

Evolutionary diversification of body form and the axial skeleton in the Gasterosteidae: the sticklebacks and their closest relatives

Windsor E. Aguirre, Seth E. Contreras, Katelyn M. Carlson, Alex J. Jagla and Lissette Arellano

Department of Biological Sciences, DePaul University, Chicago, Illinois, USA

ABSTRACT

Background: Many fishes have evolved long bodies. Decades of research have uncovered substantial variability in body form within the threespine stickleback (*Gasterosteus aculeatus*) and among its relatives in the Gasterosteidae, including the evolution of extremely long bodies. Elongation is likely to be associated with the evolution of the axial skeleton but we need studies linking variation in length and the axial skeleton.

Objectives: Examine the relationship between body form and vertebral variation in the Gasterosteidae (the sticklebacks and their closest relatives).

Methods: We examined samples of all genera of the Gasterosteidae, Aulorhynchidae, and Hypoptychidae, collected throughout their ranges. We examined body form variation using geometric morphometric methods and the fineness ratio. We obtained total vertebral number and the proportion of precaudal to caudal vertebrae from X-rays of the same specimens.

Results: Mean total vertebral number varied from 26.8 in *Gasterosteus wheatlandi* to 54.5 in *Hypoptychus dybowskii*. Body shape was significantly related to total vertebral number across taxa, with longer bodied species having more vertebrae. *Hypoptychus dybowskii*, which has more vertebrae than predicted from its relative body length, is an outlier. The number of precaudal and caudal vertebrae covary linearly across taxa. Again, *Hypoptychus dybowskii* was an exception, having an excess of precaudal vertebrae for the number of caudal vertebrae that it possesses. *Apeltes quadracus* was a second exception, having an excess of caudal vertebrae for the number of precaudal vertebrae that it possesses. In summary, changes in the relative proportions of the body are typically accompanied by corresponding changes in vertebral number. Differences in the number of caudal vertebrae appear to be particularly important among some of the morphologically more similar genera like *Gasterosteus*, *Culaea*, and *Pungitius*. Consistent with the hypothesis of pleomerism, vertebral number was also associated with body size, such that larger species have more vertebrae.

Keywords: adaptation, body shape, evolution, Gasterosteidae, geometric morphometrics, pleomerism, vertebrae.

Correspondence: W.E. Aguirre, Department of Biological Sciences, DePaul University, 2325 North Clifton Avenue, Chicago, IL 60614-3207, USA. email: waguirre@depaul.edu
Consult the copyright statement on the inside front cover for non-commercial copying policies.

INTRODUCTION

Body form and vertebral variation in fishes

The external body form of aquatic vertebrates is under strong selection for hydrodynamic efficiency given the energetic demands related to swimming in a viscous medium like water. As a consequence, body form often correlates with ecology and can be used to develop a deeper understanding of patterns of divergence among fish species (e.g. Webb, 1984; Wainwright, 1991; Ricklefs and Miles, 1994). Body elongation, an increase in the length of the body relative to its depth, is one of the most important ways in which body form evolves in fishes, and has evolved repeatedly in response to diverse conditions (Ward and Brainerd, 2007). In fact, the evolution of body elongation is often the strongest pattern of body shape divergence seen in fishes (Claverie and Wainwright, 2014).

Changes in body form are strongly associated with changes in vertebral number and length across vertebrates (e.g. Wake and Larson, 1987; Parra-Olea and Wake, 2001; Ward and Brainerd, 2007; Ward and Mehta, 2010). Body elongation is most commonly associated with increases in vertebral number, although increases in vertebral length have also led to the evolution of body elongation in some lineages (Ward and Mehta, 2010). This difference between the number and length of vertebrae may matter ecologically, since fish with more vertebrae that are short in length would presumably be more flexible than fish with fewer vertebrae that are longer, and differences in body flexibility can impact swimming performance (e.g. Brainerd and Patek, 1998). Fish also possess two highly distinct body regions with different types of vertebrae. Precaudal vertebrae are characterized by the presence of ribs and are located in the anterior part of the body, dorsal to the viscera. Caudal vertebrae lack ribs, possess haemal spines, and are located in the posterior region of the body where they provide support for the muscles used for swimming via tail propulsion. The proportion of precaudal to caudal vertebrae can change as total vertebral number evolves, depending on the specific functional demands on the body (Ward and Mehta, 2010, 2014). Body size is another morphological feature associated with variation in the number of vertebrae. Larger fish species tend to have more vertebrae. This relationship, known as pleomerism, appears to be widespread in fishes and some other vertebrates (e.g. Lindsey, 1975; Lindell, 1994), although there are exceptions (e.g. Van Damme and Vanhooydonck, 2002; Shikano and Merilä, 2011).

Although several recent reviews have examined broad scale patterns of vertebral diversity across fishes (e.g. Ward and Brainerd, 2007; McDowall, 2008; Ward and Mehta, 2010, 2014), there have been relatively few rigorous studies of the relationship between body form and vertebral phenotype variation within lineages (e.g. McDowall, 2004; Mehta *et al.*, 2010; Reece and Mehta, 2013). In this study, we examine the relationship between body form and vertebral variation in the Gasterosteidae, the sticklebacks and their closest relatives (Fig. 1). Below, we describe the major characteristics of this group, indicate the value of studying its body form and vertebral variation, and provide the specific objectives for the study.

Evolutionary diversity of the Gasterosteidae

The Gasterosteidae are a morphologically distinctive and ecologically important group of fishes found primarily at high latitudes of the northern hemisphere. It includes the sticklebacks (Gasterosteidae), the sand eel (Hypoptychidae), and the tube snouts (Aulorhynchidae) (Kawahara *et al.*, 2009). The Gasterosteidae likely represent a robust group,

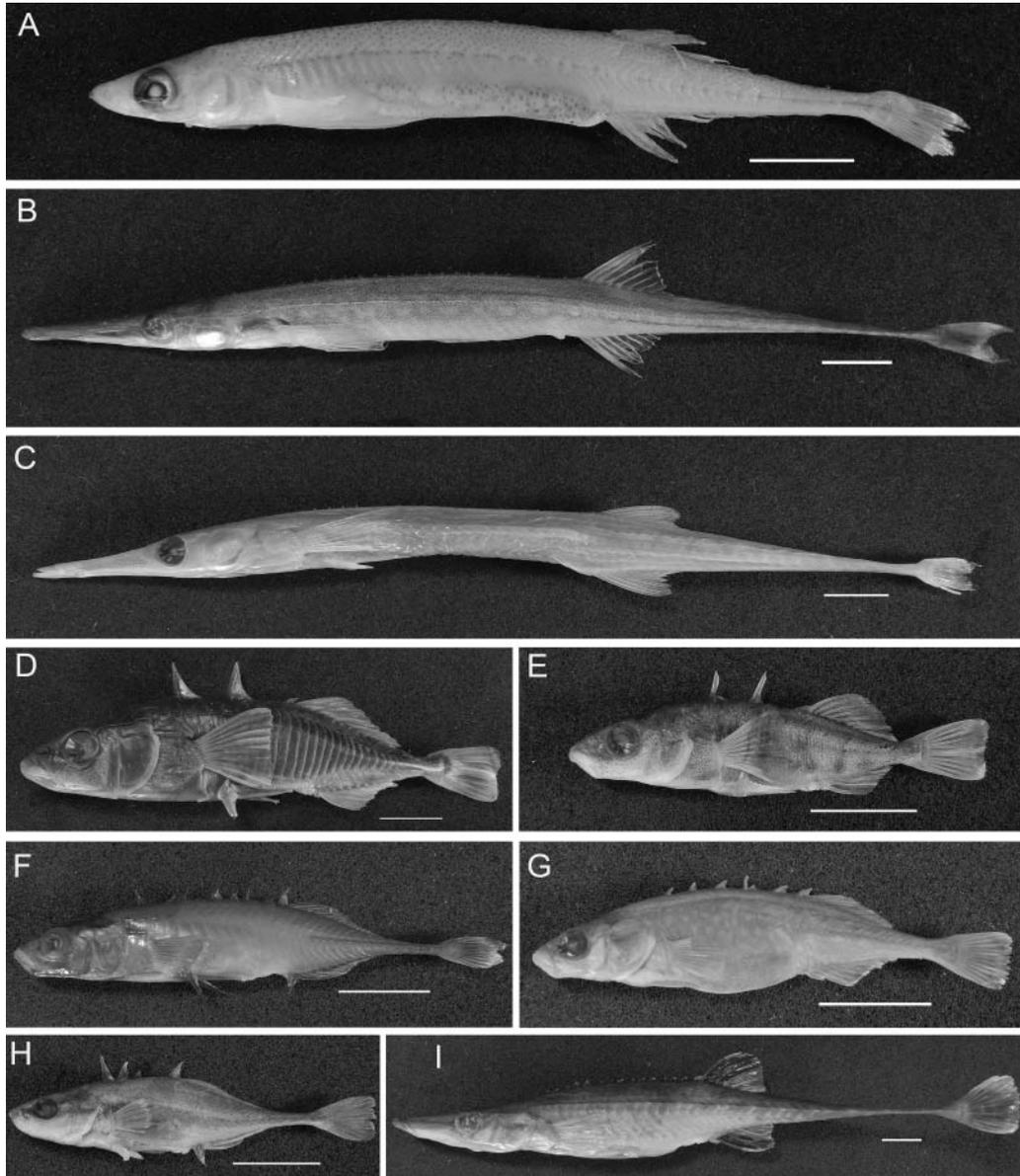


Fig. 1. Gasterosteidae included in the study. (A) *Hypoptychus dybowskii*. (B) *Aulichthys japonicus*. (C) *Aulorhynchus flavidus*. (D) *Gasterosteus aculeatus*. (E) *Gasterosteus wheatlandi*. (F) *Pungitius pungitius*. (G) *Culaea inconstans*. (H) *Apeltes quadracus*. (I) *Spinachia spinachia*. Scale bar = 10 mm.

since most members were previously clustered based on their phenotypic similarities (e.g. Wootton, 1976; Bowne, 1994), and two independent molecular phylogenies have verified their close relationships (Kawahara *et al.*, 2009; Betancur-R *et al.*, 2013). In this study, we follow the phylogeny depicted in Kawahara *et al.* (2009), which is based on whole mitochondrial genome data and 11 nuclear gene sequences.

