Evolution of axial patterning in elongate fishes

ANDREA B. WARD* and ELIZABETH L. BRAINERD†

Biology Department and Organismic and Evolutionary Biology Program, University of Massachusetts, Amherst MA 01003, USA

Within the ray-finned fishes, eel-like (extremely elongate) body forms have evolved multiple times from deeper-bodied forms. Previous studies have shown that elongation of the vertebral column may be associated with an increase in the number of vertebrae, an increase in the length of the vertebral centra, or a combination of both. Because the vertebral column of fishes has at least two anatomically distinct regions (i.e. abdominal and caudal), an increase in the number and relative length of the vertebrae could be region-specific or occur globally across the length of the vertebral column. In the present study, we recorded vertebral counts and measurements of vertebral aspect ratio (vertebral length/width) from museum specimens for 54 species representing seven groups of actinopterygian fishes. We also collected, from published literature, vertebral counts for 813 species from 14 orders of actinopterygian and elasmobranch fishes. We found that the number of vertebrae can increase independently in the abdominal and caudal regions of the vertebral column, but changes in aspect ratio occur similarly in both regions. These findings suggest that abdominal vertebral number, caudal vertebral number, and vertebral aspect ratio are controlled by separate developmental modules. Based on these findings, we suggest some candidate developmental mechanisms that may contribute to vertebral column patterning in fishes. Our study is an example of how comparative anatomical studies of adults can generate testable hypotheses of evolutionary changes in developmental mechanisms. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, **90**, 97–116.

ADDITIONAL KEYWORDS: comparative methods – elongation – modularity – vertebrae – vertebral column.

INTRODUCTION

Elongate body forms occur in all major groups of vertebrates, and previous studies have shown that elongation of the vertebral column may be associated with an increase in the number of vertebrate, an increase in the length of vertebral centra, or a combination of both (Wake, 1966; Lindsey, 1975; Parra-Olea & Wake, 2001). Because vertebral columns have at least two anatomically distinct regions (i.e. abdominal and caudal), increases in number and relative length of the vertebrae could occur regionally or globally along the length of the axial skeleton (Gadow, 1933; Goodrich, 1958; Polly, Head, & Cohn, 2001; Narita & Kuratani, 2005).

Previous studies have found that axial elongation is generally associated with an increase in the number of vertebrae (Wake, 1966; Lindsey, 1975; for a review, see Richardson *et al*., 1998), and changes in vertebral number appear to be region specific (Wake, 1966; Asano, 1977; Polly *et al*., 2001). In a study of plethodontid salamanders, Wake (1966) found that elongation in this clade usually involves an increase in the number of abdominal vertebrae but, in at least one genus, *Eurycea*, the number of abdominal vertebrae remains constant and the number of caudal vertebrae increases. Polly *et al*. (2001) found a similar dissociation between vertebral regions in snakes. Some lineages show changes in the number of abdominal vertebrae without changes in the number of caudal vertebrae, and others show changes in the number of caudal vertebrae without changes in the number of abdominal vertebrae.

Fewer studies have tested the hypothesis that elongation of the individual vertebral centra may contribute to elongation of the vertebral column. *Lineatriton* has been shown to have elongated centra relative to

*Corresponding author. Current address: Adelphi University, Department of Biology, 1 South Avenue, PO Box 701, Garden City, NY 11530-0701, USA. E-mail: award@adelphi.edu
†Current address: Department of Ecology and Evolutionary Biology, Box G-B210, Brown University, Providence, RI 02912, USA.

other plethodontid salamanders (Wake, 1966; Parra-Olea & Wake, 2001) and, in some groups of snakes, elongation of the vertebral column is associated with elongated centra (Johnson, 1955). Thus, although variation in the relative length of centra has received relatively little attention, it does appear that increases in centrum length may be associated with axial elongation in some vertebrates.

AXIAL REGIONALIZATION

The vertebral column varies in degree of regionalization across vertebrates. The vertebral column of actinopterygian fishes has two distinct regions: the pre-anal abdominal region and the post-anal caudal region (Grande & Bemis, 1998). Sarcopterygians, including tetrapods, are generally considered to have more specialized regions than actinopterygians (Romer, 1970). Tetrapods generally have cervical, abdominal, sacral, and caudal regions. The abdominal region may also be regionalized into the thoracic and lumbar regions.

Although the actinopterygian vertebral column has two primary regions (i.e. abdominal and caudal), there is diversity in vertebral form within these regions (Ford, 1937; Pietsch, 1978; Grande & Bemis, 1998; Bemis & Forey, 2001). The abdominal region may include, from anterior to posterior, occipital vertebrae that are incorporated into the skull through ontogeny, anterior vertebrae that are highly modified (e.g. Weberian apparatus in Ostariophysi and fused vertebrae in Sygnathoidei), and vertebrae that generally bear abdominal ribs (Fig. 1A). The caudal region includes vertebrae that bear haemal spines (Fig. 1B) and ural vertebrae that bear hypurals. For the purposes of the present study, we will consider the vertebral column as having only two regions (abdominal and caudal) for easier comparison with previous studies (Wake, 1966; Asano, 1977; Polly *et al*., 2001).

TESTING FOR MODULARITY

Modules are defined as discrete anatomical units that can change without affecting other structures (Raff, 1996; Gass & Bolker, 2003). Similarly, some developmental processes can be considered to be modular because one process may function and change without affecting another process (Gass & Bolker, 2003). Polly *et al*. (2001) proposed that the abdominal and caudal vertebral regions of snakes are separate modules because changes in vertebral number are inherited differentially in each region. We formalize this idea into a set of three models of vertebral number variation in the abdominal and caudal regions (Fig. 2A). Models A1 and A3 demonstrate regional modularity in the number of vertebrae. In each case, the slope of the line relating caudal to abdominal vertebral number is
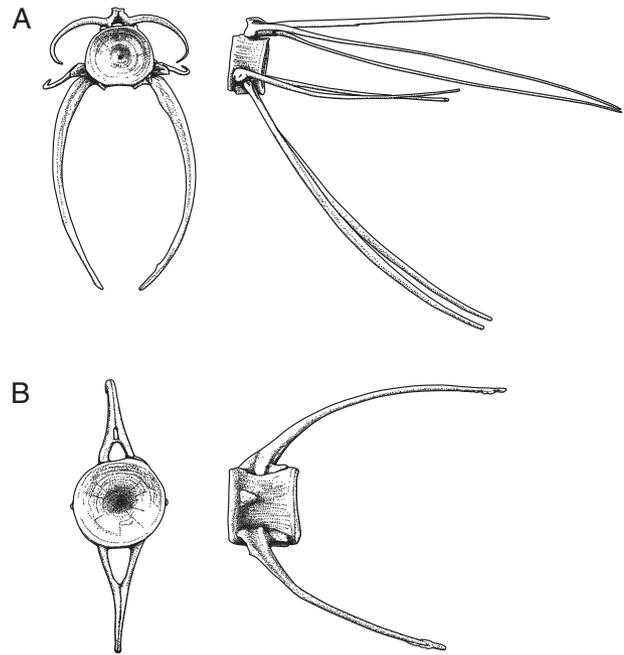
Figure 1. Vertebrae from the Atlantic tarpon, *Megalops atlanticus*. A, anterior and lateral views of an abdominal vertebra with ribs; B, anterior and lateral views of a caudal vertebra with fused haemal arch.

not equal to one, indicating that there is a disproportionate change in one region over the other. In Model A2, there is an equal change in the number of vertebrae in both regions, and the resulting slope of the line is one (Fig. 2A).

We have developed similar models to describe variation in aspect ratio (length/width) of the vertebral centra. If the changes in aspect ratio are equal in the two vertebral regions, then the slope of the line will be one (Fig. 2B, Model B2). In Models B1 and B3, the slope of the line differs from one, indicating that centrum elongation occurs more in one region of the body than in the other region.

In this study, we use vertebral counts and measurements of vertebral aspect ratio to test these models in actinopterygian fishes. Within ray-finned fishes, elongate body forms have evolved multiple times from more deep-bodied forms; therefore, Actinopterygii is a particularly good group for studying vertebral column modularity. Here, we examine seven clades in which more elongate body forms have evolved from deeper-bodied forms (Fig. 3). As reflected in our models (Fig. 2), we anticipate several possible outcomes to the study. Within each clade, body elongation may be associated with equal increases in vertebral number in both the abdominal and caudal regions (Model A2), indicating that evolutionary changes in vertebral number are constrained by a single developmental
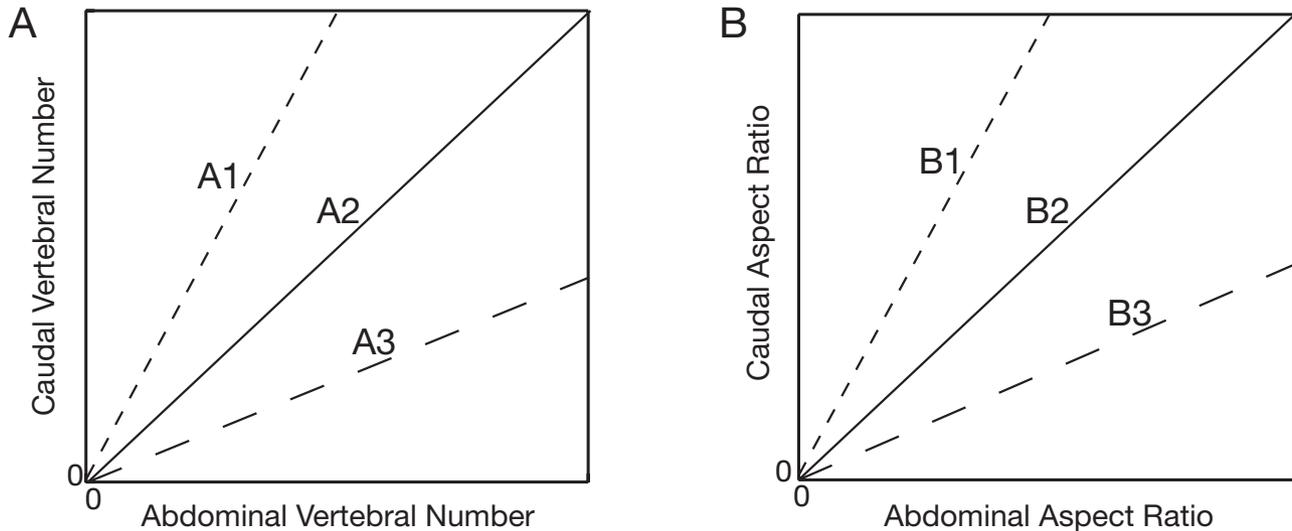
Figure 2. Models of axial patterning in fishes. A, vertebral number; B, vertebral aspect ratio (centrum length/centrum width). For an explanation of the models, see text.

module, or they may increase differentially (Models A1 and A3), indicating at least partially separate developmental control. Similarly, within each clade, vertebral aspect ratio may change equally (Model B2) or unequally (Models B1 and B3) in the two regions, with similar interpretations of these patterns. In addition, different clades of fishes may or may not exhibit the same patterns of vertebral column evolution, allowing us to determine, based on our sample of seven clades, whether vertebral developmental modules vary or are conserved within Actinopterygii.

MATERIAL AND METHODS

Two data sets were collected for this study. The first contains abdominal and caudal vertebral counts from published literature, and the second contains abdominal and caudal vertebral counts and aspect ratios measured on dried skeletal specimens from museum collections.

