

The Effect of Phylogeny on Interspecific Body Shape Variation in Darters (Pisces: Percidae)

J. MICHAEL GUILL,¹ DAVID C. HEINS,¹ AND CRAIG S. HOOD^{2,3}

¹Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, Louisiana 70118, USA; E-mail: guill@tulane.edu

²Department of Biological Sciences, Loyola University, New Orleans, Louisiana 70118, USA

³Tulane University Museum of Natural History, Belle Chasse, Louisiana 70037, USA

Abstract.— We conducted a geometric morphometric analysis of interspecific body shape variation among representatives of 31 species of darters (Pisces: Percidae) to determine whether there is evidence of a phylogenetic effect in body shape variation. Cartesian transformation grids representing relative shape differences of individual species and subspecies revealed qualitative similarities within most traditionally recognized taxonomic groups (genera and subgenera). Canonical variates analysis and a UPGMA cluster analysis were conducted to explore further the relationships among body shapes of species; both analyses revealed patterns of variation consistent with the interpretation that shape is associated with taxonomic affinities. Normalized Mantel statistics revealed a significant positive association between body shape differences and phylogenetic interrelatedness for each of four recent phylogenetic hypotheses, providing evidence of a phylogenetic effect. This result is somewhat surprising, however, given the largely incompatible nature of these four phylogenies. We provide evidence that this result may be due to (1) the inclusion of multiple sets of closely related species to represent the traditionally recognized genera and subgenera within each phylogeny and/or (2) the inclusion of several species with relatively divergent shapes and their particular positions within the phylogenies relative to one another or to the other species of darters. [Body shape variation; geometric morphometrics; phylogenetic effect; thin-plate spline.]

Variations in body form have important fitness consequences for organisms because these variations can affect the ability to occupy particular habitats successfully (Gatz, 1979a, 1979b; Douglas, 1987; Losos and Sinervo, 1989; Irschick et al., 2000), to prevail in predator–prey interactions (Toline and Baker, 1993; Carpenter, 1996; Walker, 1997; Nagel and Schluter, 1998), and to reproduce successfully (Fleming and Gross, 1989; Foster et al., 1992; Abell et al., 1999). As with all patterns of phenotypic variation, however, the effects of phylogenetic nonindependence must be evaluated as part of a complete understanding of body shape variation (Harvey and Pagel, 1991; Martins, 1996; Garland et al., 1999). To our knowledge, there has been no prior quantification of the phylogenetic effect in the context of a geometric morphometric analysis, although an effect is beginning to be assumed and preemptively controlled for (e.g., Rüber and Adams, 2001; Rosenberg, 2002).

The darters (subfamily Etheostomatinae, family Percidae) are a diverse clade of small, mostly benthic stream fishes that are endemic to North America. A great deal of research effort has been focused on their ecologies, behavioral patterns, life histories, and evolutionary relationships. One recurring theme has been the association of their generally small body size with other aspects of their biology (e.g., Page and Swofford, 1984; Paine, 1990; Bart and Page, 1992). Body shape variation— independent of size—is, however, an essentially unexplored component of darter morphology.

Many taxonomic descriptions and other studies of darters have included general qualitative assessments of body form (e.g., fusiform, deep-bodied, laterally compressed, or cylindrical), although few such studies have analyzed interspecific variation in these traits quantitatively. Two notable exceptions are the investigations by Page (1983) and Page and Swofford (1984), who examined large numbers of darter species for correlations among a suite of traditional morphological measures and various ecological and behavioral traits. These analyses

underscored the primary importance of body size in the observed patterns of variation in all of the other meristic, mensural, habitat, and behavioral characters that were examined. Although these authors attempted to explore some aspects of shape variation beyond the effects of size, the techniques employed (i.e., ratios and ordination of linear measurements) have since been shown to be problematic (Bookstein, 1991; Reymont, 1991).

Darter systematics is currently an active topic of research (Near et al., 2000; Kinzinger et al., 2001; Near, 2002), and the publication of several new broad phylogenies (Wood and Mayden, 1997; Song et al., 1998) presented the opportunity to assess darter shape variation in the context of possible phylogenetic effects (Felsenstein, 1985; Martins, 1996; Garland et al., 1999). The substantial differences between the phylogenies proposed by Wood and Mayden (1997) and Song et al. (1998; see Fig. 1) have somewhat complicated this analysis but have also afforded the opportunity to examine our results in terms of a real-world case of nontrivial phylogenetic uncertainty. In the present study therefore, we examined the correlations between these four hypotheses of darter evolutionary relationships and a set of rigorous estimates of overall body shape obtained using modern geometric morphometric techniques.

MATERIALS AND METHODS

Phylogenetic Hypotheses

The hypotheses of phylogenetic relationships employed in the present study were derived from analyses of allozymes (Wood and Mayden, 1997) and mitochondrial cytochrome *b* DNA sequences (Song et al., 1998). Both studies proposed several possible phylogenies resulting from different analytical models. In the present study, we used two trees proposed by Wood and Mayden (1997: Figs. 1, 3, hereinafter WM1 and WM3, respectively) and two proposed by Song et al. (1998: Figs. 3, 4, hereinafter SNP3 and SNP4, respectively). These four

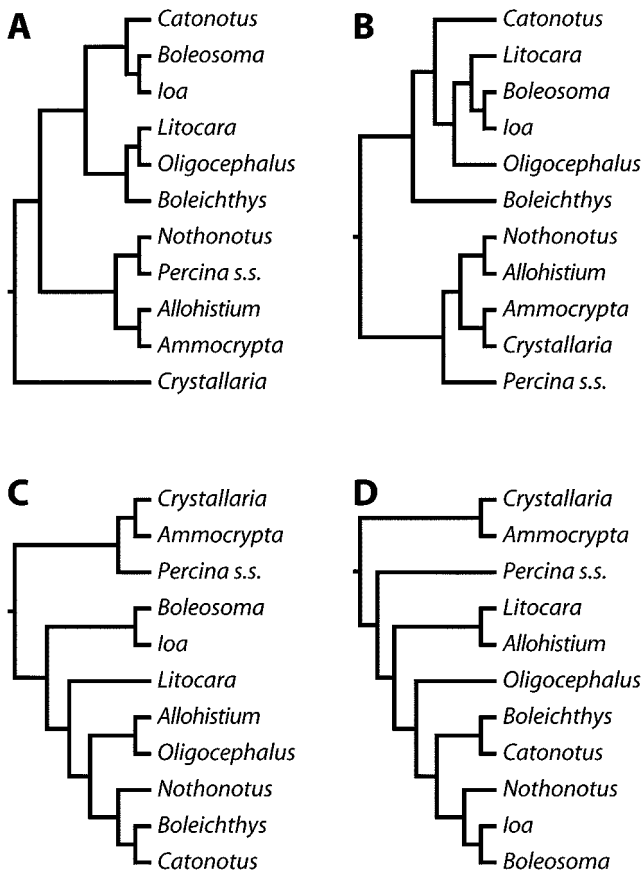


FIGURE 1. Dissimilarity of four hypotheses of darter phylogenetic relationships. These trees are abstractions to the subgeneric level of the phylogenies presented by Song et al. (1998) and Wood and Mayden (1997), limited to the subgenera common to both of those studies. The strict consensus of these trees contains only a single resolved node: *Boleosoma* + *Ioa*. (A) Song et al. (1998), Figure 3. (B) Song et al. (1998), Figure 4. (C) Wood and Mayden (1997), Figure 1. (D) Wood and Mayden (1997), Figure 3.

phylogenies differ substantially in their hypothesized evolutionary relationships of the major groups of darters (Fig. 1). The data set analyzed by Wood and Mayden (1997) addressed variation in electrophoretic mobility at 32 allozyme loci for 26 darter species and three outgroups. WM1 (not shown) is one of two equally parsimonious trees resulting from maximum parsimony analysis, and WM3 (Fig. 2A) is the most-parsimonious tree resulting from frequency analysis (FREQPARS). Wood and Mayden (1997) did not include estimates of support for nodes within these trees. Song et al. (1998) analyzed complete (1,140 base pairs [bp]) cytochrome *b* sequences of 15 darter species, 6 nondarter percids, and 7 outgroup species. SNP3 (Fig. 2B) is the single most-parsimonious solution resulting from a maximum parsimony analysis, and SNP4 (not shown) is the single shortest tree resulting from a minimum evolution analysis. SNP3 has Bootstrap values >50% for 6 of 13 internal nodes in SNP3 (Fig. 2B) and for 7 of 13 nodes for SNP4 (Song et al., 1998).

