ONTOGENETIC ALLOMETRY OF BODY SHAPE IN SQUATINA GUGGENHEIM MARINI, 1936 (ELASMOBRANCHII: SQUATINIDAE)

Mauro J. CAVALCANTI

Ecoinformatics Studio, PO Box 18123, CEP 20720-970, Rio de Janeiro, RJ, Brazil.
Corresponding author: maurobio@gmail.com

Resumo
As espécies do gênero *Squatina* são morfologicamente muito similares e pouco se conhece sobre seus padrões de variação da forma corporal. Neste trabalho, as alterações ontogenéticas na forma do corpo de *Squatina guggenheim* Marini, 1936 foram analisadas com técnicas de morfometria geométrica. Foram tomadas 16 medidas morfométricas, a partir do protocolo geométrico de redes de treliças, resultantes da associação de oito marcos anatômicos definidos com base na morfologia externa de 30 exemplares de *S. guggenheim*, com comprimento total entre 198 e 661 mm. Para cada exemplar, as coordenadas cartesianas reconstruídas dos marcos anatômicos foram alinhadas pelo método generalizado dos mínimos quadrados, sendo determinada a regressão multivariada do tamanho do centroide sobre os componentes da forma. A maior parte das modificações ontogenéticas da forma ocorre ao longo do eixo lateral do corpo e podem ser descritas principalmente por um aumento relativo na largura da nadadeira peitoral em relação à cabeça. Entretanto, análises posteriores, com um maior número de exemplares, serão necessárias para corroborar os resultados aqui obtidos quanto aos padrões de crescimento e de alteração da forma durante a ontogenia de *S. guggenheim*.

Palavras-Chave: análise multivariada, cação-anjo, morfometria geométrica ontogenia.

Abstract
Species within the genus *Squatina* are morphologically very similar, and their patterns of body shape variation remain poorly understood. This study analyzed the ontogenetic changes in the body shape of *Squatina guggenheim* Marini, 1936, using geometric morphometric techniques. Sixteen morphometric measurements were taken using a geometric truss network protocol based on eight anatomical landmarks defined from the external morphology of 30 specimens of *S. guggenheim*, with total length ranging from 198 to 661 mm. For each specimen, the reconstructed cartesian coordinates of the anatomical landmarks were aligned using the generalized least squares method, and the multivariate regression of centroid size on shape components was determined. Most of the ontogenetic changes in shape occur along the lateral axis of the body, and can be described mainly by a relative increase in the width of the pectoral fin region, in relation to the head. However, further analyses with a larger sample size will be necessary to fully elucidate the patterns of growth and shape change during the ontogeny of this species.

Keywords: Angel shark, geometric morphometrics, multivariate analysis, ontogeny.

Introduction

The genus *Squatina* comprises 22 species of cartilaginous fishes, commonly known as angel sharks, that are characterized by a flattened body, large pectoral fins not attached to the head, lateral gill openings, and mouth almost terminal (Ellis *et al.*, 2021).

The species of *Squatina* are morphologically very similar and until recently, only one species, *Squatina argentina* (Marini, 1930) was considered to occur in the Southwestern Atlantic Ocean, from southern Brazil to Argentina (Cousseau, 1973; Figueiredo, 1977). However, biochemical genetics studies have shown the existence of three sympatric species of this genus in South Brazil (Solé-Cava *et al.*, 1983; Solé-Cava & Levy, 1987), that were later demonstrated to be also morphologically identifiable (Vooren & Silva, 1991). These three species are *Squatina argentina* (Marini, 1930), *Squatina guggenheim* Marini, 1936, and *Squatina occulta* Vooren & Silva, 1991. They differ in a number of morphological and reproductive characteristics, as well as in size. More recently, Vaz & de Carvalho (2018) described a fourth species, *Squatina varii*, from specimens collected from the Northeastern Brazilian continental slope (between 251-666 m in depth). Molecular genetic analyses based on mitochondrial cytochrome b and recombinant (r)DNA sequences carried out by Garcia *et al.* (2015) have corroborated the hypothesis of population differentiation in *Squatina guggenheim* from the Southwestern Atlantic in the vicinity of the La Plata River mouth and its maritime front, including, with samples from the outer continental shelf representing a group isolated from other areas.

