# Evolutionary history of elongation and maximum body length in moray eels (Anguilliformes: Muraenidae)

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Body shape and size are important axes of organismal diversification. The elongate body form has evolved repeatedly in disparate vertebrate clades, and is associated with a variety of maximum body lengths. We used a time-calibrated phylogeny for 40 species of moray eels to analyse the evolution of elongation and the morphological mechanisms underlying variation in body shape and maximum body length. We find that body elongation in morays evolves independently of elongation of the vertebral column. In contrast, maximum body length evolves by a different mechanism: through region-specific increases in vertebral number, elongation of individual vertebral centra, and postembryonic somatic growth. We reconstruct an ancestral moray eel and provide evidence for accelerated morphological evolution in three highly elongate species that are associated with a burrowing lifestyle. We compare these patterns with those described for other vertebrates, and show that body shape and body length may evolve independently of each other and (in the case of shape) of the vertebral column. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **109**, 861–875.

ADDITIONAL KEYWORDS: axial skeleton – morphological evolution – pleomerism – size – vertebrae – vertebral aspect ratio.

## INTRODUCTION

Elongate vertebrates occupy a particularly interesting position along the body shape continuum, as they reflect one end of the extremes. The elongate body shape has evolved repeatedly in almost every vertebrate lineage, including fishes (Ward & Brainerd, 2007; Mehta *et al.*, 2010; Ward & Mehta, 2010), amphibians (Wake, 1966; Parra-Olea & Wake, 2001), reptiles (Johnson, 1955; Wiens & Slingluff, 2001; Brandley, Huelsenbeck & Wiens, 2008; Bergmann & Irschick, 2010), and mammals (Brown & Lasiewsk, 1972). Over 15% of actinopterygian fishes are considered highly elongate, with at least half of these representatives having an eel-like or 'anguilliform' body plan (Nelson, 2006; Ward & Mehta, 2010). Elongation of the body typically involves increasing the number of vertebrae or elongating individual vertebrae in different body regions (Richardson *et al.*, 1998; Ward

& Brainerd, 2007). Although the repeated evolution of elongation implies common mechanisms of morphological evolution, recent studies have revealed diversity in how the axial skeleton is altered to produce elongation in fishes (Ward & Brainerd, 2007; Mehta *et al.*, 2010).

In most fishes, the maximum recorded body length is positively correlated with mean vertebral number, a pattern more commonly known as pleomerism (Lindsey, 1975; Spouge & Larkin, 1979). As a phenomenon, pleomerism is taxonomically informative, particularly when the comparison is between fish groups that share a particular body shape. For example, although highly elongate fishes such as congrid eels reach similar mean lengths as centropomids (collectively known as snooks), they have six times as many vertebrae (Mehta *et al.*, 2010). Lindsey (1975) further explained this variation in vertebral number with the observation that when species have fewer vertebrae than would be anticipated from length alone, this pattern is typically the result of variation in the second major body axis, body depth or

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**Figure 1.** Histograms for the metrics analysed to describe the shape of the body, axial skeleton, and maximum body length in moray eels: A, elongation ratio (ER; this metric of shape divides the standard length by the second major body axis) ; B, axial elongation index (AEI); and C, maximum body length. Measurements were made on 40 species of morays and 33 species of non-moray anguilliform fishes that were selected to represent the range of anguilliform morphological diversity (species list available in Mehta *et al.*, 2010). The *x*-axis values represent the maximum value for a given bin. Individuals placed in a bin have a value between the preceding bin and the bin in which they reside. Moray eels span the majority of the variation in ER, AEI, and maximum body length present in the Anguilliformes. The moray species *Scuticaria tigrina*, *Rhinomuraena quaesita*, and *Pseudoechidna brummeri* (indicated by asterisks) represent extremes in all three histograms.

width, suggesting a deviation in shape. Specifically, the greater the variation in body shapes among species, the weaker the relationship between mean vertebral number and maximum recorded length.

Body shape and maximum length are two important axes of diversification among organisms, as each have substantial effects on organismal–environment interactions (Brown & Lasiewsk, 1972; Schmidt-Nielsen, 1984). Certain shapes may place a limit on the maximum length an organism may attain, particularly in endotherms, where metabolic rate, body size, and shape are tightly interrelated (Brown & Lasiewsk, 1972; McMahon, 1973; Heusner, 1985); however, many ectotherms such as fish show a decoupling of this relationship, and organisms with similar body shapes can exhibit a wide range of maximum body lengths. Examining how a particular body shape and its maximum length evolve among related species may provide insight into the different mechanisms underlying these changes, as well as factors that may influence basic body design.

Recent studies have quantified variation in fish body shape (Walker, 1997; Walker & Bell, 2000) and length (Nagel & Schluter, 1998), and others have identified developmental and genetic mechanisms influencing some of this variation (Peichel *et al.*, 2001; Morin-Kensicki, Melancon & Eisen, 2002; see also Gomez & Pourquié, 2009); however, few works examine how evolutionary changes in the axial skeleton are correlated with changes in body shape or how these changes may relate to maximum body length within a particular clade (McDowall, 2003, 2008). Here, we investigate body elongation and maximum body length evolution in moray eels, a group of elongate marine fishes.

Moray eels (Muraenidae), one of the largest clades within Anguilliformes, are a group of 197 species (Smith, Irmak & Özen, 2012), distributed in every ocean basin. Despite being universally elongate, morays exhibit a surprising range of body sizes in relation to both mass and total length. Moray species range in adult body mass from 4 to over 600 g, and from 10 to 400 cm in total length (FishBase, http://fishbase.org; Froese & Pauly, 2011). With respect to body shape and maximum length, morays encompass most of the diversity present among anguilliform fishes that have been studied to date (Fig. 1; Mehta *et al.*, 2010). Remarkably, mean vertebral numbers in morays show a 2.4-fold variation, comparable with the variation described across all extant snakes, a distantly related vertebrate clade with members exhibiting a convergent body plan and containing roughly five times as many species (Polly, Head & Cohn, 2001).

Our primary goals are to examine components of the axial skeleton to understand the evolution of elongation and maximum body length among 40 species of morays. To better understand body shape evolution in morays, we investigate the evolutionary relationship between body elongation and the axial skeleton, as well as the interaction between body shape and maximum body length. We then infer the ancestral body shape and maximum body length for morays and trace the pattern of evolution from that inferred ancestor to extant species to investigate the pattern and timing of morphological diversification.

