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Convergent evolution of the head retraction escape response in elongate fishes and amphibians

Andrea B. Ward*, Emanuel Azizi

Organismic and Evolutionary Biology, University of Massachusetts Amherst, 205 Morrill Science Center, 611 North Pleasant Street, Amherst, MA 01003, USA

Abstract

Aquatic escape responses have typically been described as C-starts. However, another aquatic escape response, head retraction, occurs in several elongate species, but has never been studied in detail. The goals of this study are to describe the head retraction escape response in a phylogenetically diverse sample of species, to trace the evolution of head retraction in anamniote vertebrates, and to correlate key morphological traits with escape response behavior. In analyzing the evolution of escape behavior, we found that the head retraction escape response has evolved at least six times in anamniote vertebrates. Using independent contrast analysis, the head retraction escape response was found to be correlated with an increase in the total number of vertebrae and an increase in the elongation of an animal. Results from this study indicate a correlation between head retraction as an escape response, elongation of the axial skeleton, and living in structured habitats.
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Introduction

Aquatic escape responses in vertebrates typically have been described as C-starts and are characterized by two distinct stages. In stage one, the head and tail turn away from the predator, causing the body to form a "C" (Fig. 1a). The body is then propelled away from the stimulus as this bend is propagated caudally, generating an undulatory wave that defines stage two (Fig. 1a). Owing to the stereotyped nature of the behavior and the ease of eliciting C-starts in the laboratory, data on escape response performance and neural control are readily available in the literature (e.g., Fetcho and Faber, 1988; Eaton et al., 1991; Hale et al., 2002). In addition, kinematic data from escape responses are available from a diverse sample of anamniote vertebrates including *Protopterus*, *Latimeria*, and numer-

ous actinopterygian fishes (e.g., Fricke et al., 1987; Domenici and Blake, 1997; Meyers et al., 1998).

Although C-starts have been the central focus of most studies of aquatic escape responses, other escape behaviors have been described in a few species. S-starts have been documented in *Esox* spp. and *Cyprinus carpio* (Harper and Blake, 1990; Spiers and van Leeuwen, 1999; Hale, 2002). The S-start response is characterized by the presence of two bends along the length of the body, which bend the body into an "S" shape during the initial phase of the response (Fig. 1b). During the propulsive phase of the response, the fish swims away from the stimulus by passing the bends down the body (Hale, 2002). This behavior is generally considered to be distinct from C-starts and is thought to be controlled by a different neural mechanism (Hale, 2002).

In addition to C- and S-starts, a few authors have noted the use of a withdrawal or retraction-type startle response in some elongate fish species. Eaton et al.

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*Corresponding author.

E-mail address: award@bio.umass.edu (A.B. Ward).
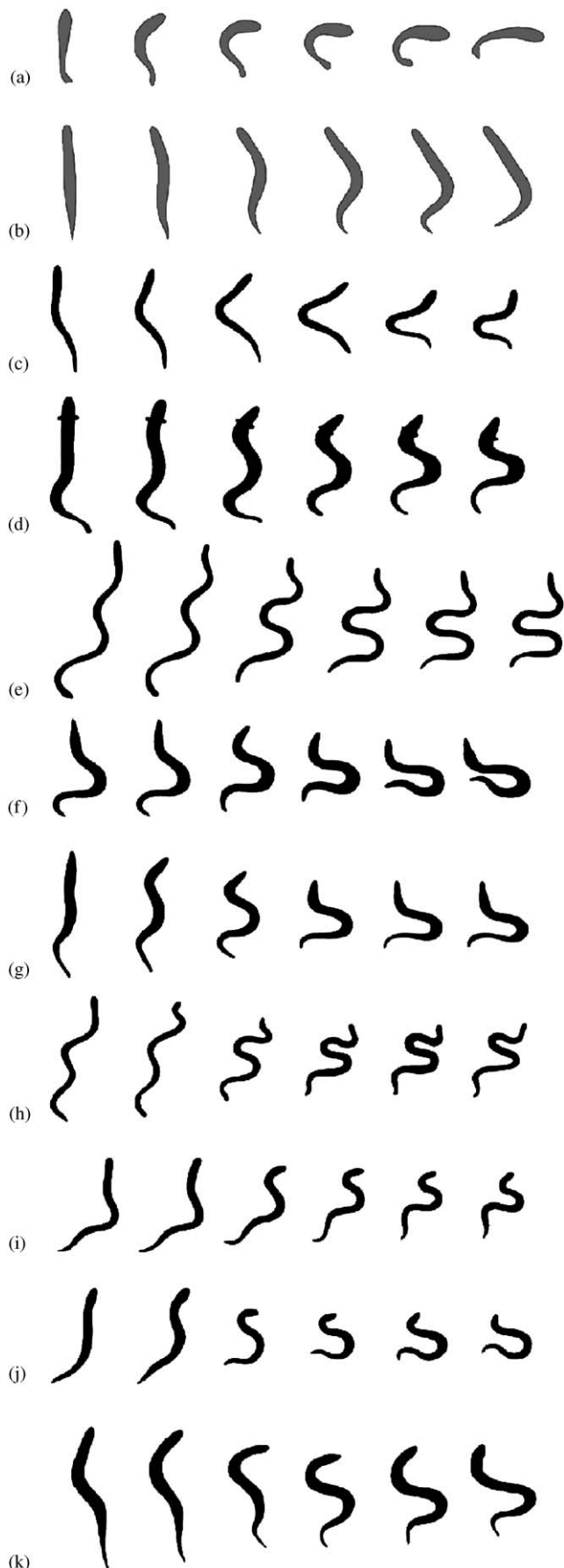
(1977) observed that the escape response of the spiny eel (*Mastacembelus loennbergi*) differs from C-starts in that the head is retracted and the propulsive phase is absent. This response has also been observed in American eels (*Anguilla rostrata*), larval lamprey (*Petromyzon marinus*), and the ropefish (*Erpetoichthys calabaricus*) (Currie and Carlsen, 1985; Meyers et al., 1998; Bierman et al., 2002). Studies by Currie and Carlsen (1985, 1987) have shown that the head retraction escape response of a larval lamprey is characterized by bilateral bending of the body resulting in a shape similar to the Greek letter Omega (Ω) (for an example, see Fig. 1c). Although this alternate startle behavior has been noted in a few species, little comparative data exist on the function, mechanics, or evolution of head retraction.

