category of mating pattern (i.e. monogamous: $N_{\text{individual/species}} = 101/5$, polygynandrous: $N_{\text{individual/species}} = 90/3$, polyandrous: $N_{\text{individual/species}} = 52/4$). Therefore, this trend needs to be cautiously interpreted. Nevertheless, overall, our results are highly encouraging for future studies to further investigate the role of sexual selection in brain size evolution.

In summary, our study demonstrates the role of feeding ecology, life history and sexual selection in brain size evolution of pipefishes and seahorses. Most importantly, our results reveal the involvement of sexual selection on females in brain size evolution. Together with existing literature on similar patterns for males in lineages with conventional sex roles (Kolm *et al.*, 2009; Kotschal *et al.*, 2012, 2014; Fusani *et al.*, 2014; Samuk *et al.*, 2014), our study adds significant credibility to the hypothesis that sexual selection is an important factor for brain size evolution.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Ecological and life history information of Syngnathidae

Table S2 Data accession number for three regions of mitochondrial gene

Table S3 Full result of the phylogenetic mixed model

Table S4 Result of the Heidelberger and Welch's convergence diagnostics

Data deposited at Dryad: doi: 10.5061/dryad.45q7h

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