The Gasterosteidae include some of the ecologically most important forage fish in coastal and inland waters at high latitudes of the northern hemisphere, and constitute critical prey for many ecologically and commercially important predatory fishes (Wootton, 1976; Reimchen, 1994). It also includes species like the threespine stickleback (*Gasterosteus aculeatus*), which has been described as a species complex – that is, a species composed of thousands of relatively closely related but morphologically divergent populations that have diversified rapidly in response to heterogeneous environmental conditions (e.g. Bell, 1976; Bell and Foster, 1994; McKinnon and Rundle, 2002; Hendry *et al.*, 2009; Cassidy *et al.*, 2013; Reimchen *et al.*, 2013). The genera *Pungitius* and *Culaea* also harbour substantial intraspecific phenotypic variability (e.g. Nelson and Atton, 1971; Nelson, 2006), with *Pungitius* consisting of several valid species (Wang *et al.*, 2015). Genera in the Gasterosteidae are extremely divergent morphologically from one another. Some, like *Gasterosteus*, *Apeltes*, and *Culaea*, have fairly typical fish-like body forms, while others, like *Spinachia*, *Aulorhynchus*, and *Aulichthys* exhibit extreme body elongation.

Although it is obvious that body form varies tremendously among the Gasterosteidae (Fig. 1) and published vertebral counts indicate substantial heterogeneity in vertebral number within the group (e.g. Wootton, 1976; Shikano and Merilä, 2011), there has been no systematic study of body form – vertebral evolution for the group. Moreover, Aguirre *et al.* (2014) recently proposed the threespine stickleback as a potential model system for studying microevolutionary diversification of the axial skeleton in fishes. The threespine stickleback is already one of the most important model systems in evolutionary biology (e.g. Bell and Foster, 1994; Östlund-Nilsson *et al.*, 2007; Wootton, 2009; Hendry *et al.*, 2013), and the repeated parallel evolution of deep-bodied (benthic) and elongate (limnetic) ecomorphs is one of the most interesting patterns of evolutionary divergence within the species (e.g. Schluter, 1996; Walker, 1997; Spoljaric and Reimchen, 2007; Berner *et al.*, 2009; Reid and Peichel, 2010; Bell and Aguirre, 2013). Studies conducted by Reimchen and Nelson (1987) and Ahn (1998) previously suggested associations between body form and vertebral variation in this species. Aguirre *et al.* (2014) confirmed that the evolution of body elongation in limnetics is associated with increased vertebral number relative to benthic and ancestral anadromous populations. The increase primarily involves the number of caudal vertebrae, indicating that adaptation to different ecological niches involves body region specific responses of the axial skeleton. Continued sampling efforts of populations with extreme body forms in Alaska indicate that the pattern of divergence in the axial skeleton is likely stronger than initially reported (W.E. Aguirre *et al.*, unpublished data). How the magnitude and pattern of the microevolutionary divergence of the axial skeleton seen in the threespine stickleback compares with that in other taxa in the Gasterosteidae is not known. Examining variation among the Gasterosteidae may thus provide an important link between studies directed towards large-scale patterns of body form: vertebral evolution across fishes (e.g. Ward and Brainerd, 2007; Ward and Mehta, 2010) and microevolutionary studies focusing on the mechanisms of change within species (e.g. Reimchen and Nelson, 1987; Ahn, 1998; Aguirre *et al.*, 2014).

Objectives

We addressed the following specific objectives in this study. (1) We conducted a geometric morphometric analysis of representatives of all genera in the Gasterosteidae to examine the major patterns of body shape variation in the group. (2) The same specimens were X-rayed and the total number of vertebrae, the number of precaudal vertebrae, and the number of caudal vertebrae were counted to describe the major patterns of variation in

vertebral number. (3) We tested whether body elongation is associated with increases in vertebral number and whether there is body region specificity. (4) Finally, we tested whether the total number of vertebrae is positively associated with body size as predicted by the pleomerism hypothesis.

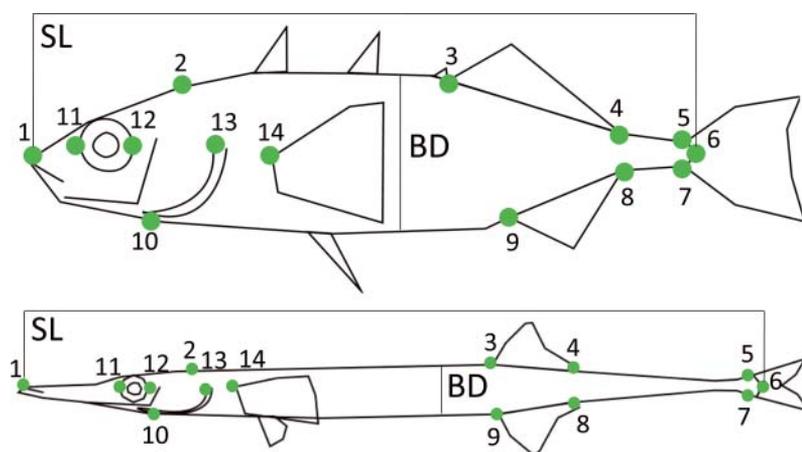
MATERIALS AND METHODS

The specimens used in this study primarily came from North American museum collections and from research collections provided by colleagues (www.evolutionary-ecology.com/data/2989Appendix.pdf). We aimed to include samples of ten specimens per lot although some lots had fewer specimens (Table 1). The study was not intended to be a comprehensive survey of variation within each of the taxa examined given the broad geographic distribution of many species and the enormous levels of morphological and ecological diversity present in some species. Samples were selected to maximize the geographic representation for the taxa included, and emphasis is placed on differences among taxa in the analyses. In addition, although considerable progress is being made (e.g. Higuchi *et al.*, 2014; Wang *et al.*, 2015), the complex evolutionary history of some genera (e.g. *Pungitius* and *Gasterosteus*) has resulted in significant uncertainty regarding how many species should be recognized. Thus, we primarily focus on variation at the level of the genus in this study and avoid designating species within the genera *Gasterosteus* and *Pungitius*, with the exception of recognizing *G. wheatlandi* because of its morphological distinctiveness and its long standing as a recognized species (Hubbs, 1929; Wootton, 1976; Buth and Haglund, 1994; Mattern, 2007). All other *Gasterosteus* included in this study were pooled under *G. aculeatus*. We note that although a sample of *Gasterosteus* from Japan is included in the study (FMNH-76260), the specimens are low-armoured and thus likely represent a freshwater resident form of *G. aculeatus* and not the recently described *G. nipponicus* from the Sea of Japan (Higuchi *et al.*, 2014). *Gasterosteus nipponicus* is completely plated and characterized by a distinctive plating pattern posterior to the pelvis. No specimens of *G. nipponicus* were included in this study, although the vertebral number range of 30–33 listed by Higuchi *et al.* (2014) is consistent with that seen in *G. aculeatus*. In total, 693 specimens from 80 lots were included in the study (Table 1; [2989Appendix.pdf](http://www.evolutionary-ecology.com/data/2989Appendix.pdf)). Taxa are ordered in tables, legends, and figures in the same order in which they appear in the phylogenetic tree of Kawahara *et al.* (2009).