## MATERIAL AND METHODS

### PHYLOGENETIC AND MORPHOLOGICAL DATA

We examined the evolution of elongation and maximum body length in moray eels using a molecular phylogeny of 40 muraenid species, pruned from a tree of 46 species (Reece, Bowen & Larson, 2010). This recently published moray time tree (Fig. 2) was calibrated with both fossil and biogeographic data (Reece *et al.*, 2010). Moray relationships were based on portions of two mitochondrial (cytochrome *b* and *cytochrome oxidase subunit 1*) and two nuclear (recombination activating protein *RAG-1* and recombination activating protein *RAG-2*) genes, corresponding to GenBank accessions HQ122450–HQ122568, HQ1442581, and HQ1442588–HQ1442590. Alcohol-preserved specimens, totalling 153 individuals, with between one and eight representatives of each species, were obtained through personal collections and museum loans (Table S1). From these specimens we measured total length, maximum body depth and
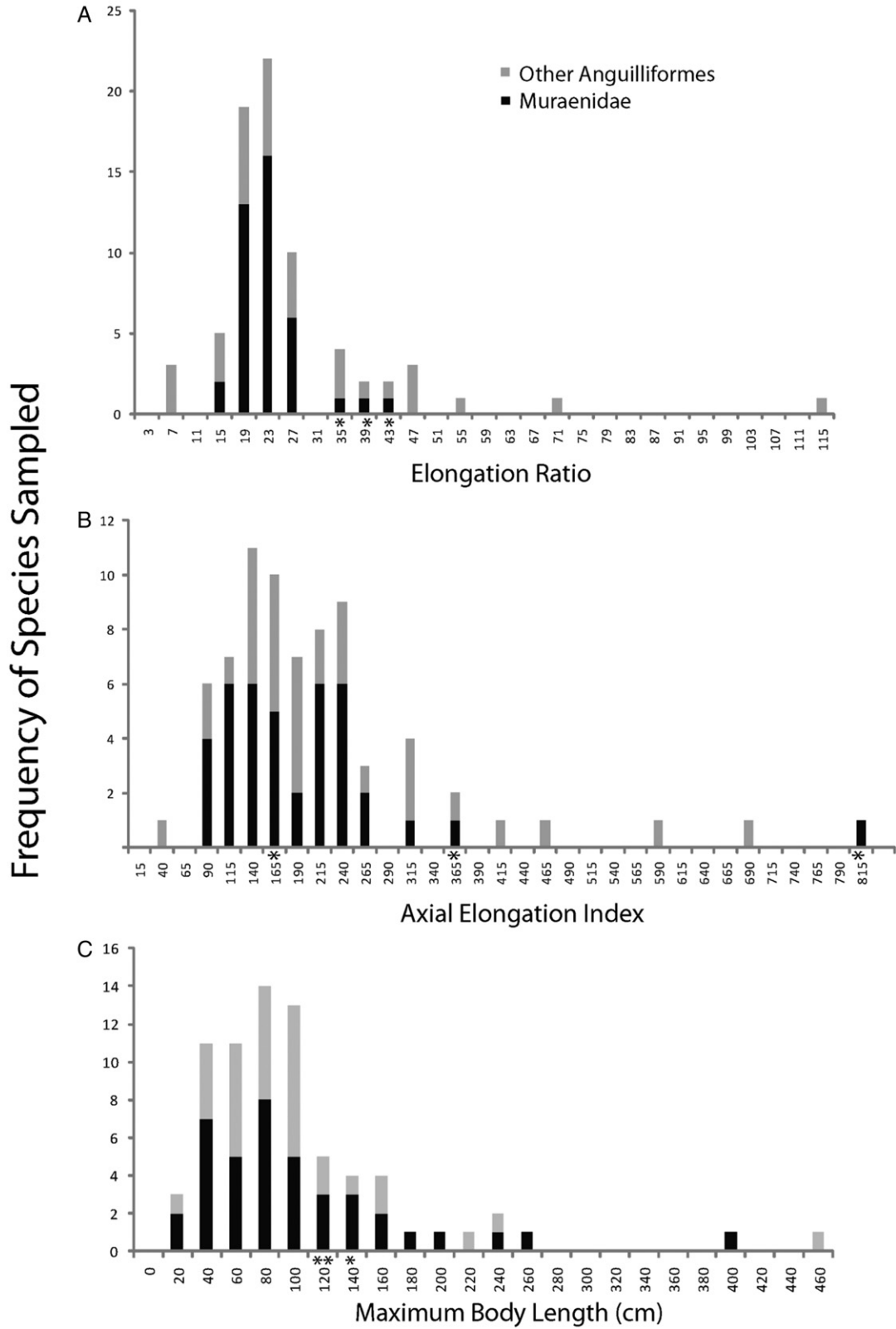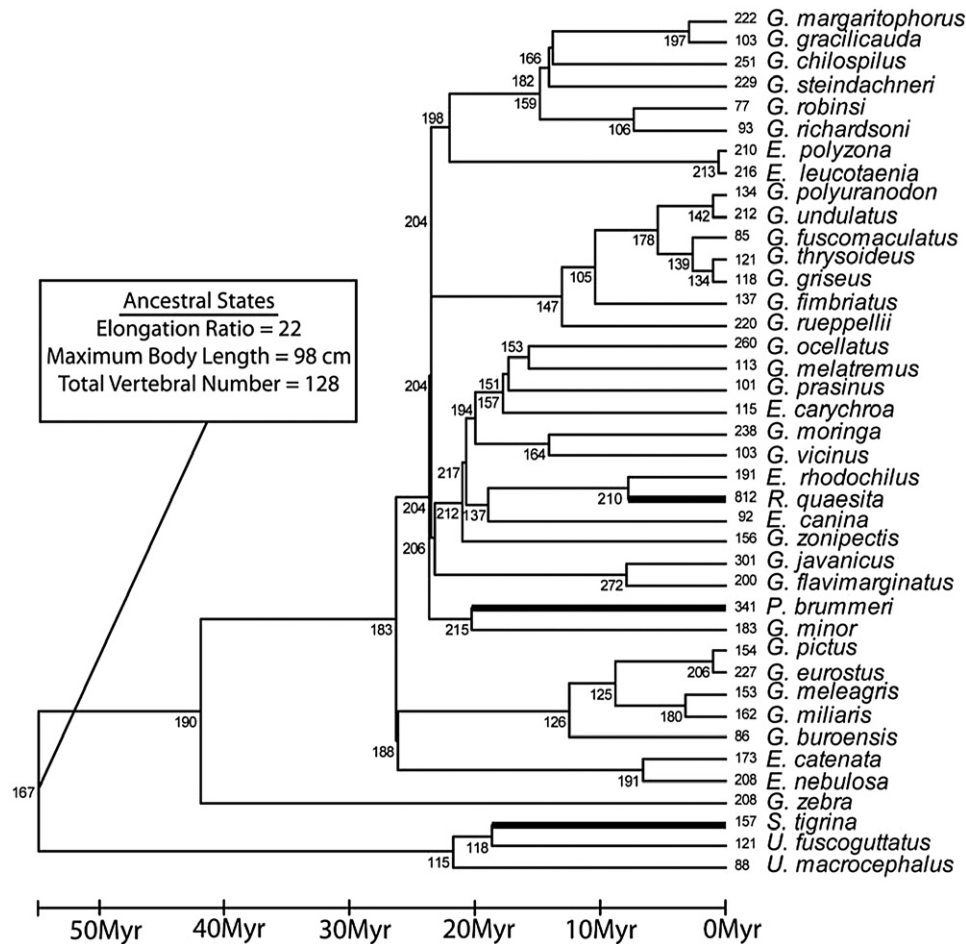
**Figure 2.** Phylogeny of selected muraenid species. Ancestral states for the root of the moray tree are given for the elongation ratio (ER), axial elongation index (AEI), maximum body length, and total vertebral number. Values at all remaining nodes are given for AEI (see Material and methods for details on ancestral state reconstructions). Branches set in bold indicate three species with elongate body forms; note that despite an elongate body shape, *Scuticaria tigrina* does not have an elongate vertebral column. Time is in Myr, with a root age of 56 Myr.

width, and head length. With these measurements we calculated a metric of body shape known as the elongation ratio (ER), defined as the total length of an individual divided by the second largest major body axis: depth or width (Ward & Azizi, 2004). Specimens were then cleared and stained following a modified protocol of Dingerkus & Uhler (1977). We recorded the following measurements on cleared and stained individuals: (1) the number of precaudal vertebrae, defined as those that were cranial to vertebrae with fused haemal arches; (2) the number of caudal vertebrae, defined as vertebrae with fused haemal arches found in the caudal region. Vertebral counts were made three times in both precaudal and caudal regions, and the average number of vertebrae in each region was used for all analyses; (3) precaudal vertebral aspect ratios; and (4) caudal vertebral aspect ratios. We calculated vertebral aspect ratio by divid-

ing vertebral centrum length by vertebral centrum width (Ward & Brainerd, 2007). Centrum lengths and widths were measured for three vertebrae from each region using digital calipers at a precision of 0.01 mm. Vertebral measurements were not taken on adjacent vertebrae in an effort to capture variation from each region without sampling extremely small vertebrae just posterior to the skull and at the tip of the tail. To characterize elongation of the axial skeleton, we adopted the axial elongation index (AEI; Ward & Brainerd, 2007). AEI is defined as (precaudal vertebral number × precaudal aspect ratio) + (caudal vertebral number × caudal aspect ratio). To understand the relationships between AEI, ER, and maximum body length, we recorded the maximum body length reported in the literature for each species as reported in FishBase (Froese & Pauly, 2011), which lists specimen data amalgamated from several major museum

collections, and also lists references for published size records. We report species averages for all metrics.

