SYMPOSIUM

Elongation of the Body in Eels

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Synopsis The shape of the body affects how organisms move, where they live, and how they feed. One body plan that has long engaged the interest of both evolutionary biologists and functional morphologists is axial elongation. There is a growing interest in the correlates and evolution of elongation within different terrestrial and aquatic vertebrate clades. At first glance, Anguilliformes may appear to exhibit a single cylindrical form but there is considerable diversity underlying this seemingly simplified body plan. Here, we explore evolution of the axial skeleton in 54 anguilliform taxa and some close relatives. We describe the diversity of axial elongation as well as investigate how characters such as head length, branchial-arch length, and shape of the pectoral fins correlate with vertebral number to possibly facilitate changes in absolute diameter of the body. Overall, we find that precaudal vertebral numbers and caudal vertebral numbers are evolving independently across elopomorph fishes. We also find that precaudal and caudal vertebral aspect ratios are evolving together across elopomorph fishes. When focusing within Anguilliformes we find striking diversity in the mechanisms of elongation of the body, including almost every trend for axial elongation known within actinopterygian fishes. The three major clades of eels we examined have slightly different mechanisms of elongation. We also find a suite of morphological characters associated with elongation in anguilliform fishes that appears to coincide with a more fossorial lifestyle such as high elongation ratios, a more posteriorly extended-branchial region, and a reduction in the size of the pectoral fins. Lastly, we point out that a diverse range of derived behaviors such as head- and tail-first burrowing, rotational feeding, and knotting around prey are only found in long cylindrical vertebrates.

Introduction

In an attempt to understand the evolution of limb reduction, Gans (1975) considered some important features associated with limblessness in terrestrial vertebrates and came to the conclusion that the common theme unifying the majority of limb-reduced or limbless body forms was elongation. Defining elongation as a reduction in relative diameter for a given mass, an increase in relative length, or both, Gans postulated that animals with these specializations would be adapted for a burrowing lifestyle. Although this may be primarily true of terrestrial vertebrates, there are numerous aquatic vertebrates that have evolved an elongate, limb-reduced body plan, and little is known about how

the elongate body form may be adapted for aquatic habits. In fact, despite current interest in understanding the molecular, developmental, and anatomical basis for elongation, few studies have explored the diversity of elongation within a clade and discussed what ecological, morphological, and behavioral patterns may be associated with this seemingly simplified body plan (Wiens and Slingluff 2001; Adriaens et al. 2002; Wiens et al. 2006; Brandley et al. 2008; Yamada et al., 2009).

With a distinct larval stage (leptocephalus) and their radiation of more than 800 species, elopomorph fishes are arguably one of the most enigmatic and interesting groups of teleosts. Elopomorph fishes are a morphologically diverse clade containing bone
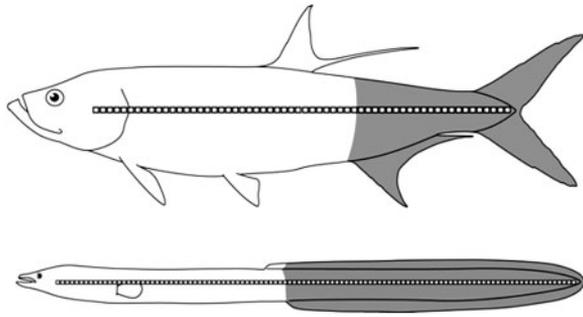
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Fig. 1 The two general body designs observed in the Elopomorpha: Top: deep-bodied species represented by Megalops atlanticus and Bottom: extremely elongate form represented by Anguilla rostrata. Shaded regions indicate the caudal region as well as the differences in vertebral number in the caudal region which contribute to overall body length in these two species.

fish, tarpon, and Anguilliformes, a large monophyletic radiation of limb-reduced eels. The extremes of body plan in Elopomorpha range from deep-bodied tarpons to extremely elongate forms (Fig. 1). The extremely elongate forms are mostly found within Anguilliformes. At first glance, anguilliform fishes exhibit a relatively uniform cylindrical body design; however, there is considerable diversity in their axial skeleton.

Ward and Brainerd (2007) examined the axial skeleton of 11 species of elopomorph fishes and found that the group appears to add vertebrae equally in both the precaudal and caudal-body regions. However, they noted that their phylogenetic sampling may have been too sparse to identify all mechanisms of axial elongation in this highly diverse group. Therefore, one of the goals of the present study is to explore mechanisms of elongation within a larger sample of elopomorph diversity.

We expect differences in the axial skeleton across anguilliform species due to their incredible ecological and morphological diversity. Anguilliforms occupy a wide range of habitats including coral and rocky reefs, sea grass beds, sandy flats, muddy bottoms, and open ocean. Members exhibit varied cranial features from long jaws with large recurved teeth to shorter jaws with numerous small recurved teeth. These differences in the jaws alone may be correlated with differences in the axial skeleton. For instance, we might expect longer jawed species such as morays to exhibit a longer precaudal region that accommodates the large prey they are known to consume (Nelson 1966; Miller 1987, 1989; Mehta and Wainwright 2007, 2008). We might also expect a similar pattern of axial design for Anguilliformes

that exhibit extreme gapes and occupy bathypelagic zones of the ocean. A sand-dwelling and tail-first burrowing congrid eel with short jaws, on the other hand, might be expected to have a shorter precaudal region and an extensive caudal region.

Elongation in vertebrates is thought to coincide with any number of the following features: a reduction in relative diameter of the body (Gans 1975; Ward and Brainerd 2007), an increase in the number of trunk vertebrae (Wake 1966; Gans 1975; Lindsey 1975; Ward and Brainerd 2007), an increase in the length of the vertebral centra (Parra-Olea and Wake 2000), an increase in length of the head (Ward and Brainerd 2007), and a reduction or complete loss of limbs (Gans 1975). However, there may be other correlates to elongation in certain clades or different combinations of those already mentioned that may provide insight into the potential benefits derived from being long. Nelson (1966) studied the branchial arches of anguilliform fishes and noted their posterior position in relation to the skull. He postulated that a reduction in head diameter in Anguilliformes may have been achieved in part by the movement of the branchial arches from within the cranium to a position posterior to it. This posterior movement of the branchial arches, which was termed "branchial displacement" (Nelson 1966), requires the loss of skeletal connections with the cranium and sometimes between the branchial arches themselves, which could augment expansibility of the pharynx. Nelson's ideas about the corollaries of elongation in anguilliform fishes have yet to be tested using a phylogenetic framework.

The goals of this article are to describe the diversity of the axial skeleton of elopomorph fishes and to identify the morphological correlates of elongation in Anguilliformes. We address the following questions: how is body elongation achieved? Are patterns of axial elongation consistent across anguilliform clades? As species get longer, what other aspects of their morphology change? Lastly, what ecological and behavioral traits are associated with elongation in anguilliform fishes?

Materials and methods

A total of 147 specimens from 56 species of elopomorph fishes were examined from museum and personal collections (for museum accession numbers, see Supplementary Appendix S1). At least two specimens were examined for each species (except where noted; see Supplementary Appendix S1). Total length, mass, maximum body depth, and maximum body width were first measured for each

formalin-fixed and alcohol-preserved specimen to the nearest 0.01 mm with digital calipers. Specimens were then cleared and stained for bone and cartilage following a modification of Dingerkus and Uhler's (1977) method. For each specimen, we counted the number of precaudal and caudal vertebrae three times and then calculated the mean number of vertebrae for each region. The precaudal region was defined as the region of the vertebral column without haemal spines while the caudal region of the vertebral column contained the vertebrae with haemal spines. As described previously in Ward and Brainerd (2007), vertebral length and width were measured at the anterior part of the vertebral centra. These measurements were taken from three vertebrae located near the center of the precaudal region and in three vertebrae located near the center of the caudal region using digital calipers. The mean vertebral aspect ratio, defined as centrum length/centrum width, was determined for vertebrae in both the precaudal and caudal regions.