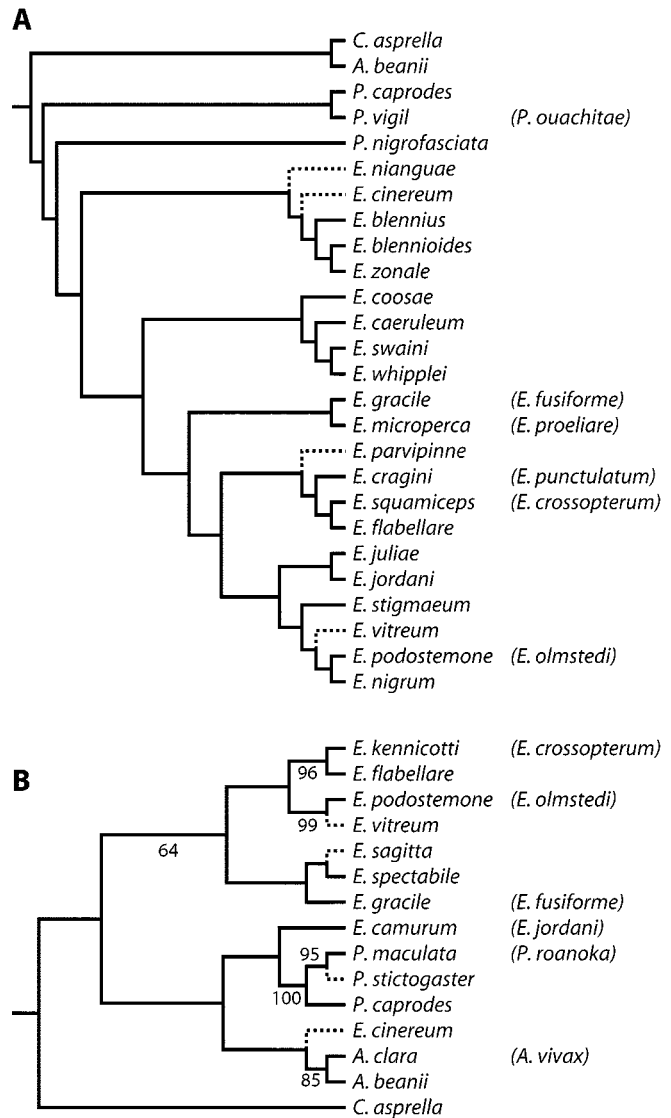


FIGURE 2. Two of the four hypotheses of the darter phylogenetic relationships. (A) Redrawn from figure 3 of Wood and Mayden (1997:269): the most-parsimonious tree resulting from frequency analysis based on 32 allozyme loci. Estimates of node support were not presented. (B) Redrawn from figure 3 of Song et al. (1998:349): the most-parsimonious tree resulting from maximum parsimony analysis of cytochrome *b* (1,140 bp) DNA sequence data. Bootstrap values (1,000 replications) are indicated. Species in parentheses are closely related substitutes for those species we were unable to represent; dotted terminal branches indicate species for which no suitable substitute could be made and therefore were not considered in the present analyses. Morphometric data were gathered for the following additional species but were not included in the phylogenetic analyses: *Percina sciera*, *Etheostoma exile*, *E. inscriptum*, *E. lynceum*, *E. radiosum*, and *E. simoterum* (including both *E. s. simoterum* and *E. s. atripinne*). C. = *Crystallaria*; A. = *Ammocrypta*; P. = *Percina*; E. = *Etheostoma*.

Data Collection

The present analyses include shape data collected from a total of 1,089 adult female specimens representing 31 species and two subspecies of darters. Adult females were used to avoid the possible confounding

effects of ontogenetic allometry and sexual dimorphism. Our choice of species generally followed those of Wood and Mayden (1997) and Song et al. (1998). For some species included by those authors, however, sufficient numbers of specimens could not be obtained (Fig. 2). For most of these cases, it was possible to substitute an adequate number of specimens of relatively uncontroversially closely related species (cf. Butler et al., 2000).

Shape data were collected and analyzed following the protocol outlined by Hood and Heins (2000) and Guill

et al. (2003) using tpsRegr (Rohlf, 1997) and NTSYSpc (Rohlf, 2001b). Eight two-dimensional landmarks were digitized, including the most anterior point on the head, the anterior junctions of the first and second dorsal fins with the dorsal midline, the junctions of the caudal fin with the dorsal and ventral midline, the anterior junction of the anal fin with the ventral midline, the point on the ventral midline between the anterior junctions of the pelvic fins with the body, and the dorsal junction of the left pectoral fin with the body (Fig. 3A).

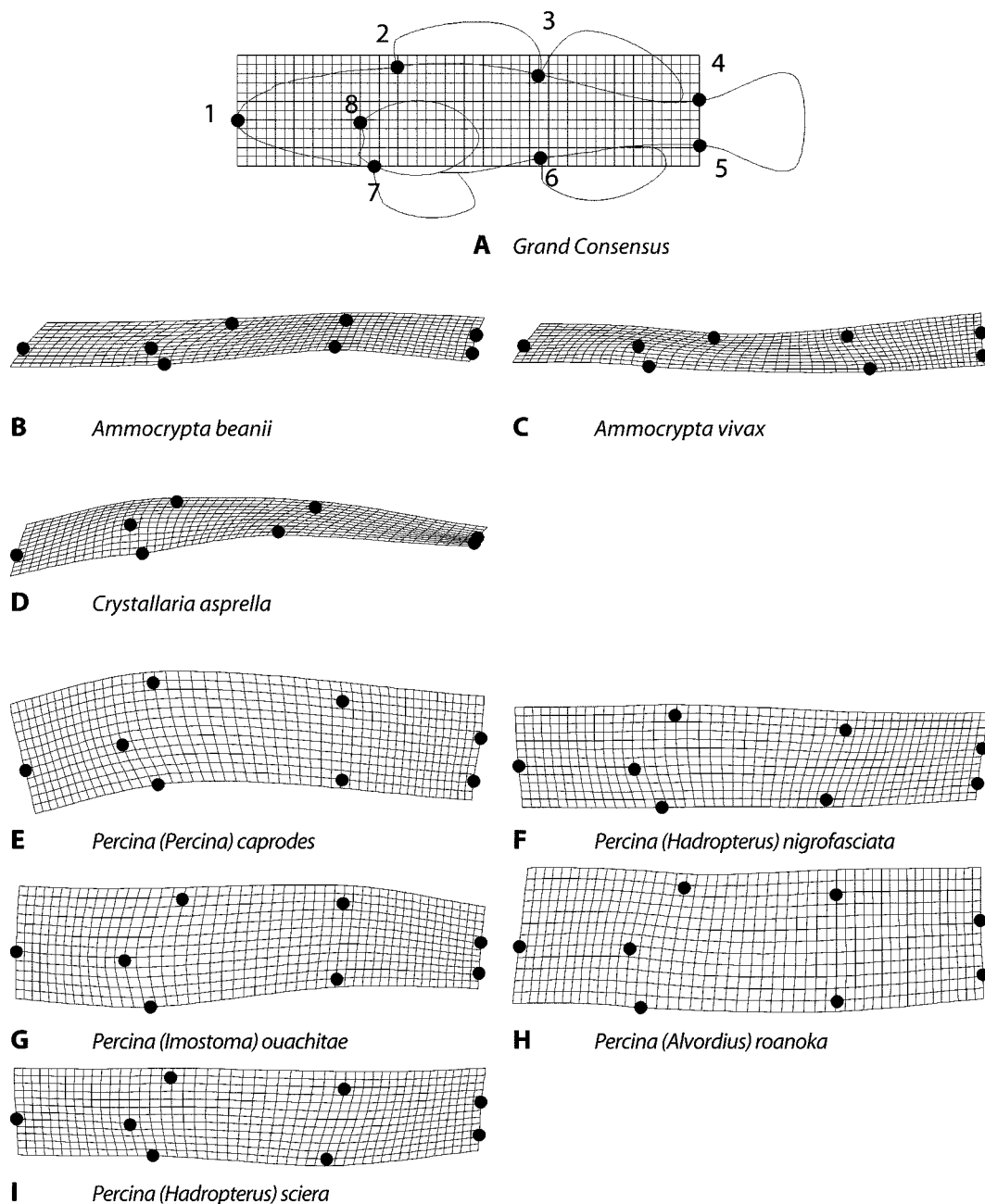


FIGURE 3. Cartesian transformation grids illustrating the average landmark configuration for 31 species and two subspecies of darters (A) and the relative shape differences of species representing the genera *Ammocrypta* (B, C), *Crystallaria* (D), and *Percina* (E–I). Deformations are exaggerated three times to aid interpretation.

The digitized landmark data were used to generate consensus configurations (average shapes) for each of the 32 taxa using the orthogonal generalized least squares method (Rohlf and Slice, 1990), and these consensus were used to generate a grand consensus of all 32 species. The grand consensus was generated using the 32 consensus rather than configurations for all of the individual specimens because species were represented by unequal numbers of specimens. Cartesian transformation grids (CTGs) were then generated to illustrate relative shape differences between each species' consensus and the grand consensus.

Statistical Analysis

The program tpsSmall (Rohlf, 1998) was used to verify the assumption that the amount of shape variation represented by the landmark data set was sufficiently small that analyses could be performed on their projections into linear tangent space. Canonical variates analysis (CVA) was used as an inferential and ordination technique to examine variation in overall body shape among species and to illustrate the associations among species of various taxonomic affiliation in the space defined by the canonical variate axes. The shape data used in the CVA consisted of the affine and partial warps scores of each individual specimen relative to the consensus of all 1,089 specimens. A UPGMA cluster analysis was conducted using the generalized (Malhalanobis) distances between all pairs of species calculated from the matrix of species' affine and partial warps scores, following Rohlf et al. (1996) and Marcus et al. (2000). Affine and partial warps scores are measures of shape differences in one configuration of landmarks relative to another (Bookstein, 1991). In the present analyses, these scores collectively describe the shape of individual specimens or species relative to the average (consensus) of all specimens or species.