LITERATURE-BASED DATA SET

To test the models proposed in Fig. 2A, abdominal and caudal vertebral counts were gathered from published literature (Springer & Garrick, 1964; Rofen, 1966; Rosen & Patterson, 1969; Tesch, 1977; Tyler, 1980; Shiogaki, 1981; De Sylva, 1984; Ahlstrom *et al*., 1984; Anderson, 1984; Collette *et al*., 1984a, b; Deelder, 1984; Fahay & Markle, 1984; Gordon, Markle & Olney, 1984; Horn, 1984; Olney, 1984; Smith-Vaniz, 1984; Stevens, Watson & Matarese, 1984; Roberts, 1986; Smith & Heemstra, 1986; Anderson, 1990; Chiu & Markle, 1990; Dewitt, Heemstra & Gon, 1990; Gon,

1990a, b, c; Hureau, 1990; Iwami & Kock, 1990; Nakamura, 1990; McCosker, 1998). The maximum number of abdominal or caudal vertebrae was used for each species when a range of values or multiple values for a species were available. A total of 813 species from 14 orders of elasmobranch and actinopterygian fishes was included (Table 1; Supplementary Material: Table S1). For this analysis, species were grouped into orders (orders as defined by FishBase; Froese & Pauly, 2004). Regressions for each order were calculated using a reduced major axis algorithm in SPSS 10.0 (SPSS, Inc., Chicago, Illinois, USA) and slopes were tested against the models proposed in Fig. 2A.

MUSEUM SPECIMEN-BASED DATA SET

Seven clades of actinopterygians were chosen based on range of body forms, availability of phylogenetic information, and availability of specimens. The groups included (and number of species examined) are: Polypteriformes ($N = 3$), Osteoglossomorpha ($N = 7$), Elopomorpha ($N = 11$), Ostariophysi ($N = 9$), Paracanthopterygii ($N = 10$), Beloniformes ($N = 6$), and Scombroidei ($N = 8$). A total of 311 specimens from 54 species were examined from museum collections (for museum numbers, see Supplementary Material: Appendix S1).

Three skeletal specimens were examined for each species (except where noted, see Supplementary Material: Table S2). The number of abdominal and caudal vertebrae was counted three times and the mean values for each region were calculated for each individual. Species means were then calculated for abdominal vertebral number (AVN) and caudal
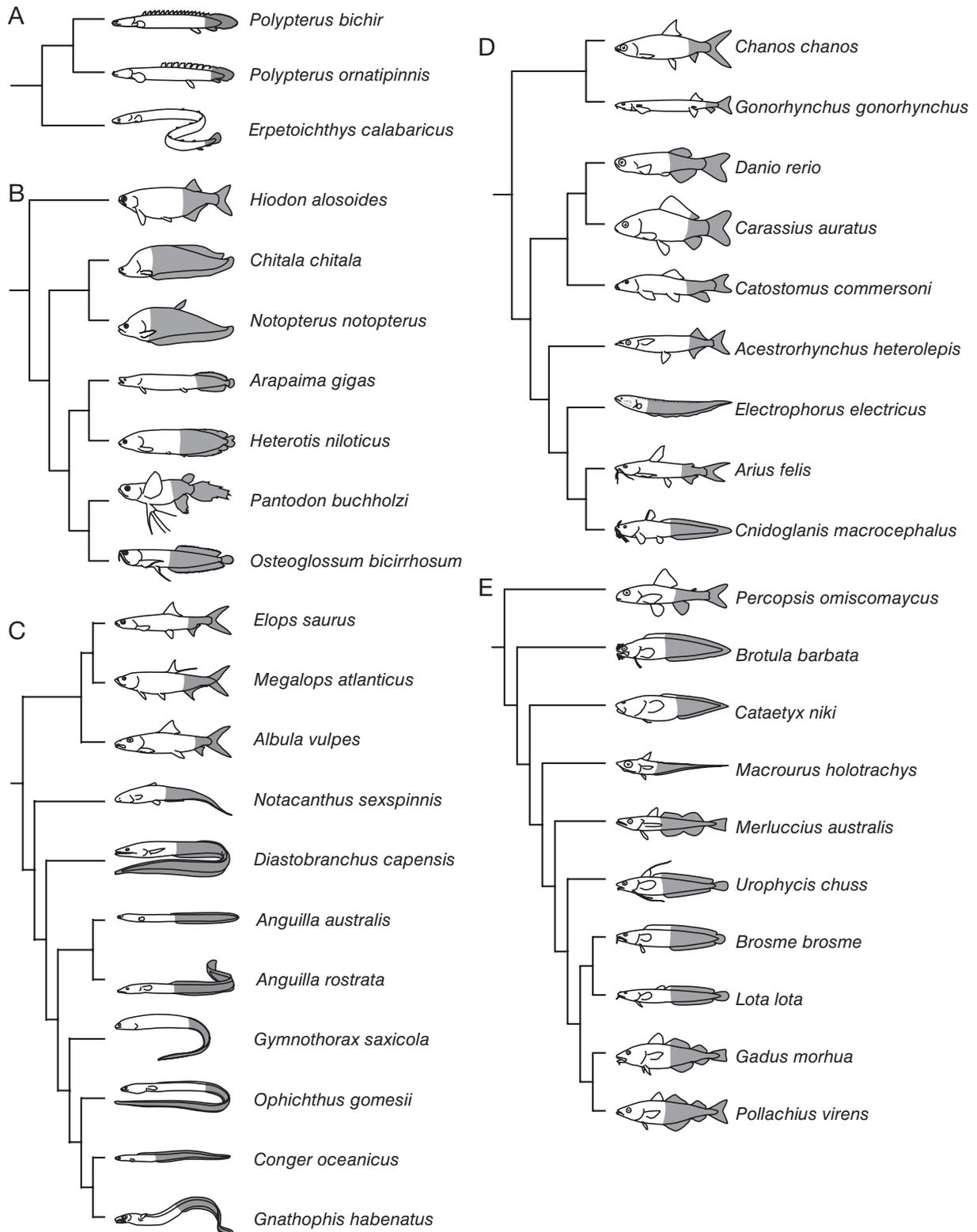
Figure 3. Intrarelationships of the seven groups included in our museum-based study. In the line drawings, the grey portion of each silhouette highlights the tail region of the body. A, Polypteriformes (Nelson, 1994); B, Osteoglossomorpha (Hilton, 2003); C, Elopomorpha (Belouze, 2002); D, Ostariophysi (Fink & Fink, 1981; Nelson, 1994); E, Paracanthopterygii (Patterson & Rosen, 1989; Endo, 2002); F, Beloniformes (Lovejoy, 2000); G, Scombroidei (Johnson & Baldwin, 1994).
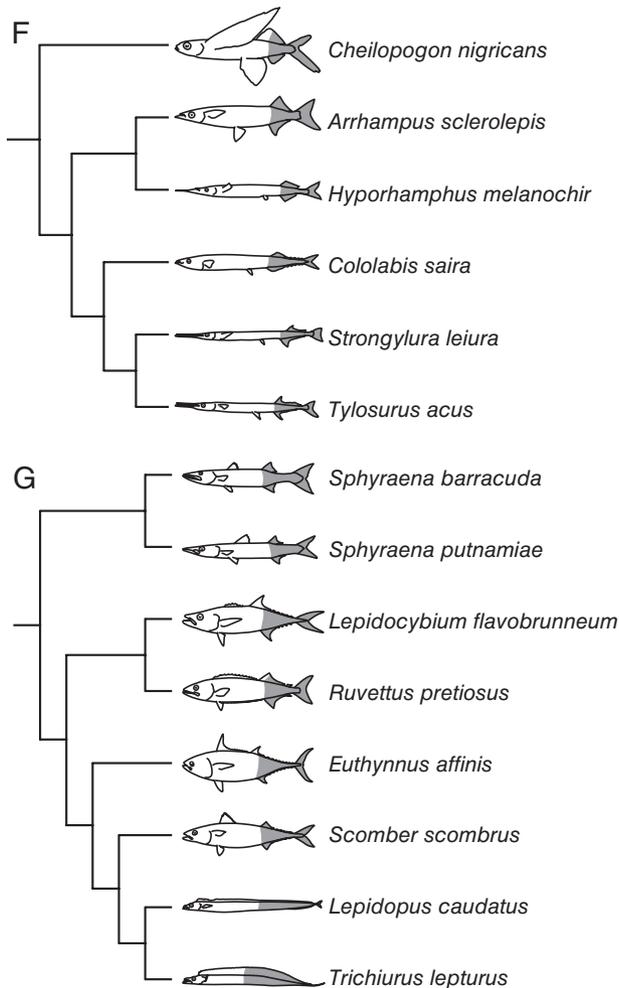
Figure 3. *Continued*

vertebral number (CVN). Abdominal vertebrae were defined as those that were cranial to vertebrae with fused haemal spines (Fig. 1A). The caudal region was defined as the region from the first fused haemal spine posterior to the last centrum including the ural centrum (Fig. 1B). Vertebral centrum length and width were measured in three vertebrae from the centre of the abdominal region and three from the centre of the caudal region. Vertebral width was measured at the anterior margin. Centrum measurements were made using digital calipers and recorded to the nearest 0.01 mm. The mean vertebral aspect ratio (AR = centrum length/centrum width) for each region was calculated for each individual. Species means were then calculated for abdominal aspect ratio (AAR) and caudal aspect ratio (CAR). We developed an index of vertebral column elongation, the 'axial elongation index' (AEI), which we defined as AEI = (AVN)(AAR) + (CVN)(CAR).

To quantify body shape in each species, the total length, standard length, maximum body depth, and maximum body width were measured in three wet specimens (alcohol preserved) of each species considered in the museum-based skeletal analysis. A measure of body shape, elongation ratio (ER), has been previously defined as total length divided by the second largest major body axis (either width or depth; Ward & Azizi, 2004). This ratio provides a size-independent view of the relationship between standard length and depth or width. Total length and standard length were measured with a ruler to the nearest 0.5 mm. Maximum body depth and width were measured using digital calipers to the nearest 0.01 mm. Because only actinopterygian fishes were included in this study, standard length was used instead of total length due to potential damage to the fin rays of caudal fins during collection and preservation.

STATISTICAL ANALYSIS

When comparing three or more species, phylogenetic information should be incorporated because species are not independent data points, but may share a similar evolutionary history (Felsenstein, 1985). To account for phylogenetic effects in relevant regressions, independent contrasts were calculated using CAIC, version 2.6.9 (Purvis & Rambaut, 1995). Branch lengths were set to unity. Regressions were run separately for each of the seven clades included in the study, and tree topologies were based on published phylogenetic analyses [for phylogenies of each group, see Fig. 3; Polypteriformes: Nelson (1994); Osteoglossomorpha: Hilton (2003); Elopomorpha: Belouze (2002); Ostariophysi: Fink & Fink (1981); Nelson (1994); Paracanthopterygii: Patterson & Rosen (1989), Endo (2002); Beloniformes: Lovejoy (2000); Scombroidei: Johnson & Baldwin (1994)]. Both the raw data and the independent contrast scores were analysed using reduced major axis (RMA) regression (SPSS 10.0), with the intercept of the contrast scores forced through zero.

Because only three species were included from Polypteriformes, a regression analysis was not conducted. Instead, species means for numbers of abdominal and caudal vertebrae were calculated. An analysis of variance (ANOVA) was used to test whether there were significant differences between the species for the abdominal and caudal regions (JMP 5.0; SAS Institute, Inc., Cary, NC, USA).