We also measured the following characters in cleared and stained individuals: head length (measured from the tip of the snout to the posterior-most edge of the neurocranium), length of the lower jaw, length of the branchial region (measured from the anterior-most point on the first branchial arch to the posterior-most end of the last branchial arch), and aspect ratio of the pectoral fins (when present) to the nearest 0.01 mm using digital calipers. We note that in previous studies of Anguilliformes, head length included the region of the gill arches (Böhlke 1989) but the two characters have been separated here to determine the length of the branchial arches themselves and to calculate relative branchial-arch length, which is the length of the branchial arches in relation to the head (length of branchial region/head length), modified from Nelson (1966). Lastly, we adopted two measures to quantify body shape and elongation of the axial skeleton: elongation ratio (ER) and the axial elongation index (AEI). ER is total length divided by the second largest body axis, which is either body width or depth (Ward and Azizi 2004). AEI is the number of precaudal vertebrae × precaudal aspect ratio + the number of caudal vertebrae × caudal aspect ratio (Ward and Brainerd 2007). ER is a measure of general body shape, while AEI characterizes only the axial skeleton.

Statistical analyses

As species are not independent data points and share a similar evolutionary history, we incorporated phylogenetic information into our analyses whenever possible (Felsenstein 1985).

We constructed a phylogeny for the elopomorph species from our morphological dataset by downloading 12S and 16S sequences in Genbank (for GenBank accession numbers, see Supplementary Appendix S2). These sequences were manually aligned to models of secondary structure (Orti et al. 1996; Wang and Lee 2002). We excluded ambiguously alignable regions from further analysis. We estimated a time-calibrated phylogeny of the taxa in our study using BEAST 1.48 (Drummond and Rambaut 2007) with the ages of three nodes assigned prior ages based upon log-normal distributions: crown Anguilliformes (min 98 MY based upon fossils from the Cenomanian of Lebanon) (Belouze et al. 2003a, 2003b), 95% upper bound 135 MY based upon the fossil albulid *Albuloideorum ventralis*; crown congrids (min 50 MY based upon *Bolcyrus bajai* and *Voltaconger latispinus* from Monte Bolca) (Frickhinger 1991) and crown muraenids (min 50 MY based upon *Eomuraena sagittidens*) (Patterson 1993). We ran the Markov chain for 50 million generations and sampled every 5000 to construct the timetree used in our comparative analyses. We visually assessed convergence using plots of model parameters and effective sample size using Tracer (Rambaut and Drummond 2007) and discarded the first 10 million generations to help insure that the chain had adequately sampled the target joint distribution. We used the remaining samples to construct a time tree based upon the maximum clade credibility tree. Our phylogenetic topology with branch lengths was imported into MESQUITE version 2.72 (Maddison and Maddison 2009) and we used the PDAP module (Garland et al. 1992) to calculate the independent contrasts (ICs) to account for phylogenetic effects in our regression analyses. To answer the different questions we propose in this study, we analyzed some of our data without taking phylogenetic information into account. For example, when analyzing vertebral characteristics within anguilliform groups to gain a finer understanding of the variation across Anguilliformes, we simply relied on RMA regressions with the raw data because we lacked a species-level phylogeny of the various anguilliform clades for which we had vertebral data. All variables, with the exception of ratios, were log transformed before statistical analyses.

Models of axial patterning

To test whether two vertebral characters in different regions were changing proportionally, we used the

95% confidence intervals of the RMA slopes to compare the measured slopes with the model slopes at the 0.05 level of significance, following Ward and Brainerd (2007). A slope of greater than 1 indicates that caudal vertebral characteristics (number of vertebrae or aspect ratio) are changing faster than are precaudal vertebral characteristics, while a slope of less than 1 would indicate the opposite. A slope of 1 indicates that caudal vertebral number and precaudal vertebral characteristics are changing equally. We then extended this method to examine the strength of the correlations between calculated contrasts for total vertebral number, ER, and other morphological features that have been proposed to accompany elongation of the body.

To identify independent axes of morphological variation between eels that occupy different marine habitats, we assigned a subset of our anguilliform species to one of two groups: reef-associated and non reef-associated. Information on habitat use was obtained from FishBase (Froese and Pauly 2009, http://www.fishbase.org). Reef-associated eels tended to be crevice-dwellers or burrowers whereas non reef-associated eels tended to occupy mid-water or the bathypelagic zone. We then conducted a principal components analysis on seven morphological variables: precaudal and caudal vertebral number, average vertebral centrum length and width, head length, length of the branchial-arch region, and fin aspect ratio. We used a correlation matrix to extract principal components as our dataset was a combination of linear and meristic variables.

Results

Body-elongation continuum

We found that elopomorph fish exhibit a wide range of forms ranging in ERs from 3.4 to 110.0 (Fig. 2). This large span in ER reveals that as some elopomorphs are attaining greater relative lengths, they are also changing in body depth, which was the second largest body axis for the majority of fish in our dataset. Only two Anguilliformes: the serrivomerid, *Serrivomer beanii*, and the nettastomatid, *Saurenchelys fierasfer*, exhibited greater body widths compared to depths. The bone fish, *Albula vulpes*, and the two-tarpon species occupied the lower left quadrant of the graph representing the deeper bodied species with the fewest number of vertebrae in this dataset. The thinnest species were the congrid, *Heteroconger hassi*, with an ER of 71 and the ophichthid, *Myrophis vafer*, with an ER of 110. We found a significant correlation between total vertebral number and ER ($R = 0.49$, $P < 0.05$). ICs also
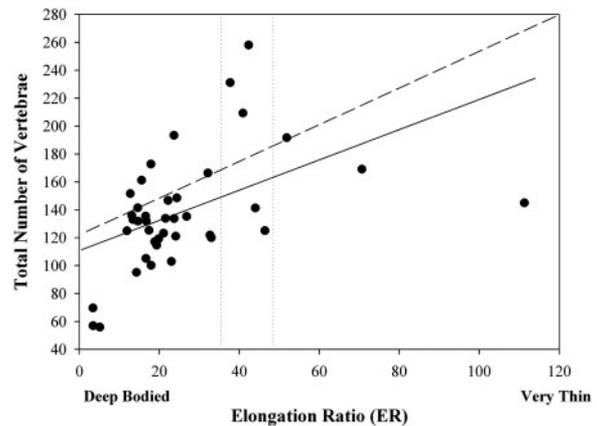

Fig. 2 The relationship between total number of vertebrae and body shape expressed in terms of ER (ER = total length / second major longest axis of the body, depth, or width). The solid line is a RMA regression based on the raw data (data points as shown) while the dashed line is an RMA regression based on independent contrasts. The dashed line reveals a positive relationship between the two variables ($R = 0.54$, $P < 0.001$, slope = 3.36). Note the wide variation in vertebral numbers for Anguilliformes in a narrow range of ERs as indicated by the horizontal dotted lines.

revealed a significant correlation between total vertebral number and ER ($R = 0.54$, $P < 0.001$) suggesting that these two characters are changing together.

Patterns of axial elongation across Elopomorpha

We found a significant relationship between caudal vertebral number and precaudal vertebral number in the raw data points ($R = 0.42$, $P = 0.05$) but no relationship in the ICs, indicating that evolutionary changes in vertebral numbers in the two regions of the axial skeleton are independent ($R = 0.04$, $P = 0.41$; Fig. 3). This marked difference between the raw data and contrast scores lead us to a more in depth investigation of regional vertebral patterns within Anguilliformes (see below). We found a strong positive relationship between precaudal aspect ratio and caudal aspect ratio in the raw data points ($R = 0.91$, $P < 0.001$) as well as in the ICs ($R = 0.52$, $P = 0.007$), revealing that these two variables are evolving together (Fig. 4). The slope of this line was not significantly different from 1.0, indicating similar variation in aspect ratios in both regions of the axial skeleton.

