

AMANDA ALVES GOMES

**Exploring ecomorphological patterns and the evolution of shape in Stomiiformes
(Actinopterygii: Teleostei): a study of deep-sea fish adaptations**

São Paulo

2023

AMANDA ALVES GOMES

**Exploring ecomorphological patterns and the evolution of shape in Stomiiformes
(Actinopterygii: Teleostei): a study of deep-sea fish adaptations**

Versão Final

A thesis submitted to the Graduate Program in Oceanography at the Instituto Oceanográfico, of the Universidade de São Paulo, in partial fulfillment for the degree of Doctor of Science.

Concentration area: Biological Oceanography.

Advisor: Prof. Dr. Marcelo Roberto Souto de Melo

Co-advisor: Dr. Rodrigo Antunes Caires

São Paulo

2023

GOMES, Amanda Alves. **Exploring ecomorphological patterns and the evolution of shape in Stomiiformes (Actinopterygii: Teleostei): a study of deep-sea fish adaptations.** A thesis submitted to the Graduate Program in Oceanography at the Instituto Oceanográfico, of the Universidade de São Paulo, in partial fulfillment for the degree of Doctor of Science. Concentration area: Biological Oceanography.

Approved in: ___/___/_____

Committee:

Prof. Dr. _____ Institution _____
President Signature _____

Prof. Dr. _____ Institution _____
Judgement _____ Signature _____

Prof. Dr. _____ Institution _____
Judgement _____ Signature _____

Prof. Dr. _____ Institution _____
Judgement _____ Signature _____

For Bruno Gonçalves Augusta, my beloved
husband, who has supported me since we met, and
for our future children.

ACKNOWLEDGMENTS

First of all, I would like to thank my two advisors, Marcelo Roberto Souto de Melo and Rodrigo Antunes Caires, and my internship supervisor, Brian L. Sidlauskas, for their friendship, support, discussions on my work, and for the advice and lessons that I will always carry with me. I am also very thankful to Marcelo for the many opportunities that he gave me in his lab, to Rodrigo for the patience and dedication, and to Brian for receiving me at Oregon State University so generously and for all the support that he and his beautiful family – Rae Sidlauskas and Fiona Sidlauskas – gave me during my stay at Corvallis. I also thank Fabio Machado for collaborating with my research with advice, discussions and with statistical analyses. I deeply thank José Lima Figueiredo for introducing me to ichthyology years ago, and for all the teachings.

I would like to thank all the financial support that made this study possible. This study was financed in part by the the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process 159146/2018-6). I also deeply thank Luke Tornabene from University of Washington (UW) and oVert: Open Exploration of Vertebrate Diversity in 3D for providing financial support for the CT Scanning (UW Budget 61-6284) at Friday Harbor Laboratories (FHL).

I deeply thank Adam Summers (UW) for receiving me at FHL and for the opportunity to scanning the Stomiiformes. I also thank Dominick Dickerson for training in the use of CT-Scan and image reconstructions, and Diana Pieples for the arrangement of my stay at FHL.

The MorphoSource platform also contributed to this study by providing additional CT-scan images. I also thank all technicians from MEV LCT-USP, especially Marco, for CT-Scan images acquisition of additional specimens used in this study, and Alberto Carvalho from the Museu de Zoologia, Universidade de São Paulo (MZUSP) for the tips on the CT-Scan procedures.

I am very thankful to all my DEEP Lab colleagues from Instituto Oceanográfico da USP (IOUSP) for the friendship, help, and for accompanying me throughout my PhD journey: Andreza C. Caieiro, Barbara Bouquerel, Barbara Uenoyama, César Santificetur Romero, Flávia T. Masumoto, Guilherme de Faria, Heloísa de Cia Caixeta, José Rui Paitio, Julia Yumi Ibanhez, Marcos R. dos Reis, Margherita Gallano, Marina de Lemos, Nathalie Amorim, Paola Lemes, Pollyana G. Roque, Rayane França. I especially thank Andreza for the help with CT-scan images, Flávia for the partnership in other projects, and Heloísa for her help and comments in

this study. I also deeply thank the Sidlauskas Lab colleagues for receiving me so kindly e for the enjoyable conversation, especially Alvaro Cortes, Hakan Aydogan, Olivia Boisen, Randy Willians and Nate Neal. I particularly thank Alvaro Cortes for his help with 3D slicer software and for his travel tips and advice during the CT-scan at FHL, and Kaitlyn Allison and Benjamin Johantgen for their help with photographs.

I greatly thank IOUSP and all its employees, professors and technicians who contributed in some way to my research, especially André L. A. Neves for the help in lab procedures. The Graduation Office of the IOSP provided substantial support during the development of my thesis, so I would like to thank everyone in this office, especially Daniel Nunes, Ana Paula Evangelista, and Paulo H. L. Ventrame. I am also deeply thankful to Carmen L.D.B. Rossi-Wongtschowski for the advice in the beginning of my career in Ichthyology and for introducing me to my advisor, Marcelo Melo.

My internship at Oregon State University (Corvallis, OR) was a key step at my journey developing a PhD, so I am deeply thankful to all the people involved in this process, including people of the international office of the Oregon State University, and people of the Graduation Office of the Department of Fisheries, Wildlife and Conservation Sciences. I am deeply thankful to Peter Konstantinidis for his support during my stay at OSU e for showing me the amazing and bizarre larvae of *Idiacanthus*. I also want to deeply thank Rebecca Freeby and JB Freeby, for receiving me so kindly at their home during all my stay in Corvallis, and for Ellie, the most incredible dog-host in the world!

The specimens from ichthyological collections were essential for the development of this study, and I would like to thank the following people and institutions for providing specimens: Aléssio Datovo, Mario C. C. de Pinna, Osvaldo Oyakawa and Michel D. Gianeti from Museu de Zoologia, Universidade de São Paulo (MZUSP), Cristiano L.R. Moreira, Marcelo R. Britto, and Paulo A. Backup from Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Brian Sidlauskas and Peter Konstantinidis from the Oregon State Ichthyology Collection (OS), Oregon State University (OSU), Meaghan Sorce and George Lauder from the Museum of Comparative Zoology, Harvard University (MCZ), Benjamin Frable from Scripps Institution of Oceanography, University of California in San Diego (SIO), Lynne Parenti and Diane Pitassy from National Museum of Natural History, Smithsonian Institution (USNM).

To participate on oceanographic cruises was an incredible experience in professional terms, and I would like to thank the crew of the R/V Alpha Crucis under the command of José Rezende during the cruises of the DEEP OCEAN Project, and the DEEP OCEAN Project team

for the incredible moments and excellent collections during the cruises. I especially thank Mayza Pompeu for being a good friend during the cruises. I also thank Marcos O.C. Santos (IOUSP) for the specimens collected by the RV Alucia.

I am very thankful to all the professors of the classes I participated during my PhD: Aléssio Datovo, Manoela Marinho, Naercio Aquino Menezes, Guilherme M. Dutra, and Luiz A.W. Peixoto from MZUSP, Joseph Harari and Sérgio Teixeira de Castro from IOUSP, Sergio Meirelles from Instituto de Biociências, Universidade de São Paulo (IB-USP), and Mauro Cavalcanti from Instituto Nacional de Pesquisas da Amazônia (INPA).

I deeply thank Mario C. C. de Pinna from MZUSP, Rosilene L. Delariva from Universidade Estadual do Oeste do Paraná (UNIOESTE), and Paulo Y. Sumida from IOUSP for their important comments from my qualifying exam committee. I also thank Pedro P. Rizzato (IB-USP), Leandro Nolé Eduardo (UFRPE), and Mayara Pereira Neves (Rice University) for their notable comments in the final version of this thesis.

I would like to thank my colleagues of the “Peixe nas Redes” Karla Soares and Kleber Mathubara for the opportunity to disseminate science, especially ichthyology, in the social media and for their friendship.

My family supported me more than I can ever count, giving me all the love and strength that I needed, so I deeply thank my mom Elizabeth Alves Gomes, my dad João Gomes Neto, my siblings Janete A. Gomes, Janelma A. Gomes, Paulo A. Gomes, and Rubens A. Gomes, my nephews and nieces, and my in-laws, especially my mother-in-law Virginia Celia Gonçalves. I can't help but thank my little girl Bella (*in memoriam*), the best dog in the world, for being by my side for almost 13 years, giving me so much love.

Finally, I would like to thank my beloved husband Bruno Gonçalves Augusta for all the love, support, friendship, and for our partnership in Zoomundo. Bruno has supported and loved me since we met, and he helped me with discussions, comments and scanning and image editing in this thesis. Thank you for being such a generous person, Bruno, I love you!

We need to respect the oceans and take care of them
as if our lives depended on it. Because they do.

(EARLE, Sylvia)

RESUMO

GOMES, Amanda Alves. **Exploring ecomorphological patterns and the evolution of shape in Stomiiformes (Actinopterygii: Teleostei): a study of deep-sea fish adaptations**. 2023. 155 f. Tese (Doutorado) – Instituto Oceanográfico, Universidade de São Paulo, São Paulo, 2023.

Stomiiformes é um grupo monofilético e diverso de peixes mesopelágicos e batipelágicos que inclui 457 espécies válidas classificadas em 52 gêneros e cinco famílias, incluindo os peixes-boca-de-cerda, os peixes-machadinha e os peixes-dragão. O objetivo deste estudo é investigar os padrões de evolução da forma do corpo e da forma do crânio entre os Stomiiformes usando morfometria geométrica em duas e três dimensões em um contexto ecológico e filogenético. Para a análise da forma do corpo, um total de 473 espécimes pertencentes a 55 espécies diferentes foram fotografados, e posteriormente, quatorze *landmarks* e 50 *semilandmarks* foram definidos nas imagens digitais. Para a investigação da forma do crânio, um total de 29 táxons de Stomiiformes foi escaneado usando um microtomógrafo, e posteriormente 33 *landmarks* foram marcados nas reconstruções tridimensionais. Os dados morfométricos foram submetidos à análise de componentes principais (PCA) e à análise de métodos comparativos (disparidade morfológica e ANOVA de Procrustes) para visualizar diferenças de variância entre clados, classes alimentares, profundidade, proporções cranianas e características dentárias, em seguida, relacionados com uma filogenia do grupo e testado quanto ao sinal filogenético. No geral, todos os clados de Stomiiformes variam em termos de formato do crânio e do corpo, mas a disparidade morfológica foi maior entre os peixes-dragão (Stomiidae) e peixes-machadinha (Sternoptychidae). Em relação à forma do corpo, os principais eixos da PCA descrevem uma transição de espécies com nadadeira dorsal no meio do corpo, nadadeira anal longa e corpo fusiforme ou alto para espécies com a nadadeira dorsal posicionada mais posteriormente no corpo e paralela a uma nadadeira anal curta, em um corpo alongado. Em relação à forma do crânio, o eixo principal de variação total descreve uma transição de um crânio alto para um crânio alongado e com maxilas grandes. Existe uma tendência de alongamento do corpo em relação à profundidade, com as espécies meso-batipelágicas apresentando corpos mais alongados do que aquelas restritas à zona mesopelágica. Além disso, as espécies piscívoras e generalistas exibem maior disparidade morfológica quanto à forma do corpo quando comparadas aos zooplantívoros. No entanto, quando inserido a filogenia, a disparidade morfológica da forma do corpo não foi significativa. Em contraste, considerando apenas o crânio, não houve correlação significativa entre a forma do crânio e as características ecológicas,

com ou sem a adição da filogenia. Além disso, houve um sinal filogenético significativo e forte na forma do crânio e do corpo entre os Stomiiformes, com táxons intimamente relacionados tendendo a se agrupar no filomorfoespaço, como resultado da história evolutiva compartilhada, que é frequentemente interpretada como conservadorismo evolutivo ou filogenético. Este é o primeiro estudo a investigar quantitativamente as mudanças no formato do corpo e do crânio dos Stomiiformes. Embora os Stomiiformes tenham uma notável disparidade morfológica nas formas do corpo e do crânio, a evolução da forma dentro dos táxons é explicada principalmente pela filogenia do grupo, como resultado de um padrão de ancestralidade compartilhado. É improvável que características ecológicas, como dieta, profundidade do oceano e migração vertical diária, desempenhem um papel importante na formação da evolução morfológica dos Stomiiformes. Outra hipótese possível é que os dados selecionados não tenham sido capazes de detectar a importância da ecologia na amostragem táxon aqui escolhida.

Palavras-chave: Disparidade morfológica. Ecomorfologia. Morfometria geométrica. Peixes-dragões. Sinal filogenético.

ABSTRACT

GOMES, Amanda Alves. **Exploring ecomorphological patterns and the evolution of shape in Stomiiformes (Actinopterygii: Teleostei): a study of deep-sea fish adaptations.** 2023. 155 p. Thesis (Doctorate Degree) – Instituto Oceanográfico, Universidade de São Paulo, São Paulo, 2023.

Stomiiformes is a monophyletic and diverse group of mesopelagic and bathypelagic fishes that includes 457 valid species classified in 52 genera and five families, including bristlemouths, lightfishes, hatchetfishes, and dragonfishes and their allies. The objective of this study is to investigate the patterns of body shape and skull shape evolution among the stomiiforms using two- and three-dimensional geometric morphometrics in an ecological and phylogenetic context. For the body-shape investigation, a total of 473 specimens from 55 different species were photographed, then, fourteen homologous landmarks and 50 semilandmarks were defined on the digital images. For the skull-shape investigation, a total of 29 stomiiforms skull were scanned using a micro-CT scan tomograph, then 33 homologous landmarks were defined on the three-dimensional reconstructions. Morphometric data was submitted to principal component analysis (PCA), and to comparative methods (morphological disparity and Procrustes ANOVA) analysis to visualize differences in variance among stomiiform clades, dietary classes, depth, skull proportions and tooth traits, then, linked with a recent phylogeny and tested for phylogenetic signal. Overall, all stomiiforms clades vary in terms of skull and body shape, but morphological disparity was higher in dragonfishes (Stomiidae) and hatchetfishes (Sternoptychidae). Regarding body-shape, the main axis of total variation describes a transition from species with a midbody dorsal fin, long anal fin, and body fusiform or deep to species with the dorsal fin positioned more posteriorly on the body and parallel to a short anal fin, in an elongated body. Regarding skull shape, the main axis of total variation describes a transition from a deep skull to an elongated skull, and large jaws. There is a trend towards body elongation in relation to depth distribution, with the meso-bathypelagic species having more elongated bodies than those restricted to the mesopelagic zone. Also, piscivorous and generalist species exhibit higher morphological disparity regarding body-shape when compared to the zooplanktivorous ones. However, when phylogeny is accounted for the body-shape morphological disparity is not significant. In contrast, regarding only the skull, there was no significant correlation between skull shape and ecological traits, with or without the effect of phylogeny. Additionally, there was a significant and strong phylogenetic signal in both skull and body shape among stomiiforms, with closely related taxa tending to cluster

together in the phylomorphospace, as a result of the shared evolutionary history, which is often interpreted as evolutionary or phylogenetic conservatism. This is the first study to quantitatively investigate changes in the body and skull shape of stomiiforms. Although stomiiforms have a remarkable morphological disparity in body and skull shapes, the shape evolution within the taxa is explained mostly by the phylogeny of the group, as a result of a shared ancestry pattern. Ecological traits, such as diet, ocean depth, and diel vertical migration are unlikely to play a major role in shaping the morphological evolution of stomiiforms. Another possible hypothesis is that the selected data was not able to detect the significance of ecology in the taxon sampling chosen here.

Keywords: Dragonfishes. Ecomorphology. Geometric morphometrics. Morphological disparity. Phylogenetical signal.

LIST OF FIGURES

Figure 1.1 The diversity of Stomiiformes, with the number of genera and species included in each family. 26

Figure 1.2. Selected synapomorphies of the Stomiiformes. (A) and (B): type 3 of tooth attachment. (A) upper and lower jaws of *Melanostomias bartonbeani*, MCZ 132357, 222.9 mm SL, micro CT-Scan, left side in medial view, with the white arrow indicating the unmineralized collagen area on the teeth posterior border, scale bar=10mm; (B) *Melanostomias* sp., MZUSP uncat., with the white arrow indicating the mineralized area at the teeth anterior border, scale bar=10 mm; (C) swim bladder scheme of *Astronesthes niger*, with the red arrow indicating rete mirabile on the posterior region (adapted from MARSHALL, 1960); (D) histology of ventral photophore of *Chauliodus sloani* (adapted from MARRANZINO, 2016). Abbreviations: l=lens, pc=photogenic chamber; r=reflector layer, p=pigment layer, scale bar=1 µm. 31

Figure 1.3. Osteological synapomorphies of the Stomiiformes: (A) dorsal portion of branchial apparatus of *Diplophos taenia*, USNM206614, 171.0 mm SL, with red arrow indicating the broad termination of the second epibranchial (adapted from FINK; WEITZMAN, 1982); (B) anterior ligaments of the suspensorium in *Diplophos taenia*, USNM206908, 184.3 mm SL, dorsal view, with the red arrows indicating the premaxillary-rostrodermethmoid ligaments (adapted from FINK; WEITZMAN, 1982); (C) hyoid arch of *Stomias brevibarbus*, right side, lateral view, with red arrow indicating the branchiostegal rays in ventral hypohyal (adapted from FINK, 1985); (D) left side of the hyoid apparatus in *Argyropelecus aculeatus*, 49.5 mm SL, lateral view, with red arrow indicating the last branchiostegal ray widely large (adapted from WEITZMAN, 1974). 32

Figure 1.4. Synapomorphies in muscular system of the Stomiiformes: (A) medial division of adductor mandibulae in *Diplophos taenia* (MCZ 55469; 117.0 mm SL), with red arrow indicating the dorsal section (adapted from FINK; WEITZMAN, 1982); (B) dorsal gill-arch of *Diplophos* with the upper red arrow indicating the component of the obliquus dorsalis 4 (Od4) attached to pharyngobranchial 4 (Pb4), and the lower red arrow indicating adductor 5 (Ad5) attached to epibranchial 4 (Eb4) (adapted from SPRINGER; JOHNSON, 2004). 33

Figure 2.1. The diversity of the Stomiiformes, with representatives of all families. A. *Diplophos taenia*, MZUSP80530, 74.4 mm SL; B. *Sigmops elongatus*, MZUSP086606, 80.1 mm SL; C. *Vinciguerrria nimbaria*, MZUSP uncat., 38.6 mm SL; D. *Maurolicus stehmani*, MZUSP080238, 34.8 mm SL; E. *Sternoptyx pseudobscura* MN30167, 49.8 mm SL; F. *Astronesthes macropogon*, MZUSP80272, 115.6 mm SL; G. *Chauliodus sloani*, MZUSP uncat., 95.6 mm SL; H. *Idiacanthus atlanticus*, MZUSP078406, 310.1 mm SL; I. *Aristostomias* sp., MZUSP uncat., 125.1 mm SL; J. *Melanostomias* sp., MZUSP uncat, 193.6 mm SL; K. *Stomias affinis*, MZUSP086572, 162.2 MM SL. Scale bars = 10 mm. 54

Figure 2.2. The position of the fourteen fixed landmarks (red) and 50 semilandmarks (green) illustrated in *Vinciguerria nimbaria*, MZUSP uncat., 38.6 mm SL. Scale bar = 10 mm. 60

Figure 2.3. The first and second axes of a Principal Component Analysis (PCA) in Stomiiformes, based on 473 specimens. Colored ellipses represent distribution of specimens within stomiiform taxonomic groups. 63

Figure 2.4 Stomiiform species at the negative and positive extremes of body shape variation along the first three principal component axes. Wireframes represent the average body shape, and vectors represent the average configuration warped to an extreme observed value of the corresponding PC axis. The percent of total variance represented by each axis is indicated between the two extreme shapes. 64

Figure 2.5 The first and second morphospace axes, color coded by (A) diet, (B) bathymetric distribution, and (C) diel vertical migration pattern (DVM). 65

Figure 2.6 Genus-level phylomorphospace based on the time-calibrated phylogeny of Rabosky et al. (2018), color coded by stomiiform taxonomic groups. 68

Figure 2.7 Time-calibrated phylogenetic tree of the Stomiiformes, adapted from Rabosky *et al.* (2018), with the ecological traits of diet (type 1 = zooplanktivorous; type 2 = generalists; or type 3 = piscivorous), depth distribution (mesopelagic; or meso-bathypelagic) and diel vertical migration pattern (migratory; or non-migratory) indicated. Color code on the cladogram indicates families. 76

Figure 3.1 The diversity of the Order Stomiiformes A. *Manducus maderensis*, MCZ61476, 91.9 mm SL; B. *M. maderensis*, MCZ 91350, 95.8 mm SL; C. *Polymetme thaeocoryla*, USNM304218, 145.1 mm SL; D. *P. thaeocoryla* MZUSP086650, 100.2 mm SL; E. *Astronesthes gemmifer*, USNM436537, 151.7 mm SL; F. *Astronesthes macropogon*, MZUSP80272, 115.6 mm SL; G. *Opostomias mitsuii*, OS014651, 123.2 mm SL; H. *O. mitsuii*, OS 014668, 116.3 mm SL; I. *Malacosteus niger*, YPMICH025708, 175.40 mm SL; J. *Malacosteus australis*, MNRJ30386, 160.8 mm SL; K. *Chauliodus sloani*, VIMS16520, 222.47 mm SL; L. *C. sloani*, MZUSP uncat., 95.6 mm SL; M. *Argyrolepecus affinis*, SIO71-191, 44.0 mm SL; N. *Argyrolepecus aculeatus*, MZUSP uncat., 47.8 mm SL. A, C, E, G, I, K and M: micro-CT Scan of the skull. Scale bars =10 mm. 93

Figure 3.2. Selected 33 fixed landmarks (3D) in *Grammatostomias flagellibarba* (MCZ164122, 202.7mm SL, left lateral view, scale bar=10 mm) and measurements. SLn (LM1-LM33): skull length; SDp (LM14-LM33): skull depth; UJLn (LM1-LM7): upper jaw length; DLn (LM9-LM11): dentary length; TLn (anterior tooth base to tooth tip): tooth Length. 99

Figure 3.3 Overall shape variation of stomiiforms skull revealed by the first and second axes resulting from the Principal Component Analysis (PCA), ordinated by clades (represented in different colors) and diet (represented by geometric forms), and warp graphics of the extreme positive and negative species in the PC1 and PC2. 103

Figure 3.4. Landmarks variation in PC1 (A, D, G, J), PC2 (B, E, H, K), and PC3 (C, F, I, L). The size of the vectors in A-C represents the landmarks variation in PC 1, PC2 and PC 3, respectively. The interpolation between extreme negative and positive stomiiforms shape in PC1, PC2 and PC3 according to the landmark's variation is represented in D-F. G-L represents landmarks, measures, and skull images from extreme and positive stomiiforms shapes in PC1, PC2 and PC3. G=*Polyipnus*; J=*Photonectes*; H=*Sternoptyx*; K=*Maurolicus*; I=*Echiostoma*; L=*Zaphotias*. 104

Figure 3.5. Overall shape variation of stomiiforms skull revealed by the first and second axes resulting from the Principal Component Analysis (PCA), ordinated by skull length (A), skull depth (B), and jaws length (C). 105

Figure 3.6. Genus-level phylomorphospace of the main axes resulting from the Principal Component Analysis (PCA) of the Order Stomiiformes combined with the time-calibrated phylogeny of Rabosky *et al.*, 2018, color coded by stomiiform taxonomic groups. 106

Figure 3.7. Time-calibrated phylogenetic tree of the Stomiiformes, adapted from Rabosky *et al.* (2018), with the ecological traits of diet (zooplanktivorous; generalists; or piscivorous), and depth distribution (mesopelagic; or meso-bathypelagic) indicated. Color code on the cladogram indicates families. 111

Figure S2.1 Outliers resulted from the Procrustes ANOVA analysis, highlighting the *Sternoptyx pseudobscura*, indicated by the red arrow, with high Procrustes distance from mean. 126

Figure S2.2 Scatterplot of the first and second axes resulting from Principal Component Analysis (PCA) among the species of Stomiiformes. A. PCA including *Sternoptyx pseudobscura*; B. PCA excluding *Sternoptyx pseudobscura*. 127

Figure S2.3 A. Phylogenetic signal (K) and p-values in stomiiforms shape, and B. Centroid Size. 128

Figure S3.1. Types of tooth attachment in stomiiforms (sensu FINK, 1981). A=Type 3 of tooth attachment in oral jaws of *Melanostomias bartonbeani*, MCZ132357, 222.9 mm SL, micro-CT Scan of the upper and lower jaws, left side in medial view, with the white arrow indicating the unmineralized collagen area on the teeth posterior border; B=Type 1 of tooth attachment in oral jaws of *Malacosteus niger*, YPMICH025708, micro-CT Scan of the upper and lower jaws, left side in medial view showing the fixed teeth. Scale bars=10 mm. 141

Figure S3.2. Phylogenetic signal (K) and p-values in stomiiforms shape (A), and Centroid Size (B). 142

Figure S3.3. Micro CT-Scan of stomiiforms skull belonging to the families Diplophidae (*Diplophos taenia*, UW115370, and *Manducus maderensis*, MCZ61476, 191.9 mm SL), and Gonostomatidae (*Margrethia obtusirostra*,

YPMICH027653, and *Zaphotias pedaliotus*, YPMICH027653). Scale bars=10mm (except in *Margrethia obtusirostra*). 143

Figure S3.4. Micro CT-Scan of stomiiforms skull belonging to the paraphyletic family Phosichthyidae (*Phosichthys argenteus*, MCZ61171, 237.2 mm SL, *Polymetme thaeocoryla*, USNM304218, 145.1 mm SL, and *Yarrella blackfordi*, MCZ126580, 154.4 mm SL). 144

Figure S3.5. Micro CT-Scan of stomiiforms skull belonging to the Sternoptychidae subfamilies Sternoptychinae (*Argyropelecus affinis*, SIO71-191, 44.0 mm SL, *Polyipnus spinifer*, USNM135514, 49.6 mm SL, and *Sternoptyx pseudobscura*, SIO66-536), and Maurolicinae (*Maurolicus weitzmani*, USNM391455, 58.3 mm SL). 145

Figure S3.6. Micro CT-Scan of stomiiforms skull belonging to the Stomiidae subfamily Astronesthinae (*Astronesthes gemmifer*, USNM436537, 151.7 mm SL, *Borostomias antarcticus*, SIO6145, 201.4 mm SL, *Heterophotus ophistoma*, SIO70341, 76.5 mm SL, and *Neonesthes capensis*, USNM454448, 153.4 mm SL). 146

Figure S3.7. Micro CT-Scan of stomiiforms skull belonging to the Stomiidae subfamily Melanostomiinae (*Odontostomias micropogon*, MCZ132152, 220.1 mm SL, *Opostomias mitsuii*, OS014651, 123.2 mm SL, *Leptostomias gladiator*, MCZ149495, 105.1 mm SL, *Thysanactis dentex*, UW150245, and *Flagellostomias boureei*, SIO76107, 178.1 mm SL). 147

Figure S3.8. Micro CT-Scan of stomiiforms skull belonging to the Stomiidae subfamily Melanostomiinae (*Chirostomias pliopterus*, MCZ132709, 157.7 mm SL, *Echiostoma barbatum*, UF168937, *Trigonolampa miriceps*, MCZ165921, 172.9 mm SL, *Grammatostomias flagellibarba*, MCZ164122, 202.7 mm SL, *Photonectes margarita*, SIO985, 122.9 mm SL, and *Melanostomias melanopogon*, MCZ132243, 149.5 mm SL). 148

Figure S3.9. Micro CT-Scan of stomiiforms skull belonging to the Stomiidae subfamilies Malacosteinae (*Aristostomias grimaldii*, MCZ93834, 99.3 mm SL, and *Malacosteus niger*, YPMICH025708), and Stomiinae (*Chauliodus sloani*, VIMS16520). 149

LIST OF TABLES

| | |
|---|-----|
| Table 2.1 List of species of Stomiiformes examined, including the number of specimens (N), minimum and maximum standard length, and depth of capture..... | 56 |
| Table 2.2 Descriptions of the fourteen homologous landmarks..... | 59 |
| Table 2.3 Procrustes variance and P-values of the morphological disparity in Stomiiformes test by diet, depth, and diel vertical migration (DVM), with and without the hatchetfish <i>Sternoptyx pseudobscura</i> | 66 |
| Table 2.4 Results from Procrustes ANOVA testing for differences in mean body shape and centroid size (CS) among the Stomiiformes classified by clades, diel vertical migration (DVM), bathymetric distribution (depth) and diet, with and without the hatchetfish <i>Sternoptyx pseudobscura</i> . Asterisks represent significant differences ($p \leq 0.05$)..... | 67 |
| Table 2.5 Procrustes variance and P-values of the morphological disparity in Stomiiformes test by diet, depth, and diel vertical migration (DVM), without and with accounting for phylogeny. Asterisks represent significant morphological disparity ($p \leq 0.05$)..... | 69 |
| Table 2.6 Results from Procrustes ANOVA testing for differences in mean body shape and centroid size (CS) among the Stomiiformes classified by clades, diel vertical migration (DVM), bathymetric distribution (depth) and diet, without and with accounting for phylogeny. Asterisks represent significant differences ($p \leq 0.05$)..... | 69 |
| Table 3.1. Stomiiformes species used in the study, including catalog number, standard length (SL in mm), parameters used in the scans: kilovolts (kv), microampere (μA) and voxel size (μm), and MorphoSource id..... | 96 |
| Table 3.2 The 33 homologous landmarks, and their respectively descriptions..... | 100 |
| Table 3.3. Stomiiforms Procrustes variance and P-values from the morphological disparity test by ecological and anatomical traits without and accounting phylogeny. Asterisks represent significative morphological disparity..... | 107 |
| Table 3.4. Stomiiforms ANOVA F and P values of means for body shape and centroid size (CS) tested by Species, Clades, Depth, Diet, Skull length, Skull depth, Jaws length, Tooth type, Toot size, and Tooth attachment. Asterisks represent significant differences ($p \leq 0.05$)..... | 107 |
| Table S2.1. Ecological traits of the Stomiiformes, encompassing their diet, diel vertical migration, and depth, with respective references from the literature regarding these traits, alongside the specific ecological traits categories employed within this investigation. DVM=Diel Vertical Migration. Type 1 diet=zooplanktivorous; Type 2 diet=generalists; Type 3 diet=piscivorous. Asterisk means that diet type was inferred based on other species or genera from the same family..... | 129 |

| | |
|---|-----|
| Table S2.2. Species of Stomiiformes used to create a genus-level phylomorphospace based on the molecular phylogeny produced by Rabosky <i>et al.</i> (2018)..... | 135 |
| Table S3.1. Ecological traits of the Stomiiformes, encompassing their diet and depth, with respective references from the literature regarding these traits, alongside the specific diet and depth categories employed within this investigation. Asterisk means that diet type was inferred based on other species or genera from the same family..... | 150 |
| Table S3.2 Species of Stomiiformes used to create a genus-level phylomorphospace based on the molecular phylogeny produced by Rabosky <i>et al.</i> (2018). | 154 |

TABLE OF CONTENTS

| | |
|---|-----------|
| 1. CHAPTER ONE: GENERAL INTRODUCTION..... | 22 |
| 1.1 THE DEEP-SEA | 22 |
| 1.2 ORDER STOMIIFORMES | 25 |
| 1.2.1 <i>General remarks</i> | 25 |
| 1.2.2 <i>Synapomorphies of the group</i> | 29 |
| 1.2.3 <i>Systematics</i> | 33 |
| 1.2.4 <i>Ecological aspects</i> | 35 |
| 1.3 ECOMORPHOLOGY AND GEOMETRIC MORPHOMETRIC METHODS (GGM) | 36 |
| 1.4. RESEARCH AIMS AND OBJECTIVES | 38 |
| 1.5. REFERENCES | 40 |
| 2. CHAPTER TWO: BODY-SHAPE EVOLUTION IN STOMIIFORMES | 52 |
| 2.1 INTRODUCTION | 53 |
| 2.2 MATERIALS AND METHODS..... | 55 |
| 2.2.1 <i>Taxonomic sampling</i> | 55 |
| 2.2.2 <i>Ecological data</i> | 58 |
| 2.2.3 <i>Geometric morphometrics</i> | 59 |
| 2.2.4 <i>Phylomorphospace, phylogenetical signal and phylogenetic ANOVA</i> | 60 |
| 2.3 RESULTS | 61 |
| 2.3.1 <i>Principal Component Analysis (PCA)</i> | 61 |
| 2.3.2 <i>Morphological disparity and Procrustes ANOVA</i> | 65 |
| 2.3.3 <i>Phylogenetic signal, phylomorphospace, and phylogenetic ANOVA</i> | 67 |
| 2.4 DISCUSSION | 69 |
| 2.4.1 <i>Body-shape patterns in Stomiiformes</i> | 69 |
| 2.4.2 <i>Phylogenetic patterns</i> | 74 |
| 2.5 CONCLUSION | 77 |
| 2.6 MATERIAL EXAMINED..... | 78 |
| 2. 7 REFERENCES | 81 |
| 3. CHAPTER THREE: EVOLUTION OF SKULL SHAPE IN STOMIIFORMES | 90 |
| 3.1 INTRODUCTION | 91 |
| 3.2 MATERIAL AND METHODS..... | 95 |

| | |
|---|------------|
| 3.2.1 Taxonomic sampling..... | 95 |
| 3.2.2 Data acquisition | 95 |
| 3.2.3 Ecological data and anatomical measurements..... | 97 |
| 3.2.4 Geometric morphometrics | 99 |
| 3.2.5 Principal Component Analysis | 100 |
| 3.2.6 Procrustes ANOVA and morphological disparity | 101 |
| 3.2.7 Phylogenetic patterns | 101 |
| 3.2.8 Procrustes ANOVA and morphological disparity in a phylogenetic framework .. | 102 |
| 3.3 RESULTS | 102 |
| 3.3.1 Principal Component Analyses (PCA)..... | 102 |
| 3.3.2 Morphological disparity and Procrustes ANOVA..... | 105 |
| 3.3.3 Phylomorphospace, phylogenetic signal and phylogenetic ANOVA..... | 106 |
| 3.4 DISCUSSION | 108 |
| 3.5 CONCLUSION | 114 |
| 3.6 REFERENCES | 115 |
| 4. FINAL CONCLUSIONS | 124 |
| SUPPLEMENTAL INFORMATION CHAPTER TWO | 126 |
| SUPPLEMENTAL FIGURES CHAPTER TWO | 126 |
| SUPPLEMENTAL TABLES CHAPTER TWO..... | 129 |
| REFERENCES SUPPLEMENTAL INFORMATION CHAPTER TWO | 137 |
| SUPPLEMENTAL INFORMATION CHAPTER THREE..... | 141 |
| SUPPLEMENTAL FIGURES CHAPTER THREE | 141 |
| SUPPLEMENTAL TABLES CHAPTER THREE..... | 150 |

If you stare into the abyss, the abyss stares back at
you.
(NIETZSCHE, Friedrich)

1. CHAPTER ONE: GENERAL INTRODUCTION

1.1 The deep-sea

The deep sea, entailing the region beneath the euphotic zone at depths exceeding 200 meters, constitutes the planet's most extensive habitat, spanning approximately 95% of the Earth's living space (COCKER, 1978; ANGEL, 1997; RAMIREZ-LLODRA, 2020). Ocean depth plays a crucial role in marine ecosystems, as it encompasses various environmental factors that significantly impact living organisms, such as the hydrostatic pressure, temperature, dissolved oxygen, and sunlight availability (THISTLE, 2003; SUMIDA, 2009). These changes in abiotic variables also lead to changes in the structure of community assemblages, delimiting true biogeographical regions in the deep sea (SUTTON *et al.*, 2017).

The vertical gradients' strength and interaction result in pronounced vertical structuring in the bathymetric distributions of both pelagic and benthic assemblages (SUTTON, 2013). The epipelagic zone comprises the euphotic zone and the seasonal pycnocline and is conventionally considered to extend to depths of 200 m. Situated beneath the epipelagic zone, the mesopelagic zone extends downward to approximately 1,000 m, serving as the upper limit delineating the deep ocean (ANGEL, 1997). The bathypelagic zone, also known as midnight zone, typically occurs between 1,000 and 4,000 m, a depth at which daylight ceases, leading to complete darkness. The boundary between the mesopelagic and bathypelagic zones coincides with the oxygen minimum and corresponds to the peak in species richness for both pelagic and megabenthic assemblages (ANGEL, 1993, 1997). Notably, the abyssopelagic zone, between 4,000 and 6,000 m resides within a layer of isothermal and isohaline water, while the hadopelagic zone, 6,000 m and beyond, only exists in the trenches, the deepest oceanic features (ANGEL, 1997; MERRET; HAEDRICH, 1997).

Environmental conditions result in significant shifts in the vertical structure of marine communities and trigger depth-specific adaptations of vision, buoyancy control, and intraspecific communication, among others (ANGEL, 1989; HADDOCK *et al.*, 2010; WIDDER, 2010). Evolutionary adaptations enabling deep-sea life to have evidently evolved independently on multiple occasions, with fish orders inhabiting this environment (WEITZMAN, 1997). The deep-sea fishes exhibit an astonishing level of diversity and possess specialized traits in terms of tooth morphology, body shape and presence of bioluminescent organs that facilitate feeding, reproduction, and camouflage of such taxa in the deep-sea habitats.

(RANDALL; FARRELL, 1997; HADDOCK *et al.*, 2010; WIDDER, 2010; MARRANZINO; WEBB, 2018).

Approximately 33% of the extant fish families have representatives dwelling in the deep-sea environment or, at the very least, species that occasionally penetrate into the deepest parts of the mesopelagic zone (ranging between 500 and 600 meters in depth) (WEITZMAN, 1997, MELO *et al.*, 2020). Bony fishes (Osteichthyes) constitute the majority of the deep-sea fish diversity, with some species occurring at depths surpassing 8,000 meters (WEITZMAN, 1997). Noteworthy examples include the cusk eel *Abyssobrotula galathea* Nielsen, 1977 of the family Ophidiidae (NIELSEN, 1977) and the snailfishes of the genus *Pseudoliparis* Andriashev, 1955, family Liparidae (LINLEY *et al.*, 2016, JAMIESON *et al.*, 2023). Nevertheless, most deep-sea species are distributed up to 2,000 meters, with a significant reduction in both diversity and biomass observed beyond 2,500 meters (PRIDE; FROESE, 2013).

There are two main distinct habitats in the deep ocean: the pelagic region and the benthic region, and the fauna of these environments is equally distinct (MARSHALL, 1979; MERRET; HAEDRICH, 1997). In the pelagic region, mesopelagic fish (200–1,000 m depth) are the most important marine vertebrate in numerical terms, presenting high biodiversity and several adaptations to inhabit the deep-sea environment (IRIGOIEN *et al.*, 2014; NELSON *et al.*, 2016). They are generally dark or silvery and often have bioluminescent organs called photophores, mainly on the ventral region of the body and on the head, which serve mainly for camouflage in the water column (MARSHALL, 1979). Some species also have large, tubular eyes, and the retina adapted for dark vision or to selectively filter out certain wavelengths for viewing their prey (MARSHALL, 1971). These species also make diel vertical migrations: during the day, they remain at greater depths where sunlight does not reach, while during the night they migrate to shallow waters in search for food (SUTTON; HOPKINS, 1996; ANGEL, 1997). This migration is only possible due to a highly developed swim bladder (a hydrostatic organ that regulates the buoyancy of fish in water) (MARSHALL, 1971). Deep-sea sharks, on the other hand, have a larger liver to enable buoyancy control (MARSHALL, 1979).

The total biomass of mesopelagic fishes has been estimated as at least 1,000 million tons, however, some authors argue that this number might be underestimated (IRIGOIEN *et al.*, 2014). These fish are key organisms in the oceanic food webs (SUTTON; HOPKINS, 1996; ANGEL, 1997; CARMO *et al.*, 2015): through the diel vertical migration, they feed near the surface, or, at least, in the upper mesopelagic region and defecate well down in the mesopelagic zone, transporting carbon from the epipelagic to meso and bathypelagic environments (SUTTON; HOPKINS, 1996; ANGEL, 1997; CARMO *et al.*, 2015). The two main groups of

deep-pelagic fishes are the Myctophiformes, commonly known as lanternfishes, and Stomiiformes, represented by the bristlemouths, lightfishes, hatchetfishes, dragonfishes, viperfishes, and fangtooths (SUTTON *et al.*, 2020).

Investigations of deep-sea habitats and consequently of the deep-sea fauna only began in the late 19th century (RAMIREZ-LLODRA, 2020). Melo *et al.* (2020) provided a detailed description of the history of oceanographic expeditions focusing in deepwater fauna, from the 19th century onwards. According to the authors, until the mid-19th century, the azoic hypothesis that there would be no animal life in marine regions below 550 meters was widely spread (FORBES, 1843; ANDERSON; RICE, 2006) given the extreme conditions in such environments. However, expeditions carried out from the end of the 19th century began to register the presence of different organisms at different depths, including in the deep-sea trenches and hydrothermal vents. It was the Challenger Expedition (1872–1876), coordinated by the Scottish naturalist Charles Wyville Thomson aboard the English corvette H.M.S. Challenger, which marked the beginning of scientific exploration of the deep ocean. A total of 362 stations were carried out around the world at depths of up to 8,183 m, which resulted in the description of 715 genera and 4,417 species of marine organisms (THOMSON, 1880; MELO *et al.*, 2020).

The Challenger expeditions also marked the exploration of the deep-sea fauna in the western South Atlantic, with the passage of the H.M.S. Challenger off the coast of Northeastern Brazil in 1873 and off the coasts of Uruguay and Argentina in 1876. Almost a century later, the German research vessel RV Ernst Haeckel operated between Southern Brazil and Argentina in 1966, and the American research vessel MIV Oregon II operated between 1957 and 1976 in northern South America, both providing new information on the deep-sea fauna of the western South Atlantic (MELO *et al.*, 2020).

In the Brazilian Exclusive Economic Zone, which includes a considerable portion of the western South Atlantic, the most important expedition of the deep-sea has initiated only after 1994, with the Programa de Avaliação do Potencial Sustentável de Recursos Vivos na Zona Econômica Exclusiva - REVIZEE, of the Brazilian Federal Government, developed between 1994 and 2004, as part of the requirements for extending the outer limit of the territorial sea to 200 nautical miles from the coast (MELO *et al.*, 2020). The REVIZEE was responsible for a substantial part of the knowledge concerning the Brazilian deep-sea fauna, especially regarding deep-sea fish (BERNARDES *et al.*, 2007; FIGUEIREDO *et al.*, 2002). From the year 2000, with the increase in efforts to explore oil and gas in Brazilian deep waters, the company Petróleo Brasileiro S.A. (PETROBRÁS) also started to invest in projects focused on the knowledge of

the deep-sea fauna (MORAIS, 2013). Recent expeditions focused on the Brazilian deep-sea ichthyofauna were conducted onboard the French RV *Antea* in 2015, 2017 and 2021, as part of the ABRACOS (Acoustics along the BRAzilian COaSt) project (BERTRAND, 2015, 2017; BERTRAND *et al.*, 2021), and onboard the Brazilian RV *Alpha Crucis* between 2019–2021, as part of the DEEP-OCEAN (Diversidade e Evolução de Peixes de Oceano Profundo) project (MELO *et al.*, 2020). So far, 712 species from 145 families and 37 orders of deep-sea fishes had been recorded in the Brazilian waters including five species of Myxini, six species of Holocephali, 81 species of Elasmobranchii, and 620 species of Actinopterygii (MELO *et al.*, 2020).

1.2 Order Stomiiformes

1.2.1 General remarks

The order Stomiiformes comprises marine bioluminescent fishes that inhabit meso- and bathypelagic regions, currently containing 53 genera and 457 species, widely distributed in all oceans (NELSON *et al.*, 2016; FRICKE *et al.*, 2023) including the bristlemouths, lightfishes, hatchetfishes, dragonfishes, viperfishes, loosejaws, and snaggletooths (FINK; WEITZMAN, 1982; HAROLD, 2002; NELSON *et al.*, 2016). Stomiiforms are traditionally divided into five families: Diplophidae, Gonostomatidae, Phosichthyidae, Sternoptychidae and Stomiidae (HAROLD, 2002; NELSON, 2006), however, recent molecular hypothesis recovers Phosichthyidae as paraphyletic (KENALEY *et al.*, 2014; BETANCUR-R *et al.*, 2017; Mirande, 2017; RABOSKY *et al.*, 2018). Stomiiforms are generally small, varying in size from 1.5 to 50 cm in standard length, and most species measure less than 10 cm in standard length (FINK; WEITZMAN, 1982).

Stomiiforms have a worldwide distribution in the Atlantic, Pacific, Indian and Southern oceans, but some species have a more restricted distribution, like the dragonfish *Tactostoma macropus* Bolin 1939, found in Pacific Ocean (ESCHMEYER *et al.*, 1983), and the snaggletooth, *Eupogonesthes xenicus* Parin & Borodulina, 1993, encountered only in the eastern Indian Ocean (PARIN; BORODULINA, 1993). Some stomiiforms are very abundant in the oceans, like the genera *Vinciguerria* Jordan & Evermann, 1896, *Maurolicus* Goode & Bean, 1896, and the bristlemouth *Cyclothone* Goode & Bean, 1883, which is regarded as the most abundant vertebrate genus in the world (HERRING, 2002; NELSON, *et al.*, 2016). In

contrast, some stomiiform taxa are rarely collected, like the diplophid *Triplophos hemingi* (McArdle 1901), the maurolicine *Araiophos eastropas* Ahlstrom & Moser, 1969, and the astronesthines *Eupogonesthes xenicus* Parin & Borodulina, 1993 and *Rhadinesthes decimus* (Zugmayer, 1911).

Stomiiforms have a high degree of morphological diversity (FINK, 1984; HAROLD; WEITZMAN, 1996), with morphological variability in the members of this group involving features such as shape and size of the body, head, mouth, eyes and fins, and presence or absence of elements as barbels, scales, lures, and adipose fin (Fig. 1.1) (HAROLD; WEITZMAN, 1996; HAROLD, 2002; KENALEY, 2009).

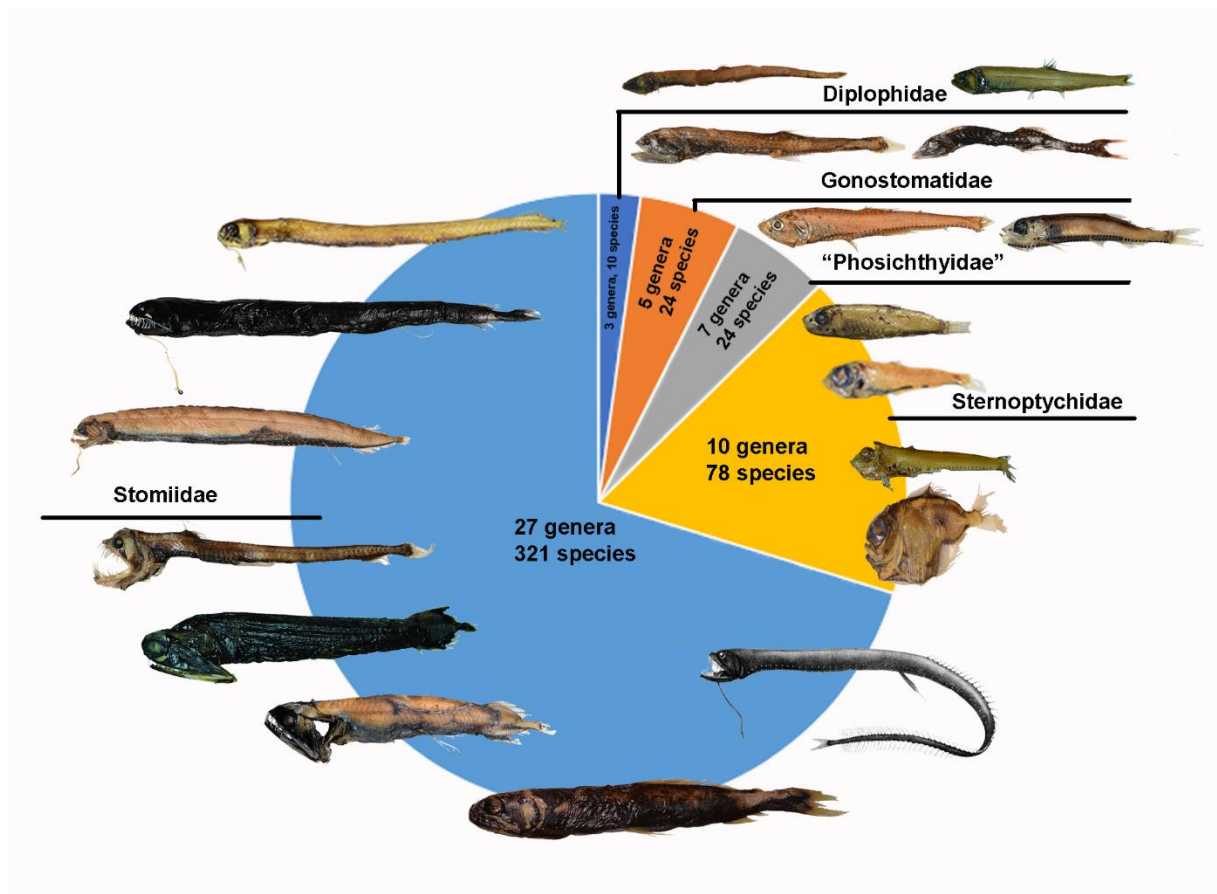


Figure 1.1 The diversity of Stomiiformes, with the number of genera and species included in each family.