TESTING THE MODULARITY MODELS

The 95% confidence intervals (CIs) of the RMA slopes were used to compare the measured slopes with the model slopes at the 0.05 level of significance. If the

Table 1. Reduced major axis regression statistics for data from the literature-based study (Fig. 5)

Group	N	Slope	95% confidence interval	R	P
Anguilliformes	37	0.23‡	0.16–0.30	0.36	0.030
Aulopiformes	39	1.16†	0.83–1.48	0.37	0.021
Beloniformes	37	0.68‡	0.49–0.86	0.60	< 0.001
Carchariniformes	56	1.19†	0.87–1.51	0.38	0.004
Gadiformes	39	1.36[NS]	0.85–1.88	0.06	0.699
Lamniformes	9	2.62[NS]	−0.96–6.19	0.52	0.144
Lampridiformes	7	2.21*	1.68–2.74	0.99	< 0.001
Ophidiiformes	38	7.77*	3.75–11.78	0.46	0.004
Orectolobiformes	6	3.49†	−4.99–11.97	0.79	0.042
Perciformes	282	3.82*	3.29–4.36	0.67	< 0.001
Pleuronectiformes	23	2.52*	0.99–4.05	0.70	< 0.001
Squaliformes	32	0.60‡	0.32–0.88	0.70	< 0.001
Synbranchiformes	9	1.16†	0.72–1.60	0.88	0.002
Tetraodontiformes	199	1.65*	1.27–2.04	0.35	< 0.001

*Slope > 1.0, caudal increase > abdominal increase. †Slope = 1.0, abdominal increase = caudal increase. ‡0.00 < Slope < 1.00, abdominal increase > caudal increase.

The sample size (N) is the number of species included from each group. For a list of species, see Supplementary Material: Table S2. The 95% interval is the 95% confidence interval on the slope.

NS, not statistically significant.

95% CI of the slope overlapped a slope of 1, then Model A2 or B2, as appropriate, was accepted (Fig. 2). If the 95% CI was higher than one, then Model A1 or B1, as appropriate, was accepted, and if the 95% CI was lower than one, then Model A3 or B3, as appropriate, was accepted.

In analysing vertebral number from the museum-based study, the models were transformed to evaluate graphs of regional number of vertebrae vs. total number of vertebrae. After transformation, Model A1 is supported if the slope of caudal vertebral number vs. total vertebral number is significantly greater than 0.5, Model A2 is supported if both slopes (abdominal and caudal) are not significantly different from 0.5, and Model A3 is supported if the slope of the abdominal regression is significantly greater than 0.5.

CHARACTER TRACING

The seven clades included in the museum-based skeletal analysis were combined into a larger phylogeny (Lauder & Liem, 1983; Nelson, 1994). Number of abdominal vertebrae, number of caudal vertebrae, and abdominal and caudal vertebral aspect ratios were optimized onto the larger actinopterygian phylogeny. Characters were traced to minimize the sum of squared changes (a squared-change parsimony algorithm) with a rooted tree using MacClade, version 4.06 (Sinauer Associates, Sunderland, MA, USA; Maddison & Maddison, 2003). Nodal reconstructions are based on Maddison (1991). *Polypterus ornatipinnis* was not

included on the aspect ratio traced phylogeny because aspect ratio information was not available.

When continuous variables are traced in MacClade, the range of possible values of the variable of interest is divided equally into ten bins. Branch colours are then based on the bin in which the reconstructed nodal value falls. Darker branches are larger values and lighter branches are at the lower end of the range. To increase the resolution of the trace of caudal vertebral number, *Electrophorus electricus* was coded as the average of the species in the sister clade. The branch for *E. electricus* was then pseudocoloured red, a colour not included on the original colour range. Adjacent branches were then pseudocoloured to reflect *E. electricus* nodal values when the original data were traced.

RESULTS

AXIAL ELONGATION

A wide range of body forms, from deep-bodied to highly elongate, are represented in the 54 species examined in the museum-based study (Fig. 3). ER ranged from less than five to more than 25 (Fig. 4). We found a significant correlation between total vertebral number and ER (mean = 9.3, $R = 0.32$, $P = 0.02$), but no statistically significant correlation between vertebral aspect ratio ER ($R = 0.18$, $P = 0.72$). Despite the lack of a significant correlation between aspect ratio and ER, combining vertebral number and aspect ratio
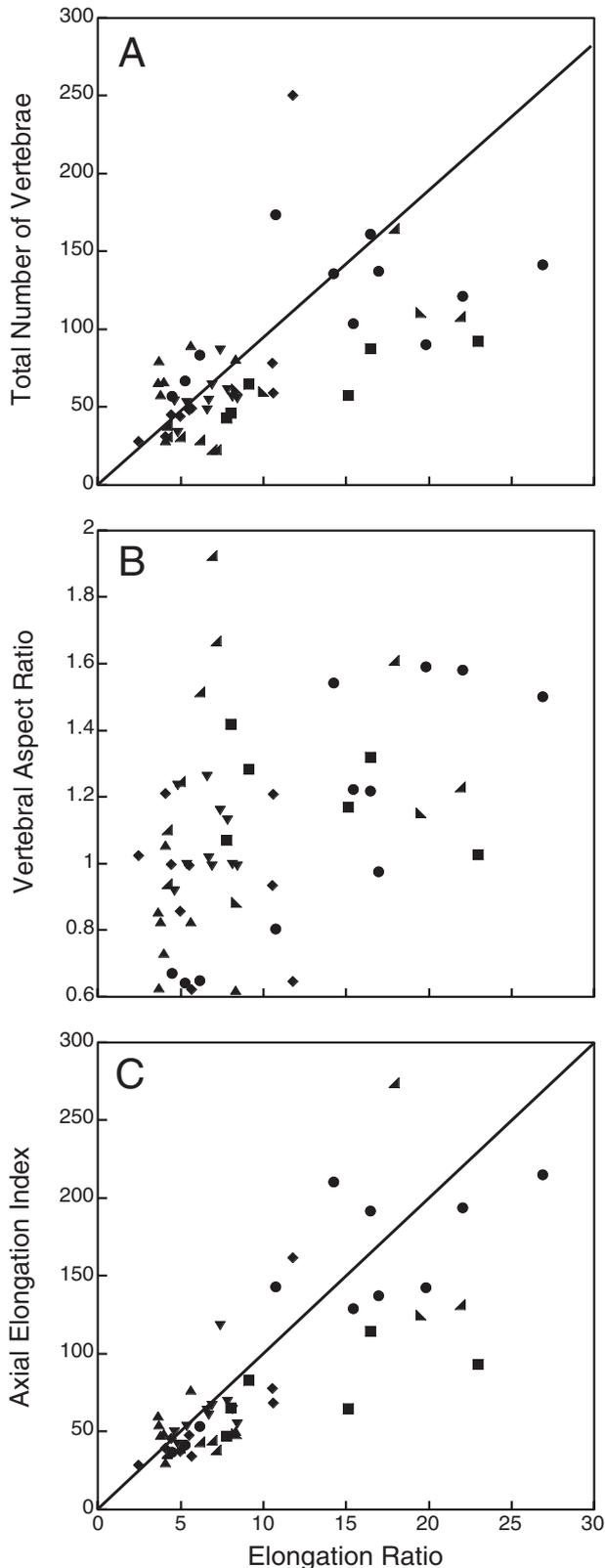
Figure 4. Contribution of increases in vertebral number and aspect ratio to overall body elongation. Elongation ratio (ER) is the standard length divided by the next largest body axis, either width or depth. The raw values plotted here were converted to independent contrast scores and a reduced major axis (RMA) regression, with the intercept forced through zero, was performed. A, total vertebral number vs. ER. Results from RMA regression on independent contrasts: $y = 9.3x$, $R = 0.32$, $P = 0.02$. B, vertebral aspect ratio (AR = centrum length/centrum width) vs. ER. Points represent the mean of abdominal and caudal ARs for each species. Results from RMA regression on independent contrasts: $R = 0.18$, $P = 0.72$. C, axial elongation index (AEI) vs. ER. AEI = (abdominal vertebral number)(abdominal AR) + (caudal vertebral number)(caudal AR). Results from RMA regression on independent contrasts: $y = 10.0x$, $R = 0.46$, $P < 0.001$. ■, Beloniformes; ●, Elopomorpha; ◆, Ostariophysi; ▲, Osteoglossomorpha; ▼, Paracanthopterygii; ◣, Polypteriformes; ◢, Scombroidei.

into an overall AEI yielded a higher correlation coefficient (mean = 10.0, $R = 0.46$, $P < 0.001$) than vertebral number alone (Fig. 4).

AXIAL PATTERNING MODELS: VERTEBRAL NUMBER

In the literature-based study, we collected abdominal and caudal vertebral numbers from 14 orders of elasmobranch and actinopterygian fishes (Fig. 5; a list of the 813 species is available in Supplementary Material: Table S1). In Lampridiformes, Ophidiiformes, Perciformes, and Tetraodontiformes, the slope of caudal vs. abdominal vertebral number was found to be significantly greater than one (Table 1), indicating a larger change in the caudal region for a given change in the abdominal region (in agreement with Model A1 in Fig. 2A). We also interpret Pleuronectiformes as conforming with Model A1, even though the lower limit of the 95% CI = 0.99, which overlaps slightly with a slope of 1.0. The slope for Aulopiformes, Carcharhiniformes, Orectolobiformes, and Synbranchiformes did not differ significantly from 1.0, indicating similar variation in both regions (Model A2), and the slope for Anguilliformes, Beloniformes, and Squaliformes was significantly less than one, indicating greater variation in the abdominal than in the caudal region (Model A3). No significant correlation between caudal and abdominal vertebral number was detected for Gadiformes and Lamniformes (Table 1).

