



**Universidade Federal do Rio Grande  
Instituto de Ciências Biológicas  
Pós-graduação em Biologia de  
Ambientes Aquáticos Continentais**



**Dimorfismo sexual e variação morfológica na  
cabeça de serpentes aquáticas e semiaquáticas  
revelados pela morfometria geométrica**

**Miriam Duarte de Oliveira**

**Orientador: Profa. Dra. Adriana Gava  
Coorientador: Prof. Dr. Daniel Loebmann**

**Rio Grande  
2024**



Universidade Federal do Rio Grande  
Instituto de Ciências Biológicas  
Pós-graduação em Biologia de Ambientes  
Aquáticos Continentais



**Dimorfismo sexual e diversidade morfológica na cabeça  
de serpentes aquáticas e semiaquáticas revelados  
pela morfometria geométrica**

**Aluno:** Miriam Duarte de Oliveira

**Orientador:** Profa. Dra. Adriana Gava

**Coorientador:** Prof. Dr. Daniel Loebmann

Dissertação apresentada ao Programa  
de Pós-graduação em Biologia de  
Ambientes Aquáticos Continentais como  
requisito parcial para a obtenção do  
título de Mestre em Biologia de  
Ambientes Aquáticos Continentais.

Rio Grande  
2024

## Ficha Catalográfica

O48d Oliveira, Miriam Duarte de.  
Dimorfismo sexual e diversidade morfológica na cabeça de  
serpentes aquáticas e semiaquáticas revelados pela morfometria  
geométrica / Miriam Duarte de Oliveira. – 2024.  
111 f.

Dissertação (mestrado) – Universidade Federal do Rio Grande –  
FURG, Programa de Pós-Graduação em Biologia de Ambientes  
Aquáticos Continentais, Rio Grande/RS, 2024.  
Orientadora: Dra. Adriana Gava.  
Coorientador: Dr. Daniel Loebmann.

1. Adaptação 2. *Erythrolamprus* 3. Evolução 4. *Helicops*  
5. Morfologia I. Gava, Adriana II. Loebmann, Daniel III. Título.

CDU 573(28)

Catálogo na Fonte: Bibliotecário José Paulo dos Santos CRB 10/2344




UNIVERSIDADE FEDERAL DO RIO GRANDE-FURG  
INSTITUTO DE CIÊNCIAS BIOLÓGICAS  
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA  
DE AMBIENTES AQUÁTICOS CONTINENTAIS  
Av. Itália s/n – Rio Grande/RS, Brasil. 96203-900 fone/fax: 53-3233.6848  
e-mail: [pgbac@furg.br](mailto:pgbac@furg.br)  
<https://ppgbac.furg.br>




---

## ATA DE DEFESA DE TESE DE MESTRADO EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS – Nº 003/2024


Às 14:00h (quatorze horas) do dia 26 (vinte e seis) do mês de abril de 2024 (dois mil e vinte e quatro), via Webconferência no endereço eletrônico (<https://conferenciaweb.mmp.br/sala/adriana-adriana-gava>), reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública da Dissertação de Mestrado da acadêmica **Miriam Duarte**. A Dissertação intitulada “**Dimorfismo sexual e variação morfológica na cabeça de serpentes aquáticas e semiaquáticas revelados pela morfometria geométrica**” foi avaliada pela Banca Examinadora composta pela Prof<sup>ª</sup>. Adriana Gava (Orientadora); Prof<sup>ª</sup>. Dra. Sônia Huckembeck (FURG), Prof. Dr. Márcio Repenning (PPGBAC/FURG) e Prof. Dr. Rodrigo Fornel (URI). Após a defesa e arguição pública, a Banca Examinadora reuniu-se para deliberação final e considerou a acadêmica **APROVADA**. Desta forma, a acadêmica concluiu mais uma das etapas necessárias para a obtenção do grau de **MESTRE EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 16:00h (dezesesseis horas) foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pela Acadêmica e pela Coordenadora do Curso.

Documento assinado digitalmente  
 **ADRIANA GAVA**  
Data: 26/04/2024 18:15:30-0300  
Verifique em <https://validar.iti.gov.br>


Prof<sup>ª</sup>. Dra. Adriana Gava (PPGBAC – orientador)

Documento assinado digitalmente  
 **MARCIO REPENNING**  
Data: 29/04/2024 18:51:55-0300  
Verifique em <https://validar.iti.gov.br>


Dr. Márcio Repenning (PPGBAC/FURG)

Documento assinado digitalmente  
 **SONIA HUCKEMBECK**  
Data: 30/04/2024 16:19:27-0300  
Verifique em <https://validar.iti.gov.br>

Prof<sup>ª</sup>. Dra. Sônia Huckembeck (FURG)

Documento assinado digitalmente  
 **RODRIGO FORNEL**  
Data: 02/05/2024 11:15:04-0300  
Verifique em <https://validar.iti.gov.br>

Dr. Rodrigo Fornel (URI)

Documento assinado digitalmente  
 **CAMILA DE MARTINEZ GASPAR MARTINS**  
Data: 24/05/2024 17:45:49-0300  
Verifique em <https://validar.iti.gov.br>

Prof<sup>ª</sup>. Dra. Camila De Martinez Gaspar Martins  
Coordenadora do Curso

## **AGRADECIMENTOS**

Essa dissertação é minha primeira contribuição original ao conhecimento científico até o momento e é fruto de muitos anos de sonhos e planejamentos sobre o dia em que eu iria oficialmente conseguir me dedicar a estudar o grupo das serpentes. Durante alguns anos da minha formação, dediquei meu tempo em desvendar algumas áreas de interesse da biologia, mas sempre tive um “pezinho” na herpetologia. Então realizar esta pesquisa foi primeiramente a realização de um sonho e, depois, o requisito para me tornar mestra.

Obviamente, um trabalho de dois anos de dedicação não se faz sozinho. Ao longo deste período algumas pessoas foram fundamentais na minha formação como cientista e também como ser humano.

Primeiramente gostaria de agradecer à minha orientadora, Dra. Adriana Gava, pelos ensinamentos, paciência e, principalmente, por ter a mente aberta e aceitar me orientar em uma área totalmente nova pra mim como foi a morfometria geométrica. Agradeço pelas reuniões de horas debatendo sobre quais metodologias utilizar e sobre quais os próximos passos a tomar. Muitas vezes discordamos sobre as ideias, mas assim se faz ciência. E por isso sou grata.

Não poderia deixar de agradecer ao meu coorientador Dr. Daniel Loebmann também, que aceitou me coorientar neste desafio sem nem antes me conhecer. Agradeço a ele por todos os ensinamentos teóricos e práticos, principalmente em campo, onde pude aprender muito também sobre as espécies de anuros da região. Minha admiração e respeito pela sua carreira são enormes.

Em seguida, esse trabalho não seria possível sem o apoio da minha mãe, pelo suporte psicológico, incentivo e compreensão que foram base para que eu conseguisse seguir em frente.

Não poderia deixar de citar também, meus amigos. Brenda e Luana são duas biólogas incríveis, com pensamentos e raciocínios únicos os quais eu tive o prazer de acompanhar desde a graduação. Obrigada por me ouvirem reclamar tantas vezes.

Ao meu amigo Luiz agradeço por me ouvir tantas vezes, pelos passeios no Cais, garrafas de vinho e térmicas de chimarrão tomadas. O teu apoio me permitiu desvirtuar o pensamento do trabalho diversas vezes e, por isso, sou grata.

Em relação ao apoio técnico, gostaria de agradecer enormemente às técnicas do laboratório de genética e biologia molecular, Daiane Carrasco e Loraine Morais, que me auxiliaram a medir os espécimes nos dias de calor de dezembro de 2022 e janeiro de 2023, aguentando o cheiro de álcool e formol por horas. Meu muito obrigado às duas!

Por último, mas não menos importante, agradeço à FURG, PPGBAC e CAPES, pela infraestrutura, apoio e suporte técnico e financeiro que possibilitaram a execução do meu trabalho.

## RESUMO

Esta dissertação possui dois capítulos que buscaram explorar a variação morfológica na cabeça de três espécies de serpentes aquáticas e semiaquáticas que ocorrem no Extremo sul do Brasil. O primeiro manuscrito testou a presença de dimorfismo sexual no tamanho e forma da cabeça de *Helicops infrataeniatus*. Constatamos que a espécie é dimórfica em relação ao tamanho da cabeça nas vistas dorsal, lateral e ventral e a forma também varia entre os sexos nas vistas dorsal e ventral. Concluímos que grande parte da variação morfológica se dá devido à alometria, o que pode estar relacionado a divergências de nicho entre os sexos. Também avaliamos como a alometria está presente em cada sexo e descrevemos a variação da forma da cabeça ao longo do desenvolvimento ontogenético. Na segunda parte, fizemos comparações intra e interespecíficas avaliando espécimes de *Erythrolamprus jaegeri coralliventris* e *Erythrolamprus poecilogyrus sublineatus*. Na primeira espécie, a cabeça das fêmeas é maior que a dos machos na vista dorsal e lateral, enquanto que na segunda apenas a região ventral é maior nas fêmeas. *Erythrolamprus poecilogyrus sublineatus* é dimórfica considerando as três vistas da cabeça: a vista dorsal é relativamente mais comprida e estreita nas fêmeas, a vista lateral é mais quadrada e os olhos são proporcionalmente menores e na vista ventral a cabeça das fêmeas é mais larga do que a dos machos. A cabeça de *E. j. coralliventris* diverge entre os sexos apenas na vista dorsal onde o focinho dos machos é mais largo que o das fêmeas. As espécies diferem entre si nas três vistas em relação a morfologia, onde *Erythrolamprus p. sublineatus* tem a vista dorsal mais arredondada e curta, olhos e boca proporcionalmente menores do que *Erythrolamprus j. coralliventris*, o que pode estar relacionado ao modo de vida das espécies.

**Palavras-chave:** Adaptação, *Erythrolamprus*, evolução, *Helicops*, morfologia

## ABSTRACT

This dissertation comprises two chapters that aimed to explore morphological variation in the heads of three species of aquatic and semi-aquatic snakes occurring in the southernmost region of Brazil. The first manuscript tested for the presence of sexual dimorphism in size and shape of the head in *Helicops infrataeniatus*. We found that the species is dimorphic in head size in dorsal, lateral, and ventral views, and shape also varies between sexes in dorsal and ventral views. We concluded that much of the morphological variation is due to allometry, which may be related to niche divergence between sexes. We also assessed how allometry is present in each sex and described variation in head shape throughout ontogenetic development. In the second part, we made intra- and interspecific comparisons evaluating specimens of *Erythrolamprus j. coralliventris* and *Erythrolamprus p. sublineatus*. In the former, females' heads are proportionally larger than males' in dorsal and lateral views, while in the latter, only the ventral region is larger in females. *Erythrolamprus p. sublineatus* is dimorphic across all three head views: the dorsal view is relatively longer and narrower in females, the lateral view is squarer, with proportionally smaller eyes, and in the ventral view, females' heads are wider than males. The head of *E. j. coralliventris* differs between sexes only in the dorsal view, in which males' snouts are wider than females. The species also differ morphologically in all three views, in which *Erythrolamprus p. sublineatus* has a more rounded and shorter dorsal view, with smaller eyes and mouth than *Erythrolamprus j. coralliventris*, which may be related to the species' lifestyles.

**Key-words:** Adaptation, *Erythrolamprus*, evolution, *Helicops*, morphology

## APRESENTAÇÃO

O estudo da morfologia dos animais é usado desde os primórdios da ciência para a formação de grupos e na classificação dos organismos. No entanto, ao longo do avanço dos estudos científicos, se mostrou necessário considerar ferramentas que permitissem analisar de maneira matemática a forma das estruturas. Nesses casos, as serpentes vêm se mostrando um grupo de grande importância na comparação de estruturas morfológicas, uma vez que possuem um corpo alongado e cilíndrico sem membros e com a quantidade limitada de caracteres corporais. Esta dissertação é composta por dois capítulos redigidos em formato de artigo científico a serem submetidos para publicação, os quais utilizaram técnicas de morfometria geométrica objetivando a comparação intra e interespecífica de três espécies de serpentes aquática e semiaquáticas. O primeiro capítulo buscou verificar a existência de dimorfismo sexual no tamanho e forma da cabeça de *Helicops infrataeniatus* utilizando a morfometria geométrica como ferramenta. Foram feitas análises de alometria ontogenética buscando elucidar as mudanças que ocorrem na morfologia da cabeça na espécie ao longo do desenvolvimento e análises de dimorfismo sexual, comparando a morfologia de machos e fêmeas. No segundo manuscrito as mesmas comparações entre os sexos foram executadas, adicionando comparações morfológicas entre *Erythrolamprus poecilogyrus sublineatus* e *Erythrolamprus jaegeri coralliventris*.



## SUMÁRIO

RESUMO .....	IV
ABSTRACT .....	V
APRESENTAÇÃO .....	VI
LISTA DE FIGURAS .....	VIII
LISTA DE TABELAS .....	X
CAPÍTULO 2.....	X
INTRODUÇÃO GERAL.....	11
ADAPTAÇÕES MORFOLÓGICAS, MORFOMETRIA LINEAR, GEOMÉTRICA E ALOMETRIA .....	11
SERPENTES E SUAS ADAPTAÇÕES .....	12
DIMORFISMO SEXUAL .....	13
ESPÉCIES FOCO .....	15
JUSTIFICATIVA .....	19
OBJETIVOS ESPECÍFICOS.....	19
HIPÓTESES .....	19
REFERÊNCIAS BIBLIOGRÁFICAS .....	20
CAPÍTULO 1.....	25
SEXUAL DIMORPHISM, ONTOGENY AND ALLOMETRY OF WATER SNAKE <i>HELICOPS</i> <i>INFRATAENIATUS</i> (SERPENTES: XENODONTINAE) REVEALED BY GEOMETRIC MORPHOMETRICS .....	25
ABSTRACT .....	28
DECLARATION OF COMPETING INTEREST .....	39
ACKNOWLEDGMENTS .....	39
CAPÍTULO 2 .....	54
EVALUATING SEXUAL AND MORPHOLOGICAL VARIATION IN HEAD SHAPE IN <i>ERYTHROLAMPRUS</i> <i>POECILOGYRUS SUBLINEATUS</i> AND <i>ERYTHROLAMPRUS JAEGERI CORALLIVENTRIS</i> (SERPENTES: DIPSADIDAE).....	54
REFERENCES .....	67
CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS .....	80
ANEXO I.....	82
GUIDE FOR AUTHORS – ZOOLOGY.....	82
ANEXO II: .....	96
INSTRUCTIONS FOR THE AUTHORS - AMPHIBIA-REPTILIA JOURNAL .....	96

## LISTA DE FIGURAS

### Introdução Geral

Figura 1: Indivíduo de *Helicops infrataeniatus*. Foto: Daniel Loebmann.

Figura 2: Padrões de coloração entre as subespécies de *Erythrolamprus poecilogyrus*. *Erythrolamprus p. caesius* (F), *E. p. poecilogyrus* (G), *E. p. schotii* (H) e *E. p. sublineatus* (I). Retirado de Entiauspe-Neto et al., 2021.

Figura 3: *Erythrolamprus poecilogyrus sublineatus* predando *Elachistocleis bicolor*.

Figura 4: Representante de *Erythrolamprus jaegeri coralliventris*. Imagem: Leandro Malta Borges, biodiversity4all.org.

### Capítulo 1.....

**Fig. 1:** Illustration of two-dimensional landmarks positioned on the head of the water snake *Helicops infrataeniatus* from the Coastal Plain of Rio Grande do Sul. The identified scales include parietal (P), frontal (F), supraocular (SO), prefrontal (PF), internasal (IN), supralabial (S) and infralabial (I). A) Dorsal view of the head. B) Lateral view of the head. C) Ventral view (see also supplementary Table 1 for descriptions of landmarks).

**Fig. 2:** Boxplot of log of the natural logarithm of centroid size centroid size across *Helicops infrataeniatus* sexes and age groups. Limits on boxes (light males and dark females) correspond to the first and third quartiles and the internal black line is the median. Divergent letters (a, b) indicate significant difference.

**Fig. 3:** Multivariate regression of head shape over size in dorsal, lateral and ventral view of females (A, C and E) and males (B, D and F) for *Helicops infrataeniatus*. Females are hollow dots and males are full. Wireframe is the variation over centroid size. Red shape is the average configuration of the head and black is the variation over the axes.

**Fig. 4:** Principal components analysis of dorsal, lateral and ventral view for juveniles (A, B and C) and adults (D, E and F) for *Helicops infrataeniatus*. Females are brown dots and males are black. Wireframe is the variation over centroid size in which red shape is de average and black is the variation over the axes.

**Fig. 5:** Principal components analysis after size correction for dorsal, lateral and ventral view for juveniles (A, B and C) and adults (D, E and F) for *Helicops infrataeniatus*. Females are brown dots and males are black. Wireframe is the variation over centroid size in which red shape is de average and black is the variation over the axes.

**Capítulo 2**.....

**Fig. 1:** Illustration of two-dimensional landmarks used in *Erythrolamprus poecilogyrus sublineatus* and *E. jaegeri coralliventris* from the Coastal Plain of Rio Grande do Sul. The identified scales include parietal (P), frontal (F), supraocular (SO), prefrontal (PF), internasal (IN), and supralabial (S), and infralabial (I). A) Dorsal view of the head. B) Lateral view of the head. C) Ventral view (see also supplementary Table 1 for descriptions of landmarks).

**Fig. 2:** Boxplot of the natural logarithm of centroid size of *Erythrolamprus jaegeri coralliventris* (A) and *E. poecilogyrus sublineatus* (B) males (brown) and females (black). Limits on boxes correspond to the first and third quartiles and the internal black line is the median. Divergent letters (a, b) indicate significant differences.

**Fig. 3:** Canonical variates analysis for dorsal (A), lateral (B) and ventral (C) view for *Erythrolamprus jaegeri coralliventris* (blue) and *E. poecilogyrus sublineatus* (green). Females are light dots and males are dark. Wireframe is the variation over centroid size in which red shape is de average and black is the variation over the axes.

**Fig. 4:** Multivariate regression of head shape over size in dorsal, lateral and ventral view of females (A, B and C) and males (D, E and F) for *Erythrolamprus jaegeri coralliventris*. Females are grey dots and males are black. Wireframe is the variation over centroid size. Red shape is the average configuration of the head and black is the variation over the axe.

**Fig. 5:** Multivariate regression of head shape over size in dorsal, lateral and ventral view of females (A, B and C) and males (D, E and F) for *Erythrolamprus poecilogyrus sublineatus*. Females are grey dots and males are black. Wireframe is the variation over centroid size. Red shape is the average configuration of the head and black is the variation over the axe.

## LISTA DE TABELAS

### Capítulo 1.....

**Table 1.** Two-way PERMANOVA on shape variables to test the influence of the age group and the sex in head shape of *Helicops infrataeniatus* considering the general dataset. Df = degrees of freedom; SS = sum of squares; MS = mean square; Rsq = R-Squared ( $R^2$ ). Significance is highlighted in italics

**Table 2.** Multivariate regressions of head shape over size data (natural logarithm of centroid size) for both male and female of *Helicops infrataeniatus* in juvenile and adult stage of development. Significance is highlighted in italics

**Table 3.** One-way PERMANOVA performed over the head shape of juveniles and adults' dataset of *Helicops infrataeniatus*. SS= sum of squares. Significance is highlighted in italics

### Capítulo 2.....

**Table 1:** Multivariate regressions values of head shape variables over size variable (lnCS) for both male and female. Significance is highlighted in italics.

**Table 2:** One-way Permutation Multivariate Analysis of Variance (PERMANOVA) of the influence of the sex and species in head shape of dataset. (SS = sum of squares. Significance is highlighted in italics.

## INTRODUÇÃO GERAL

### Adaptações morfológicas, morfometria linear, geométrica e alometria

As propriedades do ambiente e a forma com que os animais utilizam os recursos disponíveis agem como moduladores da evolução biológica, já que impõem restrições físicas aos organismos constantemente expostos à seleção natural (Martins et al., 2001; Segall et al., 2016). Além disso, as adaptações a condições ecológicas distintas são as principais responsáveis pelo surgimento e pela manutenção da diversidade de caracteres morfológicos que definem as espécies (Schluter, 2000). Dessa forma, é razoável assumir que as especificidades do ambiente ocupado e o modo de vida dos animais serão refletidos nas suas características morfológicas.

Os organismos podem desenvolver respostas distintas conforme o contexto ambiental em que estão inseridos. Quando populações da mesma espécie são expostas a pressões de seleção divergentes podem responder de duas formas: (1) conservando características morfológicas similares devido a restrições filogenéticas ou (2) diferenciando-se umas das outras por consequência do processo de adaptação por seleção natural (Klaczko et al., 2016). Por outro lado, a exposição de duas espécies filogeneticamente próximas a condições ambientais distintas pode ampliar as diferenças morfológicas entre elas, e, ao contrário, quando permanecem em situações semelhantes, pode ocorrer a evolução de caracteres análogos, processo conhecido como convergência evolutiva (Klaczko et al., 2016; Segall et al., 2016).

Ângulos e medidas lineares, como o comprimento, a largura, a altura e a profundidade das estruturas, além de índices que utilizam a combinação destas medidas são frequentemente utilizados como metodologia objetivando quantificar e comparar a variação de caracteres entre os grupos (Fernandes et al., 2004.; Mesquita, 2010). Embora úteis e bastante difundidos, estes métodos de morfometria tradicional fornecem informações lineares restritas sobre o objeto analisado, sendo incapazes de incluir a forma das estruturas nas análises, limitando a quantidade de informação que se pode obter, excluindo a possibilidade de observar a variação na forma (Zelditch et al., 2004).

A morfometria geométrica vem se popularizando nos estudos que buscam identificar diferenças morfológicas inter e intraespecíficas, já que permite análises mais detalhadas das partes corporais, possibilitando a comparação da forma e a aplicação de análises estatísticas mais completas (Zelditch et al., 2004; Tamagnini et al., 2018). A partir de imagens dos caracteres de interesse, digitalizam-se pontos de referência homólogos (marcos anatômicos = *landmarks*) e são gerados vetores com coordenadas específicas que poderão ser comparadas entre os espécimes. Para isso, estas coordenadas são sobrepostas a partir da Análise

Generalizada de Procrustes (GPA) que elimina os efeitos de rotação, escala e posição cometidos no momento da tomada das fotografias subtraindo as coordenadas do centroide das coordenadas x e y de cada marco anatômico, dividindo as coordenadas x e y pelo tamanho do centroide e rotacionando as imagens a partir de uma configuração de uma das imagens de referência (Zelditch et al., 2004). Em seguida, as coordenadas de Procrustes são expressas em uma matriz de covariância.

Somada aos estudos de forma, a alometria é uma das características comumente mensuradas em estudos que aplicam a morfometria geométrica como metodologia (Andjelkovic et al., 2016a, b; Klaczko et al., 2016; Murta-Fonseca et al., 2019; Tamagnini et al., 2018). Sendo uma condição natural presente nos animais, pode ser definida como a influência do tamanho (e.g. do corpo, da cauda, da cabeça) sobre a forma de um caractere morfológico (Klingenberg, 2016). Atualmente, existem dois ramos de estudos alométricos com pensamentos distintos sobre como tratar questões de forma e de tamanho: a escola de Huxley-Jolicoeur, a qual considera que modificações na forma do corpo são consequências do tamanho e, portanto, não podem ser separadas; e a escola de Gould-Mousimann, que afirma a relação de covariância entre tamanho e forma e estuda os dois conceitos isoladamente (Klingenberg 2016). Dependendo do contexto, a alometria pode ser classificada em evolutiva, quando a comparação da influência do tamanho sobre a forma ocorre entre duas ou mais espécies; estática, quando compara-se a alometria entre organismos da mesma espécie e mesmo sexo e/ou estágio do desenvolvimento ou em alometria ontogenética, quando a comparação se dá entre dois ou mais estágios do desenvolvimento (Klingenberg & Zimmermann, 1992; Andjelkovic et al., 2016a; Silva et al., 2017a).

### **Serpentes e suas adaptações**

A Subordem Serpentes abrange hoje cerca de 4.070 espécies de animais com grande sucesso evolutivo (Cundall & Greene, 2000; Uetz, 2024). Prova deste sucesso é a diversidade de habitats e de ambientes colonizados pelo grupo, que não pode ser encontrado apenas nas regiões polares do planeta (Campbell & Lamar, 2004). Logo, como esperado devido à grande diversidade de espécies e a variedade de nichos ocupados, as serpentes formam um grupo que apresenta ampla variedade em relação à dieta, ecologia e morfologia (Cundall & Greene, 2000; Savitzky, 1983).

Em relação aos hábitos de vida, atualmente encontramos serpentes arborícolas, terrícolas, aquáticas, marinhas e fossoriais com uma série de adaptações na cabeça que refletem as características do seu habitat (Cadle & Greene, 1993; Martins, 1993; Martins & Oliveira, 1999). Serpentes de hábitos fossoriais, por exemplo, desenvolveram modificações para melhorar a

locomoção e a escavação de túneis e tendem a apresentar a cabeça reduzida com crânio compacto, olhos reduzidos ou vestigiais e escamas fusionadas (Savitzky, 1983; de Lema, 2002). A predação e o forrageio embaixo d'água também é um comportamento desafiador para as serpentes aquáticas, já que qualquer movimento executado neste ambiente é influenciado pelas forças de inércia e de arrasto (Segall et al., 2016). Desse modo, estes animais desenvolveram particularidades relacionadas ao aperfeiçoamento do nado e do forrageamento, entre elas, uma cabeça mais estreita (Rossman, 2000; Hibbitts e Fitzgerald, 2005; Segall et al., 2016) e a localização dorsal das narinas e dos olhos em comparação com as serpentes não-aquáticas (Segall et al., 2016; Silva et al., 2017b).

Além disso, existe uma estreita conexão entre o formato da cabeça e o tipo e tamanho de presa consumido, uma vez que a morfologia desta estrutura limita a abertura máxima da boca (Silva et al., 2017b; Vincent et al., 2006). Klaczko et al. (2016) comparou serpentes do clado Xenodontine e encontrou correlações significativas entre o formato do crânio e a dieta, concluindo que as serpentes piscívoras têm o crânio e a mandíbula alongados em relação às predadoras de anuros. Tamagnini et al. (2018) também observaram este padrão morfológico quando descreveram a morfologia da cabeça da serpente anurofágica *Natrix helvetica* como mais larga e encurtada em relação à víbora *Vipera berus*, que tem hábitos alimentares generalistas.