## EVOLUTION OF ELONGATION

Our sampling of morays included some of the smallest species (e.g. *Gymnothorax robinsi*), the largest species (*Gymnothorax javanicus*), and three of the four most elongate species (*Scuticaria tigrina*, *Rhinomuraena quaesita*, and *Pseudoechidna brummeri*). Because these three species show patterns of elongation that are outside of the distribution of the remaining 37 species (Fig. 1a), we conducted the correlation analyses described below using 37 species that fit a statistically normal distribution for ER, and discuss these highly elongate species separately.

The teleost vertebral column can be divided into the trunk (precaudal) and caudal regions (Liem *et al*., 2001). Recent studies have recognized region-specific patterns in the vertebral column of fishes (Ward & Brainerd, 2007; Yamahira & Nishida, 2009; Mehta *et al*., 2010). These patterns have been attributed to modularity, the idea that body regions may act as discrete subunits with varying degrees of connectivity to other subunits (Raff, 1996; Skinner & Lee, 2009). Ward & Brainerd (2007) proposed three models for assessing modularity in vertebral number and vertebral aspect ratio in actinopterygian fishes. Reduced major axis regression was used to examine the relationship between characteristics in the precaudal and caudal region; the slope of the line was used to assess developmental modularity across the two regions. A slope of one indicated equal changes in the number and aspect ratio of precaudal and caudal vertebrae, suggesting a shared developmental module across regions. A slope greater than or less than one suggested that changes in the number or shape of vertebrae occur at a different rate in a particular body region. The lack of a linear relationship between regions indicates that vertebral number or shape evolve independently of each body region (Ward & Brainerd, 2007). Therefore, we tested for the evolution of modularity in moray vertebral numbers and aspect ratios across the two body regions. We estimated Pagel's lambda (λ, a metric of phylogenetic signal; Pagel, 1999) for each trait using the 'Geiger' package (Harmon *et al*., 2008) in R (R Core Development Team, 2009). As traits varied in their level of phylogenetic signal, we tested for correlations between precaudal and caudal vertebral numbers and between precaudal and caudal aspect ratios using: (1) phylogenetic independent contrasts (PICs; Felsenstein, 1985); (2) PICs calculated on trees uniquely transformed to reflect the phylogenetic signal (λ) of each trait; and (3) for instances of low to zero phylogenetic signal, we directly compare verte-

bral counts or aspect ratios without a phylogenetic correction. Our results were strongly consistent across all three approaches. To calculate slope, we computed reduced major axis (RMA) regressions forced through the origin using the *line.cis* command in the '(S)MATR' package (http://web.maths.unsw. edu.au/~dwarton) for R. For each regression, we used the test developed by Warton *et al*. (2006) to determine whether the slope was significantly different from 1.

Body shape, measured in terms of ER, and the axial skeleton have been shown to evolve independently of each other across Anguilliformes (Mehta *et al*., 2010); however, as Anguilliformes is comprised of several diverse clades, we tested whether this pattern would be maintained when looking closely within one of the larger and more ecologically diverse groups, such as morays. Four mechanisms of elongation have been previously suggested: regional increases in vertebral number; regional vertebral centrum elongation; elongation of the head; or reduction to the second major body axis (Ward & Brainerd, 2007). We used phylogenetic generalized least squares (PGLS) regressions (Grafen, 1989; see also Revell, 2010) to simultaneously estimate the correlation between variables and the phylogenetic signal present in that correlation. When no phylogenetic signal is present, PGLS is equivalent to a standard generalized least squares regression. We used PGLS multiple regression model building to determine which combination of the six predictors (the number of vertebrae in the caudal and precaudal region, aspect ratios in each region, and size-corrected head length and body depth) best explains variation in body elongation (ER) and maximum body length. All variables were log-transformed, and head length and body depth were size corrected by total body length using a phylogenetic size correction (Revell, 2009). We compared the fit of the models using Akaike's information criterion corrected for finite sample sizes (ΔAICc), which identifies the best fit model as the one with the lowest AICc value, and the difference between that model and the next best model is greater than four AICc units (Burnham & Anderson, 2002). We also use this method to identify which morphological variables contribute most to the variation in AEI.

## ACCELERATED RATES OF EVOLUTION

We test the hypothesis that the three highly elongate morays exhibit accelerated rates of evolution or attraction towards alternative morphological optima relative to the remaining 37 species sampled. Using our timed tree, we fitted four models of morphological evolution in the RBROWNIE module (Stack, Harmon

& O'Meara, 2011), which is an R implementation of the program BROWNIE (O'Meara, Ané & Sanderson, 2006), to each of the following: ER; AEI; the total number of vertebrae; the numbers of precaudal and caudal vertebrae; and maximum body length. For each character, we fitted a single-rate Brownian motion (BM) model (BM1), where morphological evolution is a random-walk process along a phylogenetic tree, a two-rate BM model (BM2), where the three highly elongate species share one rate of BM evolution and all remaining species share a different rate, an Ornstein–Uhlenbeck model (known as the 'rubber band' BM), where lineages evolve according to BM but with an attraction parameter towards a shared optimum (Butler & King, 2004), and an OU2 model, where the three highly elongate species are attracted towards a different adaptive optimum than the remaining 37 species. For OU2 and BM2 models, the highly elongate morays were considered to be in a different state relative to the remaining 37 species for the entire length of the branch upon which they reside. Model fitting in RBROWNIE was based on 500 equally likely phylogenetic reconstructions sampled from the prior distribution estimated in Reece *et al.* (2010). We compared the fit of models using ΔAICc, as previously described. When OU2 or BM2 models were selected, we tested whether or not this inference could be repeated by randomly selecting three species from the tree for 1000 iterations using the R package 'Picante' (Kembel *et al.*, 2010).