The first goal of this study is to describe the startle responses of nine species of elongate fishes and amphibians. The description of escape behaviors in multiple taxa will allow us to document whether head retraction is a widespread startle response in elongate aquatic vertebrates. This comparative approach will also highlight common characteristics of head retraction across a wide range of taxa, which will provide the framework for testing comparative and phylogenetic hypotheses.

The second goal is to combine the comparative survey of head retraction in anamniote vertebrates with previous studies of C-starts in order to reconstruct the phylogenetic history of aquatic escape behavior. Based on previous descriptions of head retraction in distantly related species (lamprey, American eels, and spiny eels) and the preponderance of C-start performing species within aquatic vertebrates, we hypothesize that head retraction is a convergent behavior. In this analysis, we use data from 25 terminal taxa and a vertebrate phylogeny with a well-accepted topology. This study expands the analysis conducted by Hale et al. (2002) in which character optimization was used to reconstruct the nodal character states of startle response type, motor patterns, and presence or absence of Mauthner Cells.

The final goal of this study is to correlate startle response behavior with two morphological characters. Here we focus on body shape (elongation ratio) and the total number of vertebrae present as potential factors in

Fig. 1. Axial movements during startle responses shown in dorsal view: (a) Silhouettes of *Carrasius auratus* during a C-start (modified from Eaton et al., 1977). (b) Silhouettes of *Esox musquinogy* during an S-start (modified from Hale, 2002). (c–k) Representative silhouettes of study organisms during a head retraction startle response. (c) Larval *P. marinus* shown at 16 ms intervals. (d) *A. rostrata* shown at 36 ms intervals. (e) *G. polyuranodon* shown at 24 ms intervals. (f) *M. siamensis* shown at 24 ms intervals. (g) *M. armatus* shown at 24 ms intervals. (h) *T. natans* shown at 64 ms intervals. (i) *A. tridactylum* shown at 32 ms intervals. (j) *S. intermedia* shown at 32 ms intervals. (k) *L. paradoxa* shown at 28 ms intervals. In each response, the stimulus was applied from the left.

the evolution of startle response behavior. Previous work by Meyers et al. (1998) indicates that elongation may be associated with a head retraction response. We hypothesize that head retraction will be correlated with an increase in body elongation and total number of vertebrae. To assess the correlation between behavior and morphology within a phylogenetic framework, we used an independent contrast analysis. Independent contrast analysis allows the comparison of phylogenetically corrected values (contrasts) for each of the morphological parameters in order to assess the correlation between behavior and morphology (Felsenstein, 1985). For this analysis, morphological parameters are treated as continuous and startle response behavior (head retraction or C-starts) as dichotomous (Grafen, 1989; Garland et al., 1992; Purvis and Rambaut, 1995).

Materials and methods

Study animals

Larval *P. marinus* were collected in the Connecticut River, Holyoke, Massachusetts. All other species used in this study were purchased from commercial vendors. *Gymnothorax polyuranodon* (TL = 31.5 cm), *Macrognathus siamensis* (TL = 15.9 cm) and *Mastacembelus armatus* (TL = 23.0 cm) were housed in glass aquaria and kept at $27 \pm 1\,°C$. *Amphiuma tridactylum* (TL = 29.5 cm), *Anguilla rostrata* (TL = 32.3 cm), *Lepidosiren paradoxa* (TL = 19.0 cm), *Petromyzon marinus* (TL = 13.7 cm), *Siren intermedia* (TL = 24.6 cm), and *Typhlonectes natans* (TL = 42.2 cm) were housed in glass aquaria and kept at $24 \pm 1\,°C$. All individuals were fed earthworms 1–2 times per week with the exception of *P. marinus*, which were fed ground tropical fish flakes.

Behavioral description

This description of head retraction is based on at least 14 observations of escape responses from one individual of the following nine species (number of head retraction trials): *A. rostrata* (20), *A. tridactylum* (18), *G. polyuranodon* (14), *L. paradoxa* (18), *M. siamensis* (19), *M. armatus* (16), *P. marinus* (19), *S. intermedia* (20), and *T. natans* (24) (Figs. 1c–k). Additional individuals of *A. rostrata, A. tridactylum, M. siamensis, M. armatus, P. marinus,* and *T. natans* were also observed to confirm characteristic movements of head retraction. Animals were placed in a deep plastic aquarium for filming. *T. natans* was filmed in a tank measuring 49 × 59 cm and all other species were filmed in a tank measuring 39 × 33 cm. The water temperature was adjusted to match the housing temperature for each species. All animals were acclimated to the filming tank for at least 10 min prior to observations.

Escape responses were recorded in dorsal view at 250 frames/s using a Kodak EktaPro high-speed video system. *P. marinus*, *G polyuranodon*, *A. rostrata*, *Mastacembelus armatus*, and *M. siamensis* were startled by tapping the wall closest to the animals' resting position with a metal file. Similar to previous studies, lungfishes and amphibians were startled by touching the pectoral girdle with a glass rod (Azizi and Landberg, 2002). All stimuli were directed laterally towards the animal. All species in this study were filmed under similar conditions to previous C-start species (Azizi and Landberg, 2002; Refs. in Domenici and Blake, 1997).

Videos of escape responses were analyzed using NIH Image v1.62 (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). In all responses, the tip of the snout was traced through each frame in order to track the movement of the head throughout the response. Two angle measurements were calculated to quantitatively describe angular head movements. Maximum head angle was defined as the maximum deflection of the head from the pre-stimulus position. Final head angle was defined as the difference in head angle at the end of the response relative to the pre-stimulus position of the head. Final head angle is similar to escape trajectory angle, which has been used in C-starts (Hale, 1999). Polar statistics were used to calculate the means and 95% confidence intervals of maximum head angle and final head angle for each species as well as for all head retraction responses (Zar, 1996).

Phylogenetic analysis of behavior

The vertebrate phylogeny used for our comparative analysis of startle response behavior is generally well accepted (Lauder and Liem, 1983; Benton, 1990; Nelson, 1994; Cloutier and Ahlberg, 1996; Forey et al., 1996; Grande and Bemis, 1996; Gao and Shubin, 2001). When discrepancies between phylogenies occurred, we elected to use the phylogeny with the greatest resolution. Since no strong consensus exists concerning the phylogenetic relationships within Caudata, the group was left as a polytomy (Gao and Shubin, 2001).