### **Dimorfismo sexual**

A variação intraespecífica é um dos componentes principais da evolução por seleção natural, já que permite a sobrevivência e o sucesso evolutivo dos mais adaptados às condições ambientais (Darwin, 1871). O dimorfismo sexual, por exemplo, é a variação intraespecífica nas características morfológicas de acordo com o sexo e a sua origem e modulação são comumente explicados pela seleção sexual. Nesta perspectiva, a manutenção das variações intersexuais seria garantida ao existir uma preferência de características relacionadas ao sexo que privilegiam fenótipos específicos (Darwin, 1871). Nas serpentes, a seleção direta de companheiros raramente acontece (Shine, 1993). Quando existe, ela se dá pelo combate entre os machos, que competem entre si para ter acesso a fêmeas (Shine, 1991.; Vincent et al., 2006). Logo, a seleção sexual não explica todos os tipos de dimorfismo sexual existentes nas serpentes e, com isso, outras hipóteses buscam explicar o surgimento e a manutenção das diferenças entre os sexos: a seleção relacionada à fecundidade e a divergência de nicho (Darwin, 1871;; Ford & Seigel, 1989; Kaliontzopolou et al., 2007; dos Santos et al., 2022).

A teoria relacionada à seleção de fecundidade sugere que as fêmeas com maior tamanho corporal serão privilegiadas no momento da reprodução, já que um corpo maior reflete um corpo com mais espaço para o armazenamento de ovos ou filhotes (Elgee & Blouin-Demers, 2011; Ford & Seigel, 1989). De fato, na maioria das espécies de serpentes, as fêmeas possuem maior tamanho corporal em relação aos machos (Shine, 1993). Os machos, por sua vez, possuem a cauda mais longa como adaptação para alocar o hemipenis (Regnet et al., 2022; Cundall & Greene, 2000). Por último, a hipótese de seleção por diferenças de nicho busca explicar que machos e fêmeas da mesma espécie podem utilizar os recursos do ambiente de maneiras distintas e que estas divergências de comportamento podem resultar em adaptações morfológicas (Darwin, 1871; dos Santos et al., 2022).

No entanto, o dimorfismo sexual no formato da cabeça nas serpentes só poderia ser explicado pela seleção sexual em espécies onde a cabeça desempenha um papel importante nas interações sexuais (e.g. rituais de combate entre os machos ou *display* de exibição) (Camilleri & Shine, 1990). Em outras situações, o dimorfismo sexual na forma da cabeça pode surgir a partir de divergências de nicho entre os sexos e, também, a partir de diferenças nos estímulos visuais entre os sexos desenvolvidas como defesas contra predadores e resultando no alargamento da cabeça por causa do maior desenvolvimento dos órgãos sensoriais e do cérebro em um dos sexos (Camilleri & Shine, 1990). Na maioria dos casos, contudo, o consumo de presas diferentes ou com tamanhos distintos por machos e fêmeas pode ser uma explicação mais adequada para o surgimento e manutenção da variação morfológica da cabeça neste grupo (Camilleri & Shine, 1990).

Abegg et al. (2020) encontraram diferenças relacionadas ao sexo na forma da cabeça de *Atractus reticulatus* (Boulenger, 1885), serpente especialista em predação de minhocas. Nessa espécie, a cabeça dos machos tende a ter um formato mais alongado e mais afinado com escamas frontais e pré-frontal mais amplas e pré-frontal alongada em relação às fêmeas. Por se tratar de um grupo de serpentes de hábitos especialistas, os autores atribuíram a existência de dimorfismo sexual ao consumo pelos sexos de presas de tamanhos distintos. *Crotalus polysticus* Cope, 1865 também possui dimorfismo sexual no formato da cabeça, onde os machos, que alcançam tamanhos corporais maiores e se alimentam de presas maiores, têm a cabeça maior e mais larga do que as fêmeas (Meik et al. 2012). Além disso, o alto grau de especialização no consumo de ovos pela serpente *Aipysurus eydouxii* (Gray 1849) parece ser responsável pela ausência de dimorfismo sexual no formato da cabeça, uma vez que restringe a diversidade de presas que podem ser predadas (Borczyk et al., 2021).



Alguns autores ainda pontuam que o alargamento da vista dorsal da cabeça é esperado no sexo que atinge maior tamanho corporal, uma vez que uma cabeça mais larga permite a captura de presas maiores, já que aloca mais força na mordida e que o gasto de energia para manter um corpo maior precisa ser compensado com o consumo de presas maiores ou de uma quantidade maior de refeições (Borczyk et al., 2021; Elgee & Blouin-Demers, 2011; Meik et al., 2012). Assim, por sua estreita relação com a captura e ingestão de alimento e, conseqüentemente, com a sobrevivência do animal, é possível observar que a cabeça das serpentes é a parte do corpo mais propícia a sofrer adaptações ao modo de vida, já que está constantemente exposta às pressões de seleção do ambiente (Hampton, 2011; Klaczko et al., 2016; Vincent et al., 2006).

### **Espécies foco**

A subfamília Xenodontina atualmente é composta por pelo menos 48 gêneros (Grazziotin et al., 2012). É um grupo muito bem distribuído, presente em todo o continente americano e bastante diversificado na América do Sul (Klaczko et al., 2016; Zaher, 1999). No Brasil, podem ser encontradas pelo menos 194 espécies, representando 50% das espécies de serpentes do país (Franco & Ferreira, 2002). Especialmente, a subfamília dispõe de uma ampla diversidade morfológica, ecológica e de hábitos alimentares, predando desde invertebrados até peixes, anuros, lagartos e pequenos mamíferos, o que faz deste um grupo ideal para estudos relacionados com a diversificação e o surgimento de modificações morfológicas (Klaczko et al., 2016; Zaher et al., 2009).

A tribo Hydropsini inclui os gêneros *Helicops* Wagler, 1828, *Hydrops* Wagler, 1830, e *Pseudoeryx* Fitzinger, 1826 e é um clado que engloba serpentes aquáticas endêmicas da América do Sul, possuindo bom suporte na literatura (Grazziotin et al., 2012; Zaher et al., 2009). Essas serpentes possuem características típicas de espécies de hábitos aquáticos, como as narinas e olhos posicionados na região dorsal da cabeça (Regnet et al., 2022) e escama internasal única (Moraes-da-Silva et al., 2019). *Helicops* é o mais diverso, compreendendo aproximadamente 21 espécies que podem ser distinguidas dos outros gêneros pela presença de escamas dorsais quilhadas (Moraes-da-Silva et al., 2019; Regnet et al., 2018). *Helicops infrataeniatus* Jan, 1865 (Fig. 4) é bem distribuída na América do Sul e pode ser encontrada no sul do Brasil, Uruguai, Argentina e Paraguai (Uetz et al., 2024). Se alimenta principalmente de peixes, consumindo também anuros e pode atingir até um metro de comprimento (de Aguiar & Di-Bernardo, 2005; Giraud, 2004; Quintela & Loebmann, 2009; de Aguiar & Di-Bernardo, 2010). A espécie apresenta policromatismo em relação à coloração ventral e atualmente são reconhecidas pelo menos 3 combinações de listras e coloração no grupo (Regnet et al., 2022).

Em relação ao dimorfismo sexual, as fêmeas alcançam tamanhos corporais maiores do que os machos, os quais possuem a cauda mais longa (Giraud, 2004; Regnet et al., 2022).



Figura 1: Indivíduo de *Helicops infrataeniatus*. Foto: Daniel Loebmann.

A tribo Xenodontini reúne atualmente três gêneros de serpentes da América do Sul e América Central, agrupados a partir de sinapomorfias da morfologia do hemipenis: *Erythrolamprus* Boie 1826, *Lygophis* Fitzgerald 1843 e *Xenodon* Boie 1926 (Zaher et al., 2009). *Erythrolamprus* abrange hoje espécies anteriormente classificadas nos gêneros *Umbrivaga* Roze 1964 e *Liophis* Wagler 1830 e é um dos gêneros mais diversificados em Dipsadidae, com 51 espécies. As serpentes que compõem esse grupo são de pequeno a médio porte e ocupam uma ampla gama de nichos (Grazziotin et al., 2012; Teixeira et al., 2020).

*Erythrolamprus poecilogyrus* (Wied-Neuwied, 1824) (Fig. 1) é subdividida em quatro subespécies endêmicas da América do Sul que exibem polimorfismo no padrão de coloração e ampla distribuição geográfica (Dixon & Markezich, 1992) : *Erythrolamprus poecilogyrus poecilogyrus* (Wied-Neuwied, 1824) (Fig. 1G) habita a Mata Atlântica do sudeste brasileiro, *E. p. caesius* (Cope, 1862) (cf. Dixon & Markezich 1992) (Fig. 1F) vive no Chaco, *E. p. schotii* (Schlegel, 1837) (Fig. 1H) pode ser encontrada na Amazônia, Cerrado, Caatinga e Mata Atlântica e *E. p. sublineatus* (Cope, 1860) (Fig. 1, Fig. 2) reside nos Pampas do sul brasileiro, no Uruguai e na Argentina (Dixon & Markezich, 1992; Abegg et al., 2015). *Erythrolamprus poecilogyrus sublineatus* é comumente encontrada no Rio Grande do Sul. Como adaptações à vida urbana, a espécie apresenta coloração verde-escuro, manchas pretas no dorso, podendo

medir até 70 cm de comprimento (Giraudo, 2004). Embora predominantemente terrestre, é facilmente encontrada em associação com ambientes alagados, uma vez que se alimenta majoritariamente de anfíbios anuros (54.1%, fig. 2) e peixes (42.3%), mas também preda répteis (2.7%) e pequenos mamíferos (0.9%) com menor abundância (Quintela & Loebmann, 2009; Corrêa et al., 2015).

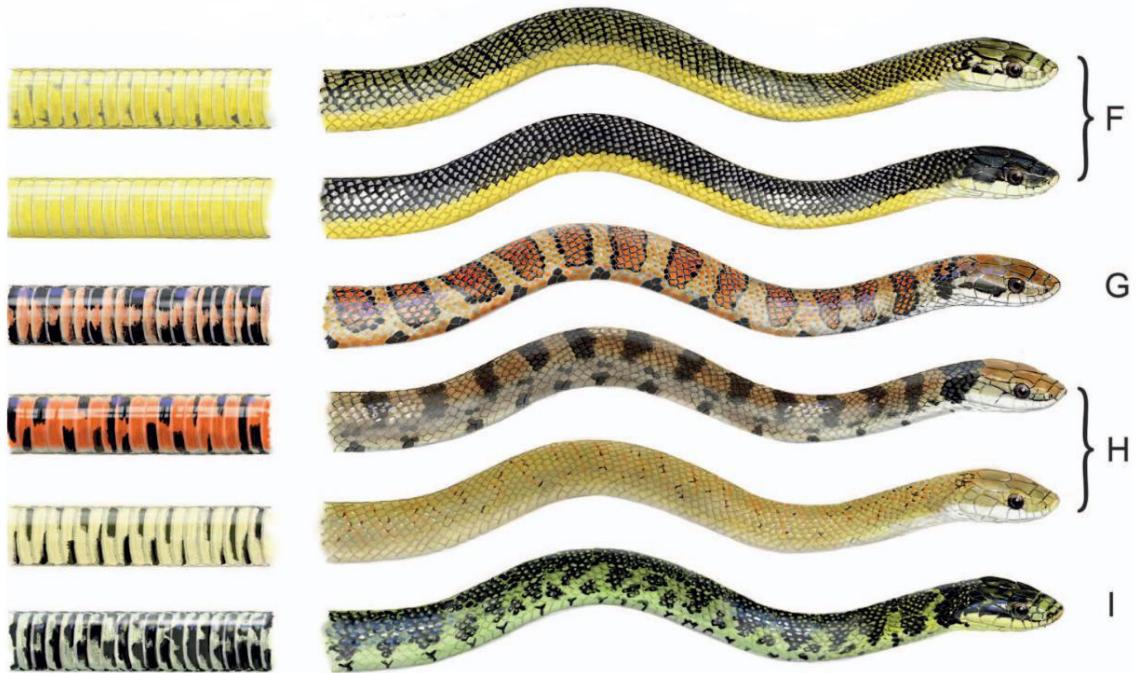


Figura 2: Padrões de coloração entre as subespécies de *Erythrolamprus poecilogyrus*. *Erythrolamprus p. caesius* (F), *E. p. poecilogyrus* (G), *E. p. schotii* (H) e *E. p. sublineatus* (I). Retirado de Entiauspe-Neto et al., 2021.



Figura 3: *Erythrolamprus poecilogyrus sublineatus* predando *Elachistocleis bicolor* (Guérin-Méneville, 1838).

*Erythrolamprus jaegeri* (Gunter, 1858) é uma serpente semiaquática de pequeno porte, muito abundante na região costeira do sul do Brasil. Atualmente são reconhecidas duas subespécies: *Erythrolamprus j. jaegeri* (Gunter, 1858), comumente encontrada em áreas abertas em associação com ambientes alagados, distribuindo-se desde o sudeste do Brasil até o Uruguai (Dixon & Markezich, 1992; Teixeira et al., 2020), e *E. j. coralliventris* (Boulenger, 1894) (Fig. 3), que pode ser encontrada no Paraguai, Argentina e sul do Brasil. Possui coloração verde-claro e uma linha dorsal na cor marrom, embora exista polimorfismo na coloração (Corrêa et al., 2015). Se alimenta principalmente de anuros (82.2%), mas também preda peixes (10.7%) e isópodes (7.1%) (Corrêa et al., 2015). Além disso, forrageia em ambientes aquáticos com bastante facilidade (de Lema, 2002; da Frota et al., 2005; Sawaya et al., 2008; Quintela & Loebmann 2009; dos Santos et al., 2010).



Figura 4: Representante de *Erythrolamprus jaegeri coralliventris*. Imagem: Leandro Malta Borges, biodiversity4all.org.

Mesmo que simpátricas e com nichos ecológicos semelhantes, as duas espécies de *Erythrolamprus* possuem distinções. *Erythrolamprus poecilogyrus sublineatus* pode atingir um comprimento corporal maior (até 70 cm) em relação à *E. j. coralliventris* (até 55 cm), característica que permite a captura de presas maiores, como pequenos mamíferos, pela primeira espécie (Corrêa et al., 2015; Giraudo, 2004). Além disso, *E. p. sublineatus* é classificada pelo índice de Levins (Krebs 1999) como uma espécie com dieta mais generalista

(anuros, peixes, répteis e mamíferos, índice de Levins = 0.61) do que *E. j. coralliventris* (anuros e peixes, índice de Levins = 0.17), uma vez que possui valor mais próximo de 1 no teste aplicado por Corrêa et al. (2015).

### **Justificativa**

Devido à estreita relação entre a cabeça das serpentes e a captura e ingestão de alimento, entender como a morfologia dessa estrutura varia entre espécies pode trazer elucidacões importantes acerca da adaptação dos organismos ao consumo dos recursos alimentares e, conseqüentemente, sobre a sua evolução (Shine, 1989). Além disso, analisar a existência de diferenças morfológicas entre machos e fêmeas pode trazer evidências sobre como o dimorfismo sexual é moldado e sobre como a seleção natural age sobre machos e fêmeas.

### **Objetivos específicos**

- Testar a existência de dimorfismo sexual no tamanho e na forma da cabeça de *H. infrataeniatus*, *E. j. coralliventris* e *E. p. sublineatus*;
- Verificar a influência do tamanho e do estágio do desenvolvimento no formato da cabeça de *H. infrataeniatus*;
- Comparar a morfologia da cabeça de *E. j. coralliventris* com a de *E. p. sublineatus*

### **Hipóteses**

- Por serem consideradas generalistas, *H. infrataeniatus* e *E. p. sublineatus* são sexualmente dimórficas em relação ao tamanho e ao formato da cabeça. Por ser classificada como especialista, *E. j. coralliventris* não tem dimorfismo no tamanho e forma da cabeça;
- Devido à estreita relação do tamanho com a forma, o estágio do desenvolvimento influencia no formato da cabeça nas três espécies;
- A cabeça de animais maiores (maior sexo ou maior espécie) refletirá em uma cabeça mais larga;
- Por ter a dieta restrita a peixes e pequenos anuros e ser mais comumente encontrada em ambientes aquáticos ou alagados, *E. j. coralliventris* terá a cabeça mais estreita na vista dorsal. Por consumir um espectro maior de presas e estar adaptada também aos ambientes urbanos, *E. p. sublineatus* tem a cabeça mais semelhante à de serpentes terrestres (mais larga na porção dorsal).

## REFERÊNCIAS BIBLIOGRÁFICAS

- Abegg, A.D., Entiauspe-Neto, O.M., Costa, H.C., Santos, P.S. (2015): *Erythrolamprus poecilogyrus* spp. (Serpentes: Dipsadidae): predação. *Herpetol. Brasileira* 4, 60-63.
- Abegg, A.D., Passos, P., da Rosa, M.C., Azevedo, W.S., Malta-Borges, L. & Budadué, J.M., (2020). Sexual dimorphism, ontogeny and static allometry of a semi-fossorial snake (genus *Atractus*). *Zoologischer Anzeiger* 287: 95-104.
- Andjelkovic, M., Blafojevic, V., Tomovic, L. & Ivanovic, A. (2016a). Ontogeny of pileus in *Natrix Tanovic* and *Natrix tessellata*. *Herpetological Journal* 26: 3-9.
- Andjelkovic, M., Tomovic, L. & Ivanovic, A., (2016b). Variation in skull and shape of two species (*Natrix Tanovic* and *Natrix tessellata*). *Zoomorphology* 135: 243-253.
- Borczyk, B., Paško, L., Kuszniierz, J., Bury, S. (2021): Sexual dimorphism and skull size and shape in the highly specialized snake species, *Aipysurus eydouxii* (Elapidae: Hydrophiinae). *PeerJ*. 9: 1-20.
- De Aguiar, L.F. & Di-Bernardo, M., (2005). Reproduction of the water snake *Helicops infrataeniatus* (Colubridae) in southern Brazil. *Amphibia-Reptilia* 26: 529-533.
- De Aguiar, L.F. & Di-Bernardo, M., (2010). Diet and Feeding Behavior of *Helicops infrataeniatus* (Serpentes: Colubridae: Xenodontinae) in Southern Brazil. *Studies on Neotropical Fauna and Environment* 39: 7-14.
- Cadle, J.E. & Greene, H.W. (1993). Phylogenetic patterns, biogeography, and the ecological structure of Neotropical snake assemblages. In: RICKLEFS, R. E.
- Camilleri, C. & Shine, R. (1990). Sexual dimorphism and dietary divergence in trophic morphology between male and female snakes. *Copeia* 3: 649-658.
- Campbell, J.A. & Lamar, W.W. (2004). The venomous reptiles of the western hemisphere. Ithaca: Cornell University Press 1: 475 p.
- Corrêa, D.M., Quintela, F.M. & Loebmann, D. (2014). Feeding ecology of *Erythrolamprus jaegeri jaegeri* (Günther, 1858) and *Erythrolamprus poecilogyrus sublineatus* (Cope, 1860) in the coastal zone of Subtropical Brazil (Serpentes, Dipsadidae). *Anais da Academia Brasileira de Ciências* 88: 293-308.
- Cundall, D. & Greene, H.W. (2000). Feeding in snakes. In K. Schwenk (Ed.), *Feeding: Form, function, and evolution in tetrapod vertebrates*. New York: Academic Press 293–333
- Da Frota, J.G. (2005). Biologia reprodutiva e dieta de *Liophis jaegeri jaegeri* (Guther, 1858) (Serpentes, Colubridae, Xenodontinae). Dissertação de mestrado, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, 43 p.

- Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex*. John Murray, London.
- De Lema, T. (2002). *Os reptéis do Rio Grande do Sul: atuais e fósseis, biogeografia e ofidismo*. Porto Alegre: EdiPucrs, 264p.
- Dixon, J.R. & Markezich, A.L. (1992). Taxonomy e geographic variation of *Liophis poecilogyrus* (Wied) from South America (Serpentes: Colubridae). *Texas Journal of Science* 44: 131-166.
- Dos Santos, M.B., Huckembeck, S., Bergmann, F.B. & Tozetti, A.M. (2010). Comportamento alimentar aquático de *Liophis jaegeri* (Günther 1858) (Serpentes, Dipsadidae) em cativeiro. *Biota Neotropica* 10: 343-346.
- Dos Santos, M.M., Klaczko, J. & Prudente, L.C. (2022). Sexual dimorphism and allometry in malacophagus snakes (Dipsadidae: Dipsadinae). *Zoology* 153: 1-14.
- Elgee, K., Blouin-Demers, G. (2011). Eastern Garter Snakes (*Thamnophis sirtalis*) with proportionally larger heads are in better condition. *Amphibia-Reptilia* 32: 424-427.
- Entiauspe-Neto, O.M., Abegg, A.D., Koch, C., Nuñez, L.P., Azevedo, W.S., Moraes, J.C.L., Tiutenko, A., Bialves, T.S. & Loebmann, D. (2021). A new species of *Erythrolamprus* (Serpentes: Dipsadidae: Xenodontini) from the savannas of northern South America. *Salamandra* 57: 196-218.
- Fernandes, D.S., Franco, F.L., Fernandes, R. (2004). Systematic revision of the genus *Lachesis* Daudin, 1803 (Serpentes, Viperidae). *Herpetologica* 60: 245-260.
- Ford, N.B. & Seigel, R.A. (1989). Relationships among body size and, clutch size and egg size in three species of oviparous snake. *Hepertologica* 45: 75-83.
- Franco, F.L., Ferreira, T.G. (2002). Descrição de uma nova espécie de *Thamnodyastes* Wagler, 1983 (Serpentes: Colubridae) do nordeste brasileiro, com comentários sobre o gênero. *Phyllomedusa* 1: 57-74.
- Giraud, A. (2004). *Serpientes de la Selva Paranaense y del Chaco Húmedo*. Buenos Aires. Ediciones L.O.L.A 328p.
- Grazziantin, F.G., Zaher, H., Murohy, R.W., Scrocchi, G., Benavides, M.A., Zhang, Y. & Bonatto, S.L. (2012). Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics* 28: 437-459.
- Hampton, P.M. (2011). Comparison of cranial form and function in association with diet in Natricine Snakes. *Journal of Morphology* 272: 1435-1443.
- Hibbitts, T.J. & Fitzgerald, L.A. (2005). Morphological and ecological convergence in two *Natricine snakes*. *Biological Journal of the Linnean Society* 85: 363-371.
- Kaliontzopoulou, A., Carretero, M.A. & Lorente, G.A. (2007). Multivariate and geometric

- morphometrics in the analysis of sexual dimorphism carination in *Podarcs* lizards. *Journal of Morphology* 268: 152-165.
- Klackzko, J., Sherrat, E. & Setz, E.Z.F. (2016). Are Diet Preferences Associated to Skulls Shape Diversification in Xenodontine Snakes? *PLoS One* 11: 1-12.
- Maneyro, R. & Carreira, S. (2012). *Guía de Anfibios Del Uruguay*. Montevidéo: Edições da Fuga 207p.
- Martins, M. (1993). Why do snakes sleep on the vegetation in Central Amazonia? *Hepertologica Review* 24: 83, 84.
- Martins, M. & Oliveira, M.E. (1999). Natural history of snakes in forest of the Manaus Region, Central Amazonia, Brazil. *Herpetological Natural History* 6: 78–150.
- Martins, M., Araujo, M.S., Sawaya, R.J. & Nunes, R. (2001). Diversity and evolution of macrohabitat use, body size and morphology in a monophyletic group of Neotropical pitvipers (*Bothrops*). *Journal of Zoology* 254: 529-538.
- Meik, J.M., Setser, K., Mociño-Deloya, E., Lawing, A.M. (2012): Sexual differences in head form and diet in a population of Mexican lance-headed rattlesnakes, *Crotalus polystictus*. *Biol. J. Linn. Soc.* 106: 633–640.
- Mesquita, P.C.M.D., Borges-Nojosa, D.M. & Bezerra, C.H., (2010). Dimorfismo sexual na “cobra-cipó” *Oxybelis aeneus* (Serpentes, Colubridae) no estado do Ceará, Brasil. *Biotemas* 23: 65-69.
- Moraes-da-Silva, A., Amaro, R.C., Nunes, P.S., Strussmann, C., Junior, M.T., Andrade, A., Sudré, V., Recorder, R., Rodrigues M.T., Curcio, F.F. (2019). Chance, luck and a fortunate finding: a new species of water snake of the genus *Helicops* Wagler, 1828 (Serpentes: Xenodontinae), from the Brazilian Pantanal wetlands. *Zootaxa* 4651, 445–470.
- Murta-Fonseca, R.A., Machado, A., Lopes, R.T. & Fernandes, D.S. (2019). Sexual dimorphism in *Xenodon neuwiedii* skull revealed by geometric morphometrics (Serpentes; Dipsadidae). *Amphibia-reptilia* 40: 461-474.
- Quintela, F.M. & Loebmann, D. (2009). *Guia Ilustrado: Os répteis da região costeira do extremo sul do Brasil*, Pelotas: USEB, 88 p.
- Regnet, R.A., Quintela, F.M., Bohme, W. & Loebmann, D. (2018). Intersexuality in *Helicops infrataeniatus* Jan, 1865 (Dipsadidae: Hydropsini). *Herpetological Journal* 28: 108-110.
- Regnet, R.A., Quintela, F.M., Rodder, D., Loebmann, D. (2022). Attributes of the population structure and ventral polychromatism of *Helicops infrataeniatus* Jan, 1865 (Serpentes, Colubridae, Dipsadinae) in subtropical Brazil. *Nort West J. Zool.* 18, 162-167.
- Rossmann, D.A. (2000). *Helicops* Wagler. pp. 122-125. In: Peters, J. A., Miranda, O. (Eds.)