### RECONSTRUCTING THE ANCESTRAL MORAY

We reconstructed ancestral states by nesting the Reece *et al.* (2010) phylogeny of Muraenidae within the best available phylogeny for Anguilliformes (Johnson *et al.*, 2012; Fig. S1). We replaced the three species that represented Muraenidae in Johnson *et al.* (2012) with the time tree of 40 species pruned from Reece *et al.* (2010). Johnson *et al.* (2012) included 39 non-muraenid species of Anguilliformes: data on ER, total number of vertebrae, and maximum body length were available from FishBase for 39, 17, and 13 of those species, respectively (Table S2), whereas data on the other character traits were not available for these out-group taxa. We reconstructed the ancestral state of Muraenidae within the anguilliform tree for these three traits using the *ace* function in Geiger and maximum likelihood. We reconstructed ancestral states for the remaining traits, the number of caudal and precaudal vertebrae, and AEI using only muraenids in 500 trees sampled from the post burn-in posterior distribution in BEAST (Drummond & Rambaut, 2007), as described in Reece *et al.* (2010). Some traits were inferred to evolve according to a BM2 model of evolution (see the Results), with an

accelerated rate estimated for the highly elongate species and a slower rate inferred for the remaining 37 species; however, ancestral state reconstructions typically assume a single BM rate. To account for the different rate on the branches leading to the three highly elongate species, we transformed these branch lengths separately for each trait by a factor equal to the accelerated BM rate divided by the BM rate for the 37 remaining species. This lengthened each of the three branches in such a way that it was appropriate to estimate ancestral states according to a single-rate BM method, as is implemented in the *ace* function. For the number of precaudal and caudal vertebrae and for AEI we report on the mean value estimated across 500 trees for the ancestral state of each node.

## RESULTS

### MORAYS ALTER BODY SHAPE WITHOUT CHANGING THE AXIAL SKELETON

We present species means for all morphological data in Table 1. The 40 moray species in our data set exhibit a two-fold variation in total vertebral numbers, ranging from 106 to 258. On average, vertebrae were distributed equally across the precaudal and caudal regions, whereas in the three most elongate species, caudal vertebrae comprised between 53 and 68% of total vertebrae. The number of precaudal vertebrae ($\lambda = 0.60$) was the only trait in these regressions with $\lambda > 0.001$. RMA regressions for all three approaches (with and without phylogenetic correction) revealed no significant relationship ($P = 0.2$; values based on direct vertebral counts without a phylogenetic correction; Fig. 3a), and thus vertebral number evolves independently across the precaudal and caudal regions. However, we find that vertebral aspect ratio evolves in a correlated fashion across the two body regions, with aspect ratios in the caudal region changing at a slightly faster rate (Fig. 3b; $P < 0.001$; slope = 1.24, 95% confidence interval = 1.09–1.46; values based on direct vertebral counts without a phylogenetic correction).

We find that ER is not significantly correlated with AEI across the 37 species of muraenids ($P = 0.7$; Fig. 4). Because ER includes head length, whereas AEI excludes any contribution of the head to the elongation of the body, we re-examined this relationship using $ER_{body}$, calculated as: (total length – head length)/(second major body axis), and found no differences in our final results. Multiple regression model building reveals that size-corrected maximum body depth explains 97% of the variation in ER. Size-corrected head length co-varies with body depth ($R^2 = 0.4$, $P < 0.001$, slope = 0.7), with deeper-bodied morays having longer heads relative to total body

**Table 1.** Raw data for the 40 species of moray eels used in this study. Genera are abbreviated after their first mention in the table. The three elongate species referred to in the text are listed at the bottom of the table. Columns include total number of vertebrae (TV), precaudal (PCN) and caudal (CN) number of vertebrae, precaudal (PCAR) and caudal (CAR) aspect ratios, elongation ratio (ER), axial elongation index (AEI), maximum body length (MBL), body length (BL, excluding head length), tail length (TL), maximum body depth (MBD), maximum body width (MBW), and maximum tail depth (MTD) and width (MTW). Mean variation among species is more than twice the mean variation in trait values among individuals of the same species