To determine the contribution of both increases in vertebral numbers and in aspect ratios to overall elongation of the body, we examined the relationship between AEI and ER and found a weak relationship in the raw data ($R = 0.2$, $P = 0.04$) and no relationship in the ICs ($R = 0.02$, $P = 0.30$). When we traced the changes in AEI on the phylogeny, several patterns
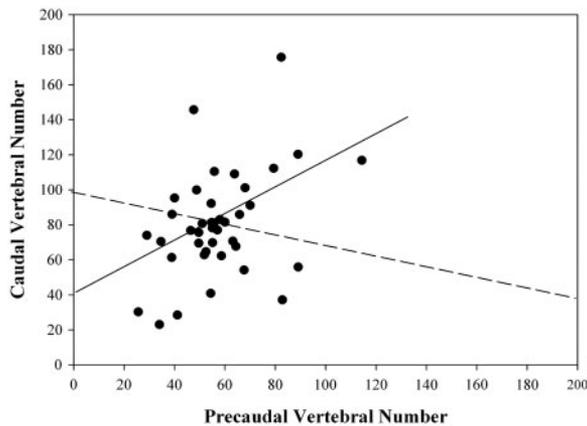
Fig. 3 The relationship between the number of precaudal and caudal vertebrae for 40 species of elopomorphs. The solid line is a RMA regression based on the raw data (data points as shown) while the dashed line is an RMA regression based on independent contrasts of precaudal and caudal vertebral numbers. While we found a significant relationship between precaudal vertebral number and caudal vertebral number in the raw data, there was no relationship in the independent contrast scores indicating that evolutionary changes in vertebral number in the two regions of the axial skeleton are independent.
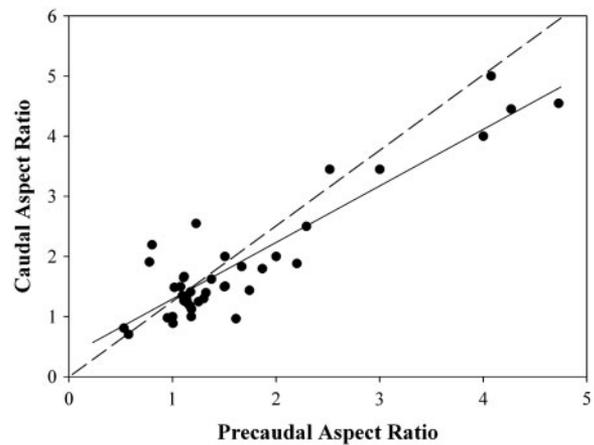

Fig. 4 The relationship between precaudal vertebral aspect ratio and caudal vertebral aspect ratio for 40 species of elopomorphs. The solid line is a RMA regression based on the raw data (data points as shown) while the dashed line is an RMA regression based on independent contrasts of precaudal and caudal vertebral aspect ratios. Both the raw data and the independent contrast scores reveal a strong relationship between vertebral aspect ratios in different regions of the axial skeleton. The dashed line has a slope of 1.27, which is not significantly different from 1, indicating similar variation in aspect ratios in both regions of the axial skeleton.

emerged (Fig. 5). At least five increases in AEI have occurred in elopomorphs. Four of these increases are in Muraenidae while another increase is found in *Serrivomer beanii*, a bathypelagic anguilliform eel. We find that species with high AEIs are nested within clades with lower AEIs, revealing that AEI has increased multiple times in elopomorph fishes. Interestingly, significant decreases in AEI have also occurred and some of these decreases occur in clades with species that exhibit the highest AEIs, suggesting that this trait is a substrate for dynamic changes in certain parts of the elopomorph tree.

Axial diversity within Anguilliformes

Within the three largest anguilliform groups, muraenids, congrids, and ophichthids, different patterns characterize the axial skeleton (Fig. 6). Muraenids are adding more caudal vertebrae than precaudal vertebrae to their axial skeleton and their caudal aspect ratios are changing slightly more than their precaudal aspect ratios. Ophichthids and congrids are adding equal numbers of vertebrae to their precaudal and caudal region. However, while the vertebral aspect ratios of ophichthids are similar across their axial skeleton, congrids exhibit greater changes in aspect ratios in the precaudal region than in the caudal region (Table 1).

Morphological correlates of axial elongation

The morphological correlates of elongation in elopomorph fishes are organized in Table 2. We found no relationship between head length and vertebral number in either the raw data ($R = 0.02$, $P = 0.88$) or the ICs ($R = 0.04$; $P = 0.76$), suggesting that longer eels do not necessarily have longer heads and that total vertebral number and head length are not evolving together (Fig. 7A). We did, however, find a strong positive relationship between lower jaw length and head length ($R = 0.90$, $P < 0.001$; $R = 0.93$, $P < 0.001$) (Fig. 7B). The relationship between relative branchial-arch length and ER was significant in the raw data ($R = 0.3$, $P = 0.05$) and in the ICs ($R = 0.42$, $P = 0.007$), indicating that the extent to which the branchial arches extend back from behind the skull is related to being relatively long and slender-bodied (Fig. 7C). Both head width and vertical-gape distance revealed a slight negative relationship with relative branchial-arch length in the raw data ($R = -0.38$, $P = 0.01$; $R = -0.24$, $P = 0.04$) but not in the ICs ($R = 0.15$, $P = 0.37$; $R = 0.16$, $P = 0.33$; Fig. 7D and E). Lastly, we found a slight negative relationship between fin aspect ratio and total vertebral number in the raw data but no relationship in the ICs, indicating that longer eels do not necessarily have smaller aspect ratios of the pectoral
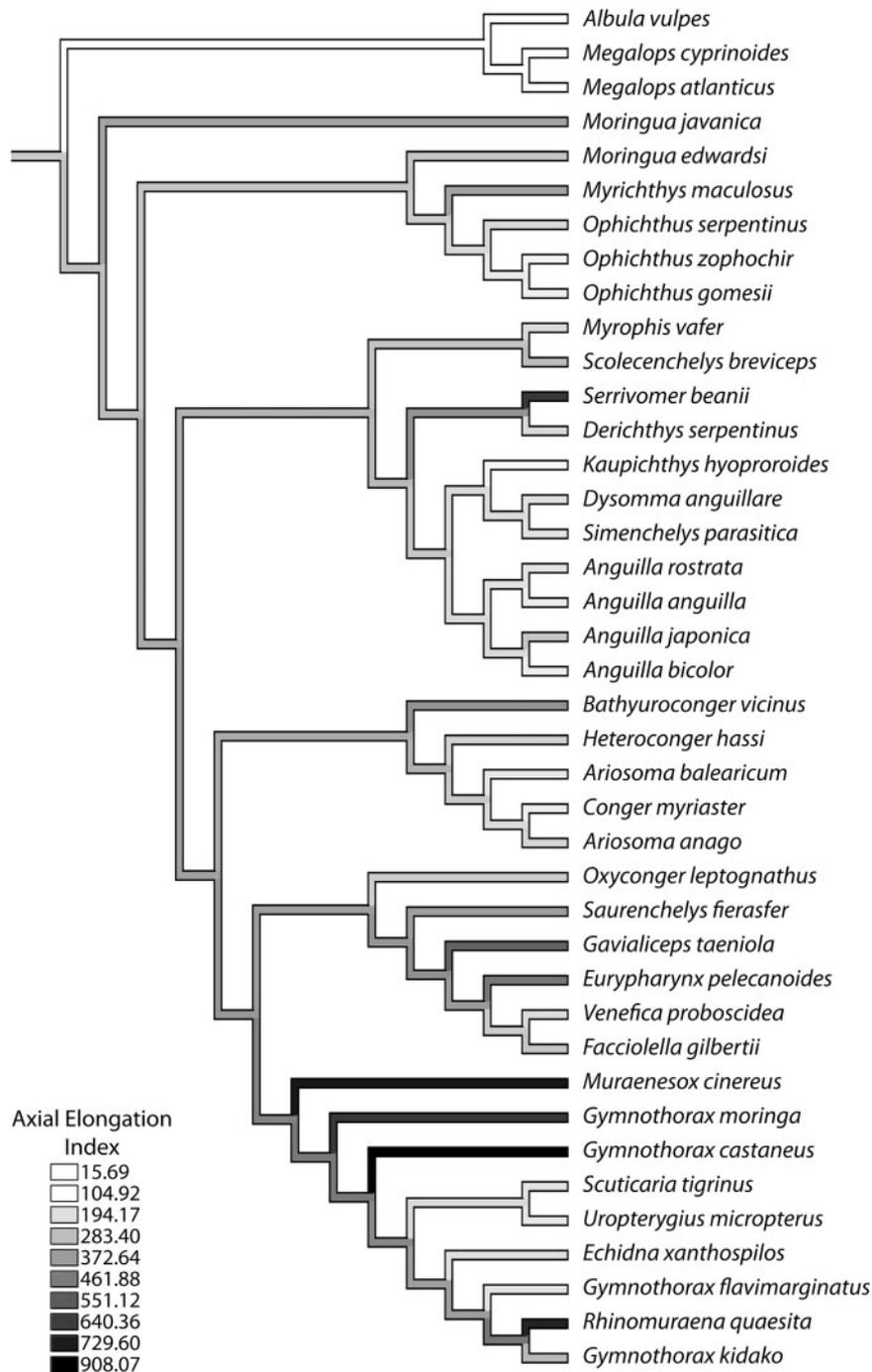
Fig. 5 Square-change parsimony traced phylogeny for AEI, a measure of the contribution of regional vertebral numbers and regional vertebral aspect ratios towards elongation (see text for details).