The method used to test for evidence of an overall phylogenetic effect in body shape variation among species was adapted from a technique used by Rohlf (2001a:2152; see also Böhning-Gaese and Oberrath, 1999). A matrix of ultrametric distances between all possible pairs of species was generated for each of the four phylogenies under consideration, which were pruned to exclude species for which shape data were not collected (Fig. 2). Branch lengths were not available for all phylogenies, and inclusion of measured branch lengths had negligible effects on the results of a preliminary analysis. Use of measured branch lengths where available would logically preclude the use of substitute species. Therefore, calculation of the phylogenetic distances between species was done with all branch lengths set to 1.

Neither phylogenetic study included all 32 taxa represented in the present study (Fig. 2); therefore, two subsets of species' consensus landmark configurations were prepared: one appropriate to WM1 and WM3 and the other appropriate to SNP3 and SNP4. Two corresponding matrices of the relevant species' affine and partial warps scores were generated (using the consensus configura-

tion of each of these subsets as reference), and matrices of Euclidian distances among all possible pairs of species were then calculated. The strength and significance of the correlations (r) between the matrices of phylogenetic and morphometric distances were measured with normalized Mantel statistics (Sokal, 1979; Smouse et al., 1986), following Rohlf (2001a). Matrix randomization was then used to estimate the probability (P) of obtaining a correlation equal to or greater than the calculated value over 10,000 random matrix permutations.

A second set of tests for phylogenetic effect were conducted to investigate how much of the observed effect was due to the inclusion of multiple species to represent the traditionally recognized subgenera of darters. We chose at random three redundant species to be excluded from the data sets based on the two phylogenies proposed by Song et al. (1998; *E. crossopterus*, *P. roanoka*, and *A. vivax*) and eight redundant species to be excluded from those based on the phylogenies of Wood and Mayden (1997; *E. olmstedii*, *E. blennioides*, *E. zonale*, *E. whipplei*, *E. swaini*, *E. juliae*, *E. proeliare*, and *E. crossopterus*). The shape data, morphometric distance matrices, and phylogenetic distance matrices were recalculated, and Mantel tests were rerun with these reduced data sets.

The shapes of several species presently considered may have been so distinct as to dominate the patterns that were detected. To explore these possible effects, the CVA, UPGMA cluster analysis, and Mantel tests for phylogenetic effect on full and reduced data sets were replicated using data sets regenerated from phylogenies and landmark data from which *A. beanii*, *A. vivax*, and *C. asprella* were excluded.

RESULTS

The CTGs (Figs. 3–6) illustrate the grand consensus body shape of all species presently considered (Fig. 3A) and the ways in which the consensus of individual species differ from the grand consensus (Figs. 3B–6I). Qualitative similarities among species within traditionally recognized higher taxa (e.g., subgenera) are readily discernable. For example, the CTGs representing two species of the genus *Ammocrypta* clearly exhibit the markedly shallow profiles characteristic of the sand darters. The profile of *C. asprella* is similarly shallow, although its tail region is much elongated and the caudal peduncle is much narrower. In contrast, a wider range of body forms is exhibited by five species representing the genus *Percina*, although they all exhibit relatively short head and tail regions (or, conversely, elongated midsections). Among these fishes, the two species (*P. nigrofasciata* and *P. sciera*) representing the subgenus *Hadropterus* are the most similarly shaped, exhibiting somewhat shallower body profiles, a posterior displacement of landmark 3, and narrow caudal peduncles (distance between landmarks 4 and 5).

The remaining 24 taxa we examined are members of the genus *Etheostoma* s.l. and represent nine traditionally recognized subgenera. Within subgenera represented by two or more species, common shape features are evident

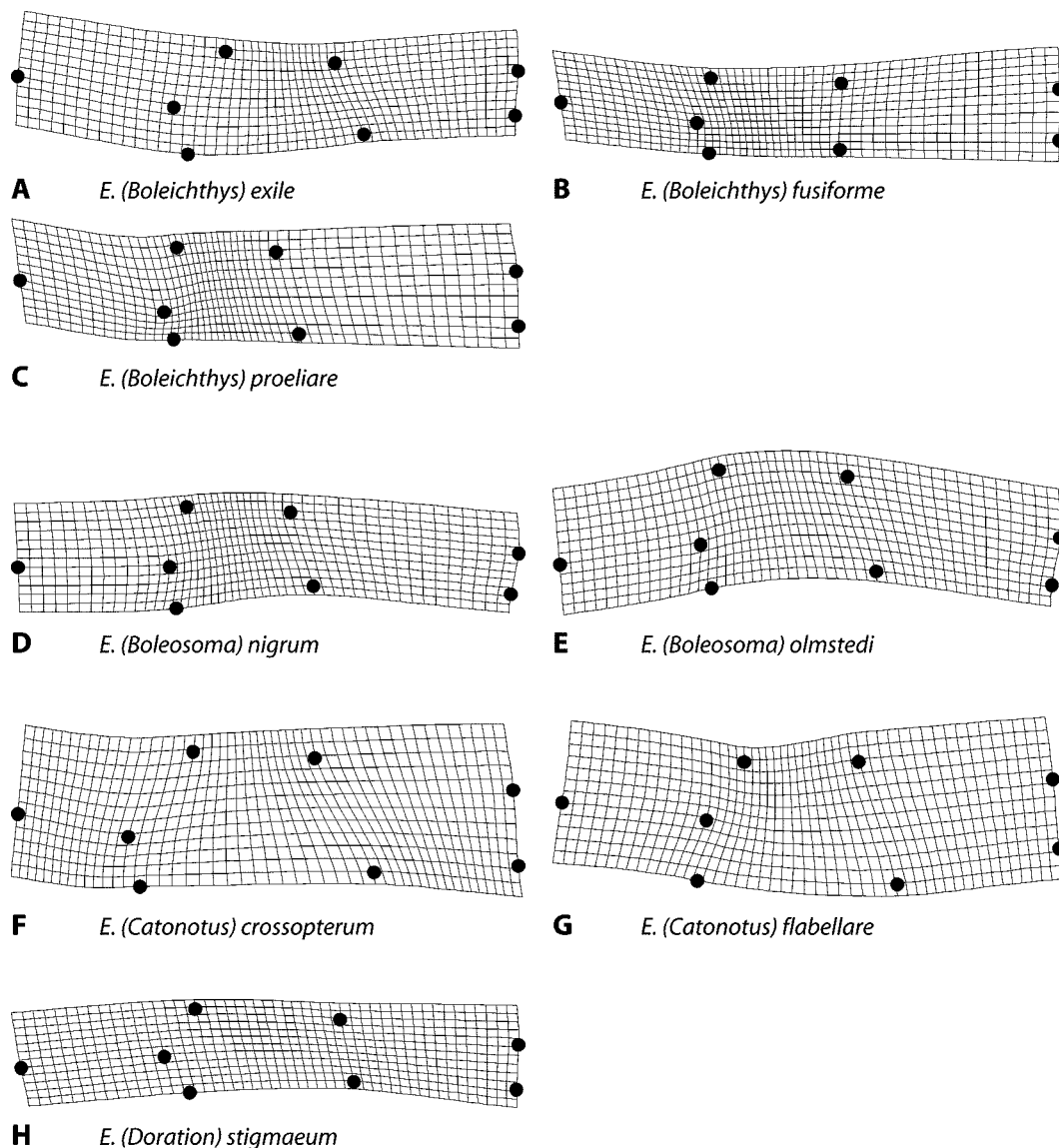


FIGURE 4. Cartesian transformation grids illustrating shape differences of species of the genus *Etheostoma*, subgenera *Boleichthys* (A–C), *Boleosoma* (D, E), *Catonotus* (F, G), and *Doration* (H). Deformations are exaggerated three times.

in most cases. The three species representing subgenus *Boleichthys* exhibit a somewhat shallow profile overall and a markedly shorter midsection than the consensus configuration. The two species representing subgenus *Boleosoma* also exhibit a shortening of the midsection in addition to a dorsal displacement of landmarks 2, 3, and 6 (particularly evident in the CTG for *E. olmstedii*) and the posterior displacement of landmark 4. Both of the species representing subgenus *Catonotus* exhibit deep body profiles, short first dorsal fins (indicated by the distance between landmarks 2 and 3), and a dorsal displacement of the anteriormost point on the head (landmark 1). In contrast, all five species representing subgenus *Etheostoma* (s.s.) have landmark 1 displaced ventrally to some extent. Additional features common to *Etheostoma* s.s. include a short head region, and relatively deep body pro-

file. The two species representing subgenus *Nothonotus* are relatively deep-bodied, with shortened tail regions. *Etheostoma juliae* exhibits a markedly dorsal displacement of landmark 1, a minor feature in *E. jordani*. The only readily apparent feature common to the two species representing subgenus *Ulocentra* is a slightly shortened head region. These species differ in displacement of landmark 1 (ventrally in *E. coosae* and somewhat dorsally in *E. simoterum*). The two subspecies of *E. simoterum* are highly similar, although minor differences are discernible regarding the dorsal displacement of landmarks 1 (*E. simoterum atripinne*) and 2 (*E. s. simoterum*) relative to the consensus and the relative depth of the caudal peduncle (landmarks 4 and 5). *Oligocephalus* is the only subgenus for which the representative species show no readily discernible common deviations from the consensus

