Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Zoologia

TAXONOMIA E MORFOLOGIA DE TRÊS ESPÉCIES DE SERPENTES DO GÊNERO *HELICOPS* WAGLER, 1828 (Serpentes: Dipsadidae)

Nathalie Queirolo Kaladinsky Citeli Coelho

Brasília - DF
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Tese de Doutorado apresentada ao Programa de Pós-graduação em Zoologia, Instituto de Ciências Biológicas, da Universidade de Brasília, como parte dos requisitos necessários à obtenção do título de Doutor em Zoologia.

Orientador: Prof. Reuber Albuquerque Brandão

Coorientadora: Profa. Julia Klaczko

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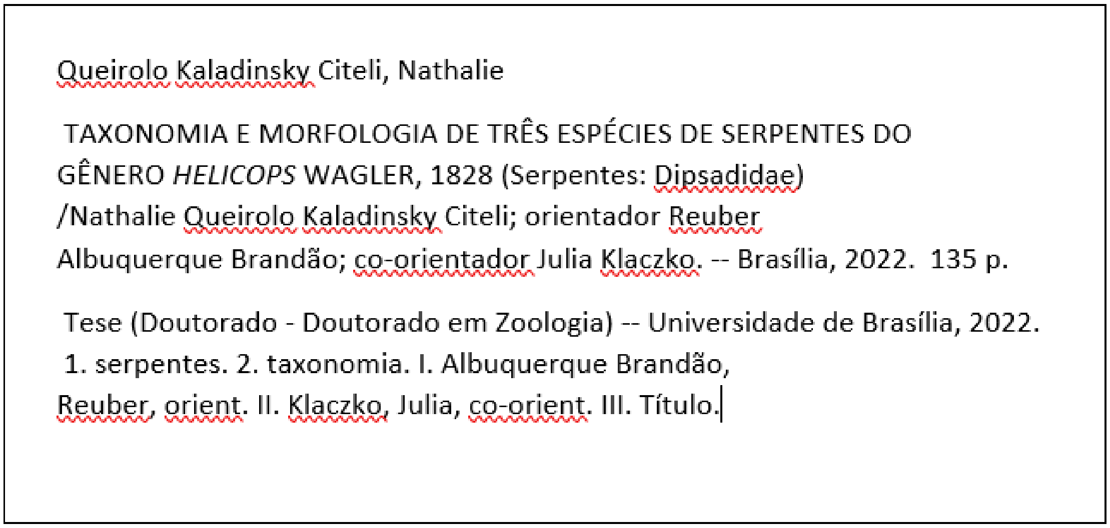

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Dedico esse trabalho aos meus pais, Sandra e Tadeu Citeli.
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“A flor envelhece, mas seu perfume e colorido serão eternos”

Tadeu Citeli

Prefácio

Essa tese está dividida em (i) introdução geral, (ii) quatro capítulos em formato de artigo, dos quais dois estão publicados, (iii) perspectivas futuras, (iv) conclusões e (v) apêndices. A introdução geral é o referencial teórico para o objeto de estudo. Os quatro capítulos consistem em estudos realizados durante o doutorado visando consolidar a taxonomia de três espécies de serpentes do gênero *Helicops* Wagler 1828. O primeiro capítulo trata da taxonomia e variação morfológica intraespecífica relacionada à alometria e ao dimorfismo sexual de *Helicops danieli* Amaral 1937; o segundo capítulo é uma ampla revisão da taxonomia da espécie amazônica *Helicops hagmanni* Roux 1910, com evidência de variação clinal; o terceiro capítulo consiste na descrição de uma nova espécie amazônica com padrão de manchas dorsais circulares, *Helicops acangussu* Moraes, Walterman, Citeli, Nunes e Curcio 2022; o quarto e último capítulo amplia o conhecimento taxonômico de *H. acangussu* pela descrição de novos exemplares, morfologia do crânio, variação do hemipênis e revisão de sua distribuição, bem como apresenta uma chave para as espécies amazônicas de *Helicops*. No apêndice estão, na íntegra, os dois capítulos publicados, I e III.

Títulos de cada capítulo são:

Capítulo I: Taxonomy, allometry, sexual dimorphism, and conservation of the trans-Andean watersnake *Helicops danieli* Amaral, 1937 (Serpentes: Dipsadidae: Hydropsini). Artigo publicado na Revista *Canadian Journal of Zoology*.

Capítulo II: Morphological review and evidence of clinal variation in *Helicops hagmanni* Roux 1910 watersnake. Manuscrito que será submetido para a revista *European Journal of Taxonomy.*

Capítulo III: A new oviparous species of *Helicops* Wagler, 1828 (Serpentes, Xenodontinae) from Brazilian Amazonia with reflections on the evolution of viviparity among hydropsine watersnakes. Artigo publicado na Revista *Zoologischer Anzeiger*.

Capítulo IV: New data on the taxonomy of *Helicops acangussu* and a key to amazonian *Helicops* Wagler, 1828 species. Manuscrito que será submetido a revista *Zootaxa.*

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VIVA A CIÊNCIA BRASILEIRA!!!

Sumário

Resumo Geral

A subordem Serpentes ocupa o planeta Terra desde a Era Mesozóica. Este grupo é composto por animais ápodes, de corpo longilíneo e crânio altamente cinético, distribuídos em virtualmente todo o planeta, com exceção das zonas mais frias. A tribo Hydropsini é composta por três gêneros sul-americanos de serpentes aquáticas, *Pseudoeryx* Fitzinger 1826, *Hydrops* Wagler 1830 e *Helicops* Wagler 1828, sendo o último deles objeto de estudo da presente tese. O gênero *Helicops* é composto por 21 espécies descritas até o momento. No entanto, a taxonomia de algumas delas é ainda frágil, sem que sua morfologia tenha sido investigada profundamente. A presente tese tem como objetivo estudar a variação morfológica de três espécies de *Helicops* distribuídas no norte da América do Sul que apresentam padrão de manchas dorsais circulares. O capítulo I teve como foco estudar as populações de *Helicops danieli*, uma espécie endêmica da região transandina da Colômbia. Para isso, examinamos 124 espécimes, de ambos os sexos e diferentes faixas etárias, provenientes de toda a sua distribuição. Com abordagem multidisciplinar, identificamos 22 caracteres diagnósticos da espécie, e diferenças na taxa de crescimento da cauda (maior em jovens), fêmeas maiores e machos com cauda mais longa, bem como altos níveis de impacto antrópico na área de sua distribuição. O segundo capítulo teve como foco a taxonomia e variação morfológica e geográfica de *Helicops hagmanni*, uma espécie amazônica. Analisamos 112 espécimes e obtivemos caracteres de morfologia interna (hemipenis e osteologia craniana), e externa (folidose e biometria) para identificação de putativa variação geográfica. *Helicops hagmanni* é a espécie do gênero com maior polimorfismo no número de fileiras de escamas dorsais (entre 23 e 29) e possui variação clinal em sua distribuição. Populações do oeste amazônico apresentam menor número de escamas dorsais, ventrais e manchas ao longo do corpo. O terceiro capítulo é a descrição da espécie *Helicops acangussu*, uma serpente amazônica com padrão de manchas dorsais circulares. Embora simpátrica com as outras espécies de padrão dorsal semelhante, *H. acangussu* é a única do gênero com a combinação de 19 fileiras de escamas dorsais, um par de escamas intergeniais e manchas circulares no dorso. O quarto e último capítulo amplia a variação morfológica conhecida e distribuição de *H. acangussu,* a partir da adição de novos 33 exemplares encontrados em coleções sul-americanas. Com esse material, descrevemos a osteologia craniana e revisamos a variação nos caracteres de folidose, biometria e hemipênis. Também apresentamos uma chave de identificação das espécies amazônicas de *Helicops*. Essa tese é uma contribuição direta para o entendimento da taxonomia, dos padrões morfológicos e da variação geográfica desse interessante grupo de serpentes neotropicais.

Palavras-Chave: biometria, especiação, folidose, Hydropsini, Squamata, vertebrados neotropicais

Abstract

The suborder Serpents occupy the Earth since the Mesozoic Era. This group is composed of apodous animals, with elongated bodies and highly kinetic skulls, virtually distributed across the entire planet, except for the coldest areas. The Hydropsini tribe is composed of three South American genera of aquatic snakes, *Pseudoeryx* Fitzinger 1826, *Hydrops* Wagler 1830 and *Helicops* Wagler 1828, the latter being the object of study of this thesis. The genus *Helicops* is composed of 21 species. The taxonomy of some of them is still fragile, and their morphology has not been deeply investigated. The present thesis aims to study the morphological variation of three spotted-dorsal species of *Helicops* distributed in northern of South America. The chapter I is focused on studying the populations of *Helicops danieli*, an endemic species from the trans-Andean region of Colombia. For this, we examined 124 specimens, of both sexes and different age groups, from its entire distribution. With a multidisciplinary approach, we identified 22 diagnostic characters of the species, and differences in the rate of tail growth (higher in juveniles), larger females and males with longer tails, as well as high levels of anthropic impact in the area of its known distribution. The second chapter focused on taxonomy, morphological and geographic variation in *Helicops hagmanni*, an Amazonian species. We analysed 112 specimens and obtained characters of internal (hemipenis and cranial osteology) and external morphology (pholidosis and biometry) to identify putative geographic variation. *Helicops hagmanni* is the species of the genus with the highest polymorphism in the number of rows of dorsal scales (between 23 and 29) and showed a clinal variation on its distribution. Populations from western Amazonia having the lowest number of spots along the body and number of dorsal and ventral scales. The third chapter is a description of the species *Helicops* *acangussu*, an Amazonian snake with a pattern of circular dorsal spots. Although sympatric with other species with a similar dorsal pattern, *H. acangussu* is unique in its genus with a combination of 19 rows of dorsal scales, a pair of intergenial scales and circular spots on the dorsum. The fourth and final chapter expands the known morphological variation and distribution of *H. acangussu*, from the addition of 33 new specimens found in South American collections. With this material, we describe cranial osteology and review variation in pholidosis, biometry and hemipenis characters. We also present an identification key for the Amazonian species of *Helicops*. This thesis is a direct contribution to the understanding of the taxonomy, morphological patterns and geographic variation of this interesting group of neotropical snakes.

Keywords: biometry, Hydropsini, neotropical vertebrates**,** pholidosis, speciation, Squamata.

1. Introdução Geral

As serpentes são animais intrigantes que habitam praticamente todo o planeta Terra, com exceção das regiões mais frias, como a Antártica (Guedes et al., 2018; Luiselli et al., 2020; Uetz et al., 2022). É um clado monofilético, originado durante a Era Mesozóica, entre os períodos Jurássico e Cretáceo, surgido entre 167–143 milhões de anos (Caldwell et al., 2015) e 128,5 milhões de anos (Hsiang et al., 2015). O grupo compreende também alguns taxa basais extintos (considerando o clado “Pan-Serpentes” ou “Ophidia”) incluindo, e.g., *Najash rionegrina*, *Dinilysia patagonica*, oriundas da Argentina e *Eophis underwoodi* do Suriname (Woodward, 1901; Zaher et al., 2009; Caldwell et al., 2015; Reeder et al., 2015) (Figura 1). Diferentes hipóteses movimentaram o posicionamento evolutivo das serpentes dentro da ordem Squamata ao longo dos anos (Bellairs e Underwood, 1951; Rage, 1982; Dois principais fatores podem estar atrelados à incerteza histórica na origem das serpentes (Rieppel, 1988). O primeiro é a visão *scala naturae* (veja detalhes em Mayr, 2000), onde a organização dos seres vivos pode ser hierarquizada a partir da existência de seres intermediários. Neste caso, alguns lagartos estariam em uma escala de perda sequencial de membros locomotores, mostrando todos os estágios que precederam a chegada do *bauplan* das serpentes (Rieppel, 1988). O segundo aspecto é a semelhança do padrão geral corporal das serpentes e alguns lagartos de hábitos fossórios, trazendo confusão ao entendimento do que seria convergência e o que seria homologia (Rippel, 1988). O primeiro entrave foi deixado para trás sob à luz do encontro de fósseis de lagartos de Varanidae e o com as suspeitas de Cope (1869) sobre o parentesco das serpentes com os Mosasauria (Répteis marinhos já extintos) (Cope, 1869; Rieppel, 1988). As relações evolutivas de serpentes com os demais membros de Squamata ainda não são completamente claras. O clado foi recuperado como grupo irmão de lagartos Anguimorpha nas hipóteses filogenéticas de Pyron e colaboradores (2013) e Tonini e colaboradores (2016), e como grupo irmão de Mosasauria por Reeder e colaboradores (2015).

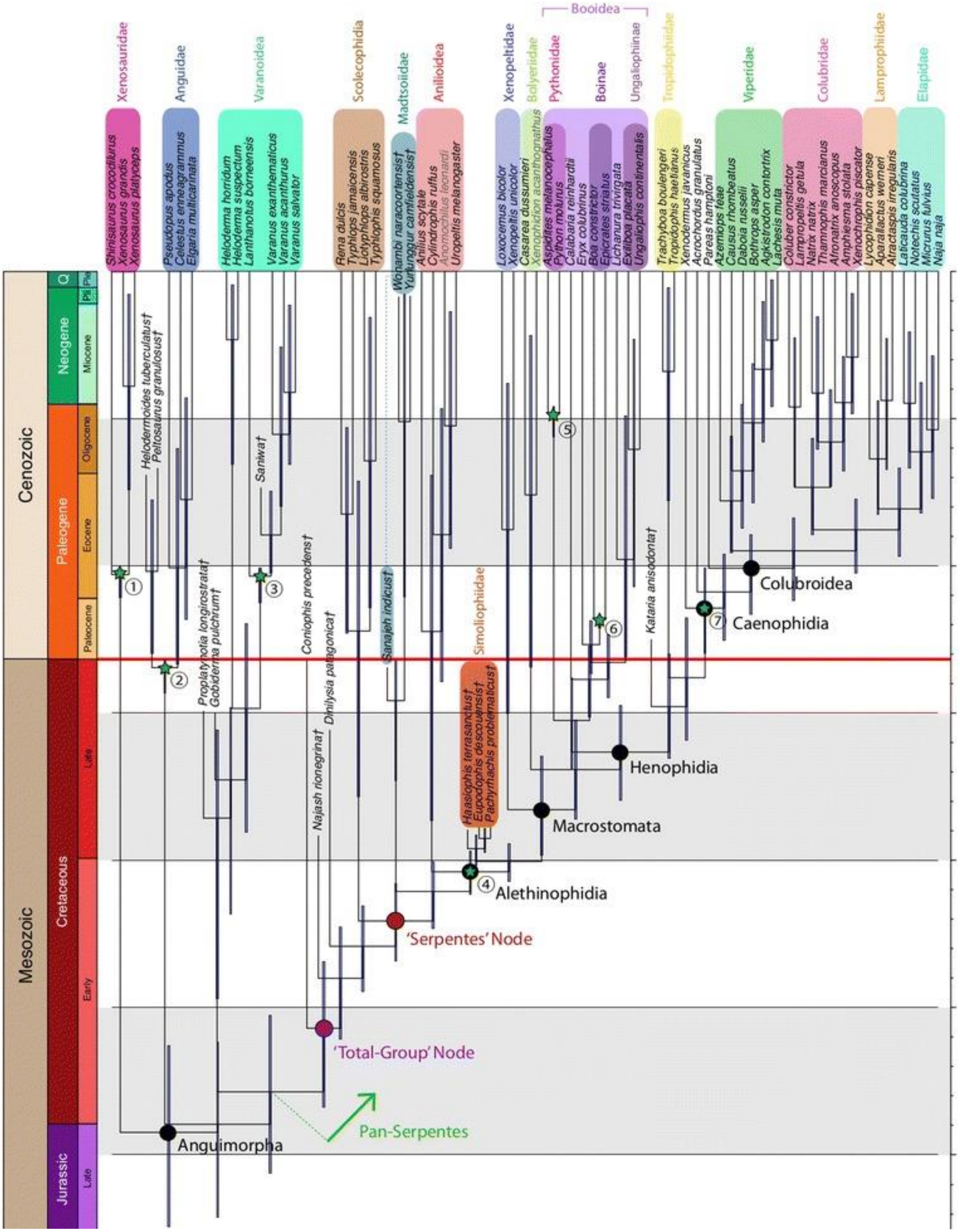

Figura 1. Origem das serpentes de acordo com Hsiang et al. (2015)

Dentre as sinapomorfias de serpentes (i.e., características exclusivas compartilhadas pela linhagem) podemos citar a posição da vesícula biliar posterior e distante do fígado, único forame mental do dentário, reposição dentária por erupção horizontal e contato entre a septomaxila e a maxila móvel (Underwood, 1967; Zaher e Rieppel, 1999; Lee e Scanlon, 2002). Provavelmente, a diversificação mais significativa do grupo ocorreu a cerca de 110 milhões de anos atrás, dando origem aos taxa mais recentes, muitos dos quais ainda viventes (Hsiang et al., 2015). Essa explosão adaptativa no período cretáceo médio originou dois grupos principais, Scolecophidia e Alethinophidia (Hsiang et al., 2015). Scolecophidia inclui as pequenas serpentes conhecidas como cobras-cegas, enquanto Alethinophidia, mais diverso, compreende todos os demais taxa, incluindo os Boidae (Pythons e Boas) e o clado derivado Caenophidia (Viperidae, Elapidae e Colubridae) (Hsiang et al., 2015; Zaher et al., 2019).

São conhecidas atualmente quase quatro mil espécies de serpentes, número possivelmente subestimado, especialmente porque as áreas com maior diversidade geralmente são as menos estudadas, associada à rápida degradação e pouco investimento em pesquisa básica nos países tropicais (Luiselli et al., 2020; Ferrante et al., 2021; Uetz et al., 2022). Dentre as famílias de serpentes que habitam a região neotropical, as mais diversas são Dipsadidae e Colubridae (Zaher et al., 2009; 2019).

Diferentes hipóteses abordaram as relações filogenéticas de Caenophidia, incluindo mudanças na posição de Dipsadidae, às vezes apontada como subfamília de Colubridae (Pyron et al., 2011; 2013; Figueroa et al., 2016). Isso decorre do fato de que a equivalência das subfamílias de Colubridae ao *status* de família ocasiona a transformação do “Colubrídeos” tradicionais na superfamília Colubroidea, considerado desnecessário, uma vez que se trata de mudanças nomenclaturais em grupos monofiléticos (Pyron et al., 2011). Por outro lado, a presença de uma fileira de espinhos laterais aumentados e lobos dos hemipênis com distintas regiões de ornamentações seriam diagnoses válidas da família Dipsadidae (Zaher et al 2009), a qual é recuperada como monofilética com robusto suporte molecular (Grazziotin et al., 2012; Zaher et al., 2019).

A tribo Hydropsini, atualmente alocada na família Dipsadidae, é representada por três gêneros de serpentes comumente encontradas em ambientes aquáticos: *Hydrops* Wagler, 1830 *Helicops* Wagler, 1828 e *Pseudoreryx* Fitzinger 1826. Embora o nome da tribo tenha surgido em Dowling & Duellman (1978), a conformação atual com os três gêneros foi estabelecida por Roze (1957), com base na presença de uma escama internasal única, além do posicionamento dorsal das narinas. Zaher (1999) sugere a relação entre os gêneros com base no desenvolvimento da musculatura adutora da mandíbula. A tribo tem sido recuperada como monofilética em hipóteses de relacionamento de Dipsadidae (Vidal et al., 2000; Zaher et al., 2009).

O gênero *Pseudoeryx* é caracterizado por possuir as escamas nasais em contato, separando a escama rostral da internasal única, além de escamas dorsais lisas arranjadas em 15 fileiras no meio do corpo (Peters e Orejas-Miranda, 1986; Schargel et al., 2007). Atualmente, é composto por duas espécies: *Pseudoeryx plicatilis* (Linnaeus, 1758), distribuída Amazônia e Pantanal, com registros nas adjacências desses biomas (Nogueira et al., 2019), e *Pseudoeryx relictualis* Schargel, Rivas-Fuenmayor, Barros, Péfaur & Navarette, 2007, restrita à bacia do lago Maracaibo, na Venezuela (Schargel et al., 2007). Os caracteres que distinguem as duas espécies são relativos à coloração, folidose e dentição (Schargel et al., 2007). *P. relictualis* possui uma banda nucal atrás das escamas parietais, ventre com manchas pretas pareadas que abrangem a borda das escamas ventrais e primeira fileira de dorsais, formando listras paralelas, segundo par de escamas geniais mais curto que o segundo, e os dois últimos dentes maxilares maiores e separados dos demais por diastema (Schargel et al., 2007). Já *P. plicatilis* não possui colar nucal, tem o ventre com manchas que não formam listras, os dois pares de geniais são subiguais em tamanho e dentes sem diferenciação de tamanho e sem diastema (Schargel et al., 2007).

O gênero *Hydrops* apresenta padrão de coloração dorsal em bandas (ausente em *Pseudoeryx*) e escamas dorsais lisas (quilhadas em *Helicops*) (Peter e Orejas Miranda, 1986; Scrocchi et al., 2007). Atualmente, o gênero conta com três espécies: *Hydrops martii* (Wagler, 1824) restrita ao bioma Amazônia, *Hydrops triangularis* (Wagler, 1824) também de distribuição na amazônica e adjacências, e *Hydrops caesurus* Scrocchi, Ferreira, Giraudo, Avila & Motte, 2005 distribuída no Pantanal e Chaco (Nogueira et al., 2019). As três espécies do gênero se distinguem principalmente por folidose. *Hydrops martii* é a única do gênero que possui 17 fileiras de escamas dorsais (Peter e Orejas Miranda, 1986; Albuquerque 2000). Já *Hydrops caesurus* e *Hydrops triangularis* compartilham 15 fileiras de escamas dorsais no meio do corpo, contudo *Hy. caesurus* tem o menor número de escamas ventrais das três espécies, embora com pequeno grau de sobreposição: 184–213, *vs.* 209–258 nas demais (Scrocchi et al., 2005).

Helicops é o gênero mais diverso da tribo, composto por 21 espécies associadas a ambientes aquáticos de todo continente sul-americano (Costa et al., 2016; Uetz et al., 2022; Moraes-da-Silva et al., 2019; 2021; 2022). Embora a descrição do gênero tenha sido atribuída a Wagler (1930) por trabalhos relevantes (e.g., Peter e Orejas-Miranda, 1986), Moraes-da-Silva (2018), em estudo de revisão taxonômica da espécie *Helicops leopardinus* Schlegel, 1837, corrige a data de proposição do gênero para Wagler (1828). O gênero foi incialmente proposto para alocar a espécie-tipo *Helicops carinicaudus* (= *Coluber carinicaudus* Wied-Neuwied 1824), descrita a partir de um exemplar proveniente da Bahia, Brasil (Wagler, 1828; Moraes-da-Silva, 2018). Contudo, apenas em 1830 Wagler apresenta a caracterização do gênero, com narinas laterais e superiores com a fissura no meio da escama, último dente longo, escamas carenadas (= quilhadas) e provenientes da América (Wagler, 1830). A presença de apenas uma escama temporal anterior (com exceções) e a internasal única também são postuladas por Peters e Orejas Miranda (1986) como características do gênero.

As espécies de *Helicops* se diferenciam pelo padrão de coloração e folidose. *Helicops angulatus* (Linnaeus, 1758), *Helicops apiaka* Kawashita-Ribeiro, Ávila & Morais, 2013 e *Helicops gomesi* Amaral, 1922 partilham o padrão dorsal de coloração com manchas em formato de “sela” (Moraes-da-Silva et al., 2021), transversais ao eixo corpóreo. Entre si, *H. gomesi* apresenta três séries de manchas dorsais irregulares, 19 escamas dorsais no meio do corpo e escamas ventrais em 123–132 (Amaral, 1922), sendo exclusiva do Cerrado e ecótonos com a Mata Altântica. *Helicops angulatus* é provavelmente um grupo de espécies crípticas ancoradas sob o mesmo nome (Murphy et a., 2020). Ainda assim, tem sido tratada como espécie única em trabalhos recentes que reconhecem sua complexidade taxonômica (Moraes-da-Silva, et al., 2021; 2022). De modo geral, *Helicops angulatus* e *H. apiaka* apresentam apenas uma série de manchas transversais em formato de “sela” no dorso (veja Moraes-da-Silva et al., 2021). Todavia, *H. angulatus* apresenta 105–123 escamas ventrais em machos e 109–123 em fêmeas, já *H. apiaka* 118–127 ventrais em machos e 124–132 em fêmeas (Kawashita-Ribeiro et al., 2013). Na descrição de *H. apiaka* o número de escamas dorsais é apontado como caráter que a distingue de *H. angulatus* (Kawashita-Ribeiro et al., 2013), onde os espécimes analisados apresentavam 21–24 dorsais anteriores, 21–22 dorsais no meio do corpo e 17–19 dorsais posteriores (*vs. H. angulatus* com 19–21 dorsais anteriores, 19–20 dorsais no meio do corpo e 17–19 dorsais posteriores; Kawashita-Ribeiro et al., 2013). Posteriormente, o número de dorsais no meio do corpo em *H apiaka* foi revisado para 19–21 (Moraes-da-Silva 2019; Moraes-da-Silva et al., 2021), invalidando esse caractere como diagnóstico para distinção da espécie e *H. angulatus* (Schöneberg e Köhler, 2021). *Helicops angulatus* é amplamente distribuída na América do Sul e *He. apiaka* é restrita a uma pequena região no norte do estado do Mato Grosso, Brasil (Nogueira et al., 2019).

Helicops carinicaudus, *Helicops trivittatus, Helicops modestus* e *Helicops infrataeniatus* são as únicas espécies do gênero que apresentam o padrão de manchas dorsais em linhas longitudinais, paralelas ao eixo corpóreo (Moraes-da-Silva et al., 2021). *Helicops carinicaudus* e *Helicops trivittatus* compartilham o padrão de manchas ventrais em fileiras com formato de meia lua. No entanto, diferem no número de fileiras de escamas dorsais, além de não serem simpátricas. *H. carinicaudus* conta com 19–17 dorsais no meio do corpo e é restrita à Mata Atlântica e *H. trivittatus* com 21–23 escamas dorsais no meio do corpo, conhecida apenas para a parte norte do ecótono cerrado e Amazônia (Rossman, 2010; Nogueira et al., 2019; Moraes-da-Silva et al., 2021). *Helicops modestus* é a única do grupo que apresenta o ventre imaculado, raramente pigmentado, sendo distribuída no Cerrado e uma porção da Mata Atlântica (Nogueira et al., 2019; Moraes-da-Silva, 2022b). *Helicops infrataeniatus* tem o padrão ventral de manchas lineares e/ou bandas, distribuída no sul da América do sul, incluindo a Mata Atlântica e o sul do Cerrado (Nogueira et al., 2019; Moraes-da-Silva, 2022b)

Helicops nentur e *Helicops tapajonicus* são distintas por apresentarem o dorso com coloração uniforme, sem manchas ou listras. Entre si, diferem na folidose. *He. tapajonicus* possui 19 escamas dorsais no meio do corpo, escama nasal semidividida e é encontrada apenas em uma pequena área do leste amazônico (leste do Rio Tapajós) (Frota, 2005; Nogueira et al., 2019). *He. nentur* possui 17 escamas dorsais no meio do corpo, escama nasal inteira e é encontrada apenas no estado de Minas Gerais, incluindo áreas de Mata Altântica e Cerrado (Costa et al., 2016; Nogueira et al., 2019).

Helicops boitata e *Helicops scalaris* diferem das demais espécies congêneres por possuírem o padrão de manchas dorsais em blocos transversais ao eixo corpóreo. *Helicops boitata* possui a escama nasal inteira, 17 dorsais no meio do corpo e é conhecida de apenas uma localidade no nordeste do Pantanal (Moraes-da-Silva, 2019). Já *Helicops scalaris* possui 19-21 escamas dorsais no meio do corpo, nasal semidividida e é endêmica da bacia do lago Maracaibo, na Venezuela, incluindo alguns registros na porção da bacia em território colombiano (Rossman, 2002a).

Helicops phantasma possui o padrão de coloração dorsal com bandas transversais ao eixo corpóreo, 19 fileiras de escamas dorsais, quilhas moderadas e hemipênis com dois espinhos maiores que os demais na base do órgão (Moraes-da-Silva et al., 2021). É endêmica da bacia hidrográfica Tocantins-Araguaia (Moraes-da-Silva et al., 2021).

Helicops acangussu, Helicops polylepis, *Helicops leopardinus*, *Helicops pastazae*, *Helicops petersi*, *Helicops danieli*, *Helicops yacu* e *Helicops hagmanni* diferem das demais congêneres por possuírem o dorso coberto por manchas circulares dispostas entre 2 a 5 fileiras longitudinais. A espécie mais recente descrita para o gênero é *Helicops acangussu*, que se distingue das demais por apresentar 19 escamas dorsais no meio do corpo, um par de escamas intergeniais separando as geniais, quilhas dorsais moderadas e, até então, distribuída apenas na margem esquerda do Rio Madeira (Moraes-da-Silva et al., 2022, veja também capítulo IV). *Helicops leopardinus* é provavelmente um complexo taxonômico que engloba mais de uma espécie (veja Moraes-da-Silva, 2018). Por ora, é ainda considerada como um único táxon para critérios operacionais nas publicações mais recentes (Moraes-da-Silva, 2021; 2022). *He. leopardinus* e *Helicops danieli* também compartilham o número de fileiras de escamas dorsais em 19 no meio do corpo, contudo, partilham as escamas intergeniais em contato, ou seja, sem um par ou mais de intergeniais (Moraes da Silva, 2018; Citeli et al., 2021; Moraes da Silva et al., 2022). As duas espécies diferem entre si pelo padrão ventral, sendo que *He. leopardinus,* amplamente distribuída na região cisandina, apresenta o ventre com barras. *Helicops danieli* apresenta o ventre com duas (raramente três) fileiras longitudinais de manchas ventrais em formato de meia lua e é endêmica da Colômbia transandina (Rossman, 2002b; Citeli et al., 2021).

As demais espécies com padrão de manchas circulares (*Helicops hagmanni, Helicops pastazae*, *Helicops petersi*, *Helicops polylepis*, e *Helicops yacu*) formam um grupo de taxonomia ainda bastante intrincada, apesar dos esforços de Rossman (1975; 1976) (ver seção abaixo sobre o grupo). Todas ocorrem em simpatria no bioma Amazônico, ainda que *He. pastazae* tenha sua distribuição majoritariamente no sopé da cordilheira dos Andes na Colômbia e no Equador (Rossman, 1976). Com exceção de *He. petersi*, todas compartilham o número de escamas dorsais em 25 fileiras, apesar de apresentarem polimorfismo neste caráter (Rossman, 1975; 1976). *He. polylepis* é distinta das demais por possuir o ventre predominantemente negro, com alguns pigmentos brancos esparsos (Rossman, 1976). Já *Helicops hagmanni* pode ter fileiras de escamas dorsais no meio do corpo entre 23 e 29, além do menor número de escamas subcaudais e, consequentemente, a menor cauda (Rossman, 1975; veja capítulo II). *Helicops yacu* é a única do grupo a apresentar escamas nasais inteiras (Rossman e Dixon 1975). *Helicops pastazae* apresenta alto número de ventrais e subcaudais e o ventre com bandas transversais completas (i.e., ocupando toda a área do ventre em faixas transversais). *Helicops petersi* costuma apresentar o menor número de fileiras dorsais (geralmente 21) e as bandas ventrais restritas à lateral do corpo, o que a diferencia de *He. pastazae* (Rossman, 1976).

Como observado, a morfologia das espécies de *Helicops* é bastante variável, exibindo polimorfismos e alto grau de sobreposição em algumas características, o que dificulta a diagnose inequívoca em alguns casos (Rossman e Abe, 1979, Moraes-da-Silva et al., 2021; 2022; veja também capítulo II). Nos últimos anos, diversos estudos têm se debruçado sobre esses aspectos, não só com o objetivo de clarificar identidades taxonômicas, mas também entender padrões evolutivos da morfologia (Scartozzoni, 2009; García-Cobos et al., 2020; 2021; Citeli et al., 2021). Nesse sentido, o complexo de espécies putativamente relacionado a *Helicops angulatus*, incluindo os taxa *Helicops gomesi* e *Helicops apiaka* já tiveram sua morfologia examinada em detalhes, (Andrade, 2017), e do mesmo modo, *Helicops leopardinus* (Moraes-da-Silva, 2018). Recentemente, as espécies de padrão dorsal “listrado” (acima mencionadas), além de *Helicops carinicaudus*, *He. tapajonicus* e *H. nentur* foram rigorosamente revisadas (Moraes-da-Silva, 2022b). É esperado que os padrões encontrados e as decisões tomadas por esses pesquisadores sejam publicadas em artigos científicos nos próximos anos, solidificando a taxonomia de *Helicops*. De modo geral, o volume de espécimes analisados tem sido crucial para caracterizar a variação morfológica do gênero, incluindo a proposição de diagnoses inequívocas e a descrição de novas espécies (Citeli et al., 2021; Moraes-da-Silva et al., 2022a). No que diz respeito ao grupo com padrão de manchas dorsais circulares, uma parte de suas espécies é objeto de estudo da presente tese.

É importante ressaltar que a caraterização dos grupos de *Helicops* com base padrão de coloração é arbitrária, visando facilitar a identificação das espécies. No entanto, essa estratégia não reflete necessariamente grupos naturais, como recuperado em hipóteses filogenéticas morfológicas e moleculares recentes (Nunes, 2006; Moraes-da-Silva et al., 2019; 2021). No entanto, essa abordagem tem sido útil para lidar com a variação morfológica e a distinção dos taxa, como é o caso das relações entre as espécies com manchas dorsais circulares, aqui abordado.

1.2. Taxonomia, conceito de espécie e variação intraespecífica.

Taxonomia é a área do conhecimento que se dedica a classificar e nomear a biodiversidade. Assim como boa parte da evolução do pensamento humano em diversas áreas do conhecimento, é de atribuição dos gregos o pioneirismo dessa ciência (Kepka e Corazza, 2018a). Aristóteles é quem comumente recebe o mérito de ser um dos primeiros pensadores a buscar uma descrição diferenciada da natureza, embora existam também textos hindus sobre a classificação dos seres vivos (Papavero e Abe, 1992; Kepka e Corazza, 2018a). Aristóteles em seu livro “A História dos Animais” apresenta classificações dos seres vivos, muitas vezes não intencionais, que teriam posteriormente grande impacto na zoologia e.g., aqueles que procriam na água e aqueles que não procriam na água (Papavero e Abe, 1992; Kepka e Corazza, 2018a).

Carl von Linné se torna o principal expoente da taxonomia quando publica o livro “Systema Naturae” em um projeto ambicioso de classificação da natureza, iniciado em três principais grupos, animais, vegetais e minerais (Linnaeus, 1758). A nível de taxa terminais, sua nomenclatura e classificação consiste basicamente em um nome duplo que pode ser mundialmente compreendido, composto pelo gênero e o epíteto específico. O sistema classificatório de Linné é utilizado de forma bastante eficiente até hoje, embora algumas correntes tenham tentado suprimi-lo em níveis hierárquicos mais altos (veja Phylocode, de Queiroz, 2012; Cantino e de Queiroz, 2020). A Linné também é atribuído o uso do conceito morfológico de espécie (=tipológico, essencialista), em que uma espécie pode ser considerada como uma classe bem definida morfologicamente (Mayr, 2000; 2001). Neste conceito pré-evolutivo, a espécie é um tipo constante, separado das demais por uma barreira instransponível (Simpson, 1951; Mayr, 2001). Entretanto, a visão de Linné como um naturalista meramente essencialista pode ser um erro de interpretação difundido por Mayr (veja Kepka e Corazza, 2018b).

Espécie é a unidade fundamental não apenas das ciências naturais como de qualquer área do conhecimento que, de alguma forma, lida com a natureza. O entendimento desse termo fomentou, e ainda fomenta, inúmeras discussões ao longo da história do pensamento científico (Simpson, 1951; De Queiroz, 2007). Diferentes conceitos de espécie foram propostos pelos naturalistas, uma vez que existem dificuldades práticas do uso do conceito tipológico. Outro marco importante é o surgimento das teorias de Darwin e Wallace sobre adaptação e seleção natural, o que acarreta mudanças no entendimento do papel da espécie na natureza e, por consequência, nos conceitos que passam a considerar a evolução (de Queiroz, 2011). Alguns desses conceitos de espécie serão comentados adiante (para detalhes, veja de Queiroz 2007).

Um dos motivos apontados por Mayr (2001) para o estabelecimento de um novo conceito em relação ao conceito morfológico, é que existem populações simpátricas de seres vivos que não diferem morfologicamente e não se reproduzem, ou seja, configurando espécies crípticas. O conceito biológico de espécie proposto de Mayr (1976; 2000; 2001), tenta suprimir esse problema. Neste caso, espécie seria um agrupamento natural de populações com capacidade de se reproduzirem, e em isolamento de outros grupos. O isolamento reprodutivo se configura como uma barreira no processo de especiação (Dobzhansky, 1950). Este conceito é comumente encontrado em livros escolares, textos jornalísticos e, provavelmente, o mais difundido entre leigos.

Simpson (1951) propôs ajustes no conceito de Mayr e aponta que uma espécie deve ser uma linhagem evolutiva em sequência ancestral-descendente evoluindo separadamente de outras. Este é conhecido como o conceito evolutivo de espécie. Van Valen (1976) entende que a evolução atua diretamente no fenótipo e que a ecologia (i.e., interações entre o meio ambiente e os seres vivos) controla a evolução a nível individual.

Assim sendo, o conceito ecológico de espécie proposto por ele considera que espécie é uma linhagem (ou algumas) que ocupam uma zona adaptativa minimamente adequada.

O conceito filogenético de Hening (1965) atribui a espécie o grupo terminal que compartilha o mesmo ancestral comum (este já inexistente), podendo ser distinto dos demais grupos. Essa abordagem difere do conceito monofilético de espécie, em que se inclui o ancestral e todos os seus descendentes (Sneath e Sokal, 1973).

Mayr (2000) havia apontado duas importantes características do termo “espécie” que precisam ser definidas *a priori*, o que é espécie conceitualmente e o que é espécie enquanto táxon. À vista disso, o conceito de espécie se refere ao papel da espécie na natureza (Mayr, 2000) e a espécie enquanto táxon é um agregado de populações. Mais tarde, de Queiroz (2007) aponta esses dois aspectos como a conceitualização de espécie e a inferência metodológica, i.e., critérios operacionais possíveis utilizados para a delimitação das espécies.

De Queiroz (2005; 2007) faz amplas revisões sobre os conceitos de espécie existentes e descreve uma proposta de síntese, dado que as abordagens anteriormente propostas não são incompatíveis, mas presumíveis etapas em um processo contínuo de especiação. Assim, o autor propõe o conceito unificado de espécie, em que a separação das linhagens pode ser detectada em diferentes níveis e com variadas linhas de evidências ao longo do tempo (e.g., isolamento reprodutivo, diferenciação fenotípica e distinção ecológica) (Figura 2). O conceito unificado considera que a espécie é uma linhagem exclusiva em evolução (de Queiroz, 2007).

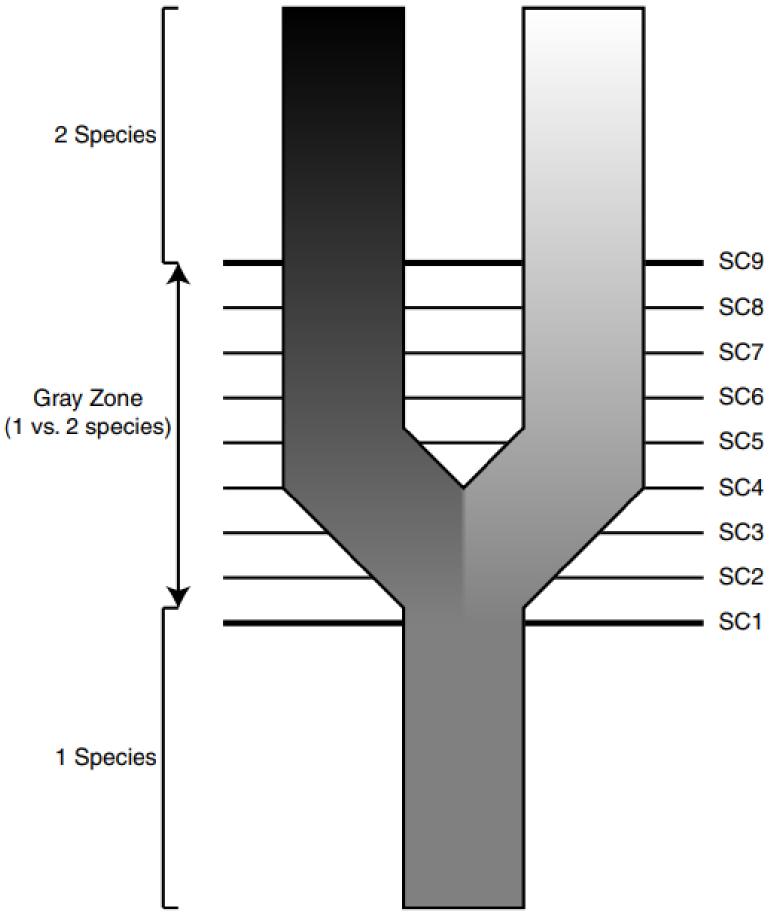

Figura 2. Diagrama representando o processo de divergência entre as linhagens de acordo com de Queiroz (2005). As linhas horizontais expressam momentos em que há aquisição de novas evidências (e.g., diferenciação fenotípica, isolamento reprodutivo etc.).

De qualquer forma, os limites para delimitação das espécies sempre se esbarram na variação intraespecífica. Virtualmente, todas as espécies apresentam esse tipo de variação, podendo essa característica ter maior evidência em uma espécie com história antiga, do que em espécies irmãs separadas recentemente (de Queiroz, 2007). Um estudo detalhado das populações ao longo de sua distribuição pode confirmar a causa desses fenômenos (Stillwell, 2010).

Huxley (1938; 1939) postulou o termo *cline* (=clina) para auxiliar a taxonomia a lidar com esse aspecto fenotípico da variação intraespecífica, intrínseco das populações que, ao longo de sua distribuição, sofrem pressões de seleção sexual, natural ou alguma combinação gênica que determine certo fenótipo, mesmo sem a diferenciação específica entre as populações. Clinas podem ser relativas ao tamanho, cores, padrão de ornamentação, e qualquer outra característica que tenha variação ao longo de uma distribuição geográfica, sendo, ou não, correspondente a algum gradiente ambiental (Huxley, 1939; Stillwell, 2010). Dois passos são apontados por Hurxley (1939) como importantes para a compreensão de clinas na natureza, o primeiro deles é a descrição da variação da característica ao longo da distribuição, e o segundo é testar a significância estatística dessa variação. Autores mais recentes também sugerem observações mais cautelosas sobre as clinas, e concordam que nem sempre são fenômenos adaptativos, refletindo a plasticidade fenotípica de um organismo (i.e., um mesmo genótipo acarretando em variados fenótipos) (Stillwell 2010; Telonis-Scott et al., 2011).

1.3. Taxonomia das espécies com manchas dorsais circulares e distribuídas no norte da América do Sul

Helicops polylepis é a mais antiga espécie descrita deste grupo (Gunther, 1861), tendo como caracteres diagnósticos a sua folidose e coloração, incluindo dois pares de escamas mentonianas, occipitais arredondadas, fileiras de escamas dorsais em 26 no meio do corpo, corpo amarronzado, com três séries de fileiras longitudinais escuras de manchas circulares e ventre predominantemente negro. A distribuição geográfica apresentada no momento de sua descrição foi “alto amazonas”. *He. polylepis* possui dois sinônimos juniores na literatura. *Tachynectes chrysostictus* foi descrita por Cope (1862) com base em um exemplar proveniente da Amazônia (Mus. Smithsonian, N° 6007). Posteriormente, a espécie foi realocada no gênero *Helicops* (=*Helicops chrysostictus*) também por Cope (1970). Jan (1865) descreveu *Helicops spixii*, que se distinguia das demais espécies conhecidas pelo alto número de escamas dorsais (=23). Jan e Sordelli (1868) apresentam a ilustração da espécie *Helicops spixii* descrita por Jan no ano anterior. Boulenger (1893) inclui os nomes *Tachynectes chrysostictus* e *Helicops spixii* na sinonímia de *Helicops polylepis*. No entanto, o exemplar de n° 6007 descrito por Cope (1862) está oficialmente documentado como perdido desde 1981 no Museu do Simthsonian (Langan, E. com. pess.). Nos anos seguintes, o taxon *Helicops polylepis* figura em trabalhos de distribuição, com acréscimos de dados morfológicos (Amaral, 1935; Cunha & Nascimento, 1978).