fin ($R = -0.23$, $P = 0.05$; $R = -0.02$, $P = 0.63$; Fig. 7F).

Ecological patterns in Anguilliformes

A PCA resulted in three axes explaining 78% of the axial and cranial variation in our 37 species of Anguilliformes with PC1 and PC2 explaining 58% of the variation among the seven morphological

variables (Table 3). No separation between reef-associated and non-reef-associated elopomorphs is obvious in this morphospace. PC1 accounted for 31.3% of the morphological variation across reef-associated and non-reef-associated species. Variables that loaded strongly and positively along PC1 were average vertebral centrum length, average vertebral centrum width, and head length. PC2 accounted
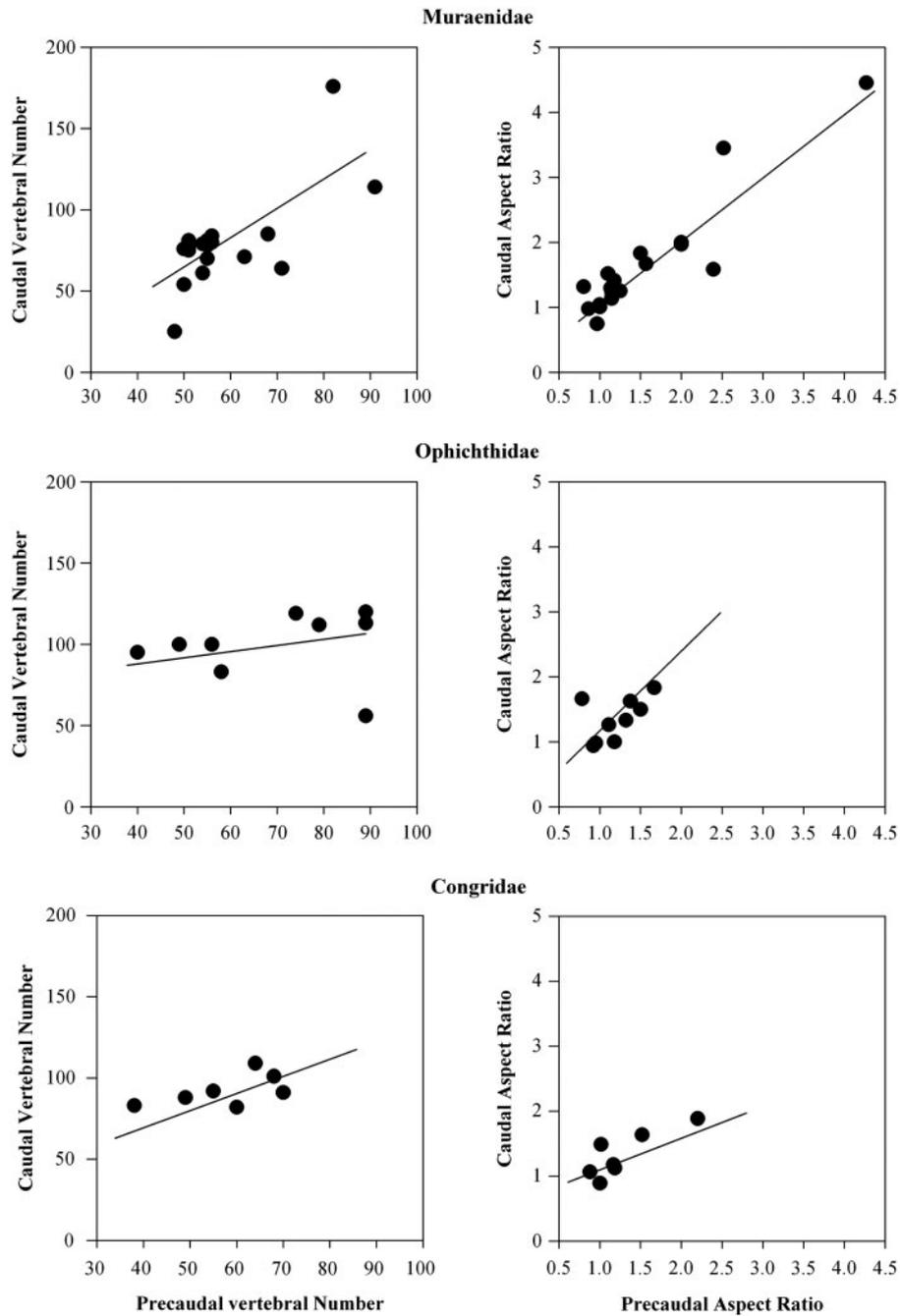
Fig. 6 The relationship between precaudal vertebral number and caudal vertebral number and precaudal aspect ratio and caudal aspect ratio in the three largest anguilliform clades: Muraenids, Congrids, and Ophichthids.

for 27% of the morphological variation across anguilliform species while branchial-arch length and fin aspect ratio loaded strongly on PC2 (Table 3). It was clear that non reef-associated species occupied a greater area of morphospace than did reef-associated species (Fig. 8).

Discussion

The variation in length and slenderness observed across the Elopomorpha comprises a continuum.

We adopted two different measures to capture overall body shape and axial elongation in elopomorph fishes: ER and AEI. ER explained the overall shape of elopomorph fishes by taking into account the changes in width and depth of the body (Fig. 2). Comparing ERs across species suggests that increases in body length are not always associated with decreases in diameter of the body in elopomorph fishes and vice versa. For example, *Rhinomuraena quaesita*, the ribbon moray, was one of the longest

Table 1 Reduced major axis regression statistics for raw vertebral numbers and ICs

Group	N	Slope	95% CI	R	P-value
Precaudal vertebral numbers versus caudal vertebral numbers					
Elopomorpha	41	1.72	1.21–2.22	0.42	0.005
Elopomorpha (ICs)	40	−0.35	−0.46 to −0.24	0.04	0.01
Muraenidae	18	2.51	1.52–3.55	0.72	0.001
Ophichthidae	9	1.07	0.12–2.04	0.11	0.783
Congridae	7	0.85	0.05–1.67	0.56	0.183
Precaudal aspect ratio versus caudal aspect ratio					
Elopomorpha	41	1.04	0.91–1.18	0.91	<0.001
Elopomorpha (ICs)	40	1.27	0.93–1.62	0.52	0.007
Muraenidae	18	1.07	0.86–1.29	0.92	<0.001
Ophichthidae	9	1.17	0.27–2.06	0.51	0.158
Congridae	7	0.73	0.20–1.25	0.78	0.001