Character optimization was used to reconstruct the aquatic escape behavior within Vertebrata using data from 25 species. This technique allowed us to determine whether head retraction evolved independently among the taxa examined. Each species used in the analysis was coded for startle response behavior (C-start, S-start, or head retraction). If a species exhibited two responses, the behavior was coded as polymorphic. Character states were unordered. Behavior was optimized using MacClade 4.05 (Maddison and Maddison, 2002).

We used independent contrast analysis to investigate possible correlations between morphological characters

and escape response behaviors. Behavior was coded as the independent variable and was regressed against elongation ratio and the total number of vertebrae. Behavior was considered a dichotomous variable (C-start or head retraction) and the most common escape behavior was selected for each species. Only two states were used for the independent contrast analysis because it is unknown how the potential character states should be ordered. The BRUNCH algorithm (CAIC v2.6.9) was used to calculate correlations between behavior and morphometric variables (Purvis and Rambaut, 1995; Nunn and Barton, 2001). Using this conservative algorithm, contrasts are calculated only when there is a transition between character states of the discrete variable (for this analysis, escape response behavior). Branch lengths were included to increase the accuracy of the analysis and were estimated from the earliest recorded fossil for a particular clade (Appendix A). The analysis was also run with branch lengths set to unity. Diaz-Uriarte and Garland (1998) describe that errors in branch lengths do not have a significant effect on results using independent contrasts. The phylogeny used for the independent contrast analysis was the same as the character optimization phylogeny except that *M. siamensis* was not included due to a lack of reliable divergence estimates between *Macrognathus* and *Mastacembelus* (Appendix A).

The two morphological variables used in the independent contrast analysis were elongation ratio and total number of vertebrae. Elongation ratio was defined as the total length of an individual divided by the second largest major body axis, either width or depth. Mean elongation ratios were gathered from three individuals of each species and used in the independent contrast analysis (Appendix B). The total number of vertebrae was determined from cleared and stained specimens, skeletal preparations, or X-rays in at least three individuals of each species (Appendix B). The average number of vertebrae was used for the independent contrast analysis.

In the BRUNCH algorithm, contrasts of the continuous variable are calculated at nodes where there is a transition in the state of the discrete variable (Purvis and Rambaut, 1995). The sign of the contrast depends on the direction of the discrete variable change. Under a null model of evolution, the discrete variable is not correlated with the continuous variable if the sum of the contrasts is equivalent to zero (Purvis and Rambaut, 1995; Nunn and Barton, 2001). A one-sample *t*-test assessed whether there was a correlation between escape response behavior (discrete variable) and the contrasts of two continuous morphological variables by testing whether the sum of the contrasts of each variable was equivalent to zero (Purvis and Rambaut, 1995; Nunn and Barton, 2001; StatView 5.0.1, SAS Institute, Cary, North Carolina). A one-way ANOVA tested whether the phylogenetically uncorrected data also differed between the two behaviors (SuperAnova 1.11, Abacus Concepts, Berkeley, California).

In addition, the CRUNCH algorithm was used to calculate contrasts of the morphological variables relative to one another. This tested whether elongation ratio and number of vertebrae were independent morphological variables. In the CRUNCH algorithm, contrasts are calculated for all continuous variables at all nodes. For this analysis, branch lengths were set to unity. Regressions were analyzed using a reduced major axis (RMA) analysis with the intercept forced through zero (SPSS 10.0, SPSS, Inc., Chicago, IL).

Results

Head retraction behavior

Behavioral observations of escape responses were made for nine species of elongate aquatic vertebrates including larval lamprey, actinopterygian fishes, a lungfish, and amphibians (Fig. 1). Observations of multiple individuals of most study species indicated that there was little qualitative variation in escape behavior. All individuals performed only head retraction when startled, with the exception of *L. paradoxa*. In three of the 21 recorded trials, *L. paradoxa* performed a C-start, and in the remaining trials it performed head retraction.

Like C-starts, head retraction is an escape response characterized by two stages. The first stage involves a rotation of the head away from the stimulus (Fig. 2). As the head rotates away from the stimulus, the anterior portion of the body moves caudally while the posterior portion of the body moves rostrally (Figs. 1c–k, Fig. 2). This movement is similar to the compression of an accordion (Figs. 1c–k). The second stage is characterized by a rotation of the head back towards the stimulus such that the final angle of the head is within 10–20° of the pre-stimulus position (Fig. 2). The only part of the body moving during stage 2 is the head. The rest of the body remains in the compressed accordion shape (Figs. 1c–k). At the end of the head retraction startle response, 2–4 axial bends are present on the body. The final resting body shape often resembles the Greek letter Ω (Figs. 1c–k). As noted previously, there is no propulsive phase associated with head retraction in these species (Eaton et al., 1977; Meyers et al., 1998).

Two head angles were measured during head retraction to describe the angular movement of the head. The maximum head angles of the nine species were not found to differ based on a comparison of the 95% confidence intervals of the mean. The head retraction mean maximum angle ($n = 168$) was 27.75° (95% Range: 22.85–32.65°, Fig. 3a). The mean final angle for each of the nine species was also found not to differ
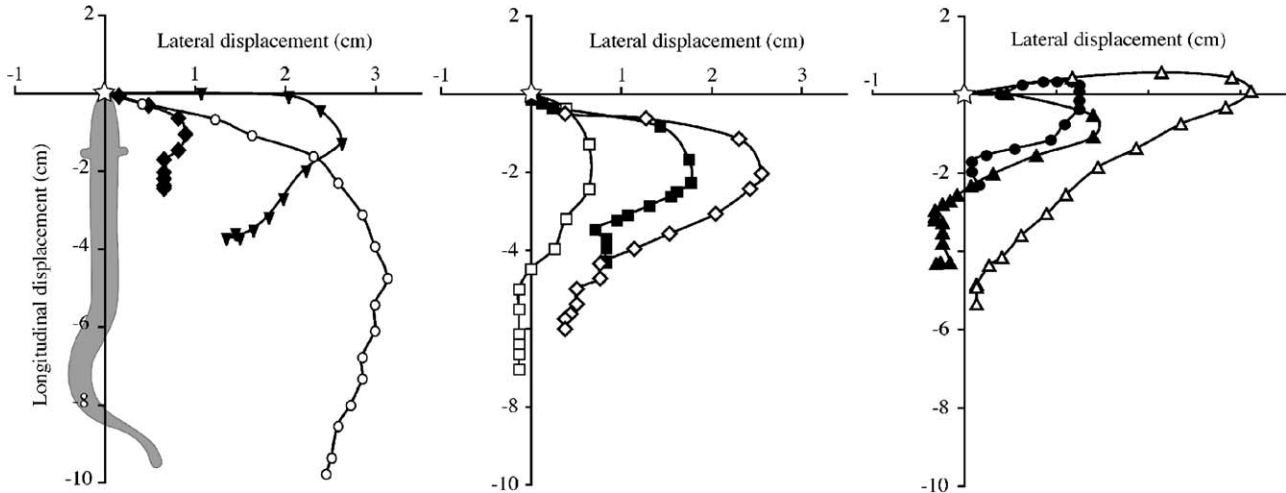
Fig. 2. Lateral and longitudinal displacement of the tip of the snout during head retraction startle responses. A representative startle response is shown for each species. The escapes are standardized so that the long axis of the animal is oriented along the *y*-axis, the initial position of the tip of the snout is at the origin (0,0) and the stimulus is applied from the left. Data points are shown at 8 ms intervals. Note that all responses are characterized by an initial turn of the head away from the stimulus, followed by a turn toward the stimulus and a rearward retraction of the head. Symbols: ● *P. marinus*; ■ *A. rostrata*; ◆ *G. polyuranodon*; ▲ *M. siamensis*; ▼ *M. armatus*; ○ *T. natans*; □ *A.tridactylum*; ◇ *S. intermedia*; △ *L. paradoxa*.