Helicops hagmanni foi descrita por Roux (1910) com base em um exemplar proveniente dos arredores de Santarém, Brasil. Na ocasião, a diagnose do táxon incluía 27 fileiras de escamas dorsais e 2 escamas preoculares. Anos mais tarde, *Helicops hagmanni* figura em três publicações do periódico "Memórias do Instituto Butantan". Gomes (1918) descreve duas fêmeas adultas do município de Peixe-Boi, estado do Pará, ambas similares ao holótipo. Hoge e Nina (1964) descrevem duas fêmeas juvenis do município de Manaus, estado do Amazonas, ambas com 25 fileiras de escamas dorsais no meio do corpo. Hoge e colaboradores (1972) descrevem um juvenil com 23 dorsais no meio do corpo, da região de Iaureté, município de Uaupés, Amazônia ocidental, Alto Rio Negro. Na ocasião, Hoge e colaboradores (1972) comentam que a coloração do espécime é mais "viva" que os demais exemplares examinados por eles em outros momentos, mas que, por se tratar de um juvenil, novos materiais da região precisam ser examinados para confirmar putativo policromatismo. Os registros de Hoge e Nina (1964) e Hoge e colaboradores (1972) parecem ser os primeiros relatos de escamas dorsais em 23 e 25 fileiras em *Helicops hagmanni*. Rossman (1975) examina três novos espécimes, um de Umarituba, estado do Amazonas, Brasil, e dois provenientes da Colômbia (município de Letícia, departamento do Amazonas e município de Florencia, departamento de Caquetá). Assim como já registrado anteriormente (Hoge e Nina 1964; Hoge et al., 1972), os indivíduos do oeste amazônico reportados por Rossman (1975) apresentam menor número de fileiras de escamas dorsais (=23), além de manchas dorsais em menor tamanho e menor número. Para Rossman (1975), mais indivíduos do Rio Negro precisariam ser examinados, uma vez que as diferenças desta população em relação ao holótipo poderiam representar uma nova subespécie.

Helicops pastazae foi descrita por Shreve (1934) como um táxon relacionado a *Helicops polylepis.* A diagnose apresentada apenas distingue a então nova espécie de *He. polylepis* com base no formato da escama frontal, primeiro par de geniais mais largo que o posterior, rostral e internasal menos frequentemente em contato, maior número de escamas ventrais e de subcaudais. Anos mais tarde, Rossman (1976) considera *Helicops pastazae* um complexo de espécies, propondo uma diagnose mais completa para esse táxon. A partir de então, *He. pastazae* passa a ser diagnosticada por escamas dorsais em 23-25 fileiras, quatro a cinco fileiras longitudinais de manchas circulares no dorso, escamas ventrais entre 130-145 em fêmeas e 121-134 em machos, alta contagem de escamas subcaudais, 77-97 em fêmeas e 93-117 em machos, ventre coberto por bandas transversais ao eixo corpóreo, ou em desenho padrão “xadrez”. Durante a revisão do “complexo *H. pastazae*”, Rossman (1976) descreve *Helicops petersi*, uma nova espécie com afinidade a *Helicops pastazae*, porém restrita ao Equador. *He. petersi* é então diagnosticada por apresentar 21-23 fileiras de escamas dorsais no meio do corpo (23 em apenas um terço das fêmeas), escamas ventrais em 137-150 em fêmeas e 135-142 em machos, subcaudais 67-73 em fêmeas e 85-91 em machos (Rossman 1976). Rossman (1976) relaciona as duas espécies em questão com a “seção” *polylepis*, agrupamento que inclui *Helicops yacu* (Rossman e Dixon, 1975). Apesar dessa classificação, do ponto de vista taxonômico, a variação interespecífica no padrão de coloração, escamação e tamanho, constitui um amplo mosaico, que pouco auxilia na determinação das afinidades do grupo (Rossman, 1976).

Helicops yacu foi descrita por Rossman e Dixon (1975) com base em quatro fêmeas da região de Loreto, no Peru. A diagnose de *He. yacu* inclui 27 fileiras de escamas dorsais, escama nasal inteira, escamas ventrais em 130-134, e subcaudais em 85-96. *Helicops yacu* foi considerada semelhante a *He. polylepis* e *He. pastazae* pelo alto número de fileiras de escamas dorsais, ventrais e subcaudais (Rossman e Dixon 1975). Anos mais tarde, Rossman e Abe (1979) reportam mais dois exemplares da espécie, incluindo o primeiro registro para o Brasil, no município de Cruzeiro do Sul, Acre (DZ – UNICAMP 127). Esse voucher, possivelmente referido a uma coleção mantida em uma extinta base da Universidade de Campinas no estado do Acre, não se encontra na coleção do Museu de Zoologia da Universidade Estadual de Campinas ‘‘Adão José Cardoso’’(ZUEC) e está provavelmente perdido (Manzani, P. com. pess.). O segundo exemplar de Rossman e Abe (1979) é o primeiro espécime macho descrito, oriundo de uma área entre Letícia, Colômbia e Iquitos, Peru. Ambos os exemplares possuíam 25 fileiras de escamas dorsais no meio do corpo, ampliando a variação morfológica até então conhecida da espécie. Depois do encontro desses dois novos exemplares, Rossman e Abe (1979) concluem que *He. yacu* é bastante semelhante a *He. pastazae*, e que a distinção entre as duas espécies não é ainda inequívoca, podendo *He. yacu* ser um sinônimo junior de *He. pastazae*. Apenas com a aquisição de mais material dessa região do Peru a taxonomia dessas espécies poderia ser clarificada.

Helicops danieli foi descrita suscintamente por Amaral (1937) com base em uma jovem fêmea proveniente de Carare, departamento de Santander, Colômbia (IBSP 9872). De acordo com o autor, o espécime possuía 19 fileiras de escamas dorsais fortemente quilhadas, 1 pré-ocular, 2 pós oculares, nasal semidividida, 143 escamas ventrais e 62 subcaudais, sendo proximamente relacionada a *He. angulatus* e *He. scalaris*. Lamentavelmente, este holótipo foi perdido durante o incêndio que destruiu boa parte da coleção herpetológica do Instituto Butantan (Citeli et al., 2021). Yuki (1994) examinou mais dois espécimes, uma fêmea e um macho, e descreveu o hemipênis da espécie, não observando variação significativa em folidose em relação ao holótipo. Rossman (2002b), após examinar 42 exemplares, apontou que a combinação do dorso com manchas circulares e ventre com linhas de marcas semilunares tornava *He. danieli* distinta das demais espécies do gênero. O autor também comentou que muitos caracteres exibiam dimorfismo sexual, alguns deles leves (e.g. comprimento relativo da cabeça) e outros pronunciados (e.g., comprimento da cauda, número de escamas subcaudais). Citeli e colaboradores (2021), após o exame de 124 exemplares de *Helicops danieli*, apresentam uma diagnose emendada com 22 caracteres, bem como a descrição do dimorfismo sexual, variação ontogenética em biometria e coloração (veja capítulo I).

Helicops acangussu foi descrita por Moraes-da-Silva e colaboradores (2022) com base em 27 exemplares provenientes da margem esquerda do Rio Madeira, estado de

Rondônia, Brasil. Os autores encontraram os espécimes da nova espécie identificados como *Helicops hagmanni* em duas coleções herpetológicas (Museu Paraense "Emilio Goeldi" (MPEG) e Universidade Federal de Rondônia (UFRO-H). *Helicops acangussu* se distingue por apresentar a combinação única do padrão de coloração dorsal com manchas circulares, 19 fileiras de escamas dorsais no meio do corpo e um par de escamas intergeniais (veja capítulos III e IV).

2. Objetivos

Revisar a taxonomia e variação morfológica intraespecífica de três espécies do gênero *Helicops* com padrão de manchas dorsais circulares, distribuídas no norte da América do Sul, a fim de clarificar a taxonomia do grupo.

- **Capítulo I:** Revisar, com base no exame de ampla amostra de exemplares, a taxonomia de *Helicops danieli*, identificando a variação morfológica ontogenética associada ao crescimento alométrico, bem como a ocorrência de dimorfismo sexual na folidose e a biometria.
- **Capítulo II:** Identificar a variação morfológica e geográfica, com a putativa ocorrência de clinas, nas populações de *Helicops hagmanni.*
- **Capítulo III:** Descrever uma nova espécie amazônica com padrão de manchas dorsais circulares, *Helicops acangussu*.
- **Capítulo IV:** Solidificar a taxonomia de *Helicops acangussu* com novos dados sobre variação morfológica, hemipênis, osteologia craniana e distribuição. Além de propor uma chave de identificação para as espécies amazônicas do gênero.

3. Resultados

Capítulo I

Taxonomy, allometry, sexual dimorphism, and conservation of the trans-Andean watersnake *Helicops danieli* Amaral, 1937 (Serpentes: Dipsadidae: Hydropsini)

Artigo publicado na revista Canadian Journal of Zoology

(texto completo no apêndice I)

Helicops danieli
Fotografia: Hernan Martinez
Caucasia, Colômbia.

Capítulo II

Morphological review and evidence of clinal variation in *Helicops hagmanni* Roux 1910 watersnake

Helicops hagmanni
Fotografia: Giovanni Chaves Portilla
Letícia, Colômbia.

(manuscrito que será submetido à revista European Journal of Taxonomy)

Morphological review and evidence of clinal variation in the Amazonian watersnake *Helicops hagmanni* Roux 1910

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Resumo

Helicops hagmanni é uma espécie de cobra d`água endêmica da Amazônia. Desde sua descrição em 1910, relatos esparsos notificaram espécimes com morfologia distinta do holótipo. O único estudo sobre a variação fenotípica da espécie utilizou apenas três exemplares. Neste trabalho, estamos interessados em identificar a variação morfológica em caracteres internos e externos de *Helicops hagmanni* ao longo de sua distribuição. Examinamos 112 exemplares de instituições estrangeiras e nacionais, coletando dados de folidose, biometria, coloração e distribuição. Testamos, com variograma, se os caracteres com maior variação apresentavam autocorrelação espacial. Propomos uma diagnose emendada para a espécie, descrevemos sua osteologia craniana e morfologia do hemipenis. Também identificamos que três caracteres merísticos (número de fileiras de escamas dorsais, número de escamas ventrais e número de manchas paravertebrais), tendem a aumentar no sentido leste – oeste ao longo da calha do rio Amazonas. Padrões de coloração também variam entre localidades. *Helicops hagmanni* é a espécie de *Helicops* com maior polimorfismo no número de fileiras de escamas dorsais (entre 23 e 29). Sugerimos análises integrativas para esclarecer a variação clinal detectada.

Palavras-chave: Krigagem, Squamata, Especiação, Variação Intraespecífica

Abstract

Helicops hagmanni is a species of watersnake endemic to the Amazon. Since its description in 1910, sparse reports have reported specimens with morphology distinct from the holotype. The only study on the phenotypic variation of the species analysed only three specimens. In this study, we are interested in identifying the morphological variation in internal and external characters of *Helicops hagmanni* along its distribution. We examined 112 specimens from foreign and national institutions, collecting data on pholidosis, biometry, coloration and distribution. We tested, with a variogram, whether the characters with the greatest variation shows spatial autocorrelation. We propose an amended diagnosis for the species, describe its cranial osteology and hemipenis morphology. We also identified that three meristic characters (number of rows of dorsal scales, number of ventral scales and number of paravertebral spots) tend to increase in an east-west direction along the Amazon River channel. Coloration patterns also vary between locations. *Helicops hagmanni* is the species of genus with the highest polymorphism in the number of rows of dorsal scales (between 23 and 29). We suggest integrative analyzes to clarify the detected clinal variation.

Keywords: Kriging, Intraspecific Variation, Squamata, Speciation.

Introduction

Snake's morphology may be under several types of pressures, such as environmental (e.g., climatic adaptations), ecological (e.g., substrate use), intra and intersexual selection (e.g., male-male combat or female choice) (Litzugs et al., 2004; Shine 1994; Brischoux and Shine, 2011; Banci et al., 2022). Therefore, describing the internal and external species morphology and its variation along populations is a crucial step toward understanding the evolutionary processes that shape biodiversity (e.g., Keogh 1999; Hibbitts and Fitzgerald 2005; Brito et al., 2008).

Helicops Wagler 1828 is a neotropical genus of 21 species of snakes associated with freshwater bodies such as rivers, lakes, and swamps (Moraes-da-Silva 2022). Some *Helicops* species shows wide variation in coloration, morphology, and scale counts (i.e., polychromatism and polymorphism). In addition, it is not unusual to find overlap in the morphological diagnosis of some species, especially in Amazonian taxa that exhibit circular dorsal spots, as *Helicops acangussu* and the species in the so-called *Helicops polylepis* group (with dorsal scales at midbody above 21), such as *Helicops pastazae*, *Helicops polylepis*, *Helicops petersi* and *Helicops yacu* (Rossman, 1976).

Helicops hagmanni was described by Roux (1910) based on a single specimen donated to the Basel Natural History Museum, coming, after dying, from the Basel Zoological Garden, both institutions in Switzerland. The individual had been taken alive to the Zoo by Dr G. Hagmann after his travel to Brazil (Roux, 1910). The new taxa honored its collector and showed 27 dorsal scales rows and two preocular scales, both characteristics differing from known congeners at the time. Also, according to the author, *H. hagmanni* would be closely related to *H. leopardinus* and *H. scalaris*, which differ in the abovementioned characteristics.

After its description, two adult females were described by Gomes (1918) from the Peixe-Boi locality, Pará state, Brazil. Later, Hoge and Nina (1964) reported two young females from Manaus, Amazonas state, Brazil, both with 25 dorsal scales rows at midbody. Hoge et al. (1972) described a young male with 23 dorsal scales at midbody from the municipality of Uaupés, western Amazon, Upper Rio Negro. This report made by Hoge seems to be the first records of individuals of *H. hagmanni* with 23 and 25 dorsal scales at midbody, respectively.

Years later, Rossman (1975) redescribes *Helicops hagmanni* using three new specimens, two from Colombia (Leticia municipality, Amazonas department and Florencia municipality, Caquetá department) and one from Umarituba, Amazonas, Brazil. The individuals described by Rossman also showed a smaller number of dorsal scales rows at midbody (= 23), as previously reported by Hoge (1972). According to Rossman (1975), individuals from Rio Negro shows marked differences in relation to the holotype, whereas suggested a circumstantial reduction in the number of midbody scale rows along the Amazon course. Rossman (1975) also makes a confusing redescription of the holotype, which is at odds with Roux's description.

Herein, we are interested in reevaluating the taxonomic status of *Helicops hagmanni* based on the analysis of a larger sample size to describe its osteology, hemipenial and geographical variation of the morphology.

Material and Methods

We examined 112 specimens of *Helicops hagmanni*, housed in eleven institutions: Instituto Butantan (IBSP), São Paulo, São Paulo, Brazil; Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, São Paulo, Brazil; Instituto Nacional de Pesquisas da Amazônia (INPA-H), Manaus, Brasil; Fundação de Medicina Tropical do Amazonas (FMT), Manaus, Brasil, Museu Paraense ‘‘Emilio Goeldi’’ (MEPG), Belém, Brasil, Instituto de Ciencias Naturales, Museo de Historia Natural, Universidad Nacional de Colombia (ICN), Bogota, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva (IAvH-R), Museo de Historia Natural de La Salle (MLS), Bogotá; Instituto Amazônico de Investigaciones Científicas (SINCHI-R), Leticia, Amazonas, Colombia; Centro de Estudos e Pesquisas Biológicas, Pontifícia Universidade Católica de Goiás (CEPB), Goiânia; Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro. We also examined by photographs the holotype deposited at Naturhistorisches Museum Basel (NMBA 6281). Institutional abbreviations follow Sabaj (2020), for the complete list of specimens see APPENDIX I

We extracted, everted, and prepared the hemipenis of seven preserved specimens following Pesantes (1994) and Zaher and Prudente (2003). The fully everted hemipenis was filled with petroleum jelly to ease the visualization of the ornamentation structures, and we followed the terminology of Zaher (1999) for the description. The organ was photographed.

We acquired the osteological characteristics of skulls from imagens provided by high-resolution micro-computed tomography (µCT) Bruker Skyscan 1273 at Instituto Alberto Luiz Coimbra de Pós-graduação e Pesquisa de Engenharia (COPPE), Laboratório de Instrumentação Nuclear, Universidade Federal do Rio de Janeiro, Rio de Janeiro. The imagens of eight skulls were visualized in CTVox for Windows 64bit version 2.6 (Bruker, µCT), and plates were made using GIMP 2.10 (Gimp, 2019). The osteological nomenclature follows Romer (1956).

We followed Dowling (1951) and Moraes-da-Silva et al. (2019; 2021) for scale counts and Moraes-da-Silva et al. (2022) for dorsal spots counts. All measurements were taken using a digital caliper to the nearest 0.01 mm, except for SVL and TL measurements, where we used a flexible ruler. The sex of individuals with no everted hemipenis was determined through an incision at the base of the tail to detect the presence of hemipenes and retractor muscles. It was not possible to use all the samples for all the analyses because some specimens were not sexed or had damage to the pholidosis. The dataset used to emended diagnosis comprised 96 individuals.

We performed a principal component analysis (PCA) to identify the external characters that showed more variation along the distribution. These analyses were performed using vegan (Oksanen et al. 2018), dismo (Hijmans et al. 2017), and rgdal packages (Bivand et al. 2019).

We modelled the geographical variation of external characters using the Kriging function (Oliver and Webster, 2014) in SAGA Gis 2.3.2. and Quantum Gis 3.18.3. This modelling procedure allowed us to estimate the external morphology of some non-analysed populations based on the available data, using interpolation weights from a semi-variogram estimated through the spatial structure of the data (Oliver 1990; e.g., Martínez-Freiría et al., 2009). The Kriging function was used as interpolation method using weights from a semi-variogram estimated through the spatial structure of the data. The magnitude of the spatial autocorrelation was observed using the spatial autocorrelation of the Moran's I coefficient (Sakizadeh 2019).

The complete list of analysed specimens, hemipenis and skulls are in Appendix 1.

Results

We analysed a sample of 96 specimens of *Helicops hagmanni* (53 females and 43 males), allowing us to describe its morphological variation throughout its distribution. We present below an emended diagnosis, redescription of the hemipenis, description of the skull, a comparison with other Amazonian *Helicops*, and the geographical variation found in some external characters.

Helicops hagmanni Roux 1910

Holotype. Adult male (NMBA 6281) collected in the vicinity of Santarém, North of Brazil (Pará state) by Dr. G. Hagmann (Roux, 1910) (Figure 1).

Emended diagnosis *Helicops hagmanni* is distinguished from all congeners by the combination of the following characters: (1) 23–29 scale rows at midbody; (2) weakly keeled dorsal scales on the first third of the body, moderated keeled scales at the midbody and strongly keeled scales on the last third of the body, including the tail; (3) 118–138 ventral scales in females and 122–135 ventral scales in males; (4) a relatively short tail for *Helicops*, with 46–57 subcaudals in females and 54–67 subcaudals in males; (5) cloacal plate always divided; (6) posterior pair of chinshields separated by small intergenial scales; (7) dorsum with two rows of paravertebral spots and two rows of paraventral blotches; (8) 25–39 paravertebral spots in females and 24–37 in males; (9) back of the head with spear mark; (10) whiteish venter with brownish irregular bands.

Intraspecific variation of meristic, morphometric and color in preservative characters (Figure 2, ABCD)

Helicops hagmanni may have anterior rows of scales as 27 (*n*=40), 23 (*n*=25), 25 (*n*=19), 24 (*n*=4), 26 (*n*=3); 29 (*n*=2), 21 (*n*=1), and 28 (*n*=1); rows of scales at midbody as 27, (*n*=47), 25 (*n*=29), 23 (*n*=13), 29 (*n*=6), and 24 (*n*=1), posterior scales rows as 21 (*n*=54), 23 (*n*=36), 22 (*n*=2), 25 (*n*=1), 20 (*n*=1), and 19 (*n*=1); single preocular (*n* = 96), rarely just one side of the head can be two i.e., asymmetric specimens (*n*=5); two postoculars (*n*=65), sometimes one (*n*=17), or divided in just one side of the head (*n*=14); temporal formula usually 2+3 (*n*= 56), 1+2 (*n*=24), and rarely 2+2 (*n*=7), 2+4 (*n*=5) or 1/2 in asymmetric specimens (*n*=4); supralabials usually eight (*n* =88), rarely seven (*n* =2), 8/7 (n=3), and 8/9 (*n*=1); fourth supralabial usually contacting orbit (*n* =95), fifth at left side of an asymmetrical specimen (*n*=1); two pairs of chinshields, the posterior pair separated by intergenial scales (*n*=96); infralabials usually 11 (*n*=68), 10 (*n*=12), 12 (*n*=5) and rarely 9 (*n*=1) or 11/12, 12/11 in asymmetrical specimens (*n*=4); first to fifth infralabials contacting anterior pair of chinshields (*n*=81), rarely first to sixth (*n*=5), first to fourth (*n*=1) or asymmetrical specimens with 4/5 (*n*=7), 5/6 (*n*=2); sixth to seventh infralabials contacting posterior pair of chinshields (*n*= 69), rarely just sixth (*n*=18), fifth to sixth (*n*=7) or just fifth (*n*=2); 118–138 ventral scales in females (*n*=53), and 122–135 ventral scales in males (*n*=43); 46–57 subcaudals in females (*n*=39), and 54–67 subcaudals in males (*n*=34); cloacal plate always divided (*n*=96); subcaudals plates divided throughout the tail (*n*=96); Largest specimen is a female with 1,070 mm in total length (SVL 850 mm, tail length 220 mm) (MPEG 13350), and the largest male with 840 mm in total length (SVL 660 mm, tail length 180 mm) (MUZUSP 18869); smallest juvenile specimen is a female with 153 mm in total length (SVL 130 mm, tail length 23 mm) (INPA 15951), and the smallest male with 200 mm in total length (SVL 150 mm, tail length 50 mm) (MPEG 22637); eye usually a half its distance to rostral scale (49.1±8.23 *n*=96); internasal triangular in shape touching the rostral (*n*=94), rarely separated by nasals (*n*=2); loreal as longer as wider (*n*=96); nasals semi-divided (*n*=96); dorsum and tail covered by two paravertebral rows of circular spots (*n*=96); first paraventral spots with a white border (=39); two rows of paraventral spots that can join in the ventral band at the last third of the body (*n*=40), in the middle of the body (*n*=24) (in the first two cases the first ones have a white border); or two rows of paraventral blotches that join the ventral bands from the first third of the body (*n*=26) (Figure 3); paravertebral spots 2–4 scales long (*n*=96), in 25–39 spots belong the dorsum in females and 24–37 in males; dorsum of the head covered by a spear-shaped pattern that starts in the supraoculars and frontal scales and extending to the occipitals (*n*=96); supralabials and infralabials brownish with darker spots in the sutures (*n*=96), with some speckled white pigments in all scales; whitish venter covered by irregular brownish bands (*n*=96) that join with paraventral blotches (as appointed above) (*n*=96); brownish gular region ending before the beginning of the ventral scales (*n*=96).

Figure 1. Dorsal and ventral views of *Helicops hagmanni* holotype - NMBA 6281

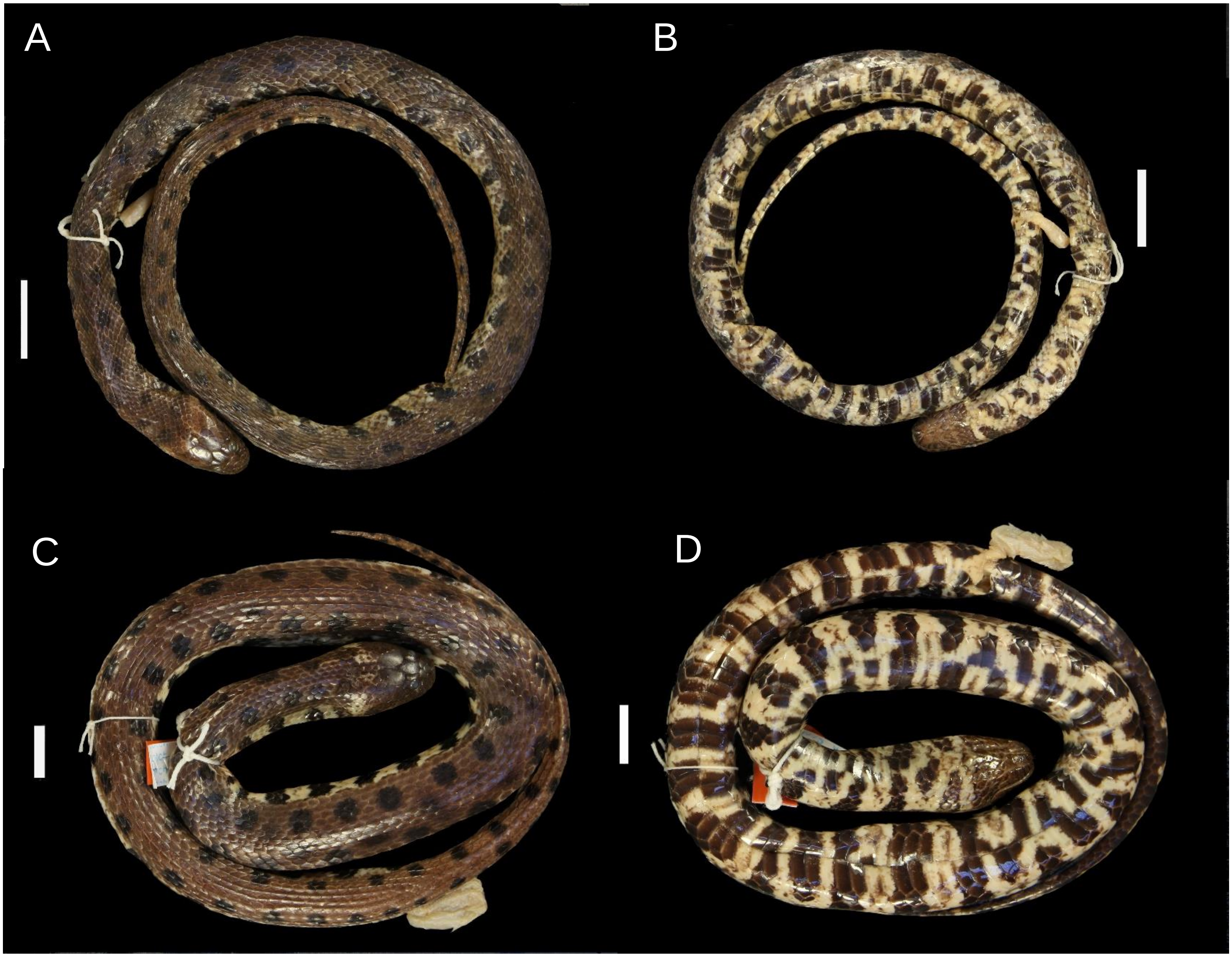

Figure 2. Morphological variation of *Helicops hagmanni.* A – B dorsal and ventral view of the specimen MPEG 6891 (east pattern); C– D dorsal and ventral view of the specimen INPA 2174 (west pattern).

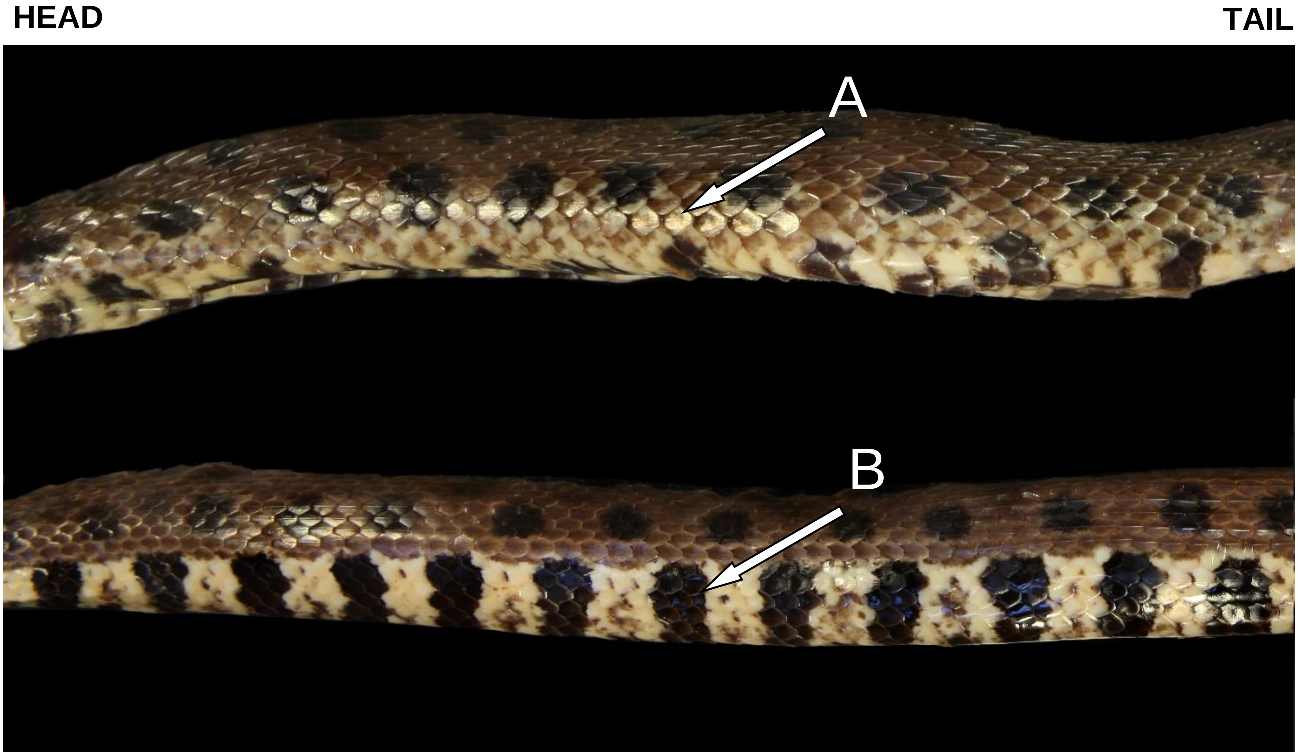

Figure 3. Lateral view of paraventral spots of *Helicops hagmanni*. A - separate from the ventral bands; B – joined to the ventral bands.

Hemipenis (n=7)

The hemipenis is strongly elongated, bilobed, calyculated and semicapitated (Figs. 4, 5 and 6); the body of the hemipenis is covered by spines on sulcate and asulcate faces, with enlarged spines in the lateral faces; the lobes are a third to one half of the body, surrounded by 4–7 rows of small spicules; the sulcate surface show spines that get larger from the base of the organ to near the base of the lobe (i.e., before capitation); intrasulcar region with small spicules that beginning in the second third of the organ; *sulcus spermaticus* bifurcates at the first third of hemipenial body; each side of the sulcus runs parallel to the middle of the organ, where they run to lateral top of each lobe; 2–3 rows of deep calyces formed around the apex (Fig. 6A), between the rows of spicules; in some regions, the walls of the calyces may decrease (Fig. 4A); the asulcate surface has the medial region full of spicules, which become less evident at the base of the lobes and then again evident at the apex of the lobes (Fig. 6B).

Figure 4. Asulcate, sulcate and lateral faces, respectively, of the right hemipenis of specimen MPEG 6811.

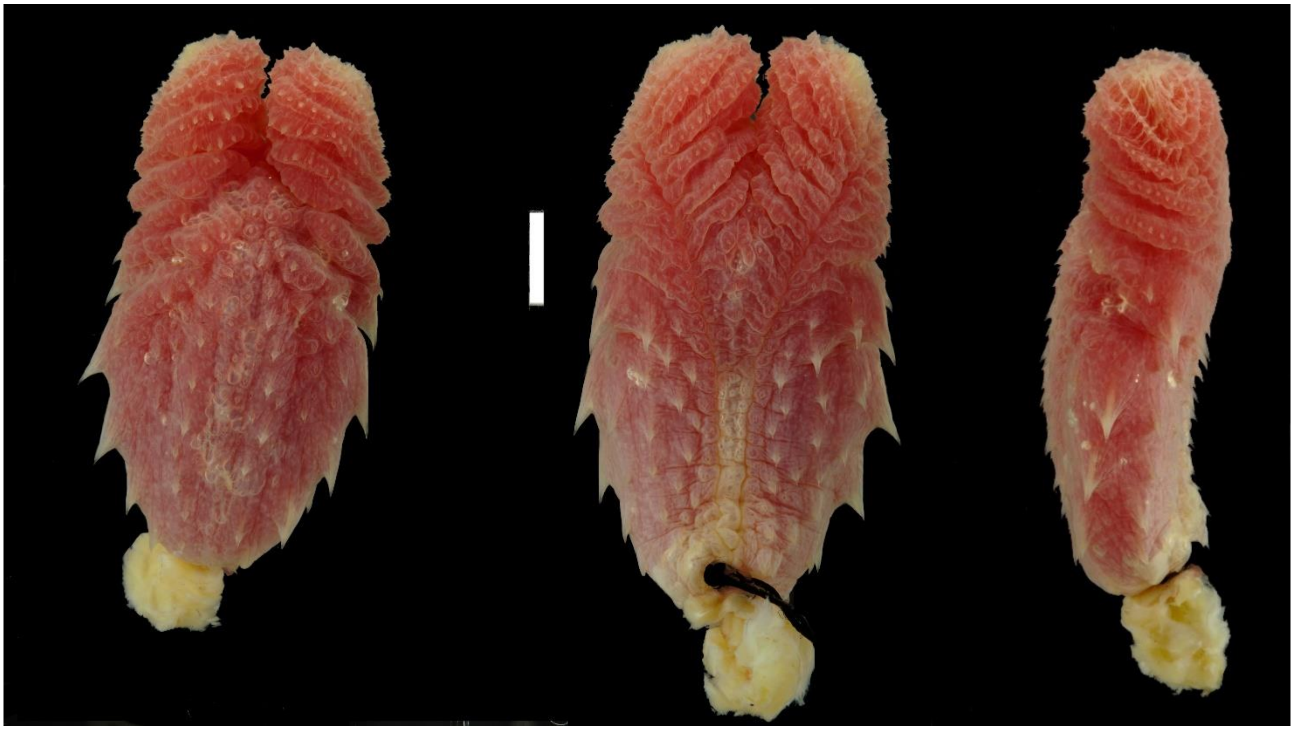

Figure 5. Asulcate, sulcate and lateral faces, respectively, of the right hemipenis of specimen INPA 2174.

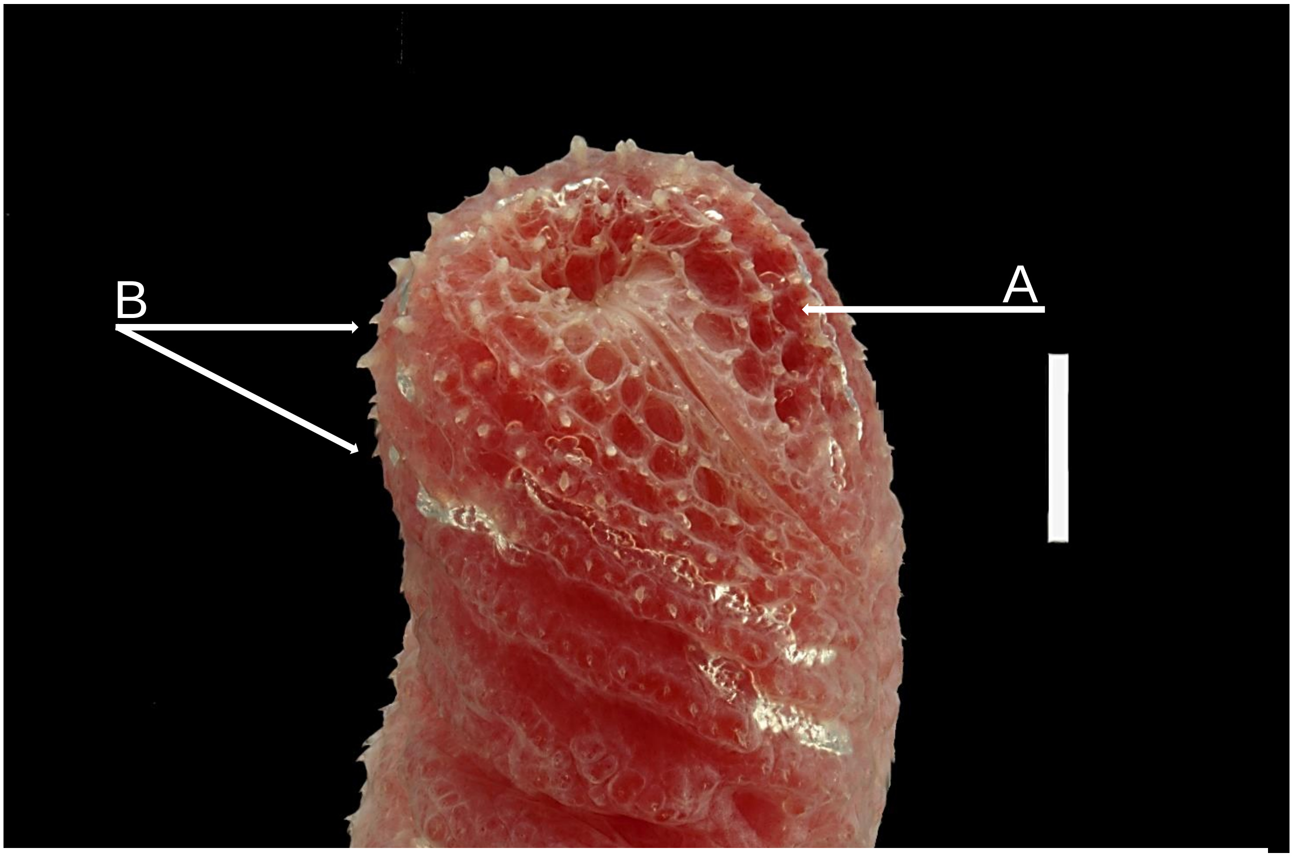

Figure 6. Lobe lateral view of the right hemipenis of specimen MPEG 4662 showing parallel rows of deep calyces (A) and decrease in spicule size followed by increase in apex (B).

Skull morphology (n= 8)

(Figure 7)

Premaxilla has a triangular shape showing two transverse processes laterally expanded, and one dorsally expanded at the base, between the vomer anterior projections; premaxilla has two sagittal projections near the beginning of the vomer; nasal dorsally triangular with two ventral projections, anterior and posterior (=caudal), the latter below the frontal, following and between vomer projections; vomer is triangular on the ventral view, with the anterior part wider and the posterior part elongated; the septomaxilla and vomer are tightly integrated; the septomaxilla has two processes; the first one is dorsally closer to the nasal side, whereas the second is ventrally almost below the frontal; frontal is square in dorsal view, the anterior region touching the prefrontals on the sides of the edges band and the entire posterior border in contact with the parietal; parietal has a triangular dorsal transverse ridge, and the front part of the bone wraps the orbits and touches half of the postorbitals on each side, which has a rounded bottom medially touching the supraoccipital; in lateral view parietal bone is also rounded; postorbital is arched with the upper half touching the parietal; the prefrontal is arched; prootics are irregular, with three foramina in lateral view; in ventral view, prootics show the anterior half covered by the basisphenoid plate and the posterior half is hidden by the basioccipital plate; supraoccipital touches the parietal end with a sagittal crest and a circular transverse crest; supratemporal elongated, touching the square bone at the height of the first vertebra (Atlas) and fitting into the top of the prootics and posterior border of the parietal; exocipital irregular with two foramens, the anterior being demarcated by the contact with the prootic; exocipital touching the supraoccipital dorsally, laterally the prootic and ventrally the basioccipital; quadrate broad and dorsoventrally flattened; maxilla laterally arched an inward triangular projection below the prefrontal, with 17 teeths; ectopterygoid convex and ventrally flattened, attached by its widest portion to the maxilla at the height of the last teeth and attached to the medial portion of the pterygoid; palatine with two rows of teeth and two lateralized projections; dentary elongated, reaching halfway through the compound bone, with a curvature towards the inside of the skull; splenial connected to dentary frontally and to angular caudally; angular bone is reduced in a triangular shape in lateral view and is connected to the compound bone, which is long and has a dorsal crest.

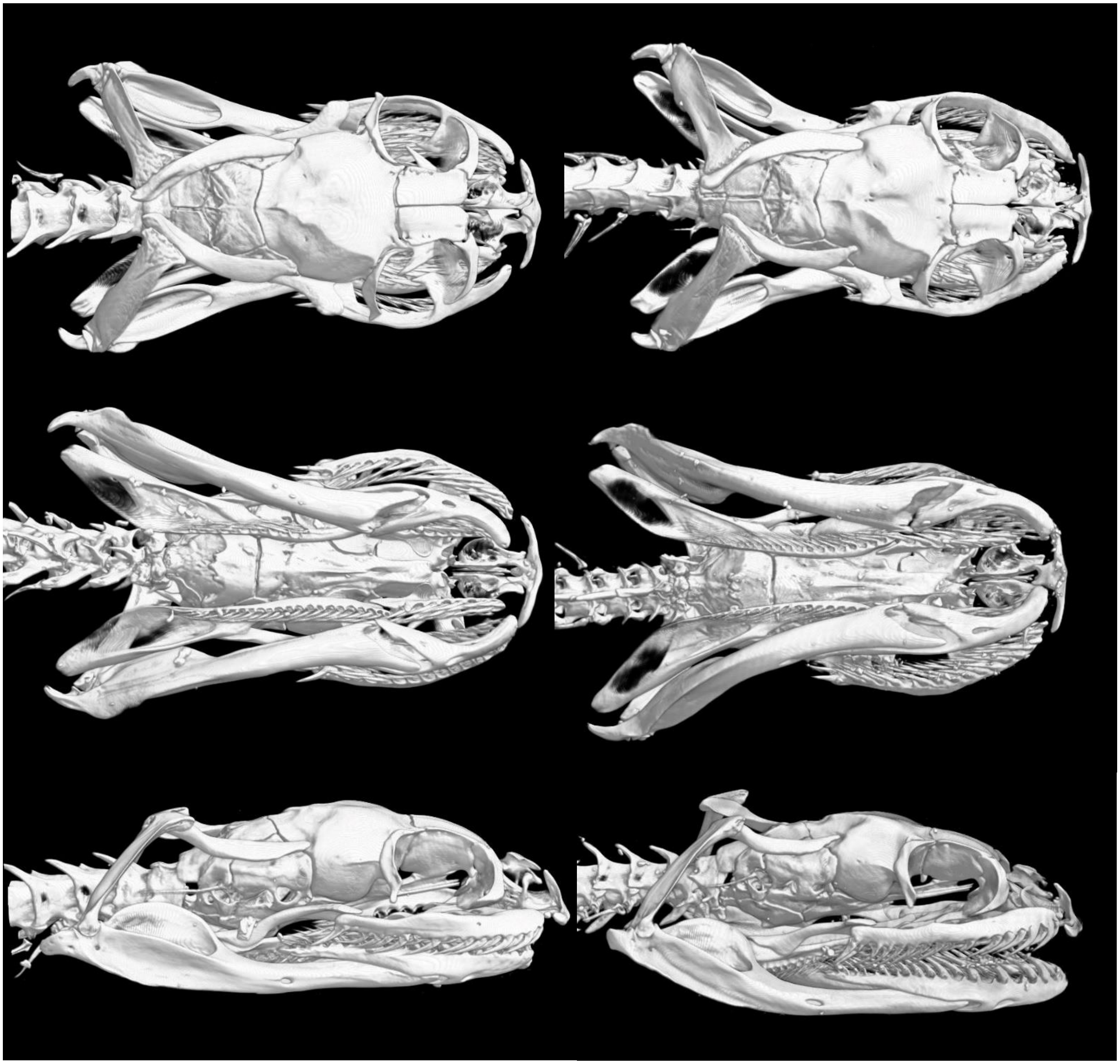

Figure 7. Dorsal (A), ventral (B) and lateral (C) view of the skull of FMT 2314. Dorsal (D), ventral (E) and lateral (F) view of the skull of MPEG 4662.

Comparisons with other Amazonian species

Helicops hagmanni is sympatric with the other ten species of *Helicops.* The dorsum pattern covered by circular spots of *H. hagmanni* allows its distinction of *H. angulatus* and *H. apiaka* (both saddle dorsal pattern), *H. tapajonicus* (uniform dorsal pattern) and *H. trivitattus* (stripped dorsal pattern). Among the *Helicops* showing spotted pattern, *H. hagmanni* differs from *H. acangussu* and *H. leopardinus* by the higher number of dorsal scales in midbody (>23 in *H. hagmanni*, 19–21 in *H. acangussu* and 19 in *H. leopardinus*) and by the presence of intergenial scales (absent in *H. leopardinus*); from *H. polylepis* differs by the ventral pattern (whitish with dark bands in *H. hagmanni* and completely blackened with white pigments in *H. polylepis*) (Citeli, unpublished data); from *H. pastazae*, *H. petersi* and *H. yacu* by the lower number of subcaudals (♀♀ 46–57, ♂♂ 54–67 in *H. hagmanni*, ♀♀ 67–73, ♂♂ 85–91 in *H. petersi*, ♀♀ 72–97, ♂♂ 93–117 in *H. pastazae*, ♀♀ 85–96 in *H. yacu*) (Rossman 1976; Rossman and Dixon, 1975, Citeli, unpublished data).