- Catalogue of the Neotropical Squamata. Part. I. Snakes. United States National Museum Bulletin.
- Savitzky, A.H. (1983). Coadapted character complexes among snakes: fossoriality, piscivory and durophagy. *American Zoologist* 23: 397-409.
- Sawaya, R.J., Marques, O.A.V. & Martins, M. (2008). Composition and natural history of a Cerrado snake assemblage at Itirapina, São Paulo state, southeastern Brazil. *Biota Neotropica* 8: 129-151.
- Schluter, D. (2000). *The Ecology of Adaptive Radiation*. Oxford University of Press, Oxford.
- Segall, M., Cornette, R., Fabre, A.C., Godoy-Diana, R. & Herrel, A. (2016). Does aquatic foraging impact head shape evolution in snakes? *Proceedings of the Royal Society of London* 283: 1-7.
- Shine, R., (1989). Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Q. Rev. Biol.* 64, 419–469.
- Shine, R. (1991). Why do large snakes eat larger prey items? *British Ecological Society* 5: 493-502.
- Shine, R. (1993). Sexual dimorphism in snakes, pp 49-86. In: Seigel, R.A., Collins, J.T. (eds), *Snakes. Ecology and Behavior*. McGraw Hill, New York, USA.
- Silva, F.M., Oliveira, L.S., Nascimento, L.R.S., Machado, F.A. & Prudente, A.L.C. (2017a). Sexual dimorphism and ontogenetic changes of Amazonian pit vipers (*Bothrops atrox*). *Zoologischer Anzeiger* 271: 15-24.
- Silva, F.M., Prudente, A.L.C., Machado, F.A., Santos, M.M., Zaher, H. & Hingst-Zaher, E. (2017b). Aquatic adaptations in a Neotropical coral snake: A study of morphological convergence. *Journal of Zoological Systematics and Evolutionary Research* 56: 382-394.
- Tamagnini, D., Stephenson, J., Brown, R.P. & Meloro, C. (2018). Geometric morphometric analyses of sexual dimorphism and allometry in two sympatric snakes: *Natrix helvetica* (Natricidae) and *Vipera berus* (Viperidae). *Zoology* 129: 25-34.
- Teixeira, V.H.S., Quintela, F.M., Loebmann, D. (2020). Reproductive biology of *Erythrolamprus jaegeri coralliventris* (Serpentes: Dipsadidae) in the Brazilian Coastal Pampa. *Anais da Academia Brasileira de Ciências* 92: 1-9.
- Uetz, P. The Reptile Database. Disponível em: <http://www.reptile-database.org>. Acesso em: 13 de março de 2024.
- Vincent, S.E., Dang, P.D., Herrel, A., Kley, N.J., (2006). Morphological integration and adaptation in the snake feeding system: A comparative phylogenetic study. *J. Evol. Biol.* 19, 1545–1554.

- Zaher, H. (1999). Hemipenial morphology of the South American Xenodontine snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of colubroid hemipenes. *Bulletin of the American Museum of Natural History* 240: 1-168.
- Zaher, H., Grazziontin, F.G., Cadle, J.E., Murphy, R.W. & Moura-Leite, J.C. (2009). Molecular phylogeny of advanced snakes (Serpentes: Caenophidia) with an emphasis on South America Xenodontines: a revised classification and descriptions of new taxa. *Papéis Avulsos de Zoologia* 49: 115-153.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink, H.L. (2004): *Geometric morphometrics for biologists: a primer*. Elsevier Academic Press, New York and London, 437p.

## CAPÍTULO 1

Sexual dimorphism, ontogeny and allometry of water snake *Helicops infrataeniatus* (Serpentes: Xenodontinae) revealed by geometric morphometrics

Manuscrito a ser submetido para publicação no periódico Zoology. Normas de formatação do periódico encontram-se no anexo um desta dissertação.

**Título:** Sexual dimorphism, ontogeny and allometry of water snake *Helicops infrataeniatus* (Serpentes: Xenodontinae) revealed by geometric morphometrics

**Autoria:** Miriam Duarte, Daniel Loebmann, Adriana Gava

**Número de palavras:** 5.099

**Figuras:** 4

**Tabelas:** 2

**Qualis Capes 2017:** A2

**Fator de impacto:** 2

**Sexual dimorphism, ontogeny and allometry in head shape of water snake  
*Helicops infrataeniatus* (Serpentes: Xenodontinae) revealed by geometric  
morphometrics**

Miriam Duarte<sup>a</sup>, Daniel Loebmann<sup>a, b</sup>, Adriana Gava<sup>a, c</sup>

<sup>a</sup> Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália km 8, CEP: 96203-900, Vila Carreiros, Rio Grande, Rio Grande do Sul, Brazil.

<sup>b</sup> Laboratório de Vertebrados, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália km 8, CEP: 96203-900, Vila Carreiros, Rio Grande, Rio Grande do Sul, Brazil

<sup>c</sup> Laboratório de Genética e Biologia Molecular, Universidade Federal do Rio Grande, Av. Itália km 8, CEP: 96203-900, Vila Carreiros, Rio Grande, Rio Grande do Sul, Brazil

**Corresponding author:** [adriana.gava@gmail.com](mailto:adriana.gava@gmail.com)

## Highlights

- Sexual dimorphism in snakes head shape and size is commonly related to diet and resources use divergences.
- *Helicops infrataeniatus* have sexual dimorphism in head size and shape and the allometry is significant in both sex for all the three views of the head (dorsal, lateral and ventral).
- The allometric trajectories are divergent between sexes, meaning that size affects males and females in different ways.
- The free-size head shape is very similar between males and females, which indicates mostly of the sexual dimorphism in head shape is due to size influences.
- The preexistent sexual body size dimorphism can explain the head shape dimorphism existent by allowing the sexes to explore different kinds of prey or different prey sizes.

## ABSTRACT

Sexual dimorphism in snakes, driven by factors like natural selection and niche divergence, manifests in characteristics such as body size and head morphology. Geometric morphometrics studies have shown that head shape in snakes is influenced by diet and niche, with generalist's species been sexually dimorphic and larger individuals having wider heads. Herein, aimed to investigate sexual dimorphism, ontogenetic changes and allometric patterns in head shape of *Helicops infrataeniatus*. We used geometric morphometric tools on dorsal, lateral and ventral view of head's by taking 2D photographs of 226 specimens available in herpetological collection of the Universidade Federal do Rio Grande. Specimens were separated by sex and age group in juveniles and adults. We computed t test, one-way PERMANOVA and PCA to investigate sexual dimorphism in size and shape, multivariate regressions of shape coordinates over centroid size to explore ontogenetic changes and to compare allometric trajectories by angular comparison. Our findings revealed size dimorphism with females having larger heads in all views. Regarding shape dimorphism, juvenile females show a relatively wider dorsal view, with wider parietal and supraocular scales than males. Otherwise, adult males have a proportionally wider head shape. Allometry is significant in both sexes in all views but female's ventral. Allometric patterns differed between the sexes in dorsal and lateral views. Age also played a role, with head shape varying between juveniles and adults and females' heads becoming shorter and wider as they grew larger. Size-free shape also is dimorphic but the scatterplot is highly overlapped, meaning that great part of head shape dimorphism is due to size influence.

*Keywords:* aquatic, Colubridae, head shape, morphometry, morphology

## Introduction

Intraspecific variation is one of the main components of evolution by natural selection, allowing the survival and evolutionary success of those best adapted to environmental conditions (Darwin, 1871). When this variation is related to sex and to traits that are not directly involved in reproduction, we refer to it as sexual dimorphism (Shine, 1993). In snakes, the evolution of sexual dimorphism is typically explained by three theories: sexual selection, fertility advantage and niche divergence (dos Santos et al., 2022). Sexual selection occurs indirectly by male-male combat or when the female chooses the male according to the presence of some trait (Darwin, 1871). The fertility-related advantage hypothesis suggests that females with larger body size carry more embryos and niche divergence explains that males and females use the environment in different ways to avoid intraspecific competition (Shine, 1989). Because there is a positive correlation between female's body size and clutch size in the offspring survival (Madsen and Shine, 1994; Borczyk et al., 2021), females tend to have larger body sizes than males in most snake's species (Shine, 1993). On the other hand, males with longer tails have an evolutionary advantage over short-tailed males due to their increased capacity for storage of hemipenis and retractor muscles (Shine, 1993). On the contrary, males can be larger when male-male combat is crucial to guaranty the assess to reproductive females (Shine, 1993).

Serpentes comprises a group with about 4.038 species of enormous evolutionary success animals (Cundall and Greene, 2000; Uetz, 2023). Due to their high diversity and the capacity to occupied different niches, snakes are a group with a very diverse carnivore diet, ecology, and morphology (Savitzky, 1983; Cundall and Greene, 2000). The South America-endemic tribe Hydropsini (*Helicops* Wagler, 1828, *Hydrops* Wagler, 1830 and *Pseudoeryx* Fitzinger, 1826) is a clade of aquatic snakes with good support in the literature (Cundall and Greene, 2000; Zaher et al., 2009). With 21 species known, *Helicops* is the most diverse genus, which are distinguished by the presence of keels on the dorsal scales (Moraes-da-Silva et al., 2019). *Helicops infrataeniatus* is a widespread non-venomous water snake abundant in lentic and lotic waters in open areas (de Lema, 1994). The species exhibits typical aquatic features, including dorsally positioned eyes and nostrils, and a single internasal scale (Rossman, 1970). The distribution of *H. infrataeniatus* encompasses southern Brazil, Uruguay, Argentina and Paraguay (Giraud, 2004; Arzamendia et al., 2019). The species may reach lengths of up to one meter, and, regarding sexual body dimorphism, females have larger body size and males have longer tails and a higher number of subcaudal scales in comparison with females (de Aguiar and Di-Bernardo, 2005; Regnet et al., 2022). This species has a generalist-piscivore diet and can feed on frogs and toads (de Aguiar and Di-Bernardo, 2010; Quintela and de Assis, 2011).

Geometric morphometrics is a methodology that utilizes landmark coordinates data to quantify and interpret differences or similarities among individuals in terms of the size and shape of their morphological structures (Bookstein, 1991). This methodology, which allows the separate study of size and shape, has gained popularity, particularly in analyzing complex structures like the head in snakes and exploring the impact of size on shape (Tamagnini et al., 2018; dos Santos et al., 2022). Indeed, allometry, a key component of animal evolution, explains the change in an organism's shape concerning its size (Klingenberg, 2016; Tamagnini et al., 2018). As with any scientific subject, allometry is studied across various contexts: between species (evolutionary allometry), among individuals of the same species and age/sex (static allometry), and in individuals at different ages (ontogenetic allometry) (Klingenberg and Zimmermann, 1992). In snake studies using geometric morphometrics, researchers have aimed to understand how factors such as diet (Meik et al., 2012; Deepak et al., 2023; Klaczko et al., 2016), ecology (Segall et al., 2016; da Silva et al., 2017, Silva et al., 2017) sexual dimorphism (Tamagnini et al., 2018; Murta-Fonseca et al., 2019; Abegg et al., 2020; dos Santos et al., 2022), ontogeny (Andjelkovic et al., 2016; da Silva, 2017) and geographic variation (Manier, 2004) influence the head and skull shape.

While sexual and fertility-related selection are responsible for dimorphism in body and tail size in snakes (Shine, 1989; Shine, 1993), other intraspecific factors might impact the head shape. Camilleri and Shine (1990) suggested that sexual dimorphism in head size and shape may have evolved as an adaptation to reduce intraspecific competition for resources, specifically through dietary niche partitioning. These authors proposed alternative hypotheses: (1) head enlargement in response to visual stimulus, probably to avoid predators, and (2) head enlargement as a reflection of selection for large sensory parts of the brain in one sex. They emphasize that, given these ideas, significant head shape dimorphism is unlikely, except for the hypothesis that dietary divergence drives shape divergence. Shine (1989) also declared that dietary differences are almost inevitable in species with sexual dimorphism in body size.

Regarding the head shape, snakes with a specialist diet tend to be less sexually dimorphic than generalists due to the homogeneity in prey size and shape that specialist snakes forage on (Abegg et al., 2020; Borczyk et al., 2021), while consuming prey of different sizes and species allows animals to explore a broader prey spectrum (Murta-Fonseca et al., 2019). Thus, large individuals with large gapes would presumably consume a more diverse range of prey and also feed on large animals (Barends and Naik, 2023). Also, larger animals require more energy to compensate for metabolic rates (Shine, 1991) and in some studies, the increase in snakes body size seems to result in the enlargement of the dorsal view of the head and skull, once it



maximizes the energy acquisition by allowing the capture of larger prey (Vincent et al., 2006; Elgee and Blouin-Demers, 2011; Borczyk et al., 2021).

Here, we aim to perform Geometric Morphometrics (GM) analyses to test for sexual dimorphism in the head size and shape of *Helicops infrataeniatus*, explore the head shape variation during growth, and compare the allometric trajectories between males and females. We hypothesize that female heads would be the largest, attributed to the overall larger body size of females than males. We predict sexual dimorphism in the head shape of *H. infrataeniatus* due to the generalist-piscivore diet of the species, which opens up the possibility of a reduction in intraspecific competition for resources, specifically through trophic niche partitioning between sexes. Theory predicts that individuals with large gapes would presumably consume a more diverse range of prey, including large animals. Consequently, females of *H. infrataeniatus* would have a head shape larger than longer, allowing the consumption of larger prey to compensate for higher metabolic rates, in comparison to the smaller males.

## **2. Material and methods**

### *2.1. Sampling*

We analyzed a total of 89 females (44 adults and 45 juveniles) and 137 males (78 adults and 59 juveniles) of *Helicops infrataeniatus*,  $n = 226$ . The specimens were collected and housed in Herpetological Collection of Rio Grande Federal University (CHFURG) in 2015 after a flood event at Laranjal Beach in the municipality of Pelotas, in the Coastal Plain of Rio Grande do Sul (31°46'S, 52°13'W, datum WGS84, ca. 2 m a.s.l.) (Regnet et al., 2017; Regnet et al., 2022). The complete list of the analyzed specimens can be found in the Supporting Information. We determined sex by examining the gonads. We took linear measurements of snout-vent length (SVL) from the tip of the snout to cloaca. The specimens were classified as juveniles when the SVL was greater than 142 mm, females were classified as adults when the SVL was larger than 486 mm and males when larger than 337 mm (de Aguiar and Di-Bernardo, 2005).

Photographs were taken along with a scale (1 cm) for the dorsal, lateral and ventral view of the head of each specimen using a Nikon Coolpix p600 digital camera placed in a standardized position and distance above the specimens. The correct alignment of camera and the specimens was verified with a spirit level. Specimens with deformations in one of the views were photographed only for the others. TPSUtil version 1.83 (Rohlf, 2013) was used to convert digital images into tps files. One of us (MD) digitalized 21 landmarks in dorsal view ( $n = 226$ ), 17 in lateral view ( $n = 142$ ) and 8 in ventral view (147) utilizing TPSDig version 2.18 (Rohlf, 2015) (Fig. 1). The measurement error was calculated by digitizing twice the LMs in a subsample of photographs. We performed a Shape Procrustes' ANOVA to compare the mean

squares (MS) of specimens with MS of Error 1 (i.e. error of digitization among the two subsamples digitalized within two weeks of difference by author MD). The software MorphoJ version 1.08 (Klingenberg, 2011) was used to perform a Generalized Procrustes Analysis (GPA) over landmarks coordinates in order to remove the scale, rotation and position effects and generate shape variables (Procrustes coordinates in lateral view and symmetric component in dorsal and ventral view) and size data (natural logarithm of centroid size (lnCS)).

### *2.2. Shape group structure sexual dimorphism and static allometry*

A permutational analysis of variance (PERMANOVA), available in Paleontological Statistical software (PAST version 4.16, Hammer et al., 2001), was conducted using the factors age (juvenile vs. adult) and sex on the general dataset (including juvenile and adults females, and juvenile and adult males) to explore whether there is group structure in our complete data. Due to the significant influence of sex and age on shape, we analyzed these factors separately in subsequent analyses (examining one at a time).

The dimorphism analyses were performed for both juveniles and adults separately. To determine if the size data fit the requirements of normality and homoscedasticity of variances, Shapiro-Wilk and Levene tests were performed using the software R 4.3.1 (R Core Team, 2016). We tested for sexual dimorphism in the natural logarithm of centroid size (lnCS) using t-test and visualized the data by boxplots. Given that size variation may differentially impact the shapes of both females and males, we examined whether there exists an association between shape change and the variation in the lnCS within each sex and developmental stage by conducting multivariate regressions and Permutational test in MorphoJ. Additionally, when allometry was detected, angles between the regressions of females and males were compared to examine similarities in the allometric trajectories of males and females.

The presence of sexual dimorphism in head shape was tested by conducting a one-way PERMANOVA on the symmetric component of Procrustes coordinates for each age group and each sex. Afterwards, the shape data were used to execute Principal Components Analysis (PCA) available in MorphoJ to explore the overall head shape variation of our data and to visualize the head shape variation. PERMANOVA was also performed over regressions residuals to verify the influence of sex after size correction. Subsequently, the residuals were also employed to perform a PCA to assess head shape variation unaffected by size.

### *2.3 Ontogeny analyses*

To understand head shape ontogenetic changes, a general dataset containing both juvenile and adult head shape information were used. Sequentially, multivariate regressions of size data

over the shape coordinates were performed for each sex on the general dataset for all the three views of the head. Permutation tests were used to test the null hypothesis of independence between size and head shape. The two-way PERMANOVA also was used to verify the influence and the contribution of age group on head shape variation. All the graphs were edited using the software Inkscape version 1.3.

### 3. Results

#### 3.1. Group structure

Exploration of whether there is group structure in our complete data revealed that shape differed significantly for all the head views of *H. infrataeniatus*, except for the ventral view (Table 1). The shape variation range explained by sex (4.87%) is bigger than the variation explained by age group (2.24%), while age group explains 4.47% vs. 3.06% of shape variance of the lateral view of head. The interaction between these factors was also significant.

#### 3.2. Sexual dimorphism and static allometry

We detected sexual dimorphism in the head size of *H. infrataeniatus*, with females exhibiting larger mean CS values than males. This size difference related to sex was observed not only in adult individuals but also when comparing the juvenile dataset for dorsal (juveniles:  $t = 5.132$ ,  $p < 0.01$ ; adults:  $t = 8.179$ ,  $p < 0.01$ ), lateral (juveniles:  $t = 4.016$ ,  $p < 0.01$ ; adults:  $t = 6.400$ ,  $p < 0.01$ ) and ventral views (juveniles:  $t = 5.018$ ,  $p < 0.01$ ; adults:  $t = 6.325$ ,  $p < 0.01$ ) (Fig. 2).

Multivariate and permutational test revealed that, in the dorsal view of the head, both sexes in both age groups exhibited significant static allometry ( $p < 0.01$ , see Table 2). In juvenile heads, size accounted for 6.40% of head shape changes ( $p = 0.0038$ ), whereas 5.64% of males head shape variation is a result of size alteration ( $p = 0.0007$ ). Comparing the regressions, the size change seemed to impact males and females differently (angle =  $60.22^\circ$ ,  $p = 0.0129$ ), since the angle between the regressions was similar to  $90^\circ$ , i.e., supporting a distinct pattern of covariation between shape and body size in both sexes. Regarding the expected morphology, larger juvenile females exhibited a broader head, with wider parietal and supraocular scales, although parietals and internasal were slightly shortened (Fig. 3a). Despite the significance of size effect over shape, the shape changes in smaller juvenile male's head to larger ones were subtle, highlighting the reduction of parietals length (Fig. 3b).

For adult females, size explained 9.49% of the dorsal head shape variation ( $p < 0.001$ ), while for males, allometry accounted for 3.15% of the total head shape variation ( $p < 0.001$ ). The allometric trajectories of adult females and males diverged (angle =  $72^\circ$ ,  $p = 0.09$ ), with the angle between the regressions similar to  $90^\circ$ . The expected shape changes in adult females, associated with larger size, also involved a relative enlargement and lengthening of parietal

scales previously reported in larger juveniles, while the rostral region became larger and shorter in dorsal view of the head (Fig. 3a). Individual adult males with higher lnCS values exhibited a relative lengthening of parietal scales, despite a slight reduction in relative head width (Fig. 3b). For this view and males, scale pattern showed relatively little size-related variation.

In the lateral view, regressions were not significant, indicating an overall lack of allometry in both sexes at both stages of development (Table 2). However, the general shape alteration in juveniles and adults involved a proportionally smaller growth of the eye which became flattened in larger females and males compared to smaller ones (Fig. 3c and 3d). Nonetheless, the head of larger juvenile females was rounder than that of smaller ones (Fig. 3c). Larger adult females exhibited a lengthening of the posterior area of the head (lateral view) and a reduction in relative length of the anterior area of the mouth (Fig. 3c). In larger males, the anterior maxilla showed an elongated shape compared to smaller animals, while the posterior maxilla exhibited a squared morphology (Fig. 3d).

In the ventral view, only the head shape of males was influenced by size, accounting for 20.77% of the total head shape variation in juveniles ( $p = 0.0001$ ) and 12.75% in adults ( $p = 0.0014$ ) (Table 2). As the animal grew, the maxilla became proportionally wider rather than longer (Fig. 3f). The variation of the shape of the female's head in the ventral view exhibited isometry, lacking static allometry in both age groups (juveniles = 17.01%,  $p = 0.0027$ ; adults = 3.69%,  $p = 0.4746$ ) (Table 2), and underwent a shape change similar to that observed in males (Fig. 3e).

Sexual dimorphism was observed in the dorsal ( $F = 10.63$ ,  $p < 0.01$ ) and lateral ( $F = 3.68$ ,  $p < 0.01$ ) views of the head of juveniles but not in the ventral portion ( $F = 2.45$ ,  $p = 0.078$ , Table 3). PC1 of dorsal view (26.89% of variance) did not differentiate between sexes, however, head showed a progressively narrower shape with slimmer parietal and supraocular scales along this axis (Fig. 4a). On the contrary, PC2 (16.83% of variance), represented a morphospace predominantly occupied by females, while males are highly distributed in the negative scores (Fig. 4a). On the positive side, the head appeared rounder and shorter with these shape changes mostly attributed to the widening of parietal and supraocular scales and the shortening of parietals (Fig. 4a). Conversely, negative part of the graph was related to the opposite head morphology (narrowed parietal and supraocular scales and longer parietal scales, Fig. 4a). In summary, females' heads were rounder and wider than males' heads, which exhibited a narrower head shape. These differences were mostly observed in parietal and frontal scales.

One-way PERMANOVA also demonstrated that sex had significant influence over the shape of dorsal ( $F = 6.83$ ,  $p < 0.01$ ) and lateral ( $F = 3.26$ ,  $p < 0.01$ ) but not the ventral view of the head

( $F = 4.33$ ,  $p = 0.012$ ) in the adult dataset (Table 3). According to the PCA of dorsal view of this dataset, the first two PCs summarized 42.52% of shape variance. Similar to juveniles, PC1 (24.9% of total variance) of adults showed a progressively narrowed head, with slender scales along positive scores (Fig. 4d). The reduction in the length of the rostral region on negative scores could also be observed, but the axes did not discriminate between sexes. Sexes were overlapped in head shape, except for the PC2 (17.6% of total variance), in which, in contrast to the juvenile's dataset, positive values represented a morphospace occupied predominantly by males, while females were more related to negative scores. Males on the positive side of PC2 tended to have rounder heads with a shape relatively wider and shorter than females (Fig. 4d). In contrast, females showed less rounded heads, with longer and narrowed parietal scales, shorter and narrower supraocular, and shorter frontal scale (Fig. 4d). In summary, females' shape associated with negative PC2 showed a longer and narrower head than the dorsal view of males. These differences were especially apparent at the parietal scale, which were slender and longer than that of males. Supraocular and frontal scales were also slender but relatively shorter when compared to the same scales of males (Fig. 4d). Scales of the anterior end of the head, internasal and prefrontal, are also shorter and slimmer, i.e., females showed a reduction in the length of rostral region, while the parietal region is relatively elongated (Fig. 4d).

Correcting for the size effect, the head shape of females and males remained divergent only for the dorsal view of juveniles ( $F = 5.43$ ,  $p < 0.01$ ) (Table 3). In agreement, PCA confirmed shape overlap of sexes for the lateral and ventral views (Fig. 5b, c, e and f), while the shape of the dorsal view partially segregated the sexes in PC2 (16.8% of variation, Fig. 5a and b). This axis of shape change showed females with parietal scales shorter and less broad than the average shape, while the prefrontal and internasal scales were longer and apparently more robust than the scales of males. Conversely, males showed a relative lengthening of parietal scales in comparison to the same scales of females. Sexual dimorphism is absent in the shape of head (all views) of adults when we removed allometry ( $F = 1.17$ ,  $p = 0.28$ , dorsal;  $F = 1.12$ ,  $p = 0.31$  ventral;  $F = 1.18$ ,  $p = 0.92$ ). Scatterplots of PC1 and PC2 scores confirmed these results as males and females did not form separated clusters (Fig. 5 b).

### 3.3. *Ontogeny*

Considering the dataset with pooled adults and juvenile specimens, two-way PERMANOVA indicated significant head shape differences regarding age group in dorsal ( $F = 5.1911$ ,  $p < 0.01$ ) and lateral views ( $F = 5.191$ ,  $p < 0.01$ ) but not for the ventral view of the head ( $F = 1.092$ ,  $p = 0.2944$ ) (Table 1). In dorsal view, females develop robust and broad heads in dorsal view during

ontogenetic growth. The changes involved elongation and widening of parietal scales, with frontal and supraocular scales becoming shorter as broader and prefrontal and internasal scales becoming shorter and smaller in larger females than smaller ones (Fig. 3a). Although significant, the changes in male head shape over ontogenetic growth are highly subtle (Fig. 3b). In lateral view, adults of both sexes have proportionally smaller eyes, shorter mouth than in juveniles (Fig. 3c and 3d). Adult females seem to develop steeper snouts, which are less squared than juveniles (Fig. 3a). In ventral portion, the head becomes wider and shorter in both sexes (Fig. 3 e and f).

The allometry was also significant for both dorsal and lateral views of the head in females and males ( $p < 0.01$ , Fig. 5). In these parts of the head, the size predicted the shape of the head of females more than that of males (dorsal = 12.41% and 2.34%, lateral = 6.70% and 3.44%, respectively). The angular comparison between females and males' regression demonstrated that the allometric trajectories were different in the dorsal view of the head since the angle is similar to  $90^\circ$  (angle =  $80^\circ$ ,  $p = 0.25$ ) but not in the lateral view (angle =  $51.29^\circ$ ,  $p < 0.001$ ). On the other hand, in ventral view, only males head shape seemed to be influenced by size (males = 12.475%,  $p < 0.001$ ; females = 5.01%,  $p = 0.049$ ).

#### **4. Discussion**

This study demonstrates significant sexual dimorphism in the head size and shape of *H. infrataeniatus*. The results confirm our specific hypothesis of size dimorphism in head, with females having larger heads than males. Female-biased sexual dimorphism in body size is common among snake species (Shine, 1993; Burbrink and Fetterman, 2019), driven by various ultimate mechanisms, including increased offspring size and success (Ford and Seigel, 1989; Rivas and Burghardt, 2001), female reproductive output (Madsen and Shine, 1994; Borczyk et al., 2021), and maximization of energy acquisition (Vincent et al., 2006; Elgee and Blouin-Demers, 2011; Borczyk et al., 2021). *Helicops infrataeniatus* is no exception to this rule (de Aguiar and Di-Bernardo, 2010; Regnet et al., 2022). Considering the gape-limited condition in snakes (Segall et al., 2016) and since larger animals require larger prey to supply energy demands (Cundall and Greene, 2000; Elgee and Blouin-Demers, 2011), an increase in body size is expected to be accompanied by an increase in head size to capture large prey. Therefore, it seems plausible that females reach larger head sizes because of the positive correlation with body sizes.