Squatina guggenheim, the species here studied, attains smaller sizes than the other three species (maximum length: 124 cm TL male; 132 cm TL female) , from which it also largely differs in reproductive parameters of growth and reproduction (Vooren & Silva, 1991). In a multivariate morphometric analysis of the species of *Squatina* of Southern Brazil, using linear discriminant functions, Vooren & Silva (1991) found that the differences in body proportions between specimens of *S. guggenheim* from different regions in their study area were of a similar magnitude. However, the patterns of intraspecific variation in body form during growth (ontogenetic allometry) in this species remains to be investigated. It has a lower fecundity and a lower body mass at birth, with a correspondingly reduced yolk reserve of the embryo. These differences in growth and reproductive parameters, coupled with little change in morphology, were suggested by Vooren & Silva (1991) as the main cause of speciation in *Squatina*.

In the present study, the ontogenetic changes in body shape of *Squatina guggenheim* are examined with techniques of geometric morphometrics, that allow the precise and detailed analysis of shape variation in organisms on the basis of coordinate data from homologous anatomical landmarks (Rohlf & Marcus, 1993; Adams *et al.* 2004, 2013). These methods represent an interesting alternative to traditional morphometrics, based on measured distances, and have demonstrated to be particularly relevant to allometric studies in fishes (Walker, 1993; Zelditch & Fink, 1995; Loy *et al.*, 1996, 1998; Reis *et al.*, 1998; Gallo *et al.*, 2002).

MATERIALS AND METHODS

Study area

The analysis was performed on the cartesian coordinates of eight two-dimensional landmarks defined on the basis of the external morphology, from 30 specimens of *S. guggenheim* (11 males and 19 females), ranging from 198 to 661 mm TL. Distance measurements based on a truss network protocol (Strauss & Bookstein, 1982; Figure 1) were taken among the landmarks with the aid of electronic calipers, accurate to 0.05 mm. The landmark coordinates for each specimen were reconstructed from these measurements by a simplified multidimensional scaling algorithm (Carpenter *et al.*, 1996), using the UNFOLD program written by H. J. S. Sommer. Specimens examined were captured along the coasts of Rio de Janeiro and Santa Catarina (southeastern Brazil) and are deposited in the fish collection of the Departamento de Zoologia, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro (UERJ). Total length was measured after Compagno (1984).

The geometric size of each specimen was estimated by centroid size, defined as the square root of the summed squared distances of all landmarks to the centroid of the configuration (Bookstein, 1991). Centroid size is the only overall size estimate entirely uncorrelated with shape in the absence of allometry (Bookstein, 1991). Two-way Analysis of Variance (ANOVA: Sokal & Rohlf, 1995) was used to test the differences in centroid size between males and females.

The landmark coordinates for each specimen were aligned and superimposed by the Procrustes generalized orthogonal least squares (GLS) method (Rohlf, 1990; Rohlf & Slice, 1990) to remove non-shape variation. In this procedure, the configurations were scaled to unit centroid size and then centered and rotated, in order to minimize the sum of squared distances between the landmarks of each configuration to the corresponding landmarks of a reference or "consensus" configuration, computed as the mean landmark configuration of all specimens.

The aligned coordinates were regressed onto centroid size, using multivariate regression (Morrison, 1990). This approach allows to regress one independent variable (centroid size) onto several dependent ones (the aligned coordinates), and is adequate to study allometric shape changes by geometric morphometric methods (Rohlf, 1998).

Deformation grids using thin-plate splines were used to graphically portray the patterns of shape variation among the landmarks.

All computations were performed on an IBM-PC compatible microcomputer, with the tpsRegrw program, version 1.45 (Rohlf, 2015) and the geomorph package, version 4.0.7 (Adams & Otarola-Castillo, 2013; Baken *et al.*, 2021), using the R software for statistical computing and data visualization, version 4.3.2 (R Development Core Team, 2023).