Distribution: *Helicops hagmanni* is widely distributed along the Amazon basin, including Brazil, Colombia, Venezuela (Paolilo, 1986) and probably Ecuador and Peru.

Geographic variation of meristic characters

Four characters showed spatial auto-correlation along the geographic distribution of *Helicops hagmanni* (p <0,05): number of dorsal scales rows at midbody, Moran I = 0,518 (Fig. 8A), number of ventral scales, Moran I = 0,200 (Fig. 8B), number of dorsal spots Moran I = 0,330 (Fig. 9A), junction region of the paraventral spot and ventral band Moran I = 0,221 (Fig. 9B) All characters showed a decrease in values from east to west along the Amazon basin.

The number of subcaudal scales (Fig. 10) was not spatially autocorrelated (p = 0,332).

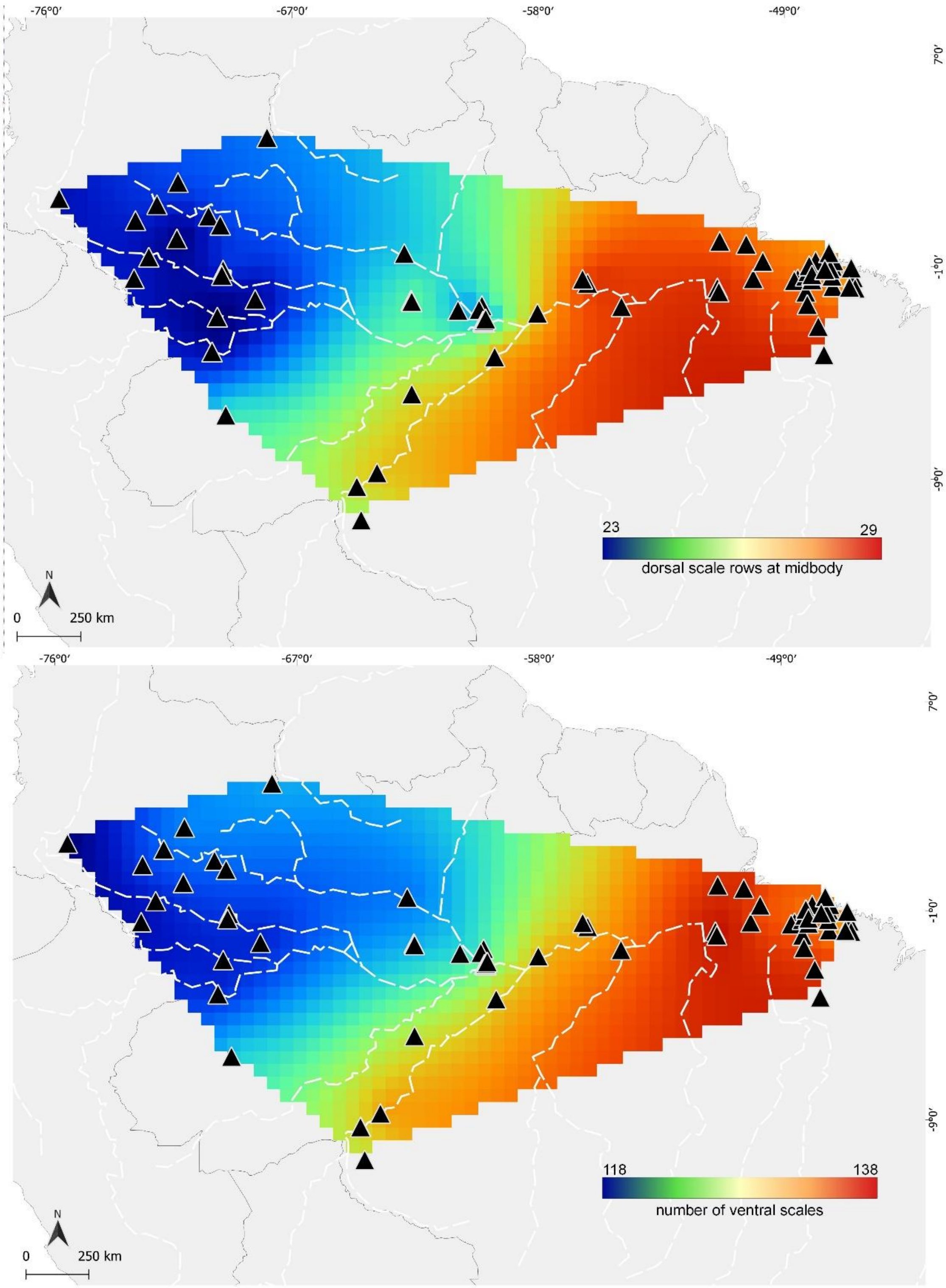

Figure 8. Spatial variation patterns in number of dorsal scales (A) and ventral scales (B) in *H. hagmanni.*

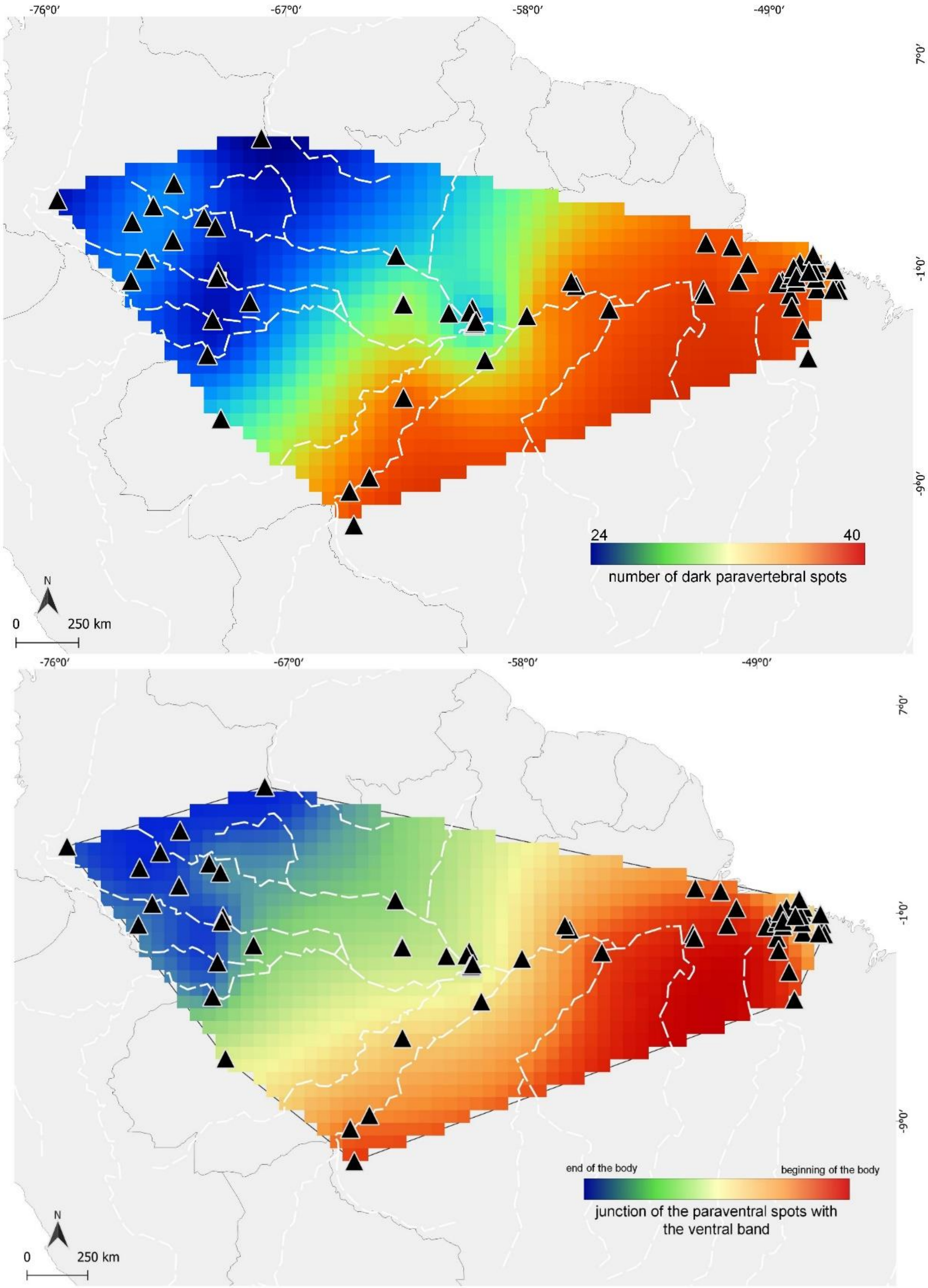

Figure 9. Spatial variation patterns in number of paravertebral spots (A) and type of paraventbral spots (joined or separate from the ventral bands) (B).

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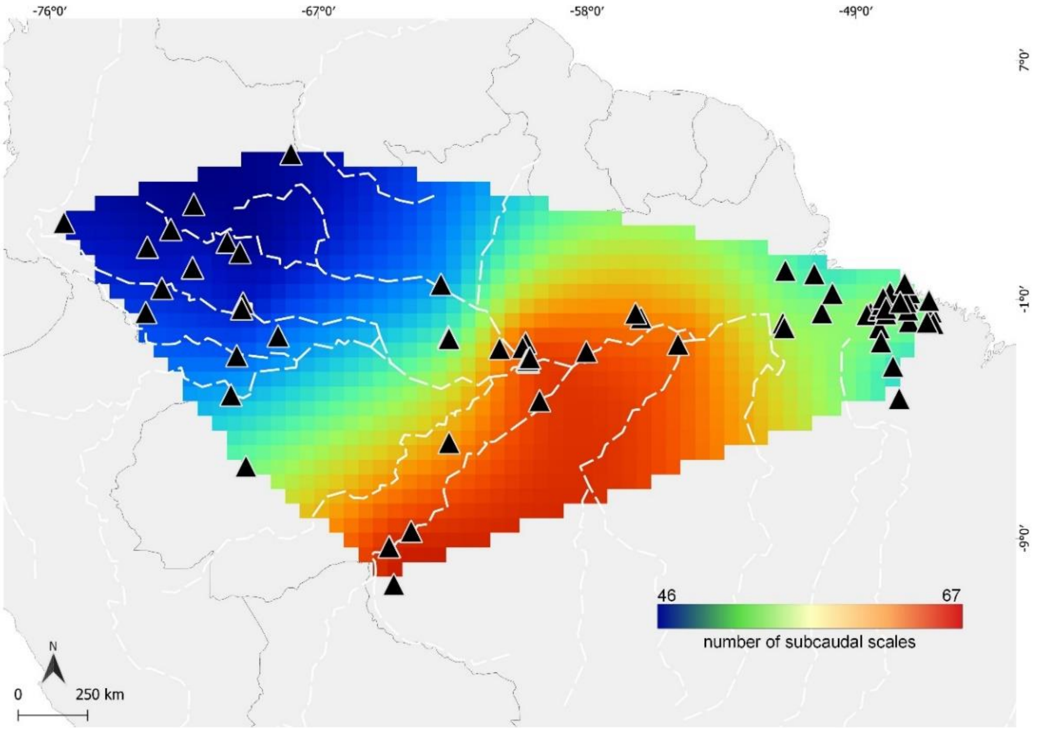

Figure 10. Spatial variation patterns in number of subcaudal scales in *H. hagmanni.*

Discussion

Until now, the morphology of *Helicops hagmanni* has not been studied in detail, and our results clarify the identity of this taxon. Previous authors addressed a putative taxonomic imprecision of *H. hagmanni* throughout its distribution. Rossman (1975) pointed out that more individuals from the lower Amazon basin (toward the headwater) would be analysed to detect if there was a decrease in dorsal scales or if the holotype was aberrant (=27 or 29 dorsal scales). Also, according to Rossman (1975), specimens from the upper Rio Negro should be analysed to confirm a putative "subspecies", since the color pattern of the individuals analysed by Rossman and by Hoge et al., (1964; 1972) were not consistent with the holotype pattern. Schöneberg and Köhler (2021), in their review of *Helicops* spp. distribution, present the dorsal scales at the midbody of *H. hagmanni* between 21–27, but it is not clear where this information was taken from, i.e., analysed specimens or from the literature. Moraes-da-Silva (2022) suggested that populations from the state of Acre, northern Brazil, named as *H. hagmanni,* should be analysed by the general pattern shown by specimens in this region. We did not find any specimen of *Helicops hagmanni* with lower number of dorsal scales at midbody (<23) in the analysed sample. On the other hand, we confirmed that individuals from upper Rio Negro and from lower Amazon River have a lower number of dorsal spots, of dorsal scales rows at midbody, ventral scales and variation in color pattern.

Intraspecific variations in morphological traits of terrestrial vertebrates are commonly observed as a response to natural selection and adaptations (Litzugs et al., 2004; Gardner et al., 2009). On the other hand, clinal variation in Amazonian groups may result from physical and climatic factors, such as rainfall, temperature, and isolation related to the width of river channels (Haffer 2008; Dickens 2021). Although we identified longitudinal variation in meristic characters, it can be an effect of individual’s body size, affecting the counting of ventral scales and of dorsal spots. The western specimens can be smaller in response to selective pressures (e.g., competition, predation, prey selection) since this is also the most diverse region of the Amazon of fishes (Oberdorff et al., 2019; Duponchelle et al., 2021), a relevant prey category for these snakes. Dorsal scales, in this case, may be related to the thickness of the body. All these characteristics are changing in association with the ventral coloration pattern, which may be indicative of ecological predation pressure or niche differentiation for hunting. The western region of the distribution of *Helicops hagmanni* has the greatest diversity of the genus, with several species occurring in sympatry (Nogueira et al., 2019).

The absence of spatial correlation in the number of subcaudals scales may be explained by sexual pressure acting along all the species distribution, as *Helicops* spp. males have larger tails (Citeli et al., 2021). In addition, none of these explanations prevents our sample may be two evolutionary units in the process of speciation without a clear geographical barrier (Haffer 2008). Molecular samples along its distribution may help to clarify these hypotheses, including a finding whether these phenotypic traits are indeed adaptive (e.g., Stillwell 2010).

However, externally, *Helicops hagmanni* is similar to the other species of *Helicops* related to *He. polylepis*, with circular dorsal spots and high number of dorsal scales (>23). According to the morphological phylogeny proposed by Nunes (2006), *H. hagmanni* is a sister group of *H. trivitattus* based on 12 osteological synapomorphies. *H. trivitattus* has a stripped dorsal pattern. On the other hand, in the phylogenetic hypothesis proposed by Moraes-da-Silva (2021), *H. hagmanni* appears as a sister group of the clade formed by *H. angulatus* and *H. gomesi,* which have 19 dorsal scales and a banded dorsal pattern. It seems clear that *H. hagmanni* is one of the most polymorphic species of the genera, and its phylogenetic positioning and divergence time must be further investigated. Despite the high polymorphism found along the species distribution, our findings allowed the unequivocal identification of *Helicops hagmanni* between spotted-dorsal *Helicops* group and other *Helicops* species.

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APPENDIX I. Specimens examined

Institution Abbreviation (follows Sabaj 2020)	Voucher Number	Locality	Municipality	Departament or State	Country	Lat (°N)	Long (°W)
IBSP	87354		Novo Airão	Amazonas	Brazil	-2.253481	-62.650962
IBSP	80594		Novo Airão	Amazonas	Brazil	-2.645359	-60.936421
CEPB	980		Porto Velho	Rondônia	Brazil	-8.763554	-63.897168
IBSP	87356		Novo Airão	Amazonas	Brazil	-2.253481	-62.650962
MPEG	10485	São Pedro	Capitão Poço	Pará	Brazil	-1.7480556	-47.2177778
MPEG	10484	São Pedro	Capitão Poço	Pará	Brazil	-1.7480556	-47.2177778
MPEG	10487	São Pedro	Capitão Poço	Pará	Brazil	-1.7480556	-47.2177778
MPEG	10627	São Pedro	Capitão Poço	Pará	Brazil	-1.7480556	-47.2177778
MPEG	10497	São Pedro	Capitão Poço	Pará	Brazil	-1.7480556	-47.2177778
MPEG	10498	São Pedro	Capitão Poço	Pará	Brazil	-1.7480556	-47.2177778
MPEG	10486	São Pedro	Capitão Poço	Pará	Brazil	-1.7480556	-47.2177778
MPEG	10908		Belém	Pará	Brazil	-1.4558333	-48.5044444
MPEG	605		Belém	Pará	Brazil	-1.4511111	-50.1333333
MPEG	26056	UHE, Usina de Jirau	Porto Velho	Rondônia	Brazil	-9.270688	-64.627871
MPEG	4784	Trombetina	Santarém Novo	Pará	Brazil	-0.9794444	-47.2386111
MPEG	7059	Trombetina	Santarém Novo	Pará	Brazil	-0.9794444	-47.2386111
MPEG	3462	Trombetina	Santarém Novo	Pará	Brazil	-0.9794444	-47.2386111
MPEG	7053	Trombetina	Santarém Novo	Pará	Brazil	-0.9794444	-47.2386111
MPEG	7060	Trombetina	Santarém Novo	Pará	Brazil	-0.9794444	-47.2386111
MPEG	21017		São Miguel do Guamá	Pará	Brazil	-3.247031	-47.751501
MPEG	7020	Puraquequara	Orém	Pará	Brazil	-1.3555556	-47.2522222
MPEG	4997	Puraquequara	Orém	Pará	Brazil	-1.3555556	-47.2522222
MPEG	7024	Puraquequara	Orém	Pará	Brazil	-1.3555556	-47.2522222
MPEG	7008	Puraquequara	Orém	Pará	Brazil	-1.3555556	-47.2522222
MPEG	4221	Puraquequara	Orém	Pará	Brazil	-1.3555556	-47.2522222
MPEG	19511	Parque Estadual de Guajará Mirim		Rondônia	Brazil	-10.539876	-64.48024
MPEG	20655		Barcarena	Pará	Brazil	-1.517244	-48.629695
MPEG	18791	Lago do Jacundá, Alter do Chão	Santarém	Pará	Brazil	-2.504701	-54.954258
MPEG	24253		São João de Pirabas	Pará	Brazil	-0.777833	-47.187476
MPEG	22488	Mutum, Barroso	Juruti	Pará	Brazil	-1.623025	-56.193987
MPEG	22637	Mutum, Barroso	Juruti	Pará	Brazil	-2.4619444	-60.061111
MPEG	23247	Mutum, Barroso	Juruti	Pará	Brazil	-2.4619444	-60.061111
MPEG	25325		Ilha de Marajó	Pará	Brazil	-0.152834	-50.390165
MPEG	21535		Benevides	Pará	Brazil	-1.349056	-48.218035
MPEG	4889	Marauá	Curuçá	Pará	Brazil	-0.7802778	-47.8488889
MPEG	4893	Marauá	Curuçá	Pará	Brazil	-0.7802778	-47.8488889
MPEG	8874	Curupati	Viseu	Pará	Brazil	-1.418283	-46.467644
MPEG	11466	Rio Gurupi, Colônia Nova, próximo do rio, BR-316	Viseu	Pará	Brazil	-1.8088889	-46.4038889
MPEG	22403	Floresta Nacional de Caxinauã	Melgaço	Pará	Brazil	-1.797143	-51.444214
MPEG	20301	Floresta Nacional de Caxinauã	Melgaço	Pará	Brazil	-1.797143	-51.444214
MPEG	6972		Santo Antônio do Tauá	Pará	Brazil	-1.1802778	-48.1291667
MPEG	1138		Santo Antônio do Tauá	Pará	Brazil	-0.8013889	-49.7819444
MPEG	15515	Ipitinga. Estrada Mojú-Acará	Acará	Pará	Brazil	-1.956845	-48.211723
MPEG	12614	Ipitinga, Estrada Mojú-Acará	Acará	Pará	Brazil	-2.425162	-48.15407
MPEG	12614	Ipitinga, Estrada Mojú-Acará	Acará	Pará	Brazil	-2.425162	-48.15407
MPEG	13350	Ipitinga, Estrada Mojú-Acará	Acará	Pará	Brazil	-2.425162	-48.15407
MPEG	672		Peixe-Boi	Pará	Brazil	-0.4730556	-47.3619444
MPEG	1820		Peixe-Boi	Pará	Brazil	-0.4730556	-47.3619444
MPEG	18438		Peixe-Boi	Pará	Brazil	-1.084243	-47.314769
MPEG	675		Peixe-Boi	Pará	Brazil	-0.4730556	-47.3619444
MPEG	10763		Augusto Corrêa	Pará	Brazil	-1.0397222	-46.5472222
MPEG	6501		Augusto Corrêa	Pará	Brazil	-1.0397222	-46.5472222
MPEG	9098		Augusto Corrêa	Pará	Brazil	-1.0397222	-46.5472222
MPEG	9916		Augusto Corrêa	Pará	Brazil	-1.0397222	-46.5472222
MPEG	11635		Augusto Corrêa	Pará	Brazil	-1.0397222	-46.5472222
MPEG	19312	Comunidade Laranjeira. Igarapé Laranjal. Baía de Caxiuanã.	Melgaço	Pará	Brazil	-1.948769	-51.39345
MPEG	8525	Santa Rosa, Estrada de Vigia	Vigia	Pará	Brazil	-0.9555556	-48.0861111
MPEG	6815	Santa Rosa, Estrada de Vigia	Vigia	Pará	Brazil	-0.9555556	-48.0861111
MPEG	8533	Santa Rosa, Estrada de Vigia	Vigia	Pará	Brazil	-0.9555556	-48.0861111
MPEG	4013	Santa Rosa, Estrada de Vigia	Vigia	Pará	Brazil	-0.9555556	-48.0861111
MPEG	4625	Santa Rosa, Estrada de Vigia	Vigia	Pará	Brazil	-0.9555556	-48.0861111
MPEG	5923	Boa Vista, Apeú	Castanhal	Pará	Brazil	-1.4891667	-47.9863889
MPEG	1998	Boa Vista, Apeú	Castanhal	Pará	Brazil	-1.4891667	-47.9863889
MPEG	1999	Boa Vista, Apeú	Castanhal	Pará	Brazil	-1.4891667	-47.9863889
MPEG	2682	Boa Vista, Apeú	Castanhal	Pará	Brazil	-1.4891667	-47.9863889
MPEG	541	Boa Vista, Apeú	Castanhal	Pará	Brazil	-1.3013889	-47.9847222
MPEG	4111	km 23 da estrada de Maracanã	Maracanã	Pará	Brazil	-1.1044444	-47.5119444
MPEG	4857	km 23 da estrada de Maracanã	Maracanã	Pará	Brazil	-1.1044444	-47.5119444
MPEG	17300	Sítio Bela Vista. PA-222.	Dom Eliseu	Pará	Brazil	-4.319125	-47.545248
MPEG	13379	Sítio Bela Vista. PA-222.	Dom Eliseu	Pará	Brazil	-4.319125	-47.545248
MPEG	5259	km 220 da BR-316, antigo km 74 (contagem de Capanema)	Viseu	Pará	Brazil	-1.7691667	-46.6130556

APPENDIX I. Specimens examined

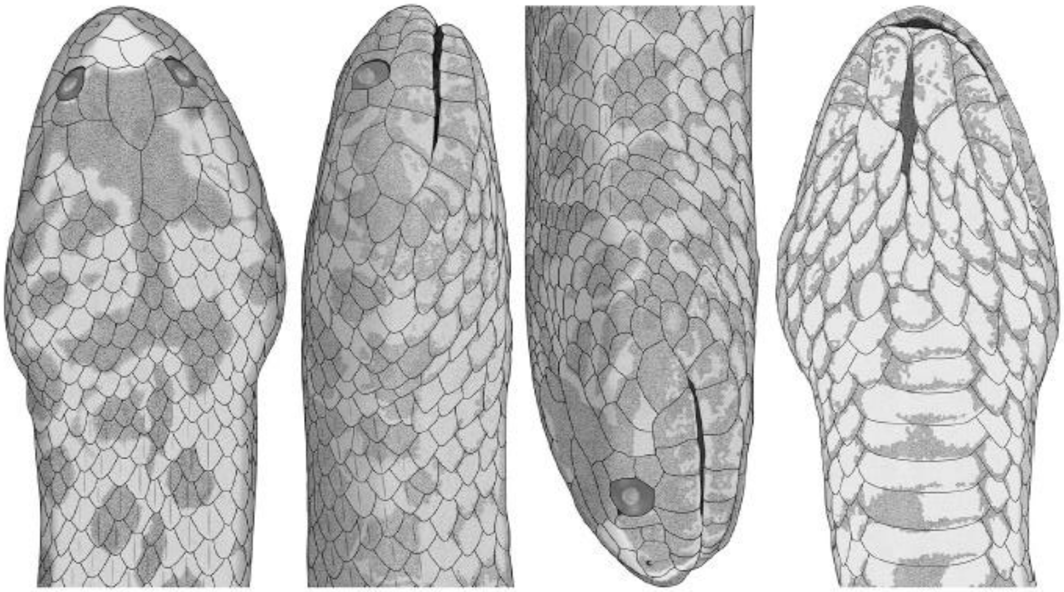
Institution Abbreviation (follows Sabaj 2020)	Voucher Number	Locality	Municipality	Departament or State	Country	Lat (°N)	Long (°W)
MZUSP	11716	Rio Maracá (boca Igarapé, Camaipi)		Amapá	Brazil	-0.037268	-51.35948
MZUSP	18869	UHE Jirau, Margem direita do Rio Madeira	Porto Velho	Rondônia	Brazil	-9.283356	-64.63839
MZUSP	7615	Reseva INPA - WWF 6		Amazonas	Brazil	-3.097647	-59.968532
MZUSP	5092		Itapiranga	Amazonas	Brazil	-2.749031	-58.02779
MNRJ	1560		Borba	Amazonas	Brazil	-4.394161	-59.593438
MNRJ	16833	Platô Aviso, Flona Sacará-Taquera, Porto Trombetas	Oriximiná	Pará	Brazil	-1.467137	-56.379362
MNRJ	10127	Ucuqui, Igarapé Javari, margem direita do Rio Acará, afluente do Rio Negro	Barcelos	Amazonas	Brazil	-0.499677	-62.899579
ICN-R	7995	Laguna Lombriz	Puerto Inírida	Guainía	Colombia	3.869689	-67.924183
ICN-R	8235	Caparú, alrededores del lago Taraira	Taraira	Vaupés	Colombia	-1.122965	-69.529989
IAvH	1881	Caserio de Timbó		Vaupés	Colombia	0.910989	-70.063061
IAvH	3215	Estación Caparú, Lago Taraira, orilla norte Río Apaporis		Vaupés	Colombia	-1.126588	-69.529974
IAvH	6945	Reserva Nacional Natural Nukak, Tomachipán, Cerro Cocui, Antiguo caserio Puinawai	San José del Guaviare	Guaviare	Colombia	2.176111	-71.181944
ICN-R	10741	Parque Nacional Natural Serranía de Chiribiquete	Solano	Caquetá	Colombia	0.741983	-72.737346
MLS	778	Caseiro Venencia		Caqueta	Colombia	1.568022	-75.529959
INPA	15951	Rio Juruá	Juruá	Amazonas	Brazil	-6.58783	-69.433616
INPA	2174	Reserva do 41, BR 174, km 41, Programa de Diversidade de Dinâmicas Florestais		Amazonas	Brazil	-2.615499	-60.163552
INPA	28824	Parna Nascentes do Lago Jari, Igarapé do Sabá		Amazonas	Brazil	-5.795098	-62.627806
INPA	20152	Reserva Ducke, Ripária 020	Manaus	Amazonas	Brazil	-2.964363	-59.92261
INPA	14686	Rio Juami		Amazonas	Brazil	-2.22868	-68.357007
INPA	39224	JAÚ - IG 31		Amazonas	Brazil	-2.313048	-62.630086
FMT	1225	INPA	Manaus	Amazonas	Brazil	-3.095391	-59.989476
FMT	1716			Amazonas (?)	Brazil	na	na
FMT	2791	BR AM Manaus AM 010 Ramal de Areia	Manaus	Amazonas	Brazil	-3.045852	-60.025428
FMT	538			Amazonas (?)	Brazil	na	na
FMT	2314	BR AM Manaus Florestal II, Cidade Nova	Manaus	Amazonas	Brazil	-3.036268	-59.985483
FMT	1849	Reserva Ducke	Manaus	Amazonas	Brazil	-2.964363	-59.92261
SINCHI-R	2654	Comunidad de Buenos Aires	Pacoa	Vaupés	Colombia	0.053907	-71.222667
SINCHI-R	2704	Comunidad de Buenos Aires	Pacoa	Vaupés	Colombia	0.053907	-71.222667
SINCHI-R	2652	Comunidad de Buenos Aires	Pacoa	Vaupés	Colombia	0.053907	-71.222667
SINCHI-R	1044		La Pedrera	Amazonas	Brazil	-1.322004	-69.578386
SINCHI-R	1047		Leticia	Amazonas	Brazil	-4.20311	-69.9359
SINCHI-R	1046	Araracuara, Isla de Los Monos	Solano	Caquetá	Colombia	-0.635734	-72.250341
SINCHI-R	1667	Los Lagos	Leticia	Amazonas	Brazil	-4.20311	-69.9359
SINCHI-R	1043		La Chorrera	Amazonas	Brazil	-1.443383	-72.788945
SINCHI-R	1042	Los Lagos	Leticia	Amazonas	Brazil	-4.20311	-69.9359
SINCHI-R	1045		La Pedrera	Amazonas	Brazil	-1.322004	-69.578386
SINCHI-R	1041	Araracuara	Solano	Caquetá	Colombia	-0.635734	-72.250341
SINCHI-R	2707	Cerca a cerro Campana	Miraflores	Guaviare	Colombia	1.337454	-71.950914
SINCHI-R	3100		Tarapacá	Amazonas	Brazil	-2.891841	-69.741923
SINCHI-R	2668	Comunidad de Buenos Aires	Pacoa	Vaupés	Colombia	0.053907	-71.222667
SINCHI-R	811		Taraira	Vaupés	Colombia	0.564349	-69.633795

Capítulo III

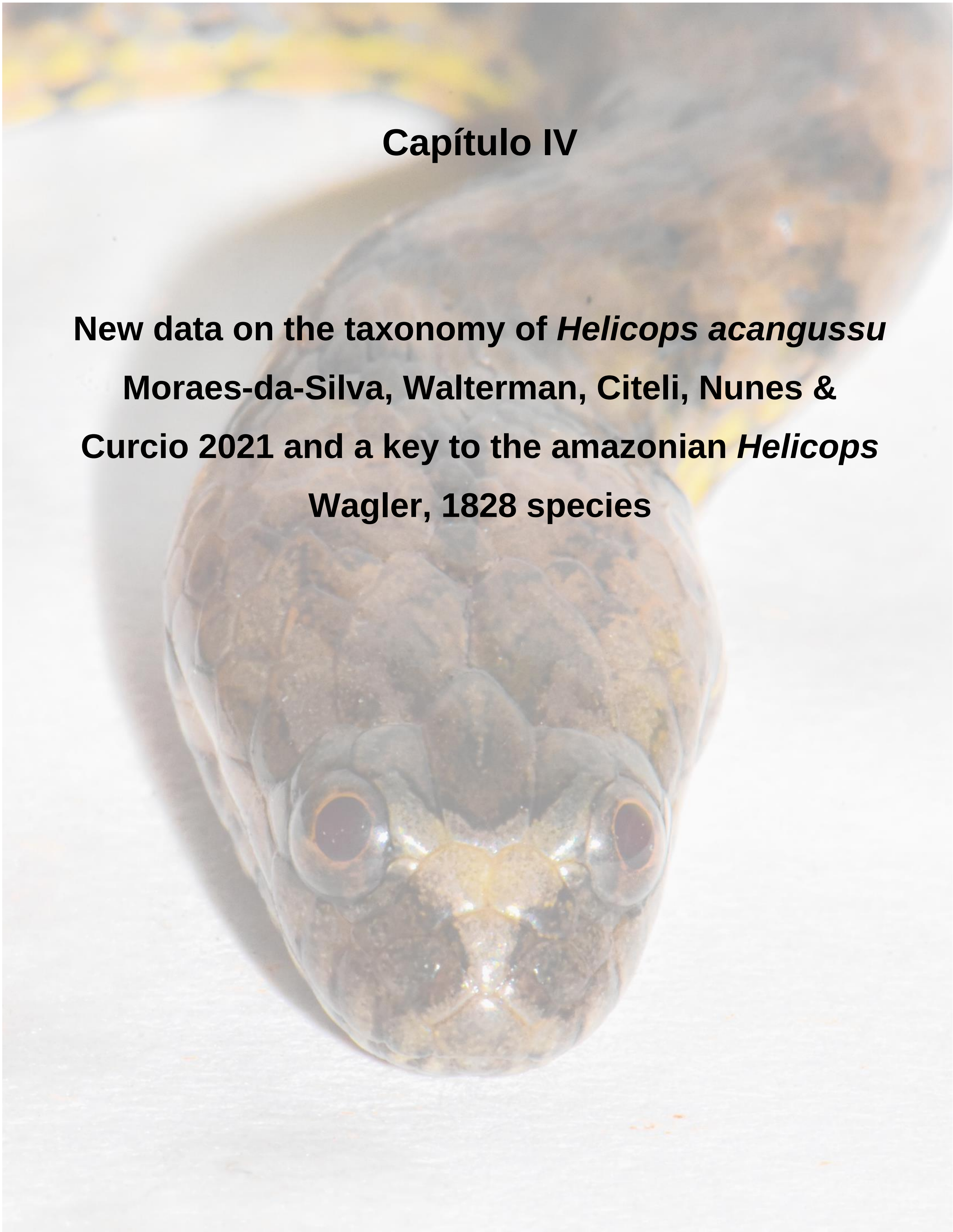
A new oviparous species of *Helicops* Wagler, 1828 (Serpentes, Xenodontinae) from Brazilian Amazonia with reflections on the evolution of viviparity among hydropsine watersnakes

Artigo publicado na revista Zoologischer Anzeiger

(texto completo no apêndice II)



Prancha do holótipo de *Helicops acangussu* (UFRO-R 2748).
Imagem extraída de Moraes-da-Silva et al. (2022).



Capítulo IV

New data on the taxonomy of *Helicops acangussu* Moraes-da-Silva, Walterman, Citeli, Nunes & Curcio 2021 and a key to the amazonian *Helicops* Wagler, 1828 species

(manuscrito que será submetido à revista Zootaxa)

New data on the taxonomy of *Helicops acangussu* Moraes-da-Silva, Walterman, Citeli, Nunes & Curcio 2021 and a key to the amazonian *Helicops* species

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Resumo

Helicops acangussu foi descrita com base em 27 exemplares identificados como *Helicops hagmanni* depositados em duas coleções herpetológicas brasileiras. Todos os espécimes provinham da margem esquerda do Rio Madeira, no estado de Rondônia, Brasil. Durante visitas em coleções sul-americanas encontramos novos 33 exemplares provenientes de regiões a cerca de 1000 km de distância da localidade-tipo, nos municípios de Letícia na Colômbia e Presidente Prudente, Amazonas, Brasil. O exame detalhado do material nos permitiu descrever a osteologia craniana, ampliação da variação morfológica externa de caracteres merísticos e biométricos, bem como a complementação da descrição do hemipenis, com a análise de três hemipenis das novas populações. *Helicops acangussu* é uma espécie de taxonomia sólida, que apresenta variação intraespecífica na morfologia do hemipenis e ampla distribuição na Amazônia. Também fornecemos uma chave de identificação das espécies de *Helicops* amazônicas.

Palavras-chave: cobra-d'água, variação do hemipenis, variação intraespecífica, osteologia.

Abstract

Helicops acangussu was described based on 27 specimens deposited in two Brazilian herpetological collections identified as *Helicops hagmanni*. All specimens came from the left bank of the Madeira River, state of Rondônia, Brazil. During visits in South American collections, we found 33 new specimens from regions about 1000 km away from the type locality, in the municipalities of Letícia in Colombia and Presidente Prudente, Amazonas, Brazil. The detailed examination of the material allowed us to describe the cranial osteology, expansion of the external morphological variation of meristic and biometric characters, as well as the complementation of the description of the hemipenis, with the analysis of three organs from the new populations. *Helicops acangussu* is a species with a solid taxonomy, which presents intraspecific variation in hemipenis morphology and a wide distribution in the Amazon. We also provide an identification key for the Amazonian *Helicops* species.

Keywords: watersnake, hemipenis variation, intraspecific variation, osteology.

Introduction

Helicops is a neotropical genus of aquatic snakes with 21 species distributed only in the South America (Nogueira et al., 2019; Moraes-da-Silva et al., 2019; 2021; 2022). The last ten years have been prolifically positive to knowledge about this genus by the significant expansion of data on morphology (Citeli et al., 2021; Garcia-Cobos, 2021), reproduction (Braz et al., 2016; Garcia-Cobos, 2020), and taxonomy (Kawashita-Ribeiro et al., 2013; Costa et al., 2016; Moraes-da-Silva et al. 2019; 2021; 2022).

The most recent species described from the genus is *Helicops acangussu* done by Moraes-da-Silva et al. (2022), based on 27 specimens from the left bank of the upper Madeira River in Rondônia state, Brazil. The individuals of the novel taxon were collected at a wildlife rescue operation associated with the Santo Antônio Dam. The authors founded 27 specimens from the new species, previously identified as *H. hagmanni* Roux 1910 in two Brazilian herpetological collections at Museu Paraense "Emilio Goeldi" (MPEG) and Universidade Federal de Rondônia (UFRO-H) (Moraes-da-Silva et al., 2022).

Helicops acangussu was tentatively allocated to the group composed by *Helicops* species bearing dorsal spotted-patterned (Moraes-da-Silva et al., 2022), being grouped with of *Helicops hagmanni*, *Helicops leopardinus* (Schlegel, 1837), *Helicops pastazae* (Schlegel, 1834), *Helicops petersi* Rossman, 1976, *Helicops polylepis* Günther, 1861, *Helicops yacu* Rossman & Dixon, 1975 (all amazonian species), and *Helicops danieli* Amaral, 1937 (restricted to the Colombian transandine region, Rossman 2002; Citeli et al. 2022). However, *H. acangussu* can be distinguished from these species mainly by (i) the number of midbody dorsal scales (19 in *H. acangussu* and 23–29 in *H. hagmanni*, 21–25 in *H. pastazae*, 21–23 in *H. petersi*, 23–25 in *H. polylepis* and 25–27 in *H. yacu*); and (ii), the presence of intergenial scales (absent in *H. danieli* and *H. leopardinus*). In addition, *Helicops acangussu* can be diagnosticated by the following combination of characters: ventral scales 121–128 in males and 124–129 in females; lowest counts of subcaudals, 52–67 in males and 40–43 in females; 35–47 dorsal spots in males, and 36–42 in females; a roughly triangular light snout spot; dorsum greenish-grey, with five rows of black rounded spots; venter background yellowish cream, with asymmetrical black squared blotches; and hemipenis semicapitated with lobes asymmetrical (Moraes-da-Silva et al., 2022).

During visits in South American herpetological collections to study Amazonian *Helicops* spp., we found 33 new specimens of *H. acangussu* from three new locations far from the originally proposed distribution for the species. After carefully analyzing the available sample of the species, we aim to expand the knowledge on (i) *H. acangussu* distribution, (ii) external morphological variation, (iii) description of skull osteology, and (iv) new data on the hemipenis morphology, complementing the preliminar description made by Moraes-da-Silva et al. (2022). Finally, to support the taxonomic identity of *H. acangussu* and the identification of other Amazonian species of the genus, we provide a key for the Amazonian *Helicops* spp.

Material and Methods

Sampling, scalation and morphometric data: We examined 33 specimens of *H. acangussu* (23 females and 10 males) housed at Instituto Nacional de Pesquisas da Amazônia (INPA-H), Manaus, Brazil; Instituto Amazônico de Investigaciones Científicas (SINCHI-R), Leticia, Amazonas, Colombia; and Museu Paraense ‘‘Emilio Goeldi’’ (MEPG), Belém, Brasil. Institutional abbreviations follow Sabaj (2020), for the complete list of specimens see APPENDIX I. We followed Dowling (1951) and Moraes-da-Silva et al. (2019; 2021) for scale counts, Citeli et al. (2021) and Moraes-da-Silva et al. (2019; 2021) for body and head traits measurements, and Moraes-da-Silva et al. (2022) for dorsal spots on dorsum counts. All measurements were taken using a digital caliper to the nearest 0.01 mm, except for SVL and TL measurements, where we used a flexible ruler. The sex of individuals with no everted hemipenis was identified through an incision at the base of the tail to detect the presence of hemipenes and retractor muscles.

Hemipenial and skull morphology:

The new data on the hemipenial morphology of *H. acangussu* was taken from three preserved specimens (SINCHI 01025, SINCHI 01026, and INPA 31415). We extracted, everted, and prepared the hemipenis following Pesantes (1994) and Zaher and Prudente (2003). We filled the fully everted hemipenis with petroleum jelly to visualize the ornamentation structures. Afterwards, we photographed and measured the organs using the ImageJ software version 1.8.0 (Rasband 2016). We followed the terminology by Zaher (1999) for hemipenis structures description.

We acquired the osteological characteristics of skulls from imagens provided by high-resolution micro-computed tomography (μCT) Bruker Skyscan 1273 at Instituto Alberto Luiz Coimbra de Pós-graduação e Pesquisa de Engenharia (COPPE), Laboratório de Instrumentação Nuclear, Universidade Federal do Rio de Janeiro, Rio de Janeiro. The imagens of two skulls (INPA 31415 and MPEG 25586) were visualized in CTVox for Windows 64bit version 2.6 (Bruker, μCT), and plates were made using GIMP 2.10 (Gimp, 2019). The osteological nomenclature follows Romer (1956).

Distribution maps

We acquired the geographic coordinates from Google Earth based on the locations informed by the collections at the municipal level. We used the software Quantum GIS 3.16 (QGIS) to produce the map using SIRGAS 2000. To identify which hydrographic basins were present in the species distribution, we used the shape of the South American basins available at HydroBASINS (Lehner & Gill 2013).

Development of species key

We followed Nogueira et al. (2019), Rossman (1976), and Moraes da Silva (2022) to identify which species of *Helicops* occur in the Amazon biome. To produce the taxonomic key we used morphological characteristics, especially diagnostic traits, available at species descriptions and taxonomic reviews, as Kawashita-Ribeiro et al. (2013) and Murphy et al. (2020) for *Helicops angulatus* (Linnaeus, 1758) and *Helicops apiaka* Kawashita-Ribeiro, Ávila & Morais, 2013; Rossman (1975) and Citeli (unpublished data) for *H. hagmanni*; Moraes-da-Silva et al. (2021, 2022) for *H. leopardinus*; Rossman (1976), Gunther (1861) Shreve (1934) and Citeli (unpublished data) for *H. pastazae*, *H. petersi* and *H. polylepis*; Frota (2003) for *H. tapajonicus*, Rossman (2010) for *Helicops trivitattus* and Rossman and Dixon (1975), Rossman and Abe (1979) and Citeli (unpublished data) for *H. yacu*.

Results

Distribution*:* We expanded the species' distribution to the municipalities of Tabatinga and Presidente Prudente, Amazonas State, Brazil, and to Letícia, Department of Amazonia, Colombia. We extended the distribution of the species to the northwestern by

818 km and to the northeast by 907 km. All occurrences are in the Amazon basin (Figure 1).