In the museum-based study, all of the clades examined showed significant correlations between total number of vertebrae and number of caudal vertebrae (Fig. 6, Tables 2, 3), with the exception of Polypteriformes, in which no correlation analysis was performed because only three species were examined. In
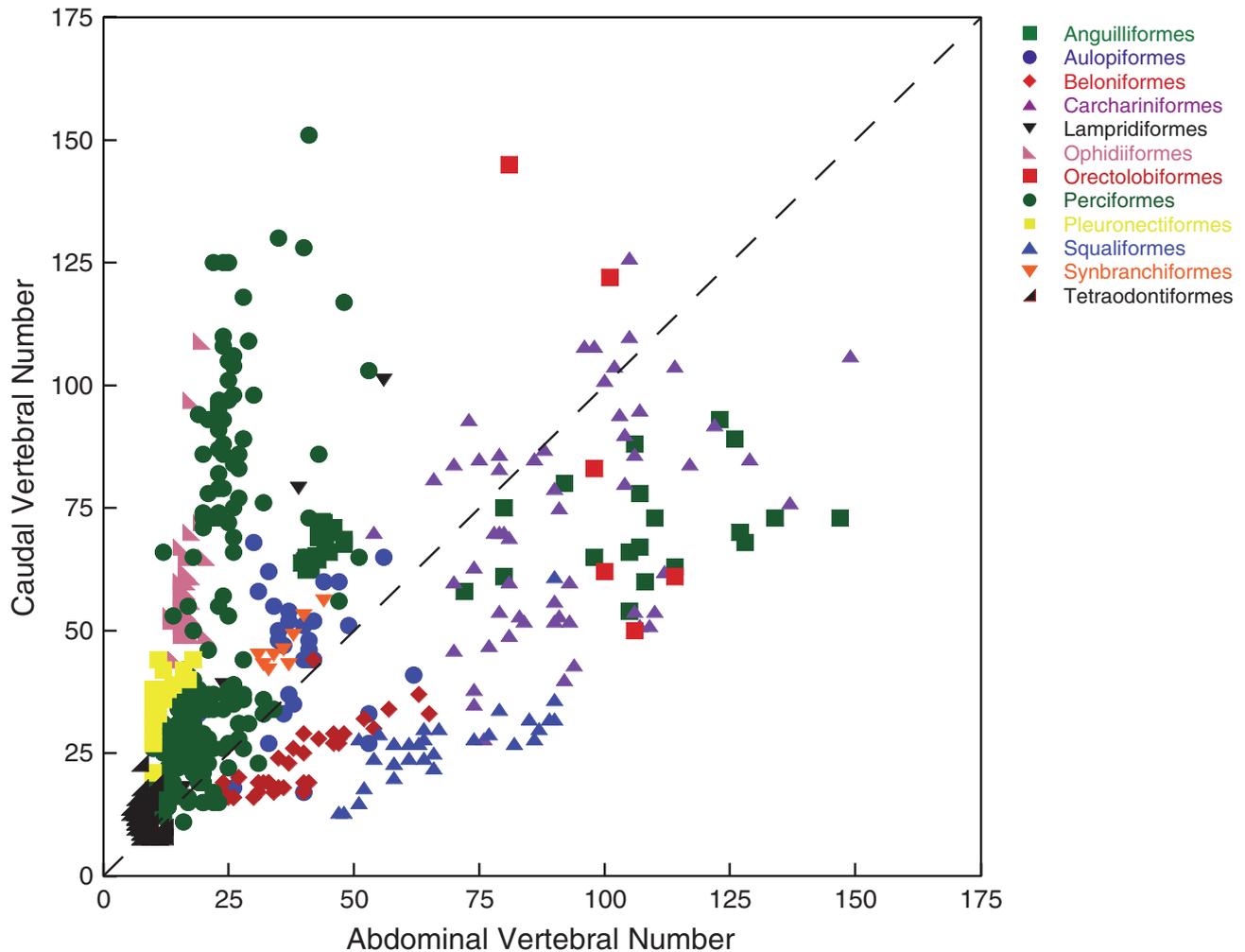
Figure 5. Number of abdominal and caudal vertebrae from our literature-based data set. Species were grouped into orders. Regression results are in Table 1, and a list of the species plotted is available in Supplementary Material: Table S1. The dotted line has a slope of one, indicating equal changes in abdominal and caudal vertebrae.

Table 2. Reduced major axis regression statistics for raw vertebral numbers (not phylogenetically corrected)

Group	Abdominal region				Caudal region			
	Slope	95% CI	R	P	Slope	95% CI	R	P
Osteoglossomorpha	0.49	−0.11–1.09	0.41	0.347	0.92	0.45–1.38	0.87	0.005
Elopomorpha	0.53	0.18–0.89	0.64	0.031	0.78	0.57–0.98	0.85	< 0.001
Ostariophysi	0.15	−0.61–0.92	0.05	0.904	1.02	0.73–1.31	0.99	< 0.001
Paracanthopterygii	0.33	−0.24–0.89	0.03	0.938	1.04	0.71–1.38	0.95	< 0.001
Beloniformes	0.61	0.50–0.73	0.99	< 0.001	0.40	0.28–0.53	0.97	< 0.001
Scombroidei	0.24	−0.01–0.49	0.91	< 0.001	0.79	0.56–1.01	0.99	< 0.001

The statistically significant slopes are shown as thick lines in Fig. 6.
95% CI, 95% confidence interval.
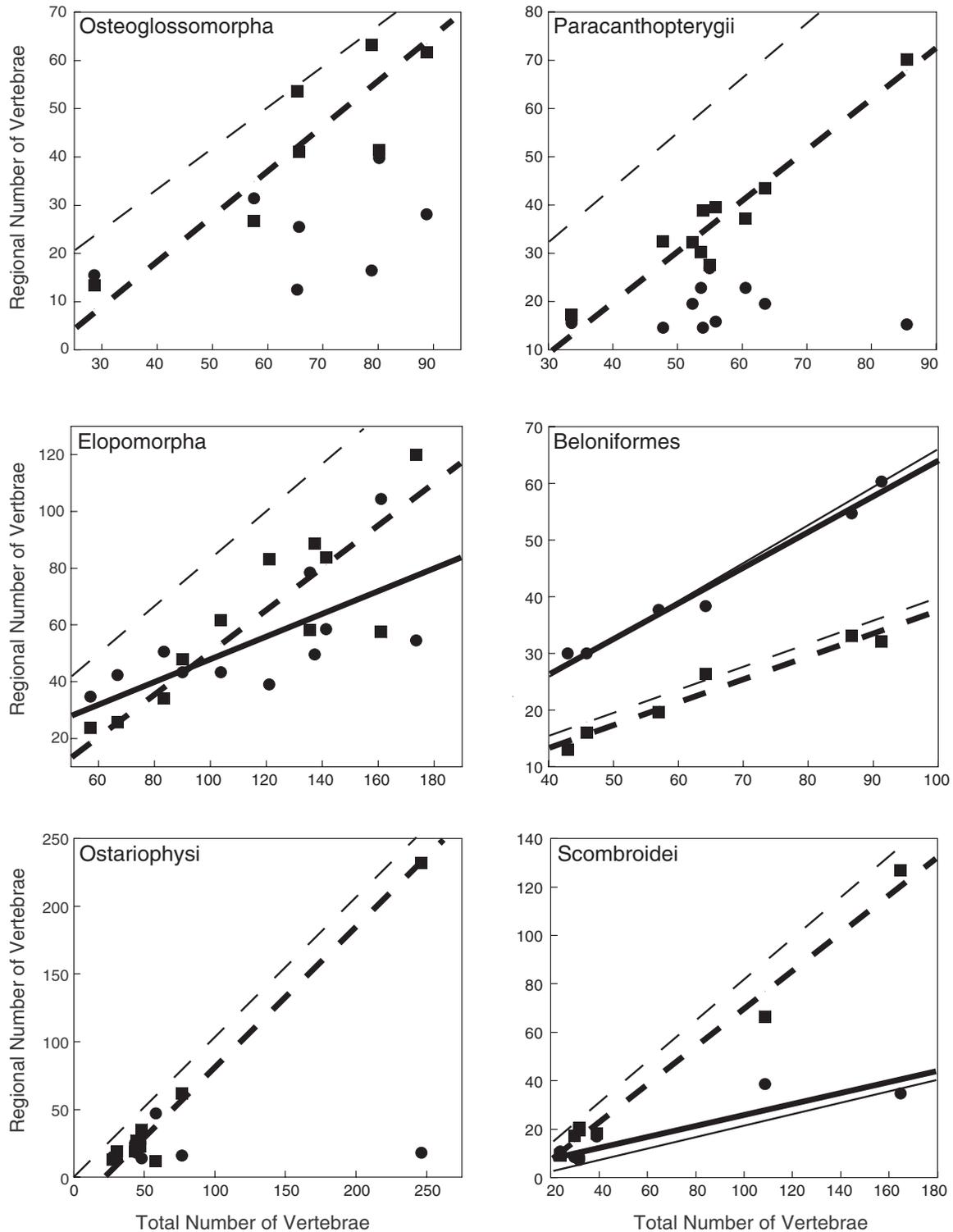
Figure 6. Number of vertebrae in each of the two regions of the vertebral column, abdominal and caudal, for six clades in the museum-based study. Solid circles (●) represent the species means for number of abdominal vertebrae, and solid squares (■) represent the species means for number of caudal vertebrae. Solid regression lines are for abdominal vertebral number and dashed regression lines are for caudal vertebral number. The thick regression lines were calculated from the raw data points shown, and the thin regression lines are based on independent contrasts. Regression statistics are given in Tables 2, 3.

Table 3. Reduced major axis regression statistics based on independent contrasts calculated for regional number of vertebrae and total number of vertebrae

Group	Abdominal region				Caudal region			
	Slope	95% CI	R	P	Slope	95% CI	R	P
Osteoglossomorpha	0.38	−0.14–0.90	0.59	0.194	0.83	0.40–1.26	0.93	0.002
Elopomorpha	0.66	0.26–1.06	0.56	0.085	0.83	0.37–1.30	0.75	0.009
Ostariophysi	0.16	−0.19–0.51	0.03	0.943	1.01	0.95–1.07	0.99	< 0.001
Paracanthopterygii	0.33	−0.01–0.67	0.15	0.697	1.10	0.89–1.31	0.95	< 0.001
Beloniformes	0.65	0.52–0.79	0.98	< 0.001	0.39	0.27–0.51	0.93	0.007
Scombroidei	0.23	0.05–0.41	0.77	0.031	0.84	0.62–1.05	0.98	< 0.001

The statistically significant slopes are shown as thin lines in Fig. 6.
95% CI, 95% confidence interval.

Ostariophysi and Paracanthopterygii, the slopes of the independent contrast scores for caudal vs. total vertebral number were found to be 1.01 and 1.10 (Fig. 6, dashed lines). Based on the 95% CIs, these slopes are not significantly different from 1.0 (Table 3), indicating that vertebral number variation in these two groups is primarily in the caudal region. (note: the modularity tests for the literature and museum vertebral number data are slightly different; see Material and methods for an explanation).

Beloniformes and Scombroidei exhibit a significant correlation between total vertebral number and both abdominal and caudal vertebral number (Fig. 6, Table 3). In Beloniformes, the slopes of the two lines are not significantly different, indicating that increases in the two regions contribute equally to axial elongation. In Scombroidei, the slopes are significantly different (Table 3). The caudal slope is 0.8 and the abdominal slope is 0.2, indicating that for every increase of ten total vertebrae, eight of them will be caudal and two will be abdominal vertebrae.

The results for Osteoglossomorpha and Elopomorpha are equivocal. In both groups, the caudal vertebral number slope is 0.83, which suggests primarily caudal elongation, but the CIs on these slopes are too large to allow us to rule out abdominal elongation or equal elongation in the two regions.

Polypteriformes were considered separately because only three species were included in this analysis: *Polypterus bichir*, *Polypterus ornatipinnis*, and *Erpetoichthys calabaricus*. *Polypterus bichir* and *P. ornatipinnis* do not differ in the number of abdominal vertebrae, having 47.4 ± 1.0 and 49.5 ± 1.6, respectively (mean ± SE; ANOVA, $P > 0.05$). *Erpetoichthys calabaricus* has 100.0 ± 1.1 abdominal vertebrae, which is double the number of the two *Polypterus* species. Caudal vertebral numbers were similar in all three species: *P. bichir*, 14.2 ± 0.78;

Table 4. Reduced major axis regression statistics for raw vertebral aspect ratio (not phylogenetically corrected)

Group	Aspect ratio			
	Slope	95% CI	R	P
Osteoglossomorpha	1.10	0.33–1.87	0.89	0.003
Elopomorpha	0.83	0.59–1.08	0.92	< 0.001
Ostariophysi	0.91	0.44–1.39	0.87	0.001
Paracanthopterygii	1.00	0.10–1.90	0.04	0.908
Beloniformes	0.89	0.15–1.64	0.72	0.082
Scombroidei	0.69	0.37–1.00	0.88	0.002

The statistically significant slopes are shown as solid lines in Fig. 7. Aspect ratio is calculated as centrum length/ centrum width.
95% CI, 95% confidence interval.