Body shape and vertebral phenotype data were collected following Aguirre *et al.* (2014). Briefly, specimens were individually tagged and a geometric morphometric analysis of body shape variation was conducted (Zelditch *et al.*, 2012). Specimens were straightened (if necessary) using insect pins or tape, and were photographed with a 10.3-megapixel Nikon Coolpix P100 digital camera. Two-dimensional coordinates were collected for 14 homologous landmarks digitized on each specimen (Fig. 2) using the program tpsDig v.2.17 (Rohlf, 2013a). The landmark data were aligned using Procrustes superimposition in Relative Warps v.1.53 (Rohlf, 2013b). A principal component analysis (PCA) was conducted in Relative Warps to examine the major patterns of body shape variation in the data. The PCA was conducted on the individual specimen data set ($N = 693$ specimens). The percentages of variation explained by the PC axes correspond to the variation among individuals. Sample means were computed from the specimen PC scores and are displayed in the plots. A discriminant function analysis (DFA) was also conducted in SPSS v.21 (IBM Corporation) on the body shape data to assess rates of correct classification of individuals by species. The shape variables (partial warp + uniform component) were entered together into the analysis, prior probabilities were

Table 1. Number of lots examined per species, total number of specimens per species (*N*), and geographic location of samples examined for each species (see [2989Appendix.pdf](#) for further details)

Species	Lots	<i>N</i>	Representative sampling localities
<i>Hypoptychus</i>	2	17	Japan
<i>Aulichthys</i>	4	22	Japan
<i>Aulorhynchus</i>	4	25	USA Pacific: California, Washington
<i>G. aculeatus</i>	19	181	USA: Alaska, California, Lake Michigan, Rhode Island; Canada: Labrador; Europe: England, N. Ireland, Wales, Germany, Sweden, Norway, Romania, Italy; Japan
<i>G. wheatlandi</i>	7	60	USA: Connecticut, Rhode Island, Massachusetts, Maine; Canada: Newfoundland
<i>Pungitius</i>	18	160	USA: Alaska, Lake Michigan, Rhode Island, Massachusetts, Maine; Canada: Newfoundland, Nova Scotia; Europe: England, Germany, Sweden, Finland; Japan
<i>Culaea</i>	7	64	USA: Minnesota, Wisconsin, Iowa, Illinois; Canada: Manitoba, Ontario
<i>Apeltes</i>	11	103	USA: Maryland, New Jersey, New York, Massachusetts, Maine; Canada: Newfoundland, Gulf of St. Lawrence
<i>Spinachia</i>	8	61	Europe: England, Ireland, France, Germany, Norway
Total	80	693	

**Fig. 2.** Landmarks and linear measures used in the study. Dots indicate the positions of the 14 landmarks used in geometric morphometric analysis of body shape variation. Lines indicate the two linear measures taken. SL = standard length, BD = body depth.

computed from sample sizes, and a leave-one-out classification cross-validation was employed. In addition to the geometric morphometric data, standard length (SL), taken from the tip of the snout to the end of the caudal peduncle, and body depth (BD), taken as the maximum body depth perpendicular to the long axis of the fish (typically measured just anterior to the dorsal fin), were also measured from the digital images in tpsDig. These were used to calculate the fineness ratio (FR), where $FR = SL/BD$. The fineness ratio is a measure

of elongation commonly used in fishes, with higher values indicating more elongate bodies (e.g. Li and Li, 2006; Walker *et al.*, 2013).

The same specimens were X-rayed at the Field Museum of Natural History using an AXR Hot Shot X-Ray Machine (Associated X-Ray Corporation). Specimens were X-rayed using the following settings: kV = 35, mA = 4, and seconds = 7–13, with the seconds varying depending on the size of the specimens. X-rays were scanned at high resolution (1200 ppi) with an HP Scanjet G4100 for transformation to a digital format (Fig. 3). An input file was created from X-ray images of individual fish and landmarks were placed on each vertebra in tpsDig v.2.17 to facilitate counting the vertebrae. The numbers of precaudal (abdominal) and caudal vertebrae were counted from the digital images and defined following Aguirre *et al.* (2014). Precaudal vertebrae have ribs and lack haemal spines, while caudal vertebrae lack ribs and have haemal spines. Transitional vertebrae with haemal arches but lacking spines were counted as precaudal vertebrae. In most species examined, the first vertebra with a definite haemal spine typically comes into contact or is in close proximity to the first anal pterygiophore, facilitating classification (Swain, 1992; Ahn and Gibson, 1999; Aguirre *et al.*, 2014). In the rare cases of vertebra with a small but visible spine that did not come in contact with the first anal pterygiophore, these were classified as precaudal vertebrae because of their greater resemblance to precaudal vertebrae with haemal arches than to caudal vertebrae with fully developed haemal spines. The urostyle was not included in the vertebral counts.

Linear correlation analysis was used to examine the association between mean vertebral counts and fineness ratios (elongation) across taxa. Species means calculated from sample means were used in these analyses. The relationship between body shape and vertebral phenotype divergence was also examined by comparing pairwise distances between species. To quantify body shape divergence, the Procrustes distance was computed between species consensus configurations for all pairs of species in Thin-Plate Spline v.1.20 (Rohlf, 2004). The Procrustes distance is a common distance measure used in geometric morphometrics. It is defined as the sum of squared distances between corresponding landmarks on two specimens after Procrustes superimposition (Zelditch *et al.*, 2012). To quantify divergence in vertebral phenotypes, pairwise Euclidean distances between taxa based on differences in the mean precaudal and caudal vertebral counts were calculated in PAST v.3.08 (Hammer *et al.*, 2001). To determine the correlation between body shape and vertebral phenotype divergence among taxa, we conducted a Mantel test on the pairwise distance matrices in PASSaGE v.2.0.11.6 (Rosenberg and Anderson, 2011). Finally, to test the hypothesis of pleomerism, we

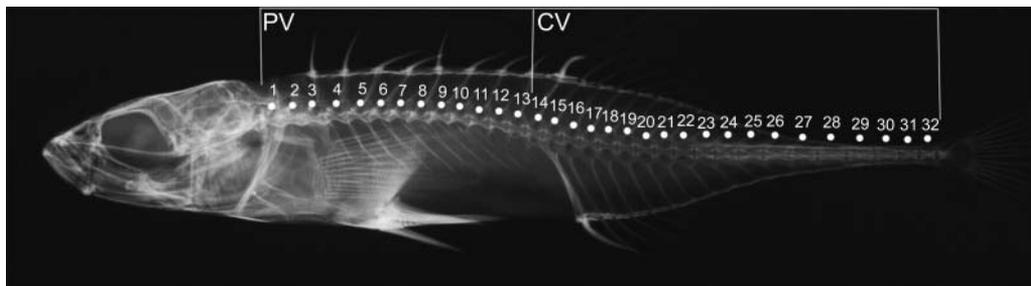


Fig. 3. Radiograph of a specimen of *Pungitius* showing the method used to count vertebrae. PV = precaudal vertebrae (possessing ribs but lacking haemal spines), CV = caudal vertebrae (possessing haemal spines but lacking ribs).

examined the correlation between mean standard length and total vertebral number. Taxon means were calculated from sample means for all taxa. Because samples were obtained from many independent collections with varying collection protocols, the body length of the samples in this study may not be completely representative of the body lengths of these taxa in nature. Therefore, we also examined the relationship between the mean number of total vertebrae and the maximum reported body length (total length) for each taxon obtained from FishBase (Froese and Pauly, 2015).

RESULTS

Body shape divergence

Body shape variation is strongly structured, with the PCA revealing a dominant PCI that accounted for 70% of the variation in body shape among individuals (Fig. 4). The first PC is associated with body length. The most elongate taxa, *Aulorhynchus*, *Aulichthys*, and *Spinachia*, are on one end of PCI, while *G. wheatlandi* and *G. aculeatus* have the most extreme scores on the other end. PCII accounted for 10.6% of the variation in body shape and is associated with the relative proportions of the body regions, such that taxa with relatively longer caudal regions have lower scores on PCII and taxa with relatively longer abdominal regions have higher scores on PCII. Divergence in body shape among taxa is relatively large and most taxa segregate well in the space formed by PCI and PCII. Even *Gasterosteus aculeatus* and *G. wheatlandi* form two distinct clusters, consistent with their long standing as separate species. The exceptions are *Pungitius*, which spans a broad area in the morphospace and overlaps with *Culaea* and *Gasterosteus*, and *Aulichthys* and *Aulorhynchus*, which are close and overlap with one another in the morphospace. Consistent with the results of the PCA, correct classification of individuals to species based on the DFA of the body shape data is generally good (2989Appendix.pdf). Correct classification is perfect for five species and over 99% for another two species, with a single specimen of *G. aculeatus* being misclassified as *G. wheatlandi* and a single specimen of *Pungitius* being misclassified as *Apeltes*. Correct classification percentages are lower for *Aulichthys japonicus* (90.9%) and *Aulorhynchus flavidus* (72%), with all misclassified individuals of *Aulichthys* being designated to *Aulorhynchus* and vice versa. The occasional misclassification of individuals of these two species is consistent with their morphological similarity and proximity in the morphospace formed by PCI and PCII (Fig. 4). Body shape divergence among the Gasterosteidae in this morphospace tracks phylogenetic relationships well (Fig. 4B). Taxa that are closely related in the phylogeny of Kawahara *et al.* (2009) tend to be close together in the morphospace, with the exception of *Apeltes* and *Spinachia*, which are still located in the same region of the morphospace.