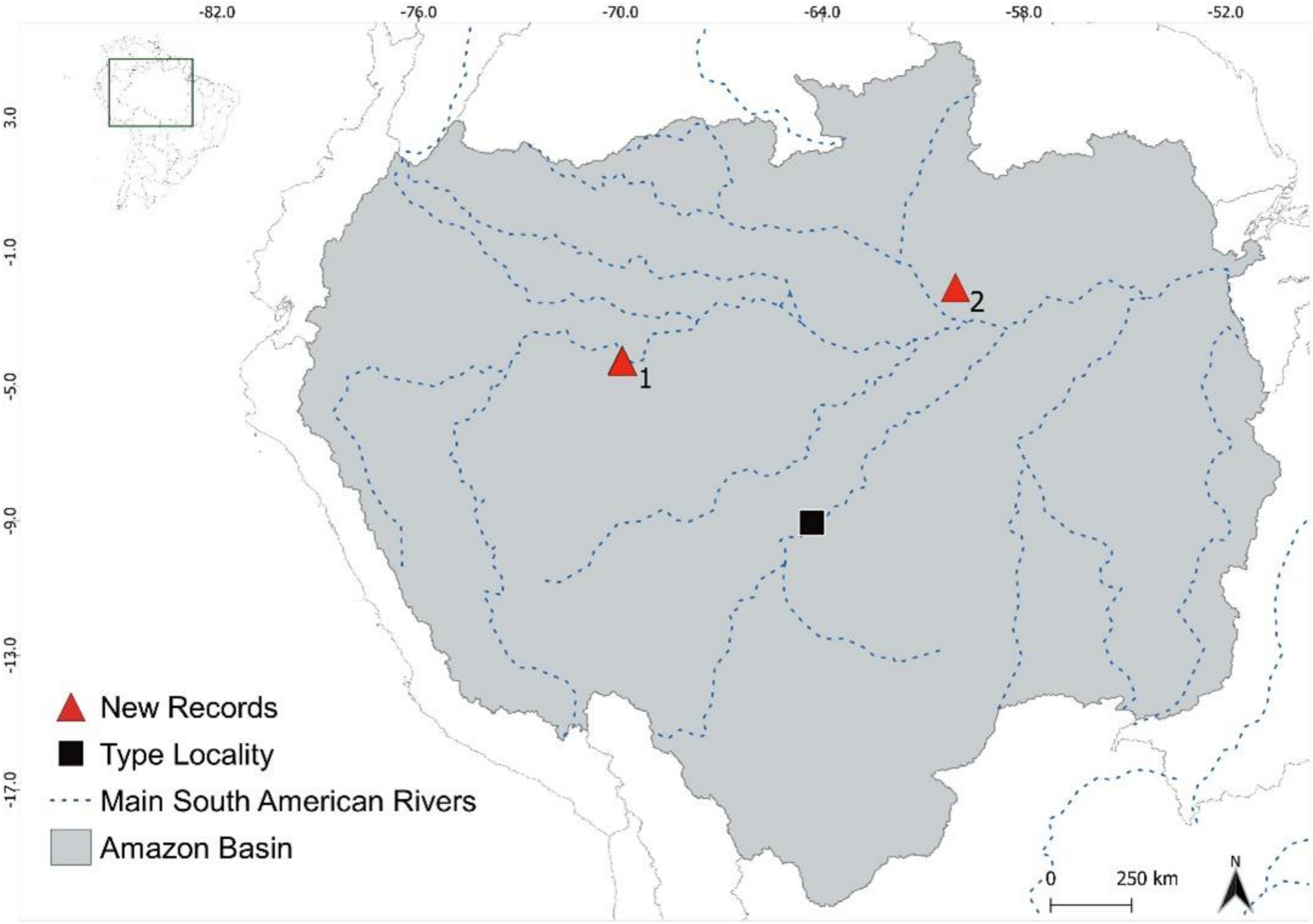

Figure 1. Map showing the new records and type-locality of Helicops acangussu

Description of the new population of Leticia - Tabatinga

(Figure 2)

We analysed a sample of 32 specimens of *Helicops acangussu* identified as *H. leopardinus* from the Amazonian region of Letícia and Tabatinga municipalities (Colombia and Brazil countries, respectively). The following characteristics obtained from this dataset are scale rows usually in 19/19/17 series (n=28), 21/19/17 (n = 2), and rarely 20/20/17 (n = 1) or 19/19/16 (n = 1); single preocular (n = 31), rarely two (n = 1); two postoculars (n = 30) and three in an asymmetric specimen (n=1); temporal formula usually 2+3 (n = 24), rarely 2+2 (n=1) and 1+3 (n=1), 3/2+3 (n=4) 2+2/3 (n=1), 1/2+3 (n=1) in asymmetric specimens; supralabials eight (n = 32); fourth supralabial usually contacting orbit; (n = 32); pairs of chinshields (n = 32); infralabials usually 10 (n = 28) rarely 11 (n = 2), 10/11 and 12/11 in two asymmetric specimens; first to fifth infralabials contacting anterior pair of chinshields (n = 26), first to fourth and first to sixth in four and two asymmetric specimens respectively; fifth and sixth infralabials contacting posterior pair of chinshields (n =27) or rarely just fifth (n= 3) or just sixth (n = 1), sixth and seventh in a asymmetric specimen; posterior separated by one pair of intergenial scales (n = 32); 126–133 ventral scales in females (n = 22), and 122–130 ventral scales in males (n = 9); 42–65 subcaudals in females (n = 22) and 56–72 subcaudals in males (n = 9); cloacal plate always divided (n=32); subcaudals divided (throughout the tail) (n = 32); nasal semidivided and separated by internasal scales. The largest specimen was a female with 620 mm and a smallest juvenile also a female with 110 mm; eye as the same size as its distance to oral border (= eye–mouth distance) (n = 32); internasal wider than long, triangular in shape (n = 32), touching the rostral (n = 32), loreal as long as wide (n = 32) dorsum and tail covered by five rows of circular darker spots (n = 32); dorsal spots 2–3 scales long (n = 30), rarely six when two rows of spots are connected (n=2); 47–57 dorsal spots in females (n= 22) and 48–60 in males (n=9); whitish belly with alternating and irregular bars (n=32); ventral bars touch the second scales row (n=32); head dorsum brownish with black pigments forming an arrow-like design, tip of head with a light snout spot slightly triangular in shape (n= 32); supralabials brownish with darker pigments more restricted to the sutures (=28) or homogeneous brownish (n=4); infralabials whitish with brownish pigments more restricted to the sutures; snout showing a triangular shaped light spot (=32).

We also found a male from Presidente Prudente municipality, Amazonas state, Brazil, identified as *Helicops hagmanni* (INPA-H 31415, Figure 3). The individual agrees with most of the features reported to the species, including the new populations described herein. However, it is the only one specimen with scales rows 21/21/17 series; lowest number of ventral scales recorded (=117); darker spots of bigger size corresponding to 3,5 – 4 scales long, and spots in smallest number along the body (=29).

Remarks

The type series showed ventral scales in females 124–129 and ventral in males 121–128, subcaudals in females 40–43 and subcaudals in males 52–67, dorsal spots on trunk in females 36–42 and 35–47 in males (see Moraes-da-Silva et al. 2022). Herein, we expanded the variation of these traits, reporting the maximum number of ventral scales in females as 133 scales and the range of ventral scales in males is 117–130; the maximum number of subcaudals in females is 65, and the maximum in males is 72; the range of dorsal spots on trunk in females is 47–57 and 29 to 60 in males.

Figure 2. *Helicops acangussu,* from Leticia, Amazonas, Colombia, photographed in life by Dario Alarcón.

Figure 3. Male specimen of *Helicops acangussu* (INPA 31425) from Presidente Prudente, Amazonas, Brazil.

Skull morphology (n=2):

Figure 4

Premaxilla triangular showing two transversally processes expanded and one dorsally expanded at the base, which comes close to the medial region of the nasal; the premaxilla has two sagittal projections near the beginning of the vomer; nasal dorsally triangular with a ventral and caudal projection, the latter below the frontal following and between vomer projections; the septomaxilla and vomer are tightly integrated; septomaxilla has two processes; the first one is dorsally closer to the premaxilla, whereas the second is ventrally at the side of the nasal projections; ventral view of vomer triangular, with anterior part wider and posterior part elongated; frontal is square in dorsal view, touching the prefrontals on the sides of the edges; parietal comprises the location of the brain, in front it wraps the orbits and touches half of the post-orbitals on each side, which has a rounded bottom medially touching the supraoccipital; parietal is rounded and touches the bottom of the prefrontal; the prefrontal is arched with the part that touches the frontal thinner in dorsal view; parietal has a triangular dorsal transverse ridge and a rounded edge behind the post-orbital; the orbital post is arched with the upper half touching the parietal, and with a caudal projection; prootics are irregular with two foramina on the ventral surface in lateral view and one at the end where the exoccipital is shaved; in ventral view, prootics show the anterior half covered by the basisphenoid plate and the posterior half is hidden by the basioccipital plate; supraoccipital touches the parietal end with a sagittal crest and a circular transverse crest; supratemporal elongated, touching the square bone at the height of the first vertebra (Atlas) and fitting into the top of the prootics where the prootics attach to the parietal; exoccipital irregular touching the supraoccipital dorsally, laterally the prootic and ventrally the basioccipital, demarcating the foramen with the prootic; quadrate broad and dorsoventrally flattened; maxilla laterally arched with an inward triangular projection; ectopterygoid convex and ventrally flattened, attached by its widest portion to the maxilla at the height of the last teeth and attached to the medial portion of the pterygoid; palatine with three rows of teeth and two lateralized projections are touched in the final portion by the pterygoid; dentary elongated, reaching halfway through the compound bone, with a curvature towards the inside of the skull; splenial connected to dentary frontally and to angular caudally; angular bone has a triangular shape in lateral view and is connected to the compound bone, which is long and has a dorsal crest and an inward projection.

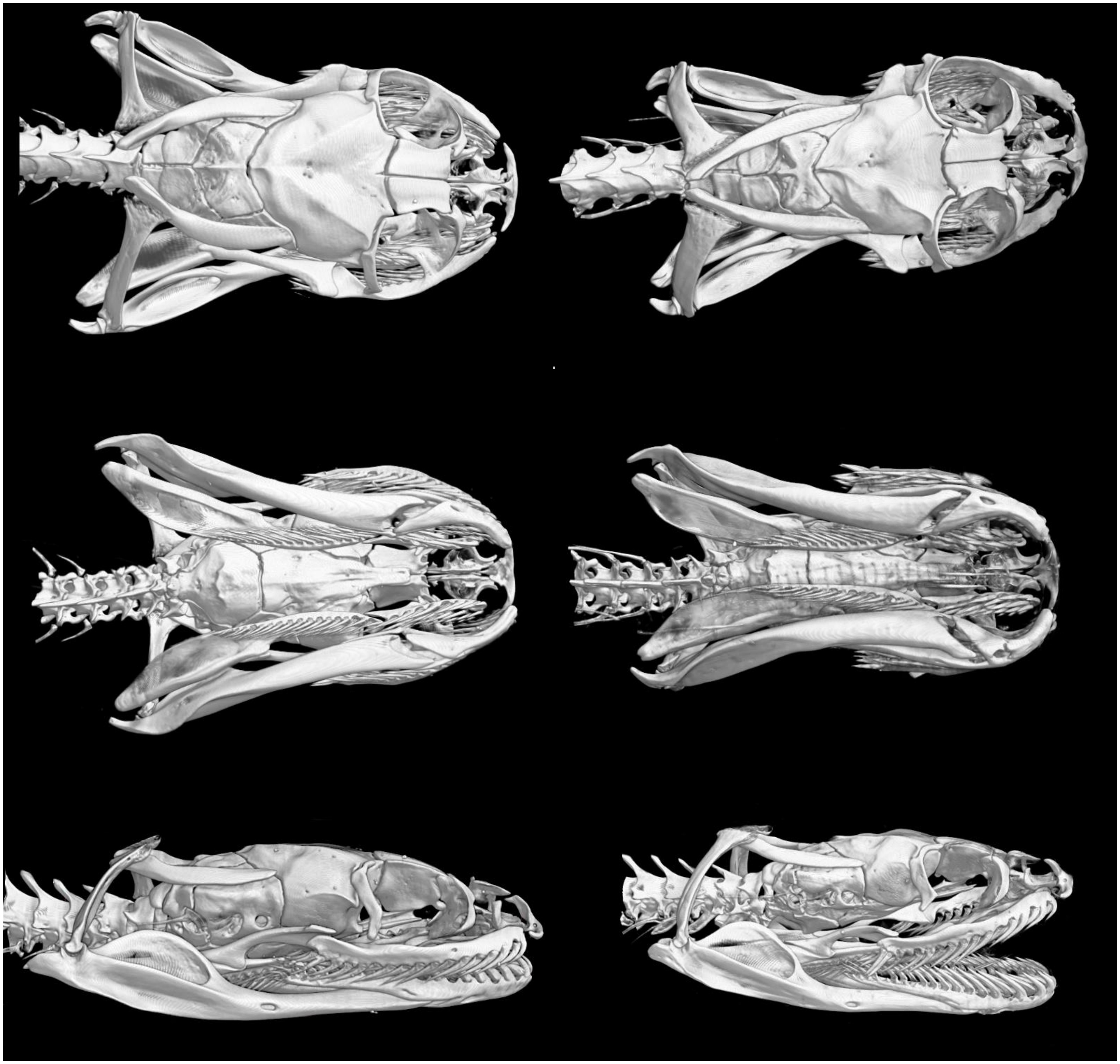

Figure 4. Dorsal (A), ventral (B) and lateral (C) view of the skull of INPA 31415. Dorsal (D), ventral (E) and lateral (F) view of the skull of MPEG 25586.

Hemipenial morphology (n=3):

Figure 5

Most of the hemipenial morphology observed in the organs of SINCHI 01025 (right) and SINCHI 01026 (right) agrees with mostly features described by Moraes-da-Silva et al. (2022). However, we found that none of the three organs showed lobes significantly asymmetrical, and we do not found rows of papillae at apex of lobes, as presented in the original description (see Moraes-da-Silva et al. 2022). The total length of SINCHI 01025 is 28.10 mm, and the width, measured at the hemipenial body and before capitation, is 10.4 mm; the mean length of the lobes is 10.8 mm, corresponding to 38% of the total length of the organ; The total length of SINCHI 01026 is 24.5 mm, and the width, measured at hemipenial body and before capitation, is 5 mm, the mean length of the lobes is 7.5 mm, corresponding to 30% of the total size of the organ. The sulcus spermaticus bifurcates at the first third of the hemipenial body in both organs.

The hemipenis of individual INPA 31415 is 16 mm in total length. Due to the imprecision of the lobe separation region, we did not measure this structure. The hemipenis of specimen INPA 31415 (right) showed more varied characteristics when compared to the species description (Figure 6). The following morphology was observed, the distinction between lobes and hemipenis body is unclear (capitation not detected); the central area of asulcate face is covered mostly with spinules that invades the base of the lobes; the base of the lobes at asulcate face are surrounded by three small spinules rows followed by two rows of papillae (the last two near the apex) (Figure 7A); irregular rows of small spinules are in the lobes and body of the hemipenis, with the exception of the base, which has the middle covered by very small spicules and large spines arranged laterally; the sulcate face has large spines arranged laterally on the basis, with evident spinules surrounding the spermatic sulcus and the intrasulcar region; the rows of spinules encircle the both lobes, changing to rows of papillae at the lobes apex.

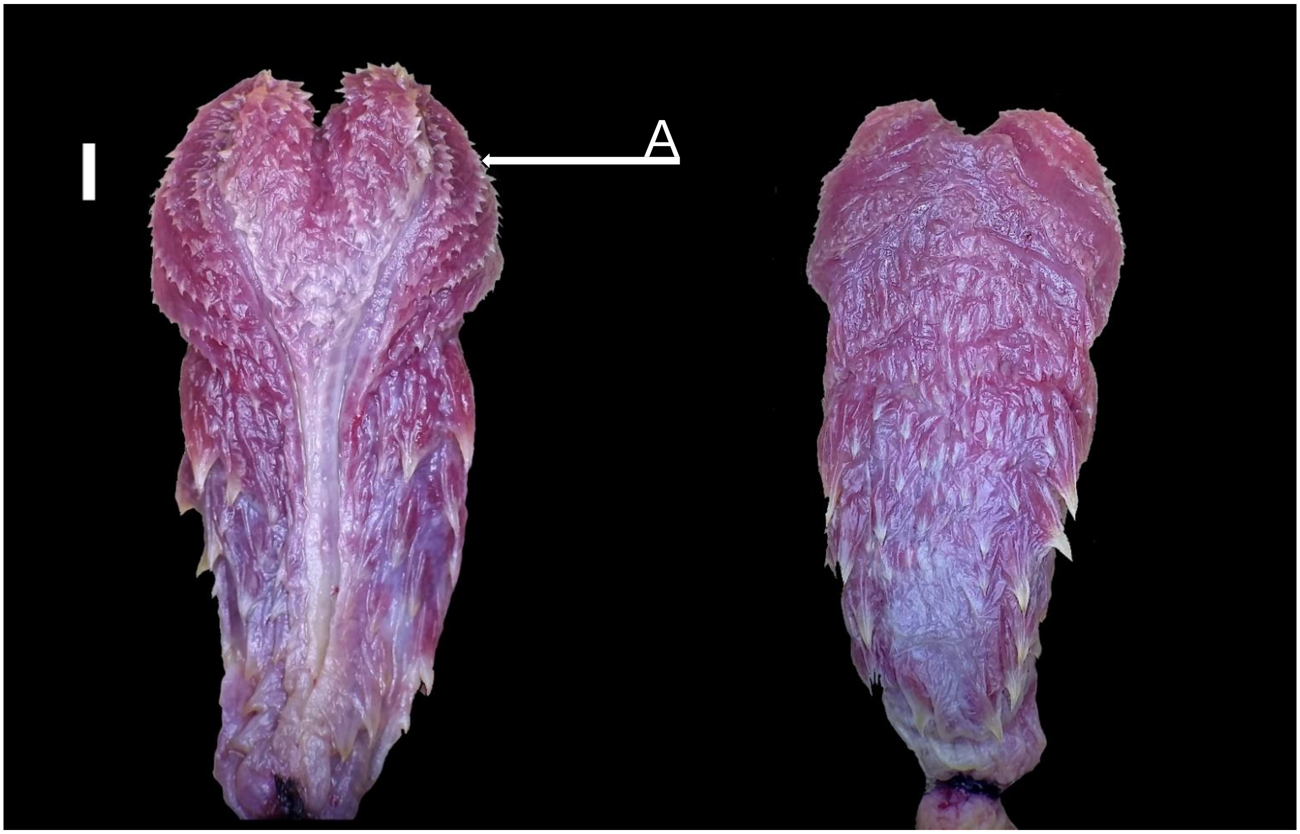

Figure 5. Sulcate and assucalte faces, respectively, of the right hemipenis of specimen SINCHI-R 1025. A – spinules at lobe apex.

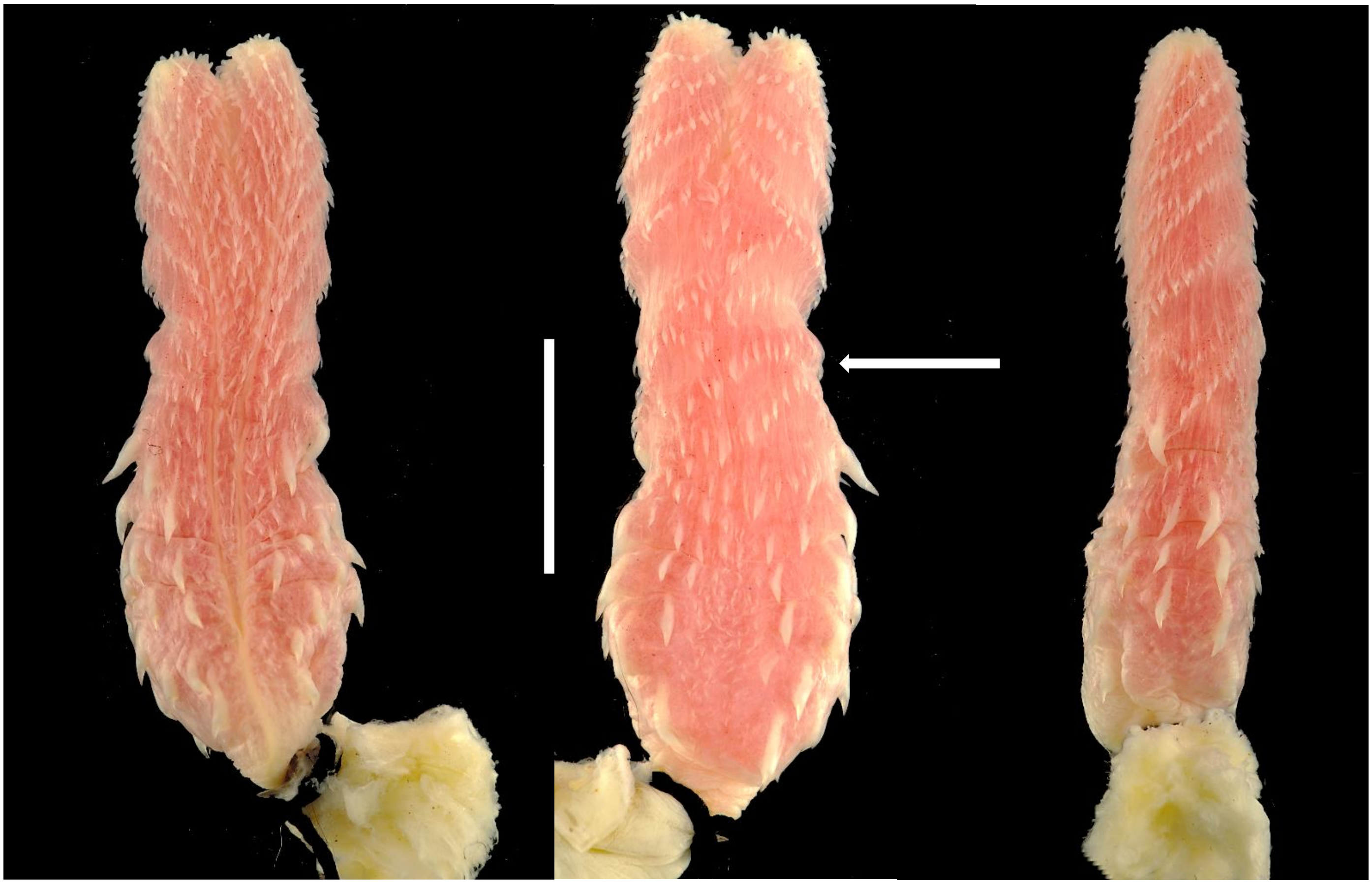

Figure 6. Sulcate, asulcate and lateral faces, respectively, of the right hemipenis of specimen INPA 31415. A - irregular rows of small spinules are in body of the hemipenis and imprecision of the lobe separation region.

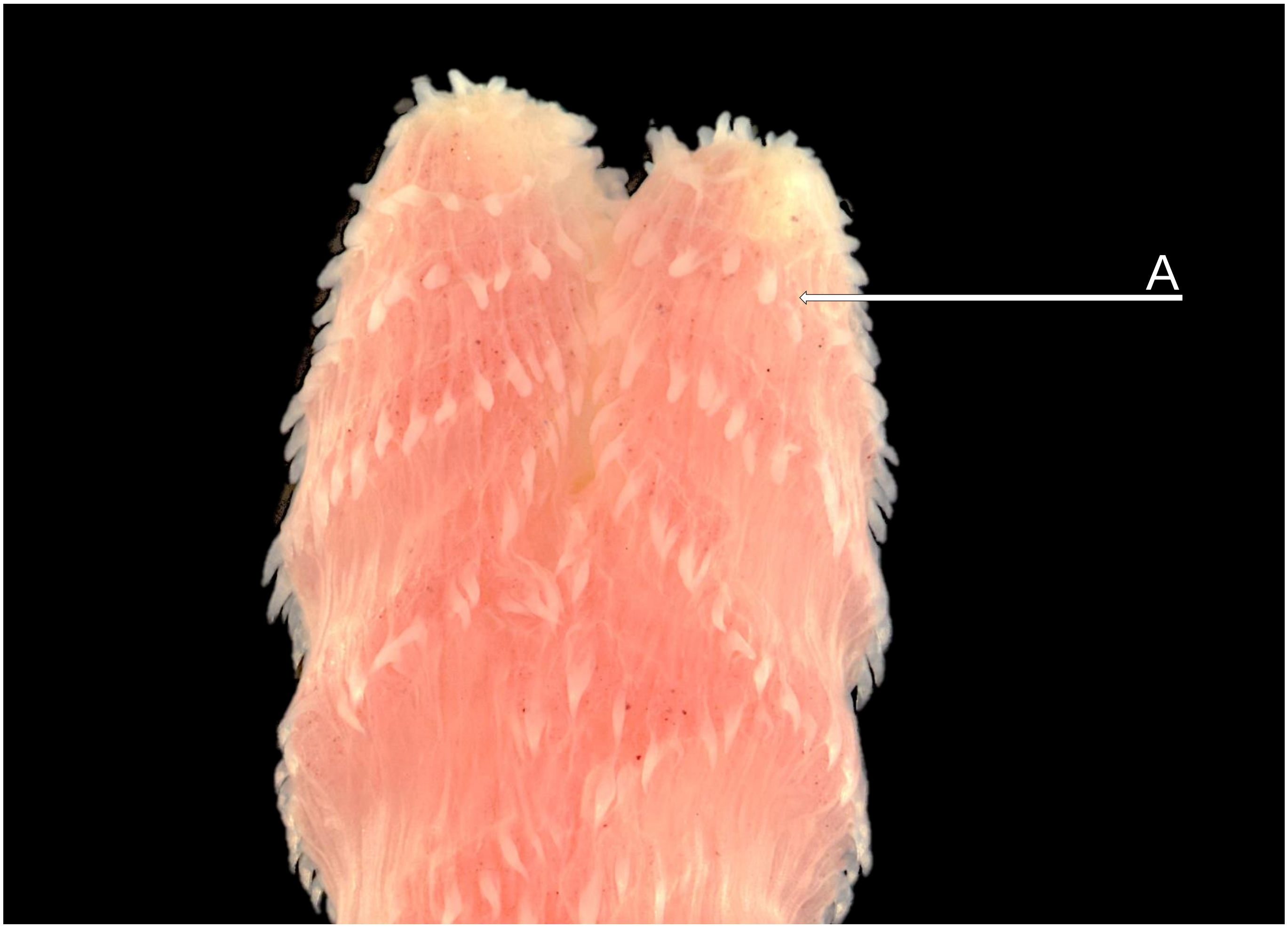

Figure 7. Lobe view of the right hemipenis of specimen INPA 31415. A - rows of papillae near the apex.

Key to the Amazonian species of *Helicops*:

1. Dorsal pattern uniform, striped or banded ………………………………...……....….. 2

Dorsal pattern composed of rounded spots (spotted-patterned) …………….………....3

2. Dorsal pattern uniform or striped ……………………………………….………..……4

Dorsal pattern banded ………………………………………………………………… 5

3. Dorsal scales at midbody equal or more than 23 rows …………………….…......…..6

Dorsal scales at midbody in 19 or 21 rows …...…………………….……………….... 7

4. Dorsal pattern uniform, white belly showing black spots forming a checkered pattern, dorsal scales at midbody in 19 rows and ventral scales between 118 and 123 ……...…………………………………………………......….***Helicops tapajonicus****.*

Dorsal pattern with light stripes, a whitish venter with black semilunar markings, dorsal scales at midbody in 21 rows and ventral scales between 116 and 125……………………………………………………………***Helicops trivitattus****.*

5. Dorsal pattern banded, a whitish venter with darker blotches, dorsal scales at midbody in 19-21 rows, ventral scales between 118–127 in males and 124–132 in females (restricted to Amazon southeastern) …...………………….…***Helicops apiaka***.

Dorsal pattern banded, sometimes polychromatic pattern, dorsal scales at midbody in 19 rows, ventral scales between 105–123 in males and 109–123 in females……………………………….………...……...…***Helicops angulatus.***

(a species complex following Murphy et al 2020).

6. Venter predominantly black with some white spots, dorsal scales at midbody 23 or 25 rows ……………………………………………………... ***Helicops polylepis****.*

Belly whitish or brownish with regular or irregular dark bands ……………...……. 9

7. Dorsal scales rows 21 at midbody (sometimes 23) scales rows, ventral bands medially interrupted, distribution known only in Ecuadorian Andean foothills………………………………………………………..***Helicops petersi***.

Dorsal scales at midbody in 19 or 21 scales rows, ventral with entire dark bands..…………………………………………...………..…………………8

8. Background of dorsal pattern grayish or brownish with five rows of dark spots, intergenial scales present, a snout light spot present……….. ***Helicops acangussu****.*

Background of dorsal pattern grayish, olive or brownish with four or five rows of dark spots, intergenial scales absent. …..…………..……… ***Helicops leopardinus*.**

9. Nasal semidivided …………………………...…………………………………………10

Nasal entire, larger tail (subcaudal scales between 85–96), dorsal scales rows at midbody 25–27 (western Amazon)..……………………………….… ***Helicops yacu.***

10. Larger tail (> 67 subcaudal scales) ……………………………………….……..11

Short tail (46–57 subcaudals in females and 54–67 subcaudals in males), dorsal scales at midbody in 23, 25, 27 or 29, brownish gular region ending before the beginning of the ventral scales, first rounded circular spots generally with a white border....………………………………………...…………***Helicops hagmanni.***

11. Dorsal scales at midbody in 21 (sometimes 23) scales rows, ventral bands medially interrupted, subcaudals scales between 67–91, distribution known only Andean foothills of Ecuador ....***Helicops petersi.***

Dorsal scales at midbody in 23 or 25 scales rows, subcaudals scales between 77–117, ventral bands touching dorsal scales in both sides…….…***Helicops pastazae*.**

Discussion

In the present study, we have expanded the knowledge on the morphology and distribution of the recently described species *Helicops acangussu.* We also provided the first data on cranial morphology and a key to clarify the identification of *Helicops* species distributed in the Amazon biome.

Moraes-da-Silva et al. (2022) suggested that *H. acangussu* are endemic to the left bank of the Madeira River and that large rivers may be essential for provide vicarious speciation event in *Helicops*. Herein, we refute the hypothesis that Madeira River act as a barrier for *H. acangussu* speciation since two new populations were found far from the type-locality. On the other hand, the formation of wide-scale hydrographic basins may have acted as vicarious events for others watersnake species, such as *H. boitata* in Paraguay Basin (Moraes-da-Silva et al., 2019) and *H. phantasma* in Tocantins-Araguaia basin (Moraes-da-Silva et al., 2021).

Some species of *Helicops* showing spotted-patterned are poorly represented in scientific collections, including records reported for remote locations (e.g., *H. yacu* and *H. petersi*) (Rossman and Dixon 1975; Rossman 1976; Rossman and Abe, 1979). Moreover, most areas of the Amazon biome are poorly sampled for snakes, and many of these places are rapidly disappearing, especially in the so-called “arc of deforestation” (Santos et al., 2021), where several processes related to habitat loss, such as deforestation, hydropower dams, and roads, are increasing in the recent decades (Dos Santos et al., 2021).

Despite the external morphological similarity, the group of spotted-patterned *Helicops* is likely an artificial cluster (Moraes da Silva et al., 2021). In this case, more data on skull morphology from other species and a molecular approach (including *H. acangussu*) may clarify kinship relationships and speciation events. Ours results showed that *H. acangussu* is sympatric not only with three spotted-patterned (*Helicops leopardinus*, *H. hagmannii*, and *H. polylepis*), as pointed by Moraes-da-Silva et al. (2022), but also with *H. yacu* and *H.* pastazae, and probably it may occur in Amazonian regions of Ecuador, Peru and Venezuela. Extensive sampling efforts carried out in these unrecorded regions, will be able to see all intraspecific variation

Finally, the asymmetry of the lobes described by Moraes-da-Silva et al., 2022 is probably an artifact of preparation, as we confirmed symmetry in three individuals. However, we cannot discredit the possibility of intraspecific variation since we also detected variation of hemipenial morphology in individuals of Presidente Prudente (Brazil) and Leticia (Colombia). Intraspecific variation in internal morphology of *Helicops* species where previously reported (Rossman, 1976), but ecological and/or evolutive pressures underlying these variations remains poorly studied.

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APPENDIX I.

Specimens examined (*skull; †hemipenis)

Brazil: AMAZONAS: Tabatinga (SINCHI-R 1023); Presidente Prudente (INPA 31415*†); RONDÔNIA: Porto Velho (MPEG 25586*); Colombia: AMAZONAS: Leticia (SINCHI-R 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1024, 1025†, 1026†, 1027, 1028, 1029, 2823, 3169, 3170).

Perspectivas Futuras

A hipótese filogenética baseada em folidose, oesteologia e morfologia hemipeniana de Nunes (2006) confirma a seção “*polylepis*” proposta por Rossman (1976), com exceção de *He. yacu*, para a qual não havia material disponível. Entretanto, o agrupamento foi recuperado como politomia, mantendo ainda desconhecidas as relações entre os táxons. As suspeitas a respeito da incerteza das relações e até mesmo sobre a complexidade taxonômica de algumas dessas espécies são recorrentes na literatura (Rossman 1976; Rossman e Abe, 1979; Nunes, 2006; Moraes-da-Silva et al., 2022).

Outro resultado relevante de Nunes (2006) é que *Helicops hagmanni* é recuperada como grupo-irmão de *Helicops trivittatus* (Nunes, 2006). Porém, no texto de descrição de *He. boitata*, Moraes-da-Silva e colaboradores (2019) propõem uma filogenia onde *Helicops hagmanni* é recuperada como grupo-irmão de *Helicops polylepis*. Na árvore apresentada na descrição de *Helicops phantasma*, *Helicops hagmanni* é novamente recuperada como proximamente relacionada a *Helicops polylepis* (Moraes-da-Silva et al., 2021). Já os posicionamentos de *Helicops acangussu* e *Helicops danieli* ainda são completamente obscuros.

Para a tomada de decisões taxonômicas futuras é estratégico um estudo morfológico descritivo com todas as espécies de *Helicops* com padrão de manchas dorsais circulares e de distribuição no norte da América do Sul (i.e., *Helicops petersi*, *Helicops polylpeis* e *Helicops yacu* (=seção *polylepis*), além de *Helicops scalaris.* Isso inclui a análise do maior número possível de exemplares, bem como visitas a coleções herpetológicas da Bolívia, Equador, Peru e Venezuela. A variação morfológica intraespecífica, i.e., policromatismo e polimorfismo, tem sido alta em *Helicops* (Murphy et al., 2020; Moraes-da-Silva, 2022; capítulo II do presente trabalho). Somente após a identificação inequívoca das espécies será possível o desenvolvimento de estudos que se proponham a explicar a evolução da morfologia externa e interna do grupo e putativas adaptações ao ambiente aquático.

A elaboração de expedições de campo em áreas amazônicas poucos amostradas para encontro de exemplares vivos e a coleta de amostras de tecidos é fundamental para auxiliar na compreensão da história evolutiva do grupo e das relações de parentesco.

Também é necessária a identificação dos padrões e processos que levaram à especiação em *Helicops*, incluindo a datação desses eventos. À exemplo de outros Squamata (e.g. Rittmeyer & Austin, 2015), o desenvolvimento de uma análise integrativa (morfológica e molecular), pode contribuir no esclarecimento das incertezas relativas às variabilidades intra e interespecíficas em *Helicops*. Considerando que filogenia e taxonomia contribuem para o reconhecimento da distribuição geográfica de espécies (e.g. Hamdan et al., 2017), uma nova percepção acerca da biogeografia regional e das áreas de endemismos poderá ser proposta.

O desenvolvimento de estudos mais amplos com *Helicops* poderá trazer novas descobertas sobre a evolução das bacias hidrográficas da América do Sul, onde o entendimento dos padrões de distribuição das espécies exerceria um papel relevante nesses estudos. Isso pode ser alcançado com a construção de modelos de distribuição potencial que projetem cenários para o passado, e.g., aspectos biogeográficos da América do Sul que ampliaram e/ou retrariam áreas de distribuição das espécies, e futuro, e.g., mudanças climáticas e persistência das espécies mediante impactos ambientais.

Conclusões

I – *Helicops danieli* é uma espécie de diagnose inequívoca, endêmica da região transandina da Colômbia. Sua distribuição se dá majoritariamente em áreas do país com maior nível de impacto antrópico. Do ponto de vista morfológico, é um táxon que apresenta crescimento alométrico (i.e., maior crescimento da cauda em jovens que em adultos) e variação alométrica de coloração (jovens com colar nucal branco que é perdido na fase adulta) e dimorfismo sexual, sendo os machos com maiores caudas e as fêmeas maiores em comprimento rostro cloacal (Capítulo I).

II – *Helicops hagmanni* é a espécie do gênero *Helicops* com maior polimorfismo no número de fileiras de escamas dorsais conhecida até o momento (entre 23 e 29). Sua distribuição é exclusivamente amazônica e quatro caracteres de sua morfologia apresentam variação clinal. Três deles (número de escamas dorsais, número de escamas ventrais e número de manchas paravertebrais) possuem a tendência de diminuição de contagens do leste ao oeste. Já o último remete ao padrão de coloração da lateral do corpo e região ventral, sendo os espécimes do leste com manchas paraventrais destacadas das barras ventrais e os do Oeste com a união dessas manchas. (Capítulo II).

III – *Helicops acangussu* é uma espécie ovípara de diagnose inequívoca, com padrão dorsal de manchas circulares e ampla distribuição amazônica. É uma espécie que apresenta variação intraespecífica na morfologia hemipeniana (Capítulo III e IV).

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Apêndice I

Canadian Science Publishing

ARTICLE

Taxonomy, allometry, sexual dimorphism, and conservation of the trans-Andean watersnake *Helicops danieli* Amaral, 1937 (Serpentes: Dipsadidae: Hydropsini)

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Abstract: The extensive lack of knowledge on the morphological aspects of South American watersnakes includes a poor understanding of phenotypic parameters, intraspecific variation, and conservation of the trans-Andean *Helicops* species, Daniel's Keelback (*Helicops danieli* Amaral, 1937). For the first time, we provide a multidisciplinary view using key features (e.g., morphology and niche modeling) to improve the taxonomic recognition of this species, as well as describing ontogenetic color changes, allometry, sexual dimorphism, and the conservation status of this poorly studied snake. First, we emended the morphological diagnosis of *H. danieli* with 23 characters and detected that juvenile tail length is positively related to allometric growth, and that juveniles differ from adults through the presence of the white nuchal collar. Females are larger than males for snout–vent length, whereas males showed proportionally longer tails and smaller head length growth. Suitable areas for *H. danieli* are restricted to the trans-Andean regions from the Magdalena drainage to the Caribbean coast, which also showed high values of anthropic impacts. Our multidisciplinary approach provided new insights into this South American watersnake's morphology, intraspecific variation, and distribution.

Key words: Daniel's Keelback, ecological niche modeling, *Helicops danieli*, linear morphometry, hemipenis morphology, intraspecific variation, ontogeny, taxonomy.

Résumé : Le vaste manque de connaissances sur les aspects morphologiques des couleuvres d'eau sud-américaines comprend une piètre compréhension de paramètres phénotypiques, de la variation intraspécifique et de la conservation d'une espèce transandine d'*Helicops* (*Helicops danieli* Amaral, 1937). Nous en présentons un premier portrait multidisciplinaire en utilisant des éléments clés (p. ex. morphologie et modélisation de la niche) pour améliorer l'identification taxonomique de l'espèce, en plus de décrire des changements de coloration ontogéniques, l'allométrie, le dimorphisme sexuel et l'état de conservation de ce serpent peu étudié. Nous modifions d'abord le diagnostic morphologique de *H. danieli* sur la base de 23 caractères et relevons que la longueur de la queue des spécimens juvéniles présente une relation positive avec la croissance allométrique et que les jeunes spécimens se distinguent des adultes par la présence d'un collet nucal blanc. Les femelles sont plus grandes que les mâles en ce qui concerne la longueur du museau à l'orifice anal, alors que les mâles ont des queues proportionnellement plus longues et une plus faible croissance de la longueur de la tête. Les secteurs qui conviennent à l'espèce se limitent aux régions transandines allant du bassin versant de Magdalena au littoral caribéen, qui présentent aussi de forts degrés d'impact de l'activité humaine. Notre approche multidisciplinaire fournit de nouveaux renseignements sur la morphologie, les variations intraspécifiques et l'aire de répartition de cette couleuvre d'eau sud-américaine. [Traduit par la Rédaction]

Mots-clés : modélisation de la niche écologique, *Helicops danieli*, morphométrie linéaire, morphologie de l'hémipénis, variation intraspécifique, ontogenèse, taxonomie.

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Introduction

In general, phenotypic traits are responses to evolutionary forces that drive adaptations to different niches or environmental constraints on nature, thus producing a myriad of morphologies and lifestyles (e.g., Pizzatto et al. 2007; Cicero et al. 2020). Snakes are very interesting models for evolutionary biology studies when considering their unique baupläne (Brandley et al. 2008), successful performance as predators (e.g., Pizzatto et al. 2009; Whitford et al. 2019), and their ability to use several, and sometimes extreme, habitats (e.g., Segura et al. 2007; Rasmussen et al. 2011; Fabre et al. 2016).

As with other vertebrates, the morphological patterns of snakes can be explained both by phylogenetic inertia (Westeen et al. 2020) and by ecological pressures (Klaczko et al. 2016). Some phenomena are usually understood as a result of intraspecific variability (sensu Klingenberg 2005; Murta-Fonseca and Fernandes 2016), such as sexual and ontogenetic differences — including allometry — between certain characteristics (e.g., Shine 1994*a*; Murta-Fonseca and Fernandes 2016; Tamagnini et al. 2018; Loebens et al. 2019; Abegg et al. 2020). On the other hand, intraspecific variation in snakes between sexes and ontogenetic trajectory show that subtle differences are selected by behavioral and ecological pressures, such as sexual selection, prey types, and habitat (or microhabitat) use. Within these intraspecific variations, there are some recurrent examples, such as greater body lengths of adult males that present male–male combat (Shine 1978, 1994*b*), different head shapes and sizes between males and females that depend on dietary preferences (Shine 1991; Vincent et al. 2004), more variable head sizes in adult males compared with females in semi-fossorial snakes (Abegg et al. 2020), and larger female aquatic snakes (Fitch 1981; Camargo et al. 2021; García-Cobos et al. 2020).

The genus *Helicops* Wagler, 1828 comprises 19 currently recognized species of watersnakes that are widely distributed across South America (Moraes-da-Silva et al. 2019, 2021; Nogueira et al. 2019). Despite their richness and relevance in aquatic ecosystems, there are deep gaps in the knowledge of their morphology, taxonomy, systematics, and conservation (Braz et al. 2016; Murphy et al. 2020; Moraes-da-Silva et al. 2021).

Amaral (1937) described Daniel's Keelback (*Helicops danieli* Amaral, 1937) based on a single specimen from Carare (= Carare River), Colombia, and proposed that the new taxon is related to another two species, the Mountain Keelback (*Helicops angulatus* (Linnaeus, 1758)) and the Ladder Keelback (*Helicops scalaris* Jan, 1865). Years later, Yuki (1994) examined two more specimens (one female and one male) and briefly described the species' hemipenis. Posteriorly, Rossman (2002*a*) examined 44 specimens of *H. danieli* and characterized the species as having dorsal scales in a 19/19/17 series; between 125–135 ventral scales in males and 130–141 ventral scales in females; between 76–86 subcaudal scales in males and 61–70 subcaudal scales in females; a spotted dorsum; and a venter with semilunar marks arranged in interrupted lines over a clear background. Currently, the species is known to be endemic to Colombia, occurring in seven country regions throughout lowlands and moderately elevated uplands, ranging from the Magdalena drainage to the Caribbean coast (Rossman 2002*a*).

Helicops danieli is the only congener that is endemic to Colombian coastal forests and is restricted to the trans-Andean region. This species has been poorly studied in terms of its morphological, anatomical, and conservation aspects (e.g., Braz et al. 2016, 2018; Moraes-da-Silva et al. 2019, 2021). The distribution of *H. danieli* has also been misunderstood, appearing only in Colombian species lists (Daniel 1949), and regional inventories (e.g., Medina-Rangel 2013; Ruíz 2014; Angarita-M. et al. 2015; Lynch 2015), with a dubious cis-Andean record in Vaupés, Colombia (Yuki and Castaño 1998). Despite the taxonomic studies developed by Yuki (1994) and Rossman (2002*a*), the diagnosis of *H. danieli* is still in its initial stages, especially considering the advances made in the taxonomic gaps and geographical ranges of the genus in the last 10 years (thus reducing the Linnean and Wallace shortfalls for South American watersnakes) (Kawashita-Ribeiro et al. 2013; Costa et al. 2016; Moraes-da-Silva et al. 2019, 2021).

Herein, we are interested in (*i*) providing new data on the taxonomy of the species and emending the *H. danieli* diagnosis; (*ii*) evaluating if the presence of the white nuchal collar varies according to age; (*iii*) testing for ontogenetic and static allometry; (*iv*) testing if *H. danieli* has sexual dimorphism, i.e., presenting females that are larger than males, which is a common pattern of other watersnakes; and (*v*) revising the distribution and conservation aspects of this enigmatic species.

Materials and methods

Sampling and morphological characteristics

We examined 124 specimens of *H. danieli* housed in the following institutions: Instituto Butantan (IBSP), São Paulo, São Paulo, Brazil; Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH), Villa de Leyva, Boyacá, Colombia; Museo de Historia Natural, Universidad de La Salle (MLS), Bogotá, Cundinamarca, Colombia; Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, São Paulo, Brazil; Museo Javeriano de Historia Natural "Lorenzo Uribe, S.J." (MPUJ), Pontificia Universidad Javeriana, Bogotá, Cundinamarca, Colombia; Universidad de Los Andes (UNIANDES-R), Bogotá, Cundinamarca, Colombia; Universidad Industrial de Santander (UIS-R), Bucaramanga, Santander, Colombia; and Instituto de Ciencias Naturales (ICN-R), Universidad Nacional da Colombia, Bogotá, Cundinamarca, Colombia. Institutional abbreviations follow Sabaj (2020). A complete list of specimens is provided in Supplementary Table S1.[1]

We followed Dowling (1951) and Moraes-da-Silva et al. (2019) for meristic and pholidosis characters: data on the congeners with trans-Andean records compared with *H. danieli* was acquired from Rossman (2002*b*) for *H. scalaris* and from Rossman (1976) for Shreve's Keelback (*Helicops pastazae* Shreve, 1934).