P. ornatipinnis, 10.5 ± 1.23;   and   *E. calabaricus*, 10.8 ± 0.87.

AXIAL PATTERNING MODELS: ASPECT RATIO

We found a significant correlation between abdominal aspect ratio and caudal aspect ratio in four clades: Osteoglossomorpha, Elopomorpha, Ostariophysi, and Scombroidei (Fig. 7, Tables 4, 5). In each of these four clades, the slope did not differ from one, indicating that variation in the two regions is closely linked (in agreement with Model B2; Fig. 2B). In Beloniformes, the slope of the independent contrast scores is marginally nonsignificant at the 0.05 level ($P = 0.085$), and the 95% CIs of the slope overlap a slope of 1.0 (Table 5). In Paracanthopterygii, there is no significant correlation between caudal and abdominal aspect ratio ($R = 0.32$, $P = 0.395$; Table 5), suggesting that
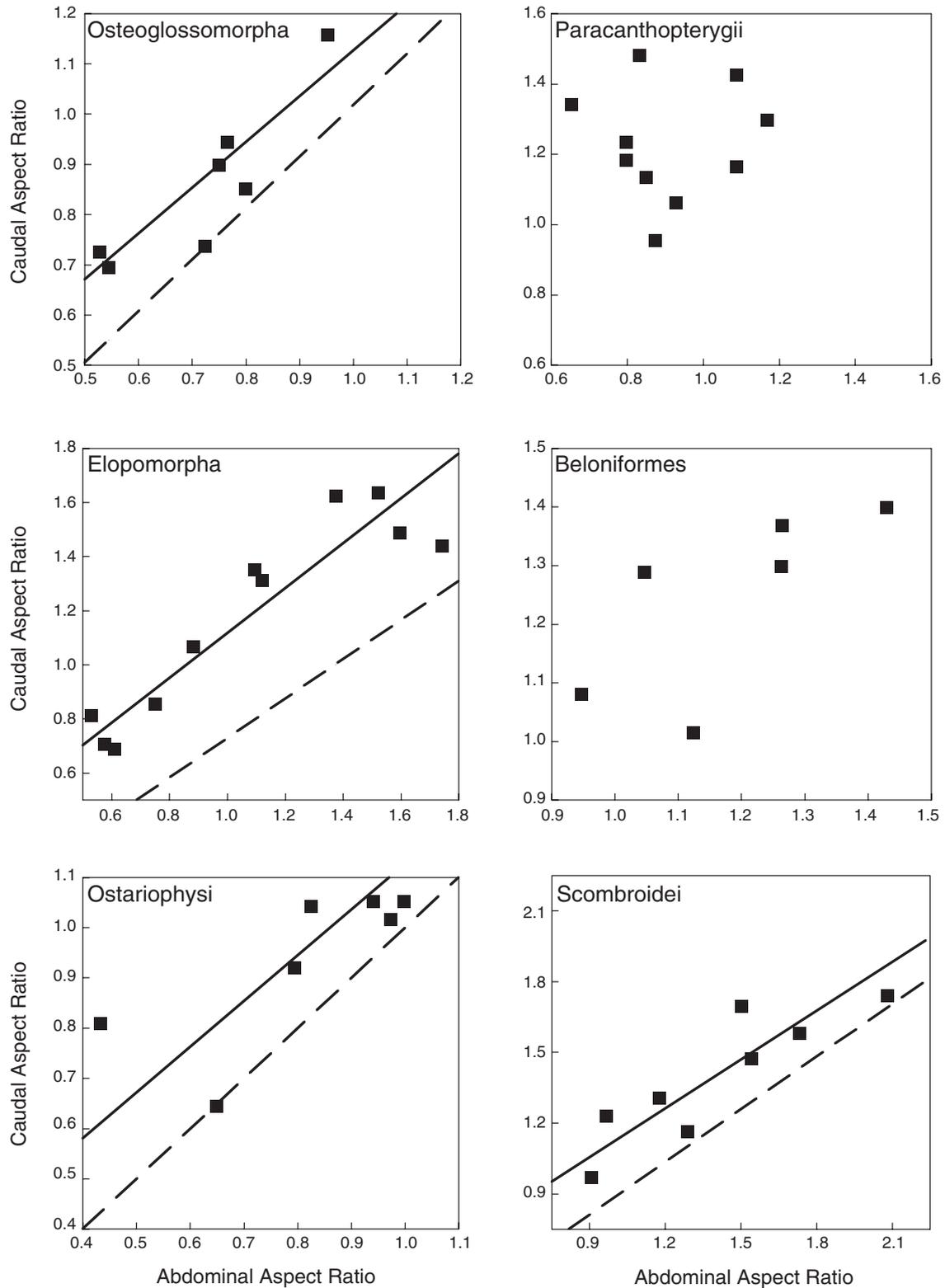
Figure 7. Vertebral aspect ratio (AR) in the abdominal and caudal regions (AR = centrum length/centrum width). Solid lines are reduced major axis (RMA) regressions based on the raw data (the data points shown), and dashed lines are RMA regressions based on independent contrasts of abdominal and caudal aspect ratio. Regression statistics are given in Tables 4, 5.

Table 5. Reduced major axis regression statistics based on independent contrasts calculated for vertebral aspect ratio

| Group | Aspect ratio | | | |
	Slope	95% CI	R	P
Osteoglossomorpha	1.02	0.65–1.40	0.89	0.008
Elopomorpha	0.73	0.44–1.03	0.79	0.005
Ostariophysi	1.00	0.53–1.46	0.84	0.005
Paracanthopterygii	0.81	0.10–1.52	0.32	0.395
Beloniformes	0.78	0.02–1.55	0.77	0.091
Scombroidei	0.84	0.32–1.36	0.83	0.013

The statistically significant slopes are shown as dashed lines in Fig. 7. Aspect ratio is calculated as centrum length/centrum width.
95% CI, 95% confidence interval.

caudal and abdominal aspect ratio vary independently in this clade.

For both aspect ratios and vertebral counts, we calculated correlation coefficients and regression slopes for both the raw data (Tables 2, 4) and for independent contrast scores (Tables 3, 5). Only one conclusion differed: in the raw data analysis for abdominal vertebral number in Elopomorpha, the correlation was significant ($P = 0.031$, Table 2) vs. marginally nonsignificant in the independent contrast analysis ($P = 0.085$, Table 3).

CHARACTER TRACING

Tracing the changes in vertebral number and aspect ratio on a cladogram yields a more detailed picture of vertebral column evolution (Figs 8, 9). Species with higher AVN or CVN are nested inside clades with lower vertebral numbers, demonstrating that vertebral number has increased multiple times (Fig. 8). A few instances of vertebral number decrease are evident, such as in the lineage leading to the osteoglossomorph *Pantodon bucholzi* but, within the seven clades studied, vertebral number increases far outweigh decreases. However, in actinopterygian fishes as a whole, the derived groups tend to have fewer vertebrae than the basal groups (Fig. 8).

In all groups except Scombroidei and Beloniformes, we found that increases in vertebral number generally have not occurred in both regions of the vertebral column (Fig. 8). For example, AVN increases but CVN remains nearly constant in the lineages leading to *E. calabaricus* (Polypteriformes) and *Diastobranchus capensis* (Elopomorpha), whereas CVN increases but AVN remains relatively constant in the lineages leading to *Notacanthus sexspinis* (Elopomorpha) and *Elec-*

trophorous electricus (Ostariophysi). Several of these groups show examples of both caudal and abdominal elongation, but these do not occur at the same nodes. In Scombroidei and Beloniformes, increases in both CVN and AVN have occurred at the same nodes (Fig. 8, nodes labelled A and B).

Vertebral aspect ratio (AR) varies greatly across actinopterygian fishes (Fig. 9), from elongate vertebral centra that are two times longer than wide (AR = 2) to squat vertebral centra that are two times wider than long (AR = 0.5). Within each species, AAR and CAR often differ from each other (Fig. 9). However, when evolutionary changes in AR have occurred, the direction of change is generally the same in both regions. For example, large increases in AAR and CAR have occurred in the lineage leading to the osteoglossomorph *P. bucholzi*, the elopomorphs *Gymnothorax saxicola* and *Gnathophis habenatus*, and several times within the scombroids. In some cases, aspect ratio decreases in both regions, as in the elopomorph *N. sexspinis*. In general, the derived groups of actinopterygian fishes tend to have higher AR than the basal groups (Fig. 9).

Counterexamples to the general rule that AAR and CAR increase or decrease together can be found in Paracanthopterygii. In the lineage leading to *Cataetyx niki*, AAR has decreased and CAR has increased, and in the lineage leading to *Macrourus holotrachys*, CAR has increased whereas AAR remained constant (Fig. 9).

INTRASPECIFIC VARIATION

Only three individuals of each species were measured in the present study, so it is not possible for us to quantify intraspecific variation. However, we did observe some intraspecific variation in vertebral number, even with our small sample sizes. For most species, variation was in the range of one to four vertebrae. Species with high vertebral counts tended to show larger variation, as in *Trichiurus lepturus*, a highly elongate species with a range of 141–182 vertebrae in the three specimens measured. In general, the number of caudal vertebrae had more variability than the number of abdominal vertebrae.