Vincent et al. (2006) concluded that for 12 snake groups, having a wider head allows for the intake of larger prey due to the increased areas for food passage. According to Borczyk et al. (2021), once wider heads facilitate the capture of larger prey, an increase in body size is

generally accompanied by an enlargement in the dorsal view of the head. The viper *Bothrops atrox* also exhibits female-biased sexual dimorphism in body size, with females developing wider heads than males (Silva et al., 2017). Female *Thamnodynastes strigatus* also develops wider parietal scales than males, while its congeneric *T. dorsatum*, exhibits wider rostral regions in females (Loebens et al., 2019). Therefore, according to the mentioned studies, a large body size implies the enlargement of heads with a broader shape in the dorsal view. A different trend was observed in the shape dimorphism of *H. infrataeniatus*. Despite the overall female-dominated head and body size, males develop wider and broader head shapes. Furthermore, size-free shape differences between sexes are minor, visually illustrating the strong influence of head size on head shape in *H. infrataeniatus*.

Given the importance of the skull to an animal's fitness, Klaczko et al. (2016) characterized the skull of 19 species to test whether diet preferences correlate with shape variation in xenodontine snakes. Indeed, the study demonstrated a high correlation between diet, prey shape, and variation in head morphology, even after accounting for the evolutionary signal over the head shape in xenodontine snakes. Comparison of anurophagus species with fish specialists among natricine snakes revealed broader head in the former, contrasting with narrower heads with longer upper and lower jaws in the latter (Brecko et al., 2011; Hampton, 2011). Meik et al. (2012) demonstrated that the species displays sexual dimorphism in head shape linked to the variation in proportions of larger prey consumed by each sex. Also, Hampton (2011) described the head of the anurophagus natricine snakes as wider and broader than those of piscivorous ones. Indeed, various studies demonstrated skull differences between fish-eating and anuran-eating snakes. For example, a piscivore diet is generally associated with the evolution of sharper teeth and elongated mandible bones (Britt et al., 2009).

However, the environment can also constrain the organisms' evolutionary pathway, leading to convergence of morphology in similar ecological contexts. Strong et al. (2020) documented adjustments in fossorial snake *Atractaspis irregularis* that are congruent to subterranean behavior, such as the reduction in maxilla as an adaptation to burrowing and moving underground. However, underwater locomotion and feeding also pose challenges and hydrodynamic constraints for various species of snakes, which have been overtaken by the evolution of slender, streamlined, narrow, and long heads. Segall et al. (2016) addressed specifically the influence of aquatic foraging in the shape of snakes' heads compared to non-aquatic species. Interestingly, they found that snakes that capture prey underwater tend to have the narrowest shape of heads, but only for the anterior shape of the head, while having a posterior part of the head enlarged compared to non-aquatic snakes. Taking that into account, it

is possible that males and females of the species are foraging in different environmental scenarios and those selective pressures for divergence are expressed in head shape differences.

Our results reveal sexual shape dimorphism in *H. infrataeniatus* with differences that resemble those of aquatic and non-aquatic snake comparisons. The trend in sexual dimorphism in shape differed from that observed in size. Regarding size, females have larger heads than males, with males exhibiting a smaller head with a shape comparatively wider than elongated. As an active forager in water, the species primarily captures fish across several strata of the water column. Nevertheless, *H. infrataeniatus* may also capture prey out of the water (de Aguiar and Di-Bernardo, 2010).

Scartozzoni, (2009) found that while *H. carinicaudus* and *H. modestus* males prefer to feed on Cyprinodontiformes, which are not preyed upon by females. In turn, females of *H. modestus* eat Perciformes fishes, a behavior not observed in males. However, there are no studies of diet aiming to compare the feeding preferences between males and females in *H. infrataeniatus*. Still, the dimorphism in head shape of *H. infrataeniatus* can also be connected to variations in prey diversity between males and females that arose from preexistent differences in body and head size between males and females, which allows sexes to explore the environmental resources in different ways. Although *H. infrataeniatus* has a preference to feed on fish's species, it also consumed anurans (40%) (de Aguiar and Di-Bernardo 2004; Quintela and de Assis 2011). Therefore, if the wide head of males is an adaptation to feed, it may indicate the higher anuran consumption rates by males.

*Helicops infrataeniatus* females and males also show significant differences in head shape in dorsal and lateral regarding the age group. This means that the morphology of these parts of the head changes over the ontogenetic growth, and that juveniles exhibits different head morphology compared to adults. Larger body sizes demand more energy (Shine, 1991) and generally result in larger heads with broad and more ample feeding structures, which allows organisms to prey on larger animals. Additionally, size impacts females and males in different ways, with adult females having a wider dorsal view of the head than juveniles, which can be explained as an adaptation to the need of females to feed on larger prey (Silva et al., 2017; Borczyk et al., 2021).

According to Outomuro and Johansson (2017), more than 38% of the geometric morphometrics studies did not consider or report the size influence on the shape. Furthermore, around 88% of the inspected studies showed that allometry was significant over the shape. Studying sexual dimorphism and correcting for the size effect is important to avoid erroneous interpretation of data once the size-free shape can produce different results. Indeed, our results



shows that there is association between head shape and size of *H. infrataeniatus*, but only the dorsal view of juveniles have shape dimorphism considering the free-size data, emphasizing the relevance of authors reporting size influence in geometric morphometrics analysis. Given the potential strong relation between diet and head shape evolution, we also suggest that future diet studies should consider to compare females and males feed preferences in order to verify the existence of intraspecific niche divergence.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### **Acknowledgments**

We would like to thank the laboratory technicians Daiane Carrasco and Loraine Moraes for their help in handling the specimens. DL thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico for researcher fellowship (Proc. 307232/2023-9), MD to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – finance code 001 and AG the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) - FAPERGS/CNPq-PRONEX number 16/0485-4.

### **References**

- Abegg, A.D., Passos, P., Mario-da-Rosa, C., Azevedo, W. dos S., Malta-Borges, L., de Moura Bubadué, J., 2020. Sexual dimorphism, ontogeny and static allometry of a semi-fossorial snake (genus *Atractus*). *Zool. Anz.* 287, 95–104.
- Arzamendia, V., Fitzgerald, L., Giraud, A., Scrocchi, G., Williams, J., 2019. *Helicops infrataeniatus*. The IUCN Red List of Threatened Species 2019: e.T15178734A15178737
- Barends, J.M., Naik, H., 2023. Body size predicts prey preference but not diet breadth in pythons. *J. Zool.* 321, 59-58
- Borczyk, B., Paško, L., Kuszniierz, J., Bury, S., 2021. Sexual dimorphism and skull size and shape in the highly specialized snake species, *Aipysurus eydouxii* (Elapidae: Hydrophiinae). *PeerJ* 9, 1-20.
- Britt, E.J., Clark, A.J., Bennett, A.F., 2009. Dental morphologies in gartersnakes (*Thamnophis*) and their connection to dietary preferences. *J. Herpetol.* 43, 252–259.
- Brecko, J., Vervust, B., Herrel, A., Damme, R. V., 2011. Head morphology and diet in the Dice snake (*Natrix tessellata*). *Mertensiella* 18, 20–29.
- Burbrink, F.T., Futterman, I., 2019. Female-biased gape and body-size dimorphism in the New

- World watersnakes (tribe: *Thamnophiini*) oppose predictions from Rensch's rule. *Ecol. Evol.*, 917, 9624-9633.
- Cundall, D., Greene, H.W., 2000. Feeding in Snakes, In: Schwenk, K. (Eds.), *Feeding: form, function and evolution in tetrapod vertebrates*. Academic Press, 293-333.
- Darwin, C., 1871. *The descent of man, and selection in relation to sex*. London: John Murray. Volume 1.
- Da Silva, F.M., Oliveira, L.S. de Nascimento, L.R. de S., Machado, F.A., Prudente, A.L. da C., 2017. Sexual dimorphism and ontogenetic changes of Amazonian pit vipers (*Bothrops atrox*). *Zool. Anz.* 271, 15–24.
- De Aguiar, L.F.S., Di-Bernardo, M., 2005. Reproduction of the water snake *Helicops infrataeniatus* (Colubridae) in southern Brazil. *Amphibi. Reptil.* 26, 527 – 533
- De Aguiar, L.F.S., Di-Bernardo, M., 2010. Diet and feeding behavior of *Helicops infrataeniatus* (Serpentes: Colubridae: Xenodontinae) in southern Brazil. *Stud. Neotrop. Fauna. Environ.* 39, 7–14.
- Deepak, V., Gower, D.J., Cooper, N., 2023. Diet and habit explain head-shape convergences in natricine snakes. *J. Evol. Biol.* 36, 399–411.
- De Lema, T. 1994. Lista comentada dos répteis do Rio Grande do Sul. *Comunicações do Museu de Ciência e Tecnologia da PUCRS, Série Zoológica* 7, 41–150.
- Dos Santos, M.M., Klaczko, J., da Costa-Prudente, A.L., 2022. Sexual dimorphism and allometry in malacophagus snakes (Dipsadidae: Dipsadinae). *Zoology* 153, 1-14.
- Elgee, K., Blouin-Demers, G., 2011. Eastern Garter Snakes (*Thamnophis sirtalis*) with proportionally larger heads are in better condition. *Amphibi. Reptil.* 32, 424–427.
- Ford, N.B., Seigel, R.A., 1989. Relationships among body size, clutch size and egg size in three species of oviparous snakes. *Herpetologica* 45, 75-83.
- Giraud, A., 2004. *Serpientes de la Selva Paranaense y del Chaco Húmedo*, first ed. L.O.L.A., Buenos Aires, Argentina.
- Hammer, Ø., Harper, D.A.T., and P. D. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Paleontol. Electron.* 4, 9pp.
- Hampton, P.M., 2011. Comparison of cranial form and function in association with diet in natricine snakes. *J. Morphol.* 272, 1435–1443.
- Klaczko, J., Sherratt, E., Setz, E.Z.F., 2016. Are diet preferences associated to skulls shape diversification in xenodontine snakes? *PLoS One* 11, 1-12.
- Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* 11, 353-357.

- Klingenberg, C.P., 2016. Size, shape, and form: concepts of allometry in geometric morphometrics. *Dev. Genes. Evol.* 226, 113-137
- Klingenberg, C.P., Zimmermann, M., 1992. Static, Ontogenetic, and Evolutionary Allometry: A Multivariate Comparison in Nine Species of Water Striders, *Source: The American Naturalist*. 140, 601-620.
- Loebens, L., Hedges, C.D., Almeida-Santos, S.M., Cechin, S.Z., 2019. Morphological variation and sexual dimorphism in two sympatric dipsadinae snakes from Southern Brazil. *Zool. Anz.* 280, 42-51.
- Madsen, T., Shine, R., 1994. Costs of reproduction influence the evolution of sexual size dimorphism in snakes. *Evolution* 48, 1389–1397.
- Manier, M.K., 2004. Geographic variation in the long-nosed snake, *Rhinocheilus lecontei* (Colubridae): beyond the subspecies debate. *Biol. J. Linn. Soc.* 83, 65-85.
- Quintela F.M., de Assis, C.L., 2011. Notas sobre a dieta de *Helicops infrataeniatus* e *Liophis poecilogyrus sublineatus* (Serpentes: Dipsadidae) na região ao sul da planície costeira do Rio Grande do Sul, Brasil. X Cong. Ecol. Brasil, 3p.
- Mebert, K., 2011. The dice snake, *Natrix tessellata* biology, distribution and conservation of a palaeartic species, first ed. Rheinbach, Germany.
- Meik, J.M., Setser, K., Mociño-Deloya, E., Lawing, A.M., 2012. Sexual differences in head form and diet in a population of Mexican lance-headed rattlesnakes, *Crotalus polystictus*. *Biol. J. Linn. Soc.* 106, 633–640.
- Mesquita, P.C.M.D., 2010. Dimorfismo sexual na “cobra-cipó” *Oxybelis aeneus* (Serpentes, Colubridae) no estado do Ceará, Brasil. *Biotemas* 23, 65–69.
- Moraes-da-Silva, A., Amaro, R.C., Nunes, P.S., Strussmann, C., Junior, M.T., Andrade, A., Sudré, V., Recorder, R., Rodrigues M.T., Curcio, F.F., 2019. Chance, luck and a fortunate finding: a new species of water snake of the genus *Helicops* Wagler, 1828 (Serpentes: Xenodontinae), from the Brazilian Pantanal wetlands. *Zootaxa* 4651, 445–470.
- Murta-Fonseca, R.A., Machado, A., Lopes, R.T., Fernandes, D.S., 2019. Sexual dimorphism in *Xenodon neuwiedii* skull revealed by geometric morphometrics (Serpentes; Dipsadidae). *Amphibi. Reptil.* 40, 461-474.
- Outomuro, D., Johansson, F., 2017. A potential pitfall in studies of biological shape: Does size matter? *J. Anim. Ecol.* 86, 1447–1457.
- Regnet, R.A., Quintela, F.M., Entiauspe-Neto, O.M., Teixeira, V. H., da Silveira, F.L., Loebmann, D., 2017. Remarkable aggregation of squamates and cecilians associated with flood events during El Niño in southern Brazil. *Salamandra* 53, 537-540.

- Regnet, R.A., Quintela, F.M., Rodder, D., Loebmann, D., 2022. Attributes of the population structure and ventral polychromatism of *Helicops infrataeniatus* Jan, 1865 (Serpentes, Colubridae, Dipsadinae) in subtropical Brazil. *Nort West J. Zool.* 18, 162-167.
- Rivas, J.A., Burghardt, G.M., 2001. Understanding sexual size dimorphism in snakes: wearing the snake's shoes. *Anim. Behav.* 62, F1-F6.
- Rohlf, F.J., 2013. tps Utility program. SUNY at Stony Brook.
- Rohlf, F. J., 2015. The tps series of software. *Hystrix-Italian J. Mammal.* 26, 9-12.
- Rossman, D.A., 1970. *Helicops* Wagler. In: Peters, J.A, Miranda O., eds., Catalogue of the Neotropical Squamata. Part. I. Snakes, U.S. Nat Mus Bull 297, pp. 122-125
- R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available in: <https://www.R-project.org> (Accessed on June 10, 2016).
- Savitzky, A., 1983. Coadapted character complexes among snakes: fossoriality, piscivory and durophagy. *Am. Zool.* 23, 397-409.
- Segall, M., Cornette, R., Fabre, A.C., Godoy-Diana, R., Herrel, A., 2016. Does aquatic foraging impact head shape evolution in snakes? *Proc. Royal Soc. B-Biol. Sci* 283, 1-7.
- Shine, R., 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Q. Rev. Biol.* 64, 419-469.
- Shine, R., 1991. Why do larger snakes eat larger prey items? *Funct. Ecol.* 5, 493-502.
- Shine, R., 1993. Sexual dimorphism in snakes, pp 49-86. In: Seigel, R.A., Collins, J.T. (eds), *Snakes. Ecology and Behavior*. McGraw Hill, New York, USA.
- Silva, F.M., Prudente, A.L.C, Machado, F.A., Santos, M.M., Zaher, H., Hingst-Zaher, E., 2017. Aquatic adaptations in a Neotropical coral snake: A study of morphological convergence. *J. Zoolog. Syst. Evol. Res.* 56, 382-394.
- Strong, C.R.C, Palci, A., Caldwell, M.W., 2020. Insights into skull evolution in fossorial snakes, as revealed by the cranial morphology of *Atractaspis irregularis* (Serpentes: Colubroidea). *J. Anat.* 238, 146-172.
- Tamagnini, D., Stephenson, J., Brown, R.P., Meloro, C., 2018. Geometric morphometric analyses of sexual dimorphism and allometry in two sympatric snakes: *Natrix helvetica* (Natricidae) and *Vipera berus* (Viperidae). *Zoology* 129, 25-34.
- Vincent, S.E., Dang, P.D., Herrel, A., Kley, N.J., 2006. Morphological integration and adaptation in the snake feeding system: A comparative phylogenetic study. *J. Evol. Biol.* 19, 1545-1554.

## Figure captions

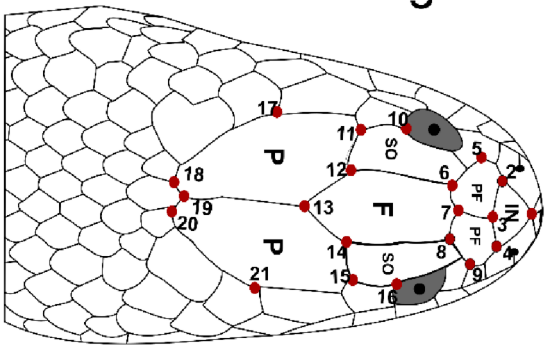
**Fig. 1:** Illustration of two-dimensional landmarks positioned on the head of the water snake *Helicops infrataeniatus* from the Coastal Plain of Rio Grande do Sul. The identified scales include parietal (P), frontal (F), supraocular (SO), prefrontal (PF), internasal (IN), supralabial (S) and infralabial (I). A) Dorsal view of the head. B) Lateral view of the head. C) Ventral view (see also supplementary Table 1 for descriptions of landmarks).

**Fig. 2:** Boxplot of log of the natural logarithm of centroid size across *Helicops infrataeniatus* sexes and age groups. Limits on boxes (light males and dark females) correspond to the first and third quartiles and the internal black line is the median. Divergent letters (a, b) indicate significant difference.

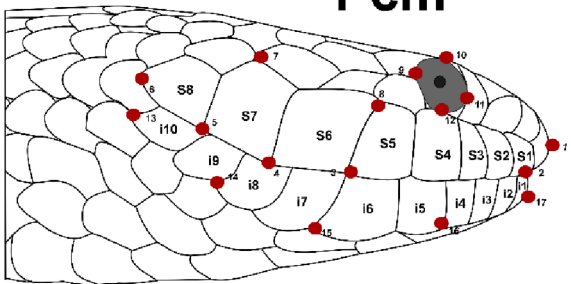
**Fig. 3:** Multivariate regression of head shape over size in dorsal, lateral and ventral view of females (A, C and E) and males (B, D and F) for *Helicops infrataeniatus*. Females are hollow dots and males are full. Wireframe is the variation over centroid size. Red shape is the average configuration of the head and black is the variation over the axes.

**Fig. 4:** Principal components analysis of dorsal, lateral and ventral view for juveniles (A, B and C) and adults (D, E and F) for *Helicops infrataeniatus*. Females are brown dots and males are black. Wireframe is the variation over centroid size in which red shape is de average and black is the variation over the axes.

**Fig. 5:** Principal components analysis after size correction for dorsal, lateral and ventral view for juveniles (A, B and C) and adults (D, E and F) for *Helicops infrataeniatus*. Females are brown dots and males are black. Wireframe is the variation over centroid size in which red shape is de average and black is the variation over the axes.