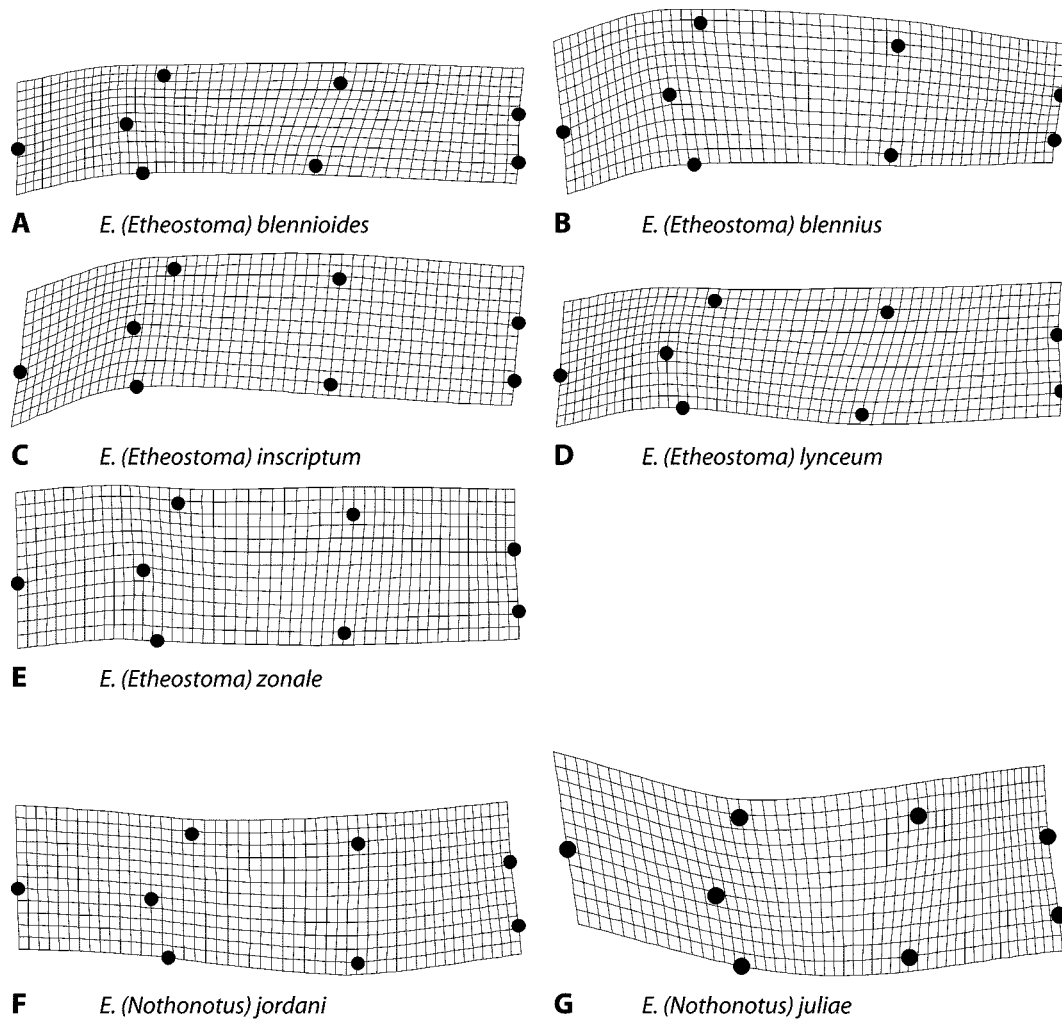


FIGURE 5. Cartesian transformation grids illustrating shape differences of species of genus *Etheostoma*, subgenera *Etheostoma* (A–E), and *Nothonotus* (F, G). Deformations are exaggerated three times.

configuration. Among the five species considered, *E. radiosum* and *E. whipplei* appear to be most similar in body form.

The regression of all pairwise distances between shapes in tangent space onto their Procrustes distances in Kendall's shape space yields a line with slope 0.999, and the two measures are perfectly correlated ($r = 1.0$); therefore, statistical analyses based on measures in tangent space should be valid. The CVA of shape variability among species clearly shows that the differences among species are highly significant (Wilks' $\Lambda = 6.14 \times 10^{-6}$; $F_{144, 8971.7} = 190.865$; $P < 10^{-8}$), and ordination plots based on the first three canonical variates axes (Fig. 7) further support the conclusion that relatively closely related species (as defined by their traditional taxonomic affiliations, i.e., genus and subgenus) are generally similarly shaped; species centroids tend to cluster by taxon. The UPGMA cluster analysis of generalized distances between species lends additional support to this conclusion (Fig. 8). Of the 11 traditionally recognized taxonomic groups (genera and subgenera) that were rep-

resented by two or more species, 5 clustered unequivocally: *Ammocrypta*, *Hadropterus*, *Boleosoma*, *Catonotus*, and *Ulocentra*. Additionally, two of the three representatives of subgenus *Boleichthys* clustered, as did three of the five representatives of *Etheostoma* s.s. and four of the five

TABLE 1. Results of Mantel matrix correlation analyses of the relationship between body shape with phylogeny based upon two phylogenies (SNP3 and SNP4) presented by Song et al. (1998) and two phylogenies (WM1 and WM3) presented by Wood and Mayden (1997); r is the normalized Mantel statistic and P is its estimated probability value. Analyses were conducted with the full data set of species (full set) and with a reduced data set with a single representative species per subgenus.

Phylogeny	Full data		Reduced data	
	r	P	r	P
SNP3	0.443	0.0009	0.334	0.0801
SNP4	0.298	0.0186	0.252	0.0869
WM1	0.212	0.0100	0.115	0.2070
WM3	0.349	0.0111	0.380	0.0158

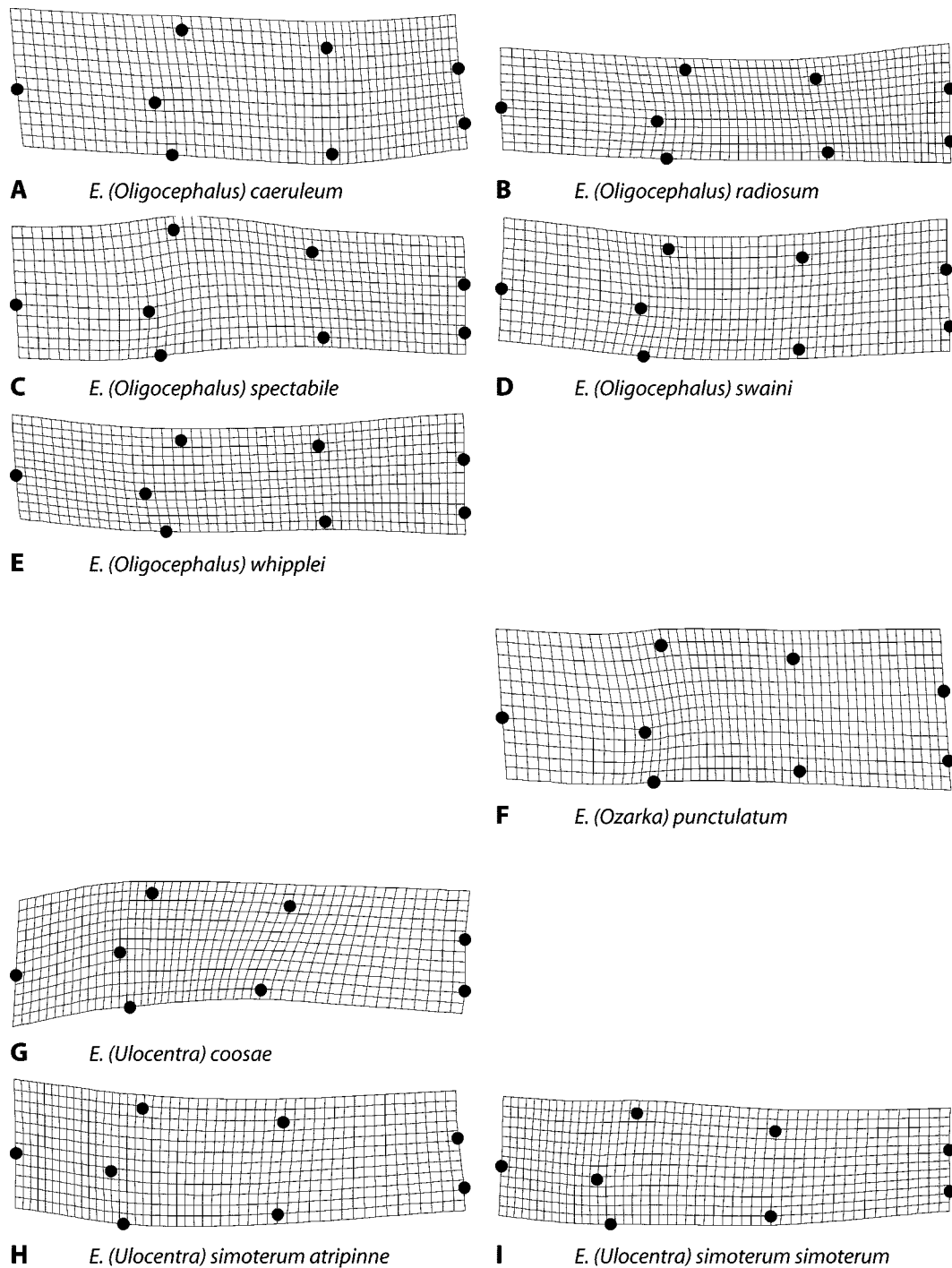


FIGURE 6. Cartesian transformation grids illustrating shape differences of species and subspecies of genus *Etheostoma*, subgenera *Oligocephalus* (A–E), *Ozarka* (F), and *Ulocentra* (G–I). Deformations are exaggerated three times.