DISCUSSION

AXIAL ELONGATION

Four types of morphological change may contribute to the evolution of elongate body forms in vertebrates: (1) an increase in the number of vertebrae; (2) an increase in the length of the vertebral centra; (3) a decrease in depth of the body; (4) and an increase in head length. In our museum-based study of seven actinopterygian clades, we found that increases in vertebral number
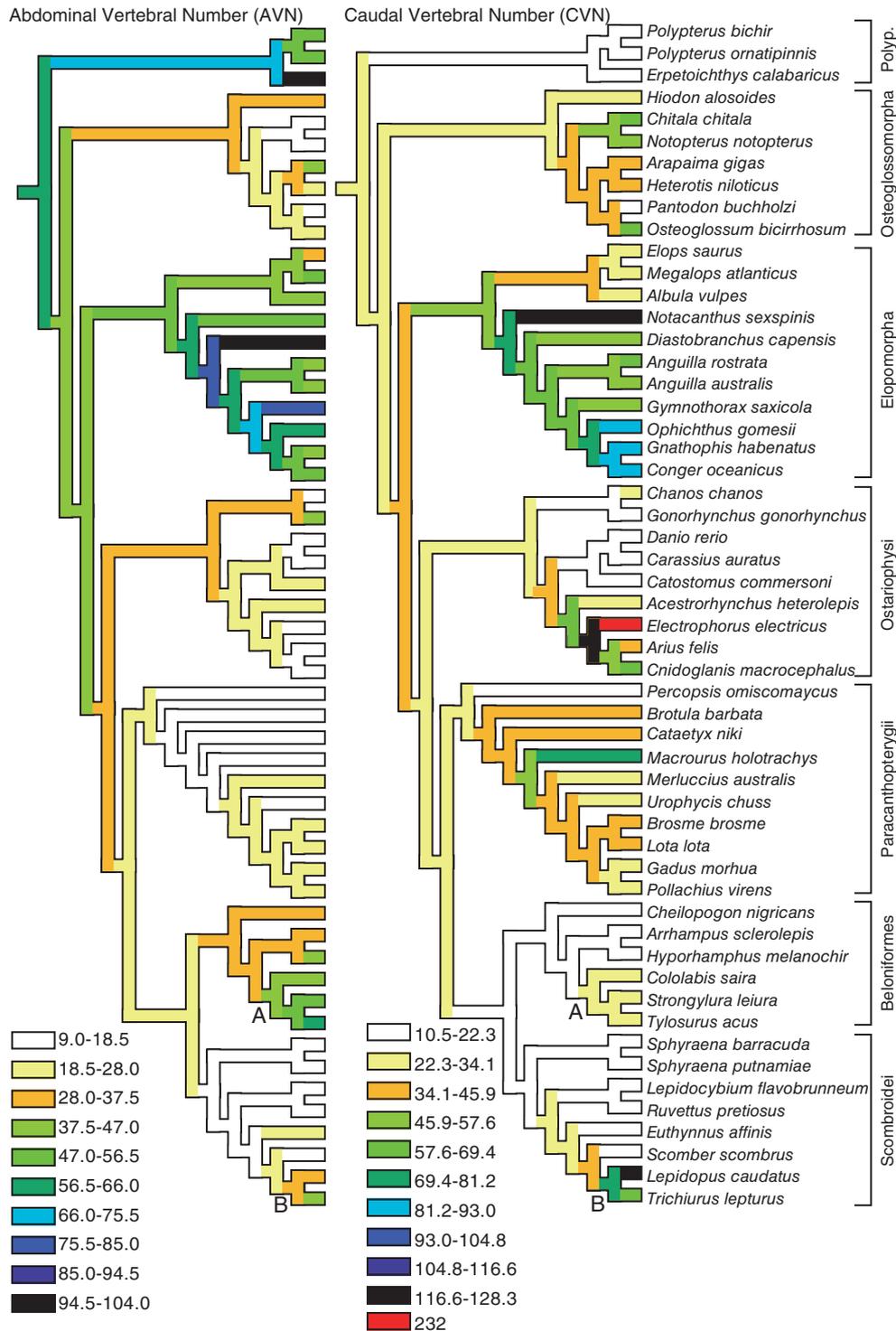
Figure 8. Squared-change parsimony traced phylogenies for number of abdominal vertebrae and number of caudal vertebrae. The interrelationships of the seven clades examined are based on Lauder & Liem (1983), and references for the intrarelationships are given in the legend to Fig. 3. Both traces are based on a squared-change parsimony algorithm in MacClade, version 4.06 (Maddison, 1991). Lighter coloured branches (white, yellow) are lower vertebral numbers and darker branches (purple, black) are higher vertebral numbers. *Electrophorus electricus* was pseudocoloured in the number of caudal vertebrae to allow for greater resolution of the caudal vertebrae trace (see Material and methods). Two nodes are labelled A and B to allow their identification in the text.
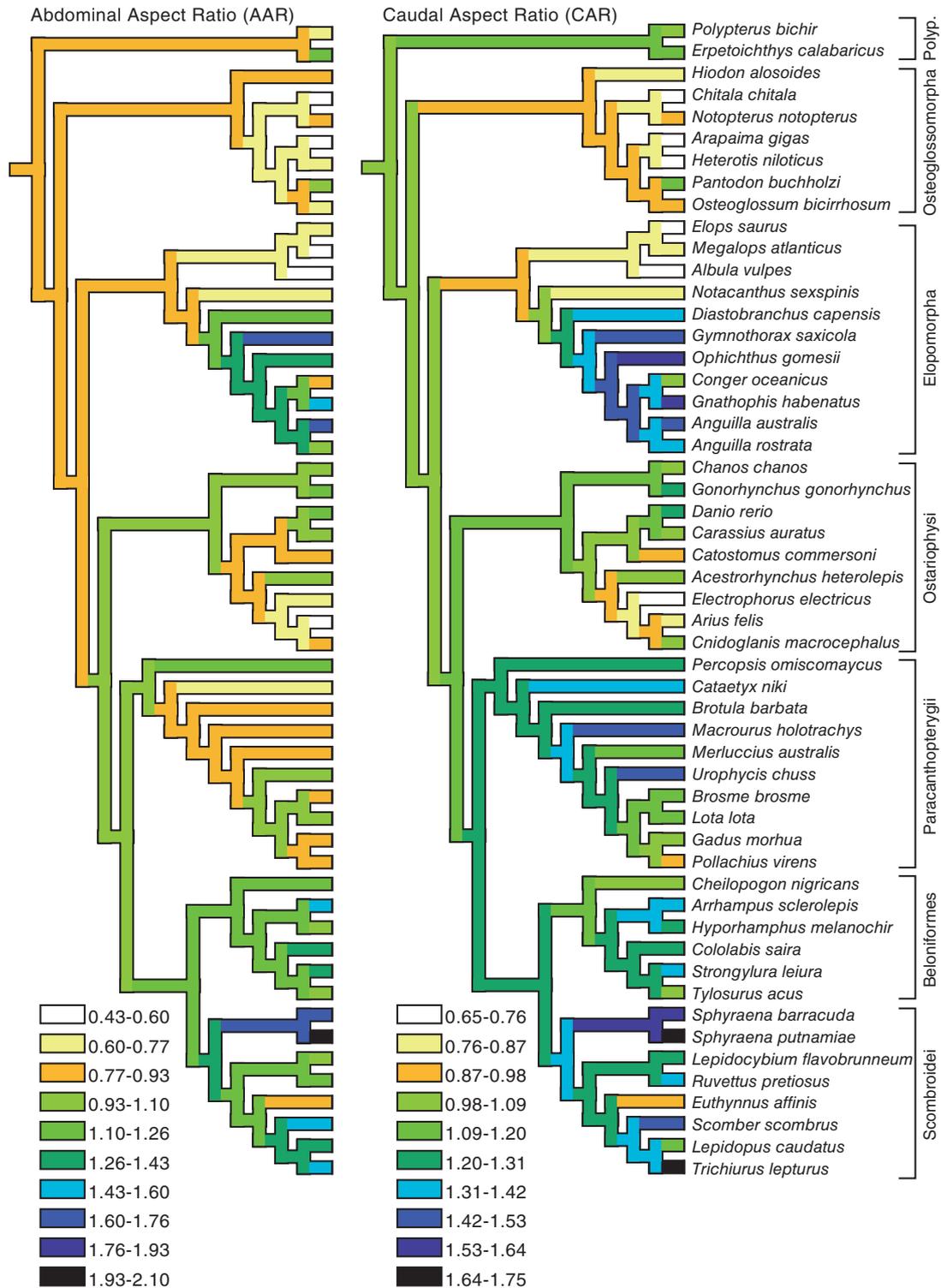
Figure 9. Squared-change parsimony traced phylogenies for abdominal aspect ratio and caudal aspect ratio. For each species, the mean of abdominal and caudal aspect ratio was calculated for this analysis. The interrelationships of the seven clades examined are based on Lauder & Liem (1983), and references for the intrarelationships are given in the legend to Fig. 3. Both traces are based on a squared-change parsimony algorithm in MacClade, version 4.06 (Maddison, 1991). Lighter coloured branches (white, yellow) are lower values of aspect ratio and darker branches (purple, black) are higher values of aspect ratio.

are associated with ER (Fig. 4A). By contrast, we found no significant correlation between vertebral aspect ratio and ER (Fig. 4B). Combining vertebral number and aspect ratio produced a stronger correlation with ER than vertebral number alone (Fig. 4C), indicating that there may be a synergistic effect between vertebral number and aspect ratio.

In Figure 4C, ER is a measure of overall body form whereas AEI is a measure of vertebral column elongation. The correlation between these variables is not tight, indicating that vertebral column elongation is not the only contributor to the evolution of elongate body forms. A decrease in the depth of the body, with no change in the vertebral column, can produce elongate body forms, and also increasing head length can contribute to elongation (our measurement of ER is based on standard length, so it includes the head). The effect of increased head length can be seen in Beloniformes, in which there is relatively less vertebral column elongation for a given increase in overall body elongation (Figs 2F, 4C, square symbols).

These results indicate that, in actinopterygian fishes, the primary morphological change associated with body elongation is an increase in vertebral number, with smaller contributions from increases in vertebral aspect ratio, decreases in body depth, and increases in head length. Previous studies on body elongation in other vertebrate groups have found that axial elongation is generally associated with an increase in vertebral number (Wake, 1966; Lindsey, 1975; Parra-Olea & Wake, 2001; Polly *et al.*, 2001). Although centrum elongation is anecdotally considered as a method for body elongation, very few studies have described morphometrics of vertebral centra. *Lineatriton* has been shown to have elongated centra relative to other plethodontids (Wake, 1966; Parra-Olea & Wake, 2001). In a study of snake vertebrae, Johnson (1955) found that elongation of the vertebral centra is associated with increasing body length. Johnson found that arboreal forms, specifically the more vine-like snakes, tend to have more elongate vertebrae than fossorial species, a morphology which may increase body rigidity when locomoting among tree limbs. In long-necked mammals, such as giraffes, neck elongation occurs by increasing the aspect ratio of a fixed number of cervical vertebrae (seven), whereas neck elongation in birds is associated with increases in vertebral number (Galis, 1999). Combined with our results, these observations indicate that future studies of vertebral column elongation should include measurements of aspect ratio as well as vertebral counts.

In addition to our analysis of vertebral number and aspect ratio across the entire vertebral column of actinopterygian fishes, we also analysed variation in the abdominal and caudal regions separately. The developmental modules that control vertebral number and aspect ratio may act globally across the entire vertebral column, or there may be separate modules controlling the abdominal and caudal regions. In the following sections, we use our data on regional elongation to develop hypotheses about developmental modules and candidate developmental mechanisms that may control axial patterning in actinopterygian fishes.

DEVELOPMENTAL MODULARITY:
NUMBER OF VERTEBRAE

In our museum-based study, we found evidence that axial elongation is associated with unequal increases in the number of abdominal and caudal vertebrae in six out of seven of the clades analysed. In Ostariophysi and Paracanthopterygii, we found increases in vertebral number primarily in the caudal region, with little change in abdominal vertebral number (Figs 6, 8). In Polypteriformes, we found a large increase in abdominal vertebral number with little change in the caudal region (Fig. 8). The results of the regression analysis for Elopomorpha and Osteoglossomorpha were equivocal (Fig. 6, Table 3). The regression analysis that we used is appropriate if there is just one pattern of elongation in a clade, either abdominal or caudal, but the Elopomorpha and Osteoglossomorpha include both patterns (Fig. 8). For example, in Elopomorpha, the lineage leading to *N. sexspinis* shows a large increase in caudal vertebral number, with no increase in abdominal vertebral number, whereas the lineage leading to *D. capensis* shows the converse (Fig. 8). These results show that the phylogenetic level of the regression analysis was too coarse for the levels at which vertebral number varies in Elopomorpha and Osteoglossomorpha. Furthermore, these results show that total vertebral number may be inferior to regional vertebral number as a phylogenetic character. Total vertebral number in *N. sexspinis* and *D. capensis* are similar, but the regional differences suggest that high vertebral number may have evolved independently in these two lineages.

By contrast to the clades discussed above, Beloniformes and Scombroidei include lineages in which both abdominal and caudal vertebral number have increased at the same nodes (Fig. 8, nodes A and B). In Beloniformes, the increases are approximately equal in the two regions (Fig. 6), whereas in Scombroidei, more vertebrae are added in the caudal than in the abdominal region. The regression slopes in Figure 6 and Table 3 indicate that, for every addition of ten total vertebrae in Scombroidei, eight are caudal vertebrae and two are abdominal vertebrae.