| Species | TV | PCN | CN | PCAR | CAR | ER | AEI | MBL | BL | TL | MBD | MBW | MTD | MTW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Echidna catenata | 122 | 62 | 61 | 1.32 | 1.51 | 20.02 | 172.93 | 165 | 22.78 | 10.28 | 1.14 | 0.50 | 0.91 | 0.65 |
| E. leucotaenia | 125 | 55 | 70 | 1.59 | 1.82 | 17.64 | 215.50 | 75 | 14.23 | 7.40 | 0.81 | 0.45 | 0.45 | 0.38 |
| E. nebulosa | 122 | 63 | 59 | 1.70 | 1.72 | 23.26 | 207.81 | 100 | 14.15 | 6.87 | 0.61 | 0.36 | 0.56 | 0.29 |
| E. polyzona | 120 | 57 | 63 | 1.79 | 1.71 | 17.68 | 209.81 | 70 | 24.75 | 12.35 | 1.40 | 0.75 | 1.08 | 0.44 |
| E. rhodochilus | 120 | 60 | 60 | 1.75 | 1.44 | 24.63 | 191.35 | 33.8 | 28.73 | 14.07 | 1.17 | 0.80 | 0.60 | 0.93 |
| Enchelycore carychroa | 131 | 57 | 75 | 0.96 | 0.82 | 24.67 | 115.31 | 34 | 17.27 | 9.03 | 0.70 | 0.37 | 0.62 | 0.37 |
| Enchelynassa canina | 141 | 70 | 71 | 0.68 | 0.63 | 15.83 | 92.00 | 250 | 33.04 | 16.68 | 2.09 | 1.46 | 1.89 | 1.21 |
| Gymnomuraena zebra | 137 | 97 | 39 | 1.41 | 1.80 | 20.75 | 207.67 | 150 | 27.67 | 8.17 | 1.33 | 0.80 | 0.92 | 0.95 |
| Gymnothorax buroensis | 111 | 53 | 57 | 0.87 | 0.68 | 17.61 | 85.87 | 35 | 16.23 | 8.37 | 0.92 | 0.49 | 0.90 | 0.44 |
| G. chilospilus | 126 | 57 | 69 | 2.06 | 1.93 | 21.66 | 250.71 | 50.5 | 17.33 | 9.30 | 0.80 | 0.31 | 0.60 | 0.45 |
| G. eurostus | 119 | 55 | 64 | 1.92 | 1.89 | 18.40 | 227.22 | 60 | 16.07 | 8.80 | 0.87 | 0.79 | 0.82 | 0.48 |
| G. fimbriatus | 121 | 57 | 65 | 1.05 | 1.19 | 17.29 | 136.60 | 80 | 17.00 | 9.27 | 0.98 | 0.55 | 0.88 | 0.50 |
| G. flavimarginatus | 134 | 67 | 68 | 1.52 | 1.46 | 17.44 | 200.34 | 240 | 15.70 | 8.60 | 0.90 | 0.50 | 0.70 | 0.43 |
| G. fuscomaculatus | 117 | 54 | 63 | 0.81 | 0.65 | 19.54 | 84.66 | 20 | 16.85 | 9.10 | 0.86 | 0.58 | 0.74 | 0.45 |
| G. gracilicauda | 127 | 55 | 72 | 0.91 | 0.73 | 21.54 | 102.77 | 32 | 18.58 | 10.23 | 0.86 | 0.50 | 0.79 | 0.48 |
| G. griseus | 134 | 62 | 72 | 0.81 | 0.94 | 26.67 | 117.76 | 65 | 21.00 | 12.55 | 0.79 | 0.51 | 0.76 | 0.45 |
| G. javanicus | 125 | 57 | 68 | 2.35 | 2.45 | 14.69 | 301.48 | 400 | 23.27 | 12.37 | 1.58 | 0.63 | 0.87 | 0.72 |
| G. margaritophorus | 131 | 57 | 74 | 1.82 | 1.61 | 19.94 | 222.32 | 70 | 20.43 | 10.85 | 1.03 | 0.49 | 0.68 | 0.46 |
| G. melatremus | 141 | 63 | 77 | 0.90 | 0.73 | 19.65 | 113.34 | 26 | 22.27 | 12.67 | 1.13 | 0.58 | 0.93 | 0.52 |
| G. meleagris | 124 | 59 | 65 | 1.22 | 1.26 | 15.15 | 152.94 | 120 | 17.93 | 10.10 | 1.18 | 0.73 | 1.18 | 0.62 |
| G. miliaris | 123 | 56 | 68 | 1.39 | 1.25 | 18.25 | 161.77 | 70 | 20.08 | 11.40 | 1.10 | 0.65 | 1.01 | 0.61 |
| G. minor | 131 | 60 | 71 | 1.37 | 1.42 | 19.17 | 182.64 | 54.5 | 32.27 | 17.57 | 1.68 | 1.22 | 1.42 | 1.13 |
| G. moringa | 136 | 60 | 76 | 1.77 | 1.73 | 19.49 | 237.65 | 200 | 18.51 | 9.98 | 0.95 | 0.41 | 0.72 | 0.43 |
| G. ocellatus | 143 | 59 | 84 | 1.77 | 1.87 | 19.59 | 260.06 | 90 | 25.22 | 14.53 | 1.29 | 0.83 | 1.04 | 0.74 |
| G. pictus | 134 | 70 | 64 | 1.11 | 1.19 | 20.38 | 153.63 | 140 | 16.17 | 7.90 | 0.79 | 0.53 | 0.54 | 0.42 |
| G. polyuranodon | 147 | 83 | 64 | 0.84 | 1.01 | 23.69 | 133.93 | 92.5 | 30.80 | 14.27 | 1.30 | 0.68 | 1.30 | 0.53 |
| G. prasinus | 137 | 64 | 73 | 0.81 | 0.67 | 18.78 | 100.70 | 91.5 | 28.80 | 15.03 | 1.53 | 1.03 | 1.48 | 0.93 |
| G. richardsoni | 114 | 56 | 57 | 0.93 | 0.72 | 20.80 | 93.13 | 32 | 23.93 | 11.65 | 1.15 | 0.63 | 0.95 | 0.58 |
| G. robinsi | 107 | 57 | 50 | 0.76 | 0.67 | 19.83 | 76.81 | 18.2 | 11.90 | 5.60 | 0.60 | 0.30 | 0.50 | 0.30 |
| G. rueppellii | 132 | 62 | 70 | 1.83 | 1.52 | 18.75 | 220.40 | 80 | 24.85 | 13.35 | 1.33 | 0.55 | 0.80 | 0.57 |
| G. steindachneri | 130 | 57 | 73 | 1.89 | 1.68 | 15.86 | 229.36 | 91 | 23.00 | 12.25 | 1.45 | 0.70 | 1.02 | 0.60 |
| G. thyrsoideus | 139 | 62 | 77 | 0.89 | 0.86 | 21.61 | 120.99 | 65 | 20.53 | 11.82 | 0.95 | 0.40 | 0.84 | 0.38 |
| G. undulatus | 131 | 60 | 70 | 1.65 | 1.60 | 20.02 | 212.33 | 150 | 22.13 | 12.06 | 1.11 | 0.72 | 0.71 | 0.55 |
| G. vicinus | 132 | 56 | 76 | 0.83 | 0.75 | 14.80 | 102.94 | 122 | 36.08 | 19.58 | 2.44 | 1.49 | 2.40 | 1.46 |
| G. zonipectis | 124 | 54 | 70 | 1.13 | 1.35 | 21.42 | 155.75 | 50 | 19.28 | 10.75 | 0.90 | 0.64 | 0.88 | 0.56 |
| Uropterygius fuscoguttatus | 119 | 61 | 57 | 1.09 | 0.95 | 24.17 | 121.50 | 30 | 14.50 | 7.55 | 0.60 | 0.35 | 0.63 | 0.33 |
| U. macrocephalus | 106 | 52 | 54 | 0.84 | 0.81 | 15.61 | 87.94 | 45 | 14.47 | 7.90 | 0.93 | 0.45 | 0.64 | 0.41 |
| Pseudoechidna brummeri | 204 | 91 | 113 | 1.52 | 1.80 | 41.91 | 341.03 | 103 | 50.08 | 28.23 | 1.20 | 0.71 | 0.82 | 0.41 |
| Rhinomuraena quaesita | 258 | 82 | 176 | 2.51 | 3.45 | 37.27 | 812.48 | 130 | 76.40 | 61.00 | 2.05 | 1.30 | 1.00 | 0.82 |
| Scuticaria tigrina | 134 | 63 | 71 | 1.15 | 1.19 | 34.29 | 156.83 | 120 | 72.00 | 32.00 | 2.10 | 1.30 | 1.65 | 1.30 |
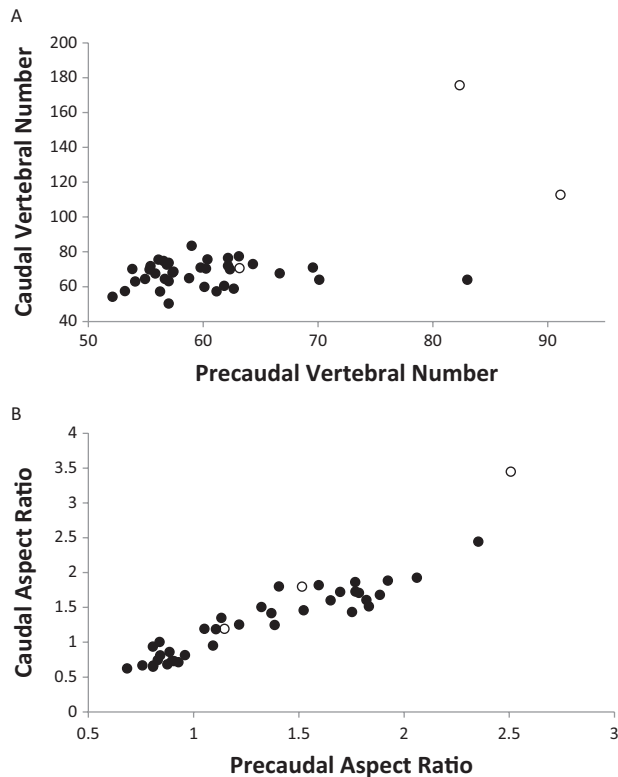
**Figure 3.** A, precaudal vertebral number plotted against caudal vertebral number; note that the axes are not on the same scale, and that caudal numbers are typically slightly higher than the precaudal numbers. Extremely elongate species are depicted by open circles, with the 37 remaining species represented by black circles. There is no linear relationship between precaudal and caudal number according to reduced major axis (RMA) regressions. B, precaudal and caudal aspect ratios; there is a strong linear relationship between aspect ratios in the caudal and precaudal regions, with caudal aspect ratios increasing slightly faster than precaudal ratios (slope = 1.24; 95% confidence interval = 1.09–1.42).

length. Therefore, rather than through changes in the vertebral column, the wide variation in moray ER has evolved primarily through changes in body depth. In fact, for all but the three most elongate species, body depth scales linearly with body length ($P < 0.001$, $R^2 = 0.78$, slope = −0.75; Fig. 5). The skinniest moray in our data set, *P. brummeri*, has the highest ER but not the highest AEI, whereas *Scuticaria tigrina* has the third highest ER but an intermediate AEI relative to all the morays sampled. A narrow range of AEI (77–121; 20% of the observed range in AEI) encompasses more than 95% of the observed variation in ER (Fig. 4).