Vertebral divergence

Total vertebral number varies from an average of 26.8 for *G. wheatlandi* to an average of 54.5 for *Hypoptychus* (Table 2, Fig. 5). Again, *G. wheatlandi* is quite distinct phenotypically from *G. aculeatus*, exhibiting almost non-overlapping vertebral counts. A single specimen of *G. aculeatus* of 181 examined had a vertebral count of 28, which overlapped with the highest count documented for *G. wheatlandi*. *Gasterosteus aculeatus*, *Pungitius*, *Culaea*, and *Apeltes* are similar in total vertebral number and exhibit overlapping counts. There is then a gap

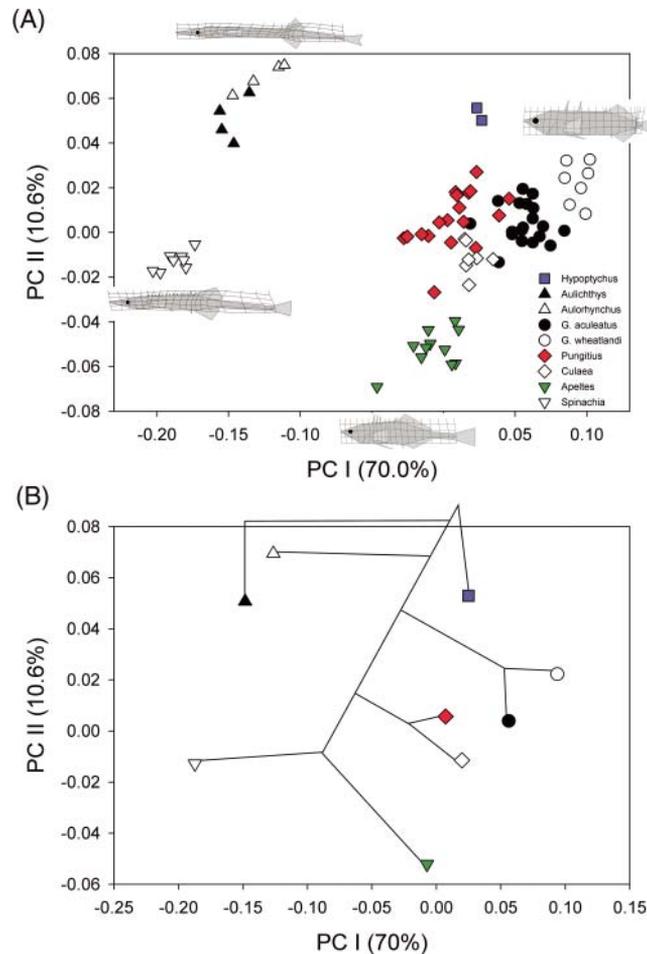


Fig. 4. Principal component analysis (PCA) of body shape variation. Numbers in parentheses indicate the percentage of variation of body shape accounted for by each PC axis. (A) Sample means plotted on PCI and PCII. Deformation grids are predicted shapes in different portions of shape space. Fins, spines, eyes, etc., are approximate and drawn to highlight important anatomical features. (B) Species means plotted on PCI and PCII. The branching pattern of Kawahara and colleagues' (2009) phylogeny is overlain on the plot.

between these taxa and *Spinachia*, with the latter exhibiting higher counts of 38–41 total vertebrae with an average of 40. This is followed by a more substantial gap between *Spinachia* and the remaining three taxa: *Aulichthys*, *Aulorhynchus*, and *Hypoptychus*. *Aulichthys* has total counts varying between 50 and 53, while *Aulorhynchus* varies between 53 and 56 and *Hypoptychus* varies between 53 and 55.

The numbers of precaudal and caudal vertebrae also appears to be linearly correlated across species (Fig. 6A). Differences in total vertebral number result from differences in both the number of abdominal and caudal vertebrae. Although the correlation between the number of precaudal and caudal vertebrae across taxa is strong and statistically significant ($r = 0.84$, $N = 9$, $P = 0.002$), two taxa – *Apeltes* and *Hypoptychus* – seemed to differ from the

Table 2. Mean number of total vertebrae, precaudal vertebrae, caudal vertebrae, and the fineness ratio

Species	Total vertebrae	Precaudal vertebrae	Caudal vertebrae	Fineness ratio
<i>Hypoptychus</i>	54.5 ± 0.05 (53–55)	28.5 ± 0.64 (28–30)	26.0 ± 0.59 (24–27)	8.7 ± 1.49
<i>Aulichthys</i>	51.3 ± 0.49 (50–53)	24.5 ± 0.52 (23–26)	26.8 ± 0.36 (26–28)	14.0 ± 1.80
<i>Aulorhynchus</i>	54.2 ± 0.42 (53–56)	25.5 ± 0.55 (25–27)	28.7 ± 0.46 (27–30)	16.3 ± 0.96
<i>G. aculeatus</i>	30.6 ± 0.89 (28–33)	14.5 ± 0.37 (13–16)	16.1 ± 0.74 (13–18)	4.1 ± 0.45
<i>G. wheatlandi</i>	26.8 ± 0.17 (25–28)	12.5 ± 0.23 (11–14)	14.2 ± 0.29 (13–16)	3.6 ± 0.15
<i>Pungitius</i>	32.1 ± 0.53 (30–35)	14.0 ± 0.49 (12–16)	18.1 ± 0.51 (16–20)	5.4 ± 0.51
<i>Culaea</i>	31.5 ± 0.31 (30–33)	14.2 ± 0.30 (13–15)	17.3 ± 0.30 (16–19)	4.1 ± 0.17
<i>Apeltes</i>	30.1 ± 0.20 (28–32)	10.4 ± 0.35 (9–11)	19.7 ± 0.29 (17–22)	4.5 ± 0.35
<i>Spinachia</i>	40.0 ± 0.28 (38–41)	17.9 ± 0.18 (16–19)	22.2 ± 0.31 (21–24)	10.3 ± 1.27

Note: Error is the standard deviation calculated from sample means and the range (in parentheses) is the lowest and highest individual vertebral count documented for each taxon.

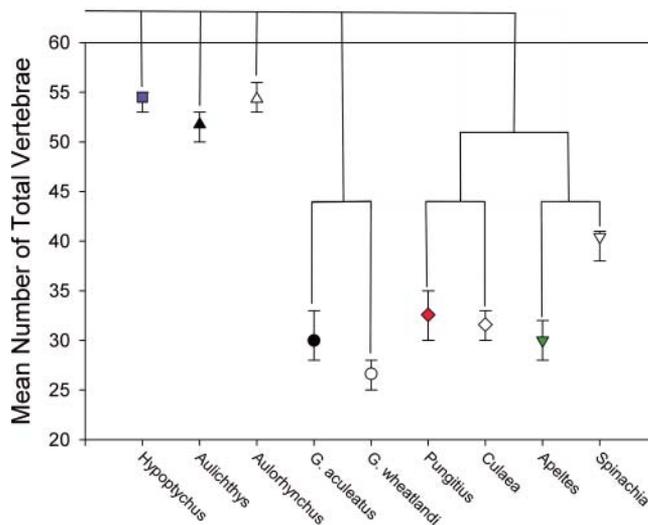


Fig. 5. Mean number of total vertebrae for the species sampled. Kawahara and colleagues' (2009) branching pattern for the phylogeny of the group is overlain on the plot. Error bars are the observed individual range in total vertebral number for each species.

rest (Fig. 6A). When these were excluded, the correlation between the number of abdominal and caudal vertebrae increased to 0.981 ($N = 7$, $P < 0.001$). In *Apeltes*, the number of caudal vertebrae is substantially elevated relative to the number of precaudal vertebrae. In *Hypoptychus*, the divergence is in the opposite direction. *Hypoptychus* exhibits an elevated number of precaudal vertebrae relative to the number of caudal vertebrae that it has. In both taxa, this deviation in the proportion of precaudal and caudal vertebrae is consistent with the divergence in external body proportions seen in the geometric morphometric analysis of body shape variation (Fig. 4).

Gasterosteus, *Culaea*, and *Pungitius*, which have broadly overlapping counts for the total number of vertebrae, exhibit an interesting pattern of divergence in the proportion of

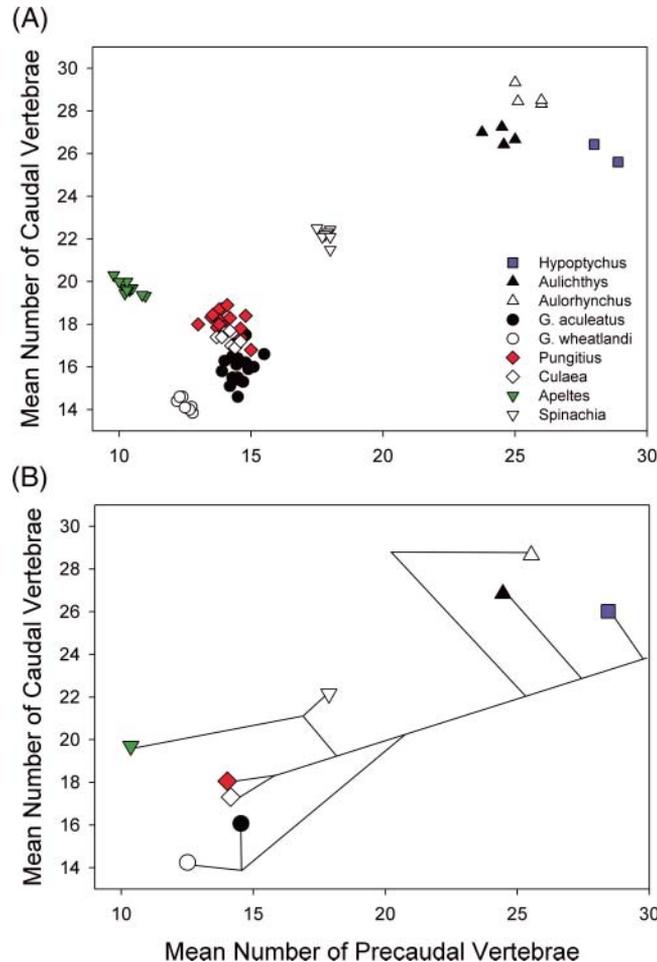


Fig. 6. Mean number of caudal vertebrae plotted against mean number of precaudal vertebrae. (A) Sample means plotted for all species. (B) Species means with the branching pattern of Kawahara and colleagues' (2009) phylogeny overlain on the plot.

precaudal to caudal vertebrae (Fig. 6B). The mean number of precaudal vertebrae is very similar across these three taxa, rising from 14.0 in *Pungitius* to 14.2 in *Culaea* and to 14.5 in *G. aculeatus* (Table 2). However, the mean number of caudal vertebrae differs much more across taxa, rising from 16.1 in *G. aculeatus* to 17.3 in *Culaea* and to 18.1 in *Pungitius*.

