

Body shape variation within and among three species of darters (Perciformes: Percidae)

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Abstract – Using geometric morphometric (GM) techniques, we quantified intra and interspecific variation in female body shape using five collections each of *Etheostoma caeruleum* Storer, *Etheostoma nigrum* Rafinesque, and *Etheostoma stigmaeum* (Jordan). Nested multivariate analysis of variance showed significant variation in body shape among populations (Wilks' $\Lambda = 0.0687$, $F_{144,7585.1} = 19.35$, $P < 0.0001$), as well as among species (Wilks' $\Lambda = 8.7 \times 10^{-6}$, $F_{24,2} = 28.18$, $P = 0.0348$). *Etheostoma caeruleum* displayed greater body depth relative to both *E. nigrum* and *E. stigmaeum*, whereas *E. nigrum* displayed a compressed arrangement of mid-body landmarks relative to both *E. stigmaeum* and *E. caeruleum*. The broader implications of these findings highlight the value of geometric morphometrics as both an exploratory and analytical approach. Conclusions drawn from comparisons among moderately differentiated darter species in future studies of body size and shape are likely robust to intraspecific variation within species, and will permit more rigorous investigations into the ecomorphology of these benthic stream fishes.

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Key words: darter; *Etheostoma*; geometric morphometrics; interspecific variation; intraspecific variation; thin-plate spline

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Un resumen en español se incluye detrás del texto principal de este artículo.

Introduction

Body shape is a major component of an organism's phenotype, and it bears directly on such important traits as feeding efficiency, locomotor performance, vulnerability to predators, and reproductive success – traits with substantial consequences for fitness. The darters (Perciformes: Percidae) are a large and diverse clade of stream fishes endemic to North America. Several previous studies (Page 1983; Page & Swofford 1984; Wood & Bain 1995) have explored the associations of darter body shape variation (as estimated by traditional morphometric techniques) and various environmental factors such as water depth, flow rate, and microhabitat use. Since the mid-1980s, however, several new methodological and analytical tools have been developed to facilitate the acquisition, interpretation and presentation of

shape data (Rohlf & Bookstein 1990; Bookstein 1991; Marcus et al. 1996). Collectively referred to as geometric morphometric (GM) techniques, these tools have been applied to a variety of questions regarding fish shape, including feeding ecology (Carpenter 1996; Caldecutt & Adams 1998), geographic variation (Corti & Crosetti 1996), sexual dimorphism and ontogenetic shape change (Hood & Heins 2000 and references therein) and systematics (Fink & Zelditch 1997). Indeed, over the past century, fishes have served prominently as models in the development of the science of morphometrics (e.g., Thompson 1952; Bookstein et al. 1985). As of yet, however, GM techniques have not been used to assess body shape variation in the darters.

Thompson (1952) noted that the study of form should progress from qualitative to the quantitative. With regards to the darter literature, taxo-

nomic descriptions and revisions of many species, as well as the encyclopedic treatments by Kuehne & Barbour (1983) and Page (1983), have typically included qualitative estimates of overall body shapes (e.g., robust, compressed, fusiform, etc.). Subsequent ecomorphological (Page 1983, Chapter 5; Page & Swofford 1984) and ontogenetic (Paine 1986) studies adopted a more quantitative approach by including measurement ratios, univariate analyses, and ordination. Data and analyses such as these are increasingly prevalent in the darter literature, though they still tend to be measurement ratios (e.g., Wood & Mayden 1993), or focus on particular aspects of shape, such as elongation of the body or fins (e.g., Williams 1975; Heins & Baker 1989), or other characteristic features such as caudal peduncle depth (e.g., Bart & Taylor 1999), rather than considering overall body shape. In the present study, we build upon this prior work with several new visualization techniques afforded by the GM approach, and begin the process of more quantitatively assessing the relative magnitudes of intra and interspecific body shape variation in three species of darters.

To a certain extent, all darters exhibit relatively similar body forms. General descriptions emphasize their diminutive size, and their elongated and fusiform profile relative to other fish (Kuehne & Barbour 1983; Page 1983). Within these general characterizations, there is nevertheless a considerable degree of variation. For example, divergent body forms are found in the extremely long and cylindrical sand darters (*Ammocrypta*), the laterally compressed species of subgenus *Nothonotus* and the extremely small species of subgenus *Microperca*. Conversely, some closely related species (e.g., *E. nigrum* and *E. olmstedii* Storer) are so similar as to require reference to particularly subtle meristic data to distinguish them reliably.