In our literature-based survey, we collected data on abdominal and caudal vertebral counts in 813 species from 14 orders of elasmobranch and actinopterygian

fishes (Fig. 5, Table 1). Our regression analysis indicated that variation in vertebral number occurs preferentially in the abdominal or caudal region in eight orders, equally in the abdominal and caudal regions in four orders, and the regression was not statistically significant in the remaining two orders. We conclude that, in most clades of actinopterygian fishes, phylogenetic changes in abdominal and caudal vertebral number are not tightly linked. The evolutionary independence of these two regions suggests that they may be controlled by separate developmental modules, as previously shown for various tetrapod groups (Polly *et al.*, 2001; Wiens & Slingluff, 2001; Narita & Kuratani, 2005).

DEVELOPMENTAL MODULARITY: ASPECT RATIO

By contrast to our findings for vertebral number, we found that increases or decreases in aspect ratio generally occur equally in the abdominal and caudal regions (Figs 7, 9; Model B2 in Fig. 2B). We conclude that, in most clades of actinopterygian fishes, aspect ratio appears to be constrained to change equally in the two regions, indicating that aspect ratio may be controlled by one developmental module acting globally across the vertebral column. We offer this global control of aspect ratio as a putative developmental constraint on the evolution of elongate vertebral columns; independent abdominal or caudal elongation can occur by the addition of vertebrae, but increases in aspect ratio will affect both regions.

The only group in which we found evidence for unequal regional changes in aspect ratio is Paracanthopterygii. In this group, there is no correlation between abdominal and caudal aspect ratio (Fig. 7), and individual lineages show increase in one region and stasis or decrease in the other region (Fig. 9). One possible explanation for these results is that the paracanthopterygian taxa included in our study do not form a monophyletic group, or that the phylogeny that we used is incorrect. The monophyly of Paracanthopterygii as a whole has been questioned (Miya *et al.*, 2003). Another alternative is that the developmental constraint imposed on aspect ratio changes has been relaxed, allowing for independent changes in aspect ratio in the two regions. The possibility that the developmental constraint on aspect ratio has been relaxed in Paracanthopterygii should be explored more fully.

CANDIDATE DEVELOPMENTAL MECHANISMS

Molecular developmental studies often use a candidate gene approach to diagnose molecular developmental changes associated with mutant phenotypes. Here, we use our evolutionary morphological results in concert with known developmental mechanisms to suggest candidate molecular changes that might be associated with the evolution of eel-like body forms. Gene networks to consider in axial patterning and elongation include Hox genes, the tail organizer, and the oscillator/wavefront mechanism of somitogenesis. Specifically, we propose that: (1) shifts in Hox gene expression boundaries may be associated with increases in abdominal vertebral number; (2) prolongation of somitogenesis in the tail may be associated with increases in caudal vertebral number; and (3) increasing the speed of the somitogenesis wavefront and/or the period of the somitogenesis oscillator may be associated with the elongation of somites, and therefore an increase in vertebral aspect ratio, along the length of the vertebral column.

Hox genes have been shown to affect anterioposterior patterning of the body, and boundaries of Hox expression domains can be correlated with key adult anatomical landmarks (Gaunt, 1994; Burke *et al.*, 1995; Prince *et al.*, 1998a; Prince, Price & Ho, 1998b; Burke, 2000). For example, the anterior boundary of the expression domain for *Hoxc6* is at the same somite level as the brachial plexus in a range of vertebrates including mice, geese, chicks, *Xenopus*, and zebrafish (Gaunt, 1994; Burke, 2000). The anterior boundary of *Hoxd12a* marks the boundary between the abdominal and caudal vertebral regions in *Danio* (van der Hoeven *et al.*, 1996). This same boundary also occurs in mice and chicks (Burke *et al.*, 1995; Burke, 2000). The boundary between abdominal and caudal vertebrae is at the proctodeum; thus, *Hoxd12* is a candidate for determining the placement of this structure. It is likely that changes in expression domains of the *Hox12* genes may be implicated in abdominal elongation. It is possible that the *Hox11* group may also be implicated in determining placement of the proctodeum since knockouts of the *Hox11* genes in mice results in posterior movement of the boundary between lumbar and sacral vertebrae (Wellick & Capecchi, 2003), but the anterior expression boundaries of the *Hox11* genes in zebrafish are located well within the abdominal region (van der Hoeven *et al.*, 1996).

Hox genes are not thought to play a patterning role in the caudal region. The caudal region appears to be controlled by a separate tail organizer that utilizes bmp, nodal, and wnt signalling (Kanki & Ho, 1997; Agathon, Thisse & Thisse, 2003). However, the signal that may stop somitogenesis in the tail region is still unknown. Potentially, turning off bmp, nodals, or wnts, may result in an end of somitogenesis and formation of the caudal skeleton. To elongate the caudal region of the vertebral column, somitogenesis must be prolonged. *Batrachoseps*, a plethodontid salamander, and *Nemichthys*, a snipe eel, have been reported to add caudal vertebrae throughout life (Noble, 1931;

Beebe & Crane, 1937). In these genera, it appears that somitogenesis is prolonged into adulthood, making their tail bud more analogous with the apical meristem of plants rather than an embryonic patterning mechanism of vertebrates. Studying such species in which somitogenesis never stops might provide clues to how the tail organizer maintains activity in species that have increased the number of caudal segments. In addition, further study of segmentation during tail regeneration in urodeles, anurans, and squamates may provide more information about the mechanisms underlying the end of somitogenesis (Goss, 1969).

We found that the vertebral aspect ratio appears to be constrained to change equally in the abdominal and caudal regions, indicating that aspect ratio may be controlled by a single developmental module acting along the length of the vertebral column. The oscillator/wavefront hypothesis of somitogenesis provides a potential explanation for such a global control of aspect ratio. During somitogenesis in zebrafish, the position of the posterior border of a somite is determined by an interaction between an oscillating level of *her1* and a posterior moving wavefront of *fgf8* (Holley, Geisler & Nusslein-Volhard, 2000; Sawada *et al.*, 2001; Holley *et al.*, 2002; Oates & Ho, 2002; for a review, see Holley & Takeda, 2002; Pourquie, 2003). When *fgf8* expression was inhibited by a pharmacological agent, somite size doubled, as a result of the faster retreat of the *fgf* wavefront (Dubrulle, McGrew & Pourguie, 2001; Sawada *et al.*, 2001). Although we do not know the effect of subsequent resegmentation on the lengthened somites (Morin-Kensicki, Melancon & Eisen, 2002), it is possible that an inhibition of *fgf8* during somitogenesis could be a mechanism for increased vertebral length. The oscillator/wavefront model also predicts that somite length could increase by slowing of the oscillator (increasing its period), but the effect of such a change has not yet been tested.

It should be noted that, although our comparative anatomical findings and proposed developmental mechanisms suggest differential control for the number and length of the vertebrae, the two characteristics are inherently linked. Given the same amount of available paraxial mesoderm, there is a reciprocal relationship between length and number of vertebrae. Therefore, in the experiment by Sawada *et al.* (2001) in which somites were experimentally lengthened, we would hypothesize that fewer somites would be formed. Further evidence to support this hypothesis is provided in work by Diez del Corral *et al.* (2003). Somite length was shortened by increased *fgf* signalling, but more somites were formed, such that the total length of the trunk paraxial mesoderm was unchanged (Diez del Corral *et al.*, 2003). Ultimately, increased body elongation of a species relative to an outgroup species must include an increase in the length of the paraxial mesoderm, which may result from continued outgrowth of the tailbud.

CONCLUDING REMARKS

Our broadly comparative study revealed that, in actinopterygians, evolutionary changes in vertebral aspect ratio are generally similar across the abdominal and caudal regions, whereas changes in vertebral number are generally region-specific. Based on these results and candidate developmental mechanisms from the literature, we propose three hypotheses:
1. Somite length changes occur equally in both the abdominal and caudal regions. We hypothesize that increased somite length could be the result of a faster retreat of the *fgf8* wavefront or slower cycling of *her1/Delta/Notch* (oscillator);
2. The number of vertebrae is controlled separately in the abdominal and caudal regions. We hypothesize that increases in the number of abdominal vertebrae may result from shifts in Hox expression domains, whereas increases in the number of caudal vertebrae may result from maintaining the tailbud organizer and delaying the end of somitogenesis; and
3. Ultimately, body elongation must involve an increase in the amount of paraxial mesoderm. Therefore, we hypothesize that elongate species will have a mechanism for maintaining the tail organizer and prolonging somitogenesis, regardless of whether body elongation is produced by the addition of abdominal vertebrate, caudal vertebrae, or the lengthening of vertebral centra.

The present study is an example of how broadly comparative, macroevolutionary studies of adults can generate testable hypotheses of evolutionary changes in developmental mechanisms. In the future, a microevolutionary approach to this problem would also be interesting. Counts and measurements of regional vertebral number and aspect ratio within a large number of fish populations would reveal the patterns of natural variation in these characters, patterns that may reflect the way that underlying developmental mechanisms can canalize the variation available for natural selection to act upon.

ACKNOWLEDGEMENTS

REFERENCES

Agathon A, Thisse C, Thisse B. 2003. The molecular nature of the zebrafish tail organizer. *Nature* **424:** 448–452.

Ahlstrom EH, Amaoka K, Hensley DA, Moser HG, Sumida BY. 1984. Pleuronectiformes, development. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 640–670.

Anderson ME. 1990. Zoarcidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean.* Grahamstown: JLB Smith Institute of Ichthyology, 256–276.

Anderson ME. 1984. Zoarcidae, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 578–582.

Asano H. 1977. On the tendencies of differentiation in the composition of the vertebral number of teleostean fishes. *Memoires of the Faculty of Agriculture Kinki University* **10:** 29–37.

Beebe W, Crane J. 1937. Deep-sea fishes of the Bermuda Oceanographic Expeditions. Family Nemichthyidae. *Zoologica* **22:** 249–383.

Belouze A. 2002. Comprehension morphologique et phylogenetique des taxons actuels et fossiles rapportes aux anguilliformes (Poissons, Teleosteens). *Documents des Laboratoires de Géologie de Lyon* **158:** 1–401.

Bemis WE, Forey PL. 2001. Occipital structure and the posterior limits of the skull in actinopterygians. In: Ahlberg P, ed. *Major events in vertebrate evolution.* London: Taylor & Francis, 350–369.

Burke AC. 2000. Hox genes and the global patterning of the somitic mesoderm. *Current Topics in Developmental Biology* **47:** 155–181.

Burke AC, Nelson CE, Morgan BA, Tabin C. 1995. Hox genes and the evolution of vertebrate axial morphology. *Development* **212:** 333–346.

Chiu TS, Markle DF. 1990. Gadidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean.* Grahamstown: JLB Smith Institute of Ichthyology, 190–191.

Collete BB, Mcgowen GE, Parin NV, Mito S. 1984a. Beloniformes: Development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 335–354.

Collette BB, Potthoff T, Richards WJ, Ueyanagi S, Russo JL, Nishikawa Y. 1984b. Scombroidei, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 591–620.

De Sylva DP. 1984. Polynemoidei, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 540–541.

Deelder CL. 1984. *Synopsis of biological data on the eel Anguilla anguilla.* FAO Fisheries Synopsis, no. 80, revision 1. Rome: FAO Fisheries

Dewitt HH, Heemstra PC, Gon O. 1990. Nototheniidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean.* Grahamstown: JLB Smith Institute of Ichthyology, 279–331.