representatives of *Percina* s.l. Three of the four traditionally recognized pairs of sister species (or subspecies) in our data set also clustered: *E. radiosum*/*E. whipplei*, *E. nigrum*/*E. olmstedii*, and *E. simoterum simoterum*/*E. s. atripinne*; the exception was *E. lynceum*/*E. zonale*.

The Mantel matrix correlations (Table 1) tested the hypothesis that closely related species tend to be sim-

ilarly shaped across the entire clade of darters, while taking into account the proposed relationships among and within the traditionally recognized genera and subgenera. Although there were the substantial differences among the phylogenies, all four tests showed a significant positive correlation between phylogenetic distances among species and their relative similarities in

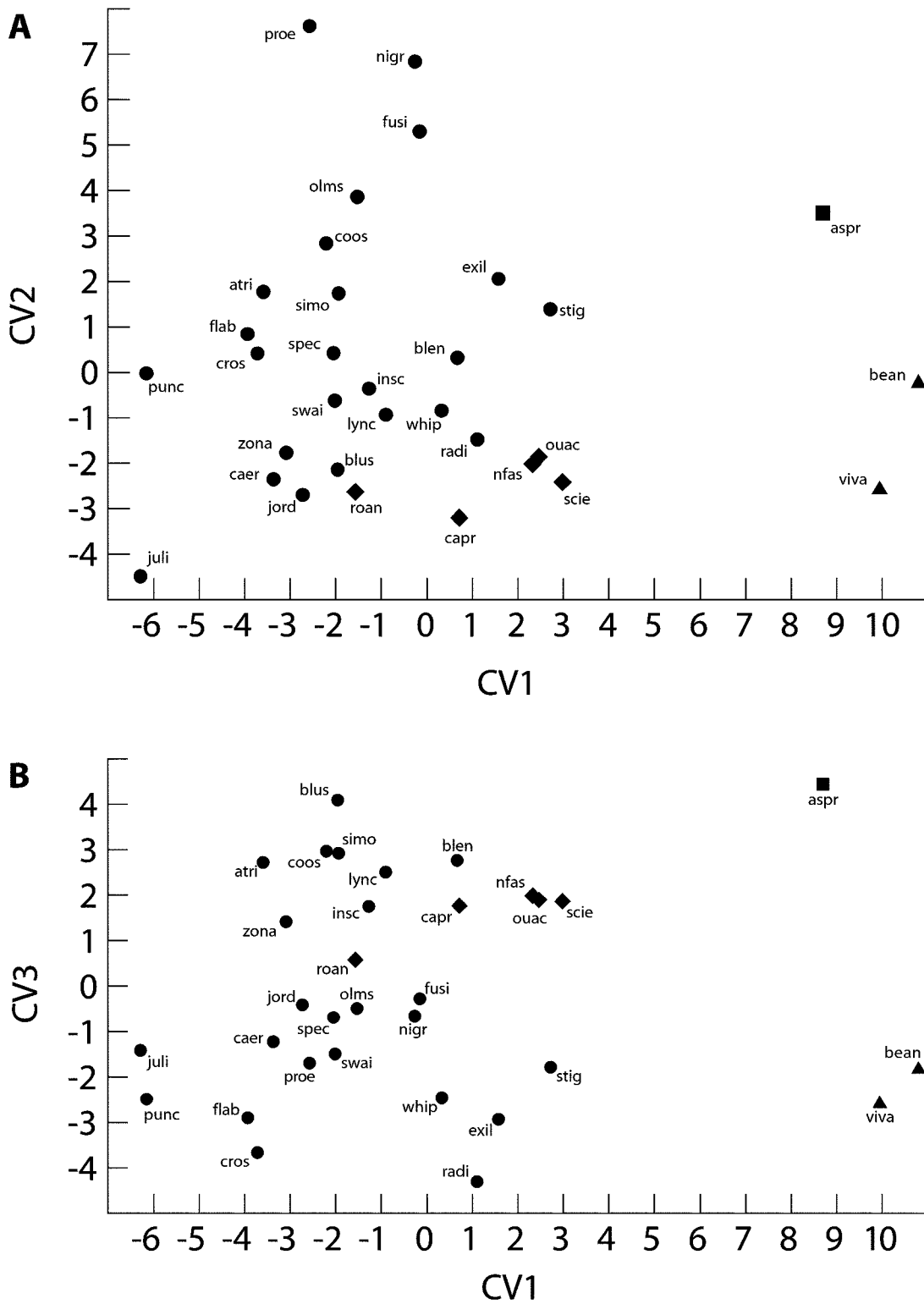


FIGURE 7. Relative body shape variation in darters as revealed by CVA. (A) First canonical variate plotted against the second. (B) First canonical variate plotted against the third. Overall similarities of body shapes are proportional to physical proximity of species' centroids in these three dimensions. Species are indicated by abbreviations consisting of the first four letters of their specific epithet (e.g., *E. proeliare* = proe), except in the cases of *E. blennioides* (blus), *E. simotermum atripinna* (atri), and *P. nigrofasciata* (nfas). Genera are represented by triangles (*Ammocrypta*), a square (*Crystallaria*), circles (*Etheostoma*), and diamonds (*Percina*).