between the species based on the 95% confidence intervals. The mean final angle was 17.71° (95% Range: 13.02–22.40°, Fig. 3b).

Phylogenetic analysis of behavior and morphology

We were unable to resolve startle response behavior at the base of Vertebrata using character optimization (Fig. 4, Node B). However, for the base of Gnathosto- mata, the C-start was determined to be the most parsimonious ancestral behavior (Fig. 4, Node C). Within vertebrates, head retraction has evolved at least six times independently: Petromyzontiformes (Node A), Dipnoi (Node E), Lissamphibia (Node D), Polypteriformes (Node F), Elopomorpha (Node G), and Percomorpha (Node H).

We examined the relationship between two morphological variables and escape behavior using a sample
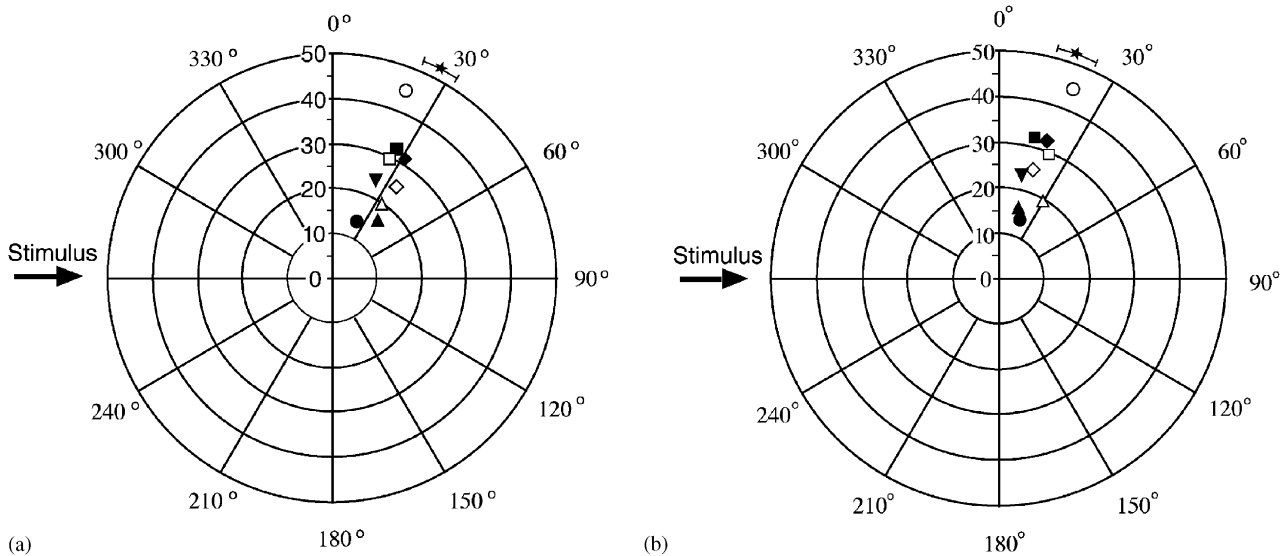
Fig. 3. Mean angular movements of the head during head retraction startle responses: (a) Maximum head angles and (b) final head angles are plotted as the circumferential axis, and total length (cm) is plotted as the radial axis. Zero degrees represent the initial position of the head and 270° represent the direction of the stimulus. All responses have been standardized to be in the clockwise direction. ★ represents the mean angular change for all head retraction trials from all nine species (*n* = 163). The error bars are the 95% confidence intervals of the mean. Symbols: ● *P. marinus*; ■ *A. rostrata*; ◆ *G. polyuranodon*; ▲ *M. siamensis*; ▼ *M. armatus*; ○ *T. natans*; □ *A. tridactylum*; ◇ *S. intermedia*; △ *L. paradoxa*.

consisting of nine head retraction species and 15 C-start species (Table 1 and Appendix B). Based on a one-sample *t*-test on contrasts of the morphometric variables, we found a significant correlation between escape