Table 2 Reduced major axis regression statistics for morphological data

Relationships examined	Slope	95% CI	R	P-value
Morphological correlates related to elongation in elopomorph fishes				
HL × total number of vertebrae	0.032	−0.04 to 0.02	0.02	0.88
HL × total number of vertebrae (ICs)	0.075	−0.89 to 1.04	0.04	0.76
Lower jaw length × HL	0.79	0.69 to 0.90	0.90	<0.001
Lower jaw length × HL (ICs)	1.065	0.92 to 1.22	0.93	<0.001
Relative branchial-arch length × ER	0.021	0.01 to 0.03	0.30	0.05
Relative branchial-arch length × ER (ICs)	10.79	3.12 to 18.47	0.42	0.007
HW × relative branchial-arch length	−0.79	−1.43 to −0.17	−0.38	0.01
HW × relative branchial-arch length (ICs)	−0.17	−0.54 to 0.21	0.15	0.37
VG × relative branchial-arch length	1.46	1.06 to 1.87	−0.24	0.04
VG × relative branchial-arch length (ICs)	0.16	−0.16 to 0.47	0.16	0.33
Fin aspect ratio × ER	−0.039	−0.05 to 0.03	−0.23	0.05
Fin aspect ratio × ER (ICs)	−0.022	−0.04 to 0.05	−0.02	0.63

HL, head length; HW, head width; VG, vertical gape distance; ER, elongation ratio; ICs, independent contrasts.

species in this dataset but had an ER of 40 due to its relatively large body depth while *M. vafer*, the worm eel (Ophichthidae), exhibited the largest ER (110) and was only one quarter of the length of *R. quaesita*. However, ER alone does not capture all of the diversity in body form in eels. For example, across a narrow range of ERs (35–48) there was a 2-fold increase in total vertebral numbers. This wide range of vertebral numbers within a narrow range of ERs suggested that other aspects of the body and/or axial skeleton, such as head length, depth of the body, and vertebral aspect ratio might also be changing in those particular species.

AEI did not exhibit a strong relationship with ER. While ER is a measure of overall body shape, taking into account total length of the organism, AEI is a measure of elongation of the axial skeleton. The lack of a strong relationship between these two variables indicates that elongation of the vertebral column is not the only variable contributing toward relative lengthening of the body. It has been suggested that a decrease in depth of the body can produce a more elongate body without changing the axial skeleton (Ward and Brainerd 2007). We found no relationship between vertebral number and head length (see below), suggesting that overall body elongation may involve increases in head length without postcranial increases and vice versa. Other morphological changes associated with elongation of the body in the Elopomorpha are an increase in vertebral number, with smaller contributions from increases in vertebral aspect ratio.
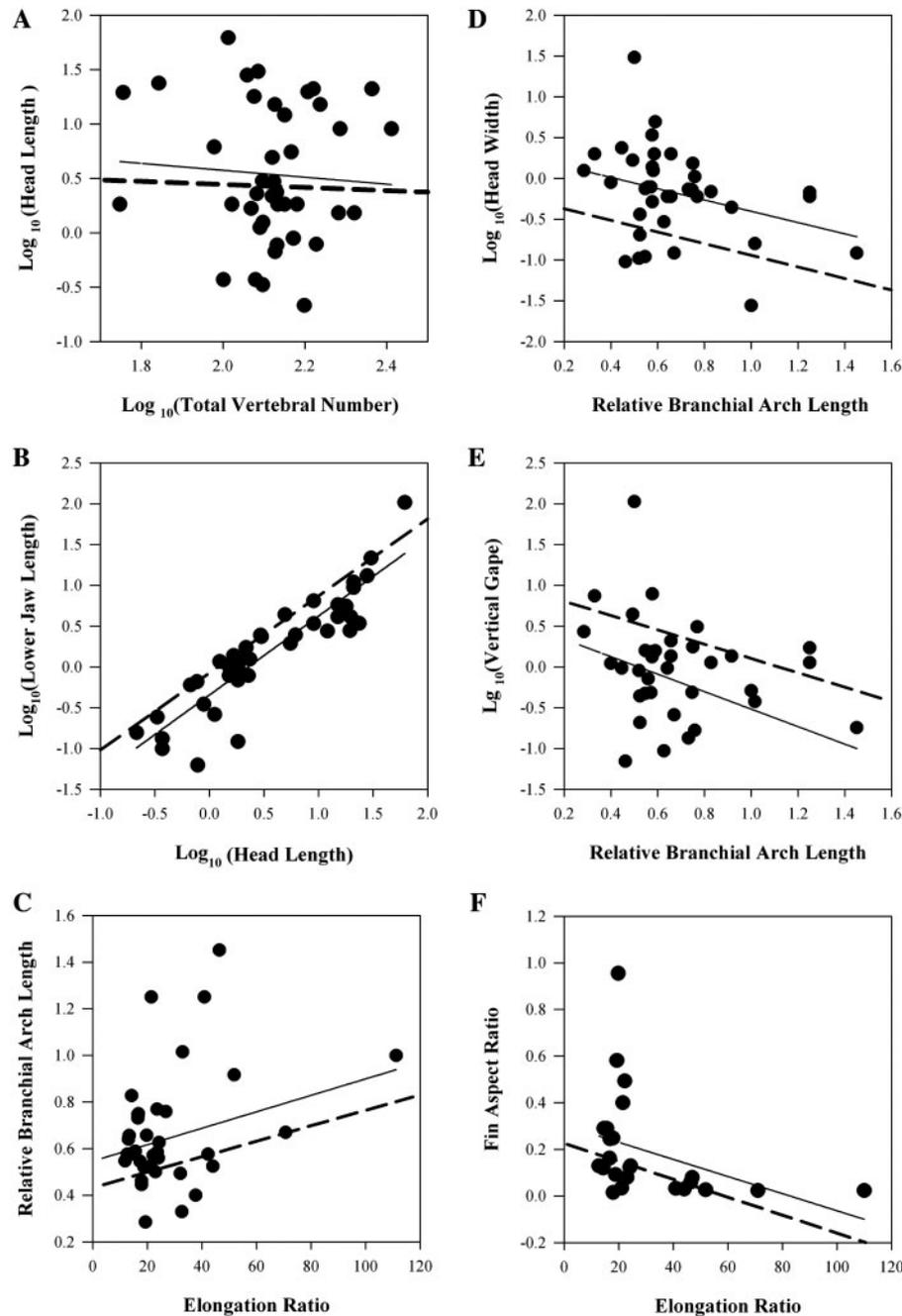
Fig. 7 The relationship between various morphological characters thought to be correlated with elongation in anguilliform fishes: (**A**) Total vertebral number and Head Length, (**B**) Head length and Lower jaw length, (**C**) Elongation ratio and Relative branchial arch length, (**D**) Relative branchial arch length and Head width, (**E**) Relative branchial arch length and Vertical gape, and (**F**) Elongation ratio and Fin aspect ratio.

Cryptic diversity in the axial skeleton

A previous survey of the literature examining precaudal and caudal vertebral numbers for 37 anguilliform species found greater variation in the precaudal region compared to the caudal region (Ward and Brainerd 2007). Overall, we found that evolutionary changes in vertebral number in the two regions of the axial skeleton are independent of one another.