The family Gonostomatidae includes 24 valid species of small to medium sized fishes commonly known as bristlemouths and it is currently classified in the following five genera: *Cyclothone* Goode & Bean 1883, *Gonostoma* Rafinesque, 1810, *Margrethia* Jespersen & Tåning, 1919, *Sigmops* Gill, 1883, and *Zaphotias* (Goode & Bean, 1898). Diplophidae includes ten valid species of small to medium sized fishes also commonly known as bristlemouths, that

were originally classified in the family Gonostomatidae, and are currently classified into three genera: *Diplophos* Günther, 1873, *Manducus* Goode & Bean, 1896, and *Triplophos* Brauer, 1902 (NELSON *et al.*, 2016; FRICKE *et al.*, 2023). Diplophids and gonostomatids are recognized by having a large mouth, with the lower jaw angle posterior to rear eye border, with numerous bristle-like teeth, equally sized, photophores with lumen, presence of one orbital photophore, and well-developed gill rakers (HAROLD; WEITZMAN, 1996; HAROLD, 2002).

The paraphyletic family Phosichthyidae embraces 24 valid species of small to medium sized fishes, included in seven genera: *Ichthyococcus* Bonaparte, 1840, *Phosichthys* Hutton, 1872, *Pollichthys* Grey, 1959, *Polymetme* McCulloch, 1926, *Vinciguerria* Jordan & Evermann, 1896, *Woodsia* Grey, 1959, and *Yarrella* Goode & Bean, 1896 (NELSON *et al.*, 2016; FRICKE *et al.*, 2023). Phosichthyids are known as lightfishes as a reference to the row of noticeable photophores in their body. Lightfishes and bristlemouths share several similarities, including an elongate body shape, a large mouth with several small teeth, and well-developed gill rakers, differing from the later by having two orbital photophores - except in *Polymetme* and *Yarrella* (HAROLD; WEITZMAN, 1996; HAROLD, 2002).

Sternoptychidae includes small to medium sized fishes with ten genera and 78 species, organized in two subfamilies: Maurolicinae, including fish with elongated body, never laterally compressed, encompassing seven genera: *Araiophos* Grey, 1961, *Argyripnus* Gilbert & Cramer, 1897, *Danaphos* Bruun, 1931, *Maurolicus* Cocco, 1838, *Sonoda* Grey, 1959, *Thorophos* Bruun, 1931, and *Valenciennellus* Jordan & Evermann, 1896, and 33 valid species; and Sternoptychinae, known as hatchetfishes by having a high body depth, extremely compressed laterally, comprising three genera: *Argyropelecus* Cocco, 1829, *Polyipnus* Günther, 1887, and *Sternoptyx* Hermann, 1781, and 45 valid species (NELSON *et al.*, 2016; FRICKE *et al.*, 2023). Sternoptychids have photophores in the body arranged in clusters, silvery color with reflective guanine pigment on the lateral side of the body, and large eyes, which are telescopic in *Argyropelecus* (HAROLD; WEITZMAN, 1996; HAROLD, 2002).

The family Stomiidae includes the largest and diverse stomiiforms, collective known as deep-sea dragonfishes, with 321 valid species allocated in 27 genera (NELSON *et al.*, 2016; FRICKE *et al.*, 2023). The Stomiidae is divided into six sub-families. Astronesthinae, commonly known as snaggletooths, comprises 16 species classified in six genera: *Astronesthes* Richardson, 1845, *Borostomias* Regan, 1908, *Eupogonesthes* Parin & Borodulina, 1993, *Heterophotus* Regan & Trewavas, 1929, *Neonesthes* Regan & Trewavas, 1929, and *Rhadinesthes* Regan & Trewavas, 1929; Stomiinae, known as scaly dragonfishes, with 10 species allocated in genus *Stomias* Cuvier, 1816; Chauliodontinae, known as viperfishes, with

11 species allocated in *Chauliodus* Bloch & Schneider, 1801; Melanostomiinae (scaleless dragonfishes): comprises 15 genera: *Bathophilus* Giglioli, 1882, *Chirostomias* Regan & Trewavas, 1930, *Echiostoma* Lowe, 1843, *Eustomias* Vaillant, 1884, *Flagellostomias* Parr, 1927, *Grammatostomias* Goode & Bean, 1896, *Leptostomias* Gilbert, 1905, *Melanostomias* Brauer, 1902, *Odontostomias* Norman, 1930, *Opostomias* Günther, 1887, *Pachystomias* Günther, 1887, *Photonectes* Günther, 1887, *Tactostoma* Bolin, 1939, *Thysanactis* Regan & Trewavas, 1930, and *Trigonolampa* Regan & Trewavas, 1930, and 223 valid species; Idiacanthinae (black dragonfishes) includes one genus *Idiacanthus* Peters, 1877, and three valid species; Malacosteinae (loosejaws) encompasses three genera: *Aristostomias* Zugmayer, 1913, *Malacosteus* Ayres, 1848, and *Photostomias* Collett, 1889, and 14 valid species.

Stomiids are sometimes named as barbeled dragonfishes by possessing a barbel attached to the hyoid arch (HAROLD; WEITZMAN, 1996; HAROLD, 2002). Malacosteines have the most elongated jaws within stomiiforms and lack the membranes that form the floor of the mouth, which increases the jaw-closing velocity, and bears the ability to greatly open their mouth at angles higher than 120° (KENALEY, 2012; SCHNELL; JOHNSON, 2017).

Most stomiiforms produce bioluminescence – a chemical reaction capable of generating ecologically functional light in a living organism (REES *et al.*, 1998), except *Cyclothone obscura*, which received the popular name of “hidden bristlemouth” (BADCOCK, 1984). Their main light organs – the photophores, have species-specific arrangements and counts of these photophores are used in taxonomic identification (BIGELOW, 1964; HAROLD, 2002).

Stomiiforms have a remarkable assemblage of photophores, exhibiting one or two rows of these organs on the body, the so-called serial photophores, positioned laterally and ventrally, minute photophores distributed on head and body, photophores in the isthmus (absent in some gonostomatids) in the orbital region (preorbitals, infraorbitals and post-orbitals in most species), in the branchiostegal region (only in Gonostomatidae and Sternoptychidae), and in the mental barbels of certain stomiids (*e.g.*, *Eustomias* and *Melanostomias*) (HAROLD; WEITZMAN, 1996; HAROLD, 2002; MARRANZINO; WEBB, 2018). The viperfish *Chauliodus* possesses a conspicuous photophore in the tip of dorsal-fin ray, which is exceptionally large and resembles the illicial lure observed in the deep-sea anglerfishes (Ceratioidei: Lophiiformes) (HAROLD; WEITZMAN, 1996; HAROLD, 2002; PIETSCH, 2009). The stomiids also have a complex of photophores in the orbital region including the antorbital and post-orbital photophores (HERRING, 2007). In some species, the postorbital photophore is sexually dimorphic, with males having postorbital photophores larger than females (*e.g.*, *Echiostoma* and *Pachystomias*) and most likely used in sexual communication (HERRING, 2000, 2007).

Besides, the malacosteines *Aristostomias* and *Malacosteus*, and the melanostomiine *Pachystomias* can produce far-red, long-wave emissions (>650 nm) from their accessory orbital photophores, a unique character among vertebrates (WIDDER *et al.*, 1984; HERRING; COPE, 2005). They also have a system and a bacteriochlorophyll-derived retinal photosensitizer that allows long-wave visual sensitivity (WIDDER *et al.*, 1984; HERRING; COPE, 2005).

Bioluminescence in stomiiforms, as well as in other bioluminescent fishes, bears several functions, including predator avoidance, prey attraction, intra and interspecific communication (*e.g.*, sexual selection), or camouflage by using counterillumination (HERRING, 1987; 2002).

1.2.2 Synapomorphies of the group

Fink and Weitzman (1982) proposed a list of synapomorphies for this group (Figs. 1.2, 1.3, 1.4A), which remained accepted years after (HAROLD; WEITZMAN, 1996; HAROLD, 1998). Springer and Johnson (2004) recognized two more synapomorphies for the group, based on dorsal gill-arch musculature (Fig. 1.4B). Currently, the following ten synapomorphies are recognized for stomiiforms:

1) Unique structure of the photophores. The photophores are non-bacterial and their walls are covered by two types of flat cells, the photocytes that produce bioluminescence, and glandular cells, which may act like filters. The granular cells are filled with guanine platelets and covered by a pigmented layer that serves as a reflector (BASSOT, 1966) (Fig. 1.2D). There are three types of photophores – Alpha, Beta and Gamma –, and their identification is based on the arrangement of the photocytes. The Beta photophore is the only type that exhibits a central lumen, which sometimes has an opening to the outside (BASSOT, 1966).

2) Unique pattern of tooth attachment within teleosts. Stomiiformes have a Type 3 of tooth attachment (*sensu* FINK, 1981) – present in most adults at both maxillary, dentary, and upper and lower pharyngeal teeth. In the type 3 tooth attachment, the anterior tooth border is not fully mineralized to the attachment bone (Figs. 1.2A, B), and acts as a hinge with an anterior axis of rotation. Stomiiforms also present type 1 and 4 of tooth attachment, but type 3 occurs exclusively in members of the order (FINK, 1981).

3) Network of blood vessels, *rete mirabile*, responsible for inflating and deflating the swim bladder, located in the posterior region of the swim bladder (Fig. 1.2C). In the other teleosts, the *rete mirabilis* is located in the middle or in the anterior region of the swim bladder (MARSHALL, 1960).

4) Second epibranchial bone having a broad termination that articulates with the second and third pharyngobranchials (Fig. 1.3A) - in other teleosts, the anterior tip of the second epibranchial is forked (ROSEN, 1973, FINK; WEITZMAN, 1982).

5) Presence of a premaxillary-rostrodermethmoid ligament that connects the dorsolateral face of the lateral ethmoid to the contralateral premaxilla or to both the contralateral and ipsilateral premaxillae (Fig. 3B) (FINK; WEITZMAN, 1982).

6) Posterior branchiostegal rays widely expanded (Fig. 3D) (FINK; WEITZMAN, 1982).

7) Branchiostegal rays articulating with the ventral hypohyals (Fig. 1.3C), except in some sternoptychids and the malacosteines, in which the branchiostegal rays reach only the anterior and posterior ceratohyals (FINK; WEITZMAN, 1982).

8) Medial section of the mandibular adductor muscle subdivided in two sections, one of them inserting dorsally directly on the maxilla and the other section inserting on the primordial ligament (Fig. 4A) (ROSEN, 1973).

9) Component of the *obliquus dorsalis* 4 (*Od4*), a muscle of the dorsal gill-arch, is attached to pharyngobranchial 4 (*Pb4*) (Fig. 4B) (SPRINGER; JOHNSON, 2004).

10) Branchial muscle adductor 5 is attached to epibranchial 4 (Fig. 1.4B) - in other teleosts, the *Ad5* is attached to *Eb5* (SPRINGER; JOHNSON, 2004).

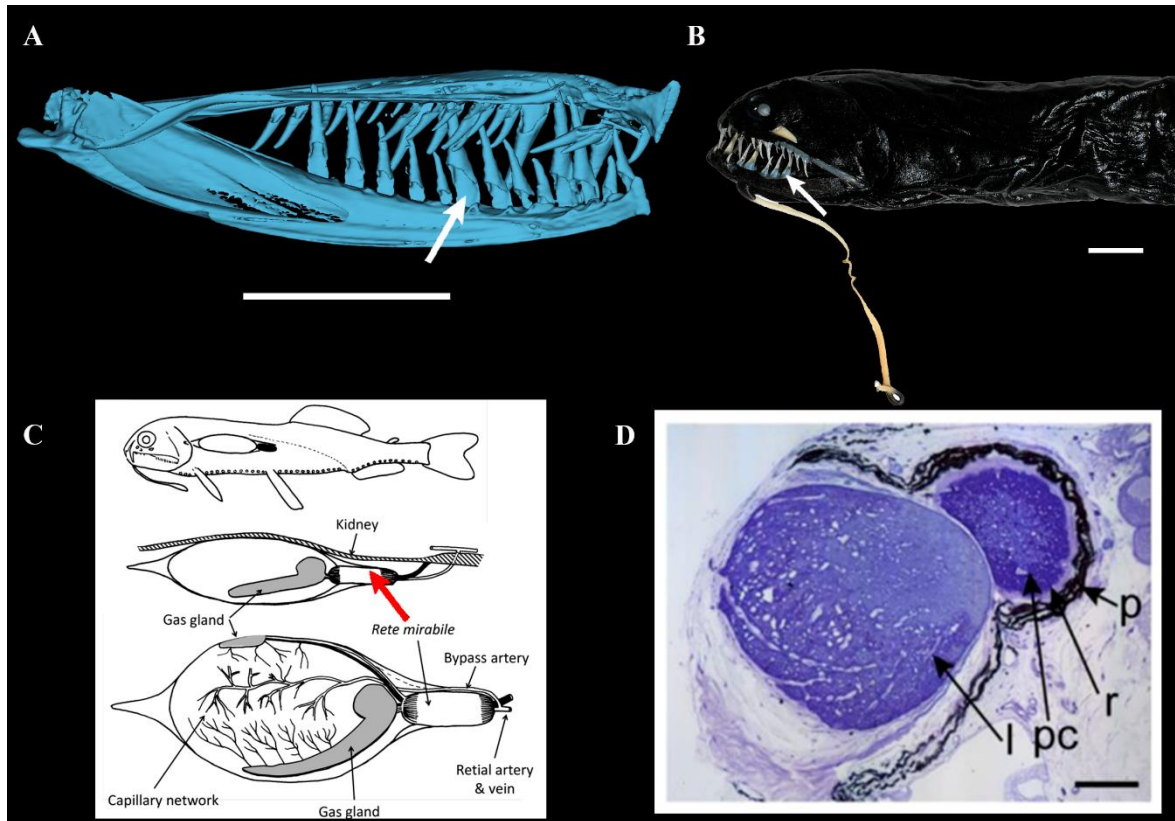


Figure 1.2. Selected synapomorphies of the Stomiiformes. (A) and (B): type 3 of tooth attachment. (A) upper and lower jaws of *Melanostomias bartonbeani*, MCZ 132357, 222.9 mm SL, micro CT-Scan, left side in medial view, with the white arrow indicating the unmineralized collagen area on the teeth posterior border, scale bar=10mm; (B) *Melanostomias* sp., MZUSP uncat., with the white arrow indicating the mineralized area at the teeth anterior border, scale bar=10 mm; (C) swim bladder scheme of *Astronesthes niger*, with the red arrow indicating *rete mirabile* on the posterior region (adapted from MARSHALL, 1960); (D) histology of ventral photophore of *Chauliodus sloani* (adapted from MARRANZINO, 2016). Abbreviations: l=lens, pc=photogenic chamber; r=reflector layer, p=pigment layer, scale bar=1 μ m.

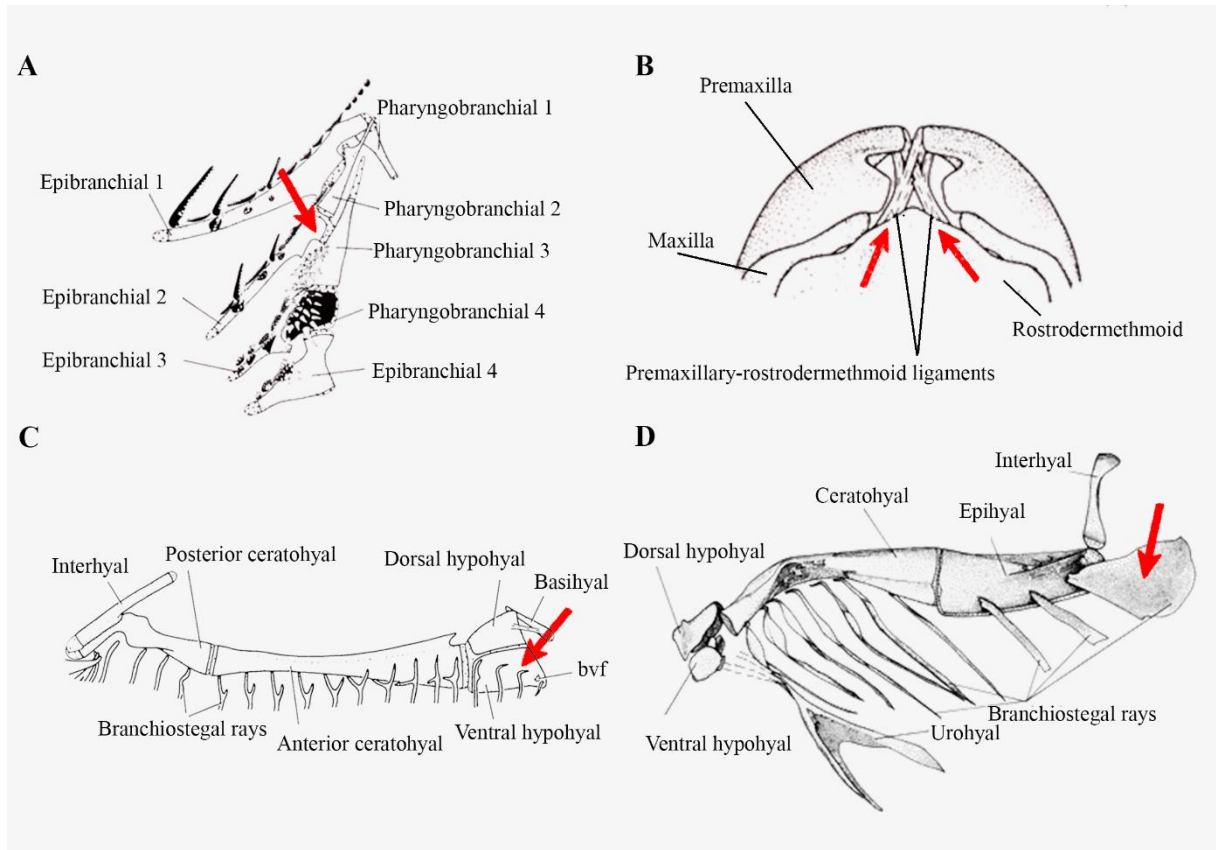


Figure 1.3. Osteological synapomorphies of the Stomiiformes: (A) dorsal portion of branchial apparatus of *Diplophos taenia*, USNM206614, 171.0 mm SL, with red arrow indicating the broad termination of the second epibranchial (adapted from FINK; WEITZMAN, 1982); (B) anterior ligaments of the suspensorium in *Diplophos taenia*, USNM206908, 184.3 mm SL, dorsal view, with the red arrows indicating the premaxillary-rostrodermethmoid ligaments (adapted from FINK; WEITZMAN, 1982); (C) hyoid arch of *Stomias brevibarbatus*, right side, lateral view, with red arrow indicating the branchiostegal rays in ventral hypohyal (adapted from FINK, 1985); (D) left side of the hyoid apparatus in *Argyropelecus aculeatus*, 49.5 mm SL, lateral view, with red arrow indicating the last branchiostegal ray widely large (adapted from WEITZMAN, 1974).

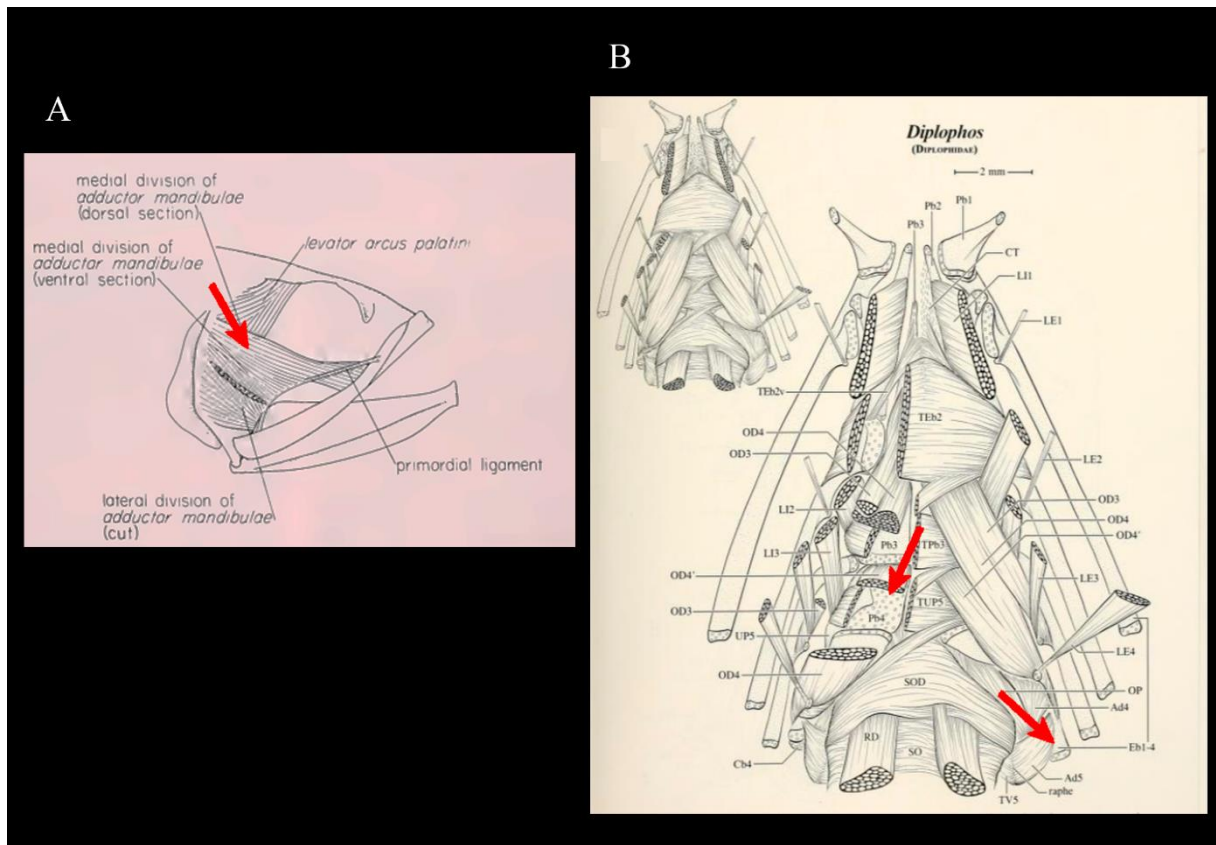


Figure 1.4. Synapomorphies in muscular system of the Stomiiformes: (A) medial division of *adductor mandibulae* in *Diplophos taenia* (MCZ 55469; 117.0 mm SL), with red arrow indicating the dorsal section (adapted from FINK; WEITZMAN, 1982); (B) dorsal gill-ach of *Diplophos* with the upper red arrow indicating the component of the *obliquus dorsalis* 4 (*Od4*) attached to pharyngobranchial 4 (*Pb4*), and the lower red arrow indicating adductor 5 (*Ad5*) attached to epibranchial 4 (*Eb4*) (adapted from SPRINGER; JOHNSON, 2004).

1.2.3 Systematics

Stomiiformes were firstly recognized and diagnosed by Brauer (1906), and at various times in the past, they have been thought to be closely related to several groups, such as the Clupeiformes (REGAN, 1923; MARSHALL, 1960), Salmoniformes (GARSTANG, 1931; BEEBE; CRANE, 1939; GREENWOOD *et al.*, 1966), and Iniomi – a now-invalid group comprising Myctophiformes and Aulopiformes (GARSTANG, 1931).

Weitzman (1967) included Stomiiformes into Osmeroidea (Osmeriiformes plus Galaxiiformes) based on the presence of an unossified mesethmoid. Such hypothesis found support on molecular evidence, with the Stomiiformes being recovered in a clade containing Osmeriformes and Galaxiiformes (LOPEZ *et al.*, 2004), or sister to Osmeriformes, forming the clade Stomiati (MIRANDE, 2017; BETANCUR-R *et al.*, 2017).

Later on, Rosen (1973) suggested that Stomiiformes were more closely related to Eurypterygii – a clade formed by Aulopiformes, Myctophiformes and Acanthomorphata (FINK; WEITZMAN, 1982). Rosen (1973) first raised stomiiforms to the order level and provided a phylogenetic diagnosis (FINK; WEITZMAN, 1982). Fink and Weitzman (1982) first recognized Stomiiformes as monophyletic and provided eight synapomorphies supporting the group, listed above.

Regarding the interrelationships among species of Stomiiformes, over the past years, the classification of genera has gone through several changes, mainly because of the extensive homoplasy within the group (FINK, 1984; HAROLD; WEITZMAN, 1996). Weitzman (1974) recognized two major clades, Gonostomata, including species with four bony pectoral-fin radials (except one in *Cyclothone*) and comprising the Gonostomatidae and Sternoptychidae; and Photichthya, including the species with three bony pectoral-fin radials (rarely 0-2 in genera with reduced pectoral fins) and comprising Stomiidae and Phosichthyidae.

Nelson (1994) followed Weitzman (1974) and recognized two suborders within Stomiiformes: Gonostomatoidei (=Gonostomata of Weitzman, 1974) and Phosichthyoidei (=Photichthya of Weitzman, 1974). This systematic proposal remains widely used so far (*e.g.*, NELSON, 2006; NELSON *et al.*; 2016). In recent years, with the popularization of the molecular techniques, new studies suggested several modifications to the systematic arrangement of the families as described next. In the molecular phylogeny proposed by Betancur-R *et al.* (2017), Phosichthyoidei was recognized as Stomiatoidei and includes Phosichthyidae, Sternoptychidae and Stomiidae, rendering Phosichthyidae as paraphyletic. Similarly, the phylogenies of Kenaley *et al.* (2014), Mirande *et al.* (2017) and Rabosky *et al.* (2018) also recovered Phosichthyidae as non-monophyletic. The genera *Diplophos*, *Manducus* and *Triplophos* comprising the family Diplophidae have been substantially involved in discussion. The family was erected by Fowler (1936), to include the genus *Diplophos*. Grey (1964) recognized *Diplophos* as belonging to the Gonostomatidae, but Fink and Weitzman (1982), Fink (1984) and Ahlstrom *et al.* (1984) agreed that this genus should not be included in the Gonostomatidae, based on myological and osteological features of the head (*e.g.*, a series of plesiomorphic characters for stomiiforms, including a large, toothed basihyal). Nelson (1994) provisionally placed *Diplophos*, *Manducus* and *Triplophos* in the subfamily Diplophinae within Gonostomatidae. Harold (1998) provided phylogenetic evidence that *Diplophos* and *Manducus* form a clade, which is a possible sister group to all stomiiforms, but not to *Triplophos*. Diplophidae was provisionally recognized by Nelson (2006) based on Harold (1998) evidence, which was followed by several authors later (*e.g.*, KENALEY; STEWART, 2015; VILLARINS

et al., 2022). Betancur-R *et al.* (2017) no longer recognized Diplophidae as a separate family, and *Diplophos* was identified as the sister of all other gonostomatids. However, the most recent molecular phylogeny of Rabosky *et al.* (2018) recovered the monophyly of the family Diplophidae.

Although recent molecular data has been addressing inter and intra relationships of the stomiiforms, there is still a lack of knowledge regarding rare species, with a considerable number of taxa not represented in the genetic banks, like the diplophid *Manducus* and the melanostomiine *Opostomias* (personal observation).

1.2.4 Ecological aspects

Stomiiforms are key organisms in the oceanic food webs (CARMO *et al.*, 2015), as they perform vertical migrations to feed on shallow waters during the night and are part of the diet of many marine vertebrates, such as tunas and dolphins (YOUNG *et al.*, 1997; GANNON *et al.*, 1998). Through the diel vertical migration (DVM), they feed near the surface, transporting carbon from the epipelagic to mesopelagic and bathypelagic zones during the daytime (SUTTON; HOPKINS, 1996; ANGEL, 1997).

Many stomiiforms perform diel vertical migration (BADCOCK, 1970; KENALEY, 2008), with very few exceptions such as the bristlemouth genus *Cyclothone* and the loosejaws genus *Malacosteus*, generally inhabiting depths below 300 meters (BADCOCK; MERRETT, 1976; MIYA; NEMOTO, 1986; SUTTON; HOPKINS, 1996; KENALEY, 2008). However, the diel vertical migration among the stomiiforms is more complex than just migration or its lack, for example, some taxa (*e.g.*, *Photostomias* and *Aristostomias*) have asynchronous diel vertical migration, where only a portion of a species' population migrates upwards at night while other individuals remain at greater depths (SUTTON; HOPKINS, 1996; KENALEY, 2008). Besides, ontogenetic variations and also physical drivers like temperature may affect the DVM (Eduardo *et al.*, 2020b).

Stomiiforms have a wide variety of feeding habits. Diplophids and gonostomatids feed on small crustaceans, especially copepods and euphausiids (HOPKINS *et al.*, 1996). Phosichthyids are usually more generalist, with some taxa feeding mostly on small crustaceans (*e.g.*, *Pollichthys* and *Polymetme*), while other taxa have diets including crustaceans and fishes (*e.g.*, *Phosichthys* and *Vinciguerria*) (HOPKINS *et al.*, 1996; MARQUES, 2001; WILLIAMS *et al.*, 2001; GASKETT *et al.*, 2010). Sternoptychids also have a diet based on small crustaceans

and fishes: *Maurolicus* feeds mostly on copepods, and hatchetfishes (*e.g.*, *Argyropelecus* and *Sternoptyx*) feed on small crustaceans (mainly amphipods) and polychaets when juveniles and small fishes when adults, and even on other hatchetfishes including cannibalism (*e.g.*, larger individuals of *A. aculeatus*) (HOPKINS *et al.*, 1996; CARMO *et al.*, 2015; EDUARDO *et al.*, 2020a). Most Stomiids feed mostly on fishes, specially myctophids (lanternfishes), except for astronesthines, whose diet is composed primarily of euphausiids and minorly of fishes, and *Malacosteus*, whose diet is based predominantly on copepods, euphausiids and decapods, and rarely on fishes (HOPKINS *et al.*, 1996; SUTTON AND HOPKINS; 1996; SUTTON, 2005; MCGONALE *et al.*, 2023; EDUARDO *et al.*, 2020b).

Stomiids have morphological adaptations for hunting relatively large prey, including elongated jaws, sharp teeth, loss of gill rakers and an occipito-vertebral gap (TRACEY; SUTTON, 1996; SCHNELL *et al.*, 2010). Some stomiids also exhibit reduction of anterior vertebral centra, such as *Chauliodus* and *Eustomias* (SCHNELL *et al.*, 2010). The occipito-vertebral gap and the reduction of anterior vertebral centra allow these fishes to bend the head to a considerable degree (SCHNELL *et al.*, 2008, 2010), and they use the occipital-vertebral hinge to create high gape angles (KENALEY, 2012). The loosejaw *Malacosteus* is also capable of reaching mouth gapes up to 120° due to the lack of a functional head joint (KENALEY, 2012; SCHNELL; JOHNSON, 2017).

1.3 Ecomorphology and Geometric Morphometric Methods (GGM)

Ecomorphology is an interdisciplinary field that intertwines ecology and morphology, investigating the intricate relationships between an organism's morphology and its environment (WAINWRIGHT, 1991). Ecomorphology is based on the fundamental premise that interspecific morphological differences may be intrinsically linked to the interaction of environmental and biological forces (WAINWRIGHT, 1991). These disparities are explored through ecomorphological attributes, which constitute patterns reflecting individual traits in relation to their ecological milieu and can serve as discernible indicators of species' adaptive strategies and habitat preferences (GATZ, 1979).

Ecomorphological studies in fishes involve two main approaches the first involves the examination of morphological traits directly related to food acquisition, encompassing parameters like mouth size, mouth position, head size, among others (ADITE; WINEMILLER, 1997; HUGUENY; POUILLY, 1999; FUGI *et al.*, 2001); the second approach relates to

attributes concerning locomotion and niche occupancy (BREDA *et al.*, 2005; WILLIS *et al.*, 2005).

In this regard, it can be inferred that the form of characters associated with food intake intricately corresponds to the composition of the dietary spectrum, where variations therein delineate the feeding aptitudes unique to each species (WAINWRIGHT; RICHARD, 1995). Similarly, attributes such as body height, body width, caudal peduncle size and configuration, fin morphology, among others, may distinctly intertwine with the structural attributes and complexity of the habitat, which includes factors like water depth and substrate composition. These variations subsequently facilitate the spatial partitioning of species across the spectrum of available habitats (WILLIS *et al.*, 2005).

Among the Stomiiformes, the use of ecomorphology focusing on morphological traits related to the diet was recently applied in the Melanostomiinae, which is the most diverse subfamily within Stomiidae family, and revealed that variation within the group was driven mostly by barbel length, vertical oral gape, and horizontal maxillary oral gape, with dietary diversity much lower than morphological diversity (MCGONALE, 2023). In contrast, Martinez *et al.* (2021) studied body-shape and niche occupancy of several lineages of deep-sea, including Stomiiformes, and concluded that body shape disparity increases with depth, driven by traits associated with locomotion.

Ecomorphology is based on a quantitative study of biological shape – or morphometrics – and such a method commonly recognizes three principal categories, each delineated by the intrinsic attributes of the data under analysis (WEBSTER; SHEETS, 2010). Traditional morphometrics entails the representation of morphology through descriptors such as linear measurements, ratios, and angles, which can be investigated in an unidimensional or multidimensional approach; landmark-based geometric morphometrics summarizes shape in terms of an assemblage of discrete anatomical loci, each characterized by Cartesian coordinates in two or three dimensions, in a multidimensional approach; outline-based geometric morphometrics captures shape by summarizing the configuration of open or closed curves (WEBSTER; SHEETS, 2010).

Geometric morphometrics provides a comprehensive approach for capturing and statistically quantifying intricate forms within a clade, facilitating the examination of evolutionary hypotheses within a phylogenetic context (ROHLF, 1990, PERES-NETO, 1995, ACERO *et al.*, 2005; GOSWAMI *et al.*, 2019). Geometric morphometric methods (GMM) differ from traditional linear morphometry for using unidirectional distances in the form of linear measurements, allowing the preservation of information about the geometry of the

organism or structure, which is difficult to recover from the records obtained by linear measurements such as distances, angles, and proportions (BOOKSTEIN, 1991; ZELDITCH *et al.*, 2004).

Geometric morphometric is based on Cartesian landmarks – which are homologous points recognizable on all specimens in the study – and can be marked in two or three dimensions (BOOKSTEIN, 1991; ZELDITCH *et al.*, 2004, WEBSTER; SHEETS, 2010). After obtaining landmarks through specific software, the next step is to translate and rotate the landmark configurations into a common position, removing variation associated with differences in landmarks location, orientation, and size, leaving only shape – an operation that called superimposition (WEBSTER; SHEETS, 2010). In addition to assessing whether groups differ in mean shape, groups can also be discriminated by categorical variables (*e.g.*, diet, geographic locality) (WEBSTER; SHEETS, 2010). Thus, geometric morphometric methods yield insights into diverse ecological components related to body-shape, such as dietary preferences, habitat utilization, selective pressures, and competition dynamics (PERES NETO, 1995).

Although geometric morphometric methods have been widely applied in studies of functional morphology of fish, only a few studies focus on ecomorphology and evolution of body shape across lineages of deep-sea fishes (*e.g.*, NEAT; CAMPBELL, 2013; FARRÉ *et al.*, 2016; MINDEL *et al.*, 2016; TUSET *et al.*, 2018, MAILE *et al.*, 2020; MARTINEZ *et al.*, 2021). Within Stomiiformes, geometric morphometrics methods were recently applied for intraspecific variability analysis of Sternoptychidae and Myctophidae (lanternfishes), the latter, one of the most notable family of mesopelagic fishes (GORDEEVA; NANOVA, 2017). The authors used a system of landmarks to describe anatomical features and configuration of the photophores, and the influence of the ecological conditions in shape considering different populations, which only was significant for myctophid populations.

1.4. Research aims and objectives

Stomiiformes is one of the most diverse groups of deep-sea fishes in terms of body shape, mouth size, dentition, feeding habitats, locomotion, and bioluminescence (HAROLD; WEITZMAN, 1996). Such morphological and ecological diversity allows a great opportunity to study the effects of intrinsic and extrinsic factors on morphological evolution.

This research investigates the patterns of diversity shape among the species of Stomiiformes, including body and skull shape, through the application of geometric morphometric methods, with data acquired in two and three dimensions, in an ecological and phylogenetic context. Therefore, this study seeks to understand the main drivers of stomiiforms diversification, testing whether such morphological variation is driven by ecological traits, such as diet and ocean depth.

This thesis is divided into three chapters. Chapter One is the General Introduction. Chapter Two, titled “Body-shape evolution among dragonfishes and their allies (Teleostei: Stomiiformes)”, is dedicated to exploring the evolutionary patterns of body shape in stomiiforms employing two-dimensional geometric morphometric methods. Furthermore, Chapter Two aims to conduct a comparative analysis of shape variation in relation to ecological traits among stomiiforms, such as diet, habitat (mesopelagic or meso-bathypelagic region), and the presence or absence of diel vertical migration pattern using multivariate methods within a phylogenetic context. The manuscript derived from Chapter Two was submitted to *Ichthyology and Herpetology*.

Chapter Three, entitled "The Evolution of Skull Morphology in Dragonfishes and their Allies (Teleostei: Stomiiformes)", is dedicated to exploring the evolutionary patterns of skull shape in stomiiforms using geometric morphometric methods based on three-dimensional models obtained from micro-CT scans. Furthermore, Chapter Three aims to conduct a comparative analysis of these shapes in conjunction with ecological (diet and habitat) and morphological traits associated with the feeding apparatus (*e.g.*, proportions of skull, jaws and type and size of the teeth), employing multivariate methods in a phylogenetic context. The main goal of Chapter Three is assessing whether the stomiiforms skull-shape is primarily influenced by ecological or anatomical traits of feeding apparatus.

1.5. References

- ACERO P., A.; TAVERA, J. J.; REYES, J. Systematics of the genus *Bagre* (Siluriformes: Ariidae): A morphometric approach. *Cybium*, v. 29, n. 2, p. 127–133, 2005. <https://doi.org/10.26028/cybium/2005-292-003>.
- ADITE, A.; WINEMILLER, K. O. Trophic ecology and ecomorphology of fish assemblages in coastal lakes of Benin, West Africa. *Ecoscience*, v. 4, n. 1, p. 6–23, 1997.
- AHLSTROM, E. H.; RICHARDS, W. J.; WEITZMAN, S. H. Families Gonostomatidae, Sternoptychidae, and associated stomiiform groups: Development and relationships, p. 184–198. In: MOSER, H. G.; RICHARDS, W. J.; COHEN, D. M.; FAHAY, M. P.; KENDALL, A. W. Jr.; RICHARDSON, S. L. (eds.). **Ontogeny and Systematics of Fishes**. American Society of Ichthyologists and Herpetologists Special Publication, Lawrence, KS, 1984.
- ANDERSON, T. R.; RICE, T. 2006. Deserts on the sea floor: Edward Forbes and his azoic hypothesis for a lifeless deep ocean. *Endeavour*, v. 30, n. 4, p. 131–137, 2006.
- ANGEL, M. V. Vertical profiles of pelagic communities in the vicinity of the Azores Front and their implications to deep ocean ecology. *Progress in Oceanography*, v. 22, n. 1, p. 1–46, 1989.
- ANGEL, M. V. What is the deep-sea? Pp. 2–41. In: RANDALL, D. J.; FARRELL, A. P. (eds.). **Deep-Sea Fishes**. San Diego, Academic Press, 1997.
- ANGEL, M. V. Biodiversity of the pelagic ocean. *Biological Conservation*, v. 7, p. 760–772, 1993.
- BADCOCK, J. The vertical distribution of mesopelagic fishes collected on the SONDA cruise. *Journal of the Marine Biological Association of the United Kingdom*, v. 50, n. 4, p. 1001–1044, 1970.
- BADCOCK, J. Gonostomatidae. In: WHITEHEAD, P. J. P.; BAUCHOT, M. L.; HUREAU, J. C; NIELSEN, J.; TORTONESE, E. (eds.). **Fishes of the north-eastern Atlantic and the Mediterranean**. v. 1. UNESCO, Paris, p. 284–301, 1984.
- BADCOCK, J.; MERRETT, N. R. Midwater fishes in the eastern North Atlantic. Vertical distribution and associated biology in 30°N 23°W with developmental notes on certain myctophids. *Progress in Oceanography*, v. 7, p. 3–58, 1976.

- BASSOT, J.-M. On the comparative morphology of some luminous organs, p. 557–610. In: JOHNSON, F. H.; HANEDA, Y. (eds.). **Bioluminescence in Progress**. Princeton University Press, Princeton, NJ, 1966.
- BEEBE, W.; CRANE, J. Deep-sea fishes of the Bermuda oceanographic expeditions. Family Melanostomiidae. **Zoologica**, v. 24, p. 65–238, 1939.
- BERNARDES, R. A.; ROSSI-WONGTSCHOWSKI, C.L.D.B.; MADUREIRA, L. S. **Prospecção Pesqueira de Espécies Pelágicas de Pequeno Porte com Rede de Meia-Água na Zona Econômica Exclusiva da Região Sudeste-Sul do Brasil**. Série documentos REVIZEE: Score Sul. São Paulo, Instituto Oceanográfico-USP, 136 p., 2007.
- BERTRAND, A. **ABRACOS** cruise, **RV Antea**, 2015. <https://doi.org/10.17600/15005600>.
- BERTRAND, A. **ABRACOS 2** cruise, **RV Antea**, 2017. <https://doi.org/10.17600/17004100>.
- BERTRAND, A.; DE SAINT LEGER, E.; KOCH-LARROUY, A. **AMAZOMIX 2021** cruise, **RV Antea**, 2021. <https://doi.org/10.17600/18001364>.
- BETANCUR-R, R.; WILEY, E. O.; ARRATIA, G.; ACERO, A.; BAILLY, N.; MIYA, M.; LECOINTRE, G; ORTI, G. Phylogenetic classification of bony fishes. **BMC Evolutionary Biology**, v. 17, n. 1, n. 162, p. 1–40, 2017. <http://doi.org/10.1186/s12862-017-0958-3>.
- BIGELOW, H. B.; SCHROEDER, W. C. **Fishes of the Gulf of Maine**. US Government Printing Office, 1964.
- BOOKSTEIN, F. L. Morphometric tools for landmark data: geometry and biology. Cambridge: Cambridge University Press, 1991. 435 p.
- BRAUER, A. Die Tiefsee-Fische. I. Systematischer Teil. In: CHUN, C. (ed.). **Wissenschaftliche Ergebnisse der deutschen Teifsee-Expedition auf dem Dampfer "Valdivia", 1898–1899**. G. Fischer, Jena, 1906.
- BREDA, L.; OLIVEIRA, E. F.; GOULART, E. Ecomorfologia de locomoção de peixes com enfoque para espécies neotropicais. **Acta Scientiarum: Biological Sciences**, v. 27, n. 4, p. 371–381, 2005.
- CARMO, V.; SUTTON, T.; MENEZES, G.; FALKENHAUG, T. BERGSTAD, O. A. Feeding ecology of the Stomiiformes (Pisces) of the northern Mid-Atlantic Ridge. 1. The Sternoptychidae and Phosichthyidae. **Progress in Oceanography**, v. 130, p. 172–187, 2015. <https://doi.org/10.1016/j.pocean.2014.11.003>.

- COCKER, J. E. Adaptations of deep-sea fishes. **Environmental Biology of Fishes**, v. 3, p. 389–399, 1978.
- EDUARDO, L. N.; LUCENA-FRÉDOU, F.; MINCARONE, M. M., SOARES, A; LE LOC'H, F.; FRÉDOU, T., MÉNARD, F.; BERTRAND, A. Trophic ecology, habitat, and migratory behaviour of the viperfish *Chauliodus sloani* reveal a key mesopelagic player. **Scientific Reports**, v.10(1): 20996, 2020. <https://doi.org/10.1038/s41598-020-77222-8>.
- EDUARDO, L. N. *et al.* Hatchetfishes (Stomiiformes: Sternoptychidae) biodiversity, trophic ecology, vertical niche partitioning and functional roles in the western Tropical Atlantic. **Progress in Oceanography**, v. 187, p. 102389, 2020a. DOI: <https://doi.org/10.1016/j.pocean.2020.102389>.
- ESCHMEYER, W. N.; HERALD, E. S.; HAMMANN, H. **A field guide to Pacific coast fishes of North America. Boston (MA, USA):** Houghton Mifflin Company, 1983.
- FARRÉ, M. *et al.* Habitat influence in the morphological diversity of coastal fish assemblages. **Journal of Sea Research**, v. 99, p. 107–117, 2015. DOI: <https://doi.org/10.1016/j.seares.2015.03.002>.
- FIGUEIREDO, J. L.; SANTOS, A. P.; YAMAGUTI, N.; BERNARDES, R. A.; ROSSI-WONGTSCHOWSKI, C. L. D. B. **Peixes da Zona Econômica Exclusiva da Região Sudeste-Sul do Brasil: Levantamento com Rede de Meia-Água.** São Paulo, Editora da USP, 248 p., 2002.
- FINK, W. L. Stomiiformes: Relationships. In: MOSER, H. G.; RICHARDS, W. J.; COHEN, D. M.; FAHAY, M. P.; KENDALL, A. W. Jr.; RICHARDSON, S. L. **Ontogeny and Systematics of Fishes.** Spec. Publ. American Society of Ichthyologists and Herpetologists, Lawrence, KS, 1984, pp. 181-184.
- FINK, W. L.; WEITZMAN, S. H. Relationships of the stomiiform fishes (Teleostei), with a description of *Diplophos*. **Bulletin of the Museum of Comparative Zoology**, v. 150, p. 31-93, 1982.
- FINK, W. L. Ontogeny and phylogeny of tooth attachment modes in actinopterygian fishes. **Journal of Morphology**, v. 167, p. 167-184, 1981.
- FINK, W. L. Phylogenetic interrelationships of the stomiid fishes (Teleostei: Stomiiformes). **Miscellaneous Publications, Museum of Zoology, The University of Michigan**, v. 171, p. 1-127, 1985.