behavior and total number of vertebrae ($p = 0.0042$). Animals with more vertebrae are more likely to perform head retraction. A significant correlation was also found between escape behavior and elongation ratio
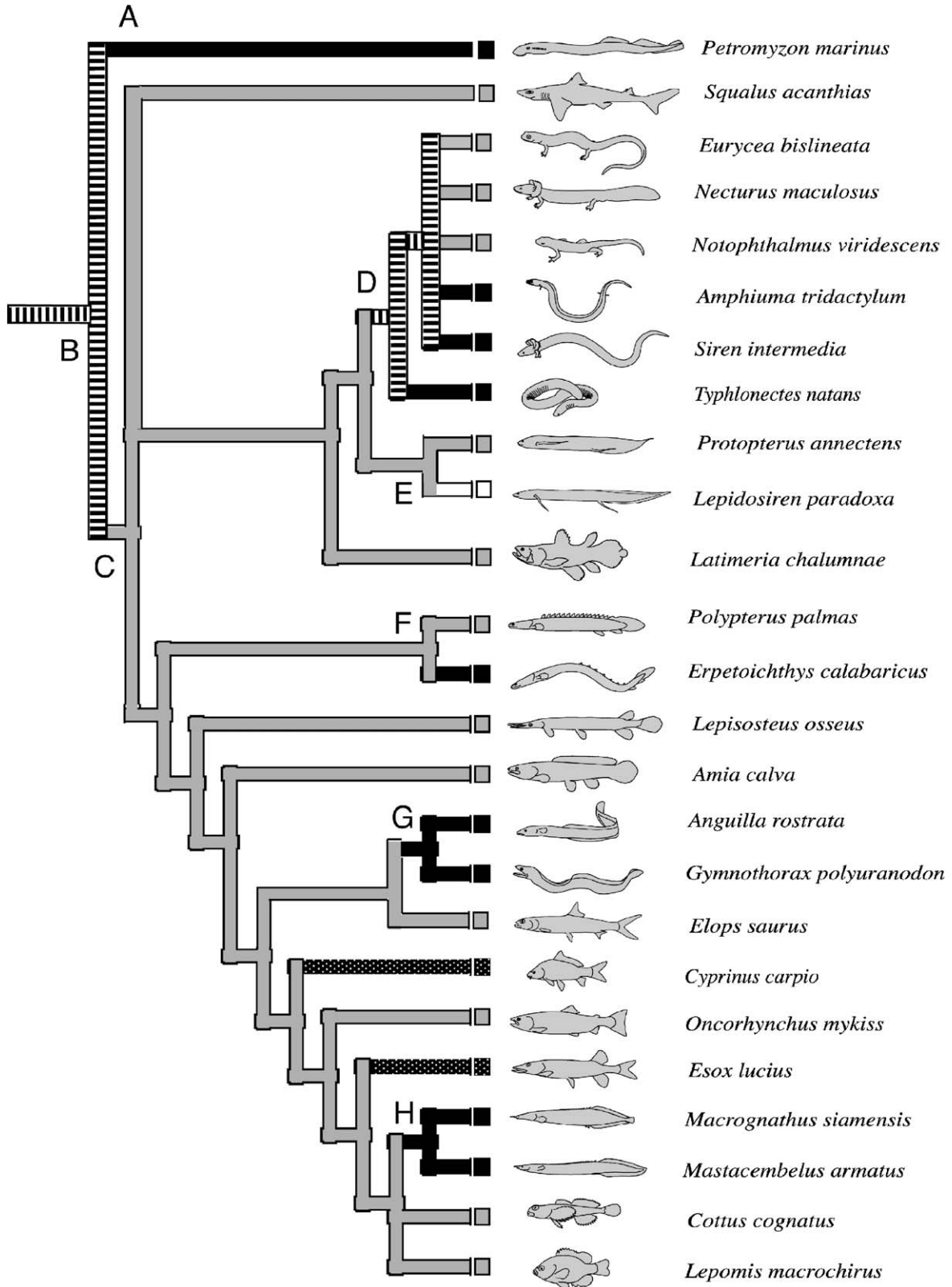
Table 1. Data used in independent contrast analysis

Species	Behavior	Elongation ratio		Total vertebrae	
		Mean	SE	Mean	SE
Amia calva	C-start	5.2	0.19	64	0.98
Amphiuma tridactylum	HR	18.9	2.54	103	1.53
Anguilla rostrata	HR	17.3	1.00	109	4
Cottus cognatus	C-start	4.3	0.14	33.3	0.88
Cyprinus carpio	C-start	3.1	0.07	34.8	0.25
Elops saurus	C-start	7.2	0.20	83.7	1.20
Erpetoichthys calabaricus	HR	19.4	0.37	109	2.08
Esox lucius	C-start	7.8	0.74	57	2.52
Eurycea bislineata	C-start	18.5	1.53	58.7	2.19
Gymnothorax polyuranodon	HR	20.8	0.97	135	6.51
Latimeria chalumnae[a]	C-start	4.1	—	94	—
Lepidosiren paradoxa[b]	HR	11.7	1.13	84.5	—
Lepisosteus osseus	C-start	19.5	1.54	57.5	3.20
Lepomis macrochirus	C-start	2.1	0.12	27	0.58
Mastacembelus armatus	HR	9.4	1.19	93.5	0.69
Necturus maculosus	C-start	10.3	0.43	43	1.53
Notophthalmus viridescens	C-start	12.1	0.53	52	2.08
Oncorhynchus mykiss	C-start	3.7	0.15	62	1.15
Petromyzon marinus[a]	HR	19.0	0.52	216	—
Polypterus palmas	C-start	9.0	0.29	56.3	0.58
Protopterus annectens	C-start	9.0	0.55	76.7	3.71
Siren intermedia	HR	14.3	0.93	76.7	1.45
Squalus acanthias	C-start	9.8	0.87	103.4	0.94
Typhlonectes natans	HR	26.1	1.70	98.7	2.19

Means and standard errors of three individuals are presented (except as noted). Elongation ratio is the ratio of total length to the second largest body axis (width or depth). References for each variable (behavior, elongation ratio, and total number of vertebrae) can be found in Appendix B. HR, head retraction; SE, standard error.

[a]Only one individual was measured.

[b]Only two skeletal specimens were available.

($p = 0.0005$) indicating that elongate species are more likely to perform head retraction. Significant correlations were also found when all branch lengths were set to unity (Vertebrae: $p = 0.0092$, Elongation Ratio: $p = 0.0012$). Similar results were obtained by performing a one-way ANOVA on the raw data. A significant difference was found between the two behaviors in total number of vertebrae ($p = 0.0004$, Fig. 5a) and in elongation ratio ($p = 0.0004$, Fig. 5b). Although elonga-

tion ratio and number of vertebrae were correlated with behavior, elongation ratio and total number of vertebrae were not significantly correlated in C-start species or in head retraction species (C-start: $p = 0.82$, Head-retraction: $p = 0.89$). The lack of correlation between elongation ratio and total number of vertebrae indicates that decreasing depth or width (by reorganization of the soft body tissue) may be a larger factor in fish elongation than an increase in total length.

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Fig. 4. Comparative analysis of the escape behavior of non-amniote vertebrates. The phylogeny used in this analysis is based on the literature (Lauder and Liem, 1983; Benton, 1990; Cloutier and Ahlberg, 1996; Forey et al., 1996; Grande and Bemis, 1996; Gao and Shubin, 2001) and all nodes have been optimized for startle behavior using parsimony. Black lines represent the taxa in which a head retraction startle response has been documented as well as ancestral nodes that are optimized as a head retraction. Grey lines represent the taxa in which a C-start has been documented as well as ancestral nodes that are optimized as a C-start. White lines represent groups that are polymorphic for escape behavior (Head retraction and C-start). Dotted lines also represent groups that are polymorphic for escape behavior (C-starts and S-starts). Dashed lines represent nodes that are unresolved for startle behavior. This phylogenetic analysis indicates that the head retraction startle response has evolved at least six times independently. Letters A–H represent key transitional nodes in startle behavior; see text for more detail. See Appendix B for references used to determine behavior in the represented species.