Given this range of darter shape variation, we selected three study species representing three subgenera within the genus *Etheostoma*: *Etheostoma (Oligocephalus) caeruleum* Storer, *Etheostoma (Boleosoma) nigrum* Rafinesque and *Etheostoma (Doration) stigmaeum* (Jordan) (see Fig. 1). These choices were based on our qualitative assessment that the variation in the body shapes among these species is intermediate relative to the full range of shape variation exhibited

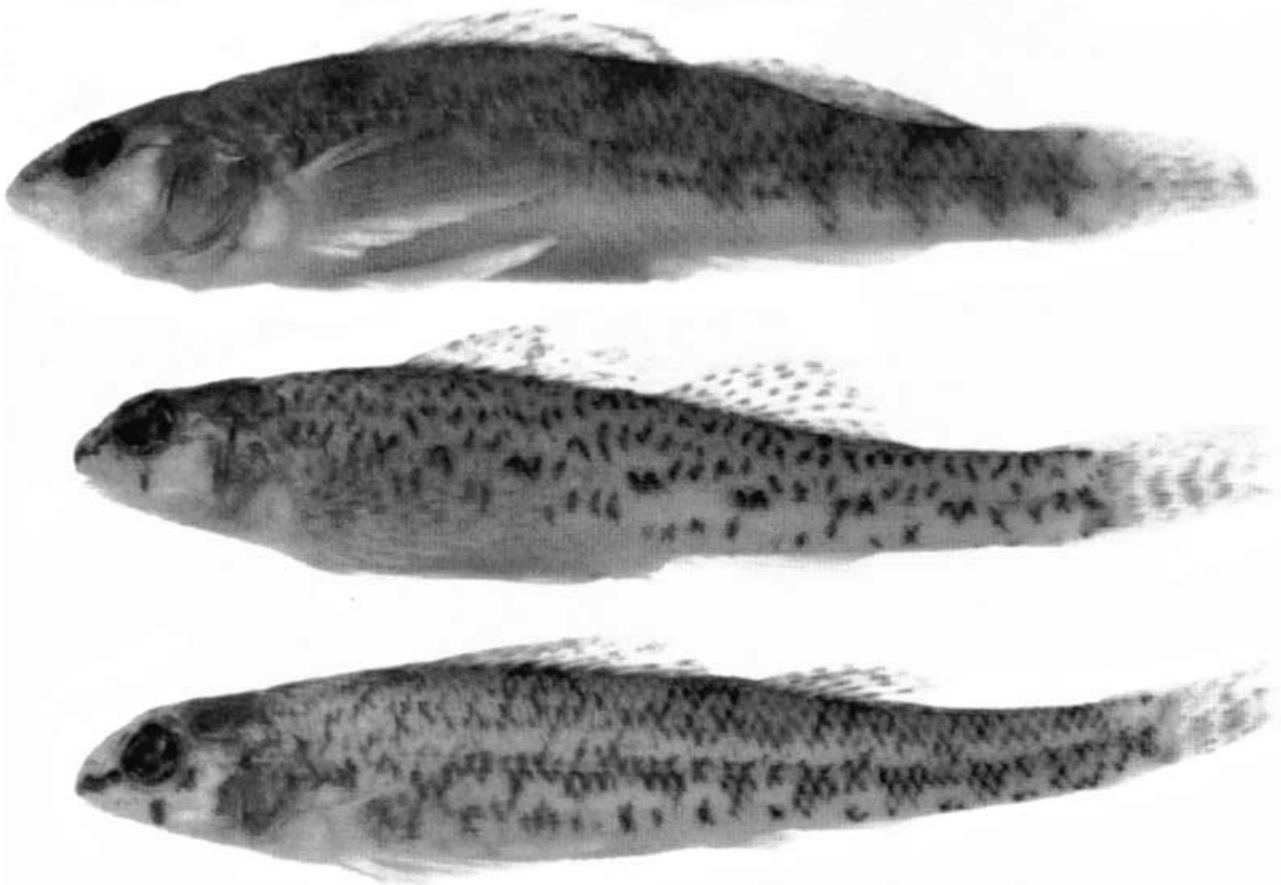


Fig. 1. Photograph of representative female specimens of each of the three species considered (top to bottom): *Etheostoma caeruleum* (TU 69866, 36.9 mm SL), *Etheostoma nigrum* (TU 69202, 34.5 mm SL) and *Etheostoma stigmaeum* (TU 76442, 38.2 mm SL).

by darters as a group. Males and juveniles were excluded from the analyses to reduce variation due to sexual dimorphism and ontogenetic allometry (cf. Hood & Heins 2000).