We followed Moraes-da-Silva et al. (2019) for the measurements of the following morphometric characters: snout–vent length (SVL), tail length (TL), eye diameter (ED), head length (HL), head width (HW), and snout length (SL) (= eye–nostril distance). We also measured eye–mouth distance (EM), from the upper tip to the lower border of the eye; head height (HH), from the medial portion of parietal scales to medial portion of chinshields (= the highest point of the head); and interocular length (IL) between the outer border of the supraocular scales. We took all measurements using a digital caliper to the nearest ±0.01 mm, except for SVL and TL measurements, where we used a flexible ruler to the nearest millimetre. The sex of individuals with no everted hemipenis was identified through an incision at the base of the tail to detect the presence of hemipenes and retractor muscles. To compare our results about dimorphism with other species of the genus, we compiled data from the literature for the morphometric traits SVL, HL, and TL.

To complement the description of the hemipenis of *H. danieli* presented by Yuki (1994), we extracted, everted, and prepared the hemipenis of the preserved specimen AMNH 97461 following Pesantes (1994) and Zaher and Prudente (2003). The fully everted hemipenis was filled with petroleum jelly to ease the visualization of the ornamentation structures, and we followed the terminology of Zaher (1999) for the description. The organ was photographed, manually drawn, and digitized. All morphological measurements were taken using the ImageJ software version 1.8.0 (Rasband 2016).

We evaluated the condition of the white nuchal collar (= juvenile collar) to test the putative relationship between collar conspicuity and age. For this, we defined three categories: (1) present, white

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[1] Supplementary table and figure are available with the article at https://doi.org/10.1139/cjz-2021-0073.

collar conspicuously visible and well defined; (2) smooth, white collar not clearly visible and poorly defined; and (3) absent, white collar absent or indistinct from the background dorsum.

Statistical analyses

It was not possible to use all the samples for all the analyses because some specimens were not sexed, had a broken tail, or had damage to the pholidosis. Therefore, we employed different datasets for each analytical approach: (i) the dataset used for the emended diagnosis and variation comprised 61 individuals (36 females and 25 males, of all ages); (ii) the dataset used for sexual dimorphism and static allometry analyses comprised only adults (32 females and 32 males); and (iii) the dataset used for ontogenetic allometry comprised 72 adults and 41 juveniles with indefinite sex. Since the analysis of ontogenetic allometry was aimed at identifying the differences between growth rates between ages, the potential sexual differences between juveniles were not considered.

Since the range of SVL related to sexual maturity among *Helicops* species is high, i.e., between 215 and 387 mm in males (Scartozzoni 2009),[2] and this information for *H. danieli* is still unknown, we employed a combined approach based on Rossman 2002*a* and our observations of the white nuchal collar, which is an associated age-specific characteristic (= ontogenetic), for age-class estimation were based on SVL. Rossman 2002*a* determines that *H. danieli* individuals with more than 230 mm are subadults and adults, and highlights the fact that the white nuchal collar may be a juvenile characteristic, fading with increasing SVL. Therefore, we used a Kruskal–Wallis test to estimate the effect of age on the presence of the white nuchal collar using SVL as a proxy for age class (see the Results section). Finally, to avoid the inclusion of subadults as adults in our analyses, we chose to use a conservative threshold, i.e., we only considered specimens with SVL of more than 250 mm to be adults.

Before performing the allometric analysis, we imputed 16 missing values (0.8% of the total dataset), to avoid substantial data loss by removing a complete set of observations for a given specimen, using the MissForest R package (Stekhoven and Buhlmann 2012). All morphometric variables were log-transformed to ensured data normality and homogeneity. Prior to the analysis, we test for data normality and homoscedasticity. Static and ontogenetic allometry were tested using the slope test function on Smatr R package (Warton et al. 2012). We estimated the allometric coefficient for each morphological trait, using the allometric equation $y = a + x^b$, where a is the intercept and b corresponds to the slope of the linear regression of the trait value against SVL. Slopes that are not statistically different from $b = 1$ indicate isometry, whereas values significantly higher or lower than $b = 1$ indicate positive or negative allometry, respectively (Warton et al. 2006; De-Lima et al. 2019). We employed ANCOVA tests to identify the differences between the regression values of adults versus juveniles and the sex regression values of males versus females (dimorphism). We also estimated an index of sexual dimorphism following Shine (1994*b*), which is expressed as positive when females are larger than males and negative when males are larger than females. All analyses and graphics were performed using R environment version 3.4.1 (R Core Team 2021).

Endemism and potential distribution modeling

We recorded all the geographical coordinates of *H. danieli* occurrences provided by the collections. For specimens without geographic coordinates (<30% of the total dataset), we georeferenced the specimen's records through a search by collection locations using Google Earth (see Supplementary Table S1).[1] We also converted all coordinates to decimal degrees using SAD69 projection and removed all duplicates. To identify the ecoregions where *H. danieli* occurs, we used the shapefile of World Ecoregions 2017 (Dinerstein 2017). Furthermore, to identify the anthropic influence values on the distribution of *H. danieli*, we used the Human Footprint (HFP) index. This index is based on a quantitative analysis of human influence across the globe, where 0 represents no impact and 50 is the maximum impact value (Venter et al. 2016*a*, 2016*b*). We extracted and calculated the median and modal values of the human footprint for each region with *H. danieli* occurrence.

To build distribution models, we downloaded 19 climate variables plus elevation, available on the WorldClim – Global Climate Data version 2.1 (https://www.worldclim.org), for the current period (between 1970 and 2000), with a resolution of 2.5 arc minutes (ca. 5 km × 5 km) (Fick and Hijmans 2017). We then performed a principal component analysis (PCA) and Pearson's correlation to choose a subset of variables that were not correlated ($r^2 < 0.7$). These analyses were performed using vegan (Oksanen et al. 2018), dismo (Hijmans et al. 2017), and rgdal packages (Bivand et al. 2019). We selected the variables according to the ecological niche parameters of *Helicops* (Ávila et al. 2006; Moraes-da-Silva et al. 2019, 2021; Murphy et al. 2020), such as temperature seasonality (BIO 04), annual temperature range (BIO 07), precipitation of wettest month (BIO 13), precipitation seasonality (BIO 15), and elevation (elev).

We used the maximum entropy algorithm "MaxEnt" (Phillips et al. 2006) to identify the potential distribution of *H. danieli*, considering its high performance and lower sensitivity to possible geographical positioning errors (Hijmans and Graham 2006; Fourcade et al. 2014; Runquist et al. 2019). Occurrences were randomly separated into 70% of all records for training and 30% for validating the model, with 100 bootstrap pseudo-replicates. To assess the model performance, we used the area under the receiver operating characteristic (ROC) curve (area under the curve (AUC)), where values closer to 1 indicate a better agreement between model outputs and the test occurrences.

Results

Taxonomy

Helicops danieli Amaral, 1937
(Figs. 1A–1D)

HOLOTYPE: Juvenile female, IBSP 9872, donated by Brother Daniel of the La Salle Catholic Order, collected on an unknown date, from Carare River, department of Santander, Colombia. The specimen was lost in the accidental fire that affected the Herpetological Collection "Alphonse Richard Hoge" of the Butantan Institute, on 15 May 2010 (F. Grazziotin, personal communication, to N. Citeli, 7 September 2020).

EMENDED DIAGNOSIS: *Helicops danieli* can be distinguished from all its congeners by the following unique combination of characters: (1) scale rows in series of 20–19/19/20–19–17; (2) preocular single; (3) postoculars two; (4) temporal formula 2+3; eight supralabials; (5) fourth supralabial usually contacting orbit; (6) two pairs of chinshields; (7) posterior chinshields in contact (= without intergenial scales); (8) 10 infralabials; (9) 130–145 ventral scales in females and 125–135 ventral scales in males; (10) 58–71 subcaudals in females and 73–86 subcaudals in males; (11) cloacal plate always divided; (12) subcaudals divided (throughout the tail); (13) keeled dorsal scales, but weakly in paraventral region; (14) eye bigger than its distance to oral border (1.4%); (15) internasal wider than long, triangular in shape touching the rostral; (16) nasals semi-divided; (17) dorsum and tail covered by four or five lines of circular spots; (18) dorsal spots 2–3 scales long; (19) posterior region of the head

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[2] R.R. Scartozzoni. 2009. Estratégias reprodutivas e ecologia alimentar de serpentes aquáticas da tribo Hydropsini (Dipsadidae, Xenodontinae). Unpublished Ph.D. thesis, Universidade de São Paulo, São Paulo, Brasil.

Fig. 1. Morphological variation of Daniel's Keelback (*Helicops danieli*): (A) uncollected specimen from Caucasia, Antioquia, Colombia, photographed in life; (B, C) dorsal and ventral views of the adult preserved specimen UIS-R 4464; (D, E) dorsal and ventral views of the juvenile preserved specimen IAvH 7757 showing a white nuchal collar. Photographs taken by, and reproduced with permission of, Hernan Martinez (A) and Nathalie Citeli (B–E). Color version online.

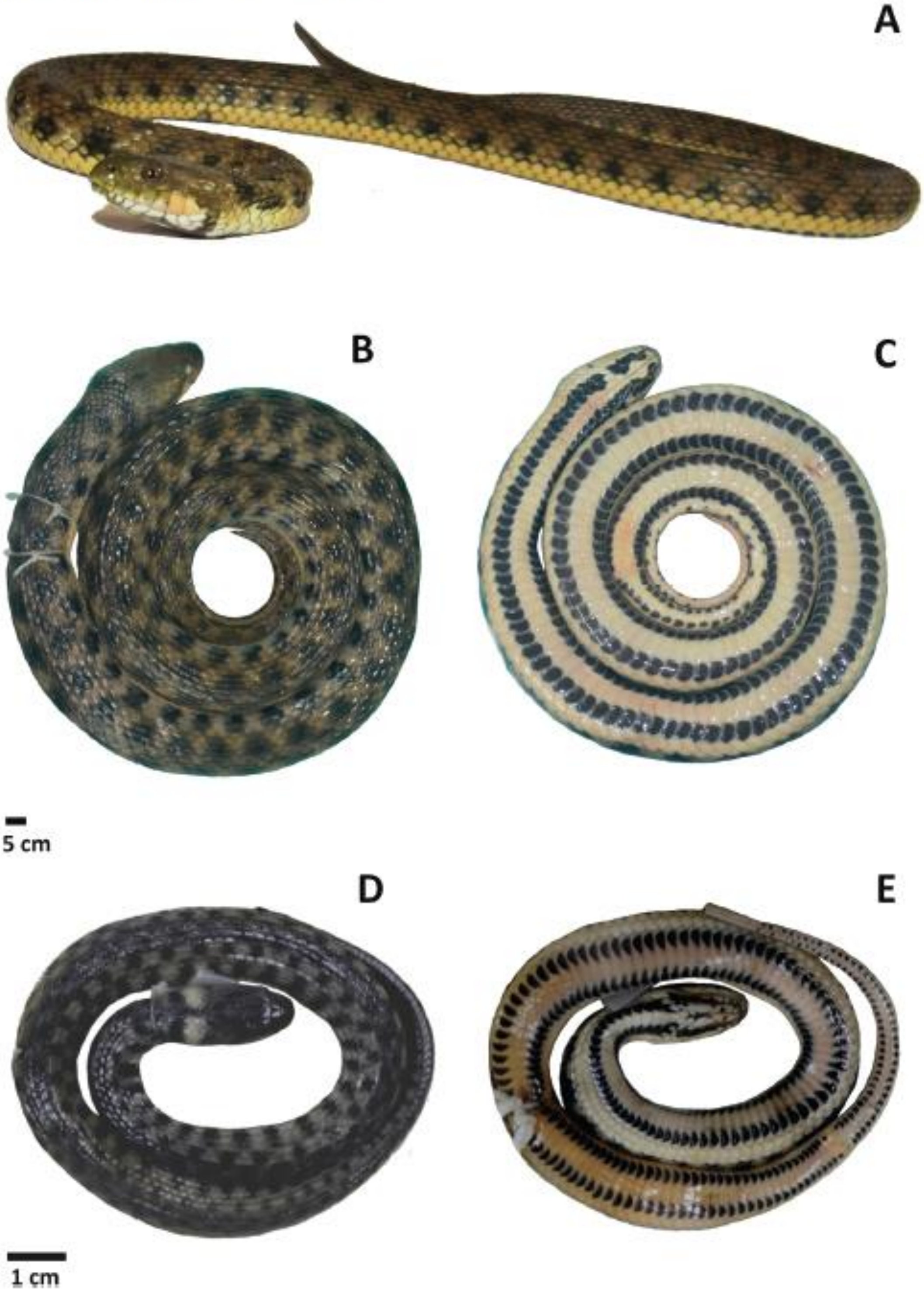
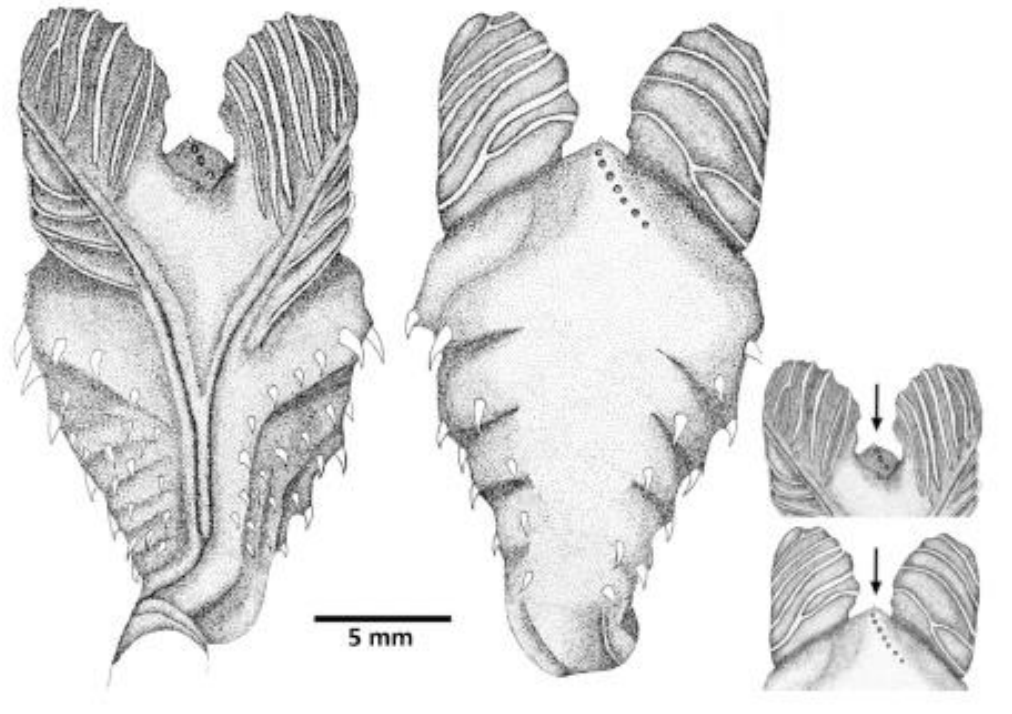


Fig. 2. Sulcate and asulcate faces, respectively, of the right hemipenis of specimen AMNH 97461 of Daniel's Keelback (*Helicops danieli*). Scale bar = 5.0 mm. In the inset, arrows point to the area between the lobes containing a longitudinal row of papillae at sulcate (top) and asulcate (bottom) faces.



with a stripe that starts from the junction of parietal scales to first dorsal spots, forming a dark nuchal band; (20) supralabials yellowish with the first ones (1–4) brownish; (21) whitish infralabials with brownish spots in the sutures and in the region of contact with chinshields; (22) yellowish-colored venter with two semilunar marks on each ventral scale; (23) whitish gular region with some brown speckled spots.

HEMIPENIS REDESCRIPTION: Hemipenis elongated, bilobed, bicalyculated, and semicapitated (Fig. 2); sulcate and asulcate faces surrounded by lateral spines that increase in size from basal region toward lobe apices; sulcate face shows 44 spines, arranged on each half of hemipenial body; intrasulcular region nude; asulcate face of hemipenial body nude, except for two longitudinal rows of lateralized spines, each composed of five to six spines; each lobe surrounded by seven to nine spiral rows of small papillae, and the area between the lobes with a longitudinal row of papillae; the total length of the organ is 22 mm, and the width, measured at hemipenial body and before capitation, is 12.34 mm; the mean length of the lobes is 5.5 mm, corresponding to 25% of the total length of the organ; the mean width of the lobes, measured in the middle of the structure, is 4.6 mm; sulcus spermaticus bifurcates at the first third of hemipenial body (3 mm from the base of the organ) and centrifugally oriented starting from the base of the organ, to median portion of sulcate face, lateral of the lobes and top of the lobes.

VARIATION: Scale rows usually in 19/19/17 series (n = 48, about 78.7%), 19/19/19 (n = 11; 18%), and rarely 20/19/17 (n = 1) or 20/19/20 (n = 1; less than 0.02%); single preocular (n = 60), rarely two (n = 1); two postoculars (n = 61); temporal formula usually 2+3 (n = 42), 2+2 (n = 15), and rarely 1+2 (n = 2); supralabials usually eight (n = 56) and rarely nine (n = 5); fourth supralabial usually contacting orbit (n = 57), rarely fourth and fifth (n = 2) or only the fifth (n = 2); two pairs of chinshields (n = 61); infralabials usually 10 (n = 50), 11 (n = 8), and rarely 9 (n = 1); first to fifth infralabials contacting anterior pair of chinshields (n = 57) or rarely first to fourth (n = 3); sixth to seventh infralabials contacting posterior pair of chinshields (n = 57) or rarely fifth to sixth (n = 3); posterior chinshields in medial contact (= without intergenial scales) (n = 61); 130–145 ventral scales in females (137.61 ± 3.08, n = 36), and 125–135 ventral scales in males (131.4 ± 3.34, n = 25); 58–71 subcaudals in females (64.52 ± 2.53, n = 24) and 73–86 subcaudals in males (77.05 ± 5.26, n = 18); cloacal plate always divided (n = 61); subcaudals divided (throughout the tail) (n = 61); total length of the smallest (juvenile) specimen is 217 mm (male) and of the largest specimen is 909 mm (female); eye is 1.4% bigger than its distance to oral border (= eye–mouth distance) (n = 55), rarely smaller (n = 6); internasal wider than long, triangular in shape (n = 60), touching the rostral (n = 51), or rarely separated by nasals (n = 9); loreal as long as wide (n = 61); nasals semi-divided (n = 61); dorsum and tail covered by four lines of circular spots (n = 40), sometimes five when a vertebral line of spots is present (n = 21); dorsal spots 2–3 scales long (n = 61); posterior region of the head with a stripe (starting from the junction of the parietal scales) and extends to first dorsal spots, forming a dark nuchal band (n = 60), a single specimen showed no stripe; white nuchal band evident in juveniles and almost absent or indistinct in adults (see ontogenetic and static allometry section); supralabials yellowish with the first (1–4) brownish (n = 42), or with speckled brownish spots on all scales (n = 19); whitish infralabials with brownish spots in the sutures and in the encounter with chinshields (n = 56), or with speckled brownish spots on all scales (n = 5); yellowish venter (anecdotal appointment, in life also with reddish tones) with pairs (n = 55) or three (n = 6) semilunar marks on each ventral scale, the third line usually starts from the second third of the body, and rarely forms two interconnected brownish lines across whole venter (n = 1); whitish gular region with some brown speckled spots (n = 61)

Table 1. Ontogenetic allometry in Daniel's Keelback (*Helicops danieli*).

			Allometry				Difference in allometric coefficient (slope)		Difference in adjusted trait size (intercept)	
Trait	Stage	Mean ± SD	b	r^2	P	Adjusted mean ± SD	F	P	F	P
Snout–vent length	J	191.22 ± 33.65	—	—	—	5.76	—	—	—	—
	A	441.78 ± 130.01				5.76				
Tail length	J	66.05 ± 20.09	1.21	0.60	**<0.001**	4.72 ± 0.03	7.50	**0.007**	3.73	0.06
	A	144.58 ± 36.23	0.73	0.58	0.057	4.78 ± 0.09				
Head length	J	13.04 ± 1.58	0.52	0.57	**<0.001**	2.83 ± 0.03	16.20	**<0.001**	0.49	0.48
	A	28.82 ± 5.72	0.79	0.93	**<0.001**	2.91 ± 0.01				
Head width	J	6.88 ± 1.09	0.63	0.48	0.38	2.24 ± 0.06	4.18	**0.04**	1.43	0.23
	A	13.19 ± 3.73	0.88	0.79	0.87	2.28 ± 0.02				
Head height	J	4.83 ± 0.81	0.75	0.64	0.53	1.95 ± 0.52	4.88	**0.03**	7.99	**<0.01**
	A	9.50 ± 3.04	0.98	0.87	0.23	1.93 ± 0.02				
Interocular length	J	3.63 ± 0.47	0.54	0.53	**<0.01**	1.56 ± 0.04	2.66	0.11	3.16	0.08
	A	5.89 ± 1.25	0.67	0.86	**<0.001**	1.56 ± 0.01				
Snout length	J	3.04 ± 0.45	0.61	0.50	0.18	1.41 ± 0.05	2.568	0.11	0.06	0.81
	A	5.53 ± 1.31	0.75	0.87	**<0.001**	1.41 ± 0.02				
Eye–mouth distance	J	1.18 ± 0.20	0.54	0.33	0.66	0.43 ± 0.07	8.72	**<0.01**	0.88	0.35
	A	2.35 ± 0.73	0.94	0.80	0.37	0.53 ± 0.02				
Eye diameter	J	1.87 ± 0.23	0.53	0.56	**<0.001**	0.89 ± 0.05	0.32	0.57	0.24	0.63
	A	2.74 ± 0.49	0.47	0.61	**<0.001**	0.85 ± 0.02				

Note: J, juveniles; A, adults; $b = 1$ indicates isometry, whereas a value significantly higher or lower than 1 indicates positive or negative allometry, respectively. Traits that showed significant differences are set in boldface type.

that may form a stripe connected to the first ventral semilunar spots ($n = 39$), and to the lateral spots of the head, forming a "sickle-shaped" spot around the mouth ($n = 30$).

COMPARISONS: Only two other *Helicops* species have trans-Andean records: *H. pastazae*, which is found in the Andean foothills, with one record in the Magdalena River Valley of Colombia (Rossman 1976) and other records from the southern portion of Maracaibo Lake (Rossman 1976; Markezich and Rossman 1992), and *H. scalaris*, which is restricted to northwestern Venezuela and adjacent to Colombia in drainages into Maracaibo Lake Basin (Rossman 2002*b*). In general, *H. danieli* differs from all its congeners due to the combination of the spotted dorsum with two lines of semilunar marks on the venter (Rossman 2002*a*). Specifically, *H. danieli* differs from *H. pastazae* and *H. scalaris* by having scale rows usually in 19/19/17 (vs. 23/23–25/19 in *H. pastazae* and 21–25/19–21/16–19 in *H. scalaris*), 58–71 subcaudals in females (vs. 72–97 in *H. pastazae* and 67–81 in *H. scalaris*), and 73–86 subcaudals in males (vs. 93–117 in *H. pastazae* and 83–95 in *H. scalaris*); venter with two lines of semilunar spots (vs. venter with dark crossbands or alternating checkered pattern in *H. pastazae* and venter with black lateral or medial stripes, sometimes with interconnected checkered pattern in *H. scalaris*). Furthermore, *H. danieli* differs from *H. pastazae* by having two pairs of chinshields in contact (intergenial scales present in *H. pastazae*), and from *H. scalaris* since females have 130–145 ventrals and males have 125–135 ventrals (vs. 113–125 in females and 110–119 ventrals in males of *H. scalaris*).

REMARKS: We found a *H. danieli* female (IAvH 84, collected on 10 November 1970) showing six well-developed embryos in the oviduct, with no sign of eggshell formation, representing the first record of litter size and thus confirming viviparity in this species. The mean size of the largest coiled embryo was 31.24 mm in diameter. The measurements of the female were SVL = 730 mm, tail length = 179 mm, head length = 35.24 mm, head height = 15.57 mm, head width = 17.25 mm, interocular length = 6.96 mm, eye–mouth distance = 3.08 mm, and snout length = 8.04 mm.

Ontogenetic and static allometry

We identified significant differences between presence and absence of a white nuchal collar between age classes ($\chi^2 = 68.068$, $P < 0.001$, $n = 106$), where 90% of juveniles showed a clear white nuchal collar, of which 10% showed a smooth band, and only 4% of adults showed a white nuchal collar, of which 36% had smooth bands and 60% completely lacked a collar. The clear white nuchal collar was found to be more related to younger snakes (less than 250 mm) (Figs. 1A–1E; Supplementary Fig. S1[1]).

We found that tail length was the only trait with positive allometry in juveniles. Head length, interocular length, and eye diameter showed negative allometry for both stages. Snout length only showed negative allometry in adults. Tail length, head length, head width, and eye–mouth distance showed significant differences in allometric coefficients between ages (see Table 1 and Figs. 3A–3H). Despite isometric growth, head height varied according to ontogenetic stage, showing different isometric growth trajectories for juveniles and adults (Table 1).

Males and females showed the same allometric pattern, with negative allometry for interocular length, snout length, eye diameter, and head length. The latter also showed differences in the allometric coefficient between sexes (see Table 2 and Figs. 4A–4H).

Dimorphism in traits

The largest female SVL was 730 mm and the largest male SVL was 490 mm. Females were generally larger than males, showing

Fig. 3. (A–H) Regressions of eight log-transformed morphometric traits vs. log-transformed body size (snout–vent length (SVL)) of Daniel's Keelback (*Helicops danieli*) for both adults (black squares) and juveniles (gray circles).

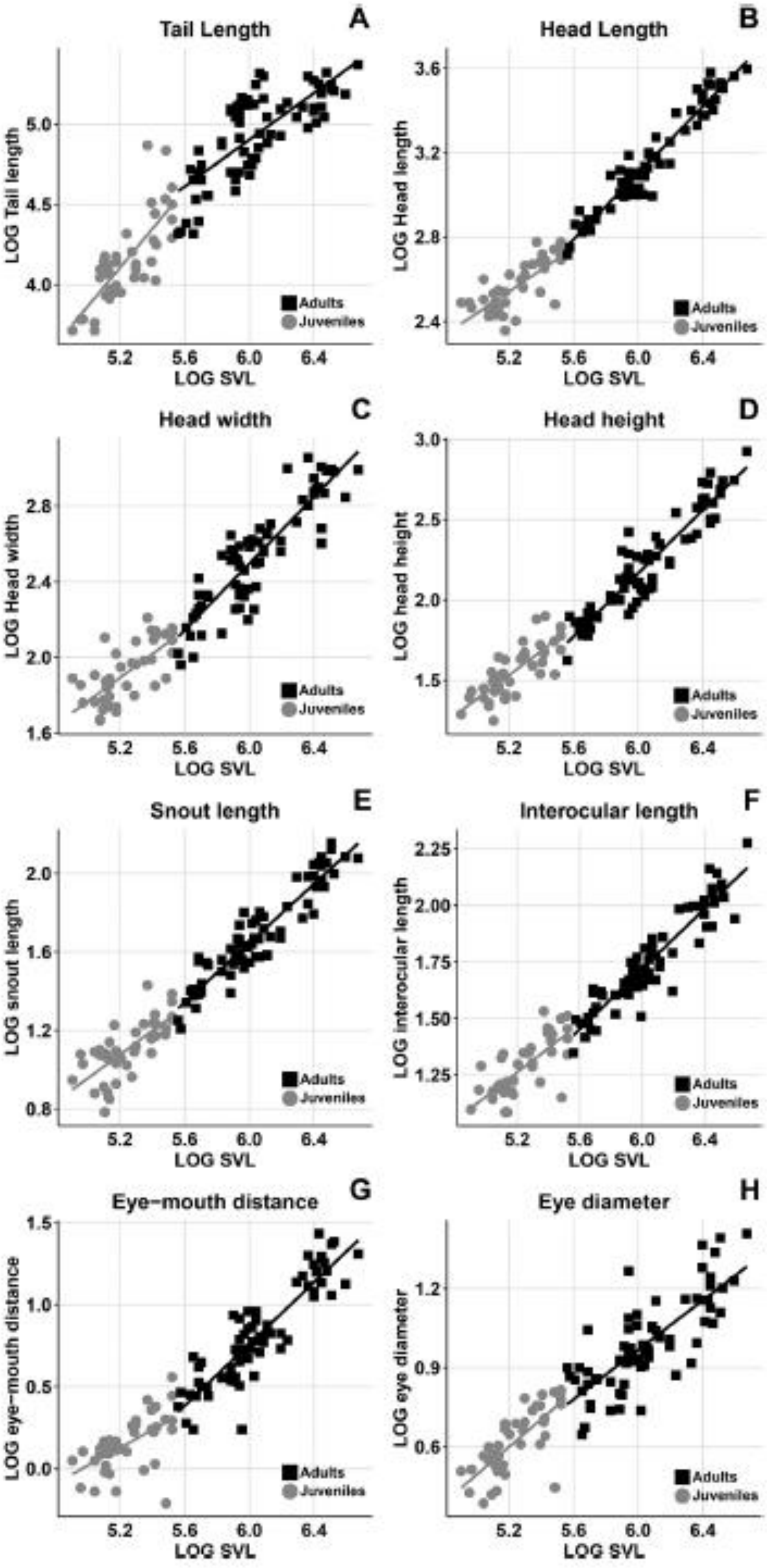


Table 2. Static allometry in Daniel's Keelback (*Helicops danieli*).

			Allometry				Dimorphism in allometric coefficient (slope)		Dimorphism in adjusted trait size (intercept)	
Trait	Sex	Mean ± SD	b	r^2	P	Adjusted mean ± SD	F	P	F	P
Snout–vent length	F	446.78 ± 164.40	—	—	—	5.91	—	—	—	—
	M	338.41 ± 80.40				5.91				
Tail length	F	133.73 ± 42.03	0.84	0.85	0.20	4.74 ± 0.027	1.89	0.18	38.89	**<0.001**
	M	136.35 ± 37.50	1.00	0.76	0.12	4.99 ± 0.029				
Head length	F	24.02 ± 7.40	0.81	0.97	**<0.001**	3.03 ± 0.014	4.57	**0.03**	0.02	0.88
	M	19.42 ± 3.50	0.68	0.83	**<0.001**	3.03 ± 0.014				
Head width	F	13.38 ± 4.86	0.89	0.85	0.63	2.42 ± 0.028	0.45	0.50	0.05	0.81
	M	10.39 ± 2.57	0.81	0.68	0.86	2.40 ± 0.030				
Head height	F	9.69 ± 3.68	0.93	0.88	0.87	2.09 ± 0.023	1.75	0.19	0.03	0.86
	M	7.43 ± 1.64	0.79	0.78	0.21	2.40 ± 0.030				
Interocular length	F	5.92 ± 1.57	0.62	0.87	**<0.001**	1.67 ± 0.016	0.27	0.60	0.20	0.65
	M	4.98 ± 0.75	0.53	0.79	**<0.001**	1.66 ± 0.016				
Snout length	F	5.55 ± 1.77	0.79	0.94	**<0.001**	1.57 ± 0.015	2.74	0.10	3.13	0.08
	M	4.67 ± 0.85	0.66	0.83	**<0.001**	1.61 ± 0.016				
Eye–mouth distance	F	2.39 ± 0.85	0.91	0.87	0.76	0.70 ± 0.025	1.22	0.27	1.00	0.32
	M	1.79 ± 0.37	0.71	0.72	0.07	0.66 ± 0.025				
Eye diameter	F	2.72 ± 0.65	0.51	0.73	**<0.001**	0.92 ± 0.020	0.56	0.46	0.65	0.42
	M	2.45 ± 0.36	0.42	0.58	**<0.001**	0.94 ± 0.021				

Note: F, female; M, male; $b = 1$ indicates isometry, whereas a value significantly higher or lower than 1 indicates positive or negative allometry, respectively. Traits that showed a significant difference for sexual dimorphism are set in boldface type.

larger SVL, head length, head width, head height, interocular length, and eye–mouth distance (Table 2). However, tail length was the only dimorphic trait when using SVL as a covariate ($F = 40.452$, $P = <0.001$, $n = 64$), and head length was equal for males and females also when using SVL as a covariate (Table 2 and for data compilation on *Helicops* spp. see Table 3). The index of sexual dimorphism for *H. danieli* was 0.32.

Distribution and conservation

We obtained 43 unique records of *H. danieli* distributed throughout five Colombian ecoregions, with 65% of the records located in Magdalena–Urabá moist forests, 16% in Sinú Valley dry forests, 7% in Chocó-Darién moist forests, 7% in Magdalena Valley montane forests, and 5% in Amazon–Orinoco–southern Caribbean mangroves (Fig. 5A). This species occurs from sea level up to 1083 m above sea level.

Our *H. danieli* samples were collected between the years 1950 and 2018, and distributed across the following departments: Antioquia, Atlántico, Bolívar, Boyacá, Caldas, Cesar, Chocó, Córdoba, Magdalena, Santander, and Sucre. The highest HFP values were found in the departments Atlántico (median 14, mode 14), Caldas (median 10.7, mode 11), Sucre (median 10.3, mode 17), and Cordoba (median 10.27, mode 13) (Fig. 5B), whereas the less impacted departments were Magdalena (median 9.6, mode 15), Cesar (median 9.5, mode 16), Santander (median 8.2, mode 10), Antioquia (median 7.4, mode 4), Boyacá (median 7.3, mode 3), Bolívar (median 6.6, mode 4), and Chocó (median 3.7, mode 4).

The generated distribution model showed high statistical performance, with a higher mean AUC = 0.963 ± 0.010. The area of suitability for the occurrence of *H. danieli* was found to be restricted to lowlands and moderate highlands in northwestern Colombia, in the surroundings of the Andes Mountain ranges, with two well-demarcated regions in the ecoregion Magdalena–Urabá moist forests and other portions around the Lake Maracaibo, in Venezuela (Fig. 5C). The variables with the greatest contribution to the model were temperature seasonality (56% of contribution) and elevation (27.4%), followed by precipitation seasonality (10.8%), annual temperature range (3.3%), and precipitation of the wettest month (2.5%).

Discussion

In this study, we used a multidisciplinary approach to improve the taxonomic, morphological, distribution, and conservation efforts for a poorly understood aquatic snake, restricted to a small biogeographical region of South America. We update the species' diagnosis, investigate two types of growth rates (i.e., ontogenetic and static allometry), and report on sexual dimorphism in *H. danieli*. We also reassessed this species' distribution, detected anthropic impacts on its range, and indicate suitable areas within its distribution range.

Our data confirmed most of the morphological variation obtained by Rossman (2002a), with the exception of greater amplitude of variation for ventrals in females (130–145 vs. 130–141 in Rossman 2002a) and subcaudals in females (58–71 vs. 61–70) and males (73–86 vs. 76–86). *Helicops danieli* is part of the *Helicops* species cluster, which has 19 dorsal scale rows in the middle of the body (sensu Moraes-da-Silva et al. 2021). This group also includes Leopard Keelback (*Helicops leopardinus* (Schlegel, 1837)) and *H. angulatus* complexes (both widely distributed in South America), Wied's Keelback (*Helicops carinicaudus* (Wied-Neuwied, 1824)) (southeastern and southern Brazil), *Helicops infrataeniatus* Jan, 1865 (southern and central-western Brazil), Sao Paulo Keelback (*Helicops gomesi* Amaral, 1921), and Olive Keelback (*Helicops modestus* Günther, 1861) (central-western Brazil), *Helicops phantasma* Moraes-da-Silva, Cecília

Fig. 4. (A–H) Regressions of eight log-transformed morphometric traits vs. log-transformed body size (snout–vent length (SVL)) of Daniel's Keelback (*Helicops danieli*) for both females (black squares) and males (gray circles).

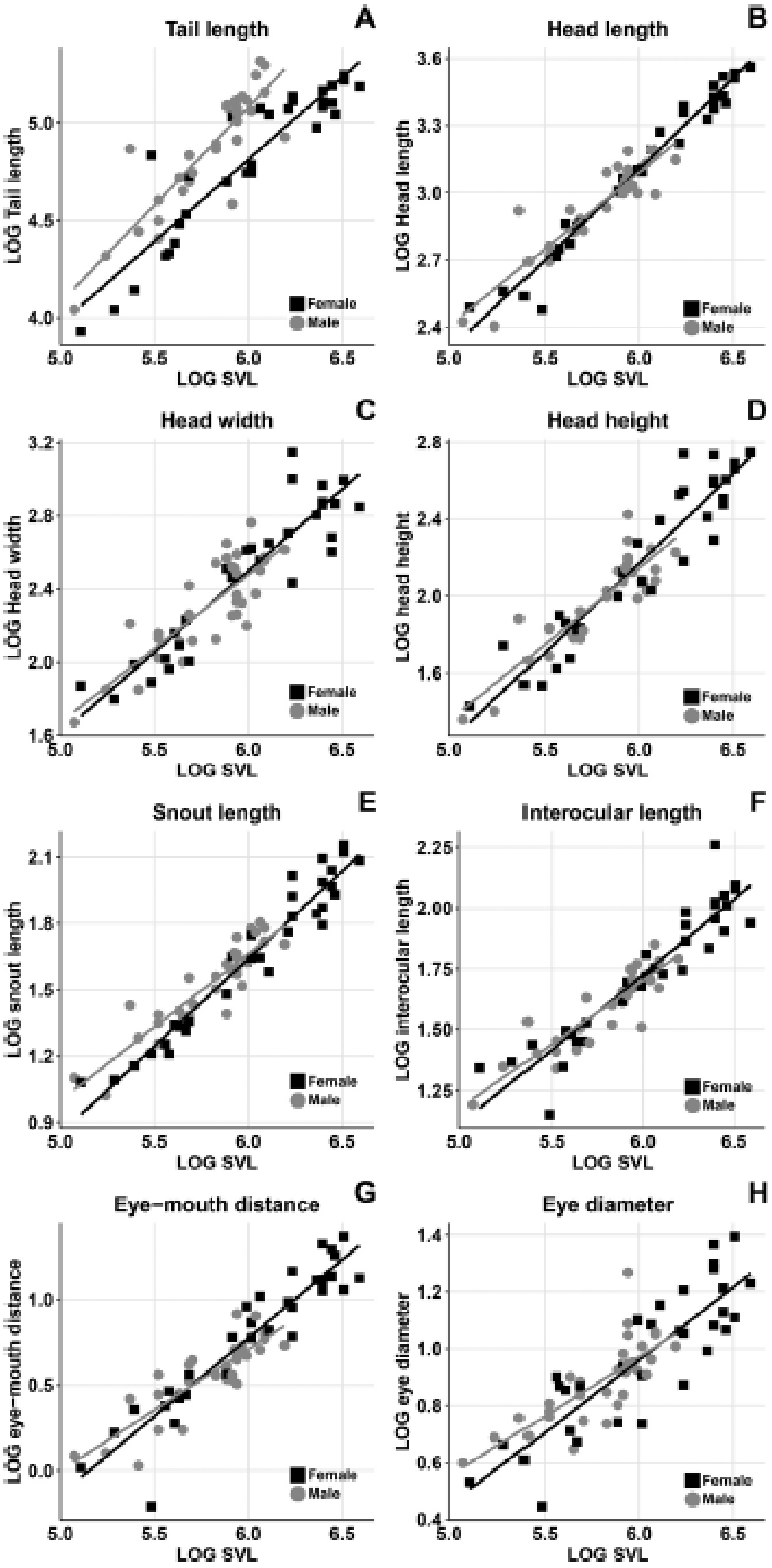


Table 3. Comparisons of sexual dimorphism in morphometric traits, snout–vent length (SVL), head length (HL), and tail length (TL) among the species of the watersnake genus *Helicops*.

	Sexual dimorphism				
Species	Morphometric traits	SVL	HL	TL	References
Mountain Keelback, *Helicops angulatus*	Yes	F > M	F = M	F < M	Scartozzoni 2009[2]; Murphy et al. 2020*
Helicops apiaka Kawashita-Ribeiro, Ávila and Morais, 2013	?	?	?	?	
Helicops boitata Moraes-Da-Silva, Cecília Amaro, Sales-Nunes, Strüssmann, Teixeira, Andrade, Sudré, Recoder, Rodrigues, Curcio, 2019	?	?	?	?	
Wied's Keelback, *Helicops carinicaudus*	Yes	F > M	F = M	F < M	Scartozzoni 2009[2]
Daniel's Keelback, *Helicops danieli*	Yes	F > M	F = M	F < M	Rossman 2002*a**; present study
Sao Paulo Keelback, *Helicops gomesi*	Yes	F > M	F > M	F < M	Scartozzoni 2009[2]
Hagmann's Keelback, *Helicops hagmanni*	Yes	F > M	F = M	F < M	Scartozzoni 2009[2]
Helicops infrataeniatus	Yes	F > M	F > M	F < M	de-Aguiar and Di-Bernardo 2004; Scartozzoni 2009[2]
Leopard Keelback, *Helicops leopardinus*	Yes	F > M	F > M	F < M	Ávila et al. 2006; Scartozzoni 2009[2]
Olive Keelback, *Helicops modestus*	Yes	F > M	F > M	F < M	Scartozzoni 2009[2]; Maia et al. 2015
Helicops nentur	?	?	?	?	
Shreve's Keelback, *Helicops pastazae*	Yes	F > M	F > M	F < M	Rossman 1976*; García-Cobos et al. 2020
Spiral Keelback, *Helicops petersi*	Yes	?	?	F < M	Rossman 1976*
Helicops phantasma	?	?	?	?	
Norman's Keelback, *Helicops polylepis*	Yes	F > M	F > M	F < M	Scartozzoni 2009[2]; Camargo et al. 2021
Ladder Keelback, *Helicops scalaris*	Yes	F > M	F > M†	F < M	Rossman 2002*b**
Helicops tapajonicus	Yes	?	?	F < M	Frota 2005*
Equatorial Keelback, *Helicops trivittatus*	Yes	F > M	F > M	F < M	Scartozzoni 2009[2]
Peru Keelback, *Helicops yacu*	?	?	?	?	

Note: F, female; M, male.

*Authors presented only descriptive data.

†Differences in head size were considered subtle by the author.

Amaro, Sales-Nunes, Rodrigues and Curcio, 2021 (Tocantins–Araguaia Basin), and *Helicops tapajonicus* Frota, 2005 (Amazonia Basin) (Nogueira et al. 2019; Murphy et al. 2020; Moraes-da-Silva et al. 2021). However, with the exception of the *H. leopardinus* complex (sensu Moraes-da-Silva et al. 2021), the spotted dorsal pattern of *H. danieli* mainly appears in *Helicops* species that are restricted to the northern portion of South America, i.e., Hagmann's Keelback (*Helicops hagmanni* Roux, 1910), *H. pastazae*, Spiral Keelback (*Helicops petersi* Rossman, 1976), Norman's Keelback (*Helicops polylepis* Günther, 1861), and Peru Keelback (*Helicops yacu* Rossman and Dixon, 1975) (sensu Moraes-da-Silva et al. 2021). The black semilunar ventral marks of *H. danieli* are shared with *H. carinicaudus*, *Helicops nentur* Costa, Santana, Leal, Koroiva and Garcia, 2016 (both from southeastern Brazil), and Equatorial Keelback (*Helicops trivittatus* (Gray, 1849)) (Amazon and Tocantins–Araguaia basins) (Rossman 2010; Nogueira et al. 2019; Costa et al. 2016).

The presence of a white nuchal collar in *H. danieli* juveniles may play a disruptive role against visually oriented predators, depending on the background of the niches exploited by developing individuals (Stevens et al. 2006; Stevens 2007). Other color traits act more strongly on small individuals, as smaller rings increase the effect of blurring with the background for visually oriented predators (Pough 1976). *Helicops danieli* and many other aquatic snakes have bright aposematic colors that are concentrated on the belly (Batista et al. 2020), these characteristics likely act as a defense mechanism against predation by carnivorous fishes in aquatic environments (Batista et al. 2020). Thus, we hypothesize that the presence of the white nuchal collar in *H. danieli*, together with bright ventral coloration, may be driven by selective forces, thereby reinforcing aposematic/disruptive non-conflicting strategies (see Titcomb et al. 2014) against terrestrial and visually oriented aquatic predators.