(a)

(b)
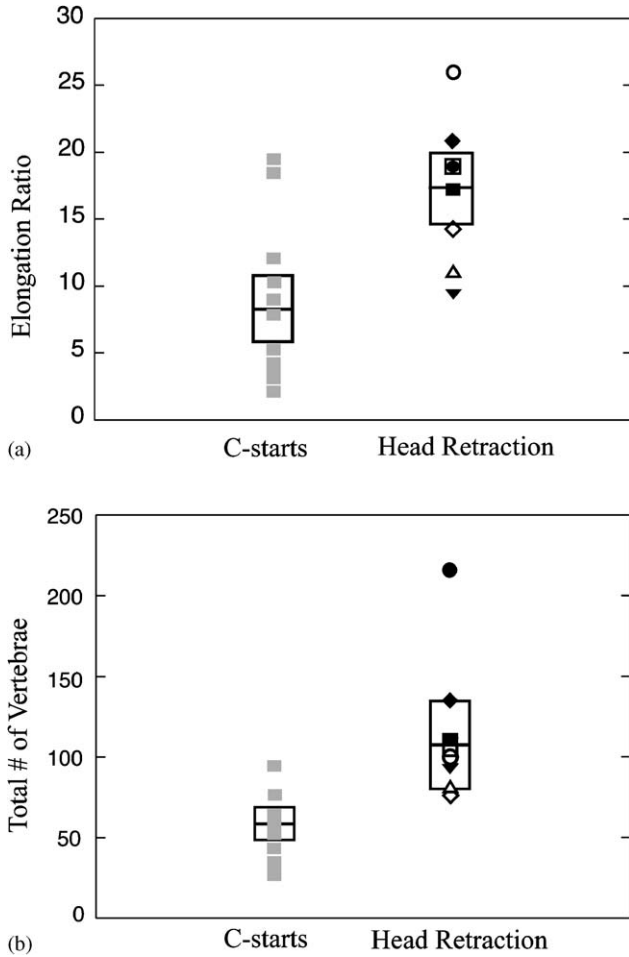
Fig. 5. A comparison of mean elongation ratio and mean total vertebral number in species that utilize a C-start or head retraction startle response: (a) Average elongation ratio (ratio of total length and the second longest body axis) for eight species that perform head retraction and ten species that perform C-starts. Boxes indicate 95% confidence ranges and lines indicate mean for the entire group. Elongation ratio is found to differ significantly in the two groups. (b) The mean total vertebral number for eight species that perform head retraction and ten species that perform C-starts. Boxes indicate 95% confidence ranges and lines indicate mean for the entire group. Total vertebral number is found to differ significantly in the two groups. A *t*-test performed on phylogenetically corrected contrasts also indicates a significant correlation between behavior and both elongation ratio ($p = 0.004$) and vertebral number ($p = 0.0005$). Symbols: ● *P. marinus*; ■ *A. rostrata*; ◆ *G. polyuranodon*; ▲ *M. siamensis*; ▼ *M. armatus*; ○ *T. natans*; □ *A. tridactylum*; ◇ *S. intermedia*; △ *L. paradoxa*.

Discussion

Escape response behaviors

Eight of the nine species in this study exhibited only head retraction when startled. In the case of *Lepidosiren paradoxa*, 3 C-starts were observed out of the 21 observed escape response trials. Head retraction can

be characterized by three movement patterns. First, the movement of the body during head retraction is along the anterioposterior axis of the body despite a laterally directed stimulus (Figs. 1c–k, Fig. 2). In contrast, the movement during C-starts is first directed laterally (Stage 1) followed by anterior propulsion (Stage 2) (Eaton et al., 1977). Secondly, during head retraction, head angle changes are rarely greater than 30° and average 17° from the pre-stimulus angle of the head to the head angle at the end of the response (Fig. 3). During stage 1 of a typical C-start, the head angle change is greater than 90° and the overall head angle change is approximately 90° (Domenici and Blake, 1997; Fig. 1a). Finally, as noted in previous studies, head retraction does not include a propulsive phase as seen during stage 2 of C-starts (Figs. 1a, c–k, Eaton et al., 1977; Domenici and Blake, 1997; Meyers et al., 1998).

Although some taxa, like *L. paradoxa*, exhibit both behaviors in their locomotor repertoire, no intermediate response was observed in any species included in this study. One potentially intermediate behavior could be the S-start, which contains components of both C-starts and head retraction. There are multiple waves on the body during an S-start, similar to head retraction (Figs. 1b, c–k). However, S-starts, by definition, have only two inflection points on the body whereas head retraction results in at least three inflection points on the body (head, midbody, and tail). Like C-starts, S-starts include a propulsive phase, which is absent in head retraction (Hale, 2002).

It is difficult to assess quantitatively the hypothesis that the S-starts are an intermediate behavior between head retraction and C-starts given the lack of comparative kinematic data on S-starts. *Esox* spp. and *C. carpio* are the only species in which S-starts have been reported (Hale, 2002; Spierts and van Leeuwen, 1999). It is, however, possible that S-starts are a behavior that is unique to *Esox*. Hale (2002) found that the S-shape of the body is the result of active muscle contraction on both sides of the body. However, similar data are not available for *C. carpio*. It is possible that the S-shape in *C. carpio* is passive and represents a temporal delay in the movement of the caudal fin due to differential hydrodynamic resistance. In the case that S-starts are unique to *Esox*, it is unlikely that S-starts would be an intermediate behavior between C-starts and head retraction. Additional taxa are necessary to reliably assess whether S-starts may be an intermediate startle response behavior. Future work on the presence of S-starts among anamniote vertebrates could reveal a continuous kinematic pattern among escape behaviors.