Body form–vertebral divergence

Body shape divergence in general, and extreme body elongation in particular, is strongly associated with divergence in vertebral phenotypes. Fish with longer bodies have more vertebrae. This is apparent in both the relationship between total vertebral number and the fineness ratio ($r = 0.88$, $N = 9$, $P = 0.002$) and in the relationship between pairwise body

shape and vertebral phenotype distance values (Fig. 7). However, there are some interesting deviations associated with particular taxa. *Hypoptychus* is an outlier. It does not follow the strong linear relationship seen between the fineness ratio and total vertebral number for the other taxa (Fig. 7A), having far too many vertebrae for the relative length of its body. Excluding *Hypoptychus*, the rest of the taxa exhibit a very strong linear correlation between

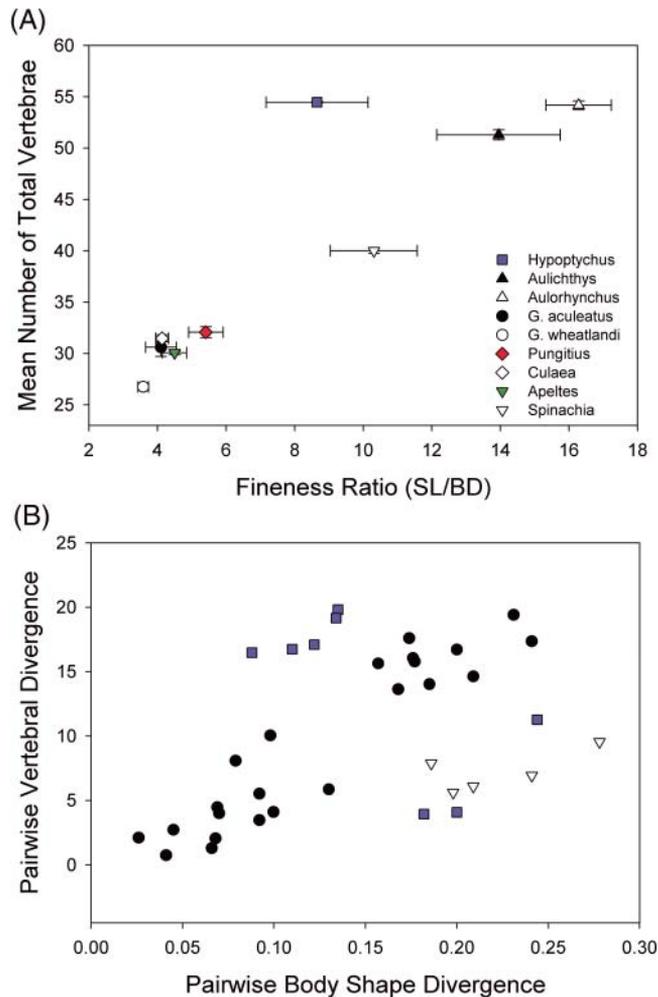


Fig. 7. (A) Mean number of total vertebrae plotted against mean fineness ratio (FR = standard length/body depth) for all species. Error bars are standard deviations calculated from sample means. (B) Relationship between body shape divergence and vertebral phenotype divergence from pairwise comparisons of all taxon means. Body shape divergence was computed from the pairwise Procrustes distance between taxon consensus configurations. Vertebral phenotype divergence was computed from the pairwise Euclidean distance between taxa based on the mean precaudal and caudal vertebral counts. Solid squares and inverted open triangles indicate perceived outliers. Solid squares indicate pairwise distances involving *Hypoptychus*. Inverted open triangles indicate pairwise distances between *Spinachia* and the other members of the family Gasterosteidae (*Apeltes*, *Culaea*, *G. aculeatus*, *G. wheatlandi*, and *Pungitius*).

body elongation as measured by the fineness ratio and total vertebral number ($r = 0.99$, $N = 8$, $P < 0.001$).

This association between body shape and vertebral divergence also held when the pairwise distances among taxa for each of these variables were compared (Fig. 7B). Including all taxa, pairwise distances in body shape and vertebral phenotypes are significantly correlated (Mantel test: $r = 0.452$, $P = 0.008$). However, *Hypoptychus* is an obvious outlier again. All pairwise comparisons between *Hypoptychus* and other taxa fall well off the linear relationship apparent between other pairwise comparisons (Fig. 7B). For three of the pairwise comparisons (*Hypoptychus*–*Aulichthys*, *Hypoptychus*–*Aulorhynchus*, and *Hypoptychus*–*Spinachia*), body shape divergence is too great relative to the divergence in vertebral counts seen. The pattern is the opposite for the other five pairwise comparisons involving *Hypoptychus* – body shape is too similar for the large difference in vertebral counts seen. Clearly, the relationship between body shape and vertebral number in *Hypoptychus* is different from that seen in the other taxa. Another five pairwise comparisons, all involving *Spinachia*, also appear to differ from the rest (Fig. 7B). For these comparisons, body shape divergence between *Spinachia* and the other five gasterosteids is too great relative to the difference in vertebral phenotypes seen.

Finally, mean standard length is strongly correlated with the mean number of total vertebrae across taxa ($r = 0.826$, $N = 9$, $P = 0.006$), consistent with the predictions of pleomerism (Fig. 8). Once again, *Hypoptychus* is an obvious outlier. Excluding it from the analysis, the correlation is almost perfectly linear, with the correlation coefficient increasing to 0.993 ($N = 8$, $P < 0.001$). The relationship between body length and total vertebral number was not statistically significant when using the maximum reported sizes from the literature (for all taxa: $r = 0.511$, $N = 9$, $P > 0.05$; excluding *Hypoptychus*: $r = 0.675$, $N = 8$, $P > 0.05$). Average standard length is also very strongly correlated with the fineness ratio, indicating that size and elongation covary in the Gasterosteidae, such that changes in body length appear to occur in conjunction with changes in body shape in this group (for all taxa: $r = 0.975$, $N = 9$, $P < 0.001$; excluding *Hypoptychus*: $r = 0.981$, $N = 8$, $P < 0.001$).

DISCUSSION

Variation in body elongation and body regionalization are the major axes of body shape divergence in the Gasterosteidae

Variation in body elongation is the major axis of body shape divergence seen among the Gasterosteidae. The PC axis associated with body elongation, PCI, accounted for a remarkable 70% of the variation in body shape among the 693 individuals included in the study. It is highly unusual for a single PC to account for this amount of variation in body shape. Similarly, the fineness ratio varied from an average of 3.6 in *Gasterosteus wheatlandi* to 16.3 in *Aulorhynchus flavidus*, or more than 4.5 times from the lowest to highest average values, indicating a tremendous amount of variation in body elongation. Although extreme, the variation in body elongation documented in the Gasterosteidae is consistent with the general trends seen in other fish groups. Body elongation has evolved repeatedly in vertebrates (e.g. Gans, 1975; Parra-Olea and Wake, 2001; Wiens and Slingluff, 2001; Ward and Brainerd, 2007) and tends to be an important source of variability in body shape across fishes. For example, Claverie and Wainwright (2014) found that variation in body elongation was the major axis of body shape divergence in an analysis spanning 2939 species in 56 families of reef fishes.

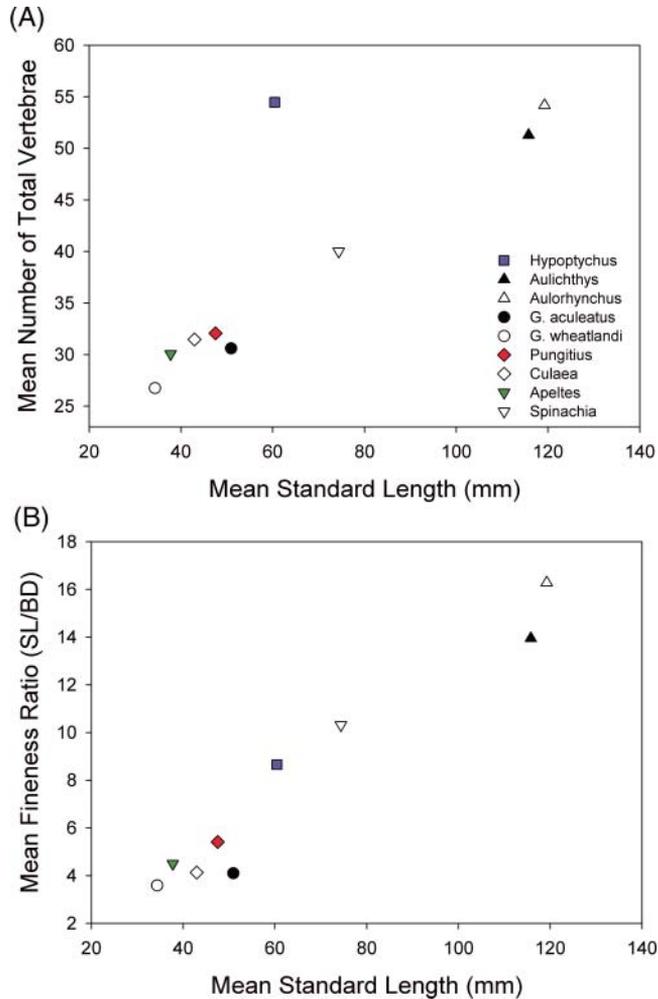


Fig. 8. (A) Mean number of total vertebrae plotted against mean standard length (SL). (B) Mean fineness ratio (FR) plotted against mean SL.