- FORBES, E. **Report on the Mollusca and Radiata of the Aegean Sea, and their distribution, considered as bearing on geology.** Report (1843) to the 13th Meeting of the British Association for the Advancement of Science: 30–193, 1844.
- FRICKE, R.; ESCHMEYER, W. N.; VAN DER LAAN, R. (eds). **Eschmeyer's Catalog of Fishes: Genera, Species, References.** 2023. Available at: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>. Access on: [July 2023].
- FUGI, R.; AGOSTINHO, A. A.; HAHN, N. S. Trophic morphology of five benthic-feeding fish species of a tropical floodplain. **Revista Brasileira de Biologia**, v. 61, n. 1, p. 27-33, 2001.
- GANNON, D. R.; CRADDOCK, J. E.; READ, A. J. Autumn food habits of harbor porpoises, *Phocoena phocoena*, in the Gulf of Maine. **Fishery Bulletin**, v. 96, p. 428-437, 1998.
- GARSTANG, W. The phyletic classification of Teleostei. **Proceedings of the Leeds Philosophical and Literary Society**, Science Section, v. 2, p. 240-260, 1931.
- GASKETT, A. C.; BULMAN, C.; HE, X.; GOLDSWORTHY, S. D. Diet composition and guild structure of mesopelagic and bathypelagic fishes near Macquarie Island, Australia. **New Zealand Journal of Marine and Freshwater Research**, v. 35, p. 469-476, 2001.
- GATZ J. R., A. J. Community organization in fishes as indicated by morphological features. **Ecology**, v. 60, n. 4, p. 711–718, 1979.
- GORDEEVA, N. V., NANOVA, O .G. Application of geometric morphometrics for intraspecific variability analysis in mesopelagic fishes of Sternoptychidae and Myctophidae families. **Journal of Ichthyology**. V.57, p. 29–36, 2017. <https://doi.org/10.1134/S0032945217010052>
- GOSLINE, W. A. Contributions toward a classification of modern isospondylous fishes. *Bulletin of the British Museum (Natural History)*: **Zoology**, v. 6, p. 325-365, 1960.
- GREENWOOD, P. H., ROSEN, D. E., WEITZMAN, S. H.; MYERS, G. S. Phyletic studies of teleostean fishes, with a provisional classification of living forms. **Bulletin of the American Museum of Natural History**, v. 131, p. 339–456, 1966.
- GREGORY, W. K.; CONRAD, G. M. Pictorial phylogenies of deep sea Isospondyli and Iniomi. **Copeia**, v. 1936, p. 21-36, 1936.
- GREY, M. Family Gonostomatidae. In: BIGELOW, B. H. et al. (Eds.). **Fishes of the western North Atlantic, Part 4 Soft-Rayed Bony Fishes: Orders Isospondyli and**

- Giganturoidei: Argentinoids, Stomiatoids, Pickerels, Bathylaconids, Giganturids.** Memoir Sears Foundation for Marine Research, 1964, p. 78-240.
- HADDOCK, S. H. D.; MOLINE, M. A.; CASE, J. F. Bioluminescence in the Sea. **Annual Review of Marine Science**, v. 2, p. 443-493, 2010.
- HAROLD, A. S. Phylogenetic relationships of the Gonostomatidae (Teleostei: Stomiiformes). **Bulletin of Marine Science**, v. 62, p. 715-741, 1998.
- HAROLD, A. S. Gonostomatidae (881–884), Phosichthyidae (885–888), Sternoptychidae (889–892), Astronesthidae (893–895), Chauliodontidae (896–898), Idiacanthidae (899–900), Malacosteidae (901–903), Stomiidae (904–906), and Melanostomiidae (907–912). In K. E. CARPENTER (Ed.) **The living marine resources of the western central Atlantic, FAO species identification guide for fishery purposes**. Vol. 2. FAO, Rome, 2002.
- HAROLD, A. S.; WEITZMAN, S. H. Interrelationships of Stomiiform Fishes. In: STIASSNY, M. L. J.; PARENTI, L. R.; DAVID-JOHNSON, G. (Eds.). **Interrelationships of Fishes**. Academic Press Inc., San Diego, CA, 1996, pp. 333-353.
- HERRING, P. J. Systematic distribution of bioluminescence in living organisms. **Journal of bioluminescence and chemiluminescence**, v. 1, n. 3, p. 147-163, 1987.
- HERRING, P. J. Bioluminescent signals and the role of reflectors. **Journal of Optics A: Pure and Applied Optics**, v. 2, n. 6, p. R29, 2000.
- HERRING, P. J. **The biology of the deep ocean**. Oxford University Press, New York, 2002.
- HERRING, P. J. Sex with the lights on? A review of bioluminescent sexual dimorphism in the sea. **Journal of the Marine Biological Association of the United Kingdom**, v. 87, n. 4, p. 829-842, 2007. <https://doi.org/10.1017/S0025315407056433>.
- HERRING, P. J.; COPE, C. Red bioluminescence in fishes: on the suborbital photophores of *Malacosteus*, *Pachystomias* and *Aristostomias*. **Marine Biology**, v. 148, n. 2, p. 383-394, 2005.
- HOPKINS, T. L.; SUTTON, T. T.; LANCRAFT, T. M. The trophic structure and predation impact of a low latitude midwater fish assemblage. **Progress in Oceanography**, v. 38, p. 205-239, 1996. [https://doi.org/10.1016/S0079-6611\(97\)00003-7](https://doi.org/10.1016/S0079-6611(97)00003-7).
- HUGUENY, B.; POUILLY, M. Morphological correlates of diet in an assemblage of West African freshwater fishes. **Journal of Fish Biology**, v. 54, p. 1310-1325, 1999.

- IRIGOIEN, X. et al. Large mesopelagic fishes biomass and trophic efficiency in the open ocean. **Nature Communications**, v. 5, n. 1, p. 3271, 2014.
- JAMIESON, A.J.; MARONI, P.J.; BOND, T.; NIYAZI, Y.; KOLBUSZ, J.; ARASU, P.; KITAZATO, H. New maximum depth record for bony fish: Teleostei, Scorpaeniformes, Liparidae (8336 m, Izu-Ogasawara Trench). **Deep Sea Research Part I: Oceanographic Research Papers**, 199, p.104132, 2023.
- KENALEY, C. P.; STEWART, A. L. Family Diplophidae. *In*: ROBERTS, A. L. *et al.* (Eds.). **The fishes of New Zealand**, vol. 2. Wellington: Te Papa Press, 2015, p. 420-422.
- KENALEY, C. P. Diel vertical migration of the loosejaw dragonfishes (Stomiiformes: Stomiidae: Malacosteinae): a new analysis for rare pelagic taxa. **Journal of Fish Biology**, v. 73, n. 4, p. 888-901, 2008. <https://doi.org/10.1111/j.1095-8649.2008.01983.x>.
- KENALEY, C. P.; DEVANEY, S. C.; FJERAN, T. T. The complex evolutionary history of seeing red: Molecular phylogeny and the evolution of an adaptive visual system in deep-sea dragonfishes (Stomiiformes: Stomiidae). **Evolution**, v. 68, n. 4, p. 996-1013, 2014. <https://doi.org/10.1111/evo.12322>.
- KENALEY, C. P. Revision of Indo-Pacific Species of the Loosejaw Dragonfish Genus *Photostomias* (Teleostei: Stomiidae: Malacosteinae). **Copeia**, n. 1, p. 175-189, 2009.
- KENALEY, C. P. Exploring feeding behavior in deep-sea dragonfishes (Teleostei: Stomiidae): jaw biomechanics and functional significance of a loosejaw. **Biological Journal of the Linnean Society**, v. 106, p. 224–240, 2012.
- LINLEY, T. D. et al. Fishes of the hadal zone including new species, in situ observations and depth records of Liparidae. **Deep Sea Research Part I: Oceanographic Research Papers**, v. 114, p. 99-110, 2016.
- LÓPEZ, J. A.; CHEN, W.-J.; ORTÍ, G. Esociform phylogeny. **Copeia**, p. 449-464, 2004.
- MAILE, A. J. et al. Marine habitat transitions and body-shape evolution in lizardfishes and their allies (Aulopiformes). **Copeia**, v. 108, p. 820–832, 2020. <https://doi.org/10.1643/CG-19-300>.
- MARQUES, A. Some Data on the Biology of *Polymetme Corythaeola* (Phosichthyidae), From Off the Portuguese South Coast, North East Atlantic. **Cybium**, v. 25, n. 1, p. 100-102, 2001.
- MARRANZINO, A. **Flow Sensing in the Deep Sea: Morphology of the Lateral Line System in Stomiiform Fishes**. Open Access Master's Theses. Paper 889. University

- of Rhode Island, 2016. Available at: <https://digitalcommons.uri.edu/theses/889>. Access: [July 2023].
- MARRANZINO, A. N.; WEBB, J. F. Flow sensing in the deep sea: the lateral line system of stomiiform fishes. **Zoological Journal of the Linnean Society**, v. 183, n. 4, p. 945-965, 2018. <https://doi.org/10.1093/zoolinnean/zlx090>.
- MARSHALL, N. B. Swimbladder structure of deep-sea fishes in relation to their systematics and biology. **Discovery Reports**, n. 31, p. 1-122, 1960.
- MARSHALL, N. B. **Explorations in the Life of Fishes**. Cambridge, Massachusetts: Harvard University Press, 1971. 204 p.
- MARSHALL, N. B. **Developments in Deep-Sea Biology**. Blandford Press, 1979. 566 p.
- MARTINEZ, C. M. et al. The deep sea is a hot spot of fish body shape evolution. **Ecology Letters**, v. 24, p. 1788–1799, 2021. DOI: <https://doi.org/10.1111/ele.13785>.
- MCGONALE, R. P.; KERSTETTER, D. W.; FENOLIO, D.; SUTTON, T. T. Ecomorphology of a predatory deep-sea fish family: does trophic specialization drive hyperspeciation?. **Frontiers in Marine Science**, v. 10, n. 1056094, 2023.
- MELO, M. R.; CAIRES, R. A.; SUTTON, T. T. The scientific explorations for deep-sea fishes in Brazil: the known knowns, the known unknowns, and the unknown unknowns. *In: Brazilian deep-sea biodiversity*. 2020. p. 153-216.
- MERRET, N. R.; HAEDRICH, R. L. Chapter one: Setting the stage. *In: MERRET, N. R.; HAEDRICH, R. L. (org.). Deep-sea Demersal Fish and Fisheries*. London: Chapman and Hall, 1997, p. 1-30.
- MINDEL, B. L. et al. Functional, size and taxonomic diversity of fish along a depth gradient in the deep sea. **PeerJ**, v. 4, p. e2387, 2016. DOI: <https://doi.org/10.7717/peerj.2387>.
- MIRANDE, J. M. Combined phylogeny of ray-finned fishes (Actinopterygii) and the use of morphological characters in large-scale analyses. **Cladistics**, v. 33, n. 4, p. 333-350, 2017. <https://doi.org/10.1111/cla.12171>.
- MIYA, M.; NEMOTO, T. Life history and vertical distribution of the mesopelagic fish *Cyclothone alba* (family Gonostomatidae) in Sagami Bay, central Japan. **Deep-Sea Research**, v. 33, p. 1053-1068, 1986.
- MORAIS, J. M. **Petróleo Em Águas Profundas: Uma História Tecnológica da PETROBRAS na Exploração e Produção Offshore**. Brasília, Petrobrás / Ipea, 424 p, 2013.

- NEAT, F. C.; CAMPBELL, N. Proliferation of elongate fishes in the deep sea. **Journal of Fish Biology**, v. 83, p. 1576–1591, 2013. DOI: <https://doi.org/10.1111/jfb.12266>.
- NELSON, J. S. **Fishes of the World**, 3^a Edition. Wiley and Sons, New York, 1994.
- NELSON, J. S. **Fishes of the world**. 4^a ed. John Wiley and Sons, Inc., Hoboken, NJ, 2006. 601 p.
- NELSON, J. S.; GRANDE, T. C.; WILSON, M. V. H. **Fishes of the World**. 5^a ed. John Wiley and Sons, Inc., Hoboken, NJ, 2016. 752 p.
- NIELSEN, J. G. The deepest living fish *Abyssobrotula galathea*: a new genus and species of oviparous ophidioids (Pisces, Brotulidae). **Galathea Report**, 1977.
- PARIN, N. V.; BORODULINA, O. D. A new mesobenthic fish, *Eupogonesthes xenicus* (Astronesthidae), from the eastern Indian Ocean. **Journal of Ichthyology**, v. 33, n. 8, p. 111-116, 1993.
- PERES-NETO, P. R. Introdução a análises morfométricas. In: PERES-NETO, P. R.; VALENTIN, J. L.; FERNANDEZ, F. A. Z. **Ecologia Brasiliensis**. vII: Tópicos em tratamentos de dados biológicos, p. 57-89, 1995.
- PIETSCH, THEODORE W. **Oceanic anglerfishes: extraordinary diversity in the deep sea**. University of California Press, California, 2009.
- PRIDE, I. G.; FROESE, R. Colonization of the deep-sea by fishes. **Journal of Fish Biology**, v. 83, n. 6, p. 1528-1550, 2013.
- RABOSKY, D. L. *et al.* An inverse latitudinal gradient in speciation for marine fishes. **Nature**, v. 559, p. 392-395, 2018. <https://doi.org/10.1038/s41586-018-0273-1>.
- RAMIREZ-LLODRA, E. Deep-sea ecosystems: biodiversity and anthropogenic impacts. In: BANET CATHERINE. **The Law of the Seabed: Access, Uses, and Protection of Seabed Resources**. Brill Nijhoff. p. 36-60, 2020.
- RANDALL, D. J.; FARRELL, A. P. **Deep-Sea Fishes**. Academic Press, 1997. 388 p.
- REES, J. F. *et al.* The origins of marine bioluminescence: turning oxygen defence mechanisms into deep-sea communication tools. **The Journal of Experimental Biology**, v. 201, p. 1211-1221, 1998.
- REGAN, C. T. The classification of the stomiatooid fishes. **Annals and Magazine of Natural History**. Series 9, p. 612–614, 1923.
- REGAN, C. T. The anatomy and classification of the teleostean fishes of the order Iniomi. **Annals and Magazine of Natural History**, Series 8, v. 7, p. 120–133, 1911.
- ROHLF, F. J.; SLICE, D. E. Extensions of the Procrustes method for the optimal superimposition of landmarks. **Systematic Zoology**, v. 39, p. 40-59, 1990.

- ROSEN, D. E. Interrelationships of higher euteleostean fishes. **Zoological Journal of the Linnean Society**, v. 53, p. 397-513, 1973.
- SCHNELL, N. K. et al. The “pseudo-craniovertebral articulation” in the deep-sea fish *Stomias boa* (Teleostei: Stomiidae). **Journal of Morphology**, v. 269, p. 513–521, 2008.
- SCHNELL, N. K. et al. New insights into the complex structure and ontogeny of the occipitovertebral gap in barbeled dragonfishes (Stomiidae, Teleostei). **Journal of Morphology**, v. 271, p. 1006–1022, 2010. <http://doi.org/10.1002/jmor.10858>.
- SCHNELL, N. K.; JOHNSON, G. D. Evolution of a functional head joint in deep-sea fishes (Stomiidae). **PLoS ONE**, v. 12, n. 2, e0170224, 2017. <https://doi.org/10.1371/journal.pone.0170224>.
- SPRINGER, V. G.; JOHNSON, G. D. Study of the dorsal gill-arch musculature of teleostome fishes, with special reference to the Actinopterygii. **Bulletin of the Biological Society of Washington**, v. 11, p. 1–235, 2004.
- SUMIDA, P. Y. G. Mar Profundo. In: PEREIRA, R. C.; SOARES-GOMES, A. **Biologia marinha**. Rio de Janeiro: Interciência, 2002, p. 631.
- SUTTON, T. T. Vertical ecology of the pelagic ocean: classical patterns and new perspectives: vertical ecology of the pelagic ocean. **Journal of Fish Biology**, v. 83, p. 1508–1527, 2013. DOI: <https://doi.org/10.1111/jfb.12263>.
- SUTTON, T. T. et al. A global biogeographic classification of the mesopelagic zone. **Deep-Sea Research Part I**, v. 126, p. 85–102, 2017.
- SUTTON, T. T.; HULLEY, P. A.; WIENERROITHER, R.; ZAERA-PEREZ, D.; PAXTON, J. R. **Identification guide to the mesopelagic fishes of the central and southeast Atlantic Ocean**. FAO Species Identification Guide for Fishery Purposes. Rome: FAO, 2020. <https://doi.org/10.4060/cb0365en>.
- SUTTON, T. T. Trophic ecology of the deep-sea fish *Malacosteus niger* (Pisces: Stomiidae): An enigmatic feeding ecology to facilitate a unique visual system? **Deep-Sea Research I**, v. 52, p. 2065–2076, 2005.
- SUTTON, T. T.; HOPKINS, T. L. Trophic ecology of the stomiid (Pisces: Stomiidae) fish assemblage of the eastern Gulf of Mexico: Strategies, selectivity and impact of a top mesopelagic predator group. **Marine Biology**, v. 127, p. 179–192, 1996. <https://doi.org/10.1007/BF00942102>.
- THISTLE, D. The deep-sea floor, an overview. In: TYLER, P. A. (Ed.) **Ecosystems of the World 28. Ecosystems of the Deep-Ocean**. Amsterdam: Elsevier, 2003. p. 5-37.

- THOMSON, C. W. General introduction to the zoological series of reports. In: Tomson, C.W. (Org.) Report on the Scientific Results of the Voyage of H. M. S. Challenger During the Years 1873-76. **Zoology**, v 1: p. 1–59, 1880.
- TUSET, V. M. et al. Morpho-functional diversity in *Diaphus* spp.(Pisces: Myctophidae) from the central Atlantic Ocean: Ecological and evolutionary implications. **Deep Sea Research Part I: Oceanographic Research Papers**, v. 138, p. 46–59, 2018. DOI: <https://doi.org/10.1016/j.dsr.2018.07.005>.
- VILLARINS, B. T. *et al.* Deep-sea dragonfishes (Teleostei: Stomiiformes) collected from off northeastern Brazil, with a review of the species reported from the Brazilian Exclusive Economic Zone. **Neotropical Ichthyology**, v. 20, 2022.
- WAINWRIGHT, P. C. Ecomorphology: Experimental Functional Anatomy for Ecological Problems. **American Zoologist**, v.31, n.4, 1991, p. 680–693, <https://doi.org/10.1093/icb/31.4.680>.
- WAINWRIGHT, P. C.; RICHARD, B. A. Predicting patterns of prey use from morphology of fishes. *In*: **ECOMORPHOLOGY OF FISHES**. Springer Netherlands, 1995. p. 97–113.
- WEBSTER, M.; SHEETS, H. A Practical Introduction to Landmark-Based Geometric Morphometrics. **The Paleontological Society Papers**, v.16, p.163–188, 2010. <https://doi.org/10.1017/S1089332600001868>.
- WEITZMAN, S. H. The origin of the stomioid fishes with comments on the classification of the salmoniform fishes. **Copeia**, v. 3, p. 507–540, 1967.
- WEITZMAN, S. H. Osteology and evolutionary relationships of the Sternoptychidae, with a new classification of stomioid families. **Bulletin of the American Museum of Natural History**, v. 153, p. 329–478, 1974.
- WEITZMAN, S. H. Systematics of deep-sea fishes. In: RANDALL, D. J.; FARRELL, A. P. (Ed.) **Deep-Sea Fishes**. San Diego: Academic Press, 1997. p. 43-77.
- WIDDER, E. A. et al. Far-red bioluminescence from two deep-sea fishes. **Science**, v. 225, p. 512–514, 1984.
- WILLIAMS, A. et al. Feeding ecology of five fishes from the mid-slope micronekton community off southern Tasmania, Australia. **Marine Biology**, v. 139, p. 1177-1192, 2001.
- WILLIS, S. C.; WINEMILLER, K. O.; LOPEZ-FERNANDEZ, H. Habitat structural complexity and morphological diversity of fish assemblages in a Neotropical floodplain river. **Oecologia**, v. 142, n. 2, p. 284-295, 2005.

YOUNG, J. W. et al. Feeding ecology and interannual variations in diet of southern bluefin tuna, *Thunnus maccoyii*, in relation to coastal and oceanic waters off eastern Tasmania, Australia. **Environmental Biology of Fishes**, v. 50, p. 275–291, 1997. <https://doi.org/10.1023/A:1007326120380>.

ZELDITCH, M. L.; SWIDERSKI, D. L.; SHEETS, H. D.; FINK, W. L. **Geometric morphometrics for Biologists: a primer**. San Diego, Elsevier Academic Press, 2004. 443p.

(...) and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been and are being evolved.

(DARWIN, Charles)

2. CHAPTER TWO: BODY-SHAPE EVOLUTION IN STOMIIFORMES

Abstract. —The diverse deep-sea order Stomiiformes includes 457 species of mesopelagic and bathypelagic fishes with remarkable diversity of structures associated with feeding, locomotion, and bioluminescence. This study investigates their patterns of body-shape evolution using geometric morphometry in an ecological and phylogenetic context. A total of 473 specimens from 55 different species in 48 genera representing all five families were photographed and 14 homologous landmarks and 50 semilandmarks were marked on the digital images. The principal component analysis (PCA) and morphological disparity analysis were used to evaluate differences in variance among species in relation to their dietary classes, habitat partitioned by depth, the presence or absence of diel vertical migration, and tested for phylogenetic and/or ecological signals. The PCA revealed that fins size and position, particularly the dorsal and anal fins, accounted for the major variations within the group. Relative body size and body depth also contribute to shape the morphological diversity, leading to a transition from species with a midbody dorsal fin, long anal fin, and body fusiform or deep to species with the dorsal fin positioned more posterior on the body and parallel to a short anal fin, and an elongated body. There is a trend towards body elongation in relation to depth distribution, with the meso-bathypelagic species having more elongated bodies than those restricted to the mesopelagic zone. The morphological disparity varies significantly considering the body shape among the clades of Stomiiformes, with the family Sternoptychidae standing out as the most morphologically diverse. Piscivorous and generalist species exhibit higher morphological disparity when compared to zooplanktivorous, and meso- to bathypelagic species exhibit higher morphological disparity when compared to strictly mesopelagic species. A high phylogenetic signal indicates that the morphological diversity among the Stomiiformes is explained mostly by their evolutionary history as a result of shared ancestry, while ecological traits, such as diet, depth, and diel vertical migration are unlikely to play a major role in shaping morphological evolution. Another hypothesis is that the current dataset lacks the statistical power to uncover any relationship that may exist between shifts in depth, diet or migration and the shape diversification of stomiiforms.

Keywords: Deep-sea fish, Ecomorphology, Morphological Disparity, Phylogenetic Signal, Phylomorphospace

2.1 Introduction

The order Stomiiformes comprises marine, bioluminescent fishes that inhabit the mesopelagic and bathypelagic regions of the world's oceans, including the bristlemouths, lightfishes, marine hatchetfishes, dragonfishes, viperfishes, loosejaws, and snaggletooths (HAROLD, 2002; NELSON *et al.*, 2016). There are currently 457 valid species allocated to the families Diplophidae (10 species), Gonostomatidae (24 species), Phosichthyidae (24 species), Sternoptychidae (78 species), and Stomiidae (321 species) (NELSON *et al.*, 2016; FRICKE *et al.*, 2023). Stomiiforms represents one of the most diversified radiations of deep-sea fishes, having a remarkable array of body shapes, sizes and structures associated with feeding, locomotion, and bioluminescence, including head length, size and position of the mouth, dentition, eye size, position of fins, presence or absence of a barbel associated to the hyoid arch, and presence or absence of an adipose fin (HAROLD; WEITZMAN, 1996; HAROLD, 2002; KENALEY, 2009) (Fig. 2.1). The luminescent organs include body photophores arranged in rows or distributed irregularly, and sometimes large orbital photophores and/or a bioluminescent lure at the tip of the barbel or at the distal tip of a modified dorsal-fin ray (HERRING, 2007, KENALEY *et al.*, 2014; MARRANZINO; WEBB, 2018). Stomiiforms also represent key pieces of the oceanic food web as they are abundant, and most species perform diel vertical migration to feed on shallow waters during the night, transporting energy and carbon to the deeper layers during daytime (YOUNG *et al.*, 1997; GANNON *et al.*, 1998; SUTTON, 2013; CARMO *et al.*, 2015; EDUARDO *et al.*, 2020).

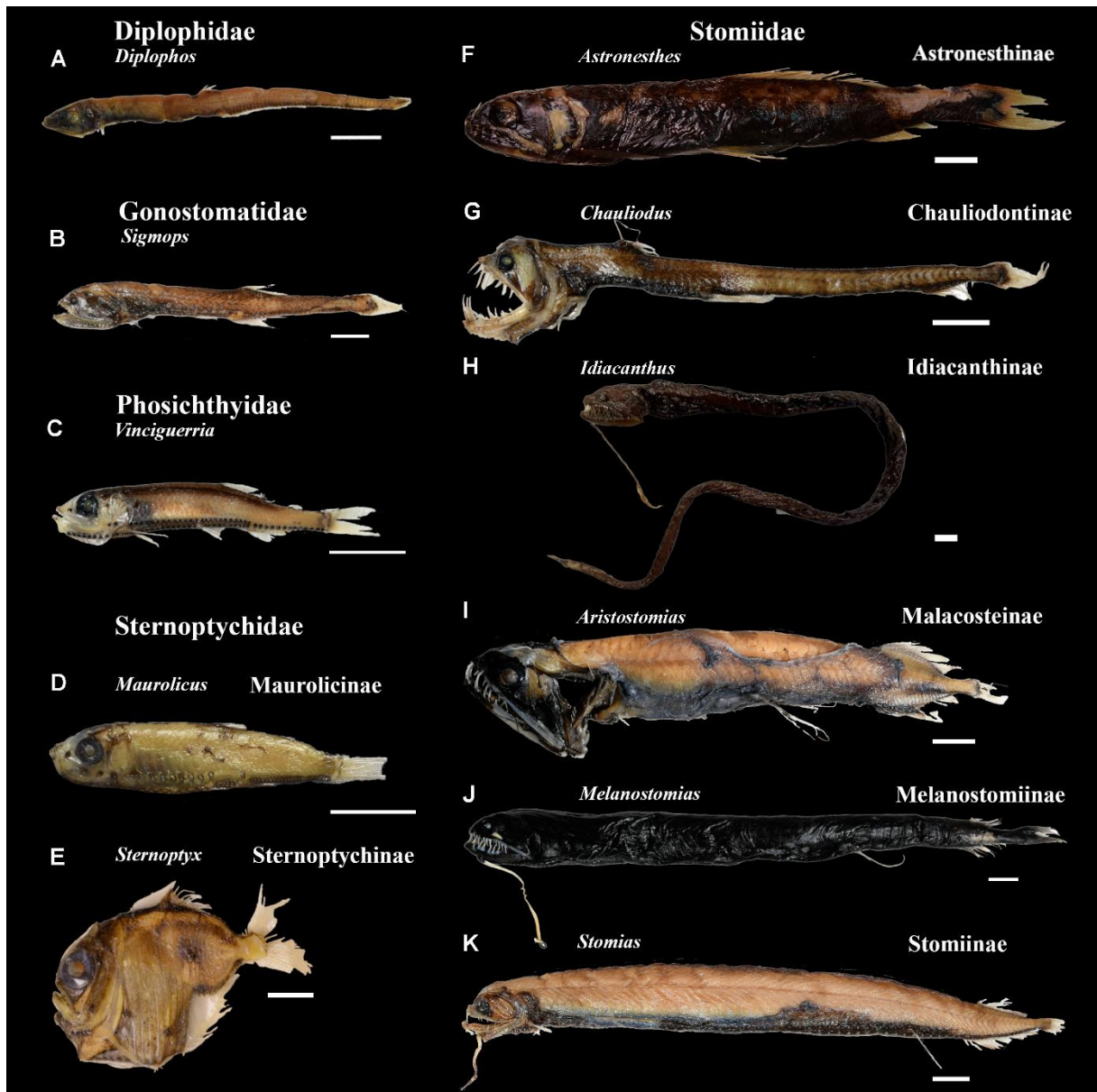


Figure 2.1. Body-shape diversity among Stomiiformes, with representatives of all families. A. *Diplophos taenia*, MZUSP80530, 74.4 mm SL; B. *Sigmops elongatus*, MZUSP086606, 80.1 mm SL; C. *Vinciguerria nimbaria*, MZUSP uncat., 38.6 mm SL; D. *Maurolicus stehmani*, MZUSP080238, 34.8 mm SL; E. *Sternoptyx pseudobscura* MN30167, 49.8 mm SL; F. *Astronesthes macropogon*, MZUSP80272, 115.6 mm SL; G. *Chauliodus sloani*, MZUSP uncat., 95.6 mm SL; H. *Idiacanthus atlanticus*, MZUSP078406, 310.1 mm SL; I. *Aristostomias* sp., MZUSP uncat., 125.1 mm SL; J. *Melanostomias* sp., MZUSP uncat, 193.6 mm SL; K. *Stomias affinis*, MZUSP086572, 162.2 mm SL. Scale bars = 10 mm.

Although the remarkable adaptations of deep-sea fishes to the environment have captivated the attention of scientists and the public for centuries, only a few studies on their ecomorphology and body-shape evolution have adopted a quantitative approach (e.g., ORLOV; BINOHLAN, 2009; NEAT; CAMPBELL, 2013; DENTON; ADAMS,

2015; FARRÉ *et al.*, 2016; MINDEL *et al.*, 2016; TUSET *et al.*, 2018, MAILE *et al.*, 2020; MARTINEZ *et al.*, 2021). Recent studies suggested that variation in depth preference strongly influences the diversification of lizardfishes (MAILE *et al.*, 2020) and deep-sea fishes generally (MARTINEZ *et al.*, 2021), increasing the morphological disparity with the depth. Variation in factors such as turbulence, habitat complexity, and sunlight levels in the deeper layers of the ocean create opportunities for the evolution of unique morphological adaptations in the deep ocean (MARTINEZ *et al.* 2021).

Indeed, because of their morphological adaptations, species richness, depth distribution, feeding habits, and migratory behavior, the stomiiforms represent an interesting case study to further understand the evolution of deep-sea organisms in relation to environmental changes (ANGEL, 1997; THISTLE, 2003, MINDEL *et al.*, 2016). In a comprehensive study about ecomorphology of deep-sea fishes, Martinez *et al.* (2021) included many stomiiform species (~70 species) but did not focus on that order, examined only eight linear traits, and a single environmental variable (depth). No modern ecomorphological study has applied geometric morphometrics to comprehensively capture and statistically quantify the intricate forms within the entire order, which would aid the examination of evolutionary hypotheses within a phylogenetic and ecological context (GOSWAMI *et al.*, 2019).

In this study, we apply geometric morphometric analyses to reconstruct patterns of body shape evolution among the stomiiforms. Specifically, the study aims to (1) elucidate the main correlates of stomiiform morphological disparity; (2) determine whether major axes of stomiiform body-shape diversity are associated with dietary preferences, bathymetric distribution, and diel vertical migration patterns; and (3) quantify the phylogenetic signal in stomiiform body shape.

2.2 Materials and methods

2.2.1 Taxonomic sampling

Specimens were obtained from the scientific collections of Museu de Zoologia, Universidade de São Paulo (MZUSP), Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Oregon State University Ichthyology Collection (OS), Museum of Comparative Zoology, Harvard University (MCZ), Scripps Institution of Oceanography,

University of California in San Diego (SIO), and National Museum of Natural History, Smithsonian Institution (USNM). Some additional specimens were collected during oceanographic cruises in the Southwest Atlantic conducted onboard the RV Alucia in 2017, and by the RV Alpha Crucis in 2019 and 2022, the latter as part of the DEEP-OCEAN Project. A total of 473 specimens from 55 species was used, including 48 out of 53 stomiiform genera representing all five families (Table 1.1).

Only adult specimens in good condition were chosen, and at least five specimens per species were selected, except in 18 (33%) cases where less than five specimens in good condition were available (Table 2.1). For each specimen, the left side was photographed using a digital SLR camera (Nikon D500 with a 60 mm lens or Nikon D3200 with an 18–55 mm lens). For each specimen, the standard length (SL) was measured using a digital caliper to the nearest 0.1 mm.

Table 2.1 List of species of Stomiiformes examined, including the number of specimens (N), minimum and maximum standard length, and depth of capture.

| Species | N | Size (mm SL) | Depth (m) |
|---|----------|---------------------|------------------|
| Diplophidae (2) | | | |
| <i>Diplophos taenia</i> Günther, 1873 | 8 | 69.8–145.7 | 132–1,300 |
| <i>Manducus manderensis</i> (Johnson, 1890) | 21 | 77.4–191.9 | 55–927 |
| Gonostomatidae (6) | | | |
| <i>Cyclothone microdon</i> (Günther, 1878) | 9 | 32.9–50.8 | 830–2,850 |
| <i>Cyclothone pseudopallida</i> Mukhacheva, 1964 | 14 | 31.0–55.7 | 1,000 |
| <i>Gonostoma atlanticum</i> Norman, 1930 | 2 | 58.6–59.0 | 3,050 |
| <i>Margrethia obtusirostra</i> Jespersen and Tåning, 1919 | 5 | 41.3–80.0 | 425–575 |
| <i>Sigmops elongatus</i> (Günther, 1878) | 24 | 51.2–159.9 | 517–1,648 |
| <i>Zaphotias pedaliotus</i> (Goode and Bean, 1896) | 8 | 38.4–69.1 | 500–2,300 |
| Phosichthyidae (7) | | | |
| <i>Ichthyococcus elongatus</i> Imai, 1941 | 4 | 27.5–93.1 | 200–2,450 |
| <i>Phosichthys argenteus</i> Hutton, 1872 | 15 | 64.5–237.2 | 517–1,141 |
| <i>Pollichthys maui</i> (Poll, 1953) | 15 | 40.6–49.7 | 445–917 |
| <i>Polymetme thaeocoryla</i> Parin and Borodulina, 1990 | 25 | 33.8–162.6 | 438–605 |
| <i>Vinciguerria nimbaria</i> (Jordan and Williams, 1895) | 23 | 31.6–44.5 | 200–1,270 |

| | | | |
|---|----|-------------|-------------|
| <i>Vinciguerria poweriae</i> (Cocco, 1838) | 8 | 30.4–36.2 | 1,480 |
| <i>Yarrella blackfordi</i> Goode and Bean, 1896 | 3 | 150.7–188.5 | 388–880 |
| Sternoptychidae (12) | | | |
| <i>Araiophos eastropas</i> Ahlstrom and Moser, 1969 | 4 | 275.0–297.0 | No info. |
| <i>Argyripnus atlanticus</i> Maul, 1952 | 10 | 49.8–71.6 | 415 |
| <i>Argyropelecus aculeatus</i> Valenciennes, 1850 | 14 | 32.6–70.3 | 445–1,000 |
| <i>Argyropelecus affinis</i> Garman, 1899 | 8 | 41.3–72.7 | 406–2,400 |
| <i>Danaphos oculatus</i> (Garman, 1899) | 3 | 33.5–42.1 | 310–2,400 |
| <i>Maurolicus stehmanni</i> Parin and Kobylansky, 1993 | 10 | 32.5–41.8 | 610 |
| <i>Maurolicus weitzmani</i> Parin and Kobylansky, 1993 | 2 | 43.5–58.3 | 219–347 |
| <i>Polyipnus laternatus</i> Garman, 1899 | 2 | 40.8–43.1 | 457–611 |
| <i>Polyipnus spinifer</i> Borodulina, 1979 | 3 | 46.4–59.1 | 465 |
| <i>Sternoptyx pseudobscura</i> Baird, 1971 | 22 | 28.4–51.3 | 904–2,450 |
| <i>Thorophos nexilis</i> (Myers, 1932) | 1 | 59.4 | 320 |
| <i>Valenciennellus tripunctulatus</i> (Esmark, 1871) | 2 | 22.4–25.1 | 830–1,000 |
| Stomiidae (28) | | | |
| <i>Aristostomias scintillans</i> (Gilbert, 1915) | 7 | 102.4–122.3 | 50–4,300 |
| <i>Astronesthes gemmifer</i> Goode and Bean, 1896 | 2 | 130.2–151.7 | 1,266 |
| <i>Astronesthes macropogon</i> Goodyear and Gibbs, 1970 | 17 | 70.9–130.0 | 635–1,799 |
| <i>Bathophilus flemingi</i> Aron and McCrery, 1958 | 12 | 56.4–67.1 | 160–2,520 |
| <i>Borostomias antarcticus</i> (Lönnerberg, 1905) | 7 | 77.1–286.5 | 1,230–2,000 |
| <i>Chauliodus macouni</i> Bean, 1890 | 13 | 67.0–190.1 | 200–1,000 |
| <i>Chauliodus sloani</i> Bloch and Schneider, 1801 | 6 | 95.6–166.6 | 599–1,089 |
| <i>Chirostomias pliopterus</i> Regan and Trewavas, 1930 | 2 | 120.3–157.7 | 1,000–1,016 |
| <i>Echiostoma barbatum</i> Lowe, 1843 | 5 | 99.3–257.7 | 760–1,332 |
| <i>Eustomias filifer</i> (Gilchrist, 1906) | 3 | 73.4–107.4 | 90–200 |
| <i>Flagellostomias boureei</i> (Zugmayer, 1913) | 6 | 72.4–184.0 | 50–1,000 |
| <i>Grammatostomias flagellibarba</i> Holt and Byrne, 1910 | 2 | 32.2–202.7 | 800–1,636 |
| <i>Heterophotus ophistoma</i> Regan and Trewavas, 1929 | 8 | 57.7–236.9 | 200–1,850 |
| <i>Idiacanthus atlanticus</i> Brauer, 1906 | 8 | 188.0–353.0 | 517–1,141 |
| <i>Leptostomias gladiator</i> (Zugmayer, 1911) | 6 | 101.0–183.6 | 170–1,354 |

| | | | |
|--|----|-------------|-------------|
| <i>Malacosteus australis</i> Kenaley, 2007 | 10 | 130.8–195.7 | 616–1,637 |
| <i>Melanostomias melanops</i> Brauer, 1902 | 9 | 153.0–206.6 | 666–1,545 |
| <i>Neonesthes capensis</i> (Gilchrist and von Bonde, 1924) | 2 | 153.4–153.6 | 592 |
| <i>Odontostomias micropogon</i> Norman, 1930 | 4 | 195.2–221.3 | 400–740 |
| <i>Opostomias mitsuii</i> Imai, 1941 | 11 | 116.3–128.1 | 50–1,500 |
| <i>Pachystomias microdon</i> (Günther, 1878) | 5 | 123.9–190.2 | 1,900–2,200 |
| <i>Photonectes margarita</i> (Goode and Bean, 1896) | 7 | 73.4–199.6 | 80–2,400 |
| <i>Photostomias guernei</i> Collett, 1889 | 6 | 80.1–107.4 | 125 |
| <i>Rhadinesthes decimus</i> (Zugmayer, 1911) | 3 | 133.9–225.8 | 1,300–2,633 |
| <i>Stomias affinis</i> Günther, 1887 | 17 | 79.9–190.9 | 506–1,200 |
| <i>Tactostoma macropus</i> Bolin, 1939 | 23 | 114.8–132.2 | 200–1,380 |
| <i>Thysanactis dentex</i> Regan and Trewavas, 1930 | 5 | 111.6–162.2 | 1,500–4,000 |
| <i>Trigonolampa miriceps</i> Regan and Trewavas, 1930 | 3 | 172.9–233.2 | 915–1,830 |

2.2.2 Ecological data

For each species, information about diet, bathymetric distribution, and presence or absence of diel vertical migration (DVM) were obtained from the literature or from the precise collection data contained on the label, coded as discrete categories according to the categories given below (Supplemental Table S2.1). Species diet was sorted into three classes: Type 1 includes zooplanktivorous that feed primarily on small crustaceans, such as copepods, euphausiids, ostracods, mysids, cladocerans and amphipods; Type 2 includes generalists that feed on both crustaceans and fishes; and Type 3 includes exclusively piscivorous. The species were divided into two categories according to the bathymetric distribution: the mesopelagic occur between 200- and 1,000-meters depth; and the meso-bathypelagic can be found both in the mesopelagic zone and deeper than 1,000 meters depth. The species were also classified as migratory or non-migratory based on the presence or absence of DVM, respectively. The environmental data were converted into factors and used as classifiers in downstream analyses. Classification to family and subfamily levels follows NELSON (2006).

2.2.3 Geometric morphometrics

A landmark-based geometric morphometric approach was used to quantify the variation in the body shape using the tps series of programs (ROHLF, 2015, 2018). We located fourteen fixed landmarks on homologous structures (Table 2.2), and interpolated 50 semilandmarks in tpsDig2 (ROHLF, 2018), thereby adapting the method of several other studies (NUNES *et al.*, 2008; PULCINI *et al.*, 2008; FARRÉ *et al.*, 2015; MAILE *et al.*, 2020), to stomiiform morphology (Fig. 2.2). We did not include a landmark on the lower jaw because of variation in the position of fixation. We excluded the ventrum because of marked shape changes due to variation in stomach fullness.

Table 2.2. Descriptions of the fourteen homologous landmarks.

| Landmark | Description |
|----------|--|
| 1 | Tip of the snout at anterior tip of premaxilla |
| 2 | Distal tip of maxilla |
| 3 | Upper eye margin |
| 4 | Lower eye margin |
| 5 | Ventralmost point of opercle |
| 6 | Dorsalmost point of preopercle |
| 7 | Dorsal margin of skull vertically aligned to the orbit |
| 8 | Dorsal-fin origin at base of the anteriormost ray |
| 9 | Dorsal-fin insertion at base of posteriormost ray |
| 10 | Pelvic-fin origin at base of the anteriormost ray |
| 11 | Anal-fin origin at base of the anteriormost ray |
| 12 | Anal-fin insertion at base of the anteriormost ray |
| 13 | Caudal peduncle at base of the anteriormost dorsal procurrent ray |
| 14 | Caudal peduncle at base of the anteriormost ventral procurrent ray |

The landmark coordinates were submitted to a Generalized Procrustes Analysis (GPA) using the geomorph package version 4.0.5 (ADAMS; OTÁROLA-CASTILLO, 2013) in R (R CORE TEAM, 2023). This analysis scales the cartesian coordinates to a common centroid size and rotates the configurations to minimize the sum of the square distances between the corresponding landmarks, thereby removing the information on isometric size, position, and spatial orientation (BOOKSTEIN, 1991).

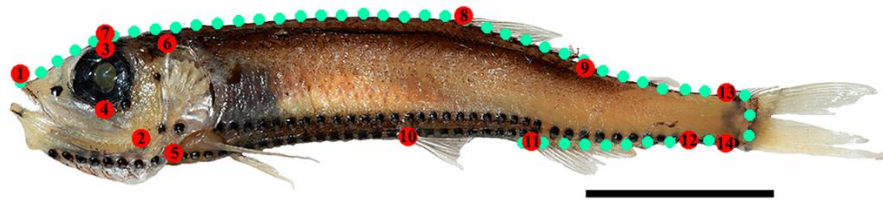


Figure 2.2. The position of the fourteen fixed landmarks (red) and 50 semilandmarks (green) illustrated in *Vinciguerria nimbaria*, MZUSP uncat., 38.6 mm SL. Scale bar = 10 mm.

Principal Component Analysis, Procrustes ANOVA and Morphological Disparity.—A Principal Component Analysis (PCA) was conducted in the geomorph package version 4.0.5 (ADAMS; OTÁROLA-CASTILLO, 2013) and plotted with ggplot2 package version 3.4.3 (WICKHAM *et al.*, 2023) according to each classifier: clades, diet, depth range, and DVM. Extremes of the negative and positive shape variation on each of the first several principal component axes were plotted using the function ‘plotRefToTarget’ from the geomorph package.

Specimen shape and centroid sizes values were averaged by species. A Procrustes ANOVA was performed to assess whether a set of species with different clades, diets, depth ranges, and migratory behaviors or in different clades differed significantly in shape. That analysis used the ‘procD.lm’ function (geomorph), with 500 permutations. This analysis also tested for an allometric effect between body shape and species size. The morphological disparity among stomiiform species in each clade, diet class, migration class and depth class were calculated using the function ‘morphol.disparity’ (geomorph) based on 1,000 randomized residual permutations. We repeated all analyses with outliers excluded (defined as species with high Procrustes distance from the mean shape).

2.2.4 Phylomorphospace, phylogenetical signal and phylogenetic ANOVA

A phylogenetic hypothesis for 99 stomiiform species was extracted from the time-calibrated phylogenetic tree of ray-finned fishes produced by Rabosky *et al.* (2018) and distributed via the Fish Tree of Life website (<https://fishtreeoflife.org/>). The phylogeny was linked to the PCA results in phytools version 1.5-1 (REVELL, 2012) to create a genus-level phylomorphospace (SIDLAUSKAS, 2008). In cases where we lacked

morphological data for the exact species in the Raboksy *et al.*'s (2018) tree but had data for a congener, the morphological data from the congener were matched to its close relative's phylogenetic position. Taxa for which we had no morphological data were trimmed using the 'drop.tip' function from the ape package version 5.7-1 (PARADIS *et al.*, 2004) and we further pruned the tree to include a single species in each genus. Supplemental Table S2.2 details the species selected for the genus-level phylomorphospace.

The phylogenetic signal of body shape and centroid size was assessed using the 'physignal' function from geomorph (ADAMS; OTÁROLA-CASTILLO, 2013). That approach uses a multivariate generalization of Blomberg's *K* which measures phylogenetic signal by calculating a ratio of the observed trait variance relative to the value estimated at the root of the tree and the trait variance expected under Brownian motion (BLOMBERG *et al.*, 2003). The resulting *K*_{mult} statistic was compared to a null distribution generated from 1,000 random permutation tests using the average shapes of species. We used phylogenetic ANOVA on the species-averaged Procrustes coordinates to test for an allometric effect between body shape and species size and for significant differences among the diet, depth, and migration classes. This analysis used the 'procD.pgls' function in geomorph with 500 permutations.

2.3 Results

2.3.1 Principal Component Analysis (PCA)

The principal component analysis (PCA) revealed significant multivariate variation in stomiiform body shape, with the first three PCA axes collectively accounting for 92.42% of the total variance among individuals, mostly on the first two principal components (Fig. 2.3). The observed shape variation includes notable shifts in the position of the dorsal and anal fins, fin sizes, and head and body proportions.

Axis 1, which explains 58.22% of the variance, describes the positions of the dorsal, anal, and pelvic fins, the length of the anal fin and head, the caudal-peduncle depth, and eye diameter. Species exhibiting midline placement of the dorsal fin, a long anal fin, pelvic fins positioned closer to the midline of the body, and a larger eye have negative PC1 values. In contrast, species with a posterior placement of the dorsal and anal fins and

a sagittiform body shape, pelvic fins located closer to the tail than the head and moderate-sized eyes exhibit positive PC1 values. The species with the most extreme negative PC1 values include the sternoptychids *Sternoptyx pseudobscura*, *Valenciennellus tripunctulatus*, *Thorophos nexilis*, *Argyripnus atlanticus*, and *Danaphos oculatus*, while those with extreme positive PC1 values include the melanostomiines *Tactostoma macropus*, *Melanostomias melanops*, *Thysanactis dentex*, and the stomiine *Stomias affinis* (Fig. 2.4).

Axis 2, which explains 25.73% of the variance, showcases variation in body depth, body length, the size and shape of the head, anal-fin size, and pelvic-fin position. Negative PC2 values reflect species with deeper bodies, pelvic fins positioned closer to the tail than the head, shorter anal fins, and larger eyes. In contrast, positive PC2 values reflect species with longer, slender bodies that are flattened dorsoventrally, pelvic fins positioned at the midline of the body, longer anal fins, and smaller eyes (Fig. 2.3). Notably, sternoptychines such as *Sternoptyx pseudobscura* and *Argyropelecus aculeatus* have extreme negative PC2 values, while the diplophid *Diplophos taenia* occupies the positive extreme of PC2 (Fig. 2.4).

Axis 3, which explains 8.47% of the shape variance, is strongly influenced by the position of the dorsal and pelvic fins. Negative PC3 values indicate species with the dorsal fin positioned more posteriorly on the body and the pelvic fin positioned near to the anal fin. In contrast, species with the dorsal fin closer to midbody and the pelvic fin more anterior have positive PC3 values. The melanostomiine *Eustomias filifer*, and the gonostomatids *Cyclothone microdon* and *C. pseudopallida* occupy the negative extreme of PC3 values while the chauliodontines *Chauliodus macouni* and *C. sloani* occupy the positive extreme (Fig. 2.4).

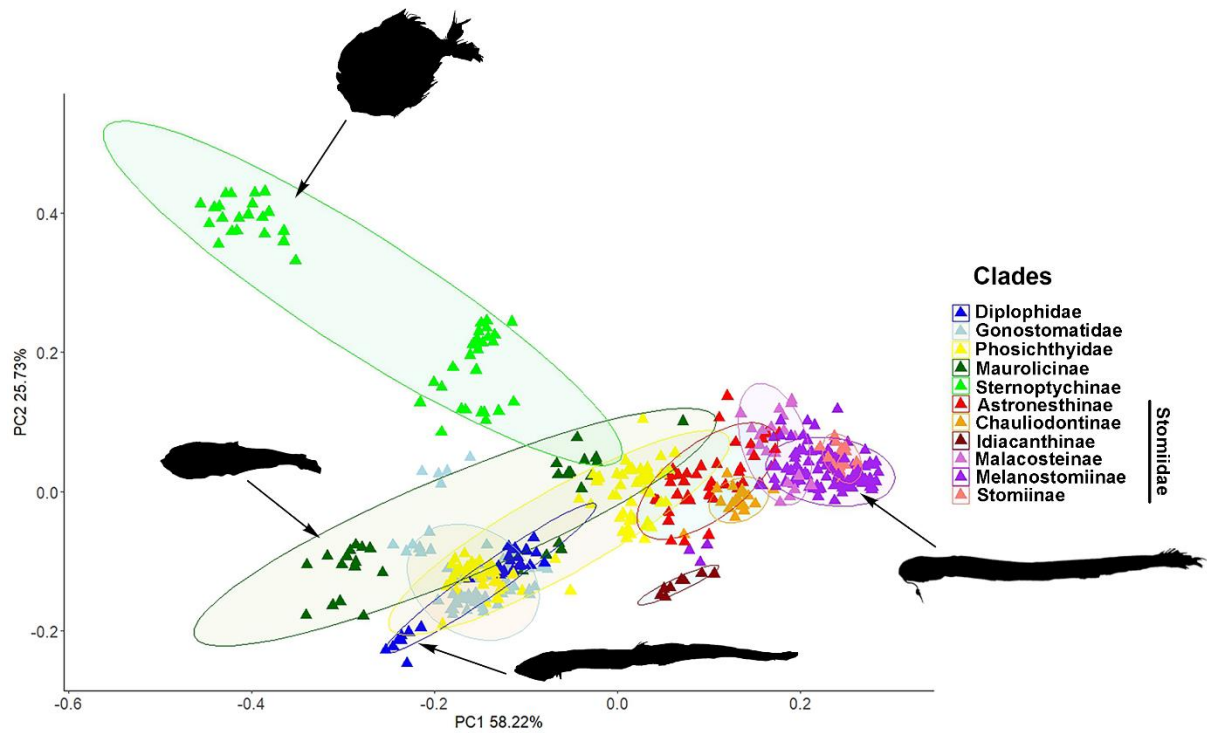


Figure 2.3. The first and second axes of a Principal Component Analysis (PCA) in Stomiiformes, based on 473 specimens. Colored ellipses represent distribution of specimens within stomiiform taxonomic groups.