RESULTS

The estimated configurations of the anatomical landmarks obtained from the multivariate regression analysis with centroid size are illustrated in Fig. 2. The regression scores of the specimens projected on centroid size indicates that shape changes in this species are allometric, that is, they show a dependence of shape on size (Fig. 3).

The ANOVA did not show significant sexual dimorphism in centroid size, but showed statistically significant differences in shape with size ($P < 0.001$) for the specimens included in the analysis (Tab. 1).

Table 1 – Results of multivariate regression for size covariation on body shape (allometry) of *Squatina*. Df, degrees of freedom; SS, sums of squares; MS, mean squares; Rsq, R-squared (coefficient of determination); F, F-values; Z, Z-values or standard score (standard deviations from the means); Pr(>|F|): probability to find F-values by chance ($^{***}P < 0.001$).

	Df	SS	MS	Rsq	F	Z	Pr(>F)
log(Csize)	1	0.013873	0.0138727	0.14664	4.8214	3.3027	0.0004***
sex	1	0.003427	0.0034272	0.03623	1.1911	0.5325	0.2978
log(Csize):sex	1	0.002495	0.0024946	0.02637	0.8670	0.0140	0.4943
Residuals	26	0.074810	0.0028773	0.79077			
Total	29	0.094605					

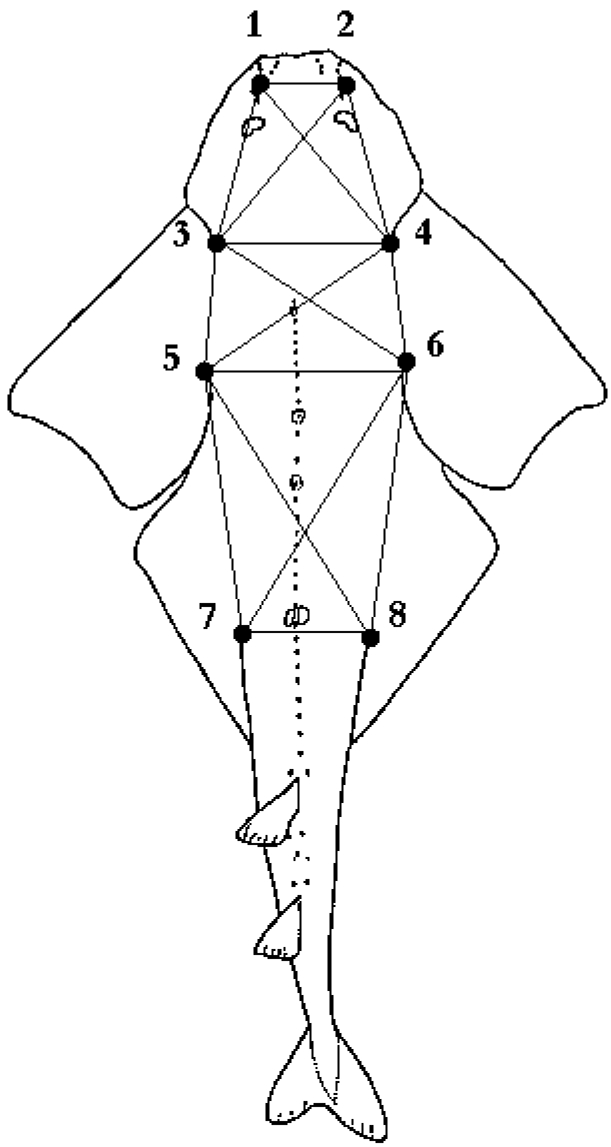

Figure 1 – Outline drawing of *Squatina guggenheim*, showing the locations of the 8 anatomical landmarks (numbered points) and distance measurements recorded on each specimen.

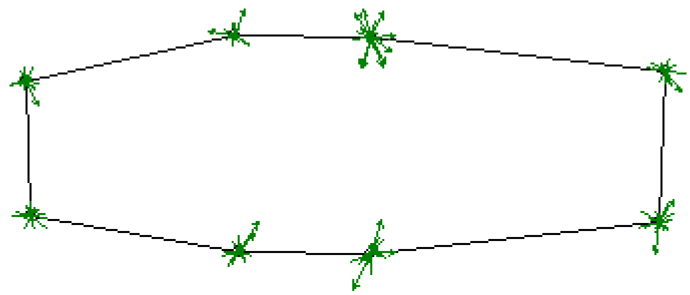

Figure 2 – Plot of mean configuration of *Squatina guggenheim* specimens with vectors indicating landmark displacements for increasing size.