They also found that two-thirds of the families surveyed diversified primarily along an axis associated with body elongation. The variation in body elongation documented for the Gasterosteidae is likely associated with important differences in ecology among taxa, although the relationship between ecology and body elongation in fishes is not a simple one. Among reef fishes, elongate bodies evolve in groups adapting to both benthic and to open water habitats (Claverie and Wainwright, 2014). Great ecological diversity has also been found among species with similarly elongate bodies in an analysis of the Anguilliformes (Mehta *et al.*, 2010).

The polarity of evolution of body elongation within the group is also unclear. According to Kawahara *et al.* (2009), *Aulichthys* and *Aulorhynchus*, with their extreme body elongation, are basal within the Gasterosteidae while the Gasterosteinae are more recently derived, suggesting that the more typical fish-like bodies seen in some of the genera in the

Gasterosteidae may be derived from ancestors with elongate bodies. This is a long-standing hypothesis and has been suggested for decades (e.g. Wootton, 1976). Furthermore, *Gasterosteus*, a genus with a fairly typical body shape, is the most basal genus within the Gasterosteidae, suggesting that body elongation may be evolving again independently within the Gasterosteidae. Interestingly, *Spinachia* appears in a derived position within the Gasterosteidae, suggesting that instead of being a transitional form between *Aulorhynchus* and *Aulichthys* and the other gasterosteids to which it appears intermediate morphologically (e.g. Wootton, 1976), its elongate body is independently derived from the more typical body forms seen in other gasterosteids. Alternatively, it is possible that past extinctions and repeated parallel evolution of body form within the group, as seen among extant populations within species (e.g. Walker, 1997; Spoljaric and Reimchen, 2007; Aguirre and Bell, 2012), make it difficult to infer the polarity of body shape evolution in the Gasterosteidae without examining the fossil record.

The second PC axis, PCII, appeared to be associated primarily with body regionalization and fin placement. Both taxa exhibiting extreme body elongation and the taxa with more typical body shapes diverged in the relative expansion of the caudal vs. the precaudal regions along this axis. *Aulorhynchus*, *Aulichthys*, *Hypoptychus*, and *G. wheatlandi* exhibit an expanded precaudal region, whereas *Spinachia* and *Apeltes* exhibit a more expanded caudal region. Fish are known to harbour three distinct body regions: the head, the precaudal (or abdominal or trunk) and the caudal region (Ward and Brainerd, 2007; Claverie and Wainwright, 2014). These regions seem to be under independent genetic control (Ward and Mehta, 2010), and the relative size of these body regions is known to vary among lineages (e.g. Ward and Brainerd, 2007; Ward and Mehta, 2014). That species in the Gasterosteidae with both typical body shapes and extreme body elongation diverged along this ‘body regionalization’ axis suggests that changes in the proportions of the body regions are likely adaptively important across large portions of morphological and ecological space in fishes.

Vertebral number variation in the Gasterosteidae is marked and associated with changes in the number of precaudal and caudal vertebrae

Consistent with the extensive variation in body shape seen in this group, the Gasterosteidae exhibit substantial variation in number of vertebrae. Mean vertebral number differs by almost 30 vertebrae between the species with the lowest mean (26.8 for *G. wheatlandi*) and the highest mean (54.5 for *Hypoptychus dybowskii*). This increase in total vertebral number is associated with increases in both the number of precaudal and caudal vertebrae. For actinopterygians and chondrichthyans, Ward and Mehta (2014) found that changes in body elongation are associated most often with changes in the number of caudal vertebrae. However, they noted that there is substantial variability among lineages. Mehta *et al.* (2010) also found diverse patterns of variation in vertebral number among major lineages within the Anguilliformes, with some primarily adding caudal vertebrae (Muraenids), and others adding precaudal and caudal vertebrae equally with elongation (Ophichthids and Congrids). The Gasterosteidae would seem to fall in line with the latter.

Within the Gasterosteidae, there is an interesting pattern of divergence in mean vertebral counts among *G. aculeatus*, *Pungitius*, and *Culaea* (Fig. 6), which are among the most similar taxa in terms of body shape and total vertebral number. Although means for the number of precaudal vertebrae are quite similar (differences of 0.2–0.3 between species), these taxa differ appreciably in the mean number of caudal vertebrae, with the mean

difference between *Gasterosteus* and *Culaea* being 1.2 vertebrae and between *Culaea* and *Pungitius* 0.8 vertebrae. This mirrors the major pattern of divergence in vertebral number seen in ecologically diverse populations of *G. aculeatus* (Aguirre *et al.*, 2014), in which more elongate limnetic populations exhibit a significant increase in the mean number of caudal vertebrae relative to deep-bodied benthic and ancestral anadromous populations, while the number of precaudal vertebrae was similar or lower. Thus there seems to be some alignment in this respect between microevolutionary and macroevolutionary axes of divergence. The caudal region is very important for swimming via caudal propulsion as well as for the bending of the body during burst swimming (Domenici and Blake, 1997; Ward and Mehta, 2014). Variation in the caudal region is often associated with divergence in body form across fishes (Ward and Mehta, 2014) and further studies on the causes and consequences of variation in the number of caudal vertebrae in the Gasterosteidae appear warranted.

Body form and vertebral number variation are strongly associated

As seen across fishes (Ward and Brainerd, 2007; Ward and Mehta, 2010), variation in body elongation was strongly associated with variation in vertebral number in the Gasterosteoidae. Excluding *Hypoptychus*, which was an outlier, the correlation between the fineness ratio and mean total vertebral number was a remarkable 0.990, indicating an almost perfect association between body elongation and vertebral number. Vertebral number was also very strongly correlated with overall body size, as measured by mean standard length, with *Hypoptychus* again being an outlier. Excluding it from the analysis, the correlation coefficient was 0.993. Vertebral number variation in the Gasterosteoidae is thus also consistent with pleomerism, the increase in vertebral number with body size (Lindsey, 1975; McDowall, 2004). However, body size and body elongation are very strongly correlated in the Gasterosteoidae ($r = 0.975$), such that species that are larger are also more elongate. This relationship even held when *Hypoptychus* was included. Because of this strong association between size and shape, it is not possible to tease these factors apart when examining the causes of change in vertebral number in the Gasterosteoidae. Body size and shape are confounded in the group, at least among the living species.

Beyond the association between body size, elongation, and vertebral number, differences in the relative size of the precaudal and caudal body regions generally also track changes in vertebral number. Changes in body shape may be associated with changes in vertebral number, length, or both (Ward and Brainerd, 2007), so an expansion of the precaudal or caudal region of the body does not necessarily have to be accompanied by a corresponding increase in vertebral number. Classic examples include the constant number of cervical vertebrae in mammals with very different neck lengths such as cetaceans and giraffes (e.g. Varela-Lasheras *et al.*, 2011), and the large differences in vertebral number in different clades of salamanders that have independently evolved, similarly elongate bodies (Wake and Larson, 1987; Parra-Olea and Wake, 2001). However, across the Gasterosteoidae as a whole, there is a clear tracking of body region size and vertebral number. Species with expansions of a particular body region in the geometric morphometric analysis exhibited an increase in the number of vertebrae in that body region. The same pattern occurs intraspecifically in ecologically divergent populations of the threespine stickleback; differences in external body form in elongate limnetics vs. deeper bodied benthic or anadromous populations clearly track changes in vertebral number in the corresponding body region (Aguirre *et al.*, 2014). This is another way in which microevolutionary and macroevolutionary patterns of divergence align in the

Gasterosteoidaei. However, the association was not perfect and there is some incongruence between body form and vertebral number.