Materials and methods

We chose 15 large series of archived specimens of three species of darters from the Tulane University Museum of Natural History, with the goal of representing a diversity of collection localities and dates: *E. caeruleum*: TU 81856, Allen Co., KY, Barren R. Dr, 4/10/73; TU 96704, Sharp Co., AR, Strawberry R. Dr, 4/6/75; TU 92578, Van Buren Co., AR, White R. Dr, 5/9/70; TU 69866, Wilkinson Co., MS, Mississippi R. Dr, 3/12/71; TU 96693, Sharp Co., AR, Strawberry R. Dr, 4/6/75. *E. nigrum*: TU 71512, Nash Co., NC, Tar R. Dr, 4/11/71; TU 69202, Franklin Co., VA, Roanoke R. Dr, 4/12/71; TU 71527, Halifax Co., NC, Tar R. Dr, 4/11/71; TU 3297, Sandusky Co., OH, Lake Erie Dr, 7/3/51; TU 72648, Franklin Co., VA, Roanoke R. Dr, 10/2/71. *E. stigmaeum*: TU 152160, Bibb Co., AL, Cahaba R. Dr, 5/12/88; TU 77022, Pickens Co., AL, Tombigbee R. Dr, 4/3/72; TU 56722, Santa Rosa Co., FL, Blackwater R. Dr, 3/7/69; TU 30222, Tuscaloosa Co., AL, Tombigbee R. Dr, 4/10/63; TU 76442, Calhoun Co., AL, Alabama R. Dr, 3/15/72. We examined a total of 910 female specimens (*E. caeruleum*: $n = 99, 62, 61, 40, 40$; *E. nigrum*: 44, 94, 130, 39, 39; and *E. stigmaeum*: 52, 54, 84, 40, 32). Such large numbers of specimens were used to provide sufficient degrees of freedom and ample statistical power for the nested experimental design described below.

Females were initially identified based on external morphology, and the identifications were later confirmed by direct examination of the gonads. Specimens were chosen at random if all adult females in a given collection were not used. We digitized eight landmarks on each specimen (Fig. 2), using a GM workstation running MorphoSys (Meacham & Duncan 1993). Data files were translated to NTS format using Morpheus (Slice 1998). The subsequent computational procedures were done using the thin-plate spline software tpsRegr (Rohlf 1997): the landmark data were aligned using least-squares superimposition (thereby removing the effects of translation, rotation, and scale) and a consensus configuration was calculated. The software was then used to generate a matrix of affine components scores and partial warps scores, which are derived variables that describe shape variation in each individual specimen relative to the consensus configuration of all specimens (Bookstein 1991, 1996; Dryden &

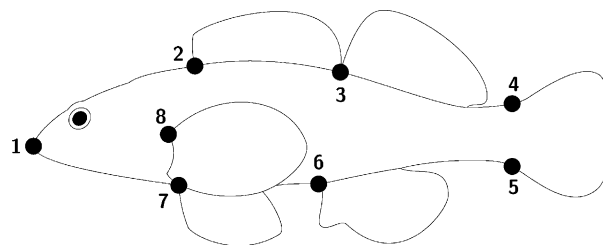


Fig. 2. Eight landmarks used in the present study of shape variation in three species of darters: (1) the anterior-most point on the head, the anterior junction of the (2) first and (3) second dorsal fins with the dorsal midline, the junction of the caudal fin with the (4) dorsal and (5) ventral midline, (6) the anterior junction of the anal fin with the ventral midline, (7) the point on ventral midline between the anterior junctions of the pelvic fins with the body and (8) the dorsal junction of the left pectoral fin with the body.

Mardia 1998). These values constituted the multivariate shape data set, upon which all further procedures and analyses were based.

Cartesian transformation grids (CTGs) were generated, depicting relative shape differences among species. This was done by regressing the shape data against dummy variable sets (scores of 1, 0, and -1 , assigned as appropriate for each contrast), and visualizing the results as deformation grids with tpsRegr (Rohlf 1997). To determine whether the apparent differences among species were significant, and, if so, to determine whether there is more variation among the three species than is explainable by variation among populations within species, the GLM procedure of SAS (1996) was used to perform a multivariate analysis of variance (MANOVA) on the shape data, with populations nested within species. Subsequent to MANOVA, a canonical centroid plot (Mardia et al. 1979, pp. 344–348) was generated using JMP (SAS Institute Inc. 1999) to depict relative shape differences among species and among populations within species.