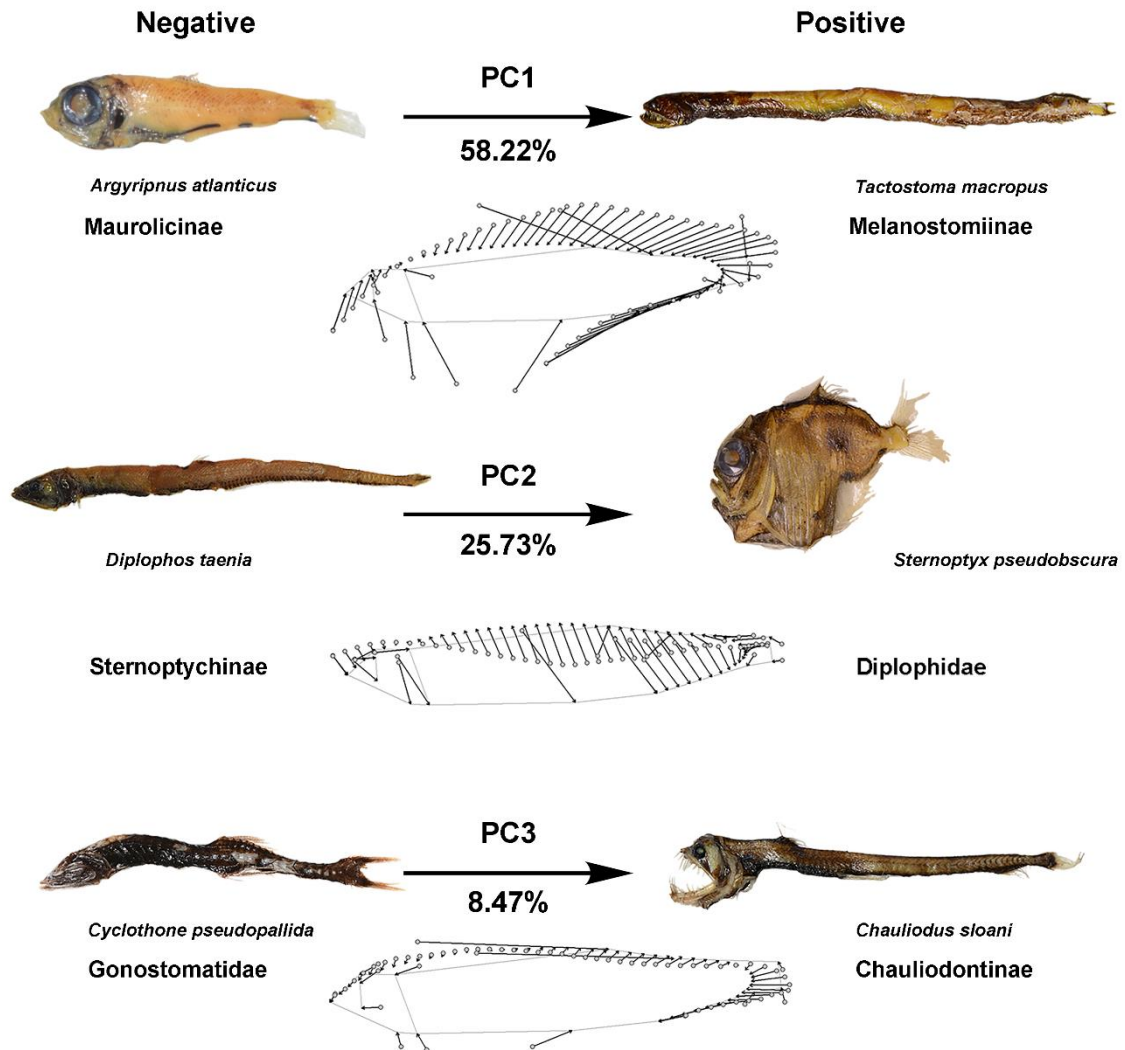


Figure 2.4. Stomiiform species at the negative and positive extremes of body shape variation along the first three principal component axes. Wireframes represent the average body shape, and vectors represent the average configuration warped to an extreme observed value of the corresponding PC axis. The percentage of total variance represented by each axis is indicated between the two extreme shapes.

Stomiiform species with different diets and bathymetric distributions tend to occupy distinct regions of morphospace (Fig. 2.5). The piscivorous stomiids (type 3 diet) occupy the positive extreme of PC1 (Fig. 2.5A) and have sagittiform body shape, large mouth, and premaxilla armed with widely spaced fangs. Zooplanktivorous species (type 1 diet) mostly occupy the lower left corner of morphospace with negative values for PC1 and PC2, including diplophids, gonostomatids, maurolicines and most phosichthyids, which have fusiform or elongated body, a moderate to large mouth armed with numerous minute teeth, and eye size varying from small to large. Generalist species that feed upon

crustaceans and fishes (type 2 diet) occur across the entire range of PC1 and have a variable morphology with a deep or fusiform body, and a moderate to large mouth armed with numerous teeth of variable sizes, including most astronesthines, and the phosichthyids *Phosichthys argenteus* and *Vinciguerria nimbaria*. Sternoptychines are also generalists but occupy the upper left quadrant of morphospace with highlight positive PC2 values due to the deep body shape.

Regarding the bathymetric distribution (Fig. 2.5B), most mesopelagic fishes have fusiform bodies with negative PC1 values, such as the maurolicines. In contrast, meso- to bathypelagic are more diverse and occupy the entire range of observed morphology, including the stomiids, gonostomatids, and most phosichthyids. In regard to DVM, no clear association with body shape emerged as most species perform DVM (Fig. 2.5C).

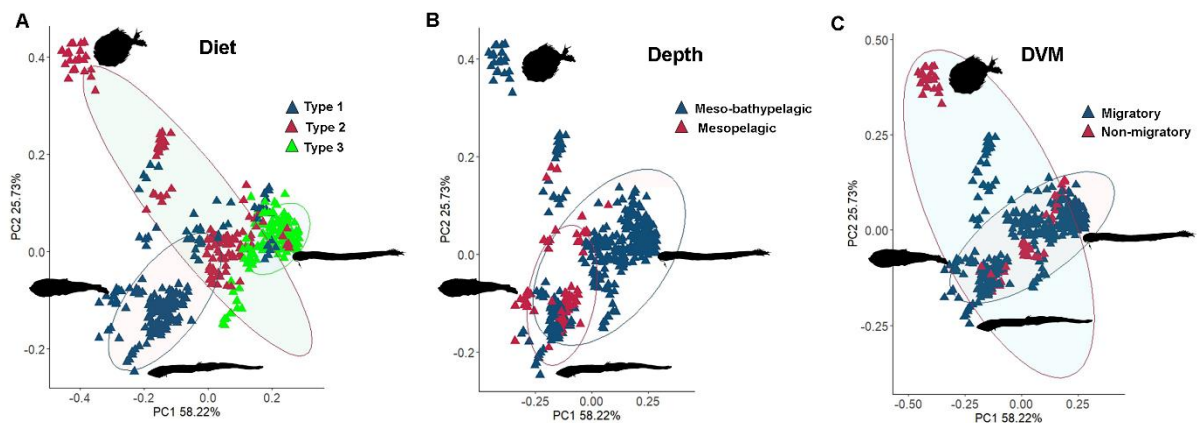


Figure 2.5. The first and second morphospace axes, color coded by (A) diet, (B) bathymetric distribution, and (C) diel vertical migration pattern (DVM).

2.3.2 Morphological disparity and Procrustes ANOVA

The morphological disparity of the astronesthines, chauliodontines, phosichthyids, and sternoptychines differs significantly ($p \leq 0.05$) from all other families and subfamilies, with sternoptychines and chauliodontines being the significantly more diverse (Procrustes variance 0.19347937 and 0.09047028, respectively), and astronesthines and phosichthyids the significantly less diverse (Procrustes variance 0.02074415 and 0.02458017, respectively).

Groups of stomiiform species with different ecologies (diet, depth, and diel vertical migration) differ in morphological disparity. Specifically, the body shape disparity of meso-bathypelagic stomiiforms exceeds that of mesopelagic, non-migratory

species are more disparate than migratory ones, and generalists are more disparate than zooplanktivores or piscivores (Table 2.3). The significance of the difference between depth classes depends on the inclusion of the outlier *Sternoptyx pseudobscura* (see below), though the other results hold whether or not that species is included.

Table 2.3. Procrustes variance and *P*-values of the morphological disparity in Stomiiformes test by diet, depth, and diel vertical migration (DVM), with and without the hatchetfish *Sternoptyx pseudobscura*.

| Ecological traits | With <i>Sternoptyx</i> | | Without <i>Sternoptyx</i> | |
|--------------------------------------|------------------------|--------------------|---------------------------|--------------------|
| | Procrustes variance | <i>P</i> -values | Procrustes variance | <i>P</i> -values |
| Diet: Zooplanktivorous | 0.048 | 0.001* / 0.042* | 0.050 | 0.001* / 0.063 |
| Diet: Generalists | 0.084 | 0.001* / 0.013* | 0.031 | 0.001* / 0.001* |
| Diet: Piscivorous | 0.063 | 0.042* / 0.013* | 0.056 | 0.063 / 0.001* |
| Depth: Mesopelagic | 0.044 | 0.009* | 0.047 | 0.864 |
| Depth: Meso– bathypelagic | 0.066 | 0.009* | 0.048 | 0.864 |
| DVM: Migratory | 0.051 | 0.001* | 0.050 | 0.001* |
| DVM: Non-migratory | 0.114 | 0.001* | 0.032 | 0.001* |

The body-shape of stomiiforms was significantly different among subclades and diet (Procrustes ANOVA, $p \leq 0.05$). In contrast, no significant relationship was found between shape and ocean depth or DVM. No significant relationships between centroid size (CS) and any classifier were uncovered (Table 2.4).

Because *Sternoptyx pseudobscura* was considered an outlier (Supplemental Fig. S2.1), we performed additional analyses excluding this species. The morphospace remains quite similar with and without *S. pseudobscura*, but the first axis explains more variance (67.14% vs 58.22%), and second axis accounts for somewhat less (15.66% vs 25.73%) (Supplemental Fig. S2.2).

Without *S. pseudobscura*, the morphological disparity among the stomiiforms was also similar, with sternoptychines and chauliodontines having the highest disparity. Morphological disparity regarding ecological traits remains significant without *S. pseudobscura*, except for the bathymetric distribution (Table 2.5). The main difference is that Procrustes variance is higher in migratory than in non-migratory species, and in

piscivorous than in generalist species, which is the opposite in comparison with the dataset containing *S. pseudobscura*. Similarly, the Procrustes ANOVA for body shape remains significant for clades and diet and non-significant for depth and DVM (Table 2.4).

Table 2.4. Results from Procrustes ANOVA testing for differences in mean body shape and centroid size (CS) among the Stomiiformes classified by clades, diel vertical migration (DVM), bathymetric distribution (depth) and diet, with and without the hatchetfish *Sternoptyx pseudobscura*. Asterisks represent significant differences ($p \leq 0.05$).

| Ecological traits | With <i>Sternoptyx</i> | | | | Without <i>Sternoptyx</i> | | | |
|-------------------|------------------------|------|----------|-------|---------------------------|------|----------|-------|
| | F-values | | P-values | | F-values | | P-values | |
| | Shape | CS | Shape | CS | Shape | CS | Shape | CS |
| Clades | 7.90 | 4.24 | 0.002* | 0.092 | 7.62 | 4.14 | 0.002* | 0.078 |
| DVM | 0.79 | 0.16 | 0.454 | 0.762 | 0.44 | 0.13 | 0.652 | 0.832 |
| Depth | 1.97 | 0.29 | 0.122 | 0.262 | 2.45 | 0.30 | 0.084 | 0.262 |
| Diet | 14.4 | 1.01 | 0.002* | 0.23 | 16.00 | 0.98 | 0.002* | 0.194 |

2.3.3 Phylogenetic signal, phylomorphospace, and phylogenetic ANOVA

Significant phylogenetic signal exists for stomiiform body shape ($K = 1.9275$, $p = 0.001$) and centroid size ($K = 1.0341$, $p = 0.03$) (Supplemental Figs. S2.3A, B). Those values indicate that body size variation closely approximates the Brownian expectation, while for body shape, closely related lineages tend to resemble each other even more than one would expect from a random walk.

When visualized in a phylomorphospace, closely related taxa clearly tend to cluster closely and distant related taxa diverge, which corroborates the numerical estimate of strong phylogenetic signal in body shape. Most stomiids occupy the same region of phylomorphospace as the other members of their families, except for the chauliodontine *Chauliodus*, the idiacanthine *Idiacanthus* and the astronesthines, especially *Astronesthes*, *Neonesthes*, and *Rhadinesthes*. Those five taxa diverge from the other members of their families in body shapes and dorsal-fin position. Chauliodontines have a dorsal fin anteriorly placed vs dorsal fin posteriorly placed in most stomiids or at midbody in astronesthines; idiacanthines have anguilliform body with long dorsal and anal fins vs elongated or fusiform bodies with short dorsal and anal fins in most of the other stomiids.

Astronesthines have a fusiform body with the dorsal fin at midbody vs elongated body in most stomiids and dorsal fin posteriorly placed, except in idiacanthines, mentioned above.

The main overlap in phylomorphospace was found between astronesthines and phosichthyids *Vinciguerria*, *Ichthyococcus* and *Phosichthys*. The remaining phosichthyids *Pollichthys*, *Polymetme* and *Yarrella* share a region of phylomorphospace with the gonostomatids. Sternoptychids have a broad distribution in the phylomorphospace, with sternoptychines occupying a distinct region, especially *Sternoptyx* (Fig. 2. 6).

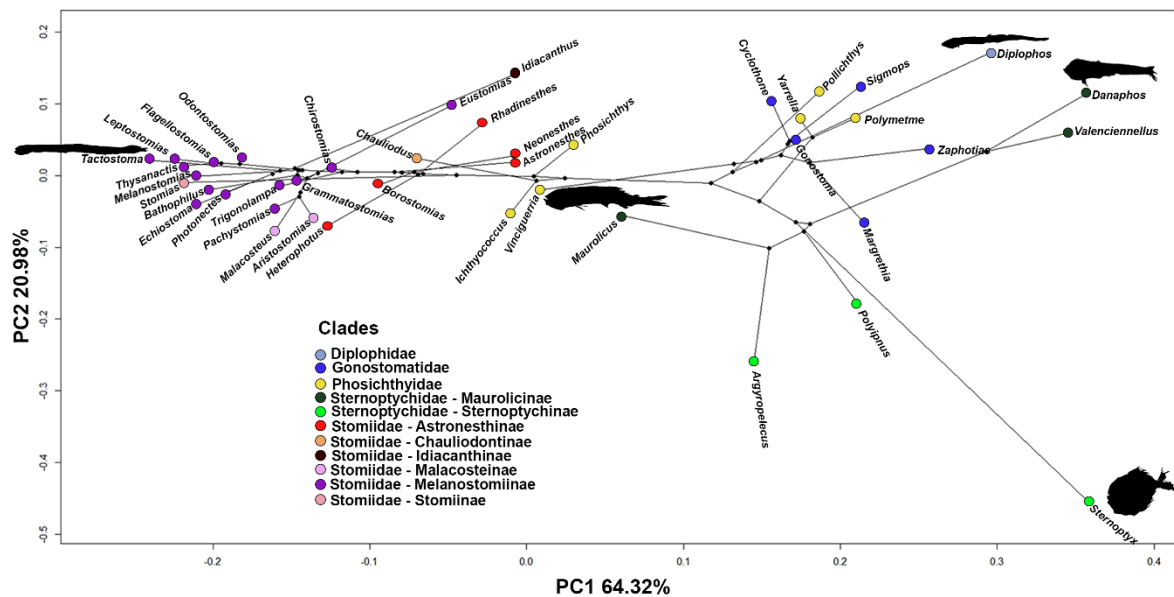


Figure 2.6. Genus-level phylomorphospace based on the time-calibrated phylogeny of Rabosky *et al.* (2018), color coded by stomiiform taxonomic groups.

Phylogenetically corrected tests for differences in morphological disparity among ecological classes and clades returned universally insignificant results ($p > 0.05$) (Table 2.5). Likewise, the phylogenetic Procrustes ANOVA uncovered no significant differences in size or shape between stomiiforms grouped by ecological traits or by subclade ($p > 0.05$) (Table 2.6). However, these tests used substantially smaller sample sizes than did the non-phylogenetic tests reported above, and the lack of significance must not be taken as proof of a lack of relationship.

Table 2.5. Procrustes variance and P -values of the morphological disparity in Stomiiformes test by diet, depth, and diel vertical migration (DVM), without and with accounting for phylogeny. Asterisks represent significant morphological disparity ($p \leq 0.05$).

| Ecological traits | Without phylogeny | | With phylogeny | |
|---------------------------------|---------------------|-----------------|---------------------|---------------|
| | Procrustes variance | P -values | Procrustes variance | P -values |
| Diet: Zooplanktivorous | 0.048 | 0.001* / 0.042* | 0.056 | 0.389 / 0.597 |
| Diet: Generalists | 0.084 | 0.001* / 0.013* | 0.039 | 0.389 / 0.677 |
| Diet: Piscivorous | 0.063 | 0.042* / 0.013* | 0.047 | 0.597 / 0.677 |
| Depth: Mesopelagic | 0.044 | 0.009* | 0.054 | 0.756 |
| Depth: Meso–bathypelagic | 0.066 | 0.009* | 0.048 | 0.756 |
| DVM: Migratory | 0.051 | 0.001* | 0.050 | 0.667 |
| DVM: Non-migratory | 0.114 | 0.001* | 0.041 | 0.667 |

Table 2.6. Results from Procrustes ANOVA testing for differences in mean body shape and centroid size (CS) among the Stomiiformes classified by clades, diel vertical migration (DVM), bathymetric distribution (depth) and diet, without and with accounting for phylogeny. Asterisks represent significant differences ($p \leq 0.05$).

| Ecological traits | Without phylogeny | | | | With phylogeny | | | |
|-------------------|-------------------|------|-------------|-------|----------------|-------|-------------|------|
| | F-values | | P -values | | F-values | | P -values | |
| | Shape | CS | Shape | CS | Shape | CS | Shape | CS |
| Clades | 7.90 | 4.24 | 0.002* | 0.092 | 0.99 | 0.60 | 0.46 | 0.58 |
| DVM | 0.79 | 0.16 | 0.454 | 0.762 | 0.51 | 0.15 | 0.73 | 0.65 |
| Depth | 1.97 | 0.29 | 0.122 | 0.262 | 0.27 | 0.00 | 0.9 | 0.97 |
| Diet | 14.4 | 1.01 | 0.002* | 0.23 | 0.74 | 0.046 | 0.65 | 0.38 |

2.4 Discussion

2.4.1 Body-shape patterns in Stomiiformes

Stomiiforms have a significant variation in body shape, with the three main PCA axes describing a significant variation in body shape associated with feeding habits and depth range. The findings indicate that the dimensions and positions of fins, particularly the dorsal and anal fins, hold sway over the primary variances in the body shapes among stomiiforms, followed by the relative body length and height. The two main PCA axes showed a transition from species with dorsal fin positioned at midbody, anterior to the level of anal-fin origin, anal fin long, and deep-bodied or fusiform body shapes present in maurolicines and sternoptychines, to species with dorsal fin posterior on body, parallel to a shorter anal fin, with elongated body present in melanostomiines and stomiines (Fig. 3). Stomiiforms were grouped in six main groups regarding body shape as described next. (1) A fusiform body shape with a long anal fin is present in the maurolicines *Argyripnus*, *Danaphos* and *Thorophos*, and in the gonostomatids *Margrethia* and *Zaphotias* at negative PC1 and PC2. (2) A fusiform body shape with a short anal fin is present in the astronesthines, in the phosichthyids *Ichthyococcus*, *Phosichthys* and *Vinciguerrria*, and in the maurolicine *Maurolicus* at consensus configuration. (3) A deep, laterally compressed body shape is present in the sternoptychines at negative PC1 and positive PC2. Additionally, there is a trend in body elongation across all stomiiforms clades, specially within the stomiids, the next three groups describe such elongation. (4) An elongated body and a long anal fin are present in the diplophids, the gonostomatids *Gonostoma* and *Sigmops*, the phosichthyids *Pollichthys*, *Polymetme* and *Yarella*, the maurolicine *Araiophos* at negative PC1 and PC2. (5) An elongated, sagittiform body shape, with the anal fin short, posterior on the body and parallel to the dorsal fin is present in stomiines, malacosteines and most melanostomiines at positive PC1. (6) An anguilliform body with an extremely elongated dorsal and long anal fin, present only in *Idiacanthus atlanticus*. at positive PC1 and negative PC2. *Idiacanthus atlanticus* clustered next to the melanostomiine *Eustomias filifer* in the morphospace, although the latter do not possess an anguilliform body shape but has a long anal fin in contrast with the short anal fin present on the other melanostomiines (Fig. 3).

The Chauliodontinae *Chauliodus* and the Astronesthinae clustered in the same region of morphospace, at positive PC1 (Fig. 3). Nevertheless, *Chauliodus* is unique in many aspects: its body shape is elongated with a long anal fin, similar to that in stomiines, malacosteines and melanostomiines, however, the dorsal fin is anteriorly positioned, with an elongated dorsal-fin ray and a bioluminescent lure at its tip – similar to the modified esca and lure present on the dorsal fin of anglerfishes (Lophiiformes) (TCHERNAVIN,

1953; CLARKE, 1982; GARTNER *et al.*, 1997; PIESTCH, 2009) –, and the adipose fin is large and posteriorly placed, parallel to the anal fin resulting on a sagittiform body shape since, presumably, the combined fin area well posterior on the body generates a large amount of burst-speed thrust for prey capture (WEBB, 1975; FINK, 1985). In contrast, most astronesthines have the dorsal fin positioned near the midbody (WEITZMAN, 1967, PARIN; BORODULINA, 2003). However, in *Heterophotus*, the dorsal fin origin is somewhat posteriorly placed, but not parallel to the anal fin (WEITZMAN, 1967, FINK, 1985), while in *Astronesthes* the dorsal-fin position varies from a midbody to posteriorly placed among the species (PARIN; BORODULINA, 2003).

Our results provide evidence that elongation is the second major axis of stomiiforms body-shape diversification, and these results are consistent with previous investigations conducted on ecologically diverse clades of teleosts, such as marine benthic / demersal teleosts (FARRÉ *et al.*, 2016; FRIEDMAN *et al.*, 2020), reef fishes (CLAVERIE; WAINWRIGHT, 2014), Carangiaria (RIBEIRO *et al.*, 2018), and characiforms (BURNS; SIDLAUSKAS, 2019). Elongation was also identified as a second major axis of morphological variation across 394 morphologically diverse teleost families (PRICE *et al.*, 2019), and the main axis of body-shape diversification across over 3,000 species of marine teleosts (MARTINEZ *et al.*, 2021).

In some of these studies investigating body-shape diversification in teleosts, body elongation was associated with habitat transition (*e.g.*, FARRÉ *et al.*, 2016; MARTINEZ *et al.*, 2021), and diet (*e.g.*, BURNS; SIDLAUSKAS, 2019). In this study, body elongation is often associated with both diet and habitat (partitioned by depth), with piscivorous stomiiforms (*e.g.*, melanostomiines, chauliodontines, and stomiines) having an elongated body-shape, with sagittiform morphology and showing the highest morphological disparity in comparison to generalists and zooplanktivorous stomiiforms when removing the morphologically distinct *Sternoptyx*, in a pattern similar to that found in characiforms (BURNS; SIDLAUSKAS, 2019). On the other hand, the generalist and zooplanktivorous stomiiforms greatly vary in body-shape, having fusiform body-shape (*e.g.*, most maurolicines, the gonostomatids *Cyclothone*, *Margrethia* and *Zaphotias*, astronesthines and the phosichthyids *Vinciguerria* and *Ichthyococcus*), elongated body-shape (*e.g.*, diplophids, the gonostomatids *Sigmops* and *Gonostoma*, and the phosichthyids *Pollichthys*, *Polymetme*, and *Yarrella*), or deep compressed body shape (*e.g.*, sternoptychines), with the dorsal fin positioned near to midbody (*e.g.*, diplophids, gonostomatids, phosichthyids, and most sternoptychids and astronesthines), long anal fin

(e.g., gonostomatids, diplophids, most sternoptychids, the phosichthyids genera *Pollichthys*, *Polymetme*, and *Yarrella*, and in the astronesthine *Neonesthes capensis*), and large eye (e.g., sternoptychids, the gonostomatids *Margrethia* and *Zaphotias*, the phosichthyids *Ichthyococcus*, *Vinciguerria*, the astronesthine *Astronesthes* and the malacosteine *Malacosteus*).

The multivariate morphological disparity is greater in meso-bathypelagic than in strictly mesopelagic stomiiforms (Table 3). Meso-bathypelagic stomiiforms include taxa of all clades. In contrast, strictly mesopelagic stomiiforms embrace the maurolicines, with a fusiform body-shape, and the sternoptychines, with deep body-shape, both zooplanktivorous or generalists. We hypothesize that the higher morphological disparity in the meso-bathypelagic species might be related to body elongation. A trend in the elongation of body shape as depth increases was also reported in recent studies, such as Neat and Campbell (2013) with deep-sea fishes from Atlantic Ocean, Farré *et al.* (2016) with demersal fish assemblages in Mediterranean Sea, Maile *et al.* (2020), with lizardfishes, and Martinez *et al.* (2021), with marine fishes inhabiting several marine environments. Our results enhance the idea that the deep sea is a hot spot of fish body shape evolution, with deep-sea fishes displaying forms mostly suited to slow swimming, whereas shallow species have shapes conferring strong, sustained swimming capacity and maneuverability (MARTINEZ *et al.*, 2021). Body elongation is usually considered an efficient strategy related to feeding and swimming performance in deep-sea ecosystems (SFAKIOTAKIS *et al.*, 1999, NEAT; CAMPBELL, 2013), with morphologies suited to low activity lifestyles. This condition is often attributed to the greater limitation of food resources as depth increases (PÈRES, 1985). Fishes having a sagittiform body shape, using the tail in a carangiform swimming pattern (SFAKIOTAKIS *et al.*, 1999), and the combined fin area well posterior on the body generates a large amount of burst-speed thrust for prey capture (WEBB, 1975), which is an advantage, since food resources are scarce.

Morphological disparity is greater in stomiiforms that do not perform diel vertical migration than those that do diel vertical migration with *Sternoptyx pseudobscura* in the analyses and is the opposite when removing this species (Table 3). However, among the specimens analyzed, only seven species (ca. 12% of the total) do not perform DVM, including the gonostomatids *Cyclothone microdon* and *C. pseudopallida*, the phosichthyids *Phosichthys argenteus* and *Yarrella blackfordi*, the sternoptychine *Sternoptyx pseudobscura*, the astronesthines *Borostomias antarcticus* and *Rhadinesthes*

decimus, and the malacosteine *Malacosteus australis*. All the non-migrant stomiiforms are generalist or zooplanktivorous feeders, but there is no correlation of position of dorsal and anal fins and body shape (PC1), caudal peduncle and eye size (PC2), or the position of the pelvic fins (PC3) in the morphospace. Even though we could not find any common trait among the non-migratory species, Schnell *et al.* (2021) revealed differences in the epaxial and hypaxial musculotendinous system between the migratory and non-migratory species. It is also important to note that the diel vertical migration among the stomiiforms is more complex than just migrate or not migrate. For example, some taxa (*e.g.*, *Photostomias* and *Aristostomias*) have asynchronous diel vertical migration, where only a portion of a species' population migrates upwards at night while other individuals remain at greater depths; there are also ontogenetic variations, among other factors (SUTTON; HOPKINS, 1996; KENALEY, 2008). Besides, several stomiiforms are rarely collected (*e.g.*, *Rhadinesthes* and *Opostomias*), which makes it difficult to assume a DVM pattern (KENALEY *et al.*, 2014).

The eye size is also related to the body shape, with fusiform and deep bodied stomiiforms having large eyes (*e.g.*, the gonostomatid *Margrethia*, the phosichthyids *Vinciguerria* and *Ichthyococcus*, the astronesthine *Astronesthes*, and all sternoptychids), and elongated stomiiforms having eye size varying from small to moderate (*e.g.*, diplophids, most gonostomatids and phosichthyids, chauliodontines, melanostomiines, and stomiines). Most stomiiforms with a large eye are strictly mesopelagic, while stomiiforms the meso-bathypelagic species have the eye size varying from small to moderate, with malacosteines being the exception. In comparison to other mesopelagic fishes, a similar pattern was found in myctophids, with species inhabiting shallower depths having the eye larger than those from greater depths (DE BUSSEROLLES *et al.*, 2013). Although this pattern has been observed in these two distinct mesopelagic fish orders, more studies are necessary to understand whether eye size is related to diet, depth, vertical migration, or even with predation avoidance.

Although Stomiidae is the most species-rich family in Stomiiformes, Sternoptychidae is the most morphologically diverse, including forms varying from fusiform body shape as *Maurolicus*, *Argyripnus*, *Danaphos* and *Valenciennellus*, to elongated species as *Thorophos* and *Araiophos* – the later includes the slenderest species within the family. However, the most remarkable forms are the hatchetfishes *Argyropelecus*, *Polyipnus* and *Sternoptyx*, which have a deep, laterally compressed body shape, something unusual for pelagic fishes and more frequent in demersal fishes (*e.g.*,

FRIEDMAN *et al.*, 2020; LAROUCHE *et al.*, 2020), and is thought to enhance maneuverability (WEBB, 1984). In terms of ecological aspects, sternoptychids include only zooplanktivorous and generalist species (HOPKINS *et al.*, 1996; CARMO *et al.*, 2015; EDUARDO *et al.*, 2020), but not strictly piscivorous species, and are either mesopelagic or meso-bathypelagic, with most species performing diel vertical migration, except *Sternoptyx* (BADCOCK, 1970; GON, 1990; MUNDY, 2005; FAHAY, 2007; EDUARDO *et al.*, 2020). Since the sternoptychine *Sternoptyx pseudobscura* has an extreme morphology, morphological disparity of this genus flattened out the differences. When this species is not included in the analyses, the relationships between the stomiiforms became more obvious. Removing *Sternoptyx* from the analysis only slightly affects disparity, and all comparisons except depth (0.864) and zooplanktivorous–piscivorous diet ($p = 0.063$) remained significantly different ($p < 0.05$) (Table 3).

2.4.2 Phylogenetic patterns

Analyzing a time-calibrated phylogeny of stomiiforms produced by Rabosky *et al.*, 2018 (Fig. 2.7), it is possible to observe a transition from a fusiform body shape with dorsal fin at midbody in phosichthyids, gonostomatids and sternoptychids to a sagittiform body shape with a posteriorly dorsal fin in most stomiids. According to relaxed-clock analysis produced by Kenaley *et al.*, (2014), Stomiiformes first appeared at the end of the Early Cretaceous at 117.3 Ma, and the diversification of crown Stomiiformes began during the Late Cretaceous (~91 millions of years ago), with the oldest known fossil taxon, *Paravinciguerria praecursor*, assumed to be a stem stomiiform (CARNEVALE; RINDONE, 2011). The family Stomiidae includes the predominantly elongated dragonfishes that appeared in the late Eocene, at ~35 Ma (KENALEY *et al.*, 2014).

Even though Phosichthyidae was not recovered as monophyletic in recent molecular phylogenies (KENALEY *et al.*, 2014; MIRANDE, 2017; Betancur-R *et al.*, 2017; RABOSKY *et al.*, 2018), this name is still in use (*e.g.*, Nelson, 2006; 2016; SUTTON *et al.*, 2020; VILLARINS *et al.*, 2023). According to the hypothesis produced by Rabosky *et al.* (2018), *Vinciguerria* is sister to all stomiiforms, *Pollichthys* is sister to a clade including Sternoptychidae, *Yarrella* and *Polymetme*, and to a clade including Stomiidae, *Ichthyococcus* and *Phosichthys*. In our results, phosichthyids occupy two distinct regions in the morphospace: one group including species with slender body, eye

small to moderate, head more slender than deep, and longer anal fin, which feed mainly on small crustaceans, especially the euphausiids, and includes the genera *Pollichthys*, *Polymetme*, and *Yarrella*; and the second group including species with a deeper body and head, larger eyes, and shorter anal fin, and that are generalist feeders, including *Ichthyococcus*, *Phosichthys* and *Vinciguerria*.

The astronesthines *Astronesthes*, *Neonesthes* and *Rhadinesthes*, and the phosichthyids *Ichthyococcus*, *Phosichthys* and *Vinciguerria* clustered around the consensus configuration in the phylomorphospace and were comparatively more restricted in body shape than other taxa, such as the sagittiform stomiids and the deep-bodied sternoptychines, which have significantly higher variance and disparity in body shape. The fusiform body shape and a mid-body position of the dorsal fin are the main traits distinguishing the astronesthine from other stomiids (WEITZMAN, 1967; FINK, 1985). A closer relationship between Astronesthinae and Phosichthyidae was suggested by Weitzman (1967) based on morphological similarities related to the skull, including the upper jaw, teeth, and the ethmoid region. However, such relationships were never recovered using molecular evidence, which rendered Astronesthinae monophyletic and within Stomiidae (MIRANDE, 2017; BETANCUR-R *et al.*, 2017; RABOSKY *et al.*, 2018). However, unlike most stomiids, the astronesthines are generalists, feeding mainly on small crustaceans such as krill and decapods, and minorly on fish (CLARKE, 1982; GIBBS; 1984; HOPKINS *et al.*, 1996; SUTTON; HOPKINS, 1996). This feeding habit is similar to the species currently allocated in Phosichthyidae (HOPKINS *et al.*, 1996; SUTTON; HOPKINS, 1996).

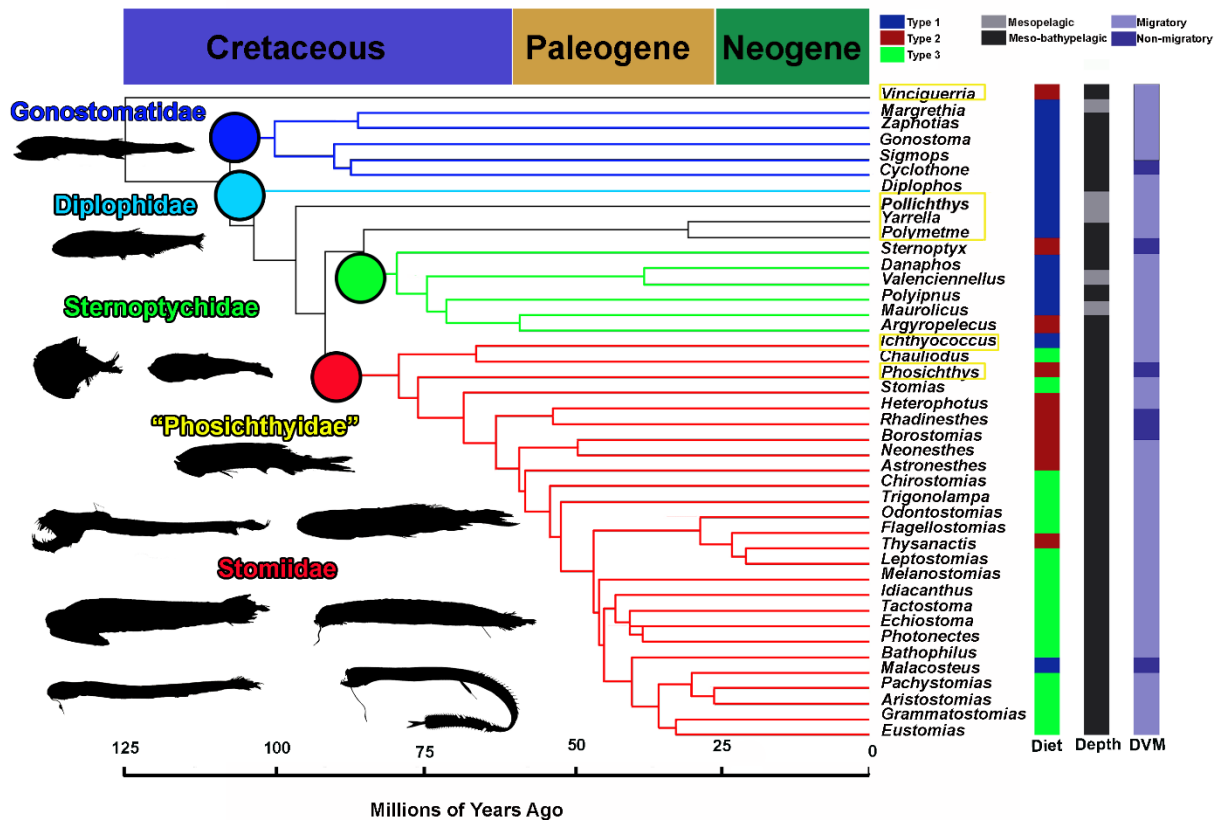


Figure 2.7. Time-calibrated phylogenetic tree of the Stomiiformes, adapted from Rabosky *et al.* (2018), with the ecological traits of diet (type 1 = zooplanktivorous; type 2 = generalists; or type 3 = piscivorous), depth distribution (mesopelagic; or meso-bathypelagic) and diel vertical migration pattern (migratory; or non-migratory) indicated. The color code on the cladogram indicates families.

The substantial phylogenetic signal in body shape and size within stomiiforms, means that closely related taxa tended to cluster in the phylomorphospace due to their shared evolutionary history (Fig.2.6). The high phylogenetic signal in body shape ($K=1.9$) substantially exceeded the expectation of $K=1$ under neutral evolution or genetic drift (KAMILAR; COOPER, 2013). Such high values are often interpreted as evolutionary or phylogenetic conservatism (LOSOS, 2008), and reflect the tendency of species to retain their ancestral traits. The existence of several distinct clusters suggests the existence of multiple adaptive peaks within the stomiiform morphospace. While this could explain why lineages tend not to leave particular regions once they have occupied them, testing that conjecture would require a more densely sampled phylomorphospace and hence a phylogeny with greater taxon sampling.

Inclusion of the phylogeny eroded the significance of differences in morphological disparity and mean shape among stomiiform ecological classes that the

non-phylogenetic analysis returned. The significant correlations between body-shape and ecological traits, which disappear after accounting for phylogeny, might suggest that: i. ecological traits are unlikely to play a major role in shaping morphological evolution; ii. ecological traits play a role only during the early evolution of the group; iii. the current dataset lacks the statistical power to uncover any relationship that may exist between shifts in depth, diet or migration and the shape diversification of stomiiforms; iv. the lack of significance could stem from the relatively few transitions among ecological classes that occurred during stomiiforms evolution; v. the lack of significance could stem from the relatively sparse taxon sampling of the phylogenetically informed analysis.

2.5 Conclusion

The complex evolutionary history of Stomiiformes to colonize the deep sea promoted diversification of body shape, from the mesopelagic generalists with fusiform body to the meso-bathypelagic piscivores with elongated sagittiform to anguilliform body, which are the most iconic living dragonfishes. The fins size and position, especially dorsal and anal fins, correspond to the major variations within the stomiiforms, followed by body size and body depth, resulting in a transition from species with dorsal fin positioned at midbody, long anal fin, and a fusiform or deep body morphology to species with dorsal fin posterior on body and parallel to a short anal fin, and an elongated body. There is a trend towards body elongation in taxa according to the depth, with the meso-bathypelagic species presenting bodies more elongated than the restricted mesopelagic. Three main patterns of body elongation are observed: the first one, related to an elongation of the anal fin, the second related to a sagittiform morphology, with dorsal and anal fin short and posteriorly placed, and the third related to an extreme elongation of the body with an elongation of both anal and dorsal fins. There is significant morphological disparity between body shape and the different stomiiforms clades, and a significant association between shape and ecological traits. Sternoptychidae is the most morphologically diverse clade, including fusiform, elongated, and deep bodied species, such as the genus *Sternoptyx*, which has the higher morphological disparity among all stomiiforms. Generalists and piscivorous stomiiforms have a higher morphological disparity than the zooplanktivorous counterparts, and generally, are meso-bathypelagic species. In contrast, zooplanktivorous includes both mesopelagic and meso-bathypelagic species.

Considerable shifts in body shape transpire across piscivorous stomiiforms, characterized by elongated bodies bearing sagittiform morphologies, large mouths, and small to moderately sized eyes. In contrast, generalist and zooplanktivorous stomiiforms have greater variability in body shape, encompassing forms such as fusiform, elongated, and deep-bodied morphologies. This spectrum includes dorsal fins positioned midbody and eyes that span a range from small to large, often trending towards magnified ocular dimensions within mesopelagic species. Both migrant and non-migrant patterns occur in all stomiiform clades and, therefore, align with additional ecological factors, notably dietary considerations, rather than being exclusively governed by morphological attributes or phylogenetic relationships. There is a phylogenetic signal in body shape among the stomiiforms, with closely related taxa tended to cluster together in phylomorphospace as the result of shared evolutionary history. The phylogenetic signal is also high and is often interpreted as evolutionary or phylogenetic conservatism. This is the first study to quantitatively investigate changes in the body shape of stomiiforms, concluding that the body-shape evolution is explained mostly by the evolutionary history, as a result of shared ancestry. The significant correlations between shape and ecological traits, which disappear after accounting for phylogeny, might be indicative that ecological traits are unlikely to play a major role in shaping morphological evolution. However, there are another hypothesis that could explain the results found, for example, the lack of significance could stem from the relatively sparse taxon sampling of the phylogenetically informed analysis. Future research should revisit this question once a detailed phylogeny of Stomiiformes becomes available.

2.6 Material examined

Araiophos eastropas: SIO 90-177 (4).

Argyripnus atlanticus: MNRJ 30552 (10).

Argyropelecus aculeatus: MZUSP 86698 (3), MZUSP 86374 (1), MZUSP 86376 (4), MZUSP 78245 (1), MZUSP 80271 (2), MZUSP uncat. (1), MZUSP uncat. (2).

Argyropelecus affinis: OSIC 19956 (1), OSIC 22684 (1), OSIC 6424 (1), OSIC 14136 (2), OSIC 19957 (2), OSIC 14155 (1).

Aristostomias scintilans: OSIC 14477 (1), OSIC 14209 (1), OSIC 14475 (1), OSIC 14701 (1), OSIC14576 (1), OSIC 14458 (1), OSIC 14557 (1).

Astronesthes gemmifer: USNM 436537 (1), USNM 45448 (1).

Astronesthes macropogon: MZUSP 78244 (2), MZUSP 80531 (1), MZUSP 80532 (1), MZUSP 80272 (1), MNRJ 30794 (5), MNRJ 30800 (2), MNRJ 30793 (2), MNRJ 30797 (2), MNRJ 30792 (1).

Bathophilus flemingi: OSIC 14605 (1), OSIC 14619 (1), OSIC 14580 (1), OSIC 14567 (1), OSIC 14294 (1), OSIC 14585 (1), OSIC 14569 (1), OSIC 14556 (1), OSIC 14616 (1), OSIC 14568 (1), OSIC 14479 (1), OS14559 (1).

Borostomias antarcticus: SIO 61-45 (1), USNM 265384 (2), USNM 300019 (1), USNM 454835 (3).

Chauliodus macouni: OSIC 014834 (2), OSIC 14153 (1), OSIC 17304 (1), OSIC 014834 (1), OSIC 14153 (1), OSIC 8673 (1), OSIC 11763 (1), OSIC 921 (1), OSIC 11347 (1), OSIC 13686 (1), OSIC 11823 (2).

Chauliodus sloani: MNRJ 42816 (1), MNRJ 42817 (2), MNRJ 42820 (1), MZUSP uncat. (2).

Chirostomias pliopterus: MCZ 132709 (1), MCZ 132707 (1).

Cyclothone microdon: OSIC 14427 (1), OSIC 11279 (4), OSIC 11346 (4).

Cyclothone pseudopallida: MZUSP uncat. (14).

Danaphos oculatus: OSIC 11913 (1), OSIC 11867 (1), OSIC 11861 (1).

Diplophos taenia: MZUSP 78235 (1), 80530 (1), OSIC 11853 (2), SIO 77-218 (2), SIO 69-334 (1), SIO 11-203 (1).

Echiostoma barbatum: USNM 235659 (2), USNM3 58644 (1), USNM 409549 (1), USNM 436522 (1).

Eustomias filifer: MCZ 153128 (2), MCZ 96167 (1).

Flagellostomias boureei: SIO 76-107 (1), SIO 74-20 (1), USNM 234681 (1), USNM 234682 (1), USNM 297019 (1), USNM 322976 (1).

Gonostoma atlanticum: OSIC 11844 (2).

Grammatostomias flagellibarba: MCZ 153118 (1), MCZI 164122 (1).

Heterophotus ophistoma: MNRJ 30914 (1), SIO 70-341 (1), SIO 20-38 (1), SIO 73-165 (1), USNM 214461 (1), USNM 258818 (3).

Ichthyococcus elongatus: OSIC 11820 (1), OSIC 11848 (1), OSIC 11872 (1), OSIC 014295 (1).

Idiacanthus atlanticus: MZUSP 086603 (2), MZUSP 78406 (6).

Leptostomias gladiator: OSIC 014575 (1), OSIC 014551 (1), MCZ 149495 (1), MCZ 155381 (1), MCZ 169550 (1), MNRJ 30806 (1).

Malacosteus australis: MNRJ 30737 (1), MNRJ 30728 (1), MNRJ 30723 (1), MNRJ 30733 (1), MNRJ 30740 (2), MNRJ 30386 (1), MZUSP uncat (1), MZUSP uncat (2).

Manducus maderensis: MNRJ 30128 (8), MNRJ 30136 (6), MNRJ 30154 (1), 30157 (1), MNRJ 30135 (2), MNRJ 30301 (1), MCZ 61476 (1), MCZ 91350 (1).

Margrethia obtusirostra: USNM 248767 (2), USNM 248768 (1), USNM 248771 (2).

Maurolicus stehmani: MZUSP 80238 (10).

Maurolicus weitzmani: USNM 391455 (1), USNM 391391 (1).

Melanostomias melanops: MNRJ 50693 (1), MNRJ 30895 (2), MNRJ 30904 (1), MNRJ 30893 (2), MNRJ 44092 (1), MCZ 148622 (1), MCZ 164745 (1),

Neonesthes capensis: USNM 454448 (2).

Odontostomias micropogon: MCZ 132152 (1), MCZ 58810 (1), USNM 326567 (1), USNM 326568 (1).

Opostomias mitsuii: OSIC 14571 (1), OSIC 14482 (1), OSIC 14686 (1), OSIC 14623 (1), OSIC 14668 (1), OS 14673 (1), OSIC 14550 (3), OS14565 (1), OSIC 14651 (1).

Pachystomias microdon: USNM 296691 (2), USNM 297922 (3).

Phosichthys argenteus: MZUSP 86731 (2), MZUSP 86653 (2), MZUSP 86652 (2), MZUSP 86420 (1), MZUSP 78249 (6), MCZ 140441 (1), MCZ 61171 (1).

Photonectes margarita: SIO 98-5 (1), SIO 97-104 (1), SIO 11-410 (1), OSIC 014658 (1), USNM 234360 (1), USNM 234374 (1), USNM 201740 (1).

Photostomias guernei: MCZ 131727 (5), MNRJ MNRJ30743 (1).

Pollichthys mauii: MZUSP (4), MZUSP 78229 (3), MZUSP 080345 (6), MZUSP 80337 (1), MZUSP 80335 (1).

Polyipnus laternatus: MCZ 40574 (1), MCZ 91010 (1).

Polyipnus spinifer: USNM 135514 (3).

Polymetme thaecoryla: MZUSP 86650 (6), MZUSP 86412 (5), MZUSP 86401 (10), USNM 407426 (1), USNM 304218 (3).

Rhadinesthes decimus: OSIC 11651 (1), OS11650 (1), MCZ 64832 (1).

Sigmops elongatus: MZUSP 86606 (2), MZUSP (1), MZUSP 80528 (1), MZUSP 80527 (1), MZUSP uncat. (8), MZUSP uncat. (1), MNRJ 46049 (1), MNRJ 46065 (1), MNRJ 46061 (1), MNRJ 42846 (3), MNRJ 46063 (1), MNRJ 30276 (1), MNRJ 30271 (1), MNRJ 30270 (1).