Morays show extensive variation in maximum body length, encompassing much of the variation in anguilliform fishes examined thus far (Fig. 1c). Multiple
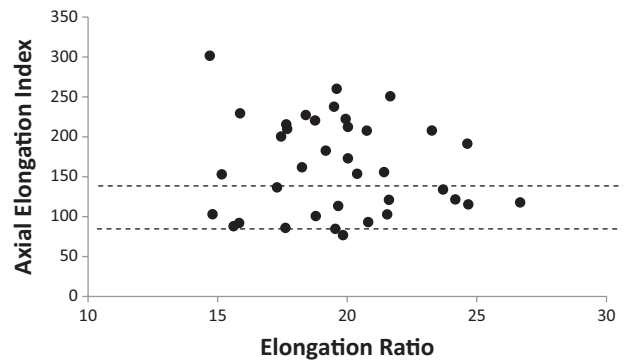


**Figure 4.** Elongation ratio (ER) and axial elongation index (AEI) for 37 species of morays in Figure 2; the three extremely elongate species are not included in this plot. Dotted lines bracket the range in AEI that encompasses more than 95% of the range in ER, emphasizing that a narrow range of vertebral elongation spans most of the range in body shape elongation. There is no linear relationship between ER and AEI according to phylogenetic generalized least squares (PGLS) regression.
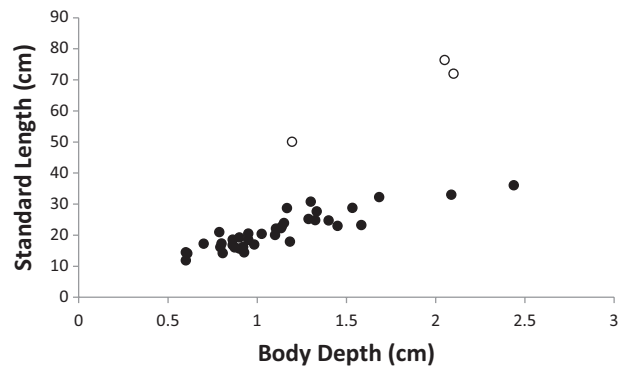


**Figure 5.** The relationship between body depth (universally the largest secondary body axis) and standard length ($r^2 = 0.78$). The three extremely elongate species are represented by open circles; all other species are represented by black circles.

regression model building yielded a model that explains 70% of the observed variation in maximum body length according to the following combination of variables (relative contributions of each variable to the coefficient of determination, or generalized $R^2$, are given parenthetically): vertebral aspect ratios (0.50); precaudal vertebral number (0.28); size-corrected head length (0.17); and caudal vertebral number (0.05) (Fig. 6). We find that ER is weakly and negatively correlated with maximum body length ($R^2 = 0.15$, $P = 0.009$, slope = −0.016), confirming that elongation does not correspond with an increase in overall length. We also find that variation in AEI is primarily driven by changes in vertebral aspect ratios
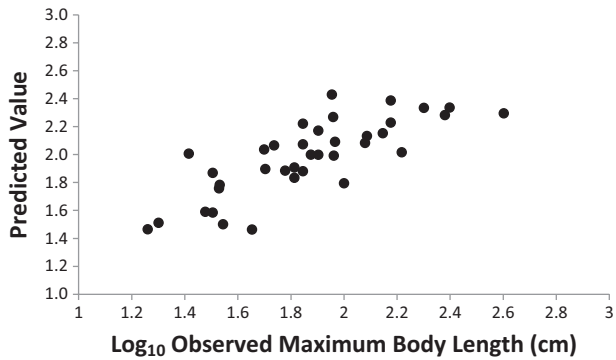
**Figure 6.** The relationship between predicted values from multiple regression model building against observed values of maximum body length. Model-predicted values encompass 70% of the variation observed in maximum body length based on the predictors of caudal aspect ratios, precaudal and caudal vertebral numbers, and size-corrected head length.

in the precaudal and caudal regions (combined $R^2 = 0.97$), and not by variation in vertebral number. The highly elongate species also exhibit elongate vertebral centra, but in the case of *P. brummeri* and *R. quaesita*, vertebral numbers, rather than increases in vertebral aspect ratios, have contributed to their high values of AEI.

MODELS AND TEMPO OF MORPHOLOGICAL EVOLUTION

Maximum body length was best fitted with an OU1 model, and is more likely to evolve according to BM with selection towards a single optimum. Total vertebral number and AEI were best fitted with BM2 models, and confirm our hypothesis that the highly elongate taxa (*S. tigrina*, *R. quaesita*, and *P. brummeri*) show accelerated rates of evolution for these variables, relative to the remaining 37 taxa (Table 2). When the caudal and precaudal vertebrae are modelled separately, the number of precaudal vertebrae fit an OU1 model, whereas caudal vertebrae fit a BM2 model. On average, the three highly elongate species are separated from each other by more than 20 Myr (Fig. 2), and represent independent evolutionary trajectories for total vertebral number, primarily through adding vertebrae to the caudal region. The ER was best fitted with an OU2 model, further supporting the idea that these three species are evolving towards an optimal body shape that is distinct from the other lineages surveyed. Our randomization procedure confirmed that OU2 and BM2 model selection could not be repeated by randomly choosing three taxa from the phylogeny, as none of the 100 iterations (each across 500 equally likely topologies) provided significant support for either model.

Ancestral states estimated by the best-fit models in RBROWNIE for ER, AEI, the total number of vertebrae (including caudal and precaudal vertebrae), and maximum body length are presented in Table 2. We also include the 95% confidence intervals from the ML reconstructions using a BM model, anguilliform outgroups (when available; Fig. S1; Table S2), and the *ace* function in Geiger. Reconstructed ancestral states for AEI at all nodes are presented in Figure 2. Based on these estimates, the ancestral moray was intermediate in body shape (ER of 22) relative to the distribution of observed ER values in Figure 1a. Most moray species evolved changes in body shape according to weak selection for a body length to depth ratio of approximately 19 : 1, and on average this ratio changed by 0.5 per million years. However, approximately 20 Mya the first two of three highly elongate lineages emerged, and were under strong selection for increased elongation, leading to the species *P. brummeri* (21 Mya), *S. tigrina* (19 Mya), and later, *R. quaesita* (8 Mya). Intermediate values for AEI (167), total vertebral number (128), and maximum body length (98 cm) are also inferred at the root of the tree, despite the presence of extant taxa with extremely high values for AEI (up to 815), and species with four times the average maximum body length.

DISCUSSION

Despite considerable work on pleomerism in fishes and interest in body shape evolution in vertebrates, few studies have addressed how evolutionary changes in the axial skeleton are correlated with changes in body shape and maximum body length. We show that moray eels are highly diverse in body shape, maximum body length, and vertebral characteristics, and that body elongation evolves independently of axial elongation, whereas maximum body length evolves through region-specific increases in vertebral number, elongation of the vertebral centra, and postembryonic somatic growth. Our phylogenetic examination of elongation and the axial skeleton in 40 species of moray eels revealed a weak association between ER and AEI, suggesting that overall body shape and the axial skeleton evolve independently within morays as well as across Anguilliformes (Mehta *et al.*, 2010). Morays exhibited ERs ranging from 15 to 43 (relatively deeper bodied to very slender), spanning most of the variation in eel body shape (Fig. 1a). We found a negative relationship between elongation of the body and elongation of the head, a surprising result in light of the positive relationship found in many elongate fish taxa (e.g. beloniform fishes; Ward & Brainerd, 2007). Body depth alone explained 97% of the variation in ER for 37 moray species (Fig. 5). Thus, we find that most