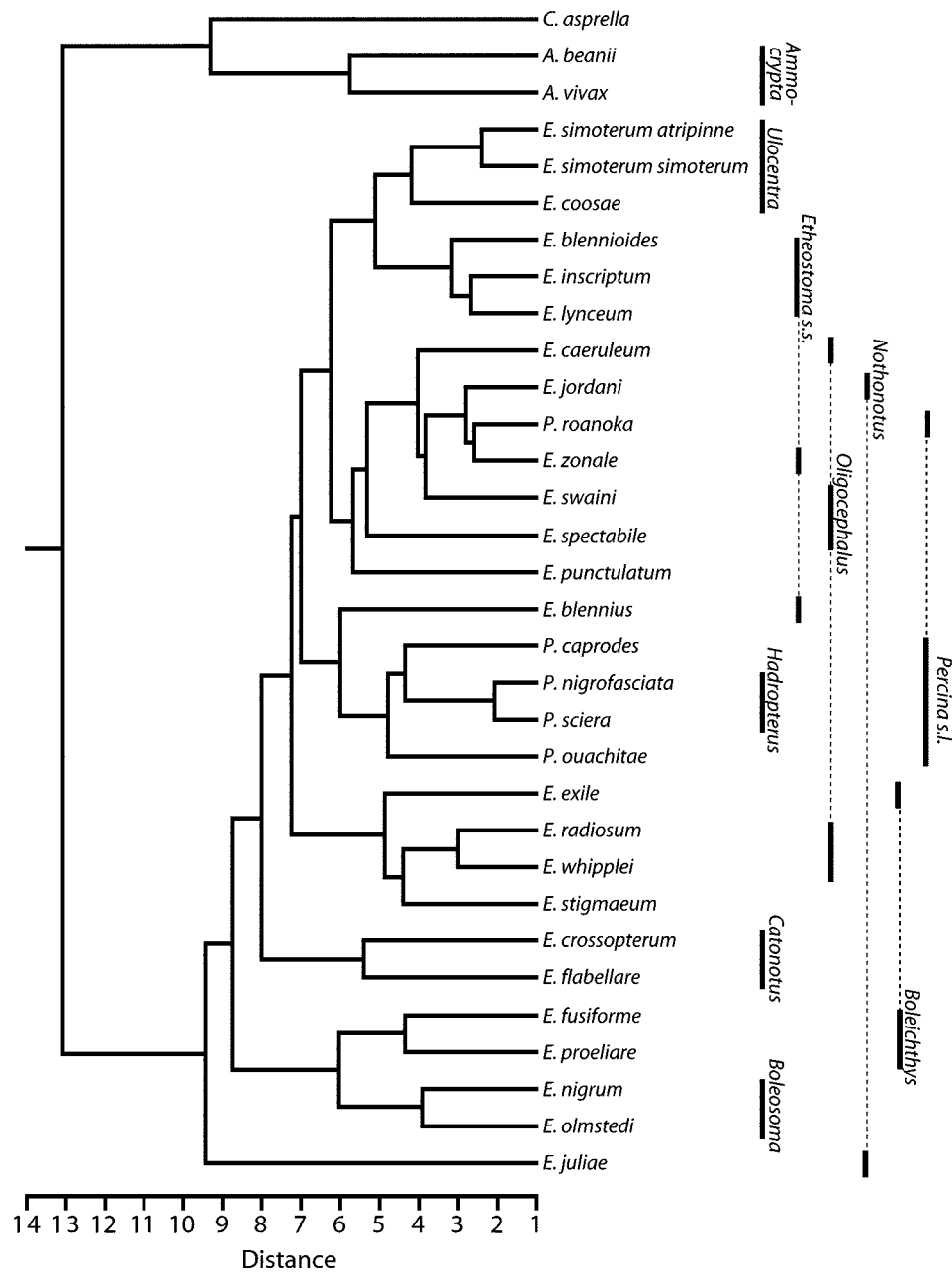


FIGURE 8. UPGMA phenogram illustrating relative similarities of body shapes among 32 taxa of darters. Solid vertical lines to the right of species names denote higher order taxa (subgenera and genera). Dashed lines connect members of higher order taxa with nonadjacent positions within the tree.

body shape. The results of the reanalyses of the reduced data sets (which in these tests include *Ammocrypta* and *Crystallaria*) are presented in the right half of Table 1. For three of the four phylogenies (SNP3, SNP4, and WM1), these reductions rendered the correlations nonsignificant ($P > 0.05$), suggesting that in these cases much of the phylogenetic effect demonstrated in the initial analyses was indeed attributable to the inclusion of multiple closely related species.

The preceding analyses were repeated with *A. beanii*, *A. vivax*, and *C. asprella* (or individuals of these species

in the case of the CVA) excluded because their extreme body shapes (see Figs. 3B–D, 7) were suspected of exerting a disproportionate influence. For the CVA and UPGMA cluster analysis, this exclusion resulted in very little changed. The differences among species revealed by CVA remained highly significant (Wilks' $\Lambda = 2.64 \times 10^{-5}$; $F_{144,8055.9} = 135.739$; $P < 10^{-8}$), and the relative locations of the remaining species' centroids in the ordination plot (not shown) of the recalculated CV2 (hereinafter CV2*) against recalculated CV1 (CV1*) were very similar to the configuration shown

TABLE 2. Results of Mantel matrix correlation reanalyses of the relationship between body shape and phylogeny, based on a full data set of species (full data) and a reduced data set with a single species per genus. The morphometrically distinct species *Ammocrypta beanii*, *A. vivax*, and *Crystallaria asprella* were excluded from both data sets.

Phylogeny	Full data		Reduced data	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
SNP3	0.189	0.0967	0.013	0.4261
SNP4	0.232	0.1314	0.272	0.1761
WM1	0.307	0.0008	0.196	0.0768
WM3	0.032	0.3885	0.030	0.4034

in Figure 7A, except rotated 90° counterclockwise. The plot of CV3* against CV1* (not shown) displayed an arrangement of species' centroids not seen previously but consistent with the interpretation that in most cases the centroids of taxonomically closely related species tend to cluster. The recalculated UPGMA cluster analysis yielded a phenogram (not shown) that differed from the arrangement of species in the original phenogram (exclusive of *A. beanii*, *A. vivax*, and *C. asprella*) in only two minor ways (cf. Fig. 8): the clades ((*E. blennioides*, *E. lynceum*) *E. inscriptum*) and (((*P. nigrofasciata*, *P. sciera*) *P. ouachitae*) *P. caprodes*). Neither of these changes alters any of the conclusions drawn from examination of the cluster analysis on the complete data set.

In contrast, substantial differences were found for the the Mantel correlations of phylogenetic and morphometric distance matrices that were recalculated from data sets from which *Ammocrypta* and *Crystallaria* had been excluded (Table 2). Only one of the correlations based on the full data sets (which included all species except *A. beanii*, *A. vivax*, and *C. asprella*) was significant, and none were significant for the reduced data set from which these species also were excluded.

DISCUSSION

We employed several complementary approaches to test how evolutionary relatedness is correlated with patterns of body shape variation among 32 taxa of darters. Readily discernible similarities were observed among species representing most of the traditionally recognized higher taxa (i.e., subgenera and genera) and were illustrated by ordination and cluster analysis. These observations generally support the descriptive accounts of previous authors (Kuehne and Barbour, 1983; Page, 1983; Page and Swofford, 1984; Bailey and Etnier, 1988). For example, Page and Swofford (1984:152) noted that *Percina roanoka* more closely resembles species of *Etheostoma* s.l. that occur in similar stream-riffle habitats than it does other members of *Percina* s.l. Their observation was confirmed by the present CTGs (Figs. 3, 5) and by the relative position of *P. roanoka* in the ordination plots (Fig. 7) and UPGMA phenogram (Fig. 8).

In addition to permitting the statistically robust quantification of size-free shape (Bookstein, 1991), our use of landmark-based geometric morphometrics has consid-

erably expanded upon previous observations regarding darter shape variation based on traditional morphometric methods. In the present study, we emphasized the unifying characteristics of shape found in most of the traditionally recognized taxa. However, the examination of CTGs readily exposes differences between otherwise relatively similarly shaped fishes such as *E. nigrum* and *E. stigmaeum* (Figs. 4D, 4H) that had not been investigated (Guill et al., 2003).

Phylogenetic Effects

Given that taxonomic categories fail to represent a large number of relationships within and among taxa, an explicitly phylogenetic approach was needed to verify these observations and to determine whether despite apparent cases of convergent evolution such as that of *P. roanoka* with other ecologically similar darters there was evidence of a phylogenetic effect across the entire set of species studied. The analysis was complicated by the existence of multiple competing phylogenies of the darters (Wood and Mayden, 1997; Song et al., 1998). Some authors have overcome similar predicaments by using composite or consensus phylogenies (e.g., Vanhooydonck and Van Damme, 1999; Jones and MacLarnon, 2001); however, such an approach for the present analyses would collapse almost all of the phylogenetically interesting nodes (see Fig. 1). We therefore opted to run analyses based upon all four phylogenies, as recommended by Harvey and Pagel (1991).

Because shape data of the sort generated by geometric morphometric analyses are inherently multivariate, measures of autocorrelation such as Moran's *I* (Gittleman and Kot, 1990) are inappropriate. The present analyses employ normalized Mantel statistics (Smouse et al., 1986) to evaluate the overall correlation of pairs of matrices representing the phylogenetic and phenotypic distances between species (Böhning-Gaese and Oberrath, 1999; Rohlf, 2001a). These correlations were significant and positive for matrices calculated from all four competing phylogenies, indicating that (1) body shape does exhibit phylogenetic effects in these species as a group as hypothesized and (2) despite their considerable differences, all four phylogenies convey sufficient phylogenetic signal to detect this effect.

These conclusions immediately raise the question of how four such different hypotheses of evolutionary relationship could all convey nontrivial phylogenetic signal. Several potential explanations were investigated through reanalysis of subsets of the data. One explanation involves the fact that each of the four phylogenies under consideration contains pairs and triplets of species that belong to traditionally recognized subgenera, and these groups invariably cluster at the tips of the phylogenies. Thus, much of the phylogenetic effect that was detected could have arisen from the fact that these species were grouped and not necessarily from the branching patterns within or among these groups. To test this proposal, we randomly excluded one or two species from each of these clusters and reran the analyses. For three

of the four phylogenies (SNP3, SNP4, and WM1), the resulting reductions in correlation and significance are consistent with the possibility that much of the phylogenetic signal was carried in these subgeneric nodes near the tips of the trees. The obvious objection that this could simply be a function of reduced sample sizes is countered by the fact that for the fourth case (WM3), the reduced data set yielded a slightly higher correlation, which remained significant at the 0.05 level.

A second possible explanation involves the potentially disproportionate effects that the inclusion of species of *Ammocrypta* and *Crystallaria* may have had on the analyses. Relative to other darters, species of these genera are highly distinct morphologically (although they are somewhat similar to each another; see Fig. 8), and in all four phylogenies, these genera are either sister to one another, partially or fully basal to the remainder of the phylogenies, or both (see Fig. 1). Reanalysis of the otherwise full data sets (from which the three species representing these genera had been excluded) yielded nonsignificant correlations for three of the four phylogenies (SNP3, SNP4, and WM3), suggesting that in these cases, one or both of these factors were responsible for much of the phylogenetic effect seen in the previous analyses that had included *Ammocrypta* and *Crystallaria*. In the fourth case (WM1), however, exclusion of these species nevertheless yielded a significant correlation that was somewhat higher than that found with them included. This correlation was rendered nonsignificant by eliminating redundant species, as was done in the previous analyses. Therefore, the phylogenetic effect originally found for each of the four phylogenies appears largely attributable to some combination of these factors, i.e., inclusion of multiple representatives for many of the subgenera and/or inclusion of species with highly distinct body shape.