The sexual dimorphism detected in *H. danieli*, where females are larger than males, is widely documented in snakes and is common in aquatic species (Shine 1978; Shine 1994*b*; Fitch 1981; for *Helicops* spp. see Table 3). Larger female body size is likely not only related to litter size and fat accumulation for embryo development, but also for the use of different niche parameters (Shine 1986, 1994*a*). Unlike many other studies with aquatic snakes (e.g., Shine 1986; Houston and Shine 1993; Shine et al. 2002) and with *Helicops* (Table 3), we did not detect females with proportionally larger heads than males. However, our results are similar to those found for the other three *Helicops* species: *H. angulatus* (which likely represents a species complex according to Murphy et al. 2020), *H. hagmanni*, and *H. carinicaudus* (Table 3). Considering that this trait may be triggered by ecological pressures related to diet and habitat use (Shine 1986; Camilleri and Shine 1990), or by phylogenetic conservatism (Westeen et al. 2020), we believe that filling in gaps in the knowledge of the natural history of *Helicops* spp. through a phylogenetic approach can clarify the morphological evolution of the group. The proportionally larger tails in males may be due to three classic and non-excluding hypotheses: (1) "The Morphological Constraint Hypothesis" — to accommodate the hemipenis (e.g., King 1989; Shine, et al. 1998; Sivan et al. 2020); (2) "The Female Reproductive Output Hypothesis" — females have relatively shorter tails as a secondary result of natural selection for increased reproductive capacity; or (3) "The Mating Ability Hypothesis" — an increased reproductive ability in males (sexual selection) (King 1989; Shine et al. 1999). However, we did not detect significant differences in the allometric coefficient between adults from both sexes for tail length, suggesting that males are born with a proportionally larger tail, or acquire this trait during the juvenile stage, thus both hypotheses should be tested in the future with neonates.

Although head height showed isometric growth in adults and juveniles, the differences in the growth coefficient of this trait (Table 1) and for the allometric coefficient of head length and head width between ages was expected, according to the proportional decrease in relative brain size during development (Gould 1966; Phillips and Shine 2006).

Paradoxically, the area with the highest concentration of *H. danieli* records is also one of the most threatened regions in South America due to the gold mining industry, in addition to other factors such as

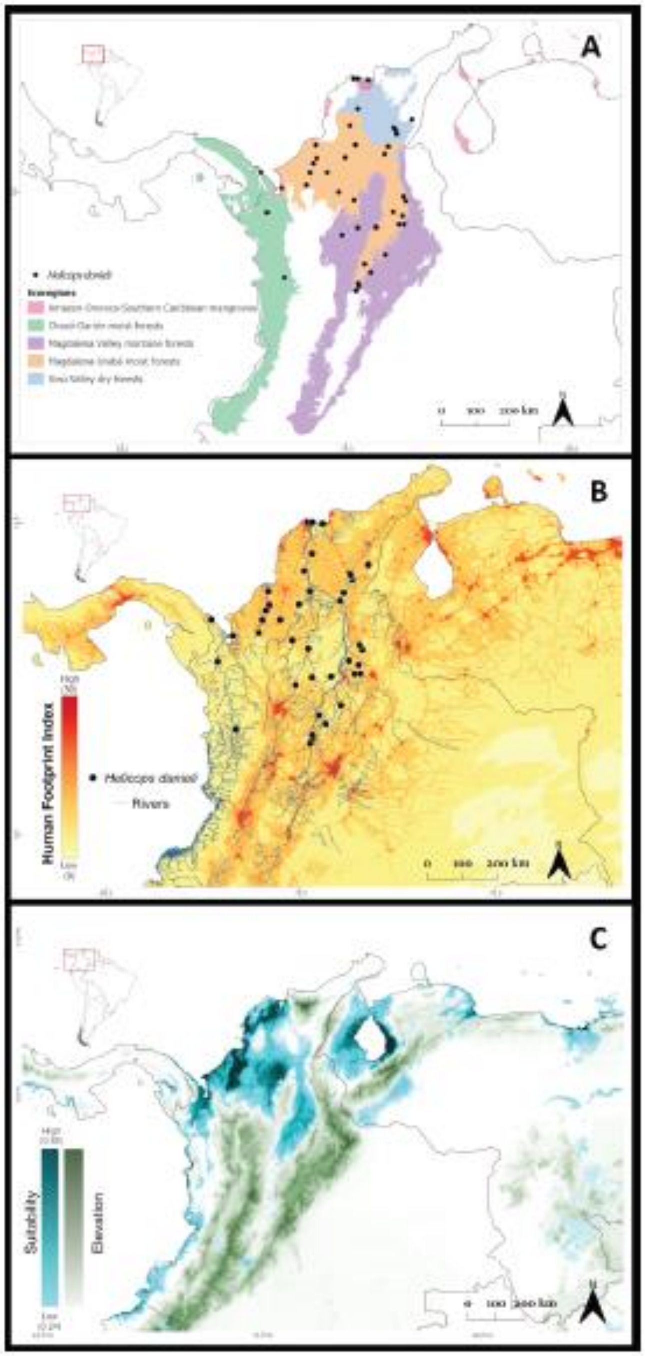


Fig. 5. Distribution maps of Daniel's Keelback (*Helicops danieli*), including (A) occurrence in five Colombian ecoregions (ecoregions base map: Dinerstein et al. 2017), (B) anthropic impact index on *H. danieli* range distribution (human footprint base map: Venter et al. 2016*a*), and (C) suitable areas for *H. danieli* potential occurrence in Colombia and Venezuela (elevation base map: Fick and Hijmans 2017). Color version online.

deforestation and river pollution as a result of agricultural practices (Sánchez-Cuervo et al. 2012; Alvarez-Berríos and Aide 2015). Aquatic snakes can act as bioindicators along River Basins (Haskins et al. 2021), as they are susceptible to wastewater and siltation. Conservation strategies for *H. danieli* should be especially directed toward the Atlantic Caribbean region, which is also the region with the highest HFP values. As our records were associated with rivers, we suggest that *H. danieli* populations should be monitored and that physiological studies on this species should be performed, with the aim of contributing to the management of water and bioaccumulation (Fontenot et al. 2000; Haskins et al. 2021).

Finally, we found that *H. danieli* occurrence in the Amazon Basin region was not suitable, even though one record (ICN-R 242) in Mitú, Vaupés, Colombia, was reported by Yuki and Castaño (1998). Rossman (2002*a*) confirmed the identification of this specimen as *H. danieli* and commented that is probably wrong in origin. However, we cannot simply discredit the possibility of the correct origin of the specimen, based on the possibility of human transportation from trans-Andean locations or even the chance that this specimen may represent an unknown species to science with a general phenotype similar to *H. danieli*. Unfortunately, we did not find the specimen in the ICN-R collection, but all our records are from Caribbean drainages and at most ca. 1000 m above sea level. We believe that the Colombian Andes (Cordillera Oriental and the Sierra de Perijá) act as important physical barriers to this species, which may also explain why we identified a high suitability for *H. danieli* around Lake Maracaibo Basin, in association with the absence of records there. A similar endemism pattern is found in other Hydropsini snakes, such as *H. scalaris* and the South American Pond Snake (*Pseudoeryx relictualis* Schargel, Rivas-Fuenmayor, Barros, Péfaur and Navarette, 2007), that are restricted to the Lake Maracaibo Basin (Rossman 2002*b*; Schargel et al. 2007).

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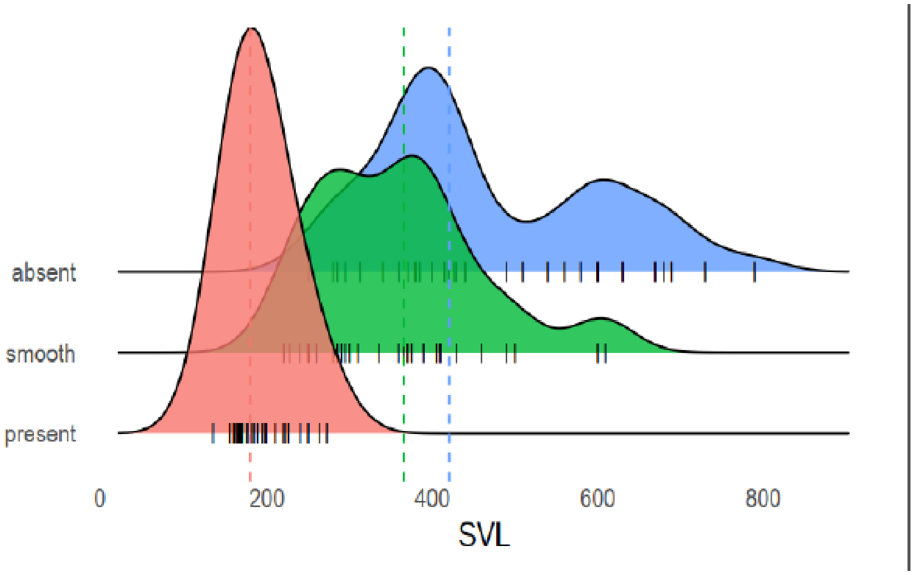

Comparison between three categories of white nuchal band and age estimated by Snout-vent length (SVL) in mm of *Helicops danieli*; Absent = white collar absent or indistinct from the background dorsum – smooth = white collar not clearly visible and poorly defined – present, white collar conspicuously visible and well defined.

Table S1. List of records.

Institution	(follows Sabaj 2020)	Voucher number	Locality	Department	Country	Latitude (°N)	Longitude (°W)	Coordinate source
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	3512	Acandí	Chocó	Colombia	8.51008	77.278983	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	3522	Acandí	Chocó	Colombia	8.51008	77.278983	*informed by the collection*
Museo Javeriano de Historia Natural "Lorenzo Uribe, S.J.", Pontificia Universidad	MPUJ	289	Agustín Codazzi	Cesar	Colombia	9.91667	73.25	***Google Earth***
Museo Javeriano de Historia Natural "Lorenzo Uribe, S.J.", Pontificia Universidad	MPUJ	288	Agustín Codazzi	Cesar	Colombia	9.91667	73.25	***Google Earth***
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	8914	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	3249	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Universidad Industrial de Santander	UIS -R	4249	Barrancabermeja	Santander	Colombia	7.12105	73.595567	***Google Earth***
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1545	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1550	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1547	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1553	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1551	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1549	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1544	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1546	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1552	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1543	Barrancabermeja	Santander	Colombia	7.12105	73.595567	*informed by the collection*
Museu de Zoologia da Universidade de São Paulo	MZUSP	2144	Barranquilha	Atlántico	Colombia	11.0027	74.807014	*informed by the collection*
Museu de Zoologia da Universidade de São Paulo	MZUSP	2145	Barranquilha	Atlántico	Colombia	11.0027	74.807014	*informed by the collection*
Museo de Historia Natural, Universidad de La Salle	MLS	736	Barranquilha	Atlántico	Colombia	11.0027	74.807014	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	116	Barranquilha	Atlántico	Colombia	11.0027	74.807014	*informed by the collection*
Universidad Industrial de Santander	UIS -R	3628	Betulia	Santander	Colombia	7.12269	73.455506	***Google Earth***
Universidad Industrial de Santander	UIS -R	3765	Betulia	Santander	Colombia	7.12804	73.454079	***Google Earth***
Museo de Historia Natural, Universidad de La Salle	MLS	2132	Caucasia	Antioquia	Colombia	7.97952	75.19856	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	11552	Cereté	Córdoba	Colombia	8.88868	75.789628	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	7757	Chimichagua	Cesar	Colombia	9.20083	73.876389	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	11551	Chimichagua	Cesar	Colombia	9.20083	73.876389	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	3821	Ciénaga de Betancí	Córdoba	Colombia	8.49991	75.966597	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	320	Cienaga La Raya	Bolívar	Colombia	7.74229	73.405518	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	211	Cimitarra	Santander	Colombia	6.31513	73.948919	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	3696	Cuatrovientos	Cesar	Colombia	9.7018	73.733789	*informed by the collection*
Museo Javeriano de Historia Natural "Lorenzo Uribe, S.J.", Pontificia Universidad	MPUJ	471	El Bagre	Antioquia	Colombia	7.77092	74.78972	***Google Earth***
Museo Javeriano de Historia Natural "Lorenzo Uribe, S.J.", Pontificia Universidad	MPUJ	470	El Bagre	Antioquia	Colombia	7.77092	74.78972	***Google Earth***
Universidad Industrial de Santander	UIS -R	924	El Banco	Madaglena	Colombia	8.9944	73.968664	***Google Earth***
Universidad Industrial de Santander	UIS -R	495	El Banco	Madaglena	Colombia	8.9944	73.968664	***Google Earth***
Universidad Industrial de Santander	UIS -R	122	El Banco	Madaglena	Colombia	8.9944	73.968664	***Google Earth***
Universidad Industrial de Santander	UIS -R	1782	El Paso	Cesar	Colombia	9.53641	73.65511	***Google Earth***
Universidad Industrial de Santander	UIS -R	4374	El Paso	Cesar	Colombia	9.63651	73.683871	***Google Earth***
Universidad Industrial de Santander	UIS -R	3814	El Paso	Cesar	Colombia	9.66612	73.703294	***Google Earth***
Universidad Industrial de Santander	UIS -R	3828	El Paso	Cesar	Colombia	9.66612	73.703294	***Google Earth***
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	11548	El Paso	Cesar	Colombia	9.66612	73.703294	***Google Earth***
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	11549	El Paso	Cesar	Colombia	9.66612	73.703294	***Google Earth***
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	11553	El Paso	Cesar	Colombia	9.66612	73.703294	***Google Earth***
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	11550	El Paso	Cesar	Colombia	9.66612	73.703294	***Google Earth***
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1560	Guarinocito, La Dorada	Caldas	Colombia	5.34991	74.733225	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	2024	sla de Salamanca, Santa Mart	Madaglena	Colombia	10.9667	74.419678	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	1934	sla de Salamanca, Santa Mart	Madaglena	Colombia	10.9667	74.419678	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5833	La Dorada	Caldas	Colombia	5.47207	74.667856	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	2001	La Dorada	Caldas	Colombia	5.47207	74.667856	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10912	La Dorada	Caldas	Colombia	5.47207	74.667856	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5819	Laguna La Palagua	Boyacá	Colombia	6.06226	74.509783	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10262	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10265	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10264	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10263	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10250	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10266	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10259	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10260	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10249	Lorica	Córdoba	Colombia	9.23756	75.813714	*informed by the collection*
Museo de Historia Natural, Universidad de La Salle	MLS	734	Magangue	Bolívar	Colombia	9.24099	74.752786	*informed by the collection*
Museo de Historia Natural, Universidad de La Salle	MLS	2235	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Museo de Historia Natural, Universidad de La Salle	MLS	2949	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Museo de Historia Natural, Universidad de La Salle	MLS	2234	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Museo de Historia Natural, Universidad de La Salle	MLS	2189	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10243	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10736	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10255	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10268	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10245	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10267	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10244	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10257	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10258	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10256	Montería	Córdoba	Colombia	8.74824	75.87845	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	123	Parque Isla de Salamarca	Madaglena	Colombia	11.0044	74.685664	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	16	Parque Isla de Salamarca	Madaglena	Colombia	11.0044	74.685664	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10253	Pueblo Nuevo	Córdoba	Colombia	8.50419	75.507475	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10251	Pueblo Nuevo	Córdoba	Colombia	8.50419	75.507475	*informed by the collection*
Instituto de Ciencias Naturales, Universidad Nacional da Colombia	ICN-R	10252	Pueblo Nuevo	Córdoba	Colombia	8.50419	75.507475	*informed by the collection*
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Universidad Industrial de Santander	UIS -R	1428	Puerto Wilches	Santander	Colombia	7.45727	73.746237	***Google Earth***
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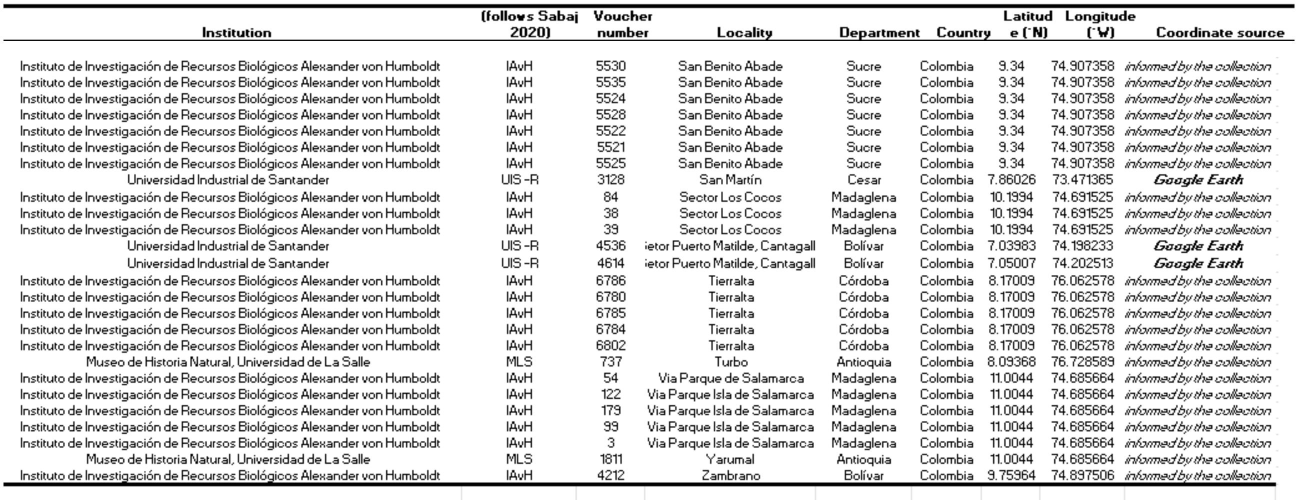
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5539	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5533	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*

Continuação

Table S1. List of records.



Institution	(follows Sabaj 2020)	Voucher number	Locality	Department	Country	Latitude (˚N)	Longitude (˚W)	Coordinate source
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Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5535	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5524	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5528	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5522	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5521	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	5525	San Benito Abade	Sucre	Colombia	9.34	74.907358	*informed by the collection*
Universidad Industrial de Santander	UIS -R	3128	San Martín	Cesar	Colombia	7.86026	73.471365	***Google Earth***
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	84	Sector Los Cocos	Madaglena	Colombia	10.1994	74.691525	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	38	Sector Los Cocos	Madaglena	Colombia	10.1994	74.691525	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	39	Sector Los Cocos	Madaglena	Colombia	10.1994	74.691525	*informed by the collection*
Universidad Industrial de Santander	UIS -R	4536	ietor Puerto Matilde, Cantagall	Bolívar	Colombia	7.03983	74.198233	***Google Earth***
Universidad Industrial de Santander	UIS -R	4614	ietor Puerto Matilde, Cantagall	Bolívar	Colombia	7.05007	74.202513	***Google Earth***
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Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	6785	Tierralta	Córdoba	Colombia	8.17009	76.062578	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	6784	Tierralta	Córdoba	Colombia	8.17009	76.062578	*informed by the collection*
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	6802	Tierralta	Córdoba	Colombia	8.17009	76.062578	*informed by the collection*
Museo de Historia Natural, Universidad de La Salle	MLS	737	Turbo	Antioquia	Colombia	8.09368	76.728589	*informed by the collection*
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Instituto de Investigación de Recursos Biológicos Alexander von Humboldt	IAvH	4212	Zambrano	Bolívar	Colombia	9.75964	74.897506	*informed by the collection*

Apêndice II

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A new oviparous species of *Helicops* Wagler, 1828 (Serpentes, Xenodontinae) from Brazilian Amazonia with reflections on the evolution of viviparity among hydropsine watersnakes

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ABSTRACT

We describe a new watersnake species of the genus *Helicops* from the left bank of the upper Madeira River, southwestern Amazonia, Brazil. Due to its spotted dorsal pattern, it resembles the sympatric species *H. hagmanni*, *H. leopardinus*, and *H. polylepis*, but a combination of features including, scale counts, prominence of dorsal keels, and hemipenial morphology allows its unequivocal recognition. The new species also exhibits oviparous reproduction, representing the fourth egg-laying lineage reported in the genus. The evolution of viviparity among the lineages of *Helicops* comprises an important debate in the field of the natural history of reptiles, especially regarding the highly unlikely possibilities of reversals from viviparity to oviparity due to constraints of genetic, ecological, and physiological nature. Based on such premises, we reconstructed the reproductive modes within the genus using a preconceived topology and discussed hypothetical scenarios for the emergence of viviparity depending on three possible positions of the new taxon. Finally, we also provide considerations on the apparent restriction of this snake to the left bank of the upper Madeira in the light of classic biogeographic evidence that points to the potential role of this major river as a vicariant element between faunas on its opposite margins.

1. Introduction

The tribe Hydropsini Dowling, 1975 comprises a clade of watersnakes belonging to three widespread South American genera, *Helicops* Wagler, 1828 (20 species), *Hydrops* Wagler, 1830 (three species), and *Pseudoeryx* Fitzinger, 1826 (two species) (Peters & Orejas-Miranda, 1970; Costa et al., 2016; Moraes-da-Silva et al., 2019, 2021; Uetz et al., 2020; Murphy et al. 2020). Recent molecular studies corroborate the current taxonomic system (see Wallach et al., 2014), consistently recovering a component formed by *Helicops* terminals as the sister group of a clade including the two latter genera (Grazziotin et al., 2012; Costa et al., 2016; Moraes-da-Silva et al., 2019, 2021).

Regarding the affinities among *Helicops* taxa, recent topologies recovered three well-supported clades revealing counterintuitive evolutionary tendencies at least concerning colour patterns (Moraes-da-Silva et al., 2019, 2021; Murphy et al., 2020). For instance, the species exhibiting rounded spots [namely *H. hagmanni* Roux, 1910, *H. leopardinus* (Schlegel, 1837), and *H. polylepis* Günther, 1861], and stripes [*H. carinicaudus* (Wied, 1825), *H. infrataeniatus* Jan, 1865, and *H. modestus* Günther, 1861] do not reflect independent monophyletic groups, denoting homoplasies associated with the two most common dorsal patterns in the genus; by contrast, the taxa with dorsal saddles sampled in these studies [namely *H. angulatus* (Linnaeus, 1758), *H. cyclops* Cope, 1868 and *H. gomesi* Amaral, 1921] did cluster in a clade, also

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sharing subcaudal keels as an additional putative synapomorphy (Costa et al., 2016; Moraes-da-Silva et al., 2019, 2021; Murphy et al., 2020).

Besides colour patterns, reproductive modes also reflect homoplasies among *Helicops* lineages. In a comprehensive study regarding the reproductive biology of Hydropsini, Braz et al. (2016) demonstrated that (i) the existence of oviparous and viviparous species of *Helicops* implies up to three independent origins of viviparity in the tribe, and (ii) at least one *Helicops* species (*H. angulatus*) exhibited a bimodal reproduction, possibly reflecting two cryptic entities yet undistinguished by traditional taxonomy. Nonetheless, the conclusions of Braz et al. (2016) relied on phylogenies which only included five *Helicops* taxa (*H. angulatus*, *H. carinicaudus*, *H. gomesi*, *H. hagmanni*, and *H. infrataeniatus*), while the combined samples of the most recent topologies compute more than twice this number (Costa et al., 2016; Moraes-da-Silva et al., 2019, 2021; Murphy et al., 2020).

Among the 15 *Helicops* species with known reproductive modes, viviparity predominates [nine lineages: *H. carinicaudus*, *H. danieli* Amaral, 1938, *H. infrataeniatus*, *H. leopardinus*, *H. modestus*, *H. phantasma* Moraes-da-Silva et al., 2021, *H. polylepis*, *H. scalaris* Jan, 1863, and *H. trivittatus* (Gray, 1849)], while oviparity appears less disseminated (three lineages: *H. cyclops*, *H. gomesi*, *H. hagmanni*, and *H. pastazae* Shreve, 1934) (Braz et al., 2016, 2018; García-Cobos and Gómez-Sánchez, 2019). In addition, the existence of at least one nominal species with bimodal reproduction [oviparous and viviparous populations documented for of *H. angulatus*] indicates that this genus may become informative in studies regarding the evolution of reproductive modes among reptiles (Braz et al., 2016; Murphy et al., 2020).

The long-standing debate on the evolution of viviparity in squamates supports independent origins of viviparous taxa as more likely than reversals to oviparity (Lee & Shine, 1998; Griffith et al., 2015). This reasoning considers that such reversals would require the re-evolution of features in the machinery of the eggshell production involving integrated morphological, physiological, and genetic parameters that could hardly be redeveloped at the same time, comprising a far more complex process than multiple transitions to viviparity [Lee & Shine, 1998; Griffith et al., 2015, but see Pyron & Burbrink (2014) for an alternative point of view].

Regarding the tribe Hydropsini, by considering that the genera *Hydrops* and *Pseudoeryx* are oviparous, as well as the biological restrictions of reversals from viviparity to oviparity (Griffith et al., 2015), it can be assumed that the emergence of viviparous species within the genus *Helicops* occurred multiple times and independently from an oviparous ancestor. In that sense, the more comprehensive sampling and the resulting topologies of recent phylogenetic studies (e.g., Moraes-da-Silva et al., 2019, 2021) paved the way to novel essays of reconstructing the reproductive modes of *Helicops*, allowing for more accurate interpretations of their evolution.

Considering the aforementioned knowledge on the reproductive biology of *Helicops* and the recent advances in the systematics of the genus (Kawashita-Ribeiro et al., 2013; Costa et al., 2016; Moraes-da-Silva et al., 2019, 2021), a new finding reported here adds further elements to the discussion. While examining spotted-patterned *Helicops* specimens housed in regional Brazilian collections, we recovered a sample containing 27 individuals from the influence area of the Santo Antônio Dam (upper Madeira River, western Brazilian Amazonia) sharing the checkered venter, 19 midbody dorsals, and moderate dorsal keels with *H. leopardinus*, but exhibiting a light triangular snout spot that does not occur in this species. More curiously, and in addition to field observations, our sample included one hatchling preserved with its eggshell indicating oviparity, which contrasts with the viviparous reproduction of *H. leopardinus*.

The only oviparous species occurring in the region possessing dorsal and ventral colour patterns that resemble the one of our sample is *Helicops hagmanni*, but the high midbody dorsal counts (23) and strong keels of this taxon did not match the pattern of our specimens (19 dorsals at midbody and moderate dorsal keels). Thus, after detailed comparisons with samples of the most similar congeners as well as literature data, we concluded that what we had at hand represented a new egg-laying species of *Helicops*, clearly distinguishable from all other counterparts. Here, we describe this species and explore its possible implications for the evolution of reproductive modes in the genus. Finally, given the importance of the Madeira River as a vicariant element in southwestern Amazonia (Wallace, 1852; Avila-Pires, 1995; Silva et al., 2005; Dias-Terceiro et al., 2015; Ferrão et al., 2018; Peixoto et al., 2020), we also discuss biogeographical aspects associated with the distribution of *Helicops* taxa in the area, hopefully encouraging future research efforts in the field of systematics, taxonomy, and phylogeography focused on widespread species of these watersnakes.

2. Material and methods

2.1. Specimens examined

Our sample includes the 27 specimens of our new species candidate (hereafter *Helicops* sp. nov.) as well as 339 additional snakes belonging to 18 *Helicops* taxa [*H. angulatus* (N = 27), *H. apiaka* Kawashita-Ribeiro et al., 2013 (N = 10), *H. boitata* Moraes-da-Silva et al., 2019 (N = 6), *H. carinicaudus* (N = 23), *H. danieli* (N = 2), *H. gomesi* (N = 4), *H. hagmanni* (N = 51), *H. infrataeniatus* (N = 17), *H. leopardinus* (N = 68), *H. modestus* (N = 21), *H. nentur* Costa et al., 2016 (N = 1), *H. pastazae* (N = 2), *H. petersi* Rossman, 1976 (N = 1), *H. phantasma* (N = 29), *H. polylepis* (N = 56), *H. scalaris* (N = 2), *H. tapajonicus* Frota, 2005 (N = 3), and *H. trivittatus* (N = 16)]; since we did not have access to specimens of *H. yacu* Rossman & Dixon, 1975, comparisons involving this species rely on data from the original description (Rossman and Dixon, 1975). The list of specimens examined appears in the Appendix and institutional acronyms follow Sabaj (2020), except for Coleção Herpetológica da Universidade Federal do Oeste do Pará (UFOPA–R, Santarém, Pará State, Brazil).

2.2. Geographic data

We obtained geographic data (coordinates and elevation of localities) directly from the institutional catalogues (when available therein) or estimated such information based on gazetteers (e.g., Paynter and Traylor, 1991) and the software Google Earth Pro version 7.3.3.7699. We constructed maps with the aid of the software QGIS 3.4 Madeira (QGIS Development Team, 2019) using the datum SIRGAS 2000.

2.3. Scalation, tooth counts, and morphometric data

We follow Moraes-da-Silva et al. (2019, 2021) in taking all measurements (SVL and tail length to the nearest 1.0 mm, and head proportions to the nearest 0.01 mm), tooth counts (on the right maxillary bone), and scale counts [except for ventrals, for which we follow Dowling (1951)]. The "right side / left side" notation denotes counts of both sides of head, while the "+" signal appears in ocular and temporal formulae on each one of the head sides (e.g., "oculars 1+2", "temporals 1+2+3"). Nonetheless, instances of asymmetry regarding oculars and temporals may also appear in the "right side

/left side" system (e.g., "oculars 1+1 /1+2"). Character states associated with keel prominence follow Moraes-da-Silva et al. (2019, 2021), with dorsal keels defined as "strong" (as for *H. angulatus*), "weak" (as for *H. modestus*) and "moderate" (all intermediate levels of prominence).

2.4. Colour pattern

Information on colour patterns in life (e.g., details regarding the tonality of spots and dorsum/venter background colouration) relies on photographs and field observations of uncollected specimens that were rescued from the influence area of the Santo Antônio Dam, on the left bank of the Madeira River (vicinities of Porto Velho, state of Rondônia, Brazil). These specimens include one adult and all 12 hatchlings of a clutch that, following the legal requirements associated with wildlife rescue operations, were released in water environments of the region, presumably free from flooding impacts. We must emphasise that only a minor proportion of the snakes obtained in the rescue operations (e.g., severely hurt or already dead individuals) were sent to the scientific collections, as it happened to most specimens of the type series. Attribution of the uncollected specimens mentioned herein to *Helicops* sp. nov. derives from features of scale counts (19 dorsals at midbody), colouration (presence of a pale triangular snout spot), and prominence of keels (moderate) taken in the field, before releasing the specimens.

Qualitative colouration parameters in our analyses include snout pattern (uniform or exhibiting a roughly triangular light snout spot), general tonality of the dorsum background, and ventral pattern. Meristic features of colouration refer to the number of spot rows at midbody, number of black spots on trunk (counted throughout the right lateral spot row, from neck to vent), number of spots on tail (counted on the right flank, from a point immediately posterior to vent until tail tip), number of blotches on venter (counted on the right margin of ventrals, from neck to vent), and number of blotches on ventral surface of tail (counted on the right subcaudal scales, from a point immediately posterior to vent until tail tip).

2.5. Sex determination and hemipenial morphology

Specimens with externally exposed hemipenes were promptly recognised as males. Nonetheless, to determine the sex of the snakes that had no externally visible genitalia, we performed a short incision between the anteriormost subcaudals to check for the internal presence of inverted hemipenes. We everted and filled the organs using the protocols of Manzani & Abe (1988), Pesantes (1994), and Zaher (1999), and used the techniques of Uzzell (1973) and Nunes et al. (2012) to stain calcareous structures. Anatomical terminology of hemipenial shape and ornaments follows Dowling & Savage (1960) and Zaher (1999).

2.6. Statistical analyses

We performed statistical analyses to compare means of continuous variables and assess sexual dimorphism, as well as to investigate possible meristic differences between *Helicops* sp. nov. and the most similar and possibly sympatric congeners (*Helicops hagmanni*, *H. leopardinus*, and *H. polylepis*). We treated these four groups as operational taxonomic units, searching for statistical evidence that could corroborate the qualitative distinction of *Helicops* sp. nov. from the other spotted-patterned *Helicops* occurring in sympatry. Since most other *Helicops* taxa differ remarkably from *Helicops* sp. nov. regarding evident qualitative (e.g., color pattern) and meristic (i.e. dorsal counts) features, we did not include these species in our statistical analyses.

The variables submitted to statistical tests for sexual dimorphism included segmental counts (ventrals and subcaudals), and tail length/total length ratio. For comparisons among operational groups, we used segmental counts, number of spots on trunk, snout-vent length (SVL), tail length, head length, and head width.

Our first approach involved the application of Kolmogorov–Smirnov and Levene tests to assess assumptions of normality and homoscedasticity. When such tests confirmed the respective assumptions ($P > 0.05$), we used the parametric Analysis of Variance (ANOVA) to compare means; alternatively, if any of such assumptions were violated ($P < 0.05$), we recurred to the Welch's ANOVA test (Sokal and Rohlf, 1995; Lix et al., 1996; McDonald, 2008; Zar, 1999). We used Tukey's HSD (for ANOVA) and Games-Howell (for Welch's ANOVA) post hoc tests to express differences between groups in pairwise comparisons.

We also performed the multivariate data-reduction procedures of Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) using meristic (scale and spot counts) and morphometric (head and body measurements) variables to assess variations that could support the distinction of *Helicops* sp. nov. from other spotted congeners. With the PCA – an exploratory technique without *a priori* definition of groups – we generated a set of principal components (hereafter PCs) that account for most of the variance in our original dataset and produced scatterplots revealing the distribution of individuals in the multivariate space (Manly, 2000). Nonetheless, we employed the DFA to assess if the variations in our meristic and morphometric features allowed the attribution of individuals to our operational groups defined *a priori* (Manly, 2000), and produced scatterplots revealing the segregation levels in the multivariate space. For multivariate treatments, we excluded juveniles (to avoid problems associated with allometry), and all adult specimens missing data for any of the variables. Based on the similar size of full-grown adults of our spotted-patterned taxa, we used *Helicops leopardinus* as a parameter for the minimum size of mature specimens [i.e., all males with SVL >230 mm and females with SVL >255 mm are considered adults (Ávila et al., 2006)]. We express descriptive statistics as "minimum–maximum ($\overline{X} \pm$ SD, sample size)", where $\overline{X}$ = mean value, and SD = standard deviation. We used the software SPSS Statistics 17.0 (SPSS Inc., 2008) to calculate descriptive statistics and perform tests of comparison of means (ANOVA and Welch's ANOVA), while the multivariate procedures were developed in the R environment (version 3.6.2; R Core Team, 2019). We tested multivariate normality using MVN packages (Korkmaz et al., 2014), while the PCAs and DFAs employed respectively the "prcomp" function from STATS package (version 3.6.2; R Core Team, 2019) and the "lda" function from MASS package (Venables and Ripley, 2002).

2.7. Reconstruction of reproductive modes

We used the topology of Moraes-da-Silva et al. (2021) to reconstruct the evolution of reproductive modes within the genus *Helicops* employing linear parsimony with the aid of the software Mesquite 3.61 (Maddison and Maddison, 2019). Besides the topology of Moraes-da-Silva et al. (2021), we inferred three distinct hypothetical scenarios in which the positions of *Helicops* sp. nov. differ (see Results). In that sense, based on similarities that include general colour pattern (e.g., presence of small rounded spots, a chequered venter, and a light triangular snout spot) and scalation (e.g., presence/absence of intergenial scales, dorsal counts, and prominence of dorsal keels), our scenarios I–III respectively assume the species *H. hagmanni*, *H. leopardinus*, and *H. polylepis* as potential sister groups of the new species candidate.

We followed Braz et al. (2016: 36) in considering "reproductive mode" as a binary unordered character, coding oviparity as '0' and viviparity as '1', with the bimodality of *H. angulatus* reflecting two closely related lineages. Nonetheless, we herein attributed '?' (missing entries) to *H. boitata* and *H. nentur* (both with unknown reproductive modes). Thus, based on Hartweg & Oliver (1940: 25), García & Ceballos (1994: 153) and Suazo-Ortuño et al. (2018) [all reporting oviparity to *Manolepis putnami* (Jan, 1863)], Marques et al. (2015) [for *Xenopholis scalaris* (Wucherer, 1861) and our terminals of Xenodontinae], Braz et al. (2016), and Moraes-da-Silva et al. (2021) (both for Hydropsini), we obtained the following final codification: *Helicops angulatus* (two terminals respectively coded as 0 and 1), *H. boitata* (?), *H. carinicaudus* (1), *H. gomesi* (0), *H. hagmanni* (0), *H. infrataeniatus* (1), *H. leopardinus* (1), *H. modestus* (1), *H. nentur* (?), *H. phantasma* (1), *H. polylepis* (1), *Hydrops triangularis* (Wagler, 1824) (0), *Pseudoeryx plicatilis* (Linnaeus, 1758) (0), Hydrodynastini Zaher et al., 2009 (0), *Manolepis putnami* (0), Pseudoboini Bailey, 1967 (0), and *Xenopholis scalaris* (0). Finally, we follow Lee & Shine (1998) in employing the terminology of "forward transitions" (i.e., transitions from oviparity to viviparity) and "reverse transitions" (i.e., transitions from viviparity to oviparity).

3. Results

3.1. Dorsal counts and qualitative characters

Qualitative characters and dorsal counts supported the recognition of four distinct operational taxonomic units among the spotted-patterned *Helicops* of the upper Madeira River, three of which attributable to the names *Helicops hagmanni*, *H. leopardinus*, and *H. polylepis*, while the fourth one included all the 27 specimens of *Helicops* sp. nov. The most informative features that distinguish the latter from these three valid taxa (character states in parentheses/brackets) include (i) the presence of a roughly triangular light snout spot (shared with *H. hagmanni*; absent in *H. leopardinus* and *H. polylepis*), (ii) 19 dorsal scales at midbody [shared with *H. leopardinus*; 23 or more in *H. hagmanni* and *H. polylepis*, but see Costa et al. (2016) and Table 1 for the possible records of 21 dorsals in *H. hagmanni*]; (iii) moderate dorsal keels (shared with *H. leopardinus*; strong in *H. hagmanni* and *H. polylepis*), and (iv) the presence intergenial scales (shared with *H. hagmanni* and *H. polylepis*; absent in *H. leopardinus*).

The oviparous mode of reproduction [confirmed by one hatchling preserved with its respective eggshell (UFRO-R 2749), as well as field observations (see notes on the section about habitat and natural history)] represented compelling evidence supporting the recognition of *Helicops* sp. nov as an independent entity; in this region of the Amazonia, *H. hagmanni* comprises the only other spotted species with the same reproductive mode, but the aforementioned morphological characters (i.e., dorsal counts and keels prominence) render both entities different. Finally, *Helicops* sp. nov. has a unique hemipenis with asymmetrical lobes, unlike any other species of the whole tribe Hydropsini with known male genitalia (see Results on hemipenial morphology).

3.2. Statistical analyses

The ANOVA and/or Welch's ANOVA tests confirmed sexual dimorphism in all the four operational taxonomic units. Males and females differed in subcaudal counts (*Helicops* sp. nov.: $F_{1,23} = 129.17$, $P = 6.48 \times 10^{-11}$; *H. hagmanni*: $F_{1,34} = 69.52$, $P = 9.85 \times 10^{-10}$; *H. leopardinus*: $F_{1,59} = 56.06$, $P = 8.92 \times 10^{-10}$; *H. polylepis*: $F_{1,27} = 112.06$, $P = 4.12 \times 10^{-11}$) and tail length/total length ratio (*Helicops* sp. nov.: $F_{1,21} = 73.82$, $P = 2.57 \times 10^{-8}$; *H. hagmanni*: $F_{1,34} = 137.85$, $P = 1.64 \times 10^{-13}$; *H. leopardinus*: $F_{1,59} = 55.49$, $P = 4.68 \times 10^{-10}$; *H. polylepis*: $F_{1,19} = 27.67$, $P = 4.50 \times 10^{-5}$). Regarding ventral counts, only three of our operational units exhibited differences between sexes (*Helicops* sp. nov.: $F_{1,23} = 6.88$, $P = 1.50 \times 10^{-2}$; *H. hagmanni*: $F_{1,\ 34} = 28.16$, $P = 7.00 \times 10^{-6}$; *H. polylepis*: $F_{1,\ 27} = 8.23$, $P = 7.90 \times 10^{-3}$); while our sample of *H. leopardinus* showed no sexual dimorphism regarding this parameter (*H. leopardinus*: $F_{1,\ 66} = 0.002$, $P = 9.69 \times 10^{-1}$). Therefore, we performed all statistical comparisons for males and females separately.

Regarding the PCA applied only to male specimens, the first two PCs accounted for 55.02% and 26.40% of the total variance,

Table 1

Comparisons of the most informative features that distinguish *Helicops acangussu* sp. nov. among all other congeners. The information combines our original dataset with the data provided by Kawashita-Ribeiro et al. (2013), Costa et al. (2016), Moraes-da-Silva et al. (2019, 2021), and Murphy et al. (2020). Bold fonts indicate parameters that differ between the new species and each of its counterparts. Asterisks denote counts from the literature that we consider doubtful [21 dorsals at midbody for *H. hagmanni*, see Costa et al. (2016); 22 dorsals at midbody for *H. leopardinus*, see Kawashita-Ribeiro et al. (2013)] and the "?" indicate unknown parameters for particular taxa.

Species	Dorsal pattern	Triangular snout spot	Dorsals at midbody	Supralabials	Infralabials	Intergenials	Dorsal keels	Reproductive mode
Helicops acangussu sp. nov.	Spots	Yes	19–21	8	10	Yes	Moderate	Oviparous
Helicops angulatus	**Saddles**	**No**	19	8–9	9–11	**No**	**Strong**	Bimodal
Helicops apiaka	**Saddles**	**No**	19–21	8–9	9–12	**No**	**Strong**	?
Helicops boitata	**Blotches**	**No**	**25**	**10**	**12–13**	**No**	Moderate	?
Helicops carinicaudus	**Stripes**	**No**	19	7–8	9–10	**No**	Weak/Moderate	**Viviparous**
Helicops cyclops	**Saddles**	**No**	19	8	?	**No**	**Strong**	Bimodal
Helicops danieli	Spots	**No**	19	7–8	10–11	**No**	Moderate	**Viviparous**
Helicops gomesi	**Saddles**	**No**	19	8	10	**No**	**Strong**	Oviparous
Helicops hagmanni	Spots	Yes	**21*–27**	8	10–12	Yes	**Strong**	Oviparous
Helicops infrataeniatus	**Stripes**	**No**	17–19	8	10–12	**No**	Moderate	**Viviparous**
Helicops leopardinus	Spots	**No**	19–22*	8–10	8–10	**No**	Moderate	**Viviparous**
Helicops modestus	**Stripes**	**No**	19	8	9–11	Yes	**Weak**	**Viviparous**
Helicops nentur	**Uniform**	**No**	**17**	8	9–10	**No**	**Weak**	?
Helicops pastazae	Spots	**No**	**21–23**	8–10	8–10	Yes	**Strong**	Oviparous
Helicops petersi	Spots	**No**	**21–23**	8	**8**	Yes	**Strong**	?
Helicops phantasma	**Bands**	**No**	19	8	10	**No**	Moderate	**Viviparous**
Helicops polylepis	Spots	**No**	**23–25**	8–9	**11–13**	**No**	**Strong**	**Viviparous**
Helicops scalaris	**Blotches**	**No**	19–21	8–9	**8–9**	Yes	**Strong**	**Viviparous**
Helicops tapajonicus	**Uniform**	**No**	19	8	9–11	**No**	**Weak**	?
Helicops trivittatus	**Stripes**	**No**	**21–23**	8–9	**11–14**	Yes	Moderate	**Viviparous**
Helicops yacu	Spots	?	**25–28**	8–9	**11–12**	Yes	?	?

respectively. Tail length represents the most related variable with PC1, while the number of dorsal spots on trunk represented the most related variable with PC2. Considering females, the PCA resulted in the first two PCs accounting for 50.52% and 30.68% of the total variance, respectively. Head length and number of dorsal spots on trunk represented the most related variables with PC1 and PC2 respectively. For both sexes (Fig. 1 A–B), the graphic combination of the first two axes derived from scores of PCAs shows considerable segregation of *Helicops leopardinus* and *H. polylepis*, while *H. hagmanni* and *Helicops* sp. nov. appear as entirely overlapping clusters.

Regarding the DFAs (i.e., with operational groups determined *a priori*), the scatterplot combining the two first axes derived from scores of discriminant functions (Fig. 1 C–D) maintained the identity of *Helicops leopardinus* and *H. polylepis* as independent groups. Nonetheless, although the scatterplot of females differed little from the one of the pertinent PCA (Fig. 1 C), most males of *H. hagmanni* and *Helicops* sp. nov. appear slightly discriminated in the pertinent graphic representation (Fig. 1 D). In both DFAs, the variables explaining the major proportion of the variance include head length (in males), tail length (in females), and number of dorsal spots on trunk (both sexes).

3.3. Taxonomic decision

Qualitative parameters and dorsal counts allow for the unequivocal distinction of *Helicops* sp. nov. not only from the three most similar and potentially sympatric congeners but also from all other counterparts. Also, although our multivariate analyses have mostly failed to show segregations between *H. hagmanni* and *Helicops* sp. nov., their results confirm the distinction of the latter from at least *H. leopardinus* and *H. polylepis*. Finally, although *Helicops* sp. nov. and *H. hagmanni* share the oviparous mode of reproduction, they differ unequivocally at least in dorsal counts (19 vs. 23–27) and prominence of keels (moderate vs. strong), representing clearly distinct morphological entities. Therefore, we herein formally recognise *Helicops* sp. nov. as a new species that we describe in the following section.