**Table 2.** For each trait, we present lambda (λ), the strength of phylogenetic signal (Pagel, 1999), and ancestral states estimated (using the model denoted) in RBROWNIE, with 95% confidence intervals estimated using a Brownian motion (BM) and maximum likelihood in the Geiger module of R. Also presented are Akaike's information criterion values, and the ΔAICc score between the preferred model and the next best fit model (values greater than 4 are considered strong support). When the preferred model of morphological evolution was an Ornstein–Uhlenbeck two-optima model (OU2) or a Brownian motion two-rate model (BM2), these models allowed for different optima or rates of BM for the 37 species versus the three burrowing species *Rhinomuraena quaesita*, *Pseudoechidna brummeri*, and *Scuticaria tigrina*. The attraction parameter for OU models (α, varies between 1 and 20, scaling with the strength of selection towards an optimum) is given, and the rate of evolution for a given character in units per million years (σ). When a BM2 model is favoured, the rate for the 37 species is presented first, followed by a forward slash and the rate for the three highly elongate species. For OU models, the optimum (OU1) or optima (OU2) are given in the same order as rates

| Metric | λ | Ancestral state | Model | AICc | ΔAICc | α | σ (Myr) | Optima |
|---|---|---|---|---|---|---|---|---|
| Elongation ratio | 0.94 | 22 (18–35)* | OU2 | 228 | 14 | 20 | 0.5 | 19/55 |
| Axial elongation index | 0.01 | 167 | BM2 | 480 | 18 | – | 9/60 | – |
| Total vertebrae | 0.01 | 128.3 (96–148)* | BM2 | 344 | 23 | – | 1/13 | – |
| Precaudal vertebrae | 0.60 | 67.3 (56–72) | OU1 | 303 | 9 | 20 | 1.8 | 67/115 |
| Caudal vertebrae | 0.01 | 61.1 (55–69) | BM2 | 304 | 44 | – | 1/11 | – |
| Maximum body length | 0.01 | 98.1 (74–131)* | OU1 | 458 | 7 | 20 | 10.6 | 115 |

*Confidence intervals with asterisks were estimated with Anguilliform out-groups; otherwise, values are estimated using data from Muraenidae only.

morays attain an elongate body by altering body depth without changing components of the axial skeleton or head length. This pattern is in contrast to those observed in other elongate vertebrates. For example, salamanders of the genus *Lineatriton* (Parra-Olea & Wake, 2001) and many arboreal snakes (Johnson, 1955) have evolved an elongate body shape through lengthening the vertebral centra. Lindsey (1975) noted that shorter fishes tended to have fewer vertebrae. Among their closest known relatives, Myrocongridae and Heterenchelyidae (Inoue *et al*., 2010; Johnson *et al*., 2012; Fig. S1), myrocongrids (five species) show a close relationship between the total number of vertebrae and ER (data in Castle, 1991 and Karmovskaya, 2006), whereas fossorial heterenchelyids (eight species) appear to show greater variation in the relationship between vertebral number and body shape (data in Smith *et al*., 2012; D. Smith, pers. comm.). The relatively weak association between vertebral characters and elongation observed in several studies (Johnson, 1955; Gans, 1975; Ward & Brainerd, 2007; Mehta *et al*., 2010; Ward & Mehta, 2010) suggests that the mechanisms underlying elongation are diverse and that similar to the pattern observed in morays, members of some lineages may simply undergo a reduction in cross-sectional area without major changes to the vertebral column.

Compared with terrestrial vertebrates, the vertebral column of fishes exhibits the simplest form of regionalization. Because elongate 'anguilliform-like' fishes tend to use their entire bodies during locomotion, we anticipate that vertebral characters may exhibit modularity across the precaudal and caudal regions (Liem *et al*., 2001). This developmental pattern provides a general framework for predicting the vertebral correlates of body shape and size evolution. When the two regions evolve according to a single regulatory module, changes should be uniform across the precaudal and caudal regions, whereas separate regulatory mechanisms allow for region-specific changes. Ward & Brainerd (2007) surveyed over 800 species of actinopterygian and chondrichthyan fishes, and found that the number of vertebrae in the precaudal and caudal regions evolved independently in most groups. For example, in Ostariophysi the number of precaudal vertebrae was conserved, whereas the number of caudal vertebrae varied. Conversely, in Polypteriformes, caudal vertebral numbers were conserved and precaudal numbers varied. Other groups such as Elopomorpha and Osteoglossomorpha showed evidence of both patterns. Our results indicate that for Muraenidae, changes in vertebral number occur independently in the precaudal and caudal regions (Fig. 3a). Similar to the pattern that has been observed across most actinopterygians

(Ward & Brainerd, 2007), we find that in morays changes in vertebral aspect ratio are correlated across the two body regions (Fig. 3b). Thus, there are likely separate developmental modules acting on vertebral numbers in the precaudal and caudal regions, and a single module affecting changes in aspect ratios of vertebrae in both regions. For anguilliform swimmers such as morays, we anticipate high vertebral numbers, which are associated with more intervertebral joints, thereby increasing body flexibility (Brainerd & Patek, 1998). In morays, there may be further functional constraints to maintain a low degree of stiffness across the body through coordinated elongation of vertebrae, as opposed to increases in the already high number of vertebrae. Evidence for such a constraint in body stiffness was also observed in Johnson's (1955) treatment of aquatic and marine snakes, which showed more elongate vertebrae (and presumably stiffer bodies) than their terrestrial counterparts.

Elongation in fishes is often associated with a burrowing lifestyle (Colin, 1973; Atkinson et al., 1987; Clark, Pohle & Halstead, 1998; Ishimatsu et al., 1998; Aoyama et al., 2005). Slender and elongate organisms are suspected to travel through sand or soil with less friction than shorter, wider organisms (Gans, 1974, 1975). Whereas most moray species inhabit reefs or rocky substrates, our sampling included three species, each representing an independent origin of sand burrowing (see Fig. 1). Specifically, these species bury their bodies in the sand and are only conspicuous by their protruding heads (McCosker & Rosenblatt, 1995; Lieske & Myers, 1996). Most morays evolved towards an optimum ER of 19, but these burrowing species showed a strong pattern of evolution towards an optimum ER of up to 55 (Table 2). In contrast to the other 37 morays examined, these specialized tail-first burrowers achieved body elongation through commensurate increases in vertebral column elongation. Heterenchelyids, which are closely related to morays, are head-first burrowers (Eagderi & Adriaens, 2010) that show considerable variation in vertebral numbers (from 129 to 160 total vertebrae; Smith et al., 2012), body size, and shape, and have more vertebrae on average than myrocongrids, another closely related clade. Like morays, elongate colubrid and elapid snakes tend to have more vertebrae than stout-bodied snakes. Unlike morays, burrowing snake species showed significantly fewer vertebrae for their size than their terrestrial or arboreal relatives (Lindell, 1994), and greater variability within species and between closely related species, suggesting a release from selection on the vertebral column in burrowing snakes (Johnson, 1955). Overall, we find no strong relationship between body elongation and vertebral number in morays, except that

burrowing species are extremely elongate and tend to have more vertebrae, particularly in the caudal region.

Similar to ER, the axial skeleton of morays encompasses the majority of the variation found in our sampling of anguilliform fishes (Fig. 1b). Axial variation, as measured by AEI, has evolved primarily through changes in the shape of individual vertebrae. Whereas AEI and total vertebral number (a component of AEI) evolved according to BM along our reconstruction of phylogenetic history, there is strong evidence for an accelerated rate of evolution among the three highly elongate sand-burrowing lineages. Most morays alter their vertebral number by approximately one vertebrae per Myr, but the highly elongate sand dwellers, *P. brummeri*, *S. tigrina*, and *R. quaesita*, all show rates of approximately 13 vertebrae per Myr, particularly within the caudal region. This translates into a 6.7-fold increase in the rate of evolution for AEI among the highly elongate sand-dwelling species relative to the remaining 37 species sampled.