Sternoptyx pseudobscura: MNRJ 50153 (1), MNRJ 30167 (8), MNRJ 30162 (1), MNRJ 30163 (1), MNRJ 30159 (1), MZUSP uncat (2), OSIC 12858 (8).

Stomias affinis: MZUSP 86719 (1), MZUSP 86574 (2), MZUSP 48982 (1), MZUSP 86575 (2), MZUSP 86572 (3), MZUSP uncat. (8).

Tactostoma macropus: OSIC 14654 (1), OSIC 9181 (1), OSIC 14558 (1), OSIC 11342 (4), OSIC 11301 (8), OSIC 14654 (6), OSIC 14653 (1), OSIC 14927 (1).

Thorophos nexilis: USNM 92326 (1).

Thysanactis dentex: USNM365806 (4), USNM365808 (1).

Trigonolampa miriceps: MCZ 124655 (1), MCZ 137986 (1), MCZ 165921 (1).

Valenciennellus tripunctulatus: OSIC 14881 (1), MZUSP uncat. (1).

Vinciguerria nimbaria: MZUSP 80330 (6), MZUSP 80287 (4), MZUSP 80287 (3), MZUSP uncat (1), OSIC 11877 (5), OSIC 11882 (1), OSIC 11874 (3).

Vinciguerria poweriae: MZUSP 80533 (8).

Yarella blackfordi: MCZ 124870 (1), MCZ 126580 (2).

Zaphotias pedaliotus: MCZ 58629 (5), SIO 63-560 (3).

2.7 References

- Adams, D. C., and E. Otárola-Castillo.** 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* 4:393–399. <https://doi.org/10.1111/2041-210X.12035>.
- Badcock, J.** 1970. The vertical distribution of mesopelagic fishes collected on the SONDR cruise. *Journal of the Marine Biological Association of the United Kingdom* 50 (4): 1001–1044. <https://doi.org/10.1017/S0025315400005920>.
- Betancur-R, R., E. O. Wiley, G. Arratia, A. Acero, N. Bailly, M. Miya, G. Lecointre, and G. Orti.** 2017. Phylogenetic classification of bony fishes. *BMC Evolutionary Biology* 17(162):1–40. <http://doi.org/10.1186/s12862-017-0958-3>.
- Blomberg, S. P., T. Garland, and A. R. Ives.** 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717–745. <http://doi.org/10.1111/j.0014-3820.2003.tb00285.x>.
- Burns, Michael D., and Brian L. Sidlauskas.** 2019. Ancient and contingent body shape diversification in a hyperdiverse continental fish radiation. *Evolution* 73 (3): 569–587. <https://doi.org/10.5061/dryad.0kf782p>.
- Bookstein, F. L.** 1991. *Morphometric tools for landmark data: geometry and biology.* Cambridge University Press, Cambridge. 435 p.

- de Busserolles, F., N. J. Marshall, and S. P. Collin.** 2014. The eyes of lanternfishes (Myctophidae, Teleostei): novel ocular specializations for vision in dim light. *Journal of Comparative Neurology* 522: 1618–1640. <https://doi.org/10.1002/cne.23495>.
- Carmo, V., T. Sutton, G. Menezes, T. Falkenhaus, and O.A. Bergstad.** 2015. Feeding ecology of the Stomiiformes (Pisces) of the northern Mid-Atlantic Ridge. 1. The Sternoptychidae and Phosichthyidae. *Progress in Oceanography* 130: 172–187. <https://doi.org/10.1016/j.pocean.2014.11.003>.
- Carnevale, G., and A. Rindone.** 2011. The teleost fish *Paravinciguerria praecursor* Arambourg, 1954 in the Cenomanian of north-eastern Sicily. *Bollettino della Società Paleontologica Italiana* 50, (1): 1–10. ISSN 0375-7633.
- Clarke, T. A.** 1982. Feeding habits of stomiatoid fishes from Hawaiian waters. *Fishery Bulletin* 80: 287–304.
- Claverie, Thomas, and Peter C. Wainwright.** 2014. A morphospace for reef fishes: elongation is the dominant axis of body shape evolution. *PloS one* 9 (11): e112732. <https://doi.org/10.1371/journal.pone.0112732>.
- Denton, J.S.S., and D. C. Adams.** 2015. A new phylogenetic test for comparing multiple high-dimensional evolutionary rates suggests interplay of evolutionary rates and modularity in lanternfishes (Myctophiformes; Myctophidae). *Evolution* 69(9): 2425–2440. <https://doi.org/10.1111/evo.12743>.
- Eduardo, L. N., A. Bertrand, M. M. Mincarone, L. V. Santos, T. Fredou, R. V. Assunção, A. Silva et al.** 2020. Hatchetfishes (Stomiiformes: Sternoptychidae) biodiversity, trophic ecology, vertical niche partitioning and functional roles in the western Tropical Atlantic. *Progress in Oceanography* 187: 102389. <https://doi.org/10.1016/j.pocean.2020.102389>.
- Fahay, M.P.** 2007. *Early Stages of Fishes in the Western North Atlantic Ocean (Davis Strait, Southern Greenland and Flemish Cap to Cape Hatteras)*. Northwest Atlantic Fisheries Organization. 1696 p.
- Farré, M., A. Lombarte, L. Recasens, F. Maynou, and V. M. Tuset.** 2015. Habitat influence in the morphological diversity of coastal fish assemblages. *Journal of Sea Research* 99: 107–117. <https://doi.org/10.1016/j.seares.2015.03.002>
- Farré, M., V. M. Tuset, J. E. Cartes, E. Massutí, and A. Lombarte.** 2016. Depth-related trends in morphological and functional diversity of demersal fish assemblages in the western Mediterranean Sea. *Progress in Oceanography* 147:22–37. <https://doi.org/10.1016/j.pocean.2016.07.006>.

- Fink, W. L.** 1985. Phylogenetic interrelationships of the stomiid fishes (Teleostei: Stomiiformes). *Miscellaneous Publications, Museum of Zoology, The University of Michigan*. 171:1–127.
- Friedman, S. T., S. A. Price, K. A. Corn, O. Larouche, C. M. Martinez, and P. C. Wainwright.** 2020. Body shape diversification along the benthic–pelagic axis in marine fishes. *Proceedings of the Royal Society B* 287 (1931): 20201053. <https://doi.org/10.1098/rspb.2020.1053>.
- Fricke, R., W. N. Eschmeyer, and R. Van Der Laan** (eds). 2023. *Eschmeyer's Catalog of Fishes: Genera, Species, References*. ([http:// researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp](http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp)). Electronic version accessed 22/April/2020.
- Gannon, D. R., J.E. Craddock, and A.J. Read.** 1998. Autumn food habits of harbor porpoises, *Phocoena phocoena*, in the Gulf of Maine. *Fishery Bulletin* 96: 428–437.
- Gartner Jr, John V., Roy E. Crabtree, and Kenneth J. Sulak.** 1997. 4 Feeding At Depth. *Fish physiology* 16: 115–193. [https://doi.org/10.1016/S1546-5098\(08\)60229-0](https://doi.org/10.1016/S1546-5098(08)60229-0).
- Gon, O.** 1990. Stomiidae. Scaly dragonfishes, p. 127–133. *In: Fishes of the Southern Ocean*. O. Gon and P.C. Heemstra (eds.). J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa.
- Goswami, A., A. Watanabe, R. N. Felice, C. Bardua, A. C. Fabre, and P. D. Polly.** 2019. High-density morphometric analysis of shape and integration: the good, the bad, and the not-really-a-problem. *Integrative and comparative biology* 59(3): 669–683. <https://doi.org/10.1093/icb/icz120>.
- Harold, A. S.** 2002. Gonostomatidae (881–884), Phosichthyidae (885–888), Sternoptychidae (889–892), Astronesthidae (893–895), Chauliodontidae (896–898), Idiacanthidae (899–900), Malacosteidae (901–903), Stomiidae (904–906), and Melanostomiidae (907–912). *In: The living marine resources of the western central Atlantic, FAO species identification guide for fishery purposes*. Vol. 2. K. E. Carpenter (Ed.) FAO, Rome.
- Harold, A. S. and S. H. Weitzman.** 1996, p. 333–353. Interrelationships of Stomiiform Fishes. *In: Interrelationships of Fishes*. M. L. J. Stiassny, L.R. Parenti, and G. David–Johnson. 1996 Academic Press Inc., San Diego, CA.

- Herring, P. J.** 2007. Sex with the lights on? A review of bioluminescent sexual dimorphism in the sea. *Journal of the Marine Biological Association of the United Kingdom*, 87(4): 829–842. <https://doi.org/10.1017/S0025315407056433>.
- Hopkins, T. L., T. T. Sutton, and T. M. Lancraft.** 1996. The trophic structure and predation impact of a low latitude midwater fish assemblage. *Progress in Oceanography* 38: 205–239. [https://doi.org/10.1016/S0079-6611\(97\)00003-7](https://doi.org/10.1016/S0079-6611(97)00003-7).
- Kamilar, J. M., and N. Cooper.** 2013. Phylogenetic signal in primate behavior, ecology and life history. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368.1618: 20120341. <https://doi.org/10.1098/rstb.2012.0341>.
- Kenaley, C. P., S. C. Devaney, and T. T. Fjeran.** 2014. The complex evolutionary history of seeing red: Molecular phylogeny and the evolution of an adaptive visual system in deep-sea dragonfishes (Stomiiformes: Stomiidae). *Evolution* 68(4): 996–1013. <https://doi.org/10.1111/evo.12322>.
- Kenaley, C. P.** 2008. Diel vertical migration of the loosejaw dragonfishes (Stomiiformes: Stomiidae: Malacosteinae): a new analysis for rare pelagic taxa. *Journal of Fish Biology* 73(4): 888–901. <https://doi.org/10.1111/j.1095-8649.2008.01983.x>.
- Kenaley, C. P.** 2009. Revision of Indo–Pacific Species of the Loosejaw Dragonfish Genus *Photostomias* (Teleostei: Stomiidae: Malacosteinae). *Copeia* 1:175–189. <https://doi.org/10.1643/CI-07-224>.
- Larouche, Olivier, Bailey Benton, Katherine A. Corn, Sarah T. Friedman, Dominique Gross, Mikayla Iwan, Brian Kessler et al.** 2020. Reef-associated fishes have more maneuverable body shapes at a macroevolutionary scale. *Coral Reefs* 39: 1427–1439. <https://doi.org/10.1007/s00338-020-01976-w>.
- Losos, J. B.** 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* 11: 995–1007. <https://doi.org/10.1111/j.1461-0248.2008.01229.x>.
- Maile, A. J., Z. A. May, E. S. DeArmon, R. P. Martin, and M. P. Davis.** 2020. Marine habitat transitions and body-shape evolution in lizardfishes and their allies (Aulopiformes). *Copeia* 108: 820–832. <https://doi.org/10.1643/CG-19-300>.
- Marranzino, A. N., and J. F. Webb.** 2018. Flow sensing in the deep sea: the lateral line system of stomiiform fishes. *Zoological Journal of the Linnean Society* 183: 945–965. <https://doi.org/10.1093/zoolinnean/zlx090>.

- Martinez, C. M., S. T. Friedman, K. A. Corn, O. Larouche, S. A. Price, and P. C. Wainwright.** 2021. The deep sea is a hot spot of fish body shape evolution. *Ecology Letters* 24: 1788–1799. <https://doi.org/10.1111/ele.13785>.
- Mindel, B. L., F. C. Neat, C. N. Trueman, T. J. Webb, and J. L. Blanchard.** 2016. Functional, size and taxonomic diversity of fish along a depth gradient in the deep sea. *PeerJ* 4: e2387. <https://doi.org/10.7717/peerj.2387>.
- Mirande, J. M.** 2017. Combined phylogeny of ray-finned fishes (Actinopterygii) and the use of morphological characters in large-scale analyses. *Cladistics* 33(4): 333–350. <https://doi.org/10.1111/cla.12171>.
- Mundy, B. C.** 2005. Checklist of the fishes of the Hawaiian Archipelago. *Bishop Museum Bulletin in Zoology* (6): 1–704.
- Neat, F. C., and N. Campbell.** 2013. Proliferation of elongate fishes in the deep sea. *Journal of Fish Biology* 83:1576–1591. <https://doi.org/10.1111/jfb.12266>.
- Nelson, J. S.** 2006. *Fishes of the world*. 4^o ed. John Wiley and Sons, Inc., Hoboken, NJ.
- Nelson, J. S., T. C. Grande, and M. V. H. Wilson.** 2016. *Fishes of the World*. 5^a ed. John Wiley and Sons, Inc., Hoboken, NJ. 752p.
- Nunes, J.L.S., N.M. Piorski, and M.E. Araújo.** 2008. Phylogenetic and ecological inference of three *Halichoeres* (Perciformes: Labridae) species through geometric morphometrics. *Cybium* 32 (2): 165–171. <https://doi.org/10.26028/cybium/2008-322-010>.
- Orlov, A., and C. Binohlan.** 2009. Length–weight relationships of deep-sea fishes from the western Bering Sea. *Journal of Applied Ichthyology* 25(2): 223–227. <https://doi.org/10.1111/j.1439-0426.2009.01215.x>.
- Paradis, E., J. Claude, and K. Strimmer.** 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20 (2): 289–290. <https://doi.org/10.1093/bioinformatics/btg412>.
- Parin, N. V. and O. D. Borodulina.** 2003. Phylogeny, systematics, and zoogeography of the mesopelagic genus *Astronesthes* (Astronesthidae, Stomiiformes). *Voprosy Ikhtiologii* 43 (5): 581–601. [In Russian. English translation in *Journal of Ichthyology* 43 (8):557–576].
- Pérès, J. M.** 1985. History of the Mediterranean biota and the colonization of the depths, p. 198–23. *In: Key environments: Western Mediterranean*. Margalef, R. (ed.) Pergamon Press, New York.

- Pietsch, Theodore W.** 2009. Oceanic anglerfishes: extraordinary diversity in the deep sea. University of California Press, California.
- Price, S. A., S. T. Friedman, K. A. Corn, C. M. Martinez, O. Larouche, and P. C. Wainwright.** 2019. Building a body shape morphospace of teleostean fishes. *Integrative and Comparative Biology* 59 (3): 716–730. <https://doi.org/10.1093/icb/icz115>.
- Pulcini, D., C. Costa, J. Aguzzi, and S. Cataudella.** 2008. Light and Shape: A Contribution to Demonstrate Morphological Differences in Diurnal and Nocturnal Teleosts. *Journal of Morphology* 269: 375–385. <https://doi.org/10.1002/jmor.10598>.
- R Core Team.** 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <https://www.R-project.org/>.
- Rabosky, D. L., J. Chang, P. O. Title, P. F. Cowman, L. Sallan, M. Friedman, K. Kaschner, C. Garilao, T. J. Near, M. Coll, and M. E. Alfaro.** 2018. An inverse latitudinal gradient in speciation for marine fishes. *Nature* 559: 392–395. <https://doi.org/10.1038/s41586-018-0273-1>.
- Revell, L. J.** 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3:217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>.
- Ribeiro, Emanuell, Aaron M. Davis, Rafael A. Rivero-Vega, Guillermo Ortí, and Ricardo Betancur-R.** 2018. Post-Cretaceous bursts of evolution along the benthic–pelagic axis in marine fishes. *Proceedings of the Royal Society B Biological Sciences* 285 (1893):20182010. <https://doi.org/10.1098/rspb.2018.2010>. <https://doi.org/10.1098/rspb.2018.2010>.
- Rohlf, F. J.** 2018. tpsDig2. Department of Ecology and Evolution, State University of New York at Stony Brook. Version 2.49. Available at: <https://life.bio.sunysb.edu/morph>.
- Schnell, N. K., and G. D. Johnson.** 2017. Evolution of a functional head joint in deep-sea fishes (Stomiidae). *PLoS ONE* 12 (2): e0170224. <https://doi.org/10.1371/journal.pone.0170224>.
- Schnell, N. K., J. Kriwet, F. A. López-Romero, G. Lecointre, and C. Pfaff.** 2021. Musculotendinous system of mesopelagic fishes: Stomiiformes (Teleostei). *Journal of Anatomy* 240 (6): 1095–1126. <https://doi.org/10.1111/joa.13614>.

- Schnell, N. K., R. Britz, and G. D. Johnson.** 2010. New insights into the complex structure and ontogeny of the occipito-vertebral gap in barbeled dragonfishes (Stomiidae, Teleostei). *Journal of Morphology* 271: 1006–1022. <http://doi.org/10.1002/jmor.10858>.
- Sfakiotakis, M., D. M. Lane, and J. B. C. Davies.** 1999. Review of fish swimming modes for aquatic locomotion. *IEEE Journal of Oceanic Engineering* 24(2): 237–252. <https://doi.org/10.1109/48.757275>.
- Sidlauskas, B.** 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. *Evolution* 62:3135–3156. <https://doi.org/10.1111/j.1558-5646.2008.00519.x>.
- Sutton, T. T.** 2013. Vertical ecology of the pelagic ocean: classical patterns and new perspectives: vertical ecology of the pelagic ocean. *Journal of Fish Biology*, 83: 1508–1527. <https://doi.org/10.1111/jfb.12263>.
- Sutton, T. T. and T. L. Hopkins.** 1996. Trophic ecology of the stomiid (Pisces:Stomiidae) fish assemblage of the eastern Gulf of Mexico: strategies, selectivity and impact of a top mesopelagic predator group. *Marine Biology* 127:179–192. <https://doi.org/10.1007/BF00942102>.
- Tchernavin, V. V.** 1953. The Feeding Mechanisms of a Deep-Sea Fish: *Chauliodus sloani* Schneider. British Museum (Natural History), London.
- Tuset, V. M., M. Pilar Olivar, J. L. Otero-Ferrer, C. López-Pérez, P. A. Hulley, and A. Lombarte.** 2018. Morpho-functional diversity in *Diaphus* spp. (Pisces: Myctophidae) from the central Atlantic Ocean: Ecological and evolutionary implications. *Deep Sea Research Part I: Oceanographic Research Papers* 138: 46–59. <https://doi.org/10.1016/j.dsr.2018.07.005>.
- Warrant, E.J., S.P. Collin, and N.A. Locket.** 2003. Eye design and vision in deep-sea fishes. *Sensory processing in aquatic environments*, 303–322. https://doi.org/10.1007/978-0-387-22628-6_16.
- Webb, P.W.** 1978. Fast-start performance and body form in seven species of teleost fish. *Journal of experimental biology*, 74(1): 211–226. <https://doi.org/10.1242/jeb.74.1.211>.
- Webb, P. W.** 1984. Form and function in fish swimming. *Scientific American* 251 (1): 72–83. <https://doi.org/10.1038/scientificamerican0784-72>.
- Weitzman, J. J.** 1967. The osteology and relationships of the Astronesthidae, a family of oceanic Fishes. *Dana Report* 71:1–54.

- Wickham, H** 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Young, J. W., T. D. Lamb, D. Le, R. W. Bradford, and A. W. Whitelaw.** 1997. Feeding ecology and interannual variations in diet of southern bluefin tuna, *Thunnus maccoyii*, in relation to coastal and oceanic waters off eastern Tasmania, Australia. *Environmental Biology of Fishes* 50:275–291. <https://doi.org/10.1023/A:1007326120380>.
- Thistle, D.** 2003. The deep-sea floor, an overview, p. 5-37. *In: Ecosystems of the World* 28. Tyler, P. A. (Ed.) *Ecosystems of the Deep-Ocean*. Elsevier, Amsterdam.
- Angel, M. V.** 1997. What is the deep-sea?, p. 2-41. *In: Deep-Sea Fishes*. Randall, D. J.; Farrell, A. P. (eds.). Academic Press, San Diego.
- Rohlf, F. J.** 2015. The tps series of software. *Hystrix* 26: 9–12. <https://doi.org/10.4404/hystrix-26.1-11264>.

So long, and thanks for all the fish.

(ADAMS, Douglas – *The Hitchhiker's Guide
to the Galaxy*, v.4)

3. CHAPTER THREE: EVOLUTION OF SKULL SHAPE IN STOMIIFORMES

Abstract. —The order Stomiiformes includes five families and 457 valid species of mesopelagic to bathypelagic fishes that exhibit a diversity of structures associated with feeding, locomotion, and bioluminescence. This study investigates the patterns of skull-shape evolution among stomiiforms using three-dimensional geometric morphometrics in ecological and phylogenetic contexts. We used CT scan models of 29 stomiiform skulls, representing 29 of the 52 genera of the order, to analyze the skull shape using 33 homologous landmarks. Morphometric data was submitted to principal component analysis (PCA), and to comparative methods (morphological disparity and Procrustes ANOVA) analyses to visualize differences in variance among stomiiform clades, dietary classes, and ocean depth, and this data was then linked to a recent phylogenetic hypothesis and tested for phylogenetic signal. Overall, all stomiiform clades vary in terms of skull shape, but morphological disparity was greater in Stomiidae and Sternoptychidae. However, a few substantial differences were observed. Significant morphological disparity in skull shape was mainly related to skull length and skull depth, with the main axis of total variation describing a transition from a tall skull, represented by the hatchetfishes, to an elongated skull, and large jaws, represented by the loosejaws and dragonfishes. There was no significant correlation between skull shape and ecological traits, with or without the account of phylogeny. Our analyses suggested that extreme morphologies in Stomiiformes might be linked to the correlated evolution of neurocranial elements, with variations in skull shape predominantly associated with head length and depth, particularly in the occipital region. There was a significant and strong phylogenetic signal in skull shape among stomiiforms, with closely related taxa tended to cluster together in phylomorphospace, as the result of shared evolutionary history, which is often interpreted as evolutionary or phylogenetic conservatism. The evolution of skull shape in Stomiiformes is explained mostly by phylogeny. Ecological traits, such as diet and depth are unlikely to play a major role in shaping morphological evolution.

Keywords: Deep-sea fish, Ecomorphology, Geometric morphometrics, Niche Conservatism, Phylogenetical Signal.

3.1 Introduction

The deep sea, encompassing the region beneath the euphotic zone at depths exceeding 200 meters, constitutes the planet's most extensive habitat, spanning approximately 65% of the Earth's surface. (COCKER, 1978, ANGEL, 1997). Ocean depth plays a crucial role in marine ecosystems, as it entails various environmental factors that significantly impact living organisms, such as the hydrostatic pressure, temperature, dissolved oxygen, and availability of light (ANGEL, 1997; THISTLE, 2003). These environmental conditions result in significant shifts in the vertical structure of marine communities and trigger depth-specific adaptations related to vision, buoyancy control, and intraspecific communication, among others (ANGEL, 1989; HADDOCK *et al.*, 2010; WIDDER, 2010). The changes associated with ocean depth gave rise to a wide array of adaptations in deep-sea organisms, and many fish have thrived through remarkable evolutionary processes in the deep-sea regions, far-reaching implications for phenotypic variation (RANDALL; FARRELL, 1997; INGRAM, 2010; MARTINEZ *et al.*, 2021). As a result, deep-sea fishes are incredibly diverse with specialized traits to feed, reproduce, and hide in deep-sea habitats, with highly variable feeding and sensory system traits and light organs (RANDALL; FARRELL, 1997; HADDOCK *et al.*, 2010; WIDDER, 2010; MARRANZINO; WEBB, 2018).

Stomiiforms represent one of most successful radiations of deep-sea fishes, currently containing 457 species that inhabit meso and bathypelagic regions of the ocean (NELSON *et al.*, 2016; FRICKE *et al.*, 2023), and they have been present within these ecosystems for at least 94 million years (CARNEVALE; RINDONE, 2011). The order Stomiiformes comprises the bristlemouths, lightfishes, hatchetfishes, dragonfishes, viperfishes, loosejaws, and fangtooths, traditionally divided into five families: Diplophidae, Gonostomatidae, Phosichthyidae, Sternoptychidae and Stomiidae (HAROLD, 2002; NELSON, 2006, NELSON *et al.*, 2016), with recent molecular phylogenies recovering Phosichthyidae as paraphyletic (BETANCUR-R *et al.*, 2017; RABOSKY *et al.*, 2018). Stomiidae is the most diverse family, comprising 320 species, divided into six subfamilies: Astronesthinae (snaggletooths), Chauliodontinae (viperfishes), Idiacanthinae (black dragonfishes), Malacosteinae (loosejaws), Stomiinae (scaly dragonfishes), and Melanostomiinae (scaleless dragonfishes) (MC GONALE *et al.*, 2023). Most stomiiforms perform a diel vertical migration (DVM) emerging from the deep waters to the surface to feed at night (SUTTON; HOPKINS, 1996). Therefore, they

are key organisms in the oceanic food webs and a significant component of the biological pump as they transport carbon from the epipelagic to meso and bathypelagic environments (SUTTON; HOPKINS, 1996; ANGEL, 1997; CARMO *et al.*, 2015).

Stomiiforms exhibit high levels of morphological diversity within the group, (FINK, 1984; HAROLD; WEITZMAN, 1996). They exhibit a great variety of body shapes and structures associated with feeding, locomotion, and bioluminescence (HAROLD; WEITZMAN, 1996) (Fig. 3.1). Notable variation within stomiiforms is present particularly in skull morphology. This variation encompasses a range of skull lengths, from short to moderate in bristlemouths (Gonostomatidae and Diplophidae) (Supplemental Fig.S3.3) and lightfishes (“Phosichthyidae”) (Supplemental Fig.S3.4), to elongate in most stomiids (Supplemental Figs. S3.6-9). The hatchetfishes of the family Sternoptychidae display high disparity in both head and body shape within stomiiforms (Supplemental Fig.S3.5). Diagnostic traits of hatchetfishes include a deep and laterally compressed body, substantial head depths, large eyes, and short jaws. The most diverse family Stomiidae, encompassing dragonfishes and their allies, is characterized by elongated and slender jaws, except for certain melanostomiines (FINK, 1985) (Supplemental Fig.S3.7).

Fish lineages that inhabit deep waters exhibit loss of skeletal elements and reduction in bone density driven by environmental conditions related to increasing depth, like hydrostatic pressure (CHILDRESS; NYGAARD, 1973; RANDALL; FARRELL, 1997; GERRINGER *et al.*, 2021). In stomiids (dragonfishes and their allies) for instance, skull bones such as the parietals, mesopterygoid, and retroarticular are absent in several taxa. The lateral ethmoids are also absent in some taxa and, when present, vary in size from large (*e.g.*, *Flagellostomias*, and *Photonectes*) to quite small (*e.g.*, *Pachystomias* and *Bathophilus*) (FINK, 1985). The postcranial skeleton of stomiids also exhibits certain reduction. All stomiid genera exhibit an occipito-vertebral gap between the skull and the first vertebra connected only by the flexible notochord (SCHNELL *et al.*, 2010). The length of the occipito-vertebral gap varies among genera, being relatively short in basal stomiids like the fangtooths (Astronesthinae), and much longer in derived taxa like the loosejaw *Malacosteus* (Malacosteinae) (Fig. 3.1I). Besides, specifically in some stomiids like the viperfish *Chauliodus*, the first vertebral centra are absent (Fig. 3.1K), and the number of absent vertebral centra varies according to species in this genus (SCHNELL *et al.*, 2010). Additionally, variations in dentition are evident across stomiiform species, with gonostomatids, phosichthyids, and sternoptychids displaying small teeth closely set

on the maxilla. Conversely, stomiids possess enlarged mouths with fang-like teeth, indicative of their specialized adaptation for capturing large prey (FINK, 1985; SUTTON, 2005).

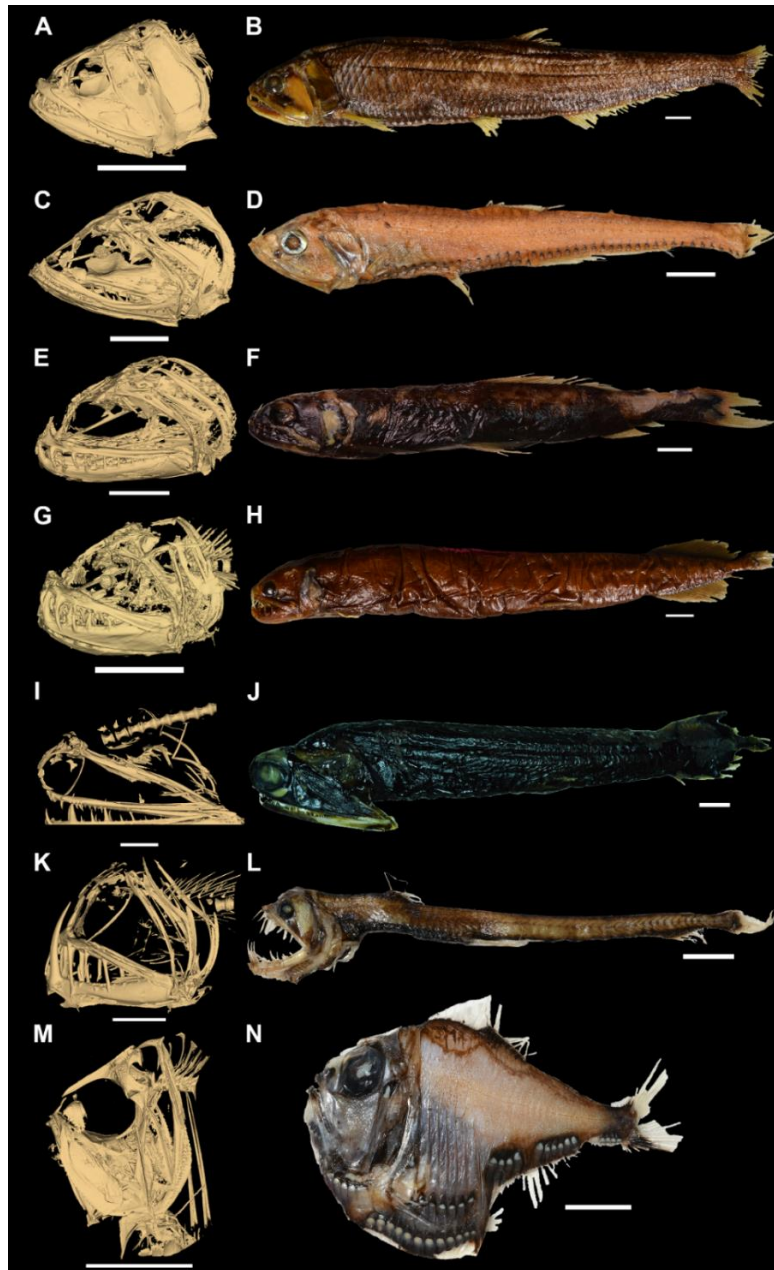


Figure 3.1. The diversity of the Order Stomiiformes. A. *Manducus maderensis*, MCZ 61476, 91.9 mm SL; B. *M. maderensis*, MCZ 91350, 95.8 mm SL; C. *Polymetme thaeocoryla*, USNM 304218, 145.1 mm SL; D. *P. thaeocoryla* MZUSP086650, 100.2 mm SL; E. *Astronesthes gemmifer*, USNM 436537, 151.7 mm SL; F. *Astronesthes macropogon*, MZUSP80272, 115.6 mm SL; G. *Opostomias mitsuii*, OS 014651, 123.2 mm SL; H. *O. mitsuii*, OS 014668, 116.3 mm SL; I. *Malacosteus niger*, YPMICH 025708, 175.40 mm SL; J. *Malacosteus australis*, MNRJ 30386, 160.8 mm SL; K. *Chauliodus sloani*, VIMS 16520, 222.47 mm SL; L. *C. sloani*, MZUSP uncat., 95.6 mm SL; M. *Argyropelecus affinis*, SIO71-191, 44.0 mm SL; N. *Argyropelecus aculeatus*, MZUSP uncat., 47.8 mm SL. A, C, E, G, I, K and M: micro-CT Scan of the skull. Scale bars =10 mm.

The diversity of skull shape in stomiiforms allows a study on the effects of intrinsic and extrinsic factors on morphological evolution, especially using geometric morphometrics methods, which provide a comprehensive approach for capturing and statistically quantifying intricate forms within a clade, facilitating the examination of evolutionary hypotheses within a phylogenetic context (GOSWAMI *et al.*, 2019). Recently, an expressive number of studies have been revealing patterns of shape diversity and evolution of the fish skull (BOYLE; HERREL, 2018; EVANS *et al.*, 2019, FORD *et al.*, 2022, EVANS *et al.*, 2023; LAROUCHE *et al.*, 2023), with some of them focused mainly on the neurocranium (*e.g.*, BOYLE; HERREL, 2018, 2018, KNAPP *et al.*, 2023), given its crucial function as protective casing for the brain and sensory organs and as a substantial support for the mobile components of the skull (HANKEN; THOROGOOD, 1993), also playing an important role in anchoring the postcranial epaxial muscles involved in locomotion, feeding, and ventilation (ALEXANDER, 1964; WINTERBOTTOM, 1974).

While geometric morphometric methods have been applied to elucidate patterns of skull evolution in freshwater fish (BOYLE; HERREL, 2018; EVANS *et al.*, 2019; FORD *et al.*, 2022) and in marine species inhabiting coastal or shallow waters (EVANS *et al.*, 2023; LAROUCHE *et al.*, 2023), limited studies have focused on the ecomorphology and evolution of skull shape across lineages residing in open ocean environments (KNAPP *et al.*, 2023), particularly deep marine habitats. These studies revealed different drivers for skull shape variation in different teleost lineages. In serrasalmids, skull shape variation might be related to the relative size of sense organs and with functional aspects of feeding (BOYLE; HERREL, 2018, 2018). In the pelagiarian fishes, skull shape reflects body shape elongation (KNAPP *et al.*, 2023).

In this study, we analyze stomiiforms skull shape diversity using geometric morphometric methods, seeking to investigate how the diversification of the skull shape is linked to ecological divergence, in a phylogenetic context. Specifically, this study main goals are as follows: (1) to elucidate the main drivers for stomiiforms skull shape disparity; (2) to investigate whether there are trends in stomiiform skull shapes that are correlated with diet, and ocean depth; (3) to investigate whether the skull shape is primarily influenced by phylogeny, ecological aspects, or functional aspects of feeding.

3.2 Material and methods

3.2.1 Taxonomic sampling

29 specimens of Stomiiformes, each belonging to a different species, representing 29 of the 52 genera and all families of the order were studied. The specimens examined are housed in the ichthyological collections of the Oregon State Ichthyology Collection, Oregon State University (OS), the Museum of Comparative Zoology, Harvard University (MCZ), the National Museum of Natural History, Smithsonian Institution (USNM), the Scripps Institution of Oceanography, University of California in San Diego (SIO), the University of Washington Fish Collection, University of Washington (UW), and the Yale Peabody Museum of Natural History, Yale University (YPM).

3.2.2 Data acquisition

The specimens were scanned and reconstructed for geometric morphometric analyses. Most specimens were scanned using the 1173 Bruker Skyscan microtomography from the Karl Liem Bioimaging Center at Friday Harbor Laboratories, Friday Harbor, WA, USA. The specimens were digitized in batches, with voltage and amperage parameters set at 55 kV and 133 μ A, respectively, and resolution of 17.7 to 35.5 μ m (voxel size). The reconstructions were performed using the NRecon software (Bruker micro-CT, Kontich, Belgium, 2016). Additionally, we included CT Scan data of nine stomiiform species from the MorphoSource platform (MorphoSource, 2022, <http://morphosource.org>). MorphoSource ID from these species is provided in Table 3.1. We selected only adults (the largest specimens) in order to avoid problems with ontogenetic dimorphism.

Images for each specimen were isolated and manipulated to reduce file size using ImageJ/Fiji software (SCHINDELIN *et al.*, 2012, <http://fiji.sc>). The resulting file for each species was later exported as a single file in Nearly Raw Raster Data (NRRD) format. The visualization and segmentation were performed using the 3D slicer software (Fedorov *et al.*, 2012, <http://slicer.org>) using the “Slicer Morph” extension. These steps were performed following a protocol adapted from Buser *et al.* (2020).

Table 3.1. Stomiiformes species used in the study, including catalog number, standard length (SL in mm), parameters used in the scans: kilovolts (kv), microampere (μA) and voxel size (μm), and MorphoSource id.

| Species | Catalogue Id | SL (mm) | kv | μA | Voxel size (μm) | MorphoSource id |
|--|------------------|---------|----|---------------|------------------------------|-----------------|
| Diplophidae (2) | | | | | | |
| <i>Diplophos taenia</i> Günther, 1873 | UW 115370 | 86.82 | 65 | 123 | 25.5 | 000S23880 |
| <i>Manducus maderensis</i> (Johnson, 1890) | MCZ 61476 | 191.9 | 55 | 133 | 27.3 | |
| Gonostomatidae (2) | | | | | | |
| <i>Margrethia obtusirostra</i> Jespersen and Tåning, 1919 | YPMICH 027653 | 43.79 | 60 | 260 | 36 | 000S32457 |
| <i>Zaphotias pedaliotus</i> (Goode and Bean, 1896) | KUBI 28490 | 53.60 | 70 | 200 | 43.1 | 424455 |
| Phosichthyidae (3) | | | | | | |
| <i>Phosichthys argenteus</i> Hutton, 1872 | MCZ 61171 | 237.2 | 55 | 133 | 35.5 | |
| <i>Polymetme thaecoryla</i> Parin and Borodulina, 1990 | USNM 304218 | 145.1 | 55 | 133 | 35.5 | |
| <i>Yarella blackfordi</i> Goode and Bean, 1896 | MCZ 126580 | 154.4 | 55 | 133 | 35.5 | |
| Sternoptychidae (4) | | | | | | |
| <i>Mauroliticus weitzmani</i> Parin and Kobylansky, 1993 | USNM 391455 | 58.3 | 55 | 133 | 27.3 | |
| <i>Argyropelecus affinis</i> Garman, 1899 | SIO71-191 | 44.0 | 60 | 110 | 20 | 0000S6607 |
| <i>Polyipnus spinifer</i> Borodulina, 1979 | USNM 135514 | 49.6 | 55 | 133 | 26.9 | |
| <i>Sternoptyx pseudobscura</i> Baird, 1971 | SIO66-536 | 26.36 | 60 | 110 | 20 | 0000S6603 |
| Stomiidae (18) | | | | | | |
| <i>Astronesthes gemmifer</i> Goode and Bean, 1896 | USNM 436537 | 151.7 | 55 | 133 | 35.5 | |
| <i>Borostomias antarcticus</i> (Lönnberg, 1905) | SIO 6145 | 201.4 | 55 | 133 | 35.5 | |
| <i>Heterophotus ophistoma</i> Regan and Trewavas, 1929 | SIO 70341 | 76.5 | 55 | 133 | 25.2 | |
| <i>Neonesthes capensis</i> (Gilchrist and von Bonde, 1924) | USNM 454448 | 153.4 | 55 | 133 | 35.5 | |
| <i>Aristostomias grimaldii</i> Zugmayer, 1913 | MCZ 93834 | 99.3 | 55 | 133 | 25.2 | |
| <i>Malacosteus niger</i> Ayres, 1848 | YPMICH 025708 | 175.40 | 55 | 200 | 100 | 000S21302 |

| | | | | | | |
|--|------------|--------|----|-----|------|-----------|
| <i>Chirostomias pliopterus</i> Regan and Trewavas, 1930 | MCZ 132709 | 157.7 | 55 | 133 | 35.5 | |
| <i>Echiostoma barbatum</i> Lowe, 1843 | UF 168937 | 290.00 | 60 | 200 | 39.1 | 51428 |
| <i>Flagellostomias boureei</i> (Zugmayer, 1913) | SIO 76107 | 178.1 | 55 | 133 | 35.5 | |
| <i>Grammatostomias</i> <i>flagellibarba</i> Holt and Byrne, 1910 | MCZ 164122 | 202.7 | 55 | 133 | 35.5 | |
| <i>Leptostomias gladiator</i> (Zugmayer, 1911) | MCZ 149495 | 105.1 | 55 | 133 | 35.1 | |
| <i>Melanostomias</i> <i>melanopogon</i> Regan and Trewavas, 1930 | MCZ 132243 | 149.5 | 55 | 133 | 35.5 | |
| <i>Odontostomias</i> <i>micropogon</i> Norman, 1930 | MCZ 132152 | 220.1 | 55 | 133 | 35.5 | |
| <i>Opostomias mitsuii</i> Imai, 1941 | OS014651 | 123.2 | 55 | 133 | 35.1 | |
| <i>Photonectes margarita</i> Regan and Trewavas, 1930 | MCZ132055 | 102.2 | 55 | 133 | 35.5 | |
| <i>Thysanactis dentex</i> Regan and Trewavas, 1930 | UW150245 | 81.15 | 55 | 143 | 28 | 000S27685 |
| <i>Trigonolampa miriceps</i> Regan and Trewavas, 1930 | MCZ165921 | 172.9 | 55 | 133 | 35.5 | |
| <i>Chauliodus sloani</i> Bloch and Schneider, 1801 | VIMS16520 | 222.47 | 70 | 200 | 72.3 | 424042 |

3.2.3 Ecological data and anatomical measurements

In order to investigate the relationship between ecological aspects and feed apparatus characteristics in skull-shape evolution among stomiiforms, we collected data on skull measurements and dentition traits. Additionally, we obtained data on diet and depth from the literature (BADCOCK, 1970; CLARKE, 1974; CLARKE, 1982; BADCOCK, 1984; GIBBS, 1984; SCHAEFER *et al.*, 1986; SCOTT; SCOTT, 1988; GON, 1990; PARIN; BORODULINA, 1990; QUÈRO *et al.*, 1990; HAROLD, 1994; SHINOHARA *et al.*, 1994; HOPKINS *et al.*, 1996; SUTTON; HOPKINS, 1996; MARQUES, 2001; WILLIAMS *et al.*, 2001; FIGUEIREDO *et al.*, 2002; HAROLD, 2002; BERNARDES *et al.*, 2005; MUNDY, 2005; FAHAY, 2007; KENALEY, 2007; GASKETT *et al.*, 2010; CARMO *et al.*, 2015; BATAGLIA *et al.*, 2018; ORLOV;

TOKRANOV, 2019; EDUARDO *et al.*, 2020; BANON *et al.*, 2021; MCGONAGLE *et al.*, 2023) (see additional details on Supplemental Table S3.1).

Diet was classified into guild 1, which includes zooplanktivorous fish, with diet focused on small crustaceans, especially euphausiids, ostracods, amphipods, and copepods; guild 2, which includes generalist stomiiforms, which feed on both crustaceans and fishes; and guild 3, which includes piscivorous stomiiforms, feeding primarily on teleosts, specially myctophids. Depth was classified into mesopelagic, which includes stomiiforms that inhabit depths up to 1,000 meters, and meso- to bathypelagic, which includes stomiiforms that inhabit depths below 1,000 meters.

Skull measurements were adapted from Buser *et al.* (2019) and defined as follow: skull length (anterior tip of premaxilla to supraoccipital crest), skull depth (quadrangular-angular joint to supraoccipital crest), upper jaw length (anterior tip of premaxilla to dorsoposterior point of maxilla), dentary length (anterior most to posterior most point of dentary) (Fig. 3.2). Measurements were used to calculate the proportional size of these structures. The size of these structures was labeled into three categories: short, moderate, and large.

Data on dentition was separated into three classes: tooth type, tooth size, and tooth attachment. Tooth type is related to the number of teeth on upper and lower jaws, and the interspace between them. This class was divided in three categories: tooth type 1, encompassing fishes that have several teeth, closely set on jaws, tooth type 2, encompassing fishes that have few teeth, widely spaced on jaws, and type 3, which include fishes that have several teeth, widely spaced on jaws. Tooth size was based on the size of the biggest premaxillary teeth, and also divided in three categories: small, moderate, and large, based on tooth proportion to head length. Type of tooth attachment on oral teeth was categorized according to Fink (1981) in the following types: type 1: fixed teeth, type 3: depressible teeth (teeth are hinged, with an anterior axis of rotation); type 1 and 3: both fixed and depressible teeth; and type 4: depressible teeth (teeth are hinged, with posterior axis of rotation). For more detail see Fink (1981) and Supplemental Fig.S3.1.

Species were also classified according to the clades in ranks of family and subfamily following Nelson (2006). Data was added in a classifier table to discriminate the samples. We use all this data to create a classifier table – sets of information that discriminate the samples. The classifiers table contains the following characters: clades,

diet, depth, head length, head depth, jaws length, tooth size, tooth type and tooth attachment.

3.2.4 Geometric morphometrics

A three-dimensional geometric morphometrics was performed in 3D slicer using sixteen paired landmarks and one unpaired landmark across the entire skull (neurocranium, suspensorium and jaws). Landmarks were captured on the image surface rendering using the “Markups” tool. The choice of landmarks was based on previous studies on feeding mechanism in fish in broad feeding guilds (*e.g.*, BUSER *et al.*, 2018; BUSER *et al.*, 2019), and adapted to the stomiiforms morphology (Table 3.2).

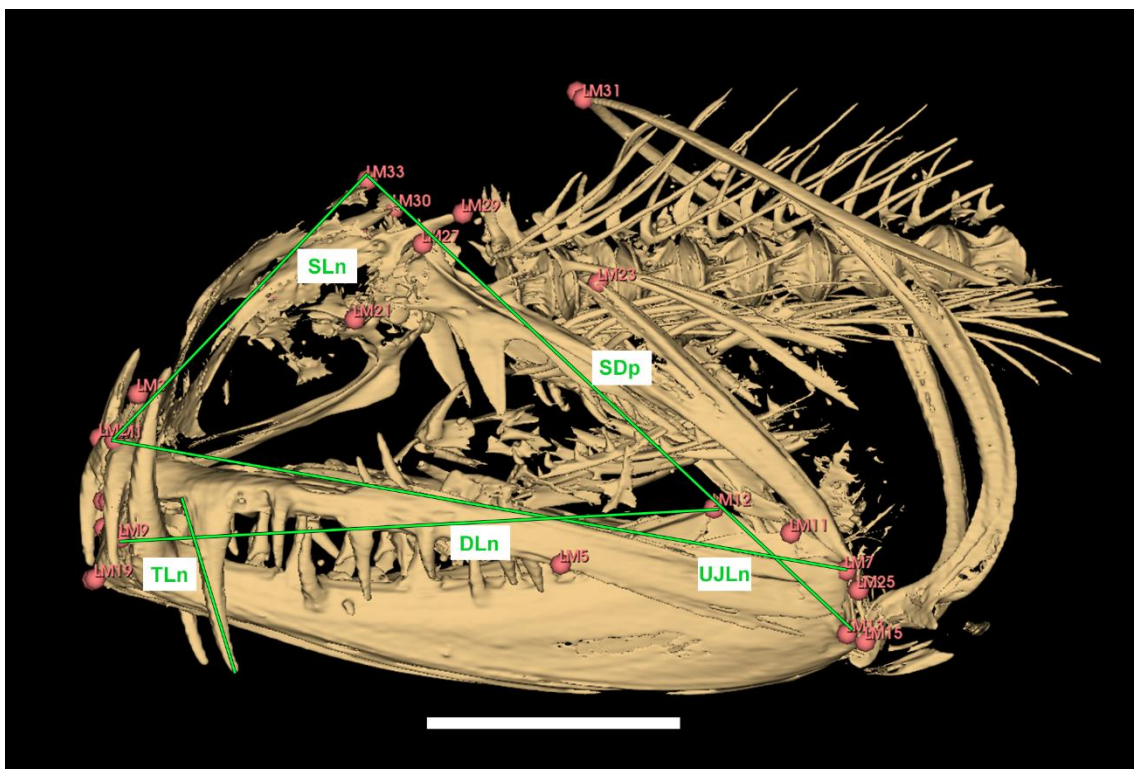


Figure 3.2. Selected 33 fixed landmarks (3D) in *Grammatostomias flagellibarba* (MCZ164122, 202.7mm SL, left lateral view, scale bar=10 mm) and measurements. SLn (LM1-LM33): skull length; SDp (LM14-LM33): skull depth; UJLn (LM1-LM7): upper jaw length; DLn (LM9-LM11): dentary length; TLn (anterior tooth base to tooth tip): tooth Length.

Each landmark was converted into Cartesian coordinates to generate a numerical matrix. The coordinate landmark data were exported in a fcsv file format for subsequent

shape analysis. All morphometric analyzes were performed in the R Core Team software (2023) using the geomorph package version 4.0.5 (ADAMS; OTÁROLA-CASTILLO, 2013). Data matrix was submitted to a Generalized Procrustes Analysis (GPA) to remove the effects of isometric size, position, and spatial orientation from the shape analysis (BOOKSTEIN, 1991).