Ecomorphology

The relationships between freshwater fish body shape variation and various ecological and functional variables have been described by many authors (Gatz, 1979a, 1979b; Douglas, 1987; Winemiller, 1992; Toline and Baker, 1993; Wood and Bain, 1995). Webb (1984) presented a classification of fish body forms based on modes of locomotion and suggested that body form associates strongly with trophic niche and predator-prey relationships. Based on their general body shapes, most darters would likely be classified as body/caudal fin transient propulsion species under Webb's (1984) model and would therefore be expected to avoid sustained swimming, specialize on nonelusive prey, and use bursts of speed and rapid turns to avoid predators. As indicated by the common name "darter," this description is a fair characterization of these species. Most darters feed almost exclusively on benthic invertebrates, gleaning small, nonelusive prey from the substrate where most species also seek refuge from predators (Kuehne and Barbour, 1983; Page, 1983). Given these overall similarities in locomotion and feeding ecology, some other factor or factors likely produced the vari-

ety of body shapes exhibited among the subgroups of darters.

Various authors (Page, 1983; Page and Swofford, 1984; Wood and Bain, 1995) have found associations between darter body shapes and environmental parameters such as current speed, water depth, substrate composition, and availability of cover (see also Fisher and Pearson, 1987; Welsh and Perry, 1998). Several of the observations made by these authors are corroborated by the CTGs generated for the present study: (1) species primarily found in high-current environments (e.g., *E. caeruleum*, *E. juliae*, *E. jordani*, and *P. roanoka*) have deep caudal peduncles and generally robust forms (Page, 1983; Page and Swofford, 1984); (2) species found in moderate to slow currents (e.g., *E. exile*, *E. fusiforme*, *E. nigrum*, and *E. stigmaeum*) tend to be more fusiform and have narrow caudal peduncles (Wood and Bain, 1995); and (3) species inhabiting sandy environments (e.g., *A. beanii*, *A. vivax*, *C. asprella*, and *E. whipplei*) tend to be especially elongate, with narrow peduncles (Page and Swofford, 1984). The results of the present study show that correction for phylogenetic effects will indeed be necessary in future investigations of darter body shape variation, because there is significant correlation between evolutionary relationships and body shape no matter which of the presently considered phylogenies is employed.

Although almost two decades of active research into the effects of phylogeny on phenotypic variation have preceded the present study, much of this research has been focused on developing and refining the appropriate methodologies. Here, we provide a robust application of these new methods. Much basic research that includes the recognition and appropriate control for phylogenetic effects remains to be conducted. The combination of comparative methods appropriate to multivariate data sets with the powerful new tools of geometric morphometrics represents a potentially fruitful avenue of investigation into the effects of body shape on the biology of organisms.

ACKNOWLEDGMENTS

We are indebted to the curators and collection managers of the museums listed in the Appendix for the loan of specimens in their care and to F. James Rohlf for advice and assistance with the statistical analyses. Henry L. Bart, Jr., Duncan J. Irschick, Thomas W. Sherry, Paul M. Magwene, and one anonymous reviewer provided valuable comments on the manuscript. This paper represents a portion of a Ph.D. dissertation by J.M.G. C.S.H. was supported by a Faculty Research Grant and the Mullahy Fund, Loyola University. Morphometric software is available at <http://life.bio.sunysb.edu/morph>.

REFERENCES

- ABELL, A. J., B. J. COLE, R. REYES, AND D. C. WIERNASZ. 1999. Sexual selection on body size and shape in the western harvester ant, *Pogonomyrmex occidentalis cresson*. *Evolution* 53:535-545.
- BAILEY, R. M., AND D. A. ETNIER. 1988. Comments on the subgenera of darters (Percidae) with descriptions of two new species of *Etheostoma* (*Ulocentra*) from southeastern United States. *Misc. Publ. Mus. Zool. Univ. Mich.* 175:1-48.
- BART, H. L., JR., AND L. M. PAGE. 1992. The influence of size and phylogeny on life history variation in North American percids. Pages 553-572 in *Systematics, historical ecology, and North American*

- freshwater fishes (R. L. Mayden, ed.). Stanford Univ. Press, Stanford, California.
- BÖHNING-GAESE, K., AND R. OBERRATH. 1999. Phylogenetic effects on morphological, life-history, behavioural and ecological traits of birds. *Evol. Ecol. Res.* 1:347–364.
- BOOKSTEIN, F. L. 1991. Morphometric tools for landmark data: Geometry and biology. Cambridge Univ. Press, New York.
- BUTLER, M. A., T. W. SCHOENER, AND J. B. LOSOS. 2000. The relationship between sexual size dimorphism and habitat use in Greater Antillean *Anolis* lizards. *Evolution* 54:259–272.
- CARPENTER, K. E. 1996. Morphometric pattern and feeding mode in emperor fishes (Lethrinidae, Perciformes). Pages 479–487 in *Advances in morphometrics* (L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice, eds.). Plenum, New York.
- DOUGLAS, M. E. 1987. An ecomorphological analysis of niche packing and niche dispersion in stream fish clades. Pages 144–149 in *Community and evolutionary ecology of North American stream fishes* (W. J. Matthews and D. C. Heins, eds.). Univ. Oklahoma Press, Norman.
- FELSENSTEIN, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125:1–15.
- FISHER, W. L., AND W. D. PEARSON. 1987. Patterns of resource utilization among four species of darters in three central Kentucky streams. Pages 69–76 in *Community and evolutionary ecology of North American stream fishes* (W. J. Matthews and D. C. Heins, eds.). Univ. Oklahoma Press, Norman.
- FLEMING, I. A., AND M. R. GROSS. 1989. Evolution of adult female life history and morphology in a Pacific salmon (coho: *Oncorhynchus kisutch*). *Evolution* 43:141–157.
- FOSTER, S. A., J. A. BAKER, AND M. A. BELL. 1992. Phenotypic integration of life history and morphology: An example from three-spined stickleback, *Gasterosteus aculeatus* L. *J. Fish Biol.* 41:21–35.
- GARLAND, T., P. E. MIDFORD, AND A. R. IVES. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *Am. Zool.* 39:374–388.
- GATZ, A. J. 1979a. Community organization in fishes as indicated by morphological features. *Evolution* 60:711–718.
- GATZ, A. J. 1979b. Ecological morphology of freshwater stream fishes. *Tulane Stud. Zool. Bot.* 21:91–124.
- GITTLEMAN, J. L., AND M. KOT. 1990. Adaptation: Statistics and a null model for estimating phylogenetic effects. *Syst. Zool.* 39:227–241.
- GUILL, J. M., C. S. HOOD, AND D. C. HEINS. 2003. Body shape variation within and among three species of darters (Perciformes: Percidae). *Ecol. Freshwater Fish* 12:134–140.
- HARVEY, P. H., AND M. D. PAGEL. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, New York.
- HOOD, C. S., AND D. C. HEINS. 2000. Ontogeny and allometry of body shape in the blacktail shiner, *Cyprinella venusta*. *Copeia* 2000:270–275.
- IRSCHICK, D. J., T. E. MACRINI, S. KORUBA, AND J. FORMAN. 2000. Ontogenetic differences in morphology, habitat use, behavior, and sprinting capacity in two West Indian *Anolis* lizards. *J. Herpetol.* 34:444–451.
- JONES, K. E., AND A. MACLARNON. 2001. Bat life histories: Testing models of mammalian life-history evolution. *Evol. Ecol. Res.* 3:465–476.
- KINZINGER, A. P., R. M. WOOD, AND S. A. WELSH. 2001. Systematics of *Etheostoma tippecanoe* and *Etheostoma denoncourti* (Perciformes: Percidae). *Copeia* 2001:235–239.
- KUEHNE, R. A., AND R. W. BARBOUR. 1983. The American darters. Univ. Press of Kentucky, Lexington.
- LOSOS, J. B., AND B. SINERVO. 1989. The effect of morphology and perch size on sprint performance in *Anolis* lizards. *J. Exp. Biol.* 145:23–30.
- MARCUS, L. F., E. HINGST-ZAHER, AND H. ZAHER. 2000. Application of landmark morphometrics to skulls representing the orders of living mammals. *Hystrix It. J. Mamm. (N.S.)* 11:27–47.
- MARTINS, E. P. (ed.). 1996. Phylogenies and the comparative method in animal behavior. Oxford Univ. Press, New York.
- NAGEL, L., AND D. SCHLUTER. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution* 52:209–218.
- NEAR, T. J. 2002. Phylogenetic relationships of *Percina* (Percidae: Etheostomatinae). *Copeia* 2002:1–14.
- NEAR, T. J., J. C. PORTERFIELD, AND L. M. PAGE. 2000. Evolution of cytochrome *b* and the molecular systematics of *Ammocrypta*. *Copeia* 2000:701–711.
- PAGE, L. M. 1983. Handbook of darters. TFH, Neptune City, New Jersey.
- PAGE, L. M., AND D. L. SWOFFORD. 1984. Morphological correlates of ecological specialization in darters. *Environ. Biol. Fish.* 11:139–159.
- PAINE, M. D. 1990. Life history tactics of darters (Percidae: Etheostomatiini [sic]) and their relationship with body size, reproductive behaviour, latitude and rarity. *J. Fish Biol.* 37:473–488.
- REYMENT, R. A. 1991. Multidimensional palaeobiology. Pergamon Press, Elmsford, New York.
- ROHLF, F. J. 1997. tpsRegr—Thin-plate spline regression software, version 1.13. State Univ. New York, Stony Brook.
- ROHLF, F. J. 1998. tpsSmall, version 1.19. State Univ. New York, Stony Brook.
- ROHLF, F. J. 2001a. Comparative methods for the analysis of continuous variables: Geometric interpretations. *Evolution* 55:2143–2160.
- ROHLF, F. J. 2001b. NTSYSpc: Numerical taxonomy and multivariate analysis system, version 2.10x. Exeter Software, Setauket, New York.
- ROHLF, F. J., A. LOY, AND M. CORTI. 1996. Morphometric analysis of Old World Talpidae (Mammalia, Insectivora) using partial-warp scores. *Syst. Biol.* 45:344–362.
- ROHLF, F. J., AND D. SLICE. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* 39:40–59.
- ROSENBERG, M. S. 2002. Fiddler crab claw shape variation: A geometric morphometric analysis across the genus *Uca* (Crustacea: Brachyura: Ocypodidae). *Biol. J. Linn. Soc.* 75:147–162.
- RÜBER, L., AND D. C. ADAMS. 2001. Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. *J. Evol. Biol.* 14:325–332.
- SMOUSE, P. E., J. C. LONG, AND R. R. SOKAL. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst. Zool.* 35:627–632.
- SOKAL, R. R. 1979. Testing statistical significance of geographic variation patterns. *Syst. Zool.* 28:227–232.
- SONG, C. B., T. J. NEAR, AND L. M. PAGE. 1998. Phylogenetic relations among percid fishes as inferred from mitochondrial cytochrome *b* DNA sequence data. *Mol. Phylogenet. Evol.* 10:343–353.
- TOLINE, C. A., AND A. J. BAKER. 1993. Foraging tactic as a potential selection pressure influencing geographic differences in body shape among populations of dace (*Phoxinus eos*). *Can. J. Zool.* 71:2178–2184.
- VANHOYDONCK, B., AND R. VAN DAMME. 1999. Evolutionary relationships between body shape and habitat use in lacertid lizards. *Evol. Ecol. Res.* 1:785–805.
- WALKER, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.* 61:3–50.
- WEBB, P. W. 1984. Body form, locomotion and foraging in aquatic vertebrates. *Am. Zool.* 24:107–120.
- WELSH, S. A., AND S. A. PERRY. 1998. Habitat partitioning in a community of darters in the Elk River, West Virginia. *Environ. Biol. Fish.* 51:411–419.
- WINEMILLER, K. O. 1992. Ecomorphology of freshwater fishes. *Natl. Geogr. Res.* 8:308–327.
- WOOD, B. M., AND M. B. BAIN. 1995. Morphology and microhabitat use in stream fish. *Can. J. Fish. Aquat. Sci.* 52:1487–1498.
- WOOD, R. M., AND R. L. MAYDEN. 1997. Phylogenetic relationships among selected darter subgenera (Teleostei: Percidae) as inferred from an analysis of allozymes. *Copeia* 1997:265–274.