Biological role of head retraction

Unlike C-starts and S-starts, the head retraction escape response lacks a propulsive phase (Eaton et al.,

1977; Currie and Carlsen, 1985; Figs. 1c–k). The absence of a propulsive phase might seem contradictory to the function of an escape response. Another contradictory aspect of head retraction as an escape response is that the primary direction of movement was never away from the stimulus, but rather directed anteroposteriorly. However, all of the elongate species in this study tend to be found in highly structured habitats (i.e. in burrows, rocks or dense vegetation) where head retraction may function as an effective predator avoidance behavior. In the first detailed description of head retraction, Currie and Carlsen (1987) noted that larval *P. marinus* tended to be partially buried in the substrate with only the anterior portions of the body exposed. When the tank was tapped, the larval lamprey retracted their heads down into the burrow.

The other species included in this study also burrow or live in highly structured environments. The two spiny eel species, *Macrognathus* and *Mastacembelus,* burrow to hide and, presumably, to avoid predation (Lowe-McConnell, 1975; Roberts, 1986). American eels (*A. rostrata*) are active at night but remain buried in mud or hidden in rocks during the daylight hours when inhabiting freshwater streams or ponds (Bigelow and Schroeder, 1953). *Amphiuma* are reported to inhabit crayfish burrows along canals and are often seen with only the anterior portions of the body extended into the water column (Cagle, 1956; Tinkle, 1959). They retract their heads back into the burrows when startled by ground vibrations (Tinkle, 1959). Like *Amphiuma*, *Siren* have been found in burrows up to forty inches below the surface of the ground (Cockrum, 1941; Duellman and Schwartz, 1958). *Typhlonectes* tend to burrow in mud and only leave their burrows after sunset (Moodie, 1978; Wilkinson and Nussbaum, 1999). Thus, the natural history of the species examined in this study supports the hypothesis that head retraction is associated with living in structured habitats.

Given that head retraction lacks a propulsive phase and all of the species studied are commonly found in structured environments, it is likely that this behavior allows an animal to escape from potential predation by retreating into its refuge. Head retraction may simply function to move the exposed portion of an organism out of the reach of a potential predator. Like tunnels or burrows, the shells of turtles provide a similar source of protection. When turtles are startled, they perform a head retraction response (Van Damme et al., 1995). Despite the lack of a propulsive phase, head retraction, in turtles and aquatic anamniote vertebrates, functions as an escape response by allowing an animal to quickly retract into its refuge and out of the reach of a potential predator.

Evolution of head retraction

In this study, behavioral observations were used to differentiate the two escape responses: head retraction and C-starts. By optimizing startle response behavior on a phylogeny, we determined that head retraction has evolved at least six times within Vertebrata. Although we were unable to determine unequivocally the plesiomorphic vertebrate escape response (Fig. 4, Node B), our analysis suggests that C-starts are the plesiomorphic condition for Gnathostomata (Fig. 4, Node C).

Head retraction has evolved at least five times within gnathostomes and in each case is associated with the evolution of an elongate body form. Head retraction has evolved at least three times within Actinopterygii: once within Polypteriformes (*E. calabaricus*); once within Elopomorpha (Anguilliformes); and once within Percomorpha (Mastacembelidae) (Fig. 4, Nodes F, G, H). Within Sarcopterygii, head retraction has evolved at least twice: once within Dipnoi and at least once within Lissamphibia (Fig. 4, Node D). It is likely, though, that head retraction has evolved at least three times within this clade (Gymnophiona, Sirenidae, Amphiumidae) based on the phylogeny proposed by Gao and Shubin (2001). However, a more fully resolved lissamphibian phylogeny is needed to confirm this hypothesis.

We calculated correlations between body shape and escape behavior using independent contrasts. Our results indicate a strong significant correlation between total vertebral number and escape behavior such that species that perform head retraction have a greater number of total vertebrae (see Fig. 5b for uncorrected data). Brainerd and Patek (1998) suggested that an increase in the number of vertebrae would increase the flexibility of the body by providing more intervertebral joints at which the animal can bend. Greater flexibility associated with an increase in vertebral number may be required to achieve the high-curvature localized bends associated with the head retraction startle response (Fig. 1). It has been shown that during undulatory locomotion, the propulsive wavelength decreases and axial curvature increases with increasing vertebral number (Long and Nipper, 1996). An increased number of vertebrae may therefore be functionally linked to the high curvature movement associated with the head retraction startle response.

Within amniote vertebrates, extreme increases in vertebral number are seen in snakes and lizards. Van Damme and Vanhooydonck (2002) report that lacertid lizards that utilize vertical habitats or densely vegetated habitats have more presacral vertebrae than lacertids that are found in open habitats. They conclude that greater axial flexibility is necessary in densely vegetated habitats (Van Damme and Vanhooydonck, 2002). Therefore, increased vertebral number may be a general theme within vertebrates that are found in highly structured habitats.

Our results also indicate a strong significant correlation between elongation ratios and escape behavior (see Fig. 5a for uncorrected data). A high elongation ratio indicates that there is an increase in total length relative to the two other dimensions (width and depth). Webb (1977, 1978)

noted the importance of body form when evaluating escape response performance across diverse taxa. He found that by amputating the median fin (thereby decreasing the body depth) the speed and distance traveled during a fast-start escape response decreased significantly. Wu (1977) suggested on theoretical grounds that the thrust produced during undulatory locomotion is proportional to the square of the depth of a fish. Therefore, if the depth is reduced, as in elongate animals, the thrust produced by a given undulatory wave may be significantly lower than that of a deep-bodied animal of similar length. These biomechanical considerations provide a possible explanation for the observed exclusion of C-starts from the locomotor repertoire of numerous elongate taxa. However, a more complete understanding of selection pressures that may have led to the evolution of head retraction and loss of C-starts in elongate lineages will require further studies on the function and evolution of escape behavior.

Concluding remarks

The evolution of aquatic escape responses provides a good model system for exploring the associations between behavior, ecology and morphology. In this study, head retraction was found to have evolved at least six times independently in association with an increase in vertebral number, elongation of the body axis and structured habitats. Future work should concentrate on smaller groups within Gnathostomata that have both elongate and non-elongate members (e.g., Polypteriformes, Blennioidei, and Gobioidei) in order to explore the selection pressures associated with the convergent evolution of these behavioral, morphological, and ecological characters.