**A****Figure 1****B**

1 cm

**C**

1 cm

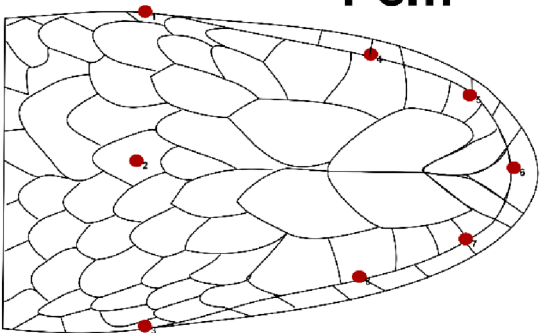


Figure 2

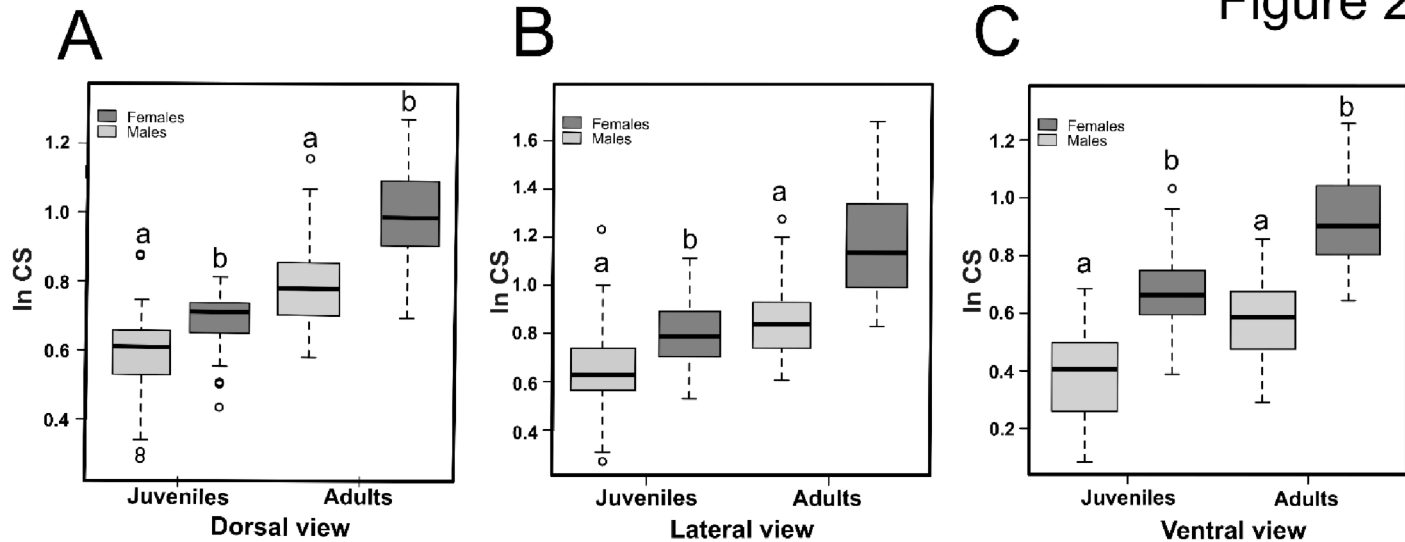


Figure 3

○ Juveniles  
● Adults

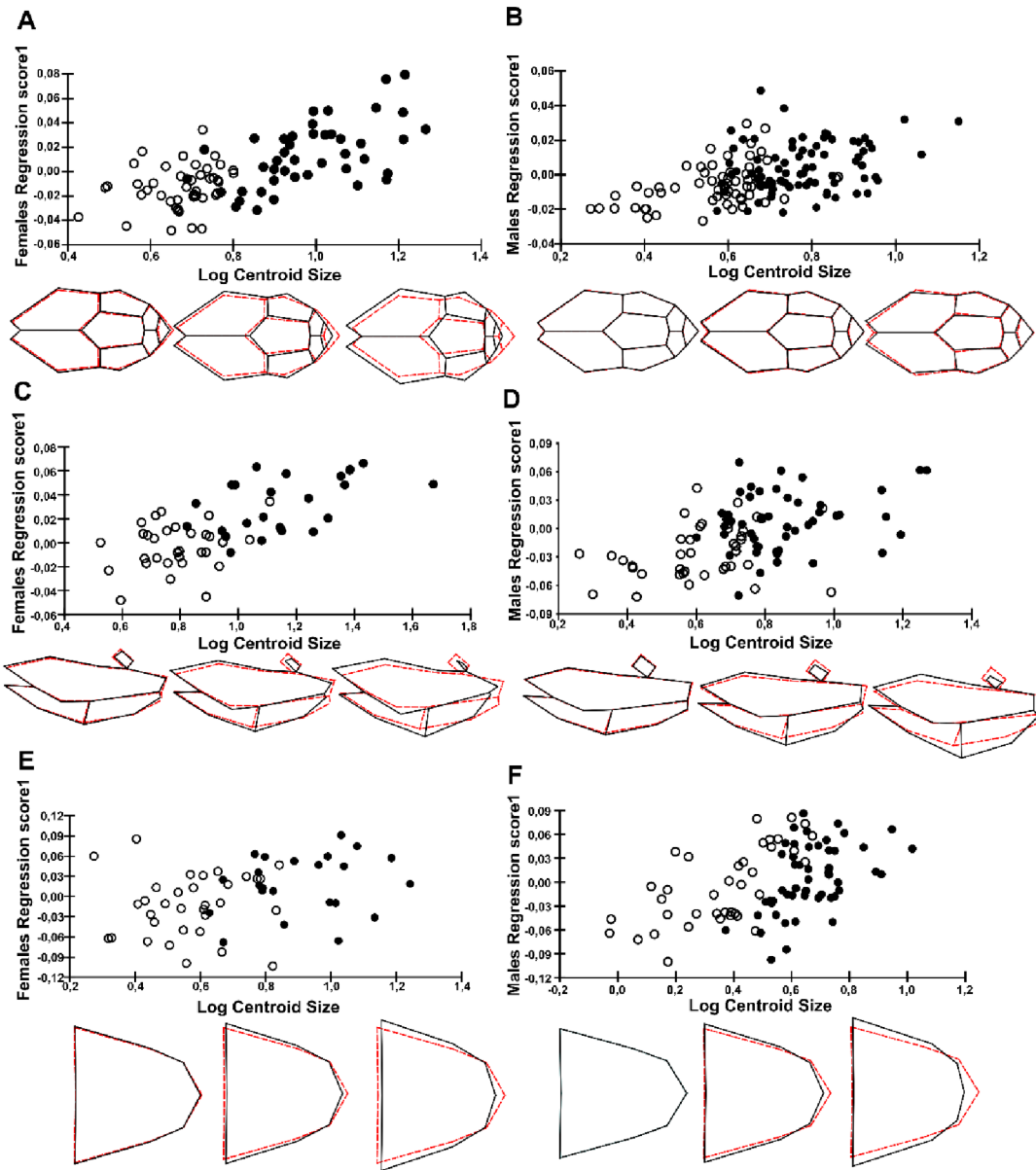




Figure 4

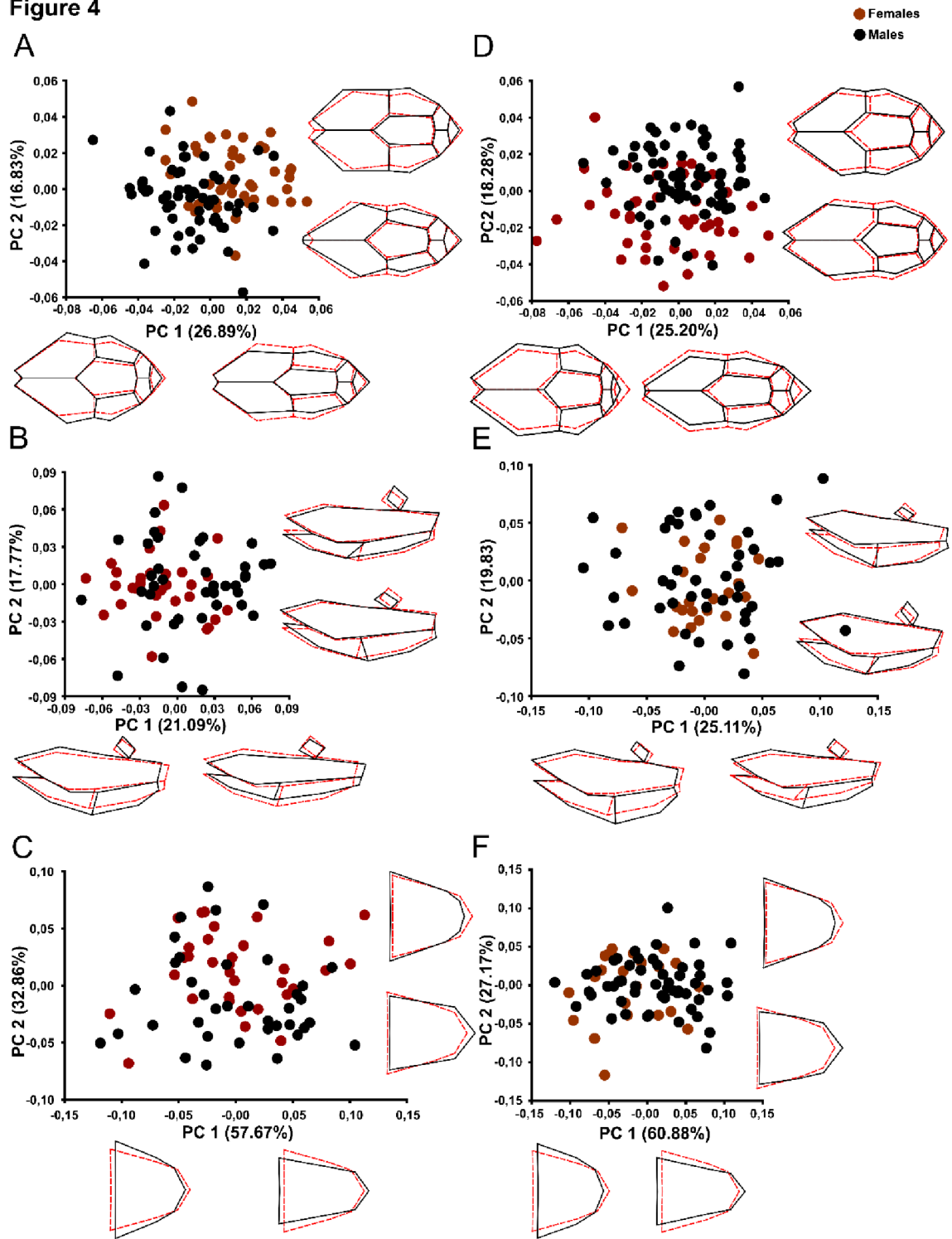
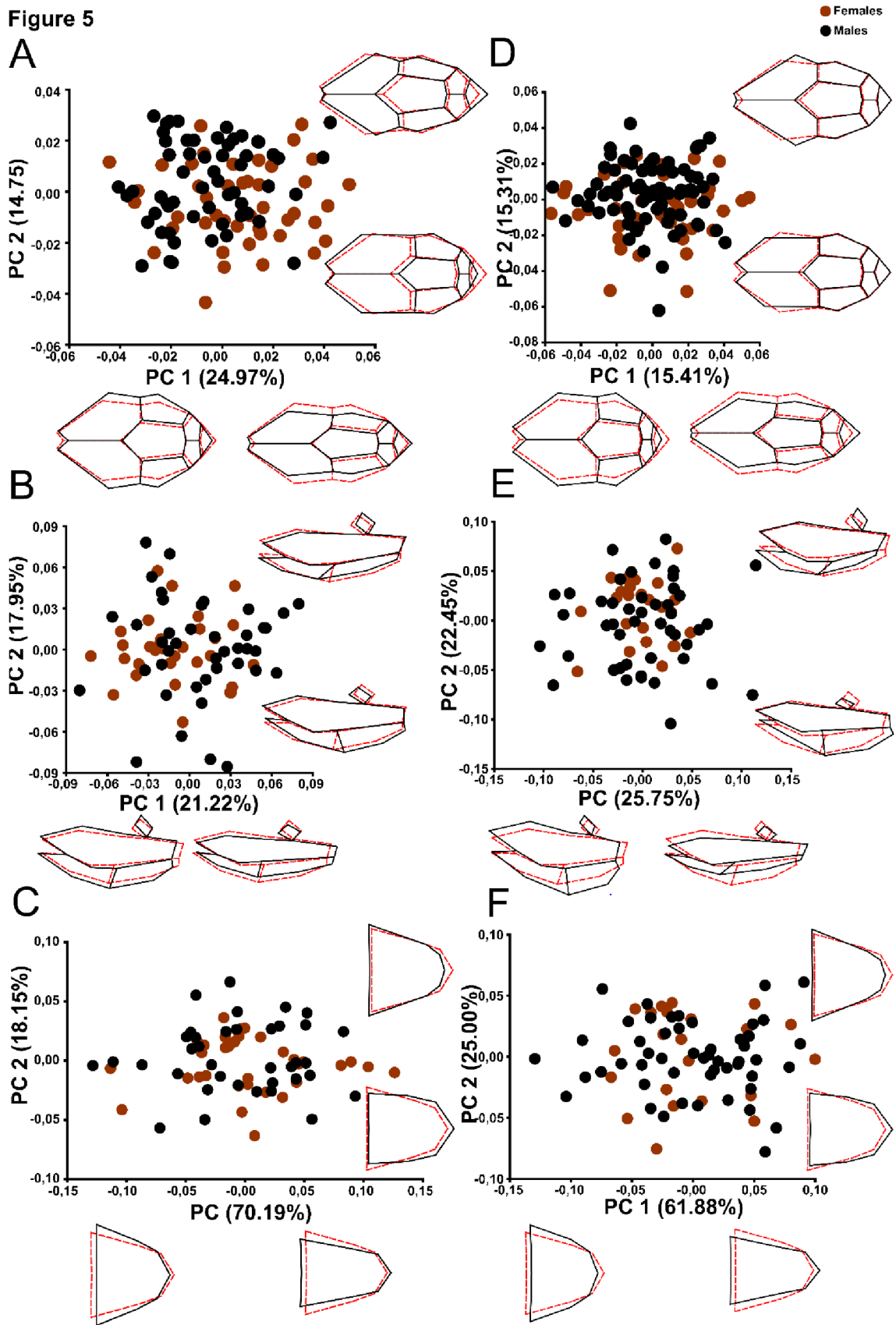


Figure 5



**Table 1.** Two-way PERMANOVA on shape variables to test the influence of the age group and the sex in the head shape of *Helicops infrataeniatus* considering the general dataset. Df = degrees of freedom; SS = sum of squares; MS = mean square; Rsq = R-Squared (R<sup>2</sup>). Significance is highlighted in italics

View	Variable	Df	SS	MS	Rsq	F	p-value
Dorsal	Age Group	1	0.0110	0.0110	0.0224	5.1911	<i>0.0002</i>
	Sex	1	0.0241	0.0241	0.0487	11.286	<i>0.0001</i>
	Residuals	222	0.4745	0.0021			
	Total	225	0.4947				
Lateral	Age Group	1	0.0110	0.0110	0.0447	5.191	<i>0.0002</i>
	Sex	1	0.0241	0.0241	0.0306	5.191	<i>0.0001</i>
	Residuals	138	0.9878	0.0071			
	Total	141	0.9717				
Ventral	Age Group	1	0.0055	0.0050	0.0080	1.092	0.2944
	Sex	1	0.0101	0.0101	0.0148	2.018	0.0977
	Residuals	142	0.7163	0.0050			
	Total	145	0.6855				

**Table 2.** Multivariate regressions of head shape over size data (natural logarithm of centroid size) for both male and female of *Helicops infrataeniatus* in juvenile and adult stage of development. Significance is highlighted in italics.

View	Sex	Dataset	Size Variable	Predicted (%)	p-value
Dorsal	Female	Juveniles	lnCS	6.40	<i>0.0038</i>
		Adults	lnCS	9.49	<i>0.0005</i>
		General	lnCS	12.72	<i>&lt;0.0001</i>
	Male	Juveniles	lnCS	5.64	<i>0.0007</i>
		Adults	lnCS	3.15	0.0122
		General	lnCS	2.32	<i>0.0022</i>
Lateral	Female	Juveniles	lnCS	3.02	0.5342
		Adults	lnCS	4.81	0.3343
		General	lnCS	6.70	<i>&lt;0.0001</i>
	Males	Juveniles	lnCS	2.51	0.4093
		Adults	lnCS	4.49	0.0464
		General	lnCS	3.44	<i>0.0062</i>
Ventral	Females	Juveniles	lnCS	17.01	<i>0.0027</i>
		Adults	lnCS	3.69	0.4746
		General	lnCS	5.01	0.0496
	Males	Juveniles	lnCS	20.77	<i>0.0001</i>
		Adults	lnCS	12.75	<i>0.0014</i>
		General	lnCS	7.79	<i>&lt;0.0001</i>

**Table 3.** One-way PERMANOVA over symmetric component and Procrustes coordinates of juveniles and adults' dataset. SS= sum of squares. Significance is highlighted in italics.

	<b>Dataset</b>	<b>View</b>	<b>SS</b>	<b>F</b>	<b>p - value</b>
With allometry	Juveniles	Dorsal	0.2082	10.64	<i>0.0001</i>
		Lateral	0.4172	3.689	<i>0.0003</i>
		Ventral	0.3246	2.452	0.0785
	Adults	Dorsal	0.2753	6.838	<i>0.0001</i>
		Lateral	0.5114	3.266	<i>0.0026</i>
		Ventral	0.3556	4.339	0.0126
Size free	Juveniles	Dorsal	0.1927	5.426	<i>0.0001</i>
		Lateral	0.4041	2.085	0.0228
		Ventral	0.2639	0.5636	0.5622
	Adults	Dorsal	0.2548	1.172	0.2812
		Lateral	0.4865	1.119	0.3138
		Ventral	0.3181	0.1774	0.9176

Supplemental Material Table 1 Description of the anatomical landmarks (LM) used head scales for *Helicops infrataeniatus*, displayed in Fig.1

View	LM	Landmark description
Dorsal	1	tip of internasal scale
	2	junction of internasal and left prefrontal scale
	3	junction between internasal and both prefrontal scales
	4	intersection of internasal and right prefrontal scale
	5	lateral-most expansion of left prefrontal
	6	intersection between anterior prefrontal, left supraocular and frontal scale
	7	intersection of frontal scale and both prefrontal
	8	intersection between anterior prefrontal, right supraocular and frontal scale
	9	lateral-most expansion of right prefrontal
	10	junction of left supraocular and posterior left eye
	11	junction of left supraocular and left parietal
	12	junction of posterior left supraocular, left parietal and frontal scale
	13	intersection of frontal and both parietal scales
	14	junction of posterior left supraocular, right parietal and frontal scale
	15	junction of right supraocular and right parietal
	16	junction of right supraocular and posterior right eye
	17	lateral-most expansion, middle of left parietal
	18	the tip of left parietal
	19	posterior intersection of both parietal
	20	tip of right parietal
	21	lateral-most expansion, middle of right parietal
Lateral	1	tip of the nose
	2	tip of the mouth
	3	inferior junction of supralabial 5 and 6
	4	junction of supralabial 5 and 6
	5	junction of supralabial 7 and 8
	6	posterior-most extension of supralabial 8

	7	superior-most extension of supralabial 7
	8	superior junction of supralabial 5 and 6
	9	posterior-most extension of eye
	10	superior-most extension of eye
	11	anterior-most extension of eye
	12	inferior-most extension of eye
	13	posterior-most extension of infralabial 10
	14	inferior junction of infralabial 8 and 9
	15	inferior intersection of infralabial 6 e 7
	16	junction of infralabial 4 and 5
	17	tip of the bottom jaw
Ventral	1	end of the mouth (right)
	2	midle of the mouth
	3	end of the mouth (left)
	4	half of head' size (right)
	5	midle between landmark 4 and 6
	6	tip of the head
	7	midle between landmark 8 and 6
	8	half of head' size (left)

## APPENDIX

Specimens examined from the herpetological collection of Universidade Federal do Rio Grande (CHFURG) Brazil, Rio Grande do Sul, captured on Praia do Laranjal: (CHFURG: 3477, 3478, 3483, 3560, 3623, 3678, 3685, 3694, 3754, 3756, 3799, 3800, 3804, 3805, 3811, 3896, 3900, 3904, 3947, 3949, 4175, 4201, 4230, 4232, 4235, 4238, 4240, 4242, 4243, 4246, 4250, 4253, 4256, 4275, 4276, 4278, 4279, 4295, 4296, 4309, 4313, 4325, 4571, 3484, 3485, 3511, 3515, 3519, 3532, 3598, 3601, 3603, 3613, 3629, 3631, 3634, 3636, 3638, 3643, 3674, 3675, 3686, 3692, 3695, 3712, 3717, 3763, 3795, 3827, 3831, 3842, 3902, 3903, 3906, 3960, 3965, 4019, 4082, 4113, 4123, 4214, 4297, 4303, 4304, 4305, 4327, 4330, 4335, 3498, 3505, 3512, 3520, 3524, 3525, 3528, 3606, 3618, 3619, 3620, 3621, 3627, 3628, 3673, 3680, 3683, 3684, 3688, 3690, 3693, 3697, 3698, 3702, 3704, 3710, 3713, 3762, 3788, 3789, 3797, 3808, 3815, 3818, 3823, 3824, 3828, 3833, 3841, 3843, 3845, 3848, 3901, 3908, 3911, 3912, 3913, 3945, 3953, 4022, 4024, 4025, 4027, 4080, 4109, 4112, 4115, 4116, 4117, 4118, 4139, 4183, 4189, 4190, 4210, 4211, 4236, 4251, 4252, 4255, 4298, 4299, 4307, 4310, 4316, 4324, 4329, 4332, 4333, 3486, 3487, 3503, 3510, 3517, 3530, 3559, 3597, 3614, 3624, 3625, 3630, 3664, 3669, 3679, 3689, 3691, 3700, 3701, 3708, 3711, 3715, 3722, 3765, 3766, 3770, 3771, 3784, 3785, 3786, 3826, 3829, 3834, 3844, 3847, 3849, 3854, 3869, 3914, 3915, 3961, 3964, 3971, 4021, 4028, 4030, 4031, 4035, 4092, 4095, 4121, 4122, 4124, 4187, 4193, 4202, 4206, 4208, 4337).

## CAPÍTULO 2

Manuscrito a ser submetido para o periódico Amphibia-Reptilia. Normas de formatação do periódico encontram-se no anexo dois desta dissertação

**Título: Evaluating sexual and morphological variation in head shape in *Erythrolamprus poecilogyrus sublineatus* and *Erythrolamprus jaegeri coralliventris* (Serpentes: Dipsadidae)**

Autoria: Miriam Duarte, Daniel Loebmann, Adriana Gava

Número de palavras: 4.588

Figuras: 7

Qualis Capes 2017: A2

Fator de Impacto: 1,6



**Evaluating sexual dimorphism and morphological variation in head shape in *Erythrolamprus poecilogyrus sublineatus* and *Erythrolamprus jaegeri coralliventris* (Serpentes: Dipsadidae)**

Miriam Duarte<sup>a</sup>, Daniel Loebmann<sup>a,b</sup>, Adriana Gava<sup>a,c</sup>

<sup>a</sup> Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália km 8, CEP: 96203-900, Vila Carreiros, Rio Grande, Rio Grande do Sul, Brazil.

<sup>b</sup> Laboratório de Vertebrados, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália km 8, CEP: 96203-900, Vila Carreiros, Rio Grande, Rio Grande do Sul, Brazil

<sup>c</sup> Laboratório de Genética e Biologia Molecular, Universidade Federal do Rio Grande, Av. Itália km 8, CEP: 96203-900, Vila Carreiros, Rio Grande, Rio Grande do Sul, Brazil

**Corresponding author:** [adriana.gava@gmail.com](mailto:adriana.gava@gmail.com)

**Abstract:** Describe and quantify animals' structures variation is important in order to understand its adaptations and evolution. Herein, we used geometric morphometric tools to analyze the presence of sexual dimorphism in head size and shape (dorsal, lateral and ventral views) both in *Erythrolamprus jaegeri coralliventris* (81 females and 70 males) and *Erythrolamprus poecilogyrus coralliventris* (56 females and 107 males). We took photographs of the head in order to evaluate static allometry and to compare head shape between species. Generalized Procrustes analysis was performed to obtain size and shape data. T test evaluated size dimorphism and two-way PERMANOVA tested over sexual dimorphism and species shape differences hypothesis. Multivariate regressions of shape over size were conducted to verify size influence on shape. Both species have size sexual dimorphism that is, females have larger heads than males. In *E. j. coralliventris*, allometry is significant only over dorsal view of females' head, while *E. p. sublineatus* have allometry in dorsal and ventral views of males' head. Considering dorsal shape, both species are dimorphic and males have wider heads and shorter snouts than females. Only *E. p. sublineatus* is dimorphic in lateral and ventral views. Males have proportionally larger eyes and slender heads in lateral view and ventral view is narrower and longer than in females. Heads' morphology also is different between species in dorsal and ventral views. Dorsally, *E. j. coralliventris* have narrower and shorter than *E. p. sublineatus*, which have wider and longer heads.

**Key words:** adaptation, environment conditions, evolution, interspecific variation, morphology

## Introduction

Up to our knowledge, morphology and anatomical features have been serving as crucial indicators of organismic similarity at least 384 B.C. Recognizing that variation in the form of morphological characters can be influenced by physical and mathematical aspects such as size, D'Arcy Thompson emerged as a trailblazer in geometric morphometric analyses. (Thompson 1945). Regarding morphometric comparisons, this tool has been utilized to elucidate similarities and differences related to sex (Tamagnini et al. 2018; Murta-Fonseca et al. 2019; Abegg et al. 2020; dos Santos et al. 2022) ontogenetic changes (Andjelkovic et al. 2016; da Silva 2017), geographic variations (Manier 2004), diet influence (Meik et al. 2012; Klaczko et al. 2016; Deepak et al. 2023) and interspecific comparisons (Ruane 2015).

In addition to studies of shape variation, evaluation of the size impact on the variation of the shape of structures is frequent. Allometry refers to a pattern of scaling relationships, i.e., the disproportionate scaling of structures or functions with body size (Lindstedt & Hoppeler 2023). Size often exerts influence on shape variation (Klingenberg 2016). Indeed, it is possible to compare these scaling relationships between sexes within the same developmental stage (static allometry), between different developmental stages (ontogenetic allometry), and between different species or taxonomic groups (evolutionary allometry) (Klingenberg and Zimmermann 1992).

Serpentes currently comprises a diverse group with 4,073 species recognized so far (Uetz 2024), with at least 436 species (10.70%) occurring in Brazil. *Erythrolamprus jaegeri coralliventris* is an aglyphous, oviparous species with a seasonal reproductive pattern, capable of attaining lengths of up to 55 cm (Giraud 2004; Teixeira et al. 2020). These animals occur from Paraguay to Argentina, predominantly in wetland ecosystems, through which they engage in foraging and resting activities (de Lema 2002). Specimens from the Brazil's Pampa coastal region exhibit specialized dietary preferences, preying primarily on anuran amphibians (82.2%) and fish (10.7% [Corrêa et al. 2014]). Dos Santos et al. (2010) observed the adaptedness of *E. j. coralliventris* to underwater locomotion and efficient submerged predation of *Cheirodon interruptus* fish, underscoring the significance of these prey in its dietary spectrum. Additionally, Schmitt & Deiques (2009) described the dorsalization of the nasal fenestrae and the presence of a swollen nasal vestibule as plausible adaptations to its semiaquatic lifestyle, facilitating

underwater breathing and preventing water entry when submerged. *E. j. coralliventris* also exhibits female-biased sexual size dimorphism in rostrum cloacal length (Teixeira et al. 2020).

*Erythrolamprus poecilogyrus sublineatus*, another aglyphous species, is distributed throughout Argentina and Uruguay, being also abundant in southern Brazil (Dixon and Markezick 1992). Attains lengths of up to 70 cm and is commonly associated with wetland environments, grasslands, wetland forests, coastal restingas and dunes, in which it displays a generalist diet, including anuran amphibians, fish, reptiles, and small mammals (dos Santos et al. 2012; Corrêa et al. 2014). Regarding sexual dimorphism, females exhibit larger rostrum-cloacal lengths compared to males (Quintela et al. 2017; Teixeira et al. 2020). Still, both *Erythrolamprus* species are sympatric and syntopic in the coastal region of southern Brazil (Quintela and Loebmann 2009, dos Santos et al. 2012) through which these species were evolving mechanisms of co-occurrence (Corrêa et al. 2014)

Serpentes exhibits considerable evolutionary success, colonizing diverse habitats ranging from terrestrial to aquatic, marine, arboreal, and fossorial environments (Cadle and Greene 1993; Martins 1993; Martins and Oliveira 1999). Consequently, one would expect morphological adaptations reflecting the ecological pressures experienced in each habitat. According to Segal et al. (2016), there is a tendency for aquatic snakes to develop more hydrodynamic heads compared to terrestrial ones, with the posterior portion of the head being broader and the anterior region narrower, thus enhancing hydrodynamics during swimming.

Furthermore, the head of snakes is a region constantly subjected to selection due to its close relationship with feeding behaviors, such as searching for food, capturing prey, and swallowing (Vincent et al. 2009; Hampton 2011; Klaczko et al. 2016). Thus, snake diet can significantly influence head morphology. Some authors have noted the predominance of a wider and shorter shape in species mainly consuming anuran amphibians, while snakes preying on fish generally exhibit a longer and thinner head (Vincent et al. 2009; Hampton et al. 2011; Murta-Fonseca et al. 2019). Additionally, Klaczko et al. (2016) demonstrated that the cranial morphology of the Xenodontinae subfamily is associated with the type of food consumed by the species. Regarding sexual dimorphism, generalist species exhibit some degree of morphological differences in head shape due to the broad

spectrum of prey consumed by males and females. On the other hand, the specialist snakes showed a trend toward homogeneous morphology and the absence of sexual dimorphism (Abegg et al. 2020; Borczyk et al. 2021).

Therefore, the present study aimed to investigate sexual dimorphism in head size and shape in *E. p. sublineatus* and *E. j. coralliventris*. We explored whether size predicts shape within each sex, and to what degree. Analyses were also conducted to compare head size and shape between the two species. We hypothesize that feeding strategy drives sexual dimorphism in head size and shape in different ways for both species: being, classified as a generalist species, *E. p. sublineatus* exhibits sexual dimorphism in head size and shape, while the specialist feeding behavior inhibits the development of morphological and size differences between sexes in *E. j. coralliventris*. Still, we hypothesize that, due to females' larger body size, their heads are also broader than males' in *E. p. sublineatus*. Finally, we hypothesize that the two species possess distinct head morphologies: *E. j. coralliventris*, being more adapted to a semiaquatic lifestyle, has a dorsal view of the head with a narrower anterior region compared to the posterior region.

## 2. Methodology

### 2.1 Sampling

We analyzed a total of 151 *E. j. coralliventris* specimens (81 females and 70 males) and 163 *E. p. sublineatus* (56 females and 107 males),  $n = 314$ . We used specimens deposited in the Herpetological Collection of Universidade Federal do Rio Grande (CHFURG) that were collected since 2010 through coastal plain of Rio Grande do Sul. The complete list of specimens used can be consulted in appendix. We determined the sex of each specimen by examining the gonads. Additionally, we distinguished adults from juveniles by taking linear measurements of snout-vent length (SVL) from the tip of the snout to the cloaca. Female *E. j. coralliventris* were considered mature when SVL were larger than 245 mm, while males were considered mature when SVL surpassed the threshold of 185 mm (Teixeira et al. 2020). Female *E. p. sublineatus* were classified as adults when SVL was greater than 333 mm, and males were mature when SVL exceeded 164 mm (Quintela et al. 2017). Due to the low representativity of juveniles, we only considered adult specimens in the analysis.

We captured photographs of dorsal, lateral, and ventral views of each specimen's head with a scale of 1 cm, included by reference, using a Nikon Coolpix p600 digital camera placed in a standardized position and distance above the specimens. The horizontal alignment of camera and the specimens was verified with a spirit level. We used the software TPSUtility version 1.60 (Rohlf 2013) to create a file with all the images. TPSDig version 2.18 (Rohlf 2015) was used to digitalize 25 landmarks in dorsal view ( $n = 314$ ) 17 in lateral view ( $n = 186$ ) and 8 in ventral view (258) (Figure 1). The software MorphoJ version 1.02 (Klingenberg, 2011) performed a Generalized Procrustes Analysis (GPA) of landmark coordinates to remove the effects of scale, rotation and position, and to extract the shape variables (Procrustes coordinates in lateral view and

symmetric component in dorsal and ventral view) used in downstream applications (Goodall 1991; Dryden and Mardia 1998). On the other hand, size data (centroid size (CS)) was obtained from row coordinates. Aiming to assess the repeatability of the landmark configurations and to avoid digitalization errors, the landmarks were digitalized twice by a single observer, with a 15-day interval. The landmarks were digitized twice by a single observer, with a 15-day interval to assess the repeatability of the landmark configurations. Procrustes ANOVA was applied to the size and shape variables of the two digitization replicates to obtain mean squares (MS). The MS variance values related to replicates are smaller than the variance of size or shape configurations among specimens within each replicate when landmarks digitalization is absent of errors.

## 2.2 Analyses

### 2.2.1 Sexual dimorphism and static allometry

Sexual size and shape dimorphism were evaluated through comparisons between females and males within each species. The normality and homoscedasticity of size data variances were assessed using Shapiro-Wilk and Levene tests within the RStudio environment (R Core Team 2016). To investigate sexual dimorphism in head size, t-tests were conducted in RStudio to compare data between sexes for each species and all tree views of the head. Visualization of size dimorphism was possible through Boxplot graphs.

Given the potential differentiated impact of size on males and females, we performed allometric analyses to elucidate the contribution of head size to variation in head shape. To this end, multivariate regressions of head shape data in relation to size information (natural logarithm of centroid size [ln CS]) for males and females of each species were performed using MorphoJ version 1.08 (Klingenberg 2011). Specifically, the Procrustes coordinates for lateral view and symmetric component for dorsal and ventral view of the head were considered in the multivariate regressions. Angles between the regression of females and males were compared to examine sexual dimorphism in allometric trajectories when size significantly influenced shape.

To investigate sexual dimorphism in head shape, a one-way PERMANOVA was conducted over shape data using sex as a factor (PAST version 4.16, Hammer et al., 2001). Cross-validation test was performed to examine the differentiation between the two groups (males and females) while permutation test was utilized to evaluate the significance of Mahalanobis distances between the mean shape of each sex. Canonical Variates Analysis (CVA) was performed for each species to visually explore head shape variability, whereas considering the sex variable.