Results

The consensus configuration and the CTGs depicting overall differences in shape between each species and the consensus are presented in Fig. 3; pairwise contrasts between species are given in Fig. 4. Inspection of these grids leads to the following general observations: *E. caeruleum* is deeper-bodied than the other two species, with a relatively shorter and deeper caudal peduncle and a disproportionately greater body depth at the origin of the anal fin. *Etheostoma stigmaeum* and *E. nigrum* are both relatively shallow-bodied, however the midsection of *E. nigrum* is relatively shorter than those of the other two species, with a much longer caudal peduncle. In

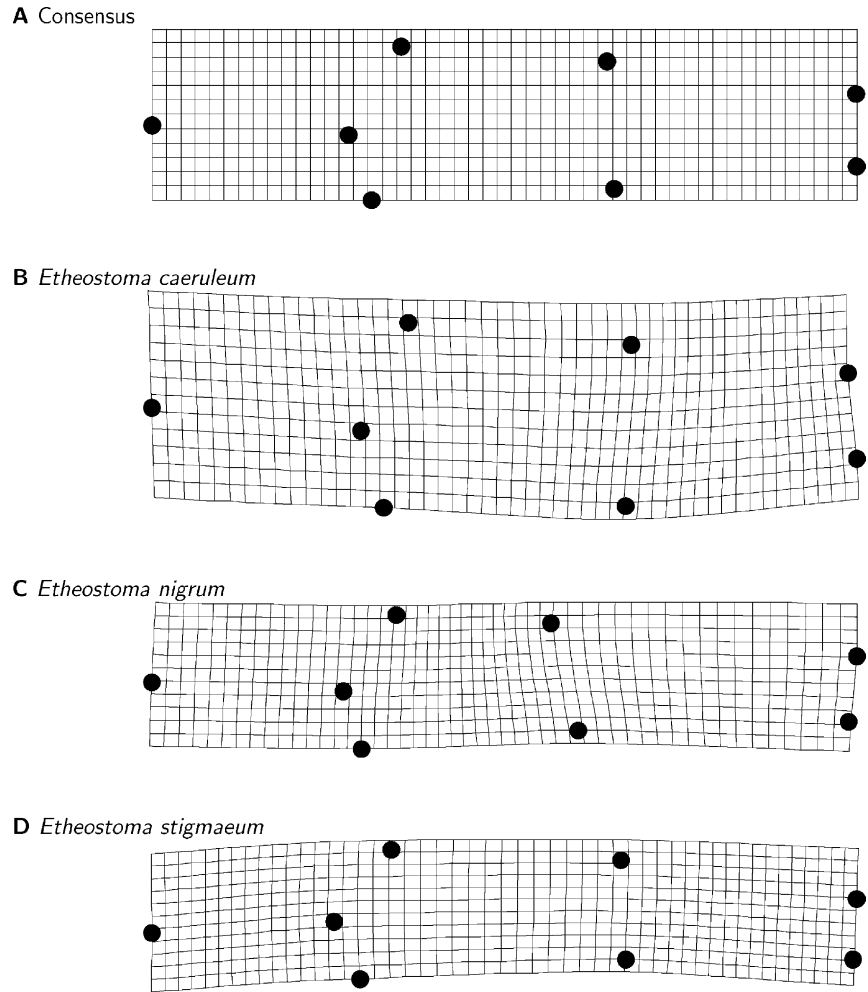


Fig. 3. Cartesian transformation grids depicting the consensus of all specimens examined (A), and the relative shape differences between the consensus and each of the three species (B–D). Deformations are exaggerated three times.