Hypoptychus is an outlier for all measures that include vertebral counts. Commonly known as the sand eel (Nelson, 2006) or Korean sandlance (Froese and Pauly, 2015), it is a marine species native to Japan and Korea (Nelson, 2006). *Hypoptychus* is divergent phenotypically from the other members of the Gasterosteoidaei and was often overlooked as a potential relative of the Gasterosteidae in historical treatments of their phylogenetic relationships (e.g. Wootton, 1976). Its relationship to the Gasterosteoidaei was established by Ida (1976), who moved it from the Ammodytoidei, which includes the sand lances, to the Gasterosteoidaei based on an analysis of osteological and reproductive characteristics. Recent molecular phylogenies are confirming its place in the Gasterosteoidaei (Kawahara *et al.*, 2008; Betancur-R *et al.*, 2013), and Kawahara *et al.* (2009) list it as the most basal species in this group. However, it is clearly quite distinctive in its vertebral phenotype. Not only does it have too many precaudal vertebrae for the number of caudal vertebrae relative to the other members of the Gasterosteoidaei (Fig. 6A), it also has too many vertebrae for both its body elongation and its size (Figs. 7, 8A). In fact, it has the highest mean vertebral count in the study, more than *Aulorhynchus flavidus* and *Aulichthys japonicus*. Inasmuch as having more vertebrae makes the body more flexible (e.g. Brainerd and Patek, 1998), *Hypoptychus* must have a highly flexible body. This may be related to its ecology. Although detailed ecological studies for this species are lacking, its superficial morphological similarity to the sand lances (Ammodytidae) may provide some clues. Sand lances are burrowing fish that have high vertebral counts (Gosline, 1963) and highly flexible bodies that allow them to quickly bury themselves in soft substrate (Robards and Piatt, 1999). Burrowing is associated with an increase in both body elongation and vertebral number across vertebrates (Gans, 1975), and expansion of the trunk (precaudal) region is particularly common in elongate burrowing tetrapods (Wiens and Slingluff, 2001). If *Hypoptychus* commonly displays burrowing behaviour like the sand lances, its high vertebral counts may be the product of convergent evolution towards a similar ecological niche.

In the analysis of the relationship between pairwise distances in body shape and vertebral number, like *Hypoptychus*, *Spinachia* also appears to be an outlier relative to the other taxa (Fig. 7B). The pairwise distances in vertebral number between it and all the other members of the family Gasterosteidae are too low relative to the body shape distances computed. That is, the precaudal and caudal vertebral counts in *Spinachia* seem too similar to those of the other gasterosteids relative to the divergence in body shape that this species exhibits. Indeed, in vertebral number, *Spinachia* is more similar to the other gasterosteids than to *Aulorhynchus* or *Aulichthys*. The minimum difference between *Spinachia* and the gasterosteid with the next highest vertebral count, *Pungitius*, was only three vertebrae (Table 2). This difference in vertebral number does not do justice to the large difference in body shape between *Spinachia* and all the other gasterosteids, including *Pungitius*. *Spinachia* exhibits fairly extreme body elongation. The significance of this discrepancy between body shape and vertebral phenotype distances in *Spinachia* is unclear. The general trend across the Gasterosteoidaei is consistent with body form–vertebral phenotype co-evolution. However, it is possible that body shape and vertebral phenotypes may evolve at different rates, such that one has a tendency to lead the other as species evolve into different portions of morphological space. Differences in genetic variation, genetic architecture, or the strength of selection on these traits could cause discrepancies in the rates at which they evolve (e.g. Schluter, 2000; West-Eberhard, 2003). The body shape and vertebral data for *Spinachia* suggest that if discrepancies do exist in the rate of evolution of these traits in the

Gasterosteioidei, it would be body shape that evolves at a faster rate. This may well be the case at the microevolutionary scale. Many studies have reported repeated parallel evolution of large differences in body shape between postglacial populations of the threespine stickleback (e.g. Walker, 1997; Spoljaric and Reimchen, 2007; Berner *et al.*, 2009; Aguirre and Bell, 2012). However, studies of divergence in vertebral phenotypes report relatively small differences in vertebral number, on the order of approximately 0.5–1 vertebrae, among ecologically and morphologically divergent populations (Reimchen and Nelson, 1987; Ahn, 1998; Aguirre *et al.*, 2014).

Future directions

The patterns documented in this study raise many questions. First, what is the functional significance of the phenotypic differences observed? The existence of highly divergent, well-characterized populations within some of the species complexes, such as *G. aculeatus* and *P. pungitius*, provides the opportunity to examine the earliest stages of the evolution of body form and the axial skeleton, and some progress on this front has already been made (e.g. Reimchen and Nelson, 1987; Swain, 1992; Ahn, 1998; Shikano and Merilä, 2011; Aguirre *et al.*, 2014). Results of tests of swimming performance and habitat use in these microevolutionary studies could then be used to inform studies between increasingly more phenotypically and evolutionarily divergent taxa. Comparative experiments along these lines have the potential to provide important insight into the process of macroevolutionary diversification couched within a microevolutionary framework that is extraordinarily rich given the great intraspecific diversity that exists within some species. Second, what is the developmental and genetic basis of phenotypic diversification in the Gasterosteioidei? How do body plans as different as those of *Gasterosteus* and *Spinachia* arise? Although these genera have likely been on independent evolutionary trajectories for tens of millions of years, they share the same basic developmental machinery inherited from a common ancestor that existed not long ago on a geological time scale. Comparative analysis of their development, especially of somitogenesis, may provide great insight into the evolution of vertebrate body plans.

ACKNOWLEDGEMENTS

This paper would not have been possible without the assistance of many people. We are grateful to Andrew Hendry and Katie Peichel for the invitation to submit the paper, and to Mike Bell, Matt Wund, and Peter Park for organizing the Eighth International Conference on Stickleback Behaviour and Evolution. We thank the curators, collection managers, and staff at the many museums that loaned us specimens for this study (2989Appendix.pdf), and to the many collectors for their efforts in the field. We are particularly grateful to the staff at the Field Museum – Kevin Swagel, Susan Mochel, and Caleb McMahan – for being generous hosts during our visits and providing assistance with the X-raying of all the specimens in the study. Mike Bell, John Baker, Paul Seear, and Tina Henrich were extraordinarily generous in providing additional samples for species from regions that were not well represented in museum collections. We thank the College of Science and Health's Graduate Research Fund program and the Department of Biological Sciences at DePaul University for funding.

REFERENCES

- Aguirre, W.E. and Bell, M.A. 2012. Twenty years of body shape evolution in a threespine stickleback population adapting to a lake environment. *Biol. J. Linn. Soc.*, **105**: 817–831.
- Aguirre, W.E., Walker, K. and Gideon, S. 2014. Tinkering with the axial skeleton: vertebral number

- variation in ecologically divergent threespine stickleback populations. *Biol. J. Linn. Soc.*, **113**: 204–219.
- Ahn, D. 1998. Factors controlling axial variation in the threespine stickleback, *Gasterosteus aculeatus* (Teleostei: Gasterosteidae): pattern of natural variation and genetic/developmental mechanisms. Unpublished DPhil thesis, University of Michigan.
- Ahn, D. and Gibson, G. 1999. Axial variation in threespine stickleback fish. *Evol. Dev.*, **1**: 100–112.
- Bell, M.A. 1976. Evolution of phenotypic diversity in the *Gasterosteus aculeatus* superspecies on the Pacific coast of North America. *Syst. Zool.*, **25**: 211–227.
- Bell, M.A. and Aguirre, W.E. 2013. Contemporary evolution, allelic recycling, and adaptive radiation of the threespine stickleback. *Evol. Ecol. Res.*, **15**: 377–411.
- Bell, M.A. and Foster, S.A., eds. 1994. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press.
- Berner, D., Grandchamp, A.C. and Hendry, A.P. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake–stream transitions. *Evolution*, **63**: 1740–1753.
- Betancur-R, R., Broughton, R.E., Wiley, E.O., Carpenter, K., López, J.A., Li, C. *et al.* 2013. The tree of life and a new classification of bony fishes. *PLOS Currents: Tree of Life* (DOI: 10.1371/currents.tol.53ba26640df0ccea75bb165c8c26288).
- Bowne, P.S. 1994. Systematics and morphology of the Gasterosteiformes. In *The Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 28–60. Oxford: Oxford University Press.
- Brainerd, E.L. and Patek, S.N. 1998. Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in tetraodontiform fishes. *Copeia*, **1998**: 971–984.
- Buth, D.G. and Haglund, T.R. 1994. Allozyme variation in the *Gasterosteus aculeatus* complex. In *The Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 61–84. Oxford: Oxford University Press.
- Cassidy, L.M., Ravinet, M., Mori, S. and Kitano, J. 2013. Are Japanese freshwater populations of threespine stickleback derived from the Pacific Ocean lineage? *Evol. Ecol. Res.*, **15**: 295–311.
- Claverie, T. and Wainwright, P.C. 2014. A morphospace for reef fishes: elongation is the dominant axis of body shape evolution. *PLOS One*, **9**: e112732.
- Domenici, P. and Blake, R.W. 1997. The kinematics and performance of fish fast-start swimming. *J. Exp. Biol.*, **200**: 1165–1178.
- Froese, R. and Pauly, D., eds. 2015. *FishBase*. World Wide Web electronic publication [www.fishbase.org, v.10/2015].
- Gans, C. 1975. Tetrapod limblessness: evolution and functional corollaries. *Am. Zool.*, **15**: 455–467.
- Gosline, W.A. 1963. Notes on the osteology and systematic position of *Hypoptychus dybowskii* Steindachner and other elongate perciform fishes. *Pac. Sci.*, **17**: 90–101.
- Hammer, O., Harper, D.A.T. and Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.*, **4**: 1–9.
- Hendry, A.P., Bolnick, D.I., Berner, D. and Peichel, C.L. 2009. Along the speciation continuum in sticklebacks. *J. Fish Biol.*, **75**: 2000–2036.
- Hendry, A.P., Peichel, C.L., Matthews, B., Boughman, J.W. and Nosil, P. 2013. Stickleback research: the now and the next. *Evol. Ecol. Res.*, **15**: 111–141.
- Higuchi, M., Sakai, H. and Goto, A. 2014. A new threespine stickleback, *Gasterosteus nipponicus* sp. nov. (Teleostei: Gasterosteidae), from the Japan Sea region. *Ichthyol. Res.*, **61**: 341–351.
- Hubbs, C.L. 1929. The Atlantic American species of the fish genus *Gasterosteus*. *Occas. Pap. Mus. Zool. Univ. Mich.*, **200**: 1–9.
- Ida, H. 1976. Removal of the family Hypoptychidae from the suborder Ammodytoidei, order Perciformes, to the suborder Gasterosteidae, order Sygnathiformes. *Jpn. J. Ichthyol.*, **23**: 33–42.
- Kawahara, R., Miya, M., Mabuchi, K., Lavoue, S., Inoue, J.G., Satoh, T.P. *et al.* 2008. Interrelationships of the 11 gasterosteiform families (sticklebacks, pipefishes, and their relatives): a