### 2.2.2 Species differentiation

Comparations between *E. p. sublineatus* and *E. j. coralliventris* were conducted comparing the general group of each species. For evaluate the presence of evolutive allometry, regressions of each species were performed and the allometric patterns between species were compared.

One-way PERMANOVA over shape variables were used to determine if the head's morphology is influenced by species factor. Cross-validation and permutation test over Mahalanobis distances were also conducted comparing species' mean shape. In sequence, we performed a PCA available in MorphoJ over shape data for each species to visualize the overall distribution of the specimens over morphospace and to assess the shape variation. If allometry was significant, regressions residuals were used to verify if there

are species differences in size-independent shape by computing a one-way PERMANOVA and performing a PCA to visualize size-free shape.

### 3. Results

#### 3.1 Sexual dimorphism and static allometry

We found sexual size dimorphism in ventral head view of *E. j. coralliventris* with mean centroid larger in females than males ( $t = 3.7304$ ,  $p < 0.01$  [fig. 2c]). Centroid have similar mean sizes between sexes for the dorsal ( $1.5731$ ,  $p = 0.1178$  [fig. 2a]) and lateral views of the head ( $t = 1.5731$ ,  $p = 0.1178$  [fig. 2b]). For *E. p. sublineatus*, mean centroid of dorsal ( $t = 4.0734$ ,  $p < 0.01$  [fig. 2d]) and lateral head views ( $3.6393$ ,  $p < 0.01$  [fig. 2e]) are larger in females than in males, whereas ventral view is not sexually dimorphic ( $t = 1.5247$ ,  $p = 0.1297$  [fig. 2f]).

In dorsal view, sexual shape dimorphism was observed in both species, as indicated by PERMANOVA results ( $p < 0.01$ , tab. 2), whereas in the lateral and ventral views shape dimorphism is solely significant for *E. p. sublineatus* ( $p < 0.001$ ) but not for *E. j. coralliventris* (tab. 2). Although mean Mahalanobis distance between the sexes of *E. j. coralliventris* was not statistically significantly different in the three views of the head, the CVA emphasized males with a rounded head shape in the dorsal view, featuring a shorter anterior part resulting from shorter prefrontal and internasal scales (fig. 3a). In contrast, females exhibited elongated morphology, characterized by longer parietal, prefrontal, and internasal regions while supraocular scales are shorter and narrower (fig. 3a). Lateral view of the head exhibits a shape change in the relative size of eyes, with females having smaller eyes and their snout is slender than males (fig. 3b). However, shape variation of ventral view between the sexes is totally overlapped (fig. 3c).

Conversely, in *E. p. sublineatus* dorsal view, females are predominantly related to CV1 negative values, while males were associated with positive values (fig. 3a). Particularly, females with negative values also displayed slightly elongated and narrower dorsal view heads, characterized by slender parietal, supraocular, and frontal scales, along with rounder prefrontal and wider internasal regions compared to males (fig. 3a). In contrast, male heads exhibited a shorter and more rounded morphology, with a reduced rostral portion in comparison to females (fig. 3a). Notably, in lateral view males exhibited significantly larger eyes, a slender head, and a shorter and thinner dentary portion compared to females on the positive side (fig. 3b). Conversely, females displayed a squared and wider morphology in this region of the head relative to males (fig. 3b).

Regarding ventral view, females exhibit highly wider and slightly shorter mandible shape, while males in positive side have intensely narrower morphology (fig. 3c).

Although the size of the head affected shape in the dorsal view of the head of female *E. j. coralliventris* specimens, accounting for 4.45% of the shape variation, males' head shape is not influenced by size (3.46%,  $p = 0.013$ , tab. 1). For this view, larger females of *E. j. coralliventris* develop longer heads, with some modifications in anterior portion of the head, where internasal, frontal, and prefrontal scales are lengthier than in smaller animals (fig. 4a). In the lateral (females: 2.654%,  $p = 0.39$ ; males: 6.91%,  $p = 0.016$ , tab. 1) and ventral view (females: 4.99%,  $p = 0.051$ ; males: 3.83%,  $p = 0.087$ , tab. 1), head shape exhibits isometry, lacking static allometry in *E. j. coralliventris*. Although there is no statistically significant influence of size in those views of the head, we observed some shape changes in the head of animals as they grew. Generally, the head lateral view of larger organisms tends to exhibit proportionally smaller eyes, with the head being flatter and shorter than smaller ones (fig. 4b). Additionally, in the ventral view, the heads of larger specimens are shorter (fig. 4c).

Conversely, in *E. p. sublineatus* specimens, only dorsal male head shape is influenced by size (4.38%,  $p < 0.01$ , tab. 1), whereas females are not affected (3.33%,  $p = 0.063$ , tab. 1). The dorsal head view of larger males of *E. p. sublineatus* shows a longer and slightly narrower head compared to smaller snakes, exhibiting longer internasal and prefrontal scales, and frontal and parietals are short (fig. 4d). Although females exhibited isometric scaling, their morphology changes in response to size increase, resulting in a squared and slimmer head, with thinner and longer parietals scales, slenderer supraocular and prefrontal scales, and prolonged internasal scales (fig. 4d).

Although allometry is not significant (females: 3.04%,  $p = 0.07$ ; males: 1.93%,  $p = 0.123$ , tab. 1) lateral shape also demonstrated some modifications in larger *E. p. sublineatus* specimens. In females, the head is wider, with the anterior portion shorter and the posterior longer (fig. 4e). Both sexes develop proportionally smaller eyes, with changes in males' heads being subtle (fig. 4d). In the ventral view, allometry is significant only in *E. p. sublineatus* males (males: 7.51%,  $p < 0.01$ ; females: 4.68%,  $p = 0.113$ , tab. 1) where, larger males' head is quite narrow compared to smaller males (Fig. 4f), and female's heads are shorter and wider (fig. 4f).



### 3.2. Interspecific analysis

Regarding species comparison, *E. p. sublineatus* and *E. j. coralliventris* differs in head size in dorsal ( $t = -11.339$ ,  $p < 0.01$ ) and ventral ( $t = -2.617$ ,  $p = 0.00931$ ) but not in lateral view ( $t = -1.068$ ,  $p = 0.2874$ ). Furthermore, PERMANOVA confirmed the presence of head shape differences for all the three views ( $p < 0.0001$ , tab. 2). Permutation test confirmed those results by computing significative p-values of Mahalanobis distances between mean head shape in dorsal, lateral and ventral view ( $p < 0.0001$ ). Although, Cross-validation test correctly identified 145 of 151 *E. j. coralliventris* (96.02%) and 160 of 163 *E. p. sublineatus* (98.15%) considering the dorsal view, 71 of 74 *E. j. coralliventris* individuals (95.94%) and 110 of 112 *E. p. sublineatus* (98.21%) analyzing the lateral and 88 of 119 *E. j. coralliventris* (73.94%) and 114 of 139 *E. p. sublineatus* (82.02%) regarding the ventral view of the head.

Analyzing the CVA scatterplot for dorsal shape coordinates, mostly *E. p. sublineatus* individuals showed negative scores for CV1, which were associated to wider and longer head shape, with wider and shorter supraocular, frontal and internasal and narrower and longer prefrontal scales (fig. 3a). On the opposite, *E. j. coralliventris* specimens are mostly associated to positive values of CV2 and are represented by narrower anterior portion of the head, with narrower and longer supraocular and frontal, narrower prefrontal and longer internasal scales (fig. 3a). Compared to *E. p. sublineatus*, *E. j. coralliventris* specimens have more triangular-shaped head and narrower snout, while *E. p. sublineatus* head is rounded (Fig. 3a).

Also, plotting the firsts two CVs of lateral view of the head CV1 discriminate between *E. j. coralliventris* and *E. p. sublineatus*. Positive values of the axis, occupied predominantly by *E. p. sublineatus*, are represented by organisms with proportionally higher heads, larger eyes, shorter snouts and longer posterior portion of the head (fig. 3b). In contrast, *E. j. coralliventris* representants are associated to negative side of the axis and have the opposite morphology characterized by the presence of slender heads, smaller eyes, longer snouts and shorter posterior portion (fig. 3b). Finally, even though the confirmation of species differences in ventral head shape pointed by PERMANOVA, in CVA graph specimens are highly overlapped in both axes (fig. 3c).

#### 4. Discussion

We confirmed in part our hypotheses since *E. p. sublineatus* exhibits sexual dimorphism in shape (across all views of the head) and size (dorsal and lateral views). These findings corroborate with previous studies that provide evidence that species with generalist diet have sexual dimorphism more pronounced than specialist ones (Abegg et al. 2020; Borczyk et al. 2021; Murta-Fonseca et al. 2019). However, we also observed sexual dimorphism in the shape of the head in the dorsal view and in the size of the head in the ventral view of *E. j. coralliventris*, which challenges the idea that a more specialized diet reduces sexual dimorphism in this body part. Regarding the size, the larger head size in female *E. p. sublineatus* specimens can be explained by their larger body size.

Considering the influence of head size on shape, no allometric pattern was observed for both species. However, allometry accounted for a significant proportion of shape variation in the dorsal view of females of *E. j. coralliventris*. In contrast, both the dorsal and ventral views of females of *E. p. sublineatus* displayed allometry. Generally, allometric changes in head shape can be explained by increases in size and the animals' need to prey on larger or different prey items compared to those consumed during their juvenile stages. As *E. j. coralliventris* exhibits a female-biased sexual dimorphism in body size (Teixeira et al. 2020) and head size (as observed in the ventral view [this study]), larger females during growth could require larger prey. This likely explains the significant covariation of size and shape in females of *E. j. coralliventris*.

In *E. p. sublineatus*, in which smaller males than females are observed (Quintela et al. 2017), significant allometry is present in males whereas females did not exhibit allometry. Similar results were found for the European adder *Vipera berus* where only males exhibit covariation of size and head shape (Tamagnini et al. 2018). In that case alteration of the head shape in relation to size is resulted of male-male combats for females (Tamagnini et al. 2018). The allometric pattern observed in *E. p. sublineatus* might be attributed to the evolution of a large head due to sexual selection. However, male to male combat is more frequent when the males are larger than females (Shine 2000). Additionally, there is no evidence obtained in the field that males of *E. p. sublineatus* physically compete for females (Senter et al. 2014).

Interestingly, females of *E. p. sublineatus* and *E. j. coralliventris* exhibit a narrower and more elongated shape of the head, with a thinner and longer snout in the dorsal view

compared to males. Conversely, the opposite pattern was observed in *Mesotes strigatus* and *Tomodon dorsatum*, in which females have wider heads and snouts (Loebens et al. 2019). Loebens et al. (2019) attributed this adaptation to the larger size of females and their need and ability to prey on larger animals. Indeed, larger snakes require more energy to survive (Shine 1991), and in some species, the larger sex assumes a wider head shape, such as in *Crotalus polystictus* (Meik et al. 2012), *Bothrops asper* (Henao-Duque and Ceballos 2013), *Aipysurus eydouxii* (Borczyk et al. 2021), and *Atractus reticulatus* (Abegg et al. 2020).

Furthermore, the head of snakes plays a crucial role in capturing, maneuvering and ingesting food; however, our results reveal a pattern contrary to the trend reported by the authors (Meik et al. 2012; Henao-Duque and Ceballos 2013; Abegg et al. 2020; Borczyk et al. 2021), as females attain larger body lengths and actually possess a narrower and more elongated dorsal view of the head than males. Similarly to *E. p. sublineatus* and *E. j. coralliventris*, females of *Dipsas mikanii*, *D. neuwiedi*, and *D. turgida* exhibit the same morphological pattern, with sexual dimorphism in these species appearing to be linked to the consumption of different prey sizes between sexes (dos Santos et al. 2022). There are no reports of different feeding preferences between males and females of *E. p. sublineatus* and *E. j. coralliventris* (dos Santos et al. 2012; Corrêa et al. 2014); thus, it is plausible that sexual dimorphism in the shape of dorsal view of the head in both species arises from distinct feeding preferences between females and males as a strategy to mitigate intraspecific competition for food resources.

Differences in head morphology are not solely due to divergent selection pressures as a result of difference in diet. It can be also driven by environmental effects according to foraging habitat and mode. *Erythrolamprus p. sublineatus* also exhibits sexual dimorphism when examining the lateral and ventral aspects of the head. In lateral view, females display proportionally smaller eyes compared to males and a more square-shaped snout. Da Silva et al. (2017) were among the few researchers who investigated intraspecific variation in a snake species – *Bothrops atrox* – focusing on the lateral head view. In contrast to our findings, the authors discovered that female vipers tend to have a higher lateral profile than males, along with wider eyes. Indeed, Segal et al. (2016) observed a trend among aquatic and non-aquatic snakes, noting that aquatic species tend to possess smaller eyes and a narrower anterior region in the dorsal head view compared

to their non-aquatic counterparts. Given these observations, one plausible explanation for the smaller eye size and narrower snout in female *E. p. sublineatus* compared to males is their potential higher affinity for aquatic habitats relative to males.

As anticipated, both the size and shape of the head vary among the analyzed species. In dorsal view, *E. j. coralliventris* exhibits a narrower and slightly shorter anterior head portion compared to *E. p. sublineatus*, which displays a more rounded morphology with a slightly elongated snout. Despite being sympatric, the species demonstrate distinct ecological traits. *Erythrolamprus poecilogyrus sublineatus*, being larger in size, is capable of preying on larger organisms such as lizards and small mammals (Corrêa et al. 2014); it is frequently encountered in urban areas, indicating its adaptability to urban environments. Conversely, *E. j. coralliventris*, being smaller, has a more restricted diet consisting mainly of anurans and fish (Corrêa et al. 2014). The species demonstrates remarkable agility in water, suggesting adaptation to the challenges posed by its aquatic habitat (Santos et al. (2010).

Snakes with aquatic behavior tend to develop narrower anterior portion of dorsal view of the head and a more dorsoventrally flattened head shape, potentially enhancing swimming hydrodynamics (Segal et al. 2016). Additionally, aquatic snakes typically exhibit smaller ocular dimensions and abbreviated oral structures compared to non-aquatic species. The narrower dorsal head of *E. j. coralliventris* view with smaller ocular portion and dorsoventral flattening suggest potential adaptations for efficient water locomotion. However, *E. p. sublineatus* also demonstrates adaptations indicative of aquatic habitat affinity, exemplified by its reduced ocular size and shorter oral morphology relative to *E. j. coralliventris*. These observations collectively indicate that both species are morphologically suited to aquatic environments.

## **5. Conclusion**

Our study provides additional insights into the intra-specific variation in both the size and shape of the heads of *E. p. sublineatus* and *E. j. coralliventris*. Due to the limited representation of juvenile specimens in our sampling, our analyses were restricted to adult organisms, which consequently constrains our ability to make assertions regarding morphological variation within and between the species. Furthermore, the morphological comparison between the species yielded valuable insights into how the organisms' lifestyles may influence the morphology and evolution of the groups.

## Acknowledgments

We would like to thank Daiane Carrasco and Loraine Moraes for their help in handling specimens used in this study. DL thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico for researcher fellowship (Proc. 307232/2023-9) and AG to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – finance code 001 and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) - FAPERGS/CNPq-PRONEX number 16/0485-4.

## References

- Abegg, A.D., Passos, P., Mario-da-Rosa, C., Azevedo, W. dos S., Malta-Borges, L., de Moura Bubadué, J. (2020): Sexual dimorphism, ontogeny and static allometry of a semi-fossorial snake (genus *Atractus*). *Zoology. Anz.* **287**: 95–104.
- Andjelkovic, M., Tomovic, L., Ivanovic, A. (2016): Variation in skull size and shape of two snake species (*Natrix natrix* and *Natrix tessellata*). *Zoomorphology* **135**: 243-253.
- Asimov, I. Breve historia de la Biología. Buenos Aires: Ed. Univ. de Buenos Aires, 1966.
- Bookstein, F.L. (1991): Morphometric tools for landmarks data: geometry and biology. Cambridge University Press, Cambridge, 435p.
- Borczyk, B., Paško, L., Kuszniery, J., Bury, S. (2021): Sexual dimorphism and skull size and shape in the highly specialized snake species, *Aipysurus eydouxii* (Elapidae: Hydrophiinae). *PeerJ.* **9**: 1-20.
- Cadle, J.E., Greene, H.W. (1993): Phylogenetic patterns, biogeography, and the ecological structure of Neotropical snake assemblages. In: RICKLEFS, R. E.
- Corrêa, D., Quintela, F.M., Loebmann, D. (2014): Feeding ecology of *E. jaegeri jaegeri* (Günther, 1858) and *E. poecilogyrus sublineatus* (Cope, 1860) in the coastal zone of Subtropical Brazil (Serpentes, Dipsadidae). *An. Acad. Bras.* **88**: 293-308.
- Da Silva, F.M., Oliveira, L.S. de Nascimento, L.R. de S., Machado, F.A., Prudente, A.L. da C. (2017): Sexual dimorphism and ontogenetic changes of Amazonian pit vipers (*Bothrops atrox*). *Zool. Anz.* **271**: 15–24.
- Deepak, V., Gower, D.J., Cooper, N. (2023): Diet and habit explain head-shape convergences in natricine snakes. *J. Evol. Biol.* **36**: 399–411.
- De Lema, T., (2002): Répteis do Rio Grande do Sul: atuais e fósseis – biogeografia – ofidismo. Porto Alegre: Edipucrs, 264p.

- Dixon, J.R., Markezich, A.L. (1992): Taxonomy and geographic variation of *Liophis poecilogyrus* (Wied) from South America (Serpentes: Colubridae). *Texas J Sci* **44**: 131-166.
- Dos Santos, M.B., Huckembeck, S., Bergmann, F.B. & Tozetti, A.M. (2010): Comportamento alimentar aquático de *Liophis jaegeri* (Günther 1858) (Serpentes, Dipsadidae) em cativo. *Biota Neotrop.* **10**: 343-346.
- Dos Santos, M.B., de Oliveira, M., & Tozetti, A. M. (2012): Diversity and habitat use by snakes and lizards in coastal environments of southernmost Brazil. *Biota Neotrop.*, **12**: 78-87.
- Dos Santos, M.M., Klaczko, J., da Costa-Prudente, A.L. (2022): Sexual dimorphism and allometry in malacophagus snakes (Dipsadidae: Dipsadinae). *Zoology.* **153**: 1-14.
- Dryden, I.L., Mardia, K.V. (1998): *Statistical shape analysis*. Wiley, Chichester.
- Giraud, A. (2001): *Serpientes de la Selva Paranaense y del Chaco Húmedo*, first ed. L.O.L.A., Buenos Aires, Argentina.
- Goodall, C.R. (1991): Procrustes methods in the statistical analysis of shape. *J. R. Statist. Soc. B.* **53**: 285–339.
- Hampton, P.M. (2011): Comparison of cranial form and function in association with diet in natricine snakes. *J. Morphol.* **272**: 1435–1443.
- Henao-Duque, A.M., Ceballos, M.V. (2013): Sex-related head size and shape dimorphism in Mapaná snakes (*Bothrops asper*) kept in captivity. *Rev. Colomb. Cienc. Pec.* **26**: 201-210.
- Klaczko, J., Sherratt, E., Setz, E.Z.F. (2016): Are diet preferences associated to skulls shape diversification in xenodontine snakes? *PLoS One.* **11**: 1-12.
- Klingenberg, C.P., Zimmermann, M. (1992): Static, Ontogenetic, and Evolutionary Allometry: A Multivariate Comparison in Nine Species of Water Striders, *Source: Am. Nat.* **140**: 601-620.
- Klingenberg, C.P. (2011): MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**: 353-357.
- Lindstedt, S. L., & Hoppeler, H. (2023): Allometry: revealing evolution's engineering principles. *J. Exp. Biol.* **226**, 24.
- Loebens, L., Hendges, C.D., Almeida-Santos, S. M., Cechin, S.Z. (2019): Morphological variation and sexual dimorphism in two sympatric dipsadine snakes from Southern

- Brazil. Zool. Anz. **280**: 42-51.
- Manier, M.K. (2004): Geographic variation in the long-nosed snake, *Rhinocheilus lecontei* (Colubridae): beyond the subspecies debate. Biol. J. Linn. Soc. **83**: 65-85.
- Martins, M. (1993): Why do snakes sleep on the vegetation in Central Amazonia? Herpetol. Rev. **24**: 83, 84.
- Martins, M., Oliveira, M.E. (1999): Natural history of snakes in forest of the Manaus Region, Central Amazonia, Brazil. Herpetol. Natural Hist. **6**: 78–150.
- Mateus, A.M. 1989: Fundamentos da Zoologia Sistemática. Lisboa: Fundação Caloste Gulbenkian.
- Meik, J.M., Setser, K., Mociño-Deloya, E., Lawing, A.M. (2012): Sexual differences in head form and diet in a population of Mexican lance-headed rattlesnakes, *Crotalus polystictus*. Biol. J. Linn. Soc. **106**: 633–640.
- Meredith, R.W., Janecka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., Goodbla, A., Eizirik, E., Simão, T.L., Stadler, T., Rabosky, D.L., Honeycutt, R.L., Flynn, J.J., Ingram, C.M., Steiner, C., Williams, T.L., Robinson, T.J., Burk-Herrick, A., Westerman, M., Ayoub., N.A., Springer, M.S., Murphy, M.J. (2011): Impacts of the Cretaceous Terrestrial evolution and KPg Extinction on Mammal Diversification. Science. **334**: 521-524.
- Murta-Fonseca, R.A., Machado, A., Lopes, R.T., Fernandes, D.S. (2019): Sexual dimorphism in *Xenodon newiedii* skull revealed by geometric morphometrics (Serpentes; Dipsadidae). Amphib. Reptil. **40**: 461-474.
- Pyron, R.A., Burbrik, F.T., Wiens, J.J. (2013): A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol. Biol. **13**: 53p.
- Quintela, F.M., Loebmann, D. (2009): Guia Ilustrado: Os répteis da região costeira do extremo sul do Brasil. Pelotas: USEB, 83 p.
- Quintela, F.M., Marques, W.C., Loebmann, D. (2017): Reproductive biology of the Green Ground Snake *E. poecilogyrus sublineatus* (Serpentes: Dipsadidae) in Subtropical Brazil. An. Acad. Bras. **89**: 2189-2197.
- Rohlf, F.J. (2013): tps Utility program. SUNY at Stony Brook.
- Rohlf, F.J. (2015): The tps series of software. Hystrix-Italian J. Mammal. **26**: 9–12.
- R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available in: <https://www.R->

project.org.

- Ruane, S. (2015): Using geometric morphometrics for integrative taxonomy: an examination of head shapes of milksnakes (genus *Lampropeltis*). *Zool. J. Linn. Soc.* **174**: 394-413.
- Segall, M., Cornette, R., Fabre, A.C., Godoy-Diana, R., Herrel, A. (2016): Does aquatic foraging impact head shape evolution in snakes? *Proc. Royal Soc. B-Biol. Sci.* **283**: 1-7.
- Senter, P., Harris, S.M., & Kent, D.L. (2014): Phylogeny of courtship and male-male combat behavior in snakes. *PLoS One* **9**.
- Shine, R. (1991): Why do larger snakes eat larger prey items? *Funct. Ecol.* **5**: 493-502.
- Shine, R., Olsson, M.M., Moore, I.T., Lemaster, M.P., Greene, M., Mason, R.T. (2000): Body size enhances mating success in male garter snakes. *Anim. Behav.* **59**, 4-11.
- Uetz, P. (editor), *The Reptile Database*, <http://www.reptile-database.org>, accessed February 29, 2024.
- Tamagnini, D., Stephenson, J., Brown, R.P., Meloro, C. (2018): Geometric morphometric analyses of sexual dimorphism and allometry in two sympatric snakes: *Natrix helvetica* (Natricidae) and *Vipera berus* (Viperidae). *Zoology.* **129**: 25–34.
- Teixeira, H.S., Quintela, F.M, Loebmann, D. (2020): Reproductive biology of *Erythrolamprus jaegeri coralliventris* (Serpentes: Dipsadidae) in the Brazilian Coastal Pampa. *An. Acad. Bras. Ciênc.* **92**: 1-9.
- Thompson, D.W. (1945): *On growth and form*. Bonner JT, ed. Cambridge University Press; 1992.
- Vincent, S.E., Brandle, M.C., Herrel, A., Alfaro, M.E. (2009): Convergence in trophic morphology and feeding performance among piscivorous natricine snakes. *J. Evol. Biol.* **22**: 1203-1211.
- Woese, C. R.; Kandler, O.; Wheelis, M. L. (1990): Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci.* **87**: 4576–4579.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink, HL. (2004): *Geometric morphometrics for biologists: a primer*. Elsevier Academic Press, New York and London, 437p.



## FIGURE CAPTIONS

**Fig. 1:** Illustration of two-dimensional landmarks used in *Erythrolamprus poecilogyrus sublineatus* and *E. jaegeri coralliventris* from Coastal Plain of Rio Grande do Sul. The identified scales include parietal (P), frontal (F), supraocular (SO), prefrontal (PF), internasal (IN), and supralabial (S), and infralabial (I). A) Dorsal view of the head. B) Lateral view of the head. C) Ventral view (see also supplementary Table 1 for descriptions of landmarks).

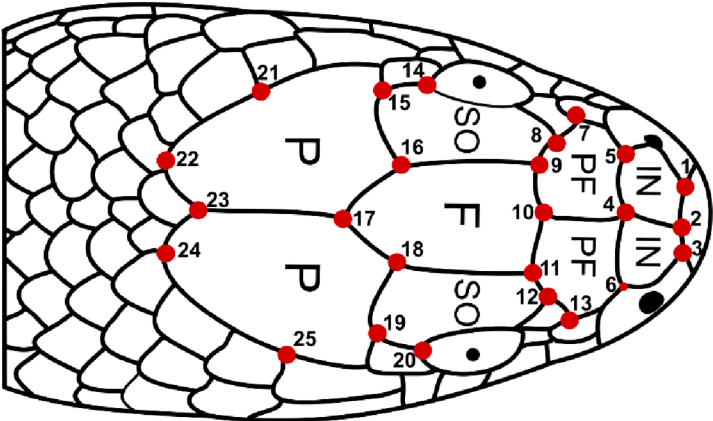
**Fig. 2:** Boxplot of log of the centroid size of *Erythrolamprus jaegeri coralliventris* (A) and *E. poecilogyrus sublineatus* (B) males (brown) and females (black). Limits on boxes correspond to the first and third quartiles and the internal black line is the median. Divergent letters (a, b) indicate significant difference.

**Fig. 3:** Canonical variates analysis for dorsal (A), lateral (B) and ventral (C) view for *Erythrolamprus jaegeri coralliventris* (blue) and *Erythrolamprus poecilogyrus sublineatus* (green). Females are light dots and males are dark. Wireframe is the variation over centroid size in which red shape is de average and black is the variation over the axes.