Diez del Corral R, Olivera-Martinez I, Goriely A, Gale E, Maden M, Storey K. 2003. Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. *Neuron* **40:** 65–79.

Dubrulle J, McGrew MJ, Pourquie O. 2001. FGF signaling controls somite boundary position and regulates segmentation clock control of spaciotemporal Hox gene activation. *Cell* **106:** 219–232.

Endo H. 2002. Phylogeny of the order Gadiformes (Teleostei, Paracanthopterygii). *Memoires of the Graduate School of Fish Science, Hokkaido University* **49:** 75–149.

Fahay MP, Markle DF. 1984. Gadiformes, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 265–283.

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

Fink SV, Fink WL. 1981. Interrelationships of the ostariophysan fishes (Teleostei). *Zoological Journal of the Linnean Society* **72:** 297–353.

Ford E. 1937. Vertebral variation in teleostean fishes. *Journal of the Marine Biology Association of the United Kingdom* **22:** 1–60.

Froese R, Pauly D, eds. .**2004.** *FishBase*, version 04/2004 [WWW document]. Available at http://www.fishbase.org.

Gadow HF. 1933. *The evolution of the vertebral column.* Cambridge: Cambridge University Press.

Galis F. 1999. Why do almost all mammals have seven cervical vertebrae? Developmental constraints, Hox genes, and cancer. *Journal of Experimental Zoology* **285:** 19–26.

Gass GL, Bolker JA. 2003. Modularity. In: Hall BK, Olson WM, eds. *Keywords and concepts in evolutionary developmental biology.* Cambridge, MA: Harvard University Press, 260–267.

Gaunt SJ. 1994. Conservation in the Hox code during morphological evolution. *International Journal of Developmental Biology* **38:** 549–552.

Gon O. 1990a. Bathydraconidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean.* Grahamstown: JLB Smith Institute of Ichthyology, 364–380.

Gon O. 1990b. Bovichtidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean.* Grahamstown: JLB Smith Institute of Ichthyology, 277–278.

Gon O. 1990c. Synaphobranchidae. In: Gon O, Heemstra PC,

eds. *Fishes of the Southern Ocean*. Grahamstown: JLB Smith Institute of Ichthyology, 102–104.

Goodrich ES. 1958. *Studies on the structure and development of vertebrates*, Vol. 1. New York, NY: Dover Publications.

Gordon DJ, Markle DF, Olney JE. 1984. Ophidiiformes, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 308–319.

Goss RJ. 1969. *Principles of regeneration*. New York, NY: Academic Press.

Grande L, Bemis WE. 1998. A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Journal of Vertebrate Paleontology* **18:** 1–690.

Hilton EJ. 2003. Comparative osteology and phylogenetic systematics of fossil and living bony-tongue fishes (Actinopterygii, Teleostei, Osteoglossomorpha). *Zoological Journal of the Linnean Society* **137:** 1–100.

van der Hoeven F, Sordino P, Fraudeau N, Izpisúa-Belmonte J-C, Duboule D. 1996. Teleost *HoxD* and *HoxA* genes: a comparison with tetrapods and functional evolution of the *HOXD* complex. *Mechanisms of Development* **54:** 9–21.

Holley SA, Geisler R, Nusslein-Volhard C. 2000. Control of *her1* expression during zebrafish somitogenesis by a *Delta*-dependent oscillator and an independent wave-front activity. *Genes and Development* **14:** 1678–1690.

Holley SA, Julich D, Rauch GJ, Geisler R, Nusslein-Volhard C. 2002. her1 and the notch pathway function within the oscillator mechanism that regulates zebrafish somitogenesis. *Development* **129:** 1175–1183.

Holley SA, Takeda H. 2002. Catching a wave: the oscillator and wavefront that create the zebrafish somite. *Seminars in Cellular and Developmental Biology* **13:** 481–488.

Horn MH. 1984. Stromateoidei, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 620–628.

Hureau JC. 1990. Harpagiferidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean*. Grahamstown: JLB Smith Institute of Ichthyology, 357–363.

Iwami T, Kock KH. 1990. Channichthyidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean*. Grahamstown: JLB Smith Institute of Ichthyology, 381–399.

Johnson RG. 1955. The adaptive and phylogenetic significance of vertebral form in snakes. *Evolution* **9:** 367–388.

Johnson GD, Baldwin CC. 1994. Accounting for endothermy in fishes. *Science* **265:** 1249–1250.

Kanki JP, Ho RK. 1997. The development of the posterior body in zebrafish. *Development* **124:** 881–893.

Lauder GV, Liem KF. 1983. The evolution and interrelationships of the actinopterygian fishes. *Bulletin of the Museum of Comparative Zoology* **150:** 95–197.

Lindsey CC. 1975. Pleomerism, widespread tendency among related fish species for vertebral number to be correlated with maximum body length. *Journal of Fisheries Research Board of Canada* **32:** 2453–2469.

Lovejoy NR. 2000. Reinterpreting recapitulation: Systematics of needlefishes and their allies (Teleostei: Beloniformes). *Evolution* **54:** 1349–1362.

Maddison WP. 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Systematic Zoology* **40:** 304–314.

Maddison DR, Maddison WP. 2003. *MacClade 4.* Sunderland, MA: Sinauer Publishers.

McCosker JE. 1998. A revision of the snake-eel genus *Callechelys* (Anguilliformes: Ophichthidae) with the description of two new Indo-Pacific species and a new callechelyin genus. *Proceedings of the California Academy of Science* **50:** 185–215.

Miya M, Takeshima H, Endo H, Ishiguro NB, Inoue JG, Mukai T, Satoh TP, Yamaguchi M, Kawaguchi A, Mabuchi K, Shirai SM, Nishida M. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **26:** 121–138.

Morin-Kensicki EM, Melancon E, Eisen JS. 2002. Segmental relationship between somites and vertebral column in zebrafish. *Development* **129:** 3851–3860.

Nakamura I. 1990. Gempylidae. In: Gon O, Heemstra PC, eds. *Fishes of the Southern Ocean*. Grahamstown: JLB Smith Institute of Ichthyology, 402–403.

Narita Y, Kuratani S. 2005. Evolution of the vertebral formulae in mammals: a perspective on developmental constraints. *Journal of Experimental Zoology* **304B:** 1–16.

Nelson JS. 1994. *Fishes of the world*, 3rd edn. New York, NY: John Wiley & Sons.

Noble GK. 1931. *The biology of the amphibia*. New York, NY: McGraw-Hill Co.

Oates AC, Ho RK. 2002. *Hairy/E(spl)-related* (*Her*) genes are central components of the segmentation oscillator and display redundancy with the Delta/Notch signaling pathway in the formation of anterior segmental boundaries in the zebrafish. *Development* **129:** 2929–2946.

Olney JE. 1984. Lampriformes, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 368–379.

Parra-Olea G, Wake DB. 2001. Extreme morphological and ecological homoplasy in tropical salamanders. *Proceedings of the National Academy of Science* **98:** 7888–7891.

Patterson C, Rosen DE. 1989. The Paracanthopterygii revisited: order and disorder. In: Cohen DM, ed. *Papers on the systematics of gadiform fishes* Natural History Museum of Los Angeles County, Science Series 32. Los Angeles, CA: Natural History Museum of Los Angeles County, 5–36.

Pietsch TW. 1978. Evolutionary relationships of the sea moths (Teleostei: Pegasidae) with a classification of gasterosteiform families. *Copeia* **1978:** 517–529.

Polly PD, Head JJ, Cohn MJ. 2001. Testing modularity and dissociation: the evolution of regional proportions in snakes. In: Zelditch ML, ed. *Beyond heterochrony: the evolution of development*. New York, NY: Wiley-Liss, Inc, 305–335.

Pourquie O. 2003. The segmentation clock: converting embryonic time into spatial pattern. *Science* **301:** 328–330.

Prince VE, Joly L, Ekker M, Ho RK. 1998a. Zebrafish *hox* genes: genomic organization and modified colinear expression patterns in the trunk. *Development* **125:** 407–420.

Prince VE, Price AL, Ho RK. 1998b. Hox gene expression reveals regionalization along the anteroposterior axis of the zebrafish notochord. *Development Genes and Evolution* **208:** 517–522.

Purvis A, Rambaut A. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analyzing comparative data. *Computer Applied Biosciences* **11:** 247–251.

Raff RA. 1996. *The shape of life: genes, development, and evolution of animal form.* Chicago, IL: University of Chicago Press.

Richardson MK, Allen SP, Wright GM, Raynaud A, Hanken J. 1998. Somite number and vertebrate evolution. *Development* **125:** 151–160.

Roberts TR. 1986. Systematic review of the Mastacembelidae or spiny eels of Burma and Thailand, with description of 2 new species of *Macrognathus*. *Japanese Journal of Ichthyology* **33:** 95–109.

Rofen RR. 1966. Family Anotopteridae. In: Olsen YH, Atz JW, eds. *Fishes of the western North Atlantic, part 5, order Iniomi, order Lyomeri.* Copenhagen: Bianco Luno's Printing, 498–510.

Romer AS. 1970. *The vertebrate body*, 4th edn. Philadelphia, PN: Saunders.

Rosen DE, Patterson C. 1969. The structure and relationships of the paracanthopterygian fishes. *Bulletin of the American Museum of Natural History* **141:** 361–474.

Sawada A, Shinya M, Jiang YJ, Kawakami A, Kuroiwa A, Takeda H. 2001. Fgf/MAPK signaling is a crucial positional cue in somite boundary formation. *Development* **128:** 4873–4880.

Shiogaki M. 1981. Redescription of the stichaeid fish *Chirolophis saitone*. *Japanese Journal of Ichthyology* **28:** 129–134.

Smith MM, Heemstra PC, eds. **1986.** *Smith's sea fishes*. New York, NY: Springer-Verlag.

Smith-Vaniz WF. 1984. Carangidae, relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 522–530.

Springer VG, Garrick JAF. 1964. A survey of vertebral numbers in sharks. *Proceedings of the United States National Museum* **116:** 73–96.

Stevens EG, Watson W, Matarese AC. 1984. Notothenioidea, development and relationships. In: Moser HG, ed. *Ontogeny and systematics of fishes.* Lawrence, KS: American Society of Ichthyologists and Herpetologists, 561–564.

Tesch FW. 1977. *The eel: biology and management of anguillid eels.* Translated by J. Greenwood. London: Chapman & Hall.

Tyler JC. 1980. *Osteology, phylogeny and higher classification of the fishes of the order Plectognathi (Tetraodontiformes).* *NOAA Technical Report NMFS Circular* **434:** 1–422.

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

Ward AB, Azizi E. 2004. Convergent evolution of the head retraction escape response in elongate fishes and amphibians. *Zoology* **107:** 205–217.

Wellick DM, Capecchi MR. 2003. *Hox10* and *Hox11* genes are required to globally pattern the mammalian skeleton. *Science* **301:** 363–367.

Wiens JJ, Slingluff JL. 2001. How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. *Evolution* **55:** 2303–2318.

SUPPLEMENTARY MATERIAL

The following material is available for this article online:

Appendix S1. Museum numbers of specimens examined.
Table S1. Species included in the literature-based study of vertebral number changes.
Table S2. Species names and number of specimens examined in the museum-based portion of this study.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1095-8312.2007.00714.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.