However, within the three large anguilliform clades (muraenids, congrids, and ophichthids), we found that different patterns emerged (Fig. 6). While morays add more caudal vertebrae to their axial skeleton, ophichthids, and congrids add equal amounts of vertebrae to their precaudal and caudal region. A similar pattern was found with vertebral aspect ratios. Although vertebral aspect ratios were

Table 3 Loadings for the seven morphological variables on the first three principal components

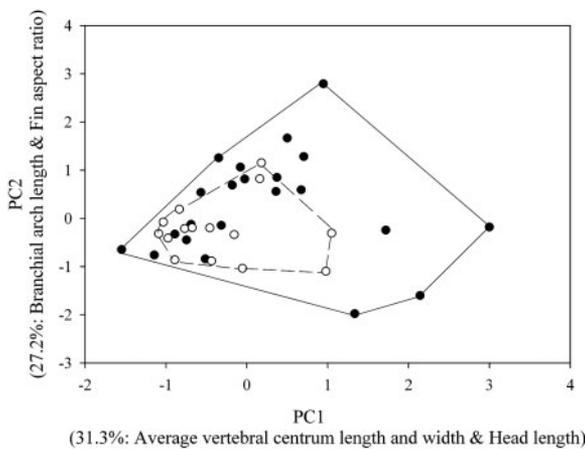| Variables	PC1 (31.3%)	PC2 (27.2%)	PC3 (19.4%)
Precaudal vertebral number	−0.22	0.07	0.74
Caudal vertebral number	0.11	0.13	0.79
Average vertebral centrum length	0.78	−0.58	0.01
Average vertebral centrum width	0.74	−0.62	−0.01
Head length	0.71	0.49	0.11
Branchial-arch length	0.66	0.63	0.09
Fin aspect ratio	0.19	0.71	−0.39

Fig. 8 A plot of principal components 1 (PC1) versus 2 (PC2) for 7 morphological variables. Anguilliformes ($N = 37$) were separated into two groups: reef-associated (open circle) and non reefassociated (filled circle). Lines connecting the outermost data points for each group indicate the perimeter of morphospace occupied. Variables that loaded strongly and positively along PC1 were average vertebral centrum length, average vertebral centrum width, and head length while branchial arch length and fin aspect ratio loaded strongly on PC2. There was no separation between these two groups although non reef-associated anguilliformes occupied a greater area of morphospace.

changing equally in both parts of the axial skeleton across elopomorphs, different patterns of variation in aspect ratios were observed within anguilliform clades. Muraenids exhibited greater changes in their caudal aspect ratios than in their precaudal aspect ratios. Ophichthids' aspect ratios were changing equally across their axial skeleton. Congrids exhibited yet a third pattern, where there were greater changes in precaudal aspect ratio than in caudal aspect ratio.

This cryptic diversity in the axial skeleton is interesting but very unexpected, particularly with respect to vertebral aspect ratio. Ward and Brainerd (2007) found variation in the precaudal and caudal aspect ratios to be closely linked in four teleost clades and concluded that in most actinopterygian clades aspect ratio appears to be constrained, changing equally in the precaudal and caudal region. They also suggested that aspect ratio may be controlled by one developmental module acting globally across the vertebral column. The changes that we are seeing may be due to different growth rates of the vertebrae in the different regions of the axial skeleton (shown to occur in *Rattus*; Bergmann et al. 2006). Further studies correcting for phylogeny within different anguilliform groups will contribute to our understanding of the evolution of aspect ratios in different regions of the vertebral column in fishes and whether vertebral aspect ratio is constrained.

Morphological correlates of elongation

We examined the relationship between two morphological features that are considered to facilitate a reduction in diameter of the body in anguilliform fishes (head width and relative branchial-arch length) and two that are thought to be related to, or contribute to, elongation in anguilliforms (head length and fin aspect ratio). In examining the two other variables, length of the lower jaw and vertical gape, we were attempting to gain insight into the diversity of eel skulls in relation to the rest of the body. Also examining the relationship between ER and relative branchial-arch length enabled us to test one of Nelson's hypotheses: that a more posteriorly placed branchial-arch region in relation to the head would aid in expansion of the pharyngeal region, especially necessary in those species that might be able to consume large prey or whole invertebrate and vertebrate prey.

Across Actinopterygii, it has been shown that head length is correlated with ER in elongate fishes, revealing that the head does contribute to lengthening of the body (Ward and Mehta, this volume). When looking across Anguilliformes, we found that head length was not correlated with ER and that in general, head length is not contributing much to increases in overall length in eels. A similar weak relationship (HL × standard length) was noted in a previous study that examined nine moray species from six different genera (Mehta 2009), suggesting that this pattern may even be consistent within diverse anguilliform groups, although future studies are necessary.

The anguilliform species included in this dataset were extremely diverse. We examined the most

members from each of the three largest anguilliform clades, Congridae, Muraenidae, and Ophichthidae and these represented 13 of the 17 recognized anguilliform groups (Nelson 2006). In our dataset head lengths ranged over 6-fold. Here we merely note the extensive variation in the different skeletal components of the head that were lengthened, reduced, fused, or oriented differently to present us with the overall notion that we were observing remarkable skull diversity in addition to the cryptic diversity already noted in the axial skeleton. In spite of this skull diversity, length of the lower jaw was strongly correlated with head length ($R = 0.9$; $P < 0.001$) and the two variables tightly evolve together.

Nelson (1966) revealed a relationship between what he termed "branchial-arch displacement" (postcranial head length/cranial head length) and degree of body elongation (total length/maximum body diameter) suggesting that longer eels tend to have more displaced branchial arches. We modified Nelson's terminology, replacing the term "branchial-arch displacement" with "relative branchial-arch length", and tested Nelson's hypothesis in a phylogenetic context. Our raw data and our IC scores revealed a positive relationship between relative branchial-arch length and ER supporting Nelson's (1966) data. When Nelson hypothesized that the relationship between relative branchial-arch length and ER had functional significance (Nelson 1966), he was specifically concerned with those eels of the anguilloid lineage. Regan (1912) coined the term "anguilloid" with respect to characteristics of the frontal bone and included in that lineage the following groups: Anguillidae, Heterenchelidae, Serrivomeridae, Nemichthyidae, Moringuidae, Chlopsidae (Xenocongridae), Dysomminidae, and Muraenidae. In examining anguilloid representatives, Nelson proposed that a reduction in head diameter would be accomplished by moving the gill arches posteriorly to a position behind, rather than within the skull, thereby facilitating the behaviors of wedging into tight crevices or burrowing. Although there appeared to be a trend for eels with narrower heads to have branchial arches that extended farther back, this relationship was not significant (Fig. 7D).

Nelson (1966) also found that gill-arch displacement in the anguilloids was not only accompanied by a loss of connections between the cranium and the gill arches but between the interconnections of the gill arches themselves, suggesting that this might aid in expansibility of the pharynx. We interpret this hypothesis to mean that eels with more posteriorly placed gill arches can laterally extend their throat regions more to accommodate large prey or whole prey, both of which would require a relatively large gape. Our data do not support Nelson's hypothesis (or rather our interpretation of his hypothesis) as we found a slight negative relationship between vertical gape distance and relative branchial-arch length in the raw data and no relationship in the IC scores. What we would like to note, however, is the high variance in size-corrected vertical gape at the lower end of relative branchial-arch length and the very few individuals with large gapes and extremely displaced branchial arches. The two Anguilliformes with large gapes at the higher end of branchial displacement are *Derichthys serpentinus* (Derichthyidae) and *Ophichthus maculates* (Ophichthidae). The majority of the moray species, which we know have a highly mobile fourth pharyngeal arch and a highly distensible pharynx, are clustered in the middle of the graph in a cloud at the more positive end of the *y*-axis. The eels with highly extended branchial arches are the two spaghetti eel species, *Moringua edwarsi* and *M. javanica*, and the ophichthid, *M. vafer*. These three species do not look like they could swallow very large prey although a detailed morphological study of the cranium of *Moringua edwarsi* revealed enlarged adductor mandibulae muscles, recurved teeth on the upper and lower jaws, and a large gape (De Schepper et al. 2005). These characteristics have been generally linked to predatory habits (Liem 1980; Herrel et al. 2002; Van Wassenbergh et al. 2004) as well as to a head-first burrowing lifestyle in the case of the moringuids (Smith and Castle 1972; Smith 1989a; DeSchepper et al. 2005). Specifically, in the case of *M. edwarsi*, enlarged adductor mandibulae muscles, recurved teeth, and a large gape may reflect adaptations for biting off chunks of food by rotational feeding rather than swallowing large prey as a whole. We did find a negative relationship between head width and relative branchial-arch length, thereby supporting Nelson's idea that a reduction in head diameter may have been partly achieved by posterior displacement of the branchial arches, although this relationship was not supported by the ICs.