Table 3.2. The 33 homologous landmarks, and their respectively descriptions.

| Landmarks | Description |
|------------------|--|
| LM1 and LM2 | Anterior-most point of the premaxilla |
| LM3 and LM4 | Dorsoposterior-most point of the ascending process of the premaxilla |
| LM5 and LM6 | Posterior most point of the premaxilla |
| LM7 and LM8 | Dorsoposterior-most tip of maxilla |
| LM9 and LM10 | Anterior-most point of the dentary |
| LM11 and LM12 | Dorsoposterior-most point of the dorsal margin of the dentary |
| LM13 and LM14 | Lowest point in the angular fossa where it articulates with the quadratus condyle to form the quadrangular-angular joint |
| LM15 and LM16 | Dorsoposterior-most point of the angular |
| LM17 and LM18 | Dorsal-most point of dentary at the symphysis |
| LM19 and LM20 | Ventral-most point of dentary at the symphysis |
| LM21 and LM22 | Postorbital process of the sphenotic |
| LM23 and LM24 | Dorsoposterior-most point of the preopercular bone |
| LM25 and LM26 | Ventroposterior-most tip of the preopercular bone |
| LM27 and LM28 | Posterior and medial-most point of hyomandibular |
| LM29 and LM30 | Postorbital process of the pterotic |
| LM31 and LM32 | Dorsal-most tip of the supracleithrum |
| LM33 | Dorsal and medial-most point of the supraoccipital crest |

3.2.5 Principal Component Analysis

The dataset underwent Principal Component Analysis (PCA) using the geomorph package version 4.0.5 (ADAMS; OTÁROLA-CASTILLO, 2013) and plotted with ggplot2 package (WICKHAM, 2016) to determine the principal axes, or principal components (PCs), representing skull shape variation among stomiiforms, with calculations based on the Euclidean distance measure. Environmental and anatomical skull traits (classifiers) were employed to discriminate the samples. Subsequently, the PCA results were visualized for each classifier, illustrating the negative and positive shape variations, utilizing the 'plotRefToTarget' function from geomorph.

3.2.6 Procrustes ANOVA and morphological disparity

In order to investigate potential differences between groups based on ecological and anatomical traits (classifiers), two types of analysis with permutation procedures (paired comparisons) were performed. First, the Procrustes ANOVA was conducted to assess statistical hypotheses describing patterns of shape variation and covariation for a set of Procrustes shape variables (skull shape vs classifiers). The 'procD.lm' function from the geomorph package was employed for this purpose, and we performed 500 iterations to test for potential allometric effects between skull shape and size. It is important to note that Procrustes ANOVA considers the overall form rather than individual and independent landmarks. Subsequently, morphological disparity was calculated using the 'morphol.disparity' function from the geomorph package to compare potential differences in the amount of skull shape variability among classifiers. These analyses were based on 1,000 permutations to ensure robustness and statistical rigor in our findings.

3.2.7 Phylogenetic patterns

The phylogenetic hypothesis based on the time-calibrated phylogenetic tree of ray-finned fish produced by Rabosky *et al.* (2018) and the stomiiforms tree from the Fish Tree of Life website (<https://fishtreeoflife.org/>) was incorporated to dataset. The Stomiiformes phylogeny from Rabosky *et al.* (2018) contains 99 species and is based on Bayesian analyses of 21 nuclear and mitochondrial genes sequences and time-calibrated using eight fossil taxa to estimate divergence times among stomiiforms. The phylogeny was integrated with scores from the PCA and used with phytools package version 1.5-1 (REVELL, 2012) in R to create a genus-level phylomorphospace (SIDLAUSKAS, 2008). For comparative analyses taxa missing in the dataset were trimmed from the phylogeny using the 'drop.tip' function from the ape package version 5.7-1 (PARADIS *et al.*, 2004) resulting in a tree for body shape analyses containing 27 of the 29 selected species (the species *Manducus maderensis* and *Opostomias mitsuui* are absent from the phylogeny). Species selected for the genus-level phylomorphospace are present in Supplemental Table S2.2. Phylogenetic signal of body-shape variation and centroid size was assessed using the 'physignal' function from phytools (ADAMS, 2014). The resulting Kmult statistic was compared to a null distribution generated from 1.000 random permutation tests using the average shapes of species.

3.2.8 Procrustes ANOVA and morphological disparity in a phylogenetic framework

Phylogenetic ANOVA for the averaged Procrustes shape variables was performed using the ‘procD.pgls’ function (geomorph) for 500 permutations testing for an allometric effect between body shape and specimen size and incorporating the Rabosky *et al.* (2018) phylogeny and the classifiers. Similarly, morphological disparity was calculated to compare potential differences in the amount of skull shape variability among classifiers in a phylogenetic context.

3.3 Results

3.3.1 Principal Component Analyses (PCA)

The first three PCA axes (Fig. 3.3) explain 73.52% of the total variability between individuals separating Stomiiformes based on skull shape. Axis 1 (41.05% of the variation) corresponds to variation related to skull length, skull depth, pre-operculum orientation, jaws length, and quadrato-mandibular joint. The negative PC1 values clearly separated species that exhibit a deep skull (large depth) with a prominent supra-occipital crest contacting posttemporal, and pre-operculum in a vertical position, from species with elongated skull (large skull length) with a short supra-occipital crest, large jaws, quadrato-mandibular joint posteriorly located, and a pre-operculum more diagonally oriented (anteriormost end located in an anterodorsal position, and posteriormost end in a posteroventral position) at positive PC1 values. The sternoptychids *S. pseudobscura* and *P. spinifer* represent the most extreme negative PC1 values and the stomiids *P. margarita*, *A. grimaldii* and *M. niger* represent the most extreme positive PC1 values (Fig. 3.4).

Axis 2 (21.38% of the variation) represents variation related to pre-operculum, pterotic and hyomandibular bones. The negative values of PC2 separated the species that have a shortened neurocranium, anteriorly in the skull, with short snout, short pterotic process, close to the dorsal most point of hyomandibular, and short occipital region from species with elongated neurocranium, posteriorly in the skull, with long pterotic process, slightly distant from the dorsal most point of hyomandibular, at positive PC2 values. The sternoptychine *S. pseudobscura* and the malacosteine *M. niger* occupy the most extreme

negative PC2, and the maurolicine *M. weitzmani* and the melanostomiine *L. gladiator* occupy the most extreme positive PC2 values (Fig. 3.4).

Axis 3 (11.09% of the variation) represents variation related to the premaxilla and dentary. The negative values of PC3 separated the species that have long premaxilla and long jaws from the species with short maxilla and dentary, at positive PC 3 values. The melanostomiine *P. margarita* represents the most extreme negative PC3 values and the gonostomatids *Z. pedaliotus* and *M. obtusirostra* represent the most extreme positive PC3 values (Fig.3.4).

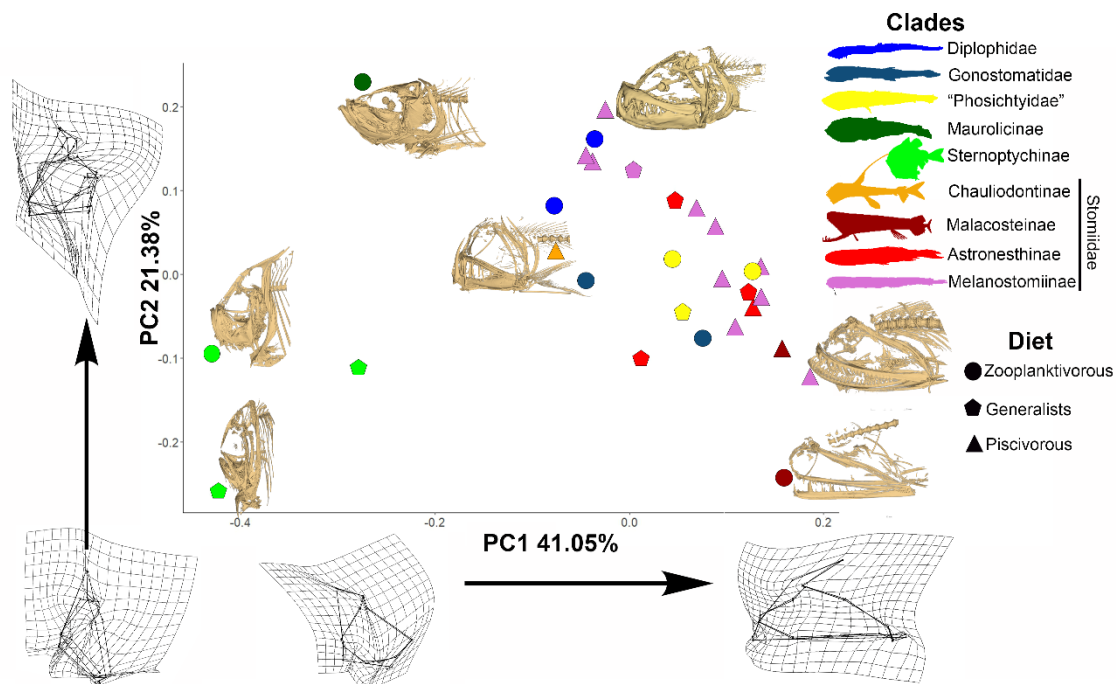


Figure 3.3. Overall shape variation of stomiiforms skull revealed by the first and second axes resulting from the Principal Component Analysis (PCA), ordinated by clades (represented in different colors) and diet (represented by geometric forms), and warp graphics of the extreme positive and negative species in the PC1 and PC2.

Stomiids and sternoptychids are the most diverse in terms of skull shape, occupying positive and negative PC1 and PC2 values (Fig. 3.3). Species exhibiting high disparity of skull shape, based on Procrustes variance, are the sternoptychids *S. pseudobscura*, *P. spinifer*, *M. weitzmani*, and *A. affinis*, and the malacosteine *M. niger*, with respectively Procrustes distance values 0.528, 0.473, 0.3989, 0.3276, and 0.3454. Most piscivorous and generalists stomiiforms cluster in the same positive PC1 values. Piscivorous stomiiforms are represented mostly by the dragonfish (Stomiidae), except the

astronesthines, while generalists include the astronesthines, the lightfish *Phosichthys argenteus* and the hatchetfishes *Sternoptyx pseudobscura* and *Argyropelecus affinis* (Fig.3.3).

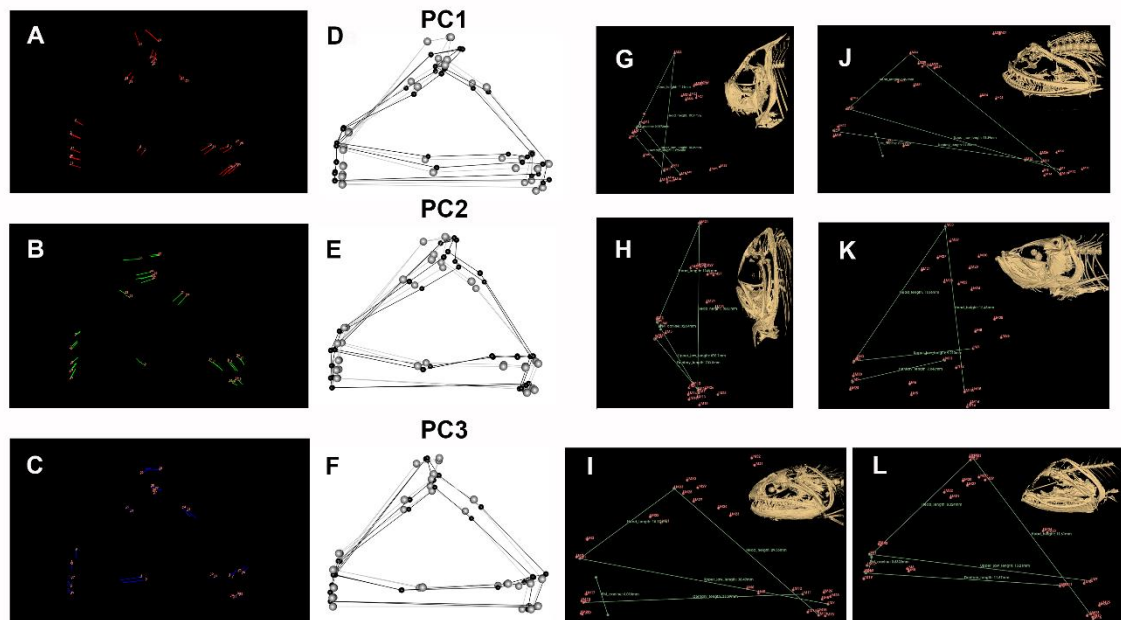


Figure 3.4. Landmarks variation in PC1 (A, D, G, J), PC2 (B, E, H, K), and PC3 (C, F, I, L). The size of the vectors in A-C represents the landmarks variation in PC 1, PC2 and PC 3, respectively. The interpolation between extreme negative and positive stomiiforms shape in PC1, PC2 and PC3 according to the landmark's variation is represented in D-F. G-L represents landmarks, measures, and skull images from extreme and positive stomiiforms shapes in PC1, PC2 and PC3. G=*Polyipnus*; J=*Photonectes*; H=*Sternoptyx*; K=*Maurolicus*; I=*Echiostoma*; L=*Zaphotias*.

The hatchetfishes (*Sternoptychinae*) and the gonostomatid *Margrethia obtusirostra* have considerable dimensions in both skull length and skull depth, and they clustered around the negative values of PC1 and PC2 (Fig 3.5A, B). In contrast, most dragonfishes, bristlemouths and lightfishes have these anatomical traits varying from short to moderate, clustering around the positive PC1, and both positive and negative PC2 values. The malacosteines (loosejaws), have a different pattern found in the other stomiids, presenting large skull depth (the largest within stomiids), and short skull length, especially *Malacosteus niger*, which represents one of the most extreme values of positive PC1 and negative PC2 (Fig. 3.5A, B). Among stomiiforms, dragonfishes exhibit the most considerable variation in jaw length, with certain species displaying exceptionally large jaws, exemplified by the loosejaws *Malacosteus* and *Aristostomias*, the melanostomiines *Photonectes*, *Grammatostomias*, and *Echiostoma*. Conversely, other dragonfishes,

specifically the melanostomiines, feature short and robust jaws, such as *Leptostomias*, *Odontostomias*, *Opostomias* and *Thysanactis* (Fig. 3.5C).

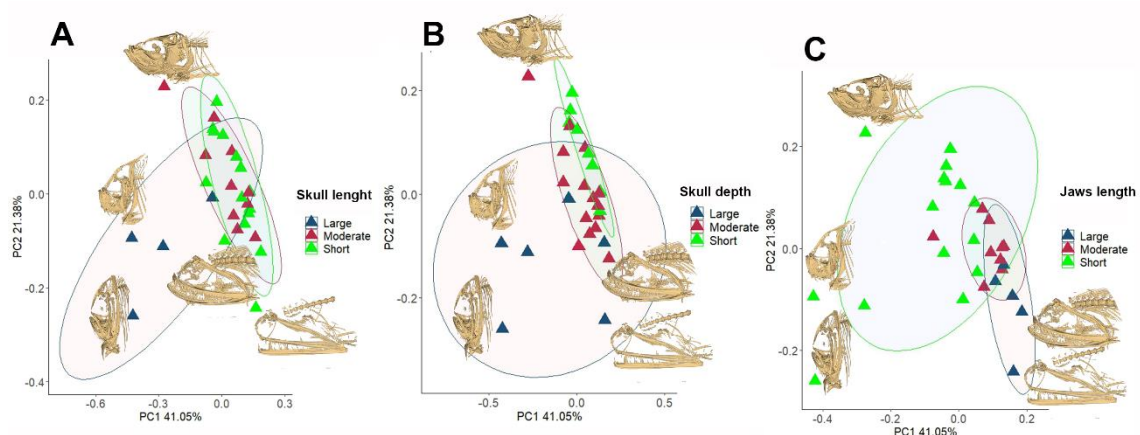


Figure 3.5. Overall shape variation of stomiiforms skull revealed by the first and second axes resulting from the Principal Component Analysis (PCA), ordinated by skull length (A), skull depth (B), and jaws length (C).

3.3.2 Morphological disparity and Procrustes ANOVA

The results on the morphological disparity demonstrate that the sternoptychids *Sternoptyx*, *Polyipnus*, *Maurolicus* and *Argyropelecus*, and the loosejaw *Malacosteus* are the species that exhibit substantial differences in skull shape within stomiiforms, with high Procrustes variance (0.26648268, 0.21605051, 0.15553753, 0.10616412, and 0.10616412, respectively), but only *Sternoptyx* exhibits significant morphological in comparison to other stomiiforms, except for the sternoptychids and *Malacosteus*. There is significant morphological disparity between skull shape and size, considering skull length and skull depth ($p < 0.05$), however, only for large skull length and large skull depth categories (Table 3.3). Large skull length present in sternoptychines and in the gonostomatid *M. obtusirostra* demonstrate the higher morphological disparity in comparison with moderate and short skull length present in other stomiiforms. Similarly, large skull depth presents in the sternoptychines, and the gonostomatid *M. obtusirostra*, and in the malacosteines *A. grimaldii* and *M. niger* demonstrate the higher morphological disparity in comparison with moderate and short skull depth present in the other stomiiforms. In contrast, there is no significant morphological disparity between shape and the other anatomic traits (jaws length, tooth size, tooth type, and tooth attachment) as

well diet, and depth ($p>0.05$) (Table 3.3). Although morphological disparity was significant only for skull length and skull depth, Procrustes ANOVA, exhibits a different pattern. Procrustes ANOVA demonstrate significant ($p<0.05$) for all classifiers considering the shape, and for skull length and skull depth considering centroid size (CS) (Table 4.4).

3.3.3 Phylomorphospace, phylogenetic signal and phylogenetic ANOVA

In most cases, closely related genera within families and subfamilies tended to cluster closer together in the morphospace, with less related taxa being more morphologically divergent. However, the melanostomiines shows a broad distribution in the phylomorphospace, with taxa presenting large skull depth and large jaws clustering with malacosteines and phosichthyids, and taxa presenting short jaws clustering with the viperfish *Chauliodus* (Chauliodontinae), with the diplophid *Diplophos* and with the gonostomatid *Margrethia*. There is a significant and high phylogenetic signal in skull shape ($K= 1.029$, $p=0.003$) and centroid size ($K= 1.553$, $p= 0.06$) among stomiiforms (Supplemental Figs. S3.2A, B). The phylogenetic signal for the first axis was 2.219818, and for the second axis was 1.712965.

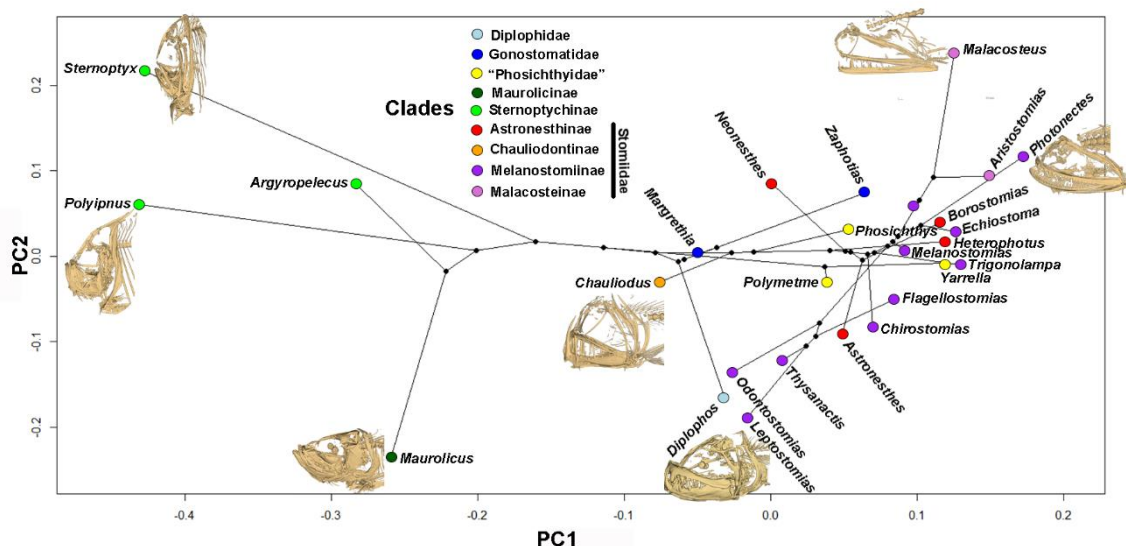


Figure 3.6. Genus-level phylomorphospace of the main axes resulting from the Principal Component Analysis (PCA) of the Order Stomiiformes combined with the time-calibrated phylogeny of Rabosky *et al.*, 2018, color coded by stomiiform taxonomic groups.

When including the phylogeny, morphological disparity results are quite similar to that without phylogeny, remaining significant only for skull length, skull depth and jaws length, and not significant for clades and, depth and diet ($p > 0.05$) (Table 3.3). These results mean that morphological disparity is similar with and without accounting for phylogeny. In contrast, the Procrustes ANOVA shows a different pattern considering the phylogeny. When accounting for phylogeny, the relationship between skull shape and classifiers is significant only for clades, skull length, skull depth, and jaws length, contrarily to the results without phylogeny, which are significant for all anatomical and ecological traits (Table 3.4).

Table 3.3. Stomiiforms Procrustes variance and P -values from the morphological disparity test by ecological and anatomical traits without and accounting phylogeny. Asterisks represent significant morphological disparity.

| Ecological traits | Without phylogeny | | With phylogeny | |
|---------------------------|-------------------|-----------------------|----------------|-----------------------|
| | PV | P -values | PV | P -values |
| Diet: Generalists | 0.073 | 0.387 / 0.912 | 0.067 | 0.438 / 0.727 |
| Diet: Piscivorous | 0.049 | 0.387 / 0.319 | 0.044 | 0.438 / 0.233 |
| Diet: Zooplanktivorous | 0.076 | 0.912 / 0.319 | 0.078 | 0.727 / 0.233 |
| Depth: Meso-bathypelagic | 0.056 | 0.175 | 0.051 | 0.081 |
| Depth: Mesopelagic | 0.089 | 0.175 | 0.096 | 0.081 |
| Skull length: Short | 0.045 | 0.862 / 0.002* | 0.041 | 0.782 / 0.004* |
| Skull length: Moderate | 0.050 | 0.001* / 0.862 | 0.047 | 0.010* / 0.782 |
| Skull length: Large | 0.158 | 0.001* / 0.002* | 0.148 | 0.010* / 0.004* |
| Skull depth: Short | 0.043 | 0.972 / 0.002* | 0.125 | 0.919 / 0.005* |
| Skull depth: Moderate | 0.044 | 0.972 / 0.001* | 0.041 | 0.1919 / 0.003* |
| Skull depth: Large | 0.136 | 0.002* / 0.001* | 0.038 | 0.003* / 0.005* |
| Jaws length: Short | 0.077 | 0.101 / 0.853 | 0.077 | 0.072 / 0.631 |
| Jaws length: Moderate | 0.035 | 0.101 / 0.328 | 0.031 | 0.367 / 0.072 |
| Jaws length: Large | 0.069 | 0.328 / 0.853 | 0.060 | 0.367 / 0.631 |
| Tooth type: Type 1 | 0.082 | 0.087 / 0.921 | 0.080 | 0.089 / 0.907 |
| Tooth type: Type 2 | 0.043 | 0.087 / 0.225 | 0.040 | 0.089 / 0.328 |
| Tooth type: Type 3 | 0.087 | 0.921 / 0.225 | 0.075 | 0.907 / 0.328 |
| Tooth size: Small | 0.086 | 0.104 / 0.068 | 0.082 | 0.077 / 0.090 |
| Tooth size: Moderate | 0.040 | 0.068 / 0.964 | 0.037 | 0.991 / 0.077 |
| Tooth size: Large | 0.042 | 0.964 / 0.104 | 0.037 | 0.991 / 0.090 |
| Tooth attachment: 1 | 0.067 | 0.384 / 0.657 / 0.096 | 0.066 | 0.287 / 0.531 / 0.120 |
| Tooth attachment: 1 and 3 | 0.041 | 0.384 / 0.818 / 0.071 | 0.036 | 0.287 / 0.851 / 0.098 |
| Tooth attachment: 3 | 0.049 | 0.657 / 0.818 / 0.088 | 0.043 | 0.531 / 0.851 / 0.116 |
| Tooth attachment: 4 | 0.155 | 0.096 / 0.071 / 0.088 | 0.147 | 0.120 / 0.098 / 0.116 |

Table 3.4. Stomiiforms ANOVA F and P values of means for body shape and centroid size (CS) tested by Species, Clades, Depth, Diet, Skull length, Skull depth, Jaws length,

Tooth type, Toot size, and Tooth attachment. Asterisks represent significant differences ($p \leq 0.05$).

| Classifiers | Without phylogeny | | | | With phylogeny | | | |
|-------------------------|-------------------|--------|---------------|-------|----------------|---------|---------------|-------|
| | Shape | | Centroid Size | | Shape | | Centroid Size | |
| | F | P | F | P | F | P | F | P |
| Species | 0 | 0.002* | 0 | 1 | 0 | 0.744 | 0 | 0.573 |
| Clades | 4.94 | 0.03* | 2.32 | 0.212 | 1.62 | 0.032 * | 1.08 | 0.386 |
| Depth | 3.11 | 0.01* | 4.28 | 0.216 | 0.53 | 0.81 | 2.87 | 0.086 |
| Diet | 2.75 | 0.002* | 1.34 | 0.524 | 0.61 | 0.798 | 0.41 | 0.614 |
| Skull length | 3.41 | 0.004* | 3.73 | 0.01* | 3.47 | 0.002 * | 3.13 | 0.068 |
| Skull depth | 2.88 | 0.002* | 2.06 | 0.01* | 2.09 | 0.034 * | 1.34 | 0.372 |
| Jaws length | 3.46 | 0.02* | 0.44 | 0.706 | 1.73 | 0.05 * | 0.30 | 0.718 |
| Tooth type | 2.36 | 0.002* | 0.69 | 0.196 | 1.75 | 0.086 | 0.72 | 0.352 |
| Tooth size | 2.73 | 0.02* | 0.51 | 0.696 | 1.34 | 0.18 | 0.04 | 0.92 |
| Tooth attachment | 2.29 | 0.002* | 0.18 | 0.618 | 2.02 | 0.04 | 0.02 | 0.992 |

3.4 Discussion

Results obtained in this study demonstrate considerable variation in skull shape among Stomiiformes, with the first three principal components describing the variation in skull shape associated with skull measurements, and ecological data (diet and depth). Overall, all stomiiform clades vary in terms of skull shape, but morphological disparity is higher in Stomiidae and in Sternoptychidae. However, a few substantial differences were observed. Significant morphological disparity in skull shape is mainly related to skull length and skull depth, with the main axis of total variation describing a transition from a deep skull, with large skull length and depth, represented by the hatchetfishes, to an elongated skull, and large jaws, represented by the loosejaws and by the dragonfish *Photonectes margarita*.

Hatchetfishes (Sternoptychinae) possess a well-developed frontal region of the neurocranium, characterized by large, vertically oriented frontal bones and the presence of parietals (which are absent in most stomiids) (FINK, 1985) (Supplemental Fig.S3.5). Additionally, hatchetfishes display a developed supra-occipital crest, with the posttemporal in contact with this crest. This enlarged supraoccipital region is believed to be linked to overall body depth, as the supraoccipital serves as the anterior origin of the epaxial musculature of the body (ALEXANDER, 1964; WINTERBOTTOM, 1974). Moreover, functional demands influencing the evolution of muscles involved in locomotion (WINTERBOTTOM, 1974) may also influence the shape of the

neurocranium, thus showing a relationship with body depth. Despite the fact that stomiiforms employ a carangiform swimming mode (SCHNELL *et al.*, 2023), with lateral undulation primarily restricted to the caudal area, and are characterized as BCF swimmers (FRIEDMAN *et al.*, 2021), relying on undulatory movements of the body and caudal fin (WEBB, 1984; SFAKIOTAKIS *et al.*, 1999), the well-developed anterior epaxial musculature in hatchetfishes likely plays a significant role in the undulatory locomotion.

Neurocranium shape plays a central role in determining the positioning of other cranial bones, consequently affecting the configuration and dimensions of cranial muscles and the biomechanics ruling feeding and ventilation (ALEXANDER, 1964; WINTERBOTTOM, 1974). For instance, neurocranium shape influences the positioning of the suspensorium, which articulates with the lower jaw, as well as the spatial arrangement of the upper jaw, opercular series, and gill arches (BOYLE; HERREL, 2018). It is possible to observe the relationship between neurocranium shape and other cranial bones examining the results, especially the skull shape of taxa exhibiting extreme morphologies, such as *Photonectes* (Melanostomiine), *Malacosteus* (Malacosteinae) and *Sternoptyx* (Sternoptychinae). In *Photonectes* and *Malacosteus*, the frontal region of the neurocranium exhibits a reduced length, with the elongation of the skull being linked to the elongation of the suspensorium and jaws. The observed shortened anterior neurocranium accompanied by elongated jaws in these species bears resemblance to the condition found in Chiasmodontidae, where the elongation of the jaws is believed to be associated with the evolution of a posterior-located quadrato-mandibular joint (KNAPP *et al.*, 2023). This correspondence aligns with our findings, as the landmarks related to the quadrato-mandibular joint exhibited one of the highest variations in PC1 (Figs. 3.5A, D, G, J).

Specifically, malacosteines and certain melanostomiines display positive values in PC1 (Fig. 3.3), indicating the presence of a posterior-oriented quadrato-mandibular joint in these taxa. Additionally, the supra-occipital crest in these taxa is poorly developed, and lacks contact with the posttemporal. The anterior region of the epaxial musculature in these stomiids appears to be less developed compared to hatchetfishes, potentially contributing to an increase in gape capability. These characteristics in stomiids are reflective of their dietary preferences and award an advantage in the mechanism of mouth gape. Notably, the lack of ossification in this region may facilitate an enhanced mouth gape. Furthermore, stomiids exhibit certain modifications in the post-cranial skeleton: certain taxa, like *Chauliodus* lacks the first vertebral centra, while *Malacosteus* exhibits

an occipito-vertebral gap (SCHNELL *et al.*, 2010) (Supplemental Fig.S3.9). This anatomical feature allows for a mouth opening angle exceeding 100° (KENALEY, 2012), due to a lack of a functional head joint, facilitating the capture of large prey in most stomiids (SCHNELL; JOHNSON, 2017) or the creation of a more efficient suction mechanism in the case of *Malacosteus*, which primarily feeds on small copepods (SUTTON; HOPKINS, 1996).

The results obtained here also describe, in the second axis of the PCA, a transition from a shortened neurocranium, anteriorly in the skull, with short snout, short pterotic process, close to the dorsal most point of hyomandibular, and short occipital region, to an elongated neurocranium, posteriorly in the skull, with long pterotic process, slightly distant from the dorsal most point of hyomandibular. Species at extreme negative PC2 values, *Sternoptyx pseudobscura* (Sternoptychinae) and *Malacosteus niger* (Malacosteinae) (Fig.3.3), drastically vary in terms of skull length, with the former having a deep skull with short jaws, and the latter having an extremely large skull, and large jaws. Notability, they share a short occipital region, with reduction of contrasting with the elongation of other skull regions (the frontal region and supra-occipital crest in hatchetfishes, and jaws and suspensorium in malacosteines e melanostomiines). Therefore, is assumed that sternoptychines and stomiids, mostly in malacosteines, may have evolved a short occipital region by evolving elongation of other skull regions. In contrast, species at extreme positive PC2 values – *Maurolicus weitzmani* (Maurolicinae) and *Leptostomias gladiator* (Melanostomiine), present a somewhat elongated neurocranium, but with a moderate to short skull length and skull depth, and short, heavy jaws. Melanostomiines clustered close to *Leptostomias* also shared with this taxon a similar head shape, with a long hyomandibular process, compared to other dragonfishes, and consequently the opercular bone posteriorly on the skull (Supplemental Fig.S3.7). Besides, these melanostomiines also have the quadrato-mandibular joint anterior-oriented, contrary to the pattern observed in other melanostomiines, such as *Photonectes* (Supplemental Fig.S3.8), and in the malacosteines.

By analyzing the time-calibrated phylogeny of stomiiforms (Fig. 3.7), it is possible to observe a transition from relatively short skulls with moderate jaw length present in diplophids, gonostomatids, and some phosichthyids and maurolicines, to larger skulls with prominent jaws predominantly found in the derived family Stomiidae.

Generally, elongated-bodied stomiiforms also exhibit elongated skulls, as evident from chapter two. The combination of an elongated body and skull is a hallmark of the

family Stomiidae. Stomiids belonging to the subfamilies Idiacanthinae, Melanostomiinae, and Malacosteinae have distinctive features such as highly elongated bodies, posteriorly positioned dorsal and anal fins, elongated skull with an occipito-vertebral gap, and large jaws with depressible teeth (FINK, 1985; SUTTON; HOPKINS, 1996, SCHNELL *et al.*, 2010). Malacosteines further exhibit other derived traits, including highly elongated jaws and the absence of membranes forming the mouth floor (FINK, 1985).

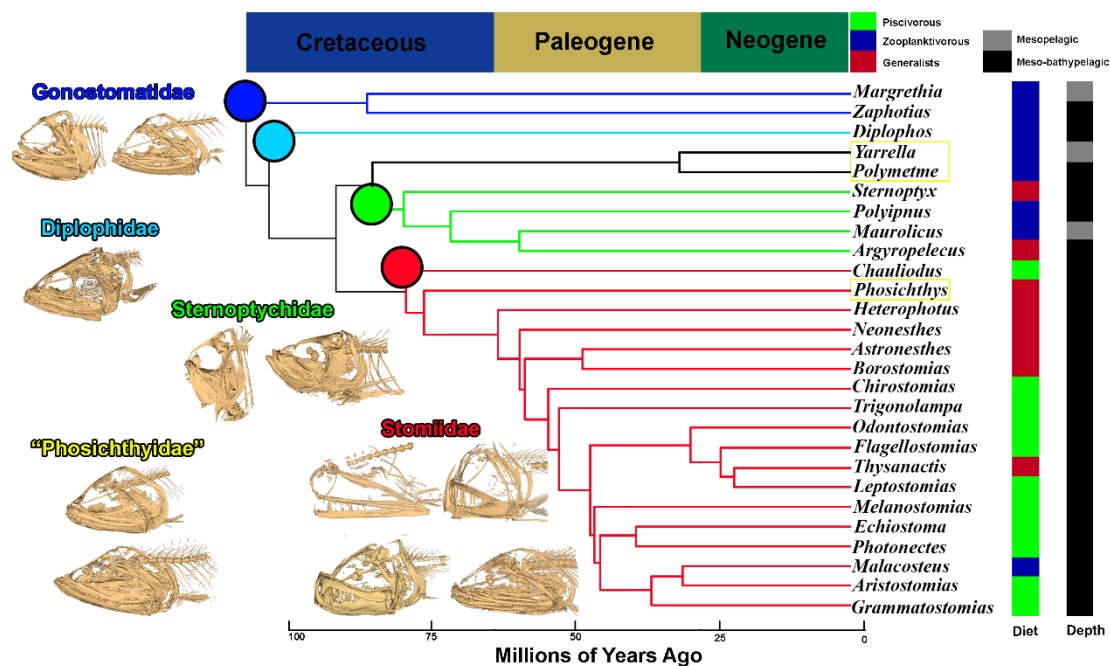


Figure 3.7. Time-calibrated phylogenetic tree of the Stomiiformes, adapted from Rabosky *et al.* (2018), with the ecological traits of diet (zooplanktivorous; generalists; or piscivorous), and depth distribution (mesopelagic; or meso-bathypelagic) indicated. The color code on the cladogram indicates families.

Stomiiforms presenting extreme morphologies, such as *Sternoptyx* and *Malacosteus* share other morphological similarities, in addition to the short neurocranium, such as the large eye size. The presence of major sensory organs supported by the neurocranium suggests a strong association between the morphological features related to these sense organs and the ecological traits of taxa, such as habitat characteristics and prey type (BOYLE; HERREL, 2018). For example, nocturnal fishes and some deep-sea fish are thought to have larger eye diameters to increase sensitivity to low-light conditions (GOATLEY; BELLWOOD, 2009; SCHMITZ; WAINWRIGHT, 2011). *Sternoptyx* and *Malacosteus* inhabit meso- to bathypelagic regions, and do not perform diel vertical migration, hunting mainly on small crustaceans. Consequently, stomiiform neurocranium

shape may vary with the relative size of sense organs and also with functional aspects of feeding and prey handling. Besides, the evolution of extreme morphologies may be closely associated with the correlated evolution of neurocranial elements.

In this study, a significant correlation between skull shape and ecological aspects was supported only by Procrustes ANOVA analysis, but not for morphological disparity analysis, which means that these factors are unlikely to play a major role in shaping its morphological evolution. These results contrast with those found in chapter one, whereas we found a significant morphological disparity between stomiiforms body shape and these same ecological traits, when excluding phylogeny. However, when accounting for phylogeny, the morphological disparity disappears, which means that ecological traits play a secondary role in stomiiforms body shape evolution. In this study, there was no significant morphological disparity even without phylogeny, indicating that ecological traits (at least diet and depth) seem to not influence the skull shape evolution.

A lack of correlation between skull shape and ecological traits was also observed in a study with fishes of the clade Pelagiaria (scombriforms, stromateoids and chiasmodontids, among others) (KNAPP *et al.*, 2023), which mostly inhabit the open ocean. Contrarily, fish that live in the shallow/coastal habitats exhibit distinct skull morphologies associated with several feeding modes (CORN *et al.*; 2022; LAROUCHE *et al.*, 2022). Besides, a recent study on the ecomorphology of Melanostomiinae revealed a lack of morphological-dietary relationships amongst such taxa, with dietary diversity much lower than morphological diversity (MCGONALE *et al.*, 2023), suggesting that other ecological traits might be the main drivers for specialization in deep pelagic waters.

The few substantial differences between skull morphological disparity in stomiiforms were also reported in pelagiarians considering the neurocranium morphology (KNAPP *et al.*, 2023). This pattern may be a result of a limitation by the structural requirements for suction feeding, which is the far more common than biting as a way to capture prey in aquatic environments (COLLAR *et al.*, 2014), being the feeding mode exhibited by stomiiforms (TCHERNAVIN, 1953). In suction feeders, there is a dependence among skull structures, known as functional integration (BOCK; VON WAHLERT, 1965). Conversely, in the biting behavior, integration between certain elements of neurocranium and splanchnocranium may weaken, leading to a remarkable diversification of skull shapes (COLLAR *et al.*, 2014). While skull shape seems not to be primarily influenced by ecological traits, at least in stomiiforms, body shape evolution of deep-sea fish seems to go in a contrary direction. Recent studies focused on body shape,

such as that of Maile *et al.* (2020) with lizardfishes, and Martinez *et al.* (2021) with several deep-sea fish lineages, found high body shape disparity with the depth increase, driven by traits associated with locomotion (MARTINEZ *et al.*, 2021). Considering all these previous studies, it is reasonable to hypothesize that deep-sea environments are hotspots for body shape diversification, but not necessarily for skull shape diversification.

There is a significant and high phylogenetic signal observed within the stomiiforms ($K=1.029$ for shape and $K=1.553$ for size), indicating that closely related taxa tend to exhibit closer clustering in the phylomorphospace. This pattern can be attributed to shared evolutionary history, as less related taxa show higher morphological divergence (Fig. 3.6). The phylogenetic signal was quantified using the K value proposed by Blomberg *et al.* (2003), where K values equal to or greater than 1 ($K \geq 1$) are considered indicative of a high phylogenetic signal. In this context, a high phylogenetic signal is typically associated with neutral evolution or genetic drift (KAMILAR; COOPER, 2013), and is also interpreted as evidence of evolutionary conservatism, where species tend to retain their ancestral traits (LOSOS, 2008). Conversely, a low phylogenetic signal suggests higher rates of trait evolution (BLOMBERG *et al.*, 2003), leading to notable differences among closely related species.

A significant phylogenetic signal was also obtained in a study on skull evolution of pelagiarian fishes although, contrarily to the stomiids, in Pelagiaria the phylogenetic signal was weak (KNAPP *et al.*, 2023), which was interpreted as a substantial degree of phenotypic convergence, suggesting that neurocranium shape in pelagiarians tends to evolve within these somewhat restricted regions of the morphospace.

Results obtained herein demonstrate that the evolution of skull shape in stomiiforms is driven mainly by the phylogeny of the group, with ecological traits like diet playing a secondary role in the diversification and morphological disparity within this group in the deep pelagic environments. A similar result was obtained in chapter two regarding the body shape, but with a more significative correlation between body shape and ecological traits without a phylogenetic context. While the open ocean is assumed to be a complex habitat that promotes phenotypic diversity considering several marine lineages (MARTINEZ *et al.*, 2021), certain lineages, such as stomiiforms and pelagiarian fishes are more conservative in terms of skull morphologies (KNAPP *et al.*, 2023), with the lesser importance of diet in the skull shape evolution, assumed to be related to the relative few feeding modes in comparison with the shallow waters, whereas certain feeding modes are related to the diversification of skull shape.

3.5 Conclusion

This study focused on the skull shape disparity within the Stomiiformes, revealing high overall shape disparity, but a limited number of distinct morphotypes. The findings indicate that the evolution of extreme morphologies in Stomiiformes may be linked to the correlated evolution of neurocranial elements. Notably, variations in skull shape are predominantly associated with skull length and skull depth, with specific regions of the neurocranium, particularly the occipital region, showing significant involvement. Landmarks exhibiting greater variation are primarily related to the occipital region, suspensorium, and preopercle.

Contrary to the expectations, there is no significant correlation between skull shape and ecological aspects such as depth and diet, even without a phylogenetic context. This contrasts with the significant correlation found in chapter two between body shape and these ecological traits without a phylogenetic context. Among the Stomiiformes clades, the Stomiidae family, represented by dragonfishes and their allies, display the most diverse range of skull shapes, occupying a substantial portion of the morphospace. Conversely, the Sternoptychidae family, encompassing hatchetfishes and their relatives, exhibit the highest variation in skull shape and demonstrated considerable disparity in head shape.

These findings shed light on the intricate patterns of shape variation within the Stomiiformes group, highlighting the potential influence of neurocranial elements on the evolution of extreme morphologies observed in species with high Procrustes Variance, such as the hatchet fish *Sternoptyx* and the loosejaw *Malacosteus*. Furthermore, the contrasting patterns observed in the correlation between skull shape and ecological traits compared to body shape emphasize the complex nature of morphological adaptations in this group. The diversification of skull shapes within the Stomiidae and Sternoptychidae families underscores their respective contributions to the overall skull shape disparity in Stomiiformes.

There is a phylogenetic signal in skull shape among stomiiforms, with closely related taxa tending to cluster together in phylomorphospace, as the result of shared evolutionary history. The phylogenetic signal is also high and often interpreted as evolutionary or phylogenetic conservatism. This is the first study to quantitatively investigate changes in the skull shape of stomiiforms, concluding that their skull shape evolution is explained mostly by the phylogeny of the group. Ecological traits, such as

diet and depth play a minor role in the diversification and morphological disparity within stomiiforms skull in the deep pelagic environments. However, there are other hypotheses that could explain our results. It is possible that ecological plays a role in stomiiforms skull shape diversification only during the early evolution of the group, or the selected data was not able to detect the significance of ecology in the taxon sampling chosen here. Finally, limitations on the chosen ecological traits might result in the lack of the statistical power to detect any pattern.

3.6 References

- Adams, D. C.** 2014. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Systematic Biology* 63: 685–697.
- Adams, D. C., and E. Otárola-Castillo.** 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* 4: 393–399. <https://doi.org/10.1111/2041-210X.12035>.
- Alexander, R. M.** 1964. Adaptation in the skulls and cranial muscles of South American characinoid fish. *Zoological Journal of the Linnean Society* 45: 169–190.
- Angel, M. V.** 1989. Vertical profiles of pelagic communities in the vicinity of the Azores Front and their implications to deep ocean ecology. *Progress in Oceanography* 22 (1): 1–46, 1989.
- Angel, M. V.** 1997. What is the deep-sea? Pp. 2–41. In: RANDALL, D. J.; FARRELL, A. P. (eds.). *Deep-Sea Fishes*. San Diego, Academic Press.
- Badcock, J.** 1970. The vertical distribution of mesopelagic fishes collected on the SOND cruise. *Journal of the Marine Biological Association of the United Kingdom* 50 (4): 1001-1044. <https://doi.org/10.1017/S0025315400005920>.
- Badcock, J.** 1984. Gonostomatidae. In: WHITEHEAD, P. J. P.; BAUCHOT, M. L.; HUREAU, J. C; NIELSEN, J.; TORTONESE, E. (eds.). *Fishes of the north-eastern Atlantic and the Mediterranean*. v. 1. UNESCO, Paris, p. 284–301.
- Bañón, R., A. de Carlos, C. Farias, N. Vilas-Arrondo, and F. Baldó.** 2021. Exploring deep-sea biodiversity in the Porcupine Bank (NE Atlantic) through fish integrative taxonomy. *Journal of Marine Science and Engineering* 9 (10): 1075.

- Battaglia, P., G. Ammendolia, V. Esposito, T. Romeo, and F. Andaloro.** 2018. Few but relatively large prey: trophic ecology of *Chauliodus sloani* (Pisces: Stomiidae) in deep waters of the Central Mediterranean Sea. *Journal of Ichthyology* 58: 8–16.
- Bernardes, R. Á. et al.** 2005. Peixes da zona econômica exclusiva da região sudeste-sul do Brasil: levantamento com armadilhas, pargueiras e rede de arrasto de fundo. Edusp, São Paulo, 295 p.
- Betancur-R, R. et al.** 2017. Phylogenetic classification of bony fishes. 2017. **BMC Evolutionary Biology** 17 (1) p. 162. <http://doi.org/10.1186/s12862-017-0958-3>.
- Blomberg, S. P., T. Garland, and A. R. Ives.** 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717–745. <http://doi.org/10.1111/j.0014-3820.2003.tb00285.x>.
- Bock, W. J., and G. von Wahlert.** 1965. Adaptation and the form-function complex. *Evolution* 19: 269–299.
- Bookstein, F. L.** 1991. Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge. 435 p.
- Boyle, K. S., and A. Herrel.** 2018. Neurocranium shape variation of piranhas and pacu (Characiformes: Serrasalminidae) in association with ecology and phylogeny. *Biological Journal of the Linnean Society* 125 (1): 93–114.
- Buser, T. J., B. L. Sidlauskas, and A. P. Summers.** 2018. 2D or not 2D? Testing the utility of 2D vs. 3D landmark data in geometric morphometrics of the sculpin subfamily Oligocottinae (Pisces; Cottoidea). *The Anatomical Record* 301 (5): 806–818.
- Buser, T. J., D. L. Finnegan, A. P. Summers, and M. A. Kolmann.** 2019. Have niche, will travel. New means of linking diet and ecomorphology reveals niche conservatism in freshwater cottoid fishes. *Integrative Organismal Biology* 1 (1): obz023.
- Buser, T. J., O. F. Boyd, Á. Cortés, C. M. Donatelli, M. A. Kolmann, J. L. Luparell, J. A. Pfeiffenberger, B. L. Sidlauskas, and A. P. Summers.** 2020. The natural historian's guide to the CT galaxy: step-by-step instructions for preparing and analyzing computed tomographic (CT) data using cross-platform, open access software. *Integrative Organismal Biology* 2 (1): obaa009.
- Carmo, V., T. Sutton, G. Menezes, T. Falkenhaus, and O.A. Bergstad.** 2015. Feeding ecology of the Stomiiformes (Pisces) of the northern Mid-Atlantic Ridge. 1. The Sternoptychidae and Phosichthyidae. *Progress in Oceanography* 130: 172–187.

- Carnevale, G., and A. Rindone.** 2011. The teleost fish *Paravinciguerra praecursor* Arambourg, 1954 in the Cenomanian of north-eastern Sicily. *Bollettino della Società Paleontologica Italiana* 50, (1): 1–10. ISSN 0375-7633.
- Childress, J. J., and M. H. Nygaard.** 1973. The chemical composition of midwater fishes as a function of depth of occurrence off southern California. *Deep Sea Research and Oceanographic Abstracts* 20 (12): 1093–1109.
- Clarke, T. A.** 1974. Some aspects of the ecology of stomioid fishes in the Pacific Ocean near Hawaii. *Fishery Bulletin* 72 (2): 337–351.
- Clarke, T. A.** 1982. Feeding habits of stomioid fishes from Hawaiian waters. *Fishery Bulletin* 80: 287–304.
- Cocker, J. E.** 1978. Adaptations of deep-sea fishes. *Environmental Biology of Fishes* 3: 389–399.
- Collar, D. C., P. C. Wainwright, M. E. Alfaro, L. J. Revell, and R. S. Mehta.** 2014. Biting disrupts integration to spur skull evolution in eels. *Nature communications* 5 (1): 5505.
- Corn, K. A., S. T. Friedman, E. D. Burrell, C. M. Martinez, O. Larouche, S. A. Price, and P. C. Wainwright.** 2022. The rise of biting during the Cenozoic fueled reef fish body shape diversification. *Proceedings of the National Academy of Sciences* 119 (31): e2119828119.
- Eduardo, L. N., A. Bertrand, M. M. Mincarone, L. V. Santos, T. Fredou, R. V. Assunção, A. Silva et al.** 2020. Hatchetfishes (Stomiiformes: Sternoptychidae) biodiversity, trophic ecology, vertical niche partitioning and functional roles in the western Tropical Atlantic. *Progress in Oceanography* 187: 102389. <https://doi.org/10.1016/j.pocean.2020.102389>.
- Evans, K. M., M. Vidal-García, V. A. Tagliacollo, S. J. Taylor, and D. B. Fenolio.** 2019. Bony patchwork: mosaic patterns of evolution in the skull of electric fishes (Apternotidae: Gymnotiformes). *Integrative and Comparative Biology* 59 (2): 420–431. <https://doi.org/10.1093/icb/icz026>
- Evans, K. M., O. Larouche, J. L. West, S. M. Gartner, and M. W. Westneat.** 2023. Burrowing constrains patterns of skull shape evolution in wrasses. *Evolution & Development* 25 (1): 73–84.
- Fahay, M.P.** 2007. *Early Stages of Fishes in the Western North Atlantic Ocean (Davis Strait, Southern Greenland and Flemish Cap to Cape Hatteras)*. Northwest Atlantic Fisheries Organization. 1696 p.