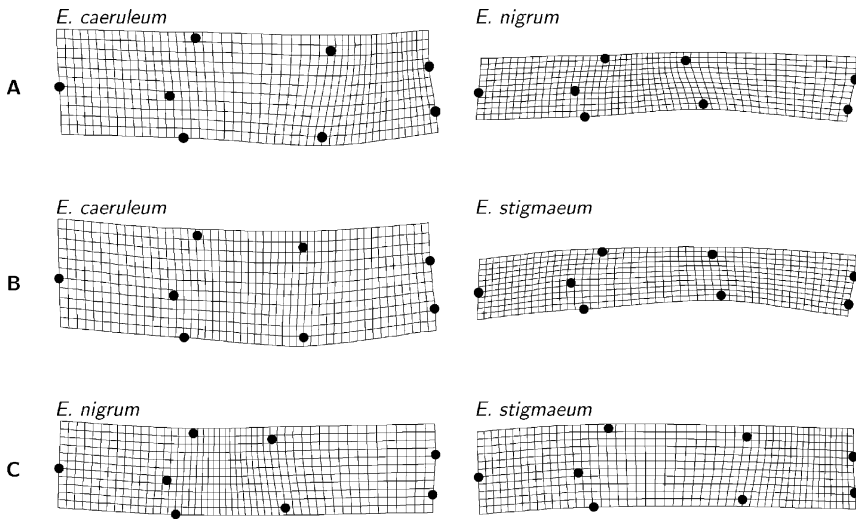


Fig. 4. Cartesian transformation grids depicting comparisons between each pair of species: (A) *Etheostoma caeruleum* versus *Etheostoma nigrum*; (B) *Etheostoma caeruleum* versus *Etheostoma stigmaeum*; and (C) *Etheostoma nigrum* versus *Etheostoma stigmaeum*. Deformations are exaggerated three times.

contrast, *E. stigmaeum* has a slightly elongated midsection, but otherwise shows little nonaffine variation from the consensus. In all three species, with the exceptions noted above, the relative positions of the origins of the pectoral, pelvic

and anal fins remains fairly consistent, as does the head length.

The nested MANOVA showed that despite significant interpopulational variation in female body shape within species (Wilks' $\Lambda = 0.0687$,

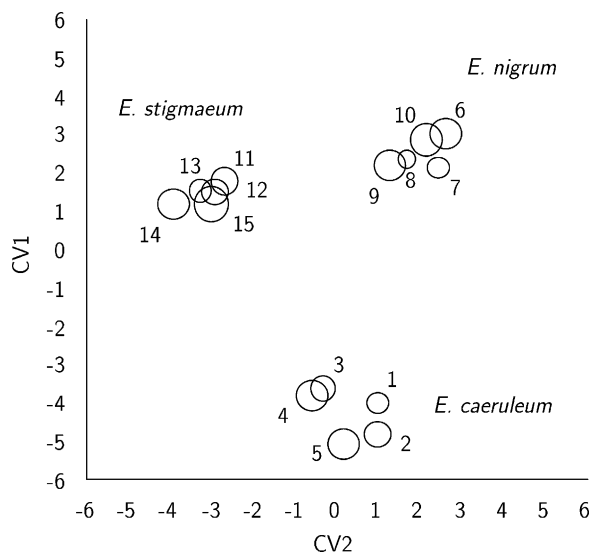


Fig. 5. Canonical centroid plot showing the relationships among the 15 sample populations with respect to the first two canonical variables. Populations are indicated by circles whose diameters represent the 95% confidence intervals around the population centroid.

$F_{144,7585.1} = 19.35$, $P < 0.0001$), there is significant variation among species (Wilks' $\Lambda = 8.7 \times 10^{-6}$, $F_{24,2} = 28.18$, $P = 0.0348$). Both of these conclusions are further supported by the canonical centroid plot (Fig. 5), which demonstrates that the population centroids vary within each species, but cluster strongly relative to those of the other two species.

Discussion

The present study of darter shape variation demonstrates significant variation within and among three species of moderately differentiated darters: *E. caeruleum*, *E. nigrum* and *E. stigmaeum*. Differences among the species fall into two broad categories: the first and most obvious reflects the significant and considerably greater body depth of *E. caeruleum* as compared to the other two species. This result is consistent with previous qualitative descriptions. Kuehne & Barbour (1983), for instance, offer terse descriptions of the body shapes of most darter species, including *E. caeruleum* (robust, p. 131) and *E. stigmaeum* (slender, p. 101). Although they do not explicitly describe the shape of *E. nigrum* in such terms, they do note the overall similarity between the subgenera to which *E. stigmaeum* (*Doration*) and *E. nigrum* (*Boleosoma*) belong.