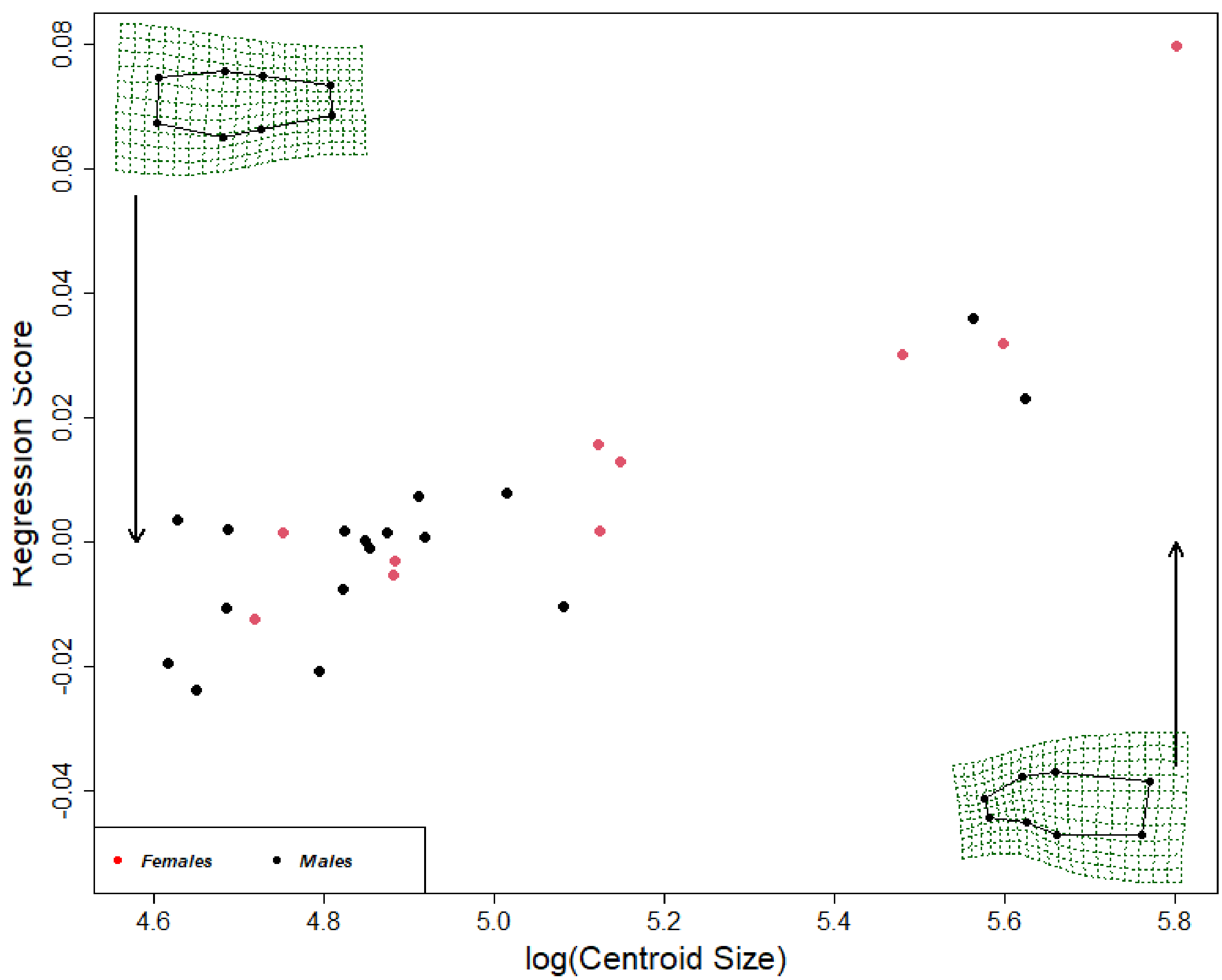

Figure 3 – Scatterplot of regression scores of *Squatina guggenheim* specimens on centroid size (log-transformed).