3.3.1. *Helicops acangussu sp. nov*

Holotype (Figs. 2 and 3).— An adult female (UFRO-R 2748) collected on September 23rd, 2011 (unknown collector), in the region of the municipality of Porto Velho, left bank of the upper Madeira River, section II of the inundation area of the Santo Antônio Dam (9°2′56.03″S, 64°18′17.70″W, 86 masl), Rondônia state, Brazil.

Paratypes.— UFRO-R 2736, 2739–40, 2743, MPEG 25581, 25584, 25587–88 (adult females); UFRO-R 2737–38, 2741–42, 2745–47, MPEG 25577–80, 25582–83, MPEG 25585 (adult males); UFRO-R 3070, 2744, 2749, 2750 (young males). All paratypes were collected in the same region of the holotype, from June 21st to November 26th of 2011 (precise coordinates available in the Appendix).

Diagnosis.— *Helicops acangussu* sp. nov. differs from all congeners by the following unique combination of characters: (1)

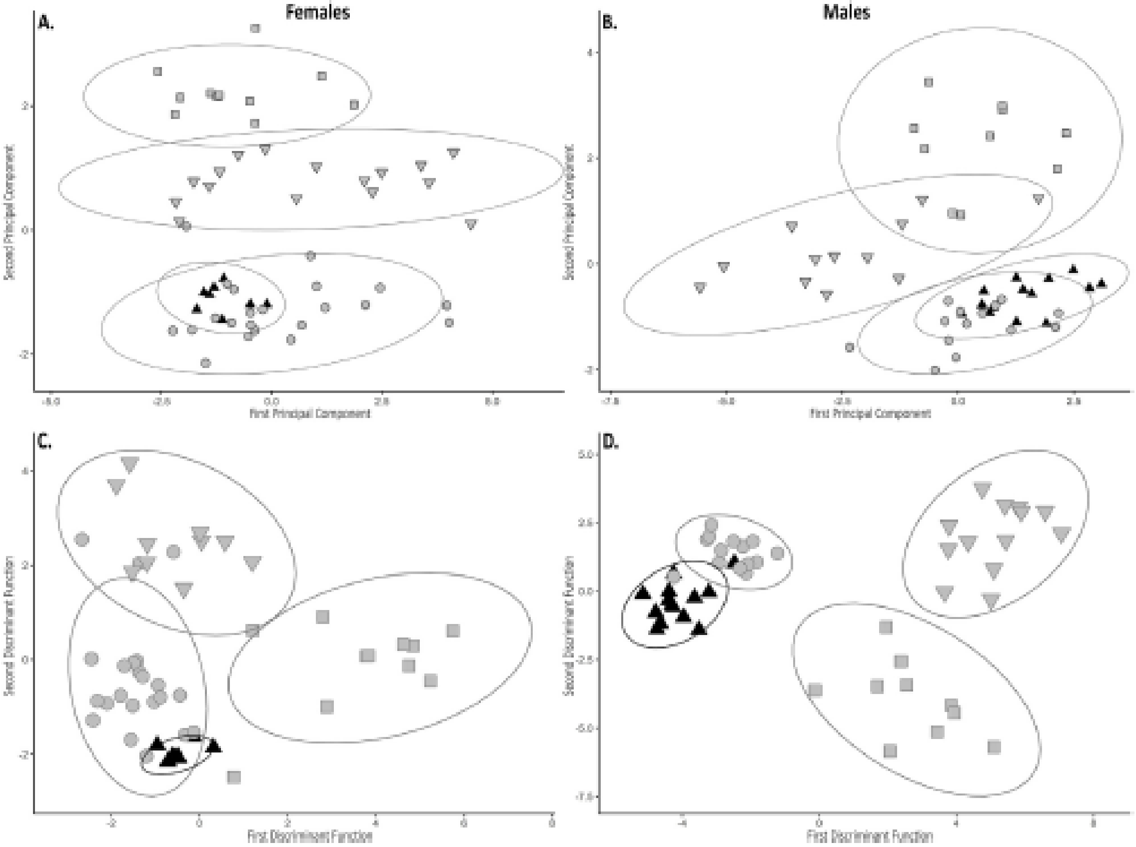

Fig. 1. Scatterplots of multivariate analyses (PCAs and DFAs) applied to the four operational taxonomic units of spotted-patterned *Helicops* recognised in this study (black triangles: *Helicops* sp. nov.; grey circles: *H. hagmanni*; grey squares: *H. leopardinus*; and inverted grey triangles: *H. polylepis*). On top, the bivariate plots with 95% confidence regions for the first two axes derived from scores of PCAs for females (A) and males (B); below, the bivariate plots with 95% confidence regions for the first two axis derived from scores of DFA for females (C) and males (D).

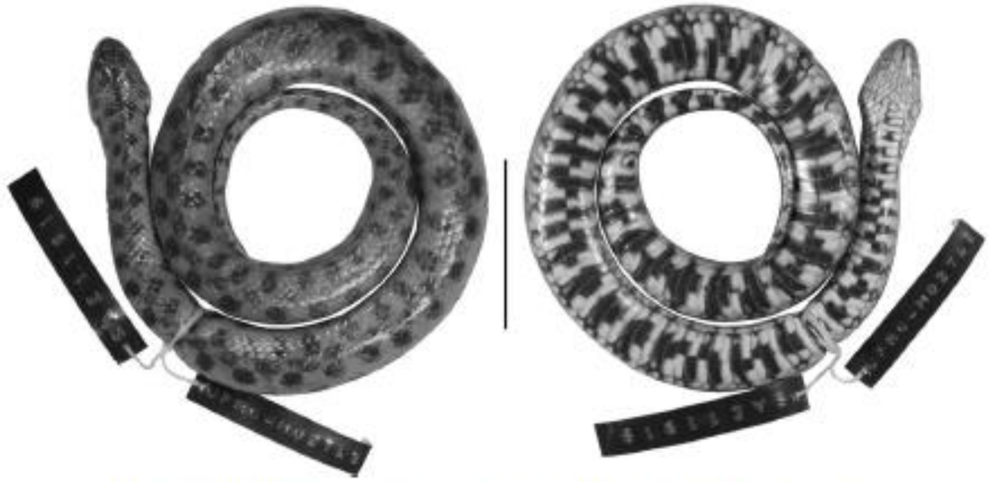

Fig. 2. Dorsal and ventral views of the holotype of *Helicops acangussu* sp. nov. Scale bars = 45 mm.

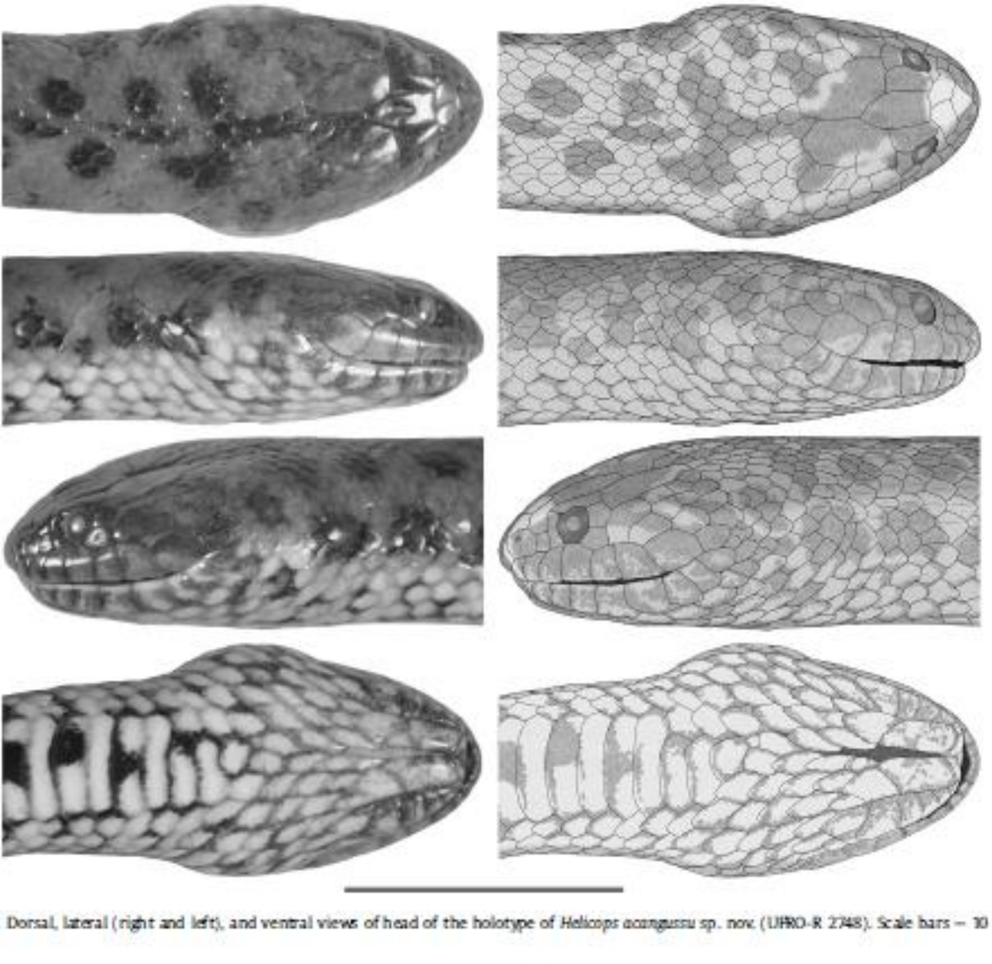

Fig. 3. Dorsal, lateral (right and left), and ventral views of head of the holotype of *Helicops acangussu* sp. nov. (UFRO-R 2748). Scale bars = 10 mm.

dorsals 19–21/19/17–18 with moderate dorsal keels throughout the whole body length; (2) ventrals 121–129 (121–128 in males, 124–129 in females); (3) subcaudals 40–67 (52–67 in males, 40–43 in females); (4) subcaudal keels absent; (5) supralabials 8/8, 4th with orbit; (6) infralabials 10/10; (7) intergenials separating the 2nd pair of genials; (8) nasal semidivided; (9) one preocular; (10) two postoculars; (11) maxillary teeth 16–18 + 2; (12) hemipenis semicapitated, lobes asymmetrical (one of the lobes distinctly longer its counterpart), with medium-sized spines dispersed on lateral surface of body; (13) snout exhibiting a triangular light spot (light tan in life) usually covering the rostral, internasals, prefrontals, and anterior borders of frontal and supraoculars; (14) dorsum greenish grey, with five rows of black rounded spots, smaller on flanks and increasing in size towards vertebral level; (15) 35–47 dorsal spots on trunk (35–47 in males, 36–42 in females), and 10–23 dorsal spots on tail (13–23 in males, 10–15 in females); (16) venter background yellowish cream, checkered with asymmetrical black squared blotches, mostly divided at midline with their halves alternately displaced in opposite directions, and (17) 37–52 black bands on venter (37–52 in males, 38–44 in females), and 10–20 black bands on tail (13–20 in males, 10–14 in females).

Comparisons.— *Helicops acangussu* sp. nov. is distinguishable from 11 out of the 20 congeners by having small and rounded black spots on dorsum (vs. saddles, in *H. angulatus*, *H. apiaka*, *H. cyclops*, and *H. gomesi*; stripes, in *H. carinicaudus*, *H. infrataeniatus*, *H. modestus*, and *H. trivittatus*; irregular bands, in *H. phantasma*, and uniform, in *H. nentur* and *H. tapajonicus*). That leaves nine nominal taxa with rounded dorsal spots or blotches (*H. boitata*, *H. danieli*, *H. hagmanni*, *H. leopardinus*, *H. pastazae*, *H. petersi*, *H. polylepis*, *H. scalaris* and *H. yacu*), which distinction from the new species must rely on other character sources (Table 1).

Dorsal counts of 19 scale rows at midbody distinguish *Helicops acangussu* sp. nov. at least from *H. boitata* (25), *H. hagmanni* (23–27), *H. pastazae* (21–23), *H. petersi* (21–23), *H. polylepis* (22–25), and *H. yacu* (25–28). Among the species sharing the same number of dorsals (*H. danieli*, *H. leopardinus*, and *H. scalaris*), *H. acangussu* sp. nov. is readily recognisable by the presence of intergenials (absent in *H. danieli* and *H. leopardinus*) and absence of subcaudal keels (present in *H. scalaris*). In addition, the moderate dorsal keels of the *H. acangussu* sp. nov. differ unequivocally from the strong keels of *H. hagmanni*, *H. pastazae*, *H. petersi*, *H. polylepis*, and *H. scalaris*. Regarding infralabial counts, *H. acangussu* sp. nov. (10 scales) appears to differ from *H. boitata* (12–13), *H. petersi* (8), *H. polylepis* (11–13), *H. scalaris* (8–9), and *H. yacu* (11–12).

Although the features compared in Table 1 already allow for the identification of *Helicops acangussu* sp. nov. among all other spotted congeners, some of these species differ remarkably from the former in qualitative parameters (e.g., *H. boitata*, with a unique ventral pattern and head shape; see Moraes-da-Silva et al., 2019), while others may require closer examination when one has mixed samples at hand. This applies more specifically to the *H. hagmanni*, *H. leopardinus*, and *H. polylepis*, all occurring in the region of the new species.

The moderate keels of *Helicops acangussu* sp. nov. (Fig. 4 A; similar to those of *H. leopardinus*, Fig. 4 B) also differ from *H. hagmanni* (Fig. 4 C) and *H. polylepis* (Fig. 4 D). In addition, the presence of a roughly triangular light snout spot (Fig. 5 A) in the new species (similar to the one of *H. hagmanni*, see Fig. 5 C) distinguishes it from *H. leopardinus* (Fig. 5 B) and *H. polylepis* (Fig. 5 D) (both bearing a uniform brown snout). Finally, although the chequered ventral pattern of *Helicops acangussu* sp. nov (Fig. 6 A). resembles the patterns present in *H. leopardinus* (Fig. 6 B) and *H. hagmanni* (Fig. 6 C), it differs remarkably from the mostly black venter with sparse pale spots of *H. polylepis* (Fig. 6 D).

Additionally to the number of dorsal rows at midbody, Table 2 shows other meristic parameters of the new species that differ from the three spotted congeners occurring in the region of the upper Madeira River. Among the four spotted congeners, *Helicops acangussu* sp. nov. has (i) the lowest counts of subcaudals, (ii) more ventrals than *H. leopardinus*, (iii) less dorsal spots on trunk than *H. leopardinus*, and (iv) more spots on trunk than *H. hagmanni*. The ANOVA also detected differences in ventral counts of *H. acangussu* sp. nov. and *H. hagmanni*, but these differences remained restricted to females.

Lastly, the hemipenes of five specimens of *Helicops acangussu* sp. nov. examined herein had one of the lobes slightly atrophied, resulting in an asymmetric morphology (Fig. 7). Such a condition is unique among the species of the whole tribe, although it occurs in at least one semi-aquatic snake of the family Colubridae (sensu Zaher et al., 2009; see Discussion). Nevertheless, among the most similar congeners, the hemipenis of *H. hagmanni* (Fig. 8 A) differs from the organ of the new species strictly due to the asymmetric nature of the latter, while *H. polylepis* (Fig. 8 B) and *H. leopardinus* [see Fig. 7 of Moraes-da-Silva 2019, 2021] exhibit major differences associated with capitation (stronger in *H. polylepis* and much less pronounced in *H. leopardinus*), shape (e.g., proportional length of body and lobes) and ornamentation (size of spines and depth of calyces).

Description of holotype (Figs. 2 and 3)**.—** Adult female, body moderately stout (midbody diameter/SVL = 0.03); tail length about one-fourth of total length (tail length/total length = 0.19); head roughly elliptical, almost twice as long as wide (head width/head length = 0.64), distinct from neck (neck width/head width = 0.59); snout short, rounded in dorsal view, slightly wider than long (snout width/snout length = 1.36), one-fourth the length of head, slightly acuminate (snout length/head length = 0.24); eyes relatively small, slightly displaced dorsally; eye diameter much shorter than head length (eye diameter/head length = 0.13) and slightly longer than one-half the distance from its anterior margin to nostril center (eye diameter/eye-nostril distance = 0.98); pupil rounded; rostral rounded, visible from above, roughly pentagonal in frontal view, not projecting over lower jaw; internasal single, triangular, slightly wider than long, lateral margins concave, posterior margin convex; internasal contacting dorsal vertex of rostral, dorsal margins of nasals, and anterior margins of prefrontals; prefrontals paired, wider than long, asymmetrical in size (left scale larger), roughly quadrangular, posterior margin in "S" shape; frontal pentagonal, longer than wide, its larger width almost one-half its length; frontal contacting posterior margins of prefrontals, medial margins of supraoculars and anterior margins of parietals; supraoculars long, twice as long as wide, roughly pentagonal, contacting posterior margin of prefrontal, lateral margin of frontal, anterior margin of parietal, dorsal margin of upper postocular and dorsal margin of eye; parietals paired, roughly trapezoidal; parietals longer than wide, their largest width slightly shorter than the length of their mutual suture; lateral margin of right parietal notched; parietals contacting posterior margins of frontal, supraocular and upper postocular, dorsal margin of upper temporals and anteriormost dorsals; nasal semidivided, roughly hexagonal, in contact with lateral margin of rostral, internasal, and prefrontals, anterior margin of loreal, and dorsal margins of 1st and 2nd supralabials; nasal suture perpendicular to ventral margin of nasal, not contacting nostril; nostril positioned near dorsal margin of nasal, entirely visible from above; loreal quadrangular, slightly higher than long, contacting posterior margin of nasal, lateral margin of prefrontal, anterior margin of preocular and dorsal margins of 2nd (right side), and 2nd–3rd (left side) supralabials; oculars 1+2; preocular much higher than long, irregular in shape, longer on upper and lower areas and distinctly narrow in front of eye; preocular contacting posterior margin of loreal, lateral margin of prefrontal, anterolateral margin of supraocular, anterior margin of eye, and upper margin of 3rd supralabial; upper postocular roughly pentagonal, almost twice as high as long, in contact posterolateral margin of supraocular, anterior margin of parietal and primary temporal, upper margin of lower postocular, and upper posterior margin of eye; lower postocular pentagonal, slightly longer than high, contacting lower margin of upper postocular, anterior margin

Fig. 4. Uncollected specimens of *Helicops acangussu* sp. nov. (A), *H. leopardinus* (B), *H. hagmanni* (C), and *H. polylepis* (D) photographed in life, allowing for comparisons of colour pattern and prominence of keels. Specimens of the three former species are from the margins of the Madeira River, area of influence of the Santo Antônio Dam, in the region of the municipality of Porto Velho, Rondônia state, Brazil, while the specimen of *H. polylepis* is from the BR-319 Road, in the Madeira-Purus interfluve, Amazonas state, Brazil. Photos: F.F. Curcio (A–C) and R. Nunes (D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 5. Detail of the head of the specimens shown in Fig. 4 [*Helicops acangussu* sp. nov. (A), *H. leopardinus* (B), *H. hagmanni* (C), and *H. polylepis* (D)] photographed in life, allowing for comparisons of snout pattern. Photos: F.F. Curcio (A–C) and R. Nunes (D).

of primary temporal, dorsal margins of 4th–6th supralabials, and upper posterior margin of eye; temporals 1+2+3 /1+3+3, primary temporal slightly higher than long, contacting posterior margins of upper and lower postoculars, anterolateral margin of parietal, anterior margins of secondary temporals, posterior upper vertex of 5th supralabial (left side) and upper margin of sixth supralabial (both sides of head); secondary and tertiary temporals roughly pentagonal, positioned between posterolateral margin of parietal and dorsal margins of 6th–8th supralabials; supralabials 8/8; 4th/3rd–4th with orbit; 6th largest; mental triangular, as long as wide; two pairs of chinshields, anterior pair three times as long as second pair; intergenials present between second pair of chinshields; infralabials 10/10, 1st–5th contacting anterior chinshields, 5th–6th contacting posterior chinshields; 6th infralabial largest, roughly

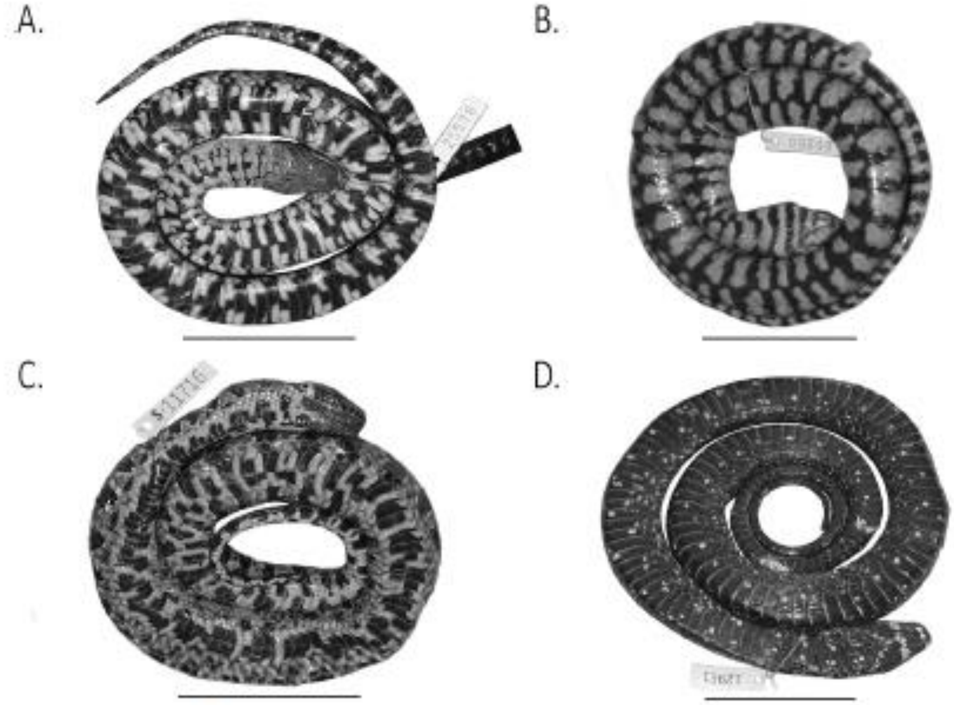

Fig. 6. Ventral views of preserved specimens illustrating the similar colour patterns of *Helicops acangussu* sp. nov. (A), *H. leopardinus* (B), and *H. hagmanni* (C), as well as their general differences from the mostly black venter with sparse pale spots of *H. polylepis* (D).

Table 2

—Comparison of quantitative characters among *Helicops acangussu* sp. nov. and the three most similar and sympatric congeners. *F* and *W's F* correspond respectively to the statistics of the ANOVA and Welch's ANOVA tests; bold fonts indicate parameters that differ between tha new species and each of its counterparts (Tukey HSD and Games–Howell probabilities follow).

	Helicops acangussu sp. nov.	*Helicops leopardinus*	*Helicops hagmanni*	*Helicops polylepis*	Overall comparison of means
Ventrals					
Females	124–129 126.88 ± 1.88, 8	**113–123** **($116.82 \pm 3.06, 11; P = 1.74 \times 10^{-7}$)**	121–138 ($130.27 \pm 3.62, 22; P = 8.00 \times 10^{-2}$)	119–132 ($127.44 \pm 3.61, 16; P = 9.80 \times 10^{-1}$)	$F_{3, 53} = 40.77$ $P = 8.45 \times 10^{-14}$
Males	121–128 124.50 ± 1.99, 14	**111–125** **($116.60 \pm 5.38, 10; P = 5.00 \times 10^{-3}$)**	120–128 ($124.57 \pm 2.17, 14; P = 1.00$)	120–130 $124.00 \pm 2.61, 13; P = 9.44 \times 10^{-1}$)	$W's\ F_{3, 23.31} = 6.52$ $P = 2.30 \times 10^{-3}$
Subcaudals					
Females	40–43 41.63 ± 1.19, 8	**60–74** **($67.64 \pm 4.46, 11; P = 2.14 \times 10^{-9}$)**	**46–64** **($52.27 \pm 3.67, 22; P = 10.00 \times 10^{-12}$)**	**72–85** **($77.94 \pm 3.99, 16; P = 8.50 \times 10^{-13}$)**	$W's\ F_{3, 26.24} = 430.95$ $P = 1.98 \times 10^{-22}$
Males	52–67 56.14 ± 3.98, 14	**70–87** **($79.10 \pm 5.19, 10; P = 5.72 \times 10^{-13}$)**	**54–67** **($62.57 \pm 3.52, 14; P = 1.71 \times 10^{-3}$)**	**89–104** **($95.38 \pm 4.89, 13; P = 5.72 \times 10^{-13}$)**	$F_{3, 47} = 216.00$ $P = 1.72 \times 10^{-27}$
Dorsal spots on trunk					
Females	36–42 22.35 ± 1.65, 8	**46–65** **($23.51 \pm 5.16, 11; P = 6.59 \times 10^{-13}$)**	**27–39** **($25.46 \pm 6.18, 22; P = 4.50 \times 10^{-3}$)**	34–46 ($26.03 \pm 7.34, 16; P = 7.70 \times 10^{-1}$)	$F_{3, 53} = 100.93$ $P = 6.65 \times 10^{-22}$
Males	35–47 39.71 ± 2.73, 14	**44–64** **($53.60 \pm 7.79, 10; P = 1.18 \times 10^{-3}$)**	**29–37** **($33.79 \pm 2.39, 14; P = 1.10 \times 10^{-5}$)**	35–46 ($39.46 \pm 2.78, 13; P = 9.95 \times 10^{-1}$)	$W's\ F_{3, 23.30} = 28.99$ $P = 4.87 \times 10^{-8}$

pentagonal; 1st pair of infralabials in contact at midline, separating mental from chinshields; dorsals 21/19/17, mostly keeled (keels virtually absent on 1st–3rd dorsal rows), without apical pits; keels intensity week in anterior one-third, and moderate on posterior two-thirds of body; anal plate divided; ventrals 128; subcaudals 43/43; maxillary teeth 16+2 diastema present; postdiastemal teeth ungrooved, distinctly larger than prediastemal ones.

Measurements of the holotype.— Total length = 502 mm (SVL = 403 mm, tail length = 99 mm); head length = 20.34 mm; head width = 13.02 mm; snout length = 4.96 mm; snout width = 5.32 mm; right eye diameter = 2.78 mm; eye-nostril distance = 2.82 mm; neck width = 7.75 mm; diameter at midbody = 13.63 mm.

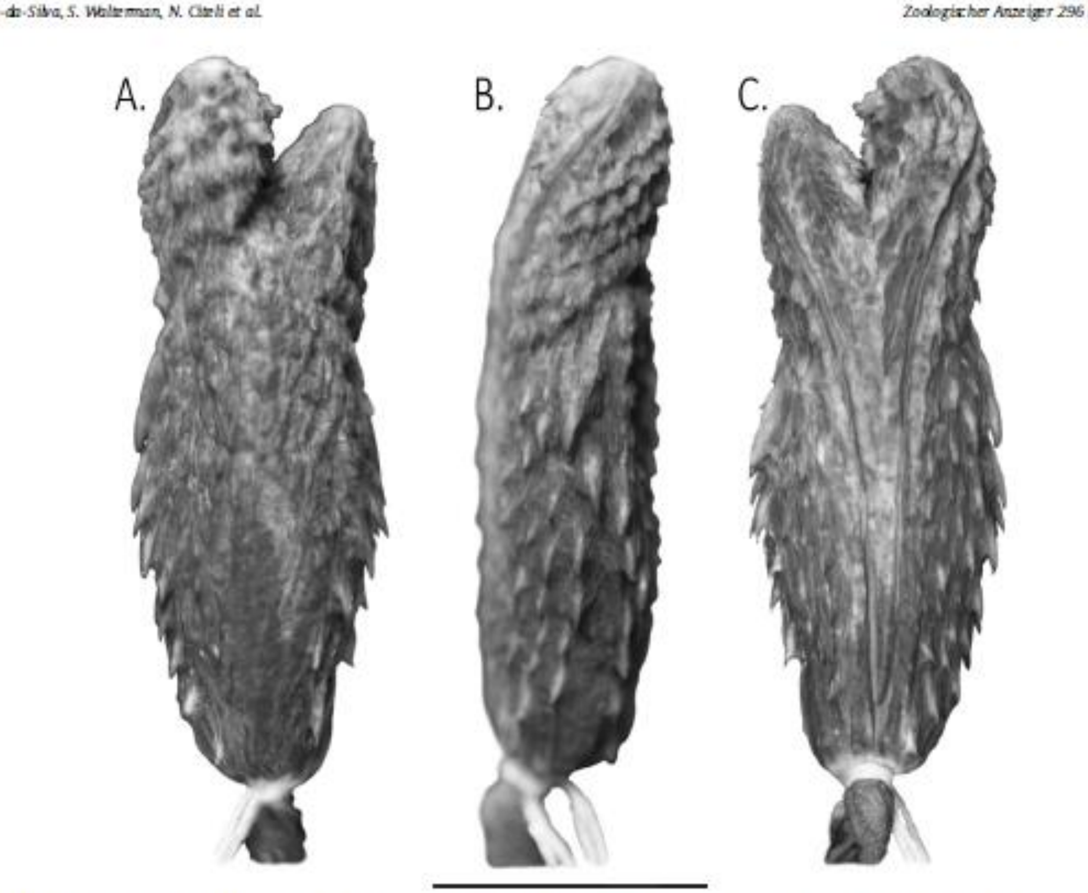

Fig. 7. Asulcate (A), lateral (B), and sulcate (C) faces of the right hemipenis of paratype UFRO-R 2742 of *Helicops acangussu* sp. nov.. Scale bar = 10 mm.

Variation of meristic and morphometric characters.— Largest specimen a female with 695 mm in total length (MPEG 25587; SVL = 587 mm, tail length = 108 mm); largest male (MPEG 25578) with 563 mm in total length; (SVL = 431 mm, tail length = 132 mm), smallest juvenile (UFRO-R 2749) a hatchling male with (SVL = 115 mm, tail length = 34 mm); preoculars 1/1 (16 specimens), 1/2 (three asymmetric specimens), and 2/2 (seven specimens); postoculars 2/2 (25 specimens), and 1/1 (one specimen); supralabials 8/8 (23 specimens), 8/9 and 7/8 (respectively two and one asymmetric specimens); 4^{th} supralabial with eye in 20 specimens, 3^{rd}–4th in four specimens, and two asymmetric specimens have 4^{th} /4^{th}–5^{th} (MPEG 25580), 3^{rd}–4^{th}/4^{th}–5^{th} (MPEG 25585) with eyes; 6^{th} supralabial the largest in 24 specimens, 6^{th}/7^{th} in two asymmetric specimens; infralabials 10/10 (n = 25), 10/11 (MPEG 25585; one asymmetric specimen); infralabials in contact with first pair of chinshields 1^{st}–5^{th} (22 specimens), and 1^{st}–4^{th} (four specimens); intergenials present only in the second pair of chinshields (n = 21), rarely absent (five specimens); dorsals 19/19/17 (n = 17), 19/19/18 (n = 1), 20/19/17 (n = 6), 21/19/17 (n = 2); ventrals 121–129 [males: 121–128 (124.6 ± 2.09, 17); females: 124–129 (126.8 ± 1.88, 8)]; subcaudals 40–67 [males: 52–67 (56 ± 3.71, 16); females 40–43 (41.4 ± 1.24, 9)]; dorsal spots on trunk 35–47 [males: 35–47 (39.8 ± 2.72, 17); females 36–42 (39.1 ± 2.08; 9)]; dorsal spots on tail in 10–23 [males: 13–23 (16.3 ± 2.55, 16); females: 10–15 (12.0 ± 1.73, 9)]; number of ventral blotches 37–52 [males: 37–52 (42 ± 4.18, 17); females: 38–44 (39.5 ± 2.0, 9)]; number subcaudal blotches 12–20 [males: 13–20 (15.75 ± 1.65; 16); females: 10–14 11.0 ± 2.32, 9)]; maxillary teeth 16+1 (five specimens), 17+2, (16 specimens), and 18+2 (three specimens). Characters that do not vary in our sample include the condition of the nasal scale (always semi divided), presence of loreals, and infralabials contacting second pair of chinshields.

Hemipenial Morphology (Fig. 7).— Description based on the fully everted organs of five specimens [UFRO-R 2737 (right organ, partly expanded), UFRO-R 2742 (right organ, fully expanded; Fig. 7), UFRO-R 2745 (left organ, fully expanded), UFRO-R 2746 (left organ, partially expanded), and UFRO-R 2747 (right organ, partly expanded)]. Inverted organ eight or nine subcaudals in length, distal end of retractor magnus divided. Hemipenis strongly bilobed, bicalyculate, semicapitate, with very shallow capitular groove; body conical, nearly two-thirds the total length of organ; lobes elongate, asymmetrical (one lobe always shorter than its counterpart), without apical disk; short lobe slightly acuminate, long lobe club-shaped and globose on tip; short lobe slightly divergent; long lobe parallel to respective margin of body; basal pocket absent; small spinules sparsely covering base, increasing in size towards lobes on asulcate face, running divergently until midpoint of body, and then converging until interlobular crotch; central area of asulcate face mostly nude, comprising an elyptical surface with sparse and very small spinules; flanks of body and outer areas of sulcate and asulcate faces ornamented by medium and large-sized spines; intrasulcar area of body lacking spines and ornamented by sparse small spinules; lobes encircled by five prominent spinulate flounces; lobe calyces shallow, irregular in shape, papillate; papillae ornamented by very small spinules; sulcus spermaticus deep, bifurcate, centrifugally oriented, dividing at a proximal point of

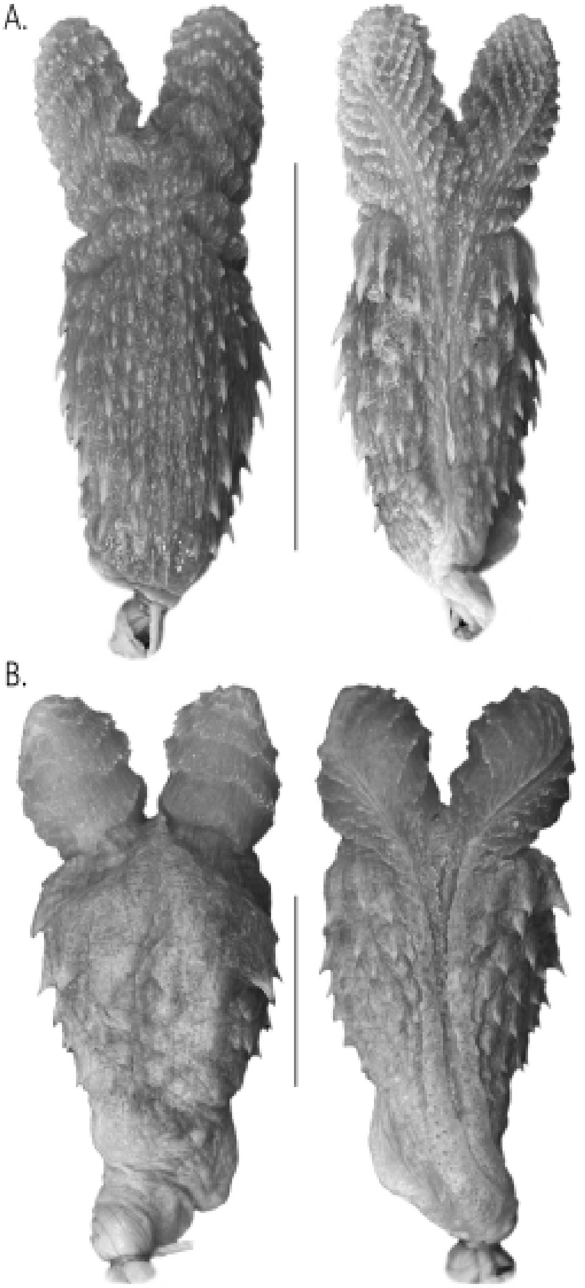

Fig. 8. Asulcate (left) and sulcate (right) faces of the fully everted and maximally expanded right hemipenis of *Helicops hagmanni* (MPEG 12371), from Bom Jesus, Bragança, state of Pará (A), and *H. polylepis* (MPEG 16715), from Cipó-Pitanga Island, Baía de Souzel, Xingu River, state of Pará, Brazil (B). Scale bars = 10 mm.

base, running throughout the median surface of body and assuming a centrifugal direction at a point proximal to lobes base, to end laterally at lobe tip.

Colouration of holotype in preservative (Figs. 2–3, 7 **A).—** Head dorsum greenish grey to copper brown, with dark brown pigment irregularly concentrated on parietals and interocular region; snout dorsum with a triangular light spot covering internasals, medial area of prefrontals, and borders of adjacent scales including lateral margins of rostral and anterior margins of nasal and 1st supralabial; anteriormost spots of paravertebral and vertebral rows connected at midline, the latter projecting anteriorly until parietals; two anteriormost pairs of spots of lateral spot rows respectively positioned on temporal region and quadrate-mandibular articulation; spots on temporal region distinctly lighter than the ones of quadrate-mandibular region and dorsum; supralabials mostly brown, 5th–7th less pigmented on central and anteroventral areas of scales; infralabials cream on background, conspicuously and irregularly marked with brown; head venter background cream, chinshields sparsely pigmented with brown, gular scales with brown pigment predominantly concentrated on posterior tips; dorsum background predominantly greenish light grey progressively fading to cream on paraventral region, at level of the three first dorsal rows; dorsum with five (two lateral, two paravertebral, and one vertebral) rows of rounded black spots; black spots increasing in size from neck to midpoint of body, and then decreasing in size towards tail tip; largest length and width of midbody dorsal spots up to 2.0 and 2.5 scales respectively; dorsal spots separated from each other by 1.5–2.0 (in a same row) and 1.0–1.5 (between rows) scales; 54 spots counted longitudinally on right lateral spot row (41 on trunk, 13 on tail); venter background light cream with alternated rectangular black blotches extending from neck to tail tip; ventral blotches covering 1.0–2.0 ventrals in length, and usually wider than half the width of one ventral shield; blotches on ventral surface of tail covering 1.5–2.5 subcaudals in length; 55 blotches counted longitudinally on right side of venter and tail (44 on trunk, 11 subcaudals).

Colouration in life [based on uncollected specimens (one adult and one juvenile)] from the left margin of the Madeira River, region of Porto Velho, state of Rondônia.— One adult specimen photographed in life exhibited a yellowish light green dorsum background, and the black spots exhibited a non-uniform distribution of black pigment, more concentrated in the suture region than on the surface of the scales (Fig. 4 A). Head pattern (Fig. 5 A) consisted of a greenish marmorated cap covering parietals, temporals, posterior tip of frontal, supralabials, oculars, loreal, and most of the nasal scales, with a distinctly darker tonality on interocular region; the snout exhibited a light tan and roughly triangular spot covering the upper tip of rostral, anterior border of nasals and 1st supralabials, most of the central area of internasal, almost the whole surface of prefrontals, as well as the anterior borders of frontal and supraocular. The nape region had a faint light collar incomplete at vertebral level and extending from the quadrate-mandibular region to the vertebral region, contacting a long black spot that extends from nape to parietals' tips (Fig. 5 A).

Juveniles have a dark brown dorsum background and the black pigment of dorsal spots more concentrated than in adults (Fig. 9 A). The head cap is distinctly darker than the dorsum background colour, with the nape collar and snout spot evident and light tan; the nape collar exhibits a pair of faint tan projections covering the temporal region on both sides of head, with its anterior limit behind the postoculars (Fig. 9 B).

Habitat and Natural History.—All specimens were observed and/or collected in forest environments with water bodies and small creeks nearby the Madeira River, during the wildlife rescue operations associated with the Santo Antônio Dam. On September 28th of 2011, a clutch with 12 eggs was found in the field (same region of collection of the holotype; Fig. 9 D) and then kept in the lab for five days until nine spotted individuals eventually hatched; all the hatchlings had 19 dorsal rows and an evident light tan triangular snout spot (Fig. 9 A–C), corroborating their attribution to *Helicops acangussu* sp. nov. One of the paratypes (UFRO-R 2749) is a newborn hatchling preserved with its respective eggshell. The adult individual responded aggressively to manipulation, promptly biting when handled for the photographic records.

Fig. 9. Records of oviparous reproduction and details of juvenile colouration features of *Helicops acangussu* sp. nov.. A five-day old specimen exhibiting the general colouration features of juveniles (A–B); a juvenile artificially removed from the egg, still connected to the yolk sac (C), a 12-egg clutch found in the field (D) from which the specimens illustrated on A–C emerged. All hatchlings of this clutch were released in the field, following a requirement from the wildlife rescue procedures in the area of influence of the Santo Antônio Dam, in the municipality of Porto Velho, state of Rondônia, Brazil. Scale bars = 20 mm.

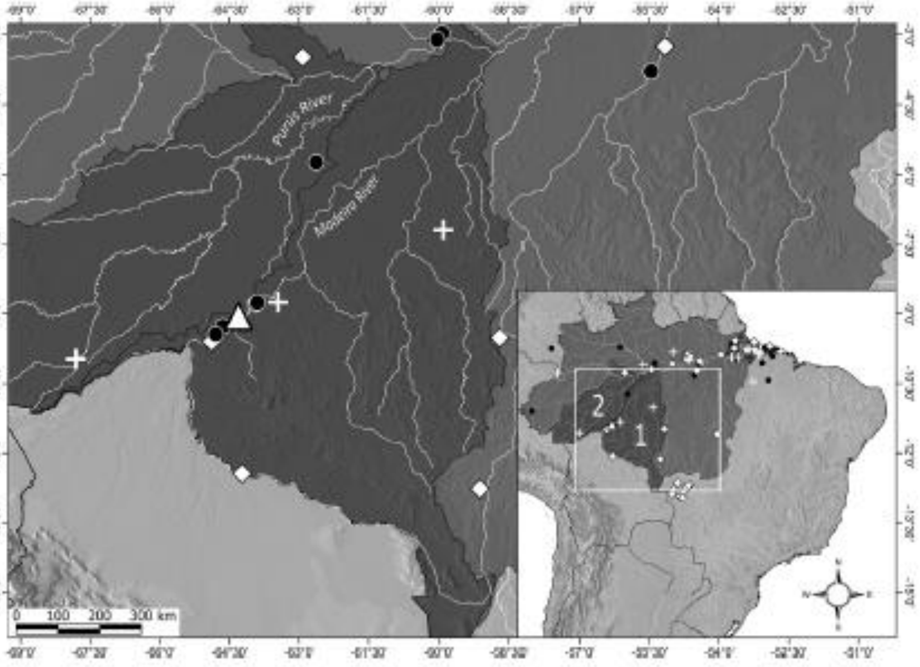

Fig. 10. Locality records of the *Helicops* specimens with spotted dorsal pattern in our sample: white triangle: *Helicops acangussu* sp. nov. (type locality); black circles: *H. hagmanni*; white diamonds: *H. leopardinus*; white crosses: *H. polylepis*. Gray shading represents the limits of Brazilian Legal Amazonia, including the Madeira (1) and Purús (2) Basins. The localities of the paratypes of *H. acangussu* sp. nov. do not appear in the map, but lie on the left bank of the Madeira River, very close to the type locality (see Appendix for precise coordinates).

Etymology.— The specific epithet is a noun in apposition derived from the Tupi-Guarani (*akãgu'su*; *a'kãga* meaning "head", *u'su* meaning "large"), a vernacular designation to the Jaguar [*Panthera onca* (Linnaeus, 1758)], a spotted South American big cat. Herein, it represents an allusion to the spotted pattern of the new species, as well as to the aggressive behaviour of most *Helicops* species that promptly bite when disturbed.

Distribution (Fig. 10).—All specimens of *Helicops acangussu* sp. nov. come from the left bank of the Madeira River, in the region of the municipality of Porto Velho, state of Rondônia, northern Brazil, in the limit zone between the Madeira and Purus basins. The whole type series was obtained during the wildlife rescue operations in the area of influence of the Santo Antônio Dam, from June to November of 2011. The localities of the paratypes lie very close to each other, and the type locality serves as a reference to the distributional area of the whole sample. Precise coordinates of all collection localities appear in the Appendix.