Reduction or loss of limbs in terrestrial vertebrates is thought to promote ease of movement below the surface (Gans 1975; Pough et al. 1998). We did find a slight negative relationship between aspect ratio of the pectoral fins and ER although ICs revealed that these variables are not evolving together. Species with high ERs and small fins, the two moringuid species and the ophichthid, *M. vafer*, tended to be burrowers (Smith 1989a; McCosker et al. 1989). The congrid, *Heteroconger halis* also had very reduced pectoral fins and a high ER. *Heteroconger halis* lives in colonies in

sandy substrata with each individual inhabiting its own burrow (Smith 1989b). If we look at the species in our dataset that do not have pectoral fins, we find that the majority are morays, the only anguilliform group whose members have universally lost the pectoral fins (Böhlke et al. 1989). The complete loss of pectoral fins in morays may have to do with their ability to consume large prey whole. Most fish are gape limited by their oral jaws, pharyngeal jaws and inter-cleitheral space. Fielitz (2002) revealed remnants of the pectoral girdle in morays; however, not having a clavicularis and a complete cleithrum enables morays to expand their bodies laterally beyond their sets of jaws. The eels with the largest pectoral fins were the four anguillids and the two congrid species, *Conger myriaster* and *Oxyconger leptognathus.*

Ecological correlates of elongation

We assigned Anguilliformes to one of two groups: those that live in or near coral reef habitats ''reef-associated'' and those that occupy bathypelagic zones or are benthic or mid-water dwellers ''non reef-associated''. We performed a PC analysis with five morphological characters of the skull and axial skeleton (HL, branchial-arch length, average vertebral aspect ratio, mean total vertebral number, and fin aspect ratio) and found no separation in morphospace between reef-associated eels and non–reef-associated eels. This was very surprising as many of the reef species either lacked pectoral fins, or exhibited very reduced ones while many of the non-reef-associated species had well-developed pectoral fins. What we managed to show was that there was a greater amount of morphological variance among non-reef-associated eels. From this analysis we conclude that there is some evidence that reef-associated eels are morphologically constrained compared to other eels. One character that would be worthwhile measuring in the future is the shape of the caudal region. We noticed that many of the reef-dwelling species had cylindrical bodies and laterally flattened tails that had a very slight taper at the end while non reef-associated species had more cylindrical tails that were highly tapered. For example, one-third of the caudal regions of *Bathyconger vicinus*, *Oxyconger leptognathus*, and all three nettastomatids were highly tapered. Laterally, compressed tails are thought to produce greater propulsive forces onto the water as compared to cylindrical bodies with tapering tails (Gillis 1996). Reef-associated species that are predaceous may gain great advantages from having a tail

capable of producing high propulsion when in pursuit of prey.

Behavioral considerations of elongation

Anguilliforms are capable of diverse behaviors such as forward and backward movements of their bodies while in confined spaces, wedging their bodies into small crevices, anchoring themselves with their tails, tying themselves into knots, and burrowing holes with their heads and with their tails. Can these behaviors shed insight into the different axial patterns we observe within anguilliform groups?

In order to understand the evolution of body elongation in aquatic vertebrates, many researchers have looked to elongate terrestrial vertebrates for inspiration. Elongation of the body and reduction of the limbs in tetrapods is associated with fossorial, crevice-dwelling, aquatic, and grass-swimming and sand-swimming lifestyles (Gans 1973, 1975). The external morphological corollaries for fossoriality and for crevice-dwelling habits exhibit additional corollaries in both the terrestrial and aquatic realm with organisms exhibiting reduction of the eyes (Gans 1975; Withers 1981; Lee 1998) and smoother scales or highly mucosal skin (Gans 1975; McCosker et al. 1989). What we have found is that Anguilliformes with the highest ERs tend to burrow either head first or tail first. We also find that these fishes have very small fin aspect ratios of the pectoral fin, perhaps resulting in less impedance to burrowing, and that they have narrower heads and greatly displaced branchial arches. The longer but thinner bodies may aid in anguilliform locomotion in the substrate.

Gape limitation is more pronounced in an elongate body (Gans 1961). Snakes and moray eels, two disparate elongate vertebrate lineages that are obligatory carnivores have evolved alternative morphological and physiological mechanisms that allow consumption of large prey whole (Mehta and Wainwright 2007, 2008). In addition to morays, other Anguilliformes have evolved behavioral adaptations for handling large prey. Rotational feeding, the act of spinning around the long axis of the body is used by morays as well as anguillid and congrid eels (Miller 1987, 1989; Helfman and Clark 1986; Helfman 1990). During this prey method of handling, an eel seizes the prey with its jaws and then performs a single twist of its body to begin rotation. Morays also appear to rotate their tails to help maintain the spinning of their bodies after initial rotation (R. S. Mehta, personal observation). Rotational feeding often results in the tearing of prey into smaller more manageable pieces but can

also be used for forcing whole pieces of prey into the mouth when the rotating body is pushing against the substrate or a nearby object. This behavior has also been studied in caecilians (Measey and Herrel 2006) and alligators (Fish et al. 2007), two other elongate tetrapod lineages.

Apparently all morays are carnivores and are able to consume large prey whole (Böhlke et al. 1989; Mehta and Wainwright 2008). However, many moray species have also been reported to take chunks from their prey. In order to do so, they first tie an overhand knot in their tail region. This knot is then slowly worked up around the precaudal region and is used for leverage around their prey (Miller 1987, 1989; R. S. Mehta, personal observation). Knotting has been compared to constriction in snakes in that it enables the moray to restrain or maintain a grip on its prey (Greene and Burghardt 1978; Miller 1987, 1989). Eels that knot may benefit from having a lengthy caudal region which may be why we see increases in vertebral numbers and increases in aspect ratios in the caudal region as morays species get longer, especially in the more piscivorous species, such as the genus *Gymnothorax*. Böhlke et al. (1989) listed the mean vertebral counts for 24 moray species and what we observe is that the larger and more piscivorous morays, on average, have longer tails. A longer tail region may also help morays anchor themselves into small crevices in coral reefs and thereby provide leverage to quickly dart out of a confined area in pursuit of prey.

Concluding remarks: how to build an eel

In this study, we addressed the following questions: How is body elongation achieved in elopomorphs? Are patterns of axial elongation consistent across anguilliform clades? As species get longer, what other aspects of their morphology change? Can we identify morphological, ecological, and behavioral correlates associated with patterns of elongation in anguilliform fishes? In general, we found that there is more than one way to build an eel and that elopomorphs are highly diverse in their mechanisms of body elongation, representing every trend previously shown for axial elongation in actinopterygian fishes. Each major lineage of Anguilliformes shows a slightly different anatomical regional pattern underlying overall elongation of the body. We also show that Angulliformes with high ERs have more displaced-branchial regions, and tend to be burrowers. However, we also found that the few morphological characters of the skull and axial skeleton that

we examined did not strongly reflect the broad marine habitats occupied by the anguilliform fishes in our study. Lastly, we observed that elongation may have opened up the potential for the evolution of extremely interesting and complex behaviors, particularly with respect to feeding. For example, rotational feeding and knotting are behaviors observed in only elongate craniates. Based on these findings, we suggest that Anguilliformes are an excellent model system for investigating the underlying anatomical basis of body elongation as well as the functional and ecological consequences of body elongation in fishes.

Supplementary Data

Supplementary data are available at *ICB* online.

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References

Adriaens D, Devaere S, Teugels GG, De Kegel B, Verraes W. 2002. Intraspecific variation in limblessness in vertebrates: a unique example of microevolution. Biol J Linn Soc 75:367–77.

Belouze A, Gayet M, Atallah C. 2003a. Les premiers Anguilliformes: I. Révision des genres cénomaniens Anguillavus HAY, 1903 et *Luenchelys* nov. gen. Geobios 36: 241–73.

Belouze A, Gayet M, Atallah C. 2003b. Les premiers Anguilliformes: II. Paraphylie du genre *Urenchelys* WOODWARD, 1900 et relations phylogénétiques. Geobios 36: 351–78.

Bergmann PJ, Melin AD, Russell AP. 2006. Differential segmental growth of the vertebral column of the rat (*Rattus norvegicus*). Zool 109:54–65.