DISCUSSION

Most of the ontogenetic shape changes in *S. guggenheim* occur along the lateral axis of the body, and can be described mainly by a large relative increase in the width of the pectoral and pelvic fins, in relation to the head, from the smallest to the largest specimens. Larger specimens have thus a relatively smaller head and relatively larger pectoral and pelvic fins (Fig. 3). This result can be interpreted functionally, considering the adaptive significance of the pectoral and pelvic fins for locomotion and prey capture in *Squatina*. The enlarged pectoral fins allow for fast moving on the bottom and for bracing the individual on the substrate when it snaps forwards or upwards to catch active prey (Compagno, 1990). This feeding behavior has been observed in the aquarium by Vooren & Silva (1991) and captured in video by Fouts & Nelson (1999).

Further analyses with a greater number of specimens will still be necessary to better elucidate the patterns of growth and shape change during ontogeny occurring in this species. Nevertheless, the results obtained in this work clearly demonstrate the potential of geometric morphometric techniques, combined with multivariate statistical analysis methods, in studies of growth and evolutionary biology of fishes.

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REFERENCES

Adams, D.C., Rohlf, F.J. and Slice, D.E. (2004), Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, Vol. 71, pp. 5-16.

Adams, D.C., Rohlf, F.J. and Slice, D.E. (2013), A field comes of age: geometric morphometrics in the 21st century. *Hystrix*, Vol. 24, pp. 7-14.

Adams, D.C. and Otárola-Castillo, E. (2013), geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, Vol. 4, pp. 393-399.

Baken, E.K., Collyer, M.L., Kaliontzopoulou, A. and Adams, D.C. (2021), geomorph v4.0 and gmShiny: Enhanced analytics and a new graphical interface for a comprehensive morphometric experience. *Methods in Ecology and Evolution*, Vol. 12, pp. 2355-2363.

Bookstein, F.L. (1991), *Morphometric Tools for Landmark Data. Geometry and Biology*. Cambridge, Cambridge University Press, 435p.

Carpenter, K.E., Sommer, H.J., III and Marcus, L.F. (1996), Converting truss interlandmark distances to Cartesian coordinates. *In:* Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P. & Slice, D.E. (eds.), *Advances in Morphometrics*. NATO ASI Series A: Life Sciences, Vol. 284. Plenum Publishing, New York, pp. 103-111.

Compagno, L.J.V. (1984), FAO Species Catalogue. Vol. 4. Sharks of the World. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. *FAO Fish. Synop.* (125), Vol. 4, Pt. 2: 251-655.

Compagno, L.J.V. (1990), Alternative life-history styles of cartilaginous fishes in space and time. *Environmental Biology of Fishes,* Vol. 28, pp. 33-75.

Cousseau, M.B. (1973), Taxonomía y biología del pez angel, *Squatina argentina* Marini (Pisces, Squatinidae). *Physis*, Vol. 32, pp. 175-195.

Ellis, J.R., Barker, J., McCully Phillips, S.R., Meyers, E.K. & Heupel, M. (2021). Angel sharks (Squatinidae): A review of biological knowledge and exploitation. *Journal of Fish Biology*, Vol. 98, No. 3, pp. 592-621.

Figueiredo, J.L. (1977), *Manual de Peixes Marinhos do Sudeste do Brasil. I. Introdução. Cações, Raias e Quimeras*. São Paulo, Museu de Zoologia da Universidade de São Paulo, 104p.

Fouts, W.R. & Nelson, D.R. (1999), Prey capture by the Pacific angel shark, *Squatina californica*: visually mediated strikes and ambush-site characteristics. *Copeia*, Vol. 1999, No. 2, pp. 304-312.

Gallo, V., Cavalcanti, M.J. & Tintori, A. (2002), Using geometric morphometrics to study ontogenetic shape changes in *Paralepidotus ornatus* (Agassiz, 1833-43) (Actinopterygii, Semionotidae). Arquivos do Museu Nacional, Rio de Janeiro, Vol. 60, no. 3, pp. 117-120.