- new perspective based on whole mitogenome sequences from 75 higher teleosts. *Mol. Phylogenet. Evol.*, **46**: 224–236
- Kawahara, R., Miya, M., Mabuchi, K., Near, T.J. and Nishida, M. 2009. Stickleback phylogenies resolved: evidence from mitochondrial genomes and 11 nuclear genes. *Mol. Phylogenet. Evol.*, **50**: 401–404.
- Li, H.W. and Li, J.L. 2006. Role of fish assemblages in stream communities. In *Methods in Stream Ecology* (F.R. Hauer and G.A. Lamberti, eds.), pp. 489–514. Burlington, MA: Academic Press.
- Lindell, L.E. 1994. The evolution of vertebral number and body size in snakes. *Funct. Ecol.*, **8**: 708–719.
- Lindsey, C.C. 1975. Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. *J. Fish. Res. Board Can.*, **32**: 2453–2469.
- Mattern, M.Y. 2007. Phylogeny, systematics, and taxonomy of sticklebacks. In *Biology of the Three-Spined Stickleback* (S. Östlund-Nilsson, I. Mayer and F.A. Huntingford, eds.), pp. 1–40. Boca Raton, FL: CRC Press.
- McDowall, R.M. 2004. Variation in vertebral number in galaxiid fishes, how fishes swim and a possible reason for pleomerism. *Rev. Fish Biol. Fish.*, **13**: 247–263.
- McDowall, R.M. 2008. Jordan's and other ecogeographical rules, and the vertebral number in fishes. *J. Biogeogr.*, **35**: 501–508.
- McKinnon, J.S. and Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.*, **17**: 480–488.
- Mehta, R.S., Ward, A.B., Alfaro, M.E. and Wainwright, P.C. 2010. Elongation of the body in eels. *Integr. Comp. Biol.*, **50**: 1091–1105.
- Nelson, J.S. 2006. *Fishes of the World*. Hoboken, NJ: Wiley.
- Nelson, J.S. and Atton, F.M. 1971. Geographic and morphological variation in the presence of the pelvic skeleton in the brook stickleback, *Culaea inconstans* (Kirtland), in Alberta and Saskatchewan. *Can. J. Zool.*, **49**: 343–352.
- Östlund-Nilsson, S., Mayer, I. and Huntingford, F.A., eds. 2007. *Biology of the Three-Spined Stickleback*. Boca Raton, FL: CRC Press.
- Parra-Olea, G. and Wake, D.B. 2001. Extreme morphological and ecological homoplasy in tropical salamanders. *Proc. Natl. Acad. Sci. USA*, **98**: 7888–7891.
- Reece, J.S. and Mehta, R.S. 2013. Evolutionary history of elongation and maximum body length in moray eels (Anguilliformes: Muraenidae). *Biol. J. Linn. Soc.*, **109**: 861–875.
- Reid, D.T. and Peichel, C.L. 2010. Perspectives on the genetic architecture of divergence in body shape in sticklebacks. *Integr. Comp. Biol.*, **50**: 1057–1066.
- Reimchen, T.E. 1994. Predators and morphological evolution in threespine stickleback. In *The Evolutionary Biology of the Threespine Stickleback* (M.A. Bell and S.A. Foster, eds.), pp. 240–276. Oxford: Oxford University Press.
- Reimchen, T.E. and Nelson, J.S. 1987. Habitat and morphological correlates to vertebral number as shown in a teleost, *Gasterosteus aculeatus*. *Copeia*, **1987**: 868–874.
- Reimchen, T.E., Bergstrom, C. and Nosal, P. 2013. Natural selection and the adaptive radiation of Haida Gwaii stickleback. *Evol. Ecol. Res.*, **15**: 241–269.
- Ricklefs, R.E. and Miles, D.B. 1994. Ecological and evolutionary inferences from morphology: an ecological perspective. In *Ecological Morphology* (P.C. Wainwright and S.M. Reilly, eds.), pp. 13–41. Chicago, IL: University of Chicago Press.
- Robards, M.D. and Piatt, J.F. 1999. Biology of the genus *Ammodytes*, the sand lances. In *Sand Lance: A Review of Biology and Predator Relations and Annotated Bibliography*, Res. Pap. PNW-RP-521 (M.D. Robards, M.F. Willson, R.H. Armstrong and J.F. Piatt, eds.), pp. 1–181. Portland, OR: US Department of Agriculture, Forest Service, Pacific Northwest Research Station.
- Rohlf, F.J. 2004. *Thin-Plate Spline, Version 1.20*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook [<http://life.bio.sunysb.edu/morph/>].

- Rohlf, F.J. 2013a. *TpsDig, Version 2.17*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook [http://life.bio.sunysb.edu/morph/].
- Rohlf, F.J. 2013b. *Relative Warps, Version 1.53*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook [http://life.bio.sunysb.edu/morph/].
- Rosenberg, M.S. and Anderson, C.D. 2011. PASSaGE: pattern analysis, spatial statistics, and geographic exegesis, Version 2. *Meth. Ecol. Evol.*, **2**: 229–232.
- Schluter, D. 1996. Ecological speciation in postglacial fishes. *Phil. Trans. R. Soc. Lond. B*, **351**: 807–814.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford: Oxford University Press.
- Shikano, T. and Merilä, J. 2011. Body size and the number of vertebrae in the nine-spined stickleback (*Pungitius pungitius*). *Biol. J. Linn. Soc.*, **104**: 378–385.
- Spoljaric, M.A. and Reimchen, T.E. 2007. 10 000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. *J. Fish Biol.*, **70**: 1484–1503.
- Swain, D.P. 1992. Selective predation for vertebral phenotype in *Gasterosteus aculeatus*: reversal in the direction of selection at different larval stages. *Evolution*, **46**: 998–1013.
- Van Damme, R. and Vanhooydonck, B. 2002. Speed versus manoeuvrability: association between vertebral number and habitat structure in lacertid lizards. *J. Zool.*, **258**: 327–334.
- Varela-Lasheras, I., Bakker, A.J., van der Mije, S.D., Metz, J.A.J., van Alphen, J. and Galis, F. 2011. Breaking evolutionary and pleiotropic constraints in mammals: on sloths, manatees and homeotic mutations. *EvoDevo*, **2**: 11.
- Wainwright, P.C. 1991. Ecomorphology: experimental functional anatomy for ecological problems. *Am. Zool.*, **31**: 680–693.
- Wake, D.B. and Larson, A. 1987. Multidimensional analysis of an evolving lineage. *Science*, **238**: 42–48.
- Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.*, **61**: 3–50.
- Walker, J.A., Alfaro, M.E., Noble, M.M. and Fulton, C.J. 2013. Body fineness ratio as a predictor of maximum prolonged-swimming speed in coral reef fishes. *PLoS One*, **8**: e75422.
- Wang, C., Shikano, T., Persat, H. and Merilä, J. 2015. Mitochondrial phylogeography and cryptic divergence in the stickleback genus *Pungitius*. *J. Biogeogr.*, **42**: 2334–2348.
- Ward, A.B. and Brainerd, E.L. 2007. Evolution of axial patterning in elongate fish. *Biol. J. Linn. Soc.*, **90**: 97–116.
- Ward, A.B. and Mehta, R.S. 2010. Axial elongation in fishes: using morphological approaches to elucidate developmental mechanisms in studying body shape. *Integr. Comp. Biol.*, **50**: 1106–1119.
- Ward, A.B. and Mehta, R.S. 2014. Differential occupation of axial morphospace. *Zoology*, **117**: 70–76.
- Webb, P.W. 1984. Form and function in fish swimming. *Sci. Am.*, **251**: 71–82.
- West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford: Oxford University Press.
- Wiens, J.J. and Slingluff, J.L. 2001. How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguillid lizards. *Evolution*, **55**: 2302–2318.
- Wootton, R.J. 1976. *The Biology of the Sticklebacks*. London: Academic Press.
- Wootton, R.J. 2009. The Darwinian stickleback *Gasterosteus aculeatus*: a history of evolutionary studies. *J. Fish Biol.*, **75**: 1919–1942.
- Zelditch, M.L., Swiderski, D.L. and Sheets H.D. 2012. *Geometric Morphometrics for Biologists: A Primer*, 2nd edn. Oxford: Elsevier Academic Press.