More explicitly, Paine (1986) analyzed variation in five linear measurements (preanal length, head length, maximum depth, caudal peduncle

depth, snout length) for different age categories (embryo through adult) in three species, two of which (*E. caeruleum* and *E. nigrum*) overlap with the present study. He found highly significant differences between adult males of these two species in all five characters, consistent with the interpretation that adult *E. caeruleum* are deeper-bodied than are those of *E. nigrum*. Similarly, Page (1983, p. 176) presents a detailed table of body and fin proportions for 23 species of darters, including the three herein analyzed. Although a statistical test was not presented, these values are consistent with the interpretation that *E. caeruleum* has a deeper body (21% of SL) than *E. nigrum* or *E. stigmaeum* (both 17% of SL). This was also true of caudal peduncle depth: *E. caeruleum* (11% of SL) versus *E. nigrum* (9%) and *E. stigmaeum* (8%).

Page (1983) found an association between relatively deep bodies and deep caudal peduncles among darters inhabiting stream riffles, and he cited *E. caeruleum* as a particularly good example. This ecomorphological relationship contrasts with the relatively shallow bodies of benthic pool inhabitants such as *E. nigrum* and *E. stigmaeum*, an association also noted by Wood & Bain (1995) for *E. stigmaeum* in relation to several other species.

The second and less readily apparent difference among the species consists of the relatively compressed mid-body landmark geometry in *E. nigrum* relative to those of the other two species. To our knowledge, this difference has not been noted in any way in prior studies. This exemplifies one important advantage of the geometric morphometric approach over the techniques available to authors of earlier studies: it does not require the investigators to decide *a priori* which measurements are likely to reveal differences (Rohlf & Marcus 1993). Thus, whereas body depth was an obvious shape difference between *E. caeruleum* and the other two species from the outset, the more subtle differences between *E. stigmaeum* and *E. nigrum* would have been much more difficult to predict, and therefore to detect. The ecomorphological relevance of this difference, if any, is unclear. Page (1983) broadly classified both *E. stigmaeum* and *E. nigrum* as pool inhabitants, though Kuehne & Barbour (1983) noted some differentiation in the size of the streams inhabited, with *E. stigmaeum* sometimes replacing *E. nigrum* in larger streams.

Our analyses showed significant body shape differences among the three species beyond what would be expected, based on the variation among populations within those species. We interpret this to mean that had any one population of each

species been chosen for study, it would probably have been sufficiently representative to draw conclusions about differences at the species level. Although adequate characterization of intraspecific variation is desirable for studies of single species or species closely related to one another, this result suggests that single collections may be generally usable for broad-brush studies of variation among relatively well-differentiated species. For reasons of practicality, many darter studies – particularly those of an ecological or life-historical nature – have been based on specimens collected from a relatively narrow range of localities (often a single locality) and/or over a relatively short period of time (often a single date).

Much work remains to be done on the systematics, ecology, ethology and life histories of darters. As pointed out over two decades ago (Gould & Lewontin 1979), architectural constraints of the sort imposed by body shape may have an underappreciated role in the evolution of these characteristics. A more quantitative approach to the study of shape and an increased appreciation for its potential importance is therefore warranted.

Resumen

1. Utilizando técnicas de morfometría geométrica, cuantificamos variaciones intra- e inter-específica en la forma del cuerpo de las hembras de cinco colecciones de trece e-species de la familia Percidae: *Etheostoma caeruleum* Storer, *E. nigrum* Rafinesque, y *E. stigmaeum* (Jordan).
2. Análisis de la varianza multivariante anidado mostró variaciones significativas en la forma del cuerpo entre poblaciones (Wilks' $\Lambda = 0.0687$, $F_{144,7585.1} = 19.35$, $P < 0.0001$) tanto como entre especies (Wilks' $\Lambda = 8.7 \times 10^{-6}$; $F_{24,2} = 28.18$, $P = 0.035$). *E. caeruleum* exhibió mayor profundidad de cuerpo relativo a *E. nigrum* y a *E. stigmaeum* mientras que *E. nigrum* mostró un diseño más comprimido del cuerpo medio respecto de ambas, *E. stigmaeum* y *E. caeruleum*.
3. Las implicaciones de estos resultados enfatizan el valor de la morfometría geométrica como aproximación exploratoria y analítica. Las conclusiones obtenidas comparando especies de Percidae moderadamente diferenciadas en tamaño y forma del cuerpo son consistentes con las variaciones intra-específicas dentro de especies y permitirán, en futuros estudios, investigaciones más rigurosas sobre la ecomorfología de estas especies bentónicas de río.

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