## THE EVOLUTION OF MAXIMUM BODY LENGTH IN MORAYS

Lindsey (1975) examined data for over 3000 fish species, and noted a strong positive association between the number of vertebrae and maximum body length. This pattern was consistent across most taxonomic families, genera, and even populations within species. Lindsey coined the term pleomerism to refer to the correlation between the meristic parts of an animal and their characteristic body sizes. In addition to Jordan's rule (Jordan, 1892), or the tendency for fish at higher latitudes to have more vertebrae than their relatives at lower latitudes (see Yamahira & Nishida, 2009), pleomerism is widely accepted as a defining characteristic in the skeletal morphology of fishes (McDowall, 2008). Pleomerism has also been found to be a general pattern in broad surveys of snakes (Lindell, 1994) and many amphibians (Jockusch, 1997). However, pleomerism has recently been challenged at both higher and lower taxonomic levels. Müller et al. (2010) showed that across 436 amniote taxa, pleomerism was not generally associated with the evolution of large body size. Instead, they found that large amniotes such as dinosaurs had the same number of vertebrae as their smaller relatives, and achieved their large body sizes through postembryonic somatic growth. Conversely, small-bodied amniotes often show high vertebral counts, implying that other ecological or behavioral traits (e.g. habitat and locomotion) might affect or be affected by vertebral number more than body size. McDowall (2003; see also McDowall, 2008) showed

that despite a general pattern of pleomerism across galaxiid fishes, body size was also strongly influenced by diadromy and swimming mode. In snakes, pleomerism explains phyletic body size increases across a wide range of species, but as noted in Müller *et al.* (2010), the largest species of snakes, such as large members of Boidae and Pythonidae, have intermediate numbers of vertebrae (250–300), and have achieved large body size primarily through postembryonic somatic growth (Head & Polly, 2007).

Over the course of their evolution, morays have evolved extensive diversity in maximum body length (Fig. 1c). We find a relatively weak influence of evolutionary history on maximum body length (Table 2), whereas phylogenetic relationships explain up to 59% of body size variation in some groups of snakes (Terribile *et al.*, 2009). Caudal aspect ratios, combined with the number of vertebrae in the precaudal and caudal regions and size-corrected head length, explain 70% of the variation in maximum body length. Interestingly, size-corrected head length modestly contributes to overall length. Slender morays tend to have shorter maximum body lengths and relatively short heads, whereas deeper bodied morays tend to have large maximum body lengths and more elongate heads. We suspect that morays with deeper bodies feed on larger prey, and thus have larger heads and longer oral jaws. Although total vertebral number is correlated with maximum body length, we find that vertebral shape plays a stronger role in the evolution of maximum body length in this group. Variation in maximum body length not explained by our model is probably a result of postembryonic somatic growth, as observed in snakes (Head & Polly, 2007) and other vertebrates (Müller *et al.*, 2010). As vertebral shape contributes to overall maximum body length, understanding the rate of vertebral growth in each axial region would provide interesting insight into the evolution of maximum body length in morays (Bergmann, Melin & Russell, 2006).

Moray eels probably appear in the fossil record between 34 and 54 Mya (Benton, 1993). The ancestral moray reached a maximum body length of approximately 98 cm. From this ancestral length, morays have reached over 4 m in length and weights of over 60 kg (the largest moray, *Gymnothorax javanicus*). Moray lineages have also decreased in length, with the smallest species that we sampled, *G. robinsi*, reaching 18 cm in length and a weight of approximately 3 g. The oldest full-bodied fossil of a moray is dated to 5.3 Mya (Arambourg, 1927; Gaudant, 2002), and is approximately 13 cm long at an unknown stage of maturity. Maximum body length evolved under weak selection for an optimum of 115 cm, on the upper edge of the distribution for extant taxa sampled (Fig. 1c), and at a rate of approximately 10.6 cm net

change in maximum body length per Myr. As a result of this rate of evolution and weak selection, variation in maximum body length steadily increased throughout moray evolutionary history. The three burrowing species, which also had the highest ERs in our data set, reach maximum lengths of just over 100 cm, which is only one-quarter of the length of *G. javanicus* (Table 1). This pattern is also observed in snakes, where burrowing species were typically shorter than their arboreal or terrestrial relatives (Lindell, 1994). The smallest species of snakes (e.g. *Leptotyphlops*, the so-called 'thread snakes') also tend to be fossorial and fairly elongate, with ERs that range from 30 to 60 (Taylor, 1939; Hahn, 1978). Whereas all three burrowing morays sampled show an increase in ER and maximum body length from their inferred ancestral states, these increases in maximum length have resulted from increases in both the number of precaudal and caudal vertebrae, elongation of individual vertebrae in the precaudal and caudal regions, and increases in relative head length. Because of the limited availability of vertebral measurements on the closest relatives of morays, we were only able to reconstruct ancestral states including out-group taxa for the traits of ER, vertebral number, and maximum body length, and thus for the remaining characters we were limited to Muraenidae (without out-groups). Our inferred ancestral state of 128 vertebrae for Muraenidae is consistent with the mean numbers in heterenchelyids and myrocongrids (average vertebral number of 147; data from FishBase and D. Smith, pers. comm.), as is ER (22 for Muraenidae, and approximately 20 for heterenchelyids and myrocongrids). Maximum body length is somewhat lower in the two out-group taxa (58.9 cm) than the ancestral state for morays (98.1 cm). Future research on patterns of evolution in these taxa, particularly with respect to vertebral aspect ratio, will contribute to a greater understanding of axial diversity and behaviours of anguilliform fishes.

## CONCLUSION

Morays exhibit great variation in body shape, vertebral characteristics, and maximum body length (Fig. 1). We show that this diversity has evolved through regionalization of vertebral development, with separate developmental modules for the numbers of vertebrae in the caudal and precaudal regions, but a shared module for vertebral aspect ratios across regions. Consistent vertebral shape in each region could potentially contribute to the functional morphology of undulatory swimming, as has been suggested for some aquatic snakes. In morays, body elongation and elongation of the vertebral column evolve independently. Throughout their

evolutionary history, moray lineages have elongated by different morphological trajectories, in contrast to shared evolutionary patterns recorded for some groups of elongate salamanders and snakes. Similar to fossorial snakes and other burrowing anguilliform fishes, burrowing species of morays are particularly elongate and show evidence for rapid evolution and strong selective forces (Table 2) relative to non-burrowing morays. Maximum body length in morays appears to evolve through pleomerism, like many other groups of fishes, but also by changes in the shape of vertebrae and postembryonic somatic growth, a pattern that also extends to large species of snakes. Finally, we reconstruct an ancestral moray and describe the timing and pattern of phenotypic evolution in body shape, maximum body length, and vertebral characters. This work reveals the importance of axial skeleton characteristics in contributing towards body shape and maximum body length in a diverse group of marine fishes, and is a step towards understanding the developmental mechanisms by which vertebrates have evolved such tremendous diversity in overall body size and shape.

## ACKNOWLEDGEMENTS

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# SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Phylogeny of Muraenidae from Reece *et al*. (2010) nested within the phylogeny of Anguilliformes from Johnson *et al*. (2012).
**Table S1.** Specimens examined and museum voucher numbers, where appropriate. Genera names are abbreviated after first mention.
**Table S2.** Anguilliform out-groups to Muraenidae sampled from Johnson *et al*. (2012) and used for ancestral state reconstructions (Table 2) of maximum body length (MBL), the total number of vertebrae (TV), and elongation ratio (ER). Data were not available for species with blank values.