- Fedorov, A., R. Beichel, J. Kalpathy-Cramer, J. Finet, J.C. Fillion-Robin, S. Pujol, C. Bauer, D. Jennings, F.M. Fennessy, M. Sonka, J.Buatti, S.R. Aylward, J.V. Miller, S. Pieper, and R. Kikinis.** 2012. 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magnetic Resonance Imaging* 30 (9): 1323–1341. <https://doi.org/10.1016/j.mri.2012.05.001>.
- Figueiredo, J.L. de, A.P. dos Santos, N. Yamaguti, R.A. Bernardes, and C.L. Del Bianco Rossi-Wongtschowski.** 2002. Peixes da zona econômica exclusiva da Região Sudeste-Sul do Brasil: Levantamento com Rede de Meia-Água. São-Paulo: Editora da Universidade de São Paulo; Imprensa Oficial do Estado, 242 p.
- Fink, W. L.** 1984. Stomiiformes: Relationships. *In*: H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, Jr., and S. L. Richardson. *Ontogeny and Systematics of Fishes*. Special Publication. American Society of Ichthyologists and Herpetologists, Lawrence, KS. pp. 181–184.
- Fink, W. L.** 1985. Phylogenetic interrelationships of the stomiid fishes (Teleostei: Stomiiformes). *Miscellaneous Publications, Museum of Zoology, The University of Michigan*. 171:1–127.
- Fink, W.L.** 1981. Ontogeny and phylogeny of tooth attachment modes in actinopterygian fishes. *Journal of Morphology* 167 (2): 167–184.
- Ford, K. L., R. Peterson, M. Bernt, and J. S. Albert.** 2022. Convergence is Only Skin Deep: Craniofacial Evolution in Electric Fishes from South America and Africa (Apterontidae and Mormyridae). *Integrative organismal biology* 4, (1): obac022. <https://doi.org/10.1093/iob/obac022>.
- Fricke, R., W. N. Eschmeyer, and R. Van Der Laan** (eds). 2023. *Eschmeyer's Catalog of Fishes: Genera, Species, References*. ([http:// researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp](http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp)). Electronic version accessed 22/April/2020.
- Friedman, S. T., Price, S. A., and Wainwright, P. C.** 2021. The effect of locomotion mode on body shape evolution in teleost fishes. *Integrative Organismal Biology* 3(1): obab016. <https://doi.org/10.1093/iob/obab016>.
- Gaskett, A. C., C. Bulman, X. He, and S. D. Goldsworthy.** 2001. Diet composition and guild structure of mesopelagic and bathypelagic fishes near Macquarie Island, Australia. *New Zealand Journal of Marine and Freshwater Research* 35 (3): 469-476. DOI: 10.1080/00288330.2001.9517016.

- Gerringer, M. E., A. S. Dias, A. A. Von Hagel, J. W. Orr, A. P. Summers, and S. Farina.** 2021. Habitat influences skeletal morphology and density in the snailfishes (family Liparidae). *Frontiers in Zoology* 18 (1): 1–22.
- Gibbs, R. H. Jr.** 1984. Astronesthidae. In P.J.P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen and E. Tortonese (eds.) *Fishes of the north-eastern Atlantic and the Mediterranean*. UNESCO, Paris. Vol. 1, p. 325-335.
- Goatley, C. H. R, and D. R. Bellwood.** 2009. Morphological structure in a reef fish assemblage. *Coral Reefs* 28: 449–457.
- Gon, O.** 1990. Stomiidae. Scaly dragonfishes. In O. Gon and P.C. Heemstra (eds.) *Fishes of the Southern Ocean*. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa, p. 127–133.
- Goswami, A.** et al. 2019. High-density morphometric analysis of shape and integration: the good, the bad, and the not-really-a-problem. *Integrative and Comparative Biology* 59 (3): 669–683. <https://doi.org/10.1093/icb/icz120>
- Haddock, S. H. D.; M. A. Moline, J. F. Case.** 2010. Bioluminescence in the Sea. *Annual Review of Marine Science* 2: 443–493.
- Hanken, J., and Thorogood, P.** 1993. Evolution and development of the vertebrate skull: The role of pattern formation. *Trends in Ecology & Evolution*, 8 (1): 9–15. doi:10.1016/0169-5347(93)90124-8
- Harold, A. S.** 1994. A taxonomic revision of the sternoptychid genus *Polyipnus* (Teleostei: Stomiiformes) with an analysis of phylogenetic relationships. *Bulletin of Marine Science* 54 (2): 428–534.
- Harold, A. S.** 2002. Gonostomatidae (881–884), Phosichthyidae (885–888), Sternoptychidae (889–892), Astronesthidae (893–895), Chauliodontidae (896–898), Idiacanthidae (899–900), Malacosteidae (901–903), Stomiidae (904–906), and Melanostomiidae (907–912). *In*: K. E. Carpenter (Ed.) *The living marine resources of the western central Atlantic, FAO species identification guide for fishery purposes*. Vol. 2. FAO, Rome.
- Harold, A. S. and S. H. Weitzman.** 1996. Interrelationships of Stomiiform Fishes. *In*: M. L. J. Stiassny, L.R. Parenti, and G. David-Johnson. 1996 *Interrelationships of Fishes*. Academic Press Inc., San Diego, CA. pp. 333–353.
- Hopkins, T. L., T. T. Sutton, and T. M. Lancraft.** 1996. The trophic structure and predation impact of a low latitude midwater fish assemblage. *Progress in Oceanography* 38: 205–239.

- Ingram, Travis.** 2011. Speciation along a depth gradient in a marine adaptive radiation. *Proceedings of the Royal Society B: Biological Sciences* 278 (1705): 613–618.
- Kamilar, J. M., and N. Cooper.** 2013. Phylogenetic signal in primate behaviour, ecology and life history. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368.1618: 20120341. <https://doi.org/10.1098/rstb.2012.0341>
- Kenaley, C. P.** 2007. Revision of the stoplight loosejaw genus *Malacosteus* (Teleostei: Stomiidae: Malacosteinae), with description of a new species from the temperate southern hemisphere and Indian Ocean. *Copeia* (4): 886–900.
- Kenaley, C. P.** 2012. Exploring feeding behavior in deep-sea dragonfishes (Teleostei: Stomiidae): jaw biomechanics and functional significance of a loosejaw. *Biological Journal of the Linnean Society* 106: 224–240.
- Knapp, A., G. Rangel-de Lázaro, M. Friedman, Z. Johanson, K. M. Evans, S. Giles, H. T. Beckett, and A. Goswami.** 2023. How to tuna fish: constraint, convergence, and integration in the neurocranium of pelagiarian fishes. *Evolution* 77 (6): 1277–1288. <https://doi.org/10.1093/evolut/qqad056>.
- Larouche, O., S. M. Gartner, M. W. Westneat, and K. M. Evans.** 2023. Mosaic evolution of the skull in Labrid fishes involves differences in both tempo and mode of morphological change. *Systematic Biology* 72 (2): 419–432.
- Losos, J. B.** 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* 11: 995 – 1007. doi:10.1111/j.1461-0248.2008.01229.x.
- Maile, A. J., Z. A. May, E. S. DeArmon, R. P. Martin, and M. P. Davis.** 2020. Marine habitat transitions and body-shape evolution in lizardfishes and their allies (Aulopiformes). *Copeia* 108: 820–832. <https://doi.org/10.1643/CG-19-300>.
- Marques, A.** 2001. Some Data on the Biology of Polymetme *Corythaeola* (Phosichthyidae), From Off the Portuguese South Coast, North East Atlantic. *Cybiurn* 25 (1): 100–102.
- Marranzino, A. N., and J. F. Webb.** 2018. Flow sensing in the deep sea: the lateral line system of stomiiform fishes. *Zoological Journal of the Linnean Society* 183: 945–965. <https://doi.org/10.1093/zoolinnean/zlx090>.
- Martinez, C. M., S. T. Friedman, K. A. Corn, O. Larouche, S. A. Price, and P. C. Wainwright.** 2021. The deep sea is a hot spot of fish body shape evolution. *Ecology Letters* 24: 1788–1799. <https://doi.org/10.1111/ele.13785>.

- McGonagle, R. P., D. W. Kerstetter, D. Fenolio, and T. T. Sutton.** 2023. Ecomorphology of a predatory deep-sea fish family: does trophic specialization drive hyperspeciation?. *Frontiers in Marine Science* 10: 1056094.
- MorphoSource.** 2022. Duke University, North Caroline, United Staes of America. Available at <http://morphosource.org>.
- Mundy, B. C.** 2005. Checklist of the fishes of the Hawaiian Archipelago. *Bishop Museum Bulletin in Zoology* (6):1–704.
- Nelson, J. S.** 2006. *Fishes of the world*. 4^o ed. John Wiley and Sons, Inc., Hoboken, NJ.601p.
- Nelson, J. S., T. C. Grande, and M. V. H. Wilson.** 2016. *Fishes of the World*. 5^a ed. John Wiley and Sons, Inc., Hoboken, NJ. 752p.
- Orlov, A. M., and A. M. Tokranov.** 2019. Checklist of deep-sea fishes of the Russian northwestern Pacific Ocean found at depths below 1000 m. *Progress in Oceanography* 176: 102143.
- Paradis, E., J. Claude, and K. Strimmer.** 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20.2: 289–290. <https://doi.org/10.1093/bioinformatics/btg412>
- Parin, N. V., and O. D. Borodulina.** 1990. Survey of the genus *Polymetme* (Photichthyidae) with a description of two new species. *Journal of Ichthyology* 30 (6):108–121.
- Quèro, J. C. H., C. Karrer, A. Post, and L. Saldanha (eds.).** 1990. Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). JNICT, Lisbon; SEI, Paris; and UNESCO, Paris. Vol. 1.
- R Core Team.** 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <https://www.R-project.org/>.
- Rabosky, D. L., J. Chang, P. O. Title, P. F. Cowman, L. Sallan, M. Friedman, et al.** 2018. An inverse latitudinal gradient in speciation for marine fishes. *Nature* 559: 392–395. <https://doi.org/10.1038/s41586-018-0273-1>.
- Randall, D. J., and A. P Farrell.** 1997. *Deep-sea fishes*. Academic Press. 388 p.
- Revell, L. J.** 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3:217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>.

- Schaefer, S., R. K. Johnson, and J. Badcock.** 1986. Photichthyidae. In: SMITH M. M.; HEEMSTRA, P. C. (Ed.). *Smiths sea fishes* Berlin: Springer-Verlag, p. 243–247.
- Schindelin, Johannes, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch et al.** 2012. Fiji: an open-source platform for biological-image analysis. *Nature methods* 9 (7): 676–682.
- Schmitz L., and Wainwright P. C.** 2011. Nocturnality constrains morphological and functional diversity in the eyes of reef fishes. *BMC Evolutionary Biology* 11: 338.
- Schnell, N. K., and G. D. Johnson.** 2017. Evolution of a Functional Head Joint in Deep-Sea Fishes (Stomiidae). *PLoS ONE* 12 (2): e0170224, 2017. <https://doi.org/10.1371/journal.pone.0170224>.
- Schnell, N. K., J. Kriwet, F. A. López-Romero, G. Lecointre, and C. Pfaff.** 2022. Musculotendinous system of mesopelagic fishes: Stomiiformes (Teleostei). *Journal of Anatomy* 240 (6): 1095–1126. <https://doi.org/10.1111/joa.13614>.
- Schnell, N. K., R. Britz, and G. D. Johnson.** 2010. New insights into the complex structure and ontogeny of the occipitovertebral gap in barbeled dragonfishes (Stomiidae, Teleostei). *Journal of Morphology* 271: 1006–1022. <http://doi.org/10.1002/jmor.10858>.
- Scott, W. B., and M. G. Scott.** 1988. Atlantic fishes of Canada. *Canadian Bulletin of Fisheries and Aquatic Sciences* 219: 731.
- Sfakiotakis, M., D. M. Lane, and J. B. C. Davies.** 1999. Review of fish swimming modes for aquatic locomotion. *IEEE Journal of oceanic engineering* 24(2): 237–252. <https://doi.org/10.1109/48.757275>.
- Shinohara, G., K. Yabe, M. Nakaya, G. Anma, S. Yamaguchi, and K. Amaoka.** 1994. Deep-sea fishes collected from the North Pacific by the T/S Oshoro-Marui. *Bulletin of the Faculty of Fisheries, Hokkaido University* 45 (2):48–80.
- Sidlauskas, B.** 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. *Evolution* 62:3135–3156. <https://doi.org/10.1111/j.1558-5646.2008.00519.x>.
- Sutton, T. T. and T. L. Hopkins.** 1996. Trophic ecology of the stomiid (Pisces:Stomiidae) fish assemblage of the eastern Gulf of Mexico: strategies, selectivity and impact of a top mesopelagic predator group. *Marine Biology* 127:179–192. <https://doi.org/10.1007/BF00942102>.

- Sutton, T. T.** 2005. Trophic ecology of the deep-sea fish *Malacosteus niger* (Pisces: Stomiidae): An enigmatic feeding ecology to facilitate a unique visual system? **Deep-Sea Research I** 52: 2065–2076.
- Tchernavin V. V.** 1953. The feeding mechanisms of a deepsea fish. Bulletin of the British Museum, 1-101 (Pl. 1-10).
- Thistle, D.** 2003. The deep-sea floor, an overview. In: TYLER, P. A. (Ed.) Ecosystems of the World 28. Ecosystems of the Deep-Ocean. Amsterdam: Elsevier, p. 5-37.
- Webb, P. W.** 1984. Form and function in fish swimming. *Scientific American* 251 (1): 72–83. <https://10.1038/scientificamerican0784-72>.
- Wickham, H.** 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.
- Widder, E. A.** 2010. Bioluminescence in the ocean: origins of biological, chemical, and ecological diversity. *Science* 328 (5979): 704-708.
- Williams, A. et al.** 2001. Feeding ecology of five fishes from the mid-slope micronekton community off southern Tasmania, Australia. *Marine Biology* 139: 1177–1192.
- Winterbottom, R.** 1974. A descriptive synonymy of the striated muscles of the Teleostei. *Proceedings of the Academy of Natural Sciences of Philadelphia* 125: 225–317.

4. FINAL CONCLUSIONS

- Overall, all stomiiforms clades vary in terms of skull and body shape, but morphological disparity was greater in dragonfishes (Stomiidae) and hatchetfishes (Sternoptychidae);

- Despite stomiiforms exhibit an overall high shape disparity, there is a limited number of distinct morphotypes;

- The fins size and position, especially dorsal and anal fins, correspond to the major variations within stomiiforms, followed by body size and body depth, resulting in a transition from species with dorsal fin positioned at midbody, long anal fins, and a fusiform or deep bodied morphologies to species with dorsal fin close to the tail, parallel to the anal fin, both shorts, with an elongated body;

- There is a trend towards body elongation in taxa according to the depth, with species inhabiting the meso and bathypelagic regions presenting bodies slenderer than those inhabiting the shallow mesopelagic regions;

- Generalist and piscivorous stomiiforms exhibit a higher morphological disparity than zooplanktivorous counterparts, and generally are meso- bathypelagic species. In contrast, zooplanktivorous stomiiforms include both mesopelagic and meso- to bathypelagic species;

- When accounting for phylogeny in body shape, there is no significant morphological disparity between body shape and ecological traits;

- Variations in skull shape are predominantly associated with skull length and skull depth, with specific regions of the neurocranium, particularly the occipital region, showing significant involvement. Landmarks exhibiting greater variation are primarily related to the occipital region, suspensorium, and preopercle;

- Contrary to the expectations, there is no significant correlation between skull shape and ecological aspects such as depth and diet, even without a phylogenetic context. This contrasts with the significant correlation found between body shape and these ecological traits without a phylogenetic context;

- It is suggested that are potential influence of neurocranial elements on the evolution of extreme morphologies observed in species with high variance, such the hatchetfish *Sternoptyx* and the loosejaw *Malacosteus*. Furthermore, the contrasting patterns observed in the correlation between skull shape and ecological traits compared to body shape emphasize the complex nature of morphological adaptations in this group;

- There is a phylogenetic signal in both body and skull shape among stomiiforms, with closely related taxa tended to cluster together in phylomorphospace, as the result of shared evolutionary history. The phylogenetic signal is also high and often interpreted as evolutionary or phylogenetic conservatism;

- This is the first study to quantitatively investigate changes in the stomiiforms shape, concluding that their shape evolution is explained mainly by the phylogeny of the group, as a result of a shared ancestry pattern;

- Ecological traits, such as diet and ocean depth are unlikely to play a major role in shaping morphological evolution, or ecological traits were only in play during the early evolution of the group. Finally, the current dataset might lack the statistical power to uncover any relationship that may exist between shifts in depth, diet or migration and the shape diversification of stomiiforms, or the lack of significance could stem from the relatively sparse taxon sampling of the phylogenetically informed analysis.

SUPPLEMENTAL INFORMATION CHAPTER TWO

Supplemental Figures Chapter Two

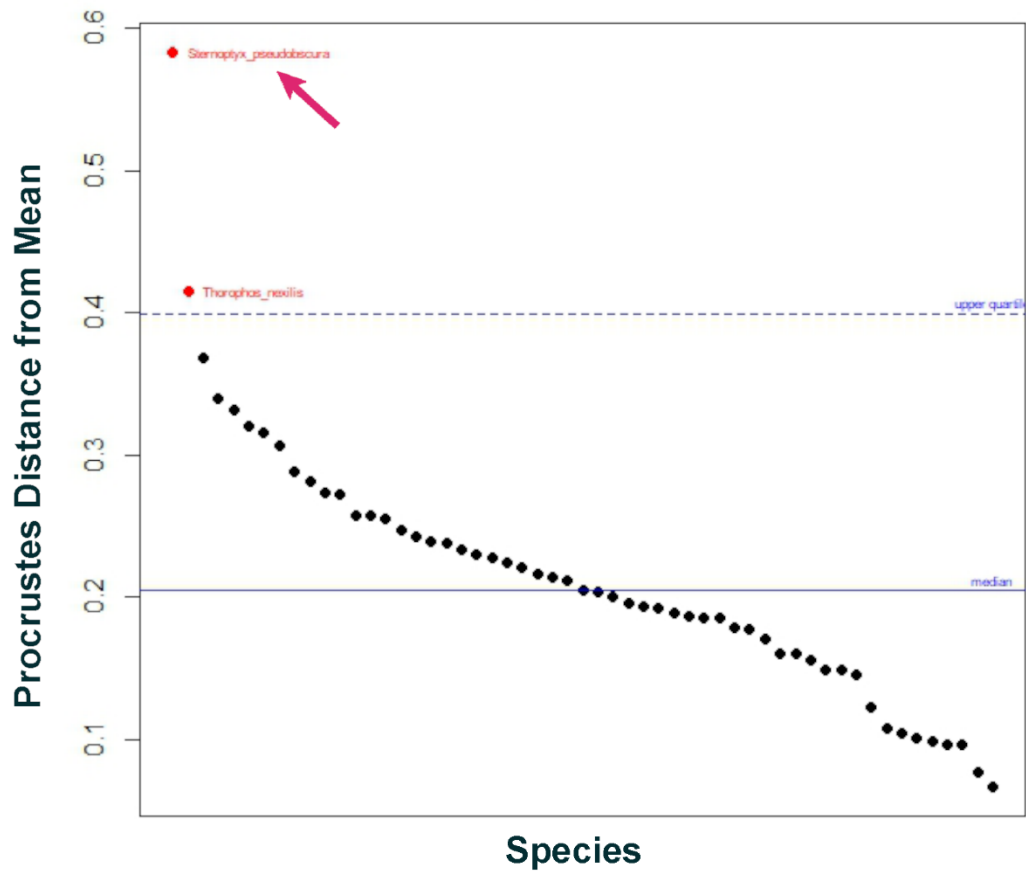


Figure S2.1 Outliers resulted from the Procrustes ANOVA analysis, highlighting the *Sternoptyx pseudobscura*, indicated by the red arrow, with high Procrustes distance from mean.

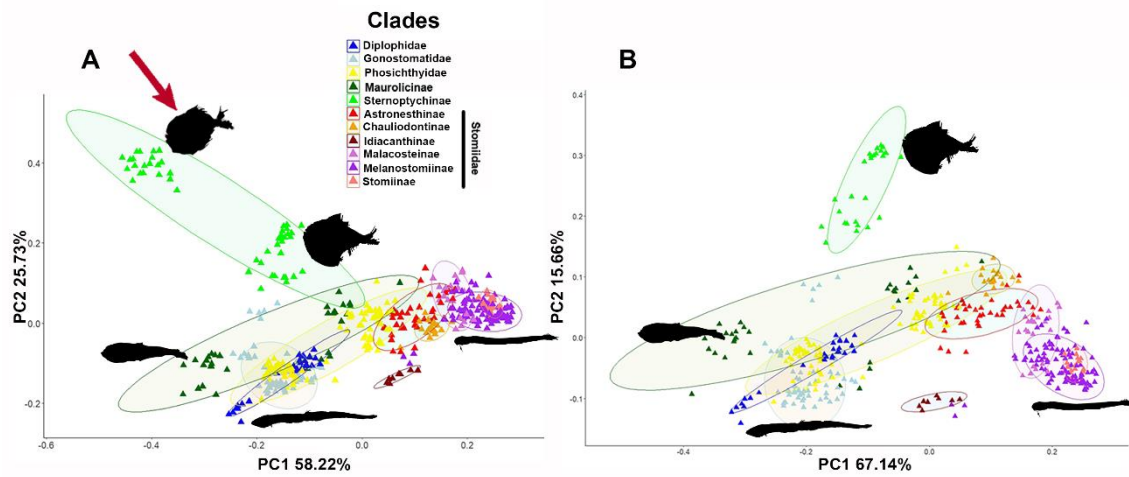


Figure S2.2. Scatterplot of the first and second axes resulting from Principal Component Analysis (PCA) among the species of Stomiiformes. A. PCA including *Sternoptyx pseudobscura*; B. PCA excluding *Sternoptyx pseudobscura*.

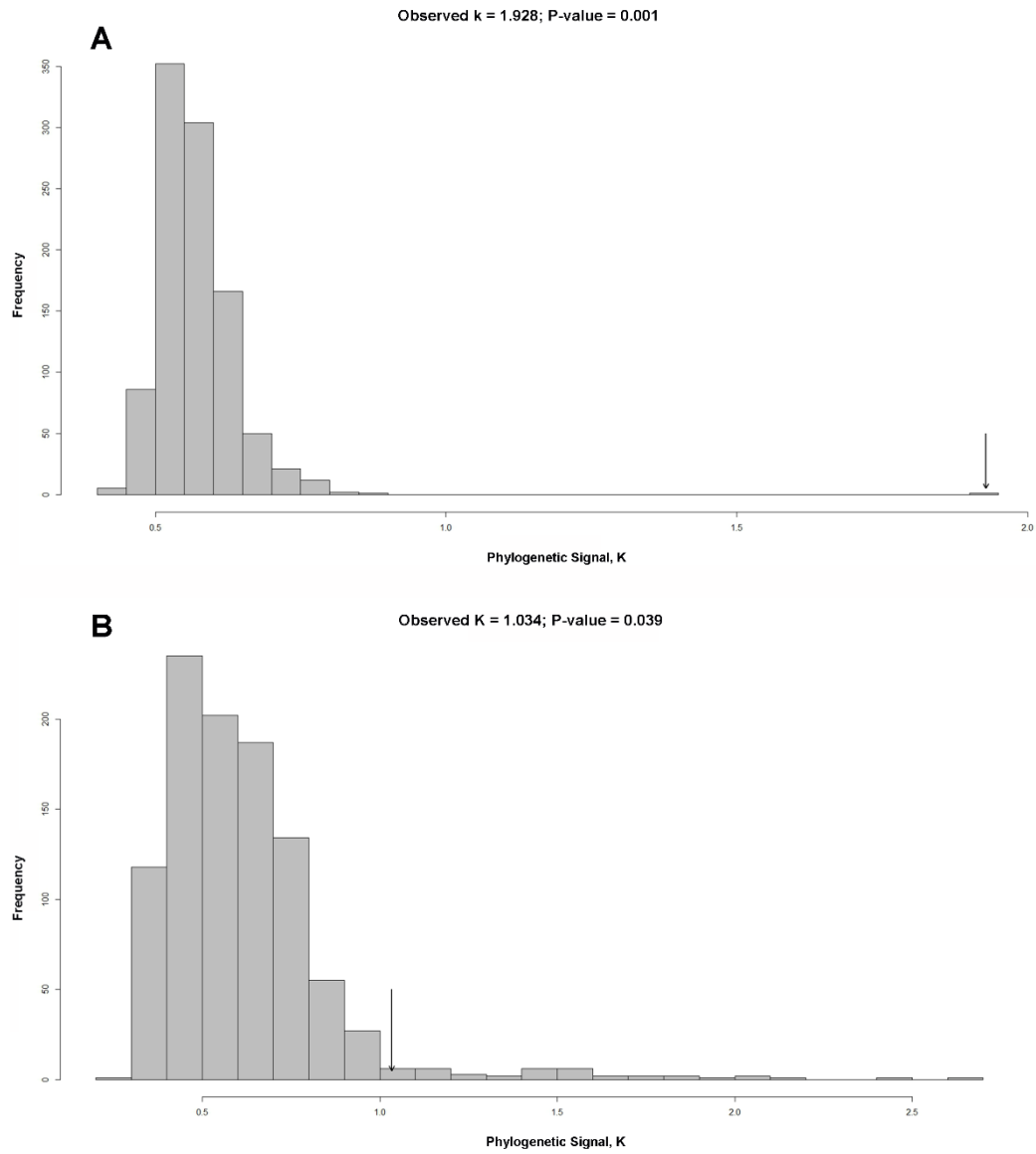


Figure S2.3. A. Phylogenetic signal (K) and p -values in stomiiform shapes, and B. Centroid Size.

Supplemental Tables Chapter Two

Supplemental Table S2.1. Ecological traits of the Stomiiformes, encompassing their diet, diel vertical migration, and depth, with respective references from the literature regarding these traits, alongside the specific ecological traits categories employed within this investigation. DVM=Diel Vertical Migration. Type 1 diet=zooplanktivorous; Type 2 diet=generalists; Type 3 diet=piscivorous. Asterisk means that diet type was inferred based on other species or genera from the same family.

| Species | Diet | Diet Reference | Diet Category | Depth Range | Depth and DVM Reference | Depth Category | DVM Category |
|---------------------------------|------------------------------------|---|---------------|-------------|---|-------------------|---------------|
| Diplophidae (2) | | | | | | | |
| <i>Diplophos taenia</i> | Mostly on copepods and euphausiids | Hopkins et al. (1996); Clarke (1982) | Type 1 | 15–1,300 | Clarke (1974); Badcock (1984); Figueiredo et al. (2002); Mundy (2005); This study | Meso–Bathypelagic | Migratory |
| <i>Manducus maderensis</i> | No information available | | Type 1* | 0–927 | Badcock (1984); Schaefer et al. (1986); Figueiredo et al. (2002); This study | Mesopelagic | Migratory |
| Gonostomatidae (6) | | | | | | | |
| <i>Cyclothone microdon</i> | Mostly on copepods | Gordon et al. (1985) | Type 1 | 500–5,301 | Mauchlinae (1988); Coad & Reist (2004); This study | Meso–Bathypelagic | Non-migratory |
| <i>Cyclothone pseudopallida</i> | Mostly on copepods | Hopkins et al. (1996); Gordon et al. (1985) | Type 1 | 300–4,938 | Badcock (1970); Badcock (1984); Shinohara et al. (1994); Mundy (2005); This study | Meso–Bathypelagic | Non-migratory |
| <i>Gonostoma atlanticum</i> | Mostly on copepods and euphausiids | Gordon et al. (1985); Clarke (1982) | Type 1 | 50–3,050 | Clarke (1974); Quèro et al. (1990); Mundy (2005); This study | Meso–Bathypelagic | Migratory |

| | | | | | | | |
|--------------------------------|---|---|---------|-----------|--|-------------------|---------------|
| <i>Margrethia obtusirostra</i> | Mostly on copepods and euphausiids | Hopkins et al. (1996) | Type 1 | 100–1,500 | Mundy (2005); Fahay (2007); Olivar & Beckley (2022); This study | Meso–Bathypelagic | Migratory |
| <i>Sigmops elongatus</i> | Mostly on copepods and euphausiids | Hopkins et al. (1996); Clarke (1982) | Type 1 | 25–1,648 | Badcock (1970); Clarke (1974); Quèro et al. (1990); Mundy (2005); This study | Meso–Bathypelagic | Migratory |
| <i>Zaphotias pedaliotus</i> | Zooplankton | Badcock (1984) | Type 1 | 100–2,300 | Scott & Scott (1988); Olivar & Beckley (2022); This study | Meso–Bathypelagic | Migratory |
| Phosichthyidae (7) | | | | | | | |
| <i>Ichthyococcus elongatus</i> | No information available | | Type 1* | 100–2,460 | Clarke (1974); Shinohara et al. (1996); Orlov & Tokranov (2019); This study | Meso–Bathypelagic | Migratory |
| <i>Phosichthys argenteus</i> | Mostly on fishes; minor on decapods and euphausiids | Williams et al. (2001); Gaskett et al. (2010) | Type 2 | 300–1,141 | Quèro et al. (1990); This study | Meso–Bathypelagic | Non-migratory |
| <i>Pollichthys maui</i> | Mostly on euphausiids | Hopkins et al. (1996) | Type 1 | 100–917 | Badcock (1984); Schaefer et al. (1986); Figueiredo et al. (2002); Fahay (2007); This study | Mesopelagic | Migratory |
| <i>Polymetme thaecoryla</i> | Mostly on euphausiids | Marques (2001) | Type 1 | 213–1,400 | Parin & Borodulina (1990); This study | Meso–Bathypelagic | Migratory |
| <i>Vinciguerria nimbaria</i> | Approximately equal parts of crustaceans and small fish; mostly amphipods and euphausiids | Hopkins et al. (1996); Clarke (1982) | Type 2 | 20–5,000 | Clarke (1974); Badcock (1984); Quèro et al. (1990); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Vinciguerria poweriae</i> | Mostly on copepods as juveniles and on euphausiids as adult | Hopkins et al. (1996) | Type 1 | 50–1,500 | Clarke (1974); Quèro et al. (1990); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Yarella blackfordi</i> | No information available | | Type 1* | 350–1,000 | Quèro et al., 1990; Fahay (2007); This study | Mesopelagic | Non-migratory |
| Sternoptychidae (12) | | | | | | | |

| | | | | | | | |
|---------------------------------------|---|---|---------|-----------|--|-------------------|---------------|
| <i>Araiophos eastropas</i> | No information available | | Type 1* | 200-? | Ahlstrom & Moser (1969) | Mesopelagic | Migratory |
| <i>Argyripnus atlanticus</i> | Crustaceans | Badcock (1984) | Type 1 | 200–500 | Mundy (2005); Fahay (2007); This study | Mesopelagic | Migratory |
| <i>Argyropelecus aculeatus</i> | Dominated by crustaceans: amphipods (mostly in Gulf of Mexico), euphausiids (mostly in Brazil), copepods, mysids, pteropods and ostracods; larger-sized individuals prey on hatchetfishes, including those of the same species (cannibalistic behavior) | Hopkins et al. (1996); Harold et al. (2002); Carmo et al. (2015); Eduardo et al. (2020) | Type 2 | 80–2,056 | Badcock (1970); Gon (1990); Shinohara et al. (1996); Mundy (2005); Eduardo et al. (2020); This study | Meso–Bathypelagic | Migratory |
| <i>Argyropelecus affinis</i> | Crustaceans (mostly), adult and larvae fishes; gelatinous invertebrates | Eduardo et al. (2020) | Type 2 | 100–2,400 | Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Danaphos oculatus</i> | Mostly copepods | Clarke (1982) | Type 1 | 183–2,400 | Shinohara et al. 1994; Mundy (2005); This study | Meso–Bathypelagic | Migratory |
| <i>Maurolicus stehmani</i> | Copepods (mostly), cladocerans, euphausiids and ostracods | Hopkins et al. (1996); Carmo et al. (2015) | Type 1 | 0–610 | Bernardes et al. (2005); This study | Mesopelagic | Migratory |
| <i>Maurolicus weitzmani</i> | Copepods (mostly), cladocerans, euphausiids and ostracods | Hopkins et al. (1996); Carmo et al. (2015) | Type 1 | 100–610 | Fahay (2007); This study | Mesopelagic | Migratory |
| <i>Polyipnus spinifer</i> | Zooplanktivorous, feeding on copepods, euphausiids, and mysids | Harold et al. (2002) | Type 1 | 150–611 | Harold (1994); This study | Mesopelagic | Migratory |
| <i>Polyipnus laternatus</i> | Planktivorous, feeding on copepods, euphausiids, and mysids | Harold et al. (2002) | Type 1 | 240–2,200 | Bright & Pequegnat (1969); Harold (1994); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Sternoptyx pseudobscura</i> | Mostly on crustaceans (amphipods and euphausiids) and polychaets in small-sized individuals; large-sized individuals prey on fish | Hopkins et al. (1996); Carmo et al. (2015); Eduardo et al. (2020) | Type 2 | 350–2,450 | Gon (1990); Fahay (2007); Eduardo et al. (2020); This study | Meso–Bathypelagic | Non-migratory |
| <i>Thorophos nexilis</i> | No information available | | Type 1* | 200–320 | Bruun (1931); This study | Mesopelagic | Migratory |
| <i>Valenciennellus tripunctulatus</i> | Mostly copepods | Clarke (1982); Hopkins et al. (1996) | Type 1 | 100–1,000 | Badcock (1970); Clarke (1974); Mundy (2005); Fahay (2007); This study | Mesopelagic | Migratory |
| Stomiidae (27) | | | | | | | |

| | | | | | | | |
|---------------------------------|--|--|---------|-----------|---|-------------------|---------------|
| <i>Aristostomias scintilans</i> | Piscivory centered on myctophids | Hopkins et al. (1996); Sutton & Hopkins (1996) | Type 3 | 0–4,300 | Clarke (1974); Sutton & Hopkins (1996); Orlov & Tokranov (2019); This study | Meso–Bathypelagic | Migratory |
| <i>Astronesthes gemmifer</i> | Mostly on euphausiids, fewest on decapods and fishes | Hopkins et al. (1996); Sutton & Hopkins (1996) | Type 2 | 58–1,266 | Clarke (1974); Sutton & Hopkins (1996); Mundy (2005); This study | Meso–Bathypelagic | Migratory |
| <i>Astronesthes macropogon</i> | Mostly on euphausiids, fewest on decapods and fishes | Hopkins et al. (1996); Sutton & Hopkins (1996) | Type 2 | 0–2,000 | Sutton & Hopkins (1996); Figueiredo et al. (2002); This study | Meso–Bathypelagic | Migratory |
| <i>Bathophilus flemingi</i> | Mostly on fishes; minor in cephalopods; Small crustaceans | Sutton & Hopkins (1996); McGonagle et al. (2023) | Type 3 | 40–2,520 | Badcock (1970); Sutton & Hopkins (1996); Mundy (2005); This study | Meso–Bathypelagic | Migratory |
| <i>Borostomias antarcticus</i> | Fishes and crustaceans | Gibbs (1984) | Type 2 | 350–2,500 | Fahay (2007); Banon et al. (2021) This study | Meso–Bathypelagic | Non-migratory |
| <i>Chauliodus sloani</i> | Piscivory centered on myctophids and on other stomiiforms; some could also feed on euphausiids | Hopkins et al. (1996); Sutton & Hopkins (1996); Bataglia et al. (2018); Clarke (1982) | Type 3 | 45–4,700 | Badcock (1970); Clarke (1974); Shinohara et al. (1994); Shinohara et al. (1996); Sutton & Hopkins (1996); Eduardo et al. (2020); This study | Meso–Bathypelagic | Migratory |
| <i>Chauliodus macouni</i> | Piscivory centered on myctophids and on other stomiiforms | Hopkins et al. (1996); Sutton & Hopkins (1996); Bataglia et al. (2018); Gordon et al. (1985) | Type 3 | 25–4,390 | Sutton & Hopkins (1996); Orlov & Tokranov (2019); This study | Meso–Bathypelagic | Migratory |
| <i>Chirotomias pliopterus</i> | No information available | | Type 3* | 500–1,016 | Scott & Scott (1988); This study | Meso–Bathypelagic | Non-migratory |
| <i>Echiostoma barbatum</i> | Piscivory; minor cephalopods | McGonagle et al. (2023) | Type 3 | 30–4,200 | Clarke (1974); Sutton & Hopkins (1996); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |

| | | | | | | | |
|--------------------------------------|--|--|---------|-----------|--|-------------------|---------------|
| <i>Eustomias filifer</i> | Piscivory | McGonagle et al. (2023); Clarke (1982) | Type 3 | 90–200 | Badcock (1970); Sutton & Hopkins (1996); Mundy (2005); This study | Mesopelagic | Migratory |
| <i>Flagellostomias boureei</i> | Piscivory | McGonagle et al. (2023) | Type 3 | 50–1,825 | Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Grammatostomias flagellibarba</i> | No information available | | Type 3* | 800–1,636 | Sutton & Hopkins (1996); This study | Meso–Bathypelagic | Non-migratory |
| <i>Heterophotus ophistoma</i> | Decapods and fishes | Clarke (1982) | Type 2 | 50–1,850 | Clarke (1974); Sutton & Hopkins (1996); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Idiacanthus atlanticus</i> | Most Fishes and minor crustaceans | Fitch & Lavenberg (1968); Borodulina (1972) | Type 3 | 500–2,000 | Badcock (1970); Clarke (1974); Gon (1990); Sutton & Hopkins (1996); This study | Meso–Bathypelagic | Migratory |
| <i>Leptostomias gladiator</i> | Piscivory | McGonagle et al. (2023) | Type 3 | 0–5,000 | Badcock (1970); Sutton & Hopkins (1996); Orlov & Tokranov (2019); This study | Meso–Bathypelagic | Migratory |
| <i>Malacosteus australis</i> | Mostly on copepods, euphausiids and decapods (rarely fish) | Hopkins et al. (1996); Sutton & Hopkins (1996); Sutton (2005); Clarke (1982) | Type 1 | 500–2,000 | Clarke (1974); Sutton & Hopkins (1996); Kenaley (2007); This study | Meso–Bathypelagic | Non-migratory |
| <i>Melanostomias melanops</i> | Piscivory centered on myctophids | Hopkins et al. (1996); Sutton & Hopkins (1996); McGonagle et al. (2023) | Type 3 | 50–1,545 | Clarke (1974); Mundy (2005); This study | Meso–Bathypelagic | Migratory |
| <i>Neonesthes capensis</i> | Fishes and crustaceans | Gibbs (1984) | Type 2 | 70–1,650 | Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Odontostomias micropogon</i> | No information available | | Type 3* | 400–740 | This study | Mesopelagic | Non-migratory |

| | | | | | | | |
|------------------------------|---|---|---------|-----------|---|-------------------|---------------|
| <i>Opostomias mitsuii</i> | No information available | | Type 3* | 50–1,500 | Mundy (2005); Orlov & Tokranov (2019); This study | Meso–Bathypelagic | Migratory |
| <i>Pachystomias microdon</i> | Piscivory | Sutton & Hopkins (1996) | Type 3 | 660–4,463 | Sutton & Hopkins (1996); Mundy (2005); Orlov & Tokranov (2019); This study | Meso–Bathypelagic | Migratory |
| <i>Photonectes margarita</i> | Piscivory | McGonagle et al. (2023) | Type 3 | 0–2,400 | Sutton & Hopkins (1996); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic | Migratory |
| <i>Photostomias guernei</i> | Decapod shrimps | Hopkins et al. (1996); Sutton & Hopkins (1996); Clarke (1982) | Type 1 | 0–3,100 | Badcock (1970); Sutton & Hopkins (1996); Fahay (2007); Kenaley (2008); This study | Meso–Bathypelagic | Migratory |
| <i>Rhadinesthes decimus</i> | Fishes and crustaceans | Gibbs (1984) | Type 2 | 500–2,633 | Quèro & Spitz (2004); This study | Meso–Bathypelagic | Non-migratory |
| <i>Stomias affinis</i> | Piscivory centered on myctophids | Hopkins et al. (1996); Sutton & Hopkins (1996) | Type 3 | 0–3,182 | Borodulina (1972); Gon (1990); Sutton & Hopkins (1996); | Meso–Bathypelagic | Migratory |
| <i>Tactostoma macropus</i> | Piscivory | Gordon et al. (1985) | Type 3 | 30–2,000 | Shinohara et al. (1994); Moser & Watson (1996); Orlov & Tokranov (2019); This study | Meso–Bathypelagic | Migratory |
| <i>Thysanactis dentex</i> | Small individuals eat mostly euphausiids and copepods and large individuals eat large fish and crustacean | Clarke (1982) | Type 2 | 75–4,000 | Clarke (1974); Mundy (2005); This study | Meso–Bathypelagic | Migratory |
| <i>Trigonolampa miriceps</i> | No information available | | Type 3* | 915–1,860 | Gon (1990); This study | Meso–Bathypelagic | Non-migratory |

Supplemental Table S2.2. Species of Stomiiformes used to create a genus-level phylomorphospace based on the molecular phylogeny produced by Rabosky *et al.* (2018).

| Family | Species |
|----------------------------|---|
| Diplophidae (1) | <i>Diplophos taenia</i> Günther, 1873 |
| | <i>Cyclothone pseudopallida</i> Mukhacheva, 1964 |
| | <i>Gonostoma atlanticum</i> Norman, 1930 |
| Gonostomatidae (5) | <i>Margrethia obtusirostra</i> Jespersen and Tåning, 1919 |
| | <i>Sigmops elongatus</i> (Günther, 1878) |
| | <i>Zaphotias pedaliotus</i> (Goode and Bean, 1896) |
| | <i>Ichthyococcus ovatus</i> (Cocco, 1838) |
| | <i>Phosichthys argenteus</i> Hutton, 1872 |
| | <i>Pollichthys maui</i> (Poll, 1953) |
| Phosichthyidae (6) | <i>Polymetme thaeocoryla</i> Parin and Borodulina, 1990 |
| | <i>Vinciguerrria nimbaria</i> (Jordan and Williams, 1895) |
| | <i>Yarrella blackfordi</i> Goode and Bean, 1896 |
| | <i>Argyropelecus aculeatus</i> Valenciennes, 1850 |
| | <i>Danaphos oculatus</i> (Garman, 1899) |
| | <i>Maurolicus muelleri</i> (Gmelin, 1789) |
| Sternoptychidae (6) | <i>Polyipnus polli</i> Schultz, 1961 |
| | <i>Sternoptyx pseudobscura</i> Baird, 1971 |
| | <i>Valenciennellus tripunctulatus</i> (Esmark, 1871) |
| | <i>Astronesthes gemmifer</i> Goode and Bean, 1896 |
| | <i>Aristostomias scintillans</i> (Gilbert, 1915) |
| | <i>Bathophilus pawneeii</i> Parr, 1927 |
| | <i>Borostomias antarcticus</i> (Lönnerberg, 1905) |
| | <i>Chauliodus macouni</i> Bean, 1890 |
| | <i>Chirostomias pliopterus</i> Regan and Trewavas, 1930 |
| | <i>Echiostoma barbatum</i> Lowe, 1843 |
| Stomiidae (24) | <i>Eustomias filifer</i> (Gilchrist, 1906) |
| | <i>Flagellostomias boureei</i> (Zugmayer, 1913) |
| | <i>Grammatostomias flagellibarba</i> Holt and Byrne, 1910 |
| | <i>Heterophotus ophistoma</i> Regan and Trewavas, 1929 |
| | <i>Idiacanthus atlanticus</i> Brauer, 1906 |
| | <i>Leptostomias gladiator</i> (Zugmayer, 1911) |

Malacosteus niger Ayres, 1848
Melanostomias margaritifera Regan & Trewavas, 1930
Neonesthes capensis (Gilchrist and von Bonde, 1924)
Odontostomias micropogon Norman, 1930
Pachystomias microdon (Günther, 1878)
Photonectes margarita (Goode and Bean, 1896)
Rhadinesthes decimus (Zugmayer, 1911)
Stomias affinis Günther, 1887
Tactostoma macropus Bolin, 1939
Thysanactis dentex Regan and Trewavas, 1930
Trigonolampa miriceps Regan and Trewavas, 1930

References Supplemental Information Chapter Two

- Ahlstrom, E. H., and H. G. Moser.** 1969. A new Gonostomatid Fish from the tropical eastern Pacific. *Copeia*:493-500.
- Badcock, J.** 1970. The vertical distribution of mesopelagic fishes collected on the SONDR cruise. *Journal of the Marine Biological Association of the United Kingdom* 50 (4): 1001–1044.
- Badcock, J.** 1984. Gonostomatidae, p. 284–301. *In: Fishes of the north-eastern Atlantic and the Mediterranean*. v. 1. Whitehead, P. J. P.; Bauchot, M. L.; Hureau, J. C; Nielsen, J.; Tortonese, E. (eds.). UNESCO, Paris.
- Bañón, R., A. de Carlos, C. Farias, N. Vilas-Arrondo, and F. Baldó.** 2021. Exploring deep-sea biodiversity in the Porcupine Bank (NE Atlantic) through fish integrative taxonomy. *Journal of Marine Science and Engineering* 9 (10): 1075.
- Battaglia, P., G. Ammendolia, V. Esposito, T. Romeo, and F. Andaloro.** 2018. Few but relatively large prey: trophic ecology of *Chauliodus sloani* (Pisces: Stomiidae) in deep waters of the Central Mediterranean Sea. *Journal of Ichthyology* 58: 8–16.
- Bernardes, R.A., J. L. Figueiredo, A. R. Rodrigues, L. G. Fischer, C. M. Vooren, M. Haimovici, and C. L. D. B. RossiWongtschowski.** 2005. Peixes da Zona Econômica Exclusiva da região sudeste-sul do Brasil. Levantamento com armadilhas, pargueiras e rede de arrasto de fundo. Edusp, São Paulo, 295 pp.
- Borodulina, O. D.** 1972. The feeding of mesopelagic predatory fish in the open ocean. *Journal of Ichthyology* 12: 692–702.
- Bright, T. J., and W. E. Pequegnat.** 1969. Deep sea hatchet fishes of the Gulf of Mexico. *Quarterly Journal of the Florida Academy of Sciences* 32(1): 26-36.
- Carmo, V., T. Sutton, G. Menezes, T. Falkenhaus, and O.A. Bergstad.** 2015. Feeding ecology of the Stomiiformes (Pisces) of the northern Mid-Atlantic Ridge. 1. The Sternoptychidae and Phosichthyidae. *Progress in Oceanography* 130: 172–187.
- Clarke, T. A.** 1974. Some aspects of the ecology of stomiatoid fishes in the Pacific Ocean near Hawaii. *Fishery Bulletin* 72 (2): 337–351.
- Clarke, T. A.** 1982. Feeding habits of stomiatoid fishes from Hawaiian waters. *Fishery Bulletin* 80: 287–304.
- Coad, B.W. and J.D. Reist.** 2004. Annotated list of the arctic marine fishes of Canada. *Canadian Manuscript Report of Fisheries and Aquatic Sciences* 2674:iv:+112 p.