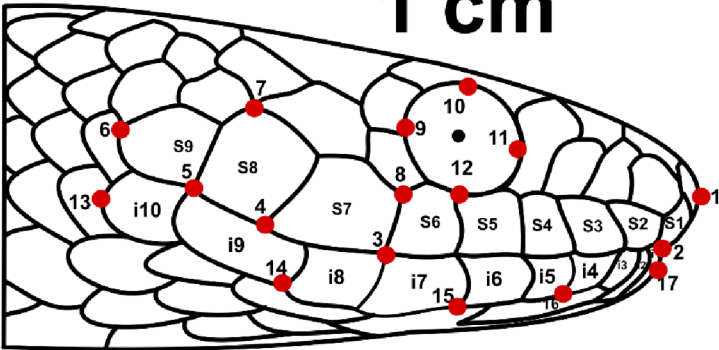
**Fig. 4:** Multivariate regression of head shape over size in dorsal, lateral and ventral view of *Erythrolamprus jaegeri coralliventris* (A, B and C) and *E. poecilogyrus sublineatus* (D, E and F). Females are grey dots and males are black. Wireframe is the variation over centroid size. Red shape is the average configuration of the head and black is the variation over the axe.

Figure 1

A



B



C

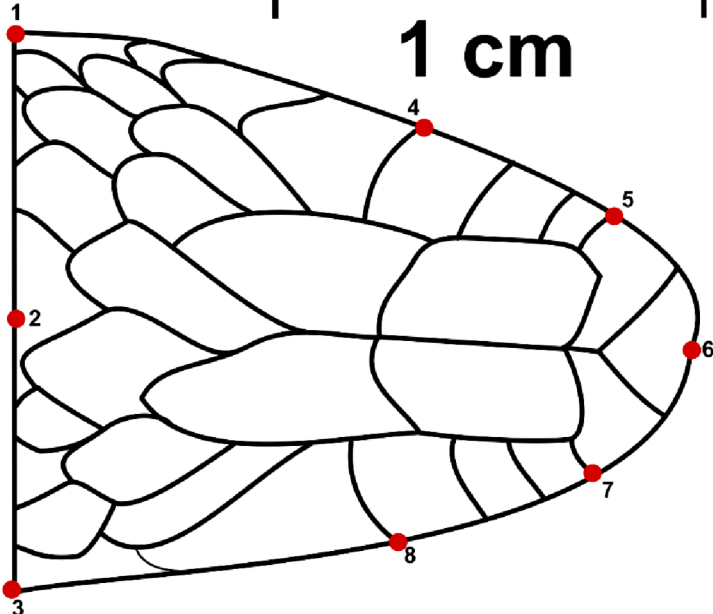


Figure 2

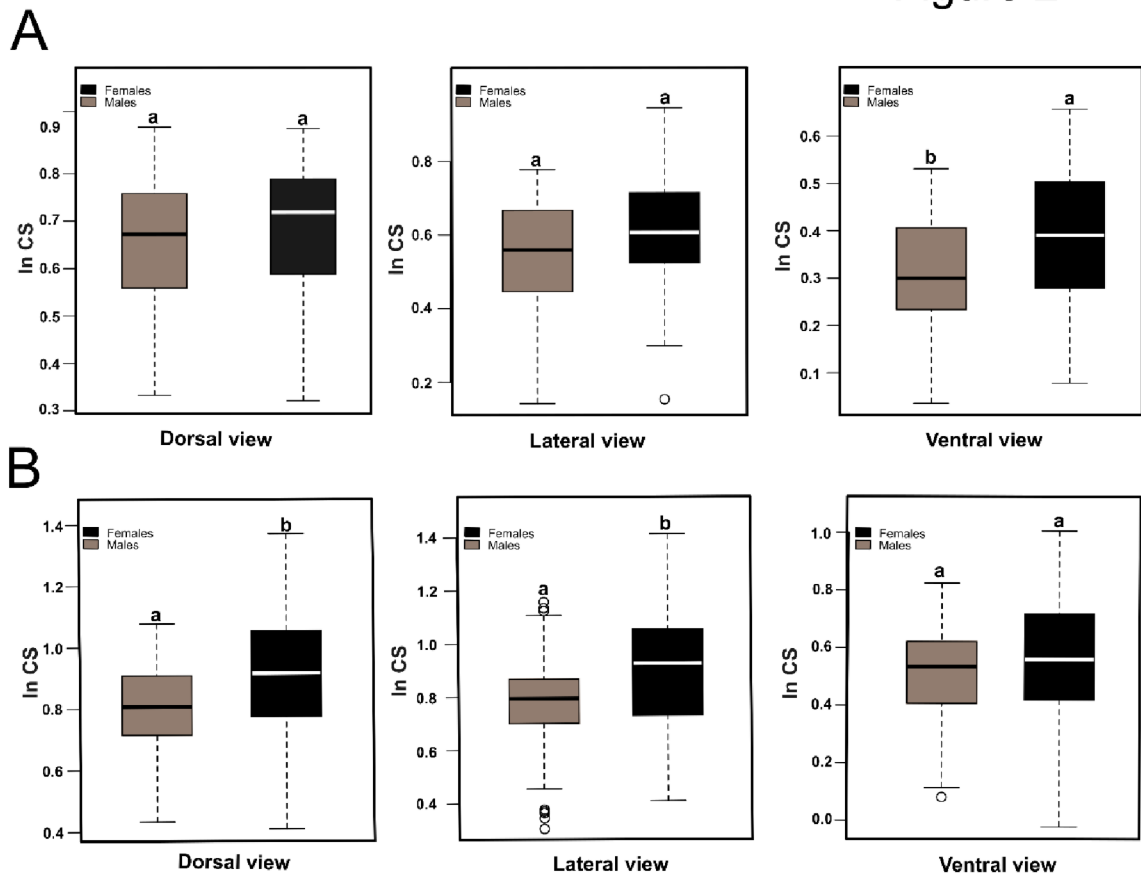


Figure 3

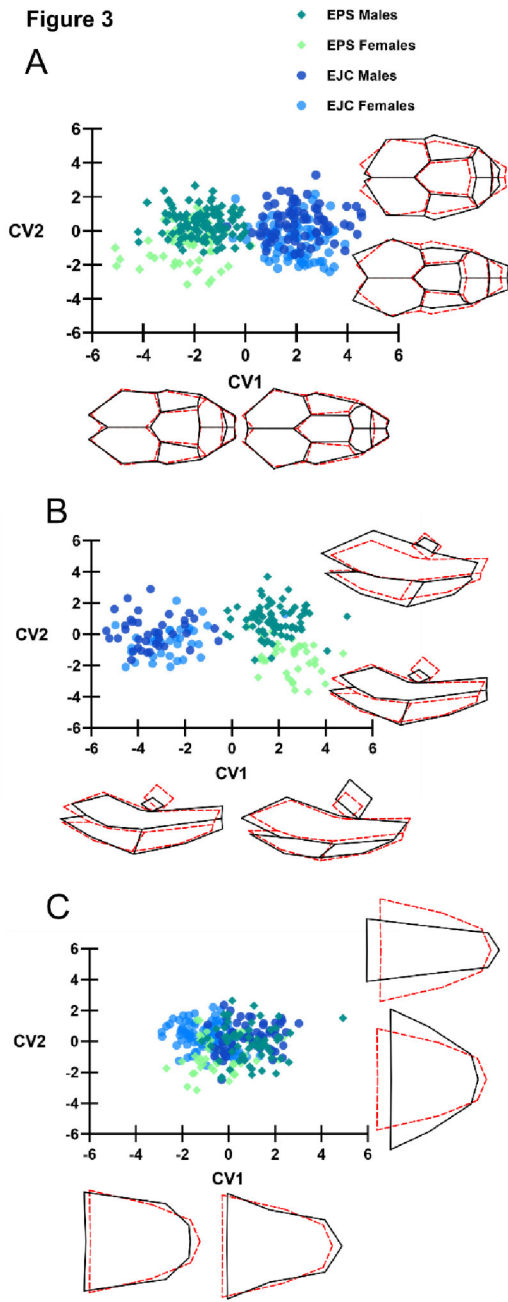
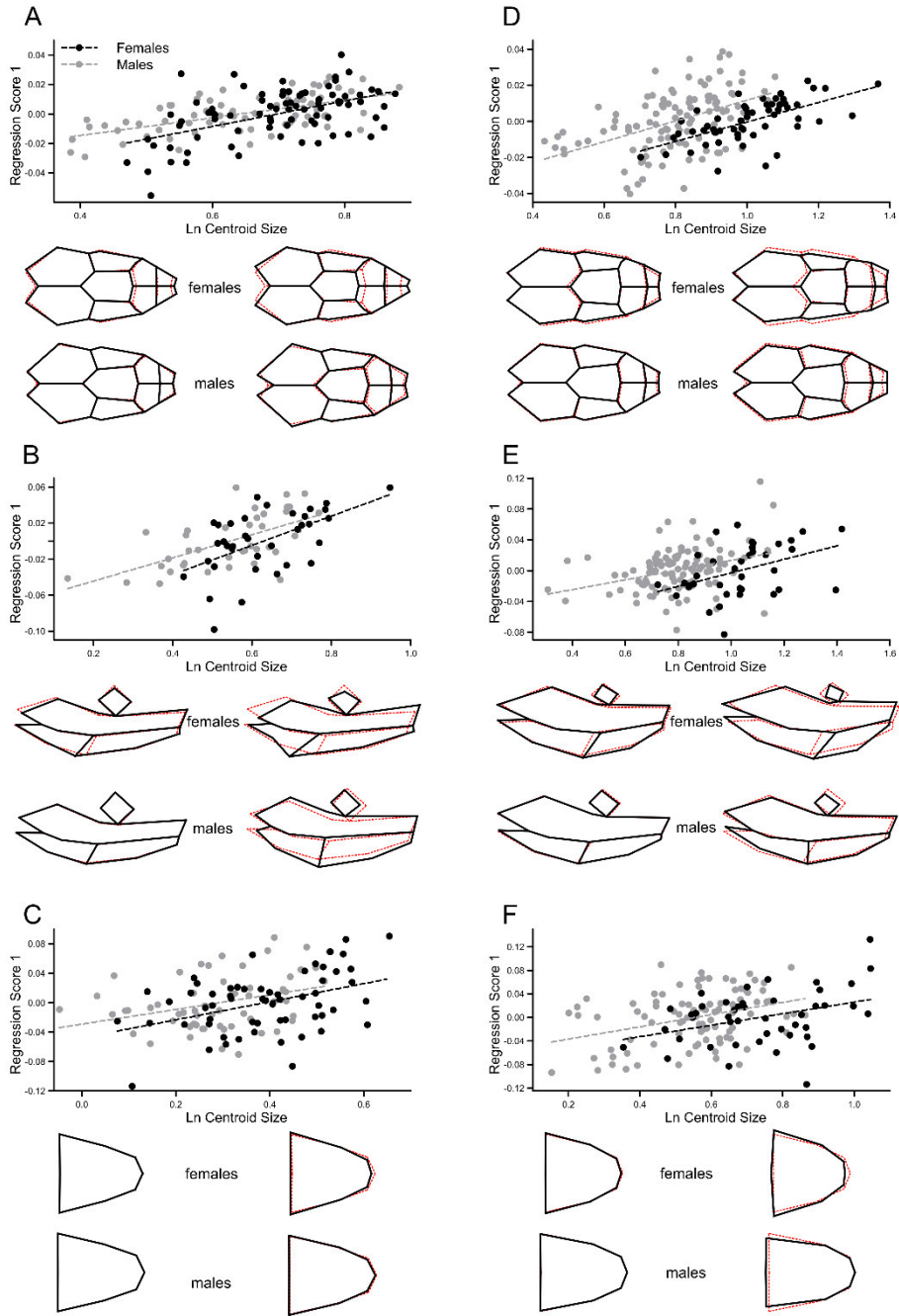


Figure 4



**Table 1:** Multivariate regressions values of head shape variables over size variable (lnCS) for both male and female. Significance is highlighted in italics

Analysis	Species	View	SS	F	p - value
Sexual dimorphism	<i>Erythrolamprus poecilogyrus sublineatus</i>	Dorsal	0.2359	4.653	<i>0.0001</i>
		Lateral	0.6751	3.867	<i>0.0011</i>
		Ventral	0.5733	31.51	<i>0.0001</i>
	<i>Erythrolamprus jaegeri coralliventris</i>	Dorsal	0.2529	2.872	<i>0.0051</i>
		Lateral	0.4003	1.4	0.167
		Ventral	0.5972	1.78	0.1516
Interspecific	<i>Erythrolamprus poecilogyrus sublineatus VS Erythrolamprus jaegeri coralliventris</i>	Dorsal	0.5002	35.83	<i>0.0001</i>
		Lateral	1.017	58.07	<i>0.0001</i>
		Ventral	1.303	21.67	<i>0.0001</i>

**Table 2:** One-way Permutation Multivariate Analysis of Variance (PERMANOVA) of the influence of the sex and species in head shape of dataset. (SS = sum of squares. Significance is highlighted in italics)

Species	Sex	Independent variable	Predicted	p - value
<i>Erythrolamprus jaegeri</i>	Females	lnCS	4.457%	<i>0.0013</i>
<i>coralliventris</i>	Males	lnCS	3.460%	0.0130
<i>Erythrolamprus</i>	Females	lnCS	3.338%	0.0579
<i>poecilogyrus sublineatus</i>	Males	lnCS	4.388%	<i>0.0001</i>

Supplemental Material Table 1 Description of the anatomical landmarks (LM) used head scales for *Erythrolamprus poecilogyrus sublineatus* and *E. jaegeri coralliventris*, displayed in Fig.1

View	LM	Landmark description
Dorsal	1	tip of internasal scale
	2	junction of internasal and left prefrontal scale
	3	junction between internasal and both prefrontal scales
	4	intersection of internasal and right prefrontal scale
	5	lateral-most expansion of left prefrontal
	6	intersection between anterior prefrontal, left supraocular and frontal scale
	7	intersection of frontal scale and both prefrontal
	8	intersection between anterior prefrontal, right supraocular and frontal scale
	9	lateral-most expansion of right prefrontal
	10	junction of left supraocular and posterior left eye
	11	junction of left supraocular and left parietal
	12	junction of posterior left supraocular, left parietal and frontal scale
	13	intersection of frontal and both parietal scales
	14	junction of posterior left supraocular, right parietal and frontal scale
	15	junction of right supraocular and right parietal
	16	junction of right supraocular and posterior right eye
	17	lateral-most expansion, middle of left parietal
	18	the tip of left parietal
	19	posterior intersection of both parietal
	20	tip of right parietal
	21	lateral-most expansion, middle of right parietal
Lateral	1	tip of the nose
	2	tip of the mouth
	3	inferior junction of supralabial 5 and 6

	4	junction of supralabial 5 and 6
	5	junction of supralabial 7 and 8
	6	posterior-most extension of supralabial 8
	7	superior-most extension of supralabial 7
	8	superior junction of supralabial 5 and 6
	9	posterior-most extension of eye
	10	superior-most extension of eye
	11	anterior-most extension of eye
	12	inferior-most extension of eye
	13	posterior-most extension of infralabial 10
	14	inferior junction of infralabial 8 and 9
	15	inferior intersection of infralabial 6 e 7
	16	junction of infralabial 4 and 5
	17	tip of the bottom jaw
Ventral	1	end of the mouth (right)
	2	midle of the mouth
	3	end of the mouth (left)
	4	half of head' size (right)
	5	midle between landmark 4 and 6
	6	tip of the head
	7	midle between landmark 8 and 6
	8	half of head' size (left)



## APPENDIX

Specimens examined from the herpetological collection of Universidade Federal do Rio Grande (CHFURG): *Erythrolamprus jaegeri coralliventris* females: 1030, 1038, 1077, 1083, 1087, 1258, 1262, 1271, 1575, 1577, 1588, 1589, 1592, 1593, 1594, 1595, 1596, 1597, 1598, 1599, 1602, 1604, 1647, 1649, 1651, 1652, 1653, 1785, 1795, 1805, 1806, 1813, 1935, 1950, 2912, 2960, 2973, 2974, 2993, 3084, 3085, 3093, 3096, 3147, 3210, 3241, 3244, 3271, 3304, 3309, 3314, 3315, 3318, 3333, 3335, 3336, 3377, 3378, 3562, 3752, 3996, 4002, 4004, 4010, 4012, 4014, 4043, 4046, 4050, 4646, 4649, 4650, 4651, 4658, 4660, 4661, 4666, 4668, 4672, 4673, 4685, 4714, 4735, 4774, 4793, 4806, 5000, 5085, 5284, 5590, 5719, 5723. *Erythrolamprus jaegeri coralliventris* males: 1028, 1034, 1081, 1092, 1272, 1578, 1579, 1581, 1654, 1656, 1720, 1737, 1801, 1803, 1807, 1808, 1814, 2018, 3242, 3338, 4013, 4538, 4647, 4663, 4667, 4669, 4675, 4677, 4699, 4715, 4717, 4737, 4739, 4794, 4807, 4892, 4915, 4916, 4992, 4995, 4998, 4999, 5001, 5061, 5063, 5227, 5311, 5443, 5467, 5472, 5479, 5481, 5487, 5489, 5490, 5491, 5492, 5494, 5496, 5501, 5502, 5503, 5504, 5505, 5569, 5570, 5597, 5599, 5601, 5602, 5722. *Erythrolamprus poecilogyrus sublineatus* females: 773, 791, 810, 812, 854, 859, 880, 897, 919, 933, 973, 975, 979, 982, 983, 985, 1000, 1001, 1002, 1003, 1250, 1259, 1263, 1527, 1548, 1608, 1630, 1633, 1634, 1635, 1638, 1640, 1641, 1642, 1644, 1645, 1669, 1690, 1735, 1784, 1788, 1789, 1815, 1951, 1955, 1956, 1957, 1958, 2309, 2311, 2322, 2389, 2411, 2509, 2556, 2560, 2972, 2982, 3081, 3136, 3141, 3142, 3145, 3159, 3245, 3268, 3269, 3270, 3322, 3325, 3405, 3407, 3408, 3415, 4322, 4382, 4393, 5042, 5043, 5048, 5050, 5088, 5139, 5140, 5142, 5144, 5147, 5148, 5593. *Erythrolamprus poecilogyrus sublineatus* males: 768, 959, 966, 968, 970, 971, 972, 977, 981, 984, 986, 988, 989, 994, 996, 997, 1005, 1007, 1037, 1074, 1260, 1269, 1270, 1443, 1444, 1445, 1540, 1625, 1626, 1628, 1629, 1631, 1632, 1639, 1673, 1674, 1675, 1676, 1692, 1729, 1730, 1731, 1733, 1793, 1802, 2024, 2147, 2314, 2372, 2544, 2778, 2820, 2836, 2876, 3091, 3112, 3135, 3137, 3139, 3140, 3143, 3144, 3157, 3207, 3320, 3321, 3323, 3324, 3401, 3403, 3404, 3409, 3410, 3413, 3414, 3416, 3417, 3419, 3420, 4053, 4388, 4893, 4894, 4895, 4896, 4922, 5034, 5035, 5036, 5037, 5038, 5044, 5047, 5052, 5054, 5141, 5143, 5145, 5326, 5327, 5442, 5457, 5462, 5463, 5588, 5592, 5594, 5667, 5727, 5924.

## CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

No presente trabalho descrevemos e quantificamos variações intra e interespecíficas na forma da cabeça de três espécies de serpentes muito comumente encontradas na planície costeira do extremo sul do Brasil. No primeiro capítulo, testamos a presença de dimorfismo sexual no tamanho e forma, exploramos a influência do tamanho na variação do formato e descrevemos a variação ontogenética na forma desta estrutura em *H. infrataeniatus*. Encontramos diferenças morfológicas entre os sexos e entre os estágios do desenvolvimento e concluímos que grande parte da variação morfológica pode ser explicada devido à pré-existência de diferenças no tamanho (do corpo e da cabeça) entre os sexos. No entanto, hipotetizamos que, devido ao dimorfismo de tamanho, fêmeas e machos podem estar ocupando nichos diferentes como forma de diminuir a competição intraespecífica por recursos e que esta distinção pode estar sendo refletida na morfologia da cabeça da espécie.

Além disso, testamos a existência de dimorfismo sexual em *E. j. coralliventris* e em *E. p. sublineatus*, a presença de alometria estática e comparamos a morfologia entre as espécies. Foram detectadas diferenças morfológicas relacionadas ao sexo nas duas espécies na vista dorsal e na vista lateral e ventral de *E. p. sublineatus*. Segundo dados da literatura já existentes, primeira espécie é classificada como tendo hábitos alimentares especialistas em anuros e peixes, enquanto a segunda possui a dieta generalista, predando anuros, peixes, pequenos mamíferos e outros répteis. Logo, os hábitos generalistas de *E. p. sublineatus* podem explicar a existência de dimorfismo na espécie, enquanto. Além disso, encontramos divergências morfológicas entre as duas espécies. Devido à sua estreita relação com a captura de alimento, é possível que os hábitos alimentares distintos possam estar contribuindo para a variação morfológica entre as espécies. Também encontramos padrões morfológicos compatíveis com o modo de vida aquático nas duas espécies, como a presença de olhos menores e organismos adultos, estreitamento da região anterior e achatamento dorsoventral da cabeça.

Dessa forma, nosso trabalho contribui para o entendimento sobre a variação morfológica entre e dentro dos grupos analisados e pode trazer *insights* relevantes sobre as adaptações e trajetões evolutivos da morfologia da cabeça no grupo das serpentes. Devido à detecção de dimorfismo sexual tanto no tamanho quanto na forma, também

pontuamos a importância de os trabalhos futuros considerarem analisar a variação na dieta considerando o sexo dos animais como forma de detectar possíveis divergências de nicho.

## ANEXO I

### GUIDE FOR AUTHORS – ZOOLOGY

#### *DESCRIPTION*

---

*Zoology* is a journal devoted to experimental and comparative animal science. It presents a common forum for all scientists who take an explicitly organism oriented and integrative approach to the study of animal form, function, development and evolution.

The journal invites papers that take a comparative or experimental approach to behavior and neurobiology, functional morphology, evolution and development, ecological physiology, and cell biology. Due to the increasing realization that animals exist only within a partnership with symbionts, *Zoology* encourages submissions of papers focused on the analysis of holobionts or metaorganisms as associations of the macroscopic host in synergistic interdependence with numerous microbial and eukaryotic species.

The editors and the editorial board are committed to presenting science at its best. The editorial team is regularly adjusting editorial practice to the ever changing field of animal biology. Recognizing the increasing importance of rapid, effective, international communication, *Zoology* offers the highest scientific standards a short review time online publication in advance of the printed journal color plates free of charge (at the editor's discretion) papers abstracted/indexed by all the major scientific indexing services online supplementary material including videos online supplementary material including videos content innovation tools including interactive phylogenetic trees, 3D molecular models, virtual microscope and MATLAB figure viewer database linking personalized link providing 50 days free access to the final published version of the article on Science Direct.

*Zoology* invites suggestions for special issues. Interested parties may contact one of the editors.

#### *GUIDE FOR AUTHORS*

---

##### *INTRODUCTION*

*ZOOLOGY* is devoted to publishing original research in experimental and comparative animal science (see aims and scope of the journal). We publish original research papers and occasionally review articles that are broad enough to generate a significant contribution to the field of animal science.

Highly specialized work and descriptive studies are not considered.

*ZOOLOGY* invites suggestions for special issues. Interested parties may contact one of the editors.

##### *Submission checklist*

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address

- Full postal address

All necessary files have been uploaded:

*Manuscript:*

- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

*Graphical Abstracts / Highlights files (where applicable)*

*Supplemental files (where applicable)*

Further considerations

- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A competing interest's statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our [Support Center](#).

## **BEFORE YOU BEGIN**

### *Ethics in publishing*

Please see our information on [Ethics in publishing](#).

### *Declaration of competing interest*

Corresponding authors, on behalf of all the authors of a submission, must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. All authors, including those *without* competing interests to declare, should provide the relevant information to the corresponding author (which, where relevant, may specify they have nothing to declare). Corresponding authors should then use [this tool](#) to create a shared statement and upload to the submission system at the Attach Files step. Please do not convert the .docx template to another file type. Author signatures are not required.

### *Declaration of generative AI in scientific writing*

The below guidance only refers to the writing process, and not to the use of AI tools to analyses and draw insights from data as part of the research process.

Where authors use generative artificial intelligence (AI) and AI-assisted technologies in the writing process, authors should only use these technologies to improve readability and language. Applying the technology should be done with human oversight and control, and authors should carefully review and edit the result, as AI can generate authoritative-sounding output that can be incorrect, incomplete or biased. AI and AI-assisted technologies should not be listed as an author or co-author, or be cited as an author. Authorship implies responsibilities and tasks that

can only be attributed to and performed by humans, as outlined in Elsevier's [AI policy for authors](#).

Authors should disclose in their manuscript the use of AI and AI-assisted technologies in the writing process by following the instructions below. A statement will appear in the published work. Please note that authors are ultimately responsible and accountable for the contents of the work.

#### *Disclosure instructions*

Authors must disclose the use of generative AI and AI-assisted technologies in the writing process by adding a statement at the end of their manuscript in the core manuscript file, before the References list. The statement should be placed in a new section entitled 'Declaration of Generative AI and AI assisted technologies in the writing process'.

*Statement: During the preparation of this work the author(s) used [NAME TOOL / SERVICE] in order to [REASON]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.*

This declaration does not apply to the use of basic tools for checking grammar, spelling, references etc. If there is nothing to disclose, there is no need to add a statement.

#### *Submission declaration and verification*

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see '[Multiple, redundant or concurrent publication](#)' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder. To verify compliance, your article may be checked by [Crossref Similarity Check](#) and other originality or duplicate checking software.

#### *Preprints*

Please note that [preprints](#) can be shared anywhere at any time, in line with Elsevier's [sharing policy](#). Sharing your preprints e.g. on a preprint server will not count as prior publication (see '[Multiple, redundant or concurrent publication](#)' for more information).

#### *Use of inclusive language*

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We suggest using alternatives that are more appropriate and (self-) explanatory such

as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

## *Reporting sex- and gender-based analyses*

### *Reporting guidance*

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/ sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the [Sex and Gender Equity in Research \(SAGER\) guidelines](#) and the [SAGER guidelines checklist](#). These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

### *Definitions*

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the [resources on this page](#) offer further insight around sex and gender in research studies.

## *Changes to authorship*

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the

manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

#### *Article transfer service*

This journal uses the Elsevier Article Transfer Service to find the best home for your manuscript. This means that if an editor feels your manuscript is more suitable for an alternative journal, you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated [Scientific Managing Editor](#), a tool assisted recommendation, or a combination. If you agree, your manuscript will be transferred, though you will have the opportunity to make changes to the manuscript before the submission is complete. Please note that your manuscript will be independently reviewed by the new journal. [More information](#).