3.4. Reconstruction of reproductive modes

Fig. 11 shows the reconstructions of reproductive modes in *Helicops* on the strict topology of Moraes-da-Silva et al. (2021) (Fig. 11 A), as well as in three hypothetical scenarios derived from it in which *H. acangussu* sp. nov. appears respectively as the sister-group of *H. hagmanni* (scenario I; Fig. 11 B), *H. leopardinus* (scenario II; Fig. 11 C), and *H. polylepis* (scenario III; Fig. 11 D). For the sake of clarity, we used the reconstructions on the original topology of Moraes-da-Silva et al. (2021) as a comparative parameter to

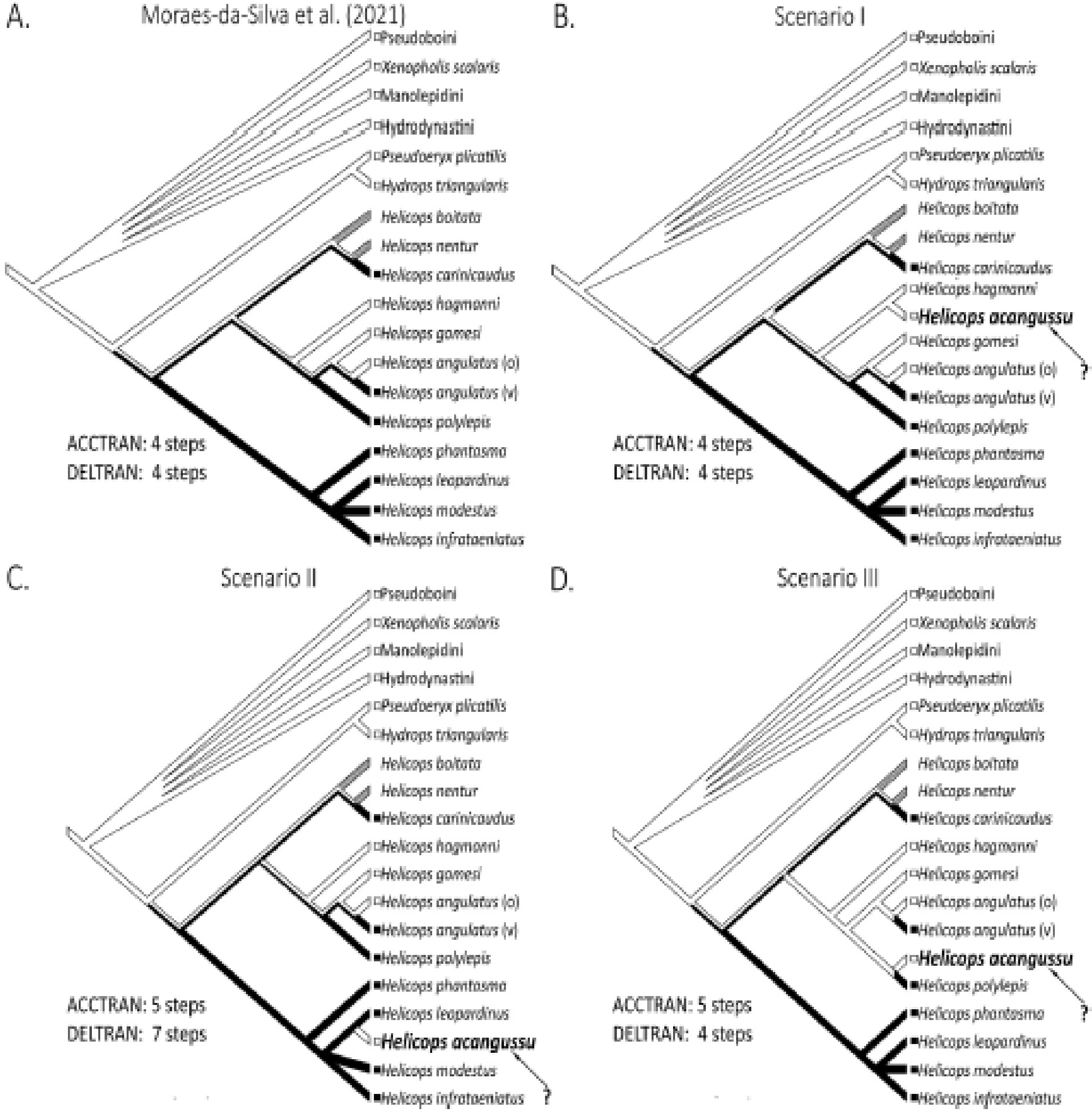

Fig. 11. Reconstructions of the reproductive modes in the genus *Helicops* mapped on the phylogenetic assumption of Moraes-da-Silva et al. (2021) (A), and in three hypothetical scenarios assuming *Helicops acangussu* sp. nov. as closely related respectively to *H. hagmanni* (Scenario I; B), *H. leopardinus* (Scenario II; C), and *H. polylepis* (Scenario III; D). Squares positioned to the left of terminals denote their respective oviparous (white squares) and viviparous (black squares) reproductive modes, following the colours of branch filling. Dashed branches denote terminals to which reproductive modes remain unknown.

assess the evolutionary unfoldings on the preconceived scenarios derived from it. All descriptions assume forward and reverse transitions as equally likely, although evolutionary constraints associated with the redevelopment of the oviparous condition will be considered in the Discussion section.

Original topology [Moraes-da-Silva et al. (2021); Fig. 11 A].— The ACCTRAN and DELTRAN optimisation systems render two equally parsimonious reconstructions (four steps). Considering an oviparous ancestor for the genus *Helicops* (DELTRAN system), viviparity must have emerged at least four times within the genus, respectively in *H. carinicaudus*, *H. polylepis*, one lineage of *H. angulatus*, and the most recent ancestor of the clade (*H. phantasma* (*H. leopardinus*, *H. modestus*, *H. infrataeniatus*)). Alternatively, if viviparity has already emerged in the common ancestor of all *Helicops* lineages (ACCTRAN system), one should expect at least three reverse transitions (*H. hagmanni*, *H. gomesi*, and one lineage of *H. angulatus*) also computing four steps.

Scenario I (Fig. 11 B)**.—** Application of DELTRAN and ACCTRAN systems to the preconceived topology of scenario I render virtually identical reconstructions to those described to the original topology (both with four steps). The only difference that deserves notice concerns the reverse transition in the most recent ancestor of the clade (*Helicops acangussu* sp.nov., *H. hagmanni*) if one assumes a viviparous origin for the genus (ACCTRAN system), while in the original topology such a reversal should be restricted to *H. hagmanni*. Morphological similarities that might support the sister-group relationship between *H. acangussu* and *H. hagmanni* include the spotted dorsal pattern, the chequered ventral colouration, and the presence of a triangular light snout spot, while the oviparous reproductive mode represents a plesiomorphic condition at least when considering the DELTRAN optimisation system.

Scenario II (Fig. 11 C)**.—** A presumed sister-group relationship between *Helicops acangussu* sp. nov and *H. leopardinus* may find support on the presence of 19 dorsals at midbody, moderate dorsal keels, and similar colour patterns (dorsum and venter), assuming that at least one of such features represents an apomorphic character state. In this context, both DELTRAN and ACCTRAN optimisation systems imply less parsimonious assumptions when compared with the original topology and scenario I. If oviparity represents the ancestral condition of the genus *Helicops* (DELTRAN system), forward transitions to viviparity should have occurred up to seven times, in *H. carinicaudus*, *H. polylepis*, *H. phantasma*, *H. leopardinus*, *H. modestus*, *H. infrataeniatus*, and in the viviparous lineage of *H. angulatus*. In contrast, although the ACCTRAN system results in fewer steps (with the emergence of viviparity in the *Helicops* ancestor and reverse transitions in *H. hagmanni*, *H. gomesi*, the oviparous lineage of *H. angulatus*, and *H. acangussu* sp. nov.), the final reconstruction of scenario II (with five steps) also reflects a less parsimonious assumption when compared with the two previous ones.

Scenario III (Fig. 11 D)**.—** A putative relationship between *Helicops acangussu* sp. nov. and *H. polylepis* could rest primarily on the superficial similarity of the dorsal pattern shared by both taxa, while most other features of colour (ventral and snout patterns) and scalation (number of dorsals at midbody and prominence of keels) of these species differ remarkably. Nonetheless, reconstructions of reproductive modes for scenario III result in more parsimonious assumptions when compared with those of scenario II, irrespective of the optimisation system adopted. Assuming an oviparous *Helicops* ancestor (DELTRAN system) implies four independent origins of viviparity (forward transitions) in *H. carinicaudus*, *H. polylepis*, one lineage of *H. angulatus*, and in the common ancestor of the clade (*H. phantasma* (*H. leopardinus*, *H. modestus*, *H. infrataeniatus*)). Alternatively, considering a recent origin of viviparity in a common *Helicops* ancestor (ACCTRAN system) implies four reverse transitions respectively in *H. hagmanni*, *H. gomesi*, the oviparous lineage of *H. angulatus* and *H. acangussu* sp. nov. rendering a final account of five steps (as parsimonious as the ACCTRAN reconstruction for scenario II, and one step more costly than both reconstructions for scenario I).

4. Discussion

As any new species assumption, the recognition of *Helicops acangussu* sp. nov. as a full species depends not only on demonstrating that it differs unambiguously from its most similar congeners (i.e., an unequivocal diagnosis), but also on objective arguments supporting its association with theoretical species concepts (Rosen, 1978; 1979; Cracraft, 1983; Donoghue, 1985; Queiroz, 2007). Therefore, in the absence of explicit phylogenetic treatments including terminals of *H. acangussu* sp. nov., we recur to the recent topology of Moraes-da-Silva et al. (2021) to discuss our new species assumption based on its potential affinities.

Altogether, the most recent phylogenetic studies have already included 12 out of the 20 valid *Helicops* taxa in their analyses, covering 60% of the genus content (Grazziotin et al., 2012; Costa et al., 2016; Moraes-da-Silva et al., 2019, 2021). In that sense, the topology of Moraes-da-Silva et al. (2021) supports the following lines of reasoning: (i) the widespread dorsal patterns of small rounded spots (seven species) and longitudinal stripes (four species) occur each in at least two independent clades; (ii) the presence of dorsal saddles represents a synapomorphy of a component including the species *H. angulatus* and *H. gomesi*, both also exhibiting subcaudal keels supporting their close kinship, and (iii) a major clade comprising the spotted-patterned *H. hagmanni* and *H. polylepis*, as well as the saddled-patterned *H. angulatus* and *H. gomesi* share strong dorsal keels as a putative (and highly consistent) synapomorphy.

The presence of a light snout spot, intergenial scales, 19 dorsals at midbody, and moderate dorsal keels comprises an unambiguous diagnosis of *Helicops acangussu* sp. nov. allowing its prompt recognition among all other congeners. Nonetheless, such external morphological features do not include unambiguous autapomorphies of the new species, failing to accommodate the parameters defended by Rosen (1978, 1979) and Donoghue (1985) in attributing species status to minimal monophyletic clusters. For instance, intergenials and a light triangular snout spot are both shared with *H. hagmanni*, while the presence of 19 dorsals at midbody and moderate keels are far from unique among all congeners. Therefore, any assessments of such features as potential autapomorphies of *H. acangussu* sp. nov. would only make sense in a phylogenetic framework.

Considering the lack of explicit phylogenetic treatments including samples of *Helicops acangussu* sp. nov., any considerations about its relationships with other congeners are necessarily speculative. Nonetheless, based on the topology of Moraes-da-Silva et al. (2021), one may discuss the evolutionary unfoldings associated with our reconstructions of reproductive modes (Fig. 11; see Results section) considering (i) potential synapomorphies reflected by morphological similarities shared by the new species and other *Helicops* taxa and (ii) the biological constraints that would hamper transitions from viviparity to oviparity (e.g., Lee & Shine, 1998; Griffith et al., 2015).

The topology of Moraes-da-Silva et al. (2021) implies three independent origins of small dorsal spots in the genus *Helicops* (namely in the taxa *Helicops hagmanni*, *H. polylepis*, and *H. leopardinus*). In that sense, since a fourth origin in *H. acangussu* sp. nov. would result in loss of parsimony, our phylogenetic discussion assumes three scenarios in which, based on similarities of dorsal pattern, the new species could represent the sister-lineage of any of the three spotted taxa. Nonetheless, we concede that future studies that include terminals of *H. acangussu* sp. nov. may reveal different topologies that would require new interpretations.

When describing the reconstructions of reproductive modes, we considered forward and reverse transitions equally likely in a strict parsimony context. Nonetheless, the literature demonstrates that transitions from oviparity to viviparity must be much more frequent than the reverse among squamates, suggesting that lineages that acquire the viviparous mode of reproduction would hardly redevelop the oviparous condition, reflecting a mild version of Dollo's law (Dollo, 1893, 1922; see Lee and Shine, 1998: 1446). Furthermore, among other highly compelling arguments, Griffith et al. (2015: 497) claim that the biological constraints that would prevent transitions from viviparity to oviparity would include the necessary – and unlikely – redevelopment of a series of morphological and physiological features related to the deposition of the eggshell, as well as the re-evolution (not the simple re-expression) of oviparity-specific genes. Thus, despite the ambiguous nature of the early reproductive condition of *Helicops* indicated in our reconstructions, we assume that the DELTRAN system (considering an oviparous *Helicops* ancestor) renders generally more likely assumptions than those assuming viviparity as the ancestral state (i.e., ACCTRAN system).

A possible sister-group relationship of *Helicops acangussu* sp. nov. and *H. hagmanni* (Fig. 11 B, scenario I) may find support on the presence of intergenials and features of colour pattern (a chequered venter and a light triangular snout spot) if such character states appear as apomorphic in a global congruence analysis. Nonetheless, such a topology would imply at least one reversal event of the strong dorsal keels – present in the more comprehensive clade including *H. hagmanni*, *H. polylepis*, *H. angulatus* and *H. gomesi* – to the moderate condition, that would then represent an autapomorphy of the new species. Finally, in this context, the oviparous condition shared by *H. acangussu* and *H. hagmanni* would represent a symplesiomorphy.

Considering an alternative scenario in which *Helicops acangussu* sp. nov. figures as the sister group of *H. leopardinus* (Fig. 11 C, scenario II), the only potentially apomorphic features of external morphology that could support their relationships include the number of dorsals at midbody (19) and the superficial similarity of dorsal and ventral patterns. Nonetheless, such a topology denotes a less parsimonious scenario than the one involving *H. hagmanni* (Fig. 11 B), implying up to seven independent origins of viviparity. Furthermore, even if one assumed the unlikely possibility of reverse transitions (ACCTRAN system, with a viviparous *Helicops* ancestor) the final cost of such a reconstruction (five steps) would still overcome the one of scenario I (four steps). However, one must mention that scenario II reflects one of the few instances in which Lee & Shine (1998: 1443, 1445) consider reverse transitions strongly supported because the oviparous terminal (*H. acangussu* sp. nov.) would appear nested within a set of viviparous lineages (*H. phantasma*, *H. leopardinus*, *H. modestus* and *H. infrataeniatus*).

Helicops polylepis represents our third potential sister group of *Helicops acangussu* sp. nov (Fig. 9 D; scenario III), based strictly on similarities of dorsal pattern, since the ventral colouration (mostly black with pale spots vs. chequered), dorsal counts (23–25 vs. 19), and the prominence of keels (strong vs. moderate) of both taxa differ remarkably. As it happens in scenario I, such a hypothesis would imply four independent origins of viviparity from an early *Helicops* ancestor, with the moderate dorsal keels representing an autapomorphy of the new species.

In contrast with external characters, hemipenial morphology corroborates more objectively our taxonomic decision because it reflects a putative unique autapomorphic condition supporting the monophyly of *Helicops acangussu* sp. nov. as an independent lineage. Although possibly suggestive of a malformation at a first sight, the asymmetric structure of the hemipenial lobes is consistently present in the five males of which we were able to examine hemipenes, representing an odd and highly peculiar feature. A similar – and much more pronounced – instance of asymmetry also occurs at least in the semi-aquatic natricine *Natriciteres fuliginoides* Günther, 1858 (Bogert, 1940: 33), and one group of alopoglossid lizards (Harris, 1994; Hernández-Morales et al., 2020) exhibits asymmetric ornamentations. Thus, these distantly related taxa indicate that genitalia asymmetries may reflect a more widespread phenomenon among squamates, requiring further investigations.

From a spatial perspective, the discovery of *Helicops acangussu* sp. nov. apparently limited to few localities in the southwestern Amazonia may reflect not only the lack of long-term surveys throughout the Amazonian lands (Duellman, 2005; Avila-Pires et al., 2009) but also biogeographic assumptions in which the Madeira River plays a role as a major vicariant agent (Avila-Pires, 1995; Silva et al., 2005; Ferrão et al., 2017). Among amphibians and reptiles, the long-known relevance of the Madeira River as a barrier separating distinct faunas in the eastern and western portions of the Amazonia (Wallace, 1852; Silva et al., 2005; Ferrão et al., 2018) receives support from recent studies focused on anurans (Dias-Terceiro et al., 2015) and lizards (Avila-Pires, 1995; Peixoto et al., 2020). Although anurans include a larger proportion of taxa restricted to one of the riverbanks, Peixoto et al. (2020) argue that part of this tendency could reflect a taxonomic bias resulting from higher study efforts in this group, while many lizard taxa await taxonomic and phylogeographic studies that might reveal undescribed and allopatric entities in the region.

The discovery of *Helicops acangussu* sp. nov. in only one of the margins of the Madeira River suggests that snakes might also include lineages restricted to opposite margins of a large riverine barrier. As it happens to lizards, *H. leopardinus*, *H. hagmanni* and *H. polylepis* – herein considered potentially related to the new species based on global similarity – comprise widespread nominal taxa that may represent species complexes to be identified with the aid of taxonomic revisions [e.g., populations tentatively attributed to *H. hagmanni* from the state of Acre, northern Brazil; see also distributional maps of Nogueira et al. (2019) and comments of Moraes-da-Silva et al., 2019, 2021)]. Thus, long-term sampling efforts of specimens and tissue samples in this particular area of the Amazonia may comprise an important source of information to assess the general distributional pattern of *Helicops* species in the region, as well as the biogeographic relevance of the Madeira River and its minor tributaries in the Madeira basin.

Lastly, we must emphasize that discussing riverine barriers as distributional limits to watersnakes implies a matter of scale. Although we concede that aquatic environments may favour the dispersal of such organisms, large and deep rivers may hamper the free exchange of small-sized individuals between two margins with at least 1,000 m of distance from each other, as it happens in this region of the upper Madeira River. Nonetheless, the geological dynamics associated with the formation of the major hydrographic basins, as well as the results of these processes in the final environmental structure could also promote events of speciation even for taxa that use aquatic environments as dispersal routes (Moraes-da-Silva et al., 2019, 2021). The finding of *H. acangussu* sp. nov. in such a unique geographical context should stimulate further research and also contribute to the debate on the conservation of the Amazonia in one of its most emblematic areas.

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.

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Appendix - Specimens Examined

All specimens of *Helicops acangussu* sp. nov. come from different localities, although very close to each other. For this species, we provide institutional numbers of each voucher, as well as the respective coordinates in parentheses, although these points do not appear as independent marks in the map (see captions for Fig. 10). For all other taxa, we provide data on localities as "**COUNTRY: Department/State/Province:** Locality: institutional identifier (latitude, longitude)".

***Helicops acangussu* sp. nov. (n = 27). BRAZIL: Rondônia**: Left bank of upper Madeira River, region of Porto Velho: UFRO-R 2736 (9°03'51"S/64°18'46"W); UFRO-R 2737 (9°08'55"S/64°22'22"W); UFRO-R 2738 (9°08'50"S/64°22'18"W); UFRO-R 2739 (9°08'49"S/64°22'16"W); UFRO-R 2740 (9°08'48"S/64°22'17"W); UFRO-R 2741 (9°08'32"S/64°22'09"W); UFRO-R 2742 (9°08'28"S/64°22'11"W); UFRO-R 2743 (9°08'30"S/64°22'14"W); UFRO-R 2744 (9°08'31"S/64°22'09"W); UFRO-R 2745 (9°08'43"S/64°22'24"W); UFRO-R 2746 (9°08'10"S/64°22'01"W); UFRO-R 2747 (9°08'09"S/64°22'00"W); UFRO-R 2748 (holotype; 9°02'56"S/64°18'17"W); UFRO-R 2749 (9°03'08"S/64°18'17"W); UFRO-R 2750 (9°03'08"S/64°18'17"W); UFRO-R 3070 (9°09'32"S/64°22'28"W); MPEG 25577 (9°00'39"S/64°14'11"W); MPEG 25578 (9°07'10"S/64°21'15"W); MPEG 25579 (9°07'45"S/64°16'17"W); MPEG 25580 (9°08'13"S/64°22'08"W); MPEG 25581 (9°07'43"S/64°21'32"W); MPEG 25582 (9°07'43"S/64°21'32"W); MPEG 25583 (9°07'44"S/64°21'33"W); MPEG 25584 (9°07'47"S/64°21'35"W); MPEG 25585 (9°07'37"S/64°21'47"W); MPEG 25587 (9°08'03"S/64°21'53"W); MPEG 25588 (9°07'33"S/64°21'46"W).

***Helicops angulatus* (*n* = 27). BRAZIL: Maranhão**: Santa Luzia do Paruá, BR-316 Road: MPEG 12815, 12820–21, 13552, 13634–36 (2°35'34"S/45°44'48"W). **Mato Grosso**: Apiacás, Igarapé do Bruno: UFMT-R 7437, 7440 (8°46'40"S/57°48'48"W); Araputanga, PCH Ombreiras: UFMT-R 2806 (15°07'50"S/58°43'48"W); Aripuanã, Cachoeira dos Patos: UFMT-R 6806 (9°52'09"S/60°16'16"W); Aripuanã, Córrego Bota Fora, right bank of Aripuanã River: UFMT-R 6669 (9°54'12"S/60°18'21"W); Aripuanã, Mining Company Geominas: UFMT-R 7201 (9°55'07"S/60°18'39"W); Alto Alegre dos Parecis, Santa Rita Farm: UFMT-R 7092 (12°07'25"S/61°51'41"W); Brasnorte, PCH Bocaiúva: UFMT-R 8019 (12°31'42"S/57°52'24"W); Cáceres, Sepotuba River: UFMT-R 929 (15°58'26"S/57°39'57"W); Campo Verde, ALT Sadia: UFMT-R 11807 (15°34'18"S/55°10'19"W); Chapada dos Guimarães, APM Manso: UFMT-R 516 (14°53'28"S/55°47'14"W); Cláudia, Continental Farm: UFMT-R 3676 (11°26'10"S/55°02'20"W); Colniza, small stream at camp site: UFMT-R 7212 (9°2'41"S/59°05'09"W); Colniza, Rio Roosevelt Ecological Station: UFMT-R 7815 (9°57'59"S/60°41'23"W); Cuiabá, Rio Claro: UFMT-R 6783 (15°28'35"S/55°53'28"W). RONDÔNIA: Machadinho d'Oeste, M6 Pedral: MZUSP 21683, 21684 (9°24'57"S/62°01'03"W); Porto Velho, Jirau Hydroelectric Power Plant, left bank of Madeira River: MZUSP 20733 (9°15'57"S/64°40'04"W); Porto Velho, Jirau Hydroelectric Power Plant, Abunã: MZUSP 21119 (9°16'43"S/64°38'38"W); Rio Mutum-Paraná: MZUSP 3227 (9°36'55"S/64°56'45"W).

***Helicops apiaka* (*n* = 10). BRAZIL: Mato Grosso**: Alta Floresta: UFMT-R 5482, 7710 (9°30'48"S/57°19'06"W); Guarantã do Norte: UFMT-R 4473, 5714 (9°56'51"S/54°56'08"W); Paranaíta, left margin (downstream) of Rio Teles Pires: UFMT-R 7975, 7983, 8004, 8169, 8180, 8244 (9°32'24"S/56°24'47"W).

***Helicops boitata* (*n* = 6)**. BRAZIL: **Mato Grosso**: Poconé, Transpantaneira Road: UFMT-R 11940 (16°25'21"S/56°40'12"W), Poconé, Transpantaneira Road, "Passagem do Boitatá", Pouso Alegre Lodge: UFMT-R 12504–06, 12520–21 (16°29'27"S/56°44'33"W).

***Helicops carinicaudus* (*n* = 23). BRAZIL: Espírito Santo**: Aracruz, Assombro, near Carretas Road: MNRJ 23844 (19°41'00"S/40°17'00"W); Aracruz, Barra do Sahy, Imetame: MNRJ 23849 (19°52'00"S/40°05'00"W); Cachoeira de Itapemirim: MNRJ 4942 (20°50'03"S/41°06'22"W); Cariacica: MZUSP 18046 (20°14'37"S/40°24'57"W); Jaguaré: MNRJ 23843 (18°54'00"S/40°04'00"W); Linhares, Goytacazes National Forest: MNRJ 23847 (19°26'53"S/40°04'51"W); Linhares, Linhares–Regência Road: MNRJ 23848 (19°24'00"S/40°04'00"W); Linhares, Road to Pontal do Ipiranga, near detour to Agrovila: MNRJ 23841–42 (19°24'00"S/40°03'00"W); Pedro Canário, Cristal do Norte: MNRJ 23846 (18°05'00"S/40°06'00"W); Presidente Kennedy, Restinga Praia das Neves: MNRJ 18712 (21°06'00"S/41°02'00"W); Aracruz, Retiro: MNRJ 23845 (19°49'00"S/40°16'00"W); Santa Teresa: MZUSP 5033 (19°56'00"S/40°36'00"W); São Mateus, BR-101 Road, near bridge over Rio São Mateus: MNRJ 23840 (22°16'00"S/41°52'00"W). **Goiás**: Goiânia: MPEG 6 (16°38'00"S/49°15'00"W). **Minas Gerais**: Cabeceira Grande MNRJ 7029–30 (15°59'14"S/47°11'53"W); **Rio Grande do Sul**: Unknown locality: MZUSP 1323. **Rio de Janeiro**: Magé, Km 133.5 from BR-116 Road: MNRJ 19159 (22°32'00"S/42°59'00"W); Cachoeiras de Macacu, Guapiaçu Ecological Reserve: MNRJ 18085 (22°27'00"S; 42°46'00"W); Nova Iguaçú, Adrianópolis: MNRJ 23628 (22°39'56"S/43°29'50"W). **São Paulo**: Guarujá: MZUSP 12213 (23°59'36"S/46°15'23"W); Itanhaém: MZUSP 1321 (24°10'59"S/46°47'21"W).

***Helicops danieli* (*n* = 2)**. COLOMBIA: **Atlántico**: Barranquilla MZUSP 2144–45 (11°00'54"N/74°48'00"W).

***Helicops gomesi* (n = 4). BRAZIL. São Paulo**: Itapura: MZUSP 1391, 1398 (20°40'02"S/51°31'02"W); Rio Passa Cinco: MZUSP 18139 (22°21'00"S/47°51'00"W); São Joaquim da Barra, Rio Sapucaí Mirim: MZUSP 14186 (20°35'00"S/47°51'00"W).

***Helicops hagmanni* (*n* = 51). BRAZIL: Acre**: Mâncio Lima, Colônia MZUSP 8001 (7°36'58"S/72°54'01"W). **Amapá**: Rio Maracá, mouth of Igarapé Camaipi: MZUSP 11716 (0°26'17"S/51°26'02"W). **Amazonas**: Barcelos, Ucuqui, Igarapé Javari, left bank of Rio Aracá, tributary of Rio Negro: MNRJ 10127 (0°45'16"S/63°11'14"W); INPA-

WWF 6 Reserve: MZUSP 7615 (2°25'25"S/59°43'02"W); Novo Airão: IBSP 80594, 87354, 87356 (2°37'17"S/60°56'39"W); Manaus, Reserva Ducke, Ripária 020: INPA-H 20152 (2°57'47"S/59°55'19"W); Manaus, Reserva do 41, BR-174, KM 41: INPA-H 2174 (3°06'07"S/60°01'30"W). Parque Nacional Nascentes do Lago Jari, Igarapé do Sabá: INPA-H 28824 (5°44'41"S/62°37'55"W). **Pará**: Augusto Corrêa, Cacoal: MPEG 6501 (1°01'18"S/46°38'06"W); Belém: MPEG 10908 (1°27'21"S/48°30'16"W); Bom Jesus, Bragança: MPEG 12371 (1°04'07"S/46°41'22"W); Capitão Poço, São Pedro: MPEG 10484–10485, 10497 (1°44'47"S/47°03'34"W); Castanhal, Boa Vista, Apeú: MPEG 541, 1998, 5923 (1°17'51"S/47°59'16"W); Curuçu, Marauá: MPEG 4893 (0°44'07"S/47°51'07"W); Dom Eliseu, Km 222, Bela Vista Ranch, old PA 70 road: MPEG 13379, 17300 (4°17'06"S/47°30'18"W); Fordlândia: MZUSP 5128 (3°49'29"S/55°29'43"W); Juruti, Mutum, Barroso: MPEG 22488 (2°09'08"S/56°05'32"W); Melgaço, Caxinauã National Forest: MPEG 22403 (1°47'50"S/51°26'07"W); Oriximiná, Aviso Plateau, Saracá-Taquera National Forest, Porto Trombetas: MNRJ 16833 (01°29'00"S/56°23'00"W); Ourém, Puraquequara: MPEG 4997, 7020, 7024 (1°33'07"S/47°06'52"W); Peixe-Boi: MPEG 1820 (1°11'31"S/47°18'44"W); Santarém, Lago do Jacundá, Alter do Chão: MPEG 18791 (2°30'13"S/54°57'09"W); Santarém Novo, Trombetinha: MPEG 3462, 3464, 4784, 7053, 7059–60, 7959 (0°58'46"S/47°14'19"W); Tomé-Açú, Moju-Acará Road, Ipitinga: MPEG 13350, 15515 (2°25'08"S/48°09'08"W); Vigia, Santa Rosa, Vigia Road (1°11'48"S/46°08'24"W; MPEG 4013, 8533); Viseu, Carupati: MPEG 8874 (1°11'48"S/46°08'24"W); Viseu, Km 220 of BR-316, old km 74, Contagem de Capanema: MPEG 5259 (1°11'48"S/46°08'24"W). **Rondônia**: Porto Velho, Jirau Hydroelectric Power Plant, right bank of Madeira River: MZUSP 18869 (9°26'14"S/64°47'58"W); Porto Velho, Jirau Hydroelectric Power Plant: UFRO-R 308–09 (8°47'21"S/63°56'26"W [approximate coordinates]); Porto Velho, Jirau Hydroelectric Power Plant: MPEG 26056 (9°16'18"S/64°38'25"W); Porto Velho: CEPB 980 (8°45'43"S/63°54'14"W). **COLOMBIA: Vaupés**: Caparú Station, Taraira Lake, northern bank of Apaporis River: IAVH-R 3215 (0°51'06"S/70°48'40"W).

***Helicops infrataeniatus* (*n* = 17). BRAZIL: Goiás**: Aparecida de Goiânia: MPEG 24239–43 (16°51'00"S/49°15'00"W); Aporé, Corrente River: MZUSP 10887–88 (18°58'15"S/51°56'05"W); Goiânia: CEPB 2270 (16°42'00"S/49°14'00"W). **Mato Grosso do Sul**: Anaurilândia, Paraná Farm: MZUSP 10615–16 (22°09'43"S/52°39'08"W); Madeira Island: MZUSP 11670 (unknown coordinates). **Rio Grande do Sul**: Taim Ecological Station: MZUSP 7430 (32°26'06"S/52°31'05"W); Viamão, Granja Pimenta: MPEG 19602 (30°04'00"S/51°02'00"W); Dom Pedro de Alcântara, Canto da Leffa: MPEG 19603 (29°22'00"S/49°51'00"W); São Leopoldo, Sinos River: MZUSP 4769 (29°47'04"S/51°10'01"W). **Santa Catarina**: Lages: MZUSP 9666–67 (27°49'59"S/50°18'59"W).

***Helicops leopardinus* (*n* = 68).** BRAZIL: **Amapá**: D.N.E.R. Reserve, Tracajatuba River: MPEG 58 (0°41'00"S/51°22'00"W); Macapá: MPEG 347, 426 (0°00'17"S/51°4'00"W). **Amazonas**: Careiro, Jacaretinga Lake MZUSP 8204 (3°28'54"S/62°55'27"W); Manaus: MZUSP 7678 (3°07'00"S/60°01'48"W); Urucará: MPEG 23773 (2°30'36"S/57°48'36"W). **Mato Grosso**: UFMT SESC Scientific Base: UFMT-R 11918 (16°15'18"S/56°36'59"W); Cáceres, Sepotuba River: UFMT-R 998 (16° 4'00"S/57°41'00"W); Cáceres, Point A10; Module Baía de Pedra: UFMT-R 7901–02 (16°30'59"S/57°50'13"W); Cotriguaçu, São Nicolau Farm, Peugeot: UFMT-R 8257 (9°30'51"S/58°40'43"W); Cuiabá, 08 de Abril Avenue, stream Mané Pinto: UFMT-R 1966 (15°34'59"S/56°04'59"W); Cuiabá, Porto: UFMT-R 1504 (15°29'20"S/55°53'22"W); Taiamã Island: MZUSP 7623–24 (16°55'00"S/56°46'40"W); Juína: MZUSP 20778–80 (12°46'08"S/59°06'17"W); Nossa Senhora do Livramento, Pirizal: UFMT-R 7792–94, 7893–94, 8344, 8470–72 (15°46'30"S/56°20'46"W); Poconé (16°15'01"S/56°37'00"W; UFMT-R 11939); Poconé, Transpantaneira Road: UFMT-R 58, 1796; 4665 (16°47'06"S/56°56'54"W); Porto Estrela, Serra das Araras Ecological Station: UFMT-R 10686 (15°19'28"S/57°13'44"W); Santo Antônio do Leverger, Repouso Ranch: UFMT-R 7769 (15°51'59"S/56°04'40"W); Transpantaneira Road: MZUSP 8053 (16°15'35"/56°37'37"W); Várzea Grande, Cristo Rei: UFMT-R 11907 (15°38'49"S/56°07'59"W); Xingu Indigenous Park: MZUSP 5511, 5516 (10°05'03"S/52°59'58"W). **Pará**: Almeirim, Canal do Navio, Projeto Araima: MPEG 16377, 19590 (1°31'49"S/52°34'08"W); Almeirim, Canal do Navio, Projeto Araima, Várzea, Amazonas River: MPEG 19591 (0°10'13"S/49°00'40"W); Aveiro (3°14'59"S/55°09'59"W; MPEG 25737); Cachoeira do Arari, do Marajó Island, Tia Bita Island: MPEG 636, 639 (1°00'58"S/48°57'01"W); Belém, MPEG Campus: MPEG 24539 (1°25'57"S/48°23'03"W); Benevides: FUNED 1998, 2000 (1°22'03"S/48°15'09"W); Chaves, Tijuquara Farm, Tijuquara River, Marajó Island: MPEG 17718–19 (0°59'60"S/49°54'60"W); Gurupá, Gurupá Canal: MPEG 15157 (1°13'04"S/51°33'19"W); Juruti: MPEG 22487; 24963 (2°9'31"S/56°5'21"W); Marajó, Igarapé Taperebá: MZUSP 3605 (1°00'55"S/49°30'00"W); Melgaço, Caxiuanã National Forest, Ferreira Pena Scientific Station, Curuá River: MPEG 19960 (1°50'45"S/51°43'28"W); Melgaço, Caxiuanã National Forest, Ferreira Pena Scientific Station: MPEG 21191 (1°59'21"S/51°47'08"W); Oriximiná MZUSP 4793 (1°45'56"S/55°51'45"W); Oriximiná, church: UFMT-R 11078 (1°45'53"S/55°51'35"W); Oriximiná, Sapucuá Lake, UFMT-R 11076 (1°37'00"S/55°56'00"W); Salinópolis: MPEG 18966 (0°36'59"S/47°19'49"W); Santa Cruz do Arari, Marajó Island: MPEG 19619 (0°40'23"S/49°9'15"W) and MPEG 19618 (0°40'6"S/49°10'19"W); Santarém: MPEG 26527 (3°17'33"S/53°38'25"W); Santarém, Carariacá: UFOPA-H 729, 739–40 (2°13'40"S/54°51'02"W); Santarém, Tapajós River: MPEG 18828 (2°24'32"S/54°42'40"W). **Rondônia:** Costa Marques: MCP 8712 (12°24'45"S/64°08'23"W); Costa Marques, Guaporé River: MCP 6258 (12°25'22"S/64°11'39"W); Porto Velho, Jirau Hydroelectric Power Plant, right bank of the Madeira River: MZUSP 19695 (9°28'01"S/63°41'02"W).

***Helicops modestus* (*n* = 21). BRAZIL: Goiás**: Cabeceiras, Lagoa Formosa: MZUSP 3848 (15°47'08"S/46°57'59"W); Aporé, Espora Hydroelectric Power Plant: MNRJ 14357–60 (18°40'00"S/51°52'00"W). **Minas Gerais**: Poços de Caldas, Alcoa: MZUSP 14063 (21°47'19"S/46°33'43"W); Santana do Riacho, Serra do Cipó: MNRJ 12590 (19°20'00"S/43°37'00"W); Perdões, José Mendes Júnior Hydroelectric Power Plant, left bank of Rio Grande: MNRJ 9447 (21°08'32"S/45°02'11"W). **Santa Catarina**: BR-101 Road, 11.6 km from detour to Itapoá: MZUSP 19577. **São Paulo**: Cotia, Caucaia do Alto: MZUSP 13238 (23°41'07"S/47°01'07"W); Cotia: MPEG 19204 (23°41'00"S/47°01'00"W); Igaratá: MPEG 19201–02 (23°12'40"S/46°08'46"W); Interlagos: MPEG 19203 (23°42'13"S/46°42'24"W); Iperó: MZUSP 13081–82 (23°21'01"S/47°41'19"W); São Roque, Valparaíso Ranch: MNRJ 14046 (23°31'00"S/47°08'00"W); Rio Claro: MNRJ 25990 (22°23'00"S/47°36'00"W); Anhembi, Rio Piracicaba: MNRJ 21920 (22°42'00"S/47°27'00"W); Anhembi, Barreirinho Ranch: MNRJ 21621–22 (21°38'00"S/46°51'00"W).

***Helicops nentur* (*n* = 1).** Brazil. **Minas Gerais**: Muriaé, District of Macuco: ZUFMS 2291 (21°08'00"S/42°23'00"W).

***Helicops pastazae* (*n* = 2). COLOMBIA: Meta**: Guejar River, north of La Macarena: MZUSP 6111(2°44'00"N/73°54'00"W). **ECUADOR: Pastaza**: Huiyayacu River, upper Corrientes River: MZUSP 14108 (2°22'00"S/76°25'00"W).

***Helicops petersi* (*n* = 1). ECUADOR: Napo**: right bank of Rio Misahualli (0°59'26"S/77°47'48"W; USNM 196360).

***Helicops phantasma* (*n* = 29). BRASIL: Maranhão:** Carolina, Estreito Hydroelectric Power Plant: MZUSP 19309–20 (7°07'14"S/47°37'15"W). **Mato Grosso:** Barra do Garças: UFMT-R 8290 (15°21'34"S/52°58'15"W). **Tocantins:** Darcinópolis, Estreito Hydroelectric Power Plant: MZUSP 19308 (6°51'22"S/47°30'40"W).

Lajeado, Luiz Eduardo Magalhães Hydroelectric Power Plant: MZUSP 14906–07, MZUSP 14911–12, MZUSP 14917 (9°44'58"S/48°23'57"W); Lajeado Hydroelectric Power Plant: IBSP 65824, 66206 (9°45'05"S/48°21'29"W); Palmas, Lajeado Hydroelectric Power Plant: IBSP 65823, 66413, (10°12'46"S/48°21'37"W), CHUNB 16176 (10°11'21"S/48°06'31"W); Porto Nacional, Lajeado Hydroelectric Power Plant: IBSP 65592–93, 66089, 66446, 66656 (10°42'29"/48°25'02"W).

Helicops polylepis **(*n* = 56). BOLÍVIA: Beni**: Trinidad: MZUSP 8314 (14°47'08"S/64°47'02"W). **BRAZIL: Acre**: Rio Branco, Km 23 of road AC. 010: MPEG 20516 (9°58'29"S/67°48'36"W). **Amazonas**: Apuí: CHUNB 38502 (07°11'50"S/59°53'29"W); Itacoatiara: MNRJ 17940 (3°09'00"S/58°23'00"W); Novo Airão: MPEG 24374 (2°37'17"S/60°56'39"W); São Sebastião, Abacaxis River: MTR 12961 (4°18'32"S/58°38'11"W); Maruim, Abacaxis River: MTR 13320 (4°01'39"S/58°20'52"W). **Mato Grosso**: Chapada dos Guimarães: MZUSP 11961, UFMT-R 506–07, 1218–19 (4°52'15"S/55°47'07"); Colniza: UFMT-R 6683, 7796 (8°55'04"S/59°27'58"W); Coxim: MZUSP 8367 (18°29'25"S/54°45'40"W); Nova Xavantina, Das Mortes River: MNRJ 7768, 9292 (14°40'09"S/52°21'10"W); unknown locality: MNRJ 215, 9285. **Pará**: Ananindeua, Pirelli Farm, Uriboca River: MPEG 18535 (1°21'56"S/48°22'20"W); Belém: MPEG 13572 (1°27'21"S/48°30'16"W); Barcarena: MPEG 16440 (01°30'21"S/48°37'33"W); Belém: MPEG 18529 (1°27'00"S/48°29'00"W); Belém, Lauro Sodré passage, Terra Firme: MPEG 18160 (1°27'00"S/48°27'00"W); Belém, Jararaca Island, near Icoraci: MPEG 17680 (1°20'00"S/48°32'00"W); Cipó-Pitanga Island, Souzel Bay, Xingu River: MPEG 16715 (unknown coordinates) Faro: MPEG 18359, 18523 (2°10'17"S/56°44'42"W); Marajó Island: MPEG 17720 (0°56'42"S/49°38'20"W); Jacundá, left bank of Tocantins River, 7–8 Km south from Jacundá: MPEG 16754 (4°27'03"S/49°06'59"W); Juruti, Capiranga Base: MPEG 21203, 22672, 23248, 23250 (2°09'08"S/56°05'32"W); Melgaço: MPEG 21855, 22140 (1°48'51"S/51°20'44"W); Melgaço, Caxiuanã National Forest, IBAMA Administrative Center: MPEG 21855–57 (1°47'52"S/51°25'9"W); Melgaço, Caxiuanã, Marinaú: MPEG 22140 (1°48'51"S/51°20'44"W); Melgaço, Caxinauã National Forest: MPEG 21188–90 (1°47'37"S/51°26'05"W); Porto de Moz: MZUSP 18645 (1°45'00"S/52°14'00"W); Viseu, Colônia Nova: MPEG 15091, 15105, 15109–10, 15115, 15118, 15120 (1°11'48"S/46°08'24"W). **Rondônia**: Cachoeira de Nazaré, Machado River: MZUSP 9037 (9°45'09"S/61°55'07"W); Candeias do Jamari, Samuel Hydroelectric Power Plant: CEPB 981 (8°45'03"S/63°27'14"W); Porto Velho, Jirau Hydroelectric Power Plant, left bank of Madeira River: MZUSP 18893 (9°15'51"S/64°40'18"W). **COLOMBIA: Amazonas**: Leticia, Amacayacu Natural National Park: UNC/MLC 8524, 8579 (3°27'31"S/70°08'43"W).

Helicops scalaris **(*n* = 6). COLOMBIA: Norte de Santander**: Cucuta, Pamplinita River: CBB 755, 762, 767 (7°53'20"S/72°29'48"W); El Zulia, Zulia River, west of Cucuta: CBB 761 (7°56'14"S/72°36'17"W). **VENEZUELA: Zulia**: Maracaibo: MBLUZ 012, 285 (10°40'00"S/21°37'00"W [approximate coordinates]).

Helicops tapajonicus **(*n* = 3)**. Brazil. **Pará**: Brasília Legal: MPEG 25735–36 (3°55'20"S/55°35'39W); Santarém, Tapari Village: MPEG 26524 (2°26'48"S/54°53'36"W).

Helicops trivittatus **(*n* = 16)**. Brazil. **Maranhão**: Carolina, Estreito Hydroelectric Power Plant: MZUSP 19299–300 (7°07'14"S/47°37'15"W). **Mato Grosso**: Barra do Tapirapés: MZUSP 3815 (10°39'00"S/50°36'00"W); Coronel Vasconcelos, Xingu: MNRJ 7784 (10°32'2"S/53°44'59"W); Diauarum, Xingu Indigenous Park: MZUSP 5506 (11°15'00"S/53°12'00"W). **Pará**: Belém, Augusto Montenegro Road: MPEG 18699, 18943 (1°27'00"S/48°29'00"W); Belém, Lago da Bolonha: MPEG 19297 (1°27'00"S/48°29'00"W); Marabá, PA 15 Road: MPEG 25933 (5°15'13"S/49°3'55"W); Melgaço, Caxiuanã National Forest, Marinaú: MPEG 21858–59 (1°48'51"S/51°20'44"W); Melgaço, Caxiuanã National Forest, Estação Ferreira Pena Scientific Station: MPEG 19971, 20482, 21187 (1°47'52"S/51°26'06"W). **Tocantins**: Palmas: MZUSP 14299 (10°12'48"S/48°21'38"W); Luís Eduardo Magalhães Hydroelectric Power Plant: MZUSP 14303 (9°45'20"S/48°22'56"W).

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