First submitted 26 August 2002; reviews returned 3 January 2003;
final acceptance 2 February 2003
Associate Editor: Junhyong Kim

APPENDIX MATERIAL EXAMINED

Material was provided by the following institutions: Tulane University Museum of Natural History (TU), University of Louisiana at Monroe Museum of Zoology (ULM), University of Michigan Museum of Zoology (UMMZ), and Cornell University Museum of Vertebrates (CU). The following species were examined: *Ammocrypta*

- beanii*: Catahoula Cr., Hancock Co., MS; Jourdan R. Dr. (collected by John Baker and David Heins: JAB 86-18; 12 April 1986). *A. vivax*: Village Cr., Hardin Co., TX; Neches R. Dr. (TU 108365; 14 July 1978). *Crystallaria asprella*: Pearl R., Marion Co., MS; Pearl R. Dr. (TU 15174; 22 January 1957). *Etheostoma blennioides*: Big R., St. Francois Co., MO; Mississippi R. Dr. (ULM 29865; 16 April 1974). *E. blennius*: Duck R., Bedford Co., TN; Tennessee R. Dr. (collected by Kyle Piller, Michael Guill, and Marcus Wasilevich: KRP 18-00; 01 April 2000). *E. blennius*: Duck R., Bedford Co., TN; Tennessee R. Dr. (collected by Kyle Piller, Michael Guill, and Marcus Wasilevich: KRP 19-00; 01 April 2000). *E. caeruleum*: Big Cr., Sharp Co., AR; Strawberry R. Dr. (TU 96693; 06 April 1975). *E. coosae*: Duck Cr., Walker Co., GA; Alabama R. Dr. (TU 40678; 14 April 1966). *E. coosae*: Talking Rock Cr., Pickens Co., GA; Alabama R. Dr. (TU 40724; 15 April 1966). *E. coosae*: Hatchet Cr., Coosa Co., AL; Alabama R. Dr. (TU 41138; 13 April 1966). *E. crossopterum*: W. Frk. Stones R., Rutherford Co., TN; Cumberland R. Dr. (collected by Kyle Piller, Michael Guill, and Marcus Wasilevich: KRP 17-00; 01 April 2000). *E. exile*: Maceday L., Oakland Co., MI; Lake St. Clair Dr. (UMMZ 81978; 10 May 1927). *E. flabellare*: Bay Fk., Allen Co., KY; Barren R. Dr. (TU 81877; 11 April 1973). *E. fusiforme*: Black Cr., Walton Co., FL; Chocktawhatchee R. Dr. (TU 105558; 11 January 1978). *E. inscriptum*: Candler Cr., Hall Co., GA; Altamaha R. Dr. (TU 116954; 01 April 1980). *E. jordani*: Chestnut Cr., Chilton Co., AL; Alabama R. Dr. (TU 52168; 23 May 1968). *E. juliae*: White R., Washington Co., AR; White R. Dr. (TU 46063; 12 June 1962). *E. lynceum*: Homochitto R., Lincoln Co., MS; Mississippi R. Dr. (collected by Michael Guill and Jonathan Barmore: JMG 000420.1; 20 April 2000). *E. nigrum*: Swift Cr., Nash Co., NC; Tar R. Dr. (TU 71512; 11 April 1971). *E. olmstedii*: Catatunk Cr., Tioga Co., NY; Susquehanna R. Dr. (TU 695; 13 May 1950). *E. proeliare*: Pearl R., Washington Parish, LA; Pearl R. Dr. (TU 56447; 07 February 1969). *E. punctulatum*: Spring R., Lawrence Co., MO; White R. Dr. (collected by Kyle Piller and Michael Guill: KRP 09-00; 11 March 2000). *E. radiosum*: Glover R., McCurtain Co., OK; Glover R. Dr. (TU 147456; 22 April 1982). *E. simoterum atripinne*: Smith Frk., Wilson Co., TN; Cumberland R. Dr. (collected by Kyle Piller, Michael Guill, and Marcus Wasilevich: KRP 15-00; 31 March 2000). *E. simoterum simoterum*: Copper Cr., Scott Co., VA; Tennessee R. Dr. (TU 71981; 13 April 1971). *E. spectabile*: Barren Frk., Miller Co., MO; Osage R. Dr. (collected by Kyle Piller and Michael Guill: KRP 05-00; 09 March 2000). *E. stigmaeum*: Six Mile Cr., Bibb Co., AL; Cahaba R. Dr. (TU 152160; 12 May 1988). *E. swaini*: Amite R., Amite Co., MS; L. Ponchatrain Dr. (ULM 49739; 27 March 1982). *E. whipplei*: Big Sand Cr., Claiborne Co., MS; Mississippi R. Dr. (collected by Michael Guill and Terenia Guill: JMG 000409.1; 09 April 2000). *E. whipplei*: Big Sand Cr., Claiborne Co., MS; Mississippi R. Dr. (collected by Michael Guill and Jonathan Barmore: JMG 000420.3; 20 April 2000). *E. zonale*: French Cr., Mercer Co., PA; Allegheny R. Dr. (CU 5927; 30 May 1935). *Percina caprodes*: Llano R., Kimble Co., TX; Colorado R. Dr. (TU 97427; 14 February 1976). *P. nigrofasciata*: Pushepatapa Cr., Washington Parish, LA; Pearl R. Dr. (TU 43467; 18 February 1967). *P. ouachitae*: Pearl R., Washington Parish, LA; Pearl R. Dr. (TU 43279; 08 February 1967). *P. roanoka*: Pigg R., Franklin Co., VA; Roanoke R. Dr. (TU 69187; 13 April 1971). *P. roanoka*: Pigg R., Franklin Co., VA; Roanoke R. Dr. (TU 69303; 13 April 1971). *P. sciera*: Strong R., Simpson Co., MS; Pearl R. Dr. (TU 32883; 17 May 1963).