- Eduardo, L. N., A. Bertrand, M. M. Mincarone, L. V. Santos, T. Fredou, R. V. Assunção, A. Silva et al.** 2020. Hatchetfishes (Stomiiformes: Sternoptychidae) biodiversity, trophic ecology, vertical niche partitioning and functional roles in the western Tropical Atlantic. *Progress in Oceanography* 187: 102389.
- Fahay, M.P.** 2007. Early Stages of Fishes in the Western North Atlantic Ocean (Davis Strait, Southern Greenland and Flemish Cap to Cape Hatteras). Northwest Atlantic Fisheries Organization. 1696 p.
- Figueiredo, J.L. de, A.P. dos Santos, N. Yamaguti, R.A. Bernardes, and C.L. Del Bianco Rossi-Wongtschowski.** 2002. Peixes da zona econômica exclusiva da região Sudeste-Sul do Brasil: levantamento com rede de meia-água. Editora da Universidade de São Paulo, São Paulo, 242 p.
- Gaskett, A. C., C. Bulman, X. He, and S. D. Goldsworthy.** 2001. Diet composition and guild structure of mesopelagic and bathypelagic fishes near Macquarie Island, Australia. *New Zealand Journal of Marine and Freshwater Research* 35 (3): 469-476. <http://doi.org/10.1080/00288330.2001.9517016>.
- Gibbs, R. H. Jr.** 1984. Astronesthidae, p. 325-335. *In: Fishes of the north-eastern Atlantic and the Mediterranean*, Vol. 1. P.J.P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen and E. Tortonese (eds.). UNESCO, Paris.
- Gon, O.** 1990. Stomiidae. Scaly dragonfishes, p. 127–133. *In: Fishes of the Southern Ocean*. O. Gon and P.C. Heemstra (eds.). J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa.
- Harold, A. S.** 1994. A taxonomic revision of the sternoptychid genus *Polyipnus* (Teleostei: Stomiiformes) with an analysis of phylogenetic relationships. *Bulletin of Marine Science* 54 (2): 428–534.
- Harold, A. S.** 1994. A taxonomic revision of the sternoptychid genus *Polyipnus* (Teleostei: Stomiiformes) with an analysis of phylogenetic relationships. *Bulletin of Marine Sciences* 54(2): 428-534.
- Harold, A. S.** 2002. Gonostomatidae (881–884), Phosichthyidae (885–888), Sternoptychidae (889–892), Astronesthidae (893–895), Chauliodontidae (896–898), Idiacanthidae (899–900), Malacosteidae (901–903), Stomiidae (904–906), and Melanostomiidae (907–912). *In: The living marine resources of the western central Atlantic*, FAO species identification guide for fishery purposes. Vol. 2. K. E. Carpenter (Ed.). FAO, Rome.

- Hopkins, T. L., T. T. Sutton, and T. M. Lancraft.** 1996. The trophic structure and predation impact of a low latitude midwater fish assemblage. *Progress in Oceanography* 38: 205–239.
- Kenaley, C. P.** 2007. Revision of the stoplight loosejaw genus *Malacosteus* (Teleostei: Stomiidae: Malacosteinae), with description of a new species from the temperate southern hemisphere and Indian Ocean. *Copeia* (4): 886–900.
- Marques, A.** 2001. Some Data on the Biology of Polymetme *Corythaeola* (Phosichthyidae), From Off the Portuguese South Coast, North East Atlantic. *Cybius* 25 (1): 100–102.
- Mauchline, J.** 1988. Growth and breeding of meso- and bathypelagic organisms of the Rockall Trough, northeastern Atlantic Ocean and evidence of seasonality. *Marine Biology* 98: 387-393.
- McGonagle, R. P., D. W. Kerstetter, D. Fenolio, and T. T. Sutton.** 2023. Ecomorphology of a predatory deep-sea fish family: does trophic specialization drive hyperspeciation?. *Frontiers in Marine Science* 10: 1056094.
- Moser, H. G., and W. Watson.** 1996. Stomiiformes. The Early Stages of Fishes in the California Current Region. *CalCOFI Atlas* 33: 244-246.
- Mundy, B. C.** 2005. Checklist of the fishes of the Hawaiian Archipelago. *Bishop Museum Bulletin in Zoology* (6): 1–704.
- Olivar, M. P., and L. E. Beckley.** 2022. Vertical distribution patterns of early stages of mesopelagic fishes along 110° E, south-east Indian Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* 201: 105111.
- Orlov, A. M., and A. M. Tokranov.** 2019. Checklist of deep-sea fishes of the Russian northwestern Pacific Ocean found at depths below 1000 m. *Progress in Oceanography* 176: 102143.
- Parin, N. V., and O. D. Borodulina.** 1990. Survey of the genus *Polymetme* (Photichthyidae) with a description of two new species. *Journal of Ichthyology* 30 (6):108–121.
- Quèro, J. C. H., C. Karrer, A. Post, and L. Saldanha (eds.).** 1990. Check-list of the fishes of the eastern tropical Atlantic (CLOFETA) Vol. 1. JNICT, Lisbon; SEI, Paris and UNESCO, Paris.

- Quèro, Jean-Claude, and J. Spitz.** 2004. Observations ichtyologiques de la faune mésopélagique du talus continental du golfe de Gascogne en 2003, p. 371–375. *In*: Annales de la Société des sciences naturelles de la Charente-Maritime, vol. 9, no. 4.
- Schaefer, S., R. K. Johnson, and J. Badcock.** 1986. Photichthyidae, p. 243–247. *In*: Smiths sea fishes. Smith M. M.; Heemstra, P. C. (eds.). Springer-Verlag, Berlin.
- Scott, W. B., and M. G. Scott.** 1988. Atlantic fishes of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences 219: 731.
- Shinohara, G., K. Yabe, M. Nakaya, G. Anma, S. Yamaguchi, and K. Amaoka.** 1994. Deep-sea fishes collected from the North Pacific by the T/S Oshoro-Marui. Bulletin of the Faculty of Fisheries, Hokkaido University 45 (2):48–80.
- Sutton, T. T. and T. L. Hopkins.** 1996. Trophic ecology of the stomiid (Pisces: Stomiidae) fish assemblage of the eastern Gulf of Mexico: strategies, selectivity and impact of a top mesopelagic predator group. Marine Biology 127:179–192.
- Williams, A., J. Koslow, A. Terauds, and K. Haskard.** 2001. Feeding ecology of five fishes from the mid-slope micronekton community off southern Tasmania, Australia. Marine Biology 139: 1177–1192.

SUPPLEMENTAL INFORMATION CHAPTER THREE

Supplemental Figures Chapter Three

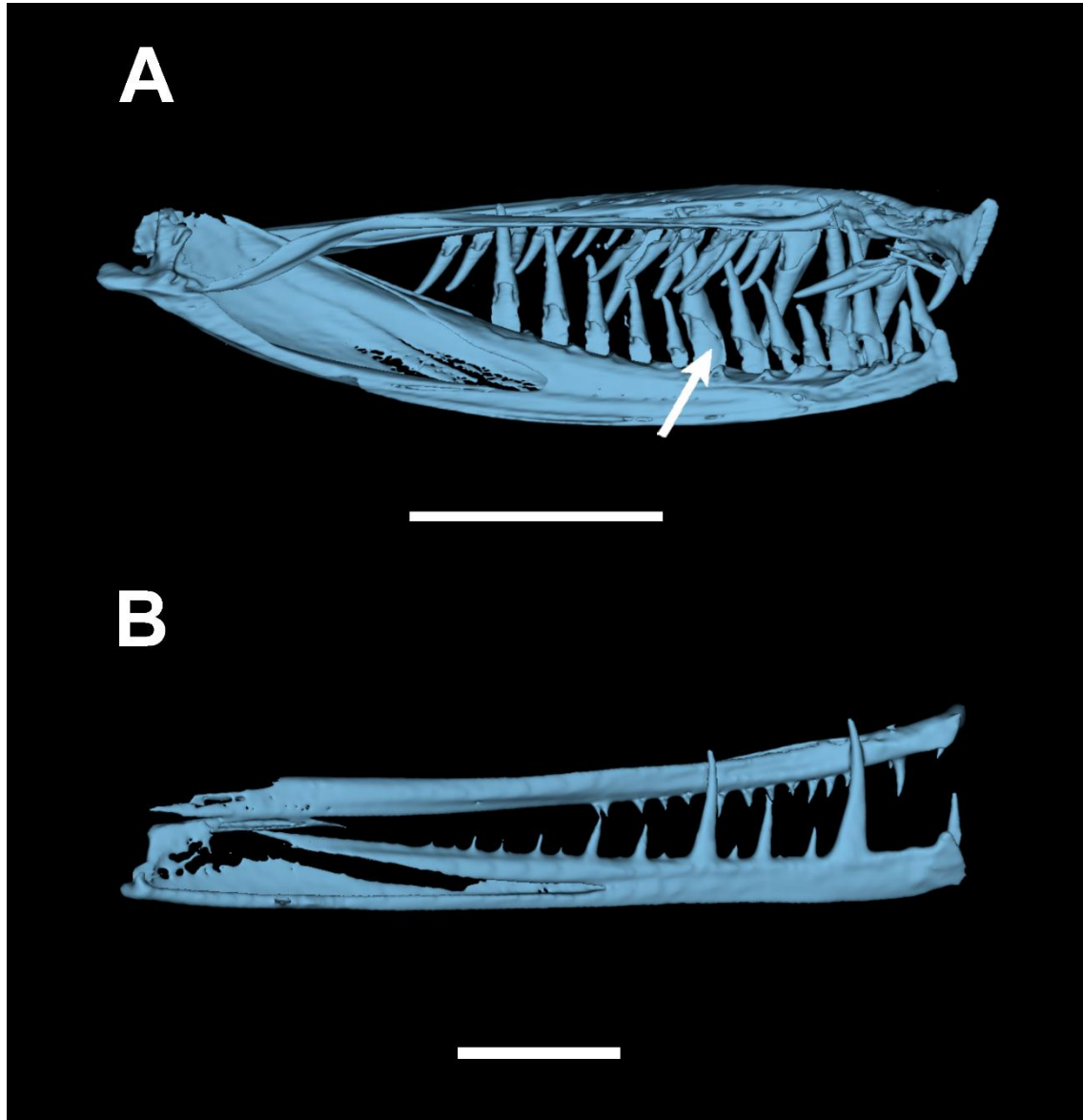


Figure S3.1. Types of tooth attachment in stomiiforms (*sensu* FINK, 1981). A=Type 3 of tooth attachment in oral jaws of *Melanostomias bartonbeani*, MCZ132357, 222.9 mm SL, micro-CT Scan of the upper and lower jaws, left side in medial view, with the white arrow indicating the unmineralized collagen area on the teeth posterior border; B=Type 1 of tooth attachment in oral jaws of *Malacosteus niger*, YPMICH025708, micro-CT Scan of the upper and lower jaws, left side in medial view showing the fixed teeth. Scale bars=10 mm

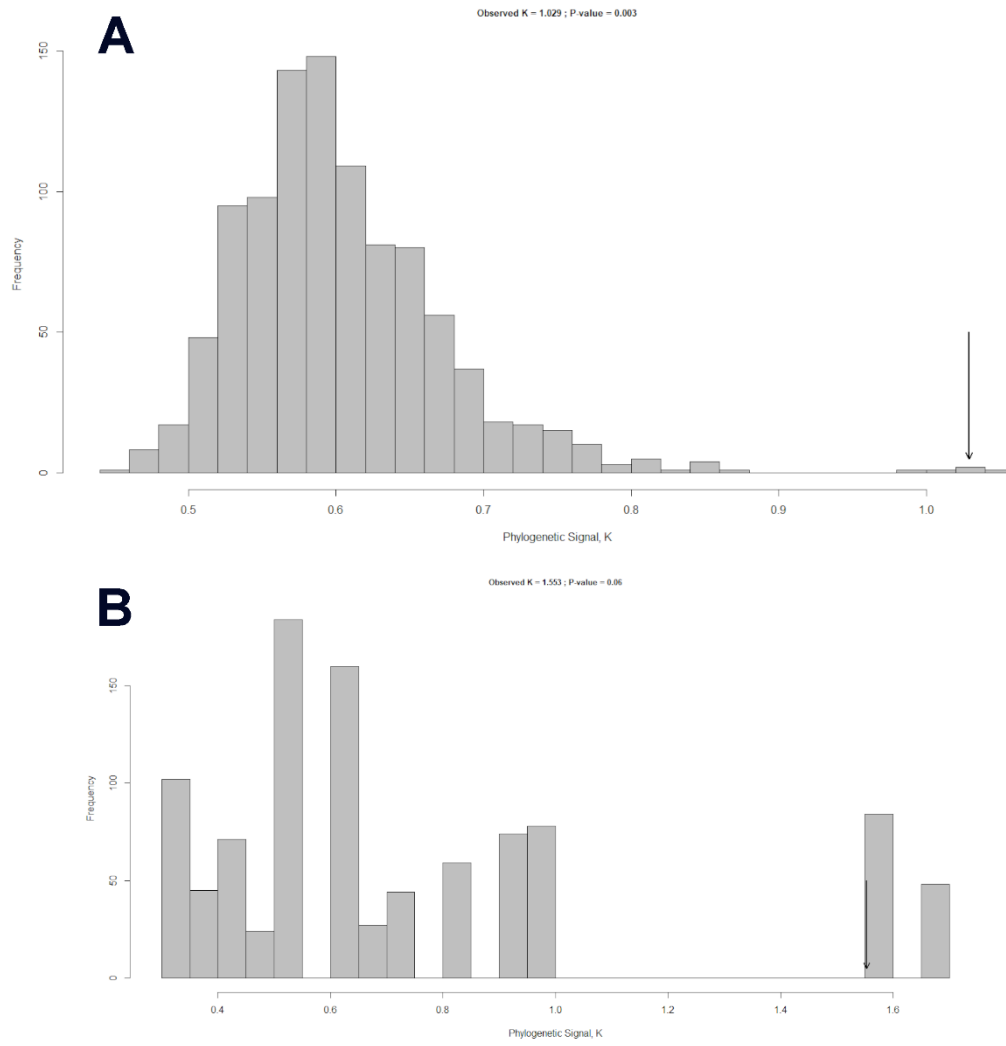


Figure S3.2. Phylogenetic signal (K) and p -values in stomiiform shapes (A), and Centroid Size (B).

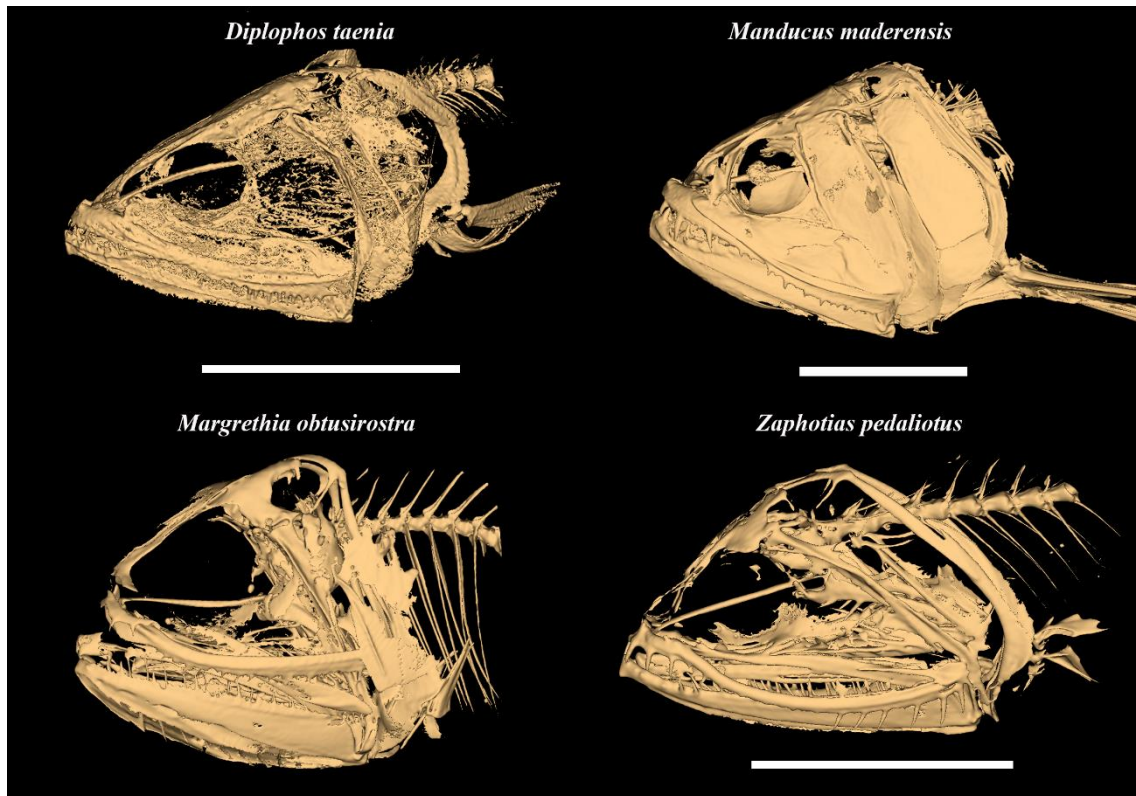


Figure S3.3. Micro CT-Scan of the skull in selected stomiiforms of families Diplophidae (*Diplophos taenia*, UW115370, and *Manducus maderensis*, MCZ61476, 191.9 mm SL), and Gonostomatidae (*Margrethia obtusirostra*, YPMICH027653, and *Zaphotias pedaliotus*, YPMICH027653). Scale bars=10 mm.

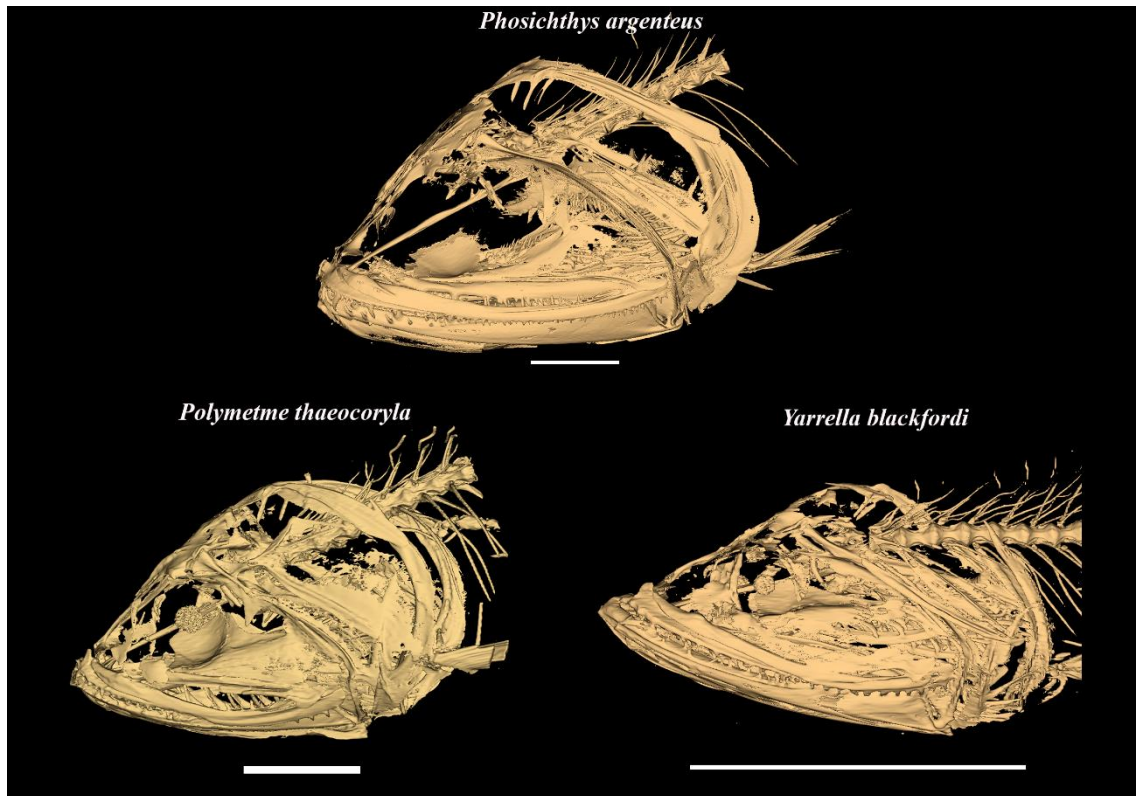


Figure S3.4. Micro CT-Scan of the skull in selected stomiiforms of the paraphyletic family Phosichthyidae (*Phosichthys argenteus*, MCZ61171, 237.2 mm SL, *Polymetme thaeocoryla*, USNM304218, 145.1 mm SL, and *Yarrella blackfordi*, MCZ126580, 154.4 mm SL). Scale bars = 10 mm.

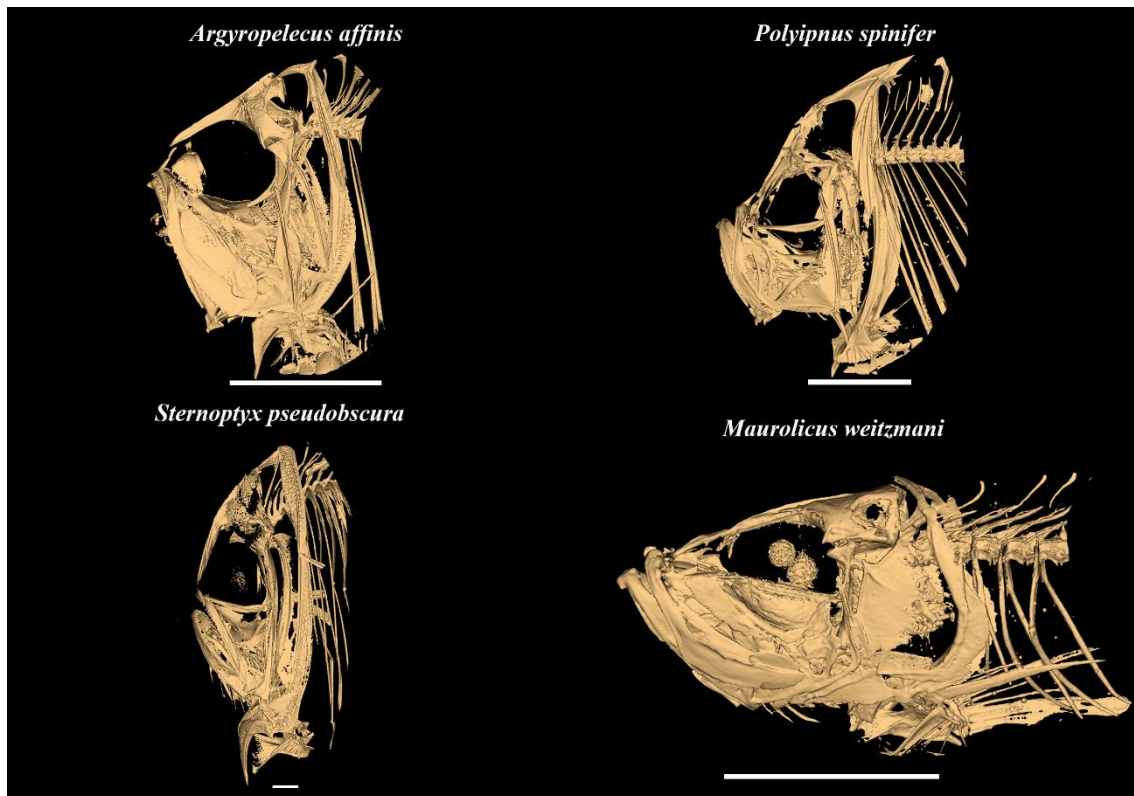


Figure S3.5. Micro CT-Scan of the skull in selected stomiiforms of the family Sternoptychidae, subfamilies Sternoptychinae (*Argyropelecus affinis*, SIO71-191, 44.0 mm SL, *Polyipnus spinifer*, USNM135514, 49.6 mm SL, and *Sternoptyx pseudobscura*, SIO66-536), and Maurolicinae (*Maurolicus weitzmani*, USNM391455, 58.3 mm SL). Scale bars = 10 mm.

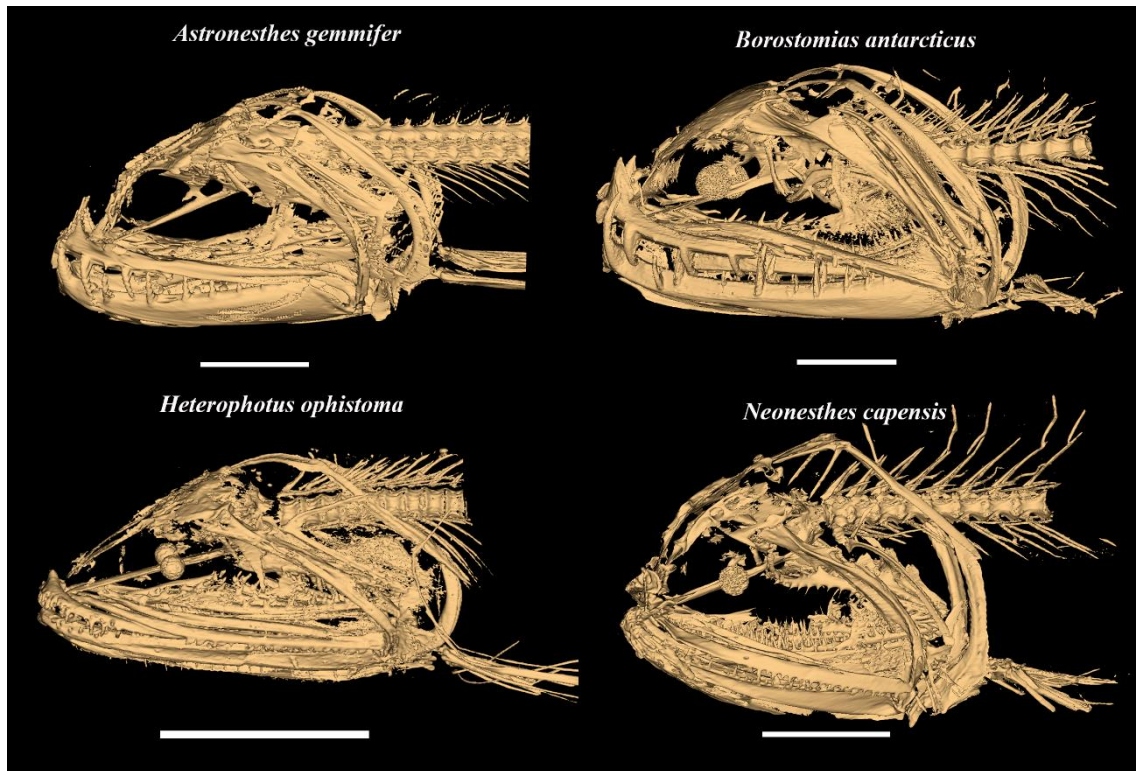


Figure S3.6. Micro CT-Scan of the skull in selected stomiiforms of the family Stomiidae, subfamily Astronesthinae (*Astronesthes gemmifer*, USNM436537, 151.7 mm SL, *Borostomias antarcticus*, SIO6145, 201.4 mm SL, *Heterophotus ophistoma*, SIO70341, 76.5 mm SL, and *Neonesthes capensis*, USNM454448, 153.4 mm SL). Scale bars = 10 mm.

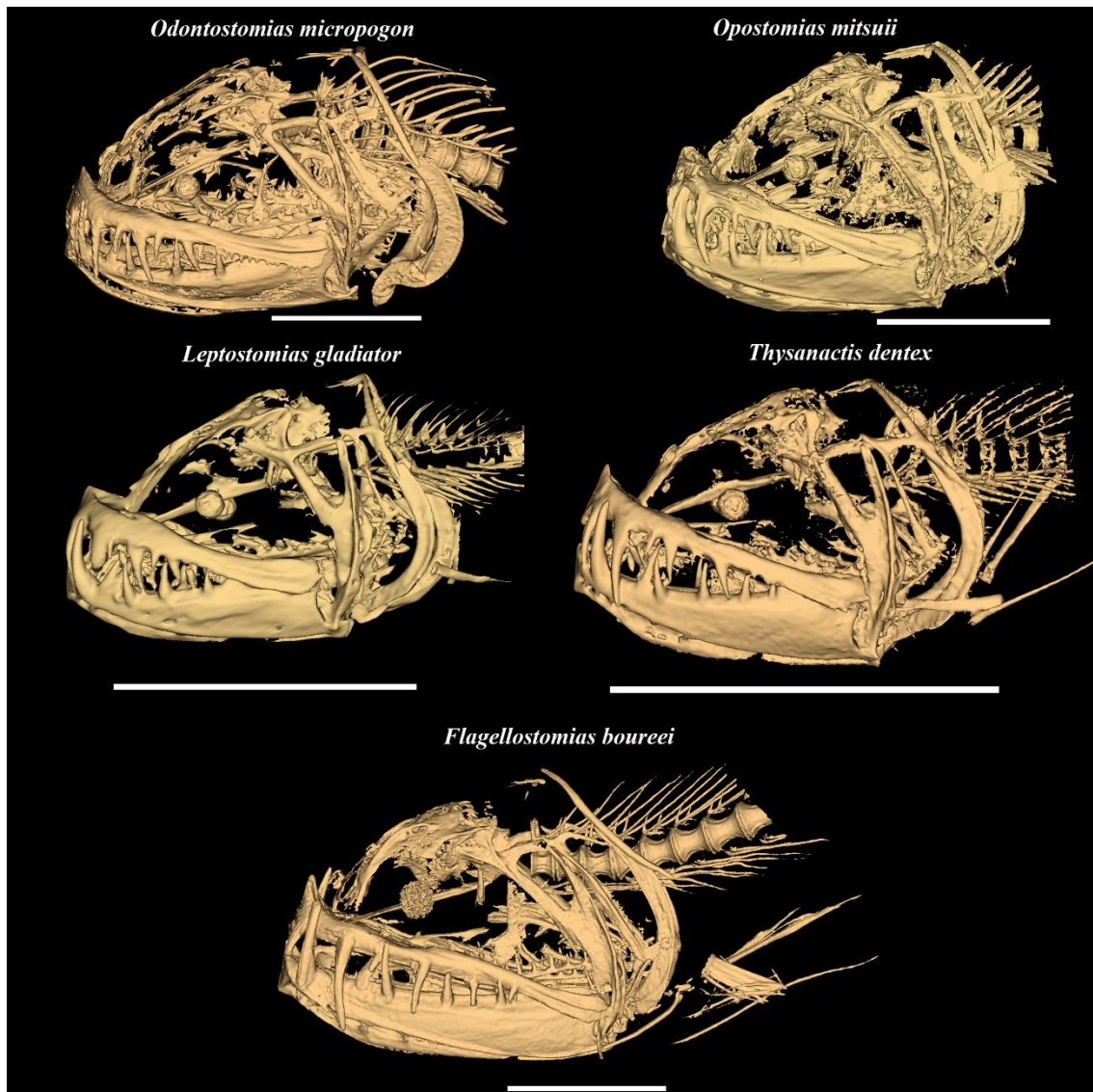


Figure S3.7. Micro CT-Scan of the skull in selected stomiiforms of the family Stomiidae, subfamily Melanostomiinae (*Odontostomias micropogon*, MCZ132152, 220.1 mm SL, *Opostomias mitsuï*, OS014651, 123.2 mm SL, *Leptostomias gladiator*, MCZ149495, 105.1 mm SL, *Thysanactis dentex*, UW150245, and *Flagellostomias boureei*, SIO76107, 178.1 mm SL). Scale bars = 10 mm.

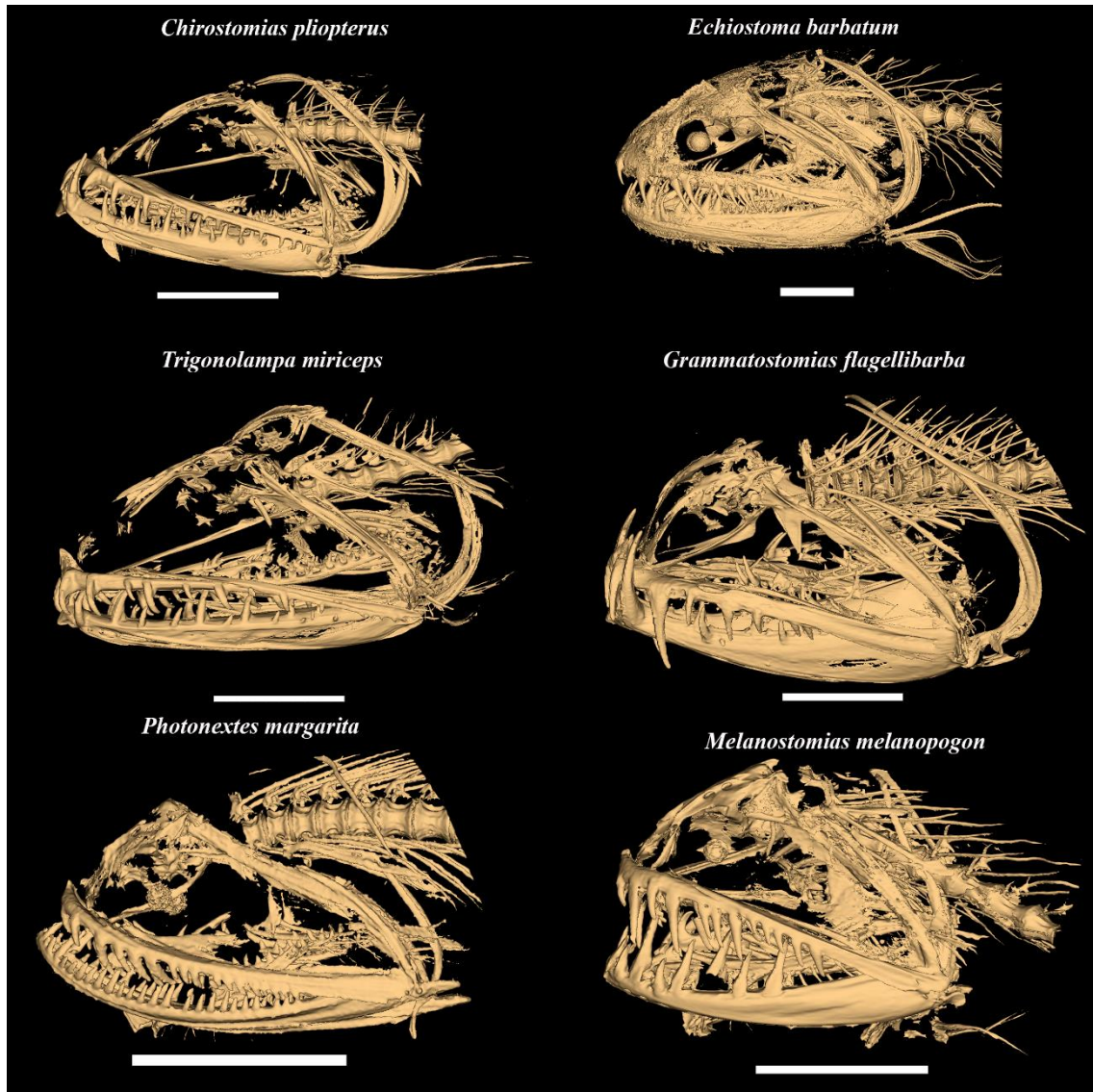


Figure S3.8. Micro CT-Scan of the skull in selected stomiiforms of the family Stomiidae, subfamily Melanostomiinae (*Chirostomias pliopterus*, MCZ132709, 157.7 mm SL, *Echiostoma barbatum*, UF168937, *Trigonolampa miriceps*, MCZ165921, 172.9 mm SL, *Grammatostomias flagellibarba*, MCZ164122, 202.7 mm SL, *Photonectes margarita*, SIO985, 122.9 mm SL, and *Melanostomias melanopogon*, MCZ132243, 149.5 mm SL). Scale bars = 10 mm.

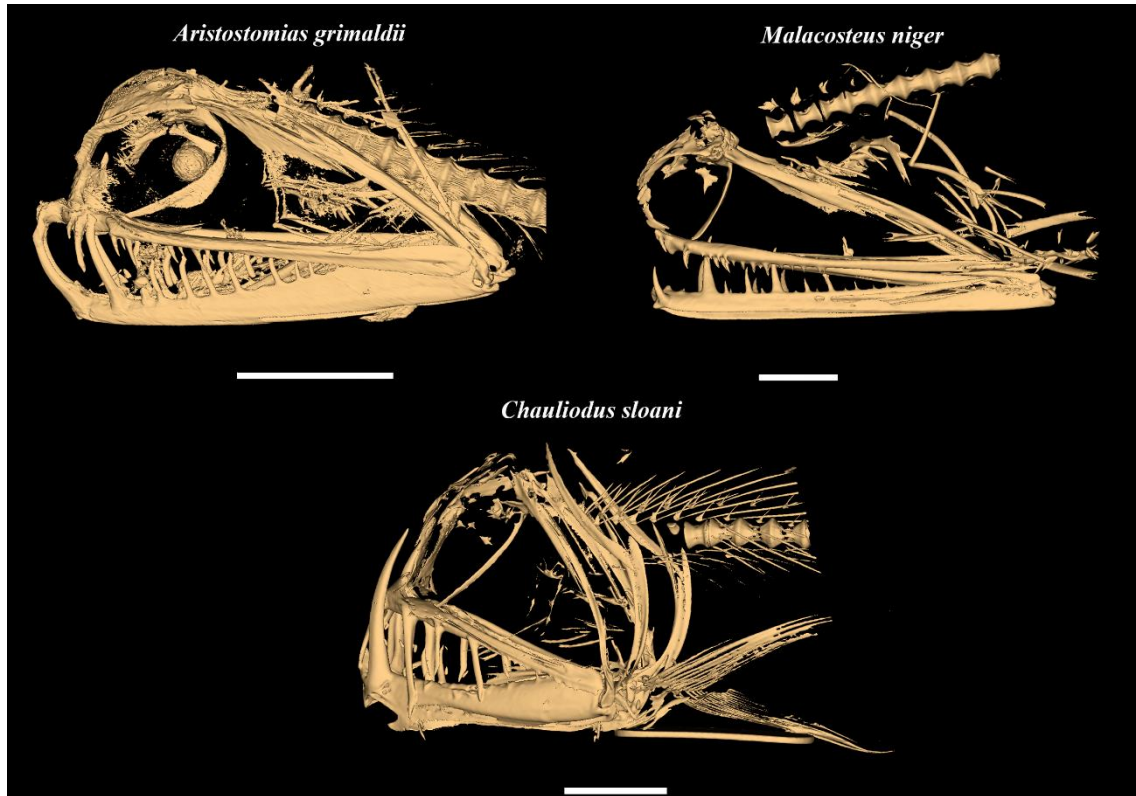


Figure S3.9. Micro CT-Scan of the skull in selected stomiiforms of the family Stomiidae, subfamilies Malacosteinae (*Aristostomias grimaldii*, MCZ93834, 99.3 mm SL, and *Malacosteus niger*, YPMICH025708), and Stomiinae (*Chauliodus sloani*, VIMS16520). Scale bars = 10 mm.

Supplemental Tables Chapter Three

Supplemental Table S3.1. Ecological traits of the Stomiiformes, encompassing their diet and depth, with respective references from the literature regarding these traits, alongside the specific diet and depth categories employed within this investigation. Asterisk means that diet type was inferred based on other species or genera from the same family.

| Species | Diet | Diet Reference | Diet category | Depth range | Depth reference | Depth category |
|--------------------------------|---|---|-------------------|-------------|--|-------------------|
| Diplophidae (2) | | | | | | |
| <i>Diplophos taenia</i> | Mostly on copepods and euphausiids | Hopkins <i>et al.</i> (1996); Clarke (1982) | Zooplanktivorous | 15-1,300 | Clarke (1974); Badcock (1984); Figueiredo <i>et al.</i> (2002); Mundy (2005); This study | Meso-Bathypelagic |
| <i>Manducus maderensis</i> | No information available | | Zooplanktivorous* | 0-927 | Badcock (1984); Schaefer <i>et al.</i> (1986); Figueiredo <i>et al.</i> (2002); This study | Mesopelagic |
| Gonostomatidae (2) | | | | | | |
| <i>Margrethia obtusirostra</i> | Mostly on copepods and euphausiids | Hopkins <i>et al.</i> (1996) | Zooplanktivorous | 100-1,500 | Mundy (2005); Fahay (2007); Olivar & Beckley (2022); This study | Meso-Bathypelagic |
| <i>Zaphotias pedaliotus</i> | Zooplankton | Badcock (1984) | Zooplanktivorous | 100-2,300 | Scott & Scott (1988); Olivar & Beckley (2022); This study | Meso-Bathypelagic |
| Phosichthyidae (3) | | | | | | |
| <i>Phosichthys argenteus</i> | Mostly on fishes; minor on decapods and euphausiids | Williams <i>et al.</i> (2001); Gaskett <i>et al.</i> (2010) | Generalists | 300-1,141 | Quèro <i>et al.</i> (1990); This study | Meso-Bathypelagic |

| | | | | | | |
|--------------------------------|---|--|-------------------|-----------|--|-------------------|
| <i>Polymetme thaecoryla</i> | Mostly on euphausiids | Marques (2000) | Zooplanktivorous | 213–1,400 | Parin & Borodulina (1990); This study | Meso–Bathypelagic |
| <i>Yarella blackfordi</i> | No information available | | Zooplanktivorous* | 350–1,000 | Quèro <i>et al.</i> (1990); Fahay (2007); This study | Mesopelagic |
| Sternoptychidae (4) | | | | | | |
| <i>Argyropelecus affinis</i> | Crustaceans (mostly), adult and larvae fishes; gelatinous invertebrates | Eduardo <i>et al.</i> (2020) | Generalists | 100–2,400 | Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic |
| <i>Maurolicus weitzmani</i> | Copepods (mostly), cladocerans, euphausiids and ostracods | Hopkins <i>et al.</i> (1996); Carmo <i>et al.</i> (2015) | Zooplanktivorous | 100–610 | Fahay (2007); This study | Mesopelagic |
| <i>Polyipnus spinifer</i> | Planktivorous, feeding on copepods, euphausiids, and mysids | Harold <i>et al.</i> (2002) | Zooplanktivorous | 150–611 | Harold (1994); This study | Mesopelagic |
| <i>Sternoptyx pseudobscura</i> | Mostly on crustaceans (amphipods and euphausiids) and polychaets in small-sized individuals; large-sized individuals prey on fish | Hopkins <i>et al.</i> (1996); Carmo <i>et al.</i> (2015); Eduardo <i>et al.</i> (2020) | Generalists | 350–2,450 | Gon (1990); Fahay (2007); Eduardo <i>et al.</i> (2020); This study | Meso–Bathypelagic |
| Stomiidae (18) | | | | | | |
| <i>Aristostomias grimaldii</i> | Piscivory centered on myctophids | Hopkins <i>et al.</i> (1996); Sutton & Hopkins (1996) | Piscivorous | 0–1,219 | Orlov & Tokranov (2019); This study | Meso–Bathypelagic |
| <i>Astronesthes gemifer</i> | Mostly on euphausiids, fewest on decapods and fishes | Hopkins <i>et al.</i> (1996); Sutton & Hopkins (1996) | Generalists | 58–1,266 | Clarke (1974); Suttom & Hopkins (1996); Mundy (2005); This study | Meso–Bathypelagic |
| <i>Borostomias antarcticus</i> | Fishes and crustaceans | Gibbs (1984) | Generalists | 350–2,500 | Fahay (2007); Banon <i>et al.</i> (2021); This study | Meso–Bathypelagic |
| <i>Chauliodus sloani</i> | Piscivory centered on myctophids and on other | Hopkins <i>et al.</i> (1996); Sutton & Hopkins (1996); | Piscivorous | 45–4,700 | Badcock (1970); Clarke (1974); Shinohara <i>et al.</i> (1994); Shinohara <i>et al.</i> | Meso–Bathypelagic |

| | | | | | | |
|--------------------------------------|--|---|-------------------|-----------|--|-------------------|
| | stomiiforms; some could also feed on euphausiids | Bataglia <i>et al.</i> (2018); Clarke (1982) | | | (1996); Suttom & Hopkins (1996); Eduardo <i>et al.</i> (2020); This study | |
| <i>Chirostomias pliopterus</i> | No information available | | Piscivorous* | 500–1,016 | Scott & Scott (1988); This study | Meso–Bathypelagic |
| <i>Echiostoma barbatum</i> | Piscivory; minor cephalopods | McGonagle <i>et al.</i> (2023) | Piscivorous | 30–4,200 | Clarke (1974); Suttom & Hopkins (1996); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic |
| <i>Flagellostomias boureei</i> | Piscivory | McGonagle <i>et al.</i> (2023) | Piscivorous | 50–1,825 | Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic |
| <i>Grammatostomias flagellibarba</i> | No information available | | Zooplanktivorous* | 800–1,636 | Suttom & Hopkins (1996); This study | Meso–Bathypelagic |
| <i>Heterophotus ophistoma</i> | Decapods and fishes | Clarke (1982) | Generalists | 50–1,850 | Clarke (1974); Suttom & Hopkins (1996); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic |
| <i>Leptostomias gladiator</i> | Piscivory | McGonagle <i>et al.</i> (2023) | Piscivorous | 0–5,000 | Badcock (1970); Suttom & Hopkins (1996); Orlov & Tokranov (2019); This study | Meso–Bathypelagic |
| <i>Malacosteus niger</i> | Mostly on copepods, euphausiids and decapods (rarely fish) | Hopkins <i>et al.</i> (1996); Sutton & Hopkins (1996); Sutton (2005); Clarke (1982) | Zooplanktivorous | 500–2,000 | Clarke (1974); Suttom & Hopkins (1996); Kenaley (2007); This study | Meso–Bathypelagic |
| <i>Melanostomias melanops</i> | Piscivory centered on myctophids | Hopkins <i>et al.</i> (1996); Sutton & Hopkins (1996); McGonagle <i>et al.</i> (2023) | Piscivorous | 50–1,545 | Clarke (1974); Mundy (2005); This study | Meso–Bathypelagic |
| <i>Neonesthes capensis</i> | Fishes and crustaceans | Gibbs (1984) | Generalists | 70–1,650 | Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic |

| | | | | | | |
|---------------------------------|---|--------------------------------|--------------|-----------|---|-------------------|
| <i>Odontostomias micropogon</i> | No information available | | Piscivorous* | 400–740 | This study | Mesopelagic |
| <i>Opostomias mitsuii</i> | No information available | | Piscivorous* | 50–1,500 | Mundy (2005); Orlov & Tokranov (2019); This study | Meso–Bathypelagic |
| <i>Photonectes margarita</i> | Piscivory | McGonagle <i>et al.</i> (2023) | Piscivorous | 0–2,400 | Suttom & Hopkins (1996); Mundy (2005); Fahay (2007); This study | Meso–Bathypelagic |
| <i>Thysanactis dentex</i> | Small individuals eat mostly euphausiids and copepods and large individuals eat large fish and crustacean | Clarke, 1982 | Generalists | 75–4,000 | Clarke (1974); Mundy (2005); This study | Meso–Bathypelagic |
| <i>Trigonolampa miriceps</i> | No information available | | Piscivorous | 915–1,860 | Gon (1990); This study | Meso–Bathypelagic |

Supplemental Table S3.2 Species of Stomiiformes used to create a genus-level phylomorphospace based on the molecular phylogeny produced by Rabosky *et al.* (2018).

| Family | Species |
|----------------------------|---|
| Diplophidae (1) | <i>Diplophos taenia</i> Günther, 1873 |
| Gonostomatidae (2) | <i>Margrethia obtusirostra</i> Jespersen and Tåning, 1919 <i>Zaphotias pedaliotus</i> (Goode and Bean, 1896) |
| Phosichthyidae (3) | <i>Phosichthys argenteus</i> Hutton, 1872 <i>Polymetme thaeocoryla</i> Parin and Borodulina, 1990 <i>Yarrella blackfordi</i> Goode and Bean, 1896 |
| Sternoptychidae (4) | <i>Argyropelecus affinis</i> Garman, 1899 <i>Maurolicus weitzmani</i> Parin and Kobylansky, 1993 <i>Polyipnus stereope</i> Jordan & Starks 1904 <i>Sternoptyx pseudobscura</i> Baird, 1971 |
| Stomiidae (18) | <i>Astronesthes gemmifer</i> Goode and Bean, 1896 <i>Aristostomias tittmani</i> Welsh, 1923 <i>Borostomias antarcticus</i> (Lönnerberg, 1905) <i>Chauliodus sloani</i> Bloch and Schneider, 1801 <i>Chirostomias pliopterus</i> Regan and Trewavas, 1930 <i>Echiostoma barbatum</i> Lowe, 1843 <i>Flagellostomias boureei</i> (Zugmayer, 1913) <i>Grammatostomias flagellibarba</i> Holt and Byrne, 1910 <i>Heterophotus ophistoma</i> Regan and Trewavas, 1929 <i>Leptostomias gladiator</i> (Zugmayer, 1911) |

Malacosteus niger Ayres 1848

Melanostomias margaritifer Regan & Trewavas 1930

Neonesthes capensis (Gilchrist and von Bonde, 1924)

Odontostomias micropogon Norman, 1930

Photonectes margarita (Goode and Bean, 1896)

Thysanactis dentex Regan and Trewavas, 1930

Trigonolampa miriceps Regan and Trewavas, 1930