Acknowledgements

Appendix A

Phylogeny and branch lengths used in the independent contrast analysis. Branch lengths based on earliest recorded fossil dates in millions of years: [A]Martin, 1984; [B]Carroll, 1988; [C]Benton, 1990; [D]Gardiner, 1993; [E]Jenkins and Walsh, 1993; [F]Patterson, 1993; [G]Schultze, 1993; [H]Forey et al., 1996; [I]Daget et al., 2001; [J]Märss and Gagnier, 2001; [K]Gao and Shubin, 2003. Branch lengths based on back calculations: [L]Back calculated based on Carroll, 1988; [M]Back calculated based on Gardiner, 1993; [N]Back calculated based on Schultze, 1993; [O]Back calculated based on Forey et al., 1996; [P]Back calculated based on known lengths of terminal branches.
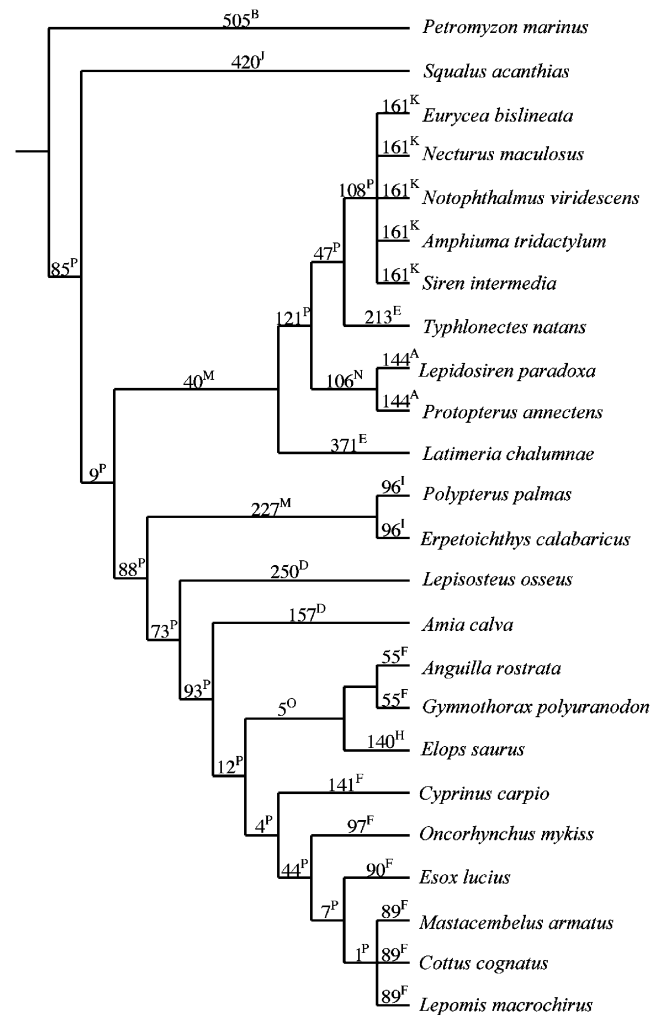
Appendix B

References for the body form and behavior independent contrast analysis data given in Table 1. Numbers in parentheses indicate the number of specimens from a given museum lot. Institutional abbreviations are as listed in Leviton et al. (1985). See Table 2.

Table 2

Species	Behavior	Elongation ratio	Vertebrae
Amia calva	Westneat et al., 1998	UMA 24-127-1 (5)	Grande and Bemis, 1998
Amphiuma tridactylum	This study	This study, FMNH 194622 (1), FMNH 194614 (1)	UMA 0729 (1), UMA uncatalogued (2)
Anguilla rostrata	Meyers et al., 1998, this study	This study, UMA 24-202-1 (2)	Tesch, 1977, this study, UMA 24-202-1 (1)
Cottus cognatus	Webb, 1978	UMA 24-511-2-30 (2), 24-511-2-34 (3)	UMA 24-511-2-39 (3)
Cyprinus carpio	Spierts and van Leeuwen, 1999, Wakeling et al., 1999	UMA 24-237-24-1 (5)	UMA uncatalogued (1), UMA 24-237-24-2 (3)
Elops saurus	Pers. Obs.	UMA 24-131-1-2 (3)	UMA F10753 (1), UMA F10756 (1), UMA F11114 (1)
Erpetoichthys calabaricus	Bierman et al., 2002	AMNH 3528 (3)	AMNH 225397 (1), FMNH 104038 (1), UMA uncatalogued (1)
Esox lucius	Weihs, 1973, Webb, 1978	UMA 24-288-1 (1), 24-288-1-1 (1), 24-288-1-2 (1)	UMA F11389 (1), F11320 (1), F10451 (1)
Eurycea bislineata	Azizi and Landberg, 2002	UMA uncatalogued (5)	UMA uncatalogued (3)
Gymnothorax polyuranodon	This study	This study, MCZ 9056 (2)	This study, MCZ 9056 (2)
Latimeria chalumnae	Fricke et al., 1987, Fricke and Hissmann, 1992	Smith, 1939	Forey, 1998
Lepidosiren paradoxa	This study	This study, AMNH 11674 (1), AMNH 55788 (1)	AMNH 36981 (1), 38137 (1)
Lepisosteus osseus	Hale et al., 2002	UMA 24-121-1-1 (1), F11394 (3)	UMA F11390, UMA F11394 (3)
Lepomis macrochirus	Webb, 1978, Jayne and Lauder, 1993	UMA 24-418-06-10 (2), 24-418-6-29 (3)	UMA uncatalogued (1), UMA 24-418-6-8 (2)
Mastacembelus armatus	This study	This study, UMA uncatalogued (3)	Roberts, 1986
Necturus maculosus	Pers. Obs.	UMA 25-1-1-4 (2), A0005 (1), A0008 (2)	Wilder, 1903, UMA A0014 (1), 25-1-1-13 (1)
Notophthalmus viridescens	Pers. Obs.	UMA A0034 (5)	UMA uncatalogued (1), UMA 25-4-1-5 (2)
Oncorhynchus mykiss	Webb, 1976	UMA 24-163-3-1 (2), 24-163-3-2 (3)	UMA F10272, UMA 24-163-3 (2)
Petromyzon marinus	Currie and Carlsen, 1985, this study	This study, UMA F0014 (4)	UMA F11355 (1)
Polypterus palmas	Westneat et al., 1998	MCZ 49096 (4)	MCZ 49096 (3)
Protopterus annectens	Meyers et al., 1998	UMA uncatalogued (5)	UMA uncatalogued (3)
Siren intermedia	This study	This study, FMNH 6812, FMNH 8013	Gillis, 1997, UMA uncatalogued (2)
Squalus acanthias	Domenici et al., 2003	UMA 21-67-1-4 (3)	Springer and Garrick, 1964 (34)
Typhlonectes natans	This study	This study, UMA uncatalogued (7)	Taylor, 1968, UMA uncatalogued (2)

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