#### *Copyright*

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see [more information](#) on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. [Permission](#) of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has [preprinted forms](#) for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete a 'License Agreement' ([more information](#)). Permitted third party reuse of gold open access articles is determined by the author's choice of [user license](#).

#### *Author rights*

As an author you (or your employer or institution) have certain rights to reuse your work. [More information](#).

#### *Elsevier supports responsible sharing*

Find out how you can [share your research](#) published in Elsevier journals.

#### *Role of the funding source*

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

#### *Open access*

Please visit our [Open Access page](#) for more information.

#### *Language (usage and editing services)*

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the [English Language Editing service](#) available from Elsevier's Author Services.



## Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

### *Submit your article*

Please submit your article via <https://www.editorialmanager.com/zool/default.aspx>.

### *Referees*

All authors must suggest 3-5 potential reviewers per submission, together with their full addresses and email details. Potential reviewers should not include anyone with whom the authors have collaborated closely. While providing reviewer suggestions, please ensure to include the following details: full name of the reviewer, affiliation, email address and reason for the suggestion.

## PREPARATION

### Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our [Support Center](#).

### Peer review

This journal operates a single anonymized review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. [More information on types of peer review](#).

### *Use of word processing software*

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the [Guide to Publishing with Elsevier](#)). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

## ARTICLE STRUCTURE

### *Subdivision - numbered sections*

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer

to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

### *Introduction*

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

### *Materials and methods*

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

### *Results*

Results should be clear and concise.

### *Discussion*

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

### *Conclusions*

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

### *Appendices*

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

## *Essential title page information*

- *Title.* Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- *Author names and affiliations.* Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
- *Corresponding author.* Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.
- *Present/permanent address.* If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

## *Highlights*

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that

capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: [example Highlights](#).

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

### *Abstract*

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

#### *Graphical abstract*

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, *Erythrolamprus p. sublineatus*, PDF or MS Office files. You can view [Example Graphical Abstracts](#) on our information site.

### *Keywords*

Immediately after the abstract, provide 3–5 keywords, avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

#### *Acknowledgements*

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

#### *Formatting of funding sources*

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

### Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

## Footnotes

Footnotes are not permitted.

### Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

## Artwork

### Electronic artwork General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Embed the used fonts if the application provides that option.
- Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Size the illustrations close to the desired dimensions of the published version.
- Submit each illustration as a separate file.
- Ensure that color images are accessible to all, including those with impaired color vision.

A detailed [guide on electronic artwork](#) is available.

You are urged to visit this site; some excerpts from the detailed information are given here. *Formats*

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

*Erythrolamprus p. sublineatus* (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.

TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi. TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.

### Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) in addition to color reproduction in print. [Further information on the preparation of electronic artwork.](#)

#### *Figure captions*

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

#### *Tables*

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

#### *References*

##### *Citation in text*

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'.

##### *Reference links*

Increased discoverability of research and high-quality peer review is ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, Crossref and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. *Journal of Geophysical Research*, <https://doi.org/10.1029/2001JB000884>. Please note the format of such citations should be in the same style as all other references in the paper.

##### *Web references*

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

##### *Data references*

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

#### *Preprint references*

Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

#### *References in a special issue*

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

#### *Reference management software*

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support [Citation Style Language styles](#), such as [Mendeley](#). Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. [More information on how to remove field codes from different reference management software.](#)

#### *Reference style*

*Text:* All citations in the text should refer to:

1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication. Citations may be made directly (or parenthetically). Groups of references should be listed first chronologically, then alphabetically.

Examples: 'as demonstrated (Allan and Jones, 1999; Allan, 1999, 2000a, 2000b). Kramer et al. (2010) have recently shown ....'

*List:* References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication. *Examples:*

Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. *J. Sci. Commun.* 163, 51–59.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. *The Elements of Style*, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, In: Jones, B.S., Smith, R.Z. (Eds.), *Introduction to the Electronic Age*. E-Publishing Inc., New York, pp. 281–304.

*For reference style 2 Harvard:*

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. <http://dx.doi.org/10.17632/xwj98nb39r.1>.

*Journal abbreviations source*

Journal names should be abbreviated according to the [List of Title Word Abbreviations](#).

### *Video*

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including [ScienceDirect](#). Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our [video instruction pages](#). Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

### *Data visualization*

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions [here](#) to find out about available data visualization options and how to include them with your article.

### *Supplementary material*

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

### *Research data*

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings, which may also include software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your

manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the [research data page](#).

#### *Data linking*

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the [database linking page](#).

For [supported data repositories](#) a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

### *Research Elements*

This journal enables you to publish research objects related to your original research – such as data, methods, protocols, software and hardware – as an additional paper in a [Research Elements journal](#).

Research Elements is a suite of peer-reviewed, open access journals which make your research objects findable, accessible and reusable. Articles place research objects into context by providing detailed descriptions of objects and their application, and linking to the associated original research articles. Research Elements articles can be prepared by you, or by one of your collaborators.

During submission, you will be alerted to the opportunity to prepare and submit a manuscript to one of the Research Elements journals.

More information can be found on the [Research Elements page](#).

#### *Data statement*

To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the [Data Statement page](#).

### **AFTER ACCEPTANCE**

#### *Online proof correction*

To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor.



Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors. If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

### *Offprints*

The corresponding author will, at no cost, receive a customized [Share Link](#) providing 50 days free access to the final published version of the article on [ScienceDirect](#). The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

### *AUTHOR INQUIRIES*

Visit the [Elsevier Support Center](#) to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch. You can also [check the status of your submitted article](#) or find out [when your accepted article will be published](#).

© Copyright 2018 Elsevier | <https://www.elsevier.com>

## ANEXO II:

### Instructions for the authors - Amphibia-Reptilia Journal

Publication of the Societas Europaea Herpetologica

#### Scope

*Amphibia-Reptilia* (AMRE) is a leading European multi-disciplinary journal devoted to most of the aspects of herpetology: ecology, behavior, evolution, conservation, physiology, morphology, paleontology, genetics, and systematics. AMRE publishes high quality original papers, short-notes, reviews, book reviews and news of the Societas Europaea Herpetologica (SEH). The SEH website is located at: [seh-herpetology.org](http://seh-herpetology.org).

#### Ethical and Legal Conditions

The publication of a manuscript in a peer-reviewed work is expected to follow standards of ethical behavior for all parties involved in the act of publishing: authors, editors, and reviewers. Authors, editors, and reviewers should thoroughly acquaint themselves with Brill's publication ethics, which may be downloaded here: [brill.com/page/ethics/publication-ethics-cope-compliance](http://brill.com/page/ethics/publication-ethics-cope-compliance).

#### Online Submission

AMRE uses online submission only. Authors should submit their manuscript online via the Editorial Manager (EM) online submission system at: [amre.editorialmanager.com/](http://amre.editorialmanager.com/). First-time users of EM need to register first. Go to the website and click on the 'Register Now' link in the login menu. Enter the information requested. During registration, you can fill in your username and password. If you should forget your Username and Password, click on the 'send login details' link in the login section, and enter your e-mail address exactly as you entered it when you registered. Your access codes will then be e-mailed to you.

Prior to submission, authors are encouraged to read the 'Instructions for Authors'. When submitting via the website, you will be guided stepwise through the creation and uploading of the various files. A revised document is uploaded the same way as the initial submission. The system automatically generates an electronic (PDF) proof, which is then used for reviewing purposes. All correspondence, including the editor's request for revision and final decision, is sent by e-mail.

#### Choosing Editors in EM

During the submission process, authors will be requested to select one of the four co-editors of the journal, according to the subject area of the manuscript:

Conservation of reptiles

Ecology

Paleontology

Other

José Carlos Brito

BIOPOLIS/CIBIO, Vairão [jcbrito@cibio.up.pt](mailto:jcbrito@cibio.up.pt)

Evolution, genetics and biogeography of reptiles

Behavior of reptiles

Morphology

Physiology

Systematics

Salvador Carranza

Institute of Evolutionary Biology (CSIC-UPF), Barcelona [salvador.carranza@ibe.upf-csic.es](mailto:salvador.carranza@ibe.upf-csic.es)

Evolution, genetics and biogeography of amphibians

Behavior of amphibians

Conservation of amphibians

Diseases

Judit Vörös

Hungarian Natural History Museum [voros.judit@nhmus.hu](mailto:voros.judit@nhmus.hu)

Each co-editor can assign the manuscripts to himself, to another co-editor or to an associate editor. When handled by an associate editor, all communications finally go through the co-editor in charge of the manuscript. Editors must be contacted using Editorial Manager.

File Formats

The submission must consist of a single text file (.doc, .docx, .odt) for the text, tables and figure legends.

For figures, .eps, .jpeg, .tiff, .gif, .pdf or .doc files should be used (with one figure per page).

Figures should not be embedded in an MS Word file but in their initial software. A figure containing several parts must be saved as a single file. To guarantee good resolution in printing, color figures should be saved as an original .tiff or .eps file with an original resolution of 600 or 1200 dpi. Files for color figures should be submitted in CMYK and not in RGB format.

If tables create problems with their placement within the main MS Word file, they can be uploaded separately. All hyperlinks and field codes (e.g., from bibliographic databases) must be removed. Manuscripts in which the track change procedure of MS Word was used must be carefully checked for final acceptance of all corrections and removal of marginal comments.

One pdf of all the separate files will be automatically generated by Editorial Manager.

#### Review Process

All manuscripts that are not editorially rejected or sent back for correction according to the instructions for authors are sent out for peer review. Manuscripts are usually reviewed by at least two external reviewers, one of the two co-editors and possibly an associate editor. Both external reviewers and members of the advisory editorial board can be selected to review a paper. Reviewers are given five days to take in charge a manuscript and then a maximum of three weeks to return their comments via the web platform. The first decision is usually made within one to two months of receipt. Authors must resubmit their manuscript within six weeks of receipt of the decision letter (4 weeks for subsequent submissions), except in cases of personal arrangements made with the coeditors. In sending their revision, authors must provide a separate letter (reply to reviewer letter) in which they paste the comments of the reviewers and their responses directly under each point raised. Revised manuscripts can be sent to reviewers again.

The average time from submission to publication is currently only 6 months. It is expected to be even shorter in the near future, when individual articles will be become available online in advance of the journal issue.

#### Supplementary Media / Data Files

To support and enhance your manuscript, *AMRE* accepts electronic supplementary material, including supporting applications, high-resolution images, background datasets, sound or video clips, large appendices, data tables and other items that cannot be included in the article PDF itself. Authors should submit the material in electronic format together

with the other manuscript files and supply a concise and descriptive caption for each file. In order to ensure that your submitted material is directly usable, please provide the data in one of the broadly accepted file formats for video, audio, etc. and limit the file size (e.g., for video: max. 3 GB). Supplementary files supplied will be published online at FigShare ([www.figshare.com](http://www.figshare.com)), to which reference is made in the published article on Brill Online Books and Journals, and vice versa.

Supplementary text, tables and figures, movies and sounds should be prepared in their final intended format by the authors. For these text files, text should be preceded by a centred title header including the following on separate lines:

- *Amphibia-Reptilia* (Times New Roman, italics, 9 pt)
- **Article title** (Times New Roman, 14 pt)
- First name + initial + family name of each author, with several authors separated by commas (Times New Roman, 12 pt)
- Authors affiliations as in the main document (Times New Roman, 9 pt)

The heading "Supplementary material" (centred, Times New Roman, bold, 12 pt) should then be followed by the supplementary text, tables and figures. Main text should be single spaced, concise, justified-aligned, in font type Times New Roman at size 12 pt. In the main text of the document, these should be referred to as

“Supplementary table/figure/text/movie/sound S1” upon first mention, and subsequently as “table/figure/text/material S1”.

The supplementary file should be submitted in .doc(x) format and will be published online in .pdf format. In the case of supplementary movie or audio files, please contact the editor for details.

#### Contact Address

For any questions or problems relating to your manuscript please contact: [amre@brill.com](mailto:amre@brill.com). For eventual questions about Editorial Manager, authors can also contact the Brill EM Support Department at: [support-em@brill.com](mailto:support-em@brill.com).

Please note, that manuscripts submitted after 20<sup>th</sup> of December will not be considered before the 10<sup>th</sup> of January next year.

#### Submission Requirements

#### Types of Contributions

There are several categories of papers:

Reviews must be written by specialists in the field and focus on hot topics or subjects not reviewed recently in the literature.

Manuscripts that are solely descriptive; purely faunistic (e.g., species check-list); provide simple range updates or report distribution novelties without analyses; based only on captive breeding; consisting only of a juxtaposition of non-connected fields; based on a too small a sample size; or contain reports of work that appear to contravene accepted principles of conservation or ethical standards, may be rejected without external review.

Manuscripts should preferably be grounded in research questions, and those based in hypothesis testing have better chances to be sent to external review.

Moreover, the research must adhere to the legal requirements of the country in which the work was carried out. As the Atlas of European Amphibians and Reptiles (NA2RE) is one of the main projects of SEH, *AMRE* welcomes update papers on European herpetofauna, but these papers should summarize the distribution of species complexes or higher level taxa (preferably genera or family level), should provide new results (e.g. SNP data) and should adhere to the above mentioned criteria. Papers providing simple range updates are recommended to be submitted to the sister journal *Herpetology Notes*. Papers describing new species are more likely to be considered if they offer broad discussion, present several new species, and are based on a sufficient number of specimens. Otherwise, editorial rejection may apply.

*AMRE* does not accept manuscripts previously submitted to preprint servers. Manuscripts that do not follow the editorial style will not be considered for publication and will be sent back to the authors. The latest instructions for authors are available on Editorial Manager ([amre.edmgr.com](http://amre.edmgr.com)), from [seh-herpetology.org/journals/amphibia-reptilia](http://seh-herpetology.org/journals/amphibia-reptilia) and [brill.com/files/brill.nl/specific/authors\\_instructions/AMRE.pdf](http://brill.com/files/brill.nl/specific/authors_instructions/AMRE.pdf).

Recent issues of *Amphibia-Reptilia* may also be consulted before submitting a manuscript. Some papers are available without charge and all papers are available free of charge after 5 years of publication on the BrillOnline Platform for the Journal at [brill.com/view/journals/amre/amre-overview.xml](http://brill.com/view/journals/amre/amre-overview.xml).

#### Language

Manuscripts should be in English, using British spelling and grammar. Spelling should be consistent throughout. If English is not an author's first language, authors may consult an English native speaker to improve and check the language of their manuscript.

## Length

Articles cannot be longer than 8000 words (with 6 tables or figures), short notes are limited to 3000 words (2 tables or figures) and reviews to 12 000 words.

If authors feel that manuscripts intended as an article will suffer severely from the requested word count threshold, they should contact one of the respective co-editors prior to submission (presubmission inquiry) in order to find out whether an exception is justified or not in a specific case.

## Manuscript Structure

### General

Manuscripts must be formatted using double-spacing, with wide margins (3 cm), and with continuous page and line numbering throughout the entire text.

It is mandatory that each manuscript is accompanied by a cover letter in which the authors state why their findings are new and important, and therefore should be published in *AMRE*.

The first page of all manuscripts must contain the title in lower case letters, the first and last names of all authors (no initials; a comma separates each author name, including the two last ones), the affiliation and address of each author, including the e-mail address of the corresponding author (manuscripts without a valid e-mail address will not be considered), the type of manuscript (article, short-note or review) and the total number of words in the whole manuscript (reference list, captions, and tables included), and in the abstract.

Short notes must be prepared without dividing the text into sections, but must contain an abstract. Articles should be assembled in the following order (after the title page): abstract, keywords, introduction, materials and methods, results, discussion, acknowledgements, references, tables, figure legends (grouped together), figures (one per page). Figures should not be embedded in the manuscript file (see below).

### Abstract

The abstract should present a brief summary of the topic, including its aims, results and the relevance of the work. It should be presented in only one language (English) and be no longer than the recommended length (maximum 250 words for articles and reviews, 150 words for short notes).

### Keywords

Four to eight keywords must be presented after the abstract. They should be different from the words used in the title of the manuscript.

### Headings

The main headings are written in bold, the second level headings in *italics*.

### New Paragraphs

Paragraphs must be indented (except after headings) and not separated from each other by an empty line.

### Italics

The scientific names of species should be in *italics*.

### Introduction

The introduction should clearly state the objectives of the study and place it within the context of previous publications. Conceptual introductions are preferred over descriptive texts. The introduction should not merely describe a study species or group but give an overview of a more general topic in herpetology and possibly other organismic groups. In other words, a paper cannot be justified just because a species is threatened or because natural history data are lacking.

### Materials and Methods

These should be presented in a smaller font than the rest of the manuscript (e.g., Times New Roman 10 vs 12). Furthermore, they should be explained in enough detail to allow replication. The sample sizes and the number of independent replicates should be clearly stated. For experimental work details on both housing and observational conditions should be stated. Environmental conditions should be controlled as much as possible to avoid biased results. The exact dates or period of sampling and observation must be given. For studies based on a small number of study sites, the geographic coordinates must be



indicated. Statistics should be explained in the methods, particularly when complex models are used.

### Results

Anecdotal results should not be presented unless they are of primary importance. Instead, they should be stated in the discussion section as personal observations. Results should focus on the main argument(s) of the manuscript. Comparisons should be tested statistically. Sample sizes should be clearly presented.

### Discussion

The results should be discussed in the context of the existing literature. The discussion should not focus only on the study species or group, but should be placed into the context of arguments about other model species to render it in a more conceptual and broad concept. The literature should be covered in sufficient detail for both the topic and the study group. Each paragraph should focus on a different idea, but very short paragraphs should be combined with other paragraphs. The discussion must not be overly long. Speculation should be avoided.

### References Text Citations

These should be presented in chronological order as follows: Petranka (1998) or (Griffiths, 1996;

Michimae and Wakahara, 2001; Schmidt, Feldman and Schaub, 2005). Where there are more than three authors, only the first should be named, followed by “et al.” (not in italics). Both the introduction and discussion must include an adequate number of citations for effective arguments to be established.

### Reference List

In the list, references should be listed in alphabetical, and then chronological order, under the first author’s name and should refer only to publications cited in the text. List references with three or more author names must be placed after those with two. Journal names must be abbreviated according to the official abbreviation. Many abbreviations are, for instance, available at: [cassi.cas.org/search.jsp](http://cassi.cas.org/search.jsp). No space must be inserted between the initials of the first names. No empty lines must be inserted between references. Volume numbers are written in bold. The two last authors or editors in a citation are

separated only by a comma. Abstracts of conferences should not be listed in the reference list, but cited in the text as unpublished data or personal observation. The references of the species description (e.g. Linnaeus, 1758) are not necessarily included in Reference List.

References must be typed in the following order and form, respectively:

- Arnold 2002
- Arnold 2003
- Arnold, Peterson 2002
- Arnold, Pfrender, Jones 2001
- Myers, E.M., Zamudio, K.R. (2004): Multiple paternity in an aggregate breeding amphibian: the effect of reproductive skew on estimates of male reproductive success. *Mol. Ecol.* 13: 1951-1963.
- Kiesecker, J.M. (2003): Invasive species as a global problem. Toward understanding the worldwide decline of amphibians. In: *Amphibian Conservation*, p. 113-126. Semlitsch, R.D., Ed., Washington, Smithsonian.
- Zug, G.R., Vitt, L.J., Caldwell, J.P. (2001): *Herpetology. An Introductory Biology of Amphibians and Reptiles*, 2nd Edition. San Diego, Academic Press.

The use of bibliographic software is recommended to format the references correctly. Independently of using such software, all references must be checked one by one accordingly to our guidelines. In particular, a great deal of attention needs to be paid to the abbreviations of journal names, as they do not depend directly on the downloadable style sheet.

### Acknowledgements

These should be kept brief, but funding agencies should be listed. If legal requirements are necessary for the study, the collecting permits must be cited with reference to the institution who issued them. Individuals are identified by their last name and the initials of their first name.

### Statistics

Means and standard errors (SE) / deviations (SD) or medians and quartiles or ranges should be given as: mean  $\pm$  SE = 5.3  $\pm$  0.3 mm. If equations or special symbols such as

the mean are used, the module MS Equation in MS Word must be used (available in “Insert Object”). Statistical symbols, such as *n*, *F*, *t*, *U*, *Z*, *r* must be indicated in italics. Degrees of freedom are indicated as a subscript to the test statistic ( $F_{2,265, t17}$ ). The name of the test should be given on its first appearance in front of the symbol (e.g., ANOVA, Mann-Whitney). The same test should be applied to the same kinds of analyses throughout the manuscript. *P* values for significant results should be quoted as below a threshold significance value ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ ). Exact probabilities should be given for non-significant results (e.g.  $P = 0.76$ ). Multiple post-hoc tests must be used with caution to avoid experimental error by chance alone. When transformations are used, they should be stated in the materials and methods. The multiple use of individuals should be controlled for or avoided. Multivariate analyses are usually requested when several explanatory variables are tested for one dependent variable or when one explanatory variable is expected to explain several dependent variables.

#### Abbreviations

The International System of Units should be used. Do not employ unexplained abbreviations for institutions, etc.

#### Ethics

Authors should explain and justify, in a cover letter and in their manuscript, all techniques which have resulted in injuries or death of animals. Failing to do so will necessitate editorial rejection of the paper. In the Materials and Methods section of the manuscript, authors should detail as precisely as possible the conditions of maintenance, transport, anaesthesia, and marking of animals. When available, references should be added to justify that the techniques used were not inappropriately invasive. When alternative techniques exist to euthanasia, but were not used, the manuscripts may not be considered for publication.

#### Figures

All illustrations should be drawn to fit into one (66 mm) or two columns (139 mm) of a journal page. Lettering and numbering should be large enough to be clearly visible when the illustration is reduced to published size. When several graphs are presented in the same figure, they should be as homogeneous as possible (i.e., the same range of values on the axes; avoidance of repetition in the different graphs). Maps must include

geographic coordinates, the indication of North, and a graphic scale. All symbols should be explained within the figure or in the legend.

Authors should upload figure files as separate files. These figure files must be uploaded as source files (.jpeg, or .tif), and not .pdfs. The quality of the figure must be suitable for printing - the resolution should be a minimum of 300 dpi (minimum 600 dpi for line art). The image itself must be sharp, and any text in the figure should be legible (at least corps 9 or larger). Figures to be printed in grey scale must not contain color. Poor quality figures may compromise acceptance. The number of illustrations should not be too excessive given the length of the text.

### Color Figures

There is no charge for full color images or figures in either the print or electronic edition.

### Tables

These should be numbered consecutively with Arabic numbers (in bold font) and submitted on separate pages. The table must be drawn using a table editor. This means that the space or tab function cannot be used. A recently published table should be used as a reference for constructing tables in the correct style. Vertical lines are not allowed, and horizontal lines must be limited to the minimum. According to their size, tables should be assembled to fit into one (66 mm) or two columns (139 mm) of a journal page. Very small tables should be avoided, and their results placed in the text.

### Copyright

The use of general descriptive names, trademarks, etc., in this publication, even if the former are not specifically identified, is not to be taken as a sign that such names are exempt from the relevant protective laws and regulations and may accordingly be used freely by anyone.

### Publication

#### Proofs

Upon acceptance, a PDF of the article proofs will be sent to authors along with a list of instructions as an attachment by e-mail to check carefully for factual and typographic errors. Corrections of the proofs are limited to typographical errors. The list of corrections

must be sent to the publisher, within two weeks of receipt, by e-mail or fax. In the absence of comments from the authors, the proofs will be considered as publishable. The costs of any other changes, involving time-consuming and expensive work, will be charged to author(s). If absolutely necessary, additions may be made at the end of the paper in a “Note added in proof”. Manuscripts will be published without proofreading by the authors if they change their e-mail address without updating their personal data in Editorial Manager, or if they do not provide their corrections on time.

#### Page Charge

There is no page charge.

#### E-offprints

A PDF file of the article will be supplied free of charge by the publisher to authors for personal use. Brill is a RoMEO yellow publisher. The Author retains the right to self-archive the submitted (pre-peer-review) version of the article at any time. The submitted version of an article is the author's version that has not been peer-reviewed, nor had any value added to it by Brill (such as formatting or copy editing). The Author retains the right to self-archive the accepted (peer-reviewed) version without any embargo period. The accepted version means the version which has been accepted for publication and contains all revisions made after peer reviewing and copy editing, but has not yet been typeset in the publisher's lay-out. The publisher's lay-out must not be used in any repository or on any website ([brill.com/page/RightsPermissions/rights-and-permissions](http://brill.com/page/RightsPermissions/rights-and-permissions)).

#### License to Publish

##### Transfer of Copyright

By submitting a manuscript, the author agrees that the copyright for the article is transferred to the publisher if and when the article is accepted for publication. For that purpose, the author needs to sign the License to Publish, which will be sent with the first proofs of the manuscript.

#### Open Access

Should the author wish to publish the article in Open Access he/she can choose the Brill Open option. This allows for non-exclusive Open Access publication under a Creative Commons license in exchange for an Article Publication Charge (APC), upon

signing a special Brill Open Consent to Publish Form. All manuscripts become open access after 5 years of publication.

More information on Brill Open can be found on [brill.com/brillopen](http://brill.com/brillopen).

A discount of 40% is given to corresponding authors who are SEH members (with membership including subscription to *AMRE* – print or online version) at the time of submission. Proof of membership (payment of SEH invoice) should be presented to the Co-Editor in Charge when the manuscript is accepted.

More on the Editorial Style, or How to Avoid Not Having a Manuscript Sent Directly for Peer Review

When a manuscript does not conform to the Instructions for Authors, it will be sent back to authors. To avoid this type of time loss in the submission process, authors are kindly requested to carefully check the Instructions for authors *before* submission of their manuscript.

The main reasons that render a manuscript unsuitable in reference to the editorial style of *AMRE* are listed hereunder, although this list is not exhaustive:

- Incorrect style of references in the text or in the reference list (e.g. no "and" between authors, volume number must be in bold, journal names must be abbreviated, mismatches between references in the text and the list; in the text: et al. for papers of more than three authors, not two).
- Incorrect style for n, P and statistical symbols (all must be in italics).
- Lack of keywords.
- Lack of abstract.
- List of authors and addresses on the title page not correctly formulated.
- Absence of double line spacing.
- Presence of empty lines between paragraphs.
- Pages and lines not numbered.
- Tables not presented as in the published papers (no vertical lines, use the table function in Word).
- Low resolution pictures (72 instead of 300-600 dpi).

- Figures that do not accord with the defined style (for maps: coordinates, North; for all figures: all symbols explained, a font that is not too small).
- Absence of acknowledgements for capture permit.
- Failure to remove comments from the right-hand column, presence of endnote fields or of automatic footnotes.

We recommend that junior, inexperienced and first time authors consult any papers that are online in open access, also see the supplementary material online.