Garcia, G., Pereyra, S., Gutierrez, V., Oviedo, S., Miller, P., & Domingo, A. (2015). Population structure of *Squatina guggenheim* (Squatiniformes, Squatinidae) from the south-western Atlantic Ocean. Journal of Fish Biology, Vol. 86, pp. 186-202.

Loy, A., Cataudella, S. & Corti, M. (1996), Shape changes during the growth of the sea bass, *Dicentrarchus labrax* (Teleostea: Perciformes), in relation to different rearing conditions. An application of thin-plate spline regression analysis. *In:* Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P. & Slice, D.E. (eds.), *Advances in Morphometrics*. NATO ASI Series A: Life Sciences, Vol. 284. Plenum Publishing, New York, pp. 399-405.

Morrison, D.F. (1990), *Multivariate Statistical Methods*. 3rd ed. New York, McGraw-Hill. 495p.

R Core Team. (2023), *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Reis, R.E., Zelditch, M.L. & Fink, W.L. (1998), Ontogenetic allometry of body shape in the Neotropical catfish *Callichthys* (Teleostei: Siluriformes). *Copeia,* Vol. 1998, pp. 177-182.

Rohlf, F.J. (1990), Rotational fit (Procrustes) methods. *In*: Rohlf, F.J. & Bookstein, F.L. (eds.), *Proceedings of the Michigan Morphometrics Workshop*. Ann Arbor, The University of Michigan Museum of Zoology, Special Publication No. 2, pp. 227-236.

Rohlf, F.J. & Slice, D.E. (1990), Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology,* Vol. 39, pp. 40-59.

Rohlf, F.J. and Marcus, L.F. (1993), A revolution in morphometrics. *Trends in Ecology and Evolution*, Vol. 8, pp. 129-132.

Rohlf, F.J. (1998), On applications of geometric morphometrics to studies of ontogeny and phylogeny. *Systematic Biology,* Vol. 47, pp. 147-158.

Rohlf, F.J. (2015), The tps series of software. *Hystrix*, Vol. 26, pp. 9-12.

Sokal, R. R. & Rohlf, F. J. (1995), *Biometry: The Principles and Practice of Statistics in Biological Research*. San Francisco, W. H. Freeman, 887p.

Solé-Cava, A.M., Vooren, C.M. & Levy, J.A. (1983), Isozymic differentiation of two sibling species of *Squatina* (Chondrichthyes) in South Brazil. *Comparative Biochemistry and Physiology,* Vol. 75B, pp. 355-358.

Solé-Cava, A.M. & Levy, J.A. (1987), Biochemical evidence for a third species of angel shark off the East coast of South America. *Biochemical Systematics and Ecology,* Vol. 15, pp. 139-144.

Strauss, R.E. & Bookstein, F.L. (1982), The truss: body form reconstruction in morphometrics. *Systematic Zoology,* Vol. 31, pp. 113-135.

Walker, J.A. (1993), Ontogenetic allometry of three-spine stickleback body form using landmark-based morphometrics. *In*: Marcus, L.F., Bello, E. & García-Valdecasas, A. (eds.), *Contributions to Morphometrics*. Museo Nacional de Ciencias Naturales, Madrid, pp. 193-214.

Vaz, D.F.B. & de Carvalho, M.R. (2018). New species of *Squatina* (Squatiniformes: Squatinidae) from Brazil, with comments on the taxonomy of angel sharks from the Central and Northwestern Atlantic. *Copeia*, Vol. 106, No. 1, pp. 144-160.

Vooren, C.M. & Silva, K.G. (1991), On the taxonomy of the angel sharks from Southern Brazil, with the description of *Squatina occulta* sp. n. *Revista Brasileira de Biologia,* Vol. 51, pp. 589-602.

Zelditch, M.L. & Fink, W.L. (1995), Allometry and developmental integration of body growth in a piranha, *Pygocentrus nattereri* (Teleostei, Ostariophysi). *Journal of Morphology,* Vol. 223, pp. 335-341.