Brandley MC, Huelsenbeck JP, Wiens JJ. 2008. Rates and patterns in the evolution of snake-like body form in squamate reptiles: evidence for repeated re-evolution of lost digits and long-term persistence of intermediate body forms. Evolution 62:2042–64.

Böhlke EB. 1989. Methods and terminology. In: Böhlke E, editor. Fishes of the western North Atlantic: orders Anguilliformes and Saccopharyngiformes. Vol. 1. New Haven (CT): Sears Foundation for Marine Research. p. 1–7.

Böhlke EB, McCosker JE, Böhlke JE. 1989. Family muraenidae. In: Böhlke E, editor. Fishes of the Western North Atlantic: orders Anguilliformes and Saccopharyngiformes, Vol. 1. New Haven (CT): Sears Foundation for Marine Research. p. 104–206.

De Schepper N, Adriaens D, De Kegel B. 2005. *Moringua edwardsi* (Moringuidae: Anguilliformes): Cranial Specialization for Head-First Burrowing? J Morph 266:356–68.

Dingerkus G, Uhler LD. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstrating cartilage. Stain Technol 52: 229–32.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Felsenstein J. 1985. Phylogenies and the comparative method. Am Nat 125:1–15.

Fielitz C. 2002. Previously unreported pectoral bones in moray eels (Anguilliformes: Muraenidae). Copeia 2002:483–8.

Fish FE, Bostic SA, Nicastro AJ, Beneski JT. 2007. Death roll of the alligator: mechanics of twist feeding in water. JEB 210: 2811–8.

Frickhinger K. 1991. *Bolcyrus bajai and Voltaconger latispinus*, both from Monte Bolca. In: Fossilien Atlas Fische. Verlag für Natur- und Heimtierkunde. Melle (Germany): Mergus.

Froese R, Pauly D, editors. 2009. FishBase. www.fishbase.org, version (11/2009).

Gans C. 1961. The feeding mechanism of snakes and its possible evolution. Amer Zool 1:217–27.

Gans C. 1973. Locomotion and burrowing in limbless vertebrates. Nature 242:414–5.

Gans C. 1975. Tetrapod limblessness: evolution and functional corollaries. Amer Zool 15:455–67.

Garland T Jr, Harvey PH, Ives AR. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Syst Biol 41:18–32.

Gillis GB. 1996. Undulatory locomotion in elongate aquatic vertebrates: Anguilliform swimming since Sir James Gray. Amer Zool 36:656–65.

Greene HW, Burghardt GM. 1978. Behaviour and phylogeny: constriction in ancient and modern snakes. Science 200:74–6.

Helfman GS. 1990. Mode selection and mode switching in foraging animals. Adv Stud Behav 19:249–98.

Helfman GS, Clark JB. 1986. Rotational feeding: overcoming gape-limited foraging in Anguillid eels. Copeia 1986:679–85.

Herrel A, Adriaens D, Verraes W, Aerts P. 2002. Bite performance in clariid fishes with hypertrophied jaw adductors as deduced by bite modeling. J Morph 253:196–205.

Lee MSY. 1998. Convergent evolution and character correlation in burrowing reptiles: towards a resolution of squamate relationships. Biol J Linn Soc 65:369–453.

Liem KF. 1980. Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. In: Ali MA, editor. Environmental physiology of fishes. New York: Plenum. p. 299–334.

Lindsey CC. 1975. Pleomerism, widespread tendency among related fish species for vertebral number to be correlated with maximum body length. J Fish Res Brd Can 32:2453–69.

Maddison WP, Maddison DR. 2009. Mesquite: a modular system for evolutionary analysis. Version 2.72. http://mesquiteproject.org.

McCosker JE, Böhlke EB, Böhlke JE. 1989. Family Ophichthidae. In: Böhlke E, editor. Fishes of the Western North Atlantic: orders Anguilliformes and Saccopharyngiformes. Vol. 1. New Haven (CT): Sears Foundation for Marine Research. p. 254–412.

Measey JG, Herrel A. 2006. Rotational feeding in caecilians: putting a spin on the evolution of cranial design. Biol Lett 2:485–7.

Mehta RS. 2009. Ecomorphology of the moray bite: relationship between dietary extremes and morphological diversity. Physiol Biochem Zool 82:90–103.

Mehta RS, Wainwright PC. 2007. Raptorial pharyngeal jaws help moray eels swallow large prey. Nature 449:79–82.

Mehta RS, Wainwright PC. 2008. Functional innovation in the pharyngeal jaw apparatus of moray eels. J Morph 269:604–19.

Miller TJ. 1987. Knotting: a previously undescribed feeding behavior in muraenid eels. Copeia 1987: 1055–7.

Miller TJ. 1989. Feeding behavior of *Echidna nebulosa, Enchelycore pardalis*, and *Gymnomuraena zebra* (Teleostei: Muraenidae). Copeia 1989:662–72.

Nelson GJ. 1966. Gill arches of teleostean fishes of the order Anguilliformes. Pac Sci 20:391–408.

Nelson JS. 2006. Fishes of the world. 4th Edition. New York: John Wiley & Sons.

Orti G, Petry P, Porto JIR, Jegu M, Meyer A. 1996. Patterns of nucleotide change in mitochondrial ribosomal RNA genes and the phylogeny of piranhas. J Mol Evol 42:169–82.

Parra-Olea G, Wake DB. 2001. Extreme morphological and ecological homoplasy in tropical salamanders. Proc Natl Acad Sci USA 98:7888–91.

Patterson C. 1993. *Eomuraena sagittidens*, referenced in Osteichthyes: Teleostei. In: Benton MJ, editor. The Fossil Record 2. London: Chapman and Hall. p. 621–56.

Pough HF, Andrews RM, Cadle JE, Crump ML, Savitzky AH, Wells KD. 1998. Body support and locomotion. In: Pough HF, editor. Herpetology. Englewood Cliffs (NJ): Prentice Hall.

Rambaut A, Drummond AJ. 2007. Tracer 1.4.

Regan CT. 1912. The osteology and classification of the teleostean fishes of the order Apodes. Ann Mag Nat Hist 8:377–87.

Smith DG. 1989a. Family Moringuidae. In: Böhlke EB, editor. Fishes of the western North Atlantic. New Haven (CT): Sears Foundation for Marine Research. p. 55–71.

Smith DG. 1989b. Family Heterenchelyidae. In: Böhlke EB, editor. Fishes of the western North Atlantic. New Haven (CT): Sears Foundation for Marine Research. p. 48–54.

Smith DG, Castle PHJ. 1972. The eel genus *Neoconger* Girard: systematics, osteology, and life history. Bull Mar Sci 22:196–249.

Van Wassenbergh S, Herrel A, Adriaens D, Aerts P. 2004. Effects of jaw adductor hypertrophy on buccal expansions during feeding of airbreathing catfishes (Teleostei, Clariidae). Zoomorphology 123:81–93.

Wake DB. 1966. Comparative osteology and evolution of the lungless salamanders, Family Plethodontidae. Memoirs of the Southern California Academy of Science 4:1–111.

Wang H-Y, Lee S-C. 2002. Secondary structure of mitochondrial 12S rRNA among fish and its phylogenetic applications. Mol Biol Evol 19:138–48.

Ward AB, Azizi E. 2004. Convergent evolution of the head retraction escape response in elongate fishes and amphibians. Zool 107:205–17.

Ward AB, Brainerd EL. 2007. Evolution of axial patterning in elongate fishes. Biol J Linn Soc 90:97–116.

Wiens JJ, Slinghuff JL. 2001. How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. Evolution 55:2303–18.

Wiens JJ, Brandley MC, Reeder TW. 2006. Why does a trait evolve multiple times within a clade? Repeated evolution of snake-like body form in squamate reptiles. Evolution 60:123–41.

Withers PC. 1981. Physiological correlates of limblessness in fossorial lizards. Copeia 1:197–204.

Yamada T, Sugiyama T, Tamaki N, Kawakita A, Kato M. 2009. Adaptive radiation of gobies in the interstitial habitats of gravel beaches accompanied by body elongation and excessive vertebral segmentation. BMC Evol Biol 9:145, 14p.